# **E16 -QUANTITATIVE ANALYSIS BY SPECTROSCOPIC METHODS**

In this experiment you will learn how to create an absorption spectrum, and prepare a calibration curve of absorbance vs. concentration. You will then use the established relationship to determine the concentration of a KMnO4 solution of unknown concentration by spectrophotometric means. For more information and illustration of this process observe the simulation at <http://phet.colorado.edu/sims/html/beers-law-lab/latest/beers-law-lab_en.html>

**INTRODUCTION**

When a beam of ordinary white light is passed through a prism or a diffraction grating, it is separated into a variety of colors that comprise the visible spectrum. 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

  Equation 16.1

where *h* = Planck’s constant, 6.626 X 10-34 J-s, and *c* is the velocity of light, 3.00 X 108 m/s. λ is the wavelength and, 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/or to 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(s) of visible 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 transmitted or reflected wavelengths. The colors of a rainbow can be laid out in order as a color wheel, shown in Figure 16.1. Beginning with the lowest energy red at the top and moving around the circle counterclockwise, the energy increases through orange, yellow, etc., to the highest 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 substance has absorbed. When red is absorbed, the substance appears green; when blue is absorbed, the substance appears orange etc. Also, a substance can display a pure color by transmitting or reflecting only a single wavelength (or a narrow band centered on one maximum wavelength). For example, if all light except 410-430 nm is absorbed, the substance appears violet. Alternatively, a substance can display a composite color by transmitting or reflecting several colors that then mix. For example, a substance that absorbs all colors except both red and blue appears purple (like a mixture of those paint colors).



**Figure 16.1 – A color or artist’s wheel**

Many substances can be identified by their colors. For example, Co2+ (aq) is red, while Ni2+ (aq) is green. Therefore, a solution of Co2+ (aq) absorbs primarily green light while a solution of Ni2+ (aq) absorbs primarily red light. (Remember the flame tests in Experiment 8? Remember comparing the apparent color of the hydrogen lamp to its emission line spectrum?) The color of our solution in this experiment is due to the aqueous permanganate ion and you will justify its apparent color by examining its absorption spectrum in the visible light portion of the electromagnetic spectrum.

Not only can substances be identified by color, but the intensity of the color can also 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, which in turn depends on the concentration of the colored species. A spectrophotometer is an instrument that is used for the analysis of solutions. The components of a spectrophotometer are shown in 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 and interacts 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 (*Io*). The ratio of transmitted light intensity to the incident light intensity is the transmittance, *T*:

  Equation 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 the proper wavelength to be absorbed by the substance, varying amounts will be absorbed depending on the concentration of the colored species. 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):

 $-log\_{10}T=A=ε•l•C$ Equation 16.3

Here *ε (the Greek letter epsilon)* is a proportionality constant called the molar extinction coefficient (also known as the absorptivity) and *A* is the absorbance 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 more efficiently absorbs light and converts the light energy into internal energy. 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.

## **PROCEDURE**

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

1. Determine the wavelength at which a selected substance (KMnO4) will absorb best.
2. Establish a standard absorption curve to derive the relationship between concentration and absorbance, from which the extinction coefficient can be calculated.
3. Analyze an unknown solution (KMnO4) for concentration.

**Part 1 Maximum Absorbance Wavelength:**

Obtain 10 mL of 0.0040 M potassium permanganate. Keep your sample protected from light (dark) as much as possible. Using a graduated 1.0-mL pipette and a pipetting bulb, transfer 1.00 mL of the KMnO4 into a 25.00-mL volumetric flask. Use good lab techniques by rinsing the pipette with a small sample of KMnO4 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 KMnO4 is in the volumetric flask, fill the flask half full with deionized water. Add 1.0 mL of 3.0 M H2SO4 and mix the contents. Fill the flask completely to the mark with deionized water and mix the contents again.

Note which instrument (SPEC 20 or SPEC 200) you are using and be sure to use the same instrument throughout the entire part of the experiment. All instruments will need to be calibrated once they are warmed up so the following information corresponds to that instrument. All spectrophotometers must first be calibrated using a blank solution. A blank solution is one that contains all the components of a solution except the colored one of interest. In this case, the blank should be H2SO4 and deionized water. However, since aqueous sulfuric acid is known to be “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. Return it clean and drained at the end of the experiment. DO NOT confuse it with an ordinary test tube!

Your instructor will demonstrate the use of the spectrophotometer.

SPEC 200 INSTRUCTIONS (FOR STUDENT USE IN PART 1)

Turn on the spectrophotometer with the power switch located on the back panel.

### **Preparing the instrument**

* 1. Ensure that any cuvettes have been removed from the sample compartment and that the sample compartment is closed. Then press the  key.
	2. The spec will run several self-tests. If an error message is displayed on the screen,

notify a TA/the stockroom. If not, the Home Menu will appear after the spec has finished running self-tests, indicating it is ready for use.

* 1. Press the  key with ‘SPEC 200E Modern Interface’ highlighted.
	2. Use the keys to highlight ‘Application;’ set it to ‘Live Display’ using the keys if not already set.
	3. Select ‘Measurement Mode’ and use the keys to toggle between %T and Abs. measurement settings.
	4. Select ‘Measurement λ’ and use the keys or the λ knob to set the desired wavelength. The λ knob changes the wavelength by units of 10; pushing the knob in will allow the wavelength to change by units of 1.
	5. Use the keys to select ‘Go,’ and press the  key.
	6. Place a blank solution cuvette in the sample holder and press the key to blank the spec at the set wavelength. The screen will display a **Performing Auto Zero** message and will return to the live display when complete.
	7. Remove the blank solution and place a sample solution cuvette in the holder. The spec will give a live reading of %T/Abs. that updates every 2-5 seconds.
	8. To measure at a different wavelength, remove the sample, change the desired wavelength using the λ knob, place a blank solution in the sample compartment; press the key. After the spec performs an auto zero, replace the blank solution with a sample solution for live measurement readings.
	9. To return to the Home menu, press the  key.

## **Scanning the colored compounds**

* 1. Turn on the spec 200E, follow the instruction on the screen, it will do initialization.
	2. Select SPEC 200E Modern Interface, press  key.
	3. Highlight Application (show green color, otherwise black).
	4. Press key three times to change the “Application” to Scan mode.
	5. Press key to highlight “Measurement Mode”, press key to change to ABS/%T. For example, if you want to find the analytical wavelength, you can choose ABS.
	6. Press key to highlight “low λ” ， use the knob or key to set up the lowest scan wavelength.
	7. Press key to highlight “High λ”, use the knob or to set up the highest scan wavelength.
	8. Press key to highlight “Next”, press  key.
	9. The screen will display a blank graph.
	10. Open the cuvette compartment, put blank in, close the compartment.
	11. Press auto key, screen will display “**Performing Auto Zero** ” wait 1 min for completing

the process.

* 1. After auto zero, open the compartment and remove the blank, then place your sample, close the compartment.
	2. Press  key, screen will display “**scanning…**.” Wait 1 min, the screen will automatically show you the graph.
	3. Use the knob or press the key to change the wavelength you choose; the screen will show you the corresponding ABS on the graph. Take a picture of the graph appears in instrument screen or graph the data in excel as directed by your TA.
	4. The wavelength at the peak of the graph is the λmax or analytical wavelength for each color
	5. Finish test, press  key, back to the home screen

A plot shows 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 KMnO4 absorbs best. Record that value which is to be used as the one and only wavelength setting for the remainder of the experiment.

SPEC 20 INSTRUCTIONS (FOR STUDENT USE IN PARTS 2 and 3)

15 minutes prior to use, turn on the SPEC 20 using the front left knob.

1. Calibrate the instrument with your blank, the directions are located on the SPEC 20.
2. With the instrument calibrated, remove the blank and replace it with a second cuvette containing the prepared KMnO4 solution. The display will now show the absorbance. Record the result.
3. Remove the KMnO4 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.
4. Replace the blank with the KMnO4 solutionand again record the absorbance.
5. Repeat this process until all wavelength settings have been examined and results recorded.

**Part 2 Standard Absorbance Curve:**

It is necessary to prepare several solutions of KMnO4 at a variety of different concentrations. One such solution was prepared in Part 1, and its concentration can be determined using the dilution equation, M1V1 = M2V2 or, alternatively, CdilVdil = CconcVconc. The absorbance value obtained for this concentration at your chosen wavelength represents your first data point for Part 2. Additional solutions should be prepared in exactly the same way except progressively smaller amounts of 0.0040 M KMnO4 are used. The recommendations are 0.80 mL, 0.60 mL, 0.40 mL, and 0.20 mL of KMnO4. For each solution be sure to add the H2SO4 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 Spec 20 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 additional recommended solutions. (It is not necessary to repeat the calibration with the blank since the same wavelength is used for all of Part 2.)

Calculate the concentration of each additional diluted solution.

Make an Excel 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. (It should extrapolate to the origin; zero concentration of colored species = zero absorbance since the instrument was calibrated to read zero for the blank.) Determine the slope of the line. The Beer-Lambert Law (Equation 16.3) states that *A = εlC*. 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, *ε*.

Submit your Excel Standard Absorbance Curve to your instructor as part of the lab report. S/he will indicate whether you are to upload an electronic copy or to print the graph for submission.

### **Part 3 Concentration of an Unknown:**

Obtain from the instructor about 10 mL of a solution of KMnO4 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 H2SO4 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 reverse application to relate the now known Cdil to the original Cconc.

Do two or more trials to get an average concentration of the unknown. With 3-5 trials, use the Q test to evaluate any suspect trials.

There is one potential difficulty in this plan which is easily overcome. It is possible to have a solution of KMnO4 so concentrated that even after dilution as instructed above, 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.

# **E16 - QUANTITATIVE ANALYSIS BY SPECTROSCOPIC METHODS**

**REPORT SHEET**

Section\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Name\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Is the KMnO4 solution purple or violet? (What is the difference?) Explain:

**PART 1 - MAXIMUM ABSORBANCE WAVELENGTH**

Take a photograph of the spectrum of KMnO4 on the Spec 200 with your phone. Upload your photograph (as a jpg, gif or png file) to the lab Canvas shell.

**Maximum Absorbance Wavelength (From Graph) \_\_\_\_\_\_\_\_\_\_\_\_\_**

**PART 2 - DATA FOR STANDARD ABSORBANCE CURVE**

| **Volume Taken, mL** | **Diluted Concentration, M** | **Absorbance** |
| --- | --- | --- |
| 1.00 |  |  |
| 0.80 |  |  |
| 0.60 |  |  |
| 0.40 |  |  |
| 0.20 |  |  |

Stock solution = 0.0040 M KMnO4

**Path Length (1) \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**Extinction Coefficient (ε)** \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Submit your Excel calibration curve. Show your calculation for ε here:

### **PART 3 - DATA AND CALCULATION FOR UNKNOWN KMnO4 CONCENTRATION**

| **Trial** | **Volume of KMnO4, mL** | **Absorbance** |  **Concentration, M****After Dilution Before Dilution** |
| --- | --- | --- | --- |
| **1** |  |  |  |  |
| **2** |  |  |  |  |
| **3** |  |  |  |  |

**Unknown Number \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

Mean concentration of unknown solution (Before Dilution) \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Show one example of your calculations here:

### **QUESTIONS:**

1. Suppose that a student accidently set an incorrect wavelength in Parts 2 and 3. Instead of the wavelength of maximum absorbance, s/he set a slightly higher wavelength where the permanganate does not absorb light energy as efficiently. How would this error affect the absorbance values throughout Parts 2 and 3? How would this error affect the extinction coefficient calculated in Part 2? How would this error affect the calculated concentration of the unknown solution?
2. What is the molar absorptivity (or, extinction coefficient) of the following compound as measured at its wavelength of maximum absorbance in a 1.00 cm cuvette?

Concentration: 25.0 μM Absorption: 0.5874