

PRINCIPLES AND APPLICATIONS OF CENTRIFUGATION



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UNIT

I:MICROTOMY,MICROSCOPY,CENTRI
FUGATION

1.3-PRINCIPALS AND APPLICATIONS
OF CENTRIFUGATION

CONTENT

- ▣ INTRODUCTION
- ▣ DEFINATION
- ▣ BASIC PRINCIPLES OF CENTRIFUGATION
- ▣ INSTRUMENTATION:
 - LOW SPEED CENTRIFUGATION
 - HIGH SPEED CENTRIFUGATION
 - ULTRA CENTRIFUGE
- ▣ APPLICATIONS OF CENTRIFUGATION
- ▣ CARE OF CENTRIFUGES AND ROTORS
- ▣ CONCLUSION
- ▣ REFERENCES

INTRODUCTION

- ▣ WHAT IS CENTRIFUGE?

- ▣ WHAT IS CENTRIFUGATION?

DEFINATION

- ▣ “An apparatus that rotates at high speed and by centrifugal force separates substances of different densities.”

BASIC PRINCIPLES OF CENTRIFUGATION

- ▣ A particle, whether it is precipitate, a macromolecule or cell organelle when rotated at high speed is subjected to a centrifugal force.
- ▣ Centrifugal force is defined as
- ▣ $F = mw^2r$
- ▣ Where F = intensity of centrifugal force
- ▣ m = effective mass of sedimenting particle
- ▣ w = angular velocity of rotation
- ▣ r = distance of migrating particles from central axis of rotation

□ A more common measurement of F, in terms of Gravitational force g, is Relative Centrifugal Force RCF, Is given as

$$\text{RCF} = (\text{rpm})^2(r)$$

Thus this equation indicates that RCF varies with r, (the distance of the sedimenting particles from axis of rotation). Thus it gives idea of only basic principle, it does not take into account other factors i.e. mass, shape, density of medium.

□ Thus centrifugal force felt by particle is defined as

$$m = m_0 - m_0 \bar{v}^2 / p$$

INSTRUMENTATION FOR CENTRIFUGATION

- It consist of two components,an electric motor to spin the sample and a rotor to hold tubes.
- Here we describe 2 types,the low speed,the high speed.



- LOW SPEED CENTRIFUGE

- HIGH SPEED CENTRIFUGE

- ULTRA CENTRIFUGE

LOW SPEED CENTRIFUGES

- ▣ LOW SPEED CENTRIFUGE
- ▣ 1) Most laboratories have a standard low-speed centrifuge used for routine sedimentation of heavy particles
- ▣ 2) The low speed centrifuge has a maximum speed of 4000-5000rpm
- ▣ 3) these instruments usually operate at room temperatures with no means of temperature control.
- ▣ 4) two types of rotors are used in it, Fixed angle and swinging bucket.
- ▣ 5) it is used for sedimentation of red blood cells until the particles are tightly packed into a pellet and supernatant is separated by decantation.

TD4A-WS/TD4Z-WS Table-top Low-speed Centrifuge



HIGH SPEED CENTRIFUGATION

▣ HIGH SPEED CENTRIFUGES

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



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.

APPLICATIONS OF CENTRIFUGES

- ▣ There are two techniques on which the application of centrifuge is based on
- ▣ 1) **PREPARATIVE TECHNIQUE**

VELOCITY SEDIMENTATION CENTRIFUGATION

PREPARATIVE DIFFERENTIAL CENTRIFUGATION

ANALYTICAL TECHNIQUES

- ▣ All analytical techniques require use of an ultra centrifuge and can be classified as
 - ▣ 1) DIFFERENTIAL CENTRIFUGATION
 - ▣ 2) DENSITY GRADIENT CENTRIFUGATION
 - ▣ A) ZONAL CENTRIFUGATION
 - ▣ B) ISOPYCNIC CENTRIFUGATION

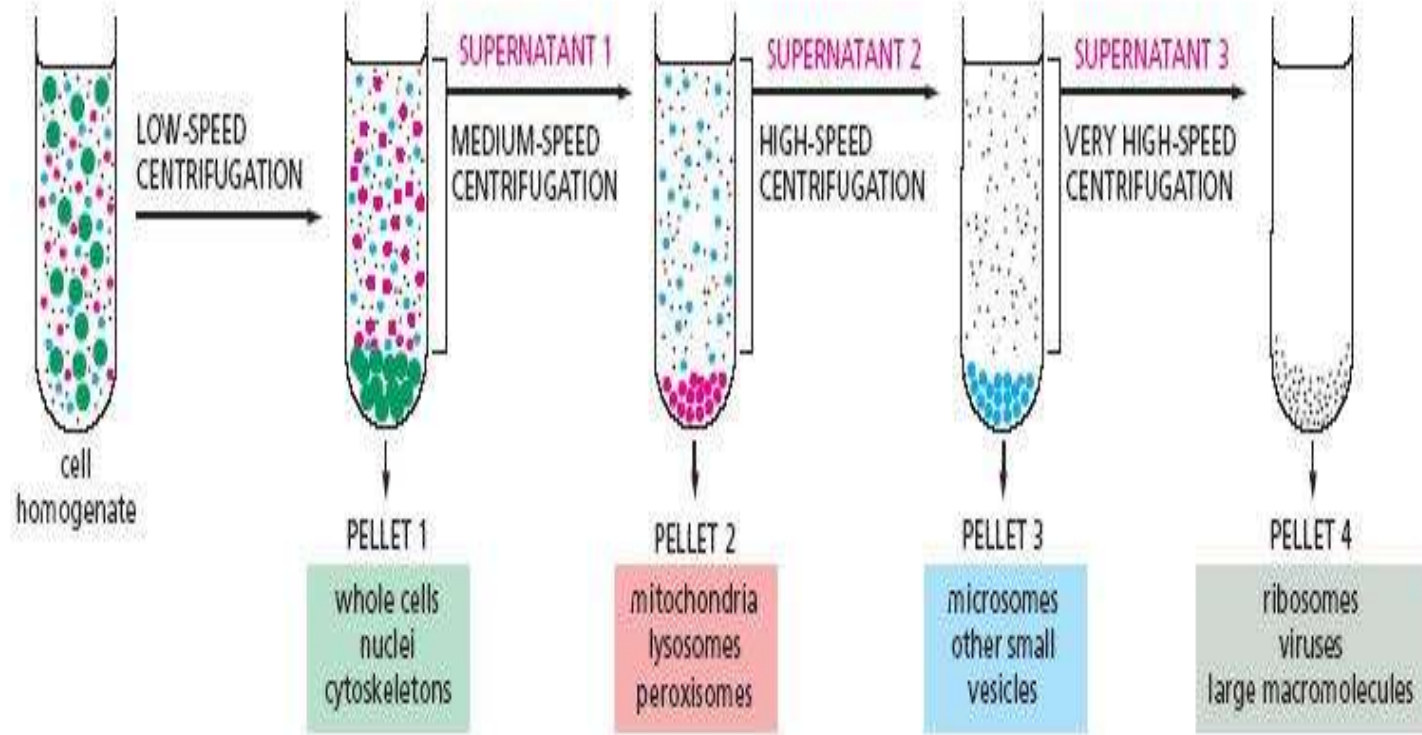
DIFFERENTIAL CENTRIFUGATION

- ▣ 1) DIFFERENTIAL CENTRIFUGATION
- ▣ a) Differential centrifugation is a technique commonly used by biochemists.
- ▣ b) tissue such as liver is homogenised at 32 degree in a sucrose solution that contains buffer
- ▣ c) the homogenate is then placed in a centrifuge and spun at constant centrifugal force at constant temperature.
- ▣ d) after sometime a sediment forms at the bottom of centrifuge called pellet and overlying solution called supernatant.
- ▣ e) the overlying solution is then placed in another centrifuge tube which is then rotated at higher speeds.

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, a portion called the supernatant.



DENSITY GRADIENT CENTRIFUGATION

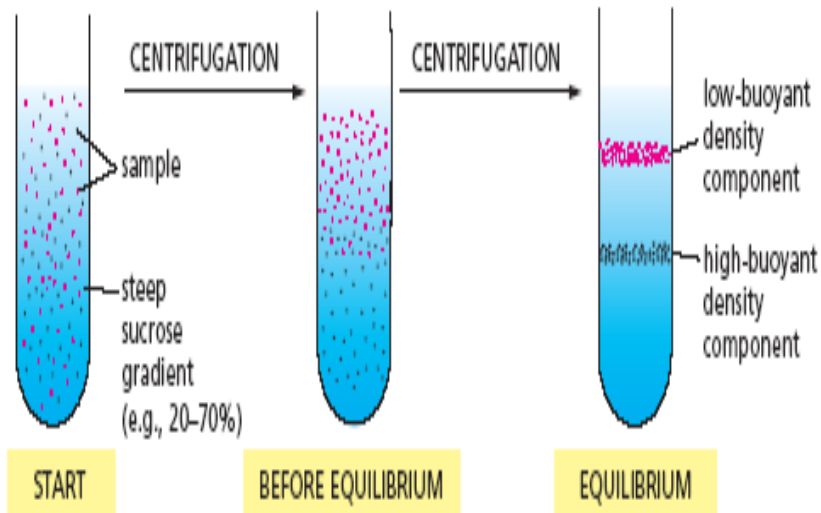
- ▣ DENSITY GRADIENT CENTRIFUGATION
- ▣ 1) This type of centrifugation is mainly used to purify viruses, ribosomes, membranes etc.
- ▣ b) A sucrose density gradient is created by gently overlaying lower concentrations of sucrose on higher concentrations in centrifuge tubes
- ▣ c) the particles of interest are placed on top of the gradient and centrifuge in ultra centrifuges.
- ▣ d) the particles travel through the gradient until they reach a point at which their density matches with the density of surrounding sucrose., the the fraction is removed and analyzed.
- ▣

EQUILIBRIUM SEDIMENTATION

The ultracentrifuge can also be used to separate cell components on the basis of their **buoyant density**, independently of their size or shape. The sample is usually either layered on top of, or dispersed within, a steep density gradient that contains a very high concentration of sucrose or cesium chloride. Each subcellular component will move up or down when centrifuged until it reaches a position where its density matches its surroundings and then will move no further.

A series of distinct bands will eventually be produced, with those nearest the bottom of the tube containing the components of highest buoyant density. The method is also called **density gradient centrifugation**.

The sample is distributed throughout the sucrose density gradient.



At equilibrium, components have migrated to a region in the gradient that matches their own density.

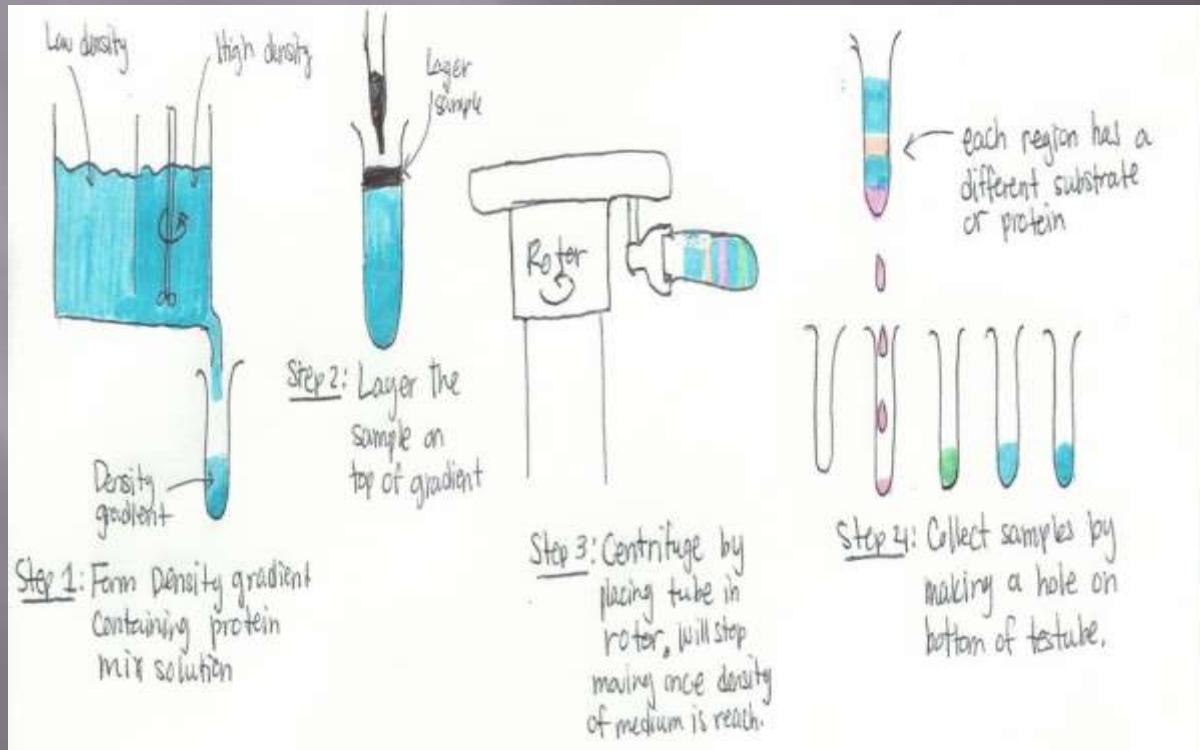
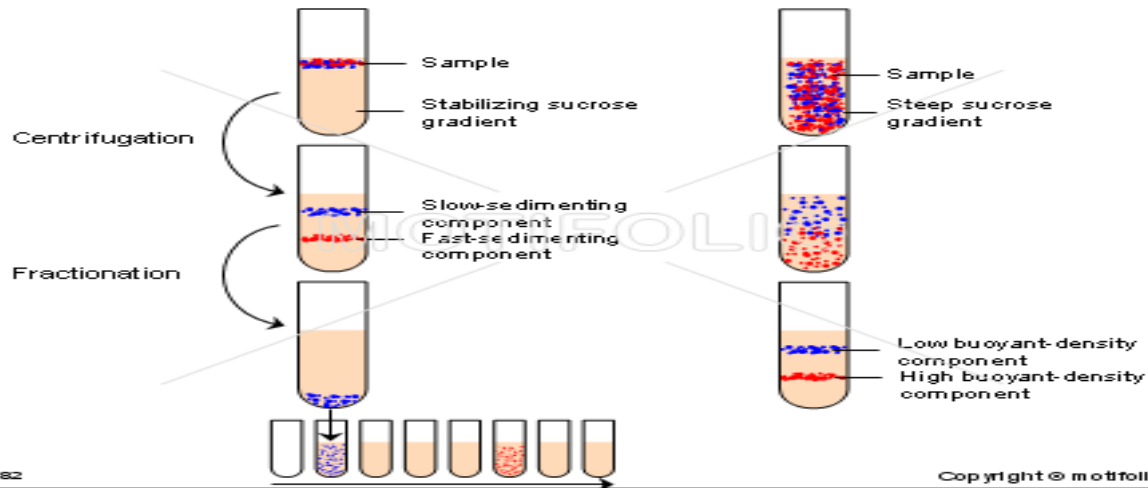
A sucrose gradient is shown here, but denser gradients can be formed with cesium chloride that are particularly useful for separating the nucleic acids (DNA and RNA).

The final bands can be collected from the base of the tube, as shown above.

ZONAL CENTRIFUGATION

- ▣ ZONAL CENTRIFUGATION
- ▣ a) zonal centrifugation is also known as band or gradient centrifugation
- ▣ b) it relies on the concept of sedimentation coefficient (ie movement of sediment through liquid medium)
- ▣ c) in this technique a density gradient is created in a test tube with sucrose and high density at the bottom.
- ▣ d) the sample of protein is placed on the top of the gradient and then centrifuged.
- ▣ e) the proteins sediment according to their sedimentation coefficient and the fractions are collected by creating a hole at the bottom of tube.

Rate-zonal centrifugation versus equilibrium density gradient centrifugation

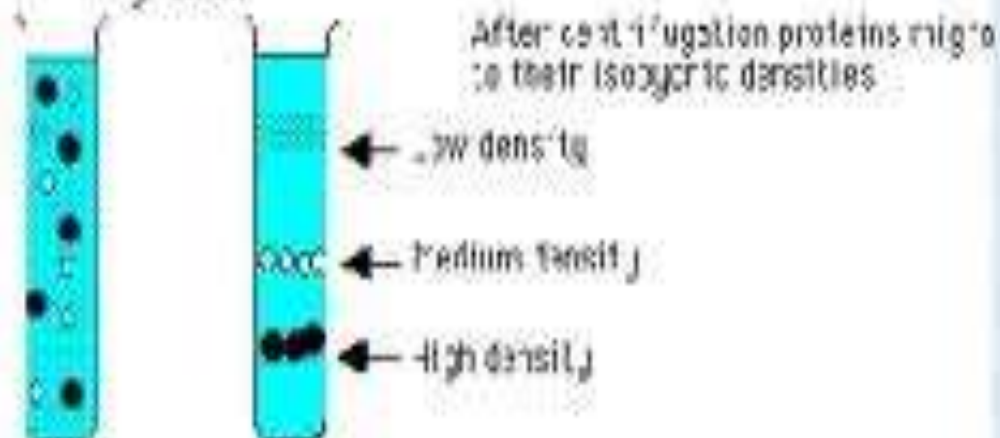


ISOPYCNIC CENTRIFUGATION

- ▣ ISOPYCNIC CENTRIFUGATION
- ▣ a) It is also called as density gradient centrifugation.
- ▣ b) the solution of biological sample and cesium salt is uniformly distributed in a centrifuge tube and rotated in an ultra centrifuge.
- ▣ c) under the influence of centrifugal force the cesium salts redistribute to form a density gradient from top to bottom.
- ▣ d) the sample molecules move to the region where their density equals to the density of gradient.

ISOPYCNIC-ZONAL CENTRIFUGATION

Figure 4 Isopycnic separation with a self-generating gradient



The sample is evenly distributed throughout the centrifuge tube before centrifugation.

CARE OF CENTRIFUGE AND ROTORS

- ▣ 1) Carefully read the manual before using centrifuges
- ▣
- ▣ 2) Select proper operating conditions
- ▣
- ▣ 3) Check rotor for cleanliness and for damage
- ▣
- ▣ 4) Select proper rotor of definite size
- ▣
- ▣ 5) Be sure the rotor is clean and undamaged
- ▣
- ▣ 6) keep accurate record of centrifuge and rotors
- ▣
- ▣ 7) carefully clean rotors after centrifugation.

SIGNIFICANCE

- ▣ 1) THE CENTRIFUGATION IS A MODERN AND EASY TECHNIQUE OF SEPERATION
- ▣ 2) DUE TO CENTRIFUGATION IT IS EASY TO SEPARATE CELLULAR AND SUB CELLUAR COMPONENTS
- ▣ 3) IT IS USED TO STUDY THE EFFECTS OF CENTRIFUGAL FORCES ON CELLS

REFERENCE

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RODNEY BOYER
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WALKER& WILSON

THANK YOU

THE
END