

CELL FRACTIONATION (Notes from Biochemical Engineering Fundamentals (2nd Ed), James E. Bailey and D. F. Ollis, McGraw-Hill Book Co. 1986)

Differential Centrifugation Method

Obtaining organelles from a given cell type in sufficient quantity for analysis usually requires that a large number of organelles be isolated from a large number of cells.

1. A cell suspension is homogenized using a pestle or ultrasonic sound. The goal is to break the cells apart without significantly disrupting the organelles.
2. Fractionation of the resulting suspension is the next step. Standard centrifugation techniques for fractionation of cell organelles rely on physical characteristics: size, shape, and density.
3. Analysis of centrifugation is presented below.

Given: spherical particle of radius R , density ρ_p placed in a centrifuge tube containing fluid medium of density ρ_f and viscosity μ . Place tube in centrifuge and spin at angular velocity ω .

Find: calculate the particle motion employing the force balance:

$$\text{Drag force on particle} = \text{buoyancy force} \quad (1)$$

$$6\pi\mu R v_r = \frac{4\pi R^3}{3} G(\rho_p - \rho_f) \quad (2)$$

where v_r is the velocity in the r direction: $v_r = dr/dt$ (3)

and G is acceleration due to centrifugal force: $G = \omega^2 r$ (4)

Stoke's Law has been used in eqn. (2) to express the drag force since particle velocities (and therefore particle Reynold's numbers) are usually very low. (Gravitational force does NOT appear in (2) because the r direction is normal to the force of gravity.)

Rotation of the centrifuge at high speed, however, produces acceleration G usually in the range of 600 – 600,000 g .

Integration of eqn. (2) with respect to time and distance traveled by the particle gives the time required for movement of the particle from r_1 to r_2 .

$$6\pi\mu R (dr/dt) = \frac{4\pi R^3}{3} \omega^2 r (\rho_p - \rho_f) \quad (5)$$

$$dt/dr = 6\pi\mu R \frac{3}{4\pi R^3 \omega^2 r (\rho_p - \rho_f)} \quad (6)$$