Series Editor's Preface

I am glad to introduce this new volume on *Chemical Test Methods of Analysis* by Yu.A. Zolotov, V.M. Ivanov and V.G. Amelin. The reason for having such a book in the *Comprehensive Analytical Chemistry* series is quite obvious: on-site analysis has grown considerably in the last few years as a consequence of the need to develop fast methods of screening in the field. By performing such tests, a considerable number of samples can be screened rapidly and an appropriate selection can be made for further analytical investigations. So, overall chemical test methods of analysis can save a lot of time, either in environmental monitoring programs, or in clinical or medical applications where a large number of samples should be processed quickly.

The *Comprehensive Analytical Chemistry* series has already covered this topic in one of its earlier titles, Volume X: *Organic Spot Tests Analysis*. By publishing this volume now we focus the attention that is needed on this topic in analytical chemistry. This book contains chapters that cover the general future of tests systems including the general requirements and metrology, chemical principles of test methods and different methods and tools for analysis of liquid samples. Particular attention is given to application areas, with a comprehensive list of determinations of both inorganic and organic components in water and soil.

The book can be used as an academic text and as reference book both for those working in routine chemical analytical laboratories who will probably learn some new chemical assays and for those who want to start with on-site analytical techniques. Overall this book covers an important technique, sometimes underestimated in the field of analytical chemistry, although there is no doubt about the usefulness of the technique for solving everyday problems in chemical analysis.

Finally I would like to thank all three authors of this book for their time and effort in preparing this excellent and useful book on chemical test methods of analysis.

D. Barceló

Authors' Preface

Chemical analysis moves from the laboratory to the site where samples to be analysed are located (aptly termed "on-site analysis"). A popular dictum today is "from sample transfer to information transfer", and this is very true. Besides, owing to the ever-increasing number of samples to be analysed, preliminary screening and selection of samples seems to be necessary, even in the laboratory.

Rapid test methods of chemical analysis can solve both these tasks. Thus, this book is devoted to test methods that are widely used in environmental, industrial, clinical, forensic, medical, and other areas and which allow rapid, simple and cost-effective analysis – qualitative, semi-quantitative and quantitative — to be performed by trained and untrained personnel alike.

Some general characteristic features of test methods and test systems are described in the book, e.g. definitions, advantages and limitations, chemical and physical principles of operation, procedures and protocols, and methodological aspects.

An extensive overview is given of the application of test methods in various areas, and the test means and test tools pertinent for solving each concrete analytical task are discussed, e.g. paper strips, indicator powders and tubes, tablets, etc. The most important applications of test methods are: testing for inorganic and organic components in water (mostly for purposes of environmental control), monitoring of toxic gases and alcohol vapours, detection of narcotics and explosives, determination of glucose, cholesterol, and other components of medical importance.

The help of our colleagues and co-workers in developing new test systems and preparing this book is gratefully acknowledged.

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Chapter 1

General Features of Test Systems

1.1 FIELDS OF USE AND ADVANTAGES OF TEST SYSTEMS, TERMINOLOGY

1.1.1 Terminology

We begin with a brief overview and definition of some basic terms used in this book.

Testing (test) in chemical analysis — a quick and simple estimation of the presence of chemical components and/or their concentrations in the sample under study.

Test tools — compact, light and usually cheap single devices, kits, or sets of such single devices used for testing.

Test systems for chemical analysis — combinations of simple, portable, light, and cheap analytical tools and corresponding rapid procedures for detection and determination of substances that do not require or involve complex sample treatment (sometimes even sampling), complicated stationary instruments, laboratory equipment, a laboratory itself, complicated treatment of results or trained personnel. In most cases, self-contained and single-used tools are applied.

Test procedure (instruction) — describes the way of performing test operations including sampling (if necessary), detecting, and determining a component or a parameter.

Test form — an analytical form of a reagent or a combination of reagents and various additives adapted to testing conditions and ready for use under such conditions.

Essentially all chemical test methods are based on analytical reactions and reagents in certain forms which, under chosen test conditions, provide observable and easily measurable effects, such as, for example, a colour change of test paper or the length of the coloured part of a tube. The reagents and different additives are used in the form of ready-to-use solutions (in ampoules or droppers) or immobilized on a solid support, e.g., paper, silica gel, polyurethane foam, etc. In fact, indicator papers, indicator tubes, tablets, powders, and other test systems are widely used in test methods.

1.1.2 Fields of use and advantages of test methods

In many cases test methods are used for a preliminary estimate of a component's presence or concentrations. In these cases a screening methodology is indicated (see Chapter 5). Quite valuable are test systems for determining total parameters, e.g., chemical oxygen demand (COD) or sum content of heavy metals in waters. It may be that as test methods become more and more reliable they will acquire the status of a sole and ultimate method of chemical analysis on a par with established instrumental methods of chemical analysis.

Test methods may be used for regulating dosage appliances, e.g., insulin pumps for diabetic patients. A special group of chemical dosimeters are used for estimating the amount of a substance accumulating in a medium under study over a given time period. Of especial importance are test methods for the so-called on-site (out-of-the-lab) analysis.

For ages, beginning with alchemists, chemical analysis has been performed in laboratories. This is so because a special chemical glassware had to be used, special equipment, e.g. muffles, and, later, measuring instruments. Toxic substances necessitated the use of good ventilation. Time and labour-intensive complicated operations of substance separation had to be performed. All these factors are in operation to this day, and this is why an innumerable host of chemical analyses is carried out in analytical laboratories, including those for physical and biological studies. Thus, any one chemical, geological, or metallurgical research institute has its own chemical analytical laboratory equipped with sophisticated and expensive analytical instruments, and the same applies to any chemical, oil refining, pharmaceutical or metallurgical plant.

The situation has begun to change of late: chemical analysis is gradually moving from the laboratory to the place where the object to be tested is located. This is a major development trend of modern analytical chemistry, evidenced, for example, by the creation of a new journal highlighting the problems of field analysis (*Field Analytical Chemistry and Technology*, J. Wiley and Sons) or by international conferences ("On-site Analysis... the Lab comes to the Field", since 1980).

The truth of the matter is that there exists a vast and as yet unsatisfied demand for out-of-the-lab chemical analysis. Here is a rather incomplete list of areas where such analysis is already being employed, or is just emerging.

- Rapid control of technological processes;
- Detection of methane in coal mines;
- Detection of gas leakages in gas pipe lines;
- Detection of carbon monoxide and hydrocarbons in automobile exhausts;
- Rapid field analysis in geological prospecting;
- Rapid soil analysis (pH, nitrogen, phosphorus);
- Foodstuffs control at market places;
- Detection of alcohol in the driver's breath;
- Determination of sugar in blood and urine of diabetic patients at home, and other rapid clinical tests;
- Operative analysis of water (including drinking water) by the consumer;
- Air analysis in work zones and in the street;
- Ozone determination in the stratosphere;
- Space research (the moon and planets);
- Narcotics detection in airports;
- Detection of chemical weapons;
- Detection of rockets' liquid fuel in the polluted soil at sites of rocket landing and at disaster areas;
- Detection of explosives.

An advantage of on-site analysis is that much time and cost are saved when the need for the sample to be shipped to the laboratory and a costly laboratory analysis is eliminated. Besides, less well trained personnel can do the job, as simple and easy-to-use test methods are employed. However, the most important advantage is that sometimes laboratory analysis cannot be performed (or, if performed, is meaningless) due to the degradation or change of components during shipment; this can be deduced from the examples already cited. On-site analysis is a real, or practically real-time analysis permitting immediate elimination of sources or causes of dangerous situations without waiting for the results of a laboratory investigation.

It is evident that the role of the laboratory tends to diminish while that of on-site analysis grows. Field analytical methods become vitally important and such organizations as the US Environment Protection Agency (EPA) issue pertinent documentation on the use of such methods, e.g. EPA-542-R-97-011, and many others. *Current Protocols in Field Analytical Chemistry* is published quarterly [1]. The dictum much in use nowadays is: From Sample Transfer to Information Transfer.

It is important to note that the advances in analytical chemistry, analytical instrumentation and related areas make possible such an analysis and make it very effective in a number of cases. Several groups of tools without strictly defined boundaries can be used for carrying out on-site analysis.

Among these are mobile laboratories organized on automobiles, motorboats, aircraft, etc., where more or less traditional chemical analysis procedures and instruments are used. Usually the instruments are made more vibration and dust resistant and autonomously powered, but, in the main, they are traditional laboratory equipment. These mobile laboratories are manufactured by a number of firms. In St. Petersburg a motorboat chemical laboratory has been designed, and in Moscow an on-car laboratory has been developed; an aeroplane version of such a laboratory is used for aerosol analysis. In USA mobile laboratories are leased by On-site Instruments, Inc.

Of a more widespread use are portable hand-held battery-powered analytical instruments, often, but not always, monofunctional, i.e. intended for determination of one given substance — analyzers, monitors, and indicators. In these devices, an analytical procedure is usually built into the design and cannot be changed. Sometimes no sample collection is needed. Of late, portable multifunctional instruments have begun to be used, e.g., chromatographs, but they are still not of portable weight and, more importantly, require trained personnel to operate.

Instruments of this type are various and are widely used. For example, dissolved oxygen was determined by means of a portable DO-meter New Rapid Pulse™ (YST, Yellow Springs, Ohio) during a large-scale screening of river water in Western Connecticut [2].

A comparatively new trend in instrument-making has emerged, viz., multifunctional on-chip devices (micro devices), which have already been used in capillary electrophoresis and flow injection analysis. If this trend gains momentum, a revolutionary change in all analytical instrumentation is easy to predict. There will be a dramatic decrease in materials and reagents expenditure, power consumption, amount of test sample needed, and workplace area.

In the near future arrays of chemical sensors will come into use. A chemical sensor is usually a small, sometimes miniature, transducer placed in the analysed medium for the continuous and reversible monitoring of a concentration of a given component in this medium as in the case of a hygrometer registering humidity or a thermometer measuring temperature. Chemical sensors are in prospect for sensitive out-of-the lab diagnostics, control and monitoring, say, chemical reactors or some systems in aircraft. But as yet sensors are rarely used for on-site analysis.

Finally, widely used and low-cost tools of on-site analysis are, without doubt, test systems. Practically all tasks of out-of-the-lab analysis mentioned above can be solved or are being solved by means of test systems. These include a procedure and an appropriate simple test means — paper test strips, powders, tubes, tablets, ampoules, droppers, etc. Occasionally, a test system includes a portable, usually pocket-sized, instrument.

Test methods make possible large-scale screening of, for example, environmental samples. Negative samples are sorted from positive ones; the latter should be subjected to a more thorough analysis, including that with the use of sophisticated laboratory equipment. Another area is the flow control of technological processes; often what is needed is a quick estimate of a concentration close to permissible just at the technological unit. It is meaningless to send labile time-dependent samples to the laboratory; they have to be measured on site. Test means are important for clinical monitoring; thus, they are invaluable for a quick measurement of glucose in the blood of diabetic patients in out-of-the-lab conditions.

Test systems have a long-standing reputation in air monitoring of factory work zones, especially those based on indicator tubes. They are also employed for detecting chemical weapons and narcotics.

1.2 SOME HISTORY

A great many chemical methods for qualitative analysis can be considered as test methods; some have been known from times immemorial.

Pliny the Elder (Caius Plinius Secundus, 23–79 AD) wrote that in order to determine iron in vinegar the Romans used papyrus impregnated with an extract of acorns. The same extract allowed iron sulphate to be distinguish from copper sulphate by a black colour formed in the presence of iron and a green colour formed in the presence of copper. This method was in use for a very long time.

In the 17th century Robert Boyle used extracts of violets, besides acorns, to identify acids and alkalis by the green colour of the extract which develop in the presence of alkalis. He also used extracts of cornflowers and cochineal; litmus was also used. Boyle noted that the black colour intensity of the acorn extracts depended on the iron content; this was a first step towards colorimetric analysis.

One of the oldest and most widely used methods of gold detection in alloys and gold articles is that using the touchstone. The method in its initial form was described by Theophrastus (372–287 BC) in his treatise “On stones”. The method was significantly improved in the middle ages; in the 16th century 23 test needles each with a different gold content were introduced. Details of this test in its modern version are given in Chapter 11.

Galen (ca. 200–130 BC) used hens’ eggs for a rough determination of salt content in water, viz., the density of salt water.

In 1767 Lewis obtained litmus paper and used it for detecting acids and alkalis. In 1817 Prout reported the use of litmus paper for measuring alkalinity of urine.

Runge (1745–1807), while studying dyes, elaborated a series of drop reactions on paper and cloth, and also on porcelain plates. He immersed pieces of paper or cloth in the solution under study and made the necessary estimates by the speed with which the tested liquid or dye ascended the test strip. He reported these experiments in “The Chemistry of Dyes” (1850) and in his other works; he also published an album of natural spots formed by dyes on filter paper with different components.

In 1834 Runge developed a near-modern test for the rapid determination of free chlorine in the form of paper impregnated with starch and potassium iodide; when the paper interacted with the test solution iodine was liberated and a blue colour appeared (a well known iodine–starch reaction).

Schiff proposed a test in 1859 for uric acid using paper impregnated with silver carbonate. Test paper for detecting glucose in urine was proposed in 1883 (G. Oliver).

In chemistry history books one can find a multitude of examples of determinations, performed virtually by test methods.

Göppelschröder (1862–1910) was close to the creation of the method nowadays known as paper chromatography, but he stopped short of spot development, confining himself to a visual detection of naturally formed coloured zones. He called his method the "capillary analysis method".

In 1909 Kharichkov proposed a simple drop test for revealing hydrogen peroxide. Filter paper turned pink on impregnation with a solution of cobalt naphthenate in petrol. It became deep olive-green on contact with a hydrogen peroxide solution due to oxidation of Co(II) to Co(III); 0.33 wt % of H_2O_2 could be detected [3].

Simple chemical methods under the general term of "spot tests" were developed by Feigl in Austria and by Tananaev in Russia. Feigl collected and classified (and developed) a host of methods for determining inorganic and organic compounds (for reviews see Refs. [4,5]). An update of this method of analysis was made by Yungreis in two editions of his monograph [6,7]. In the second edition of this excellent book a great many test methods are described. A relatively new approach to testing was provided by a ring-oven technique proposed by Weisz [8–10].

It should be noted, however, that the majority of old or comparatively old test methods could be used only in the laboratory. The methods were predominantly qualitative and those that were quantitative were not sufficiently sensitive.

Reviews of later works, including the previously mentioned books by Yungreis, can be found in Refs. [11–15], in a book by Schwedt [16], and in Merck Co. catalogues [17]. Paper-based tests have recently been reviewed by Amelin [18].

1.3 CLASSIFICATION OF TEST METHODS

Classification of test methods of chemical analysis can be done in several ways, depending on the classification criterion adopted.

Proceeding from the nature of processes generating an analytical signal, test methods may be divided into physical, chemical, biochemical and biological. There are few physical methods and they are not important in chemical analysis.

Biochemical methods are usually based on the use of enzymes and immunoassay systems. The isolated natural enzymes, especially immobilized ones, acquire in some measure properties of chemical reagents, therefore these methods can be called chemical regardless of the enzymes' chemical specificity, origin, conditions of storing and activity preservation times; immuno-based methods are, in our opinion, closer to biological. In this book enzyme methods are considered together with chemical ones. As to immunoassay methods, they are considered briefly in the practical part of the book (due to their importance in practical analysis); for the rest they are treated rather sketchily in the main body of text.

Biological methods based on the use of microorganisms, organs, tissue, and even highly organized organisms and whole populations are mentioned only in the section devoted to determination of sum parameters (biotests).

As the title of the book implies, it deals primarily with the chemical methods of analysis.

Yet another classification can be based on the forms in which test reagents are used. These are, primarily, ready-to-use solutions and "dry reagents", viz. those immobilized on a solid support, or simply powders and tablets of the reagents proper. There are various ways of working with solutions, e.g. via droppers or self-filling ampoules prepared under vacuum, etc. Still more diverse are tests on solid supports; the most well known example – indicator papers with reagents or active reacting groups, or indicator tubes for gas analysis, in which the support contains a chromogenic reagent changing colour on contact with the target gas.

Reagent supported methods differ in regards to the nature of the support, viz., cellulose, synthetic polymers, silica gel, etc., and the manner of immobilization, viz. adsorption or covalent binding. All the classifications given are more or less fully reflected in the table of contents of this book.

1.4 GENERAL REQUIREMENTS AND METROLOGY

The characteristic features of test methods discussed so far may be viewed as the general requirements that test methods and test means have to meet. Only a few additional remarks remain to be made.

1. As test methods are often used for preliminary estimations and for screening, when estimating the presence of a test component, a false positive statement is to be preferred to a false negative statement.
2. As one of the merits of test methods is their speed, the chemical reactions used must have high rates.

3. As test methods are intended for a wide use by chemically unqualified personnel and simply laymen, the number of test operations should be made minimal.
4. To make the visual detection reliable and unambiguous, the boundaries between differently coloured zones should be sharp and the colour of the zones well defined (of good contrast). In other words, the probability of ambiguous interpretation of results should be reduced to a minimum.

Now let us consider in some greater detail some metrological aspects of quantitative determinations.

The metrological aspect is, of course, very important when developing and using test systems; the results obtained should be trustworthy especially when small concentrations of target components are determined. The accuracy of test methods is usually verified by comparison with instrumental methods of analysis. This procedure is always used when developing test methods but the user of test methods is advised to undertake such a verification in order to have greater confidence in his test results. Many test systems are not universal and are intended for determination of components in fixed samples.

Reproducibility of visual measurements is characterized by the relative standard deviation (RSD). For colour reactions, it is usually 10–50%. That is why test methods should be considered as semi-quantitative. But sometimes we have cases where reproducibility is much better.

Amelin reported data on reproducibility of test results for indicator papers [19]. He characterized the reproducibility with RSD. He considered various methods for determination of concentration. Near the determination limits, RSD ≤ 0.1 for test titration and test tubes. When concentrations were determined using the paper coloured (discoloured) zone length or area, RSD ≤ 0.3 was obtained, and for a visual estimate of colour intensity of a liquid, RSD was 0.4 or less. The least precision was noted for indicator papers (RSD ≤ 0.5). These data were obtained for the case where real samples were studied at a level of determination limits and the confidence level of 0.95. As a rule, at greater concentrations RSD values are lower. The use of portable instruments measuring coefficients of diffuse reflectance (R) permits a considerable improvement of reproducibility (RSD ≤ 0.08). The results were quantified using the Gurevich–Kubelka–Munk function ($F = (1 - R)^2/2R$).

As regards sensitivity (detection limit) of test methods one should correlate it with the practical demands of real life taking into account the general trend in designing more sensitive techniques. For example, for environmental monitoring one should have tests permitting detection of specified substances at levels somewhat lower than permissible ones.

Sensitivity depends on a number of factors, such as type of test means, reagents used, analysis procedure. There are very sensitive tests, e.g. down to 0.005 mg/l of copper can be detected with indicator papers [20]. When 20 ml of test solution is passed through a test paper using a simple concentrating device, copper detection limit comes to 0.001 mg/l. Of special interest are kinetic, in particular, enzymatic methods allowing still greater sensitivities to be obtained; when detecting mercury by its inhibiting effect on horseradish peroxidase a record detection limit of $1 \cdot 10^{-5}$ mg/l was achieved. A research group in Khar'kov proposed a method for estimating detection limits of test methods based on principles developed by Komar [24].

1.5 LIMITATIONS OF TEST METHODS

Martina Unger-Heumann is of the opinion that the majority of test systems based on chemical and physicochemical principles are unable to detect trace and ultratrace amounts of substances [25,26]. She notes that even sensitive photometric methods can determine concentrations only in the range of 1 mg/l. This may be so, but in some cases detection limits in test methods are much lower than that, especially if immunoassay techniques are applied.

As a rule, test methods allow only one component or one parameter of a system to be determined. There are cases when two or more components can be revealed, but these are few in number. Simultaneous use of several test methods for a multicomponent analysis is, as a rule, inconvenient.

REFERENCES

1. D. Barcelo, W. Beckert et al. (Eds.), *Current Protocols in Field Analytical Chemistry*, Wiley, New York.
2. *Am. Environ. Lab.*, No. 2 (1999) 17.

3. K.B. Kharichkov, see *Zh. Ros. Khim. O-va im. D.I. Mendeleeva*, 41 (1999) 1821.
4. F. Feigl, *Spot Tests in Organic Analysis*, Elsevier, Amsterdam, 1960.
5. F. Feigl and V. Anger, *Spot Tests in Inorganic Analysis*, Elsevier, Amsterdam, 1972.
6. E. Yungreis, *Spot Test Analysis. Clinical, Environmental, Forensic, and Geochemical Applications*, Wiley, New York, 1985.
7. E. Yungreis, *Spot Test Analysis. Clinical, Environmental, Forensic, and Geochemical Applications*, 2nd Edn., Wiley, New York, 1997.
8. H. Weisz, *Microchim. Acta* (1954) 140.
9. H. Weisz, S. Pantel and R. Giesin, *Anal. Chim. Acta*, 101 (1978) 187.
10. H. Weisz, *Microanalysis by Ring-Oven Technique*, Pergamon Press, Oxford, 1970.
11. V.M. Ostrovskaya, *Zh. Anal. Khim.*, 32 (1977) 1820.
12. L.J. Holcombe, *Environ. Testing Anal.,* Jan.-Feb. (1994) 30.
13. Yu.A. Zolotov, *Ann. Chim. (Rome)*, 87 (1997) 284.
14. Yu.A. Zolotov, *Vestn. Ros. Akad. Nauk*, 67 (1997) 508.
15. Yu.A. Zolotov, *Khim. Prom-st. (Moscow),* No. 6 (1997) 48.
16. G. Schwedt, *Mobile Umweltanalytik*, Vogel, Würzburg, 1995.
17. *Schnelltest Handbuch*, Merck, Darmstadt, 1986.
18. V.G. Amelin, *Zh. Anal. Khim.*, 55 (2000) 902.
19. V.G. Amelin, *Zh. Anal. Khim.*, 55 (2000) 411, 532.
20. V.M. Ostrovskaya, *Zh. Anal. Khim.*, 54 (1999) 1126.
21. M.S. Kravchenko, M.S. Fumarova and A.A. Bugaevski, *Int. J. Environ. Anal. Chem.*, 33 (1988) 257.
22. R.P. Pantaler, N.B. Lebed', L.I. Avramenko and A.B. Blank, *Zh. Anal. Khim.*, 52 (1997) 643.
23. R.P. Pantaler, L.A. Egorova, L.I. Avramenko and A.B. Blank, *Zh. Anal. Khim.*, 52 (1996) 997.
24. N.P. Komar', *Osnovy Kachestvennogo Khimicheskogo Analiza (Fundamentals of Qualitative Chemical Analysis)*, Vol. 1, Khar'kov. Gos. Univ., Khar'kov, 1955.
25. M. Unger-Heumann, *Fresenius' J. Anal. Chem.*, 354 (1996) 803.
26. M. Unger-Heumann, in H.-M. Kuss and U. Telgheder (Eds.). *Modern Methods of Analytical Chemistry, Proceedings 5, ARGUS, 1997.* G. Merkator Univ. Duisburg, Germany, 1997, pp. 444–452.

Chapter 2

Chemical Principles of Test Methods: Reactions and Reagents

2.1 SELECTION OF REACTIONS AND REAGENTS

2.1.1 Some general remarks

For the most part, the tests described in this book are based on chemical analytical reactions. In spite of the common trend to use highly sensitive and selective reactions, more or less known reagents and reactions are usually used in test procedures. Challenges, which in many cases are difficult to meet, consist in selecting reactions with regard to special requirements imposed by test methods and adapting these reactions to test procedures.

For example, a conventional laboratory analysis may involve a series of consecutive procedures. These are separation, preconcentration, masking, oxidation–reduction reactions, and others. When performing tests, all or many operations should be performed almost simultaneously; all, or almost all of the required reagents should be included in a test kit (usually at the manufacturing stage). However, there are several reagents that were specially prepared for test kits.

The test chemistry is essentially based on colour reactions, e.g., redox reactions or complexation. Designers and manufacturers of test kits devote their efforts to searching for an optimal combination of reagents, stabilizing mixtures of reagents and solutions, and lowering interferences by masking. Their main purpose is to develop rapid and easy-to-use test systems.

Apart from colour reactions, chemical interactions resulting in luminescence are also used.

Analytical effects can be detected not only visually, but also measured with portable (usually pocket-type) devices. Most often the measured signal is transmission, diffuse reflectance, or luminescence.

The basic requirements imposed upon the reactions used in test procedures can be summarized as follows:

1. Selectivity to a single or several components to be detected or determined.
2. Sufficient sensitivity depending on the problem to be solved. For example, in environmental analysis, detection limits should be lower than the maximum allowable concentrations (MACs) or should be close to them.
3. Colour reactions should be characterised by a high contrast and a high rate of colouring when the compound to be determined is present.
4. Reagents should be easily transformed into a suitable form, i.e. in modifying a silica gel or cellulose surface.
5. Reagents should be stable in storage in the very form in which they are used in test procedures.
6. The analytical effect (colour, luminescence, etc.) should be stable for a sufficient time.

Nearly all types of chemical reactions are used in test systems and test tools: (1) acid–base reactions, (2) oxidation-reduction reactions, (3) complexation, and (4) various organic synthesis reactions. Catalytic reactions, especially enzyme ones, which is a somewhat separate case, are also of considerable importance in test procedures. Reagents of various mechanisms of action and of various natures, respectively, are used.

2.1.1.1 Acid–base reactions

Acid–base reactions are of great importance for pH determination. The determination of proton concentrations in analysis of natural and waste waters, technological solutions, and biological liquids is one of the most widely performed analytical operations. Despite the development of potentiometric techniques, pH determination with acid–base indicator papers remains a most common procedure. This procedure has several advantages: it is simple and rapid, and it requires no instrumentation. As a consequence, analyses are cheap and can be performed in almost any place.

The chemistry of acid–base indicators is well known [1]. However, such indicators should meet certain requirements and criteria imposed by test procedures. When an indicator is immobilized on the paper, the manner of immobilization is of prime importance [2]. Usually indicators are adsorbed on paper. However, this does not always ensure a strong immobilization. Therefore, the time of contact of the paper with the analysed solution should be very short. Direct (or substantial) indicator dyes (Congo Red or Brilliant Yellow) are immobilized through adsorption better than other indicators, because linear and non-coplanar molecules of these dyes bind to linear molecules of cellulose more firmly. The strongest binding of indicators to cellulose is ensured by covalent interaction [2].

2.1.1.2 Oxidation-reduction reactions

Oxidation-reduction reactions are also widely used in test procedures. When solid reagents are used, it is important to know whether redox potentials change after immobilization on solid matrices. There is no general solution to this problem; much depends on the way of immobilization, the nature of matrices, etc. We found that the redox potential of molybdophosphoric acid changed slightly after immobilization in silicic-acid xerogels.

The reaction of cobalt with 1-(2-pyridylazo)-2-naphthol (PAN) is highly selective [3]. It occurs in acidic media (pH 2–3) in the presence of KIO_4 that oxidizes cobalt(II) in the complex to cobalt(III). The resulting complex has an anomalous green colour. In this case, simultaneous preconcentration of cobalt on silica gel S-120 takes place. Therefore, this reaction is very sensitive: the detection limit is 0.004 mg/l. In this test procedure, silica-gel tablets are used, which contain all the necessary components. On destruction of a tablet in the analysed aqueous solution, cobalt(II) reacts with PAN, oxidises, and the complex is adsorbed on the matrix.

Among other examples, we can mention the reduction of gold and silver to the elementary state with the Mohr salt in the presence of complexants (to decrease the Fe(III)/Fe(II) potential). Gold forms blue spots on a paper strip while silver forms brown spots. Concentrations of the elements are proportional to spot diameters (analytical range for gold is 4–24 μg and for silver 2–12 μg) [4,5].

2.1.1.3 Reactions of complex formation

Complexation reactions are widely used in numerous test procedures for detection of metal ions, and less frequently in determining organic substances. There is rarely any one specific complexation reaction, which is why many test procedures involve pH adjustment, masking, and other ways of improving selectivity.

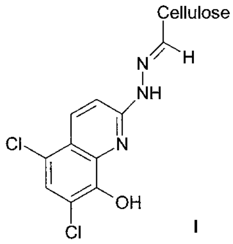
Dithizone is a widely used reagent [6]. It forms coloured complexes with many metal ions. According to their stability, complexes can be arranged in the following order: Ag > Hg > Pd > Pt > Au > Cu > Bi > In > Sn > Zn > Cd > Co > Pb > Ni > Fe(II) > Mn > Tl(I). Although dithizone is a group reagent for about 30 cations, a selective determination can be performed using variations in pH, masking, and substitution reactions. Thus, with thiourea and sodium acetate present, dithizone on an indicator paper was applied for determination of total heavy metals (with detection limit of 0.5 mg/l). A test system proposed for determination of zinc consisted of a paper strip impregnated with a mixture of dithizone (50 mg), thiourea (1 g), sodium carbonate (8 g), gelatine (2 g), ethanol (50 ml), and water (50 ml). This system permits the detection limit of zinc to be made as low as 0.5 mg/l. For determination of mercury and cadmium, cube-shaped polyurethane foam impregnated with dithizone and lead dithizonate was used. The cubes coloured after contact with the test solution. The analytical range for mercury was 0.2–10 mg/l; for cadmium 0.1–10 mg/l.

Trihydroxyfluorones [7], oximes [8], and dithiocarbamates [9] can be potentially used in test systems. These reagents are widely applied in photometry. They exhibit sufficient sensitivity and under proper conditions, selectivity. It will suffice to mention the reactions between trihydroxyfluorones and germanium, dimethylglyoxime and nickel, and dithiocarbamates and copper.

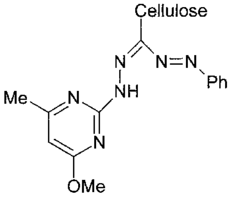
Amongst numerous reagents successfully used in test procedures for the determination of many metal ions, let us consider the reagents of two classes of organic compounds: new formazans and heterocyclic azo compounds [10].

Rigid-structure formazans provide a very selective interaction with a number of metal ions. Ostrovskaya found that the optical properties of their complexes permitted low detection limits. Compounds **I–IV** provide an example.

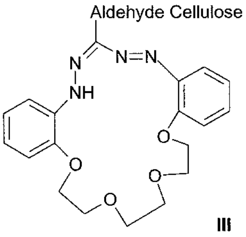
Reagent **I** was used for determination of Fe(II) and Fe(III) at pH 2–3. The proposed indicator paper strips provide for the determination of iron within three concentration ranges (by different procedures). The



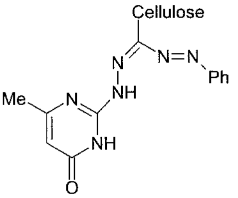
I



II



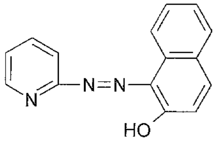
III



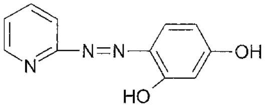
IV

detection limit for iron is 0.01 mg/l. Reagent **II** permits determination of zinc at pH 8 with a detection limit of 0.001 mg/l. A highly selective procedure for determination of mercury is based on macrocyclic formazan **III** at pH 2. Copper (down to 0.001 mg/l) can be determined with reagent **IV**. As for other reagents, they were added to cellulose. Cd, Co, Fe, Pb, and Ni do not interfere with the determination of copper at pH 1–5.

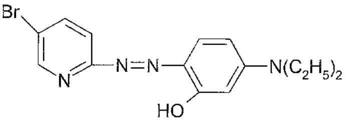
Heterocyclic azo compounds evidence a very high sensitivity in the determination of metal ions. The molar absorptivities of the complexes are as high as 3×10^5. The complexation reactions of these compounds are high-contrast ($\Delta\lambda \leq 150$ nm). The reagents can be easily immobilized on organic and inorganic supports [10]. In test procedures for determination of metal ions, silica gel tablets modified with heterocyclic azo compounds are used (e.g. made of Silochrom S-120). In this case, silica gel serves also for preconcentration of ions as complexes with azo compounds. In several cases, detection limits achieved were 2–3 orders of magnitude lower than MACs of ions in environmental objects; an example is provided by a test procedure for determination of cobalt with 1-(2-pyridylazo)-2-naphthol (PAN). The detection limit is 0.01 mg/l. PAN, 4-(2-pyridylazo)resorcinol (PAR), and 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol (5-Br-PAAP) immobilized on various supports are also used as test reagents:



PAN



PAR



5-Br-PAAP

PAN was immobilized on a Silochrom S-120 silica gel [3,11–15], a KU-2 cation-exchange resin [12], polyurethane foams [12], polyacrylonitrile fibres [16], organic polymer ion-exchange resins [17,18], naphthalene [19], and various celluloses (microcrystalline, carboxymethylcellulose, and epichlorohydrin triethanolamine cellulose (ECTEOLA cellulose)) [13]. The authors of Ref. [13] point out that silica supports exhibit the best properties. Adsorption interactions are primarily due to the most reactive surface silanol groups. Fully hydroxylated silica contains about 8 $\mu m/m^2$ of OH groups. PAR was immobilized on an S-120 Silochrom silica gel [13,15,20,21], polyurethane foams [20], and various celluloses [13]. Complete sorption was achieved in 30–60 min. For cellulose, the maximum degree of sorption was at pH ~5. For silicas, the maximum sorption was at pH 1–2 [13]. 5-Br-PAAP was immobilized on silica S-120 [15,22–24]; 4-(2-thiazolylazo)resorcinol (TAR), a thiazole analogue of PAR, was immobilized on silica S-120 [25].

Many reactions of heterocyclic azo compounds are selective. With sodium thiosulphate and sodium fluoride at pH 6 and 60°C, nickel forms a chelate with PAN. At room temperature this chelate is not formed. In contrast, zinc and cadmium complexes with PAN are formed under the same conditions but at room temperature. Fe(III) does not interfere with the determination. With bromide ions present, red-coloured Hg(II) complex with PAN on silica gives a yellow colour. Test reactions for copper, uranium, mercury, and palladium were proposed. The detection limits of the reactions are lower than MACs by the factor of 2–250 (Table 2.1).

Organic reagents immobilized on various supports can be classified as follows [26,27] (matrices and target elements are given in brackets).

Nitrogen-containing reagents: 2,2′-dipyridyl, 1,10-phenanthroline, bathophenanthroline, bathocuproine (silicas, cation-exchanger resins Dowex 50W-X2 and KU-23, polyurethane foams, and polyvinylchloride) — Fe(II) and Cu(I)).

TABLE 2.1

Test procedures for determination of metal ions with silica tablets (Silochrom S-120) impregnated with reagents

Tested ion	Reagent	pH	Detection limit (μg/ml)
Co(II)	PAN	3.0–5.0	0.004
	TAR	4.5–8.0	0.005
Pd(II)	PAN	3.0–4.0	0.1
	TAR	0.5–1.0	0.03
Hg(II)	PAN	3.0–5.0	0.003
U(VI)	PAN	6.9–8.2	0.1
	PAR	5.8–8.2	0.1
	5-Br-PAAP	4.5–5.5	0.1

Oxygen- and nitrogen-containing reagents: nitrogen-containing heterocyclic azo compounds (PAN, PAR, and others), Basic Blue K (KU-2) — Pb(II); dimethylglyoxime, benzyldioxime (S-120 and KU-2) — Ni; anthrazochrome (an anion exchanger AV-17) — Al(III); Eriochrome Black T (AV-17) — Zr(IV); zincon (Dowex 1X2) — Cu(II)); nitroxaminazo (AV-17) — Pd(II); sulphonitrophenol (capron membrane) — Pd(II); sulphochlorophenol S (AV-17) — V(V) and Mo(VI); Rhodazol X (capron membrane) — Cu(II), Ag(I), Au(III), Pb(II) and Pd(II); diphenylcarbazide (Dowex 50W-X2, fibrous materials filled with AV-17) — Cr(VI); 2,2′-diquinolyl-4,4′-dicarboxylic (bicinchoninic) acid (Sephadex QAEA-25) — Cu(I); *p*-nitrosodiethylaniline (KU-2) — Pd(II); nitroso-R salt (Sephadex QAEA-25) — Co(II); 5-bromosalicylhydroxamic acid (Sephadex QAEA-25) — V(V)).

Oxygen-, nitrogen- and sulphur-containing compounds: thiazole azo compounds (TAN, Br-TAN, TAN-3,6-S, TAAP are analogues of pyridine azo compounds) (KU-2, AV-17) — Cu(II), Fe(III), Ni(II), Pd(II), and U(VI); 4-phenolazo-3-aminorhodanine (polyacrylonitrile fibres filled with KU-2) — Hg(II).

Nitrogen- and sulphur-containing reagents: dithizone (fabric with vinylpyridinium groups) — Zn(II); diethyldithiocarbamate (polyacrylonitrile fibres filled with AV-17) — Cu(II); pyrazolinedithiocarbaminate (Amberlite A-27) — Fe(III).

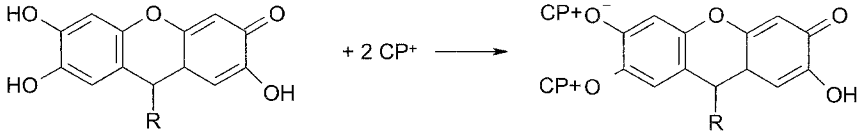
Oxygen-containing reagents: aluminon (Silasorb) — Cu(II); Catechol Violet (AV-17) — Mo(VI); Eriochromecyanine R (S-120 and cellulose) — Al(III); chromotropic acid (Dowex 1 × 8) — Ti(IV); phenylfluorone (fibrous material filled with AV-17) — Mo(VI); Tiron (LIK-21 silica gel) — Fe(II, III); sulphosalycilic acid (LIK-21 and AV-17) — Fe(III).

The classification of reagents based on the type of donor atoms is relative, because pH conditions of sorption depend on adsorbed ions. The nature of ions determines the form of the reagent and the complexation mechanism. Not all of the above support–reagent combinations are used in test procedures. However, the found optimum conditions for the sorption of metal ions permit us to perform the reactions in a test variant. Several examples are discussed below.

The selectivity of determination can be improved either through masking (as in photometric determination of these ions in solutions) or by using sufficiently selective reagents, e.g. dimethyl- or benzyldioxime (determination of nickel [28]), Tiron (determination of iron [29]), and sulphosalycilic acid (determination of iron [30]).

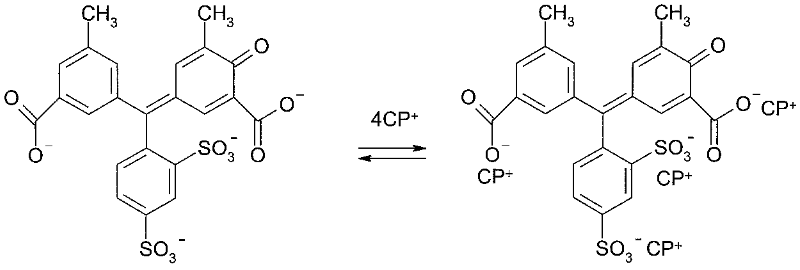
Reactions based on selective substitution of an ion in the complex with another ion are especially selective. Either the initial complex or the formed one should be coloured. In these cases, one can use the colour decay as an external effect in test procedures. For example, mercury(II) replaces copper selectively in copper dithiocarbamate (yellow-brown). This effect was used for a test determination of mercury [31]. For detection of copper, the colourless lead diethyldithiocarbamate was used. Lead is replaced selectively by copper. The resulting complex is coloured [32].

In several cases, modifiers of chromogenic reagents are used, especially cationic surfactants. Amelin [33] noted that ion associates thus formed provided for a higher sensitivity and, in several cases, better selectivity [34–37]. From this standpoint, associates of trihydroxyfluorones **V** and triphenylmethane reagents **VI** with cetylpyridinium chloride are efficient.



V

where R = C_6H_5, $C_6H_4NO_2$, C_6H_4COOH, $C_6H_3(SO_3H)_2$.



VI

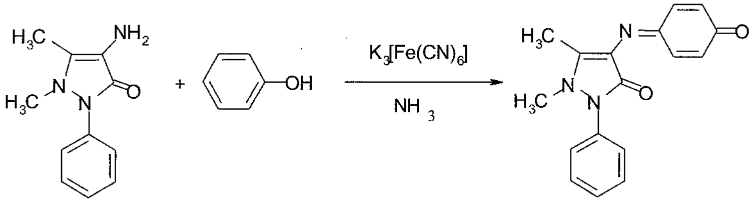
The associates are either poorly soluble in water and are adsorbed well on solid supports or are highly soluble and may be washed-off. Thus, they are used in various ways. Reactions of these associates with metal ions are also high-contrast. This approach made it possible to lower detection limits of Al, Ti(IV), Ge(IV), Sb(III), Mo(VI), W(VI), and other elements by a factor of 50–100 as compared with unmodified reagents [34–37].

2.1.1.4 Reactions of organic synthesis

Reactions of organic synthesis are less common. However, there exist examples of their application even in inorganic analysis, e.g. the determination of nitrite ions via diazotization and azo coupling reactions. Ivanov et al. [38–43] proposed non-toxic *p*-nitroaniline as a diazo component, and resorcinol or chromotropic acid as an azo component. Another example is a hydroxamation reaction used for the determination of a number of organic compounds. Hydroxamic acids formed react with Fe(III) giving an intense colour.

Tables 2.2 and 2.3 give several reagents used in indicator tubes for determining gases and vapours (manufactured by Dräger Company (Germany) and Hach Chemical Company (USA)).

Various synthetic reactions can be used for the determination of organic compounds. For example, these are oxidative condensation reactions:



VII

or azo coupling:

1-naphthol; 8-hydroxyquinoline; 2-naphthol; 2,7-dihydroxynaphthalene

TABLE 2.2
Organic reagents used in Dräger indicator tubes

Test compound	Reagent	Reaction product
Acetone	2,4-Dinitrophenylhydrazine	Yellow hydrazone
Acrylonitrile	Methyl Red + HCl	Red product
Aniline	Furfural	Dianiline derivative of hydroxyglutacondialdehyde
Benzene	Formaldehyde + H_2SO_4	*p*-Quinoid compound
Carbon disulphide	NHR_2 + Cu	$Cu(SCSNH_2)_2$
Chloroform	*o*-Tolidine	Yellow-orange product
Chlorine	*o*-Tolidine	Yellow-orange product
Nitrogen dioxide	Diphenylbenzidine	Blue-grey product
Ozone	Indigo	Isatin

TABLE 2.3
Organic reagents used in Hach Tests

Test compound	Reagent
Al	Eriochrome Cyanine R
Cr	Diphenylcarbazide
Cu	Porphyrin Bicinchonine
Fe	1,10-Phenanthroline
Mn	PAN
Mo	Mercaptoacetic acid
Ni	PAN
Zn	Zincon
Detergents	Toluidine Blue O
Hydrazine	*p*-Dimethylaminobenzaldehyde
Phenols	4-Aminoantipyrine
Dissolved O_2	Indigo Carmine

Oxidative condensation was applied for the determination of phenols and aniline. 4-Aminoantipyrine (VII) was used as an analytical reagent in the presence of $K_4[Fe(CN)_6]$. Diazotization followed by azo coupling in solution was used for the determination of aniline. 1-Naphthol in a xerogel matrix was used as a reagent.

2.2 VARIANTS OF REAGENT APPLICATIONS

There are at least three main ways in which analytical reagents may be applied in test systems:

1. as preliminary prepared and packed solutions;
2. as immobilized on a solid matrix (support);
3. as weighed and packed powders, globules, tablets, etc.

2.2.1 Preliminary prepared solutions

Aqueous solutions are widely used. Manufacturers of test kits produce solutions in ampoules, droppers, and sealed tubes. Reagents in such solutions should be stable over prolonged periods of time. Concentrations of the reagents should correspond to expected concentrations of a component to be determined. A test kit often contains solutions of several concentrations. Sometimes, solutions contain not only a reagent proper but other necessary compounds as well. Examples are Merck or CHEMetrics test kits.

2.2.2 Auxiliary agents

Besides analytical reagents, test kits usually contain auxiliary substances, namely reductants or oxidants, buffers, masking, wetting and fixing agents, etc.

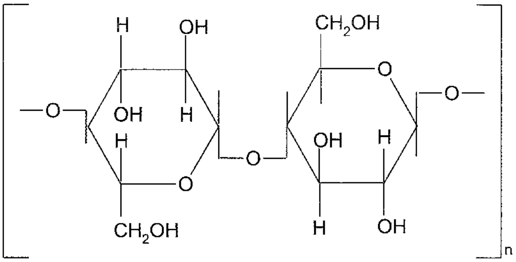
For example, reactive papers for determination of copper in water can be prepared as follows. Filter paper is impregnated with a reducer solution (hydroxylamine hydrochloride, ascorbic acid, or their mixture) for the reduction of Cu(II) to Cu(I). Next, a weak acid is added. After drying, the paper is treated with an organic solution of the analytical reagent and an emulsifier to determine copper(I) (cuproine, neocuproine, or bathocuproine). Mixtures of $Na_2B_4O_7$–H_3BO_3 or H_2CO_3–NH_3 can be added for improving the sensitivity of determination of copper. Examples of the application of surfactants were discussed above.

2.2.3 Reagents on solid matrices

Test systems based on solid supports (papers, fabrics, synthetic organic polymers, silica gels, etc.) are probably more widespread. The nature of the support, the way of its preparation and immobilization of reagents are of great importance. A reagent is immobilized through adsorption, solvent evaporation after impregnation with a reagent solution, other physical methods, or chemical (covalent) immobilization. The basic drawback of test systems based on physically fixed reagents is a relatively weak fixation of the reagent, and as a consequence, its partial washing-off on contact with solution. Chemical bonding (immobilization) improves immobilization strength of the reagent on the support. However, physical immobilization, as a rule, is much easier to perform. That is why it is widely used.

2.2.3.1 Reagents on papers

Amelin [35] gave a detailed review of papers as matrices. The base of the paper is cellulose, which is a natural polymer. The chemical composition and structure of macromolecules are determined by peculiarities of biochemical synthesis. This polymer is continuously reproduced in nature. Its resource is inexhaustible. The polymer consists of repeatable 1,5-anhydroglucopyranose groups bonded to each other via 1,4-β-glycoside bonds.



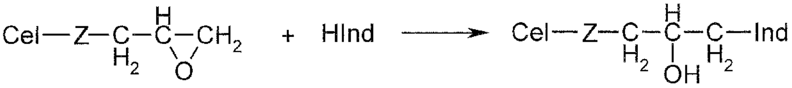
Glucose residues are attached to very long linear molecules, which contain from 100 to 20000 units. For cotton pulp, experiments showed the number of units to be 2000–36000. In crystalline domains, these macromolecules are arranged parallel to each other to form bunches. The macromolecules are bonded to each other mainly through hydrogen bonds provided by free hydroxyl groups. The molecules are different in length; thus their tails are not in line. In amorphous domains, macromolecules are not parallel and free areas are formed. A crystalline domain consists of non-sharp lattice structures with intervals between them. Macromolecules and bunches extend from crystalline to amorphous domains. Several authors think that crystalline bunches of cellulose macromolecules form connectors, and amorphous areas form node points of the three-dimensional lattice. On other evidence, amorphous cellulose glues crystalline bunches to form fibres, which can be seen with an electron microscope. In natural cellulose, which makes up cell walls, such microscopic fibrils combine into fibres. The fibres form coaxial plates around a longitudinal axis of a cell. In filter papers, fibres produced from cell walls or cell fragments are flattened. On the one hand, they are held together by friction forces; on the other hand, ground fibres are hydrogen bonded. Hydrogen bonds are formed on drying of wet paper. In paper, pores are between fibres and inside them. This fact significantly complicates the explanation of taking up water by paper.

Almost without exception, chromatographic paper is made of cotton fuzz. Examining cotton fibres under a microscope, one can see that they look like multiply twisted bands through which a wide channel passes. The fibres are 0.015–0.040 mm in diameter; their lengths depend on the type of the raw cellulose and on the degree of grinding. Individual fibres can remain intact, but under no circumstances should be crushed or split lengthwise. All papers are finely porous and strong. Absorbed water is hydrogen-bonded by hydroxyl groups at the free surface of cellulose macromolecules.

Procedures of physical immobilization (essentially on paper) are described in Ref. [35]. A solid support is immersed into a reagent solution for a certain time. Next, the support is dried. The immobilization can proceed in one or several steps. Many steps are required to make a protective layer or immobilize another reagent. In the latter case, the support is repeatedly immersed, for example, in a solution of polyvinyl, gelatine, or other film-forming substances. Next, the support is dried and immersed in a solution of another reagent. In this case, the first immobilized reagent is separated from the other with a polymer film. This approach makes it possible to separate several reagents and prevent premature chemical reactions between them. It makes it possible also to perform multiple-step reactions. When immobilized, the multi-layer reagent contacts the test solution, the substance to be determined reacts with the “external” reagent, passes through a polymer layer, and reacts with the “inside” reagent to form a coloured product.

A two-step impregnation with reagent solutions is used for the on-support synthesis of an analytical chromogenic reagent. In this case, a matrix is modified with the reagent, fixing agent, buffers, and wetting agents. First, the matrix is impregnated with an aqueous solution of inorganic substances. After drying, it is impregnated with an organic solution of an indicator.

For indicator papers, the chemical immobilization is of great importance [44–48]. Paper strips were impregnated with ethanol solutions of Methyl Red, Ethyl Red, Butyl Red, and other reagents. After drying, the strips were subjected to heating at 140°C for 0.5–1 h. Next, the strips were impregnated with ethanol and dried again. The following processes occurred in this case:



$$\mathrm{Cel{-}Z{-}\underset{H_2}{C}{-}\overset{H}{C}{-}CH_2}\ (\text{epoxide } \mathrm{O}) + \mathrm{HInd} \longrightarrow \mathrm{Cel{-}Z{-}\underset{H_2}{C}{-}\overset{H}{\underset{OH}{C}}{-}\underset{H_2}{C}{-}Ind}$$

where Z = –O– or $–OCH_2–C(CH_3)_2–COO–$; Cel is cellulose.

Epoxidized chromatographic papers [48] or papers based on aldehyde cellulose (AC) were used for chemical immobilization of formazans and hydrazones (**I–IV**) with different groups [49–51].

Such papers were used for a rapid determination of iron (**I**), copper (**II**), mercury (**III**), and zinc (**IV**). In a similar way, indicator papers with pyrazol ring and formazan group were suitable for determination of Bi(III), Fe(II, III), In(III), Cd, Co(II), Cu(II), Hg(II), Pb(II), and Zn at their MAC or dangerous levels [50,54]. The reactions are of good contrast and selective.

Procedures were proposed for chemical immobilization of benzidine on epoxidized paper or silica gel [55], of 1-naphthylamine on dialdehyde cellulose [56], 8-hydroxyquinoline [57], and polyamide groups [58]. These procedures are discussed in detail in Section 2.3.2.

2.2.3.2 Reagents immobilized on synthetic organic polymers

Synthetic organic polymers are widely used as matrices for solid test reagents.

Physical immobilization is the basic way of modification of such polymers. However, there are examples of chemical immobilization. For pH determination, test systems were produced by condensation of polyacrolein with amino compounds (4-aminoazobenzene, 4-amino-2,3-dimethylazobenzene, and others) [59], polycondensation of phenolphthalein with formaldehyde, urea, and phenol [60], and polymerisation of 4-*p*-aminophenylazophenylmethacrylate in the presence of azodiisobutyronitrile [61]. The test reagents produced are highly stable in acidic and alkaline media. Their reactions are reversible.

Among organic polymeric supports, polyurethanes and synthetic ion-exchangers (cation- and anion-exchangers) appear to be of prime importance.

Polyurethane foams are widely used as matrices for organic reagents in test methods. Polyurethane foams can retain chelate reagents, inorganic precipitators, enzymes, etc. After adsorption of coloured compounds or their formation immediately on the polyurethane surface, a colour develops that is characteristic for a compound to be determined. Test procedures using polyurethane foams are performed in one of three ways. One approach is based on the formation of a coloured compound in the analysed solution followed by its adsorption on an unmodified foam. Another variant is to make a coloured compound to form immediately on a sorbent surface as a result of the reaction between the compound to be determined and a hydrophobic reagent immobilized on the surface. Another way is to make the end groups of polyurethane molecules to participate in complexation, oxidation–reduction, or organic synthesis.

Procedures for modification of polyurethane foams with organic reagents were developed. They are based on preliminary plasticization of the foam tablets followed by treatment with a small volume of acetone solution of the reagent. This approach ensures a strong retention of reagents and a homogeneous distribution of them in the tablet. With such systems, test procedures for the determination of Ni and Cr(VI) were developed. Surfactants, phenols, Co, Fe and Ti were determined with procedures of the first type (without coating) [62–69].

Test procedures for the determination of Zn, Pb, Co, Cu, Cd, Hg, Ni and Cr were developed [63,70,71]. Reagents were immobilized on plasticized open pores of polyurethane foam shaped as cubes with a 4-mm side. The cubes were shaken with 1–2 ml of analysed solution for 1–2 min. A change in colour was compared with the reference scale made of coloured cubes. The analysed solution can be passed through a column packed with cubes. Concentration is determined from the length of the coloured zone. In this case, detection limits were lowered by a factor of 5–10 [70,71].

Polyurethane foams were also used for adsorbing a coloured compound formed in the analysed solution after adding reagents [72]. With this procedure, cobalt, iron, and titanium with potassium thiocyanate, cationic surfactants with Bromothymol Blue, and phenols with tetrabromo-4-nitrophenyldiazonium were determined.

The authors of Refs. [63,65,66,73] proposed diphenylcarbazide, Rhodamine 6G, 2,3-diaminonaphthalene, and 8-hydroxyquinoline immobilized on polyurethane for spectrophotometric determination of Cr(VI), Se, and Y, respectively. These systems can also be used in test procedures.

Examples of the application of solid matrices based on **polymer ion-exchangers** are considered in [74–76] and in a review by Lobanov [77].

A large group of test systems are based on filled fibrous materials used for the determination of metal traces [78–83]. A reagent was adsorbed on an ion-exchanger resin. Varying the nature of the anion-exchanger and of the reagent and reaction conditions, it is possible to find the most selective system for adsorption and determination of the target metal ion. The analysed solution passes through the strip-shaped material modified with the reagent. The optical effect is measured by diffuse reflectance or detected visually. The analysis takes 10 min.

2.2.3.3 Reagents on silica gels

Silica gel is one of the most widely used inorganic polymer matrices. Silica gels are modified with various reagents and often used in tubes for air analysis. For example, chromium(VI) oxide in the presence of sulphuric and phosphoric acids is used for determination of methanol and ethanol in air and COD in water [84,85]. Bromocresol Green was used for determination of SO_2 in air [86]. Fluoresceine with potassium bromide was applied for determination of chlorine [87]. Nitrogen oxides were determined with potassium iodide and starch [88]. Residual chlorine in water ($5 \cdot 10^{-5}$%) was determined with *o*-tolidine [89]. Silica gels noncovalently modified with Xylenol Orange or PAR were used for determination of iron and copper. Concentrations were determined by the length of the coloured zone after pumping the tested liquid through the sorbent [90,91]. Determination of cobalt in water at MAC levels is based on the formation of a coloured associate of tetracyanate acido-complexes with didecylaminoethyl-β-tridecylammonium bromide immobilized on silica gel [92]. The sorbent was immersed into the analysed sample for 30–60 min. Next, the colour intensity was compared with a standard colour scale. Heterocyclic azo compounds immobilized on silica were used for test determinations of Co, Pd, U(VI) [25], Fe(III), Cr(III), Mn(II), Ni(II), Co(II), and Zn(II) [13]. Concentrations were determined by colours of the sorbents immersed into analysed solutions. Tablet-shaped silica gel (Silochrom-120) modified with PAR, TAR, or PAN was proposed for determination of Co, Hg, Pd, and U with detection limits of 0.003–0.1 mg/l [13,25].

Sometimes inorganic compounds (e.g., calcium, barium, and magnesium carbonates and barium sulphate) are used as matrices. For example, finely powdered fuchsine or Methyl Violet was deposited on the salts. The systems were used for the determination of free water in hydrocarbons [93].

Modification with inorganic reagents is performed mainly via adsorption or precipitation of metal hydroxides, oxides, and salts on the sorbent [23].

2.2.3.4 Selection of optimum conditions of testing

Optimum conditions for adsorption of complexes on sorbents (or ions to be determined on matrices modified with organic reagents) and complexation conditions in aqueous solutions are usually similar. Complexation conditions for both cases are also similar. Therefore, conditions found for complexation in solutions can be used for the adsorption variant. Apart from spectrophotometry, diffuse reflectance spectroscopy is finding increasing use for optimising conditions of determination. In the last few years, chromaticity measurements are being used [94].

Characterisation of compounds with traditional molecular VIS spectroscopy mainly consists in measuring absorbance, intensity of luminescence, diffuse reflectance, etc., and finding vibronic and vibrational maxima.

Colour differences are commonly not used for these purposes. For the equal contrast CIE 1976 chromaticity system, the most important analytical parameters, which depend exponentially on the concentration of the coloured compound, are ΔL (lightness), ΔS (saturation), and ΔE (difference in hue). ΔL parameter is not selective. It characterizes only changes in colour intensity (with respect to the luminosity of a reflected or transmitted luminous flux). ΔS characterizes changes in colour. The equation for the total colour difference (or the difference in hue) is used for instrumental or visual estimation of colour changes in developing test scales. Test scales are based on the dependence of the total colour difference (or the differences in hue, ΔE) on the concentration of the tested element. That is why the increment in concentration is usually selected, for which $\Delta E = 10$, i.e. the geometric progression is used. In developing test scales, it is important to remain within the limits of saturation ΔS of test samples (20–60%). Otherwise, differences will be hardly recognizable by the human eye.

Using indium complex with 5-Br-PAAP as an example, it was found that the optimum pH value depends on the function used: it is 4.5–4.8 when ΔE, A, and T are measured, and 3.5–5.0 when ΔL is measured. For solutions, the pH range determined by spectrophotometry was 3.5–7.0. This fact is probably attributable to steric hindrances when passing from solutions to solid supports [23]. It was found that it is better to measure the colour of wet sorbates [23]. For example, for the Gurevich–Kubelka–Munk function (ΔF), and lightness (ΔL), calibration curves have the following form (c is the total concentration of indium in sorbate, μg):

$$\text{wet sorbates:}\quad \begin{aligned} \Delta F &= 0.89c + 0.80 \\ \Delta L &= 1.07c + 1.89 \end{aligned}$$

$$\text{dry sorbates:}\quad \begin{aligned} \Delta F &= 0.19c + 0.12 \\ \Delta L &= 0.77c + 0.70 \end{aligned}$$

though in this case, blank functions change proportionally. The RSD values are similar for all the cases (0.05–0.08).

2.3 IMMOBILIZATION OF CHEMICAL REAGENTS. MATRICES AND METHODS

Analytical matrices with various reagents can be classified by the method of immobilization, the type of modifier, and the type of support.

Examples of modifiers (organic and inorganic reagents) have been discussed above. As previously discussed, ion-exchanger resins, cellulose, polyvinylchloride membranes, fine silica gels (silicas and xerogels), and polyurethane foams are used as supports.

Possibilities for changing physicochemical properties of sorbents through their modification are virtually unlimited. For the fixation (immobilization) of an analytical reagent on a support, a number of methods are used, among them: immobilization through physical adsorption or impregnation, and synthetic approach based on covalent binding of a modifier. Among other sorbents, chemically modified silicas (CMS) are widely used in hybrid and coupled analytical methods. The most part of covalently modified sorbents are chemically and mechanically resistant. They provide for a rapid establishment of equilibrium even for small concentrations of test substances ($\leq 10^{-5}$ M). They do not swell in water and organic solvents. These advantages often compensate drawbacks resulting from difficulties in synthesis.

2.3.1 Immobilization through physical adsorption (physical immobilization)

The manner of physical immobilization most often depends on the support, although the chemical nature of the modifier and adsorbed ion (complex) is also important. The main drawback of the physical immobilization is a weak immobilization of adsorbed water-soluble reagents on the surface of a sorbent and, as a consequence, partial washing-off of these complexes on contact with water. Reagents poorly soluble in water are preferable, because they form more storage-stable test forms which are sparingly washed off from the test matrix on its contact with the analysed solution. When reagents poorly soluble in water are immobilized, their organic solutions or finely powdered reagents are used.

Multiple-step successive impregnation of paper with solutions of source reagents for on-matrix synthesis of analytical chromogenic reagent was proposed in Refs. [35,106–109]. For example, test determination of chloride was based on discolouration of a support impregnated with a brownish–red Ag_2CrO_4. The support was first impregnated with a silver salt. After drying, the matrix was impregnated with sodium chromate [107,108]. In this case, poorly soluble Ag_2CrO_4 is adsorbed in the pores of the support. In a similar way, a test system containing poorly soluble zirconium complex with alizarin S is used. The support was first impregnated with an aqueous solution of a zirconium salt. After drying, the support was impregnated with an ethanol solution of alizarin S. If fluoride ions are present, the red zirconium complex is destroyed. In an acidic medium, a yellow colour characteristic of free alizarin appears [107, 108].

Let us consider in greater detail some supports and peculiarities of physical immobilization.

2.3.1.1 Supports

Fibrous materials filled with AV-17 and AV-31 anion-exchangers [78,110–112], silica gels, plates made of Silasorb or Sorbfil, xerogels [113,114], polyurethane foams, cellulose based paper, cation- and anion-exchangers are used as supports. If ion-exchangers are modified, it is important to prevent adsorption on functional complexing groups. Otherwise, though immobilization is successful, the test system will not be suitable because of the absence of colour effects and complexation. For example, an AV-17 anion-exchanger was modified with Arsenazo III [116], Eriochromecyanine R [117], Lumogallion IREA, Magneson IREA [115] without the loss in reactivity of the modifiers. Similarly, a KU-2 cation-exchanger was modified with PAR [20] and PAN [12]. When supports of the same type are used, which differ in pore size, their adsorption capacity will depend on the size of modifier molecules. It was found [13] that adsorption of PAR and PAN on silica depends not only on adsorption forces, but also on the silica structure, namely on its porosity and specific surface. Adsorption isotherms for cationic forms of PAN and PAR are of Langmuir type. Using these isotherms, Gibbs energy changes were estimated (Table 2.4).

On the other hand, S-80 is preferable for adsorption of larger PAN molecules (because of a larger pore size); S-120 is preferable for adsorption of PAR [13] (Table 2.5).

Steric hindrances can manifest themselves when complexes produced in solutions are adsorbed on various matrices.

It was found that nitroso-R-salt forms complexes in solutions with Co(II), Ni(II), and Cu(II) with metal-to-ligand ratios of 1:1 (Ni), 1:1 and 1:2 (Cu), and 1:3 (Co). However, only Ni(II) and Cu(II) chelates are adsorbed on an AV-17 anion-exchanger. This fact can be used for a test determination and separation of cobalt and nickel (or copper). The procedure is based on observing the colour of the sorbent before and after adsorption. This procedure is not yet viewed as a test variant.

The optimum conditions for complexation in solutions are not always the same as for adsorption of coloured compounds. For example, iron(III) sulphosalycilates of composition 1:1, 1:2, and 1:3 are adsorbed. However, absorption maxima do not coincide with the maxima of complexes in solution. For solutions, the absorption maxima are at 490 (1:1), 460 (1:2), and 420 nm (1:3). For sorbates, a hypsochromic shift is observed: the corresponding maxima are at 460 (1:1) and 420 nm (1:2). On a LIK-21 silica with aminopropyl groups only complex 1:2 (but not 1:3) is sorbed [30].

TABLE 2.4

Gibbs energy changes (ΔG) in adsorption of PAN on Silochrom S-80 (sorbent mass 0.4 g, solution volume 10 ml, pH 1.0)

Fraction S-80 (μm)	a_m (μmol/g)	$K \cdot 10^{-4}$	ΔG (kJ/mol)
100–200	28.5	2.8	24.9
315–500	22.4	3.0	25.1

a_m is the monolayer capacity (μmol/g); K is the adsorption constant.

TABLE 2.5

Thermodynamic parameters of adsorption of cationic forms of PAR and PAN on silicas with different adsorption capacity and pore size (particle size 200–355 μm) at 20°C (sorbent mass 0.4 g, solution volume 10 ml, pH 1.0) [13]

Thermodynamic parameters	S-80		S-120	
	PAR	PAN	PAR	PAN
a_m (μmol/g)	13.0	27.3	19.0	23.4
$K \cdot 10^{-4}$	0.82	1.675	0.385	2.36
ΔG (kJ/mol)	21.9	23.7	20.1	24.5

a_m is the monolayer capacity (μmol/g); K is the adsorption constant, and ΔG is the Gibbs energy change.

When complexes formed in solution are adsorbed, a strong bathochromic shift can occur, which is similar to the effect of surfactants. For example, molybdenum(VI) complex with Catechol Violet in solution is characterized by an absorption maximum at 550 nm. The complex adsorbed on AV-17 has a maximum at 660 nm [119]. The pH range of complex stability also changes, being 3–4 for solutions and 1.5–4.0 for the adsorbed complex. For the indium complex with PAR, the optimum pH values found by various workers are 3.5–4.5, 3.5–8.0, and 6.5–8.5. Evidently, different complexes with PAR were studied. Protonated complexes are formed in a more acidic medium. Complexes containing dissociated *p*-hydroxy groups are formed at pH ≥ 6. For the complex adsorbed on Silochrom S-120, the optimum pH range is from 4.5 to 4.8. While the metal-to-ligand ratio for the adsorbed complex is 1:2, complexes of compositions 1:1 and 1:2 are formed in solution [21].

TABLE 2.6

Selected characteristics for complexes with PAN and complexes adsorbed on S-120 Silochrom [120]

Ion	$\varepsilon\cdot10^{-4}$ (λ, nm)	$H\cdot10^{-4}$ (λ, nm)	Adsorption pH	Adsorption time (min (°C))	c_{min} (μg/ml)	MAC (μg/ml)
Cr(III)	1.28 (555, 600)	0.30 (540, 570)	2.5–4.5 6.5–9.0	40–60 (80)	0.10	0.5
Fe(III)	1.7 (750)	0.32 (540, 570)	5.0–6.5	15 (20)	0.3	0.5
Mn(II)	4.07 (546)	0.36 (540, 570)	5.0–7.5	15–25 (20)	0.05	0.1
Ni(II)	5.0 (520, 570)	1.60 (540, 560)	6.0–9.0	15 (30)	0.03	0.1
Cu(II)	4.4 (564)	1.64 (540, 550)	5.0–8.0	15 (20)	0.10	1.0
Zn(II)	4.75 (514, 550)	0.65 (540)	7.5–10.0	15 (20)	0.03	5.0

In most cases absorption-band parameters for PAN complexes in solutions and complexes adsorbed on S-120 are similar, and absorptivities correlate with the sensitivity coefficient H (Table 2.6) [120]. The ratio ε:H is about 5:1.

The characteristics of the adsorbed complexes were determined using diffuse reflectance spectroscopy. Optimum conditions for test determinations were proposed.

Contrastingly, for TAR (a thiazole analogue of PAN) it was found that the optimum pH range and absorption maxima for complexes in solution (**I**) and adsorbed complexes (**II**) do not always agree (Table 2.7) [25].

In several cases, there are no data on the mechanism of immobilization (chemical or physical) of a reagent. To illustrate, the mechanism of immobilization of reagents on polyurethane foams is not yet completely clear, whereas for silica supports, several authors suggest the chemical nature of reagent immobilization.

2.3.1.2 Support adsorption capacity

Support adsorption capacity for a modifier is of great importance depending on the role of the modifier, especially if the modifier is

TABLE 2.7

Selected characteristics of complexes with TAR in solutions and complexes adsorbed on S-120 Silochrom modified with TAR

Ion	λ_{max} (nm)		Optimum pH	
	I	II	I	II
U(VI)	540	510	3.0–8.0	7.0–8.5
Cu(II)	510	510	5.0–12.0	6.5–8.5
Ni(II)	510	510	4.0–6.0	5.2–6.8
Zn(II)	500	500	8.0–10.0	5.6–7.4
Co(II)	510	510	7.5–8.0	4.5–8.0
Pd(II)	525	510	3.0–8.0	5.5–8.5
Fe(III)	485	550	6.5–8.2	3.0
Fe(II)	500	540	8.0–8.7	4.5–5.5

coloured [121]. For sorbents with high sorbent capacities, analysis becomes more difficult because colours become more saturated. For sorbents with low sorbent capacities, the role of random factors in preparing a visual scale for the determination of an element becomes of primary importance. Chromaticity techniques were proposed for the optimisation of modification. With this technique, a test scale with an increment $\Delta E = 10$ can be prepared. The scale permits a very precise visual estimation of differences in colour. To illustrate, for uranium(VI) complex with 5-Br-PAAP adsorbed on Silochrom S-120, the scale was prepared using solutions containing 5, 10, 30, 70, 150, and 220 μg of uranium in a 20-ml volume of solution (for $\Delta E = 10$).

To determine adsorbent capacity of sorbents modified with heterocyclic compounds, diffuse-reflectance spectroscopy [13] and photometry [121] were used. The latter procedure was based on the oxidation of an azo compound with cerium(IV) followed by measuring absorbances of unexpended Ce(IV). For instance, the capacity of S-80 was (μmol/g) 57.8 ± 0.4 for PAR, 36.6 ± 0.3 for TAR, 4.1 ± 0.4 for PAN, and 7.5 ± 0.5 for TAN. In spite of the lower hydrophobicity of PAR and TAR as compared with naphthol analogues, the sorbent capacity for these reagents is higher than the capacity for PAN and TAN. It was noted in [121] that PAN was immobilized better on Silochrom S-80, whereas PAR was immobilized better on Silochrom S-120.

To select an optimum sorbent, diffuse-reflectance spectroscopy and chromaticity measurements can be also used. This approach worked very well in designing a test reaction for determination of aluminium with Eriochromecyanine R [117]. Diffuse-reflectance spectra and pH curves of chromaticity functions revealed that microcrystalline cellulose was more preferable than other sorbents studied (various types of Silochrom and AV-17), because it did not adsorb the reagent. Moreover, diffuse-reflectance spectra are shifted bathochromically compared with other sorbents. Similar behaviour was found for Chromaton N-Super. However, the latter reagent is difficult to obtain.

2.3.1.3 Selectivity

When adsorption is used, selectivity of determination of metal ions usually improves. Improvement in selectivity stems from the fact that in a number of cases immobilized organic reagents change their complexing properties, e.g. their dentate numbers. This is due to geometric peculiarities of immobilization of the reagent on the sorbent surface. It is believed that modified sorbents extract most efficiently those metal ions that form ion associates or complexes with the immobilized reagent of the ratio 1:1. In this case, steric hindrances resulting from immobilization of the reagent on the sorbent surface are minimized. The advantages of this approach lie not only in combining preconcentration and production of a suitable analytical form of a concentrate for the following testing, they also lie in the increased kinetic stability of the adsorbed compound in comparison with a dissolved one. As a consequence, selectivity of the chromogenic reagent improves: it is due to an increase in the rigidity of molecules immobilized on a polymeric sorbent. This makes it possible to use either aqueous solutions of preliminary adsorbed organic reagents insoluble in water or reactions leading to the formation of poorly soluble compounds or even colloidal systems. For conventional photometry, such reagents and reactions are hardly practicable.

For test procedures, selective reagents are usually used or conditions for selective determination are provided. For instance, virtually all test systems for nickel contain dimethylglyoxime; virtually all the test systems for iron(III) contain hexacyanoferrate(II). As a rule, in the case of physical immobilization on solid sorbents the selectivity of reagents does not change, whereas in the case of chemically immobilized reagents, their selectivity often improves. It was found [59,59] that when hydrazones and formazans are immobilized on paper, their selectivity parameters improve significantly—by nearly two orders of magnitude as compared with non-immobilized reagents. This is so because the inner cavities of a chromogen molecule become accessible only to certain ions. Moreover, only complexes of composition 1:1 are formed. All this makes it possible to apply such indicator papers for the determination of Fe(II, III), Zn, Cu(II), Cd, and Pd(II) in natural and wastewaters without preliminary sample preparation and masking.

The selection of poorly soluble compounds for paper impregnation and a selective determination of a given element is based on solubility constants series [122] of inorganic salts of silver, diethyldithiocarbamates, and dithizonates [123].

For reactions of low selectivity, masking and isolation of interfering elements directly on the sorbent are used. Apart from reagents, impregnating solutions contain masking agents. Addition of masking agents directly to the analysed solution is less common. Multilayer papers or films containing masking agents in a layer (e.g. EDTA, citric acid, etc.) are quite effective [124–127]. When the analysed solution is applied onto such a multilayer matrix, interfering elements are eliminated before the solution reaches the layer with the reagent.

It is important to maintain definite optimum conditions for reactions with given components. It is provided for by adding buffer substances directly at the stage of preparation of test systems or adding various solutions to the analysed liquid. $KHSO_4$, citric acid, $Na_2B_4O_7$, Na_2CO_3, KH_2PO_4, HCl, HNO_3, NaOH, and others are often used for these purposes.

Silica gels, silica gels modified with hydrophobic organic radicals, glasses, quartz, and other silica-based materials are widely used to produce test tools using solid matrices.

2.3.1.4 Reversed-phase silica gels modified with various groups

Morosanova et al. (Moscow State University) studied covalent immobilization of many analytical reagents on reversed-phase silica gels modified with groups (C_{16}, Phenyl, CN). A wide variety of reagents was studied, namely di-(2-ethylhexyl)phosphoric acid (HDEHP), diethyldithiophosphoric acid (HDEDTP), phosphoryl-containing podand (PCP), lead diethyldithiocarbamate (Pb(DDTC)), lauryl sulphate (LS), azo compounds (PAN, TAN, sulphochlorophenolazorhodanine (SCPAR), bromobenzothiazo (BBT), Lumogallion (LG), Cadion, derivatives of triphenylmethane and xanthene (Eriochromecyanine R (ECC), Xylenol Orange, and Crystal Violet (CV)), hydroxyquinoline and its derivatives (8-hydroxyquinoline (8-HQ), 2-methyl-8-hydroxyquinoline (MHQ), and 5-phenylazo-8-hydroxyquinoline (PAHQ)), oximes (nioxime, dimethylglyoxime, and salicylaldoxime (SAO)), β-diketones (benzoylacetone (BA), thenoyltrifluoroacetone (TTA), and 1-phenyl-3-methyl-4-benzoylpyrazolone-5 (PMBP)), macrocyclic compounds (octaethylporphyrin (PtOEP) and diazo-18-crown-6 (D-18-C-6), hexaoxacycloazochrome (HOCAC), 1-nitroso-2-naphthol (NN), 2,2′-dipyridyl (Dip), 1,10-phenanthroline (Phen), bathophenanthroline (BP), Chromopyrazole I, *p*-dimethylaminobenzylidenerhodanine (DMABR), and diphenylcarbazone.

Sorbent capacities and pH ranges, within which capacities of sorbents and of columns are constant and the retention of metals is reproducible (ranges of hydrolytic stability), are determined by the nature of the surface functional groups and the composition of the modifier solution (Tables 2.8 and 2.9). The partition mechanism determines the retention process. Within the framework of each class of reagents, interdependence is found between the capacity of the hydrophobic silica gel for a reagent and the hydrophoby of this reagent

TABLE 2.8

Adsorption capacities (Q), distribution constants, and pH ranges of hydrolytic stability of dynamically modified hydrophobic silica gels ($n = 4$, $P = 0.95$)

Reagent	Q (μmol/g)		log$P$ ($CHCl_3$)	pH ranges of hydrolytic stability
	SG-C_{16}	SG-Ph		
8-Hydroxyquinoline	4.3 ± 0.3	4.0 ± 0.2	2.81	3.6–9.0
2-Methyl-8-hydroxyquinoline	4.6 ± 0.1	—	3.22	4.0–9.0
5-Phenylazo-8-hydroxyquinoline	160 ± 20	38 ± 3	>3.22	1.0–9.0
Nioxime	Not retained	Not retained	–0.96	—
Dimethylglyoxime	Not retained	Not retained	–0.92	—
Salicylaldoxime	3.7 ± 0.4	—	2.10	4.5–8.5
Benzoylacetone	2.9 ± 0.2	1.7 ± 0.1	3.60	4.2–9.0
Thenoyltrifluoroacetone	0.80 ± 0.02	—	1.73	4.0–8.0
1-Phenyl-3-methyl-4-benzoylpyrazolone-5	3.2 ± 0.1	2.5 ± 0.3	3.61	1.0–4.1
Octaethylporphyrin	240 ± 10	420 ± 60	—	1.0–9.0

TABLE 2.9

Adsorption capacities (Q) and pH ranges of hydrolytic stability of dynamically modified SG-Ph silica gel ($n = 4$, $P = 0.95$)

Reagent	Q (μmol/g)	pH ranges of hydrolytic stability
Xylenol Orange	1.25	0–9.0
1-(2-Pyridylazo)-2-naphthol	10	0–9.2
1-Nitroso-2-naphthol	39	1.5–8.4
Hexaoxacycloazochrome	2.7	1.2–8.5
p-Dimethylaminobenzylidenerhodanine	18	1.0–7.4
Cadion	6.0	1.0–8.8
Chromopyrazole I	5.0	1.5–7.4
1,10-Phenanthroline	5.5	2.3–6.9
Diphenylcarbazone	7.9	2.2–7.4

(namely, $\log P(\mathrm{CHCl_3})$) (Table 2.9). For example, for β-diketones (PMBP, TTA, and BA), Q (μmol/g) is 1.20; $\log P$ ($\mathrm{CHCl_3}$) is 1.28; the correlation coefficient is 0.994.

For surfaces with alkyl groups, the capacity decreases as the number of carbon atoms decreases (silica gels were studied from SG-C_{16} to SG-C_1). In most cases, the capacities of silica gels with phenyl functional groups are higher than for silica gels with alkyl groups. In several cases, this interaction is predominant. Immobilization of HOCAC, XO, and CD is possible only on hydrophobic phenyl-containing surfaces (Table 2.10).

The capacity depends on the composition of the modifier solution, and primarily on the concentration of ethanol, which was used for the preparation of most initial solutions of modifier reagents. Maximum capacities of sorbents are achieved at ethanol concentrations of 5–7%. An increase in the concentration to 20–30% worsens the retention of reagents. To eliminate completely any reagent studied, it is sufficient to pump 0.2–1.5 ml of ethanol through the column.

Using poorly water-soluble reagents (PAN, DMABR, and nitrosonaphthols) as examples, advantages of micellar solubilization with non-ionic surfactants for increasing the capacities of sorbents and columns were demonstrated.

For highly water-soluble reagents (Xylenol Orange, Cadion, and HOCAC), which favour protolytical reactions, the retention is signi-

TABLE 2.10

Conditions for preparation of modified xerogels and their characteristics; accelerator of gel formation is ammonium hexafluorosilicate

Reagent (R)	Solvent	$c_R \cdot 10^3$ (M)	Microwave power (W)	Factor F
PAN	EtOH	0.5–17	1100	5.0
PAR	H_2O	1.25–12.5	1100	6.2
TAN	EtOH	0.1	1100	5.3
Lumogallion	H_2O	1.0–5.0	1100	2.9
Salicylaldoxime	H_2O	1.0	1100	3.1
Bromobenzothiazo	EtOH	1.0–7.0	1100	15.0
Cadion	H_2O	10.0	1100	2.8
SCPAR	H_2O	0.08–5.0	1100	3.1
HOCAC	H_2O	0.05–0.2	1100	7.2
Xylenol Orange	H_2O	1.25–12.5	1100	1.8
Eriochromecyanine	H_2O	0.1–5.0	1100	1.0
Morin	EtOH	0.1–5.0	1100	1.2
Diphenylcarbazone	EtOH	0.2–3.0	1100	8.4
5.7-Dibromo-8-hydroxyquinoline	EtOH	0.2–2.5	1100	12.1
1,10-Phenanthroline	EtOH	0.2–3.0	1100	7.3
Bathophenanthroline	EtOH	0.2–2.0	1100	15.0
Crystal Violet	H_2O	5.0	1100	12.8
Chromopyrazole I	H_2O	0.5–4.0	1100	15.0
Cu(II)	H_2O	30–200	1100	5.4
Co(II)	H_2O	30–200	1100	6.1
Fe(III)	H_2O	30–200	1100	14.8
Vavele reagent	H_2O	Saturated solutions	700	—
HMP	EtOH	Saturated solutions	700	—
NH_4MP	—*	—	700	—
8-Hydroxyquinoline	EtOH	0.75–2.75	1100	10.3
1-Naphthol	EtOH	0.75–2.75	440	2.0
2-Naphthol	EtOH	0.75–2.75	440	1.8
2.7-DON	EtOH	0.75–2.75	660	3.2
4-AAP	EtOH	5.0	110	6.9

Note: Full reagent names are given on pages 37 and 41.
*Ethanol suspension; **NH_4F as accelerator.

ficantly influenced by the pH conditions. The retention is highest within the pH ranges where molecular forms of reagents are dominant. This fact confirms the significant role of the partition mechanism in retention.

Methodological approaches for online modification and regeneration of columns. It takes 30–120 s to immobilize via columns nitroso-naphthols, PAN, DMABR, Chromopyrazole I, and diphenylcarbazone. After a single modification, columns can operate in a continuous-flow fashion (adsorption–desorption mode) during 2–15 h.

2.3.1.5 Xerogel sorbents

Morosanova et al. [90,91,128–132] developed procedures for immobilization of analytical reagents on xerogels of silicic acid. Such modified xerogels (either as powders or packed in tubes for analysis of water and other liquids) were used for many test procedures. Modified xerogels of silicic acid were prepared by sol–gel technology involving hydrolysis of tetraethoxysilane (TEOS) in a water–ethanol solution in the presence of a reagent to be immobilized and gel formation. Next, after drying, a "dry gel" (xerogel) was formed.

Immobilization of many reagents was studied, namely azo compounds (PAR, PAN, TAN, SCPAR, BBT, Lumogallion, and Cadion), derivatives of triphenylmethane and xanthene (Eriochromecyanine R, Xylenol Orange, Crystal Violet), 5,7-dibromo-8-hydroxyquinoline (5,7-dibromo-HQ), many other organic reagents (see above), salts of Cu(II), Co(II, III), and Fe(III), molybdophosphoric acid (HMP), ammonium molybdophosphate (NH_4MP), Vavele reagent (a mixture of 12- and 18-heteropoly acids), and reagents entering the reactions of azo or oxidative coupling (1-naphthol, 2-naphthol, 2,7-dihydroxynaphthalene (2,7-DON), and 4-aminoantipyrine (4-AAP).

Depending on the solubility, solutions of reagents to be immobilized were injected into a mixture for hydrolysis, aqueous or ethanol solutions or a stable ethanol suspension (prepared under ultrasound action).

Effects of the following factors on physicochemical properties of xerogels were studied: the ratio of TEOS, water, and ethanol in the hydrolysing mixture, the nature and concentration of the additive accelerating the gel formation, the nature and concentration of the reagent to be immobilized, and the procedure for drying wet gels. The optimum ratio of TEOS, water, and ethanol was found to be 2:2:5.

With viscosimetry and turbidimetry, effects of the concentration of ammonium fluoride, which is a most efficient catalyst, and ammonium hexafluorosilicate, which is a new agent for accelerating gel formation, on the rate of gel formation, were studied. The time of hydrolysis, sol–gel transformation, and complete ripening is less in the presence of ammonium hexafluorosilicate than for ammonium fluoride, other factors being equal. An increase in concentrations of accelerators within the range from $2 \cdot 10^{-3}$ to $22 \cdot 10^{-3}$ M accelerates significantly the gel formation.

Optical properties of gels prepared in the presence of ammonium fluoride and hexafluorosilicate are different. Gels prepared with hexafluorosilicate are transparent. Gels prepared in the presence of fluoride are opaque. For xerogels prepared with ammonium hexafluorosilicate, specific surface area increases on increasing its concentration in the hydrolysing mixture from $2 \cdot 10^{-3}$ to $17 \cdot 10^{-3}$ M. In this case, the average pore diameter does not change. An increase in the concentration of ammonium fluoride from $2 \cdot 10^{-3}$ to $11 \cdot 10^{-3}$ M results in lowering the specific surface area and an increase of the average pore diameter.

To dry wet gels, microwave radiation of different power was used (300–1000 W). Advantages of this approach were demonstrated in comparison with drying at high temperatures. Microwave radiation permits a deep drying of wet gels. Depending on the radiation power, it takes from several minutes to 2 h to dry gels. It takes much more time to dry gels by usual heating (1–10 h). An increase in the power of microwave radiation results in a significant increase in the specific surface area of xerogels. IR spectroscopy showed that in the presence of ammonium fluoride and hexafluorosilicate, hydrolysis of TEOS is complete. On drying in a microwave field, ethanol is eliminated completely. However, a three-dimensional spatial pattern of amorphous silica includes adsorbed water.

On drying xerogels modified with reagents that are non-resistant to oxidation (4-aminoantipyrine, 1- and 2-naphthol, and 2,7-dihydroxynaphthalene), 400–600 W microwave radiation was used. Gels modified with other reagents were dried with microwave radiation of the maximal power (1100 W).

Correlation between the slope of the function of the reagent concentration in xerogel vs. its concentration in the hydrolysing mixture (F in Table 2.10) and characteristics of fixed reagents (hydrophoby, solubility, molecule size, and capability of the formation of hydrogen and ionic bonds with silanol groups of silica) were studied (Table 2.10).

Sol–gel technology is a versatile method for preparation of modified silicas. The specific character of the retention of immobilized reagents with xerogel matrices (where mechanical capture is rather significant) results in extending the range of immobilized reagents, and enables one to prepare materials with higher concentrations of fixed reagents with a wider range of hydrolytic stability in comparison with silica gels modified under static or dynamic conditions (Table 2.11).

TABLE 2.11

Comparison of procedures for noncovalent modification of silicas

Reagent	Support	Immobilization procedure	Maximal reagent concentration	mmol/ g×10^2
PAN	SG-Ph	A	0.1	2–8
	Silochrom S-120	B	2.3	1–3
	Xerogel	C	8.2	0–10
PAR	SG-Ph	A	Not retained	—
	Silochrom S-120	B	1.8	1–3
	Xerogel	C	3.7	0–10
Cadion	SG-Ph	A	0.25	0–7.4
	SG-C_{16}	A	Not retained	
	Xerogel	C	0.6	0–10
Crystal Violet	SG-Ph	A	1.7	2–10
	Silochrom S-120	B	2.1	2–10
	Xerogel	C	3.2	0–10
Bromobenzothiazo	SG-Ph	A	Not retained	—
	Xerogel	C	5.6	0–10
SCPAR	SG-Ph	A	0.3	0–7
	Xerogel	C	0.6	0–10
8-Hydroxyquinoline	Silochrom S-80	B	0.2 ± 0.02	3.1–9.0
	SG-C_{16}	B	0.1 ± 0.02	3.1–9.0
	SG-C_{16}	A	0.43 ± 0.03	3.6–9.0
	SG-Ph	A	0.42 ± 0.02	3.6–9.0
	Xerogel	C	1.57	0–10
Xylenol Orange	SG-Ph	A	1.2 ± 0.2	1–9
	SG-C_{16}	A	Not retained	—
	Xerogel	C	1.57	0–10
Cu(II)	Silochrom S-120	A	Not retained	—
	Silochrom S-120	B	Not retained	—
	Xerogel	C	200	2–10

A = Dynamic modification; B = static modification; C = sol–gel procedure.

TABLE 2.12

Acidity constants of reagents in aqueous solutions and in xerogels ($n = 3$, P = 0.95)

Reagent	pK_a (Solution)	pK_a (Xerogel)	ΔpK_a
PAN	1.9 ($H_2L^+ \rightarrow HL$)	1.7 ± 0.3	0.2 ± 0.3
PAR	5.5 ($H_2L \rightarrow HL^-$)	4.9 ± 0.3	0.6 ± 0.3
Bromobenzothiazo	13.3 ($H_2L \rightarrow HL^-$)	10.9 ± 0.5	2.4 ± 0.5
Lumogallion	5.7 ($H_2L^{2-} \rightarrow HL^{3-}$)	4.8 ± 0.3	0.7 ± 0.3
Xylenol Orange	10.4 ($H_2L^{5-} \rightarrow HL^{6-}$)	9.0 ± 0.4	1.4 ± 0.4

Acid–base, complexing, and redox properties of analytical reagents immobilized on xerogels were studied using solid-phase spectrophotometry. Their ability to take part in azo and oxidative coupling was also examined.

Acid–base properties of immobilized reagents. Immobilization on xerogels results in increasing acidic properties of immobilized reagents (Table 2.12). This is probably connected with interactions of labile protons of reagents with deprotonated silanol groups of the matrix. As a result, the dissociation of immobilized reagents becomes easier.

Complexing properties. Heterogeneous complexation reactions on modified xerogels were studied under static and dynamic conditions via atomic absorption spectroscopy and solid-phase spectrophotometry.

Immobilized chelating analytical reagents. Spectrophotometric characteristics of immobilized organic chelating reagents and their metal complexes were studied. For reagents forming with metal ions complexes of composition 1:1 and 1:2, absorption maxima do not change on immobilization. For complexes of composition 1:3, absorption maxima for immobilized and non-immobilized complexes do not coincide. For xerogels, hypsochromic shifts were observed. This was presumably due to a decrease in the number of coordinated ligands because of steric hindrances in xerogels.

For static and dynamic conditions, optimum pH ranges for the reactions of metals with xerogels modified with analytical reagents were found (Table 2.13).

For immobilized bromobenzothiazo, the conditions of the reaction with cadmium change significantly. It is likely to be the result of a significant increase in acidic properties of the reagent due to immobilization.

TABLE 2.13

Optimum pH ranges for reactions of immobilized and non-immobilized reagents with metal ions

Reagent	Metal	Xerogel		Solution
		Dynamic conditions	Batch conditions	
PAN	Cd	7.4–9.0	—	5–6
	Co(II)	5.0–9.0	5.0–8.0	<4
	Cu(II)	5.0–9.0	4.8–8.0	3–8
	Mn(II)	8.0–10.0	8.0–9.0	8–10
PAR	Cd	7.4–8.5	—	6.0–11.5
	Zn	>8.2	—	5–6.5
	Pb	9–10	8.5–9.5	8.0–10.0
Bromobenzothiazo	Cd	9.0–10.0	8.8–9.2	<13.7
SCPAR	Ag	<1	2.0–7.0	1.0–7.0
Lumogallion	Mo(VI)	—	3–5.8	1–4
Xylenol Orange	Fe(III)	2–7	1–4	1–8

For the determination of fluoride and chloride ions, the possibility of performing ion-exchange reactions in xerogels was studied. A solution of halide was added with a metal ion (in plenty). Next, a xerogel modified with an organic chelating reagent was added:

$$x\mathrm{An}^- + (k+1)\mathrm{Me}^{n+} + n\overline{\mathrm{R}} = \overline{\mathrm{MeR}_n} + k\mathrm{MeAn}_x$$

The best results were obtained for systems Xylenol Orange–Zr(IV)–F^- and diphenylcarbazone–Hg(II)–Cl^-.

Immobilized metal ions. Spectrophotometric characteristics for immobilized metal complexes with various ligands change somewhat in comparison with complexes in aqueous solutions. Hypsochromic shifts of absorption maxima (in comparison with solutions) can be presumably explained by the decrease in the valent state of the bonds between donor atoms and ligands or by the formation of other compounds. Acidity effects on reactions of immobilized metal ions with ligand solutions were investigated. It was found that the pH ranges, within which the maximal absorbances of xerogels were observed, are shifted to higher pH values as compared with the optimum pH values for chelating in solutions (Table 2.14).

TABLE 2.14

pH ranges of interaction of immobilized metal ions with organic chelating reagents

System	Xerogel	Solution
Cu(II)–PAR	3–5	0.05–0.5 M H_2SO_4
Co(III)–2N1N	5–9	>4
Fe(III)–Phenylhydroxamic acid	2.5–5.5	1–7

TABLE 2.15

Composition of complexes and equilibrium constants of heterogeneous complexation in xerogels of silicic acid

Immobilized reagent	Adsorbed compound	Ratio of components in the complex		pK
		Solution	Xerogel	
PAN	Cu(II)	1	0.8 ± 0.2	1.7 ± 0.5
	Ni(II)	2	1.8 ± 0.3	5.2 ± 0.3
	Fe(III)	2	1.9 ± 0.1	3.2 ± 0.4
	Mn(II)	2	2.0 ± 0.1	10.3 ± 0.3
PAR	Pb(II)	1	1.1 ± 0.2	3.7 ± 0.6
	Co(II)	2	1.97 ± 0.05	5.0 ± 0.1
	Zn(II)	2	2.0 ± 0.3	5.5 ± 0.8
Bromobenzothiazo	Cd(II)	2	2.3 ± 0.5	12.9 ± 1.9
HOCAC	Pb(II)	1	0.9 ± 0.2	1.0 ± 0.3
SCPAR	Ag(I)	0.5; 1; 2	1.29 ± 0.03	2.0 ± 0.1
Lumogallion	Mo(VI)	1	1.1 ± 0.1	0.6 ± 0.2
8-Hydroxyquinoline	Ce(IV)	4	2.9 ± 0.3	9.6 ± 0.9
5,7-Dibromo-8-hydroxyquinoline	Ce(IV)	4	2.8 ± 0.1	9.5 ± 0.5
Phenanthroline	Fe(II)	3	2.4 ± 0.2	–15.9 ± 0.3
Bathophenanthroline	Fe(II)	3	2.3 ± 0.2	–17.2 ± 0.4
Co(III)	1N2N	3	2.8 ± 0.3	–0.6 ± 0.2
	2N1N	3	2.9 ± 0.2	3.4 ± 0.4
Cu(II)	PAR	1	1.0 ± 0.1	1.81 ± 0.06
Fe(III)	Benzohydroxamic acid	2	1.8 ± 0.3	6.5 ± 0.5
	Benzohydroxamic acid		2.1 ± 0.5*	8.7 ± 0.9*

*In the presence of ethanol (40 vol. %).

The composition of compounds formed in xerogels and complexation constants was determined (Table 2.15)

$$Me^{n+} + \overline{nHR} = \overline{MeR_n} + nH^+$$

$$nHR + \overline{Me^{n+}} = \overline{MeR_n} + nH^+$$

(The mark denotes the compound in a xerogel phase.)

The calculated equilibrium constants for heterogeneous reactions of complexation change symbately with the corresponding equilibrium constants in solutions.

The kinetics of heterogeneous reactions was studied. It was shown that within a wide concentration range for reagents immobilized in xerogel the equilibrium time remained constant. This fact gives grounds for thinking that the rate-determining step is diffusion. Rate constants for diffusion processes are given in Table 2.16. These heterogeneous reactions can be intensified by microwave radiation, as is illustrated by the data presented in Table 2.17.

TABLE 2.16

Results of mathematical treatment of kinetic functions for various analytes with modified xerogels

System	Equilibrium time (min)	Half-reaction time (min)	Diffusion constant (min^{-1})
Co(II)–PAN/XG	28	4	0.16 (ext.); 0.07 (int.)
Cu(II)–PAN/XG	15	2	0.27 (ext.)
Cd–BBT/XG	25	10	0.11 (ext.)
Pb–CP I/XG	30	12	0.19 (int.)
Pb–TAN/XG	45	10	0.13 (ext.)
Ag–SCPAR/XG	20	5	0.23 (ext.)
Mo(VI)–LG/XG	25	8	0.21 (ext.)
1N2N–Co(III)/XG	13	6	0.37 (ext.); 0.27 (int.)
HPH–Fe(III)/XG	20	10	0.26 (ext.)
Aniline–1-naphthol/XG	20	7	0.14 (ext.)
Phenol–4-AAP/XG	25	5	0.13 (ext.)

TABLE 2.17

Equilibrium times and half-reaction times under usual conditions and in the microwave field (power 600 W)

System	Equilibrium time (min)		Half-reaction time (min)	
	Usual conditions	Microwave field	Usual conditions	Microwave field
Co(II)/PAN–XG	28	9	3.5	2
Cd/BBT–XG	25	6	10	4
Hydrazine/HPA–XG	35	13	19	7.5

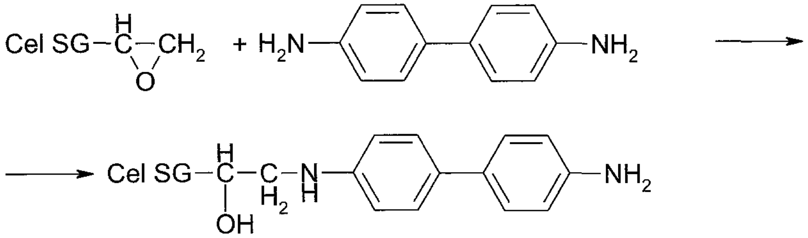
2.3.2 Chemical immobilization

For chemical immobilization (with covalent bonding), paper, organic polymeric sorbents, and silica gels are used. However, the number of sorbents used is less in comparison with the physical immobilization.

The procedure for the preparation of acid–base papers with a covalent fixation of an indicator on a paper support was described as early as 1964 [44]. An amino compound with an active vinylsulphone group was diazotized and azo coupled with various azo compounds (1-naphthol, phenol, and dimethylaniline). In so doing, active indicators were formed. Indicator papers with covalently bonded reagents were prepared by treatment of cellulose in an alkaline medium. The similar synthesis and covalent immobilization of azo dyes for the pH determination was described [45,46]. The easier procedure for the preparation of indicator papers with covalently immobilized reagents was proposed in Ref. [47]. Chromatographic paper modified with glycidyl methacrylate and epichlorohydrin was used as a support [48].

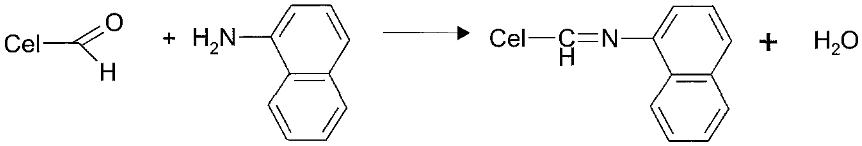
The covalent immobilization of reagents, unlike adsorption immobilization, provides for the multiple use of such papers after the decomposition of complexes formed (e.g. by washing with water or alcohol). However, a multistage and complicated synthesis, toxicity of reagents used, and their inaccessibility restrict the application of such systems.

A procedure for chemical immobilization of benzidine on epoxidized paper or silica gel (SG) was proposed [55]:

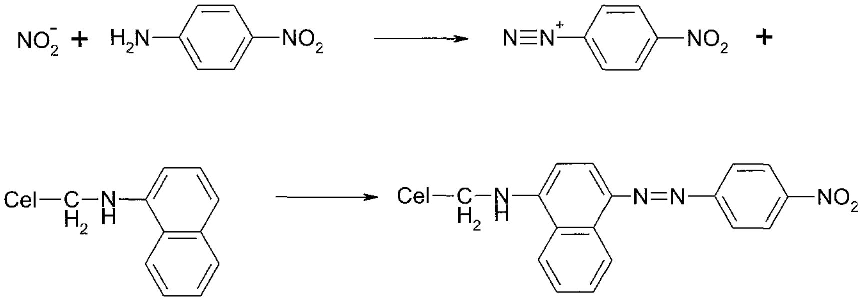


The prepared test systems were used for the determination of oxidizers (Cl_2, H_2O_2, and $K_3[Fe(CN)_6]$) and (after synthesis of diazonium salts) of aniline and phenols.

For the determination of small amounts of nitrates and nitrites (the determination limit is 0.01 mg/l), the chemical immobilization of 1-naphthylamine on dialdehyde cellulose was proposed [56]:

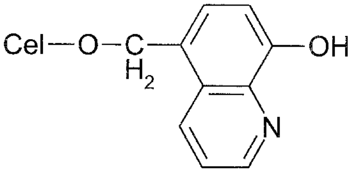


On reacting with diazonium salts, a colourless paper becomes red as the result of synthesis of the azo compound:



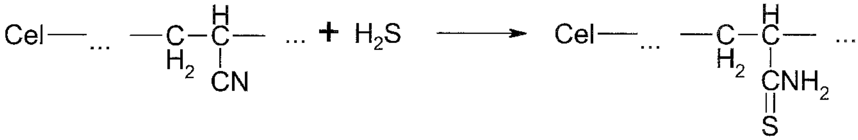
The prepared paper is highly stable in air. Moreover, unlike 1-naphthylamine, it is not very toxic.

The synthesis of cellulose ether containing 8-hydroxyquinoline was performed. The product was formed on interaction of alkaline cellulose with 5-chloromethyl-8-hydroxyquinoline:



The synthesized ether was used for the separation of mixtures (e.g. Cu(II), Ni(II), and Co(II)) [57].

As a result of the treatment of a cellulose–polyacrylonitrile copolymer with hydrogen sulphide, thioamide groups are formed. These groups are capable of complexation with mercury and platinum ions [58]:



$$\text{Cel}-\ldots-\underset{H_2}{C}-\overset{H}{\underset{CN}{C}}-\ldots + H_2S \longrightarrow \text{Cel}-\ldots-\underset{H_2}{C}-\overset{H}{\underset{\underset{\underset{S}{\|}}{C}NH_2}{C}}-\ldots$$

Ostrovskaya et al. investigated thoroughly a great number of chemically modified reagent indicator papers (RIP) [52,53,59]. Several examples are presented in Table 2.18. The chemical names of the majority of reagents are not given. They were characterized also as polydentate. Test tools proposed by the authors provide online pre-

TABLE 2.18

Reagent indicator papers with covalently immobilized analytical reagents [52]

Ion	pH	Analytical range (mg/l)	Colour of adsorbed complex	λ_{max} (nm)
Bi(III)	1	0.01–0.2	Green	655
Fe(II,III)	3–4	0.01–200	Greyish-green	400–700
In(III)	1	0.01–0.2	Cyan	605
Cd(II)	6	0.03–1.0	Brown	560
Co(II)	6–7	0.05–1.0	Brownish-red	—
Cu(II)	4–7	0.001–500	Bright blue	580
Hg(II)	3	0.02–2.0	Greyish-black	—
Pb(II)	6	0.01–2.0	Yellowish-green	—
Zn(II)	6–10	0.001–100	Dark-red	500
NO_2^-	4–5	0.5–50	Reddish-violet	490

concentration and semiquantitative estimation of concentration of the tested component with a pocket concentrating device.

Reagent indicator papers for the determination of SO_4^{2-}, Cl^-, CrO_4^{2-}, oxidants (Cl_2), aromatic amines, hydrazine, ketones (acetone, methyl butyl ketone, acetoacetic acid), and phenols were developed. All reagent indicator papers were characterized as to their selectivity, applications, and shelf life. The detection limits were compared with the MACs.

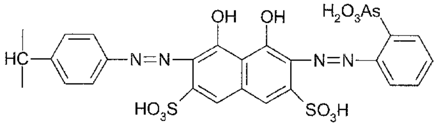
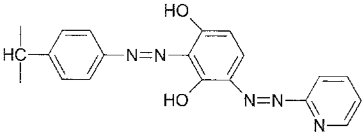
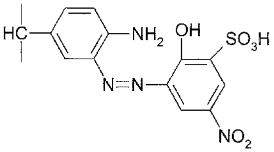
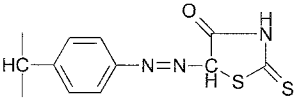
Several reagent indicator papers are highly selective. To illustrate, a RIS-Iron(II,III)-Test permits the determination of 1 mg/l Fe(III) (pH 3–4) in the presence of the following elements (mg/l): Ca, Cd, Cr(III), K, Na, Ni, Mg, and Zn (1000); Mn(II) (500); Al, Ba, B, Ce, Cr(VI), Co, Ga, Li, Ag, Sr, Tl, Th, Sn(II),Ti, W, and Y (100); Sn(IV) and Zr (30); Pd (20); Hg(II) (5); Be (4); Cu and V (3); NO_3^-, NO_2^-, $S_2O_8^{2-}$, SO_4^{2-}, SO_3^{2-}, $S_2O_3^{2-}$, and Cl^- (1000); CH_3COO^-, $Cr_2O_7^{2-}$, BrO_3^-, I^-, MoO_4^{2-}, MnO_4^-, SCN^-, salicylate, EDTA (100); ascorbate, $C_2O_4^{2-}$, and citrate (5); tartrate (4); S^{2-} and PO_4^{3-} (1). A RIS-Indium-Test at pH 1–2 permits highly selective determination of indium. It does not react with alkaline and alkaline-earth metals and rare-earth elements. At pH 4–7, the selectivity of the reactions falls an the degree of colour contrast decreases. For a number of elements, analytical ranges are very wide thanks to the use of several colour scales.

Chelating sorbents are of great interest for test systems. They are three-dimensional cross-linked polymers exhibiting chelating properties or simultaneous ion-exchanging and chelating properties which are determined by functional analytical groups in polymer molecules. Sorbents based on styrene-divinylbenzene copolymers and chemically modified silica gels receive the widest application. Such sorbents make it possible to concentrate target ions, to separate them from the matrix and interfering ions, and to measure them in the sorbent phase either via diffuse-reflectance spectroscopy or solid-phase spectrophotometry, or, by any one method, after desorption. As sorbates are coloured, the intensity of the colour can be estimated either visually or with pocket test devices. Examples of chelating sorbents based on copolymer matrices are presented in Table 2.19.

Chemically modified silica was described above (Section 2.3). It also contains functional groups for different ions. They are widely used for separation and preconcentration. However, chemically modified silicas are not applied in test systems, though this is possible. Both the sorbate and matrix are colourless. The adsorbed component even of a high

TABLE 2.19

Chelating sorbents based on linear polystyrene [133]

Reagent	Sorbent linkage with chelating groups	Adsorbed elements
Polystyrene-azo–Arsenazo		Be, Hf, Mo, Nb, Pa, Pu, Sc, Th, U, rare-earth elements
Polystyrene-azo–PAR		Cu, Hf, Mo, Nb, U, V, rare-earth elements
Polynitroxaminazo		Au, Pd
Polystyrene–azorhodanine		Au, Ir, Pd, Pt, Ag, Rh

concentration cannot be seen. Testing can be performed if the sorbate is treated with a reagent that forms a very stable coloured complex. Another application involves preconcentration in a small volume of a sorbent (for increasing the sensitivity) followed by desorption with a small volume of an eluent (usually with an acid for determining metals) and testing in an aqueous solution. Chemically modified silicas are known, which contain mono- and polyamine groups, complexones (iminodiacetic and ethylenediaminetriacetic acids), hydroxamic acids, neocuproine, 1,10-phenanthroline, diethyldithiocarbamate, and formazans [95].

2.4 CATALYTIC REACTIONS

2.4.1 Enzymes

Enzymes (biological catalysts) are significantly different from the usual chemical reagents. As a rule, they exhibit activity in only a few processes. That is why they are characterized by a high, sometimes unique, selectivity. Catalytic activity of enzymes is usually very high. Therefore, only very small amounts and concentrations of enzymes are needed for analytical purposes. However, the activity proper depends on many factors, viz. the source from which the reagent was prepared, time and conditions of storage, separation efficiency, and conditions of employment.

For developing test systems, immobilized enzymes are more convenient than native (free) ones. The most stable and relatively inexpensive enzymes are needed (certainly, enzymes are almost without exception more expensive than the usual chemical reagents). The use of the following enzymes has been reported: peroxidases [134–159], glucose oxidase [137–139,141,145–147,160,161], urease [162,163], choline esterase [164,165], monoamine oxidase [166], alcohol oxidase [167,168], lactate dehydrogenase [169], lipase [146], esterase [170], diaphorase [171–173], catalase [172,173], and dehydrogenase [171].

In selecting enzymes for developing test systems, the possibility of obtaining a distinct analytical signal is taken into account.

For immobilization of enzymes and developing test systems, various supports are used, viz. papers and polymers, e.g. polyurethanes. A convenient procedure for immobilization involves the formation of a solid solution in film-forming substances (e.g., in chitosan). Chitosan is a polysaccharide prepared from chitin through alkaline hydrolysis. Chitosan is soluble in water and buffer solutions. Other film-forming substances that preserve the enzyme activity can be used, e.g. polyvinyl alcohol, gelatine, etc. Enzymes can also be chemically immobilized on supports [33].

For chemical immobilization of enzymes in test systems aldehyde cellulose is most often used. Free groups of enzyme are linked with aldehyde groups of cellulose. In a similar way, immunoglobulins are immobilized on paper for immunoenzyme reactions. To prevent washing off of enzymes from the matrix, the latter is cross-linked with glutaric aldehyde. Alternatively, glutaric aldehyde is used as a cross-linking agent between a matrix with amino groups and an enzyme.

TABLE 2.20

Enzymatic test systems

Reagent, indicator system	Test component	Conc. range	Material	Ref.
Peroxidase, glucose oxidase, *o*-tolidine, carboxymethylcellulose	Glucose	1–40 mmol	Blood	[137]
Peroxidase, glucose oxidase, sodium ascorbate, *o*-tolidine	Glucose	1–10 mmol	Urine	[139,145]
Peroxidase, glucose oxidase, *o*-tolidine, glucose	Catalase	—	Biological liquids	[147]
Urease, Brilliant Yellow	Urea	—	Blood, plasma	[163]
Alcohol oxidase, peroxidase, *o*-dianisidine	Ethanol, aliphatic alcohols	—	Solutions	[148]
Esterase (I), buffer compounds	Organophosphorus pesticides	—	—	[170]
Diaphorase, catalase, alcohol oxidase, Methylene Blue	Ethanol, aliphatic alcohols	100–1500 mg/l	Solutions	[172,173]
Dehydrogenase, diaphorase, NADP, buffers and colourants	Alkaline-phosphatase	—	Blood	[171]

Despite chemical immobilization leading to a decrease in enzyme activity, it provides for more stable test forms with a shelf life of not less than a year at room temperature.

A wide range of test systems are based on cholinesterase, especially systems developed for detection of poison gases [49]. Systems for the determination of glucose in blood based on glucose oxidase are most widely used.

In most of enzymatic test systems, papers treated with enzymes and auxiliary reagents are used. Intensity of colour is detected visually. Several examples are given in Table 2.20. Clinical applications of test systems are discussed more comprehensively in Chapter 10.

Immunoenzyme procedures based on reagent indicator papers were developed [174,175]. Using fluorogenic substrates and groups as markers, reagents for immunoenzyme analysis were immobilized on paper strips [176,177]. Indicator papers were developed for the determination of morphine in blood in the analytical range 5–1000 ng/ml [178].

2.4.2 Catalytic non-enzymatic reactions

A procedure for the visual determination of cobalt(II) based on an autocatalytic reaction exemplifies the kinetic method [182]. Time for discolouration of a coloured inert compound (cobalt(III) bis[2-(5-bromo-2-pyridylazo)-5-(N-propyl-N-sulphopropylaminophenol ate]) serves as an analytical signal. The compound is decomposed through oxidation with peroxymonosulphate. On oxidative decomposition of this compound, cobalt ions are released. They serve as a catalyst of the complex decomposition. Decomposition time is inversely proportional to the logarithms of the initial concentration of cobalt(II). The determination is performed in the presence of some free azo reagent. There are several variants of the procedure, namely a macro (3 ml of an analysed solution), a micro (on a plate, sample volume 30 µl), and a spot variant (sample volume 1.5 µl). For the latter procedure, 5.0 µl of a reagent solution and 1.0 µl of water are applied onto a teflon plate. The plate is mechanically shaken. On simultaneous addition of 2.5 µl of a 2% solution of potassium peroxymonosulphate (Oxone) and 1.5 µl of the sample solution, the reaction occurs. Time of the discolouration of the violet Co(III) complex is measured with a stopwatch. During the reaction, the temperature should be kept constant (25°C). The procedure was used for the determination of cobalt in urine.

The kinetic procedure based on Landolt-type reaction, which is catalysed by some ions, was developed and used for the determination of vanadium and molybdenum [183,184]. The reaction based on catalytic substitution was applied for a visual determination of the total amount of heavy metals in fresh water [185].

The characteristics of catalytic reactions performed on a sorbent often differ significantly from the characteristics of those in solution. This, for example, is the case of the determination of manganese on paper [186]. Mn(II) is a catalyst for the oxidation of 3,3′,5,5′-tetramethylbenzidine (TMB) by periodate in an aqueous solution and on filter paper unmodified or modified with diethyltriaminotetraacetate (for preconcentration of manganese). For equal manganese concentrations, papers demonstrate a higher initial rate of reaction. A procedure for a rapid determination of manganese with visual detection was developed.

Similar differences were observed for other reactions. To illustrate, these are reactions for the determination of phosphorus-containing pesticides on plates for thin-layer chromatography [187], copper(II) by its catalytic activity in the oxidation of hydroquinone by hydrogen peroxide (paper and solution were compared) [188], and for the determination of cadmium with bromobenzothiazo noncovalently immobilized on silica gel or paper [189].

The test procedure for the determination of Cu(II) is based on the catalytic activity of copper in the reaction of oxidation of TMB by ammonium persulphate in the presence of γ-picoline as an activator [190]. The concentration of copper is determined by colour matching the reaction product against a reference scale. The reaction can be performed either on paper or on a Silochrom S-80 silica gel. The detection limit is 0.1 μg/ml.

Another above-mentioned procedure [188] for rapid determination of copper is based on the fact that copper(II) is a catalyst in the reaction of oxidation of hydroquinone with hydrogen peroxide in the presence of 2,2′-dipyridyl and malonic-acid dinitrile. The latter compound reacts with intermediate *p*-benzoylquinone. In so doing, the colour of the reaction product changes from pink to orange to greyish-green to cyan depending on the ratio of quinone to malononitrile, i.e. finally on the concentration of copper. All the reagents are deposited by a pipette on a paper filter. The analytical range is $1 \cdot 10^{-5}$–0.1 μg of copper for solution volume 1 μl.

Amines (mono-, di-, and tri-substituted pyridines and 2,2′-dipyridyl) can be determined by their influence on the catalytic activity of copper(II) in the above reaction of oxidation of hydroquinone with hydrogen peroxide [191]. The reaction is performed in a drop variant on Sorbfil plates. The detection limits for amines are within the range of 10^{-6}–10^{-10} mol.

2.5 IMMUNOASSAY PROCEDURES

Immunoassay test procedures are not of a chemical nature and we do not consider them in this book. However, it is necessary to pay some attention to these methods because they are important tools of out-of-laboratory analysis. For several decades immunotests have been widely used in medicine and, to some extant, in food analysis. In the 1990s, they became used in environmental analysis [192,193]. Based on these methods, various kits are produced and used for the determination of petroleum hydrocarbons, polyaromatic hydrocarbons, polychlorobiphenyls, pentachlorophenol, numerous pesticides, and other substances in water, food, and soils.

The US Environmental Protection Agency (USEPA) pioneered a number of immunoassay methods, mostly for on-site screening. Based on enzyme immunoassay analysis (EIA), procedures for the determination of BTX (benzene, toluene, xylene), polychlorobiphenyls (PCB), polychlorophenols (PCP), pesticides in water and soil, cocaine, and heroin were developed and came into practice. The procedures are very suitable for preliminary characterization of samples when only a limited number of components is to be determined. EIA tests are simple, rapid (take several minutes), and relatively inexpensive (though they are more expensive than chemical tests). Procedures are designed for determination of numerous toxic organic compounds. These systems often provide for very low detection limits.

EIA methods exhibit several drawbacks. It takes a long time to analyse many samples; each compound requires definite reagents and procedures and this increases analysis time if many components have to be determined. It is not easy to ensure proper quality control under out-of-laboratory conditions. For a number of organic toxicants, test kits are not easily available or do not exist at all.

The enzyme-linked immunosorbent assay (ELISA) is the most widely used among immunoassay methods.

Merck Company produces immunotests for the determination of 2,4,6-trinitrotoluene (the TNT Explosive Test), aromatic hydrocarbons (benzene, toluene, ethylbenzene, and xylene) (the BTEX-Test), polyaromatic hydrocarbons (the PAH-Test), and polychlorobiphenyls (the PCP-Test). All the tests are intended for on-site analysis. They are used in combination with DTECH® Immunological Field Tests.

Immunotests are most often used for the determination of pesticides. Thus, the Ohmicron Corp. (USA) markets a great number of test kits (Penta RaPID) based on ELISA for the determination of various pesticides (Table 2.21). This company also produces EPA approved tests for determination of toxic industrial substances (PCBs, PCP, TNT, and petroleum aromatic hydrocarbons). In our opinion, the company produces test system selective to benz(a)pyrene and other carcinogenic PAHs.

Millipore EnviroGard Test Kits were designed for screening waters, soils, and foods for pesticides, industrial toxicants (more than 40

TABLE 2.21

Rapid Assay Kits for determination of pesticides

Baker Rapid Assay	Art. no.	Concentration range (ppb)	Detection limit (ppb)
2,4-D	2931	0.7–50	0.7
Alachlor	2924	0.05–5	0.05
Aldicarb	2922	0.25–100	0.25
Atrazin	2923	0.04–5	0.04
High Sensitivity Atrazin	2942	0.015–1	0.015
Benomyl	2933	0.1–5	0.1
BTEX	2972	0.02–3	0.02
Captan	2932	0.01–3	0.01
Carbaryl	2936	0.25–5	0.25
Carbofuran	2926	0.06–5	0.056
Carcinogenic PAH	2957	0.2–10	0.2
Chlorpyrifos	2938	0.1–3	0.1
Chlorothalonil	2935	0.07–5	0.07
Cyanazine	2925	0.04–3	0.035
Metolachlor	2930	0.05–5	0.05
Methomyl	2954	0.45–15	0.45
PAH	2944	0.7–50	0.7
Paraquat	2941	0.02–0.5	0.02
PCB	2939	0.1–5	0.1
Pentachlorophenol	2937	0.06–10	0.06
Procymidone	2934	0.8–100	0.8
Silvex	2955	1.4–250	1.4
TNT	2973	0.07–5	0.07
Trichloropyridinol	2956	0.25–6	0.25
Trichlopyr	2946	0.1–3	0.03

compounds), and for petroleum hydrocarbons in soil. Analyses are performed almost without sample preparation and with a minimal number of solvents. The kits do no require trained personnel. Test forms are various, including tubes and plates. In several cases, mini photometers are used. Several tests are very sensitive. To illustrate, the detection limits for 2,4-D and dicyanol in water are 0.1 ppb and 0.002 ppm, respectively.

EnSys Environmental Products Inc. developed Penta RiSc Test System including kits for the determination of PCB. The information on test kits is available from EPA Bulletins: *EPA/540/MR–95/514, EPA/540/MR–95/515, EPA/540/MR–95/517, EPA/540/MR–95/518, EPA/540/MR–95/521,* and *EPA/540/MR–95/528.*

A great number of immunoassay kits for environmental analysis is supplied by the Strategic Diagnostics Inc.: *PCB 4020, PAH 4035, Carcinogenic PAH 4035, TPH 4030, PCP 4010, TNT 4050, TNT 8515, RDX 4051, RDX 8510, Toxaphene 4040, Chlordane 4041, DDT 4042,* and *2,4-D 4015.*

For obtaining analytical signals in immunoassay analysis, portable photometers or reflectometers are often used [192]. A portable photometer–reflectometer for qualitative immunoassay determination was recently designed [193]. The instrument operates in absorbance and reflectance modes. It can be used for field analysis. Potentialities of immunoassay methods for testing are discussed in greater detail in [194–199].

REFERENCES

1. E. Bishop, *Indicators*, Pergamon, Oxford, 1972.
2. V.M. Ostrovskaya and N.A. Fomin, *Vysokochist. Veshchestva*, No. 4 (1987) 183.
3. V.M. Ivanov, S.A. Morozko and Yu.A. Zolotov, *Zh. Anal. Khim.*, 48 (1993) 1389.
4. V.M. Ivanov, N.V. Erokhina and V.N. Figurovskaya, *Vestn. Mosk. Univ., Ser. 2: Khim.*, 35 (1994) 65.
5. V.M. Ivanov and N.V. Erokhina, *Vestn. Mosk. Univ., Ser. 2: Khim.*, 35 (1994) 269.
6. G. Iwantscheff, *Das Dithizon und Seine Anwendung in der Mikro- und Spurenanalyse*, 2 Ed., Verlag Chemie, Weinheim, 1972.
7. V.A. Nazarenko and V.P. Antonovich, *Trihydroxyfluorones*, Nauka, Moscow, 1983 (in Russian).
8. V.M. Peshkova, V.M. Savostina and E.K. Ivanova, *Oximes,* Nauka, Moscow, 1977 (in Russian).
9. V.M. Byr'ko, *Dithiocarbamates, Nauka,* Moscow, 1984 (in Russian).
10. V.M. Ivanov, *Heterocyclic Nitrogen-Containing Azo Compounds*, Nauka, Moscow, 1982 (in Russian).
11. S. Tong, F. Lui and K. Li, *J. Environ. Sci.*, 1 (1989) 121.
12. V.M. Ivanov and S. Massud, *Vestn. Mosk. Univ., Ser. 2: Khim.*, 34 (1993) 572.

13. S.A. Morozko and V.M. Ivanov, *Zh. Anal. Khim.*, 51 (1996) 631.
14. V.M. Ivanov and N.I. Ershova, *Vestn. Mosk. Univ., Ser. 2: Khim.*, 38 (1997) 396.
15. N.I. Ershova and V.M. Ivanov, *Anal. Chim. Acta*, 364 (1998) 235.
16. O.P. Shvoeva, S.B. Savvin and L.M. Trutneva, *Zh. Anal. Khim.*, 45 (1990) 476.
17. K. Yoshimura, Y. Toshimitsu and S. Ohashi, *Talanta*, 27 (1980) 693.
18. J. Chwastowska and E. Mozer, *Talanta*, 32 (1985) 574.
19. N.M. Alfonso, N.E. Lacomba and S. Arpadjan, *Dokl. Bulg. Akad. Nauk*, No. 9, 43 (1990) 53.
20. V.M. Ivanov and S. Massud, *Vestn. Mosk. Univ., Ser. 2: Khim.*, 35 (1994) 350.
21. V.M. Ivanov and N.I. Ershova, *Vestn. Mosk. Univ., Ser. 2: Khim.*, 39 (1998) 101.
22. S.A. Morozko, V.M. Ivanov and S. Massud, *Vestn. Mosk. Univ., Ser. 2: Khim.*, 37 (1996) 253.
23. V.M. Ivanov and N.I. Ershova, *Vestn. Mosk. Univ., Ser. 2: Khim.*, 39 (1998) 170.
24. V.M. Ivanov, S.A. Morozko and S. Massud, *Zh. Anal. Khim.*, 50 (1995) 1280.
25. V.M. Ivanov and O.V. Kuznetsova, *Zh. Anal. Khim.*, 50 (1995) 498.
26. O.A. Zaporozhets, O.M. Gawer and V.V. Sukhan, *Usp. Khim.*, 66 (1997) 702.
27. O.V. Kuznetsova, *Cand. Sc. Dissertation*, Moscow State University, Moscow, 2000 (in Russian).
28. V.M. Ivanov and N.I. Ershova, *Vestn. Mosk. Univ., Ser. 2: Khim.*, 40 (1999) 22.
29. O.V. Kuznetsova,V.M. Ivanov and N.V. Kazennov, *Vestn. Mosk. Univ., Ser. 2: Khim.*, 38 (1997) 53.
30. V.M. Ivanov, O.V. Kuznetsova, N.A. Nosova and T.V. Polenova, *Vestn. Mosk. Univ., Ser. 2: Khim.*, 38 (1997) 85.
31. V.M. Ivanov and G.A. Kochelaeva, *Vestn. Mosk. Univ., Ser. 2: Khim.*, 42 (2001) 17.
32. V.M. Ivanov and G.A. Kochelaeva, *Vestn. Mosk. Univ., Ser. 2: Khim.*, 42 (2001) 103.
33. V.G. Amelin, *Zh. Anal. Khim.*, 55 (2000) 902.
34. V.G. Amelin and V.M. Ivanov, *Zh. Anal. Khim.*, 55 (2000) 411.
35. V.G. Amelin, *Doctoral Sc. Dissertation*, Moscow State University, Moscow, 1998 (in Russian).
36. R.K. Chernova, S.N. Shtykov, L.A. Agranovskaya and V.D. Bubelo, *USSR Inventor's Certificate* 1555667, *Byull. Izobret.*, No. 13 (1990).
37. R.P. Pantaler, A.K. Timchenko, L.I. Avramenko and A.B. Blank, *Zh. Anal. Khim.*, 52 (1997) 384.

38. V.M. Ivanov and A.G. Shirinova, *Vestn. Mosk. Univ., Ser. 2: Khim.*, 33 (1992) 63.
39. A.G. Shirinova, G.V. Prokhorova, V.M. Ivanov and E.A. Osipova, *Zh. Anal. Khim.*, 48 (1993) 176.
40. A.G. Shirinova, T.V. Rodionova, V.M. Ivanov, M.K. Beklemishev and Yu.A. Zolotov, *Zh. Anal. Khim.*, 48 (1993) 55.
41. A.G. Shirinova and V.M. Ivanov, *Zh. Anal. Khim.*, 49 (1994) 266.
42. A.G. Shirinova and V.M. Ivanov, *Vestn. Mosk. Univ., Ser. 2: Khim.*, 37 (1996) 267.
43. A.G. Shirinova and V.M. Ivanov, *RF Pat.* 2038579, *Byull. Izobret.* No. 18 (1995).
44. E. Pfeil, *Ger. Pat.* 1256445, 1964.
45. K. Neisius, *Ger. Pat.* 1698247, 1980.
46. P. Rosental and H. Roesgen, *Ger. Pat.* 2362859, 1975.
47. V.M. Ostrovskaya and N.A. Fomin, *RF Pat.* 2095779, *Byull. Izobret.* No. 31 (1997).
48. V.M. Ostrovskaya, N.A. Fomin, M.S. Aksenova, T.S. Kazakova, T.D. Popova and E.T. Prishchep, *USSR Inventor's Certificate* 1651204, *Byull. Izobret.*, No. 19 (1991).
49. Yu.A. Zolotov, *Ann. Chim.(Paris),* 87 (1997) 285.
50. V.M. Ostrovskaya, *Fresenius' J. Anal. Chem.*, 361 (1998) 303.
51. V.M. Ostrovskaya and O.V. Ivanov, *Vysokochist. Veshchestva*, No. 4 (1987) 178.
52. V.M. Ostrovskaya, *Reactive Indicator Tools for Multielement Tests*, 1-ya Obraztsovaya tipografiya, Moscow, 1992 (in Russian).
53. V.M. Ostrovskaya, *Zh. Anal. Khim.*, 5 (1996) 987.
54. V.M. Ostrovskaya, M.S. Aksenova and R.L. Medvedev, *Zh. Anal. Khim.*, 42 (1987) 2197.
55. V.M. Ostrovskaya, Yu.A. Zolotov, E.I. Morosanova and D.Yu. Marchenko, *Fresenius' J. Anal. Chem.*, 361 (1998) 300.
56. V.G. Amelin and I.S. Kolodkin, *in VII All-Russian Conference on Organic Reagents in Analytical Chemistry*, Saratov, 1999, (in Russian).
57. Lin Yan, V.A. Derevitskaya and Z.A. Rogozin, *Vysokomol. Soedin.*, No. 1, 1 (1959) 157.
58. Z.A. Rogozin and L.S. Gal'braikh, *Chemical Reactions and Modification of Cellulose*, Khimiya, Moscow, 1979 (in Russian).
59. V.M. Ostrovskaya, *Zh. Anal. Khim.*, 22 (1977) 1820.
60. S. Horiguchi and M. Makamura, *Jap. Pat.* 73–02930, 1973.
61. H. Tanaka, K. Sigiyama, T. Nakaya and M. Imoto, *Macromol. Chem.*, 175 (1974) 1855.
62. S.G. Dmitrienko, O.A. Kosyreva, V.K. Runov and Yu.A. Zolotov, *Mendeleev Commun.*, No. 2 (1991) 75.
63. S.G. Dmitrienko, Yu.A. Zolotov, O.A. Kosyreva, V.K. Runov and E.S.

Frolova, *RF Pat.* 1803839, *Byull. Izobret.* No. 11 (1993).

64. S.G. Dmitrienko, Yu.A. Zolotov, V.K. Runov and E.S. Frolova, *RF Pat.* 1803837, *Byull. Izobret.* No. 11 (1993).
65. S.G. Dmitrienko, E.M. Loginova and V.K. Runov, *Zh. Anal. Khim.*, 50 (1995) 420.
66. E.M. Loginova, S.G. Dmitrienko, V.K. Runov, T.G. Jordanidi and Yu.A. Zolotov, *Zh. Anal. Khim.*, 50 (1995) 423.
67. S.G. Dmitrienko, L.N. Pyatkova, L.P. Bakhaeva, V.K. Runov and Yu.A. Zolotov, *Zh. Anal. Khim.*, 51 (1996) 493.
68. S.G. Dmitrienko, L.N. Pyatkova and V.K. Runov, *Zh. Anal. Khim.*, 51 (1996) 600.
69. S.G. Dmitrienko, E.N. Myshak, V.K. Runov and Yu.A. Zolotov, *Chem. Anal. (Warsaw),* 40 (1995) 291.
70. A.G. Hamza, A.B. Farag and A. Al-Herthani, *Microchem. J.*, 32 (1985) 13.
71. A.B. Farag, M.E. Hassouna and M.H. Abdel-Rahman, *Bull. Soc. Chim. Belg.*, 96 (1987) 147.
72. Yu.A. Zolotov, *Khim. Prom-st. (Moscow),* No. 6 (1997) 48.
73. V.A. Nazarenko, Zh.N. Grabovskaya, S.V.Tsigankova and S.V. Bel'tyukova, *Zh. Anal. Khim.*, 48 (1993) 61.
74. M. Fujimoto, *Chem. Analyst (Cambridge, U.K),* 49 (1966) 4.
75. O. Samuelson, *Ion Exchange Separations in Analytical Chemistry*, Wiley, New York, 1963.
76. W. Rieman and H.F. Walton, *Ion Exchange in Analytical Chemistry*, Pergamon Press, Oxford, 1970.
77. F.I. Lobanov, G.K. Nurtaeva and N.V. Makarov, *Usp. Khim.*, 43 (1979) 1453.
78. L.M. Trutneva, O.P. Shvoeva and S.B. Savvin, *Zh. Anal. Khim.*, 44 (1989) 1804.
79. S.B. Savvin, L.M. Trutneva, O.P. Shvoeva and K.A. Effendieva, *Zh. Anal. Khim.*, 46 (1991) 709.
80. O.P. Shvoeva, L.M. Trutneva, E.A. Likhonina and S.B. Savvin, *Zh. Anal. Khim.*, 46 (1991) 1301.
81. O.P. Shvoeva, L.M. Trutneva and S.B. Savvin, *Zh. Anal. Khim.*, 49 (1994) 574.
82. S.B. Savvin, O.P. Shvoeva, V.P. Dedkova, S.V. Rudakov and A.V. Mikhailova, *Zh. Anal. Khim.*, 51 (1996) 308.
83. V.P. Dedkova and O.P. Shvoeva, in *International Congress on Analytical Chemistry*, Moscow, 1997, Vol. 2, K-13 (in Russian).
84. M.M. Kushner, N.A. Klassovskaya and B.P. Zemskii, *USSR Inventor's Certificate* 1698756, *Byull. Izobret.*, No. 46 (1989).
85 *Process Eng.* (Austral.), No. 8, 20, 21 (1993).
86. A.G. Balekaev and M.A. Balayan, *USSR Inventor's Certificate* 1797054, *Byull. Izobret.*, No. 7 (1993).

87. A.G. Balekaev and M.A. Balayan, *USSR Inventor's Certificate* 1817022, *Byull. Izobret.*, No. 7 (1993).
88. L.M. Nikolaeva, V.G. Putilov and O.M. Zaichkina, *USSR Inventor's Certificate* 1673958, *Byull. Izobret.*, No. 32 (1991).
89. I. Kobayasi and H. Takeno, *Kogyo Josui*, No. 85, (1965) 36.
90. I.M. Maksimova, E.I. Morosanova, A.A. Kukhto, N.M. Kuz'min and Yu.A. Zolotov, *Zh. Anal. Khim.*, 49 (1994) 1210.
91. E.I. Morosanova, I.V. Pletnev, V.Yu. Solov'ev, N.V. Semenova and Yu.A. Zolotov, *Zh. Anal. Khim.*, 49 (1994) 676.
92. V.V. Sukhan, O.Yu. Nadzhafova, O.A. Zaporozhets and L.I. Savranskii, *Khim. Tekhnol. Vody*, No. 2, 16 (1994) 139.
93. W. Beil, A. Hoeppener, J.H. Wolf and C.H. Beil, *BRD Pat.* 1961811, 1971.
94. S.A. Morozko and V.M. Ivanov, *Zh. Anal. Khim.*, 52 (1997) 858.
95. G.V. Lisichkin (Editor), *Modified Silicas in Adsorption, Catalysis and Chromatography*, Khimiya, Moscow, 1986 (in Russian).
96. V.M. Ivanov, G.V. Lisichkin, G.V. Kudryavtsev and P.N. Nesterenko, *Determination of Small Concentrations of Elements*, Nauka, Moscow, 1986 (in Russian).
97. G.V. Lisichkin, G.V. Kudryavtsev, V.M. Ivanov and V.N. Figurovskaya, *Zh. Vses. Khim. O-va im. D.I. Mendeleeva*, 24 (1979) 294.
98. G.V. Kudryavtsev, V.M. Ivanov and G.V. Lisichkin, *Dokl. Akad. Nauk SSSR*, 250 (1980) 635.
99. T.F. Rudometkina, V.M. Ivanov, G.V. Kudryavtsev and G.V. Lisichkin, *Zh. Anal. Khim.*, 37 (1982) 2207.
100. G.V. Kudryavtsev, G.V. Lisichkin and V.M. Ivanov, *Zh. Anal. Khim.*, 38 (1983) 22.
101. V.M. Ivanov, G.N. Gorbunova, G.V. Kudryavtsev and G.V. Lisichkin, *Zh. Anal. Khim.*, 39 (1984) 504.
102. P.N. Nesterenko, V.M. Ivanov, G.V. Kudryavtsev and G.V. Lisichkin, *Zh. Anal. Khim.*, 39 (1984) 456.
103. I.P. Alimarin, P.N. Nesterenko and V.M. Ivanov, *Dokl. Akad. Nauk SSSR*, 271 (1983) 627.
104. T.F. Rudometkina, E.P. Chertenko, V.M. Ivanov, G.V. Lisichkin and G.V. Kudryavtsev, *Zh. Anal. Khim.*, 40 (1985) 292.
105. T.F. Rudometkina, N.V. Pergunova and V.M. Ivanov, *Zh. Anal. Khim.*, 40 (1985) 1039.
106. G. Pollakowski and J.A. Keiler, *GDR Pat.* 205010, 1983.
107. V.G. Amelin, *Zh. Anal. Khim.*, 53 (1998) 868.
108. P.J.M. Sloan, G. Beevers and F.E. Baxter, *Clin. Chem. (Winston-Salem, N.C.)*, 30 (1984) 1705.
109. V.G. Amelin, *RF Pat.* 2093823, *Byull. Izobret.*, No. 29 (1997).
110. S.B. Savvin, L.M. Trutneva and O.P. Shvoeva, *Zh. Anal. Khim.*, 48 (1993) 502.

111. V.P. Dedkova, O.P. Shvoeva and S.B. Savvin, *Zavod. Lab.*, No. 12, 64 (1998) 7.
112. V.P. Dedkova, O.P. Shvoeva and S.B. Savvin, *Zavod. Lab.*, No. 3, 64 (1998) 12.
113. M.P. Volynets, R.F. Gur'eva and T.V. Dubrova, *Zh. Anal. Khim.*, 46 (1991) 1595.
114. L.E. Zel'tser and A.V. Bychenko, *Zh. Anal. Khim.*, 48 (1993) 1659.
115. V.M. Ivanov, A.V. Rybakov, V.N. Figurovskaya, G.V. Prokhorova and G.A. Kochelaeva, *Vestn. Mosk. Univ., Ser. 2: Khim.*, 39 (1998) 258.
116. V.M. Ivanov, N.I. Ershova and V.N. Figurovskaya, *Zh. Anal. Khim.*, 54 (1999) 1153.
117. N.I. Ershova and V.M. Ivanov, *Anal. Chim. Acta,* 408 (2000) 145.
118. Yu.A. Barbalat and V.M. Ivanov, *Vestn. Mosk. Univ., Ser. 2: Khim.*, 37 (1996) 48.
119. Yu.A. Barbalat, V.M. Ivanov, T.V. Polenova and N.V. Fedorova, *Vestn. Mosk. Univ., Ser. 2: Khim.*, 39 (1998) 174.
120. S.A. Morozko and V.M. Ivanov, *Zh. Anal. Khim.*, 50 (1995) 629.
121. V.M. Ivanov and S.A. Morozko, *Zh. Anal. Khim.*, 51 (1996) 1083.
122. N.A. Tananaev, *Spot Analysis*, Goskhimizdat, Moscow, Leningrad, 1954 (in Russian).
123. V.G. Amelin, *Zh. Anal. Khim.*, 53 (1998) 958.
124. H.P. Andersen, *US Pat.* 3511608, 1970.
125. K.A. Blake, J.E. Christner and L.J. Lucchese, *PCT (WO),* 92/177768, 1992.
126. H. Matsumoto, *Jap. Pat.* 48-14273, 1973.
127. V.M. Ostrovskaya, *RF Pat.* 2009486, *Byull. Izobret.*, No. 5 (1994).
128. D.Yu. Marchenko, I.A. Morozkin, E.I. Morosanova, N.M. Kuz'min and Yu.A. Zolotov, *Zh. Anal. Khim.*, 52 (1997) 1292.
129. D.Yu. Marchenko, E.I. Morosanova, N.M. Kuz'min and Yu.A. Zolotov, *Zh. Anal. Khim.*, 52 (1997) 1289.
130. E.I. Morosanova, A.A. Velikorodnyi, N.M. Kuz'min and Yu.A. Zolotov, *RF Pat.* 2139243, *Byull. Izobret.*, No. 28 (1999).
131. E.I. Morosanova, A.A. Velikorodnyi and Yu.A. Zolotov, *RF Pat.* 2139244, *Byull. Izobret.*, No. 28 (1999).
132. E.I. Morosanova, A.A. Velikorodnyi, Yu.A. Zolotov and V.I. Skornyakov, *Zh. Anal. Khim.*, 55 (2000) 1265.
133. G.V. Myasoedova and S.B. Savvin, *Chelating Sorbents*, Nauka, Moscow, 1984 (in Russian).
134. E.B. Nikol'skaya, G.A. Evtyugin and T.N. Shekhovtsova, *Zh. Anal. Khim.*, 49 (1994) 452.
135. T.N. Shekhovtsova, S.V. Chernetskaya, E.B. Nikol'skaya and I.F. Dolmanova, *Zh. Anal. Khim.*, 49 (1994) 862.
136. T.N. Shekhovtsova, S.V. Chernetskaya, N.V. Belkova and I.F. Dolmanova, *Zh. Anal. Khim.*, 50 (1995) 538.

137. A.P. Filippov, I.K. Mel'nik, K.B. Yatsimirskii, V.P. Komissarenko and A.S. Efimov, *USSR Inventor's Certificate* 1155944, *Byull. Izobret.*, No. 18 (1985).
138. A.P. Filippov, I.K. Mel'nik, A.S. Efimov, K.B. Yatsimirskii, V.P. Komissarenko and O.I. Sinyavskaya, *USSR Inventor's Certificate* 1269021, *Byull. Izobret.*, No. 41 (1986).
139. H. Tanaka, *Jap. Pat.* 05-03799, 1993.
140. R.F. Zuk, V.K. Ginsberg, T. Houts, J. Rabbie, H. Merrick, E.F. Ullman, M.M. Fisher, C.C. Sizto, S.N. Stiso and D.J. Litman, *Clin. Chem. (Winston-Salem, N.C.)*, 31 (1985) 1144.
141. F. Arai, K. Yazawa, H. Takeuchi and M. Kitajima, *US Pat.* 4783315, 1998.
142. H. Stec, J. Ciba and Z. Gregorowicz, *Pol. Pat.* 101167, 1979.
143. D. Barham and P. Trinder, *Analyst (Cambridge, U.K)*, 97 (1972) 142.
144. M. Mueller-Frohne, J. Aumann, B. Neumann, H. Ullman, J. Kies, A. Mueller and M. Scholz, *Ger. Pat.* 237678, 1986.
145. H. Ito, *Jap. Pat.* 05-223812, 1993.
146. W. White, *Eur. Pat.* 25227, 1981.
147. A.M. Gronberg, *Ger. Pat.* 2727347, 1977.
148. M. Marek, Z. Pavlicek and B. Polezal, *Czech. Pat.* 261636, 1984.
149. E. Barzana, A.M. Klibanov and M. Karel, *Anal. Biochem.*, 182 (1989) 109.
150. M. Kamiyama, S. Kawakatsu, T. Akashi and K. Okaniwa, *Jap. Pat.* 01-289495, 1989.
151. R. Bauers and T.A. Magers, *Eur. Pat.* 164008, 1985.
152. M. Watanabe, T. Suzuki and M. Kanegama, *Jap. Pat.* 60-172298, 1985.
153. I.F. Dolmanova, E.B. Nikol'skaya, T.N. Shekhovtsova and S.V. Chernetskaya, *RF Pat.* 2013770, *Byull. Izobret.*, No. 40 (1994).
154. T.N. Shekhovtsova and I.A. Veselova, *in International Congress on Analytical Chemistry*, Moscow, 1997, Vol. 2, P-18 (in Russian).
155. D. Noble, *Anal. Chem.*, 65 (1993) 1037A.
156. S.V. Muginova, N.A. Akovbyan and T.N. Shekhovtsova, *Zh. Anal. Khim.*, 54 (1999) 645.
157. T.N. Shekhovtsova, S.V. Muginova and N.A. Bagirova, *Anal. Chim. Acta*, 344 (1997) 145.
158. T.N. Shekhovtsova, S.V. Muginova and N.A. Bagirova, *Mendeleev Commun.*, (1997) 119.
159. I.A. Veselova and T.N. Shekhovtsova, *Mendeleev Commun.*, (1999) 248.
160. K.H. Macho and K.D. Hungerberg, *US Pat.* 5096836, 1990.
161. V. Svoboda, E. Hrboticka and B. Sedlinska, *Czech. Pat.* 260408, 1989.
162. Miles Laboratories, *US Pat.* 3395089, 1968.
163. K.H. Kallies, *Ger. Pat.* 2831083, 1979.
164. E.B. Nikol'skaya, G.A. Evtyugin and A.V. Svyatkovskii, *Zh. Anal. Khim.*, 49 (1994) 374.
165. O.V. Vashkevich and A.N. Ustinova, *RF Pat.* 1709824, *Byull. Izobret.*, No.

29 (1995).
166. A.Yu. Budantsev, E.G. Litvinova and M.A. Kovaleva, *Zh. Anal. Khim.*, 52 (1997) 539.
167. V.M. Mizgunova, G.A. Zolotova and I.F. Dolmanova, *Analyst (Cambridge, U.K.)*, 121 (1996) 431.
168. T.N. Shekhovtsova and S.V. Chernetskaya, *Anal. Lett.*, 27 (1994) 2864.
169. N.V. Gist-Brocades, *Ger.Pat.* 2831083, 1979.
170. O.V. Vashkevich and A.I. Ustinova, *RF. Pat.* 1709824, *Byull. Izobret.*, No. 29 (1995).
171. M. Kamiyama, *Jap. Pat.* 63-137697, 1998.
172. M. Watanabe, T. Suzuki and M. Kagenama, *Jap. Pat.* 60-196198, 1985.
173. M. Watanabe, T. Suzuki and M. Kagenama, *Jap. Pat.* 60-188097, 1985.
174. T.T. Ngo and H.M. Lenhoff (Editors), *Enzyme-Mediated Immunoassay*, Plenum, New York, 1985.
175. H. Friemel (Editor), *Immunologische Arbeitsmethoden*, Gustav Fiescer, Jena, 1976.
176. A.C. Greenquist, B. Walter and T.M. Li, *Clin. Chem. (Winston-Salem, N.C.)*, 27 (1981) 1616.
177. R.J. Tyhach, P.A. Rupchock, J.H. Pendergrass, A.C. Skjold, P.J. Smith, R.D. Johnson, J.P. Albarella and J.A. Profitt, *Clin. Chem. (Winston-Salem, N.C.)*, 27 (1981) 1499.
178. D.J. Litman, R.H. Lee, H.J. Jeong, H.K. Tom, S.N. Stiso, N.C. Sizto and E.F. Ullman, *Clin. Chem. (Winston-Salem, N.C.)*, 29 (1983) 1598.
179. C. Glad and A.O. Grubb, *Anal. Biochem.*, 85 (1978) 180.
180. D.J. Litman, T.M. Hanlon and E.F. Ullman, *Anal. Biochem.*, 136 (1980) 223.
181. R.F. Zuk, V.K. Ginsberg, T. Houts, J. Rabbie, H. Merrick, E.F. Ullman, M.M. Fisher, C.C. Sizto, S.N. Stiso and D.J. Litman, *Clin. Chem. (Winston-Salem, N.C.)*, 31 (1985) 1146.
182. M. Endo, S. Abe, Y. Deguchi and T. Yotsuyanagi, *Talanta,* 47 (1998) 349.
183. Q. Cai, B. Gu and Y. Zhang, *Talanta,* 36 (1989) 665.
184. S. Kawakubo, K. Ogihara and M. Iwatsuki, *Bunseki Kagaku,* 45 (1966) 965.
185. S. Abe, A. Kanno, K. Hasegawa and M. Endo, *Int. J. Environ. Anal. Chem.*, 42 (1990) 45.
186. M.K. Beklemishev, T.A. Stoyan and I.F. Dolmanova, *Analyst (Cambridge, U.K.)*, 122 (1997) 1161.
187. A.L. Kapanadze, M.K. Beklemishev and I.F. Dolmanova, *Zh. Anal. Khim.*, 54 (1999) 1182.
188. Yu.Yu. Petrova, M.K. Beklemishev, N.A. Bazhanova, A.A. Druzhinin and I.F. Dolmanova, *Zh. Anal. Khim.*, 55 (2000) 318.
189. M.K. Beklemishev, T.A. Stoyan and I.F. Dolmanova, *Fresenius' J. Anal. Chem.*, 367 (2000) 17.

190. M.K. Beklemishev, Yu.Yu. Petrova and I.F. Dolmanova, *Analyst (Cambridge, U.K.),* 124 (1999) 1523.
191. Yu.Yu. Petrova, *Cand. Sc. Dissertation,* Moscow State University, Moscow, 2000 (in Russian).
192. C. Bordier and P. Ryter, *Anal. Biochem.*, 152 (1986) 113.
193. Yu.L. Shishkin, A.V. Zherdyaev, B.B. Dzantiev and Yu.A. Zolotov*, Prikl. Biokhim. Mikrobiol. (Appl. Biochem. Microbiol.),* 36 (2000) 497.
194. E.P. Diamandis and T.K. Christopoulos (Editors), *Immunoassay*, Academic Press, New York, 1996.
195. R. Niessner, *Anal. Methods Instrum.*, No. 3, 1 (1993) 134.
196. K.R. Carter, No. 2 (1992) 39.
197. P. Marsden, *Environ. Testing Analysis*, Jan.–Feb. (1995) 58, 60, 61, 85, 86.
198. B.D. Hammock and R.O. Mumma, in *Pesticide Analysis Methodology*, J. Harvey and G. Zweig (Editors), ACS Symposium Series, Washington, 1980, No. 136, 321.
199. J.M. Van Emon (Editor), *A User's Guide to Environmental Immunoassay Analysis*, ACS Symposium Series, Washington, 1994, No. 442.

Chapter 3

Methods and Tools for Analysis of Liquid Samples

The main testing tools for water, aqueous solutions, and other liquid media are indicator paper strips, self-filling ampoules, indicator tubes, tablets, and the simplest titration devices. However, other tools are also available. Certainly, the procedures used depend on the type of tool.

3.1 PAPER STRIPS AND THEIR ANALOGUES

Test systems, in which an analytical reagent is immobilized on solid carriers, especially cellulose papers, show much diversity in how test tools are designed and how concentrations are determined. The amount of a component is determined from the hue or intensity of the colour appearing after contact of a carrier with an analysed liquid, by comparing it with a colour scale or by the area of coloured or discoloured zone on an indicator paper.

3.1.1 Indicator papers for determining the concentration by immersing into the solution and measuring the results

The simplest way to determine concentrations using indicator papers was first implemented in 1767 by Lewis, who measured acidity with a thick writing paper treated with litmus [1]. To date, indicator paper is produced as rolls or strips of indicator bands. To determine pH, a paper strip is immersed in the test liquid, and the resulting colour is compared with a standard colour scale (pH indicator meter, E. Merck; PEHANON®, Macherey-Nagel). Sometimes, two or three indicators

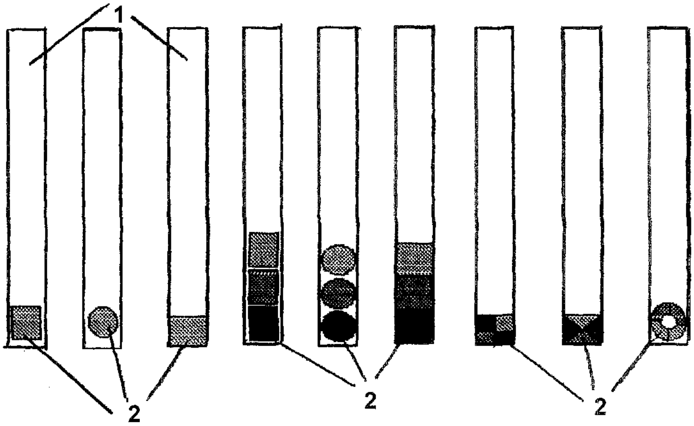


Fig. 3.1. Layouts of indicator papers on polymer holders: (1) the polymer holder; (2) the indicator paper.

are separately introduced to the same paper (DUAL-TINT®, J.T. Baker; DUOTEST®, TRITEST, Macherey-Nagel). The drawback of such a method of using indicator papers is that the experimenter cannot avoid dirtying his hands with indicators. At the same time, an indicator paper can be polluted by the experimenter's hands.

To avoid these drawbacks, square pieces of indicator paper are glued to edges of polymer handle bands in one, two, or several rows [2–5] (Fig. 3.1). Not only is it convenient to determine concentrations with such indicator papers with handles, but also the indicator paper absorbs a certain volume of the test liquid, which provides more stable analysis results.

A polymer handle may hold square pieces of the indicator papers (usually 3 to 4) impregnated with various regents and giving substances of various colours with the reagent [6–8] (Fig. 3.1). The precision of the determination of the concentration in this case is higher as the intensities of colour of several indicator zones are compared. Similarly, several components can be determined simultaneously with a single strip.

To provide the sampling of a certain amount of the test solution, uniform impregnation, and retention of this solution on the indicator paper, the square pieces of papers can be pasted at the edge of the plastic strip with an uncoloured gauze film [9,10].

If several reagents are used for the detection of substances, and they cannot be impregnated simultaneously to a single substrate (to prevent their reactions or to separate the stages of the reaction), multilayer indicator papers are used [11–15]. Such a complex test system contains at least two paper layers. A drop of the test solution with the determined component goes through the first layer, and the component is transformed into a certain uncoloured compound. Next, the drop goes through the second layer, and the compound is transformed into a coloured substance. The colour of the paper is observed from the side opposite to that at which the drop was placed. Also, a powdered reagent can be placed between paper strips.

Merck company produces a wide range of test strips for the analysis of liquids (Table 3.1).

The concentration of the component is estimated from the intensity of the colour of the reaction zone (usually, its diameter is no more than 6 mm). In this case, the product is concentrated at the paper, and the sensitivity of determination increases considerably—usually by a factor of 100–1000—compared to the approaches discussed above. In this case, papers with reagents (formazans and hydrazones) covalently immobilized on cellulose are used, which are not washed out from the paper after pumping 20–40 ml of the sample (Table 3.2).

It was proposed to use indicator papers having a series of zones with subsequently increasing concentrations of a reagent [17–20]. The colour of the reagent changes only if the concentration of the test substance exceeds a certain value. For instance, different amounts of the reagent are deposited on a cellulose substrate with 12 sectors [19]. The central circular part does not contain the reagent, and it is used to introduce a sample solution. The sample diffuses through the paper to the reagent sectors, and the intensity of the colour increases from sector to sector depending on the concentration of the reagent. Knowing the number of the sector (the concentration of the reagent), from which no further increase is observed, one can estimate the concentration of the test component in the sample.

To prevent the oxidation of a reagent with air oxygen and pollution from the environment, indicator papers are laminated into a polymer film or foil [15,21–23]. Such test strips are opened in a certain place to make a contact of their surface impregnated with reagents with the test solution.

A more complex device for analysis of liquid samples is a paper placed in two polymer layers [24]. This strip has a window for the

TABLE 3.1

Merckoquant® test strips (E. Merck) for liquid samples [16]

Test component	Reagent	Concentration range (mg/l)
Al(III)	Aluminon	10–250
NH_4^+	Nessler reagent	10–400
As(III,V)	$HgBr_2$ (for AsH_3)	0.1–3
Ascorbic acid	Molybdophosphoric acid	50–2000
Ca(II)	Glyoxal-bis-(2-hydroxyanil)	10–100
Cl^-	Ag_2CrO_4	500–3000
Cl_2	Barbituric acid	4–120
CrO_4^{2-}	Diphenylcarbazide	3–100
Co(II)	NaSCN	10–1000
Cu(II)	4,4′-Dicarboxy–2,2′-diquinolyl	10–1000
CN^-	Barbituric acid	1–100
Ag(I)	CdS	0.5–10
Formaldehyde	4-Amino-3-hydrazino-5-mercapto-1,2,3-triazole	10–100
Fe(II)	2,2′-Dipyridyl	3–500
Pb(II)	Rhodizonic acid	20–500
Mn(II)	Oxidation to MnO_2 with an organic indicator	2–100
Mo(VI)	Toluene-3,4-dithiol	5–250
Ni(II)	Dimethylglyoxime	10–500
NO_3^-	N-(1-Naphthyl)ethylenediamine, sulphanilic acid, cadmium	10–500
NO_2^-	N-(1-Naphthyl)ethylenediamine, sulphanilic acid	0.1–3
H_2O_2	Horseradish peroxidase and an organic indicator	100–1000
PO_4^{3-}	Ammonium molybdate with a reductant	10–500
K	Dipicrylamine	250–1500
SO_4^{2-}	Thorin chelate of barium	200–1800
Zn(II)	Dithizone	10–250
Total hardness of water	EDTA, Eriochrome Black T	4–25°d

introduction of the test sample and a vessel with a reagent solution. The test liquid is introduced into the window and is quickly absorbed by the paper. Next, the mechanical pressure over the reagent vessel destroys its inner walls, and the reagent solution impregnates the

TABLE 3.2

Parameters of test systems for determining concentrations by the intensity of colour of indicator papers after pumping 20 ml of a test solution [25,27]

Test substance	Optimum pH	Concentration range (mg/l)	Colour changes
Ag(I)	0.5	0.05–10	Yellow–blue
Cd(II)	6–7	0.003–1	Yellowish orange–brown
Co(II)	5–6	0.01–1	Dark yellow–black
Cu(II)	3–5	0.001–1	Yellow–violet
Fe(II, III)	2.5–3	0.01–2	Yellow–greyish green
Hg(II)	2–5	0.05–2	Yellow–red
Pd(II)	1.5–2.5	0.01–5	Orange–dark green
Zn(II)	8–9	0.001–1	Orange–red
Bi(III)	1–2	0.01–0.2	Yellow–green
In(III)	1–2	0.01–0.2	Yellow–bluish green

paper. The test liquid and the reagent solution are gradually mixed up and diffuse to another edge of the strip through an optical window. The intensity of the characteristic colour formed as a result of the interaction of the test substance and the reagent is a measure of the concentration.

3.1.2 Preconcentration of the reaction products

Indicator papers [25–27] and corresponding preconcentration devices [28–30] for pumping a certain volume of the test liquid through the indicator paper are proposed.

A simpler device for preconcentrating reaction products on an indicator paper is proposed [31]. It consists of two details made as hollow truncated circle cones. The truncated cone has a smaller diameter and can be inserted into the another cone to a depth of 3–5 mm, if cones are attached by their bases. Indicator paper is attached to cones when they are attached to each other by their bases. Inner diameters of the bases of the smaller and the larger cones are 2–7 and 4–9 mm, respectively. The inner diameter of the tops of the truncated cones is 0.5–1 mm. The length of both cones can be 4–6 mm, and does not play an important role.

This device can be attached to a pumping device, for instance a disposable syringe without any other devices, by inserting the top of one of the cones into the inlet by screwing. The pumping rate of the liquid can be controlled by the number of drops appearing from the test device. With a change of the indicator paper, the test device can be used an unlimited number of times.

Also, a simpler approach to preparing indicator papers used in this device is proposed [32–37]. Mainly, it uses poorly soluble reagents and complexes precipitated on the paper and able to be exchanged with ions when the test liquid is pumped through the paper. For instance, a paper impregnated with poorly soluble uncoloured zinc diethyldithiocarbamate (DDTC) was used for determining copper(II). When 20 ml of the test solution containing copper is pumped through this paper with a preconcentrating device, a brown $Cu(DDTC)_2$ is formed. Table 3.3 shows parameters of test systems for determination of concentrations using this test device [32–37].

The proposed preconcentrating device and complex indicator papers make it possible to use "displacement" and "amplification" reactions for component determinations. To implement this, multilayer indicator papers are used. For instance, the determination of iron by the displaced copper:

$$3Cu_2[Fe(CN)_6] + 4Fe^{3+} = Fe_4[Fe(CN)_6]_3 + 6Cu^{2+};$$

$$Cu^{2+} + Zn(DDTC)_2 = Cu(DDTC)_2 + Zn^{2+}.$$

At the first paper strip 4 mols of iron(II) displace 6 mols of copper(II) and the paper becomes blue. The displaced copper appears at the second strip impregnated with $Zn(DDTC)_2$, and the brown colour of $Cu(DDTC)_2$ appears. The determination can be based on the intensity of the colour of both the first and the second papers, this improves the objectivity of estimation of the concentration, and, sometimes, decreases the lower calibration limit by a factor of 2–3 (Table 3.4) [35].

3.1.3 Paper Chromatography

The dependence of the area of the coloured spot on the concentration is used in paper chromatography [38]. Mori [39] shaped the paper in such a manner that the spot of the test compound appeared at the narrowed part of the paper ("a quantitative bridge"), and the spot length is

TABLE 3.3
Parameters of test systems for determining concentrations by the intensity of colour of indicator papers after pumping 20 ml of the test solution

Test component	Reagent	Optimum pH	Conc. range (mg/l)	RSD ($n = 5$)	Max. of the diffuse reflectance spectra of the paper (nm)	
					Absent*	Present*
Al	Thiazolylazo-catechol–cetylpyridinium	3–5	0.01–0.5	0.4–0.2	430	560
Ti(IV)	Phenylfluorone–cetylpyridinium	3–5	0.001–0.1	0.4–0.3	420	580, 600
Mo(VI)	Phenylfluorone–cetylpyridinium	3–5	0.01–1	0.5–0.2	420	560
W(VI)	Phenylfluorone–cetylpyridinium	3–5	0.01–1	0.5–0.2	420	550
V(V)	4-(2-Thiazolylazo)-catechol–cetylpyridinium	3–5	0.01–2	0.4–0.3	430	570
Fe(II,III)	$Cu_2[Fe(CN)_6]$, $Cu_3[Fe(CN)_6]_2$	1–3	0.01–1	0.4–0.2	440	670
Ag	Cu–dithizone	1–7	0.01–1	0.5–0.3	450	480, 630
Cd	Zn–dithizone	3–7	0.005–1	0.5–0.3	530	440
Cu(II)	$Pb(DDTC)_2$	1–14	0.001–0.1	0.3–0.2	—	460, 580
Zn	Cr, Mn–dithizone	4–8	0.01–1	0.4–0.2	450	530
Pb(II)	ZnS	0.1 M NaOH	0.01–1	0.5–0.3	—	460, 630
Hg(II)	Cu–dithizone	0.5–2	0.005–1	0.5–0–3	450	520
HS^-	AgI	4–6	0.01–1	0.3–0.2	—	470, 640
NO_3^-	Cellulose with chemically immobilized 1-naphthylamine, anaesthesine, zinc	0.5–1	0.1–10	0.4–0.3	—	530
NO_2^-	Cellulose with chemically immobilized 1-naphthylamine, anaesthesine	0.5–1	0.01–1	0.4–0.3	—	530
H_2O_2	Horseradish peroxidase, 4-chloro-1-naphthol	5–8	0.01–1	0.4–0.3	—	580
Total hardness of water	Eriochrome Black T, magnesium complexonate	9–12	0.01–5 mM	0.4–0.3	680	550

*Absent = In the absence of the test component; Present = In the presence of the test component.

TABLE 3.4

Examples of application of replacement (amplification reactions in test systems)

Test ion	Determination method: (A) by a single strip; (B) by a dual-layer strip	Reagents on strips 1 and 2	Lower determination limit (mg/l)	RSD (n = 5)
Fe(III)	A	$Cu_2[Fe(CN)_6]$	0.01	0.4
	B	1 – $Cu_2[Fe(CN)_6]$, 2– Zn(DDTC)	0.003	0.3
Hg(II)	A	$Cu(DDTC)_2$	0.005	0.5
	B	1–$Cu(DDTC)_2$, 2–$Pb(DDTC)_2$	0.005	0.2
Hg(II)	A	ZnS	0.01	0.4
	B	1–ZnS, 2-$Mn(Dz)_2$	0.005	0.3

measured at this place. The same principles are used in test methods. Contrary to paper or thin-layer chromatography, where the sample is introduced to a plate, one edge of which is placed into a mobile phase (usually a mixture of organic solvents), test methods use reagent-containing substrates, and the analysed liquid is used as a mobile phase. In a number of test methods [15,34,40–43], the principles of precipitation paper chromatography [44] are implemented. As the test liquid rises through the indicator paper laminated in a polymer film due to capillary forces, the test component forms a poorly soluble compound with the reagent impregnated on a paper and is retained where it is formed. It results in the formation of a coloured or a discoloured zone on a paper strip, the length of which is proportional to the concentration (Fig. 3.2). Other variants of contact of the test solution with the indicator paper are also possible: for instance, it can be implemented through a round orifice or a slit in the film (see Fig. 3.3) [45].

The main processes in such test systems, as in planar chromatography, are firstly the movement of the front of the analysed solution in a planar system, and secondly the formation and movement of the front of the coloured zone. This zone is formed on contact of a solution containing the analysed impurity with a planar test system containing an analytical reagent, which forms a coloured or uncoloured compound with the test substance.

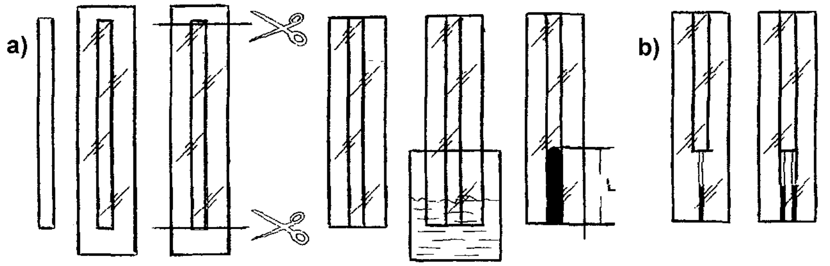


Fig. 3.2. Method for development and application of (a) test strips and (b) shaped test strips.

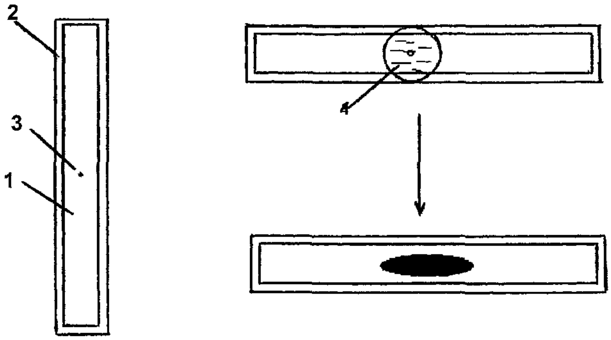


Fig. 3.3. Method of preparation of an indicator test strip and its application for the determination of concentrations: (1) the indicating layer; (2) a polymer film; (3) a hole in the test strip; (4) analysed solution.

The movement of the front of the analysed solution in a test system is described with an equation [46] previously deduced in planar chromatography for describing the movement of the upper boundary of the eluent [47]:

$$z^2 = \chi t \tag{3.1}$$

where z is the distance from the line of contact of the test solution with the planar system to the front of this solution is this system; χ is the constant depending on the flow; t is the process time.

Note that the regularity (3.1) may not be obeyed in test methods as, firstly, a test solution, contrary to an eluent in thin-layer chromatography, is not pure, and, secondly, physicochemical properties of the planar system change as a result of impregnation with reagents. However, the studies made in Ref. [46] for several systems (paper and thin-layer chromatography) show that Eq. (1) is obeyed in the practical analysis.

The relationship of parameters of the forming coloured zone (for instance, length) in a planar system and the concentration of the test component of the solution and its volume pumped through the zone with the indicator reagent is the most important parameter. Based on the equation of material balance, the authors of Ref. [46] deduced an equation for the test concentration of the impurity. The determined concentration of the test substance c_i may be expressed as

$$c_i = \frac{m_c}{V_l} = \frac{b_s l_s \rho_c}{z b_s \rho_l} = \frac{l_s \rho_c}{z \rho_l} \tag{3.2}$$

where m_c is the total amount of the test components in the coloured zone, V_l is the total volume of the test solution pumped through the front of the coloured zone, b_s is the zone width, l_s is the zone length, ρ_c is the load of the selective reagent, which is expressed, for instance, in milligrams of the test component required to form 1 mm^2 of the coloured zone; ρ_l is the specific capacity of the indicator strip to the analysed solution, which is expressed, for instance in millilitres of this solution that wets 1 mm^2 of the used planar system.

As results from Eq. (3.2), the sensitivity of the analytical system increases (the value c_i decreases) with an increase in the sensitivity of the reaction used, i.e. with a decrease in ρ_c (the specific load of the selective reagent) and an increase in ρ_l (the capacity of the system to the analysed solution), and also with an increase in z. Hence, substituting Eq. (3.1) in Eq. (3.2), we obtain:

$$c_i = \frac{l_s \rho_c}{\rho_l \sqrt{\chi}\sqrt{t}} = K_{ps} \frac{1}{\sqrt{t}} \tag{3.3}$$

where

$$K_{ps} = \frac{l_s \rho_c}{\rho_l \sqrt{\chi}}. \tag{3.4}$$

It results from Eq. (3.3) that c_i decreases with an increase in the time of impregnation of the used system with the test solution (this conclusion

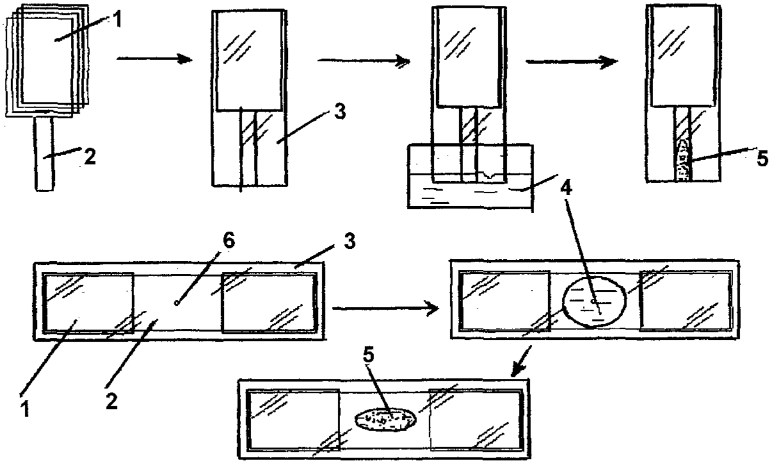


Fig. 3.4. Method of preparation of an indicator test strip and its application for the determination of concentrations: (1) a multilayer paper filter; (2) an indicator paper; (3) a polymer film; (4) analysed solution; (5) a coloured or discoloured zone; (6) a hole in the film.

is obtained with the assumption that l_s = const). For instance, an increase in the determination time from 10 to 100 min decreases the determined concentration by approximately 30%.

Thus, significantly better analytical parameters were obtained in Ref. [46] when a principally novel system was used. Contrary to existing approaches, it implements firstly the separation of the analytical and pumping systems, and secondly a more capacious pumping system with a small hydraulic resistance (for instance a shorter system consisting of a package of paper filter strips) is used. This system is serially connected analytical and pumping systems (Fig. 3.4). For instance the first (relative to the movement of the test solution) may be an indicator system (a paper strip impregnated with a reagent, for instance of 1.5 cm in length), and the second is a porous system, which is characterised with (1) an increased capacity to the analysed liquid and (2) a high rate of impregnation (e.g. a multilayer paper filter). Table 3.5 summarizes the main parameters of the proposed analytical system. Compared to conventional single-layer systems, a binary system is characterized by a higher sensitivity (approximately by a factor of five).

TABLE 3.5

Dependence of the length of the coloured zone of a test strip on the concentration of copper(II) for (A) single-layer and (B) dual-layer test systems. The area of the absorbing surface is (A) 3.2 and (B) 45 cm^2 [46].

c_{Cu} (mg/l)	The length of the coloured zone (mm)	
	A	B
0.01	—	0.5
0.05	—	1
0.1	0.5	2
0.5	1	5
1	2	7
2	3	10
5	4	22

Our experience of using this approach to determine the concentration allowed us to define the requirements for the selection of reagents impregnated on the paper [33,41]:

1. Low solubility of reagents to be immobilized in water (no more than 10^{-3}–$5 \cdot 10^{-3}$ mol/l at various pHs and ionic strengths of the solution).
2. Low solubility of the reaction product formed at the paper ($K_s \leq 10^{-10}$). The formed reaction product should be retained in the paper pores at the place of its formation and not be washed away with the flow of a liquid.
3. In the case of coloured reagents and substances, the reaction product should be either colourless or coloured with a degree of contrast of $\Delta\lambda \geq 60$ nm. A lower degree of contrast hinders visual estimation of the length of the coloured zone of the reaction product.
4. The reaction rate should allow the reaction products to form in at least 3–5 min.

These requirements were used for developing reagent systems immobilized on paper including poorly soluble organic reagents, their associates with cetylpyridinium, complexes of inorganic ions with organic ligands, and poorly soluble complex and inorganic salts (Table 3.6).

TABLE 3.6

Reagent mixtures for the impregnation of indicator papers

Type of impregnated reagent	Reagent	Test elements
Poorly soluble organic reagents	Trihydroxyfluorones	Ti, Zr, Hf, Mo, W, Ge, Sb, Sn, Ta
	Catechol azo compounds	Al, Ga, V, Ge, Mo, W, Ti
	1-(2-Pyridylazo)naphthol	Ni, Co, Fe, Cu, Zn, total heavy metals
	Dimethylglyoxime	Ni
	Dithizone	Zn, Cd, Hg, Ag, Pb, total heavy metals
	Nitroso-naphthols	Co, Fe, Cu
	Dimethylaminobenzylidene-rhodanine	Ag, An, Pt, Hg
Poorly soluble associates	Trihydroxyfluorones–surfactants	Ti, Zr, Hf, Mo, W, Ta, Sn, Sb, Ge
	Catechol azo compounds–surfactants	A1, W, V, Mo, Ge, Ga
Poorly soluble chelates of inorganic ions with organic ligands	Metal chelates with trihydroxyfluorones	Cationic surfactants
	Metal chelates with catechol azo compounds	Cationic surfactants
	Dithizonates	Zn, Cd, Hg, Ag, CN^-
	Diethyldithiocarbamates	Cu, Hg, Ag, Co, Bi, Fe
	Hydroxyquinolinates	V, Tl, Ni, Al, In, Bi
	Cupferronates	Fe, Ga, Cu, In
	Alizarinates	F^-, PO_4^{3-}, SO_4^{2-}
Poorly soluble complex salts	Hexacyanoferrates(II)	Fe(III)
	Hexacyanoferrates(III)	Fe(II), Cl^-, Br^-, I^-, SCN^-
Poorly soluble inorganic salts	Silver salts	Cl^-, Br^-, I^-, SCN^-
	Sulphides	H_2S, $[Fe(CN)_6]^{4-}$, Pb, As, Ag, Cu, Hg

Poorly soluble organic reagents (Table 3.6) are used for group determination of Ni(II), Co(II), Fe(III), Cu(II), Zn, Hg(II), Ag, Cd, and Pb(II). Selective determination requires adjusting the solution pH, using masking agents, or separating interfering elements. The use of selective reagents seems promising. The low determination limit using the reagents from this group is 1–5 mg/l.

Poorly soluble associates of cationic surfactants with trihydroxyfluorones or azo-derivatives of catechol (Table 3.6) impregnated on paper are sensitive reagents for Ti(IV), Zr(IV), Mo(VI), W(VI), Ta(V), Sn(IV), Sb(III), Ge(IV), Al, V(V), and Ga. The sensitivity of determination is enhanced by a factor of 50–100 compared to the reagents of the first group. The degree of contrast is also increased by 20–50 nm, which is important for visual estimation of the length of the coloured zone. The possibility of selective determination of elements is enhanced due to a difference in optimum acidity ranges of chelation of ions.

The low determination limit of the reagents of the last three groups of Table 3.6 is 0.1–0.5 mg/l. The differences in stability and solubility of compounds with these reagents make it possible to propose compositions for selective ion determination with due regard to the solubility rows [48]. For instance, solubility rows for silver salts, diethyldithiocarbamates, and dithizonates are as follows:

Silver salts:

$SO_4^{2-} > CH_3COO^- > C_2O_4^{2-} > CO_3^{2-} > CrO_4^{2-} > PO_4^{3-} > SO_3^{2-} > [Fe(CN)_6]^{3-}$
$> AsO_4^{3-} > SeO_3^{2-} > Cl^- > [Fe(CN)_6]^{4-} > NCS^- > Br^- > I^- > S^{2-}$

Diethyldithiocarbamates:

Mn(II) > Fe(III) > Sb(III) > In(III) > Zn(II) > Cd(II) > Pb(II) > Co(II) >
Bi(III) > Ni(II) > Cu(II) > Ag(I) > Pd(II) > Hg(II)

Dithizonates:

Fe(II) > Ni(II) > Pb(II) > Co(II) > Zn(II) > Cd(II) > In(III) > Bi(III) >
Cu(II) > Pd(II) > Hg(II) > Ag(I)

According to the rule of solubility rows, the transformation of precipitates is directed toward the lower value of the solubility of the precipitate. The use of this rule to poorly soluble compounds immobilized on paper with due regard to the experience accumulated for such papers allows us to define the following rules:

1. Ions located in the row to the left of the precipitate impregnated on the paper show lower interference with the determination of ions located to the right. For example, the determination of chloride with paper impregnated with $Ag_3[Fe(CN)_6]$ will not be interfered with at least 1000-fold amounts of SO_4^{2-}, CH_3COO^-, $C_2O_4^{2-}$, CO_3^{2-}, CrO_4^{2-}, PO_4^{3-}, and SO_3^{2-}.
2. If a coloured zone is formed on a paper impregnated with a colourless substance, ions forming colourless compounds and located in the row before the determined ion do not interfere with its determination. For instance, determination of copper (a brown colour) with a paper impregnated with colourless $Zn(DDTC)_2$ is not interfered with Cd(II), Pb(II), Co(II), Bi(III), and Ni(II), which form colourless or weakly coloured precipitates.
3. If ions located between immobilized and determined ions form differently coloured zones, the formation of several zones is possible. For instance, a paper impregnated with zinc dithizonate reveals a brown zone of copper and an orange zone of cadmium.

It was also shown that cellulose papers with chemically immobilized 1-naphthylamine can be used for determining nitrates, nitrites, and aromatic amines [49].

To increase the sensitivity of determination, shaped test strips are proposed. They provide a two- to fivefold decrease in the limit of detection of components due to diminishing the area of contact of the paper with the test liquid [41]. Simultaneous determination of several components from their mixture is also possible: it is provided by test strips impregnated with various mixtures of reagents or composed test strips [33,41]. The selection of simultaneously determined elements was based on optimum acidity ranges. For instance, simultaneous determination of F^-, Hg(II), Cu, and Fe(II, III) is possible in acidic solution, and Zn, Cd, Cu, and Cl^- can be determined in a neutral solution.

Dependences of lengths of coloured or discoloured zones of test strips on the concentration are non-linear and are usually described by a power function. Table 3.7 summarizes parameters of test systems implementing this approach of determining concentrations [33].

TABLE 3.7

Parameters of test systems for determining concentrations by the length (area) of the coloured or discoloured zones on test strips

Test component	Reagent	Optimum pH	Conc. range (mg/l (mg/kg))	RSD ($n = 5$)	Colour change: initial colour/ zone colour	Test materials
Al	4-(2-Thiazolylazo)-catechol–cetyl-pyridinium	3–5	1–50	0.3–0.1	Yellow/red	Wastewater, solutions
V(V)	4-(2-Thiazolylazo)-catechol–cetyl-pyridinium	3–5	0.1–10	0.2–0.1	The same	The same
Fe(II,III)	$Cu_2[Fe(CN)_6]$ $Cu_3[Fe(CN)_6]_2$	1–3	0.1–40	0.3–0.05	Reddish brown/blue	Natural and wastewaters, soils, atmospheric precipitations
Total heavy metals	Dithizone	6–8	$(2–500) \cdot 10^{-6}$ M	0.3–0.1	Yellow/red	Natural and wastewaters
Cd	Zn-dithizone	3–7	0.1–200	0.3–0.2	Magenta/ orange	The same
Cationic surfactants	Mo(VI) – 4-(2-Thiazolylazo)catechol	4–8	1–1000	0.3–0.05	Orange/lilac	The same
Cu(II)	$Pb(DDTC)_2$	1–14	0.1–100	0.2–0.05	Colourless/ brown	Natural and wastewaters, soils
Bi(III)	$Cu(DDTC)_2$	1–5	1-100	0.3–0.2	Brown/ colourless	Wastewater, solutions
Ni(II)	Dimethylglyoxime	6–10	1–500	0.2–0.05	Colourless/ red	The same
Ag	Cu–dithizone	1–7	0.5–100	0.3–0.1	Brown/red	The same
Hg(II)	Cu–dithizone	0.5–2	0.1–200	0.3–0.1	The same	Wastewater, soils
Pb(II)	ZnS	>14	1–500	0.3–0.2	Colourless/ black	Potable and wastewater, soils
Zn	Cr(III),Mn(II)–dithizone	4–8	0.1–200	0.3–0.05	Green/ magenta	Wastewater
Ti(IV)	Phenylfluorone–cetylpyridinium	3–4	0.01–2	0.2–0.1	Yellow/blue	The same
Ge(IV)	Phenylfluorone–cetylpyridinium	3–4	0.01–1	0.3–0.1	Yellow/red	Wastewater, silicates
Mo(VI)	Phenylfluorone–cetylpyridinium	3–4	0.05–10	0.3–0.2	The same	The same

continued

Test component	Reagent	Optimum pH	Conc. range (mg/l (mg/kg))	RSD ($n = 5$)	Colour change: initial colour/ zone colour	Test materials
W(VI)	Phenylfluorone–cetylpyridinium	3–4	0.1–10	0.4–0.2	Yellow/ red	Wastewater, silicates
I^-	AgCl	3–10	1–1000	0.3–0.1	Colourless/ yellow	Solutions
Cl^-	Ag_2CrO_4	4–8	0.2–1000	0.2–0.05	Brown/ colourless	Water, atmospheric precipitations, soils
SCN^-	AgCl	3–8	1–500	0.3–0.2	Colourless/ brown	Wastewater, solutions
Br^-	AgCl	4–8	1–500	0.3–0.2	The same	Solutions
CN^-	Cu–dithizone	3–6	1–500	0.3–0.1	Brown/ colourless	The same
SO_4^{2-}	Ba–Rhodizonate	2–4	10–800	0.3–0.1	Red/colourless	Water, atmospheric precipitations, soils
F^-	Zr–Alizarin	1–3	0.5–500	0.3–0.1	The same	The same
PO_4^{3-}	Ce–Alizarin	4–8	1–500	0.3–0.1	The same	Wastewater. soils
NO_3^-	Cellulose with chemically immobilized 1-naphthylamine, anaesthesine, zinc	0.5–1	1–100	0.3–0.2	Colourless/ lilac	Water, soils
NO_2^-	Cellulose with chemically immobilized 1-naphthylamine, anaesthesine	0.5–1	0.1–100	0.3–0.2	Colourless/ lilac	Water, soils
H_2O_2	Horseradish peroxidase, 4-chloro-1-naphthol	5–8	0.1–300	0.3–0.2	Colourless/ blue	Water, atmospheric precipitations
Aromatic amines	Cellulose with chemically immobilized 1-naphthylamine, anaesthesine, nitrites	0.5–1	1–100	0.3–0.2	Colourless /lilac	Solutions
Total hardness of water	Eriochrome Black T, magnesium complexonate	9–12	0.5–50 mM	0.3–0.2	Green/red	Water
Total alkalinity	Congo Red, citric acid	6–8	0.5–50 mM	0.3–0.2	Cherry-coloured / red	Water

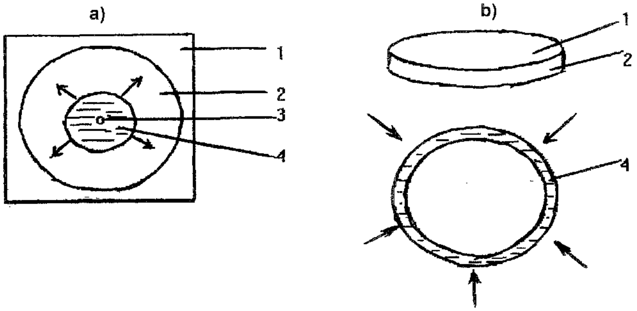


Fig. 3.5. Method of preparation of an indicator test strip and its application for the determination of concentrations by the area of the coloured or discoloured zone. The movement of the analysed liquid is denoted by arrows. (1) A polymer film; (2) an indicator paper; (3) a hole in the film; (4) a coloured or discoloured zone. (a) Radial chromatography; (b) antiradial chromatography.

Paper discs laminated in a polymer film for determining halides (chlorides, bromides, and iodides) are produced by Saltesmo and Macherey-Nagel. The approach to determining concentrations is based on radial chromatography (Fig. 3.5). The test liquid impregnates the indicator paper through a small hole in the film. This results in a coloured or discoloured zone on a paper, its area is proportional to the concentration 250–5000 mg/l of chlorides [50, 51]. Starp et al. [51] used this method for determining cationic surfactants, nickel(II), H_2O_2, and sulphates (Table 3.8) and proposed the principle of antiradial chromatography. In this method, the test liquid impregnates along the perimeter of the disc. The area of formed coloured or discoloured zone is also proportional to the concentration of the test compound.

TABLE 3.8

Test strips for determining concentrations by the principle of radial chromatography [51]

Test component	Impregnated reagent	Concentration range (mg/l)
SO_4^{2-}	Barium rhodizonate	50–5000
H_2O_2	Horseradish peroxidase, 3,3′,5,5′-tetrametyldiphenyl-4.4′–diamine	1–10000
Ni(II)	Dimethylglyoxime	10-5000
Cationic surfactants	Bromothymol Blue or Bromocresol Green	50–2000

TABLE 3.9

Test systems with separate reaction steps used for analysis of natural and waste waters

Test ion or compound	Reagent on A and B strips	Concentration range (mg/l)	RSD (n=5)	Colour change
NO_2^-	A–Sulphanilic acid B–8-Hydroxyquinoline	0.1–1	0.3–0.2	Yellow/red
Fe(III)	A–Hydroxylamine B–Bathophenanthroline	0.8–6	0.4–0.2	Colourless/ red
NH_4^+	A–Nitroprusside, $CaOCl_2$ B–8-Hydroxyquinoline	2–20	0.3–0.1	Yellow/green
O_2	A–NaOH B–$MnSO_4$	2–10	0.5–0.3	Colourless/ brown
Ge(IV), Ta(V), Sn(IV)	A–Cetylpyridinium B-Phenylfluorone	0.1–5	0.4–0.2	Yellow/red

A method for determining concentrations by the intensity of the appearing colour of the indicator paper laminated in a polymer film and impregnating a certain volume of the test liquid is described [52]. The indicator zone is located at the paper B attached to the strip A at which the test liquid is moving. The distance from the zone to the strip edge depends on the composition of immobilized reagents and test components. Such an approach to determining concentrations is usually used in two-stage reactions or to prevent premature reactions of reagents on the substrate. For instance, in the case of determination in water, the strip at which the liquid is moving is impregnated with sodium hydroxide, and the indicator zone contains manganese(II) sulphate. When the solution reaches the indicator zone, Mn(II) is oxidized by the oxygen to Mn(IV), and a brown colour appears. This approach is proposed for determining nitrates and nitrites, Fe(III), Ge(IV) [33,52, 53] (Table 3.9).

Glad and Grabb [54] developed a method of immunocapillary migration for quantitative determination of antigens. The method is based on capillary migration of an antigen in a porous carrier contacting immobilized antibodies. Interactions of antigen with immobilized antibodies decreases the migration rate. As the concentration of the antigen increases, an increasing amount of immobilized antibodies interacts with it. Thus, a part of the antigen molecules migrate farther, and the migration distance is proportional to the concentration of the antigen in the sample. To reveal the zone with the antigen, the strip is treated with its antibodies with a fluorescing or enzymatic mark. Such antigens as C-reactive protein can be determined quantitatively in 15 min.

A combination of immunocapillary migration and the principle of enzymatic channels [55] resulted in the method of enzymatic chromatography for quantitative determination of substances. This method used (1) antibodies immobilized on a 4 × 90 mm paper strip; (2) an enzyme solution containing glucose oxidase and a ligand marked with peroxidase; (3) a solution of substrates containing glucose and 4-chloro-1-naphthol. In analysis, the test solution is added to the enzyme solution, the edge of the indicator paper with immobilized antibodies is immersed in this solution, and the solvent moves up the strip. After 10 min, the paper strip is removed and fully immersed in the substrate solution. In 5 min, a coloured zone appears, its height is proportional to the concentration of the ligand in the sample. Test strips for determining theophylline in blood by enzymatic immunochromatography are proposed, the determination range is 2.5–40 mg/l [56].

A method for determining 5–50 mg/l Hg(II) and 1–10 mg/l Ni(II) is proposed. The method is based on estimating the intensity of the colour of reagent zone on a paper test strip with a length of 12 cm, in the moments when the test liquid raises to heights of 15, 30, and 45 mm. The narrow reagent zone is located in the lower part of the strip and is 5–6 mm from its edge. The test strip is immersed to the liquid by 2–3 mm. Poorly soluble reagents not washed away with the movement of the liquid (CdS for Hg(II) and dimethylglyoxime for Ni(II)). The products formed, HgS (black) and nickel dimethylglyoximate (red), are also poorly soluble and remain when they are formed. The intensity of the colour appearing depends on the volume of the impregnated liquid and on the concentration of determined component. It is compared with a colour scale.

In the evaporation method, the larger part of the test strip is placed in a vial with a stopper containing the analysed liquid; the smaller part of the strip is outside. The outside edge of the strip has an indicator zone. When the liquid rises up the strip and vaporizes, a certain amount of the test solution moves through this indicator zone. This approach was used for determining nickel with dimethylglyoxime within the range 2–20 mg/l and iron(III) with hexacyanoferrate(II) within the range 0.05–0.65 mg/l [6]. The concentration is estimated by the intensity of the appearing colour.

3.1.4 Reaction rate measurements

In many cases, kinetic methods show high sensitivity and selectivity, which is also used in test methods. The concentration is determined from the time of the change of the colour of the indicator substrate after placing a test solution on it.

The determination of mercury with the lower determination limit of $1\cdot10^{-7}$–$1\cdot10^{-5}$ mg/l is based on its inhibiting effect on horseradish peroxidase in the reaction of oxidation of amines with hydrogen peroxide [57,58]. Peroxidase is immobilized on paper. The lower limit of determination of organomercury compounds is 0.02 μM [59].

A paper test for determining Mn(II) in potable water is proposed. The paper contains KIO_4 with $BaSO_4$, 1,10-phenanthroline, nitrilotriacetic acid, Malachite Green, Rhodamine C, and buffering and masking agents. In the process of analysis, potassium periodate oxidizes catalytically Malachite Green to colourless products. As a result, the green colour of Malachite Green decreases and pink colour of Rhodamine C appears [59]. Nitrilotriacetic acid and 1,10-phenanthroline are activators. The limit of detection of manganese in potable water is 0.03 mg/l.

Catalytic methods for the determination of copper(II) and manganese(II) on paper after their preconcentration on this substrate are proposed [61,62]. The paper with immobilized hexamethylenediamine, diethylenetriamine, and diethylenetriamine tetraacetate are used. To determine copper(II) in the range $1\cdot10^{-6}$–0.1 mg/l, the reaction of oxidation of hydroquinone with hydrogen peroxide in the presence of 2,2′-dipyridyl is used. Manganese(II) in the range $5\cdot10^{-6}$–10^{-3} mg/l is determined by its promoting effect on the reaction of 3,3′,5,5′-tetramethylbenzidine with periodate.

3.1.5 Test titrations with papers

A method for testing titration on paper impregnated with the titrant and indicator is proposed. Test liquid is added dropwise to this paper until the indicator changes its colour. A paper filter is impregnated with Methyl Orange of $K_2Zn_3[Fe(CN)_6]_2$ to determine 1–50 mM HCl and 1–10 mg/l Fe(III), respectively. Test liquid is added to a certain area of such a paper until its colour changes. The concentration is estimated by the number of drops added, the concentration range is predetermined using standard solutions of HCl and Fe(III) [6].

In the case of titration with indicator papers, the paper with an immobilized indicator is placed in the analysed solution, and paper pieces (1 cm^2) with an immobilized titrant are added under stirring. The number of pieces added is proportional to the concentration. EDTA, carbonates, tetraborates, hydrosulphates, citric acid and other titrants immobilized on paper are used as titrants. The following indicators are proposed: Methyl Orange for determining total alkalinity of water, phenolphthalein for determining total acidity of water, Eriochrome Black T for determining total hardness of water, and ion associates of Xylenol Orange with cetylpyridinium for determining rare-earth elements [6,33].

3.1.6 Colorimetry with indicator papers

The mixtures of reagents for impregnating indicator papers for determining ammonium, aniline, phenol, pH of atmospheric precipitates, active chlorine, chromium(VI), nickel, and nitrites are proposed (see Table 3.10) [33]. The determination is based on measuring the intensity or hue of the analysed liquid after immersing the indicator paper in it or after pumping the analysed liquid through the indicator paper using a test device. The primary requirement to the reagents immobilized on paper is their ability to be washed off from the substrate after stirring the solution for 2–5 min.

3.2 INDICATOR POWDERS

Indicator powders are either mixtures of reagents for determining substances or reagents immobilized on powdered carriers.

In the former case, the concentration is determined by the intensity of the colour of the liquid after introducing the indicator powder and its dissolution in the analysed solution. For instance, the indicator powder consisting of N-(1′-naphthyl)ethylenediamine : sulphanilamide : potassium hydrogen sulphate = 1:300:300 is proposed for determining nitrites. A 0.3-g portion of this mixture is added to 10 ml of the sample and mixed; the concentration of nitrite is determined by the colour intensity [63].

TABLE 3.10

Characteristics of test systems for the determination of concentration based on the colour intensity of a liquid after immersing reactive papers

Test ion or compound	Reagent	Absorption maximum (nm)	Conc. range (mg/l (mg/kg))	RSD (n=5)	Analysed samples
Aniline	Tetrafluoroborate 4-nitrophenylazo-diazonium, cetylpyridinium	520	0.05–5	0.3–0.2	Wastewater
Phenol	The same	525	0.05–5	0.3–0.2	The same
Ge(IV)	Phenylfluorone–cetylpyridinium	530	0.01–1	0.4–0.1	The same
Ta(V)	Phenylfluorone–cetylpyridinium	540	0.05–2	0.3–0.1	The same
Zr(IV)	Phenylfluorone–cetylpyridinium	540	0.02–3	0.4–0.2	The same
NH_4^+	8-Hydroxyquinoline, nitroprusside, $CaOCl_2$	700	0.5–20	0.3–0.1	Natural and wastewaters, atmospheric precipitations, soils
Ni(II)	Dimethylglyoxime, $(NH_4)_2S_2O_8$	450	0.05–10	0.4–0.2	The same
NO_2^-	8-Hydroxyquinoline, sulphanilic acid, cetylpyridinium	540	0.1–5	0.3–0.1	The same
pH	Bromocresol Purple + Methyl Red	Depends on pH	pH 4–6	0.1–0.08	Atmospheric precipitations
Cl_2	KI + starch	630	0.3–8	0.3–0.1	Waste and drinking waters
Cr(VI)	Diphenylcarbazide	550	0.05–5	0.3–0.1	Waste and drinking waters

In the latter case, indicator powder is introduced to the sample and mixed, and the concentration is determined by the intensity of the powder colour (Fig. 3.6) [64].

The use of modified xerogels provides a combination of sorption preconcentration of the analysed component from dilute solutions and its visual determination on the surface of the indicator powder. High

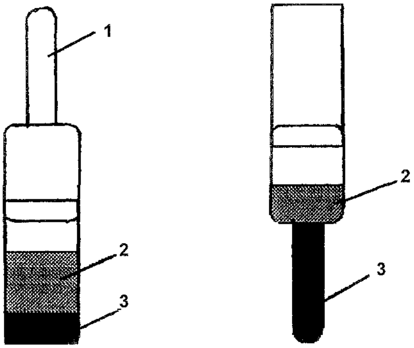


Fig. 3.6. Device for test determinations using indicator powders: (1) colorimetric tube; (2) analysed solution; (3) indicator powder.

transparency of indicator xerogel powders prepared using ammonium hexafluorosilicate as an accelerating agent provides high sensitivity of determination. It was found that an increase in the specific surface increases the sensitivity of determination. The specific surface of xerogel powders used in analytical procedures is 600–950 m^2/g [64–70].

The effect of the concentration of the reagent in xerogel on sensitivity is complicated. It depends on spectrophotometric parameters of reagents and reaction products and also the specific effects of the existence of the reagent in the hydrolysing mixture at the stage of preparation of modified xerogels on structural parameters of produced sol–gel materials.

The procedures for visual tests using modifies xerogels are proposed for metal ions, halides, and organic substances (see Table 3.11) [64–70].

Test determination of fluoride and chloride are based on non-covalent immobilization of Arsenazo I, Alizarin Red, Xylenol Orange and diphenylcarbazone by their inclusion in silicic acid xerogels and silica modification. The determination of fluoride and chloride are based on exchange complexation reactions in systems of immobilized Xylenol Orange–zirconium(IV) and immobilized diphenylcarbazone–mercury(II) [69].

Test determination of total phenolic compounds and halogen derivatives of phenols, alkyl-substituted anilines, naphthols, and various esters are based on reactions of these compounds with chromogenic reagents included in silicic acid xerogels [65,68]. The determination of phenols is based on their ability to enter the reaction of oxidative condensation with 4-aminoantipyrine included into xerogel resulting

TABLE 3.11

Characteristics of test procedures based on indicator powders

Test compound	Indicator powder	Concentration range (mg/l)	RSD (c, mg/l)	Analysis time (min)
Cd	Bromobenzothiazo/xerogel	0.05–1	0.05 (0.1)	20
F^-	Xylenol Orange–Zr(IV)/xerogel	1–10	0.06 (8.0)	35
Cl^-	Diphenylcarbazone–Hg(II)/ xerogel	3–20	0.04 (10)	15
Hydrazine	Molybdophosphoric acid–Cu(II)/xerogel	0.3–3	0.10 (0.25)	10
Ascorbic acid	Molybdophosphoric acid–Cu(II)/xerogel	10–400	0.02–0.1	10
Esters	Fe(III)/xerogel	40–450	0.09 (60)	15
Phenol	4-Aminoantipyrine/xerogel	0.03–0.5	0.09 (0.2)	15
Active chlorine	N,N-diethyl-*p*-phenylendiamine/ diasorb-SO_3H	0.005–0.1	0.2–0.3	10

in coloured indophenols. The reaction of azo coupling of naphthol-1 immobilized in xerogel with anilines is used for their determination. The determination of naphthols includes their nitrosation followed by the formation of chelates with cobalt(III) ions included into the xerogel. The determination of esters includes their preliminary transformation into hydroxamic acids and the interaction of the products of the hydroxamation reaction with iron(II) included in the xerogel [65].

N,N-Diethyl-*p*-phenylenediamine immobilized on Diasorb-SO_3H was used for determining active chlorine in water in the range of 0.005–0.1 mg/l [69].

Adsorption of 1-(2-thiazolylazo)-2-naphthol, dithizone, and zinc dithizonate on the surface of highly dispersed silica (Silpearl UV 254) was used to prepare indicator powders for determining Zn(II), Co(II), Hg(II), Ag(I), and Pb(II) [71, 72] (see Table 3.12).

Simple, selective and rapid procedures for the determination of the above metals in wastewaters and natural and potable waters have been developed. The relative standard deviation of measurements does not exceed 30%.

Heterocyclic azo compounds immobilized on silica were used as test materials for determining Co(II), Pd(II), V(V) [73], Fe(III), Cr(III), Mn(II), Ni(II), Co(II), and Zn(II) [74]. The concentration is determined by the colour of sorbents after their contact with the analysed solution.

TABLE 3.12

Indicator powders based on fine silicas

Test ion	Indicator powder	pH	Concentration range (mg/l)
Zn(II)	1-(2-Thiazolylazo)-2-naphthol/Silpearl	5.5–6.8	0.02–0.4
Co(II)	1-(2-Thiazolylazo)-2-naphthol/Silpearl	3.2–4.6	0.008–0.2
Cu(II)	1-(2-Thiazolylazo)-2-naphthol/Silpearl	3.3–4.6	0.007–0.1
Hg(II)	Dithizone/silica gel	4.4–5.1	0.05–1.0
	Dithizone/Silpearl	0.0–0.5	0.005–0.2
Ag(I)	Dithizone/silica gel	1.0–2.0	0.05–2
Pb(II)	Dithizone/silica gel	7.0–9.0	0.25–5

3.3 INDICATOR TUBES

Glass tubes are filled with a sorbent with an immobilized reagent. A certain volume of the analysed liquid is pumped through the tube; it can be implemented using a syringe, by a hydrostatic pressure or by capillary forces immersing the tube into the analysed liquid and waiting up to the end of the sorbent layer in the tube. The interaction between the immobilized reagent and the analyte results in a compound with a colour different from the colour of the sorbent layer. The length of the coloured zone depends on the analyte concentration.

Probably the first application of indicator tubes for analysis of liquids was described in Ref. [75]. Glass tubes of 2.4–2.6 i.d. filled with silica with immobilized *o*-tolidine were used for determining chlorine in water. The length of the coloured zone is proportional to the chlorine concentration in the rang 0.5–10 mg/l.

A Quantab device is proposed for the determination of chlorides by the length of the discoloured zone [76–79]. This device consists of a plastic strip with a T-shaped capillary tube glued to it. The long vertical part of the tube contains Ag_2CrO_4, while the horizontal part has an indicator to water. The long part of the tube has a scale denoting chloride concentration. The test device is immersed into the analysed liquid, which raises the capillary tube, reaches the vertical part and colours the indicator indicating the end of the analysis. The length of the AgCl column in the long tube is proportional to the concentration of chlorides in the sample.

Another device [80,81] uses an indicator tube of 60 mm in length, o.d. of 7.1 mm and i.d. of 4.1 mm with two zones. One zone has a reagent powder and the other imitates the colour of the test component corresponding to a certain concentration. A liquid is pumped through the tube until the intensity of the colour in the first zone becomes equal to the intensity of the colour in the second zone. The concentration of the component is determined from the volume of the pumped liquid.

Another development implements indicator tubes or flat test materials for detecting substances in aqueous solutions: tubes or strips contain a layer of a chromatographic sorbent and a layer of a sponge made of a hydrophilic material (cellulose) used as a reservoir for the analysed liquid. The determination of iron(III) uses tubes with a diameter of 6 mm closed at one end with a porous material with 3 cm of cellulose. Attached to the cellulose is a paper cartridge impregnated with $K_4[Fe(CN)_6]$. To use the tub, its end with a porous material is immersed to the analysed liquid for 6 s [82].

An indicator tube filled with an ion-exchanger with immobilized Chromazurol S was used for determining 1–150 mg/l of beryllium in wastewaters [83]; SO_3^{2-} ions were determined using an indicator tube 1.8 × 110 mm filled with cellulose powder with immobilized Malachite Green [84].

Sol–gel technology was used to include 1,10-phenanthroline, 1-nitroso-2-naphthol, Alizarin to silica, which were used for determining iron(II), cobalt(II), aluminium(III) and pH [85,86] (Table 3.13). Capillaries of 1.7 × 100 mm were filled with indicator powders, the ends of capillaries were stopped with paper filters (about 3 mm). Concentrations were determined by the length of the coloured zone of the sorbent after the analysed solution penetrated the layer due to capillary forces or was pumped through it with a syringe or a hydrostatic pressure. In the two latter cases, a plastic tube (2 × 200 mm) was attached to the indicator tube, it was used to determine the volume of the pumped solution.

The volume of the analysed solution pumped through the indicator tube was calculated from

$$V = \pi(l_c r_c^2 + l_a r_a^2)$$

where l_c and r_c are the length and radius of the capillary with the indicator powder and l_a and r_a are the length and radius of the attached plastic tube.

TABLE 3.13

Concentration ranges and reagents in determining ions with indicator tubes based on raising an analysed liquid by capillary forces [85,86]

Test ion	Reagent	Concentration range (mg/l)
Fe(II)	1,10-Phenanthroline	25–500 0.5-5*
Co(II)	1-Nitroso-2-naphthol	30–300
Al(III)	Alizarin	15–300
H^+	Alizarin	10–60

*The volume of an analysed solution passed through an indicator tube was 0.5 ml (hydrostatic pressure 0.05 atm was provided by dropping the indicator tube into the liquid to the depth of 50 cm).

In this case, the relative length of the coloured zone *R* of the indicator powder depends on the volume of the pumped solution as

$$R = 100Sr_c^2(l_c r_c^2 + l_a r_a^2)$$

$$R = 100S/l_c \tag{3.5}$$

where *S* is the absolute length of the coloured zone of the indicator tube.

Equation (3.5) was used in the absence of the attached plastic tube. It was found that *R* is proportional to the concentration of iron(II) and described with the equation $R = 0.07 + 4.91c$ for the range 25–500 mg/l and $R = 0.23 + 27.6c$ for the range 0.5–5 mg/l (*c* is the concentration of iron in mg/l). The relative standard deviation of the results of determination of iron for the concentration of 500 mg/l is 10%, the value of 30% was reached for the concentration of 5 mg/l.

In the case of capillary forces, a tube absorbs 0.02 ml of the analysed solution; in the case of a hydrostatic pressure (0.05 atm) this value is as high as 0.5 ml. This increases the sensitivity of determination by a factor of 50 (see Table 3.13).

The authors of Refs. [87–95] proposed two variants of generation of analytical signals using indicator tubes (1–3 mm in diameter): the formation of a coloured compound directly in a tube as a result of pumping the sample through a tube filled with an indicator powder (Table 3.14) and the formation of the coloured compound in solution followed by pumping it through the tube (Table 3.15).

TABLE 3.14

Coloured compounds formed immediately in an indicator tube

Test compound	Indicator powder	pH	Zone colour (background)
Fe(II)	1,10-Phenanthroline/silica gel-phenyl or xerogel	4–9	Red (white)
	2,2′-Dipyridyl/silica gel-phenyl	4–9	Red (white)
Fe(III)	1-Nitroso-2-naphthol/silica gel-C_{16}	6–9	Green (yellow)
	Xylenol orange/silica gel-phenyl or xerogel	2–5	Violet (orange)
Co(II)	1-Nitroso-2-naphthol/silica gel-C_{16} or xerogel	6–9	Red (yellow)
Cu(II)	1-(2-Pyridylazo)-2-naphthol/silica gel-phenyl or xerogel	3–5	Violet (orange)
	Diethyldithiocarbamate/silica gel	3–9	Brown (white)
Total Cu(II), Co(II), Ni	1-(2-Pyridylazo)-2-naphthol/silica gel-phenyl	7–9	Red (violet)
Cd	Bromobenzothiazo/xerogel	9	Blue (orange)
Cl^-	Diphenylcarbazone, Hg(II)/xerogel	3–4	Pink (blue)
Hydrazine	Molybdophosphoric heteropoly acid/xerogel	0.4 M H_2SO_4	Blue (yellow)

TABLE 3.15

Coloured compounds formed in solutions

Test compound	Indicator reagent or mixture	Sorbent	pH	Zone colour (background)
Pb	Rhodizonate	Silica gel	3–5	Brown (white)
Cd	1,10-Phenanthroline, Pyrogallol red	Silica gel-phenyl	9	Blue (white)
	1,10-Phenanthroline, Bromopyrogallol Red	Silica gel-phenyl	9	Blue (white)
Nitrite	Chromotropic acid, Sulphanilic acid	Silica gel-SO_3^-	4–5	Red (white)
Nitrate	Chromotropic acid, Sulphanilic acid, zinc	Silica gel-SO_3^-	4–5	Red (white)
Molybdate	Lumogallion	Silica gel	1–4	Reddish-orange (white)
Phenols	4-Aminoantipyrine, oxidant	Silica gel	8–9	Red (white)

Indicator powders were prepared by noncovalent immobilization of analytical reagents on reversed-phase silica and their inclusion in silicic-acid xerogels. The following requirements for the reagents used in the method of indicator tubes should be met: a high degree of contrast of the reaction, a high rate of interaction with the determined component, strong retention of the reagent and product on the indicator powder, sensitivity, and selectivity. The effect of the injection mode of the analysed solution to the indicator tube, physicochemical parameters of indicator powders, the length and the inside diameter of tubes, and solution pH on the length of the coloured zone was studied.

A decrease in the concentration of the immobilized reagent in the indicator powder increase the sensitivity of determination. However, starting from certain values (individual for each system) a decrease in the concentration of the reagent significantly degrades the degree of the contrast of the zone and reproducibility of measurements (Table 3.16).

TABLE 3.16

Concentration ranges and slopes (*S*) in determining Cu(II) and Fe(III) with indicator tubes depending on the reagent concentration in indicator powders. Cu(II): indicator tubes 1×50 mm; Fe(III): indicator tubes 2×70 mm

Test ion	Reagent	Concentration of a reagent in an indicator powder (μmol/g)	Concentration range (mg/l)	*S* (mm·l/mg)
Cu(II)	1-(2-Pyridylazo)-2-naphthol	0.2	0.3–5.6	7.2
		0.6	0.6–11.3	3.6
		1.9	1.5–30.0	1.4
		3.1	2.0–40.0	1.0
		15.6	15.0–300.0	0.1
Fe(III)	Xylenol orange	0.3*	—	—
		0.6*	—	—
		1.1	1.0–35.0	2.0
		2.1	1.0–40.0	1.4
		5.6	1.0–45.0	1.3
		10.0	5.0–80.0	0.8
		13.5	10.0–120.0	0.5

*Under these conditions, it is impossible to determine the length of a coloured zone because of the insufficient contrast of the reaction.

One should keep pH values providing quantitative sorption of determined components by the indicator powder; the specific surface of indicator powders based on modified silica should not exceed 200 m^2/g. To provide a distinct edge of the coloured zone, the pumping rate of the analysed solution to the indicator tube should not exceed 0.25 ml/min.

A decrease in the inside diameter of the tube in the range 1.0–3.0 mm increases the sensitivity of determination. In the immersion mode, indicator tubes of about 40 mm in length are expedient; the pumping mode (a syringe), the expedient length is 70 mm.

Based on experimental data, a mathematical simulation of indicator tubes was made. This model was used for explaining the dependence of performance parameters on the rate of the heterogeneous analytical reaction and the flow rate of the analysed solution in the indicator tube. To provide a high accuracy of determination, the residence time of the analysed solution in the indicator tube should be 10- to 20-fold greater than the time of the reaction.

Procedures for determining many metal ions, a number of anions, hydrazines, and phenols were developed using indicator tubes (Tables 3.14, 3.15 and 3.17). Performance parameters of these determinations are comparable with the characteristics of instrumental methods. High

TABLE 3.17

Characteristics of indicator tubes manufactures by Ekoanalitika Association (Moscow)

Test substance	Trademark	Concentration range (mg/l)	RSD (c, mg/l)
Fe	EA-Fe-Tr, 14-99	0.05–50	0.33–0.07
Co(II)	EA-Co-Tr, 15-99	0.06–6.0	
Cu(II)	EA-Cu-Tr, 10-99	0.1–65	0.25–0.05
Cd(II)	EA-Cd-Tr, 11-99	0.3–3.0	0.3 (1.0)
Sn(II)	EA-Sn-Tr, 12-99	10–200	0.05 (40)
Cl^-		20–200	0.06 (100)
NO_3^-	EA-NO_3-PTr, 1-99	10–80	
NO_2^-	EA-NO_2-PTr, 2-99	0.5–20	
Total metals	EA-Total Metals-Tr, 9-99	$(1–50)\cdot10^{-6}$ M	
Cr(VI)	EA-Cr-Tr, 13-99	0.5–50	
Sulphur-containing reductants	EA-Sred-PTr, 3-99	3–330	
Active chlorine	EA-Cl_2-PTr, 4-99	0.5–3	

selectivity of determination results from the selection of relatively selective chromogenic reactions, the selection of experimental conditions, and the use of masking agents.

Determination of Cd(II) and Cu(II) was based on the use of polyurethane-foam cubes (side length 4 mm) and dithizone and $Pb(DDTC)_2$ as reagents. Cubes are placed into a glass tube and a volume of 100 ml of the analysed liquid is pumped through the tube. The concentration is determined from the length of the coloured zone of cubes in the range of 0.01–0.05 mg/l [96].

3.4 TABLETS AND SIMILAR FORMS

The use of prepared mixtures of reagents as tablets or pills is very useful in the field. A certain volume of the analysed liquid is sampled to a vial, a dry mixture of reagent is added, and the intensity of the colour appearing is estimated after stirring the solution [97,98].

Rapid tests for metals implement Silochrom S = 120 tablets with heterocyclic azo compounds [73,99,103]. Silica is used as a tool for ion preconcentration. In a number of cases, the lower determination limits were as low as 2–3 orders lower than the minimum allowable concentrations of test ions in environmental materials [99–105] (see Table 3.18). As an example, a test for cobalt with 1-(2-pyridylazo)-2-naphthol has the determination limit of 0.004 mg/l.

A mixture of silica and sucrose (25–30%) containing organic reagents (0.1–1.2%) was proposed instead of pure silica. Tablets (100

TABLE 3.18

Test procedures for the determination of metal ions with silica gel tablets (Silochrom S-120) modified with reagents

Test ion	Reagent	pH	Determination limit (mg/l)
Co(II)	1-(2-Pyridylazo)-2-naphthol	3–5	0.004
Hg(II)	Dithizone	3–5	0.003
Pd(II)	1-(2-Pyridylazo)-2-naphthol	3–4	0.1
U(VI)	1-(2-Pyridylazo)-2-naphthol	6.9–8.2	0.1
	4-(2-Pyridylazo)resorcinol*	5.8–8.2	0.1
	2-(5-Bromo-2-pyridylazo)-5-diethylaminophenol	4.5–5.5	0.1

*On a KU-2 cation-exchanger.

pieces) are placed into a microcomparator cell. A certain volume of the analysed solution is put on the tablet, and the developing colour is compared with a scale of pre-made tablets.

For instance, while preparing tablets for the determination of nitrate, 2.5 g of sucrose is mixed with 0.01 g of naphthylethylenediamine and 0.1 g of sulphanilic acid in acetone, 0.9 g of carbonic acid, and 6.5 g of silica. After removing acetone by evaporation, 0.06 g of zinc dust is added to the mixture and thoroughly stirred and ground. Tablets are pressed out and placed into comparator cells. A drop of the analysed liquid is put into a tablet. If nitrates are present, a colour from pale pink to dark magenta appears. The colour is compared with a standard scale prepared using known concentrations of nitrate.

Another test for nitrates is worth mentioning [98,104,105]. It was based on the azo coupling with non-toxic *p*-nitroaniline as the starting material for the diazo component and resorcinol or chromotropic acid as an azo coupling component. In the latter case, it is possible to carry out the diazotization and azo coupling at the same pH, under room conditions, and without organic solvents.

Rapid tests for the detection of Au(III) and Ag(I) are based on the reactions of reduction of these metals to the elementary state [106].

Polyurethane foams are widely used as substrates for organic reagents. They are foamed polymers based on ethers and esters. A part of the solid phase is “replaced” with a gas, which exists in the polymer as small bubble cells. Usually, the gas takes over 90% of the total volume of the polymer. If, during the synthesis, 1/6 of cell surface is decomposed, polyurethane foams of open-cellular type are formed, and the surface available for adsorbing substances increases significantly. Polyurethane foams can retain chelating agents, inorganic precipitators, or enzymes. As a result of sorption or formation of coloured substances at the surface, polyurethane foams show the colour characteristic of these compounds.

Rapid tests using polyurethane foams are implemented in three variants. The first is based on the formation of the coloured compound in the analysed solution followed with the sorption with unmodified polyurethane foam. Another variant is implemented when the analysed substance interacts with terminal toluidine groups of polyurethane. The third case is based on the formation of the coloured compound directly in the sorbent phase as a result of interaction between the substance and a hydrophobous reagent immobilized of the polyurethane foam.

A method of modification of polyurethane foam with organic reagents includes pre-plasticization of foam tablets followed with treatment with a small volume of the reagent solution in acetone. Tablets are immersed in a plasticizer (tri-*n*-octylamine) for a day. The excess amount of tri-*n*-octylamine is then removed by drying the tablet between sheets of a paper filter. Next, the tablet is treated with 0.2–0.3 ml of an acetone solution of the reagent to be immobilized. After acetone is evaporated, the tablets can be used. This method provides strong retention of reagents and uniform distribution throughout the tablet. Polyurethane foams modified according to this approach were used in developing rapid tests of nickel and chromium(VI) [107–109]. Polyurethane foams without a modification are used for determining cobalt(II), iron(III), titanium(IV) [110], surfactants [111, 112], and phenols [113] (see Table 3.19). The sensitivity of the latter case is lower, although the selectivity increases.

Nickel is determined after it is adsorbed from an ammonium buffer solution (pH 9) at a polyurethane foam modified with dimethylglyoxime. The determination of 0.08 mg/l of nickel is not interfered with Ca, Mg, acetate and tartrate at $5{\cdot}10^3$ excess amounts, fluoride and ascorbate at $1{\cdot}10^3$, Cd, Cu, and Zn at 100, Pb at 10 and Co at twofold amounts.

Chromium(VI) is determined after its interaction with immobilized diphenylcarbazide. At pH 1–5, tablets are coloured violet. The determination of 0.1 mg/l of chromium is not interfered with Ca, Mg, chloride, sulphate, and hydrogen phosphate at $1{\cdot}10^3$ excess amounts, Cu and Zn at 100 and Co and Ni at 10-fold amounts.

Tablets made of polyurethane foam as carriers for immobilizing horseradish peroxidase were used in developing a rapid test for 0.008–1000 μM organomercury compounds (methyl-, ethyl- and phenylmercury) [123]. The determination is based on their liberative effect on the enzyme inhibited with phenylthiourea in the reaction of oxidation of *o*-dianisidine or their effect on the duration of the induction period in the reaction of oxidation of 3,3′,5,5′-tetramethylbenzidine in the presence of sodium diethyldithiocarbamate. The preparation of peroxidaze immobilized on polyurethane foam retains its catalytic activity for 1.5 years; in the case of a chromatographic paper the lifetime is six months.

Several tests are proposed for a number of ions (Table 3.20), which implement reagents immobilized on plasticized open pores of polyurethane foams. They are shaped in the form of a cube with a side

TABLE 3.19

Test tablets made of polyurethane foams (analysed volume 25 ml, tablet mass 0.05 g)

Test component	Concentration range (mg/l)	Determination conditions
Co(II)	0.1–4	Adsorption of thiocyanate complexes from 0.01 M HCl in the presence of 1 M NaF
Fe(III)	0.02–0.6	Adsorption of thiocyanate complexes from 1 M HCl
Ti(IV)	0.03–0.5	Adsorption of thiocyanate complexes from 1 M HCl in the presence of 1 M NaF
Cr (VI)	0.01–0.2	Adsorption from 1 M H_2SO_4 on polyurethane foams modified with diphenylcarbazide
Ni(II)	0.2–4	Adsorption from an ammonium buffer solution on polyurethane foams modified with dimethylglyoxime
Perchlorate	0.03–0.4	Reaction with reduced $H_4SiMo_{12}O_{40}$ immobilized on polyurethane foams in 4 M H_2SO_4
Bromate	0.3–20	Reaction with reduced $H_4SiMo_{12}O_{40}$ immobilized on polyurethane foams in 0.1 M H_2SO_4
Dichromate	0.1–9	Reaction with reduced $H_4SiMo_{12}O_{40}$ immobilized on polyurethane foams in 1 M H_2SO_4
Nitrite	0.3–5.0	Reaction with polyurethane foams in 1 M HCl
Phenols	0.01–0.80	Adsorption of azo compounds from 0.2 M Na_2CO_3 in the presence of cetyltrimethylammonium
Nitrophenols	0.005–0.05	Adsorption from an aqueous solution on polyurethane foams modified with in the presence of cetyltrimethylammonium
1-Naphthol	0.02–0.4	Adsorption of azo compounds from 0.2 M Na_2CO_3
Cationic surfactants	0.4–10	Adsorption of associates with Bromophenol Blue from 0.4 M Na_2CO_3
Anionic surfactants	1–30	Adsorption of associates with $[Fe(Phen)_3]^{2+}$
Aniline	0.04–0.4	Adsorption of azo compounds from 0.2 M Na_2CO_3 followed by the treatment of polyurethane foams with 30% $[(C_4H_9)_4N]OH$
Active chlorine	0.2–8.0	Reaction with polyurethane foams in 0.2 M H_2SO_4
Ascorbic acid	0.3–2.4	Reaction with oxidised $H_4SiMo_{12}O_{40}$ immobilized on polyurethane foams in 1 M HCl

TABLE 3.20

Cube-shaped polyurethane test systems (analysed volume 25 ml, tablet mass 0.05 g)

Test ion	Reagent immobilized on polyurethane foams	Concentration range (mg/l)	Ref.
Cd(II)	Dithizone	0.1–10	96
Co(II)	Amberlite LA-1, thiocyanate	0.3–500	115
Cr(VI)	Diphenylcarbazide	0.1–5.0	116
Cu(II)	Diethyldithiocarbamate	0.02–10	96, 115, 117, 118
	Diacetylmonoxime–benzothiazolylhydrazone	0.1–20	
Fe(III)	Alamine 336, thiocyanate	0.1–20	119
Ni(II)	Dimethylglyoxime	0.05–50	120
Pb(II)	Zinc dithizonate	0.2–100	115
Pd(II)	Diacetylmonoxime–benzothiazolylhydrazone	0.5–50	118
Hg(II)	Lead dithizonate	0.2–10	121

length of 4 mm. The cubes are shaken for 1–2 min with 1–2 ml of the analysed solution. A change in colour is compared with a scale made of similar cubes.

Reagents for rapid tests can be immobilized on other artificial polymer matrices. A polyvinyl chloride film with adsorbed bathophenanthroline [122] or disodium 2,5-(nitro-2-pyridylazo)-5-N-propyl-N-sulphopropylamino)phenol [123] was proposed for iron(II) determination. The film is immersed into a solution with pH 3–6 for 5–20 min, and a change in colour is estimated visually or with a spectrophotometer. Films with adsorbed reagents are prepared by adding indicators in solution of polymer materials [124,125] or in the process of polymerisation [125,126]. This approach is used in tests for pH, proteins, ketones, nitrites and Fe(II) [124,126]. Pastes from polyvinyl acetate of polyvinyl propionate containing magnesium dipicrylaminate and morin are used for determining potassium and aluminium in drilling cores by the intensity of the developed colour after the paste is applied to the analysed surface [127].

A large group of optical sensing systems for determining trace metals using rapid tests is developed. It is based on packed fibrous materials [128–130]. Sorbents are prepared adding finely dispersed cation- and anion exchangers of various types (particle size 5–10 μm) to

TABLE 3.21

Main characteristics of test procedures for the determination of heavy metals [128–130]

Test ion	Reagent	Change in disk colour	Concentration range (mg/l)
Cr(VI)	Diphenylcarbazide	White–lilac	0.01–0.25
Co(II)	1-(2-Pyridylazo)-2-naphthol	Orange–green	0.05–0.5
Zn(II)	Dithizone	Orange–cream	0.1–0.5
Cu(II)	Diethyldithiocarbamate	White–yellow	0.05–1
Ni(II)	Dimethylglyoxime	White–red	0.1–1
Fe(III)	Potassium thiocyanate	White–red	0.1–1
Th(IV)	Arsenazo III	Lilac–green	0.01–0.08
Pd(II)	Nitrosodiethylaniline	Yellow–red	0.01–0.1
U(VI)	Arsenazo III	Lilac–blue	0.1–0.5

fine threads of polyacrylonitrile fibre (diameter 30–10 μm) directly in the process of its formation. Next, the fibre is treated with a reagent solution usually used for photometric determination of the desired element. The reagent is retained in the ion exchanger due to absorption. Depending on the nature of the ion exchanger and the reagent, the latter may lose its ability to react with the given ion (the reaction centre of the reagent molecule is blocked) or unequally change its reactivity to various ions. Thus, varying the nature of the ion exchange and the reagents as well as reaction conditions, it is possible to find the most selective system for adsorption and determination of the required metal cation. The material with the immobilized reagent is shaped as a disc, and analysed solution is pumped through it. Optical signal is measured visually or by diffuse-reflectance spectroscopy. Table 3.21 summarizes parameters of rapid tests for determining metal ions for the visual estimation of concentrations. The analysis time is 10 min, error is 20–60%.

3.5 REGULAR AMPOULES AND DROPPERS

Ready-to-use solutions or organic solvents for rapid tests are stored and sold in soldered ampoules or droppers. Soldered ampoules are usually used for organic solvents, concentrated acids, and the whole

TABLE 3.22

Composition of polytests for determination of narcotics

Narcotic	Reagent mixtures in ampoules	Colour mark
Morphine, heroin, codeine, promedolum, amphetamines	2% Formaldehyde in concentrated sulphuric acid	Violet (morphine, heroin), bluish-violet (codeine), red (promedolum), orange (amphetamines)
Barbiturates	(1) 5 % Cobalt nitrate in isopropanol (2) 10% piperidine in isopropanol	Lilac
Heroin	(1) Bromophenol Blue in a buffer solution, pH 5; (2) chloroform	Purple
Cocaine	(1) 2% Cobalt thiocyanate (2) chloroform	Blue-coloured bottom layer
Amphetamines	(1) 1% Sodium nitroprusside in 5% aqueous solution of acetone (2) 2% potassium carbonate	Dark-pink
Ephedrine	(1) 0.5 M Copper sulphate (2) *n*-butanol	Violet-coloured top layer
LSD	5% *p*-Dimethylaminobenzaldehyde	Reddish-violet
Morphine, heroin, codeine	5% Ammonium molybdate in concentrated sulphuric acid	Violet
Barbiturates	(1) 0.5% copper sulphate in 5% piperidine solution (2) chloroform	Blue-coloured bottom layer

contents of an ampoule is used in the analysis. For instants, tests for narcotics use polyethylene cartridges with semitransparent reaction containers, glass ampoules filled with reactants and polyethylene holders. When pressing a holder, ampoules are decomposed, and its contents is transferred to the container with the sample of the test material [131].

The results of titration is considered positive if the major colour of the reaction mixture is coincident with the colour mark on the package. The list of tests for several groups of narcotics and the chemical composition of reagents in ampoules is summarized in Table 3.22 [131].

There is wide variety of kits of reagent solutions in droppers. Merck company proposes Aquaquant®, Aquamerck®, Microquant®, and Spectroquant® analysis kits, Macherey-Nagel produces Visocolor® comparator, Visocolor® alpha, Visocolor® ECO, and Visocolor® HE [132,133].

These systems are based on colorimetric comparison. Aquamerck® uses a test-tube, and reagents are added with a special dropper. The colour of solutions is compared with a scale printed on cardboard, the test-tubes are examined from above. Aquaquant® system is similar to Aquamerck®, but an increase in the optical pathlength at the stage of colour estimations is provides higher sensitivity, a 10- to 100-fold increase compared to Aquamerck®.

In a Microquant® system, test-tubes are examined from one side using a simple comparator with a scale applied to a rotating disc.

The Visocolor® comparator system uses glass test-tube cells divided into two parts. One part bears a colour scale. Analysed liquid is placed into another part, required reagents are added with a dropper, and the resulting colour (after stirring) is compared with the colour scale.

Spectroquant® and Visocolor® systems implement portable photometers for estimating the intensity of the developed colour after adding reactants to the analysed solution.

As an example, Table 3.23 summarizes linear calibration ranges for several ions using various systems. Visocolor® HE, Aquaquant®, and Spectroquant® analysis systems are characterized by the maximum sensitivity.

TABLE 3.23

Concentration ranges for several ions in water using various Merck and Macherey-Nagel test kits

Ion	Visocolor®			Aquaquant®	Microquant®	Spectroquant®
	ECO	Comparator	HE			
NH_4^+	0.2–3.0	0.2–10.0	0.02–0.5	0.025–0.4	0.2–8.0	0.01–3.5
Cu(II)	0.1–1.5	0.1–3.0	0.04–0.5	0.05–0.5	0.3–10.0	0.05–8.0
CN^-	—	0.05–1.0	0.002–0.04	0.002–0.03	0.03–5.0	0.002–0.5
Cl_2	0.1–2.0	0.1–2.0	0.02–0.6	0.01–0.3	0.1–2.0	0.05–5.0
SO_4^{2-}	—	25–200	—	25–300	25–300	10–600
PO_4^{3-}*	0.2–5.0	0.1–1.5	0.01–0.25	0.015–0.14	0.2–3.0	0.02–5.0

*Calculated to phosphorus.

3.6 SELF-FILLING AMPOULES

These ampoules are patented, produced, and sold by CHEMetics Company (USA) [134–137]. Ampoules contain solutions of reagents and auxiliary substances in required quantities. Ampoules with plucked and slightly cut tip are prepared *in vacuo*, and they have some amount of an inert gas. When ampoules are placed in a beaker with an analysed liquid, the ampoule tip is broken under a slight push, and a certain amount of the liquid fills the ampoule due to the vacuum. The reaction takes place, and its result is estimated either visually using comparators or with a pocket-size photometer CHEMets® and VACUettes® system, while Vacu-vials® are proposed for instrumental control.

The determination of low concentrations (below 1 mg/l) with CHEMets® and VACUettes® is made with a cylindrical comparator, where the colour intensity is examined along the whole length of an ampoule. For higher concentrations, a flat comparator is used, and ampoules are examined from one side. A small amount of the analysed liquid is placed in the ampoule via a special capillary, and the ampoule is then filled with a diluting solution.

CHEMets® and VACUettes® systems use ampoules of 7 mm in diameter; the Vacu-vials® system uses 13-mm ampoules.

Self-filling ampoules from CHEMetrics are widely used in practical analysis. They are frequently used, e.g. for determining low concentrations of dissolved oxygen (in power engineering), for analysing natural and wastewaters, in the control of technological processes, and in education. The company produces kits for determining total alkalinity and hardness of water, ammonium, chlorides, cyanides, copper, iron, formaldehyde, nitrates, nitrites, phenol, zinc, and other components. Kits are usually designed for a certain concentration range. As an example, Table 3.24 shows the data from the company catalogue (1999) implementing the determination of some compounds with various reagent systems. The most sensitive are CHEMets® systems.

3.7 TITRATION KITS

Test titration usually includes the following procedure. The analysed liquid is placed in a glass flask; the indicator is added. Next, the titrant solution is added dropwise using a calibrated syringe or a dropper

TABLE 3.24

Concentration ranges (mg/l) for several components in water using self-filling ampoules

Analyte	Reagent	CHEMets®	Vacu-vials®	VACUettes®
NH_4^+	Nessler reagent	0.05–1.0	0.2–7.0	1.25–25
		1.0–10.0	0.4–14.0	25–250
		0.2–2.0		2.5–50
		2.0–10.0		50–500
				5–100
				100–1000
				50–1000
				1000–10000
Cl_2	Dialkyl-*p*-phenylenediamine	0.02–0.2	0.1–4.0	1.25–25
		0.05–1.0		25–125
		1.0–5.0		2.5–50
				50–250
				5–100
				100–500
				50–1000
				1000–5000
Cr(VI)	Diphenylcarbazide	0.05–1.0	0.1–7.0	1.25–25
		1.0–10.0	0.2–14.0	25–250
				2.5–50
				50–500
				5–100
				100–1000
				1000–10000
Cu(II)	Bathocuproine	0.05–1.0	0.1–7.0	
		1.0–10.0	0.2–14.0	—
		0.1–1.0		
Phenol	4-Aminoantipyrine	0.05–1.0	0.25–8.0	1.25–25
		0.05–12.0	0.5–16.0	1.25–300
				2.5–50
				2.5–600
				5–100
				5–1200
				50–1000
				50–12000
Fe(II,III)	1,10-Phenanthroline	0.05–1.0	0.08–2.5	1.25–25
		1.0–10.0		25–250
		0.1–1.0		2.5–50
				50–500
				5–100
				100–1000
				50–1000
				1000–10000

under stirring until the indicator changes colour. The concentration of the analysed component is determined either by the number of titrant drops or by the volume of the titrant measured using the syringe.

Macherey-Nagel Company produces Visocolor® ECO titration and Visocolor® titration systems [133]. The former system implements the determination of concentrations from the number of drops; the latter system used titrant volume. Similar kits, Aquamerck®, are produced by Merck [132].

The Titrets® titration system from CHEMetrics company uses self-filling ampoules with a capillary [135]. An ampoule contains the indicator solution. It is placed in a special device and the end of the capillary is immersed into the analysed liquid. Pressing a lever on the device allows the analysed solution to be transferred to the ampoule in portions. After adding each portion, the contents of the ampoule are stirred, the titrant is added again, the contents are stirred again and again until the indicator in ampoule changes its colour. The concentration is determined from the volume of the solution in the ampoule.

Test titrations were used for determining alkalinity and acidity, calcium, carbonate hardness, chlorides, total hardness, oxygen, and sulphites in water (Table 3.25).

TABLE 3.25

Concentration ranges, indicators, and titrants used for analysis of water in Merck and CHEMetrics test kits

Parameter	Indicator	Titrant	Aquamerck®	Visocolor® titr
Acidity	Phenolphthalein	NaOH	0.1–10.0 *mM*	0.2–7 *mM*
Alkalinity	Methyl Red	HCl	0.1–10.0 *mM*	0.2–7.0 *mM*
Carbonate hardness	Methyl Red	HCl	0.1–35 *mM*	0.2–7.0 *mM*
Cl^-	Fe(III)	$Hg(SCN)_2$	2–250 mg/l	5–500 mg/l
Total hardness	Calcichrome	EDTA	0.1–35 *mM*	0.01–3.6 *mM*
SO_3^{2-}	Starch	Iodate	0.3–50 mg/l	2–200 mg/l

3.8 OTHER TOOLS

Multilayer film systems

Multilayer film systems were first used in glucose determination [138] followed by urea, amylase, bilirubin, and triglycerides [139,140] in blood serum. These tools consist of at least two layers. The analysed liquid is placed on the upper layer. It usually consists of a porous layer of titanium dioxide (to better reflect the light when the colour is observed from the lower side). The next layer contains reagents. Actually, it may be several layers depending on the test reaction. To prevent premature reactions in these layers, they are separated by semipermeable membranes made of cellulose acetate. The lower layer is made of a transparent polymer material. Below is the layout for an indicator element for urea according to the reaction:

$$H_2N\text{–}CO\text{–}NH_2 + H_2O \xrightarrow{\text{urease}} 2NH_3 + CO_2$$

$$NH_3 + HR \longrightarrow NH_4^+ + R$$

where R is Bromocresol Green.

The analysed solution penetrates the layer of titanium dioxide and appears in the reagent layer. The ammonia formed in the reagent layer diffuses through the membrane to the indicator layer and the characteristic colour of the indicator appears. The colour is observed through the transparent polymer base.

The principle of multilayer devices is used in clinical analysis using Eastman-Kodak Ektachem® and Fuji Drichem® systems; the measurement of the analytical signal is implemented using reflectance microphotometers.

Testing pencils and pens

These tools are rods pressed from reagent mixtures with a binder (usually barium sulphate). For instance, pencils for determining neuroparalytic poison gases consist of 80% of 4,4′-bis(diethylamino)benzophenoneoxime, 10% of sodium cyanide, and 10% of barium sulphate. In the presence of tabun and sarin vapours at the concentrations below 1 μg/l, the lines drawn with these pencils turn orange to red [141].

Pencils for determining lewisite consist of Michler's thioketone and barium sulphate. The pale brown colour of such pencils turns to a bluish green when contacting liquid lewisite or in the presence of its high concentrations in the air [141].

A device for rapid colorimetric determination of trace metals at the surface has been patented [142]. It consists of a cylindrical plastic case and a working head (tampon) from a fibrous material. The case is filled with an inert carrier material with a reagent. It also contains one or more thin-wall ampoules with activator solvent or a dye solution. If the case is pressed, ampoules are broken, the liquid impregnates the carrier and moistens the tampon. The moistened tampon contacts the studied surface. If the test element is present, the tampon or case body shows the characteristic colour.

REFERENCES

1. E. Bishop, *Indicators*, Pergamon, Oxford, 1972.
2. H.K. Macho and K.D. Hungenberg, *US Pat.* 5096836, 1992.
3. F. Arai, K. Yazawa, H. Takeuchi and M. Kitajima, *US Pat.* 4783315, 1988.
4. A. Jina, L. Larson and J. Smith, *US Pat.* 5526120, 1996.
5. M. Rapkin, *US Pat.* 4301115, 1981.
6. H. Weisz and H. Lepper, *Anal. Chim. Acta*, 172 (1985) 265.
7. V.G. Amelin, *Zh. Anal. Khim.*, 55 (2000) 532.
8. C. Osnowski, *Pol. Pat.* 92803, 1977.
9. H.R. Lange, E. Geisler and W. Werner, *Ger. Pat.* 3048799, 1982.
10. H. Lange, W. Rittersdorf, H.G. Rey and P. Rieckmann, *US Pat.* 3802842, 1978.
11. H.P. Andersen, *US Pat.* 3511608, 1970.
12. K.A. Blake, J.E. Christner and L.J. Lucchese, *PCT (WO)*, 92/177768, 1992.
13. H. Matsumoto, *Jpn. Pat.* 48-14273, 1973.
14. V.M. Ostrovskaya, *RF Pat.* 2009486, *Byull. Izobret.*, No. 5 (1994).
15. K.A. Blake, J.E. Christner and L.J. Lucchese, *US Pat.* 5330715, 1994.
16. *Merckoquant®. Chemical microchips for analysis you take in your stride*, Merck KgaA, 1999.
17. H. Clifford, *US Pat.* 3510263, 1970.
18. C. Orley, *Fr. Pat.* 1603510, 1971.
19. Ly Uy-Yu, *US Pat.* 4654310, 1987.
20. B.A. Popov, *USSR Inventor's Certificate* 205360, *Byull. Izobret.*, No. 23 (1967).
21. W. Gunkel and H. Krauß, *Ger. Pat.* 3616105, 1987.
22. *Jpn. Pat.* 80-147174, 1980.
23. R. Bauer, C.O. Rupe and R.G. Rosenfield, *Ger. Pat.* 2015271, 1970.
24. L. Hammond, *UK Pat*, 2201241, 1988.
25. V.M. Ostrovskaya, *Fresenius' J. Anal. Chem.*, 361 (1998) 303.
26. V.M. Ostrovskaya and O.V. Ivanov, *Vysokochist. Veshchestva*, No. 4 (1987) 178.

27. V.M. Ostrovskaya, *Reactive Indicator Tools for Multielement Tests*, 1-ya Obraztsovaya tipografiya, Moscow, 1992 (in Russian).
28. V.M. Ostrovskaya, *RF Pat.* 2095779, *Byull. Izobret.*, No. 31 (1997).
29. T.N. Shelkovaya, S.A. Fetisova, V.M. Ostrovskaya, N.A. Golikov and A.S. Aksenova, *USSR Inventor's Certificate* 732739, *Byull. Izobret.*, No. 17 (1980).
30. G.G. Chernyavskii, M.S. Kravchenko and S.P. Bushtets, *USSR Inventor's Certificate* 1386893, *Byull. Izobret.*, No. 13 (1988).
31. V.G. Amelin, *RF Pat.* 2119666, *Byull. Izobret.*, No. 27 (1998).
32. V.G. Amelin and V.M. Ivanov, *Zh. Anal. Khim.*, 55 (2000) 411.
33. V.G. Amelin, *Doctoral Sc. Dissertation*, Moscow State University, Moscow, 1998 (in Russian).
34. V.G. Amelin, *Zh. Anal. Khim.*, 53 (1998) 868.
35. V.G. Amelin, *Zh. Anal. Khim.*, 54 (1999) 651.
36. V.G. Amelin, *Zh. Anal. Khim.*, 54 (1999) 753.
37. V.G. Amelin, *Zh. Anal. Khim.*, 54 (1999) 1088.
38. J. Hais and K. Macek (Editors), *Papirova Chromatografie* (Paper Chromatography), Praque, 1959 (in Czech).
39. Y. Hashimoto and L. Mori, *J. Pharm. Soc. Jap.*, 72 (1952) 1532.
40. V.G. Amelin, *RF Pat.* 2093823, *Byull. Izobret.*, No. 29 (1997).
41. V.G. Amelin, *Zh. Anal. Khim.*, 53 (1998) 958.
42. V.G. Amelin, *RF Pat.* 2103677, *Byull. Izobret.*, No. 3 (1998).
43. V.G. Amelin, *RF Pat.* 2103678, *Byull. Izobret.*, No. 3 (1998).
44. K.M. Ol'shanova, V.D. Kopylova and N.M. Morozova, *Precipitation Chromatography*, AN SSSR, Moscow, 1963 (in Russian).
45. V.G. Amelin and V.G. Berezkin, *RF Pat.* 2161304, 1999.
46. V.G. Berezkin, V.G. Amelin, I.S, Kolodkin and A.A. Rodionov, *J. Planar Chromatogr.*, 13 (2000) 254.
47. F. Geiss, *Fundamentals of Thin-Layer Chromatography (Planar Chromatography),* Huetig Verlag, Heidelberg, 1987.
48. N.A. Tananaev, *Spot Analysis*, Goskhimizdat, Moscow, Leningrad, 1954 (in Russian).
49. V.G. Amelin and I.S. Kolodkin, *Zh. Anal. Khim.*, 56 (2001) 120.
50. K. Cammann, *DE Patent*, 1998, Application 19839705.4.
51. H. Starp, N. Bushmann and K. Cammann, *Fresenius' J. Anal. Chem.*, 368 (2000) 203.
52. V.G. Amelin and A.V. Spirin, *RF Pat.* 2058547, *Byull. Izobret.*, No. 11 (1996).
53. V.G. Amelin and E.P. Grishina, *Izv. Vyssh. Uchebn. Zaved., Khim. Khim. Tekhnol.*, No. 5, 40 (1997) 50.
54. C. Glad and A.O. Grubb, *Anal. Biochem.*, 85 (1978) 180.
55. D.J. Litman, T.M. Hanlon and E.F. Ullman, *Anal. Biochem.*, 106 (1980) 223.

56. R.F. Zuk, V.K. Ginsberg, T. Houts, J. Rabbie, H. Merrick, E.F. Ullman, M.M. Fisher, C.C. Sizto, S.N. Stiso and D.J. Litman, *Clin. Chem. (Washington, D. C.),* 31 (1985) 1144.
57. T.N. Shekhovtsova, S.V. Chernetskaya, E.B. Nikol'skaya and I.F. Dolmanova, *Zh. Anal. Khim.*, 49 (1994) 862.
58. T.N. Shekhovtsova, S.V. Chernetskaya, N.V. Belkova and I.F. Dolmanova, *Zh. Anal. Khim.*, 50 (1995) 538.
59. T.N. Shekhovtsova, S.V. Muginova and N.A. Bagirova, *Anal. Chim. Acta,* 344 (1997) 145.
60. L.V. Gudzenko, R.P. Pantaler, L.I. Avramenko and A.B. Blank, *Zh. Anal. Khim.,* 51 (1996) 993.
61. M.K. Beklemishev, Yu.Yu. Petrova and I.F. Dolmanova, *Analyst (Cambridge, U.K.),* 124 (1999) 1523.
62. M.K. Beklemishev, T.A. Stoyan and I.F. Dolmanova, *Analyst (Cambridge, U.K.),* 122 (1997) 1161.
63. K. Chaimaneewong, E. Nakamura and , H. Namiki, *Sci. Rep. Yokohama Nat. Univ.*, Sec. 1, No. 35 (1988) 45.
64. E.I. Morosanova, A.A. Velikorodnyi and Yu.A. Zolotov, *Fresenius' J. Anal. Chem.,* 361 (1998) 305.
65. A.A. Velikorodnyi and E.I. Morosanova, *Zh. Anal. Khim.,* 55 (2000) 808.
66. E.I. Morosanova, A.A. Velikorodnyi, E.A. Reznikova and N.M. Kuz'min, *Zh. Anal. Khim.,* 55 (2000) 423.
67. E.I. Morosanova, E.A. Reznikova, A.A. Velikorodnyi and Yu.A. Zolotov, *Zh. Anal. Khim.*, 56 (2001) 195.
68. A.A. Velikorodnyi, E.I. Morosanova and Yu.A. Zolotov, *Zh. Anal. Khim.,* 55 (2000) 1105.
69. E.I. Morosanova, A.A. Velikorodnyi, O.V. Myshlyakova and Yu.A. Zolotov, *Zh. Anal. Khim.*, 56 (2001) 320.
70. D.Yu. Marchenko, E.I. Morosanova, N.M. Kuz'min and Yu.A. Zolotov, *Zh. Anal. Khim.*, 54 (1999) 455.
71. O.A. Zaporozhets, N.I. Petruniock and V.V. Sukhan, *Talanta,* 50 (1999) 865.
72. O.A. Zaporozhets, N.I. Petruniock, O. Bessarabova and V.V. Sukhan, *Talanta,* 49 (1999) 899.
73. V.M. Ivanov and O.V. Kuznetsova, *Zh. Anal. Khim.*, 50 (1995) 498.
74. S.A. Morozko and V.M. Ivanov, *Zh. Anal. Khim.*, 50 (1995) 629.
75. I. Kobayasi and H. Takeno, *Kogyo Josui,* No. 85, (1965) 36.
76. J.M. Figg, *Concrete (London),* No. 9 (1975) 38.
77. V.E. Headley, *Feedstuffs,* No. 8 (1967) 60.
78. T.I. Headrick and S.H. Schanderl, *J. Dairy Sci.*, No. 1, 49 (1966) 114.
79. J.M. Sloan, G. Beevers and F.E. Baxter, *Clin. Chem. (Winston-Salem, N.C.),* 30 (1984) 1705.
80. J. Gleisberg, *Ger. Pat.* 2620923, 1977.

81. J. Gleisberg, *Ger. Pat.* 2720073, 1979.
82. K. Habenstein, H. Kohl and A. Walch, *Ger. Pat.* 3043608, 1982.
83. A. Chiba and T. Ogawa, *Denki Kagaku oyobi Kogyo Butsuri Kagaku,* 56 (1988) 630.
84. F. Goshima and I. Ichihara, *Bunseki Kagaku,* 30 (1981) 796.
85. I. Kuselman, B.I. Kuyavskaya and O. Lev, *Anal. Chim. Acta,* 256 (1992) 65.
86. I. Kuselman and O. Lev, *Talanta,* 40 (1993) 749.
87. Yu.A. Zolotov, *Ann. Chim.(Rome),* 87 (1997) 284.
88. I.M. Maksimova, A.A. Kukhto, E.I. Morosanova, N.M. Kuz'min and Yu.A. Zolotov, *Zh. Anal. Khim.*, 49 (1994) 628.
89. I.M. Maksimova, A.A. Kukhto, E.I. Morosanova, N.M. Kuz'min and Yu.A. Zolotov, *Zh. Anal. Khim.*, 49 (1994) 1090.
90. D.Yu. Marchenko, E.I. Morosanova, N.M. Kuz'min and Yu.A. Zolotov, *Zh. Anal. Khim.*, 52 (1997) 1162.
91. D.Yu. Marchenko, I.A. Morozkin, E.I. Morosanova, N.M. Kuz'min and Yu.A. Zolotov, *Zh. Anal. Khim.*, 52 (1997) 1167.
92. E.I. Morosanova, N.M. Kuz'min and Yu.A. Zolotov, *Fresenius' J. Anal. Chem.* 357 (1997) 853.
93. V.R. Andreev, N.S. Pliss, E.I. Morosanova and Yu.A. Zolotov, *Nauch. Priborostroenie,* No. 3, 9 (1999) 116.
94. E.I. Morosanova, A.A. Velikorodnyi, I.V. Nikulin, E.A. Puganova and Yu.A. Zolotov, *Zh. Anal. Khim.*, 55 (2000) 486.
95. Zh.M. Azarova, E.I. Morosanova and Yu.A. Zolotov, *Zh. Anal. Khim.*, 55 (2000) 714.
96. A. G. Hamza, A.B. Farag and A. Al-Herthani, *Microchem. J.* 32 (1985) 13.
97. A.G. Shirinova and V.M. Ivanov, *Zh. Anal. Khim.*, 49 (1994) 266.
98. A.G. Shirinova and V.M. Ivanov, *RF Pat.* 2038579, *Byull. Izobret.* No. 18 (1995).
99. S.A. Morozko and V.M. Ivanov, *Zh. Anal. Khim.*, 50 (1995) 629.
100. V.M. Ivanov, S.A. Morozko and S.V. Kachin, *Zh. Anal. Khim.*, 49 (1994) 857.
101. V.M. Ivanov, S.A. Morozko and Yu.A. Zolotov, *Zh. Anal. Khim.*, 48 (1993) 1389.
102. V.M. Ivanov and S. Massud, *Vestn. Mosk. Univ., Ser. 2: Khim.*, 34 (1993) 572.
103. V.M. Ivanov and S. Massud, *Vestn. Mosk. Univ., Ser. 2: Khim.*, 35 (1994) 350.
104. A.G. Shirinova and V.M. Ivanov, *Zh. Anal. Khim.*, 49 (1994) 268.
105. A.G. Shirinova and V.M. Ivanov, *Vestn. Mosk. Univ., Ser. 2: Khim.*, 33 (1992) 63.
106. V.M. Ivanov and N.V. Erokhina, *Vestn. Mosk. Univ., Ser. 2: Khim.*, 35 (1994) 269.

107. S.G. Dmitrienko, Yu.A. Zolotov, O.A. Kosyreva, V.K. Runov and E.S. Frolova, *RF Pat.* 1803837, *Byull. Izobret.* No. 11 (1993).
108. S.G. Dmitrienko, Yu.A. Zolotov, O.A. Kosyreva, V.K. Runov and E.S. Frolova, *RF Pat.* 1803838, *Byull. Izobret.* No. 11 (1993).
109. S.G. Dmitrienko, Yu.A. Zolotov, O.A. Kosyreva, V.K. Runov and E.S. Frolova, *RF Pat.* 1803839, *Byull. Izobret.* No. 11 (1993).
110. S.G. Dmitrienko, O.A. Kosyreva, V.K. Runov and Yu.A. Zolotov, *Mendeleev Commun.*, No. 2 (1991) 75.
111. S.G. Dmitrienko, L.N. Pyatkova, L.P. Bakhaeva, V.K. Runov and Yu.A. Zolotov, *Zh. Anal. Khim.*, 51 (1996) 493.
112. S.G. Dmitrienko, L.N. Pyatkova and V.K. Runov, *Zh. Anal. Khim.*, 51 (1996) 600.
113. S.G. Dmitrienko, E.N. Myshak, V.K. Runov and Yu.A. Zolotov, *Chem. Anal. (Warsaw),* 40 (1995) 291.
114. I.A. Veselova and T.N. Shekhovtsova, *Anal. Chim. Acta,* 392 (1999) 151.
115. T. Braun and A.B. Farag, *Anal. Chim. Acta,* 73 (1974) 301.
116. A.B. Farag, A.M. El-Wakil and M.S. El-Shahawi, *Analyst (Cambridge, U.K.),* 106 (1981) 809.
117. T. Braun, J.D. Navratil and A.B. Farag, *Polyurethane Foam Sorbents in Separation Science and Technology*, CRC Press, Boca Raton, FL, 1985.
118. A.B. Farag, M.A. Morsi, S.A. Ibrahim, *Indian J. Chem., Sect. A: Inorg., Bio-inorg., Phys., Theor. Anal. Chem.*, 25A (1986) 882.
119. A.B. Farag, A.M. El-Wakil and M.S. El-Shahawi, *Ann. Chim. (Rome),* 72 (1982) 103.
120. A.B. Farag, A.M. El-Wakil and M.S. El-Shahawi, *Talanta,* 29 (1982) 789.
121. A.B. Farag, M.E. Hassouna and M.H. Abdel-Rahman, *Bull. Soc. Chim. Belg.*, 96 (1987) 147.
122. T. Tanaka and K.Z. Hiiro, *Anal. Chem.*, 275 (1975) 15.
123. H. Katsuyama, *US Pat.* 5186894, 1993.
124. R. Lucas, *Austr. Pat.* 360961, 1981.
125. I. Jaunakais, *US Pat.* 5620658, 1997.
126. W. A. Bullard and D.B. Stahlman, *US Pat.* 3963442, 1976.
127. W. Hennige and H.D. Pflug, *Ger. Pat.* 1243422, 1967.
128. O.P. Shvoeva, V.P. Dedkova, A.G. Gitlits and S.B. Savvin, *Zh. Anal. Khim.*, 52 (1997) 89.
129. V.P. Dedkova, O.P. Shvoeva and S.B. Savvin, *Zavod. Lab.*, No. 3, 64 (1998) 11.
130. V.P. Dedkova, O.P. Shvoeva and S.B. Savvin, *Zavod. Lab.*, No. 12, 64 (1998) 7.
131. V.I. Sorokin, A.V. Gaevskii, E.V. Degtyarev and S.K. Volkov, *Applications of Rapid Tests in Examinations of Narcotics and Strong Medicines, the Expert Forensic Centre of the Ministry of Internal Affairs of the Russian Federation*, 1996 (in Russian).

132. *Aquamerck®. Aquaquant®. Microquant®. Ferdgtests fur die Mobile Analytik*, E. Merck, Darmstadt, 1993.
133. *Rapid Tests*, Macherey-Nagel GmbH & Co. KG, 2000.
134. *US Pat.* 3634038.
135. *US Pat.* 4332769.
136. *US Pat.* 4537747.
137. *US Pat.* 4569780.
138. H. Curme, R.L. Columbus, G.M. Dappen et al., *Clin. Chem. (Winston-Salem, N.C.)*, 24 (1978) 1335.
139. R.W. Spayd, B. Bruschi, B.A. Burdick. et al., *Clin. Chem. (Winston-Salem, N.C.)*, 24 (1978) 1343.
140. A. Ohkubo, S. Kamei, M. Yamanaka, F. Arai, M. Kitajima and A. Kondo, *Clin. Chem. (Winston-Salem, N.C.)*, 27 (1981) 1287.
141. V.N. Aleksandrov and V.I. Emel'yanov, *Poison Gases*, Moscow, 1990 (in Russian).
142. J. Stone Marcia, *US Pat.* 5039618, 1991.

Chapter 4

Systems of Measurements and Registration

4.1 REQUIREMENTS FOR METHODS OF ANALYSIS OF LIQUIDS

The most general requirements the test methods are expected to meet are ease and speed of operation, small size and low weight of device, and autonomous power supply (e.g. from batteries). For a long time visual techniques of detection and measurement were used in test methods; in the main, these were visual evaluation of colour intensity, or the measurement of the length of the coloured zone on a test means. The next step was made when simple devices came into use for comparing and assessing colour, such as comparators and colorimeters. In recent times due to the advances of microelectronics, physics and technology, the possibility has emerged to build portable pocket-size instruments, among them photometers, reflectometers, luminometers, turbidimeters. Recently, measurement of colour and colorimetric methodology has become accepted practice. In addition, simple titrimetric techniques continue to be used.

4.2 VISUAL METHODS

Visual approaches in test methods remain to this day the main ones due to their simplicity and low cost. A widely known approach is a visual observation of colour change produced by reaction of a test means with the target component. Examples include: pH determination using indicator test strips, coloration of a test tube by alcohol vapour if present in the driver's breath or measuring the length of the coloured zone in a Dräger test tube, used for detecting chloroform in the air of chemical plants. These examples show that visual methods are employed both for revealing substances (e.g. alcohol) and for semi-quantitative, and sometimes quantitative, substance detection. For the latter calibration is needed; thus, colour scales are used or length scales directly printed on indicator tubes.

Visual methods include the so-called drop analysis, especially if performed not in the usual laboratory format.

The accuracy of visual methods is not in most cases very high, usually being on the borderline between "yes" and "no", and precision of quantitative determination is no better than 10%, yet all this does not vitiate the usefulness of the visual methods.

4.3 COLORIMETRIC VISUAL METHODS

In principle these are also visual methods but in this case the human eye is assisted by an instrument, viz., a comparator or colorimeter. The usual procedure here is colour matching.

Such an approach is realized in Merck systems, e.g. Aquamerck, in its colorimetric variant. One or more reagents giving a colour reaction with the target component are added to the tested liquid sample placed in a special vessel. A colour develops, the intensity of which is proportional to the component's concentration. The colour is compared with the colour scale on the water-resistant plastic card. Colours of different hue and lightness are printed on the card with corresponding concentration values. The best match between the colours is found ny moving the vessel along the colour scale. By using blanks the sliding comparator developed for the purpose automatically takes the intrinsic coloration of the sample into account. In addition to colour cards, there are test vessels with integrated fields for colour comparison.

Colorimetric procedures in the Aquamerck system have been elaborated for the determination of residual hardness of water, pH, ammonia, chlorine, formaldehyde, iron, magnesium, nitrate, nitrite, and phosphate.

The firm Palintest (UK) produces test systems including a comparator with discs, square cross section cells and reagents in tablet form packed like medicines in blisters (Fig. 4.1). The test system can be used by anyone: just put the water to be analysed in the cell, add the tablet reagent to the test sample, place in the comparator and match against the appropriate disc. Colour discs are available for a wide range

Fig. 4.1. System of discs with a comparator from Palintest company.

of water test parameters. Each disc is intended for a definite component — ammonia, nitrate, etc. Discs are produced by the technology enabling the colour to be printed from a palette of over two million different shades, using colourfast inks and light-resistant materials. There should be no loss of colour integrity of the standard with time.

4.4 USE OF MORE SOPHISTICATED BUT STILL COMPACT INSTRUMENTS

At present there is a variety of easy-to-handle analytical instruments of the pocket type with an autonomous power supply. They can be tentatively subdivided in two groups.

Instruments of the first group are more or less universal, and they are employed in combination with ready-to-use test kits. That is, such instruments are part of the system, the internal one (the instrument proper), and the external one (the test means). In the context of the present book, instruments of this type will be the focus of our attention.

The second and probably larger group includes portable instruments in which the concrete analytical protocol is built into the instrument as its software. For these no peripheral chemistry is needed. They are analyzers of specialized application. They are also very simple and easy to use, sometimes even simpler than those of the first group. Several of these will be reviewed later.

The second group of instruments will become more and more universal. Already there are pocket-size analyzers capable of determining several gases, being provided with appropriate sensors. In principle, instruments in the second group have more chance of becoming widely produced and used.

As time goes on the very notion of "test method" will undergo change and will ultimately mean a simple determination using a simple device by unqualified personnel independent of whether there is or there is not the peripheral chemistry; or, to be more exact, if the "chemistry" remains, it will preferably be in the instrument proper. The dividing line between test-means, mini- as well as micro-analyzers will become thinner and thinner. As concerns micro analyzers, as those in the form of microchips, they will be both either special one-purpose or multipurpose measuring devices, used in chromatography, capillary electrophoresis, flow injection analysis and the like.

Let us now consider some concrete examples. As was mentioned above, portable (pocket size) easy-to-use photometers have been constructed. They are usually sold equipped with test means proper, i.e., test strips, tablets, ampoules, etc. The protocol and calibration data are inside the instrument and need not concern the user, his task being only to place the ampoule with the reacted reagents in the instrument and read the analysis results on the instrument's display screen.

A photometer model 5000 from the firm Palintest (UK) is used with test kits in tablet form. The system "tablet-photometer" allows determination of a number of components in potable, sewage and other waters. The Palintest kits allow determination of about 40 components. Any combination of tests out of the whole set can be supplied with the instrument, that is, any tablets with indicator reagents. The instrument weighs 500 g, measures 170 × 130 × 53 mm, and can be used both in laboratory and in the field. The test procedure simply involves dissolving a reagent tablet in a sample, inserting the sample test tube in the photometer, pressing a button and noting the digital readout. Comparing the readout to a chart reveals the level of the parameter being tested.

Palintest Co. markets a pocket Palintest Micro 1000 Colorimeter equipped with sample tubes for determining residual chlorine in potable water. Just place the tube in the meter and press the ON key. The instrument has automatic blank recognition and zero setting.

Noteworthy are test kits marketed by Tintometer GmbH (Dortmund). For every given component there is a pocket-type photometer (Sovibona® Single-Parameter PCcheckit®). Chemical reactions are performed using either a tablet in aluminum foil or a small test tube with more or less common reagents included in the test kit. The photometers have automatic zero setting, digital readout and allow very quick measurements (3 s); the instrument is automatically switched off 5 min after a measurement. Test methods for a large number of components are listed in Table 4.1. Of special interest is COD (Chemical Oxygen Demand). There are also tests for total alkalinity, calcium hardness of water, hydrazine, hydrogen peroxide, urea, pH, and turbidity.

Riedel-de Haën AG manufactures test kits for water analysis AQUANAL®-Fischwater Lab. Using test kits it is possible to measure water impurities directly at the water with quick results. Test kits include tools for the determination of ammonium, nitrate, nitrite, carbonate, phosphate, iron, oxygen, pH, and total hardness. Newer versions allow determination also of chloride, sulphate, sulphide, cyanide, as well as magnesium, chromium, copper, nickel, phenol. Actually, the kits are a portable laboratory also including a simple pocket photometer used with test tubes.

The British Professional Test Systems Co. produces and sells a portable test kit for on-site testing of drinking water according to international standards. The test kit includes an incubator and a membrane filtration device for bacteria analysis, a photometer for chemical analysis and meters for pH, conductivity, turbidity and temperature measurement, the whole placed in a hand-carried case.

ENV-1A test kit includes a chemical analysis module (electronic photometer with starter/refill packs for chlorine, ammonia, nitrate and nitrite) and hand-held meters for pH, conductivity, temperature and turbidity measurement.

ENV-1C and ENV-1D is similar to ENV-1A but with simple visual comparison test methods for chlorine, ammonia, nitrate and nitrite, simple pocket meters for pH, conductivity and temperature and a simple visual comparison tube for turbidity measurements. The company sells simple instruments separately, viz.:

- pocket pH meter Model pH-1, range 0–14 pH, resolution 0.1 pH;
- pocket pH meter Model pH-2, range 0–14, resolution 0.01 pH;
- conductivity meter Model TDS-3, range 10–1990 μS;
- conductivity meter Model TDS-4, range 100–19,9 mS;
- turbidity tube for visual analysis, range 5–500 JTV;
- chemical analysis modules and accessories (Table 4.2).

TABLE 4.1

PCcheckit® test methods

Test	λ (nm)	Range	Method
Alkalinity M (total)	605	52–200 mg/l	ALKA-M-PHOTOMETER
Alkalinity P	528	5–500 mg/l	ALKA-P-PHOTOMETER
Aluminium	528	0.05–0.3 mg/l	ALUMINIUM No.1/No.2
Ammonia	660	0.02–1; 0.2–10 mg/l*	AMMONIA No.1/No.2
Bromine	528	0.25–13 mg/l	DPD No.1
Calcium hardness	528	50–500 mg/l	CALCHECK
Chloride	528	0.5–25; 5–250 mg/l*	CHLORIDE T1/T2
Chlorine DPD(1)	528	0.05–6 mg/l	DPD No.1/No.3/No.4
Chlorine DPD(2)	528	0.05–6 mg/l	DPD Liquid Reagents
Chlorine HR	470	5–200 mg/l	CHLORINE HR (KI) ACIDIFYING GP
COD(3)	430	15–150 mg/l	Supplied without vial tests and reactor
	605	100–1000 mg/l	
	605	1000–10000 mg/l	
COD	430	15–150 mg/l	Supplied with vial tests (3 ranges) and reactor
	605	100–1000 mg/l	
	605	1000–10000 mg/l	
Copper	528	0.05–5 mg/l	COPPER No.1/No.2
Copper/zinc	580	0.02–1 mg/l	COPPER/ZINC LR/EDTA
Hydrazine	470	0.05–0.5 mg/l	HYDRAZINE TEST POWDER(5)
Hydrogen peroxide	528	0.03–3; 0.3–30 mg/l	HYDROGENEPEROXIDE LR
Iron	528	0.02–1; 0.2–10 mg/l*	IRON LR
Nitrite LR	528	0.02–0.5 mg/l	NITRITE LR
Nitrite HR	528	10–1500 mg/l	NITRITE HR No.1/No.2
Ozone	605	0.02–1 mg/l	OZONE
Phosphate LR	660	0.05–4 mg/l	PHOSPHATE LR No.1/No.2
Phosphate HR	470	10–100 mg/l	PHOSPHATE HR P1/P2
pH value	528	6.5–8.4	PHENOLRED-PHOTOMETER
Silica	580	0.05–4 mg/l	SILICA No.1/No.2/PR

continued

Test	λ (nm)	Range	Method
Sodium sulphite	528	5–100 mg/l	SULFITE P
Sulphate	528	5–200 mg/l	SULFATE T
Swimming pool analysis⁽⁶⁾			
Total hardness	528	50–500 mg/l	HARDCHECK
Turbidity⁽⁷⁾	875	0.1–2000 mg/l	NTU (FNU,TE/F)
Urea		0.1–3; 0.2–6 mg/l	AMMONIA No.1/No.2
Zinc (see Copper)	580	0.02–1 mg/l	COPPER/ZINC LR/EDTA

* High range by dilution.
(1) Unit supplied with reagent tablets.
(2) Unit supplied with liquid reagents.
(3) PCcheckit basic unit COD.
(4) Reagent 2 is not included in the Test Kit and must be ordered separately.
(5) The sample must be filtered. Accessories for filtration are not part of the Test Kit.
(6) Swimming pool analysis: see leaflet L 351.
(7) Turbidity: see leaflet L 860.

Special-purpose pocket colorimeters for water analysis are manufactured by Hach Co. Among them are instruments for determination of fluoride, nitrate, chlorine, bromine, and dissolved oxygen. Results are expressed in mg/l, and no standard samples or graduation data are needed. On sale are spectrophotometers and turbidimeters for rapid, but sufficiently accurate testing of samples in the laboratory and in the field. Among them are, for example, portable photometers DR-800, DR-820, DR-850, DR-870 and others, suitable for use in the field in conjunction with packed reagents supplied by the same firm. The tests are programmed and are selected by pushing a button. Results are displayed in concentration units, absorption or transmission. Analysis results are stored in the instrument's memory (up to 50 tests). Light emitting diodes (LEDs) with precision of wavelength setting ±1 nm are used, and absorption is measured in the range 0-1 A with a precision of 0.005 A. The instrument weighs 450 g, measures 23.8 × 8.7 × 4.7 cm. Model DR-890 allows determination of 90 water parameters; some examples are given in Table 4.3.

An original simple instrument for colorimetric tests was presented in a recent Brazilian paper [1]. It includes a low cost serial multimeter and a device for light absorption (an optical sensor).

Among portable photometers one may mention those of LASA (Dr. Lange, Germany). LASA1 is a filter photometer for various tests with

TABLE 4.2

Chemical analysis modules and accessories supplied by Professional Test Systems Co.

Modules, accessories	Features
Chemical Analysis Module	Chemical analysis module (consisting of electronic photometer with starter packs for chlorine, ammonia, nitrate and nitrite)
Chlorine Starter/Refill Pack	Free, total and combined. Range 0–5 mg/l (Cl) 50 tests
Chlorine Starter/Refill Pack	Free, total and combined. Range 0–5 mg/l (Cl) 250 tests
Ammonia Starter/Refill Pack	Range 0–1 mg/l (N), 0–1.3 mg/l (NH_4), 50 tests
Ammonia Starter/Refill Pack	Range 0–1 mg/l (N), 0–1.3 mg/l (NH_4), 250 tests
Nitrate Starter/Refill Pack	Range 0–1 mg/l (N)/0–4.4 mg/l (NO_3), 50 tests
Nitrate Starter/Refill Pack	Range 0–1 mg/l (N)/0–4.4 mg/l (NO_3), 250 tests
Nitrite Starter/Refill Pack	Range 0–0.5 mg/l (N)/0–5 mg/l (NO_2), 50 tests
Nitrite Starter/Refill Pack	Range 0–0.5 mg/l (N)/0–5 mg/l (NO_2), 250 tests
Spare Test Tubes	Pack of 5 with caps
Test Tube Rack	Holds up to 8 tubes
Photometer Accessories Pack	Comprises stirring rods × 10, test tubes and caps × 5, tube cleaning brush × 1, batteries × 1 set
Chlorine Pocket Kit	Simple, self-contained visual colorimetric test kit with reagents for 50 tests. Range 0–2 mg/l Cl_2
Chlorine Refill Pack	250 tests for above
Ammonia Pocket Kit	Simple, self-contained visual colorimetric test kit with reagents for 50 tests. Range 0–1.6 mg/l (N)/0–2 mg/l (NH_4)
Ammonia Refill Pack	250 tests for above
Nitrate Pocket Kit	Simple, self-contained visual colorimetric test kit with reagents for 50 tests. Range 0–17 mg/l (N)/0–75 mg/l (NO_3)
Nitrate Refill Pack	250 tests for above
Nitrite Pocket Kit	Simple, self-contained visual colorimetric test kit with reagents for 50 tests. Range 0–0.6 mg/l (N)/0.2 mg/l (NO_2)
Nitrite Refill Pack	250 tests for above

solutions (analysis of water, sewage, etc.). It has nine interchangeable filters. LASA-10 and LASA-20 are photometers with a sensor array design, error recognition for clouding or discoloration of the sample by simultaneous wavelength measurement, reference beam method,

TABLE 4.3

Examples of water parameters tests using Hach photocolorimeter DR-890

Water parameter	Analytical range (mg/l)
Alkalinity ($CaCO_3$ form)	10–4000
Hardness (carbonate)	10–4000
Dissolved oxygen	0–15
Aluminium	0–0.8
Nitrogen (monochloramine and free ammonia, elemental nitrogen form)	0–0.5
Iron total	0–3.0
Manganese	0–0.7
Copper	0–210
Nitrates	0–30
Nitrites (nitrogen form)	0–0.35
Sulphate	0–70
Sulphide	0–0.7
Phosphate	0–0.25
Fluoride	0–2.0
Chlorine active	0–5.0
Chlorine total	0–5.0

automatic zero calibration. Wave lengths: 240, 440, 500 and 600 nm. Suitable for measurement in round cuvettes, power supply is from NiCd batteries, weight 0.5 kg. Instruments are pre-programmed for many cuvette tests.

Suitable for work with colour charts is Color Pen (Fig. 4.2), produced by the same firm (Dr. Lange) — the simplest and lightest differential colorimeter. With one battery it is possible to perform several thousand tests of colour in colour units in conformity with basic international standards. Switch on Color Pen, set in place, read off the result, store and/or print.

The Hydrocheck water testing system from WPA (UK) allows rapid on-site analysis by non-laboratory staff using 40 test kits. COD, ammonia and chlorine can be determined quickly and easily.

Thomas Scientific Co. produces photometers for analysis of water, sewage, soil and air, including cheap ones for use in the field. PCcheckit

Fig. 4.2. Color Pen (Dr. Lande).

photometer from Tintometer (Germany) is intended for measuring COD, which is used as a measure of the oxygen equivalent of organic matter content that is susceptible to oxidation by a strong chemical oxidant (dichromate). The instrument is used in combination with cuvette tests. It is possible to determine 15-10000 mg/l COD.

A number of instruments use reflectometry instead of transmission spectroscopy; for the rest, these are identical instruments. Reflectometers are employed with opaque samples, such as indicator test strips. There are also instruments capable of working both in reflectance and transmission modes.

Merck Co. markets reflectometers Reflectoquant and DTECHTOK, the latter mainly for use with immunochemical diagnostic kits DTECH (Figs. 4.3 and 4.4). The instrument is easy to use, is battery powered, small in size (17 cm in length), and weighs 170 g; the light source produces a spark which illuminates simultaneously the sample studied and the reference sample. The difference of reflected fluxes is found and the result given in relative reflection coefficients (percents). The memory stores data of 127 tests (date, time, test number, results).

The Moscow-based firm Kostip produces a portable photometer Multiecotest [2] with red and green LEDs and a sensitive photoreceptor for work with indicator test strips and polyurethane tablets. The instrument measures the reflection from a colour spot on a test strip, processes data according to a given algorithm and shows the results as

Fig. 4.3. DTECHTOK reflectometer (Merck).

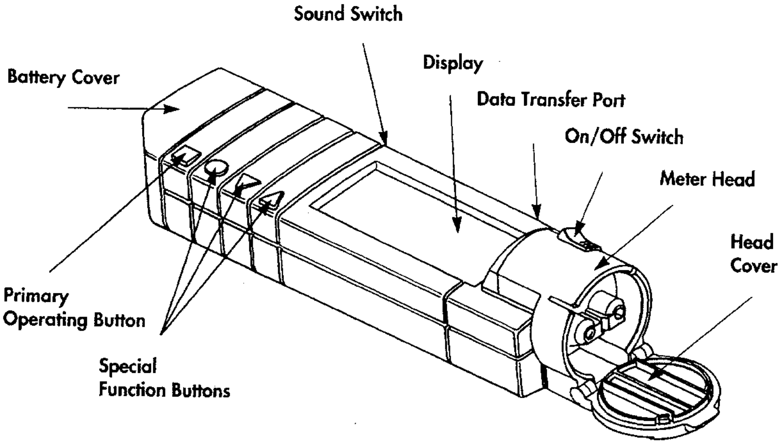


Fig. 4.4. The scheme of a DTECHTOK reflectometer.

concentration (mg/ml) on the display screen. A test strip 10 mm wide and 0.2–0.5 mm thick with a water-proof reagent is used. The instrument includes a photometric unit, a processing and displaying module and a voltage source. The photometric unit is a box with a slip lid under which is a slot for exit of light falling on the strip. It contains light sources (LEDs), light receptors (photodiodes) and an amplifier for raising the signal to a level sufficient for processing by the analog/digital board. The instrument cannot work with liquid or gaseous samples.

In our laboratory a different colorimeter–reflectometer was constructed, suited for work with coloured and opaque solutions, coloured and grey surfaces, acid or basic gases [3]. It includes two optrons (light source + photoreceptor) differentially connected, thus forming two optical channels — one for the sample studied and the other for the reference sample. Either of the channels can be used for measurement in transmission or reflectance modes. The instrument is supplied with different attachments allowing work with samples of any nature or form, including liquids, solids or gases [4,5].

The American firm ESECO-Speedmaster produces small-size handy reflectometers called PocketPal.

Among instruments for luminometry one should note luminometer ToxAlert® marketed by Merck Co. It is used for determining toxicity of purified and raw sewage waters, and of lake and river waters.

Among turbidimeters suitable for tests in the field one may mention 2100P Portable Tubidimeter (microprocessor based) with an optical part measuring the difference signal of two detectors. Different samples can be analysed, including coloured solutions. Yet another turbidimeter — Nephla — was developed by Dr. Lange Co. It weighs 1.5 kg, supply connections battery (8 × mignon); it is intended for use in the laboratory or in the field in conformity with the German standard DIN 38404.

4.5 MEASUREMENT OF COLOUR

As stated above, many test methods proceed from visual observation of colour changes of chromogenic reagents which are matched against certain colour scales. Yet the performance of the human eye is limited, leaving aside the subjective (human) factor. This explains difficulties met when trying to work out objective metrological criteria for the visual test methods. The way out of this situation may be found not only by using the instruments described above, but also by adopting another methodology, namely that of colour measurement.

This is a well-developed area, especially for the dyeing or printing (and allied) trades. Goethe, Ostwald, Maxwell and many other illustrious scientists contributed much to this branch of science. The application of colour measurement methods to test methods was pioneered by V.M. Ivanov, S.A. Morozko and others [6–8]. They showed, for example, the possibility of optimising the content of chromogenic reagents in test-forms (on substrates).

Colour measurement science deals in its own basic concepts, such as vector colour space, colour coordinates, and such basic colour characteristics as hue, saturation, and lightness.

4.6 TITRIMETRIC METHODS

A standard set for titrimetry — a burette, a pipette, a flask — are not ideal for use with test methods. Yet more convenient tools and original approaches have been developed for titration work in the field. It is possible to just count drops or work with a plastic cylinder.

Aquamerk system from Merck includes titrating pipettes, a precision dropper and droplet bottles. The principle of the procedure involves addition of a reagent to the sample reacting with the substance to be determined. The end of the reaction is signalled by a sudden change of the colour on an indicator. The amount of the substance can be deduced from the quantity of the reagent added. When using the droplet bottle or precision dropper, the number of added drops are counted until a change in colour occurs. Thus each drop corresponds to a definite amount of the component to be determined. When using titrating pipettes the quantity of reagent added can be read directly from the scale on the pipette as the concentration of the determined substance.

Titrimetric test methods of the Aquamerck system were developed for determining acidity and alkalinity, carbonate hardness, total hardness, and oxygen according to the Winkler method, chloride, calcium and other water parameters.

The Titrets system of CHEMetrics Co. is based on a somewhat different device (Fig. 4.5). The procedure employed is:

- Step 1: Lift the control bar and insert the assembled Titret cell into the body of the Titrettor device.
- Step 2: With the sample pipe of the Titret cell in the sample, press the control bar firmly but briefly to allow entry of a small amount of sample into the ampoule. After each addition, rock the entire assembly to mix the contents of the Titret cell. Repeat until a colour change is noticed.

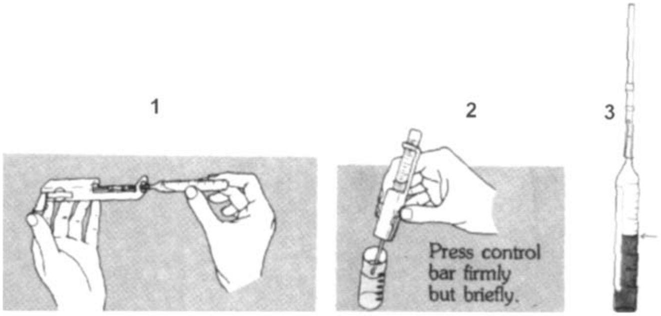


Fig. 4.5. Titration operations in a Titrets system (CHEMetrics).

- Step 3: When a colour change occurs, hold the Titret cell upright and read the ppm concentration from the scale of the side of the ampoule that corresponds to the liquid level.

For titration, V.G. Amelin used pieces of paper impregnated with a titrant [9].

App-Chem (UK) Co. manufactures tests based on simple droppers (Titrimetric drop test kits). The number of drops added until colour change occurs is proportional to the concentration. The average error for these tests is 10%. The test tools are placed in an ABS plastic case. The simplest Macro Drop Single Parameter Test is intended mainly for water supply stations and water distribution systems. There exist also multi-component test sets.

4.7 CHEMICAL DOSIMETERS

This is a special group of instruments designed to accumulate tested substances during a given time interval with simultaneous registration of the amount of collected matter. Their modes of action are different, but more often than not it boils down to detecting the colour change of the indicator reagent on reaction with the tested compound. Another possibility is the change of the length of the absorbing layer in a tube, and other variants. Passive dosimeters are being especially actively developed for determination of gases and vapours in air.

The majority of known dosimeters are personal ones. They are produced on a large scale by such firms as Dräger (Germany), MSA (USA), Khimanalit (Russia). Thus, Dräger Co. produces dosimeters for detection of formaldehyde and ozone, MSA Co. — those for carbon monoxide, mercury vapours and many others.

Two types of chemical dosimeters are known — passive and active ones, the first type being the most widely used. In this case the sorption process is controlled only by diffusion, therefore several hours are needed for sufficient substance accumulation. Their strong points are simplicity of design and operation, low weight and quick measurement proper. We shall now consider those dosimeters that do not require desorption procedures. Some models of such dosimeters have been described [10], including their advantages and shortcomings as compared with other systems of gas detection, and perspective trends in dosimeter control and monitoring of gaseous contamination of work zones [11]. Dosimeters for determining at least 25 volatile organic compounds are known [12,13].

One can judge the concentration of a tested substance by the colour change of the indicator layer, its size and intensity. As a rule dosimeters of this type are used for semi-quantitative gas determination by colour matching against a standard colour scale. For example, dosimeter for amines represents a paper sheet or a layer of Al_2O_3 or SiO_2 impregnated with a water solution of 1,2-naphthoquine-4-sulphoacid and then dried. The layer is covered with a porous membrane with a hole in it. Depending on the pH of the solution used, one can determine the sum total of aliphatic and aromatic amines ($pH > 5$) by a red-brownish coloration of the layer, or only aromatic amines ($pH < 4.5$) by a bright-red coloration. The dosimeter for ammonia consists of a glass tube containing a paper strip impregnated with a chromogenic reagent. Concentration is judged by the length of the coloured zone.

An original approach has been realised in the construction of a dosimeter for formaldehyde. It is a tube with a finely powdered reagent. Formaldehyde diffuses and forms centres of crystallization. After the tube has been treated with over-saturated reagent solution the growth of crystals takes place, the length of the visible zone being proportional to the exposure time and thus the gas concentration.

Dosimeters may have a variety of shapes and forms [14,15]. Most often they are designed as cylinders resembling a pencil that can be carried in the pocket. There are also dosimeters in the form of boxes, plates, and indicator tubes.

Any one construction of passive dosimeters includes an indicator and a diffusion layer. For example, in the simplest case the diffusion layer may represent a set of several monolayers of some porous hydro-

TABLE 4.4

Examples of personal passive chemical dosimeters with visual determination of concentration

Substance to be detected	Parameters	Ref.
H_2S, SO_2, Cl_2	—	16
NH_3	A tube; analytical range 1–200 ppm	17
O_2	Indicator tube; analytical range 0.5–10 ppm	18
HCN	Polystyrene ribbon; 3–5 and 5–150 $mg{\cdot}h/m^3$	19
Formaldehyde	A tube; 0.5–5.0 and 3.0–30 ppm/h	20
Vinyl chloride, ethylene oxide	—	21
Amines	Paper	22

phobic material with a high average pore diameter to the monolayer thickness ratio (to make the rate of sample accumulation diffusion controlled; the 1st Fick's law), the ratio of the length of diffusion channels to their diameter must at least equal 8. The indicator monolayer may be formed of an analogous porous but hydrophilic material containing reagents sensitive to gaseous components, so that determinations can be performed visually on a semi-quantitative basis. The indicator layer may be formed by a solid, e.g., Tenax (for detecting poly aromatic hydrocarbons), activated coal, or a mixture of sorbents.

Examples of known passive dosimeters for various gases are given in Table 4.4.

Man uses up about 20 litres of air per minute. The amount of air passing through the passive dosimeter by diffusion during a work shift is much less than this figure. To get a representative sample probe, the air must be pump-driven through the dosimeter. Therefore active type dosimeters are being developed. A portable battery-fed pump fastened on a dosimeter allows pumping at the rate of 2 litres of air per hour.

4.8 MINI DEVICES WITH BUILT-IN SOFTWARE METHODS

The "philosophy" of simple autonomously powered instruments was outlined above. Strictly speaking, the pocket special-purpose instruments free of "peripheral chemistry" are not the subject of this book. Nevertheless, taking into consideration the fact that no sharp dividing

Fig. 4.6. Pocket pH meters by Milwaukee.

line can be drawn between the two classes of instruments named above and, specifically, noting the tendency towards their integration, it would be appropriate to mention at least some of them.

These mini devices can be placed in two groups: (i) those for water analysis and (ii) those for gas analysis. Their advent has become possible due to the recent advances of microelectronics and other branches of science.

Among the devices for water analysis are numerous mini pH meters. They are all based on potentiometric method of measurement with a glass electrode. Often the same instruments can also measure conductivity. Pocket pH meters proper are marketed by such firms as Milwaukee (Fig. 4.6), Trans Instruments, Eutech Instruments (CyberScan series), Behr Labor–Technik, and others. Somewhat larger instruments with peripheral electrodes similarly oriented are produced by many firms, viz., Horiba, Orion Research, Inc., Hydrolab, WTW, Amel Instruments, Jenway Ltd., Corning, Mylon L. Co., Behr Labor-Technik, and others. Pocket meters are usually sold with two vessels for graduating solutions, precision of pH measurement is about ±0.1, battery life is 3000 h (Milwaukee) or less (80–300 h for meters from Trans Instruments, series Aqua-pal), weight 60 g.

Devices of a similar type have been developed and marketed for determining dissolved oxygen by nearly the same companies, at least by Horiba, Eutech Instruments, Trans Instruments. A meter of the said type is described below; it is produced by Palintest (micro 900 D0 Meter).

Oxygen in the test solution passes through a thin permeable Teflon membrane to the sensor element, which comprises a glass-encapsulated platinum cathode and a silver wire anode. The probe also features a built-in thermistor sensor for temperature measurement and linear temperature compensation. Calibration of the meter takes only a couple of minutes. It has been simplified to a push button operation with the probe placed in saturated air. The large screen simultaneously displays the dissolved oxygen result, in mg/l or percent air saturation, and temperature. The meter is supplied complete with two spare probe membranes and probe electrolyte solution. The instrument comes in a rigid carrying case complete with probe with 4-meter cable length. Optionally, the meter can be supplied with a probe with a 10-meter cable length.

Quite a few mini devices are designed to identify individual gases and vapours. They employ sensors of differing nature and mode of action. Let us give some examples. A number of gases in the work zone can be detected using small size instruments from Dräger Co., Environmental Instruments (Anagas CD 98 for CO_2). Portable Hygrometers also belong here for measuring relative air humidity, e.g., those from Control Company. Many instruments can do without calibration.

At present there are many mini devices allowing determination of not one, but several gases. For example, a personal gas analyzer MicroPac from Dräger Safety Technology allows determination of carbon monoxide, Hydrogen sulphide and oxygen while the meter MiniWarn of the same firm is capable of detecting four different gases. The instruments can work with interchangeable sensors, and thus the number of detected gases is drastically increased. The Single Gas Detection Instrument Pac 111 of the same firm can determine up to 35 gases. Its weight is 185 g and it measures 110 × 66 × 32 mm.

Multi gas monitors are produced by Industrial Scientific Corporation and Gas Tech Inc. In Russia the institute Khimanalit (St. Petersburg) and its associated firms developed a hazardous air components analyzer ANT-2M, capable of detecting several dozen gases (the instrument is calibrated optionally to the order of the client).

In a number of cases the instruments play the role of alarm alerts generating a sound or light signal when the concentration of a given substance reaches a threshold value. In this way operates the meter from Matheson Co. which monitors the content of oxygen in air; the signal is given when the oxygen content falls below 19.5%. H_2S and CO alerts from Quest Technologies and meters ToxiBEE from Lumidor Safety Products operate in a like manner. One may also note the meter GasBadge which is a personal gas alert for potentially hazardous levels of CO, H_2S, as well as O_2.

Metrosonics Co. (USA) developed an extremely simple-to-use single or two-gas monitor, The Patriot. It gives immediate warning about the presence of potentially hazardous concentrations of carbon monoxide and/or hydrogen sulphide. The Patriot's small size and weight make it very convenient for putting into a shirt pocket or attaching to a collar or a lapel.

4.9 TEST CONCENTRATORS INCORPORATING INDICATOR POWDERS

Convenient test-concentrators with indicator powders have been proposed for use in the field conditions (Fig. 4.7). They are tightly stoppered flasks in which a tube has been placed. The solution to be tested is poured into the concentrator up to the mark then the powder is

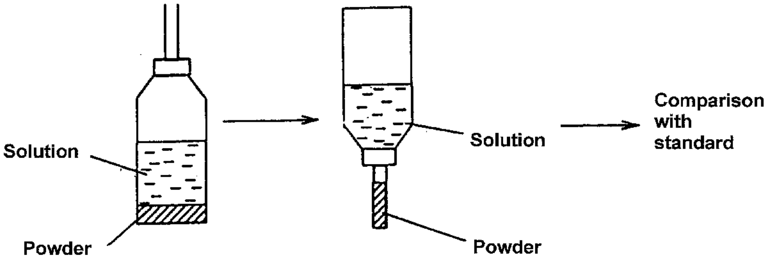


Fig. 4.7. Device for tests using indicator powders.

added and all is mixed (agitated) for some 5-15 minutes. Then the flask is turned upside down, the powder being collected in the tube. Then the content of the target component is determined using the colour of the powder matched against a standard colour scale.

REFERENCES

1. A.V. Rossi, X. He and M. Tubino, *Anal. Lett.*, 33 (2000) 1885.
2. *Instruction Manual: Multiecotest Photocolorimeter*, Kostip Co, Moscow, 1995.
3. Yu.L. Shishkin, *RF Pat.* 2154260.
4. Yu.L. Shishkin, A.V. Zherdev, B.B. Dzantiev and Yu.A. Zolotov, *Prikl. Biokhim. Mikrobiol. (Appl. Biochem. Microbiol.),* 36 (2000) 497.
5. Yu.L. Shishkin, A.V. Zherdev et al., *Application of Field Photometer-Reflectometer 'Ecotest-2020' in Immunoassay Analysis*, Guidelines, Moscow, 2000.
6. V.M. Ivanov and O.V. Kuznetzova, *Usp. Khim.*, 70 (2001) 915.
7. S.A. Morozko and V.M. Ivanov, *Zh. Anal. Khim.*, 51 (1996) 631.
8. V.M. Ivanov and S.A. Morozko, *Zh. Anal. Khim.*, 51 (1996) 1083.
9. V.G. Amelin, *Doctoral Sc. Dissertation*, Moscow State University, Moscow, 1998 (in Russian).
10. Y. Namiesnik et al., *Sci. Total. Environ.*, 38 (1984) 225.
11. V.M. Isaev, I.V. Korablev, V.P. Arsenkin, Nauch.-tekh. Ref. Sb. *Rational, Proposals and Advanced Industrial Expertise*, Automation of Chemical Industries series, NIITEKHIM, No. 12, (1989) 7.
12. A.R. Neuman, *Anal. Chem.,* 63 (1991) 237.
13. P. Lioy, *Environ. Sci. Technol.*, 24 (1990) 938.
14. F.R. Davis, *US Pat.* 4144032.
15. Y. Yanagisawa and M. Hishimura, *US Pat.* 5168068.
16. L. Anders and D.C. Weigel, *US Pat.* 4328181.
17. E.S. McKee and P.W. McConnaughey, *Am. Ind. Hyg. Assoc. J.*, 46 (1985) 407.
18. S.D. Saratova, V.I. Ostoushko et al., *Zavod. Lab.*, 56, No. 3 (1990) 11.
19. H. Schaffernicht, *Z. Gesamte Hyg. Genzgeb.*, 29 (1983) 17.
20. S. Day, *Book of Abstracts, Pittcon 1985*, New Orleans, 1985, p. 923.
21. L.A. Gonzalez and M.V. Sefton, *Am. Ind. Hyg. Assoc. J.*, 46 (1985) 591.
22. A.J. Attar, *US Pat.* 4840919.

Chapter 5

Methodology and Application Areas of Test Systems

5.1 FIELDS OF USE

The main areas where test systems are currently used (or may be used) are the following [1–3]:

- environmental monitoring, determination of the most important state regulated components in water, soil extracts, air (mainly in the field);
- assessing the quality of food, including drinking water and beverages, in the first place the presence of contaminants in them;
- analysis of blood, urine, sweat for medical purposes, at home and other places;
- solving tasks in forensic medicine, law protection agencies and military service (drugs of abuse, explosives, poisons);
- control in industry, transport, e.g., detection of gas leakages;
- teaching chemistry, ecology, etc. in schools and other educational establishments.

Test methods can be used in any analytical laboratory for preliminary testing and sorting out analysed material (see below about sample screening); but more important is their use in out-of-the-lab conditions, for example in environmental monitoring or at home, when blood or urine has to be tested or when the quality of tap water has to be quickly assessed.

Test methods may become indispensable in emergency situations for urgent determination of components in air, water and other objects after explosions, industrial or natural accidents. They are indicated for large-scale inspections of living quarters and industrial premises, e.g. the control of mercury vapours, formaldehyde, phenol and other pollutants.

5.2 ATTESTATION OF METHODS AND THEIR OFFICIAL ENDORSEMENT

Methods officially approved for environmental control or control of food products must be able to reveal, or at least semi-quantitatively determine, target components at levels corresponding to their admissible concentrations. In each country the norms may differ although a number of countries accept EPA norms and regulations. In Europe the

TABLE 5.1

Drinking water parameters

Test parameters	European Council directive 98/83/EC of 3.11.98 on potable water quality for human consumption	Sanitation rules and norms SanPiN 2.1.4.559-96
Colour (deg)	Acceptable for consumption, without anomalies	20
Turbidity (mg/l)	The same	1.5 (by kaolin) 2.6 (by formazin)
Aluminium (mg/l)	0.2	0.5
Odour (level)	Acceptable for consumption, without anomalies	< 2
Chlorides (mg/l)	250.0	350.0
Sulphates (mg/l)	250.0	500.0
Cadmium (mg/l)	0.005	0.001
Chloroform (mg/l)	—	0.2
Formaldehyde (mg/l)	Norm absent	0.3
Oxidizability (mg/l)	5.0	5.0
Chromium (mg/l)	0.05	0.05
Cyanides (mg/l)	0.05	0.035
Arsenic (mg/l)	0.0001	0.05
Iron (mg/l)	0.2	0.3
Lead (mg/l)	0.01	0.03
Copper (mg/l)	0.002	1.0
E. Coli (cl)	0 in 100 ml	0 in 200 ml
Total microbial titer (cl)	Without anomalies	< 50 cells in 1 ml
Coliphages (cl)	Norm absent	0 in 100 ml
Enterococcus (cl)	0 in 100 ml	Norm absent
Clostridium (cl)	0 in 100 ml	0 in 20 ml

norms established by the European Commission (the European Union) are enacted. European and Russian norms for potable water are given as an example in Table 5.1 which lists both chemical and microbiological parameters.

For a relatively long time the test methods were not among those officially approved, e.g., those for environmental or food quality control. But in recent years in the USA, Russia and some other countries they have become officially recognized as methods of wide use. In the USA a number of test methods were approved by the Environment Protection Agency (EPA). As an example, method 8023 can be cited: determination of total chromium using 1,5-diphenylcarbohydrazine and test systems from Hach Co. (Table 5.2).

TABLE 5.2

Sample collection scheme for total chromium determination

	Liquid samples	
Expected conc. chromium (mg/l)	Sample amount (ml)	Analysis volume (ml)
0.05-2.0	40.0	20.0
0.20-8	20.0	10.0
0.75-33	10.0	5.0
7.5-330	5.0	1.0
75-3300	1.0	0.500

	Oil samples	
Expected conc. chromium (mg/kg)	Sample amount (g)	Analysis volume (ml)
8-330	0.25	20.0
20-820	0.25	10.0
50-2200	0.15	5.00
350-16000	0.10	1.00

	Solid samples	
Expected conc. chromium (mg/kg)	Sample amount (g)	Analysis volume (ml)
4.0-165	0.500	20.0
10-410	0.400	10.0
25-1100	0.300	5.00
190-8200	0.200	1.00
750-33000	0.100	0.500

Sample instructions for Hach Kit test procedures (chromium)

1. Select the sample amount from tables below and digest according to procedure in Section II. Note: If sample cannot be analysed shortly after sampling, see section IV for storage and preservation information. Note: This is an EPA-approved method only if preceded by an EPA-approved nitric acid digestion. The Digesdahl digestion procedure is not EPA approved and cannot be used for permit reporting purposes.
2. Use analysis volume in the tables below that corresponds to the sample amount selected in Step 1. Pipette analysis volume into a 25-ml mixing graduated cylinder. If aliquot is more than 0.5 ml, pH adjust according to the last step in the digestion procedure in Section II. Dilute to the 25-ml mark with deionized water, if necessary. Pour contents of cylinder into a 25-ml sample cell. Note: for proof of accuracy, use a 0.25 mg/l chromium standard solution (preparation given in the Accuracy Check) in place of the sample.
3. Fill a second 25-ml sample cell with deionized water to the 25-ml mark (the reagent blank).
4. Add the contents of one Chromium I Reagent Powder Pillow to each cell. Swirl to mix. Place cells in a boiling water bath and wait for 5 minutes. Note: If a precipitate forms while heating, add a second Chromium 1 Reagent Powder Pillow and continue heating.
5. Remove cells from the water bath and cool to 25°C under tap water. If necessary, add deionized water to the 25-ml mark of the sample cell.
6. Add contents of one Chromium 2 Reagent Powder Pillow to each cell. Swirl to mix. Note. Add the contents of a second Chromium 2 Reagent Powder Pillow if a second Chromium I Reagent Powder Pillow was added in Step 5.
7. Add the contents of one Acid Reagent Powder Pillow to each cell. Swirl to mix. Note: Test results will not be affected if a small portion of this reagent does not dissolve. Add contents of a second Acid Reagent Powder Pillow if a second Chromium 1 Reagent Powder Pillow was added in Step 5.
8. Add contents of one ChromaVer 3 Chromium Reagent Powder Pillow to each cell. Swirl to mix Note: A purple colour will develop if chromium is present. The Pour-Tru Cell can be used.

9. Wait 5 minutes for the colour to develop. Note: Do not wait more than 20 minutes before completing Steps 11 to 12.
10. Zero instrument with reagent blank using settings below. Read the mg/l chromium of other cell. Device DR/3000 Program No. 13 Wavelength 540 nm. Device DR/2000 Program No. 100 Wavelength 540 nm. Device DR/700 Module No. 55.01 Wavelength 550 nm. Note: See Section I for information on instrument standardization.
11. Calculate the total chromium concentration of the sample using the following formula:

    total mg/l Cr = $A \times 2500/(B \times C)$

    where A = mg/l read (Step 11), B = ml(g) sample amount (Step 1), C = ml analysis volume (Step 2). Note: For solid and oil samples express the resulting concentration as mg/kg and not as mg/l.

A large number of test methods are described in *Current Protocols in Field Analytical Chemistry*, regularly issued by EPA [4], and other EPA publications, e.g. Refs. [5,6].

In 1999 in Russia the State Committee on Standardization and Metrology introduced a new list of approved methods — a list of chemical analysis test systems. In it are included the first 25 test systems developed and attested in the Moscow State University, some of which are given in Table 5.3.

5.3 SAMPLE SCREENING

There is an ever-growing need for chemical analysis owing to emerging new areas of research and control and to a deepening understanding of the importance of traditional ones. The latter group includes, e.g., monitoring of environment, control of foodstuffs, medicinal preparations, biological fluids. As a result, there is a drastic increase in the number of samples that have to be analysed. To meet this challenge automated analytical methods can be resorted to, especially if only one type of sample with a restricted number of components has to be analysed. In addition, screening methodologies can be employed on a larger scale. They are especially useful when the target component is present not in all of the samples or, if present, then in amounts below the permissible levels. For example, the normative documents

TABLE 5.3

Test systems officially approved for use in the Russian Federation

Substance to be determined	Test tool	Mode of detection	Analytical range (mg/l)
Aluminum	Indicator tube	Coloured zone length	0.27–3.0
Chromium(VI) (EA-Cr-Tr, 13-99)	Indicator tube	Coloured zone length	0.5–50
Manganese(II) (EA-Mn-P, 6-99)	Indicator powder	Visual	0.5–5.0
Iron (EA-Fe-Tr, 14-99)	Indicator tube	Coloured zone length	0.05–50
Cobalt (EA-Co-Tr, 15-99)	Indicator tube	Coloured zone length	0.06–6.0
Nickel	Indicator tube	Coloured zone length	0.2–30
Copper (EA-Cu-Tr, 10-99)	Indicator tube	Coloured zone length	0.1–65
Cadmium (EA-Cd-Tr, 11-99) (EA-Cd-P, 5-99)	Indicator tube Indicator powder	Coloured zone length Visual	0.3–3.0 0.001–0.01
Tin(II) (EA-Sn-Tr, 12-99)	Indicator tube	Coloured zone length	10–200
Lead	Indicator powder	Solid phase spectroscopy (SPS)	0.01–1.0
Fluoride	Indicator powder	Visual	1.0–10
Chloride	Indicator tube Indicator powder	Coloured zone length Visual	20–200 3.0–20
Nitrate (EA-NO_3-RTr, 1-99)	Indicator tube	Coloured zone length	10–80
Nitrite (EA-NO_2-RTr, 2-99)	Indicator tube	Coloured zone length	0.5–20
Sulphur-containing reductants (EA-Sred-RTr, 3-99)	Indicator tube	Coloured zone length	3.0–330
Active chlorine (EA-Cl_2-RTr, 4-99)	Indicator tube	Coloured zone length	0.5–3.0
Hydrazines (EA-Hydrazine-P, 7-99)	Indicator powder	Visual	0.3–3.0
Aniline (EA-Aniline-P, 8-99)	Indicator powder	Visual	0.05–10
Phenols (with sorption enrichment)	Indicator powder	Visual, SPS	0.5–5 0.05–0.5 0.01–5 0.001–0.01

stipulate that foods should be tested for lead, cadmium and mercury, but these heavy metals are seldom found in foods in concentrations harmful for consumers, viz., their actual contents are lower than permissible ones. From this follows that samples should be first sorted out (screened) by some simple analytical means and only those in which the target component has been found in tangible amounts should be subjected to detailed analysis.

Screening essentially boils down to the following [7]. The preliminary inspection should preferably be inexpensive, simple, requiring no skilled personnel and, in principal, amenable to large-scale performance wherein a great number of samples can be measured. Two kinds of results are thus obtained — positive and negative. The negative result is considered to be final and is discarded. The positive samples are further studied using more sophisticated methods whose results may be, if necessary, used in arbitration. As an example one may cite the inspection of drivers for the presence of alcohol in their breath, which is performed by the traffic policeman using an indicator tube. If the result is negative the driver is released. The positive result, though justifying the withdrawal of a driving license, will have no validity in the court if not corroborated by blood analysis in stationary conditions accompanied by a pertinent medical document.

The fact that the negative result is viewed as accurate and final makes one think of raising the reliability of test means employed for screening. Simplicity and low cost of testing may be very good but it will serve no useful purpose if a positive sample is thereby tested as negative.

Screening can be performed in a common analytical laboratory but it would be more appropriate if carried out on-site, taking into account that the preparation of a large number of samples for analysis (e.g., acidifying) and their shipment to the lab is in itself no simple task. In other words, reliable field screening procedures and appropriate test means are needed.

REFERENCES

1. Yu.A. Zolotov, *Ann. Chim. (Rome)*, 87 (1997) 285.
2. Yu.A. Zolotov, *Vestn. Ross. Akad. Nauk (Bull. Russ. Acad. Sci.)*, 6 (1997) 508.
3. Yu.A. Zolotov, *Khim. Prom-st. (Chemical Industry) (Moscow)*, 6 (1997) 48.

4. D. Barcelo, W. Beckert et al. (Eds.), *Current Protocols in Field Analytical Chemistry,* Wiley, New York.
5. *EPA Document* No. EPA-542-R-97-001.
6. *EPA Document* No. EPA-542-B-97-001.
7. Yu.A. Zolotov, *Zh. Anal. Khim.*, 56 (2001) 794.

Chapter 6

Determination of Inorganic Components in Water and Soil

In this chapter water and soil are considered to be part of the environment. Examples are given of testing waters of different origin and hence with different pollutant content, among them surface and sea waters, potable and underground waters of varying mineralization, and industrial (drainage) ones. When choosing an indicator reaction for a target component and appropriate test means one should take into consideration its detection limit and a probable content of accompanying components. In soils only pollutants are targeted. Usually soil extracts are obtained using appropriate solutions answering the concrete testing task. For example, when migrating components are being detected, water extraction is used or that with 0.1 *M* solutions of $NaNO_3$, NaCl, NH_4NO_3, NH_4Cl and other electrolytes. Elements are extracted with HCl and HNO_3 solutions of different concentration. The colour of the extract is important as it can be indicative of the presence of humic acids which can form stable complexes with metal ions; the latter have to be broken down. The above is a minimal sample preparation that can be easily performed on the site.

Depending on the substance's content to be determined, test tools are chosen which can, if need be, ensure preconcentration or separation of the substance from other components. A number of such test means and test devices have been discussed in Chapters 3 and 4.

Water and soil extracts analysis is carried out in steps outlined in this chapter. Testing is aimed at finding out whether or not the target component is present and at what concentrations: above or below the admissible levels. If above, then its exact concentration does not need to be known. Examples of quantitative measurements by test means are also given, their accuracy and precision being checked by other methods, such as diffuse reflection spectroscopy, because the same analytical form of the sample is used by both methods.

While testing, it is important to know the pH of the tested solution because it determines the ionic form of compounds in solution. Thus, high acidity excludes the presence of such anions as S^{2-}, CO_3^{2-}, PO_4^{3-}, BO_2^-, and others and low acidity excludes the presence of such anions as HPO_4^{2-}, HCO_3^-, HSO_3^-, and others.

There are some group test reagents which, under certain conditions, allow determination of either a total pollutants content or the content of some of them if masking agents are used. Such reagents are valuable due to their economical use but they require strict observance of proper testing conditions and strict control of the colour change. By way of example, a description of some determinations is given below. In most cases the test procedure is uniform and is conditioned by the given test means, the instruction being printed on the package box. Despite the great number of known test systems [3], it should be noted that quite a few components [2] cannot be measured by the known test means [1].

6.1 SUM PARAMETERS

To quickly assess the quality of natural, technical, drainage and potable waters sum total parameters are used such as hardness, pH, acidity, alkalinity, chemical and biological oxygen demand, sum content of heavy metals or carbon, and some others. In some cases a group substance determination would be sufficient and, if the result is negative or of low value, no further testing for separate components is needed.

In the field or out-of-the lab conditions water quality is quickly assessed by test methods for such parameters as total hardness, acidity, alkalinity, sum content of heavy metals, pH and different forms of chlorine.

6.1.1 Water hardness

Total hardness of water is linked to the presence in it of alkaline earth metals, viz., calcium and magnesium. These elements find their way into water due to the action of carbon dioxide on carbonate minerals or as a result of biochemical processes in humid soil layers.

The amount of Ca and Mg equivalent to the amount of carbonates and hydrocarbonates in water is called carbonate hardness. Non-carbonate hardness is found as the difference between total and carbonate hardness and shows the amount of alkaline earth cations stemming from mineral acids containing chloride, sulphate, nitrate anions, and others. Separate values of carbonate and non-carbonate hardness are calculated from total hardness and alkalinity of water. The result is expressed in *mM*/l or degrees: °d (German), °e (English), °f (French) or in mg/l $CaCO_3$ (USA), the relationship between them being

$1°d = 1.25°e = 1.78°f = 0.178\ mM = 17.8$ mg/l $CaCO_3$.

To determine total hardness by test methods, visual chelatometric test titration is used (CHEMetrics Titrets®, VISICOLOR®, Cal Ver®11, etc.), as well as observing colour intensity of indicator papers and coloured zone length of test strips (Table 6.1). As indicators in test titration and reagent papers, Eriochrome Black T (ECRT), Eriochrome Blue-Black R (ECRR), calmagite, calcichrome and zincon are employed. The two latter reagents are employed for determining calcium hardness.

The procedure for preparing indicator test papers is rather simple. For example, in Ref. [6] the following preparation protocol is described of the paper for hardness measurement using the length of the colour zone on the test strip or the colour intensity of the spot on the paper after passing a given volume of tested liquid through it. In all cases the paper is impregnated with water solutions of ECRT or ECRR, 5–8 g/l sodium tetraborate and 0.3–0.4 g/l magnesium complexonate solutions. The latter is added to enhance the colour transition of the reagents in the presence of calcium. Alkaline earth metals induce the colour change from blue to violet. For visual determinations to be more exact and colour sensitive, the impregnating solution is spiked with 3–4 g/l Methyl Orange. Calcium and magnesium ions cause the paper colour to change from green to grey to red.

The calcium and magnesium complexes with ECRT or ECRR are well adsorbed by the cellulose paper and are not washed out by water volumes of up to 20 ml [10]. The analytical range of the test strips for alkaline earth metals constitutes 0.05–40 *mM* (coloured zone length 1–70 mm) and that for flow-through test devices is 0.001–0.1 *mM* (solution volume 20 ml).

Heavy metal ions interfere when their content exceeds 0.1 mg/l. For their exclusion one has to add sodium sulphide or diethyldithiocarbamate (DDTC). When working in the field it is convenient to use a dried reagent paper measuring 1×1 cm impregnated with 20–50 g/l DDTC. One piece of such paper placed in 20 ml of tested solution masks

TABLE 6.1

Test methods for the determination of total hardness of water

Indicator system	Principle of determination	Linear calibration range (m*M*)	Ref.
Eriochrome Black T, EDTA	Appearance of the colour on a single (or several) of 24 zones containing different amounts of EDTA	0.5–10	1
Eriochrome Black T, EDTA, $MgCl_2$, NH_4Cl, KCl	Visual estimation of the colour intensity of a liquid after introducing indicator powders or tablets	0.1–20	2
Eriochrome Black T, hydroxyethylenediaminotetraacetic acid	Visual estimation of the colour intensity of indicator papers after their contact with the test liquid	0.5–20	3
Eriochrome Black T, aminosalicylphosphonic acid	The same	0.5–20	4
Eriochrome Black T, EDTA, Methyl Red	Titration with papers	0.1–10	5
Eriochrome Black T, EDTA, $MgCl_2$, $Na_2B_4O_7$	Determination of the length of the coloured zone of the indicator strip	0.05–40	6
Eriochrome Black T, EDTA, $MgCl_2$, $Na_2B_4O_7$	Estimation of the colour intensity of indicator papers after pumping 20 ml of the test solution through them	0.001–0.1	6
Calcichrome	Visual estimation of the colour intensity of the liquid in the ampoule after filling it with the test solution (CHEMets®)	0.002–0.01	7
Calmagite, EDTA	Test titration (Titrets®)	0.02–25	7
Zincon, EDTA (calcium hardness)	Test titration (Titrets®)	50–1000 mg/l $CaCO_3$	7
Eriochrome Black T, $MgCl_2$, $Na_2B_4O_7$, an increasing concentration of EDTA	Estimation of intensity of the colour of indicator papers (AQUADUR®)	0.5–5	8
Hydroxynaphthol Blue, EDTA, a buffer solution with pH 12–13	Test titration (Cal Ver® II)	0.1–40	9
Eriochrome Black T, EDTA	Test titration (VISO-COLOR®)	0.01–3.6	8

up to 10 mg/l Zn, Pb(II), Fe(III), Ni, Mn(II), Cu(II), Co, Cd, Hg(II). Sodium tetraborate in the impregnating solution ensures an optimal pH 10–11, while the tested solution may have pH 6–11.

Test strips were used for total hardness determination of natural, drinking and technical waters. Analysis time was 10–15 min, relative standard deviation (RSD) was under 0.3. To find the hardness of distilled or deionized water its 20 ml volume was passed through a flow-through device during 3–5 min. A standard colour scale was used to measure the intensity of the colour spot on the indicator paper. The RSD in this case was in the range 0.1–0.3.

6.1.2 Total alkalinity and acidity of water

Alkalinity of water is the content in it of substances reacting with strong acids; the latter's uptake expresses the total alkalinity of water. As a rule, alkalinity in common natural waters depends primarily on hydro carbonates of alkaline earth metals. In this case the pH of water is not greater than 8.3. Total alkalinity practically equals carbonate hardness and is conditioned by hydro carbonates. Soluble carbonates and hydroxides make the pH of water greater than 8.3. That part of the total alkalinity corresponding to the amount of acid needed to bring pH to 8.3 is called the free alkalinity of water.

Alkalinity is determined by titrating water with a strong acid until pH 4.5 is reached using combined indicators Methyl Red and Bromocresol Green.

Acidity of water is the content in it of substances reacting with strong bases; the uptake of the base expresses the total acidity of water. In common natural waters acidity depends in most cases on the content of dissolved carbon dioxide. A natural part of acidity may be due to humic and other weak organic acids. In all these cases the pH of water does not fall below 4.5.

Acidity of water is determined by titrating it with a strong base. The amount of base used to bring the pH to 4.5 is a measure of the free acidity, and that needed to bring it to 8.3 corresponds to the total acidity. As an indicator methyl orange is used in the first case, and phenolphthalein in the second.

Test titrations using test methods are conducted with phenolphthalein, Methyl Red–Bromocresol Green (Hach Co., E. Merck, Macherey-Nagel, CHEMetrics). The analytical range for acidity and alkalinity is 0.2–7 *mM*.

Alkalinity and acidity can also be measured using indicator papers. Thus, to measure total alkalinity use was made of the paper impregnated with 0.5% water–ethanol solution of Bromophenol Blue containing different amounts of $KHSO_4$ (1.0, 1.5 and 2.0 mg/l). The indicator paper was arranged on a polymer support in three layers: the upper with a low content of $KHSO_4$ changed colour from yellow to lilac in the range 0–1 *mM*; the second layer in the range 0.7–7 *mM*; and the bottom layer, with the greatest $KHSO_4$ content, in the range 3–30 *mM* [6].

Bromophenol Blue changes colour from yellow to violet in the pH interval 3.0–4.6. The ions OH^-, HCO^{3-} CO_3^{2-}, NH_3 and others determining alkalinity react with HSO_4^- resulting in pH lowering and indicator colour change. Three indicator paper strips on a polymer support and a standard colour scale are used to assess during 2–3 min alkalinity of natural, technical and drinking waters. The coefficient of variation of measurements (RSD) is no greater than 0.2.

To find total acidity the paper was impregnated with 0.5% water–ethanol solution of Thymol Blue containing different amounts of Na_2CO_3 (0.2, 0.3, and 0.4 mg/l). The pH interval of the indicator colour change from blue to yellow is 8.0–9.6. The organic base ions, H^+, NH_4^+, weak acids, and others determining the acidity of water, react with Na_2CO_3, thus raising the pH of the tested solution and inducing colour change from blue to yellow. The top indicator strip with a lesser Na_2CO_3 content allows measurement of total acidity in the range 0.1–1.0, the second one in the range 1–2, and the bottom, with the greatest Na_2CO_3 content, in the range 2–5 *mM*.

An original method of total alkalinity and acidity measurement using the coloured zone length of a test strip glued between two polymer films was proposed in [6]. A filter paper strip is impregnated with Congo Red and a citric acid solution (alkalinity measurement) or sodium tetraborate (acidity measurement). When alkalinity in the range 0.2–30 *mM* is being determined a red zone 1–75 mm long appears on the violet test strip; when acidity is being determined in the range 0.5–20 *mM* a blue zone 2–50 mm long appears on the red test strip.

6.1.3 Total contents of heavy metals

To find the total content of heavy metals, group reagents can be used such as dithizone and 1-(2-pyridylazo)-2-naphthol which under certain conditions react with many heavy metal ions (Table 6.2). Their total content can be found either by measuring the coloured zone length of

TABLE 6.2

Test methods for determining sum of heavy metals

Test metal ions	Indicator system	Test method	Analytical range (*mM*)	Ref.
Ag, Cd, Co, Cu, Hg (I, II), Ni, Pb, Tl(I), Zn	Dithizone, thiourea	Estimation of the colour of indicator paper	Not indicated	11
Cd, Co, Cu, Fe(II, III), Mn(II), Ni, Pb, Zn	1-(2-Pyridylazo)-2-naphthol	As above	0.1–1.5	12
Ag, Cd, Co, Cu, Fe, Hg, Mn, Ni, Pb, Zn	Cellulose bound chromogenic reagent	As above	20–500 mg/l	13
	As above	Visual estimation of the colour of the indicator paper after passing test solution through it	0.005–0.1 mg/l	13
Ag, Co, Cu, Hg (II), Ni, Pb, Zn	Dithizone	Coloured zone length of test strip	0.002–0.5	6
Co, Cu, Hg(II), Mn (II), Ni, Pb, Zn	1-(2-Pyridylazo)-2-naphthol	As above	0.002–0.5	6
Ag, Co, Cu, Hg(II), Ni, Pb, Zn	Dithizone	Visual estimation of the colour of indicator paper after passing 20 ml test solution through it	0.00005–0.001	6
Co, Cu, Hg(II), Mn(II), Ni, Pb, Zn	1-(2-Pyridylazo)-2-naphthol	As above	0.0001–0.002	6

test strips with adsorbed dithizone and 1-(2-pyridylazo)-2-naphthol, or by the colour intensity of analogous indicator papers after passing the test solution through them.

A red zone appears on the green test strip with immobilized dithizone at pH 6 (acetate buffer solution) in the presence of Zn, Pb, Hg(II), Ag, Ni, Cu(II), Co(II), a brown zone in the presence of Fe(III) and an orange one if Cd is present. A lilac zone is formed on a yellow indicator paper containing PAN at pH 7–9 (borate buffer solution) in the presence of Zn, Pb(II), Ni, Cu(II), Hg(II), Mn(II), and a green one in the presence of Co(II).

There is a non-linear dependence of the coloured zone length on metal content. When 10^{-5} *M* heavy metals are determined no interference (no change in coloured zone length) is observed from 500 mg/l alkali and alkaline earth metals, chlorides, acetates, 50 mg/l sulphates, phosphates, 10 mg/l oxalates and citrates, 5 mg/l Fe(III) (if dithizone is used Fe(III) is masked by sodium tartrate).

The working range of the flow-through device for 20 ml test solutions is $(0.5–10)10^{-7}$ *M* if dithizone is used and $(1–20)\cdot10^{-7}$ *M* if PAN is used. The time of analysis is 5–10 min, RSD no greater than 0.3.

Determination of heavy metals: Heavy metals total content [Cu, Co, Ni, Cd, Zn, Pb, Mn] in drinking, drainage, natural and atmospheric waters can also be determined using indicator tubes [14]. The method is based on the colour reaction of metal ions with 1-(2-pyridylazo)-2-naphthol immobilized non covalently on hydrophobized silica gel. The test means is a glass tube 50 mm long and 2 mm in diameter filled with indicator powder. On passing a test sample through the tube using a syringe a coloured zone develops, the length of which is directly proportional to the content of the target component in solution, which is found via a standard length scale or a graduation graph equation. The test means includes:

1. indicator tube filled with indicator powder.
2. ampoule with 0.5 ml borate buffer solution, pH 9.0 (solution 1).
3. syringe.
4. test glass with a ground stopper.

Analysis: To conduct the analysis, solution 1 is added to the test solution and mixed. The mixture is introduced into the tube using the syringe. The length of the violet colour zone formed is measured and the metal content found via the length scale or the graduation equation.

The method characteristics: analytical range 0.01–1 *mM*, RSD 0.06–0.08. No interference is observed from multiple quantities of: K, Na, Ca (10^3), Al, Mg (500), Fe(III) in the presence of 0.3 *M* NaF (100).

Accuracy and precision: Accuracy and precision were checked by spiked solutions and by analysing standard solutions for metals total content.

6.1.4 Active chlorine

The so-called active chlorine is determined in drinking water after its disinfection, in swimming pools and drainage waters that have to be chlorinated and in some types of waters contaminated with chlorine and compounds liberating chlorine. The term "active chlorine" implies the total content in water of free chlorine, hypochlorous acid HClO, hypochlorite ions ClO^-, and chloramines NH_4Cl, $NHCl_2$, NCl_3.

To determine chlorine using test methods reagents are employed that easily oxidize forming coloured products, such as *o*-tolidine or N,N-diethyl-1,4-phenilenediamine (DPD). DPD is used most often in test methods due to bright colour products of its oxidation (red violet). The active chlorine content is found via the colour intensity of the test solution formed on adding to it the reagent solution. To quantify results, colour discs in a comparator (E. Merck, Macherey-Nagel) are employed or a mini photometer (Table 6.3).

To determine active chlorine a new reagent FACTS (syringaldazine) has been proposed [17]. It was used for impregnating filter paper. Under the action of active chlorine the paper turns lilac.

A method was proposed for preparing indicator papers for active chlorine containing as indicator 3,3′,5,5′-tetramethylbenzidine. Polymer supports were used with an oval hole at one end covered with indicator paper. Chlorine in the range 0.05–25 mg/l could be determined by using a water insoluble indicator, phosphate buffer solution (pH 7.0), polyester as stabilizer and viewing the colour intensity change through a hole in the support [19].

An original method of active chlorine detection is described in [20]. The method is based on the reaction of chlorine with toluidine groups of polyurethane foam tablets. The latter turn yellow when coming into contact with the test solution. Using diffuse reflection spectroscopy one can determine 0.2–6 mg/l of chlorine.

Active chlorine content can be found via indicator test strips either by the length of coloured zone or by the colour intensity change of test solution after immersing the indicator paper in it. In the first case the paper was impregnated with 0.01 and 0.05% water–ethanol solution of *o*-toluidine, and in the second with water solutions of KI and starch [6]. Interference from Fe(III) was eliminated by adding EDTA and Na_2HPO_4 to the impregnating or test solution. Determination is hampered by NO_2^- ions.

TABLE 6.3

Test methods for active chlorine determination

Indicator system	Measurement principle	Analytical range (mg/l)	Ref.
1-(2-Hydroxynaphthalene)-benzene-4-sulphonate	Visual estimation of the decolouration rate of indicator paper	5–100	15
2,4-Dinitro-1-naphthol	Visual estimation of the degree of decolouration of indicator paper	5–100	16
FACTS	Visual estimation of colour intensity of the indicator paper on its contact with the test solution	0.5–10	17
Michler thioketone	As above	0.1–3	18
KI, starch	As above	2–100	13
3,3′,5,5′-Tetramethylbenzidine	As above	0.05–25	19
Polyurethane foam	Diffuse-reflectance spectroscopy	0.2–6	20
N,N-Diethyl-1,4-phenylenediamine	Visual estimation of colour intensity of the test solution after adding indicator powder	0.01–0.3 0.1–2 0.25–15	21
o-Tolidine	Estimation of the length of coloured zone of the test strip	5–500	6
KI, starch	Estimation of colour intensity of the test solution after immersing indicator paper	0.3–8	6
N,N-Diethyl-1,4-phenylenediamine	Estimation of colour intensity of indicator paper (QUANTOFIX®)	1–100	8
N,N-Diethyl-1,4-phenylenediamine	Estimation of colour intensity of test solution using a miniphotometer (NANOCOLOR®)	0.02–10	8
N,N-Diethyl-1,4-phenylenediamine	System VISOCOLOR®HE	0.02–0.6	8

Depending on the concentrations of impregnating solutions, a yellow or an orange zone appears on the indicator paper with *o*-tolidine under the action of active chlorine. The analytical range of the method is 5–500 mg/l at optimal pH 4–7. At 0.01% *o*-tolidine concentration of the impregnating solution the length of the yellow colour zone on the test strip changes from 1 to 65 mm, and at 0.05% from 1 to 40 mm. The test strips were used to determine active chlorine in disinfecting solutions, precision of analysis was checked by titration. The time of testing was 10–15 min, RSD ≤ 0.2.

Lower active chlorine concentrations (0.3–8 mg/l) were determined by colorimetry, after immersing in a 1 ml test solution a 1×1 cm reagent paper impregnated with KI and starch. After mixing for 1 min the liquid was colour-matched against a standard colour scale. The precision in this case was poorer (RSD < 0.3) [6].

Determination of active chlorine via polyurethane foam tablets [22]
The test means is proposed for qualitative and semi-quantitative evaluation of active chlorine in tap water and disinfecting solutions. The method is based on the oxidation of end toluidine groups of polyurethane by active chlorine resulting in the colour change of the tablet from white to yellow; the chlorine content is determined by colour matching against a standard colour scale. Detection range is 0.2–8 mg/l. The test means includes:

1. polyurethane foam tablets (ether based) 16 mm in diameter, 6 mm thick, weighing approx. 0.04 g.
2. sulphuric acid, 0.5 *M* solution;
3. test glasses with ground stoppers for mixing;
4. syringes, a cylinder;
5. glass rod;
6. colour scale;
7. filter paper.

Analysis: The test glasses are filled with 20 ml of tested water and spiked with 5 ml of 0.5 M H_2SO_4. A polyurethane tablet is placed in each test glass, pressed with the glass rod until air bubbles are removed and agitated for 15 min. Test glasses are decanted and tablets dried between two sheets of filter paper, and then colour matched against the colour scale.

Selectivity: Selectivity was characterized by a "selectivity factor", i.e., the limit ratio of accompanying component to active chlorine at which the error of measurement does not exceed 15%; 4 mg/l of active chlorine can be determined without interference from multiple quantities of H_2O_2, Cl^-, (10^4), ClO_4^- ($4 \cdot 10^3$), K, Na, Ca, Mn, Zn, Cu(II), $S_2O_8^{2-}$, BrO_3^-, NO_3^- (100), Fe(III) (10).

Accuracy and precision: The test means allow screening of water samples for active chlorine and shows its presence for amounts greater than 2 mg/l.

The time needed for a white polyurethane tablet to turn yellow depends on the active chlorine content in the test sample:

Active chlorine content (mg/l)	Time for colour to develop (min)
2	15
4	7–8
6	5
10	0.5
20	0.5

Full extraction of the target compound into the tablet takes 15 min.

The precision of the method depends on the active chlorine content in the sample. Relative standard deviation is RSD 0.20–0.30 for chlorine contents in the interval 4–10 mg/l; and RSD 0.4–0.5 for the contents out of this interval ($n = 6$). Precision is improved if a portable reflectometer MULTIECOTEST of Kostip Co. is used. The graduation curve was obtained using a series of standard solutions and employed for chlorine determination. In this case RSD 0.05–0.1 for chlorine contents in the interval 3–10 mg/l and RSD = 0.15 for contents out of this interval ($n = 6$). Precision of the method was checked by spiked solutions using a model tap water sample containing 3.2 mg/l of active chlorine. The found value was 3.1 ± 0.6 mg/l ($n = 6$, RSD = 0.08).

6.1.5 pH measurement of water solutions

Determination of acidity and alkalinity (pH measurement) of water solutions (e.g. of soil extractions) is carried out using indicator papers and by colorimetric and photometric methods (Macherey-Nagel, E. Merck, Hach Co.). The colour scale may be graded in 1, 0.5, 0.3 or 0.2 pH units. Table 6.4 gives pH gradation of indicator papers produced by Macherey-Nagel and E. Merck companies.

Acid–base indicators are immobilized on the paper either covalently [23] or by physical adsorption [24]. Usually mixtures of the following indicators are used: Cresol Red, Bromocresol Blue, Dimethyl Yellow [25], Bromothymol Blue, Methyl Orange, Phenolphthalein, Alizarin Red, Thymol Blue [26], Bromocresol Purple, and Methyl Red [5]. The

TABLE 6.4

Colour scale gradations of indicator papers for pH measurement

Indicator paper	Gradation
DUOTEST® pH 5–8	5.0–5.3–5.6–5.9–6.2–6.5–6.8–7.1–7.4–7.7–8.0
PEHANON® pH 5.2–6.8	5.2–5.5–5.7–5.9–6.1–6.3–6.5–6.8
PEHANON® pH 6.0–8.1	6.0–6.3–6.6–6.9–7.2–7.5–7.8–8.1
PEHANON® pH 7.2–8.8	7.2–7.4–7.6–7.8–8.0–8.2–8.5–8.8
VISICOLOR® pH 4.0–10	4.0–4.5–5.0....10.0
pH-Fix 5.1–7.2	5.1–5.4–5.7–6.0–6.3–6.6–6.9–7.2
pH-Fix 7.5–9.5	7.5–7.9–8.2–8.4–8.6–8.8–9.1–9.5
pH-test	7.1–7.4–7.6–7.8–8.0–8.2–8.5–8.8

indicator mixture for determining pH of soil extracts consists of Bromocresol Green, Bromocresol Purple, and Bromocresol Red (0.05%, 0.1%, and 0.02%, respectively) [27].

6.2 CATIONS

6.2.1 Silver

In acid solutions *n*-dimethylaminobenzylidenerhodanine forms a poorly soluble silver compound [28]. The silver salt formed with a yellowish-brown reagent has a reddish-brown colour. This reaction is used for determining silver via paper test; 0.5 ml/l Ag can be detected in 0.2 M HNO_3 (detection limit). Analysis is hampered by mercury, gold, platinum, palladium, and copper salts [1].

Tests using the reaction of silver with cadmium sulphide were developed [29–31]. In Russia reagent paper IS-1 is manufactured for semi-quantitative silver determination in fixer solutions in the range 0.5–10 g/l. For fixer solutions methods for silver detection have been patented (range 0–10 g/l) based on the formation of a black Ag_2S on filter paper [32,33].

Paper impregnated with copper dithizonate can also be used for silver detection [34]. In neutral medium a lilac zone is formed on the test strip and a red one in the acid medium. The zone length in both cases (0.1 M HNO_3) is practically the same. In the range 0.5–100 mg/l the graduation equation $y = 1.03\ x^{0.72}$ can be used. Mercury and chlorides interfere with the analysis.

Silver, along with mercury, forms the most stable complexes with DDTC. A test was developed for semi quantitative silver detection using paper impregnated with $Cu(DDTC)_2$. In an acid medium (0.05–0.1 M HNO_3) decolouration of the brown test strip is observed [35]. Detection of Ag in 0.1 M HNO_3 is not hampered by copper and 5-multiple amounts of mercury. Detection limit of the proposed test method is 0.5 mg/l.

To determine silver in natural waters indicator paper was used impregnated with copper dithizonate [36]. After passing 20 ml of analysed solution through the test device the colour intensity of the reaction spot was matched against the colour scale in the range 0.01–2 mg/l.

6.2.2 Aluminium

Aluminium is determined by test methods using organic reagents known in photometry: Chromazurol S, aluminon, Eriochromecyanine R, thiazolylazo pyrocatechol in the presence of cetylpyridinium chloride. Reactions are run at a fixed pH maintained by buffer solutions. Reactions with aluminon are run at pH 13.3–14.0. The following ions interfere with analysis: Be^{2+}, >10 mg/l Cu^{2+}, >50 mg/l $Fe^{2+/3+}$, >100 mg/l MnO_4^-, PO_4^{3-}, SO_3^{2-}, S^{2-}, >250 mg/l F^-, >500 mg/l CN^-, CrO_4^{2-}, VO_3^-, citrates, SO_4^{2-}. With analysis using Chromazurol S or Eriochromecyanine R, such ions as Co^{2+} (>5 mg/l), Cr^{3+}, Cd^{2+} (>10 mg/l), $Fe^{2+/3+}$, Cu^{2+}, $Mn^{2+,}$ Zn^{2+} interfere. The methods are summarized in Table 6.5.

A pink and, at large concentrations, a red colour spot is formed on the yellow indicator paper impregnated with the 4-(2-thiazolylazo)-pyrocatechol–cetylpyridinium associate on passing through it 20 ml of aluminium salt solution. The reaction was conducted at pH 3–5 (acetate–ammonium buffer solution). The analytical range for Al is 0.01–0.5 mg/l [38]. Under these conditions no interference is observed from 1000-multiple amounts of alkali and alkaline-earth metals, Zn, Cd, Cl^-, SO_4^{2-}. Ions Fe(III), PO_4^{3-}, Mo(VI), W(VI), Sb(III), Ga, Sn(IV), Ge(IV), Zr(IV), Ti(IV), V(V) interfere. Interference from 100-multiple quantities of Fe(III) can be excluded by reduction with ascorbic acid. This system was used for detecting aluminium in tap (drinking) water. To 20 ml of water were added 1 ml of 0.1% ascorbic acid solution and 5 ml of acetate buffer solution (pH 4.5); all was mixed, sucked into a

TABLE 6.5

Test methods for aluminium determination

Reagent	Analytical range (mg/l)	Test system	Ref.
Aluminon	10–250	Merckoquant® system	37
Aluminon	5–500	QUANTOFIX® system	8
4-(2-Thiazolylazo)pyrocatechol–cetylpyridinium	1–50	Estimation of the coloured zone length	5
4-(2-Thiazolylazo)pyrocatechol–cetylpyridinium	0.01–0.5	Estimation of colour intensity of the indicator paper after passing 20 ml of test solution	38
Chromazurol S	0.07–6	Microquant ® system	38
Eriochromecyanine R	0.01–1	NANOCOLOR ® system	8

syringe and passed through a test device. A standard colour scale was used to assess aluminium concentration. Analysis time was from 10 to 20 min.

6.2.3 Ammonium

The Nessler method is the most common and widely used for ammonia and ammonium compounds analysis. It is based on the formation of a reddish-brown colloid according to the reaction

$$2NH_3 + 2HgI_4^{2-} = NH_2Hg_2I_3 + 5I^-$$

The coloured product absorbs in a broad spectrum range. For $NH_4^+ < 0.2$ mg/l it absorbs at 400–425 nm and for $NH_4^+ = 1$ mg/l — at 550–580 nm.

A more sensitive method than this is the indophenol method. Phenol treated by alkali metal hypochlorite in the presence of ammonia gives the indophenol blue:

$$NH_3 + NaOCl = NH_2Cl + NaOH$$

$$C_6H_5OH + NH_2Cl + NaOH = HO\text{-}C_6H_5\text{-}NH_2 + NaCl + H_2O$$

p-Aminophenol is oxidized with hypochlorite to *p*-benzoquinonimine:

$HO\text{-}C_6H_4\text{-}NH_2 + NaOCl \Rightarrow O{=}C_6H_4{=}NH + NaCl + H_2O$

This compound is chlorinated by hypochlorite giving *p*-benzoquinonechloroimine:

$O{=}C_6H_4{=}NH + NaOCl \Rightarrow O{=}C_6H_4{=}NCl + NaCl$

In alkaline medium *p*-benzoquinonechloroimine interacts with phenol giving Indophenol Blue:

$O{=}C_6H_4{=}NCl + C_6H_5\text{-}OH + 2NaOH \Rightarrow O{=}C_6H_4{=}N\text{-}C_6H_4\text{-}ONa + NaCl + H_2O$

Sodium nitroprusside is used as a catalyst for this reaction. Instead of phenol 1-naphthol, 2-methyl-8-hydroxyquinoline, guaiacol, cresols, salicylic acid, 8-hydroxyquinoline, Thymol and others can be used. The method is selective and allows us to determine ammonium ions without preliminary refluxing of waste and natural waters. Melamine, dicyanamide, carbamide, cyanuric acid and nitrogen containing compounds do not interfere. Sulphides, H_2S, and hypochlorite reductants do interfere. The above methods are used to test water and soil extracts for ammonium ions (Table 6.6).

TABLE 6.6

Test methods for ammonium determination

Reagent	Analytical range (mg/l)	Test system	Ref.
Nessler reagent	10–400	QUANTOFIX® test strips	8
8-Hydroxyquinoline, nitroprusside, $CaOCl_2$	2–20	Estimation of colour intensity of the indicator paper	5
8-Hydroxyquinoline, nitroprusside, $CaOCl_2$	0.5–20	Estimation of the colour intensity of the solution after immersing the indicator paper	5
Nessler reagent	0.5–20	VISOCOLOR® system	8
Thymol, chloramine	0.2–3	VISOCOLOR® ECO system	8
Salicylic acid, chloramines	0.2–10	VISOCOLOR® system	8
Salicylic acid, chloramines	0.2–0.5	VISOCOLOR® HE system	8
Nessler reagent	0.05–10000	CHEMetrics systems	7
Nessler reagent	0.05–10	Aquaquant® system	21
Salicylic acid, chloramines	0.025–10	Aquamerck®, Aquaquant®, Microquant®	21

6.2.4 Arsenic

All the test methods for revealing arsenic are based on its recovery from water and soil extracts as arsine using Zn and $CuSO_4$. Arsenic(V) is reduced to arsenic(III) in acid medium by KI. Gaseous AsH_3 acting on the filter paper impregnated with $NaNO_3$ or HgBr makes it turn yellow or brown [5,8,30]. Using a colour scale, arsenic is detected in the range 0.05–3 mg/l; devices for obtaining arsenic have been [5,39] proposed consisting of a glass or plastic vessel containing Zn, $CuSO_4$, KI and an ampoule with an acid. After adding the analysed solution the Ampoule is broken and the vessel is closed with a stopper provided with a gas outlet tube. The tube is covered with indicator paper whose colour is changed by the evolving gaseous arsine passing through the paper. Interfering are antimony, germanium, phosphates. These are removed by placing in the gas outlet tube some cotton impregnated with lead acetate for absorbing phosphine.

6.2.5 Beryllium

To reveal beryllium in the range 1–50 mg/l in wastewaters with accuracy of 10% an indicator tube was proposed filled with an ion-exchanger resin impregnated with Chromazurol S [40].

6.2.6 Bismuth

Indicator papers RIS-Bismuth-Test were proposed in the form of a paper strip with a covalently bound reagent [13]. A light-yellow reagent gives with bismuth ions a light-green complex. The analytical range at pH 1.0 is 0.01–0.2 mg/l for 20 ml of test solution passed through the concentrating test device. The reaction is highly selective at pH 1–2.

A test method for bismuth was proposed making use of a paper impregnated with iron diethyldithiocarbamate [35]. The length of decolorised zone on the brown test strip was a measure of the bismuth(III) content at pH 1–5 in the range 1–500 mg/l. The method is non-selective, and lead, cobalt, copper, silver, mercury interfere. This method can be used in the absence of these elements in bismuth containing solutions or after removing (masking) them.

6.2.7 Calcium

To reveal calcium in the presence of magnesium using both indicator papers and test-titration, Glioxal-bis-2-hydroxyanil was used

(QUANTOFIX®, Macherey-Nagel; Merckoquant®, E. Merck). Analytical ranges are 10–100 mg/l for indicator papers and 3–40 mg/l for test-titration. 1000 mg/l of Mg^{2+} do not interfere. The method is used for analysing wastewaters and soil extracts. The determination of total calcium–magnesium content was described in Section 6.1.1.

6.2.8 Cadmium

Polyurethane foam cubes soaked in dithizone were proposed for cadmium detection [41]. The cubes are placed in a 1–2 ml solution, and if cadmium is present they change their colour from green to orange red. Semi-quantitative determination in the range 0.1–10 mg/l is carried out using a colour scale made up of tablets in the form of 4×4 mm cubes. To reveal 0.01–0.05 ml/l Cd a 100 ml solution is passed through a column filled with the cubes. Cr(VI), Mn(VII), Ag, Zn, Pb, Hg, and sulphites interfere.

Cadmium can be determined using indicator paper RIS-Cd-Test [13]. The test is based on the reaction of a chromogenic reagent strongly bound to the cellulose paper whose colour changes from yellow-orange to brown. The absorption maximum of the reagent is 460 nm, and that of the complex –560 nm. The analytical ranges are 0.003–0.01–0.03–0.05–0.1 mg/l when a 20 ml solution at pH 6 is passed through an indicator paper zone 6 mm in diameter using a concentrating device. Not interfering are: 1000 mg/l K^+, Na^+, SO_4^{2-}, Cl^-, 500 mg/l Ca^{2+}, 100 mg/l Al^{3+}, Ba^{2+}, $V^{4+/5+}$, Ga^{3+}, $Fe^{2+/3+}$, La, Mg, Mn, Sn^{2+}, $Pt^{2+/4+}$, Pb, Ag, Ti, $Cr^{3+/6+}$, NO_3^-, NO_2^-, PO_4^{3-}, F^-, 5 mg/l Pd, Hg. Interference from Zn ions is excluded by 0.01 *M* potassium ferrocyanide solution, that from copper by 5% solution of thiourea or a borate buffer.

A more selective test method for cadmium was proposed using paper impregnated with zinc dithizonate [34]. The pH of test solution was 3–7. In the presence of cadmium an exchange reaction takes place:

$$Zn(DTZ)_2 + Cd^{2+} = Cd(DTZ)_2 + Zn^{2+}$$

and the paper changes colour from crimson to orange. Diffuse reflection maximum of the reagent paper is 530 nm and that of the reacted paper is 440 nm. When a 20 ml test solution is passed through the paper an orange zone is formed whose intensity is proportional to cadmium concentration in the ranges 0.005–0.01–0.05–0.1–0.5–1.0 mg/l. It should be noted that there is no interference from zinc.

TABLE 6.7

Test methods for cadmium determination

Reagent	Analytical range (mg/l)	Test system	Ref.
Bromopyrogallol Red, 1,10-phenanthalin	1–10	Indicator tube colour zone length	42
Dithizone	0.1–10	Visual estimation of polyurethane-foam colour	41
Dithizone	0.01–0.05	Estimation of coloured zone length of polyurethane cubes in a column	41
Cellulose with formazan groups	0.03–1.0	Estimation of paper colour intensity	13
Zinc dithizonate	0.1–200	Estimation of the coloured zone length of the test strip	34
Zinc dithizonate	0.005–1.00	Estimation of colour intensity of indicator paper after passing 20 ml of the test solution	34
Cadion	0.1–2.0	NANOCOLOR® system	8
Dithizone	0.002–0.5	NANOCOLOR® system	8

In the case of the test strips glued in a polymer film it is the strip's coloured zone length that is proportional to the cadmium concentration (Table 6.7).

Cadmium determination using indicator powder [43]

For revealing Cd in potable, natural, waste, and atmospheric waters indicator powder was proposed in the form of a silica-based sorbent non-covalently modified by a complex-forming analytical reagent bromobenzothiazo. After coming into contact with the test solution the powder turns blue-violet. The colour intensity is directly proportional to the cadmium concentration in solution and can be evaluated by visual methods using colour scales or by diffuse reflection spectroscopy. For colour measurement a portable reflectometer Multiecotest from Kostip Co., Russia, was used. The test means includes:

1. indicator powder, 0.15 g;
2. ampoule with 5 ml of buffer solution (solution 1);
3. test glass with ground stopper;
4. dosage pipette.

A portable photometer is needed for analysis.

Solution 1 and the indicator powder is added to 25 ml of analysed sample, and all mixed for 20–25 min; using the dosage pipette the sorbent is separated from the solution and its absorbance or diffuse reflection measured on the portable photometer at 620 nm. Concentration is read on a graduation graph.

The method parameters are: analytical range 0.05–0.5 mg/l, do not interfere (in multiple amounts): K, Na, ($1.0 \cdot 10^6$), Ca ($1.5 \cdot 10^4$), Zn ($3.0 \cdot 10^3$), Fe(III) (500). Interference from copper is excluded by thiourea. The accuracy and precision was determined by the spiked solutions.

As model solutions samples of natural, tap water and ash content of milk were analysed in which were found 0.090 ± 0.008, 0.035 ± 0.003, and 0.008 ± 0.003 mg/l Cd, respectively.

Cadmium determination using indicator tubes [44]

Indicator tubes were proposed for revealing cadmium in technological solutions. The test means consist of a glass tube 50 mm long, 1 mm inner diameter, filled with indicator powder. A coloured zone proportional to the cadmium concentration develops in the tube after adding complexing agents to the test sample and passing the obtained coloured solution through the tube via a syringe. This concentration is quantified using a calibrated length scale or a calibration relationship. The method is based on a colour reaction of cadmium with 1,10-phenanthroline and Bromopyrogallol Red in solution and sorption of the coloured product in the indicator tube. The test means includes:

1. indicator tube with indicator powder;
2. ampoule with 0.3 ml of $2.5\ 10^{-5}$ *M* Bromopyrogallol Red solution (solution 1);
3. ampoule with 0.3 ml of $9\ 10^{-5}$ *M* 1,10-phenanthroline solution (solution 2);
4. ampoule with 0.4 ml of borate buffer (solution 3)
5. syringe
6. test glass with ground stopper for mixing.

Analysis: To a 1 ml test solution solutions 1, 2, and 3 are added and mixed. The obtained coloured solution is passed via the syringe through the indicator tube. The length of the violet pink zone in the tube is measured and cadmium content found via the length scale or a calibration relationship. Analytical range is 1–10 mg/l and the relative standard deviation 0.06–0.08. Not interfering (in multiple amounts): Al

(50), Ni (25), Zn, Co, Mn(II) (10), Pb (2). The accuracy and precision of the test were checked by the spiked solutions and by comparison with instrumental methods of analysis.

Good agreement between the two approaches was found.

6.2.9 Cobalt

To reveal cobalt, indicator papers are used containing thiocyanate, 1-nitroso-2-naphthol, 1-(2-pyridylazo)-2-naphthol, 4-(2-thiazolylazo)-resorcinol or cellulose with chemically immobilized formazan (Table 6.8). For the latter the greatest sensitivity was achieved (0.01 mg/l) when 20 ml of test solution was passed through the concentrating test

TABLE 6.8

Test methods for cobalt determination

Reagent	Analytical range (mg/l)	Test system	Ref.
KSCN, $Na_2S_2O_3$	10–1000	Merckoquant® system	45
KSCN	0.1–4.0	Estimation of colour intensity of polyurethane-foam tablets	46
KSCN, Amberlite LA-1	0.3–500	Estimation of colour intensity of polyurethane-foam tablets	47
1-(2-Pyridylazo)-2-naphthol	0.05–0.5	Estimation of colour intensity of paper discs after pumping the test solution	48
1-(2-Pyridylazo)-2-naphthol	0.1–1.0	Estimation of colour intensity of support	49
1-Nitroso-2-naphthol	0.06–0.6	Coloured zone length of indicator tube	50
Nickel complex with 1-nitroso-2-naphthol	0.3–5	Estimation of colour intensity of indicator paper	51
4-(2-Thiazolylazo)resorcinol	0.06–2	Estimation of colour intensity of support	52
Cellulose with formazan groups	0.01–100	Estimation of colour intensities of indicator papers with and without test components	13

device and the colour on the indicator paper evaluated. Cu^{2+}, $Fe^{2+/3+}$ interfere; for their exclusion thiosulphate and sodium phosphate were used.

Cobalt determination using polyurethane foam tablets [53,54]
This test means is intended for qualitative and semi-quantitative detection of cobalt in natural waters. It is based on sorption of blue thiocyanate complexes by a polyurethane foam tablet. The presence of cobalt in the sample is revealed when the white tablet becomes blue and its concentration in the sample is quantified by visual matching of the tablet against a colour scale prepared beforehand. Analytical range of the method is 0.1–4 mg/l. The test means include:

1. ether-based polyurethane foam tablets 16 mm in diameter, 6 mm thick, weighing ca. 0.04 g;
2. potassium thiocyanate in 1.2 g batches and sodium fluoride in 0.1 g batches;
3. sulphuric acid, 2 *M* solution;
4. test glasses with ground stoppers for mixing and shaking;
5. syringes, a cylinder;
6. glass rod;
7. colour scale;
8. filter paper.

Analysis: To a 20 ml water sample in the test glasses are added 0.2 ml of 2 M H_2SO_4 (5–7 drops), then weighed amounts of KSCN and NaF. The solution is mixed and shaken until the salts are fully dissolved. In each test glass a polyurethane tablet is placed, pressed with the glass rod until all air bubbles are removed, and shaken for 15 min. The solutions are decanted, the tablets dried between two sheets of filter paper, and the tablet colour matched against the colour scale.

Selectivity: The selectivity of the method was characterized by a selectivity factor, i.e., the ratio “accompanying component/cobalt” at which the error of measurement is no greater than 15%. Detection of 2 mg of cobalt is not hampered by multiple amounts of Cr(III) ($3 \cdot 10^5$), Mg, Ca, Sr, Ba, Ni (10^5), Tl(I) ($8 \cdot 10^3$), Cu(II), Cd(II), Hg(II), Pb(II), Fe(III), As(III), Mn(II) (10^3), Zn(II) (50), Cu(II) (10).

Precision and accuracy: The test means allows screening of water samples to be carried out for cobalt and reveal it for concentrations higher than 0.1 mg/l. The time needed for the blue colour of the tablet to develop depends on the cobalt concentration as follows:

Cobalt concentration (mg/l)	Time for the colour to develop (s)
0.1	30
0.5	20
1.0	10
2.0	5
4.0	5

Quantitative extraction of the target component into the tablet takes 15 min.

Precision of the visual test method depends on the cobalt content in the sample. The relative standard deviation is 0.1–0.2 at 0.5–2 mg/l contents; 0.3–0.4 at contents less than 0.5 and greater than 2 mg/l (n = 6). The precision can be improved by using a portable reflectometer–colorimeter "Multiecotest" from Kostip Co. (Russia). The calibration relationship was obtained using a series of standard solutions. Precision in this case was 0.05–0.1 at 0.1–2 mg/l Co concentrations; 0.15 at concentrations higher than 2 mg/l (n = 6).

The precision of the method was verified by analysing two water standards: COB-1 containing according to specification 1 mg/ml, of each: Ag, Co, Ni, Cu, Zn, Cd, Al, 2 mg/l of Fe, 4 mg/l of Pb; and GSORM-2, containing 1 mg/ml Co, Ni, REE, Cu, Cr, Fe, diluted 1000-fold. The revealed cobalt amounts were: 1.03 ± 0.03 and 0.95 ± 0.1 μg/ml, respectively (n = 6, RSD = 0.05).

6.2.10 Chromium

Chromium(VI) can be revealed qualitatively and semi-quantitatively in natural waters using polyurethane foam tablets. The method is based on sorption of Cr(VI) from 1 M H_2SO_4 solution containing $2 \cdot 10^{-4}$ M sodium tetraphenyl borate by a polyurethane tablet modified with diphenylcarbazide in the presence of a plasticizer. If Cr(VI) is present, the tablet changes colour from light pink to blue; the Cr content is quantified using a standard colour scale. Do not interfere 10-fold amounts of Cu(II), Zn(II), Co(II), Ni(II), Cd(II), Fe(III). Analytical range is 0.01–0.2 mg/l [55,56].

6.2.11 Copper

A variety of reagents were proposed for qualitative and semi-quantitative copper determination by test method. For example, the following solution for impregnating paper was used: 2 g of hydroxylamine chloride, 0.4 g of ascorbic acid, 4 g of tartaric acid, 0.8 g of sodium citrate, 100 ml of water, all mixed and brought up to pH 2.6 by a 2 *M* NaOH solution; a second impregnating solution contains 0.25 g of 2,2′-diquinoline, 5 g of nonylphenolpolyglycol ether, 100 ml of methanol, mixed with 0.3% of 10 *M* HCl [57].

Besides cuproine (2,2′-diquinoline), as complexing agents are used neocuproine, bathocuproine, 2,2′-dipyridyl and others [58,59]. Detection limit for copper is 0.5–5 mg/l.

Cuprizon [oxalic bis(cyclohexylidenehydrazide)] is recommended as a specific and sensitive reagent for copper [59]. Its chelate copper compound has a deep blue colour. A standard colour scale obtained by immersing the reagent paper in a series of standard solutions covers the range 2–200 mg/l. Do not interfere 500 mg/l of Ca, Mg, 100 mg/l of Cd, K, Na, Ni, Zn, Al, Fe(II,III), Ba, V, Bi(III), In, Y, CO, La, Li, Mn(II), Mo, Sn(II), Ag, Pb, Sc, Sr, Sb(III), Ti(IV), Cr(III,VI), Zr, 5 mg/l of Pd, Hg(II), 1 mg/l of Be, 100 mg/l of Br^-, Cl^-, NO_3^-, NO_2^-, SCN^-, PO_4^{3-}, SO_3^{2-}.

For detecting small quantities of copper a technique was proposed of impregnating polyurethane foam tablets (PUF) of cubic form with lead diethyldithiocarbamate [41,60]. A PUF tablet is added to 1–2 ml of test solution with a copper content 0.05–10 mg/l and agitated. The tablet colour changes from colourless to yellow red. 100 ml samples containing 0.01–0.05 mg/l of copper are passed through a column filled with PUF cubes. The length of the coloured cubes in the column is proportional to the copper concentration. Silver, mercury ions, and iodides interfere.

A method was patented for rapid determination of 0.05–0.9 mg/l of copper in liquids using iminodiacetate resin (IR) and sodium diethyldithiocarbamate as chelating reagents. The analysed solution (50–200 ml) at pH 2–7 is flowed at the rate 0.08–0.33 ml/s through a glass tube filled with 15 g of IR with particle size 0.3–1 mm. The copper content is quantified by matching the obtained brown colour of the resin against a colour scale.

Indicator paper impregnated with Michler's ketone [62] was proposed for revealing copper in 0.05–2 mg/l solutions. Do not interfere 10 mg/l of Fe, Ni, Co, Cd, Zn, Pb, Al, Cr, V, Hg, and other elements. The method was used for determining copper in drinking water.

Reagent indicator test strips RIS-Copper-Test [13,63] were proposed for copper detection. The method is based on a colour reaction of copper ions with a reagent strongly bound to the cellulose paper. The

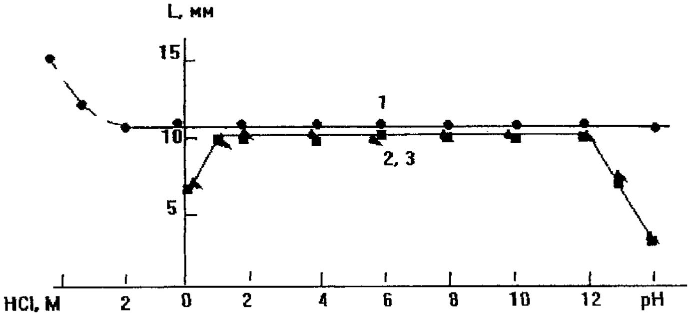


Fig. 6.1. Influence of acidity on the coloured zone length of test strips for copper determination with $Pb(DDTC)_2$ (1), $Zn(DDTC)_2$ (2) and $Fe(DDTC)_2$ (3); copper concentration is 10 mg/l.

diffuse reflection maximum of the reacted paper lies at 580 nm and is constant at pH 4–7. Solutions containing 0.001–0.1 mg/l of copper are analysed by passing 20 ml of the sample through a reaction zone 6 mm in diameter of a concentrating device. In natural waters organic substances may have a masking effect on copper, therefore a preliminary treatment of the sample is needed to destroy the complexes. Determinations are conducted at pH 4–5.

In the above methods copper concentration is judged by the colour intensity of indicator paper immersed in the tested liquid. Test strips impregnated with diethyldithiocarbamates of Fe(III), Zn and Pb [34] were proposed for copper detection using the length measurement of the brown colour zone on the strip. On all the papers a brown zone with distinct boundaries was formed in the presence of copper. Acidity of the medium does not affect the copper determination using paper impregnated with $Pb(DDTC)_2$. In 2 *M* HCL and up to pH 14 the length of the coloured zone remains constant (Fig. 6.1).

In acid media (>2 *M* HCl) the coloured zone becomes blurred and is difficult to measure. For the papers impregnated with $Zn(DDTC)_2$ and $Fe(DDTC)_2$ the optimal pH range is 1–12. In all the three systems the coloured zone length dependence on concentration is identical in the range of copper content 0.05–500 mg/l, $y= x/(0.71+0.014x)$. The lower detection limit is 0.05 mg/l (the coloured zone length is 0.3–0.5 mm). The method is quite selective, interfere only mercury, palladium and silver which hamper the formation of the coloured zone. Organic substances (humic and fulvic acids) present in natural waters influence detection of copper at pH 6–9. Adjusting the pH of solution to 1.0 by adding HCl results in destroying complexes of copper and returning it to its reactive form.

The developed test systems were used to rapidly determine copper in various objects. To a 1 ml sample 1–2 drops of 2 *M* HCl were added, mixed and the test strips immersed in the liquid. To determine migrating forms of copper in soils, to 10 g of dried and powdered soil 10 drops of 2 *M* HCl and 10 ml of water were added, mixed for 10–15 min and the test strip immersed in the mixture. To determine copper in natural waters paper impregnated with copper diethyldithiocarbamate was used. After passing 20 ml of analysed solution through such paper (pH 1–2) using a concentrating device a brown spot is formed on the paper whose intensity is proportional to copper concentration in the range 0.001–1 mg/l [36].

Copper detection using indicator tubes

To determine copper(II) in wastewaters, natural, potable, atmospheric waters, technological solutions, ashed foodstuffs, and biological liquids indicator tubes were proposed [64] representing a glass tube (50 ml long, inner diameter 1–2 mm) filled with indicator powder. A coloured zone proportional to the copper concentration develops in the tube on passing through it the analysed sample using a syringe. The copper concentration is quantified via a length scale or a calibration equation. The method is based on a colour reaction of copper with 1-(2-pyridyl-azo)-1-naphthol present in the indicator powder. High selectivity is obtained by conducting the reaction in acid medium. The test means includes:

1. indicator tube filled with powder;
2. ampoule containing 0.2 ml 0.1 *M* HCl (solution 1);
3. syringe;
4. test glass with ground stopper for mixing.

Analysis: Solution 1 is added to 2 ml of analysed liquid and mixed. The mixture is passed through the tube via the syringe. The violet colour zone length is measured and copper content calculated using a length scale or graduation equation.

Characteristics of the method: The analytical range is 0.06–0.3 mg/l for tubes with 1 mm inner diameter, and 0.12–0.4 mg/l for those with 2 mm inner diameter. Relative standard deviation is 0.06–0.08. Not interfering: multiple amounts of K, Na (10^4), Ca (300), Mg (75), Pb (50), Ni (40), Zn (30), Al (25), comparable amounts of Mn (II) and Co (II). The accuracy and precision of the analysis was checked by the spiked solutions.

6.2.12 Iron

For iron reagent papers were proposed containing, as a rule, 1,10-phenanthroline, bathophenanthroline or potassium ferro(ferri)cyanides [65]. Detection limit is 3 mg/l.

In the test for Fe(III) [66] the paper is impregnated with a 3% solution of ethylenediaminedihydroxybenzylphosphonic acid. In the presence of 5 mg/l Fe the paper colour is light pink, of 50 mg/l Fe it is pink, of 500 mg/l Fe red, and of 1000 mg/l Fe brown red.

A plate for detecting Fe(II) in the presence of copper and zinc ions was proposed [67,68]. On a transparent plate two layers are consecutively deposited: the reagent layer and the detecting layer. The detecting layer contains a disodium salt of 2-(5-nitro-2-pyridylazo)-5-(N-propyl-N-sulphopropylamino)phenol. The reagent layer contains a reagent masking copper and zinc and a reductant for Fe(III). Iron concentration is assessed by the colour intensity of the plate after its contact with the sample.

Iron can be determined using a chromogenic reagent strongly bound to the cellulose paper [13,69]. The colour change is from light yellow to grey-green. A broad diffuse reflection maximum lies at 400–700 nm. The analytical range is 10–200 mg/l for Fe(II,III), the iron content is found by colour matching against a standard colour scale. The test is selective.

A rapid semi-quantitative test for detecting iron in drinking water was proposed [70]. The test employs phenylfluorone and cetylpyridinium chloride bound on paper. Detection limit is 0.2 mg/l. 10 mg/l Mo, W, V, Pb, Al, Cu, Co, Ni, Cd, Sn, and Zn do not interfere.

A sensitive test method for revealing iron in natural waters is known [13,69]. To a 100 ml sample 1 ml of concentrated HCl is added and treated with acetate buffer solution (pH 3–4). The 20 ml mixture is passed through a 6 mm zone of the reagent paper based on irregular 4-chloro-1-isoquinolone-3-carbonylhydrazonyl-α-cellulose or 8-hydroxy-5,7-dichlorquinoline-1-hydrazon-α-cellulose using a concentrating device (RIS-Iron-Test(II,III)). In the presence of iron a grey violet or violet colour develops. The diffuse reflection maximum of the reagent paper is 420 nm, that of the reacted paper 580 nm. Analytical ranges

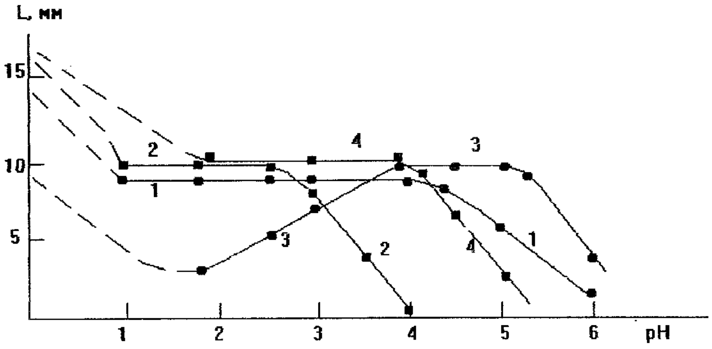


Fig. 6.2. Dependence of the coloured zone length on pH value; the paper is impregnated with $Co_2[Fe(CN)_6]$ (1), $Cu_2[Fe(CN)_6]$ (2), $Pb_2[Fe(CN)_6]$ (3), and $Ag_4[Fe(CN)_6]$ (4); iron concentration 10 mg/l.

are 0.01–0.025–0.05–0.1–0.2 mg/l. Many metal ions present in natural waters do not interfere.

A test for Fe(II,III) was proposed allowing determination of iron by the length of the coloured zone on a test strip [71] made of filter paper impregnated with precipitates of $Ag_4[Fe(CN)_6]$, $Pb_2[Fe(CN)_6]$, $Co_2[Fe(CN)_6]$, $Cu_2[Fe(CN)_6]$. The first two papers were white, the third was lilac and the fourth brownish-red. In the presence of Fe(III) blue zones with well defined boundaries appear on the test strips. For the paper impregnated with $Co_2[Fe(CN)_6]$ pH 1–4 is the optimal interval; pH 1–2.5 for the paper with $Cu_2[Fe(CN)_6]$; pH 4–5 for the paper with $Pb_2[Fe(CN)_6]$; and pH 2–4 for that with $Ag_4[Fe(CN)_6]$ (Fig. 6.2).

When iron is determined with the $Co_2[Fe(CN)_6]$ paper copper interferes (coloured zone), and Zn and Pb if their concentrations are higher than that of iron (discoloured zone). In the case of the lead salt impregnated paper Zn and Cd interfere if their amounts surpass that of iron (discoloured zone).

Because of the fact that iron is better determined in acid media (especially in natural and wastewaters), the best results were shown by the $Cu_2[Fe(CN)_6]$ paper which can be used at pH 1.0. The test is highly selective. When 1 mg/l iron is being detected no interference is observed from 1000 mg/l Cu, Zn, Pb, Cd, alkali and alkaline earth metals, Ni, Co, chlorides, sulphates, acetates, 500 mg/l Cr(III), Al, Mn(II), nitrites, 100 mg/l V(V), 5 mg/l phosphates, citrates, fluorides, Hg, oxalates, 1 mg/l sulphides. The same indicator paper was used for detecting iron in the range 0.01–1 mg/l. The analysed solution was acidified and 20 ml of it passed through the indicator paper using a concentrating test device. The iron content was judged by colour intensity of the reaction zone [36].

To determine Fe(III) at pH 1 paper impregnated with $Cu_2[Fe(CN)_6]$ was proposed. A blue coloured zone is also formed in the presence of Fe(II); 1 mg/l Fe(II) can be found without interference from 100 mg/l Fe(III). The test characteristics are analogous to those of the $Cu_2[Fe(CN)_6]$ paper [71].

Iron determination via polyurethane foam tablets [53, 72, 73]

The test means is intended for qualitative and semi-quantitative detection of iron(III) in natural waters. It is based on sorption of red coloured thiocyanate complexes by a polyurethane foam tablet. The presence of iron is revealed by the change of the tablet's colour from white to red; the iron in the sample is quantified by colour matching of the tablet against an appropriate colour scale. Analytical range is 0.02–1 mg/l. The test means includes:

1. ether-based polyurethane foam tablets 16 mm in diameter, 6 mm thick, weight ca. 0.04 g;
2. potassium thiocyanate in 1.5 g batches;
3. sulphuric acid, 2 *M* solution;
4. test glasses with ground stoppers for mixing and shaking;
5. medical pipettes, a cylinder;
6. a glass rod;
7. a colour scale;
8. filter paper.

Analysis: A batch of potassium thiocyanate is placed in the test glass and 10 ml of water and 0.2 ml of 2 M H_2SO_4 (5–7 drops) are added. All is mixed until full salt dissolution. A polyurethane foam tablet is immersed in the solution, pressed with the glass rod until all air bubbles are removed and agitated for 20 min, after which the tablet is removed and discarded. To the potassium thiocyanate solution now freed from all traces of iron, 15 ml of the water sample are added. In each test glass a PUF tablet is introduced, pressed with the glass rod until all air bubbles are removed and agitated for 15 min. The solutions are discarded and the tablets are dried between two sheets of filter paper and colour-matched against a standard colour scale.

Selectivity: The selectivity of the method was characterized by a selectivity factor, i.e., the ratio "accompanying component/iron" at which the error of measurement is no greater than 15%. Detection of 0.2 mg of iron(III) is not hampered by multiple amounts of Cr(III) (10^4), Mg, Ca, Sr, Ba ($5 \cdot 10^5$), Tl(I) (10^3), Mn(II) (500), Cd, Pb (250), Zn (75), Co, Ni (25), Cu, Hg(II), As(III) (5).

Accuracy and precision: The test means allows screening of water samples for iron(III) to be carried out and the establishing of its presence, provided its content is higher than 0.02 mg/l.

The time needed for the red colour of the tablet to develop depends on the iron(III) concentration as indicated below:

Iron(III) concentration (mg/l)	Time for the colour to develop (min)
0.05	10
0.1	5
0.2	3
0.5	1.5–2.0
1.0	1.5–2.0

Quantitative extraction of the target component into the tablet takes 15 min.

Precision of the visual test method depends on the iron content in the sample. The relative standard deviation is 0.1–0.2 at 0.05–0.2 mg/l Fe(III) contents; RSD = 0.3–0.4 at contents less than 0.05 and greater than 0.5 mg/l ($n = 6$). The precision can be improved by using a portable reflectometer "Multiecotest" from KOSTIP Co. (Russia). The calibration relationship was obtained using a series of standard solutions. Precision in this case was 0.05–0.1 at 0.0–0.2 mg/l Fe(III) concentrations and 0.15 at concentrations higher than 0.5 mg/l ($n = 6$).

The accuracy and precision of the method was verified by analysing a standard water sample GSORM-2 containing according to specification 1 mg/ml, of each: Co, Ni, REE, Cu, Cr, and Fe, diluted 1000-fold. The revealed amount of iron was: 1.0 ± 0.2 ($n = 6$, RSD = 0.05).

Determination of Fe(II) via indicator tubes [74]

To determine Fe(II) in natural and wastewaters, atmospheric precipitates and technological solutions indicator tubes can be used. The determination is based on a redox reaction between Fe(II) and 2,6-dichlorindophenol non covalently immobilized on a hydrophobic silica gel. The test means is a glass tube 50 mm long, 2 mm inner diameter, filled with indicator powder. After adding a buffer solution and a masking agent to the test solution the tube is inserted into the solution. The latter is driven into the tube by the capillary forces. After the solution fills the tube the length of the discoloured zone, which is proportional to the iron content, is measured. The element is quantified using a length scale or a calibration graph equation. The test means includes:

1. indicator tube filled with indicator powder;
2. ampoule with 0.2 ml of acetate buffer (solution 1);
3. ampoule with 0.2 ml of 0.2 *M* EDTA (solution 2);
4. test glass with ground stopper for mixing.

Analysis: Solutions 1 and 2 are added to 5 ml of test solution, mixed and one end of the tube is immersed in the solution. After the liquid has filled the tube it is removed from the solution and the discoloured zone length measured. Iron(II) is quantified using the length scale or the calibration graph equation.

Method characteristics: Analytical range is 0.1–3.0 mg/l, RSD = 0.07–0.09. Not interfering: Cu, Mn(II), Zn up to 10 mg/l, 1000-multiple amounts of SO_4^{2-}, Cl^-, NO_3^-, K, Na, NH_4^+, Ca, Mg.

Accuracy and precision: Accuracy and precision of determination were checked by the spiked solutions and by comparison with instrumental methods of analysis. Satisfactory agreement between the two approaches was found.

Determination of Fe(II) using a modified silica gel indicator powder [74]

To determine Fe(II) in drinking and natural waters one can use a hydrophobic silica gel non covalently modified with cetyltrimethylammonium chloride and 2,6-dichlorindophenol. The latter's reduction, leading to the powder decolouration, forms the basis of the test method. The test means includes:

1. test-concentrator;
2. indicator powder, 0.05 g;
3. ampoule containing 0.5 ml of 0.2 *M* EDTA solution (solution 1);
4. ampoule containing 0.5 ml of acetate buffer solution with pH 4.5–6.5 (solution 2).

Analysis: 10 ml of test solution are placed in the concentrator, solutions 1 and 2 and the indicator powder are added and all is mixed for 10 min. The concentrator is turned so that its beaker is pointing downwards and the colour of the powder in the beaker is matched against the colour scale.

Method characteristics: Analytical range is 0.05–2.0 mg/l, relative standard deviation 0.2–0.25. Not interfering: Cu, Mn(II), Zn up to 10 mg/l, 10^3-multiple amounts of SO_4^{2-}, Cl^-, NO_3^-, K, Na, NH_4^+, Ca, Mg.

Accuracy and precision: Accuracy and precision were verified by the spiked solutions and by other (instrumental) methods of analysis.

6.2.13 Germanium

For drop analysis of Ge(IV) a reagent paper with phenylfluorone [75] and another with disulphophenylfluorone and cetylpyridinium chloride were proposed [76]. In the first case the reaction is run in 0.5–2 *M* HCl with detection limit of 1.2 mg/l and in the second case sulphuric acid is employed with detection limit of 1.0 mg/l. Not interfering: 100-multiple amounts of Ti, Nb, Bi, Fe, Zr, V, Sb; Ta (50), Mo (45), Sn (1.5), Cr and W (6).

A more sensitive semi-quantitative method was proposed using a reagent paper impregnated with phenylfluorone and cetylpyridinium chloride [5]. Phenylfluorene as the least soluble of the three trioxyfluorones gives the most sensitive reaction on the paper. Ethanol solution containing 0.001 *M* phenylfluorone and 0.05 *M* cetylpyridinium was used for impregnating the paper. The paper turns yellow in the presence of germanium. To increase sensitivity 2 *M* H_2SO_4 was employed. Colour scales were prepared using standard solutions with concentrations in the range 0–0.5–1–2–3–5 mg/l. The proposed tests were applied to detect germanium in neutralizing solutions of blanks in the production of light-emitting diodes. To a 1 ml sample 10 drops of 6 *M* sulphuric acid were added and the indicator paper measuring 7 × 7 mm glued to a polymer strip was immersed in the solution for 1–2 s. In 5 min the paper was colour matched against a standard scale.

6.2.14 Mercury

To reveal mercury a paper is used impregnated with diphenylcarbazide or diphenylcarbazone producing with mercury complexes ranging in colour from violet to blue [28]. In neutral or weakly acid solutions some metals (Cu, Fe, Co, etc.) hamper analysis. To increase selectivity the reaction is usually run in 0.1 *M* HNO_3 solution. Detection limit is 2 mg/l.

Filter paper impregnated with CuI exhibits deep coloration from orange to red when acted upon by an acidified mercury solution. The reaction

$4CuI + Hg^{2+} = Cu_2[HgI_4] + 2Cu^+$

is used to selectively detect mercury [28]. Interfering are salts of Ag, Au, Pt, which interact with CuI giving partially black metallic films. Detection limit is 0.1 mg/l.

A composition made up of dithizone, thiourea and sodium acetate is often used [77,78]. Mercury is detected together with Zn, Ag, Cd, Pb, Tl(I), Cu(I), Ni, Co. Detection limit is 0.5 mg/l.

Paper impregnated with $Cu(DDTC)_2$ and $Fe(DDTC)_2$ for semi-quantitative mercury detection by test method was proposed [35]. The paper was impregnated at the first stage with 0.0005 *M* solution of $CuSO_4$ or $FeCl_3$ and at the second stage with a 0.001 *M* DDTC solution.

The paper impregnated with $Cu(DDTC)_2$ allows mercury detection at pH 3–5 and that with $Fe(DDTC)_2$ at pH 1–4. The brown test strips become decolorised in the presence of mercury. Detection limit is 1 mg/l (discoloured zone length is 0.5 mm). In the first case (the $Cu(DDTC)_2$ paper) detection is hampered by Ag; in the second by Bi(III), Sb(III), In, Pb(II), Co, and sulphides.

A rapid test for detecting 0.2 ppm Hg in solutions at pH 1–6 is described in Ref. [60] based on colour change of polyurethane foam modified with lead dithizonate. Two variants, static and dynamic, were proposed. In the first variant 1–2 ml of the analysed solution are mixed with the foam; in the second 100 ml of the solution are passed through a column filled with the foam. The element is quantified by colour matching the foam against a standard colour scale prepared using 0.2–1.0–5.0–10 ppm Hg(II) standard solutions. Bi and Ag interfere.

To determine mercury and diphenylmercury a polyurethane sheet 35 × 10 × 2 mm impregnated with dithizone was proposed [70]. To a 100 ml sample masking agents are added and agitated with the sheet for 10 min. Then the sheet's colour is measured visually or on a spectrophotometer at 485 nm. Detection limits of Hg and phenylmercury are 0.005 and 0.01 mg/l, respectively.

A highly mercury selective reagent indicator paper RIS–Hg–Test was proposed [13] based on a colour reaction of Hg(II) with a covalently bound cellulose reagent. The paper colour changes from light yellow to grey-black. A 40 ml analysed solution is passed during 10 min through a 6 mm diameter reaction zone using a concentrating device. The colour of the reaction zone is matched against a standard colour scale. Analytical ranges are 0.02–0.1–0.3–1–2 mg/l. Ten-multiple amounts of chlorides interfere.

A more mercury sensitive and chloride selective paper was proposed [36]. This is very important as in natural waters there is a large variation of chlorides content. A filter paper was impregnated with a copper salt and dithizone solution. Detection of mercury was done in 0.2–0.5 *M* HCl as ensuring selectivity. To a 20 ml sample 30–40 drops of 2 *M* HCl were added, mixed, sucked into a syringe and passed through a test device with the dithizonate impregnated paper. The paper was colour matched against a colour scale. Analytical ranges were 0.001–0.005–0.1–0.5–1 mg/l.

Dithizonates of iron, zinc, nickel, and cadmium precipitated on paper are destroyed in acid medium while those of copper are not [35]. Taking into consideration that acid medium is preferable for detecting heavy metals in natural and wastewaters as in it metal-organic complexes are destroyed, copper dithizonates were chosen for mercury detection. The paper impregnated with copper dithizonate has a grey colour. In the presence of Hg(II) a red zone develops on the test strip glued in a polymer film. In 0.1–1 *M* HCl, H_2SO_4 or HNO_3 solutions the length of the coloured zone remains practically constant. If the acids' concentration is higher than 1 *M*, the red mercury zone becomes blurred, and for HCl, HNO_3 concentrations lower than 0.1 *M* the length of the coloured zone decreases. The graduation graph equation is $y = 1.95\ x^{0.62}$ in the range 0.1–200 mg/l Hg. The test system is highly selective; no interference is observed from the following multiple quantities of ions: alkali and alkaline earth elements (1000), Al, Sc (1000), Zn (200), Cd (500), Ti(IV) (100), Zr(IV) (50), PO_4^{3-} (100), Cl^- (1000), SO_4^{2-} (1000), Sb(III) (50), Cu (1000), Ni (200), Pb(II) (1000), NO_3^- (1000), CH_3COO^- (1000). Ag and sulphides interfere.

A number of tests were developed based on Hg(II) inhibiting effect on the immobilized horseradish peroxidase in the reactions of oxidation by H_2O_2 of *o*-dianisidine, *o*-phenylenediamine or 3,3′,5.5′-tetramethylbenzidine (TMB) [80]. The detection limits of the systems are: *o*-dianisidine/polystyrene, 10 ng/l; *o*-phenylenediamine/polystyrene, 50 ng/l; *o*-phenylenediamine/paper, 40 ng/l; TMB/polystyrene, 0.1 ng/l; TMB/paper, 100 ng/l. One can also use non-catalytic systems: silica gel-based tablets with dithizone, detection limit (c_{min}) being 3.0 mg/l (pH 3–5) or ion exchanger AV-17 with PAR, $c_{min} = 0.01$ mg/l (pH 5.9–8.8).

Detection of methyl-, ethyl-, and phenylmercury [81]

One of the variants is based on a liberating action of these compounds in oxidation of *o*-dianisidine by H_2O_2 on the phenylthiourea inhibited

TABLE 6.9

Test methods for the determination of organomercury compounds (variant A)

Compound	Immobilized HRP support	Analytical range (μ*M*)	Detection limit (μ*M*)	RSD
Methylmercury	Polystyrene	20-200	20	0.23
	Paper	1-100	1	0.19
	PUF	0.007-0.5	0.007	0.11
Ethylmercury	Paper	10-1000	10	0.18
	PUF	0.01-0.5	0.01	0.12
Phenylmercury	Paper	10-1000	10	0.18
	PUF	2.5-500	.5	0.11

TABLE 6.10

Test methods for the determination of organomercury compounds (variant B)

Test compound	Analytical range (μ*M*)	Detection limit (μ*M*)	RSD
Methylmercury	5-1000	5	0.3
	0.01-5	0.01	0.16
Ethylmercury	100-1000	100	0.26
	1-50	1	0.09
Phenylmercury	100-1000	100	0.23
	75-1000	75	0.11

horseradish peroxidase immobilized on various supports: polystyrene plates, chromatographic paper, polyurethane foam (variant A). Pertinent information about the methods is given in Table 6.9.

Another variant (variant B) is based on the interaction of mercury compounds with diethyldithiocarbamate — a second peroxidase substrate in the reaction of oxidation of 3,3′,5,5′-tetramethylbenzidine; inclusion of this substrate in the indicator reaction causes an induction period to appear on kinetic curves whose length is inversely proportional to the concentration of mercury organic compounds. The reaction is conducted on paper with the immobilized enzyme (Table 6.10).

6.2.15 Indium

Indium detection is based on a colour reaction with formazans chemically bound to the cellulose paper [13]. A yellow indicator paper becomes blue-green in the presence of indium (diffuse reflection maximum at 605 nm). Analytical range is 0.01–0.2 mg/l in the method of passing a 20 ml test solution through the indicator paper. The reaction is selective at pH 1–2; alkali, alkaline and rare earth metals do not interfere.

6.2.16 Potassium

To determine 250–1500 mg/l potassium in water and soil indicator paper with dipicrylamine was proposed (Merckoquant®, E. Merck). After treating the reaction zone of the paper with an alkaline test solution it is acted upon by nitric acid; an orange colour develops in the presence of potassium. To determine 10–250 mg/l K in natural waters croconic acid is immobilized on the filter paper [82]. Analysis is hampered by sodium.

A phototurbidimetric approach was realized in the VISOCOLOR® system (Macherey-Nagel) using tetraphenylborate producing a white precipitate with potassium [8].

6.2.17 Manganese

To determine Mn(II) its complex reactions with organic compounds are used, as well as kinetic reactions in which Mn(II) catalyses oxidation of organic reagents, and the reaction of Mn(II) oxidation to permanganate.

Formaldoxime forms with manganese a colourless complex rapidly acquiring a red brown colour due to oxidation by air oxygen giving the complex $[Mn(CH_2NO)_6]^{2-}$; the reaction is used in a VISOCOLOR® system (Macherey-Nagel) for detecting Mn(II) in the range 0.03–4 mg/l. Do not interfere 300 mg/l Ca and Mg, 200 mg/l Zn and phosphates, 20 mg/l Fe, 2 mg/l Cr, Co, Cu.

To determine manganese in the range 0–0.3 mg/l 1-(2-pyridylazo)-2-naphthol is used forming with Mn(II) a red complex (HANNA INSTRUMENTS Inc.).

Oxidation of Mn(II) with periodate is used in test kits for water analysis (Hach Co, Hellige Co, CHEMetrics):

$3H_2O + 2Mn^{2+} + 5IO_4^- = 2MnO_4^- + 5IO_3^- + 6H^+$.

The colour intensity is measured using a miniphotometer or visually via a comparator with colour discs. Analytical range is 0.1–50 mg/l.

In kinetic methods Mn(II)-catalysed oxidation of Malachite green by KIO_4 is used. Thus, the indicator paper contains a mixture of KIO_4 with $BaSO_4$, gelatine, phosphate buffer, 1,10-phenanthroline, nitrilotriacetic acid, triton X-100, Malachite green, Rhodamine 6G and dimethylglyoxime [83]. The time needed for the colour change of the indicator zone is measured. The test was applied for detecting Mn(II) in drinking water in the range 0.03–0.5 mg/l.

The indicator paper for detecting Mn(II) in the ranges 5–25–100–500 mg/l contains *o*-tolidine, tartaric acid and barium sulphate (Merckoquant®, E. Merck). To detect Mn(II) the paper indicator zone is first immersed in the test solution, then in 1–2 *M* NaOH solution for a few seconds and lastly in 10% acetic acid solution. In the presence of Mn(II) the paper turns blue [37].

A rapid Mn(II) determination was proposed based on Mn-catalysed oxidation of 3,3′5,5′-tetramethylbenzidine by periodate. The analysed solution is pumped through a DETATA filter, reagent solutions are applied to the filter and 3 min later the colour is observed and matched against a colour scale. Analytical range is $5\cdot10^{-6}$–$5\cdot10^{-4}$ μg/ml in which concentrations of Mn differing by half an order of magnitude can be distinguished. Do not interfere multiple amounts of Zn(II) (150), Cl^- (500), K, Na, Ca, Mg, Al, Fe(II,III), Cu, Br^-, SO_4^{2-} (700–1000).

Drop analysis: The principle of the method is the same as above. An aliquot of the test solution is placed on top the plate for thin layer chromatography (Sorbton-8 with grafted C_8-groups) and then one proceeds as above. In the range $4\cdot10^{-3}$–0.3 μg/ml Mn concentrations differing threefold can be visually distinguished.

6.2.18 Molybdenum

Drop reactions on paper were proposed for molybdenum and wolfram [75]. Molybdenum is revealed by a carmine-red coloured spot on the paper impregnated with salicylfluorone and KF after applying to it a strongly acidic test solution. Wolfram detection is based on its reduction by $SnCl_2$ to blue-coloured oxides of the lower oxidation states.

An indicator plate for semi quantitative detection of Mo was proposed [85]. It has the form of a water insoluble support impregnated with a mixture of hydroxylamine and gallic acid. In the presence of molybdates an orange to brown colour develops depending on molybdenum concentration.

Paper-immobilized ion associates of 4-(2-thiazolylazo)pyrocatechol (TAP) and cetylpyridinium (CP), viz. TAC$(CP)_2$ and those of CP and phenylfluorone (FF), viz. FF$(CP)_2$ can be used for Mo(YI) detection via the length of the test strip colour zone [85]. For the paper impregnated with FF$(CP)_2$ a constant length of the pink-lilac zone on the yellow test strip is observed at pH 2–5 (acetate–ammonium buffer). Test strips containing TAP$(CP)_2$ allow detection of molybdenum by a red zone on the yellow paper. Molybdenum can be revealed in the presence of 0.01 *M* NaF and 0.01 *M* EDTA without interference from 100-multiple amounts of alkali and alkaline earth elements, Al, Zn, Cd, Fe; interfere V(V), Ge(IV), Sn(IV), Sb(III), Bi(III). For molybdenum detection one can also use paper bound FF; at pH 3–5 (acetate buffer) a pink red colour develops on the yellow reaction zone in the presence of Mo(VI) which is visually observable in the ranges 0.01–0.05–0.1–0.5–1 mg/l.

Indicator tubes with indicator powder, e.g. hydrophobic silica gel impregnated with Lumogallion allow detection of 4–12 mg/l of molybdenum via the length of a red violet complex zone.

6.2.19 Sodium

Croconic acid forms with sodium a red precipitate. Visual evaluation of the paper colour can be done in the range 40–750 mg/l Na [82]. Analysis is hampered by other alkali metals.

A method of revealing sodium, calcium and lanthanum salts in natural and wastewaters using indicator tubes was proposed [82]. The tubes are filled with ion exchanger of particle size 10–500 μm treated by Quinaldine Red and thymolphthalein. The test solution is passed through the tube in which consecutive coloured zones are formed (ranging from 0.5 to 25 mm) whose length is proportional to the ions concentration.

6.2.20 Nickel

In all the known nickel test methods dimethylglyoxime or diacetyldioxime are used which form with Ni(II) in alkaline medium a poorly soluble red complex and with Ni(III) a brown soluble complex. In the latter case ammonium peroxodisulphate, bromine or iodine are used as oxidants [5,87,88]. The reagents are employed either in the form of powders, solutions or are immobilized on paper or, less frequently, on polyurethane foam. There are various modes of Ni contents quantification, e.g.: paper immersion in test solution and reading the result; visual estimate of the colour intensity of test solutions after adding reagents; estimate of the coloured zone length on test strips glued in a polymer film; estimate of the area of the coloured spot on a paper disc glued in a polymer film and contacting the test solution through a hole in the film. In colorimetric methods the nickel's analytical range is 0.02–10 mg/l, in those of area or length estimates it is 1–500 mg/l, and 10–500 mg/l in methods of paper immersion and reading the result.

Indicator papers Merckoquant® (E. Merck), QUANTOFIX® (Macherey-Nagel) are consecutively impregnated with 0.1–1% dimethylglyoxime solution, 5–12% sodium thiosulphate solution, 3–8% KF solution, 5–10% tartaric acid solution and 0–3% hydroxylamine solution. This paper is noted for its high selectivity and broad range of action (pH 2–7) [87]. Test methods are used for detecting nickel in natural waters and soil extracts.

Nickel determination using polyurethane foam tablets [89]

The test means can be used for qualitative and semi-quantitative testing for nickel in natural waters. It is based on nickel sorption from ammonium buffer solution by the PUF tablet modified with dimethylglyoxime in the presence of a plasticizer. The presence of nickel is evidenced by a colour change of the PUF tablet from white to pink; nickel is quantified by colour. Analytical range is 0.2–4 mg/l. The test means includes:

1. ether-based PUF tablets 16 mm in diameter, 6 mm thick, weight approx. 0.04 g, modified with dimethylglyoxime in the presence of plasticizer (trioctylamine);
2. ammonium buffer solution with pH 9;
3. test glasses with ground stoppers for mixing;
4. medical pipettes, a cylinder;
5. glass rod;
6. colour scale;
7. filter paper.

Analysis: In each test glass a 15 ml water sample is placed and 10 ml of buffer solution added. One PUF tablet is put in each test glass, pressed with the glass rod until all air bubbles are removed and agitated for 30 min. Solutions are discarded and the tablets dried between sheets of filter paper and colour matched against the colour scale.

Selectivity: Selectivity was characterized by the limit ratio: accompanying compound/nickel at which the error of measurement is under 15%. Do not interfere (1 mg/l Ni) multiple amounts of Ca, Mg, acetate, tartrate (5000), fluorides (1000), oxalate (500), Cu(II), Zn(II), Cd(II), Cr(III), Fe(III), hydrogen phosphate (100), Pb(II) (10), Co(II) (5).

Accuracy and precision: The test means allows to screen water samples for nickel and confirm its presence at concentrations higher than 0.1 mg/l.

The time needed for the pink colour of the PUF tablet to fully develop is dependent on the nickel content in the sample:

Nickel content (mg/l)	Time of colour development (min)
0.2	10
0.5	5
1	1–2
2	0.5
4	0.5

Extraction of nickel into the tablet is completed in 30 min.

Precision of the test method depends on the nickel content in the sample. At nickel content 0.2–1 mg/l the relative standard deviation RSD = 0.2–0.3, at nickel content out of this range RSD = 0.4–0.5 ($n = 6$). Precision of the method can be improved by the use of a portable reflectometer "Multiecotest" from Kostip Co. (Russia). Using a calibration graph equation RSD = 0.05–0.10 was found for the range 0.2–1 mg/l and RSD = 0.15 for the nickel content out of this range. The validity of the test method was confirmed by the analysis of a standard water sample GSORM-2, containing according to specification 1 mg/ml of each: Co, Ni, Sr, Cu, Cr, Fe and diluted 1000-fold. The amount of nickel found was $C_{Ni} = 1.03 \pm 0.06$ μg/ml ($n = 6$, RSD = 0.05).

6.2.21 Lead

Lead, along with other heavy metals, was determined by means of a reagent paper impregnated with a solution containing 10 mg dithizone, 0.5 mg thiourea, 6 g sodium carbonate, and 1 g polyvinyl alcohol in 50 ml ethanol and 50 ml water [77,78]. Detection limit was 0.5 mg/l.

Lead in the range 20–500 mg/l is detected using filter paper with sodium rhodizonate wherein a violet or purple red precipitate is formed. Detection limit is 2 mg/l. Ba, sulphides and sulphites in amounts greater than 10 mg/l (Merckoquant,® E. Merck) interfere.

The system NANOCOLOR® Lead 5 uses 4-(2-pyridylazo)resorcinol forming with lead a red complex. To mask interfering elements cyanides are employed. Analytical range is 0.1–5 mg/l.

The system NANOCOLOR® Lead detects lead in the range 0.005–1 mg/l using extraction-photometric dithizone method in the presence of cyanides.

A method using the coloured zone length of a test strip with the formation of a black lead sulphide was proposed [5]. The solubility series for the sulphides is given below:

$MnS > FeS > NiS > CoS > ZnS > SnS > CdS > PbS > Sb_2S_3 > Bi_2S_3 > CuS > Ag_2S > HgS$.

Of all the precipitates suitable for use in test systems the most acceptable is white zinc sulphide in which zinc is replaced by lead giving a black lead sulphide. The test strips measure 3×80 mm. The coloured zone length is constant at pH 4–8 and decreases at pH > 8 and pH < 4. At an optimum pH the dependence of the length of the coloured zone on lead concentration was found for the range 1–500 mg/l. The graduation graph equation has the form $y = 0.81\, x^{0.60}$. The system is non-selective, Sb, Bi, Cu, Ag, Hg interfere, also forming with lead black precipitates. The method can be used if the interfering elements are absent or have been removed.

A test method using reagent paper RIS-Lead-Test was proposed [13]. The paper has a yellow colour changing to a brown colour in the presence of lead. A 20 ml test solution is passed through the paper reaction zone 6 mm in diameter by means of a concentrating device. Analytical ranges are 0.01–0.02–0.05–0.1–0.2 mg/l. The test is poorly selective, interfere Cu, Zn, Cd, phosphates, sulphides.

A more selective and easy to prepare reagent paper was proposed [36]. The paper is soaked in 0.04 M $ZnSO_4$, dried and soaked in 0.04 M Na_2S solution; Zn is precipitated. On passing a 20 ml test solution containing lead through such a paper a deep brown colour develops whose intensity is proportional to the lead concentration.

Contrary to other poorly soluble compounds, lead sulphide is formed in acid and strongly alkaline media:

$Pb^{2+} + ZnS = PbS\downarrow + Zn^{2+}$

$Na_2PbO_2 + ZnS = PbS + Na_2ZnO_2$

Taking this into account, the reaction was conducted in alkaline medium. The maximum coloration of the reaction zone was observed in 0.05–0.1 *M* NaOH. To separate hydroxides from other metals a filter paper gasket was used in the test device, whereby insoluble hydroxides of copper, silver, mercury, bismuth and antimony are arrested by the filter paper and their 1 mg/l amounts do not hamper the analysis. 500-multiple amounts of alkali and alkaline earth elements, Al, Zn, Cd, chlorides, nitrates, 50-multiple amounts of phosphates and sulphates do not interfere.

A standard colour scale was prepared for lead concentrations 0.01–0.05–0.1–0.5–1 mg/l. The system was employed to reveal lead in natural water. To a 20 ml sample 1 ml of 1 *M* NaOH is added, mixed, sucked into a syringe and passed through a test device at the rate of 5–8 drops/s. In the test device two paper gaskets are mounted: the first for holding up the precipitate and the second impregnated with ZnS. The test device is taken apart and the colour of the second paper compared with that of the colour scale.

Lead determination by means of indicator tubes [90]

Indicator tubes 50 mm long and 1 mm inner diameter filled with indicator powder were proposed for detecting lead in wastewaters and technological solutions. The test is based on a colour reaction between lead and sodium rhodizonate followed by sorption of the coloured product by the tube's indicator powder. After adding a complexing agent to the sample under test and passing the obtained coloured solution through the tube via a syringe a coloured zone appears in the tube whose length is proportional to the lead content in the sample. The latter is quantified using a length scale or a graduation graph equation. The test means includes:

1. indicator tube filled with indicator powder;
2. ampoule containing 0.5 ml $5\cdot10^{-4}$ sodium rhodizonate solution (solution 1);
3. ampoule containing 0.5 ml of tartrate buffer solution with pH 3.6–3.8 (solution 2);
4. syringe;
5. test glass with ground stopper for mixing.

Analysis: To 2 ml of test sample solutions 1 and 2 are added and mixed. The obtained coloured solution is passed through the tube by means of the syringe. The length of the violet pink zone is measured and the lead content quantified using a length scale or a calibration graph equation.

Method characteristics: Analytical range is 10–80 mg/l; relative standard deviation is 0.06–0.08. Multiple amounts of Na, K, Ca, Mg, Ba (2500), chlorides (1000), Zn, Sr (100), Fe, Mn, Cd, Cu, sulphates (10) do not interfere.

Accuracy and precision: Accuracy and precision of the test system were checked by the spiked solutions and by the use of instrumental methods of analysis. Good agreement between the two approaches was found.

6.2.22 Palladium

Palladium can be revealed by a visual estimate of the colour intensity of indicator papers with chemically immobilized formazan groups or of that of polyacrylonitrile discs with immobilized nitrosodiethylaniline after passing through them a 20 ml test solution. Analytical range is 0.01–5 mg/l Pd [13,48].

To determine 0.06–0.7 mg/l Pd an indicator powder with immobilized 4-(2-thiazolylazo)resorcinol was used. The Pd content is determined by the colour intensity of the powder after its contact with the test solution [62].

6.2.23 Titanium

A test means employing polyurethane foam tablets was developed for qualitative and semi-quantitative determination of titanium in water samples [46]. It is based on sorption of yellow titanium thiocyanate complexes by the PUF tablet, whereby the latter goes from white to yellow. The titanium content is quantified by colour matching against a colour scale. Do not interfere multiple amounts of Mg, Ca, Sr, Ba ($5 \cdot 10^5$), Mn(II) ($8 \cdot 10^3$), Ni ($4 \cdot 10^3$), Cd(II), Pb(II) ($2.5 \cdot 10^3$), As(III) (500), Co(II) (150), Bi(III) (100), Cr(III) (10). Analytical range is 0.03–0.5 mg/l Ti.

6.2.24 Uranium

As test reagents PAN, PAR and 5-Br-PAAP [91–93] were proposed, as well as TAR from the thiazole-group compounds [52]. In the presence of TGTA, NaF and sulphosalycilic acid at pH 6–8 the reaction is practically selective. The detection limit is 0.1 mg/l but it can be lowered by an order of magnitude via sorption of a U(VI) complex with, e.g., PAR on cation exchanger KU-2; using 0.3 g KU-2 and 10 ml of water sample one can get the enrichment factor of about 30. When using PAN and 5-Br-PAAP the complex can be sorbed on silica gel S-120. To reveal very low uranium contents, for example, in natural waters a few grains of KU-2 can be used for concentrating the uranium-PAR complex while viewing the colour through a lens.

6.2.25 Zinc

For zinc determination a paper was proposed impregnated with a solution containing 50 mg dithizone, 1 g thiourea, 8 g sodium carbonate, 2 g gelatine, 50 ml ethanol, 50 ml water [78]. Detection limit is 0.5 mg/l.

Reagent papers Merckoquant® (E. Merck) were proposed for zinc determination in the ranges 10–40–100–250 mg/l [37]. The test is based on the formation in alkaline medium of a Zn-dithizone complex.

In Refs. [13] and [94] the zinc determination is based on a colour reaction of Zn with a polydentate reagent strongly bound to the cellulose paper whose colour changes from yellow orange to dark red depending on the zinc content (at pH 6–10). The diffuse reflection maximum of the reagent paper lies at 440 nm and that of the reacted paper at 500 nm. Analytical ranges are 5–10–20–50–100 mg/l for a paper dipped into the test solution.

From different solubilities of dithizonates, it was found out that of all the dithizonates precipitated on paper only Cr(III) and Mn(II) dithizonates can be decomposed by zinc resulting in a scarlet zone on the test strip glued in a polymer film [34]; 2 mg/l of Zn can be detected without interference from 1000 mg/l of alkali and alkaline-earth metals, 500 mg/l of aluminium, chlorides, sulphates, 100 mg/l of lead, phosphates, fluorides, ammonium, 10 mg/l of iron(II,III), copper, 2 mg/l of nickel, and 1 mg/l of cadmium. Mercury and silver interfere. If the test solution contains more than 10 mg/l of iron and copper, two coloured zones are formed — the lower brownish and the upper a pink one corresponding to zinc; the latter's length is equal to that formed in the absence of interfering elements. This makes possible determination of zinc using the pink zone even if more than 10 mg/l Fe and Co are present.

A test method using polyurethane foam cubes with immobilized dithizone was proposed [65]. The cube with a 4 mm rib is shaken with 1–2 ml of analysed solution at pH 6.2 for 1–2 min. The red colour produced is matched against the colour scale. Detection limit is 0.05 mg/l.

Zinc can also be determined using the paper RIS FMOPF-6-TS [13, 95]. A covalently immobilized reagent of yellow-orange colour gives with zinc a dark red colour at pH 6–10. The bathochromic shift in the diffuse reflection is 60 nm. Analytical ranges are 0.001–0.005–0.01–0.05–0.1 mg/l for a 20 ml solution passed through a paper reaction zone 6 mm in diameter using a concentrating device. Interference from Ni, Co, and Cu is excluded by adding KCN or a 5-fold amount of EDTA. Cadmium also gives colour reactions (a red-pink colour). Analytical ranges are 0.001–0.005–0.01–0.05–0.1–0.5–1 mg/l. The reagent paper impregnated with chromium(III) dithizonate was used for testing samples of natural waters and atmospheric precipitates.

6.3 ANIONS

6.3.1 Bromides

To determine bromides the reaction of bromine with fluoresceine is employed [28]. First bromides are oxidized to free bromine with lead dioxide in acetic acid medium. Bromine transforms yellow fluoresceine into red tetrabromofluoresceine. Chlorides do not interfere. Iodides form a red tetraiodofluoresceine. Sensitivity of the reaction is not high, detection limit is 30 mg/ml.

Paper impregnated with silver chromate, silver ferricyanide and silver chloride can be used to determine bromine via the coloured zone length [96]. The first and the second papers can be used if the interfering chlorides are absent. The third is used for bromide detection in the presence of chlorides. For the paper impregnated with silver chromate the optimum pH range is 4–8, for that with silver ferricyanide pH is 3–10.

The above systems can be used in the absence of chlorides and iodides or for detecting their sum total with bromides. To exclude interference from chlorides one can use a paper impregnated with AgCl in which case 1000-multiple amounts of phosphates, sulphates, selenites, chlorides, equal amounts of thiocyanates and ferrocyanides do not interfere. Iodides and sulphides do interfere. The method can be applied to determine bromides in exhausted developing solutions and wastewaters. To detect high bromide concentrations a test SALTESMO (Macherey-Nagel) was proposed based on radial chromatography [8]. Analytical range is 0.45–8.8 g/l NaBr.

6.3.2 Chlorides

To determine chlorides papers impregnated with Ag_2CrO_4 are often used. In Ref. [97] the paper was treated with 2% potassium dichromate solution, dried and then separate drops of 0.1, 0.05, 0.025, 0.12 *M* $AgNO_3$ solution were deposited on it. A 3 μl test solution was applied to each spot and disappearance of the red spot was monitored. Relative error of analysis is 2%.

To detect chlorides in solutions a "Quantab titrator" is sometimes used representing a T-shaped capillary tube [98–101] whose short end is filled with an indicator signalling the end of reaction by the change of its colour. The longer part of the tube is filled with silver chromate. The chloride concentration is determined by the length of the discoloured capillary zone on its immersion in the test solution. Compositions have been developed for determining chlorides in the ranges 30–180 mg/l, 30–600 and 300–6000 mg/l.

To detect chlorides in natural, waste and technological waters indicator reagent test strips RIS-Chloride-Test were proposed [13]. Chloride ions interact with a black brown complex forming a yellow coloured indicator. Analytical ranges at pH 1.5–2 are: 5, 10, 20, 30, 50, 100, 250, 500 and 1000 mg/l. In the range from 50 to 1000 mg/l the indicator paper is dipped into the analysed solution and then colour matched with a colour scale. In the range 5–50 mg/l a 3 ml test solution is passed through the paper's reaction zone 6 mm in diameter using a concentrating device and then the obtained colour matched against a colour scale.

To determine chlorides by means of the length (area) of the test strip glued in a polymer film filter paper impregnated with silver chromate and silver ferricyanide was used [96]. In the first instance the paper had a red brown colour and in the second an orange one. The chlorides detection limit depends on the concentration of impregnating solutions:

the lesser is the silver chromate and silver ferricyanide content in the paper the lower chloride content can be detected. On the other hand, the minimum content of the silver salt on the paper is limited by the possibility of viewing the colour zone.

Acidity of the analysed solution influences the detection of chlorides. In acid and alkaline media ($8 < pH < 4$) Ag_2CrO_4 is decomposed and the paper discoloured as in the case of AgCl formation. For the precipitate $Ag_3[Fe(CN)_6]$ this happens in the range of $pH > 10$ and $pH < 3$. The pH range 4–8 should be used for detecting chlorides by means of the paper impregnated with Ag_2CrO_4 and the pH range 3–10 when the paper impregnated with $Ag_3[Fe(CN)_6]$ is used. For the latter detection limit is 50 mg/l and for the former it is 0.1 mg/l with the discoloured zone length equalling 0.5 mm. Chloride detection is hampered by ferrocyanides, thiocyanates, bromides, iodides; these anions form discoloured zones on the test strip. Not interfering are active chlorine (up to 3 mg/l) and organic substances present in natural and wastewaters (COD_5 up to 100 mg O_2/l). No sample preparation and filtering are needed for detecting chlorides in natural, wastewaters and atmospheric precipitates.

To determine chlorides in soil 10 g of air-dried and powdered soil is collected, 10 ml of distilled water added and one end of the test strip dipped into the obtained suspension. After the liquid has reached the upper end of the test strip the latter is withdrawn and the discoloured zone length measured. The chloride content is quantified using a calibration graph or a table.

In the systems VISOCOLOR® (Macherey-Nagel) and Aquamerck® (E. Merck) mercurimetric titration was used with an iron(III) salt as indicator:

$$2Hg(SCN)_2 + 6Cl^- = HgCl_2 + HgCl_4^{2-} + 2SCN^-$$

$$SCN^- + Fe^{3+} = Fe(SCN)^{2+} \text{ (red)}$$

Analytical range is 1–60 mg/l.

In systems of colorimetric analysis VISOCOLOR® ECO, NANOCOLOR® (Macherey-Nagel) and Aquamerck®, Microquant® (E. Merck) an analogous reaction is used; analytical ranges are 5–500, 0.5–50, and 0.2–125 mg/l.

In test systems Tirets® (CHEMetrics), Hach Chemical Co. diphenylcarbazone is used as indicator forming a complex with Hg(II) which is decomposed on introduction of chloride anions.

In test methods the Mohr method has also been used with $AgNO_3$ as titrant and sodium chromate as indicator (CHEMetrics and Hach Co.).

To determine high chloride contents the method of radial chromatography SALTESMO (Macherey-Nagel) was used for the range 0.25–5 g/l NaCl. The indicator paper was impregnated with Ag_2CrO_4. The content was found by the discoloured area of the indicator paper glued in a polymer film after its contact with the test solution through a hole in the film.

6.3.3 Cyanides

Cyanides can be determined using reagent paper impregnated with CuS [28]. In the presence of cyanides the black precipitate of CuS becomes discoloured:

$$2CuS + 10KCN \Rightarrow 2K_3[Cu(CN)_4] + (CN)_2 + 2K_2S$$

Iodides, bromides, chlorides, thiocyanates, ferrocyanides do not interfere. Detection limit is 25 mg/l cyanides.

For detecting cyanides by the coloured zone length papers were proposed impregnated with copper (zinc) dithizonate [34]. In the presence of cyanides at pH 6–10 a yellowish almost colourless zone appears. Analytical range is 0.5–500 mg/l CN^-. Free cyanides can be detected without interference from complex cyanides, thiocyanates, chlorides, sulphates, nitrates. The test systems were used to detect cyanides in washing waters of galvanic baths.

There are sensitive tests based on combinations pyridin–pyrazolon (or pyridine)–barbituric acid inducing the formation of polymetin dyes. At first chlorine evolving from chloramines T oxidizes cyanides to chlorocyan: $2CN^- + Cl_2 = 2CNCl$ which reacts with pyridine and after hydrolysis of the resulting compound glutaconic aldehyde is obtained:

$$CNCl + C_5H_5N \Rightarrow C_5H_5NCN^+ + 4H_2O \Rightarrow O{=}CH\text{-}CH{=}CH\text{-}CH_2\text{-}CH{=}O$$

Glutaconic aldehyde reacts with pyrazolone to form a blue dye. The system is used in colorimetric kits of Hach Co., HANNA Instruments. Cyanide analytical ranges are: 0.01–0.2 and 0.005–0.2 mg/l.

The methods employing pyridine and barbituric acid are used in colorimetric kits of such firms as VISOCOLOR® (0.002–0.4 mg/l CN^-), NANOCOLOR® (0.001–0.5 mg/l CN^- and in indicator papers Merckoquant® (1–30 mg/l CN^-). In CHEMets® (CHEMetrics) isonicotinic and barbituric acids are used (0.005–0.1 mg/l).

6.3.4 Chromates

Chromium(VI) is detected by test methods most often using diphenylcarbazide (DFC), e.g. immobilized on ion exchanger [28] or polyurethane foam [102]. In the first instance Cr(VI) is detected by depositing a drop of test solution on paper impregnated with resin; a red violet colour develops in the presence of Cr(VI). Detection limit is 0.5 mg/l. In the second case a chromium complex is absorbed from test solution by the PUF. Colour intensity is measured by diffuse reflection spectroscopy. Detection limit is 0.05 mg/l.

To determine 0.1–500 mg/l Cr(VI) RIS–Chromate–Test was proposed [13]. Chromates oxidize a colourless indicator to a brown-black product.

A test allowing detection of 0.02 mg/l Cr(VI) was proposed [5]. The paper is impregnated with a saturated alcoholic DFC solution, dried and cut in 7 × 7 mm pieces. To a 1 ml test solution 4 drops of 2 *M* HCl solution are added and one paper piece placed into the solution. In the presence of Cr(VI) the liquid assumes a lilac violet colour which is matched against a colour scale prepared for Cr(VI) concentrations 0.02–0.05–0.1–0.5–1–3–5 mg/l. In acid medium, 1000-fold amounts of alkali and alkaline earth metals, Al, Zn, Cd, Fe, 100-fold amounts of Mo, Hg, V, Co, and Ni do not interfere. Chlorides, sulphates, phosphates, fluorides, acetates, and nitrates do not interfere. The test was used for detecting Cr(VI) in waste and natural waters.

In systems VISOCOLOR®, NANOCOLOR® (Macherey-Nagel), CHEMetrics diphenylcarbazide is used. For oxidizing Cr(III) ammonium peroxodisulphate is employed. Detection limit is 0.01 mg/l CrO_4^{2-}.

6.3.5 Fluorides

Decolouration of zirconium complexes by chromogenic reagents, e.g. alizarin red C is used in test methods as well as in photometry to detect fluorides [28,103]. Zirconium complex with alizarin in acid solutions has a red-brown colour in an excess of reagent and a violet colour in excess of zirconium. The complex becomes decolorised in the presence of fluorides, phosphates, arsenates, sulphates, thiosulphates, oxalates and other organic acids. Detection limit for paper tests is 20 mg/l.

An indicator system zirconium-alizarin red C was proposed for detecting fluorides via the length of the discoloured zone of test strips glued in a polymer film [96].

Fluorides can be revealed in the pH range 1–3 where a pH independent decolouration zone is observed. At pH < 1 the zirconium–alizarin complex decomposes and the paper loses colour. At pH > 3 the discoloured zone length shortens and at pH > 5 the effect is not seen at all. The test was conducted at pH 1.0 as in acid medium many fluoride complexes decompose. Analytical range is 0.05–50 mg/l. At pH 1.0 the following do not interfere: 1000-multiple amounts of alkali and alkaline earth metals, Co, Ni, Cu, Zn, Cd, nitrates, 500-multiple amounts of Hg, chlorides, phosphates, Sc, Cr(III), Mn(II), 100-multiple amounts of sulphates, thiosulphates, 20-multiple amounts of ammonium, Fe, 10-multiple amounts of oxalates and tartrates. The test method has been recommended for detecting fluorides in natural, wastewaters, and soils. To 10 g of dried and powdered soil 8 drops of 2 *M* HCl solution and 10 ml of water are added and mixed for 10–15 min. Then test strips are dipped in the mixture.

A NANOCOLOR® (Macherey-Nagel) system of fluoride detection is based on decolouration of lanthanum-alizarin complex by fluorides in the range 0.1–2 mg/l, while the use of an indicator paper (Macherey-Nagel) is based on decolouration of aluminium–organic reagent complex (2–200 mg/l F^-) [8].

6.3.6 Iodides

For semi-quantitative determination of iodides a test was proposed [62,83] in which the paper is impregnated with a mixture of the following solutions: 10 g Ce(IV) in 10 ml 10% H_2SO_4, 5 g sodium arsenate in 100 ml water, 0.025 *M* ferroin and saturated ammonium chloride in the ratio 3:2:3:2. The paper strip changes colour from blue to orange. The test is based on a catalytic reduction of Ce(IV):

$$2Ce^{4+} + AsO_3^{3-} + H_2O = 2Ce^{3+} + AsO_4^{3-} + 2H^+$$

Bromides, chlorides, sulphates, and nitrates interfere if small quantities of iodides are being determined. Analysis is hampered by cyanides of mercury, silver, and manganese. Detection limit is 1 mg/l iodides.

Filter paper impregnated with Ag_2CrO_4, $Ag_3[Fe(CN)_6]$, AgCl, and $Ag_4[Fe(CN)_6]$ and glued in a polymer film was used to detect iodides via

TABLE 6.11

Characteristic features of test methods for iodide determination

Impregnated reagents	Optimum pH of analysis	Analytical range (mg/l)	Interfering ions
Ag_2CrO_4	3–8	0.2–1000	Cl^-, Br^-, PO_4^{3-}, SCN^-, S^{2-}
$Ag_3[Fe(CN)_6]$	3–10	30–1000	Cl^-, Br^-, SCN^-, S^{2-}
AgCl	3–7	1–1000	Br^-, SCN^-, S^{2-}
$Ag_4[Fe(CN)_6]$	3–10	1–1000	Br^-, SCN^-

the length of the test strip coloured zone [96]. Data on the test systems is given in Table 6.11. The ions cited in Table 6.11 interfere only if their concentration surpasses that of iodides.

When using the paper impregnated with ferrocyanide it has to be held up to light at least 20–30 min after passing through it the test solution. A brown coloured zone appears on the white test strip in the presence of iodides. In the case of silver impregnated paper, a yellow zone develops against the grey background of the test strip 15–20 min after passing the solution. The silver chromate impregnated paper is the most sensitive (0.2 mg/l) but, owing to the fact that the silver chloride impregnated paper is more selective, it is the latter that is recommended for detecting iodides in wastewaters and technological solutions [96].

To detect large iodide concentrations (0.7–14.2 g/l) the radial paper chromatography is used (SALTESMO, Macherey-Nagel) employing a paper glued in a polymer film and contacting the studied solution through a hole in the film [8].

6.3.7 Nitrates and nitrites

Detection of nitrates and nitrites is of great practical importance in analysis of wastewaters of plants, drinking water and soil.

The most widely used methods are those based on reactions of diazotization and azo coupling giving azo dyes; thus, in the Griss method sulphanilic acid and 1-naphthylamine are used [103]. Because it was proven that the latter compound has carcinogenic properties, it is being replaced by other compounds like 1-naphthylamine-7-sulpho-acid, N-(1-naphthyl)ethylenediamine and others. The sensitivity of the

TABLE 6.12

Test methods of nitrite determination

Indicator system	Analytical range (mg/l)	Test system and the principle of detection	Ref.
Sulphanilic acid, 1-naphthylamine	1–80 100–3000	Test strips QUANTOFIX®	8
Sulphanilic acid, 1-naphthylamine	0.05–1	System VISOCOLOR®	8
Sulphanilic acid, 1-naphthylamine	0.02–0.5	System VISOCOLOR® ECO	8
Sulphanilic acid, 1-naphthylamine	0.005–0.1	System VISOCOLOR® HE	8
Sulphanilic acid, chromotropic acid	0.5–200	Coloured zone length estimate of indicator tubes	104
3-Hydroxy-1,2,3,4-tetrahydro-(h)quinoline, sulphanilic acid	0.5–50		105
8-Hydroxyquinoline, sulphanilic acid, cetylpyridinium	0.1–5	Colour intensity estimate of the test liquid after placement of indicator paper	5
Cellulose with 1-naphthylamine groups, Anesthesin	0.1–100	Estimation of coloured zone length of test strips	106
Cellulose with 1-naphthylamine groups, Anesthesin	0.01–3	Estimation of colour intensity of indicator paper after passing 20 ml of the test solution through it	106
Chromotropic acid, *p*-nitroaniline	0.2–2.3	Estimation of colour intensity of the test liquid after adding the indicator powder	107, 108

Griss method depends on the reagents and test systems used and conditions of determination (Table 6.12).

The currently known test methods of nitrate determination are also based on the Griss reaction and use 1-naphthylamine and its derivatives as an azo component (Table 6.13). Apart from the named components the impregnating solutions contain an organic acid (citric or tartaric) and metallic zinc or cadmium for reducing nitrates to nitrites.

Nitrite can be determined in natural and drinking waters, atmospheric precipitates and foodstuffs by means of indicator tubes. The method consists in obtaining a coloured compound in solution and subsequent use of an indicator powder, e.g. hydrophobic silica gel

TABLE 6.13

Test methods of nitrate determination

Indicator system	Analytical range (mg/l)	Test system and manner of determination	Ref.
Sulphanilic acid, 1-naphthylamine, Cd	10–50	Test strips, QUANTOFIX®	8
Sulphanilic acid, 1-naphthylamine, Cd	4–120	System VISOCOLOR® ECO	8
Sulphanilic acid, 1-naphthylamine, Cd	1–50	System VISOCOLOR®	8
Sulphanilic acid, 1-naphthylamine, Cd	0.1–5	System VISOCOLOR®	8
Sulphanilic acid, N-(naphthyl-1)ethylene-diamine	0.05–250	System CHEMetrics	7
3-Hydroxy-1,2,3,4-tetrahydro-(h)quinoline, sulphanilic acid, Cd	10–500	Estimation of colour intensity of indicator papers	108
Cellulose with 1-naphthylamine groups, anesthesin, Zn	1–100	Estimation of coloured zone length of the test strip	106
Cellulose with 1-naphthylamine groups, anesthesin, Zn	0.1–10	Estimation of colour intensity of indicator paper after passing 20 ml of the test solution through it	106
N-(Naphthyl-1)ethylene-diamine, sulphanilamide, Zn	0.1–10	Estimation of colour intensity of the test liquid after adding the indicator powder	109
8-Hydroxyquinoline, sulphanilic acid, $NiSO_4$, Zn	20–2000	Estimation of colour intensity of indicator papers	110
Chromotropic acid, sulphanilic acid, Zn	0.5–200	Estimation of coloured zone length of indicator tubes	104

noncovalently modified with cetyltrimethylammonium. The reagents used are a mixture of chromotropic, sulphanilic and citric acids and a catalyst of diazotization. The tubes are dipped into the test solution. The analytical range is 2.5–50 mg/l; it is 0.5–20 mg/l if a 1 ml solution is passed through a tube of a similar design.

Nitrite determination using polyurethane foam tablets [111]
The test means can be used for qualitative and semi-quantitative testing for nitrite of natural waters. It is based on interaction between nitrite and end toluidine groups of polyurethane. The presence of nickel is evidenced by the colour change of the PUF tablet from white into yellow; it is quantified by colour matching against a standard colour scale. Analytical range is 0.3–5 mg/l. The test means includes:

1. ether-based PUF tablets 16 mm in diameter 6 mm thick, weight approx. 0.04 g, modified with dimethylglyoxime in the presence of a plasticizer (trioctylamine);
2. hydrochloric acid, a 5 *M* solution;
3. test glasses with ground stoppers for mixing;
4. medical pipettes, a cylinder;
5. glass rod;
6. colour scale;
7. filter paper.

Analysis: In each test glass a 20-ml water sample is placed and 5 ml of 5 *M* HCl solution are added. One PUF tablet is put in each test glass, pressed with the glass rod until all air bubbles are removed and agitated for 15 min. Solutions are discarded and the tablets dried between sheets of filter paper and colour matched against a colour scale.

Selectivity: Selectivity was characterized by a limit ratio: accompanying compound/nitrite at which the error of measurement is no greater than 15%; 0.7 mg/l nitrate can be determined without interference from 100-multiple amounts of K, Na, Ca, Mn, Zn, Fe(III), Cu(II), NO_3^-, SO_4^{2-}, and Cl^-.

Accuracy and precision: The test means allows us to screen water samples for nitrite and confirm its presence at concentrations higher than 0.3 mg/l.

The time needed for the lemon yellow colour of the PUF tablet to fully develop is dependent on the nitrite content in the sample:

Nitrite content (mg/l)	Time for colour development (min)
0.3	5
0.5	4
3	3
4	1
5	0.25

Extraction of nitrite into the tablet is fully completed in 15 min.

Precision of the nitrite determination depends on its content in the sample. At nitrite content 0.5–4 mg/l the relative standard deviation is 0.2–0.3, at nitrite content out of this range RSD = 0.4–0.5 (n = 6). Precision of the method can be improved by the use of a portable reflectometer "Multiecotest" from Kostip Co. (Russia). Using a calibration graph equation, RSD = 0.05–0.10 was obtained for the range 0.5–4 mg/l and RSD = 0.15 for the nitrite content out of this range. The validity of the test method was confirmed by the analysis of a model sample based on tap water containing 0.67 mg/l nitrite. The amount of nitrite found was 0.67 ± 0.01 mg/l (n = 6, RSD = 0.05). The method was applied to testing water from an underground well in the Volgograd region. Nitrite content was found to be 18±1 mg/l (n = 6, RSD = 0.06). The result is in good agreement with that obtained spectrophotometrically using the Griss reaction (19±1 mg/l, n = 6, RSD = 0.05).

Nitrate determination using indicator tubes [112]

To determine nitrates in natural, drinking and wastewaters, and atmospheric precipitates indicator tubes were proposed whose action is based on a colour reaction of diazotising–azo coupling of nitrate ions with components of the reagent powder followed by sorption of the resulting dye in the indicator tube. The test means is a glass tube 40–50 mm long, 1–2 mm inner diameter, filled with indicator powder. After adding reagent powder to the test solution the indicator tube is dipped into the obtained red coloured solution. The latter is driven into the tube by the capillary forces and a coloured zone develops in the tube whose length is proportional to the content of the target component. The latter is quantified using a length scale or a calibration graph equation. The reagent powder contains Zn powder, diazotization catalyst, substances for ensuring optimum pH, sulphanilic acid as diazo component, and disodium salt of chromotropic acid as azo component. The test means includes:

1. indicator tube filled with indicator powder;
2. ampoule with 20 mg of reagent powder;
3. test glass with ground stopper for mixing.

Analysis: To a 2 ml test solution the reagent powder is added and mixed; 10 min later the indicator tube is dipped into the red coloured solution. After the liquid has reached the upper end of the tube it is withdrawn and the coloured zone length measured. The nitrate is quantified by means of a length scale or a calibration graph equation.

Method characteristics: Analytical range is 10–80 mg/l, relative standard deviation 0.07–0.09. The following do not interfere in multiple amounts: Cl^- (10^4), SO_4^{2-} ($5 \cdot 10^3$), Ca, Mg (10^3), Fe(III), Mn(II), Al (100), Co, Ni, Cu (20).

Accuracy and precision: Accuracy and precision of the test were confirmed by the spiked solutions and comparison with results obtained by instrumental methods of analysis. The indicator tubes were used to determine nitrate in children urine. On adding 79, 4.2 and 3.4 mg/l NO_3^- was found 86±7; 3.7±0.4, and 3.3±0.3 mg/l NO_3^-, respectively.

Nitrate determination using indicator powders [112]

To determine nitrates in natural, drinking and wastewaters, atmospheric precipitates and soil extracts indicator powders were proposed whose action is based on a colour reaction of diazotising–azo coupling of nitrate ions with components of the reagent powder followed by sorption of the resulting dye by the indicator powder. After adding the reagent powder to the test solution a red colour develops whose intensity is directly proportional to the nitrate content in solution. The latter is quantified using a colour scale or a spectrophotometer. The reagent powder contains Zn powder, diazotization catalyst, substances for ensuring optimum pH, sulphanilic acid as diazo component, and disodium salt of chromotropic acid as azo component. The test means includes:

1. ampoule with 20 mg of reagent powder;
2. test glass with ground stopper for mixing.

Analysis: To a 10 ml test solution the reagent powder is added and mixed; 10 min later the solution having a red colour is compared with a colour scale or its absorption is measured at 520 nm. The nitrate is quantified by means of a colour scale or a calibration graph equation.

Method characteristics: The analytical range is 5–200 mg/l for visual detection, RSD = 0.25–0.30; or it is 0.5–60 mg/l when a photometer is used (RSD = 0.07–0.1). The following do not interfere in multiple amounts: Cl^- (10^4), SO_4^{2-} ($5 \cdot 10^3$), Ca, Mg (10^3), Fe(III), Mn(II), Al (100), Co, Ni, Cu (20).

Accuracy and precision: Accuracy and precision of the test were confirmed by the spiked solutions.

6.3.8. Phosphates

To determine phosphates the reaction of molybdophosphoric heteropoly acid formation and its further oxidation to molybdenum blue is used:

$$2Na_2MoO_4 + H_2PO_4^- + 24H^+ \rightarrow [H_2PMo_{12}O_{40}]^- + 24Na^+ + 12H_2O$$

$$[H_2PMo_{12}O_{40}]^- + 2e \rightarrow [H_2PMo_{12}O_{40}]^{3-}$$

Benzidine, $SnCl_2$, sulphites, ascorbic acid or methol are used as reductants. Phosphates are quantified by visual or instrumental methods using the colour that is produced after adding reagents to the test solution. Comparators with a colour disc, a 5-step cell with a colour scale are employed (Hach Co., E. Merck, Hellige Co., Macherey-Nagel, and others). Analytical ranges are 0.05–1 mg/l, 0.1–1,5 mg/l, 0.2–2.5 mg/l (VISOCOLOR®, Macherey-Nagel), 0.03–5 mg/l, 0.5–15 mg/l, 0.5–50 mg/l (NANOCOLOR®, Macherey-Nagel), 0.05–1 mg/l, 2–10 mg/l, 50–500 mg/l (CHEMetrics) [7–9].

The formation of a yellow vanadomolybdophosphoric acid is also used (NANOCOLOR®, Macherey-Nagel, CHEMets®, CHEMetrics). Analytical ranges are 0.5–100 mg/l, 2–150 mg/l.

In NANOCOLOR® systems total phosphates are determined after decomposing polyphosphates by heating in acid medium [8].

Multilayer papers were proposed for phosphate detection [65]. The first paper strip is impregnated with citric acid and ammonium molybdate, and the second with $SnCl_2$ solution. In the presence of phosphates it turns blue in the range 1–50 mg/l PO_4^{3-}.

Indicator papers were proposed for the ranges 3–100 mg/l (QUANTOFIX®, Macherey-Nagel), 10–500 mg/l, 10–500 mg/l (Merckoquant®, E. Merck).

In the above systems, interference from silicates is excluded by adding tartaric acid to the indicator composition.

6.3.9 Sulphides

Sulphides are determined using reagent papers containing sodium nitroprusside [28] with red violet colour formation. Detection limit is 20 mg/l; sulphites interfere.

To determine large concentrations of H_2S and other sulphides a paper is produced impregnated with silver iodide in the form of test strips glued in a polymer film [5]. The sulphide solubility series shows that the least soluble is Ag_2S (HgS is excluded due to its toxicity). On the other hand, one can see from the silver salts solubility series: $Ag_2SO_4 > Ag_2CO_3 > Ag_2CrO_4 > Ag_3PO_4 > Ag_2SO_3 > Ag_3[Fe(CN)_6] > AgCl > Ag_4[Fe(CN)_6] > AgSCN > AgBr > AgI > Ag_2S$ that sulphides will substitute sulphur for I in the AgI precipitate and thus all other anions (bromides, thiocyanates, etc.) will not hamper the analysis. Taking this into consideration a paper was prepared impregnated at first with 0.1% silver sulphate solution, dried and then impregnated with 0.1% KI solution. Then the paper was washed and dried again. On passing through the colourless paper a 20 ml test solution it turned brown. There are two broad diffuse reflection maxima at 470 and 640 nm. Sulphides can be detected at pH 4–10. Analytical ranges are 0.01–0.05–0.1–0.5–1 mg/l. Sewage waters were analysed for sulphides. To do this the water was filtered through a "black ribbon" filter, sucked into a 20 ml syringe and passed through a test device with a AgI paper/filter paper gasket. The total content of all three forms was determined (H_2S, HS^-, S^{2-}) referred to H_2S.

In systems VISOCOLOR®, NANOCOLOR® (Macherey-Nagel) the formation of Methylene blue is used. Hydrogen sulphide and sulphide ions form in acid medium with N,N′-dimethyl-*p*-phenylenediamine an intermediate which is oxidized by Fe(III) to Methylene blue. Analytical range is 0.01–1 mg/l S^{2-} [8].

6.3.10 Thiocyanates

To determine thiocyanates, a number of drop reactions were proposed which can be conducted on paper.

A simple cyanide determination in the presence of iodides is based on the fact that in a solution of Fe(III) salt containing large quantities of Fe(II) salts the Fe^{3+} ion oxidizes only the SCN^- ions and not the I^- ion; that is explained by the lowering of the redox potential of the system Fe^{3+}/Fe^{2+} under such conditions. A drop of the test solution slightly acidified with acetic acid is applied onto the filter paper and then a reagent solution added (1 g $FeCl_3$ and 1 g $FeSO_4$ in 100 ml diluted HCl). In the presence of thiocyanates a red brown spot or a ring appears. Up to 6 mg/l SCN^- can be determined.

Cobalt(II) salt acetone solution in the presence of thiocyanates acquires a blue colour. This colour reaction is used both for cobalt and cyanide determination. Nitrites interfere as they form nitrozylthiocyanate.

Test strips impregnated with AgCl and glued in a polymer film can be used for detecting thiocyanates. A discoloured zone forms on the grey background of the strip after its contact with the tested liquid and holding it in the light for 30–40 min [5]. The optimal pH range for SCN^- detection is 3–8. The lower detection limit is 1 mg/l SCN^- (a discoloured zone of 0.5 mm) and the highest one is 500 mg/l (a discoloured zone of 55 mm). bromides, iodides, and sulphides interfere; also ferrocyanides in quantities greater than SCN^-.

In a NANOCOLOR® system reaction of thiocyanate with iron(III) is used giving a red colour whose intensity is proportional to the concentration in the range 0.5–50 mg/l SCN^- [8].

6.3.11 Silicates

Silicates are determined using the reaction of formation of molybdosilicic heteropolyacid with further reduction to molybdenum blue as in the case of phosphate determination. Interference from phosphates is eliminated by means of citric acid. Do not interfere < 10 mg/l Fe, Al, < 20 mg/l PO_4^{3-}, < 100 mg/l Ca. Analytical ranges are 0.2–5 mg/l SiO_2 (VISOCOLOR®), 0.005–10 mg/l SiO_2, (NANOCOLOR®), 0.3–10 mg/l Si (Microquant®), 0.01–0.25 mg/l Si (Aquaquant®) [8,21]).

6.3.12 Sulphates

Sulphates are usually determined using the formation of a poorly soluble barium sulphate [13,28]. The reagent paper is impregnated with a coloured barium complex with a suitable reagent, which gives the paper its colour when liberated by sulphate. Thus, the paper with barium rhodizonate has a red-brown colour, which is lost in the presence of sulphate. Detection limit is 10 mg/l [28]. A green-blue barium complex with sulphonazo III formed on filter paper turns violet or green-blue under the action of sulphate [62]. Detection limit is 2 mg/l SO_4^{2-}. In the case of indicator test strip RIS–Sulfate–Test sulphate reacts with a black-blue complex liberating a pink-violet reagent. Analytical ranges at pH 4–7 are: 50–100–250–500–1000 mg/l.

In the method of detecting sulphates by the coloured zone length of a test strip glued in a polymer film a paper is used impregnated with barium rhodizonate having a red-brown colour. The optimum pH range for sulphate detection is 2–4. At pH < 2 barium rhodizonate is decomposed while at pH > 4 the discoloured zone on the test strip becomes indistinct. Multiple amounts of chlorides, nitrates, acetates, thiosulphates (1000), fluorides (50), phosphates (10), and chromates (5) do not interfere. The test system was used for detecting sulphate in natural and sewage waters. To a 0.5 ml sample 5 drops of acetone or ethanol with pH 2 were added, mixed, and the test strip was dipped into the mixture; after the strip was wetted its discoloured zone was measured. Analysis time was 20–30 min, RSD was less than 0.3.

In systems Merckoquant® (E. Merck), QUANTOFIX® (Macherey-Nagel) four reaction zones are accommodated on a polymer support having different contents of barium complex with thoron. Sulphate is quantified using a colour scale in the range 200–1600 mg/l.

A turbidimetric method is used in test kits from Hach Co., VISOCOLOR®, NANOCOLOR® (Macherey-Nagel). Sulphate is quantified in the range 10–200 mg/l by the clouding of the solution on adding barium chloride [8,9].

6.3.13 Sulphites

To determine sulphite in sewage a titrimetric method is used based on titrating excess iodine with sodium thiosulphate:

$$SO_3^{2-} + H_2O + I_2 \rightarrow SO_4^{2-} + 2H^+ + 2I^-$$

$$I_2 + 2S_2O_3^{2-} \rightarrow 2I^- + S_4O_6^{2-}$$

This reaction is used in the system VISOCOLOR® (Macherey-Nagel) for the sulphite range 2–100 mg/l. A more selective method of sulphate detection in the range 0.05–10 mg/l is that using thiodibenzoic acid (NANOCOLOR®) [8].

6.4 OTHER INORGANIC COMPONENTS

6.4.1 Hydrazine

Hydrazine detection is based on its condensation with 4-dimethylaminobenzaldehyde in acid medium giving a yellow-orange product.

Analytical ranges are: 0.002–1.5 mg/l N_2H_4 (NANOCOLOR®), 0.05–2 mg/l (Vacu-Vials®), 0.02–0.7 mg/l (CHEMetrics) [7,8].

Reduction of molybdophosphoric acid to molybdenum blue with hydrazine was used to detect the latter by the coloured zone length of the indicator tube (0.03–2 mg/l N_2H_4) [104] or by the colour intensity of indicator paper after passing through it 20 ml of the analysed solution (0.005–0.2 mg/l [42].

Hydrazine detection by means of indicator tubes [104]

To determine total hydrazine in natural and wastewaters and soil extracts one can use indicator tubes (50 mm long, 1 mm inner diameter) filled with indicator powder. The tube is dipped into the acidified test solution, the liquid enters the tube under the action of capillary forces and a coloured zone develops, whose length is proportional to the hydrazine content. The latter is quantified using a length scale or a graduation graph equation. The test is based on a redox reaction between hydrazine and silicon oxide gel modified with a molybdophosphoric heteropoly compound resulting in the latter's reduction and blue coloration of the gel. The test means includes:

1. indicator tube filled with indicator powder;
2. ampoule with 0.2 ml 4 M H_2SO_4 solution;
3. test glass with ground stopper for mixing.

Analysis: To a 2 ml test sample the content of the ampoule is added and the tube is dipped into the mixture. After the liquid has reached the upper end of the tube it is withdrawn and 10 min later the length of the blue zone measured and the hydrazine quantified using a length scale or a graduation graph equation.

Method characteristics: Analytical range is 0.02–1 mg/l, RSD is 0.09–0.13. Other reducing agents like tin salts, sulphur containing anions, dithiocarbamates, xanthogenates, tannates, and ascorbic acid can also react with the immobilized complex inorganic compound included in the indicator powder. But the above listed reductants are prone to rapid decomposition in natural environment under the action of air oxygen and the sun. Besides, the rate of formation of the blue reaction products and their spectral properties are reductant dependent, thus enabling one to choose a sufficiently selective protocol of hydrazine detection. Accordingly, the length and diameter of the tube, hydrophobicity and the grain size of the indicator powder are chosen such as to give time for hydrazine to react but not time sufficient for other reductants to react. This makes the proposed methods of hydrazine detection sufficiently selective in the analysis of natural and wastewaters.

Accuracy and precision of the test system was confirmed by the spiked solutions and by comparison with instrumental methods of analysis. Good agreement between the two approaches was found.

Hydrazine determination using indicator powders [104]

To detect hydrazines in natural and wastewaters indicator powders can be used. The test is based on a redox reaction between hydrazines and silicon oxide gel modified by a molybdophosphoric heteropoly compound resulting in the reduction of the latter to a blue coloured product. The colour intensity is proportional to the hydrazine content in solution and can be measured visually via colour scales or using spectrophotometric equipment. The test means includes:

1. indicator powder;
2. ampoule containing 1.1 ml conc. sulphuric acid (solution 1);
3. test glass with a ground stopper;
4. dosage pipette.

A portable photometer (reflectometer) is required for conducting the test.

Analysis: To 50 ml of test sample solution 1 and indicator powder are added and mixed for 20–25 min. Then the sorbent is separated from the solution via the pipette and its absorbance or diffuse reflection are measured using a photometer. Absorbance is measured at 670 nm and diffuse reflection at 620 nm.

Method characteristics: Analytical range is 0.2–6 mg/l, RSD is 0.09–0.13 for solid phase spectrometry. The selectivity is similar to that obtained with indicator tubes. Accuracy and precision of analysis was confirmed by the spiked solutions: 1 mg/l hydrazine and 0.40 mg/l phenyl hydrazine were added; 0.9 ± 0.1 and 0.4 ± 0.06 mg/l, respectively, were found.

6.4.2 Hydrogen peroxide

Hydrogen peroxide is formed in the upper atmosphere and the earth surface waters under the action of the UV rays, and it plays an important role in turning atmospheric sulphur dioxide into sulphate, as well as in various redox reactions in water reservoirs. Its content in atmospheric precipitates varies in the range 0.03–0.3 mg/l and in surface waters in the range 0.003–0.3 mg/l. To detect hydrogen peroxide in the said media photometric techniques are mainly used employing horse radish peroxidase catalysed reactions of oxidation with H_2O_2 of N,N-dimethyl-*n*-phenylenediamine [113,114] or 2,2′-azinodi(3-ethylbenzthiazoline-6-sulphonate) [115,116]. The analytical range of these methods is 0.003–0.3 mg/l.

Indicator papers were proposed for detecting H_2O_2 in the range 0.5–25 mg/l containing either easily oxidized organic reagents forming coloured reaction products [117] or enzyme-catalysed reactions similar to those used in photometric methods [118]. H_2O_2 concentration is judged by the colour change of the indicator paper after its brief contact with the test solution. The error of analysis is 30–50%. These papers are not used in the analysis of natural waters due to their low sensitivity.

Indicator papers were proposed based on HRP catalysed reaction of oxidation of 4-chloro-1-naphthol or *o*-tolidine [119]. To immobilize the latter (or *o*-toluidine) and HRP, cellulose or aldehyde cellulose paper was used. The H_2O_2 was quantified by the coloured zone length of a test strip (3×80 mm) glued in an adhesive polymer film after its contact with the test solution. Analytical range is 0.1–300 mg/l and 0.005–1 mg/l in the case where 20 ml of test solution are passed through the paper. Rapid methods were developed for H_2O_2 determination in atmospheric precipitates, sewage and natural waters (RSD < 0.3).

Enzyme reactions of H_2O_2 detection are used in systems VISOCOLOR®, NANOCOLOR® (Macherey-Nagel). Detection limit is 0.03 mg/l H_2O_2 [8]. In CHEMetrics systems is used the reaction of oxidation of Fe(II) to Fe(III) in the presence of thiocyanate giving a red thiocyanate Fe(III) complex. Detection limit is 0.05 mg/l H_2O_2 [7].

6.4.3 Oxygen

Oxygen in water is quantified using the Winkler method (Macherey-Nagel, E. Merck). In the sample under test a Mn(II) salt and alkali are introduced; Mn(II) hydroxide is precipitated which, being oxidized by the dissolved oxygen, turns into Mn(IV) hydroxide:

$$2Mn(OH)_2 + O_2 + 2(n-1)H_2O \rightarrow 2MnO_2 \cdot nH_2O$$

The precipitate formed is dissolved in an acid and KI is added. The liberated iodine is titrated with thiosulphate (VISOCOLOR®, Aquamerck®) or the resulting colour is measured (NANOCOLOR®). Analytical range is 0.1–12 mg/l O_2 [8,21]. In CHEMetrics systems colorimetric methods based on rhodazine D and indigocarmine are used (0.03–15 mg/l O_2) [7].

6.4.4 Ozone

Ozone is employed for disinfection of sewage and natural waters. For its detection the same methods are used as for detection of active chlorine, namely oxidation of N,N′-diethyl-1,4-phenylenediamine (CHEMetrics, Macherey-Magel). Analytical ranges are 0.07–3 mg/l, 0.05–2 mg/l [7,8].

REFERENCES

1. H. Clifford, *US Pat.* 3510263, 1970.
2. E. Sandorn and Hun. *Pol. Pat.* 170775, 1978.
3. J. Siepak and H. Witkowski, *Pol. Pat.* 141087, 1987.
4. J. Siepak and H. Witkowski, *Pol. Pat.* 141088, 1987.
5. V.G. Amelin, Doctoral Sc. Dissertation, Moscow State University, Moscow, 1998 (in Russian).
6. V.G. Amelin, *Zh. Anal. Khim.*, 55 (2000) 359.
7. *Perfecting Simplicity in Water Analysis.* CHEMetric (1999) 40.
8. *Rapid Tests,* Macherey-Nagel. Macherey-Nagel & Co. KG (2000) 118.
9. Hach Co. (2000) 56.
10. V.G Amelin, *Zh. Anal. Khim.,* 53 (1998) 958.
11. D. Schmitt, W. Baumer and A. Stein, *US Pat.* 3843325, 1974.
12. R.P. Pantaler, N.B. Lebed, L.I. Avramenko and A.B. Blank, *Zh. Anal. Khim.* 52 (1997) 643.
13. V.M. Ostrovskaya, *Reactive Indicator Tools for Multielement Tests*, 1-ya Obraztsovaya tipografiya, Moscow, 1992 (in Russian).
14. E.I. Morosanova, N.M. Kuzmin and Yu.A. Zolotov, *Fresenius' J. Anal. Chem.,* 357 (1997) 853.
15. E. Halamex, F. Prikryl and J. Sonak, *Czech. Pat.* 239708, 1987.
16. E. Halamex, F. Prikryl and J. Sonak, *Czech. Pat.* 239710, 1988.
17. Ch.O. Rupe and R. Bauer, *US Pat.* 4092115, 1978.
18. R.P. Pantaler, L.A. Egorova, L.I. Avramenko and A.B. Blank, *Zh. Anal. Khim.* 51 (1996) 5213.
19. T.A. Magers and D.I. Tabb, *US Pat.* 4290773, 1981.
20. S.G. Dmitrienko, O.A. Sviridova, L.N. Pyatkova, V.A. Zhukova and Yu.A. Zolotov, *Anal. Chim. Acta,* 405 (2000) 231.

21. *Test Kits Aquamerck®, Microquant® for Mobile Analysis.* E. Merck, Darmstadt, 1994, 28 p.
22. S.G. Dmitrienko, Doctoral Sc. Dissertation, Moscow State University, Moscow, 2001 (in Russian).
23. V.M. Ostrovskaya, N.A. Fomin and Yu.P. Adler, *Zh. Anal. Khim.,* 42 (1987) 1716.
24. L. Roman, R. Craciunescu, E. Floreau and C. Crihalmeanu, *Roum Pat.* 89539, 1986.
25. L. Roman, R. Craciunescu, E. Floreau and C. Crihalmeanu, *Roum. Pat.* 86542, 1986.
26. L. Roman, R. Craciunescu, E. Floreau and C. Crihalmeanu, *Roum. Pat.* 86489, 1985.
27. M.L. Jackson, *Soil Chemical Analysis,* Prentice-Hall, Englewood Cliffs, NJ, 1958.
28. F. Feigl and V. Anger, *Spot Tests in Inorganic Analysis.* Elsevier, Amsterdam, 1972.
29. *Ger. Pat.* 2038651, 1979.
30. *Brit. Pat.* 1281817, 1972.
31. *US Pat.* 3661538, 1972.
32. *Ger. Pat.* 205010, 1984.
33. *GDR Pat.* 238276, 1986.
34. V.G. Amelin, *Zh. Anal. Khim.*, 54 (1999) 753.
35. V.G. Amelin, *Zh. Anal. Khim.*, 54 (1999) 1088.
36. V.G. Amelin, *Zh. Anal. Khim.*, 54 (1999) 651.
37. Mercoquant. *Chemical Microchips for Analysis You Take in Your Stride.* E. Merck, 1999. 37 p.
38. V.G.Amelin, V.M. Ivanov, *Zh. Anal. Khim.,* 55 (2000) 411.
39. *US Pat.* 3741727, 1973.
40. Atsushi Chiba and T. Ogawa, *Inf. Mater. Energy Theory Liff.*, 56 (1988) 630.
41. A.G. Hamza, A.V. Farag and A. Al-Herthani, *Microchem. J.*, 32 (1985) 13.
42. Yu.A. Zolotov, *Vestn. Ross. Akad. Nauk,* 67 (1997) 508.
43. E.I. Morosanova, A.A. Velikorodny and Yu.A. Zolotov, *Fresenius' J. Anal. Chem.,* 361 (1998) 305.
44. Yu.A. Zolotov, *Khim. Prom-st (Moscow),* No. 6 (1997) 48.
45. *US Pat.* 3697225, 1972.
46. S.G. Dmitrienko, O.A. Kosyreva, V.K. Runov and Yu.A. Zolotov, *Mendeleev Commun.,* 2 (1991) 75.
47. T. Braun and A.B. Farag, *Anal. Chim. Acta,* 73 (1974) 301.
48. O.P. Shvoeva et al., *Zh. Anal. Khim.,* 52 (1997) 89.
49. V.M. Ivanov, S.A. Morozko and Yu.A. Zolotov, *Zh. Anal. Khim.*, 48 (1993) 1389.
50. E.I. Morosanova and I.M. Maksimova, *Zh. Anal. Khim.,* 49 (1994) 602.

51. R.P. Pantaler, A.K. Timchenko, L.I. Avramenko and A.B. Blank, *Zh. Anal. Khim.*, 53 (1998) 3.
52. V.M. Ivanov and O.V. Kuznetsova, *Zh. Anal. Khim.*, 50 (1995) 498.
53. S.G. Dmitrienko, O.A. Kosyreva, V.K. Runov, *Modern Methods of Analytical Control in Industry*, Moscow, 1991 (in Russian).
54. S.G. Dmitrienko et al., USSR Inventor's Certificate 1673922, *Byull. Izobret.,* No. 32 (1991).
55. S.G. Dmitrienko et al., USSR Inventor's Certificate 1803837, *Byull. Izobret.,* No. 11 (1993).
56. S.G. Dmitrienko et al., USSR Inventor's Certificate 1803839, *Byull. Izobret.,* No. 11 (1993).
57. *Swiss Pat.* 550403, 1974.
58. *Ger. Pat.* 2162122, 1980.
59. *Ger. Pat.* 2038651, 1979.
60. A.B. Farag, M.E. Hassouna and M.H. Abdol-Rahman, *Bull. Soc. Chim. Belg.*, 96 (1987) 147.
61. *GDR Pat.* 238541, 1986.
62. R.P. Pantaler, L.A. Egorova, L.I. Avramenko and A.B. Blank, *Zh. Anal. Khim.* 51 (1996) 997.
63. M.S. Kravchenko et al., *Zh. Anal. Khim.,* 42 (1987) 263.
64. I.M. Maksimova et al., *Zh. Anal. Khim.,* 49 (1994) 1210.
65. V.M. Ostrovskaya, *Zh. Anal. Khim.,* 32 (1977) 1820.
66. J. Siepak and H. Witkowski, *Chem. Anal.*, 31 (1986) 669.
67. *US Pat.* 5186894, 1993.
68. J. Siepak and H. Witkovski, *Chem. Anal.*, 32 (1987) 1035.
69. V.M. Ostrovskaya, M.S. Aksenova and R.L. Medvedeva, *Zh. Anal. Khim.*, 42 (1987) 2197.
70. R.P. Pantaler, A.K. Timchenko, L.I. Avramenko and A.B. Blank, *Zh. Anal. Khim.*, 52 (1997) 384.
71. V.G. Amelin, *Zh. Anal. Khim.*, 54 (1999) 991.
72. S.G. Dmitrienko and V.K. Runov, USSR Inventor's Certificate 1732224, *Byull. Izobret.,* No. 7 (1992).
73. S.G. Dmitrienko et al., USSR Inventor's Certificate 17373170, *Byull. Izobret.,* No. 20 (1992).
74. E.I. Morosanova, N.M. Kuzmin and Yu.A. Zolotov, *Fresenius' .J. Anal. Chem.*, 357 (1997) 853.
75. V.A. Nazarenko and V.P. Antonovich, *Trioxifluorones*, Nauka, Moscow, 1973 (in Russian).
76. *USSR Inventor's Certificate* 1555667, 1989.
77. *US Pat.* 3843325, 1974.
78. *Ger. Pat.* 2162122, 1980.
79. M.R. Abbas et al., *Anal. Lett.*, 22 (1989) 2575.
80. T.N. Shekhovtsova et al., *Zh. Anal. Khim.*, 50 (1995) 538.

81. T.N. Shekhovtsova, S.V. Muginova and N.A. Bagirova, *Anal. Chim. Acta,* 344 (1997) 145.
82. M.S. Kravchenko and M.Sh. Fumarova, *Zh. Anal. Khim.,* 50 (1995) 1288.
83. L.V. Gudzenko, R.P. Pantaler, L.I. Avramenko and A.B. Blank, *Zh. Anal. Khim.*, 51 (1996) 993.
84. M.K. Beklemishev, T.A. Stoyan, I.F. Dolmanova, *Analyst,* 122 (1997) 1161.
85. *US Pat.* 5106581, 1992.
86. *Ger. Pat.* 2720073, 1978.
87. *Ger. Pat.* 2043600, 1972.
88. *Pol. Pat.* 101167, 1979.
89. S.G. Dmitrienko, Yu.A. Zolotov et al., USSR Inventor's Certificate 1803838, *Byull. Izobret.* No. 11 (1993).
90. I.M. Maximova, E.I. Morosanova, N.M. Kuzmin, Yu.A. Zolotov, *Zh. Anal. Khim.*, 51 (1996) 398.
91. V.M. Ivanov and S. Massud, *Vestn. Mosk. Univ., Ser. 2, Khim.,* 35 (1994) 350.
92. V.M. Ivanov, S.A. Morozko and S. Massud, *Zh. Anal. Khim.,* 50 (1995) 1280.
93. S.A.Morozko, V.M. Ivanov and S. Massud, *Vestn. Mosk. Univ., Ser. 2, Khim.,* 37 (1996) 253.
94. V.M. Ostrovskaya, *Fresenius' J. Anal. Chem.*, 361 (1998) 303.
95. V.M. Ostrovskaya et al., *Vysokochist. Veshchestva,* No. 6 (1988) 196.
96. V.G. Amelin, *Zh. Anal. Khim.,* 53 (1998) 868.
97. *Fr. Pat.* 1603510, 1972.
98. J.M. Figg, *Concrete,* 9 (1975) 38.
99. V.E. Headley, *Feedstuffs* (1967) 60.
100. T.I. Headrick and S.H. Schanderl, *J. Dairy Sci.*, 49 (1966) 114.
101. J.M. Sloan, G. Beevers and F.E. Baxter, *Clin. Chem.* (Winston-Salem, N.C.), 30 (1984) 1705.
102. S.G. Dmitrienko et al., USSR Inventor's Certificate 1803839, *Byull. Izobret.,* No. 11 (1993).
103. W.J. Williams, *Handbook of Anion Determination*, Butterworth, London, 1979.
104. D. Marchenko et al., in: *International Congress on Analytical Chemistry*, Moscow, 1997, Vol. 2. K-15.
105. *RF Pat.* 2009486, 1994.
106. V.G. Amelin and I.S. Kolodkin, *Zh. Anal. Khim.*, 56 (2001) 206.
107. A.G. Shirinova and V.M. Ivanov, *Zh. Anal. Khim*, 49 (1994) 266.
108. *RF Pat.* 2038579, 1995.
109. E. Jungreis, *Microchem. J.*, 32 (1985) 143.
110. *RF Pat.* 1775667, 1992.
111. S.G. Dmitrienko et al., *Zavod. Lab.*, 66 (2000) 10.

112. D. Marchenko et al., in: *International Congress on Analytical Chemistry*, Moscow, 1997, Vol. 2, K-15.
113. H. Bader et al., *Water Res.*, 22 (1988) 1109.
114. M. Tokuwame et al., *Chem. Pharm. Bull.*, 37 (1989) 202.
115. N. Hasegawa et al., *Bunseki Kagaku* 30 (1981) 470.
116. I.D. Eremina, L.K. Shpigun and Yu.A. Zolotov, *Zh. Anal. Khim.*, 44 (1989) 341.
117. *US Pat.* 4956146, 1991.
118. *Merck Schnelltest Handbuch.* E. Merck, Darmstadt, 1986, 288 p.
119. V.G. Amelin, I.S. Kolodkin and Yu.A. Irinina, *Zh. Anal. Khim.*, 55 (2000) 419.

Chapter 7

Determination of Organic Components in Water and Soil

7.1 SUM PARAMETERS

Because of the great diversity of organic compounds encountered in water and soil, it is difficult to determine each of them separately by conventional methods. Therefore, often only the overall contents of these compounds are estimated.

7.1.1 Chemical oxygen demand (COD)

The chemical oxygen demand (COD) is the amount of oxygen (or another oxidant calculated in relation to oxygen) in mg/l required for a complete oxidation of organic compounds present in a water sample in which carbon, hydrogen, sulphur, phosphorus, and other elements (except nitrogen) present in organic compounds are converted into the higher oxides (CO_2, H_2O, P_2O_5, and SO_3), while nitrogen is converted into the ammonium ion. Determination of COD is usually based on oxidation of organic compounds by potassium dichromate in an acid medium

$$Cr_2O_7^{2-} + 14\ H^+ + 6\ e = 2\ Cr^{3+} + 7\ H_2O$$

and subsequent estimation of the colour intensity either visually or using a photometer. For COD of 40, 160, and 300 mg/l, determination is based on the colour of $K_2Cr_2O_7$, while for COD values of 1500 and 15000 mg/l, the colour of chromium(III) is evaluated.

In the presence of a catalyst, e.g. silver sulphate, oxidation of organic compounds is accelerated and covers virtually all organic compounds. Oxidation does not take place for pyridine and its homologues, pyrrole, pyrrolidine, proline, nicotinic acid, or some other nitrogen-containing compounds; benzene, toluene, or other aromatic hydrocarbons; paraffin, naphthalene. If the sample under analysis contains inorganic reducing agents, the amount of these compounds determined separately (and calculated in relation to oxygen) should be subtracted from the result of COD determination.

The interfering influence of chloride ions (which are oxidised to elemental chlorine during determination) is eliminated by masking them by mercury(II) sulphate.

Each available kit for COD determination includes a device for heating the reaction mixture (148–150°C, 2 h).

The existing kits for COD determination are mainly employed to analyse waste waters because their sensitivity is relatively low. The ranges of COD that can be determined are listed in Table 7.1.

TABLE 7.1

Test systems for determination of COD

System	Analytical range (mg/l)	Ref.
Process Eng. (Australia)	50–150	1
	20–400	
	50–2000	
CHEMetrics	10–150	2
	30–1500	
	300–15000	
HACH Co.	2–40	3
	10–150	
	100–1500	
	1000–15000	
NANOCOLOR®	2–40	4
	15–160	
	15–300	
	100–1500	
	1000–15000	

7.1.2 Biochemical oxygen demand (BOD)

The biochemical oxygen demand is the amount of oxygen in milligrams required for the oxidation of organic compounds present in 1 litre of water upon biological processes occurring in water under aerobic conditions; BOD does not include the consumption of oxygen for nitrification. The biochemical oxygen demand is determined from the difference in the oxygen content before and after incubation of the sample under standard conditions. The standard conditions chosen imply incubation for 5 days at 20°C away from air and light (BOD_5).

In the NANOCOLOR® BOD_5 system (Macherey-Nagel), the content of oxygen is determined by the Winkler method (VISOCOLOR® Oxygen). The range of contents determined is 2 to 300 mg/l of O_2. Nitrification is prevented by adding N-allylthiourea to the solution under analysis.

A method for quantification of oxygen in the determination of BOD_5 in the range of 2–1400 mg/l of O_2 has been proposed (Dr. Bruno Lange GmbH). Oxygen is determined by photometry based on the red colour of an alkaline solution of a catechol derivative in the presence of iron(II).

7.1.3 Total organic carbon (TOC)

Determination of COD and BOD_5 is supplemented by determination of organic carbon. In this analysis, the carbon contained in organic compounds is also oxidised under conditions where oxidation occurs especially vigorously. Determination of organic carbon is most often needed in calculation of balances (for example, in determination of gain or loss of organic mass, the relative contents of biogenic elements, and so on).

The procedure for determination of the total organic carbon includes combustion of organic substances on treatment with an oxidising mixture in the presence of a silver salt as a catalyst to give carbon dioxide, which is then absorbed by sodium hydroxide. The colour intensity of an indicator is measured using a photometer. The Dr. Bruno Lange company has proposed a test kit for determining total organic and inorganic carbon. The test kit comprises two glass bottles having a common plugged neck and separated by a membrane. One bottle contains an oxidising mixture and the sample under analysis, the second one contains the absorbing solution with an indicator. The CO_2 evolved passes through the membrane and changes the colour of the indicator. The ranges of contents covered by this procedure are 2–65 mg/l, 60–735 mg/l (organic carbon); 12–75 mg/l, 75–750 mg/l (total carbon), and 10–73 mg/l, and 15–150 mg/l (inorganic carbon).

The Hach Co. markets a colorimetric test for determination of total organic carbon in potable and waste waters [3].

7.1.4 Total nitrogen

Total nitrogen is the sum of nitrogen contained in nitrates, nitrites, ammonia, and organic compounds. The content of nitrogen is found after converting all the compounds into ammonia or nitrates. The results are expressed in mg/l of N.

In the NanOx® N system (Macherey-Nagel), nitrogen compounds are oxidised to nitrates by potassium peroxodisulphate, which is added to a 5-ml sample and the mixture is kept in a microwave oven or in a furnace for 1 h at 100°C or for 30 min at 120°C. After that, nitrates are quantified using the NANOCOLOR® Nitrate system [4]. The ranges of concentrations determined are 0.5–220, 0.3–22.0, and 4–60 mg/l of N.

7.1.5 Adsorbable organically bound halogens (AOX)

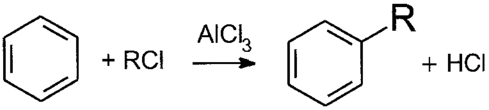
Due to the increased use of halogenated organic compounds in industry, sale, agriculture and household, substantial amounts of these compounds are now present in the environment, in particular, in waste, surface, and underground waters. Organic compounds of halogens are often hazardous substances.

The total content of organic halogens in water can be expressed in terms of the parameter referred to as Adsorbable Organic Halogens (AOX); determination of this value includes adsorption of the substances on active carbon or some other adsorbent.

Organic compounds contained in water are adsorbed on the Nanosorb adsorbent. After oxidative decomposition of the sample by heating at 120°C or in a microwave oven, the chlorides formed are determined by a photometric method (NANOCOLOR® AOX 3, Macherey-Nagel). Analytical ranges are 0.1–3.0 and 0.01–0.3 mg/l of adsorbable organic halogens [4].

Dr. Bruno Lange GmbH produces special carbon disks (Carbodisk) for adsorption of halogen-containing organic compounds.

The company HNU Systems Inc. produces test kits for determination of crude oil, fuel and waste oil in soil and water. The method is based on the Friedel–Crafts alkylation of aromatic hydrocarbons present in petroleum products with alkyl halides to give coloured products



The reaction is catalysed by anhydrous aluminium chloride [5,6].

To analyse soil, a 5-g sample is placed in a weighing bottle, 10 ml of an organic solvent is added from an ampoule, and the mixture is stirred for 3 min. The resulting extract is transferred into a tube and an alkyl halide and a catalyst are added from an ampoule. After stirring for 3 min, the colour of the sample is examined against a reference colour scale. When analysing water, extraction is performed using 500 ml of water sample.

Depending on the component to be determined, the following colours of the extract can be observed:

- benzene: from yellow to orange
- toluene, ethylbenzene, xylene: from yellow-orange to bright orange
- petrol: from beige to red-brown
- diesel fuel: from beige to green.

Colour scales have been prepared in the ranges 1–10–50–100–200–500–1000 mg/kg for soils and 0.1–1–5–10–20–50–100 mg/l for water.

The RemediAid™ Total Petroleum Hydrocarbon Test Kit (AZUR Environmental Ltd.) is a rapid, simple field test for measuring petroleum hydrocarbon contamination in soil. The test is based on the Friedel–Crafts reaction with that fundamental difference that it is a coloured intermediate formed in the solvent that is measured [2]. A sample of soil is added to a reaction tube which contains anhydrous sodium sulphate as a drying agent. Dichlorometane (20 ml) is then added to the reaction tube. This organic solvent extracts the petroleum hydrocarbons from the soil sample. Finally, a pre-determined volume of the hydrocarbon-containing solvent is drawn into a vacuum-sealed ampoule with aluminium chloride. Interference from polar organic

TABLE 7.2

Detection limits of aromatic hydrocarbons

Compound	LD (mg/l)	Compound	LD (mg/l)
BTEX	0.6	Ethylbenzene	0.6
Toluene	0.6	*o*-Xylene	0.6
m-Xylene	1.4	*p*-Xylene	1.3
Benzene	1.2	Benzo[a]pyrene	6
Chlorobenzene	1.2	1,2-Dichlorobenzene	5
Naphthalene	11	*o*-Cresol	5
4-Ethyltoluene	5	Nitrobenzene	5

substances is eliminated by using a silica-packed column mounted on the tip of a vacuum-sealed ampoule. The hydrocarbons in the solvent react with aluminium chloride to give a soluble coloured product directly proportional to the petroleum hydrocarbon concentration in the sample. The absorbance of the sample is measured using a portable, battery-powered LED-based colorimeter, and the resulting value is used to determine the hydrocarbon content in the soil in mg per kg using a calibration formula.

Immunoassays for determination of benzene, toluene, ethylbenzene, and xylene (BTEX) in soil were proposed (D TECH® BTEX, E. Merck; OHMICRON's RaPID Assay, Supelco (USA) [7]).

The ranges of BTEX concentrations that can be determined by means of the D TECH® BTEX system are 0.6–10 mg/l for water and 2.5–35 mg/kg for soil. Extraction is performed with isopropanol; a small volume of the extract is then dissolved in a buffer solution and the immunoenzyme reaction is carried out. The colour intensity can be estimated both visually and using a mini photometer (DTECHTOR). Detection limits of aromatic hydrocarbons obtainable in D TECH® system are listed in Table 7.2.

The OHMICRON system includes a soil collection kit, a sample extraction kit, a magnetic rack, a box with reagents, and the OHMICRON analyser (a laboratory benchtop, single-wavelength, microprocessor based analyser). The analysis is based on covalent binding of antibodies to magnetic particles. The first stage is an immunochemical reaction between antibodies/magnetic particles and the compound present as antigen. The second stage is separation in which the magnetic field is applied in order to separate the magnetic particles with the compound. The particles remain attached to the tube wall, while excess reagents are decanted. The particles are washed twice. The last step is colour development. For this purpose, a colour reagent is added. After a short incubation period, the colour development is arrested. The analyte concentration is quantified with the OHMICRON analyser. Using the analyser, it is possible to measure absorbances of calibrators and samples and to perform computation; sample absorbances and sample concentration with their uncertainties can be read from the instrument. Data obtained with immunochemical techniques were analysed using the STATISTICA program for Windows.

The DEXSIL Co. and Millipore companies offer test kits for determination of petroleum hydrocarbons in soil (PetroFlag™, EnviroGard™ Petroleum). The analysis includes sample collection, extraction of hydrocarbons from samples into an organic solvent, filtration through a filtering material, and measurement of the absorbances on a mini spectrophotometer. Analytical ranges 3–100 mg/kg (EnviroGard™) and 10–100 mg/kg (PetroFlag™).

The general engineering design department (Moscow, Russia) has proposed a device for rapid monitoring of petroleum products in waste, natural, and potable waters (Liliya 1). The device consists of a sampling tool, a pump, a sensing device (which includes an adsorption cartridge, a cylindrical ampoule, and an indicator tube) and a measuring chamber with a scale. A water sample to be analysed is passed through the adsorption cartridge to adsorb the petroleum products present in water. Subsequently, a solvent is passed through the absorbing cylinder and through the indicator tube. The indicator tube is filled with a special chemical blend indicating the content of petroleum products. After the solution has been passed through the tube, the length of the coloured zone is determined using the scale of the measuring chamber. The length of the coloured zone in the indicator tube is proportional to the concentration of petroleum products. The concentration of petroleum products in water in the range from 0.05 to 50 mg/l is found from a chart, depending on the length of the coloured zone and the volume of the water sample. The amounts of samples taken for this analysis are 5, 50, or 350 ml.

7.3 POLYAROMATIC HYDROCARBONS (PAH)

The systems proposed for determination of polycyclic aromatic hydrocarbons (PAH) include D TECH® PAH (E. Merck) and OHMICRON's RAPID Assay (Supelco, USA).

The methods involve an immunological approach. Polycyclic aromatic hydrocarbons react with a specific antibody bound to a latex particle (D TECH®) or to magnetic particles (OHMICRON), competing with an enzyme-labelled antigen (competitive enzyme immunoassay). A blue colour develops after the addition of a colour reagent. The PAH concentration can be determined semi-quantitatively with the help of a DTECHTOR (D TECH®) or OHMICRON analyser. The more intense is the blue colour, the lower is the concentration of PAH in the sample. The tests are designed in such a way as to eliminate interference from other substances as fully as possible.

The ranges of PAH concentrations determined in this way are 8–250 μg/l for water and 0.6–25 mg/kg for soil (D TECH®). Detection limits of various PAH for the D TECH® system are listed in Table 7.3.

TABLE 7.3

Detection limits of polycyclic aromatic hydrocarbons of the D TECH® system

Compound	Detection limit (μg/l)
Naphthalene	1766
Acenaphthylene	311
Acenaphthol	311
Fluorene	106
Phenanthrene	421
Anthracene	10
Fluoranthene	5
Pyrene	10
Benzo(a)anthracene	42
Chrysene	8
Benzo(b)fluoranthene	53
Benzo(a)pyrene	10
Dibenzo(a,h)anthracene	1060
Benzo(g,h,I)perylene	42
Indeno(1,2,3-cd)pyrene	8

Benzene, ethylbenzene, toluene, xylene, pentachlorophenol, and the mixture of polychlorinated diphenyls Arochlor 1254 do not interfere with determination.

7.4 ORGANOCHLORINE COMPOUNDS

HNU-Henby (USA) manufactures environmental test kits for detection and quantification of halogenated solvents in water and soil. The method is quick and easy. The whole procedure takes less than ten minutes. Sensitivity of the test is from 0.5 ppb to 100 ppm, depending on the compounds present in the sample.

QuickTest® for volatile organic halides in water (Envirol Inc., USA) were developed for determination of trichloroethylene, perchloroethylene, trihalomethanes, and carbon tetrachloride. The analysis includes extraction of the sample, recovery of the extract, and quantitative determination. A water sample is taken and mixed with the solvent extracting the analyte from the sample.

A Teflon® tape is used in the extraction process to separate the solvent from the water sample and minimize analyte volatilisation. The Teflon tape is removed and the extraction solvent containing the analyte is sucked into a syringe. The extract is then mixed with a reagent and exposed to ultraviolet light in the Envirometer™. Concentration of the analyte (in ppb) is displayed on the Envirometer display. The range of concentrations covered by this method is 5–2000 ppb. Benzene, methanol, toluene, oxalic acid, glyoxylic acid, sodium trichloroacetate, or sodium dichloroacetate (more than 2000 ppb), 2,2,2-trichloroethanol and vinyl chloride (more than 200 ppb) and pentachlorophenol (20 ppb) do not interfere with analysis.

The Dräger Liquid Extraction method (DLE) is an easy rapid test for detection of volatile contaminants (like perchloroethylene or trichloroethylene) in water. The measuring system comprises a specially calibrated gas-washing bottle, corresponding Dräger tubes, and a gas detection pump. The Dräger tubes are designed and calibrated for air analysis. Therefore, direct quantitative analysis of water, e.g. by drawing in water samples, is impossible. To make the tube technique applicable to detection of harmful substances in water, the contaminant should be transferred from the liquid to the gas phase.

A colorimetric test HYDROCLOR-Q® was proposed by the DEXSIL company for determination of organochlorine compounds in waste waters in the range of 200–4000 mg/l. Inorganic chlorine compounds do not interfere with analysis.

7.5 POLYCHLORINATED BIPHENYLS (PCB)

Two types of test kits for determination of polychlorinated biphenyls (PCB) in water and soil were proposed. In one type of test kit, determination is based on either visual or mini photometic estimation of the colour intensity of the compound formed upon the Friedel–Crafts alkylation of PCB with alkyl halide (see Section 7.2). Test kits based on this principle are, mainly, those developed by the HBU System Inc. and DEXSIL Co. (CLOR-N-SOIL®) [5,6,8]. The lower limits of measurable PCB concentrations are 0.5 mg/l for soil and 0.05 mg/l for water. In the presence of PCB, the colour of the extract varies from gentle pink to coral.

The second type of test kits makes use of immunoenzyme reactions; it is represented by OHMICRON RAPID Assay (Supelco, USA), D TECH® PCB (E. Merck), and Dräger EnviCheck PCB (Dräger, FRG) [9].

The ranges of PCB concentrations measurable in soil by the D TECH® PCB system are 0.5–25 mg/kg if the DTECHTOR mini photometer is used and 1–25 mg/kg when a colour scale is employed. The limits of detection are presented in Table 7.4.

The PCB concentrations in different soil samples were determined using a Dräger EnviCheck PCB test kit according to the following

Table 7.4

Detection limits for cross-reacting PCB mixtures of the D TECH® and Dräger EnviCheck PCB kits

Arochlor PCB mixture	Detection limit (mg per kg of soil)	
	D TECH® PCB	Dräger EnviCheck PCB
1016	5.7	4.0
1232	9.0	4.0
1242	1.5	2.0
1248	0.8	1.0
1254	0.5	0.4
1360	0.5	0,4

procedure. Soil samples (10 g) are weighed into an extraction vial and 20 ml of methanol is added. The vial is vigorously shaken for one minute with five stainless-steel beads for extraction. After one minute of sedimentation, the supernatant layer is filtered through a plastic filter syringe to remove soil particles. A sample (60 µl) is diluted with 540 µl of methanol. To obtain 1:10 dilution, 60 µl of the resulting mixture is diluted with 540 µl of methanol. A 30 µl-sample of the standard solution is buffered prior to immunoanalysis. The test is performed as a direct competitive enzyme immunoassay in test tubes coated with PCB-specific antibodies. Standard solutions of PCB and the samples are incubated in different antibody-coated test tubes for 10 min, and three drops of an enzyme tracer solution are added. After 5 min, the tubes are washed to remove unbound reagents. For substrate–chromogen reaction, five drops of H_2O_2 solution and the same amount of tetramethylbenzidine solution are incubated for 2.5 min. The reaction is terminated by addition of five drops of H_2SO_4, and the colour intensity is measured at 450 nm. The intensity of the resulting yellow colour is inversely proportional to the PCB concentration in the soil. Determination of unknown PCB concentrations in soil was carried out by comparing the absorption values obtained for samples and for standards. A soil sample containing PCB in a 1 mg/kg concentration served as standard. Each sample was measured in 1:1 and 1:10 dilutions, and absorbancies were compared with those of the standard. The samples were classified according to three concentration ranges

- less than 1 mg/kg soil
- 1 to 10 mg/kg soil
- at least 10 mg/kg soil

The sample whose absorbance was lower than that of the standard was regarded as positive. A positive 1:1 dilution implies the PCB concentration of at least 1 mg/kg, while a positive 1:10 dilution points to the 'at least 10 mg/kg soil' range. When the sample showed a higher absorption value than the standard, the PCB concentration in the soil was below 1 mg/kg. Twelve test kits for immunochemical evaluation of PCB (DRÄGER EnviCheck PCB) are available. Each kit comprises four single tests for determination of PCB concentration in soils. According to the manufacturer's information, cross reactions occur with several PCB mixtures.

The following do not interfere with analysis: 1000 mg/kg amounts of 1-chloroanthracene, 2- chloroanthracene, 9-chloroanthracene, 9,10-dichloroanthracene, 1,2-dichloroanthracene, 1.3-dichloroanthracene, 1,4-dichloroanthracene, 1,2,3-trichloroanthracene, 1,2,4-trichloroanthracene, 1,2,5-trichloroanthracene, 1,2,4,5-tetrachloroanthracene, pentachlorphenol, DDT, or 2,4-dichlorophenylbenzenesulphonate.

7.6 VARIOUS PESTICIDES

Organophosphorus pesticides are determined using enzyme test methods. Indicator strips contain choline esterase enzyme, acetylcholine as substrate and an acid-base indicator. Enzymatic hydrolysis of the substrate slows down in the presence of organophosphorus compounds:

choline esterase

$$(CH_3)_3NCH_2CH_2OCOCH_3 + H_2O \rightarrow (CH_3)_3NCH_2CH_2OH + CH_3COOH$$

In the presence of organophosphorus compounds, the rate of enzymatic reaction decreases, resulting in a decrease in the amount of the acid evolved and a change in the colour of the indicator.

A colorimetric test method for determination of methyl and dimethyl carbamates and the sum of organic phosphorus-containing esters based on inhibition of acetylcholine esterase enzyme was (NANOCOLOR® Pesticides 35, Macherey-Nagel). Results are expressed in μg/l of paraoxon-ethyl equivalents (POE). Analytical range is 0.5–35 μg/l POE [4]. A method of preparation of indicator paper strips for determination of organic pesticides was proposed. The procedure includes impregnation of paper with an alkaline borate buffer (pH 9.5–10) and esterase-1 enzyme of 0.3–0.4 E/ml concentration; a 0.008 to 0.012% formaldehyde solution is used for immobilization and 0.48–0.52% gelatine for stabilization [10].

A very sensitive test for organophosphorus pesticides using two detector strips was developed. The test is based on inhibition of choline esterase by organophosphorus compounds [11–13]. One strip (A) is impregnated with choline esterase from horse-liver acetone treated powder. It metabolises the 1-naphthyl acetate present on the second strip (B) to naphthol. The latter couples with Fast Blue B in diazotization reaction to form a magenta-coloured compound. Methyl parathion and other organophosphorous compounds inhibit this reaction and give rise to a white spot on a detector strip.

For the analysis, one drop of the test solution is dropped onto indicator strip A using a microcapillary pipette and the spot is dried in a stream of air. Then the strip is put horizontally on a clean glass microslide and wetted with distilled water. The slide is heated for 2 min at 40°C. Then a 2.5-cm^2 portion of indicator strip B is placed on another glass microslide and wetted. Strip A is put below strip B between the two slides. The presence of organophosphorus compounds is indicated by a white spot formation. The detection limit is 0.1 μg for methyl paraoxon and omethoate and 0.5 μg for fenitrooxon [14].

A new specific spot test for detection of malathion in water is performed by shaking the sample with 100 mg of activated charcoal for 2–3 min. The charcoal is then separated by centrifugation and shaken with 2 ml of ethanol containing 1–2 mg of KOH. Acetic acid (6 or 7 drops) is added and the mixture is heated for 5 min at 135°C. A red colour develops if malathion is present. Detection limit is 1 mg/l [15].

A capillary spot test for detection of trace levels of organophosphorus insecticides in water and soil is carried out with decomposition of the sample by heating it with a 0.25% aqueous solution of NH_4Cl and zinc dust. The vapour evolved during heating is collected in a capillary tube loaded with a cotton plug impregnated with equal volumes of a 10% solution of dimethylaminobenzaldehyde and a 20% solution of trichloroacetic acid (both in benzene). The solvent is removed by drying with hot air. Colouring of the plug indicates the presence of the analyte. The method was used for detection (at microgram levels) of, e.g., malathion, formothion, thiometon, dichlorvos, methyl parathion, dimethoate, and phosphamidon in tap water and soil [16].

A direct spot test for carbaryl (1-naphthol-*N*-methylcarbamate) in water [17] is based on spotting a sample on paper impregnated with NaOH. The paper is then immersed in a 0.1% benzene solution of 2,6-dichloroquinone-4-chloroimine, a violet-blue colour indicating the presence of carbaryl. This method can be made semi-quantitative by controlling the volumes of the sample and the reagent solution and by comparing the colour with the colour of a standard. The detection limit is 1 ppm. The method was applied to river and tap water.

A test using 8-hydroquinoline (brown colour), hexacyanoferrate (violet), and sodium nitrite and sulphanilic acid (pink) as indicators was for field determination of carbaryl and mancozeb pesticides in water and soil.

Immunoenzyme test kits available for determination of pesticides in water and soil include the EnviroGard™ test kits (Supelco, Inc., USA) and RaPID Assay® (Millipore, USA) [19-21]. In the immunoassay, an antibody is generated which recognizes very selectively the specific antigen. Antibodies are formed on exposure of specialised cells to a specific antigen (analyte) of interest. These cells then produce large quantities of antibodies specific to this particular analyte. When the antibody is exposed to a mixture of analytes, for example, pesticides or toxic organic compounds in soil or food extract or in a water sample, it will recognise and bind only one specific analyte that induced its original formation.

In the RaPID Assay® kit, these analyte-specific antibodies are attached to a solid support (magnetized particles) to facilitate analyte binding. Note that the antibody–antigen complex cannot be destroyed (unless the buffer pH or the ionic strength are sharply changed), and this should be considered to be a permanent bond within the assay. To attain a maximum sensitivity of immunoassay, RaPID Assay® test kits are produced in the competitive format. This means that the analyte in the sample and an enzyme-labelled analyte conjugate compete for the antibody binding sites. A coloured solution is used to react with the enzyme conjugate generating colour that can be read with a spectrophotometer at 450 nm. The colour developed is inversely proportional to the amount of the analyte in the sample. RaPID Assay® systems were proposed for determination of alachlor, aldicarb, atrazine, captan, carbaryl, carbofuran, chlorothalonil, chlorpyrifos, cyanazine, 2,4-D, methomyl, metolachlor, paraquat, procymidone, trichloropyridinol, and triclopyr. The EnviroGard™ test kits are intended for determination of ametryn, atrazine, cyanazine, de-ethylatrazine, de-isopropylatrazine, prometon, prometryn, propazine, simazine, simetryn, terbuthylazine, terbutryn, trietazine, and 6-OH-atrazine. The lower limits of determination vary from 0.01 to 0.3 ppb, depending on the particular pesticide.

A simple method for detection of photosynthesis-inhibiting herbicides was proposed as a technique for practical environment monitoring. The method combines a test system based on inhibition of thylakoid membrane activity in microtiter plates with sample preconcentration on solid-phase cartridges. The C_{18} cartridge provides for an acceptable recovery (>85%) of atrazine, simazine and cyanazine over a broad range of concentrations. The proposed method permits detection of photosynthesis-inhibiting herbicides in the concentration range of $1 \cdot 10^{-10}$ mol/l to $2 \cdot 10^{-5}$ mol/l. Three procedures for immobilization of thylakoid membranes on microtiter plates were compared in order to improve stability of thylakoid membranes. The procedure in which poly(vinyl alcohol) with grafted styrylpyridinium groups was used for immobilization of thylakoid membranes was found to be the most promising. Due to the simplicity of manufacture of the test kits, they can be considered to be a practical tool for detection of trace quantities of photosynthesis-inhibiting herbicides in potable water [22].

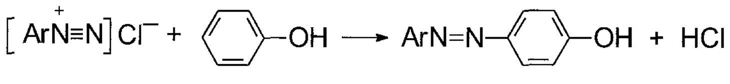
7.7 OTHER ORGANIC COMPOUNDS

7.7.1 Phenols

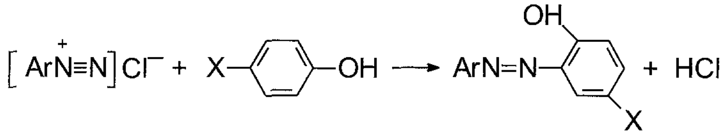
Detection of phenol and its derivatives in test analysis is mainly based on azo dye formation. The following procedure is most often used. The first stage is diazotization of primary aromatic amine with sodium nitrite in an acid medium, resulting in the formation of a diazonium salt

$$ArNH_2 + NaNO_2 + 2\ HCl = [ArN^+{\equiv}N]\ Cl^- + NaCl + 2H_2O$$

and the second stage is azo coupling of the diazonium salt with phenol in an alkaline medium giving an azo compound



If the *para*-position is occupied, an *ortho*-azo compound is formed

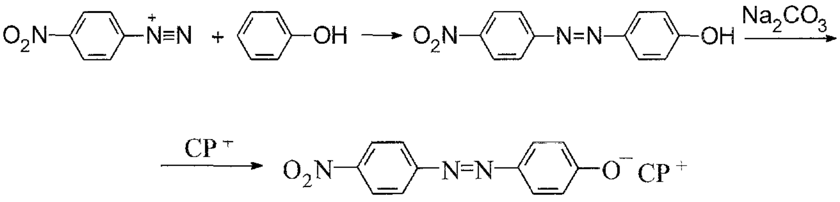


Azo coupling with hydroxy compounds, which are most active as phenoxide anions, is normally carried out at pH 8–11. The diazonium salts are unstable in aqueous solutions and gradually decompose to give phenols and nitrogen; therefore, the main task in developing test methods for determination of phenols and amines is to prepare stable diazo compounds.

The complex salt, 4-nitrophenyldiazonium tetrafluoroborate (NPD), was for determination of phenol as a reagent stable during storage [23]:

For determination of phenol, a square piece (1×1 cm) of a filter paper impregnated with NPD and a square piece of paper impregnated with a mixture of sodium carbonate and cetylpyridinium (CP) chloride are added to 1 ml of the liquid to be analysed.

In the presence of CP, a more extensive colour develops, due to the formation of an ion associate with the dissociated hydroxy group.

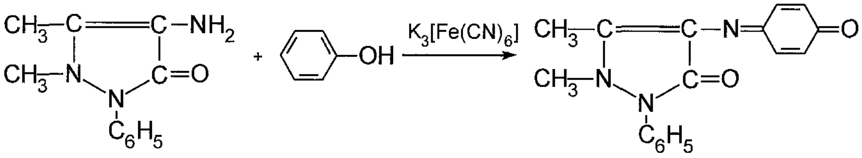


The presence of a 50-fold excess of aniline does not interfere with determination. 2,4,6-Trisubstituted phenol derivatives, 2,4-disubstituted 1-naphthol derivatives, and 1-substituted 2-naphthol derivatives do not hamper the analysis either. The ranges of phenol concentration that can be determined by this method are 0.05–0.1–0.3–0.5–1–3–5 mg/l. These tests were employed to determine phenol in waste waters.

In NANOCOLOR® Phenol (Macherey-Nagel) system, 4-nitroaniline is used as a diazo component. The range of phenol concentrations determined is 0.01–7.0 mg/l.

For qualitative or semi-quantitative estimation of the content of phenol in natural water, pellets made of polyurethane foam were proposed [24,25]. The method is based on adsorption of red-violet-coloured associates of 4-nitrophenylazo-derivatives of phenol with cetyltrimethylammonium cation by a polyurethane foam pellet. The presence of phenols is indicated by the change of the pellet colour from yellow to red-violet. The content of phenols is determined by visual comparison of the colour of the pellet with a colour scale. The impurities that do not interfere with the determination of phenols include 1000-fold amounts of alkali and alkaline earth metals, Cu(II), Pb(II), Fe(III), Cl^-, NO_3^-, SO_4^{2-}, HPO_4^{2-}, CH_3COO^-, tartrate and citrate ions; 800-fold amounts of Co(II); 500-fold amounts of Ni(II), Cr(III,VI); 300-fold amounts of S^{2-}; 200-fold amounts of Al(III); 100-fold amounts of Mn(II), humic acids, and sulphonic acids; 10-fold amounts of Hg(II); and stoichiometric amounts of 1- and 2-naphthol, 2- and 3-cresol, and a 3-fold excess of 4-cresol. Concentrations of phenol derivatives ranging from 0.1 to 0.8 mg/l (calculated in relation to phenol) can be determined in this way.

4-Aminoantipyrine is used most often as the reagent in test methods (Macherey-Nagel, Hach Co., CHEMetrics). Phenol and its derivatives form coloured compounds with 4-aminoantipyrine in the presence of potassium hexacyanoferrate(III) at pH 10.



4-Aminoantipyrine virtually does not react with 4-cresol and para-substituted phenols containing alkyl, aryl, nitro, benzoyl, nitroso and aldehyde groups. The range of phenol concentrations determined using the NANOCOLOR® Phenol, Hach Co. and CHEMetrics systems is 0.1–5.0 mg/l [2–4].

Indicator tubes for phenol determination in natural and waste waters and in industrial solutions were proposed [26]. A coloured compound can be formed in the tube between the indicator powder and the hydrophobized silica gel modified non-covalently by 4-aminoantipyrine. The range of concentrations determined for 1 ml of an analyte passed through the tube is 0.01–0.1 mg/l.

7.7.2 1-Naphthol

A test system for qualitative and semiquantitative estimation of the content of 1-naphthol in natural water was proposed [27]. The method is based on sorption of a blue-coloured 4-nitrophenylazo compound of 1-naphthol with a foamed polyurethane pellet. The presence of 1-naphthol is indicated by the change in the colour of foamed polyurethane from white to blue; the content of 1-naphthol is determined by visual examination of the colour intensity against a colour scale prepared beforehand. The determination of 1-naphthol is not hampered by 3500-fold amounts of $H_2PO_4^-$; 2000-fold amounts of Ca, Mg, and Co(II); 1000-fold amounts of Li, Na, K, Ba, Fe(III), Ni, Cd, Cu(II), Cr(VI), F^-, Cl^-, Br^-, I^-, NO_3^-, SO_4^{2-}, CH_3COOH, citrate, and tartrate; 500-fold amount of Zn, NH_4^+, aniline, and Al (in the presence of 0.01 M $H_2PO_4^-$); 300-fold amounts of Mn, Cr(III), and Pb (in the presence of 0.01 M $H_2PO_4^-$); 10-fold excess of Hg(II); and stoichiometric amounts of phenol and 2-naphthol. The method allows determination of 1-naphthol in the concentration range of 0.02–0.4 mg/l.

7.7.3 Nitrophenols

Determination of nitrophenols is based on sorption from aqueous solutions by polyurethane foam pellets [28]. The presence of nitrophenols is judged by the change in the colour of the pellet from white to yellow. The content of nitrophenols is determined by visual examination of the intensity of the colour of the pellet against a colour scale prepared beforehand. The method allows determination of nitrophenols in the concentration range from 0.005 to 0.05 mg/l.

7.7.4 Anionic surfactants

Determination of anionic surfactants (ASurf) is based on the formation of associates with Methylene Blue or Toluidine Blue, which are dissolved in chloroform to give blue solutions (Macherey-Nagel, Hach Co., CHEMetrics, Hellige Co.). Analysis is based on evaluation of the intensity of the developed colour using a comparator with a colour disk or using a mini photometer. The ranges of concentrations determined (calculated in relation to the standard, methyl dodecyl benzenesulphonate) are 0.02–5 mg/l (NANOCOLOR® Detergents anionic), 0.05–1 mg/l (Hach Co.), and 0.1–3 mg/l (CHEMetrics).

Yet another method for determination of anionic surfactants is based on sorption of red-coloured outer-sphere associates $[Fe(Phen)_3](ASurf)_2$ formed by anionic surfactant and iron(II) phenanthrolinate complex cation by a foamed polyurethane pellet [29,30]. The presence of anionic surfactants is indicated by the change in the foamed polyurethane colour from white to red; the content of anionic surfactants is found by visual comparison of the colour of the pellet with a colour scale prepared beforehand. The presence of 350-fold amounts of PO_4^{3-}, 200-fold amounts of Cl^-, or 100-fold amounts of NO_3^{3-} or SO_4^{3-} do not interfere with the determination. The test system permits determination of anionic surfactants in concentrations from 1 to 30 mg/l.

7.7.5 Cationic surfactants

Determination of cationic surfactants (CSurf) is similar to that of anionic surfactants except that the basic dye is replaced by an acidic one, namely, Bromphenol Blue, which forms with CSurf coloured associates soluble in chloroform (Macherey-Nagel). Determination is based on the intensity of the developed colour using a comparator with a colour disk or a mini photometer. The analytical range of the NANOCOLOR® Detergents cationic system (calculated in relation to the standard — N-cetyl-N,N,N-trimethylammonium bromide) is 0.05 to 5 mg/l.

Indicator paper impregnated with Bromothymol Blue or Bromocresol Green for determination of CSurf in the range 50–2000 mg/l was proposed [31]. Disks of indicator paper 3.8 cm in diameter sealed into a polymer film were used. A small hole was made in the middle of the disk. Analysis is based on the area of spots produced after the solution to be analysed has been absorbed through the hole in the film.

Yet another method proposed for qualitative and semi-quantitative determination of the contents of cationic surfactants in natural waters is based on sorption by a polyurethane foam pellet of blue-coloured ion associates formed by cationic surfactants with a double-charged anion of Bromophenol Blue, $(CSurf)_2BPB$ [32]. The presence of cationic surfactants is indicated by a change in the colour of the pellets from white to blue; the content of cationic surfactants is determined by visual comparison of the colour intensity with a colour scale prepared beforehand. Determination of cationic surfactants is not hampered by $4\cdot10^4$-fold amounts of Na^+ and CO_3^{2-} in the presence of EDTA, 100-fold amounts of Li, K, NH_4^+, Mg, Ca, Zn, Cd, Al, Fe(III), Co(II), Ni, Cu(II), F^-, Cl^-, SO_4^{2-}, and CH_3COO^-; a 200-fold amount of PO_4^{3-}, 100-fold amounts of I^- and NO_3^-, a 10-fold amount of Triton X-305, and an equal amount of Triton X-100. The test method provides for determination of 0.4 to 10 mg/l of cationic surfactants.

A method for determination of CSurf based on the length of the coloured zone on a test strip impregnated with metal chelates and organic reagents and sealed in a polymer film was proposed [33]. Clear and sharp zones are produced on the test strip in the presence of cationic surfactants in the molybdenum–thiazolylazocatechol system. The length of the coloured zone formed on the strip is proportional to CSurf concentration in the range of 1–1000 mg/l. In the presence of surfactants, a red zone develops on a yellow test strip. Determination of cationic surfactants is not interfered with by 100-fold amounts of anionic or non-ionic surfactants, 1000-fold amounts of alkali and alkaline earth metal elements, Al, Zn, Cd, Mo, Cl^-, or 50-fold amounts of Ge, V, W, Ni, or Co.

The content of CSufr was determined using the colouring of paper after 20 ml of the solution was passed through it using a concentrating device. An antimony complex with phenylfluorone and a molybdenum complex with 4-(2-thiazolylazo)pyrocatechol immobilized on paper were used in this test [33]. In the former case, the orange reaction zone is coloured pink-red in the presence of CSurf, while in the latter case, the red-orange area is coloured lilac. The lower limit of determination of cetylpyridinium (CP) chloride in the Sb–phenylfluorone system was 0.01 mg/l, that for the Mo–4-(2-thiazolylazo)pyrocatechol system was 0.1 mg/l.

The determination of CP using a Sb–phenylfluorone-impregnated paper is best carried out at pH 4–6. The colour of the reaction zone can be observed visually for ranges of CP concentrations of 0.01–0.05–0.1–0.5 mg/l. The presence of a 200-fold amount of a non-ionic surfactant does not interfere with the determination of cationic surfactants.

7.7.6 Non-ionic surfactants

Indicator paper for determination of non-ionic surfactants in waste waters was proposed. The paper is impregnated with aluminium, lanthanum, or zirconium complexes with sodium 2-aminonaphthalene-(1-azo)benzene-2-carboxylate [34]. To determine a non-ionic surfactant, a drop of the water to be analysed is applied to the indicator paper and the colour of the spot (margin) is examined against the reference scale composed using standard non-ionic surfactants. The range of concentrations determined in this way is 30 to 5000 mg/l. The analysis can be performed in the presence of 100 mg/l of calcium, magnesium, manganese(II), zinc, lead, copper, cobalt, nickel, cadmium, aluminium, iron(III), chromium(III), or chromium(VI), or anionic surfactants. The presence of molybdenum(VI) hampers the analysis.

7.7.7. Aniline

Indicator tubes were developed for determining aniline in waste waters and industrial solutions [35]. A coloured compound is formed in the tube filled with an indicator powder, e.g. silica gel modified non-covalently with N,N-diethyl-*p*-phenylenediamine; the reagents used are N,N-diethyl-*p*-phenylenediamine and potassium dichromate. For a 1 ml solution passed through the tube, the range of concentrations determined is 0.02–2.5 mg/l.

Indicator powder, viz. silica gel modified non-covalently with N,N-diethyl-*p*-phenylenediamine, was proposed for solid-phase spectrophotometric determination of aniline in industrial solutions and environmental objects. The range of concentrations covered by this method is 0.02–0.6 mg/l.

Indicator paper strips with chemically immobilized 1-naphthylamine were proposed for determination of aromatic amines including aniline [36]. Test strips sealed in a polymer film were employed. Sodium nitrite was added to the water under test in order to prepare diazotized aniline and then the test strips were used. On the liquid being absorbed, a red zone appeared on the white test strip whose length was proportional to the concentration of aniline in the range of 0.5 to 1000 mg/l.

7.7.8 Sum total content of sulphur-containing reductants

For determination of the total amount of sulphur-containing reductants (diethyl dithiocarbamate, ethyl xanthate calculated in relation to sulphite) in natural waters, atmospheric precipitates, and industrial solutions, indicator tubes were proposed [37]. A coloured compound is formed in solution. Silica gel is used as indicator powder and Methylene Blue triiodide is used as reagent. When a 1-ml sample is passed through the tube, the range of concentrations covered is 1–10 mg/l.

7.7.9 Organomercury compounds

Polyurethane foam (PUF), a new support for immobilization of peroxidase from horse-radish roots, was used to develop a test procedure for determining 0.008 to 1000 μmol/l of organomercury compounds (methyl-, ethyl- and phenylmercury) based on a liberating action of these compounds on a phenylthiourea-inhibited enzyme in the oxidation of *o*-dianisidine, or on the influence of these compounds on the duration of the induction period in the oxidation of 3,3′,5,5′-tetramethylbenzidine in the presence of sodium diethyldithiocarbamate (DDTC) [38,39]. Metrological characteristics of the determination of organomercury compounds are presented in Tables 6.9 and 6.10.

7.7.10 Diethylhydroxylamine

Determination of diethylhydroxylamine (DEHA) in boiler water is based on reduction of iron(III). Iron(II) thus formed reacts with ferrozine to give a purple-coloured chelate. The colour intensity is estimated either visually or with a mini photometer. The ranges of DEHA concentrations covered by the procedure are 0.05–1 mg/l (NANOCOLOR® DEHA), 0.02–3 mg/l (CHEMetrics), and 0.01–1.7 mg/l (Hach Co.).

REFERENCES

1. Wastewater COD Pollution Test, *Process Eng.* (Austral.), 21 (1993) 20.
2. *CHEMetrics, Inc. Perfecting Simplicity in Water Analysis,* Catalog, 2000.
3. *HACH Co. Test Kits and Portable Laboratories*, Catalog.
4. *Macherey-Nagel. Rapid Tests*, Catalog, 2000.
5. J.N. Driscoll and L. Julin, *Int. Env. Tech.*, 9, 1999, 42.
6. V.D. Roe, M.J. Lacy, J.D. Stuart and G.A. Robbins, *Anal. Chem.*, 61 (1989) 2584.
7. G. Plaza, K. Ulfid and A.J. Bevolo, *Int. Env. Tech.*, 9 (1999) 11.
8. *DEXSIL Co. Environmental Field Test Kits*, Catalog.
9. S. Pullen, G. Haiber, I.I.E. Scholer and B. Hock, *Intern. J. Environ. Anal. Chem.*, 65 (1996) 127.
10. O.V. Vashkevich and A.I. Ustinova. *RF Pat.* 1709824, *Byull. Izobret.*, No. 29 (1995).
11. C.E. Mendoza, *Residue Rev.* 43 (1972) 105.
12. C.E. Mendoza, P.J. Wales, H.A. McLeod and W.P. McKinley, *Analyst (London),* 93 (1968) 34.
13. K. Nanguneri, V. Nanda, K. Visweswaraiah and S.K. Majumdar, *J. Agric. Biol. Chem.,* 40 (1976), 431.
14. K. Nanguneri, V. Nanda and Y.P. Devi, *J. Assoc. Off. Chem.,* 64 (1981) 841.
15. S.R. Sharma, H.S. Rathore and S.R. Ahmed, *Anal. Abstr.,* 46 (1984) 3H69.
16. H.S. Rathore, I. Ali and A. Kumar, *Anal Abstr.,* 52, (1990) 7G13.
17. H.S. Rathore and S.K. Saxena, *Anal. Lett.,* 23 (1990) 93.
18. U.S. Rathore, R. Sharma and S. Mital, *Water, Air and Soil Pollut.* 97 (1997) 431.

19. R.J. Bushway et al., *Food Chem.,* 35 (1990) 51.
20. W.H. Newsorae, *J. Agric. Food Chem.,* 33 (1985) 528.
21. R.A. Leavitt et al., *Bull. Environ. Contam. Toxicol,.* 46 (1991) 22.
22. E.V. Piletskaya, S.A. Piletsky, T.A. Sergeyeva, A.V. El'skaya, A.A. Sozinov, J.-L. Marry and R. Rouillon. *Anal. Chim. Acta.,* 397 (1999) 1.
23. V.G. Amelin, Doctoral Sc. Dissertation, Moscow State University, Moscow, 1998 (in Russian).
24. S.G. Dmitrienko, E.N. Myshak, V.K. Runov and Yu.A. Zolotov, *Chem. Anal. (Warsaw),* 40 (1995) 291.
25. S.G. Dmitrienko, E.V. Loginova, E.N. Myshak and V.K. Runov, *RF Pat.* 2078333, *Byull. Izobret.*, No. 12 (1997).
26. Yu.A. Zolotov, *Vestn. Ross. Akad. Nauk,* 67 (1997) 508.
27. S.G. Dmitrienko, E.N. Myshak, A.V. Zhigulev, V.K. Runov and Yu.A. Zolotov, *Anal. Lett.*, 30 (1997) 2541.
28. S.G. Dmitrienko, Doctoral Sc. Dissertation (Chemistry), Moscow, 2001.
29. S.G. Dmitrienko, Yu. A. Zolotov, L. N. Pyatkova and V.K. Runov, *RF Pat.* 2041460, *Byull. Izobret.*, No. 22, 1995.
30. S.G. Dmitrienko, L.N. Pyatkova and V.K. Runov, *Zh. Anal. Khim.*, 51 (1996) 600.
31. H. Starp, N. Buschmann and K. Cammann, *Fresenius' J. Anal. Chem.*, 368 (2000) 203.
32. S.G. Dmitrienko, L.N. Pyatkova, L.P. Bakhaeva, V.K. Runov and Yu.A. Zolotov, *Zh. Anal. Khim*, 51 (1996) 493.
33. V.G. Amelin and V.M. Ivanov, *Zh. Anal. Khim.*, 55 (2000) 411.
34. V.I. Kuznetsov, G.N. Puliko and V.V. Gorshkov, *USSR Inventor's Certificate* 1567975, *Byull. Izobret.*, No. 20 (1990).
35. D.Yu. Marchenko, I.A. Morozkin, E.I. Morosanova, N.M. Kuzmin and Yu. A. Zolotov., *Zh. Anal. Khim.*, 52 (1997) 1167.
36. V.G. Amelin and I.S. Kolodkin, *Zh. Anal. Khim,* 56 (2001) 206.
37. D.Yu. Marchenko, E.I. Morosanova, N.M. Kuz'min and Yu.A. Zolotov, *Zh. Anal. Khim.*, 52 (1997) 1162.
38. T.N. Shekhovtseva, I.A. Veselova and I.F. Dolmanova, *RF Pat.* 2143683, *Byull. Izobret.*, No. 36, 1999.
39. I.A. Veselova and T.N. Shekhovtsova, *Mendeleev Commun.* No. 6 (2000) 98.

Chapter 8

Analysis of Air and Vapours

The goals of air analysis are very diverse. They include continuous sanitary chemical monitoring of work zones, industrial gas emissions, technological processes, chemical survey under emergency situations due to chemical and environmental accidents, geological survey, chemical monitoring of fire- or explosion-prone works, and individual safety control. This results in a great diversity of test methods, their combinations, analytical ranges, and complexity of instrumentations, tools, and devices for air analysis. Besides gaseous components like carbon oxide, ozone, nitrogen dioxide, ammonia, and chlorine, it is usually necessary to monitor the concentration of vapours of liquids like acetone, benzene, chloroform, gasoline, mercury, and organomercury compounds, or of solid substances like aniline, formaldehyde, etc. Air analysis also includes determination by chemical methods and biological indicators of solid atmospheric precipitations like dust, microbiological pollution, priority chemical pollutants including acids (determination of Σc/MAC ratio).

Methods to determine hazardous air components have been described in many publications [1–6]. The majority of hazardous substances in air are monitored by laboratory methods requiring sample collection followed by shipment to the laboratory for indoor analysis. This does not always guarantee effective measures for providing safe working conditions. Thus, the development of rapid, cheap, and simple methods, including rapid tests, seems topical. Test methods can be in the form of indicator tubes, pocket analysers, and others.

8.1 ANALYSIS USING INDICATOR TUBES

The advantages of analysis using indicator tubes are due to its quickness and the possibility of obtaining results directly on site. The method's instrumentation is quite simple enabling untrained persons to use it. Devices are compact, light and cheap. The method's sensitivity is rather high, its performance parameters are quite satisfactory, no preliminary calibration of analytical instruments, or sources of electrical or heat power and needed.

8.1.1 Principles of operation and design of indicator tubes

Indicator tubes are sealed glass tubes filled with a solid sorbent treated with an analytical reagent (Fig. 8.1). Various powdered materials are used as reagents supports: silica, aluminium oxide, porcelain, glass, and chromatographic sorbents. The structure and nature of the sorbent significantly affect the properties of indicator powder.

Before use, the majority of sorbents are treated with acids (usually with boiling HCl), dried or heated at a certain temperature, and ground to the required particle size. Next, indicator powder is prepared by applying a reagent solution with a given concentration; auxiliary components provide for optimum conditions of subsequent detection and determination and to enhance selectivity. Finally, the indicator

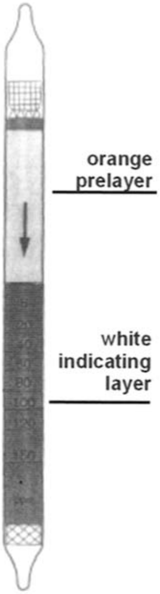


Fig. 8.1. Indicator tube for determining gases with a scale of lengths of coloured zones.

powder is dried at a required temperature until a friable state of the powder is achieved.

The indicator powder is located in the indicator tube between tampons of atmosphere-proof materials like hygroscopic adsorbent cotton, fibreglass, or porous teflon pads. Sometimes, a silica layer is placed between the powder and tampons to exclude their direct contact.

Indicator tubes are hermetically sealed. Directly before use, the ends are broken, and an air sample of a given volume is pumped through the tube.

8.1.2 Chemical composition of indicator powders

Only compounds quickly interacting with the target component and selectively forming intensely coloured reaction products can be used as reagents. Also, the colour of the formed products should be different from the colour of the indicator powder. Selectivity is usually enhanced by the use of additional oxidative, drying, or filtering tubes or tubes equipped with sorbents for absorbing components interfering with the main determination.

Examples are: chamotte treated with copper sulphate (the latter interacts with ammonia and hydrogen sulphide, but not with phosphine), chamotte with silver(I) nitrate and mercury(I) sulphate that can be used to determine gasoline in the presence of unsaturated aliphatic and aromatic hydrocarbons, glass powders with barbituric acid interacting with ammonia but not with aromatic amines, and a glass powder treated with ammonium acetate which absorbs formaldehyde but does not react with acrolein.

Tables 8.1 and 8.2 present data on the main components of indicator powders, principles of colour reactions and analytical ranges. Analytical ranges can be changed either by varying concentrations of the main components of indicator powders or the volume of tested samples (number of pumping cycles; each cycle samples 100 cm^3 of air). In Tables 8.1 and 8.2 linear analytical ranges are expressed in ppm units ($ppm = mg/m^3$).

In some cases, it is required to determine the sum total of air components that are close in their physical or chemical properties. The filtering tampon cannot be of help as it is poorly selective. Nevertheless, such tubes may still be used for sanitary control of the air of industrial enterprises. For instance, determination of methanol, ethanol, isopropanol, isobutanol, diethyl ether, and butyl acetate is

TABLE 8.1

Some Dräger indicator tubes for rapid determination of hazardous substances in air

Compound to be detected	Reagent or reaction	Analytical range (ppm)
Acrylonitrile	Formation of HCN, interaction with $HgCl_2$ and Methyl Red	5–30
Ammonia	Bromophenol Blue, acid	5–70; 50–700
Aniline	Furfural, acid	1–20
Nitrogen dioxide	Diphenylbenzidine	0.5–10; 5–25
Dimethyl sulphate	4-(4-Nitrobenzene)pyridine	0.2–5
Diethyl ether	Chromates, H_2SO_4	100–4000
Mercaptans	Copper(II) compounds	2–100
AsH_3	Gold(III) compounds	0.05–3
Carbon disulphide	Copper(II) compounds, amine	13–288
Toluene	I_2O_5, H_2SO_4	5–400
Phenol	2,6-Dibromoquinone-4-chlorimide	5
Chlorine	*o*-Tolidine	0.2–3; 2–30
Epychlorohydrin	Oxidation to Cl_2, *o*-tolidine	5–50

based on one and the same indicator powder, viz. that with chromium acid anhydride in sulphuric acid. Unreacted chromium(VI) then reacts with diphenylbenzidine. In the case of trichloroethane, trichloroethylene, and epichlorohydrin, they are first oxidised to elementary chlorine, which is followed by the reaction with *o*-tolidine or diphenylbenzidine. Indicator tubes for ammonia, hydrazine, HCl and triethylamine contain Bromophenol Blue and an acid.

Some indicator tubes use reagents that will decompose during long storage. Therefore, the components are separated and mixed directly before use. One component is a solution in an ampoule placed in the indicator tube. When such a tube is used, before or after pumping the sample, the ampoule is broken with a special device resulting in the reagent reacting with the tested substance and colour development.

In some cases, a sealed ampoule with liquid reagent is placed in a plastic tube connecting two parts of the indicator tube. Before the test, the tube is bent, the ampoule breaks, and its contents interact with the tested compound.

TABLE 8.2

Some Krismas+ (St. Petersburg) indicator tubes for rapid determination of hazardous substances in the air

Compound to be detected	Reagent or reaction	Analytical range (ppm)
Sulphur dioxide	KIO_3 (by the formation of free I_2)	5–14
Nitrogen dioxide	Diphenylamine	2–200
Nitrous oxide	*o*-Dianisidine	2–100
Carbon oxide	I_2O_5 (by the formation of free I_2)	5–50; 500–60000
Petroleum hydrocarbons (C_5–C_{11})	KIO_3 (by the formation of free I_2 and asphaltization products)	100–300
Ozone	By the discolouration of Indigo Carmine	0.1–2.0
Chlorine	By the formation of tetrachlorofluorescein	0.5–200
Hydrogen sulphide	Lead acetate	1–50
Ammonia	Bromophenol Blue	5–100; 10–1000
Carbon dioxide	By the formation of the coloured associates of leuco-base of Crystal Violet with tetraethylenepentamine	200–10000

8.1.3 Methods of concentration determination

The concentration of the tested component is quantified either via the change in the intensity of the colour of the indicator powder or by comparing it with colour scales using the length of the coloured layer. The former option is more subjective as its results depend on the keenness of the colour vision of the operator, time of day, brightness of the background, and stability of the colour scale which are either printed on the ampoule or supplied separately. The latter option has been called linear-colorimetric [7–11]. The method is based on the colour change of indicator powder as a result of interaction with a hazardous substance in the analysed air pumped through the tube. The length of the coloured zone of the reaction product is proportional to the concentration of the target component.

Several factors affect the length of the coloured zone: nonuniformities of the inside diameter of the glass tube, rate of pumping, indistinct boundaries of the coloured zone due to washout effects, changes in

TABLE 8.3

Some Dräger indicator tubes of prolonged action

Component to be detected	Analysis time (h)	Volume range (μl)	Analytical range (ppm) for the maximum analysis time
Ammonia	4	10–100	2.5–25
Benzene	4	20–200	5–50
Vinyl chloride	8	10–50	1.3–6.3
Nitrogen dioxide	8	10–100	1.3–13
Sulphur dioxide	4	5–50	1.3–13
Carbon oxide	8	5–500	6.3–63
Hydrogen sulphide	8	5–60	0.63–7.5
Prussic acid	8	10–120	1.3–15
Hydrochloric acid	8	10–50	1.3–6.3
Toluene	8	200–4000	25–500
Trichloroethylene	4	10–200	2.5–50
Carbon dioxide	4	1000–6000	250–1500
Hydrocarbons	4	100–300	25–750
Chlorine	8	1–20	0.13–2.5
Chloroprene	4	5–100	1.3–25

humidity and temperature, uncertainties in calibration of indicator tubes, compliance to conditions of use and time of storage, and the state (quality) of the air sample. To increase the accuracy and precision of measurements, tables of temperature corrections or correction factors are used [2,12,13].

The relative standard deviation (RSD) of measurement may vary in the range 0.20–0.50 [3,14]. This might be due to a partial irreproducibility of tube parameters. For instance, unequal packing density of indicator powder, slight variations in layer length, variations in tube diameter, non-uniform modification of the sorbent by the reagent, unequal particle size of the sorbent, and unequal aerodynamic resistance of tampons in the tubes make it impossible to obtain an RSD better than 0.05.

Table 8.3 shows some examples of tubes produced by Dräger and based on linear colorimetric determination of concentration and long-time signal accumulation.

The development of indicator tubes of prolonged action is topical for industrial sanitary monitoring.

Besides tubes based on a linear colorimetric approach, there are tubes of the “yes-no” type as well as passive dosimeters. For instance, Dräger produces so-called “indicator tickets”, which are passive dosimeters for formaldehyde and ozone, and indicator tubes for ammonia, dimethylamine, nitrogen oxides, phenylacetic acid and phenols for clinical use. There exist individual dosimeters of the chemical action, indicator tubes for determining total hazardous substances as time-weighted averages over a work shift. There are tubes for amino compounds (aromatic amines and hydrazines) based on the use of benzoxazole derivatives as selective reagents [15].

8.1.4 Air samplers

Devices for air sampling and pumping should enable work with accuracies no worse than 5%, should be air-resistant, simple, portable, and provide for a precise indication of pumping time. Devices of various designs are used which differ in their parameters. Semiquantitative determinations or detection of hazardous substances can even be performed with a syringe. For quantitative measurements, bellows or plunger pumps are used. In the case of small-volume bellows pumps, the number of required compressions of the bellows in one sampling is 1–10; in the case of larger bellows, this number is 1–3. Air samplers with larger bellows enable higher rarefaction than devices with smaller bellows. Thus, the former are used with indicator tubes having high aerodynamic resistance, while the latter are used with tubes of low aerodynamic resistance. The majority of air-sampling devices make it possible to pump air through indicator tubes at different rates: at the beginning of the pumping process the rate is high, decreasing towards the end of the process. At a high initial pumping rate the length of the coloured zone increases and as the pumping rate continuously decreases, so does the length of the coloured zone; at the end of analysis, at low pumping rate, it remains practically constant, while its boundaries become more distinct. Usually, air samplers are included in air analysis kits.

Plunger pumps are usually used in kits for chemical survey and rapid detection of poisonous gases in air under field conditions.

Recently, sampling pumps (aspirators) made of metal have become widely used. They can be rapidly recharged in the field enabling many samples to be collected. Such pumps are included in kits for rapid monitoring of air along with indicator tubes, rapid tests, and indicator elements. They can also be used by mobile analytical units, laboratories of environmental and sanitary control, and by services specializing in emergency situations. For instance, reusable air samplers produced by AO Analittsentr provide for sampling of gas–vapour mixtures by a solid-phase sorbent with a flow rate of up to 1.2 m^3/h under conditions of high dust content in the environment, as compared with 0.012–0.030 m^3/h of conventional liquid absorbers. This precludes distortion of analysis results due to losses in absorbing solutions and opens the possibility of sampling under any meteorological conditions.

8.1.5 Indicator tube kits and their applications

AO Analittsentr (St. Petersburg, Russia) produces mini laboratories based on the use of indicator tubes for rapid determination of ammonia, gasoline, benzene, SO_2, CO_2, CO, xylenes, nitrogen oxides, H_2S, petroleum hydrocarbons, HF, formaldehyde, chlorine, and toluene. Sensitivity of determination is at levels of 0.5 MAC, time of analysis no more than 10 min. The kit also includes indicator elements and a plunger pump as air sampling device. Indicator elements are used for determining ammonia, SO_2, H_2S, CS_2, HCN, HF, Cl_2 at the level of 1 MAC. The kit case measures 0.38×0.30×0.10 m, weighs no more than 3 kg. The kit produced by the same company for determining NO_2, NH_3, phenol and formaldehyde was proposed for monitoring the air of industrial premises and living quarters. It operates in temperature range from –30°C to +30°C. The kit also includes miniature metallic samples, restorable specific adsorbents, and flasks for preservation and delayed treatment of samples.

Krismas+ Company (St. Petersburg, Russia) produces indicator tubes 69 of which have been certified for detection of 47 components. The company also produces indicator tube kits for CO, NO, SO_2, NH_3, and H_2S for monitoring of industrial gas ejections, kits for detecting alcohol vapours in the breath of drivers and working staff, rapid tests for alarm control of air pollution by ammonia, SO_2, H_2S, CS_2, HF, Cl_2, and HCN, as well as tests for control of air polluted with ammonia, NO_2, and mercury vapours. They are equipped with air samplers of AM type. Several examples are given in Table 8.4. Tubes are used at temperatures of 10–50°C and relative humidity of 30–95%; they are

TABLE 8.4

Krismas+ indicator tubes

Tube model	Tested compound	Analytical range (mg/m^3)
TI-1	Ammonia	2.5–100
TI-2	Hydrogen sulphide	2.5–50
TI-3	Sulphur dioxide	10–130
TI-4	Nitrogen dioxide	2.5–200
TI-5	Nitrous oxide	2.5–200
	Total nitrogen oxides*	2.5–200
TI-6	Chlorine	0.5–200
TI-7	Hydrogen chloride	2.5–150
TI-8	Carbon dioxide	700–10000
TI-9	Acetone*	100–1600
TI-10	Benzene*	20–200
TI-11	Gasoline	100–4000
TI-12	Total petroleum hydrocarbons*	100–4000
TI-13	Kerosene*	100–1200
TI-14	Toluene*	50–1600
TI-15	Xylenes*	25–300
TI-16	Acetic acid	2.5–2000
TI-17	Ozone*	0.1–3.0
TI-18	Bromine	0.5–80
TI-19	Butanol and isobutanol	10–200
TI-20	Propanol and isopropanol	10–200
TI-21	Ethanol	250–5000
TI-22	White spirit*	300–4000
TI-23	Trichloroethylene	5–150
TI-25	Formaldehyde	2.5–100
TI-26	Diethyl ether*	100–2500
TI-27	Acetylene	200–6000

*Used with filtering tubes.

125 mm in length, with an inside diameter of 4.5 mm; their service life is 12–24 months. The tubes have been certified and approved for use in Russia.

TABLE 8.5

Krismas+ special-purpose indicator tubes

Tube model	Tested compound	Analytical range
IT-2 T, 205-03	Nitric acid vapours	0.005–10.0 mg/m^3
IT-G 1, 207-04	Unsymmetrical dimetylhydrazine	0.00010–0.010 mg/m^3
IT-46, 201-01	BZ aerosols	From $(3.0–5.0)\cdot 10^{-4}$ mg/l
IT-49, 203-02	CS	From $2.0\cdot 10^{-5}$ mg/l
IT-36, 211-05	Mustard gas	From 0.002 mg/l
IT-45, 209-06	Phosgene	From 0.005 mg/l
	Diphosgene	From 0.005 mg/l
	Prussic acid	From 0.005 mg/l
	Chlorocyan	From 0.005 mg/l
IT-13-3, 213-07	Lewisite	0.002-1.3 mg/l
	Nitrous yperite	0.001–0.25 mg/l
IT-15-30, 215-08	Adamsite	0.002–0.2 mg/l
	Chloroacetophenone	0.0001–0.1 mg/l
IT-28, 225-18	Carbon oxide	50.0–7000 mg/m^3
IT-48, 223-17	SR	From $(3–4)\cdot 10^{-5}$ mg/l
		From $(1–2)\cdot 10^{-3}$ mg/l
IT-51, 217-09	Sarin	From $(1.0–2.0)\cdot 10^{-6}$ mg/l
		From $1.0\cdot 10^{-4}$ mg/l
	Soman	From $(1.0–2.0)\cdot 10^{-6}$ mg/l
		From $1.0\cdot 10^{-4}$ mg/l
	VX	From $(1.0–2.0)\cdot 10^{-6}$ mg/l
		From $1.0\cdot 10^{-4}$ mg/l

A recent catalogue of this company gives data on indicator tubes for arsine, hydrogen bromide, dimethyl- and diethylamines, dimethyl ether, oxygen, mineral oil (aerosol), methanol, methyl- and ethyl mercaptans, nitroglycerine, CO, total nitrogen oxides, mercury vapours, styrene, phenol, phosphine, HF, furfural, ClCN, and HCN.

A special kit is worth mentioning intended for chemical surveying and monitoring of acutely hazardous and poisonous substances in air (Table 8.5).

Kitagawa indicator tubes for determination of hazardous substances in air (Komyo Rikagaku Kogyo K.K.) have been produced since 1946. Unfortunately, among 150 tubes for determining 67 substances, the majority is unusable in Russia as MAC values in Russia are lower than in Japan. Only tubes for 17 substances are recommended for use in Russia, viz. those for ammonia, benzene, vinyl chloride, NO_2, xylenes, NO, CO, styrene, H_2S, SO_2, toluene, phosgene, chlorine, hydrogen chloride, ethylene, ethanol, and prussic acid. Several companies in Bulgaria, Poland and Czech Republic produced or continue producing indicator tubes. The best known US firms specialising in indicator tubes are Bacharach Industrial Instrument Co., Blaw-Knox, Pennsylvania; Davis Engineering Equipment Co. Inc., Baltimore, Maryland; Union Industrial Equipment Corp., Culver City, California; Mine Safety Appliances Co., Pittsburgh, Pennsylvania; Acme Protection Equipment Corp., Emerson New Jersey; National Dräger Inc., Pittsburgh, Pennsylvania.

The products of Dräger Company are probably the most numerous [16]. This company produces kits for determining gases in air (also in water and soil) and a large number of indicator tubes for instant and prolonged measurement of concentrations of hazardous components; diffusion tubes with direct indication, badges with direct indication, sampling tubes and systems for instant and prolonged measurement, and tubes for determining pollution in compressed gases.

The system comprises an indicator tube and an aspirator. Each indicator tube has a highly sensitive reagent ensuring a reliable and readable result, provided the technical parameters of the aspirator used are well correlated with reaction kinetics of the reagents in the indicator tube. Some examples of the systems are given in Table 8.6.

The company produces 160 types of tubes for instant measurements. Among areas of applications are determination of instant (e.g. peak) concentrations, measurement in the zone of danger to man's respiratory organs; detection of sites of gas leakage (e.g. in pipe lines or at gas plants); control of air composition in closed areas (e.g. in channels, tunnels, or shafts). Table 8.6 gives a selected list of tested compounds, mainly of those that require determination in a wide range of concentrations.

The kit for prolonged measurements (Table 8.7), equipped with a Polymeter aspirator and an automated explosion-proof constant-air-volume pump, is designed for detection of inorganic and organic vapours and gases both for personal use and for use under stationary

TABLE 8.6

Dräger indicator tubes for instant measurements (20°C, pressure 1013 gPa)

Tested component	Analytical range (ppm)	K (1 mg/m^3 = $K \times$ ppm)	Number of pump cycles	Analysis time (min)
Acrylonitrile	1–15	2.62	20	4
	5–50		10	2
	0.5–10	2.21	20	4
	1–20	2.21	10	2
	5–30	2.21	3	0.5
Ammonia	0.25–3	0.71	10	1
	5–70	0.71	10	1
	2–30	0.71	5	1
	5–100	0.71	1	10 s
	50–700		1	6 s
Benzene	0.5–10	3.25	40	15
	0.5–10	3.25	20	20
	2–60	3.25	20	8
	5–40	3.25	15	3
	5–50	3.25	20	8
	15–420	3.25	20	4
Water vapours	0.1–1.0 mg/l		3	2
	1–18 mg/l		2	1.5
	1–15 mg/l		2	0.5
	20–40 mg/l		1	20 s
	1–40 mg/l		10	40 s
SO_2	0.5–5	2.66	20	6
	1–25	2.66	10	3
	20–200	2.66	10	3
	50–500	2.66	10	3
	400–8000	2.66	10	15 s
H_2S	0.2–5	1.42	10	5
	0.5–15	1.42	10	6
	1–20	1.42	10	3.5
	10—200		1	20 s
	2–20	1.42	10	3.5
	20–200		1	20 s
	2–60	1.42	1	0.5
	5–60	1.42	10	4
	100–2000	1.42	1	0.5

TABLE 8.7

Dräger indicator tubes for prolonged measurements (20°C, pressure 1013 gPa)

Test component	Concentration range (ppm)		Maximum analysis time (h)	K (1 mg/m^3 = $K \times$ ppm)
	Analysis time of 1 h	Maximum analysis time		
Ammonia	10–100	2.5–25	4	0.71
Acetone	500–10000	62.5–1250	8	2.41
Benzene	20–200	10–100	2	3.25
Vinyl chloride	10–50	1–5	10	2.60
Nitrogen dioxide	10–100	1.25–12.5	8	1.91
Sulphur dioxide	2–20	0.5–5	4	2.66
Methylene chloride	50–800	12.5–200	4	5.53
Nitrose gases	5–50	1.25–12.5	4	
Perchloroethylene	50–300	12.5–75	4	6.98
Carbon sulphide	10–100	1.25–12.5	8	3.16
Toluene	200–4000	25–500	8	3.83
Trichloroethylene	10–200	2.5–50	4	5.46
Prussic acid	10–120	1.25–15	8	1.12
Hydrochloric acid	10–50	1.25–6.25	8	1.52
Hydrocarbons	100–3000	25–750	4	
Carbon dioxide	1000–6000	250–1500	4	1.83
Carbon monoxide	10–100	2.5–25	4	1.16
Acetic acid	5–40	1.25–10	4	2.50
Chlorine	1–20	0.13–2.5	8	2.95
Ethanol	500–8000	62.5–1000	8	1.92

conditions. For prolonged individual measurements, the company produces diffusion indicator tubes with direct indication (Table 8.8).

For determining pollutants in compressed gases, the company produces the tests listed in Table 8.9.

TABLE 8.8

Dräger diffusion tubes with direct indication (20°C, pressure 1013 gPa)

Tested component	Concentration range (ppm)		K (1 mg/m^3 = $K \times$ ppm)
	Analysis time of 1 h	Analysis time of 8 h	
Ammonia	200–1500	2.5–200	0.71
Butadiene	100–300	1.3–40	2.25
Water vapours	5–100 mg/l	0.6–12.5 mg/l	
Nitrogen dioxide	10–200	1.3–25	1.91
Sulphur dioxide	50–150	0.7–19	2.66
Carbon dioxide	500–20000	65–2500	1.83
Carbon monoxide	50–600	6–75	1.16
Olefins	20–1500	2.5–200	
Hydrogen sulphide	10–300	1.3–40	1.42
Prussic acid	20–200	2.5–25	1.12
Hydrochloric acid	5–100	1.3–25	1.52
Perchloroethylene	200–1500	25–200	6.89
Toluene	100–3000	13–380	3.83
Trichloroethylene	200–1000	25–125	5.64
Acetic acid	10–200	1.3–25	2.50
Ethanol	1000–25000	125–3100	1.92
Ethyl acetate	500–10000	65–1250	3.66

TABLE 8.9.

Tests for determining pollutants in compressed gases

Component to be determined	Analytical range
Water vapours	2–450 mg/m^3
Carbon dioxide	100–3000 ppm
Oils	2.5–10 mg/m^3
Carbon oxide	2.5–150 ppm
Nitrose gases	0.25–1 mg/m^3

8.2 APPLICATION OF POCKET ANALYSERS

As discussed in Section 8.1, detection of air components is effected by means of indicator tubes. Sampling requires the use of pumps (aspirators) of various design including handheld devices like bellows pumps. They are easy to use, made of a durable material, and allow multiple measurements. While using a bellows pump, one hand should be free. One-hand operated pumps are called "accuro". The Dräger company proposed a very convenient Accuro 2000 automatic pump: a manual aspirator is put into the device, the door is shut, the indicator tube inserted, the number of working cycles set (no more 199), and the device is ready for work. A convenient LCD screen displays the end of preset working cycles.

To determine a single gas, Dräger produces simple devices of two types: Mini Pac and Pac II. Mini Pac is intended for 1000 h of continuous work. It is powered by one 9-V alkaline AC battery. A display continuously shows concentration of the determined gas. If the upper or lower concentration thresholds are reached or if the working voltage drops below the preset minimum, the device gives a visual and sound alarm. The device can be used for determining CO, H_2S, SO_2, NO_2, and O_2 at temperatures from –20°C to +40°C.

Pac II is a gas-measuring device of the second generation. It is also designed for measuring a single gas, viz. CO, H_2S, SO_2, NO, O_2, NH_3, HCN, Cl_2, phosphines, CO_2, or phosgene at –20°C to +40°C. It has a single alkaline AC 9-V battery as a power source, but can be powered from a lithium or accumulator nickel–cadmium battery. The device gives very distinct alarm signals, a preliminary one and the main one. Results measured during 8 h are memorised.

Portable devices of the Multiwaru family (Dräger) are used for determining concentrations of several gases simultaneously: H_2S and O_2; CO and O_2; CO, CH_4 and O_2; CO_2, H_2S, and O_2; CO_2 and CO. Results are indicated on three lit screens.

The company also produces sets of non-aspiratory diffusion tubes and badges with direct indication provided with special holders for fixing them onto clothing. Depending on the type, the measurement time is 1–8 h. For instance, a badge for phosgene can measure 0.2–4.8 mg/m^3 of phosgene for a 0.5 h measurement and 0.01–0.3 mg/m^3, if the analysis takes 8 h. The same applies to phosphines.

A GKh-4 chemical gas analyzer was produced in the USSR, it was designed for rapid determination of trace gases via indicator tubes;

measured gases included CO (0–0.002 vol.%), SO_2 (0–0.007 vol.%), H_2S (0–0.0066), nitrogen oxides (0–0.005 vol.%). Among other gas analyzers were GKh-5 (<50 mg/m^3 of CO_2) and GKh-6 (<21 mg/m^3 of O_2) models. All these gas analyzers were equipped with an AM-3 aspirator of the bellows type with cycle volume of 100 ml. Indicator tubes were used in linear colorimetric determinations. Also, linear colorimetric determinations of hazardous gases and vapours in air were made using versatile portable gas analyzers of UG type.

Nowadays, Krismas+ Company produces kits consisting of pumps and tubes for determination of iron(II, III), mercury, cobalt, nickel, chromium(VI), chlorides, petroleum products, nitrogen dioxide in gases, aerosols, solutions (the "Pchelka" series). The experimental metrological laboratory of the GosNII Khimanalit State Research Chemical Analytical Institute (St. Petersburg, Russia) produces a UPGK versatile instrument for environmental-monitoring safety services. The instrument weighs 5 kg and can work autonomously from an accumulator battery; power consumption is under 30 W. It can detect poison gases like sarin and soman ($\geq 2 \times 10^{-3}$ mg/m^3), V-gases ($\geq 5 \times 10^{-3}$ mg/m^3), mustard gas (2–300 mg/m^3), BZ (0.3–150 mg/m^3), phosgene, diphosgene, HCN, ClCN (5–800 mg/m^3), and 29 other hazardous substances in a wide range of concentrations. Besides the above-mentioned hazardous substances, it is possible to determine adamsite, nitric yperite, amidol, heptyl, lewisite, pronite, chloroacetophenone, and 53 other hazardous substances (UPGK–LIMB series of instruments). The instrument is equipped with a control unit connected to a sampling unit.

Dräger Company has started production of PAC III gas analysers of the third generation. It includes 14 electrochemical sensors for determining 35 toxic vapours and oxygen. Sensors are easily inserted into corresponding slots, are pre-calibrated, and their data are written into electronic memory of the sensor. The instrument automatically recognizes the sensor type, the analytical range, and alarm thresholds. Sensors are not disabled or damaged by high concentrations of gases.

The same company also produces microprocessor-based Multiwarn II instruments and on-chip measurement systems. The latter make it possible to determine vapours of organic substances (benzene, toluene, perchloroethylene, and vinyl chloride) and inorganic gases (NH_3, HCl, NO_2, Cl_2, and H_2S). Today, Dräger produces Bio-Check tests for ozone, formaldehyde, vapours of organic solvents, pentachlorophenol, and ethanol. In these devices, biological sensors are used as sensing devices, and the concentration of the tested element is determined using a special colour scale. These test means are designed for non-professional users.

REFERENCES

1. E.A. Peregud, M.S. Bykhovskaya and E.V. Gernet, *Rapid Methods for Determination of Toxic Compounds in Air*, Khimiya, Moscow, 1970 (in Russian).
2. E.A. Peregud and D.O. Gorelik, *Instrumental Methods for Monitoring of the Atmosphere Pollution*, Khimiya, Leningrad, 1981 (in Russian).
3. W. Leithe, *Die Analyse der Luft und ihrer Verunreinigungen in der Freien Atmosphaere und am Arbeitsplatz*, Wissenschaft Verlagsgesellschaft, Stuttgart, 1974.
4. S.I. Murav'eva, M.D. Babina, A.G. Atlasov et al., *Sanitary Chemical Control of the Industrial-Plant Air*, Meditsina, Moscow, 1982 (in Russian).
5. Yu.S. Drugov and V.G. Berezkin, *Gas-Chromatographic Analysis of the Polluted Air*, Khimiya, Moscow, 1981 (in Russian).
6. S.I. Murav'eva, M.I. Bukovskii and E.K. Prokhorova, *A Guide on Monitoring of Toxic Pollutants in the Working-Area Air*, Khimiya, Moscow, 1991 (in Russian).
7. E.D. Filyanskaya, T.N. Kozlyaeva and I.G. Vorokhobin, *Linear Colorimetric Technique for Analysis of Toxic Vapours and Gases in the Industrial-Plant Air*, Profizdat, Moscow, 1958 (in Russian).
8. M.I. Kolesnik, V.I. Zhukov and M.I. Bukovskii, *Methods for Determination of Toxic Compounds in the Air with Indicator Tubes*, NIITEKhIM, Moscow, 1983 (in Russian).
9. A.A. Voloskovets, D.D. Mysakh and G.P. Khudosovtseva, *Gig. Truda Profzabolevanii*, No.7 (1978) 56.
10. V.A. Simonov, *Zh. Anal. Khim.*, 37 (1982) 2225.
11. V.A. Simonov, *Zh. Anal. Khim.*, 41 (1986) 2050.
12. K. Leichnitz, *Am. Ind. Hyg. Ass. J.*, 38 (1977) 707.
13. K. Leichnitz, *Drägerheft*, No. 311 (1978) 3.
14. K. Leichnitz, *Drägerheft*, No. 313 (1979) 17.
15. M.I. Evgen'ev, I.I. Evgen'eva and N.A. Moskva, *Methods of Analysis of Toxicants in Biologically Active Substances, Wastewaters and Air*, Kazan Institute of Chemical Technology, Kazan, 1992 (in Russian).
16. *Dräger-Tube Handbook*, 11 Ed., Drägerwerk Aktiengesselschaft, 1997.

Chapter 9

Detection of Alcohol Vapours, Narcotics, Chemical Weapons and Explosives

9.1 ALCOHOL VAPOURS

The most common methods for detecting alcohols are those based on enzyme reactions, alcohol oxidase being a most selective enzyme [1]. Reagents changing colour during the tests participate in redox reactions and include *o*-dianisidine, 3,3′,5,5′-tetramethylbenzidine, 2,6-dichlorophenolindophenol, 1,7-dihydroxynaphthalene, 4-aminoantipyrine, 3,5-dichloro-2-hydroxybenzenesulphonic acid, 3-methyl-2-benzothiazolinehydrazone, Methylene Blue, Nitrotetrazolium Blue, etc.

If paper strips are used, then, depending on the chromogenic reagent taken, in the presence of alcohols, the indicator strip is coloured orange (with *o*-dianisidine), blue (3,3′,5,5′-tetramethylbenzidine), green (2,6-dichlorophenolindophenol, a mixture of 1,7-dihydroxynaphthalene with 4-aminoantipyrine, or a mixture of 3,5-dichloro-2-hydroxybenzenesulphonic acid with 3-methyl-2-benzothiazolinehydrazone), or yellowish-green (bilirubin).

The maximum catalytic activity is observed for aldehyde dehydrogenase (ADH) in the oxidation of ethanol; therefore ethanol can be determined in the presence of other lower aliphatic alcohols. The simplest test systems consist of ADH, nicotinamide-adenine dinucleotide (NAD^+), a chromogenic reagent, an electron-transferring agent (e.g., 1-methoxyphenazinemetasulphane), and certain antioxidants [2,3]. Reagents are applied to a filter paper in a definite order and then the paper is dried. The presence of ethanol in the range from 3 to 65 *mM* in biological liquids is determined by the bluish-violet colour that appears on the strips containing the tetrazolium salt as chromogenic reagent.

In some cases, the paper is additionally impregnated with a polymer [4], e.g., dehumidifying polymer Water-Lock L413. The ethanol detection threshold is 17 μ*M* ($1 \cdot 10^{-3}$ vol.%).

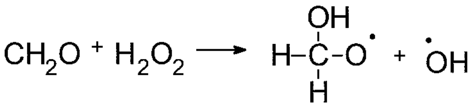
Mixtures of two enzymes can also be used [5,6], for example, alcohol oxidase and peroxidase or diaphorase [6,7]. The aldehyde formed due to the oxidation of alcohol with aldehyde dehydrogenase is subsequently oxidized with ADH, and the resulting NADH reacts with *p*-nitrotetrazolium violet to give a coloured product. This reaction is catalysed by diaphorase. A mixture of alcohol oxidase with peroxidase was used in a number of instances [8–13]. The detection threshold depended on the chromogenic reagent chosen. The use of a multi-enzyme system, viz., alcohol oxidase–aldehyde dehydrogenase–diaphorase–catalase, in ethanol assays has been reported [14,15]. Acetaldehyde formed upon enzymatic oxidation of ethanol is oxidized with NAD^+ in the presence of aldehyde dehydrogenase. The resulting NADH oxidized the colourless form of Methylene Blue in the presence of diaphorase to give a coloured product. Catalase catalyses decomposition of hydrogen peroxide formed upon oxidation of alcohol. This method is advantageous due to a higher detection sensitivity for ethanol in comparison with two-enzyme systems (detection limit was 2–30 *mM*); however, the complexity of preparation of the indicator strips limits considerably its applicability.

A test procedure for determining methanol in blood plasma based on a multi-component enzyme mixture was developed [16]. Plasma was incubated for 4 min at room temperature with a reaction mixture containing alcohol oxidase, dehydrolipoamide dehydrogenase, formaldehyde dehydrogenase, and nitrotetrazolium blue in a phosphate buffer solution. The content of methanol was then evaluated visually by comparison of the colour of the resulting solution with that of reference solutions.

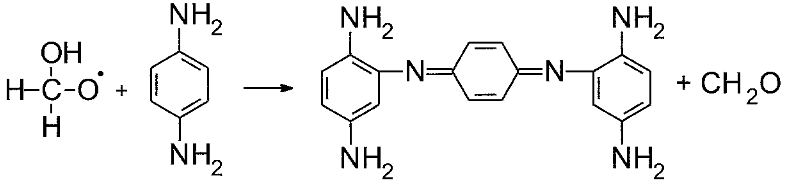
Ethanol and methanol were determined separately using alcohol oxidase (AO). It is known that alcohols are oxidized by atmospheric oxygen to give corresponding aldehydes

$$RCH_2OH + O_2 \xrightarrow{AO} RCHO + H_2O_2$$

The resulting aldehydes catalyse oxidation of *p*-phenylenediamine, used as a chromogenic reagent, with the hydrogen peroxide evolved. This gives the Bandrovsky base



$CH_2O + H_2O_2 \longrightarrow H\text{-}\overset{OH}{\underset{H}{C}}\text{-}O^{\bullet} + {}^{\bullet}OH$



Methanol and ethanol were identified in the cells of a polystyrene plate by adding dropwise the following reagents in a definite order: a phosphate buffer solution (pH 7.0), solutions of *Pichia Pinus* yeast alcohol oxidase with an activity of 14 units/mg, *p*-phenylenediamine ($7 \cdot 10^{-2}$ *M*), an alcohol, and a 0.225 *M* solution of hydrogen peroxide. One minute after the addition of hydrogen peroxide, the colours of the solutions in the cells were compared. The solutions containing ethanol turn pink, while those containing methanol turn dark-violet.

Enzymatic reactions were helpful in solving the selectivity problem in testing for alcohols. These reactions can be used for testing both biological liquids and vapours present in the breath of a supposedly drunk person. The existing test is based on CrO_3 reduction with the exhaled alcohol vapours. The CrO_3 applied on fine-grain silica gel oxidizes alcohol in the presence of sulphuric acid to give green chromium(III) compounds. The reaction is non-selective as chromium(VI) also oxidizes acetone, esters, aldehydes, petrol, and lower alcohols. Therefore, a positive test for ethanol of biological liquids requires confirmation by other test reactions [17]. However, this test is still being used.

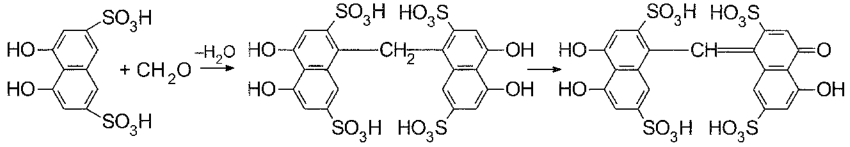
Instead of CrO_3 and concentrated H_2SO_4, one can use the reaction of ethanol (and other lower alcohols) with $K_2Cr_2O_7$ in sulphuric acid, which can be carried out in indicator tubes. An orange solution turns green in the presence of alcohols:

$$3C_2H_5OH + 2K_2Cr_2O_7 + 8H_2SO_4 = 3CH_3COOH + 2Cr_2(SO_4)_3 + 11H_2O + 2K_2SO_4$$

In order to determine ethanol in urine, a 1-ml sample is placed in a indicator tube; a Whatman filter strip carrying one drop of H_2SO_4 is inserted in a indicator tube and heated for 2 min in a boiling water bath. If ethanol is present, the strip turns green. The detection limit is 40 mg % [18].

Testing for ethanol in urine can also be carried out using indicator strips containing horse-radish alcohol oxidase and a chromogenic reagent such as 3-methylbenzothialinone hydrazone hydrochloride and 3-dimethylaminobenzoic acid. A sample of urine (1–5 ml) is placed in a 15-ml centrifuge tube, and a indicator strip is mounted above the solution. The closed tube is heated for 5 min at 65°C and the colour is noted [19].

When identifying the cause of poisoning, it is very important to distinguish methanol from ethanol. Methanol is oxidized in an acid medium to give formaldehyde, whereas ethanol gives acetaldehyde. The reaction of formaldehyde with chromotropic acid enables reliable identification of methanol in the presence of ethanol [18,20]:



Chromotropic acid Methylenedichromotropic acid The semiquinoid form of methylenedichromotropic acid

One drop of a 25% solution of $K_2Cr_2O_7$ in 50% H_2SO_4 is added to 1 ml of urine, and the mixture is kept for 5 min at room temperature. Ethanol (1 drop) and chromotropic acid (a few milligrams) are added. Afterwards, concentrated H_2SO_4 is added until a layer is formed at the bottom of the indicator tube. If methanol is present, the mixture turns violet.

Macherey-Nagel (GmbH and Co., KG) produce Nanocolor® Ethanol 1000 indicator tubes for the determination of 0.10–1.00 g/l (0.013–0.13 vol.%) of ethanol. The reaction product is blue. If necessary, the optical density of the solution can be measured on a photometer if the testing has shown a positive result. Alcohol oxidase and an appropriate chromotropic compound are used as reagents. The test can be used to determine ethanol in vodka, beer, and fruit juices. The reagent is stable for one year at 0°C.

Best Sensors Co., Ltd. has developed a portable pocket device enabling determination of alcohol vapours in exhaled air. The device employs a solid-state semiconductor sensor based on SnO_2.

9.2 NARCOTICS

Classification of narcotic-based drugs and methods of systematic testing were reviewed in a monograph [21] and are partly considered in Chapter 10. Narcotics are isolated from plants (hemp, poppy), or synthesized (narcotic analgesics). They include phenylpiperidine derivatives, alkaloid type compounds, barbiturates, amphetamines, opiates, benzodiazepines, and indoles.

Narcotic compounds and drastic medicines are often separated using TLC on commercial chromatographic plates produced by Merck, Sorbfil, Lenkhrom (St. Petersburg), Plastmash (Krasnodar) and developed for viewing using various reagents: strong blue B, Dragendorff's reagent, ninhydrin, strong black K, mercury(II)-diphenylcarbazone complex, and *p*-dimethylaminobenzaldehyde.

An example given below shows detection characteristics of tests for poppy alkaloids using formaldehyde and the Mandelin reagent (NH_4VO_3 in concentrated H_2SO_4) (Table 9.1) [22]. A toluene–acetone–ethanol–25% NH_3 mixture 45:45:62 was used as mobile phase.

This method has a drawback, as the alkaloid-related colouring does not last long (no longer than 10 min), and it cannot be determined

TABLE 9.1

Colours of poppy alkaloid zones and reaction sensitivity on plates treated with formaldehyde and the Mandelin reagent

Alkaloid	Zone colour	Detection limit in the zone (μg)	
Narceine	yellow	5	3
Morphine	violet-blue	1–2	1
Codeine	blue	1	0.5
Reticulin	violet	1	0.7
Thebaine	orange	5	2.5–3.0
Narcotoline	orange	2	1
Papaverine	blue	2	1
Narcotine	orange	3	1

quantitatively by densitometry. However, in selectivity for narcotic components of poppy, this method is superior to other methods suggested earlier for this purpose.

Polytests for narcotics (Polytest-1, Polytest-2) consist of a polyethylene case with semi-transparent reaction containers, two glass ampoules with chemical reagents, and two polyethylene stoppers [22]. When the stoppers are depressed, the ampoules break and their contents enter the reaction container with the sample. Some of the test kits are additionally equipped with standard tube-droppers containing aqueous solutions of potassium carbonate of various concentrations. The test kit for opium and poppy straw is equipped with an extraction container and that containing ampoules with concentrated acids is supplied with alumina (in ampoules) for neutralization of the medium.

A list of tests for certain groups of narcotics, as well as the chemical compositions of reagents in the ampoules and tube-droppers are given in Table 9.2.

A methodology for testing objects of plant origin and pharmaceutical products has been developed. It includes 18 tests making use of pure chemical reagents and their mixtures.

Analyses of narcotics are discussed in Refs. [23,24].

The Helling (GmbH) company makes Expray kits for detection of various narcotics, such as heroin (the Herosol kit), cocaine (Coca-Test), hashish and marijuana (Cannabispray) and many others (HKG-NiK). The scientific-production association Vilar, together with VNIKhFI, produce Toksi-Lab kits consisting of reagents and accessories which allow rapid identification of dozens of narcotics and drastic medicines without use of reference samples. The tests are suitable for analysing raw plant products. A selective test for heroin was proposed [25]; the kit comprises a saturated solution of Bromophenol Blue in dichloromethane applied onto an indicator paper sealed in a polymer film with an indicator hole. A test for opiate group narcotics has been proposed [26].

Morphine in blood and urine (0.005–1 mg/l) can be detected using paper moistened with a solution containing antibodies against morphine, glucose oxidase, 4-chloro-1-naphthol, sodium phosphate, albumin, the peroxidase–morphine conjugate, and glucose [27,28]. In a test for amphetamine, cocaine, marijuana, and morphine, paper is treated with a mixture of basic bismuth nitrate, KI, and K_2PtCl_4 [29]. In a test for hashish and marijuana, silica gel is impregnated with Strong blue B and NaOH [30].

TABLE 9.2

Composition of polytests [22]

Narcotics type	Reagent composition	Colour
Marijuana, hashish	Powder of Strong blue B; a chloroform–CCl_4 mixture (1:3); 10% K_2CO_3	Orange to purple-red
Opium, poppy straw	10% formaldehyde in sulphuric acid, $CHCl_3$; 5% K_2CO_3	Violet colouring of the top layer
Morphine, heroin, codeine, promedol, amphetamines	2% formaldehyde in sulphuric acid, Al_2O_3 (neutralizing agent)	Violet (morphine, heroin); bluish-violet (codeine); red (promedol); orange or brown (amphetamines)
Barbiturates	5% $Co(NO_3)_2$ in isopropanol; 10% piperidine in isopropanol	Lilac
Heroin	Bromophenol blue in a buffer solution (pH 5), $CHCl_3$	Purple
Cocaine	2% cobalt thiocyanate, $CHCl_3$	Light-blue colouring of bottom layer
Amphetamines	1% sodium nitroprusside in 5% aqueous acetone; 2% K_2CO_3	Dark pink
Ephedrine	0.5 M aqueous $CuSO_4$; *n*-butanol; 10% K_2CO_3	Violet colouring of top layer (ephedrine, pseudo-ephedrine)
LSD	5% *p*-dimethylaminobenzaldehyde in an ethanol – H_3PO_4 mixture (1 : 1); Al_2O_3	Reddish-violet
Morphine, codeine, heroin	0.3% ammonium molybdate in conc. H_2SO_4; Al_2O_3	Violet (morphine, heroin); bottle-green (codeine)
Barbiturates	0.5% $CuSO_4$ in 5% piperidine; $CHCl_3$	Light-blue colouring of the bottom layer

9.3 CHEMICAL WEAPONS

The majority of chemical compounds of natural and synthetic origin are toxic but their toxicities are very different. Highly toxic compounds suitable for military usage for damaging the enemy's manpower are referred to as chemical toxic agents (CTA). Chemical toxic agents were used on a large scale during World War I. The total losses due to the use of CTA amounted to 700–800 thousand people [31].

The toxic effect of CTA is based on inhibition of enzyme systems in the organism. For instance, hydrogen cyanide, which is a typical inorganic compound, inhibits Warburg's iron-containing redox systems, initially causing intracellular hypoxia and then paralysis of respiratory centres. Lewisite and other lacrimators rapidly react with sulphhydryl groups of various enzymes thus disabling them. Yperite inhibits hexokinase and affects the nuclear apparatus of cells. Phosphorous-containing CTA, such as tabun, sarin, soman, etc., cause very fast phosphorylation of choline esterase, thus inhibiting its function in the nerve impulse transfer. Some CTA react with enzymes through their nucleophilic groups: NH_2, OH, SH, and others. The initial step in the reactions of such toxic agents as HCN, AsH_3, and PH_3 involves complex formation with the iron atom of blood haemoglobin or iron-containing enzymes.

Chemical toxic agents belong to various classes of chemical compounds. Compounds that correspond to different chemical classes may be similar in their effects. For example, chloropicrin, chloroacetophenone, bromobenzyl cyanide, and acrolein are lacrimators (tear-gases). Therefore, toxic agents are classified according to their physiological effect on the organism rather than their chemical nature. Five groups of CTA are distinguished: nerve agents (diisopropyl fluorophosphate, sarin, soman, tabun, phosphorylthiocholines), blood agents (HCN, CNCl, CO, AsH_3, PH_3), vesicants (yperite, trichlorotriethylamine, phosgene oxime), suffocants (Cl_2, phosgene, diphosgene, aminochlorocarbonates, phosgene oxime), and irritants (bromobenzyl cyanide, chloroacetophenone, chloropicrin, acrolein, haloacetates, diphenylchloroarsine, diphenylcyanoarsine, adamsite, capsaicin).

A tactical classification has been proposed which is based on the behaviour of toxic agents on the ground under military application conditions. According to this classification, there are non-persistent CTA, persistent CTA, and toxic smoke-forming compounds.

Identification of CTA is primarily important during combat operations to quickly detect the starting moment of attack and to establish the type and concentration of the toxic agent used. It is also important for civilians in the area of combat operations. At present, when procedures for chemical weapons destruction are being implemented, safety should be ensured at every stage of the process [32,33]. Elaboration of scientifically and technically substantiated systems for monitoring CTA both for technological and ecological purposes is also a topical issue. Standard indicating devices are available for field conditions; they include the simplest tools for chemical reconnaissance and chemical monitoring, semi-automatic and automatic gas indicators and gas analyzers, and dedicated army chemical laboratories.

In turn, the simplest tools for chemical reconnaissance include a number of devices, such as the army chemical reconnaissance device, the semi-automatic instrument for chemical reconnaissance, the semi-automatic army gas indicator, automatic gas alarms, and field chemical laboratories.

Below, we consider test tools for detection of CTA, such as indicator papers, indicator tubes and indicator films.

There are three types of *indicator papers* for CTA: those for detection of liquid CTA on surfaces; those for CTA vapours in the air; and those for CTA dissolved in water.

The paper for detecting CTA drops is used by blotting the suspicious drops found on various surfaces, protective clothing, etc., or by applying the tested substance to the indicator paper, e.g., in the laboratory in order to establish the type of an unknown CTA. There are two options for detection of CTA vapours or toxic gases in the air: (i) either to hang, attach or arrange the paper in certain places to expose it to the natural effect of toxic atmosphere and then examine the result, or (ii) to force the toxic atmosphere through the paper by means of a compressor, a rubber bulb, or a bicycle pump. If air is forced through paper, analyses are more sensitive and fast.

Certain test papers are suitable for CTA detection in various environments. In many cases, the concentration of CTA in air can be estimated by examining the colour the test paper acquires after a particular exposure time, or, *vice versa*, by recording the time it takes for a certain colour to develop. Rather high accuracy can be achieved by measuring the volume of air that has been pumped through. Sensitivity and accuracy of measurement are much affected by air humidity.

Examples of standard test papers and films are presented in Table 9.3.

Each test paper included in the set has a limited specificity; however, combination of test papers and indicator tubes as well as a comprehensive analysis of results enable more reliable detection of CTA with these simple tools.

Test papers for chlorine, phosgene, HCN, chloropicrin, sulphur mustard, nitric yperites, phosphorus-containing CTA, ethyldichloroarsine, and CO have also been proposed. They are based on a variety of reactions.

TABLE 9.3

Characteristics of standard indicator papers (TP) and films (TF)

CTA to be detected	Reagents	Size of detectable CTA particles (μm)
VX	Bromocresol green	>20
Tabun	*p*-nitrobenzaldehyde, potassium and sodium carbonate	>200
Yperite	Crystal violet, copper(II) chloride, mercury iodide	>200
Yperite	Michler ketone, mercury(II) chloride	>200
Nitric yperite	Pinaverdol and picric acid	>200

For example, a number of test papers for detection of chlorine were proposed, such as the iodide–starch, fluorescein, and *o*-tolidine papers; they provide for chlorine detection limits of 0.006 mg/l, 0.03 mg/l, and 0.03 mg/l, respectively. However, the task of increasing selectivity is still topical. Indeed, the iodide–starch paper, which is very sensitive to chlorine and maintains its indicating capacity for an unlimited period of time if stored under appropriate conditions, reacts in the same way with bromine, ozone, and nitrogen oxides. Bromine hampers determination of chlorine with fluorescein paper. Lately, civilian objects (including potable water, waste water, water for swimming-pools, disinfectant solutions) have often been tested using papers based on Michler thioketone [34], vanillin derivatives [35], dinitronaphthol [36], azo compounds [37,38], and benzidine [39]. The majority of tests for chlorine are based on its high oxidizing ability.

Reactions used in testing other CTA are more diverse. For example, HCN is detected using papers with benzidine–copper acetate, $HgCl_2$ with Methyl Orange, and $AgNO_3$ with Congo Red mixtures, sodium picrate and iron(II). Some CTA are detected using complex mixtures of components. For example, for testing nitric yperites, the paper is impregnated with a solution containing $Co(NO_3)_2$, NaSCN, $ZnCl_2$, NH_4NO_3, and $TiCl_3$. Detection of some CTA is based on the formation of new organic compounds. These are reactions of phosphorus-containing CTA with diisonitrosoacetone, ethyldichloroarsine with 2,4-dinitrophenol or with *m*-dinitrobenzene, chloropicrin with *p*-dimethylaminobenzaldehyde, etc.

Indicator tubes are mostly used for detecting CTA under field conditions. They are also widely used for the purposes of professional hygiene, in industrial toxicology, in industrial monitoring, and in toxicological studies. A common feature of all indicator tubes is the presence of a sorbent packing (usually silica gel) where a colour reaction of a CTA with the reagent occurs.

In one type of tube, after pumping toxic air through silica gel, i.e., when the CTA has been absorbed, silica gel is withdrawn from the tube, extracted, stirred, and divided into portions, and each portion is tested for a certain CTA. The CTA can also be extracted from silica gel using appropriate solvents. Portions of the extract are then used to determine different CTA. These tubes, which are mostly used for sampling, can be packed with activated coal as a sorbent.

In another type of tube, a reagent solution is fed into the tube after pumping toxic air through it; the reagent reacts with a group of toxic agents or specifically with one compound to give a coloured product.

Quantitative determination of chemical toxic agents in indicator tubes can be carried out by juxtaposing the effect observed with the velocity of the air pumped through the tube; for example, the length of the coloured zone can be related to the volume of air passed, or the resulting colour can be compared with a coloured reference specimen. When calibrating the tubes against air containing a known concentration of CTA, it is important to establish the dependence of the length of the coloured zone (or the colouring intensity) on the air flow rate.

There are five groups of indicator tubes for chemical toxic agents.

Group I includes tubes intended for ionic reactions which are fast and stoichiometric. Such tubes are easy to calibrate since their readings are independent of the flow rate over a wide range of flow rates. A unit length of the coloured zone corresponds to a certain amount of the toxic agent.

Group II: The tubes of this group are similar to Group I tubes, but coloured rings are formed instead of coloured zones.

Group III: These are tubes where non-stoichiometric reactions of toxic agents with organic reagents are carried out. In this case, the tube readings dependence on the flow rate remains constant until very high flow rates are achieved.

Group IV: The readings depend on the flow rate over the whole range but the character of the dependence changes; so an appropriate calibration range has to be determined for each particular case.

Group V: Such tubes are calibrated by comparing the colour that appears with that of a reference. The length of the coloured zone is correlated with the CTA concentration over a small section of the curve. As the flow rate increases, the reaction is no longer quantitative, as the amount of CTA sorbed from a unit volume starts to decrease.

Table 9.4 shows examples of indicator tubes for some toxic agents.

TABLE 9.4

Characteristics of standard indicator tubes

CTA to be detected	Test format	Detection limit (mg/l)	Comments
Chloroacetophenone	Ampoule: a solution of KOH in ethanol; packing: silica gel impregnated with *m*-dinitrobenzene	$1 \cdot 10^{-4}$	Compounds that interfere: bromobenzyl cyanide, acetophenone, petrol, H_2S, smokes
Adamsite	Ampoule: a solution of $Hg(NO_3)_2$ in concentrated H_2SO_4; packing: glass wool	$2 \cdot 10^{-4}$	Diphenylamine and smoke give similar colouring
o-Chlorobenzylidene-malonodinitrile	Ampoule: a solution of ninhydrin in ethanol; packing: silica gel impregnated with a solution of $NaHCO_3$	$5 \cdot 10^{-4}$	Phosgene oxime and halocyanes react similarly
Dibenz(*b*,*f*)-1,4-oxazepine	Ampoule: a solution of pyridine in HCl, a solution of 1-naphthylamino-ethylene-diamine dichloride; packing: silica gel modified with $NaNO_2$	$4 \cdot 10^{-4}$	Smoke interferes
Nitric yperite	Ampoule: a solution of bismuth iodide in a KI solution; packing: silica gel	$1 \cdot 10^{-3}$	Yperite, VX and BZ give similar colouring
Lewisite	Ampoule: a solution of NaOH in ethanol; packing: silica gel impregnated with a solution of $NaHCO_3$ and a mixture of copper thiosulphate and $Na_2S_2O_3$	$2 \cdot 10^{-3}$	The reaction is almost specific
Quinuclidinyl 3-benzylate (BZ)	Ampoule: a solution of paraform in H_2SO_4; packing: glass	$3 \cdot 10^{-4}$	Acidic smoke interferes

The calibration marks on the tubes can be used only if the same pump is used.

The AP-1 set of *indicator films* serves for the determination of Vx-type toxic agents. These films are polyethylene strips coated with a reagent. The mixture is based on Bromocresol Green — an acid–base indicator which changes from yellow to bluish-green upon exposure to Vx. The films make it possible to identify Vx in isolated drops; the analysis takes 30 and 80 seconds at temperatures above and below zero, respectively.

Gas indicators and *gas analyzers* using indicator tubes are widespread tools for chemical reconnaissance in the army. In many cases, they are compact (their weight ranges from 1.8 to 2.8 kg) and enable rapid determinations (from 2 to 7 min) of the content of toxic agents in air, on land, equipment, and other objects. Detection limits vary from $1 \cdot 10^{-7}$ to $1 \cdot 10^{-3}$.

Instruments operating in automatic and semi-automatic modes are available. The instruments of this group can be used for continuous automatic monitoring of air at various objects. Detection limits are $2 \cdot 10^{-6}$ to $2 \cdot 10^{-4}$ mg/l; the time required for the test is 0.5 to 7 min. The operation is based on ionization, ionization sensor, or biochemical principles. Their weight is from 15 to 40 kg. The instruments provide for light and sound alarms.

At present, modernization of these instruments is in progress, mainly, in regard to miniaturization. For example, the Laboratory of biochemical methods at the State Research Institute Khimanalit (St. Petersburg) has developed, in the context of the program for the destruction of chemical weapons and missile fuel components, a personal army chemical monitoring kit, GO.2.84.00, for detection of organophosphorus compounds, yperite, and lewisite in air and water. Detection limits for organophosphorus compounds are $n \cdot 10^{-8}$ mg/l in air and $n \cdot 10^{-7}$ mg/l in water (for direct determination) or $n \cdot 10^{-9}$ mg/l in air and $n \cdot 10^{-9}$ mg/l in water (after sample preparation). The same company has developed the GO.2.77.00 chemical monitoring kit for detection of drops and aerosols of V gases, soman, sarin, and yperite and the GO.2.77.00 personal kit for the chemical monitoring of air contaminated with organophosphorus compounds, yperite and lewisite.

This company manufactures a personal gas alarm for equipping inspection groups working at the facilities where chemical weapons are stored and destroyed. The instrument operates in continuous automatic air monitoring mode and gives a light and audible alarm if the

CTA content in air exceeds a given value. The indication block with a battery weighs not more than 400 g; its size is 37×43×220 mm. The sensitivity thresholds of the gas analyzer for CTA vapours are: the first threshold, $(3–8)\cdot 10^{-5}$ mg/l, and the second $(1–2)\cdot 10^{-4}$ mg/l; the response time is less than 5 s; the operation temperature range is –20 to +50°C; the service life is no less than 5 000 h. The weight of the power source does not exceed 600 g.

9.4 EXPLOSIVES

Chemical compounds or mixtures of compounds capable of very fast transformation with liberation of a great amount of heat and gaseous products are referred to as explosives. The most important individual compounds include nitro compounds, particularly aromatic polynitro compounds and their derivatives, viz., trinitrotoluene (TNT), trinitrophenol (picric acid), trinitroxylene, dinitronaphthalene, etc.; nitroamines such as cyclotrimethylenetrinitroamine (hexahydro-1,3,5-trinitro-1,3,5-triazine, hexogen), cyclotetramethylenetetranitroamine (octogen), trinitrophenylmethylnitroamine (Tetryl), ethylenedinitroamine (EDNA), dinitrooxydiethylnitroamine (DINA), etc.; nitrates of polyhydric alcohols and carbohydrates, e.g., pentaerythritol tetranitrate (TEN), glycerol trinitrate (nitroglycerol), nitrocellulose (pyroxylin), etc.; nitrates, e.g., NH_4NO_3; azides, e.g., lead azide.

It is common practice to prepare explosives from compounds that can undergo oxidation (combustible compounds) and compounds containing much oxygen (oxidants). The most important mixtures include gunpowder, nitroglycerol-based explosives, ammonia–saltpetre mixtures (ammonites), chlorate and perchlorate explosives, mixtures and alloys of nitro compounds, and other explosives.

A specific feature of nitro compounds often used for testing is that they are soluble in acetone. Even the solubility of the least soluble nitro compound, hexogen (RDX) is as high as 1 g in 25 ml of acetone. Nitrocellulose and nitro starch are well dispersible as colloids. After extraction, the solvent is usually evaporated; the residue is treated with water and tested for inorganic ions.

The test for polynitroaromatic compounds includes two reagents. The first one is a 2.5-20% solution of tetraalkylammonium or tetraalkylphosphonium hydroxides in a mixture of 60% DMSO and 3% methanol; the second one is the Griss reagent, e.g., sulphonylamide

TABLE 9.5

Reagents used in tests for explosive organic nitro compounds

Explosive	Reagents	Ref.
Dinitrotoluene	Tetramethylammonium hydroxide	44
Nitrocellulose	Benzoin, Griss reagent	45
Nitroglycerol	Diphenylbenzidine	44
Tetryl	Hexamethylenetetramine, Griss and Nessler reagents	44
2,4-Dinitrotoluene, 2,4,6-Trinitrotoluene	Tetramethylammonium hydroxide, Griss and Nessler reagents	46

and *N*-1-naphthylenediamine. Upon explosion, polynitroaromatic compounds are converted into nitrite ions reacting with the Griss reagent [40].

Testing for hexogen is carried out by pyrolytic oxidation [41], by the reaction with J-acid [42], and by the reaction with thymol in a sulphuric acid medium [43]. Hexogen is colourless and insoluble in water and benzene but soluble in acetone. Heating hexogen in the presence of MnO_2 results in formaldehyde and nitrous acid. This reaction is carried out in a indicator tube heated in a glycerol bath (180°C). The tube neck is closed with filter paper wetted with the Nessler and Griss reagents. A brown or black spot with the Nessler reagent is evidence for the formation of formaldehyde; a red spot with the Griss reagent is indicative of the formation of HNO_2.

The J-acid test is based on hexogen decomposition on treatment with concentrated H_2SO_4 followed by the reaction of the resulting formaldehyde with J-acid to give a cationic dye of the xanthene series, which shows yellow fluorescence under UV irradiation.

Table 9.5 presents information on some tests for nitro compounds. The reagents listed in Table 9.5 are used after appropriate sample preparation, which normally involves heating of the test objects in a glycerol bath at temperatures of 150°C and above. The decomposition results in formaldehyde, ammonia, or nitrous acid.

Sugar (sucrose) is a common reducing agent for the preparation of explosives at home. After explosion, sugar can be detected as follows [44]. Two drops of the test solution are placed on a spot plate, then one drop of 15% solution of 1-naphthol in ethanol and two drops of concentrated H_2SO_4 are added. A blue or purple-blue colouring indicates that sugar is present. The same reaction carried out before the explosion results in purple colouring.

Explosives in fire-arms can be identified based on metals (lead, antimony, and barium) present in combustion products.

Lead is easily detected with sodium rhodizonate. The violet-coloured $Pb(C_6O_6)Pb(OH)_{2}\_H_2O$ precipitate is formed in neutral media, while a scarlet-red $2Pb(C_6O_6)Pb(OH)_2 \cdot H_2O$ precipitate is formed in weakly acidic media [21]. The detection limit is 0.1 μg Pb.

Barium gives a reddish-brown precipitate of barium rhodizonate [47]. The detection limit is 0.25 μg Ba.

Testing for antimony is performed using triphenylmethylarsonium iodide as a reagent; it forms an orange precipitate of an ionic associate [48].

Alkaline metal dichromates are used as oxidants for home preparation of explosives. The remaining chromium can be detected using a test reaction with diphenylcarbazide. Detection of magnesium, nitrates, perchlorates, permanganate, phosphorus, zinc, and other inorganic ions is described in the relevant parts of this book.

In recent years, the use of enzyme reactions in test methods for detection of explosives has started. A review has been published [49] on testing 2,4,6-trinitrotoluene and thirteen other nitroaromatic compounds and polycyclic aromatic hydrocarbons. Testing of trinitrotoluene in potable water using an enzyme reaction on indicator strips has been proposed [50,51]. This reaction involves glucose oxidase and 3-[(3-cholamidopropyl)dimethylamino]-1-propanesulphonate, which form apoglucose oxidase. The sample and apoenzyme preparation are rather complex. However, the low detection limit (0.7 μg/l) and the approach to the selection of a sensitive system on indicator strips make the method attractive. The reaction selectivity is high enabling to determine trinitrotoluene in the presence of all inorganic components of the potable water.

The Merck company has developed immunoenzyme tests for trinitrotoluene (TNT) and hexogen (RDX) DTECH®TNT/RDX, DTECH®TNT, and DTECHTO, which provide for determinations in the ranges of 0.5–5.0 mg/kg (soil) and 5–60 μg/l (water). Other nitrogen-containing organic compounds, nitrates, nitrites, and ammonium do not hamper the analysis.

REFERENCES

1. V.G. Amelin, *Zh. Anal. Khim.,* 55 (2000) 901.
2. D. Felscher and K. Lehmann, *GDR Pat.* 237223, 1986.
3. D. Felscher and K. Lehmann, *GDR Pat.* 256196, 1988.
4. D.A. Kidwell, *Anal. Biochem.*, 182 (1989) 257.
5. A. Oonishi, M. Kamiyama and K. Okaniva, *Jpn. Pat.* 02109998, 1990.
6. M. Watanabe, T. Suzuki and M. Kagenama, *Jpn. Pat.* 154409, 1985.
7. S.Y. Chung, J.R. Vercellotti and T.H. Sanders, *J. Agric. Food. Chem.*, 43 (1995) 1545.
8. M. Marek, Z. Parliček and B. Doležal, *Czech. Pat.* 261636, 1989.
9. E.C. Adams, *US Pat.* 4786569, 1988.
10. E. Barzana, A.M. Klibanov and M. Karel, *Anal. Biochem.*, 182 (1989) 109.
11. M. Kamiyama, S.S. Kawakatsu, T. Akashi and K. Okaniwa, *Jpn. Pat.*, 01289495, 1989.
12. R. Bauer and T.A. Magers, *Eur. Pat.* 164008, 1985.
13. M. Watanabe, T. Suzuki and M. Kagenama, *Jpn. Pat.* 60172298, 1985.
14. M. Watanabe, T. Suzuki and M. Kagenama, *Jpn. Pat.* 60196198, 1985.
15. M. Watanabe, T. Suzuki and M. Kagenama, *Jpn. Pat.* 60188097, 1985.
16. H.G. Giles, M. Hirst, E. Hoffmann and B.M. Kapur, *Clin. Chem. (Washington, D.C.),* 39 (1993) 693.
17. A.E. Uspenskii, *Khimiya i Zhizn' – 21 vek* (1997) 60.
18. E.G.C. Klarke, *Isolation and Identification of Drugs*, Pharmaceutical Press, London, 2 (1975) 893.
19. K. Verebey and S.J. Mule, *J. Anal. Toxicol.*, 10 (1986) 167.
20. E. Eegrive, *Mikrochim. Acta*, 2 (1937) 329.
21. E. Jungreis, in: *Spot Test Analysis* (J.D. Winefordner, ed.), John Wiley and Sons, Inc., New York, 1997.
22. V.I. Sorokin, A.V. Gaevskii, E.V. Degtyaryov and S.K. Volkov, *Ispol'zovanie ekspress-testov pri issledovanii narkoticheskikh i sil'nodeistvuyushchikh sredstv (Use of Rapid Tests in Investigation of Narcotics and Drastic Medicines)*, Ekspertno-kriminalisticheskii tsentr MVD RF, Moscow (1996) 72 (in Russian).
23. T.A. Gouch (Ed.), *Analysis of Drugs of Abuse,* John Wiley and Sons, Inc., New York, 1991.
24. S.K. Eremin, B.N. Izotov and N.V. Veselovskaya, *Analiz Narkoticheskikh Sredstv (Analysis of Narcotics),* Mysl', Moscow, 1993 (in Russian).
25. T.A. Gorokhova, Yu.A. Fedorov, V.A. Kobylyanskii and L.I. Savvateeva, *RF Pat.*, 2126964, *Byull Izobret.*, No 6 (1999).
26. R.P. Pantaler, T.I. Ivkova and A.B. Blank, *Ukr. Pat.* 17252 A, 1997.
27. T.T. Ngo and G. Lenhoff (Eds.), *Immunofermentnyi analiz (Immunoenzyme Assay),* Mir, Moscow, 1988 (Rus. Transl.)

28. Tsv. Todorova, B. Mandova and T. Zhecheva, *Godishn. Vis. Khim.-Tekhnol. Inst. (Sofiya)*, 23 (1977/1978) 241.
29. J.J. Gibson, *US Pat.* 5523051, 1996.
30. J. Valkmann, S. Ullver, M. Muehlenberg and M Torf, *Ger. Pat.* 19607646, 1997.
31. A.A. Stepanov, In: *Kratkaya khimicheskaya encyclopediya (Concise Chemical Encyclopeadia)*, Sovetskaya encyclopediya, Moscow, 3 (1964) 814 (in Russian).
32. S.V. Petrov, *Ros. Khim. Zh.*, 37 (1993) 5.
33. I.V. Astaf'ev and V.I. Kholstov, *Ros. Khim. Zh.*, 37 (1993) 3.
34. V.V. Romana and K.P. Yamuna, *Pittsburg Conf. Anal. Chem and Appl. Spectrosc.*, New Orleans, 1995, p. 981.
35. R. Bauer, C.D. Rupe and R.G. Rosenfield, *Ger. Pat.* 2015271, 1970.
36. E. Halamek, F. Pžikryl and J. Souček, *Czech. Pat.* 239710, 1988.
37. E. Halamek, F. Pžikryl and J. Souček, *Czech. Pat.* 239708, 1988.
38. E. Halomek, Z. Kobliha and T. Capoun, *Czech. Pat.* 281603, 1986.
39. V.M. Ostrovskaya, Yu.A. Zolotov, E.I. Morosanova and D.Yu. Marchenko, *Fresenius' J. Anal Chem.*, 361 (1998) 300.
40. B. Glattstein, *Aust. Pat.* Au602734, 1987.
41. F. Feigl and D. Hagenauer-Castro, *Chemist-Analyst*, 51 (1962) 5.
42. E. Sawicki, T.W. Stanley and J. Pfaff, *Chemist-Analyst*, 51 (1962) 9.
43. S.A. Adams and H.J. Yallop, *Analyst (London)*, 94 (1969) 828.
44. *General Information Bulletin*, Federal Bureau of Investigation, Washington, DC, 74–8 (1974) 4.
45. F. Feigl and E Liebergott, *Anal. Chem.*, 36 (1964) 132.
46. A.H. Amas, H.J. Yallop, *Analyst (London)*, 91 (1966) 336.
47. F. Feigl, *Mikrochemie*, 2 (1924) 188.
48. H.C. Harrison and R. Gibroy, *J. Forensic Sci.*, 4 (1959) 184.
49. P.M. Krämer, *Anal. Chim. Acta*, 376 (1998) 3.
50. C. Keuchel and R. Niessner. *Fresenius' J. Anal. Chem.*, 350 (1994) 538.
51. C. Heiss, M.G. Weller and R. Niessner, *Anal. Chim. Acta*, 396 (1999) 309.

Chapter 10

Use of Test Methods in Medicine

Blood and urine are the most frequent biological fluids studied in medicine. For example, a usual practice in diabetes diagnostics is to determine glucose levels in blood and urine. Since chemical and biochemical compositions of blood and urine are different, procedures of sample preparation in the chemical analysis of these two biological liquids are also different and are rather complicated in both cases. For example, urine may contain proteins, ketone bodies, bilirubin, urobilinogen, nitrites, leucocytes, erythrocytes, and minor concentrations of about a thousand other components including metal ions as complex formers. The chemical composition of blood is equally complicated. It depends notably on the time of keeping the samples prior to analysis, and temperature. The state of urine is strongly influenced by its pH, which varies depending on the type of disease.

The composition of urine provides important information concerning the state of health and the incipient abnormalities. The first investigations of urine for these purposes date back to the golden ages of the ancient Babylon and Sumer. Even in primitive medicine, diabetes was diagnosed based on urine examination. The appearance of glucose in urine in the case of diabetes was noted in ancient India. In the Middle Ages, simple urine tests were carried out, viz., its colour, odour, consistency, and the presence or absence of a sediment were examined. Even at that time, a strange smell of children's urine was thought to be associated with mental retardation. Currently, the appearance of a strange smell of urine is explained by the formation of pyruvic acid, indicative of phenylketonuria. It was not until the 18th century that a scientific approach to urine examination was proposed: a dark colour of urine was attributed to alkaptonuria, and proteins were determined using the deproteinization reaction, which takes place on boiling urine specimens with acids. A correlation between a sweet taste of urine and the level of diabetes was observed. In this connection, measurements of urine specific gravity began.

Determination of urine acidity using litmus paper (Prout, 1817 [1]) and detection of uric acid by means of a paper strip wetted with a solution of silver salt and carbonate are believed to be the earliest tests in urine examination. In 1837, Babington reported the use of litmus paper for the investigation of fresh mucus. It is believed [2] that before 1837, Babington had reported on the possibility of using reagents supported on a solid material. In 1841, Trommer suggested determining glucose in urine based on the reduction of copper(II) by glucose in a hot alkaline solution. A decade later, Fehling modified this method. In 1850, Maumene described the detection of glucose in urine using a strip of white merino wool impregnated with tin(II) chloride [3]. The same investigator reported that it is impossible to conduct this test for glucose using paper support because paper reacts with $SnCl_2$ producing a false effect. Oliver [4] used a paper strip impregnated with indigo carmine; prior to test, the strip was wetted with an alkali solution and used to determine the glucose content in urine. Nowadays, impregnated fibres or multilayer filters are used most often to test for glucose and proteins in urine and blood [2].

10.1 DETERMINATION OF GLUCOSE

The presence of glucose in urine (glucosuria) is the most important chemical symptom of diabetes. For early diagnosis of diabetes or for the conclusion about a patient's recovery, it is necessary to determine very low contents of glucose in urine; in addition, the patient should be able to do it himself.

The chemistry of virtually all the tests used to determine glucose in urine is based either on specific enzymatic catalysis of glucose oxidation by atmospheric oxygen or on copper(II) reduction by sugar in a hot alkaline solution (Benedict's reaction). The reaction of 2,2′-bicinchoninic acid used for detection of copper(I) is 10^4 times as sensitive as the reduction of copper(II) by sugar. This reagent is prepared by mixing equivalent amounts of two solutions, one containing 2,2′-bicinchoninic acid in the presence of sodium carbonate and bicarbonate and the other containing copper(II) in the presence of L-serine.

Probably, the first tests based on impregnated Clinistix® and Tes-Tape® fibres and meant for glucose detection in urine were those produced by Ames Division, Miles Laboratories Inc. and Eli Lilly companies (US, 1956) [2]. Free et al. [5] used glucose oxidase and peroxidase as reagents and *o*-tolidine as substrate, while Comer [6] employed tartrazine as a coloured reagent for the same purpose. 4-Aminoantipyrine, 3,5-dichloro-2-hydroxybenzenesulphonic acid, and tetramethylbenzidine were proposed as chromogenic reagents [7]. The crucial factor governing the selectivity of glucose determination is not the chromogenic reagent, which changes colour in its presence, but rather selectivity of the enzyme towards glucose. For example, oxidation of D-glucose by atmospheric oxygen in the presence of glucose oxidase (a selective enzyme for glucose) affords β_1-D-gluconolactone and hydrogen peroxide. It is the latter product that oxidizes the chromogenic reagent in the presence of peroxidase contained in the test-reagent kit rather than the initial glucose, the latter being inert to this reagent. The oxidation product has a specific colour, the intensity of which is used to estimate the content of glucose. This test usually requires only one drop of blood. However, it implies elimination of the influence of plasma and separation of serum by means of specially prepared test strips or simple devices.

Glucose tests are produced by Kyoto Daiichi (Glucopat™) and Ames Div. Miles Labs Inc. (Visidex™, Visidex II™) [2].

The Clinitest® manufactured by Ames Company (Elkhart, Indiana) contains pellets of modified Benedict reagent, viz. $CuSO_4$, NaOH, Na_2CO_3 and citric acid. In an alkaline medium, sugar reduces the blue $CuSO_4$ to give a reddish insoluble copper(I) oxide. The colour intensity of the precipitate is proportional to the sugar content in urine. However, this test is not specific for glucose. The same effect is caused by lactose, galactose, fructose and other reducing compounds different from sugars, for example, ascorbic acid or salicylate-containing drugs. A pellet mixed with 5 drops of urine is used to determine 0–2% of sugar. When 2 drops of urine are used, 0–5% of sugar can be determined. Depending on the content of sugar in urine, its colour varies in the following way:

Sugar content (%):	0	trace	0.5	1	2	3	5
Colour:	blue	blue-green	light green	olive green	dark brown	light brown	orange

The pellet test gives a false result if the patient takes methyldopa.

The same company offers tests for glucose in blood or urine (Dextostix® and Azostix®); these tests imply separation of small water-soluble molecules from high-molecular-weight proteins using a semi-permeable filter. The same approach is used the Hakmo-Glucotest® and Reflotest®-Urea tests (Boeringer-Mannheim).

Increasing demands for rapid, inexpensive and reliable methods of determination of glucose in physiological liquids, mainly in the blood, have stimulated a huge effort devoted to elaboration of appropriate methods and technical tools [8]. Blood glucose determination deserves particular attention since it is of great importance for diabetic patients. A viable glucose test system should meet a number of requirements — it should be relatively inexpensive, easy to use, amenable to mass production [9], stable and easy to calibrate, and capable of tackling small samples of undiluted whole blood. It should also be of small size and disposable. Several examples of glucose determination are given in Table 10.1. Filter paper impregnated with appropriate reagents is mostly used as support and the glucose content is estimated visually.

To eliminate interference from blood components, special expedients are used: for example, the influence of erythrocytes can be eliminated by applying dextran with a molar mass of 10^4–$5{\cdot}10^4$ to the test strip prior to analysis [19]. Dextran prevents erythrocytes from penetrating into the area occupied by the reagents for glucose. Nylon membranes were immersed in a citrate buffer solution containing EDTA, glucose oxidase, peroxidase, dextran (20 wt%) with molar mass of $4{\cdot}10^4$ and tartrazine. Then the membranes were dried, immersed in a solution of *o*-tolidine and 3,3′,5,5′-tetramethylbenzidine in methanol, and used as test strips to determine glucose in the blood.

In the method for simultaneous and separate determination of glucose and oligosaccharides, strips were used with one end impregnated with reagents for glucose and the other end containing an enzyme for oligosaccharides. The strips made of cellulose acetate or polyethylene terephthalate were coated with a 10 μm-thick layer of a solution containing invertase, mutarotase, toluidine dichloride, and chromium(III) acetate. Then a layer of a 8% solution of gelatin containing glucose oxidase, peroxidase, 1-(4′-phenoxy-3′-sulphophenyl)-3-stearyl-4-isopropylpyrazolone-5-*N*-butyl-*N*-(4-sulpho butyl)-1,4- phenylenediamine, and chromium(III) acetate was applied [20]. After drying, the test strips were used for determination of sucrose in the presence of glucose; sucrose did not interfere with the determination of glucose.

TABLE 10.1

Glucose test methods

Indicator system, reagent	Analytical range (mM/l)	Material	Ref.
4-Aminoantipyrine, substituted pyrazolones	200–30000 mg/l	Solutions	10
4-Aminoantipyrine, 3-methyl-2-benzothiazolinehydrazone, glucose oxidase	200–30000 mg/l	Solutions	10
4-Aminoantipyrine, 3,,5-dichloro-2-hydroxybenzene, peroxidase, tetramethylbenzidine	1–40	Blood	11
4-Aminoantipyrine, glucose oxidase, peroxidase, 1,7-dihydroxynaphthalene	—	Solutions	12
Glucose oxidase, peroxidase, *o*-tolidine, carboxymethylcellulose	1–40	Blood	13
Glucose oxidase, peroxidase, *o*-tolidine, polyvinyl acetate, cellulose triacetate	1–40	Blood	14
Glucosoxidase, peroxidase, *p*-diethylenephenylenediamine	1–40	Blood, plasma	15
Glucose oxidase, peroxidase, sodium ascorbate, *o*-tolidine	1–10	Urine	16, 17
Glucose oxidase, lipase, peroxidase, 1-naphthol, phosphates	c_{min} = 250 mg/l	Solutions	18

Preparation and use of porous membranes of sodium dodecylbenzenesulphonate [21]; a mixture of an ester and polyvinyl alcohol [22]; starch, acrylic acid copolymers, and other components supported on $Ca_3(PO_4)_2$ or hydroxyapatite [23] were reported. Very complex mixtures of reagents were proposed for testing glucose in blood on a TiO_2-laminated plastic support [24] or a polyamide support [25].

GLUCODIAGNOSTIK test strips [Farmatsevticheskii Tsentr (Pharmaceutical Centre)] were designed for rapid determination of glucose in urine based on visual examination using a colour scale. These are plastic strips with a yellow-coloured sensor element fixed on them. The range of glucose concentrations in urine covered by this method is 0.1–2%. Fifty strips are packed in a box. The assay is performed with freshly collected urine (no more than 2 h prior to test). Strips removed from the box but not used lose their activity within 4 h; they are sensitive to sunlight and moisture.

Glucochrom D diagnostic test strips (State Research Institute of Biological Instrument Making) enable detecting, within 2 to 3 min, 1 to 44 *mM* of glucose in capillary blood applied onto a strip. The same manufacturer in co-operation with Boeringer-Mannheim (Germany) and Electronica-77 (Hungary) turns out the Decont-3 glucose meter adapted for the Glucochrom D test strips. In a 2 to 3 min assay, this device determines 1.5 to 25 *mM* of glucose in blood. The glucose meter and the test strips operate according to the photometer principle; if desired, the operator can compare the results read on the instrument display with those obtained using a colour scale enclosed in the box with test strips. The State Research Institute of Biological Instrument Making manufactures a compact device (130 × 52 × 18 mm, 200 g weight) suitable for determination of 2.2–25 *mM* of glucose in blood. The kit includes the Glucochrom D test strips and a lancet for finger puncturing. The device is very economical: one battery lasts about three years.

Good metrological characteristics were demonstrated by the Auto Analyser® reflectance meter used with test strips (Technicon Co., Tarrytown, New York) [26]. For example, for known contents of 20, 100, 250, and 600 mg of glucose in 100 ml of blood, the instrument's readings were 18, 93, 235, and 510, respectively.

The use of reflectance meters manufactured by various companies was reviewed in the literature [27,28]. The relative standard deviation of reported methods does not exceed 0.122 [27].

A miniature disposable amperometric glucose biosensor based on an electropolymerized polypyrrole film was recently developed by Quinto et al. [8] for determination of glucose in serum. A commercially available three-electrode system assembled on a flat corundum ceramic base was used as a biosensor substrate; the working platinum electrode was modified by an electrically generated overoxidized polypyrrole film. Immobilized glucose oxidase was used as catalyst. Analysis can be done on a sample as small as 50 μl. One of the two sensors developed can be used in flow injection analysis of glucose.

The private joint-stock company Diaplus produces Self-Stick 10L, Self-Stick 4, Self-Gluceto, and Self-Glucose kits for glucose determination in urine.

10.2 TESTING FOR CHOLESTEROL

In the laboratory, cholesterol is determined by using an enzymatic colorimetric reaction and an automated analyser. Either whole blood, or serum, or plasma can be used for the assay. It is clear that this method is unsuitable for home use, as it requires participation of professional clinical analysts. Therefore, the development of test methods and test reactions continues to be a topical task [29].

In 1985, the National Institute of Health (US) developed a program of national education devoted to the cholesterol problem (NCEP).

The ChemTrak Diagnostics company (Sunnyvale, US) was among the first to start manufacturing cholesterol meters (AccuMeter®) [10]. The testing device includes a filter made of glass fibre combined with a microporous membrane (Fig. 10.1). Plasma is passed through the filter

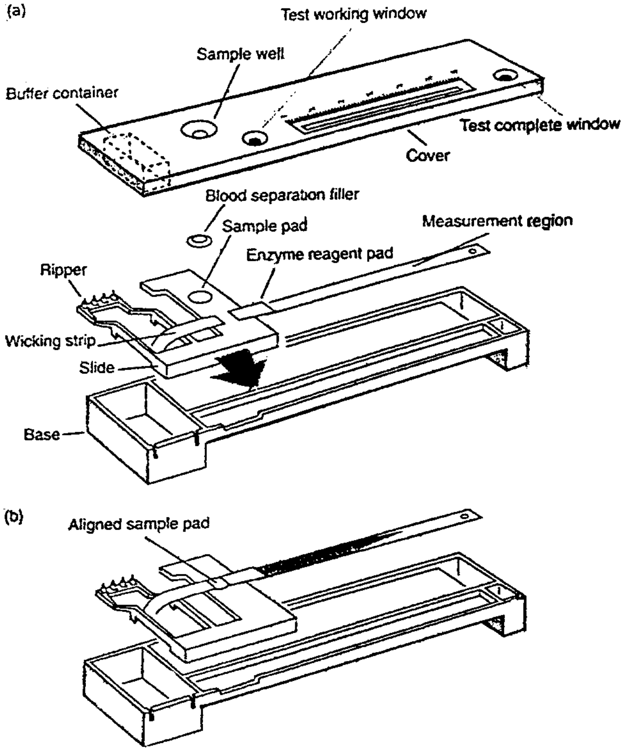


Fig. 10.1. Exploded view of the Accumeter home cholesterol test.

onto a chromatographic paper strip having a definite thickness and a known liquid absorption capacity. For example, if a 5 μl volume of plasma was placed on the filter and analysed, the relative standard deviation of analysis was about 0.02. The paper strip in a plastic bag was kept for 1 min in a slide until saturation. Then, the reaction with the paper impregnated with a cholesterol-sensitive enzyme took place; after that, the paper was brought into contact with an indicator strip wetted by the leuco form of the reagent. Development of a purple colour was then monitored.

The Auto HDL™ device (Pointe Scientific, Inc., Michigan, USA) for determination of cholesterol in blood plasma or serum was designed. It includes a set of three reagents; reagent 1 contains a polyanion and a polymer in a liquid, reagent 2 is a detergent in a liquid, and reagent 3 is a lyophilized powder of cholesterol esterase, cholesterol oxidase or horse-radish peroxidase. The method permits determination of high-density lipoproteins against the background of low-density lipoproteins. The test kit can be stored at 2 to 8°C for a long period of time; when unpacked, it can be stored for not more than 3 weeks. In our opinion, this device can be used only in the laboratory because sample preparation requires centrifugation. The authors themselves consider this to be a test intended for automated analyser. The calibration plot is linear in the range from 25 to 94 *mM*/dL of high-density lipoprotein. The correlation coefficient is 0.96, the relative standard deviation is less than 0.015 for the freshly cooled blood and less than 0.038 for the blood that has been stored for 20 days. Reagents used in the procedure are 4-aminoantipyrine and phenol; on treatment with H_2O_2 they form red-coloured quinoneimine with an absorption maximum at 500 nm.

The company produces numerous reagents for determination of cholesterol and other components and a set of standard reference samples.

A test procedure not requiring centrifugation of samples implies the use of magnesium dextran sulphate (with a molecular weight of about $5 \cdot 10^4$), which precipitates lipoproteins with very low and low densities, only high-density lipoproteins being left in the solution [30]. This reagent is stable within 90 days at 2 to 8°C. Polyethylene glycol can also be used as a precipitating agent. The preparation is stable at room temperature.

Yotsuyanagi et al. [31] developed a simple, very sensitive visual colorimetric method for determination of protein in urine using a membrane filter. Immediately after filtrating the solution containing the sample and also Bromochlorophenol Blue and drying for 10 min at 60°C, the initial greenish-yellow colour of the membrane filter changes to dark-blue. In the presence of protein, an ionic associate with excess reagent is formed and adsorbed on the membrane filter; after drying, it acquires a blue colour characteristic of the doubly charged anion of the dye.

Determination of cholesterol appears to be more difficult than that of glucose due to lower selectivity of the available test reagents for cholesterol. Therefore, many additional components are included in the kits for cholesterol testing, for example: enzymes such as lipoprotein lipase, cholesterol esterase, cholesterol oxidase or peroxidase; components of buffer solutions such as KH_2PO_4, $Na_2B_4O_7$; chromogenic reagents, viz. the leuco form of a dye and $K_4[Fe(CN)_6]$; support modifiers which isolate some components such as gelatin, polyethylene terephthalate, polyethylene glycol (with molar mass of $5 \cdot 10^4$). The supports used in this case are also more complex; they may include methylcellulose fibres, fabrics, cellulose acetate membranes, or TiO_2 [33,34].

Several other publications [35–37] and a monograph [38] have also been devoted to determination of cholesterol in blood in clinical tests.

10.3 OTHER MEDICAL APPLICATIONS OF TEST METHODS

Glucose and cholesterol are not the only components determined in clinical blood and urine analyses. Parameters determined in a blood examination include haemoglobin and lactates and choline esterase activity. Urine specific gravity, pH, proteins, ketone bodies, bilirubin, urobilinogen, blood traces, haemoglobin, phenyl ketones, L-amylase, leucocytes, ascorbic and lactic acids, nitrite and chloride ions, and copper(II) are determined in urine analyses. Test methods for determination of each of these components have been developed [38]. We shall consider some of them.

The tests for urine proteins are second in importance after those for glucose. As early as 1827, Bright found a first correlation between the albumin content in urine and renal insufficiency. He induced coagulation of urine albumin by adding various acids and used turbidimetry as a method of monitoring the procedure. The best results in protein separation and coagulation were attained when using sulphosalicylic acid.

Pellets with sulphosalicylic acid as a first albumin test for urine (Bumintest®) have been produced by Ames Co. since 1950. The action of the pellets is based on the Bright reaction. Results can be affected by tolbutamide metabolites, γ-globulin and in the case of urine by a high pH. The Albustix® test proposed by the same manufacturer makes use of the reaction of the potassium salt of tetrabromophenolphthalein ethyl ester with urine albumin promoted by acetic acid. Upon introduction of acetic acid, the blue colour of the reagent solution changes to a green one in the presence of albumin or to a yellow one when excess albumin is present. The test enables detection of 0.5 μg of albumin, casein, haemoglobin, clupein, or salmine; 1 μg of gliadin; and 5 μg of edestin. Methods for protein testing in urine were overviewed in a monograph [38].

For determination of NO_2^- ions in urine, the Griss reaction is used (Nitur-Test®, Boeringer-Mannheim). Diazotized sulphanilic acid can be coupled with *N*-amino-3-hydroxypyridine [39] or 3-hydroxy-1,2,3,4-tetrahydrobenzoquinoline.

When measuring pH, it should be borne in mind that pH of urine varies from 4.5 to 8.0 (the normal value is 5–6). The increase of pH to 7 or 8 indicates a probable disease. The first tests for urine pH were performed using litmus paper. Today, paper strips impregnated with a mixture of methyl red and Bromothymol Blue are used, changing colour from orange to blue in pH range of 5–9.

Determination of chlorides in urine is performed using test strips wetted with a complex of silver with 2-(2-thiazolylazo)-*p*-cresol [40], prepared in 2-(*N*-morpholine)ethanesulphonate and methanol in the presence of gum arabic.

For copper in urine, it was recommended to use test strips containing 4-(3,5-dibromo-2-pyridylazo)-*N*,*N*-dialkylaniline, ascorbic acid, surfactants, and buffer substances (pH 4.5–7.5) [41,42].

A test reagent for calcium used for examining physiological liquids contains 3,3-bis[di(carboxymethyl)aminomethyl]-*o*-cresolphthalein as a chromogenic reagent, 8-hydroxyquinoline-5-sulphonic acid to mask magnesium, and sodium or potassium 3-cyclohexylamino-1-propanesulphonate for maintaining a given pH [43].

Some test methods not covered in the monograph [38] are given in Table 10.2.

The private joint-stock company Diaplus produces test-strip kits for semiquantitative determination of urine leucocytes, pH, ketone bodies, urobilinogen, nitrites, occult blood, and bilirubin (Self-Stick 10L);

TABLE 10.2

Tests for components in medicinal samples

Type of sample	Component to determine	Analytical range (mg/l)	Indicator system, reagent	Ref.
Urine	Protein	0–100	Octahalosulphophthalein, polyethylene glycol	44
		0–100	Protein antibodies, marker	45
	γ-Glutamyl transpeptidase,	—	*p*-Nitroaniline	46
	Lactates	0.4 *mM*	Dichloroindophenol, lactate dehydrogenase	47
	Urobilinogen		4-Dimethylamino-benzaldehyde, brilliant blue, $CuSO_4$, potassium tartrate, phenol	
Saliva	Chloride ions	c_{min}= 2 mg/l	Ag_2CrO_4	49
Physiological liquids	Chloride ions	30–6000 mg/l	Ag_2CrO_4	50,51
	pH	7–10	Phenolphthalein	52
	Haemoglobin		3,3,5,5-Tetramethylbenzidine	53
	Catalase		Peroxidase, *o*-tolidine, glucosoxidase, glucose	54
Serum	γ-Glutamine transpeptidase		*p*-Nitroaniline	46
Blood	Urea	—	Brilliant yellow, urease	55

occult blood and pH (Self-Stick 4); and ketone bodies (Self-Gluketo), and for diagnosing urolithiasis and determination of the composition of calculus-forming salts (the Litos kit). The same manufacturer offers test kits for determination of indoor, pollen, food (B and C groups) and mould allergens (Top Screen), and immunodot techniques. Group C food allergens can be encountered in celery, tomatoes, carrot, hazelnut, shrimp, crabs, and fish; Group B allergens may be found in cow milk, eggs, peanut, wheat, and soy-bean. Plasma serum and blood can be used for the analysis.

The State Research Institute of Biological Instrument Making manufactures test strips Polytest for semi-quantitative determination of biochemical characteristics of human urine. The assay of ten characteristics (protein, bilirubin, glucose, ketones, nitrates, erythrocytes, leucocytes, pH, urobilinogen, density) takes not more than 2 min. The device requires a 220-V power supply; it is convenient for use under extreme conditions (military field hospitals, natural disasters, emergency ambulance service).

10.4 DRUG CONTROL

Pezza et al. [56] reported that in 1998, Brazilians were shocked by the news of numerous adulterations of common commercial drugs. The first forgery discovered concerned some contraceptives and preparations for prostate cancer therapy (the active components were found to be completely replaced by wheat flour) and antibiotics, which gave no therapeutic effect. A short time later, it was discovered that adulteration had become widespread — it was found in virtually all types of pharmaceuticals. Even the most common and simple drugs, both solid and liquid — e.g., those containing dipyrone — attracted the adulterators. This state of affairs has not changed crucially after these disclosures, and it is not only in Brazil. In the opinion of the Pezza [56], the same situation exists in the USA and Europe.

This accounts for the necessity to develop fast, easy-to-use, and inexpensive methods for testing medicinal preparations, in particular, dipyrone, that would be amenable to mass production and application without loss of sensitivity and reliability.

It was proposed to detect dipyrone by making use of the test developed by F. Feigl for detection of pyramidon (1-phenyl-2,3-dimethyl-4-dimethylamino-5-pyrazolone), which is a close analogue of dipyrone (the sodium salt of 1-phenyl-2,3-dimethyl-4-dimethylamino-methanesulphonate-5-pyrazolone). The Feigl test was adapted to the new task in such a way that it could be used in an ordinary drugstore even by unqualified personnel.

The following procedure was used. A very small portion of powder (about 1 mg) was placed in a micro-test-tube or on a plate for drop reactions together with 10–15 mg of disodium salt of chromotropic acid. A drop of concentrated sulphuric acid was added, and a drop of water was added to the resulting suspension. The mixture thus formed was prone to self-heating, and spontaneous boiling was observed. Immediate appearance of colour indicates the presence of dipyrone. The colour may vary from intense bright-blue to red-violet, depending on the dipyrone content in the sample. If this is low, it is desirable to conduct a blank experiment. In the case of liquid preparations, a drop of concentrated sulphuric acid is added to 10–15 mg of disodium salt of chromotropic acid. A drop of the test solution is added to the resulting mixture. The limit of detection is about 125 μg of dipyrone for both solid and liquid drugs.

The test is selective; many medicinal reagents and filling materials do not interfere with it. It is inapplicable in the presence of pyramidon, urotropine, diazepam, or guanine or tetracycline hydrochlorides. The test can be made quantitative; the colour is estimated either visually or using a simple reflectance meter.

Development of selective test methods for detection of drugs (including cases of drug abuse) in complex samples such as urine, blood, and living tissues is quite topical, especially in view of the instability of some drugs *in vivo* and the use of compound drugs containing several components.

As a result of testing 43 of the most common drugs by means of nine reagents, the following classification of drugs was suggested [57].

1. ***Alkaloids***: atropine, caffeine, cocaine hydrochloride, codeine phosphate, ephedrine sulphate, heroin, lysergide, mescaline hydrochloride, morphine sulphate, nicotine salicylate, psilocin, psilocybin, quinine sulphate, scopolamine hydrobromide, strychnine, yohimbine hydrochloride.

2. ***Compounds giving a positive response to the alkaloid test***: lidocaine hydrochloride, meperidine, methadone hydrochloride, methapyrilene hydrochloride, methaqualone, methylphenidate hydrochloride, pentazocine hydrochloride, phencyclidin hydrochloride, and propoxyphene napsylate.

3. ***Barbiturates***: aminobarbital, phenobarbital (Na salt), secobarbital.

4. ***Amphetamines***: amphetamine sulphate, methamphetamine hydrochloride.

5. ***Blended drugs***: aspirin, benzocaine, cannabidiol, cannabinol, diphenylhydantoin (Na salt), glutethimide, meprobamate, tetrahydrocannabinol.

The systematic course of the analysis is shown in Fig. 10.2.

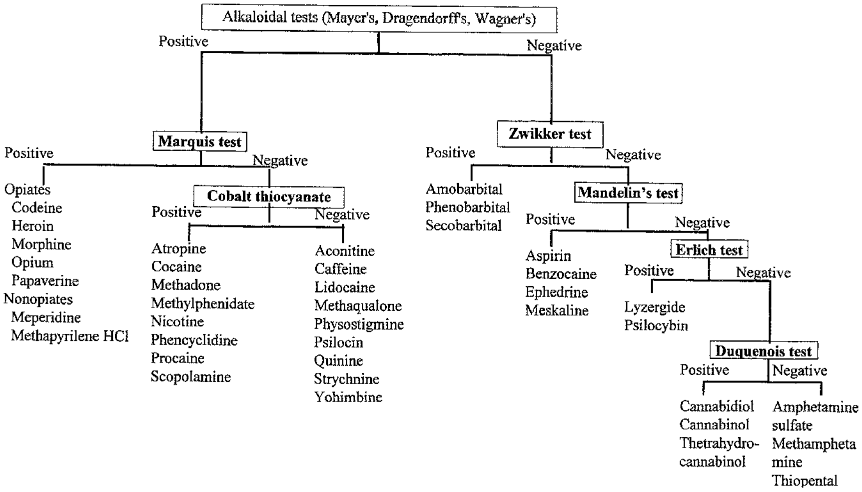


Fig. 10.2. Flowchart for drug testing.

Methods of preparation of test reagents are outlined below.

Mayer's Reagent. $HgCl_2$ (0.68 g) and KI (2.5 g) are dissolved in water and diluted by water to 100 ml.

Dragendorff's Reagent. Solution A: $Bi(NO_3)_3$ (0.85 g) is dissolved in 50 ml of 20% CH_3COOH. Solution B: KI (8.0 g) is dissolved in 20 ml of water. Five parts by volume of solution A are mixed with 2 parts by volume of solution B. The working solution is prepared by diluting 10 ml of this mixture with water to 100 ml.

Wagner's Reagent. A mixture of I_2 (1.27 g) and KI (2 g) is dissolved in water and diluted with water to 100 ml.

Marquis Reagent. Concentrated H_2SO_4 (10 ml) is mixed with 8 to 10 drops of a 40% solution of formaldehyde. A freshly prepared solution should be used.

Cobalt thiocyanate. A 2% solution is prepared.

Zwikker's Test. Solution A is a 1% solution of cobalt acetate in methanol. Solution B is a 5% solution of isopropylamine in methanol.

Mandelin's Test. A 1% solution of NH_4VO_3 is added to concentrated H_2SO_4 until an orange colour develops.

Ehrlich Reagent. A 5% solution of $FeCl_3$ (0.2 ml) is added to a 0.2% solution of *p*-dimethylaminobenzaldehyde in 65% H_2SO_4 (100 ml). The reagent is unstable; a fresh solution should be prepared every week.

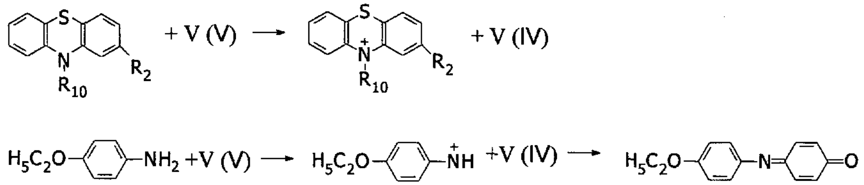
Duquenois Reagent. Acetaldehyde (5 drops) and vanillin (0.4 g) are added to USP grade ethanol (20 ml). The reagent should be stored in a glass bottle plugged with a glass stopper in the dark. The reagent becomes unusable when its colour has changed to dark yellow.

For example, among the alkaloids, only heroin and morphine give a positive reaction with Mayer's reagent; caffeine, heroin, and morphine give a positive reaction with Dragendorf's reagent; and caffeine, heroin, and psilocin, with Wagner's reagent. Ephedrine, lysergide, mescaline, and psilocybin do not give positive reaction with any of the above-listed reagents. However, most of the non-alkaloid drugs do give positive reaction with these reagents. Therefore, testing of any possible drug should include the use of all the test reagents described above.

Comprehensive tests for drugs were reported in Ref [57] and in a monograph [38]. We cite them here in order to demonstrate that these tests make use of well known and frequently encountered reagents. Some of the tests for narcotics are covered in Section 9.2.

A systematic procedure of analysis for determining drugs in urine was developed [58]. Methods for testing and determination of drugs are covered in Refs. [59–61].

Several reactions were proposed for testing phenothiazines in solutions or powders [62], viz. oxidation by vanadium(V) to a radical cation exerting a catalytic effect in the oxidation of *p*-phenetidine by vanadium(V). Reactions are carried out on a glass or plastic plate in the presence of sulphosalicylic acid. The limits of detection are 0.5 and 0.02 μg of phenothiazine in a solution drop for the first and the second reactions, respectively.



A reaction based on destruction of a coloured ionic associate of copper(II) diphenylcarbazonate with chromazurol S by barbiturates was proposed for determination of barbiturates [62].

Apparently, the test for NO_3^- based on its reduction to NO_2^-, diazotization of rivanol followed by azo coupling with phenols can also be used as a test for rivanol.

Test for barbiturates with Zwikker's reagent [57]. Several drops of solution A and several drops of solution B (Zwikker's reagent) are added successively to several milligrams of a drug (or the residue resulting from evaporation of water from a solution of the drug). In the presence of barbiturates, a blue-violet colour develops. This reaction is observed for aminobarbital, phenobarbital (Na salt), and secobarbital. In some versions of the analysis, when testing barbiturates and hydantoin, 2,6-dimethylmorpholine, cyclohexylamine or isobutylamine is used instead of isopropylamine for alkalization and increasing the selectivity [64–66].

According to another procedure, 1–2 ml of a 1% ethanol solution of $[Co(NH_3)_2]^{2+}$ and one or two NaOH grains are added to 1–2 ml of the test sample dissolved in ethanol. In the presence of barbiturates, a blue colour appears.

The positive reaction is also observed for bemegride, glutethimide, phenytoin, primidone. When LiOH is used in place of NaOH, this reaction becomes a selective test for barbiturates.

When testing barbiturates in urine, a 50-ml specimen of urine is acidified by 10% H_2SO_4 to pH 4–5 and extracted with 50 ml of ether; the ethereal extract is washed with water and concentrated to dryness; and the residue is dissolved in 1 ml of chloroform. Two drops of a 1% solution of LiOH in methanol are added. In the presence of barbiturates, a blue ring is formed.

For determination of 10^{-4}–10^{-5} M of biogenic amines (tryptamine, serotonin, tyramine, dophamin, noradrenalin), paper test strips impregnated with nitrotetrazolium blue and deaminating monoaminooxido reductase are used [67].

Some of the tests for narcotic drugs are described in Section 9.2 (devoted to the detection of narcotics).

REFERENCES

1. L. Wershub, *Urology from Antiquity to the Twentieth Century*, Green, St Louis, 1970, 139.
2. A. Zipp and W.E. Hornby, *Talanta*, 31 (1984) 863.
3. M. Maumene, *Compt. Rend.*, 30 (1850) 314.
4. G. Oliver, *Lancet*, 1 (1883) 858.
5. A.H. Free, E.C. Adams, M.L. Kercher, H.M. Free and M.H. Cook, *Abstr. Int. Cong. of Clinical Chem.*, New York, 1956, p. 236.
6. J.P. Comer, *Anal. Chem.*, 28 (1956) 1748.
7. D. Barham and P. Trinder, *Analyst*, 97 (1972) 142.

8. M. Quinto, I. Losito, F. Palmisano and C.G. Zambonin, *Anal. Chim. Acta*, 429 (2000) 9.
9. M. Alvarez-Icaza and U. Bilitewski, *Anal. Chem.*, 63 (1993) 525A.
10. V. Svoboda, E. Hrboticka and B. Sedlinska., *Czech. Pat.*, 260408, 1989.
11. D. Barham and P. Trinder, *Analyst*, 97 (1972) 142.
12. F. Arai, K. Yazawa, H. Takeuchi and M. Kitajima, *US Pat.* 4783315, 1988.
13. A.P. Fillipov, I.K. Mel'nik, K.B. Yatsimirskii, V.P. Komissarenko and A.S. Efimov, *USSR Inventor's Certificate*, 1155944, *Byul. Izobret.*, No. 18, 1985.
14. A.P. Fillipov, I.K. Mel'nik, A.S. Efimov, K.B. Yatsimirskii, V.P. Komissarenko and O.I. Sinyavskaya, *USSR Pat.* 1269021, *Byul. Izobret.*, No. 41, 1986.
15. M. Mueller-Frohne, J. Aumann, B. Neumann, H. Ullman, J. Kies, A. Mueller and M. Scholz, *GDR Pat.* 237678, 1986.
16. H. Tanaka, *Jpn Pat.* 05-03799, 1993.
17. H. Ito, *Jpn Pat.* 05-223812, 1993.
18. W. White, *Eur. Pat.* 25227, 1981.
19. C.B. Maddox, *US Pat.* 5212060, 1993.
20. W. Herrmann, H. Weise, D. Plaschnik and G. Hilderband, *Ger. Pat.* 294344, 1991.
21. K. Hilderband, K. Wehling, H. Pudleiner and H. Engelhard, *Eur. Pat.*, 407800, 1990.
22. H. Macho, R. Lerch, H. Harttig, V. Zimmer, *Eur. Pat.,* 571940, 1993.
23. Tsuji Noboyuki and Oka Motohiro, *Jpn. Kokai Tokkyo Koho JP*, 0349695, 1991.
24. Taya Keiko and Kurosaki Kazuko, *Jpn. Kokai Tokkyo Koho JP*, 03152463, 1991.
25. E.J. Kiser, E. Rice and M.F. Tomarro, *Eur. Pat.*, 41567, 1991.
26. M.K. Vickers, *Med. Lab. Technol.*, 30 (1973) 235.
27. J.C. Forest, F. Rousseau, J. Talbot, P. Douville and M. Gosselin, *Clin. Chem. (Winston-Salem, N.C.),* 33 (1987) 2312.
28. J.A. Hunt and N.C. Alojado, *Diabetes Res. Clin. Pract.*, 7 (1959) 51.
29. D. Noble, *Anal. Chem.*, 65 (1993) 1037 A.
30. *Product Catalog*, Pointe Scientific, Inc., 1998–1999.
31. T. Yotsuyanagi, E. Kaneko, M. Ishihara, A. Ishida, Y. Deduchi, H. Mizuguchi and K. Yoshimoto, *10th Russian–Japanese Joint Symposium on Anal. Chem.*, Moscow and St. Petersburg, Russia, 2000, Book of Abstracts, p. 44.
32. M.P. Allen, A. Delizza, U. Ramel, H. Jeong and P. Singh, *Clin. Chem. (Winston-Salem, N.C.),* 36 (1990) 1591.
33. M. Yoshihiko, T. Kaoru and H. Naofum, *Eur. Pat.*, 571940, 1993.
34. A. Rothe and H. Eikmeier, *Ger. Offen DE*, 4001155, 1991.
35. M.P. Allen and H.J. Jeong, *Eur. Pat.*, 441325, 1991.
36. P.J. Patel, M.P. Allen and P. Singh, *US Pat.*, 5215886, 1993.

37. J.L. Aronowitz, *PCT Int. Appl., WO* 94/12879, 1994.
38. E. Jungreis, in: *Spot Test Analysis* (J.D. Winefordner, ed.), John Wiley and Sons, New York, 1997.
39. B.S. Garg, Y.L. Mehta and M. Katyal., *Talanta,* 23 (1976) 71.
40. Tobari Jinen, *Jpn. Kokai Tokkyo Koho JP,* 02134567, 1990.
41. Umezawa Takehiko and Akiyoshi Yasushi, *Jpn. Kokai Tokkyo Koho JP,* 04110663, 1990.
42. Umezawa Takehiko and Akiyoshi Yasushi, *Jpn. Kokai Tokkyo Koho JP,* 05999929, 1993.
43. Tanako Mitsutoshi and Okanoe Yutaka, *Jpn. Kokai Tokkyo Koho JP,* 02136746, 1988.
44. V. Rittersdorf, V. Verner, H.-G. Rei and P. Rikmann, *USSR Inventor's Certificate* 635501, *Byul. Izobret.*, No. 42, 1978.
45. D. Kutter, J. Thoma, A. Kremer, S. Hansen and R. Carl, Eur. *J. Clin. Chem. Clin. Biochem.*, 33 (1995) 243.
46. A. Szewszuk and M. Mulezuk, *Pol. Pat.* 84464, 1976.
47. Gist-Brocades N.V., *Ger. Pat.* 2831083, 1979.
48. Sh. Yasuda, *Jpn. Pat.* 59-148868, 1984.
49. H.M. Emrich, *Ger. Pat.* 2631864, 1978.
50. J.M. Sloan, G. Beevers and F.E. Baxter, *Clin. Chem.*, 30 (1984) 1705.
51. J.M. Figg, *Concrete,* 9 (1975) 38.
52. I. Jaunakais, *US Pat.* 5620658, 1997.
53. M. Watakabe, *Jpn. Pat.* 01-65453, 1989.
54. A.M. Gronberg, *Ger. Pat.* 2727347, 1977.
55. K.H. Kallies, *Ger. Pat.* 2843485, 1979.
56. L. Pezza, M. Tubino, C.B. Melios and H.R. Pezza, *Anal. Sci.,* 16 (2000) 313.
57. A.N. Masoud, *J. Pharm. Sci.*, 64 (1975) 841.
58. E.G.C. Clarke, *Isolation and Identification of Drugs,* Pharmaceutical Press, London, 1975, vol. 2.
59. G. Dale, *Analytical Aspects of Drug Testing,* Wiley, New York, 1989.
60. T.A. Gough (Ed.), *Analysis of Drugs of Abuse,* Wiley, New York 1991.
61. *Chemical Spot Test Kits for Preliminary Identification of Drugs of Abuse,* US Dept. of Justice, Washington, 1978.
62. R.P. Pantaler, O.V. Gaiduk, L.V. Gudzenko and A.B. Blank, *All-Ukrainian Conference on Analytical Chemistry,* Absts., Khar'kov, May 15–19, 2001, p. 164.
63. R.P. Pantaler and T.I. Ivkova, *RF Pat.* 1628697, *Byul. Izobret.,* No. 16, 1993.
64. M.J. de Faubert Maunder, *Analyst (London),* 100 (1975) 878.
65. A. Bult and H.B. Klasen, *Pharm. Weekbl. Ned.*, 109 (1974) 389.
66. F. Schmidt, *Dtsch. Apoth. Ztg.*, 113 (1978) 443.
67. A.Yu. Budantsev, E.G. Litvinova and M.A. Kovalyova. *Zh. Anal. Khim.*, 52 (1997) 539.

Chapter 11

Some Other Applications of Test Methods

There are other areas where test methods are being employed but which have not been mentioned so far in this book. Two of them — determination of noble metals by the use of the touchstone and application of test methods in the teaching process — are briefly considered in this chapter.

11.1 DETERMINATION OF GOLD AND SILVER BY THE TOUCHSTONE

This technique has been known since ancient times. Probably, the first mention of such a method of gold assessment was made in Theophrastus's work "On Stones" (372–287 B.C.). The method has been in use for many centuries. In the early 1600s it was improved by the introduction of reference samples, viz. 23 needles containing different amounts of gold from 1 to 23 carats and an additional one made of pure gold.

Procedure

To test gold specimens, a strip of uniform geometry and density 15–20 mm long and 2-3 mm wide is formed on the touchstone by scratching with the tested golden specimen. A similar strip is made in close vicinity with an appropriate reference needle. The strips are wetted width-wise by a special gold reagent using a glass rod and the reaction progress is closely watched for 15–20 seconds, after which the reagent is gently removed with filter paper, the test and reference strips are finally dried and colour-matched against each other. If the colours of the reaction spots on the strips are more or less identical then the gold contents of the tested and reference samples are assumed to be also identical. If the colour intensity of the test strip is lower (or higher) than that of the reference one then the gold content of the test specimen is lower (or higher) than that of the reference sample. The method has the advantage over other methods requiring dissolution of the sample that it is very quick and simple and causes almost no damage to the tested gold articles. It allows not only qualitative but also quantitative determination of gold, silver, platinum in alloys and golden articles. Its accuracy is high, e.g. when testing a gold article of 583 per mille (p.m.) gold content the measurement error may be 2–5 p.m.

11.1.1 The touchstone and test needles

The touchstone refers to silicon slate, is black in colour, has a uniform small-grained composition without quartz films or cracks, is sufficiently hard, and allows obtainment of uniformly dense strips of noble metals. It is chemically stable and is resistant to mineral acids (HNO_3, HCl, H_2SO_4) and their mixtures.

The test needles are alloys of gold, silver and platinum taken in different but strictly defined proportions. The gold needles are usually alloys of gold, silver, copper, and less frequently those of gold, silver or gold, copper. For testing gold articles the needles of the following p.m. gold content are used: 333, 375, 500, 578, 583, 750, 900, 916 and 958. Additionally, needles with intermediate gold contents are prepared (Table 11.1). Silver needles are made of silver-copper alloys of the following p.m. gold content: 500, 550, 600, 650, 700, 750, 800, 875, 900, 916. The 500 and 900 p.m. needles are used to test coin alloys, and 750, 800, 875, and 916 p.m. needles are used for testing silver articles manufactured in Russia.

11.1.2 Reagents

Reagents for gold are prepared in the following manner. Gold chloride solutions are obtained by dissolving 23 g $H[AuCl_4]\ 4H_2O$ in 1000 ml distilled water. It is used for testing alloys and articles of the 583 p.m. gold content and lower. Those of the 375–958 p.m. gold content are tested with acid reagents whose composition is indicated in Table 11.2. Although the touchstone is employed for testing silver and platinum articles, besides gold ones, in this book attention is given only to its use with golden alloys and articles just for the sake of an example.

TABLE 11.1

Chemical composition of test needles used for testing gold articles and alloys with the touchstone (%)

Gold	Silver	Copper	Gold	Silver	Copper
Needles of 375 p.m. gold content			Needles of 750 p.m. gold content		
37.5	62.5	—	75.0	25.0	—
37.5	50.0	12.5	75.0	18.7	6.3
37.5	37.5	25.0	75.0	12.5	12.5
37.5	25.0	37.5	75.0	6.2	18.8
37.5	12.5	50.0	75.0	—	25.0
37.5	—	62.5	75.0		
Needles of 500 p.m. gold content			Needles of 833 p.m. gold content		
50.0	50.0	—	83.3	8.3	8.4
50.0	40.0	10.0	Needles of 916 p.m. gold content		
50.0	30.0	20.0	91.6	4.2	4.2
50.0	20.0	30.0	Needles of 958 p.m. gold content		
50.0	10.0	40.0	95.8	4.2	—
50.0	—	50.0	95.8	2.1	2.1
			95.8	—	4.2

TABLE 11.2

Composition of acid reagents for testing gold articles and alloys

Gold contents (p.m.)	HNO_3 (sp.w. 1.4)	HCl (sp.w. 1.19)	Water
375	59.5	—	40.5
500	100.0	—	—
750	59.3	1.1	39.6
833	68.7	1.3	30.0
900	78.7	1.3	20.0
958	69.2	2.0	28.8

The above-mentioned highly sensitive and specific gold chloride solution is employed for testing, besides gold alloys, two-component gold–silver or gold–copper alloys, as well as three-component gold–silver–copper ones with less than 583 p.m. gold content.

The scientific technology centre Tekhnokom-AS (Moscow) has developed a portable instrument "Gold Detector" for detecting gold in alloys and gold wares. The instrument scale has nine divisions corresponding to gold contents from 300 to 750 p.m., it shows the presence of platinum or a complete absence of gold (RF Patent 2057410).

11.2 USE OF TEST METHODS IN EDUCATION

One of the main objectives of ecological education is to overcome the passive attitude of pupils and students to ecological problems and inculcate in them an active and creative desire to defend the environment. Making them participate in research projects aimed at environmental monitoring and control raises their awareness, develops individual skills and awakens an interest in scientific work. The analyses and tests performed by them with their own hands and the results obtained are the best argument in favour of the necessity to protect and improve the environment.

Test systems are an excellent means of teaching chemistry and ecology in schools and other educational institutions. They are convenient to use in the chemistry schoolroom, for out-of-the-class studies, expeditions, and excursions. They are quite valuable for teaching students ecological and foodstuffs control, and ecological monitoring of lakes and rivers during expeditions. Students of non-chemical departments learn the simplest methods of soil assessment, of bio- and mineral testing.

As an example, one may cite the teaching test kits manufactured by Krismas+ (St. Petersburg, Russia) — Pchelka-U, Pchelka-U/M, and Pchelka-U/Khim. For example, the "Pchelka-U" kit allows the following experiments to be carried out:

- rapid analysis by means of indicator tubes of such air pollutants as nitrogen oxides and sulphur dioxide;
- rapid non-aspiratory analysis of ammonia vapours;
- rapid analysis of contaminants in water and water extracts, e.g. active and total chlorine, pH, nitrates and chromates;
- rapid analysis of soil contamination using soil extracts;

- rapid analysis of salts and fertilizers;
- rapid analysis of nitrates in beverages and fruits.

The Pchelka-U/Khim kit allows determination of carbonates and hydro carbonates, chlorides, sulphates and total water hardness. The kit's weight is no more than 3 kg; it is a hand-carried case measuring 330 × 170 × 160 mm. It includes five indicator tubes for air analysis, test means for 100 analysis of each specified water parameter.

There are also ecological kits for practical work intended for use by students in Forms 9–11 of schools and supplementary learning establishments. The practical work kit is a modular-based didactic methodological complex including equipment and tools for carrying out experiments in ecology, environmental monitoring, geography, chemistry, biology with elements of ecology, and also the methodological literature. The practical work kit covers the main themes included in the ecological practical course, namely:

- assessment of water bodies' ecological status using test-kits and tests; the tested parameters are: organoleptic characteristics, pH, sulphates, carbonates, chlorides, dissolved oxygen, nitrates, nitrites, total hardness, chromates, copper, potassium, sodium, active chlorine, and others;
- assessment of the ecological status of air; determination of carbon dioxide, sulphur dioxide, nitrogen oxides, dust, bio indication of atmospheric precipitates;
- assessment of the ecological status of soil; determination of pH, salt content in water extracts;
- modelling of chemical contamination of air and water media and their rapid chemical analysis;
- determination of nitrates in vegetables, fruits, beverages, and water.

Modular design of the practical work kit creates favourable conditions for carrying out expedition tasks, field and outdoor practical work of all or selected directions. A complex ecological monitoring can be performed by students using the kit in combination with such instruments as microscope, noise meter, lux meter, radio dosimeter, psychrometer and others.

Ecology and environmental protection have long been an integral part of school curricula, especially in chemistry and biology. The Aquamerck® test kit system and, especially, the Compact Laboratory, are ideal tools for illustrating ecological principles and the potential effect of our interfering with nature. The test procedures can be learned quickly and pupils soon begin to participate actively and even carry out their own measurements.

Aquamerck® test kits can also be used highly effectively and in a very impressive manner to demonstrate reaction mechanisms and analytical methods. Apart from the various ecological aspects involved, the Aquamerck® test kits can be used to illustrate other principles, e.g. the solubility of gases in liquids using oxygen as an example.

Many of the Aquamerck® kits are used in technical colleges; they are excellent for explaining complex technical processes and concepts. Merck also provides the schooling and "Nitrogen — a key element of life" (Junger Verlag, Offenbach: available currently only in German). Using nitrate as an example, the origin and potential effects of an environmental problem are illustrated in such a way as to cover a number of interdisciplinary curriculum subjects.

The Somerset Educational Co. (SAR) manufactures laboratory equipment kits Microchem® for use in school chemistry curricula, among them a MicroScience® Minilab kit for 100 analyses of water. Analogous kits are produced by ECOTECH (Moscow) in which are included reagents for field analyses of Fe(II), Cu, total and calcium hardness, chlorides, sulphates, fluorides at maximum admissible concentration levels.

A range of educational material and pertinent test systems under the rubric "Monitoring and protection of the environment" has been developed and marketed by the Vladimir State University (Vladimir, Russia) for pupils of Forms 9–11 of general learning institutions attending classes in ecology, chemistry, biology, and for faculty and group studies [3]. Emphasis is placed on developing the practical skills of pupils in analyzing the environment and drawing the right conclusions. The program corresponds with that for ecological practicum of general learning establishments. The test systems are reagent papers impregnated with appropriate ingredients and showing the presence of the target component by the change of the test strip coloured or discoloured zone length or area. Test systems are easy to use, do not contain carcinogenic substances and can be used in the field, at home and in other out-of-lab conditions. The kit includes test systems without liquid or solid reagents, syringes, glass flasks, and a dropper with a 7% HCl solution. User instructions are printed on the package cover; 50 tests can be performed with one kit batch Table 11.3).

TABLE 11.3

Parameters of test systems for field environmental monitoring

Component to be detected	Analytical range (mg/l)	Analyzed medium
Aluminum	0.01–50	Drinking water
Ammonium	0.5–20	Natural and waste waters, soils, atm. precipitates
Aniline	0.1–5	Waste waters, air
Bromides	1–500	Waste waters, solutions
Ascorbic acid	50–1000	Foodstuffs
Iron	0.01–1	Natural waters, atmospheric precipitates
Iron	0.1–40	Natural and waste waters, solutions, soils
Total hardness	0.1–10, 1–20 m*M*	Water, atmospheric precipitates
Iodides	1–1000	Waste waters
Cadmium	0.005–1	Natural waters
	0.1–200	Waste waters, soils
Cationic surfactants	0.01–1000	Waste waters
Acids	0.1–100 m*M*	Solutions
Copper	0.001–0.1	Natural waters and atmospheric precipitates
	0.1–100	Natural, atmospheric and waste waters, soils
Arsenic	0.05–3	Natural, atmospheric and waste waters, soils
Nickel	0.05–10	Natural, atmospheric and waste waters, soils
Nitrates	40–800	Natural and waste waters, soils, foodstuffs
Nitrites	2–60	Waste waters, soils, foodstuffs
Nitrites	0.05–5	Natural and waste waters, atmospheric precipitates
Total acidity	0.1–10 m*M*	Waste waters
Total alkalinity	0.1–10 m*M*	Natural waters
pH	4–6	Atmospheric precipitates
pH	4–8	Soils
pH	6–8	Natural waters

continued

TABLE 11.3 (*continuation*)

Component to be detected	Analytical range (mg/l)	Analyzed medium
Mercury	0.005–1	Natural and waste waters
	0.1–200	Waste waters, soils
Lead	0.01–1	Natural and drinking waters
	1–500	Waste waters, technological solutions
Silver	0.005–1	Natural waters
	0.5–100	Waste waters, technological solutions
Hydrogen sulphide	0.01–1	Waste waters, solutions, atmospheric precipitates
Sulphates	10–800	Water, atmospheric precipitates
Thiocyanides	1–500	Waste waters, solutions
Phenol	0.05–5	Waste waters
Phosphates	1–50	Natural and waste waters, soils
	2–50	Waste waters, soils
Fluorides	0.5–500	Natural and waste waters, atmospheric precipitates, soils
Active chlorine	0.3–8	Drinking and waste waters
Chlorides	0.5–500	Natural and waste waters, atmospheric precipitates, soils
Chromium(VI)	0.05–500	Natural and waste waters, atmospheric precipitates, soils
Cyanides	1–500	Waste waters and technological solutions
Zinc	0.01–1	Natural waters, atmospheric precipitates
	0.1–200	Natural and waste waters, soils

Using the test methods one can quickly detect the presence or absence of a harmful component in different media and its approximate content. The test kit was employed in the field for such tasks as:

- detection and assessment of different components in waste, natural, drinking, atmospheric waters, technological solutions, foodstuffs, and bio-liquids;
- assessment of the degree of purification of drinking water using household filters;
- control of wastewater purification;
- teaching pupils ecology, chemistry, biology in field conditions;

From 1994 to 2000 the students of Vladimir educational institutions carried out the following experiments using test systems:

- monitoring water quality in wells and springs of Vladimir;
- monitoring water quality in the river Rpen;
- quality control of vegetables and fruits by measuring their ascorbic acid, nitrate and nitrite content;
- monitoring the tap water quality of the eastern district of the city;
- assessment of atmospheric air contamination by detecting heavy metals in the foliage;
- assessment of atmospheric air contamination by chemical analysis of atmospheric precipitates;
- nitrogen biocycle in the atmosphere;
- micro-elemental composition of human hair and man's living conditions.

The results of this work were presented at international, all-Russian and district ecological Olympiads where the pupils received high esteem and recognition.

REFERENCES

1. E.A. Marenkov and D.G. Grebenkin (Eds.), *A Guide on Assay*, Gosfinizdat, Moscow, 1953 (in Russian).
2. S.V. Alekseev, N.V. Gruzdeva, A.G. Muraviev and E.V. Gushchina, *Practical Course on Ecology*, AO MDS, 1996 (in Russian).
3. V.G. Amelin, *Ecology and Industry of Russia,* 1997, 43.

Chapter 12

Conclusions and perspectives

Making the chemical analysis cheap and simple is a noble and worthy task but, regrettably, the solution of many analytical problems requires the use of expensive and sophisticated analytical equipment. Fortunately, recent advances in chemistry, physics, microelectronics, and computing make possible the development of new tools of chemical analysis that are progressively more and more miniature, low-cost and simple to use and at the same time quite comparable in their performance to the more sophisticated modern analytical instruments. Test systems, without doubt, can be considered to be one of this family.

The material presented in this book demonstrates that, firstly, the test methods are very much in demand; secondly, quite a few test systems of various types and fields of use have already been produced; thirdly, the majority of tests are based on sensitive and selective chemical reactions; fourthly, the results can be quantified either visually, using the simplest procedures: measuring the coloured zone length, counting the number of drops, or by means of mini devices that are simple to operate.

Although test methods are also used in the laboratory, e.g. for sample screening, it would be more advisable to use them in the field, all the more so if one considers the fact that on-site analysis is an important and rapidly growing field of analytical chemistry.

Among the areas where test methods have already secured a firm footing are:

- environmental monitoring;
- quality control of foodstuffs;
- clinical analysis;
- forensic medical analysis;
- military service;

- some branches of industry;
- precious metals analysis.

For developing reliable, sensitive and selective tests, the achievements of classical analytical chemistry are used (reactions and reagents). However, the search of novel approaches is more important.

There is a methodological question that should be considered in greater detail. Namely, where is the borderline between test methods employing simple mini devices, on the one hand, and traditional automated analytical methods – photometric, luminescent, electrochemical ones, using portable instruments, on the other? Undoubtedly, these two areas are drawing ever closer; the analysis protocols are more and more often built into the instrument proper, making the analysis very simple. The trend here is to use ever more compact instruments (leaving aside such multifunctional sophisticated methods of chemical analysis as GC-MS, ICP-AES, ICP-MS, and the like). Thus the borderline mentioned earlier is gradually becoming more and more blurred. The situation here is analogous to that in the computer industry where a small pocket calculator can replace a cumbersome computer, or as in the medical profession where the blood pressure that earlier had to be measured in a clinic now can be measured at home.

Chemical analysis is becoming more and more accessible to the broader public. In this context, test methods will occupy, if not the first place, certainly not the last.

Appendix

Producers and Suppliers of Test Kits

Amel Instruments Srl, Via S. Giovanni Battista de la Salle 4, 20132 Milan, Italy. Tel.: +39-2-27203060. Fax: +39-2-2564832.

Ames Division, Bayer Diagnostics UK Ltd, Stoke Court, Stoke Poges, Slough SL2 4LY, England.

Anachem Ltd., 20 Charles Street, Luton, Beds. LU2 OEB, UK. Tel.: 44 (0) 1582 745000. Fax: 44(0) 1582 391768.

Analit-tsentr (Analyt-Center), 14, Prospekt Dobrolyubova, 197198, St. Petersburg, Russia. Tel.: +7 312 238-9558. Fax: +7 812 233-8989.

App-Chem Ltd., Hugmore Lane, Llanypwll, Wrexham, North Wales, LL13 91E, UK. Tel.:+44(0)1978 661 551. Fax: +44(0)1978 661 611. E-mail: info@app-chem.com; Website: www.app-chem.com.

Argument, NTTS, P.O. Box 459, Moscow 121374, Russia. Tel.: +7 095 954-0179, +7 095 440-1455. Fax: +7 095 954-3719.

Aurora Scientific, Braysdown Works, Braysdown, Peasedown, St. John, Bath BA2 8LL, UK. Tel.: 44(0) 1761 439139. Fax: 44(0) 1761 433393.

Behr Labor-Technik GmbH, Spangerstrasse 8, Dusseldorf-Reisholr, D-40599, Germany. Tel.: +49(0) 211-748-4732. Fax: +49(0) 211-748-4748.

Best Sensors Co. Ltd., 178-8 Ohguchi-Nakamachi, Kanagawa-ku, Yokohama-shi 221, Japan. Tel.: 81-(45)-402-0068. Fax: 81-(45)-402-1161.

BioOrbit Oy, P..O. Box 36, FIN-20521, Turka, Finland. Tel.: +358-2410-1100. Fax: +358 2410 1123. E-mail: bioorbit@bioorbit.com.

Bioscience Inc., 1550 Valley Center Parkway, STE 140, Bethlehem, PA 18017, USA. Tel.: 1 610 974 9693. Fax: 1 610 691 2170.

Boehringer Mannheim GmbH, Mannheim, Germany.

CAE EXPRESS (A Division of Clean Air Engineering), Palatine, USA.

Camlab Limited, Cambridge, UK.

Campro Scientific B.V., Veenendaal, The Netherlands.

The Capital Controls Group Ltd., Park Lane, Minworth, Sutton Coldfield, B76 9BL, UK. Tel.: 44 (0)121 313 2300. Fax: 44 (0)121 313 1938. Website: http://www.capital-centrols.com.

Cecil Instrument Ltd., Milion Technical Centre, Milton, Cambridge CB4 6AZ, UK. Tel.: 44 (0)1223 420821. Fax: 44 (0)1223 420475.

Central Company, 308 West Edgewood, Frindswood, Texas 77546, USA. Tel.: (281) 432-1714. Fax: (281) 482-9448. E-mail: info@control.3.com. Website: http://www.control.3.com.

CHEMetrics Inc., Route 28, Calverton VA 20138, USA. Tel.: (703) 788-9026, (800) 356-3072. Fax: (703) 788-4856, (540) 788-4856. E-mail: prodinfo@chemetrics.com. Webbsite: http://www.chemetrics.com.

Chromato-sud, 15 Rue d'Artiguelongue, 33240 Saint Antoine, France. Tel.: 33 57 940626. Fax: 33 57 940620. Website: http://www.chromatosud.com.

Cole-Parmer International, USA. Fax: (847) 549-1700. E-mail: export@coleparmer.com.

Corporation Scientifique Claisse Inc., Sainte-Foy (Quebec), Canada.

DEXSIL Corporation, One Hamden Park Drive, Hamden, CT 06517, USA. Tel.: (203)288-3509. Fax: (203)248-6523. Website: http://www.dexsil.com.

Dr. Lange, Dr. Bruno Lange GmbH Berlin, Industriemesstechnik, Willstatterstrasse 11, 8-40549 Dusseldorf, Germany. Tel.: (0211) 52830. Fax: (0211) 5288-175.

Drägerwerk AG, Moislinger allee 53/55, D-23542 Lübeck. Tel.: +49 451 832-3771. Fax: +49 451 882-3900.

Ekoanalitika Association, Moscow State University, Department of Chemistry, Leninskie Gory, Moscow, Russia.

Ekotest (Ecotest), 10 Eniseiskaya ul., App. 308, Moscow 129344, Russia. Tel.: +7 095 471-5226; +7 095 461-5301.

Ele International Ltd., Eastman Way, Hemel Hempslead, Herts, HP2 7HB, UK. Tel.: 44 (0)1442 218355. Fax: 44 (0)1442 252474. E-mail: ele@eleint.co.uk.

EnSys Inc., Research Triangle Park, NC, USA.

Envirol Inc., 1770 Research Park Way, Suite 160, North Logan, Utah 84341. Tel.: (435) 753-7946. Fax: (435) 787-2878.

Environmental Instruments, Sovereign House, Queensway, Leamington Spa CV31 3JR, UK. Tel.: +44(0) 1926 470101. Fax: +44(0) 1926 338110. E-mail: ei@geotech.co.uk.

Environmental Remediation Equipment Inc., Lafrenaie, Canada.

Environmental Test Systems Inc., Elkhart, Ind., USA.

ESECO-Speedmaster, One Eseco Rd., Cushing, OK 74023, USA.

Eutech Instruments Pte Ltd. 55 Ayer Rajad Crescent, # 04-16/24 Singapore 139949. Tel.: (65) 778-6876. Fax: (65) 773-0836. E-mail: marketing@eutechinst.com. Website: http://www.eutechinst.com.

Gas Tech Inc., 8407 Central Ave., Newark, CA 94560-3431. Tel.: (510) 745-8700.

GreyHound Chromatography, Birkenhead, UK.

HACH Company, 5600 Lindbergh Drive, PQ Box 389, Loveland, Colorado 80539-0389, USA. Tel.: 1 (970) 669-3050. Toll-Free: 1-800-227-4224. Fax: 1-970-669-2932. E-mail: intl@hach.com. Website: http://www.hach.com.

Hanna Instruments, 584 Park East Drive, Woonsocket, RI 02895, USA. Tel.: (800) 426-6287. Website: www.hanna-inst.com.

Helling Kommanitgesellschaft für Industrieprodukte und Anlagenbau, Sylvesteallee 2, D-22525 Hamburg. Tel.: (040) 547180. Fax: 542061.

HNU Systems Inc., 160 Charlemont Street, Newton, MA 02161-9987, USA. Tel.: (617) 964-6690. Fax: (617) 558-0056.

HNU-Nordion Ltd OY, Helsinki, Finland.

ImmunoLab GmbH, Kassel, Germany.

Industrial Test Systems Inc., 1875 Langston Street, Rock Hill, SC 29730, USA. Tel.: (800) 861-9712, (303) 329-9712. Fax: (803) 329-9743. E-mail: its@cetlink.net. Website: www.sensafe.com.

Iris Instruments, Orleans, Cedex 2, France.

J.T. Baker Inc., 222 Red School Lane, Phillipsburg, NJ 08865, USA, Fax: (908) 859-9318.

Jenway Ltd., Gransmore Green, Felsted, Dunmow, Essex CM6 3LB, UK.

Jepson Bolton and Co. Ltd., Watford, UK.

Johnson & Johnson Co. Milpitas, California 95035, USA.

K and M Environmental, 2421 Bawland Pkwy Unit 102, Virginia Beach, Virginia 23454, USA. Tel.: (800) 808-2234, (804) 431-2260. Fax: (804) 431-2255.

Khimanalit Institute (Chemanalit), 150 Naberezhnaya Obvodnogo Kanala, St. Peterburg 198020, Russia. Tel.: +7 812 186-6749.

Khimavtomatika, Selskokhozyaistvennaya ul., 12 a, Moscow.

Kimoto Eletric Co., Ltd., Osaka, Japan.

Kostip, ul. Narodnogo Opolcheniya, 38, buil. 2, Moscow 129293, Russia. Tel/Fax: (095) 943-6348.

Kristmas+ Co., 102 Naberezhnaya Fontanki, 191180 St. Petersburg, Russia. Tel.: +7 812 112-4114; +7 812 164-6042; +7 812 164- 3101. Fax.: +7 812 112-4114.

Lamotte Company, Chestertown, MD, USA.

Lumidor Safety Products, 11221 Interchange Circle South, Miramar, FL 33025. Tel.: (305) 433-7000. Fax: (305) 433-7730.

M.P.K. Omega Co, 3615 Carson, Amarillo. Tex. 79109.

Macherey-Nagel GmbH & Co. KG, P.O. Box 10 13 52. D-52313 Duren, Germany. Tel.: (02421) 969-0. Fax: (02421) 969-199, Website: http://www.macherey-nagel.com. E-mail: sales@macherey-nagel.de.

MCB Reagents, 480 Democrat Rd. Gibbstown, N.J., USA.

Merck KGaA, P.O. Box 4119, D-64271 Darmstadt, Germany. Tel.: +49 6151 727143. Fax: +49 6151 723880. E-mail: environ.analysis(@merck.de. Website: http://www.merck.de.

Millipore Corp., 80 Ashley Rd., Bedford, MA 01730, USA. Tel.: 275-9200. Fax: (617) 275-5550.

MSA: Mine Safety Appliances Co.

M-Tech Diagnostics Ltd., Warrington UK.

Myron L, Fax: 619/931-9189.

NeCi, The Nitrate Elimination Company Co. Inc. (USA).

Ohmicron Environmental Diagnostics Inc., 375 Pheasant Run, Newtown, PA 18940, USA. Tel.: (215) 860-5115. Fax: (215) 860-5213.

Orion Research Inc. 500 Cumming Center, Beverly, MA 01915-6199, USA. Tel.: 978-232-6000. Toll Tel.: 800-225-1480. Fax Int'l: 978-232-6031.

Oxord Limited, Wave Road, Basingstoke, Hants., RG24 8PW, UK. Tel.: 44 (0)1256841144. Fax: 44 (0) 1256 463388. Telex: 858793 INIPAT G.

Palintest Ltd., Palintest House, Kingway Team Valley, Gateshead, Tyne and Wear, NE11 0NS, UK. Tel.: 0191 491 0808. Fax: 0191 482 5372, E-mail: palintest@palintest.com.

Petrolab Corp., 874 Albany-Shaker Rd., Latham, NY 12100, USA. Fax: (518) 783-5185.

Pierce, a Perstorp Biotec Company, 3747 N. Meridian Road, P.O. Box 117, Rockford, IL 61105, USA. Tel.: (800) 874-3723, (815) 968-0747.

Pointe Scientific Inc., 1025 Joh A. Papalas Drive, Lincoln Park, Michigan 48146, USA. Tel.: (800) 445-9853, (313) 383-7220. Fax: (313) 383-7841.

Professional Test Systems, Summer Court, Manafon, Welshpool, Powys SY21 8BJ, UK. Tel.: (01686) 650170. Fax: (01686) 650170. E-mail: sales@proftest.com. Website: www.proftest.com.

Quadrex Scientific, Weybridge, UK.

Quest Technologies Inc., 510 South Worthington Street, Oconomowoc., Wl 53066, USA Matheson.

Randox Laboratories Ltd., 55 Diamond Road, Grumlin, Country Antrim, UK BT29 4QY. Tel.: +44 1819 422413. Fax: +44 1819 452912. E-mail: randox@compusere.com.

Riedel-de Haen, Postfach 100262, D-30918 Seelze, Germany. Tel.: (5137) 999-273. Fax: (05137) 999-502.

Robin Instruments Ltd., Bagshot, UK.

Supelco, Inc. Supelco Park. Bellefonte, PA 16823-0048, USA. Phone 300-247-6623 / 8143593441. Fax: 800-447-3044 / 814-359-3044.

SDI-Ensys Europe Ltd., Alton, UK.

SECOMAM, Domont, France.

The Tintometer Ltd., Salisbury, UK.

Thomas Scientific, .P.O. Box 99, Swedesboro, NJ 08085-0099, USA.

Tintometer GmbH, Schleefstrasse 8 a, 44287 Dortmund, Germany. Tel.: +49 (0)231 94 51 00. E-mail: technik@tintometer.do.eunet.de.

Toyo Roshi International, Inc. 7005. Flourr St. Suite 190I, Los Angeles, CA 90017, USA. Tel.: (213) 489-3232.

Trans Instrument, No 8 Aljunied, Ave 3, Oakwell Building, Singapore 389933. Tel.: (65) 742-0367. Fax: (65) 742-5082. E-mail: transins@pacific.net.sg.

Tudor Seilentific Glass Co 555 Edgexield Rd., Belvedere., S.C. 29841. (803) 279-4666.

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Waterra (UK) Ltd., Solihull, UK.

Witratemp - Industrievertretungen, D-92256 Hahnbach, Wüstenauer Weg 13, Suss, Germany. Tel.: 99664-1543. Fax: 09664-404. (Products of Dr. Lange Co.)

WPA Ltd., The Old Station, Linton, Cambridge, CBI 6NW, UK. Tel.: +44 (0)1223 892688. Fax: +44 (0)1223 894118. E-mail: sales@wpaltd.co.uk. Website: www. wpaltd.co.uk.

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