YILDIZ TECHNICAL UNIVERSITY

BIOMEDICAL ENGINEERING DEPARTMENT

BME2901- BIOCHEMISTRY COURSE

2020-2021 FALL SEMESTER

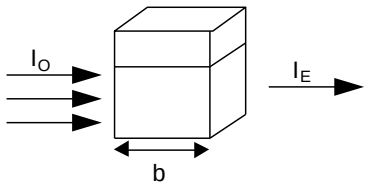
EXPERIMENT 2

SPECTROPHOTOMETRICAL ANALYSIS

1. THEORETICAL KNOWLEDGE

Spectrophotometry is the measurement of the interaction of light with matter. The most common application of this technique in biochemistry is in the measurement of the concentration of a compound in solution.

Compounds that absorb visible light appear colored. Many colorless compounds also absorb light in the region of the spectrum that is not visible to the naked eye. Light absorption of molecules is due to the nature of their chemical bonds. While molecules absorb some of the light that passes through a specimen, some of the light is transmitted. Spectrophotometer is the device that effectively "counts" the number of photons that enters a sample and compares it with the number of photons that exits a sample. Thus, it can detect the amount of light absorbed by molecules in a solution.



I_O = Intensity of incident light
I_E= Intensity of exiting light
b = path length of sample

$$T= I_0/I_E$$

Where:

T: Transmittance

$$A = - \log_{10} T$$

The quantitative relation for absorption as a function of concentration of an absorbing species was formulated by Beer and Lambert and known as Beer-Lambert Law. This law states that "the proportion of light absorbed by a medium is independent of the intensity of incident light" and "the absorbance of light is directly proportional to the concentration of the absorbing medium and the thickness or path length of the medium". It is formulated as the following equation:

$$A = \varepsilon \times b \times c$$

Where:

A: absorbance

ε : molar absorptivity (molar extinction coefficient)

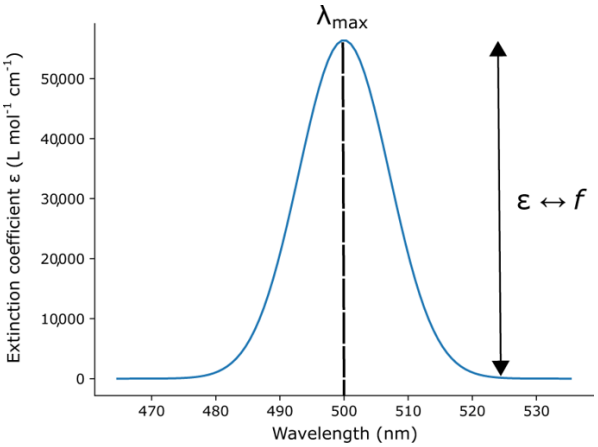
b: length of the path

c: concentration

It follows from this equation that absorbance is directly proportional to concentration. Thus, when ε and b are known, the concentration of the absorbing species in a solution can be determined by measuring its absorbance.

However, the wavelength of light at which maximum absorption takes place (λ_{max}) differs for various molecules and should be used to detect the concentration of a specific type of molecule in a solution. For example, while DNA absorbs highest energy at 260 nm, proteins absorb at 280 nm, both in the range of ultraviolet light. Pigments and dyes, on the other hand, absorb visible light.

The spectrophotometer is also able to take white light and separate it into its constituent colors (i.e. somewhat like a prism), allowing the user to examine the absorption spectrum of the molecule and to find λ_{max}. Absortion spectrum of a molecule looks like below:



According to Beer-Lambert Law, the absorbance versus concentration curve should yield a straight line, the slope of which is E x b. The procedure used to achieve such a plot is called the Construction of a Calibration Curve. A calibration curve is constructed using known concentrations of the sample from which an unknown concentration can be derived using its absorbance value.

In this laboratory class, the absorption spectrum of a $KMnO_4$ solution will be found by a wavelength scan from 200 to 700 nm by 20 nm intervals. This will be done by taking absorbance values of the solution throughout the wavelength region between 200 and 700 nm. This absorption spectrum will give an estimate of the region where the λ_{max} will be. Wavelength scan will then be repeated around the maximum wavelength with 2 nm intervals. After this, λ_{max} will be found with a high precision. A calibration curve for $KMnO_4$ will be then constructed by measuring the absorbances of a series of $KMnO_4$ solutions of different

concentrations at the predetermined λ_{max}. Finally the concentration of $KMnO_4$ in an unknown solution will be calculated using this calibration curve.

2. MATERIALS

1) $4x10^{-5}$ M $KMnO_4$ Solution.

Take 0.0316 g $KMnO_4$ into a 500 mL volumetric flask, dissolve in water and dilute to the mark.

2) 0.5 N H_2SO_4.

Add 7 mL of concentrated H_2SO_4 (sp. gr. = 1.84 g/mL, purity= 96 %) on 493 mL of water slowly, while stirring continuously.

3. PROCEDURE

a. Absorption Spectrum of $KMnO_4$

Take 5 mL of the $4x10^{-5}$ M $KMnO_4$ solution into a test tube, add 2 mL of 0.5 N H_2SO_4, and mix. Rinse and fill one of the spectrophotometer cuvettes with this diluted $KMnO_4$ solution and another with 0.5 N H_2SO_4. Measure the absorbance of $KMnO_4$ against H_2SO_4 at 20 nm intervals throughout the wavelength region between 400 and 660 nm. Take additional readings at 2 nm intervals around the peak value. Plot the absorption curve as absorbance versus wavelength.

b. Calibration Curve for $KMnO_4$

Prepare a series of $KMnO_4$ solutions with different concentrations as follows:

i) 2 mL $4x10^{-5}$ M $KMnO_4$ + 2 mL 0.5 N H_2SO_4 + 6 ml dH_2O

ii) 3 mL $4x10^{-5}$ M $KMnO_4$ + 2 mL 0.5 N H_2SO_4 + 5 ml dH_2O

iii) 4 mL $4x10^{-5}$ M $KMnO_4$ + 2 mL 0.5 N H_2SO_4 + 4 ml dH_2O

iv) 5 mL $4x10^{-5}$ M $KMnO_4$ + 2 mL 0.5 N H_2SO_4 + 3 ml dH_2O

v) 6 mL $4x10^{-5}$ M $KMnO_4$ + 2 mL 0.5 N H_2SO_4 + 2 ml dH_2O

vi) 7 mL $4x10^{-5}$ M $KMnO_4$ + 2 mL 0.5 N H_2SO_4 + 1 ml dH_2O

vii) 8 mL $4x10^{-5}$ M $KMnO_4$ + 2 mL 0.5 N H_2SO_4

Read the absorbances of these solutions at the chosen wavelength, and plot a calibration curve. Calculate Ɛ x b from the slope of your curve.

c. Determination of $KMnO_4$ Concentration in a Sample

Read the absorbance of the solution given at the chosen wavelength. Calculate the concentration of $KMnO_4$ in the sample using the calibration curve.