Physics of Medical Devices

Ninth lecture

Centrifuge

Msc. Eman Ahmed

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Department of medical physics

Al-Mustaqbal University-College

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Introduction

Centrifugation is a mechanical process that utilizes a spinning medium to separate one or more components of a sample according to density or size. While gaseous or immiscible liquids can be separated by centrifugation, the majority of applications involve sedimentation of solid particles in a liquid medium. Centrifugal separations may be classified as either analytical or preparative. In 'analytical centrifugation', the objective is to monitor particle sedimentation behavior in order to characterize particle properties, e.g., molecular weight, shape, and association. In 'preparative centrifugation', the objective is to separate and recover one or more components from a sample mix. Preparative centrifugation encompasses the vast majority of centrifugal applications.

Centrifuge definition

A centrifuge is a device used to separate components of a mixture on the basis of their size, density, the viscosity of the medium, and the rotor speed.

- The centrifuge is commonly used in laboratories for the separation of biological molecules from a crude extract.
- In a centrifuge, the sample is kept in a rotor that is rotated about a fixed point (axis), resulting in strong force perpendicular to the axis.

- There are different types of centrifuge used for the separation of different molecules, but they all work on the principle of sedimentation.

Relative Centrifugal Force (RCF)

- Relative centrifugal force is the measure of the strength of rotors of different types and sizes.
- This is the force exerted on the contents of the rotor as a result of the rotation.
- RCF is the perpendicular force acting on the sample that is always relative to the gravity of the earth.
- The RCF of the different centrifuge can be used for the comparison of rotors, allowing the selection of the best centrifuge for a particular function.

The formula to calculate the relative centrifugal force (RCF) can be written as:

$$\text{RCF (g Force)} = 1.118 \times 10^{-5} \times r \times (\text{RPM})^2$$

where **r** is the radius of the rotor (in centimeters), and **RPM** is the speed of the rotor in rotation per minute.

Types of centrifuges

1. ***Benchtop centrifuge***
2. ***Continuous flow centrifuge***
3. ***Gas centrifuge***
4. ***Hematocrit centrifuge***
5. ***High-speed centrifuge***
6. ***Low-speed centrifuge***
7. ***Microcentrifuge***
8. ***Refrigerated centrifuges***
9. ***Ultracentrifuges***

Types of centrifugation

1. Analytical Centrifugation

Principle of Analytical Centrifugation

- Analytical centrifugation is based on the principle that particles that are denser than others settle down faster. Similarly, the larger

molecules move more quickly in the centrifugal force than the smaller ones.

- Analytical ultracentrifugation for the determination of the relative molecular mass of a macromolecule can be performed by a sedimentation velocity approach or sedimentation equilibrium methodology.
- The hydrodynamic properties of macromolecules are described by their sedimentation coefficients. They can be determined from the rate that a concentration boundary of the particular biomolecules moves in the gravitational field.
- The sedimentation coefficient can be used to characterize changes in the size and shape of macromolecules with changing experimental conditions.
- Three optical systems are available for the analytical ultracentrifuge (absorbance, interference, and fluorescence) that permit precise and selective observation of sedimentation in real-time.

Uses of Analytical Centrifugation

- Analytical centrifugation can be used for the determination of the purity of macromolecules.

- It can also be used for the examination of changes in the molecular mass of supramolecular complexes.
- Besides, it allows the determination of the relative molecular mass of solutes in their native state.

2. Density gradient centrifugation

Density gradient centrifugation is the separation of molecules where the separation is based on the density of the molecules as they pass through a density gradient under a centrifugal force.

Principle of Density gradient centrifugation

- Density gradient centrifugation is based on the principle that molecules settle down under a centrifugal force until they reach a medium with the density the same as theirs.
- In this case, a medium with a density gradient is employed, which either has to decrease density or increasing density.
- Molecules in a sample move through the medium as the sample is rotated creating a centrifugal force.

- The more dense molecules begin to move towards the bottom as they move through the density gradient.
- The molecules then become suspended at a point in which the density of the particles equals the surrounding medium.
- In this way, molecules with different densities are separated at different layers which can then be recovered by various processes.

Uses of Density gradient centrifugation

- Density gradient centrifugation can be applied for the purification of large volumes of biomolecules.
- It can even be used for the purification of different viruses which aids their further studies.
- This technique can be used both as a separation technique and the technique for the determination of densities of various particles.

3. ***Differential centrifugation***

Differential centrifugation is a type of centrifugation process in which components are separately settled down a centrifuge tube by applying a series of increasing centrifugal force.

Principle of Differential centrifugation

- Differential centrifugation is based upon the differences in the sedimentation rate of biological particles of different size and density.
- As the increasing centrifugal force is applied, initial sedimentation of the larger molecules takes place.

- Further particles settle down depending upon the speed and time of individual centrifugation steps and the density and relative size of the particles.
- The largest class of particles forms a pellet on the bottom of the centrifuge tube, leaving smaller-sized structures within the supernatant.
- Thus, larger molecules sediment quickly and at lower centrifugal forces whereas the smaller molecules take longer time and higher forces.
- In the case of particles that are less dense than the medium, the particles will float instead of settling.

Uses of Differential centrifugation

- Differential centrifugation is commonly used for the separation of cell organelles and membranes found in the cell.
- It can also be used for low-resolution separation of the nucleus.
- As this technique separates particles based on their sizes, this can be used for the purification of extracts containing larger-sized impurities.

4. Isopycnic centrifugation

Isopycnic centrifugation is a type of centrifugation where the particles in a sample are separated on the basis of their densities as centrifugal force is applied to the sample.

Principle of Isopycnic centrifugation

- Isopycnic centrifugation is also termed the equilibrium centrifugation as the separation of particles takes place solely on the basis of their densities and not on their sizes.
- The particles move towards the bottom, and the movement is based on the size of the particles. And, the flow ceases once the density of the particle becomes equal to the density of the surrounding medium.
- The density in the gradient increases as we move down the tube towards the bottom. As a result, the particles with higher densities settle down at the bottom, followed by less dense particles that form bands above the denser particles.
- It is considered as a true equilibrium as this depends directly on the buoyant densities and not the sizes of the particles.

5. Rate-zonal density gradient centrifugation/ Moving Zone Centrifugation

Rate-zonal density gradient centrifugation is a type of centrifugation that separates particles on the basis of their shape as size and works on the same principle of density gradient centrifugation but works in a different way. It is also called the moving zone centrifugation.

Principle of Rate-zonal density gradient centrifugation

- Rate zonal centrifugation fractionates particles by both size and shape.
- The procedure is to layer a sample in a restricted zone on top of a pre-poured density gradient. The density gradient is then centrifuged.
- All particles migrate into the density gradient because the density gradient has only densities much lower than the densities of the particles being centrifuged.
- The particles are fractionated primarily by size and shape. The larger a particle is, the more rapidly it sediments.
- The more spherically symmetrical a particle is, the more rapidly it sediments.
- The particles sediment through the gradient at a rate that is a function of their sedimentation coefficient.
- Unlike differential centrifugation where the sample is distributed throughout the medium, in rate-zonal centrifugation, the sample is initially present only on top of the gradient as a narrow band.