Phosphate: Its Importance in Water and Its Measurement

Phosphorus in the form of phosphate (PO_4^{-3}) is an essential plant nutrient and is a major component of most fertilizers. Erosion and runoff can result in large amounts in bodies of water and lead to eutrophication, the depletion of oxygen from excessive aquatic growth. Animal die-off, toxins and foul water can also result. These are some of the primary reasons that the monitoring of phosphate levels in water is extremely important.

This unit is challenging and chemistry-intensive. It is recommended for high school students with a background in chemistry.

Although the pre and post discussions are necessary to provide important information about each topic, it is the activity that is most vital to this unit. Be sure to allow plenty of time to complete the activity.

Pre-Activity Discussion (Answers can be found in the Background Information section below)

Before beginning the activity, ask students:
1) What is phosphate and what are some of its uses?
2) Why is the level of phosphate in the water important? How does it affect living things?
3) How does the presence of phosphate in water cause oxygen depletion?
4) What human activities affect the level of phosphate in the water?

The Activity

Equipment needed

--Small table
-- Hanna HI 736 pocket spectrophotometer
--Distilled water for cleaning the cuvette which comes with the spectrophotometer
--Long-handled dipper, if needed, to sample from a steep bank
--Work/data sheets
--Data form and clipboard

Site selection and set-up

Choose a site with room for a table and easy access to the stream. Be sure it is upstream from activities that may cause turbidity (BMI collection, stream speed) and free of obstructions. Avoid sites with nettles, thorny plants or poison ivy. The water should be deep enough to collect samples without getting sediment from the stream bottom.

The Hanna HI 736 Spectrophotometer: How to Ensure an Accurate Measurement

1. The sample must contain no debris.
2. Whenever the cuvette is placed into the measurement cell the outside must be dry and free of fingerprints, oil, and dirt. Use the cloth supplied to clean the cuvette.
3. Shaking the cuvette can cause bubbles, resulting in high readings. For accurate readings, remove bubbles by swirling or gently tapping the cuvette.
4. Do not let the reacted sample stand for too long. This will affect the results.

5. After the reading, it is important to discard the sample immediately and rinse the cuvette with distilled water or the cuvette may be permanently stained.

Phosphate determination with the Hanna HI 736 pocket spectrophotometer

The minimum phosphate as P concentration that triggers algae blooms is only 0.05 mg/L (50 ppb, parts per billion). An instrument of very high sensitivity is therefore needed if one is to obtain environmentally significant results. The Hanna Instruments HI 736 ultra low range phosphorus checker has a range from 0 to 0.20 mg/L (0 to 200 ppb) phosphate as P, so fills the bill. It is a battery-operated fixed wavelength (525 nm) spectrophotometer that uses a light-emitting diode light source and a silicon photocell light detector.

Procedure

1. Turn the meter on by pressing the button (there's only one). All display segments will be shown. When the display shows **Add Press C.1** the meter is ready.
2. Fill the cuvette with 10 mL of unreacted sample and replace the cap. (The outside of the cuvette MUST be clean and dry.) Place the cuvette into the meter and close the meter's cover.
3. Press the button. When the display shows **Add C.2** with **Press** blinking, the meter is zeroed.
4. Remove the cuvette from the meter and unscrew its cap. Add the contents of one packet of HI 736-25 reagent. Replace the cap and shake the cuvette gently for 2 minutes until the powder is completely dissolved. Place the cuvette back into the meter and close the meter's cover.
5. Press and hold the button until the timer is shown on the display; the display will show the countdown prior to the measurement.
6. The meter then displays the concentration of phosphate as P in ppb. The meter will then turn off automatically after 2 minutes.

If your reading is a flashing **200**, the P concentration is greater than 200 ppb. Try taking a new sample, 5 mL of actual sample and 5 mL of distilled water, and then proceed as above. If this works, the P concentration of the water sample is twice the value given by the meter. If you again get a flashing **200** reading, try 2.5 mL of sample and 7.5 mL of distilled water.

Error and Warning messages that may be shown on the display:

L.Hi Light high. There is too much light to perform a measurement. Please check the preparation of the zero cuvette.

L.Lo Light low. There is not enough light to perform a measurement. Please check the preparation of the zero cuvette.

Inu Inverted cuvettes. The sample and the zero cuvettes are inverted.

0 Under range. A blinking 0 indicates that the sample absorbs less light than the zero reference. Check the procedure and make sure you use the same cuvette for reference (zero) and the measurement.

200 Over range. A flashing 200 indicates that the reading is over range—the sample contains a concentration of P that is greater than 200 ppb. Dilute the sample as described above and try again.

bAL Battery low. The battery must be replaced soon

bAd (or bAL) Dead battery; unscrew the Phillips head screw in the bottom of the instrument and replace the battery with a 1.5 V AA battery. This interrupts the normal operation of the instrument, so it must be restarted.

Post-activity discussion questions

1. On the basis of the phosphate reading, what can you conclude about the water in the stream? Are there any visible signs in the stream of high phosphate levels?
2. What are some ways that phosphate gets into the watershed?
3. How can people keep the amount of phosphate in a stream to a healthy level?

Background Information

Phosphorus in the form of phosphate (PO_4^{-3}) is an essential plant nutrient. Phosphate in the form of "superphosphate"--calcium hydrogen phosphates [$CaHPO_4$ and/or $Ca(H_2PO_4)_2$]--is a major component of many fertilizers. Excessive concentrations of soluble reactive phosphate (SRP, a.k.a. soluble reactive phosphorus) are often responsible for eutrophication of lakes, reservoirs, and streams--excessive aquatic plant growth, including massive algae blooms that can result in fish kills and foul odors when the algae die and decay, using up the dissolved oxygen in the water and generating smelly, toxic gases like hydrogen sulfide. Algae blooms may contain blue-green algae (cyanobacteria) that release toxins into the water, as happened in Lake Erie in the summer of 2014. This made the water from the Toledo water treatment plant unfit to drink for several days. Phosphate itself is not toxic. The concentration of soluble reactive phosphate that may trigger an algal bloom is quite low—around 0.05 mg/L. (Imagine a couple of grains of table salt in a quart of water!)

Phosphate occurs in runoff from fields treated with phosphate-containing fertilizers, runoff from livestock and poultry-feeding operations, runoff from fertilized lawns and golf-courses, pet wastes, food-processing wastes, wastewater from the pulp and paper industry, and partially treated or untreated sewage.

Phosphate used to be found in household detergents and other cleaning materials, but this has been greatly reduced in recent years. Modern sewage treatment plants remove phosphate from wastewater by precipitating it with ferric chloride, $FeCl_3$, or aluminum sulfate, $Al_2(SO_4)_3$. Some communities, such as Ann Arbor, have placed restrictions on the use of phosphate-containing fertilizers to limit eutrophication. Tap water often contains ~ 300 ppb of phosphate as P; this is added as a rust inhibitor and to prevent toxic lead and copper from dissolving from the plumbing.

Other uses of phosphates include: fire retardants, building materials, latex paint, industrial cleaners, metal treatment, ceramics, bakeware and a variety of foods and beverages.

In discussing phosphate in water the words phosphate and phosphorus are often used interchangeably, and the concentrations of soluble reactive phosphate (SRP) and total phosphate are often reported in mg/L of phosphate as phosphorus. 1 mg/L of phosphate as P = 3.07 mg/L of phosphate as PO_4. 1 mg/L of phosphate as PO_4 = 0.326 mg of phosphate as P. (Problem: prove this.)

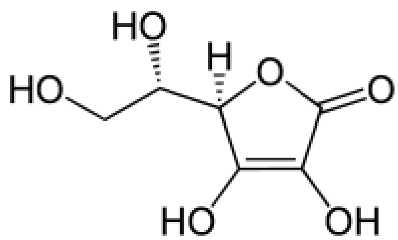
The chemistry of phosphate in nature is rather complex. Soluble reactive phosphorus (SRP) is orthophosphate [PO_4^{-3}], hydrogen phosphate [HPO_4^{-2}], dihydrogen phosphate [$H_2(PO_4)^-$], and (under quite acidic conditions) phosphoric acid [H_3PO_4]. A large group of polymeric phosphoric acids and their salts (polyphosphates) exists. Another group of organic phosphorus compounds are of great importance in biological processes involving energy conversion (ATP) and in genetics (DNA, RNA). Phosphorus is found in teeth and bones mainly as hydroxyapatite, $Ca_5(PO_4)_3(OH)$. And phosphate rock (the principal phosphate ore) contains the minerals hydroxyapatite and fluorapatite, $Ca_5(PO_4)_3F$.

Phosphate in water may be present as soluble reactive phosphate, as organic compounds from living organisms, as polyphosphates, and in suspended sediment, either adsorbed on clay particles or actually chemically combined in, for example, ferric phosphate, $FePO_4$. The phosphate tied up in sediment is not available for use by plants. However, if a lake or reservoir undergoes thermal stratification (less dense warm water floating on top of more dense cooler water) during the summer months, the cool bottom water may become anaerobic as decay processes use up its dissolved oxygen reserves. Under anaerobic conditions, ferric phosphate is converted to ferrous phosphate, $Fe_3(PO_4)_2$, which is much more soluble in water. Phosphate is then released from the sediment into the bottom water. Should cool weather, strong winds, or a heavy rain then occur, the phosphate-laden bottom water will be mixed into the upper layers of the lake. With phosphate and plenty of sunlight in the upper layers, an algae bloom quickly forms.

Analysis of ortho-phosphate (SRP). Ammonium molybdate-ascorbic acid method.

In the ammonium molybdate-ascorbic acid method ortho-phosphate (PO_4^{-3}) reacts with ammonium molybdate, $(NH_4)_6Mo_7O_{24}$, to produce ammonium phosphomolybdate, $(NH_4)_3PMo_{12}O_{40}^{-3}$. This is then reduced with ascorbic acid, to produce intensely colored molybdenum blue. The blue color arises because the near-colorless phosphomolybdate anion, $PMo_{12}O_{40}^{3-}$, can accept more electrons (i.e. be reduced by the ascorbic acid) to

L-Ascorbic acid



$C_6H_7O_6$, (5R)-[(1S)-1,2-Dihydroxyethyl]-3,4-dihydroxyfuran-2(5H)-one

form an intensely colored mixed-valence complex. The reduction process is reversible and the structure of the anion is essentially unchanged.[4]

$PMo(VI)_{12}O_{40}^{3-} + 4e \rightleftharpoons PMo(V)_4Mo(VI)_8O_{40}^{7-}$ (molybdenum blue).

The blue complex has an absorption maximum at a wavelength of 880 nm.

For this and all other units, advanced level information is available if desired. Contact the HRWC and request an electronic version of the unabridged manual.