VERIFICATION OF BEER'S LAW

PROJECT REPORT

Submitted by

KEERTHANA MANOJ

Register Number: AB19PHY030

Under the guidance of

Dr. SANTHI A, Associate Professor

Department of Physics, St. Teresa's College (Autonomous),

Ernakulam, Kochi-682011

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BACHELOR DEGREE OF SCIENCE IN PHYSICS

ST. TERESA'S COLLEGE(AUTONOMOUS)

ST. TERESA'S COLLEGE(AUTONOMOUS)

ERNAKULAM

CERTIFICATE

This is to certify that the project report entitled '**VERIFICATION OF BEER'S LAW**' is an authentic work done by KEERTHANA MANOJ, St. Teresa's College Ernakulam, under my supervision at Department of Physics, St. Teresa's College for the partial requirements for the award of Degree of Bachelor of Science in Physics during the academic year 2021-22. The work presented in this dissertation has not been submitted for any other degree in this or any other university.

Supervising guide

Dr. SANTHI A

Associate Professor

Head of the Department

Dr. PRIYA PARVATI AMEENA JOSE

Associate Professor

PLACE: Ernakulam

DATE: 04-05-2022

ST. TERESA'S COLLEGE(AUTONOMOUS)

ERNAKULAM

B.Sc. PHYSICS

PROJECT REPORT

Name : KEERTHANA MANOJ

Register Number : AB19PHY030

Year of work : 2021 - 2022

This is to certify that this project work entitled ' **VERIFICATION OF BEER'S LAW** ' is an authentic work done by KEERTHANA MANOJ.

Staff member in-charge

Dr. SANTHI A

Head of the Department

Dr. PRIYA PARVATI AMEENA JOSE

ST. TERESA'S COLLEGE ERNAKULAM DEPT. OF PHYSICS

Submitted for the University examination held at St. Teresa's College, Ernakulam.

DATE: 04-05-2022

EXAMINERS:

DECLARATION

I, KEERTHANA MANOJ (Register Number: AB19PHY030), final year B.Sc. Physics Students, Department of Physics, St. Teresa's College, Ernakulam do hereby declare that the project work entitled '**VERIFICATION OF BEER'S LAW'** has been originally carried out under the guidance and supervision of Dr. SANTHI A, Associate Professor, Department of Physics, St. Teresa's College (Autonomous), Ernakulam in partial fulfilment for the award of the degree of Bachelor of Physics. I further declare that this project is not partial or wholly Submitted for any other purpose and the data included in the project is collected from various sources and is true to the best of our knowledge.

ACKNOWLEDGEMENT

I express my sincere gratitude to all those who helped me to achieve this moment of satisfaction. Firstly, I thank the lord almighty for the immense grace at every stage of the project. I am highly indebted to my project guide Dr. Santhi A, Associate Professor, St. Teresa's College, Ernakulam for her valuable guidance and constant supervision as well as for providing necessary information regarding the project. I would like to express my gratitude to all the staff members of the Physics Department, St. Teresa's college, for their valuable guidance and suggestions for completing the project and for all those who have willingly helped me out of their abilities.

ABSTRACT

The report is presented in the aim of the **Verification of Beer's Law**. Beer's law also known as Beer- Lambert law relates to the absorption of light to the properties of the material through which the light is travelling. The law states that when light passes through a solution of a given thickness the fraction of incident light absorbed is dependent not only on the intensity of light but also on the concentration of the solution. It is done by an experiment where the absorption spectrum of a sample of solution of eosin yellow is measured and verified. The law is commonly applied to chemical analysis measurements and used in understanding absorption in physical optics, for photons, neutrons, or rarefied gases.

CONTENTS

CHAPTER 1

MECHANISM OF ABSORPTION AND EMISSION

1.1 INTRODUCTION

Atoms are the basic building blocks of all existing matter. They are responsible for the characteristic behavior of the basic different types of matter, that is, solid, liquid and gas. The term ‘Atom’ was derived from a Greek word ‘Atomos’ which means ‘non-divisible’. Many physicists did a lot of experiments to figure out if the speculations regarding the ‘un-cuttable’ nature of atoms was correct. The first atomic theory was given by John Dalton in 1808 and he thought of the atom as the ultimate particle of matter. Through his theory, he was able to explain the law of conservation of mass, law of constant composition and law of multiple proportion successfully but failed to explain the dissimilarities in the properties of some elements. Some of the other models were Thomson’s plum pudding model, Rutherford’s Nuclear Model of Atom, Bohr’s model, and so on. Many of these proposed models were able to explain the structure of atoms to a certain extent but could not account for several other important factors such as stability, existence of double spectral lines, etc. However, through the evolution of time and scientific techniques, scientists found that atoms are composed of smaller particles, such as electrons, protons and neutrons which are collectively known as ‘elementary particles’.

According to Bohr’s theory, electrons revolve around the nucleus in certain orbits of different energy levels. Energy levels show the distance of electrons from the nucleus of a particular element. Electrons are distributed to different shells of different levels corresponding to their energy. There are K, L, M…shells corresponding to the principal

quantum numbers n = 1, 2, 3, … Each shell can only hold a fixed number of electrons and after filling a certain energy level, it jumps to higher energy levels, if provided with sufficient energy. The K shell lies closest to the nucleus and it can accommodate two electrons only. The L shell can hold a maximum of 8 electrons. The n^{th} shell can hold $2n^2$ numbers of electrons.

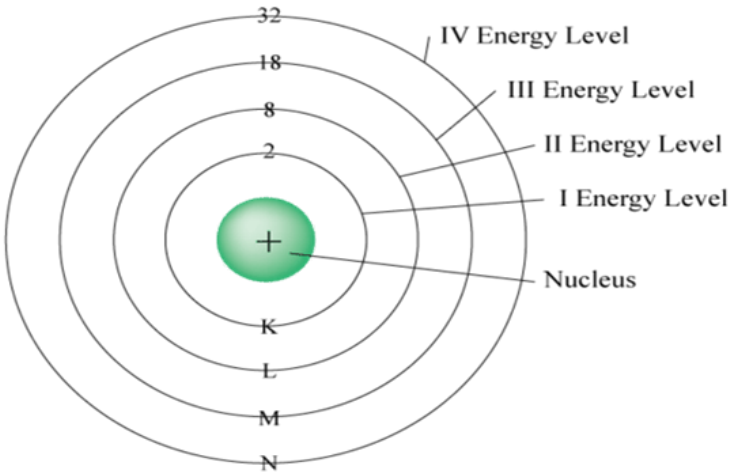


Figure 1(a) : Energy levels of an atom

1.2 ABSORPTION AND EMISSION

The binding energy of electrons to their parent nucleus is higher for the shells closest to it. As the energy level increases, the electrons are less tightly bound to the nucleus. The electrons at the outermost energy level are known as the valence electrons. They have more chances of becoming a free electron under the application of an external energy. When an incident photon having a frequency $\boldsymbol{\nu}$ hits an atom, certain changes happen in its energy states. Its electrons absorb the quanta energy from the photon and excite to higher energy levels. This process is known as absorption of light. The excited electron stays at that

particular higher energy level for a very short time in the range of a few nanoseconds.

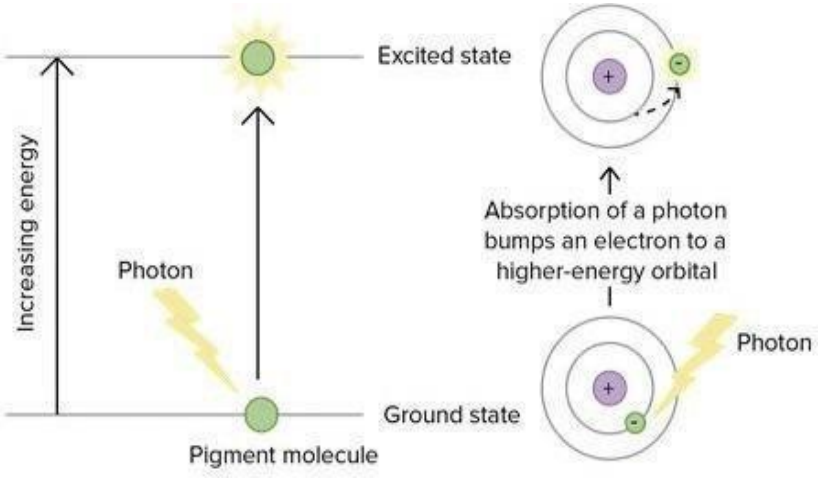


Figure 1(b): Absorption of a photon when it incidents on an atom

As the excited state is unstable, the electron de-excites back to its original state after some time. During de-excitation, electrons emit radiation in the form of photons and this process is known as Emission. That is, an atom can absorb or emit one photon when an electron makes a transition from one energy level to another. Conservation of energy determines the energy of the photon and thus the frequency of the emitted or absorbed light.

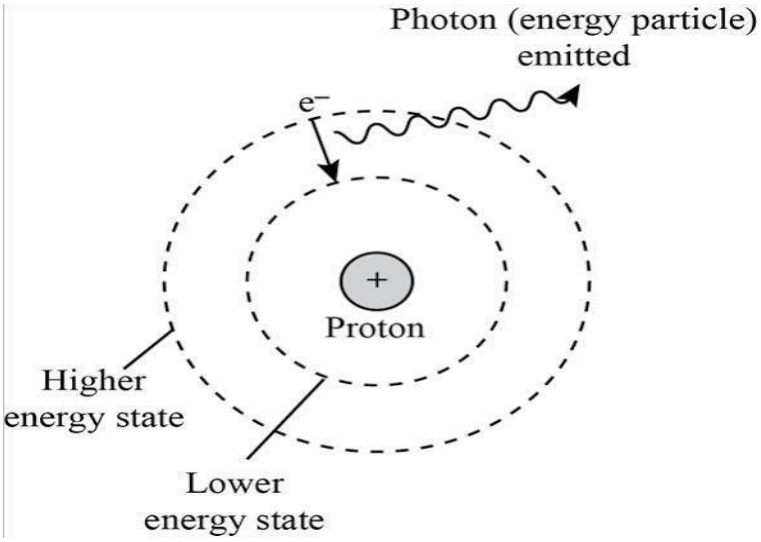


Figure 1(c) : De-excitation of an electron causing the emission of a photon

1.3 PHOTOLUMINESCENCE

When certain energy is absorbed by a molecule, it becomes excited and its electrons are raised to higher energy levels. The electrons do not remain there for a longer time and return to lower and more stable energy levels. This can occur by non-radiative process or through a radiative process. The radiative process involves the emission of electromagnetic radiations. This process of emitting radiation is collectively called luminescence. Photoluminescence is the emission of light which is caused by the irradiation of a substance with other light. The term embraces both fluorescence and Phosphorescence, which differ in the time after irradiation over which the luminescence occurs. The radiated light is often visible but can also be in the ultraviolet or infrared spectral region.

1.4 FLUORESCENCE AND PHOSPHORESCENCE

Fluorescence is the phenomenon in which a material absorbs light of a particular wavelength followed by a short-lived emission of light of a longer wavelength. This process involves a light source to excite the molecule, which is then transformed from a ground to an excited state. As the molecules return to the ground state, energy is released in the form of heat and light. The phenomenon of fluorescence is instantaneous and starts immediately after the absorption of light and stops as soon as the incident light is cut off. The fluorescent materials generally emit the radiation within 10^{-6} to 10^{-4} seconds of absorption. Thus, the lifetime of fluorescence is generally small. Also, no change in Spin state of the electron involved during the process of fluorescence.

Phosphorescence is the process in which the radiations are incident on certain substances, they absorb them and then emit light continuously for a long time even after the incident light is cut off. In this process the direction of the electron Spin may change when the electrons move to a lower energy state. In Phosphorescence the molecule does not return immediately to the ground state. Instead, it goes through a metastable state, a state where electrons stay for a longer period of time. This transition is known as intersystem crossing. The life-time of Phosphorescence is therefore much longer.

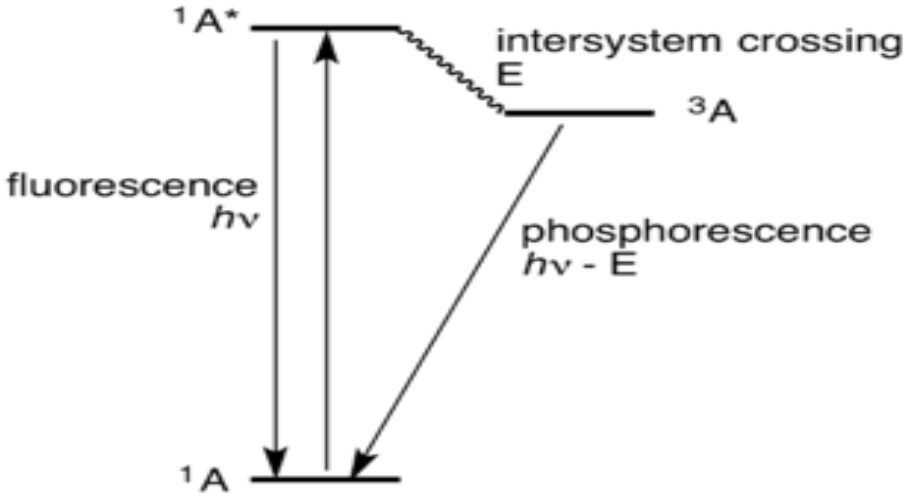


Figure 1(d) : Energy level diagram depicting fluorescence and phosphorescence

1.5 CLASSIFICATION OF DE-EXCITATION

Atomic de-excitation is the process by which an atom transfers from an excited electronic state back to the ground state electron configuration. It often occurs by emission of heat and light. Electronically excited states are unstable. Electrons drop back to their ground states. At the same time, the excitation energy is released again. One distinguishes between radiative and non-radiative decay processes. Most of the time,

the decay is non-radiative, for example through vibrational relaxation, quenching with surrounding molecules, or internal conversion.

Sometimes, a radiative decay can occur in the form of fluorescence and phosphorescence. The energy is emitted as electromagnetic radiation or photons. The emitted light has a longer wavelength and a lower energy than the absorbed light because a part of the energy has already been released in a non-radiative decay process. This is the reason that an emission in the visible spectrum can be achieved by excitation with non-visible UV-radiation.

CHAPTER 2

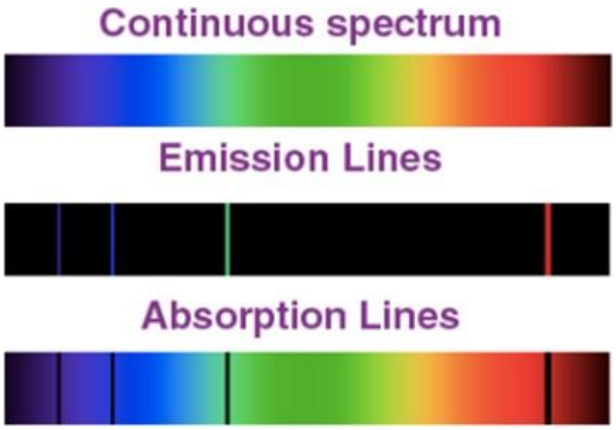
ABSORPTION SPECTRUM

In 1801 William Wollaston observed a rainbow in close detail and noticed tiny dark lines in the visible spectrum. In later years Fraunhofer took an even closer look at the Sun's spectrum (IR, UV and visible) by expanding the spectrum onto a large wall. As a result, he found thousands of slices that were missing. These are known as absorption lines or 'Fraunhofer lines'.

2.1 WHAT IS ABSORPTION SPECTRUM ?

Absorption spectra (singular spectrum) of chemical species (atoms, molecules, or ions) are generated when a beam of electromagnetic energy (i.e. light) is passed through a sample, and the chemical species absorbs a portion of the photons of electromagnetic energy passing through the sample. This spectrum is constituted by the frequencies of light transmitted with dark bands when the electrons absorb energy in the ground state to reach higher energy states. When light from any source is passed through the solution or vapour, a pattern comprising dark lines is obtained. This pattern is analysed using the spectroscope. Depending on the nature of the chemical or element, certain radiation is absorbed by the chemical or element when passed through it and dark line pattern is seen exactly in the same place where coloured lines are seen in the emission spectrum. The spectrum thus obtained is known as the absorption spectrum.

Emission spectra can emit all the colours in an electromagnetic spectrum, while the absorption spectrum can have a few colours missing due to the redirection of absorbed photons. The wavelengths of light absorbed help figure out the number of substances in the sample.



2.2 THEORY OF BEER-LAMBERT LAW

The Beer-Lambert law, known by various names such as the Lambert-Beer law, Beer-Lambert–Bouguer law or the Beer's law states that,

When light passes through a solution of a given thickness the fraction of incident light absorbed is dependent not only on the intensity of light but also on the concentration of the solution.

This law is taken from two other laws that laid the foundation to Beer-Lambert law. They are,

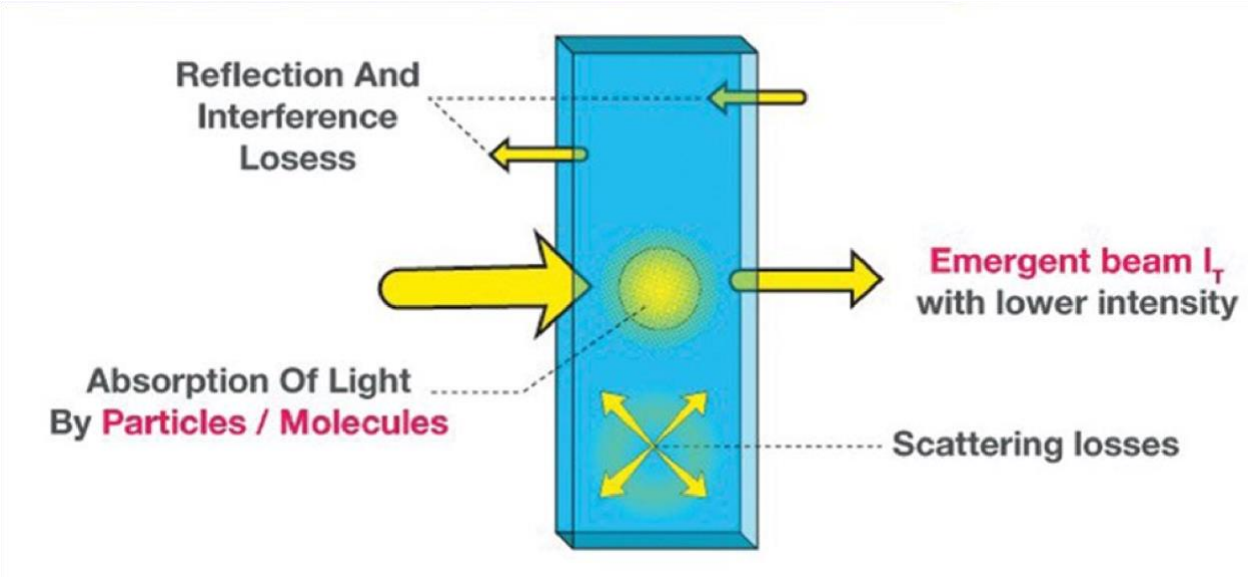
●Beer's law stated by August Beer : concentration and absorbance are directly proportional to each other

●Lambert law stated by Johann Heinrich Lambert : absorbance and path length are directly proportional

If material bodies are exposed to radiation, part of the incident radiation is absorbed, a part is scattered and a part is transmitted. As a result of absorption the intensity of light passing through material bodies, i.e. the intensity of transmitted light, decreases. The fraction

of incident light absorbed depends on the thickness of the absorbing medium.

When a monochromatic light of initial intensity Io passes through a solution in a transparent vessel, some of the light is absorbed so that the intensity of the transmitted light I is less than Io.There is some loss of light intensity from scattering by particles in the solution and reflection at the interfaces, but mainly from absorption by the solution.The relationship between I and Io depends on the path length of the absorbing medium, l, and the concentration of the absorbing solution, c. These factors are related in the laws of Lambert and Beer.



From the law we know that the decrease in intensity of light with thickness of the absorbing medium at any point is directly proportional to the intensity of light. If we express mathematically;

$$-dI / dx \propto I \qquad (1)$$

Where dI is a small decrease in intensity of light upon passing through a small distance dx and I is the intensity of the monochromatic light just before entering the medium.

Equation (1) can be written as

$-dI / dx = aI$ (2)

Where $-dI/dx$ is the rate of decrease of intensity with thickness dx , **a is called the absorption coefficient**.

Integration of equation (2) after rearrangement gives,

$-\ln I = ax+C$ (3)

Where C is a constant of integration. At x=0, I=Io. So, C = – ln Io.

Introducing this in equation (3) we get,

$\ln I/ Io = -ax$ (4)

Equation (4) can also be written as,

$I = Io\ e^{-ax}$ (5)

Equation (5) can also be written

as, $\log I/ Io = - a x / 2.303$

(6) or, $\log I/ Io = -a` x$

(7)

Where a` (= a /2.303) is called extinction coefficient and -ln I/ Io is termed absorbance of the medium. Absorbance is represented by **A**.

Lambert's law was extended by Beer gives;

$-dI/dx \propto c$ (8)

The two laws may be combined to write

$-dI/dx \propto I \times c$

Or, $-dI/dx = b \times I \times c$ — — — — — (9)

When the concentration, c, is expressed in mol /L, b is called the molar absorption coefficient.

As in the case of Lambert's law equation (9) may be

transformed into, $\log I/Io = -b/2.303 \times c \times x$ — — — — —

(10) **$\log I/Io = -\in \times c \times x$** — — — — — (11)

Where $\in$ ($= b / 2.303$) is called the molar extinction coefficient which is expressed in L/mol/cm.

The molar extinction coefficient $\in$ is dependent on the nature of the absorbing solute as well as on the wavelength of the incident light used. The expression (equation 11) is commonly known as Beer-Lambert's law.

$A = -\in \times c \times x$ where A is the

absorbance of the sample

The Beer-Lambert law allows you, the scientist, to measure the absorbance of a particular sample and to deduce the concentration of the solution from that measurement! In effect, you can measure the concentration of a particular chemical species in a solution as long as you know the species absorbs light of a particular wavelength.

2.3 FACTORS THAT AFFECT ABSORBANCE

These are the factors that affect absorbance:

- The concentration of a sample is one factor that affects its absorbance. As the concentration rises, more radiation should be

absorbed, increasing the absorbance. As a result, the concentration and absorbance are directly proportional.

- The length of the path is a second consideration. The longer the path length, the more molecules in the path of the radiation beam, and thus the absorbance increases. As a result, the length of the path is proportional to the concentration.

- The molar absorptivity is the third factor when the concentration is expressed in moles/litre and the route length is expressed in centimeters. This is more commonly referred to as the extinction coefficient in some sectors of study.

We may construct the Beer-Lambert law (commonly referred to as Beer's Law) to show this relationship because concentration, path length, and molar absorptivity are all directly proportional to absorbance.

The absorbance of a transition depends on two external assumptions:

- The absorbance is directly proportional to the concentration (c) of the solution of the sample used in the experiment

- The absorbance is directly proportional to the length of the light path (l), which is equal to the width of the cuvette.

2.4 LIMITATIONS OF BEER-LAMBERT LAW

This law can be used to study the absorptivity coefficient of the sample when the concentration is low ie; <10mM, but as the concentration becomes high ie; >10mM there is a deviation as the electrostatic interactions become more.

CHAPTER 3

SAMPLE PREPARATION OF BEER'S LAW

The solute taken for the experiment is Eosin Yellow. It is a water-soluble dye and is a red crystalline powder. The purity of the dye content is 88%. The chemical formula of Eosin Yellow is $C_{20}H_6Br_4Na_2O_5$. Its molecular weight is 691.8515 g/mol. Stock solution is a solution which is prepared by weighing out an appropriate portion of a pure solid and placing it in a suitable flask, and diluting to a known volume. The steps to get the necessary sample and to obtain the absorption spectrum is as follows:

- To measure the mass of the solute:

  Using butter paper tared on a balance, we carefully weigh the amount of Eosin Yellow required for the stock solution. We only need a very small scoop of material. Mass of Eosin Yellow content is measured using a digital weighing scale.

  Weight of Eosin Yellow dye taken = 0.032g

- To make Stock solution :

  0.032 g is dissolved in 10ml of distilled water in a beaker. This is taken as the Stock solution.

  Number moles of solute, n = (Measured weight of solute) / (Molecular weight of solute)

Volume of distilled water used to make the Stocks solution, V = 10 mln = (0.032g) / (691.8515g/mol) = $4.62527 * 10^{-5}$mol

691.8515 g dissolved in 1L gives one molar (1 M)

i.e, 6.918515 g dissolved in 10 ml gives one molar

Therefore, there are (0.032g) / (6.918515g/mol) numbers of moles dissolved in 10 ml.

Molarity of the solution, M = Number of moles / Volume of the solvent

M =(0.032g) / (6.918515g/mol) * (10 ml) =M = 0.4625 g/L (0.032/ 6. 9185)*(1000/10)

- Diluting the stock solution to make the standard solutions:

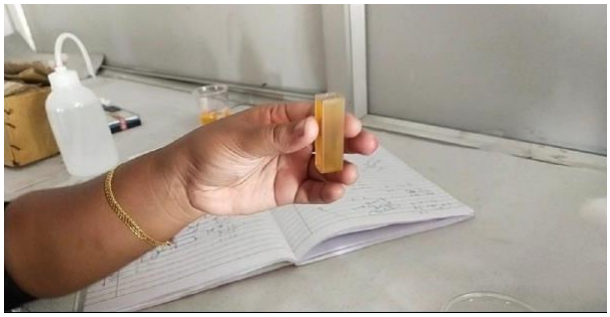
  10 samples are to be prepared. 1 ml of the stock solution is taken in a clean test tube using a syringe. Different concentrations of its diluted solution gives us the required 10 samples. 1 ml of stock solution diluted in 15 ml is taken as the first sample and 1 ml of stock solution in 16 ml is taken as the second sample. Likewise, each of the ten samples are obtained by adding 1 ml into the previously diluted solution. Maximum concentration of the solute is found in the first sample and its concentration gradually decreases after each sample solution.

  Concentration of first sample is obtained by

$(0.032/0.69185)$ g in (15 + 1)ml ==(2.89 g/L$0.032/0.69185$)* (1000/16)

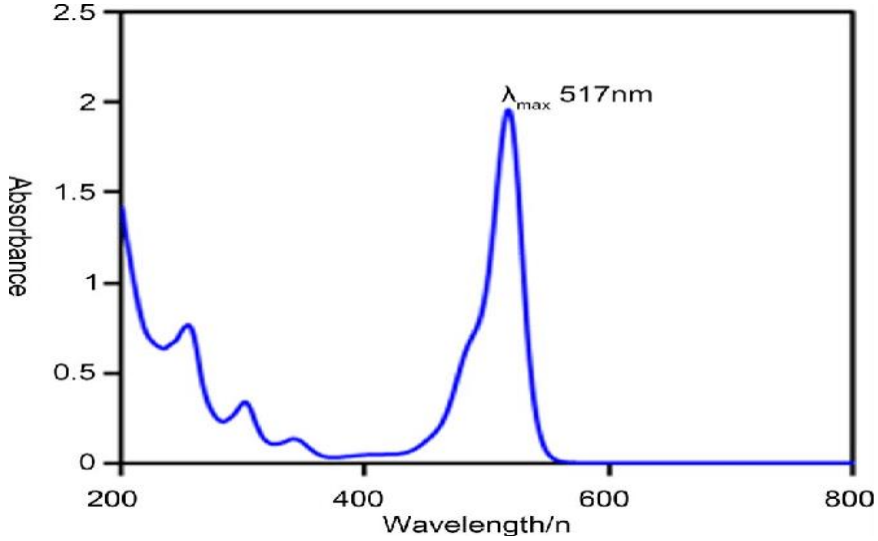
Similarly, concentrations of 10 samples can be calculated.

- Getting absorption spectrum from Spectrophotometer :



Each of the samples is taken in a cuvette and kept inside the device for obtaining required absorption spectrum. Absorption spectrum is obtained for 10 samples.

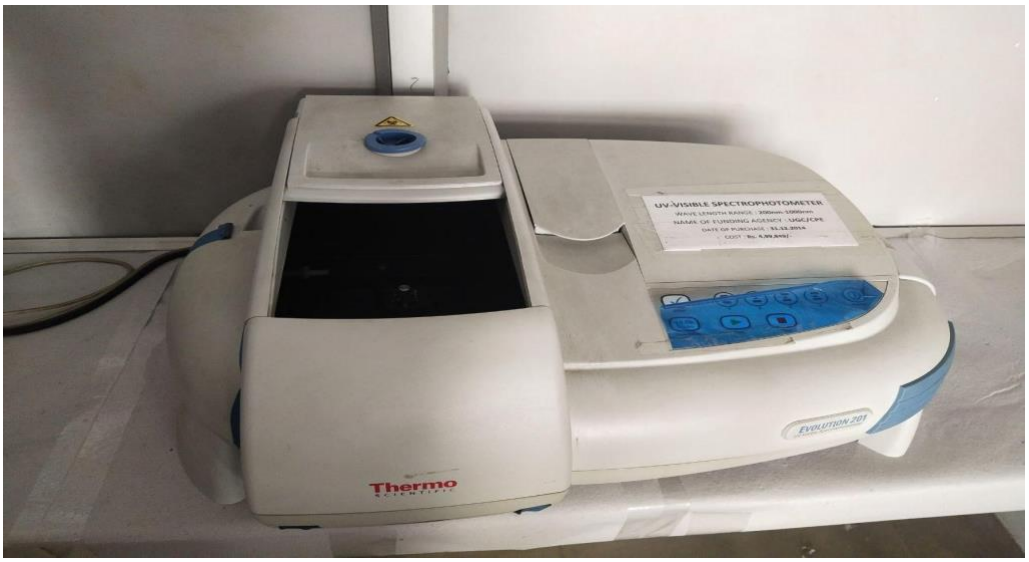
Eosin Y is a chemical compound with an absorbance peak at 517 nm. The actual absorption spectrum of a sample solution of eosin yellow is shown below :



CHAPTER 4

MEASUREMENT OF ABSORPTION SPECTRUM

4.1 SPECTROPHOTOMETER



Every chemical compound has the ability to absorb, transmit or reflect electromagnetic radiation over a certain wavelength. Spectrophotometry is a method to determine how much a chemical substance absorbs light by measuring the intensity of light as a beam of light passes through a sample solution. Absorption spectroscopy is widely used in chemical analysis, for determining unknown samples from a given mixture, infrared gas analyser (which identifies pollutants in the air), remote sensing (for detecting the presence of hazardous elements in a mixture) and so on. A colored solution absorbs all the colours of white light and selectively transmits only one colour.

A spectrophotometer is an instrument that measures the amount of photons (the intensity of light) absorbed after it passes through sample solution. With the spectrophotometer, the amount of a known chemical substance (concentrations) can also be determined by measuring the intensity of light detected. Depending on the range of wavelength of light source, it can be classified into two different types:

- **UV-visible spectrophotometer**: It uses light over the ultraviolet range (185 - 400 nm) and visible range (400 - 700 nm) of electromagnetic radiation spectrum.
- **IR spectrophotometer**: uses light over the infrared range (700 - 15000 nm) of electromagnetic radiation spectrum

4.2 DEVICE COMPONENTS

Light Source

Unlike Deuterium or tungsten-halogen lamps, which provide a constant light source, a Xenon flash lamp emits light for an extremely short time, in flashes. Since it emits only for a short time and only during sample measurement, it has a longer life. The sample is only irradiated with light at the time of measurement. This short illumination time makes the Xenon flash lamp suitable for measuring samples that may be sensitive to photobleaching. Photobleaching can be observed on sensitive samples when exposed to a constant long exposure by a continuous light source. The Xenon flash lamp emits high intensity light from 190-1100nm, which means no secondary light source is required . The Xenon flash lamp may be used for many years before requiring replacement, which makes it a popular choice compared to

systems using D2 or tungsten halogen lamps. An extra benefit is that it does not require warm up time, unlike D2 or tungsten-halogen lamps.

Cuvettes

Monochromator source is used; before reaching the sample, light is divided in two parts of similar intensity with a half mirror splitter. One part travels via the cuvette having the solution of material to be examined in transparent solvent. Second beam or reference beam, travels via a similar cuvette having only solvent. Reference and sample beam containers have to be transparent towards the passing beam.

Photosensitive Detector

The detectors are devices that convert radiant energy into electrical signals. It should be sensitive, and has a fast response over a considerable range of wavelengths. The electrical signal produced by the detector must be directly proportional to the transmitted intensity.

4.3 PRINCIPLE

A light beam is passed through an object and wavelength of the light reaching the detector is measured. The measured wavelength provides important information about chemical structure and number of molecules (present in intensity of the measured signal). Thus, both quantitative and qualitative information can be gathered. Information may be obtained as transmittance, absorbance or reflectance of radiation in the 190 to 1100nm wavelength range. The absorption of incident energy promotes electrons to excited states. For this transfer to occur, photon energy must match the energy needed by the electron to be promoted to the next higher energy state.

This process forms the basic operating principle of absorption spectroscopy. When light having specific wavelength and energy is focused onto the sample, it absorbs some energy of the incident wave. A photodetector measures energy of transmitted light from a sample, and registers absorbance of the sample. The absorption or transmission spectrum of the light absorbed or transmitted by the sample against the wavelength is formed. Bouguer−Beer law or the Lambert−Beer rule is the basic principle of quantitative analysis, and it establishes that absorbance of a solution scales directly with analyte concentration.

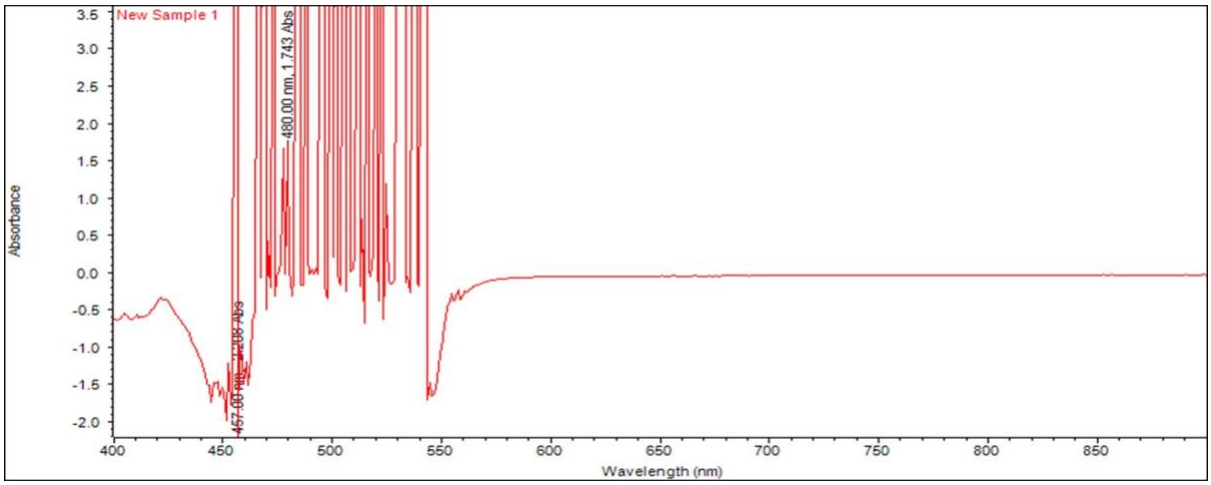
4.4 WORKING OF UV-VISIBLE SPECTROPHOTOMETER

Ultraviolet-visible spectrophotometer system focuses electromagnetic radiation from the light source to the sample. Depending on the configuration set in the system, light is transmitted through the sample or reflected off it. Then, the light is collected from the sample through reading.

Initially, light is focused into the entrance slit of the monochromator from the source. Monochromator uses dispersing elements, namely optical grating to separate the light by wavelength. The light is passed into a charged coupled device (CCD), which is made up of individual tiny detectors, hence the intensity of light at each wavelength will be measured. CCD is read-off to a computer and the result obtained is a spectrum, which shows the intensity of each wavelength of light. Spectrophotometers are able to measure the electromagnetic radiation from ultraviolet to infrared. Spectrum will show the intensity of light versus the wavelength.

The absorption spectrum of 10 samples are as follows:

Sample 1:



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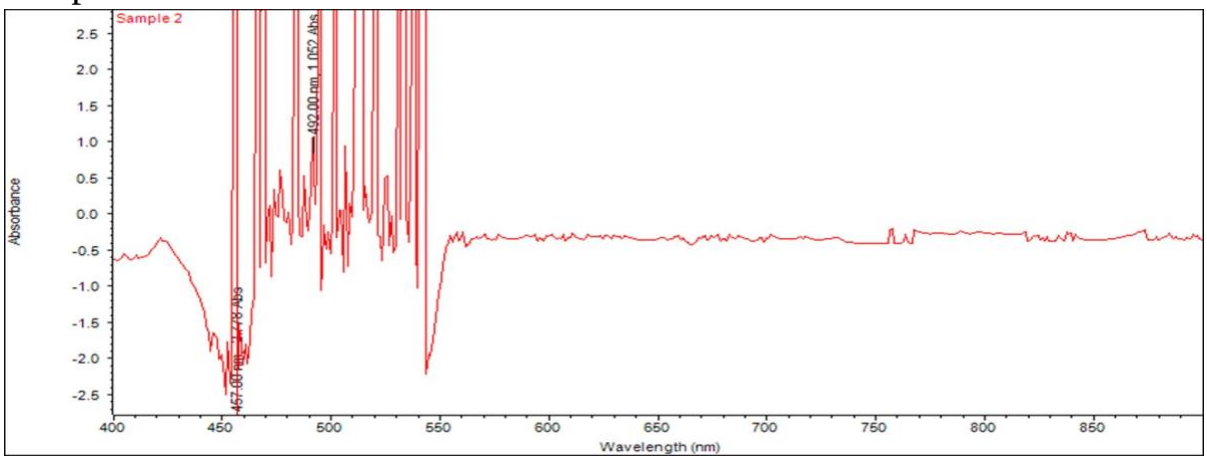

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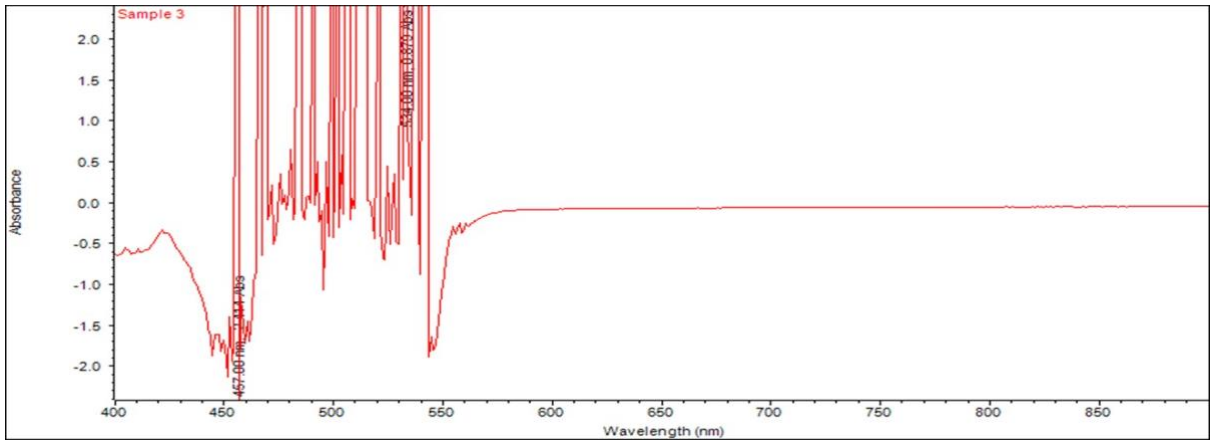

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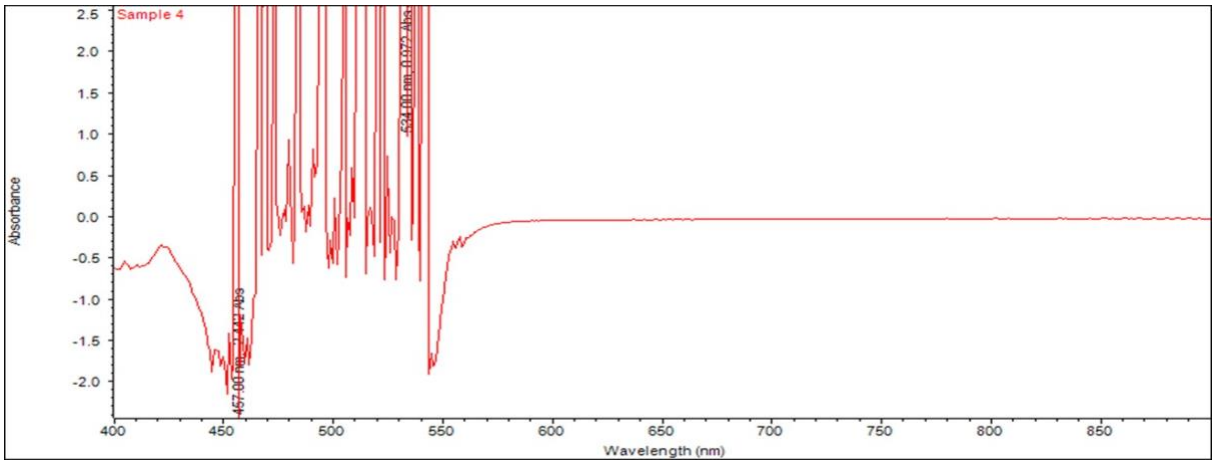

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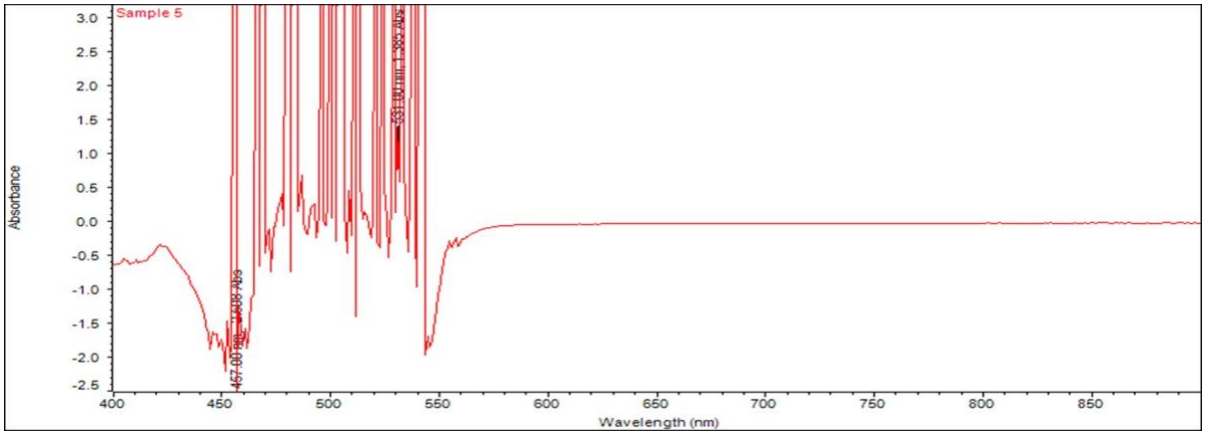

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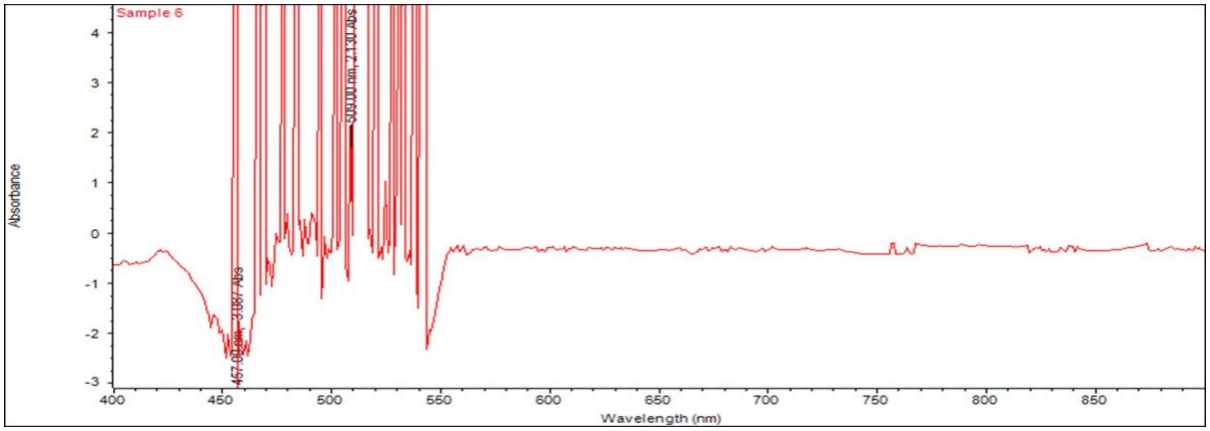
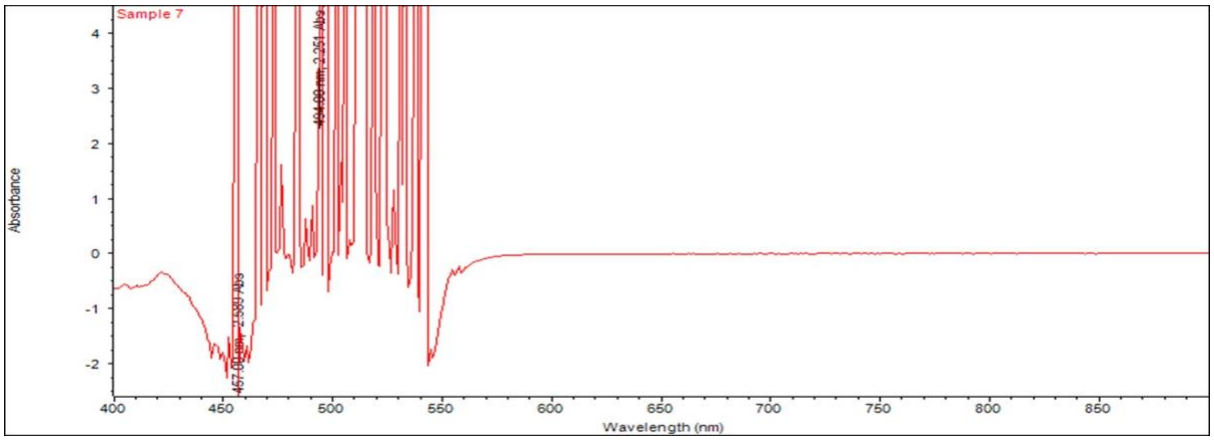


Sample 7:



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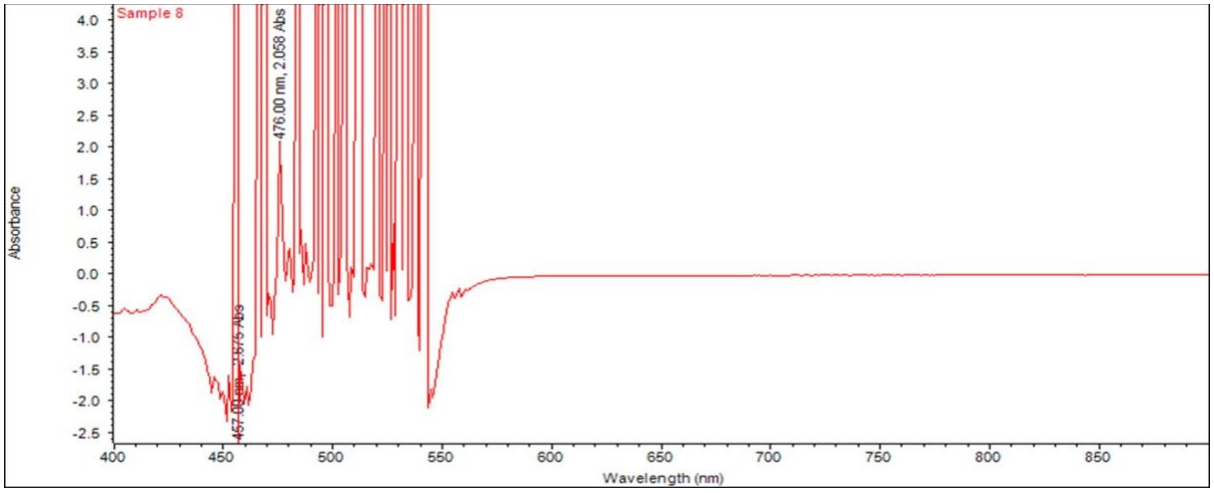

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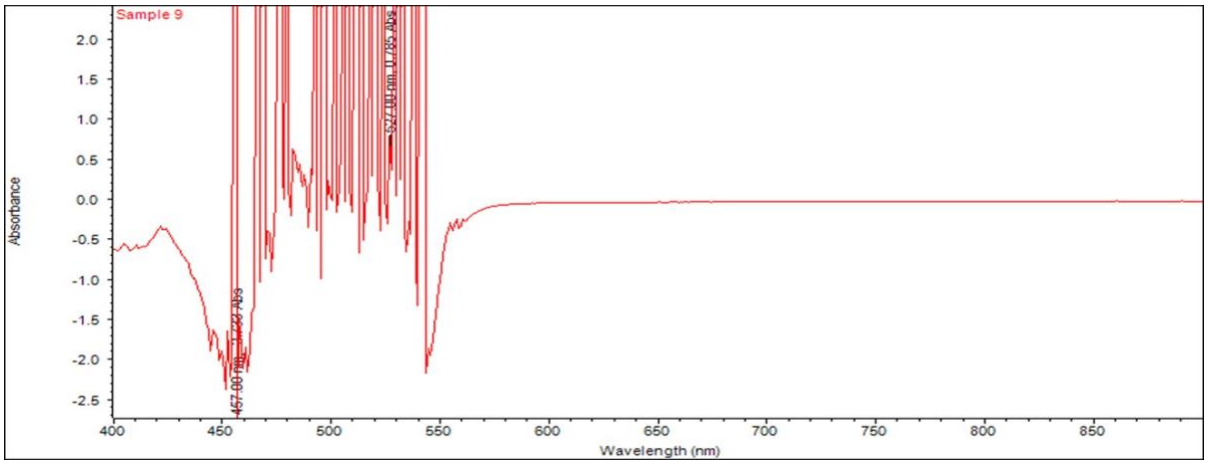

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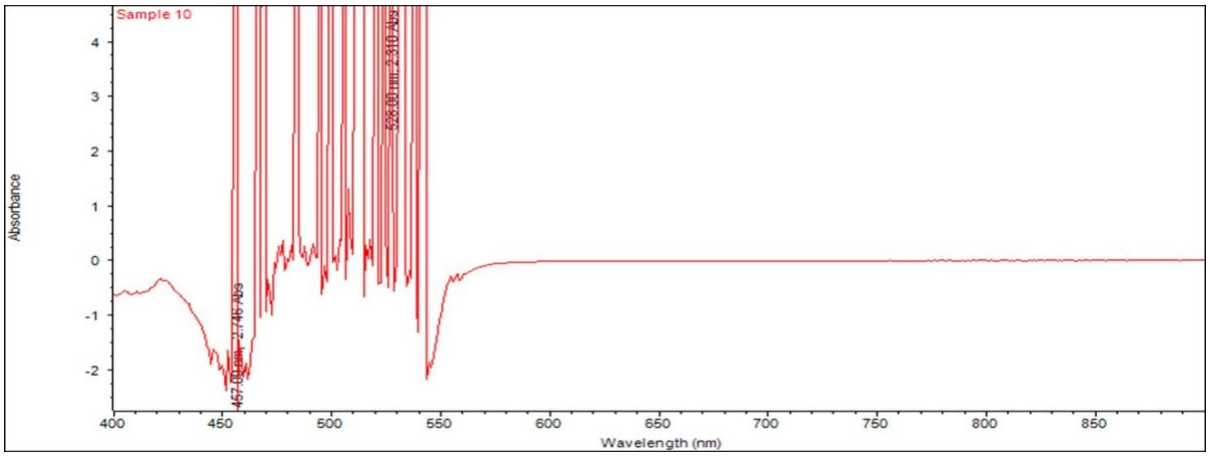


4.5 APPLICATIONS

●The absorption of a reactant or product at constant wavelength provides a means of monitoring the progress of a chemical reaction. Clinical studies using indirect measurement of reactions characterised by enzymes can also be carried out.

●DNA or Deoxyribonucleic acids are involved in protein synthesis in living cells and preserve genetic information. Changes in pH or heating

can lead to denaturation with the resultant increase in absorption at 260 nm. Monitoring absorption at this wavelength is useful for denaturation monitoring.

●Appearance of additional peaks due to presence of impurities can help establish purity of standard materials.

●Absence or presence of functional groups results in absence of presence of their characteristic absorption bands.

●Identification and quantitative determination of polynuclear aromatic compounds

●Non – absorbing molecules can be estimated by combination with derivative forming molecules that produce new spices with absorption characteristics in the UV – VIS region

●Quantitative estimation of ionic solutions of transition metals or complex forming ligands

CHAPTER 5

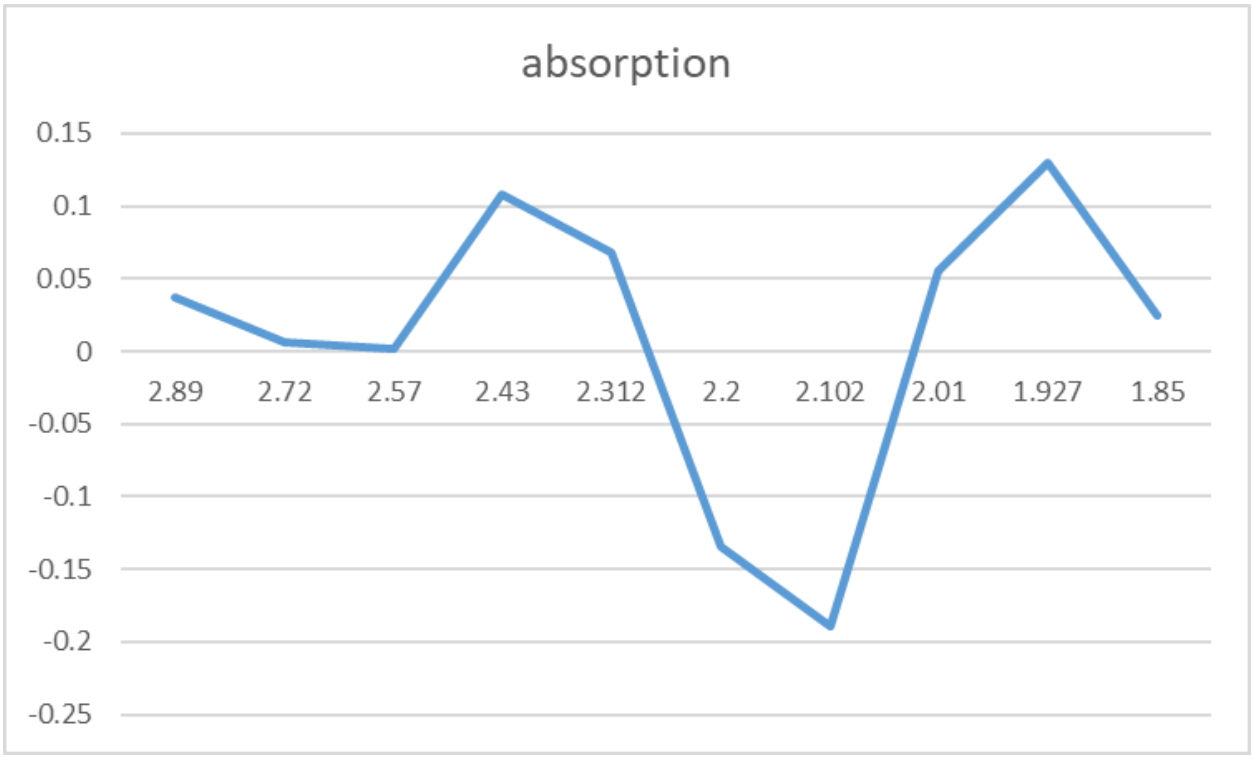
RESULTS AND CONCLUSION

Absorption peak of the chemical compound Eosin yellow is at wavelength 517 nm. Absorbance (A) at this certain wavelength is noted from each of the samples and is plotted against their corresponding Concentrations (C).
They are given as follows:

Sample	Absorption (A)	Concentration (C)
1	0.03716	(0.0.032/0.69185)× (1000/16) = 2.89
2	0.005833	(0.032/0.69185)×(1000/17) = 2.72
3	0.00197	(0.032/0.69185)×(1000/18) = 2.57
4	0.108443	(0.032/0.69185)×(1000/19) = 2.43
5	0.068485	(0.032/0.69185)×(1000/20) = 2.312
6	-0.134944	(0.032/0.69185)×(1000/21) = 2.20
7	-0.18949	(0.032/0.69185)×(1000/22) = 2.102
8	0.055654	(0.032/0.69185)×(1000/23) = 2.01

9	0.129347	(0.032/0.69185)×(1000/24) = 1.927
10	0.025064	(0.032/0.69185)×(1000/25) = 1.850

The absorption versus concentration graph is given below:



CONCLUSION

In this project, an attempt has been made to verify Beer's law by taking the measure of absorption spectrum of the sample of eosin yellow solution. But the result was not completely in favour of the aim of the project since the absorption spectrum has noise. This has occurred due to few errors such improper baseline setting in the device, high concentration, impurities in the sample and scattering. This causes unclear absorption peaks. Therefore, the corresponding absorbance extracted from the data set has anomalies. This could be rectified by careful sample preparation of sample, optimization of concentration and proper initial settings in the spectrometer.

Beer’s law plays a very significant part in industries as it is most commonly used for chemical analysis, to determine the properties, components and concentration of liquids. One of the major uses in forensic! By comparing the spectra of suspected toxins with those from the crime scene, the nature of the poison can be determined. Once the identity of the poison is determined, Beer’s law can be used to determine the concentration of poison or even any liquid in concern. It is used to find out the concentration in terms of normality of an unknown solution. There are many more applications of this law in real life and also have the potential for more applications in many more fields.

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