# Instrumental Methods of Chemical Analysis

**Introduction:** It is well known that Analytical Chemistry is the science, which deals with methods for determining the chemical composition of samples of matter (elements or compounds). This may be achieved either by classical or instrumental analytical methods.

**Classical methods of chemical analysis.** These methods involve separating the components in a sample by precipitation, extraction or distillation. In **qualitative classical methods**, the separated components treated with reagents can yield products recognized by their colors, boiling points, melting points, solubility's in a series of solvents, odors, optical activities or their refractive index. In **quantitative classical methods**, the amounts of components are determined by gravimetric or titration methods. In **gravimetric analysis,** the mass of components is determined**.** In **titrimetric analysis**, the volume or mass of a standard reagent, required to react completely with sample components is measured.

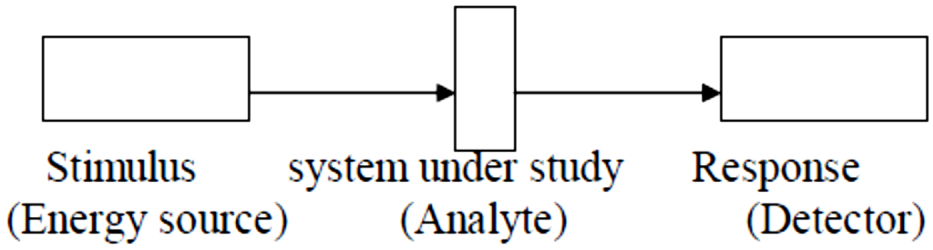
**Instrumental methods of chemical analysis.** In these methods other phenomena than those used for classical methods are exploited for solving analytical problems. Thus, measurement of physical properties of analysts such as- conductivity, electrode potential, light absorption or emission, mass-to-charge ratio and fluorescence, began to be used for quantitative analysis of a variety of inorganic, organic and biochemical analysis. Furthermore, highly efficient chromatographic techniques began to replace distillation, extraction and precipitation for the separation of components of complex mixtures prior to their qualitative and quantitative determination. These newer methods are called **Instrumental methods of chemical analysis**. The most characteristic properties that are used for instrumental chemical analysis are listed in table (1).

In addition, there is a group of instrumental methods used for separation and resolution of closely related compounds called chromatography. The methods listed in table (1) are used following chromatographic separations.

Table (1) Chemical and Physical properties Employed in Instrumental Methods

| Characteristic property | Instrumental methods |
|---|---|
| Interaction with radiation | Spectroscopy methods (UV visible, IR, x-ray and NMR spectroscopy, -- etc.) |
| Electrical | Potentiometry, conductometry, - -etc. |
| Mass-to-charge ratio | Gravimetry, mass spectrometry, - -etc |
| Rate of reaction | Kinetic methods |
| Thermal characteristics | Thermal gravimetry TG, Differential thermal analysis DTA, differential scanning calorimetry DSC, - -etc. |
| Radioactivity | Activation and isotope analysis methods |

**Instruments for chemical analysis**. Generally**,** instruments for chemical analysis comprise three basic components:




1-The stimulus (energy source), usually in the form of electromagnetic,electrical or thermal energy
2- System under study (analyte)
3- Response (detector) that converts changes in properties of the analyte to a number that is proportional to the relevant chemical or physical occurred through interaction with the stimulus.

4- Readout system: meter, plotter or computer.

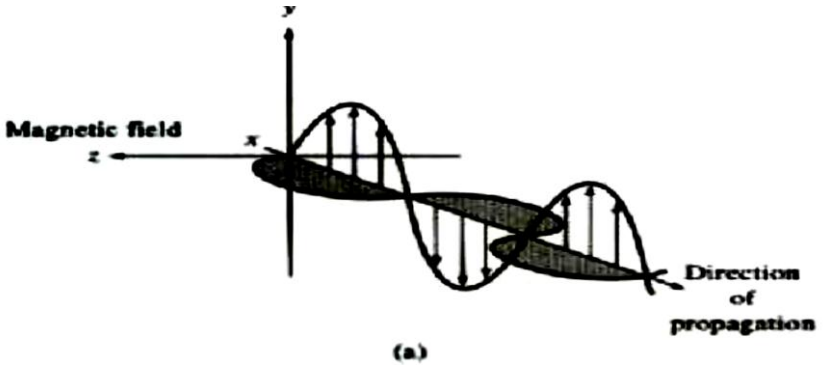
The detection system converts information to electrical signals by photodiodes, photomultipliers, - etc. Then the electrical signal converts information to numeric or graphic output of a photographic plat, recording paper, or computers. The information appears as the blackening of a photographic plate, a tracing on a recorder or computer output. Most modern analytical instruments contain computers. The intensity of light is determined before and after its interaction with the sample, and the ratios of these intensities (I/Io) provides a measure of the analyst concentration.

## Optical Methods

A major class of analytical methods is based on the interaction of electromagnetic radiation with matter. The most widely used spectroscopic methods are based on electromagnetic radiation, which is a type of energy that takes several forms. The most readily recognizable radiations are light and radiant heat.
In fact, the spectroscopic methods that employ not only visible but also gamma rays and x-rays as well as ultraviolet, infrared, microwave and radiofrequency radiation are often called *optical methods* despite the fact that the human eye is sensitive to neither of the later types of radiations. This might be due for the similarities of the ways of interaction of these types of radiation with matter.

### Nature of electromagnetic radiation

Any wave is essentially just a way of shifting energy from one place to another. Relatively small local movements in the environment transfer the energy. The energy of radiation travels because of local fluctuation changes in electrical and magnetic fields- hence electromagnetic radiation.
In contrast to other wave phenomenon, such as sound, electromagnetic radiation requires no supporting medium for its transmission and thus passes readily through a vacuum.
As indicated in figure ( a) and as the name implies, an electromagnetic wave has an electric component and a magnetic component. The two components oscillate in plane perpendicular to each other and perpendicular to the direction of propagation. Only the electric component is active in ordinary energy transfer interaction with matter.
Henceforth, in our discussion of wave behavior, we will consider only the electric component. Figure ( b) is a two dimensional representation of the electric component of the ray.



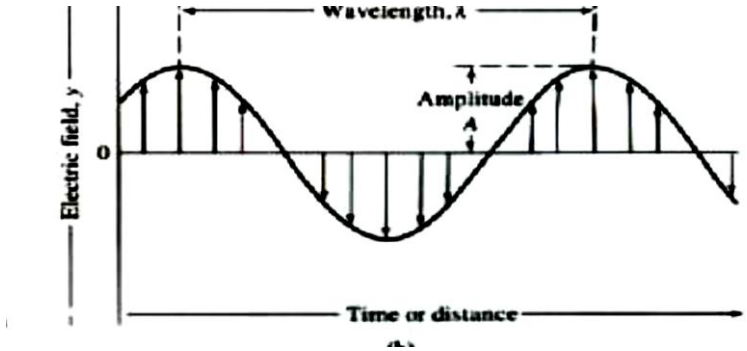
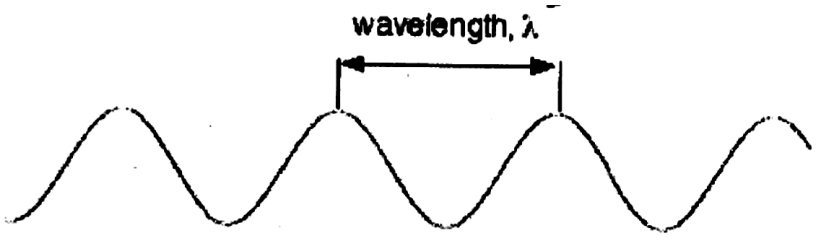




Fig . 1 An electromagnetic wave

Radiant energy can be described in terms of three parameters:

Wavelength (λ) is the linear distance between any two equivalent positions on the wave (e.g., successive maxima or minima). The units of wavelength are the micrometer (1 μm = $10^{-6}$ m), usually called micron. The unit widely used in spectroscopy is the nanometer ( 1 nm = $10^{-9}$ m ) or angstrom (1A = $10^{-10}$ m).




Wave number ($\bar{\nu}$) is the number of waves per unit distance ($\bar{\nu}$ = 1/ λ )
The unit most commonly used for wave number is the reciprocal cm (cm-1).

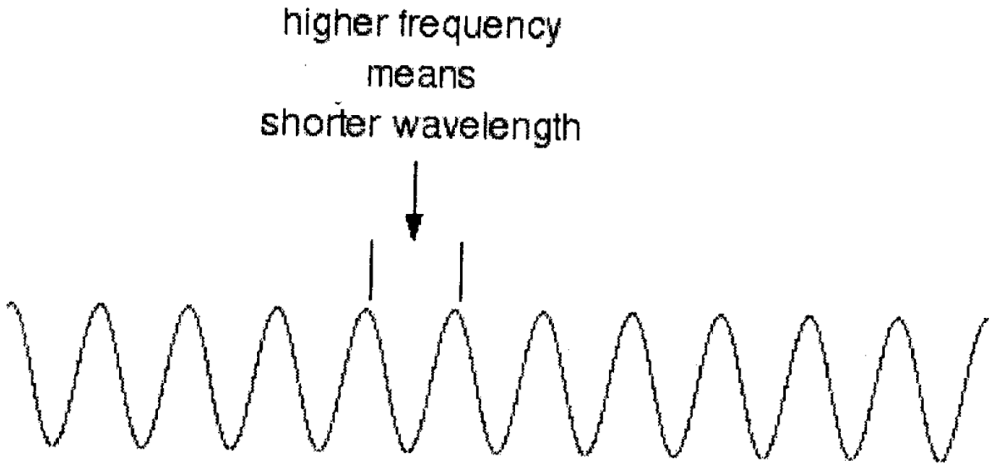
Frequency (ν) is the number of complete wavelength units which pass a fixed point per unit of time. The units of frequency are cycles per second ( $s^{-1}$) or Hertz (Hz).

Light has a constant speed through a given substance, e.g., it always travels at a speed of approximately $3\times10^8$ m/sec in a vacuum. This is actually the speed that electromagnetic radiation travels.

The wavelength (λ) and frequency (ν) are related to the velocity (C) of light in vacuum by the expression:

$$\nu = c/\lambda = c\bar{\nu}$$

These relationship means that if the wavelength is longer, the frequency is lower




**Particle properties**

To describe how electromagnetic radiation interacts with matter, consider the beam of radiation as a train of photons. The energy of each photon is proportional to the frequency of radiation given by the relationship:

$$E = h\nu = h\,C/\lambda = h\,C\bar{\nu}$$

Where, E = energy of the photon (ergs)
ν = frequency of electromagnetic radiation (Hz)
h = plank's constant = $6.624 \times 10^{-27}$

The higher the frequency, the higher the energy of radiation (i.e.) a photon of high frequency (short wavelength) has higher energy content than one of lower frequency (longer wavelength).

The intensity of a beam of radiation is proportional to the number of photons and is independent of the energy of each photon. Since energy per unit time is power, Intensity is often referred as the radiant power emitted by the source.

## Electromagnetic Spectrum

The electromagnetic spectrum is composed of a large range of wavelengths and frequencies (energies). It varies from the highly energetic gamma rays to the very low energy radio-waves. The entire range of radiation is commonly referred to as the electromagnetic spectrum.
Table (2) lists the wavelength ranges for the regions of the spectrum that are important in analytical purposes and, also gives the names of the various spectroscopic methods associated with each and the types of transitions that serves as the basis for the various spectroscopic techniques.

### Table (2) Regions of electromagnetic spectrum

Electromagnetic Spectrum

| Type of Radiation | Frequency Range (Hz) | Wavelength Range | Type of Transition |
|---|---|---|---|
| Gamma-rays | $10^{20}$-$10^{24}$ | $<10^{-12}$ m | nuclear |
| X-rays | $10^{17}$-$10^{20}$ | 1 nm-1 pm | inner electron |
| Ultraviolet | $10^{15}$-$10^{17}$ | 400 nm-1 nm | outer electron |
| Visible | 4-7.5x$10^{14}$ | 750 nm-400 nm | outer electron |
| Near-infrared | 1x$10^{14}$-4x$10^{14}$ | 2.5 μm-750 nm | outer electron molecular vibrations |
| Infrared | $10^{13}$-$10^{14}$ | 25 μm-2.5 μm | molecular vibrations |
| Microwaves | 3x$10^{11}$-$10^{13}$ | 1 mm-25 μm | molecular rotations, electron spin flips* |
| Radio waves | <3x$10^{11}$ | >1 mm | >1 mm |

### Absorption and Emission of Electromagnetic of Radiation

Electromagnetic radiation can interact with matter in a number of ways. If the interaction results in the transfer of energy from a beam of radiant energy to the matter, it is called **"absorption".** The reverse process in which a portion of the internal energy of matter converted into radiant energy is called **"emission".** In emission process, species in an excited state can emit photons of characteristic energies by returning to lower energy states or ground states.

In other words, absorption of potassium permanganate solution results in a violet color. This color is due to absorption of the green component in the white light. The combination of transmission of the red and blue components results in the production of the violet color. Since the green is being absorbed, the difference in the energy levels between the ground ***($E^{\circ}$)*** and excited state ***($E^{*}$)*** corresponds to about 500 nm ( exactly 525 nm ).

Similarly, when sodium is heated in a flame, the atoms are excited ***($E^{\circ}$)*** to ***($E^{*}$)***. These, excited atoms emit yellow light corresponding to a wavelength of about 600 nm (exactly 589 nm). Thus the fundamental difference between emission and absorption is that the deactivation of an electron leads to emission of energy, while promotion of an electron leads to absorption of energy.

The questions that should be asked at this particular point are what is the experimental results of such transitions, what types of spectra are obtained, and what are their physical appearance?

## Differences in the origin and appearance of atomic and molecular spectra

### Absorption of radiation

When radiation passes through a layer of solid, liquid or gas, certain frequencies may be selectively removed by ***absorption,*** a process in which electromagnetic energy is transferred to the atoms, ions, or molecules composing the sample. Absorption promotes these particles from their normal room temperature state, or ground state to one or more higher-energy excited states. According to quantum theory, atoms, molecules, or ions have only a limited number of discrete, energy levels. For absorption of radiation to occur, the energy of the exciting photon must exactly match the energy difference between the ground state and one of the excited states of the absorbing species. Since these energy differences are unique for each species, a study of the frequencies of absorbed radiation provides a means of characterizing the constituents of a sample of matter. A plot of absorbance as a function of wavelength, frequency or wave number called the ***absorption spectrum***. The nature of the spectrum is influenced by differences between absorption spectra for atoms and those for molecules

### Atomic spectra

The passage of electromagnetic ultraviolet radiation through a medium that consists of mono-atomic particles, such as sodium vapor, results in the absorption of few well-defined frequencies as in figure 3.

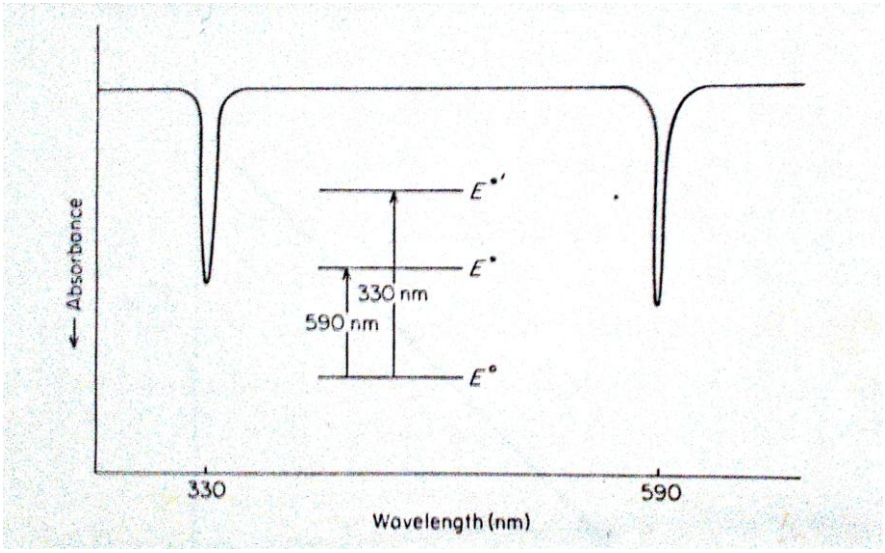



Fig. 3 Absorption spectrum of sodium vapor

Excitation can occur only by an electronic process in which one or more of the electrons of the atom are raised to a higher energy level. For example, all the atoms in sodium vapors are in the ground state under ordinary conditions. Their valence electrons lie in the 3S level. If irradiated with a beam of energy, the outer electrons of many of the atoms will absorb photons and accelerate to the 3P levels. The excited electron has a strong tendency to return to its normal 3S state and in so doing emits a photon. This emitted photon posses a definite amount of energy, dictated by the spacing of the energy levels. As a result, the spectrum of the sodium vapor exhibits sharp absorption peak in the yellow region of he visible spectrum at 589 nm. If the electron is given more energy, it may be raised to some higher level than 3P such as 4P or 5P resulting for an ultraviolet peak at about 330 nm.

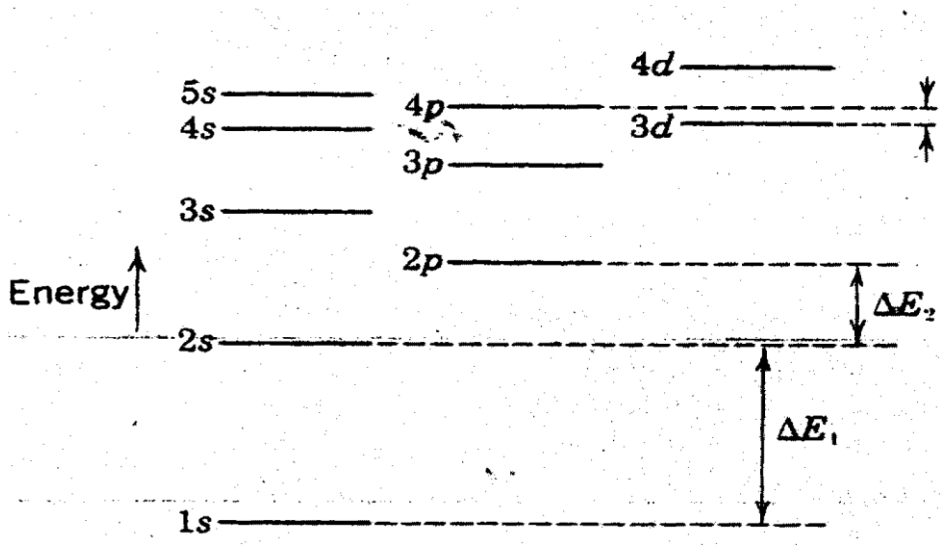



Figure (4)- Energy level diagram for sub-shells in poly electron atom

The vertical lines labeled ΔE1, ΔE2 and so on indicate allowed transitions

between energy levels. Such transitions can occur only if photons of exactly the same energy are available; otherwise, no absorption can occur.

Generally, in atomic spectra, since excitation occurs due to only electronic transition **($E_{ectronic}$)** their spectra are characterized by sharp lines (or peaks) and also known as line spectra.

With a highly energetic source of excitation, many electrons (not only the outer most) in any element can be excited to varying degrees, and the resulting emitted radiation may contain up to several thousand discrete and reproducible wavelengths mostly in the UV and visible regions. If even more energy is available for excitation, an inner electron can be torn entirely away from the atom. An electron from some higher level will then drop to fill the vacancy. The radiation emitted will be of much greater energy. This describes the emission of X-rays from atoms subjected to bombardment by a beam of fast moving electrons.

**Molecular spectra**

The absorption of radiation by a molecule is far more complex than absorption by individual atoms. The total energy state of a molecule includes electronic, vibration and rotational components. That is

***E = E ectronic + E vibrational + E rotational***

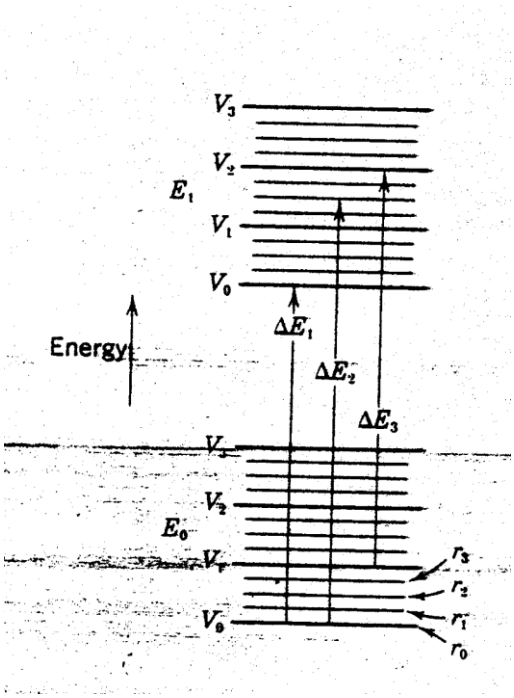



Figure (5) molecular electronic, vibrational and rotational energy levels

For each electronic level, there will be several sublevels corresponding to vibration states; and the later further subdivided into rotational levels. For example, in figure (5), ΔE1, ΔE2 and ΔE3 all represent electronic transitions involving the same two electronic levels but different vibration and rotational

levels. Each absorption thus correspond to energy transfer from radiation of a given frequency or wavelength. As can be seen from figure (5), the energy difference between the ground state and an electronically excited state is large relative to the energy differences between vibration levels in a given electronic state.

The following Figures are typical examples for molecular spectra of some inorganic and organic molecules. Since the spectra is due electronic, vibrational and rotational transition between ground and excited states within the molecule they appear as broad bands (or peaks). So, the molecular spectra is known as band spectra in comparison with line spectra for atoms**.**

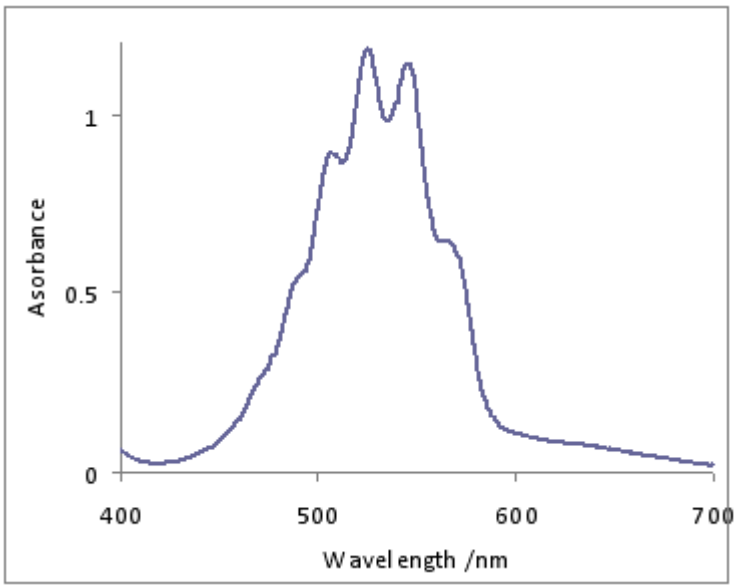



Fig.6 Absorption spectra of $KMnO_4$ in aqueous solution. λ max. at 525 nm

Absorption spectrum of an aqueous solution of potassium permanganate. The spectrum consists of a series of overlapping lines.

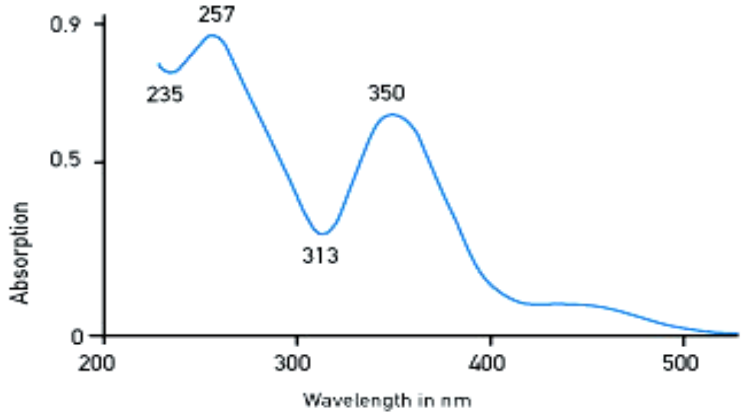



Fig. 7 Absorption spectra of potassium dichromate

Potassium dichromate dissolved in perchloric acid solution is a highly suitable method to check for photometric accuracy (absorbance) in the UV region. Potassium dichromate as shown in fig. 7 gives a spectral scan containing characteristic peak maxima at 257 nm and 350 nm and minima at 235 nm and 313 nm.

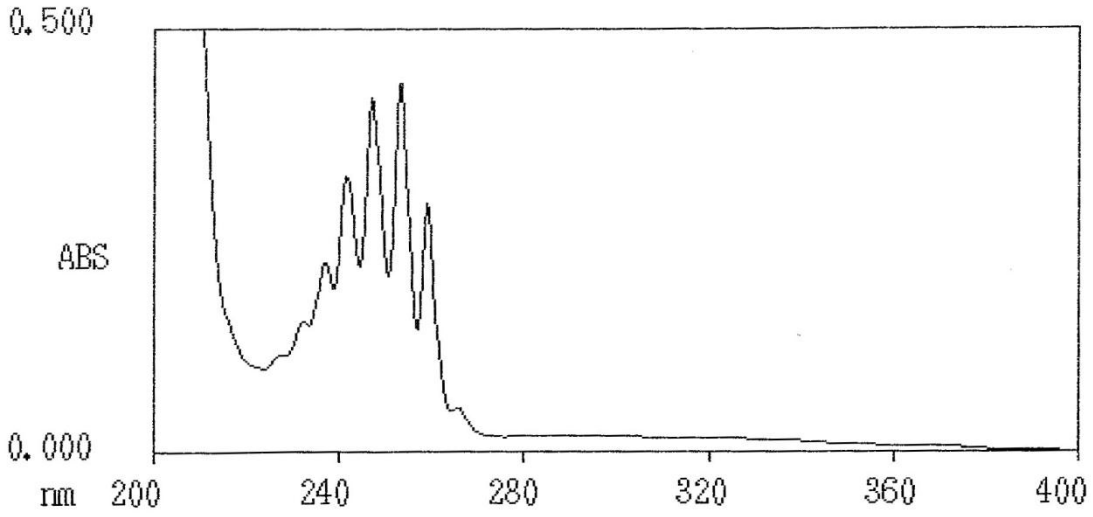



Fig. 8 Absorption spectrum of benzene vapor

Absorption spectrum of benzene vapor shows multiple peaks in the ultra violet region ( 230 nm – 270 nm ). No peaks appear in the visible region.

Structure of hemoglobin

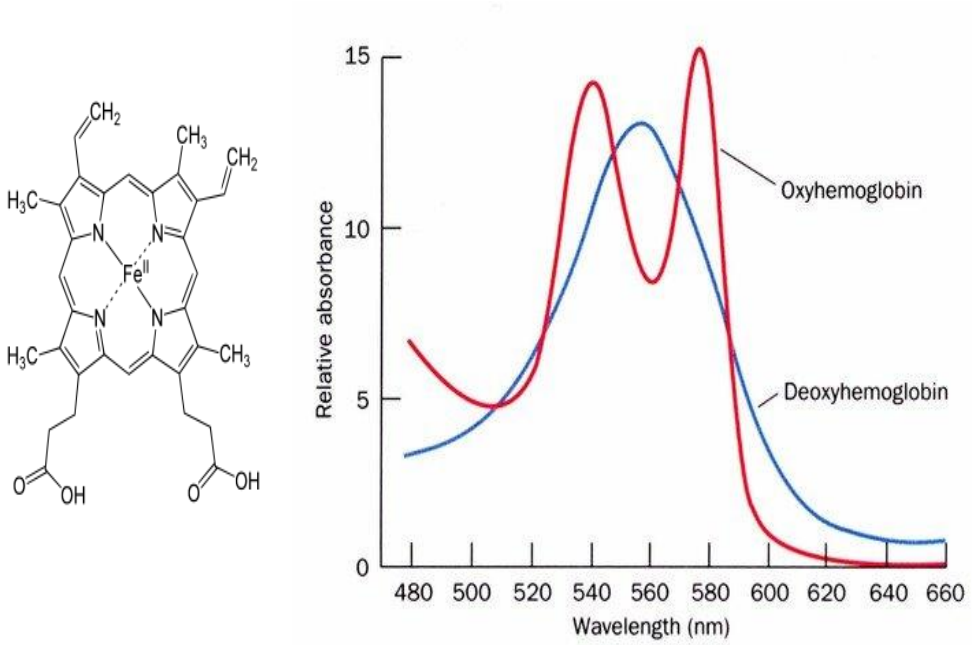



Fig.9Hemoglobin structure and absorption spectrum of oxyhemoglobin and deoxyhemoglobin.

Oxyhemoglobin shows two well defined absorption peaks at 540 nm and 580 nm. However, deoxyhemoglobin shows only one absorption peak maximum at 558 nm.

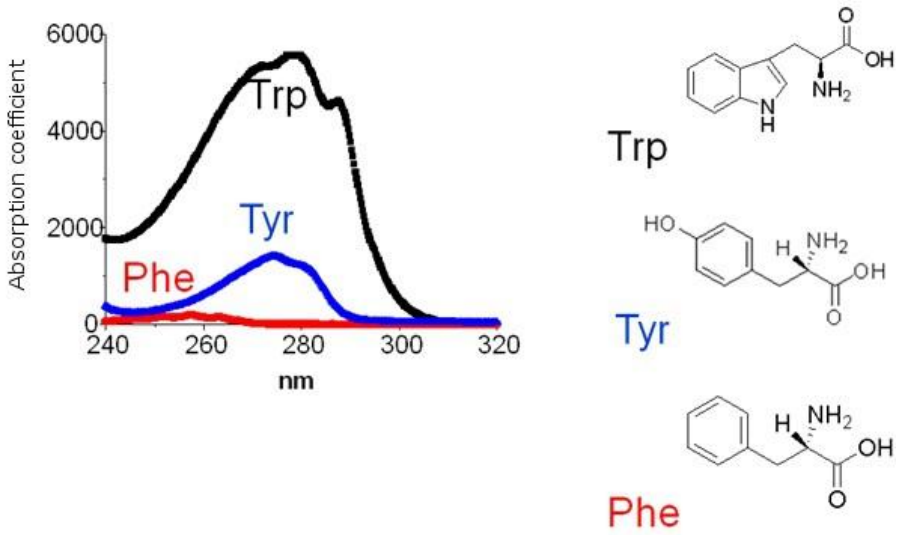



Fig.10Absorption spectra of some three amino acids. Phenylalanine(Phe), Trosine(Tyr) and Tryptophan(Trp). The peak at 280 nm is caused by the absorption of aromatic amino acids

**Emission radiation**

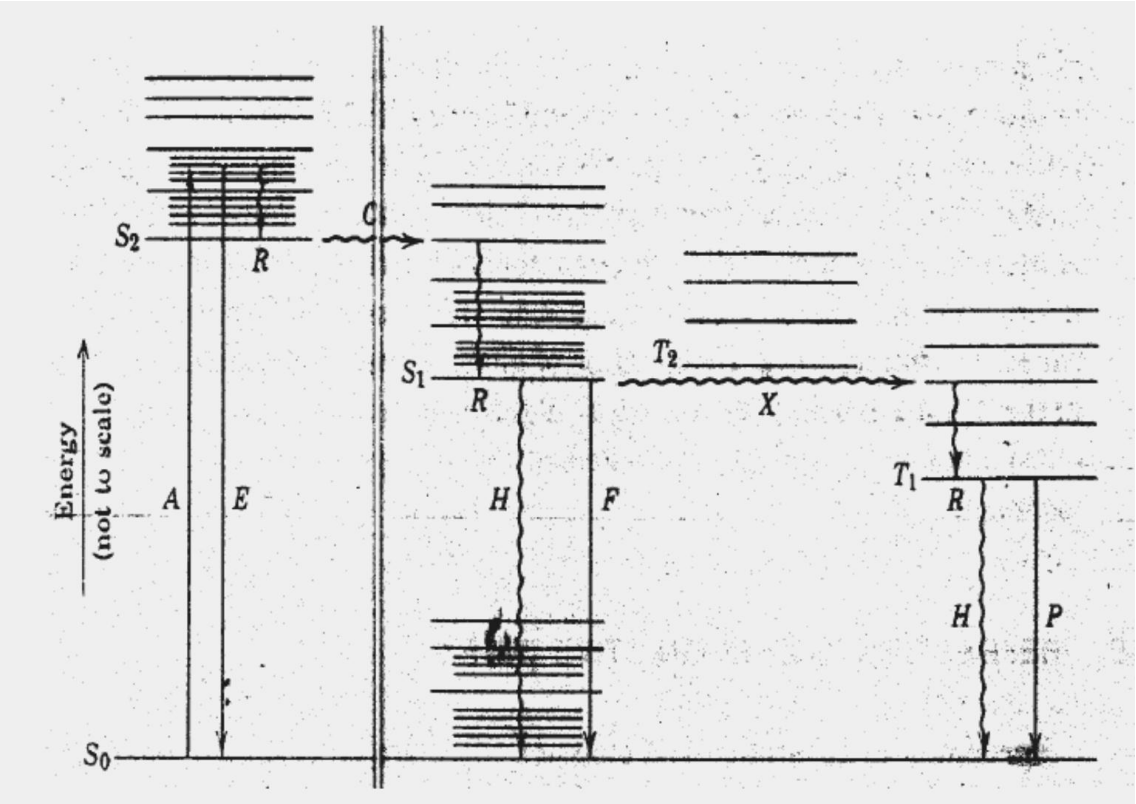



Fig. 11 Energy level diagram of singlet and triplet states of a molecule

The interaction of electromagnetic radiation with matter is a reversible phenomenon. Let us examine the events, which might follow the absorption of radiation raising the energy of a molecule from its ground state, So, to an excited vibration level of an excited singlet state, S2, (Arrow A). The molecule may lose the acquired energy through one of several alternate pathways:

(a) The process might be reversed immediately in which the emitted radiation is identical in frequency to the radiation employed for excitation (Arrow E). This process called **Resonance Fluorescence.**

(b) The excitation energy is more likely that it is converted to kinetic energy by collisions with other molecules and fall to the lowest vibrational level of the S2 state, resulting in a minute increase in the temperature of the system. A process called **Vibrational Relaxation**, (Arrow R).

(c) A transition from the lowest S2 state to the next lower singlet state S1 is highly favored, and is called **Internal Conversion**, (Arrow C). The molecule then rapidly loses energy through additional collisions until it reaches the lowest level of the lowest singlet state S1. The molecule may return directly from the S1 level to the ground state by emitting a photon, termed **normal fluorescence** (Arrow F). The frequency of normal fluorescence will be lower than the resonance fluorescence. Many organic and some inorganic compounds fluorescence in the visible region when they are irradiated with ultraviolet light. Another possibility that the molecule may return to the ground state by further collisions, dissipating the energy as non radiated heat (Arrow H). A third possibility is that the molecule can shift from the singlet state to the corresponding triplet state (S1 T1), a phenomenon called **Intersystem Crossing** (Arrow X). This crossing involves unpairing of the two electrons, leaving the molecule in an excited vibrational level. The lifetime of the T1state is relatively long (> 10 sec) and its energy is lower than the S1 state, therefore a triplet molecule is more likely tolose energy through collisions.

However, some substances do return from the triplet state to the ground state via photon emission (Arrow P), a process called **Phosphorescence**. The duration of phosphorescence depends on the lifetime of the T1 state and may last as long as 10 sec. Only a few type of molecules exhibit phosphorescence.

## Instruments for Spectroscopy

### Introduction

The instruments that are used to study the absorption or emission of electromagnetic radiation as a function of wavelength are called spectrometers or spectrophotometers.

The essential components of a spectrophotometer include:

1- A stable source of radiant energy

2- Monochromators to resolve the radiation into component wavelengths or bands of wavelength. In addition of a system of lenses, mirrors, and slits which define, collimate (make parallel) and focus the beam
3- A transparent container to hold the sample.
4- Radiation detector
5- Readout system (meter, digital recorder, plotter or computer).

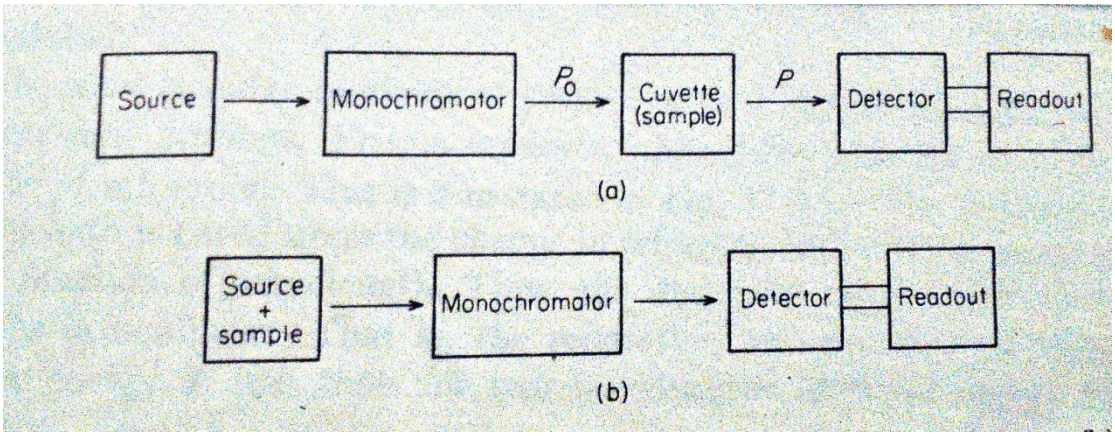



Fig. 12 Block diagram of a spectrophotometer. (a) Absorption spectrophotometrer; (b) Emission spectrophotometer.

Note that in absorption spectrophotometer the source of radiation and sample are separated as shown in Fig.12a. In comparison,an emission spectrophotometer combines the source and sample in one unit as shown in Fig.12 b.

**Sources of radiant energy**

Sources of radiant energy consist of materials that are excited by a high voltage electric discharge or by electrical heating. As the materials return to lower energy states, they emit photons of characteristic energies corresponding to ΔE, the energy difference between the excited and lower quantum states.

**Sources of Ultraviolet Radiation**

The hydrogen lamp and deuterium lamp are the most common sources of UV radiation. They consist of a pair of electrodes which are enclosed in a glass tube provided with a quartz window and filled with hydrogen or deuterium gas at low pressure. When a stabilized high voltage is applied to the electrodes, an electron discharge occurs which excites other electrons in the gas molecules to high energy states. As the electrons return to their ground states they emit radiation in the region roughly between 180 and 350nm.

**Sources of Visible Radiation**

A tungsten (W) filament lamp is the most satisfactory and inexpensive source of visible radiation. The filament is heated by a d-c power supply, or by a

storage battery. The tungsten filament emits continuous radiation in the region between 350 and 2500 nm.

**Sources of Infrared Radiation**

The Globar and Ernst glower are the primary sources of infrared radiation. The Globar is a silicon carbide (SiC) rod heated to approximately 1200 0C. It emits continuous radiation in the (1-40) μm region.

**Monochromator.** The monochromator is used to separate polychromatic radiatin into a suitable monochromatic form. Several important advantages are gained by the use of monochromatic radiation.

1- A closer adherence to Beer‘s law should be expected, since Beer‘s law is based on monochromatic radiation.
2- The sensitivity of the measurement will be increased and the interference due to adverse compounds will be decreased.

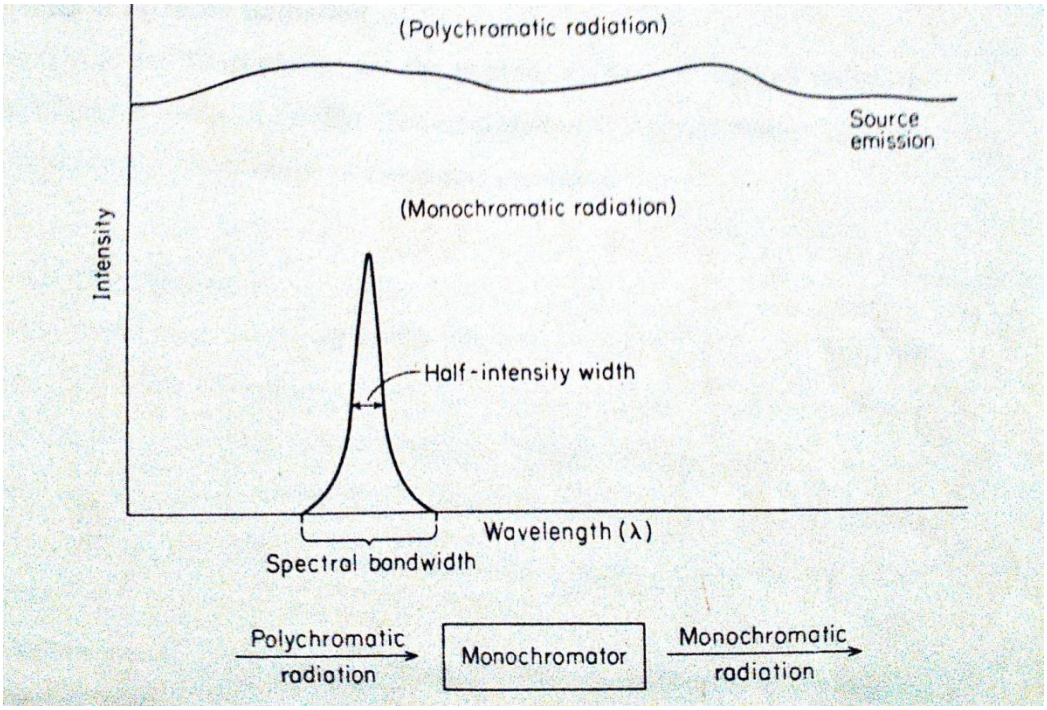
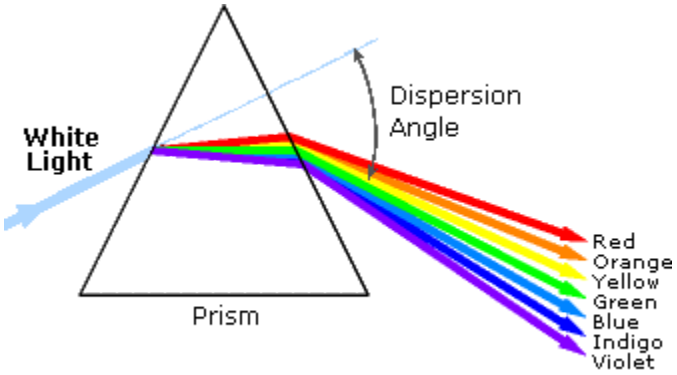



Fig.13 Comparison of monochromatic and polychromatic radiation.

The polychromatic radiation is the radiation incident to the monochromator, while the monochromatic radiation is the energy transmitted by the monochromator.

The monochromatic unit consists of the following: 1- focusing lens, 2- an entrance slit, 3- a dispersing (or resolving) device, and 4- an exit slit. The useful dispersing devices are prisms and gratings. There are, however, monochromators which use filters, such as colored glass.

The most popular methods of producing monochromatic radiation are prisms and gratings. Prisms separate white light into its components by means of refraction.



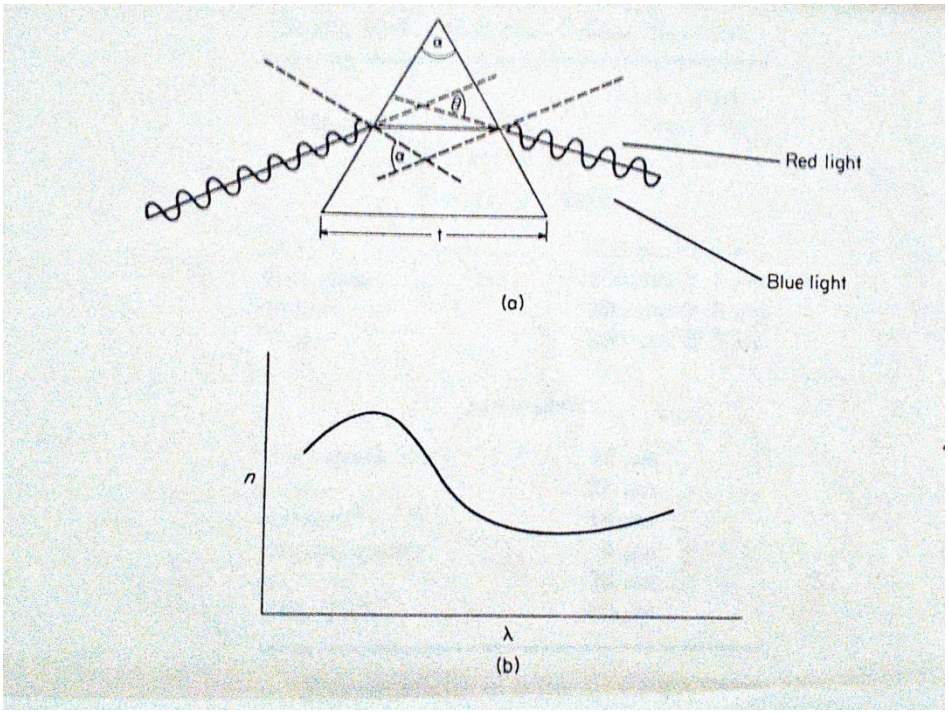




Fig.14 Interaction of prism with radiation. (a) Refraction of radiation by a prism is shown where **α** is the refractive angle of the prism and **ɵ** is the angle of refraction; (b) change in the refractive index with wavelength.

The separation of the wavelength is based upon the change in refractive index of the prism material as a function of wavelength. **Thus, the dispersion of the individual wavelengths is nonlinear.** That is, the separation between two wavelengths at higher energy is less than for two wavelengths of lower energy.

Grating are prepared by cutting grooves in transmitting or reflecting plates. A typical surface would have between 2500 and 60,000 lines/inch (2.5 cm), and its physical appearance is essentially an indistinguishable set of fine parallel straight lines. **One of the main advantages of the grating is that it provides a linear dispersion with λ.**

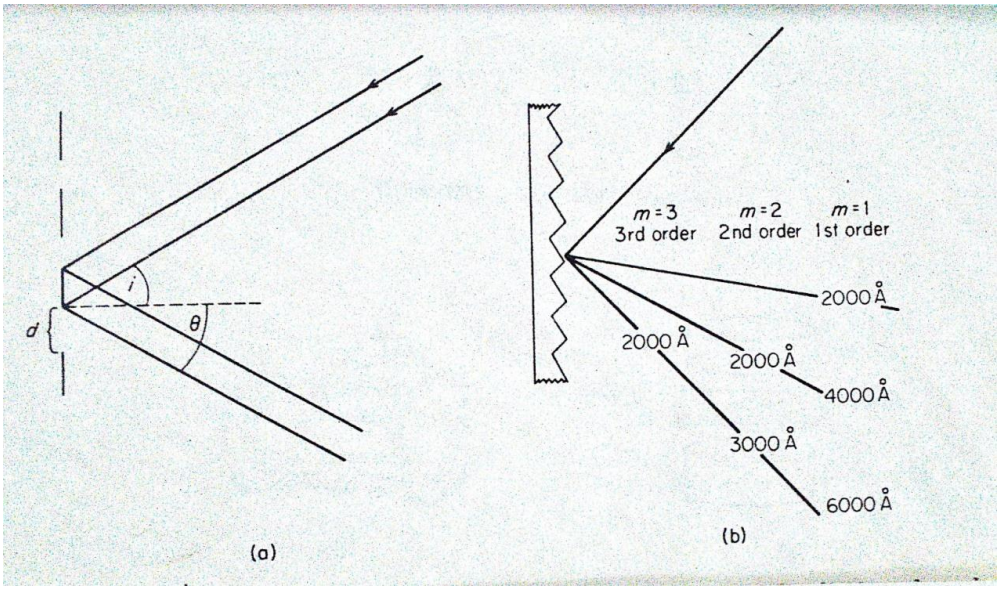



Fig.15 Diffraction of radiation by a grating. (a) The diffraction of radiation where ***d*** is the groove spacing, I is the incident angle, and **ɵ** is the angle of diffraction; (b) the different orders observed in diffraction.

A prism or grating type instrument has two advantages over a filter type instrument. First, the former can be used to scan an absorption spectrum (cover a wide range of wavelengths), while filer is used to measure absorption at one wavelength. The second advantages is that prism or grating instruments will provide more detail in the spectrum due to their high resolution.

## Sample Containers

The cells or cuvettes that hold the samples must be made of material that is transparent to radiation in the spectral region of interest. Quartz or fused silica is required for work in the ultraviolet region (< 350 nm). Plastic containers have also found application in the visible region. Crystalline sodium chloride (NaCl) is the most common substance employed for cell windows in the infrared region.

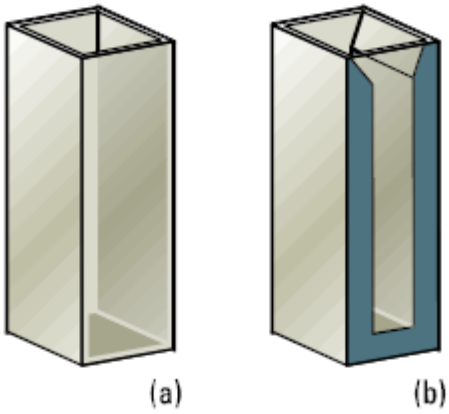



Fig 16 (a) Rectangular standard cell **,**
(b) Rectangular cell used for limited sample volume

**Radiation Detectors**

Any detector absorbs the energy of the radiation and converts this energy to a measurable quantity such as the darkening of a photographic plate, electric current or thermal changes. Any detector must generate a signal which is quantitatively related to the radiant power striking it. The noise of a detector refers to the **background signal** or **dark current** generated when no radiant power from the sample reaches the detector.

**Phototubes** ultraviolet and visible radiation, possess enough energy to cause photo ejection of electrons when they strike surfaces which have been treated with specific type of compounds. Their absorption may also cause bound, non-conducting electrons to move into conducting bands in certain semiconductors. Both processes generate an electric current which is directly proportional to the radiant power of the absorbed radiation.

**Photomultiplier tube** if the ejected electron is accelerated by an electric field, it acquires more energy; and if it strikes another electron-active surface, it may transfer some of its energy, ejecting several more electrons. These electrons may in turn be accelerated to another surface and produce even more electrons, and so on. After nine stages of amplification, the original photon has been amplified by a factor of approximately 106.

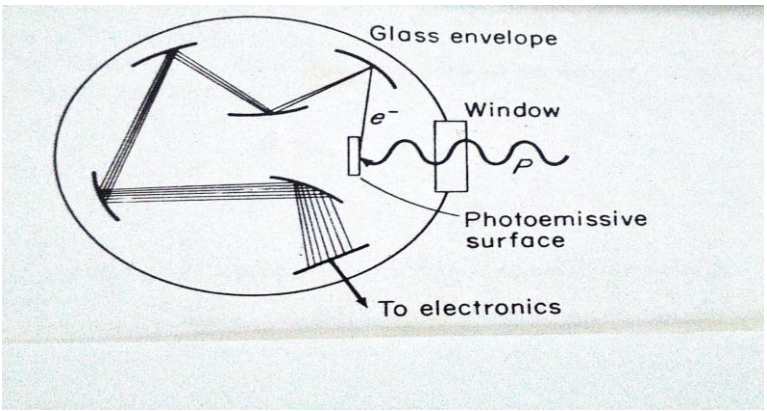



Fig.17 Photomultiplier

**Photoconductivity detectors** These are semiconductors whose resistance decreases when they absorb radiation in the region (0.75 to 3 μm). The application of photoconductors is important in Fourier Transform Infrared instrumentation FT- IR. Absorption of radiation by semiconductor materials promotes some of their bound electrons into an energy state in which they are free to conduct electricity. The resulting change in conductivity can then be measured.

**Thermal detectors**

In thermal detectors, the radiation absorbed is converted to thermal energy(heat) and a corresponding temperature change is noted. There are various types of rapid response thermometers such as thermocouples, resistance thermometers (bolometer) and gas thermometers.

**Processors and readout**

The electronic signal generated by any radiation detector must be translated into a form that can be interpreted. This process is typically accomplished with amplifiers, ammeters, potentiometers and potentiometer recorders.

**Amplifiers** The amplifier takes an “input” signal from the circuit of the sensing component and, through a series of electronic operations, produces an “output” signal which in many times larger than the “input”. The amplification factor (ratio of output to input) is called “**the gain”** of the amplifier.

**Readout devices** Several types of readout devices are found in modern instruments. Some of these devices include the digital meters and computers. The instrument is calibrated so that there are 100 units on the meter from (It =0) to (I = I0) and these units are linear with respect to It. When an absorbing sample is substituted for the “blank”, the detector response will show between 0 and 100 units on the meter.

## Types of UV-Visible Instruments

The instrument used in the UV / VIS is called a spectrophotometer. There are two types of spectrophotometer:. A single beam and double beam spectrophotometer.

### (a) Single beam spectrophotometer

It consists of the radiation source, a filter or monochromator for wavelength selection, matched cells that can be imposed alternately in the radiation beam, the photodetector, an amplifier and readout device.

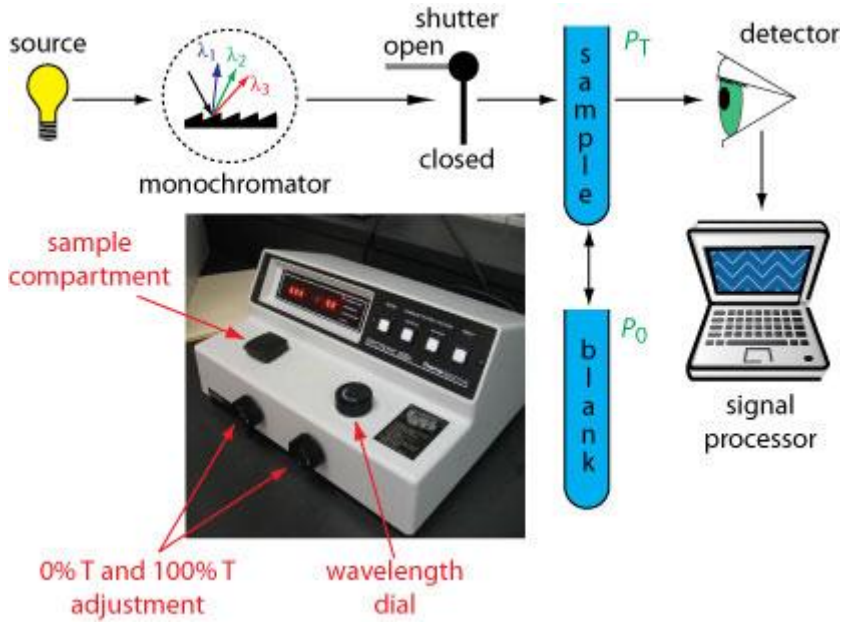



Fig.17 Single-Beam Spectrophotometer

The illustration here shows a diagram of a single-beam spectrophotometer that uses a monochromator to select the wavelength. The analyst manually selects the wavelength by adjusting the wavelength dial. A fixed wavelength single-beam spectrophotometer, such as the one illustrated here, is not practical for recording a spectrum because manually adjusting the wavelength and recalibrating the spectrophotometer is awkward and time-consuming.

### Colorimeters

The image below shows the simplified operation of a typical colorimeter. In this example, light of a substance-specific wavelength is passed through a solution containing the substance. (This wavelength is one known to be strongly absorbed by the substance.) The transmitted light strikes a photocell which

measures the amount of light absorbed and outputs this figure as absorbance. By comparing the figure to a reference curve, an operator can determine the concentration of the substance within the solution.

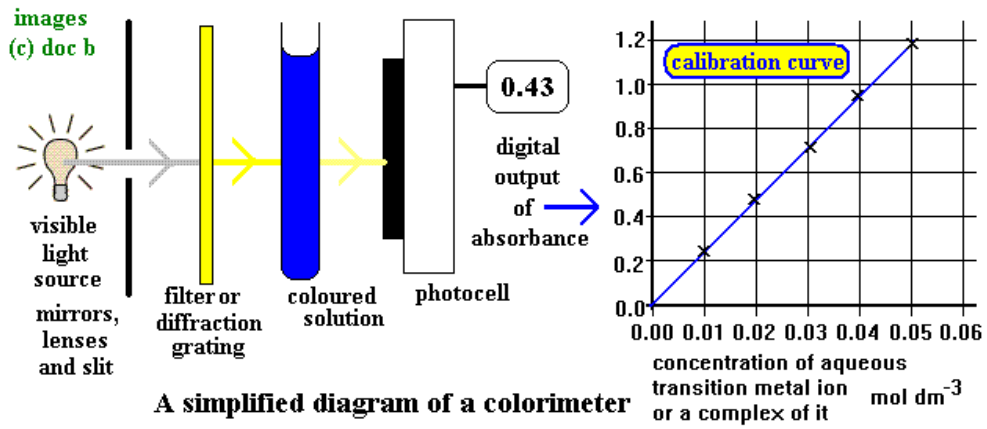



Fig. 18 A simplified diagram of a colorimeter

Device Selection Criteria between spectrophotometer and colorimeter

Colorimeter tungsten lamp as light radiation source. In uv.vis. spectrophotometers tungsten and deuterium lamp
Wavelength selector filter & prism or diffraction grating.

- **Accuracy required**: spectrophotometers are more.
- **User skill**: colorimeters generally require experienced staff. Digital spectrophotometers require little skill to operate.
- **Operation**: colorimeters are robust and user-friendly despite being less accurate than photometers.
- In addition light source in colorimeter is tungsten lamp, while in spectrophotometer is deuterium.
- Wavelength resolving in colorimeter is a filter , while in spectrophotometer is a prism or a diffraction grating

**(b) Double Beam spectrophotometer (UV-Vis. Spectrophotometers)**

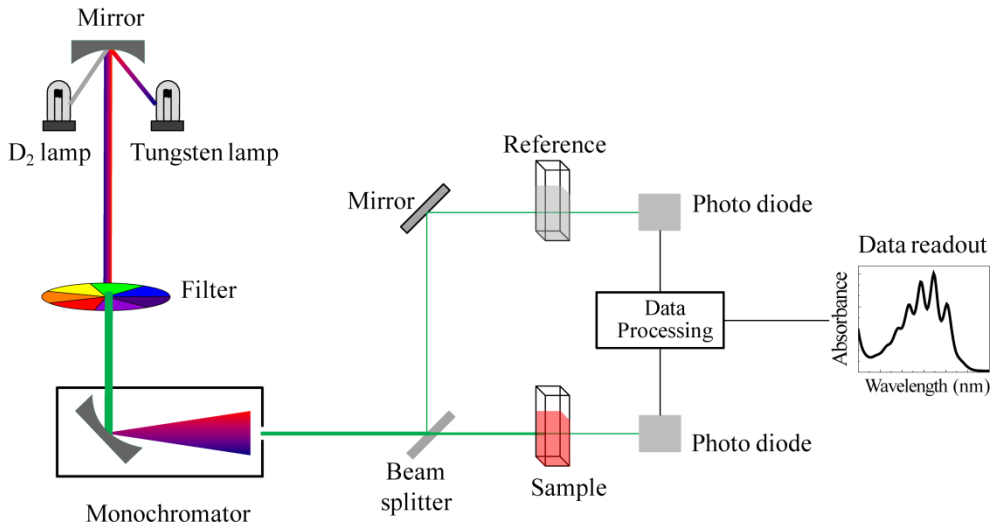



Fig.19 Schematic (I) of a uv-visible spectrophotometer

Two beams are formed in space by a beam splitter. One beam passes through a reference solution to a detector, and the second simultaneously traverses the sample to a second, matched detector. The two outputs are amplified, and their ratio (or the log of their ratio) is determined electronically and displayed by the readout device.

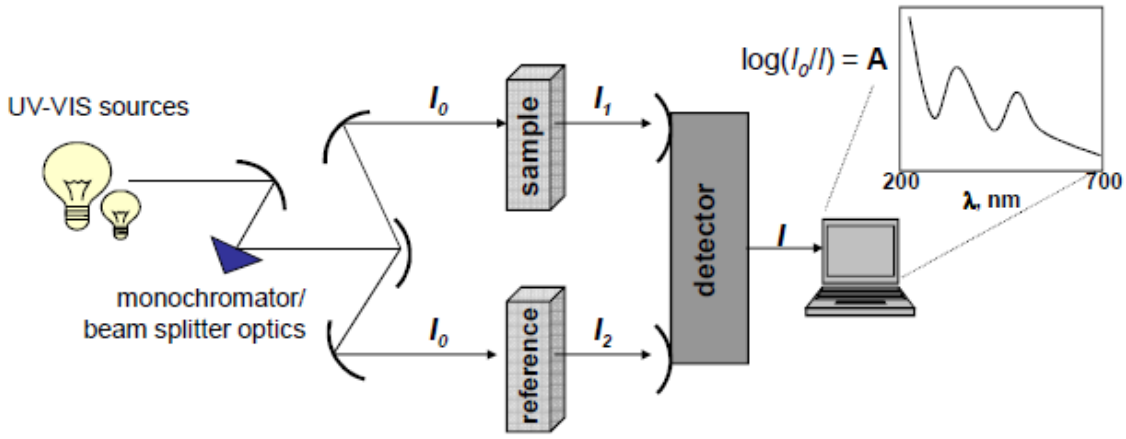



Fig. 20 Schematic (II) of a UV-visible spectrophotometer

To obtain information on the absorption of a sample it is inserted in the optical path of the apparatus. Then, UV light and / or visible light at a certain wavelength (or range of wavelengths) is passed through the sample. The spectrophotometer measures how much light was absorbed by the sample. The intensity of the light before passing through the sample is symbolized by I◦, and intensity of light after passing through the sample is symbolized by I . The

transmittance of the sample is defined by the ratio I/ Io which is usually expressed in percent transmittance %T = I/Io × 100%. From this information, the absorbance of both is determined for that certain wavelength or as a function of a range of wavelengths. The most sophisticated spectrophotometers usually do this automatically.

Although samples can be solid (or even gas), they are usually liquid. A cell transparent (i.e. does not absorb radiation in the wavelength range used), commonly called a *cuvette,* is filled with sample liquid and placed in the spectrophotometer. The path optical by the sample is then the width of the cuvette. Spectrophotometers simple (economic) use with cylindrical vials (vials), however, more sophisticated use rectangular cuvettes, usually with a width. For only visible spectroscopy, single cuvette glass may be used, but ultraviolet spectroscopy requires special cuvettes made of a material that (unlike glass) does not absorb light UV, such as quartz.

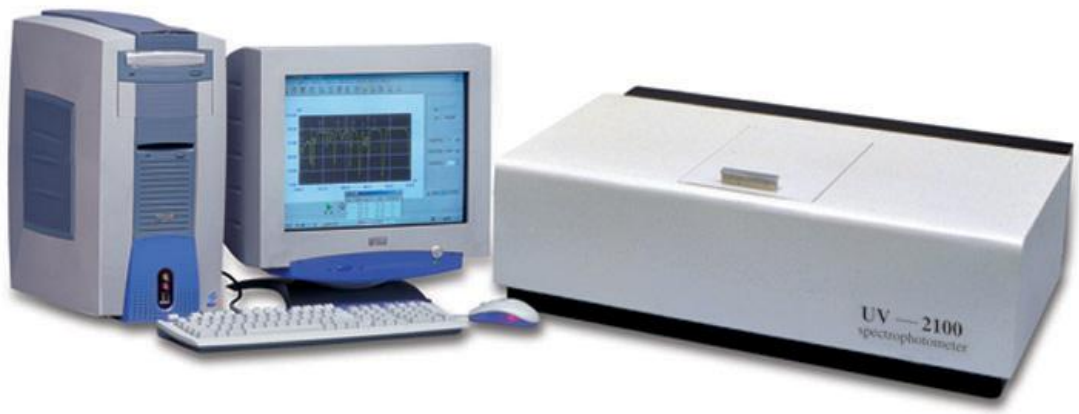



Image for a UV-Vis spectrophotometer

Instrument's specification

- Double beam, fully automated scanning system.
- Compatible PC controlled, rich analytical software.
- Wavelength Scan: Scanning range within 190-900nm.
- Three scanning speed: Fast, Middle and Slow selectable, with Min. sampling interval of 0.04nm.
- Detector : photomultiplier
- Light source : Tungsten and deuterium lamp

# Quantitative Spectral Analysis

## Beer's Law (Beer's- Lambert's Law)

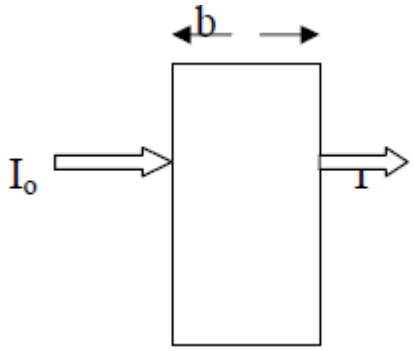



Fig. 21 Absorbing solution of concentration (c)

If a beam of white light passes through a glass container (cuvette) filled with liquid, the emergent radiation is always less powerful than the entering. The loss is due in part to "reflections" at the surfaces and to scattering by any suspended particles present. But in the absence of such particles, it is primarily accounted for "absorption" of the radiant energy by the liquid.

Figure (6) depicts a beam of parallel radiation b before and after it has passed through a medium that has a thickness of (b) cm and a concentration Io I of (c) of an absorbing species. As a consequence of interaction between the photons and absorbing atoms or molecules, the power of the beam is attenuated from Io to I.
The transmittance T of the medium is then the fraction of incident radiation transmitted by the medium:

$$\mathbf{T = P(I) / Po\ (Io)}$$

Transmittance is often expressed as a percentage or

$$\mathbf{\%T = I/Io \times 100\%}$$

The Absorbance (A) of a medium is defined by the equation:

$$\mathbf{A = \log Io/I = - \log T}$$

Note that the ratio in contrast to transmittance, the absorbance of a medium increases as attenuation of the beam becomes greater.

Since percent transmission and absorbance are used extensively, the relation between these two terms can be written as follows:

$$\mathbf{A = 2 - \log \% T}$$

## Presentation of the Data : Spectrum and Concentration

The criterion for presentation of a spectrum must follow a plot of energy (absorbed or emitted) versus a function of absorption of radiation. The most common methods are to plot either absorbance, percent transmission, or log ϵ versus wavelength, frequency or wavenumber as shown in fig. 22.

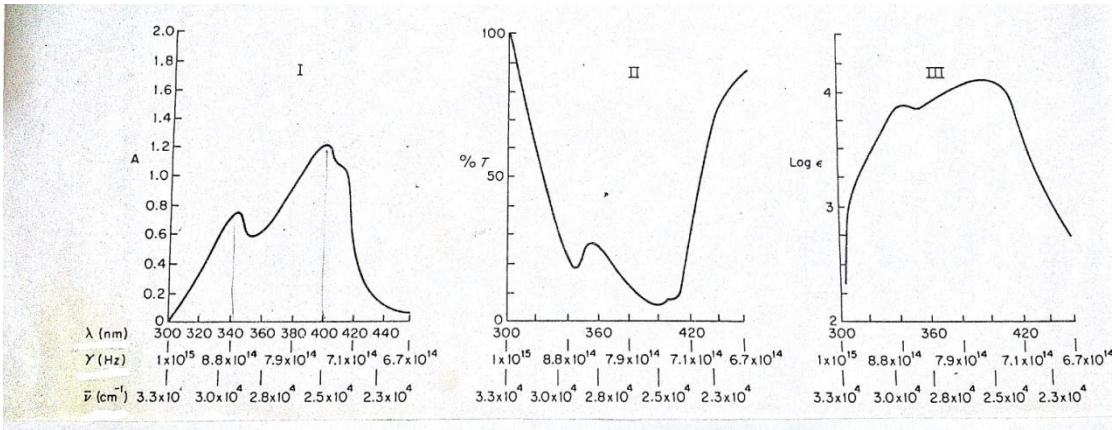



Fig. 22 Presentation of spectral data. (I) Absorbance vs energy; (II) percent transmission vs energy; (III) log ϵ vs energy.

## Beer's Law

For monochromatic radiation, absorbance is directly proportional to the path length of radiation through the medium (b) and the concentration (c) of the absorbing species,

**A** (absorbance) = log Io/I **= a b c**

Where (a) is the proportionality constant called the absorptivity, units (L.g-1.cm-1). When the concentration is expressed in moles per liter, the absorptivity is called the molar absorptivity and is given the symbol(ϵ)

**A = ϵ b c** (units of ϵ = L.mole-1.cm-1)

Note:- Absorptivity (ϵ) is a property of a substance (intensive property), whereas absorbance (A) is a property of a particular sample (extensive property) and will therefore vary with the concentration and length of light path through the sample container.

In Fig.23 absorption curves, a, b, c, and d.represent increasing concentrations of the sample material. As the concentration increases, the absorbance (A) increases while the percent transmission (%T) decreases.

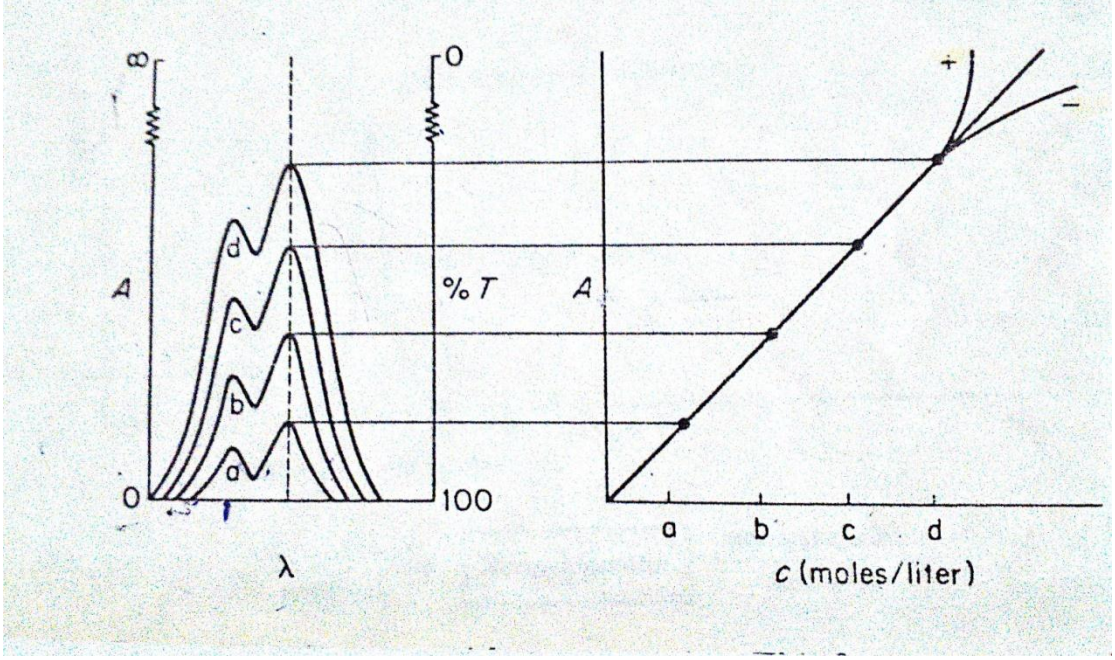



Fig.23 Correlation of spectra with concentration. This figure represent the conversion of spectral data into a Beer's Law plot. Note that deviations can occur in a positive (+) or negative (-) direction.

An example of a Beer's Law is also shown in the previous figure. A linear function as predicted by Beer's Law is found when A is plotted vs concentrations a, b, c, and d at a wavelength of the absorption maxima. The slope of the line is the molar absorptivity (ϵ) provide the pathlength is 1 cm. A calibration curve of %T vs concentration can also be used, but has the disadvantage of not providing a linear relationship.

**Deviation from Beer's Law**. A deviation from Beer's is observed when a plot of concentration vs absorbance is nonlinear as shown in Fig. Deviation toward the ordinate is known as a positive deviation, while deviation toward the abscissa is known as a negative deviation. In either case, strict adherence to Beer's law (absorbance being proportional to concentration) does not exist in all regions. However, in most systems there is a region of concentration in which a linear relationship exists.
The more important factors that produce deviation are :

1- Environment, such as temperature, pressure and solvent.
2- Instrumental errors, such as stray radiation, stability of the radiation source, detector, wavelength selector and reliability of the optical parts.
3- Chemical deviations, including changes in chemical equilibrium such as pH, presence of complexing agent, competitive metal ion reactions and concentration dependence.

There are several useful ways of handling the absorption data in analysis. Although the absorbance scale covers the range of zero to infinity, the best accuracy is obtained in the absorbance range of 0.1 to 1.0. Therefore, the experimental conditions should be designed to give absorbance data in this range. If the solutions provide too high of absorbance, they should be diluted.

Similarly, if the absorbance is too low, the solutions should be concentrated. Usually, these decisions are based on a preliminary spectrophotometric measurements ( calibration curve construction and data analysis). Under favorable experimental and instrumental conditions the error in a quantitative determination can be expected to be less than 2%.

As will be illustrated in the following example, Beer's Law can be applied to quantitative determinations with many variations. All of the calculation with the exception of one, depend upon a linear correlation between absorbance and concentration of the absorbing species. Only the calibration method can be used when a deviation from Beer's law occurs.

**Standard Comparison.** The absorbencies for the unknown, $A_{unk}$ and the standard, $A_{std}$ are described through Beer's law by

$$A_{unk} = \epsilon_1 b_2 C_{unk}$$
$$A_{std} = \epsilon_2 b_2 C_{std}$$

Thus,

$$\frac{A_{unk}}{A_{std}} = \frac{\epsilon_1 b_2 c_{unk}}{\epsilon_2 b_2 c_{std}}$$

However, $\epsilon_1 = \epsilon_2$ ( the same compound ) and $b_1 = b_2$ (the same used for both measurements; often 1 cm ) and

$$\frac{A_{unk}}{A_{std}} = \frac{C_{unk}}{C_{std}}$$

Example 1. A 1.00 g sample of steel is dissolved in $HNO_3$. The trace Mn is oxidized with $KIO_3$ to $KMnO_4$ and diluted to 100 ml. The absorbance of this solution in a 1.0 cm cell at 525 nm was 0.70. A $1.52 \times 10^{-4}$ M solution of $KMnO_4$ served as a standard and under the same conditions its absorbance was 0.350. What is the percent Mn in the steel sample? ( Atomic mass of Mn =54.94)

**$C_{unk} = A_{unk}/A_{std} \times C_{std}$**

**$C_{unk} = 0.7/0.35 \times 1.52 \times 10^{-4} = 3.04 \times 10^{-4}$ M**

**$[KMnO_4] = [Mn] = 3.04 \times 10^{-4}$ M**

**$M \times V_{mL}$ = mass/molar mass $\times$ 1000**

**$3.04 \times 10^{-4} \times 100$ = mass/54.94 $\times$ 1000**

**Mass = $3.04 \times 10^{-4} \times 100 \times 54.94 / 1000$**

**Mass = $1.67 \times 10^{-3}$**

**% Mn in the sample = mass of Mn (g)/ mass of sample (g) x 100**

**= $1.67 \times 10^{-3}$ /1.0 X 100 = 0.167 %**

**Standard Addition (spiking method).** When a suitable standard is not available, it may be possible to use the element to be determined as its own internal standard. This is sometimes more convenient than the use of a different element.

$$\frac{I_1}{I_2} = \frac{C_1}{C_1 + C_2}$$

$$\frac{I_1}{I_3} = \frac{C_1}{C_1 + C_2 + C_3}$$

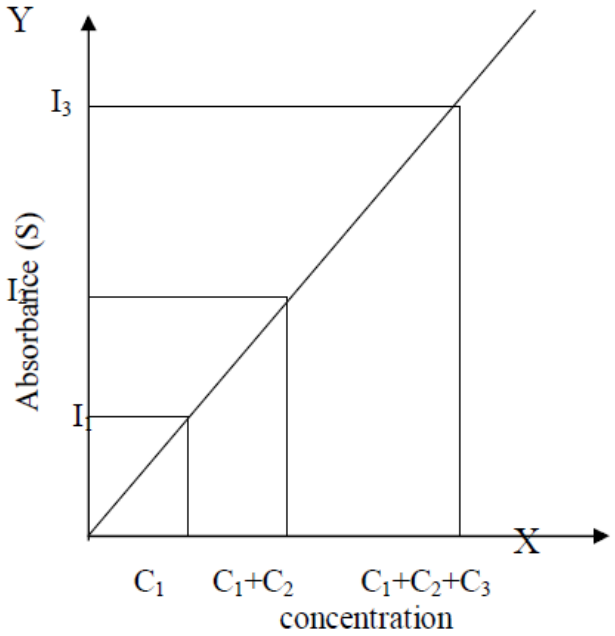



Fig. 23 principle of spiking procedure

A small quantity (C2) of the element to be analyzed is added to the sample, and the ratio of the count rates before and after addition are used to estimate the initial concentration (C1). If the concentration / count rate is linear, the net count rate from an element is proportional to its concentration, and addition of more of the same element should cause the count rate to increase proportionally. Fig.23 illustrates the concept and it can be seen that net count rate (I1) is proportional to the concentration (C1). By addition of (C2) of the analyzed element to give a new concentration of (C1+C2) the net count rate increases to (I2), and with more additions (C1+C2+C3) the net count rate increases to I3 and so on. This method is useful for the determination of single elements in very complex matrices, and where suitable standards are not available.

**Direct Application of Beer's Law.** The concentration of an absorbing solution can be calculated with Beer's law providing the molar absorptivity (ϵ) is known.

Example 2. From the date given the previous example, find the molar concentration of Mn

$$\epsilon = A / bc = \frac{0.350}{1.0 \times 1.52 \times 10^{-4} M} = 2300 \text{ L mol}^{-1} \text{ cm}^{-1}$$

When this constant and the absorbance of the unknown A = 0.700 are known, then the molar concentration of Mn can be calculated.

$$c = A / \epsilon b = \frac{0.700}{2300 \times 1.0} = 3.04 \times 10^{-4} \text{ M}$$

Example 3 Antimycine , an experimental fungicide , has an absorption maximum at 320 nm . If a $1.1 \times 10^{-4}$ M solution of this compound produces an absorbance of 0.52 , calculate the molar absorbitivity of this compound. (assuming 1.0 cm pathlength).

**$A = \varepsilon bc$**

**$\varepsilon = A/bc$**

**$= 0.52 / 1.0 \times 1.1 \times 10^{-4} = 4727$ liter $mol^{-1}$ $cm^{-1}$**

Example 4 An aqueous solution of a colored compound has a molar absorptivity (ε) of 3200 at 520 nm. Calculate the absorbance and percent transmission of a $3.4 \times 10^{-4}$ M solution if a 1.cm cell is used.

**$A = \varepsilon bc$**

**$= 3200 \times 1 \times 3.4 \times 10^{-4} = 1.09$**

**$A = 2 - \log \% T$**

**$1.09 = 2 - \log \% T$**

**$\text{Log } \% T = 2 - 1.09 = 0.91$**

**% T = shift log Ans. = 8.13**

**Calibration.** In this method, a series of standard solutions containing known concentrations of the absorbing species are prepared. Their absorbances are measured and plotted against concentration. Subsequently, an unknown is treated similarly and its absorbance is used to read the concentration directly from the calibration curve.

The calibration method is used more than any other method for a quantitative determination. This method offers the advantage of averaging a number of values to obtain a line which best fits the data. Thus, the determination will be more accurate than the obtained by using only one absorbance reading for one standard concentration.

Samples which are this easily handled are not often encountered in practice since a sample generally has other elements which will interfere with the analysis in one of several ways. For example, other species in the solution may absorb at the same wavelength.

Example 5. From the data in example 1 and the calibration curve in Fig.24 calculate the percent Mn in the sample.

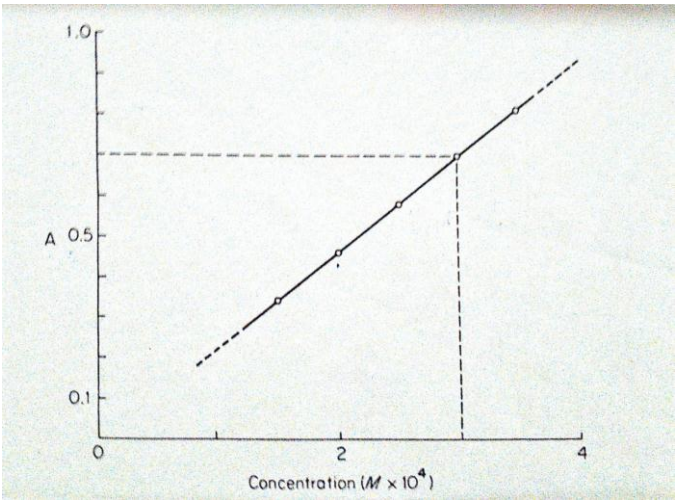



Fig.24 Beer's law calibration plot for potassium permanganate

Since the absorbance of the unknown ( $A_{unk}$) equals 0.700 ,from the calibration curve a concentration of 3.00 x $10^{-4}$ M is obtained. The percent Mn is then calculated as shown in Example 1.

The standard known concentrations of $KMnO_4$ 1.5 x $10^{-4}$, 2.0 x $10^{-4}$, 2.5 x $10^{-4}$, 3.0 x $10^{-4}$ and 4.0 x $10^{-4}$ M ( 10 ml each ) used to construct the previous calibration curve were prepared by :

1- Dissolving 0.158 g of $KMnO_4$ in 100 ml of distilled water to obtain$10^{-2}$ M solution used as stock. The of mass of $KMnO_4$ was obtained from the relation:

$$\text{Mass} = M \times V_{ml} \times \text{molar mass} / 1000$$
$$= 10^{-2} \times 100 \times 158 / 1000 = 0.158 \text{ g}$$

2- Applying the dilution law to determine the required volume from stock to prepare the standard concentration.

$$(M_1 \times V_2)_{\textbf{before dilution}} = (M_2 \times V_2)_{\textbf{after dilution}}$$

$$(10^{-2} \times V_2) = (1.5 \times 10^{-4} \times 10)$$

So, $V_2$ =0.15 ml, diluted with 9.85 ml of the solvent to obtain 1.5 x $10^{-4}$M $KMnO_4$
$V_2$=0.20 ml, diluted with 9.80 ml of the solvent to obtain 2.0 x $10^{-4}$M $KMnO_4$
$V_2$ =0.25 ml, diluted with 9.75 ml of the solvent to obtain 2.5 x $10^{-4}$M $KMnO_4$
$V_2$=0.30 ml, diluted with 9.70 ml of the solvent to obtain 3.0 x $10^{-4}$M $KMnO_4$
$V_2$=0.40 ml, diluted with 9.60 ml of the solvent to obtain 4.0 x $10^{-4}$M $KMnO_4$

Example 5. Alcuronium, a muscle relaxant, has an absorption maximum at 292 nm. A series of standard solutions were prepared and the absorbances determined as shown below:

| Concentration (M) | Absorbance |
|---|---|
| $5.00 \times 10^{-6}$ | 0.22 |
| $1.00 \times 10^{-5}$ | 0.43 |
| $2.00 \times 10^{-5}$ | 0.85 |
| Unknown | 0.73 |

If the same cell (1.00 cm) was used for all determination, calculate the concentration of the unknown solution.

Method 1 :

$$\frac{A_{unk}}{A_{std}} = \frac{C_{unk}}{C_{std}}$$

$$\frac{0.73}{0.85} = \frac{C_{unk}}{2.0 \times 10^{-5}\ M}$$

$$C_{unk} = 1.72 \times 10^{-5}\ M$$

Method 2 :

$$A = \epsilon\, b\, c$$

$$\epsilon = A / bc = \frac{0.850}{1.0 \times 2.00 \times 10^{-5} M} = 4.3 \times 10^{4}\ L\ mol^{-1}\ cm^{-1}$$

$$c = A / \epsilon\, b = \frac{0.73}{4.3 \times 10^{4} \times 1.0} = 1.70 \times 10^{-5}\ M$$

**Analysis of Organic Compounds**

A large number of organic compounds absorb radiation in the ultraviolet and visible region. Those has have high molar absorptivities can be determined directly. Those that do not can be converted chemically into derivatives which have high molar absorptivities.

Tetracyclin hydrochloride, is a drug used as antimicrobial medicinal and as a broad spectrum antibiotic in animals. It can be determined spectrophotometrically. The absorption spectrum of this compound gives three peaks at 220 nm, 268nm and 355nm.

If the percent tetracycline in a tablet is to be determined, the following experimental techniques would be carried out. A serious of standards are prepared in 0.10 M HCl solution and the absorbance is determined at 355 nm for each standard.

To prepare the sample ten tablets are crushed and homogeneously mixed. A portion of this is accurately weighed, diluted to the appropriate volume, and the absorbance determined.

Example 6. Ten tablets of tetracycline hydrochloride (M.W 480.9) are crushed and homogeneously mixed. A portion weighing exactly 0.450 g is dissolved in one liter of 0.10 M HCl. Exactly 10 of the solution is subsequently diluted to

100.0 ml in a volumetric flask. The absorbance of this solution using a 1.00 cm cell is 0.940. From the data below calculate the percent tetracycline hydrochloride in the tablet.

| Standard | Conc. [M] | Absorbance | b (cm) |
|---|---|---|---|
| 1 | $2.50 \times 10^{-5}$ | 0.46 | 1.00 |
| 2 | $4.20 \times 10^{-5}$ | 0.70 | 1.00 |
| 3 | $5.00 \times 10^{-5}$ | 0.90 | 1.00 |
| 4 | $6.40 \times 10^{-5}$ | 1.15 | 1.00 |

If the Beer's law plot is linear in the region where the unknown sample is to be determined, a standard comparison method can be used. Hence,

$$\mathbf{C_{unk} = A_{unk}/A_{std} \times C_{std}}$$

$$\mathbf{C_{unk} = 0.940 / 0.900 \times 5.00 \times 10^{-4} = 5.22 \times 10^{-5} M}$$

In order to insure that the calculation is correct, the sample can be compared with the other standards and the values averaged.

| Standard | Concentration of unknown (M) |
|---|---|
| 1 | $5.11 \times 10^{-5}$ |
| 2 | $5.19 \times 10^{-5}$ |
| 3 | $5.22 \times 10^{-5}$ |
| 4 | $5.23 \times 10^{-5}$ |

$$C_{unk\ (average)} = 5.18 \times 10^{-5}\ M \pm 0.04 \times 10^{-5}$$

Mass of TC.HCl in the sample = $\dfrac{M \times V_{ml} \times d_f \text{ (dilution factor)} \times M.W}{1000}$

$$= 5.18 \times 10^{-5} \times 1000 \times 10 \times 480.9 / 1000 = 0.249\ g$$

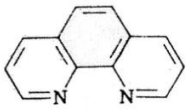
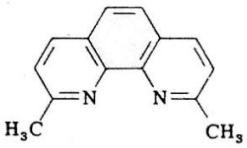
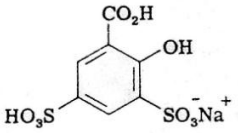
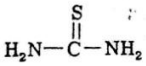
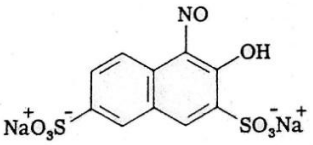
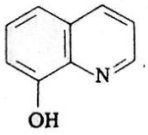
% TC.HCl in the tablet = $\dfrac{\text{mass of TC.HCl (g)}}{\text{Mass of the sample (g)}}$

$$= 0.249 / 0.450 \times 100 = 55.3\ \%$$

## Application of Absorption Spectrophotometry in Inorganic Analysis

A common way of converting a nonabsorbing species into an absorbing species is through a complexation reaction. If the chelating agent (the ligand forming a ring with the taget metal ion) is properly chosen, large molar are obtained. Consequently, spectrophotometry employing ligands is often applied to trace metal ion determinations.

Several complexing agents are used in spectrophotometric analysis of metal ions, the following are some examples:

| Reagent | Structure | Ion analyzed[b] |
|---|---|---|
| 1,10-Phenanthroline |  | Fe(II) |
| 2,9-Dimethyl-1,10-phenantholine |  | Cu(I) |
| Sulfosalicylic acid |  | Al(III),Ti(IV) |
| Thiourea |  | Bi(III), Os |
| Nitroso R salt |  | Co(II) |
| 8-Hydroxyquinoline |  | Zn(II), Al(III), Ce(III), Ga(III), In(III), Mg(II), Sc(III), others |

Generally, some requirements are essential for success in a spectrophotometric determination using a complexing agent, these include the following:

1-The complexation reaction must be complete and stoichiometry.

2-The formed metal complex must be stable

3-The complex must absorb in the visible region.

4-The absorption spectrum of the complex should not overlap with the absorption spectra of the ligand or the metal ion.

Two additional advantages are gained by converting the metal ion to a complex. For example, a chelating agent will often react only with a few metal ions, thus providing selectivity. Second, even when several metal ions form complexes with the same reagent, the absorption characteristics may differ enough to allow determination of one metal in presence of the others.

Moreover, in evaluating spectrophotometric procedures as shown in the previous examples it is necessary to look for certain characteristics. The following parameters are the more important and should be achieved and discussed in the procedure.

1-Molar absorbitivity.
2- Stability and sensitivity with respect time and temperature.
3- Effect of pH.
4-Absorption spectra of reactants and products.
5- Nature of the reaction which includes establishing the stoichiometry and other experimental details.
6- Beer's law plot and concentration range in which the linear relationship is obeyed.
7- Interferences and how they are eliminated.
8- Accuracy and reproducibility of the results.

-

# Atomic Spectrometry

**Principle**. The basic principle upon which Atomic Spectroscopy works is based on the fact that ***:" Matter absorbs light at the same wavelength at which it emits light."*** Based on this principle a number of instruments are used for determination of the concentration of metal ions in solution, where energy is absorbed or emitted by the target metal atom. Among these instruments:

1- Flame photometry (FP) or Flame Atomic Emission Spectrometry (FAES).
2- Flame Atomic Absorption Spectrometry (FAAS).
3- Graphite Furnace – Atomic Absorption Spectrometry (GF-AAS)
3- Inductive Coupled Plasma- Optical Emission Spectrometry (ICP-OES).

## 1- Flame Photometer or Flame Atomic Emission Spectrometry

When the atoms of samples are excited to higher electronic energy levels in flames they emit radiation in the visible and UV regions of the electromagnetic spectrum. Emission intensities may be measured to analyze for metals, especially alkali and alkaline earth elements.

### Principles and Instrumentation of Flame Photometry

The basis of low temperature flame photometry is the same as that of the simple qualtitative analytical flame test. This exploits the fact that compounds of the alkali and alkaline earth metals are thermally dissociated into atoms at the temperature of a Bunsen burner flame and that some of these atoms produced are further excited to a higher energy level. When these 'excited' atoms return to the ground state, they emit radiation, which for the elements of these two groups lies mainly in the visible region of the electromagnetic spectrum. The wavelength of the light emitted from the flame is characteristic of the particular element.

Table 1. Flame excited lines of some metals along with their colors

| Metal | Emission wavelength/nm | Flame Color |
|---|---|---|
| Lithium | 670 | Red |
| Sodium | 589 | Orange–yellow |
| Potassium | 766 | Red |
| Magnesium | 285 | UV |
| Calcium* | 622 | Orange |
| Strontium | 461 | Scarlet red |
| Barium | 554 | Green |

*calcium is measured by using the calcium hydroxide band emission at 622 nm. However, the main atomic emission occurs at 423 nm.

The other elements of group I and II in the periodic table also give a characteristic and sometimes intense band of emitted radiation when introduced into a flame (as shown below). However, if these are to be utilized analytically then factors such as the need for higher flame temperatures and measurements outside the visible spectrum should be considered.

| Metal | Emission wavelength/nm | Flame Color |
|---|---|---|
| Cesium | 852 | ** |
| Rubidium | 780 | Violet |

** The main emission peak for Cs is outside the visible spectrum, however, modern detectors are sensitive enough to enable Cs and Rb to be measured in the absence of interferents, e.g. potassium.

The flame may be produced by burning various gas mixtures ,as listed in Table *2*.

Table 2 Gas mixtures (fuel – oxidant) used in flame photometry.

| Fuel | Oxidant | Temperature (°C) |
|---|---|---|
| Acetylene | oxygen | 3100 - 3200 |
| Acetylene | nitrous oxide | 2900 - 3000 |
| Natural gas | oxygen | 2700 - 2800 |
| Hydrogen | oxygen | 2500 - 2700 |
| Acetylene | air | 2100 - 2400 |
| Hydrogen | air | 2000 – 2100 |
| Propane | air | 1900 – 2000 |
| Butane | air | 1800 – 1900 |
| Natural gas | air | 1700 – 1800 |

The analysis of Na, K, Li, Ba and Ca are typically determined at low temperatures, i.e. 1500-2000°C, therefore suitable mixtures are propane/air, butane/air and natural gas/air.

The structure of the flame comprises an **inner cone**, which is the primary reaction zone for combustion, and the **outer cone or mantle** where secondary reactions occur. For the best results, the optical axis is arranged to pass through the flame at the junction of the inner and outer cones. The supply of fuel and oxidant is adjusted to give an optimum **burning velocity. The processes that occur to transfer the sample to the flame, and excitation of the target atoms may be summarized as follows:**

(i) Nebulization. Aspiration of the sample solution, formation of aerosol and mixing fuel, oxidant with the aerosol.
(ii) removal of solvent MA(aq) → MA (solid)
(iii) vaporization of sample MA(solid) → MA(vapour)

(iv) atomization MA → M• + A•
(v) excitation M• → M*
(vi) emission M* → M•

The emitted radiation is isolated by an optical filter and then converted to an electrical signal by the photo detector. The intensity of these radiation, in most cases, proportional to the absolute quantity of the species present in the flame at any moment, i.e. the number of atoms returning to the ground state is proportional to the number of atoms excited, i.e. the concentration of the sample.

The intensity of the emitted light increases with concentration, and the relationship is usually linear:

$$I = K\,c$$

Thus, unknown concentrations can be determined by comparison with one or a series of standards in the same manner as described for the molecular techniques in UV-Vis Absorption spectroscopy.

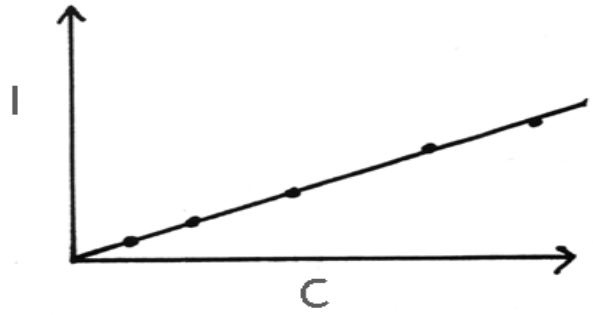



Fig.2 A plot of intensity (I) vs concentration (C) representing quantitative analysis using flame photometer

**Instrumentation** A simple flame photometer consists of the following basic components:

1- A burner to maintain the flame in a constant form and at a constant temperature. A premix type burner is used where the sample is nebulized and mixed with the fuel and oxidant prior to introduction into the head of the flame.

2- Nebuliser and mixing chamber for transporting an homogeneous solution into the flame at a steady rate. The role played by the nebulizer unite is as follows:

- sucks up the liquid sample (aspiration).
- creates a fine aerosol (fine spray) for introduction into flame.
- mixes aerosol, fuel and oxidant thoroughly, creates a homogeneous mixture.

3- A simple color filter for isolating light of the wavelength to be measured from that of extraneous emissions.

4- A photo detector for measuring the intensity of radiation emitted by the flame.

In more detail flame atomic emission spectrometers have similar optical systems to those of UV-visible spectrometers, but **the source of radiation is provided by the sample itself.** A **flame photometer** is a simpler instrument employing a filter in place of a monochromator Fig.3 The sample is prepared as a solution, which is drawn into a **nebulizer** by the effect of the flowing oxidant and fuel gases. The fine droplets produced pass into the flame where sample atoms are progressively excited. The emitted radiation passes through the filter and is detected by a photocell or photomultiplier tube.

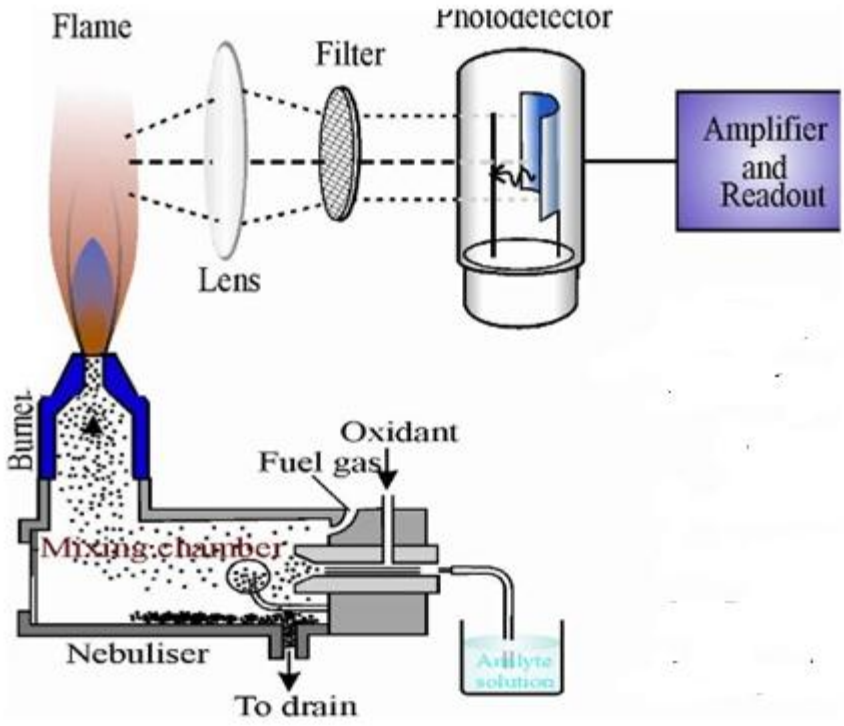



Fig.3 A schematic representation of flame photometer

**Interferences** An interference in a flame is observed when the number of excited species is caused to increase or decrease. Interferences can be classed into two categories, chemical and spectral.
In order to determine the concentration of a metal ion in solution, it is necessary to determine the extent of both spectral and chemical interferences. For most samples the effect is minimized by the addition of the interference to the standards or by a standard addition method.

**Chemical interference** occurs when a species in a flame reacts with the atoms, thus decreasing the emission. An example of this is the reaction between calcium and phosphorous containing molecules. If a solution of calcium and a soluble phosphorous compound is nebulized into a flame, the concentration of calcium atoms would be decreased due to the formation of stable molecules of calcium phosphate $Ca_3(PO_4)_2$ in the flame. Thus the

emission intensity of calcium decreases as the phosphorous concentration is increased. To overcome this problem the following can be proceed:

- Use of another fuel-oxidant mixture (acetylene-oxygen) to obtain higher temperature.

- Addition of chelating agent such as EDTA to form Ca-EDTA complex.

$$Ca_3(PO_4)_2 + 3\ EDTA \rightarrow 3\ Ca(EDTA) + 2\ PO_4^{3-}$$

- Addition of a releasing agent such as $LaCl_3$ to release Ca ions

$$Ca_3(PO_4)_2 + 2\ LaCl_3 \rightarrow 3\ CaCl_2 + 2\ LaPO_4$$

Another example is the analysis of a sample for calcium. Calcium chloride completely atomizes, but calcium sulfate does not. Thus if both of these calcium salts are present in a sample, incomplete atomization would lead to incorrect measurements. The solution for this problem is to add a substance to the sample which would free calcium from the sulfate matrix. The perfect example of this added substance is the element lanthanum. It may seem an unusual application of this inner-transition metal, but lanthanum sulfates are more stable than calcium sulfates, and thus with lanthanum ions present in the solution, the sulfate binds with the lanthanum and calcium ions are free to atomize. Lanthanum is used in a significant number of determinations for this reason.

$$3\ CaSO_4 + 2\ LaCl_3 \rightarrow La_2(SO_4)_3 + 3\ CaCl_2$$

**Spectral interferences** can be observed when the emission of a second species in the flame occurs at the same wavelength as the compound being measured. As an example, consider a solution of calcium and sodium nebulized into the flame where the sodium is to be determined. The sodium emission is measured at 589 nm. From the intensity of emission, it appears that there is more sodium than was actually placed in the solution. The reason for this is that another species, CaO, which is produced by the flame, is also emitting at this wavelength. Combustion products from the fuel and oxidant also have a tendency to interfere with the formation of metal atoms in the flame by converting the atoms into metal oxides and hydroxides. There often very stable molecular species and thus, reduce the metal atom concentration appreciably.
The most common method of solving this problem is to use a different spectral line for the element of interest so that there is no overlap. So-called secondary lines can be found in the literature.

**Applications.** The analysis of alkali and alkaline earth metals by flame photometry (FP) has two major advantages.

1. Their atoms reach the excited state at a temperature lower than that at which most other elements are excited.
2. Their characteristic wavelengths are easily isolated from those of most other elements due to wide spectral separation.

Actually, FP is applied to determine Na, K, Ca, Ba and Li in

- Plant materials, food and beverages.
- Electrolyte in serum.
- Body fluids, blood and urine.
- Nutrient solutions for cultivation of antibiotics.
- Water: natural, spring, waste and river.
- Soil

The above applications include the areas of agriculture, food, mining, metallurgy, pharmaceuticals, pathology, pollution monitoring and research laboratories.

The following is an image for flame photometer along with its description

.

An image for flame photometer

Description

Flame photometry is an atomic emission method for the routine detection of metal salts, principally Na, K, Li and Ca. Quantitative analysis of these species is performed by measuring the flame emission of solutions containing the metal salts. Solutions are aspirated into the flame. The hot flame evaporates the solvent, atomizes the metal, and excites a valence electron to an upper state. Light is emitted at characteristic wavelengths for each metal as the electron returns to the ground state. Optical filters are used to select the emission wavelength monitored for the analyte species. Comparison of emission intensities of unknowns to either that of standard solutions, or to those of an internal standard, allows quantitative analysis of the analyte metal in the sample solution.

Flame photometry is a simple, relatively inexpensive, high sample throughput

method used for clinical, biological, and environmental analysis. The low temperature of the natural gas and air flame, compared to other excitation methods such as arcs, sparks, and rare gas plasmas, limit the method to easily ionized metals. Since the temperature isn't high enough to excite transition metals, the method is selective toward detection of alkaline and alkali earth metals. On the other hand, the low temperatures renders this method susceptible to certain disadvantages, most of them related to interference and the stability (or lack thereof) of the flame and aspiration conditions. Fuel and oxidant flow rates and purity, aspiration rates, solution viscosity, concomitants in the samples, etc affect these. It is therefore very important to measure the emission of the standard and unknown solutions under conditions that are as nearly identical as possible.

## Atomic Absorption Spectrometry

Atomic absorption spectrometry (AAS) is an analytical technique that measures the concentrations of elements, mainly transition metals. The technique makes use of the wavelengths of light specifically absorbed by the target element. Atomic absorption is so sensitive that it can measure trace concentrations, parts per billion (µg /L) of the element in a sample.

**Principles**: The absorption of electromagnetic radiation by atoms allows both qualitative and quantitative determination of a wide range of elements.

In more detail, the energy levels of atoms are specific and determined by the quantum numbers of the element. If ground state atoms are excited, some will be promoted to higher energy levels, the transitions being characteristic of the element involved. Atoms may be excited by incident UV or visible electromagnetic radiation, and if the wavelength (or frequency) corresponds to that of the transition, it will be absorbed. The degree of absorbance will depend on concentration, in the same way as with other spectrometric techniques. This technique is known as **atomic absorption spectrometry (AAS)**. The sample is generally volatilized by a flame or furnace. **The temperature is not usually sufficient to produce ionization, so that the vapor contains largely atoms. These atoms absorb the characteristic incident radiation resulting in the promotion of their electrons to an excited state.**

As implied previously, only a very small number of the atoms in the flame are actually present in an excited state at any given instant. Thus there is a large percentage of atoms that are in the ground state and available to be excited by some other means, such as a beam of light from a light source. AA takes advantage of this fact and uses a light beam to excite these ground state atoms in the flame. Thus AA is very much like molecular absorption spectrophotometry in that light absorption (by these ground state atoms) is measured and related to concentration. The major differences lie in instrument design, especially with respect to the light source, the "sample container," and the placement of the monochromator.

The light source, called a hollow cathode tube, is a lamp that emits exactly the wavelength required for the analysis (without the use of a monochromator). The light is directed at the flame containing the sample, which is aspirated by the same method as in FP. The flame is typically wide (4-6 inches), giving a reasonably long pathlength for detecting small concentrations of atoms in the flame. The light beam then enters the monochromator, which is tuned to a wavelength that is absorbed by the sample. The detector measures the light intensity, which after adjusting for the blank, is output to the readout, much like in a single beam molecular instrument. Also as with the molecular case, the absorption behavior follows Beer's Law and concentrations of unknowns are determined in the same way. All atomic species have an absorptivity, a, and the width of the flame is the pathlength, b. Thus, absorbances (A) of standards and samples are measured and concentrations determined as with previously presented procedures, with the use of Beer's Law ($A = a\ b\ c$ ).

**Table 1: Comparsion of AA and FP**

| | AA | FP |
|---|---|---|
| **Process measured** | Absorption (light absorbed by unexcited atoms in flames) | Emission (light emitted by excited atoms in aflame) |
| **Use of flame** | Atomization | Atomization and excitation |
| **Instrumentation** | Light source | No light source (independent of flame) |
| **Bear' s Law** | Applicable | No applicable (I= KC) |
| **Data obtained** | A vs. C | I vs. C |

**Mode of Working**

Atoms of different elements absorb characteristic wavelengths of light. Analyzing a sample to see if it contains a particular element means using light from that element. For example with lead, a lamp containing lead emits light from excited lead atoms that produce the right mix of wavelengths to be absorbed by any lead atoms from the sample.

In AAS, the sample is atomized – i.e. converted into ground state free atoms in the vapor state – and a beam of electromagnetic radiation emitted from excited lead atoms is passed through the vaporized sample. Some of the radiation is absorbed by the lead atoms in the sample. The greater the number of atoms there is in the vapor, the more radiation is absorbed. The amount of light absorbed is proportional to the number of lead atoms. A calibration curve is constructed by running several samples of known lead concentration under the same conditions as the unknown. The amount the standard absorbs is compared with the calibration curve and this enables the calculation of the lead concentration in the unknown sample.

The calibration curve shows the concentration against the amount of radiation absorbed (Fig. 8(a)). The sample solution is fed into the instrument and the unknown concentration of the element – e.g. lead – is then displayed on the calibration curve (Fig. 8(b))

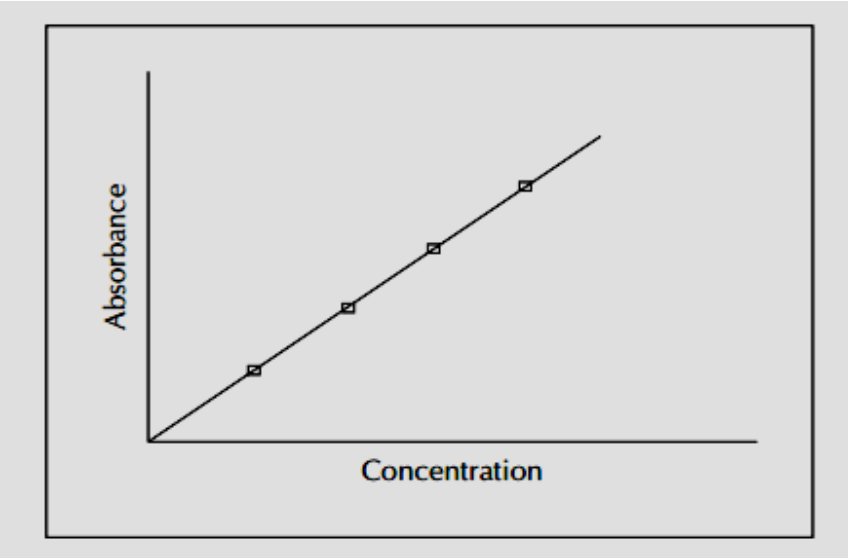



Fig. 8.a

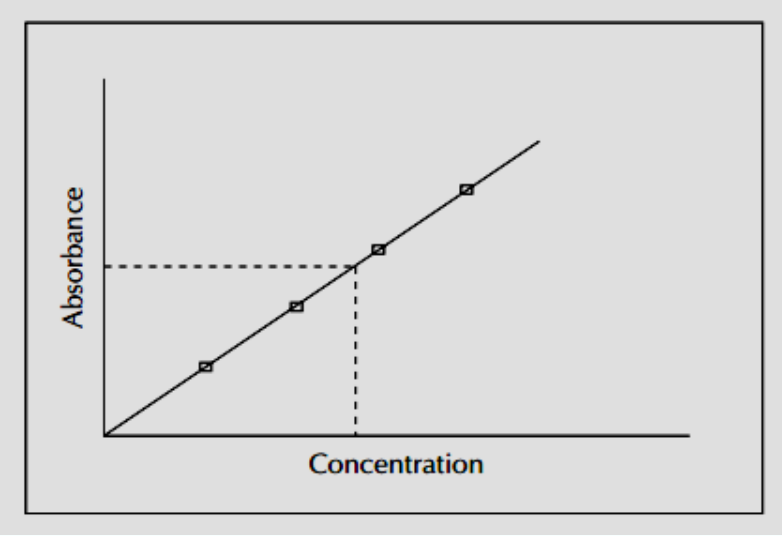



Fig. 8.b

Consequently an atomic absorption spectrometer incorporates the following main components:

A light source; a system to produce gaseous atoms; and a means of measuring the specific light absorbed.

**1- A light source.** Hollow cathode lamp ( HCL)

The hollow cathode lamp is the common source of light (Fig. 1).

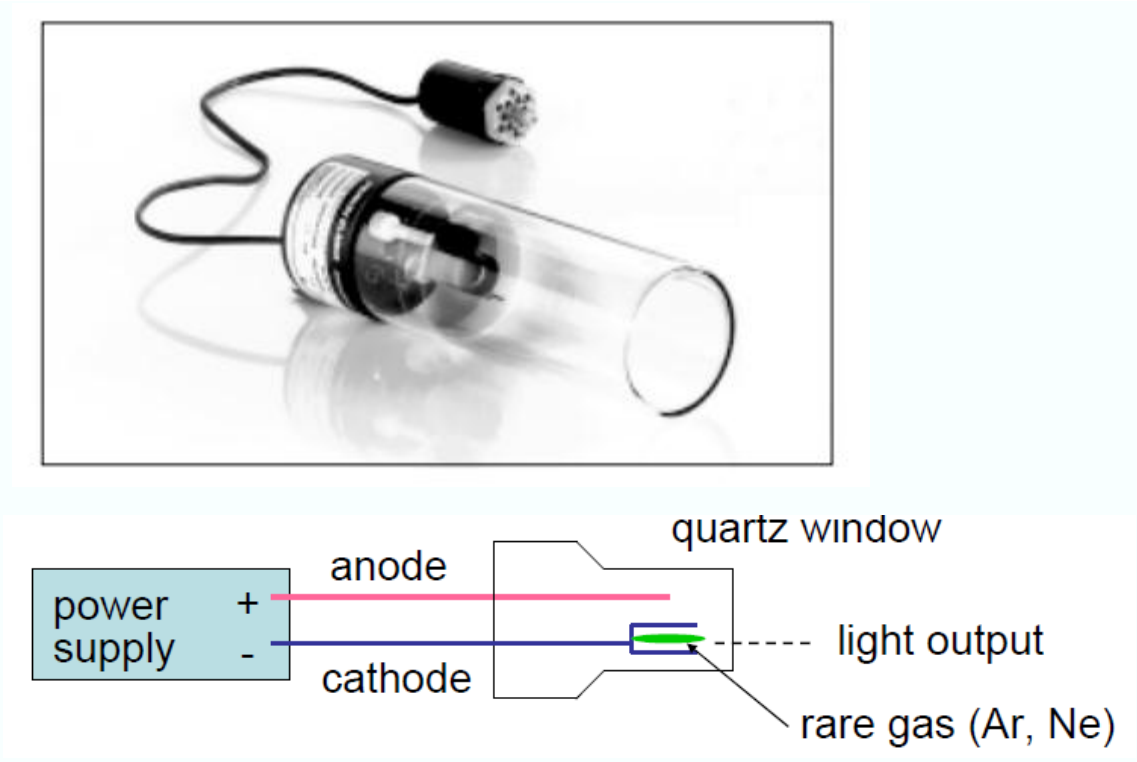



Fig. Hollow cathode lamp (HCL)

HCL contains a tungsten anode and a cylindrical hollow cathode made of the element to be determined. These are sealed in a glass tube filled with an inert gas ( Neon or Argon) at a pressure of between 4.0 – 10 atm. The ionization of some gas atoms occurs by applying a potential difference of about 300–400 V between the anode and the cathode. These gaseous ions bombard the cathode and eject metal atoms from the cathode in a process called sputtering. Some sputtered atoms are in excited states and emit radiation characteristic of the metal as they fall back to the ground state – e.g. Pb* → Pb + h (Fig. 2).

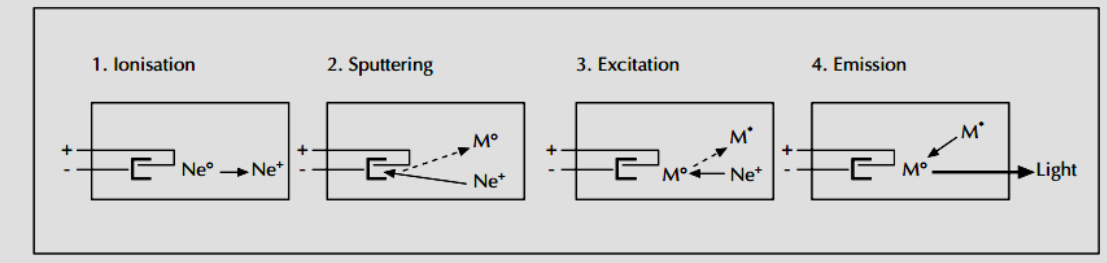



Fig. 2 Production of a characteristic light by the HCL ( the cathode made from the same element to be analyzed)

The shape of the cathode concentrates the radiation into a beam which passes through a quartz window. A typical atomic absorption instrument holds several lamps each for a different element. The lamps are housed in a rotating turret so that the correct lamp can be quickly selected.

**Hollow cathode lamp(HCL) is a source of electromagnetic radiation specific for the element to be analyzed . HCL is a glass envelop with a window of silica consists of Anode (Tungesten wire) & Cathode ( made from the same metal to be determined) , filled with inert gas He or Ar under pressure 4-10 torr. The ionized gas produced due the high voltage between the cathode and anode will be directed towards the cathode, hitting its surface causing elimination of some metallic atoms in the excited state. These excited atoms on going to ground state will emitte specific radiation which will be absorbed selectively by the metal atoms aimed to be determined**.

## 2- The optical system and detector

A monochromator is used to select the specific wavelength of light – i.e. spectral line – which is absorbed by the sample, and to exclude other wavelengths. The selection of the specific light allows the determination of the selected element in the presence of others. The light selected by the monochromator is directed onto a detector that is typically a photomultiplier tube. This produces an electrical signal proportional to the light intensity (Fig. 3).

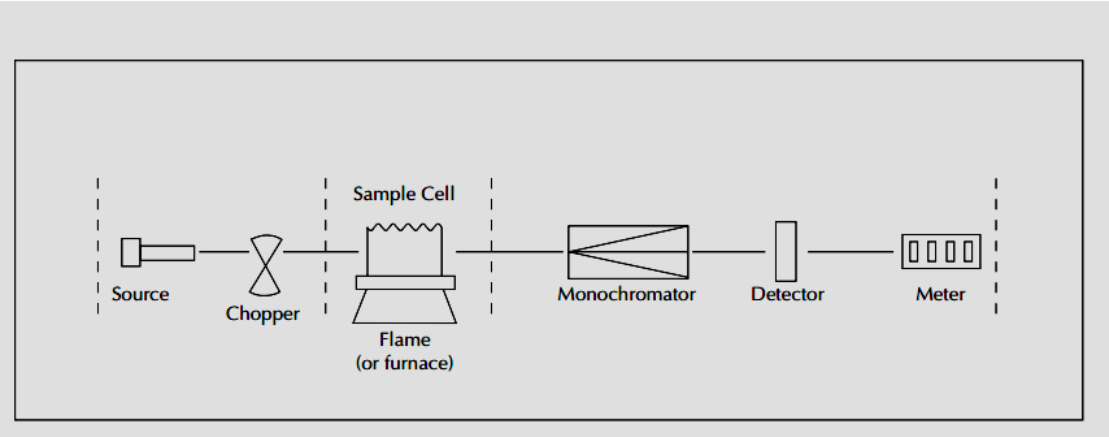



Fig. Optical system and detector in a a single beam AAS.

Double beam spectrometers

Modern spectrometers incorporate a beam splitter so that one part of the beam passes through the sample cell and the other is the reference (Fig. 4). The intensity of the light source may not stay constant during an analysis. If only a single beam is used to pass through the atom cell, a blank reading containing no analyte (substance to be analyzed) would have to be taken first, setting the absorbance at zero. If the intensity of the source changes by the time the sample is put in place, the measurement will be inaccurate. In the double beam instrument there is a constant monitoring between the reference beam and the light source. To ensure that the spectrum does not suffer from loss of sensitivity, the beam splitter is designed so that as high a proportion as possible of the energy of the lamp beam passes through the sample.

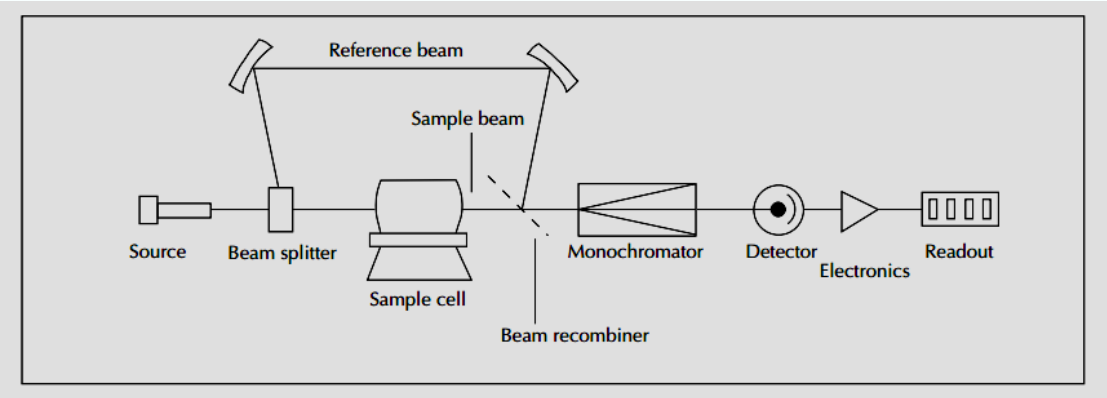
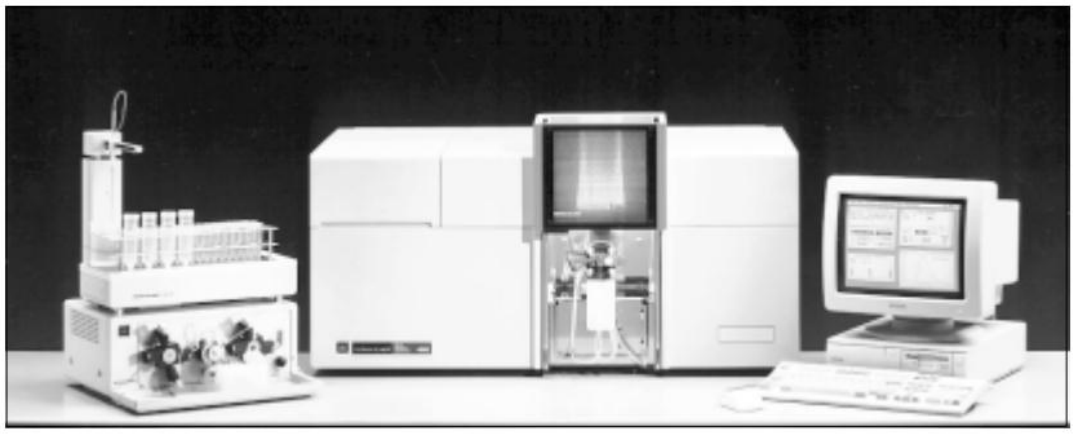



Fig. Schematic representation of a double beam AAS



An image of a Flame Atomic Absorption Spectrometry (FAAS)

**2- A system for atomization of sample solution**

Atomization of the sample

In flame vaporization, the **sample** is usually prepared as a solution that is sprayed into the burner.

A flow spoiler removes large droplets, and the sample undergoes a similar sequence of events to that described in flame photometry, converting it into gaseous atoms.

The signal reaches a constant value proportional to the concentration of the analyte element in the sample.

The **flame** is generally produced using one of the gas mixtures given in Table 2. Air-acetylene gives a flame temperature of about 2400 K, while air-propane is cooler (~1900 K), and nitrous oxide-acetylene hotter (~ 2900 K).
The structure and temperature of the flame is most important, as is the alignment of the optical path with the region of the flame in which an optimum concentration of the atoms of analyte is present.

Variations in flame temperature, including those caused by cooling due to the sample,

will affect the sensitivity of the technique. Flame sources have advantages for analysis where large volumes of analyte are available.

An alternative vaporization method is to use a **graphite furnace,** which is an open-ended cylinder of graphite placed in an electrically heated enclosure containing argon to prevent oxidation. Temperatures in the region of 2500 K are achieved, and the heating program is designed to heat the sample, deposited on a smaller tube or **L'Vov platform,** by radiation. The graphite furnace produces a peak signal whose area is proportional to the total amount of vaporized element in the sample.

The use of graphite furnace atomic absorption (**GFAA)** has a number of advantages:
● it avoids interactions between the sample components and the flame since atomization takes place in an inert gas stream;
● it gives increased sensitivity because of the longer residence time of the sample in the beam from the source;
● the sensitivity is further increased because a higher proportion of atoms are produced;
● it has the ability to handle small volumes of samples, down to 0.5-10 *m*l, such as clinical specimens;
● the results are more reproducible than flame AAS.
One disadvantage is that it is rather slower that flame AAS.

Another approach is **hydride generation,** as certain elements, such as arsenic, tin and selenium, have volatile hydrides. By removing all organic matter by oxidation, and then reducing the sample with sodium borohydride, $NaBH_4$, the volatile hydride is produced and can be swept out into the radiation path using argon.

Atomization of the sample

Two systems are commonly used to produce atoms from the sample. Aspiration involves sucking a solution of the sample into a flame; and electrothermal atomization, where a drop of sample is placed into a graphite tube that is then heated electrically.

Some instruments have both atomisation systems but share one set of lamps. Once the appropriate lamp has been selected, it is pointed towards one or other atomisation system.

Flame aspiration Figure 5 shows a typical burner and spray chamber. Ethyne/air (giving a flame with a temperature of 2200–2400 °C) or ethyne/dinitrogen oxide (2600–2800 °C) are often used. A flexible capillary tube connects the solution to the nebuliser. At the tip of the capillary, the solution is 'nebulised' – ie broken into small drops. The larger drops fall out and drain off while smaller ones vaporise in the flame. Only ca 1% of the sample is nebulised.

Electrothermal atomization

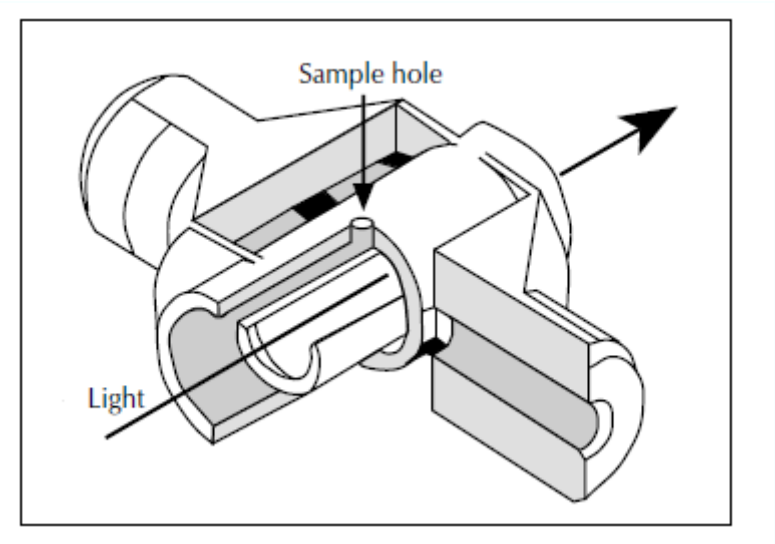



Fig. A hollow graphite tube incorporated GF-AAS

Figure 6 shows a hollow graphite tube with a platform. 25 µl of sample (ca 1/100th of a raindrop) is placed through the sample hole and onto the platform from an automated micropipette and sample changer. The tube is heated electrically by passing a current through it in a pre-programmed series of steps. The details will vary with the sample but typically they might be 30–40 seconds at 150 °C to evaporate the solvent, 30 seconds at 600 °C to drive off any volatile organic material and char the sample to ash, and with a very fast heating rate (ca 1500 °C s-1 ) to 2000– 2500 °C for 5–10 seconds to vaporize and atomize elements (including the element being analysed). Finally heating the tube to a still higher temperature – ca 2700 °C – cleans it ready for the next sample. During this heating cycle the graphite tube is flushed with argon gas to prevent the tube burning away. In electro thermal atomization almost 100% of the sample is atomized. This makes the technique much more sensitive than flame AAS

Sample preparation

Sample preparation is often simple, and the chemical form of the element is usually unimportant. This is because atomization converts the sample into free atoms irrespective of its initial state. The sample is weighed and made into a solution by suitable dilution. Elements in biological fluids such as urine and blood are often measured simply after a dilution of the original sample.

Figure 7 shows an image for a flame atomic absorption spectrometer with an auto sampler and flow injection accessory.

**Interferences.** Other chemicals that are present in the sample may affect the atomization process. For example, in flame atomic absorption, phosphate ions may react with calcium ions to form calcium pyrophosphate. This does not dissociate in the flame and therefore results in a low reading for calcium. This problem is avoided by adding different reagents to the sample that may react with the phosphate to give a more volatile compound that is dissociated easily. Lanthanum nitrate solution is added to samples containing calcium to tie up the phosphate and to allow the calcium to be atomized, making the calcium absorbance independent of the amount of phosphate. With electro thermal atomization, chemical modifiers can be added which react with an interfering substance in the sample to make it more volatile than the analyte compound. This volatile component vaporizes at a relatively low temperature and is removed during the low and medium temperature stages of electro thermal atomization.

**Applications:** Atomic absorption spectrometry has many uses in different areas of chemistry.

**Clinical analysis.** Analyzing metals in biological fluids such as blood and urine.

Environmental analysis. Monitoring our environment – e.g. finding out the levels of various elements in rivers, seawater, drinking water, air, petrol and drinks .

**Pharmaceuticals.** In some pharmaceutical manufacturing processes, minute quantities of a catalyst used in the process (usually a metal) are sometimes present in the final product. By using AAS the amount of catalyst present can be determined.

**Industry.** Many raw materials are examined and AAS is widely used to check that the major elements are present and that toxic impurities are lower than specified – e.g. in concrete, where calcium is a major constituent, the lead level should be low because it is toxic.

Mining. By using AAS the amount of metals such as gold in rocks can be determined to see whether it is worth mining the rocks to extract the gold.

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**Method of Standard Additions**

Due to the effects of other constituents in a sample, such as we have just noted in the previous section and in previous chapters, it is always desirable to match the blank and standards to the sample as much as possible. With AA, the sample preparation is frequently so simple that samples to be tested are aspirated directly into the flame and measured. One can imagine, for example, an environmental water sample (from a well, creek, pond, etc.) being brought into the lab and aspirated directly into the flame. In a case such as this, one may have no quantitative idea as to what the total, or even partial matrix composition might be, and thus blank and standards compositions which do not match the sample matrix are prepared, and the analysis results cannot be considered reliable.

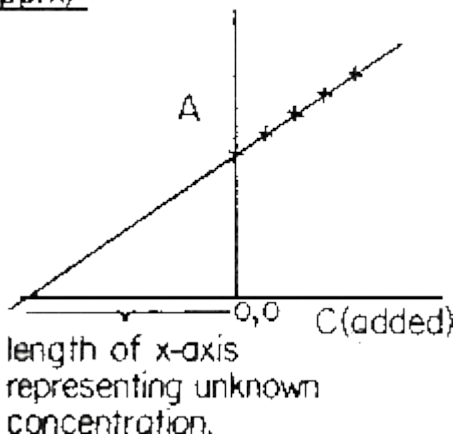
The solution to this problem is to use the method of standard additions. In this method, small amounts of a standard solution of the element being determined are added to the sample and the absorbance measured after each addition. In this way, the sample matrix is always present, and interfering sample components affect the observance equally with each measurement. Therefore there is no effect on the outcome and the total sample composition need not be known.

The plotting procedure and the use of the graph for obtaining the sample concentration is altered somewhat, however, The Beer's Law plot is a graph of A vs. concentration *added.* Since the element being determined is present in the sample from the start, a bona fide absorbance reading is measured for the sample to which nothing has been added. As more and more analyte is added, the absorbance reading simply increases (linearly) so that a graph, which does not intersect zero (at zero added concentration) is plotted. (as seen below.) Extrapolation of the graph to zero absorbance, as shown, results in a length of x-axis, on the negative*side* of zero added, which represents the concentration in the unknown.

Sample Data

| A | C(added)(ppm) |
|---|---|
| 0.210 | 0 |
| 0.256 | 1 |
| 0.298 | 2 |
| 0.341 | 3 |
| 0.378 | 4 |

Sample Graph




Sample data and graph for a "standard addition" experiment in AA.

**SUMMARY of Atomic Techniques**

A discussion of specific applications of the variety of atomic techniques presented in this paper and the application of the various atomic techniques is briefly summarized here.

The most important and obvious point to be made is that these techniques are indeed *atomic.* This means that they cannot be applied to analytes that are molecular in nature. Atomic techniques are limited to ions of metals-those species, which can be atomized. Ions of nonmetals can be analyzed too, but only by an indirect method. An example would be the determination of chloride by measuring the silver ion before and after precipitation of the chloride. Silver can be measured directly; chloride cannot.

**Flame AA**

This technique has been the most popular of all atomic techniques, and continues to be so, given the expense of the improved techniques, such as ICP. The instrumentation technology has been in place for a long time and advantages, disadvantages, and sensitivities for particular metals are well known.

**Graphite Furnace AA**

This technique should be used only when the sample size is small and/ or when a greater sensitivity is needed. It should not be used when ordinary flame AA would do as well, since there are disadvantages relating to sample size and precision.

**Flame Photometry**

There is no real clear-cut advantage or disadvantage of this technique. In terms of sensitivity, some metals are better analyzed by FP than by AA. However, there is an equal number that are better analyzed by AA. Also, there are a number of metals that are analyzed with about equal sensitivity.

**ICP**

As previously stated, this technique offers many advantages over the others. Increases in sensitivity and linear range are the most important. The instruments, however, are more costly.

**Atomic Fluorescence**

This technique does offer some advantages, especially in terms of sensitivity, in a few cases but has not "caught on," since the other instruments are so available and popular.

**Spark or Are Emission**

This technique requires a solid sample and is very useful for qualitative analysis. Quantitative analysis procedures, however, have been documented, but are less popular than the others, given the need for a solid sample and difficulties in preparing homogeneous solid standards. Also, reproducing excitation conditions for a series of standards and the samples is difficult.

**Disadvantages of Flame Atomic Absorption Spectroscopy**

- only solutions can be analysed
- relatively large sample quantities required (1 – 2 mL)
- less sensitivity (compared to graphite furnace)
- problems with refractory elements

**Advantages**
 inexpensive (equipment, day-to-day running)
 high sample throughput
 easy to use

 high precision

**Graphite furnace atomic absorption spectroscopy**

Sample holder: graphite tube

Samples are placed directly in the graphite furnace which is then electrically heated.
Beam of light passes through the tube.

Three stages:
1. drying of sample
2. ashing of organic matter
3. vaporization of analyte atoms

to burn off organic species that would interfere with the elemental analysis.
Molecules have broad absorption bands!

**Stages in Graphite Furnace**
typical conditions for Fe:

drying stage: 125・C for 20 sec

ashing stage: 1200・C for 60 sec

vaporization: 2700・C for 10 sec

**The cathode contains the element that is analysed.**

・ Light emitted by hallow-cathode lamp has the same wavelength as the light absorbed by the analyte element.

・ Different lamp required for each element (some are multielement)

**Hollow-cathode lamps are discharge lamps that produce narrow emission from atomic species.**
Atomic absorption and emission linewidths are inherently narrow. Due to low pressure and lower temperature in the

lamp, lines are even narrower than those of analyte atoms.

Table 1. Comparison of **AA and FP**

| | AA | FP |
|---|---|---|
| Process Measured | absorption (light absorbed by unexcited atoms in flames) | emission (light emitted by excited atoms in a :flame) |
| Use of Flame | atomization | atomization and excitation |
| Instrumentation | light source | no light source (independent of flame) |
| Beer's Law | applicable | not applicable (I=kc) |
| Data Obtained | A vs. c | I vs. c |

## ATOMIC ABSORPTION SPECTROPHOTOMETRY

As implied previously, only a very small number of the atoms in the flame are actually present in an excited state at any given instant. Thus there is a large percentage of atoms that are in the ground state and available to be excited by some other means, such as a beam of light from a light source. AA takes advantage of this fact and uses a light beam to excite these ground state atoms in the flame. Thus AA is very much like molecular absorption spectrophotometry in that light absorption (by these ground state atoms) is measured and related to concentration. The major differences lie in instrument design, especially with respect to the light source, the "sample container," and the placement of the monochromator.

The light source, called a hollow cathode tube, is a lamp that emits exactly the wavelength required for the analysis (without the use of a monochromator). The light is directed at the flame containing the sample, which is aspirated by the same method as in FP. The flame is typically wide (4-6 inches), giving a reasonably long pathlength for detecting small concentrations of atoms in the flame. The light beam then enters the monochromator, which is tuned to a wavelength that is absorbed by the sample. The detector measures the light intensity, which after adjusting for the blank, is output to the readout, much like in a single beam molecular instrument. Also as with the molecular case, the absorption behavior follows Beer's Law and concentrations of unknowns are determined in the same way. All atomic species have an absorptivity, a, and the width of the flame is the pathlength, b. Thus, absorbances (A) of standards and samples are measured and concentrations determined as with previously presented procedures, with the use of Beer's Law (A = a b c ).

# PART (2)

# Electrochemical Method

## الطرق الكهروكيميائية

## المعايرات الجهدية

### Nernst Equation : قانون نرنست

$$E = E^{\circ} + \frac{RT}{nF} \; ln \; \frac{[oxidized\ form]}{[Reduced\ form]}$$

| | | |
|---|---|---|
| الجهد القياسى : $E^{\circ}$ | , | فرق الجهد : E |
| درجة الحرارة الكلفينية : T | , | الثابت العام للغازات: R |
| كولوم F : 96500 | , | عدد الالكترونات : n |
| نواتج الاختزال : Reduced form , | | نواتج الأكسدة : oxidized form |

لقياس الجهد لقطب معين فى خلية كهربية لابد من استخدام قطب قياسي فى هذه الخلية ومحصلة جهد الخلية يساوى:

$E_{\text{الخلية}}$ = $E_{\text{القطب القياسى}}$ $\pm$ $E_{\text{القطب المجهول}}$

تقاس من الجهاز — معلومة — ??

### Reference Electrodes : الأقطاب القياسية

### 1- قطب الهيدروجين : Hydrogen electrode

$$H_2 \underset{red}{\overset{oxi}{\rightleftharpoons}} 2H^+ + 2e^-$$

$$E = E^{\circ} + \frac{RT}{nF} \; in \; [H^+]^2 \; / \; P_{H_2}$$

$$E = 0.0592 \log[H^+]$$

$$E = -0.0952 \; pH$$

### 2- قطب الزئبق Mercury Electrode ( Colomel )

– له ثلاث أنواع وهو أكثر أنواع الأقطاب القياسية شهرة والأنواع هي:

( 0.1 M KCl , 1 M KCl / محلول مشبع KCl )

$$Hg_2Cl_2 + 2e^- \underset{oxi}{\overset{red}{\rightleftharpoons}} 2Hg° + 2Cl^-$$

$$E = E^\circ + \frac{RT}{F} ln\ K_{sp} - \frac{RT}{F} \ln[Cl^-]$$

**أقطاب تستخدم لقياس الرقم الهيدروجيني :**

**1-** قطب الهيدروجين **(كما سبق)**

**2-** قطب الانيتمون

$$Sb_2O_3 + 6H+ + 6e^- \rightarrow 2Sb_{(s)} + 3H_2O$$

$$E = E^\circ - 0.0592\ pH$$

3- قطب الكين هيدرون

$$E = E^\circ + \frac{RT}{F} \ln[H^+]$$

$$E = E^\circ - 0.\mathbf{0592\ pH}$$

**4-** القطب الزجاجى وهو من الأقطاب اختيارية

هو عبارة عن انبوبة من الزجاج بداخل الأنبوبة محلول مائي حامض حيث يحدث انتشار الأيونات الهيدروجين من الخارج (المحلول الخارجي) ( المجهول التركيز ) إلي الداخل المعلوم التركيز أو العكس حسب المنطقة الأكثر تركيزاً والأقل تركيزاً أثناء عملية الانتقال هذه ينشأ فرق في الجهد يطلق عليه ( $E_{glass}$ )

أننا نتعامل مع أيونات هيدروجين فهذا يشبه قطب الهيدروجين . لذلك يعتبر

$$E^\circ = 0$$

$$E_{glass} = \frac{RT}{F} \ln \frac{[H^+]_1}{[H^+]_2} \quad (n = 1)$$

$$E_{glass} = 0.0592\ log\ \frac{[H^+]_1}{[H^+]_2}$$

$$E_{glass} = 0.0592\ log\ ([H^+]_1 - log\ [H^+]_2)$$

$$E_{glass} = 0.0592 \ ( PH_2 - PH_1 )$$

## التحليل الجهدي : Potentiometer Analysis

## ***( تفاعلات الترسيب ) Precipitation titrations:***

$$A_gNO_3 + NaCl \rightarrow AgCl\downarrow NaNO_3$$

$$Ag\,Cl(s) \rightleftharpoons Ag^+ + Cl^-$$

$$K_{sp} = [\,Ag^+\,]\,[\,Cl^-] = 1.8 \times 10^{-10}$$

## 100 ml 0.1 N $Ag\,NO_3$ with 1N NaCl :

### (a) عند بداية المعايرة At the start

$$E = E^0 + 0.0592 \log [Ag^+]$$

$$E^0 = 0.8\ V \rightarrow \quad E = 0.8 + 0.0592 \log [Ag^+]$$

$$[AgNO_3] = [Ag^+] = 0.1 \rightarrow E = 0.741 \ \text{Volt}.$$

### (b) قبل نقطة النهاية : Before End point

لحساب التركيز الغير متفاعل من الفضة = الحجم الغير متفاعل x تركيزه / الحجم الكلى

نفترض اضافة 1 مل من كلوريد الصوديوم فنجد ان:

(1ml) NaCl يكافئ ( 10ml ) $AgNO_3$

ويكون الحجم الغير متفاعل 90 مل من الفضة ونعوض فى العلاقة السابقة:

$$[Ag^+] = \frac{90 \times 0.1}{101} = 0.738\ volt$$

وبالمثل نفترض اضافة 5 مل و 6 مل و .... وهكذا

**(c) At end point : عند نقطة النهاية**

اضافة 10 ml ≡ 100 mL $AgNO_3$

$K_{sp} = [Ag^+]\,[Cl^-] = 1.8 \times 10^{-10}$

$[Ag^+] = [Cl^-] \neq [AgCl]$ .

$[Ag^+]^2 = 1.8 \times 10^{-10}$ , $[Ag+] = \sqrt{K_{sp}}$

**(d) After end point : بعد نقطة النهاية**

اضافة ( 11ml ) excess of NaCl

نستخدم العلاقة = الحجم الزائد x تركيزه / الحجم الكلى

$$[Cl\text{-}] = \frac{1 \times 1}{111} = 9 \times 10^{-3}\text{N}$$

$$K_{sp} = [Ag+][Cl\text{-}] \rightarrow [Ag+] = \frac{K_{sp}}{9 \times 10^{-3}}$$