Analytical Technologies in Biotechnology

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Module - 5
Centrifugation Techniques
Lecture - 3
Types of centrifuges and analytical ultracentrifugation method

In previous lecture, we have discussed about the general overview and the basic principles of centrifugation technique. If you could recall, we have discussed about the various factors, which are involved in the centrifugation of a particular particle. Now, this depends on like the size or we can say, mass of the particle, density of the particle whether it deviated from a spherical molecule that is whether it is ideally a spherical molecule or a spherical molecule or non hydrated or hydrated molecule.

All these factors play an important role in the sedimentation of or the sedimentation rate of a particular particle in the under the influence of centrifugal field. Also we have discussed about the sedimentation coefficient, where it is expressed in terms of Svedberg units. We have discussed about how different types of particles say bio molecules or sub cellular organelles have different sedimentation coefficient value in terms of Svedberg unit.

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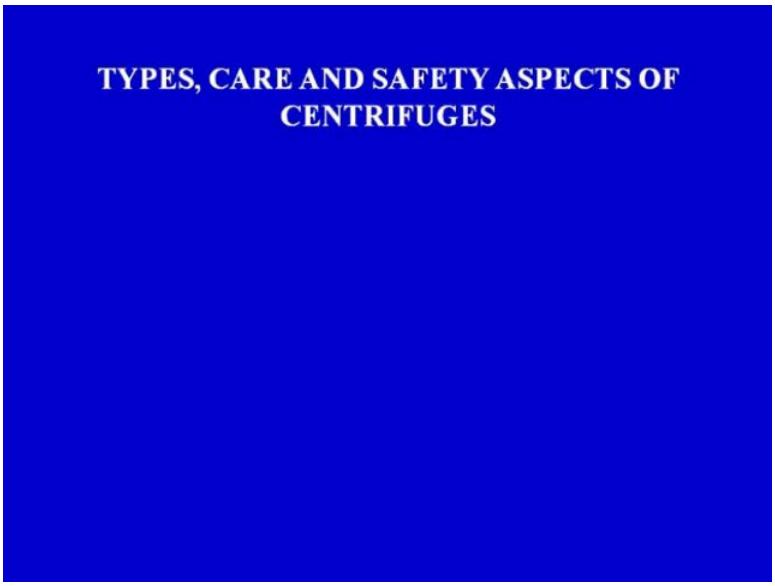

In this lecture, we are going to extend our discussion to the types of the different types of centrifuges, types of rotors, which are used in these centrifuges. Then care and safety aspects of centrifuges as well as rotors used in them, we will be discussing all these things in coming lectures.

In this lecture, we are going to discuss about different types of centrifuges used for various applications. Now, centrifuges are the devices used in a variety of scientific and technical applications. Now, these centrifuges, what they do is they in particular spin, carrier vessels or we can call them centrifuges tubes actually at a very high rotational speed or you can say at very high applying very high centrifugal force. Now, the centrifugal force generated in here is proportional to the rotational rate of the rotor in terms of rpm and also the distance between the rotor and the centrifuges tubes.

So, both these factors affect that is how fast it is rotating a tube is being rotated in terms of revolutions per minute. What is the distance between the rotor centre that is the axis of rotation and the centrifuges tube? Now, there could be like each centrifuge, which could be of different types. We are going to discuss about them. They can carry multiple types of rotors of different sizes, so that the volume of sample, which could be analyzed or which could be processed is like you have flexibility in choosing the centrifugation conditions for particular type of analysis or a procedure. Now, the various applications that can be performed depend on the relative centrifugal field.

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So, you can see here. There are some examples of very small micro centrifuges or micro fuse, you can say a quick spin type centrifuges. There is a table top centrifuge, which is a refrigerated centrifuge. These are little higher floor model centrifuges. So, there could be various kinds of centrifuges for various kinds of applications with lot of flexibility.

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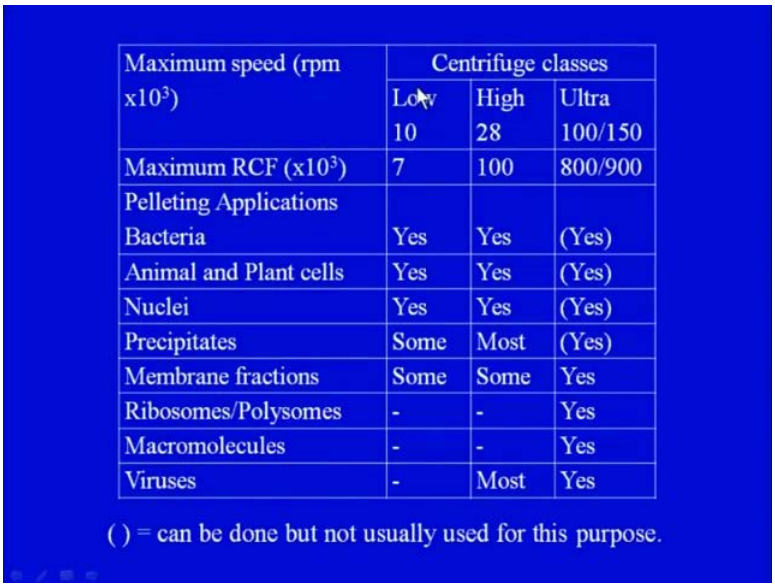

Now, in terms of the kind of speed that is in revolutions per minute and the maximum relative centrifugal field, which could be carried, the centrifuges could be classified into say low speed centrifuges, high speed and ultra centrifuges. Now, low speed you go along with the various parameters given here. If you have low a speed centrifuges, you will have revolutions per minute around 10,000. High speed will have 28,000 revolutions per minute. These are approximate values. Ultra centrifuges will have say 100,000 revolutions per minute.

So, what you see approximately? So, corresponding RCF values are also given in here, which is like 7,000 g force hundred thousand or 800,000 g force approximately. So, what kind of applications could be like pelleting applications could be performed with these kinds of speeds? Here, if you see there are like these are listed in here say bacteria can be pelleted at all these. But, you do not really require ultra centrifuges for this. Though you can do it, but it could be done at low and high speed centrifuges. Likewise, animal plant cells can be pelleted at up to high speed centrifuges nuclei, then certain precipitates.

Now, in nuclei, you can always use any of these certain precipitates like high speed centrifuges are quite good. For that, maybe low speed is not enough. You can also do it like mostly; they are not used like in brackets. We have put in it can be done, but not usually used for this purpose.

Now, if you go further down here, you can see that the membrane fractions ribosome, certain macro molecules viruses, these all require ultra centrifugation. So, you can see that as the size goes smaller, you require much higher relative centrifugal field for sedimentation of these molecules particularly, bio molecules. So, you require very high speed or in terms of rpm or in terms of relative centrifugal field. So, this just summarizes the kind of applications, which can be done on low high or ultra centrifuges.

So, before we go on each type of centrifuges, the working of centrifuges essentially requires a material to be spun in centrifuges. So, whichever material needs to be spun is placed in centrifuges tube, which is then placed in a rotor. Now, rotor and this I have shown you earlier in earlier lectures. Now, rotor is generally a dense metal, which can dissipate heat and could take the stress. This is because of centrifugal force. It has sufficient mass that it can generate momentum. It can spin without any problem.

Now, work they could be like centrifuges, where you require vacuum particularly for ultra centrifuges. Many of the centrifuges are refrigerated also not all of them. Nowadays, many of these are centrifuges have refrigeration system. This reduces the heating cost by frictional forces as the rotor spins. Now, rotors are usually stored before you are going to start an experiment. You can store them in refrigerator or refrigeration unit, so that they could be near the operating temperature. Also, many macro molecules, they could be damaged or they may not remain in their active form. So, many times, refrigeration is required when you are working with biological molecules.

Now, centrifuges come in all shapes and sizes, the rotors also vary. The universal and transferrable unit of centrifugation here will be centrifugal force like earlier. Also, like we were discussing that ultimately it is a relative centrifugal field, which needs to be taken into account. Then you can through that like through a monogram; we have shown you can calculate the revolutions per minute. Then whatever centrifugal force is required that needs to be should be applied on the particular material for a particular application.

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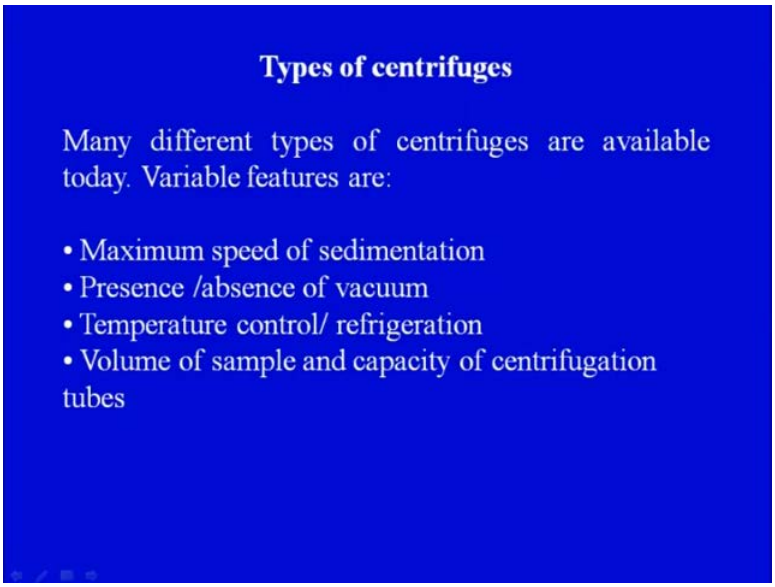

Now, there are many different types of centrifuges available. They will include variable features actually some of might have like say there is a maximum speed of sedimentation, which a particular centrifuges could achieve. There could be a presence or absence of vacuum like I said ultra centrifuges in particular have to have the rotor chamber has to be evacuated temperature control. Refrigeration is required in many of them as we have discussed.

Then, another important factor is how much volume of the sample could be taken by particular rotor type. That means what is the capacity a sample capacity or the volume of sample and a particular capacity of centrifugation tubes. For example, if you can put 5 tubes, what is the volume, which could be kept in there? We will be discussing these aspects as we go along.

So, let us start with different types of centrifuges here. Now, small bench centrifuges, these are very simple. These are very simple and least expensive centrifuges. They have many types of designs actually nowadays available. Now, these are like when we say small, you can simply put them on your bench top. They are small. They could be put anywhere. They are for smaller volumes actually. So, they are used to collect small amounts of material and those sediments rapidly. For example, it could be cells like yeast cells or RBCs that could be pelleted or collected. Maximum speed is 4,000 to 6,000

revolutions per minute, which could be achieved in here and corresponding relative centrifugal field is around 3,000 to 7,000 g force.

These can operate at ambient temperature. Certain centrifuges like latest ones might have refrigeration systems here to keep rotors cool and prevent denaturation of bio molecules particularly proteins. Now, these small centrifuges here, they are also available with more speed or maximum speed like as we have seen 4,000 to 6,000 revolutions per minute for certain centrifuges.

They are available as in speed range of 8,000 to 13,000 or 14,000 revolutions per minute also and may be more also. The relative centrifugal field may go up to 10,000 g. So, lot of different kinds of small bench centrifuges are available for rapid sedimentation of small amount of materials. Now, these are very useful for sedimenting very small volumes like I said up to say 1.5 ml. So, they are sometimes called like micro fuses. There is a rotor, which contains centrifuges tubes around 1.5 or so and so it is for mostly small volume.

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So, these are examples here. This is very small centrifuges that are small bench type centrifuges. This could be containing only rotor, which can be small tubes are in there. There could be centrifuges tubes; rotors are also available in here. So, small bench type rotors are routinely available in lot of different research and teaching labs.

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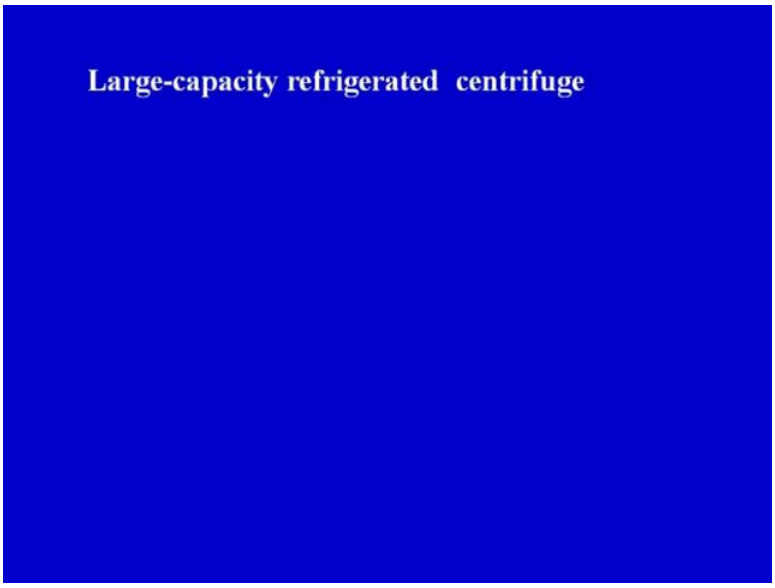

Then, there are large capacity refrigerated centrifuges. As it says large capacity, it can take larger amount of sample or more volume of sample. Maximum speed of these centrifuges could go up to 6,000 revolutions per minute. Maximum RCF corresponding to that or relative centrifugal field is 6500 g. These are refrigerated. So, rotor chamber is refrigerated.

So, their carrying capacity is much higher than small bench type centrifuges. There are inter changeable rotors available for this. There are swinging bucket rotors and fixed angle rotors for these centrifuges. We will be discussing swinging bucket and fixed angle rotors in later chapter. Separation could be achieved in like 10, 50 or 100 ml tubes. Now, these are different like it can go up to total volume or total volume capacity. It could be say 4 to 6 litres are available in these, but there are different capacities available.

Now, we are working on this. We are spinning these tubes here. We said accurate balance needs to be done, so that these tubes are balanced. Rotor imbalance is not there. They use to like they pellet a substance and the sediment rapidly. For example, RBCs or certain bulky precipitates or say yeast cells nucleic nuclei and chloroplast, a lot of other things could be worked upon here for certain applications.

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This is typical high capacity refrigerated centrifuges as you can see. This can carry like 4 to 6 litres of material. These could vary in capacity and various types are available in this range. So, we move onto high speed refrigerated centrifuges. Now, high speed refrigerated means they could go to a very high speed. Maximum speed could go approximately up to 25,000 revolutions per minute.

The relative centrifugal field corresponding to that is around 60,000 g. Now, you can see that why one must work with relative centrifugal field rather than rpm. This is because when you have lower rpm, they are almost equal to the RCF value. Here, as it goes up, you can see 25,000 revolutions per minute. RCF is quite lot here. 60,000 g force is applied.

In high speed refrigerated centrifuges, the capacity here is not like high capacity refrigerated centrifuges, but it could go up to 1.5 litres or so. This also can take both fixed angle and swinging bucket rotors. They are interchangeable like you can put either of them on the drive shaft and screw them. Then both could be worked upon. Of course, the speeds of swinging bucket and fixed angle rotors will be different and that could only be known by specifications of the rotor.

Now, these are used to collect say micro organisms, cellular debris, larger cellular organelles, protein precipitated by ammonium sulphate. So, lot of applications are there, even differentiation, separation of say nucleus, mitochondria or protein precipitates, large

interact organs can be done. So, there are lot of applications of high speed refrigerated centrifuges, but they cannot be utilized for or they do not have sufficient centrifugal force is not generated here to effectively sediment like viruses. If you could recall in the table for viruses or ribosome or smaller organelles, you require ultra centrifugation. So, high speed refrigerated centrifuges are good enough for many applications or sedimentation of many different kinds of materials. There is not like they are not good for say viruses or ribosome or other smaller organelles.

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It is a very typical high speed refrigerated centrifuges; they could be like all these centrifuges could be obtained from different companies, lot of makes are available. One can compare and acquire one or many of them to work upon. Now, as we were seeing all these centrifuges, these were simple centrifuges, where you keep your volume and work upon them. There are also other centrifuges like continuous flow centrifuges.

Now, continuous flow as name suggests, there is a continuous flow of material from there is an inlet and there is an outlet. These are relatively simple centrifuges. They could go to high speed. So, these are high speed centrifuges. Now, rotor is quite different. Here, it is long tubular and non inter changeable rotor. The particles or the material, which is being used in here or being processed in here, particles suspend in a medium flow continuously through the rotor.

So, as medium enters the rotator like rotor chamber, the particles will sediment on its wall actually and access is clarified. The medium overflows through an outlet port. So, there is an inlet port. There is outlet port as the medium containing the cells or other material. Particles enter the rotor. That material on the basis of centrifugal force will pellet on to the walls or sediment onto the walls. Medium overflows through the outlet port. So, this is done for very large volumes and major applications are like harvesting of bacteria, yeast cells from very large volumes of cultures few litres to many like 100 litres or 500 litres cultures are used. We will be discussing about continuous flow centrifuges continuous flow rotors actually later on.

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This could be a typical centrifuge for that. Now, as we have seen in our table, the very high speed centrifuges are ultra centrifuges.

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They could achieve very high speeds like say in terms of 70,000 rpm or above actually. So, the ultra centrifuges are made for both preparative and analytical applications actually. So, you can do both analytical applications could be performed in here. We can analyze a particular purified or partially purified sample for its sedimentation behaviour or other properties. It could also be preparative actually, where you can get after the centrifugation. You can get material for further biochemical analysis.

Rotors run in high vacuum. They are like chamber where rotors are kept. It is evacuated, refrigerated and it is sealed chamber. So, this requires much higher advancement or like say there is an armoured chamber and all those things for because it is spinning at a very high speed. So, much more sophistication is required in terms of vacuum or refrigeration in here.

Now, maximum speed here, as it is evacuated, it is not limited by air friction. The rotors do not have to be aerodynamically designed as compared to other centrifuges. So, these can reach very high speeds in here. Like I said, the maximum speed, angular velocities or revolutions per minute could reach up to a nexus of 70,000 rpm. It could achieve the g force of 500,000 or above actually. It could be used for membrane fractionation. We were talking about viruses or ribosome, which cannot be done by high speed centrifuges. They could be pelleted in here. They could be sedimented in ultra centrifuges. A typical ultra centrifuge is very schematic. It is a simple schematic diagram of an ultra centrifuge.

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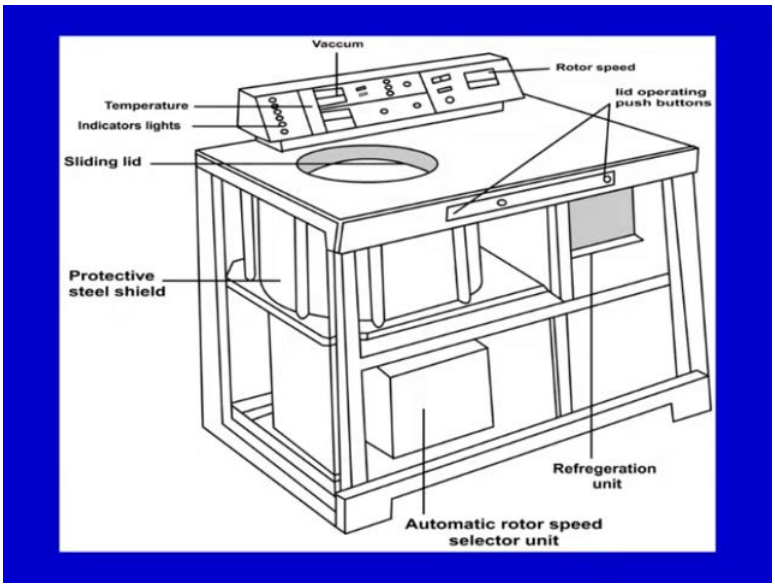

As you can see, there are lot of different things, which are important in here. There is an arrangement for vacuum. You can use switches or these are like controls for vacuum, temperature, indicator lights, and rotor speed. Here, these are lid operating push buttons. Then there is automatic rotor speed selection unit is here. There is a protective steel shielding.

There is a sliding lid, which we can put in things in here. There is a refrigeration unit. So, you can say it is a typical ultra centrifuge. It might look like this actually. It has lot of different things like I said protective steel shielding, good refrigeration system, high vacuum conditions, automatic rotor speed selector unit, imbalance, unit like imbalance recognition; all these things are available in here.

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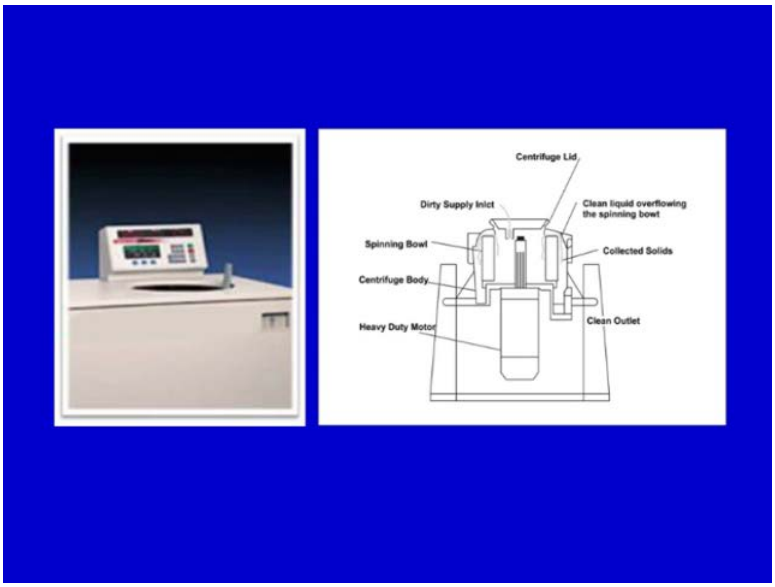

This is like one typical ultra centrifuges here with all these controls and other things. This is a simple diagram for that for an ultra centrifuges actually. There is a heavy duty motor and lot of other things as we have discussed in here. Now, ultra centrifuges can be of 2 types like we were discussing.

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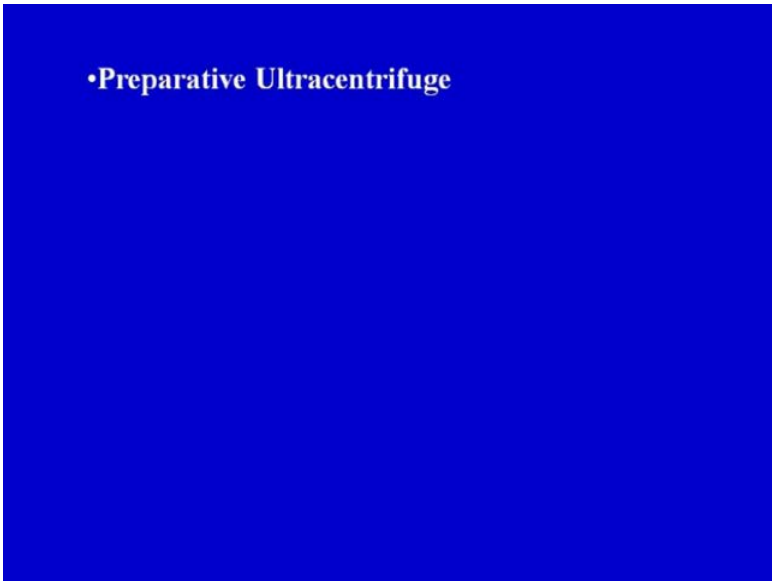

One is preparative ultra centrifuges and another is analytical ultra centrifuges. So, let us start with preparative ultra centrifuges. In preparative ultra centrifuges, maximum rotor speed could go up to somewhere around or may be more also 80,000 revolutions per

minute. It can go even much higher in bench top ultra centrifuges like 150,000 revolutions per minute generating a RCF value of say 600,000 g.

So, this is for floor models. Actually, these are like I said, these are sealed evacuated refrigerated rotor chamber is available in there. Excessive rotor temperature, which might be generated by frictional resistance between air and spinning rotor, is reduced quite a lot in here. This is because of evacuation.

There is a temperature monitoring system, which is highly sophisticated, which employs infrared temperature sensors and other advanced techniques technology to monitor rotor temperature and control the refrigeration system. This is because the speeds are so high that it has to have these kinds of advanced modern systems. There is an over speed control system. So, whatever you set that will be maintained. Actually, there are electronic circuits to detect rotor imbalance and control refrigeration system.

So, there are whole lot of mechanisms. So, these centrifuges could be operated without any hitch. There is a flexible drive shaft system in these centrifuges to maximize, to minimize the vibration arising due to rotor imbalance. There is accurate balance has to be done within 0.1 gram. There are sensors to detect imbalance. So, rotor will not run if there is an imbalance. Rotor chamber of high speed and ultra centrifuges are enclosed in very heavy armour plating like we have seen protective shielding actually. So, rotor chamber rotors are kept in a protective shielding.

Even if something happens, it does not escape the ultra centrifuges. There are other centrifuges called air fuse. Actually, these are air driven table top preparative centrifuges, which are available. They are called air fuse also. These are capable of accelerating to a magnetically suspended rotor and accommodating say certain like 1.5, 1.75 mm cube tubes, 175 mm cube tubes. This could be 7, like it could be 6 or so tubes of around; you can say small centrifuges tubes could be put in there. It is virtually a friction free cushion of air.

These are in non evacuated chambers actually. So, those could also be utilized. Maximum speed could go to 100,000 revolutions per minute; around 160,000 g force in very short period of time. Now, there are table top ultra centrifuges available, which could go up to 150,000 rpm. Lot of small samples, volume samples could be for preparative purpose, could be processed in those centrifuges. Essentially, it is also

important to note that all these like ultra centrifuges are quite expensive and need to be maintained in a particular lab. So, those things also have to be taken in to account.

Preparative ultra centrifuges are very useful. Like, we were saying for table top or small ultra centrifuges, which are also preparative centrifuges, these are also very important and small volumes are required. High centrifugal force could be applied and lot of biochemical and clinical studies could be done on there.

So, here what is done is you can do separation, isolation and purification of lot of things like whole cells, sub cellular organelles, chromatic nucleic acids and other things, macro molecules or ligand binding kinetic studies could be performed. Steroid hormone separation of the major lipoprotein fractions from plasma and de proteinization of physiological floats for amino acid analysis and lot of other things could be performed on these ultra centrifuges, which are small and take small volumes.

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So, this is a typical ultra centrifuge, a preparative ultra centrifuges floor model, which is for say lot of applications can be done on here. So, preparative ultra centrifugation technique is quite widely used for lot of different applications. For example, if you would like to separate ribosome from the cells, then it could only be done in preparative ultra centrifuges. So, likewise I told you many viruses or many small sub cellular organelles could only be separated in preparative ultra centrifuges. So, there are certain applications where it is a must actually; though I said it is quite expensive instrument.

So, from preparative ultra centrifuges, let us move on to analytical ultra centrifuges. Now, analytical ultra centrifuges are also very important. They also operate at very high speeds, which are approaching say 70,000 revolutions per minute with RCF value of 500,000 g. Now, these consist of a motor rotor inside a particular protective chamber, which is refrigerated and evacuated. This is like I said earlier armoured chamber, which is you can say protective shield around the rotor.

Analytical ultra centrifuges unlike preparative centrifuges will also contain a system for detection. That is optical system for study of sedimentation characteristic of biological macro molecules and molecular structure and other like to determine say concentration distribution in the sample or particularly the sedimentation characteristics need to be analyzed in here of purified or partially purified samples actually. So, there needs to be a particular type of rotor and particular arrangement. So, that optical system could be put in here for real time analysis as the operation is going on.

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A typical analytical ultra centrifuges you can say looks like this. It has all the controls and other things. Also, it will contain an optical system here. So, these are just 2 representations of that. Now, depending on the type of optical systems used in analytical ultra centrifugation sedimentation, in an ultra centrifuges is observed in that particular way. It could be like concentration based or say concentration gradient as a function of

radius. Both ways it could be seen. There are different types of optical systems available. Analytical ultra centrifuges can come with those kinds of optical system.

So, 3 types of optical systems are available. One is absorbance, optical system absorbance. Optical system displays the boundary in terms of optical density as a function of radius. So, if you have certain sample say for example, protein or nucleic acid sample and as you layer it or it is presented in the whole of the solution as it sediments. There will be area where it moves down and concentration increases. A particular peak will be obtained in here. As the concentration will increase, certainly the peak will also be different.

So, what you would do is that measurement of sample concentration will be done. It will be analyzed at wavelengths, which could be from ultraviolet region, a visible region say 200 to 800 nanometre. Particularly nucleic acids and proteins could be analyzed in u v region. Many of these macro molecules may contain strong chromo force. So, they could be analyzed on that basis through u v wavelengths or like wavelengths in ultraviolet or visible region. The concentration differences could be recorded and they could be analyzed in real time.

Then, there is alternative Schlieren system. So, like earlier in absorption system, it was like particular wavelengths are absorbed by these macro molecules like say nucleic acid absorbs at 260 nanometres or protein absorbs at 280 nanometre. They might contain certain chromo force, which absorb at other certain wavelengths. These peaks or the data could be generated. So, in alternative Schlieren system, it detects the changes in refractive index of the solution.

So, it will display the boundary in terms of refractive index gradient as a function of radius. So, what does that mean is when you have taken a solution in a particular sector cell or a tube in analytical ultra centrifuges, when the solution is homogeneous, you will not see any boundary. There is no boundary as such. There will not be any gradient of refractive index. So, nothing, no signal will come. As the centrifugation starts, the macro molecule or whichever is the material starts sedimenting.

Then, a boundary or a zone is created which moves in the direction of centrifugal force actually. This boundary which is created is detected in terms of refractive index gradient as it moves down the cell. So, here what is done in alternative Schlieren system? That is

refractive index of the solution or you can say measurement sample concentration based on refractive index changes is performed.

This could analyze macro molecules, which are lacking intense chromo force. For example, they cannot be seen in absorption system like polysacrides or carbohydrates are mostly analyzed with a detector that is a refractive index measuring detectors rather than u v monitor or absorption based detector. So, these samples require a refractive index detector.

Now, there is a third system, which is rally interference optical system. Rally interference optical system is an improved version for alternative Schlieren system. What you have is that the sensitivity of rally interference optical system is much higher as compared to alternative Schlieren system. Here as the concentration is higher, many systems utilize rally interference optical system.

So, essentially what you have is you have 3 systems; one is absorption system where particular macro molecule or a chromophore can absorb the wavelength either in say u v rang or visible range. That will be recorded and analyzed. The data could be analyzed in alternative Schlieren system. Rally interference optical system will be detecting the changes in the refractive index of the solution.

As far as rotors are concerned, rotors for analytical ultra centrifugation must be capable of withstanding enormous gravitational stresses and under these conditions. Lot of like a mass of 1 gram experiences an apparent weight of say 250 kg that is kind of stress here. So, rotor must allow also. Not only that, it could take stress as well as it has to allow the passage of light through the spinning sample. That is you have to install optical system in here. Some mechanism must be available for this and also you have to control the temperature.

A rotor is solid in this analytical ultra centrifugation with holes to hold the cells that contain samples. It is suspended on a wire coming from drive shaft of a high speed motor. The tip of the rotor contains a thermistor for measuring temperature. I will show this in a little while. The simplest rotor has 2 cells. One is called analytical cell and another is called counter poise cell. Now, counter poise cell have 2 holes to facilitate the calibration of distances in the analytical cell. Varieties of analytical cells are available with volume capacities, which could go from 0.02 to 1 ml.

There are sector shaped cells essentially in velocity experiments. These sector shaped cells could be single sector like 2 degree or 4 degree sector shape could be there or to prevent convection, an optical path length, which can vary from say 1.5 mm to 30 mm. Usually with 12 mm of path lengths, centre pieces are available. There are also single sector cells could be there. There are double sector with say 2.5 degree sectors in optical path length, which can vary from 12 millimetres to 30 millimetres. So, various types of cells are available.

Double sector cells permit us to account for absorbing components in the solvent to correct for the redistribution of solvent components, measurement of differences, sedimentation coefficient, and diffusion coefficients. Counter poise cell is designed that when correctly aligned in the rotor, the walls will be parallel to the lines of centrifugal force like swinging bucket rotor, which is extended outwards. So, there is no accumulation of materials against the wall of the analytical cell unlike the sector like it kind of spreads out as it goes away from the centre of the rotation.

Analytical cells have upper and lower plane windows of quartz or synthetic sapphire. These are to withstand very high g force here. So, rotor chambers have an upper condensing lens and a lower collimating lens in optical system. Upper condensing lens together with camera lens focuses light on to a photographic plate or a photo multiplier. Lower collimating lens collimate light. So, the sample cell is illuminated by a parallel light. So, this is how the arrangement or optical system is installed in here. In more advanced instruments, photographic detector could be replaced by electronic scanning system and data could be collected right away and could be analyzed. It is seen as the experiment is going on. So, this is very simple schematic here you can go through.

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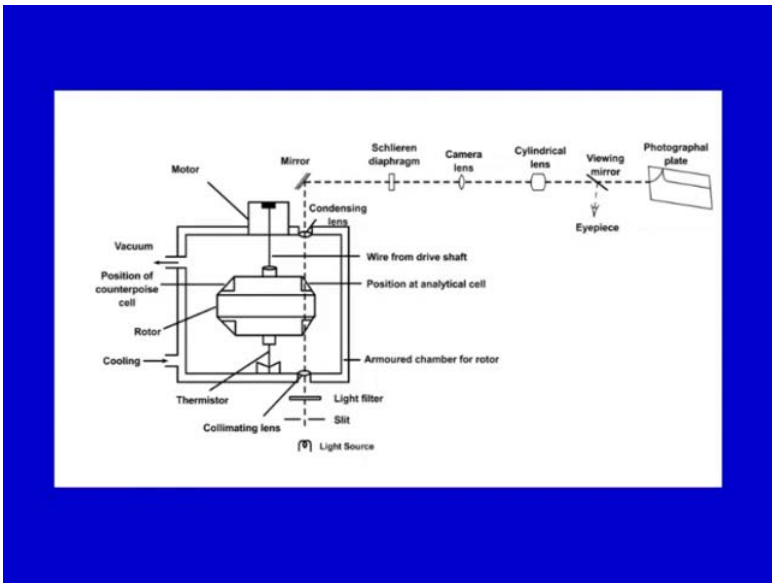

If you can see here, this is a slit here. There is a collimating lens. I was talking about these. This is a solid rotor, which contains a counter poise cell and analytical cell. This is placed in here where the collimating lens light comes from collimating lens, passes through the analytical cell, and then collected by condensing lens. It is reflected by mirror. There is a system of Schlieren diaphragm, camera lens, cylindrical lens and finally, photographic plate where peak or the data is being recorded. The photographic film could be replaced by an electronic scanning system. This rotor is kind of suspended through a wire here, which kind of balances this. There is a thermistor here to sense the temperature. There is a cooling and vacuum system to evacuate the chamber, where rotor is placed. There is armoured shielding here, which is you can say armoured chamber for rotor.

So, what you have is a system where rotor is placed in an evacuated refrigerated and armoured chamber. There is a temperature sensor. This rotor is a solid rotor with 2 places or 2 holes where a counter poise cell and the analytical cell could be placed in. So, it is below the rotor. At another tip, there is a wire or there is a thermistor, which senses the temperature, there is a well placed optical system. It passes through analytical cell and analyzes the sample or the sedimentation characteristics or different applications as the experiment is going on.

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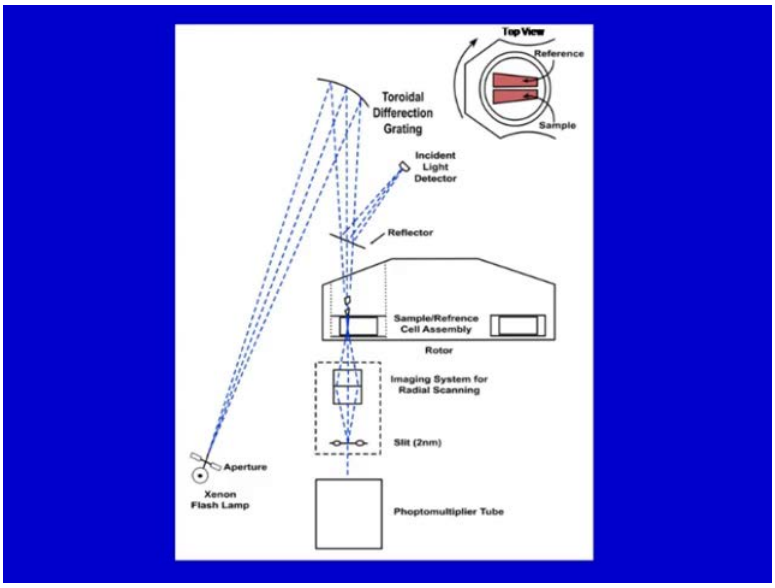

There is another representation of this whole thing, where you can see that there is a lamp. Here, aperture light is like there is a toroidal diffraction rating incident light detector is here and reflector. There is a sample cell. These are 2 cells in here in this particular rotor chamber. So, place for 2 cells and here, there is imaging system. Finally, photo multiplier tube is utilized in place of photographic film. If you see here, there is a reference cell like sector shape. They are here. If you can see the diameter or this particular part increases as it goes from one side to another so that there are no wall effects or sedimentation on walls. Actually, they are like acting as swinging bucket rotors only. So, this is how these are placed and the analysis could be performed in here.

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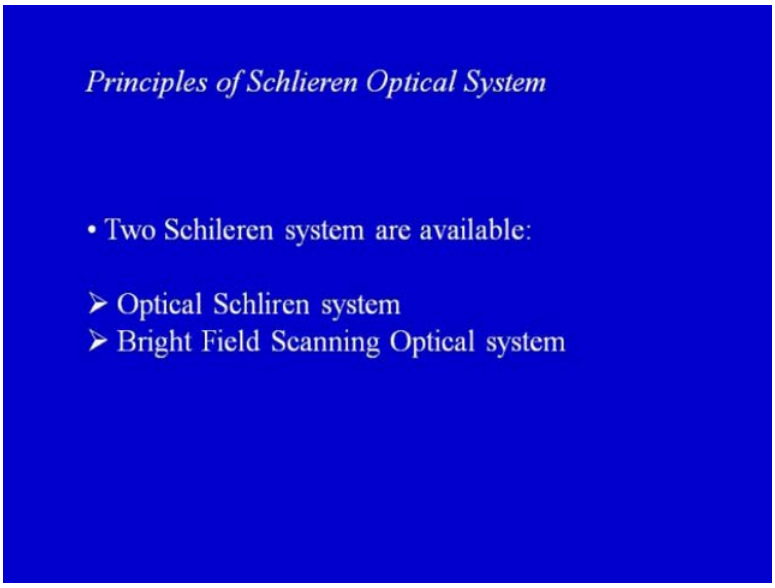

So, if we see principles of Schlieren optical system for here, if light passes through a solution of uniform concentration, it does not deviate. If it is a homogeneous solution, there are no boundaries and no deviations, but passing through solutions of different densities, it is refracted at the boundary between these zones. The optical system records the changes in the refractive index of the solution, which will vary as the concentration changes.

So, 2 Schlieren systems are available; one is optical Schlieren system called OSS or bright field scanning optical system. In first one optical Schlieren system, the deviated light is passed through an inclined Schlieren diaphragm and a cylindrical lens. As I have shown you, the sedimenting particle or material in analytical cell forms a boundary between the solvent, which is cleared of particles and the remainder of the solution containing the sedimentation material. So, what happens? This boundary behaves like a refractive lens, which results in the production of a peak in the final image on the photographic plat or detector system.

Now, peak is an exact record of the refractive index gradient and area beneath. It is proportional to the concentration of the solute. Rate of shift of the peak will give a measure of the rate of particle sedimentation. After a period of the sedimentation, the peak height diminishes and the width increases. Why this is because you have a sector shaped cell as I have shown you that width of the cell is wider at the centrifugal end.

So, OSS or optical Schlieren system plots the refractive index gradient against distance along the analytical cell, which makes it useful for locating boundaries and sedimentation velocities measurements. Now, in bright field scanning optical system, the light is deviated or you can say deviated light is interrupted by a knife edge. The resultant image is scanned with a photo multiplier. The resultant refractive gradient appears as a dark band in bright background. So, this is how it takes place through refractive index detector. It would be same for absorption system. The analysis is performed. Let me show you on your screen what is going on in here.

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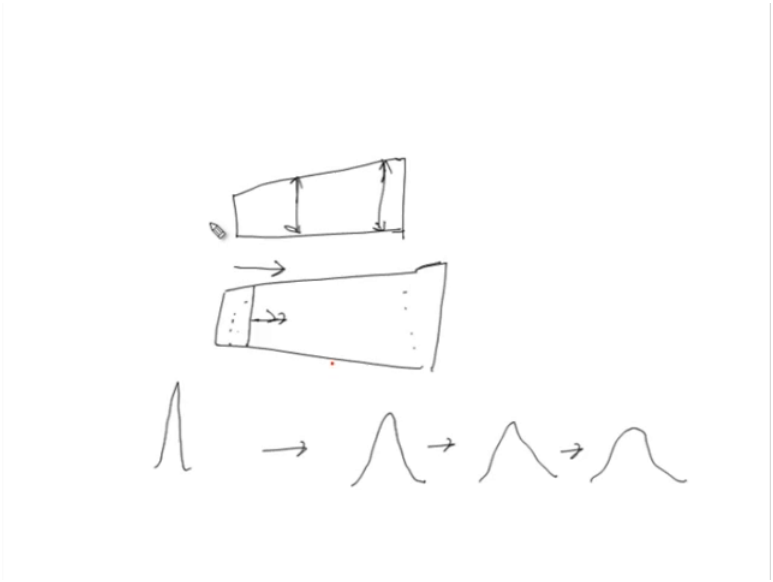

So, what you have? You have a cell or which is somewhere in this shape. You can see this that it is wider at this place than at this place. So, what happens is when this is filled in with homogeneous solution, then there is no refractive index or there is no boundary as such and so no refractive index is registered. Actually, no changes are registered, but as centrifugation occurs and centrifugal force is in this direction, the material or a macro molecule or whatever it is, it starts moving towards the centrifugal end because of the centrifugal force, boundary is created. This area, which is in here is devoid now is devoid of the sample. So, this boundary kind of acts as a lens and a refractive index gradient occurs where you have more concentration in here as compared to this.

So, as this peak moves in here, you will get this peak it moves. So, the refractive index gradient or it moves also like peak also moves. So, what you get is you get as you start

you get a peak which is sharp, but as you act as this peak moves and comes in, this peak will start, the height of the peak start diminishing and you get. Subsequently, what you get is the peak spreads out. Finally, it is quite spread actually because of this shape here of the cell.

The Schlieren system is very widely used likewise the absorption system depending on your application. Now, the third one rally interference optical system like I said it is like alternative Schlieren system, but it detects changes. You can say it is highly sensitive or more sensitive to detect small concentration as compared to alternative Schlieren system.

So, it employs double sector cell in which one sector cell contains the solvent and the other solution. The optical system measures for the differences in the refractive index between the reference solvent and the solution by the displacement of the interference fringes caused by slits placed behind the 2 liquid columns.

Now, each fringe is tracing a curve of the refractive index gradient against distance in the cell. Now, since the position of the fringes is determined by solute concentration, it is possible to measure the concentration of solute at any point along the cell. So, for higher sensitivity, many systems will utilize rally interference optical system in place of alternative schliren system.

There are lot of applications of ultra centrifugation. These determine sample purity. You can say through analysis of sedimentation boundaries, determination of relative molecular mass by sedimentation velocity method and sedimentation equilibration method. They characterize, you can characterize assembly and disassembly mechanism of bio molecular complexes. Determine sub unit stoichiometries. You can detect and characterize macro molecular conformational changes by differences in sedimentation velocity of the sample. One can measure equilibrium constants and thermodynamic parameters for the self and hetero associating systems.

So, there could be lot of applications of this analytical ultra centrifugation technique. To summarize this lecture, in this lecture, we have discussed about different types of centrifuges. Right from simple bench top centrifuges to high capacity refrigerated centrifuges, high speed refrigerated centrifuges, ultra centrifuges, where we have discussed about preparative and analytical ultra centrifuges. Also, we have discussed

about different optical systems, which are employed in analytical ultra centrifuges with their applications.

In the next lecture, we will be discussing about different methods of preparative methods, which are utilized in centrifugation techniques, particularly preparative ultra centrifugation techniques. Example is density gradient centrifugation or differential centrifugation. Also, we will be discussing the different types of rotors are being used and the care taken for these rotors. So, we will stop here in this lecture. Thank you for your attention.

Thank you.