Analytical Technologies in Biotechnology
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Module - 5
Centrifugation techniques
Lecture - 6
Types of Rotors cont. and Care of Rotors

In previous lecture, we started with the Types of Rotors and we discussed about three different types of rotors that is the fixed angle rotor, swinging bucket rotor and vertical type rotors. All three rotors are quite widely used, particularly fixed angle rotor and swinging bucket rotors are mostly used quite a lot, as compared to vertical type of rotors. And we discussed about that how centrifugation takes place and various parameters regarding or concerning the solution which is placed and how the palatine or sedimentation takes place. Now, for larger volumes and to minimize wall effects, there are rotors known as zonal rotors. So, in this lecture, we are going to discuss about these zonal rotors as well as we will discuss about care of rotors. That is also very important.

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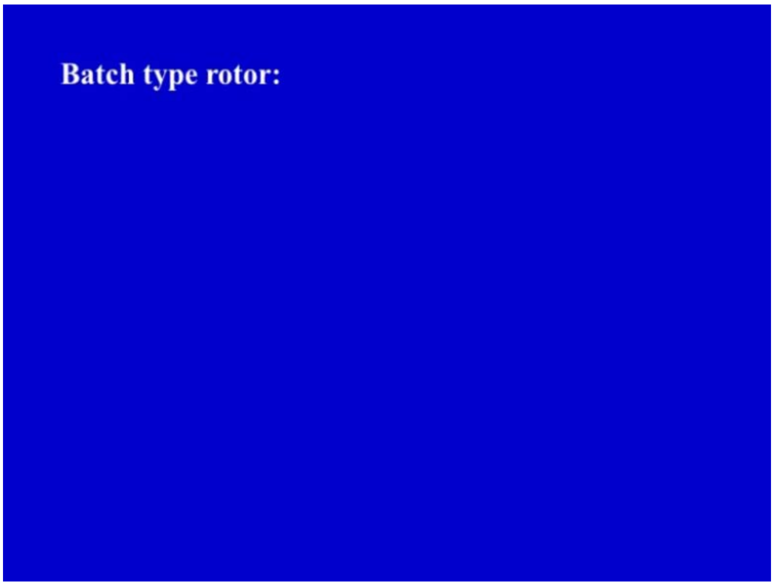

So, in zonal rotors, there are two kinds of rotors, one batch type rotors and others the continuous flow rotors. So, let us start with batch type rotors, now batch type rotors are more extensively used, they have less wall effects and you can use more or higher sample size in these types of rotors. Now, these are based on sample loading and unloading type, where there are two types, we will discuss as we go along.

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Now, there could be batch type rotors, which are low speed batch rotors and there could be ((Refer Time: 02:19)) high speed batch rotors. Now, in low speed batch rotors, it is like 5000 revolutions per minute it can go up to, these are mostly made up of aluminium or prospects with and could have thick transparent top and bottom, to permit direct examination of particles sedimentation during centrifugation. So, low speed batch rotors are utilized for lot of different kinds of applications then there are high speed batch rotors. Now, high speed batch rotors, the speed could go up to say, 60,000 revolutions per minute, these are also made up of either aluminium or titanium alloy. And they have thick transparent top and bottom to permit direct examination or particle sedimentation during the centrifugation.

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A typical batch rotor as you can see, will look something like here. There are lot of different parts of this rotor. As you can see, there is a seal unit, it is used for injecting density gradients and sample into a running rotor. And also for collecting, it can be cooled, like we will see that, how the samples are loaded and unloaded in the running rotor, which are of dynamic mode. Then there are cap used for high speed operation, there are guard plate, this is plate supporting the seal unit, this cover and there are other like rotor, which looks something like this.

So, a rotor is like I said, it is could be a cylindrical or bowl shaped rotor, so in zonal centrifugation, density gradients are produced within the zonal rotor running at lowest speed. Next sample to be separated is injected through the centre, as we will see in detail then the zonal rotor is separated at high speed, the separation will take place in here. After separation, the separated sample will be taken out through the centre by injecting high density liquid from the outside wall of the zonal rotor, while the rotor is running.

So, like here, we will see that, there are two types of rotors, batch type rotors, one where loading and unloading is done while rotor is spinning and one, where the unloading is done when rotor is at rest. Now, body of a typical batch rotor is, either a large cylindrical container or a hollow bowl, where the rotor volume varies through the square of the radial distance, so from the centre of the rotation. So, here the rotor volume will vary, as the square of the radial distance from centre of the rotation.

Centre of the rotor has a core, to which is attached the vein assembly, that derives or that divides the rotor internally into four sector shaped compartments. So, centre of the rotor like, as I will show you, has a core and there is a vein assembly, which will divide the rotor internally into four sector shaped compartments and this minimizes the swirling of the rotor components. So, dividing the rotor into four compartments, certainly minimizes swirling to a larger extent.

Now, these veins or septa which are there, have radial ducts to allow gradient to be pumped to the periphery of the rotor from the centre core. Let us discuss these two types of rotor, batch type rotors, which is standard core dynamic method rotor and the static method, rotors in a static method. So, rotor is enclosed by a threaded lid and capacity may range from 300 ml to say, 2 litres with gradient material filling the entire enclosed space. So, rotors like I said, there are two types, rotor core are of two types based on type of operation. The standard core, it is a dynamic method, where loading and unloading of the rotor while it is spinning and then it is a reorientation or reorienting gradient core, it is a static method, where loading and unloading is done while rotor is at rest.

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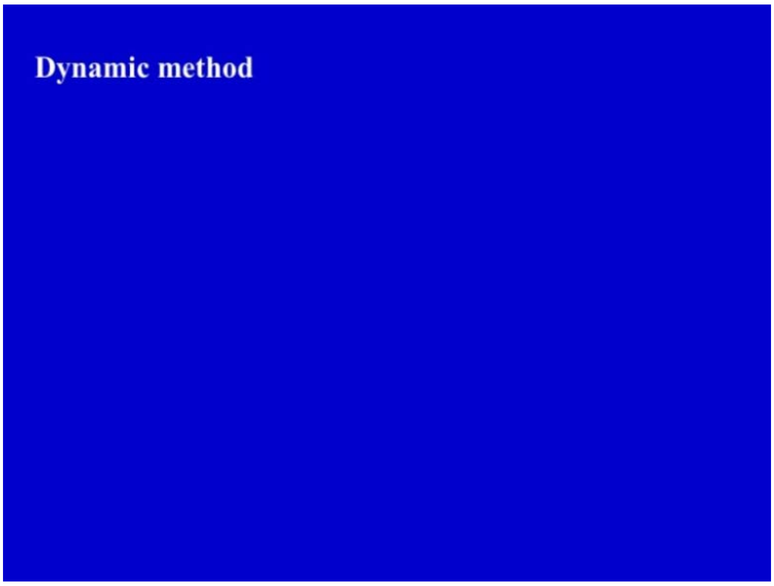

So, let us discuss first one that is, the dynamic one method here, in this mode of operation, loading of the standard core type rotor is achieved while rotor is revolving at approximately 2000 revolution per minute. Now, what is done is, the lighter end of the preformed gradient will be pumped first into the rotor through a fixed or removable seal, as I have shown you. And it emerges at the periphery, it is pumped through the central seal and it will be pumped to the periphery of the rotor and form a uniform layer held in a vertical orientation against the outer rotor wall by centrifugal force.

Remember this here, rotor is spinning at 2000 revolutions per minute, now subsequently what is done, that successive addition of the density gradient material will result in continuous displacement of the lighter gradient pumped in earlier towards rotor core. So, the lighter gradient material, which was pumped earlier will be pushed towards the central core like that is, centripetal end and a new addition, which is higher density material will be replacing it.

So, once this is done like, as you keep the pumping of the gradient into the rotor is done, finally the rotor is filled with this gradient material and this is a continuous or this is like, as you put in, it would be a different concentrations of gradients you have put in. after this, fluid cushion which is denser than heaviest end of the preformed gradient, is pumped to fill the rotor completely. Now here, both ways it could be done, like it could be a continuous gradient, which could be pumped in here or it could be step gradient, which many times the lines or which is separating will be disappearing here.

Now, this simple then after this, once the gradient materials is pumped in and rotor is occupied completely, the sample is then introduced by the fluid line leading to the centre of the rotor and then displaced and overlaid by low density liquid. So, once you put in the sample from the centre then it will be overlaid by a very low density liquid. After removal of the gradient lines to the rotor, the rotor is accelerated to operating speeds. So, it is already operating or it is already running at 2000 revolutions per minute and so it will be accelerated to the operating speed, which could be upto 60000 revolutions per minute, but it could be less also.

So, that will depend on a particular application and it will be accelerated for a particular period of time to give either a rate zonal or isopycnic a separations. So, recovery of once this centrifugation is over after a limited period of time, the recovery of the separated particles and gradient is done by deaccelerating the rotor to it is original speed that is, 2000 revolutions per minute. And then rotor content is displaced, where lighter end is displaced first by introducing additional cushion to the periphery of the rotor. A modified rotor core is available, which allows fractions to be recovered at the rotors edge as well as at it is centre.

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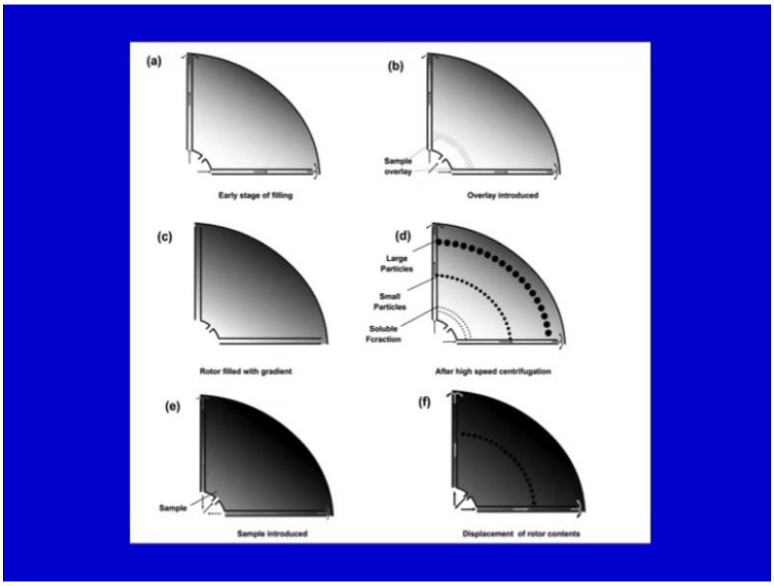

To understand the whole thing, which we have discussed, if you see here, the hollow bowl or cylindrical form of rotor like I said is separated into four compartments, this is one of the compartment and this is the central area here. So, if you can see here, the central area there is opening here and what is done from the top, central area sample. So, if you have this already and this can go from this side or so as you push the gradient material, it will be overlaid in here and on all four compartments it will lead.

And then finally, you have got the gradient, so sample will be pushed in and sample is placed into over the gradient material and then it will be overlaid by the lighter gradient material. Now, what is done, centrifugation is performed at operating speeds and then as the centrifugation like rotor is put in, after centrifugation what you will get, you will get separation of the different mixture or different particles in the sample. So, larger particle as rule we have seen, will sediment faster towards the end and the lighter particles will be towards centripetal end.

And so you will find that, different particle on the basis of their centrifugal force, which is applied onto them, they will separate in here. Now, once they are separate then what is done, some things has to be removed, so again what is done is, it could be you can push another liquid and then things could be coming out of this central core here. And finally, displacement of rotor contents can be achieved in here by displacing it, there could be many more methods for recovering like I said, it could be from edge or it could be from centre. So, this is how, in dynamic mode or in this particular, where rotor is spinning during loading as well as unloading, the samples could be applied. Gradient material could be filled in as well as after the centrifugation, the sample or the different particles could be recovered, while the rotor is spinning.

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Now, next method is the static method, which is reorienting gradient core method or static method, where the rotor is at rest while loading and unloading. So, in this method, which uses reorienting gradient core, the sample is layered on top of a density gradient in a rotor, while a rotor is at rest. Now, once it is done, the rotor is then slowly accelerated to around 1000 revolutions per minute. Now, this is done to prevent mixing of the rotor contents and also gradient layers reorient under centrifugal force.

Now, as the speed is increased to the operating speed, zones approach a vertical orientation and at very high speed, zones becomes vertical. Now, particle separation occurs at rotors operating speed and then after completion of the separation then again as done earlier, rotor is deaccelerated to 1000 revolutions per minute and then very slowly brought to rest. So, the contents can be displaced from rotor and recovered by drawing the contents from the bottom of the rotor or by displacing the gradient out through the top, both ways it could be done, whichever is convenient.

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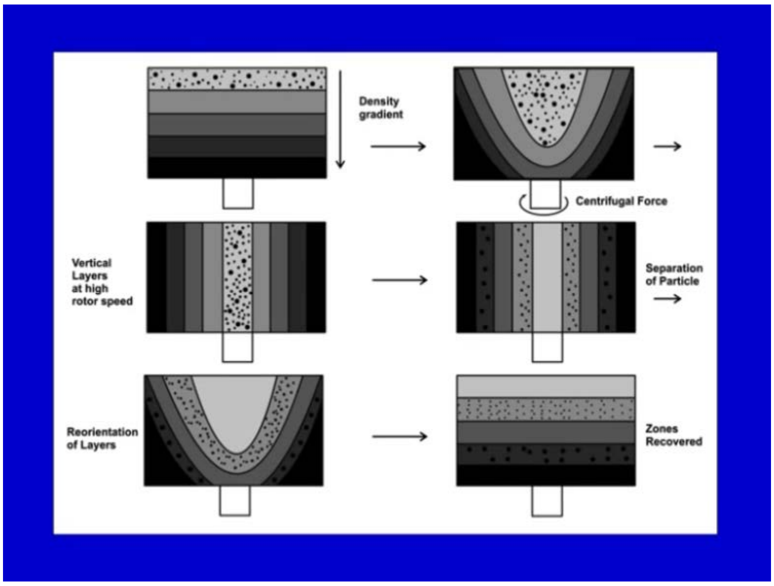

So, what is done, as you can see on your screen, so what you have done is, first thing is that is, the sample is layered on top of the gradient. And you can see the gradient here, the densest material is here of gradient, lightest one lighter side of the gradient is this and the denser side is this. Now, as the rotor is started and brought to very slowly to 1000 revolutions per minute, you can see that, reorientation happening and this cores different zones becoming vertical. And at a very high speed that is, operating speed towards that, they are becoming totally vertical and then separation takes place.

After separation then the process is reverse that is, it is slowed down to 1000 revolutions per per minute and then slowly brought to the rest and you can get different particles in different zones here. So, they can be recovered like I said from the top or from the bottom, so this is how, in a static loading and unloading of the rotor, it is quite suitable like. So, it is like done at rest, unlike where in dynamic rotor, where it is done while rotor is spinning.

Now, static loading and unloading of the rotor is quite suitable for the isolation of long fragile molecules. For example, DNA strands which may be due to seal like, they could be damaged by the rotating seal assembly used in dynamic method. So, many times certain molecules may be better separated in static method, now both static and dynamic methods give very good resolutions. Also the gradient emerging from the rotor can be passed through suitable monitoring device to aid in zone isolation.

Now, batch type zonal rotors have been used to remove lot of different things like contaminating proteins from different preparations and for isolation of hormones, enzymes, macroglobulins, ribosomal sub units, viruses, sub cellular organelles, animal or plant tissue homogenates and likewise, there could be many different types of materials could be used for separation here.

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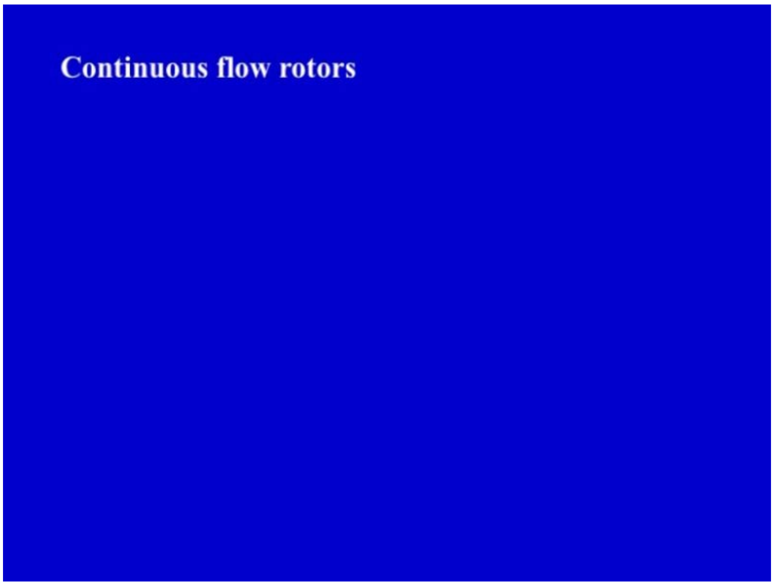

So, these were two batch type rotors, these were two zonal rotors, batch type rotors, one dynamic method, another is static method. Now, there are apart from batch type rotors, there could be continuous flow rotors also. In continuous flow rotors, they are designed for high speed separation of, rather relatively small quantities of solid matter from very large volumes of suspension. Now, this is useful for harvesting of cell and large scale isolation of say, viruses or other material.

Now, it is similar to batch type zonal rotor, but differ in design of core, because of different fluids flow patterns in the rotor, as we will discuss. In operation, suspension is continuously fed into the rotor during centrifugation at a flow rate and at a particular flow rate, and what happens is, as it is done, the sedimenting material moves into the rotor, while particle free affluent leaps the rotor through the outlet line. So, what is happening that, as the materials is continuously pumped in, particles sediment, while the particle free affluent leaves the rotor.

Now, rotor may be operated with or without density gradient in here and like, it could be used for lot of different application. Now, one of the examples we will take here of continuous flow rotor is, elutriator rotor. Elutriator rotor is like illusion type of rotor you can say, now this is a type of continuous flow rotor and it contains a single conical shaped separation chamber. Now, in this separation chamber, apex of this separation chamber points away from the axis of rotation and a bypass chamber on the opposite side of the rotor, which serves as a counter balance also and to provide the fluid outlet.

So, on one side, there is a fluid inlet and on other side, there is a fluid outlet and it is also a counter balancing the other side. Now, there are arrangements which allows the rotor chamber content to be visualized. There could be like a transparent window or different arrangement for visualizing it, for visualizing the rotor contents. Now, particles suspended in a uniform low density medium are pumped into the rotor chamber at it is peripheral as, via a rotating seal assembly when rotor is spinning at particular speed say, it may not be spinning at very high speed, but could be like say, 3000 revolutions per minute or so.

Now, since the separation chamber is conically shaped, a gradient of liquid flow velocity gradually decreases, as the diameter of the chamber increases towards it is centripetal end that is, towards axis of rotation. So, what is it is like, it has a conical shape and in conical shape, when a liquid flows, which is a gradient of liquid is flowing from inlet and it is going towards other end.

So, the velocity will certainly gradually decrease, as the diameter in the conical shaped chamber, as the diameter increases of the chamber increases, so the flow or the velocity will decrease and we will see what is the significance? So, as a result, gradient of liquid flow velocity forms, that opposes the applied centrifugal field. So, the centrifugal field or applied centrifugal field is outward, whereas liquid is being float towards centripetal end. So, these will certainly oppose each other that is, the velocity towards the centripetal end and the centrifugal field that is outward.

Now, the tendency of particles of different sedimentation rate will sediment in the centrifugal field and it will be balanced against the controlled flow of liquid being pumped through the separation chamber in the opposite direction towards it is centripetal end. So, what will happen, particles bend in the chamber at a position, where their sedimentation velocity is balanced by the liquid flow rate in opposite direction.

So, there are one has to understand that, there are two forces acting in here, one is the flow that is velocity, which is towards centripetal end and another is the applied centrifugal field, which is in opposite direction, which is outwards actually. So, the larger particles accumulate towards the centrifugal end, as could be understood and where the liquid flow velocity is high and a smaller particles will accumulate, where the liquid flow velocity is low that is, towards centripetal end.

Now, either by a stepwise decrease in rotor speed or by a stepwise increase in the liquid flow rate through the separation chamber, collection of the separated uniformly sized particles can be made centripetally. In order to, of successive increasing diameter by elutriation from the chamber, so that is why it is it is kind of illusion, that is why it is called elutriator rotor.

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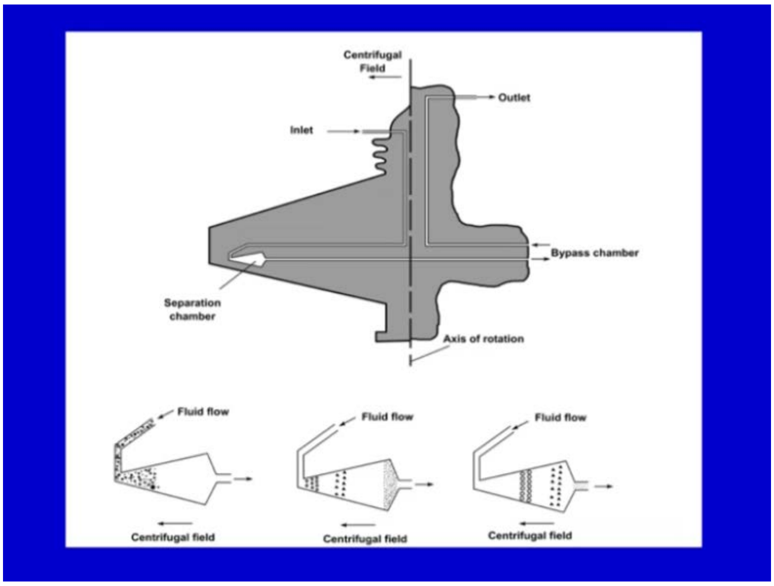

So, if you can see on your screen, this is like what is meant here, this is the rotor actually and you can see the very small rotor chamber in here. Now, this rotor chamber is, you can see this is conical in shape, the diameter is more towards centripetal end, rather that towards centrifugal end actually. Now, if you can see that, inlet is here, it goes all the way and it is into this conical chamber and outlet is on the other side of the rotor, which is also, this part is also counter balancing this one.

So, what are the different stages in here, so there are two forces, one is from inlet, you are pumping in the material containing the particles. And there is an outlet, so when you are forcing something from here, there is a velocity or there is a flow here towards the centripetal end and the outwards force is centrifugal force, that is applied centrifugal field. So, both will oppose each other and on that particular basis, this particular separation takes place.

So, you can see here these three figures, so fluid is flowing in here and as it is flowing in here, there will be like separation of the particles and larger particle will be on this side and smaller particles on other side. And as it goes on, this will be like finally, there will be separated where like I said, larger particles accumulate towards the centrifugal end and where the liquid flow velocity is high and the smaller particles will move towards or will be towards, where the velocity is low.

And so once the separation is taken place, either by stepwise decrease in rotor speed or by increase in the liquid flow, there could be, like these separated uniformly side particles can be successively taken out and it could be said, they could be eluted from the rotor actually, so this is how, the separation takes place in elutriator rotor. Now, our continuous flow rotors, so continuous flow rotors are used for separation of different types of monocytes or lymphocytes from human blood to purify koofers. And endothelial cells from sinusoidal liver cells and fat storing cells from rat liver, fractionation of yeast cell population. And many other applications are there, this is something looks like real way.

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It looks something like this, there is a big rotor, on one side there will be of like elutriator rotor we have described, so the continuous flow rotor is also having applications in different areas of different separations. So, what we have discussed in zonal rotors is, two types of rotors that is, batch type of rotors and we have discussed about the continuous flow rotors. Let us move on, so this was about all different types of rotors, as we have discussed in previous lecture and this lecture.

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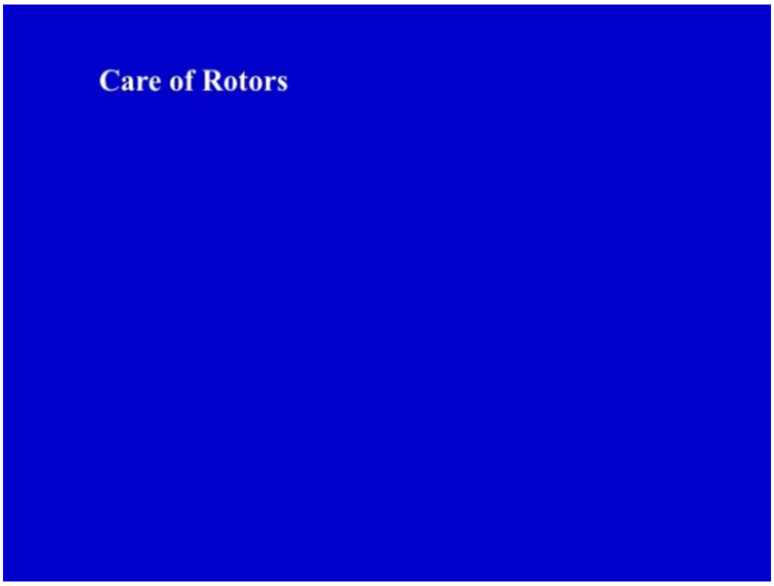

Now, let us extend our discussion to care of rotors, that is also of paramount importance, that for prolonging the life and to maintain the rotors, one has to take proper care of rotors. It is important to take care of rotors to reduce the wear and tear and chances of failure, there are lot of instructions, which are given in manuals for rotor care and they are rotor care guides. One thing is that, one should use only compatible rotors, one should routinely check for rotor damage, the tubes which are put in the rotor should not be overfilled.

This tube should be kept or closed, safe high speed spin requires nearly perfecting balanced load like, as we have seen the rotors have even number of slots. And once you put one tube, there should be counter balancing tube as well, check that rotor is correctly seated on dry spindle, that is very important and it should be locked in properly or screwed in properly so that, it does not create problems. Rotor should be run always at less than it is maximum safe speed or around maximum safe speed, it should not be run higher than that.

It should be that, one should stay there until full speed is reached, when you are operating a centrifuge and once it is reached then you should be around also. One should stop the centrifuge, if any thing seems unusual, like if some sound is coming or just could be certain other things then the centrifuge should be stopped immediately. One should never open the centrifuge, until the rotor is stopped and manual break should not be put in. Rotor should be cleaned gently many times, the spilling might occur or other problems or it is wet, it should be dried completely. Rotor should be stored upside down, maintain a careful rotor lock also, derate and retire rotors for say, age or overuse actually.

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Now, there is another problem of corrosion, so integrity of rotors can be compromised by corrosion or fatigue, many rotors are made from either titanium or aluminium alloy to prevent corrosion. Now, although aluminium rotors do not provide high degree of protection and should be handled with care to prevent scratching. When corrosion occurs, the metal is weakened and less able to bear the stress from the centrifugal force exerted during operation. Combination of stress and corrosion causes the rotor to fade more quickly and at a lower stress level.

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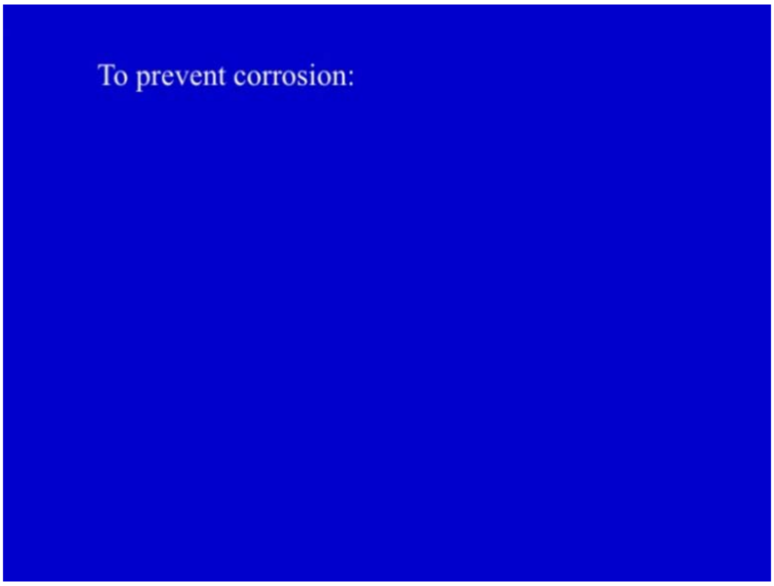

Now, to prevent corrosion, there could be few steps like keep rotors clean and dry, wash immediately if spills have taken place, like example, salts or other corrosive material have been used and it should be cleaned right away. One has to be gentle with aluminium rotors like avoid harsh detergents or bottle brushes with sharp wire ends, inspect rotor regularly. If there are rough spots or certain other indications then do not run the rotor and it should be taken into account, what has happened and if corrective measures could be applied in there.

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Now, mechanical stress here, all rotors are designed to carry a particular or maximum load at a maximum speed. And based upon the rotor tubes or bottles filled with a solution, whose density is no greater than a particular like 1.2 gram per centimetre cube. So, reduction in maximum density is required, if density of solution exceeds this value, so mechanical stress is also very important.

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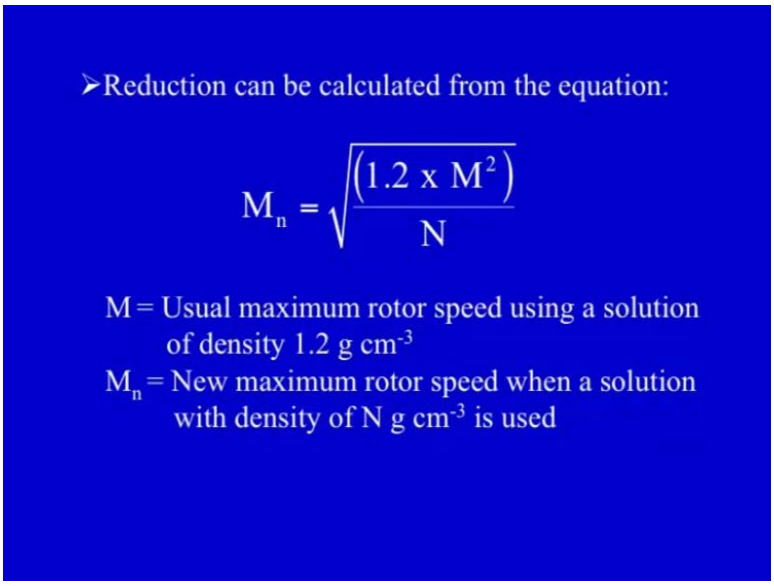

And reduction can be calculated from this equation here, where M is usual maximum rotor speed using a solution of a particular density like that is, 1.2 gram per centimetre cube. And M n is the new maximum rotor speed when a solution with density of N gram per centimetre cube is used. So, speed reductions are required for stainless steel caps and tubes and to prevent recrystallization of high density salt solutions. To prevent possible damage to the drive shaft of the centrifuge due to vibration, always ensure that, load are evenly distributed that is, evenly balanced before a run. Always one has to observe the manufacturers maximum speed and sample density ratings, many centrifuges have automatic rotor detections, also always observe speed reductions when running high density solutions, plastic adaptors or stainless steel tubes.

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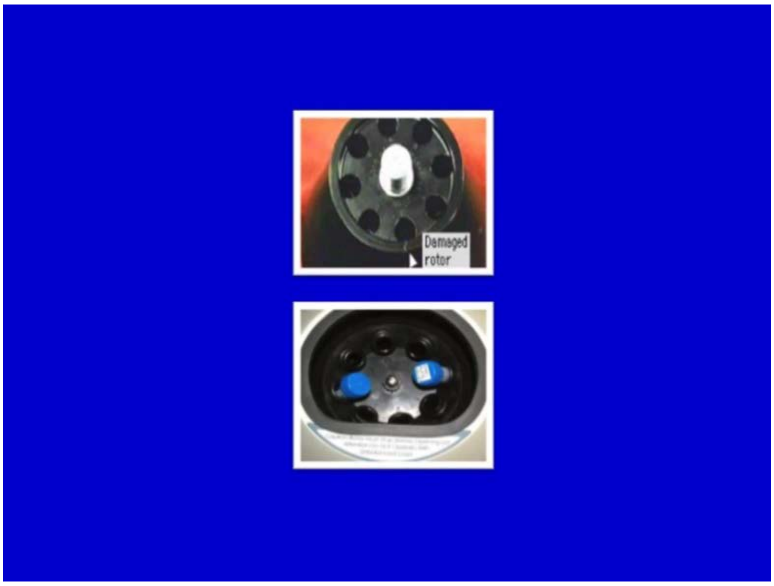

As you can see here, these are the rotors which are damaged here and if you compare here with this rotor, so damaged rotor should not be used or should not be run. During acceleration and deacceleration of the rotor, like cycling, stretching and relaxing of the metal causes fatigue and failure of the rotor. And to avoid over stressing, this should be kept of it is total usage, record of total usage and number of runs, etcetera should be kept and could be the specification of rotor could be compared with in the catalogue or given by the particular centrifuge company.

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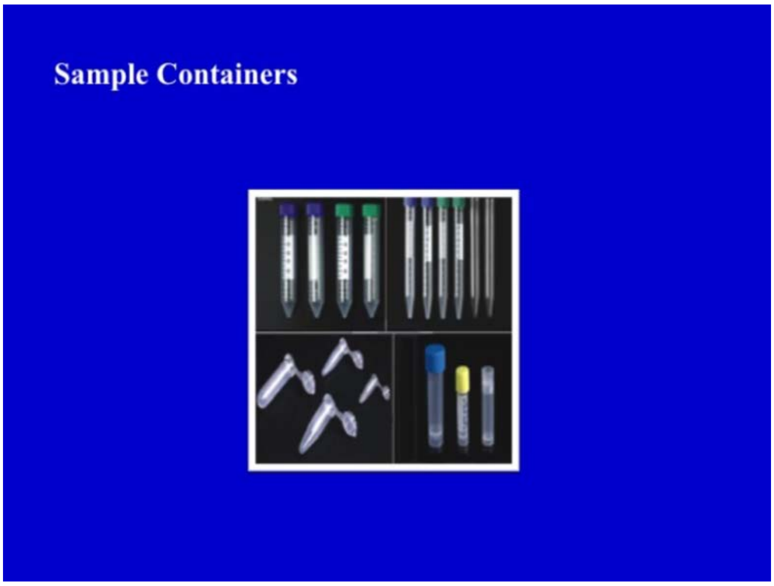

So, the sample containers, which are used like centrifuge tubes or bottles are manufactured in a range of different sizes. They could be like from few ml to say litre or so or more than that, with varying thickness and rigidity and from wide variety of materials. They could be like sample containers can be made from glass, plastics, it could cellulose, esters, nylon, stainless steel and so on. Now, if you compare these, the glass can go upto limited RCF or you can say, low RCF and it is not recommended for say, bacteriological work or for a higher RCF.

Stainless steel, it is inert, can be heat sterilized and can withstand high RCF, likewise plastic can also withstand very high RCF depend on it is rigidity or it is strongness. Thin wall tubes can be used in swinging bucket rotors, while thick walled tubes are used in fixed angle and vertical rotors. So, if you see here, tube tie up and rotor compatibility then a thin wall open. thin walled tubes are good for swinging bucket rotors and thick wall are good for a fixed angle and swinging bucket and others. So, likewise like say, thin walled sealed are used for vertical rotors, so likewise there is a compatibility for different types of rotors.

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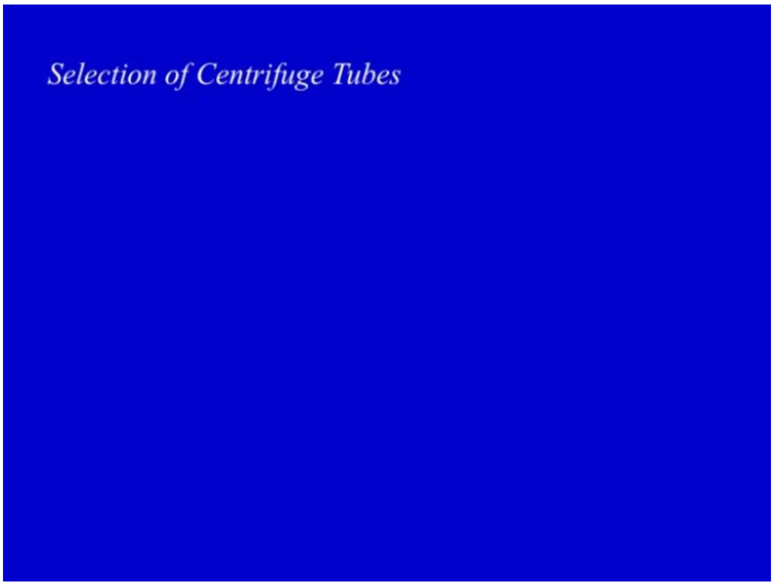

While selecting the type of centrifuge tubes, correct choice of sample container is essential for achieving desired degree of separation of particles from a sample mixture. Now, factors to be considered for selection of sample containers are, one is that, one should consult manufacturer's technical literature to determine limitation of material used for making container. One prevent, it should be seen that, sample leakage or loss will not be there, chemical compatibility had to be ensured, it should be that you can recover the sample easily.

Proper capping and sealing of the tubes should be available, type of container used will depend on factors such as, the nature volume of the sample to be centrifuged, type of rotor to be used, available centrifuge what centrifugal force is being used, chemical resistance to various solvents, temperature limits, etcetera. Like we discussed, certain materials cannot be used for high speed centrifugation and likewise for certain types of rotors. Now, major factor in selection of a tube material could be, another criteria could be the clarity that, you can see through then strength that is, chemical and heat resistance and also sealing mechanisms.

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Tube Plastic type	Clarity	Chemical Resistance*
Polypropylene (PP)	Opaque	Good
Polyallomer (PA)	Opaque	Good
Polycarbonate (PC)	Clear	Poor
Polyethylene terephalate (PET)	Clear	Poor

So, the chemical compatibility of a popular tube materials if you see, if you see polypropylene, these are clarity wise they are opaque, but their chemical resistance is good. Then polyallomer, they are opaque, but chemical resistance is good, polycarbonate they are clear, but the chemical resistance is poor. Likewise, polyethylene these are clear, but chemical resistance is poor, so likewise, one has to select a particular type of tube material.

What we have discussed about till now, is the care of rotors as well as the type of material, which is being used in here. So, if you see here, like one has to choose the type of material or the tube material, which is to be used, based on different factors like we have seen that is, whether it can take up the stress, whether it is chemically and physically compatible, it does not interfere or react with the sample, there is no problem in recovering of the sample, all these things has to be taken care.

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Now, if you compare the safety aspects in the centrifuge, that is the last part of our lecture here. The safety aspects are very important in centrifuge, while you are working on a particular centrifuge. A centrifuge user should strictly observe the precautions, which are given in the literature or given by particular company. And they should follow strictly those precautions, like manufacturers manual should be strictly followed, this is very important for the safety of a person working on there and as well as for centrifuge.

Rotor should be always stored in proper containers, attention should be given to imbalanced detectors, this is very important. If there is an imbalance then the rotor operation will be hampered, will be not correct and it can harm the rotor also. Rotor speed should not exceed the assigned value that is, whatever the maximum speed is assigned for a particular type of rotor, rotor should not be, never should be run higher than the maximum safe speed, which can completely like damage the rotor.

Then, lid of the rotor chamber should remain locked during operation, this is very important, many times people forget to lock the lid or put the lid and lock it. And this could be very, very dangerous for the centrifuge and even for the person, if it is not completely secured, a centrifuge is not completely secured. So, in this case, the rotor many times could come of the spindle and could be hitting the walls of the centrifuge. While using hazardous material like pathogenic organisms, a infectious material, viruses, etcetera, samples should be kept air tight and in leak proof containers, if these things are spilled, they will be very, very dangerous.

So, even it could be like, centrifuge could be marked for this kind of work in isolated or in a particular lab place. To avoid the rotor failure, manufacturer's instructions regarding the rotor care and use should always be followed. So, this is like, one need to strictly follow this particular safety instructions given in manufacturer or in literature, so this completes our section on centrifuge. To summarize the whole part, we started with the basic principle of centrifugation and in basic principle we have seen that, the sedimentation rate depends on applied centrifugal field, the size of the particle, density of the particle and the spherical nature, whether it is a spherical or aspherical.

And we have seen various parameters, which play a role in the centrifugation of a particular particle. The centrifugation, the sedimentation rate or velocity could also be expressed in terms of svedberg unit in honour of the scientist, who did a pioneering work and it was the unit 1 S was 10 raised to power minus 13 seconds. So, larger is the molecule, it will have a larger value of S and it will also indicate, how fast this particular particle will sediment.

Now, they were as we have gone along, we have discussed about different types of centrifuges. If you could recall, we have discussed about a benchtop centrifuges, high capacity refrigerated centrifuges, high speed refrigerated centrifuges, ultra centrifuges, continuous flow centrifuges and so on. We have discussed about different types of rotors also and also we have discussed about different types of preparative techniques and analytical techniques.

In analytical techniques, we have discussed about analytical ultra-centrifugation, where there is a provision for an optical system to be incorporated to monitor the run as in real time. And through different optical system, which could be absorbing system or based on refractive index measurements, in analytical centrifugation, the centrifugational properties or sedimentation behaviour of the particle could be analyzed. As well as like other applications could be, to determine the purity of the sample, to see interactions or conformational changes.

Likewise in preparative ultra-centrifugation methods, we have seen differential centrifugation and density gradient centrifugation. Then we have discussed about types of rotors, in types of rotors, we have gone through the operation, as it happens in three main types of rotor types. One is swinging bucket rotor, fixed angle rotors and vertical type rotors and all three we have discussed in detail that, how the different factors affect that.

Also we have discussed about zonal rotors, which can take larger volumes and can minimize wall effects to a larger extent. Now, these are like cylindrical or hollow bowl rotors and which have a particular design to minimize the wall effects. We have talked about batch type rotors, which are of two types, one is the dynamic method or which are like loading and unloading is done, while the rotor is spinning and another one was static type, where loading and unloading is done at rest.

And different types of materials like fragile material could be better separated on a static than dynamic, but both give good resolutions and different types of materials could be separated on both rotors and in rate zonal or isopycnic gradient separations. And then we have discussed about continuous flow rotors and we have taken one example of elutriator rotors, which has a conical shape chamber in the rotor and there is a inlet and outlet on the other side.

And so there are two forces, one that is the flow of the material and this rotor is utilized for large volumes, where particles or the solid material is very low, but volumes are large. So, it is continuously flown into the rotor, the particles sediment at different as we have discussed and the affluent without the particle will move out. So, these were the rotors then we have discussed very important part that is, care of rotors and the safety aspects to be followed in the centrifuges.

Care of rotors is very important, because if rotor, these are expensive materials, both rotors and centrifuges, they are very expensive and proper care should be taken. So that, you can prolong the life of these rotors and you do not have to spend too much money on reacquiring those rotors, if they are damaged. So, proper care and maintenance of rotors is a must and likewise, the safety aspects, which are given, which could be of common sense thing or which are like manufacturer's instruction should be strictly followed for the safe operation of the centrifuge.

So, with this, we complete the section on centrifugation techniques, in the next lecture we will start with a new topic that is, spectroscopy techniques, where we are going to discuss about different types of spectroscopic techniques. And that will include like say, UV vis or say fluorescence spectroscopy, circular dichroism, NMR, X ray and other methods.

Thank you.