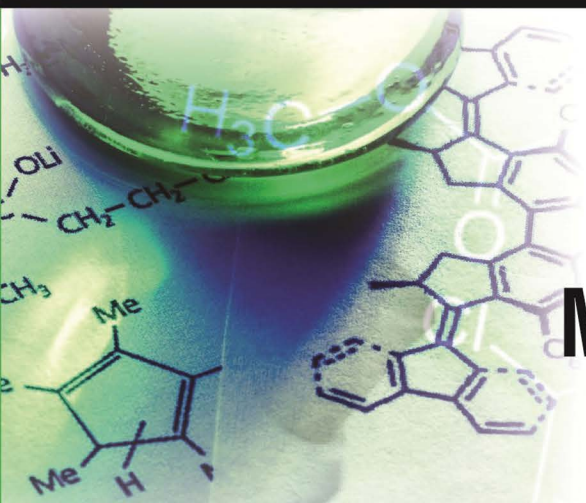
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# Manipal Manual of Clinical Biochemistry



Shivananda Nayak B

5th Edition

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# 3 UNIT

# Centrifugation Techniques

## Learning Objectives

***At the end of this unit, the learner should be able to understand:***

- Principle of centrifugation.
- Different types and advantages of centrifugation.

### INTRODUCTION

Centrifugation is a separation technique commonly used in clinical and research laboratories. It is based on the behavior of particles in an applied centrifugal field.

### PRINCIPLE

Particles that differ in density, size or shape, sediment at different rates in a centrifugal field. The particles will tend to sediment under the influence of gravity. If the particles suspended in a liquid are so small or have a density so close to that of the liquid, then the force of gravity fails to sediment the particles into a separate layer. So the basis of centrifugation techniques is to exert a larger force than the gravitational force to enhance the effective sedimentation force for the separating such particles from the liquid.

In centrifugation, the particles are normally suspended in a specific liquid medium, held in tubes, which are located in a rotor. The rotor is positioned centrally on the drive shaft of the centrifuge. Particles that differ in density, shape or size can be separated since they sediment at different rates in the centrifugal field, each particle sedimenting at a rate, which is proportional to the applied centrifugal field.

The rate at which the sedimentation occurs in centrifugation is expressed in terms of sedimentation coefficient and is given by the formula:

$S = V / \omega^2 r$

V = Migration (sedimentation) of the molecule

$\omega$ = Rotation of the rotor in radians/sec (angular velocity)

r = Distance in cm, from the center of rotor

The sedimentation coefficient has the units of seconds. It was usually expressed in units of 10–13 seconds, (since several biological macromolecules occur in this range) which is designated as one svedberg unit, i.e., 1 svedberg unit = $10^{-13}$ seconds. For instance, the sedimentation coefficient of hemoglobin is $4 \times 10^{-13}$ seconds or 4S; ribonuclease is $2 \times 10^{-13}$ seconds or 2S. Conventionally, the subcellular organelles are often referred to by their 'S' value, e.g., 70S ribosome.

#### Centrifuges

The equipment used to perform centrifugation is called "centrifuge". The centrifuge should essentially have a rotor to keep the sample tube. The rotor can be of three types:

1. Fixed angle rotor **(Fig. 3.1)**
2. Vertical tube rotor **(Fig. 3.2)**
3. Swinging bucket rotor **(Fig. 3.3)**

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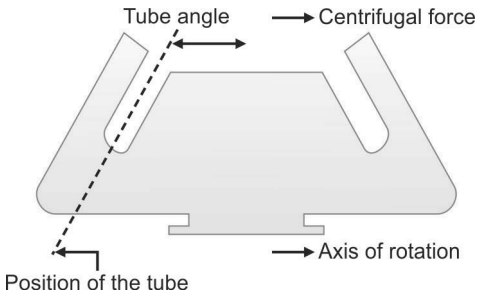



**Fig. 3.1:** Fixed angle rotor.

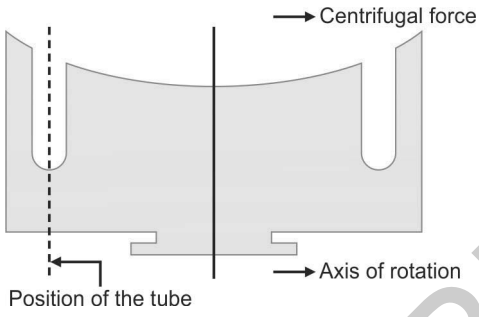



**Fig. 3.2:** Vertical tube rotor.

### *Fixed Angle Rotor*

In this, tube will be in a fixed position in a specific slanting position throughout the process **(Fig. 3.1)**.

### *Vertical Tube Rotor*

In this, tube will be in a fixed position, but its axis will be parallel to the axis of rotation throughout the process **(Fig. 3.2)**.

### *Swinging Bucket Rotor*

In this type of rotor, at rest the tube axis will remain parallel to axis of rotation. However, during centrifugation the tube swings and will have an angle of 90° with the axis of rotation **(Fig. 3.3)**.

Aluminum alloy or titanium alloys have been used as rotor materials as they can withstand the higher stress force generated during high speed centrifugation. Aluminum alloys rotors, although less expensive, are far more susceptive to corrosion by acids and alkalis. Titanium alloy rotors can withstand nearly twice the centrifugal force of rotors made from aluminum alloy.

## Types of Centrifuges

Centrifuges may be classified into four major groups:

1. Small bench centrifuges
2. Large capacity refrigerated centrifuges
3. High speed refrigerated centrifuges
4. Ultracentrifuges—which are of two types—preparative and analytical.

### *Small Bench Centrifuges*

These are simplest and least expensive and available in many designs (may be driven by mechanical force or electrical force). These centrifuges generally will have a maximum speed of 4000–6000 revolutions per minute (RPM) with maximum relative centrifugal field (RCF) of 3000 to 7000 g. They are often used to

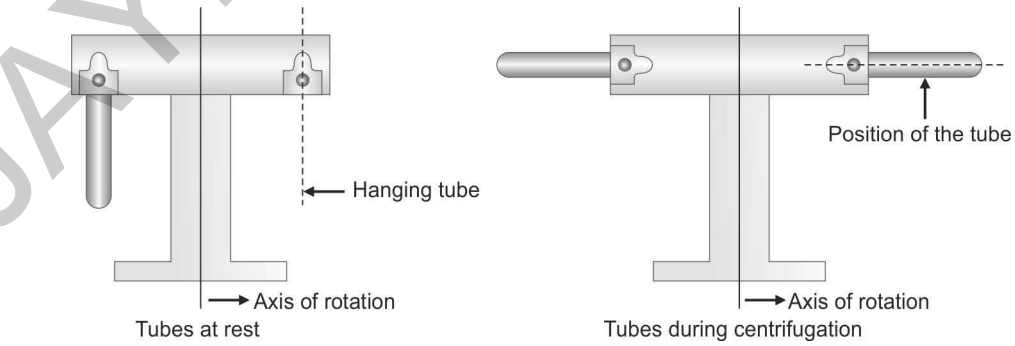



**Fig. 3.3:** Swinging bucket rotor.

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collect small amounts of material that rapidly sediment (yeast cells, erythrocytes, coarse precipitates).

### *Large Capacity Refrigerated Centrifuges*

These have a maximum speed of 6000 RPM and produce a maximum relative centrifugal field approaching 6500 g. These have a refrigerated rotor chambers and vary only in their maximum carrying capacity. These instruments are most often used to collect substances that sediment rapidly, for example, RBCs, bulky precipitates, yeast cells, nuclei and chloroplasts.

### *High Speed Refrigerated Centrifuges*

These are available with maximum rotor speeds in the region 25000 RPM (corresponding to a RCF of 60000 g). These can be used to collect microorganisms, cellular debris and large cellular organelles. But these cannot generate sufficient centrifugal force to sediment viruses or smaller organelles, such as ribosome.

### *Preparative Ultracentrifuges*

These are capable of spinning rotors to a maximum speed of 80000 RPM and can produce a relative centrifugal field of up to 600000 g. The rotor chamber is refrigerated, sealed, evacuated to minimize excessive rotor temperatures generated by friction between air and spinning rotor. The temperature monitoring system is more sophisticated than simpler instruments, employing an infrared temperature sensor. An overspeed control system is also incorporated. For safety reasons, rotor chamber is enclosed in heavy armor plating. And these preparative ultracentrifuges are used to separate, isolate and purify whole cells, subcellular organelles, plasma membranes, polysomes, ribosomes, chromatin, nucleic acids, lipoproteins and viruses.

### *Analytical Ultracentrifuges*

These are capable of operating at a speed of 70,000 RPM (corresponding 500,000 g). These will have a rotor contained in a protective armored chamber, which is refrigerated and evacuated. These instruments will also have a special optical system to observe the sedimenting of material and to determine concentration distributions within the sample at any time during centrifugation. The optical system records the change in refractive index of the solution which will vary as the centrifugation changes. This behaves like a refraction lens resulting in the production of a peak on the photographic plate, which is used as a detector system. These centrifuges are mainly used to study pure macromolecules, their sedimentation characteristics and molecular structure.

### Application of Centrifugation

a. Separation of thick precipitates from solution.
b. Separation of serum from clotted blood.
c. Separation of plasma from anticoagulant added blood.
d. Precipitating proteins from serum during colorimetric estimation of serum urea, creatinine. Trichloroacetic acid is added to serum sample, which denatures proteins and precipitates. The precipitate is separated by centrifugation. The protein free filtrate is used for further analysis.
e. Separation of erythrocytes from oxalated (or heparinized) blood.
f. To determine packed cell volume (PCV) or hematocrit values. The oxalated blood is centrifuged in hematocrit

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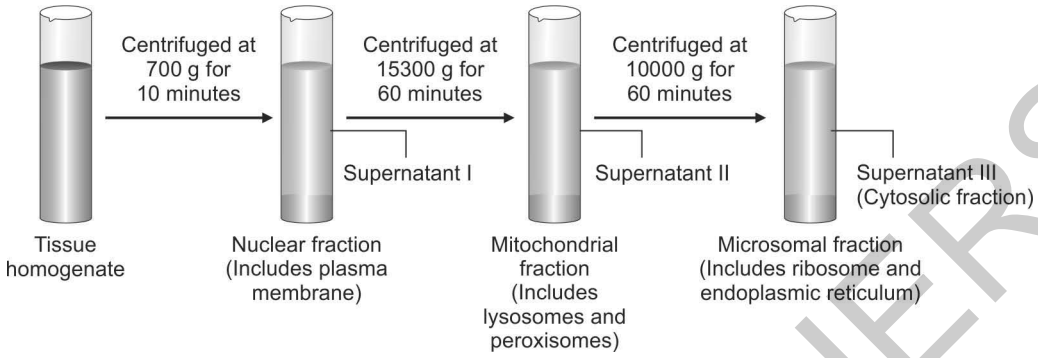



**Fig. 3.4:** Isolation of subcellular organelles.

tubes (110 mm long and 3 mm in bore diameter) at 3000 RPM for 30 minutes. The proportionate volume of RBCs, settled in the bottom layer below the supernatant layer of clear plasma in the tube, is read from the graduations of the tube as PCV (normally it averages 45% in humans).

g. *Isolation of subcellular organelles:* The cells are subjected to disruption by sonication or by osmotic shock or by the use of homogenizer. This is usually carried out in an isotonic (0.25M) sucrose solution. The subcellular particles can be separated by differential centrifugation **(Fig. 3.4)**.
The purity (or contamination) of the subcellular fraction can be checked by the use of marker enzymes. Example—DNA polymerase is the marker for nucleus, glutamate dehydrogenase for mitochondrion, glucose-6-phosphatase for ribosome, and hexokinase for cytosolic fraction.
h. Determination of molecular weight (in the fields of protein and nucleic acid chemistry) by studying their sedimentation characteristics.
i. Estimation of purity of macromolecules, i.e., purity of DNA preparations, viruses and proteins.
j. Detection of conformational changes in macromolecules, such as DNA and proteins.

## SELF TEST

1. Name the different types of centrifuges used in the clinical biochemistry laboratory.
2. Mention the different types of rotors.
3. Mention the application of centrifugation.

## MULTIPLE CHOICE QUESTIONS

**1. Select the unrelated term in the following:**
   a. Rotor
   b. Drive shaft
   c. Monochromatic light
   d. Relative centrifugal field

**2. Separation of particles in a centrifuge depends on the following property:**
   a. Charge
   b. Density
   c. Fluorescence
   d. Color

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3. **One of the following is not an application of centrifugation:**
   a. Separation of thick precipitates from solution
   b. Separation of serum from clotted blood
   c. Separation of plasma from anticoagulant added blood
   d. Separation of inhibitors.
4. **All of the following are the applications of centrifuges, *Except:* It is used:**
   a. To separate serum from clotted blood
   b. To separate erythrocytes from oxalated/heparinized blood
   c. To isolate sub cellular organelles
   d. To separate subunits of proteins.
5. **Concerning the different types of rotors, one of the following statements is INCORRECT:**
   a. Fixed angle rotor fixed in a specific position when swinging
   b. Vertical tube rotor fixed in and its axis parallel to axis of rotation
   c. In swinging bucket rotor, the tube axis will remain parallel to axis of rotation
   d. Horizontal tube rotor remains horizontal during rotation.

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# Manipal Manual of Clinical Biochemistry

***Salient Features***

- Thoroughly updated
- Furnish the readers a logical sequence of events that aids learning and memorize
- Written in a simplified and easy-to-understand format and divided into 25 chapters
- Lucid diagrams, flowcharts and tables will help the learners to understand and remember easily
- Provides list of laboratory test, normal ranges and their clinical use
- This is the unique title comprising preparation of solutions, sample collection, quality control and functional tests in detail
- Each chapter begins with an objective, introduction and ends with summary and self-assessment questions which involve short answer questions, case studies and multiple choice questions (MCQs).

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