TECHNIQUES IN BIOCHEMISTRY
BIOCHEM-505 4 (2+2)

Block 1: Unit 4

CENTRIFUGATION

(HYDRODYNAMICS METHOD FOR SEPARATION OF BIOMOLECULES)

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CENTRIFUGATION

(HYDRODYNAMICS METHOD FOR SEPARATION OF BIOMOLECULES)

LECTURE NO- 5 to 6

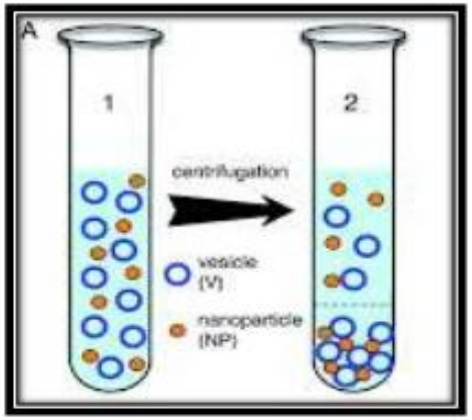
Objective-

- **Introduction**
- **Principle**
- **Instrumentation**
- **Application**

DEFINE: CENTRIFUGATION

- Centrifugation is a technique of separating substances which involves the application of centrifugal force.
- The particles are separated from a solution according to their size, shape, density, the viscosity of the medium and rotor speed.

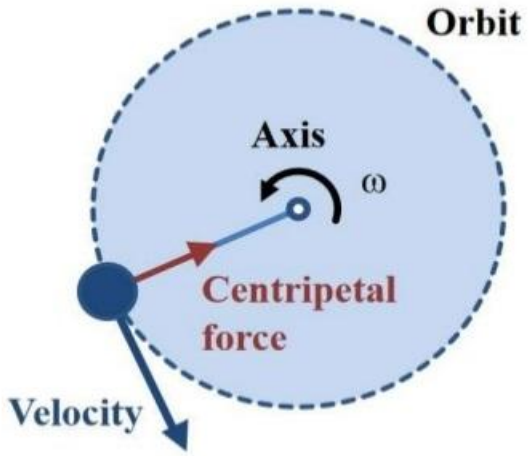
"**Centrifugation** is the process where a mixture is separated through spinning by using a centrifuge."



Principle of Centrifugation

- In a solution, particles whose density is higher than that of the solvent sink (sediment), and particles that are lighter than it floats to the top.
- The greater the difference in density, the faster they move. If there is no difference in density (isopycnic conditions), the particles stay steady.
- To take advantage of even tiny differences in density to separate various particles in a solution, gravity can be replaced with the much more powerful “centrifugal force” provided by a centrifuge.
- A centrifuge is a piece of equipment that puts an object in rotation around a fixed axis (spins it in a circle), applying a potentially strong force perpendicular to the axis of spin (outward).

- The centrifuge works using the sedimentation principle, where the centripetal acceleration causes denser substances and particles to move outward in the radial direction.
- At the same time, objects that are less dense are displaced and move to the center.
- In a laboratory centrifuge that uses sample tubes, the radial acceleration causes denser particles to settle to the bottom of the tube, while low- density substances rise to the top.



Types of Centrifuge

1. LOW-SPEED CENTRIFUGE
2. HIGH-SPEED CENTRIFUGES
3. ULTRACENTRIFUGES

LOW-SPEED CENTRIFUGE

1) Most laboratories have a standard low-speed centrifuge used for routine sedimentation of heavy particles
2) The low-speed centrifuge has a maximum speed of 4000-5000rpm
3) These instruments usually operate at room temperatures with no means of temperature control.
4) Two types of rotors are used in it- Fixed angle and

   Swinging bucket.
5) It is used for sedimentation of red blood cells until the particles are tightly packed into a pellet and supernatant is separated by decantation.

HIGH-SPEED CENTRIFUGES

- High-speed centrifuges are used in more sophisticated biochemical applications, higher speeds and temperature control of the rotor chamber are essential.
- The high-speed centrifuge has a maximum speed of 15,000 – 20,000 RPM
- The operator of this instrument can carefully control speed and temperature which is required for sensitive biological samples.
- Three types of rotors are available for high-speed centrifugation-

1. Fixed angle
2. Swinging bucket
3. Vertical rotors

ULTRACENTRIFUGES

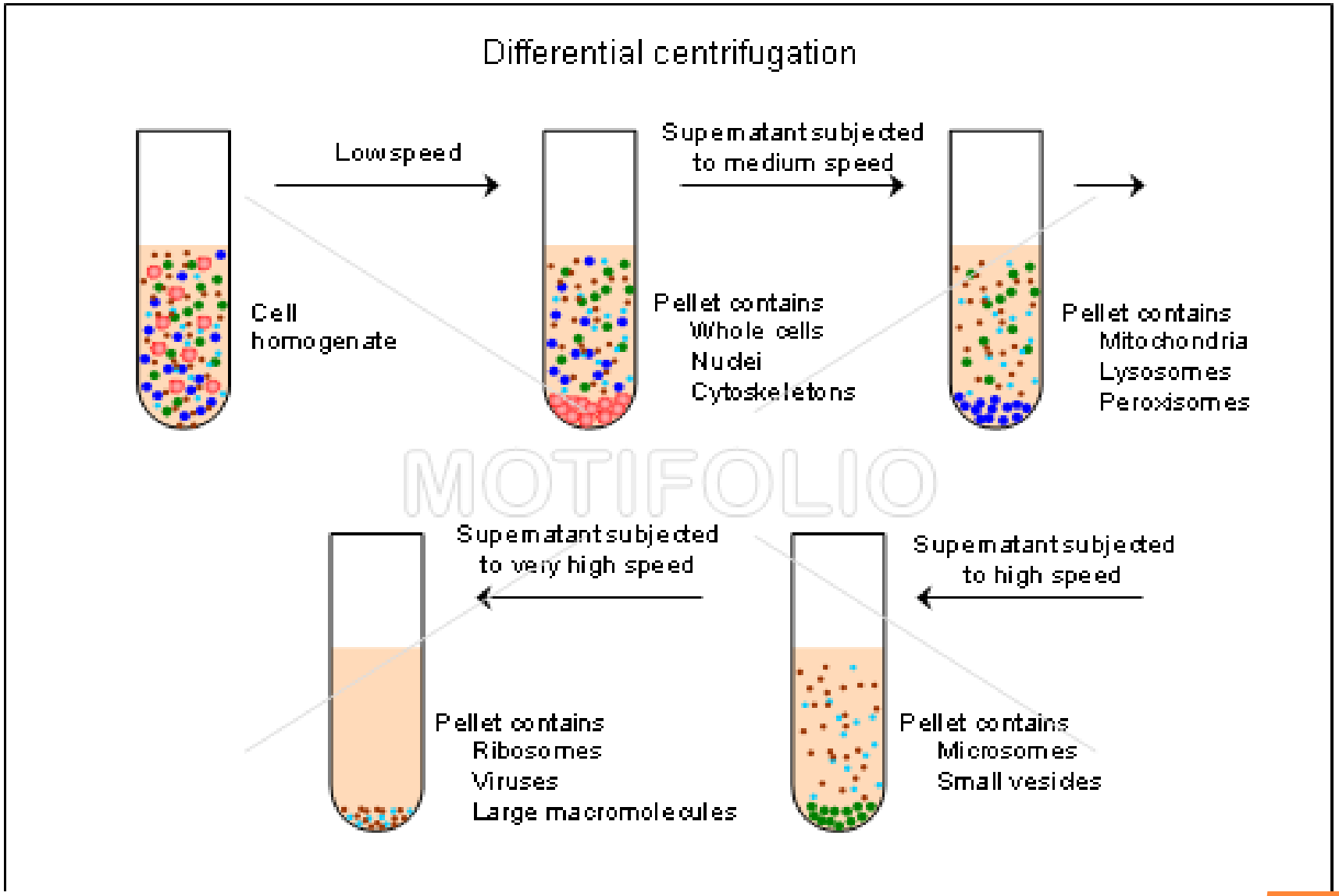
- It is the most sophisticated instrument.
- Ultracentrifuge has a maximum speed of 65,000 RPM (100,000's x g).
- Intense heat is generated due to high speed thus the spinning chambers must be refrigerated and kept at a high vacuum.
- It is used for both preparative work and analytical work

Types of Centrifugation

1. **Differential Pelleting (differential centrifugation)**
2. **Density Gradient Centrifugation**
3. **Rate-Zonal Density-Gradient Centrifugation**
4. **Isopynic Centrifugation**

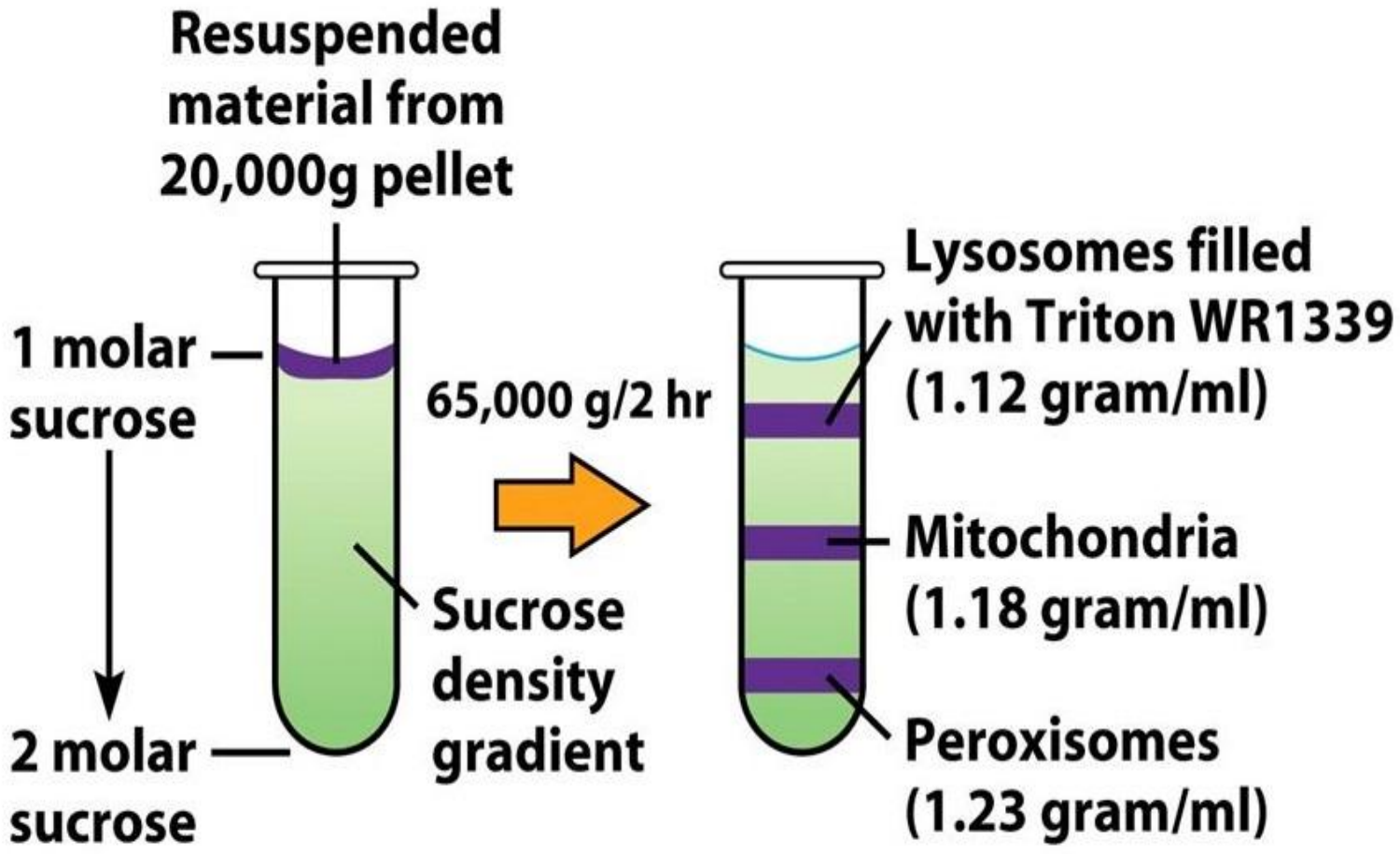
Differential Pelleting (differential centrifugation)

- It is the most common type of centrifugation employed.
- Tissue such as the liver is homogenized at 32 degrees in a sucrose solution that contains buffer.
- The homogenate is then placed in a centrifuge and spun at constant centrifugal force at a constant temperature.
- After some time a sediment forms at the bottom of a centrifuge called pellet and an overlying solution called supernatant.
- The overlying solution is then placed in another centrifuge tube which is then rotated at higher speeds in progressing steps.


Differential centrifugation
Low speed
Cell homogenate
Supernatant subjected to medium speed
Pellet contains
Whole cells
Nuclei
Cytoskeletons
Pellet contains
Mitochondria
Lysosomes
Peroxisomes
Supernatant subjected to very high speed
Pellet contains
Ribosomes
Viruses
Large macromolecules
Supernatant subjected to high speed
Pellet contains
Microsomes
Small vesicles

Density Gradient Centrifugation

- This type of centrifugation is mainly used to purify viruses, ribosomes, membranes, etc.
- A sucrose density gradient is created by gently overlaying lower concentrations of sucrose on higher concentrations in centrifuge tubes
- The particles of interest are placed on top of the gradient and centrifuge in ultracentrifuges.
- The particles travel through the gradient until they reach a point at which their density matches the density of surrounding sucrose.
- The fraction is removed and analyzed.



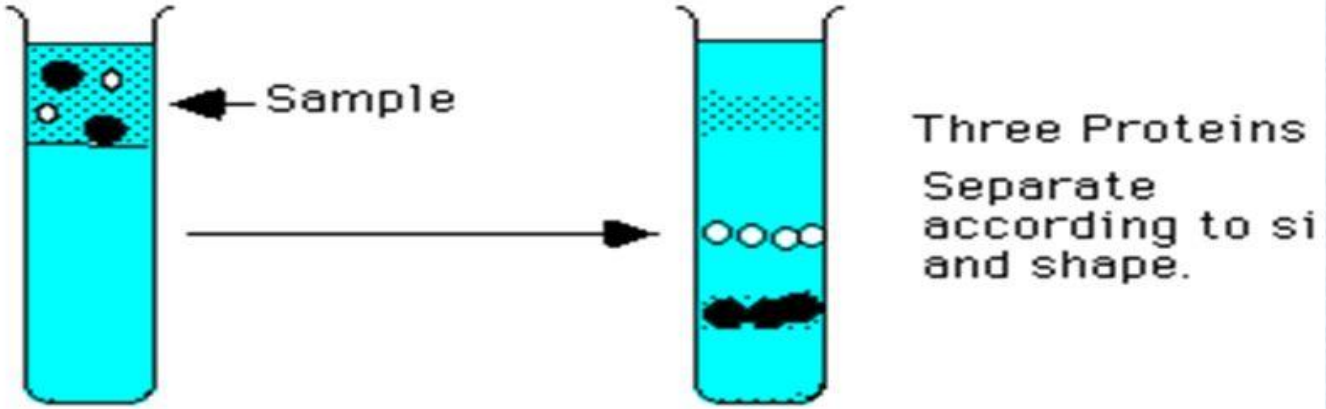
Density Gradient Centrifugation

Rate-Zonal Density-Gradient Centrifugation

- Zonal centrifugation is also known as band or gradient centrifugation
- It relies on the concept of sedimentation coefficient (i.e. movement of sediment through the liquid medium)
- In this technique, a density gradient is created in a test tube with sucrose and high density at the bottom.
- The sample of protein is placed on the top of the gradient and then centrifuged.
- With centrifugation, faster-sedimenting particles in sample move ahead of slower ones i.e. sample separated as zones in the gradient.
- The protein sediment according to their sedimentation coefficient and the fractions are collected by creating a hole at the bottom of the tube.

RATE-ZONAL CENTRIFUGATION

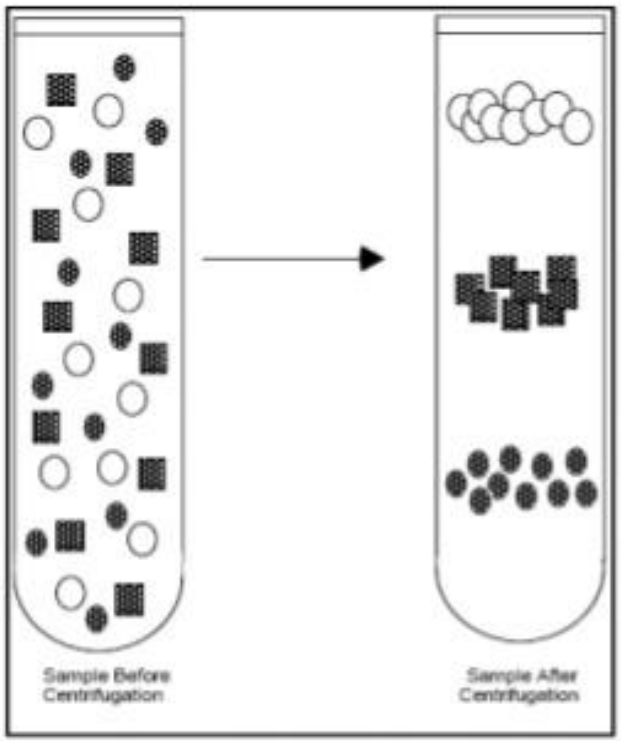
Figure 3b: Rate zonal centrifugation.



Isopynic Centrifugation

- The sample is loaded into the tube with the gradient-forming solution (on top of or below pre-formed gradient, or mixed in with self-forming gradient)
- The solution of the biological sample and cesium salt is uniformly distributed in a centrifuge tube and rotated in an ultracentrifuge.
- Under the influence of centrifugal force, the cesium salts redistribute to form a density gradient from top to bottom.
- Particles move to point where their buoyant density equals that part of gradient and form bands. This is to say the sample molecules move to the region where their density equals the density of gradient.
- It is a "true" equilibrium procedure since depends on bouyant densities, not velocities
- Eg: CsCl, NaI gradients for macromolecules and nucleotides – "self-forming" gradients under centrifugal force.

Isopynic or sedimentation equilibrium centrifugation



- In Isopycnic centrifugation separation of particles occurs into zones on the basis of their density differences, independent of time.

Applications of Centrifugation

1. To separate two miscible substances
2. To analyze the hydrodynamic properties of macromolecules
3. Purification of mammalian cells
4. Fractionation of subcellular organelles (including membranes/membrane fractions) Fractionation of membrane vesicles
5. Separating chalk powder from water
6. Removing fat from milk to produce skimmed milk
7. Separating particles from an air-flow using cyclonic separation
8. The clarification and stabilization of wine
9. Separation of urine components and blood components in forensic and research laboratories
10. Aids in the separation of proteins using purification techniques such as salting out, e.g. ammonium sulfate precipitation.