EXPERIMENT 7: PREPARATION OF ANALYTICAL SOLUTIONS II AND ANALYSIS OF THEIR CONCENTRATION BY UV-VIS ABSORBANCE DATA.

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PURPOSE

Provide a practical experience in the preparation of analytical solutions starting from solids and stock solutions. Understand the use of analytical standards for the preparation of calibration curves. Prepare a series of solutions to determine the concentration of an unknown sample of Methylene Blue using the standard addition method. You will gain experience in the preparation of solutions using the successive dilutions method. You will be graded in terms of the precision and accuracy in the preparation of your sample solutions.

PRELABORATORY EXERCISE

1. Explain the term matrix.
2. When can you use the standard addition method?
3. What is the useful range of a standard addition curve?
4. What are the advantages and limitations of the standard addition method?
5. Distinguish between the terms interpolation and extrapolation.

APPARATUS AND MATERIALS

Beckman DU-640 UV-Vis Spectrophotometer
Methylene Blue standard
Unknown Methylene Blue sample
1.0 - 5.0, 10, 50.0 mL transfer pipets (Class A)
100.0 mL volumetric flasks
200.0 mL volumetric flask

EXPERIMENTAL

NOTE: If you have kept solutions labeled as Stock and Unknown from Experiment IV proceed to step c.

a. Preparation of the Stock Solution:

1. Get from your instructor the Methylene Blue standard and unknown samples.
2. Use the standard Methylene Blue to accurately prepare a 0.001M stock solution, in a 100.0 mL volumetric flask. Label it as **Methylene Blue Stock**.

b. Preparation of the Unknown Sample Solution:

1. Transfer 5.00 mL of the unknown sample of Methylene Blue and quantitatively transfer it into a 100.0 mL volumetric flask. Label it as **MB-Unknown 1**.

c. Preparation of Sample Aliquots for Standard Addition (SA) Method:

1. Prepare a calibration blank.
2. Pipet 2.0 mL aliquots from the Unknown solution into four 100 mL volumetric flasks. Label them as **REFERENCE, SA-1, SA-2, and SA-3. Do not fill to the mark yet**.

3. Based on the average concentration obtained for the unknown in the previous experiment, accurately add a volume of standard close to 50% of the unknown concentration expected for the reference sample to flask SA-1, 100% into flask SA-2 and a 150% into flask SA-3. **Do not add any standard to the reference solution.**

   Note: Remember that you have a dilute solution of your unknown in each flask. Your unknown concentration estimates must be based on the diluted unknown concentration.

4. Complete the solutions to the mark with distilled water.

PREPARATION OF A CALIBRATION CURVE USING KNOWN STANDARDS:

1. Turn on the UV-VIS Spectrophotometer as described by your instructor. The standard operating procedures for the instrument are posted on the bulletin boards of each *Instrumentation Room.*

2. Using the **SA-2** sample, determine the wavelength at which your analyte, methylene blue, exhibits its maximum absorbance.

3. Starting from the least concentrated sample read triplicate sets of absorbance, at this wavelength, for all your solutions and register your results in your laboratory notebook.

CALCULATIONS

A. For Standard Addition Method:

1. Report the relative standard deviation of the absorbance for each replicate solution. Comment about the precision in the preparation of your sample solutions.

2. Construct a calibration curve for the replicate set of sample solutions (Average absorbance Vs Added Standard Concentration). Do a linear regression analysis on the data.

3. Use the calibration curve to calculate the concentration of methylene blue in the unknown aliquot.

4. Determine the concentration of Methylene Blue in the original sample.

5. Determine the propagated uncertainty of the unknown.

6. Determine the RSD of the unknown in parts per thousand (ppt).

QUESTIONS

1. Based on your results, distinguish between the direct calibration and the standard addition methods in terms of their advantages and disadvantages.
2. Why did you select the wavelength of maximum absorbance for the analysis?
3. Explain why you extrapolated your data to determine the concentration of the unknown?
4. What is the physical meaning of the slope and intercepts (x and y) values for the calibration curves used in this experiment? How do they differ from the ones of the previous experiment?
5. What are the limitations of a standard additions analysis over a direct calibration one?