Spectrophotometric Determination of Phosphates in Water

Background

Controlled concentration of phosphorus in water is critical for a stable ecosystem. Aquatic plants are dependent on a certain amount of phosphorus in order to survive; however, excess phosphorus leads to eutrophication, the over enrichment of a water body with nutrients. This can lead to a surge in biomass growth manifesting as an algal bloom that blocks sunlight from other aquatic plants and starves the water of oxygen adversely effecting the environment. The EPA Water Quality Criteria recommends a maximum phosphorus concentration of $100\,\mu g/L$ in rivers and streams and $25\,\mu g/L$ in lakes in order to prevent eutrophication from occurring. Phosphorus is also found in tap water where it is added in order to inhibit corrosion of piping - a thin layer of phosphorus builds up on the inside of the pipes and prevents any metals from seeping into the water. A maximum phosphorus concentration of $0.14\,\mu g/L$ is mandated by the California Water Resources Control Board. In this experiment, you will measure phosphorus concentrations in water from several locations by spectrophotometry with a molybdate complex that turns dark blue in the presence of phosphorus.

The phosphorus cycle is a simple method to comprehend the role phosphorus plays in the environment. Since phosphorus has limited methods to enter vapor form, the cycle predominately moves through organisms and ground/water phases. Phosphorus is essential to various biological compounds in organisms, such as DNA and proteins. When organisms die, the organic phosphorus taken up by the organism returns to the soil. Various bacteria and fungi initiate processes that then break down the phosphorus which is either picked up by ground water or rainfall and swept into rivers and lakes, or it may build up as sediment in a mineralization process leading to phosphorus rich rocks and minerals that may erode later on. Alternatively, plants can uptake the phosphorus from the soil directly.

Phosphorus is present mainly in the form of phosphates. These phosphates exist in three forms. Organic phosphates exist in biological systems and play a role in the life cycle of the organism. An example would be adenosine triphosphate (ATP) that provides energy to cells. Metaphosphates are found in inorganic structures such as metals and salts. Orthophosphates are the simplest form of phosphate. Unlike organic phosphates and metaphosphates which are not readily available, orthophosphates are reactive forms of phosphate in water and can be directly measured.

Phosphorus can seep into the environment from both natural sources and man-made sources. Natural sources include rocks, minerals, and sediment while man-made sources include fertilizers and detergents. Many US states have banned phosphate detergents leading to most detergent companies reformulating their products. Phosphate-based fertilizers are especially effective at growing crops and are therefore used on many fields. Any rainfall over a field will drain into a body of water and lead to high concentrations of phosphorus. This can be extremely problematic in agricultural regions such as the central valley where a significant portion of land gets fertilized causing large increases in phosphates in surrounding rivers and streams.

Water Sources

The Arboretum Waterway extends throughout the UC Davis Arboretum along what was once the north fork of Putah Creek which was diverted away from Davis in the 1870s to prevent farmland flooding - this waterway is only connected to Putah Creek if its water levels are very high and water is allowed to escape
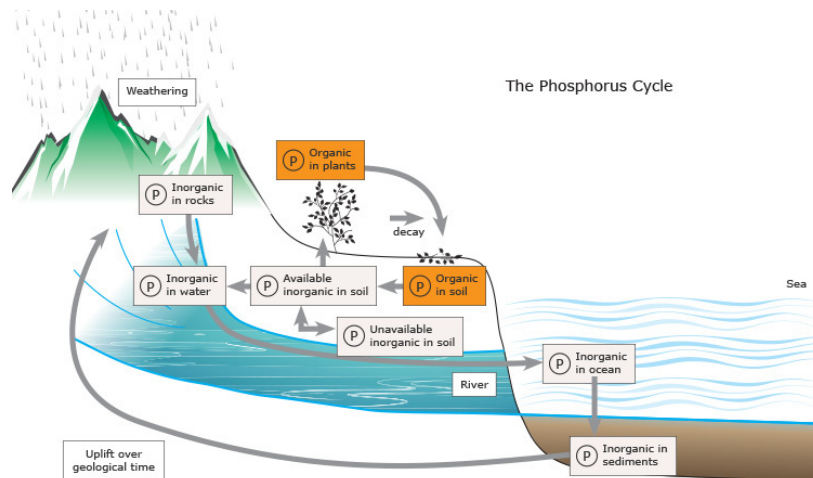
Figure 1: Schematic of the phosphorus cycle illustrating the mechanism by which phosphorus naturally enters rivers and lakes

and drain into the creek. Today the Arboretum Waterway is effectively a storm water basin collecting rain water that falls onto the central UC Davis campus.

Arcade Creek flows through 16.2 mile through mostly urban areas in Sacramento County. Because its watershed is almost completely urbanized, Arcade Creek is also susceptible to water quality issues making it a suitable water source to study in this experiment.

Spectrophotometry

The method we will use to measure the concentration of pollutants in the water is called Spectrophotometry, which is a procedure that determines how much a chemical compound absorbs and transmits light. Having the light absorbance profile of a solution, we can compare it with a known sample and identify which compounds are present and also their quantities. Spectrophotometry has an immense importance in the scientific community, being one of the most widely used methods for quantifying compounds in different fields such as chemistry, physics, and biology. One of the most common uses of spectrophotometry is the analysis of water samples, which will be explored in this experiment.

A spectrophotometer measures the intensity of light that passes through a solution. This is accomplished by shining light at a specific wavelength through a solution and comparing the intensity with a reference blank. Figure 2 depicts a simple spectrophotometer setup. The spectrophotometers in this lab differ slightly from that depicted - a series of LEDs outputting light at specific wavelengths replaces the white light source and monochromator. Fundamentally the mechanism at play is unchanged.

Spectrophotometers are only able to emit and detect light within a specific wavelength range and are classified accordingly. For example, a common type is the UV-visible spectrophotometer that emits and absorbs photons with wavelengths in the ultraviolet range ($185\,\text{nm}$ to $400\,\text{nm}$) and visible range ($400\,\text{nm}$ to $700\,\text{nm}$), while another type, the IR spectrophotometer, uses wavelengths in the infrared range ($700\,\text{nm}$ to $1500\,\text{nm}$).

The most accessible of these types is the visible light spectrophotometer, which will be used in this experiment. Particularly for visible light Spectrophotometry, the solutions color can be used as an indicator of the solutions light absorptivity and transmissivity. For example, if a solution absorbs red light it will
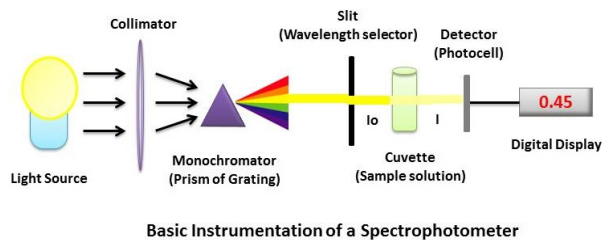
Figure 2: Basic schematic of a spectrophotometer, from https://chem.libretexts.org

appear green since these are complementary colors. Certain chemical compounds absorb light at specific wavelengths unique to the system. If the solute is present in a higher concentration, more light will be absorbed as described by the Beer-Lambert equation, also known as Beer's Law. This expression relates the absorbance to the solute concentration in a given solution

$$A = eLc \qquad (1)$$

where A is the compounds absorbance (unitless), e is the molar absortivity (L/(mol cm)), L is the path length (cm) and c is the compound concentration (mol/L). Beer's law is dependent on several assumptions that are explored in the pre-lab section. In order to obtain quantitative absorption data a set of standards with known concentration must be analyzed in order to create a list of values to compare with the unknown sample.

Experimental

In this experiment, orthophosphates react with ammonium heptamolybdate to form a phosphomolybdic acid. This complex then is reduced by ascorbic acid in the presence of potassium antimony tartrate to form molybdenum blue. Consequently, measured concentration of molybdenum blue by spectroscopy stoichiometrically determines the concentration of orthophosphates in the water sample. The basic chemical reactions describing this color change are the formation of phosphomolybdic acid:

$$72\,H^+ + PO_4{}^{3-} + 12\,Mo_7\,O_{24}{}^{6-} \rightarrow 7\,P\,Mo_{12}\,O_{40}{}^{3-} + 36\,H_2O \tag{2}$$
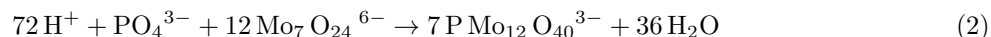
which is followed by the formation of molybdenum blue from phosphomolybdic acid:

$$P\,Mo_{12}\,O_{40}{}^{3-} + 2\,C_6H_8O_6 \rightarrow PMo_{12}O_{40}{}^{7-} + 2\,C_6H_6O_6 + 4\,H^+ \tag{3}$$
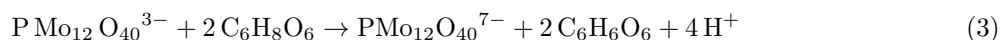
Materials

1. 10 mL of each phosphorus calibration sample (0.0625, 0.125, 0.25, 0.5, and 1 mg/L)
2. 10 mL of Arboretum Waterway sample
3. 10 mL of Arcade Creek sample
4. 10 mL of tap water sample
5. 90 mL reagent
6. 9 cuvettes
7. 10 mL graduated cylinder
8. DI Water

Procedure

1. Rinse graduated cylinders and cuvettes with DI water.

2. In each cuvette add 6 mL reagent and 6 mL of calibration sample. Remember to label each cuvette. Repeat for the 3 water samples.

3. For the blank, add 6 mL of DI water and 6 mL of reagent to a cuvette.

4. Allow the solutions to sit for 30 minutes for the color to develop.

5. Start up spectrophotometer

6. Record absorptivity for the blank and calibration samples in table 1.

7. Record absorptivities for the water samples in table 2.

8. Construct the calibration curve by plotting absorptivity versus concentration.

9. Determine the equation of best linear fit.

4

Table 1: Standard Absorptivity

Standard Concentration	Absorbance

Table 2: Samples

Sample	Absorptivity

Experimental Questions

These questions are intended to be answered while you are waiting for the blue color to develop.

1. If you are unfamiliar with the spectrophotometer equipment and software, take this time to review the equipment procedure.

2. What do you expect your calibration curve to look like?

3. What is turbidity? What is the influence of turbidity in your water samples?

4. What color is your reagent? How would the color of the reagent influence your data measurement? How do you account for the reagent color in your measurement?

Post Lab Questions

1. What does your standard curve look like? Does this agree with your expectations for the standard curve? How will this impact the results of your experiment?

2. It is likely that as the phosphorus concentration in the sample gets larger the absorbance points will stop falling on a straight line. What may be the cause for such a deviation from a linear fit?

3. Using your standard curve, calculate the phosphate concentration in the three water samples collected. Be sure to include appropriate units.

Sample	Phosphate Concentration

4. Which species of phosphorus are measured (hint: look at reaction equations 2 and 3)? Is this the total phosphorus? If not, why not?

5. Which water source had the highest phosphate concentration? The lowest?

6. Why might the phosphate concentration differ between sources?

7. What are the ramifications of the given phosphate concentrations on the water sources? (Hint: Refer to background section)

8. Suppose all water samples were treated with chemicals that break down organic phosphorus into orthophosphate. How would the absorptivity be affected, if at all? Discuss.

References

1. http://www.water.ncsu.edu/watershedss/info/phos.html

2. E. A. Nagul, I. D. McKelvie, P. Worsfold, and S. D. Kolev, Analytica Chimica Acta 890, 60 (2015).

3. https://www.britannica.com/science/phosphorus-cycle

Reagent Preparation

Once fully mixed, the reagent for the molybdenum blue assay is good for several hours, starting out mostly transparent and turning darker blue with age. For best results, the reagent should be prepared as separate parts, to be combined directly prior to running the test. Unmixed, these components should have a longer shelf life. To make 1L of mixed reagent, three separate parts should be prepared:

1. 800 mL of 0.5 M HCl (dilute with DI or purified water)

2. 1.9 g ammonium heptamolybdate tetrahydrate added to 100 mL DI water

3. 0.044 g potassium antimony tartrate trihydrate added to 100 mL DI water (serial dilutions may help, depending on the accuracy of available scales)

Mix all three components together and let sit for a few minutes prior to testing phosphate levels. The mixture should be a faint yellow or blue color. If the solution turns dark blue shortly after mixing, then the cause is likely phosphate contamination either from the water used in preparing solutions or from glassware and containers. It is recommended to use distilled or purified water if available.

Water Samples

This experiment gives a lot of room to explore, especially with the water samples studied. We chose to look at water from the UC Davis Arboretum, Arcade Creek near American River College (ARC), and tap water from ARC. One potential experiment could be based around taking water from several spots along the Sacramento river. Water could be taken from before Shasta Lake, after the lake, and at several more spots as the river travels through more and more agricultural land. Questions could be crafted pertaining to the amount of phosphorus accumulated as a function of farmland the river traverses. Alternatively, water could be taken from the same spot at several different times over the course of a year to monitor phosphorous concentration as a function of time.