CH 2212
PHOTOMETRIC DETERMINATION OF PHOSPHATE

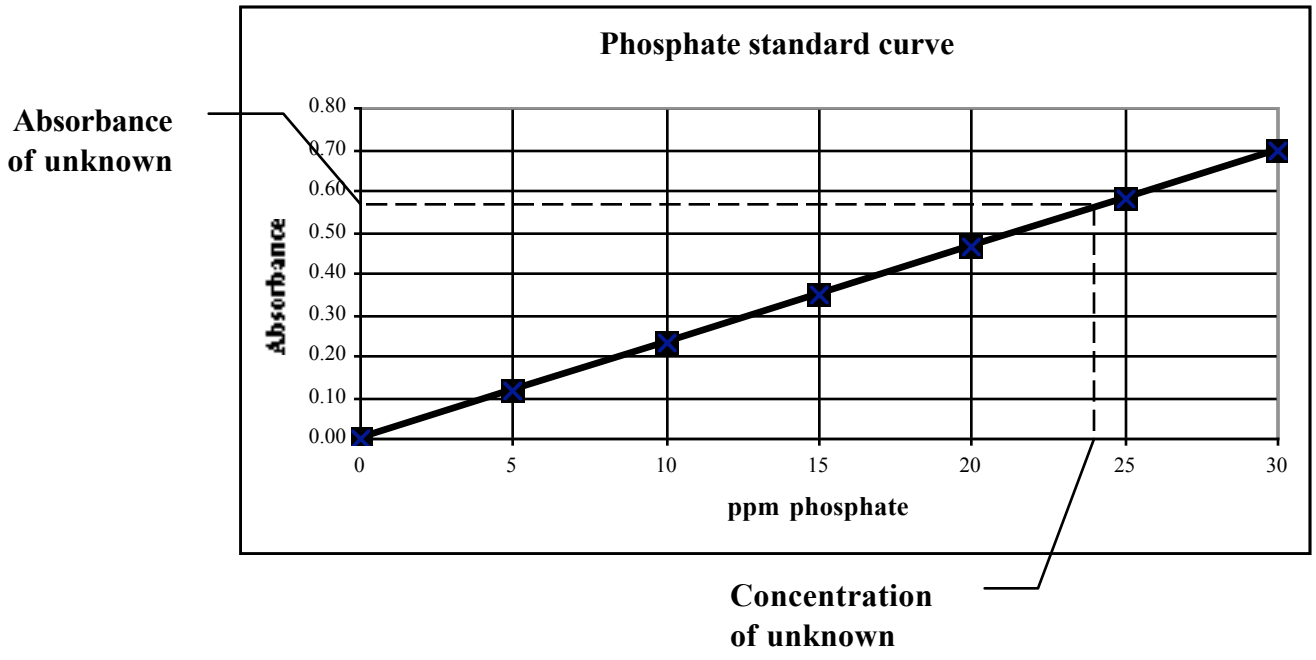
BACKGROUND:

Phosphate occurs in trace amounts in many natural waters, and often in appreciable amounts during periods of low biologic productivity. Traces of phosphate increase the tendency of troublesome algae to grow in reservoirs. Phosphate analyses are made primarily to control chemical dosage, or as a means of tracing flow or contamination.

The principle of this determination is that in dilute phosphate solutions, ammonium molybdate reacts in an acid medium to form a heteropoly acid, molybdophosphoric acid. In the presence of vanadium the vanadomolybdophosphoric yellow color is formed. The intensity of the yellow color at 410 nm is proportional to the phosphate concentration in the solution. The structure of the vanadomolybdophosphate is unknown, but it has been demonstrated that in dilute, acidic solutions with an excess of the molybdate species present, Beer's Law is obeyed with respect to phosphate. Since the colored species formed is such an intense absorber of light the minimum detectable concentration is 0.6 mg/L (0.6 ppm) PO_4^{-3} in 1-cm cells.

Beer's Law is frequently written as: $A = \varepsilon b C$

where A is the absorbance, ε is the molar absorptivity, b the path length in cm, and C the concentration of absorbing species in moles per liter. If Beer's Law is obeyed, a linear plot of A vs C results. If one prepares a Beer's Law plot with standard solutions, an unknown can be determined by comparison to the standard curve. See the figure below.



A phosphate solution of known concentration will be used to prepare a set of standards from which a Beer's Law plot can be made. The unknown is then determined by comparison with the Beer's Law plot.

In this experimental procedure positive interference is caused by silica and arsenic only if the sample is heated. Negative interferences are caused by arsenate, fluoride, thorium, bismuth, sulfide, thiosulfate, thiocyanate, or excess molybdate. Blue color is caused by ferrous iron but this does not affect results if the Fe(II) is less than 100 mg/L. If nitric acid is used in the test, chloride interferes at 75 mg/L.

The development of the vanadomolybdophosphoric yellow color requires that the color developing agent be added to all phosphate standards, samples and blanks. Consequently, there is a dilution effect which must be taken into account when calculating the actual phosphate concentrations. The way this lab exercise is set up, all your standards and samples will be diluted by a factor of two (25 mL standard or sample + 10 mL color reagent + 15 mL distilled water). Each of the standards in the calibration curve on the previous page, for instance, were diluted 2X for measurement in the spectrophotometer. The concentrations plotted are the measured concentrations after dilution. The absorbances plotted are those of the corresponding diluted solutions.

PRELIMINARY CALCULATIONS:

1. Find the absorbance of a solution that is 1.23×10^{-3} M in the absorbing species if ε is 6.20×10^{2} liters $mole^{-1}$ cm^{-1} and the path length is 1.50 cm.

2. Find the corresponding %T value for #1.

3. The absorptivity for the vanadomolybdophosphoric yellow is 2.6×10^{3} L/mole PO_4^{-3} - cm. If a 1.00-cm cell is used, what concentration range of phosphate should be used to cover the absorbance range from 0.2 to 0.9?

4. Convert the concentration range in #3 from molarity to mg phosphate per liter (ppm).

5 Describe how one would prepare from a stock solution that contains 500 mg phosphate per liter, five standards solutions of 100 mL each that will effectively cover the concentration range of #4 (after dilution by a factor of two for measurement).

Answers:

1) 1.14 AU
2) 7.18% T
3) C (0.2 AU) = 7.69×10^{-5} M; C (0.9 AU) = 3.46×10^{-4} M
4) C (0.2 AU) = 7.3 ppm; C (0.9 AU) = 32.9 ppm
5)

mL 500 ppm stock	$[PO_4^{-3}]$ ppm before dilution	$[PO_4^{-3}]$ ppm as measured
3.2	16	8
6	30	15
8	40	20
12	60	30
14	70	35

PROCEDURE:

1. Obtain an unknown

Turn in a labeled 250.0 mL volumetric flask to the instructor. A measured volume of liquid unknown will be dispensed into this flask. The unknown solution is to be diluted to the mark with distilled water and mixed well.

2. Prepare standard phosphate solutions

Obtain approximately 50 mL of the standard 500 ppm phosphate solution. Rinse out a 50 mL buret with a small portion of the standard solution and then pour the remainder into the buret. Calculate the volumes of the standard phosphate solution which, when diluted to 100 mL (and then subsequently diluted by a factor of two), will produce absorbances ranging from 0.2 to 0.9 absorbance units.

Using the buret, deliver the appropriate volumes into 100 mL volumetric flasks and dilute to the mark with distilled water.

3. Develop the vanadomolybdophosphate color and measure the absorbance

a) Pipet a 25.0 mL aliquot of each standard into a clean 250 mL Erlenmeyer flask or beaker. Add 10.0 mL of vanadate-molybdate solution and mix thoroughly by swirling. Add an additional 15.0 mL of distilled water and mix. All additions must be made with pipets since the total volume must be accurately known.

b) Pipet a 25.0 mL aliquot of the unknown solution into a clean 250 mL Erlenmeyer flask or beaker and treat as for the standard solutions.

c) Prepare a blank solution by treating 25.0 mL of distilled water in the same manner as the standards, i.e., the blank contains the color developing reagent, but no PO_4^{-3}.

d) Set up the Ocean Optics spectrophotometer as instructed. Bring to the spectrophotometer station: a waste beaker, wash bottle with distilled water, the standard and sample solutions. Take a reference spectrum using the blank solution. Take a dark spectrum. Switch to absorbance mode. Adjust the wavelength range to 300 – 500 nm. At the end of the series of measurements, check the blank reading to evaluate instrument drift.

e) Ten minutes or more after mixing but in the same lab period, transfer the solutions individually to the cuvette and measure the absorbance at 410 nm. Make absorbance readings for each of the five standards. The standard curve should be run twice, from the lowest concentration to the highest. Rinse the cuvette well between measurements with the next solution to be measured. Wipe the outside of the cuvette with a Kim-Wipe before each measurement. This will prevent droplets of water from changing the path of the light beam. The unknown should be run after each standard series.

The unknown must be prepared and measured at the same time as the standards using the same spectrophotometer without readjustment of any parameters. All measurements should be completed in a single lab period.

4. Plot the standard Beer's Law curve

Plot a rough calibration curve in your lab notebook immediately after taking your absorbance readings. Use this plot to judge the validity of your absorbance values. If obvious deviation from the expected is found, inspect your notebook entries and fix the error. You can use the absorbances of your standards to check your calculation procedure. Compare the calculated concentration (based on the absorbance measurement and calibration curve) to the known concentration for the corresponding standard.

Prepare a high quality calibration curve using EXCEL (or other suitable plotting program) by plotting absorbance on the vertical axis and mg phosphate/liter on the horizontal axis. Do not "connect the dots". You may hand-draw a best-fit straight line through your data points. This line should be straight if Beer's Law is followed and it should pass through the origin. Tape the calibration curve into your notebook. Make a second copy to hand in with your results.

5. Find the concentration of the unknown in ppm phosphate

From the Beer's Law plot find the phosphate composition. Report the PO_4^{-3} concentration in ppm for the unknown solution in the 250-mL volumetric flask.