Analytical Technologies in Biotechnology

Prof. Dr. Ashwani K. Sharma
Department of Biotechnology
Indian Institute of Technology, Roorkee

Module - 5
Centrifugation Techniques
Lecture - 2
Basic Concepts 2

In the previous lecture, we have discussed about the basic features of centrifugation. So, we started this topic and we have discussed about and we have taken an overview of this particular topic. Now, we have discussed about the, that this particular separation technique is based on the behaviour of particles of different size, shape, or density in applied centrifugal field.

Now, if you could recall, we have discussed that how the different particles of different size or different density and shape will behave and sediment according to these physical properties and applied centrifugal force. We have discussed about the basic principle of centrifugation. Now, rate of sedimentation depends on the applied centrifugal field, so when if you could recall, we started this here, so the applied centrifugal field which could be given in terms of angular velocity.

(Refer Slide Time: 01:44)

$$G = \omega^2 r$$

$$\omega = \frac{2\pi \text{ rev min}^{-1}}{60}$$

$$G = \frac{4\pi^2 (\text{rev min}^{-1})}{3600}$$

Now, angular velocity, if I say, G is the angular velocity then sorry; if G is the applied centrifugal field, then it is given by the square of the angular velocity and the radial distance from the centre of rotation. So, sedimentation rate will depend on one of the factors will be the applied centrifugal field. Now, this applied centrifugal field, that is can also be expressed in terms of revolutions per minute. Here, you have 1 revolution equals 2 pi radians.

So, what you can do is you can express this like, if you have to convert this angular, which is expressed in radian per second into revolutions per minute. You can do it by 2 pi revolution per minute upon 60, so this could be substituted in above equation and which becomes 4 pi square revolutions per minute upon 3,600. Now, this particular quantity that is applied centrifugal field could also be expressed in terms of relative centrifugal field, or in terms of multiples of the g value that is the gravitational pull.

(Refer Slide Time: 03:09)

$$G = \omega^2 r$$

$$\omega = 2\,\Pi \text{ rev min}^{-1} / 60$$

$$G = 4\,\Pi^2 (\text{rev min}^{-1})^2\, r / 3600$$

$$RCF = 4\,\Pi^2 (\text{rev min}^{-1})^2\, r / 3600 \times 981$$

$$RCF = (1.118 \times 10^{-5})\, (\text{rev min}^{-1})^2\, r$$

So, if you substitute this with particular, like say in terms of multiples of gravitational force, then it could also be written as and here you have to put r that is the radial distance. Also revolution per minute into 981 that is the 981 is the gravitational force. Here, that is 981 centimetre per square per second square. So, in multiples, of in multiples of gravitational force you can calculate and this is RCF or relative centrifugal field.

The basic part is, one is, that relative centrifugal field will play an important role in determining the rate of sedimentation. Now, two parameters of considerable significance; when you are selecting particular centrifuge; one is G force or the applied centrifugal field or you can also say relative centrifugal field. Now, G force is in multiples of g actually, so that force relative centrifugal force which is acting on particles here is exponential to the speed of the rotation, as you have seen in the equation.

Now, doubling the speed of rotation will increase the centrifugal force by a factor of 4. Likewise, the centrifugal force also increases with the distance from the axis of rotation, like it is directly proportional to r that is the radial distance from the centre. So, as the distance increases, so the centrifugal force also increases. These two factors needs to be considered and are significant, when we are selecting particular centrifuse.

Now, when you select a particular centrifuse, the protocols for centrifugation will typically specify the amount of the centrifugal force, in terms of relative centrifugal field to be applied to the sample. They will, it is rather a practice that in particularly high speed centrifuses rather than the rotational speeds or revolutions per minute, the centrifugal forces are more specified or given.

Now, this distance is important and this particular distinction is important. So, this distinction is important, because two rotors with different diameters. That is, because as they will have different diameters, so will be the radial distance actually, of the particle where it is placed in that centrifuse. So, when they are running at the same rotational speed, they will be subjected, a sample will be subjected to different accelerations or different centrifugal force, because the radial distance will be different in 2 centrifuses.

So, during circular motion the acceleration is the product of the radius and the square of the angular velocity and the centrifugal field relative to G that is what we said, so this is relative centrifugal field. Most of the time relative centrifugal field is taken or is specified for a particular centrifuse. For example, many centrifuses can go up to a particular G force only or particular centrifugal field only, because they are the rotor, is meant rotor is made in such a way that it cannot take stress more than that. So, relative centrifugal field is quite important here.

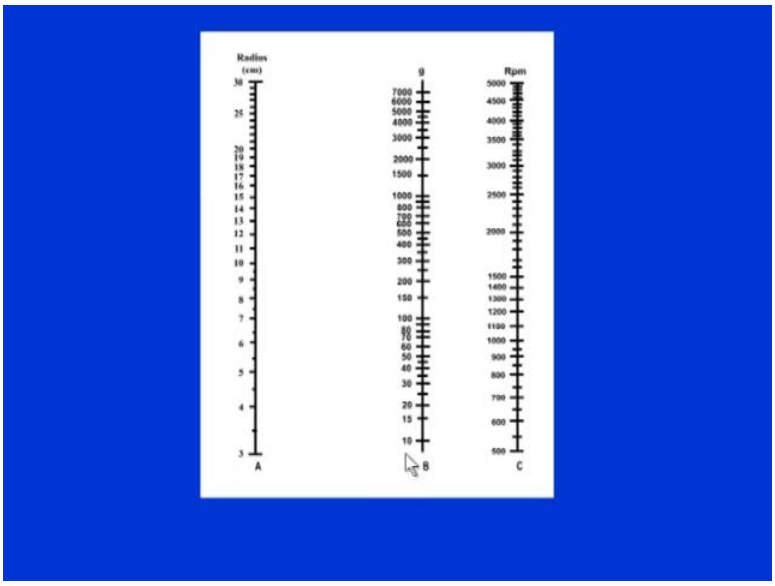
Now, many times it is very easy or convenient to work in terms of revolutions per minute and for that a nomograph can be used to obtain the speed of a centrifuse rotor, necessary for a desired RCF or relative centrifugal field. For example, if you want to do a particular centrifugation, perform a particular centrifugation method and particular protocol has given that you have to use RCF value particular RCF value. Since, you would like to program it your centrifuse, in terms of revolutions per minute then the nomograph can help, which gives an idea about like nomograph, gives you both RCF, the revolutions per minute, rotational speed and also the radius of the centrifuse. So, all these things could be related, like I will tell you how.

So, this quick estimate is useful for low speed centrifugation applications, however it is more accurate. So, many times you know revolutions per minute could be utilized for low speed applications. But, for higher applications speed applications RCF calculations for the speeds has to be done or performed and you need accurate RCF values for that matter.

So, what is done, most of the centrifuses might have nomograph instructions. It is a measure, like what you have to do is measure the radius from the centre of the centrifuse to the rotor, centrifuse rotor to the end of the test tube carrier. Many times it is given actually in the rotors, so you do not really have like, there are particular specific numbers of rotors and that could be matched here then. So, then you have to find out what is the relative centrifugal force required for the application.

You know two things, one you know either you know the rotor number of a particular company. And you know the relative centrifugal force of field which needs to be applied, then there will be revolutions per minute, also straight line connecting the value of the radius with the relative centrifugal force, value will enable the speed of the rotor to be read off the column in nomograph, in a nomogram.

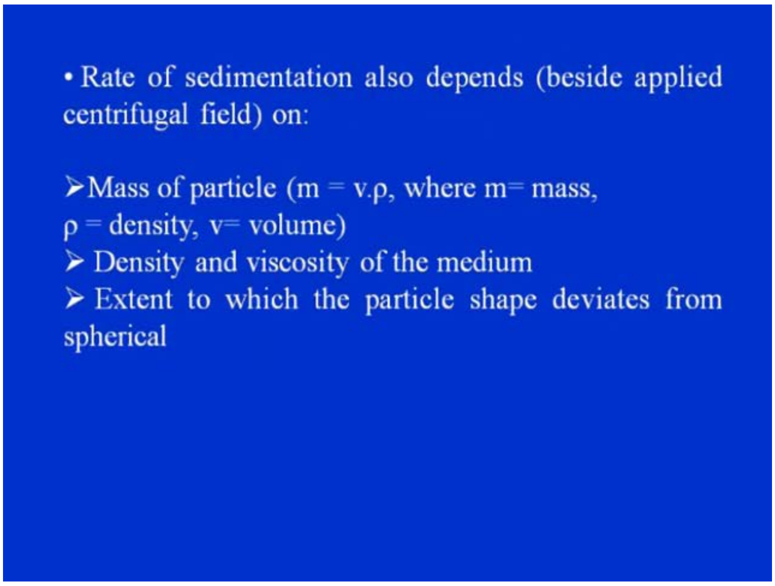
(Refer Slide Time: 10:03)



So, this is a typical nomogram for estimation of rpm setting and here as you can see three scales in here. One is for the radius, actually of the rotor like I said, from the centre to where the tubes are placed tip of the tube. Now, here many times rather than these radius, the centrifuse, a table might contain the number of particular commercial specification of the rotor.

Now, from here, so you know the radius of the rotor and you know the this gives you the relative centrifugal field. So, if you know, say this is your radius is 6 centimetre and your relative centrifugal field is 150, then if you draw a line from here and continue that then you will be, your line will touch the point where the rpm number would be or rpm setting will be known. So, that way you can do the rpm setting, that way you fulfil the requirement of the particular relative centrifugal force. So, the relative centrifugal field or means in terms of g multiples of g or in very simple terms applied centrifugal field is one important determinant of the rate of sedimentation.

(Refer Slide Time: 11:37)

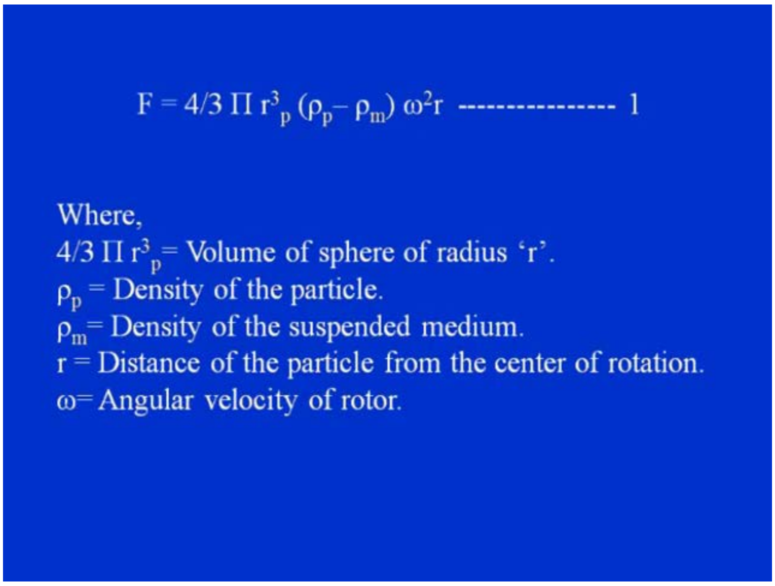


Now, rate of sedimentation also depends besides applied centrifugal field on other factors; one is that is mass of the particle. Now, mass is the density into the volume, multiplied by the density, that is your mass of the particle. Then it also depends on density and viscosity of the medium in it, in which it is suspended. Remember, most of the, almost all the experiments in centrifugation is done where particles are suspended or they are put in a liquid medium, it could be different types of liquid medium.

Also, one factor, so one is mass of the particle another is density and viscosity of the medium and another is extent to which the particle shape deviates from the spherical. That is a particle is an ideal spherical molecule or it is say elongated or some other kind of shapes it takes. Here, spherical, a spherical needs to be remembered.

A spherical like elongated or also spherical, here is considered in terms of non hydrates and aspherical in terms of hydrated molecule. So, if you consider that, it is assumed if a particle, which is, which needs to be centrifused is assumed to be a sphere of known volume and density, then the net force experienced when the centrifugal force at an angular velocity of say, omega radians per second is given, that was volume into density.

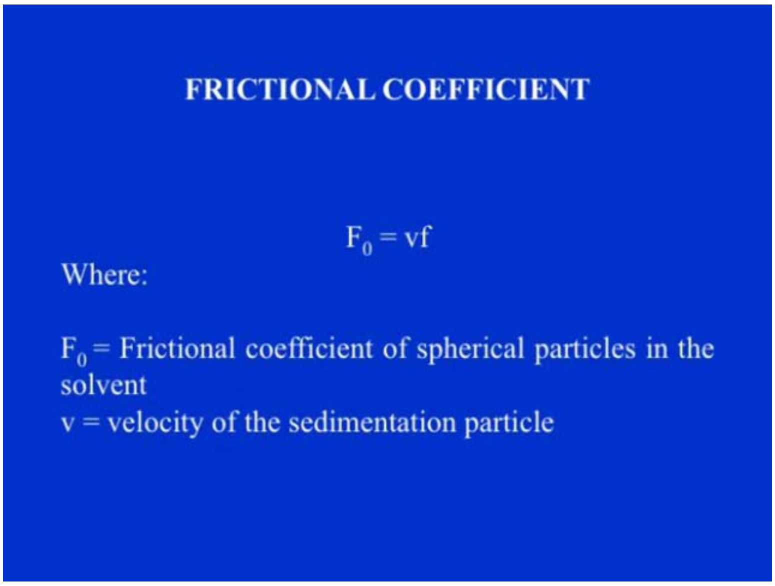
(Refer Slide Time: 13:18)



The force which will be applied or force which will be experienced will be 4 3 pi r cube and here r p is for the particle. Actually, it is different from the radial distance in here and then for correcting for buoyancy. Here, this is density of the particle and this is density of the medium, so that is a rho p minus rho m is also taken into account. So, this is the force which will be experienced by a particle of a particular mass or we can say volume into density.

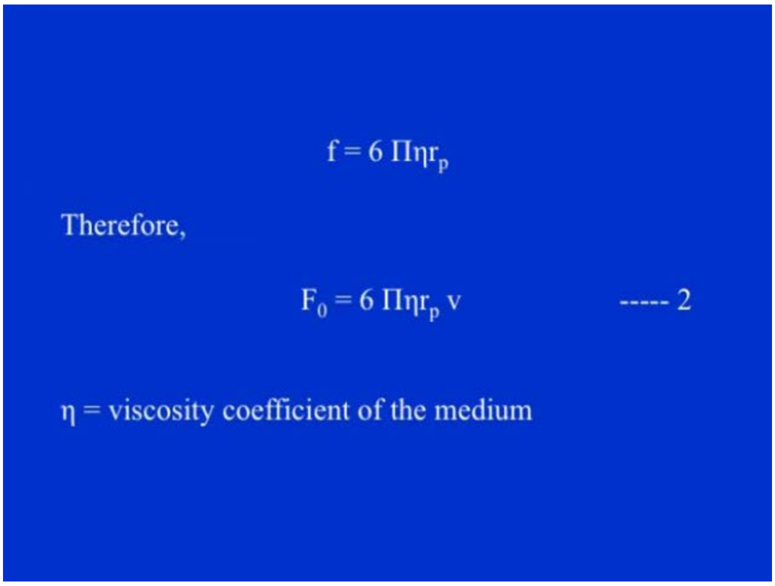
So, this will also affect the force experienced by the particle. Now, there is another force, which will also come into play when particles move through a medium. They will also generate a friction as they migrate through the solution. So, if you could recall in electrophoresis, also we were talking about the frictional force which opposes the forward motion of the particle. So, in the same way here also a particle is moving through a solution. It moves through a solution with a particular velocity this will also experience an opposing force that is frictional force and it is also called frictional coefficient.

(Refer Slide Time: 14:37)



So, if the particle is spherical and is moving at a known velocity then it is frictional force appearing that is opposing the forward motion will be given by v f where v is the velocity of the sedimentation particle, sedimenting particle and f is the frictional ((Refer Time: 15:00)) coefficient. So, here this is like the force which will be experienced by or the opposing force which will be experienced by the particle. Now, frictional coefficient of a particle is a function of its size shape and hydration and also, viscosity of the medium and by the stokes equation for an unhydrated spherical particle. So, that is the ideal one we are talking about.

(Refer Slide Time: 15:34)

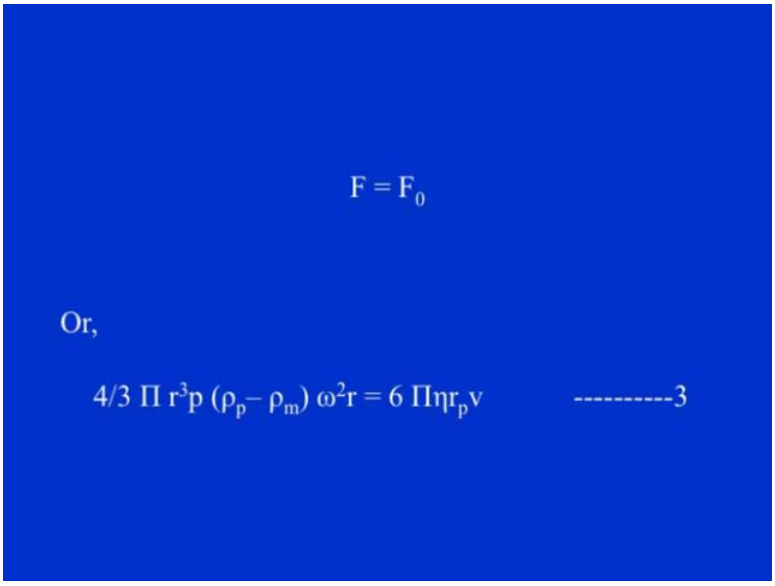


This is given by this particular equation that is frictional coefficient equals 6 pi neta r, which r p is the radius of the particle. So, your force, opposing force or frictional force becomes this particular equation, where you have put in the velocity. Also, frictional coefficient into velocity that is the viscous drag you can say, comes into play, as soon as the particle starts accelerating into the solution due to the applied centrifugal field.

Now, so this particle of known volume and density, which is present in a medium of constant density will be accelerated in a centrifugal field. So, as the centrifugal field is applied that is a centrifuse is switched on after putting your sample into the centrifuse, the particle will start moving or accelerating in that particular solution of particular of constant density.

Now, as the particle accelerates there is a opposing force, which starts developing and more is the acceleration more will be the resisting force. So, the net force on the particle after certain period of time equals the force resisting its motion through the medium. So, as particle accelerates and after very fast actually this force is equalled by a resisting force and then particle moves with a constant velocity without acceleration.

(Refer Slide Time: 17:16)



So, then what you have is you have the force that is forward force due to this applied centrifugal force, which is being experienced by the particle and this is the opposing force, which is frictional force. So, these becomes equal so the values for both these could be substituted in here for F and for a frictional force and when you would like to calculate the velocity or rate of sedimentation here.

So, the balancing of this force occurs quickly as it happens very fast and result is that particle will sediment at a constant rate or it will have a constant velocity it will achieve constant velocity after certain period of time. Now, this sedimentation of particle it could include lot of the different kinds of particles and this could be explained by the stokes equation and which describes the movement of a sphere in a gravitational field.

(Refer Slide Time: 18:26)

$$v = \frac{dr}{dt} = \frac{2r_p^{\,2}(\rho_p - \rho_m)\,\omega^2 r}{9\eta} \quad \text{----------- } 4$$

Now, the equation here given calculates the velocity of sedimentation and these are various parameters which have been utilized. So, velocity which is a change actually, it is a rate of motion per unit time actually. So, here you can see these are like parameters which are involved is like your particle radius the densities of the particle and the medium angular velocity, the radial distance then this eta value which comes from frictional coefficient or this is viscosity of the medium. So, all these factors play a role.

Now, this we were talking about this equation is for an ideal spherical molecule which is unhydrated spherical molecule. Now, supposing there is a non-spherical molecule, as in case of say rod like molecules or elongated or flattened molecules. For example, DNA is a rod like or like molecule, it is not a sphere spherical molecule many proteins I was talking about, like many structural protiens they are not spherical molecules like myosin or actin or for example, collagen these are not spherical molecule. So, they will experience considerable frictional resistance as compared to a spherical molecule.

So, the frictional coefficient of the this molecules has to be compared with the frictional coefficient of a similar spherical or a sphere here. So, the equation here will be different from the previous equation which we have seen for ideal spherical molecule and that is unhydrated molecule.

Now, here this will be different, the resultant is that particle will sediment at a lower rate and equation will become this one, where you have to add one another factor. That is the frictional coefficient of the particular elongated molecule or non spherical molecule compared to that of a sphere here, frictional coefficient of that. So, this has to be taken into account and where this difference has to be taken for and this will depend on particular kind of molecule or a particular unhydrated or sorry hydrated a spherical molecule and all these factors has to be taken into account.

Now, the from equations it is very clear that sedimentation rate of a particle is dependent on many factors. Now, these include one is it is directly proportional to the size of the particle, where I was telling you the radius r p actually of the particle. Since, the equation involves the square of the particle radius, so it is apparent that the size of the particle has greatest influence upon sedimentation.

So, if you could recall, here it is r square, actually or r p square, which is radius of the particle. It has quite a lot of effect on the sedimentation, that is a particle is doubled then sedimentation rate will be more. It is also directly proportional to the difference in density between the particle and the medium, so one like if you see this equation. Here, there is like a density of the particle and density of the medium. So, this is the difference between the density of the particle and medium is very important, what is this mean?

Here, that the sedimentation will be or you can say the rate of sedimentation will be zero when the density of the particle and the medium are equal. If both are equal then sedimentation becomes zero, there will not be any sedimentation and many times when you are doing density gradient centrifugation. And if the particle density is lower than the density of the medium then the particle floats on the density on the medium and does not move further. You may apply any amount of centrifugal force, but it will not move.

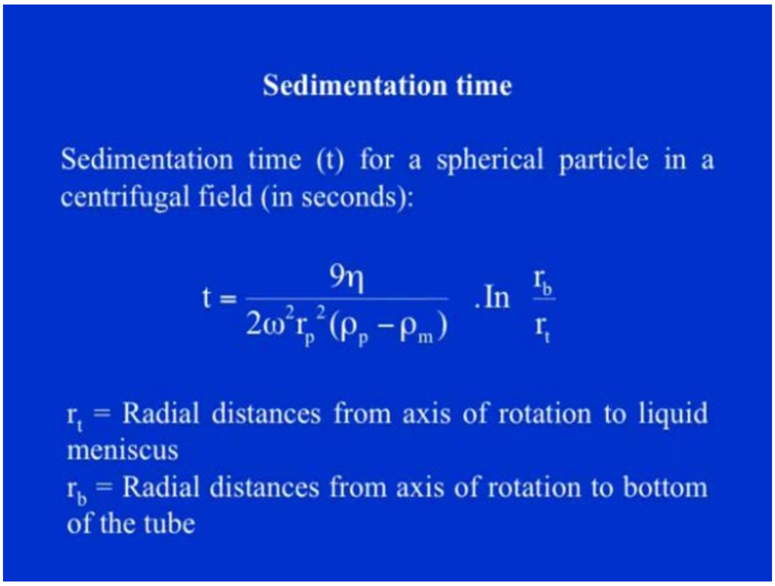
Likewise, the particle density the particles of similar density, but only slightly different sizes can have large differences in their sedimentation rate.

So, if you compare medium, like one is that it will be zero, when the density of the particle and the medium are equal like and when it is like medium's density is more than it will just float on the this, if it is like higher than this then it will move faster in there. So, then medium will have any effect on that.

Now, particles of similar density, but only slightly different size can have large differences in their sedimentation rate. Also, the sedimentation rate will decrease when the viscosity of the medium increases. If you could recall, it is inversely proportional, that is the velocity or the rate of sedimentation is inversely proportional to the viscosity of the medium. So, these factors play an important role and apart from that as we have seen applied centrifugal field, where omega square r or angular velocity, in terms of square of the angular velocity and the radial distance will also play a role in determining the sedimentation rate.

So, the sedimentation rate or velocity also decreases. Like in the last equation, we have seen the frictional coefficient ratio. So, it will decrease as the frictional coefficient ratio increases and it is approximately one for a spherical molecule. So, all these factors will play a role in the velocity of sedimentation.

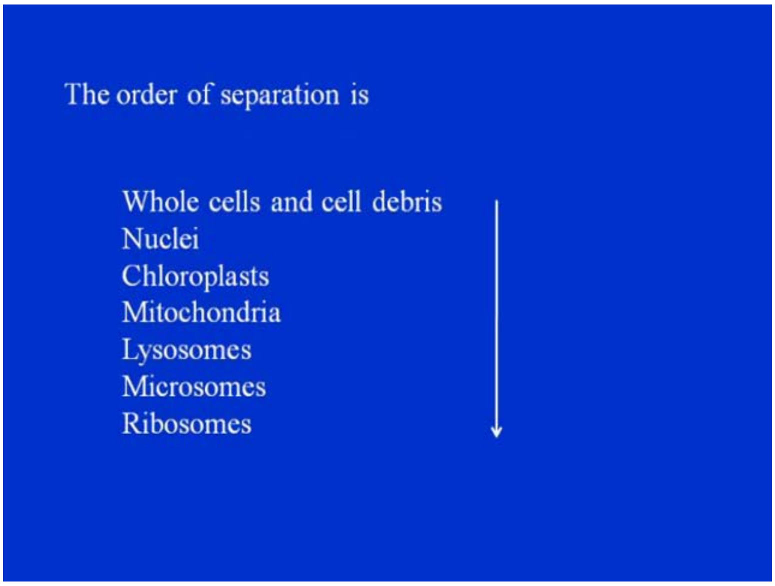
(Refer Slide Time: 24:58)



Now, there is another term we can determine that is sedimentation time that is how much time a particle will take to sediment to a certain n extent, so the sedimentation time for a spherical particle in a centrifugal field. In terms of in seconds it is given by this particular equation this is derived from the earlier equation, where t that is the time of sedimentation in seconds is given by this particular equation and this is log r b upon r t. This is r b upon r t now r t is the radial distance from the axis of rotation to the liquid meniscus and r b is the radial distance of the axis of rotation to the bottom of the tube. So, this equation gives you the sedimentation time here.

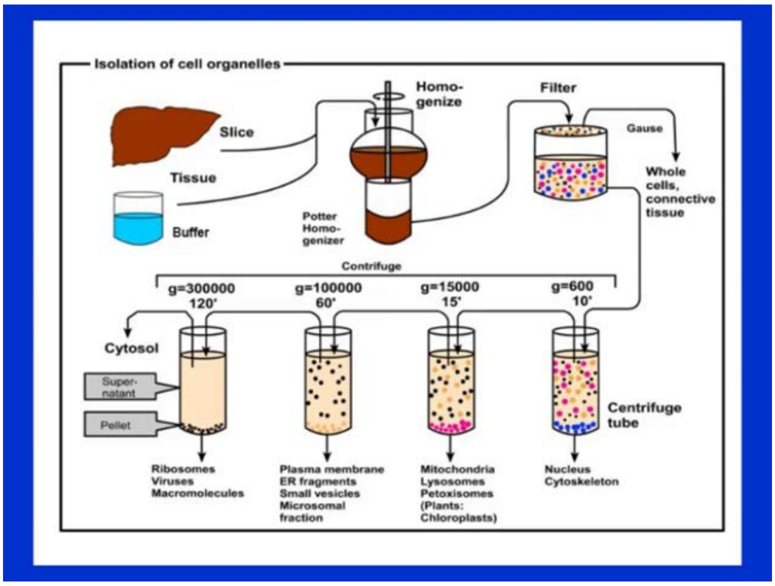
Now, when we say sedimentation time that is how much time a particular particle takes for sedimentation. So, a mixture of if we say heterogeneous approximately spherical particles is taken and they can be separated by centrifugation on the basis of various factors. Like as we have mentioned many times density and size that is the most important shape will also come in then, time required for their sedimentation or extent of sedimentation after a given time. So, how much time they require for sedimentation or you can take that after a particular time how much, what is the extent of sedimentation.

(Refer Slide Time: 26:44)



Now, if we take certain examples the order of separation for say the constituents of cell or whole cell and it is like sub cellular organelles. Other parts then order is somewhere whole cell and cell debris will settle down first and then comes the nuclei chloroplast then mitochondria lysosomes. So, this is the order microsomes, ribosomes this is the order that first whole cell and ribosome will be last to sediment.

(Refer Slide Time: 27:07)

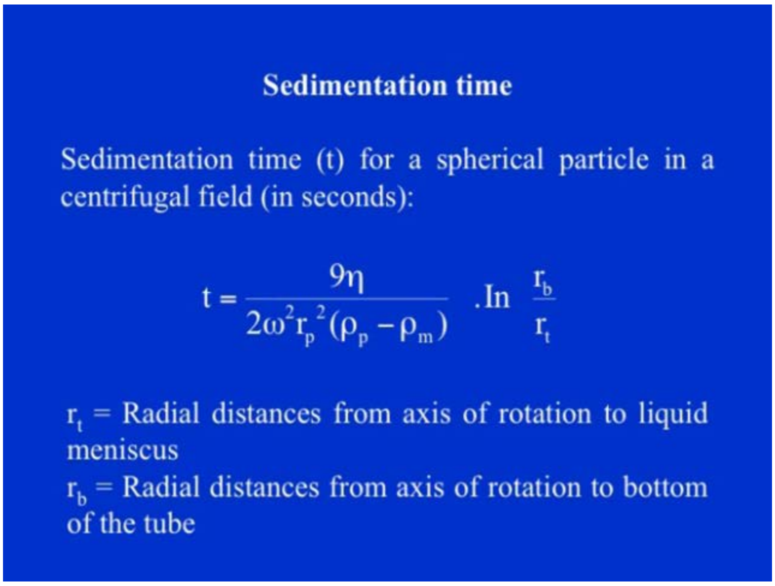


As we can see, in the figure here very clearly, if you can see on the screen that you have taken a particular tissue and you have sliced and you done the processing, that is homogenization and other things. Now, as this whole thing comes in here and you start sedimentation process then whole cells or the cell debris will be the first to sediment. Then as you go on centrifusing after each step you will find that the few things, as we have seen in order nucleus and other things will come first, after the cell debris as I have shown here like whole cell nuclei chloroplast. Here, you can see after certain period of time nucleus and cytoskeleton is sedimented or you can say pelted.

As you go along, you for more time then mitochondria, lysosomes, peroxisomes, chloroplast will sediment after certain point certain time, then more time will be taken by plasma membrane, E R fragments microsomal fractions to sediment. Then finally, ribosomes at very high speed and after longer period of time like ribosomes viruses macromolecules will sediment.

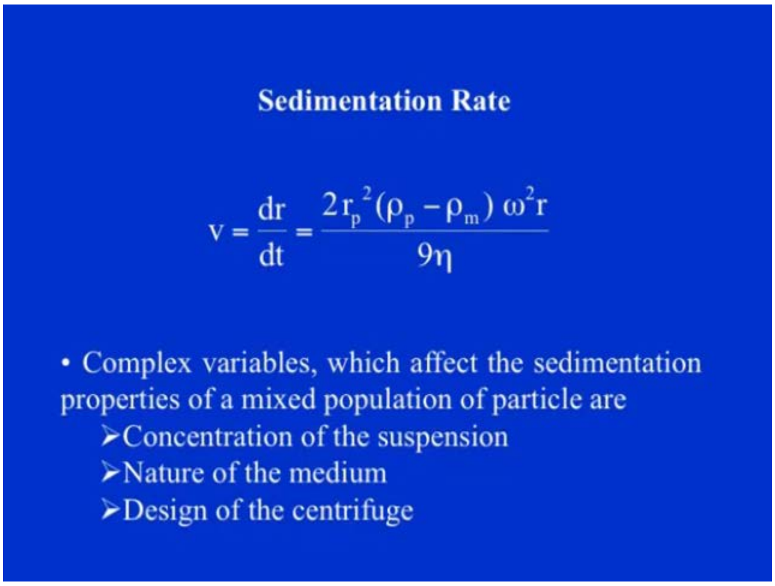
So, you can see for different things or different constituents of cells. You need more time and this G force also is increased so the extent of the time taken for sedimentation, or you can say time required for the sedimentation could be a basis for separation, because they separate differently and take different times for sedimentation.

(Refer Slide Time: 29:05)



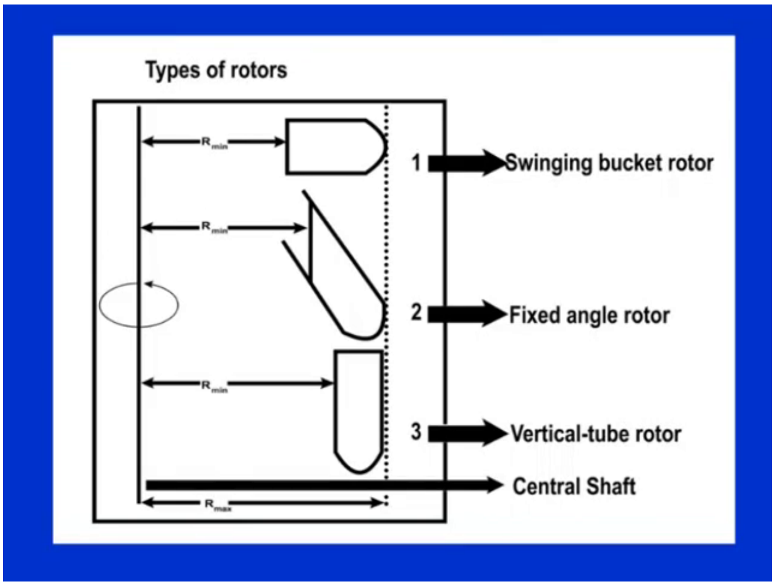
So, that is also another important part that you can calculate, the sedimentation time a spherical particle and you can also work for the same thing for aspherical particle. This could be a basis for, you can say how much time spherical particle can be separated or different particles could be separated by a centrifuse the centrifugation step, all right?

(Refer Slide Time: 29:32)



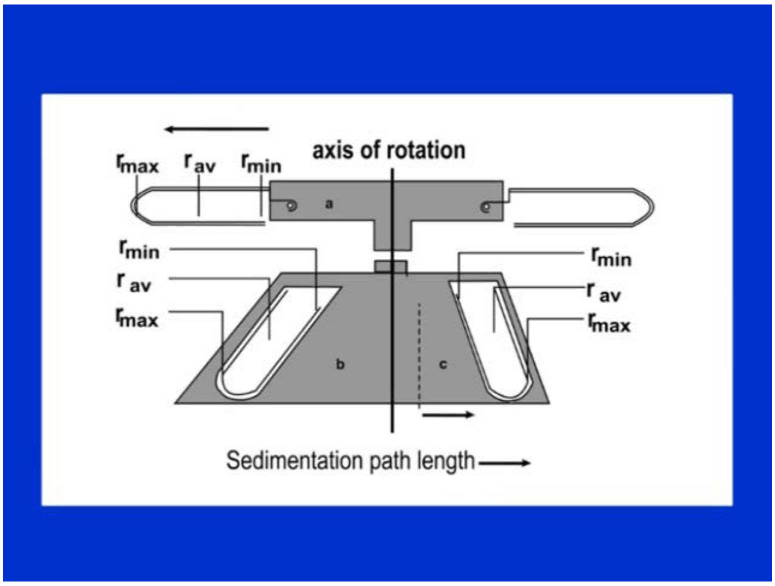
Now, another term like sedimentation rate, we were talking about as we have discussed and we have talked about this equation here. These complex variables which affect the sedimentation properties of a mixed population of particles, they also depend on say concentration of the suspension nature of the medium design and handling of the centrifuse.

(Refer Slide Time: 30:03)



All these things affect that. For example, we were talking about the radial distance and if you see here there are three types of rotors, here one is swinging bucket rotor, one is fixed angle rotor, another is vertical tube rotor. Now, they have when the centrifuse is switched on, they will have different like a swinging bucket rotor, will extend out as in the direction of the centrifugal field, which is outward radially outward. These angular ones they will remain as such, because they are kind of put in a fixed angle and vertical also are fixed in a fixed angle. So, they will now, the particles at different places will experience different centrifugal force.

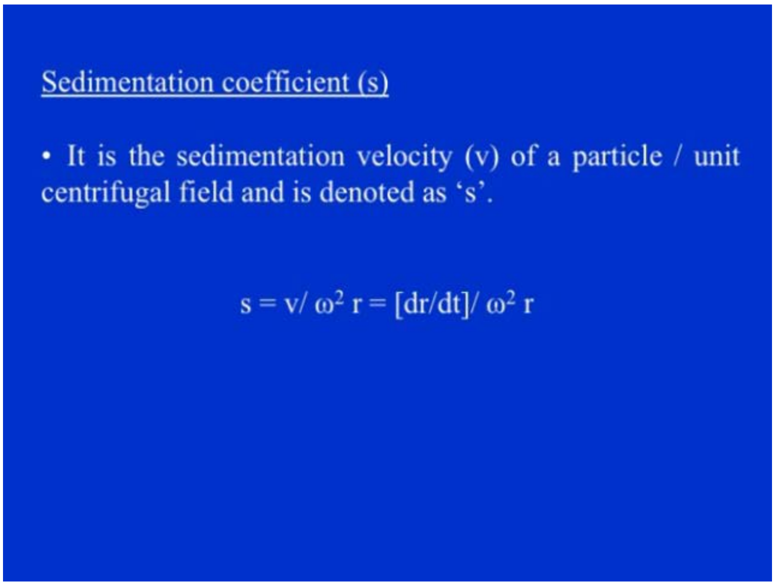
(Refer Slide Time: 30:46)



Now, if you can see here, this is very clear in here, this is the axis of rotation and this we are just showing for this particular one only, so this is axis of rotation, you have this is for this swinging bucket rotor. You can see that particle will be here as it is in solution mixed in solution then there will be different centrifugal force at r minimum r average and r maximum, so many times when you want to calculate the centrifugal field for a particular sample. May you can take r average or r effective r could be taken in here r average could be taken in here. Now, likewise when you are considering the spherical particles and if you are not very sure about the radius of the particle like we are utilizing, then r effective could be taken in there.

So, here since this will also affect geometry of rotors will also affect and the particle, where it is in suspension will affect its sedimentation rate, will be affected by that, because there will be experienced different centrifugal force. Likewise in angled rotors also, you have three like distances from the axis of rotation, that is r minimum here and at this place it is r maximum. So, r average could be taken for calculations different various calculations here.

(Refer Slide Time: 32:21)

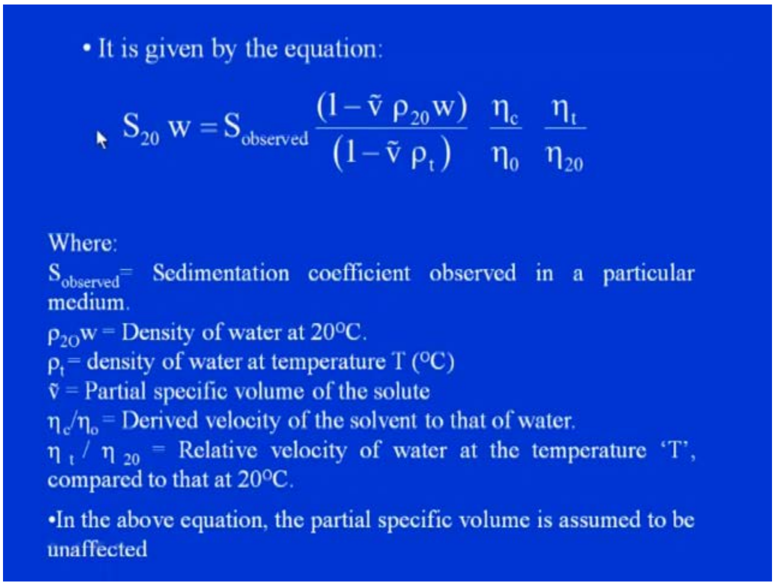


Now, there is another term which is quite important, that is sedimentation coefficient. Sedimentation coefficient is the sedimentation velocity of a particle per unit centrifugal field. So, sedimentation coefficient could be expressed as sedimentation rate or sedimentation velocity of a particular particle per unit centrifugal field and it could be denoted by this particular equation that is small s, v that is velocity upon omega square r. So, sedimentation coefficient is another important part and sedimentation coefficient as we will see the sedimentation rate could be expressed in terms of sedimentation coefficient.

Now, many times since this is like can be done in different ways. There is another term called standard sedimentation coefficient. Now, standard sedimentation coefficient of a substance in water at 20 degree celcius is taken. Now, since sedimentation rate studies may be performed using a wide variety of solvent and solute systems and even at different temperatures. When you do that, the sedimentation coefficient might come different. So, it could be like corrected to a constant value of standard sedimentation coefficient, which is taken in water at 20 degree Celsius.

Now, the measured value of sedimentation coefficient, which is affected by temperature solution viscosity and density is often connected or it is connected to a value that could be obtained in a medium with viscosity and density of water at 20 degree celsius. This could be expressed as a sedimentation coefficient in water or standard sedimentation coefficient.

(Refer Slide Time: 34:16)



This is given by this particular equation, so when it is corrected for this, so as 20 w, it should be for water. So, as observed and these parameters are taken to correct this, where you can see as observed is the sedimentation coefficient observed in a particular medium then this is rho, that is density of water at 20 degree Celsius, then density of water at temperature T particular temperature T.

You have taken this is partial specific volume of the solute, this is partial specific volume of the solute. This terms here that is derived velocity of the solvent to that of the water and this one here. This term is relative velocity water at temperature T compared to that at compared to that at 20 degree celsius. So, all these things could be like sedimentation could be corrected to standard value here and this could be calculated for certain very accurate calculations.

So, now sedimentation coefficient is usually very small for most biological molecules, so this particular one, now basic unit for convenience here is taken as 10 raise to power minus 13 second. Now, it is denoted as 1 Svedberg unit or 1 S S, we can call it, so this like when you have different kinds of constituents different kinds of particles they sediment at different rates. As we have seen sedimentation coefficient, we have taken that is per unit field, it is taken velocity per unit field. So, for convenience and for like it is ery like we said sedimentation coefficient is very small for most biological molecules 1 S equals 10 raise to power minus 13 second. So, what does that means?

It means that a molecule possessing a sedimentation coefficient of say 5 into 10 raise to power minus 13 power seconds will have a value of 5 S. So, like as the sedimentation coefficient increases, it means the sedimentation rate also increases. So, this gives an idea about the size of the molecule as it is influenced by shape size and density of the particle.

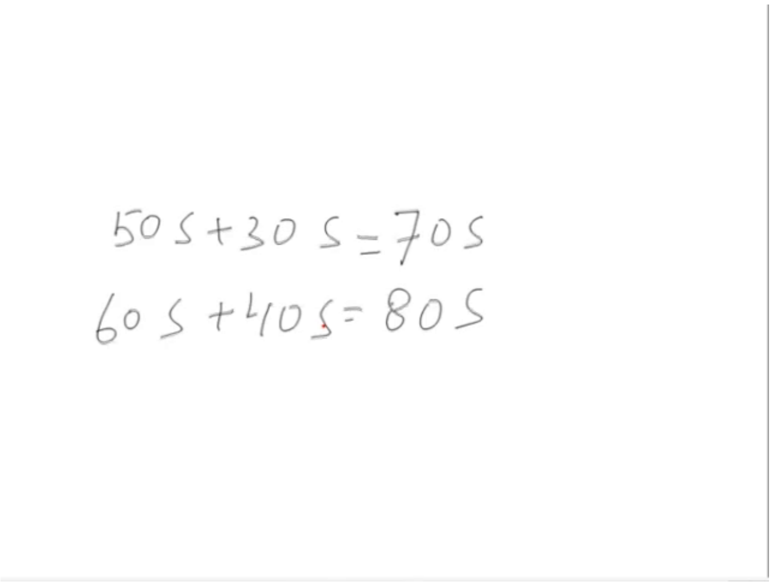
Therefore larger the particle larger is its S value. Now, this S value is comes from the is in honour of a scientist Svedberg. As we have discussed in the history of centrifugation development of centrifugation method and in his honour the name is given for his pioneer, pioneering work. So, this gives an idea about size of the molecule and it is influenced by various factors as we have discussed about.

Now, larger the particle, so you have larger particle and larger will be the S value, that is its Svedberg unit and faster would be its sedimentation, so if you consider sedimentation coefficient in Svedberg units, the enzymes and soluble proteins have only small values that is from 2 to 25 S. Now, as you go along bigger like say sub cellular organelles, this will increase. For example, nucleic acids have S value in range of 3 to 100 S, depending on where from you are isolating this.

Ribosomes and polysomes might have 20 to 200 S of value, then viruses, the value could range from 40 to 1,000 S in membranes, which could range the value or Svedberg unit value could range from 100 to 100 into 10 raise to power 3 S. Likewise for mitochondria it could range, it could range from 20 into 10 raise to power 3 to 17 into 10 raise to power 3, that is around 17,000 S and in nuclei like other like for nuclei it could be between 4, 010 raise to power 3 to 40, 010 raise to power 3.

Earlier, we have talked about nucleic acid that is 3 to 100 S, not nuclei it is nucleic acids. So, you have range of sedimentation coefficient here and which will vary from very small for soluble proteins or enzymes to very large for say nuclei or whole cells. Remember sedimentation coefficients are not additive.

(Refer Slide Time: 39:57)



For example, we have ribosomes which are bacterial ribosomes, prokaryotic ribosomes are 70 S and the eukaryotic ribosomes are 80 S. Now, if you consider both of them then individual ribosomes are for prokaryotes, it is 50 S plus 30 S. So, it does not equate to 70 S.

So, when the two ribosomal sub units, they are separate they sediment at different rate as compared to the the ribosomes full unit, when they come together they sediment at a rate which is cannot be added here. Likewise, this is also the individual units for eukaryotic ribosomes is 60 S and 40 S, which does not equals to 100 S. Rather, it is 80 S for that matter, so what does that mean? That is the sedimentation coefficients or S units in terms of svedberg unit. They are not additive as such and so they have to be calculated separately.

(Refer Slide Time: 41:11)

Protein	S value (Svedberg units)	Molecular weight
Pancreatic trypsin inhibitor	1	6,520
Cytochrome *c*	1.83	12,310
Ribonuclease A	1.78	13,690
Myoglobin	1.97	17,800
Trypsin	2.5	23,200
Carbonic anhydrase	3.23	28,800
Concanavlin A	3.8	51,260
Malate dehydrogenase	5.76	74,900
Lactate dehydrogenase	7.54	146,200

Now, this is a table which shows S values of and molecular weights for various protein fractions and as you see here as the molecular weight increases, so is the S values, because their size will increase and they will be sedimenting at a higher rate. Like I have shown you here or like I have told you for different sub cellular organelles or cells, as the size increases and density or other factors increases, the S value also increases. So, this was basic principle and the various equations which we have discussed for centrifugation process of centrifugation.

So, in this lecture to summarize, we have discussed about the basic principle of centrifugation where we have discussed how the rate of sedimentation or the velocity of sedimentation is affected by various factors. Including one factor was applied centrifugal field that is the applied centrifugal field could be expressed in terms of either angular velocity into radial distance square of angular velocity into radial distance or it could be also expressed in terms of relative centrifugal field.

Now, most of the time the relative centrifugal field is utilized, another important factor that we have discussed about is that the sedimentation or the velocity will also depend on the mass of the particle the viscosity and density of the medium. Also whether a particle is a sphere or a spherical is it spherical and non hydrated or aspherical and hydrated molecule. So, depending on that these sedimentations rate will differ.

Now, as the particle moves in a medium or a solution when applied centrifugal field is switched on or centrifugation centrifuse is switched on and there is a centrifugal force on the particle. Then a frictional force opposing force also develops and this opposing force opposes the motion of the particle that is forward motion of the particle, or you can say outward motion of the particle and this is like after accelerating for certain time. Like very quickly the opposing force, that is forward force and the opposing force become equal and particle moves in the solution with a constant velocity.

You can also, one can also calculate the time of sedimentation or the extent of sedimentation in particular time. As we have discussed a spherical particles or hydrated particles which are like elongated or rod shaped particles, will experience more friction. Therefore, rate of sedimentation is lower and one has to like this particular factor where the frictional coefficient for that is ratio of f frictional coefficient experienced, if frictional coefficient of sphere needs to be taken into account.

Also, we have discussed about that the sedimentation coefficient that is sedimentation rate or sedimentation velocity per unit field actually. Centrifugal field and form sedimentation coefficient we have derived in the honour of scientist T Svedberg. The Svedberg unit, which is 10 raise to power minus 13 seconds, now it is smaller for or quite small for say proteins or soluble proteins and other enzymes, so it is expressed in terms of Svedberg units. 1 Svedberg unit is 10 raise to power minus 13 seconds. So, you can compare different particles or different molecules to be separated by a molecules to be separated or sub cellular organelles to be separated on the basis of Svedberg unit.

So, it gives an idea about size like greater the size greater would be the units and so as the, like say if you have 5 S as the units increase like from say 1 S to 100 S to 1000 S. The rate of sedimentation also increases and so you will have an idea that the particle is smaller or larger and densities size and shape all these things could be looked into that yes higher the Svedberg units then higher the sedimentation rate, because of particular nature of that particle in terms of physical properties. We have discussed.

We have discussed, so in these two lectures one earlier than this, we have discussed, we have given an overview and in this lecture, we have discussed about the basic principle of the centrifugation technique. In the next lecture, we are we will be starting we will start discussing like different types of centrifuse different types of rotors the care needs to be taken a for the rotor the safety aspects which are involved while using centrifugation technique. Also me for the preparative methods in terms of say differential centrifugation or density gradient centrifugation. So, coming lectures will include all these all this material as we go along.

Thank you.