Lab Exercise: Light Absorption, Spectrophotometer, and the Pulse Oximeter

Objectives

- Determine which characteristics of a medium are important for light absorption.
- Explain how absorption properties of blood allow for a pulse oximeter to measure oxygen content and the pulse of a patient.
- Explain why bromothymol blue solution can be used as an analog for blood, based on its light absorption characteristics.

Equipment

- Vernier spectrophotometer
- Distilled water
- Salt
- Bromothymol blue solution
- Beaker
- Cuvette tissue for cleaning cuvette surfaces
- Cuvettes for spectrophotometer
- Petri dish or clear plastic cup
- Pipette
- Simulated pulse oximeter device
- Vernier light sensors

Introduction

The Medical Field

By the end of this lab you should have a good idea of how a pulse oximeter, a device commonly used in hospitals to measure vital signs of patients, is able to noninvasively measure the change in oxygen content in blood. Due to the difficulties in working with blood, we will instead use bromothymol blue in the lab to make the relevant observations necessary to understand how the pulse oximeter works. Bromothymol blue is a compound that is used as a pH indicator for weak acids and bases. When in a basic solution, Bromothymol blue will appear blue; when it is in a neutral solution it will appear green; and when in an acidic solution it appears yellow. These changes in color can be observed both visibly and with a spectrophotometer. A spectrophotometer is a device that measures the absorbance or percent transmittance of light through a solution. Simply breathing on a solution of Bromothymol blue will introduce carbon dioxide (CO_2) from your breath into the solution. The CO_2 reacts with the solution to form carbonic acid (H_2CO_3), which increases the acidity of the solution. As the pH of the solution changes, the color of the solution also changes. In this lab, we will examine the change both visually and with a spectrophotometer, linking observations to the spectral characteristics of deoxygenated and oxygenated blood as measured by a pulse-oximeter.

Light Absorption

The electromagnetic spectrum is composed of wavelengths, which range from short to long. In this lab, we will ignore the wavelength extremes at both ends and instead focus on the wavelengths most familiar to us - visible and near visible light. Human vision is capable of detecting wavelengths of electromagnetic radiation from about 380 nm-750 nm. This is called the *visible light range*. In this lab, you will also examine light at wavelengths near-infrared, which have a wavelength that is slightly longer than those observed in the visible light range.

When light passes through a substance, some of it is absorbed due to interactions with the atoms and molecules in the medium. This can be quantified by defining the term *absorbance* (*A*). Absorbance is given by the following equation:

$$A = \log (I_0/I_1),$$

where I_o is the intensity of the light before it enters the substance and I_1 is the intensity of light after it is transmitted through a substance. *Intensity* (I) is defined as the amount of energy transmitted in a unit area in a unit time, or power (P) per area (A_s).

$$I = P/A_s.$$

The absorbance is wavelength dependent and will also vary depending upon the medium (air, water, glass, etc.) through which light passes. The medium can be characterized by the molar *absorptivity*, ε (in m^2/mol), by the concentration, c (in mol/m^3), and by the length of the medium the light passes through, l (in m). The molar absorptivity has one value for a particular material, e.g., there is one value of ε for salt. Of course, the medium may consist of various substances; for instance, a solution may have multiple solutes each with their own absorption coefficient and concentration. The absorption coefficient and concentration can be combined into a single coefficient, the absorption coefficient α (in m^{-1}). The dependence of absorbance on these variables is called Beer's Law and is given by the following equation:

$$A = \varepsilon cl = \alpha l.$$

The loss in intensity through a medium is known as *attenuation*.

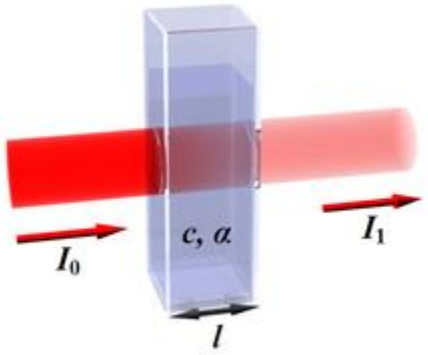


Figure 1- Absorption due to light passing through a medium

Part One: Using the Spectrophotometer with Varying Concentrations of a Solution

The spectrophotometer measures the amount of visible light absorbed by a solution. To obtain a reading, the spectrophotometer will direct a known amount of light through a cuvette filled with solution and will measure the amount of light that passes through the cuvette.

For this experiment you will compare the relative intensity measured for two different concentrations of saltwater solutions.

- **Q1: What is the relation between energy of light and its wavelength (λ)? What happens to the frequency as λ decreases?**

PROCEDURE

Saltwater Solution:

1) In a beaker, mix one part sodium chloride (common table salt) with five parts water (by volume). Label this solution A. Transfer solution A to a cuvette. Also, mix one part table salt with twenty parts water, label this solution B. Transfer this solution to a cuvette and make sure the solution is well mixed by shaking the cuvette. Your instructor may have already done that for you, so check in with her/him.
2) Plug the Spectrophotometer into the USB port in the computer and open Logger–Pro. Make sure the light sensor used in Part Four of this lab is NOT connected to the LabPro or it will interfere with the Spectrophotometer. Using a cuvette filled with distilled water, calibrate the Spectrophotometer: Position the cuvette with distilled water so that the side with a smooth surface is aligned with the ►on the spectrophotometer. You may want to clean any fingerprints off the cuvette surface with tissue first. From the **Experiment** menu click **Calibrate** → **Spectrometer 1.** After waiting 90 seconds for the lamp to warm up, click on **Finish Calibration.**
3) After the Spectrophotometer is calibrated, select **OK** and replace the distilled water cuvette with the cuvette containing the saltwater solution A.
4) Click **COLLECT.** After a graph is produced, click **STOP**. Make sure to **STOP** data collection or you will lose your data when you switch solutions.
5) Repeat data collection for saltwater solution B. A prompt will ask you if you would like to erase the prior data, choose "Save Latest Run". This will allow you to analyze both experiments on the same graph.
6) Save graphs and absorbance curves from each experiment to answer the following questions:

Q2: How did the absorbance for the saltwater solution with a greater concentration (solution A) differ from that of the saltwater solution with a lesser concentration (solution B)?

Q3: For the above experiment, you measured the absorbance of various wavelengths of light by the solution. How does absorbance relate to the intensity of light that is transmitted through the solution and measured by the detector? Using just salt and water, how could you decrease the intensity of light measured in the detector? If the intensity of light decreases as measured by the detector, does this mean the absorbance has increased or decreased?

Q4: Suppose you repeated this experiment with red fruit punch as your solution. Which wavelengths of light would you expect to be strongly absorbed? Which wavelengths are not strongly absorbed? Which wavelengths of light have the greatest intensity as measured by the spectrophotometer?

Q5: Write the equation for Beer's Law. Which variables in Beer's law changed by increasing the amount of salt?

PART TWO: SPECTROPHOTOMETER - BROMOTHYMOL BLUE

PROCEDURE

Bromothymol blue is a pH indicator that changes color with a change in pH – although it appears blue in an alkaline solution, its color is green in a neutral solution. In this experiment, you will utilize bromothymol blue to simulate the color change that occurs as oxygen binds to hemoglobin and to illustrate how absorption characteristics change with color. Standard safety precautions must be used when working with any chemical. Do not ingest and avoid contact with eyes. Wash hands after contact.

The hemoglobin molecule is quite complex and made up of many atoms. We will not go into detail about how the molecule changes when bound with an oxygen molecule, as it is sufficient for us to consider that the absorption characteristics of the molecule will change depending upon whether it is oxygenated or not. Similarly to how we observe hemoglobin as red when bound with an oxygen molecule (and more purple if it is reduced), CO_2 in the form of carbonic acid reacts with the bromothymol blue molecule so that we observe a change in color from green to a more yellow color. Over time, the solution reacts with oxygen in the surrounding air and the pH will change, resulting in another change in color - returning to the initial shade of green.

Neutral Solution:

- Fill cuvette with prepared bromothymol blue solution (it should be in its neutral green phase). Place cuvette in Spectrophotometer. Position cuvette so that the side with a smooth surface is aligned with the ►on the spectrophotometer. **COLLECT** data. After graph is produced, click **STOP**.(failure to click **STOP** will result in loss of data.)

Acidic Solution:

- Pour bromothymol blue into a cup and gently blow on it (carefully, so as not to splatter the solution.) Wait 30-60 seconds to allow time for the reaction to occur and the solution to change color - note that the color change should be distinct. If you do not detect a color change, continue to blow until change is observed. Once the color change has happened, transfer the solution into a cuvette (to avoid spills, transfer solution with a funnel over a sink.) Place cuvette in Spectrophotometer, again positioning cuvette so that the side with a smooth surface is aligned with the ►on the spectrophotometer. **COLLECT** data. A prompt will ask you if you would like to erase the prior data, choose "Save Latest Run". This will allow you to analyze both experiments on the same graph.

Print out and save a copy of the absorption graphs. Label, either in LoggerPro or by writing on the graph, which solution is which. You will use the graph again for later questions.

ANALYSIS

Q6: Use the graphs you produced to evaluate which wavelengths of light are most strongly absorbed.

- **Neutral: _____**
- **Acidic: _____**

Q7: Compare the results obtained for both the neutral and acidic solutions. Which wavelengths of light in the 500 – 700 nm range are absorbed more in the neutral solution? Which wavelengths are absorbed more in the acidic solution?

Q8: Bromothymol Blue solution was used for this experiment because of its reactive properties. List the ways that Bromothymol Blue can be compared to hemoglobin in the human body.

PART THREE: PULSE OXIMETRY

INTRODUCTION

We all know that if we do not breathe, we die - but how do we know whether or not someone is receiving enough oxygen? Regardless of whether a person's respiratory rate is normal, the amount of oxygen being delivered throughout the body could be insufficient. For years, health care professionals would subject patients to endless poking and prodding to obtain blood samples from them - a method that was not only uncomfortable, but also ineffective. Health care workers had to wait for the results of the blood test before they were able to assess a patient's condition, and often by the time they received the results, the patient's condition had changed.

Many people in the health care industry recognized the need for an easier way to assess a patient's peripheral blood oxygen saturation levels (SpO2) and in the 1970's a new and improved form of pulse oximetry was developed. This provided an expedited, more convenient, and less invasive way to measure a patient's SpO2. The device known as a 'pulse oximeter' incorporates a finger probe equipped with a light emitting diode (LED) and photoreceptor to measure the difference in light absorption of oxygenated and deoxygenated (or reduced)

hemoglobin. Pulse oximetry uses a method called photoplethysmography (PPG) in which light and analysis of the properties of absorption provide a reliable determination of SPO2 levels in a person's blood. PPG's are a non-invasive, economical way to provide an accurate appraisal of SPO2, as well as a way to measure changes in the volume of blood to provide heart rate information (another common way of obtaining the heart rate is by using an electrocardiogram (EKG) sensor that measures the electrical activity of the heart).

PROCEDURE

Turning the device on:

Locate the ON/OFF switch on the side of the device and switch to the ON position. Initially the display will read "finger out"; gently place your index finger into the finger clamp so that the tip of your finger covers the LED light. Two numbers will display on the screen - one labeled "SPO2" and the other "BPM"; these numbers represent the "spot oxygen saturation" (blood oxygen saturation) level and the number of heart beats per minute (pulse), respectively. (As an energy saving mechanism, the pulse ox screen may go dark at times; to retrieve data simply tap the display.)

EXPERIMENT

Once you have placed the pulse oximeter on your fingertip, remain still for 30 seconds. If pulse readings appear inconsistent or if SPO2 reading is below 90%, try repositioning finger in probe device to obtain a better reading.

- **Record the data displayed - at rest:**
    - **SPO2 _____ %**
    - **BPM _____**

Next, use the pulse oximeter and determine how these numbers change when you hold your breath. Try holding your breath for a comfortable amount of time, not exceeding 30 seconds.

- **Record the data displayed - breath held:**
    - **SPO2 _____ %**
    - **BPM _____**

Next, test how movement affects the results. Wave your arm in the air for 5 - 10 seconds.

- **Record the data displayed - movement:**
    - **SPO2 _____ %**
    - **BPM _____**

ANALYSIS

From the varying pulsatile components, the device is able to measure the frequency of the heartbeat, which we call the pulse when measured in beats per minute. You can do this yourself by measuring the number of peaks in the graphical pulsatile component shown on the bottom of the device and the amount of time between these

peaks. First count the number of peaks **after** the first, *N,* and measure the time it took between these peaks in seconds. The frequency of the heart will is the total cycles divided by the time they occurred:

$$f = \frac{N}{t}$$

The pulse is the frequency given in cycles per minute:

$$Pulse = \frac{N}{t} * \frac{60\ s}{1\ min}.$$

APPLICATION IN A CLINICAL SETTING

The number that is displayed as "SPO2" represents the percentage of blood that is oxygenated relative to the maximum percentage that could potentially be oxygenated. On one side of the finger clamp there is a small LED light and on the side opposite the LED is a light sensitive detector. Two beams of light are emitted, one in the red visible light range (approx. 650 nm) and the other in the infrared range (approx. 900nm). As the light travels through tissue, bone and blood, the emission is attenuated resulting in only a portion of the light reaching the sensor. Of these components blood is the only variable (both bone and tissue remain constant). Therefore, only the amount of arterial blood, in which hemoglobin is bound to oxygen, will change from moment to moment. Because of this, we know that the readings will vary as the degree of oxygen bound to hemoglobin varies. The device accuracy is limited to its calibration range, which is typically between 70 and 100% for SPO2.

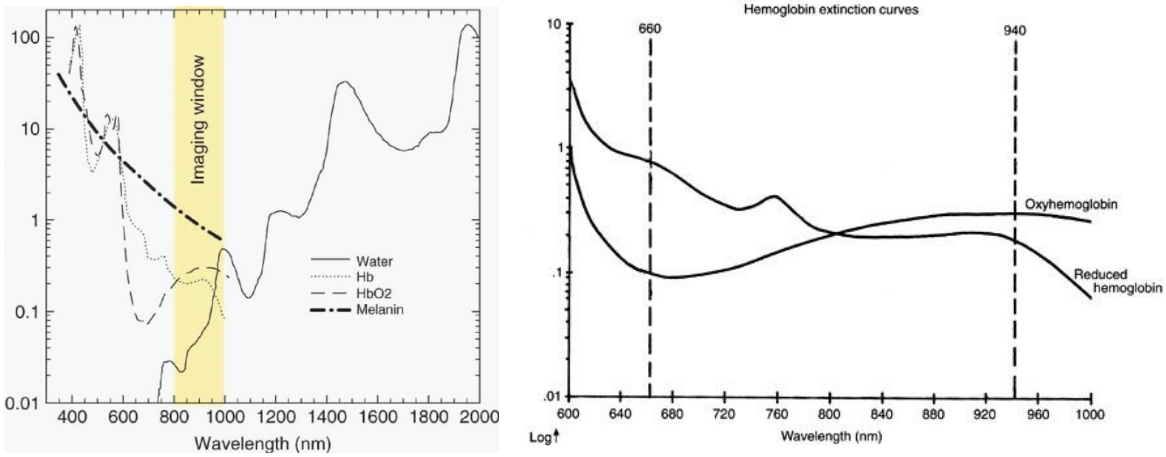


Figure 2- Absorption coefficient, α in cm^{-1} as a function of wavelength in nm.

Q9: From Figure 2, why do you think red and infrared lights are used in pulse oximetry? Could blue (475nm) or green lights (550 nm) be used? Why or why not?

Q10: Patients' body types and bone densities vary significantly from one person to the next; how does this affect the readings obtained when using a pulse oximeter?

SPO2 readings vary, on average, from 94 - 100% for a healthy adult. These results are based on numerous clinical studies that analyzed oxygen saturation readings from healthy subjects. Data are integrated to produce a complex algorithm used to effectively measure SPO2 with a pulse oximeter based on the absorption of red and infrared light. These readings may be affected by movement, inadequate blood flow (including effects from shock, cold temperatures, and medications), external light interference, venous pulsation, and nail polish or fake fingernails. Inaccurate readings may also occur if a patient is anemic. Anemia is a decrease in the number of red blood cells or amount of hemoglobin in the blood. These conditions can limit the accuracy of the readings obtained when using a pulse oximeter.

Q11: Why is a pulse-ox device overestimating the amount of oxygen carried by the blood for a patient with anemia?

Q12: What conditions may cause a person to have decreased SpO2 levels?

PART FOUR: SIMULATED PULSE OXIMETRY

In PART ONE of this lab you performed experiments with white light that illustrate how light absorption changes as solution concentration varies. PART TWO of this lab demonstrated how the color of a solution impacts the absorption of different wavelengths of light. We will now consider how absorbance at different wavelengths can be used to give information about a solution.

Figure 2 shows how absorbance varies for oxygenated and reduced hemoglobin. A pulse oximeter measures the absorbance of two wavelengths of light through blood to calculate the oxygen content of hemoglobin based on the absorbance measured for a given person. And now for something completely different: You have just discovered alien life!!! This creature uses not blood, but bromothymol blue to transport CO_2 throughout its body. How can you use what you know about the absorbance spectrum of bromothymol blue to noninvasively measure the CO_2 content of this creature's circulatory system? In your lab, you have red and green LEDs that you can use to take measurements.

(You will be using a light sensor from Vernier that measures in the unit of lux. One lux is one lumen per meter squared. Ideally measurements would be made in SI units of intensity for light (W/m^2). However, due to limitations of the sensor, different wavelengths of light will give different values for the same intensity. This is also true for our eyes as they are more sensitive to some wavelengths than others; in particular, our eyes are most sensitive to green light. For our purposes in this lab, we can safely make the assumption that a higher value of lux will correspond to a higher intensity and therefore we will not need to worry about this detail for the remainder of the lab.)

Q13: Look at the graph you generated for Part Two and the graph in the right of Figure 2. Given a red (~700 nm) and green (~565 nm) LED, would you measure a difference in absorbance for neutral and acidic (CO_2 rich) bromothymol blue solution? How would this be similar to or different from measuring the absorbance of oxygenated or reduced hemoglobin?

There are several complications in pulse oximetry that we will summarize here as a matter of course; some have already been touched upon. In pulse oximetry, absorbance measurements at two wavelengths of light are taken at wavelengths that have significantly different absorption coefficients for deoxygenated hemoglobin (Hb) and oxygenated hemoglobin (HbO). Looking at the ratio of these light intensity measurements using Beer's Law removes the path length dependence *l* of the measurements. However, for Pulse Oximetry to be effective and accurate it must also measure the absorbance of the pulsatile component of blood apart from the constants that are always present such as tissue, bone, and non-pulsatile blood. A pulse-ox device is designed to subtract out the absorbance of unchanging tissue components. Additionally, scattering effects of light in body tissues can make readings inaccurate. Scattering effects can be reduced by measuring at multiple wavelengths, where an elaborate scattering calculation must be made. Alternatively, many measurements of different patients can be made so that an empirically derived average scattering effect is determined and removed. To convert from an absorbance

measurement to a value for SPO2, all of these problems must be accounted for in the empirically generated algorithm used in the device or in the functional properties of the device itself.

In the next section of the lab, we will use prior experimental data that relates absorption to a desired variable (pH) similarly to how absorption coefficients are related to oxygen content in human blood. In particular, we will be looking at two different wavelengths of light and show that we can determine the pH of a substance which is itself linked to the amount of $C0_2$ in the solution.

PROCEDURE

Imagine you have a sample of neutral Bromothymol Blue sampled from a healthy alien and a sample that appears to be more acidic from an unhealthy alien. You want to determine the pH of the circulatory fluid for each creature. To do this, place the cuvette filled with Bromothymol Blue solution in the light shielding part of the simulated pulse oximeter. Connect the Vernier Light Sensor to Channel 1 of a LabPro board connected to the computer. If you still have the previous data from Part Two open, unplug the SpectroVis from the computer and in LoggerPro, under the **File** menu select **New** and click on **No** when prompted to save the spectrometer data. Otherwise, simply open LoggerPro. You will be measuring the amount of light that makes it through the solution with the light sensor.

- Make sure that the light detector range switch is set at the middle position, 0-600 lux.
- Flip switch to turn on the green LED (~565 nm) and shine light through cuvette ensuring that it is properly aligned with sensor.
    - Click COLLECT
    - Record data- use the peak value of the intensity
- Replace the neutral Bromothymol Blue solution with the acidic solution and repeat
- Repeat procedure by flipping the switch so that the LED emits red light (≈700 nm) and take measurements for both the neutral and acidic solutions
- Complete the data table below with the intensity values collected and the calculations listed

Assume that the bromothymol blue intensity measurements you have made represent the pulsatile component of the newly discovered alien's "blood." Absorbance has a logarithmic relationship to intensity (see Introduction), so you must take the log of your measurements. You will need to look at the ratio of these values to remove the dependence that absorbance has on path-length *l*. Finally, you will convert the absorbance ratio to a "blood" pH value that is related to CO_2 content, just as absorbance is used in the pulse oximeter to determine blood oxygen content.

DATA

	Green LED, ~565 nm	**Red LED, ~700 nm**		**Green LED, ~565 nm**	**Red LED, ~700 nm**
Measured Intensity, Neutral Solution (Lux)			**Measured Intensity, Acidic Solution (Lux)**		
Log(Intensity)			**Log(Intensity)**		

Ratio for neutral solution, Log(Green)/Log(Red)		**Ratio for acidic solution, Log(Green)/Log(Red)**	

Q14: Does a large light intensity (in lux) correspond to a large or small absorption coefficient?

Q15: Which color is best absorbed by the acidic bromothymol blue solution? Which color is least absorbed by the acidic bromothymol blue solution?

Q16: Which color is best absorbed by the neutral bromothymol blue solution? Which color is least absorbed by neutral bromothymol blue solution?

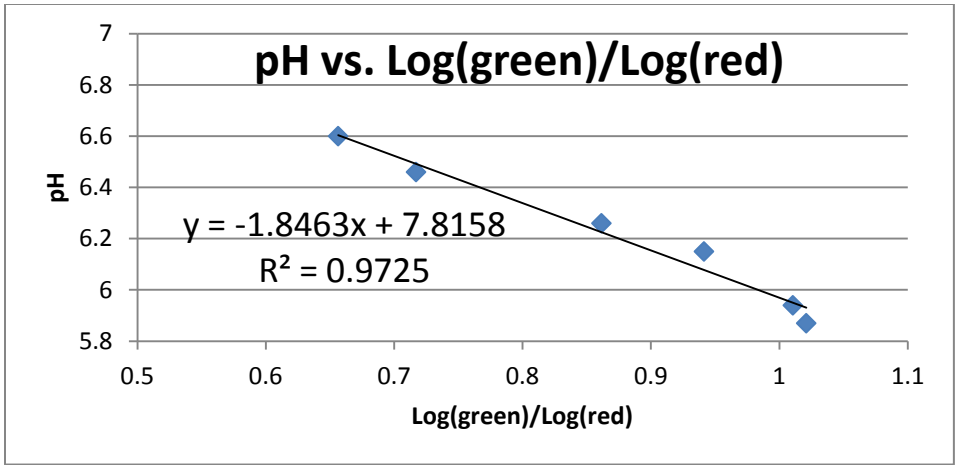
Q17: Which color has the least variation between the neutral and acidic bromothymol blue solution? Which color would this correspond to in the actual pulse oximeter in terms of absorption variation? Refer to figure 2.

Q18: Which color has the most variation between the neutral and acidic bromothymol blue solution? Which color would this correspond to in the actual pulse oximeter in terms of absorption variation?

Q19: From the graphs you generated using the spectrometer and the bromothymol blue solutions look at the absorption characteristics of the two solutions at the wavelengths of light emitted by the LED. Do the absorption graphs and the intensity we measured using the simulated pulse oximter in this section corroborate each other? At what wavelength of light would you measure to get the highest variation in intensity between the two solutions?

Q20: Economics played a role in determining the LED we used. It was much simpler and cheaper to buy a common single LED with the green/red wavelengths that we used than to pick the ideal colors with the most and least variation. Were there similar decisions made by the designers of the pulse oximeter?

Q21: The graph below shows an empirically derived relationship between the ratio you calculated in the table and pH. Use this relationship to determine the "blood" pH of the healthy and ill aliens' blood. Publish your results, and become famous!



Q22: Use the pH sensor provided to test the pH of the acidic solution. Calculate the percent error between the measured pH and the result derived from the graph.

Return your lab set-up to how it was before you started working. Make sure to flip the LED switch so it is set to "off" in the middle position.