Experiment 8: [http://genchemlab.wordpress.com/8-beers-law/](http://genchemlab.wordpress.com/8-beers-law/)

**Objectives**
- To understand absorbance and transmittance of light.
- To understand the Beer's Law equation, its uses, and its limitations.
- To determine the phosphate levels in water samples.
- To understand the concept of dilution.

**In the Lab**
- Students will work in pairs.

**Waste**
- Waste will be disposed of in the aqueous waste container.

**Safety**
- No special considerations.

Susan Jacobs was out on her evening walk and was about to return home because the recent thunderstorm had left the air muggy and walking miserable. As she turned, she saw a body lying in the tall grass and it wasn’t moving.

Moments later, a car appeared from around a curve in the road. Susan began waving her arms to get their attention. “Help! Stop! Help!”

As the car pulled over, Susan ran towards it. “There’s someone lying in the grass. I don’t know if they are alive or dead. Can you call the police?” Susan started to run back toward the body while the driver, an older man named Peter Edwards, found his phone and
started dialing 9-1-1. The operator picked up and Peter began to explain what was happening. “There’s a body lying on the side of the road. Maybe…”

Susan interrupted him to say “It’s a man and he’s not breathing.”

Peter continued, “He’s not breathing, send an ambulance. I guess he was hit by a car. We’re on Clark Road near the old gas station.” After answering the rest of the operator’s questions, Peter hung up and came over to Susan where she was trying to do CPR but it appeared he had been there a while because his clothes were wet. They continued CPR until the ambulance arrived and the paramedics took over, but the man was showing no signs of life.

The police arrived, talked to Susan and Peter, and then took Susan home. The body was taken to the coroner’s office, where the coroner began his examination. The next day, Susan saw the headline on the front of the paper: “Local Businessman Dead, Foul Play Suspected.” The autopsy of the man’s body revealed that he had a large quantity of water in his lungs and the cause of death was listed as drowning and that he had been dead about an hour before the body was found. Police labeled his death a homicide.

Three suspects quickly emerged—the man’s three adult children: the socialite daughter, a rancher son, and another son who is a mining engineer. All of his children would inherit substantial fortunes upon their father’s death and all three were known to have recently argued with him. Knowing the location of the drowning may help police identify the killer so police started gathering water samples from the three bodies of water within an hour of where he was found. Samples were collected from the swimming pool at the local country club; a large pond on one son’s ranch; and a mine-waste settling pond. Chemical analysis of the water from the man’s lungs reflected abnormally high concentrations of phosphates. In order to determine the location of the man’s death, the phosphate level of the three water sources must be determined along with the level in the water found in the man’s lungs.

**Phosphate Concentration**

The phosphate concentration can be determined spectrophotometrically by examining how light interacts with both known and unknown solutions. The phosphate, which will not absorb light in the visible region, in both the known and unknown solutions will be reacted with ammonium molybdate to create a blue-colored complex which will absorb light in the visible region. The intensity of the color will be proportional to the amount of the blue complex that forms, which, in turn, indicates the amount of phosphate present in the solution.

**Beer’s Law**

Light is a form of electromagnetic radiation and behaves like a wave when it interacts with matter. The region of visible light in the electromagnetic spectrum occurs between 400–700 nm. The combination of light in the visible region (red, orange, yellow, green, blue, indigo, and violet) creates white light, which is called polychromatic radiation. Light of a single wavelength is known as monochromatic radiation. Sources of monochromatic radiation include LEDs (light emitting diodes) and lasers (Light Amplification by Stimulated Emission of Radiation). While LEDs generally only produce light of a single wavelength, some lasers produce multiple, but distinct, wavelengths of light. When only one wavelength of light is needed from a laser, filters can be used to remove the unwanted wavelength(s) of light. Filters may also be used to remove ranges of wavelengths from polychromatic light.

The color of a substance is determined by its interaction with light. We can see blue because a sample absorbs all colors of light except blue, which is reflected. Alternatively, we can see blue because its complementary color, orange, is absent from the light. Complementary colors are any two colors that produce white light when added together. For example, combining red and green light, two complementary colors, will generate white light.

The amount of light absorbed or transmitted by a substance can be determined by measuring the intensity of the incident light and the intensity of light that is transmitted through the sample. The difference between the incident light and the light which is transmitted
through the sample is the absorbance. Absorbance (typical values are less than one) and transmittance (reported as a percent) are related by the following equation

\[ A = \log \left( \frac{1}{T} \right) \]

Beer’s Law allows us to correlate the absorbance to the concentration of a sample. However, a calibration curve must be constructed using the absorbance values of standard solutions which are solutions of known concentrations. For a Beer’s Law plot, the calibration curve is a straight line of the formula

\[ A = \varepsilon l c \]

Where
- \( A \) = absorbance (light absorbed by the sample)
- \( \varepsilon \) = molar absorptivity (a constant for a given substance at a given wavelength of light)
- \( l \) = path length (distance light travels through the sample)
- \( c \) = concentration of the solution

Compared to the formula for a line, we can make the following comparison:

\[ A = \varepsilon l c \]
\[ y = mx + b \]

Since we will create a calibration curve with solutions of known concentration, the value for the molar absorptivity will be the same for all solutions. Likewise, the path length will be the same for all samples. As a result, the slope of the line will be equal to their product (\( \varepsilon l \)). The concentration of the solution is the independent variable and corresponds to \( x \) in the formula for a line. \( A \) is the dependent variable and corresponds to \( y \). The formula for Beer’s Law does not show an intercept (i.e., \( b = 0 \)) which makes sense because if the concentration of the species being studied is zero, we should have an absorbance of zero. Once we have created a calibration line, the concentration of unknown solutions can be determined based on the graph and formula of the calibration line.
There are some limitations of Beer’s Law that must be considered.

1. Beer’s Law is only valid for concentrations below 0.01 M. At higher concentrations, the molecules are closer together and they may interact with each other, influencing the absorbance values. Solutions which are too concentrated can be diluted before use.

2. The system under investigation should be free of other species that may interfere with the absorbance of the species being studied.

3. The molar absorptivity relates to the probability of an electronic transition. Beer’s Law assumes that the absorptivity is constant for a given substance at a given wavelength.

**Cuvettes**

In doing a Beer’s Law experiment, there are two options for ensuring the same path length. Use the same cuvette for all samples or use a set of matched cuvettes (also known as matched cells). They are called matched cells because their size and optical properties are identical. Due to the specifics of these cells, they are rather expensive. Using a single cuvette accomplishes the same task and eliminates the need for matched cells.

**Colorimeters**

In this experiment, we will use a colorimeter to measure the absorbance values of both standard solutions and unknowns in order to determine the concentration of the unknown solutions. The colorimeters (see Figure 8.3) used emit one of three wavelengths of light. The light passes through the sample and the detector measures the amount of light transmitted through the sample (see Figure 8.4). We will be doing what is known as dual-beam colorimetry because there are two beams of light that will go through two paths. One of the beams will go through a cuvette containing the solvent or other appropriate solution (cell holder “R”) to account for any light absorbance by the cuvette and/or the solution/solvent. This will be known as the blank or reference solution. The other beam will go through the sample (cell holder “S”) being studied so the reported value will actually be a reporting of the difference in the amount absorbed between the two samples. The use of a dual beam eliminates variations in the signal due to changes in the intensity of the light. Pay attention to the following issues when using a colorimeter:

- Always put a cap on the cuvette.
- All cuvettes should be wiped clean and dried on the outside with a lint-free tissue.
- All solutions should be free of bubbles.
- Rinse the cuvette with the solution you are about to use and then refill it to take the measurement.
Experiment 8 • Application of Beer’s Law

Color Development
The phosphate itself will not absorb light so placing the phosphate solutions into the colorimeter will result in a near zero absorbance value regardless of the phosphate concentration. However, we can add other reagents, ammonium molybdate and stannous chloride, which will result in the formation of a blue species that absorbs light. The concentration of that species will be the same as the phosphate concentration because phosphate will be the limiting reagent.

Materials
ammonium molybdate solution
stannous chloride solution
20.0 ppm phosphate solution
10 mL graduated cylinder
50 mL graduated cylinder
6 inch test tubes
droppers
2 cuvettes and caps
colorimeter
lint-free tissues
test tube rack
beakers

Procedure
1. Wash all glassware to be used in hot water and rinse with distilled water prior to use.

2. Connect the colorimeter to the workstation. Make sure no other probes are connected to the workstation.

3. After verifying that your TA has logged you in to the workstation, press “MAIN MENU” on the workstation.

4. Select “COLORIMETRY/FLUOR/TURB” and then select “COLORIMETRY.”

5. Select “RED LED” to use the 635 nm wavelength.

6. Select “KINETICS” on the workstation.

7. Obtain approximately 15 mL of the 20.0 ppm (parts per million) stock phosphate solution in a small beaker.

8. Prepare the standard solutions according to the following directions. Always record the exact volumes used since it is unlikely that you will get the exact amounts. For example, instead of 1.00 mL, you might have 1.08 mL. It is very important that you record the exact volume so that your concentrations will be correct in the graph which will be used to determine the concentration of the unknown.

   a. Use the 10 mL graduated cylinder and a dropper to measure 1 mL of the 20.0 ppm stock phosphate solution. Record the exact volume and pour into the 50 mL graduated cylinder.

   b. Add distilled water to a total volume of 20 mL. Record the exact total volume in the cylinder and pour into a clean, dry 6-inch test tube.

   c. Calculate the concentration of the solution \( M_1V_1 = M_2V_2 \) and label the test tube with the exact concentration.

   d. Repeat a–c for all five standard solutions using the volumes of phosphate and water shown in Table 8.1. Record the exact volumes used for each solution.

Table 8.1. Standard solutions.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Approximate Amount of 20.0 ppm phosphate solution (mL)</th>
<th>Approximate Amount of Water (mL)</th>
<th>Total Volume (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>19</td>
<td>20</td>
</tr>
<tr>
<td>B</td>
<td>2</td>
<td>18</td>
<td>20</td>
</tr>
<tr>
<td>C</td>
<td>3</td>
<td>17</td>
<td>20</td>
</tr>
<tr>
<td>D</td>
<td>4</td>
<td>16</td>
<td>20</td>
</tr>
<tr>
<td>E</td>
<td>5</td>
<td>15</td>
<td>20</td>
</tr>
</tbody>
</table>

9. Obtain 20 mL of distilled water in one of the 6-inch test tubes and label it “blank.”

10. Obtain approximately 10 mL of the ammonium molybdate solution in a small beaker.
The following steps are time sensitive. Wait 5 minutes after preparing the solutions before collecting data and then collect the data quickly, but carefully.

11. To all six test tubes (five standards and blank), add 20 drops of ammonium molybdate using a dropper and mix well.

12. Add 2 drops of the stannous chloride solution to all six test tubes and mix well.

13. Record the time. **Wait 5 minutes** after the addition of stannous chloride to begin collecting data. This insures that the formation of the blue complex is complete.

14. Rinse one of the cuvettes twice with the blank solution. Rinsing involves pouring a small amount of a solution in a cuvette and pouring it out. This removes any residual liquids from the cuvette to make sure the concentration of the solution is not affected by those residual liquids.

15. Fill the cuvette with the blank solution and cap the cuvette. Use a lint-free tissue to wipe the sides of the cuvette being careful to hold the cuvette by the top to avoid fingerprints on the sides.

16. Place the cuvette with the blank solution in the cell holder marked “R.” Use care when inserting the cell in the holder as it is designed to hold the cuvette by the corners only (see Figure 8.5).

17. Repeat steps 14 and 15 with the second cuvette and the blank solution.

18. Place the second cuvette with the blank solution in the cell holder marked “S.”

19. Slide the colorimeter lid closed and press “ENTER.” The minimum and maximum percent transmittance will be adjusted at this time and will take approximately 10 seconds to complete.

20. After this is complete, select “DISPLAY.”

21. Leave the blank solution in the cell holder marked “R” and remove the blank solution from the cell holder marked “S.”

22. Rinse the second cuvette with your 1 ppm standard (solution A).

23. Fill the cuvette with the 1 ppm solution and cap the cuvette. Use a lint-free tissue to wipe the sides of the cuvette being careful to hold the cuvette by the top to avoid fingerprints on the sides.

24. Place the cuvette with the 1 ppm solution into the cell holder marked “S.” Use care when inserting the cell in the holder.

25. Wait until the value has stabilized and then, in your lab notebook, record the absorbance for the solution.

26. Pour the solution into a waste beaker.

27. Repeat steps 22–26 with each of the remaining standards (solutions B, C, D, and E).

28. Pour the solutions from both cuvettes into a waste beaker.

29. Push the “MAIN MENU” key and then select “OTHER.”

30. Select “MANUAL ENTRY.”

31. Key in the value of the concentration for your first standard solution and press “ENTER.”
32. Key in the value of the absorbance for your first standard solution and press “ENTER.”

33. Repeat for each of the standard solutions. If you need to change the data, use the arrow keys to select a data point and then key in the new value and press “ENTER.”

34. Press “FILE OPTIONS” on the workstation and select “SAVE DATA.” When prompted for a file name use “001.” You must use the correct file name so that the data is associated with your Chem21 account. If you save your file with the wrong name, repeat the save with the correct name.

35. Check with your TA to make sure the file was saved and uploaded correctly.

36. Before continuing, verify with your TA that your standard absorbance curve is acceptable.

37. Press “MAIN MENU” on the workstation.

38. Select “COLORIMETRY/FLUOR/TURB” and then select “COLORIMETRY.”

39. Select “RED LED” to use the 635 nm wavelength.

40. Select “KINETICS” on the workstation.

41. Label test tubes for each of the known location samples and the unknown for the deceased businessman. Obtain approximately 20 mL of each of the four unknowns in clean, dry 6-inch test tubes.

42. Add approximately 20 mL of distilled water to a test tube to make a new blank solution.

43. To all five test tubes (four unknowns and blank), add 20 drops of ammonium molybdate using a dropper and mix well.
44. Add 2 drops of the stannous chloride solution to all five test tubes and mix well.

45. Record the time. **Wait 5 minutes** after the addition of stannous chloride to begin collecting data.

46. Repeat steps 14–27 with each of the four unknowns.

47. Dispose of all solutions in the aqueous waste container.

**Data Analysis**

1. Determine the exact concentrations of your standard solutions based on the exact volumes used in the experiment. *You completed this step in lab so that the concentrations could be manually entered in MeasureNet at steps 30–33.*

2. View the graph of the known concentration on Chem21. Using Beer’s Law:
   a. determine the slope
   b. plot the y-intercept
   c. determine R² value

These values can be found on your graph. **R² tells us how well the linear regression fits the data points.** R² = 0 means the data points are spread widely around the line. R² = 1 means the line fits on the data path (Figure 8.7).

3. Determine the actual phosphate concentrations of each of the four samples. Use the y-intercept as determined in the linear regression. In your discussion, discuss any differences between the actual value of the y-intercept and its theoretical value.

4. Determine the location where the drowning occurred.