Centrifugation

BCH 332 lecture 14

Sedimentation

- When particles are forced through a solution, they experience resistance to movement which depends on properties of the particle such as mass, shape and density and properties of the solvent such as its temperature, viscosity, density and composition.
- A commonly-used experimental format where this occurs is *sedimentation in a centrifugal field which is* also called *centrifugation.*
- *This can be used as a preparative* strategy to separate complex mixtures present in biological samples.
- Alternatively, it can also be used *analytically to* determine the mass, shape or density of particles.

- If a solution of large particles is allowed to stand, then the particles will tend to sediment under the influence of gravity.
- For a given particle the rate or the velocity at which it sediments is proportional to the force applied so that the particles sediment more rapidly when the force applied is greater than the gravitational force.
- The basis for centrifugation is to exert a larger force than does the earth's gravitational field thus increasing the rate at which particles sediment.

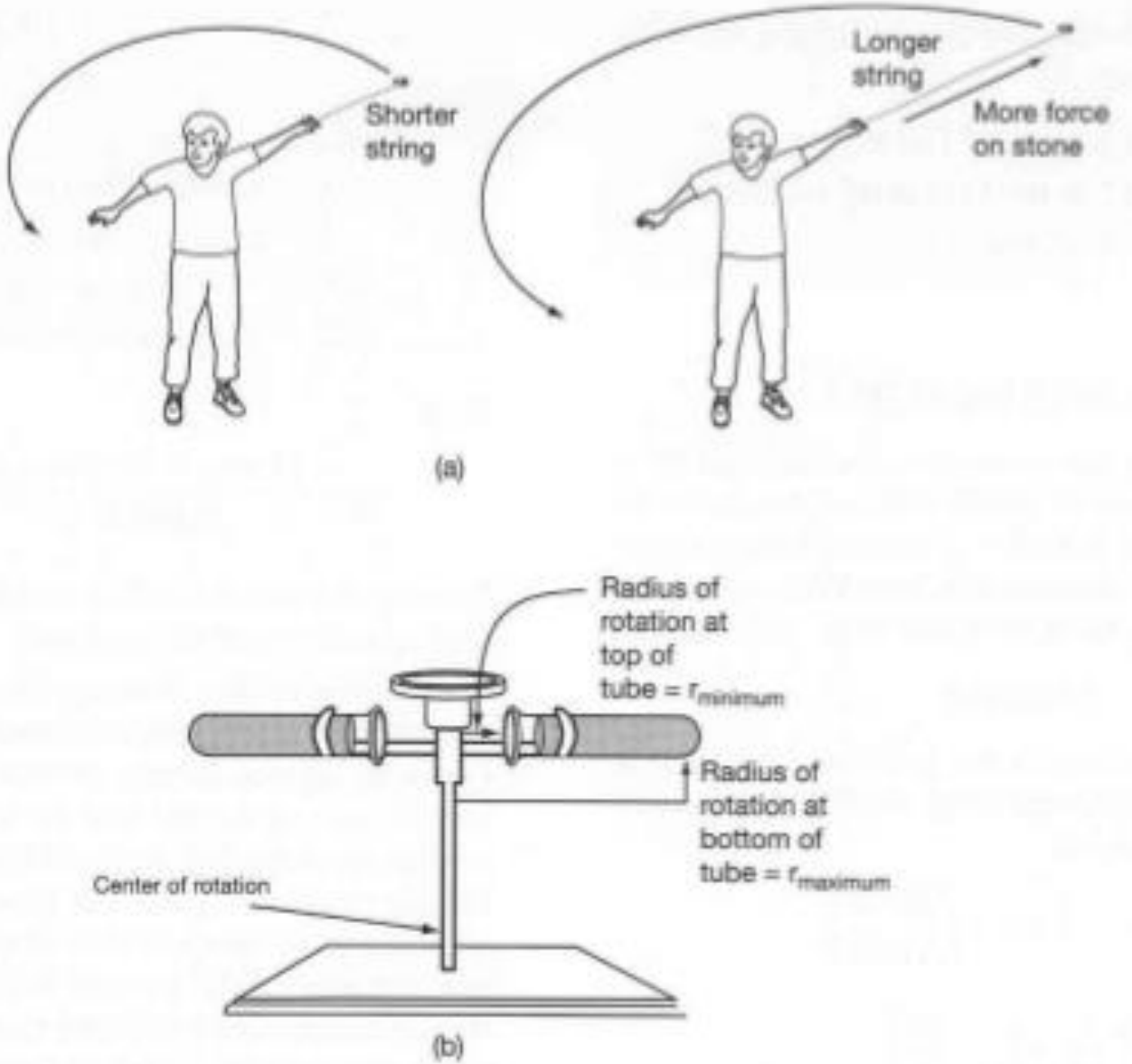


Figure 26.3. The Radius of Rotation. a. The longer a string being whirled, the greater the radius of rotation and the more force that is experienced by the stone. **b.** In a centrifuge, the further a particle is from the center of rotation, the more force it experiences.

- Particles that differ in density, shape or size can be separated because they sediment at different rates in the centrifugal field each particle sedimenting at a rate that is directly proportional to the applied centrifugal field.

Types of Centrifugation Techniques

- Preparative centrifugation techniques are concerned with the actual separation, isolation and purification of whole cells, subcellular organelles, plasma membranes, polysomes, ribosomes, chromatin, nucleic acids, lipoproteins and viruses for subsequent investigation.

- Analytical Centrifugation Techniques are devoted mainly to the study of pure or virtually pure macromolecules. They are concerned primarily with the study of the sedimentation characteristics of biological macromolecules and molecular structures.

Principle

A particle, whether it is a precipitate, a macromolecule, or a cell organelle, is subjected to a centrifugal force when it is rotated at a high rate of speed. The **centrifugal force,** F, is defined by Equation 7.1.

$$F = m\omega^2 r \qquad \textbf{Equation 7.1}$$

where

F = intensity of the centrifugal force

m = effective mass of the sedimenting particle

ω = angular velocity of rotation in rad/sec

r = distance of the migrating particles from the central axis of rotation

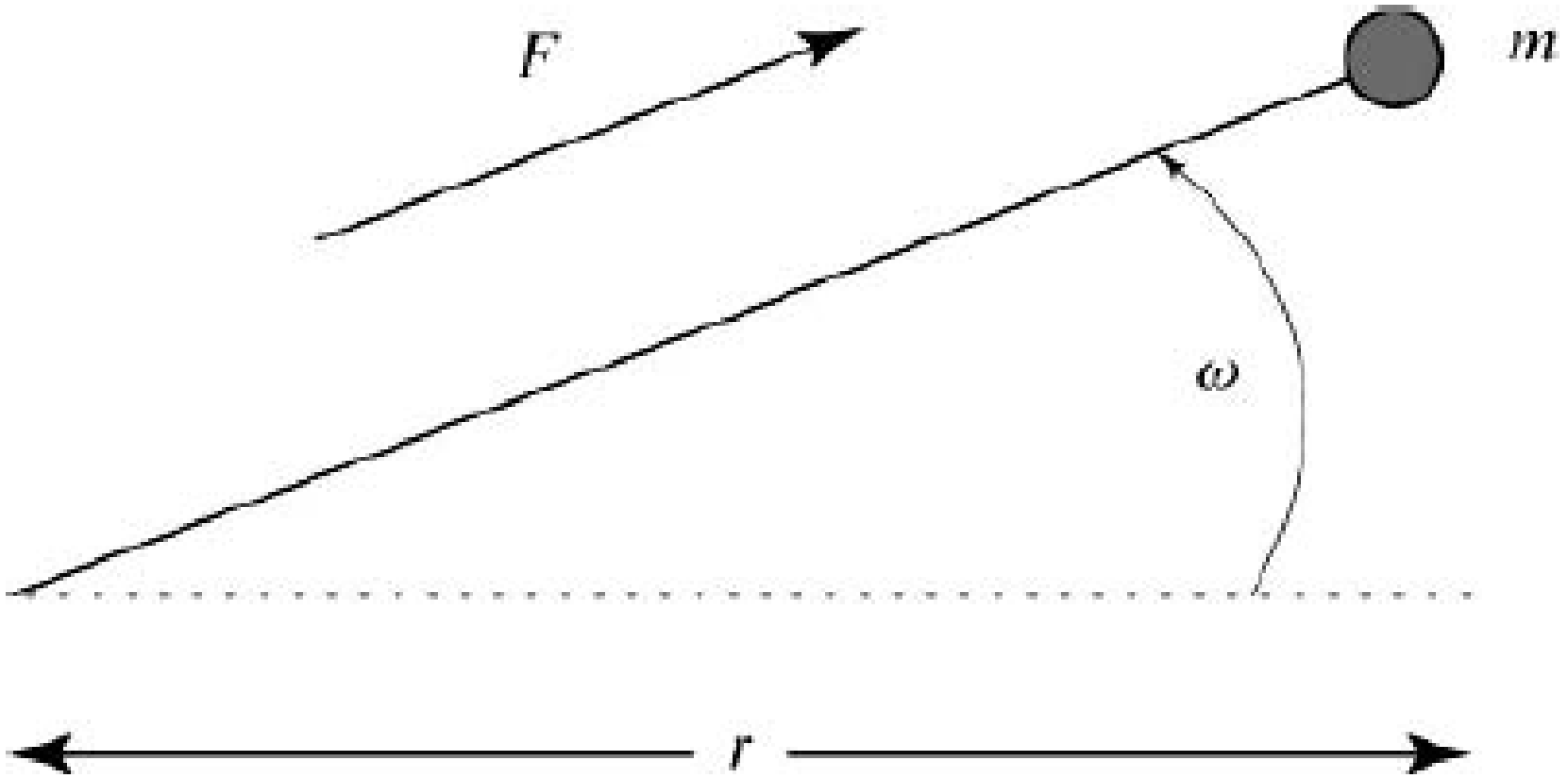


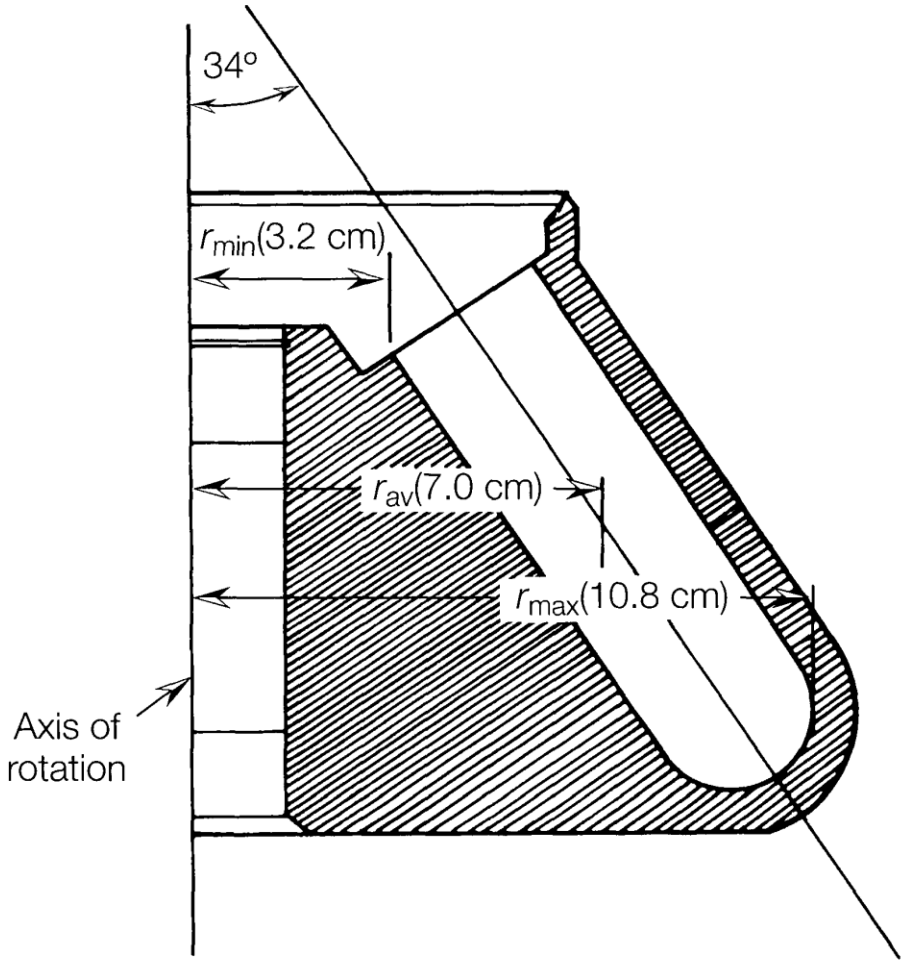
Figure 7.5. Centrifugal force. A particle of mass, m, experiences a centrifugal force, F, when centrifuged at an angular velocity, ω, around a point from which it is distant by a radius, r.

The force on a sedimenting particle increases with the velocity of the rotation and the distance of the particle from the axis of rotation. A more common measurement of F, in terms of the earth's gravitation force, g, is **relative centrifugal force,** RCF, defined by Equation 7.2.

$$\text{RCF} = (1.119 \times 10^{-5})(\text{rpm})^2(r) \qquad \textbf{Equation 7.2}$$

- This equation relates RCF to RPM.
- Centrifuged particles migrate at rate that depends on mass, shape and density of the particle and density of the medium.

A diagram illustrating the variation of RCF with *r,* the distance of the sedimenting particles from the axis of rotation. *Courtesy of Beckman Instruments, Inc.*



The goal of many centrifugation experiments is the measurement of *s*. This value is important because it can be used to calculate the size (molecular weight, kilo base pairs, etc.) of a molecule or cell organelle.

The term s is most often defined under standard conditions, 20°C and water as the medium, and denoted by $s_{20,\,w}$. The *s* value is a physical characteristic used to classify biological macromolecules and cell organelles. Sedimentation coefficients are in the range 1×10^{-13} to $10{,}000 \times 10^{-13}$ second. For numerical convenience, sedimentation coefficients are expressed in Svedberg units, S, where $1\ S = 1 \times 10^{-13}$ second. Human hemoglobin has an *s* value of 4.5×10^{-13} second or 4.5 S. The value of S for several biomolecules, bacterial cells, and cell organelles is shown in Figure 7.3.

- The sedimentation coefficient is defined as the ratio of a particle’s sedimentation velocity to the acceleration applied to it.
- The sedimentation coefficient is expressed as Svedberg unit.

- The more massive particle tends to move faster than a less massive one.
- The denser particle moves faster than a less dense one.
- The denser the solution the more slowly a particle will move.
- The greater the frictional coefficient, the more slowly the particle moves.

Instrumentation for Centrifugation

The basic centrifuge consists of two components, an electric motor with drive shaft to spin the sample and a **rotor** to hold tubes or other containers of the sample. A wide variety of centrifuges is available, ranging from a low-speed centrifuge used for routine pelleting of relatively heavy particles to sophisticated instruments that include accessories for making analytical measurements during centrifugation. Here we will describe three types, the low-speed or clinical centrifuge, the high-speed centrifuge, and the ultracentrifuge. Major characteristics and applications of each type are compared in Table 7.1.

Types of Centrifuges and Applications

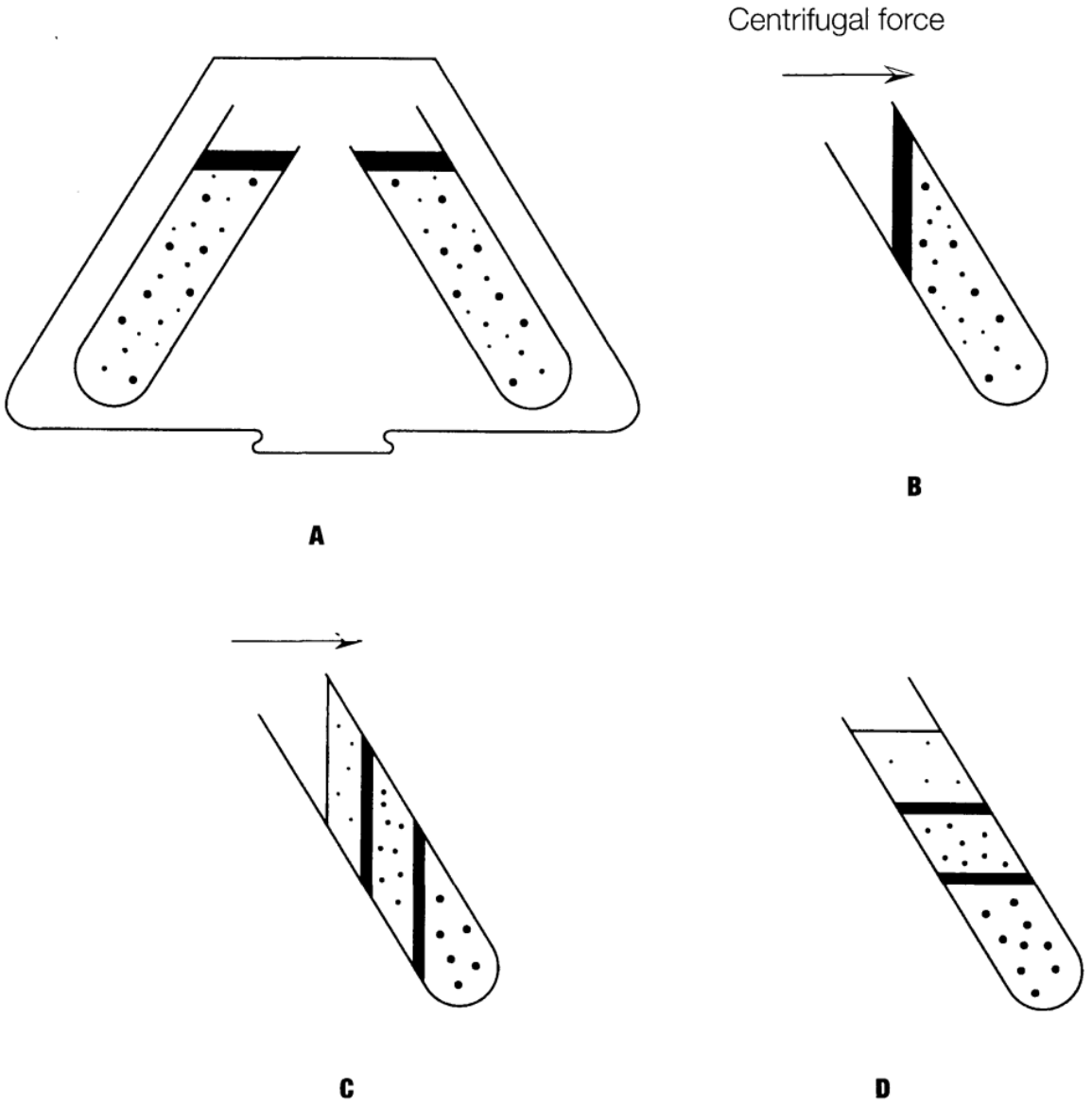
Characteristic	Type of Centrifuge		
	Low-speed	High-speed	Ultracentrifuge
Range of speed (rpm)	1–6000	1000–25,000	20–80,000
Maximum RCF (*g*)	6000	50,000	600,000
Refrigeration	Some	Yes	Yes
Applications			
Pelleting of cells	Yes	Yes	Yes
Pelleting of nuclei	Yes	Yes	Yes
Pelleting of organelles	No	Yes	Yes
Pelleting of ribosomes	No	No	Yes
Pelleting of macromolecules	No	No	Yes

Low Speed Centrifuges

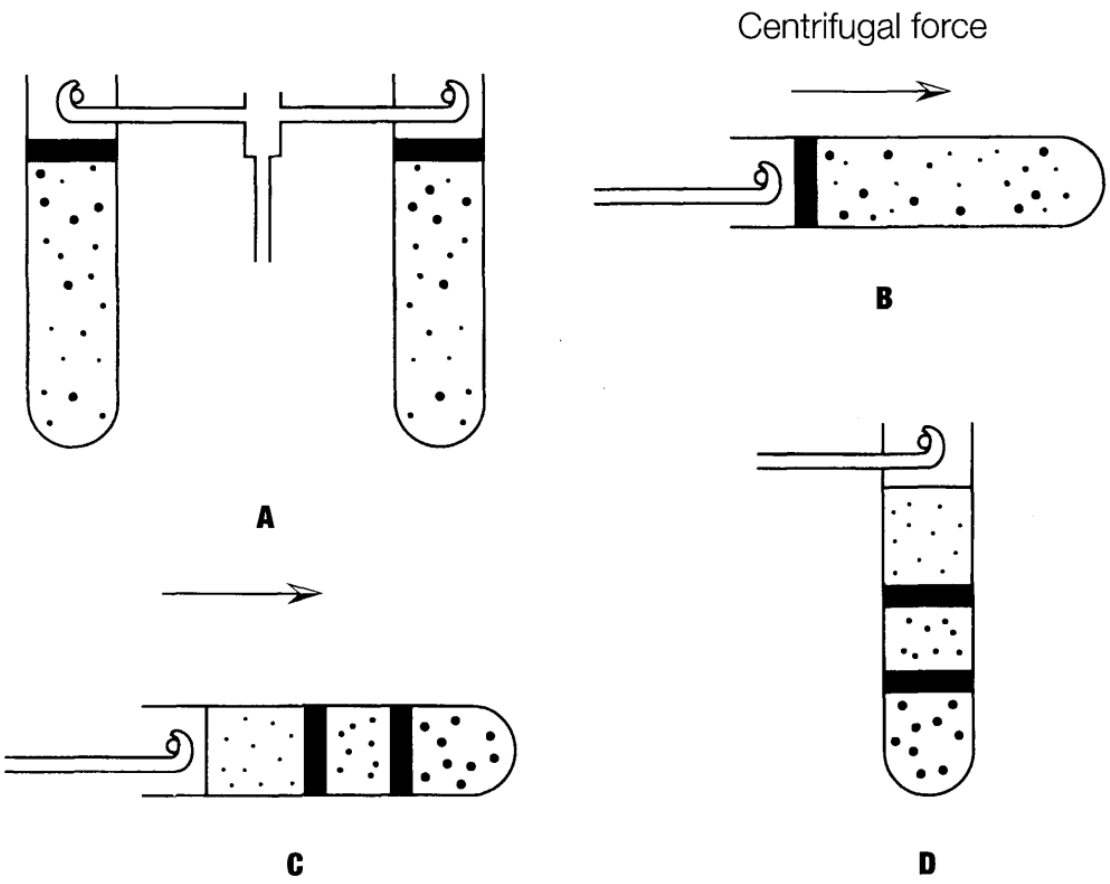
Most laboratories have a standard low-speed centrifuge used for routine sedimentation of relatively heavy particles. The common centrifuge has a maximum speed in the range of 4000 to 5000 rpm, with RCF values up to 3000 × *g*. These instruments usually operate at room temperature with no means of temperature control of the samples. Two types of rotors, **fixed angle** and **swinging bucket,** may be used in the instrument. Centrifuge tubes or bottles that contain 12 or 50 mL of sample are commonly used. Low-speed centrifuges are especially useful for the rapid sedimentation of coarse precipitates or red blood cells. The sample is centrifuged until the particles are tightly packed into a **pellet** at the bottom of the tube. The upper, liquid portion, the **supernatant,** is then separated by decantation.

Figure 7.6

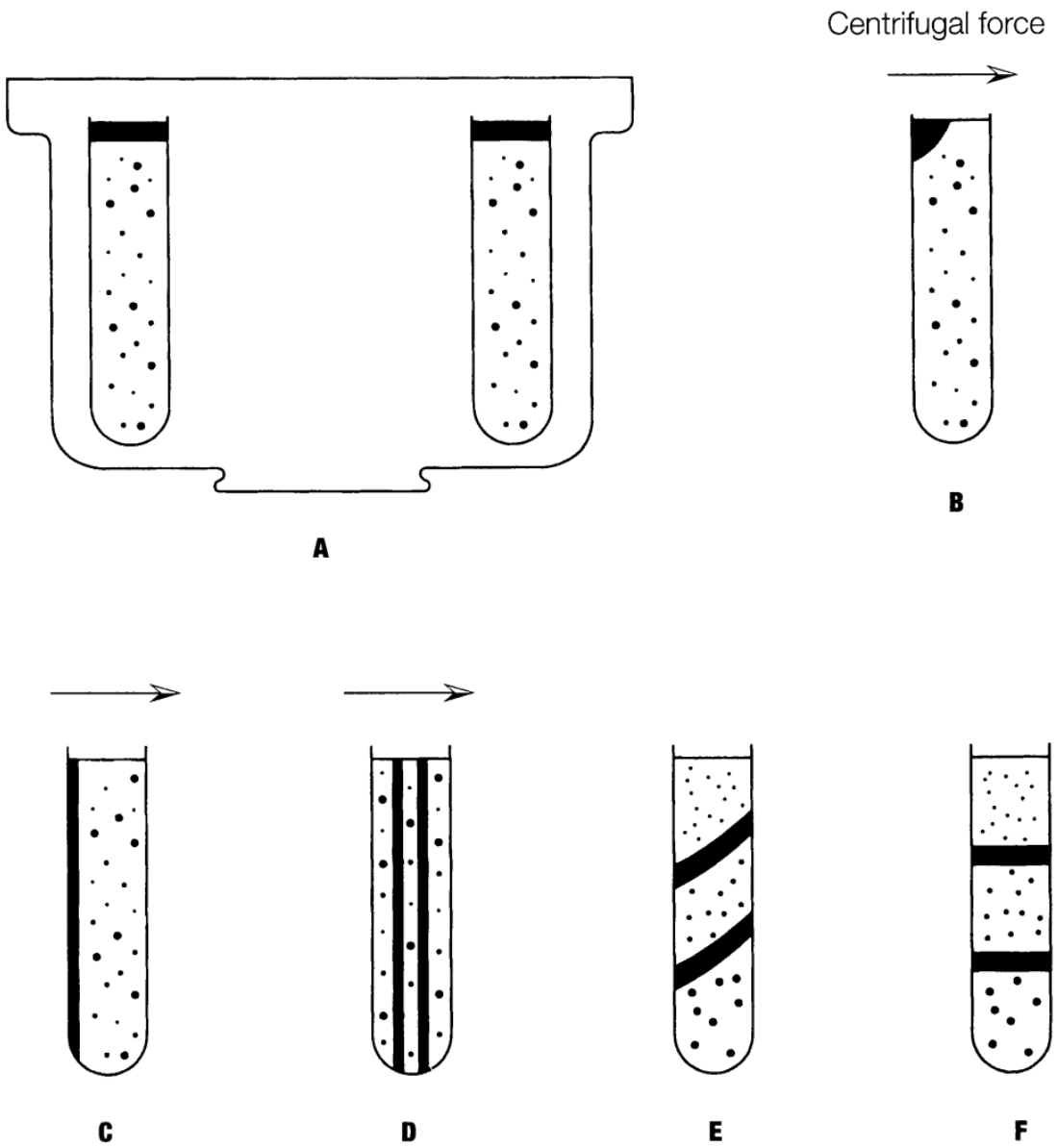
Operation of a fixed angle rotor. **A** Loading of sample. **B** Sample at start of centrifugation. **C** Bands form as molecules sediment. **D** Rotor at rest showing separation of two components.



Operation of a swinging-bucket rotor. **A** Loading of sample. **B** Sample at start of centrifugation. **C** Sample during centrifugation separates into two components. **D** Rotor at rest.



Operation of a vertical rotor. **A** Loading of sample. **B** Beginning of centrifugation. **C, D** During centrifugation. **E** Deceleration of sample. **F** Rotor at rest.



High Speed Centrifuge

- For sensitive biochemical operations.
- High speed and temperature control chamber are essential.
- Temperature is maintained at 4^0C.

Three types of rotors are available for high-speed centrifugation, the fixed-angle, the swinging-bucket, and the vertical rotor (Figure 7.5A–C). Fixed-angle rotors are especially useful for differential pelleting of particles (Figure 7.6A). In swinging-bucket rotors (Figure 7.5B), the sample tubes move to a position perpendicular to the axis of rotation during centrifugation, as shown in Figure 7.7. These are used most often for density gradient centrifugation (see below). In the vertical rotor (Figure 7.5C), the sample tubes remain in an upright position (Figure 7.8). These rotors are used often for gradient centrifugation.

Widely used in the category of medium-speed centrifuges is the "microfuge" (Figure 7.9). These instruments, which are designed for the benchtop, are used for rapid pelleting of small samples. Fixed-angle rotors are available to hold up to eighteen 1.5- or 0.5-mL tubes. The maximum speed of most commercial microfuges is between 12,000 and 15,000 rpm, which delivers a force of 11,000–12,000 $\times$ *g*. Some instruments can accelerate to full speed in 6 seconds and decelerate within 18 seconds. Most instruments have a variable speed control and a momentary pulse button for minispins.

The preparation of biological samples almost always requires the use of a high-speed centrifuge. Specific examples will be described later, but high-speed centrifuges may be used to sediment (1) cell debris after cell homogenization, (2) ammonium sulfate precipitates of proteins, (3) microorganisms, and (4) cellular organelles such as chloroplasts, mitochondria, and nuclei.

Ultracentrifuge

- Most sophisticated and used for analytical and preparative work.
- Because of enormous heat generated, the rotor chamber is refrigerated and placed in vacuum to avoid friction.

Preparative Ultracentrifuges

- Produce centrifugal field of 600000 g.
- The rotor chamber is refrigerated, sealed

Analytical Ultracentrifuges

- Speed up to 70000 rpm (500000 g).
- Consist of rotor which is refrigerated.
- Optical system to observe the sendimenting material.

Analytical Ultracentrifugation

- Analytical ultracentrifugation is the study of the behavior of macromolecules in solution under the influence of a strong gravitational force.
- Most macromolecules have a different density from the solvent surrounding them and so will sink (or float) in a strong enough field.
- Observations of dynamic behavior ("sedimentation velocity") or of systems in equilibrium ("sedimentation equilibrium") can provide information about size, shape, density and conformational changes in proteins and other macromolecules.
- The technique can be applied to materials ranging in size from peptides to viruses and living cells.

Applications of Analytical Ultracentrifugation

- Determination of protein homogeneity in solution (or a mixture of forms, e.g. monomer/dimer or aggregates).
- Determination of conformational changes associated with oligomerization or binding of another component
- Determination of molecular weights or subunit stoichiometry (monomer, dimer, trimer etc) in solution using sedimentation equilibrium.
- The analytical ultracentrifuge provides information about the oligomeric state of a protein and is more accurate than gel filtration.