Cellular Biophysics
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Part: 01
Sedimentation and Centrifugation

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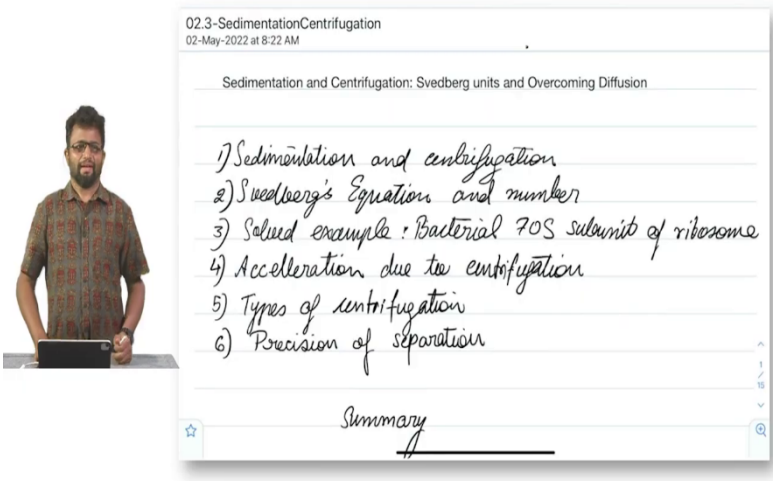

Hi, welcome back. We have been so far talking about fluids, fluid mechanics, viscosity and in the last segment, we spoke about the strange world of cells at Reynolds numbers. Today, we are going to continue in that sense. But on a slightly more practical note, in respect to the fact that the same properties of fluids that we have been discussing so far are some things we can exploit as cell, molecular biologists, biochemist, protein biologists, and molecular biologists in general. And that is something that I think many of you who may have taken a course in biology are familiar with namely centrifugation. So, that is the crux of this module.

And I am going to cover a couple of topics, which some of you may have heard of, but may not know the biophysics under it. And this is really to highlight the principle that illustrates, elucidates and gives clear insight into how centrifugation works, so let us get to it. So, we will cover sedimentation and centrifugation, Svedberg's equation, and Svedberg's number. We are going to use some solved examples.

Many of you know that monkey Venkat Ramakrishna's Nobel Prize along with other unit was one for solving the structure of the ribosome. So, the 70S bacterial large subunit ribosomal is a very important component of this. S stands for Svedberg units will ask a little bit of a question, what does it mean? We will then get to acceleration due to certification, something

that you see in a practical sense when you go to an actual centrifuge and do such an experiment. And then talk about the types of centrifugation and precision versus separation and summarizes some topic.

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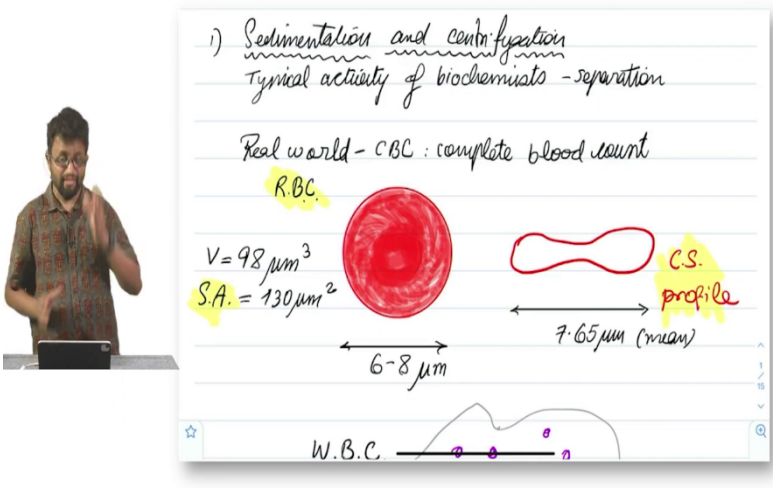

So, what is sedimentation and centrifugation? In the real world, if you have a health problem, and now we are in continuing to be in the pandemic of Corona, many times, clinicians, doctors, medical doctors will prescribe to you a tests to be done which is called CBC. So, those of you know the full form know that this is a complete blood count. And complete blood count involves sampling various aspects. And it depends on which country you are in about what all is involved in CBC, one of the important components are red blood cells, you see this here and I think you can follow my pen.

These red blood cells are often approximate volume of 98 micrometer cube. And I say approximate because there is a variation between people, individuals and a surface area of 130 micrometer square. Their sizes are between 6 and 8 microns. Now, I think you recall that in some of the previous lectures, we said that we treat the E Coli bacterial cell as a hydrogen atom or ideal model cell. In the same fashion, we treat red blood cells as an ideal model cell. From a biological perspective, this is a little strange, of course, because an ideal cell should have all the basic components of a cell.

For those of you who have taken more biology already know this erythrocytes or red blood cells in the human bloodstream, mature blood cells lack an important component that most people agree should be in every cell. Yes, DNA. They are enucleated. In fact, they do not

have a nucleus. This little dip in the center is a biconcave structure, which is why the cross sectional profile of the red blood cell looks like a doughnut in a way without the hole. The size of around 7.65 or between 6 and 8 micrometers is the mean diameter.

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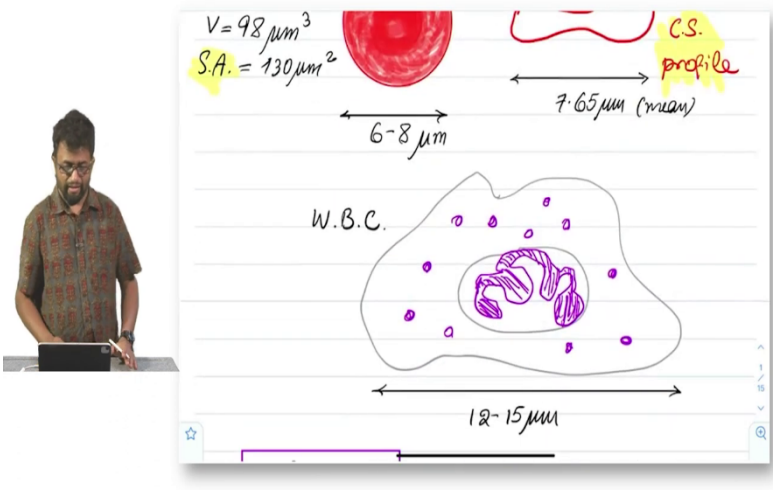

And contrast this with the white blood cell which is much bigger, it is about 12 to 15 microns. So, you could say roughly twice as large in terms of diameter.

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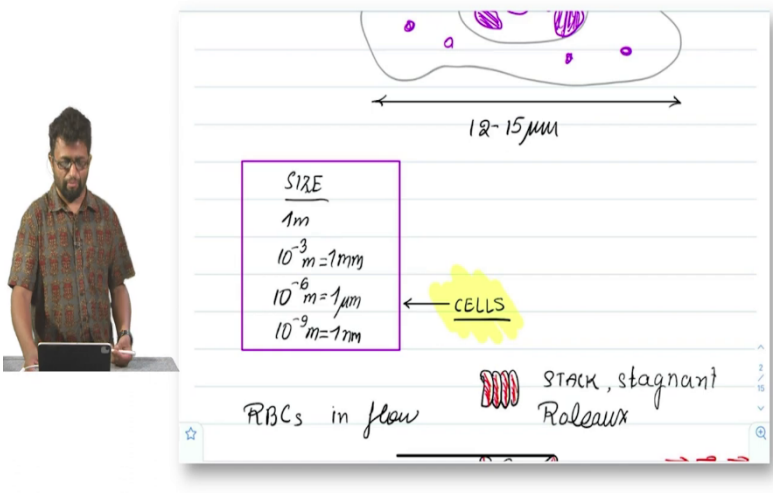

This means that because we have a fairly good idea of how big cells are and how many they are, we can use the blood count itself as a qualitative measure of the health of the individual. And this is fundamental to hematology, blood physiology and human physiology in general. This is why your doctor prescribes complete blood counts because they tell us a lot of things.

They are not what we can call objective indicators of what is going on, but they give a hint that something is off.

Just as an aside to remind you of units, conversions and conversion factors, we use the decimal system and the base 10 value system, because it has an advantage and so, as you see here, when we go from size scales, 1 meter that is 100 centimeters to 1 millimeter, that is by a factor of 10 to the power minus 3, meaning to say one thousandth of a meter is 1 millimeter. One thousandth of a millimeter is a micrometer, one thousandth of a micrometer is a nanometer, where do cells exist in this size scale, and that is exactly the point where we are going to be discussing and have been discussing so far, namely, cells are at the micrometer scale.

I would like you to remember this, because, in the same fashion, that if I ask you, what is the size of a cow, you may not be a farmer, you may not know how big a cow is. But at least you can imagine that a cow is bigger than a dog and smaller than an elephant. And since you are training to be scientists, you want a slightly more precise answer.

And so you can argue that a cow is about maybe the height of a human being maybe one meter high, maybe one meter wide, maybe one and a half. And because we are doing biophysics, we want to simplify, remember, I said this at the beginning also simplification is an important part of the process of making physical, intuitive, logical arguments about biology. In such a case, you may even argue that a cow can be treated as a spherical object in vacuum.

Now, this has been treated as a joke often, but it is important because it allows you to clear all the other parts away and think purely in terms of numbers, dimensions, densities, mass and simple physical properties. And what helps you to do that is this comparative size scale, we will keep coming back to this in different contexts. And I hope you will make sense of it when we arrive at the conclusions that we can get from it.

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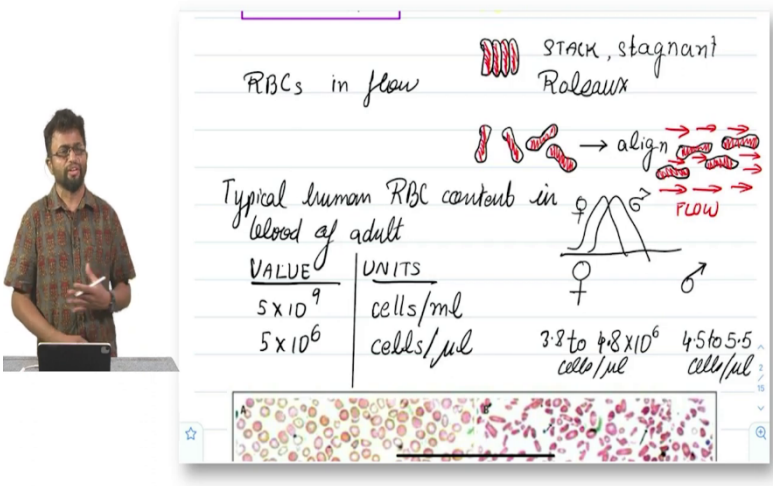

So, to continue with our blood cell study, red blood cells, when they flow in a fluid, they may undergo at least a couple of various, a couple of orientations. One is that they may become stagnant, they may start forming these roleaux, as they are sometimes called discs. It is like when you put all the plates one on top of the other, the plate has a little slightly convex or concave shape, depending on whether you look from top or below. And so if you put all the plates on top of each other, even your thali has a little lip, it goes upwards. So, all those lips aligned to each other and they stack up and that is what this rolo means.

On the other hand, if the RBCs are not sticking to each other, and the flow is of a certain speed, they may so it is like when there is a strong wind, all the trees there, the branches align in one direction and this is the same idea that when there is a strong flow, the RBCs align in one direction. The process by which all these happens is related to the mechanical properties of the red blood cell and the fluid properties of the medium in which they are which is blood indeed. And understanding these again as I was saying earlier, gives us many interesting insights and medical useful inferences into the physiological state of the person.

So, what is the red blood cell count in blood? This is for two units cells per milliliter, 5×10^9 and 5×10^6 per micro liter. Obviously, milli to micro is 10^{-3} meaning one thousandth therefore, the value is just simply one thousandth of the other. There is also a slight difference between human adult males and human adult females.

Now, if you are very careful and you have studied some biostatistics or statistics in general, you will notice that the value of human female or women's blood counts is 3.8 to 4.8 into 10

to the power 6 or million cells per micro liter, tens of lakhs, whereas for me it is 4.5 to 5.5 which means there will be some women with higher blood counts then some men will have lower than female blood counts.

This also means that there is a distribution that is overlapping in terms of the female and male RBC counts. And these come to frequency distributions, which we will have some opportunity to discuss, and how to distinguish between them, which is a topic that biostatistics normally covers. And I urge you if you are interested in reading more about this.

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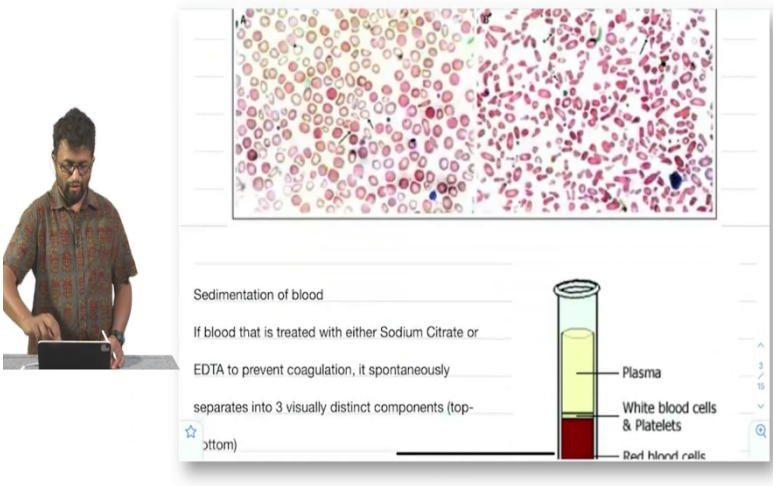

Now, if you actually do a blood smear, then this is what you will typically see the arrows mark slightly unusual shapes, and potentially harmful entities like plasmodium that may be in your blood. This is the thin smear technique that is used, even now in most laboratories as a gold standard for testing for blood pathogens. So, knowing blood cells, their concentration, their mobility is important. One of the key methodologies that is used to perform this kind of blood characterization is sedimentation.

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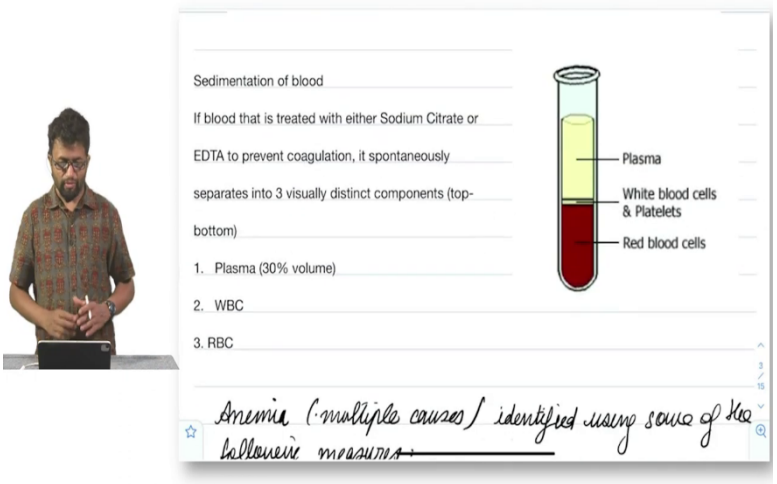

So, the idea is that if the blood is treated with sodium citrate or EDTA, this prevents coagulation. It will, over time spontaneously separate roughly an hour into three visually distinct components from top to bottom plasma white blood cells and platelets and red blood cells. The plasma is about 30 percent of the volume, the remaining 70 percent consists of WBCs and RBCs and platelets and other cells.

The height, relative height of the red part, the RBC settled sedimented red blood cells, compared to the total length as a percentage is used as a measure of the proportion of red blood cells in your blood. This is a very crude measure, you will argue. But interestingly enough, this is since the nineteen hundreds, one of the most reliable measures of serious hematological readout of disease, and all this coming from simple fluid behavior.

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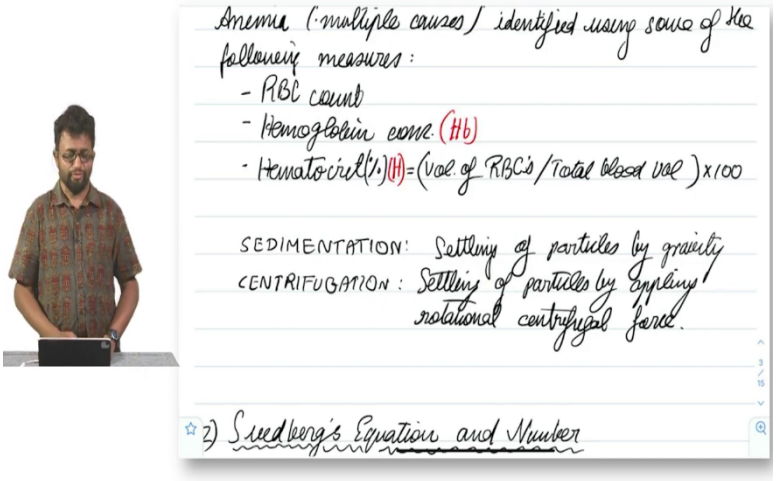

So, anemia has multiple causes, and it can be identified using some of the following measures RBC count, hemoglobin, hematocrit which is percent of volumes of RBCs as a percentage of total blood, and you both may needs sedimentation, which is the easy thing, settling of particles by gravity or centrifugation. And so now we are going to dive a little bit into the details of each of these.

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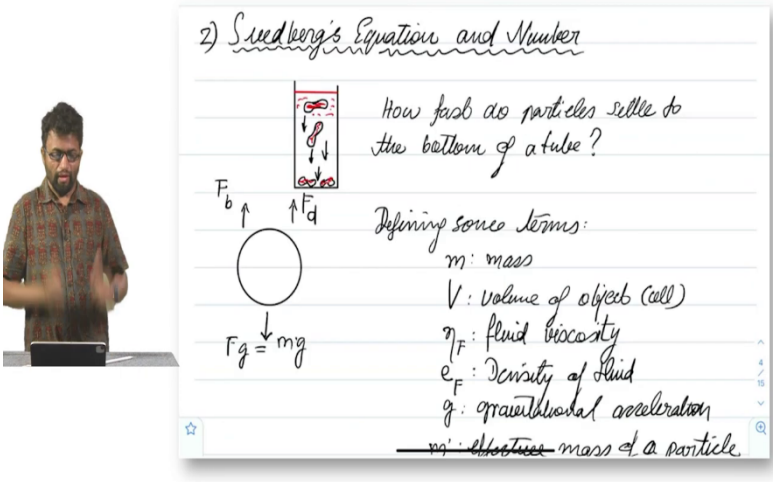

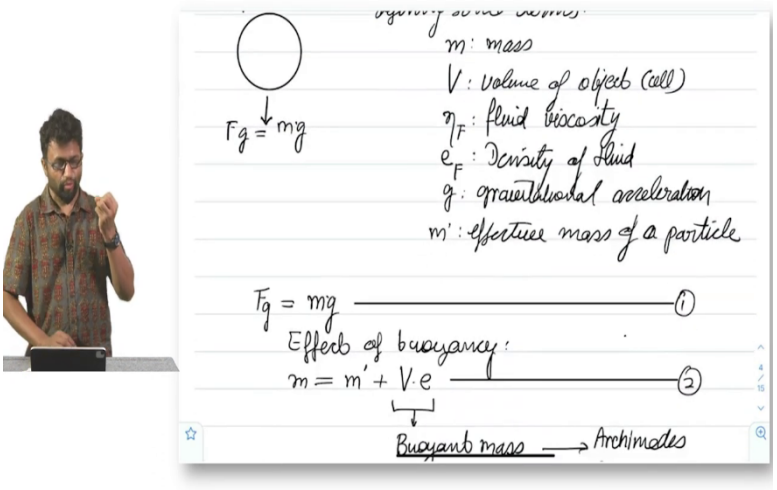

So, as we were saying, one of the important measures of sedimentation and centrifugation is Svedberg's number and the equation that describes this process of sedimentation and certification is Svedberg's equation. So, the question that Svedberg asked was how fast do particles settle to the bottom of a tube and what determines? And it is a very simple question. And when I was a student of probably some of your ages, this was a question that really bothered me. And I read the textbook and it did not make full sense. So, I hope I will make a little bit more sense than I had at that time.

So, we need to define some terms. This is biophysics class, so we actually talk about symbols. M is your mass. It is the objects mass that actually defines the product of the volume and density. Remember, there is a distinction between mass and weight, weight is mass into gravitational acceleration mg, it is in Newton's, we are conventionally used to measuring weight that is compared to actual mass.

So, in physics, we distinguish a little bit more finely between these two correct. Then there is volume of the object or cell if it is that the object that you are trying to centrifuge, ρF, I am sorry, η F is the fluid viscosity, ρ f is the density of the fluid, g is the gravitational acceleration, m prime is the effective mass of the particle. The force due to gravity acting on an object of mass m is m times g, mg. This is kind of obvious.

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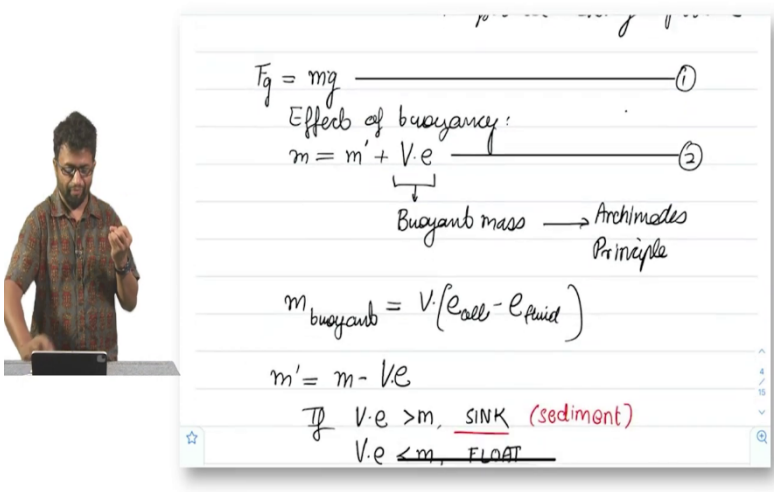

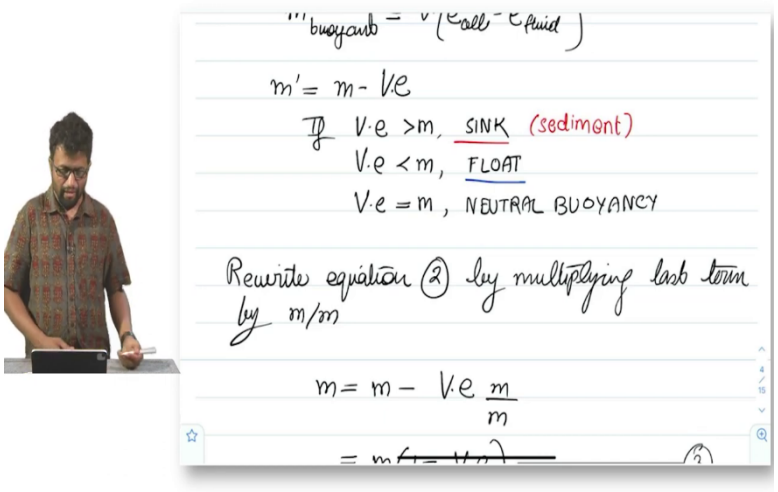

The effect of buoyancy is to modify the mass with the effective mass and the buoyant mass. And we say that, therefore the buoyant mass is equal to the volume into the difference between the cells density and the fluids density. You could argue it is the force acting upwards. That is what I have drawn here the Fb. The m prime therefore becomes m minus V times ρ. And if V ρ that is the product of the volume and the density is greater than the mass as felt by the object it sinks. And this is nothing but your Archimedes principle, if it is less than it floats and if it is equal, it is called neutral buoyancy.

So, if you look at fish that are swimming in the ocean, they are usually at a specific position they neither go down nor up unless they want to. And they have very complex mechanisms by which they can regulate, when they dive and when they come up. And some of you may

know that the very large mammals that live in the ocean, the whales, they have mechanisms complex, volume, density, compensation mechanisms in their bladders. So, while we are talking about centrifugation, you can also use these principles just as well for ecology and outdoor and large animal physiology. So, these principles apply universally, Archimedes was right.

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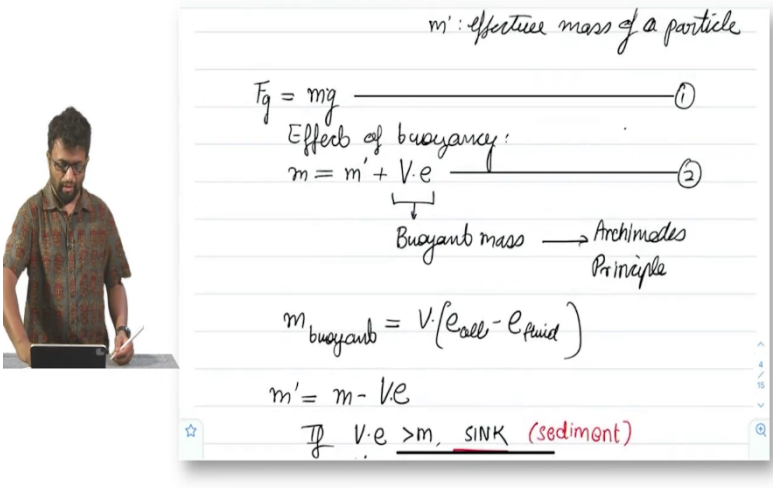

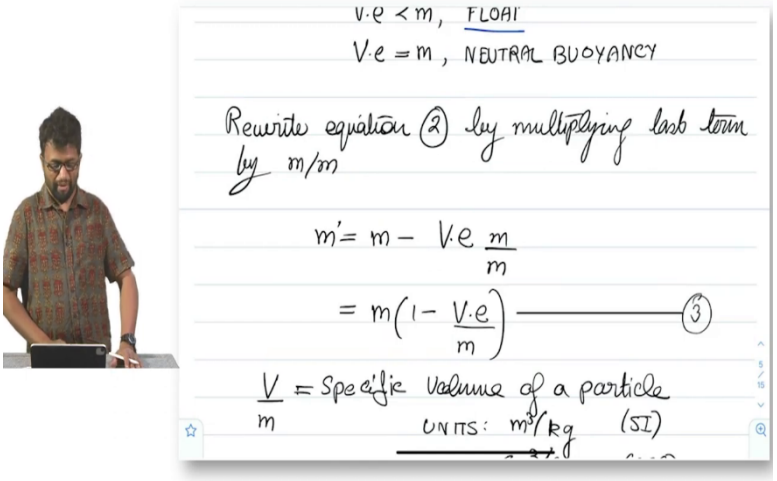

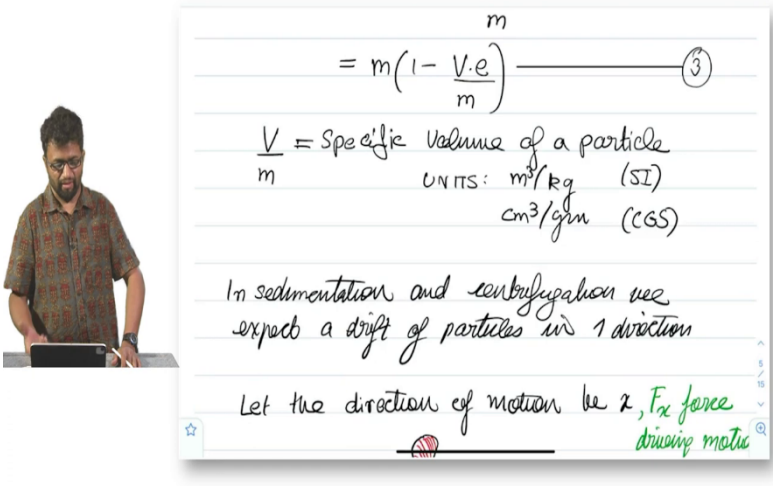

Rewrite the equation number two, according to the multiplication of the term m by m, which is basically getting rid of m and m cancel each other out. So, we can do this and when we do that, we can get take m common and we get m is equal to m. So, this should be m prime is equal to m into 1 minus v ρ upon m in brackets. So, the specific volume of the particle is something now we can define we can call it the ratio of the volume by the mass with units of meter cube per kg in SI units or centimeter cube per gram in CGS units.

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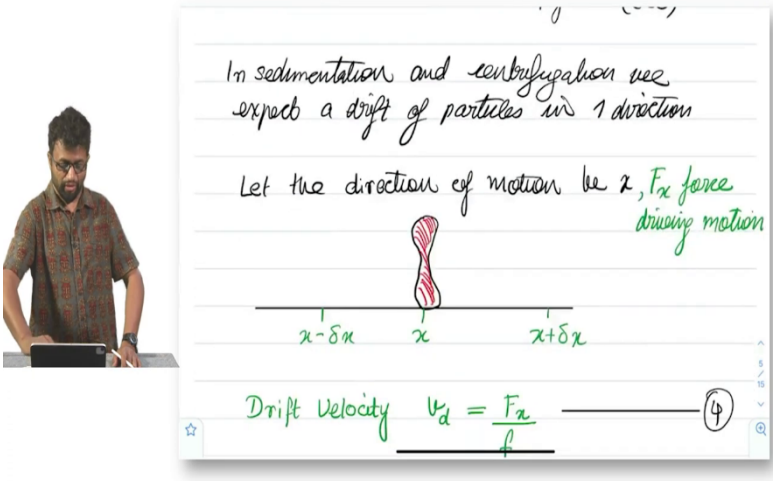

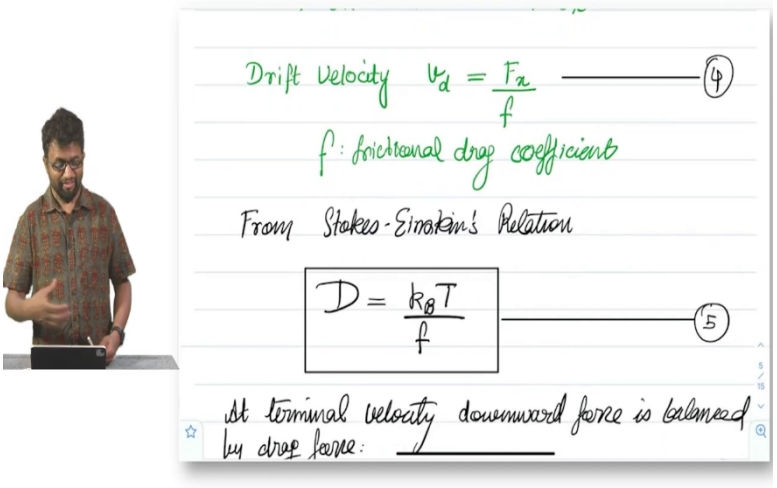

So, in centrifugation and sedimentation, we expect that the particle will move in a certain direction. So, I mean obviously, we expect sedimentation means the particle will sediment to the bottom the red blood cell for example. So, let the direction of the motion be x and Fx be the driving force that is driving this motion, then the drift velocity vd will just simply be Fx meaning to say the driving force and the opposing force, the opposing force is opposing tendency is nothing but fluid viscosity.

And if you remember from last few lectures, we talked about drag coefficient and this is the Stokes drag coefficient f. From Stoke Einstein's relation we also know so, Einstein as an Albert Einstein did a couple of different three or four magical works, which made him made those his so called miracle years, Wunderjahr in German, that was the time he worked on quantum electrodynamics relativity and diffusion and this diffusion equation is originated from that period of 1905.

The equation combined with Stokes drag coefficient gives us the answer, that the diffusion coefficient of a particular molecular object is the ratio of the thermal energy term k_BT upon f. If you recall in the entropic calculations that we talked about last week, we discussed that the energy due to ambient temperature meaning temperature around us from statistical mechanical theory is the product of the constant k times the temperature. kb is used as an honorific to honor the work of Ludwig Boltzmann who came up with this concept. So, D is equal to k_BT by f.

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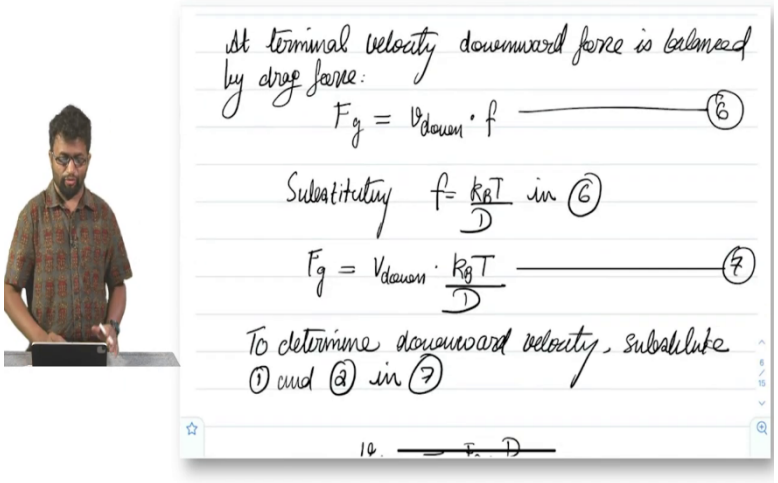

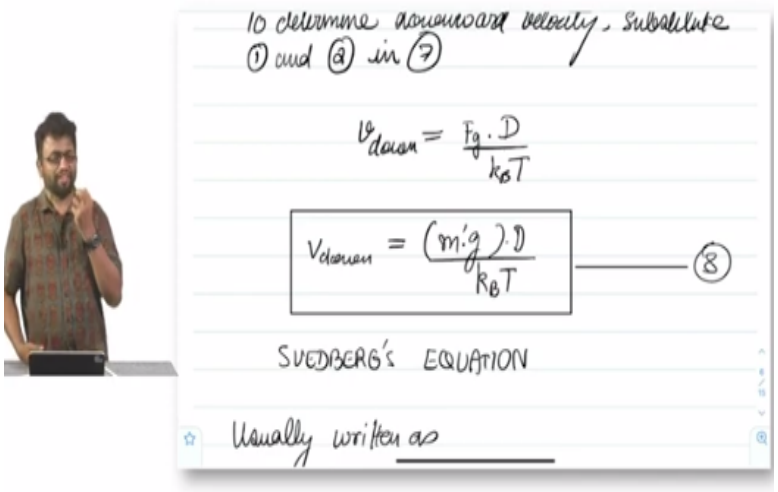

So, at terminal velocity the downward force is balanced by the drag force. So, we say that we can equate F_g gravitational force or D downward gravitational force as f v down times f. Now, substituting f by F is equal to k_BT by D in 6, which is basically a rearranged rearrangement of this equation number 5, we can get the downward velocity by substituting one and two in 7 and eventually get v down is equal to effective mass into gravitational acceleration into diffusion coefficient divided by k_BT. This is Svedberg's equation.

Please stare at it for once, because what this implies is that if the diffusion coefficient is very high, then the velocity downward will be higher. If the mass effective mass is very high, the downward velocity will be very high but if the thermal energy if the temperature is very high, then the velocity downward will reduce. So, there is a direct and inverse proportion of these

right hand side terms depending on whether in numerator or denominator. This is Svedberg's equation.

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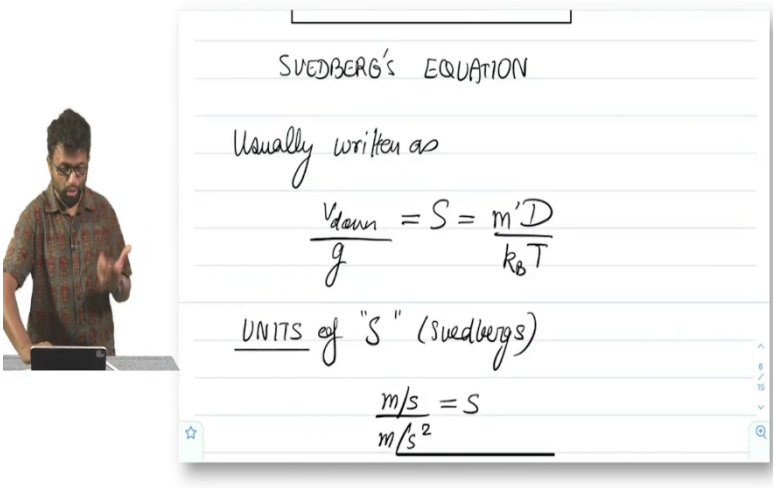

It is usually written as the ratio v down by g. That is to say, relative to the acceleration and that is replaced by symbol S, which is the Svedberg unit that is so famous. So, Svedberg unit has the units meters per second divided by meters per second square, meter-meter cancels out and you end up with seconds.

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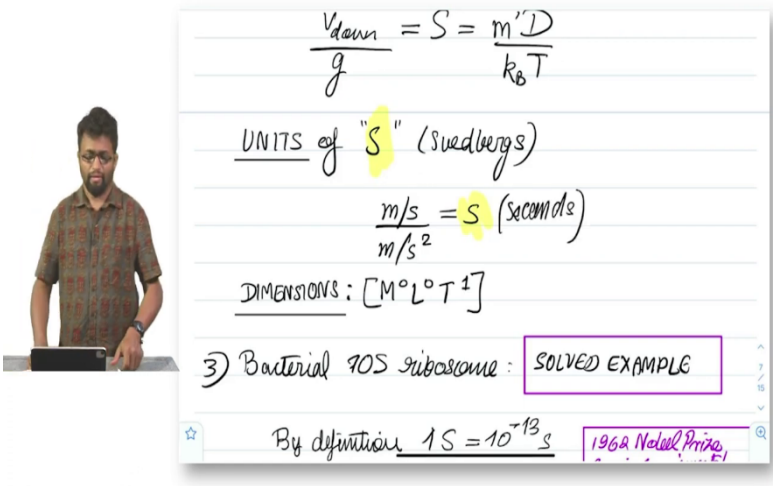

So, in fact, the dimensions are time to the power one M^0 L^0 and the units are time units which in SI units we use as seconds represented by the symbol S, just not to confuse this S is capital, the one representing Svedberg units and this s is small, in other words, seconds.