6. Sedimentation

Sedimentation

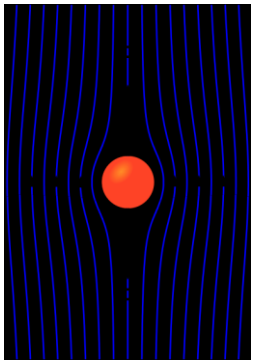
- **Definition**
  - The movement of particles or macromolecules in an inertial field
- **Accelerations range**
  - 1 x g to 100,000 x g
- **Applications in bioprocessing**
  - Clarification of broths and lysates
  - Collection of cells and inclusion bodies
  - Separation of fluids having different densities

6.1. Sedimentation Principles

Equation of Motion

- **Spherical Particle**
  - Radius a, density ρ, mass (4/3) $\pi a^3 \rho$
- **Centrifugal acceleration**
  - $\omega^2 R$
    - ω : angular velocity (rad/s)
    - R: The distance of the particle from the center of rotation
- **Forces in the R direction**
  - $m\, dv/dt = F_{iR} + F_{bR} + F_{dR}$

$= (4/3)\, \pi a^3\, \rho \omega^2 R - (4/3)\, \pi a^3\, \rho_0 \omega^2 R - 6\, \pi \mu a v$



    - i: inertial acceleration
    - b: buoyancy due to the density of the medium ρ_0
    - d: the Stokes drag force
      - Proportional to the velocity (v) and viscosity (μ) under conditions of creeping flow

Equation of Motion

- Centrifugal velocity
  - At steady state $dv/dt=0$
  - $v=[2a^2(\rho-\rho_0)\,\omega^2 R]/9\mu$
- Sedimentation only in the presence of gravity
  - $v=[2a^2(\rho-\rho_0)\,g]/9\mu$

    g = 9.8 m/s^2
- Moving particle
  - R is not constant $v=dR/dt$
  - $dR/R=\{[2\,a^2(\rho-\rho_0)\,\omega^2 R]/\,9\mu\}\,dt$
  - Integration, at $t=0$, $R=R_0$
  - $\ln(R/R_0)=[2a^2(\rho-\rho_0)\,\omega^2 t]/\,9\mu$
    - Relationship between time and travel distance of the particle

Sensitivities

- **Reynolds number**
  - Re = $2avp/\mu$, typically <0.001
  - ρ_0 =*1.0* g/cm^3, μ=*1.0 cp*

- **Sedimentation velocities**

Particle	a (*μm*)	ρ (g/cm^3)	Dimensionless acceleration (G), $\omega^2 R/g$	*V* (*cm/h*)	Re
Yeast cell	2.5	1.1	1	0.5	7×10^{-6}
Bacterial cell	0.5	1.1	1	0.02	6×10^{-8}
Protein	0.05	1.3	10^4	0.06	2×10^{-9}

Sensitivities

- **Isopycnic or equilibrium sedimentation**
  - When $\rho=\rho_0$
  - Used for determination of molecular densities and separation of living cells using density gradient or density shelf
  - E.g. separation of lymphocyte
    - Density shelf with a density around 1.07 g/cm (combination of Ficoll, hypaque)
    - Sedimentation of RBC and floating of WBC

TABLE 5.2
Measured Values of the Density of Representative Cells, Organelles, and Biomolecules

Cell, organelle, or biomolecule	Density, ρ (g/cm^3)
Escherichia coli	1.09[a]
Bacillus subtilis	1.12
Arthrobacter sp.	1.17
Saccharomyces pombe	1.09
Saccharomyces cerevisiae	1.11[a]
Amoeba proteus	1.02
Murine B cells	1.06[a]
Chinese hamster ovary (CHO) cells	1.06
Peroxisomes	1.26[a]
Mitochondria	1.20[a]
Plasma membranes	1.15[a]
Proteins	1.30[a]
Ribosomes	1.57[a]
DNA	1.68[a]
RNA	2.00[a]

[a]Average value.

Sensitivities

- **Hindered settling**
  - Increase in the concentration of sedimenting particles → decrease in v
    - $v_c = v(1-\Phi)^n$
      - v_c : sedimentation velocity of particles in a concentrated suspension
      - v: velocity of individual particles
      - Φ : the volume fraction of the particles
      - n: a function of the shape of the particle and of the Re
        - » Re <0.2, r=4.65

TABLE 5.3
Effect of Particle Volume Fraction ϕ on the Particle Sedimentation Velocity for Spherical Particles

ϕ	v_c/v
0.01	0.95
0.05	0.79
0.10	0.61
0.20	0.35

6.2. Methods and Coefficient

Equilibrium Sedimentation

- **Methods to generate concentration gradient**
  - Step by step layer of solutions of decreasing density
  - Centrifugation at extremely high speed
    - Isothermal stratification of a density forming solute like CsCl
  - Gradient mixing
    - Linkage of two cylindrical containers with high and low density solutions
    - Time-dependent solute concentration

      $C(t) = c_{1,0} + [Q(c_2-c_{1,0})/(2V_0)]\ t$

      - » Q : outflow rate, pumping or gravity feed
      - » V_0 : initial volume
      - » c_2 : concentration in the nonmixed chamber (constant)
      - » $c_{1,0}$: Initial concentration in the mixed chamber

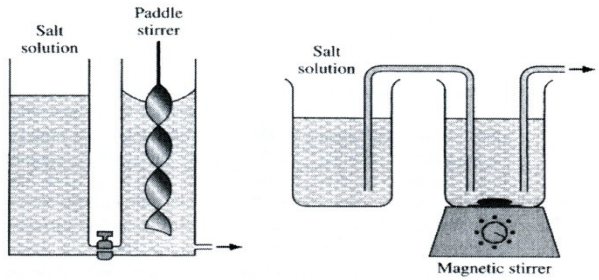


Figure 5.1 Two types of linear gradient mixer.

Sedimentation Coefficient

- **Sedimentation coefficient (s)**
  - $s = v/(\omega^2 R)$
  - $v = [2a^2(\rho - \rho_0)\ \omega^2 R]/9\mu$
  - $s = 2a^2(\rho - \rho_0)\ /9\mu$
    - Defined in terms of the properties of the particle and the medium
  - $s_{20,w}$ (s)
    - At 20°C, under conditions of pure water
    - 10^{-13}s = 1 svedberg unit (S)
      - Svedberg: inventor of the ultracentrifuge

Example : Time required for sedimentation

- **Conditions**
  - 70S ribosome
  - Centrifuge at 10,000 rpm
  - R_o=4 cm, R=5 cm
- **Time for complete sedimentation**
  - $s = v/(\omega^2 R) = dR/dt \bullet 1/(\omega^2 R)$
  - Integration, at t=0, $R = R_o$
  - $\omega^2 st = \ln (R/R_0)$
  - t=8.1 h

Equivalent Time

- **Equivalent time**
  - Used to assess the approximate properties of a particle type to be separated
  - Dimensionless acceleration $G = \omega^2 R/g$
  - Equivalent time = $Gt = \omega^2 R/g \bullet t$
    - t : time required for sedimentation
    - Eukaryotic cells: 0.3 x 10^6 s
    - Bacteria: 9 x 10^6 s
    - Ribosome : 1100 x 10^6 s
  - Scale up
    - Assume constant equivalent time
    - (Gt)1 = (Gt)2

Sigma Analysis

- **Sigma Analysis**
  - Used for engineering analyses and scaling up
- **Q: volumetric flow rate of feed flow**
  - $Q = \{v_g\}[\Sigma]$
  - $v_g = 2a^2(\rho-\rho_0)g/9\mu$ (1)
    - Sedimentation velocity at 1 x g
    - Properties of the particle and the fluid
  - Σ
    - Represents the geometry and speed of the centrifuge
    - Cross-sectional area equivalent of the centrifuge
    - Properties of the centrifuge
  - $\ln(R/R_0) = [2a^2(\rho-\rho_0)\omega^2 t]/9\mu$ (2)
  - $v_g = [g \ln(R/R_0)] / \omega^2 t$ (1) + (2)
    - Can be determined in the lab
      - t: the minimum time to clarify the sample
      - R: distance from the center of rotation to the top of the packed solids
      - R_0: distance from the center of rotation to the top of the liquid

3. Production Centrifuges

Common Types of Production Centrifuge

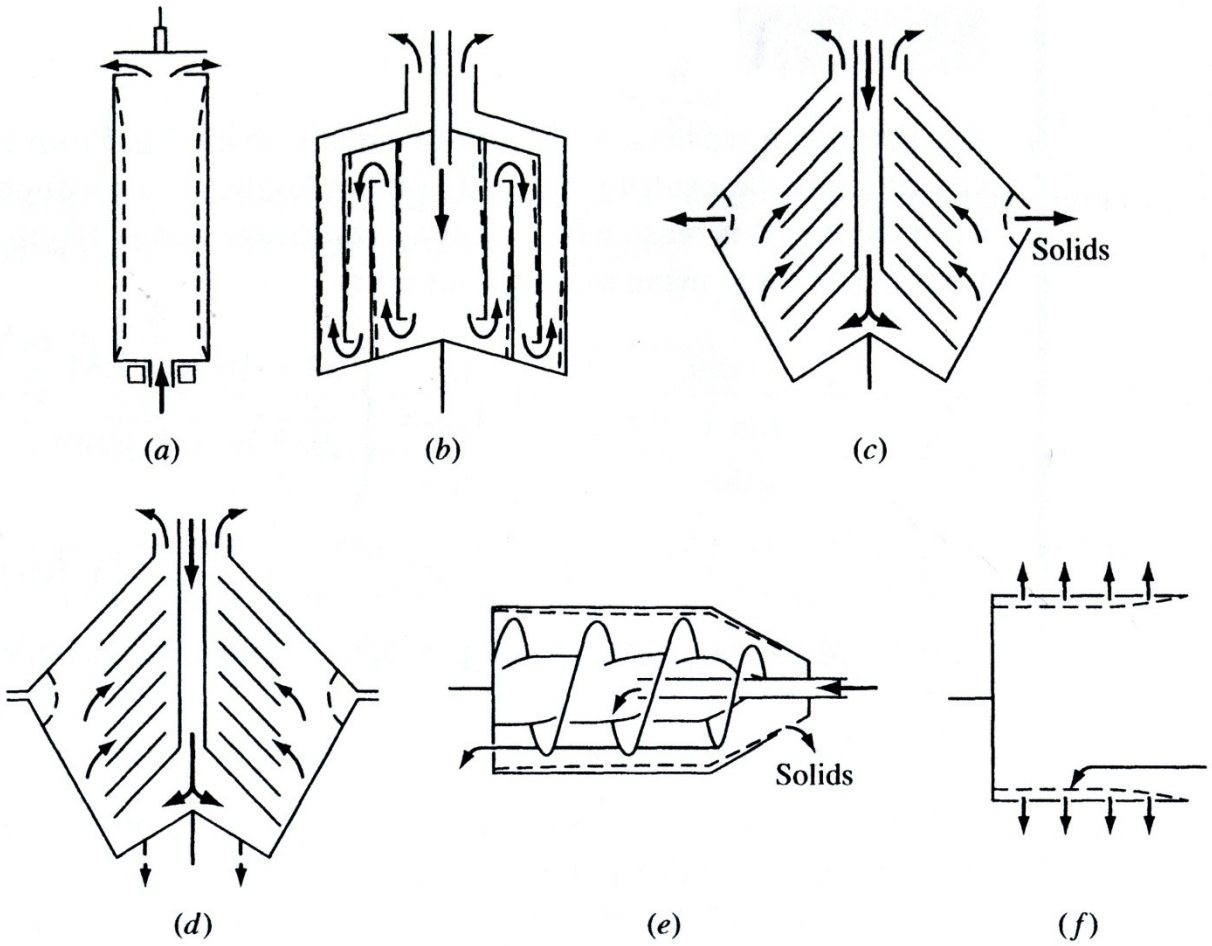
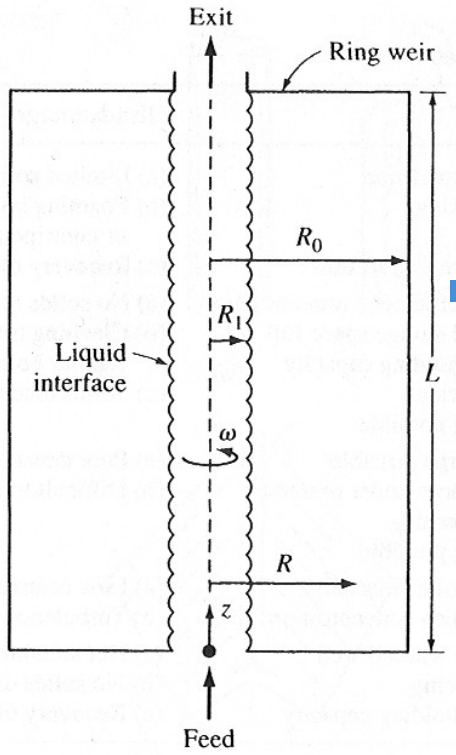


Figure 5.2 Common types of production centrifuge: (*a*) tubular bowl, (*b*) multichamber, (*c*) disk, nozzle, (*d*) disk, intermittent discharge, (*e*) scroll, and (*f*) basket. Arrows indicate the path of the liquid phase; dashed lines show where the solids accumulate.

Common Types of Production Centrifuge

- **Tubular centrifuge**
  - For particles of relatively low sedimentation coefficient
  - e.g. protein precipitate
- **Disk centrifuge**
  - Large sedimentation area
  - Continuous or intermittent solids discharge is possible
  - e.g. Cells and cell lysates
- **Scroll and basket centrifuges**
  - For particles of relatively high sedimentation coefficient
  - e.g. Antibiotic crystal

Tubular Bowl Centrifuge



Q: flow rate

- Trajectory of sedimented particles
  - In the radial direction (1)
    - $dR/dt = [2a^2(\rho-\rho_0)\,\omega^2 R]/9\mu$
  - In the axial direction (2)
    - $dz/dt = Q/A = Q/[\pi\,(R_0{}^2 - R_1{}^2)]$
- Combination of (1) and (2)
  - $(dR/dt)/(dz/dt) = dR/dz$
  - Integration
  - $Q = \{[2a^2(\rho-\rho_0)]/9\mu\}\,[\pi\,L(R_0{}^2 - R_1{}^2)\omega^2/\ln(R_0/R_1)]$
    - $Q = \{v_g\}[\sum]$
    - $v_g = 2a^2(\rho-\rho_0)g/9\mu$
  - $\sum = \pi\,L(R_0{}^2 - R_1{}^2)\omega^2/[g\ln(R_0/R_1)]$
- Practical usage
  - To get an optimal centrifuge
    - Determine v_g using a benchtop centrifuge
    - Calculate Q from process requirement
    - Determine $\sum$ to get a proper centrifuge

4. Ultracentrifugation

Ultracentrifugation

- **Ultracentrifugation**
  - 50,000 to 100,000 g
  - Very small particles and macromolecules can be sedimented
- **Types**
  - Analytical centrifugation
    - < 0.1 ml volume sample
    - Monitoring of centrifugation in an optical cell
  - Preparative centrifugation
    - Up to 50 ml sample
    - Collection of the sample by puncturing the bottom of the centrifuge tube

Determination of Molecular Weight

- Determination of molecular weight by the combined measurement of sedimentation and diffusion coefficient
- $m\ dv/dt = F_{iR} + F_{bR} + F_{dR}$
- $V(\rho-\rho_0)\ \omega^2 R - 6\pi\mu a v = 0$ at steady state
  - V: volume of a single molecule
- If $V = m\bar{V} = m/\rho$
  - $m = 6\pi\mu a v / [(1-\bar{V}\rho_0)\ \omega^2 R]$ (1)
    - m: mass of a single molecule
    - $\bar{V}$: specific volume
  - Stokes-Einstein equation:
    - $\mathcal{D}\mu/kT = 1/6\pi a$
    - $6\pi a\ \mu = kT/\mathcal{D} = RT/\mathcal{N}\mathcal{D}$ (2)
      - k : Boltzmann's constant = R (gas constant)/$\mathcal{N}$(Avogadro number)
  - $s = v/(\omega^2 R)$ (3)
- $M = sRT/\mathcal{D}(1-\bar{V}\rho_0)$: Substituting (2) and (3) into (1)
  - M : molecular weight