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DEPARTMENT OF BIOTECHNOLOGY
FACULTY OF ENGINEERING & TECHNOLOGY

LT 25. Centrifugation: Theory & Principles

Content Outline

1. Introduction
2. Usage of centrifugation in biology
3. Factors affecting centrifugation
4. Types of centrifugation procedure
5. Principle of Centrifugation
6. Sedimentation coefficient

TOPIC

Centrifugation

Biological centrifugation is a process that uses centrifugal force to separate and purify mixtures of biological particles in a liquid medium. The particles are suspended in a liquid medium and placed in a **centrifuge tube**. The tube is then placed in a **rotor** and spun at a define speed. Rotation of the rotor about a central axis generates a **centrifugal force** upon the particles in the suspension.

First analytical ultracentrifuge was developed by Svedberg in the late 1920s and the technical refinement of the preparative centrifugation technique by Claude and colleagues in the 1940s.

Usage of centrifugation in biology

•It is a key technique for isolating and analysing cells, subcellular fractions, supramolecular complexes and isolated macromolecules such as proteins or nucleic acids.

Factors affecting centrifugation

The separation of particles from a solution is based on their size, shape, density, viscosity of the medium and rotor speed. Key factors affecting centrifugation are

•Density of both samples and solution

•Temperature/viscosity

•Distance of particles displacement

•Rotation speed

Types of centrifuge procedure

There are two types of **centrifuge procedures**; one is preparative and another one is analytical. Analytical centrifugation is mainly involves in measuring physical properties of the sedimenting particles. It is concerned with the study of purified macromolecules or isolated supramolecular assemblies.

Preparative centrifugation methodology is devoted to the actual separation of tissues, cells, subcellular structures, membrane vesicles and other particles of biochemical interest

Basic principles of centrifugation

•Generally Stokes' law is used in order to describe the sedimentation process of a solid particle within a centrifugal field and is the starting point for the theoretical understanding of all sedimentation processes. As a rotor spins in a **centrifuge**, a centrifugal force is applied to each particle in the sample; the particle will then sediment at the rate that is proportional to the centrifugal force applied to it. The viscosity of the sample solution and the physical properties of the particles also affect the sedimentation rate of each particle.

•At a fixed centrifugal force and liquid viscosity, **the sedimentation rate of a particle is proportional to its size** (molecular weight) and to the difference between the particle density and the density of the solution.

•Biological particles moving through a viscous medium experience a frictional drag, whereby the frictional force acts in the opposite direction to sedimentation and equals the velocity of the particle multiplied by the frictional coefficient. The frictional coefficient depends on the size and shape of the biological particle.

Centrifugal field and relative centrifugal field

In a centrifuge, the rate of sedimentation is dependent upon gravitational field, G, that is determined by the radial distance, r, of the particle from the axis of rotation (in cm) and the square of the angular velocity, ω of the rotor (in radians per second):

$G = \omega^2 . r$ (5.1)

The average angular velocity of a rigid body that rotates about a fixed axis is defined as the ratio of the angular displacement in a given time interval. Accordingly, the angular velocity in rads per second of the rotor can be expressed in terms of rotor speed s as:

$$\omega = \frac{2\pi s}{60} \quad (5.2)$$

Putting value of ω in equation 5.1, we get

$$G = \frac{4\pi^2 \left(\text{rev min}^{-1}\right)^2 r}{3600} \quad (5.3)$$

Relative centrifugal field

Relative centrifugal field (RCF) describes and compares the strength of the fields generated by different size rotors and different operating speeds. The relative centrifugal field is generally expressed in multiples of the gravitational field, g (981 cm s–2). The relative centrifugal field (g), RCF, which is the ratio of the centrifugal acceleration at a specified radius and the speed to the standard acceleration of gravity, can be calculated from the following equation:

$$\mathrm{RCF} = \frac{4\pi^2(\mathrm{rev\ min}^{-1})^2 r}{3600 \times 981} = \frac{G}{g} \qquad \textbf{(5.4)}$$

RCF units are therefore dimensionless (denoting multiples of g) and revolutions per minute are usually abbreviated as r.p.m.: RCF = 1.12 × 10-5 r.p.m^2 . r

How to convert between times gravity (×g) and centrifuge rotor speed (RPM)?

The relationship between RPM and RCF is as follows:

$g = (1.118 \times 10^{-5}) \times R \times S^2$

Where g is the relative centrifugal force, R is the radius of the rotor in centimeters, and S is the speed of the centrifuge in revolutions per minute.

In a suspension of biological particles, the rate of sedimentation is dependent not only upon the applied centrifugal field, but also on the nature of the particle, i.e. its density and radius, and also the viscosity of the surrounding medium. Stokes' Law describes these relationships for the sedimentation of a rigid spherical particle:

$$\nu = \frac{2}{9} \frac{r^2(\rho_p - \rho_m)}{\eta} \times g$$

where ν is the sedimentation rate of the sphere, 2/9 is the shape factor constant for a sphere, r is the radius of particle, ρ_p is the density of particle, ρ_m is the density of medium, g is the gravitational acceleration and η is the viscosity of the medium.

Accordingly a mixture of biological particles exhibiting an approximately spherical shape can be separated in a centrifugal field based on their density and/or their size. The time of sedimentation (in seconds) for a spherical particle is:

$$t = \frac{9}{2} \frac{\eta}{\omega^2 r_p^2 (\rho_p - \rho_m)} \times \ln \frac{r_b}{r_t}$$

where t is the sedimentation time, η is the viscosity of medium, r_p is the radius of particle, r_b is the radial distance from the centre of rotation to bottom of tube, r_t is the radial distance from the centre of rotation to liquid meniscus, ρ_p is the density of the particle, ρ_m is the density of the medium and ω is the angular velocity of rotor.

Sedimentation coefficient

The sedimentation rate or velocity of a biological particle can also be expressed as its sedimentation coefficient (s), whereby:

$$s = \frac{v}{\omega^2 r}$$

The sedimentation coefficients of biological macromolecules are relatively small, and are usually expressed, as Svedberg units, S. One Svedberg unit equals 10^{-13} s.

Significance

•Biological particles with a different molecular mass, shape or size migrate with different velocities in a centrifugal field

•It can be used to determine the conformation of protein. For example, a macromolecule that changes its conformation into a more compact structure decreases its frictional resistance in the solvent. In contrast, the frictional resistance increases when a molecular assembly becomes more disorganised.

Test your Understanding

Centrifugation refers to

a. Spinning of sample in circle Spinning of sample in circle
b. It uses centrifugal force to separate and purify mixtures of biological particles in a liquid medium.
c. It can be used to study sedimenatation rate of biological particle
d. All of these

Which of the following are usage of centrifugationm in biology?

a. Isolation of particle from cellular homogenates
b. Fundamental study on hydrodynamics property of biological samples
c. Both (a) and (b)
d. none of the above

What are various factor that affects centrifugation?

a. Rotation speed
b. Density of both samples and solution
c. Viscosity
d. All of the above

Choose the correct statement regarding preparative centrifugation

a. It is concerned with the study of purified macromolecules or isolated supramolecular assemblies
b. It is concerned with separation of biological particle from cellular homogenate.
c. Preparative centrifugation was developed by Svedberg
d. None of the above statements are correct

Choose the correct statement regarding Analytical centrifugation

a. It is concerned with the study of purified macromolecules or isolated supramolecular assemblies
b. It is concerned with separation of biological particle from cellular homogenate.
c. Preparative centrifugation was developed by Svedberg
d. None of the above statements are correct

References & Further reading

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3. Biochemical Methods of Analysis, Saroj Dua And Neera Garg : Narosa Publishing House, New Delhi.
4. Bioanalytical Techniques, M.L. Srivastava, Narosa Publishing House, New Delhi.