RESEARCH CENTRIFUGE- ADVANCED TOOL SEPERATION

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Abstract-

A centrifuge is a critical piece of equipment for the laboratory. Purpose of this study was to study research centrifuge in detail, its applications, uses in different branches and silent features. Their are two types of research centrifuge study here revolutionary research centrifuge and microprocessor research centrifuge. A centrifuge is a device that separates particles from a solution through use of a rotor. In biology, the particles are usually cells, sub cellular organelles, or large molecules, all of which are referred to here as particles. There are two types of centrifuge procedures; one is preparative, the purpose of which is to isolate specific particles, and the other is analytical, which involves measuring physical properties of the sedimenting particles. The revolutionary research centrifuge having silent features more than other centrifuge.

Key words- research centrifuge, silent feature, applications in different branches.

Introduction

Centrifugation is a process that 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. Chemists and biologists may increase the effective gravitational force on a test tube so as to more rapidly and completely cause the precipitate ("pellet") to gather on the bottom of the tube. The remaining solution is properly called the "supernate" or "supernatant liquid". The supernatant liquid is then either quickly decanted from the tube without disturbing the precipitate, or withdrawn with a Pasteur pipette The rate of centrifugation is specified by the angular velocity measured in revolutions per minute (RPM), or acceleration expressed as *g*. The conversion factor between RPM and*g* depends on the radius of the sample in the centrifuge rotor. The particles' settling velocity in centrifugation is a function of their size and shape, centrifugal acceleration, the volume fraction of solids present, the density difference between the particle and the liquid, and the viscosity. The most common application is the separation of solid from highly concentrated suspensions, which is use in the treatment of sewage sludges for dewatering where less consistent sediment is produced

Separation of particles by sedimentation is one of the most powerful tools in biology. Even though sedimentation using centrifugation is not a new technology, it is essential for cutting edge genomic and proteomic research by providing purified particles of interest. In a survey at the US National Institutes of Health, over 65% of research workers replied that using centrifugation to purify cells, subcellular organelles, viruses, proteins, and nucleic acids is an integral part of their work. Density gradient centrifugation is a technique that allows the separation of particles on the basis of their size, shape, and density. A density gradient is typically created by layering media of increasing density in a centrifuge tube. When a sample is layered on top of a density gradient and centrifuged, the various particles move through the gradient at different rates. The particles appear as bands or zones in the gradient with the more dense and larger particles migrating furthest.

This sedimentation of particles, including cells, can be explained by the Stokes equation, which describes the movement of a sphere in a gravitational field.1 The equation calculates the velocity of sedimentation utilizing five parameters

$$v = \frac{d2(p - L)3g}{18n}$$

v = sedimentation rate or velocity of the sphere

d = diameter of the sphere

p = particle density

L = medium density

n = viscosity of medium

g = gravitational force

Microprocessor Research Centrifuges

Compufuges model PR-23 & PR-24 are Microprocessor controlled centrifuges used in auxiliary laboratories in industry, Educational & Research institutions, Bio Technology, Medical Laboratories , Hospitals , Blood Banks , Pharmaceutical laboratories and agriculture soil testing for determination of moisture equivalent of soil. It is also suitable for determination of settlement of paints, pastes, cosmetics and food products. Centrifuge rotors & carriers are also suitable for use with a variety of Falcon & Vacutainer tubes .

PR-23 & PR-24 have soft touch keypads for fast and accurate setting of run parameters. Basic runs are easy with setting of speed, time, temperature, brake, acceleration and deceleration. Press 'start' key and Compufuge does the rest for you.

Brushless induction motor with frequency drive & unique automatic rotor identification ensures accurate speed setting for individual rotors. Over current overheating safety cutoff circuit is provided for motor protection. Safety lid interlock on the centrifuge ensures that the rotor does not run with cover open while the cover cannot be opened unless rotor comes to complete halt.

Salient Features:

• User friendly microprocessor control with Interactive LCD display

• Menu driven ten programme memory

• Choice of High brake, low brake and coasting

• Imbalance high detector with cutoff

• Safety lid interlock to prevent cover openingduring centrifugation

• Automatic unique safety rotor identification system

• Speed holding accuracy 100 RPM

• Self-diagnosis for errors

Revolutionary Research Centrifuges

R-23 & R 24 centrifuges are widely used in auxiliary laboratories in industry, Educational & Research institutions, Bio Technology, Medical Laboratories, Hospitals, Blood Banks, Pharmaceutical laboratories and agriculture soil testing for determination of moisture equivalent of soil. It is also suitable for determination of settlement of paints, pastes, cosmetics and food products. Centrifuge rotors & carriers are also suitable for use with Variety of Falcon & Vacutainer Tubes.

Salient Features:

• Stepless speed regulator with zero start interlock

• Digital speed indicator

• Dynamic brake

• 0-99 minutes digital count down timer

• Imbalance detector with cutoff

• Safety lid interlock to prevent cover opening during centrifugation

Applications of research centrifuge in seperations -

1. Pelleting Bacteria Using Large Volume Carbon Fiber Rotors

Bacterial cells grown in tissue culture media for extracting nucleic acids is frequently harvested with conventional superspeed centrifuges using metal fixed angle rotors, which carry bottles with volumes up to 500 mL. These rotors weigh approximately 40-49 pounds (19-22 kg). The total run time to pellet the bacteria and solids in these heavy metal rotors can be up to 20 minutes per run.

2. Separation of Whole Cells

Cell separation and cell harvesting methods have been reported for a variety of cell types: blood, cultured tumor cells, bacteria, algae, yeasts and cells of tissues such as brain, lymph node and spleen. The density of most mammalian cells falls between 1.06 and

1.12 g/mL. Some cells with large amounts of cytoplasm may have densities as high as 1.29 g/mL. Tabletop centrifuges (general purpose and microcentrifuges) are used for differential centrifugation (pelleting) of small amounts of these cells.

3. Isolation of Lipoproteins

Lipoprotein classification is important to human cholesterol studies. The various classes of serum and plasma lipoproteins differ from one another in both densities and sedimentation; differential flotation is the most commonly used method to separate them. Usually the starting solution is first centrifuged at approximately 15,000 x g for 30 min at the normal serum density to allow large chylomicrons to float to the top. A subsequent

step will allow for the separation of very low-density lipoprotein (VLDL) which is removed at the end of the centrifugation. The VLDL cholesterol is then quantified meanwhile the low-density lipoproteins (LDL) and the high-density lipoproteins (HDL) remains in solution in the tube. The remaining solution in the tube is adjusted with salts (sodium chloride, sodium bromide or potassium bromide) to the density needed to float the next classes of lipoprotein. Using the Fiberlite F50L-24x1.5 rotor in an ultracentrifuge (i.e. Sorvall WX Series), the following protocol can be used for the isolation of VLDL protein

4. Rapid Isolation of Synaptosomes from Rat Brain Tissue

When brain or other nerve rich tissue is homogenized, the disrupted nerve endings form vesicles called synaptosomes. These synaptosomes consist of the plasma membranes and

the organelles of the nerve endings (i.e. mitochondria and synaptic vesicles). The synaptic vesicles are small secretory vesicles, which store the various neurotransmitters. Isolation

of synaptosomes, synaptic vesicles and synaptic membranes, are usually separated with Ficoll, Percoll, Metrizamide or Nycodenz gradients because of the osmotic shock of the

synaptosomes by sucrose gradients.

5. Nucleic Acid DNA

DNA is commonly extracted by homogenizing tissues, cells and subcellular fractions, bacteria or viruses by treatment with detergent such as Triton X-100 or sodium dodecyl sulfate (SDS) with or without lysozyme. In some cases less gentle methods cells disruption such as boiling or alkali treatment may be suitable for the isolation of smaller plasmids, as

reported by Maniatis et al.. (1982) The material may then be extracted with phenol or chloroform, and the aqueous phase containing the DNA collected after low speed centrifugation. Any detergent used in the isolation procedure can be removed by dialysis. The DNA in the solution may then be precipitated with ethanol and the precipitate collected as sediment using a tabletop micro centrifuge 20 at 13,000 x g.

6. Separation of Sarcoplasmic Reticulum

Previous studies by Kai, Y. et al., reported that glycolytic enzymes were associated with sarcoplasmic reticulum membrane vesicles and metabolism through these enzymes was capable of supporting 45Ca transport (Kai et al, 1995). Sealed right-side-out SR vesicles were isolated by step sucrose gradient using a swinging bucket rotor to purify the SR vesicles from rabbit skeletal and cardiac muscle. Intravesicular 45Ca transport was measured after the addition of glycolytic substrates and cofactors specific for each of the glycolytic reactions were studied or after the addition of exogenous ATP and was expressed as transport sensitive to the specific Ca2+- ATPase inhibitor thapsigargin. The authors found that the entire chain of glycolytic enzymes from aldolase, including aldolase, GAPDH,

phosphoglycerate kinase (PGK), phosphorglyceromutase, enolase, and pyruvate kinase (PK), was associated with SR vesicles from both cardiac and skeletal muscle. In the centrifugation method for purifying the SR membranes, the Fiberlite carbon fiber fixed angle F50L-8x39 can be used with a differential centrifugation method to collect the supernatant containing the SR vesicles and purify the SR membrane vesicles by density gradient centrifugation. The total run time for the SR preparation and purification is less than 3.5 hours when the F50L – 8 x 39 rotor was used with a Thermo Scientific Sorvall Ultracentrifuge. A total of 18 hours with a swinging bucket rotor with similar tube volumes were needed to accomplish the same purification of the SR membranes

7. Nucleic Acid Isolation in Fixed Angle, Vertical and Near Vertical Tube Rotors and RNA Sample Contamination

DNA is commonly extracted by homogenizing tissues, cells, subcellular fractions, bacteria or viruses by treatment with detergent such as Triton® X-100 or sodium dodecyl sulfate (SDS) with or without lysozyme. In some cases less gentle methods, such as boiling or alkali treatment may be suitable for cell disruption for the isolation of small plasmids (Maniatis et al, 1982). The material may then be extracted with phenol or chloroform. The aqueous phase contains the DNA after low speedcentrifugation. Any detergent used in the isolation procedure can be removed by dialysis. The DNA in the solution may then be precipitated with ethanol and the precipitate collected as sediment using a tabletop microcentrifuge at 13,000 x g. Maniatis et al. (1982) reported that DNA could be isolated via CsCl/EtBr isopycnic gradients using an ultracentrifuge at a force field of 200,000-300,000 x g for 16 hours (overnight). Much of the preliminary sample preparation can be eliminated when this method is used. The starting tissue is ground in SDS solution and solid CsCl is added releasing the DNA. When this mixture is centrifuged using a fixed angle rotor at high speed, the proteins form a precipitate at the meniscus, and the RNA (because it is denser than the CsCl) pellets to the bottom of the tube. The isolated DNA is banded as a zone in the middle of the tube and can be collected by puncturing the tube with a hypodermic needle and syringe at the area where the DNA zone is visible. Studies have shown that when plasmid DNA with high RNA concentration is harvested from bacterial cell lysates the RNA from the sample

sediments on the tube wall when vertical and near vertical tube rotors are used. At the end of such a run this RNA falls from the tube wall and re-contaminates the purified DNA zones.

Conclusion-

The different research centrifuge was studied, and it was concluded that the revolutionary centrifuge having silent features more than other centrifuge. This was add new applications in separation and it gave accurate results.

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