

LECTURE NOTES

ON

CHM 303 INSTRUMENTAL METHOD OF ANALYSIS-2UNITS

DEPARTMENT OF CHEMISTRY,

UNIVERSITY OF AGRICULTURE, ABEOKUTA(UNAAB)

Topics:

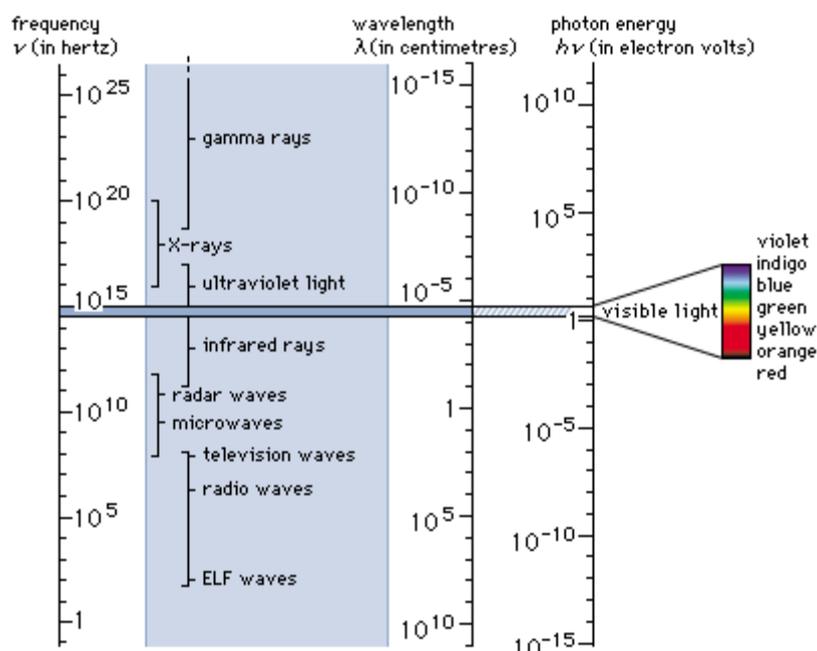
1. General Principles of Spectrometer
2. Ultraviolet-Visible Spectrophotometry

LECTURER

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GENERAL PRINCIPLE OF SPECTROMETERS

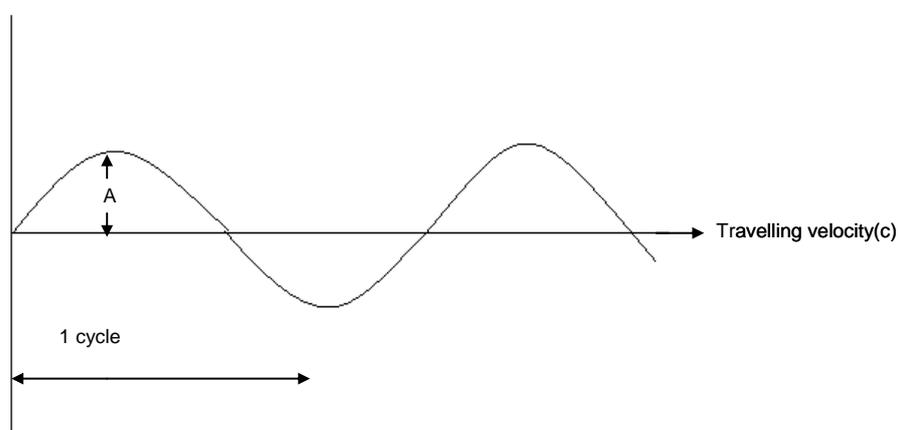
Introduction



Electromagnetic spectrum. The small visible range (shaded) is shown enlarged at the right.

Electromagnetic radiation is the type of energy that is transmitted through space at enormous velocities. It is composed of oscillating electric and magnetic fields that have the ability to transfer energy through space. It is seen as light, radiant heat, X-ray, Ultraviolet, microwave and radiofrequencies. The electromagnetic spectrum covers a range of wavelengths or energies.

$$\frac{1}{\text{Wavelength}(\lambda)} = \text{Wavenumber}(\bar{\nu}) = \frac{\text{frequency}(\nu)}{\text{Velocity - of - light}(c)}$$



Definition of Terms

Frequency is the number of cycles per seconds(s^{-1})

Wavenumber is the number of waves per cm(cm^{-1})

Wavelength is the linear distance between successive maxima or minima of a wave or distance between the peaks in cm.

Spectroscopic method of analysis is a group of techniques in which the extent of interaction between matter and emr is measured(extent of interaction and the conc of the matter).

Spectroscopy is the study of the interaction of emr with matter.

Photometry is the measurement of light using an optical filter.

Spectrophotometry is light measurement after spectral selection using a monochromator.

Spectrometer is the instrument used to measure the interaction.

Absorption spectroscopy: The atom absorbs emr to promote an electron from one energy level to the other [$E=h\nu$, $E=mc^2$, $E=hc/\lambda$]

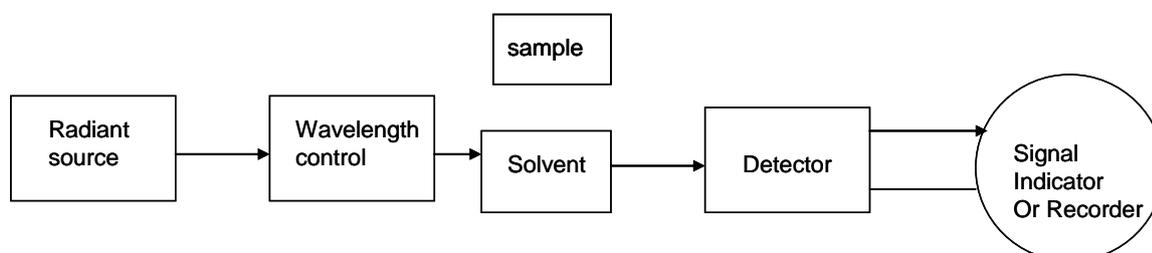
Emission Spectroscopy: When an atom absorbs a thermal energy, it is excited . emr is emitted as the electrons fall back to the ground state.

Fluorescence spectroscopy: This is a form of emission, the difference is that the original source of the emission is the emr.

Basic Components of Spectrometer

The apparatus used to accept light, separate it into its component wavelengths, and detect the spectrum is called a spectrometer. Production and analysis of a spectrum usually require the following:

- (1) a source of light (or other electromagnetic radiation)
- (2) a disperser or a spectral selection device to separate the light into its component wavelengths or select specific wavelength(a device that permits the use of a restricted wavelength region).
- (3) transparent containers for samples or solvents
- (4) a detector to sense the presence of light after dispersion. It converts the radiant energy to a measurable signal (usually electrical)
- (5) a signal indicator.



A Schematic Diagram of a Spectrometer

Source of Radiation

For Absorption and Fluorescence spectrometry, it is radiation source, while for emission spectrometry, it is excitation source.

A source of radiation must meet the following requirements;

1. have high intensity
2. stable with little or no short and long term drifts.

3. should be relatively cheap.
4. easily available
5. cover a wide spectrum

Examples:

Type of Spec.		Example of Radiation Source	Notes
Absorption	Colorimeter	Xenon lamp etc	
	Photometer	Tungsten filament lamp	The tungsten filament is an excellent source of visible and near-IR light. It operates at a temp of 2900K and produces useful radiation in the range of 320-2500nm which covers the entire Vis., parts of Ir and UV region. Do not have enough output in the mid-IR region(4000-200cm ⁻¹) to be used for IR spec. The deuterium arc lamp is employed for UV spec. Here, the electric discharge causes D ₂ to dissociate and emit UV light over the range of 160-375nm.
	Spectrophotometer UV-Vis	Deuterium arc lamp, Tungsten filament lamp	
	Infrared	Electric heating of an inert solid eg silicon carbide called Globar(heated to 150K) or Nernst glower(Zr and Yt oxides)	
Atomic absorption spectral methods	Flame and non flame AAS	Hollow cathode lamp	
Atomic emission spectral methods	Arc spec.	Sample in arc	
	Spark spec.	Sample in Spark	
	Flame emission spec.	Sample in flame	
	Atomic fluorescence spec.	Discharge lamp	

Spectral Selection Device.

The small portion of wavelength which is required for absorption by the analyte is selected from a wide range of spectrum. Examples are optical filters, prisms, diffraction gratings. Prisms and diffraction gratings are called **monochromators** and they are used to disperse light into its component wavelength. A light of one wavelength is said to be monochromatic.

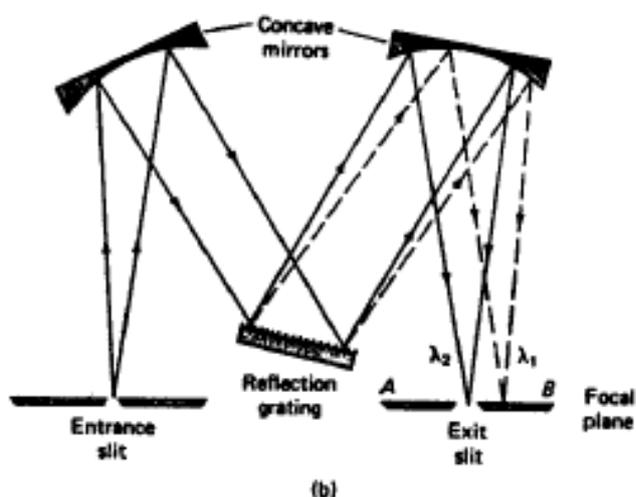
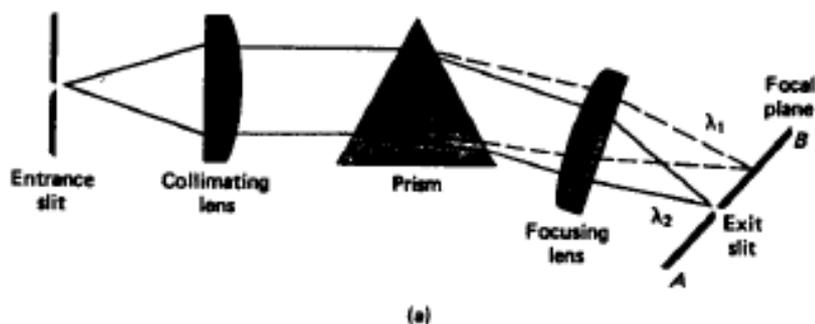
Optical filters: This function by absorbing large portions of the spectrum while transmitting relatively limited wavelength regions. It is used when the spectral purity of the radiation is not important. It is used in colorimeters, photometers, emission spec. etc. A colorimeter usually comes with a number of filters and the appropriate one is selected. The filter is normally the colour

complement of the solution of the analysis. The sensitivity of the measurement is dependent on the filter. It has the advantages in the instrument in that (1) it is very simple (2) it has a low cost (3) it has high light transmittance.

Monochromator: All monochromators contain

1. an entrance slit
2. a collimating lens or mirror to produce a parallel beam of radiation
3. a prism or grating as a dispersing element
4. a focusing element which projects a series of rectangular images of the entrance slit upon a plane surface (the focal plane)
5. Most monochromators have entrance and exit windows, which are designed to protect the components

In any type of monochromator, the width of the exit slit determines what range of wavelengths is passed on to the sample. The narrower the slit width, the narrower is the bandwidth (range of wavelength) emerges from the monochromator. Sometimes an instrument may have double monochromator that is; 2 DG or 2 P or 1P and 1 DG. FAES, Colorimeter and photometer uses optical filters. Non-flame AES uses a prism. FAAS uses diffraction grating.



Two types of monochromators: (a) Bunsen prism monochromator, and (b) Czerny-Turner grating monochromator.

- a. *Prisms*: Here, the radiation is admitted through an entrance slit, collimated by a lens and then strikes the surface of the prism at an angle. Refraction occurs at both faces of the prism. The

dispersed radiation is then focused on a slightly curved surface containing the exit slit.

The desired wavelength can be caused to pass through this slit by rotation of the prism. The spectral purity of the radiation energy is determined mainly by the dispersion character of the prisms. Prism has some limitation due to changes in ambient temperature. The angular dispersion $\frac{d\theta}{d\lambda}$ is a function of temp. When light passes through a prism the light bends. As a result, the different colors that make up white light become separated. This happens because each color has a particular wavelength and each wavelength bends at a different angle.

- b. *Diffraction gratings*: The grating is ruled with a series of closely spaced parallel grooves and coated with aluminum to make it reflective. On top of the aluminum is a thin protective layer of silica SiO_2 to prevent the metal surface from tarnishing due to oxidation which would reduce its reflectivity. One advantage of this monochromator is that the dispersion along the focal plane of the exit slit is very nearly independent of the wavelength, thus a given slit setting will provide the same band width regardless of the spectral region. Gratings give exceptionally high resolutions of spectral lines. Spectrum of white light by a diffraction grating. With a prism, the red end of the spectrum is more compressed than the violet end.

Sample Containers

The sample containers are usually called cells or cuvette. The sample container should not absorb the radiation. The most common cuvettes for measuring Vis and UV spectra are made of quartz – 1.000cm pathlength. Glass cells are suitable for Vis light measurement but not for UV spec. because glass absorbs UV light while quartz is transparent through the normally accessible UV-Vis regions.

For IR spec. with liquid samples, the cells are commonly constructed of NaCl, KBr or AgCl which transmit IR radiation. For solid sample, they are ground into fine powder, mixed with nujol(mull). The mull is pressed between two IR windows (KBr) or the sample could be mixed directly in KBr.

Since sample cells contribute significantly to the observed transmittance, qualitative spec, requires a suitable reference sample. This must be a solvent or a reagent blank containing all reagents (except the analyte) in a cell identical to the sample cell.

For atomic spec., no special sample containers are used since the samples have to be atomized. For non-flame AES, sample is placed on the electrode. For FAES and FAAS sample is aspirated. For non-flame AAS, the sample is pipetted unto heated surface.

Detector

It is used to turn a level of illumination or photons into an electrical signal so that the sample's transmittance or reflectance can be measured. A detector must have the following requirements:

1. A radiation detector must respond over a broad wavelength range
2. It should be sensitive to low levels of radiant power
3. It should respond rapidly to the radiation
4. It should produce an electrical signal that can be readily amplified
5. It should have a relatively low noise level.

There are different types: Vacuum tube type eg phototube and photomultiplier and solid state devices e.g. barrier layer cell.

Phototubes: This is made of a photocathode in the form of a half cylinder with an anode rod at its axis and it is enclosed in a glass envelope or silica. It is used in majority of single beam spectrometer since it has the advantage of (1) offering good sensitivity (2) enabling modest slit width (3) long life (4) good stability (5) requires simple electronics.

Photomultiplier: This is used in most double beam scanning spectrophotometer. It is made up of photocathode which has an additional electrode (called dynode) which is held at about 70-100V positive with respect to the cathode, which attracts the photoelectrons. The dynode is coated with antimony, cesium or beryllium-copper alloys. It emits several secondary electrons, usually four or five for each photoelectron that strikes it. These emitted electrons are attracted to a 2^o dynode with a further current increase and so on for more dynodes where even more electrons are knocked off and accelerated towards a third dynode. This process is repeated several times with the result that more than 10⁶ electrons are finally collected for each photon (photoelectron) striking the first surface.

However photomultiplier tubes are easily damaged by exposure to strong radiation and can only be used for measurement of low radiation energy. This can cause either saturation of the detector or permanent damage of either the photocathode or dynode surface. This problem can be normally avoided by the spectrometer having a solenoid or mechanically operated shutter which interrupts the light beam.

Barrier layer cells (photovoltaic cells): It is a very simple photovoltaic device which generates a voltage when its semiconductor type surface is illuminated. It is made up of a flat copper or iron electrode, upon which is deposited a layer of semiconductor material such as copper(I) oxide or selenium, with a sputtered surface coating of silver or gold which faces the illumination (acting as a collector electrode). The radiation through the very thin sputtered silver coating promotes electrons at the silver-selenium interface to a conducting state. These electrons

then migrate to the collector electrode on the silver surface a voltage is generated and the current is proportional to light intensity, hence a transmitter reading is readily obtainable.

Disadvantages: it has low sensitivity and restricted to visible region

Advantages: It has low cost, it is rugged, no external source of electrical energy is required

Thermocouple: Infrared radiation is detected by measuring the temperature rise of a blackened material placed in the path of beam. The following devices are used: thermocouple (a junction between two different electrical conductors) or a thermopile (a group of thermocouple) and a Bolometer (a resistance wire or a thermistor whose resistance varies as a function of temperature).

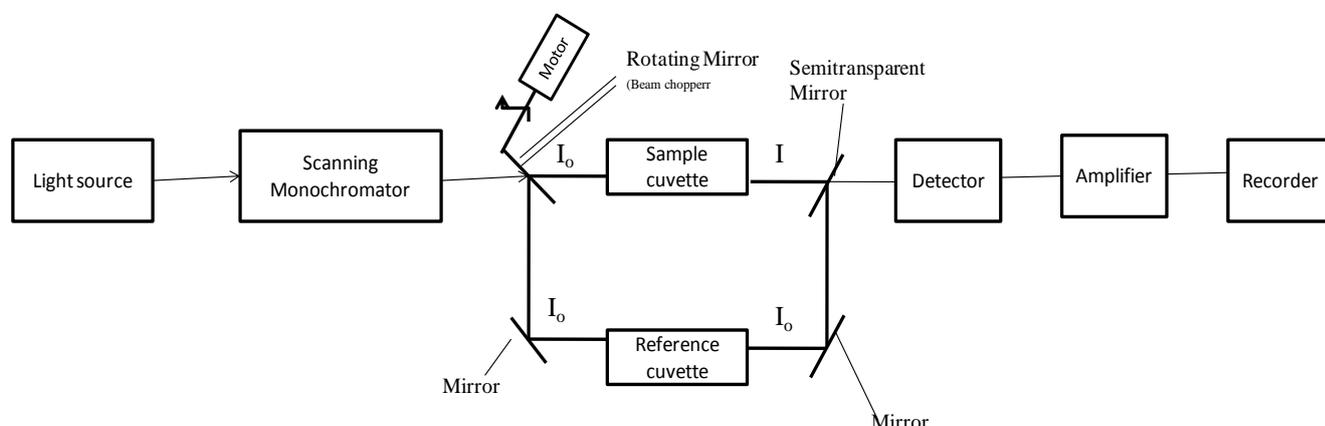
Non flame AES uses a camera- photo plate as output device. FAES uses a barrier layer cell. FAAS uses a photomultiplier.

Readout devices

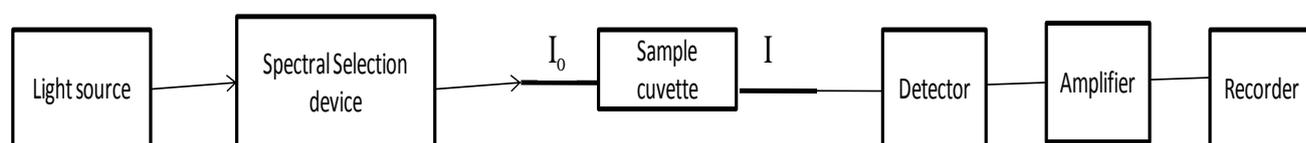
1. Analogue meter: The value that is displayed is proportional to the magnitude of signal from the detector and its proportional to the concentration of the analyte.
2. Digital meter. Show actual value
3. Chart recorder: keeps permanent records of the signals and also gives record of background noise.
4. Computer readout
5. Camera e.g. in AES.

Design of Spectrometer

It has two main designs: Single and double beam.



Schematic diagram of a double beam scanning spectrophotometer



Schematic diagram of a single beam spectrophotometer

Single beam designs are very simple and have very efficient intensity with high resolution:

In the double beam design, there is compensation for changes in lamp intensity and may require less frequent re-zeroing than a single beam instrument. However the double beam system is more complex resulting in a loss of light efficiency.

The double beam instrument makes an essentially continuous measurement of the light emerging from the sample and the reference cells. Here light passes alternatively through the sample and the reference cuvettes. This is done by a motor that rotates a mirror into and out of the light path. The incident beam is passes alternately through the sample and reference cuvette by the rotating beam.

ULTRAVIOLET-VISIBLE SPECTROPHOTOMETRY:

Principle:

When light is absorbed by a sample, the radiant power or intensity of the beam is decreased. The radiant intensity I refer to the energy per second per unit area of the beam. The light passes through a monochromator (diffraction grating) to select a wavelength. Light of this wavelength with I_0 strikes a sample of length l . The intensity of the beam emerging from the other side of the sample is I which is measured by a detector, usually photomultiplier. Some of the light may be absorbed by the sample so $I < I_0$.

Beer-Lambert's Law

The Beer-Lambert's Law states that absorbance is proportional to the concentration of the absorbing specie (analyte).

$$A \propto c$$

$$A = \epsilon c l$$

Limitations of Beer-Lambert's Law

The law works very well for dilute solutions ($\leq 0.01M$) of most substances. When solutions are concentrated, the solute molecules begin to influence each other due to their proximity, affecting their electrical properties, absorption of light, hence the graph of A against c will no longer be a straight line.

Origin of Absorption Spectra

The absorption of radiation is due to the fact that molecules contain electrons which can be raised to higher energy levels by the absorption of radiation. This is why UV-Vis spec. is sometimes called electronic spectroscopy. Electrons in a molecule can be classified in three ways;

1. Electrons with a single bond (σ bond). This needs radiations of high energy and short wavelength.
2. Electrons attached to atoms such as Cl, O or N as lone pairs. This can be excited at a lower energy and longer wavelength
3. Electrons in double or triple bonds (π orbitals) which can be excited relatively easily. They are delocalized and require less energy for excitation so the absorption rises to higher wavelength.

When a substance contains what is termed a **Chromophore**, its absorption of radiation is enhanced. A chromophore is simply "any functional group that absorbs emr whether or not a colour is thereby produced. Examples are $-C=O$, $-C=C$, $R-NO_2$, $-C \equiv N$, benzene etc.

An **auxochrome** is defined as a group that could enhance the colour imparting properties of a chromophore without being itself a chromophore. Examples are $-OR$, $-NH_2$, $-NR_2$. Their effect

is to displace the absorption maximum to a longer wavelength due to their electron-donating properties. This shift to longer wavelength is called a bathochromic shift (red shift). Hypsochromic shift (blue shift) is a shift to a shorter wavelength. A hyperchromic effect is one that leads to increased intensity of absorption. A hypochromic effect is the reverse or opposite.

Characteristics of Spectrophotometer

Spectrophotometer has the following important characteristics:

1. Wide applicability: Numerous inorganic and organic species absorb in the UV-Vis range. Also non-absorbing species can be analysed after conversion to absorbing species by suitable chemical treatments eg complexing agents.
2. High sensitivity. Analysis for concentration in the range of 10^{-4} to 10^{-5} M are common.
3. Moderate to high selectivity. Under some certain condition, it may be possible to locate a wavelength region in which the analyte is the only absorbing component in a sample.
4. Good accuracy: Here the relative error in concentration measurements lies in the range of 1 to 3%. This can be decreased to a few tenths of a percent.
5. Ease and convenience. They are easily and rapidly performed with the modern instruments

Procedure for Carrying out Spectrophotometric Analysis

Before spectrophotometric analysis can be undertaken, it is necessary to develop a procedure;

1. Selection of Wavelength
2. Variables that influence absorbance

This includes the following

- a. The nature of the solvent
 - b. The pH of the solution
 - c. The temperature
 - d. High electrolyte concentration
 - e. Presence of interfering species
3. Determination of the relationship between absorbance and concentration
 - a. Calibration Curve--- It is a way of determining the relationship between absorbance and concentration (at different concentrations)
 - b. Standard addition--- It is a way of determining the relationship between absorbance and concentration. Here standards are prepared. Increasing volume of the standards (starting from 0 mL) are added to fixed amount of the sample solution and made up to a uniform mark. This method eradicates matrix effects due to difference in standards and sample

Sources of Errors in Ultraviolet-Visible Analysis

1. Choosing the wavelength and bandwidth
 - a. Beer's law applies for monochromatic radiation. The wavelength of the maximum absorption should be used. This renders the effect of using light that is not perfectly monochromatic to insignificance, since the molar absorptivity is fairly constant over a small range of wavelength
 - b. The monochromator bandwidth should be as large as possible but small compared with the band being measured giving a smaller signal to noise ratio.
2. Instrumental errors: Reliability for most spectrophotometers are between $A \approx 0.4-0.9$.
 - a. Too low absorbance means I_0 coming through the sample is smaller to the intensity coming through the reference so large relative uncertainty in the measured difference between them. T high absorbance, too little light reaches the detector, so loe signal-to-noise ratio and and reduced precision.
 - b. Also, instrumental noise also called dark current noise occurs which is caused by factors such as thermal motion of electrons in electronic components, noise in the readout device, etc
 - c. Also, the placement and cleaning of the cuvette(fingerprints, dust etc)are large sources of error
 - d. Another error special to only UV-Vis spec. is Stray light which is light with wavelengths outside the narrow bandwidth expected from the monochromator. The source of the stray light is majorly radiation source or improperly closed instruments.
3. Others include errors arising from sample preparation, uncertainties in the wavelength setting and source fluctuations.