

Clinical Instruments

Most of the pathological processes result in chemical changes in the internal environment of the human body. These changes can generally be detected by the analysis of various samples taken from the body. The analysis not only helps in the diagnosis of various ailments but also in determining the progress of treatment and for making a prognosis. Samples taken from the body are analyzed in three different areas within the clinical laboratory set up

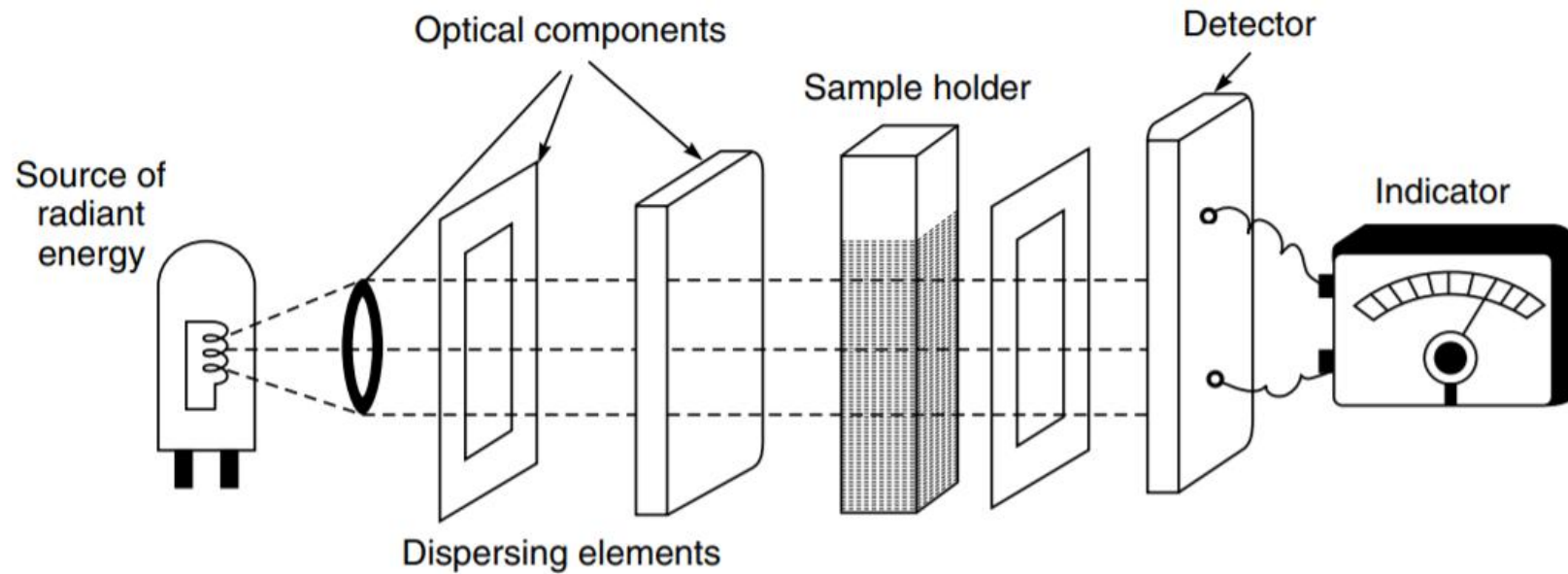
Chemistry section deals with the analysis of blood, urine, cerebrospinal fluid (CSF) and other fluids to determine the quantity of various important substances they contain. Most of the electronic instruments in the clinical laboratory are available in this section.

Haematology section deals with the determinations of the number and characteristics of the constituents of the blood, particularly the blood cells.

Microbiology section in which studies are performed on various body tissues and fluids to determine the presence of pathological micro-organisms.

Spectrophotometer

Spectrophotometer instruments



➤ **Fig. 14.2** *Various components of a spectrophotometer type instrument*

Components of spectrophotometer

- A source of radiant energy, which may be a tungsten lamp, a xenon-mercury arc, hydrogen Or deuterium discharge lamp, etc.
- Filtering arrangement for the selection of a narrow band of radiant energy. It could be a single wavelength absorption filter, an interference filter, a prism or a diffraction grating.
- An optical system for producing a parallel beam of filtered light for passage through an absorption cell (cuvette). The system may include lenses, mirrors, slits, diaphragm, etc.
- A detecting system for the measurement of unabsorbed radiant energy, which could be the human eye, a barrier-layer cell, phototube or photo-multiplier tube.
- A readout system or display, which may be an indicating meter or a numerical display.

Source of Radiation

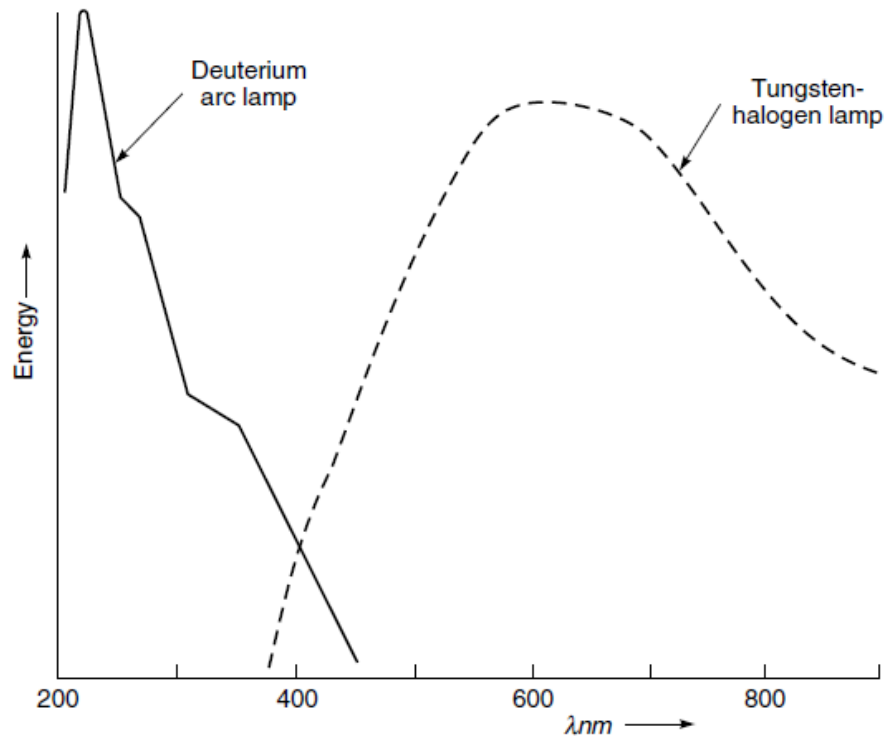
- Tungsten lamp is the most commonly used for radiation in the visible region. Tungsten filament is sealed inside the glass envelope, it emits radiation in the infrared regions also and this causes the heating effect and heat absorbers need to be used before sample holder
- Tungsten lamp is not suited for infrared region as it emits visible radiation
- Tungsten -Halogen lamp can also be used for high intensity radiation
- Deuterium lamp is preferred for UV radiation. 190nm to 380 nm. Quartz or silica gel is used as envelope for the lamp

Optical Filters

- Filter may be considered as any transparent medium which by its structure, composition or colour enables the isolation of radiation of a particular wavelength.
- Ideal filters should be monochromatic, i.e. they must isolate radiation of only one wavelength. A filter must
(a) high transmittance at the desired wavelength and (b) low transmittance at other wavelengths.

Practical filters transmit a broad region of the spectrum.

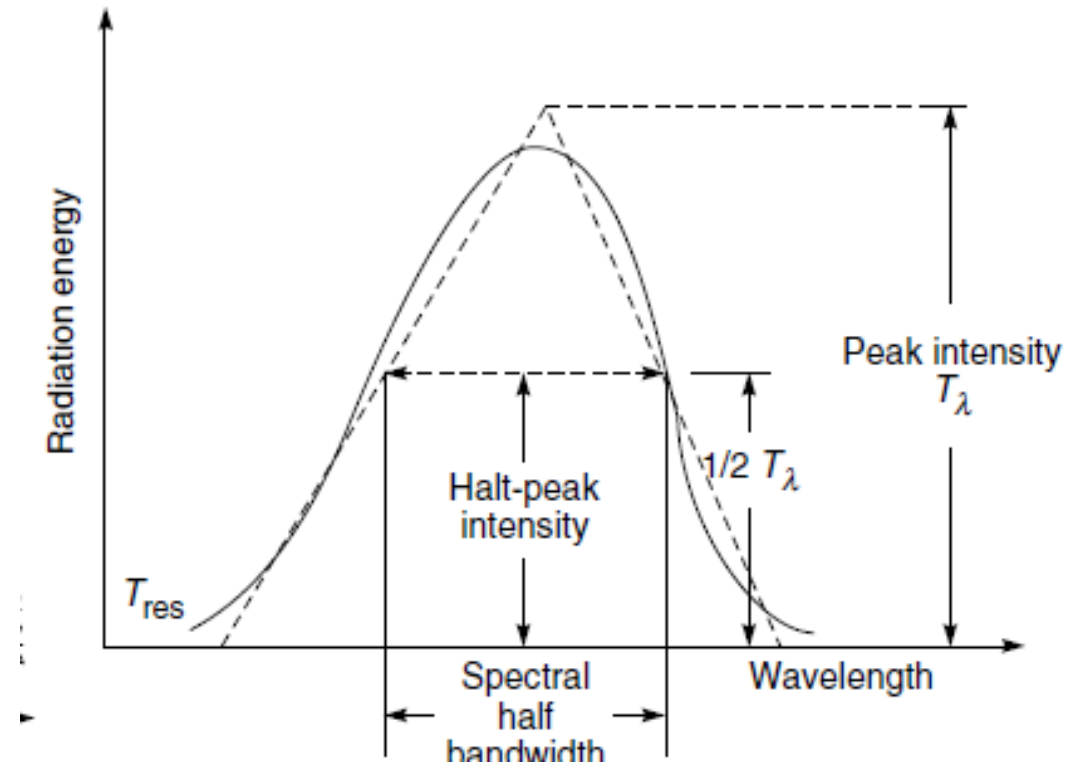
Emission Spectrum of Radiation sources



► Fig. 14.3 Energy output as a function of wavelength for deuterium arc lamp and Tungsten-halogen lamp

Optical Filter continued...

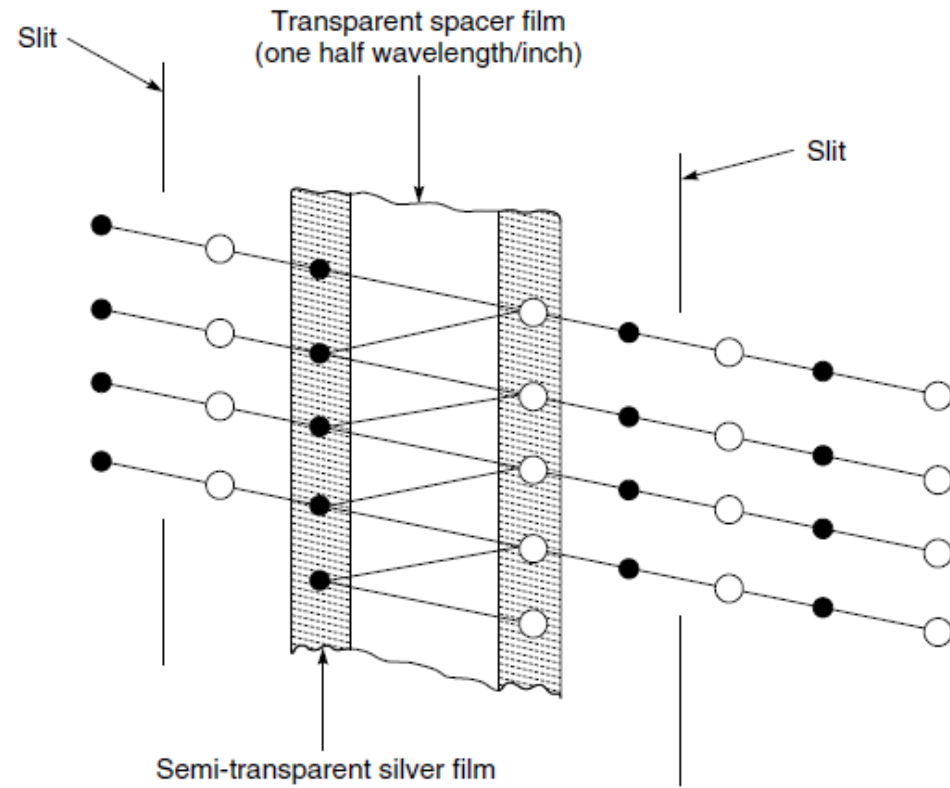
Optical Filters should allow the Maximum wavelength and the Half bandwidth wavelengths



Types of Optical Filters

- Absorption filters and Interference filters
- **Absorption filters** will be coloured glass/gel which absorb the half transmittance wavelength. They have very poor transmittance only upto 25%
- **Interference filters** are layers of silver coated glass separated from each other. The space between the layers is covered with a low refractive index substance, the thickness of this dielectric substance determines the wavelength of the radiation that is transmitted
- The distance of the path in between one wavelength or multiples of wavelength constructive interference takes place and the radiation passes through otherwise destructive interference takes place and the radiation does not pass through

Interference filter



► Fig. 14.5 Path of light rays through an interference filter

Monochromators

- They provide better isolation than filters. They are used when a very narrow band of radiation is required
- They make use of quartz prism or diffraction grating system as the dispersing media
- The efficiency of such monochromators is much better than that of filters and spectral half-bandwidths of 1 nm or less are obtainable in the ultraviolet and visible regions of the spectrum.

Optical Components

Different types of optical components are used in the construction of analytical instruments based on the radiation/ absorption principle. They could be windows/mirrors

The material used in the construction of these components

- Depends largely on the range of wavelength of interest. normally, the absorbance of any material should be less than 0.2 at the wavelength of use.

Some of the materials used are:

- Ordinary silicate glasses : 350 to 3000 nm.
- Special cortex glass: 300 to 350 nm
- Quartz: : Below 300 nm and the limit is 210 nm. (UV)
- Fused silica: 80 to 210 nm,

Photosensitive Detectors

Radiation can be measured by making it fall on a photosensitive detector, which converts the optical energy into electrical energy. The electrical current can be measured by a deflecting galvanometer. There are various types of photosensors. **The photosensor should have a linear spectral response for the radiation of interest.**

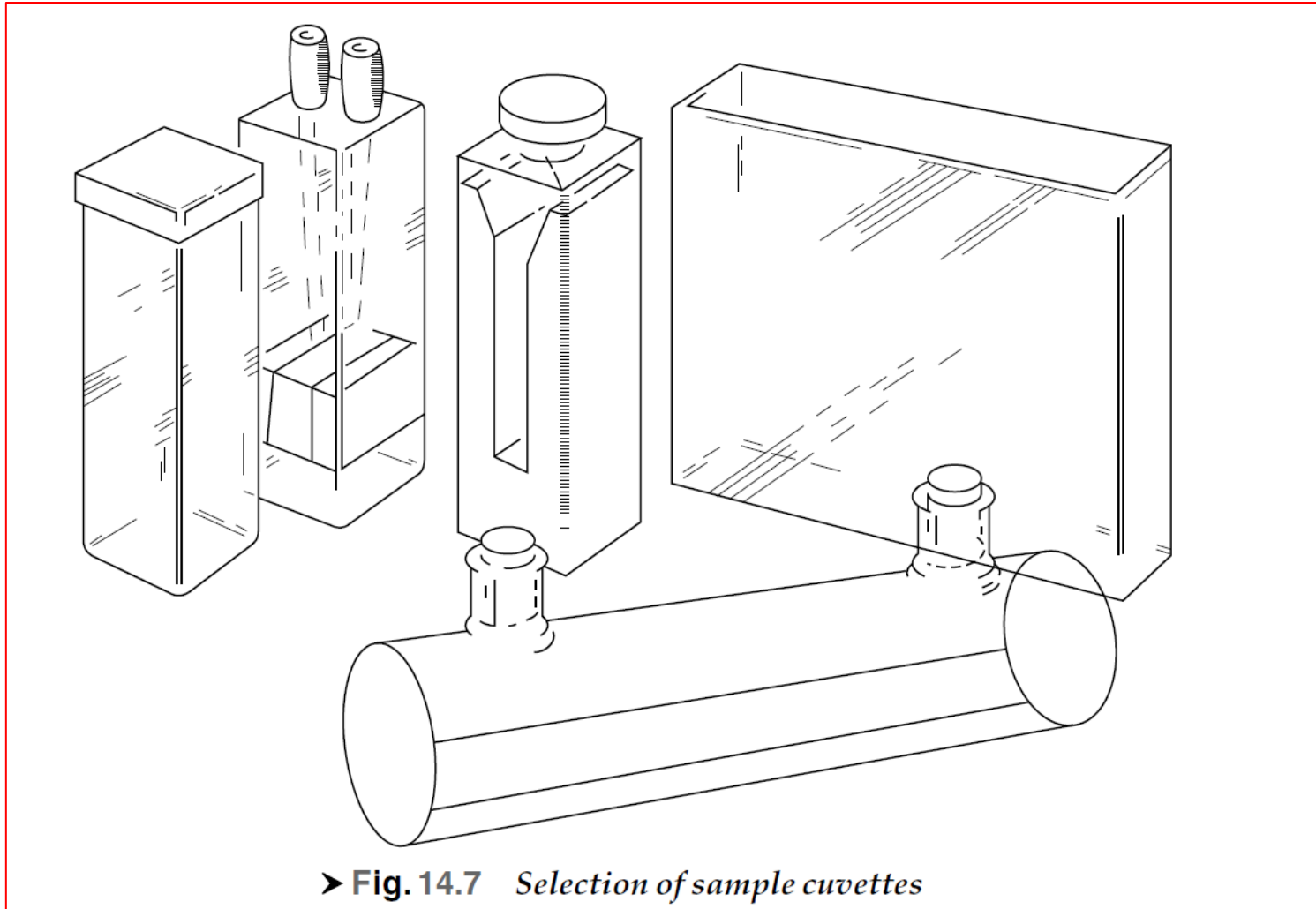
Sample Holders

- Liquids may be contained in a cell or cuvette made of transparent material such as silica, glass or perspex. The faces of these cells through which the radiation passes are highly polished to keep
- reflection and scatter losses to a minimum. Solid samples are generally unsuitable for direct spectrophotometry. It is usual to dissolve the solid in a transparent liquid. Gases may be contained in cells which are sealed or stoppered to make them air-tight. The sample holder is generally inserted somewhere in the interval between the light source and the detector. For the majority of analyses, a 10 mm path-length rectangular cell is usually satisfactory.
- In analyses where only minimal volumes of liquid samples are practical, microcells, which have volumes as small as 50 μ l, can be employed. Most of the rectangular liquid cells have caps and, for the analyses of extremely-volatile liquids, some of the cells have ground-glass stoppers to prevent the escape of vapour.

Sample Holders continued....

- When the traces of components are to be absorbed sample cells of long path are required **50 cm path length and 300 ml** of sample is required

Different Types of sample holders



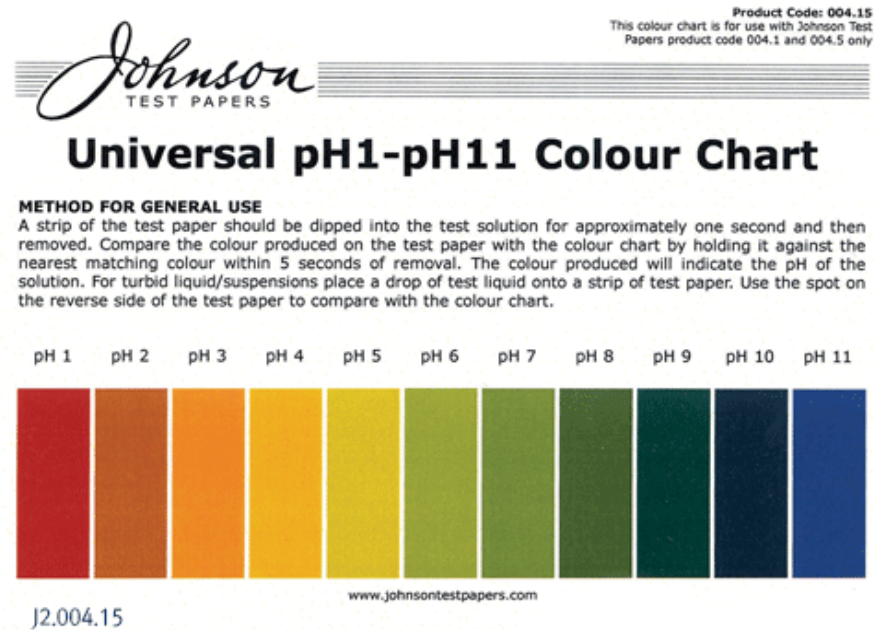
Calorimeter

Colorimeter

- A visual means of matching of colours, with a series of standard solutions and the colour produced by an unknown solution. This method will involve nearly 5-25% of error
- Visual inspection of the colour is termed as colorimeter
- The photoelectric method of measurement has replaced the colorimetric method and eliminates the trouble of preparing the standard solutions

Colour chart

www.ALLforLAB.com



pH 1~11(만능) 표준 색상표

Measurement

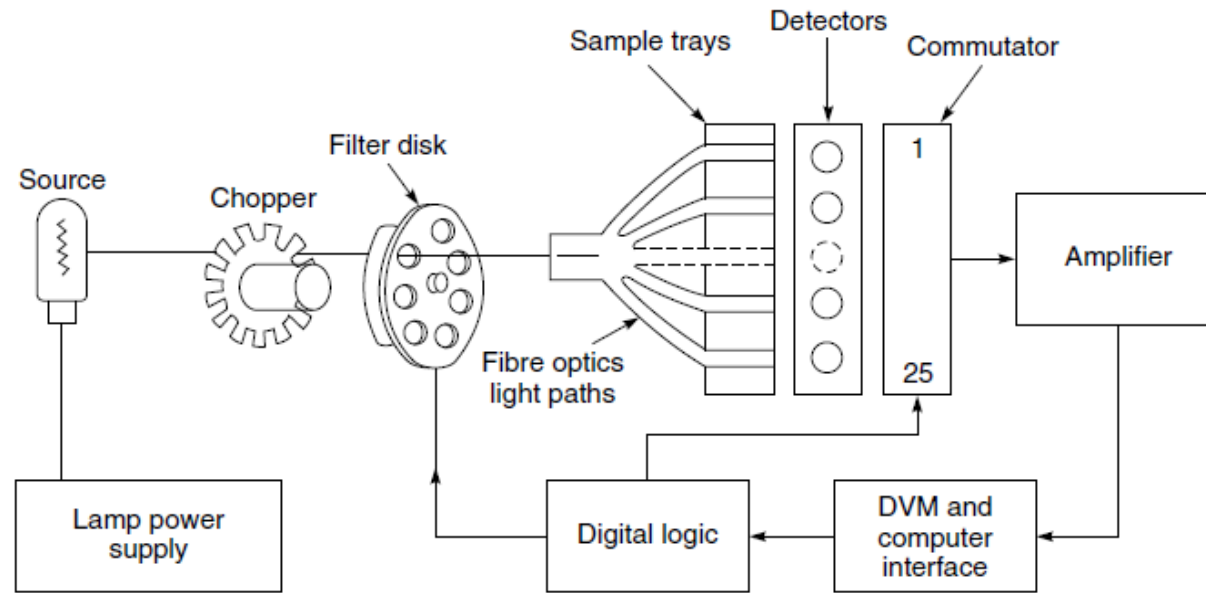
- Sample concentration = Standard concentration * Sample reading/Reference Reading
- With Reference reading a shutter is moved in the light path and adjusted until the meter gives a full scale deflection indicating 100% Transmittance. And then the sample cuvette is moved in place and the transmittance is measured as a percentage of reference reading
- A chart of standard series of colours are required in calorimeter and there would be a comparison for matching of the colours and there will be error 5-25%

The photodetectors can replace the charts and the calorimeter will be called as photometer or spectrophotometer

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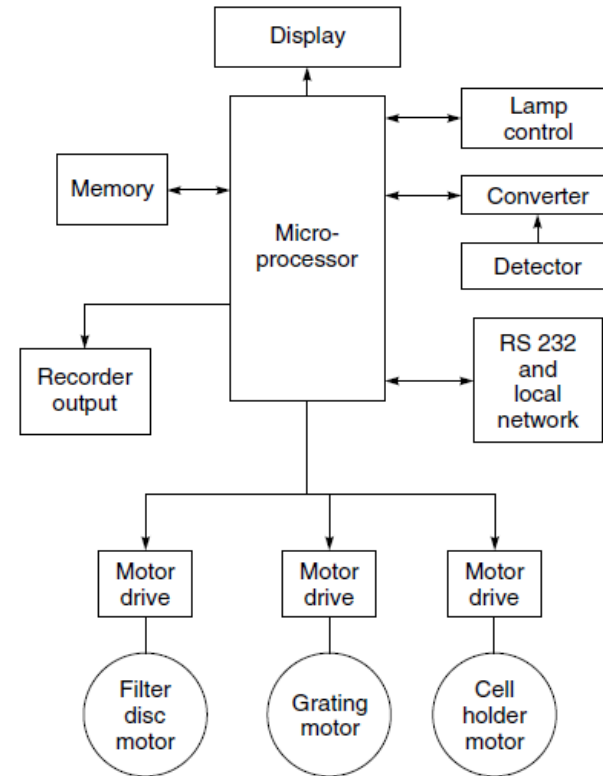
- Simple in construction but the disadvantage is it requires large number of colour filters and colour filters will allow a band of spectrum which will be larger than the absorption spectrum

Multi channel Photometer



➤ Fig. 14.8 Schematic of a multi-channel photometer

Microprocessor Based Spectrophotometer



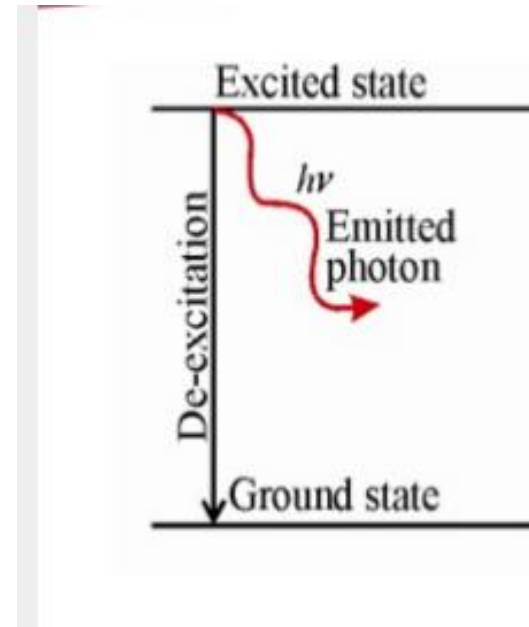
➤ Fig. 14.10 Block diagram of a microprocessor controlled spectrophotometer

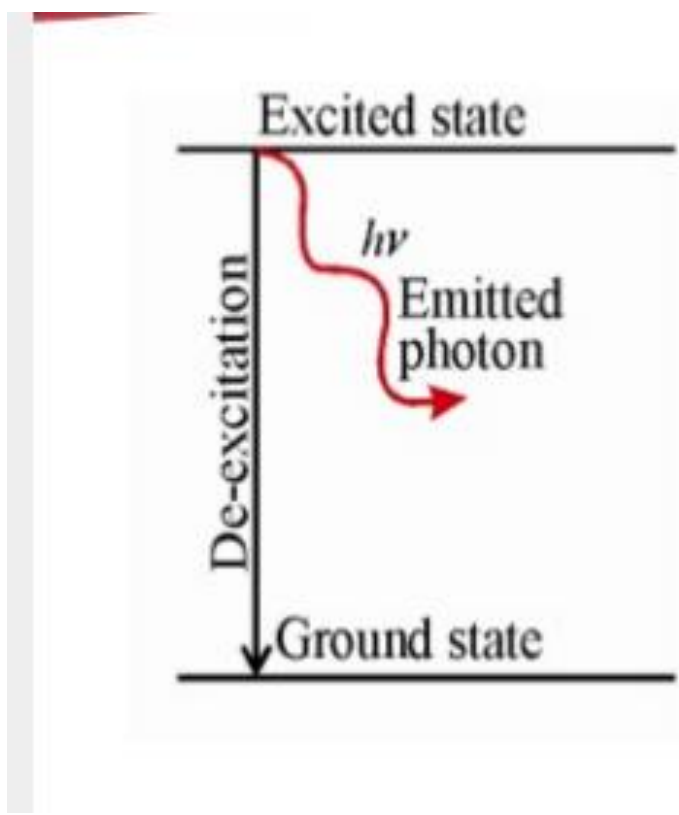
Flame Photometry

- The flame photometer is one of the most useful instruments in clinical analyses. This is due to the suitability of the flame photometer for determining sodium, potassium and calcium, which are of immense importance in the development of the living being and are indispensable for its physiological functions. In the clinical analysis of sodium and potassium, the flame photometer gives, rapidly and accurately, numerous differential data for normal and pathological values.

Principle

- The matters absorb energy at the same wavelength at which they emit the radiation. When a metal solution is burnt it emits a coloured radiation , which is characteristic of the element. The flame photometry can be used to detect the presence or absence of a metal.





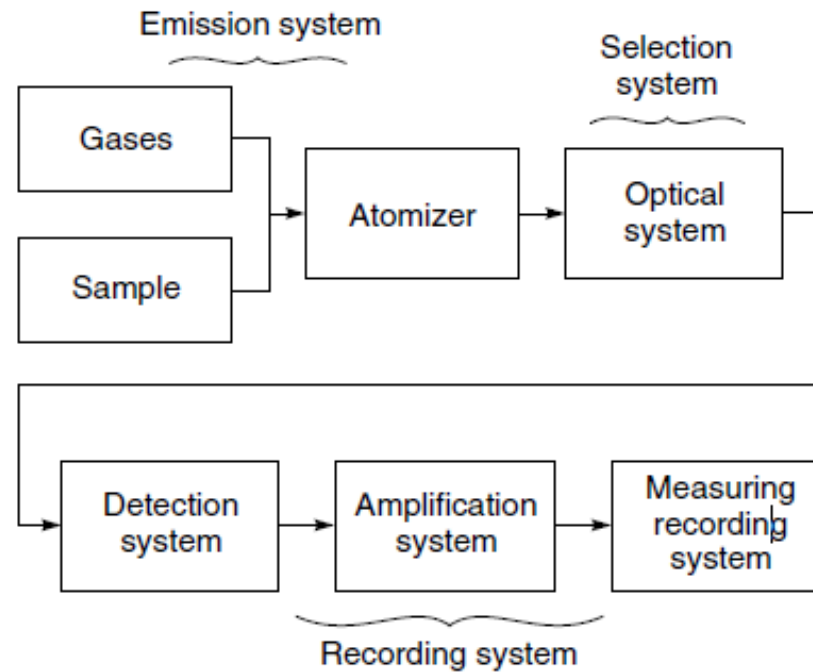
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Procedure

- A solution of the sample to be analyzed is prepared.
- A special sprayer operated by compressed air or oxygen is used to introduce this solution in the form of a fine spray (aerosol) into the flame of a burner operating on some fuel gas, like acetylene or hydrogen.
- The radiation of the element produced in the flame is separated from the emission of other elements by means of light filters or a monochromator.
- The intensity of the isolated radiation is measured from the current it produces when it falls on a photocell.
- The measurement of current is done with the help of a galvanometer, whose readings are proportional to the concentration of the element.
- Galvanometer are calibrated with solutions of known composition and concentration
- The measured intensity is correlated to the intensity of a given spectral line of the unknown sample, with the amount of the same element present in a standard solution.

Components of Flame Photometer



➤ Fig. 14.16 *Essential parts of a flame photometer*

Emission

Emission System:

- (i) **Fuel gases and their regulation:** comprising the fuel reservoir, compressors, pressure regulators and pressure gauges.
- (ii) **Atomizer:** consisting, in turn, of the sprayer and the atomization chamber, where the aerosol is produced and fed into the flame.
- (iii) **Burner:** receives the mixture of the combustion gases.
- (iv) **Flame:** the true source of emission.

Optical System:

It consists of the optical system for wavelength selection (filters or monochromators), lenses, diaphragms, slits etc.

Recording System: It includes detectors like photocells, photo-tubes, photomultipliers, etc.

- and the electronic means of amplification, measuring and recording.

Continued.....

- The sample is mixed with compressed air or oxygen and gets sprayed as aerosol from the atomiser
- The aerosol is sprayed into the burning flame with fuel gases acetylene or hydrogen. The flame has to be maintained at a constant temperature
- The radiation of the element from the flame is separated using monochromators or other filters
- The intensity of radiation is detected by the photo detector

Dedicated instruments for the simultaneous analysis of sodium, potassium and lithium are available. In these instruments, sample handling is automatic, as the system has a turntable, which will hold up to 20 samples in cups and an automatic positive piston displacement dilutor, that dilutes the sample prior to entering the spray chamber.

Flame spray Chamber and Burner

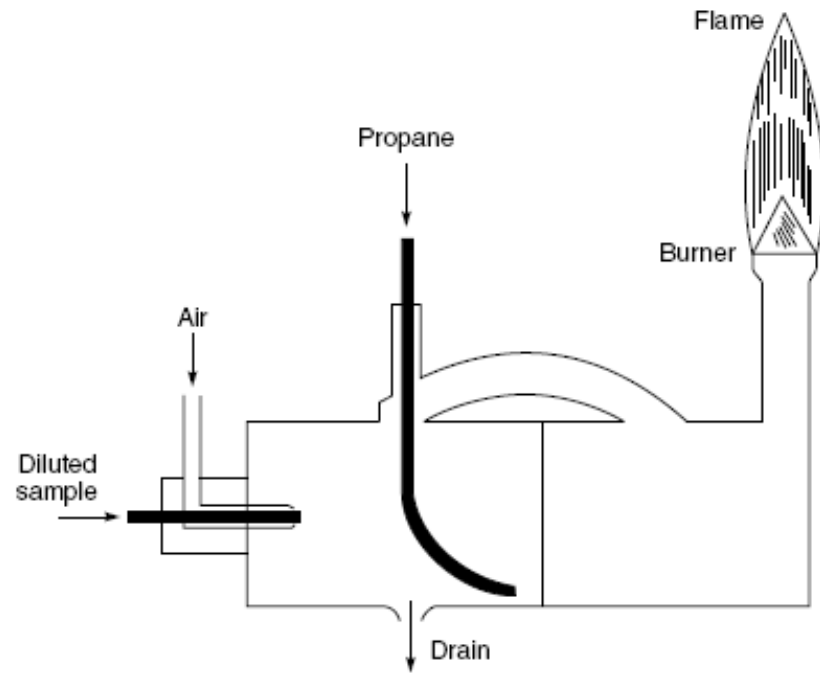




Fig. 14.17 Flame spray chamber and burner of KLiNa flame-photometer (Courtesy: M/s Beckman Instruments, USA)

Advantages

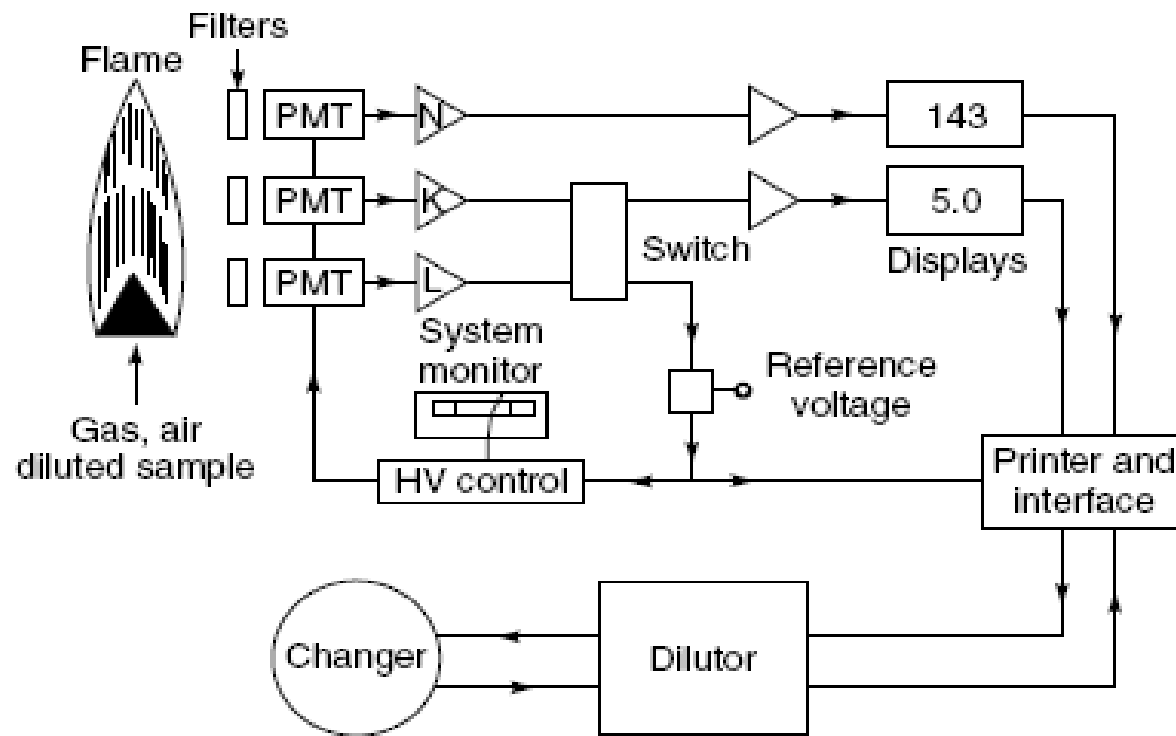
- Both quantitative and qualitative flame analysis can be carried out
- The determination of alkaline and alkaline earth metals can be performed easily and accurately to parts per million (ppm) or even to parts per billion (ppb)
- The concentration of Na^+ , K^+ ions in the body fluid present in muscles and heart can be determined by using the blood sample for flame photometry
- Can be used to determine the Calcium, Sodium, Potassium, lithium lead etc. from the samples of serum, urine and CSF

Disadvantages

- Spectral interferences can occur among different wavelengths can not be resolved from each other. This can happen between the flame and the emitted radiation
- The detailed chemical analysis shows that at high temperature there will be interference among different molecules
- And ionisation can happen and the ions emit different radiation than that of the parent atom
- Non alkali metals can not be determined as they do not emit radiation

Name of the element	Emitted wavelength range (nm)	Observed color of the flame
Potassium (K)	766	Violet 
Lithium (Li)	670	Red 
Calcium (Ca)	622	Orange 
Sodium (Na)	589	Yellow 
Barium (Ba)	554	Lime green 

Flame Photometer



➤ Fig. 14.18 Schematic diagram of a flame photometer

Working

- The flame burns with samples and the optical filters selectively allows the chosen metal. For **Sodium the filter** is narrow band selector **centered at 589 nm** and the detector will respond only to this radiation. For **Potassium the detector will respond to 766nm** and for **Lithium the detector responds to 671 nm**.
- The system accuracy is ± 0.2 mmol/litre for potassium and lithium
- and ± 2.0 mmol/litre for sodium.
- Potassium and lithium both show linearity to 20 mmol/litre, while sodium is linear to 200
- (mole= the amount of any chemical substance that equals the no. of atoms in 12 gms. Of C-12, ex: a blood glucose level of 8.4 **mmol /L**)

Changer and Diluter

- Diluter

The diluter is a motor driven cam-programmed system that functions through a cycle of operations.

These pick-up sample and transport to an internal mixing cup and the addition of a measured volume of diluent, mixing to ensure a properly prepared sample and couples it to the spray chamber so that sample aspiration occurs and finally washing and draining of the mixing cup .

Changer

The automatic changer enables automatic presentation to the diluter sample probe up to 20 successive samples. It is a turntable, which rotates stepwise to locate each sample cup under the extended and down position of the diluter sample probe. The probe is extended from the diluter, once for each sample determination. The probe tip enters the sample and a measured volume is taken for transport to the diluter mixing cup. Individual sample trays are placed on the changer turntable. Each Table can handle upto 20 sample holder

Ion Selective Electrode based Analyser

There are different ions present in body fluids both anions and cations.

The principal blood plasma constituents are Na^+ , K^+ , Ca^{2+} Mg^{2+} and anions are Cl^- and HCO_3^-

The alteration in the concentration is going to vary the blood pH which is around 7.3 to 7.4

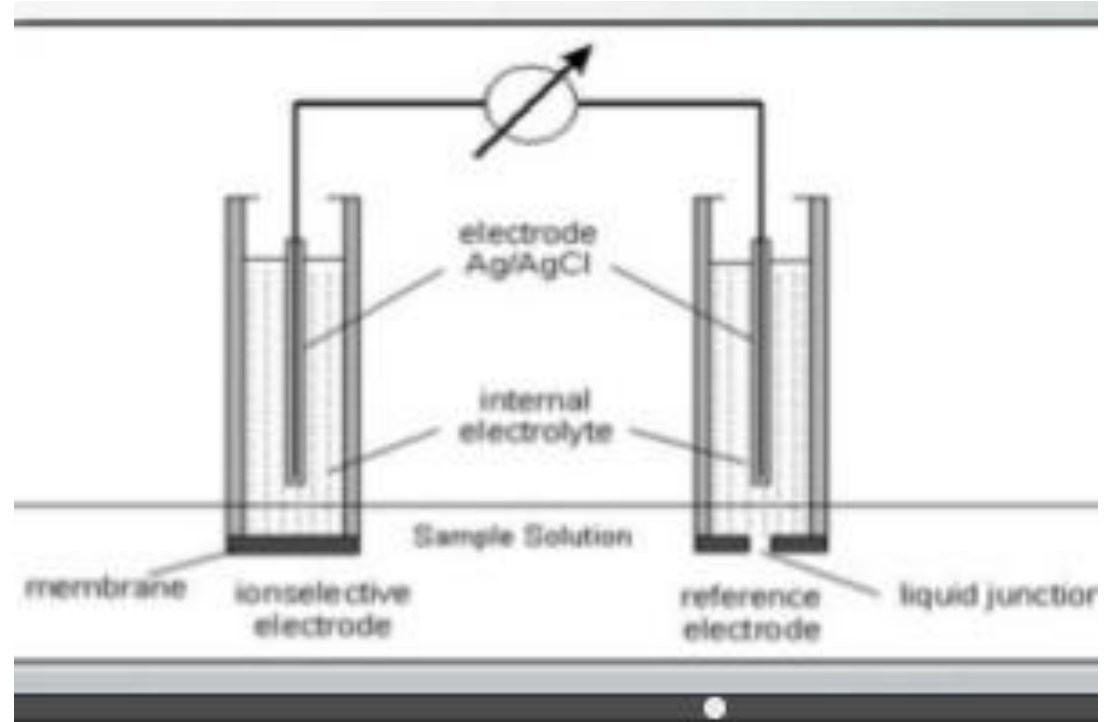
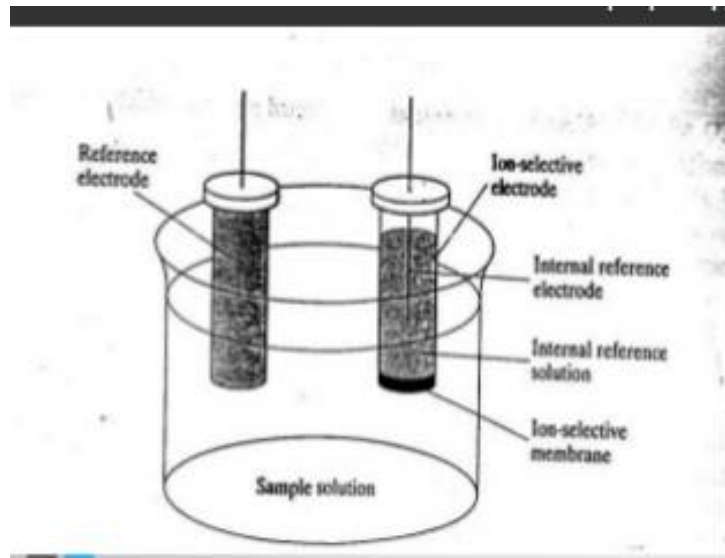
Medically measurement of these ions especially the K and Ca ions are important and this is established through the ion selective electrodes

- Over the past decade, the pH meter has been at the centre of a most important change in the field of analytical measurements due to the introduction of selective-ion electrodes. As their name implies,
- these electrodes are sensitive to the activity of a particular ion in solution and quite insensitive to the other ions present. As the electrode is sensitive to only one ion, a different electrode is needed for each ion to be studied. Approximately 20 types of selective-ion electrodes are presently available.

Types of Electrodes

- **Solid State Electrodes:** These electrodes use single crystals of inorganic material doped with a rare earth material. Such electrodes are particularly useful for **fluoride, chloride, bromide and iodide ion analysis**.
- **Liquid-Liquid Membrane Electrodes:** These electrodes are essentially liquid ion-exchangers, separated from the liquid sample by means of a permeable membrane. This membrane allows the liquids to come in contact with each other, but prevents their mixing. Based on this principle, cells have been developed that are **selective to calcium and magnesium**. These cells are used for measuring water hardeners.
- **Gas Sensing Electrodes:** These electrodes respond to the partial pressure of the gases in the sample. The most recent of these to be developed are the gas sensing electrodes for ammonia and Sulphur dioxide. **Ammonia or sulphur dioxide** is transferred across a gas permeable membrane, until the partial pressure in the thin film of filling solution between the glass electrode membrane and the probe membrane equals that in the sample. The resultant pH change is measured by a combination pH electrode. A potential is developed related to the partial pressure and hence the ammonia or sulphur dioxide concentration is measured.
- **Applications** using ion-selective electrodes are many, most being time saving and simple to use. The electrodes are now used for the continuous monitoring of important electrolytes in the **blood such as sodium, potassium, calcium, chloride, etc.**

Typical Electrode setup

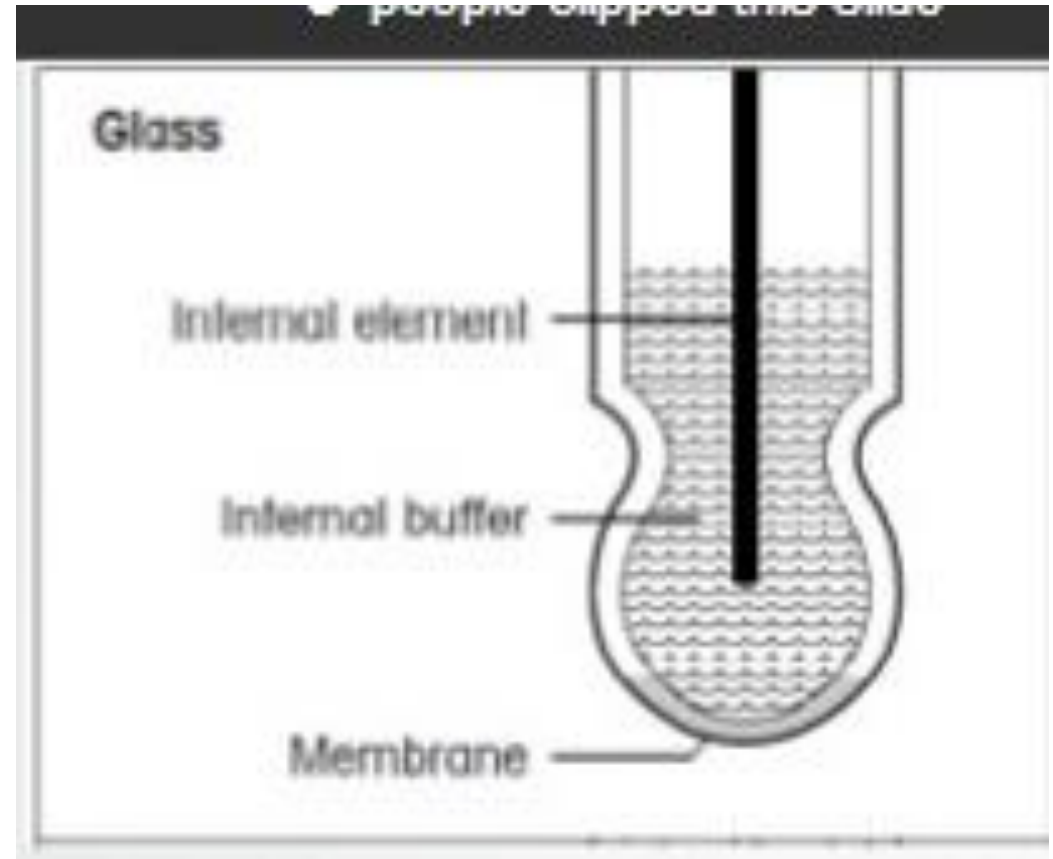


Glass Electrode

Glass Electrodes: The first glass ion-selective electrode developed is the one sensitive to hydrogen ions. Glasses containing less than 1% of Al_2O_3 are sensitive to hydrogen ions (H^+) but almost insensitive to the other ions present. Glasses, of which the composition is Na_2O –11%, Al_2O_3 –18%, SiO_2 –71% is highly selective towards sodium, even in the presence of other alkali metals. Glass electrodes have been made that are selectively sensitive to sodium, potassium, ammonium and silver.

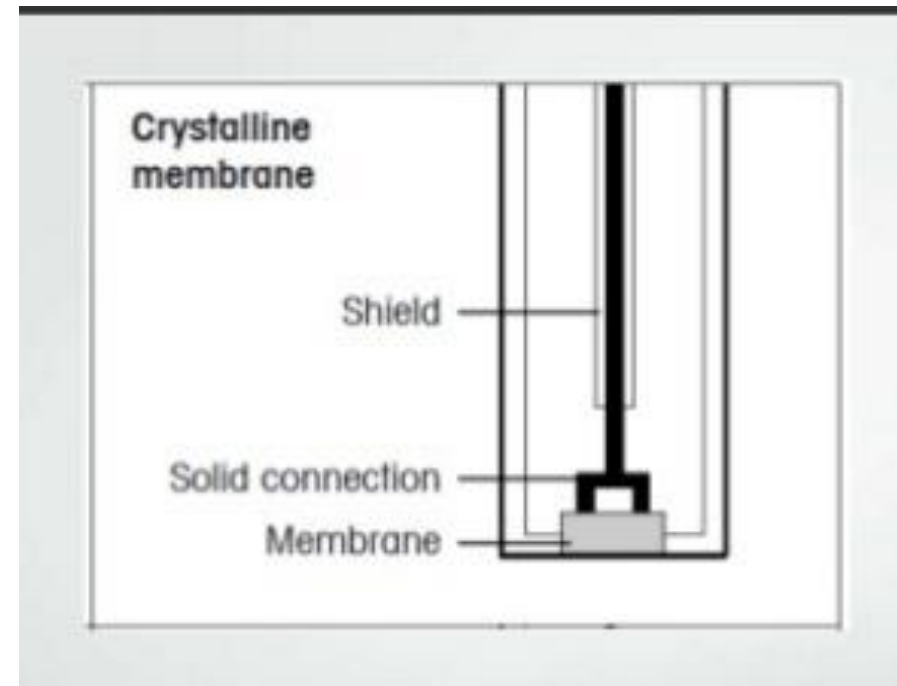
Glass Electrode

- Glass electrode is made up of Silicon dioxide with negatively charged Oxygen ions. HCl forms the internal buffer solution. The electrode is Ag coated with AgCl. The electrode is immersed in the sample solution



Solid State Electrode

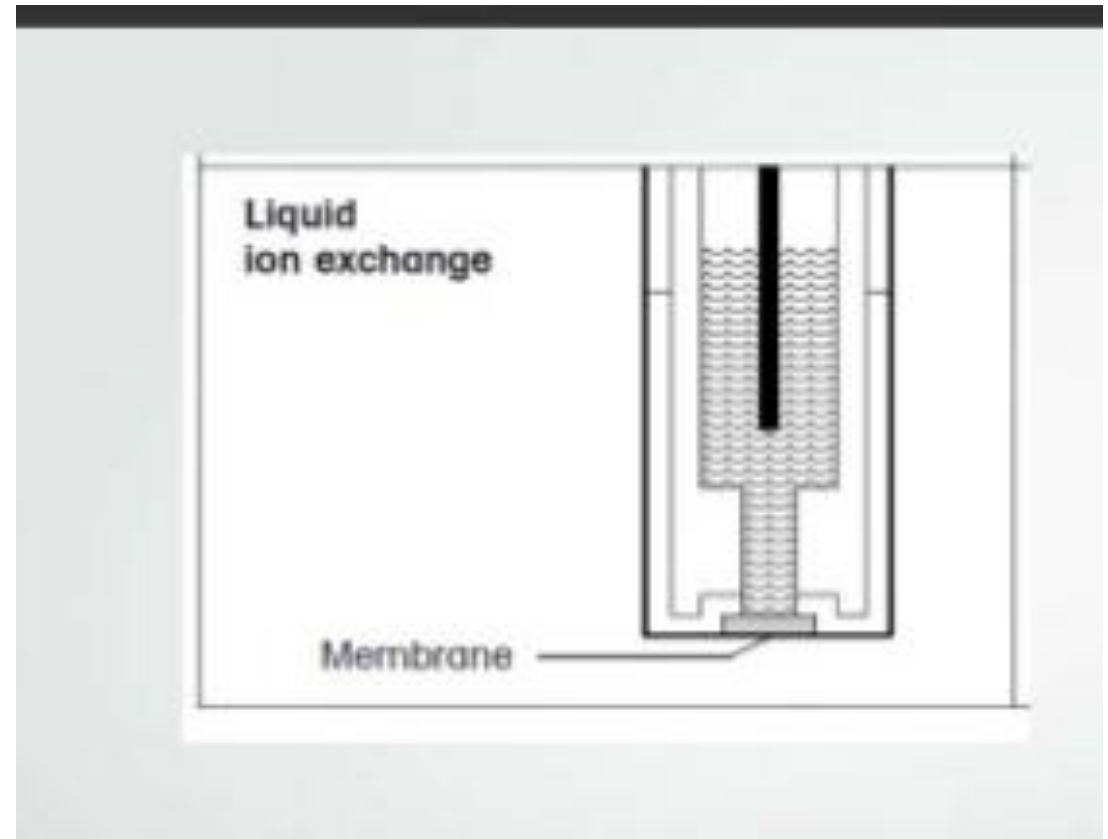
The electrode is crystalline made up of AgI/
Ag₂S
Sensitive to anions



Liquid-Membrane Electrode

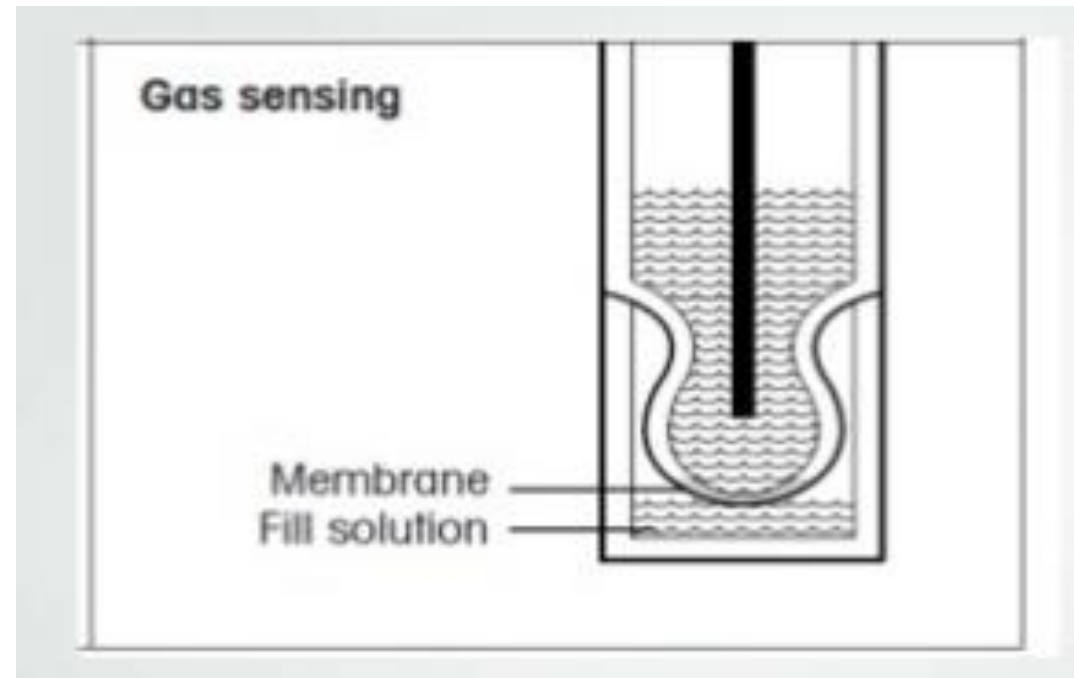
- Water immiscible liquid substance made of polymeric membrane PVC
- Used for cations and anions, especially for Ca ions.

The internal solution is a chloride solution for the internal reference electrode



Gas Electrode

- Measurement of Ammonia, Carbon dioxide and nitrogen dioxide
- This does not require an
- External reference electrode



Ion Analysers

- $C_x = C_s \times \Delta E / S$
- where, C_x = concentration of the unknown solution
- C_s = concentration of the standard solution
- ΔE = difference between the observed potential in the sample solution and the observed potential in the standard solution
- S = electrode slope (change in electrode potential per ten-fold change in concentration)

Nernst Electrode Equation

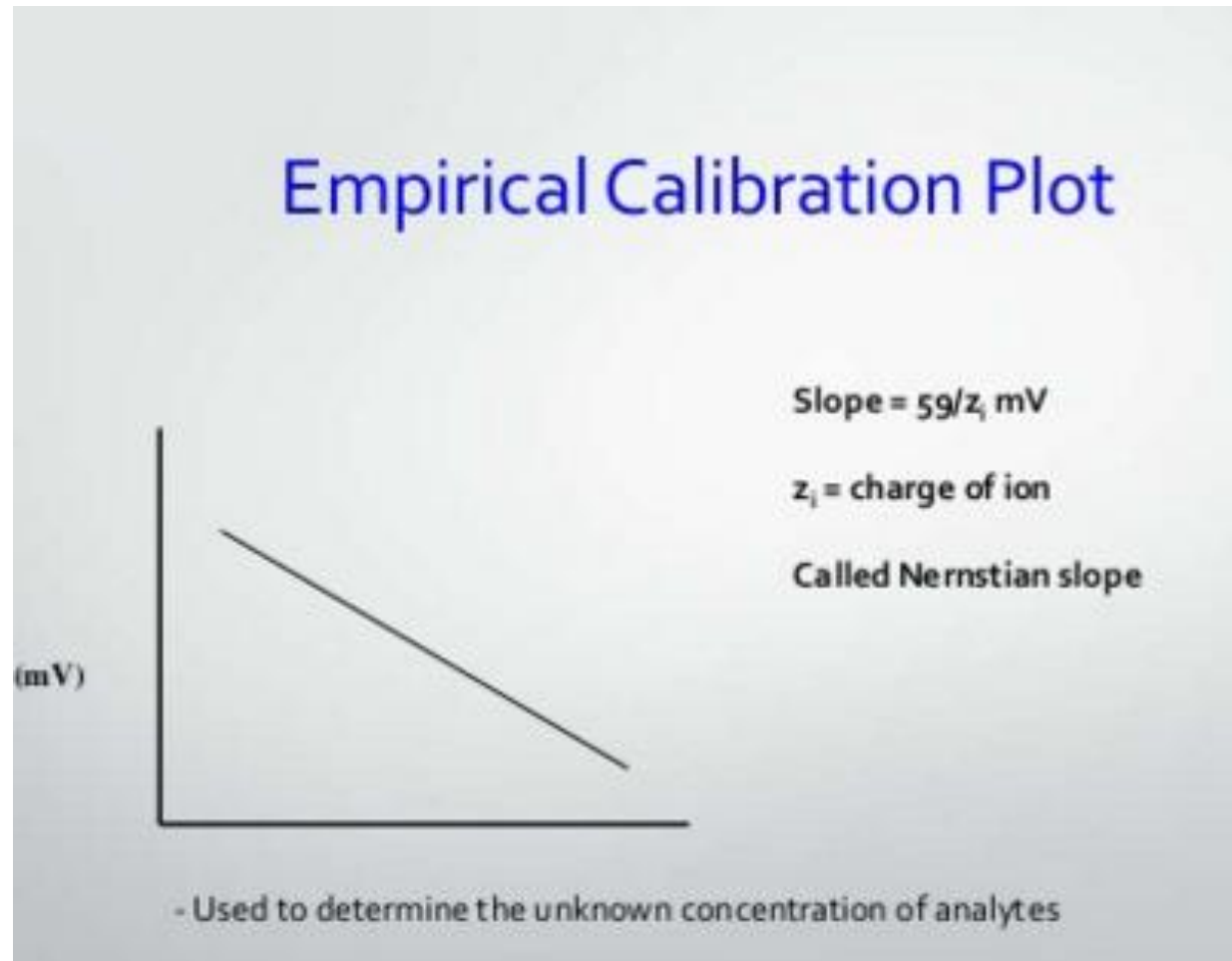
- $E_x = E_o + S \log(C_x + C_b)$

E_x = Electrode concentration

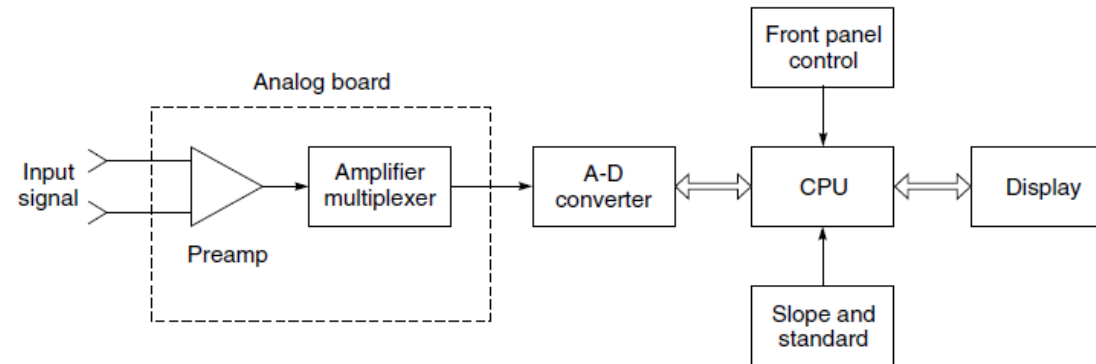
E_o = Constant

C_b = Blank Correction

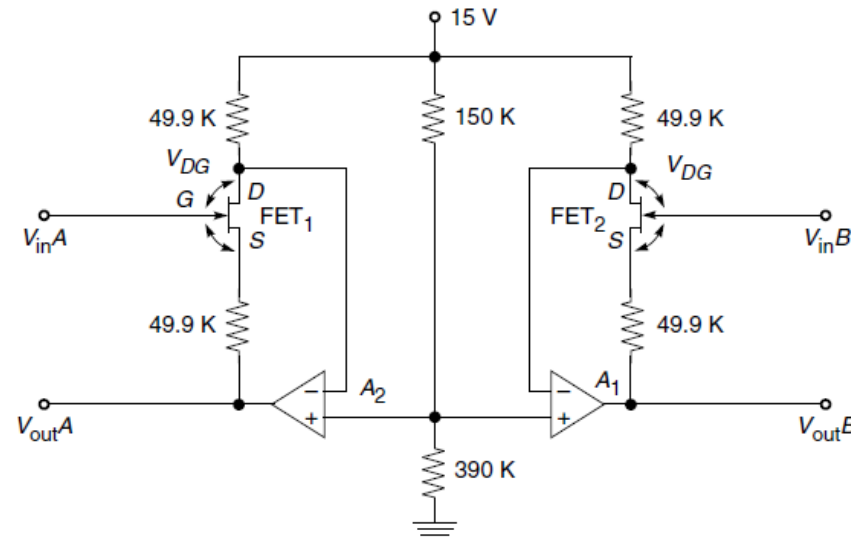
Calibration Slope



Microprocessor Based Ion Analyser



► Fig. 14.19 Block diagram of a microprocessor based ion-analyzer



► Fig. 14.20 Input-buffer amplifier of an ion analyzer