EXPERIMENT 11

UV/VIS Spectroscopy and Spectrophotometry: Spectrophotometric Analysis of Potassium Permanganate Solutions.

Outcomes

After completing this experiment, the student should be able to:

1. Prepare standard solutions of potassium permanganate.
2. Construct calibration curve based on Beer's Law.
3. Use Beer's Law to determine molar absorptivity.
4. Explain the fundamental principal behind spectrophotometric analysis

Introduction

Most analytical methods require calibration (a process that relates the measured analytical signal to the concentration of *analyte*, the substance to be analyzed). The three most common analysis methods include the preparation and use of a *calibration curve*, the *standard addition method,* and the *internal standard method*. In this experiment we will use *spectrophotometry* to prepare a calibration curve for the quantitative analysis of $KMnO_4$.

Spectrophotometry

Spectrophotometry is a technique that uses the absorbance of light by an analyte (the substance to be analyzed) at a certain wavelength to determine the analyte concentration. UV/VIS (ultra violet/visible) spectrophotometry uses light in UV and visible part of the *electromagnetic spectrum*. Light of this wavelength is able to effect the excitation of electrons in the atomic or molecular ground state to higher energy levels, giving rise to an absorbance at wavelengths specific to each molecule. The complex formed between ASA and Fe^{3+} is intensely violet coloured, and therefore can be determined by spectrophotometry using the visible part of the electromagnetic spectrum.

The human eye is able to "see' light in the wavelength range 400-700 nm (nanometer or 10^{-9} m). To the human eye, wavelength appears as colour, as shown in the following table.

Table: Correlation between wavelength, colour, and complementary colour

Wavelength, nm	Colour	Complementary colour
400-430	Violet	Yellow-green
430-490	Blue	Yellow
490-550	Green	Purple
550-590	Yellow	Blue
590-630	Orange	Green-blue
630-700	Red	Blue-green

When we see an object as purple, in fact it absorbs light in the "green" region of the spectrum: only blue and red wavelengths reach the eye, which we experience as the purple colour. The ASA-Fe^{3+} complex formed in the analysis of aspirin absorbs wavelengths in the blue, green and yellow regions of the spectrum, as shown in figure 1. Only violet and red light are "transmitted". As a result, the human eye sees the transmitted light as a mixture of violet and red, which we experience as a deep purple.
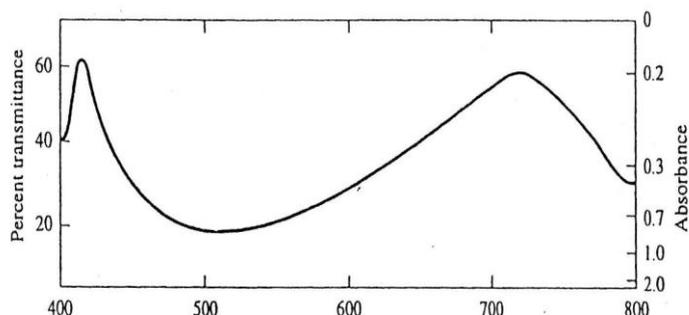
Figure 1: The absorption spectrum of the Fe^{3+}-salicylate complex.

In Fig. 1, note that % Transmittance T is plotted on the left (linear scale), Absorbance A on the right (logarithmic scale). The absorbance maximum is at approximately 510 nm (λ_{max}).

The spectrophotometer takes the place of the human eye by accurately measuring the intensity of the light transmitted at each wavelength. For spectrophotometry in the visible region the light source is a common tungsten light bulb emitting "white" light. The light is collimated and focused on an entrance slit, and then falls on a monochromator, that separates the white light in its constituent wavelengths. The monochromator can be a glass prism, but in modern instruments it will be a grating. From the monochromator the light is sent through the sample, and finally reaches a photocell that measures the intensity of the light at each specific wavelength.

Spectrophotometers have many different designs. In the simplest instrument, for each wavelength λ set by the monochromator, the user first inserts a blank solution in place of the sample. The photocell then records the intensity at that wavelength. This intensity is called $I_o(\lambda)$. Next the blank solution is replaced by the sample solution, the photometer measures the new intensity called $I(\lambda)$. The *Transmittance*, T is then displayed on the screen or spectrophotometer output:

$$T(\lambda) \quad = \quad \frac{I(\lambda)}{I_o(\lambda)} \tag{1}$$

Often the Transmittance is expressed as a percentage:

$$\%T(\lambda) = \frac{I(\lambda)}{I_o(\lambda)} \times 100 \ \% \tag{2}$$

This procedure is then repeated for a number of wavelengths.

More sophisticated instruments "scan" the spectrum over the required wavelength range automatically, and record the transmittance as a function of wavelength. In a "double beam" scanning spectrophotometer, part of the light is reflected to a separate blank cell, and the intensities I and I_0 are measured simultaneously at each wavelength, and automatically compared to yield a direct output of T vs λ. Another modern form of the UV/VIS spectrophotometer is the "Diode Array" spectrophotometer. In this instrument the photocell, which measures light intensity at one wavelength at a time, is replaced by a CCD detector similar to the detector in your digital camera, and the instrument can record the spectrum over the full wavelength range (typically 200−700 nm) within one second!

In each fractional layer of the sample the intensity of the incoming light will decrease proportionally to the concentration of the analyte. As a result, the Transmittance T decreases exponentially with increasing pathlength l . This is expressed in the Beer Lambert law, which states:

$$-\log \frac{I}{I_o} = \varepsilon.\ell.C \tag{3}$$

ε is called the "Molar Absorptivity" of the compound. It is a function of wavelength specific for each molecule. With the pathlength l normally given in cm, and C in Molarity units, mol/L ε has the units $L.mol^{-1}cm^{-1}$. If alternatively C is in mol/mL, then ε will have the units cm^2mol^{-1}.

The Absorbance, A, is defined as

$$A = -\log \frac{I}{I_o} \qquad (4)$$

Thus, the Beer-Lambert law, more commonly called *Beer's law*, can be written as:

$$A = \varepsilon . l . C \qquad (5)$$

Finally, the relation between A and T is given by:

$$A = -\log T \qquad (6)$$

Note that A is a dimensionless quantity. Because A is directly proportional to the analyte concentration, it is more often used than T or %T. Most spectrometers can record either A, T, or %T for a given wavelength.

The calibration curve method

To use the calibration curve technique, several standards containing exactly known concentrations of the analyte are introduced into instrument, and the instrumental response is recorded. Ordinarily this response is corrected for instrument output obtained with a blank. Ideally, the blank contains all of the components of the original sample except for the analyte. The resulting data are then plotted to give a graph of corrected instrument response versus analyte concentration, and this graph in turn can be used to find the concentration of an unknown.

Solutions of analyte may absorb light of different wavelengths. When the analyte to our eyes is a coloured solution, this means that out of the white light visible to the eye certain wavelengths are absorbed by the analyte molecules, letting the other colours of the white light pass through, giving rise to the characteristic colour of the analyte in solution. For instance, the analyte in this experiment, potassium permanganate or $KMnO_4$ absorbs light in the green region of the visible spectrum, letting red light and blue light pass through. The mixture of blue and red is perceived by the eye as the typical purple colour of $KMnO_4$.

A spectrum shows how much light is absorbed for a range of wavelengths. In UV/VIS spectrophotometry we plot the *Absorbance* A of a solution (A is a measure of how much light is absorbed, see below), against the wavelength of the light reaching the solution, λ. This is called the *absorption spectrum*.

The higher the analyte concentration, the more light at a certain wavelength will be absorbed. The relation between Absorbance A and analyte concentration C is given in equation 5:

In a *UV/VIS spectrophotometer* a *light source* emits light at all wavelengths of the UV and visible spectrum. Via a system of mirrors the light is guided to a *monochromator* that selects light of only a certain wavelength. After the monochromator, the light passes through the analyte sample solution held in a *cuvette* in the *sample holder*, and finally reaches the *detector* (a *photocell*), where the intensity of the light reaching it is measured, and recorded as the *Absorbance* A or the *Transmission* T (or %T). The relation between A, T and %T is given in the spectrophotometry section of Experiment 10, but in this experiment we will only use the Absorbance at different wavelengths, $A(\lambda)$.

In a *single beam spectrophotometer* (as used in this experiment), we first place a cuvette filled with solvent only (water in this case), to measure the intensity of the light source at a certain wavelength. Next, we place a cuvette filled with sample solution in the cell holder. The instrument will record light intensity and absorbance relative to the light intensity passes through solvent (water) alone. This must be repeated at different wavelengths to obtain the spectrum of the dissolved solute ($A(\lambda)$ vs. λ).

From the spectrum we find the *wavelength with the highest absorbance*, the wavelength of the absorption peak, λ_{max}. At this wavelength the spectrophotometric method is most sensitive for the analyte. Next, we determine the absorbance A at λ_{max} for a number of standard solutions of different concentration, always starting with the lowest concentration. From these absorbance values, we plot a calibration curve for the analyte ($KMnO_4$ in our case), which we can then use to determine the concentration of an analyte solution of unknown concentration.

Safety Precautions

Follow all safety rules described in experiment 1.

Materials and Equipment

UV/VIS spectrophotometer and polystyrene cuvettes, 2   100 mL volumetric flask, 1000 μL micropipette, 100 μL   pipet, Hot plate, solid $KMnO_4$, 1   10 mL graduated cylinders, 6   10 mL volumetric flasks, 2 125 mL Erlenmeyer flasks or 150 mL beakers

Procedure

Preparing the stock solution and four standard solutions.

1. Preparation of 100 mL of a stock standard solution of 0.008M $KMnO_4$: Accurately weigh 126 mg solid $KMnO_4$. Transfer quantitatively to a 100 mL volumetric flask and fill to the mark with water. This is the *stock solution*.

2. Prepare four standards in 10.o mL volumetric flask with concentrations of 0.00008 M (solution #1), 0.00016 M (solution #2), 0.0004 M (solution #3) and 0.0008 M (solution #4) by diluting the stock solution prepared in Step 1 as following.

For the 0.00008, 0.00016 and 0.0004 M standards use the 100 μL micropipets (100 μL = 0.1 mL; calculate how many 100 μL samples stock solution are needed in each case) to make 10 mL standard solution.For the 0.0008 M standard use the 1 mL (1000 μL) micropipet, Mark the four 10 mL flasks with standard solutions #1-4 (lowest concentration #1 = 0.00008 M, highest concentration #4 = 0.0008 M).

Measuring the absorption spectrum and determining λ_{max}

This part of the experiment may be done by all students together, with each pair of students determining the absorbance at one wavelength. Each pair of students should record all absorbances at each wavelength and draw the absorption spectrum.

3. Rinse one of the cuvettes with distilled water and fill it with water. Put the cuvette in the sample compartment. This is the reference solution. Set the wavelength to 400 nm, then set the Absorbance to zero.

4. Rinse a second cuvette once with distilled water and once with standard solution #1, then fill it with standard solution #1 (0.0008 M $KMnO_4$). Place the cell in the sample compartment, measure the Absorbance at 400 nm and record in your notebook.

5. Repeat this procedure (steps 3 and 4 above) for the two cuvettes at wavelengths 420, 440, 460,…….600 nm, first setting A = 0 for the cuvette with water, then measuring A for the cuvette with 0.0008 M $KMnO_4$, recording the absorbance at each wavelength. Record in data table.

6. Prepare a graph of absorbance A vs. wavelength λ and determine λ_{max} (maximum wavelength). Attach this graph to the lab report.

The calibration curve
This part of the experiment must be done by each pair of students separately.

7. Set the wavelength at 525 nm (λ_{max}). Place the cuvette with distilled water in the cell compartment and again set the Absorbance to zero.

8. Measure and record the Absorbance of each of the four standard solutions, starting with the most dilute standard. After each measurement, rinse the cuvette with the next standard, not with distilled water!

9. Draw a plot having X-axis as concentration (mole/L) and Y-axis as Absorbance at λ_{max} (525 nm).

10. Use Beer's law to calculate ε for $KMnO_4$, given the cell width (path length *l*) to be 1 cm.

Experimental General Chemistry 1

Experiment 11: Spectrophotometric Analysis

Laboratory Data Sheet

Name: _____ Section: _____

1. Stock solution

(1)   Mass of $KMnO_4$ = _____ g

(2)   Molar mass of $KMnO_4$ = _____ g/mol

(3)   Moles $KMnO_4$ = mass/molar mass   = _____ mol $KMnO_4$

(4)   Molarity of <u>stock solution</u> = mol $KMnO_4$/0.100L = _____ mol/L

2. Preparation of standard solutions

Standard solution 1 (dilute 1.0 mL stock to 10 mL).
$KMnO_4$ molarity = (1.0/10)x(stock solution concentration)   = _____ mol/L

Same for concentrations of solutions "2", "3" and "4" with 500, 200, and 100 μL stock. *Remember that 100 μL is 0.1 mL.*

$KMnO_4$ molarity for standard solution 2   = _____ mol/L

$KMnO_4$ molarity for standard solution 3   = _____ mol/L

$KMnO_4$ molarity for standard solution 4   = _____ mol/L

3. The absorption spectrum of $KMnO_4$:

S. #	Wavelength	Absorbance
1	400	
2	420	
3	440	
4	460	
5	480	
6	500	
7	520	
8	540	
9	560	
10	580	
11	600	

Make a graph of A vs λ at 20 nm wavelength intervals from 400 nm to 600 nm (as measured by the whole laboratory group). Attach the graph with this lab report.
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4. The calibration curve at λ$_{max}$ = 525 nm

Standard	Concentration	Absorbance
1		
2		
3		
4		

Make a graph of A (y-axis) vs M (x-axis). Attach the graph to this report.

5. Calculation of the molar extinction coefficient ε at 525 nm.
Use the A-value of solution 1:

$$\varepsilon = A/(C. \, l), \text{ with } l = 1 \text{ cm} \qquad\qquad \varepsilon = \underline{\hspace{2cm}} \quad \underline{\hspace{1.5cm}}$$

Example: how to make a graph in Excel.

Standard	Concentration	Absorbance
1	0.00008	0.124
2	0.00016	0.239
3	0.00040	0.614
4	0.00080	1.160

In Excel, enter the concentration values e.g. in cells b1-b4, and enter the absorbance values in cells c1-c4. Now continue as follows
1. Insert choose scatter then from the types of graphs choose the one with only your data points. Your graph will appear.
2. Now you want to show the best straight line through the points:
3. On "layout" select "trendline", choose "linear". The line will appear but it will not extend to the y-axis (where x = 0)
4. Now right click on the line. Choose "formal trendline"
5. Under "forecast" click backward and 0.00008. Also click the box "display equation on chart"

Your chart is now complete, but still in Layout you have to insert the chart title and horizontal and vertical axis titles (include units, e.g. for the x-axis title write Concentration (mol/L). The final graph is shown on the next page. Notice the many ways in which Excel allows you to edit the data points, the trendline, titles, sub- and superscripts, etc.

KMnO₄ calibration curve

$y = 1442.7x + 0.0149$
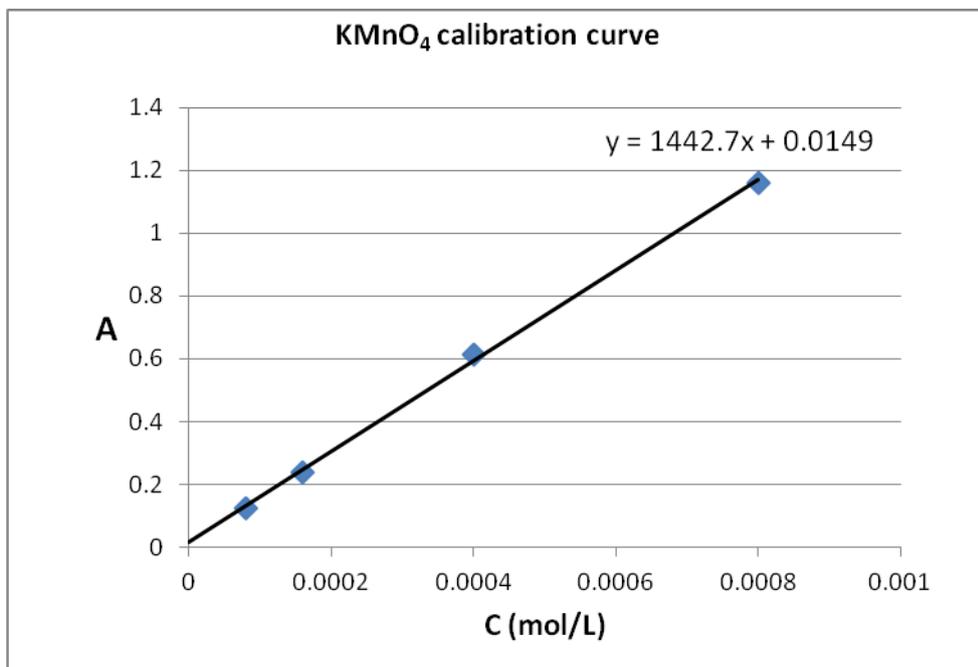
On the graph you can see that the trend line does not pass exactly through zero. This is as expected: it may be due to the statistics of the data point, which are not exactly on the straight line due to random errors in the concentration and/or the absorbance reading, or to the fact that there is a remaining solution absorbance (relative to the blank) for the standards. For these reasons, you should not choose the "set intercept" box when formatting the trendline.

Finally, note that the trend line slope is the Molar Extinction Coefficient (Molar Absorptivity)! The equation $y = 1442.7x + 0.0149$ for this application should be read as $A = \varepsilon.C + \text{constant}$, with the constant = intercept.