EXPERIMENT 6: PREPARATION OF ANALYTICAL SOLUTIONS I AND ANALYSIS OF THEIR CONCENTRATION BY UV-VIS ABSORBANCE DATA. Error! Bookmark not defined.

De Jesús M. A.; Reyes L.; Vera M.; Padovani J. I. (2010); University of Puerto Rico; Department of Chemistry; P. O. Box 5000, Mayagüez, P. R. 00681.

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 standard solutions to determine the concentration of an unknown sample of Methylene Blue using the direct calibration method.

PRELABORATORY EXERCISE

1. Define the following units of concentration: molarity, molality, parts per million, parts per billion, percent by weight and by volume.
2. Describe the preparation of all the standard solutions for this experiment and determine their concentrations if you were using KHP 99.99% pure (FW = 204.22 g/mol).
3. Describe the preparation of 25.0 mL of a solution that contains 1.0 ppm Cu, 2.0 ppm Pb, 3 ppm Cd, 5% HNO_3 and 30 ppm Hg from 1000 ppm stock solutions of Cu, Cd, Pb, and Hg; and 36% HNO_3.
4. Define the following concepts related to UV-Vis spectroscopy: chromophore, absorbance, Beer's Law.
5. Read the MSDS of all the reagents used in this experiment and prepare a brief yet succinct summary on their proper handling, disposal and any health-related precautions.

APPARATUS AND MATERIALS

Beckman DU-640 UV-Vis Spectrophotometer
Standard Methylene Blue (MM: 319.85)
Unknown Methylene Blue sample
1.0 - 5.0, 10, 50.0 mL transfer pipets (Class A)
100.0 mL volumetric flasks (12)
200.0 mL volumetric flask

EXPERIMENTAL

I. SAMPLE PREPARATION:

IT IS EXTREMELY IMPORTANT TO INCLUDE ALL YOUR CALCULATIONS FOR SAMPLE PREPARATION IN YOUR NOTEBOOK PRIOR TO YOUR LAB SESSION. CONSULT BEFORE GETTING TO THE LAB, IF YOU HAVE DOUBTS. THIS IS EXTREMELY IMPORTANT IF YOU WANT TO FINISH YOUR EXPERIMENT ON TIME.

a. Preparation of 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 Calibration Standards:

1. Prepare five standard solutions in the $1x10^{-6}$-$9x10^{-6}$ M range into five 100.0 mL volumetric flasks. Complete to the mark with distilled water. Label these solutions as **MB-Standards A-E**. Use 1 mL pipets, or larger, to prepare all the solutions.
2. Prepare a calibration **blank**.

HINT: You may need to prepare a methylene blue solution of intermediate concentration in order to use 1 mL or larger pipets!

c. 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**.

d. Preparation of Unknown Serial Dilutions:

1. Pipet a 50 mL aliquot of **Unknown 1** into a 100.0 mL volumetric flask. Complete to the mark with distilled water. Label this solution **Unknown-2**.
2. Pipet a 50 mL aliquot of **Unknown 2** into a 100.0 mL volumetric flask. Complete to the mark with distilled water. Label this solution **Unknown-3**.

II. PREPARATION OF A CALIBRATION CURVE USING KNOWN STANDARDS:

1. Turn on the UV-VIS Spectrophotometer as described by your instructor. The specific operating procedures for the instrument are included next.

2. Using **Standard C solution**, determine the wavelength at which your analyte, methylene blue, exhibits its maximum absorbance (around 661 nm).

3. Read triplicate sets of absorbance for all the standard and unknown solutions, at this wavelength. Record your results in your laboratory notebook. Remember to set the absorbance of the blank to zero (*autozero*) before any absorbance determination.

4. Read the absorbances of all your solutions, starting with the blank, followed by the least concentrated of the **standards** and then the solutions prepared in **steps c-d**.

CALCULATIONS

1. Calculate the average absorbance for each solution.

2. Report the relative standard deviation of the absorbance for each of the standard and unknown solutions. Comment about the precision in the preparation of your samples.

3. Construct a calibration curve from the standard solutions data (Average absorbance Vs concentration of Methylene Blue). Do a linear regression analysis on the data.

4. Use the calibration curve to calculate the concentration of Methylene Blue in the unknown aliquots **Unknown 1-3**. Comment on your signal reproducibility relative to the preparation of each unknown solutions.

5. Determine the concentration of Methylene Blue in the original unknown based on the concentration of each **unknown (1-3)**.

6. Determine the standard deviation of the three unknown concentrations and compare it with the propagated uncertainty obtained for each individual calculation. Are they significantly different? Explain your findings.

7. Calculate the RSD of the unknown in parts per thousand.
8. Plot a bar chart of the concentrations of each unknown. Since they were prepared from the same sample the results should be the same. What are the sources for the differences in concentration between each unknown sample.

Questions

1. Describe the cuvette used in this experiment (in terms of size, material, and shape.) Would it be adequate to use these cuvettes for analysis in the UV region? Justify your answer.

2. Why do you use Standard C solution to determine the wavelength of maximum absorbance?

3. What is the physical meaning of the slope and intercept values in this experiment?

4. What is the relation between the standard deviation and the precision of an analytical method?

5. Explain in your own words the meaning of the following statement: “The validity of a chemical analysis ultimately depends on measuring the response of the analytical procedure to known standards”.

OPERATING INSTRUCTIONS FOR THE BECKMAN DU 640 UV-VIS SPECTROPHOTOMETER

INSTRUMENT SET UP:

1. Turn ON the computer monitor and the instrument (spectrophotometer), in this order.
2. When the instrument turns on, it performs a diagnostic test. If all tests are passed, close the diagnostic window, by pressing <QUIT>.
3. Turn ON the visible and ultraviolet lamps of the instrument pressing <VIS OFF> and <UV OFF> in the menu bar localized at the screen left bottom part. The visible lamp turns on immediately, but the ultraviolet lamp needs approximately 30 seconds to warm up before it turns on. Wait until the ultraviolet lamp turns on before continuing.
4. **Let the instrument warm up for approximately 30 minutes before running your samples.**

PARAMETERS OF METHOD STORED FOR THE ACQUISITION OF THE FULL UV-VIS SPECTRUM OF KHP, SAVED AS <[A:/]KHP>.

Method in use: A:/KHP .
Read Mode: [Abs]
Start wavelength: 250nm
End wavelength: 700nm
Scan per sample: 1
Interval Time: 35.00 [sec]
Upper [Scan] limits: 1.0000
Autoscaling: [No]
Lower [Scan] limits: 0.0000
Autoprint: [No]
Autosave: [No]
Overlay scans: [No]
Autosave file name: [A:/]Quim3055L
Function: VIEW
Scan speed: 600 nm/min (lower scan speeds improve the spectral resolution)

TO RUN FULL SPECTRA (DETERMINE MAXIMUM ABSORPTION WAVELENGTHS):

1. Select the <WAVELENGTH SCAN> option; it appears at the screen's upper left hand corner.
2. Select <METHOD NAME> to select the corresponding method: e.g. **<[A:/]KHP>.** Check the parameters set correspond to the desired method.
3. Place your **BLANK** in the quartz cuvette. *Avoid fingerprints on the quartz cuvette walls by holding the cells by the non-transparent sides.*
4. Select <BLANK SCAN> (located at the bottom left corner of the screen) to run your blank.
5. Place your **Standard C solution** in the cuvette.
6. Select <READ SAMPLE> to initiate the run under the same conditions as the BLANK was run.
7. Select <FUNCTION>. Select <PEAK PICK> and **1 for KHP** (the number of peak maxima) the instrument will display on the left window. Annotate the wavelength corresponding to the maximum absorption for <u>your KHP Standard C</u> solution.
8. Select <EXIT>.

TO READ ABSORBANCES AT A FIXED WAVELENGTH:

1. Select the <FIXED WAVELENGTH> option (at upper left hand corner of screen).
2. Select <METHOD NAME> to select the corresponding method (e.g. <[**A:/]KHPFW**>. Check the method is set to read the absorbance at the wavelength previously determined as the absorption maxima for the sample of interest.
3. Place your BLANK in the quartz cuvette.
4. Select <BLANK > (located at the bottom left corner of the screen) to run your blank.
5. Place Standard A, your lower concentration sample, in the cuvette.
6. Select <READ SAMPLE>. Record the absorbance of the sample at the given wavelength.
7. Repeat Step 5 and 6, for each sample, beginning with the blank, the more diluted standard to the most concentrated one, and the more diluted unknown to the most concentrated one. Follow your instructor's recommendations.
8. Repeat Steps 3 to 7 <u>three times</u>, until you have obtained <u>three independent sets of absorbance readings</u> for each of your samples.

NOTE: **The correct analytical procedure is to obtain <u>three independent sets of readings</u>. Samples must be read in the following order. <u>It takes more time this way, but it is the correct way. Think why?</u>**

Set 1: Blank, Standards A-E, Unknowns 3-1
IMPORTANT: Rinse the cuvette very well between sets since you will be going from a high concentration solution to the blank, which contains no $CuSO_4$.
Set 2: Blank, Standards A-E, Unknowns 3-1
Set 3: Blank, Standards A-E, Unknowns 3-1.

SHUTDOWN PROCEDURE:

1. When you have finished the experiment, press <QUIT> to exit the method.
2. Turn off the visible and ultraviolet lamps of the instrument. Click on <VIS ON> and <UV ON> in the menu bar localized at the screen left bottom corner to turn OFF.
3. Turn off the instrument power switch and the computer monitor.
4. Rinse the sample cuvette(s) and return to the instructor.
5. Clean the instrument work area.

REMEMBER NOT TO PLACE SOLUTIONS ON TOP OF THE INSTRUMENT. ANY SPILL MAY DAMAGE THE ELECTRONICS AND OPTICS.