Experiment Details

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| Department Name | Biotechnology engineering |
| Class | SY BTech |
| Semester | 3 |
| Subject Name | Biochemistry |
| Experiment No. | 1 |
| Experiment Name | Spectroscopy |

Version History

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| --- | --- | --- | --- | --- |
| Sr. No. | Version Number | Created By | Approved By | Date |
| 1 | v1.0 | Rameshwari | Asst.prof Atish Chavan | 16/10/2020 |
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AIM: To determine maximum absorption wavelength for given chemical compound (KMnO4) and verify Beer Lamberts law.

THEORY:

Optical analysis is one of the analytical methods to detect component of interest quantitatively and to determine the concentration of component of interest. In this type of analysis, the component (atom or molecule) interacts with light of specific wavelength.

When a chemical compound (i.e. molecule) is to be detected from a mixture the molecular absorption spectrometry is used widely. In this type of spectrophotometry, the molecule absorbs a specific wavelength of light in different spectral regions maximally. Therefore, it is important to find out that specific wavelength by scanning the major part of the electromagnetic spectrum. In this practical using monochromatic light of different wavelength the absorption is studied with the help of plot of wavelength versus absorbance, the maximum specific wavelength for given molecule is found out. When a ray of monochromatic light of initial intensity I0 passes through a solution, in a transparent vessel light is transmitted. I is less than I0. There is some loss of light intensity due to scattering by particles in the solution and the reflection at the interface and also from absorbance by the solutions. The relationship between I0 and I depend on the path length of absorbing medium and concentration of absorbing solution.

Extinction/Absorbance/Optical Density: -

If logarithms are taken of the equation i.e. I = I0e - K3cl

log (I0/I) = K3cl

log10 (I0/I) = K3cl/2.303 where, K3=extinction coefficient

c = concentration of solution (mg/ml)

l = path length (mol-1cm-1)

1)Lambert's law - when a ray of monochromatic light passes through an absorbing medium, its intensity decreases exponentially as the length of the absorbing medium increases.

2)Beer's law - when a ray of monochromatic light passes through an absorbing medium, its intensity decreases exponentially as concentration of absorbing medium increases.

Beer's law is obeyed keeping 'l' constant. The graph of extinction/absorbance against concentration is more convenient than graph of transmittance Vs concentration. With the help of this, standard curve can be drawn. With this the concentration of unknown solution can be easily determined from its extinction absorbance.

PRE TEST:

1. What is the wavelength range for UV spectrum of light
2. **10nm to 400nm**
3. 400nm to 700nm
4. 0.01nm to 1nm
5. Beer Lamberts law gives the relation between which of the following?
6. Reflected radiation and concentration
7. Scattered radiation and concentration
8. **Energy absorption and concentration**
9. In which of the following ways, absorption is related to transmittance
10. **Absorption is the negative logarithm of transmittance**
11. Absorption is multiple transmittance
12. Absorption is the logarithm of transmittance
13. **Beer’s law states that the intensity of light decreases with respect to \_\_\_\_\_\_\_\_\_\_\_**

**a)Concentration**  
b)Distance  
c)Composition  
d) Volume

1. **Lambert’s law states that the intensity of light decreases with respect to \_\_\_\_\_\_\_\_\_\_**

a)Concentration  
b)Distance  
c)Composition  
**d) Volume**

PROCEDURE:

1. One cuvette was filled with distilled water also called as blank cuvette or reference solution
2. The solution of potassium permanganate (KMnO4) (1 mg/ml) was prepared.
3. It was diluted with water accordingly to dilution shown to generate ascending concentration range.
4. The spectrophotometer was switched on.
5. Blank cuvette was inserted in cell holder & its absorbance was measured.
6. The absorbance reading was made to ‘0’ for taking a standard result by using the knob of the spectrometer..
7. The absorbance of each solution was read and recorded at λ max =520nm .
8. Graph of absorbance versus wavelength was plotted to obtain λmax

POST TEST:

**1.The representation of Beer Lambert’s law is given as A = abc. If ‘b’ represents distance, ‘c’ represents concentration and ‘A’ represents absorption, what does ‘a’ represent?**

a)Intensity  
 b)Transmittance  
 **c)Absorptivity**  
 d) Admittance

2. **What is the unit of molar absorptivity or absorptivity which is used to determin absorbance A in Beer Lambert’s formula?**

**a)Lmol-1 cm-1** b)gm-1 cm-1  
 c)Cm  
 d) No unit

3. **Which of the following is not a type of Spectroscopy?**

a)Gammaray  
b)Xray  
**c) Sound**

REFERENCES:

* + - 1. Instrumentationtools.com
      2. KIT Biotechnology engineering department S.Y.Btech