UNIT IV

ATOMIC ABSORPTION SPECTROSCOPY (AAS)

PRINCIPLE

Atomic absorption spectroscopy (**AAS**) is a spectroanalytical procedure for the quantitative determination of chemical elements using the absorption of optical radiation (light) by free atoms in the gaseous state.

The atomic absorption based on the principle that a ground state atom is capable of absorbing light of the same characteristic wavelength as it would emit if excited to a higher energy level.

In flame atomic absorption a cloud of ground state atom is formed by aspiration a solution of the sample into flame of a temperature sufficient to convert the element to its atomic state. The degree of absorption of characteristic radiation produced by a suitable source will be proportional to the population of ground state atoms in flame and hence to the concentration of the element in the sample.

The technique makes use of absorption spectrometry to assess the concentration of an analyte in a sample. It requires standards with known analyte content to establish the relation between the measured absorbance and the analyte concentration and relies therefore on the Beer-Lambert Law.

In short, the electrons of the atoms in the atomizer can be promoted to higher orbitals (excited state) for a short period of time (nanoseconds) by absorbing a defined quantity of energy (radiation of a given wavelength). This amount of energy, i.e., wavelength, is specific to a particular electron transition in a particular element. In general, each wavelength corresponds to only one element, and the width of an absorption line is only of the order of a few picometers (pm), which gives the technique its elemental selectivity. The radiation flux without a sample and with a sample in the atomizer is measured using a detector, and the ratio between the two values (the absorbance) is converted to analyte concentration or mass using the Beer-Lambert Law.

In analytical chemistry, Atomic absorption spectroscopy is a technique for determining the concentration of a particular metal element in a sample. Atomic absorption spectroscopy can be used to analyze the concentration of over 62 different metals in a solution

The technique typically makes use of a flame to atomize the sample, but other atomizers such as a graphite furnace are also used. Three steps are involved in turning a liquid sample into an atomic gas.Three steps are involved in turning a liquid sample into an atomic gas:

- **Desolvation** – The liquid solvent is evaporated, and the dry sample remains
- **Vaporisation** – The solid sample vaporises to a gas.
- **Volatilization** – The compounds making up the sample are broken into free atoms

The flame is arranged such that it is laterally long (usually 10cm) and not deep. The height of the flame must also be monitored by controlling the flow of the fuel mixture. A beam of light passes through this flame at its longest axis (the lateral axis) and hits a detector.

The light that is focused into the flame is produced by a hollow cathode lamp. Inside the lamp is a cylindrical metal cathode containing the metal for excitation, and an anode. When a high voltage is applied across the anode and cathode, the metal atoms in the cathode are excited into producing light with a certain emission spectrum.

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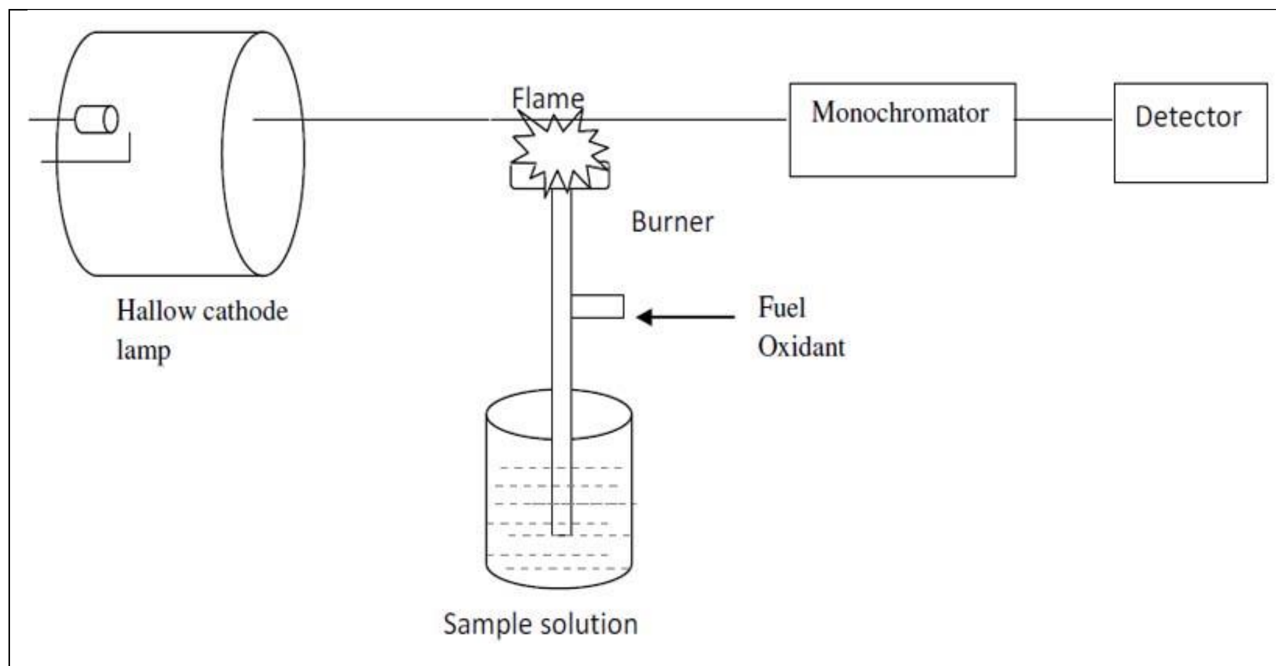
The method involves the spraying of sample in solution state over a burner. This leads to evaporation of solvent and leave fine dry residue behind which is nothing but neutral atoms in ground state. To these atoms, a light of specific wave length is passed and the un-absorbed light is recorded over a detector.

INSTRUMENTION

Atomic absorption spectroscopy unlike other spectroscopy methods has two additional requirements like a specially designed lamp to produce light of desired wave and a burner to prepare the sample for absorption of light.

The instrumentation includes:

1. The burner to dry the sample and produce atoms.
2. Sample container.
3. Fuel and oxidant to burn the sample by heat.
4. Hallow cathode lamp to produce light of desired wave length.
5. Detector to detect the absorption intensity.
6. Amplifier and data recorder.



The burner: Here sample from the capillary rises to the tip of burner where it is burned with flame produced by the fuel and oxidant combination. The sample after evaporation leaves a fine residue of neutral atoms.

Sample container: This is a beaker like container of sample which is placed below the burner preferably. A capillary tube drains the sample to the tip of burner.

Fuel and oxidant: This is a very important part of entire process to be remembered. If the heat produced is not sufficient then the sample doesn't form neutral atoms. If the heat of burner is more, the sample molecules may ionize instead of forming atoms. So both are undesirable for experimentation. Hence a proper combination of fuels and oxidants are to be used to produce recommended temperatures. Commonly used flues include propane, Hydrogen and acetylene ad oxidants are mostly air or oxygen.

Hallow cathode lamp: This is another primary requirement in the entire process. If the element to be analyzed is magnesium, the cathode lamp made of magnesium is used and so for all the other metal elements analyzed like Na, Ca, K, Zn etc.

Detector: The used can be a simple photo multiplier tube or photo cell. The current or potential recorded for the sample absorption is recorded in a computer software and then analyzed.

ATOMIC ABSORPTION SPECTROSCOPY APPLICATIONS:

1. Atomic spectroscopy is used for quantitative analysis of metal elements in any sample.

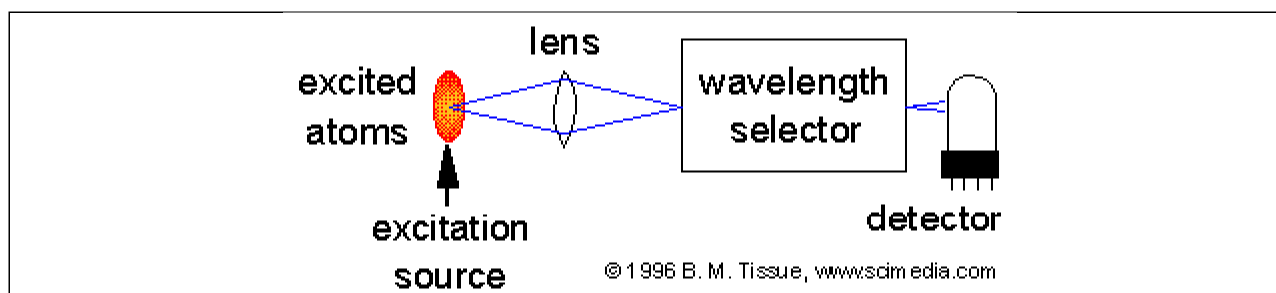
2. It is especially useful to analyze trace metal elements in plasma and other body fluids.

3. To determine metal elements in food industry.

4. To estimate Lead in petroleum products.

ATOMIC EMISSION SPECTROSCOPY (AES) OR FLAME PHOTOMTERY

Atomic emission spectroscopy (AES) uses quantitative measurement of the optical emission from excited atoms to determine analyte concentration. Analyte atoms in solution are aspirated into the excitation region where they are desolvated, vaporized, and atomized by a flame, discharge, or plasma. These high-temperature atomization sources provide sufficient energy to promote the atoms into high energy levels. The atoms decay back to lower levels by emitting light. Since the transitions are between distinct atomic energy levels, the emission lines in the spectra are narrow. The spectra of multi-elemental samples can be very congested, and spectral separation of nearby atomic transitions requires a high-resolution spectrometer. Since all atoms in a sample are excited simultaneously, they can be detected simultaneously



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INSTRUMENTION

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- 2. Sample container.
- 3. Fuel and oxidant to burn the sample by heat
- 4. Detector to detect the absorption intensity.
- 5. Amplifier and data recorder

FLAME EXCITATION SOURCE

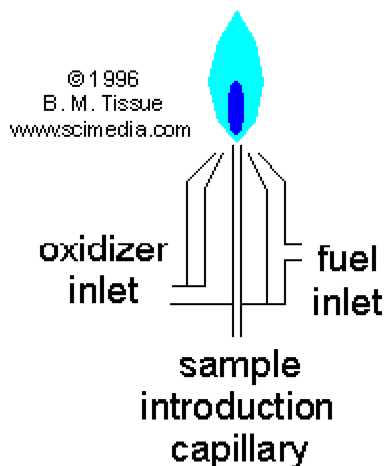
A flame provides a high-temperature source for desolvating and vaporizing a sample to obtain free atoms for spectroscopic analysis. In atomic absorption spectroscopy ground state atoms are desired. For atomic emission spectroscopy the flame must also excite the atoms to higher energy levels. The table lists temperatures that can be achieved in some commonly used flames.

Temperatures of Some Common Flames

Fuel	Oxidant	Temperature (K)
H_2	Air	2000-2100
C_2H_2 (ACETYLENE)	Air	2100-2400
H_2	O_2	2600-2700
C_2H_2	N_2O	2600-2800

TOTAL CONSUMPTION BURNER

Total consumption burner in which the sample solution is directly aspirated into the flame. This flame design is common for atomic emission spectroscopy. All desolvation, atomization, and excitation occur in the flame. Other flame designs nebulize the sample and premix it with the fuel and oxidant before it reaches the burner. Atomic-absorption instruments almost always use a nebulizer and also use a slot burner to increase the path length for the sample absorption



II. Premix long path burner/
Laminar flow burner
- Gases are mixed and sample is atomized before being burned.

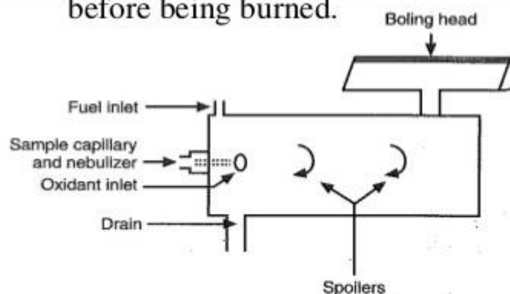


Figure 3-13 Laminar flow burner.

TOTAL CONSUMPTION BURNER LAMINAR FLOW BURNER

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1-Total consumption burner and

2-**laminar flow burner**

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FLUORIMETRY

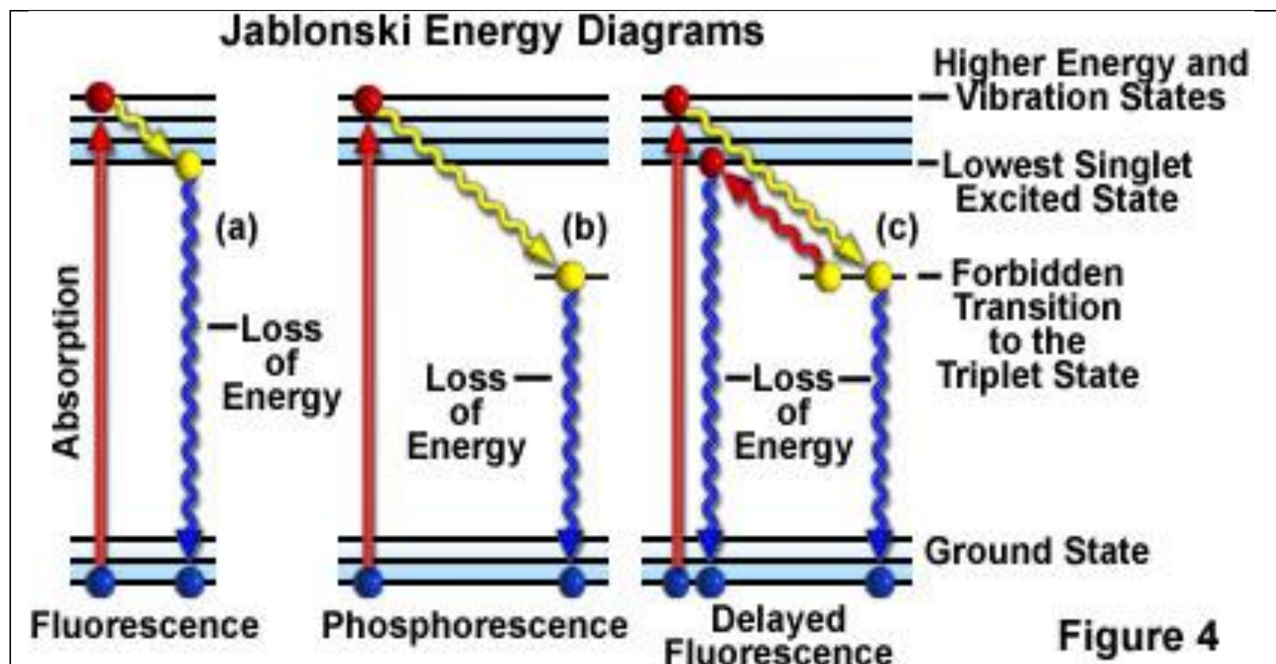
A **fluorometer** or **fluorimeter** is a device used to measure parameters of fluorescence: its intensity and wavelength distribution of emission spectrum after excitation by a certain spectrum of light. These parameters are used to identify the presence and the amount of specific molecules in a medium. Modern fluorometers are capable of detecting fluorescent molecule concentrations as low as 1 part per trillion.

Fluorescence is the emission of visible light by a substance that has absorbed light of a different wavelength. The emitted photon has a longer wavelength.

Phosphorescence is related to fluorescence in emitting a photon; however, a phosphorescent material does not immediately re -emit the radiation it absorbs.

As the excitation of the molecule is due to the absorption of a photon (light), these types of luminescence are called photoluminescence

Chemiluminescence is another phenomenon that falls in the category of luminescence. This refers to the emission of radiation during a chemical reaction. However, in such cases the excited state is not a result of absorption of electromagnetic radiation. The oxidation of luminol (3 - aminophthalhydrazide) in an alkaline solution is an example of chemiluminescence.



At the ground state, the molecular orbital's are occupied by two electrons. The spins of the two electrons in the same orbital must be antiparallel. This implies that the total spin, S, of the molecule in the ground state is zero [½ + (½)]. This energy state is called "**SINGLET STATE"** and is labeled as S 0. The electron spins in the excited state achieved by absorption of radiation may either be parallel or antiparallel. Accordingly, this may be a **TRIPLET (PARALLEL)** or a **SINGLET (ANTIPARALLEL) STATE.**

Activation and Deactivation • The absorption of a photon of suitable energy causes the molecule to get excited from the ground state to one of the excited states. This process is called as excitation or activation. According to this principle, the electronic transition takes place so fast (~10-15 s) that the molecule does not get an opportunity to execute a vibration, – i.e., when the electrons are excited the internuclear distance does not change. The basis for the principle is that the nuclei are very massive as compared to the electrons and therefore move very slowly.

The deactivation processes can be broadly categorized into two groups given below.

- Nonradiative deactivation
- Radiative deactivation

FACTORS AFFECTING FLUORESCENCE AND PHOSPHORESCENCE

The common factors affecting the fluorescence are as follows.

- **Unsaturartion**
- **Temperature**
- **pH**
- **Dissolved oxygen**
- **Solvent**

Unsaturartion and substituent

- Photoluminescence and Structure The presence of the benzene ring and the nature of substituents on it seem to favour the fluorescent behavior of the molecule.
- The halogen substituents tend to decrease the fluorescence and shift the fluorescence bands to longer wavelengths; the effects increase with increase in the atomic mass of the substituted halogen. Compounds with fused ring are found to be especially fluorescent, and the extent of fluorescence is found to be directly proportional to the number of rings in the molecule.
- The structural rigidity in a molecule favours fluorescence Eg- fluorescein and phenolphthalein

The fluorescence observed with rigid cyclic molecules with pi-bonds is found to be enhanced by electron donating groups e.g., −NH2, OR, – OH and OCH3, The electron withdrawing groups such as COOH, NO2, N=N and Br, I and CH2COOH tend to reduce it. On the other hand the nonrigid molecules do not fluoresce much, as these rapidly lose the absorbed energy through nonradioactive means like, vibrational relaxation or even degradation.

Temperature

A rise in temperature is almost always accompanied by a decrease in fluorescence. The change in temperature causes the viscosity of the medium to change which in turn changes the number of collisions of the molecules of the fluorophore with solvent molecules. The increase in the number of collisions between molecules in turn increases the probability for deactivation by internal conversion and vibrational relaxation.

pH

Relatively small changes in relatively small changes in pH can sometimes cause substantial changes in the fluorescence intensity and spectral characteristics of fluorescence. – For example, serotonin shows a shift in fluorescence emission maximum from 330 nm at neutral pH to 550 nm in strong acid without any change in the absorption spectrum. In the molecules containing acidic or basic functional groups, the changes in pH of the medium change the degree of ionization of the functional groups. This in turn may affect the extent of conjugation or the aromaticity of the molecule which affects its fluorescence. – For example, aniline shows fluorescence while in acid solution it does not show fluorescence due to the formation of anilinium ion. Therefore, pH control is essential while working with such molecules and suitable buffers should be employed for the purpose.pH can sometimes cause substantial changes in the fluorescence intensity and spectral characteristics of fluorescence. – For example, serotonin shows a shift in fluorescence emission maximum from 330 nm at neutral pH to 550 nm in strong acid without any change in the absorption spectrum. In the molecules containing acidic or basic functional groups, the changes in pH of the medium change the degree of ionization of the functional groups. This in turn may affect the extent of conjugation or the aromaticity of the molecule which affects its fluorescence. – For example, aniline shows fluorescence while in acid solution it does not show fluorescence due to the formation of anilinium ion. Therefore, pH control is essential while working with such molecules and suitable buffers should be employed for the purpose.

Dissolved oxygen:

The paramagnetic substances like dissolved oxygen and many transition metals with unpaired electrons dramatically decrease fluorescence and cause interference in fluorimetric determinations. The paramagnetic nature of molecular oxygen promotes intersystem crossing from singlet to triplet states in other molecules. The longer lifetimes of the triplet states increases the opportunity for radiationless deactivation to occur.

Presence of dissolved oxygen influences phosphorescence too and causes a large decrease in the phosphorescence intensity. It is due to the fact that oxygen which is in triplet state at the ground state gets the energy from an electron in the triplet state and gets excited. This is actually the oxygen emission and not the phosphorescence. Therefore, it is advisable to make phosphorescence measurement in the absence of dissolved oxygen.

SOLVENT

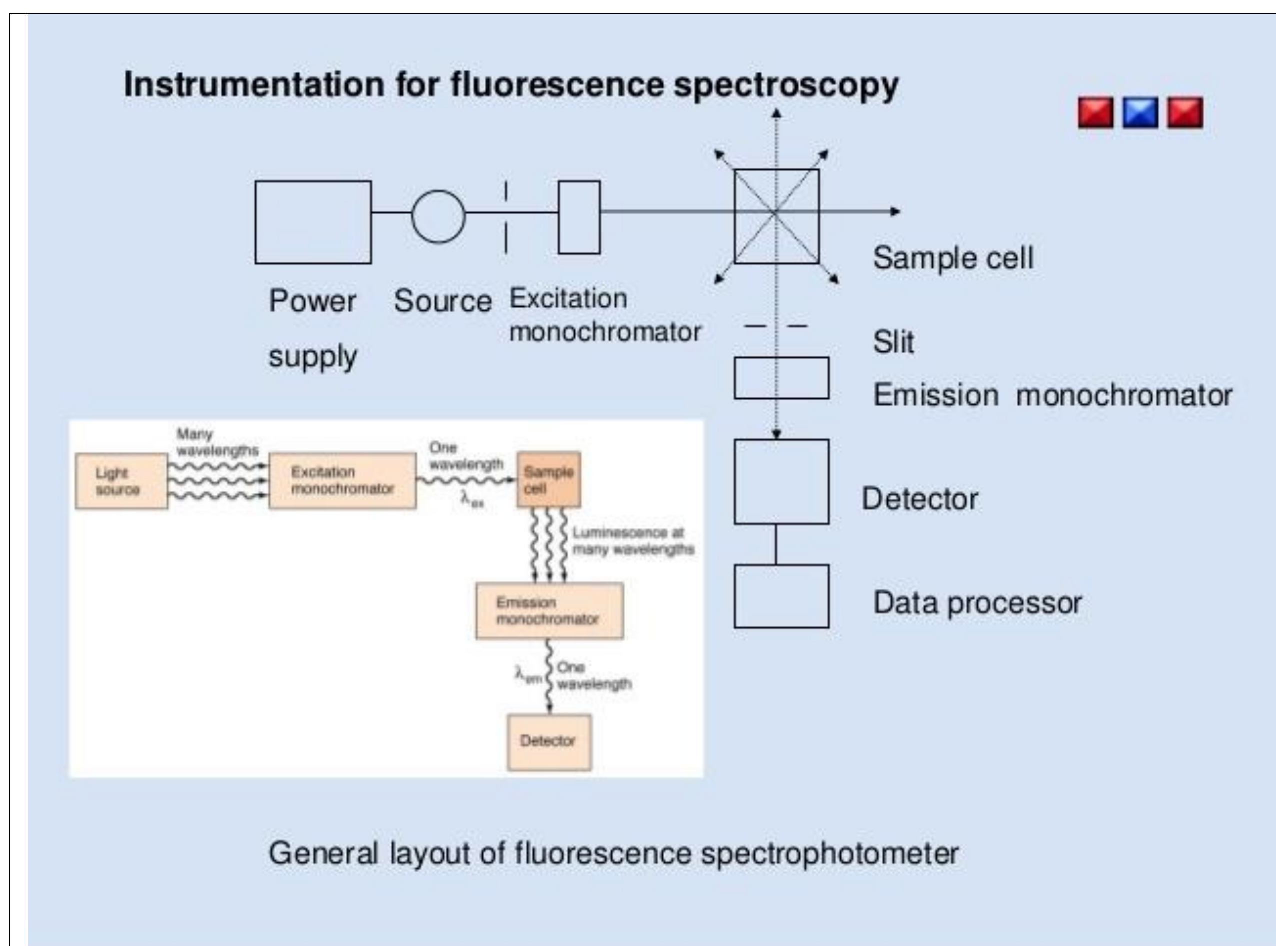
Solvent the changes in the “polarity” or hydrogen bonding ability of the solvent may also significantly affect the fluorescent behavior of the analyte. The difference in the effect of solvent on the fluorescence is attributed to the difference in their ability to stabilise the ground and excited states of the fluorescent molecule. Besides solvent polarity, solvent viscosity and solvents with heavy atoms also affect fluorescence and phosphorescence. Increased viscosity increases fluorescence as the deactivation due to collisions is lowered. A higher fluorescence is

observed when the solvents do not contain heavy atoms while phosphorescence increases due to the presence of heavy atoms in the solvent.

INSTRUMENTATION

The essential components of an instrument used to measure fluorescence of the sample are:

- Excitation light sources
- Filters or Monochromators
- Sample holder
- Detector
- Readout device



General layout of fluorescence spectrophotometer

Excitation light sources. Tungsten lamp

Filters or Monochromators

Primary filter – It allows only U.V. Radiation and check visible radiation.

Secondary filter- It checks U.V. radiation and allow visible radiation.

Sample holder: It is made up of glass.

Detector: Photomultiplier Tube

APPLICATION OF FLUORIMETRY

1-Analysis of Gaseous Pollutants

2-Determination of NO – NO_2

In order to determine the amount of NO, the gas is passed through the reactor in which it reacts with ozone. Initially the excited * NO_2 species is produced as per the reaction given below. NO + O_3 * NO_2 + O_2 The activated * NO_2 then gives chemiluminescence broadband in the visible to infrared range (600 – 2800 nm) and reverts back to a lower energy state. The emitted photons are proportional to the amount of NO present and are measured with the help of a photomultiplier tube (PMT).

3-Determination of SO_2

4-Analysis of Water Pollutants

The analysis of metal pollutants like Al, Zn, and the anion, − F in the aquatic environment by fluorescence methods.

5-Analysis Of Fluoride

GEL ELECTROPHORESIS

Gel electrophoresis is a method for separation and analysis of macromolecules (DNA, RNA and proteins) and their fragments, based on their size and charge. It is used in clinically biochemistry and molecular biology to separate a mixed population of DNA and RNA fragments by length, to estimate the size of DNA and RNA fragments or to separate proteins by charge.

Nucleic acid molecules are separated by applying an electric field to move the negatively charged molecules through a matrix of agarose or other substances. Shorter molecules move faster and migrate farther than longer ones because shorter molecules migrate more easily through the pores of the gel. This phenomenon is called sieving. Proteins are separated by charge in agarose because the pores of the gel are too large to sieve proteins. Gel electrophoresis can also be used for separation of nanoparticles. The electric field consists of a negative charge at one end which pushes the molecules through the gel, and a positive charge at the other end that pulls the molecules through the gel

"Electrophoresis" refers to the electromotive force (EMF) that is used to move the molecules through the gel matrix. By placing the molecules in wells in the gel and applying an electric field, the molecules will move through the matrix at different rates, determined largely by their mass when the charge to mass ratio (Z) of all species is uniform. However, when charges are not all uniform then, the electrical field generated by the electrophoresis procedure will affect the species that have different charges and therefore will attract the species according to their charges being the opposite. Species that are positively charged will migrate towards the cathode which is negatively charged (because this is an electrolytic rather than galvanic cell). If the species are negatively charged they will migrate towards the positively charged anode.

Types of gel

The types of gel most typically used are agarose and polyacrylamide gels. Each type of gel is well-suited to different types and sizes of analyte. Polyacrylamide gels are usually used for proteins, and have very high resolving power for small fragments of DNA (5-500 bp). Agarose gels on the other hand have lower resolving power for DNA but have greater range of separation, and are therefore used for DNA fragments of usually 50-20,000 bp in size, but resolution of over 6 Mb is possible with pulsed field gel electrophoresis (PFGE).[5] Polyacrylamide gels are run in a vertical configuration while agarose gels are typically run horizontally in a submarine mode. They also differ in their casting methodology, as agarose sets thermally, while polyacrylamide forms in a chemical polymerization reaction.

Agarose

Agarose gel electrophoresis

Agarose gels are made from the natural polysaccharide polymers extracted from seaweed.
Agarose gels are easily cast and handled compared to other matrices, because the gel setting is a

physical rather than chemical change. Samples are also easily recovered. After the experiment is finished, the resulting gel can be stored in a plastic bag in a refrigerator.

Agarose gels do not have a uniform pore size, but are optimal for electrophoresis of proteins that are larger than 200 kDa. Agarose gel electrophoresis can also be used for the separation of DNA fragments ranging from 50 base pair to several megabases (millions of bases), the largest of which require specialized apparatus. The distance between DNA bands of different lengths is influenced by the percent agarose in the gel, with higher percentages requiring longer run times, sometimes days. Instead high percentage agarose gels should be run with a pulsed field electrophoresis (PFE), or field inversion electrophoresis.

"Most agarose gels are made with between 0.7% (good separation or resolution of large 5–10kb DNA fragments) and 2% (good resolution for small 0.2–1kb fragments) agarose dissolved in electrophoresis buffer. Up to 3% can be used for separating very tiny fragments but a vertical polyacrylamide gel is more appropriate in this case. Low percentage gels are very weak and may break when you try to lift them. High percentage gels are often brittle and do not set evenly. 1% gels are common for many applications.

POLYACRYLAMIDE

Polyacrylamide gel electrophoresis (PAGE) is used for separating proteins ranging in size from 5 to 2,000 kDa due to the uniform pore size provided by the polyacrylamide gel. Pore size is controlled by modulating the concentrations of acrylamide and bis-acrylamide powder used in creating a gel. Care must be used when creating this type of gel, as acrylamide is a potent neurotoxin in its liquid and powdered forms.

Traditional DNA sequencing techniques such as Maxam-Gilbert or Sanger methods used polyacrylamide gels to separate DNA fragments differing by a single base-pair in length so the sequence could be read. Most modern DNA separation methods now use agarose gels, except for particularly small DNA fragments. It is currently most often used in the field of immunology and protein analysis, often used to separate different proteins or isoforms of the same protein into separate bands. These can be transferred onto a nitrocellulose or PVDF membrane to be probed with antibodies and corresponding markers, such as in a western blot.

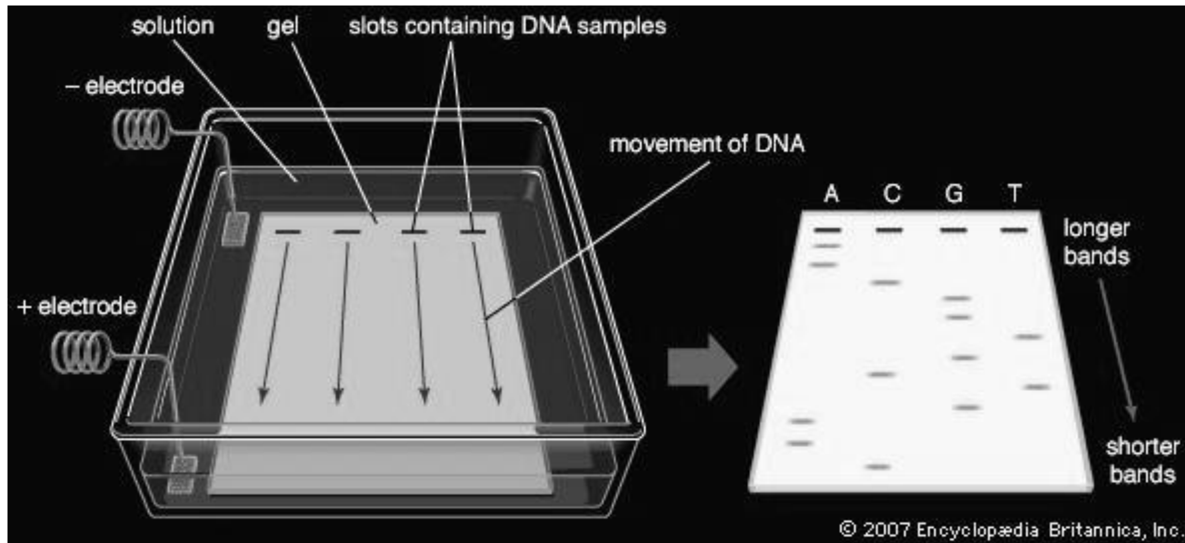
Applications

Estimation of the size of DNA molecules following restriction enzyme digestion, e.g. in restriction mapping of cloned DNA.

- Analysis of PCR products, e.g. in molecular genetics diagnosis or genetic fingerprinting
- Separation of restricted genomic DNA prior to Southern transfer, or of RNA prior to Northern transfer.

Gel electrophoresis is used in forensics, molecular biology, genetics, microbiology and biochemistry. The results can be analyzed quantitatively by visualizing the gel with UV light and a gel imaging device. The image is recorded with a computer operated camera, and the intensity

of the band or spot of interest is measured and compared against standard or markers loaded on the same gel. The measurement and analysis are mostly done with specialized software.

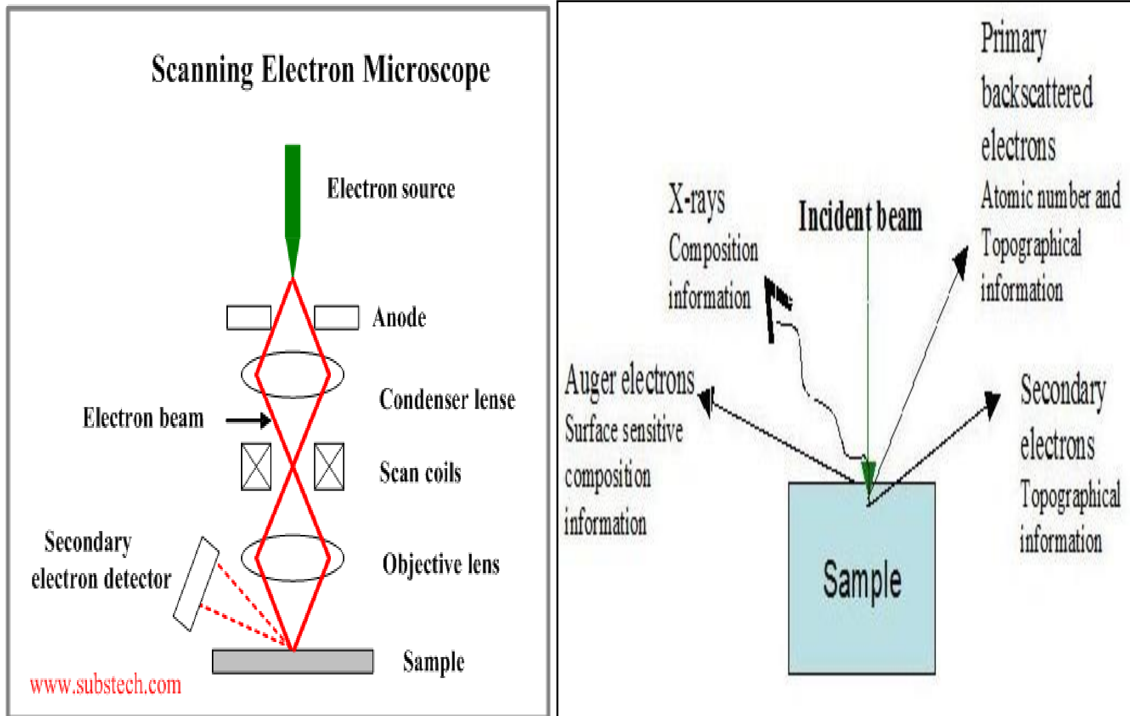


SCANNING ELECTRON MICROSCOPE

The basic principle is that a beam of electron generated by suitable source typically a tungsten filament or a field emission gun. The electron beam is accelerated through a high voltage (20 kv) and pass through a system of aperture and electromagnetic lenses to produces a thin beam of electron. Then the beam scan the surface of the specimen and the electron are emitted from specimen by the action of scanning beam and collected by a suitably positioned detector.

The electrons interact with atoms in the sample, producing various signals that contain information about the sample's surface topography and composition.

The most common SEM mode is detection of secondary electrons emitted by atoms excited by the electron beam. The number of secondary electrons that can be detected depends, among other things, on the angle at which beam meets surface of specimen i.e. on specimen topography. By scanning the sample and collecting the secondary electrons that are emitted using a special detector, an image displaying the topography of the surface is created.



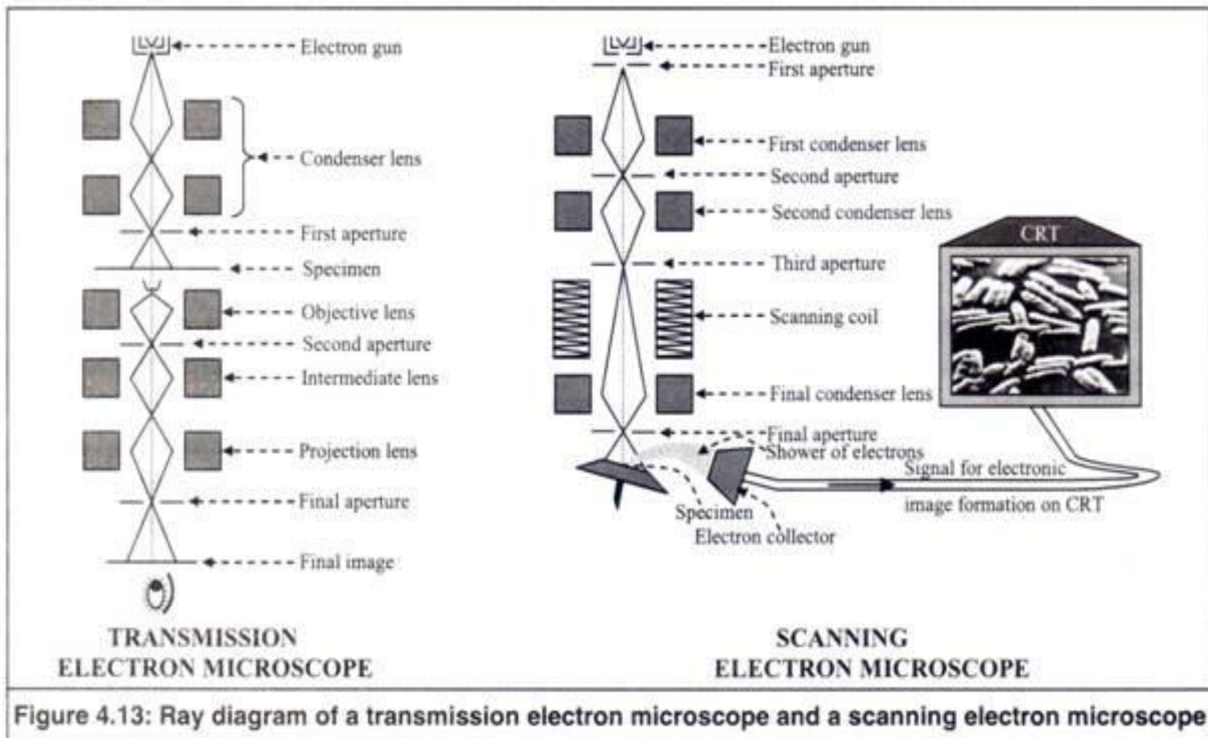


Figure 4.13: Ray diagram of a transmission electron microscope and a scanning electron microscope

PRINCIPLE

The types of signals produced by an SEM include secondary electrons (SE), reflected or back-scattered electrons (BSE), photons of characteristic X-rays and light (cathodoluminescence) (CL), absorbed current (specimen current) and transmitted electrons. Secondary electron detectors are standard equipment in all SEMs, but it is rare that a single machine would have detectors for all other possible signals.

The signals result from interactions of the electron beam with atoms at various depths within the sample. In the most common or standard detection mode, secondary electron imaging or SEI, the secondary electrons are emitted from very close to the specimen surface. Consequently, SEM can produce very high-resolution images of a sample surface, revealing details less than 1 nm in size. Back-scattered electrons (BSE) are beam electrons that are reflected from the sample by elastic scattering. They emerge from deeper locations within the specimen and consequently the resolution of BSE images is generally poorer than SE images. However, BSE are often used in analytical SEM along with the spectra made from the characteristic X-rays, because the intensity of the BSE signal is strongly related to the atomic number (Z) of the specimen. BSE images can provide information about the distribution of different elements in the sample. For the same reason, BSE imaging can image colloidal gold immuno-labels of 5 or 10 nm diameters, which would otherwise be difficult or impossible to detect in secondary electron images in biological specimens. Characteristic X-rays are emitted when the electron beam removes an inner shell electron from the sample, causing a higher-energy electron to fill the shell and release energy. These characteristic X-rays are used to identify the composition and measure the abundance of elements in the sample.

Due to the very narrow electron beam, SEM micrographs have a large depth of field yielding a characteristic three-dimensional appearance useful for understanding the surface structure of a sample. This is exemplified by the micrograph of pollen shown above. A wide range of magnifications is possible, from about 10 times (about equivalent to that of a powerful hand-lens) to more than 500,000 times, about 250 times the magnification limit of the best light microscopes.

Sample preparation

All samples must be of an appropriate size to fit in the specimen chamber and are generally mounted rigidly on a specimen holder called a specimen stub. Conductive materials in current use for specimen coating include gold, gold/palladium alloy, platinum, osmium,[12] iridium, tungsten, chromium, and graphite. Additionally, coating with heavy metals may increase signal/noise ratio for samples of low atomic number (Z). The improvement arises because secondary electron emission for high-Z materials is enhanced.

Biological samples

For SEM, a specimen is normally required to be completely dry, since the specimen chamber is at high vacuum. Hard, dry materials such as wood, bone, feathers, dried insects, or shells (including egg shells) can be examined with little further treatment, but living cells and tissues

and whole, soft-bodied organisms usually require chemical fixation to preserve and stabilize their structure. Fixation is usually performed by incubation in a solution of a buffered chemical fixative, such as glutaraldehyde, sometimes in combination with formaldehyde and other fixatives, and optionally followed by postfixation with osmium tetroxide.

APPLICATION

- Gunshot residue analysis
- Firearms identification (bullet markings comparison)
- Investigation of gemstones and jewellery
- Examination of paint particles and fibres
- Filament bulb investigations at traffic accidents
- Handwriting and print examination / forgery
- Counterfeit bank notes
- Trace comparison
- Examination of non-conducting materials
- High resolution surface imaging

TRANSMISSION ELECTRON MICROSCOPY (TEM)

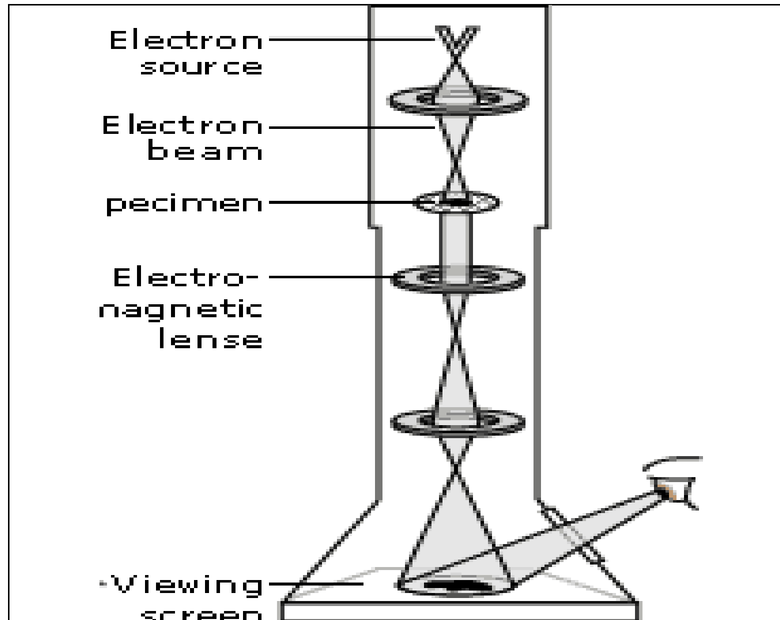
Transmission electron microscopy (**TEM**) is a microscopy technique in which a beam of electrons is transmitted through an ultra-thin specimen, interacting with the specimen as it passes through it. An image is formed from the interaction of the electrons transmitted through the specimen; the image is magnified and focused onto an imaging device, such as a fluorescent screen, on a layer of photographic film, or to be detected by a sensor such as a charge-coupled device.

TEMs are capable of imaging at a significantly higher resolution than light microscopes, owing to the small de Broglie wavelength of electrons. This enables the instrument's user to examine fine detail—even as small as a single column of atoms, which is thousands of times smaller than the smallest resolvable object in a light microscope. TEM forms a major analysis method in a range of scientific fields, in physical, chemical and biological sciences. TEMs find application in cancer research, virology, materials science as well as pollution, nanotechnology, and semiconductor research.

1. Illumination system. It takes the electrons from the gun and transfers them to the specimen giving either a broad beam or a focused beam. In the ray-diagram, the parts above the specimen belong to illumination system.
2. The objective lens and stage. This combination is the heart of TEM.
3. The TEM imaging system. Physically, it includes the intermediate lens and projector lens.

The diffraction pattern and image are formed at the back focus plane and image plane of the objective lens. If we take the back focus plane as the objective plane of the intermediate lens and projector lens, we will obtain the diffraction pattern on the screen. It is said that the TEM works

in diffraction mode. If we take the image plane of the objective lens as the objective plane of the intermediate lens and projector lens, we will form image on the screen. It is the image mode.



Transmission Electron Microscopy (TEM)

TEM APPLICATION

- **3D tissue imaging**
- **Biological production and viral load monitoring**
- **Cryobiology**
- **Cryo-electron microscopy**
- **Diagnostic electron microscopy**
- **Drug research**
- **Electron tomography**
- **Food science**
- **Particle analysis**
- **Protein localization**
- **Structural biology**
- **Toxicology**
- **Virology**
- **Vitrification**

MATERIALS RESEARCH

- **Device testing and characterization**
- **Electron beam-induced deposition**
- **Materials qualification**
- **Medical research**
- **Nanometrology**
- **Nanoprototyping**

INDUSTRY

- **2D & 3D micro-characterization**
- **Chemical/Petrochemical**
- **Direct beam-writing fabrication**
- **Dynamic materials experiments**
- **Forensics**
- **Fractography and failure analysis**
- **High-resolution imaging**
- **Macro sample to nanometer metrology**
- **Mining (mineral liberation analysis)**
- **Particle detection and characterization**
- **Pharmaceutical QC**
- **Sample preparation**