Spectrophotometric Analysis


OBJECTIVES

• Practice calculating and performing dilutions of solutions.
• Determine the concentration of phosphate in a water sample by spectrophotometric analysis.
• Construct and utilize an absorbance and calibration curve.
• Explore the dynamics of working with a larger group of students

BACKGROUND

• Spectrophotometric Analysis and the Determination of Phosphate
• The Absorbance Curve
• The Calibration Curve

Chromogenic Reaction of Phosphate

\[
\text{NH}_4\text{VO}_3 + \text{MoO}_4^{2-} + \text{PO}_4^{3-} \rightarrow (\text{NH}_4)_3\text{PO}_4 \cdot \text{NH}_4\text{VO}_3 \cdot 16\text{MoO}_3
\]

Chromogenic Reagent, AV solution

Spectrophotometric Analysis and the Determination of Phosphate(1)

Spectrophotometric Analysis and the Determination of Phosphate(2)
**Spectrophotometric Analysis and the Determination of Phosphate (3)**

**Color absorbed or transmitted?**

**Complementary color**

**Beer-Lambert Law**

\[
\epsilon = \frac{A}{b c} = -\log_{10}(\text{T})
\]

\[
T = \frac{P}{P_0} \times 100\%
\]

**PROCEDURE-- Part A.**

- **Organizing your group**
  - Prepare a group of phosphate solutions with concentrations range from \(1.00 \times 10^{-5}\) M to \(4.00 \times 10^{-4}\) M.
  - Each student is responsible for making at least one of the solutions and measuring at least one data point of Absorbance.

**PROCEDURE-- Part B.**

- **Preparation of Standard Solutions**
  1. Pipet 0.00, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06 mL 1.00 \(\times 10^{-3}\) M phosphate stock solution into 1 - 6# 50-mL volumetric flasks, respectively.
  2. Pipet 2.00 mL 2M HNO₃ solution into each 1 - 6# 50-mL volumetric flask.
  3. Pipet 1.00 mL of the ammonium vanadomolybdate stock solution into each 1 - 6# 50-mL volumetric flask.
  4. Dilute the solution by filling the volumetric flask until the meniscus reach the mark.
PROCEDURE-- Part C.

- **Adjusting the Spectrophotometer**
  1. Turn on the spectrometer to warm-up (15min).
  2. Adjust the wavelength to 400nm. Use a blackblock to adjust T=0%.
  3. Wash and rinse the cuvettes. Insert a cuvette filled with ¾ blank solution to set T=100%.

PROCEDURE-- Part E.

- **Making the Absorbance Curve**
  1. Rinse another cuvette and ¾ fill the rinsed cuvette with the 6th solution.
  2. Insert the cuvette into the spectrometer. Measure and record A in 400-450nm every 10nm.
  3. Find $\lambda_{max}$.

PROCEDURE-- Part F.

- **Making the Calibration Curve Using the standard Solutions**
  1. Rinse the same cuvette, ¾ fill the rinsed cuvette with the 1st solution.
  2. Insert the cuvette into the spectrometer. Measure and record A at $\lambda_{max}$.
  3. Repeat above step for 2nd-5th solutions.
  4. (All data points for a given curve must be measured with the same cuvette)

PROCEDURE-- Part G.

- **Determination of Unknown Concentration**
  1. Pipet 5.00 mL of the unknown, 2.00 mL HNO$_3$ and 1.00 mL of the ammonium vanadomolybdate solution into 7th 50-mL volumetric flask.
  2. Dilute the solution by filling the volumetric flask until the meniscus reach the mark.
  3. Half-fill the rinsed cuvette with the unknown solution. Use the spectrometer to measure A.
  4. Determination of unknown concentration by using the calibration curve.

Data?

- Make the curves using a computer
- Softwares, such as excel and origin

![Data Graph](image)