PASCO

Introduction to Spectroscopy in the Teaching Lab Using Ocean Optics Spectrometers

A Guide for use with Ocean Optics' SpectraSuite Software and PASCO Scientific Xplorer GLX

A Collection of Activities as Provided by Ocean Optics Educational Grant Recipients

Monde Qhobosheane, Ph.D.
Ocean Optics. Inc.

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Preface

Students can now study the basic scientific principles on the same world-class equipment used by leading researchers in the university and government labs and more excitingly, NASA. With advances in electro-optics and their continuous impact on the sensing community, 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 marketing of scientific instruments and methods have changed in an equally dramatic way; in the past, cutting-edge instrumentation started with expensive research devices that were 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 settings.

Our knowledge of spectroscopy has been based upon years of experimentation in a wide array of disciplines ranging from art to applied physics. All of the fields have strong roots in education, more specifically teaching and learning the basics of the field. It has been the experience of thousands of science educators that have utilized the Ocean Optics spectrometers with there own specific real world exciting experiments that the students in there classes lives have been enriched and have a greater 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 scientist to study the fundamentals of spectroscopy using modern research/industry instrumentation.

Part A of this manual is a series of activities to teach the basic ideas and use-cases of spectroscopy. Part B is a series of experiments that can be incorporated into traditional curriculum while still exposing students to optical sensing.

I would like to offer special thanks to the educators who contributed to this lab manual as part of the ongoing Ocean Optics grant program. Also my deepest appreciation to Kristin Detwiler for her ground breaking work in this project.

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

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Part A: 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 also known as electromagnetic radiation. Light consists of photons, little packets of energy that have properties of both particles and waves. All waves have a wavelength, the length of one complete cycle of the vibration. For light, the range of possible wavelengths is enormous. Figure 1 gives the range of known wavelengths of electromagnetic radiation and the common names given to radiation of particular wavelengths.

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

The problem faced by chemists, biochemists and microbiologists is to gain an understanding of what happens at the atomic and molecular level without actually being able to "see" atoms and molecules. Even though we can't make images of atoms and molecules, we can use light to learn about the structures of atoms and molecules. This is because atoms and molecules can absorb or emit light. By looking at the light that is absorbed or emitted we can learn about the species involved. The technique for looking at such light is referred to as *spectroscopy*. It is the only tool available to astronomers to collect information about the cosmos. It is the most powerful tool available to scientists to study atoms and molecules, a technique that is universally used in science and engineering disciplines.

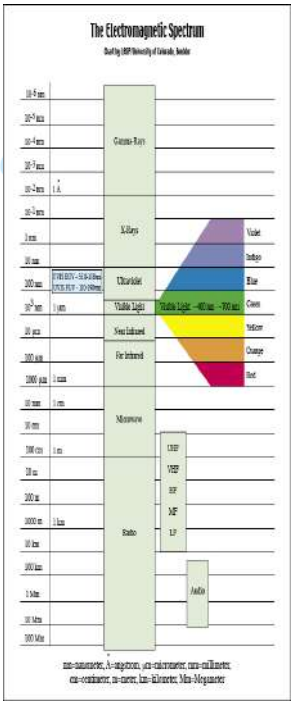

Figure 1. Wavelength Ranges

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Wavelength and Energy

Our understanding of the nature of light is a rather recent development. For a long time the different forms of electromagnetic radiation were thought to be different 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 related (inversely) to its wavelength.

$$E = \frac{hc}{\lambda} \qquad \text{Eqn. 1}$$

Where:

E = energy of the photon in joules

λ = wavelength in nanometers

h = Plank's constant

c = speed of light

The shorter the wavelength, the higher the energy of the photon.

Because the wavelength range of known electromagnetic radiation is enormous, the difference in energy of photons is also enormous. And it is this energy that will determine the effect of the photon when it interacts with matter. The radio frequency photons have very small energies which is why we can saturate our atmosphere with them without affecting our environment. The amount of energy they impart to the atoms that absorb them is almost negligible. 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

To be more correct, it is usually the electrons in atoms and molecules that absorb and emit photons of light (Gamma rays are energetic enough to interact with atomic nuclei, but we'll leave that topic to the physicists to pursue). An electron can absorb low energy photons, like radio frequencies, by flipping its "spin". This effect is used to create nuclear magnetic resonance spectroscopy (NMR) and magnetic resonance imaging (MRI). An electron can absorb infrared, visible and ultra violet photons by changing its 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", 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.

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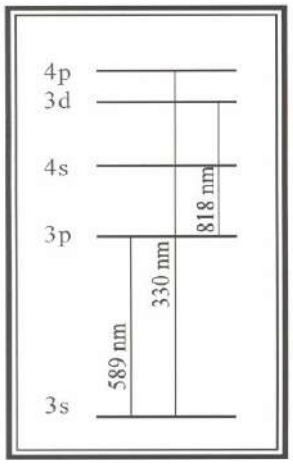

Figure 2

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 one of interest here is 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. Notice that an electron in the ground state cannot emit any photons as it already has the least 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 bonds between carbon, hydrogen, oxygen and nitrogen atoms that predominate in organic compounds. Thus, infrared is a key tool of the organic chemist. Infrared spectra can indicate the presence of particular structures 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 particularly useful for studying certain kinds of organic compounds and elements that have electrons in d-orbitals, such as transition metals. *Ultraviolet spectroscopy* is useful for studying certain kinds of organic compounds that predominate in biological contexts. All proteins have useful ultraviolet spectra as do DNA and many reaction co-factors. Many biochemical reactions can be effectively monitored in the ultraviolet 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 interactions 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 wavelengths of light that interact with atoms and molecules. The other is the measurement of the amount of light being absorbed or emitted at any particular wavelength. Both determinations require separating a light source into its component wavelengths. Thus, a critical component of any spectroscopic measurement is the breaking up of light into a spectrum. For each of these aspects there are two ways observations can be made: the light that is *absorbed* by atoms and molecules, and the light that is *emitted.* This creates a total of four different kinds of spectroscopy: absorption, emission, qualitative and quantitative.

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Absorption Spectroscopy

Absorption spectroscopy is the study of light absorbed by molecules. In it, white light is caused to pass through a sample and then through a device (such as a prism) that breaks the light up into a spectrum. You will recall that white light is a mixture of all wavelengths of visible light. When such light is passed through a sample, under the right conditions, the electrons of the sample will absorb those wavelengths of light that can change them to other levels. Thus, the light coming out of the prism will be missing those wavelengths corresponding to the allowed energy levels of the electrons in the sample. We will see a spectrum with black lines 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. This time, however, we will see only a dark field with colored lines that correspond to the electron transitions. Figure 3 shows the view through the telescope of the 589 nm Helium emission line with a scale superimposed. Notice that the absorption and emission spectra of the same substance will have the same values for wavelength. In the absorption spectrum these values will appear as black lines on a colored field whereas in the emission spectrum they will be colored lines on a black field.

Figure 3

Qualitative Spectroscopy

One of the 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. Thus, the spectrum of a species can be thought of as a fingerprint for that species. *Qualitative spectroscopy* is used to identify chemical species by making a spectrum and comparing it with known spectra 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 1868the French astronomer, Pierre-Jules-Cesar 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 quick 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.

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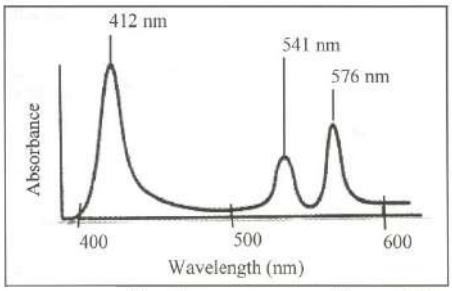
Quantitative emission spectroscopy requires samples be heated very hot to enable electrons to emit light. Most often, this is done by feeding the sample into a burner flame. As a result, it is not practical for use with most molecular compounds. It is frequently employed for elemental analysis. A quantitative emission technique, flame photometry, is employed in clinical labs to determine sodium and potassium levels in blood plasma and urine.

Creating a flame emission.

Figure 4

Because quantitative absorption spectroscopy can be done at room temperature, it is the more common technique. It is usually performed on samples dissolved in solution. In clinical labs, determinations of the amounts of compounds like glucose and cholesterol in blood and urine samples employ this technique.

Absorption spectroscopy is performed by passing light of all wavelengths through a sample and measuring how much of 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 complex interactions of atoms and molecules with water molecules in solution 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 trace.* Figure 5 is an example of such a trace. Absorbance traces are like fingerprints. Each compound has its own unique trace. In some cases this can be used to identify the presence of certain compounds in a sample. More often, it is used to determine the amount of compound present.



Absorbance spectrum of hemoglobin in the visible region.

Figure 5

Color and Wavelength

The visible region is a good place to begin an investigation of spectroscopy because it is a critical feature of our everyday world. This phenomenon is known as color vision. 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. Thus, 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 *spectrophotometers.*

White light is an equal mixture of light of all wavelengths (colors). When such 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, as 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.

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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 6. 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. Thus, 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.

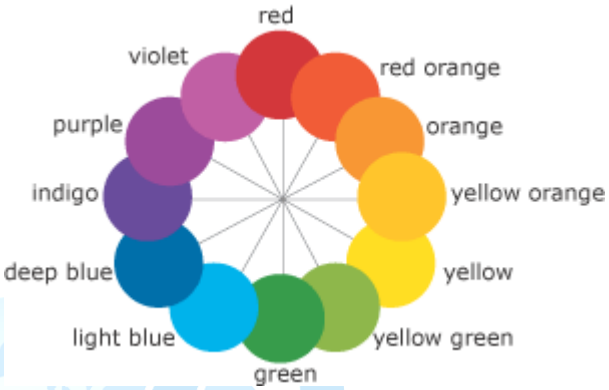

Figure 6. Color Wheel

Instruments that Generate Spectra

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

Spectroscopes

A *spectroscope* is simplest of spectroscopic instruments. Its function is to take light from any source and spread it into a spectrum for viewing with the unaided eye. Figure 7 is a diagram of a simple spectroscope. The light from the source passes through the slit and into the prism where it is spread 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. Figure 3 shows a view through a spectroscope's telescope. 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.

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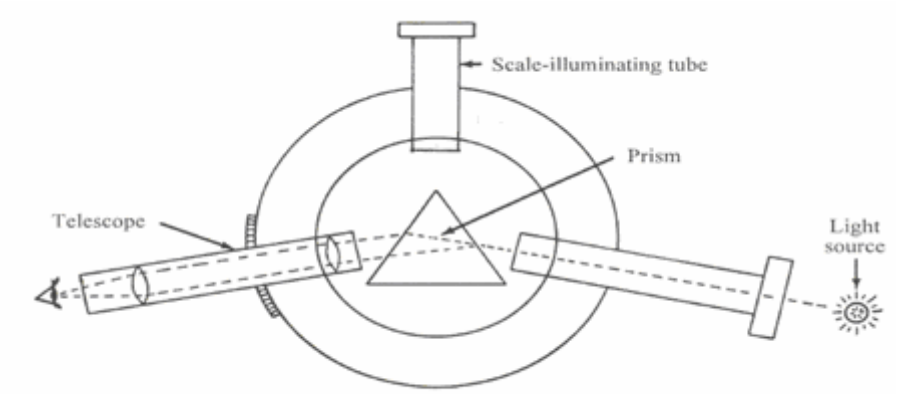

Figure 7. Diagram of a spectroscope.

Spectrometers

A *spectrometer* is a spectroscope that has some sort of meter attached that can measure the *amount* of light (number of photons) at specific wavelengths. Thus, it is designed to provide a numerical measure of the amount of light emitted or absorbed at a particular wavelength. It is 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. In this way it is possible to learn which wavelengths of radiation are present and in what relative amounts. Spectrometers are common in astronomy where they are used to evaluate the 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.

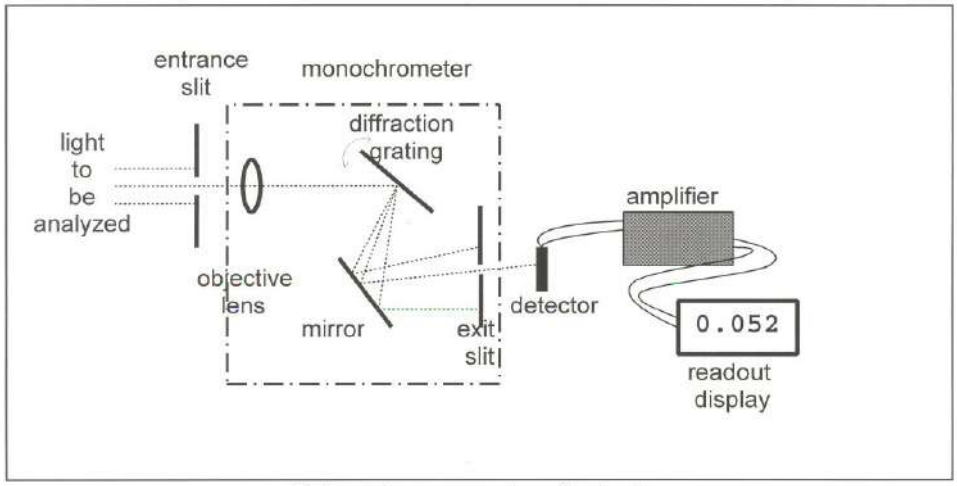

Figure 8 Schematic representation of a simple spectrometer.

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 which 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 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 useful fashion such as intensity.

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As with all electronic devices, the design and operation of spectrometers had been greatly impacted by the developments of the latter half of the 20th century. Perhaps the most crucial was the development in the early 70's 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. Charged Couple Devices 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 9 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.

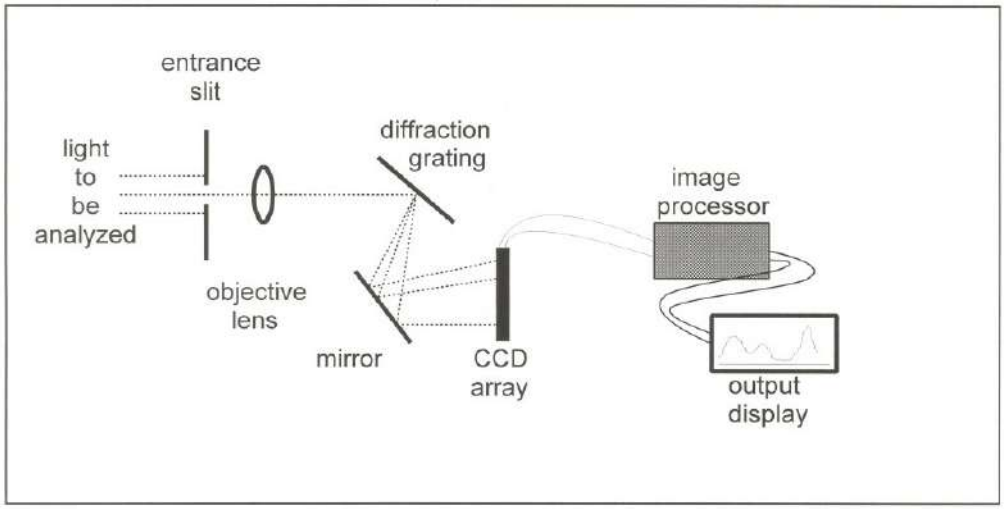

Figure -9 Schematic representation of a CCD spectrometer.

Spectrophotometers

Since spectrometers measure the amount of light entering the instrument, they are most often used for emission spectroscopy. In order to perform absorption spectroscopy, a light source of known intensity is required. An instrument that includes such a light source is known as a *spectrophotometer.* It is constructed so that the sample to be studied can be irradiated with light of known wavelength and intensity. The wavelength can be varied and the amount of radiation absorbed or transmitted by the sample determined for 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 by a sample and then convert this to more useful measures. One is the ratio of the transmitted light (I) to the incident light (I_o), expressed as a percent. This is known as the percent transmittance (% T).

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

Eqn. 2

The %T calculation is easy to design into a spectrophotometer and was a common output before the advent of computer chips. A more useful quantity is the absorbance, A or Abs, because it is directly related to the molar concentration of the chemical species doing the absorbing. Absorbance values can be obtained from % T values using the following expression.

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

Eqn. 3

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. Samples containing solid material, or which are cloudy, 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.

The other assumption is that light might be scattered or absorbed by the container used to hold the solution. Care must be taken to ensure that the *sample cells* do not affect the measurement. The cells must be constructed of absolutely clear glass. If measurements are to be made below 350 nm, they must be made of quartz glass. Regular glasses are opaque below 350 nm.

The Beer-Lambert Law

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

$$\mathrm{A} = \varepsilon \mathrm{bc}$$

Eqn. 4

Where:

A = measured absorbance,

c = concentration of the absorbing species,

b = path length of the sample (width of the cuvette)

ε = a proportionality constant known as the *molar absorptivity* with units of ($M^{-1}cm^{-1}$).

The molar absorptivity is constant for a specific chemical compound and 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 visible spectrum of hemoglobin (Figure 5). There are three wavelengths in the visible range that would be suitable: 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),

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measure the absorbance there and measure the path length. The above equation can be rearranged to solve for ε (ε = A / bc) and the value computed from the experimental measurements. The result, however, may not be reliable. For example, the *Spec 20,* one of the spectrophotometers available for your use, produces reliable results only in the absorbance range of to 0.01 to 1.5. A value outside this range will have questionable meaning. It is also possible for the instrument to malfunction or to be operated improperly. Therefore, multiple measurements of a number of samples under a variety of conditions are required to provide a believable 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.* Beer's law is a linear equation of the form

$$y = mx + b$$

A = εbc
↓ ↓ ↓
Y = m x

(b, the y intercept, is zero and therefore does not appear in the Beer's law equation.) A plot of absorbance vs. concentration should produce a straight line with a slope equal to εb. Figure 10 is a representation of such a plot. Because the intercept is zero, a concentration value of zero should produce a zero absorbance and the origin of the plot (0, 0) should be a point on the plot.

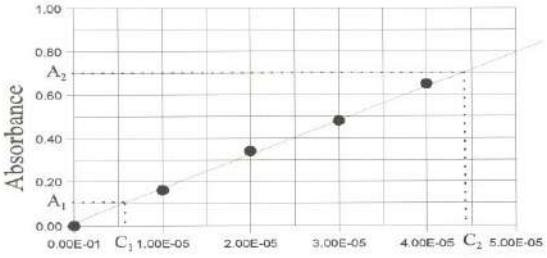

Figure 10 Sample concentration (M)

Determining E requires determining the slope of the best-fit line through the data points. Consider the data graphed in Figure 10. 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.

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

As long as the *path length* through the sample can be measured, ε can be calculated from the slope. One way to measure the path length is 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 path length from Beer's Law.

Note that the value of b 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 diameter in all directions. To maintain optimal accuracy, one should always use the same cuvette and make sure it is oriented the same way every time it is 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 11, an absorbance reading of 0.45 produces a concentration of 2.7×10^{-5} M.

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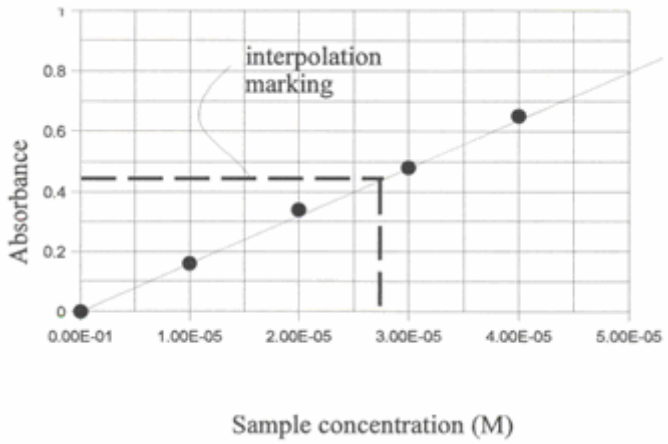

Figure 11. Interpolating Concentrations from a Beer's Law Plot

Instrumentation

Three different instruments are currently available for you to use to make spectroscopic observations and measurements. They are described here.

Figure _12 Spectroscope

Gaertner® Spectroscope

This instrument is little changed from the spectroscopes used by Gustav Kirchhoff and Robert Bunsen (the father of the burner that carries his name) to develop the science of emission spectroscopy during the 1850's. It is pictured in Figure 12 and is schematically represented in Figure 7. It can be used to perform qualitative emission spectroscopy on sufficiently bright light sources. The following is an outline of its operation.

1. Note the narrow slit in the end of the light collection tube. Aim this directly at the light to be studied. Looking through the telescope, and grasping it by its base, move the spectroscope back and forth until the light you see in the spectroscope is brightest. If you see no light, you many need to move the telescope back and forth using the positioning control directly underneath. (It's best to begin with the telescope in the middle of its range.)
2. Turn on the desk lamp and shine it through the scale illuminating tube. Look into the telescope and observe the scale. If it is not exactly horizontal in the field of view, gently twist the end of the scale illuminating tube until it is.
3. Move the telescope lens in or out to bring the position scale into focus. Once the spectroscope is aligned, avoid moving it.
4. Move the telescope back and forth using the knob below it to view the entire spectrum. Look down the telescope and read the positions of the colored lines on the illuminated scale. Record these readings in your notebook. You should be able to interpolate the positions to 0.01 (there are no units). The best strategy is this: instead of trying to determine the position of the center of a line, find the position of the sharp edge. Each line will have a sharply defined edge on the left side and a diffuse edge on the right.

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Ocean Optics USB4000 Spectrometer

This is a quick and easy-to-use instrument for generating UV and visible region spectra from any light source. The spectrophotometer (mirrors, grating, slit, and detector) are housed in an optical bench that's small enough to fit into the palm of your hand.

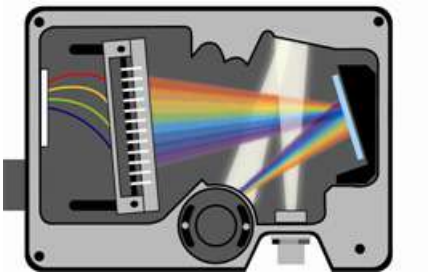

Figure 13. Ocean Optics Spectrometer **Toshiba Linear Detector**

The spectrometer accepts light energy transmitted through an optical fiber or free spaced and disperses it via the fixed grating across the linear CCD detector that is designed to provide output readings at 3648 evenly-spaced locations in the wavelength range of choice. The output from the detector is then fed into the computer via USB to software, processed, and displayed on the monitor as "counts" per millisecond. (One "count" is equivalent to one photon hitting the chip). Thus, the display you see is the result of more than 3500 different outputs 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".

Emission Measurements Using Ocean Optics' SpectraSuite Software

1. Start Spectrasuite by clicking the **Start** button, then selecting **All Programs | Ocean Optics | SpectraSuite |** **SpectraSuite**. Figure 14 is the toolbar that will appear at the top of the screen.

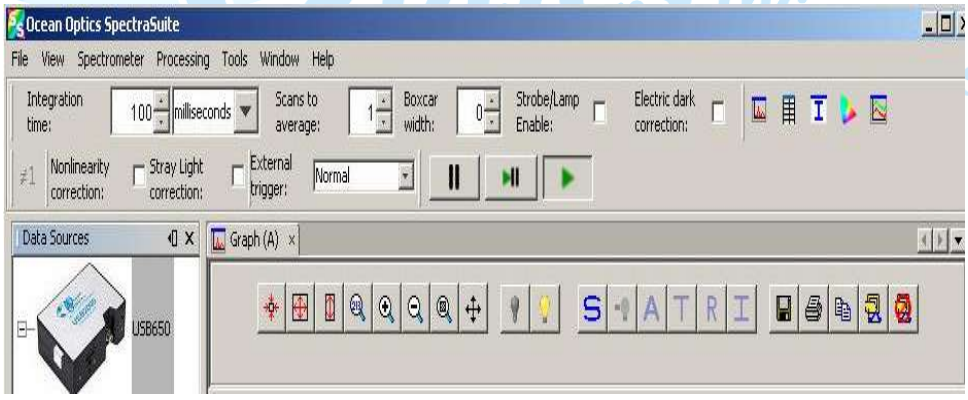

Figure 14. SpectraSuite toolbar

2. Turn on the high voltage source to light the Helium discharge tubes. Adjust the voltage control until the tubes are glowing steadily (if applicable). Do not use maximum output without consulting your instructor.

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CAUTION

The power supply that lights the discharge tube generates a voltage of from 5000 to 7000 volts. Avoid nasty shocks by not touching exposed metal connections.

3. Align the optical cable on the discharge tube and adjust it until the lines in the SpectraSuite window have their maximum height.
4. Inspect the spectrum. The top should be near the top of the window, but not off scale. If it is too big or too small, it will be necessary to adjust the *integration time* to produce the best possible spectrum. The integration time is the time, in milliseconds, that the instrument counts photon for display on the screen. Finding the best integration time is a process of trial and error. Find the integration time control on the left side of the toolbar and adjust it until you are comfortable that the maximum signal is the right size. For emission spectra, it may be necessary to use a large integration time to identify very weak peaks.
5. When you are happy with the spectra click the Save icon ( ) in the SpectraSuite toolbar. Name and save the file. Then click the Overlay Spectral Data icon ( ) from the SpectraSuite toolbar to allow you to view and analyze the captured spectra.
6. Click on the screen to activate the cursor. This will be used to identify wavelengths and signal strengths for each line or peak you are interested in. Click on the screen and the cursor will move to that location. Identify the wavelengths of all the lines that you can identify. Record both the wavelengths and signal intensities in your notebook.
7. Carefully move the helium discharge tube aside and replace it with the hydrogen discharge tube. Adjust the optical cable so that the lines are as bright as possible. Again, use the cursor to read the positions and intensities of the lines. There will be a smear of color in the orange-to-yellow region. Do not record readings for any lines you may observe in this region. These lines arise from a different phenomenon than the other lines and do not fit the mathematical treatment.
8. Repeat Steps 2 through 6 using the hydrogen discharge tube.

Ocean Optics USB4000 as a Spectrophotometer

The USB spectrometer can also make absorbance measurements when used in conjunction with the tungsten light source and cell holder pictured in Figure 16. To measure absorbance, the instrument must be calibrated. This is done by first recording the number of counts at each of the 2048 elements from the pure light source as it passes through a reference solution (water). Next, the number of counts when the light source is blocked is recorded. Both of these operations are automatically performed by the software when the appropriate buttons are pushed. Once the instrument has been calibrated and a sample is inserted in the holder, the computer calculates the ratio of the counts hitting the detector to the stored reference counts for each of the 2048 elements, converts these to absorbance values, and plots the result on the screen. Again, the computer operates at a speed that makes all this appear to happen instantaneously.

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Figure 15

Using SpectraSuite to Make Absorption Measurements

1. Start Spectrasuite by clicking the **Start** button, then selecting **All Programs | Ocean Optics | SpectraSuite |** **SpectraSuite**. Figure 16 is the toolbar that will appear at the top of the screen.

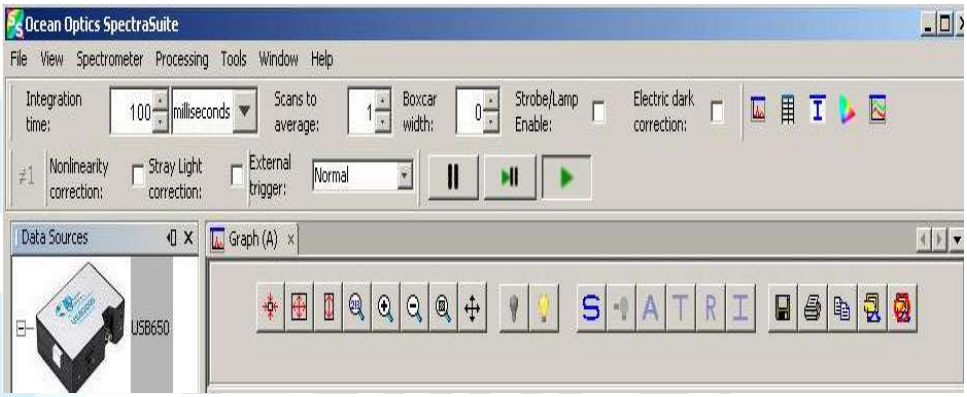

Figure 16. Spectrasuite toolbar

2. Record a spectrum of a cuvette filled with the solvent (water) and save it as the reference spectrum. To do this, select **File | Store | Store Reference Spectrum** or click in the graph toolbar.
3. Completely block off the light source by inserting a piece of dark paper between the light source and your cuvette. Select **File | Store | Store Dark Spectrum** from the menu to save a dark spectrum to a file. You can also click in the graph toolbar. Then, remove the paper you inserted in front of the light source.
4. Click on the Absorbance mode icon ( ). This will put the spectrophotometer into the Absorbance mode. You are now ready to generate absorbance spectra.
5. You can adjust the display parameters using the Scale Graph Height to Fill Window ( ) and Manually Set Numeric Ranges ( ) functions. For this experiment, you will want to use the "set scale" function to reset the wavelength scale to from 400 to 700 nm.

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6. Rinse the cuvette with the solution to be tested and fill it with the same solution. Insert the cuvette in the chamber. Take a snapshot of the spectrum by selecting **File | Screen Capture**, then clicking the **Accept** button in the dialog box that appears. This freezes the spectrum on the screen. Print your data by clicking on the printer icon ( ) on the SpectraSuite toolbar.
7. Repeat Step 6 until all the dyes have been tested.

Spectronic 20® Spectrophotometer

This is the best known and most widely used spectrophotometer ever built. First introduced over 40 years ago, this rugged and reliable instrument can provide accurate absorbance readings in the 325 to 650 nm range. What it does not do is provide is complete spectra: it only provides single readings at single wavelengths.

The Spec 20® employs the same simple spectrometer design as shown in the schematic of Figure 17. The light source is a tungsten lamp and the slit is about 20 nm wide.

Figure 17. Spec 20®

The output of the lamp and the response of the detector differ greatly over the visible spectrum. Unlike the Ocean Optics USB4000 Spectrometer, it does not store reference spectrum and dark current information. Thus it is necessary to re-zero the instrument every time the wavelength is changed. Once the instrument is at a particular wavelength, it is most efficient to measure the absorbance of all solutions before moving on to a new wavelength.

The Spec 20® should work reliably at wavelengths between 400 and 640. They often work as low as 325 nm. For wavelengths below 400 and above 640, if you can get the instrument to zero, you should go ahead and make the measurements.

Using the Spec 20®

The following is an explanation of the instruction outline that appears on most instruments (Figure 18).

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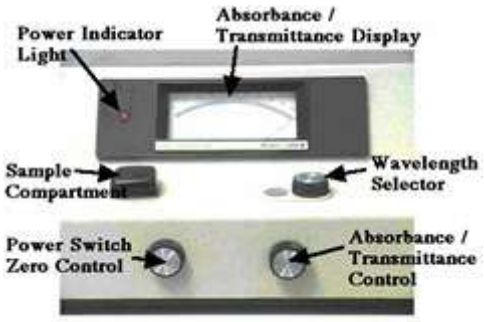

Figure 18

1. Set the wavelength to the desired value using the knob next to the wavelength window.
2. With nothing in the sample compartment, set the infinite absorbance adjustment. This is done by adjusting the left front dial until the meter reads infinite absorbance.
3. The Spec 20 uses round cuvettes; 4" test tubes are the ideal size. Since they are not uniform in diameter, they should always be aligned the same way when inserted in the sample holder.
4. Set the zero absorbance adjustment. Insert the reference cuvette (a clean cuvette containing distilled water) into the sample compartment making sure it is properly aligned. Make sure the sample compartment lid is closed. Adjust the right front dial until the meter reads zero absorbance. Note that this control will be adjusted many full turns over the range of wavelengths used.
5. Replace the reference cell with a sample cuvette. Align the cuvette. Make sure the sample compartment lid is closed. Read and record the absorbance.

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Part B: The Basics

Projecting Spectrum with an Overhead Projector

I Materials

- Overhead projector
- 2 file folders or 8" x 11" cardboard pieces
- 4" x 5" piece of Flinn C-Spectra®
- Colored filters -red, blue, green, and magenta

II Procedure

1. Cut a 4" by 5" square from Flinn's C-Spectra® sheet, remove the protective film, and tape it to the lens of the overhead projector. The grating is ruled vertically parallel to the 5" side. The 4" side must therefore be on top when taping the grating to the overhead projector lens. Prepare a slit on the stage of the overhead projector by placing two pieces of cardboard or two file folders roughly ¼" apart. When the overhead projector is turned on, a continuous spectrum should be projected symmetrically on the screen. Adjust the width of the slit in a manner that gives a sharp spectrum on both sides of the central maximum, which is white.
2. Place a red filter over the slit. Its absorption spectra should be displayed on the screen with a portion of the continuous spectrum below. Note that this red filter absorbs all colors but the red. Note that the blue and green portions of the spectrum appear black. Also note that the red color from the filter is uniform over the entire portion of its spectrum. That is, the orange and most of the yellow colors are now replaced by the same red hue.
3. Repeat the experiment by placing a green filter below the red filter. Leave the red filter on the screen and display a portion of the continuous spectrum for comparison purposes. Note that both the red and blue portions of the spectrum are absorbed. Also note that the green color of the filter is uniform over the entire portion of its spectrum. Areas which were once turquoise and yellow are now green.
4. What happens when you overlap the red and green filters? Overlap portions of both red and green filters. No light is transmitted. One filter absorbs the red and the other absorbs the green.
5. Repeat the experiment by replacing the green filter with a blue filter. Again, all of the colors are absorbed except blue.
6. Replace the blue filter with a magenta filter. Green is absorbed and red and blue are transmitted.
7. Replace the magenta filter with a piece of cobalt glass. Note that the glass does not transmit light in the yellow portion of the spectrum. Since sodium is a common impurity in many samples, cobalt glass is used to remove the yellow sodium flame from a mixture so that potassium or another element can be identified.

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III Notes

- While Arbor Scientific filters were chosen in this presentation, many pieces of colored cellophane or acetate work equally well. The physics teacher may have a collection of Wratten filters or other colored materials. The stage director may have a set of colored filters which you may borrow. Test each filter before demonstrating it to your students to determine which filters are best suited for this demonstration.
- In addition to plastic filters, you can add two or three drops of food coloring to a 50 -ml tissue culture flask or clean PETG bottle. Fill the bottle with water until no air is remains when the bottle is sealed. The food coloring alone is interesting, but you can also prepare mixtures of two different food colors such as red and green. Do you get yellow? No - black. The red absorbs all but red and the green absorbs all but green. The result is that almost no light is transmitted.

IV Discussion

The color of an object is the color it reflects or transmits. In this demonstration, the red filter is red because the entire light incident upon it is absorbed except red. Note the transmission spectrum of this red filter. Likewise, the green filter appears green because it absorbs all the colors except green. The blue filter is blue because it absorbs all the colors but blue. Observe their transmission spectra below.

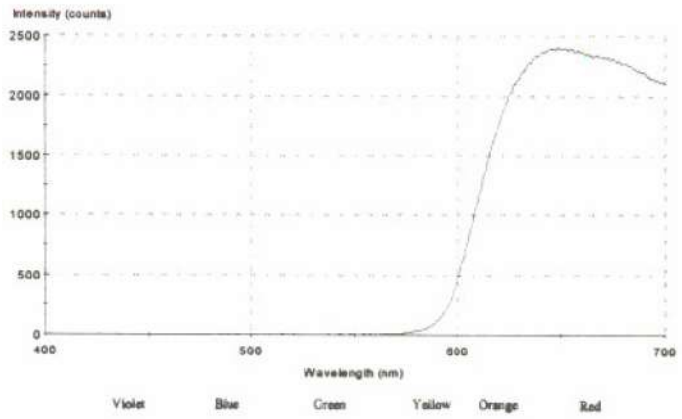

Figure 19. Arbor Scientific Red Filter

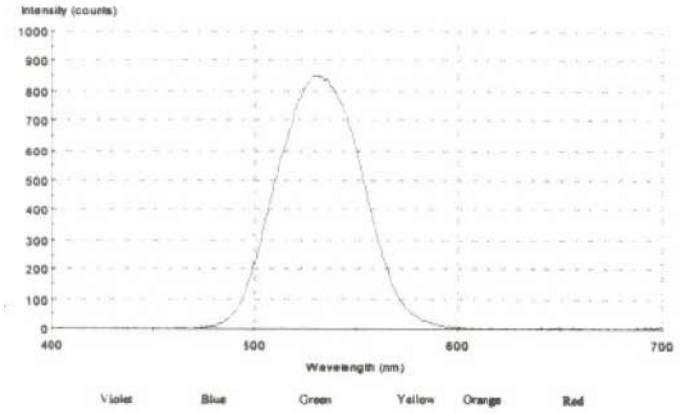

Figure 20. Arbor Scientific Green Filter

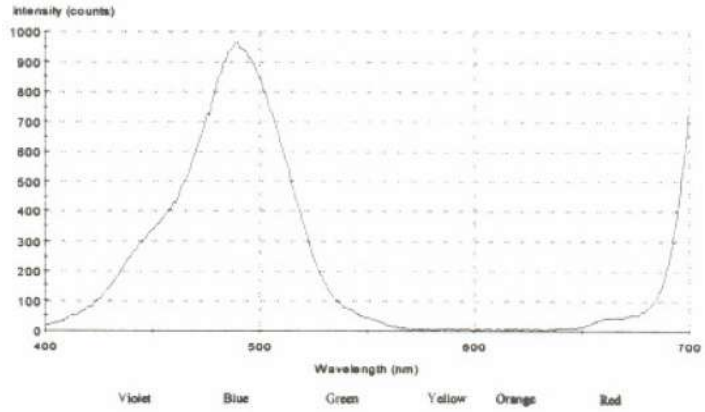

Figure 21. Arbor Scientific Blue Filter

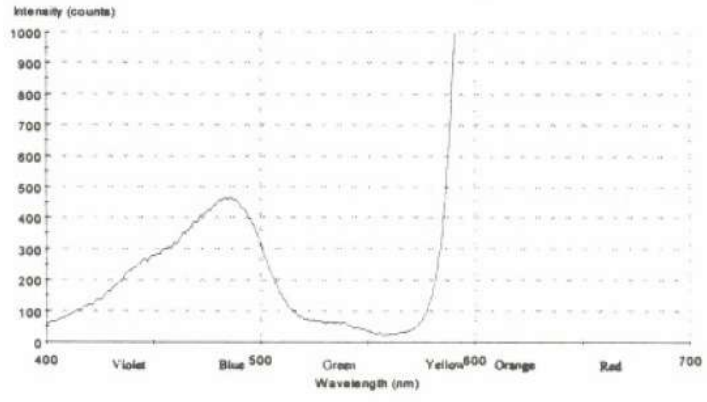

Figure 22. Arbor Scientific Magenta Filter

What about the magenta filter? The magenta filter absorbs only green, allowing both the red and blue light to pass through. The result is that the complementary color to green, magenta, is observed.

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Why were the colors of the red and green filters uniform over the entire portion of their spectrums?

Human beings can see only three colors, red, green, and blue since there are only three different types of cones in the retina. Each cone contains one of three light-sensitive pigments which enable the eye to respond to each of the primary colors -red, green, and blue. We perceive both yellow and orange by averaging red and green together. If the green portion of the spectrum is removed, only the red contribution to yellow is observed. The actual color is different, but it is our perception that the red has the same continuous hue since we do not have the green to detect the subtle variations in color.

Likewise, if red is removed from the spectrum, we see only the green contribution to the yellow part of the spectrum. The same pattern exists in the green-blue part of the spectrum.

What happens to the light absorbed by the filter?

It is converted into heat. The plastic filters curl after several minutes of heating on the overhead projector.

How can you predict the color transmitted or reflected from a filter or piece of paper by its color?

A color rosette or color wheel allows you to predict the color transmitted or reflected in more complicated mixtures of color. The color reflected or transmitted is the complementary color to the color absorbed. For example, in this demonstration, green light is absorbed from the magenta filter so that a magenta-colored light is transmitted. In a cyan (blue-green) filter, the red light is absorbed so that blue-green or cyan light is transmitted.

Figure 23. A Color Rosette

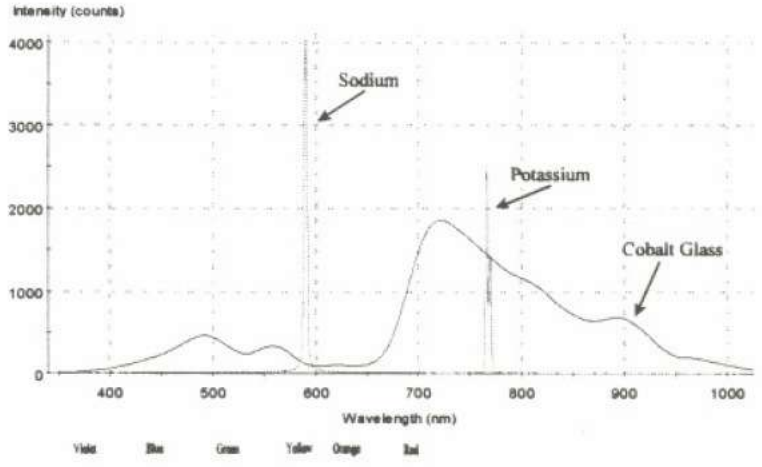

Figure 24. The Emission Spectrum of Pyrex Glass Tubing Superimposed on a Cobalt Glass Spectrum

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What about Cobalt Glass?

Cobalt glass is similar to ordinary plate glass only it contains a small amount of cobalt oxide as a colorant. Cobalt glass is used to study the emission spectrum of potassium in the presence of sodium. Sodium is one of the most common impurities in many substances and its intense light tends to mask the less intense potassium lines in a mixed emission spectrum. Cobalt salts have the ability to absorb visible light in the 600 nm region and transmit light above that value. Since the principal emission of sodium light is at 589 nm and the principal emission of potassium is at 767 nm, the sodium light is absorbed and most of the potassium light passes through the filter unimpeded. See the spectrum in Figure 24.

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Emission Spectroscopy

Observations of chemical phenomena are not always easy to make. One area where such difficulties are encountered is in the study of atomic structure. Because of these difficulties, it wasn't until the 20th century that scientists began to understand the nature of things as small as atoms. One of the reasons that things on the atomic level are so difficult to observe is because we cannot "see" them. They are too small to reflect electromagnetic radiation (light). Thus, we cannot produce an image of such things, even under extreme magnification.

We can, however, make use of light to learn about the structures of atoms and molecules. Under certain conditions, an atom or molecule can absorb or emit light. By looking at the light that is absorbed or emitted we can learn about the species involved. The technique for looking at such light is referred to as *spectroscopy*. This experiment is designed to introduce you to the concept of spectroscopy and to show you the kind of information it provides. Please review the information on spectroscopy at the beginning of this manual.

I Introduction

Atoms consist of a nucleus surrounded by a number of electrons. Although the electrons are normally described as occupying their "ground state" energy levels, they can also occupy higher levels. In order for an electron to move from its current 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.

One of the ways an electron can gain or lose energy is by the *absorption* or *emission* of light. You will recall that the term "light" refers to all electromagnetic radiation. (That part of the spectrum our eyes respond to is referred to as "visible light.") Light is not continuous, but is composed of small units referred to as *photons*. Each photon represents a specific amount of energy. The energy of a photon is related to its wavelength.

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$$E = \frac{hc}{\lambda}$$ Eqn. 6

Where:

E = energy of the photon in joules

λ = is the wavelength in nanometers

h = Plank's constant

c = speed of light

An electron can absorb a photon of light that strikes it only if that photon has exactly enough energy to change the electron to a higher energy level that is allowed for that electron. An electron that has somehow been promoted to a higher level can emit a photon of light having exactly enough energy to change that electron to one of the lower allowed levels. Notice that an electron in the ground state cannot emit any photons as it already has the least possible energy.

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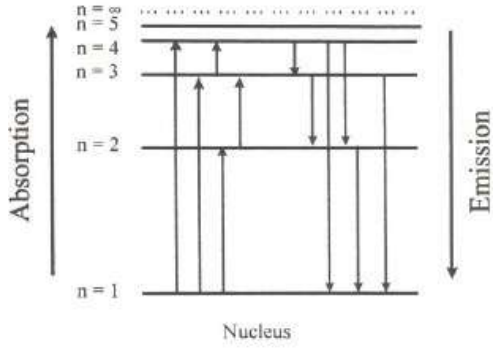

Figure 25

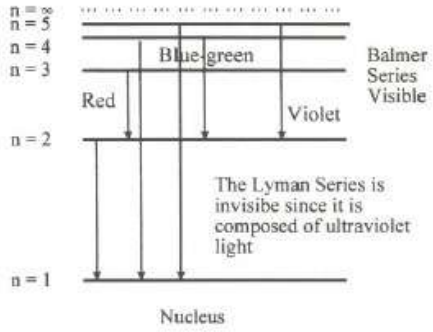

Figure 26

The efforts to explain the relationship between observed atomic spectra and the electronic structure of atoms was a major focus of physical chemists in the latter part of the 19th century. Initial efforts focused on the hydrogen atom due to its simplicity. Johann Balmer and Johannes Rydberg became famous for developing an equation that relates the wavelengths found in the hydrogen emission spectrum with the various quantum levels of the hydrogen atom. In Rydberg's honor, the equation is named after him.

$$\frac{1}{\lambda} = R\left(\frac{1}{n_x^2} - \frac{1}{n_y^2}\right)$$

Eqn. 7

In this equation, λ is the wavelength of an observed line, R is Rydberg's constant ($1.0974 \times 10^7 m^{-1}$).

n_x is the lower quantum level and n_y is the higher quantum level. The series of lines in the hydrogen spectrum that arise when n_x has a value of 2 are known as the Balmer series.

In this experiment you will have the opportunity to observe emission spectra. You will use an Ocean Optics spectrometer interfaced to a computer or the Pasco Xplorer GLX.

You will begin by looking at the emission spectra produced by gas discharge tubes. A high voltage power supply is used to pass high energy electrons through the length of the discharge tube. Inside the tube is a small amount of a pure gas. As the high-energy electrons collide with the atoms of the gas, the electrons in the gas atoms absorb energy and move to much higher energy states. You will observe the photons emitted by the gas atoms as their electrons return toward the ground state.

First, you'll view the emission spectrum of a helium discharge tube. The wavelengths of the emission lines will be determined and compared with literature values. From this comparison, the precision of the spectrometer will be determined. Next, light from a hydrogen tube will be observed and the wavelengths of the observed emission lines are determined. These are then compared with those predicted from the Rydberg equation. Finally, a comparison of the deviations will allow you to determine the accuracy of the theory.

The second part of the experiment involves observing the emission spectra of some simple metal ions. Salts containing these cations will be heated in a flame. The heat will produce hot, gaseous ions. At these elevated temperatures some electrons in the metal ions will be promoted to higher energy states. As the ions rise out of the flame and cool, the electrons in the ions will return to the ground state, emitting photons in the process. You will observe the characteristic colors of these ions and then attempt to identify the composition of an unknown sample based on your observations.

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II Procedure

Procedure using Ocean Optics' SpectraSuite Software

1. Click the **Start** button, then select **All Programs | Ocean Optics | SpectraSuite |** **SpectraSuite**. Figure 27 is the toolbar that will appear at the top of the screen.

Figure 27 : SpectraSuite Toolbar

2. Turn on the high voltage source to light the Helium discharge tubes. Adjust the voltage control until the tubes are glowing steadily (if applicable). Do not use maximum output without consulting your instructor.

   CAUTION: The power supply that light the discharge tube generate a voltage of from 5000 to 7000 volts. Avoid nasty shocks by not touching exposed metal connections.

3. Align the optical cable on the discharge tube and adjust it until the lines in the SpectraSuite window have their maximum height.

4. Inspect the spectrum. The top should be near the top of the window, but not off scale. If it is too big or too small, it will be necessary to adjust the *integration time* to produce the best possible spectrum. The integration time is the time, in milliseconds, that the instrument counts photon for display on the screen. Finding the best integration time is a process of trial and error. Find the integration time control on the left side of the toolbar and adjust it until you are comfortable that the maximum signal is the right size. For emission spectra, it may be necessary to use a large integration time to identify very weak peaks.

5. When you are happy with the spectra click the Save icon ( ) in the SpectraSuite toolbar. Name and save the file. Then click the "open as overlay" icon from the SpectraSuite toolbar allowing you to view and analyze the captured spectra.

6. Click on the screen to activate the cursor. This will be used to identify wavelengths and signal strengths for each line or peak you are interested in. Click on the screen and the cursor will move to that location. Identify the wavelengths of all the lines that you can identify. Record both the wavelengths and signal intensities in your notebook.

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7. Carefully move the helium discharge tube aside and replace it with the hydrogen discharge tube. Adjust the optical cable so that the lines are as bright as possible. Again, use the cursor to read the positions and intensities of the lines. There will be a smear of color in the orange-to-yellow region. Do not record readings for any lines you may observe in this region. These lines arise from a different phenomenon than the other lines and do not fit the mathematical treatment.
8. Repeat steps 2 through 6 using the hydrogen discharge tube.

Procedure using Pasco's Xplorer GLX

Spectra

1. Turn on the Xplorer GLX and plug in the spectrometer. Wait for the spectrometer to initialize.
2. Turn on the high voltage source to light the Helium discharge tubes. Adjust the voltage control until the tubes are glowing steadily (if applicable). Do not use maximum output without consulting your instructor.

   CAUTION: The power supply that light the discharge tube generate a voltage of from 5000 to 7000 volts. Avoid nasty shocks by not touching exposed metal connections.
3. Inspect the spectrum in the set-up window on the Xplorer GLX. The top of the spectrum should be near the top of the window, but not off scale. If it is too big or too small, it will be necessary to adjust the *integration time* to produce the best possible spectrum. The integration time is the time, in milliseconds, that the instrument counts photon for display on the screen. Finding the best integration time is a process of trial and error. Find the integration time control on the left side of the toolbar and adjust it until you are comfortable that the maximum signal is the right size. For emission spectra, it may be necessary to use a large integration time to identify very weak peaks. Push the Check button to highlight the integration time and type in a value, then press the Check button again to accept the change. It should be noted that often in emission spectroscopy, varying the distance between the light source and the fiber optic cable can be used to control saturation.
4. Choose F4, which in this view will close the set-up window. Then push the start/stop button to monitor the spectra of the discharge tube.
5. When you are happy with your spectrum, push the Start/Stop button. This will capture the last view of the spectra.
6. Under the Tools menu, choose the Smart Tool with the Check button. This will be used to identify wavelengths and signal strengths for each line or peak you are interested in. Use the left and right arrow buttons to move the curser to each peak. Identify the wavelengths of all the lines that you can identify. Record both the wavelengths and signal intensities in your notebook.

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Cation Spectra

1. Place the bottoms of five glass Petri dishes on fire resistant ceramic fiber squares or wire gauze with ceramic fiber centers in the center of your workstation. Add roughly 10 grams of potassium chloride to the first, 10 grams of copper (II) chloride to the second, 10 grams of sodium chloride to the third, 10 grams of strontium chloride to the fourth, and 10 grams of lithium chloride to the last Petri dish. Label the Petri dishes so that you know which is which.
2. Add about 15 milliliters of methanol and 5 milliliters of water to each Petri dish, close the alcohol container and remove it from the area. Place four of your Petri dishes away from the center of your workstation while keeping one out for the first step.
3. Ignite the alcohol in the first dish with an "Aim-n-flame" or other long handled igniter. The alcohol will burn with a blue flame until the salts are hot enough to emit their characteristic spectra. Record the color observed visually in your notebook.
4. Increase the integration time to about 200ms in order to capture this spectra.
5. Point the optical cable near the flame above the Petri dish so that it is horizontal and pointing through the flame. The flame will be unsteady, so be prepared to push the Start/Stop button (GLX) or the Save () button (SpectraSuite) when you see large peaks.
6. Record both the wavelengths and signal intensities of your spectrum in a table or on the printed spectra.
7. After the alcohol is consumed or the flames are nearly gone, cover the Petri dishes with their tops to extinguish any remaining flame until the dishes cool enough to be handled. Alternately, water may be poured onto the burning salt/alcohol mixture to extinguish the flames.
8. Repeat steps 4 through 10 until you have obtained the spectra for each of your salts.
9. Acquire spectra of an unknown solution and label the peaks as with your known solutions.

III Data Analysis and Discussion Questions

1. You are to use your helium measurements to determine the accuracy of your spectrometer. Table 1 provides the commonly accepted values for these transitions. You are to compare your experimental helium wavelengths with these literature values in the following manner:

   a. For each line, determine the deviation of your experimental value from the literature one.

   $$\text{deviation} = \left| \text{experimental value} - \text{theoretical value} \right| \qquad \text{Eqn. 8}$$

   b. For each deviation, determine the percent relative deviation.

   $$\%\ \text{relative deviation} = (\text{deviation} / \text{theoretical value}) \times 100\% \qquad \text{Eqn. 9}$$

   c. From the set of percent relative deviations, calculate a standard deviation.

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2. Compare your measured hydrogen emission lines to those predicted by Rydberg and Balmer. First, use Rydberg's equation to calculate the predicted values.

$$\frac{1}{\lambda} = \left(1.0974 \times 10^{7}\ m^{-1}\right)\left(\frac{1}{2^2} - \frac{1}{n_y{}^2}\right) \qquad \text{Eqn. 10}$$

3. Use Equations 3 and 4 to calculate deviations and % relative deviations for these values:

Table 1. Known helium emission lines in the visible range.

Wavelength (nm)	Line Color	Relative Intensity
728.1	red	3
706.5	red	7
667.8	red	10
587.6	yellow	100
501.6	green	10
492.2	green	5
471.3	blue	4
447.1	violet	10
438.8	violet	3
402.6	violet	7

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4. You observed the emissions of a number of known metal cations. You also observed the emissions of a solution containing unknown cations. Based on these observations you are to determine which two ions are in your unknown and construct a defense of your conclusions by citing your observations.
5. Discuss what the standard deviations reveal regarding the accuracy of the technique and of Rydberg's equation.
6. Discuss the apparent accuracy of the flame emission technique and its applicability.

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Absorbance of Light vs. Concentration

I Introduction

When light is passed through a sample of material, the compounds in the sample may absorb some of the light. When this occurs, the intensity of the light beam that exits the sample will be less than the intensity of the light beam that entered the sample.

A *spectrophotometer* is an instrument that measures the intensity of the light entering a sample and the light exiting a sample and compares the two intensities. Information about the two intensities can be expressed as transmittance (the ratio of the intensity of the exiting light to the entering light), % transmittance (transmittance x 100%), or absorbance.

Different materials absorb different wavelengths of light. Therefore, the wavelength of maximum absorption λ_{max} of a material is one of the characteristic properties of that material. In this experiment, the λ_{max} of several food dyes will be determined.

It is also possible to relate absorbance at a given wavelength to the concentration of the absorbing material present in a solution. This relationship is known as *Beer's Law*.

$$A = \varepsilon b c \qquad \text{Eqn. 11}$$

Where:
A = absorbance of the solution
ε = molar absorptivity (L/mol•cm, specific to the chemical species and wavelength of light used)
b = cell path length (cm)
c = concentration of absorbing species (mol/L)

The more concentrated the light-absorbing material in the solution, the more light is absorbed at the A_{max} and the less light is transmitted. This behavior is investigated in this exercise and used to determine the concentration of a dye in a commercial drink.

II Procedure

Procedure using Ocean Optics' SpectraSuite Software

Several samples of commercial food dyes are available for this lab. Work in groups of two to three to test λ_{max} of the food dyes. The Ocean Optics Spectrophotometer will be used to test the absorbance of the dye over a selected wavelength range.

1. Click the **Start** button, then select **All Programs | Ocean Optics | SpectraSuite |** **SpectraSuite**. Figure 28 is the toolbar that will appear at the top of the screen. The functions of the controls will be discussed in the following text.

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Figure 28: SpectraSuite Toolbar

2. Record a spectrum of a cuvette filled with the solvent (water) and save it as the reference spectrum. To do this, select **File | Store | Store Reference Spectrum** or click in the graph toolbar.
3. Completely block off the light source by inserting a piece of dark paper between the light source and your cuvette. Select File | Store | Store Dark Spectrum from the menu to save a dark spectrum to a file. You can also click in the graph toolbar. Then, remove the paper you inserted in front of the light source.
4. Click on the Absorbance mode icon (A). This will put the spectrophotometer into the Absorbance mode. You are now ready to generate absorbance spectra.
5. You can adjust the display parameters using the Scale Graph Height to Fill Window ( ) and Manually Set Numeric Ranges ( ) functions. For this experiment, you will want to use the Manually Set Numeric Ranges function to reset the wavelength scale to from 400 to 700 nm.
6. Rinse the cuvette with the solution to be tested and fill it with the same solution. Insert the cuvette in the chamber and acquire the absorption spectrum. Take a snapshot of the spectrum by selecting **File | Screen Capture**, then clicking the **Accept** button in the dialog box that appears. This freezes the spectrum on the screen. Print your data by clicking on the printer icon ( )on the SpectraSuite toolbar.
7. Repeat step 6 until all the dyes have been tested.
8. Attach the print-out for absorbance vs. wavelength or draw the graph in your notebook. You must record the λ_{max} (the wavelength at which maximum absorbance occurs) for each dye.

Procedure using Pasco's Xplorer GLX

1. Turn on Xplorer GLX and plug in the Ocean Optics spectrometer. Wait for the initialization process to complete.
2. While the set-up window, insert a cuvette filled with a sample of the darkest solution you expect to use in the experiment and adjust the integration time so that the highest peak is not clipped at the top. Do this by pushing the Check button to highlight the integration time and typing in an appropriate value. Then push the Check button to accept the change.
3. Insert a cuvette filled with the solvent (water) and save it as the reference spectrum by clicking on the Save Reference (F2) button.

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4. Use the Arrow button on the Xplorer GLX to highlight the lamp tab. Push the Check button to turn off the light source, then press the Save Dark (F1) button. Now push the Check button to turn the light back on.
5. Hit the Close (F4) button, and then push the Start/Stop button on the Xplorer GLX. Push the Check button to highlight Intensity on the y-axis. Push the Check button while Intensity is highlighted and scroll down to absorbance. Push the Check button again to accept this change. Click on the Absorbance Mode button. You are now ready to generate absorbance spectra.
6. Rinse the cuvette with the solution to be tested and fill it with the same solution. Insert the cuvette in the chamber and acquire the absorption spectrum by pushing the Start/Stop button. This freezes the spectrum on the screen. Record the identity of the sample and the run # from the Xplorer GLX.
7. Repeat step 6 until all the dyes has been tested.
8. Disconnect the Xplorer GLX and connect it to a printer. From the Data Files menu (from the home screen), open each run as a graph and print the spectra by pushing Print in the Graphs submenu (F4).
9. Attach the print-out for absorbance vs. wavelength or draw the graph in your notebook. You must record the λ_{max} (the wavelength at which maximum absorbance occurs) for each dye.

Determine the Relationship of Absorbance to Concentration at λ_{max}

1. Obtain a 20 mL sample of one of the food dyes. (Each student needs to investigate one dye.)
2. Label the undiluted sample Solution A and record its concentration.
3. Make a series of dilutions of the sample using the dye and distilled water. Record the volume used for each dilution. When diluting a sample of solution A to make a new solution, the amount of solute does not change. Therefore, the concentrations of the new solutions can be determined by the following relationship:

$$M_A V_A = M_B V_B \qquad \text{Eqn. 12}$$

   a. Solution B: Pipette 5.00 mL of Solution A into a 10.00mL volumetric flask. Dilute to the mark with water. Mix thoroughly and label as Solution B and its concentration.
   b. Solution C: Pipette 2.00 mL of Solution A into another 10.00mL volumetric flask. Dilute, mix as before and label.
   c. Solution D: Pipette 1.00mL of Solution A into a third 10.00 mL volumetric flask. Dilute, and mix as before and label.

3. Set the wavelength on the spectrophotometer at the λ_{max} for the dye using the curser in SpectraSuite or the Smart Tool in the tools menu of the Xplorer GLX.
4. Determine the Absorbance for solutions D, C, B, and A at λ_{max}. It is advisable to begin with the least concentrated solution.
5. Obtain a sample of one of the commercial drinks that contains the dye you are investigating. Take the absorbance spectrum of the sample and record the absorbance.

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III Data Analysis

1. Use a Microsoft Excel spreadsheet to graph the absorbance versus concentration.
2. By linear regression using Excel or the graphing calculator, determine the equation for the "best fit" line through your data points and record the value of R_2. An R_2 value close to 1.00 indicates that the line fits the data points well.
3. Determine the equation for the best fit line of the graph of absorbance versus concentration:

$$Y = mx + b \qquad \text{Eqn. 13}$$

Where:
y = absorbance
x = concentration
m = slope of the line
b = y intercept

4. Calculate absorbance for the sample of the commercial drink. Report the concentration of the dye in the commercial drink using your Beer's Law standard curve (the best fit line equation) to relate absorbance to concentration.

IV Discussion Questions

1. Discuss the relationship between absorbance and concentration-based using the data from this experiment. This relationship is called Beer's Law.
2. Student Problem

   A soft drink company found that stores in their market area were not buying as many cases of their product as usual. Investigation showed that the stores had purchased the same number of cases but not from the company representative. The company was sure someone was counterfeiting their labels and selling imitations of their products.

   The chemical analysis lab for which you work has been asked to submit a bid for the job of analyzing the suspected merchandise. Your job as a chemist is to work with samples of a commercial soft drink containing two food dyes and to develop a method to:

   - Determine the food dyes in a drink
   - Determine the concentration of each food dye in the drink
   - Separate the food dyes in the drink samples so that further analysis by an organic laboratory can be done on each dye separately

NOTE: A nonpolar column must be used for this problem.

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Part C: Applications

Not All Light Bulbs are the Same

I Introduction

The spectrum of incandescent light more or less mimics sunlight except it is shifted slightly towards the infrared. The high infrared content results in much energy being lost as heat lowering its lighting efficiency. Fluorescent lamps are tailored to contain almost no infrared and therefore, are much more efficient at converting electricity into light, but not all fluorescent lamps are the same.

The 38 W GE represents the newer tri-color fluorescent lamp. These lamps are more efficient than normal fluorescent lamps with few, very intense lines. Our brain averages out the spectrum to give the nearly white light expected. All fluorescent lamps, however, contain the characteristic lines of mercury, the excitation source for all fluorescent lamps.

How are halogen lamps different?

Halogen bulbs are similar to ordinary incandescent bulbs in that their light source is a hot tungsten filament. The difference is the halogen bulb contains the tungsten filament enclosed in a small quartz housing within an outer bulb. The outer bulb helps insulate the hot inner quartz jacket. The filament in a halogen bulb operates at a much higher temperature than in an ordinary incandescent bulb, giving both a bluer, and hence, whiter and a much more intense light.

Halogen bulbs also contain a small amount of either bromine or iodine. The halogen atoms react with tungsten atoms which have sublimed from the filament producing gaseous tungsten-halogen molecules. For this reason, they are known as "scavengers." When these scavenger species encounter the hot filament they disintegrate, depositing the tungsten back on the filament. The halogen molecules also prevent tungsten metal from being deposited on the inside of the bulb as it cools since they are easily vaporized when the lamp is lighted.

In this experiment, the emission spectra of several different types of light bulbs will be taken using an Ocean Optics spectrophotometer and compared.

II Procedure

Procedure using Ocean Optics' SpectraSuite Software

1. Click the **Start** button, then select **All Programs | Ocean Optics | SpectraSuite |**  **SpectraSuite**. Figure 29 is the toolbar that will appear at the top of the screen.

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Figure 29: SpectraSuite Toolbar

2. Locate the blue fiber optic cable coming from the spectrometer. Make sure it is routed in a manner that will prevent it from developing kinks or other potential signal interruptions. Make sure it is pointed it at the light bulb to be evaluated.
3. Inspect the spectrum. The top should be near the top of the monitor, but not off scale. If it is too big or too small, it will be necessary to adjust the *integration time* to produce the best possible spectrum. The integration time is the time, in milliseconds, that the instrument counts photon for display on the screen. Finding the best integration time is a process of trial and error. Find the integration time control on the left side of the toolbar and adjust it until you are comfortable that the maximum signal is the right size. For emission spectra, it may be necessary to use a large integration time to identify very weak peaks.

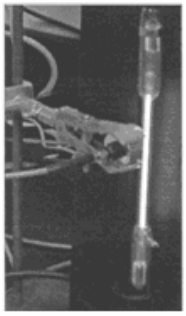

Figure 30 OOI emission optical cable holder

4. When you are satisfied with your spectrum, take a snapshot of it by selecting **File | Screen Capture**. Change the image format to **JPG** and click **Accept**. Then save it by clicking the Save icon ( ).
5. If your computer is connected to a printer, you can print copies of your spectra. Click on the Print icon ( ) on the toolbar.
6. You can also save your data to a file. Click on the Save icon ( ) to save the wavelength values and intensities in a file format that can be opened in most spreadsheet programs (including EXCEL). Note that it saves values for all 3648 elements.
7. Repeat steps 2 through 6 with each of the different light bulbs provided.

Procedure using Pasco's Xplorer GLX

1. Turn on the Xplorer GLX and plug in the spectrometer. Wait for the spectrometer to initialize.
2. Turn on the high voltage source to light the Helium discharge tubes. Adjust the voltage control until the tubes are glowing steadily (if applicable). Do not use maximum output without consulting your instructor.

CAUTION: The power supply that light the discharge tube generate a voltage of from 5000 to 7000 volts. Avoid nasty shocks by not touching exposed metal connections.

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3. Inspect the spectrum in the set-up window on the Xplorer GLX. The top of the spectrum should be near the top of the window, but not off scale. If it is too big or too small, it will be necessary to adjust the *integration time* to produce the best possible spectrum. The integration time is the time, in milliseconds, that the instrument counts photon for display on the screen. Finding the best integration time is a process of trial and error. Push the Check button to highlight the integration time and type in a value, then press the Check button again to accept the change. (Another option in emission experiments is to vary the distance between the light source and the fiber optic cable until the spectrum is satisfactory.)
4. Choose F4, which in this view will close the set-up window. Then push the Start/Stop button to monitor the spectra of the discharge tube.
5. When you are happy with your spectrum, push the Start/Stop button. This will capture the last view of the spectra.
6. From the Home screen on the Xplorer GLX, choose **Table** to see the spectra as a table. Then under F4, choose **Export all data** to save data to a flash memory stick that has been inserted into the USB slot on the side of the Xplorer GLX. This will save the data as a text file to be opened and viewed in Microsoft Excel.
7. Repeat steps 2 through 6 with each of the different light bulbs provided.

III Data Analysis

Compare the spectra of each light bulb and discuss the largest differences between them.

IV Discussion Questions

1. What is the difference between clear and frosted incandescent light bulbs?
2. What are two negative properties about halogen lamps?

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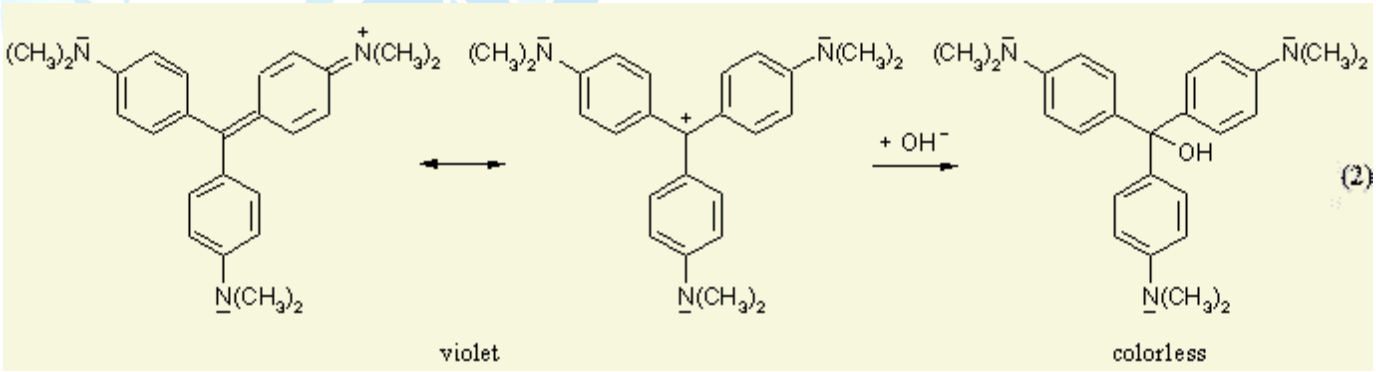
Kinetics of Crystal Violet Bleaching

I Introduction

Chemists are always interested in whether a chemical reaction can occur and exactly how it occurs. The first question is answered though thermodynamics, as you saw in an earlier experiment, while the second is the domain of kinetics. In a kinetics experiment, a chemist attempts to understand the step-by-step transformation of reactants to products. Taken together these *elementary steps* give us the *mechanism* by which the reaction proceeds. Note that a reaction's kinetics are very much tied to the pathway the reactants take to the products (i.e., the mechanism), which is very different from the reaction's thermodynamic properties (i.e., DH, DS and DG) that do not depend on the path. While the thermodynamics and kinetics of a reaction may at times seem complementary, and at other times seem contradictory, it is always important to have a detailed understanding of both.

In this experiment you will determine the *rate law* for a chemical reaction. The rate law is a mathematical expression that relates the amount of time it takes a reaction to happen to the concentrations of the starting materials. The disappearance of reactant over time depends on the *rate constant*, and the concentration of each reactant raised to some power. This power is known as the *order of reaction* with respect that reactant. The sum of the individual orders is the *overall order of the reaction*. The order of reaction with respect to each reactant, as well as the rate law itself, cannot be determined from the balanced chemical equation; it must be found experimentally. The rate law is the basic equation of kinetics and it will be the standard against which we judge possible mechanisms.



Scheme 1. Reaction of crystal violet with OH^-.

In this experiment, you will determine the rate law for the reaction of a dye, crystal violet (CV) with OH^- in aqueous solution according to the balanced net ionic equation given in Scheme 1. We will define the rate of reaction as the disappearance of the colored CV over time, which can be expressed in differential form as $d[CV]/dt$. So, the rate law for this reaction can be written as shown in Eqn. 14 in terms of the concentration of CV and OH^- and the rate constant for the reaction, k. In writing this equation we assume that both CV and OH^- are involved in the reaction (that is x and y are both not zero and are likely integers), but only the experiment will tell us whether these assumptions are valid.

$$rate = -\frac{d[CV]}{dt} = k[CV]^x[OH^-]^y \qquad \text{Eqn. 14}$$

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The point of any kinetics experiment is to determine the order with respect to each reactant (i.e., find x and y) and to find the value of k. This is a problem if we have more than one reactant, as is the case here. When there is more than one reactant, the *isolation method* is often used, which entails making the concentration of all but one of the reactants very high (so their concentrations do not change appreciably over the course of the reaction). The order with respect to the isolated reactant is then determined. The process is then repeated, isolating each of the other reactants in turn, until all of the orders have been determined.

In this experiment we will make the [OH^-] very large and, therefore, essentially constant. We can then simplify Eqn. 14 to Eqn. 15, where we have defined a new rate constant, k_{obs}, which is the observed rate constant at some specific [OH^-]. The relationship between k_{obs} and the intrinsic rate constant, k, for this reaction is given by Eqn. 16.

$$rate = k_{obs}[CV]^x \qquad \text{Eqn. 15}$$

$$k_{obs} = k[OH^-]^y \qquad \text{Eqn. 16}$$

Under conditions of high, constant [OH^-], the order with respect to CV can be determined by graphically applying the integrated rate laws. Since the absorbance of a CV solution is directly proportional to the concentration of CV, according to Beers' Law, the actual [CV] can be replaced by A_{max}, the solution's maximum absorbance (somewhere around 600 nm). A graph of A_{max} as a function of time will give a straight line if the reaction is zero-order in CV ($x = 0$). If the reaction is first-order in CV ($x = 1$), then a graph of $\ln(A_{max})$ as a function of time is linear. And finally, if a graph of $1/Amax$ as a function of time is linear, it indicates that the reaction is second-order with respect to CV ($x = 2$). In each case, if a particular relationship is linear, then the slope of that graph can be used to determine k_{obs}. Note that only one of these three graphs will be linear!

In some instances it is not possible to isolate one of the reactants, because the concentration of that reactant must remain high for the system to behave predictably. In this reaction the [OH^-] must remain high, but the order of the reaction with respect to OH^- and k, can still be found. First we need to change Eqn. 16 into an easily-graphed form by taking the natural logarithm of both sides to give Eqn. 17. To determine the order with respect to OH^- and k, we first perform the kinetics experiment at different, albeit still high, OH^- concentrations and then graph $ln(k_{obs})$ for these reactions as a function of $ln[OH^-]$. The slope of this graph is y, the order with respect to OH^-, and the intercept is $ln(k)$.

$$\ln(k_{obs}) = y\ln[OH^-] + \ln(k) \qquad \text{Eqn. 17}$$

Procedure using Ocean Optics' SpectraSuite Software

1. Click the **Start** button, then select **All Programs | Ocean Optics | SpectraSuite |**  **SpectraSuite**. Figure 31 is the toolbar that will appear at the top of the screen.

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Figure 31: SpectraSuite toolbar

2. Record a spectrum of a cuvette filled with the solvent (Sodium Hydroxide) and save it as the reference spectrum by increasing the integration time to near saturation and then clicking on the Store Reference ( ) icon.
3. Completely block off the light source by inserting a piece of dark paper between the light source and your cuvette. Click on the Store Dark ( ) icon. Remove the paper you inserted in front of the light source.
4. Click the Absorbance mode icon ( ). This will put the spectrophotometer into the Absorbance mode.
5. From the menu, select **File | New | Strip Chart**. In the dialog box, check the box **Stop after this amount of time** and select **10 minutes**. On the right side of the dialog box under **Range Selection** select **One Wavelength** and choose **565** nm (NOTE: do not click Accept).
6. Obtain about 10ml of crystal violet solution and about 10ml of sodium hydroxide solution.
7. Mix the two solutions into one of the graduated cylinders and swirl. Pour some of the mixed solution into a cuvette until 2/3 full. Place cuvette into the cuvette holder of the spectrometer and click the Accept button in the SpectraSuite dialog box. A graph of absorbance vs. time will be generated.
8. At the conclusion of the time run click the Save icon ( ) on top of the graph. Highlight the trend, enter a filename for it, then click the **Save** button.
9. Open the file in Excel, create a column for the ln(absorbance) and plot a graph of ln(abs) vs. time. If this graph is linear, the reaction is first order as described in your text.

Procedure using Pasco's Xplorer GLX

1. Connect the spectrometer to the Xplorer GLX and wait for the initialization process.
2. Insert a cuvette filled with the solvent-background (sodium hydroxide) and adjust the integration time such that the peak near 565nm is near the top of the graph.
3. Click on Save Reference.
4. Using the cursor keys, tab over to the lamp tab and choose turn off lamp and push the **Save Dark** key. Then turn lamp back on.
5. Tab over again to the Time Acquisition tab and change from Scope mode to Time Acquisition mode.

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6. Move the cursor down and select the wavelength using the Check button. Type in 565nm for the wavelength to monitor.
7. Enter **3** for the Bandwidth. Then click the Close button which will open the full graph screen on the Xplorer GLX. Use the Check button to highlight the y-axis and hit the check button again and select absorbance.
8. Obtain about 10 ml of both crystal violet and sodium hydroxide solutions.
9. Mix the two solutions into a single graduated cylinder and swirl. Pour the mixed solution into a cuvette until the cuvette is about 2/3 full.
10. Place the cuvette into the spectrometer and wait about 2 minutes. Then push the start-stop button on the Xplorer GLX to gather data. Run the experiment for about 10 minutes.
11. The data can be saved to a flash drive by choosing the table view from the home screen and then pushing F4 and selecting “export all data.” The data can be analyzed directly on the Xplorer GLX by choosing the Calculator from the home screen and creating a formula for lnABS. This transformed data can then be graphed vs. time by choosing the graph view from the home screen. Clicking the check button will highlight the y-axis. Clicking the check button again will allow you to choose lnABS as the new y variable which will give you a graph of ln ABS vs. time.

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Kinetics of Methylene Blue Reduction by Ascorbic Acid

I Introduction

In the first part of this laboratory experiment, you explore methods for measurement of kinetic rate laws and determine the order of a simple chemical reaction. The reaction is that of a dye molecule, methylene blue with ascorbic acid in the presence of hydrochloric acid (a catalyst to this reaction). You will determine the dependence of the rate on each of these reagents.

The Reaction

A schematic representation of the chemical reaction is shown in Figure 32.

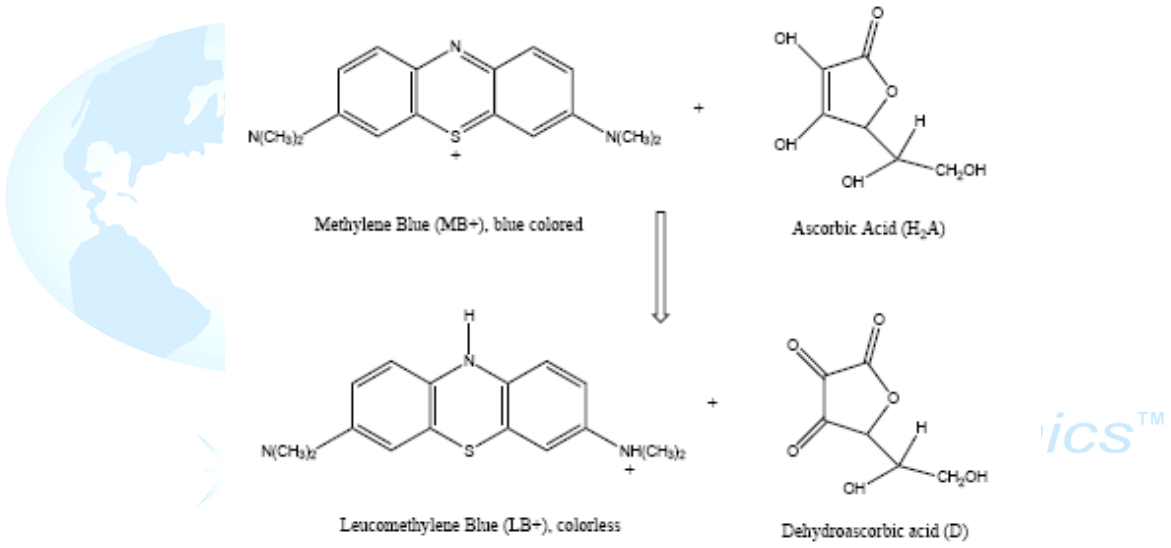

Figure 32. The reaction of methylene blue with ascorbic acid

The overall reaction involves transfer of two electrons and two protons (H^+) between the reagents. The MB^+ dye is *reduced* (electrons added), and the ascorbic acid (H_2A) is *oxidized* (electrons removed). The stoichiometry of the overall reaction is 1:1, and using shorthand is written:

$$MB^+ + H_2A \rightarrow HMB^{2+} + D \qquad \text{Eqn. 18}$$

Numerous mechanisms have been proposed with the primary difference between the mechanisms being the timing of the electron and proton transfers. In some mechanisms, the electrons are transferred before the protons, and in others, the timing is reversed.

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Reaction Rates

We will also be determining whether this reaction is first or second order. Remember that the rate of a chemical reaction is usually dependent on the concentrations of the reactants. The *rate law* is an equation of the rate of reaction as a function of the reagents. For the reaction in this experiment, the general rate law would be:

$$\text{Rate} = k[MB^+]^x\,[H_2A]^y \qquad \text{Eqn. 19}$$

Where:
k is the *rate constant* of the reaction.

If the rate is only dependent on a single reagent and the power that that reagent is raised to is one, then the reaction is considered a *first-order* reaction. If the rate is dependent on two reagents, both raised to a power of one, or on a single reactant raised to a power of two, then the reaction is considered a *second-order* reaction.

The simplest means of differentiating between a first-order and a second-order reaction is to create plots for analyzing the relationship between concentration and time. A first-order reaction has a linear plot when the natural log of the concentration X is plotted versus time, as in Figure 33. In this case, the slope of the line is equal to –k. A second-order reaction has a linear plot when the reciprocal of the concentration X is plotted against time, as in Figure 34. Here the slope of the line is equal to k.

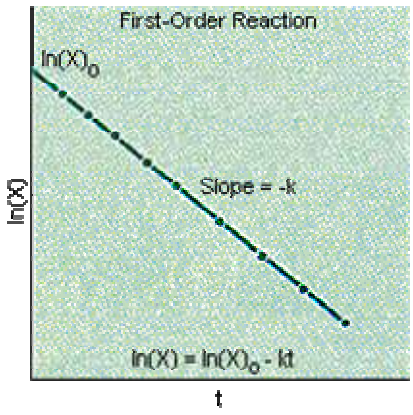

Figure 33. First-Order reaction plot.

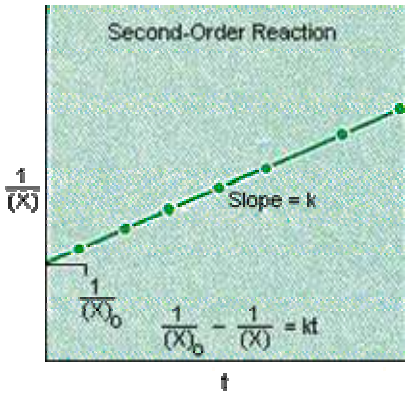

Figure 34. Second-order reaction plot.

Both first and second order reactions can be described by a half-life, which is the time for the reagent to decrease by a factor of 2. For a first-order reaction, the half-life, $t_{1/2}$, is given by

$$t_{1/2} = 0.693 / k \qquad \text{Eqn. 20}$$

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For a second-order reaction, the half-life is given by the equation

$$t_{1/2} = 1 / k[A]_o \qquad \text{Eqn. 21}$$

Where:

$[A]_o$ = the initial concentration of the reagent.

Thus, only for a first-order reaction is the lifetime independent of the starting concentration of the reagent.

II Procedure

Procedure using Ocean Optics' SpectraSuite Software

You will record absorbance versus time plots using the Ocean Optics, Inc. spectrophotometers.

1. You will prepare stock solutions in our laboratory and then move to one of the spectrometers to take the data. The stock solutions have the following concentrations:

Solution	Stock Concentration
Methylene Blue (MB^+)	4.0×10^{-4} mol L^{-1}
Ascorbic Acid (H_2A)	0.10 mol L^{-1}
Hydrochloric Acid (HCl)	1.0 mol L^{-1}

2. Wash your glassware in 0.1 M HCl with a 10-minute soaking period to remove contaminants from the glass. Prepare these stock solutions using deionized water very carefully in volumetric flasks.
3. You will need 10 mL of the ascorbic acid and HCl solutions, and 5 mL of the MB stock. After preparing these stocks, calculate the amounts of each reagent required to make 2.0 mL solutions under the following 15 conditions. Check your answers with your instructor.

Condition #	HCl / (mol L^{-1})	H_2A / (mol L^{-1})	MB^+ / (mol L^{-1})	Total Reaction time / sec
Set A: Varying MB^+ with others constant				
A1	0.3	0.025	3.50×10^{-5}	120
A2	0.3	0.025	2.50×10^{-5}	120
A3	0.3	0.025	1.50×10^{-5}	120
A4	0.3	0.025	9.00×10^{-6}	120
A5	0.3	0.025	6.00×10^{-6}	120
Set B: Varying Asc concentration				
B1	0.2	0.000	1.50×10^{-5}	600
B2	0.2	0.004	1.50×10^{-5}	600
B3	0.2	0.008	1.50×10^{-5}	360
B4	0.2	0.016	1.50×10^{-5}	180
B5	0.2	0.036	1.50×10^{-5}	120

4. Your first experiments will be run at room temperature. Be sure to record the room's temperature and keep this thermometer (labeled) in our hood.

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5. For each run, you will use the SpectraSuite program to record the absorbance of the MB+ versus time. The maximum absorption of MB+ is at 665 nm (in the red). Click the Start button, then select **All Programs | Ocean Optics | SpectraSuite |** **SpectraSuite**. Figure 35 is the toolbar that will appear at the top of the screen.

Figure 35: SpectraSuite toolbar

6. Record a spectrum of a ***blank*** cuvette filled with all the same compounds as the solution to be measured except the compound of interest. The blank should contain 0.3 mol L^{-1} HCl, 0.025 mol L^{-1} H_2A, and the dilution water (but no MB^+). Save the spectrum as the reference spectrum by clicking on the Store Reference icon ( ).
7. Completely block off the light source either by inserting a piece of dark paper between the light source and your cuvette. Click on the Store Dark icon ( ). Remove the paper you inserted in front of the light source.
8. Click the Absorbance mode icon ( ). This will put the spectrophotometer into the Absorbance mode.
9. From the menu, select **File | New | Strip Chart**. In the dialog box, check the box **Stop after this amount of time** and select **2 minutes**. On the right side of the dialog box under **Range Selection** select **One Wavelength** and choose **665** nm (NOTE: do not click Accept).
10. Wrap with parafilm and invert to mix then put into the cuvette holder. Place the cuvette in the spectrometer and click the **Accept** button in the SpectraSuite dialog box. Wait 2 minutes for the data to be recorded. At the conclusion of the time run click the Save icon ( ) on top of the graph. Highlight the trend, enter a filename for it, then click the **Save** button. You will be able to pull up your data in Microsoft Excel for analysis.
11. Repeat step 10 for each of the conditions in Set A and in Set B. Remember to change the filename that the data will be saved to for each run. Also remember to adjust the total duration of the acquisition for each condition in set B.

Procedure using Pasco's Xplorer GLX

1. Do steps 1-4 above. Connect the Spectrometer to the Xplorer GLX and wait for the initialization process.
2. Fill a cuvette with all the same compounds as the solution to be measured except the compound of interest in order to have a reference spectra. For part A, the blank will contain 0.3 mol L^{-1} HCl, 0.025 mol L^{-1} H_2A, and the dilution water (but no MB^+). Click on Save Reference.

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3. Using the cursor keys, tab over to the lamp tab and choose turn off lamp. Then push the Save Dark key. Then turn lamp back on.
4. Tab over again to the Time Acquisition tab and change from Scope mode to Time Acquisition mode.
5. Move the cursor down and type in 665nm as the wavelength to monitor.
6. Enter **3** for the Bandwidth. Then click the close button which will open the full graph screen on the Xplorer GLX. Use the check button to highlight the y-axis and hit the check button again and select **Absorbance**.
7. Wrap with parafilm and invert to mix then put into the cuvette holder. Place the cuvette in the spectrometer and click the Start/Stop button on the Xplorer GLX. Wait 2 minutes for the data to be recorded.
8. The data can be saved to a flash drive by choosing the table view from the home screen and then pushing F4 and selecting Export All Data.
9. Repeat Step 7 for each of the conditions in Set A and in Set B. Remember to change the filename that the data will be saved to for each run. Also remember to adjust the total duration of the acquisition for each condition in set B.

III Data Analysis

1. Create four plots in order to determine the extent to which the reaction rate is dependent on each reagent. You should have two plots for MB^+ and two for H_2A: one of the natural log of the concentration versus time and one of the reciprocal of the concentration versus time.
2. After you determine the order of the reaction, calculate the rate constant, k, from the appropriate plot.
3. Determine half-life of each reagent, using whichever equation is appropriate for the order you determine for each.

IV Discussion Questions

1. Discuss the differences between a first-order and a second-order reaction, including differences between the half-life for each.
2. Describe how you determined the rate order for each reagent. What is the rate order for the overall reaction?
3. Why can a rate law not be determined from the reaction stoichiometry?

V References

Mowry, S, and P. J. Ogren, "Kinetics of Methylene Blue Reduction by Ascorbic Acid" J Chem. Ed., 76 (7), 970-974 (1999).

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Spectrophotometric Determination of an Equilibrium Constant

I Introduction

In this experiment, you will study the reaction between aqueous iron (III) nitrate, $Fe(NO_3)_3$, and potassium thiocyanate, KSCN. They react to produce the blood-red complex $[Fe(SCN)]^{2+}$.

$$Fe^{3+} + SCN^- \rightarrow [Fe(SCN)]^{2+}$$

The equilibrium constant expression may be expressed as:

$$K = \frac{[Fe(SCN)]^{2+}}{[Fe^{3+}][SCN^-]}$$

You will prepare a series of standard solutions that contain known concentrations of $[Fe(SCN)]^{2+}$ and will determine their absorbance's at 447 nanometers. The concentrations and absorbance values will be used to construct a calibration graph for $[Fe(SCN)]^{2+}$.

In the second part of the experiment, various combinations of $Fe(NO_3)_3$ and KSCN will be combined. The amount of product formed, $[Fe(SCN)]^{2+}$, will be determined from the calibration graph prepared earlier. From the original amounts of reactants for each trial and the amount of product formed, the concentration of all species at equilibrium may be determined. When these concentrations are substituted into the equation for the equilibrium constant, values for the equilibrium constant are determined. An average value for the constant will then be determined.

II Purpose

The purpose of this experiment is to determine a value for the equilibrium constant for the reaction between iron (III) nitrate and potassium thiocyanate.

III Equipment/Materials

- 0.00200 M KSCN
- 0.00200 M $Fe(NO_3)_3$
- burets or pipettes
- cuvettes
- 0.200 M $Fe(NO_3)_3$
- 0.05 M HNO_3
- 50 mL beakers

IV Safety

An apron and goggles should always be worn in the lab.

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V General Procedure

The chart below provides the volumes of reactants needed to prepare the standard solutions. Notice that the concentration of the iron solution is much greater than that of the KSCN solution. This is to ensure that all of the KSCN is used up in the reaction. The concentration of the product will be determined from the volume and concentration of the KSCN used in each trial.

Solution	0.00200 M KSCN	0.200 M $Fe(NO_3)_3$	0.05 M HNO_3
1	5.0 mL	5.0 mL	15.0 mL
2	4.0 mL	5.0 mL	16.0 mL
3	3.0 mL	5.0 mL	17.0 mL
4	2.0 mL	5.0 mL	18.0 mL
5	1.0 mL	5.0 mL	19.0 mL

Procedure using Ocean Optics' SpectraSuite Software

1. Click the **Start** button, then select **All Programs | Ocean Optics | SpectraSuite |** **SpectraSuite**. Figure 36 is the toolbar that will appear at the top of the screen.

Figure 36: SpectraSuite toolbar

2. Record a spectrum of a cuvette filled with the solvent (0.05 HNO_3) and save it as the reference spectrum by increasing the integration time to near saturation and then clicking on the Store Reference ( ) button.
3. Completely block off the light source by inserting a piece of dark paper between the light source and your cuvette. Click on the Store Dark ( ) button. Remove the paper you inserted in front of the light source.
4. Click the Absorbance mode button ( ). This will put the spectrophotometer into the Absorbance mode. You are now ready to generate absorbance spectra.
5. You can adjust the display parameters using the Scale Graph Height to Fill Window ( ) and Manually Set Numeric Ranges ( ) functions. For this experiment, you will want to record your absorbance values at 447 nm.

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6. Construct a calibration curve for the standard solutions made using the absorbance data collected from samples. Plot absorbance on the y-axis and concentration of [Fe(SCN)2+] on the x-axis. Draw a best-fit line though the data points. This graph will be used to determine the amount of product formed in the next step.
7. Use a burette or pipette to measure the volumes of the reactants listed below. Note that this set of combinations uses the more dilute $Fe(NO_3)_3$ solution.

Solution	0.00200 M $Fe(NO_3)_3$	0.00200 M KSCN	0.05 M HNO_3
1	5.0 mL	1.0 mL	4.0 mL
2	5.0 mL	2.0 mL	3.0 mL
3	5.0 mL	3.0 mL	2.0 mL
4	5.0 mL	4.0 mL	1.0 mL
5	5.0 mL	5.0 mL	0

8. After the above combinations have been mixed, samples should be poured into clean cuvettes. The absorbance of each solution should be measured and recorded in the data table. Using your calibration graph, determine the concentration of $[Fe(SCN)^{2+}]$ for each of the trials.
9. From the concentration of [Fe(SCN)2+] produced and the original concentrations of the reactants, construct tables to determine the equilibrium concentrations of all species. Use these values to calculate the equilibrium constant for each trial.

Procedure using Pasco's Xplorer GLX

1. Connect the spectrometer to the Xplorer GLX and wait for the initialization process.
2. Insert a cuvette filled with the solvent-background (sodium hydroxide) and adjust the integration time such that the peak near 565nm is near the top of the graph.
3. Click on "save reference."
4. Using the cursor keys, tab over to the lamp tab and choose turn off lamp. Then push the "save dark" key. Then turn lamp back on.
5. Tab over again to the Time acquisition tab and change from "scope mode" to "time acquisition mode."
6. Move the cursor down and type in 447nm as the wavelength to monitor.
7. Enter **3** for the Bandwidth. Then click the close button, which will open the full graph screen on the Xplorer GLX. Use the check button to highlight the y-axis and hit the check button again and select absorbance.
8. Hit the Close button to see the full-screen. The axis will be Intensity vs. time. Push the Check button to highlight the y-axis, then push the Check button again and select absorbance.

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VI Data

Part 1

For this part of the experiment assume $[SCN^-] = [FeSCN^{2+}]$

Trial	$[SCN^-]$	$[FeSCN^{2+}]$	Absorbance
1.			
2.			
3.			
4.			
5.			

Part 2

Trial	1	2	3	4	5
Absorbance					
$[FeSCN^{2+}]$ (from graph)					
Volume Fe^{3+}					
$[Fe^{3+}]$ initial					
$[Fe^{3+}]$ final					
Volume SCN^-					
$[SCN^-]$ initial					
$[SCN^-]$ final					

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VII Calculations

For each of the trials calculate a value for the equilibrium constant. At the end, report the average value for the constant.

Trial 1: $K = \frac{[FeSCN^{2+}]}{[Fe^{3+}]\,[SCN^{-}]}$

Trial 2: $K = \frac{[FeSCN^{2+}]}{[Fe^{3+}]\,[SCN^{-}]}$

Trial 3: $K = \frac{[FeSCN^{2+}]}{[Fe^{3+}]\,[SCN^{-}]}$

Trial 4: $K = \frac{[FeSCN^{2+}]}{[Fe^{3+}]\,[SCN^{-}]}$

Trial 5: $K = \frac{[FeSCN^{2+}]}{[Fe^{3+}]\,[SCN^{-}]}$

Average K = _____

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VIII Questions

1. Why is the experiment run at a wavelength of 447 nm?

2. What is the relationship between concentration and absorbance in Part I of the experiment?

3. Why is the Fe^{2+} concentration so much higher than the KSCN in Part I of the experiment?

4. Extra Credit: Create a spreadsheet using Excel that will calculate the equilibrium constants for this experiment.

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Light Emitting Diodes

I Laboratory Goals

In this week's lab you will:

- Fabricate a circuit containing a light emitting diode (LED)
- Investigate the effect of chemical composition and temperature on the emission properties of LEDs.

II Introduction

Light-emitting diodes (LEDs) are used in a wide range of applications as compact and efficient light sources. Unlike incandescent sources, which emit a broad range of visible and infrared frequencies according to the spectrum of a blackbody at the high temperature of the glowing filament, LEDs emit light only over a narrow spectrum corresponding to the band gap of the semiconductor material that is used as the active component. Accordingly, LEDs emit useful visible light much more efficiently than is possible for ordinary light bulbs.

In this laboratory, you will construct a circuit that can be used to drive a set of LEDs with different semiconductor compositions. The effects of the composition and temperature on the emission spectrum of the LEDs will be observed and recorded visually, and by using the Ocean Optics spectrograph/CCD detector system. The emission spectrum reports changes in the band gap that arise from changes in orbital overlap with composition and structure.

III Background Reading

There is a good section on the band theory for materials in *Chemistry and Chemical Reactivity* by Kotz & Treichel, section 10.4 (pages 407-410), which you should read prior to coming to the laboratory. Section 3 in Chapter 1 of *What Science Is and How It Works* by Derry describes the efforts to understand the motion of electrons in solids using quantum mechanical models that ultimately led to the discovery of band structure in solids.

IV Theory

As shown in some of the earlier laboratory exercises in this course, molecules and atoms emit light at wavelengths corresponding to the transition energies between a pair of energy levels. These energy levels arise from the molecular or atomic orbitals that are resident on single molecules or atoms. In crystals, however, the energy levels arise from a vast number of interacting atoms, which are arranged in repeating sequences according to the structure of the crystal. In a real sense, when one holds a crystal of NaCl in the hand, just as an example, one is holding an enormous single molecule. The overlapping atomic orbitals in a crystal lead to delocalized orbitals that extend over the entire crystal, and so the energy levels of the atoms are said to merge into bands. Whereas the energy levels of an atom or molecule are sharp, being at discrete energies, the bands of a crystal are a continuum of energies (see Figure 37). These bands are the origin of the high electrical conductivity of metals. The electrons in a metal are only weakly associated with the nuclei; in a way, bonding in metals can be described as a sea of delocalized and mobile valence electrons that serve to hold the positively charged metal nuclei together. The delocalized orbitals permit electrons to move from one part of the crystal to another, so that an electrical impulse is transmitted across the material.

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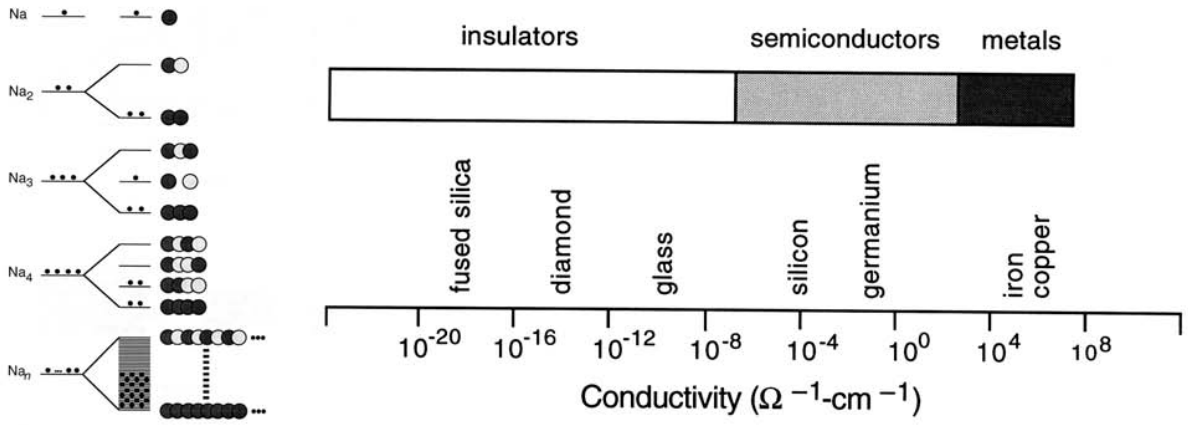

Figure 37. *Left panel:* Atoms, such as Na(*g*), and small molecules, such as Na_2(*g*), Na (*g*), and Na_4(*g*), have well-separated energy levels. In crystalline solids, large numbers of atoms interact to produce bands of delocalized orbitals that have a relatively narrow distribution of energies. The figure shows how combinations of the 3*s* orbitals of Na atoms lead to production of 3*s* bands in the crystalline limit, indicated here as Na_n. The shading of orbitals (large circles) indicates the sign of the wave function; the lower, valence band for Na_n arises from bonding (constructive interference) overlaps, but the upper, conduction band arises from antibonding (destructive interference) overlaps. *Right panel:* Electrical conductivities for a range of solid materials.

Not all materials conduct electricity as well as metals. Insulators have very low electrical conductivity compared to metals, and semiconductors conduct electricity well only in the presence of an electric field. The conductivity (σ) is the current density in A/m^3 established in a solid when an electric field of 1 V/m is applied. In a way, the conductivity is an electrical analog of the optical transmittance. The quantity that is typically measured instead of conductivity or its reciprocal, resistivity (ρ), is resistance (*R*). The resistance of a wire is related to the conductivity or resistivity by the equation $R = L / (\sigma A) = \rho LA$, where *L* represents the length of the wire / and *A* stands for its cross-sectional area. Over the range of materials that is known, the conductivity varies over thirty orders of magnitude (see Figure 37).

The high conductivity of metals arises from the partial occupancy of the upper or *conduction band* by the valence electrons that are available. Figure 38 shows in a schematic way how insulators and semiconductors differ from metals. In an insulator, the conduction band is essentially empty. The *band gap*, the energy gap between the *valence* and *conduction bands*, is large enough that very few electrons can be promoted thermally from the valence band to the conduction band. A semiconductor, on the other hand, has a small enough band gap that thermal promotion of electrons to the conduction band is possible. Lastly, the band gap for metals is small enough that the valence band overlaps somewhat with the conduction band, allowing electrons in the valence band to occupy the conduction band directly.

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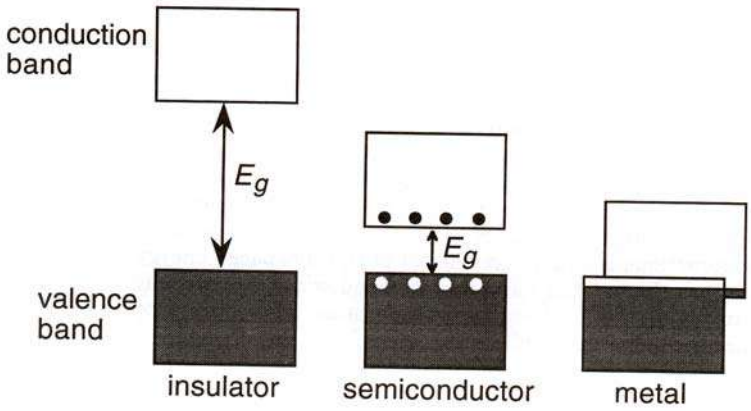

Figure 38. Trend in the band gap for insulators, semiconductors, and metals. The band gap energy, E_g, is shown as the double-headed arrow between the top of the valence band and the bottom of the conduction band. Electron-hole pairs are shown for a semiconductor as filled circles depicting electrons in the conduction band and open circles representing holes in the valence band.

One can distinguish between metals and semiconductors by characterizing the temperature dependence of the electrical conductivity. Metals exhibit a conductivity decrease with increasing temperature; conversely, the electrical conductivity of semiconductors increases. On a molecular level, electrical resistance in all conducting materials arises from free electrons being scattered by the vibrating nuclei. As the temperature increases, higher vibrational levels are populated resulting in an increase in both the frequency and amplitude of vibrational motion, which in turn increases the incidence of electron scattering and decreases the conductivity. In both metals and semiconductors, the number of conduction electrons also increases with temperature. However, the increase in conduction electrons in metals with temperature is relatively small and is overshadowed by the increased scattering of electrons by the vibrating nuclei. In semiconductors, the increase in the number of charge carriers (electron-hole pairs) with temperature dominates to produce increased conductivity.

The magnitude of the band gap depends mostly upon the degree of overlap between orbitals on adjacent atoms. As the internuclear separation increases, the orbital overlap decreases, and concomitantly the splitting between the bonding (valence) and antibonding (conduction) bands derived from the atomic orbitals decreases. The internuclear separation in a crystal is controlled by the size of the *unit cell*, the repeating structure that forms the crystal. As one goes down the periodic table, the unit cell size increases owing to expansion of the atomic radius. As an example, the unit cell's lattice constant (the spacing between adjacent repeating units) for crystals formed from Si is 5.43 Å; the unit cell increases in size so that the lattice constant is 5.66 Å for crystals formed from Ge, the next element below Si in the periodic table. Accordingly, the band gap decreases from 1.1 eV (corresponding to an 1100-nm photon) for Si to 0.66 eV (corresponding to a 1900-nm photon) for Ge.

It turns out that it is possible to form solid solutions of materials that have comparable crystalline structure and atoms of comparable size. An especially important type of solid solution is the mixture of the so-called *zinc blende* semiconductors GaAs and GaP.

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These solutions exhibit tunable band gaps that depend on the relative composition of the components. The zinc blende structure is one resembling that of ZnS, in which the anion S^{2-} forms a face-centered cubic arrangement and the cation Zn^{2+} occupies half of the tetrahedrally shaped holes (see Figure 39). A large number of compounds isoelectronic to pure Si or pure Ge with the stoichiometry called AZ form this type of structure; for instance, the series BN, AlP, GaAs, CdSe, and AgI illustrate the sequence of isoelectronic pairs of elements that are centered on the group IV elements C, Si, Ge, and Sn in the periodic table. Because As has a larger atomic radius than P, the lattice constant is 5.65 Å for GaAs compared to 5.45 Å for GaP. By mixing GaP and GaAs in different relative compositions to form solid solutions, one obtains a material in which the lattice constant can be linearly tuned from 5.65 Å to 5.45 Å, with a resulting scan of the band gap from 1.4 eV (a 890-nm photon) for pure GaAs to 2.3 eV (a 540-nm photon) for pure GaP.

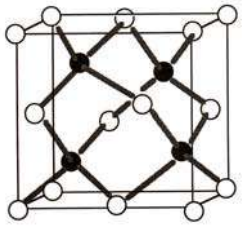

Figure 39: Drawing of a unit cell of a crystal in which all of the atoms are bonded to 4 other atoms. When all of the atoms are the same element, this is the structure of crystalline diamond. When the lighter colored spheres are different elements than the darker colored spheres, the structure has AZ stoichiometry and is called zinc blende.

In semiconductors like GaAs or GaP, it is possible to determine the band gap energy by observing the emission of light that arises as electrons promoted to the conduction band by an electric field (from a battery or other source of electrical potential energy) recombine with holes created in the valence band. The recombination of a conduction electron with a hole in the valence band results in production of a photon or, alternatively, dissipation of the energy in terms of vibrations of the crystal lattice (phonons). The emission spectrum of an LED constructed from GaAs, GaP, or a mixture of the two is typically narrow (though not nearly as narrow as an atomic emission line). Even though electrons can be created well above the bottom edge of the conduction band and holes can be created well below the top edge of the valence band, the electrons and holes rapidly come to the band edge by dissipating the excess energy in terms of phonons, which effectively raise the temperature of the crystal. So the electron-hole recombination emission occurs at a photon energy that is more or less exactly equal to the band gap. Some LEDs emit multiple emission peaks; these LEDs have been doped with impurities in order to optimize the efficiency of the conversion of electrical energy into light.

V Laboratories

Construction of an LED Circuit

In this laboratory you will construct a pair of simple electronic circuits that are capable of driving an LED. Figure 40 illustrates the circuit to be constructed. The LED will be run by a 9V transistor-radio battery. A 1 kΩ resistor will be placed in series with the battery and LED to limit the current dissipated.

1. First, connect one of the leads of the battery snap to a 1 kΩ resistor by winding the stripped end of the lead around one end of the resistor. Use solder and the soldering iron to make the connection permanent. To do this, heat the junction between the resistor and the battery snap (where the stripped lead is wrapped) with the soldering iron until the solder melts and flows. (Do not try to heat the solder directly.)

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CAUTION: The soldering iron's tip is really hot, and molten solder may cause burns of the skin. Keep your safety glasses on and work carefully with the soldering iron. Be patient; it takes a little practice to learn how to solder properly. A good junction is typically shiny and relatively smooth; a bad, nonconducting junction looks rough or crystalline. You may want to reheat your solder junction if you suspect the junction is bad; the solder will reflow around the parts, leading to a good electrical connection when it cools down.

2. Solder the other lead of the resistor to one of the two terminals on the LED socket. It will help here if you use a pair of forceps from your equipment drawer to hold the plastic part of the *resistor* LED socket while making contact between the LED socket and resistor leads. Another pair of forceps could be used to hold the resistor itself (but not on the lead, since the metal forceps would conduct heat away from the connection).

   Again, use the soldering iron to heat the resistor lead at the connection point until solder flows around the resistor lead and the socket terminal. Be careful to hold the LED socket still until the molten solder solidifies (a few seconds).

3. Connect the other lead of the battery snap directly to the open terminal on the LED socket. Solder the two together using the techniques described above.

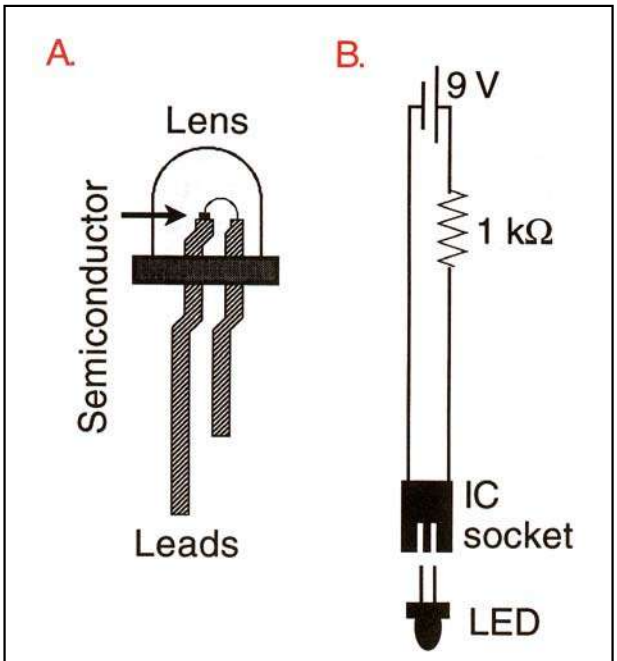

Figure 40: (a) A close-up view of an LED, showing the tiny semiconductor chip connected in series with the two electrical leads on the exterior case. (b) The test circuit to be constructed, consisting of an integrated- circuit (IC) socket or LED socket connected to a 9-V transistor radio battery with a 1 kΩ load in series.

Emission of LEDs at Room Temperature

Using the 1-kΩ circuit constructed following the instructions given above, you will obtain a series of emission spectra and voltage-drop readings with a range of GaP/GaAs LEDs of different compositions.

1. Snap a 9-V transistor radio battery into the battery snap of the 1-kΩ circuit.
2. Insert an LED into the socket. This completes the circuit across the LED from one battery terminal to another. The LED should light up. Does it matter which way the LED is inserted? Why would this be expected? (To answer these optional questions, you will have to read about how LEDs are constructed from *p*-*n* junctions in Chemical Principles: The Quest For Insight by Atkins/Jones.) What is the apparent color of the emission from the LED?

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3. With the LED lit up in the circuit, look at the LED emission using the Ocean Optics spectrograph/CCD system. Determine the range and extent in wavelength of the spectrum that is observed. Where is the emission maximum?
4. Repeat Steps 2–3 with the other LEDs that are available, noting the composition each time. There are two examples (from different manufacturers) for some of the LED compositions; do you observe any differences in the spectra obtained for a given quoted composition?
5. Place an LED into the socket. Then, measure the voltage drop across the LED by holding the probes of the multimeter on the two LED-socket terminals (or the solder junction on the terminals). Note the magnitude of the voltage drop (the sign will depend on the order of the two multimeter probes; note that reversing them causes the sign to reverse). This is a direct measurement of the band gap of the LED material. NOTE: If the measured voltage drop is greater than 3 V, then no current is flowing across the LED because it is inserted backwards into the LED socket. If this is the case, remove and invert the LED in the socket, and then repeat the measurement.
6. Repeat Step 5 with the other LEDs that are available, noting the composition each time.
7. To analyze your results, plot the band gap obtained from the two types of measurements (in eV) as a function of the lattice constant for the unit cell. You can determine the lattice constant from the information given above in the theory section; assume that there is a linear relationship between the lattice constants for the GaP and GaAs unit cells. Compare the band-gap estimates for the LEDs, as obtained by the emission and voltage-drop methods. (Note that the voltage-drop measurement also obtains a contribution from thermally excited electrons flowing in the LED, but the shift as a function of lattice cell constant will exhibit the correct trend.) Determine the change in band-gap per 0.001 angstrom expansion in unit lattice constant, as determined using the two methods.

   NOTE: Your answer should be in units of eV/A.

Emission of LEDs at 77 K

In this part of the experiment, you will compare the LED emission spectrum that you observed at room temperature to the spectrum observed at or near the temperature of boiling liquid nitrogen (77 K). Owing to thermal contraction of the LED's semiconductor crystal, one would predict that the band gap for the semiconductor will increase with a concomitant blue shift of the emission spectrum as the temperature is lowered. As an example, a decrease in the temperature from room temperature (300 K) to 77 K results in a decrease of the lattice constant for GaP from 5.451Å to 5.447 Å.

1. Obtain a styrofoam coffee cup full of liquid nitrogen from the instructor.

   CAUTION: Liquid nitrogen is extremely cold. Do not play around with the liquid nitrogen. Wear your safety goggles at all times when working with liquid nitrogen. Do not allow your eyes, skin or clothes to come into contact with spilled liquid nitrogen. The skin is easily frozen or burned by liquid nitrogen. If you spill liquid nitrogen on your clothing, quickly remove the clothing so that the absorbed or trapped liquid nitrogen will boil away before freezing your skin.

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2. Using the 1-kΩ circuit, light up an LED by inserting it into the socket. Carefully dip the LED only, if possible, just into the surface of the liquid nitrogen in the foam cup. Does the color of the emission change in a noticeable way?
3. Record the emission spectrum from the LED using the Ocean Optics spectrograph/CCD setup. ***Be careful: DO NOT insert the fiber optic cable into the liquid nitrogen***. Hold the tip of the fiber optic cable just above the LED.
4. Remove the LED from the liquid nitrogen, and allow it to warm back up to room temperature. Are the temperature-induced changes in the emission spectrum reversible?
5. Repeat Steps 2–4 for each of the LEDs. Analyze the results as suggested above. Can you determine from your results the apparent contraction of the unit cell that occurs as a result of the lowering of the temperature to 77 K?

Composition of the Four Colored LEDs

The LEDs that you will use in this laboratory have the following approximate compositions:

Color	Composition
RED	$GaPo._4AsO._{60}$
Yellow	$GaPo.65AsO._{35}$
Orange	$GaPo._{.85}AsO._{.15}$
Green	$GaPo_{1.0}AsO$

VI Lab Write-up

In pairs, answer the assorted questions posed in the previous portions of the lab. You are encouraged to answer in paragraph form addressing the individual groupings of questions together.

Acknowledgment

This laboratory procedure, introductory materials, and figures are adapted from materials provided by the Institute of Chemical Education at the University of Wisconsin, Madison. Source materials for this laboratory can be found in the following:

Ellis, A. B.; Geselbracht, M. J.; Johnson, B. J.; Lisensky, G. C.; Robinson, W. R. *Teaching General Chemistry: A Materials Science Companion*; American Chemical Society: Washington, DC, 1993.

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Quantitative Analysis of Phosphates

I Introduction

Phosphates enter lakes, ponds, rivers, estuaries, and the ocean from various primary sources such as inorganic fertilizers, wastewater treatment from municipal sources, runoff from feed lots, soaps and detergents, and industrial processes. Certain types of detergents can introduce a high concentration of phosphate ions into bodies of water. In detergents, tripolyphosphates are used to stabilize dirt particles and complex Ca2+ and Mg2+ to prevent combining with the detergent molecule resulting in superior cleaning ability. However, when the soluble detergents are rinsed away, the resulting wash water has high concentrations of phosphates which act as environmental "nutrients."

Phosphate itself is colorless and not easily determined from water samples with typical spectrophotometric techniques (which determine concentration based on color intensity.) Due to the reactive nature of phosphates, one can easily convert them to a colored compound through chemical reaction which can then be directly detected. In this lab we will be using an ammonium vanadomolybdate reagent. This reagent is made from ammonium metavanadate (NH4VO3) and molybdate (MoO42-) in an acidic solution. This ammonium vanadomolybdate reagent will react with phosphates to produce a yellow heteropoly acid compound with an uncertain formula (though it is thought to be a (NH4)3PO4·NH4VO3·16MoO3.) This is the compound which is used colorimetrically for analytical work.

Color Intensity and Concentration

Analytical chemistry is that branch of chemistry that deals with the quantitative description of a sample, i.e., how much of what is there? The quantitative aspect of this branch makes it applicable to a broad range of disciplines–from microbiology to human biology, from assessing environmental health here on earth to sampling the atmospheres and soil of other planets, and so on. Analytical chemists have a wide array of techniques available to them to analyze their samples, and one of the most powerful is spectrometry–the use of light absorption to measure concentration.

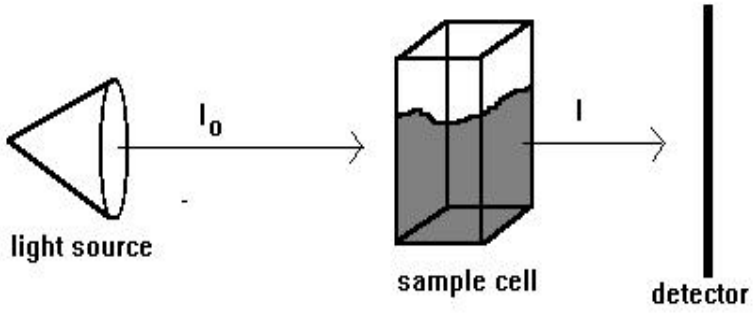

Figure 41: Schematic of a spectrometer

All types of spectroscopy (spectrometry is the use of light to measure concentrations–spectroscopy is the more general study of how matter interacts with light) deal with the interaction of light and the compounds under investigation. Our particular interest is to have this interaction of light tell us how much of a compound there is. The basic idea is fairly straightforward--if your substance is going to absorb light, then the more absorbent molecules or ions you have, the greater the absorption of light. To perform an experiment like this, indeed to perform any spectroscopic experiment, you need three things: a light source, a sample, and a detector. This scheme is shown in Figure 41, which is a representation of a typical spectrometer (sometimes also called a spectrophotometer):

The light incident upon the sample, Io, has three options when it interacts with the sample molecules. It can:

- Hit a molecule and scatter.
- Be absorbed by the molecule (if it has the correct λ of light to excite the molecule to a new energy level.)
- Go through the sample without interacting with any of the sample's molecules.

Clearly, the more molecules that are "packed" into the sample cell, the more likely it is that a molecule will interact with the light if it can. So, the amount of light that is absorbed by the solution is proportional to the concentration. Furthermore, we expect that if we use a smaller sample cell there will be fewer molecules in the path of the light, and the absorption should therefore be smaller than if we used a larger cell. So the amount of absorbed light should also be proportional to the pathlength of the light through the sample. Finally, we might also expect that some molecules are better at absorbing light than others; the probability of a species absorbing a given wavelength of light might not be the same as that of different species. All of these dependencies are accounted for in the Beer-Lambert Law:

$$A_\lambda = \varepsilon bc$$

Where:

$A\lambda$ = absorption at some wavelength λ,

ε = probability that a sample molecule will absorb light of wavelength λ (called the molar absorbtivity coefficient),

b = pathlength of the light through the sample, and c is the concentration.

Of course, absorbances are meaningless without some reference, and in this case I_0 is an obvious choice. The spectrometer measures both the intensity of the incident light and the light transmitted through the sample, I, (separately if there is only one beam, simultaneously if the beam has been split into two) and displays the ratio I/I_0. This ratio is related to absorbance by:

$$A = -\log(I/I_0) = \log(I_0/I) = \varepsilon bc$$

(the "λ" subscript has been left off for clarity). A related quantity also displayed by your spectrometer is the percent transmittance, %T. The relation between %T and absorbance is:

$$A = 2 - \log(\%T) = 2 - \log[(I/I_0)*100]$$

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Returning to Beer's Law, notice that log(I/I0) is directly proportional to concentration for a given molecule if the path length is not varied. Similarly, both absorbance and log(%T) (but not %T) are proportional to concentration. Therefore, graphs of A vs. c and log(%T) vs. c should result in straight lines for solutions obeying Beer's Law. This fact alone will enable the determination of concentration of your unknown solution.

Your lab this week will involve using standards (i.e., solutions of known concentration)of phosphate to create plots of absorption vs. concentration (these are called calibration curves). These calibration curves will then allow you to determine the concentrations of unknown samples of phosphates. These unknowns will be from collected natural water samples or from soaps.

II Procedure

General Procedure

1. Make a set of standard solutions of known phosphate concentration to develop your calibration curve. A 0.001M stock solution will be provided. You should use this to make dilutions for concentration of approximately 1.00×10^{-3} M, 5.00×10^{-4} M, 2.50×10^{-4} M, 1.00×10^{-4} M, 5.00×10^{-5} M, and 2.50×10^{-5} M. Remember that you will need to add the necessary amount of concentrated solution to a volumetric flask and then exactly enough water to fill the flask until the bottom of the meniscus is tangent to the mark of the flask. These solutions should be thoroughly mixed before using it for any further dilutions.
2. In order to measure the absorbance of the known concentration solutions, you will take 10.0 mL of your phosphate standard and add in 5.0 mL of the ammonium vanadomolybdate reagent. This should produce the yellow heteropoly acid product that will be measured. For your analyses you will need to determine the wavelength with the maximum absorption. You will need to use the same λ for all your readings.

Procedure using Ocean Optics' SpectraSuite Software

1. Click the **Start** button, then select **All Programs | Ocean Optics | SpectraSuite |** **SpectraSuite**. Figure 42 is the toolbar that will appear at the top of the screen.

Figure 42: SpectraSuite toolbar

2. Record a spectrum of a cuvette filled with the solvent (water) and save it as the reference spectrum by increasing the integration time to near saturation and then clicking on the Store Reference () button.
3. Completely block off the light source by inserting a piece of dark paper between the light source and your cuvette. Click on the Store Dark () button. Remove the paper you inserted in front of the light source.
4. Click the Absorbance mode button (). This will put the spectrophotometer into the Absorbance mode.
5. Once you have your data you should roughly graph it out to ensure that you obtain a straight line for your data. If you do not have a straight line, then you should remeasure your standard solutions (did you have the cuvette facing the same direction?). If the data remains unchanged, then talk to your instructor. The relationship described by Beer's Law ONLY holds true for concentrations that give a linear relationship to absorbance. Also, it is important to realize that absorbance values over about 1.7 are not reliable (over 90% of the incident light is being absorbed). Lower values (below 1.5) are considered more reliable.
6. Now find the absorption for your unknown samples. As always, record all your data in a clearly labeled data table. If the absorbance value for your sample (prepared by adding 10 mL of your sample to 5.0 mL of the ammonium vanadomolybdate reagent) does not have an absorbance value below 1.5 you may find it necessary to use dilutions to get an accurately measurable value. All dilutions should be recorded so that you can accurately determine the original phosphate concentration. There are other possible control experiments that might be necessary for you to perform to try to answer your specific question (and accurately test your sample.) For example, other colored compounds in commercial soaps may also absorb the 400 nm wavelength light and you will have to test for any such compounds (their presence does not mean that the phosphate concentration cannot be tested.) Note too, that opaque soaps can potentially scatter the incoming light and to give an inaccurate reading (how can you deal with this?) You will have to come up with some manner to overcome such problems; your instructor may be able to provide some specific suggestions, but you will have to present your own thoughts first.

Procedure using Pasco's Xplorer GLX

1. Turn on Xplorer GLX and plug in the Ocean Optics spectrometer. Wait for the initialization process to complete.
2. While the set-up window, insert a cuvette filled with the solvent (water) and adjust the integration time so that the highest peak is not clipped at the top. Do this by pushing the Check button to highlight the integration time and type in an appropriate value, then push the Check button to accept the change.
3. Push the Save Reference (F2) button.
4. Use the Arrow button on the Xplorer GLX to highlight the lamp tab. Push the Check button to turn off the light source, then press the Save Dark (F1) button. Now push the Check button to turn the light back on.

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5. Hit the Close (F4) button, then push the Start/Stop button on the Xplorer GLX. Push the Check button to highlight Intensity on the y-axis. Push the Check button again while Intensity is highlighted and scroll down to absorbance. Push the Check button once more to accept this change. Click on the Absorbance mode button. You are now ready to generate absorbance spectra.
6. Rinse the cuvette with the solution to be tested and fill it with the same solution. Insert the cuvette in the chamber and acquire the absorption spectrum by pushing the Start/Stop button. This freezes the spectrum on the screen.
7. Determine the λ max by using the smart tool located in the tools menu. This will be the wavelength used for the calibration curve.
8. Under the tools menu, choose the Ocean Optics set-up. In the set-up screen, tab over to the Time Acquisition tab and change Scope mode to Time Acquisition.
9. Scroll down to the wavelength and select with the check button. Type in the value of maximum absorbance determined from the initial scan.
10. Enter **3** for bandwidth and hit the close button.
11. From the home screen, choose digits display to read the absorbance values of your calibration solutions. Record these values in your lab book.
12. Record the absorbance of your unknown sample.

III Calculations

To find the concentration of all of your unknown samples you need to construct calibration curves for the known phosphate solution. To do this, plot A vs. concentration for all your standard solutions. Perform a least squares analysis on each calibration curve, and report the slope and intercept for each. Use the best fit line to calculate the concentration of your unknown solution. Report this value and its standard deviation using proper significant figures.

IV Questions

1. Determine ε for the heteropoly acid at what you feel is the λ with the greatest absorbance nm. There are two ways of doing this:
   - By plugging values into Beers law
   - By using the slope of the calibration curve (how should the molar absorptivity relate to this slope?)
2. Use both these methods to find ε if the path length is 1.0cm. If you end up with an intercept that is not at zero (or very nearly) what does that actually mean?

V Chemicals

Phosphate stock solution ($1x10^{-3}$ M), Ammonium vanadomolybdate solution

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VI Chemical Disposal

Any solution containing the ammonium vanadomolybdate must be put into the waste bottle in the hood. These heavy metal compounds should not go down the drain as they are toxic. Any phosphate solution, soap or water samples without the ammonium vanadomolybdate added may go down the drain

VII Equipment

Ocean Optics spectrometers, cuvettes, volumetric glassware.

References

1. K. Spotz "Spectrophotometric Analysis: Phosphate in Water" in *Hands on Chemistry*, McGraw Hill; New York, 2001.
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4. D. Ebbing, *Experiments in General Chemistry*, Houghton Mifflin Company, Boston, 1987.
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Spectrophotometric pH Determination Using Bromocresol Green

I INTRODUCTION

UV-VIS Spectrophotometry

This experimental technique relies upon the absorption of ultraviolet and visible light. Atoms and/or molecules in the sample absorb incident energy, enter an excited state, and may dissipate their absorbed energy via thermal, radiant, or chemical processes. Beer's Law is a simple formulation relating the absorbance of incident energy to the particular chemical species, the concentration of that species in solution, and the distance the incident energy must travel through the solution.

$$A = \varepsilon\, b\, c \qquad \text{Eqn. 22}$$

Where:

A = absorbance of the solution

ε = molar absorptivity (L/mol•cm, specific to the chemical species and wavelength of light used)

b = cell path length (cm)

c = concentration of absorbing species (mol/L)

Traditionally, measurements using Beer's Law have been made at particular wavelengths of light where there exists a linear relationship between concentration and absorbance.

Acid-base Equilibrium and Spectroscopy

The indicator bromocresol green, by definition, has an acid and a base form, with their concentrations depending upon the pH of the solution.

$$HIn + H2O \leftrightarrow H3O+ + In- \qquad \text{Eqn. 23}$$

The equilibrium expression is:

$$K_a = \frac{[H_3O^+][In^-]}{[HIn]} \qquad \text{Eqn. 24}$$

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The two forms (HIn and In-) absorb differently, thereby making the compound analytically useful for determining the pH of a solution.

The two forms of bromocresol green have different molar absorptivities at all wavelengths but one. This wavelength is called the isosbestic point. The existence of the isosbestic point proves that there exist two interconvertible absorbing forms of a species with overlapping spectra. An example of an isosbestic point is shown in Figure 43. Note that, at about 475 nm, the absorbance is independent of pH.

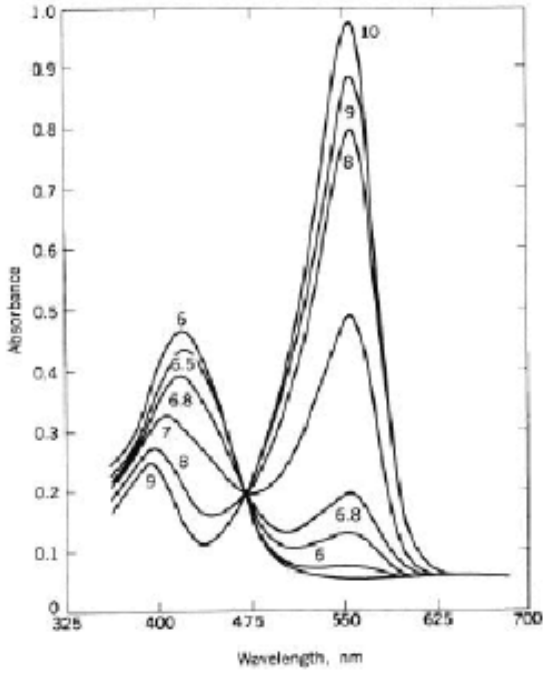

Figure 43. The absorption spectra of phenol red depends on the pH of the solution at all wavelengths except the isosbestic point.1 All solutions are made using a constant concentration of indicator.

II Procedure

1. Prepare 5 solutions of a known and constant concentration of bromocresol green. Each solution will be prepared in a different pH buffer. You will need to include the buffers on the extreme ends of the pH scale to insure that all of the indicator has been converted to a single form (either all HIn or all In-). You will prepare the other three solutions near the pKa of the indicator.
2. Prepare your unknown sample by adding bromocresol green of a known concentration and measure an absorption spectrum. You will use this spectrum to determine the pH of the unknown solution

Procedure using Ocean Optics' SpectraSuite Software

1. For each run, you will use the SpectraSuite program to record the absorbance spectrum. Click the **Start** button, then select **All Programs | Ocean Optics | SpectraSuite |** **SpectraSuite**. Figure 44 is the toolbar that will appear at the top of the screen.

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Figure 44: SpectraSuite toolbar

2. Record a spectrum of a cuvette filled with the solvent (water) and save it as the reference spectrum by increasing the integration time to near saturation and then clicking on the Store Reference ( ) button.
3. Completely block off the light source by inserting a piece of dark paper between the light source and your cuvette. Click on the Store Dark ( ) button. Remove the paper you inserted in front of the light source.
4. Click the Absorbance mode button ( ). This will put the spectrophotometer into the Absorbance mode.
5. You can adjust the display parameters using the Scale Graph Height to Fill Window ( ) and Manually Set Numeric Ranges ( ) functions. For this experiment, you will want to use the Manually Set Numeric Ranges function to reset the wavelength scale to from 180 to 800 nm
6. Rinse the cuvette with the solution to be tested and fill it with the same solution. Insert the cuvette in the chamber and acquire the absorption spectrum by clicking the "snapshot" button. This freezes the spectrum on the screen.
7. You will save your spectra in on the 3.5" High Density Diskette that you brought to lab today. Click on the save button and you will be able to save the wavelength values and intensities in a file format that can be opened in most spreadsheet programs (including Excel).
8. Repeat Steps 6 and 7 until all the samples have been measured.
9. Determine the pH of each of the samples using a pH meter.

Procedure using Pasco's Xplorer GLX

1. Turn on Xplorer GLX and plug in the Ocean Optics spectrometer. Wait for the initialization process to complete.

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2. While the set-up window, insert a cuvette filled with a sample of the darkest solution you expect to use in the experiment and adjust the integration time so that the highest peak is not clipped at the top. Do this by pushing the check button to highlight the integration time and type in an appropriate value, then push the check button to accept the change.
3. Insert a cuvette filled with the solvent (water) and save it as the reference spectrum by clicking on the “save reference” (F2) button.
4. Use the arrow button on the Xplorer GLX to highlight the lamp tab. Push the check button to turn off the light source, then press the “save dark” (F1) button. Now push the check button to turn the light back on.
5. Hit the close (F4) button, and then push the start/stop button on the Xplorer GLX. Push the check button which will highlight Intensity on the y-axis. push the check button while highlighted and scroll down to absorbance and push the check button again to accept this change. Click on the "absorbance mode" button. You are now ready to generate absorbance spectra.
6. Rinse the cuvette with the solution to be tested and fill it with the same solution. Insert the cuvette in the chamber and acquire the absorption spectrum by pushing the start/stop button. This freezes the spectrum on the screen. Record the identity of the sample and the run # from the Xplorer GLX.
7. After recording the run, choose the table view from the home screen. Insert a USB flash memory stick into the Xplorer GLX. From the Table menu (F4) choose export all data which will copy your data as a text file to be opened in Excel.
8. Repeat with all of the solutions.

III DATA ANALYSIS

1. Print out two plots of your spectra: the first plot showing the five solutions at various pH, and the second showing the unknown spectrum.
2. Calculate εHIn and εIn- at the absorption maximum for the low and high pH extremes respectively.
3. Calculate Ka and pKa of bromocresol green with the intermediate pH buffers using equation 3. You will need to determine [In-] / [HIn] in each buffer first.

$$\frac{A_{In^- @ lowpH}}{A_{HIn @ highpH}} = \frac{\varepsilon_{In^-} \bullet b \bullet [In^-]}{\varepsilon_{HIn} \bullet b \bullet [HIn]}$$

Eqn. 25

4. Calculate the pH of the unknown solution.
5. Calculate the molar absorptivity at the isosbestic point. Remember the concentrations of the contributing species are additive.

$$A_{Isobestic} = \varepsilon_i * b_i * c_i$$

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IV DISCUSSION

1. Comment on how your results for the Ka and pKa compare with literature values. How useful would this method be for determining pH values?
2. Discuss how pH might be determined using a calibration curve if the [Bromocresol Green] was not known or if the path length of the cell was not known.
3. What possible practical advantage is there in operating a spectrophotometric analytical method at an isosbestic point?

V REFERENCES

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2. Ocean Optics, Inc. http://www.oceanoptics.com/products/pc2000.asp

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Spectrophotometric Analysis of Commercial Aspirin

I Purpose

We will determine how much active ingredient, Acetyl Salicylic Acid (ASA), (in mass %), is contained in the commercially available aspirin tablets using visible spectrophotometer.

II Theory

Concentration of ASA can be determined spectrophotometrically by the percent transmittance (%T) of visible light at a given wavelength,

%T = (I_t/I_o) x 100%, where I_t = Intensity of the beam transmitted by the solution, I_o = Intensity of the light beam before it is impinging on the sample solution.

The absorbance of the analyte in solution is related to the transmittance (T) as follows:

$$A = -\log (I_t/I_o) = -\log T = -\log (\%T/100) = -\log (\%T) + 2 = 2 - \log (\%T) \quad \text{Eqn. 26}$$

Beer's Law says that

$$\mathbf{A = \varepsilon \times b \times [\, c\,]} \quad \text{Eqn. 27}$$

Where:

ε = molar absorptivity of the particular absorbing species in 1/(M.cm),

b = path-length of the light through the solution in cm, and

[c] = concentration of the absorbing species in moles/L.

From the above Equation (1) and (2), we can determine the concentration [c], in mole/L for the absorbing species, if the percent transmission (%T) can be determined from the spectrophotometer.

To determine the concentration of the absorbing species in solution, one needs to construct the Standard Beer's Law Plot. This is done by preparing a series of known concentrations of the analyte and reading the percent transmission of each standard solution at the maximum absorbing wave length, λ , in the visible region.

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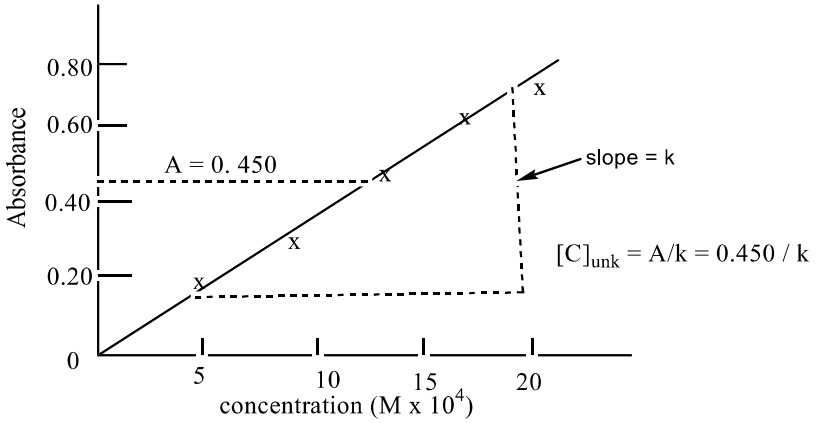

Figure 45

The resulting plot is a straight line plot known as the Beer's Law Plot shown in Fig. 45.

Plot of Absorbance vs. concentration (M) gives a straight line plot passing through the origin (0,0). The slope (k) of the straight line can be obtained from a linear regression or the least-squares fit method and is equal to the k =A / [c], where k = ε x b, k = the slope of the Beer's law plot, ε = molar absoptivity (1/M.cm), b = path-length of the light (cm).

If the absorbance of the unknown solution is known, then the concentration of the ASA complex can be calculated by the formula,

$$[c] = A/k \qquad \text{Eqn. 28}$$

The analyte of interest in this experiment, Acetyl Salycylic Acid (ASA) complex ion, is formed by hydrolyzing the ASA or Aspirin sample in NaOH solution and complexing it with Fe^{3+} ion in acid solution to bring out the color as shown below. The complex displays a maximum absorption at a wavelength (λ) of 530 nm, and appears crimson red to our eyes.

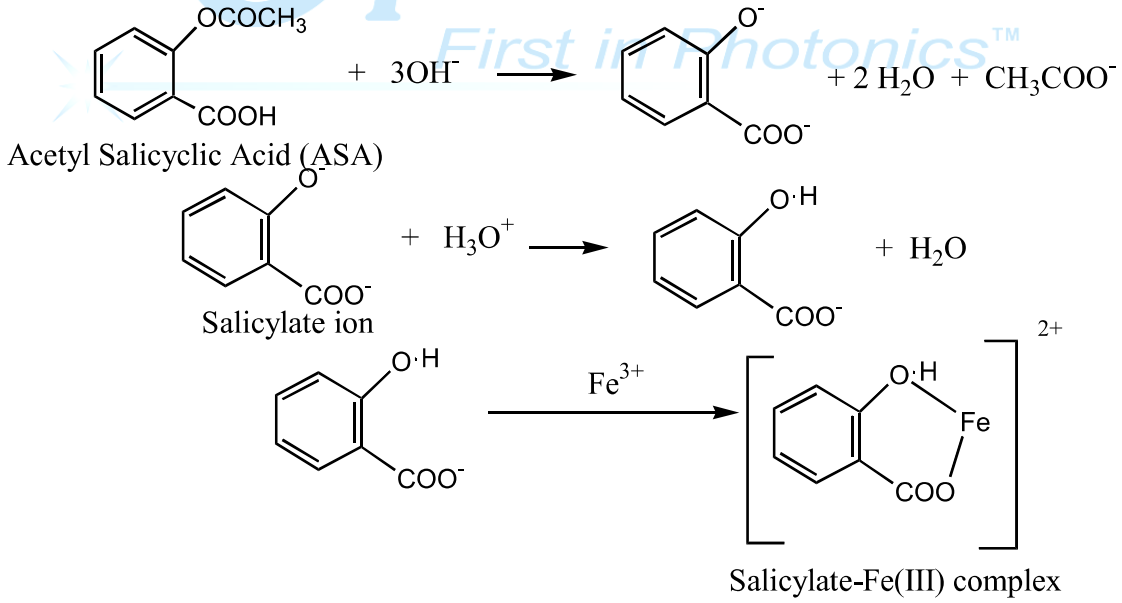

Figure 46

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III Prelab Questions

Construct a Beer's Law plot for the following experimental data, using a computer graphics or precision graph paper.

Concentration of Cobalt (III) Complex (Standard sol'n, M)	%T	A
3.00×10^{-4} M	76.91	_____
6.00×10^{-4}	59.16	_____
1.20×10^{-3}	35.08	_____
1.50×10^{-3}	27.10	_____
1.80×10^{-3}	20.65	_____
Unk. Sol'n	42.57	_____

1. Calculate the absorbance of each solution using Eqn. 26.
2. Plot absorbance on the y-axis vs. concentration on the x-axis for the five standard solutions. Use computer graphics or precision graph paper.
3. From the above plot (Beer's Law plot), obtain the slope (k). Use the linear regression to find the best slope, if you are using the computer graphics. The straight line should pass through the origin (0,0), otherwise a large error may incur.
4. Determine the molar concentration of the unknown cobalt (III) solution, using Eqn. 28.

IV Procedure

Part 1. Hydrolyzing the ASA and Aspirin samples

Work in pairs to prepare the standard Acetyl Salicylic Acid (ASA) solution and aspirin sample solution simultaneously.

1. Weigh to the nearest mg (±0.001 g) on a piece of weighing paper or weighing boat approximately 0.4 g of reagent grade ASA (acetyl salicylic acid). Transfer the sample to 125 mL Ehrlenmeyer, labeled "flask #1". Record the exact mass of ASA and weighing boat or paper on your Data Sheet 1.
2. Record your unknown aspirin brand name and weigh each aspirin tablet to the nearest milligram (±0.001 g) as before. Record this mass on Data Sheet 2. Repeat above procedure for the aspirin sample and label it Ehrlenmeyer "Flask #2".

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3. Measure 10 mL of 1.0 M NaOH solution in a clean, dry, graduated cylinder. Add the NaOH to the ASA in the 125 mL Ehrlenmeyer "Flask #1".
4. Repeat the same for Flask #2.
5 Heat the mixtures (i.e., Flask#1 and Flask#2) to a mild boil for five minutes on a hot plate to hydrolyze the ASA. Be careful to avoid splattering and do not let the solution dry up to prevent loss of contents. Rinse the inside of walls of the flasks with small amount of DI water to ensure complete chemical reaction of ASA.
6. Quantitatively transfer solution of sodium salicylate in flask #1 to a 250 mL volumetric flask through a glass funnel. Thoroughly rinse the flask and funnel with DI water so that the rinse water flows into the volumetric flask. Add DI water to the solution in the flask until the bottom of the meniscus touches the index mark of the flask neck. Stopper the flask. While firmly holding the stopper, with your fore finger, invert the flask 10 times to thoroughly mix the solution. Repeat the process for Flask#2 containing aspirin sample.
7. Transfer these solutions into 250 mL Ehrlenmeyer flasks with rubber stoppers and label "Standard" for one and "Aspirin" for the other. Store these flasks in your drawer until needed. (Check with your instructor whether you may proceed beyond this point.)

Aspirin solutions may have milky appearance due to starch fillers. Some buffering agents such as aluminum hydroxide will not dissolve completely in base. In such cases, allow the solution to settle to the bottom of the flask.

NOTE: If a precipitate is present, use your pipette to remove solution from the top portion of the liquid so that you will not draw any precipitate into your pipette.

Part 2: Preparing Standard and Aspirin Solutions

1. Clean your pipette with small portions of the solution you are trying to measure by drawing with suction bulb and discarding the solution.
2. Pipette a 2.40 mL portion of the standard solution into a clean 50 mL volumetric flask. Allow the solution to drain and gently allow the tip of the pipette to touch the side of the flask. Add 0.020 M $FeCl_3$/KCl/HCl solution (pH=1.6) to the 50 mL vol. flask until the bottom of the meniscus touches the index mark on the flask. (You should see the solution turn red at this point. If no color appears after adding $FeCl_3$ solution, notify the instructor.) Stopper the flask. Mix thoroughly by inverting the flask 10 times. Label this flask Standard Solution A.
3. In a similar fashion, prepare standard solutions B, C, D and E by diluting 2.00, 1.60, 1.00, 0.40 mL portions of the sodium salicylate stock solution (**Standard**) with the $FeCl_3$/KCl/HCl solution. Transfer these standard solutions to large test tubes with stoppers and label them clearly.
4. Prepare your unknown aspirin solution in two (2) trials as follows:
5. Using a clean, well-rinsed pipette, transfer 1.20 mL portion of the aspirin stock solution into a 50 mL volumetric flask, and dilute it to the mark with 0.020 M $FeCl_3$/KCl/HCl solution and mix thoroughly as described earlier. Make a second trial aspirin sample, this time using 1.60 mL of the aspirin stock solution. Transfer aspirin solutions to large test tubes with stoppers and label them clearly.

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Part 3. Measuring the % Transmittance using Spectrophotometer

Procedure Using and Ocean Optics' SpectraSuite Software

1. Click the **Start** button, then select **All Programs | Ocean Optics | SpectraSuite |** **SpectraSuite**. Figure 47 is the toolbar that will appear at the top of the screen.

Figure 47: SpectraSuite Toolbar

2. Record a spectrum of a cuvette filled with the solvent ($FeCl_3$/KCl/HCl) and save it as the reference spectrum by clicking on the Store Reference ( ) button.
3. Completely block off the light source by inserting a piece of dark paper between the light source and your cuvette. Click on the Store Dark ( ) button. Remove the paper you inserted in front of the light source.
4. Click on the Transmission button ( ). This will put the spectrophotometer into the Transmission mode.
5. Place your curser at 530nm and record the percent transmittance of the standard solutions A, B, C, D and E and the two trials of the aspirin samples on Data Sheet 1 and 2.

   NOTE: Your aspirin sample solutions should have % Transmittance between 30% and 70%. If not, make appropriate dilutions or additions so that they fall within the range. Remember to record the dilution or addition factor used to prepare your aspirin samples should you decide to make concentration adjustment.

Procedure Using Pasco's Xplorer GLX

1. Turn on Xplorer GLX and plug in the Ocean Optics spectrometer. Wait for the initialization process to complete.
2. While the set-up window, insert a cuvette filled with a sample of the darkest solution you expect to use in the experiment and adjust the integration time so that the highest peak is not clipped at the top. Do this by pushing the check button to highlight the integration time and type in an appropriate value, then push the check button to accept the change.
3. Insert a cuvette filled with the solvent ((FeCl3/KCl/HCl)) and save it as the reference spectrum by clicking on the "save reference" (F2) button.

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4. Use the arrow button on the Xplorer GLX to highlight the lamp tab. Push the check button to turn off the light source, then press the “save dark” (F1) button. Now push the check button to turn the light back on.
5. Hit the close (F4) button, then push the start/stop button on the Xplorer GLX. Push the check button which will highlight Intensity on the y-axis. Push the check button while highlighted and scroll down to transmittance and push the check button again to accept this change. Click on the "transmission mode" button. You are now ready to generate absorbance spectra.
6. Following this procedure, place your curser at 530nm and record the percent transmittance of the standard solutions A, B, C, D and E and the two trials of the aspirin samples on Data Sheet 1 and 2.

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Data Sheet 1

Preparing Standard Solutions:

Weighing boat or paper, g _____

Mass of ASA + weighing boat or paper, g _____

mass of ASA, g _____

Concentration of ASA complex in solution, M

Solution	Concentration, M	% Transmittance	Absorbance, A
A	_____	_____	_____
B	_____	_____	_____
C	_____	_____	_____
D	_____	_____	_____
E	_____	_____	_____

Slope of the Beer's Law plot = _____

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Data Sheet 2

Analyzing Commercial Aspirin Tablets:

Sample ID _____

mass of tablet + weighing boat or paper, g _____

mass of weighing boat or paper, g _____

mass of tablet, g _____

percent Transmittance, %T	_____	_____
absorbance of solution, A	_____	_____
concentration of aspirin in solution, M	_____	_____
mass of ASA in tablet, g	_____	_____
percent ASA in tablet, %	_____	_____

mean percent of ASA, % _____

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V Calculations

1. Find the slope (k) from your Beer's Law plot.

2. Convert the percent transmittance (%T) to the equivalent absorbance (A) using Eqn. 26.

3. From the calculated absorbance and the Beer's Law plot slope (k), determine the concentration of ASA in your aspirin solution using Eqn. 28.

4. Calculate the mass of ASA in each tablet, using Eqn. 29 below.

Mass of ASA in grams = [ASA]*(180.2 g /mol ASA)(0.0500 L)(250 mL/1.60 mL*) Eqn. 29

* ... Use your experimental values of [ASA] in mole/L and the volume of the "original" Aspirin solution in mL.

5. Find the percent ASA in each tablet, using Equation (5).
% ASA in tablet = (mass ASA in tablet, g/ mass of tablet, g) x 100% Eqn. 30

6. Calculate the mean percent ASA in your commercial brand of aspirin, using Eq. (6).

Mean % ASA per tablet ={[ASA (trial #1)] + [% ASA (trial#2)] }/2 Eqn. 31

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VI Post Lab Questions

(Use separate sheets for answers.)

1. Explain why the Fe^{3+}/H^{+} solution was used as a reference solution. Suggest a procedure you could follow to determine whether it was necessary to use the solution as a reference or whether de-ionized water would have been just as satisfactory.

2. Most commercial aspirins claim to contain 5.0 grains of ASA per tablet, where a grain is an old apothecary unit of measurement for mass, equal to 65 mg . Compare your calculated value of ASA per tablet with respect to the advertised value (i.e., 5.0 grains) and determine the percent error.

3. Compare the results of the class data, if available, for different commercial brands of aspirin. Is there any difference between the different brands of aspirin? Support your answer using simple statistics.

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