Introduction to Spectroscopy and Applications

Preface

Students can now study basic scientific principles on the same world-class equipment used by leading researchers in university and government labs like NASA. Advances in electro-optics, high-speed array detectors, inexpensive optical fibers and powerful computers have made optical spectroscopy the sensing technique of choice for many real-world applications.

The development and availability of scientific instrumentation and methods have changed in an equally dramatic way. In the past, cutting-edge scientific instruments were expensive research devices accessible only to well-funded research and development enterprises. Gradually, the technologies filtered into general laboratory use, application-specific instruments and now into the educational setting.

Our knowledge of spectroscopy is based on more than 20 years of experimentation in a wide array of disciplines ranging from art to applied physics. All of these fields have their roots in education with educators teaching their students the basics of the field. Thousands of science educators have utilized Ocean Optics spectrometers to create real-world, exciting experiments to teach their students and enrich their lives with a greater understanding and appreciation for science.

It is important that today's science and engineering students appreciate the capabilities of optical sensing, the fundamental physics of the measurement process, the design trade-offs inherent in selecting and integrating components and the discipline required to produce quality results. The goal of this lab manual is to provide a vehicle to allow future scientists to study the fundamentals of spectroscopy using modern instrumentation.

We would like to offer special thanks to the educators who contributed to this lab manual as part of the ongoing Ocean Optics grant program.

Note to Educators: If you would like to contribute to future compilations, please send an email to info@oceanoptics.com.

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Table of Contents

Spectroscopy Concepts

Overview

Scientific discoveries are based on observations. Scientists look for patterns in what they see, hear, feel, smell and taste to formulate theories and make predictions. Originally, scientists depended solely on their own senses to make observations. But as science has evolved, scientists have developed instruments to extend their observational powers beyond our sensory limits. Telescopes have enabled astronomers to see more of the sky and vastly improve our understanding of the heavens. Likewise, microscopes have enabled biologists to view ever smaller parts of living organisms in their quest to understand living systems.

Astronomers are only limited by the size of the telescopes they can build and the distorting effects of the earth's atmosphere. As technological developments have allowed for bigger mirrors and space-based platforms, astronomers have been able to see ever further into space and make more and more discoveries. Unfortunately, the situation is very different going in the other direction. There is a physical limit to the size of objects that can be "seen." This limit is due to the nature of light itself.

Light

Light is a type of electromagnetic radiation consisting of little packets of energy called photons with both particle and wave-like properties. As shown in the complete electromagnetic spectrum in Figure 1, light in the visible region (~400 to 700 nm) makes up only a small region of the entire spectrum of electromagnetic radiation.

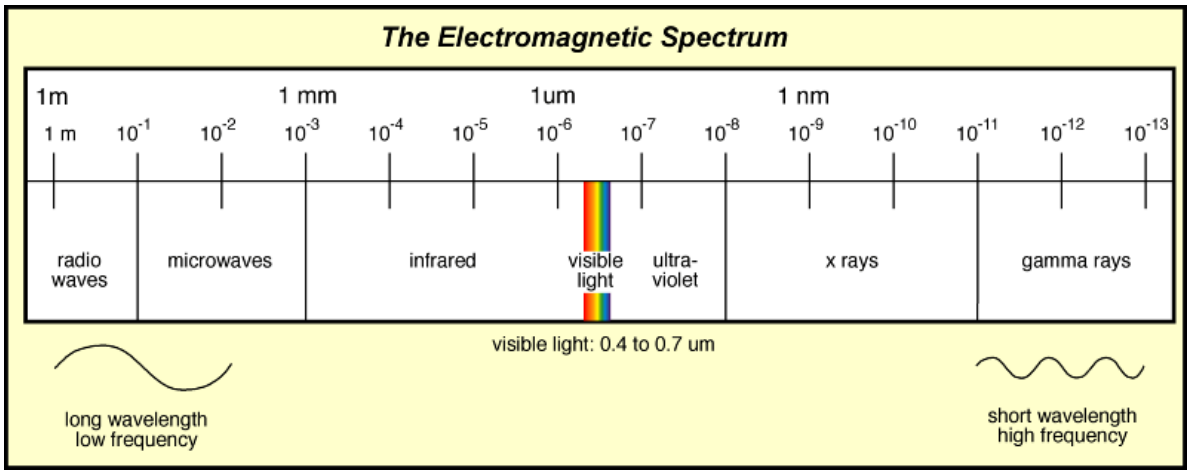


Figure 1: Electromagnetic Spectrum
http://www.columbia.edu/~vjd1/electromag_spectrum.htm

It is the wave properties of light that limit our ability to use light to create images. For any given wavelength, images can be formed for objects larger than the wavelength of light used to visualize the image. Therefore, for visible light (wavelengths between 4 x 10^{-7} and 7 x 10^{-7} m) it is impossible to form images of atoms with sizes on the order of 10^{-9} m. Very large molecular assemblies such as chromosomes (DNA molecules coated with protein molecules) are the smallest objects we can image with visible light.

The challenge faced by chemists, biochemists and microbiologists is studying what happens at the atomic and molecular level without actually being able to physically visualize atoms and molecules. Fortunately, even though we cannot capture images of atoms and molecules, we can use light to learn more about them. This is because atoms and molecules interact with light providing detailed information on their structure, composition and interactions. The technique for measuring the interaction of light with matter is referred to as spectroscopy. It is arguably the most powerful tool available to scientists to study the molecular world around them. Spectroscopy techniques are used universally in science at the intersection of the disciplines of chemistry, biology, engineering and physics.

Wavelength and Energy

Our understanding of the nature of light is a relatively recent development. For a long time the different forms of electromagnetic radiation were thought to be individual phenomena. Thus, we have the collection of common names ending in *-wave* and *–ray* for the various wavelength

ranges. This is because the energy of a photon is inversely related to its wavelength. The shorter the wavelength, the higher the energy of the photon.

Where:

E = Energy of the photon in joules
λ = Wavelength in nanometers
h = Planck's constant
c = Speed of light

$$E = \frac{hc}{\lambda}$$

The enormous wavelength range for known electromagnetic radiation yields photons with energy covering an equally wide range. It is this energy that determines the effect of the photon when it interacts with matter. For example, radio frequency photons have very small energies enabling us to saturate our atmosphere with them without affecting our environment. The amount of energy they impart to whatever absorbs them is almost negligible (you don't get dents in your car from listening to one of the many available radio stations). Infrared photons have enough energy to heat objects and, as a result, they make great heat lamps. Ultraviolet photons have enough energy to break chemical bonds and can cause molecular rearrangements resulting in effects like sunburn and genetic damage. X-rays are very energetic and readily break even the strongest bonds causing significant molecular destruction. For this reason the medical use of X-rays is destructive of living tissues and must be done carefully and only in extremely small doses.

The Interaction of Light with Matter

It is the electrons in atoms and molecules that typically absorb and emit photons of light. It is worth noting that gamma rays are energetic enough to interact with atomic nuclei and generate photons of light, but we'll leave that to the physicists to pursue. When an electron absorbs low energy photons, like radio frequencies, the "spin" of the electron is flipped. This effect is used in nuclear magnetic resonance spectroscopy (NMR) and can also be used to generate the images from magnetic resonance imaging (MRI). When an electron absorbs infrared, visible and ultraviolet photons they change energy level. All electrons have a series of energy levels they can occupy. The lowest energy level is referred to as the "ground state." The highest level is the "ionization energy" or the energy required to completely remove the electron from the influence of the nucleus. In order for an electron to move from one level to a higher level it must absorb energy equal to the difference in the levels. Likewise, to move to a lower level the electron must give up energy equal to the difference. Because there are a

limited number of levels the electron can occupy, there are limited amounts of energy it can absorb or give up.

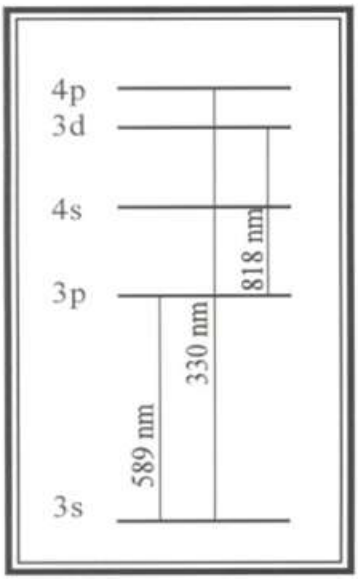


Figure 2: Common Electronic Transitions for Sodium

A more detailed discussion can be found in *Chemistry and Chemical Reactivity,* Chapter 7 by Kotz and Treichel. Their figure 7.13 is a detailed presentation of the *electronic transitions* possible for the simplest atom, the hydrogen atom. Figure 2 is a diagram of the most common transitions possible for a sodium atom. The 3s to 4p transition is in the ultraviolet range. The 3p to 3d transition is in the infrared range. And the 3s to 3p transition is in the orange region of the visible spectrum. This line is the source of the characteristic color of sodium vapor lamps.

There are a number of ways an electron can gain or lose energy. The ones of interest here are the absorption or emission of light. An electron can absorb a photon of light that strikes it only if that photon has the exact energy to change the electron to a higher allowed energy level. An electron already at a higher level can emit a photon of light having exactly enough energy to change that electron to one of its lower allowed levels. Note that an electron in the ground state cannot emit any photons as it already has the lowest possible energy.

The magnitude of the difference in allowed energy levels determines which kinds of light can be used to study particular atoms and molecules. While spectroscopy is conducted in nearly all regions of the electromagnetic spectrum, practical considerations make the infrared, visible and ultraviolet regions the most useful in chemical laboratories.

Infrared spectroscopy is particularly useful for studying the vibration of bonds between carbon, hydrogen, oxygen and nitrogen atoms that predominate in organic compounds. Thus, infrared spectroscopy is a key tool of the organic chemist. Infrared spectra can indicate the presence of particular functional groups in unknown organic compounds by the presence of characteristic features. They can also be used to confirm the identity of compounds by comparison with known spectra. Reference books containing thousands of spectra of known organic compounds are available for this purpose.

Visible light spectroscopy is useful for studying some organic compounds and elements that have electrons in d-orbitals, such as transition metals.

Ultraviolet spectroscopy is useful for studying some organic compounds and most biological samples. All proteins have useful ultraviolet spectra as do nucleic acids. Furthermore, UV spectroscopy can be used to follow biochemical reactions and this tool is commonly found in biochemical laboratories. In clinical laboratories, ultraviolet spectroscopy is often the means for making quantitative determinations on plasma and urine samples.

Types of Spectroscopy

Spectroscopy is the study of the interaction of light with matter. There are two distinct aspects of this interaction that can be used to learn about atoms and molecules. One is the identification of the specific wavelengths of light that interact with the atoms and molecules. The other is the measurement of the amount of light absorbed or emitted at specific wavelengths. Both determinations require separating a light source into its component wavelengths. Thus, a critical component of any spectroscopic measurement is breaking up of light into a spectrum showing the interaction of light with the sample at each wavelength.

Light interacts with matter in many ways. Two of the most common interactions are light that is *absorbed* by the atoms and molecules in the sample and light that is *emitted* after interacting with the atoms and molecules in the sample.

Absorption Spectroscopy

Absorption spectroscopy is the study of light absorbed by molecules. For absorbance measurements, white light is passed through a sample and then through a device (such as a prism) that breaks the light up into its component parts or a spectrum. White light is a mixture of all the wavelengths of visible light. When white light is passed through a sample, under the right conditions, the electrons of the sample absorb some wavelengths of light. This light is absorbed by the electrons so the light coming out of the sample will be missing those wavelengths corresponding to the energy levels of the electrons in the sample. The result is a spectrum with black lines at the wavelengths where the absorbed light would have been if it had not been removed by the sample.

Emission Spectroscopy

Emission spectroscopy is the opposite of absorption spectroscopy. The electrons of the sample are promoted to very high energy levels by any one of a variety of methods (e.g., electric discharge, heat, laser light, etc.). As these electrons return to lower levels they emit light. By collecting this light and passing it through a prism, it is separated into a spectrum. In this case, we will see a dark field with colored lines that correspond to the electron transitions resulting in light emission. In Figure 3, hydrogen absorption and emission spectrum lines are shown with a continuous spectrum for reference (top spectrum). The absorption (middle spectrum) and emission (bottom spectrum) of a substance share the same wavelength values. In the absorption spectrum, wavelengths of absorbance appear as black lines on a colored field. In the emission spectrum, the wavelengths of emission appear as colored lines on a black field.

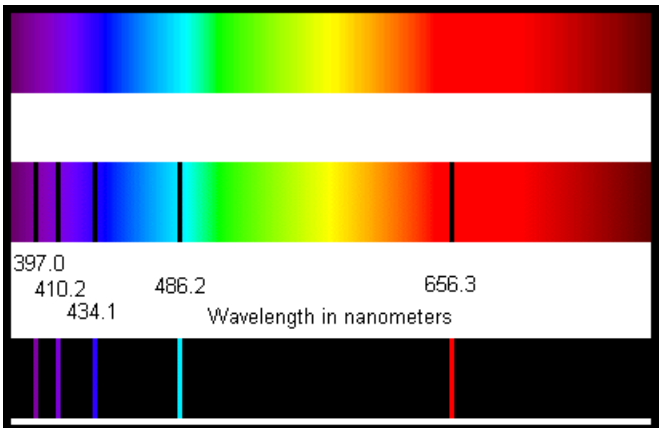


Figure 3: Hydrogen Emission and Absorption
http://www.astro.washington.edu/courses/astro211/Lectures/hydrogen_atom.html

Qualitative Spectroscopy

One of the most useful aspects of spectroscopy derives from the fact that the spectrum of a chemical species is unique to that species. Identical atoms and molecules will always have the same spectra. Different species will have different spectra. For this reason, the spectrum of a species can be thought of as a fingerprint for that species. *Qualitative spectroscopy* is used to identify chemical species by measuring a spectrum and comparing it with spectra for known chemical species to find a match.

As an example, consider the discovery of the element helium. It was first observed, not on the earth, but in the sun! In 1868 the French astronomer, Pierre Jules César Janssen, was in India to observe a solar eclipse when he detected new lines in the solar spectrum. No element known at that time would produce these lines and so he concluded that the sun contained a new

element. This initiated a search for the new element on planet earth. By the end of that century, the new element had been identified in uranium ores and was named helium after the Greek word for the sun (Helios). Today, spectroscopy finds wide application in the identification of chemical species.

Quantitative Spectroscopy

Quantitative spectroscopy is one of the quickest and easiest ways to determine how many atoms or molecules are present in a sample. This is because the interaction of light with matter is a stoichiometric interaction. At any given temperature, the same number of photons will always be absorbed or emitted by the same number of atoms or molecules in a given period of time. This makes spectroscopy one of the few techniques that can provide a direct measure of the number of atoms or molecules present in a sample.

Quantitative emission spectroscopy requires heating samples to very high temperatures to enable electrons to emit light. Most often, this is done by feeding the sample into a burner flame. As a result, it is not practical for use with most molecular compounds but is frequently employed for elemental analysis.

Absorption spectroscopy is performed by passing light of all wavelengths through a sample and measuring how much of the light at each wavelength is absorbed. The statement made above that "the absorption spectrum will appear as black lines on a colored field" is a considerable oversimplification. The interactions of atoms and molecules with water molecules make the absorbance of light in solutions a very complex phenomenon. Nevertheless, the patterns are repeatable and predictable, thus making them useful. By making absorbance measurements at various wavelengths and then plotting the result, one can create what is known as an *absorbance spectrum.*

In Figure 4 the absorbance spectra for different heme-containing proteins is shown. Even though the proteins are closely related, the absorbance spectra are distinct enough to enable discrimination of the different proteins. Absorbance spectra are like fingerprints. Each compound has its own unique spectrum. In some cases the spectrum can be used to identify the

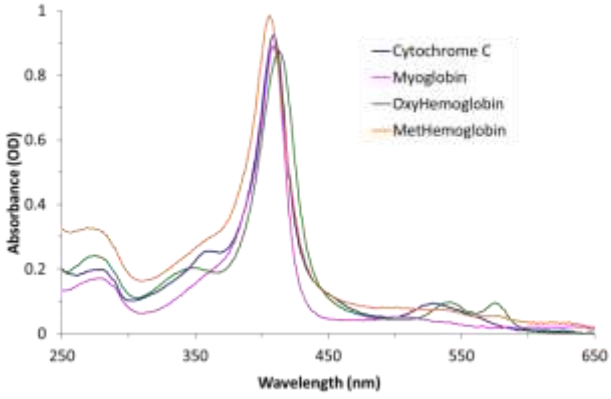


Figure 4: UV-Vis Absorbance Spectra for Heme Proteins

presence of certain compounds in a sample. More often, it is used to determine the amount of a compound present.

Due to the nature of the electronic changes that give rise to absorption spectra, the peaks are generally broad. Therefore, absorption spectroscopy is less useful than other molecular techniques for the purpose of identifying compounds. For example, infrared or Raman spectroscopy is much better for identifying the component species when compared to UV-Visible absorption spectroscopy.

Color and Wavelength

The visible region is a good place to begin a discussion of spectroscopy because color vision is a critical feature of our everyday world. Our perception of *color* is the eye's response to light of different wavelengths. When photons of a narrow wavelength range interact with our retina, we perceive the effect as color. The apparent color of an object is due to the wavelengths of the photons of light reaching our eyes from that object. This is true whether the object is emitting its own light or reflecting light from another source. In a sense, our eyes operate like a *spectrophotometer.*

White light is a mixture of light of all wavelengths (colors). When white light strikes an object and is completely reflected, we see equal amounts of light of all colors and perceive the object to be white. When all light striking an object is absorbed, no light enters our eyes and we perceive the object to be black. A sheet of paper is white because all light striking it is reflected and none is absorbed. The print on the paper is black because all light striking it is absorbed. None is reflected. We perceive color when some wavelengths of light are reflected (or transmitted in the case of a solution) more than others.

There is a rather complex pattern to the absorption of light by colored objects. The statement that "an object appears red because all red light is reflected and all other light is absorbed" is a considerable oversimplification. In fact, varying amounts of light of different wavelengths are absorbed in most colored objects and the color we perceive is more closely related to the color that is most absorbed rather than to the color that is reflected.

The brain assigns color to an object by a process known as *complementary color vision.* According to this theory, all colors of light have a complementary color. This is often displayed through the use of a "color wheel" like the one shown in Figure 5. A color and its complementary color are opposite each other on the color wheel. The perception of color

occurs when the optic nerve and the brain compare the amount of light of a particular color with the amount of its *complementary* color. If the two amounts are the same, we see gray or white. If not, we see color. A fire extinguisher appears red in white light because more blue-green light (the complementary color of red) is being absorbed than any other color. Of course, this also means that more red light is being reflected than its complementary color, blue-green. For all other colors, relatively equal amounts of each color, and its complement, are being reflected.

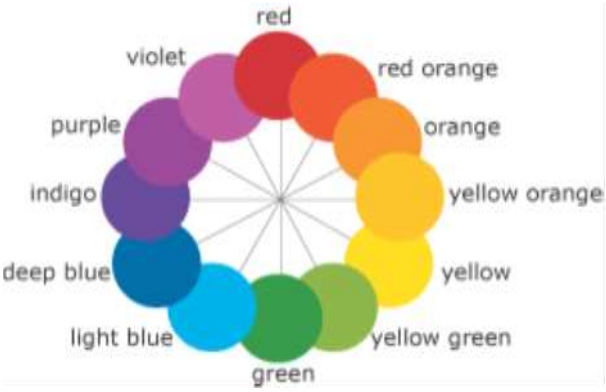


Figure 5: Color Wheel

Spectroscopy Instrumentation

A large variety of instruments are used to perform spectroscopy measurements. They differ greatly in the information they provide. What they all have in common is the ability to break light up into its component wavelengths.

Spectroscopes

A *spectroscope* is the simplest spectroscopic instrument. It functions to take light from any source and disperse it into a spectrum for viewing with the unaided eye. In Figure 6, a diagram of a simple spectroscope is shown. The light from the source passes through the slit and into the prism where it is dispersed into a spectrum. The telescope is used to focus on the light coming out of the prism. The third arm contains a wavelength scale that can be superimposed over the spectrum by shining a white light into it. Spectroscopes are useful for determining what wavelengths of light are present in a light source, but they are not very useful for determining the relative amounts of light at different wavelengths. Spectroscopes are most commonly used for qualitative emission spectroscopy.

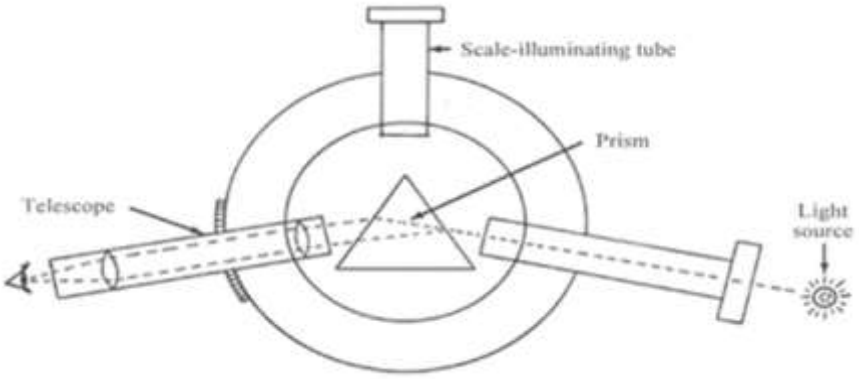


Figure 6: Diagram of a spectroscope

Spectrometers

A *spectrometer* is a spectroscope with a meter or detector so it can measure the *amount* of light (number of photons) at specific wavelengths. It is designed to provide a quantitative measure of the amount of light emitted or absorbed at a particular wavelength. Some spectrometers are constructed so that the wavelength can be varied by the operator and the amount of radiation absorbed or transmitted by the sample determined for each wavelength individually. Others have a fixed light dispersing element (e.g., diffraction grating) that disperses multiple wavelengths of light onto a multi-element detector. Using a spectrometer, it is possible to measure which wavelengths of light are present and in what relative amounts. Spectrometers are common in astronomy where they are used to evaluate light collected by telescopes. They are the only source of information we have about the chemical composition of the universe outside our own solar system.

The diagram of a simple spectrometer is shown in Figure 7. Light enters the spectrometer via the entrance slit and then passes through several parts: an objective lens, a grating, and an exit slit. This combination of parts functions as a *monochromator,* a device that selects only one color (actually, a narrow band of wavelengths) from all of the wavelengths/colors present in the source. A particular wavelength is selected, using the wavelength control, by adjusting the angle of the grating. This works because different wavelengths of light reflect off the grating at different angles. The net result is the separation of white light into a "rainbow," much like the effect of light transmitted through a prism of glass. The selected wavelength is at the center of the narrow band of wavelengths passing through the slit. The light then strikes a detector that generates a voltage in proportion to the intensity of the light hitting it. That voltage is then used to drive a read-out device that is designed to provide data in a format such as intensity.

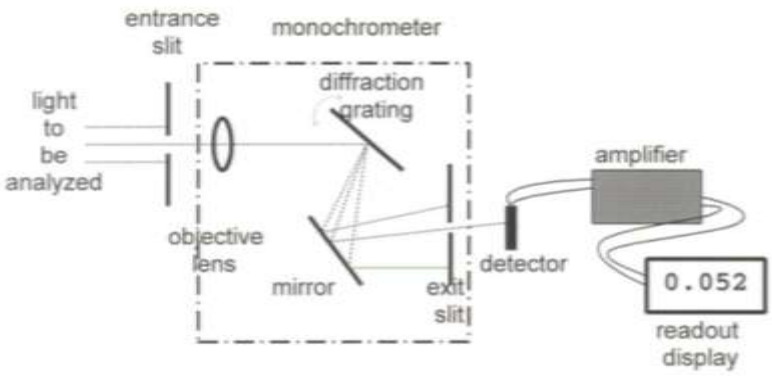


Figure 7: Diagram of a simple spectrometer

As with all electronic devices, the design and operation of spectrometers has been greatly impacted by the developments of the latter half of the 20th century. Perhaps the most crucial was the development in the early 1970s of the *Charged Coupled Device (CCD).* Originally conceived as a new mode of data storage, it was soon discovered that CCDs held great promise as imaging devices. An imaging device is something that electronically mimics what photographic film does. CCDs consist of a number of elements between which charge can be shifted. In an image sensor, light falling on the array of elements produces a pattern of charges corresponding to the image. This image can then be electronically transported to some other location, such as a monitor, and reconstructed. CCDs were first employed to replace photographic plates in telescopes. The first such device was installed on the I-meter telescope at Kitt Peak National Observatory in 1979. Today, CCDs are the detectors that make digital cameras not only possible, but affordable.

Soon after its successful application to astronomical problems, it was determined that CCDs could greatly enhance the performance of spectrometers. This was achieved by replacing both the exit slit and detector with a CCD array. Now, it was no longer necessary to measure light intensity one wavelength at a time. The number of wavelengths that can be monitored simultaneously is determined by the number of elements in the CCD array. Figure 8 is a schematic of a spectrometer outfitted with a CCD array. The array generates an output that can be used to reconstruct the intensity of light striking each of the elements in the array. This output can be sent to a monitor or a printer for display. The output is instantaneous across the spectrum. No longer is it necessary to "scan" back and forth across the spectrum to identify light intensity at individual wavelengths.

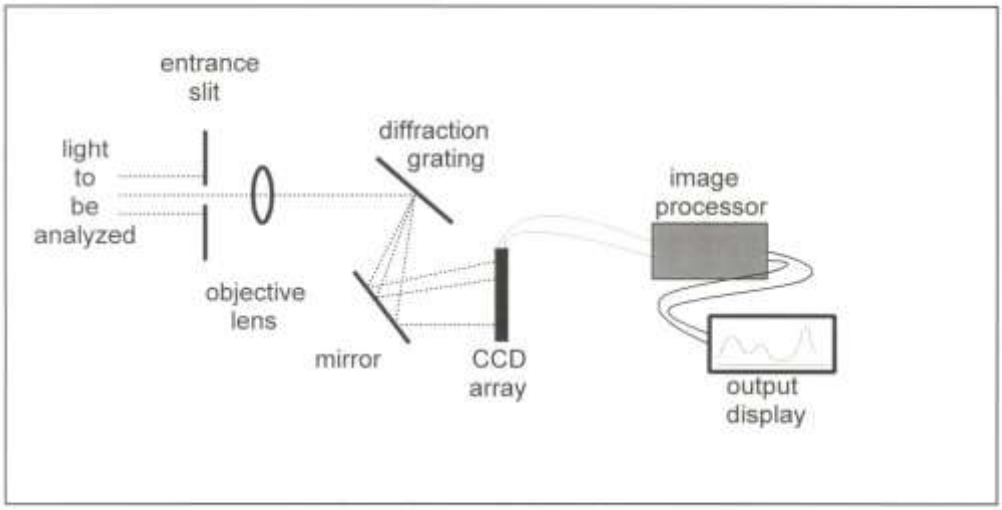


Figure 8: Diagram of a CCD Spectrometer

Spectrophotometers

An instrument that includes a light source is known as a *spectrophotometer.* It is constructed so that the sample to be studied can be irradiated with light. The wavelength of light incident on the sample can be varied and the amount of light absorbed or transmitted by the sample determined at each wavelength. From this information, an absorption spectrum for a species can be obtained and used for both qualitative and quantitative determinations.

Spectrophotometers measure the amount of light transmitted through a sample. Once the transmission for a sample is measured, it can be converted into other values. Percent transmittance (%T) is the ratio of the transmitted light (I) to the incident light *(*I_o) expressed as a percent.

$$T = \left(I \diagup I_o\right) X 100\%$$

The %T calculation is easy to design into a spectrophotometer and was a common output in primitive early spectrometers. Percent transmittance can also be converted to absorbance, A or Abs, which is directly related to the molar concentration of the chemical species in the sample. Absorbance values are calculated from % T values using the following equation.

$$\text{A} = \text{Log}\left(\frac{1}{\%T}\right) = \log\left(\frac{I_o}{I}\right)$$

There is an assumption inherent in the calculation of either %T or absorbance. The assumption is that all light not transmitted to the detector is absorbed by the chemical compounds in the solution. Two other possibilities exist. One is that the light is being scattered by the solution. Light interacting with any particle that is larger than its wavelength can scatter light. Because of this, samples containing solid material or that are cloudy or turbid in nature are difficult to analyze using a spectrophotometer. Samples encountered in the commercial world (biological fluids, soil solutions, etc.) are often cloudy and extra steps must be employed before analysis by absorption spectrophotometry can begin.

Another consideration is that light can be scattered or absorbed by the container used to hold the solution. Care must be taken to ensure that the *sample cells* are clean and free from fingerprints so they do not affect the measurement. The cells must also be constructed of absolutely transparent material free of defect. If measurements are to be made below 350 nm, the cells must be made of quartz or other materials that readily transmit UV light.

The Beer-Lambert Law

The relationship between absorbance and concentration is known as the *Beer-Lambert Law,* or sometimes simply *Beer's Law,*

$$A = \varepsilon \, l \, c$$

Where:

A = Measured absorbance,
c = Concentration of the absorbing species,
l = Pathlength of the sample (width of the cuvette)
ε = Proportionality constant known as the *molar absorptivity* with units of ($M^{-1}cm^{-1}$).

The molar absorptivity (ε) is constant for a specific chemical compound at a specific wavelength. For most compounds, there is typically at least one wavelength where ε reaches a maximum. This wavelength is often chosen to carry out absorption spectrophotometry of that compound. For example, consider the absorbance spectra for hemoglobin shown in Figure 4. There are three wavelengths in the visible range that would be suitable for characterizing the absorbance of hemoglobin: 412, 541 and 576 nm.

If the molar absorptivity is known at a particular wavelength, the concentration of a chemical compound present in a transparent sample can be calculated from the measured absorbance

using Beer's Law. The simplest way to determine ε is to take a solution of known concentration, select the wavelength for which the value of ε is desired (usually the wavelength where the absorbance has its greatest value), measure the absorbance there and measure the pathlength. The above equation can be rearranged to solve for ε (ε = A /lc) and the value computed from the experimental measurements.

It is important to remember that spectrometers are limited in their ability to measure absorbance accurately; therefore, the results for very concentrated samples (with high absorbance values) may not be reliable. For example, an inexpensive spectrometer may produce reliable results only in the absorbance range of 0.01 to 1.5. Absorbance values outside this range are not reliable due to instrument limitations. For the best results, multiple measurements of a number of samples under a variety of conditions are required to provide an accurate answer.

A more accurate method to determine ε is to measure the absorbance of a number of solutions of different concentrations and construct a *calibration plot* or *standard curve.* Beer's Law is a linear equation of the form

$$A = \varepsilon l c \qquad \boxed{\begin{array}{c} A = \varepsilon l c \\ \downarrow \quad \downarrow \downarrow \\ Y = m x \end{array}}$$

$$y = mx + b$$

(b, the y intercept, is zero and therefore does not appear in the Beer's Law equation.)

A plot of absorbance versus concentration should produce a straight line with a slope equal to ε l. A sample standard curve is shown in Figure 9. With a zero intercept, a concentration value of zero should produce a zero absorbance and the origin of the plot (0, 0) should be included as a point on the plot.

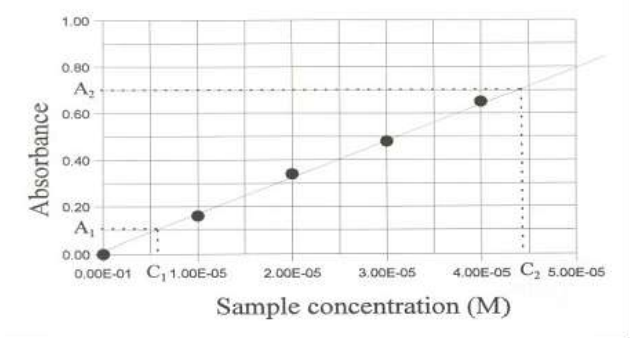


Figure 9: Absorbance Standard Curve

Determining ε requires calculating the slope of the best-fit line through the data points. Consider the data graphed in Figure 9. By selecting two points on the line and reading their coordinates, the slope can be calculated. To avoid biasing the readings, the points selected for this determination should not be the same as any of the data points.

$$\varepsilon l = \text{slope} = \frac{A_2 - A_1}{C_2 - C_1} = \frac{0.70 - 0.10}{4.4 \times 10^{-5}\ \text{M} - 0.60 \times 10^{-5}\ \text{M}} = 1.6 \times 10^{4}\ \text{M}^{-1}$$

As long as the *pathlength* (l) through the sample can be measured, ε can be calculated from the slope. A quick measurement of pathlength can be made with a ruler. A more rigorous method is to measure the absorbance of a *standard solution* having a known concentration and molar absorptivity and then calculate the pathlength from Beer's Law.

Note that the value of l may vary from cuvette to cuvette. It will also vary with the orientation of the cuvette in the sample holder if the cuvette does not have a uniform pathlength in all directions. To maintain optimal accuracy, one should always use the same cuvette or at the very least make sure cuvettes are oriented the same way every time they are placed in the spectrophotometer.

It is possible to read the concentration of an unknown sample directly from a calibration plot by *interpolation* using the measured absorbance of the unknown sample. In the example shown in Figure 10, an absorbance reading of 0.45 produces a concentration of 2.7 x 10^{-5} M.

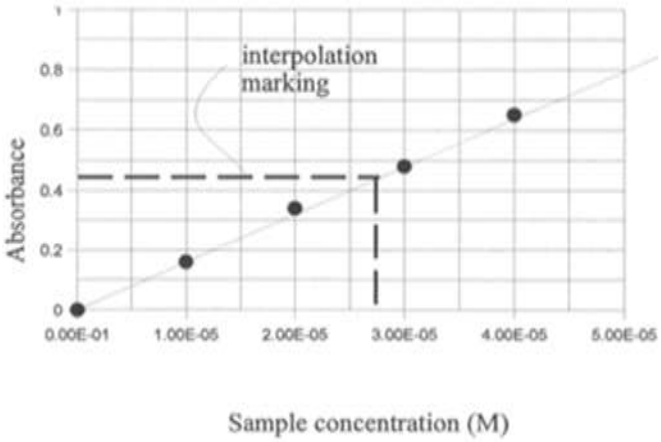
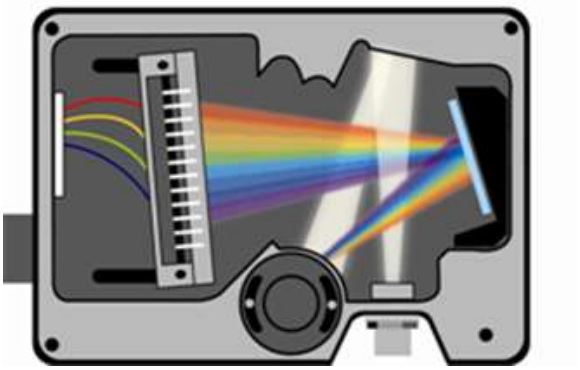


Figure 10: Interpolating Concentrations from a Beer's Law Plot

Instrumentation

Ocean Optics Spectrometers

The Ocean Optics spectrometer is a quick and easy to use instrument for generating spectra in the UV, visible and near-infrared (near-IR) regions of the electromagnetic spectrum. The spectrometer (mirrors, grating, slit and detector) are housed in an optical bench that is small enough to fit into the palm of your hand.



The spectrometer accepts light energy – either transmitted through an optical fiber or in free space -- and disperses it via a fixed grating across the linear CCD detector. The detector is designed to provide an electronic output for its wavelength range. The output from the detector is then fed into the computer to software, processed, and then displayed in the appropriate units. The spectrum you see is the result of multiple detector elements being fed into the computer and processed. This happens fast enough for you to be looking at the spectra generated by the instrument in real time.

Ocean Optics Spectrophotometers

Ocean Optics spectrometers can also be used to make absorbance measurements when used in conjunction with a light source and cell holder as shown in Figure 11. To measure absorbance, the instrument must be calibrated. This is done by first measuring the number of counts at each of the detector elements from the light source as it passes through a reference solution (solvent without the analyte of interest). Next, the background or number of counts when the light source is blocked (no light is entering the spectrometer) is measured. Both of these operations are automatically performed by the software when the appropriate buttons are pushed. Once the instrument has been

Figure 11: Ocean Optics Flame Spectrometer with Vis-NIR Integrated Sampling System

calibrated and a sample is inserted into the holder, the computer calculates the ratio of the counts hitting the detector to the stored reference counts for each of the detector elements and converts these to absorbance values displayed as an absorbance spectrum.