DETERMINATION OF PHOSPHORUS IN RIVER WATER USING ABSORPTION SPECTROPHOTOMETRY

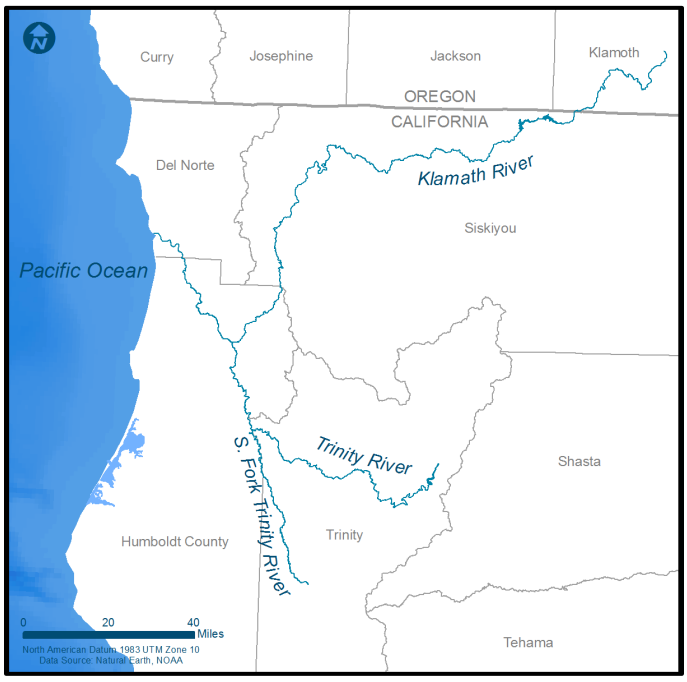
Objective

The objective of this experiment is to determine the concentration of phosphorus in local river water using absorption spectrophotometry. The levels of phosphorus in water samples collected last summer from the Klamath and Trinity Rivers will be measured and compared. The analysis will be accomplished by creating a Beer's Law plot, or standard curve, using solutions containing known amounts of phosphorus. This lab will provide hands on experience performing a spectrophotometric analysis and will also give an introduction to how chemistry is used in the field of water quality. The results from all of the lab group will be compiled and used to obtain a true value for phosphorus in our local rivers.

Background: A Water Quality Investigation

The Klamath and Trinity Rivers are the two main tributaries that make up the Klamath River Basin, which is the drainage for a large swath of land in southern Oregon and northern California. The confluence of the two rivers is located in Weitchpec, Humboldt County, California. The combined waters of these two tributaries continues to flow to the Pacific Ocean as the Klamath River (Figure 1).

The Klamath and Trinity River watersheds are very different in their land use and geology. The Trinity River watershed supports a sparse human population in a rugged mountain terrain. The Klamath River has similar usage and terrain in some areas, but it also extends into the high desert of southeast Oregon where the land has been converted to agricultural use, despite the lack of water. Excess fertilizer applied to the farmland contains nitrogen and phosphorus which is transported through water runoff to the Klamath River. The geology also contains minerals that are rich in phosphorus. Both nitrogen and phosphorus are considered nutrients and will encourage the formation of algal blooms in the river. Because of the differences in the land use and geology, the chemistry and



water quality (i.e., health) of the two rivers can differ significantly.

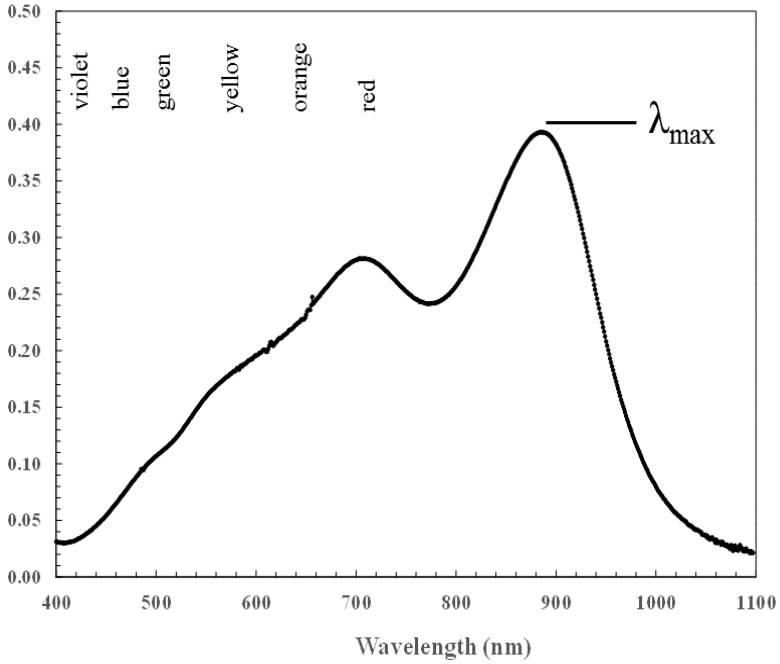
Nutrient levels are a very important chemical component affecting water quality in these rivers. All living things need nutrients to flourish, but excess nutrients can cause a water body to succumb to toxic conditions. One common condition that occurs with excess nutrients is eutrophication. Under eutrophic conditions, excess nutrients cause the development of large algal blooms, which in turn fuel other processes that deplete the oxygen in the water, stressing or killing organisms such as fish and invertebrates. It is the addition of the *limiting nutrient* to the ecosystem that induces eutrophication. The lack of the limiting nutrient, most often nitrogen or phosphorus, affects all biological growth in an aquatic ecosystem. In river water, phosphorus is commonly the limiting nutrient, unless the river system has been disturbed by high anthropogenic inputs of phosphorus.

Figure 1: Map showing the Klamath and Trinity River.

The U.S. Environmental Protection Agency (EPA) has set the limit for phosphorus at a concentration of 0.030 mg/L (ppm or parts per million) in river water. Phosphorus at or below this level is insufficient to allow eutrophication. The primary goal of this laboratory experiment is to use absorption spectrophotometry to determine whether the phosphorus levels in the Klamath and Trinity Rivers meet the EPA regulation.

Introduction to Absorption Spectrophotometry

When white light is passed through a solution containing a colored compound, the molecules absorb certain wavelengths of light. The wavelengths of light that are absorbed are unique to each molecular structure; therefore, each molecule has its own "fingerprint", which is called an absorption spectrum. For example, an absorption spectrum of a blue dye, usually a large molecule, is shown in Figure 2. As can be seen in the spectrum, the blue dye predominantly absorbs in the red region of the spectrum. The wavelengths in the blue region are not absorbed, but rather transmitted through the solution, and results in the molecule (or a solution containing the molecule) appearing blue in color. The wavelength where maximum absorbance occurs is called λ_{max} (lambda max), and for the molybdenum blue dye this occurs at 880 nm. Molybdenum blue is the colored molecule used to detect phosphorus in this experiment.



An instrument that is capable of measuring the absorption spectrum of a molecule is called a spectrophotometer (or sometimes spectrometer). This device measures the fraction of light that is transmitted through a solution. An incident beam of radiation is passed through a solution and onto a detector, where the intensity of light before the sample (I_0) is compared to the intensity of light after some of it has been absorbed by the molecules in the solution (I). The ratio of these two quantities is defined as transmittance (T) and is shown in equation 1a. Additionally, the transmittance of incident radiation can be related to the absorbance of the molecule by equation 1b.

Figure 2: Absorption Spectrum of Molybdenum Blue

$$T = \frac{I}{I_0} \qquad \text{(Eq 1a)}$$

$$A = -\log T \qquad \text{(Eq 1b)}$$

The absorbance of light can be used to quantitatively investigate how many light absorbing molecules are present in a solution. The more light a solution absorbs, the higher the concentration of light-absorbing molecules; therefore, concentration and absorbance are directly proportional. This proportionality is converted to equality by Beer's Law, which relates the amount of light absorbed by a molecule to the concentration of that molecule in solution, as shown in equation 2:

$$A = \epsilon \cdot b \cdot [C] \qquad \text{(Eq 2)}$$

where ε (Greek letter epsilon) is the molar absorptivity coefficient with units of $M^{-1}cm^{-1}$, $[C]$ is the concentration of the absorbing molecule in molarity (M), and b is the path length of light absorbed, which is almost always 1 cm and is the width of the container (cuvette) placed inside the spectrometer. The molar absorptivity coefficient is unique to each molecule and depends on wavelength; therefore it is important to specify the molar absorptivity coefficient at a certain

wavelength, which is typically done at the λ_{max} value when using Beer's Law. For example, the compound absorbing light in this experiment is molybdenum blue, which has a molar absorptivity coefficient of 2.8×10^4 $M^{-1}cm^{-1}$ at 880 nm.

Equation 2 (i.e. Beer's Law) reveals that when absorbance (A) is plotted on the y-axis and concentration in molarity is plotted on the x-axis a straight line is observed (for absorbance values less than 1 and a path length of 1 cm), where the slope of the line is the molar absorptivity coefficient (ε). By generating a Beer's Law Plot (or standard curve) as shown in Figure 3, the relationship between absorbance and concentration can be determined over a wide range of values. Once a standard curve is created, the absorbance of an unknown sample can be converted to concentration, such as the amount of phosphorus in a river water sample.

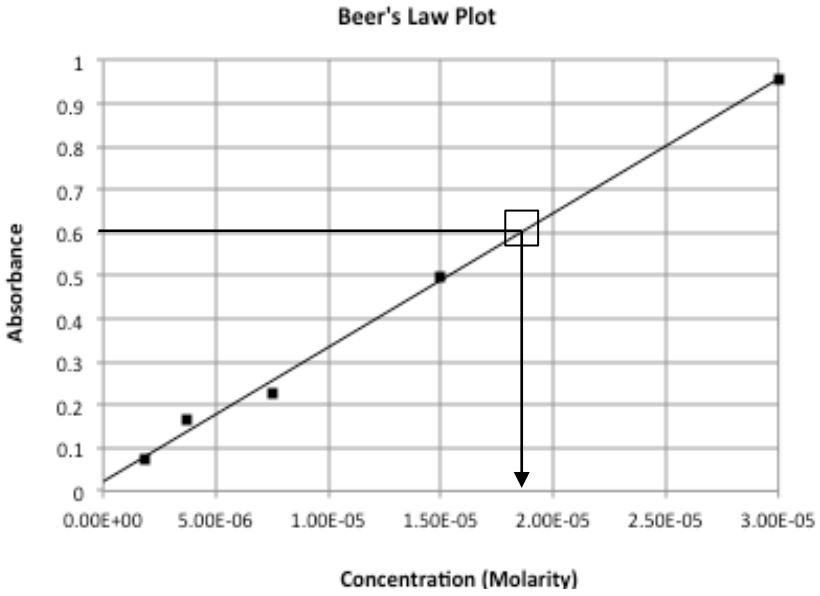


Figure 3: Beer's Law Plot. The black squares are from standard solutions; the open square represents an unknown sample concentration determined from its absorbance on the standard curve.

Procedure

Work in pairs. Obtain from the stock room and your drawer the equipment listed below. Locate the reagents that you will mix to obtain the coloring reagent, the phosphorus standard for the preparation of the standard curve, and the samples (Klamath River, Trinity River, Unknown Sample). Make sure that all your glassware is clean and that you do not contaminate any of the reagents or samples.

Note: The unknown sample was prepared by the stockroom and will test the accuracy of your results. Analyze the unknown sample alongside the river water samples.

To save time, one lab partner should work on Part 1 while the other lab partner works on Part 2.

Chemicals:	ascorbic acid ammonium molybdate sulfuric acid potassium antimonyl tartrate phosphorus stock solution (prepared using potassium dihydrogen phosphate)
Equipment and Supplies:	10 test tubes, medium size (18 x 150 mm) 1 mL volumetric pipette (stock room) 5 mL volumetric pipette (stock room) pipette bulb (stock room) Vernier LabQuest 2/Spectrophotometer (stock room) other miscellaneous glassware

Safety and Waste Disposal:

Any solutions containing ascorbic acid, ammonium molybdate, sulfuric acid or potassium antimonyl tartrate need to be disposed of in the waste container located in the laboratory. **Do not** dispose of these chemicals down the sink.

Part 1 – Preparation of Standard Solutions and Samples

Clean and dry 10 medium-sized (18 x 150 mm) test tubes. If they do not appear greasy, there is no need for soap. Rinse the 3 times with tap water and another 3 times with RO water. Shake dry. **Do not** dry the inside of the test tubes with paper towels. Any beakers used to hold water or reagents should be cleaned similarly.

Label 7 test tubes as "stock solution", "blank" and the others as #1-5. Using a volumetric pipette, aliquot 5.00 mL of RO water into 5 clean and dry test tubes (blank, label #2-5). Obtain about 15 mL of the phosphorus stock solution (1 delivery from the bottle dispenser) and place in the clean test tube labeled "stock solution". Using the 5.00 mL volumetric pipette, give the pipette a rinse with the solution and discard. Then remove 5.00 mL of the stock solution and add to the first test tube (label #1) not containing RO water. This is your most-concentrated phosphorus standard. Also deliver 5.00 mL to the second test tube (label #2) containing RO

water, and mix thoroughly using a stir rod. This is your second most-concentrated standard. From here, you will prepare the next standards using the serial dilution method.

To create serial dilutions, take 5.00 mL of the diluted solution from test tube #2, and transfer it the third test tube (label #3) containing 5.00 mL of RO water. Mix thoroughly. Repeat the serial dilution procedure 2 more times (label #4-5). You should now have 5 standard solutions that are all half the concentration of the preceding solution, plus another solution containing 5.oo mL of RO water that will be used to blank the instrument (labeled "blank").

Record the concentration, in molarity, of the phosphorus stock solution directly from the dispenser bottle and enter the value in the Data Sheet. This is the concentration of your first standard (label#1). Calculate the concentration of the 4 standard dilutions (label #2-5), and record values in Table 1.

Rinse the 5.00 mL pipette with RO water. Label separate test tubes for the Klamath River (KR), Trinity River (TR) and Unknown Sample (US). Use a clean 50 mL beaker and rinse the beaker with a small amount of sample (~ 10 mL). This rinse solution can be discarded down the drain. Fill the beaker with about 10 mL of sample, starting with the river samples and finally the Unknown Sample. Rinse the beaker and pipet with sample solution between samples. Deliver 5.00 mL of each sample to the labeled test tubes.

<u>Part 2 – Prepare the Combined Reagent (Coloring Reagent) and Calibration of the Spectrophotometer</u>

The coloring reagent will need to be added to each standard solution and sample. Prepare the coloring reagent by taking a clean 50 mL beaker and dispensing a full delivery of: (1) sulfuric acid, (2) potassium antimonyl tartrate, (3) ammonium molybdate, and (4) ascorbic acid. Mix after the addition of each reagent using a stir rod. The combination of these chemicals in the presence of phosphorus creates a very intensely colored molecule called molybdenum blue. This chemical reaction is very specific and only phosphorus, in the form of phosphate, causes the color formation. The coloring reagent will be added to each solution (standards, sample and blank).

Add 1.oo mL of coloring agent to 5.00 mL of RO water (test tube labeled "blank"). Communicate with your partner to determine the correct test tube to use for the blank. Stir thoroughly with a clean stir rod. Use this blank solution to calibrate the spectrophotometer. The instrument will be blanked so that a zero baseline at $\boldsymbol{\lambda}_{max}$ = 880 nm can be established.

Fill the cuvette about ¾ of the way full with the blank. Wipe the clear sides of the cuvette with a Kimwipe and avoid touching the clear sides of the cuvette with your fingers. Dust, grease, and/or oil from your fingers and/or the room will affect the measurement. Note that the

orientation of the cuvette as you place it in the spectrophotometer. FOLLOW THE INSTRUCTIONS ON THE LAMINATED SHEET to finish calibrating the spectrophotometer.

Part 3 – Beer's Law

Absorbance measurements of Standard Solutions

Add 1.00 mL of coloring reagent, stir thoroughly, to each test tube containing either a standard (#1-#5). Wait 10 minutes for optimal color formation, but no more than 30 minutes. Do not add the coloring reagent unless you are at least 10 minutes away from analyzing them on the spectrophotometer. After the addition of the coloring reagent, it could be that some solutions are not substantially colored. Remember that the spectrophotometer can detect color formation better than the human eye.

Once the spectrophotometer is blanked, start with your most dilute standard solution (label #5). Rinse the cuvette with a very small amount of this solution and discard to waste. Then transfer enough liquid to fill the cuvette about ¾ of the way full. Wipe the clear sides of the cuvette with a Kimwipe to remove any residual liquid, and place it in the spectrophotometer. The same orientation of the cuvette used to measure the blank should be used for all samples. After recording the absorbance at λ_{max} (880 nm) in Table 1 in the Data Sheet, transfer the standard from the cuvette back to its original test tube. Next, place the standard in test tube (label #4) into the cuvette and repeat. Continue until the measured absorbance of all 5 standards (label #1-5), going from most dilute to most concentrated is complete.

Review the absorbance values of each standard solution in Table 1. The absorbance reading of each standard solution should be roughly twice as large as the previous reading. If this is not the case you may need to make the standard solutions again or measure the absorbance again. DO NOT proceed until your absorbance data are satisfactory. Consult the lab instructor if you are unsure.

Measure Absorbance of the River Water and Unknown Samples

Run the samples (Klamath River, Trinity River, Unknown Sample) as you did the standards. Add 1.00 mL of coloring reagent to each sample and allow 10 minutes for full color formation, but no more than 30 minutes. Rinse the cuvette well with RO water and measure the absorbance of each sample. Measure the least colored sample first and work towards the most colored. Record the absorbance in Table 2 of the Data Sheet and calculate the concentration for each sample. The concentration of each sample can be determined by using the Beer's Law Plot (standard curve).

Construct a Beer's Law Plot and Determine the Concentration of Phosphorus in Samples

When all of the data have been recorded power down the LabQuest, disconnect the spectrophotometer, power it back on and proceed with the next step. This procedure of powering down, disconnecting, and powering back on will save the lamp from drastic power changes and make generating the Beer's Law plot more straightforward as the LabQuest will be reset to nominal conditions for making graphs.

Another laminated set of instructions has been provided to make the Beer's Law plot or standard curve. Use these instructions and generate the plot (see Fig. 3), and record your fitted equation in the form of y = mx + b in the Data Sheet. DO NOT use your river or unknown samples in making the Beer's Law plot. The slope of your best-fit line (i.e. the experimentally determined molar absorptivity coefficient) should be very close to the molar absorptivity coefficient of 2.8×10^4 $M^{-1}cm^{-1}$ at 880 nm found in the literature. Consult with your lab instructor if these values are not close. Use the equation in the form of y = mx + b calculated above and use the absorbance (y) for each sample and solving for concentration (x).

Dispose of all solutions containing coloring reagent in the proper waste container. Ask your laboratory instructor if you are uncertain whether a particular solution is waste.

Data Sheet

Name: _____

Lab Day:_____ Time:_____

Lab Partner:_____

Record your experimental data in the appropriate spaces below. You must include units and the correct number of significant figures for full credit.

Table 1 – Standard Solutions

Standard Solution	Molarity (M)	Absorbance (A)
Standard 1 (Stock)		
Standard 2		
Standard 3		
Standard 4		
Standard 5		

Table 2 – Samples

Sample	Absorbance (A)
Trinity River	
Klamath River	
Unknown Sample	

Beer's Law Plot

Working range of standard solution absorbance (min → max):

_____

Record your best-fit equation in the form of $y = mx + b$ in the space below.

From the best-fit equation, report the value of the molar absorptivity coefficient (ε) at λ_{max} = 880 nm with proper units in the space provided.

_____

Results

a. Calculate the concentration, in molarity, of phosphorus in each sample using the equation for the best-fit line on the Beer's Law plot. Report your values in Table 3. SHOW CALCULATIONS.

   NOTE: If the absorbance of the sample is below the working range listed on page 7, the concentration of phosphorus in the sample cannot be accurately determined. In cases like these, the concentration for the sample should be reported as NR, or not reported. This does not suggest there is no phosphorus, but it does indicate that the concentration in the sample is below the range that the chemical method can accurately measure. It is best practice to only quantify a concentration in a sample if it falls within the working range of the standards.

b. Very common concentration units used by scientists and engineers in the field of water quality are parts-per-million (ppm) and parts-per-billion (ppb). These units can be expressed in many ways, but ppm is commonly reported in mg/L. Likewise, ppb is reported as µg/L. In the space below, convert the molarity (mol/L) of phosphorus (molar mass = 30.97 g/mol) for each sample to units of ppm and ppb. SHOW CALCULATIONS. Record your values in Table 3. If the molarity of phosphorus in a sample is NR, the ppm and ppb will also not be reported (see NOTE above).

Table 3 – Phosphorus Levels in Samples

Standard Solution	Molarity	ppm (mg/L)	ppb (µg/L)
Trinity River			
Klamath River			
Unknown Sample			

As a group (you and your partner), come to a consensus on the reported values in Table 3. Record your values, in mg/L, for each sample on the sheet located at the front of the laboratory.

Over 200 groups from both CHEM 107 and 109 will determine the phosphorus levels in these river samples this semester. The phosphorus concentrations will be compiled in an effort to determine the true amount of phosphorus in the Klamath and Trinity Rivers. The information will allow local agencies and scientists to assess the water quality of the rivers during this past summer when the samples were collected. The unknown sample value will also be recorded to help verify that your analysis and calculations were performed properly.

Questions

1. The EPA limit for total phosphorus in river water is set at 0.03 mg/L, a level considered not to contribute to eutrophication. Using the EPA limit as a guideline, is there any concern about the levels of phosphorus and water quality in the Klamath or Trinity Rivers? Explain. To properly answer this question, convert the molarity of your lowest standard (Standard #5) to units of ppm (mg/L).

   ppm (mg/L) of lowest standard (Standard #5) _____

2. It is common that the limiting nutrient in a river system is either nitrogen or phosphorus. Both nutrients are required in the production of algal biomass during photosynthesis. Below is the complete reaction for photosynthesis with products of algal biomass and oxygen gas.

$$106\ CO_2 + 16\ NO_3^- + H_2PO_4^- + 122H_2O + 17\ H^+ + \text{sunlight} \rightarrow (CH_2O)_{106}(NH_3)_{16}\ H_3PO_4 + 138\ O_2$$

The addition of the limiting nutrient (i.e., limiting reactant) to a river system initiates the production of biomass and can lead to algal blooms that cause eutrophic conditions. Therefore, any addition of the limiting nutrient drives the photosynthesis reaction to form the products and the reaction continues until the limiting nutrient is completely depleted from the water.

On the day that the Klamath and Trinity River samples were collected last summer, algal blooms were not observed in either river. Base on this observation taken during the sample collection and the concentration of phosphorus measured in each river sample, speculate whether nitrogen or phosphorus is the limiting nutrient in these rivers? Explain.

	Limiting Nutrient	
Klamath River	_____	briefly explain:
Trinity River	_____	briefly explain:

3. The principle chemical species containing phosphorus in natural waters is phosphate ion (PO_4^{3-}). The chemical method used in this experiment estimated the amount of phosphorus through the reaction of PO_4^{3-} with the coloring reagent to form molybdenum blue. Using the ppm (mg/L) as phosphorus (P) from your Unknown Sample (Table 3), calculate the concentration of phosphate (PO_4^{3-}) in the Unknown Sample in units of ppm (mg/L).