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EXPERIMENTS BASED ON INSTRUMENTAL TECHNIQUES HANDLING

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GENERAL INTRODUCTION RELATED TO PHARMACEUTICAL ANALYSIS

INTRODUCTION TO ANALYTICAL TECHNIQUES

IMPORTANT DEFINITION[1]

Analysis

Pharmaceutical analysis or chemical analysis a technique to identify and or quantify any sample, substance, compound by using manual, chemical or instrumental techniques by qualitative or quantitative method

Qualitative Analysis

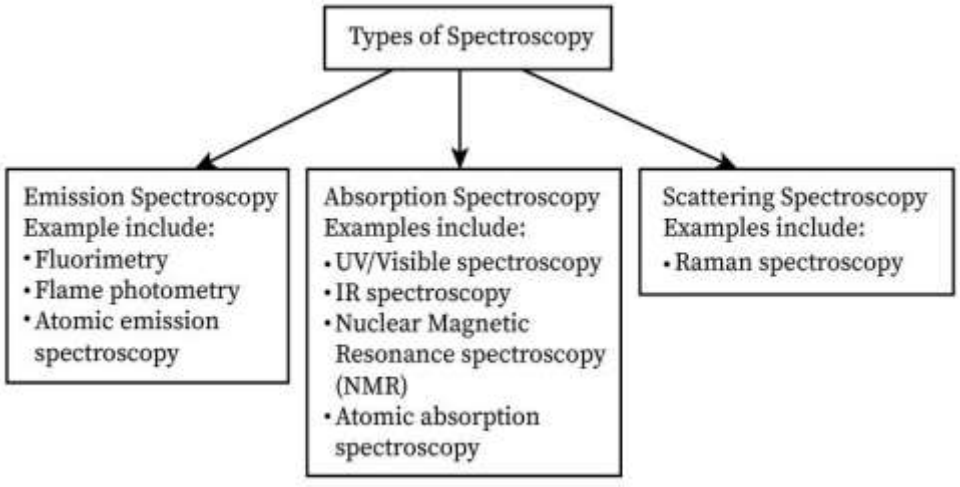
When Completely unknown sample is analysed to determine the presence or absence of the particular substance in the sample, and the technique is known as qualitative analysis. Ex. Phenolphthalein indicator gives light pink color in alkaline solution will be either present or absent.

Quantitative Analysis

Determination of the quantity in numbers weight, length or any other measurement parameter is carried out under quantitative analysis. Specific components are quantified in the sample by using quantitative ,method are available to determine the sample i.e titrimetry ,volumetry, gravimetry, thermal, electro-chemical, spectral analysis etc.

Spectroscopy:

Spectroscopy is defined as the branch of science which studies the introduction of matters with light or electromagnetic radiation, in this method electromagnetic waves of particular wavelength or range of wavelength are use to identify the qualitative and quantitative analysis of matter.



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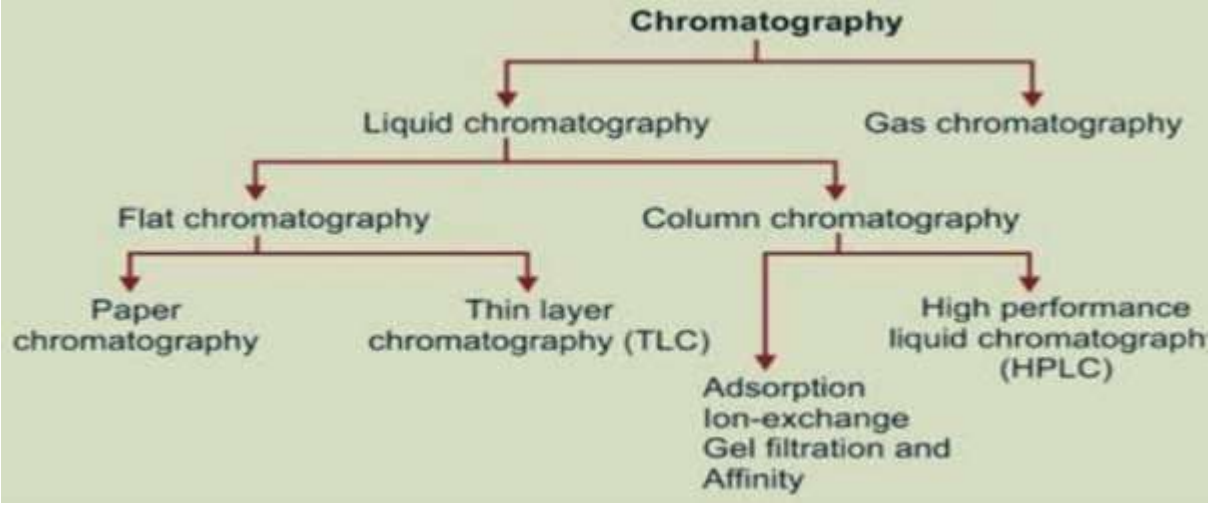
Chromatography[2]

Chromatography is a physical method for the separation of mixture components. The mixture (sample)is dissolved in a fluid called mobile phase. Which flow through the stationary phase. Chromatography can be analytical or preparative. It can't gas , mobile phase can be liquid or gas but can't be solid.



Pharmaceutical consideration in developing QA / QC systems [3]

pharmaceutical QA/QC procedures requires resources expertise and time In is expected developing any QA / QC system, Resources allocated to QC for different source categories and the completion process.

Elements of a QA/QC system

Inventory Agency

The inventory agency is responsible for coordinating QA/Qc activities for the national inventory. The inventory agency is also responsible for ensuring that the QA/QC plan is developed and implemented.

QA/QC Plan

AQA/QC plan is a fundamental element of a QA/QC system and it is good practice to develop one. The plan should in general outline QA/QC that will be implemented and include a schedule time frame that follows inventory preparation from its initial development through to final reporting in any year.

General QC Procedures

The focus of general QC techniques is on the processing, handling, documenting, archiving and reporting procedures that are common to all the inventory source categories. General Inventory Level QC Procedures, lists the general QC checks that the inventory agency should use routinely throughout the preparation of the annual inventory..

QA procedures

Good practice for QA procedures requires on objective review to assess the quality of the inventory and also to identify areas. Where third party reviewers outside the inventory agency are not available staff from another part of the inventory agency not involved in the portion of the inventory being reviewed can also fulfill QA ro QC.

Documentation, Archiving and Reporting

Internal documentation and archiving As part of general QC procedures, it is good practice to document and archive all information required to produce the national emissions inventory estimates

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Reporting[4]:
It is good practice to report a summary of implemented QA/QC activities and key findings as a supplement to each country's national inventory. However, it is not practical or necessary to report all the internal documentation that is retained by the inventory agency.

CALIBRATION OF ANALYTICAL INSTRUMENT [11]:
Introduction: Calibration is a comparison between measurements one of known magnitude or correctness made or set with one device and another measurement made in as similar a way as possible with a second device. The device with the known or assigned correctness is called the standard. The second device is the unit under test, test instrument, or any of several other names for the device being calibrated.

Calibration may be called for
- A new instrument.
- After an instrument has been repaired or modified when a specified time period has elapsed.
- When a specified time period has elapsed.
  Before and/or after a critical measurement.

Calibration of IR spectrophotometer
The calibration of IR Spectrophotometer consists of five tests. It is done in intervals of every 3 months or after every heavy usage of the instrument. The tests are as follows
1. Power spectrum. 2. Wavenumber accuracy test. 3. Resolution. 4. Wavenumber reproducibility. 5. Transmittance reproducibility.

Calibration of HPLC
1. Tests for pump. 2. Tests for autosampler. 3. Tests for detector. 4. Tests for heating system.

Preventive Actions :
1. Trending and monitoring of calibration data.
2. Minimizing the calibration intervals.
3. Minimizing the Preventive maintenance intervals.
4. Unused/ less used instruments- calibrate before use.
5. Instrument history cards to be sincerely maintained with all details.
6. Proper training on calibration/ instrument handling.
7. Analyst qualification calibration.
8. Experienced person shall be responsible for calibration and related activities.

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Experiments Based On Instrumental
Techniques Instrument handling

Potentiometer[14] 1. Before connecting the mains keep the controls as under (a) SELECTOR switch to 'O' (b) STIRRER switch to "MIN". (to its built-in "OFF" position) (c) SET POINTER to about the middle of its full range. 2.Connect the mains supply (230 Volts 50 HZ) and switch "ON" the MAINS switch. The red pilot lamp will then light up and the display will show some reading near about to Omv 3. Rotate the STIRRER control knob and adjust the stirrer speed. 4. Connect the pins of the sensors to the sockets provided on the machine on both sides of the pled rod. (Calomel to black socket) and lower the sensors with the spring clamp. So that the tips of the sensors are well immersed in the solution in the beaker. Care should be taken to see that the tips may not get smashed by the rotating magnetic rotor. Tum SELECTOR to 'POT-G' or 'POT-M' as the case may be. To 'POT-G if Class/Calomel pair is used and to "POT-M' for Metal/Calomel pair. 5.The display will now show some positive or negative reading depending on the nature and strength of the solution. Adjust it near to "O" by SET POINTER knob. Note: The instrument is provided with COARSE and FINE" SET POINTER controls. Fine control can be used to adjust the milli-volts reading precisely to O on the digital display. While coarse control is used for major change in the milli-volt reading. When "SELECTOR" is in 'O' or 'O-R' position, both FINE and COARSE "SET POINTER" will have no control on Digital display. 6. Add some drops of solution from the burette and note the reading on the digital display. Add fixed volume of solution from the burette (say Iml, 0.5 ml OR 0.1 ml) every time and note the reading of "CHANGE IN MILLIVOLTS" for each addition. It will be found that for the same addition every time the display will[15]

Conductivity meter[16] 1: Switch ON the main switch in switch board 2: Press the ON/OFF switch of the instrument 3: It will show Cell K value, Battery level and Temperature 4: It will go to 'AUTORANGING mode and show 'rAnd. 5: Then it will show the conductivity of the solution in the beaker in which the sensor is dipped (generally DM water). 6: Wash the beaker and pour the sample solution in it to measure its conductivity (ensure the sensor is dipped in the soln.). 7: The conductivity will be displayed on the screen. 8: Press "MODE' to get the values of Salinity and TDS of the soln. 9: Wash the beaker, fill it with DM water and dip the sensor in it 10: Press the ON/OFF switch of the instrument to turn it OFF Step 11: Switch OFF the main switch in switch board

HPLC[17]

- Read the SDS for all materials.
- Prepare the analyte solution in a fume hood.
- Make sure the solvent reservoirs are filled before using the instrument.
- Make sure that you are using the correct mobile phase. If you are changing the mobile phase, completely wash all lines and columns before use.
- Ensure that the pump lines have been purged of air bubbles and check the system for leaks before beginning the analysis.
- Turn on the HPLC and equilibrate the mobile phase solution.
- Make sure that the pressure is behaving normally and is well below the maximum pressure for the HPLC system.
- Constantly monitor the solvent levels in the solvent mobile phase bottles, NEVER let them run dry.
- Click the 'injection' icon button (or the equivalent on your instrument). Inject the background into the instrument.
- Once the background has stabilized, then inject your sample into the instrument.
- The HPLC column should be washed for at least 30 minutes after each run to ensure that it is properly cleaned. The procedure for washing the column and inject will vary depending on the instrument and on the sample that is being analyzed.
- Turn off the HPLC.
- Dispose of all waste in the appropriate hazardous waste containers.

FTIR 1. Switch on the FTIR, the power switch is located on the lower left side of theinstrument.2. Open the main tank valve on the nitrogen cylinder. The flow-rate should be preset to approximately 50 ml/min. To purge the sample chamber of air the nitrogen should flow for approximately 20 minutes before running a sample. 3. Press the (Proceed) button-soft key located at the top right of the FTIR control panel. 4. Log on to the computer located to the left of the FTIR. Log on as (username= analab) (password = Letmein!). 5. Start the (Spectrumv2.00) software from the desktop. 6. Insert the sample. If you are using the Durascope see the section on Durascope operation. After inserting the sample wait 5 minutes before scanning to purge the sample chamber. 7. Using the Spectrum software, go to (instrument) and click on (scanbackground). Label file as desired. 8. Save background as new stored background. 9. Click on (Print) under (File Option) menu to print the background scan. 10, Insert the sample you wish to scan in the FTIR sample holder. Close cover and wait 2 minutes to re-establishing nitrogen purge. 11. Go to (instrument) select (scan sample). 12. Name sample. Verify the (Ratio vs. File) option is selected.13. Scan sample. 14. When sample is scanned, select the background sample on the lower left hand corner of the screen. Go to (File) (Close) this will close the background

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scan and display the spectra of your sample as compared to the background. 15. Select the sample scan by highlighting the filename in the lower left hand corner of the window. Go to (View) and click on (Full Scale). 16. To label peaks go to (View) and click on (Label Peaks). 17. To remove peak labels go to (View) and click on (Clear All Peak Labels) 18. To add comments go to (View) and click on (Add/Edit Text). 19. To view peak area/ height or to change the view from absorbance to transmittance go to tools and click on (A). (T) or the peak icon as desired. 20. Print from the print icon or (File) (Print). 21. To save go to (File) (Save As). Use ASCII file format which can be converted into Excel

Interpretation of various spectra and graph

UV Spectroscopy[18]

Has been widely used for the identification and quantification of organic compounds, the aim of this text is to describe some important topics related to this theme. Both qualitative and quantitative analysis, including the direct and the derivatization procedures, are dealt with. Since, in some cases, the compounds to be analyzed are present in very low concentrations, some pretreatment sample techniques are mentioned. Additionally, different kinds of detectors used in UV–Vis spectrophotometry. (EX: RIZATRIPTAN)

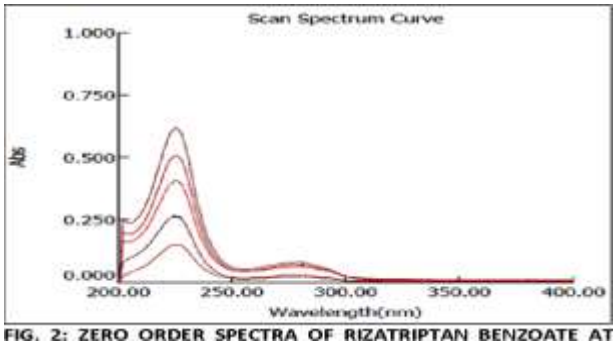
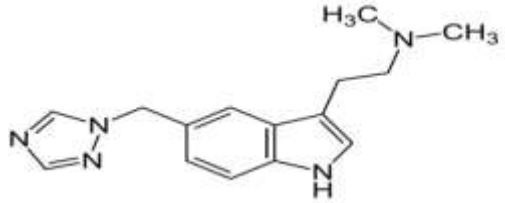


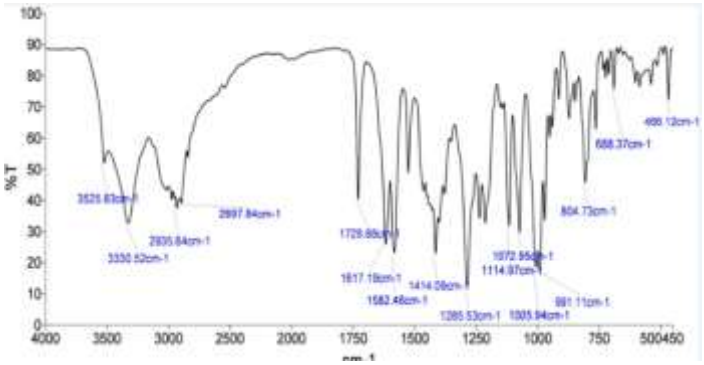
FIG. 2: ZERO ORDER SPECTRA OF RIZATRIPTAN BENZOATE AT

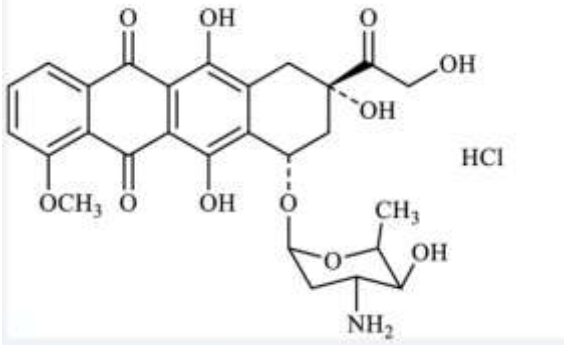


RIZATRIPTAN

Infra Red Spectroscopy[19]

Monitors the interaction of functional groups in chemical molecules with infrared light resulting predictable vibrations that provides a "fingerprint" characteristic of chemical or biochemical substances present in the sample. (EX: DOXORUBICIN HYDROCLORIDE)





DOXORUBICIN HYDROCLORIDE

NMR Spectroscopy[20]

NMR spectroscopy is a physicochemical analysis technique that is based on the interaction of an externally applied radiofrequency radiation with atomic nuclei. During this interaction there is a net exchange of energy which leads to a change in an intrinsic property of the atomic nuclei called **nuclear spin**.

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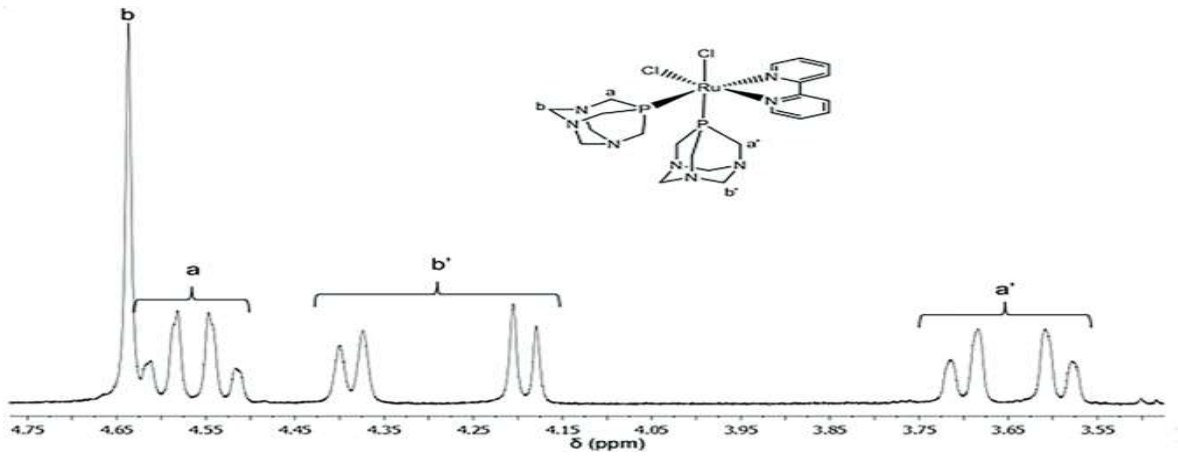
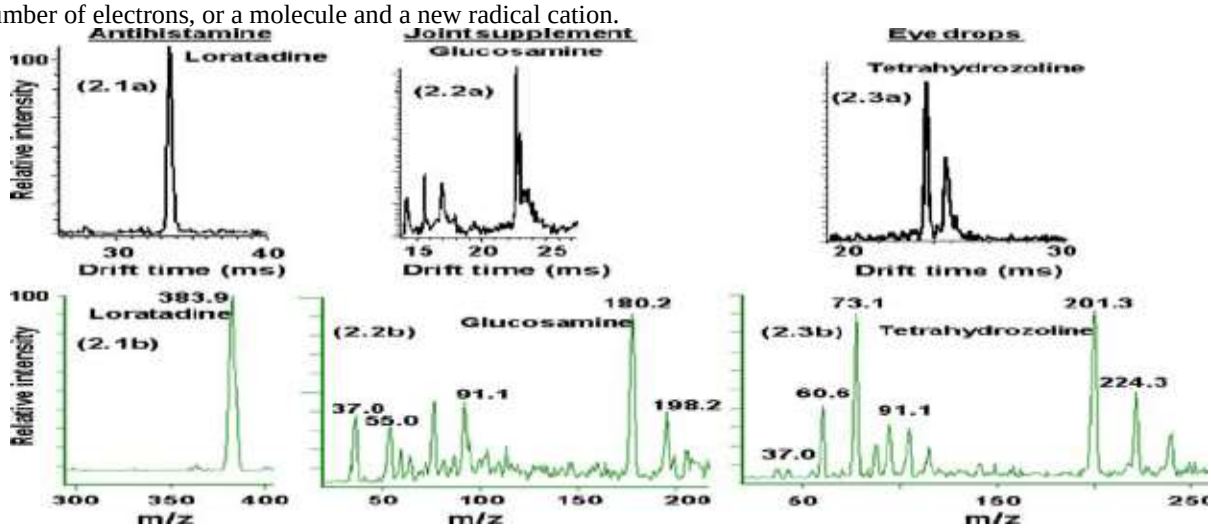


(Neutral 1,3,5-Triaza-7-phosphaadamantane-Ruthenium(II) Complexes as Precursors for the Preparation of Highly Water Soluble Derivatives.)

Mass Spectroscopy[21]

Mass spectrometry's characteristics have raised it to an outstanding position among analytical methods: unequalled sensitivity, detection limits, speed and diversity of its applications. The first step in the mass spectrometric analysis of compounds is the production of gasphase ions of the compound, for example by electron ionization: M + e− −→ M•+ + 2e− This molecular ion normally undergoes fragmentations. Because it is a radical cation with an odd number of electrons, it can fragment to give either a radical and an ion with an even number of electrons, or a molecule and a new radical cation.



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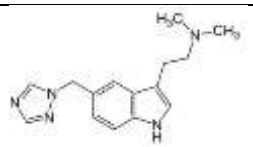
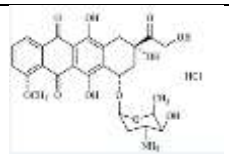
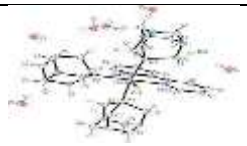
Sr. No	Instrument name	Molecular Changes	Detection (Graph)	Example	Drug Structure
1	UV Spectroscopy	Electronic transition lower state to higher state	Absorption VS Wave length	Rizatriptan	
2	IR Spectroscopy	Stretching and Bending vibration	% Transmittance VS Wave number	Doxorubicin hydrocloride	
3	MNR Spectroscopy	Change Spine Orintation	Applied radio frequency VS Absorption	Neutral 1,3,5-Triaza-7-phosphaadamantane-Ruthenium	
4	Mass Spectroscopy	Ionization and fragmentation of molecule	Reletive intensity VS Drift time	Loratadine	

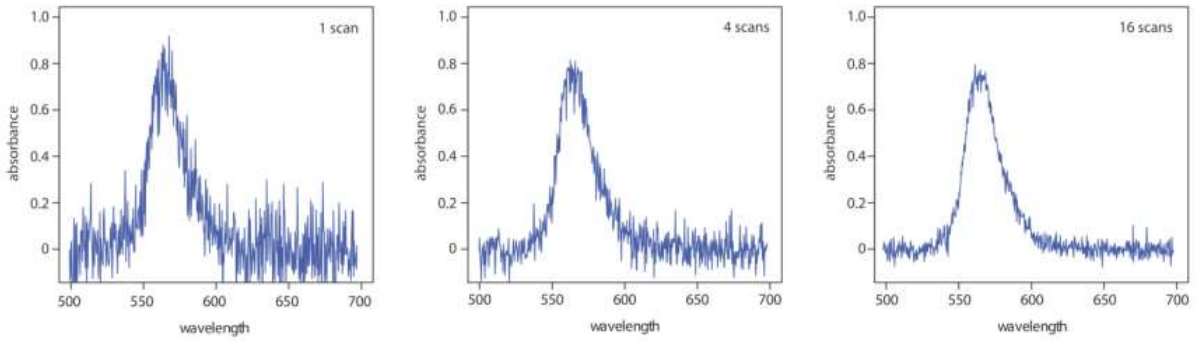
Interpretation of Result :- Structural educidation by physical method.[18, 19,20, 2]

Instrument :-

Uv-Spectroscopy Introduction:-[23] The alternative title for this technique is electronic spectroscopy, since it involve the promotion of electron of (σ π n^*) the group state to the higher energy state. Visible and ultra-violet spectra electronic excitation occurs in the range of 200-800 mμ and involve the promotion of electron to the higher energy molecules orbits.

5.1 Calibration Graph

5.1.1 Absorption vs Wavelenght Qualitative application/ absorption



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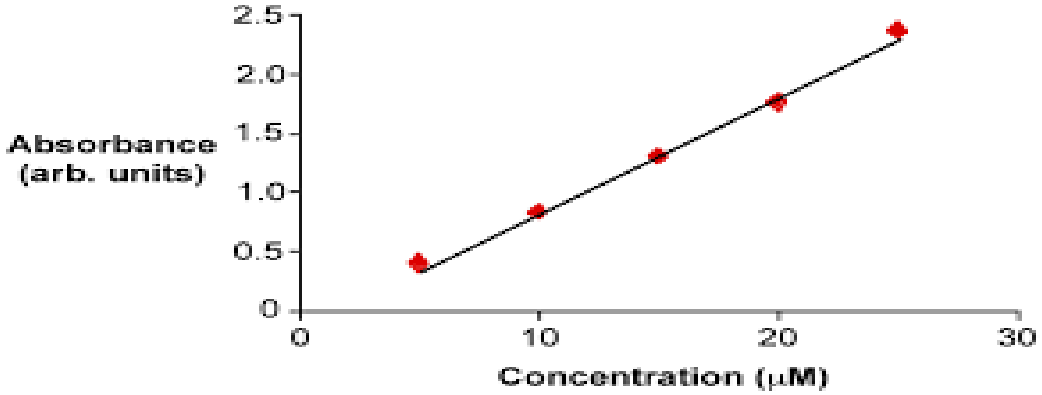
5.1.2 Absorption vs concentration:- Quantitative application. To interpret the results from AAS, a calibration graph needs to be made. A line of best fit is drawn between all the data points and is used to work out the concentration of the sample from its detected absorbance level[24].



UV visible absortion lows[25]

- Beers law absortion and concentration relation.
- Lambert law absortion and path length relationship.

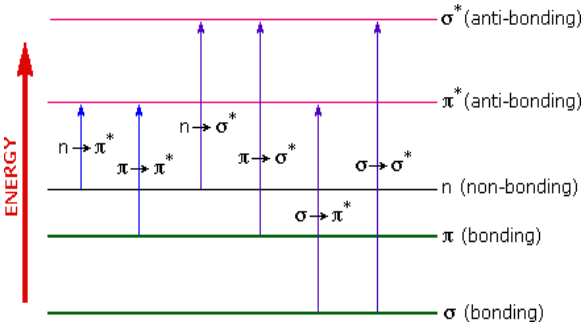
Beers law:- Whan a beam monochromatic radiation passes through a homogeneous absorption substance the rate of intensityof radiation (-DI) with concentration absortion solution is proportional to the intensity of Incident radiation.
Acc to the law = -DI/DI ∝ I. $I = I_0e^{-kc}$. $A \propto C$. $A = \varepsilon C$ A = Absortion
ε = molar extinction coefficient. C = Concetration.

5.2.2 Lambert's law:- Whan a beam monochromatic radiation passes through a homogeneous absorption substance the rate of intensity of radiation (-DI) with thickness of absortion solution is proportion to the intensity of the incident radiation Acc to this law = -DI/DI $\propto I_o$ and $I = I_0e^{-kl}$. $A \propto L$. $A = \varepsilon L$
Beers law and Lambert's law used combined from in UV spectroscopy $A = logI_0/I = \varepsilon CL$ I_0 = Intensity of incident light

I = intensity of transmitted light C = concentration of solution in mol litre^{-1} . L = Path length of the sample (usually 1 CM)

Both law combine $I = I_0e^{-kcl}$ and A=εCL

Electronic transition[26]:- A] σ $(sigma)electron$:- they required amount of energy for their excitation and do not show absorption in UV region. Absorption band is appeared in vaccum UV region. These electrons are involve in satural bond. Example saturated hydrocarbon are transparent inear UV region and thus they can be used as solvent.



B] π $(Pia)electron$:- They are found in multiple bond. They are generally mobile electron. Since π bond are weak bonds they energy produced by UV visible radiation can excite π electron to higher energy level. These electron are involved in unsaturation hydrocarbon.
C] N $(non\ bonding)$ electron:- Valance electron which do not participat in chemical bonding. In molecule are called as non bonding electron or pia electron. These are located principle in atomic orbital of N, O, S, and halogen as a lone pair of electron.

Energy required for the excitate $\sigma \rightarrow \sigma^* > n \rightarrow \sigma * > \pi \rightarrow \pi * > n \rightarrow \pi *$

$\sigma \rightarrow \sigma^*$ Note excited in the UV visible spectra because they required high amount of energy for excitation.

Chromophore[26]**:-** It is defined as any covalently bonded group that shows a characteristic absorption of electromagnetic absortion of in the UV visible region. The part of a molecule responsible for absorbing radiation is called a chromophore, and it generally include unsaturation group such as C=C C=O, N=N, -NO_2 or the benzene ring.

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Auxochrome[26]:- The colour of a molecule may be intensified by group called auxochrome which generally do not absorb significantly in the 200-800 nm region but will affect the spectrum of the chromophore to which it is attached the most and their properties are acidic(phonolic) or basic. The actually effect of an auxochromes on a chromophore depends on the polarity of the auxochromes e.g group like CH_3. In generally it should be possible to prediction the effect of non polar or weakly polar auxochromees but the effect of an auxochrome.

A] Bathochromic shift or effect or red shift Absorption maxima is shifted towards longer wavelength due to presenceof auxochrome or by the change of auxochrome or by the change of solvent. Extended conjugation and the addition of ring shift the lambda max towards longer ʎ. Trans isomer of olefin absorb at longer ʎ with more intensity than cis isomer.

B] Hypsochromic shift or effect or blue shift. Absorption maxima shigted toward shorter wavelength it may be caused by the removal of conjugation and also by changing the polarity of te solvent[4].

C] Hyperchromic effect Intensity of absorption maxima increase i.e E max increase[4].

D] Hypochromic effect intensity of absorption maxima decrease ε max decrease[4]

Absorption band[27]:- K-band, B-band, R-band, E-band.

K- band- Intensity of the k-band is usually > 10^4/10000. Occurs due to ---------- transition. Conjugation system like dienes polumers enones etc. Aromatic compound substituted by a chromophore. Affected by changing the polarity of solvent.

R-Band:- Intensity of the R-band is usually less than 100. Occurs due to---------- transition, R-band are also called forbidden band

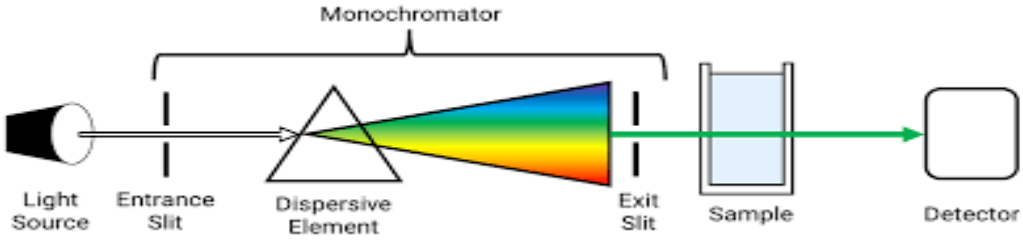
B-Band Occurs due to ------trasition in aromatic or heteroaromatic compound, Ex Benzene, Toluene, Phenol.

E-Band:- Occurs due to electronic trasition in the benzenoid system of three ethylene bond which are in closed cyclic conjugation. These are further characterized as $E_1$and E_2 bands of benzene at 184 nm and 204 nm respectively.

Transition probability:- Extinction coefficient $0.87 \times 10^{20} \times P \times A$ where P- transition probability with values from 0 to 1. A- Taeget area of the absorption system, usually called chromophore**.**

Used solvent. Water, Ether, Methanol, Chloroform, Carbone, tetrachloride, benzene, tetrahydrofuran**.**

INSTRUMENT USED IN UV VISIBLE SPECTROSCOPY[28]



Photometer:- Measured relative intensity. Illuminometer, Isolate a narrow wavelength region[6].

Spectrophotometer:- Measured the ratio, Isolate a large wavelength region have dispersing element. Commonly used 10 single beam spectrophptpmeter and double beam spectrophotometer[6].

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colorimeter:- Used visible radiation only.

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Sample cell[29]: The containers for the sample and reference solution must be transparent to the radiation which will pass through them. Quartz or fused silica cuvettes are required for spectroscopy in the UV region. These cells are also transparent in the visible region. Silicate glasses can be used for the manufacture of cuvettes for use between 350 and 2000 nm.

Applcations Of Uv-Vis Spectroscopy: UV –vis spectroscopy has many different application 1. Detection of impurities 2. Structural elucidation of organic compounds 3. Quantitative analysis 4. Qualitative analysis 5. Chemical analysis 6. Quantitative analysis of pharmaceutical substance 7. Dissociation constant of acids and bases 8. Molecular weight determination 9. As HPLC detector 10. Deviations from the Beer-Lambert law

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4. *REVIEW EDITORS Carlos M Lpez Cabrera (Cuba) and Leo A Meyer (Netherlands) Expert Group: Quality Assurance and Quality Control (QA/QC) CO-CHAIRS Kay Abel (Australia) and Michael Gillenwater (USA) AUTHOR OF BACKGROUND PAPER Joe Mangino (USA) on page no 8.8 - 8.17*
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