Chem.243-Exp.9 (2019)

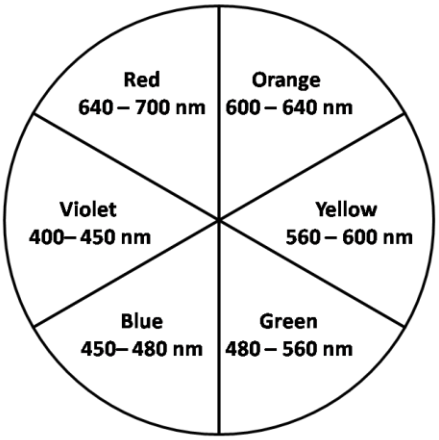
Measurement of absorption spectrum & Spectrophotometric determination of Ni (II)

Introduction:

The radiant energy emitted by the sun (or other stars) contains all possible wavelengths of the electromagnetic radiation. Light is electromagnetic radiant energy and depending upon the point of view, we can think of light as having either wave properties or particle properties. For example, when we refer to photons, we are speaking of the particle-like aspects of light. Electromagnetic radiation is composed of both electric and magnetic fields whose waves vibrate in mutually perpendicular planes. Light from the sun is composed of a continuum of energies and thus of a continuum of waves and frequencies most of which is invisible to us. The portion of this radiation to which the Humans' retinas respond is called the visible light region. The wavelengths of visible light extend from about 800 nm to about 400 nm. The electromagnetic spectrum is divided into regions called bands. In addition to visible region, ultra violet, infrared, microwave and radio are examples of the different bands. Seen together, the wavelengths of light in the visible region appear white, but if this band of visible light is separated into narrower bands of wavelengths, by prism for example, we can see the seven different components colors of the visible light: red, orange, yellow, green, blue, indigo and violet (ROY G BIV).

When light impinges on a substance, one or a combination of things can happen to the light. The light can be scattered, reflected, transmitted, or absorbed by the substance. These interactions have been studied in detail and can account for phenomena such as the blue color of the sky. The absorbed light energy causes such changes as atomic and molecular rotations, vibrations, and electron transitions. As a result of the absorption, specially designed instruments may sense these changes as heat, fluorescence, phosphorescence, or color. The pattern of absorbed and reflected/transmitted light energies is a characteristic property of a substance and can be a useful "fingerprint"

When light interacts with matter in the visible region, one of the following can happen: if a substance absorbs all wavelengths in the visible range, none of the light is reflected back to our eyes and the substance appears black; if the substance absorbs none of the visible range waves, all wavelengths in the visible range is reflected back to our eyes and it appears white or colorless because all light is transmitted; if the substance absorbs light principally in one wavelength range (generally, a number of wavelengths on both sides of the principal absorption wavelength are also absorbed, so a broad absorption band results), the color we see will be a mixture of all the wavelengths which are NOT absorbed. For example, the indigo color in the blue jeans has maximum absorbance in the 500- 650 nm range. Because this absorbance is in the red-to-green region, the wavelengths which are not absorbed are observed to be blue-violet. Chlorophyll reflects primarily yellow and green wavelengths and absorbs mainly in the blue-violet and the red regions.



Color wheel with approximate wavelength values for different color light.

A color wheel shown above, illustrates the approximate complementary relationship between the wavelengths of light absorbed and the wavelengths reflected. In the example of a blue substance, there would be a strong absorbance of the complementary color of light, orange. For this case, the absorption spectrum of a blue solution would have a maximum absorbance at a wavelength corresponding to orange light.

Spectroscopy is a basic analytical technique that utilizes the interaction between matter and the electromagnetic energy. A spectrophotometer is an instrument that separates electromagnetic radiation according to wavelengths, passes these separated wavelength bands through a sample, and detects the intensity of the transmitted light. The graphical plot of the intensity of absorption of radiation versus the wavelength absorbed is called a substance's absorption spectrum. All spectrophotometer have the following fundamentals parts: a source of radiant energy to emit light, a prism or grating to isolate radiant energy to narrow wavelength regions, sample holder and a detector for measuring the light intensity. Spectrophotometers measure both the percent of transmittance (%T) and absorbance (A) of light passing through the sample. The percent transmittance is the ratio of the amount of light transmitted (passed through), I, to the amount that initially strikes the sample, I_o:

$$\%T = (I / I_o) \times 100\ \%$$

Absorbance is the amount of light absorbed expressed in logarithmic terms as:

$$A = \log (I_o / I)$$

In addition, the absorbance of a substance is related to the concentration of the absorbing species by Beer's Law:

$$A = \varepsilon b c$$

Where A is absorbance (no units, since $A = \log (I_o / I)$

ε is the molar absorptivity with units of L mol^{-1} cm^{-1}

b is the path length of the sample - that is, the path length of the cuvette in which the sample is

contained. usually expressed in centimeters.
c is the concentration of the compound in solution, expressed in mol L^{-1}

In this experiment you will be introduced to the fundamental operations of a spectrophotometer and you will use it to determine the wavelengths of visible light absorbed by substances in solutions, then you will plot and absorption spectrum. After identifying the wavelength of maximum absorption and preparing a series of standard solutions you will plot a calibration curve for absorbance versus concentration of the standards and finally you will determine the concentration of the substance in an unknown solution. Specifically, you will obtain the absorption spectrum of nickel(II) sulfate salt as in the following reaction:

$$NiCl_2.6H_2O \rightarrow Ni(H_2O)_6^{+2} + 2\ Cl^-$$

Procedure:

- Your instructor will demonstrate how to use the spectrophotometer
- 2 cuvettes will be provided; make sure there are the same brand and please return them to your instructor when you finish

Part I: calibrating the instrument with the blank sample (distilled water)

1. Turn on the instrument to warm up for at least 20 minutes
2. Wash and rinse the cuvettes with distilled water. Rinse the cuvette with sample solution and then add fresh sample solution to measure the absorbance. Wipe the outside of the cuvette with a tissue and handle the cuvette only on its top sides
3. Set the desired wavelength with wavelength selector knob
4. With no cuvette in the instrument, set the readout display to 0% transmittance using the left hand knob
5. Place a cuvette approximately half filled with the reference blank (distilled water) in the instrument sample holder. Align the front of the cuvette with the mark on the front of the sample holder. The top of the holder must be shut except when you are loading or unloading a sample to prevent stray light from entering the instrument
6. Set the readout display to 100% transmittance (0 absorbance) using the right hand knob
7. Remove the reference blank. The display should read zero percent transmittance. If it does not, repeat steps 4 through 7 or ask your instructor for help
8. To obtain an absorbance reading, place the cuvette containing the sample in the instrument, close the lid, and record the absorbance and the wavelength

The instrument must be calibrated every time you change the wavelength. This is not necessary if you merely change the sample but not the wavelength.

Part II: the absorption spectrum using the 0.20xx M standard (stock) solution

1. Prepare 0.20xx M nickel sulfate solution using 100 mL volumetric flask. Make sure not to exceed the mark on the flask. This is your standard or stock solution

2. Obtain a second cuvette, clean it and rinse it with standard solution. Fill about two-thirds (2/3) of the cuvette with the standard solution.
3. Set the wavelength to 330 nm and calibrate the spectrophotometer according to the directions in part I. Read the absorbance of your sample at that wavelength. Remove the cuvette from the spectrophotometer
4. Increase the wavelength by 20 nm, calibrate the spectrophotometer, and take an absorbance reading of your sample. **Remember to calibrate the spectrophotometer every time you change the wavelength.**
5. To obtain the absorption spectrum, continue to take absorption readings of your sample at 20 nm intervals up to 550 nm.
6. Identify the maximum absorbance and its corresponding wavelength (λ_{max}) from your data. Take additional absorbance readings at 1 nm intervals 10 larger and for 10 smaller than your initially identified λ_{max} to increase the likelihood that you have found the true λ_{max}
7. Plot absorbance versus wavelength (nm) for your standard samples using Microsoft Excel.
8. Identify the principal wavelength(s) absorbed and relate this to the color you observe.

Part III: Absorbance and concentration

1. Use the same solution investigated in parts I and II above as your stock solution
2. Use distilled water as your blank sample (100 % transmittance; 0 absorbance)
3. From your stock solution (0.20xx solution) prepare 10 mL of 0.10 M, 0.05 M, 0.025 M, and 0.0125 M. Calculate the necessary volumes needed using the dilution formula: $M_{conc}V_{conc} = M_{dil}V_{dil}$. Check your calculation with your instructor before you proceed to prepare the standard solutions. You should have a total of 6 samples (blank, 0.0125 M, 0.025 M, 0.05 M, 0.1 M, and 0.2 M)
4. Set the spectrophotometer to the λ_{max} you found from part II and calibrate the instrument
5. Read the absorbance of each of the 7 standard solutions at the λ_{max} beginning with the lowest concentration to the highest concentration. Use the same cuvette for each solution, rinsing the cuvette first with distilled water and then with the solution to be used before it to take a reading.
6. Obtain a sample (the same absorbing substance as you used in parts II and III) of an unknown concentration from your instructor. Measure its absorbance at the wavelength of maximum absorption. Compare the visual intensity of the color of your unknown to the standards solutions; can you approximate its concentration?
7. Plot the absorbance versus the concentration for each standard solutions using Microsoft Excel
8. Draw the best fit straight line for your data which should show a linear relationship between absorption and concentration of the absorbing substance
9. Determine the concentration of the unknown sample using your graph
10. After your instructor reports the true concentration of the unknown, calculate the percent error
11. Attach the two graphs with your lab report

Pre lab assignment: Measurement of absorption spectrum & spectrophotometric determination of Ni (II)

1. What are the approximate wavelengths (in nm) in the spectrum of visible sunlight that correspond to the following colors: red, yellow, green and blue?

2. What are the main components of the spectrophotometer? What is the purpose of each of these components?

3. Why do you measure the absorbance for the standards at the maximum wavelength?

Data and calculations: Absorption spectroscopy

Name: _____ID:_____

Partner's name: _____Date: _____

Data table 1 from part II: the absorption spectrum

Wavelength (nm)	Absorbance
330	…….……..
350	…….……..
360	……...
390	……...
410	……...
430	…….……..
450	…….……..
470	…….……..
490	……...
510	……...
530	……...
550	……...
570	……...

Data table 2 from part II: the expanded absorption spectrum at the maximum wavelength

Wavelength (nm)	absorbance	Wavelength (nm)	Absorbance

Data table 3 from part III: absorbance for standard solutions and the unknown

Concentration (M)	Absorbance
0.00 (distilled water)	
0.0125	
0.025	
0.05	
0.1	
0.2	
Unknown letter: _____	