Spectroscopic Analysis

Introduction

In this experiment you will be performing a spectroscopic analysis. You will be using the GENESYS™ 20 to find the concentration of an unknown potassium permanganate solution. A question you should be asking is, how does this work? How can light be used to determine the concentration of a solution? Simply put, when you pass light through a sample, a certain amount of light is absorbed by the molecules in solution and the rest passes through. In fact, materials have specific color due to certain wavelengths or colors of the visible spectrum that are being absorbed by the molecules in the material. The color you attribute to the material is actually all the remaining reflected light. If we measure the amount of light that is going into the sample and we measure the amount of light that comes out, we can determine to what extent the sample is absorbing the light. In this experiment you will carefully analyze the concentration of a $KMnO_4$ solution. To do this you will use a spectrophotometer, an instrument which measures the fraction of light (I/I_O) that is absorbed by a sample. The sample is placed in a tube called a cuvette and is then irradiated with an incident beam of light (I_O) of a specific wavelength. A detector then measures the amount of light that is transmitted through the sample (I). A schematic representation of a spectrophotometer is shown below in Figure 1.

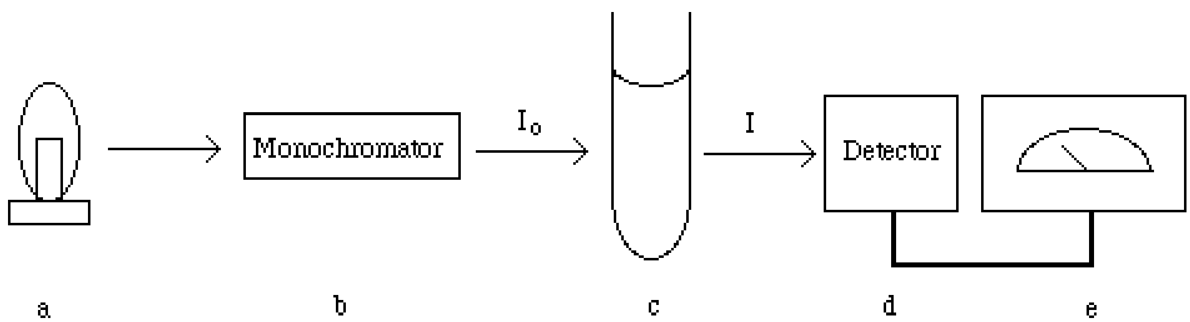


Figure 1. A schematic representation of a spectrophotometer.

(a) A light source produces a polychromatic beam of light. The source used for this experiment produces light of wavelength ranging from 340-1000 nm.

(b) The monochromator selects a particular wavelength for the incident beam of light (I_O).

(c) This is the sample cell. Treat the Cuvettes with care.

(d) The detector measures the intensity of the transmitted light (I).

(e) The readout is supplied by a meter.

For any substance, the amount of light absorbed depends on: 1) the concentration of the absorbing species, 2) the length of solution that the light passes through (commonly called the path length), 3) the wavelength of incident light, and 4) the identity of the absorbing species present.

One of the more common ways of expressing the amount of light absorbed is called percent transmittance, %T, which is defined for a particular wavelength as

$$\%T = (I/I_0) \times 100 .$$

Since the solvent itself may absorb light of the wavelength selected, the value of I_O will be chosen as the intensity of light transmitted when the cuvette is filled with solvent only. Another common method of expressing the amount of light absorbed is called absorbance. Absorbance is defined by

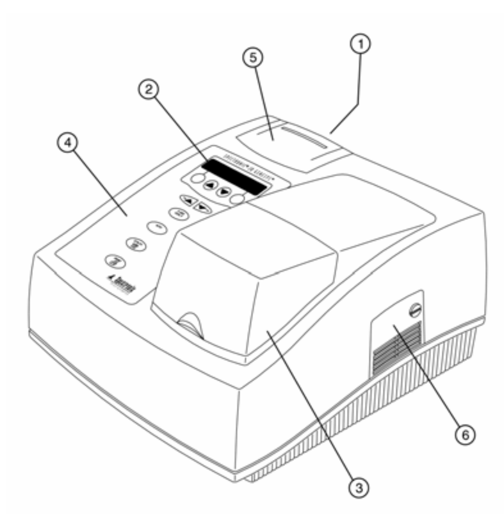
$$A = \log_{10} (I_o/I) = \log_{10} (100 / \%T) .$$

Consider a completely transparent sample. In this case the percent transmittance would be 100 which corresponds to an absorbance of 0. Also note that a percent transmittance of 50 corresponds to an absorbance of 0.301. Although absorbance may at first appear complex, it is a very useful measurement. This is because it is directly related to concentration as is shown in the following equation, called Beer's Law:

$$A = \varepsilon l c$$

where A is the absorbance, c is the molar concentration, l is the path length, and ε is the molar absorptivity or molar extinction coefficient. Note that a plot of A vs. c will yield a straight line which has a slope of εl. Be aware that Beer's Law does not necessarily hold for all substances or at all concentrations; for example, it often fails at high concentrations.

In this lab you will be finding the concentration of an unknown solution of $KMnO_4$. Seems simple enough - find the absorbance, plug it in to the equation and ... wait! What is εl? In what table do you look that up? Well, if εl is the slope of the line for the graph of A vs. c, then you should be able to find it experimentally! That is, if you find the absorbance of $KMnO_4$ at different concentrations and plot this set of data, you should get a linear plot that has a slope of εl. So it really isn't that difficult after all!



1. On / Off switch
2. LCD display
3. Sample compartment door
4. Keyboard
5. ----
6. Lamp compartment door

Figure 2: GENESYS™ 20

It is important that you understand the operation of the GENESYS™ 20 before you begin this laboratory. The GENESYS™ 20 is an expensive and sensitive instrument and must be operated carefully and intelligently.

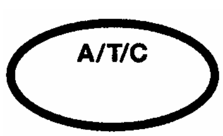
The light source in a GENESYS™ 20 is an ordinary tungsten lamp whose radiation extends over the entire visible range. The light from the lamp passes through an entrance slit and is dispersed by a diffraction grating. The grating can be rotated so that a small band of selected wavelengths from the dispersed beam passes through an exit slit, and then through the cell (cuvette) containing the sample. The cuvettes used with a GENESYS™ 20 have a path length (internal diameter) of 1.00 cm. The light transmitted through the sample strikes a solid-state silicon detector that generates an electrical signal proportional to the radiant power (light intensity). The signal from the detector drives a meter that can be calibrated to read transmittance or absorbance.

The calibration procedure entails setting 0 Absorbance at a given wavelength with a cuvette containing a reference or blank solution. The blank solution is missing the component of interest, but is otherwise as identical as possible to the solution to be analyzed for the component of interest. Typically, the blank solution is just the solvent. This is required since the output of the lamp and the sensitivity of the detector varies with wavelength. The electronics of the instrument automatically sets 100% absorbance. . An identical cuvette containing the solution of interest is then inserted into the spectrometer, and the absorbance is read from a meter on the instrument. Both the calibration and the reading must be done at the same wavelength. The reading for the solution then represents the absorbance at the chosen wavelength due to the component of interest. The calibration has accounted for any absorption (or reflection or scattering) of light by the cuvette and other species in the reference solution.

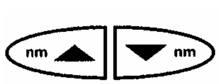
GENESYS™ 20 Operation

The power switch is located on the bottom left in the back of the instrument. When you turn on your GENESYS™ 20 spectrophotometer, it performs its power-on sequence. This sequence includes checking the software revision, initializing the filter wheel and the monochromator. The power-up sequence takes about two minutes to complete. ***Allow the instrument to warm up for 30 minutes before using it.***

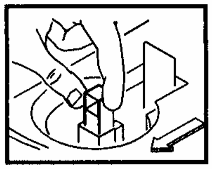
Note: Be sure the cell holder is empty before turning on the instrument.



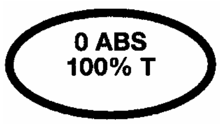
1
Press **A/T/C** to select the absorbance or % transmittance mode. The current mode appears on the display.



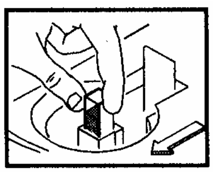
2
Press **nm** ▲ or **nm** ▼ to select the wavelength.
Note: *Holding either key will cause the wavelength to change more quickly.*



3
Insert your blank into the cell holder and close the sample door.
Note: *Position the cell so the light (indicated by the arrow in drawing) passes through the clear walls.*



4
Press **0 ABS/100%T** to set the blank to 0 A or 100%T.



5
Remove your blank and insert your sample into the cell holder. The sample measurement appears on the LCD display.

<u>Error Messages</u>
This section lists the messages generated to alert you of errors or other abnormal conditions. The instrument recognizes two types of errors. With the first type, the instrument is still functional; with the second, the instrument is not functional until the condition is resolved.

<u>Flashing Data Display</u>
This condition indicates that the sample has an absorbance or a transmittance value below or above the photometric range of the instrument. The display flashes until the condition is resolved.

<u>Sample too dark</u>
This condition indicates that the instrument has been asked to zero a sample with a high absorbance at a low energy point. The instrument beeps three times to announce the message, the message remains on the display for two seconds, then the normal display returns.

<u>Sample too bright</u>
This condition indicates that the instrument has been asked to zero a sample while the door of the sample compartment is open. The instrument beeps three times to announce the message, the message remains on the display for two seconds, then the normal display returns.

Safety: All waste solutions may be disposed of by rinsing them down the drain. Treat the GENESYS™ 20 with great care as it is an expensive instrument. Wear your goggles.

Procedure

Work in pairs on this experiment.
Each student must collect data and submit a separate report.
The actual data analyses and the written reports must be done entirely independently of your lab partner or other students. Make sure that you avoid unauthorized collaboration and plagiarism. All suspected violations of the Code of Academic Conduct will be referred to Student Judicial Affairs.

1. Be sure that the GENESYS™ 20 spectrophotometer is turned on and allow the instrument to warm up for 30 minutes before use.

Making a Potassium Permanganate Solution:

2. Calculate the amount of solid you will need to make 250 mL of an about 0.04 M potassium permanganate ($KMnO_4$) solution. You can find the molecular weight of $KMnO_4$ on the bottle. In a clean weighing boat, weigh out the correct amount of $KMnO_4$. Be sure you record the weight precisely in your lab notebook (use four significant figures). Please note that you do not need to make the solution exactly 0.04 M, but that you must know the exact concentration of the solution that you make. Clean any $KMnO_4$ spills immediately as it is extremely corrosive and will damage the balance pans.

3. Fill a 250 mL volumetric flask with approximately 150 mL of deionized water. Carefully place your pre-weighed $KMnO_4$ into the flask. You may use your wash bottle to wash down any $KMnO_4$ that may adhere to the neck of the flask. Cover the top of the flask with Parafilm and place your hand over the Parafilm. With your hand in this position, invert the flask several times to dissolve the solid and to insure a homogeneous solution. You may also want to swirl the flask to help dissolve the solid $KMnO_4$. Once you are sure all of the solid has dissolved, then add deionized water close to but below the 250 mL mark on the flask. Ask your TA if you are unsure about the location of the mark. Mix the solution well. Finally, use your wash bottle to *carefully* add water to the mark. Make sure the bottom of the meniscus is touching the mark. Be sure you don't add too much water or you will have to re-make the solution.

Question A: The solution is very dark and yet the solution is dilute. Do you expect the ε to be large or small for this compound? Explain.

Question B: Calculate the theoretical concentration of your solution and record it in your lab notebook.

Diluting the Potassium Permanganate

Before performing a spectroscopic analysis, you need to dilute your solution. As you will observe, the solution you have made is dark purple. Can you see through it? If you made your solution correctly, the answer should be no. Remember that Beer's law doesn't work for solutions that are too concentrated. We need to dilute the solution in order to get good data. Figure 3 illustrates how you are going to perform what is called a *serial dilution*.

Question C: Draw Figure 3 in your notebook and fill in the molarities for each dilution. Show all calculations in the "Calculations" section of your notebook.

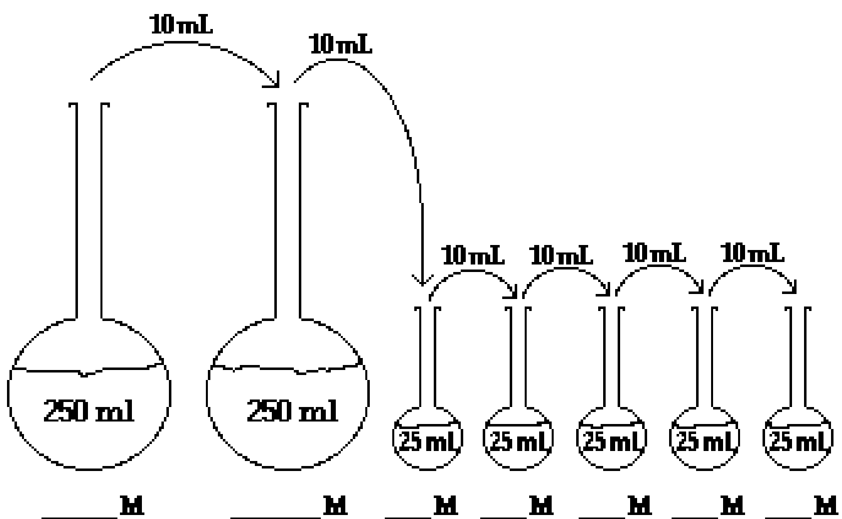


Figure 3: Dilution Scheme

4. Pour the KMnO4 solution you prepared into a **clean and dry** 400 mL beaker. If any solid remains in the flask, pour some of the solution back into the flask and swirl to dissolve it. Then pour the solution back into the beaker. Repeat this procedure a few times.

Question D: Why is it important to use a dry 400 mL beaker?

5. Rinse the 250 mL volumetric flask found in you locker thoroughly with deionized water until no KMnO4 remains, and discard the solution. Fill the flask with approximately 150 mL of water. Carefully rinse a 10 mL pipet with deionized water. Then carefully condition the pipet with a few mL of the solution in the 400 mL beaker and discard. Do not contaminate your solution. Remove a small quantity of solution from your 400 mL beaker to be used for conditioning the pipet. When conditioning the pipet, rinses the pipet three times with small portions of the solution you will be measuring. Be sure the all surfaces of the pipet are properly rinsed with solution. Now accurately transfer 10 mL of the solution you prepared from the 400 mL beaker to the 250 mL volumetric flask. The pipet is calibrated "to

deliver". That means **do not** blow out the drop left in the tip. It has been calibrated to hold that last drop. Refer to the "Introduction" section of this manual for a discussion on how to properly use a pipet. Add deionized water up close to the mark and swirl until a homogeneous solution is obtained. Finally, use your wash bottle to bring the bottom of the meniscus up to the mark. You have completed the first dilution! Do not throw out the solution in the 400 mL beaker! If you make a mistake in your dilutions, you will need this solution to continue.

Question E: Why is it not necessary to dry the volumetric flask when you had to dry the beaker in Step 4?

6. Now you are ready for the second dilution. Obtain **TWO** 25 mL volumetric flasks from the shelf. Rinse a 25 mL volumetric flask with deionized water and fill it with 10 mL of deionized water. Rinse your 10 mL pipet with deionized water and then condition by rinsing it with approximately 1-2 mL of the $KMnO_4$ you just prepared. Repeat this second rinse a couple of times. Now using the 10 mL volumetric pipet, transfer 10 mL of the new $KMnO_4$ solution from the 250 mL flask to the 25 mL flask. Swirl the contents to obtain a homogeneous solution. Use your water bottle to raise the meniscus up to the mark. Again, swirl the contents to obtain a homogeneous solution.

7. Once the solution has been prepared, place about 5 mL of it into a dry and clean test tube. Label the test tube 2nd Dilution. Place the rest of the solution into a dry and clean beaker.

8. Now you are ready for the third dilution. Clean the 25 mL volumetric flask. Rinse your 10 mL pipet with deionized water, and then with approximately 1-2 mL of the $KMnO_4$ which you just prepared in the beaker. Place 10 mL of deionized water into the 25 mL volumetric flask. Using the 10 mL volumetric pipet, transfer 10 mL of the new $KMnO_4$ solution from the beaker to the 25 mL volumetric flask. Swirl the contents to obtain a homogeneous solution. Use your water bottle to fill the 25 mL flask to the mark. Swirl the contents to obtain a homogeneous solution. Once the solution has been prepared, place about 5 mL of it into a dry and clean test tube. Label the test tube 3rd Dilution. Place the rest of the solution into a dry and clean beaker. Note: Some laboratories may have extra volumetric flasks which can be used to aid this process.

9. Repeat Step 8 three more times to prepare the 4th, 5th and 6th Dilutions. You will then have completed the serial dilution and you will have prepared 5 standards (2nd-6th Dilutions). You will use these standards to make a standard curve.

   When you are done using the two 25 mL volumetric flask, **please rinse them with deionized water and return them to the laboratory shelf**. Do not put them in your locker.

Determining the Blank Cuvette

10. Set the wavelength at 524 nm. This is the wavelength of light of highest absorbance for $KMnO_4$ and is commonly called λ_{max}. You might want to take a few minutes now to become familiar with the various operating controls. The GENESYS™ 20 and the location of the controls are shown in Figure 2. Please be extremely careful with your spectrophotometer. It is a very sensitive and expensive instrument, and must be operated carefully and intelligently.

11. Obtain a pair of cuvettes. One will be used for the blank solution and the other will be used for the standard and unknown samples.
12. Fill both cuvettes with the deionized water (blank solution) and wipe the outside with a Kimwipe to make sure it is clean and dry (no fingerprints!). Be sure to always add enough solution to reach the to within 1/8 of the triangular mark on the cuvette. This will insure that all incident radiation passes through the solution. It is also critical that the triangular mark on the cuvette be facing you when inserted in the sample holder for all measurements.

13. Insert one filled cuvette into the sample holder that is located under the hood to the left. Close the hood.

14. Calibrate to 0 absorbance by pressing the 0 ABS/100%T key. Remove the cuvette, insert the other cuvette, and read the absorbance for this second cuvette. If the reading is less than 0 absorbance, then this second cuvette will be used as the blank cuvette; if the reading is greater than 0 absorbance, then the first cuvette will be used as the blank cuvette.

Collecting Data for a Standard Curve – Measuing absorbance of standard solutions

16. Now you are ready to calibrate your instrument. Rinse and fill the cuvette chosen for the blank with solvent, which in this case is deionized water.

17. Now you will calibrate the GENESYS^{TM} 20 using your "blank". First wipe all fingerprints from the "blank" using a Kimwipe. Fingerprints on the cuvette will cause a decrease in the amount of transmitted light and thus an error in your readings. Insert your "blank" into the cuvette holder and closing the hood. Calibrate your instrument by pressing the 0 ABS/100%T key. When the LCD Display reads 0.000 absorbance remove the blank.

18. Rinse the second cuvette with deionized water then condition this cuvette by rinsing it again with a small amount (less than 1 mL) of the $KMnO_4$ solution you will be analyzing (solution from 2nd dilution). Be sure all sides of the cuvette have been rinsed by the solution. Now fill the cuvette with this solution which to be analyzed

Question F: If you touched the cuvette and left your fingerprints, would you expect the absorbance reading to be too high or too low?

19. Now put the cuvette containing your sample into the cuvette holder. Again, remember to place the triangular mark facing you.. Measure the absorbance and record it in your notebook.

20. Repeat Steps 18 and 19 for the other standards (3rd-6th Dilutions).

Measuring absorbance of Unknown Solution - Determining the Concentration

21. Your TA will assign you an unknown. Record the unknown letter in your notebook. Repeat steps 18 and 19 with your unknown solution. You will not need to recalibrate your instrument if you take the absorbance measurements of your unknown solution when you measure the absorbance for your standard solutions.

22. Repeat the measurement with a fresh sample of unknown.

Making your Beer's Law Plot: Standard Curve

24. Make a plot of Absorbance vs. Concentration using your concentrations and absorbance readings of your standard solutions. This is called the standard curve. You should make a rough plot during the laboratory period to see if you have a reasonably straight line (your graph should be linear). If you don't, then you can quickly remeasure a point that does not fit the line. Make a clear plot on a full page of graph paper and with labeled axes. Draw the best fit straight line for your data. Find the slope of your graph. Using 1.0 cm for *l*, calculate the value of ε in units of $M^{-1}cm^{-1}$. Show your calculations in your notebook.

25. Take the average of the absorbance reading of your unknown solution and, using your standard curve, determine the concentration of $KMnO_4$ in your sample.

Clean Up. All solutions can go down the drain with plenty of water. Be sure that you have returned the 25 mL volumetric flasks to the laboratory shelf. Keeping them in your locker will cause a shortage the following lab period.

Write-Up. Your lab report should include a graph of your spectrophotometric measurements and the concentration of your unknown.