Example Lab Report

Spectrophotometric Analysis

CEE 341
Fluid Mechanics for Civil Engineers

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For:

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1.0 Objective

Spectrophotometric analysis for determining the amount of an inorganic compound in solution involves a reaction between an organic reagent and an analyte to form a colored complex. The reaction can be used to determine analyte concentrations assuming the color intensity and absorbance is proportional to the analyte concentration, the complex is stable, and the reagent does not significantly react with other constituents thereby causing interferences. A spectrophotometer is the specific device which measures the absorption of a monochromatic light beam by a sample and added reagent. The objective of this laboratory exercise is to become familiar with a typical spectrophotometric analysis and to examine the effect of an interfering substance. The inorganic analyte being considered in this particular analysis is phosphate and the interfering substance is arsenic.

2.0 Theory

The first portion of a spectrophotometric analysis consists of preparing six standard solutions, each with a known phosphate concentration. By measuring the absorbance of each standard and added reagent with a spectrophotometer and plotting its value relative to the known phosphate concentration, a standard phosphate curve can be developed [Standard Methods, 1992]. The second portion of this type of experimental analysis involves measuring the absorbance water samples and reagent having unknown phosphate concentrations. These samples typically consist of canal water filtered through acid-washed GF/C filters, canal water stored without filtration for one day and then filtered through nonwashed GF/C filters, filtered canal water with 1.0 mg/L of arsenic added, and a distilled water blank. The standard curve can be used with the measured absorbance to determine the unknown phosphate concentration in each sample. This specific method of analysis is often referred to as the ascorbic acid method for determining phosphate concentrations.

Beer's law can be described by the following expression [Standard Methods, 1992]:

$$A = k_2 C \tag{1}$$

where k_2 is Beer's proportionality constant, A is absorbance, and C is concentration. According to Beer's Law, the relationship between absorption and concentration is linear.

A statistical analysis is used to evaluate consistency and performance in spectrophotometric analysis. The standard deviation, a common measure of variability, is evaluated using the following expression given [Hogg and Ledolter, 1987]:

$$s_x = \sqrt{\frac{\sum (X_i - X_{AVG})}{n-1}} \tag{2}$$

Where X_i is the computed concentration value, X_{AVG} is the mean concentration value, n is the number of samples analyzed, and s_x is the standard deviation.

The coefficient of variation, which is used to normalize the standard deviation to the mean, is determined by:

$$CV = \frac{s_x \times 100}{X_{AVG}} \tag{3}$$

A large coefficient of variation indicates widely scattered or varied results, and thus precision has been somewhat sacrificed in the analysis.

3.0 Anticipated Results

The following is a short list of anticipated results and trends which will be confirmed by this laboratory exercise:

- An intensely colored solution of sample and reagent should yield a higher absorbance and, in turn, a higher phosphate concentration.
- Since arsenates also react with the molybdate reagent, the presence of arsenic will most likely cause distinct interferences in the determination of phosphate concentrations.
- The phosphate concentration in stored samples should be higher than those that were not stored due to the formation and persistence of orthophosphates over time.
- Beer's Law is expected to be observed within the working range since the standard curve will be approximated as a straight line.
- The detection limit is expected to be approximately at the lower end of the working range.

4.0 Apparatus

A schematic of the apparatus used for this laboratory exercise is shown in Table 1 on page 6, if one uses their imagination. The device on the left is seated at a desk, while the device on the right

is standing. There are several sheets of paper attached to the apparatus and one of the devices is applying lead marks to one of them.

Figure 1: Spectrophotometric Analysis Apparatus

5.0 Procedure

The first step in the ascorbic acid method for determining phosphate concentrations was to develop the standard curve using six working standards. The standard curve could then be used with values of absorbance measured by the spectrophotometer in order to analyze four samples of unknown phosphate concentration.

5.1 Working Standards

The development of a standard phosphate curve was necessary to establish a relationship between absorbance and phosphate concentration for the spectrophotometric analysis. First, a 30-ml beaker was filled with a standard phosphate solution of which 1.0-ml contained 2.5 μg-P. To each of six 100-ml flasks, the following volumes of the standard phosphate solution were added using an autopipette and then diluted to the 100-ml mark: 0.4-ml, 0.8-ml, 1.6-ml, 2.4-ml, 3.2-ml and 4.0-ml. These diluted mixtures thus contained 10, 20, 40, 60, 80 and 100 μg-P/L, respectively, and were used as working standards for the analysis. After sufficient mixing, 50-ml of each working standard was transferred to a labeled 250-ml Erlenmeyer flask using a 100-ml graduated cylinder. The transfer began with the lowest phosphate concentration and continued in order of increasing

concentration. The flasks were then set aside as the measured absorbance and known phosphate concentrations of these working standards would be used to develop the standard curve.

5.2 Sample Description

Four samples of unknown phosphate concentrations were to be analyzed within this laboratory exercise. These samples included (1) canal water filtered through acid-washed GF/C filters, (2) canal water stored without filtration for one day and then filtered through nonwashed GF/C filters, (3) filtered canal water with 1.0 mg/L arsenic, and (4) a filtered distilled water blank. Note that samples no. 1 and no. 3 were identical except that no. 3 contained 1.0 mg/L of arsenic and that samples no. 1 and no. 2 were the same except that no. 2 was stored for one day and filtered through a nonwashed filter. First, 50-ml of deionized water was added to a 250-ml Erlenmeyer flask and was used as a blank for the analysis. To the remaining four 250-ml Erlenmeyer flasks, 50-ml of each sample was added using designated 100-ml graduated cylinders.

5.3 Sample Analysis

To this point, each laboratory group had prepared eleven flasks filled with standard and sample solutions, respectively. To each flask, one drop of phenolphthalein indicator was added and mixed well. Sample no. 3 developed a slight red color which was discharged by the addition of one drop of 5 N H2SO4 solution. Next, 8-ml of premixed combined reagent was added to each flask using an autopipette. Each solution was mixed well following the addition of the combined reagent. The reagent consisted of 5N sulfuric acid, potassium antimonyl tartrate solution, ammonium molybdate solution and 0.01 M ascorbic acid.

The laboratory groups were careful in coordinating the addition of combined reagent since it was necessary to wait at least ten minutes for color development and no longer than thirty minutes before measuring the absorbance of each sample. To measure absorbance, all the samples and standards were transferred into labeled, clean 5-cm vials. The spectrophotometer was set to 880 nanometers and zero percent transmittance, and was then used to measure absorbance. The deionized water blank was inserted into the vial holder on the spectrophotometer and the cover placed over the vial. Using the blank, the device was calibrated to zero absorbance. Next, the absorbance of each working standard, starting with the lowest concentration, was measured and

recorded. Once complete, the same procedure was used to measure the absorbance of each sample. Following the measurement of all samples and standards, the deionized water blank was reinserted into the spectrophotometer to confirm that the device was still calibrated. The recorded data used to develop the phosphate standard curve and evaluate the phosphate concentration of each sample are presented in Section 6.0 on page 8.

6.0 Results

Using methods previously described, a record of measured absorbance for each of the four samples and the five standard solutions is shown in Table 1. Note that the absorbance for sample no. 3 was too high for the spectrophotometer to recognize at the specified settings.

Table 1: Measured Absorbance by Spectrophotometric Analysis

Sample No.	Identification	Measured Absorbance
Blank	Distilled Water Blank	0.000
1	Filtered Canal Water	0.031
2	Stored, Nonfiltered Canal Water	0.059
3	Filtered Canal Water w/ Arsenic	*
4	Filtered Distilled Water	0.003
5	10 μg-P/L Standard	0.013
6	20 μg-P/L Standard	0.022
7	40 μg-P/L Standard	0.059
8	60 μg-P/L Standard	0.071
9	80 μg-P/L Standard	0.119
10	100 μg-P/L Standard	0.139

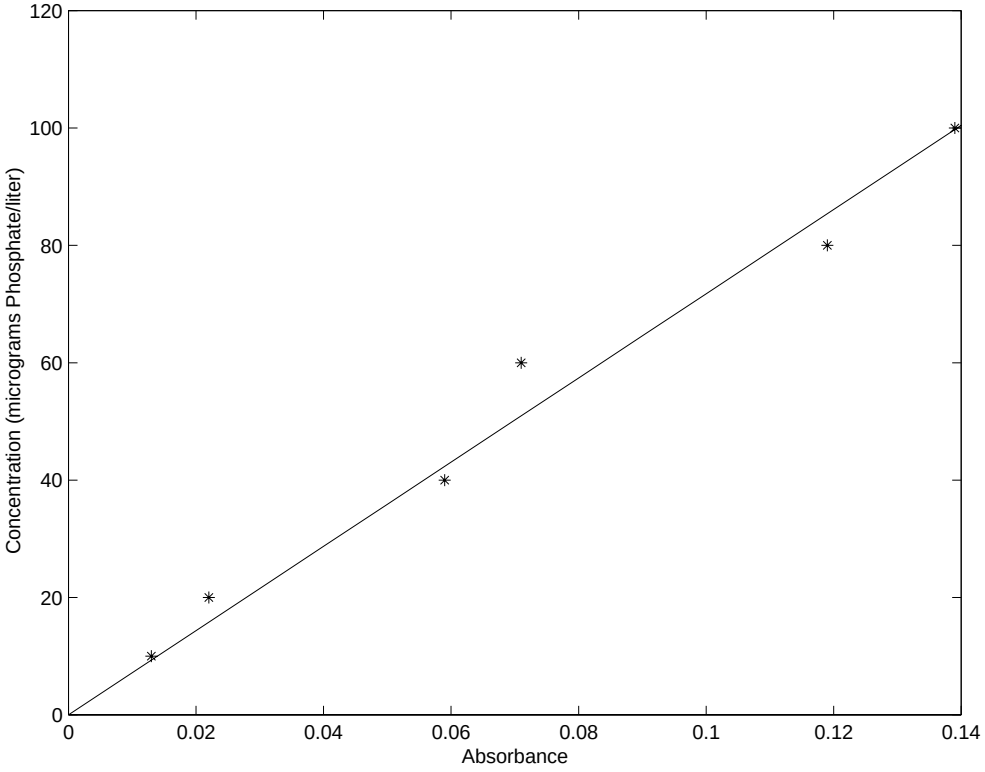
* Concentration too high for measurement (>> 100 μg-P/L)

Using the measured absorbance values for the working standards, listed as samples no. 5 through 10 in Table 1, the standard curve shown in Figure 2 was developed. This curve simply plots the measured absorption of a monochromatic light in the spectrophotometer relative to the known phosphate concentration. A straight line was approximated through the data points using linear regression. As shown in the figure, the equation of the approximated line is best described as:

$$[\text{Concentration}] = 717.58 \times [\text{Absorbance}] \quad (4)$$

The line has a slope of 717.58 and an R2 value of 0.9779 from a linear fit.

Figure 2: Standard Curve for Phosphate Analysis



Using the developed equation (4) for the standard curve, phosphorus concentrations for samples no. 1 through 4 could be determined. Table 2 shows the concentrations found by substituting the measured absorbance value of each sample into the standard curve equation. Note that the table shows the results for all five laboratory groups, each of which developed a slightly different standard curve.

Table 2: Analytical Phosphate Concentration in g/L

Sample No.	Identification	Group 1	Group 2	Group 3	Group 4	Group 5
1	Filtered Canal Water	21.15	13.57	22.05	22.24	16.70
2	Stored, Nonfiltered Canal Water	42.31	42.50	36.76	42.34	30.60
3	Filtered Canal Water w/ Arsenic	*	*	*	*	*
4	Filtered Distilled Water	0.00	2.14	0.00	2.15	0.00

* Concentration too high for measurement (>> 100 μg-P/L)

Using results for samples no. 1 and 2 from each of the five laboratory groups and equations (2) and (3), a statistical reduction provided the mean, standard deviation and coefficient of variation of phosphate concentrations. Statistical results are shown in Table 3 and a qualitative evaluation of these data will be further discussed in Section 7.0

Table 3: Statistical Reduction of Data

Sample No.	Identification	Mean Conc. g-P/L	S(Xi-Xmean)2	Std. Deviation	Coeff. of Variation (%)
1	Filtered Canal Water	19.14	59.10	3.84	20.08
2	Stored, Nonfiltered Canal Water	38.90	109.89	5.24	13.47

7.0 Discussion

This exercise was helpful in gaining an understanding of spectrophotometric techniques and its usefulness in determining concentration of an inorganic compound. In the following section, the data obtained is briefly discussed from a qualitative viewpoint, specific questions posed in the laboratory manual are addressed, and general trends and relationships are presented.

As expected, a more intensely colored solution of sample and reagent yielded a higher absorbance and, in turn, a higher apparent phosphate concentration. Sample no. 3 gave the most intense blue color following the addition of the combined reagent. As shown in Tables 1 and 2, the absorbance and concentrations were too high to measure or determine within this particular working range. It is evident, however, that arsenic has interfered with the phosphate determination in sample no. 3. In other words, the intense blue color is not the sole cause of high phosphate concentration since arsenic reacts with the molybdate reagent in a similar manner to that of phosphate.

Specific problems within the laboratory exercise were limited. Further, the reasonable coefficients of variation shown in Table 3 demonstrate that the results did not vary significantly from one laboratory group to another. Regardless, one possible cause of variations in mean concentrations may be the room for possible error in reading spectrophotometer. Reading the measured absorbance values from the meter is subject to a certain amount of personal judgment and experience, and results could therefore vary slightly between laboratory groups. Also, a lack of precision may arise from the fact that the analysis consisted of many volumetric transfers of solution from one container to another.

For this experimental analysis, the standard curve developed from working phosphate standards was approximated by a straight line. The approximated line was described by equation (4), rewritten here as:

$$[\text{Concentration}] = 717.58 \times [\text{Absorbance}] \quad (5)$$

The observed Beer's proportionality constant for this analysis is then (1/717.58) or 1.39 x 10-3. Since the working standards were created using concentrations varying from 10 to 100 μg-P/L, the working range for this analysis is also 10 to 100 μg-P/L. It can then be said that for this analysis, Beer's Law is observed for the standard curve within the working range. Above the working range, it is expected that the absorption-concentration relationship may significantly deviate from a linear correlation. Note that if the straight line had not been used to approximate the relationship between absorbance and concentration, the correlation would not have been linear and thus Beer's Law would not truly apply. This analysis essentially manipulates the data slightly in order to allow application of Beer's Law. Items that may cause a significant deviation from Beer's law include using a non-monochromatic light, the existence of an unstable solution, or the interference from other ions present in solution.

From the statistical values presented in Table 3, it is evident that mean phosphate concentrations for samples no. 1 and 2 for the five laboratory groups are not the same. The mean phosphorus concentration for sample no. 2 is approximately twice that of sample no. 1. Note that the samples are identical except that sample no. 2 was stored for one day and then filtered through a non-washed GF/C filter. The practical interpretation of these results is that storing the sample for one day and filtering through a nonwashed filter has allowed the natural formation and existence of additional orthophosphates over time. This results in an increased phosphorus concentration as evidenced in the results for sample no. 2. Note that the coefficient of variation for samples no. 1 and 2 are 13.47% and 20.08%, respectively. For this analysis in which exact methodology between laboratory groups could vary significantly, this particular indication of result scatter seems acceptable.

Working standards for this analysis were created using concentrations varying from 10 to 100 μg-P/L. Therefore, the working range is also approximately 10 to 100 μg-P/L. Outside this range,

the linear standard curve has been extrapolated and accuracy will begin to be sacrificed. Based on the working range, the acceptable detection limit for this analysis is approximately 10 μg-P/L. Below this level, which is outside the working range, one cannot be confident in the accuracy of determined concentrations [Standard Methods, 1992].

Samples No. 1 and No. 3 were identical in nature except that No. 3 contained 1.0 mg/L of arsenic. Therefore, any concentration differences in the results for these samples are attributable to the presence of the arsenic. The mean phosphate concentration of sample no. 1 for the five groups was 19.14 μg-P/L. However, the mean concentration for sample no. 3 was too high for measurement by methods prescribed in this analysis. Hence, arsenic has greatly interfered with this phosphate analysis. Since the working range for this exercise is 10 to 100 μg-P/L, as determined earlier, sample no. 3 can be described as having a concentration of >>100 μg-P/L. Then the interference due to the presence of 1.0 mg/L of arsenic is at least (100 - 19.14) μg-P/L or >> 80 μg-P/L. The reason for the interference is that arsenic reacts with the molybdate combined reagent, in a similar manner to that of phosphate, to produce a blue color. The intense blue color observed in sample no. 3 was not caused solely by the presence of phosphorus, but also by the presence of arsenic [Standard Methods, 1992].

The initial intent of this laboratory experiment was to analyze samples no. 1 and 3 by ion chromatography following the spectrophotometric analysis. However, the ion chromatograph was inoperable at the time of this exercise. In speculation of the results, ion chromatography is not based on the determination of concentration by monochrome light absorption or color intensity of a solution. Therefore, regardless of the fact that arsenic reacts with the combined reagent to cause a more intensely colored solution, ion chromatography should eliminate the arsenic interference.

8.0 Conclusion

This laboratory exercise successfully facilitated an understanding of spectrophotometric analysis techniques and the effect of an interfering substance. Therefore, the objective of this experiment has been satisfied. Other conclusions that can be drawn include:

- A more intensely colored solution of sample and combined reagent is indicative of a solution that yields a higher absorbance and that contains a higher phosphate concentration.
- Arsenic significantly interferes with the phosphate analysis since arsenic reacts with the molybdate reagent in a similar manner to that of phosphate. The interference was quantified as >> 80 μg-P/L.
- The phosphate concentration in a sample stored for one day is greater than that of an identical sample that has not been stored for a period of time. This effect is due to the formation and persistence of orthophosphates over time.
- Beer's law, which describes a linear relationship between absorbance and concentration, was essentially observed for this analysis within the working range since the standard curve was approximated with a straight line.
- The working range for this analysis was 10 to 100 μg-P/L as determined by the working standards, and the minimum detection limit for phosphate concentration was best approximated as 10 μg-P/L.
- Though it was not verified by experimental methods within the laboratory exercise, it is expected that ion chromatography would eliminate the interference caused by arsenic. This statement is based on the fact that ion chromatography is not dependent on light absorption and color intensity, while spectrophotometry is dependent on these parameters.
- Precision of this analysis seemed acceptable as evidenced by the relatively low coefficients of variation for phosphate concentrations as determined by the five laboratory groups.

9.0 Critique

The lab manual was extremely helpful due to its clarity and attention to detail. The apparatus and procedures were described in detail and were plain even to the uninitiated. However, the objective of the experiment did not become clear even after reading and studying the theory behind it. However, after processing the data to complete the lab, the relationship between absorbance and concentration became clear. This sample lab report really helped me to understand what is required in terms of style and layout. Use your own thoughts and ideas about the manual, apparatus, procedure, and methods used in the lab.

10.0 Appendix

10.1 References

1.Greenberg, A., Clesceri, L. and Eaton, A., *Standard Methods for the Examination of Water and Wastewater*, 18th ed., American Public Health Association, American Water Works Association, and Water Environment Federation, Washington, DC, 1992.

2.Hogg, R. and Ledolter, J., *Engineering Statistics*, Macmillan Publishing, New York, NY, 1987.

10.2 Sample Calculations

10.3 Original Data Sheets