Cellular Biophysics
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Part: 02
Sedimentation and Centrifugation

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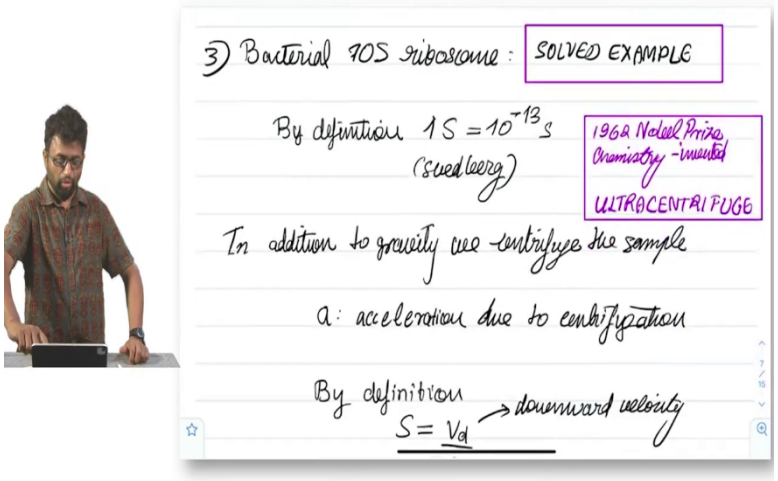

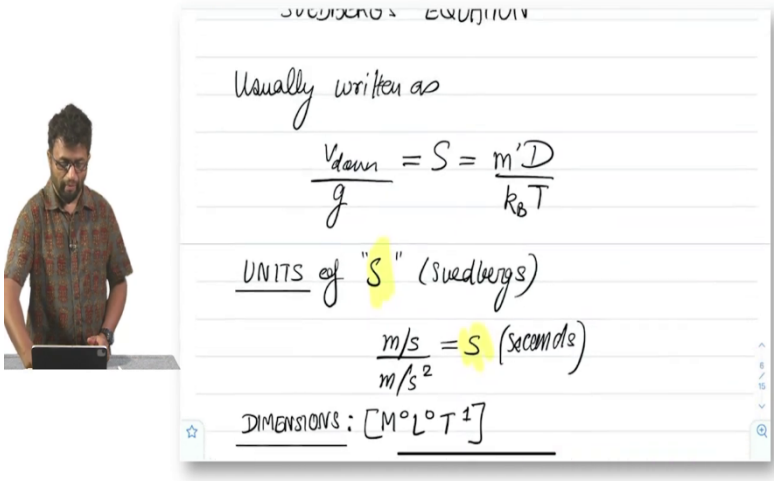

Now, the bacterial 70S ribosome unit this is something that you have all read in your biology textbooks maybe even in high school I do not know. And since many of you may be attracted to structural biophysics and may have heard about the work of Ada Yonath and Venki Ramakrishnan, you at least have read about this subunit. Certainly, it is important as a marker for phylogenetic analysis in terms of the RNA sequence. But suffice to say that, we will try to look at it from a sedimentation perspective, because that S is something we want to know

what does it tell us? What, does it mean, ribosome large ribosomal subunit of E. coli is 70S. That is the question we want to answer.

So, let us go at it. So, by definition, we say that 1 Svedberg unit is equal to 10 to the power minus 13 seconds, this is a constant. And this is defined from here. Now, Svedberg by the way, in fact, obtained was awarded the 1962 Nobel Prize for Chemistry for his invention of the ultra centrifuge. Some of you again, in biology lab may have had the good fortune of seeing one or using one even those of you who have not, you are welcome to go back and read the Nobel lecture, because all these are online.

Because it tells us a lot about the fact that many people at the early days of modern biology, were inventors, they were making things because when they faced a problem, when they saw something that was not functioning or did not address the need that they had for doing certain kinds of experiments, they made up a device, they invented a device.

And so this is something that you can also do, especially if you know the principles. And I know sometimes you feel, I am not an engineer, I am not a physicist, I am not a mechanics trained person, I am not a technician, but you have to be curious and you talk to the right people, and you can figure this out. Because the means are today so easy, that you can actually be both a scientist and inventor.

So, in addition to gravity, when we centrifuge the sample, we add acceleration to it and this is acceleration due to nothing but centrifugal energy. This is why it is called a centrifuge. If I take a thread and I swing it, you see that the thread becomes horizontal to the axis of the plane of our planet, which means that it is again acting against gravitational acceleration, in fact opposing successfully opposing the motion, you know this when you are on a merry go round and you went very, very fast, you were hanging out at times. So, centrifugal force can be very powerful, it can even be more powerful than the gravitational acceleration the thing that keeps us on the ground.

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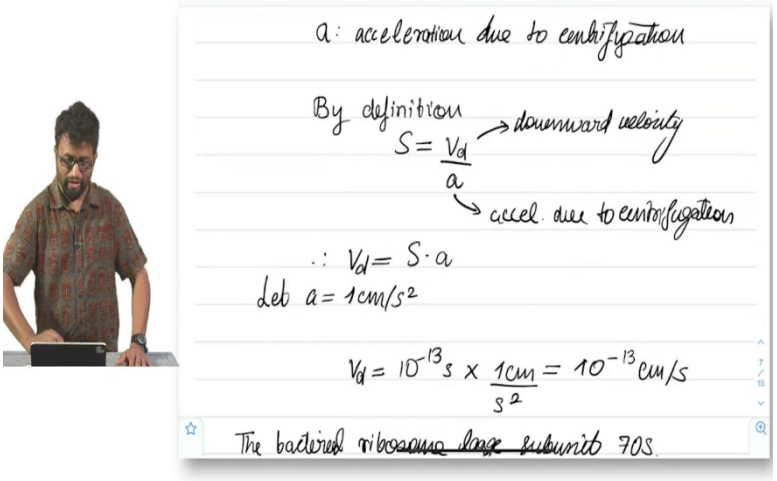

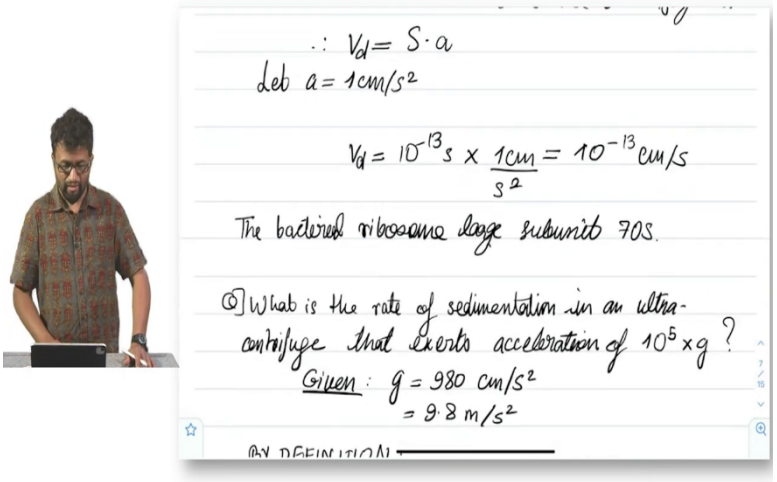

So, acceleration due to centrifugation by definition, means that S is equal to Vd by a, where a is now whatever factor of gravitational acceleration that we are interested in, means that we can rewrite this as Vd that is downward velocity is equal to S into a. And if we assume for a moment, that a is equal to one centimeter per second square, then Vd is 10 to the power minus 13 centimeters per second. So, for an acceleration of 1 centimeter per second square, downward velocity will be 10 to the power minus 13 centimeters per second. Again, this is coming from definition of Svedberg unit. But the bacterial ribosome's subunit is 70S.

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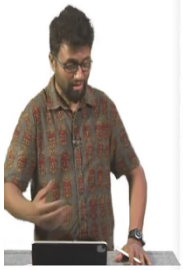
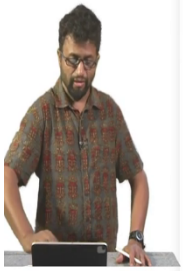


Q] What is the rate of sedimentation in an ultra-centrifuge that exerts acceleration of $10^5 \times g$?

Given: $g = 980\ cm/s^2$
$= 9.8\ m/s^2$

BY DEFINITION:

$$V_d = S \cdot a$$
$$= 70S \cdot (10^5 \times g)$$
$$= (70 \times 10^{-13})\ s \times 10^5 \times 980\ \frac{cm}{s^2}$$
$$= 6.86 \times 10^{(-13+4+5)}$$
$$= 6.86 \times 10^{-4}\ cm/s$$



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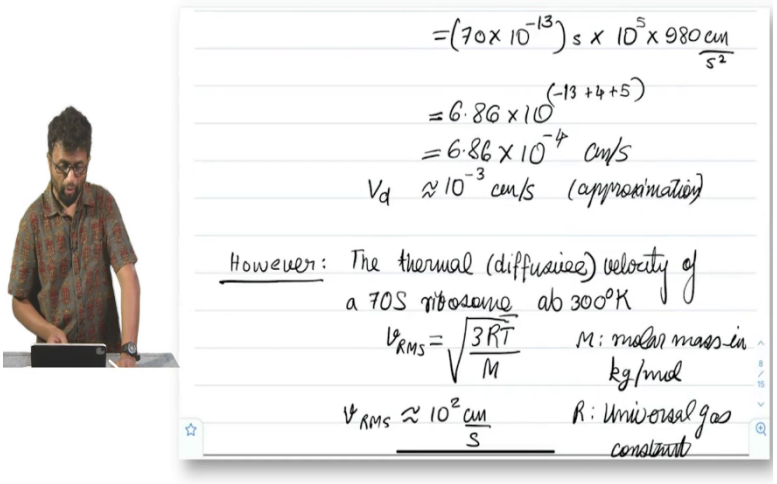
$$= 6.86 \times 10^{-4}\ cm/s$$
$$V_d \approx 10^{-3}\ cm/s \quad \text{(approximately)}$$

However: The thermal (diffusive) velocity of a 70S ribosome at 300°K



So, what will the rate of segmentation be? This is what we asked ourselves. So, we need a few numbers, we need gravitational acceleration, all of us know it is 9.8 meters per second square or 980 centimeters per second square. I am going to use the CGS units for the moment.

By definition, V downward is equal to S into a as we said, so, the 70S become 70S into 10 to the power 5 g and because we are centrifuging it at one lakh g that is 10 to the power of 5 g, this is what an ultra centrifuge can achieve by the way and that is why they are expensive, that is why they need good maintenance and they need modern ultra centrifuges have very sophisticated circuits to create a vacuum. Very high vacuum in millibar ranges of pressure and they have very well lubricated rotors.

The centrifuges are made of light but strong alloys typically aluminum and the tubes are standardized so that there are almost no imbalances when you are at that speed even tiny imbalances can cause catastrophic damage. Because this is partly why what we call balancing the tubes is critical in ultra centrifugation. If you thought that running it at 5000g was bad enough, think about it that you are going at one lakh.

So, in this case, we substitute the numbers Vd is equal to 70S into 10 to the power 5 g and we get an answer, which is that Vd is equal to 10 to the power minus 3 centimeters per second, which is approximated from the exact answer which is 6.86 into 10 to the power minus 4 centimeters per second.

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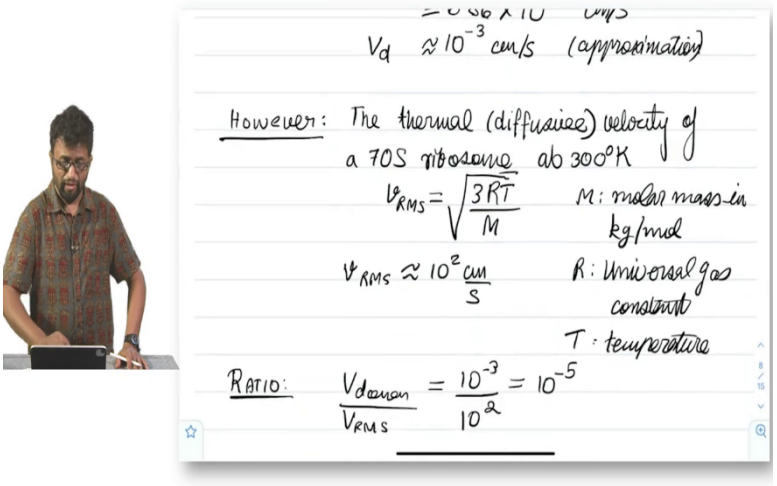

However, thermal diffusion also has velocity associated with it and we will come back to this later, but just to remind you, the velocity of the rms velocity the thermal motion random motion Brownian motion of a particle means that at any given instant any object has a certain velocity by which remove which is proportional to the thermal energy, ambient energy which is given by the Boltzmann's constant or the universal gas constant and is inversely proportional to the mass when we substitute the numbers for 7S ribosome subunit based on its molecular weight, we get a rms velocity of 10 to the power 100 centimeters per second.

Now, please compare this 10 to the power minus 3 centimeters per second to 100 centimeters per second that is to say one thousandth of a centimeter per second is the rms velocity, I am sorry is the downward velocity whereas 100 centimeters per second that is to say 10,000 times larger is the rms velocity than the centrifugal velocity. So, that means, we should never be able to centrifuge because the velocity of one is much greater than the other.

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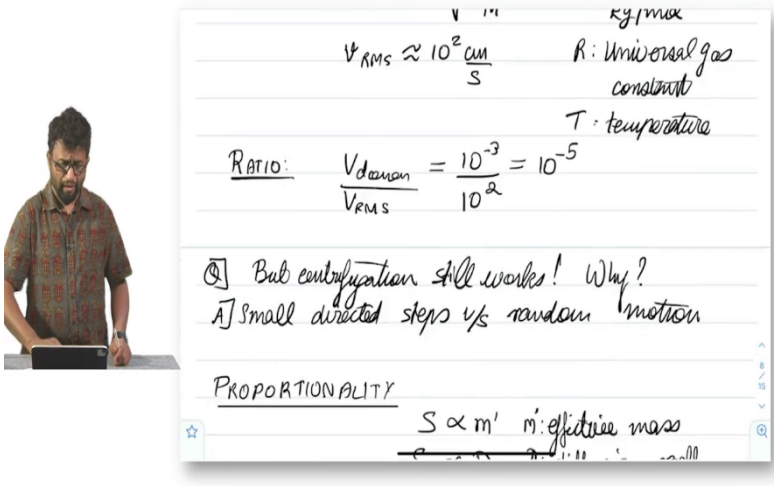

The funny part is that centrifugation still works, so, if we go to a lab and do the experiment, and I will not be able to show you that, but I will do a demonstration to suggest that sedimentation works and so, we know this. So, why does it work when all the other factors seem to affect it, it turns out that random walks and Brownian motion which we will have an opportunity to discuss in great detail in the next section are non directional, they do not have any sustained direction in which they work.

As a result even though the motion is very fast, it is undirected and overtime it has very little or no effect on the motion of these particles, so, long as there is a sustained downward velocity due to centrifugation or sedimentation as the case might be.

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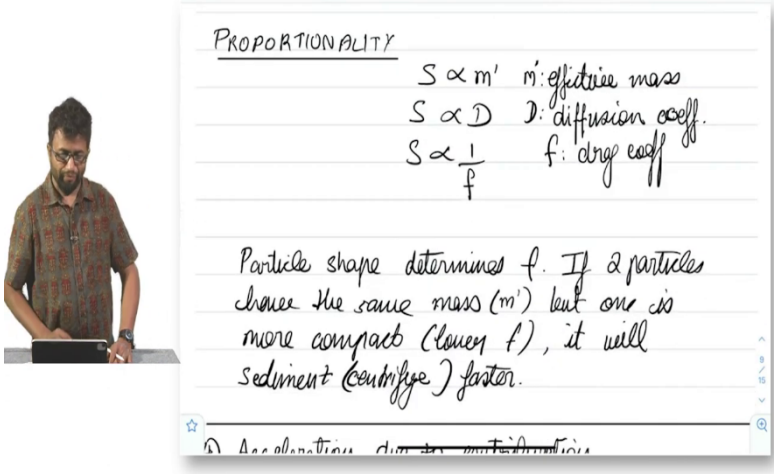

So, to summarize the proportionality of the Svedberg unit, Svedberg constant is directly proportional to the effective mass, directly proportional to diffusion coefficient and inversely proportional to the drag coefficient. In a sense, the drag coefficient is a measure not just of the viscosity, but also the shape of the object. So, if two particles have the same effective mass, but one is more compact than the other, then the, then it will have a lower effective drag coefficient and therefore, it will be faster at sedimenting or a higher S rate because of the shape of the object itself.

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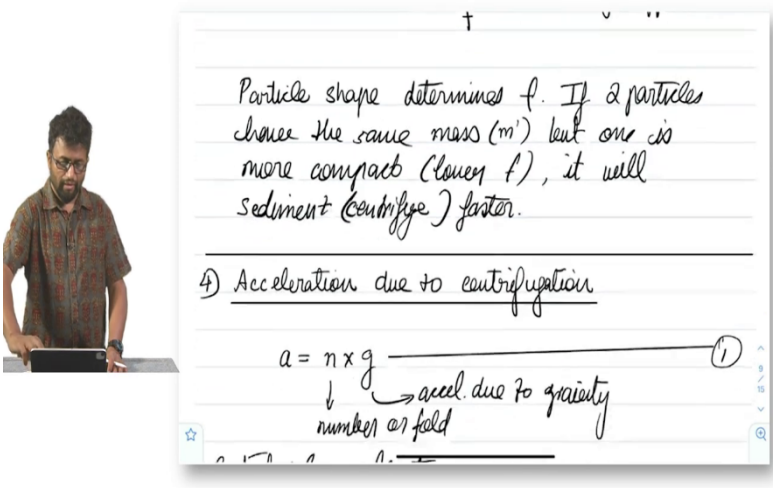

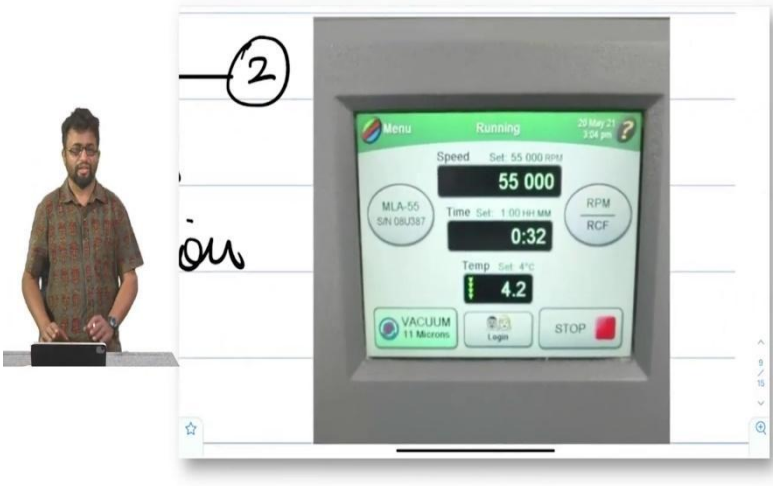

So, we go on to acceleration due to centrifugation. This is a practical aspect, which I just want to highlight here is illustrated in this display. So, this is a display of an ultra centrifuge, you remember I mentioned vacuum, so, the vacuum that the ultra centrifuge can exert is up to

1.1 micrometer Mercury height, it also gives you time, it gives you the temperature so, they also cooling because at lower temperatures, there is less chance of thermal motion so you cool them so, you improve your separation, we will get to that in a minute and you can set the speed and the speed can be set in terms of RPM, that is rotations per minute or RCF and this is the subject of what I want to talk about for this segment right now.

So, acceleration can be considered to be a factor of the gravitational acceleration that is a is equal to n into g, n is the number of fold of g. So, typically if you ask people who are working with certification for many years, how many g's are you centrifuging for this is the typical answer that you will get all the dada's and didi's the PhD students was who talks, scientists who work in a lab. And, and this is important, because this is of exactly what I was saying earlier, a factor of the gravity how many times of gravitational acceleration are you using because we know that gravitational acceleration is constant, it is universal.

So, surgical acceleration itself that is to say how fast you how much acceleration you get in terms of a centrifuge is dependent on r into omega square (ω^2), where omega is the angular velocity of rotation. You remember your high school physics, which is in terms of radians per second.

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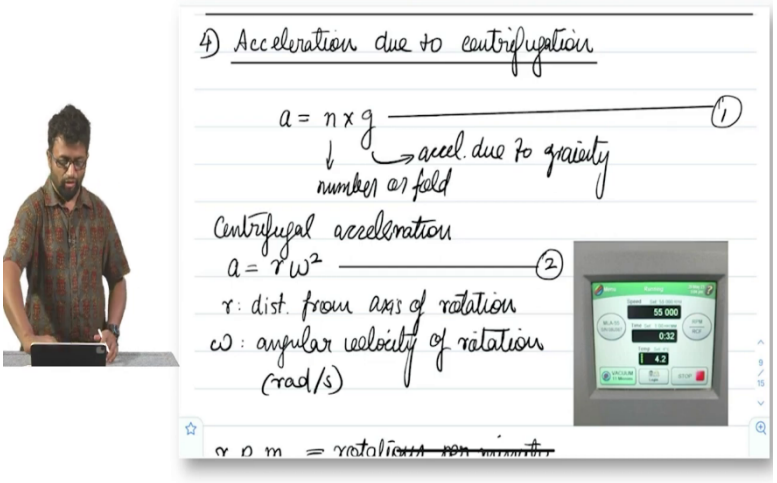

So, rpm stands for rotations per minute and rps stands for rotations per second this is kind of obvious answer, one is in terms of per unit minute and the other is in terms of per unit second, which means one rps corresponds to 60 rpm obviously.

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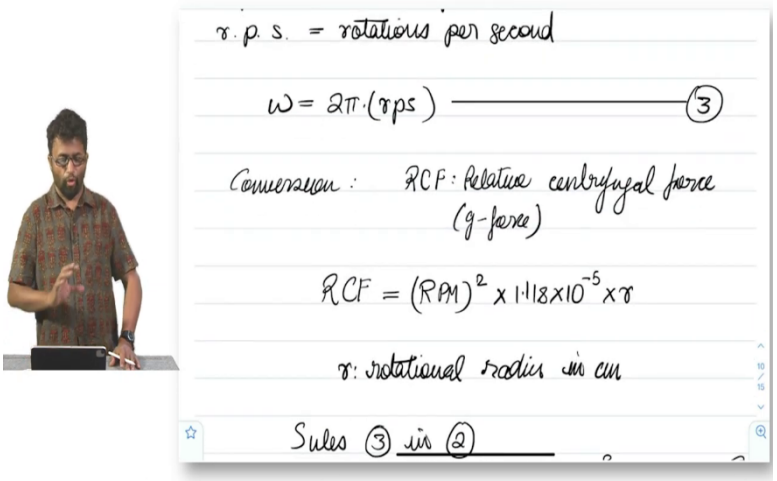

ω is equal to 2 π into rotations per second. Now, we want to know what is the conversion of this g, g factor how many g's from rpm. So, in order to do that, we need to equate RCF and RPM if you Google search this question you will come up with a lot of manufacturers of centrifuges, thermo scientific, Beckman Coulter and companies who have put these numbers online and they tell you RCF is equal to RPM square into 1.118 into the 10 to power minus 5 into r.

$$RCF = (RPM)^2 \times 1.118^{-5} \times r$$

How did they get this number, how? So, that is what we are going to try to explain in terms of derivation.

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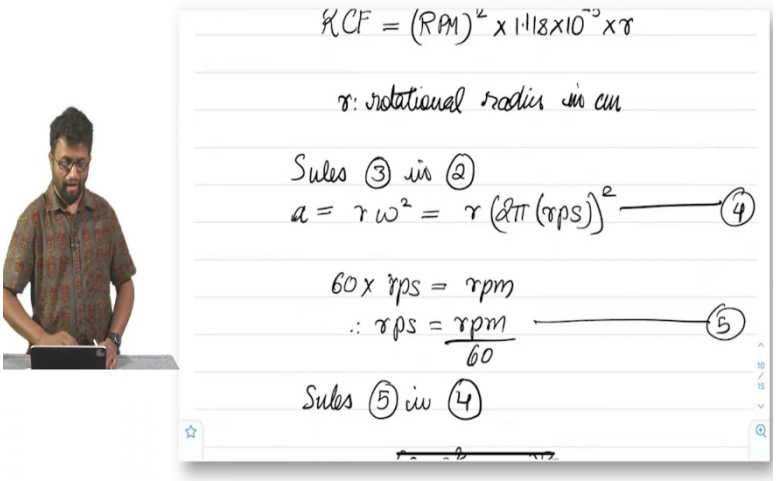

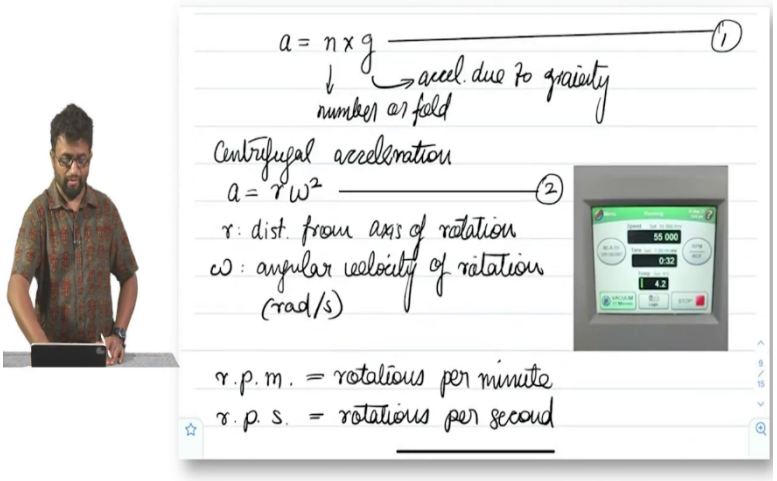

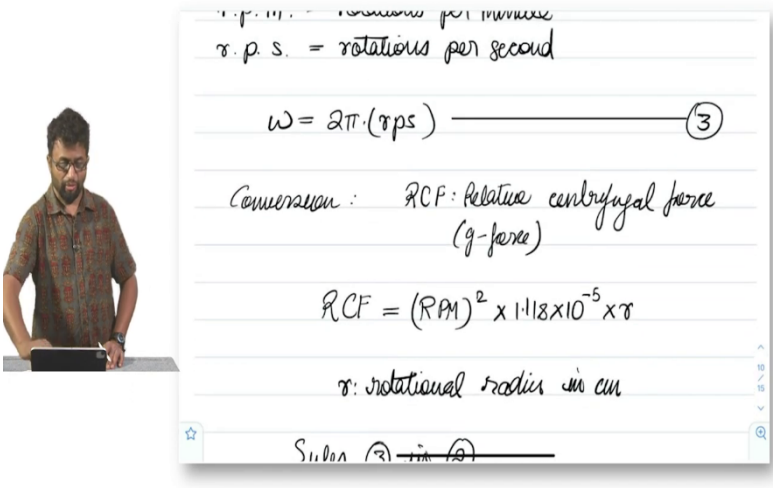

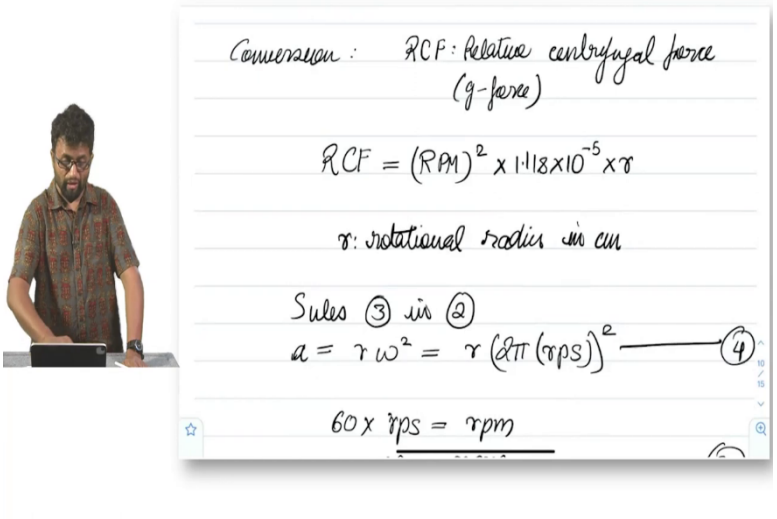

r here is the rotational radius in centimeters meaning to say if your rotor is one centimeter or other the sample is held at one centimeter from the center of rotation or one meter from the center of rotation that is what this r is defining for you.

$$a = r\omega^2$$

So, a is equal to r omega square, this is from simple rotational motion as we said earlier, equation number two and when we substitute omega as 2 π rps which we said is by definition from equation 3, then we end up with the possibility to now write out the conversion from rpm to rps as I said 60 rpm is one rpm, therefore, rps is rpm by 60 and substituting we get because, we want to convert rpm to rps remember.

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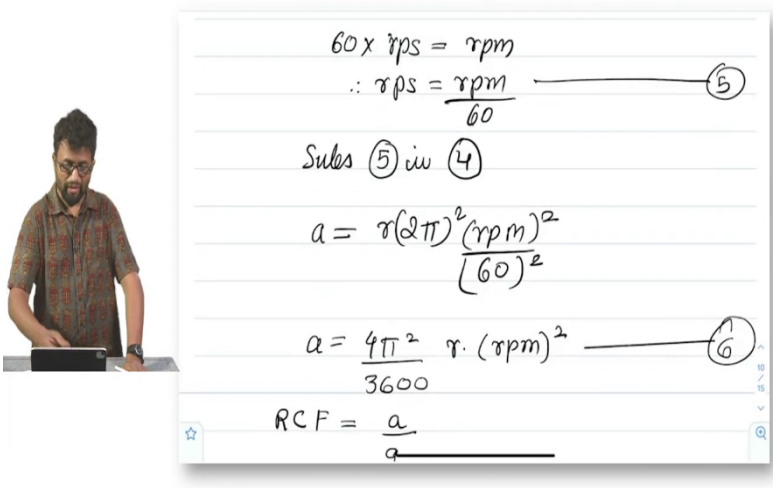

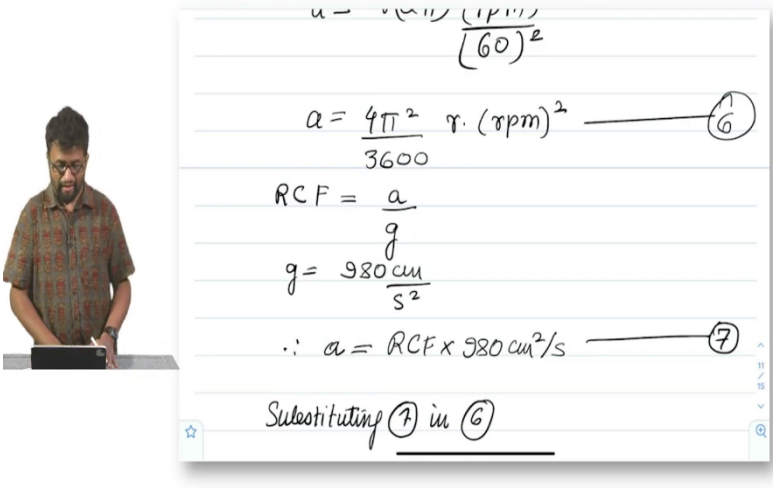

So, we substitute and we get a is equal to 2 pi square whole square rpm square by 60 square all this leaves us with a number which is a is equal to 4 pi squared upon 36,000, 3600, sorry into r into rpm square. RCF on the other hand, rotational centrifugal force is nothing but the fold g of acceleration we said that right how many g's remember.

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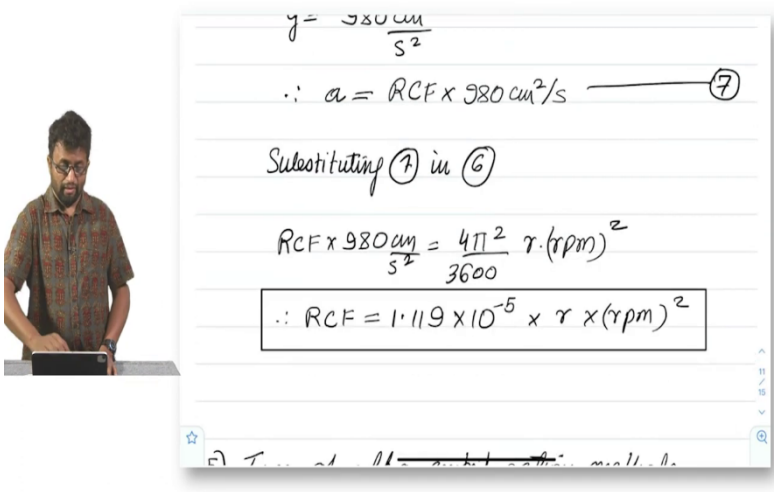

So, when we now take this equation and say therefore, that a is equal to RCF into 980 centimeters square per second using CGS units then RCF into 980 centimeters squared per second is equal to 4 π^2 upon 3600 into numerator r into rpm square. Rearranging the terms with RCF on the left side gives us 1.119 into 10 to the power minus 5 you can use your calculators to check into r into rpm square, this is how we get the number.

So, we now have a conversion factor for relative gravitational acceleration RCF and RPM and if you know the rpm, you can get your RCF and if you need to know the rpm for unknown RCF you need to rearrange and take square roots on the right side. So, this is your simple derivation of a very practical use and day to day requirement for centrifugation and centrifugation.

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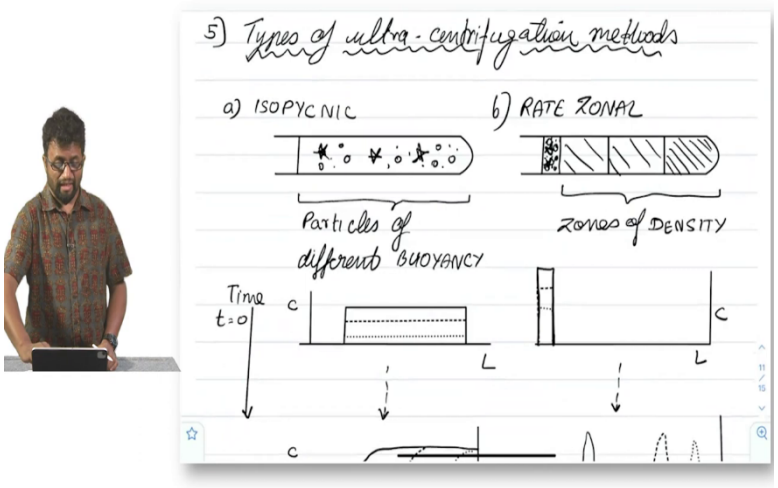

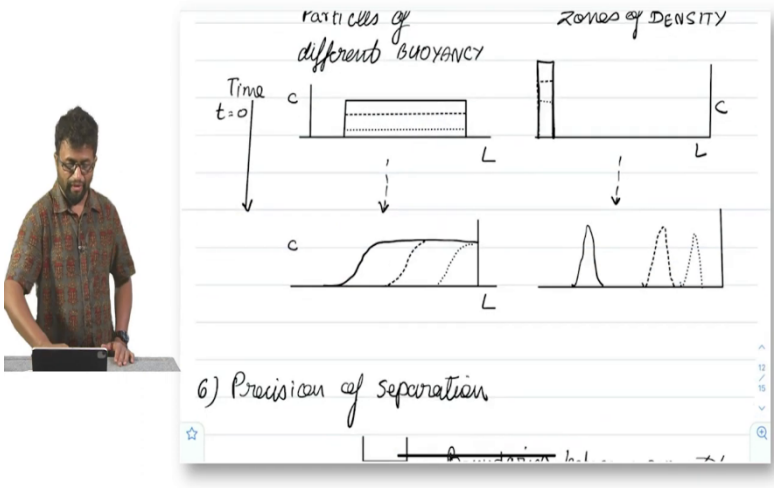

So, what are the types of ultra centrifugation methods that we can typically see? So, the two that we are going to discuss right now are isopycnic and rate zonal. So, in isopycnic, the idea is that you can centrifuge mixed sample based on the fact that you already know that the party that you want to centrifuge let us say it is nuclei, you have crushed some cancer cells, you made a lysate, say resuspended in buffer and that the nucleus has a different buoyancy as compared to the membrane as compared to the endoplasmic reticulum as compared to the Golgi.

So, knowing this, if you now centrifuge this mixture fast enough, over time, you will get different zone different regions in which the different organelles will form. And this is allowing you to separate them out. On the other hand, rate zonal centrifugation says that the

density of different reagents let us say ficoll or sucrose can be used to find that position at which the density is matched to the object that you are trying to separate.

In a way we know that the density of the object and the density of the fluid are related to the buoyancy. So, you could argue that these two methods are not very different from each other. But in terms of practical implementation, in rate zonal centrifugation, we layer our sample on top of the gradient over here where you see the yellow mark, and then centrifuge it.

So, over time we get clean zones that differentiate the different segments that we want to look at. This assumes that you know that the object that you are trying to centrifuge. Let us say chloroplasts from plant cell extracts that they come at a certain density, their density matched concentration of ficoll or sucrose must be present in your gradient, so that you can then go back and take it out. And you need some spectral methods to test whether what you have done is right. So, that was regard to typical methods of ultra centrifugation.

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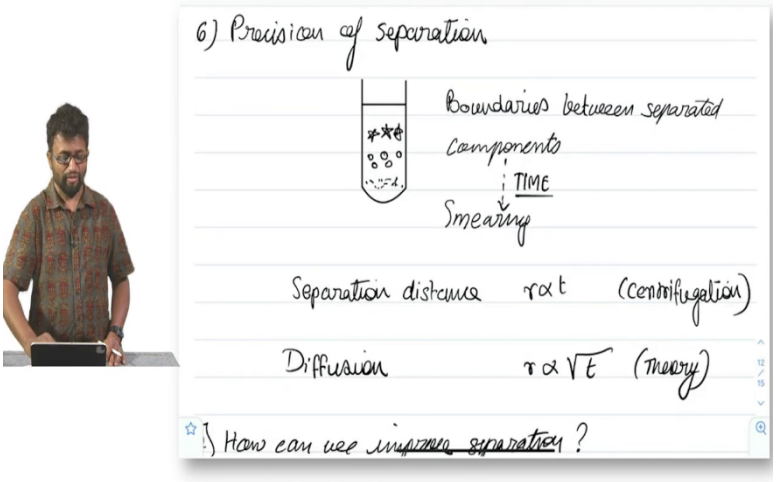

Finally, I want to talk a little bit about the precision of centrifugation. And what can cause differences in how well we separate a material. So, the answer to that lies in the question how clear the boundaries between the separated regions, when you take out a tube and you look and you have, like we talked about at the beginning, about sedimentation of red blood cells or blood cells from blood, we see clear separation boundaries, we expect to see clear separation boundaries.

So, in this sense, what we do not want to see a smearing over time. So, if you leave that tube for a while, you expect that the boundaries will blur from one another. And we know that the

separation distance is proportional to the centrifugation time and for diffusion, it is proportional to the square root of the time, meaning to say it is slower, with the same amount of time, it only increases as a square root of it. So, if in four seconds, the separation distance increases by a factor of 4, then the diffusion causes the separation in by a factor of 2, slower.

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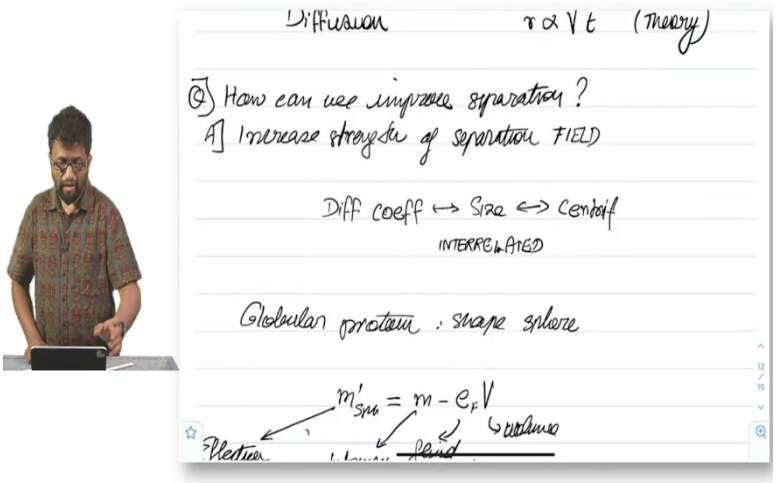

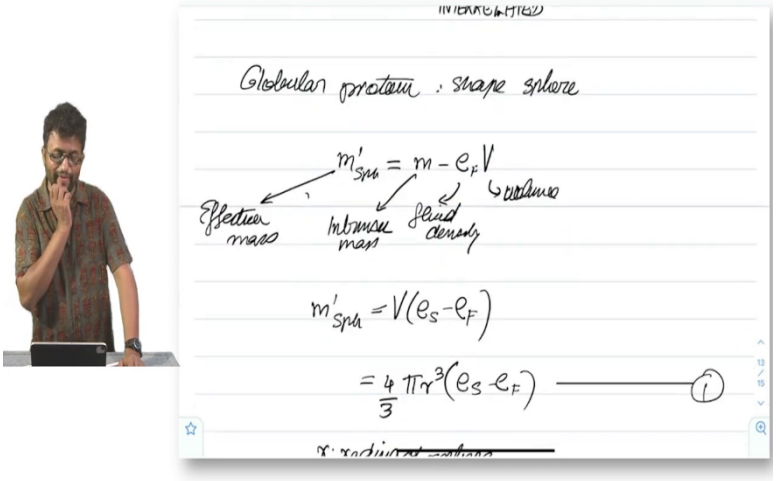

How can we improve separation? The simple answer is to increase the strength of the separation field. Because diffusion portion size and centrifugal force or tendency are interrelated to each other. So, for globular struck proteins of a shape that we can approximate as a sphere, we can say that the effective mass of the sphere is equal to mass, the intrinsic mass of the sphere minus the fluid density and the volume.

And the fluid density itself is in terms of in terms of the difference between the I am sorry, the effective mass of the sphere is in terms of the product to the volume and the difference between the solid density and the fluid density, which if we substitute for a sphere, the volume of the sphere equation four thirds pi r cube using r as the radius.

$$Vol = \frac{4}{3}\pi r^3$$

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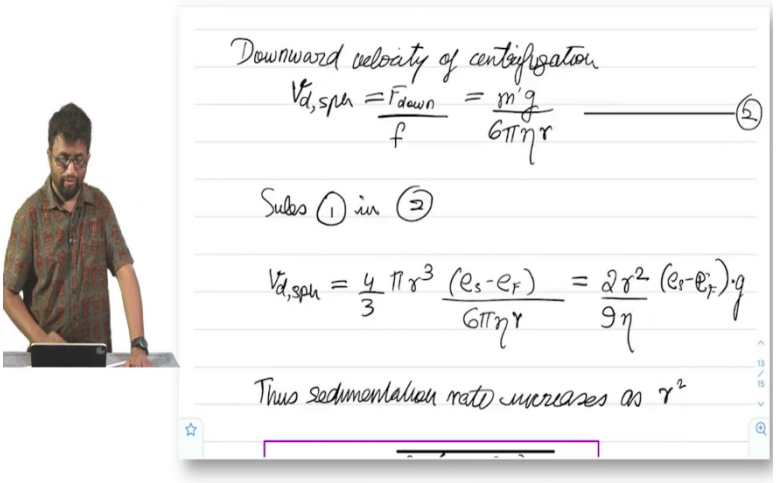

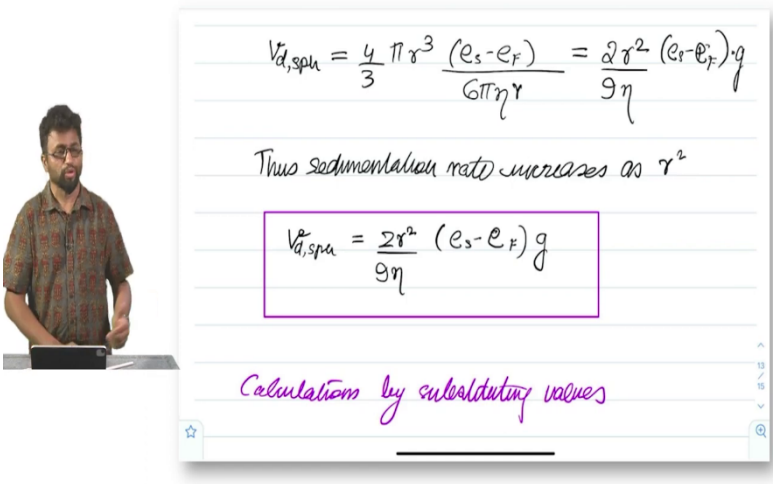

$$\frac{F_{down}}{f} = \frac{m'g}{6\pi\eta r}$$

We get a downward velocity of centrifugation, which is F down by f, which is m prime g upon 6 pi eta r Stokes drag coefficient in the denominator and m prime g in the numerator, substituting this equation into 2, we get a substitution which looks a little messy, but if you write it out, you should be able to check that the downward velocity of a sphere is equal to 2 into r square upon 9 eta all into the product of the difference between the solid and fluid densities multiplied by the g.

$$V_{down} = \frac{2r^2(\rho_s - \rho_f)g}{9\eta}$$

So, this is why ultra centrifugation is so useful because you get higher g's you get higher acceleration so, you are directly proportional to the velocity of downward motion. So, with that we end this segment on centrifugation.

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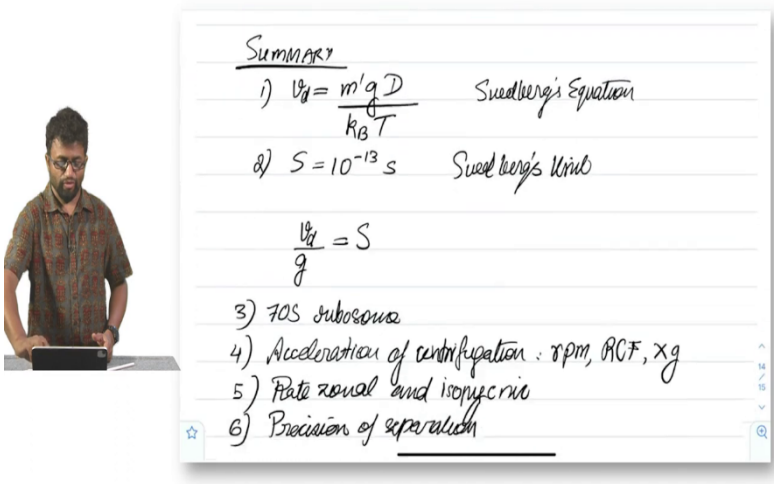

And I am going to summarize what we have covered so far. So, in summary, the download velocity or centrifugation is equal to the effective mass into gravitational acceleration with different quotients upon $k_B T$ this is Svedburg's equation, Svedburg unit is S is equal to 10 to the power minus 13 seconds is by definition, Vd by g is S which is the Svedberg symbol. The 70S ribosome is based on the speed at which it centrifuges in a certain gravitational acceleration and the acceleration of the centrifugation we said can be used to infer interrelationships between RCF, rpm and how many g's.

Rate zonal and isopycnic centrifugation are used differentially depending on whether we know the buoyancy or the density of the object. And we can also infer that we can improve the precision of separation through higher forces of centrifugation.