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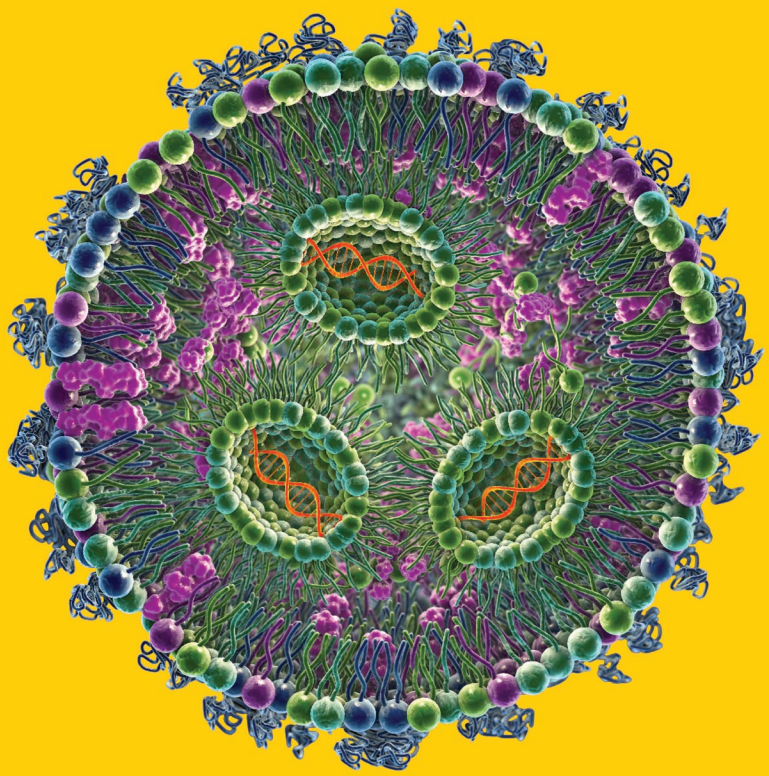

Concise Textbook of BIOCHEMISTRY for Paramedical Students

for BPT and Other Paramedical Courses

DM Vasudevan
Sukhes Mukherjee

3rd Edition

JAYPEE

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25

CHAPTER

Clinical Laboratory Techniques

LEARNING OBJECTIVES

The learner will be able to:

- Enumerate and discuss the principles, uses and types of electrophoresis
- List the types and uses of chromatography
- Describe adsorption chromatography
- Explain partition chromatography
- Mention the different types of immunoassays
- Describe in detail on enzyme-linked immunosorbent assay (ELISA)
- Discuss on the chemiluminescent immunoassay (CLIA)
- Explain the principles and applications of colorimetry
- List salient features of spectrophotometer
- Osmosis, osmotic pressure
- Reverse osmosis
- Dialysis
- Filtration
- Microscopes; light, electron microscope

ELECTROPHORESIS

Electrophoresis is the migration of charged particle or molecule in a medium under the influence of an applied electric field. The usual purpose of electrophoresis is to determine the number, amount, and mobility of component in a given sample or to separate them. Tiselius first performed electrophoresis.

The positively charged particles migrate toward cathode and the negatively charged particles migrate toward anode. The rate of migration depends on the number of charges each particle carries. As a result of different rates of migration, a mixture of substance can be separated into number of fractions upon suspension in an aqueous solvent. Many important biomolecules such as nucleotides, nucleic acid, amino acid, peptides, proteins, etc., acquire either positive or negative charges.

Factors Affecting Electrophoretic Mobility

- **Sample:** Charge/mass ratio of the sample dictates its electrophoretic mobility. The mass depends on the size and shape of the molecule.
- **Charge:** The higher the charge of the molecule, the greater is the electrophoretic mobility. The charge, however, is dependent on the pH of the medium.
- **Size:** The bigger the molecule, greater are the fractional and electrostatic forces exerted up on it by the medium. So, the larger particles have a smaller electrophoretic mobility compared to the smaller particles.
- **Electric field:** An increase in potential gradient increases the rate of migration. The current in the solution placed between two electrodes is carried mainly by the buffer ions. An increase in the potential difference increases the current. Resistance to flow of current is inversely proportional to the rate of migration. The power pack can control the current to a constant level without the production of heat.
- **Support medium:** An inert supporting medium is chosen for electrophoresis.

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But even this inert supporting medium can exert adsorption or molecular sieving effect on the particle, thereby influencing the rate of migration.

- **Composition of buffer:** Commonly used buffers are phosphate, citrate, acetate, tris, and barbitone. The choice of buffer depends on the type of sample being electrophoresed. The buffer can affect the electrophoretic mobility, if it is able to bind to component of the sample being separated.
- **Ionic strength of buffer:** Increased ionic strength of the buffer means a larger share of the current being carried by the buffer ions and or smaller proportion by the sample ions. This will lead to slower migration of sample component. Decreased ionic strength of the buffer means a larger share of the current being carried out by the sample ions, leading to faster separation. But this will cause diffusion of the smaller component that tends to be high with concomitant loss of resolution. The ionic strength used is usually between 0.05 and 0.1 M.
- **pH of buffer:** The direction and extent of migration of sample particles are dependent on pH. Buffers ranging from pH 1 to 11 can be used to produce the required separation.

Types of Electrophoresis

Microelectrophoresis

This technique involves the observation of motion of small particles (such as RBCs, neutrophils, bacteria, etc.) in an electric field under a microscope. The suspension is contained in a closed system composed of a thin-walled section for optical observations with suitable electrodes.

Moving Boundary Electrophoresis

In moving boundary electrophoresis, a buffered solution of macromolecules is placed under a layer of pure buffer solution contained in a U-shaped observation cell. The whole cell may then be immersed in a constant temperature bath insulated from vibrations. The power is switched on to generate an electric field between the electrodes. The macromolecule bearing a net negative charge and will move toward anode, while the macromolecule bearing a net positive charge will move toward the cathode.

Zone Electrophoresis

Here the molecules are immobilized by fixation in different zones. The molecules are then detected by staining them on the supporting medium. The other methods to detect the separated molecules are as follows:

- Visualization by ultraviolet (UV) light
- Detection by enzymatic reaction
- Detection by radioactivity if the molecules are radiolabeled.
- The separated component can be eluted from the medium for further studies.

The zone separation uses stabilizing media, such as paper, or gels, such as starch, agar, agarose, polyacrylamide, etc. In zone electrophoresis, the molecules in the sample, during their migration, are separated into many different zones, as the sample contains different migrating components.

Paper Electrophoresis

Filter paper contains 95% of cellulose and possesses only a slight adsorption capacity. The equipment consists of a power pack and an electrophoresis cell. The power pack provides a stabilized direct current and can be programmed to give either constant voltage or constant current. The electrophoretic cell contains the electrodes, buffer reservoirs, a support for a paper, and a transparent insulating cover. The electrodes are usually made of platinum **(Fig. 25.1)**.

Two buffer reservoirs are usually used, and each is partitioned into two interconnected sections. One containing the electrode and the other in contact with the supporting medium.

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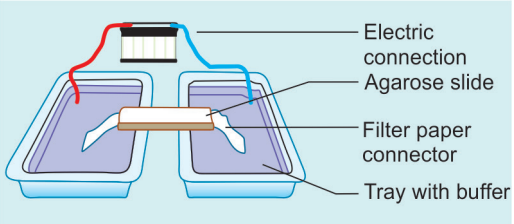

Fig. 25.1: Horizontal paper electrophoresis.

Separate compartments are necessary, so that any change in pH occurring at the electrodes does not affect the buffer in contact with the supporting medium. Wicks are used to establish contact between the buffer reservoir and the supporting medium **(Fig. 25.1)**. Wicks are made of several layers of filter paper or gauze. The paper is placed on an insulating material usually a Perspex sheet. The whole electrophoretic unit is then covered with an insulating material to minimize evaporation during run and to assure electrical insulation. The fitter strips are commonly arranged, either horizontally or vertically.

The sample may be applied as a spot (about 0.5 cm in diameter) or as a narrow uniform streak. Special devices are available commercially for this purpose. The sample is applied to the paper and the paper has been equilibrated with the buffer. Then current is switched on. Devices providing stable voltage or current are available. Overheating of the equipment can be avoided by placing the entire equipment in a cold room. The process does not usually take longer than 2 hours. The power is switched off after the run and before the paper is removed. Once removed, the paper is dried in a vacuum oven at 110°C.

Detection

- **Fluorescence:** Staining with ethidium bromide and subsequent visualization of the electrophoretogram under UV light makes DNA and RNA fluoresce and thus facilitates their detection.
- **Ultraviolet adsorption:** Proteins, peptides, and nucleic acid absorb in the range of 260–280 nm.

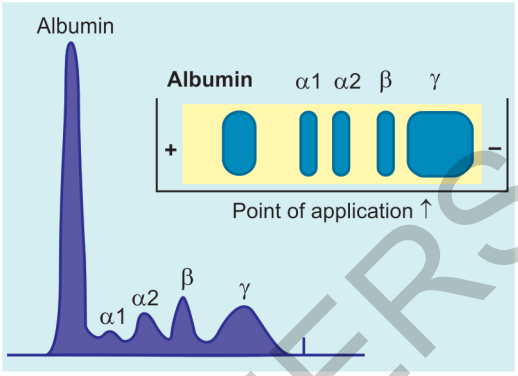

Fig. 25.2: Electrophoresis of normal human serum.

- **Staining:** Different dyes are used for detecting different components. For example, bromophenol blue or Coomassie brilliant blue is used for proteins **(Fig. 25.2, right side)**.

Quantitative Estimation

The same may be passed through a scanner to produce a graphic representation, from which the area under each peak can be noted; this will be proportional to the quantity of protein present in that peak **(Fig. 25.2, left side)**. Alternatively, the compound is eluted from the electrophoretogram The zone is excised from the paper and placed in a suitable solvent and estimated. Abnormal patterns of electrophoresis are shown in **Figure 14.1**.

Gel Electrophoresis

In paper electrophoresis, charge of the molecule was the major determinant for its electrophoretic mobility. The gels, however, are porous and the size of the molecule determines whether the molecule will enter the pore and be retarded or will bypass it. The separation thus depends on not only the charge of the molecule but also its size.

Types of Gels

Starch Gel

Potato starch is hydrolyzed in acidified acetone at 37°C. The suspension is then neutralized with sodium acetate and washed with large

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amount of distilled water and dried with acetone. This hydrolyzed starch when heated and cooled in an appropriate buffer set as a gel. The pore size in starch gel cannot be controlled and this is the biggest drawback.

Agar

Agar consists of two galactose-based polymers named as agarose and agaropectin. A solution of agar at 80°C is mixed with an equal volume of 40% polyethylene glycol which precipitates the agarose. The latter is collected, washed with distilled water, and dried with acetone. Agar solubilizes in aqueous buffers above 40°C and sets to form a gel at about 38°C. Agar is being used to separate high-molecular weight macromolecules like proteins and nuclear acids. **Figure 25.2** shows agarose gel electrophoresis.

Polyacrylamide

It is a known neurotoxin and thus care must be taken while preparing the gel. Acrylamide monomers are polymerized under specified conditions when the gel will have known porosity. The running of the electrophoresis is done as discussed above.

Immunoelectrophoresis

The technique exploits the specificity of reaction between an antigen and antibody as well as the molecular sieving of the gel. Immunoelectrophoresis is performed using a double-diffusion chamber. The chambers may be prepared by coating a glass microscope slide with agarose and punching appropriate holes in the supporting medium **(Fig. 25.3)**.

The antigen is filled in the small round well. The current is switched on and the electrophoresis is allowed to continue for 1–2 hours. Immediately after disconnecting the voltage supply, the rectangular well is filled with appropriate antiserum and the gel incubated overnight at room temperature to permit diffusion of antigen and antibody toward each other. This leads to the formation of precipitin bands at the site lateral to the position where the desired component has separated during electrophoresis **(Fig. 25.3)**. The major advantage of this method is its increased resolving ability due to the combination of electrophoresis with immune specificity. The technique can be made quantitative and has been used to detect particular antigens in sera, tissue, or cell extracts and culture filtrates. It has also been used to determine the purity of a given antigen.

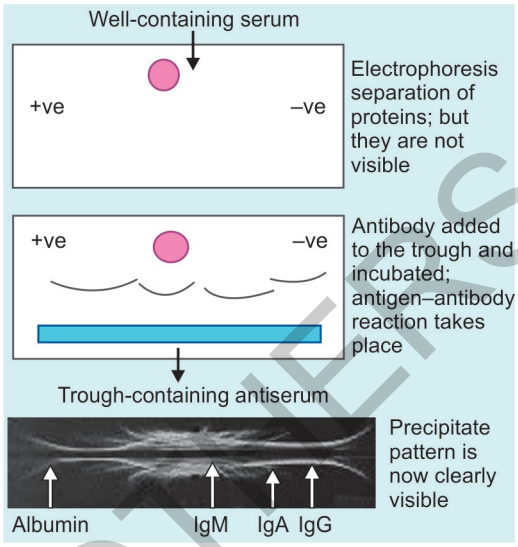

Fig. 25.3: Immunoelectrophoresis.

CHROMATOGRAPHY

Chromatography is a method of separation and purification of compounds. It is first used for separating pigments and hence, the name. Types of chromatography are:

- Adsorption chromatography
- Partition chromatography
- Paper chromatography
- Thin-layer chromatography
- Gas–liquid chromatography (GLC)
- High-pressure liquid chromatography (HPLC)
- Ion exchange chromatography
- Affinity chromatography
- Gel filtration chromatography

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Adsorption Chromatography

In this technique, the separation is based on differences in adsorption at the surface of a solid stationary medium. The common adsorbing substances used are alumina, silicates, or silica gel. These are packed into columns and the mixture of proteins to be separated is applied in a solvent on the top of the column. The components get adsorbed on the column of adsorbent with different affinity. The fractions slowly move down; the most weakly held fraction moves fastest; followed by others, according to the order of tightness in adsorption. The eluent from the column is collected as small equal fractions and the concentration of each is measured, in each fraction **(Fig. 25.4)**.

Partition Chromatography

Partition chromatography is commonly done for separation and identification of amino acids, sugar, etc., in body fluids. These compounds are soluble in both water and organic solvents and can be readily separated by this technique. The compound is partitioned between two immiscible solvents. One type of solvent, usually water, is held on the stationary supporting phase which is in the form of a thin film of inert material. The other phase consists of a mobile organic liquid that flows through the stationary phase. The components of the sample are separated depending on their partition coefficient.

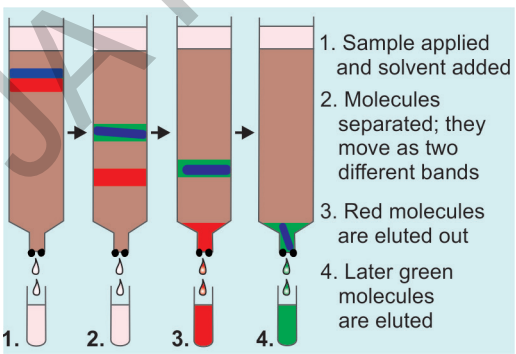

Fig. 25.4: Adsorption chromatography.

Paper Chromatography

In paper chromatography, the mixture is spotted onto the filter paper and dried, then the solvent is allowed to flow along the paper either by gravity (descending) or by capillary action (ascending). The solvent front is marked and then the paper is dried, the position of the compound (solute) present in the mixture is visualized by a suitable staining reaction. The ratio of the distance moved by the solute and that moved by the solvent (Rf value) is more or less constant for a particular solvent system **(Fig. 25.5)**. Hence, compounds can be identified by running pure standards along with unknown mixture.

Thin Layer Chromatography (TLC)

This is another version of liquid-liquid chromatography. A thin layer of silica gel (Kieselguhr) is spread on a glass plate; a biological sample is applied as a small spot; the plate is placed in a trough containing the solvent. The stationary water phase is held on the silica gel and the mobile phase of the non-polar solvent moves up. In the case of paper chromatography, it takes 14–16 hours for the separation of components to be separated. But in the case of TLC, it takes only 2–4 hours.

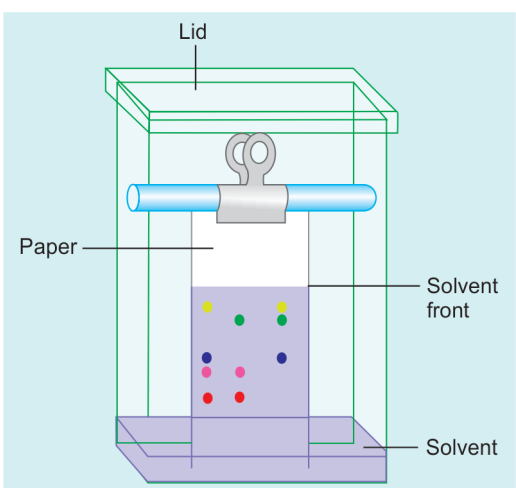

Fig. 25.5: Paper chromatography.

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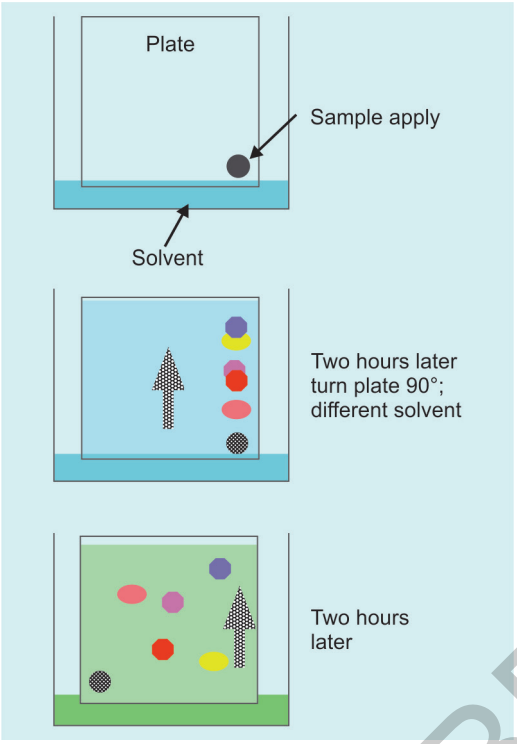

Fig. 25.6: Thin layer chromatography (two dimensional).

That is a distinct advantage for TLC. TLC can be performed as two-dimensional **(Fig. 25.6)**.

After the chromatographic run is over, when the paper or glass plate is dried, it is sprayed with ninhydrin reagent (for locating amino acids and proteins). The spots may be identified by the Rf value of the unknown substance and compared with those of pure standards. (Rf = ratio of fronts). The Rf value is the ratio of the distance travelled by the substance (solute) to the distance travelled by the solvent. The Rf value is a constant for a particular solvent system at a given temperature.

Gas–Liquid Chromatography (GLC)

This is another type of partition chromatography where the stationary phase is a liquid, and the mobile phase is gas. The stationary liquid phase is supported by a column of inert material such as silica in a long narrow column. The mixture of substances to be separated is made volatile at one end of the column and the vapors are swept over the column by an inert carrier gas like argon or nitrogen. The fractions emerging from the column are detected and quantitated by a detecting device. This is more suitable for compounds (e.g., lipids) that resist degradation at high temperatures.

High-pressure Liquid Chromatography (HPLC)

HPLC is now widely used for the separation of all types of compounds. Incompressible silica or alumina microbeads are used as the stationary phase. The technique allows high flow rates. The liquid phase passes through this column under high pressure (1,000 times atmospheric pressure). The column may be packed with materials for adsorption, partition, or ion exchange. The method is, therefore, based on the same principle as for those types already described, but separation is achieved with better resolution and high speed (within minutes). HPLC can resolve mixtures of lipids whose solubility differs only slightly. In **reversed phase HPLC**, hydrophobic polymers are used as stationary phase; this is generally used to separate peptides.

Ion Exchange Chromatography

In this method, the separation is based on electrostatic attraction between charged biological molecules to oppositely charged groups on the ion exchange resins. These resins are cross-linked polymers containing ionic groups as part of their structure. The polymer must be sufficiently cross-linked to have negligible solubility, but porous enough for the ions to diffuse freely through it.

The resins are either cation exchange resins or anion exchange resins. The ionic groups in cation exchange resins are sulphonic and carboxylic groups, whereas **anion exchange** resins have quaternary nitrogen (N^+), e.g., Amberlite IRC- 50 (weak cation); Dowex-3

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(weak anion); and DEAE-Cellulose (strong anion). The separation is based on the ionic character of proteins and amino acids (isoelectric point). The **cation exchange** particles carry acid groups, e.g., $COO^-.Na^+$. When cations (C^+) are passed through the column, Na^+ in the resin is replaced by C^+; thus C^+ particles are adhered in the column, while negatively charged particles are eluted out easily **(Fig. 25.7)**.

Affinity Chromatography

The technique is based on the high affinity of specific proteins for specific chemical groups. Thus, coenzymes can be used to purify the enzymes. For example, NAD is used to purify dehydrogenases. By using antibodies, antigens could be easily separated **(Fig. 25.8)**. Conversely, antibodies can be purified by passing through a column containing the antigen.

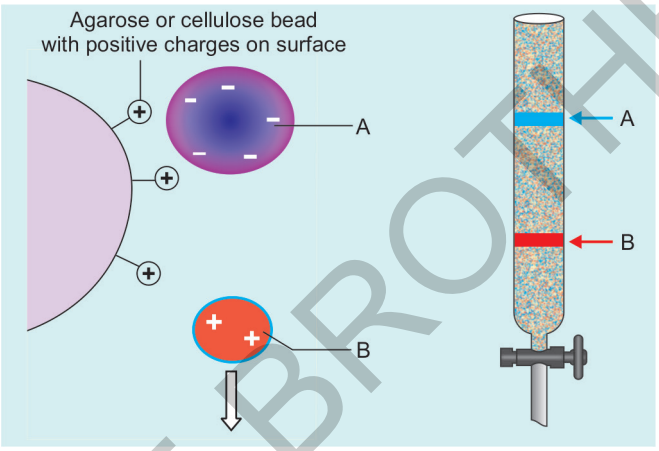

Fig. 25.7: Ion-exchange chromatography.

(A: negatively charged molecules attach the beads and so move slowly;
B: positively charged molecules repel the beads, so move faster in the column)

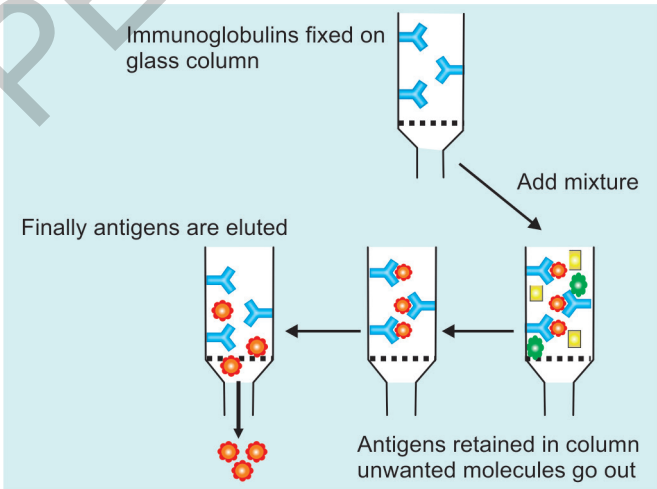

Fig. 25.8: Affinity chromatography.

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Gel Filtration (Size Exclusion) Chromatography

It is also called molecular sieving. Hydrophilic cross-linked gels like acrylamide (Sephacryl), agarose (Sepharose) and dextran (Sephadex) are used for the separation of molecules based on their size. Sephadex is widely used, and the range of separation is based on pore size designated by the symbols G-10 to G-200. The gel is packed in a column. The gel particles are porous in nature. These pores will allow small molecules (e.g., insulin, mol. wt., 5,700 D) to enter the gel. But larger molecules (e.g., immunoglobulin, mol. wt., 150,000 D) could not enter pores of the gel and so are excluded. Suppose a mixture of insulin plus immunoglobulin is passed through the column. The small molecules can enter the gel particles, then come out, and re-enter into another particle. Thus, the insulin molecule has to travel a long distance inside the gels. Thus, small molecules are held back. But the large immunoglobulin molecules cannot enter the pores and sidetrack the gel particles; so, they move in the column rapidly **(Fig. 25.9)**. In short, **larger molecules will come out first, while smaller molecules are retained in the column.** The gel filtration technique is used for: (a) separation of protein molecules; (b) purification of proteins; and (c) molecular weight determination.

ENZYME-LINKED IMMUNOSORBENT ASSAY

ELISA is the abbreviation for enzyme-linked immunosorbent assay. The ELISA techniques are widely used not only for hormone measurements but also for detecting other growth factors, tumor markers, bacterial or viral antigens, antibodies against microbes, and any other antigens or antibodies in biological fluids. This test is commonly employed to detect antigens or antibodies present in very small quantities in tissues or blood.

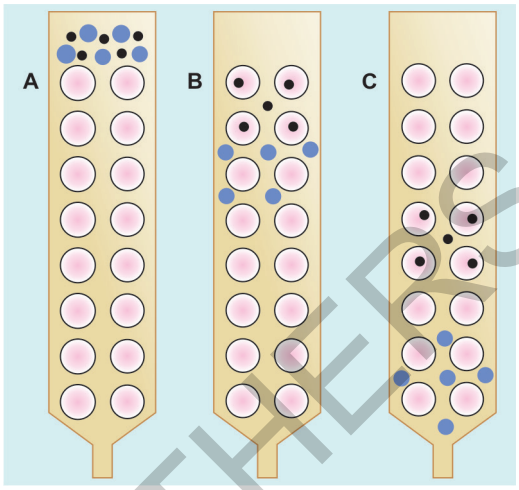

Fig. 25.9: Sephadex (gel filtration) chromatography.

(A: protein solution is added on the top of the column; B: small proteins get inside the beads, and so take a longer time to reach the bottom; C: larger molecules cannot enter the beads, so travel quickly, and reach the bottom faster)

Antibody Detection by ELISA

This is useful to detect small quantities of antibodies in the blood. A good example is the test for the detection of HIV antibody. In patients with AIDS, the human immunodeficiency virus (HIV) produces specific antibody. To detect the HIV antibody, the following method is used.

Antigen from HIV is coated in the wells of a multiwell (microtiter) plate. Patient's serum is added and incubated. If it contains the antibody, it is fixed. The wells are washed. This is to remove excess antibodies in the serum. Next a second antibody (antibody against human immunoglobulin) conjugated with horseradish peroxidase (HRP) is added. Then a color reagent containing hydrogen peroxide and diaminobenzidine is poured over. If a brown color develops, it means that the antibody was originally present in the patient's serum **(Fig. 25.10)**. Here, the color developed is proportional to the antibody. So, the quantity of the antibody can be calculated.

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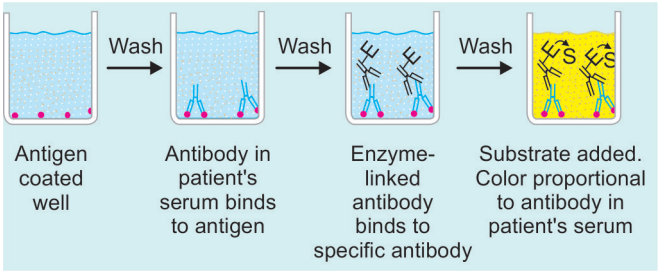

Fig. 25.10: Indirect enzyme-linked immunosorbent assay to detect antibody.

HIV antibody is an example, any antibody could be detected by using the specific antigen.

Antigen Detection by ELISA Method

At first, the specific antibody is fixed to the well of a microtiter plate **(Fig. 25.11)**. A good example is the assay of thyroid hormone T4. The patient's serum is added in the well, and incubated for 30 minutes at 37°C. By this time, antigen (T4 in this example) present in the serum is fixed on the antibody. Excess antigen and other unwanted proteins are washed out.

Then, specific antibody (antibody against T4, tagged with enzyme HRP) is added. If the antigen is already fixed, the antibody–enzyme conjugate will be fixed in the well. Then a color reagent containing hydrogen peroxide (H_2O_2) and diaminobenzidine (DAB) are added **(Fig. 25.11)**. The hydrogen peroxide will oxidize the DAB to produce a color. The intensity of the color thus produced will be proportional to the antigen (T4) present in the patient's blood. So, quantitation is easy.

OSMOSIS

Osmosis is an important property of particles in solution. It is the spontaneous flow of a solvent into solution when a semipermeable membrane separates the two. It is also the flow of a solvent from a more dilute solution to a relatively concentrated solution through a semipermeable membrane. Osmosis refers strictly to the flow of solvent, i.e., passive movement.

Experiment

A beaker is partitioned by a semipermeable membrane. Both sides are filled with equal volume of water **(Fig. 25.12A)**. A known quantity of solute is dissolved in the left compartment. Water molecules tend to passively move toward the left compartment. Once the equilibrium is reached, the water level in the left compartment increases **(Fig. 25.12B)**. Eventually, the force of the column of water on the hypertonic side of the semipermeable membrane will equal the

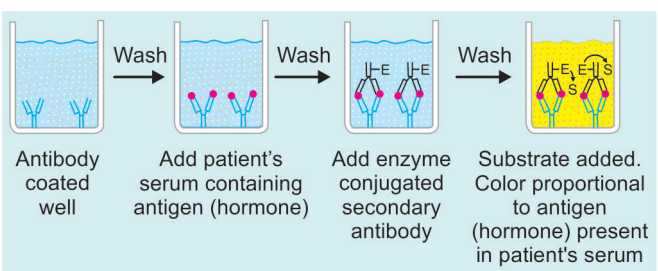

Fig. 25.11: Antigen detection by ELISA. The five sequential steps are explained in the text.

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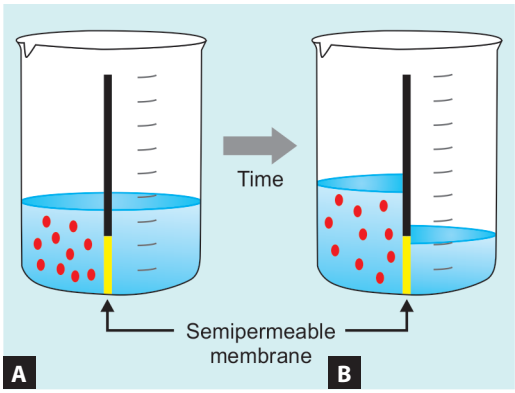

Figs. 25.12A and B: Osmosis.

force of diffusion on the hypotonic (the side with a lesser concentration) side, thereby creating equilibrium. When the equilibrium is reached, water continues to flow, but it flows both ways in equal amount and force, therefore stabilizing the solution.

Osmotic Pressure

Osmotic pressure is defined as the excess pressure applied to the solution to prevent the passage of the solvent through the semipermeable membrane separating the two solutions. The osmotic pressure of a solution is directly proportional to the concentration of the solute and in turn depends on the number of molecules or ions contained in the solution. Although dependent on the number of molecules of the solute, osmotic pressure is independent of the nature of the solute. A substance of lower molecular weight will have more molecules per unit volume than a substance of higher molecular weight and will therefore exert greater osmotic pressure, e.g., albumin has lower molecular weight than globulin. Hence, albumin exerts greater osmotic pressure than globulin.

Tonicity

Living cells have selectively permeable membrane that are permeable to water and some low-molecular-weight solutes but much less permeable to other substances. This leads to the inflow of some solute molecule along with the solvent, while others are retained. Under these conditions, the solution exhibits only that fraction of its total osmotic pressure, due to the solutes, which are retained. This fraction of total osmotic pressure of a solution is termed as its ***tonicity***.

Isotonic Solution

A pair of solutions, which produce no net flow through a semipermeable membrane, is said to be isotonic. If the cell is kept in hypotonic solution, the plasma membrane will allow water to pass through it and will setup an excess pressure in the interior of the cell causing the cell wall to be tight. This condition is known as "turgor" and the cell is said to be turgid. If the cell is immersed in a concentrated solution (hypertonic solution), water will pass out of the cell. The cytoplasm will then shrink and detach itself from the cell wall. This phenomenon is said to be "***lysis***".

Role of Osmosis in Physiological Processes

- **Regulation of blood volume:** The osmotic pressure of plasma proteins regulates water to flow from the interstitial fluid to the blood vessels. The osmotic pressure of protein is referred to as oncotic pressure.
- The cells in the body are isotonic with the tissue fluid and blood plasma [Living red blood cells (RBCs) if suspended in 0.92% sodium chloride solution, they neither gain nor lose water, i.e., intracellular fluid of red cell is isotonic with 0.92% NaCl solution]. An appreciable osmotic dilution of plasma would create a dangerous hydrostatic pressure in the RBCs and cells, which would take in water to achieve osmotic equilibrium. This does not occur in the body because water or salt are excreted by the kidneys, so as to keep the blood isotonic with the cells.

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- Absorption from the gastrointestinal tract.
- Fluid interchange in various compartments of the body follows the principle of osmosis. Water moves spontaneously depending on the osmotic pressure.
- **Destruction of RBC by hemolysis:** When RBCs are placed in a hypotonic solution, the RBCs swell, owing to water passing into the cell osmotically (**endosmosis**). If the solution is sufficiently hypotonic, the cell may even rupture with the cell content diffusing into the surrounding fluid. This is called hemolysis.
- If the RBCs are placed in hypertonic solution, the water passes out of the cell (exosmosis) and the RBCs will shrink. This process is called **crenation**.

Reverse Osmosis

Suppose a solution is separated from pure solvent by a semipermeable membrane and if the pressure applied on the solution is more than the osmotic pressure, the solvent will start flowing from the solution toward the pure solvent. This phenomenon is known as reverse osmosis. This principle is being employed for getting drinking water from seawater.

DIALYSIS

The process of separating crystalloids from colloids by means of diffusion of crystalloid through a membrane is called **dialysis** and the apparatus used for separation is called **dialyzer**. This method is based on the fact that the pores of the membrane are very small and allows the free passage of only the ions of the solution, but the colloidal particles are too big to pass through these pores and hence, they are retained inside the bag.

Dialysis consists of parchment or cellulose acetate bag immersed in water. The impure solution (mixture of crystalloids and colloids) is placed in the dialyzer and the outside water is continuously renewed. The crystalloids slowly pass freely through the membrane into the outside water, while the colloidal solution is left inside the dialyzer. Dialysis is a very slow process. The membrane used for dialysis is semipermeable; therefore, it allows selectively the passage of only crystalloids and not the colloidal particle.

Electrodialysis

Applying a potential difference across the membrane can accelerate the process of diffusion of crystalloid impurities from colloidal solution. Under the influence of electric field, the crystalloids migrate toward oppositely charged electrodes at a much faster rate. This process is called ***electrodialysis***.

Medical Importance of Dialysis

- Separation of proteins from small solutes.
- Formation of biological ultrafiltrates such as interstitial fluid, cerebrospinal fluid, and glomerular filtrate.
- Dialysis by artificial kidney.

Acute or chronic loss of function of kidney is a threat to life and requires removal of toxic waste products and restoration of body fluid volume and composition toward normal. This can be accomplished by dialysis with an artificial kidney. In acute renal failure, an artificial kidney may be used to tide the patient over until the kidney resumes its normal function.

Dialysis cannot maintain completely normal body fluid composition and cannot replace all the multiple functions performed by the kidney. Thus, better treatment of permanent loss of kidney function is to restore functional kidney tissues by means of kidney transplantation.

KNOW MORE

Principle of Artificial Kidney

The basic principle of artificial kidney is to pass blood through minute blood channels bounded by a thin membrane. On the other side of the membrane is a dialyzing fluid into which the unwanted substances in the blood pass by diffusion.

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FILTRATION

Filtration is exclusion of particle having a larger diameter than the pores of the separation medium. It has often been used for the separation of solid from liquid. Two types of filtrations are used to separate particle from liquid, i.e., surface filtration and depth filtration.

- **Surface filtration (screen filtration)** is performed with filter paper or membranes. Surface filtration retains the solid material on the filter medium and filtrate (the liquid that passes through the filter paper) to be collected or discarded as required.
- **Depth filtration:** In this, the separation medium allows the particle to be retained by the body of the filter medium as well as on the surface. Depth filters are usually made up of cotton, fiberglass, asbestos, etc. The depth filter is used as a prefilter to trap larger particles, thus lengthening the life of the secondary surface filter. Combining depth and surface filters into a single filter is useful for purifying the solutions.
- Filtration may be conducted under gravity, pressure, or in vacuum. The **vacuum filtration** is used to accelerate the filtration rate.
- Many filtrations in the clinical laboratory are carried out with **filter paper**. Different types of filter paper include low-ash or ash-less filter paper as well as various grades related to thickness. The membrane filters are made from homogeneous polymeric materials such as cellulose ester, cellulose acetate, polyvinyl chloride (PVC), etc.
- **Ultrafiltration** is a technique for removing dissolved particles using an extremely fine filter.

MICROSCOPES

The human eyes have limited power to distinguish small objects. The capacity of the eye to distinguish two closely placed points is the resolving power of the eye. The resolving power of the human eye is about 100 microns, i.e., two points closer than 100 microns cannot be perceived by human eye as two separate points but as a single point. So naturally cells remain invisible to our naked eye and study of the cell cannot be possible without the aid of microscope, which is having a higher resolving power.

Compound Light Microscope

An ordinary compound microscope uses visible light to illuminate the object. It consists of lenses that magnify and resolve the image of the object and focus the light on the retina of the observer's eye. In its simplest form, the compound microscope consists of two lenses, one at each end of a hollow tube. The lens closer to the eye of the observer is called the **eyepiece** and that closer to the object is called the **objective**. The object mounted on a glass slide is placed on a stage beneath the objective lens. The light source is beneath the object and often a third lens called the **condenser** is placed between the object and the light source to focus the light on the object. In modern compound microscope, instead of a single lens, a combination of a number of lenses is used for each objective and eyepiece.

When the light rays pass through the object, different regions of the object will refract the light rays at different angles producing contrast between the structures. The objective lens and the eyepiece lens form a magnified image of the object which falls on the retina of the eye. If the object is very small in comparison with the wavelength of the light beam, the object cannot be resolved. The limit of resolution of the compound microscope is 0.2 micron.

Different types of light microscopes with increased resolving power include dark-field microscopes, phase-contrast microscopes, polarization microscopes, interference microscopes, and fluorescence microscopes.

Electron Microscope

Instead of using visible light to illuminate the object, the electron microscope uses a beam

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