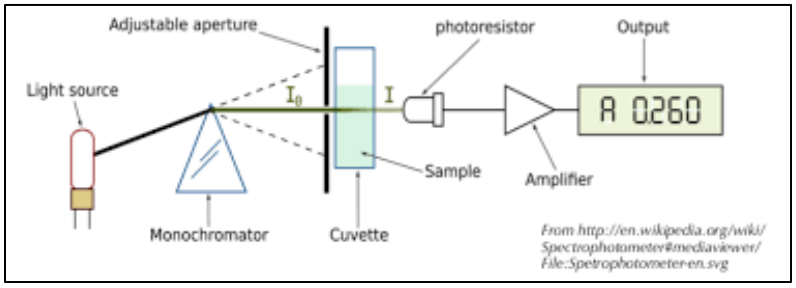
Quantitative Analysis of Transition Metal Salts by UV/Vis Spectroscopy

Introduction:

The purpose of this experiment is to learn the operation of UV/Vis Spectrophotometer and its application in quantitative determination of the transition metals' content in aqueous solution.

The molecular spectroscopy is based on the measurement of the *transmittance* **T** or the *absorbance* **A** of solutions placed in transparent cell *(cuvette)* having a path length of ***b*** cm (typically 1cm). As the monochromatic (single wavelength) radiation with the *original intensity* $\boldsymbol{I_0}$ emanates from the source, it passes through a layer of a solution with *thickness* ***b*** and some *concentration* ***c***. As the beam traverses through the solution, its photons are absorbed by the transition metal complex particles dissolved in the cuvette's solution attenuating $\boldsymbol{I_0}$ to ***I***.



The amount of light that is absorbed by the sample is linearly related to the amount of transition metal complex (*analyte)* present in the solution. The mathematical relationship of absorbance as a function of analyte's concentration is defined by Beer's Law:

$$\mathbf{A} = -\log \boldsymbol{T} = \log \boldsymbol{I_0}/\mathrm{I} = \boldsymbol{abc}$$

Where ***T*** is the transmittance of the solution, and ***a*** is a proportionality constant called *absorptivity*.

When the units of concentration ***c*** are moles per liter, the proportionality constant is called *molar absorptivity* and is expressed as $\boldsymbol{\epsilon}$:

$$\mathbf{A} = \boldsymbol{\epsilon bc}$$

Since $\boldsymbol{\epsilon}$ and ***b*** are constants, the absorbance of the analyte is directly related to its concentration. Therefore, five solutions containing known concentrations of a transition metal complex will be prepared by you and their corresponding absorbances will be measured by the UV\Vis spectrophotometer. These five data points plus the blank datum point will be used to plot the *calibration curve* of **Absorbance** versus **Concentration** in the Logger Pro® software.

The procedure that follows, consists of two parts. In **Part I**, the instrument will be optimized to find its maximum absorbance *lambda max* (λ_{max}) for a particular metal complex.

In **Part II**, a concentration of the unknown amount of the metal salt will be calculated based on the calibration curve.

Equipment, Glassware, and Reagents:

GENESYS 30 Spectrophotometer, Analytical Balance, Cuvettes, Kimwipes®, 50-mL Volumetric Flask, 10-mL Mohr pipet, 4 test tubes (20 x150mm), Cobalt (II) Chloride Hexahydrate salt, Distilled/Deionized water.

Procedure:

Preparation of Standard Stock Solution:

Using glassine weighing paper, weigh (to the nearest 0.1mg = 0.0001g) about 0.84g of metal hydrate salt into 50 mL volumetric flask; use a funnel to ensure quantitative transfer of the solid. Rinse the funnel well, dissolve the metal hydrate salt in about 20 mL of distilled/deionized water, dilute to the mark, and mix thoroughly.

Chemical Name of Transition Metal Salt _____

Molecular Formula of Transition Metal Salt _____

Molar Mass of Transition Metal Salt _____ g/mol

Mass of Metal Hydrate Salt _____ g

Volume of Solution _____ L

Molarity of Standard Stock Solution _____ mol/L

Blank Solution:

Use distilled/deionized water.

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Part I:

Signal Optimization Procedure (Selection of λ_{max}):

Note:
A Signal Optimization step is always performed in the beginning of any photometric or spectrophotometric analysis to establish a wavelength with maximum absorption value. At this λ_{max}, a spectrophotometer will realize the maximum sensitivity towards the analyte of interest and thereby quantitatively generate more accurate and precise measurements.

1. To generate the absorption spectrum of a metal complex, on the **Home Page**, choose the **Scan** mode by using the navigation keys  and press  to start the application.

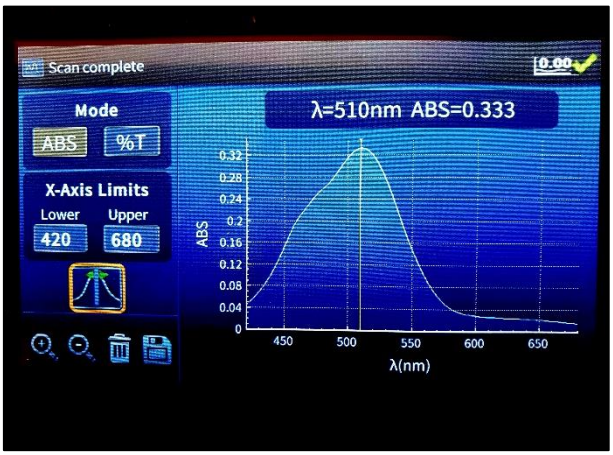
Home Page:

2. Select the data mode, **ABS**.
3. In the **X-Axis Limits**, enter 420 nm in the lower wavelength limit field and 680 nm as an upper limit.
4. Place the cuvette with a blank solution into the cuvette holder of the instrument with ***the transparent side oriented to your left and right*****.** Close the lid and press 

.
5. After the blank scan has been completed, remove the blank cuvette. Place the standard stock solution cuvette in the measurement position, close the lid and press  .
6. Wait until the spectrum is generated. To find the highest point on the spectrum, use  or  to move the cursor line left and right.

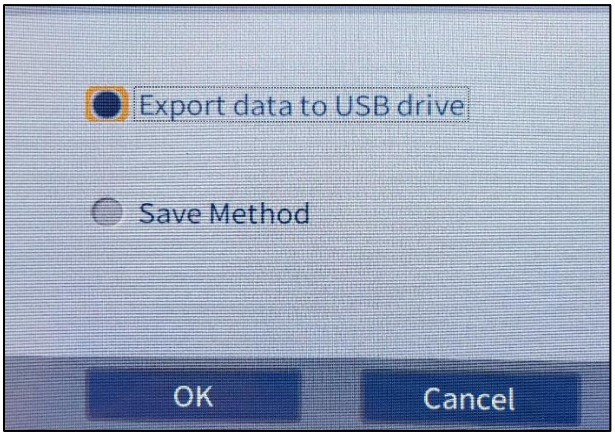
7. The cursor position and corresponding ABS value displayed above the spectrum plot. As you move the cursor across the recorded spectrum, identify and record the wavelength with the largest absorbance value (see spectrum below for reference).

Spectrum with Selected λ_{max}:



Record λ_{max} from your spectrum: _____ nm

8. To obtain a printout of the generated spectrum, place a thumb drive in the instrument's USB port (on the lower right side towards the back) and select  to save your data. Select **Export data to USB drive,** as indicated in the picture below, by pressing  . Choose **OK** and press ↵.



9. Insert the thumb drive into the laboratory computer (underneath the left side of the monitor), locate the saved data file and double click on it. Microsoft Excel® will launch as the default program to display the raw data. Copy the absorbance and wavelength data and paste the data into Logger Pro® **X** and **Y** columns.
10. To create a title and to label the axes in the Logger Pro®, double click on the graph, the **Graph Options** menu will display. Enter the title and check off the **Point Symbols** box. Under the **Axes Options** adjust the absorbance (**Top** and **Bottom** fields) and the wavelength **(Left** and **Right** fields) scales. To label the **Y-Axis**, in the **Label** field enter: Absorbance. To label the **X-Axis**, at the bottom of the graph, right click on the **X** , choose **Column Options→Data Set/**X. Under **Label** and **Units**, in the **Name** field, enter: Wavelength, nm. Press **Done** to display the spectrum.

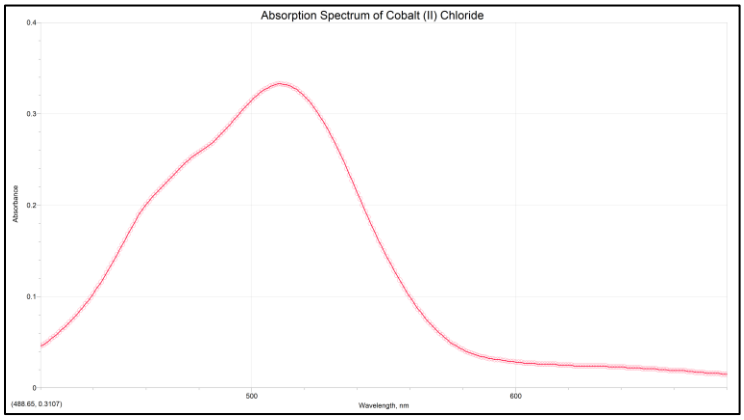


Figure 1. Absorption Spectrum of Cobalt (II) Chloride generated in Logger Pro®.

11. Print a copy of the spectrum in a landscape mode for each member of the group.

Part II:

Quantitative Analysis:

1. Prepare dilutions from the Standard Stock Solution, in test tubes, by measuring and delivering the appropriate volumes as depicted in the table below using the Mohr pipet:

Table 1. Preparation of Dilution Standards

Dilution #	Volume of Standard Stock Solution (mL)	Volume of Distilled Water Added (mL)
1	2.0	8.0
2	4.0	6.0
3	6.0	4.0
4	8.0	2.0

2. Mix the Dilution solutions by using the vortex machine on the lab bench.

3. Go to the home screen by pressing  .
4. On the home screen choose **Live Display**.
5. Set the spectrophotometer to the λ_{max} which was determined in the Signal Optimization part and press  .
6. Select the **Mode**, **ABS**

> **Note:**
> To avoid cross contamination, rinse the cuvette with at least three portions of the solution you are planning to use next (each time filling ~half of the cuvette). Start with Dilution#1.

7. Place your blank cuvette in the cuvette holder, close the lid and press 

.
8. Remove the blank cuvette. Place the cuvette with the Dilution #1. Live measurements will begin automatically displaying the flashing **ABS** value.
9. To make measurements of additional samples, simply put the cuvette with the new sample in the measurement position, close the lid and wait for the displayed value to flash. The flashing **ABS** value indicates that the measurement has been made.
10. Record the absorbance values in Table 2.

Table 2. Absorbance and Concentration Values

Sample Name	Concentration (M)	Absorbance
Blank		
Dilution#1		
Dilution#2		
Dilution#3		
Dilution#4		
*Stock Solution		
Unknown#		

*Use the absorbance value at λ_{max} generated in Part 1.

11. Calculate concentrations of Dilution Standards using the following formula:

$$\mathbf{M_1{}^x V_1 = M_2{}^x V_2}$$

Where $\mathbf{M_1}$ is the concentration of the Standard Stock Solution, $\mathbf{V_1}$ is the volume of the Standard Stock Solution (e.g. 2.0 mL, 4.0 mL, 6.0 mL, and 8.0 mL) used to prepare Dilution#1-4, $\mathbf{M_2}$ is the molarity of Dilution#1-4, $\mathbf{V_2}$ is the total volume of Dilution#1-4 (10.0 mL).

12. Plot the graph **Absorbance** vs. **Concentration**. To create the graph title and to label the axes follow the instructions outlined in Part 1, step 10.
13. To obtain the equation for the calibration curve, click on **Analyze → Linear Fit**. The equation of the calibration curve will be displayed.

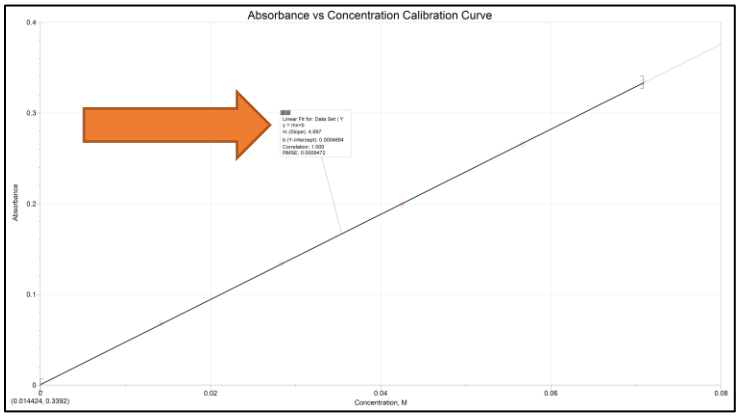


Figure2. Absorbance vs. Concentration, Calibration Curve.

14. Print the calibration curve in the landscape mode for each group member.
15. From *your* calibration curve, use the values of **m** (Slope) and **b** (Y-intercept) to find the **x** (analyte's concentration in your unknown sample). The example below demonstrates this calculation:

    From the calibration curve depicted above:

    m (Slope): 4.697
    b (Y-intercept): 0.0004694

    If the absorbance value of the unknown sample: **0.164 AU**, then the equation of the line can be used to determine the concentration **x** of the unknown sample as follows:

$$\mathbf{y = mx + b} \rightarrow \mathbf{x} = \frac{\boldsymbol{y-b}}{\boldsymbol{m}} \rightarrow \mathbf{x} = \frac{\mathbf{0.164-0.0004694}}{\mathbf{4.697}} = \mathbf{0.0348M}$$

16. Report the concentration of your unknown sample in Table 2.

Post-Lab Questions:

1. How would the determined concentration of your unknown sample be affected (increased, decreased, or stayed the same) if during the transfer of the metal salt into the 50 mL volumetric flask you accidently spilled some of it? Explain.

2. Would the determined concentration of your unknown sample increase, decrease, or stay the same if during the preparation of the Standard Stock Solution you accidently added more water than required to the 50 mL volumetric flask? Explain.

3. Would the determined concentration of your unknown sample increase, decrease, or stay the same if instead of the hydrated salt you accidently used anhydrous salt to prepare your Standard Stock Solution? Explain.

4. How would the determined concentration of your unknown be affected (increased, decreased, or stayed the same) if you accidently read your blank solution with the opaque side facing the source? Explain.