

The Use of the Spectrophotometer and Beer's Law

Purpose

To become familiar with using a spectrophotometer and gain an understanding of Beer's law and its relationship to solution concentration.

Introduction

Scientists use many methods to determine the identity and quantity of a substance in samples. Spectroscopy is a simple and powerful method for performing both qualitative and quantitative analyses. Each chemical species has a unique spectral fingerprint based on where electrons are located with respect to the nucleus. For example, a solution of sodium ions sprayed into a flame will change the flame's color to a bright yellow, while a solution of lithium ions will cause the flame to burn a deep red color. These flame tests reveal the solution's emission spectrum – the wavelength (or color) of light revealed by the flame is due to excited electrons within atoms and ions in the solution relaxing to a lower energy state, emitting photons. A photon is a packet of light energy, the first indication that light may have particle-like properties. The flame provides the energy used to excite the electrons within the metal ions. The wavelength of radiation emitted can then be used to determine the energy lost by the electron as it relaxes.

Since electrons can occupy only discrete energy states, the way radiation interacts with matter can indicate its chemical identity. Chemists commonly use absorbance spectroscopy, or how a substance absorbs photons of light, to obtain both qualitative (identity) and quantitative (amount) information. The quantitative measurement is achieved because each photon of light absorbed corresponds to the excitation of a single electron.

Of course, in the laboratory, analyses are performed on large numbers of atoms or molecules, therefore a relationship must be established to obtain quantitative information. Initial spectrophotometric studies measured transmittance, which is defined as the fraction of light that passes through the sample:

$$T = \frac{I}{I_0}$$

$$\%T = T \times 100$$

where I_0 is the intensity of the light passing through the solvent and I is the intensity of light that passes through the sample solution. Percent transmittance (%T) is simply the transmittance fraction multiplied by 100. A more useful quantity in performing analyses is the absorbance or the negative log of transmittance ($A = -\log T$).

A linear relationship exists between absorbance and concentration known as Beer's Law ($A = \epsilon b c$), where b is the length of the path traveled by light through the sample, c is the concentration and ϵ is a molar absorptivity constant that depends on both wavelength and substance. This linear relationship between concentration and absorbance allows scientists to use spectroscopy for quantitative measurements of unknown samples. Using a calibration curve prepared from standard solutions (solutions of known concentration), the concentration of an unknown solution can be interpolated by linear regression.

Before Beer's law may be used as an analytical tool, it is necessary to select a suitable wavelength and determine whether Beer's law is valid (linear) at the wavelength selected. The most suitable wavelength is that at which a maximum absorbance is observed (called λ_{\max}). The λ_{\max} will always be at the same wavelength for a

The Use of the Spectrophotometer and Beer's Law

given species (even if the calibration on the instrument dial is in error) and can be found by any experimenter under any conditions.

In this experiment, the absorption spectrum of potassium permanganate will be measured between 400 and 600 nm. Based on this spectrum, λ_{\max} will be determined, and a set of standard solutions will be analyzed to obtain a Beer's Law plot (calibration curve). The concentration of permanganate in an unknown will then be determined using this plot.

Procedure

Part A: Obtaining an Absorbance Spectrum to Determine λ_{\max}

1. Obtain 8 cuvettes. Make sure the cuvettes are clean and dry.
2. Fill one cuvette with water, leaving a 3 mm gap at the top. This will be the "blank". It must be used to zero the spectrometer each time the wavelength is changed.
3. Fill the remaining cuvettes with each of the standard and unknown KMnO_4 solutions provided. Make sure the transparent sides are wiped clean of any fingerprints. If there are air bubbles in the cuvette, use a pipette to agitate them from the solution.
4. Using the cuvette filled with 3.00×10^{-4} M KMnO_4 solution, measure the absorbance between 400 and 600 nm in increments of 25 nm. (If the older analog instruments are used, record %T then convert to absorbance). Be certain to re-zero the spectrometer at each wavelength using the blank solution.
5. Record these values in Table 1 of the data sheet.
6. Once the region from 400 to 600 nm has been measured, identify the wavelength with the highest absorbance.
7. In increments of 5 nm, choose four wavelengths below and four wavelengths above your highest absorbance wavelength. Record the absorbance at these new wavelengths.
8. The wavelength with the highest absorbance is λ_{\max} and should be used in Part B for the Beer's law plot.

Part B: Preparation of a Beer's Law Plot

1. Set the spectrometer to the selected λ_{\max} , and re-zero using the blank. Remember the point where absorbance and concentration both have a value of 0.00 is a data point.
2. Now obtain samples of the 2.40×10^{-4} , 1.20×10^{-4} M, and 0.600×10^{-4} M KMnO_4 solutions. At the selected λ_{\max} , measure the absorbance for each solution and record these on your data sheets. *You do not need to re-zero the spectrometer between readings.*

The Use of the Spectrophotometer and Beer's Law

Part C: Determining the Concentration of Unknown Solutions

1. Keeping the spectrometer set at λ_{\max} , measure and record the absorbance values for your unknown solutions. *You do not need to re-zero the spectrometer between readings.*
2. When you have completed the linear plot of Absorbance vs. Concentration, then you can determine the concentration of your unknown solutions from your graph. To determine the concentration of an unknown, use the absorbance of the unknown solution. Find that value on the absorbance scale, then move across the graph parallel to the concentration axis until you meet the straight line. At that point move directly down to the concentration axis and read the concentration of the unknown.
3. Repeat this for each unknown solution analyzed.

Graphs and Calculations

- a Construct a graph of absorbance vs. wavelength (used to determine λ_{\max}) using all the values Listed in Tables 1 and 2. See Figure 1 below for an example. Make sure to label your axes and provide a descriptive title.
- a Construct a Beer's Law Plot from the data in Table 3. See Figure 2 below for an example. Make sure to label your axes and provide a descriptive title.
- a Calculate the concentration of your unknown samples by using your Beer's Law Plot. If you used the equation of the best fit line, show a sample calculation. If you just estimated from your graph, show your points on the graph.

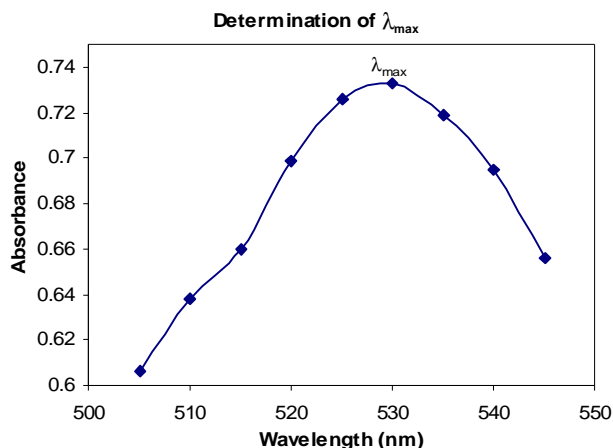


Figure 1: Sample Graph for Determination of Maximum Wavelength

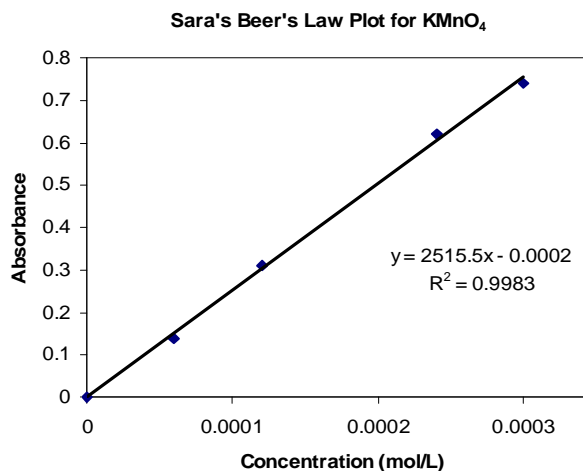


Figure 2: Sample Beer's Law Plot

The Use of the Spectrophotometer and Beer's Law

Calculations.

The Use of the Spectrophotometer and Beer's Law

Data Sheet

Name: _____

Lab Partner: _____

Part A: Determination of λ_{\max}

Table 1:

Wavelength (nm)	%T	A
400		
425		
450		
475		
500		
525		
550		
575		
600		

Table 2:

Wavelength (nm)	%T	A

Wavelength selected for Beer's Law plot _____.

(Select an increment of 5 nm such as 570, 575 or 580. Do not select a value such as 577 nm.)

Part B: Data for Beer's Law Plot

Table 3: Calibration Curve Data

Concentration (mol/L)	%T	Absorbance	ϵ
0.00			
0.600×10^{-4}			
1.200×10^{-4}			
2.400×10^{-4}			
3.000×10^{-4}			

Part C: Analysis of Unknowns

Table 4: Unknown Absorbance Data

Unknown #	%T	Absorbance	Concentration (mol/L)

The Use of the Spectrophotometer and Beer's Law

Post-Lab Exercises

Name: _____

1. Why is absorbance used instead of transmittance in spectroscopic methods?
2. What physical process is being observed using the spectroscopic techniques used in this experiment?
3. What is the relationship between the color of a solution and the wavelengths the solution absorbs?
4. The 3.000×10^{-4} M KMnO_4 solution was used to prepare the four standard solutions (see Part B) you used for the Beer's Law plot. If 50.0 mL of each standard solution is needed, what volume of 3.000×10^{-4} M KMnO_4 is required to make each of the four standard solutions? Show your work.