EXPERIMENT 16

Quantitative Analysis by Spectrophotometric Methods

PURPOSE:
The purpose of this experiment is to determine the concentration of a solution of $KMnO_4$ by spectrophotometric means.

INTRODUCTION:
When a beam of ordinary white light is passed through a prism or reflected off of a diffraction grating, it is separated into a variety of colors. This happens in nature when sunlight passes through water droplets on a rainy day to cause a rainbow.

These rainbow colors, visible to the human eye, can be described in terms of their wavelengths as well as their colors. The wavelength is a function of the energy of the photon. Our eyes detect the light and our brain interprets these different wavelengths and energies as different colors of light. Short wavelengths of about 350 nm are high energy and are correspond with violet. As the wavelength increases to give lower energy light, all the colors of the rainbow are exhibited ending with red light at about 750 nm. Shorter wavelengths, such as ultra-violet light and x-rays, and longer wavelengths, such as infrared and radio waves, are not visible. The energy of a photon is

$$E_{photon} = \frac{hc}{\lambda} \qquad 16.1$$

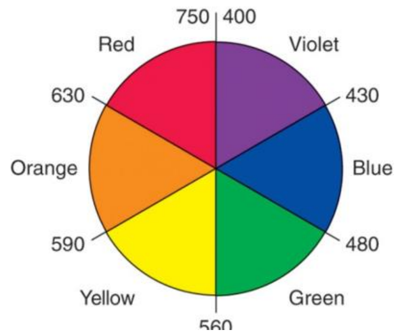
where *h* = Planck's constant, 6.626×10^{-34} J/s, and *c* is the velocity of light, 3.00×10^{8} m/s. λ is the wavelength, although usually expressed in nanometers, must be in meters in this equation.

When this visible white light strikes a substance, the substance may (1) reflect the light if it is opaque, (2) transmit the light if it is transparent to the light striking it, or (3) absorb the light. Substances absorb light by changing their electronic and vibrational quantum states or modes. These modes, which are related to harmonic variations of bond lengths and angles and electronic configurations, have energies on the order of ultraviolet and visible (UV-vis) light. Absorbed UV-vis light can increase the energy of the mode where other forms of electromagnetic radiation cannot. Each mode is sensitive to the slightly different wavelength of light and will only absorb that wavelength.

The color of a substance depends on which wavelength of light it absorbs. If a substance absorbs red light only, it will appear green in color, green being the average color of all the unabsorbed wavelengths. The colors of a rainbow can be laid out in order as a color wheel, shown in Figure 16.1. Beginning with the lower energy red at the top and moving around the circle counter clockwise, the energy increases through orange, yellow, etc, to the higher energy violet. The wheel becomes useful by noting that the color a substance appears to be is directly across the wheel from the color of light that substances has absorbed. When red is absorbed the substance appears green. Many substances can be identified by their colors. For example

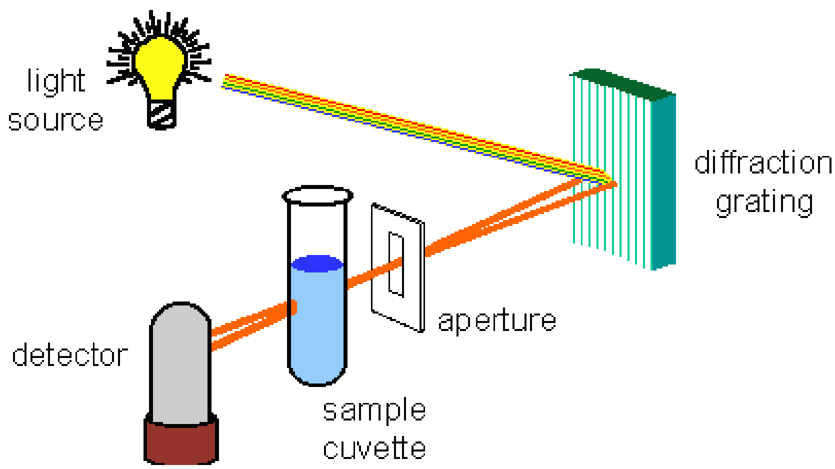
Co^{2+} (aq) is red, while Ni^{2+} (aq) is green. Therefore, a solution of Co^{2+} (aq) absorbs primarily green light while a solution of Ni^{2+} (aq) absorbs primarily red light.

Figure 16.1 – A color or artist's wheel



Not only can substances be identified by color, the intensity of the color can indicate the concentration of that substance when it is in solution. The intensity of the color is directly related to the amount of light absorbed by the substance. A spectrophotometer is an instrument that is used for the analysis of solutions. The components of a spectrophotometer are shown in the following diagram, Figure 16.2.

Figure 16.2 – Schematic diagram of a spectrophotometer (single-beam)



White light produced by the light source is transmitted off a diffraction grating through an aperture which narrows the beam before incidence with the sample. Adjusting the angle of the diffraction grating allows one to select the wavelength of the incident light which strikes with the sample. If light is absorbed by the sample, the intensity of the transmitted light exiting the sample (I) will be less than the intensity of the incident light (I_o). The ratio of transmitted light intensity to the incident light intensity is the transmittance, T:

$$T = \frac{I}{I_0} \qquad 16.2$$

The extent to which the light is absorbed by the sample is due to the concentration of the sample, *C*. If the light is of the proper wavelength to be absorbed by the substance, varying amounts will be absorbed depending on the concentration. When the concentration is high, many absorbing molecules will be in the path of the light and will absorb it, and less light will be transmitted. When the concentration is low, fewer absorbing molecules will be struck by the light and little light will be absorbed; thus more light is transmitted in this case. Another factor that affects the intensity of the light exiting the sample is the path length, *l*, which is the distance the light travels through the sample. The greater the distance the light must travel through the solution (path length), the greater the number of absorbing molecules the light will strike. The relationship between transmittance, and those factors that directly affect the intensity, *l* and *C*, is logarithmic and proportional. The relationship is expressed in the Beer-Lambert Law (or simply Beer's Law):

$$-\log_{10} T = A = \varepsilon l C \qquad 16.3$$

Here ε is a proportionality constant called the molar extinction coefficient (also known as the absorptivity) and *A* is the <u>absorbance</u> of light by the sample. The extinction coefficient depends on the structure of the species absorbing the light and the wavelength of the light absorbed. A larger extinction coefficient means that substance absorbs more light. Each pure substance has its own unique extinction coefficient. The absorbance, *A*, is a direct experimental measure of the light absorbed by the sample. In this experiment you will measure the absorbance directly to determine the molar concentration of a sample of potassium permanganate, and the molar extinction coefficient of potassium permanganate.

<u>PROCEDURES:</u>

In this experiment there will be three basic tasks to accomplish using the spectrophotometer.

1. Determine the wavelength at which a selected substance ($KMnO_4$) will absorb best.
2. Establish a standard absorption curve from which the extinction coefficient can be calculated.
3. Analyze an unknown solution ($KMnO_4$) for concentration.

1. **<u>Maximum Absorbance Wavelength</u>:** Obtain 10 mL of 0.0040 M potassium permanganate. Keep your sample dark. Using a graduated 1.0-mL pipette and a pipetting bulb, transfer 1.00 mL of the $KMnO_4$ into a 25.00-mL volumetric flask. Use good lab techniques by rinsing the pipette with a small sample of $KMnO_4$ before using. Be certain all glassware is clean. Also be careful not to get solution into the pipetting bulb. It would be good to practice with deionized water until the operation of the bulb is mastered. After the $KMnO_4$ is in the volumetric flask, fill the flask half full with deionized water. Add 1.0 mL of 3.0 M H_2SO_4 and mix the contents. Fill the flask completely to the mark with deionized water and mix the contents again.

Calibrate a spectrophotometer using a blank solution. Note which instrument you are using and be sure to use the same instrument throughout the entire experiment. A blank solution is one that contains all the components of a solution except the one of interest. In this case, the blank should be H_2SO_4 and deionized water. However, since aqueous sulfuric acid is "transparent" in the visible region, it is sufficient to use deionized water only as your blank. Rinse a cuvette with deionized water 2-3 times, then fill with deionized water. Wipe the outside dry using a soft tissue, and place the cuvette in the spectrophotometer.

The cuvette is a glass tube made of high quality glass designed especially for the spectrophotometer. It is important that the cuvette be kept clean and free of scratch marks. Wash and rinse it thoroughly each time it is used but not with abrasives or brushes. Your instructor will demonstrate the use of the spectrophotometer.

With the instrument calibrated, remove the blank and replace it with a second cuvette containing the prepared $KMnO_4$ solution. The display will now show the absorbance. Record the result. Remove the $KMnO_4$ cuvette from the instrument and replace it with the blank. Select the next highest wavelength setting and recalibrate the instrument to read 100% transmittance. Each time a new wavelength is selected, the instrument must be recalibrated. Replace the blank with the $KMnO_4$ solution and again record the absorbance. Repeat this process until all wavelength settings have been examined and results recorded. Plot the absorbance data versus the wavelength, with absorbance on the vertical axis and wavelength on the horizontal axis. A visible absorption spectrum will be developed. Find the wavelength value which corresponds to the maximum absorbance. This is the wavelength at which $KMnO_4$ absorbs best. Record that value which is to be used as the one and only wavelength setting for the remainder of the experiment.

2. **Standard Absorbance Curve:** It is necessary to prepare several solution of $KMnO_4$ at a variety of different concentrations. One such solution was prepared in part 1, and its concentration can be determined using the dilution equation, $M_1V_1 = M_2V_2$. The additional solutions should be prepared in exactly the same way except progressively smaller amounts of 0.0040 M $KMnO_4$ are used. The recommendations are 0.80 mL, 0.60 mL, 0.40 mL, and 0.20 mL of $KMnO_4$. For each solution be sure to add the H_2SO_4 and dilute to exactly 25.00 mL. Complete all the work on one solution before preparing the next. After the 0.80 mL solution is prepared, set the spectrophotometer to the wavelength maximum discovered in Part 1 and calibrate using the blank. Examine the 0.80 mL solution for the absorbance and record the findings. Proceed in a similar way for each of the recommended solutions.

   Make a plot of the absorbance (vertical axis) verses concentration (horizontal axis). A straight line relationship will result, from which you can determine the equation for this straight line. Determine the slope of the line. The Beer-Lambert Law (Equation 16.3) states that $A = \varepsilon l C$. So the slope of the line will equal εl. The path length (approximately l cm) through the cuvette is constant since the same cuvette is used for all solutions; however, you must measure the path length to the nearest 0.01 cm. Use calipers to measure this path length by measuring the inside diameter of your cuvette. Record it and use the slope of the line and this measurement to determine the molar extinction coefficient.

3. **Concentration of an Unknown:** Obtain from the instructor about 10 mL of a solution of $KMnO_4$ of unknown concentration. The concentration of the unknown will be determined from a measurement of the absorbance of the solution. That absorbance along with the path length value and the extinction coefficient determined in Part 2 will allow the calculation of the concentration by means of the Beer-Lambert Law. Because this calculation uses ε from

Part 2, the unknown solution must be treated in the same manner as those solutions in Part 2 to make the calculation valid. Use 1.0 mL of unknown, dilute with water and 1.0 mL H_2SO_4 to 25.00 mL and obtain the absorbance of that solution. To get the concentration of the unknown before it was diluted, use the dilution equation in a reverse way.

There is one potential difficulty in this plan which is easily overcome. It is possible to have a solution of $KMnO_4$ so concentrated that even after dilution so much light is absorbed that the readings are inaccurate. The Beer-Lambert Law breaks down at high concentration. A second dilution may be necessary. If absorbance values are greater than 1.0, a second dilution is recommended. If necessary, take 1.0 mL of the diluted solution and dilute that in a similar manner. Record all observations and results in your notebook.

NAME_______________________________________ SECTION _______

REPORT SHEET
EXPERIMENT 16

Spectrometry

TABLE 1 – DATA FOR MAXIMUM ABSORBANCE WAVELENGTH

Wavelength	Absorbance
____________	______________
_____________	______________
_____________	______________
_____________	______________
_____________	______________
_____________	______________
_____________	______________
_____________	______________
_____________	______________
_____________	______________

Maximum Absorbance Wavelength (From Graph) ______________
(Please include your graph in your report.)

TABLE 2 – DATA FOR STANDARD ABSORBANCE CURVE

0.0040 M $KMnO_4$

Volume (mL)	Concentration	Absorbance
1.00	____________	___________
0.80	____________	___________
0.60	____________	___________
0.40	____________	___________
0.20	____________	___________

Path Length (1) _______________

Extinction Coefficient (ε) _______________

Show your calculation for ε here:

TABLE 3 - DATA AND CALCULATION FOR UNKNOWN $KMnO_4$ CONCENTRATION

Unknown Number __________________

Do at least two trials.

Trial	Vol of $KMnO_4$	Absorbance	Concentration After final Dilution	Concentration Before Dilution
1	__________	____________	___________	__________
2	__________	_____________	___________	__________
3	__________	_____________	___________	__________

Mean concentration (Before Dilution) _________________

Show one example of your calculations here: