Phosphate: It Isn't Just an Environmental Problem Anymore

Introduction

All living organisms require phosphate to make two of the most important organic macromolecules: deoxyribonucleic acid (DNA) and adenosine triphosphate (ATP). The macromolecule DNA you know as the molecule holding your genetic code. Physically, DNA is comprised of two mirror image molecular strands twisting around each other in a double helix, a shape resembling a spiral staircase. The backbone of each strand is made of molecules of the sugar deoxyribose alternating with phosphates. ATP is the molecule that powers all work within a cell. As the name implies, ATP is three phosphates bonded to the nucleic acid adenosine. Breaking a phosphate off an ATP releases energy that a cell can use to power movement, chemical reactions, and other functions. You need so much ATP that you burn an amount roughly equivalent to your body weight every day. Luckily, your body is good at making more, using energy derived from the digestion of glucose.

The importance of phosphates make them an essential nutrient. Animals easily meet their phosphate needs by eating other living things. Plants, on the other hand, must absorb phosphate from the ground and often have difficulty getting enough. To make up this deficiency, most fertilizers, whether for house plants or commercial crops, include phosphate.

Commercial Uses for Phosphates

Fertilizer manufacturing is a huge business and consumes much of the commercially produced phosphate. However, phosphates are good at removing dirt and so are a key ingredient in some detergents, although fewer now than in times past, for reasons we will discuss below.

Phosphates can also be found in some foods. Phosphoric acid acts as a flavor enhancer in foods with caramel. Colas manufacturers also use phosphoric acid for flavor, and to help retain carbonation.

The Significance of Phosphates in the Environment

Phosphate's role in promoting plant growth actually makes it a dangerous pollutant when dumped in excessive quantities into aquatic ecosystems. As mentioned above, plants have difficulty obtaining phosphates. In fact, plants have so much difficulty that the chemical is a limiting nutrient. The rate at which plants can grow and reproduce is limited by the amount of usable phosphate in the soil or water (for aquatic plants). When humans add extra phosphorous to water, they create a condition called eutrophication that can wipe out aquatic ecosystems. Eutrophication is characterized by a rapid growth in the plant population (an algal bloom). With more living plants comes more dead plants for decomposition. The bacteria that decompose the dead plants use oxygen, and eventually burn up so much that not enough remains to support fish, insects, mussels, and other animals, leading to a massive die-off.

The presence of phosphates in virtually every detergent, including household cleaners and laundry soap, used to contribute significantly to eutrophication. In the United States, the problem was moderated somewhat in the 1970’s when the Clean Water Act and other laws mandated the removal of phosphate from many detergents. Next time you do laundry, look on the side of the box and you might find the phrase “Contains No Phosphates” -- although many industrial cleaners and some specialty detergents still use phosphates. The responses of some
concerned citizens on both sides of the issue in Spokane, Washington, are featured in the newspaper article posted [here](http://www.epa.gov/owow/nps/dosdont.html). A more recent article about the chemical, engineering, environmental, and marketing aspects of replacement formulations for phosphate-based detergents is linked on the [American Chemical Society website](http://www.epa.gov/owow/nps/dosdont.html).

Besides the dishwasher detergent pollution mentioned in these articles, fertilizer run-off from agriculture and landscaping continues to be a major source of phosphate pollution. This website details steps you can take to minimize run-off from your lawn and garden: [http://www.epa.gov/owow/nps/dosdont.html](http://www.epa.gov/owow/nps/dosdont.html). Animal wastes can also add significant amounts of phosphate to water. For many years, Kansans have debated the pros and cons of development of large corporate hog farms. People fighting the farms include among the list of reasons for their opposition a fear of eutrophication caused by phosphates (and other nutrients) in animal wastes that could leak into area streams and water tables. If you wish, you may click on the commercial web links below to learn more about this dialogue.

- [http://www.inmotionmagazine.com/hogmyth.html](http://www.inmotionmagazine.com/hogmyth.html)

The concept of eutrophication will be discussed in greater detail during the upcoming Water Quality Analysis lab.

**Using our Spectrophotometer with Phosphate Standards to Determine Concentration**

Under appropriate conditions, a substance's concentration and its absorbance are proportional: a high-concentration solution absorbs more light than a solution of comparably lower concentration. For this lab experiment, we will create a series of solutions containing various known concentrations of phosphate. After treating each of these analytical standards with reagents that convert all constituent phosphate species to a blue-colored substance, we will measure absorbance. An Excel plot of absorbance vs. concentration will afford a line of best fit that serves as a calibration curve, or standard curve. This provides a convenient method for determining the phosphate concentration of samples of cola, surface water, and other aqueous solutions of interest. (Review the online write-up for the Enzyme Kinetics Lab and your corresponding lab notebook entries for help with the calibration curve concept.)

**Pre-lab**

**Safety**

- Always, always wear safety glasses in the laboratory.
- The molybdate solution is acidic; be careful with this solution as acids can be hazardous if splashed on clothing, exposed skin or in the eyes. Prolonged exposure of the skin to even fairly dilute solutions of acid can cause serious burns. If acid is splashed on skin or clothes, remove the affected clothing and flush the affected areas thoroughly with cold water.
- All of the solutions used in this laboratory are dilute and can be discarded down the drain.
(1) Describe how you will prepare the 1.00 x 10^{-3} M phosphate solution mentioned in Part 1, Step 1, of the Procedure. (Hint: you must use appropriate volumetric glassware!)

(2) Calculate the volumes of reagents your group will have to measure to perform the dilutions mentioned in Part 1, Step 2, of the Procedure.

(3) The phosphate ion, PO_{4}^{3-}, can exist in aqueous solutions if the pH is sufficiently high. If the pH of such a phosphate-containing solution were gradually lowered by addition of HCl or some other strong acid, what three protonated phosphate species would be generated? (Specify the chemical formula and name of each.)

**Procedure**

*Crucial procedural steps are shown below. Specific details, such as quantities of material you must combine to generate the solutions of known concentration, are not provided. Use the information below, along with your previous experience in chemistry lab, to decide how to proceed.*

**Part 1 – Calibration Curve**

1. Using the phosphate stock solution provided (1.00 x 10^{-1} M), prepare 200 mL of 1.00 x 10^{-3} M phosphate solution. *A variety of volumetric pipettes and volumetric flasks are available.*

2. From your 1.00 x 10^{-3} M phosphate solution, prepare 100 mL each of five standard solutions: 2.00 x 10^{-5}, 5.00 x 10^{-5}, 1.00 x 10^{-4}, 2.00 x 10^{-4}, and 5.00 x 10^{-4} M. *These will serve as your analytical standards,* which means that their phosphate concentrations must be precisely known.

*Phosphate will react with ammonium molybdate to form the compound (NH_{4})_{3}PO_{4}•12 MoO_{4}. This is reduced with a mild reducing agent; in this experiment we use 1,2,4-aminonaphtholsulfonic acid to form a blue complex.*

*Free (unbound) molybdates will not reduce under these conditions so only the molybdate that is bound with phosphate will form a blue compound. The intensity of this blue color can then be recorded spectrophotometrically: the blue has a maximum absorption of light, \( \lambda_{\text{max}} \), at a wavelength of approximately 690 nm.*

**How will you prepare a blank for your spectrometer?**

3. Deliver 5.00 mL of each phosphate standard solution into a small beaker or large test tube.

4. Add 1.00 mL of the ammonium molybdate solution to each using the plastic pipette attached to the side of the stock bottle. Mix gently.

5. Add 0.40 mL of the aminonaphtholsulfonic acid reagent to each using the *graduated glass pipette.* Mix gently.

6. Allow the solutions to stand for 5 minutes.

7. Measure the absorbance at 690 nm starting with the least concentrated solution.
8. Use Excel to construct a calibration curve. If you do not recall how to do this, review the Introduction section of this write-up

**Part 2 – Determination of Phosphate in Soda Pop**

Often, an industrially- or environmentally-derived sample must be altered in one or more ways before it is analyzed.

**Why would it be difficult to reliably obtain a 10.0 mL sample from a freshly opened can of soda pop? How will the soda pop sample preparation, described in steps #1 and #2 below, improve the reliability of your measurements?**

1. Measure out approximately 50 mL of your soda sample into a beaker and heat it to boiling, or nearly boiling for 1 minute. Cover the beaker with a watch glass to reduce evaporation.
2. When cool, take a sample of your soda and dilute it 50-fold with ultra-pure water.
3. Deliver a 5.00 mL-size sample of your diluted soda pop to a small beaker or large test tube.
4. Add 1.00 mL of the molybdate reagent and 0.40 mL of the aminonaptholsulfonic acid reagent in the manner described for Part 1; allow to stand for 5 minutes prior to absorbance measurement.
5. Use your calibration curve to solve for the phosphate concentration in soda pop.

**How will you account for the fact that you performed a 50-fold dilution?**

**Part 3 – Determination of Phosphate in a Water Sample**

Phosphate functions as a food additive, detergent, and fertilizer, and it has many other essential roles. However, phosphate can also be regarded as an environmental contaminant; thus, it is important that we have reliable methods for determining the concentration of phosphate in groundwater, surface water, and drinking water. (During the last two weeks of Chemistry 188 lab, students obtain surface water samples from locations of their choosing and analyze them for turbidity, pH, ...and phosphate concentration!)

Using the scheme shown in Part 2 as a guideline, analyze the phosphate unknown provided by your TA. This unknown water sample contains phosphate at a concentration similar to that of some ponds that are overtaken by rapidly growing “seaweed” and algae.

**Is it necessary to boil your unknown water sample prior to analysis, as in Part 2, Step 1?**

**Must you dilute your unknown water sample prior to analysis?**