EXPERIMENT E3

Spectrophotometric Analysis: Phosphates in Water

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(Modified version of Kristen Spotz and University of Michigan General Chemistry Laboratory Manual)

I. OBJECTIVES

- Practice calculating and performing dilutions of solutions.
- Determine the concentration of phosphate in a water sample by spectrophotometric analysis (see Figure 1 spectrophotometry illustration).
- Construct and utilize a calibration curve.
- Explore the dynamics of working with a larger group of students.

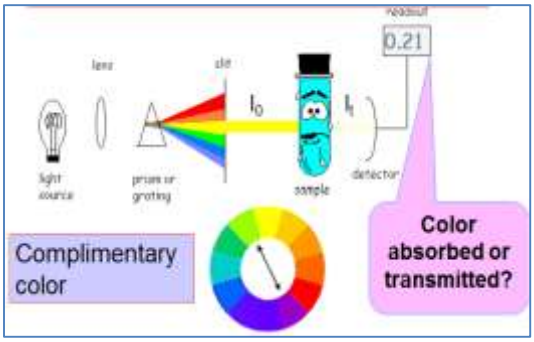

Figure 1: Spectrophotmetric analysis illustration

II. INTRODUCTION

Imagine a time when the lakes and rivers are no longer safe for swimming or boating, or when the ocean is no longer a source of food. Coastal zones and estuaries, some of the most productive ecosystems in the word, are in danger. The problem of eutrophication, is affecting the water supply of towns across the nation making the water unsafe for consumption and hazardous to the wildlife that depend upon it.

The source of the eutrophication problem is an excessive input of nutrients into rivers, lakes and the seas because of the extensive use of fertilizers, the combustion of fossil fuels and waste from animal feedlots. This excessive nutrient input stimulates the growth of algae and bacteria, robbing the water of precious oxygen. The resulting algal blooms, red tides and deterioration of sea grass makes the waters uninhabitable for most fish and coastal wildlife.

What role will you play as a future scientist' or citizen in ensuring the protection of our valuable water resource?

III. BACKGROUND

Phosphates are one of the major groups of contaminants affecting our nation's water supply. Phosphates are found in environment, not only in the form have you seen in your chemistry book (PO_4^{3-}), but also as polyphosphates (such as $P_2O_7^{4-}$ or $P_3O_{10}^{5-}$) or as organic phosphates which are eventually hydrolyzed to form PO_4^{3-}. The primary means by which humans introduce phosphates into the environment is through the use of fertilizers and detergents. In particular, tripolyphosphates ($P_3O_{10}^{5-}$) have been used in soaps and detergents to combat the problem of hard water. Phosphates are also a major component of fertilizers, because phosphorus is a necessary plant nutrient and is crucial for seed formation, root development, and crop maturation. These phosphates eventually enter the water supply leaving lakes, rivers, and seas with an abnormally high phosphate concentration.

A. Spectrophotometric Analysis and the Determination of Phosphate

Spectrophotometric analysis relies on the fact that the amount of light absorbed by a sample shows a linear dependence upon the concentration of the compound present in the solution. You have probably seen this phenomenon for yourself before. Just hold up two glasses of juice made from powdered concentrate; one made with three scoops and one made with one scoop. The more concentrated drink absorbs more light and is darker (see Figure 2). The problem with using spectrophotometric analysis in our case is that phosphates are colorless and therefore do not absorb light in the visible portion of the electromagnetic spectrum. However, due to the reactive nature of phosphates, one can easily color them using an ammonium vanadomolybdate reagent. This reagent includes ammonium metavanadate (NH_4VO_3) and molybdate (MoO_4^{2-}) and reacts with the phosphate to the form a yellow compound (called "heteropoly acid" from here on). The formula of the yellow compound is uncertain but thought to be $(NH_4)_3PO_4 \cdot NH_4VO_3 \cdot 16MoO_3$. The brightness of the resulting yellow solution is directly proportional to the concentration of phosphate in the water.

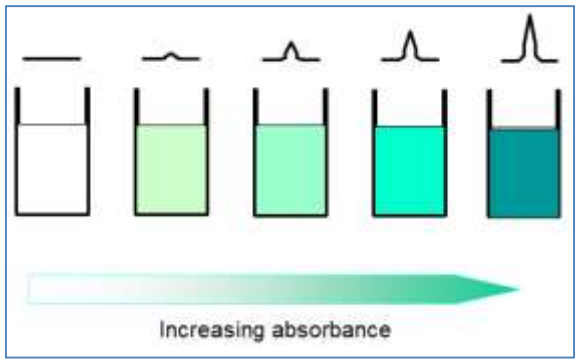

Figure 2. Concentrated solutions absorbs more light

Scientists use an instrument called a spectrometer to quantitatively determine the amount of light absorbed by a solution. The primary inner parts of a typical spectrometer are illustrated in Figure 3. The spectrometer has a light source that emits light which is focused with a small slit. The wavelength of interest is then selected using the monochrometer ("mono" meaning one and "chromate" meaning color) and an additional slit. The selected light then reaches the sample and depending on how the photons interact with the compound of interest, the light is either absorbed or passes straight through. By comparing the amount of light entering the sample (I_o) with the amount of light reaching the detector (I), the spectrometer is able to tell how much light is absorbed.

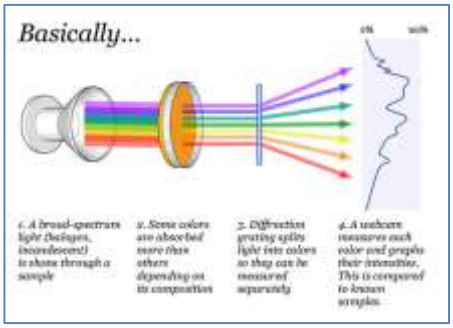

Figure 3. Working principle of spectrometer

Scientists quantify the amount of light passing through the sample in terms of percent transmittance (%T). Using Beer-Lambert law, percent transmittance is calculated as the fraction of original light intensity (I / I_0) that passes through a sample (Equation 1).

$$\%T = \left(\frac{I}{I_o}\right) \times 100 \qquad \text{Equation 1}$$

Equation 2 shows how percent transmittance (%T) can easily be converted into a quantity known as absorbance (A). Though most spectrophotometers give readings in terms of both %T and A, measurements should be made in %T and mathematically converted to A because %T can be determined more accurately.

$$A = -\log 10\left(\frac{\%T}{100}\right)$$ or A = - Log_{10}(%T/100) = 2 – Log_{10}(%T) Equation 2

The absorbance of a sample is important because of the previously mentioned linear relationship between absorbance the concentration of the sample. This relationship is known as Beer-Lambert law (Equation 3).

$$A = \varepsilon bc$$ Equation 3

The amount of the light that is absorbed depends on several variables (see Figure 4):

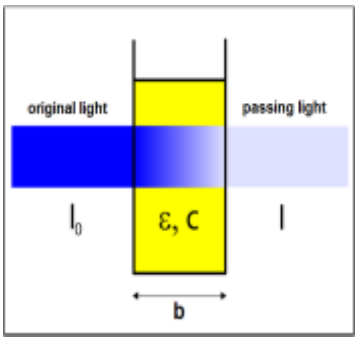

Figure 4. Light absorbance Beer-Lambert law

- "*A*" is the absorbance of the sample, which in this experiment is due to the interaction of phosphate, in the form of heteropoly acid, with the photons of light. Although the compound being studied may, in general, absorb light over a fairly broad range of wavelengths, there is only one region where the light is absorbed most strongly. This wavelength is known as λ_{opt} (pronounced "lambda optimum"). The absorbance of the sample should be measured at this wavelength to optimum reading (see illustration in Figure 8). Erratic absorbance readings may be observed if meter wave length is set near the bottom of a curve of absorbance vs. wavelength. In this case, it is recommended to change the spectrometer to a better detection method.

- "ε" is the molar absorptivity with units L.mol^{-1}.cm^{-1}. The molar absorptivity is a constant representing the efficiency by which the substance absorbs light. The greater the value of "ε" the more strongly the substance absorbs light resulting in a more intense color.

- "*b*" represents the solution path length. It is the distance that the light must travel through the sample and is measured as the width of the sample holder (also called a cuvette). "*b*" is a constant for each experiment (typically 1 cm).

- "*c*" represents the molar concentration of absorbing species in the sample (mol/L).

One can easily determine the unknown concentration of a sample from Equation 3 after measuring the absorbance of the sample and using the molar absorptivity of the compound and the path length of cuvette. If the molar absorptivity of the compound is not known, the concentration of an unknown can still be found by constructing a calibration curve.

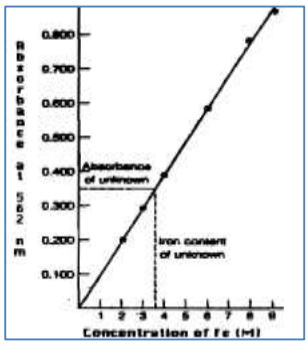

Figure 5. Fe(II) sample absorbance @562nm

B. The calibration Curve

A calibration curve allows scientists to determine the unknown concentration of a known species. According to Beer - Lambert law, as long as we account for a blank solution in our studies, a plot of absorbance versus concentration gives a straight line with slope = "εb" and y-intercept

= 0. For example, the calibration curve in Figure 5 is used to determine the concentration of an unknown solution of iron. The graph is constructed from six points that are made from a stock solution of iron having a known concentration. The experimentally measured absorbance of each of the six solutions is then plotted as a function of concentration and a line best fit is drawn through the points. As expected, the absorbance of the sample increases linearly as the concentration increases. The absorbance value of 0.357 was then measured for the unknown iron solution of interest. To relate the absorbance to the unknown concentration we can either use the equation of the line of best fit or we can extrapolate from the graph (as shown in Figure 5). This absorbance value was found to correspond to a concentration of 3.59 M of iron in the unknown sample.

The calibration curve in Figure 5 is an example of a successfully constructed graph. The title is labeled above the graph with both the axes clearly labeled using the independent (x-axis) and the dependent (y-axis) variables in the experiment. After plotting each of the data points on the graph, a line of best fit is drawn. Although, the points do not have to fall directly on the line, a good agreement is expected and needed for accurate determination of the concentration of your unknown.

C. OVERVIEW

In this experiment, students will work in groups to first prepare a series of six standard solutions of known phosphate concentration by dilution of a stock solution. Using λ_{max} of 400nm, the absorbance of the five standard phosphate solutions will be measured and used to construct a calibration curve. The absorbance of a sample of unknown phosphate concentration will then be determined. The calibration curve will be used to relate the absorbance to the unknown concentration of phosphate in sample.

IV. EXPERIMENTAL PROCEDURES

"Make sure you take photos of your favorite lab work for use in your final PPT presentation assigned by your TA about one of the experiments E1-E5. Also closely review the lecture presentation for this E3 as posted on Canvas"

Part A. Preparation of Standard Solutions

Chemicals used	Materials used
Phosphate stock solution (1.00×10^{-3} M) 2M HNO_3 Ammonium vanadomolybdate (AV) solution Two water samples (A & B) of unknown phosphate concentration	Spectrophotometer 50- mL Volumetric flask 1, 2, 5- mL Pipets and pipet bulb Cuvettes (1 per group of students) 500- mL Beakers

1. Students will work in groups as assigned to construct a single calibration curve consisting of 6 data points having phosphate concentrations in the range 2.00×10^{-5} M to 1.00×10^{-4} M. Each student will be responsible for making at least one of the solutions (see Figure 6 demonstration) and measuring the absorbance of at least one data point.

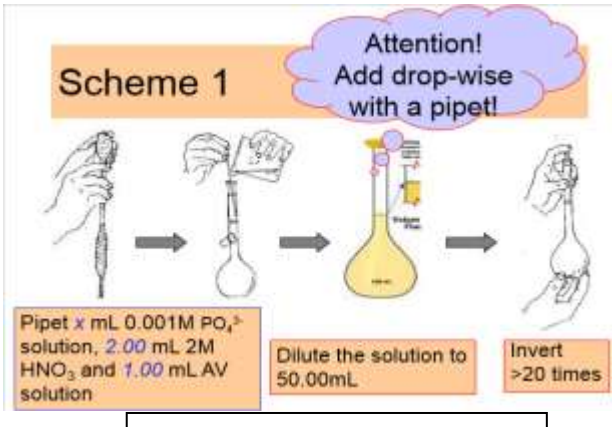

Figure 6. Dilution demonstration.

Show your instructor your calculations for making your 50- mL standard solutions from the 1.00×10^{-3} M phosphate stock solution before your group goes on part B. Remember, solutions must be made using only the available volumetric flask and pipets. Glassware including pipets and volumetric flasks must be washed & rinsed with distilled water as instructed. **Do not contaminate reagent bottles and follow the instructor's procedure**.

Make the following data sheet to record your data:

Sample	$1^{\#}$	$2^{\#}$	$3^{\#}$	$4^{\#}$	$5^{\#}$	$6^{\#}$	Unknown $7^{\#}$ A	Unknown $7^{\#}$ B
V(mL)	0.00	1.00	2.00	3.00	4.00	5.00		
Conc.(M)								
Absorb. A								

2. Based on your calculation from **step 1**, pipet 1.00mL of 1.00×10^{-3} M phosphate stock solution, , into a 50-mL the volumetric flask labelled as $2^{\#}$.
3. Pipet 2.00 mL 2M HNO_3 solution into the same volumetric flask.
4. Pipet 1.00 mL of the ammonium vanadomolybdate (AV) stock solution into the same flask.
5. Dilute the stock solution by filling the volumetric flask until the meniscus reaches the mark (Figure 7).
6. Repeat steps 2 to 5 for each of the six standard solutions $2^{\#}$ - $6^{\#}$ but pipet the corresponding phosphate volume from the table instead of that shown by Step 2.
7. For the preparing the blank sample $1^{\#}$ repeat steps 3 through 5 (skip step 2 so do not add any phosphate stock).

Figure 7. Proper dilution to meniscus

Now you have prepared six standard solutions $1^{\#}$ through $6^{\#}$, complete their corresponding data in above data sheet.

Procedure Part B. Adjusting the Spectrophotometer

1. Turn on the spectrometer (Figure 8) by rotating the power control clockwise. Allow the spectrophotometer to warm-up for 10 minutes before using.

2. Adjust the wavelength to 400 nm. With no sample in the spectrometer (use only the black block and do not insert any cuvette), turn the zero adjust so the meter reads 0% T. Each member of the group should verify all readings.

Figure 8. Typical spectrophotometer

3. *Always use the same cuvette and rinse with a few mLs of solution whenever you are using a new solution.* Discard the rinsing solution according to your instructor's directions. Three-quarters fill the rinsed cuvette with the blank solution from the standard sample labelled $1^{\#}$. Insert the cuvette into the sample holder of the spectrometer and adjust the light–control knob so 100% transmittance is read. Use always the same slot of the

sample holder for the remains of the experiment. Your instrument is now calibrated until completion of the experiment properly.

Attention: If wavelength is changed for any reason, then you may have to calibrate the meter in transmittance setting again at either 0% or 100% mode (more quick to use 0% mode with the black block to prevent light transmittance. Check with instructor.

Procedure Part C. Making the Absorbance Spectrum & Finding λ_{OPT} using a Standard Solution

1. Rinse the same cuvette you used for your blank with about 1 mL of your standard solution 6#. Three-quarters fill the rinsed cuvette with the sample solution. Insert the cuvette into the spectrometer. Measure and record the percent absorbance A in the range of 400-450nm but measure the data at every 10nm increments.

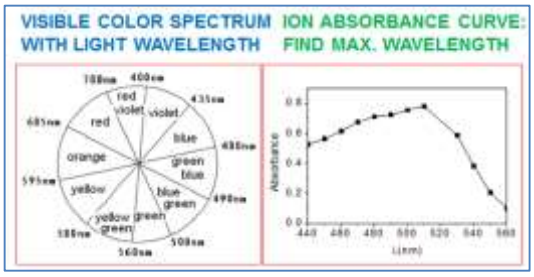

Figure 9. Determining λ_{OPT}.

2. Record your data on the table and the datasheet. Find λ_{OPT} corresponding to the maximum A (similar to data in Figure 9). All data points for a given curve must be measured with the same cuvette. All phosphate solutions should be discarded according to your instructor's directions. Diagram here shows typical absorbance with wave length using same sample solution. **Your curve may look different than that shown by Figure 9.**

λ (nm)	400	410	430	450
A				

Important Notes: Maximum Absorbance Optimum Wavelength (λ_{opt})

1. After calibration in Procedure Part B, remove cuvette and rinse in distilled water followed by sample 6# solution. Must use same cuvette, then change mode to absorbance.
2. Insert a 3/4 full of sample 6# solution into the same slot as during calibration with λ = 400nm, then read absorbance (make sure knob is pulled to the location slot of the cuvette).
3. Find the optimum wavelength when maximum absorbance is observed by varying λ=400nm-450nm, always increase knob in same direction and do not go backward until this Procedure Part C is completed. Do not re-calibrate the meter when changing wavelengths.

Important note: the spectrometers in the chemistry building are operating near the low ends of the Gaussians distribution curve of a vs λ, so higher than 400nm will produce erroneous absorbance data that can go up and down but higher that that at 400nm. Therefore, λ_{opt}= 400nm and the remaining absorbance data can be ignored.

Many factors can effect this erratic behavior such as: meter light sensitivity near the bottom of the Gaussians curve, accuracy of wavelength dial mechanism, accuracy of dial reading, cuvette condition such as rinsing, cleaning, bubbles, touching, solution reproduction etc.

Additional notes:

a. Make sure to use same cuvette and same slot inside spectrometer compartment
b. Before measuring, rinse cuvette with distilled water 2-3 times and then followed with Sample 6# again 2-3 times.
c. Only handle cuvette with the opaque side and wipe dry before inserting into spectrometer.

Procedure Part D. Making the Calibration Curve Using the standard Solutions

All data points for a given curve must be measured with the same cuvette using the same spectrometer. Before using any glassware with each new solution, the glassware must be rinsed with de-ionized water and about 1 mL of the new solution and as instructed during the lab.

1. Rinse the same cuvette you used for your blank with about 1 mL of your standard solution $2^{\#}$. Three-quarters fill the rinsed cuvette with the sample solution.
2. Insert the cuvette into the spectrometer. Measure and record the percent absorbance A in the range at λ_{OPT} once.
3. Repeat step 1 for the standard samples solutions $3^{\#}$ through $5^{\#}$ but only measure 1 absorbance for each sample 2# - 5#.
4. Record your absorbance data on the table shown by procedure Part A & on the datasheet.

Procedure Part E. Determination of Unknown Concentration

1. Pipet 5.00 mL of the unknown phosphate solution, 2.00 mL of 2M HNO_3 and 1.00 mL of the ammonium vanadomolybdate solution into the $7^{\#}$ 50-mL volumetric flask.
2. Dilute the solution by filling the volumetric flask until the liquid reaches the meniscus mark.
3. ¾ fill the rinsed cuvette with the unknown solution. This is Unknown $7^{\#}$ A. Use the spectrometer to measure its absorbance A. Using solution and same cuvette (empty), repeat procedure here to make Unknown $7^{\#}$ B and then measure its absorbance.
4. Now determine the unknown concentration by using the calibration curve (for further information, see references at the end of this experiment).
5. **Before you leave,** make sure everyone in your group has recorded on the datasheet, the concentration and the absorbance A for each of the various phosphate solutions. ***Please make the curves by using software such as origin or excel.***

E3: Spectrophotometric Analysis: Phosphates in Water

Name:	**Lab instructor:**
Date:	**Lab section:**

V. PRE-LABORATORY EXERCISE (PLE)

1. Define the underlined words in the **BACKGROUND** section.

2. In your own words, summarize the purpose of a calibration curve.

3. To prepare yourself for performing the dilutions required in this laboratory experiment, read the section on dilutions in your textbook (Chapter 4, section on dilution). What volume of 1.00×10^{-3} M phosphate stock solution is required to make 25.0 mL of a 4.00×10^{-5} M solution?

4. Using the spectrophotometer, a sample was analyzed and found to have a percent transmittance of 85%.

    a) What percent of light was actually absorbed by the sample?

    b) Calculate the absorbance (A) of the sample.

E3: Spectrophotometric Analysis: Phosphates in Water

Name:	**Lab instructor:**
Date:	**Lab section:**

VI. RESULTS AND POST-LABORATORY QUESTIONS (PLQ)

1. Attach a copy of your data table from today's experiment. Your table should include the concentration of phosphate in each standard solution, the measured %T and your calculated absorbance. You may copy the table shown in the Procedure – Part A.

2. Attach a copy of your calibration curve. What is the equation of the best-fit line?

3. Determine the concentration of phosphate in your unknown solution by extrapolation of the calibration curve (refer back to Figure 2) and by using the equation for the line of best fit. The extrapolation should be shown on your attached calibration curve. The calculation using the line of the best fit should be shown below.

4. The U.S. Public Health Service has set the maximum value of phosphate in the drinking water at 0.30 mg phosphate/liter. Did your unknown water sample violate this standard? Show your work.

SAMPLE DATASHEET FOR A LABORATORY SECTION

VC211 DATASHEET FOR EXPERIMENT: E3 SPECTROPHOTOMETERY OF PHOSPHOROUS IN THE FORM OF PO_4^{3-}

SECTION: TA: **GROUP EFFORTS &INDIVIDUAL REPORTS: EACH STUDENT SHOULD PREPARE AT LEAST ONE SAMPLE SOL'N, 1 CALIBRATION DATA & COMPLETE ROW DATA**

PART A: PREPARE 6 STANDARD CAL. SAMPLES + 1 UNKNOWN SAMPLE ← ----- **PARTS A, D, & E:** SAMPLES PREPARED WITH SHOWN VOLUMES (mL) ----->

PART B: CALIBRATION @ 400nm 0%T(black block)&100%T(Sample 1#) ←**PART A:** CALIBR. SAMPLES WITH SHOWN STNDRD VOLUME SOL'NS→ ←**PART E:** UNKNOWN ABSORB @λ_{OPT}→

←--- **PART C:** FIND λOPT of 6# SAMPLE → ←**PART D:** ABSORBANCE OF PREPARED SAMPLES @λ_{OPT}→

GRP #	NAME	ID #	λ1	λ2	λ3	λ4	λ5	λ_{OPT}	1#	2#	3#	4#	5#	6#	ABSORB. 7#A	ABSORB. 7#B	CONC. (M) 7#A	CONC. (M) 7#B
			400nm	410nm	420nm	430nm	450nm	.....nm	0.00mL	1.00mL	2.00mL	3.00mL	4.00mL	5.00mL	5.00mL	5.00mL	5.00mL	5.00mL
	ABSORBANCE (A)		A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13	A14	CONC'N	CONC'N
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Maximum Absorbance Optimum Wavelength (λ_{opt})

1. After calibration in Procedure Part B, remove cuvette and rinse in distilled water followed by sample 6# solution. Must use same cuvette, then change mode to absorbance.
2. Insert a 3/4 full of sample 6# solution into the same slot as during calibration with λ = 400nm, then read absorbance (make sure knob is pulled to the location slot of the cuvette).
3. Find the optimum wavelength when maximum absorbance is observed by varying λ=400nm-450nm, always increase knob in same direction and do not go backward until this Procedure Part C is completed. Do not re-calibrate the meter when changing wavelengths.

Important note: the spectrometers in the chemistry building are operating near the low ends of the Gaussians distribution curve of a vs λ, so higher than 400nm will produce erroneous absorbance data that can go up and down but higher that that at 400nm. Therefore, λ_{opt}= 400nm and the remaining absorbance data can be ignored. Many factors can effect this erratic behavior such as: meter light sensitivity near the bottom of the Gaussians curve, accuracy of wavelength dial mechanism, accuracy of dial reading, cuvette condition such as rinsing, cleaning, bubbles, touching, solution reproduction etc.

Additional notes:

a. Make sure to use same cuvette and same slot inside spectrometer compartment
b. Before measuring, rinse cuvette with distilled water 2-3 times and then followed with Sample 6# again 2-3 times.
c. Only hanndle cuvette by the opaque 2 sides and wipe dry carefully withproper tissue before inserting into its slot inside the spectrometer rack.

Procedure Part A: each student prepare 2 samples, group leader 1 sample
Procedure Part B: each student verify calibration
Procedures Part C, D, E: each student measure and record the remaining rows data (A1-A13(or A1-A12 & A14))

1. Wash & rinse all glassware.
2. Rinse each pipet & drive out all excess water then wipe dry the outside. 4 pipets available color coded: 1-mL (yellow tag for AV), 2-mL(black tag for 2M HNO_3), 5-ml (red tag) for 0.001M Na_3PO_4), 5-ml(red tag) for unknown ?M PO_4^{3-}). Keep pipets on the rack each at its original location.
3. Rinse pipet with stock solution (2-3 times). Minimize waste of reagents.
4. Using the pipet 1 mL AV stock solution into each of the 7 volumetric flasks.
5. Repeat step 4 by using 2mL 2M HNO_3 .
6. Repeat step 4 by pipet x mL 0.001M Na_3PO_4 standard solution. See (x) values above. For the unknown molarity sample of PO_4^{3-} dilute 5 mL in a 50.00-mL volumetric using either unknown samples A or B but not both
7. Dilute to 50.00mL mark
8. Measure absorbance of each sample at the optimum λ, make sure you rinse cuvette with each corresponding sample 2-3 time

AV = ammonium vanadomolybdate

ADDITIONAL REFERENCES: For reference & reading only but no need to copy this section into any part of your After-Lab Report (ALR)

REFERENCE 1

Doubts about E3

Sat, Mar 22, 2014 01:14 PM
Dear Professor:
I'm Michael again, and I want to ask a question on behalf of our lab team.
When we were finding the maximum wavelength corresponding to the maximum A, we had a problem which can't be solved.
First I want to show our data when carrying out Part 3:
λ(nm) 400 405 410 415 420 425 430 435 440 445 450
A 0.3 0.271 0.242 0.217 0.195 0.177 0.162 0.145 0.136 0.126 0.114
As is shown above, when λ is 400nm, A becomes the largest. But since 400 is the smallest in the range of 400~450, we can't
determine whether A will become even larger when λ further decreases. Therefore, we further decreased λ to examine A
continuously, and we found the following data:
λ(nm) 400 395 390 385…
A 0.3 0.332 0.371 0.415…
And the result is just as we expected.
So we still hold the doubt that whether we should use bigger λ to examine A in the following experiments? Is the range 400~450nm
too narrow for determining the λmax? Does this have something to do with the visible light spectrum whose range of
wavelength is 390~700nm?
Above is our current question, and I have put it in the Discussion part of my post-lab report.
Thank you so much for checking this out!
Have a nice weekend!
Michael Liu/JI Student Mar 22, 2014
Xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx

REFERENCE 2

TO STUDENTS: this section is for reference only & no need to copy into your report.

FERRIC SOLUTION SPECTROPHOTOMETRIC ANALYSIS

"The phosphate calibration solution used by SJTU chemistry department (easier to prepare) has disadvantage of not having a precise λ_{MAX} (no peak with bell shape trend). Instead & in the future, it is preferred to use the following alternative iron solution standard (using similar experimental procedure). Fe solution displays a nice shaped bell peak for λ_{MAX} as shown in the reference paper at the end of this experiment."

Procedure Part A. Preparation of Standard Solutions

1. Pipet 0.00, 1.00, 2.00, 3.00, 4.00, 5.00mL 20μg·mL^{-1} ferric stock solution into into labelled 1$^{\#}$ through 6$^{\#}$ 50-mL volumetric flasks, respectively. Record data in the following table and in your report.

Sample	1$^{\#}$	2$^{\#}$	3$^{\#}$	4$^{\#}$	5$^{\#}$	6$^{\#}$	Unknown 7$^{\#}$ A	Unknown 7$^{\#}$ B
V(mL)	0.00	1.00	2.00	3.00	4.00	5.00		
Conc.(M)								
Absorb. A								

2. Pipet 1.00 mL 10% hydroxylamine hydrochloride and 5.00mL 1.0M NaAc solutions into each of the labelled $1^{\#}$ - $6^{\#}$ 50 mL volumetric flasks, respectively.
3. Pipet 2.00 mL 0f 0.15% of Phenanthroline stock solution into each $1^{\#}$ - $6^{\#}$ 50-mL volumetric flasks.
4. Dilute the solution by filling the volumetric flask until the liquid reached the meniscus mark.

Procedure Part B. Adjusting the Spectrophotometer

1. Turn on the spectrometer to warm-up (15min).
2. Adjust the wavelength to 440nm. Use a black block to adjust T = 0%. Do not insert any cuvette in sample holder.
3. Wash and rinse the cuvettes. Insert a cuvette filled up to ¾ volume with solution from the $1^{\#}$ prepared solution to set T = 100%. Only insert the cuvette either on the left hand side or the right hand side space and keep using the same position throughout the entire experiment.

Procedure Part C. Making the Absorbance Spectrum Using a Standard Solution

1. Rinse another cuvette and ¾ fill the rinsed cuvette with solution from the $6^{\#}$ prepared solution.
2. Insert the cuvette into the spectrometer. Measure and record absorbance A in the range of 440-560nm but measure the data at every 5nm increments.
3. Find λ_{OPT} corresponding to the maximum A.

Procedure Part D. Making the Calibration Curve using the Standard Solution

- All data points for a must be measured with the same cuvette using the same spectrometer

1. Rinse the same cuvette, ¾ fill the rinsed cuvette with the $2^{\#}$ solution.
2. Insert the cuvette into the spectrometer. Measure and record A at λmax once.
3. Repeat above step for $3^{\#}$-$5^{\#}$ solutions but only measure 1 absorbance for each sample $2^{\#}$ - $5^{\#}$.

Procedure Part E. Determination of Unknown Concentration

1. Pipet 5.00 mL of the unknown ferric solution, Pipet 1.00 mL 10% hydroxylamine hydrochloride, 5.00mL 1.0M NaAc, and 2.00 mL 0f 0.15% of Phenanthroline stock solution into the $7^{\#}$ 50-mL volumetric flask.
2. Dilute the solution by filling the volumetric flask until the liquid reaches the meniscus mark.
3. ¾ fill the rinsed cuvette with the unknown solution. Use the spectrometer to measure absorbance A.
4. Now determine the unknown concentration by using the calibration curve. (for further information, see references at the end of this experiment). **Please make the curves by using software such as origin or excel.**

REFERENCE 3

Plot of the standard curve: showing the linear relation between light absorption and concentration of the standards
New Delhi, February 2000
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New Delhi – 11 00 16 India
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with
HALCROW, TAHAL, CES, ORG & JPS

1. Introduction to Absorption Spectroscopy

Absorption Spectroscopic methods of analysis rank among the most widespread and powerful tools for quantitative analysis. The use of a spectrophotometer to determine the extent of absorption of various wavelengths of *visible* light by a given solution is commonly known as *colorimetry*. This method is used to determine concentrations of various chemicals which can give colours either directly or after addition of some other chemicals. As an example, in the analysis of phosphate, a reaction with orthophosphate is made, to form the highly coloured molybdenum blue compound. The light absorption of this compound can then be measured in a spectrophotometer.
Some compounds absorb light in other than the visible range of the spectrum. For example, nitrates absorb radiation of 220 nm wave length in the UV region.

2. Absorption Spectroscopy Theory

Absorption Spectroscopic methods of analysis are based upon the fact that compounds ABSORB light radiation of a specific wavelength. In the analysis, the amount of light radiation absorbed by a sample is measured. The light absorption is directly related to the concentration of the colored compound in the sample.

The wavelength (λ) of Maximum Absorption is known for different compounds. For example, the colored compound formed for analysis of Phosphate (molybdenum blue) has maximum light absorption at λ= 640 nm. Conversely, a minimum amount of light is transmitted through the compound at λ= 640 nm. This is shown schematically in Figure 1.

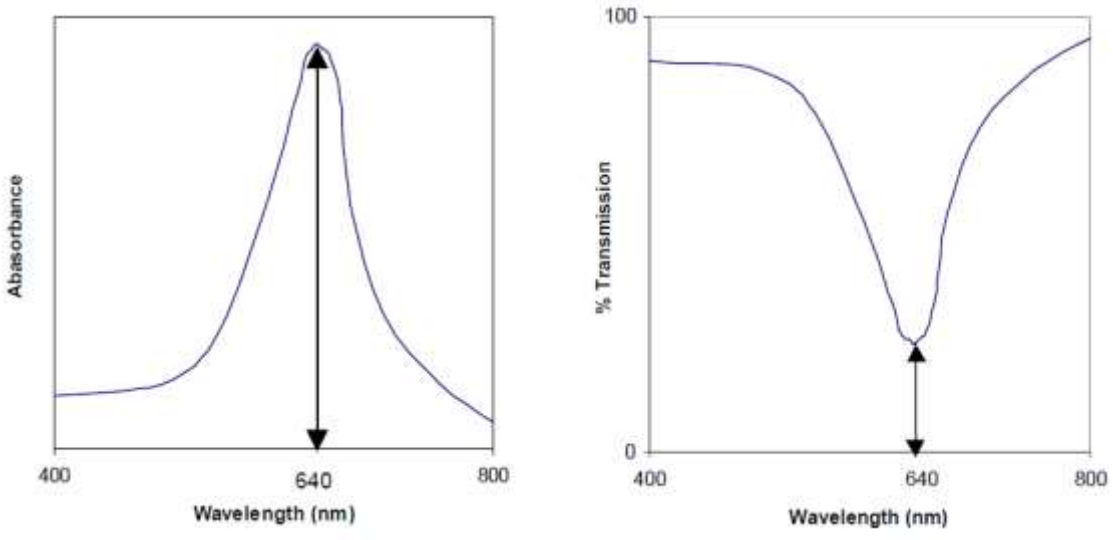

Figure 1: Light Absorption and Transmission by Phosphate-molybdenum blue compound. Schematic diagram showing maximum light absorption (and minimum light transmission) at λ= 640 nm.

Due to the fact that the overall composition of the sample is seldom the same as that of the calibration standard, in some cases, the absorption characteristics of the two may differ. Where such discrepancy is suspected, the standard addition approach may be used. Here, a known amount of analyte is added to a second aliquot of the sample. The difference in absorbance is used to calculate the analyte concentration of the sample as illustrated in

Example 1

A 25 mL sample after treatment with reagents to generate colour for measurement of phosphate yielded absorbance of 0.428. Addition of 1.00 mL of a solution containing 5.0µg phosphorus to a second 25 mL aliquot and development of colour resulted in an absorbance of 0.517. Calculate µg phosphorus in each mL of sample.

Solution:
Correct absorbance for dilution:
Corrected absorbance = 0.517 (26.0/25.0) = 0.538
Absorbance caused by 5µg phosphorus = 0.538 - 0.428 = 0.110
Therefore, phosphorus in the sample = (5.0/0.11) 0.428
= 19.5µg, or 19.5/25 = 0.7µg/mL

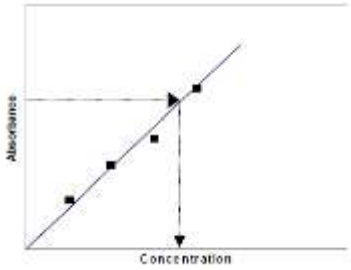

Figure 6: Finding the concentration of an unknown sample from the standard curve.

Overview of individual methods

The general procedure can be followed for all spectrophotometer analyses. For analysis of specific compounds, the method of preparation of the colored compound, and the wavelength of maximum light absorption will vary. An overview is given in Table 1.

Table 1 Overview of specific methods used for analysis of water quality parameters, and the wavelength of maximum light absorption

Parameter	λ	Method Name	SAP
Aluminum	535	Eriochrome Cyanine R Spectrophotometric	1.30
Boron	540	Curcumin Spectrophotometric	1.3
Chlorophyll a	750, 664, 65	Acetone Extraction Spectrophotometric	1.5
Flouride	570	SPADNS Spectrophotometric	1.11
Iron	510	Phenanthroline Spectrophotometric	1.13
Manganese	525	Persulphate Spectrophotometric	1.34
NH_3-N	640	Phenate Spectrophotometric	1.15
NO_3-N	220, 275	UV Spectrophotometric	1.16
NO_2-N	543	Sulphanilamide Spectrophotometric	1.17
-PO_4	880	Ascorbic Acid Spectrophotometric	1.20
Total P	880	Digestion + Ascorbic Acid Spectrophotometric	1.39
Silica	815	Ammonium Molybdate Spectrophotometric	1.38
Sulphate	420	NOTE: preferred method for sulphate analysis is with nephelometer	1.26

Spectrophotometric analysis has:
• wide applicability
• high sensitivity: detection limit 10^{-5}M to 10^{-4}M range
• moderate to high selectivity
• good accuracy: relative error 1 to 3%
• ease and convenience, lends to automation

REFERENCE 4:

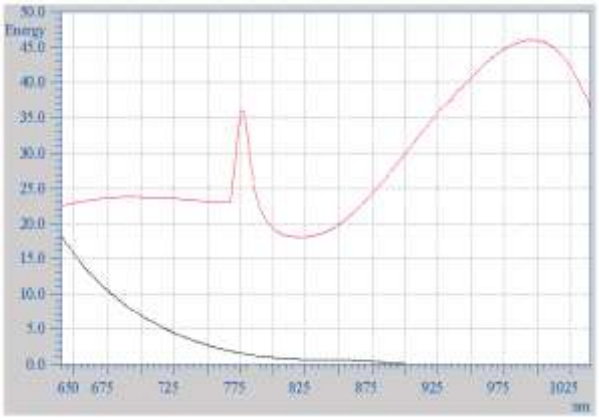

Figure 1: Comparison of the Cintra 10e raw light scan with a conventional spectrometer raw light scan

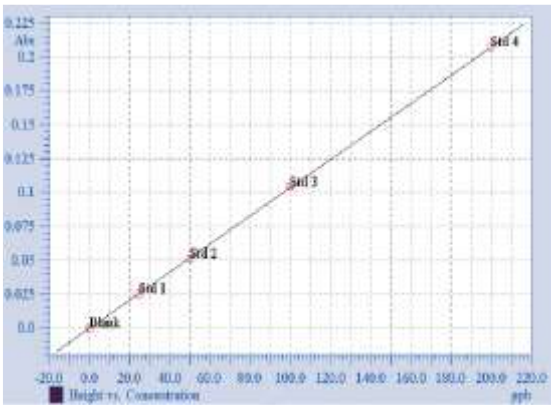

Figure2: Calibration graph of standards from 25 to 200 ppb Phosphorus.

Sensitivity from 600–1,200 nm, Analysis of Phosphorus to Sub-ppb Levels

application note

Cintra 10e—Enhanced Sensitivity from 600–1,200 nm, Analysis of Phosphorus to Sub-ppb Levels

by Paul A. Liberatore
Marketing Product Manager
UV-Vis Spectroscopy

Introduction

The analysis of phosphorus is of importance in environmental and industrial type monitoring applications. Phosphorus has been traditionally measured in solutions using a UV-Visible Spectrometer at the compromised 470 nm or the 690 nm wavelengths[1]. These methods have poor detection limits and suffer from the phosphorus-complex formation taking a relatively long time to stabilize as well as producing unstable complexes.

The most sensitive 830 nm wavelength has not been commonly utilized due to very low light throughput of conventional spectrometers at this wavelength.

The reason for this is that spectrometers, that utilise a photo multiplier detector usually have an upper wavelength range of 900 nm. These instruments have very poor light throughput in the 600 to 900 nm range and hence cannot utilise the most sensitive 830 nm wavelength.

The Cintra 10e uses a Silicon Photodiode and advanced optics design, which enables a wavelength range up to 1,200 nm. The Cintra 10e also has significantly increased light throughput in the important 830 nm wavelength range.

The Molybdenum Blue phosphorous method[2] is a very sensitive method for the determination of phosphorus. When this method is used in conjunction with a spectrometer with ample light throughput at 830 nm, such as the Cintra 10e, then phosphorus analyses can be determined at sub ppb concentrations. The Molybdenum Blue phosphorous method is not only easier to use than conventional methods but the blue phosphorous-molybdenum complex formed is produced very quickly and is stable for over 24 hours. This enables quick turn around time for sample analysis because long stabilization times required for complex formation (as occurs in conventional methods), are not required. As the blue phosphorous-molybdenum complex is very stable, samples do not have to be analysed quickly, so large sample batches may be analysed. Conventional methods produce unstable complexes, necessitating fast analysis of small sample batches.

Equipment

Cintra 10e.
Spectral Quantify Application.
1 cm quartz cell.

For the automated analysis of up to 270 samples, use the SDS-270 auto-sampler and the auto-sipper with flow through cell.

Notes—UV-Visible 35

Cintra 10e enhanced energy above 630 nm

A Raw Light Scan is the best way to visually determine the light throughput to the detector at various wavelengths. The photomultiplier gain is kept constant and light scanned across the wavelength range. Figure 1 graphically illustrates the superiority of the Cintra 10e over conventional spectrometers in regards to light throughput above 630 nm. The Cintra 10e has a flat energy response from 630 to 760 nm, then it peaks at 770 nm drops in intensity at 800 nm and then peaks again at 1,000 nm. The energy response for a conventional spectrometer is already low at 600 nm and drops exponentially up to 900 nm.

At 830 nm the energy response of the Cintra 10e is 18.4%. Compare this to a conventional spectrometer which has an energy response at 830 nm of 0.4%. The energy response at 830 nm is nearly 50 times higher in the Cintra 10e compared to a conventional spectrometer. This increase in energy at 830 nm will result in decreased photo multiplier voltage required to amplify the signal and a subsequent decrease in noise. The decrease in noise will also lead to improved detection limits in the case of phosphorus determination.

The increased energy at 830 nm coupled with an inherently sensitive phosphorus method enables in the measurement of sub ppb phosphorus concentrations.

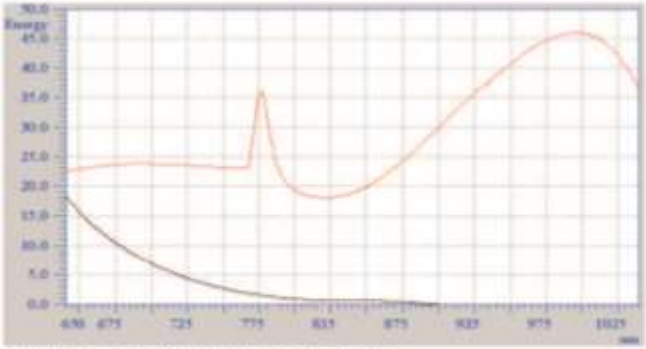

Figure 1: Comparison of the Cintra 10e raw light scan with a conventional spectrometer raw light scan

Molybdenum Blue Method

Orthophosphate and molybdate ions condense in acidic solution to form molybdophosphoric acid (phosphomolybdic acid). Upon selective reduction, (e.g., with hydrazinium sulphate) a blue colour is produced due to Molybdenum Blue of uncertain composition. The intensity of the blue colour is proportional to the amount of phosphate initially incorporated into the heteropoly acid. If the acidity at the time of reduction is 0.5 M in sulphuric acid and hydrazinium sulphate is the reductant then the resulting blue complex exhibits a maximum absorbance at 820–830 nm.

Ions which form heteropoly acids, such as silicate, arsenate, germanate and tungstate should be absent. Silicate may be removed by fuming with perchloric acid to dehydrate the silicic acid and render it insoluble. Arsenate may be volatized as arsenic (III) bromide from a hydrobromic-acid sulphuric acid solution. Tin and germanium are also volatilized simultaneously.

Reagents

1. ***Molybdate solution.***

   12.5 g of Analytical Reagent sodium molybdate ($Na_2MoO_4.2H_2O$) was dissolved in 5 M sulphuric acid and diluted to 500 mL with 5 M sulphuric acid.

   This solution is to be prepared fresh monthly.

2. ***Hydrazinium sulphate solution.***

   1.5 g of Analytical Reagent hydrazinium sulphate was dissolved in deionised water and diluted to 1000 ml..

   This solution is to be prepared fresh monthly.

3. ***Standard Phosphate solution (10 ppm P)***

   0.04393 g of Analytical Reagent Potassium Dihydrogen Phosphate was dissolved in deionised water and diluted to 1000 mL. 1 mL of solution = 0.01 mg P.

   This solution is to be prepared fresh weekly.

4. ***Calibration Standards***

   The calibration standards were prepared as per table 1. These are to be prepared fresh daily.

Volume of 10 ppm P	Final Volume	Concentration P (ppb)
0.000	50	0
0.125	50	25
0.250	50	50
0.500	50	100
1.000	50	200

Table 1: *Dilution scheme for calibration standard preparation*

Procedure

The sample solution should not contain more than 400 ppb of phosphorus present as the orthophosphate and should be neutral. Solutions, which have greater than 400 ppb phosphorus should be diluted or alternatively higher concentration standards can be prepared and used.

Transfer 25 mL of the solution to a 50 mL volumetric flask. Add 5.0 mL of the molybdate solution, followed by 2.0 mL of the hydrazinium sulphate solution. Make to volume with distilled/deionised water and mix well. Immerse the stoppered flasks in boiling water for 10 minutes. Remove the flasks and cool rapidly.

Shake the flasks and measure the absorbance at 830 nm against a reagent blank.

Note that the sample heating for 10 minutes ensures that the reaction has gone to completion. Studies undertaken showed that the blue colour of the phosphorus-molybdenum complex formation was complete after the 10 minute heating and no further reaction occurred. As a stable reading is obtainable immediately after the cooling step, samples can be analysed immediately unlike conventional methods such as the Vanadomolybdophosphoric acid method at 470 nm, which has a long stabilization time.

Laboratory productivity is significantly enhanced using the Molybdenum Blue method.

The blue phosphorous-molybdenum complex was found to be stable after 24 hours. This gives the analyst the flexibility that;

1. Samples do not have to be analysed immediately after they are prepared
2. Sample integrity is maintained if large batches of samples are prepared and then analysed.

In contrast, conventional methods such as the Vanadomolybdophosphoric acid method at 470 nm do not form stable coloured complexes. Hence with these methods, as samples must be analysed immediately after preparation, large sample batches cannot be to be analysed.

Results

Table 2 lists the standard results obtained. Figure 2 graphically illustrates this data.

Even at the low phosphorus concentrations, a linear correlation was obtained between concentration and absorbance.

Concentration P (ppb)	Absorbance
0.0	0.0000
25	0.0253
50	0.0513
100	0.1042
200	0.2071

Table 2: *Calibration results obtained*

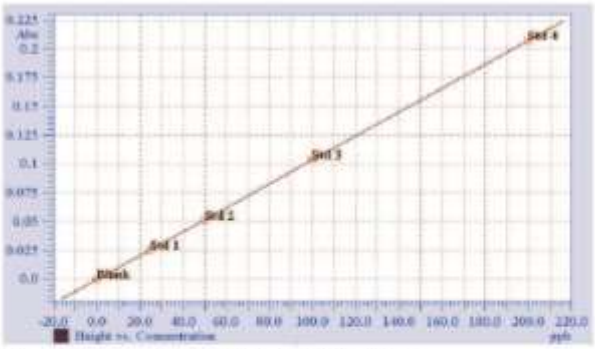

Figure2: *Calibration graph of standards from 25 to 200 ppb Phosphorus.*

The detection limit was calculated by analysing a 25 ppb standard and reagent blank 10 times. Using a 3σ confidence limit, a detection limit of 0.2 ppb was obtained. This is 1000 times more