



CENTRIFUGATI ON

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CONTENTS

- ◉ Definition
- ◉ Introduction
- ◉ History
- ◉ Principle
- ◉ Centrifuge
- ◉ Instrumentation
- ◉ Types of Centrifugation Techniques
- ◉ Calculation of Centrifugal Field
- ◉ Calculation of Angular Velocity
- ◉ Applications in Water Treatment
- ◉ Other Applications
- ◉ References

DEFINITION

Centrifugation is a process used to separate or concentrate materials suspended in a liquid medium. The theoretical basis of this technique is the effect of gravity on particles (including macromolecules) in suspension. Two particles of different masses will settle in a tube at different rates in response to gravity.

ILLUSTRATION OF CENTRIFUGATION



INTRODUCTION

- **Centrifugation** is a process which involves the use of the centrifugal force for the sedimentation of heterogeneous mixtures with a centrifuge, used in industry and in laboratory settings.
- This process is used to separate two immiscible liquids.
- More-dense components of the mixture migrate away from the axis of the centrifuge, while less-dense components of the mixture migrate towards the axis.

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HISTORY

In 1923 Theodor Svedberg and his student H. Rinde had successfully analyzed large-grained sols in terms of their gravitational sedimentation.

Sols consist of a substance evenly distributed in another substance, also known as a colloid. However, smaller grained sols, such as those containing gold, could not be analyzed.

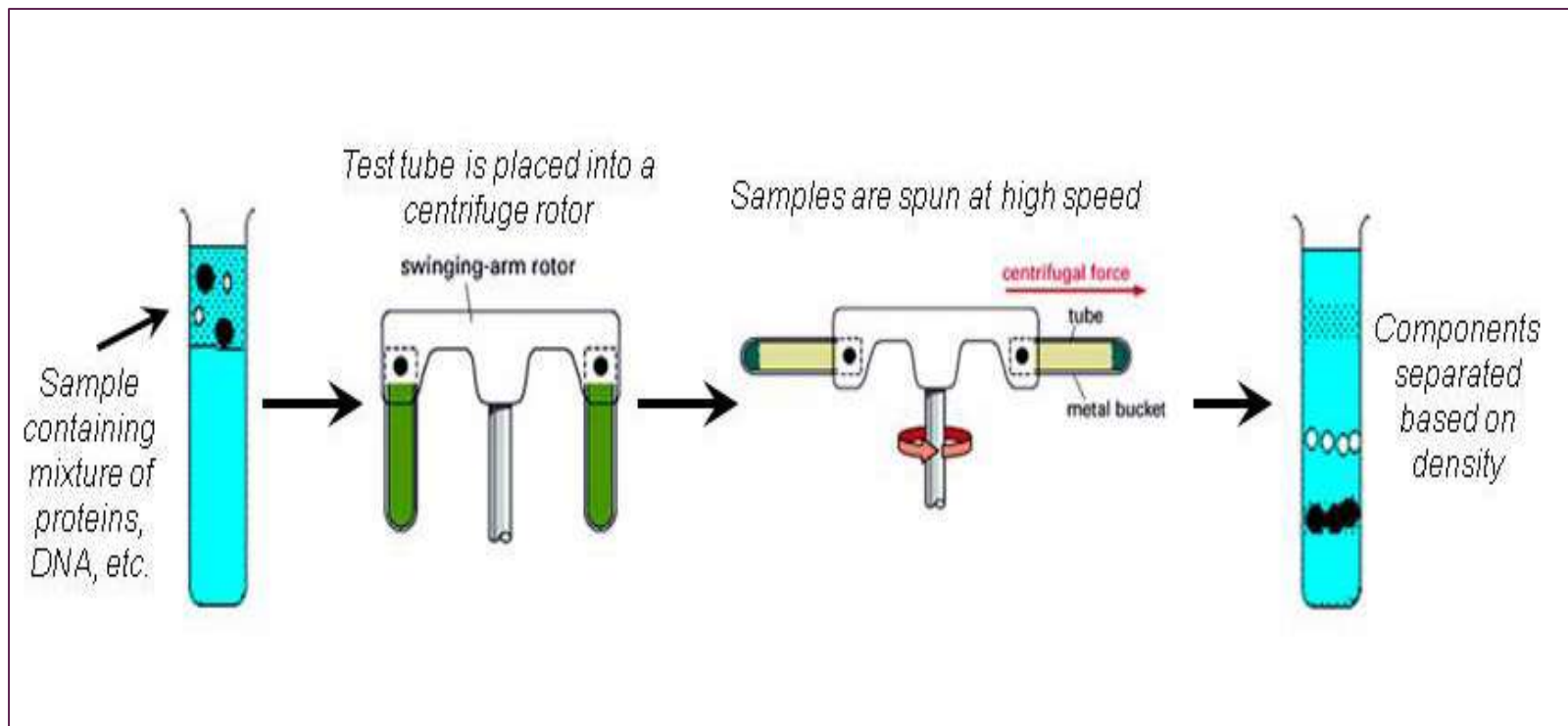
To investigate this problem Svedberg developed an analytical centrifuge, equipped with a photographic absorption system, which would exert a much greater centrifugal effect.



PRINCIPLE

- ⦿ A centrifuge is a device for separating particles from a solution according to their size, shape, density, viscosity of the medium and rotor speed.
- ⦿ In a solution, particles whose density is higher than that of the solvent **sink (sediment)**, and particles that are lighter than it **float to the top**.
- ⦿ The greater the difference in density, the faster they move. If there is no difference in density (isopyknic conditions), the particles stay steady.

- To take advantage of even tiny differences in density to separate various particles in a solution, gravity can be replaced with the much more powerful “**centrifugal force**” provided by a centrifuge.



CENTRIFUGE

- ◉ A **centrifuge** is a piece of equipment that puts an object in rotation around a fixed axis (spins it in a circle), applying a potentially strong force perpendicular to the axis of spin (outward).
- ◉ The centrifuge works using the sedimentation principle, where the centripetal acceleration causes denser substances and particles to move outward in the radial direction.
- ◉ At the same time, objects that are less dense are displaced and move to the center. In a laboratory centrifuge that uses sample tubes, the radial acceleration causes denser particles to settle to the bottom of the tube, while low-density substances rise to the top.

INSTRUMENTATION

- It consist of two components, an electric motor to spin the sample and a rotor to hold tubes.

LOW SPEED CENTRIFUGE

HIGH SPEED CENTRIFUGE

ULTRA CENTRIFUGE

LOW SPEED CENTRIFUGE

- Most laboratories have a standard low-speed centrifuge used for routine sedimentation of heavy particles.
- The low speed centrifuge has a maximum speed of 4000-5000rpm.
- These instruments usually operate at room temperatures with no means of temperature control.
- Two types of rotors are used in it, fixed angle and swinging bucket.
- It is used for sedimentation of red blood cells until the particles are tightly packed into a pellet and supernatant is separated by decantation.

**LOW SPEED
CENTRIFUGE**



HIGH SPEED CENTRIFUGE

- ⦿ High speed centrifuges are used in more sophisticated biochemical applications, higher speeds and temperature control of the rotor chamber are essential.
- ⦿ The operator of this instrument can carefully control speed and temperature which is required for sensitive biological samples.
- ⦿ Three types of rotors are available for high speed centrifugation-fixed angle, swinging bucket, vertical rotors

HIGH SPEED CENTRIFUGE

2334



ULTRA CENTRIFUGE

- It is the most sophisticated instrument
- Intense heat is generated due to high speed thus the spinning chambers must be refrigerated and kept at high vacuum.
- It is used for both preparative work and analytical work.

ULTRACENTRIFUGE



BECKMAN
COULTER

Optima™ MAX Ultracentrifuge
130,000 RPM

VWR
500 ml

CENTRIFUGE ROTOR

- A centrifuge rotor is the rotating unit of the centrifuge, which has fixed holes drilled at an angle. Test tubes are placed inside these holes and the rotor spins to aid in the separation of the materials.



TYPES OF ROTOR



swing-bucket
Rotor



fixed-angle
Rotor



vertical rotor

SWING-BUCKET ROTOR

- A swing-bucket rotor usually supports samples ranging in volume from 36 mL to 2.2 mL. Swing-buckets can support two types of separations: rate-zonal and Isopycnic.
- Swing-buckets are preferred for rate-zonal separations, because the distance between the outside of the meniscus and the outside of the bottom of the tube is long enough for separation to occur.

**swing-bucket
Rotor**



FIXED-ANGLE ROTOR

- ⦿ Fixed-angle rotors are usually used for pelleting applications to either pellet particles from a suspension and remove the excess debris, or to collect the pellet. Rotor cavities range from 0.2 mL to 1 mL.
- ⦿ The most important aspect in deciding to use a fixed-angle rotor is the K factor. The K factor indicates how efficient the rotor can pellet at maximum speed. The lower the K factor, the higher the pelleting efficiency.

Fixed-angle Rotor



VERTICAL ROTOR

- Vertical rotors are highly specialized. They are typically used to band DNA in cesium chloride. Vertical rotors have very low K factors, which is useful if the particle must only move a short distance until it pellets. Run time on vertical rotors is short.

Vertical Rotor



Types of Centrifugation Techniques

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graph TD; A[Types of Centrifugation Techniques] --> B[Density gradient centrifugation]; A --> C[Differential centrifugation]; A --> D[Ultra centrifugation];
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Density
gradient
centrifugation

Differential
centrifugation

Ultra
centrifugation

DENSITY GRADIENT CENTRIFUGATION

- It allow separation of many or all components in a mixture and allows for measurement to be made
- There are two forms of Density gradient centrifugation :

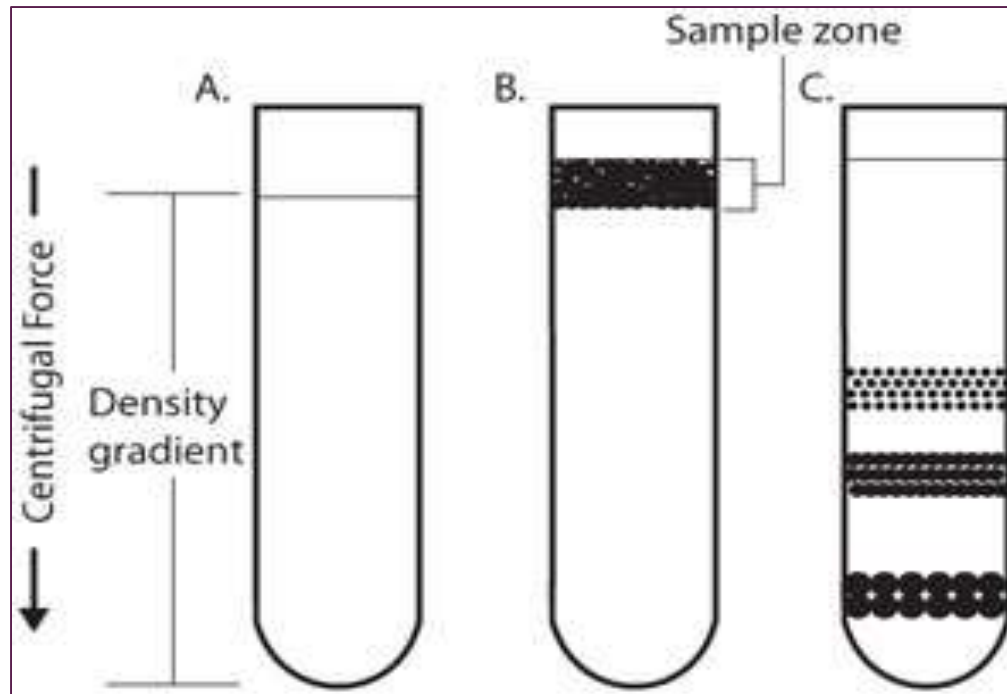
Rate zonal centrifugation

Isopycnic or sedimentation equilibrium centrifugation

I. RATE ZONAL CENTRIFUGATION

- ◉ In Rate zonal centrifugation the solution have a density gradient. The sample has a density i.e. greater than all the layers in the solution.
- ◉ The sample is applied in a thin zone at the top of the centrifuge tube on a density gradient. Under centrifugal force, the particles will begin sedimenting through the gradient.

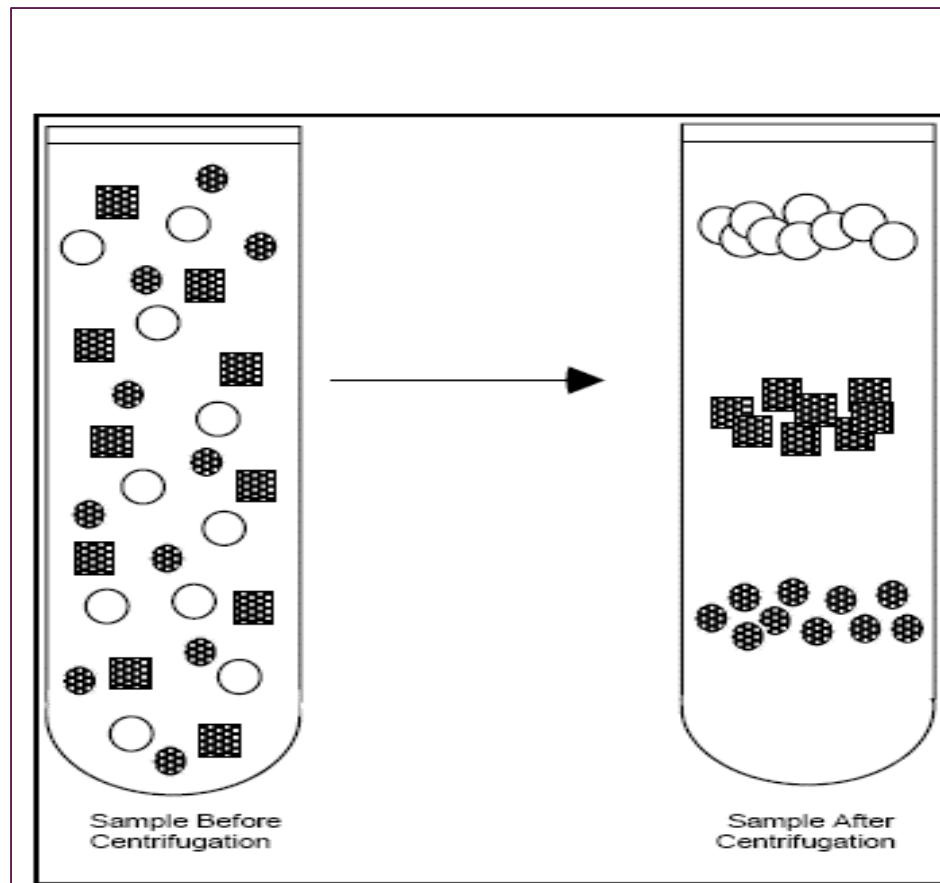
- The particles will begin sedimenting in separate zones according to their size shape and density.



II. ISOPYCNIC OR SEDIMENTATION EQUILIBRIUM CENTRIFUGATION

- In this type of centrifugation , the solution contains a greater range of densities.
- The density gradient contains the whole range of densities of the particles in the sample.
- Each particle will sediment only to the position in the centrifuge tube at which the gradient density is equal to its own density.

- In Isopycnic centrifugation separation of particles occurs into zones on the basis of their density differences, independent of time.



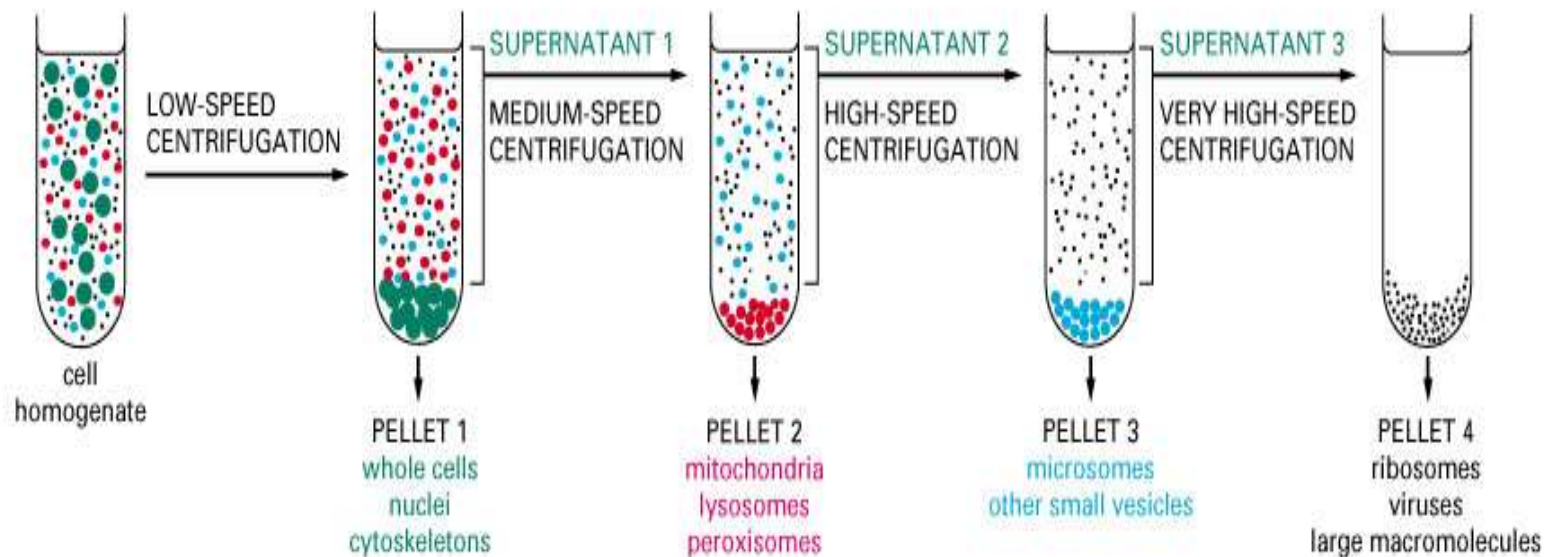
DIFFERENTIAL CENTRIFUGATION

- ⦿ Differential centrifugation is a common procedure in microbiology and cytology used to separate certain organelles from whole cells for further analysis of specific parts of cells.
- ⦿ In the process, a tissue sample is first homogenized to break the cell membranes and mix up the cell contents.
- ⦿ The homogenate is then subjected to repeated centrifugations, each time removing the pellet and increasing the centrifugal force.

DIFFERENTIAL CENTRIFUGATION

Repeated centrifugation at progressively higher speeds will fractionate cell homogenates into their components.

Centrifugation separates cell components on the basis of size and density. The larger and denser components experience the greatest centrifugal force and move most rapidly. They sediment to form a pellet at the bottom of the tube, while smaller, less dense components remain in suspension above, called the supernatant.



ULTRACENTRIFUGATION

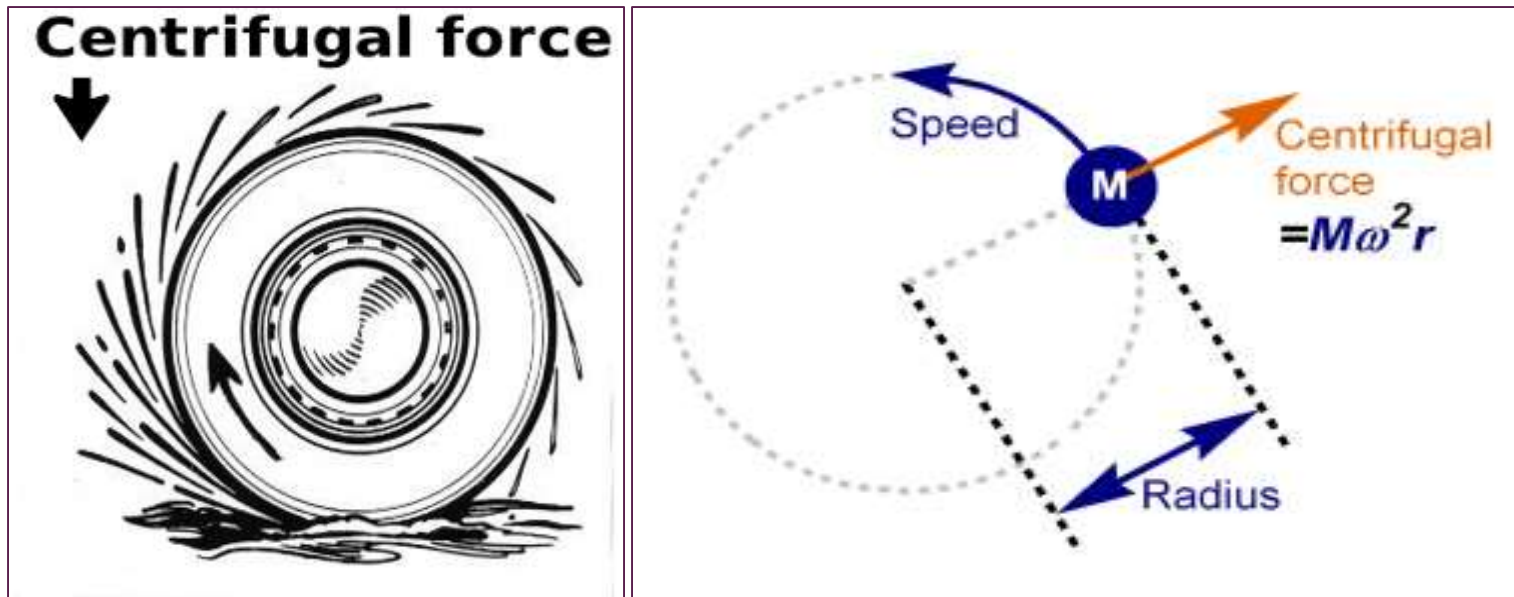
- Svedberg coined the term “ultracentrifuge”. He was colloid chemist.
- He used the ultracentrifuge to determine the MW and subunit structure of hemoglobin , studies which changed the ideas concerning the structure of proteins.
- The first commercial ultracentrifuge was produced in 1940 by SPINCO.

- An important tool in biochemical research is the centrifuge, which through rapid spinning imposes high centrifugal forces on suspended particles, or even molecules in solution, and causes separations of such matter on the basis ULTRACENTRIFUGATION of differences in weight.
- Example:

Red cells may be separated from plasma of blood, nuclei from mitochondria in cell homogenates, and one protein from another in complex mixtures

CALCULATION OF CENTRIFUGAL FORCE

- "A real or "reactive" centrifugal force occurs in reaction to a centripetal acceleration acting on a mass." So basically, it is the opposing force to Centripetal force.



CALCULATION OF ANGULAR VELOCITY

- ⦿ The **angular velocity** is defined as the rate of change of angular displacement and is a vector quantity (more precisely, apseudovector) which specifies the angular speed (rotational speed) of an object and the axis about which the object is rotating.
- ⦿ The SI unit of angular velocity is radians per second, although it may be measured in other units such as degrees per second, degrees per hour, etc. Angular velocity is usually represented by the symbol omega (ω , rarely Ω).

- The rate of change of angular displacement of the particle in a given time is called angular velocity.

- It is expressed as

$$\omega = \frac{d\theta}{dt}$$

- Where $d\theta$ is change in angular displacement, dt is change in time t .

- ◉ Angular Velocity Formula is given by

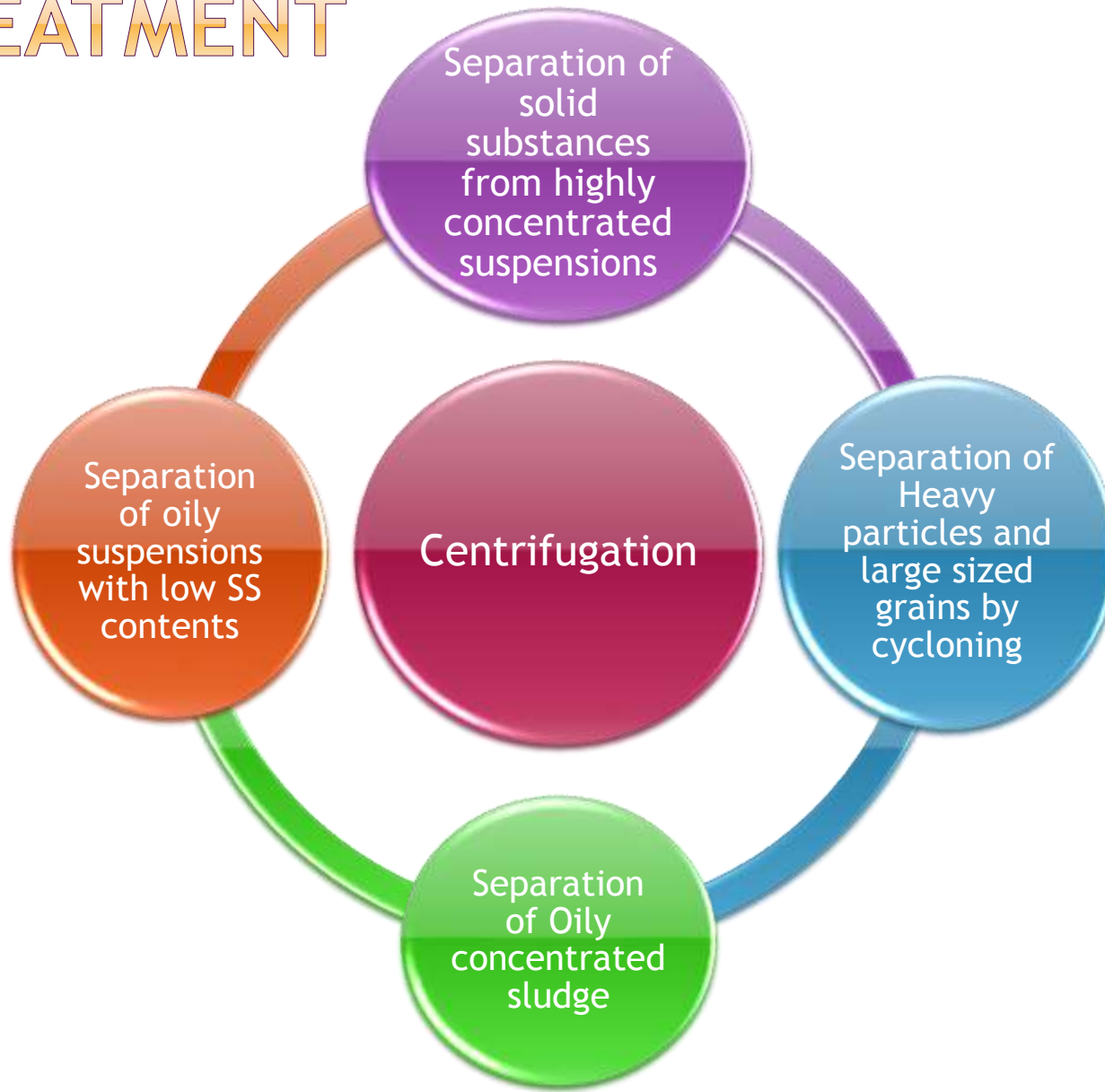
$$\omega = \frac{\theta}{t}$$

- ◉ Where θ is angular displacement and t is the time taken.
- ◉ The linear Velocity and angular velocity is given by the formula

$$\omega = \frac{v}{r}$$

- ◉ Where V is the linear velocity r is the radius of circular path.
- ◉ Angular velocity is expressed in radian per second (**rad/s**). Angular Velocity formula is used to calculate the angular velocity of any moving body.

APPLICATION IN WATER TREATMENT



OTHER APPLICATIONS

- ⦿ Separating chalk powder from water
- ⦿ Removing fat from milk to produce skimmed milk
- ⦿ Separating textiles
- ⦿ Removing water from lettuce after washing it in a salad spinner
- ⦿ Separating particles from an air-flow using cyclonic separation

- ◉ The clarification and stabilization of wine
- ◉ Separation of water particles from clothes while spin-drying in washing machines
- ◉ Separation of urine components and blood components in forensic and research laboratory



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THANK YOU!

A vibrant, 3D-style graphic of the words "THANK YOU!". The text is rendered in a bold, sans-serif font with a white fill and a thick, multi-colored gradient border. The letters are arranged in two lines: "THANK" on top and "YOU!" on the bottom. The colors of the gradient border transition through a spectrum: purple, blue, green, yellow, orange, and red. The graphic is set against a white background with a subtle radial pattern of thin, golden lines emanating from behind the text. Scattered around the text are several colorful balloons in shades of red, green, blue, and orange, along with small, five-pointed stars in yellow, red, and blue. The overall effect is celebratory and cheerful.