The absorbancy index, a_s is defined as :

$$a_s = \frac{A}{CL}$$

[where C = concentration of absorbing material in gms/litre

L =distance in cms travelled by the light in solution.]

If one wishes to express the light absorption in terms of the molar concentration of the absorbing material, the molar absorbancy index, a_m will be equal to :

$$a_m = a_s M$$

[where M = molecular weight of the absorbing material.]

Spectrophotometer. A spectrophotometer has two fundamental parts : a source of radiant light and a monochromator. Fig. 35–10 outlines Beckman spectrophotometer. It consists of a prism. This disperses the radiant energy into a spectrum. A slit is also fixed which selects a narrow portion of the spectrum. The standard cell or cuvette is placed in a light-tight-unit. The incident light strikes the standard cell and emergent light passes into a photocell. The photocell changes the emerging light energy into measureable electrical energy.

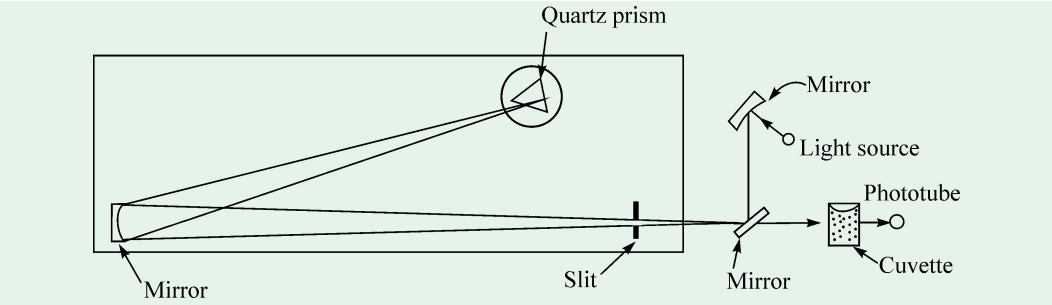

Fig. 35–10. Beckman spectrophotometer

(*Adapted from Conn EE and Stumpf PK, 1966*)

Applications. The technique finds many applications :

(*a*) It can be used in determining the concentration of a compound by measuring the optical density, provided the absorbancy index, a_s is known.

(*b*) The course of a reaction can be determined by measuring the rate of formation or disappearance of a light-absorbing compound.

(*c*) A compound can be identified by determining its absorption spectrum in the visible and ultraviolet region of the spectrum.

ELECTROPHORESIS

Principle. Migration of ions in an electric field at a definite *p*H is called **electrophoresis**. This method was developed by Arne W.K. Tiselius in 1937 and is based on the principle that the proteins migrate in an electric field except at the *p*H of their isoelectric point (refer page 218). And in a mixture of proteins, each protein with its characteristic electrical charge will respond differently to an applied electric potential. The rate of this electrophoretic migration (or 'mobility') depends on the pH of the medium, strength of the electric field, magnitude of the net charge on the molecule and the size of the molecule. A generalized diagram explaining the principle of gel electrophoresis for analyzing and sizing proteins is presented in Fig. 35–11.

ARNE W.K. TISELIUS
(LT, 1902-1971)

Tiselius, a Swedish physical biochemist, won the **1948 Nobel Prize in Chemistry** for the discovery of proteins inblood serum and for the development of electro-phoresis as a technique for studying proteins.

Electrophoresis apparatus. Tiselius electrophoresis apparatus consists of compartmented cells forming a U tube, connected to an anode and a cathode compartment. The protein solution is placed at the bottom of the U tube. The U tube is kept immersed in a water bath at 4°C to minimize convection currents and the movement of the proteins is visualized by a Schlieren optical system.

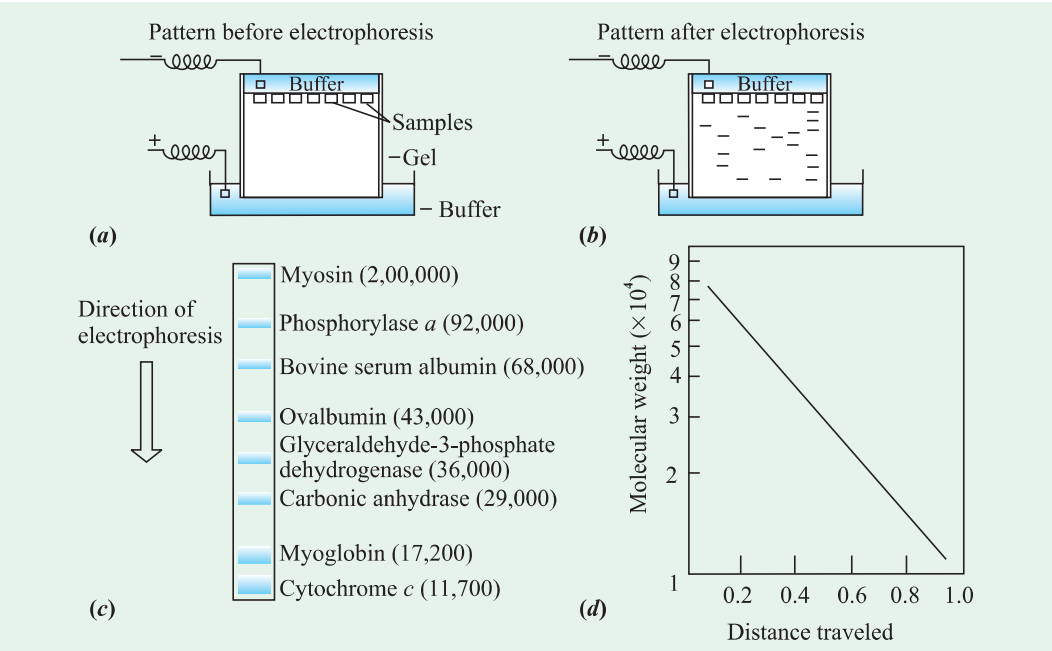

Fig. 35–11. Gel electrophoresis for analyzing and sizing proteins

(***a***) Apparatus for slab-gel electrophoresis. Samples are layered in the little slots cut in the top of the gel slab. Buffer is carefully layered over the samples, and a voltage is applied to the gel for a period of usually 1-4 h.

(***b***) After this time, the proteins have moved into the gel at a distance proportional to their electrophoretic mobility. The pattern shown indicates that different samples were layered in each slot.

(***c***) Results obtained when a mixture of proteins was layered at the top of the gel in phosphate buffer, pH 7.2, containing 0.2% SDS. After electrophoresis, the gel was removed from the apparatus and stained with Coomassie Blue. The protein and its molecular weight are indicated next to each of the stained bands.

(***d***) The logarithm of the molecular weight against the mobility (distance traveled) shows an approximately linear relationship.

(*Source: Data of K Weber and M Osborn*)

Consider that the protein solution is composed of 3 components, A, B and C and that these components carry charges of the same sign (either + or –) but of different magnitude. Before the electric field is applied, the solution will be homogeneous from ascending boundary to descending boundary. On the flow of current, the 3 components will separate. If the magnitude of the electric charge is in the order A > B > C, the components will separate as shown in Fig. 35–12. The faster moving component A will be present in pure form in the ascending boundary, as will C in the descending boundary. The component B will, however, always be mixed with A or with C. The rate of migration of the protein is measured by observing the movement of the boundary as a function of time. During electrophoretic migration, the concentration gradients will be set up at the boundaries. These gradients can be measured by optical systems since most of the proteins are colourless. Migration of coloured proteins like hemoglobin is, however, readily observed. The method described above is usually referred to as **free boundary electrophoresis** or simply **free electrophoresis** (*i.e.*, in free solution).

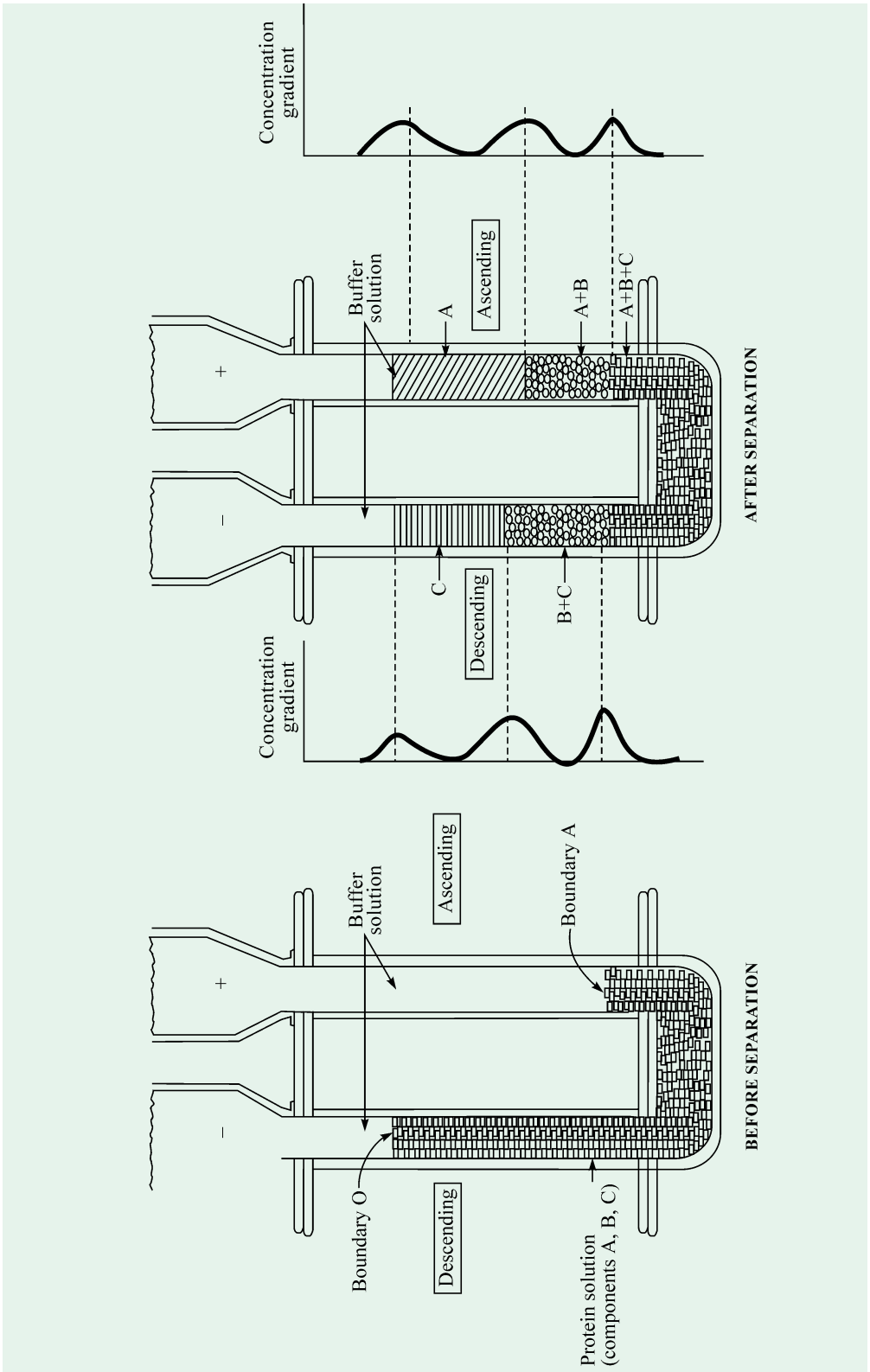

Fig. 35–12. U tube of an electrophoresis cell before and after electrophoretic separation

Modification. An important modification of the electrophoretic technique described above is the migration of proteins (and of other charged molecules) in an electric field passing through a solution supported by inert materials such as moistened filter paper, starch gel, silica gel, cellulose sponges or glass powder. This method, termed as **zone electrophoresis** or **ionophoresis**, allows ready separation of components of different mobility into zones. After separation, the different zones may be located by staining with dyes. The individual components can also be extracted from these zones. *Ionophoresis technique is, thus, a combination of electrophoresis and chromatography.* A schematic diagram of the zone electrophoresis apparatus using gel appears in Fig. 35–13.

Zone electrophoresis has proved much useful for the study of serum proteins and the cleavage products of proteins and of nucleic acids.

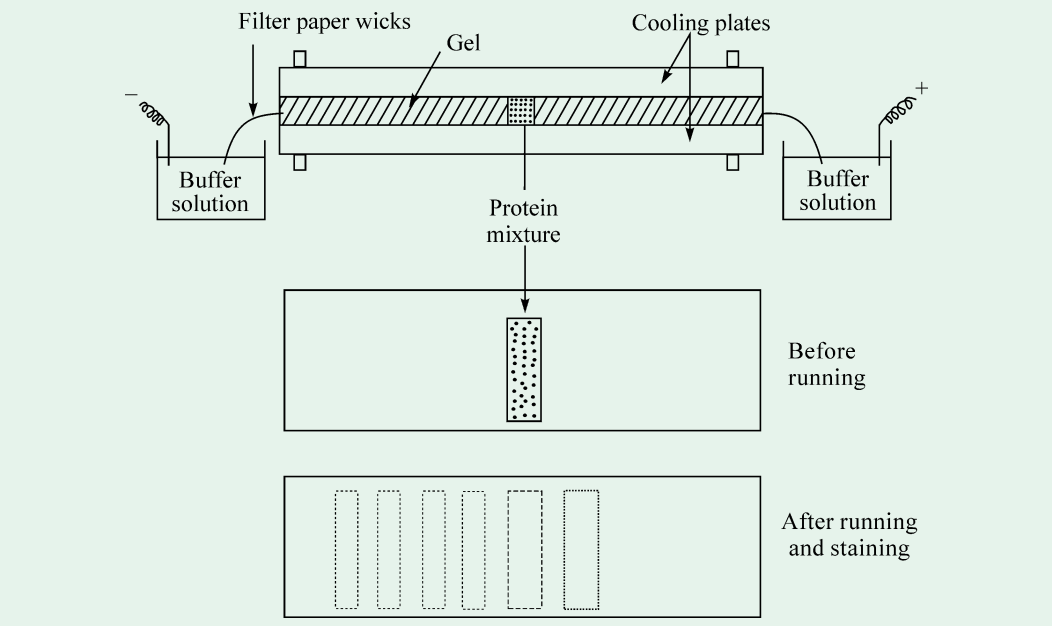

Fig. 35–13. Schematic representation of gel electrophoresis apparatus

[The two lower figures represent separation of protein mixtures.]

(*Redrawn from Fairley JL and Kilgour GL, 1966*)

ULTRACENTRIFUGATION

Principle. The ultracentrifuge method for determining the molecular weights of proteins was developed by Svedberg. In this method, the protein molecules are subjected to gravitational (centrifugal) forces greater in magnitude than the thermal forces whereby causing them to diffuses. Protein molecules are large-sized and hence described as macromolecules. Under high centrifugal forces, these molecules can be made to sediment toward one end of a centrifuge tube. By means of photographic and optical systems similar to those used in electrophoresis, it is possible to follow the rate of sedimentation. The rate of sedimentation for a protein under a certain centrifugal force depends on the density, shape and size of the molecule.

THEODORE SVEDBERG
(LT, 1884–1971)

Svedberg, a pioneer Swedish physical chemist, was responsible for much of the theoretical and practical development of centrifugation as a tool for studying large molecules. He was awarded the coveted **1926 Nobel Prize in Chemistry** for his work on colloids and macromolecular compounds. He also worked on nuclear chemistry, radiation biology, photographic processing and also worked out a method of making synthetic rubber during World War II.