BASIC PRINCIPLE OF SEDIMENTATION

Sedimentation is the process of allowing particles in suspension to settle down out of the suspension under the effect of gravitational field. The particles that settle down from the suspension are called sediment like mud settles from muddy water. For sedimentation to occur, it is required for particles to be heavier than the solution. In this process, the Brownian particles attain a certain velocity under the action of gravitational field (external field), which is known as sedimentation or settling velocity. For small particles, the sedimentation velocity in the earth's gravitational field is very small, and sedimentation can only be observed by artificially increasing the gravitational field by means of centrifugation. The sedimentation, under the influence of gravitational field or centrifugal field in centrifugation, occur in a specific rate in solution depending upon the various factors, such as mass and size of the Brownian particles, and gravitational field as well. The difference in sedimentation velocity for particles of different mass and size are applied as means to separate different species of Brownian particles.

In centrifugation process, the rate of sedimentation is dependent upon the applied centrifugal force (g) being directed readily outwards, which is determined by the square of the angular velocity of the rotor (ω in radians S^{-1}) and the radians (r, in centimeters) of the particle from the axis of the rotation. Therefore, according to the equation;

$$G = m^2 r$$

Since, one revolution of the rotor is equal to 2 radians, its angular velocity, in radians S^{-1}, can be expressed in terms of revolutions per minute (rev.min^{-1}). Therefore, speed of rotor will be;

$$m = \frac{2\pi \; rev.min^{-1}}{60}$$

Therefore, the centrifugal force in terms of rev.min1^{-1} is then

$$G = \frac{4\pi 2 (rev.min^{-1})^2}{3600} \times r$$

and is generally expressed as a multiple of the earth's gravitational force (g = 981 cm s^{-1}), i.e., the ratio of the weight of the particle in the centrifugal force to the weight of the same particle when acted on by gravity alone, and is then referred to as the relative centrifugal force (RCF) or more commonly as the 'number times g'. Hence, from the above equation,

$$rcf = \frac{4\pi 2 (rev.min^{-1})^2}{3600} \times r \times 981$$

which may be shortened to give rcf as;

$$rcf = (1.118 \times 10^{-5} (rev.min^{-1})^2 \times r$$

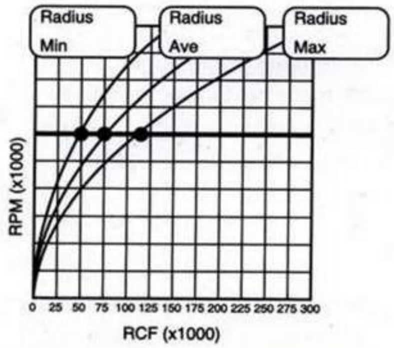


Fig. 5.1: Relation between RCF and RPM

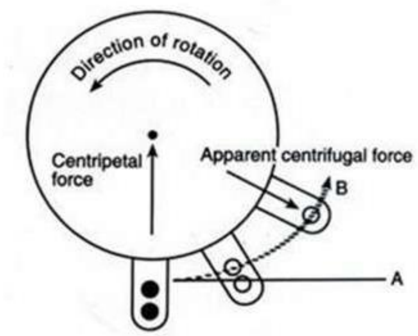


Fig. 5.2: Different forces during centrifugation

Since rotors are different from various manufacturers, rcf is used to represent centrifugal force. When conditions for the centrifugal separation of particles are reported, therefore, rotor speed, radial dimensions and time of operation of the rotor must all be quoted. Since biochemical experiments are usually conducted with particles dissolved or suspended in solution, the rate of sedimentation of a particle is dependent not only upon the applied centrifugal field but also upon the mass of the particle, which may be expressed as the product of its volume and density, the density and viscosity of the medium in which it is sedimenting and the extent to which its shape deviates from spherical.

When particle sediments it must displace some of the solution in which it is suspended, resulting in an apparent up-thrust on the particle equal to the weight of the liquid displaced. If a particle is assumed to be spherical and of known volume and density, the latter being corrected for the buoyancy due to the density of the medium, then the net outward force (F) it experiences when centrifuged at an angular velocity of ω radians s^{-1} is given by

$$F = \frac{4}{3}\pi . r_p^3 (\rho_p - \rho_m) \times m^2 \times r$$

Where $'\frac{4}{3}\pi . r_p^3'$ is the volume of a sphere of radius **'r_p'**, **'ρ_p'** is the density of the particle, $\boldsymbol{\rho_m}$ is the density of the suspending medium, and **'r'** is the distance of the particle from the center of rotation. Particles, however, generate friction as they migrate through the solution. If a particle is rigid and spherical and moving at a known velocity, then the frictional force **'F_o'** opposing motion is;

$$F_o = v \times f$$

where, **'v'** is the velocity or sedimentation rate of the particle, and **'f'** is the frictional coefficient of the particle in the solvent. The frictional coefficient of a particle is the function of its size, shape and hydration, and of the viscosity of the medium, and according to the Stokes equation, for an un-hydrated spherical particle, the frictional coefficient is;

$$F = 6\pi . \eta . r_p$$

where η is the viscosity coefficient of the medium.

But, for the asymmetric and/or hydrated particles, the actual radius of the particle is replaced by the effective of Stokes radius, r_{eff}. When an unhydrated, spherical particle of known volume and density, present in a medium of constant density is accelerated in a centrifugal field, its velocity increases until the net force of sedimentation equals the frictional force resisting its motion through the medium, i.e.,

$$F = F_o \quad \text{or} \quad \frac{4}{3}\pi . r_p^3 (\rho_p - \rho_m) \times m^2 \times r = 6\pi . \eta . r_p \, v$$

In practice, the balancing of these forces occurs quickly and the particle reaches a constant velocity because the frictional resistance increases with the velocity of the particle. Under these conditions, the net force acting on the particle is zero. Hence, the particle no longer accelerates but achieves a maximum velocity, with the result that it now sediments at a constant rate. Therefore, rate of sedimentation (v) is then

$$v = \frac{dr}{dt} = 2r_p^2 (\rho_p - \rho_m) \times m^2 \times r = 9\&$$

It is evident from this equation that the sedimentation rate of a given particle is proportional to its size, to the difference in density between the particle and the medium and to the applied centrifugal force. It is zero when the densities of the particle and medium are equal; it decreases when the viscosity of the medium increases, and increases as the force increases. However, since the equation involves the square of the particle radius, it is apparent that the size of the particle has the greatest influence upon its sedimentation rate.

Therefore, sedimentation coefficient is

$$\frac{dv}{dt} = 0$$

$$m^2 . r(\rho_p - \rho_m) - f . v = 0$$

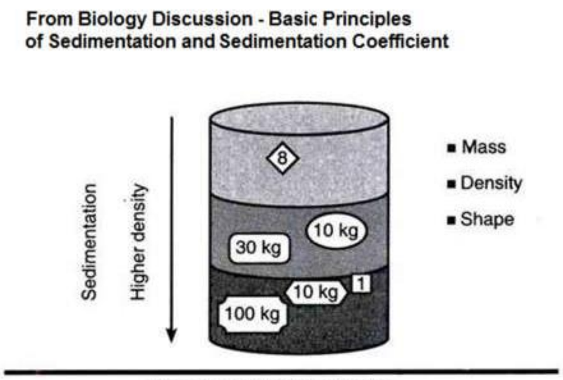
$$S = \frac{v_t}{m^2 . x} = \frac{m(1 - \bar{\upsilon} V_t)}{m^2 . x}$$

Where 'S' is a terminal velocity/unit acceleration, 'm' is the mass of particle, 'f' is a frictional coefficient of the particle in the solvent, 'ρ' is the density of solution, and 'v' is the velocity of particle.

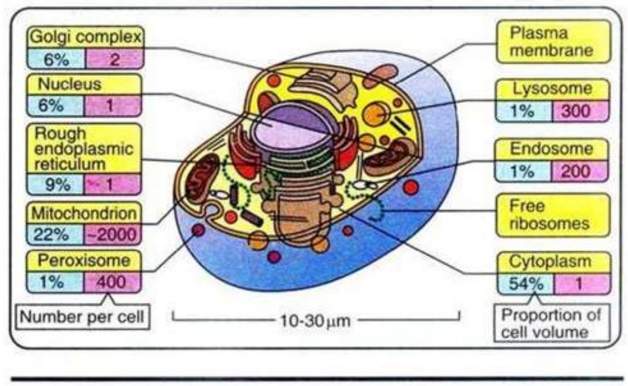
Character of a sedimentation coefficient

Sedimentation coefficients have units of sec.10^{-13} sec is called 1 Svedberg (or 1 S). There are following properties of sedimentation coefficient;

– S is increased for particle of larger mass.



Separation by Sedimentation



Distribution of various organelles in cells

- S is increased for particle of arger density (equal volume).
- S is increased for more compact structure (shape) of equal particle mass (frictional coefficient
- S is increased with the rotational speed.