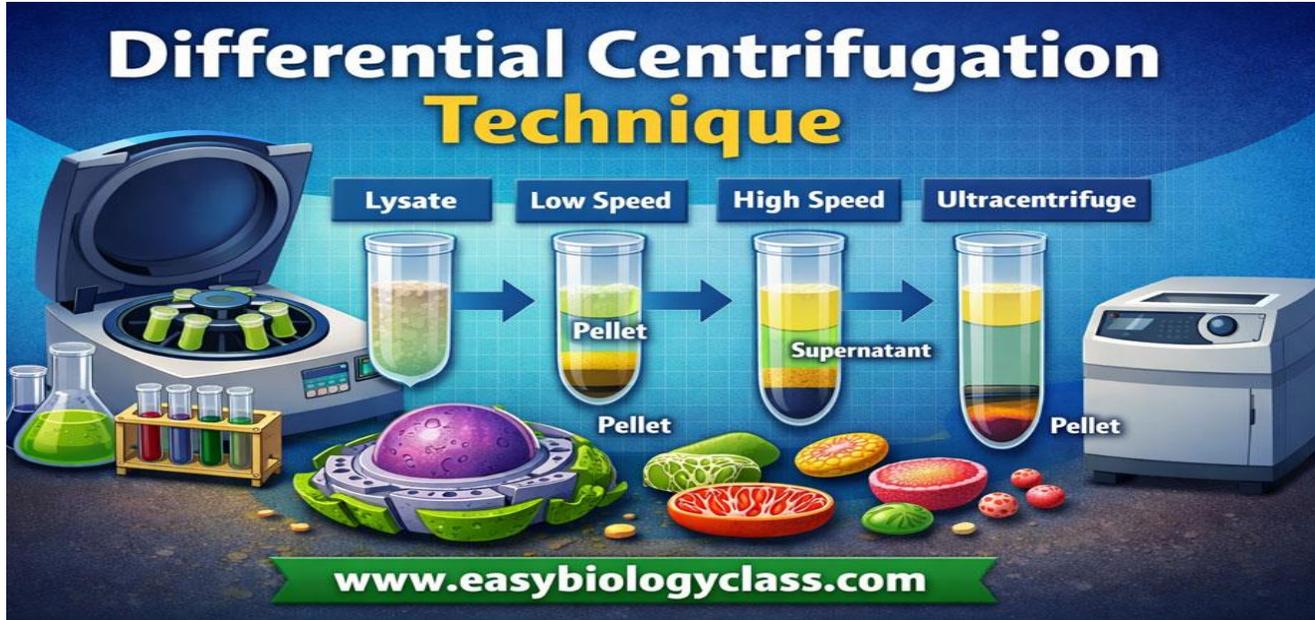


Differential Centrifugation Technique: Principles & Applications



Introduction

The **differential centrifugation technique** is a widely used laboratory method in biochemistry and cell biology. It helps scientists separate cellular components based on their sedimentation rate.

This method is essential for studying cell organelles such as nuclei, mitochondria, and ribosomes. Moreover, researchers use it to analyze the internal organization of cells.

In addition, the technique is useful for separating non-living particles such as viruses, nanoparticles, and colloidal particles. Because of its simplicity and efficiency, it is one of the most common cell fractionation methods used in biological laboratories.

Definition

Differential centrifugation technique is a laboratory method used to separate cellular organelles and particles according to their size, density, and sedimentation rate by applying increasing centrifugal forces during repeated centrifugation steps.

Differential Centrifugation Technique in Cell Biology

The **differential centrifugation technique** is mainly used to isolate cell organelles for biological analysis. It separates particles step-by-step using increasing centrifugal speeds.

First, a tissue sample is disrupted to break the cell membrane. As a result, cellular components are released into a liquid suspension called a lysate.

Next, the lysate undergoes repeated centrifugation cycles. During each cycle:

- Larger particles settle faster and form a **pellet** at the bottom of the tube.
- Smaller particles remain suspended in the **supernatant**.

After every centrifugation step, the supernatant is transferred to a new tube and centrifuged again at a higher speed. Consequently, different organelles separate progressively.

However, this method provides only **crude separation**. For more precise purification, scientists often use density gradient centrifugation.

Principle of Differential Centrifugation

The separation in this technique occurs due to differences in the **sedimentation rate** of particles.

Several factors influence how quickly particles settle in a centrifugal field:

- Gravitational or centrifugal force
- Density difference between particle and medium
- Viscosity of the surrounding fluid
- Size and shape of particles

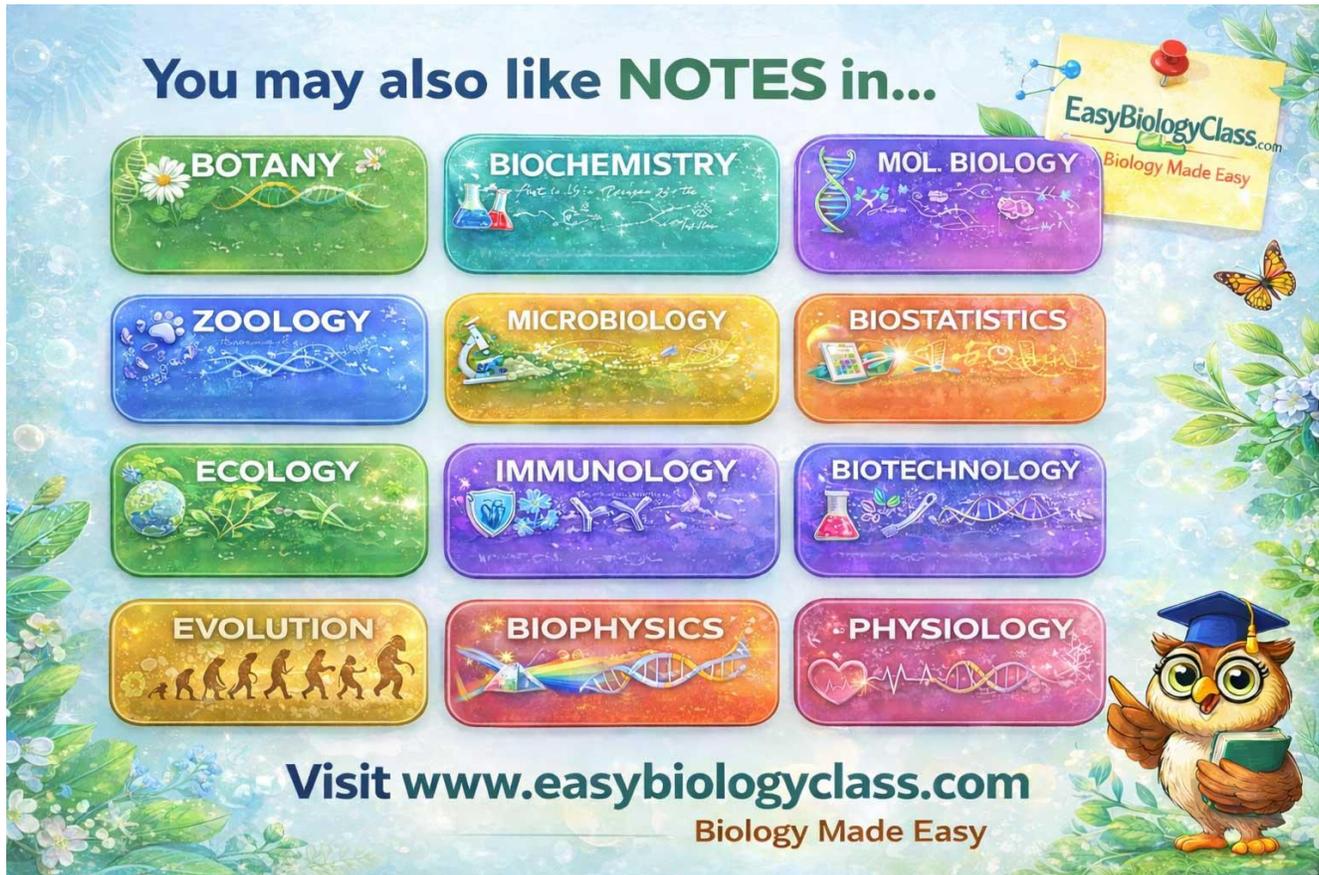
Larger and denser particles settle faster than smaller ones. Therefore, they form pellets at lower centrifugal speeds.

On the other hand, particles with lower density than the surrounding fluid do not sediment. Instead, they float even under strong centrifugal force. A common example is fat droplets in water.

Thus, the **differential centrifugation technique** separates particles mainly based on **size, density, and shape**.

Sedimentation Theory

Sedimentation occurs when particles move through a fluid under the influence of centrifugal force.



However, the movement of particles depends strongly on the physical properties of the system.

Key factors include:

- Fluid viscosity
- Density difference between particle and medium
- Rotational speed of the centrifuge
- Distance from the center of rotation

High centrifugal force accelerates sedimentation. Consequently, even very small particles can settle faster than the rate of Brownian motion.

In practical centrifugation systems, **Stokes' law** is modified to account for changes in centrifugal force during rotation.

This theoretical understanding helps scientists determine the appropriate centrifugation speed and duration.

Procedure of Differential Centrifugation

The **differential centrifugation technique** follows a series of controlled centrifugation steps. Each step separates a specific cellular component.

1. Cell Lysis and Homogenization

First, cells must be broken open to release internal structures.

Gentle homogenization methods are preferred because they preserve organelle integrity. For example, a **Dounce homogenizer** is commonly used.

Excessive mechanical force may damage organelles. Therefore, careful homogenization is important.

The resulting mixture is known as the **cell homogenate**.

2. First Centrifugation (Low Speed)

The homogenate is centrifuged at a low speed.

Typical conditions include:

- **100 × g for 5 minutes**

During this step:

Pellet contains

- Intact cells
- Large cell debris

Supernatant contains

- Cytoplasm
- Smaller organelles

3. Second Centrifugation (Medium Speed)

The supernatant from the previous step is centrifuged again at a higher speed.

Typical conditions include:

- **600 × g for 10 minutes**

This step separates the **nuclei** from other cellular components.

Pellet contains

- Nuclei

Supernatant contains

- Cytosol
 - Other organelles
-

4. Third Centrifugation (High Speed)

The next centrifugation step occurs at a much higher force.

Typical conditions include:

- **15,000 × g for 20 minutes**

At this stage:

Pellet contains

- Mitochondria
- Chloroplasts
- Lysosomes
- Peroxisomes

Supernatant contains

- Cytosol
- Microsomes

This fraction is often called the **post-mitochondrial supernatant**.

5. Fourth Centrifugation (Very High Speed)

The supernatant undergoes another centrifugation at very high speeds.

Typical conditions include:

- **50,000–100,000 × g for 60 minutes**

This step isolates:

Pellet contains

- Plasma membrane fragments
- Microsomal fractions
- Large polyribosomes

Supernatant contains

- Cytosol
 - Ribosomal subunits
 - Enzyme complexes
-

6. Ultracentrifugation Step

Finally, the remaining supernatant is centrifuged using an **ultracentrifuge**.

Typical conditions include:

- **50,000–100,000 × g for 120 minutes**

This step separates very small particles.

Pellet contains

- Ribosomal subunits
- Small polyribosomes
- Some soluble enzyme complexes

Supernatant contains

- Cytosolic proteins
-

Advantages of Differential Centrifugation

The method offers several benefits in biological research:

- Simple and widely used technique
- Requires basic laboratory equipment
- Effective for separating major cell organelles
- Allows further biochemical analysis of isolated fractions

Moreover, isolated organelles often retain normal biological activity if handled carefully.

Limitations of the Technique

Despite its usefulness, the method has some limitations.

- Provides **crude separation** rather than pure fractions
- Organelles may mix between fractions
- Requires additional purification methods for precise separation

Therefore, scientists often combine it with **density gradient centrifugation** for improved resolution.

Conclusion

The **differential centrifugation technique** is a fundamental method in cell biology and biochemistry. It separates cellular components by applying increasing centrifugal forces during repeated centrifugation steps.

Because the technique relies on differences in particle size and sedimentation rate, it effectively isolates organelles such as nuclei, mitochondria, and ribosomes. Consequently, researchers can study the structure and function of cellular components in detail.

For more notes and study materials, please visit

www.easybiologyclass.com



You may also like...

- Biology PPTs
- Video Tutorials
- Biology MCQ
- Question Bank
- Difference between
- Practical Aids
- Mock Tests (Online)
- Lecture Notes
- Mock Tests (Online)
- Biology Exams
- Biology Exams

Visit www.easybiologyclass.com
— Biology Made Easy —