

Electron Microscope:

It is a microscope that uses accelerated electrons as a source of illumination. Because the wavelength of an electron can be up to 100,000 times shorter than that of visible light photons, the electron microscope has a higher resolving power than a light microscope and can reveal the structure of smaller objects.

Electron microscopes are used to investigate the ultrastructure of a wide range of biological and inorganic specimens including microorganisms, cells, large molecules, biopsy samples, metals, and crystals.

Industrially, it is often used for quality control and failure analysis. Modern electron microscopes produce electron micrographs, using specialized digital cameras or frame grabbers to capture the image.

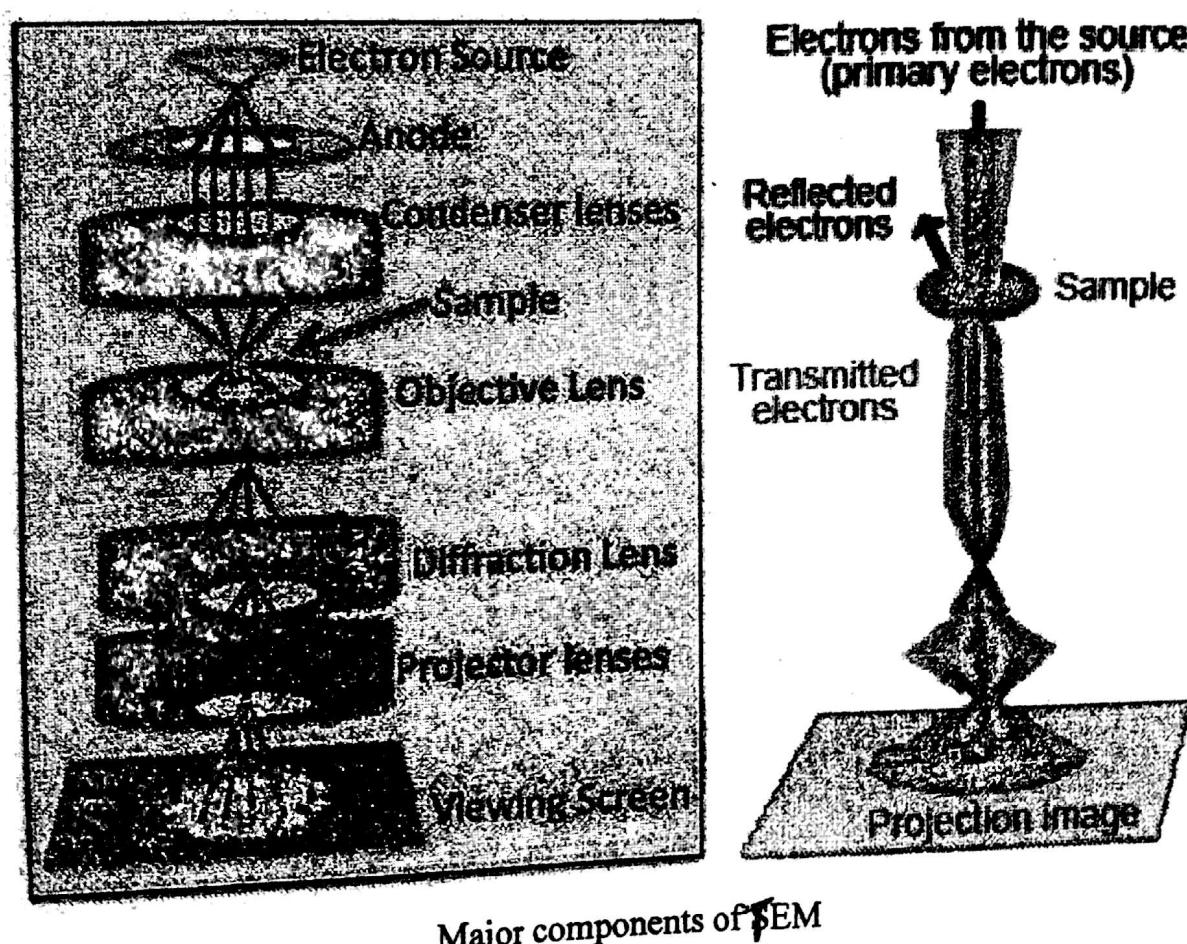
Difference between SEM & TEM:

- SEM is based on scattered electrons while TEM is based on transmitted electrons.
- SEM focuses on the sample's surface and its composition and morphology whereas TEM provides the details about internal composition.
- The sample in TEM has to be cut thinner whereas there is no such need with SEM sample. TEM has much higher resolution than SEM.
- SEM allows for large amount of sample to be analyzed at a time whereas with TEM only small amount of sample can be analyzed at a time.
- SEM is used for surfaces, powders, polished & etched microstructures, IC chips, chemical segregation whereas TEM is used for imaging of dislocations, tiny precipitates, grain boundaries and other defect structures in solids
- In TEM, pictures are shown on fluorescent screens whereas in SEM, picture is shown on monitor.
- SEM also provides a 3-dimensional image while TEM provides a 2-dimensional picture.
- Specimen sample in SEM need not be a single crystal or even a polycrystalline powder.
- Generally, the image resolution of an SEM is at least an order of magnitude poorer than that of a TEM.
- Another advantage of SEM is its variety called environmental scanning electron microscope (ESEM) that produces image of sufficient quality and resolution with the samples being wet or contained in low vacuum or gas. This greatly facilitates imaging biological samples that are unstable in the high vacuum of conventional electron microscopes.

Transmission electron microscope (TEM):

TEM can achieve better resolution and magnifications than most of the light microscopes. It uses a high voltage electron beam to create an image. The electron beam is produced by an electron gun, commonly fitted with a tungsten filament cathode as the electron source. The electron beam accelerated by anode is focused by electrostatic and electromagnetic lenses, and transmitted through the specimen. These electron optical lenses are analogous to the glass lenses of an optical light microscope. When it emerges from the specimen, the electron beam carries information about the structure of the specimen that is magnified by the objective lens system of the microscope. The "image" may be viewed by projecting the magnified electron image onto a fluorescent viewing screen coated with a phosphor or scintillator material such as zinc sulfide. Alternatively, the image can be photographically recorded by exposing a photographic film or plate directly to the electron beam, or a high-resolution phosphor may be coupled by means of a lens optical system or a fibre optic light-guide to the sensor of a CCD (charge-coupled device) camera. The image detected by the CCD may be displayed on a monitor or computer.

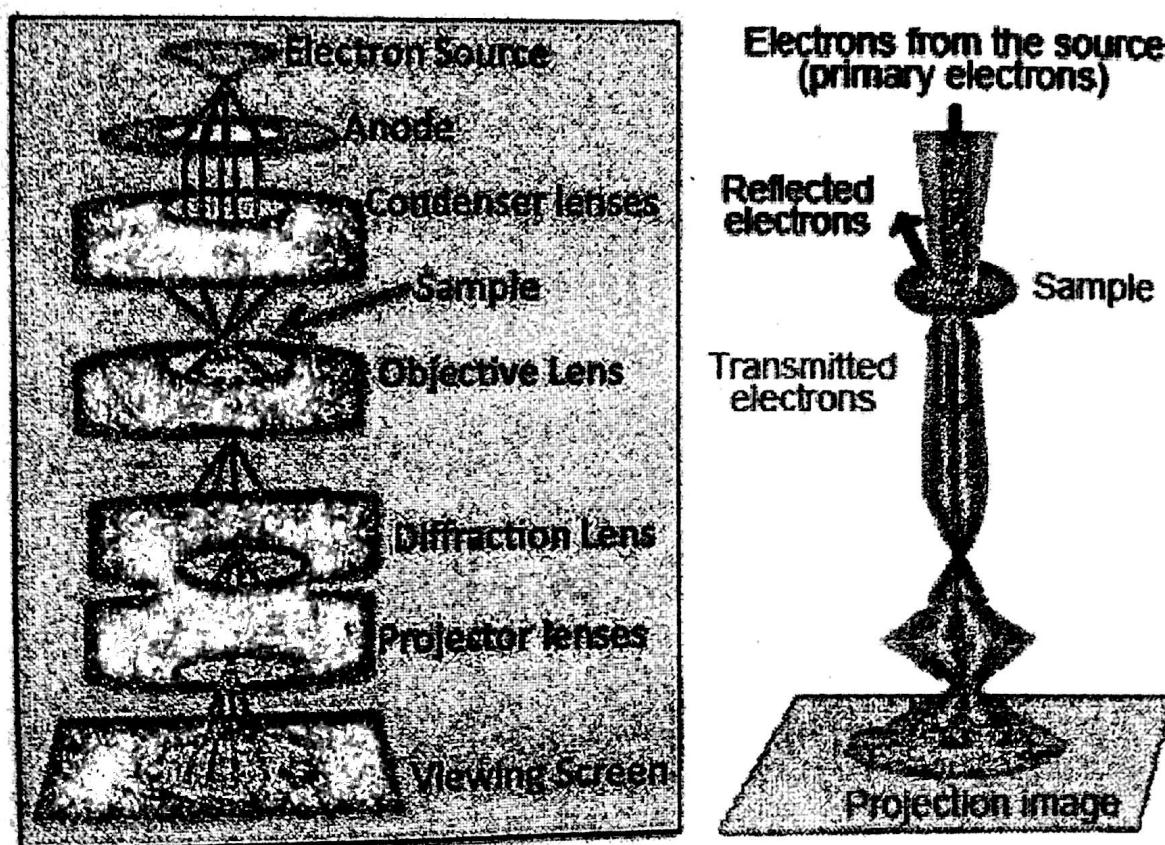
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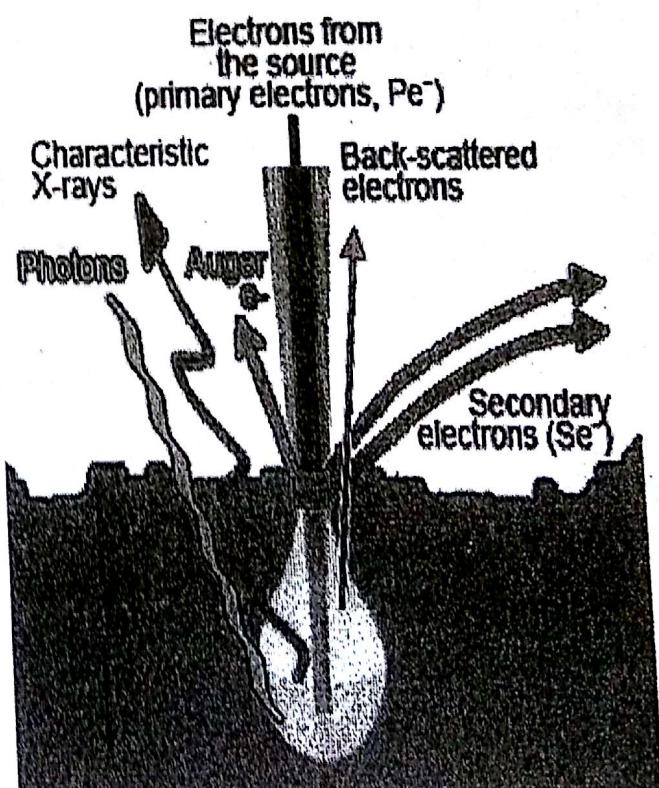
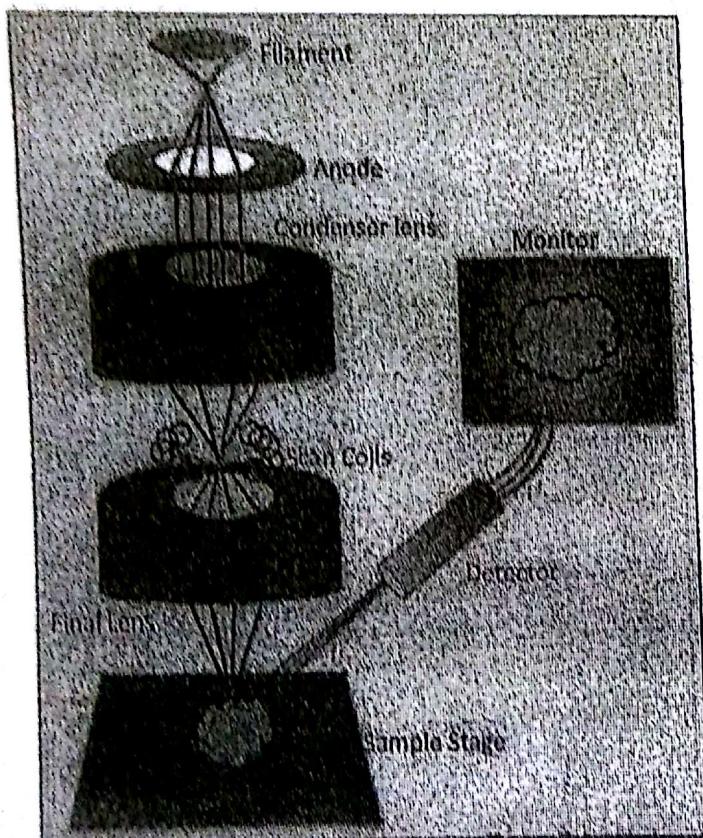


Major components of TEM

Scanning electron microscope (SEM):

Unlike the TEM, where electrons of the high voltage beam carry the image of the specimen, the electron beam of the scanning electron microscope (SEM) does not carry a complete image of the specimen at any time.

The SEM produces images by probing the specimen with a focused electron beam that is scanned across a rectangular area of the specimen (raster scanning). When the electron beam interacts with the specimen, it loses energy by a variety of mechanisms. The lost energy is converted into alternative forms such as heat, emission of low-energy secondary electrons and high-energy backscattered electrons, light emission (cathodoluminescence) or X-ray emission, all of which provide signals carrying information about the properties of the specimen surface, such as its topography and composition. The image displayed by SEM maps the varying intensity of any of these signals into the image in a position corresponding to the position of the beam on the specimen when the signal was generated.



Major components of SEM

CHAPTER 17 COLORIMETRY AND SPECTROPHOTOMETRY

17.1 GENERAL DISCUSSION

The variation of the colour of a system with change in concentration of some component forms the basis of what the chemist commonly terms **colorimetric analysis**. The colour is usually due to the formation of a coloured compound by the addition of an appropriate reagent, or it may be inherent in the desired constituent itself. The intensity of the colour may then be compared with that obtained by treating a known amount of the substance in the same manner.

Colorimetry is concerned with the determination of the concentration of a substance by measurement of the relative absorption of light with respect to a known concentration of the substance. In **visual colorimetry**, natural or artificial white light is generally used as a light source, and determinations are usually made with a simple instrument termed a **colorimeter**, or colour comparator. When the eye is replaced by a photoelectric cell (thus largely eliminating the errors due to the personal characteristics of each observer) the instrument is termed a **photoelectric colorimeter**. The latter is usually employed with light contained within a comparatively narrow range of wavelengths furnished by passing white light through filters, i.e. materials in the form of plates of coloured glass, gelatin, etc., transmitting only a limited spectral region: the name '**filter photometer**' is sometimes applied to such an instrument.

In **spectrophotometric analysis** a source of radiation is used that extends into the ultraviolet region of the spectrum. From this, definite wavelengths of radiation are chosen possessing a bandwidth of less than 1 nm. This process necessitates the use of a more complicated and consequently more expensive instrument. The instrument employed for this purpose is a **spectrophotometer**.

An optical spectrometer is an instrument possessing an optical system which can produce dispersion of incident electromagnetic radiation, and with which measurements can be made of the quantity of transmitted radiation at selected wavelengths of the spectral range. A photometer is a device for measuring the intensity of transmitted radiation or a function of this quantity. When combined in the spectrophotometer the spectrometer and photometer are employed conjointly to produce a signal corresponding to the difference between the transmitted radiation of a reference material and that of a sample at selected wavelengths:

The chief advantage of colorimetric and spectrophotometric methods is that they provide a simple means for determining minute quantities of substances. The upper limit of colorimetric methods is, in general, the determination of constituents which are present in quantities of less than 1 or 2 per cent.

The sensitivity can, however, be improved if the technique of derivative spectrophotometry (Section 17.12) is employed. The development of inexpensive photoelectric colorimeters has placed this branch of instrumental chemical analysis within the means of even the smallest teaching institution.

In this chapter we are concerned with analytical methods that are based upon the absorption of electromagnetic radiation. Light consists of radiation to which the human eye is sensitive, waves of different wavelengths giving rise to light of different colours, while a mixture of light of these wavelengths constitutes white light. White light covers the entire visible spectrum 400–760 nm. The approximate wavelength ranges of colours are given in Table 17.1. The visual perception of colour arises from the selective absorption of certain wavelengths of incident light by the coloured object. The other wavelengths are either reflected or transmitted, according to the nature of the object, and are perceived by the eye as the colour of the object. If a solid opaque object appears white, all wavelengths are reflected equally; if the object appears black, very little light of any wavelength is reflected; if it appears blue, the wavelengths that give the blue stimulus are reflected, etc.

Table 17.1 Approximate wavelengths of colours

Ultraviolet	< 400 nm	Yellow	570–590 nm
Violet	400–450 nm	Orange	590–620 nm
Blue	450–500 nm	Red	620–760 nm
Green	500–570 nm	Infrared	> 760 nm

It must be emphasised that the range of electromagnetic radiation extends considerably beyond the visible region. The approximate limits of wavelength and frequency for the various types of radiation, including the frequency range of sound waves, are shown in Fig. 17.1 (not drawn to scale); this may be regarded as an electromagnetic spectrum. It will be seen that γ -rays and X-rays have very short wavelengths, while ultraviolet, visible, infrared and radio waves have progressively longer wavelengths. For colorimetry and spectrophotometry, the visible region and the adjacent ultraviolet region are of major importance.

Electromagnetic waves are usually described in terms of (a) wavelength λ (distance between the peaks of waves in cm, unless otherwise specified), (b) wavenumber $\tilde{\nu}$ (number of waves per cm), and (c) the frequency ν (number of waves per second). The three quantities are related as follows:

$$\frac{1}{\text{Wavelength}} = \text{Wavenumber} = \frac{\text{Frequency}}{\text{Velocity of light}}$$

$$\frac{1}{\lambda} = \tilde{\nu} = \frac{\nu}{c}$$

The units in common use are:

$$1 \text{ \AAngstrom unit} = 1 \text{ \AA} = 10^{-10} \text{ metre} = 10^{-8} \text{ cm}$$

$$1 \text{ nanometre} = 1 \text{ nm} = 10 \text{ \AA} = 10^{-7} \text{ cm}$$

$$1 \text{ micrometre} = 1 \mu\text{m} = 10^4 \text{ \AA} = 10^{-4} \text{ cm}$$

$$\text{Velocity of light} = c = 2.99793 \times 10^8 \text{ m s}^{-1}$$

The potentiostat is then caused to make a voltage sweep in reverse, starting from the potential used in the electrolysis. This means that a gradually increasing positive potential is applied to the H.M.D.E., which is now the anode of the cell. If the current is measured and plotted against the voltage (a recorder is used), then initially a gradually increasing current corresponding to the residual current of conventional polarography, and due mainly to the ground solution, is observed. As the potential approaches the oxidation potential of one of the metals dissolved in the mercury, then ions of that metal pass into solution from the amalgam and the current increases rapidly and attains a maximum value when the potential has a value approximating to the appropriate oxidation potential. The metal is said to be 'stripped' from the amalgam, and if the potential were held at the value corresponding to the maximum current, all of the metal would eventually be returned to the solution. In actual fact, however, the potential is not held stationary, and as the potential sweep continues, the current declines from its maximum value and settles down to a new approximately steady value. In other words, the curve shows a peak. With continuing rise in the anodic potential, fresh peaks will be produced in the curve as the oxidation potentials of the different metals contained in the amalgam are reached: by analogy with polarogram, the resulting curve is termed a **voltammogram** (or **stripping voltammogram**). A typical result with a single metal concentrated in the mercury is shown in Fig. 16.13.

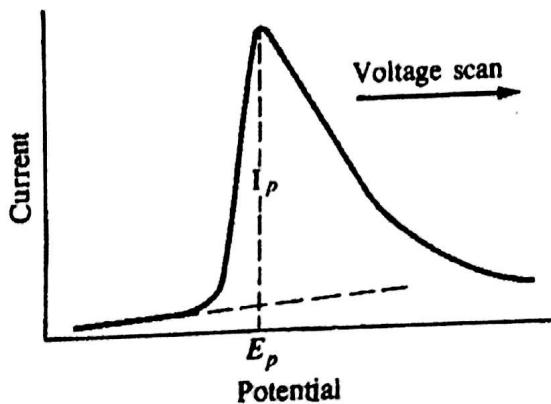


Fig. 16.13

The peaks are characterised by the peak potential E_p , by the peak current I_p (i.e. the height of the peak) and by the breadth b (i.e. the voltage span of the peak at the point where the current is $0.5I_p$): these parameters are, however, dependent upon characteristics of the electrode and upon the rate of the voltage sweep during the stripping process. The magnitude of the peak current is proportional to the concentration in the amalgam of the metal being stripped, and is, therefore, proportional to its concentration in the original solution.

From the nature of the process described above it has been referred to as 'stripping polarography', but the term 'anodic stripping voltammetry' is preferred. It is also possible to reverse the polarity of the two electrodes of the cell, thus leading to the technique of cathodic stripping voltammetry.

In just the same way as differential pulse polarography represents a vast improvement over conventional polarography (see Section 16.10), the application of a pulsed procedure leads to the greatly improved technique of differential pulsed anodic (cathodic) stripping voltammetry. A particular feature of this