COURSE CODE: *CHM 303*

COURSE TITLE: *Instrumental Method 0f Analysis*

NUMBER OF UNITS: *2 Units*

COURSE DURATION: *Two hours per wee*

COURSE DETAILS:

Course Coordinator: **Prof O. Odukoya** B.Sc.,M.Sc., Ph.D

E mail: **ooodukoya@yahoo.com**

Office Location: **B215, COLNAS**

Other Lecturer(s) **Dr. (Mrs) C.A Akinremi**

COURSE CONTENT:

General Principles of Spectrometer, Ultraviolet-visible absorption spectroscopy: theory, quantitative application of UV measurement, IR spectrophotometry:basic theory, solid, liquid and gas samples, Group frequencies and quantitative uses, Molecular fluorescence spectroscopy, Atomic spectroscopy, absorption and emission, flame atomization

COURSE REQUIREMENTS:

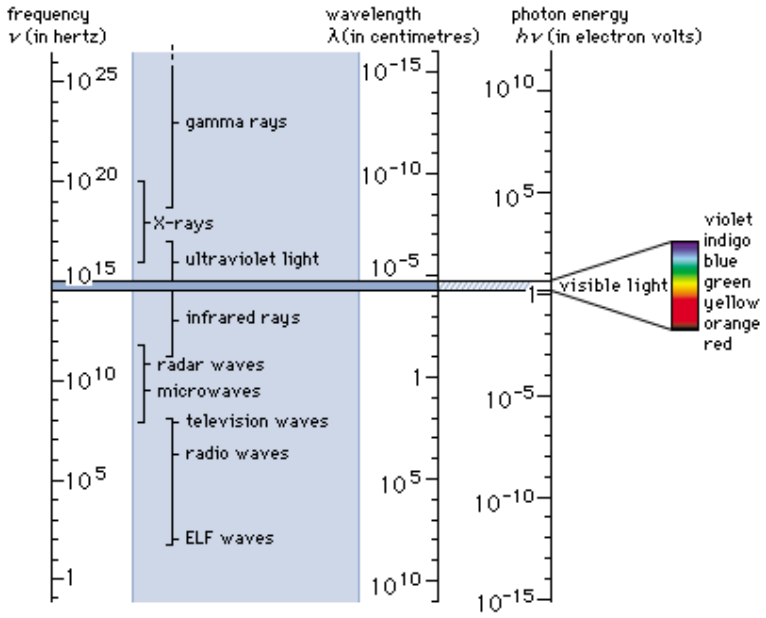
This is a compulsory course for all 300 LEVEL Chemistry students. In view of this students are expected to participate in all the course activities and have minimum of 75% attendance to able to write the final examination.

READING LIST:

G.H. Jeffrey, J. Basset, J. Mendham and R.C. Denney: *Vogel's textbook of quantitative chemical analysis* (5^{th} Ed) Longman Group, UK Ltd, UK.pp 519-779, (1989).

GENERAL PRINCIPLE OF SPECTROMETERS

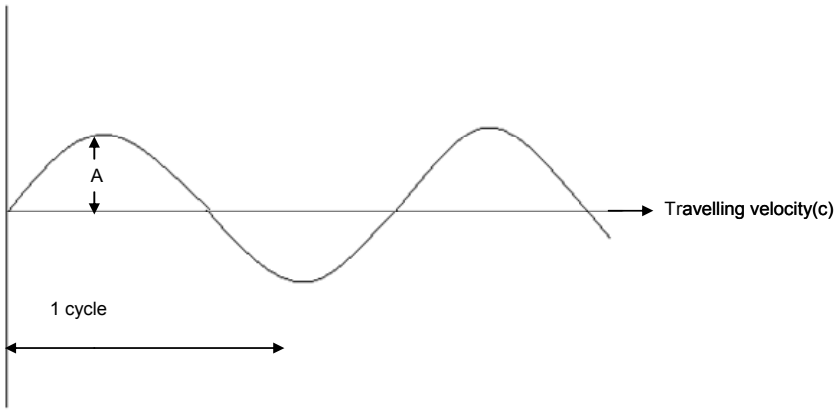
Introduction



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.

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



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 enrgy level to the other [$E=h/v$, $E=mc^2$, $E=hc/\lambda$]

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:

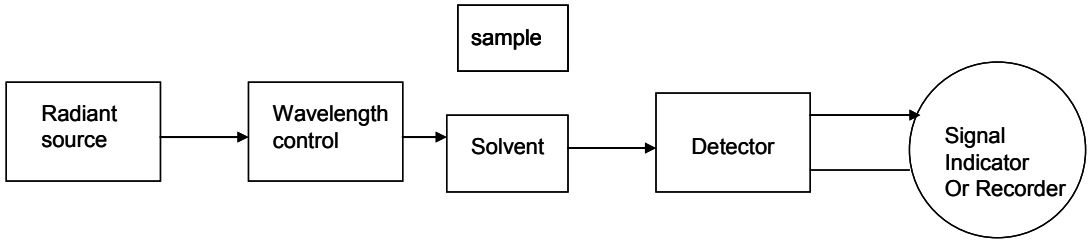
(1) a source of light (or other electromagnetic radiation)

(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 atemp 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 diperse light into its component wavelength. A light of one wavelength is said to be monochromatic.

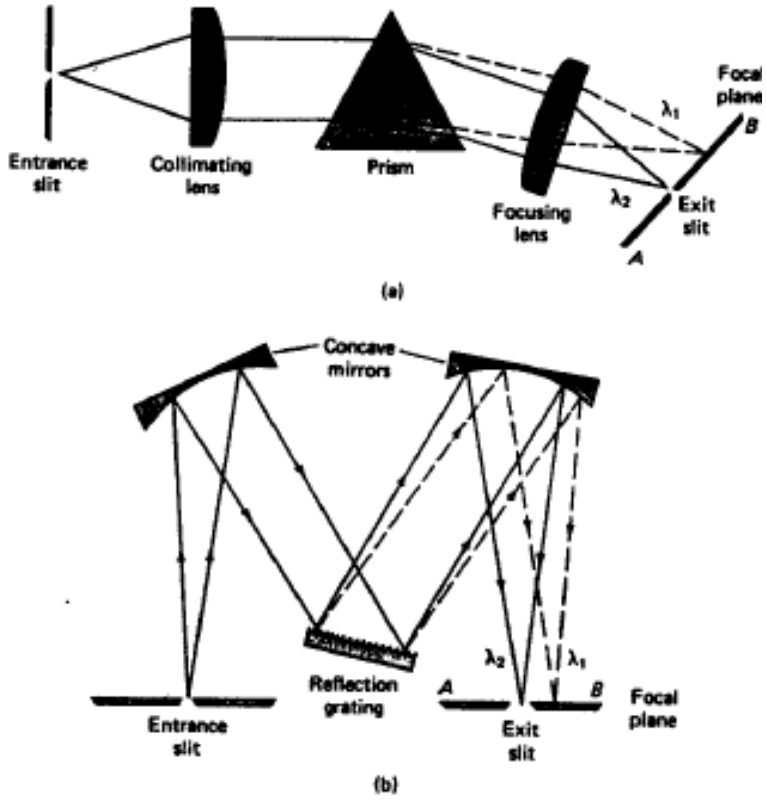
Optical filters: This function by absorbing large portions of the spectrum while transmitting relatively limited wavelength regions. It is used when the spectral purity of the radiation is not

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) Czerney-Tumer 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 $d\theta/d\lambda$ 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: T his 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 2º 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 10^6 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 coper(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 transmitor 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).

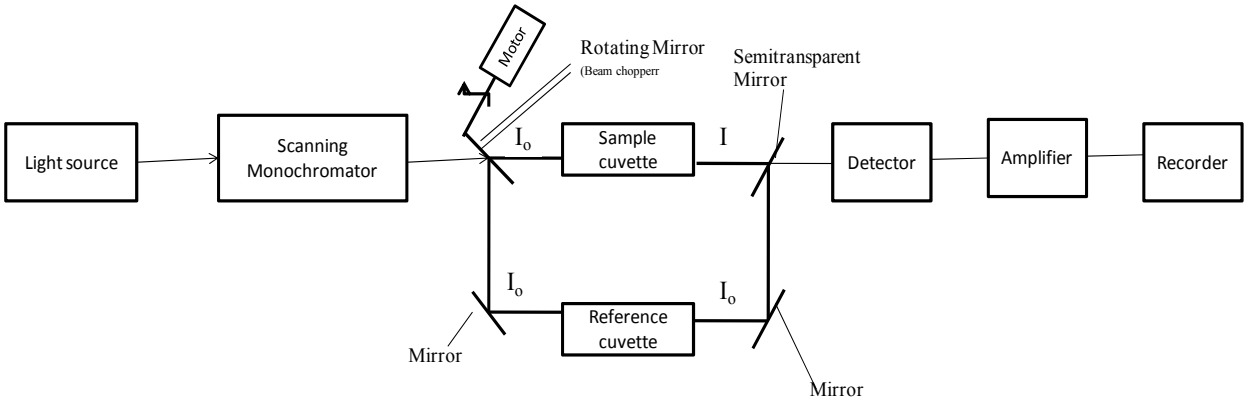
Non flame AES uses a camera- photo plate as output device. FAES uses a barrier layer cell. FAAS uses a photomultiplier.

Readout devices

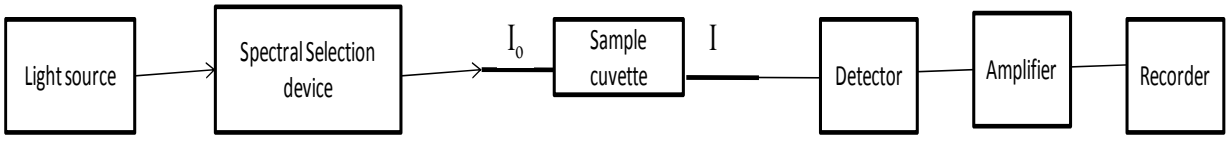
1. Analogue meter: The value that is displayed is proportional to the magnitude of signal from the detector and its proportional to the concentration of the analyte.
2. Digital meter. Show actual value
3. Chart recorder: keeps permanent records of the signals and also gives record of background noise.
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_o 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 $I < I_o$.

Beer-Lambert's Law

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

$$A \propto c$$

$$A = \varepsilon c l$$

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 absorbs 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 cmmon.
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 components 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 Carrrying 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 standard are prepared. Increasing volume of the standards (starting from 0mL) 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

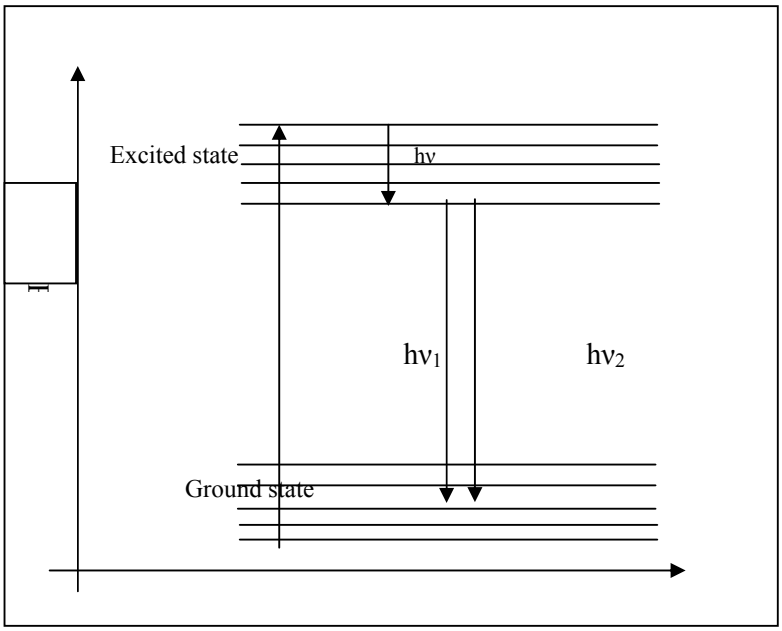
1. Choosing the wavelength and bandwidth
    a. Beer's law applies for monochromatic radiation. The wavelength of the maximum absorption should be used. This renders the effect of using light that is not perfectly monochromatic to insignificance, since the molar absorptivity is fairly constant over a 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≈0.4-0.9.
    a. Too low absorbance means I_o coming through the sample is smaller to the intensity coming through the reference so large relative uncertainity 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, uncertainities in the wavelength setting and source fluctuations.

FLUOROMETRY

Molecular Fluorescence

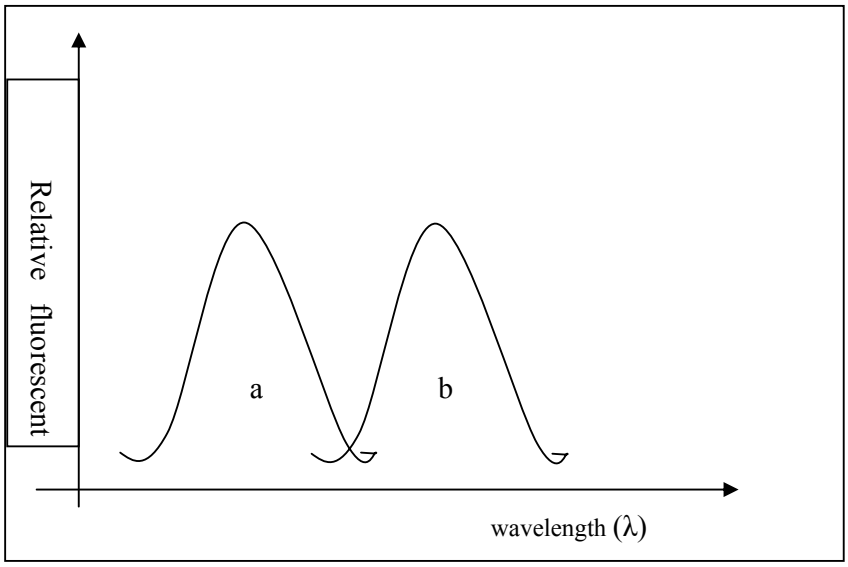
When a molecule absorbs electromagnetic energy, the energy is usually lost as heat by collision process for certain molecules, (5-10%) especially when absorbing high energy radiation like UV, only part of the energy is lost by collision; the electron then drops back to ground state by emitting a photon of lower energy (longer wavelength) than the one absorbed.

The emitted radiation is usually in the visible region and at right angles to the incident radiation; only visible rarely when absorbed radiation is of visible high energy (short wavelength) in U.V region is the emitted radiation in U.V region. Molecules at room temperature are in ground electronic state. It absorbs energy and goes to excited electronic state. The groups of lines represent vibrational state. The entire process takes place in a very short time ($\approx 10^{-12} - 10^{-9}$ s). The absorption requires about 10^{-15} s while the collision takes about 10^{-8} s.



The molecules emit energy hν by collision and drops to the lowest vibrational level of the excited state. The probability of return to ground state with emission of photon is greatest at this point. The electron thus emits a photon of energy $h\nu_2$ at a longer wavelength which is lower than $h\nu_1$ absorbed. This emitted

light is “fluorescence”. In some cases, the electron crosses over to a triplet state (i.e. it becomes unpaired) before emitting a photon. This takes a longer time $>10^{-9}$ s. the emitted radiation is then called phosphorescence. Both processes are called luminescence.



a – excitation b – fluorescent

Generally, Beer’s law applies to power of radiation transmitted by a substance or solution but modification becomes necessary for fluorescence since radiation is emitted and not transmitted

Beer’s law: $\ln T = \ln P/Po = - abc$

$A = \ln 1/T = \ln Po/P = abc.$

Fluorescence

$F = k (Po - P)$ …….. (1)

Where F is the intensity of fluorescent radiation, k is constant (quantum yield), Po is incident radiation and P is transmitted radiation.

I.e. $F \propto (Po - P)$ i.e. radiation is absorbed.

From Beer’s law,

$P = Po\ e^{-abc}$

Where Po is the power of incident radiation and P is the power of transmitted radiation.

$Po – P = Po – Po\ e^{-abc}$ …… (2)

$= Po (1 - e^{-abc})$…… (3)

$F = K (Po - P) = KPo (1 - e^{-abc})$……. (4)

From (1), if no light is transmitted, i.e. all incident radiation is absorbed,

$Fo = KPo$ …… (5)

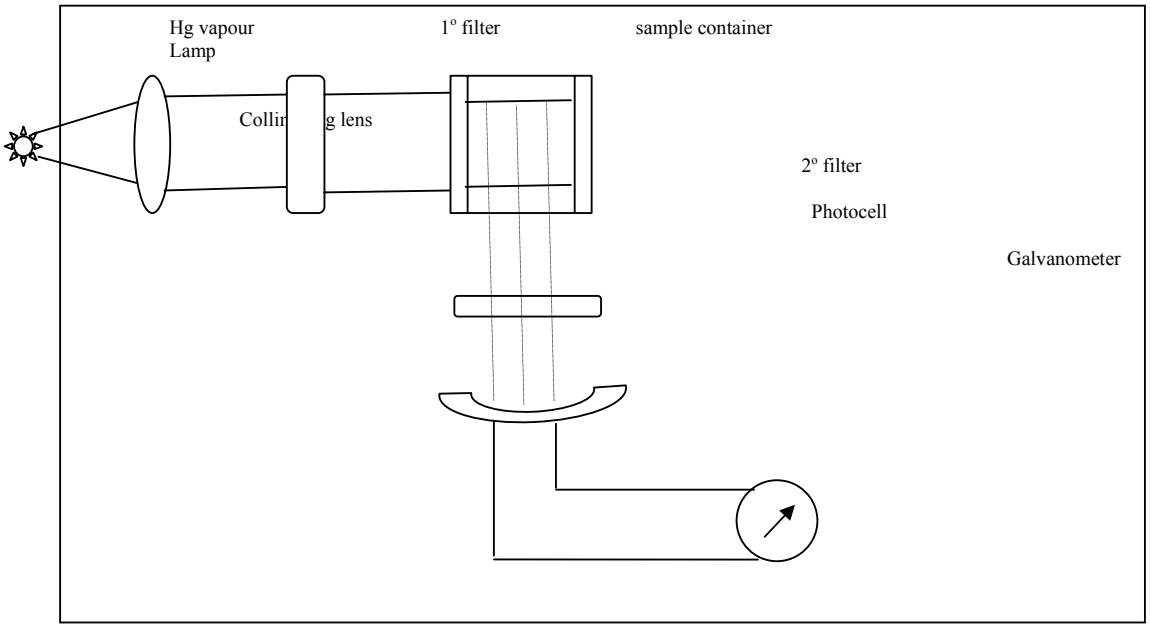
From (4), $F = Fo - Fo\ e^{-abc}$ …… (6)

$Fo - F/ Fo = e^{-abc}$

$\ln Fo - F/ Fo = -abc$

$\ln Fo/ Fo - F = abc$ ……. (7)

K is the fraction of incident radiation that is absorbed and depends on factors such as the dimensions of the light beam, area of the solution irradiated, transmission band of filter before photocell, spectral response of photocell etc. "a" is constant – absorptivity.



"b" is the path length (thickness) of cell, "c" is concentration. When abc is small and negligible compared to 1, (≤ 0.01), equation (4) becomes

$F = 2.303\ KPo\ .\ abc$ …… (8) (Proved)

$= K'C$ …… (9)

i.e. $F \propto C$ if $abc \leq 0.01$. K' is constant for a particular substance in a given instrument.

Equation (8) holds only for dilute solutions (when most of the radiation is transmitted, 92%) and breaks down at higher concentration. As usual, there should be no dissociation or association of molecules.

Chemical Structure and Fluorescence

In principle, any molecule that absorbs UV light radiation should fluoresce but not all do. The greater the absorption by a molecule, the greater its fluorescence intensity. Many aromatic and hetero compounds especially if they contain certain substituted groups. Compounds with multiple conjugated double bonds are more favourable to fluoresce. Presence of one or more electron donating group enhances fluorescence e.g. –OH, $-NH_2$, $-OCH_3$ etc. Polycyclic compounds like vitamin K, purines and nucleosides and conjugated polyenes like vitamin A are fluorescent. Groups like $–NO_2$, -COOH, $-CH_2COOH$, -Br, -I, and azo groups tend to inhibit fluorescence. The nature of other substituent may affect the degree of fluorescence. The fluorescence of many molecules is pH dependent as only the ionized or un-ionized form may be fluorescent.

A compound that is not fluorescent may be converted to a fluorescent derivative e.g. non-fluorescent steroids may be converted to fluorescent compounds by dehydrating with conc. H_2SO_4 which convert these cyclic alcohols to phenols. Similarly, dibasic acids e.g. maleic acid can be reacted with β-naphtha in conc. H_2SO_4 to form a fluorescent derivative.

White *et al* developed fluorometric methods for many metals by forming chelates with organic ligands. Antibodies can be made fluorescent by condensing them with fluorescein isocyanate which reacts with free amino groups of the proteins.

Fluorescence Quenching

Some substances quench fluoresce. These substances compete for electronic excitation energy and decrease the quantum yield (i.e. decrease the rate of conversion of absorbed energy to fluorescent radiation). I^- is a very effective quencher. I^- and Br^- substituent groups decrease quantum yield. These substances may even be determined by measuring the extent of fluorescence quenching. Some molecules do not fluoresce, whose bond dissociation energy is less than that of incident radiation. Instead of getting excited, a bond is

broken. Also, coloured species in solution with fluorescing species may interfere by absorbing the fluorescent radiation. This is "inner filter" effect. For example, in Na_2CO_3 solution, $K_2Cr_2O_7$ has absorption peaks at 245 and 348 nm, which overlap with excitation (275 nm) and emission (350 nm) peaks of tryptophan and interferes.

Limitations

This arises because fluorometry is an extremely sensitive technique and can detect at ppb level and the method is in fact limited to trace levels (a few ppm). Problems include instability of dilute solutions due to adsorption onto container surface which leads to significant errors. The problem is negligible in more concentrated solutions. Organic substances at < 1 ppm in organic solvents are adsorbed onto glass surfaces. Addition of small amount of more polar solvent may decrease it.

Quantitative Procedure

A series of standard solutions are prepared with slightly different concentration. The fluorescent intensity (power) is measured and a calibration curve is plotted. The intensity of the sample solution is also measured and concentration is read from calibration curve.

Infrared Spectrophotometry

The infrared region extends from a wavelength of 780nm to 1500nm (1.5µm) for near I.R and 1.5µm to 300µm for the far I.R, but the most useful region is from 2.5µm to 25µm which is most frequently used for analysis.

Not all molecules can absorb in I.R region but only those with a change in dipole moment (polarity) of the molecule. A diatomic molecule must have a permanent dipole although bigger molecules do not. For example, NΞ N, H-H, O=O, without dipoles cannot absorb in I.R region. C Ξ O has dipole moment and will absorb CO_2 is symmetrical and has no net dipole and not expected to absorb in I.R but by vibration, it develops dipole and absorbs. In the vibration mode (a), there exist symmetry and no absorbance while in mode (b), there exist no symmetry and it absorbs.

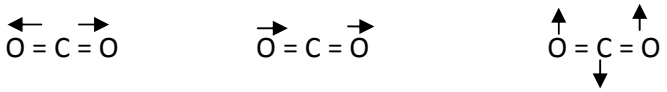
O <= C => O (a) O <=C <= O (b)

I.R. Spectra and Molecular Structure

Vibrating groups absorbing in the I.R. region do so within certain wavelength region but the exact wavelength depends on neighbouring groups. The peaks are sharper than in UV or visible regions and easier to identify. Each molecule has a complete absorption spectrum unique to it which is equivalent to a fingerprint of the molecule. Hence, the molecule can be identified.

Vibrational Transitions

Two types of molecular vibrations occur; stretching and bending, e.g. CO_2.



I **II** **III**

I and II are stretching while III is bending. I will not lead to IR absorption while II and III will. Bending may involve movement of a group of atoms within a molecule relative to the rest of the molecule. Different types of bending occur: twisting, rocking, wagging, scissoring e.t.c.

IR absorption due to II and III of CO_2 occur at "fundamental frequencies". These are frequencies at which intense absorption bands occur for complex molecules (ν_1, ν_2 etc). Less intense bands called "overtones" may occur at multiples of the fundamental frequencies, e.g. ($2\nu_1$, $2\nu_2$ etc). There could also be combination tones at frequencies corresponding to sums of fundamental frequencies e.g. ($\nu_1 + \nu_2$) etc.

Structure of Frequency

Vibrational frequency of the bond between given atoms or groups of atoms is characteristic and not affected by molecular environment. It is therefore possible to obtain structural or functional group information from IR spectra e.g. carbonyl group =C=O in alkanals and alkanones absorbs at wave number 1700 cm^{-1} or wavelength 5.9μm. Structural changes can cause minor shifts and changes in the absorption bands, which can provide additional information. Correlation is possible between absorption frequency and types of bonds or chemical groups.

There are four general regions in the IR spectrum;

1. Hydrogen stretching region (2.7 – 4.0 μm = λ). This includes stretching bands of O-H, N-H, C-H and S-H bands.
2. Triple bond stretching region (λ = 4.5 – 5.0 μm). This includes stretching bands of C Ξ C and C ΞN. Cumulated double bonds (C = C = C) also absorb in this region.
3. Double bond stretching region (λ = 5.4 – 6.4 μm). This include C = C, C = O, C = N with C = O at 5.9 μm.

Acids (-C=O-OH) and esters (-C=O-OR) absorb at lower wavelength while amides (-C=O-NH_2) absorb at longer wavelength with two peaks, hence discrimination is possible. These three regions are the "functional group" region. The fourth region is the "fingerprint" region. This is the single bond stretching and bending region. This includes not only C-H and N-H stretching and bending but also vibrations of single bonds that connect groups such as methyl (-CH_3), (-CH_2) and amine (-NH_2) groups. Absorption in this region is very much dependent on molecular environment and is unique for each molecule and leads to identification of the molecule.

IR is mainly used for qualitative analysis i.e. for identification and structural analysis. It can also be used for quantitative analysis of complex mixtures of similar compounds because some absorption peaks for each compound occur at definite and selective wavelength and have intensities directly proportional to the concentration of absorbing species.

Cells

Most common cells are cells of NaCl windows. The solvent used must not attack the windows of the cell. NaCl cells must be protected from moisture and are stored in desiccators. They require periodic polishing to remove "fogging" due to moisture condensation.

AgCl windows are used for wet samples or aqueous solutions. These are soft and must be protected from light as they slowly darken in visible light.

When samples therefore exist as pure liquid, they are run without dilution. This allows for identification or confirmation of an unknown or new compound. The cell length must be short (0.01 – 0.05 mm) to keep the absorbance within the optimum region. If solution of sample is to be prepared, a fairly high concentration is required because no solvent is completely transparent in the IR region and this keeps solvent absorbance at a minimum. So, short path length ≤ 0.1mm.

Solids may be run as suspension or thick slurry in a viscous liquid having approximately same refractive index to reduce light scattering. The sample is ground in the liquid, usually Nujol (a mineral oil). If Nujol masks any C-H band, then chlorofluorocarbon greases are used. This method is only for qualitative work since the slurry is difficult to reproduce for quantitative work. Alternatively, the sample may be ground with KBr (transparent in IR region) and pressed into a pellet for mounting.

For gases, a long path length (≈10cm) is required.

ATOMIC SPECTROMETRY

Emission

The principle depends on measurement of emitted radiant energy from sample solution aspirated into the flame.

The instrument consists of the nebulizer, the burner, the optical system, detector, amplifier, scale or recorder.

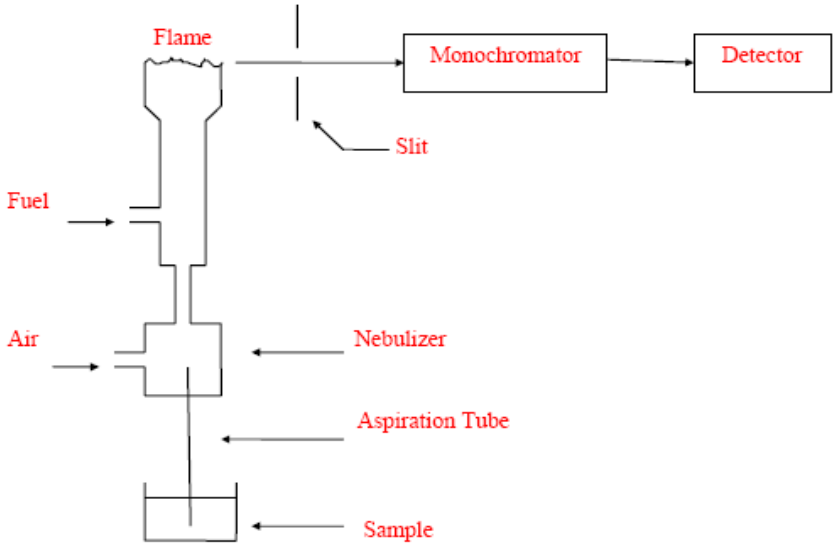
Nebulizer: This breaks the solution into a fine spray and introduces the spray into the flame at a stable and reproducible rate. It must not be attacked by corrosive solutions and must be easily cleaned. It is usually made of plastic.

Burner: It must provide a steady flame.

Optical System: This collects light from the steadiest part of the flame, renders it monochromatic and focuses it onto the surface of a photosensitive detector. It consists of a concave mirror placed behind the flame with its centre of curvature in the flame thereby almost doubling the intensity of emitted radiation. For simple routine use, absorption filters used for elements with simple spectrum and in absence of background emission from flame transmits wide band as it cannot absorb radiation close to analytical line. Interference filters are better but monochromators containing quartz prism or diffraction grating and narrow slit-width, with very sensitive detecting circuits and amplifiers are best.

Flame Photometer

A simple flame photometer consists of a nebulizer, flame, lens, a screen with a slit, filter, detector and galvanometer.



Air is drawn in at a given pressure and passed into a nebulizer where it creates a partial vacuum resulting in suction which sucks in the sample solution as a fine spray into a small mixing of the chamber at the burner. Here it mixes with the fuel gas at a specific pressure into the flame. Emitted radiation from the flame passes through a lens which renders it parallel and through a slit to produce a narrow beam. It then passes through a filter which allows only the line of the test element to pass through to the detector (photocell) and a galvanometer which gives the reading. The flame is surrounded by a chimney to protect it from drought. It is primarily used for analysis of sodium, potassium, calcium and lithium.

Flame and Flame Temperature:

- It transforms sample from liquid or solid state to gaseous state.
- It decomposes molecular compounds into simpler molecules or atoms.
- It excites atoms to emit radiation.

Gases in flame are CO, H_2, CO_2 and H_2O (and N_2, if air is one of the gases), with smaller quantities of H, O and OH

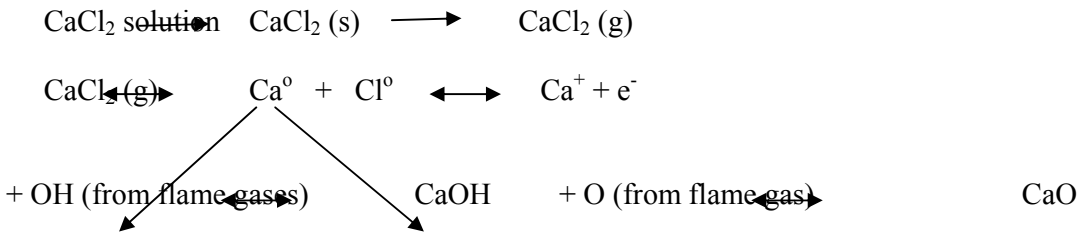
Temperature of flame depends on fuel gas and supporter.

Fuel gas	**Air**	**Oxygen**
Illuminating gas	1700	2700
Propane	1925	2800
Butane	1900	2900
Hydrogen	2100	2780
Acetylene	2200	3050
Cyanogens	2330	4550

Emission Spectra

On aspiration into flame following in rapid succession;

- Water or solvent vaporized, leaving minute particles of dry salt.
- Salt vaporized; part or all gaseous molecules dissociated into ground state atoms.
- Some atoms combine with radicals or atoms in flame gases.
- Vapours of ground state metal atoms or molecules containing the atoms absorb energy from flame – excited. Some ionization may occur.
- Species return to ground state – emit excess energy. E1 – E2 = hv. Return may be in one step or in several steps. Most prominent line is equivalent to the lowest excited level and ground state.



Applications

Simple flame photometers using butane air flame with element filters are used to routinely determine easily excitable elements, K, Na, and Ca – elements with low ionization energies.

However, with hotter flames like oxyacetylene flame and use of spectrophotometers and very narrow slit widths, more elements (up to 70) can be determined.

Spectral Interference

In relatively cool flames, refractory molecular oxides and hydroxides form. Molecules have energies and energy levels of rotational, vibrational and electronic excitation. Each electronic transition is

accompanied by a whole lot of vibrational and rotational ones - a broad emission spectrum rather than a narrow band. The bands interfere with and make measurement of analytical line difficult and inaccurate if adjacent to or overlapping analytical line. Examples of such molecules are CaOH, SrOH, BaOH, MnOH, CaO etc. Also, background emission from flame due to –OH, CO, O_2, CH, C_2, and H_2O.

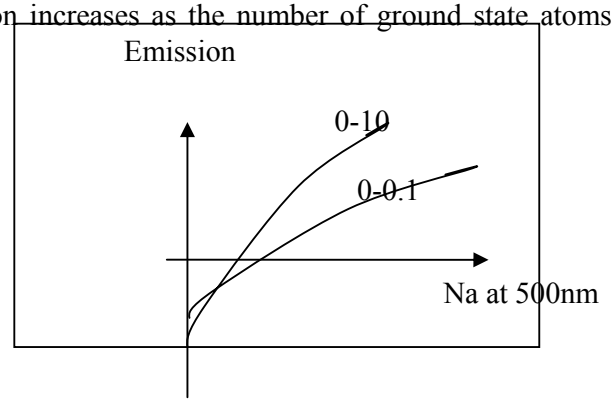
Usually prevalent with filters and reduced by monochromators. If improved, resolution cannot correct it, another line must be found.

Background Emission

This is emission both from flame and sample matrix. This must be corrected to avoid serious errors. If only from flame gases, aspirate pure solvent to zero. If monochromator, used, background measured in presence of test element and subtract background signal.

Self – Absorption

Excited atoms release excess energy in discrete amounts. The radiant energy travels some distance in flame before getting out. Collides with other ground state atoms and get absorbed which leads to decrease in signal. Self – absorption increases as the number of ground state atoms in flame increases. Work at low concentration.



Ionization

Some atoms ionized rather than being excited if flame is hot enough. Increase in the number of atoms and decrease in signal.

$\longleftarrow M \longrightarrow \quad Mn^{+} + ne$

Add a second easily ionizable element e.g. Na or K. Excess electrons drive equilibrium to left hand side. More metal atoms, higher signal. This is more easily observed in acetylene-air or oxy-acetylene flames.

Acetylene air

Effect of Na on K emission

	+20 ppm Na	+100 ppm Na	+1000 ppm Na	+2000 ppm Na	+5000 ppm Na
K 5 ppm	+17	+56	+92	+96	+97

Add large amount of easily ionizable element to standardize the sample.

Effect of Anions (Refractory Compound Formation)

Some anions from acids or salts depress signals of metallic emission. Significant above 0.1M H_2SO_4, HNO_3 and in particular H_3PO_4 are very prominent. For example, Ca and other alkaline earth metals depressed by $PO4^{3-}$ and Al_2O4. The compound formed is refractory and does not volatilize or decompose.

Use releasing agent e.g. La^{3+} or protective chelation – polyhydroxy alcohols (glycerol) or EDTA.

Procedure for Analysis

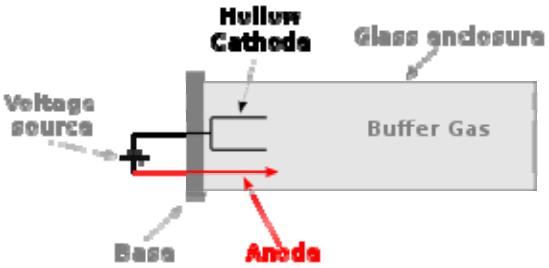
Calibration curve and standard addition.

ATOMIC ABSORPTION SPECTROPHOTOMETRY

Principle

Measurement of light absorbed at wavelength of a resonance line by unexcited atoms of element. It is useful for elements which cannot be excited by flame. It can also be used for some excitable ones since about 99% of all atoms remain unexcited in normal air – acetylene flame. The flame is like a trough or sample cell of the absorbing gas and the absorption follows Beer's law i.e. proportional to path length of flame and concentration of atomic vapour in flame.

Instrumentation



Source

This provides the resonance line of element. It is usually a hollow cathode lamp which emits the specific monochromatic wavelength.

Cylindrical hollow cathode made of the element to be measured or an alloy of it. Anode is tungsten. It is enclosed in glass tube with quartz window (most wavelengths in UV region) reduced pressure and filled with inert gas, Ar or Ne. high voltage across tube, electrons released by anode, ionize gas , positive gas ions accelerated to cathode. They bombard the cathode, cause metal to sputter and vapourise. Metal atoms excited by collision with more ions. They return to ground state and emit characteristic wavelength. Filler gas also emits line but not close enough to interfere. It is passed through flame and get absorbed. Most absorbed is usually but not always the resonance line. This is equivalent to transition from ground state to lowest excited state.

Multi element Lamp

These are alloys of several elements. They emit lines of all the elements. They are used for two or three elements but have shorter life span. Radiation lines from hollow cathode lamp narrower width than absorption line of atom in flame.

Burner and Nebulizer

Nebulizer breaks the solution into a fine spray and introduces the spray into the flame at a steady and reproducible rate.

There are two types of nebulizer:

1. The one with total internal combustion or direct aspiration; aspirates all the solution into the flame.
2. The one with premix chamber in which the solution goes through a chamber where the large drops are removed and only the fine droplets mix with the flame gases and go into the flame.

The advantages and the disadvantages are as follows.

Total Internal Combustion

Disadvantages

a) It has a shorter path length.
b) Large droplets are not completely vapourized; leaves solid particles in light path which scatter light which is recorded as absorption (error).
c) Nebulization efficiency is greatly affected by viscosity of sample.

Advantages

a) Absorption is proportional to gas flow than in pre-mix.
b) Viscous liquid and high solids can be aspirated.

Pre-mix

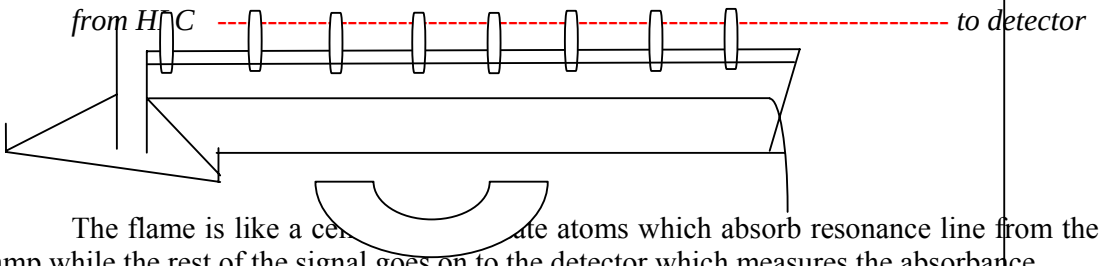
Advantages

a) Fine droplets which are easily vapourized.

b) Nebulization efficiency is greater.
c) Path length is longer.
d) Combustion is very quiet while total internal combustion is noisy.

Therefore, pre-mix is better.

Burner head, mostly longer than optical path length.



The flame is like a cell containing ground state atoms which absorb resonance line from the hollow cathode lamp while the rest of the signal goes on to the detector which measures the absorbance.

Flames are the same as emission.

Oxy – acetylene 3060^{o}C

Nitrous oxide-acetylene 2955^{o}C. They are the highest used.

Interferences

1. **Spectral Interference**

Similar to emission. Refractory molecular band emission with d.c. source but is eliminated with a.c. If molecule absorbs source radiation, positive interference in AAS is minimized by using line source. Light scatter by solid particles result to positive interference especially less than 300 nm with high salt solution. Measure absorbance at a line close to line of element to get background absorption – subtract, since interference over broad area.

2. **Ionization Interference**

Similar to emission.

3. **Refractory Compound Formation**

Similar to emission.

Compounds formed with flame gases e.g. MO, MOH etc. use hotter flames to decompose e.g. nitrous oxide-acetylene or air-acetylene.

Application

Get solution of sample. If interferences absent, chemical form does not matter. Hence, it is used in biological samples blood, urine, csf etc. Aspirate directly or after suitable dilution into flame to prevent clogging of burner.