

UV/Vis Spectroscopy

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Water Analysis Using LAMBDA UV/Visible Spectrophotometers: Ortho-Phosphate Determination

Introduction

In surface waters, phosphorus commonly exists in its phosphate

form. A high concentration of phosphate in water is indicative of domestic waste, industrial effluent, and agricultural runoff which can lead to eutrophication. Eutrophication causes an increase in plant and algal growth, which decreases the dissolved oxygen in the water, often leaving the water uninhabitable to organisms. Most phosphates from these human sources are either polyphosphates or organically bound, which eventually degrade to ortho-phosphates (PO_4^{3-}).¹

In this application, the quantitative analysis of ortho-phosphate was performed using the LAMBDA 265™ UV/Vis spectrophotometer and CHEMetrics ortho-phosphate cell test kit.

Principle

Ortho-phosphates react with ammonium molybdate and ammonium vanadate in acidic solution to form a yellow colored complex which can be detected spectrophotometrically at 420 nm and is directly proportional to the phosphate concentration. This method is suitable for the concentration range of 0 – 80 mg/L phosphate in water, allowing its concentration to be determined without the use of a calibration curve by incorporating the measured absorbance at 420 nm into a known equation.

Reagents and Apparatus

1. CHEMetrics phosphate Vacu-vials® kit (K-8503) - containing 30 vials and sample cup
2. PerkinElmer LAMBDA 265 PDA UV/Visible Spectrophotometer
3. UV Lab™ software
4. Phosphate standard solution (1000 +/- 4 mg/L)
5. Deionised (DI) water
6. Volumetric flasks (100 ml)
7. Micropipettes

Method

A stock solution of phosphate (1000 mg/L) in water was used to prepare a 40.00 mg/L phosphate solution in a 100 ml volumetric flask by dilution with DI water.

Following preparation of solutions, the sample cup was filled with the 40.00 mg/L phosphate solution up to the 25 mL mark. The tip of the Vacu-vial ampule was placed in the sample cup, snapped, and the ampule then inverted several times to promote mixing. The ampule was dried and left to stand for five minutes and the absorbance measured in the spectrophotometer. This technique was also carried out for the reference sample supplied in the test kit.

Using the UV Lab software, the LAMBDA 265 instrument parameters were set, as shown in Figure 1, to measure the absorbance at 420 nm, and an equation set up to calculate the phosphate concentration as shown in Equation 1. Following measurement of the blank, the absorbance of the known phosphate solution in the Vacu-vial was recorded.

Equation 1.

$$\text{Phosphate concentration (mg/L)} = 70.2 (A_{420}) - 0.19$$

Results

Figure 2 shows spectra from three repeat runs of the 40.00 mg/L phosphate sample, with the results shown in Table 1. The mean absorbance at 420 nm was determined to be 0.610, which corresponded to a calculated concentration of 40.92 mg/L phosphate. The results obtained had a good level of accuracy and repeatability with a relative standard deviation of 0.11%.

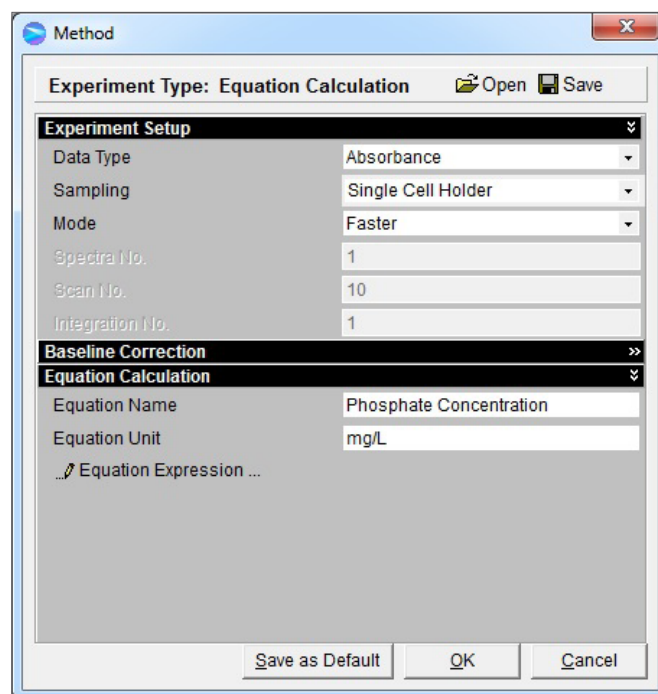


Figure 1. Instrument parameters and method setup.

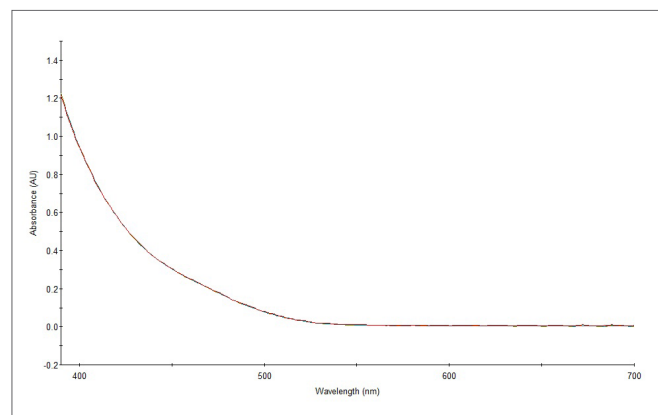


Figure 2. Overlaid UV/Vis spectra of repeat measurements of phosphate solution.

Table 1. Results for repeat measurements.

Phosphate Solution	Absorbance at 420 nm	Phosphate Concentration (mg/L)
Repeat 1	0.610	40.89
Repeat 2	0.610	40.89
Repeat 3	0.611	40.99
Repeat 4	0.609	40.88
Repeat 5	0.610	40.90

Conclusion

Quantitative analysis of ortho-phosphate in water was achieved rapidly with a high level of repeatability and accuracy using the LAMBDA 265 UV/Vis spectrophotometer and UV Lab software. The CHEMetrics phosphate cell test kit allows for simple determination of phosphate concentration, avoiding exposure to hazardous chemicals and measurement of calibration standards.

References

1. <http://water.epa.gov/type/rsl/monitoring/vms56.cfm>
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