Experiment-1

Fundamentals of Spectrophotometer

Assay of sodium salicylate by calibration curve method

Outcomes: -

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

1. Prepare standard solutions of sodium salicylate.
2. Construct a calibration curve based on Beer's Law.
3. Use Beer's Law to determine molar absorptive.
4. Explain the fundamental principle 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 a spectrophotometer to prepare a calibration curve for the quantitative analysis of sodium salicylate.

Spectrophotometer: -

It is a technique that uses the absorbance of light by an analyte at a certain wavelength to determine the analyte concentration. A UV/VIS (ultraviolet/visible) spectrophotometer uses light in the UV and visible parts of the electromagnetic spectrum. Light of this wavelength can affect 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.

In UV/VIS spectrophotometer we plot the **Absorbance** (**A)** of a solution against the wavelength of the light reaching the solution (**λ**), this is called the **absorption spectrum**, the higher the analyte concentration, the light at a certain wavelength will be absorbed more, the relation between Absorbance **A** and analyte concentration **C** is given in equation (Beer's Law):

$$\mathbf{A = \varepsilon\, l\, C}$$

Spectrophotometers have many different designs, in the simplest instrument (a single beam) for each wavelength **λ** set by the monochromator, the user first inserts a **blank** solution in place of the sample a **cuvette**, the photocell then records the intensity at that wavelength, this intensity is called $\mathbf{I_o}$, next the blank solution is replaced by the sample solution, the photometer measures the new intensity called **I**, the transmittance (**T**) is then displayed on the screen or spectrophotometer output:

$$\mathbf{T = \frac{I}{I_o}} \quad \textbf{or} \quad \mathbf{T\% = \frac{I}{I_o} x100}$$

This procedure is then repeated for several 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 in a separate blank cell (intensities "$\mathbf{I_o}$") and sample cell (intensities "**I**") 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 (**charge-coupled device**) 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 path-length **l**, this is expressed in the **Beer-Lambert law**, which states**:**

$$\mathbf{-\log \frac{I}{I_o} = \varepsilon\, l\, C}$$

ε is called the "Molar Absorptivity" absorption factor of the compound, it is a function of wavelength specific for each molecule, with the path-length **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:

$$\mathbf{A = -\log \frac{I}{I_o}}$$

Thus, the Beer-Lambert law, more commonly called **Beer's law**, can be written as**:** $\mathbf{A = \varepsilon\, l\, C}$ Finally, the relation between **A** and **T** is given by:

$$\mathbf{A = -\log T}$$

Note that **A** is a dimensionless quantity because **it 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 absorption spectrum:

We place a cuvette filled with a sample solution in the cell holder (blank solution than sample solution), the instrument will record the light intensity and absorbance relative to the light intensity that passes through the blank solution alone (the

Absorbance to zero), this must be repeated at different wavelengths to obtain the spectrum of the dissolved solute (**A** vs. **λ**) **absorption spectrum**, from **absorption 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 several standard solutions of different concentration.

The calibration curve method:

To use the calibration curve method, several standards containing exactly known concentrations of the analyte are introduced into an 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, always starting with the lowest concentration, from these absorbance values, the resulting data are then plotted to give a graph of corrected instrument response versus analyte concentration, and this graph (**calibration curve**) in turn can be used to find the concentration of an unknown.

Sodium salicylate is a sodium salt of salicylic acid. It can be prepared from sodium phenolate and carbon dioxide under higher temperatures and pressure. Historically, it has been synthesized by refluxing methyl salicylate (wintergreen oil) with an excess of sodium hydroxide. Sodium salicylate is of the salicylate family. It is used in medicine as an analgesic and antipyretic. Sodium salicylate also acts as a non-steroidal anti-inflammatory drug (NSAID) and induces apoptosis in cancer cells and necrosis. It is also a potential replacement for aspirin for people sensitive to it. It may also be used as a phosphor for the detection of vacuum ultraviolet radiation and electrons.

Materials and Equipment:

UV/VIS spectrophotometer and polystyrene cuvettes or quartz cuvettes (Absorption cells), pipette, 5.00mL volumetric flasks**,** beakers, Ferric nitrate, Nitric acid, sodium salicylate.

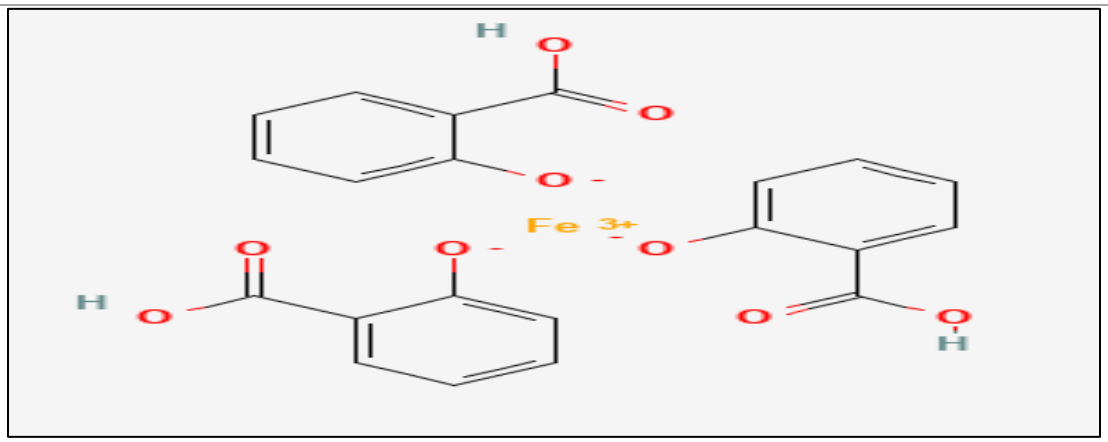

Figure (1): Chemical structure of complex

Procedure:

Preparing the stock solution and standard solutions: -

1) **Stock solution of sodium salicylate:** Weight 0.2 g of sodium salicylate in the beaker, dissolve the solid by the addition of D.W., then transfer to the volumetric flask (100mL), and continue adding D.W. to the mark on the volumetric flask (2000ppm).
2) **Ferric Nitrate:** Dissolve 1.0 g ferric nitrate in 99 mL of water to make a 1% ferric nitrate solution. (Total volume = 100 mL of 1% ferric nitrate).
3) **Nitric Acid I:** Prepare 100 mL of 0.07 M nitric acid.
4) **Standard solution:** Transfer 1.00 mL of stock solution (2000ppm) to a volumetric flask (20mL), then dilute with D.W. to mark (Its concentration is ….mg/L).
5) **Dilute Ferric Nitrate:** Mix 5 mL of 1% ferric nitrate with 4 mL of 0.07 M HNO3 (nitric acid I) and label the container "dilute ferric nitrate."
6) Prepare solutions in 5mL volumetric flasks, then fill to the mark with D.W., as follows: -

Type of Soln.	Vol. of Standard Soln. (mL)	Vol. of $FeNO_3$ dilute (mL)	Concentration (mg/L)	Absorbance (A)
Blank	**0**	**1**		
No.1	**1**	**1**		
No. 2	**2**	**1**		
No. 3	**3**	**1**		
No. 4	**4**	**1**		

Unknown 2 mL	**0**	**1**		

Table (1) for calibration curve

Measuring the absorption spectrum and determining λ_{max}:-

In this part of the experiment, each pair of students should record all absorbance at each wavelength and draw the absorption spectrum.

1. Rinse one of the cuvettes with blank solution, put the cuvette in the sample compartment, this is the reference solution, set the wavelength to 270 nm, then set the Absorbance to zero.
2. Rinse a second cuvette standard solution No.4, place the cell in the sample compartment, measure the Absorbance at 250 nm, and record in your notebook.
3. Repeat this procedure (steps 1 and 2 above) for the two cuvettes at wavelengths 255, ……,340 nm, first setting A = 0 for the cuvette with blank, then measuring A for the cuvette with standard solution No.4, recording the absorbance at each wavelength, record in a data table (at absorbance begin larger reduce wavelength intervals to 5nm).

Prepare a graph of absorbance (**A)** vs. wavelength (**λ)** and determine λ_{max} (maximum wavelength). Attach this graph to the lab report, (*Plotting* Use the program Excel to plot the absorption spectrum and determine λ_{max}).

The calibration curve:

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

1. Set the wavelength at (λ_{max}), place the cuvette with a blank in the cell compartment, and again set the Absorbance to zero.
2. Measure and record the Absorbance of each of the four standard solutions & unknown, starting with the most dilute standard, after each measurement, rinse the cuvette with the next standard, not with blank!
3. Draw a plot having the X-axis as concentration (mg/L) and the Y-axis as Absorbance at λ_{max} (*Plotting* Use the program Excel to plot the calibration curve**).**
4. Use Beer's law to calculate **ε** for Sodium Salicylate, given (path length l) to be 1 cm.
5. Use a calibration curve to calculate the concentration of an unknown solution.
6. Find an application for a calibration curve equation, to calculate the concentration of unknown solution.