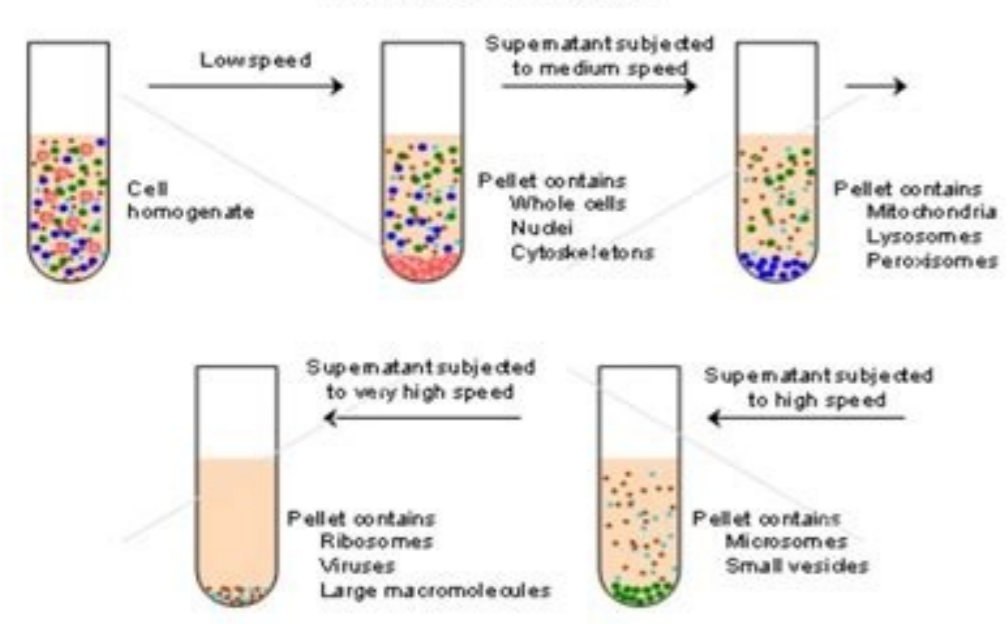


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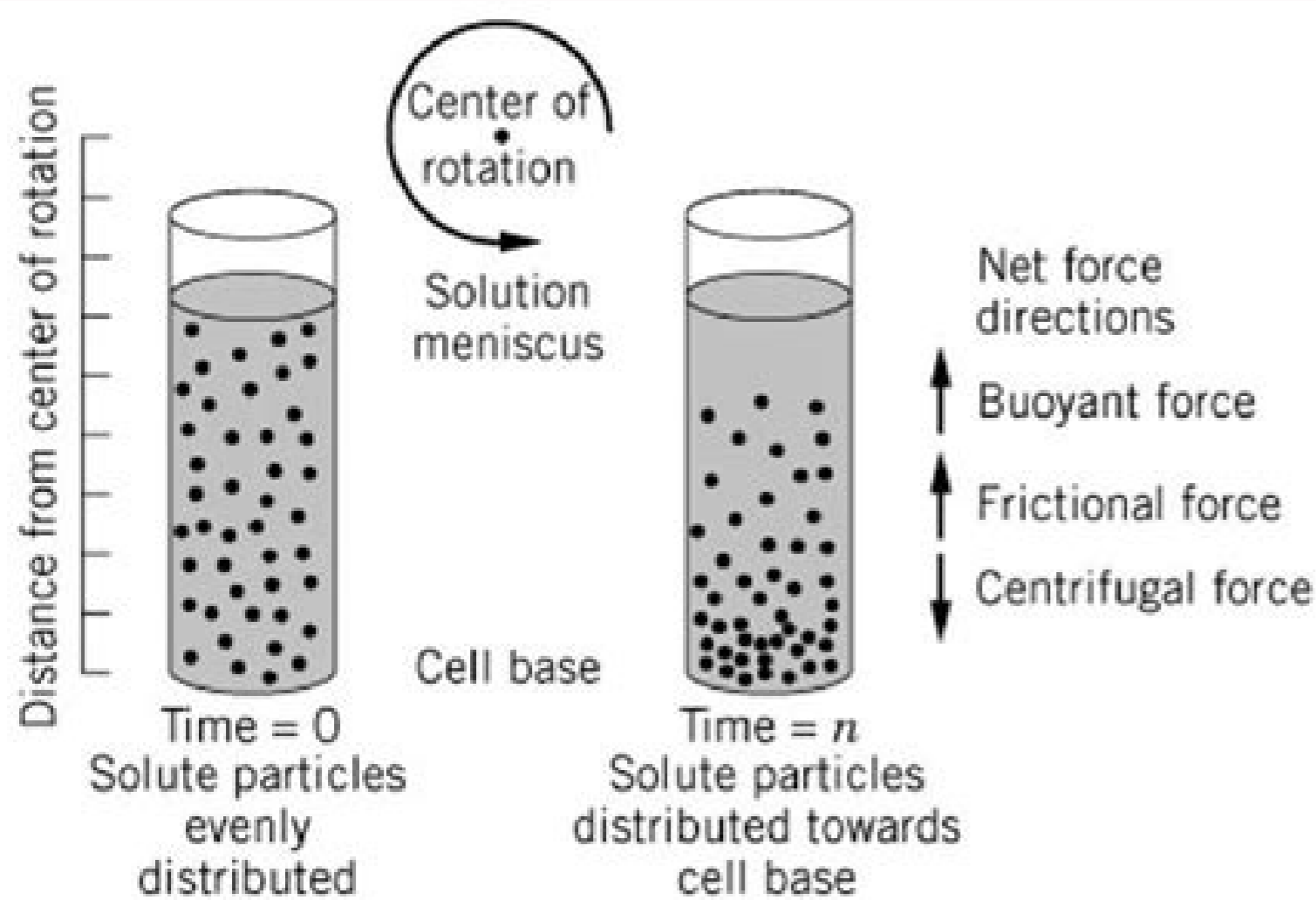
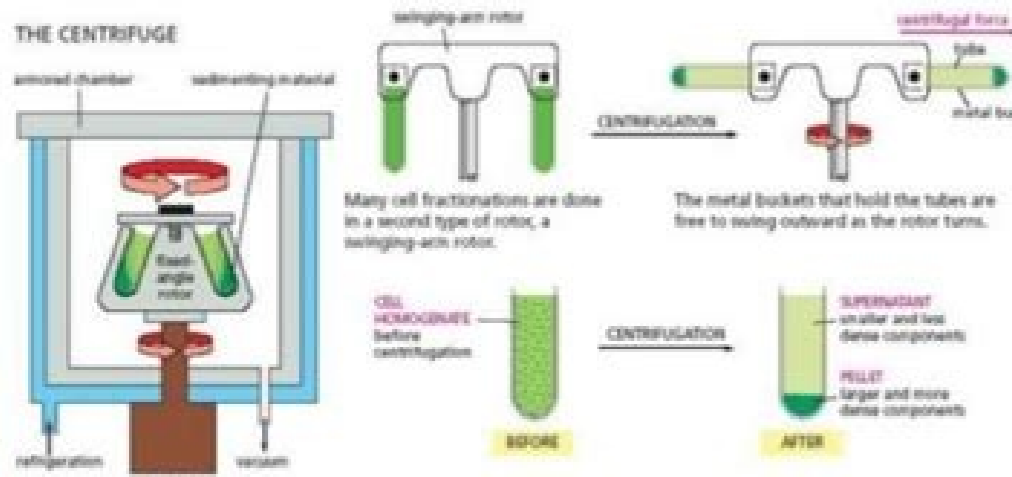
DIFFERENTIAL CENTRIFUGATION

Differential centrifugation



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
- Undergoes tissue disruption and cell lysis.
- Finally centrifugation



Principle of centrifugation

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_c is denoted by equation

$$F_c = m\omega^2 r$$

Where

- F_c = intensity of the centrifugal force
- m = effective mass of the sedimenting particle
- ω = angular velocity of rotation
- r = distance of the migrating particles from the central axis of rotation

A more common measurement of F_c in terms of the earth's gravitation force, g , is rotor is the rotating portion of the centrifuge that is rotated by the motor. So, you can say that the number of complete rotations that a rotor makes in a minute is known as RPM. The RPM of the centrifuge can range from around 300 – 1,50,000. So, based on the necessity (application), various centrifuge machines have been designed. What is RCF in Centrifuge? RCF stands for Relative Centrifugal Force. It is also known as the g-force. As the name suggests, it is a type of gravitational force, that is created when the rotor is spinning around. This force always acts away from the center. Relationship between RPM and RCF The relationship between RPM and RCF can be stated by the given formula, $RCF = 11.2 \times \text{Radius} \times (\text{RPM}/1000)^2$ where Radius is given in cm. The radius refers to the distance from the center of rotation to the sample. So, it includes the radius of the rotor and the distance to the midpoint of the test tube, bucket, etc. for holding the sample. What is the difference between RPM and RCF? As stated before, RPM denotes the speed of the rotor which is spinning around. It does not give us an idea of the g's force being exerted into the sample. The RCF (g force) shows the gravitational force being exerted into the sample for sedimentation. The value of RCF is more important than that of RPM. For two different sizes of rotors, the RPM can be constant but RCF will vary. Hence, even by keeping the RPM fixed, the result obtained by centrifuging the samples will differ. So, instead of setting the RPM in the machine, you need to set RCF. In such a case, you will obtain better results from the centrifuge. Some of the centrifuges will only have the RPM function. However, in advanced machines, there can be the option for setting both the RPM and the RCF. Based on the budget and your requirement choose a proper centrifuge machine. What are the types of centrifuge? There are various kinds of centrifuges available in the market. They can be differentiated into a number of types based on a number of factors. Some of them are as follows. 1. On the basis of motor The centrifuge can be differentiated into two types on the basis of the motor. The motor can operate by making use of the carbon brush. Besides that, it may operate without the application of the carbon brush. The centrifuge machines which have got the carbon brush in them are called a brushed centrifuge. While the other forms of centrifuges are called brushless centrifuge. The brushless centrifuges are cheap and require periodic maintenance. On the other hand, the brushless centrifuge may be expensive but are almost maintenance-free. 2. On the basis of size On the basis of the size, the centrifuge can be differentiated into two types. They are the benchtop centrifuge and the floor centrifuge. The benchtop centrifuges are smaller in size. They are adjustable on the bench. Since the size of the machine is not big, it can only hold a small volume of samples. They are simple and cheaper. The floor centrifuges are bigger in size. So, they are fixed on the floor. Since the size of the machine is bigger in comparison to the benchtop model, it can hold a larger volume of samples. The sample can be stored in the larger test tube or the buckets. The floor centrifuge can be further classified into two types. One of them is the refrigerated centrifuge, while the other one is the non-refrigerated centrifuge. The refrigerated centrifuge consists of a compressor. Thus, it provides cooling action. The temperature can go up to -20°C to + 40°C. Thus, even heating is possible in these kinds of centrifuges. 3. On the basis of control On the basis of the control system, there are two types of centrifuges. One is the analog centrifuge such as the doctor centrifuge. The other one is the digital centrifuge. Except for the doctor centrifuge, almost every other kind is digital in nature. 4. On the basis of the application Based on the purpose of the machine, they can be given different names. For different purposes, the machine can have different sizes, RPM/RCF range, etc. Some of them are the doctor centrifuge, laboratory centrifuge, mini centrifuge, microcentrifuge, ultracentrifuge, blood bank centrifuge, research centrifuge, cyto-centrifuge, etc. Where do we use centrifuge? (What are the uses of centrifuge?) The centrifuge machine can be used in different sectors. Some of them are as follows. In hospitals and clinics, it is used for blood separation. Blood separation refers to the separation of the blood cells (RBC, WBC, platelets) from the protein (serum). After the blood separation, the serum can be used in applications such as biochemistry. In blood bank, it is used for blood separation. Unlike in hospitals, the blood cells such as RBC, WBC, and platelets are also separated in the blood bank by the process of differential centrifugation. Useful for the analysis of DNA, RNA, viruses, antibodies, proteins, etc. Useful in the chemistry and the biology labs of the educational institutes. For separating the isotopes for the programming of the nuclear weapons and nuclear power program. Useful in pharmaceutical companies. In industries for separating the particles such as glass, silicon, graphite, etc from the water. It is also used for wastewater management. In astronomy. Separation of fat from the milk in dairy farm. Household applications such as washing machines. Types of Centrifuge Rotors The centrifuge consists of a motor. The motor is connected to the rotor head. The rotor head is responsible for the holding of the sample solution. So, as the rotor rotates, the sample will also rotate along with it. 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Similarly, the fluid medium (known as the supernatant), settles at the upper surface of the solution. The path traveled by the particles to the side of the walls will be short. Hence, it reduces the time for centrifugation. Since the fixed angle rotor does not swing, it does not experience high metal stress. Thus, it can withstand higher g force. In other words, it can spin at a higher RPM. Generally, it provides centrifugation of more no. of samples with a smaller volume. In comparison to the swing bucket rotor, it provides low sample separation. A fixed angle rotor can be useful in the separation of macromolecules such as DNA, RNA, protein, etc. 2. Swing Bucket Rotor Swing Bucket Rotor of Centrifuge The swing bucket rotor is a type of rotor that swings during the time of centrifugation. Initially, the swing bucket rotor aligns itself to the position of the angle rotor. However, during the time of centrifugation, the rotor raises itself perpendicular to the axis of rotation. 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Thus, it can not be operated at higher g-force or RPM. It holds less no. of samples with a larger volume. As the RPM of the rotor decreases, the rotor will begin to return back to the original stage. During this movement, the pellets formed may be disrupted. A swing bucket rotor is useful in differential centrifugation in blood banks. Differences between swing bucket rotor and fixed angle rotor Swing Bucket Rotor Operates at lower RPM (RCF). Operates at higher RPM (RCF). If all the parameters are constant, the time for centrifugation is longer. If all the parameters are constant, the time for centrifugation is shorter. Able to centrifuge less no. of samples with a larger volume. Able to centrifuge more no. of samples with lower volume. The pellet accumulates at the center of the base of the container. The pellet accumulates at the sidewalls and the corner of the base of the container. Provides better sample separation. The sample separation is not as good as that of the swing bucket rotor. There are many other types of rotors available for different purposes. Some of them are the Vertical Rotor, Zonal Rotor, Elutriator Rotor, etc. However, in the case of the health sector, those rotors are not much used.



Purpose of Centrifugation

- Since ours is a fermentation based bio process, the ISOLATION and the purification of the Insulin is the immediate goal once the fermentation step is concluded.
- In Insulin production, used to separate desired proteins from unwanted biomass.

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Sedimentation is the process of settlement of the suspended particles of the fluid to the base. The heavier particles have a higher rate of sedimentation than the lighter particles. So, if you leave any sample such as whole blood for some time, the denser particles will accumulate at the base followed by the lighter particles. Thus, this leads to the separation of the particles. But, if the particles have a very insignificant mass, then the rate of sedimentation will be very low. Hence, to accelerate the rate of sedimentation, the centrifuge machine has been used. The centrifuge works on the principle of centrifugal force. What is the principle of centrifuge? Principle of Centrifuge The centrifuge works on the principle of centrifugation. Centrifugation is the process of sedimentation of the particles (materials) present within the container (test tube, buckets, etc.) using the angular motion. So, it helps in the separation of particles having different sizes and shapes. When the particle is moving with a certain angular velocity, the particle will experience a centripetal force towards the center of rotation. In our case, the centripetal force is due to the upthrust of the fluid media and the electrostatic repulsion due to the charged particles present on the surfaces of the sample particles. To balance the inward centripetal force, the pseudo force (known as the centrifugal force) acts away from the center. During this process, the particle begins to move away from the center. This is due to the inertia of the particles. Different particles can have different sedimentation rates. A higher sedimentation rate means that the particle will quickly settle at the base. Thus this helps to separate the particles. Sedimentation Coefficient The rate of sedimentation is directly proportional to the sedimentation coefficient. In other words, the particle with a higher sedimentation coefficient settles quickly. There are few factors that affect the sedimentation coefficient. These factors are stated below. The sedimentation coefficient is directly proportional to the mass of the particles. So, the particle having a higher mass will have a higher sedimentation rate. Similarly, the particle having a lower mass will have a lower sedimentation rate. The frictional coefficient of the particle is inversely proportional to the sedimentation coefficient. The frictional coefficient of the particle is dependent upon the shapes of the particles. More is the uniformity of the particle, lesser will be the coefficient of friction. For example, the perfectly spherical particle will have the least friction coefficient. The particle with higher density undergoes quicker sedimentation than the particle with lower density. The density of the fluid medium is also responsible for sedimentation. If the density of the fluid is greater than that of the particle, then the particle will experience greater upthrust. The upthrust causes the floatation of the particles. Thus, it will not undergo any sedimentation. If the density of the fluid medium is lower than that of the particle, then the weight of the particle will be greater than the upthrust exerted by the fluid. Hence, the particles will undergo sedimentation and form a pellet. Similarly, if the fluid and the particles present in it have the same density, then the particles will not move at all. Components of Centrifuge Here we will discuss some of the components of the machine. But, all of these components may not be relevant in every type of centrifuge. Motor:- Responsible for the spinning of the rotor. Rotor:- Holds the sample and rotates at the speed of the motor. Speed Regulator:- Controls the RPM of the motor. Speed Sensor (Optional):- Identifies the speed of the rotor and displays it. If the speed goes beyond the set value, the machine will give you an error signal on the display. Door Sensor (Optional):- Identifies whether the door is opened or closed. If the door is open, the machine will show the error on the display. Unbalance Sensor (Optional):- If the rotor is unbalanced due to improper sample loading, the machine will show the error on the display. The error may also occur if the machine is inclined or rotates at the maximum speed. Then, the machine will stop rotating. Rotor Sensor (Optional):- Identifies the type or model of the rotor. If the rotor is mismatched, the centrifuge will not operate. Compressor (Optional):- In the case of the cooling/freezing centrifuge, the compressor is used. What is RPM in Centrifuge? RPM stands for Rotation Per Minute. The RPM of the centrifuge can range from around 300 – 1,50,000. So, based on the necessity (application), various centrifuge machines have been designed. What is RCF in Centrifuge? RCF stands for Relative Centrifugal Force. It is also known as the g-force. 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Useful for the analysis of DNA, RNA, viruses, antibodies, proteins, etc. Useful in the chemistry and the biology labs of the educational institutes. For separating the isotopes for the programming of the nuclear weapons and nuclear power program. Useful in pharmaceutical companies. In industries for separating the particles such as glass, silicon, graphite, etc from the water. It is also used for wastewater management. In astronomy. Separation of fat from the milk in dairy farm. Household applications such as washing machines. Types of Centrifuge Rotors The centrifuge consists of a motor. The motor is connected to the rotor head. The rotor head is responsible for the holding of the sample solution. So, as the rotor rotates, the sample will also rotate along with it. There are various types of rotors. The rotors may hold the container such as test tube, blood bag, cuvette, centrifuge tube, etc. So, based on the application different types of rotors have been designed. 1. 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As the RPM of the rotor decreases, the rotor will begin to return back to the original stage. During this movement, the pellets formed may be disrupted. A swing bucket rotor is useful in differential centrifugation in blood banks. Differences between swing bucket rotor and fixed angle rotor Swing Bucket Rotor Operates at lower RPM (RCF). Operates at higher RPM (RCF). If all the parameters are constant, the time for centrifugation is longer. If all the parameters are constant, the time for centrifugation is shorter. Able to centrifuge less no. of samples with a larger volume. Able to centrifuge more no. of samples with lower volume. The pellet accumulates at the center of the base of the container. The pellet accumulates at the sidewalls and the corner of the base of the container. Provides better sample separation. The sample separation is not as good as that of the swing bucket rotor. There are many other types of rotors available for different purposes. 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