Analysis of the uv visible spectroscopy biology essay

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Purpose of this term paper is to describe working principle, instrumentation, data collection and data analysis of the UV-Visible spectroscopy which is also known as “ electron spectroscopy”. In working principle, Beer-lambert law correlate absorption of light to concentration of substance in solution. Parts of UV-Visible spectrometer have been described. Data analysis and data collection section describe how data collected by system and what type of information we can get from the data collected from UV-Visible spectroscopy. In the end we conclude what we learn from the project.

Introduction:

In spectroscopy, matter is been exposed to radiative energy (light, high energy electron, high energy ions etc.), at that time it can interact with matter according to atoms or molecule are present in system. Depending on its interaction with material gives different information about the matter. In short, spectroscopy is the study of the interaction between matter and radiated energy.

Absorption, emission, elastics scattering, inelastic scattering are major interaction between radiative energy and matter. In absorption, radiated energy is absorbed by matter. In emission, electron will excite and goes to higher energy level, then when it goes to ground state it will emits electromagnetic waves. When electron and radiative energy interact, but they don’t exchange energy, this type of interaction are called elastic scattering, and if electron and radiative energy exchange energy, then this type of interaction are called nonelastic interaction.

The selection of the spectroscopy to analysis the sample is depending on what is present in the sample. For example, if atoms of sample are targeted to analysis, X-ray spectroscopy and XRF (X-ray fluorescent) are useful. If molecules of sample are targeted to analysis, Infrared, Raman, visible, UV-visible spectroscopy, and fluorescence spectroscopy are useful. Raman and Infrared spectroscopy are complementary to each other. Same way, UV-visible spectroscopy is complementary to fluorescence spectroscopy. UV-Visible spectroscopy usually used for quantitative analysis of different molecule like transition metal ions, highly conjugated organic compound, and biological macromolecule.

UV-Visible spectroscopy use UV light and visible light for analysis of sample. Range of Visible light is 400nm to 800nm. Where UV light has range from 400nm to 200nm which usually used in spectroscopy. Energy associate with 800 nm visible light is 36 kcal/mole. Energy associated with 400 nm visible light is 72 kcal/mole. UV light used in UV-Visible spectroscopy is up 200 nm because smaller then this range it is difficult to handle. So, UV light which has energy less than 200 nm doesn’t use frequently. UV light which has wavelength 200 nm, energy associated with it is 143 kcal/mole.

Figure 1: Energy band diagram of molecule here it shows HOMO and LUMO (bonding and anti-bonding) energy level

Figure 1 is showing general energy band diagram which has energy level HOMO (highest occupied molecular orbital), LUMO (lowest unoccupied molecular orbital). HOMO is also known as bonding energy level. And LUMO is also known as anti-bonding energy level. In this diagram, shows some exciting possibility in molecular electron from lower energy orbital to higher

energy orbital. However, from UV-Visible spectroscopy, electron gain only enough energy to excitation from Ï€ (bonding) to Ï€* (anti-bonding) energy level or from n (non-bonding) energy to Ï€* (non-bonding) energy level. For other excitation, it will require even higher energy that UV or Visible light can provide. So, using UV-Visible spectroscopy we can measure first two left hand sides to excitation from diagram. UV-Visible spectroscopy is also known as " electronic spectroscopy" because it measure absorption of light by electron.

When sample molecules are exposed to light having an energy that equals a possible electronic transition within the molecule from HOMO to LUMO, some of the light energy will be absorbed as the electron is promoted from lower energy Ï€ orbital to a higher energy orbital like Ï€* orbital. An optical spectrometer records absorption at each wavelength and present as graph of absorbance vs. wavelength. Range of absorbance ranges from 0 (no absorption) to 2 (99% absorption) calculate by spectrometer.

Here in UV-Visible spectroscopy, Beer-Lambert law has been used to find concentration of absorbing solute in the solution. When a light passes through a solution, due to interaction with material some of the light might be absorbed and the remaining light transmitted through the solution. The ratio of the initial intensity (entering the sample (Io)) and final intensity (exiting the sample (It)) of light at a certain wavelength is defined as the transmittance (T). Most of the time it has been expresses as percent transmittance. And the absorbance (A) of a sample is the negative logarithm of the transmittance.

Equation : Beer-Lambert law which correlate transmittance to initial intensity and final intensity

Equation : Beer-Lambert law which correlate Absorption to transmittance

Here, Io is initial intensity (entering intensity) and It is final intensity (exiting intensity), T is transmittance, A is absorption.

The absorbance of a sample at a given wavelength is equal to the absorptivity of the substance, path length and concentration of the substance. Value of the absorptivity of the substance depends on the wavelength. For different wavelength, value of the absorptivity is different. The path length is the distance the light travels through the sample.

Equation : Beer-Lambert law which correlate absorptivity, path length, concentration of substance

Here, is absorptivity of the substance, l is path length; and c is concentration of the substance.

Commonly, and l are constant for experiment because depending on material is fix value and experiment length of path (l) is also fix for each experiment. So, using these equations we can calculate the concentration of substance in given sample.

Instrumentation:

Figure 2: Working principal of UV-Visible spectroscopy [3]

Ultraviolet (UV) and Vis light spectroscopy has been shown is figure-1. This device contains UV light source and visible light source, slits, Diffraction grating, filters, mirrors, reference cuvette, sample cuvette, lenses and detectors.

Light source: This device has two light sources. Depending on the sample either UV or Visible light source will be used. Using Mirrors light will be concentrated on Diffraction gritting. Usually UV light source has range from 200nm to 400nm, and visible has range of 400nm to 800nm. For UV light source, Hg bulb is used. And For Visible light source, Tungsten is uses.

Diffraction gritting and filter: Diffraction gritting converts light source into its component wavelength light. Prism can be used instead of diffraction gritting. Then Created single wavelength light is given to half mirror

Half mirror: Half mirror is special kind of mirror which can provide two same intensity output from single input sources. In our device, it will be used to provide same intensity single wavelength light to the reference cuvette, and sample cuvette which contain only solvent. Solvent can also interact with the sample. So it is necessary to measure light interaction with solvent which later can be remove from sample which contain solvent and solute to measure light interaction with solute which is area of interest.

Reference Cuvette and Sample Cuvette: In UV- Vis spectroscopy, it is very important to compare intensity to get transmission. Light is passes through the both cuvette. Absorption is done at this stage; level of absorption will depend on the sample and the reference themselves.

Lens and Detector: Lenses will be used to focus and magnify the output reference beam (I0) and output sample beam (I). Here I0 should be absorbed just little Detector will be used to detect these signals and convert into electrical signals which can be further understand using software and computer.

Figure 3: Shimadzu 1650PC, UV-visible Spectrophotometer. [2]

Data Collection [4] [5]:

Instrument was SHIMADZU UV 1601. Absorption of liquid and thin film can be measured by this instrument.

Liquid Sample

For liquid or solution, cuvette is used. It is required reference solution containing cuvette, and sample containing cuvette. In reference cuvette, it will have only solvent. Using this data, absorption for cuvette and solvent can be understood. Using data of sample containing cuvette, absorption for cuvette, the sample and solvent can be understood. Surface of cuvettes must be cleaned after filling the liquid to make sure surface does not have any dust particles.

Using software, Method is needed to be defined. In our Method, wavelength range is 300 nm to 1100 nm; scan speed is medium; sampling interval is 1 nm; scan mode is single.

A 1st need to do is measure reference (baseline, solvent only). It is also important that reference cuvette is inserted in reference stage not in sample

stage. Mounting of cuvette is very important. Cuvette has two types of surface. Cuvette has two transparent sides and two semi-transparent sides. Transparent sides need to be aligned so light beam can enter and exit from transparent sides. Load the reference sample. Define wavelength range (1100-300nm) scan in software. It will take about 2 min to finish scan for reference. It is also important to do it reference scanning process again if you change the type of the sample, cuvette or solvent.

Secondly, low concentration seldibrdge (2. 9*10^-6 mole/L) chromophore sample will be loaded in another cuvette and it will be loaded in to sample stage. In software, start scanning button is clicked. Scanning of the sample will also take about 2 min. When scanning is finished new window will appeared and will ask for file name and file description. Now on screen you will able to see typical graph absorption spectrum of " seldibrdge chromophore". Computer will assign some peaks. To access this information peak button on screen will be pressed. After that computer will provide table which include Wavelength and Absorption. This table and concentration of the sample is useful to calculate extinction coefficient or molar absorptivity. This calculation will be done by Beer-Lambert Law.

Third, high concentration seldibrdge chromophore (2. 9*10^-5 mol/L) sample will be loaded in another cuvette and it will be loaded in to sample stage. Concentration is almost 10 times more than low concentration. It will be loaded in to sample stage. In software, start scanning button is clicked. Scanning of the sample will also take about 2 min. Data can be stored by using Data Print Table extension and it will information in notepad.

Thin film

Sample is dissolved in polymer matrix and applied on glass substrate. For this process, glass substrate is used as sample. It is very important to not to touch surface of the glass substrates. It is also required to make sure it does not have any dust particles on it. Then load reference sample in reference stage and press baseline button on computer screen. After that, sample is put on sample stage. It is important to orient the sample surface side, so light can directly interact with thin film first rather than glass substrate. Then press start on computer screen. Data table and Spectrum chart will be provide by computer. Data can be saved as the above description. In thin film, concentration is unknown, so extinction co-efficient cannot be calculated. For thin film maximum absorption is important factor.

Data analysis:

Chromophore is part of molecule which is responsible for its color. So, during UV-Visible spectroscopy electrons in chromophore are interacting with light. Table 1 is giving data about different chromophore. Chromophore may be present in solvent. So, we require selecting proper solvent to measure chromophore of solute. Not all the solvent can be used in UV-Visible spectroscopy. For example, oxygen non-bonding electrons in alcohols do not give rise to absorption above 160 nm. So, we can use as solvent for UV-Visible spectroscopy. Common solvent can be used in UV-Visible spectroscopy are Hexane (alkane), ethanol (alcohol), water. However, if we used UV light which has wavelength are lower than 200 nm, then we can’t use alcohol because it create very sharp peak.

Chromophore

Example

Excitation

Î» max, nm

É›

Solvent

C= C

Ethene

Ï€__> Ï€*

171

15, 000

Hexane

Câ‰¡C

1-Hexyne

Ï€__> Ï€*

180

10, 000

Hexane

C= O

Ethanal

n __> Ï€*

Ï€__> Ï€*

290

180

15

10, 000

hexane

hexane

N= O

Nitromethane

n__> Ï€*

Ï€__> Ï€*

275

200

17

5, 000

ethanol

ethanol

C-X X= Br

X= I

Methyl bromide

Methyl Iodide

n__> Ïƒ*

n__> Ïƒ*

205

255

200

360

hexane

hexane

Table 1: Some measured data by UV-Visible spectroscopy.

As describe in introduction, using transmittance, we can calculate concentration peak. However, all the data in computer give form of absorbance vs. wavelength or É› vs. wavelength. Sometime value of log É› is

taken in place of É›. There are some graph obtain from UV-Visible spectroscopy. Both É› (molar absorptivity) and A (absorption) are changing with different wavelength. Figure-4 is absorbance vs. wavelength graph for C5H6O. Figure 5 is graph of É› vs. wavelength for different conjugate compound which has same chemical formula. In graph, each peak represents a certain excitation. Figure 7 shows two different excitation, first excitation is from Ï€ to Ï€* and second excitation is from n to Ï€*. Both excitations have pick value at different wavelength. For excitation Ï€ to Ï€*, maximum É› value is around 250 nm. And for excitation n to Ï€*, maximum É› value is around 300 nm. From figure 5 and 6 we can say that as number of Chromophore, curve shift to longer wavelength; however number of picks doesn’t change if Chromophore is same. However, for aromatics compound, as ring increase, number of pick are increasing. Due to different Chromophore present in molecule, it will effect on maximum absorption wavelength. There different terms are assigned for different type of shift which can see in table 2.

Figure 4: UV-Visible spectroscopy results for C = C and C = C Chromophore for particular chemical compound at specific pH, and solvent. [1]

Figure 5: UV-Visible spectroscopy results for conjugated compound. [1]

Figure 6: UV-Visible spectroscopy results for conjugated compound [1]

Figure 7: UV-Visible spectroscopy results for C = C and C = O Chromophore for particular chemical compound at specific pH, and solvent. [1]

pH of system is also effect on absorption peak. Diluted Copper sulfate solution is very light blue. However, if you add ammonia which will change pH more than 7, color of solution will change due to change in absorption peak and intensity. Following figure 8, is UV-Visible spectra of phenolphthalein at different pH value.

Figure 8: UV-Visible spectra of phenolphthalein (0. 103 mmol cm-3) at pH 13 solid light line, pH 9 solid dark line, pH 8 dash light line, pH 4 dashed dark line

Nature of Shift

Descriptive Term

To Longer Wavelength

Bathochromic

To Shorter Wavelength

Hypsochromic

To Greater Absorbance

Hyperchromic

To Lower Absorbance

Hypochromic

Table 2: Terminology for Absorption Shifts [1]

2

3

1

Figure 8 : Result obtain for Low concentration Liquid seldibrdge chromophore (1), high concentration Liquid seldibrdge chromophore (2), and unknown concentration of seldibrdge chromophore dissolved in polymer matrix and applied on glass substrate (3). [4], [5]

Wavelength (in nm)

Absorption

780

0. 604

510

0. 103

475

0. 101

342

0. 174

1063

. 001

540

0. 085

492

0. 097

389

0. 060

For low concentration Liquid seldibrdge chromophore, concentration is 2. 9*10^-6 mol/L. peaks and absorption has been shown in following Table.

Table 3: Wavelength and absorption for low concentration Liquid seldibrdge chromophore, concentration. [4][5]

For higher concentration liquid seldibrdge chromophore, concentration is 2. 9*10^-5 mol/L. Absorption of this sample is out of spectroscopy range. So it is required to dilute for further understanding and calculation. For unknown concentration of seldibrdge chromophore dissolved in polymer matrix and applied on glass substrate, concentration is unknown, so further calculation of extinction coefficient cannot be done, but peak absorption can be find. For this sample peak absorption is 0. 512 at 808 nm wavelength. Using equation

3, we calculated É› (extinction coefficient or absorptivity) is around 2. 1E5 1 / M * cm at maximum absorption.

Conclusion:

UV-Visible spectroscopy is good for finding concentration or molar absorptivity of of biological macromolecule, organic molecule, transition metal, conjugated organic compound.. However, we need to make sure about pH of system and solvent before taking sample analysis. Using this spectroscopy, we find molar absorptivity or extinction coefficient of 2. 9*10^-6 mol/L concentration Liquid seldibrdge chromophore for maximum absorption at given wavelength.