CENTRIFUGATION

1. Introduction

- centrifuge: device for separating particles from a solution according to their size, shape, density, viscosity of the medium and rotor speed
- effect of gravity
- centrifugal force
- pellet and supernatant
- used to separate particles or macromolecules

II. Types of Centrifugation

a) Preparative :

1. Differential Centrifugation
2. Density gradient Centrifugation
    - 2a. Rate-Zonal
    - 2b. Isopycnic

b) Analytical

a) Preparative Centrifugation

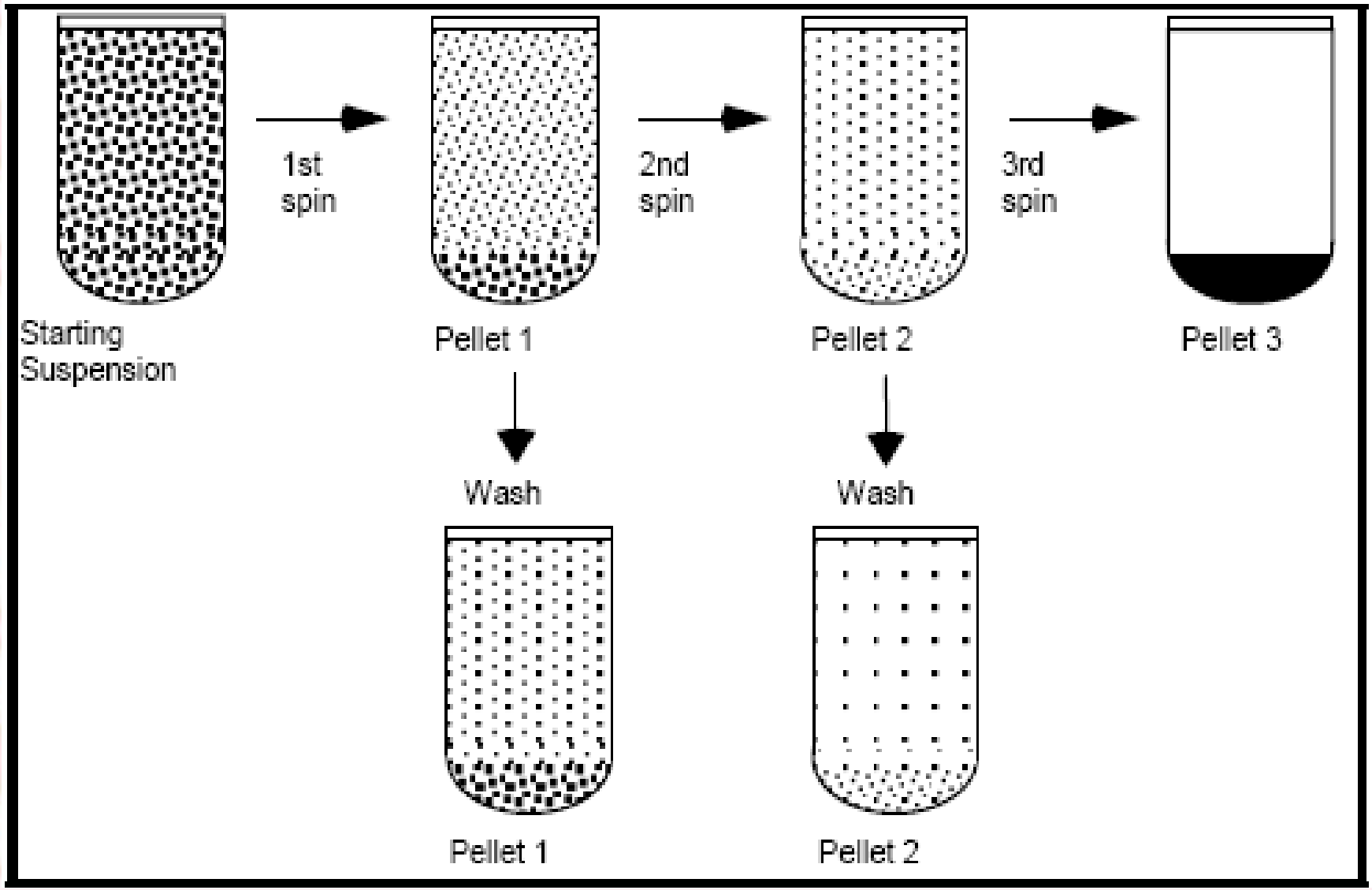
- used to separate organelles and molecules
- can handle larger liquid volumes
- no optical read-out
- Separation methods used in preparative ultracentrifugation:

1. Differential Centrifugation- pelleting,
2. Density Gradient Centrifugation

1.Differential Centrifugation

- based on the size of the particles
- used for simple pelleting, for the separation of sub cellular organelles and macromolecules
- first, sample must be homogenised
- ultra centrifugation
- sedimentation depends on mass, shape and partial specific volume of a macromolecule, as well as solvent density, rotor size, rate of rotation.
- Usually uses a fixed angle rotor

Figure 1. Differential Centrifugation



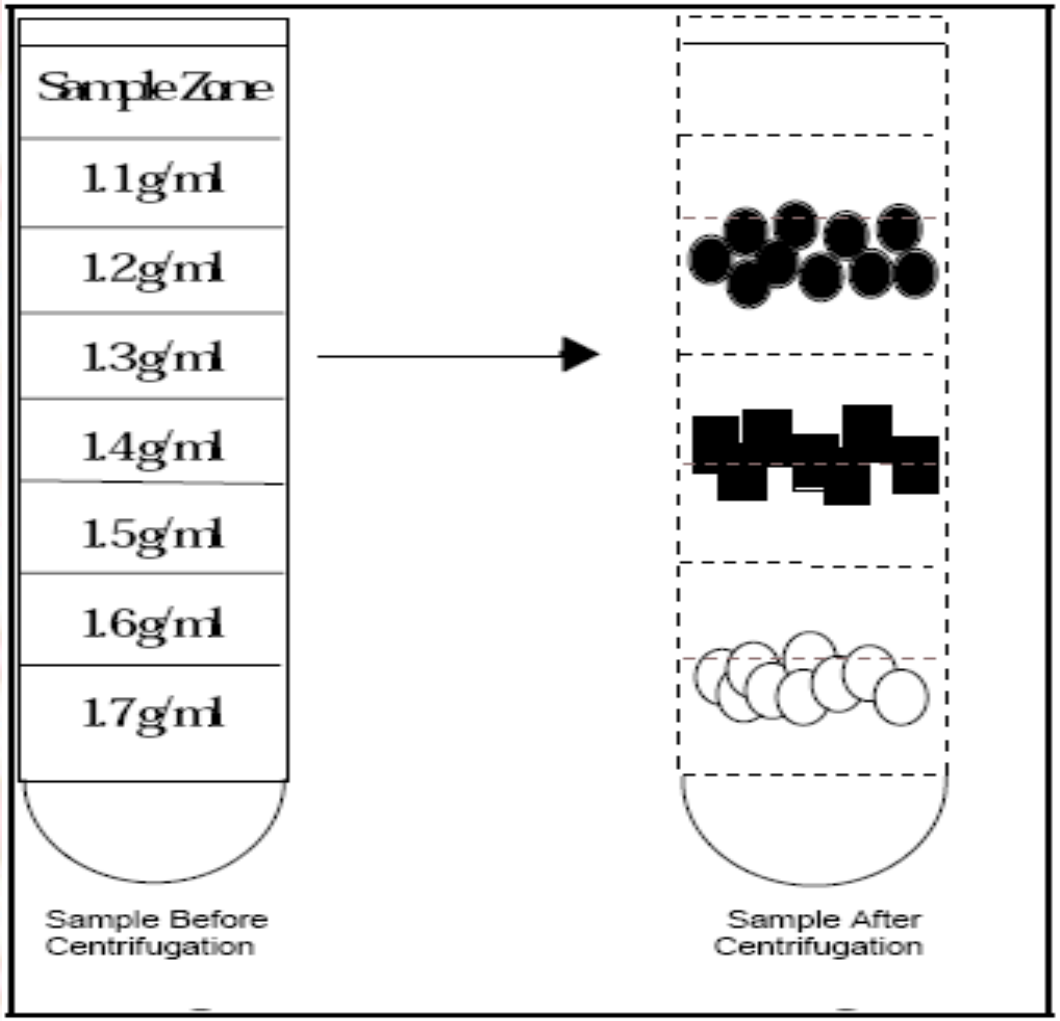
2.Density Gradient Centrifugation

- method to purify subcellular organelles and macromolecules.
- density gradients generated by placing layer after layer of gradient media
- Density gradient centrifugation classified into two:
  - 2a. Rate-Zonal separation (size)
  - 2b. Isopycnic Separation (Density)

2a.Rate- Zonal Centrifugation

- use of continuous density gradient of solvent such as sucrose.
- density increases towards the bottom of the tube
- sample layered on the top
- molecules form discrete bands after centrifugation
- separation based on size of the molecules
- Swinging bucket rotors

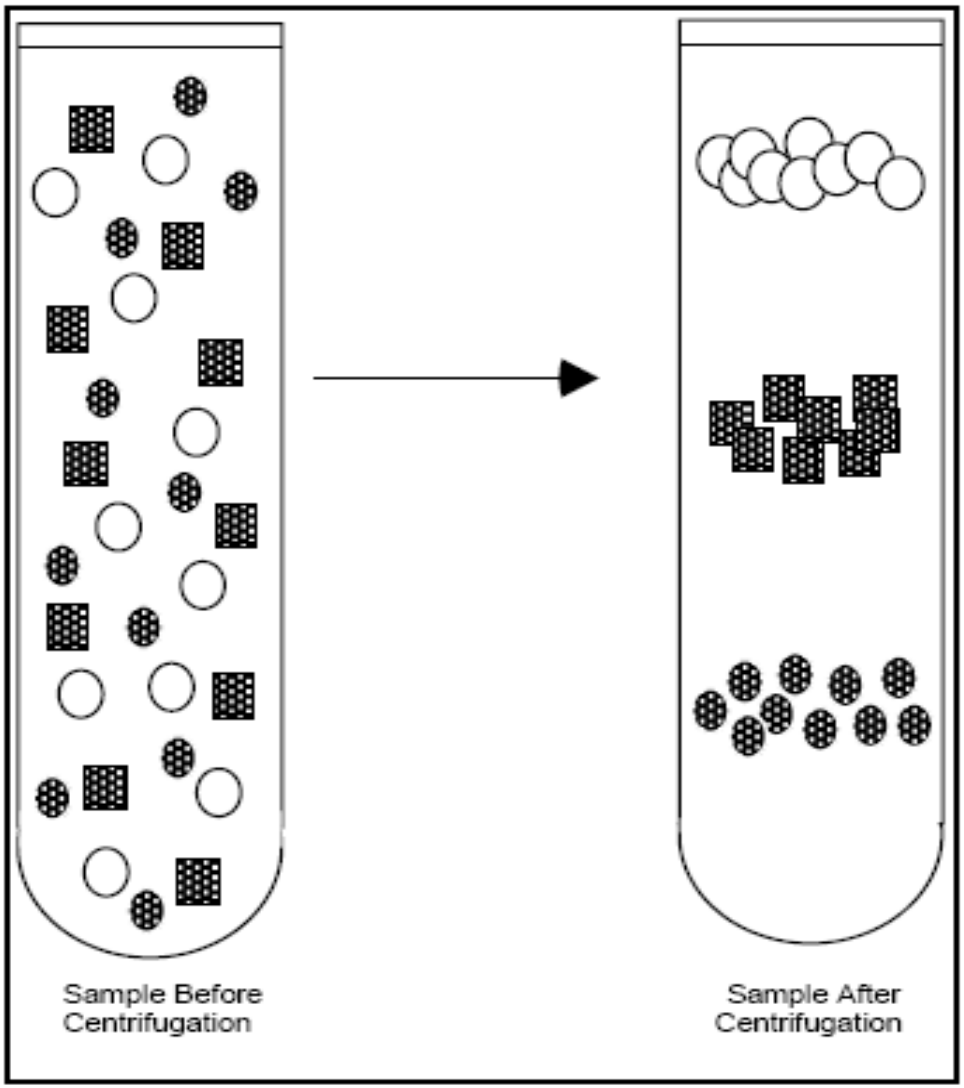
Figure 2. RATE-ZONAL (SIZE) SEPARATION



2b. Isopycnic Separation

- based on the density of the molecules
- Mix gradient material with the sample molecule (CsCl)
- molecules move to the position where their density is same as the gradient material (isopycnic position)
- inorder to generate a gradient, we select a CsCl concentration that will give a range of densities that includes the range of molecules that have to be separated.
- used for the separation of DNA
- Swinging bucket or fixed angle rotor

Figure 3. ISOPYCNIC (DENSITY) SEPARATION



b) Analytical Centrifugation

- uses small size samples
- built-in optical system
- uses relatively pure sample

III. Theory of Centrifugation

- Centrifugation:any object moving in a circle at a steady angular velocity is subject to an outward directed force, F. The magnitude of this force depends on the angular velocity in radians, w, and the radius of rotation, r, in centimeters.

$$F = w^2 r$$

F= gravitational force and also referred to as the relative centrifugal force, RCF

Where the earth's gravitational field ($g=980\ cm/s^{-2}$).

$$RCF = w^2 r / 980$$

- Sedimentation of a molecule influenced by
  > properties of the molecules (Size, shape, density)
  > properties of the solvent, or the gradient material(density, viscosity, temperature)
  > interactions between the solute molecules and the solvent gradient molecule
  -As the rotor spins, centrifugal force is applied to each molecule in the sample:
  Centrifugal force= $M\omega^2 r$
  M=mass(molecular weight),
  ω=angular velocity(radius/sec),
  r= distance from the axis of the rotation

-Two forces act to counteract the centrifugal force

> buoyant force (displacement force)

> frictional force

Buoyant force=$M\omega^2 rV\rho$

V=partial specific volume of the solute,

ρ=density of the solvent(g/ml)

- suspended particles also generate friction as they migrate through the solution

Frictional Force=f(v)=f(dr/dt)

F=Frictional coefficient unique to the molecules in question,

dr/dt=rate of sedimentation expressed as a change in the axis of the rotation with time

- For a spherical molecule, $f = 6\pi\eta r_m$

η = viscosity of the medium

r_m = radius of the molecule

Sedimentation coefficient:

$$S = v/\omega^2 r$$

where, v =velocity

v can be given as (dr/dt)

$$S = (dr/dt)/\omega^2 r$$

IV. Types of Centrifuges

- Desktop clinical centrifuges: below 3000rpm
- High speed centrifuges : 20,000 to 25,000rpm
- The Ultracentrifuge :75,000rpm
    a. Drive and speed control
    b. Temperature control
    c. Vacuum System
    d. Rotors

V. Types of Rotors

- Swinging bucket rotors
- Fixed angle
- Vertical