

EMMAWELL

INSTRUMENTATION IN MEDICAL LABORATORY SCIENCE (MLS 331)

ION SELECTIVE ELECTRODE

An ion-selective electrode (ISE), also known as a specific ion electrode (SIE), is a transducer (or sensor) that converts the activity of a specific ion dissolved in a solution into an electrical potential, which can be measured by a voltmeter or pH meter. The voltage is theoretically dependent on the logarithm of the ionic activity, according to the Nernst equation. The sensing part of the electrode is usually made as an ion-specific membrane, along with a reference electrode. Ion-selective electrodes are used in analytical chemistry and biochemical/biophysical research, where measurements of ionic concentration in an aqueous solution are required, usually on a real time basis.

Types of ion-selective membrane

There are four main types of ion-selective membrane used in ion-selective electrodes (ISEs): glass, solid state, liquid based, and compound electrode.

Glass membranes

Glass membranes are made from an ion-exchange type of glass (silicate or chalcogenide). This type of ISE has good selectivity, but only for several single-charged cations; mainly H^+ , Na^+ , and Ag^+ . Chalcogenide glass also has selectivity for double-charged metal ions, such as Pb^{2+} , and Cd^{2+} . The glass membrane has excellent chemical durability and can work in very aggressive media. A very common example of this type of electrode is the pH glass electrode.

Crystalline membranes

Crystalline membranes are made from mono- or polycrystallites of a single substance; They have good selectivity, because only ions which can introduce themselves into the crystal structure can interfere with the electrode response. Selectivity of crystalline

membranes can be for both cation and anion of the membrane-forming substance. An example is the fluoride selective electrode based on LaF_3 crystals.

Ion-exchange resin membranes

Ion-exchange resins are based on special organic polymer membranes which contain a specific ion-exchange substance (resin). This is the most widespread type of ion-specific electrode. Usage of specific resins allows preparation of selective electrodes for tens of different ions, both single-atom or multi-atom. They are also the most widespread electrodes with anionic selectivity. However, such electrodes have low chemical and physical durability as well as "survival time". An example is the potassium selective electrode, based on valinomycin as an ion-exchange agent.

Construction

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These are a development from "A Calcium Ion-selective Electrode Based on a Liquid Ion-exchanger in a Poly(Vinyl Chloride) Matrix. Analyst, 1970, Vol. 95, 910-918, by J.D.R. Thomas, with G.J. Moody and R.B. Oke" These (modified, based on original 1970 technology) electrodes are prepared from glass capillary tubing approximately 2 millimeters in diameter, a large batch at a time. Polyvinyl chloride is dissolved in a solvent and plasticizers (typically phthalates) added, in the standard fashion used when making something out of vinyl. In order to provide the ionic specificity, a specific ion channel or carrier is added to the solution; this allows the ion to pass through the vinyl, which prevents the passage of other ions and water.

One end of a piece of capillary tubing about an inch or two long is dipped into this solution and removed to let the vinyl solidify into a plug at that end of the tube. Using a syringe and needle, the tube is filled with salt solution from the other end, and may be stored in a bath of the salt solution for an indeterminate period. For convenience in use, the open end of the tubing is fitted through a tight o-ring into a somewhat larger diameter tubing containing the same salt solution, with a silver or platinum electrode wire inserted. New electrode tips can thus be changed very quickly by simply removing the older electrode and replacing it with a new one.

Applications

In use, the electrode wire is connected to one terminal of a galvanometer or pH meter, the other terminal of which is connected to a reference electrode, and both electrodes are immersed in the solution to be tested. The passage of the ion through the vinyl via the carrier or channel creates an electric current, which registers on the galvanometer; by calibrating against standard solutions of varying concentration, the ionic concentration in the tested solution can be estimated from the galvanometer reading.

In practice there are several issues which affect this measurement, and different electrodes from the same batch will differ in their properties. Leakage between the vinyl and the wall of the capillary, thereby allowing passage of any ions, will cause the meter reading to show little or no change between the various calibration solutions, and requires that the electrode be discarded.

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Similarly, with use the ion-sensitive channels in the vinyl appear to gradually become blocked or otherwise inactivated, causing the electrode to lose sensitivity. The response of the electrode and galvanometer is temperature sensitive, and also 'drifts' over time requiring recalibration frequently during a series of measurements, ideally at least one calibration sample before and after each test sample. On the other hand, after immersion in the solution there is a 'settling time' which can be five minutes or even longer, before the electrode and galvanometer equilibrate to a new reading; so that timing of the reading is critical in order to find the most accurate 'window' after the response has settled, but before it has drifted appreciably.

Enzyme electrodes

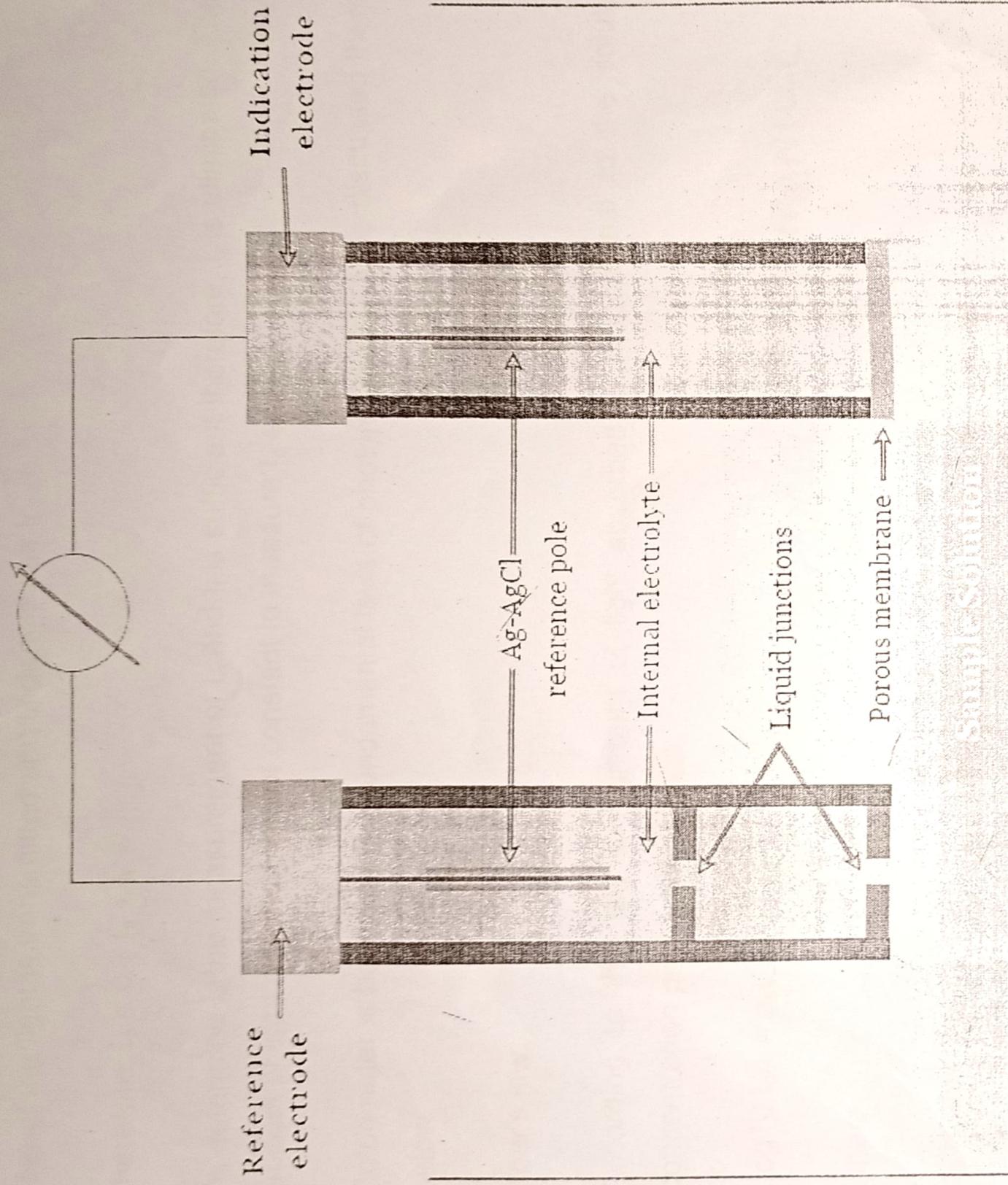
Enzyme electrodes definitely are not true ion-selective electrodes but usually are considered within the ion-specific electrode topic. Such an electrode has a "double reaction" mechanism - an enzyme reacts with a specific substance, and the product of this reaction (usually H^+ or OH^-) is detected by a true ion-selective electrode, such as a pH-selective electrodes. All these reactions occur inside a special membrane which covers the true ion-selective electrode, which is why enzyme electrodes sometimes are considered as ion-selective. An example is glucose selective electrodes.

Interferences

The most serious problem limiting use of ion-selective electrodes is interference from other, undesired, ions. No ion-selective electrodes are completely ion-specific; all are sensitive to other ions having similar physical properties, to an extent which depends on the degree of similarity. Most of these interferences are weak enough to be ignored, but in some cases the electrode may actually be much more sensitive to the interfering ion than to the desired ion, requiring that the interfering ion be present only in relatively very low concentrations, or entirely absent. In practice, the relative sensitivities of each type of ion-specific electrode to various interfering ions is generally known and should be checked for each case; however the precise degree of interference depends on many factors, preventing precise correction of readings. Instead, the calculation of relative degree of interference from the concentration of interfering ions can only be used as a guide to determine whether the approximate extent of the interference will allow reliable measurements, or whether the experiment will need to be redesigned so as to reduce the effect of interfering ions.

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The nitrate electrode has various ionic interferences, i.e. perchlorate, iodide, chloride, and sulfate. These interferences vary markedly in the extent to which they interfere. Thus, perchlorate gives a response which is about 50,000x as great as an equal amount of nitrate, while 1000x as much sulfate produces about a 10% error in the reading. Chloride causes a 10% error when present at about 30x the nitrate level, but can be removed by the addition of silver sulfate. Alternatively, nitrate can be determined by using an ammonia gas sensing electrode. This technique allows the user to determine both ammonium and nitrate ions sequentially. The procedure makes use of the reducing ability of titanium chloride. Trivalent titanium reduces any nitrate ion, up to 20 ppm, to ammonium ion (i.e., reverse nitrification). At pH 12-13, any ammonium ion in the sample is converted to ammonia gas and is ultimately detected by the electrode.



COLORIMETER

A colorimeter is a device that is used in Colorimetry. It refers to a device which helps specific solutions to absorb a particular wavelength of light. The colorimeter is usually used to measure the concentration of a known solute in a given solution with the help of the Beer-Lambert law. The colorimeter was invented in the year 1870 by Louis J Duboscq.

Principle of Colorimeter

It is a photometric technique which states that when a beam of incident light of intensity I_0 passes through a solution, the following occur:

- A part of it is reflected which is denoted as I_r

- A part of it is absorbed which is denoted as I_a
- Rest of the light is transmitted and is denoted as I_t

Therefore, $I_o = I_r + I_a + I_t$

To determine I_a the measurement of I_o and I_t is sufficient therefore, I_t is eliminated. The amount of light reflected is kept constant to measure I_o and I_t .

Colorimeter is based on two fundamental laws of photometry. We have discussed them below:

Beer's law:

According to this law the amount of light absorbed is proportional to the solute concentration present in solution.

$$\log_{10} \frac{I_o}{I_t} = a_s c$$

where,

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a_s is absorbency index

c is the concentration of solution

-- Lambert's law:

According to this law the amount of light absorbed is proportional to the length as well as thickness of the solution taken for analysis.

$$A = \log_{10} \frac{I_o}{I_t} = a_s b$$

Where,

A is the test absorbance of test

a_s is the standard absorbance

b is the length / thickness of the solution

1. Light Source:

The light source should produce energy at sufficient intensity throughout the whole visible spectrum (380-780nm). Tungsten lamp is frequently used.

2. Slit:

It allows a beam of light to path and minimize unwanted light.

3. Condensing lens:

Give parallel beam of light.

4. Monochromator:

It is used to produce monochromatic radiation (one wavelength band) from polychromatic radiation (white light) produced from light source. It allows required wavelength to pass through it. gelatin fibres or interference filters can be used.

5. Sample Holder (Cuvette):

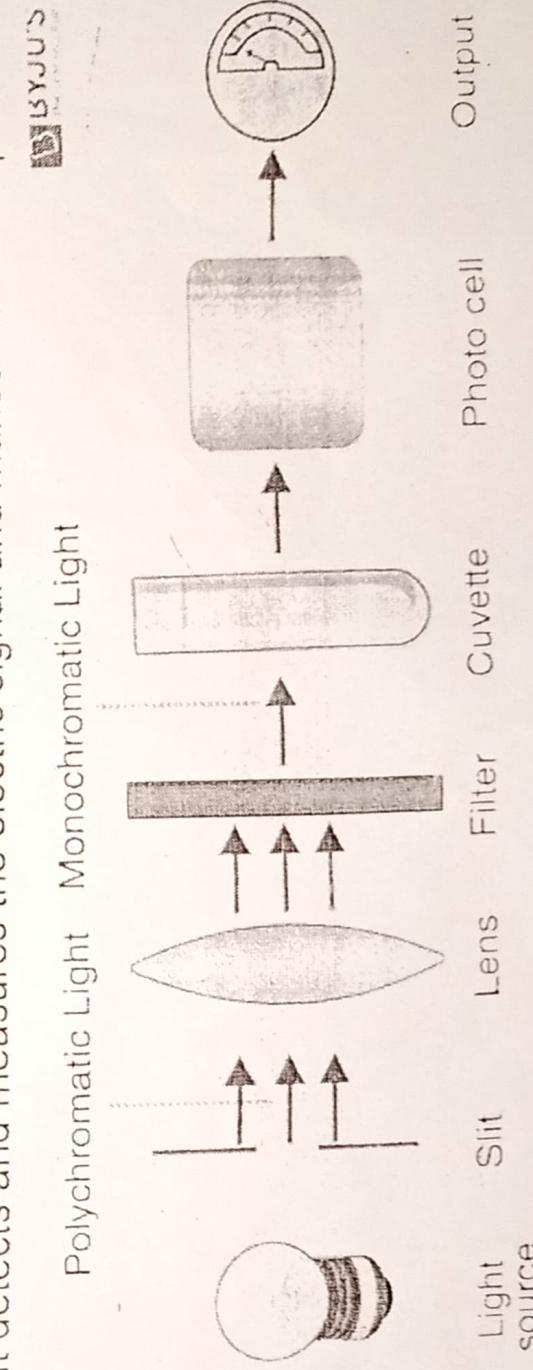
Must be transparent. Glass or clear plastic cuvettes are preferred.

6. Photo detectors:

Detector of colorimeter basically receives the resultant light beam once it has passed through the sample and converts it into electrical signal. Selenium photocell, silicon photocell, phototube, photomultiplier tube etc are used.

7. Display:

It detects and measures the electric signal and makes visible output.



Working of Colorimeter

Step 1: Before starting the experiment it is important to calibrate the colorimeter. It is done by using the standard solutions of the known solute concentration that has to be determined. Fill the standard solutions in the cuvettes and place it in the cuvette holder of colorimeter.

Step 2: A light ray of a certain wavelength, which is specific for the assay is in the direction of the solution. The light passes through a series of different lenses and filters. The coloured light navigates with the help of lenses, and the filter helps to split a beam of light into different wavelengths allowing only the required wavelength to pass through it and reach the cuvette of the standard test solution.

Step 3: When the beam of light reaches' cuvette, it is transmitted, reflected, and absorbed by the solution. The transmitted ray falls on the photodetector system where it measures the intensity of transmitted light. It converts it into the electrical signals and sends it to the galvanometer.

Step 4: The electrical signals measured by the galvanometer are displayed in the digital form:
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Step 5: Formula to determine substance concentration in test solution.

$$A = \epsilon cl$$

For standard and test solutions

ϵ and l are constant

$$A_T = C_T \dots \dots \text{(i)}$$

$$A_S = C_S \dots \dots \text{(ii)}$$

From the above two equations,

$$A_T \times C_S = A_S \times C_T$$

$$C_T = (A_T/A_S) \times C_S$$

Where,

C_T is the test solution concentration

A_T is the absorbance/optical density of test solution

C_S is the standard concentration

A_S is the absorbance / optical density of standard solution

Uses of Colorimeter

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- It is used in laboratories and hospitals to estimate biochemical samples such as urine, cerebrospinal fluid, plasma, serum, etc.
- It is used in the manufacturing of paints.
- It is used in textile and food industry.
- It is used in the quantitative analysis of proteins, glucose, and other biochemical compounds.
- It is used to test water quality.
- It is used to determine the concentration of haemoglobin in the blood.

Advantages and disadvantages of Colorimeter

Some benefits are as follows:

It is an inexpensive method, widely used in the quantitative analysis of coloured samples, easy to carry, and transport.

Some disadvantages are as follows:

Analysis of colourless compounds is not possible, does not work in IR and UV regions.

Colorimeter Applications

Few of the applications of colorimeters are explained here.

- Colorimeters are widely used to monitor bacterial growth or yeast culture.
- They provide highly accurate and reliable results when used for the assessment of colour in bird plumage.

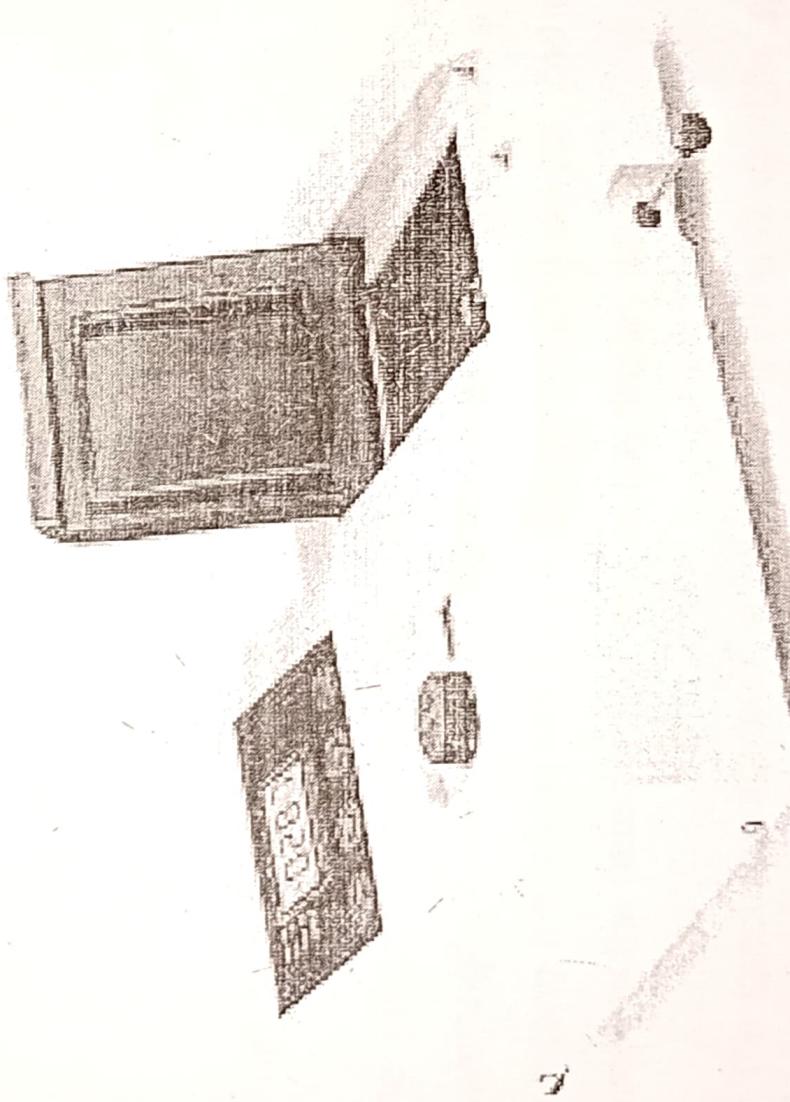
- They are also used to monitor and measure the colour of various foods and beverages, including sugar and vegetable products. Certain colorimeters can even measure the colours used in fax machines, copy machines, and printers.
- A colorimeter is used for basic research in chemistry laboratories, in addition, colorimeters have numerous practical applications including testing water quality by screening chemicals such as fluoride, zinc, chlorine, cyanide, iron, dissolved oxygen, molybdenum, and hydrazine.
- Also, they are used to determine the plant nutrient concentrations such as nitrate, ammonia, and phosphorus in soil or the haemoglobin in the blood.
- Besides, Colorimetry is a process used in textile manufacturing, colour printing, and paint manufacturing for precise quality inspection.

ANALYSE

ANALYSE

SPECTROPHOTOMETER

The spectrophotometer is an instrument which measures the amount of light that a sample absorbs. The spectrophotometer works by passing a light beam through a sample to measure the light intensity of a sample.

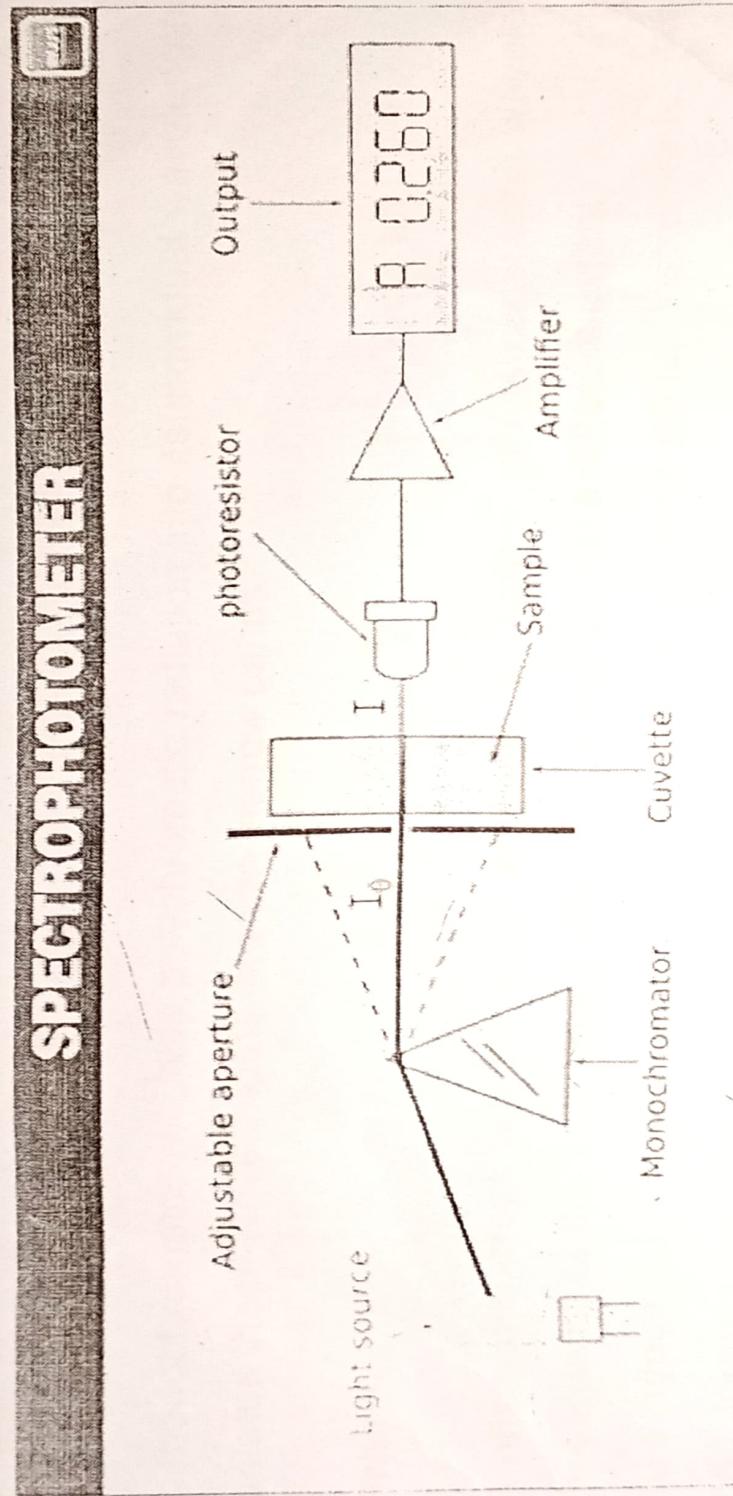


A spectrophotometer is made up of two instruments: a spectrometer and a photometer. The spectrometer is to produce light of any wavelength, while the photometer is to measure the intensity of light. The spectrophotometer is designed in a

way that the liquid or a sample is placed between spectrometer and photometer. The photometer measures the amount of light that passes through the sample and delivers a voltage signal to the display. If the absorbing of light changes, the voltage signal also changes.

The basic spectrophotometer instrument consists of a light source, a digital display, a monochromator, a wavelength selector to transmit a selected wavelength, a collimator for straight light beam transmission, photoelectric detector and a cuvette to place a sample.

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Principle of Spectrophotometer

The spectrophotometer technique is to measure light intensity as a function of wavelength. It does this by diffracting the light beam into a spectrum of wavelengths, detecting the intensities with a charge-coupled device, and displaying the results as a graph on the detector and then on the display device.

1. In the spectrophotometer, a prism (or) grating is used to split the incident beam into different wavelengths.
2. By suitable mechanisms, waves of specific wavelengths can be manipulated to fall on the test solution. The range of the wavelengths of the incident light can be as low as 1 to 2nm.

3. The spectrophotometer is useful for measuring the absorption spectrum of a compound, that is, the absorption of light by a solution at each wavelength.

Instrumentation of Spectrophotometer

The essential components of spectrophotometer instrumentation include:

1. A table and cheap radiant energy source
 - Materials that can be excited to high energy states by a high voltage electric discharge (or) by electrical heating serve as excellent radiant energy sources.
2. A monochromator, to break the polychromatic radiation into component wavelength (or) bands of wavelengths.
 - A monochromator resolves polychromatic radiation into its individual wavelengths and isolates these wavelengths into very narrow bands.

Prisms:

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- A prism disperses polychromatic light from the source into its constituent wavelengths by virtue of its ability to reflect different wavelengths to a different extent
- Two types of Prisms are usually employed in commercial instruments. Namely, 600 cornu quartz prism and 300 Littrow Prism.

Grating:

- Gratings are often used in the monochromators of spectrophotometers operating ultraviolet, visible and infrared regions.
- 3. Transport vessels (cuvettes), to hold the sample
 - Samples to be studied in the ultraviolet (or) visible region are usually glasses (or) solutions and are put in cells known as "CUVETTES"
 - Cuvettes meant for the visible region are made up of either ordinary glass (or) sometimes Quartz.
- 4. A Photosensitive detector and an associated readout system

Application of Spectrophotometry

Spectrophotometry is widely used for quantitative analysis in various areas (e.g., chemistry, physics, biology, biochemistry, material and chemical engineering, clinical applications, industrial applications, etc) and depending on the wavelength, they can be classified into

- UV-visible spectrophotometer (wavelength of 185 – 400nm)
- Visible spectrophotometer (wavelength of 400 – 700nm)

The differences between Colorimetry and Spectrophotometry

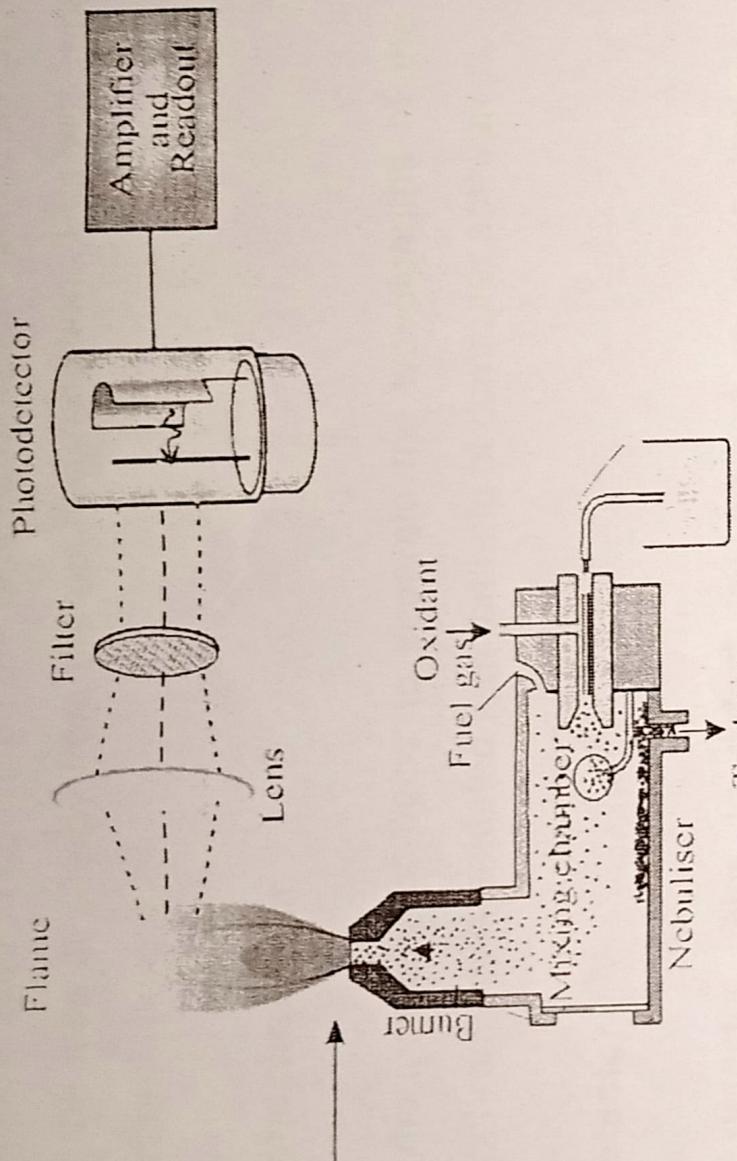
- A colorimeter quantifies color by measuring three primary color components of light (red, green, blue), whereas spectrophotometer measures the precise color in the human-visible wavelengths.
- Colorimetry uses fixed wavelengths, which are in the visible range only, but spectrophotometry can use wavelengths in a wider range (UV and IR also).
- Colorimeter measures the absorbance of light, whereas the spectrophotometer measures the amount of light that passes through the sample.

FLAME EMISSION SPECTROSCOPY ANYAMELE EMMANUEL

Flame emission spectroscopy provides us atomic emission spectrum based on the excitation of atoms from a lower energy state to a higher state. When these atoms return to their original state, they emit radiations which are used to draw the graph.

This technique is widely used to determine the presence of unknown elements like alkali and alkali earth metals in the sample. This is supported by the fact that different metals have different band gaps and they will show different emission spectrums.

Instrumentation of flame emission spectroscopy



1. Sample

The sample is kept in a sample chamber which is connected to a tube with a nebulizer. The sample may be liquid or solid. The solid sample is first dissolved in a solvent and then analyzed. The sample cannot be a gas.

2. Nebulizer

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The nebulizer is a closed chamber connected with the sample through a tube. It also has a continuous supply of fuel and oxidants for the smooth flow of the flame. The nebulizer also contains inert gas which acts as a carrier of the sample molecules toward the flame.

3. Burner/Flame

The burner has a **continuous glowing flame** that is connected to the nebulizer. The flame is continuously glowing through the continuous flow of fuel and oxidants.

It is on the flame in which the atomization of the sample molecule takes place that the atoms of the molecules gas from the lower energy level to the higher energy level and then return to the ground state by emitting the radiation of a suitable wavelength.

4. Lens

The lens is used to converge the emitted radiations into a single path. This is done to avoid the loss of energy from the emitted radiation. This will improve the accuracy of the graph.

5. Monochromator

The monochromator helps to separate the wavelengths of the emitted radiation. A monochromator is adjusted with the help of computer software.

6. Detector

The detector helps to detect the emitted radiation that is passed through the monochromator. A good detector should be able to detect even a weak signal to produce better accuracy.

7. Computer

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A computer is an integral part of the flame emission spectrometer. It helps us to draw the graph of the emission spectrum for various atoms of the sample. It is done with the help of suitable software.

Working of flame emission spectrometer

1. Desolvation

The sample is mixed in the solvent in the sample chamber. So, in this step, the metal particles are dehydrated in the flame thus evaporating the solvent. Now only metal particle remains in the flame.

2. Vaporisation

The metal particles are further dehydrated and they get converted into gaseous particles. That's why this process is known as vaporization.

3. Atomization

This is the most crucial step. In this step, the metal ions or molecules get bifurcated into an individual atom with the application of extreme temperature.

Different metals have different atomization temperatures, some of them require only less temperature whereas some of them require temperature up to 1000 °C.

4. Excitation

In this step, the atoms absorb energy from the flame and get transferred from the lower energy state to the higher energy state. The excitation energy will be different for different metals as different elements have different band gaps.

5. Emission

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Finally, when the atoms return to their ground state they emit the absorbed radiation. The emission radiation has different wavelengths by different elements.

This is finally passed through a monochromator and detector and a graph or **emission spectrum** is drawn. The emission spectrum will be different for different metals.

Application of flame emission spectroscopy

1. It is used for the analysis of ferrous and non-ferrous alloys.
2. It is used for the determination of trace metal impurities in alloys, metals, reagents, and solvents.
3. It is used for the analysis of metals in geological, environmental, and biological materials.

ATOMIC ABSORPTION SPECTROSCOPY

Atomic absorption spectroscopy principle

Atomic absorption spectroscopy (AAS) and atomic emission spectroscopy (AES) principle is based upon the absorption and emission of light by atoms in the gaseous state. It was first observed by Fraunhofer while studying dark lines in the solar spectrum.

Atomization, hollow cathode lamp, monochromator, detector, and recorder are the main components in atomic absorption spectroscopy instrumentation. Elements with low excitation energy can be determined by flame emission while high excitation energy can be determined by atomic absorption spectroscopy.

Atoms absorbed light at a definite wavelength depending on the nature of chemical elements.

- Sodium is absorbed in 589 nm
- Uranium is absorbed in 589 nm
- Potassium is absorbed in 766.5 nm

Light at this wavelength has absorbed energy to excite another electronic state. The electronic transition is specific to a particular element. From the ground state of an atom is excited to a higher energy level by absorption of energy.

Atomic spectra are identified by sharp lines which can be distinguished from broadband spectra associated with molecules. Usually, lines arising from the ground state are almost important in atomic absorption spectroscopy. These are called resonance lines.

Atomic absorption spectroscopy flame

We used fuel and oxidant to create an atomic absorption spectroscopy flame. Usually, natural gas, propane, butane, hydrogen, and acetylene are used as fuels to create a flame.

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Air, oxygen, nitrous oxide, and a mixture of nitrous oxide and acetylene are used as an oxidant for flame creation in atomic absorption spectroscopy.

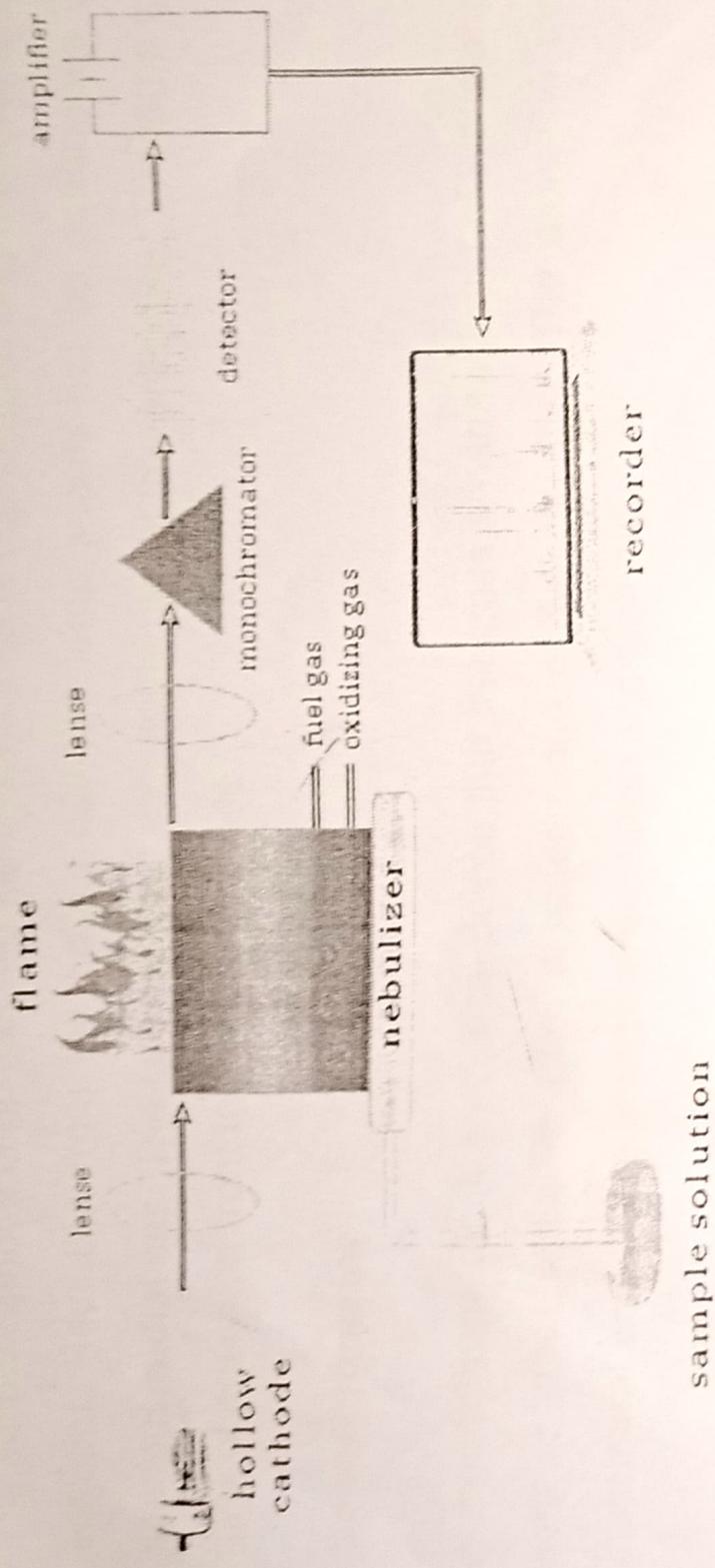
We used low temperatures for metals like copper (Cu), lead (Pb), zinc (Zn), and cadmium (Cd). These metals are easily vaporized at low temperatures.

Some metals are not easily atomized or vaporized. Therefore, we need a high temperature for the vaporization of such metals. Such high temperatures can be attained by using an oxidant in the flame along with fuel gas in atomic absorption spectroscopy.

For example, we used oxyacetylene flame for the analysis of aluminum, titanium, and rare earth elements in an AAS instrument.

Atomic absorption spectroscopy instrumentation

Atomic absorption spectroscopy



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For instrumentation, flame, non-flame, and graphite furnace are available in atomic absorption instruments. Any atomic absorption spectroscopy instrumentation has the following types of components,

- Atomization
- Hollow cathode lamp
- Monochromator
- Detector
- Recorder

Atomization

Atomization can be carried out either by a flame or furnace. Heat energy is utilized in atomic absorption spectroscopy to convert metallic elements to atomic dissociated vapor. The temperature should be controlled very carefully for the conversion of atomic vapor. At too high temperatures, atoms can be ionized.

Fuel and oxidant gases are fed into a mixing chamber which passes through baffles to the burner. A ribbon flame is produced in the AAS instrument. The sample is aspirated through the air into the mixing chamber.

Hollow cathode lamp

The hollow cathode lamp has two electrodes, one is cup-shaped and made of a specific element. Radiation from the hollow cathode lamp should not be continuous due to spurious radiations. The lamp is filled with noble gas at low pressure. The lamp forms a glow of emission from the hollow cathode.

Monochromator

A monochromator is an optical device that transmits a narrow band of wavelengths of light or other radiation from a wider range of wavelengths. The atoms in the AAS instrument accept the energy of excitation and emit radiation.

Detector

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A detector can convert light coming from a monochromator to a simplified electrical signal. Generally, we used a photomultiplier tube as a detector in the atomic absorption spectroscopy instrument. A detector can be tuned to respond by a specific wavelength or frequency.

Recorder

The recorder can receive electrical signals from the detector to convert them into a readable response. In atomic absorption spectroscopy instrumentation, today we used a computer system with suitable software for recording signals coming from the detector.

Application of atomic absorption spectroscopy

Today, the atomic absorption spectroscopy technique is the most powerful tool in analytical chemistry, forensic science, environmental analysis, and food industries. It is popular for analysts due to several advantages.

- The most important advantage is the speed of analysis. It can analyze various samples within a day.
- Secondly, it is possible to determine all elements at trace concentration.
- Thirdly, it is not always essential to separate the element before analysis because AAS can be used to determine one element in presence of another.
- The atomic absorption spectroscopy principle or instrumentation can be used to analyze sixty-seven metals and several nonmetals such as phosphorus and boron

CHROMATOGRAPHY

Chromatography (*/kroʊmə'tgrəfi/*; from Greek *χρῶμα chroma* which means "color" and *γράφειν graphein* "to write") is the collective term for a set of laboratory techniques for the separation of mixtures. The mixture is dissolved in a fluid called the *mobile phase*, which carries it through a structure holding another material called the *stationary phase*. The various constituents of the mixture travel at different speeds, causing them to separate. The separation is based on differential partitioning between the mobile and stationary phases. Subtle differences in a compound's partition coefficient result in differential retention on the stationary phase and thus changing the separation.

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Chromatography may be preparative or analytical. The purpose of preparative chromatography is to separate the components of a mixture for more advanced use (and is thus a form of purification). Analytical chromatography is done normally with smaller amounts of material and is for measuring the relative proportions of analytes in a mixture. The two are not mutually exclusive.

CHROMATOGRAPHY TERMS

- Chromatograph - equipment that enables a sophisticated separation, e.g Gas chromatography or Liquid chromatography
- Eluent - Fluid entering column/ solvent that carries the analyte.
- Eluate - Mobile phase leaving the column.
- Stationary phase - Immobilized phase, Immobilized on the support particles or on the inner wall of the column tubing. Examples : Silica layer - Thin Layer Chromatography