Drug Delivery Principles and Engineering
Prof. Rachit Agarwal
Department of BioSystems Science and Engineering
Indian Institute of Science, Bengaluru

Lecture – 09
Research Paper Discussion and Diffusion Controlled Systems

Hello everyone, welcome to another lecture for Drug Delivery Engineering and Principles.

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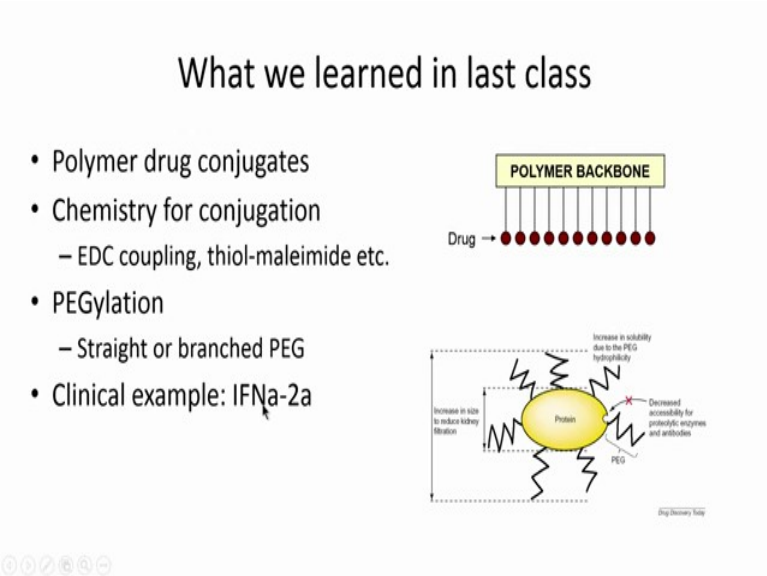

So, what we learned in the last class? We mostly talked about polymer drug conjugates. So, these are polymers that can be conjugated to the drugs, either in this format where are long polymer bone gets conjugated to small polymer to small drugs. Or you can have a large drug like proteins and you can conjugate polymers on to. What is the advantage of this? That one of the advantages is of course, that it increases the size for these small drugs.

So, there residence time increases as we discussed in terms of elimination that kidney cannot clear anything above 6 to 10 nanometers. So, it will have enhanced circulation as well as it also prevents any degradation by external proteases and all of that. So, these are some of the advantages here.

Then we also talked about chemistry that we can use for different conjugations. EDC coupling is one to conjugate carboxyl's to amine we had thiol and maleimide, click chemistry which is very widely used to conjugate sh group to a maleimide group and then we talked about several others as well.

Then one of the polymer drug conjugate we talked about is PEGylation, very widely used in the literature. These could be a single chain of PEG or could be a branch chain of PEG. Branch chain we found out is more effective just because it is a lot more area that it can cover as eventually wiper. And then we gave a clinical example, something that is being used in clinic for IFN alpha-2a. Where we showed that if you conjugate it with the branch to PEG it circulates much longer into the blood compared to just the free drug alone.

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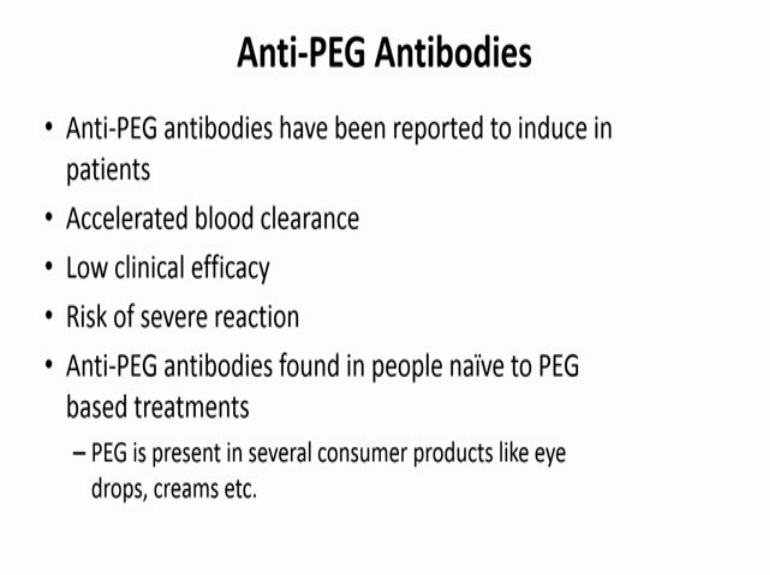

So, today we are going to talk about some of the challenges with PEGylation, and one of the major challenge that has come is the anti PEG antibodies, these are antibodies that are generated against the PEG. So, about 4-5 years ago this was reported, before then it was considered that PEG is a fairly inert molecule. And it does not really induce any kind of immune response in the system; but in the last 5 to 6 years more and more people are reporting that there are antibodies against this PEG polymer that is being generated and induced in patients. Of course, the one of the major purpose of the antibodies is to cause rapid clearance of anything foreign. So, these antibodies bind to their targets and

once the antibody is bound to the target, the immune system clears those things up, they take this up through Fc receptors and various kinds of other mechanisms. So, what will happen is, whatever drug you are conjugating with the PEG will now; instead of remaining longer it will actually get removed much faster.

This will result in low clinical efficacy and also result in risk of severe reaction. Because these antibodies then once they bind to their receptors they generate quite a lot of immune response, lots of cytokines and mean secreted by the immune cells. And so, all of this will cause severe reaction and this will manifest in form of high temperature, fever, pain and all those effects, so again not very desirable. What is even more concerning is that the anti PEG antibodies have been found in people that are naive to PEG. And when I say that is basically; that means, that these people have never been treated with any of these polymer drug conjugate with PEG.

And even then they have antibodies why do they have these antibodies? Is because PEG is also very widely used in several consumer products like eye drops and creams and even though we may never have been given any IFN-alpha treatment with the conjugated PEG, we are still exposed to the PEG, on the basis of these drops and creams. And our body has been exposed to the PEG which has generated these antibodies. So, even the first time we will give these PEG drug conjugates, we will see that they are getting cleared very rapidly.

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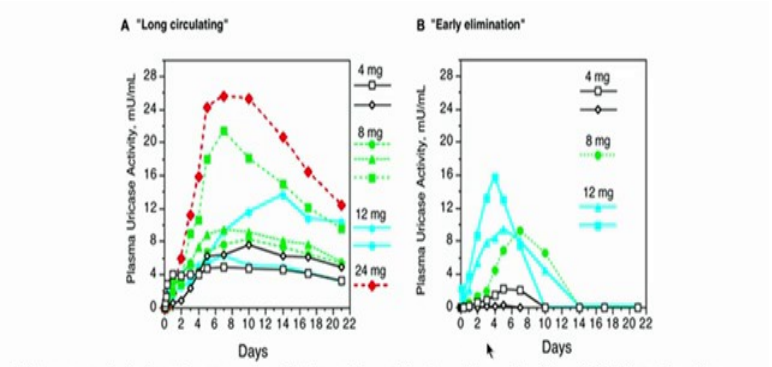

Uricase activity in plasma over 21 days in subjects with and without PEG antibodies. (A) Subjects without PEG antibodies had "long circulating" uricase after a single injection, as indicated by detectable plasma uricase activity for 21 days. (B) In contrast, subjects with anti-PEG antibodies displayed "early elimination" of uricase. Each line in panels A and B represents an individual patient receiving either 4 (black), 8 (green), 12 (blue), or 24 (red) mg uricase.

Ganson NJ et al. *Arthritis Res. Ther* **2006**, *8* (1), R12,DOI: 10.1186/ar1861

So, here is just one example here. So, you see in case of long circulation. So, you have different kinds of drugs that are conjugated to PEG. In this case this is a uricase, which has an activity over twenty one days in subjects with or without the PEG antibodies, but what happens is, when the subjects are giving the PEG related product you get quite a lot of uricase activity which last quite a long. If they do not have these antibodies, but when they do have these antibodies these gets cleared very rapidly.

So, as you can see, let us compare the twelve milligram example, in this twelve milligram blue curve, you see that the PEG products are being circulating for more than 22 days; however, once you have given; once these patients have been exposed and they have generated the antibodies, when you give them again you see that they get rapidly cleared within 10 days itself. So, these are some of the issues that have started to come up with the PEG conjugation.

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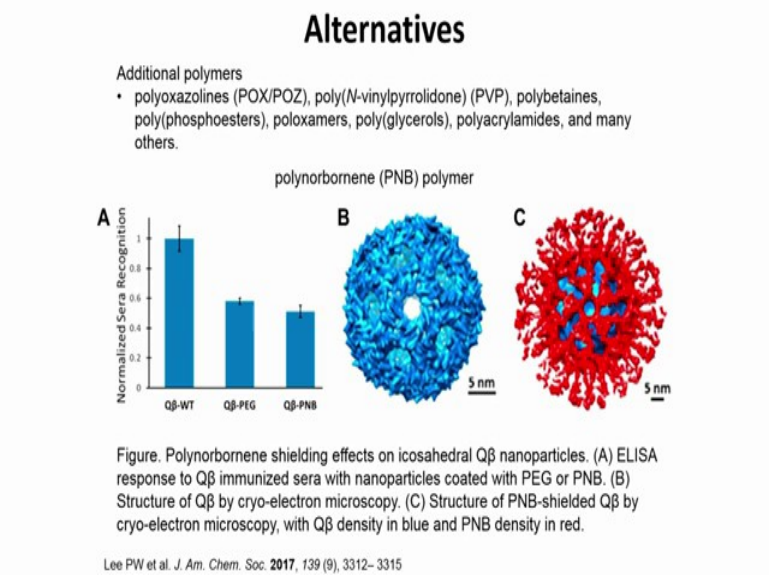

So, then what are the alternatives? So, we do have other polymers as well we have polyoxazolines, PVP, polybetaines and other kinds of polymers that are also being explored. So, far none of these antibodies have been reported against these polymers, but having said that they have not been used as much as the PEG was. So, more and more research will tell us whether these will last or whether the body will also start generating antibodies against these particular polymers.

One example here is norbornene, which has started to come as a good alternative. So, if you look at the norbornene graphs, you would see that it covers the protein fairly completely. The protein here is in blue and then the red is essentially your polymer coating. So, it can act as a good alternative, but again as I said, only time will tell whether once this started to use more and more widely, whether our body also generates antibody against norbornene.

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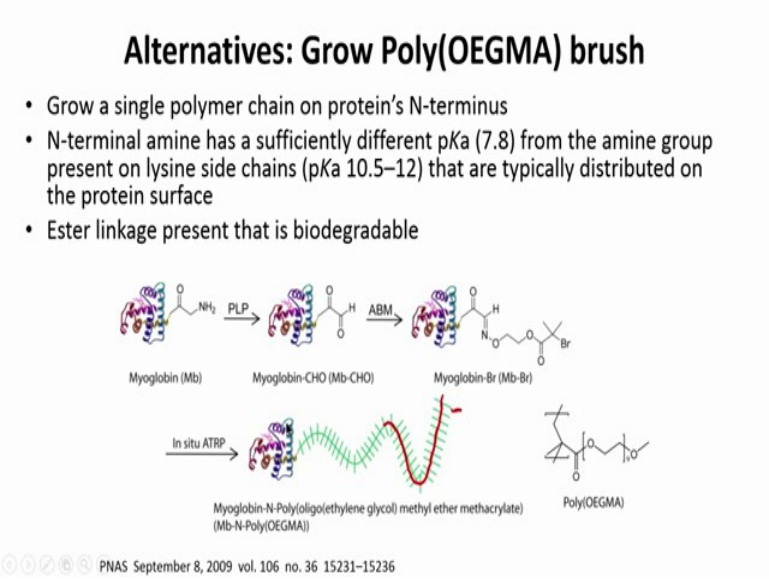

One of the alternative is very similar polymer to PEG is called poly OEGMA and what it is, is essentially and this functional group which is very similar to PEG if you look at this unit this PEG itself, but in this case what is happening is, a small unit of this is growing. So, the polymerization is on this end. So, previously if you were seeing PEG there was being written as this. In this case if you see this is only 9 units or you can vary this unit around, but the polymerization is actually happening on this backbone. So, instead of having a long polymer like this, with all of PEG, what do you have is a long polymer like this, with a small PEG units hanging on it.

So, this is something that is being proposed. And more and more research is now going into it and atleast the initial data is suggesting that this could be an alternative to PEG. And so one of the paper here describe the use of growing this polymer on to a single polymer chain of protein. So, what they use, is they use the fact that the N-terminal amine will have a different pKa. Then rest of the amine and present in the protein core

and. So, you they use this pH differential to form a biodegradable bond at that site. So, in this case they used different kinds of chemistry a different pH to utilize only the N-terminal and then they grew this long poly OEGMA chain with it. Remember these small pendants are the PEG chain and then the basic unit here this is not PEG, but this is what is getting polymerized.

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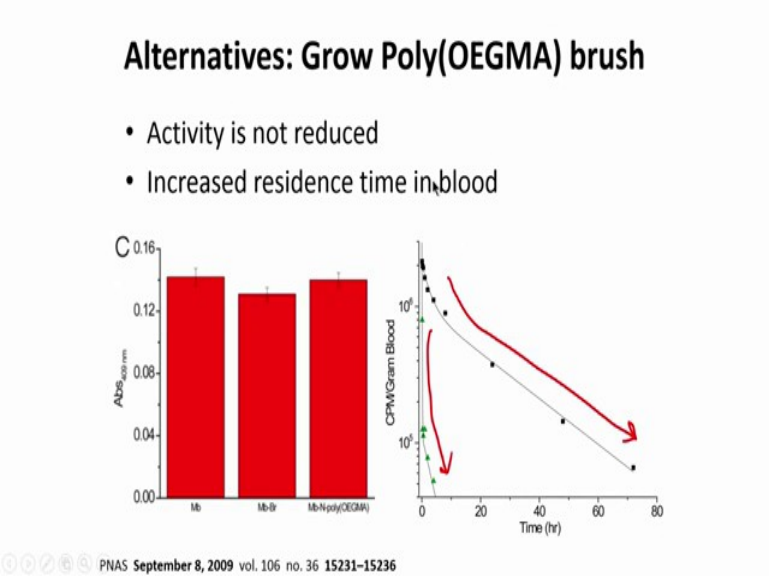

And then they went ahead to show that the activity of this does not really change with the growing polymer chain. In fact, it is almost the same as the native protein itself and like the PEGylation, it also has quite a long resinous time. So, the free drug gets cleared very rapidly, but once you conjugate it with this polymer it stays for much longer.

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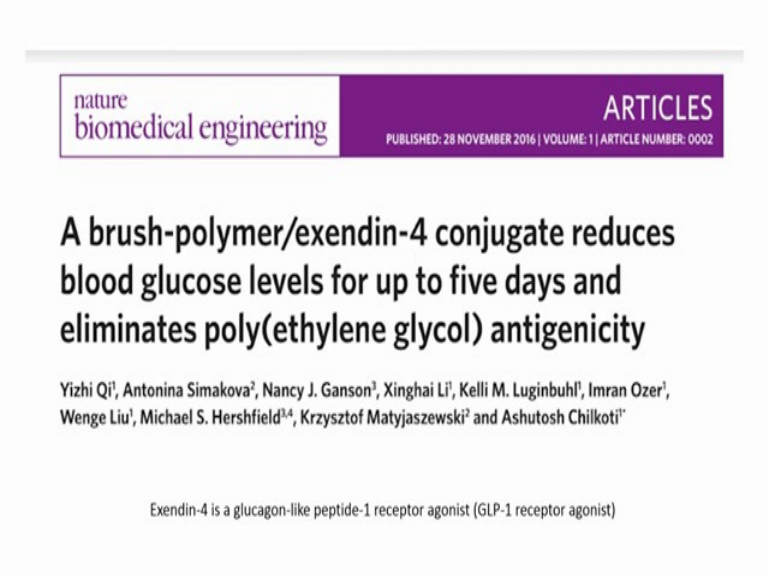

So, further to this, then they started evaluating whether such strategies can be used to prevent any antibody generation. So, here is another paper by and Chilkoti group, we are going to talk a little bit about this in this paper. So, what they have they have used protein called exendin-4 which is a small peptide which is used in case of diabetes to secrete insulin.

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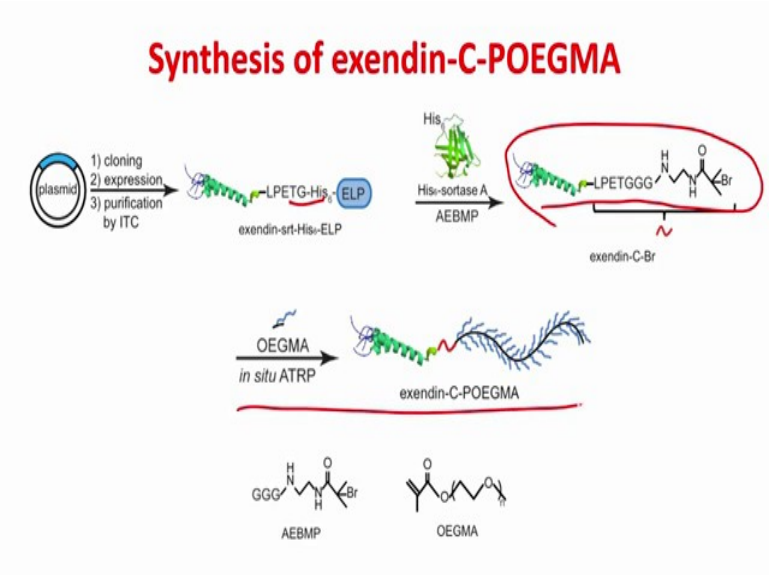

And what they have done is that they have made a recombinant protein of this, to this protein what they have done is they have added a his tag and this his tag is essentially

used to purify this protein in downstream processes. And they have also added a small peptide sequence to it. So, what they can do then is they can do their chemistry on to the small peptide sequence by using some enzymes which are fairly very specific.

So, in this case they have now conjugated an initiator onto this C-terminal of this protein where this his-tag was attached. So, now, instead of the his-tag you have this whole protein containing the initiate and once there is a initiator you can do your OEGMA reaction to essentially get a long chain as described previously.

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Table 2 | Pharmacokinetic parameters of exendin and the exendin-C-POEGMA conjugates, calculated from data in Fig. 6c,d.

Exendin/conjugates	$t_{1/2a}$ (h)	$t_{1/2e}$ (h)	C_{max} (nM)*	t_{max} (h)*	AUC (h × nM)†
Exendin	0.7±0.1	1.7±0.2	37.1±3.8	1.78±0.1	217.5±36.5
54.6 kDa EG9	6.2±0.5	42.4±2.9	56.4±3.9	27.1±0.4	4,795.5±440.7
55.6 kDa EG3	7.6±0.7	61.2±5.0	44.0±2.7	28.5±2.3	4,775.0±482.9
71.6 kDa EG3	9.0±1.7	61.5±3.2	37.7±5.0	32.4±3.9	4,411.2±499.6

*Derived from curve fitting. †Calculated from t = 0 to ∞ from curve fitting. Values are reported as mean ± SEM. t_{max}: time required to attain C_{max}.

So, very similar strategy to what because there, but then in this paper then they went ahead and showed that first of all the half life is increased. So, if you have only protein you only have a half life of less than an hour whereas, as you increase these polymer chains, molecular weight you get quite a lot of half life.

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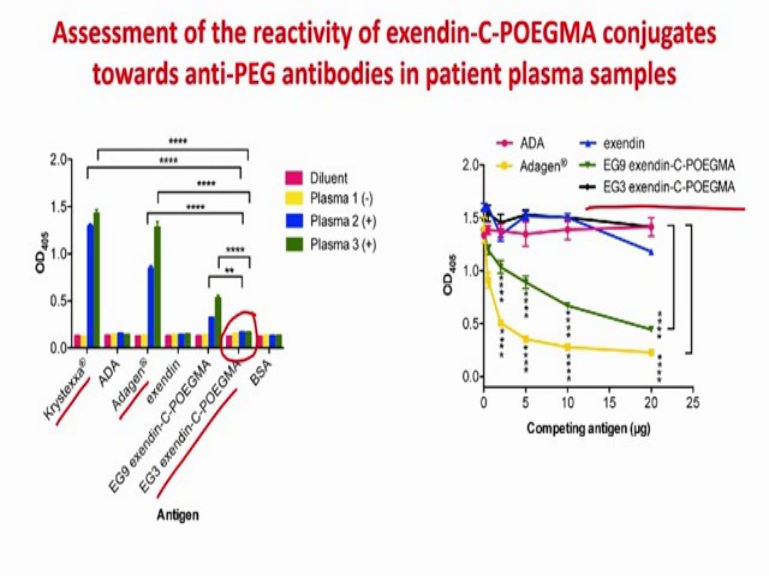

And then finally, then they showed that if they compare it to some PEG products in the market with the patient samples, what they find is, these two product the Krystexxa and Adagen are PEG based products which are already being used in the market and if you expose them to patients containing antibodies against PEG if you see quite a lot of antibody binding to these products.

Whereas, if they use their polymer, they do not see any effect on the antibody binding. And this is again showed in terms of another essay here, where there EG3 extendin poly OEGMA does not really show any decrease.

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So, that was on polymer drug conjugates, as we go along we are going to talk more about controlled drug lease and there are several systems for this. One is a diffusion control system which is a basically a reservoir system where you create a reservoir and then release the drug on the basis of diffusion. Or this could be a matrix as well, which is non erodible. You can have a solvent controlled systems these could be osmotic pumps. So, there is some sort of swelling happening because of the presence of the solvent and that causes the drug to come out.

Or this could be chemically controlled. One example of this we already learned in terms of polymer drug conjugates. These could be bio erodible as well. So, as the chemical bonds in the membrane degrades, you will see that more and more drug is coming out. So, we will talk about the diffusion controlled systems first and before we actually do that we need to learn some of the laws of diffusion.

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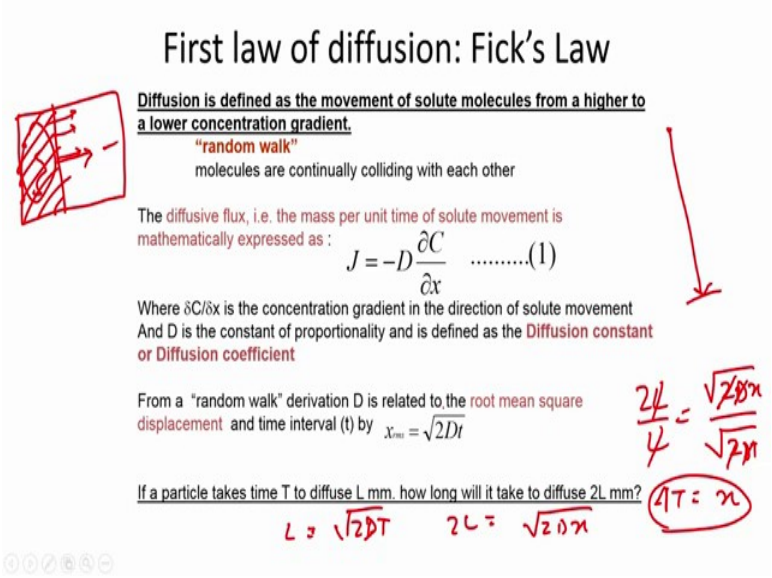

So, the first thing we are going to talk about is Fick's law of diffusion. And what it is? It is just a kind of an estimate of how the molecules diffuse, it is a model based on the random walk. And so, what typically happens let us say if I have a sample a containing high concentration of drug on one side and low concentration on the other side. These molecules are constantly moving and colliding with each other. So, the collisions will be more in this area than in this area. So, as there are more collisions they will tend to move because of these random collisions towards the low collision area. So, that is essentially what is defined as diffusion in this case, through this model.

And the diffusive flux, which is essentially the rate of the movement of the solute into the rest of the media, is then defined as J which is nothing but, is equal to the diffusion coefficient multiplied with change in concentration.

$$J = -D\frac{\partial C}{\partial x}$$

So, this is how it is mathematically expressed, D is the diffusion coefficient which is going to be constant for a certain solute in a certain solvent.

So, for a random walk another term that is defined as a root means quite displacement which basically means that if a molecule is just freely diffusing from one place how long will it take for it to reach a certain distance or how much time it would take for it to

reach a certain distance. So, this X^{rms} which is the root mean square displacement is defined as

$$x_{rms} = \sqrt{2Dt}$$

So, that is again here, D is a diffusion coefficient then T is just the time. So, if I ask you that if a particle takes time T to diffuse a distance L millimeter how long will it take to diffuse 2L millimeter. So, you can use the above defined equation to answer that,

$L = (2DT)^{1/2}$

In this case the T is capital; then what will be 2L? How much time will be take for 2L. So, the time taken will be what. So, let us say 2L time taken is x.

$2L = (2Dx)^{1/2}$

I can just essentially divide these two equations. So, it will give

$2L/L = (2Dx/2DT)^{1/2}$

So, essentially if I square both sides I will get

$4T = x$

So, it will essentially take four times x because this is a root relationship.

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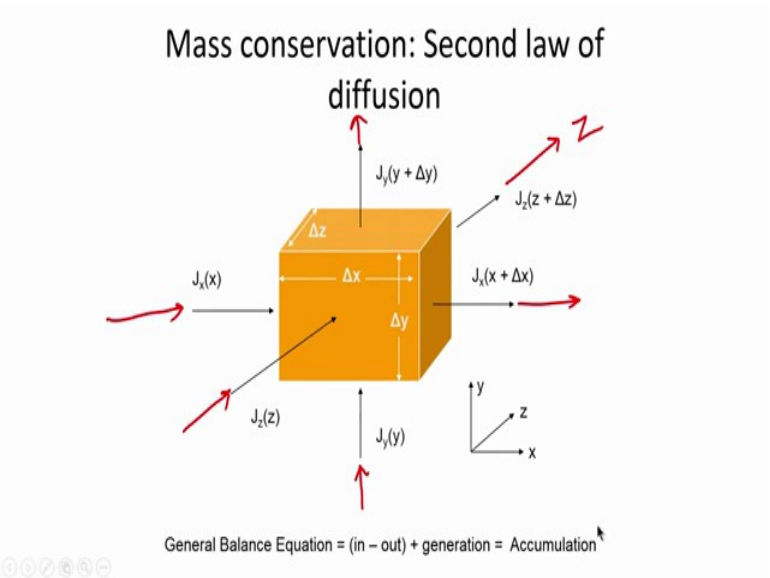

So, then we define the second law of diffusion and that is nothing, but mass conservation. So, what it says is let us say if we take a small volume, then its general mass balance equation which basically means it whatever of that particular solute is coming in, in any of the direction. So, it could be in the z direction it could be in the x direction or it could be in the y direction, then if you subtract whatever is going out.

So, let us say and this is the going out in the x, this is the going out in the y and this is the going out in the z, plus if there is any generation within that volume. So, let us say if a reaction is taking place also in there small volume, then that term will also get added up as generation that should all be equal to what is the total accumulation in this particular region.

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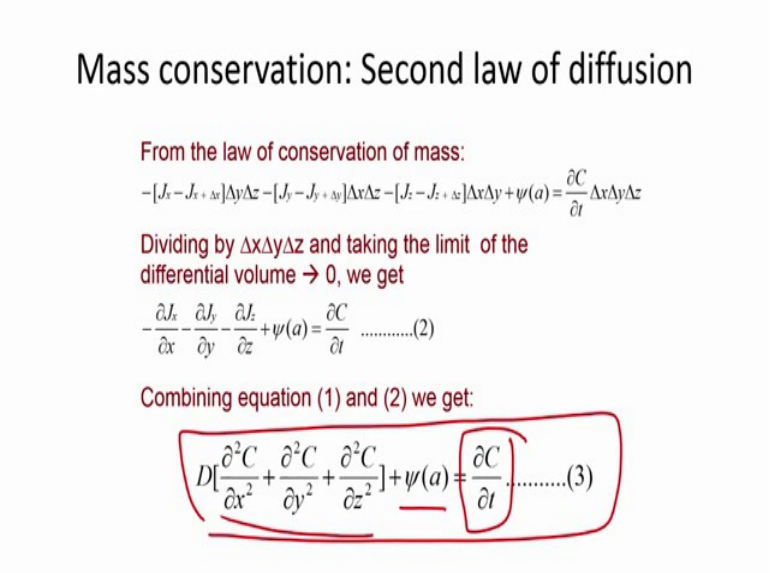

So, if I then express it mathematically you essentially get equation which is this, which is nothing but, this is essentially saying in minus out the rate of change of C in each of the directions with the generation term and this will then tell you how much over time the concentration is changing at that particular volume. And if I decreased volume to very small then it essentially will tell you at what is the concentration at that point.

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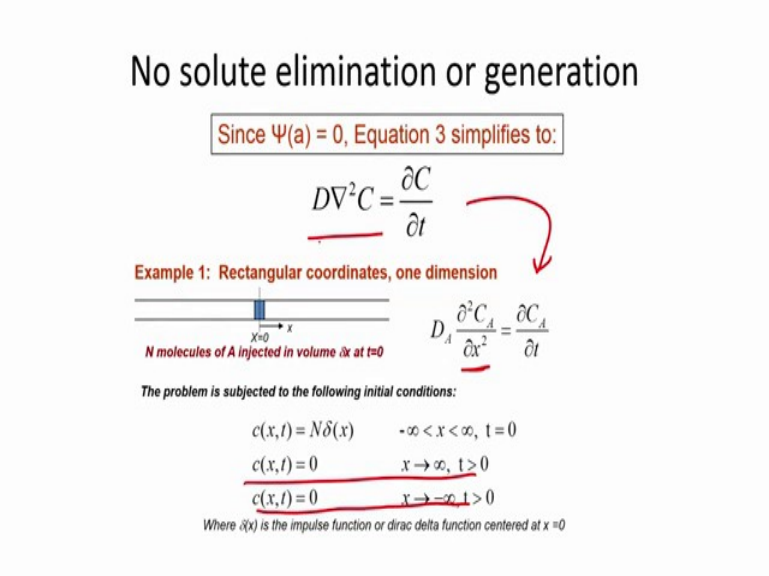

So, again this is also written as

$$D\nabla^2 C = \frac{\partial C}{\partial t}$$

if I assume that there is no generation which is going to be the case in our case where we will design devices and put them in the body and see how they are diffusing through in the body.

So, let us take a simple case in this case. So, instead of having an x y and z we will just say it is a one dimension system and. So, only diffusion that can happen is in one direction. So, in that case, this equation simplifies to this where the only x term is remaining the y and the z are gone and then if you apply some boundary conditions. And so what are the boundary conditions here? So, what are we doing here? So, we are given we have given a amount of drug let us say c0 at this particular time at x0 at a particular time at x0 at time t equal to 0 let us say.

So, what are the boundary conditions. Boundary conditions will essentially be that at x equal to infinity and x equal to minus infinity which is very far away from here, there will be no concentration of this particular molecule at that large distances at anytime. Whether the time is 0 or 1 hour whatever.

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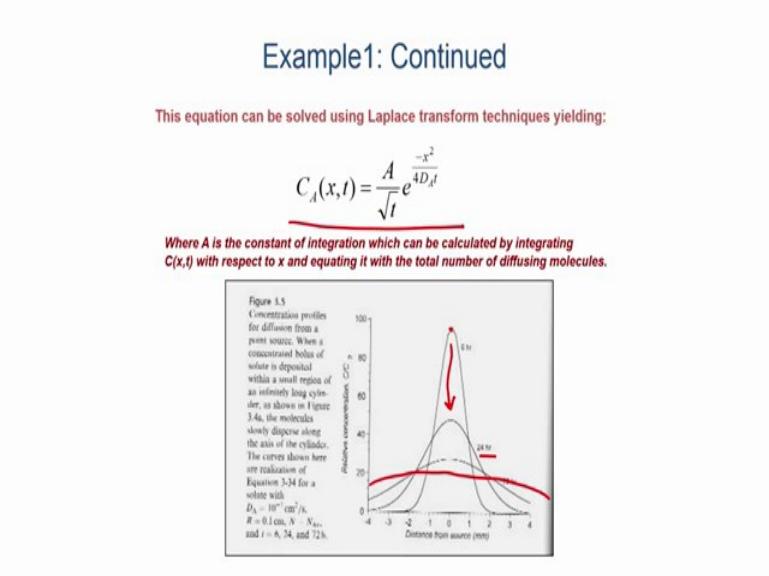

And if I define through these equations what I will get is, if I solve this, I will get a function which is mathematically expressed as this. And if you start plotting it over time or over distance from the source and different time points you will get something like this. At certain time you will get a very high concentration of the source and as you move away from the source it decreases and at a time which is greater than the previous time this concentration will decrease.

Because you already have quite a lot of it diffused out into the surrounding medium and the surrounding medium will start to increase and essentially is going to follow a similar trend. So, next will be something like this we will continue to go like this till it becomes equal everywhere.

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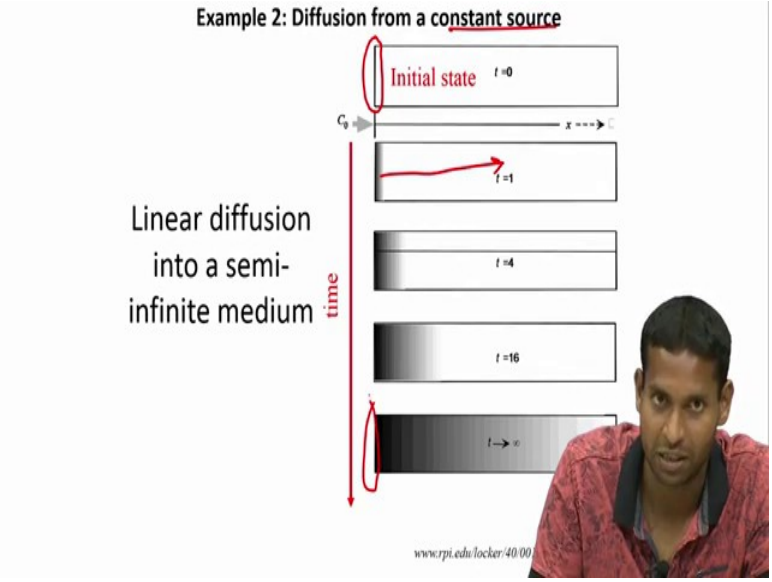

So, another difference to that is a different state to that is something when we say the diffusion is from a constant source. So, in this case we were saying that the source is not constant, what you have put is essentially going to diffuse out in the media and the concentration at that point is going to decrease. But now we are saying that the source is constant; that means, that whatever your put at that particular time is not going to change at all. So, if that is the case then what will happen is the concentration at this point will always remain C0, which is the concentration that we have invested and as time increases you have more and more solute starting to diffuse into the medium. But still the concentration at this point will always be C0.

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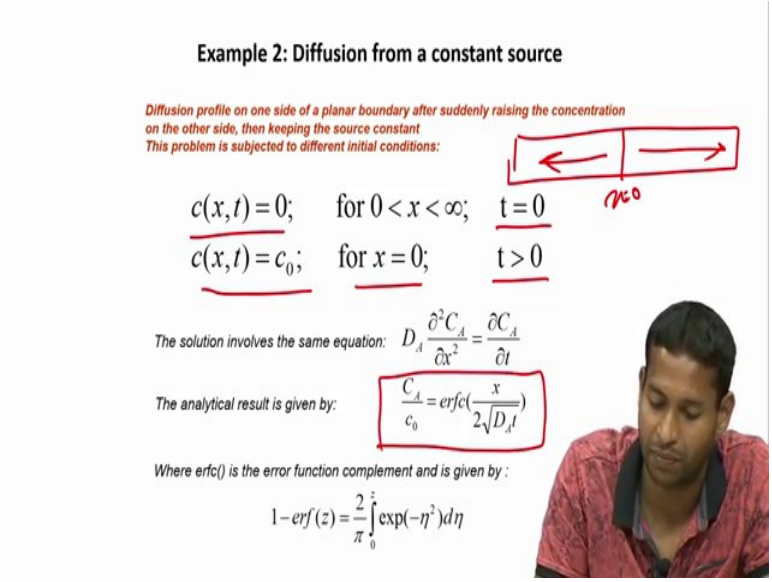

So, if you go ahead and put these boundary conditions so in now in this case what you are saying is essentially for any time the concentration is always going to be c0 at x equal to 0 for any time greater than 0 and of course, at t equal to 0 you had this to be 0 at any point away from the source. So, basically essentially saying that if this was represented again if this is x equal to 0 at time t equal to 0 there is no concentration here or here so, this is what is represented here. So, if you solve this essentially gives you an analytic result as this equation.

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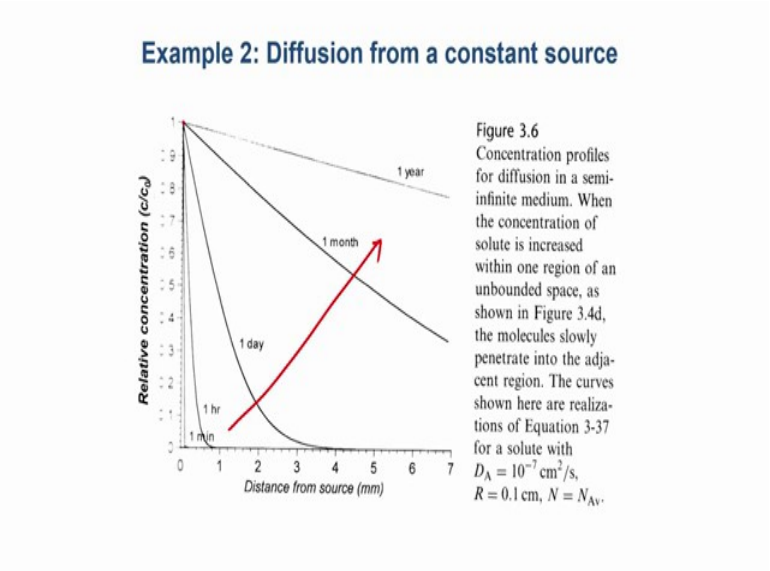

And if you plot it mathematically like we did in the first case you will essentially get at source the concentration will always remain the same. And as time increases, this is going to give more and more into the surrounding medium and with increase in time the concentration in the surrounding medium will also increase, at all time at all different locations.

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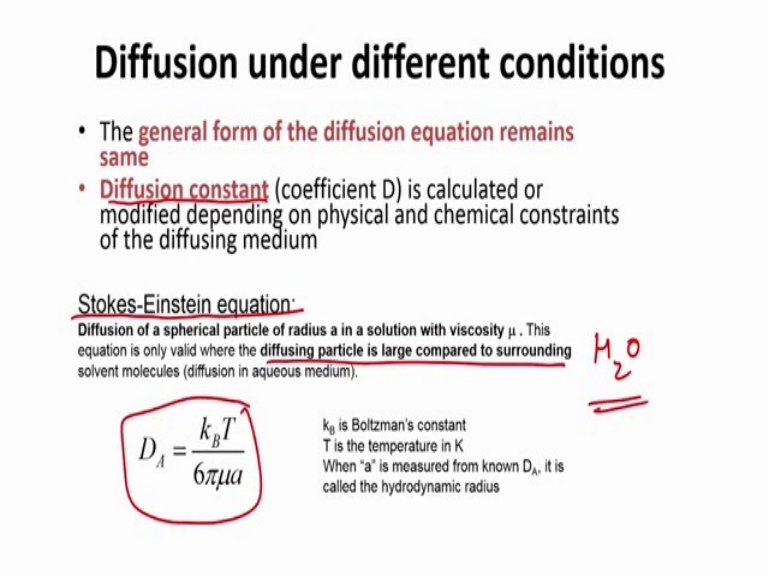

So, this was all for diffusion under aqueous conditions what happens in cases where let us say the solvent is not equals, but let us say the solvent is viscous. So, then as I described earlier, the diffusion constant is essentially constant for a particular solute in a particular solvent. Now, if you are going to change the solvent then the diffusion constant will also change. But the general form the diffusion equation will remain the same its just the diffusion coefficient is going to start to change.

So, how will the diffusion coefficient or diffusion constant is going to change as the solution becomes more and more viscous. So, basically as you might have guessed already, since its becoming more and more viscous it will be harder for it to diffuse, the diffusion coefficient is actually going to decrease. So, the same things applies for several other factors and if I define this using a Stokes-Einstein equation which is used for defining diffusion coefficient of a solute, it essentially boils down to this where kB is the Boltzmann constant, T is the temperature in Kelvin and μ is the viscosity of the medium and then finally, A is the hydrodynamic radius of the solute molecule.

So, this equation is only valid if you are saying that the diffusing particle is large compared to the surrounding solvent molecules, for all drug delivery applications we are essentially talking about the surrounding molecules to be water which is fairly small and most of our drugs are going to be much larger than water. So, we can use this particular stokes Einstein equation in cases where the viscosity of the water is changing due to some solvents.

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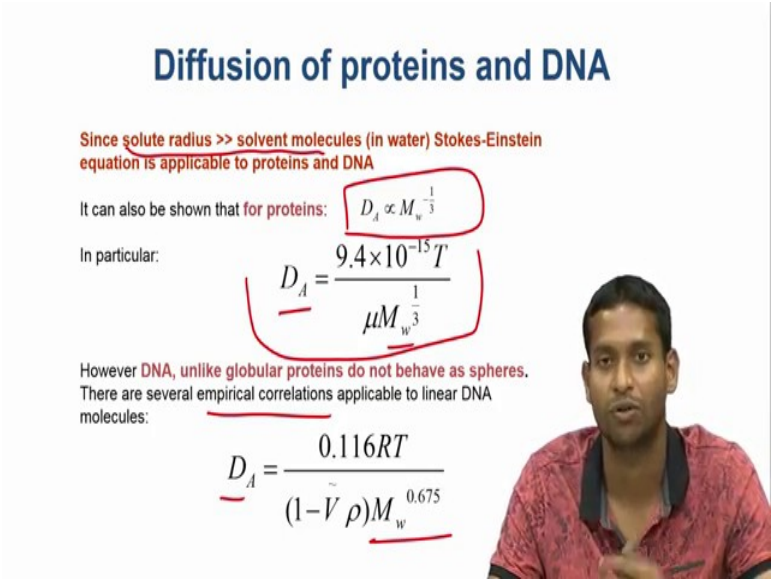

So, as I said in cases of protein we can assume that the sense the solute radius is much greater than the solvent this still holds true and then we can further show that this diffusion coefficient is related to the molecular weight as

$$D_A \propto M_w^{-\frac{1}{3}}$$

and in particular this is how it is defined for proteins. This equation changes a little bit for DNA, again you can assume most of the time that the DNA is small enough that it be spherical, but we know that the DNA is a linear polymer.

So, for DNA which is not a globular protein, it typically do not behave as spheres people have done some empirical correlations to find out what is the relation between the D_A and the molecular weight and what they found is its related with the certain power over the molecular weight. So, again this is mostly for information this was empirically derived, but I just wanted you guys to have it in case you are trying to model the rate of

diffusion of DNA which is we are starting to use more and more in terms of micro RNA and all those kinds of drugs. So, you have this term there.

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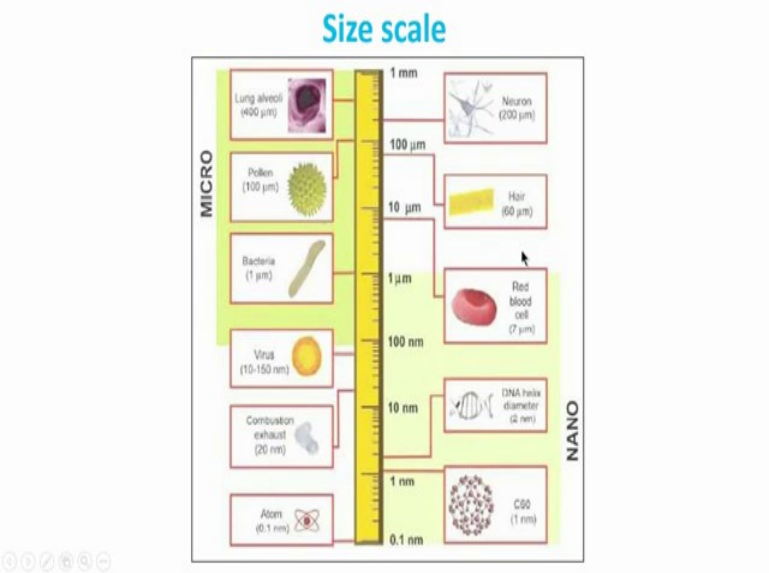

Here, we are going to little bit discussed on the size scale as well since now I am saying that the protein molecule is extremely large compared to solvent molecule, which is water. Let us talk about the size ranges of what we are talking about for proteins here especially. So, I mean this is a classic size scale, you have different sorts of objects to basically give you an idea. So, here we are saying that viruses are 10 to 150 nanometer, DNA can be anywhere between two to ten nanometer, atoms are 0.1 nanometer. So, all of this kind of listed here. Hairs are essentially in microns close to about hundred microns. So, this kinds of helped us define what we are talking about in terms of size.

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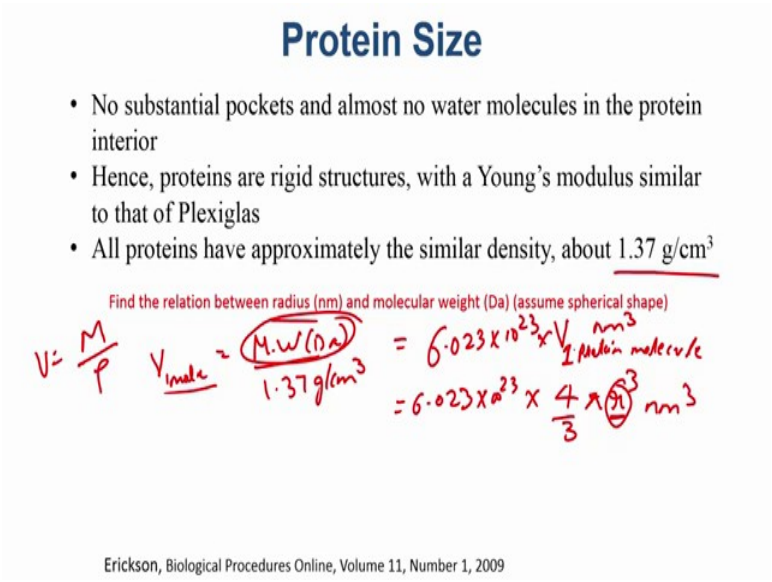

So, let us do some protein size estimates. So, if we assume that proteins have no substantial pockets. So, essentially they are very closely packed and almost no water molecule is present in the protein interior which we can assume for the most part, even if there is water molecule is very few compared to the size of the protein. So, hence the proteins are fairly rigid structures where the young modulus is similar to that of almost Plexiglas and they have a density of about 1.37 gram per centimeter cube.

So, now if I have to find the relation between radius of the protein and its molecular weight assuming it is a spherical shape then what will I do. So, let us continue with this particular equation and try to solve this. So, if I say that what is the relation between the radius and the molecular, but to find that I have to first find out what is the relation between the volume and the molecular weight right. And so we know that

Volume = Mass/Density

So, how do we define mass here? So, let us say we are talking about one mole of protein. So, in terms of one mole of protein, we are saying the volume of 1 mole of protein is equal to molecular weight. So, let us say molecular weight and the density which was found out is 1.37. So, this molecular weight is in Daltons.

Now, since we have this, let us further define what is this volume in one mole? So, let we can say that this is also equal to number of particles and number of atoms or number of

molecules in a mole, which is (6.023 x 10^{23}) x (volume of one protein). And now that we define that, we can further expand this volume. So, this volume is going to be depending on how we are defining how we are define the radius.

So, if we are saying nanometer then it is going to be nanometer^3. So, this then further becomes equal to (6.023 x 10^{23}) x (volume of each particle). Volume of each particle is $4\pi r^3/3$, where r is the radius in nanometer right. So, now, you have a relation between r and the molecular weight everything else is constant. So, you can solve that and if you go ahead and solve that what you will find is; this is already done.

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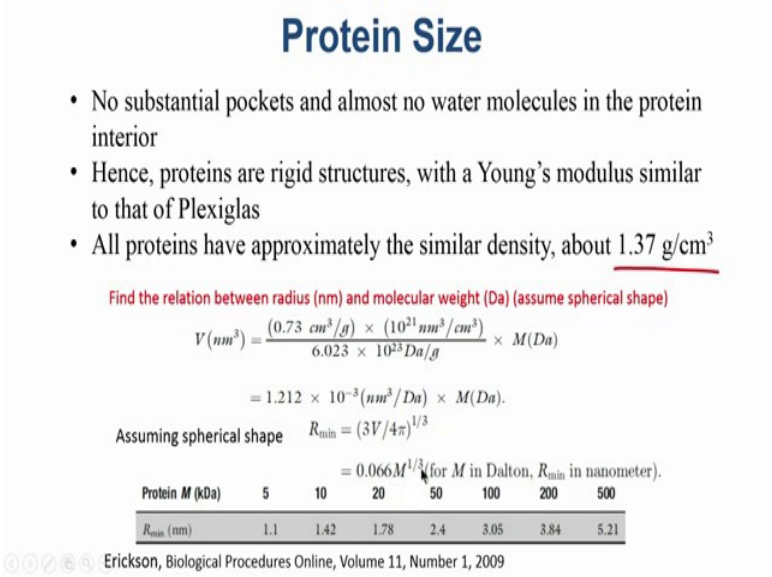

And what you will find is the relation comes out to be somewhere around

R = 0.066$M^{1/3}$ where the molecular weight is in Dalton and R is in nanometer.

And if we do this calculation of various things what you will find is a protein with a molecular weight of 5 kilo Dalton is about 1.1 nanometer and even if you increase the molecular weight by almost 100 times to 500, the radius only increases a little bit to 5.21 and that is because it is the relation is on that cube rule.

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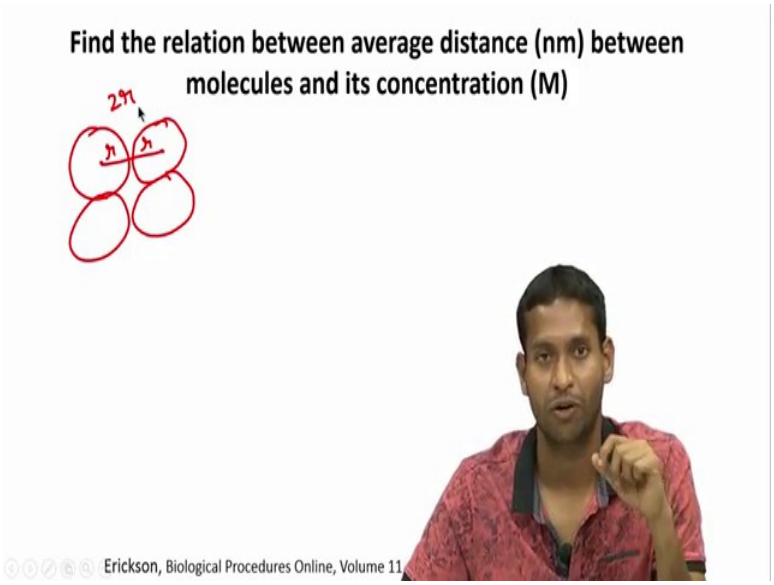

So, let us do another calculation. Let us do a relation between the average distance between the molecules and its concentration. So, if I say that a protein is at a concentration of a certain x molar then what is the distance between the two protein molecules in that solution?

So, how will it go about this. I will give you a moment to think before I start proceeding with the answer. So, in this case what we will do is we will make an assumption. So, let us say we make an assumption that for any particular concentration, the protein is completely tightly packed and there is no space. So, in that case let us say we are saying that the protein is fairly tightly packed and what we are now trying to find is the distance between the centers of these tightly packed spheres right. So, let us say this is r and this is r then distance between average since for a certain concentration is 2 r right. So now, if we have this assumption, how we can go about this.

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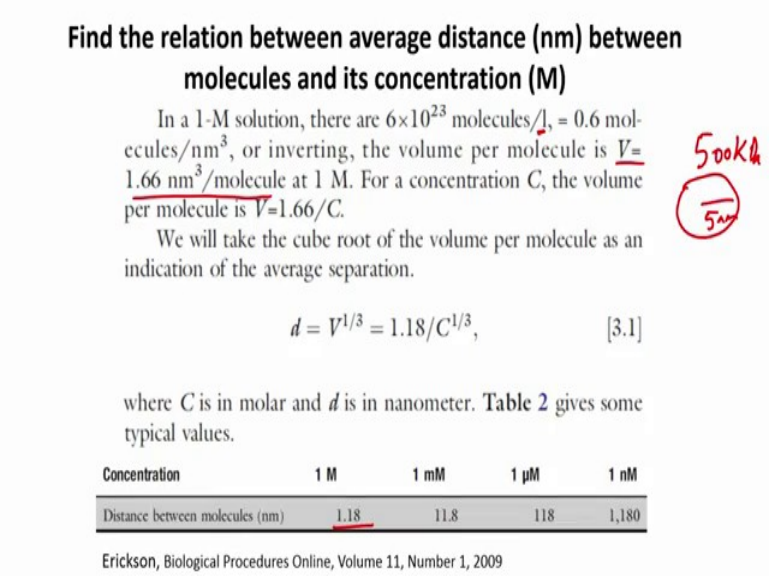

So, we can essentially assume that in a one molar solution there are 6 to the power 6 into 10 to the power 23 molecules; that means, that there are 0.6 molecules per nanometer cube. Right because we know that these many are per liter and if you do the conversion it will come out to be volume is essentially 1.66 nanometer cube per molecule.

And then you can essentially and do the similar calculation as we did before and what you will find is the concentration, if we are saying the concentration is 1 molar then the average distance between molecules is only 1.18 in nanometers. So, then that begs the question is can you make one molar concentration of a protein which is let us say 500 kilo Daltons. So, in the last slide we know that this size of a protein molecule which is 500 kDa is approximately 5 nanometer.

So, something like that cannot be physically PEG in a solution. So, that is why you have solubility limits along with other factors as well. So, that helps you kind of defining how much distances are we talking about. So, in proteins we are talking about typically nano molar some micro molars and. So, that gives you an idea of how far different protein molecules are and that helps you in terms of diffusion kinetics and all. So, we will stop here we will continue in the future classes to talk about more on the diffusion control systems as well as other controlled release systems.

Thank you.