Trilogy™
Laboratory Fluorometer

Application Note:
Phosphate

Introduction

This note will provide background knowledge of the different applications phosphate can be used for, identify sources, and describe in detail how to measure inorganic phosphate levels in water systems using absorbance.

Background

Phosphate (PO_4^{3-}) can be found as a free ion in water systems, as a salt in terrestrial environments, used in detergents as water softeners, or can be found in rock deposits. The phosphate identified in these sources is referred to as inorganic phosphate because it has not been incorporated into biological tissue. Inorganic phosphate, which is typically limiting, is one of the three necessary nutrients used for primary production by terrestrial and aquatic plants. It is used in fertilizers to enhance agricultural growth and production and when introduced into aquatic systems can cause algal blooms.

Why is measuring phosphate important?

Measuring phosphate in aquatic environments can be a very important tool in understanding the health of a system or it's "water quality". Phosphates in fertilizers, rock, or soil seep into water systems. Natural events such as heavy rainfall will wash agricultural fields of their topsoil and introduce phosphate salts into rivers, lakes and ultimately coastal/oceanic environments, which will increase phosphate concentration causing algal blooms that could lead to fish kills or impacts on other organisms. Wastewater released into the environment, that has not been properly treated, can potentially contain phosphates from detergents and can cause similar effects.

Monitoring of phosphate levels in water can help identify possible sources for phosphate introduction to aquatic systems. Precautions can be taken to minimize these inputs and can prevent any harmful effects that can occur as a result eutrophication.

Phosphate Analysis

Overview

Phosphate concentrations in water samples can be measured using absorbance. This process involves a chemical reaction, creating an *in situ* reduced heteropoly acid complex that produces a blue solution, which has extinction at 885-nanometer wavelength (nm). The intensity of the blue solution is related to the concentration of phosphorus in the water samples.

Apparatus

Absorbance measurements are made using the Turner Designs Trilogy Laboratory Fluorometer (P/N: 7200-0000) with the phosphate absorbance kit (P/N: 7200-070). The Trilogy is a solid state instrument that uses a Light Emitting Diode (LED), filter, and photodiode combination to measure phosphate extinction coefficients at 885 nm. Borosilicate 12x75mm glass cuvettes are recommended for phosphate measurements because there is minimum absorption of light by the glass material. ***The following method is adapted from Strickland and Parsons (1968), <u>Determination of Reactive Phosphorus.</u>***

Reagents Required:

Ammonium paramolybdate	$[(NH_4)_6 Mo_7O_{24} \bullet 4H_2O]$
Sulfuric acid	$[H_2SO_4]$
Ascorbic acid	$[C_6H_8O_6]$
Potassium antimonyl-tartrate	$[C_4H_4KO_7Sb.0.5H_2O]$
Anhydrous potassium dihydrogen phosphate	$[KH_2PO_4]$
Chloroform	$[CHCl_3]$

Reagent Preparation (Strickland and Parsons 1968)

Ammonium molybdate solution

Dissolve 15 grams of ammonium paramolybdate in 500 milliliters of distilled water. Store in dark plastic bottle. The solution is stable indefinitely.

Sulfuric acid solution

Add 140 milliliters of concentrated sulfuric acid to 900 milliliters of distilled water. Allow solution to cool and store it in a glass bottle. The solution is stable indefinitely.

Ascorbic acid solution

Dissolve 27 grams of ascorbic acid in 500 milliliters of distilled water. Store in freezer in plastic bottle so that solution is frozen. Thaw for use and refreeze at once. The solution is stable for many months. (*Note: should not be kept for more than a week at room temperature)*

Potassium antimonyl-tartrate solution

Dissolve 0.34 grams of potassium anitmonyl-tartrate in 250 milliliters of distilled water. Store in glass or plastic bottle. The solution is stable for many months

Mixed Reagent

Mix together in a dark glass bottle, 100 milliliters ammonium molybdate, 250 milliliters sulfuric acid, 100 milliliters ascorbic acid, and 50 milliliters potassium antimonyl-tartrate solution. This solution will be stable for up to six hours after preparation**. Do not use this solution after 6 hours**. These volumes are suitable for 50 samples.

Potassium dihyrogen phosphate (standard)

Dissolve 0.816 grams of anhydrous potassium dihyrogen phosphate in 1000 milliliters of distilled water. Store in a dark bottle with 1 ml of chloroform. The solution is stable for many months.

Calibration

- Snap in the phosphate absorbance module making sure that the correct filter paddle is being used and turn the Trilogy Fluorometer on
- Choose the absorbance mode and verify that the correct snap in module has been loaded
- Press the calibrate function on the touch screen, you will be prompted to insert a blank
- Insert your blank (*Deionized water with mixed reagent added*) and press ok, the Trilogy has now been blanked
- Choose a working range, typical of samples, to calibrate (*Note: accuracy increases as your working range decreases*)
- Dilute the solution of potassium dihydrogen phosphate (Calibration Standard) to make solutions of known concentrations that can be used to create a calibration curve for your working range (*Note: accuracy increases as you increase the number of calibration points used)*
- Make sure samples are at room temperature before adding mixed reagent
- Make a 10:1 mixture of sample:mixed reagent
- Wait at least 5 minutes, then measure absorbance (*Absorbance measurements should be made within 2 hours after the addition of the mixed reagent*)
- Graph concentration of calibration standards vs. Absorbance units (Au) for each standard
- Draw a regression through the points, use the equation of the regression (Y = mx + b) to calculate the concentration of phosphate in water samples that fall within that working range by plugging in the absorbance values for ‘x’ in the equation
- Any samples that exceed the calibrated working range need to be diluted to fit the calibrated working range for accurate estimation of phosphate concentration

Sample Processing

1) Make sure the samples are at room temperature prior to addition of mixed reagent
2) When adding mixed reagent make a 10:1 mixture of sample:reagent (example: to 100 ml of sample add 10ml of reagent)
3) After adding mixed reagent to samples, allow at least 5 minutes before measuring absorbance. After 2 hours the sample will expire and will not yield accurate phosphate estimates
4) Use the regression equation ($Y = mx + b$) from your calibration curve to calculate the concentration of phosphate in your sample by plugging in the absorbance values for 'x' in the equation

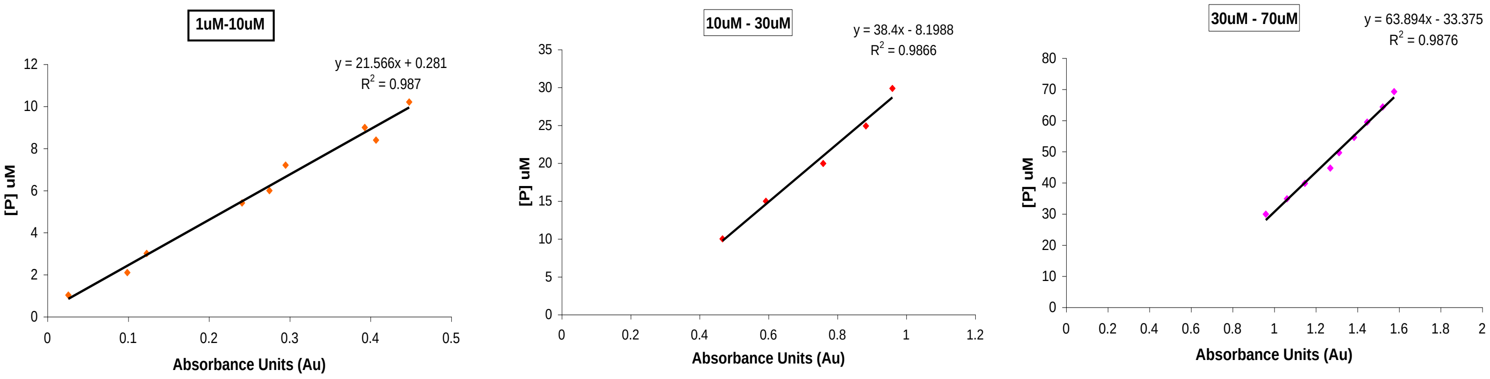

Figure 1: The above graphs are **EXAMPLES** of working ranges (1-10uM, 10-30uM, and 30-70uM) for phosphate concentrations and their respective regression equations.

Reference:

J. D. H. Strickland and T. R. Parsons, 1972. A practical handbook of seawater analysis. Second Edition, Bulletin 167. Fisheries Research Board of Canada, Ottawa

Timothy R. Parsons, Maita Y. and Lalli C. M., 1984. A manual of chemical and biological methods for seawater analysis. First Edition. Pergamon Press Ltd., Great Britain