Drug Delivery Principles and Engineering
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Lecture – 22
Nano and Micro particles – V Liposomes

Hello everyone, welcome to another lecture for Drug Delivery Engineering and Principles. We were talking about particles and their synthesis method and what different types of particles are there. We are going to continue that discussion about what different types of particles do we have.

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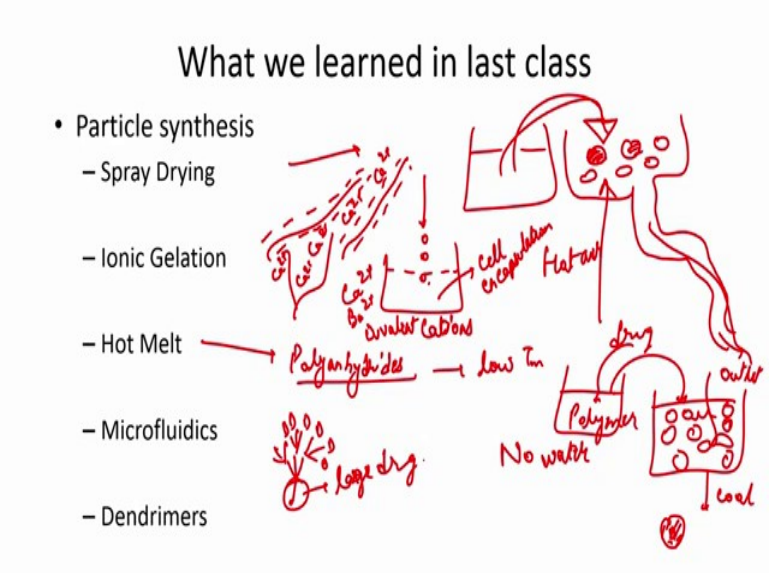

And before that we will talk about what different types of synthesis we talked about in the last class. So, we talked about spray drying which is basically nothing, but you have a solution that is being pumped through a nozzle. Let us see, this is a nozzle into a heated chamber and then, this is some sort of an outlet.

So, you can continuously spray this and you will have hot air being blown to sort of whatever droplets are being formed through this nozzle, they get dried and whatever polymer is there just gets condensed into particles, which are then collected; so that is spray drying. Then we talked about ionic gelation, which is typically used for hydrogel.

So, you have polymers, which are highly anionic and contains lots and lots of anion charge. And then what you do is you put them through a nozzle and these droplets you drop them into calcium or other barium divalent ions. And what will happen is the calcium is going to then go ahead and bind to different chains; and causes polymerization. And essentially, whatever is the size of the droplets that you are making will result in polymerization typically, very widely used for cell encapsulation as this is a very mild process. Then, we talk about a hot melt used for polyanhydrides quite a lot. And why is it used for polyanhydrides? Because, first of all polyanhydrides have low Tm. So, what you can do is, you can heat the polymer at a lower temperature and make it liquid and then, all you have to do is just mix drug and form emulsion. Let us say, this is your polymer solution; in this case, the polymer is the solvent, you add your drug and then, you mix this whole thing in oil with some stirring. In these polymer droplets will be formed, which then, you can cool to result in a solid drug containing polyanhydrides or other polymers. And another advantage is throughout this process there is no water being used.

So, the Polyanhydrides as we know is liable to get degraded by water. So, that way you can prevent any sort of change in the polymer structure. And then, we talked about micro fluidics. So, essentially there is several variations to this in the literature, but all of the involves some sort of a droplet formation in presence of oil and then, polymerization to happen this could be either through cross linker or this could be just on the basis of time.

When, you mix the polymers this could be if you increase the temperature downstream or change the pH or whatever it might be that you might be doing, but essentially cause this polymerization trigger to happen. And finally, we talked about dendrimers, which are essentially these hierarchical structures with multiple branching out and you can use that for surface conjugation of your drug predominantly or technically, you can also encapsulate large drug into the core.

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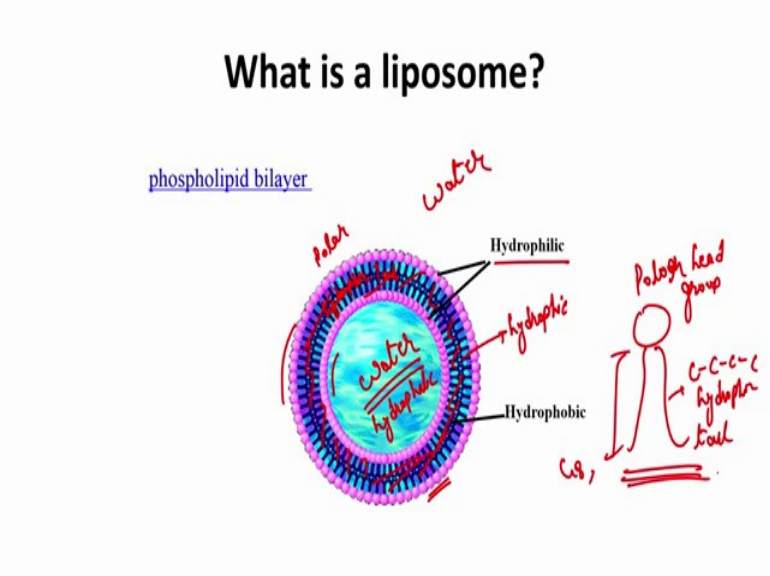

So, let us continue today with different classes of particle. Today, we are going to talk about liposomes, which is one of the very very widely used particle in the literature and what it is is very similar to your cell vesicle or a cell membrane. You have this lipid bilayer that has polar group on the outside and hydrophobic tail on the inside. And from this lipids bilayer; so, what do you have here is because the polar group is outside as well as inside your hydrophilic moieties interacting with the external water as well as the internal water. And then, you have hydrophobic domain which is sort of hidden in between these two hydrophilic domains.

And why is it so widely used? First of all, its very simple system and there is really no oil or nothing that involved at the time when it is finished; as well as what you have is a capability to encapsulate drugs, which are hydrophilic in this water phase as well as hydrophobic drugs within this hydrophobic domain. So, it gives you capability to do both of these and it is very very similar to your cell structure itself. So, it is fairly compatible and all these lipids that are seen here.

So, essentially if I zoom into individual lipids. What you have is a polar head group and then, you have a sort of carbon based hydrophobic tail. And then, these tail length can vary; this could be C18, this could be something else. It just depends on what lipid you are interested in but essentially, where the biocompatibility lies is all of these polar head groups are actually nothing, but cell membrane phospholipids.

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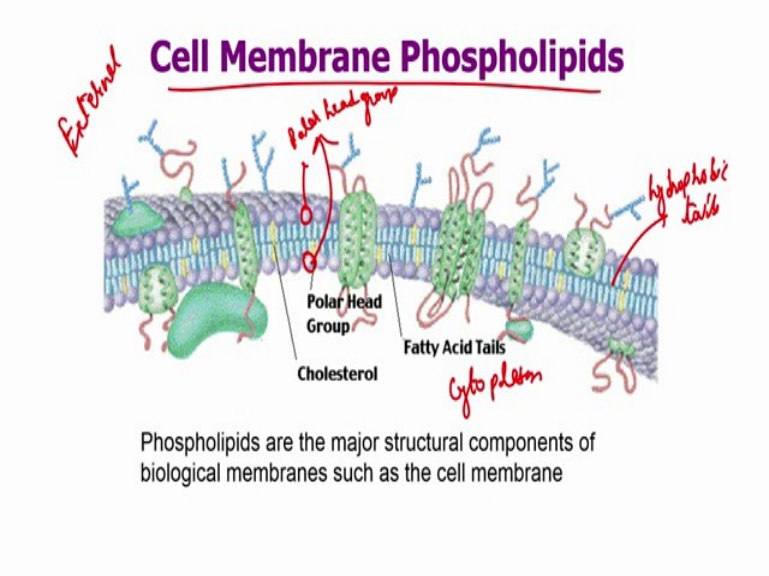

So, a cell structure is also very similar except they have lots of proteins as well. So, if you look at here. So, all of these is nothing, but the polar head group. So, this is a zoomed-in image of a cell. You have these hydrophobic tails and again there is a polar head group. And then, this is where the cell cytoplasm is and this is you have extra-cellular space.

So, there are several types of phospholipids or cell contained. So, you can use extract these polymers or these phospholipids out from the cell and use that to make liposome. So, essentially you are using the same material and that the cell itself has. So, they are extremely biocompatible in that scenario.

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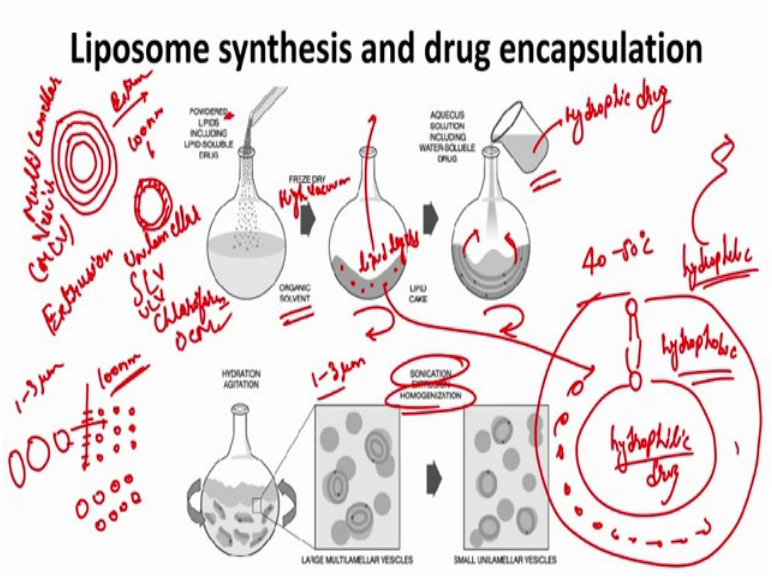

And here's basically, how the synthesis process typically goes on. So, a round bottom flask is very widely used. Although, there are other types of flasks there are also being used. And so, what do you have is you have these powdered lipids, which are then derived from your water phase or from the cell phase and these are added at a certain ratio to an organic solvent. So, this could be again chloroform or something else, DCM and then, what do you do? You can either freeze dry it or you can sort of use high vacuum. And what will happen with that is this chloroform or DCM will evaporate and while you are doing that you also sort of spin this so that you get a very uniform coating. So, in this case, If you have any hydrophobic drug, you may want to add it into this organic solvent. So, what you will get is your hydrophobic drug is entrapped within these lipid layers.

So, all of this lipid, which is not going to evaporate will just form a full on the base of these round bottom flask. Once, this is done you can come in with your aqueous solvent. So, in this case, if you want to encapsulate any hydrophilic drug you can put the hydrophilic drug into this aqueous solvent and you just add it there and start mixing it. You can also heat it up. So, most of the time the lipids that are chosen as such that they have a melting temperature at about let us say just above the room temperature or just above the body temperature.

So, let us say about 40 to 50 degree Celsius. So, you can heat it up; so that they start becoming more and more mobile and start to come off. So, now, what will happen is as you are giving this agitation as well as this hydration these lipid membranes will start to peel off, but they do not really interact with water very well because there is also a hydrophobic domain and hydrophilic domain. So, what they do is they form this lipid bilayer to minimize its interaction. And essentially, you get this lipid bilayer that is formed, all the hydrophobic drug that you had added here, gets here (in hydrophobic region), because this hydrophobic drug has nowhere else to go, everything else is hydrophilic and whatever hydrophilic drug that you added is going to end up inside or outside.

So, that way now you have been able to achieve encapsulation of both; of course, with the hydrophilic drug you have lost on the drug which is outside, which is not encapsulated. So, it will just get diffused out into the media, but most of your hydrophobic drug is in here and some of the hydrophilic drug also gets inside. So, that is how you get these vesicles that are peeled off and then, what you can do is if you want a certain size range - so typically you will depending on the lipids and sort of the agitation that you are giving, you get these in the range of 1 to 3 micron, but then what you can do is you can pass these. So, let us say these are my lipid vesicles that are being formed; you can take them and pass them through a membrane with a certain pore size. So, what will happen? As they pass through this porous membrane, they squeeze through this they will break and reform and they will end up being smaller and more monodisperse.

So, you can get it down to even 100 nanometer from 1 to 3 micron; through this process called extrusion. Other way, you can break them down is using a high powered sonication or homogenization. And what that will do is they will just give enough energy. So, these are again; these are very sort of fragile lipid vesicles. They are not very strong; they will break, if you give them too much energy just as they do here. Say we give them too much energy, they will eventually break down into smaller vesicles and that way you can also make individual vesicles that are up down to about 100 nanometer. One thing to note is with the lipid bilayer structure that it is; once you get down to 100 nanometer… so, initially when, you are saying 1 to 3 micron, they are not actually a single vesicle, but what they are is. So, you have one lipid bilayer; this could have multiple lipid bilayers over a single particle depending on the size and all, but once you

do this extrusion process and bring them down to 100 nanometer, what eventually happens is, it is not physically possible to have multiple bilayer. So, they will only be one bilayer; when you down to it about 100 nanometer.

So, these are called unilamellar; whereas, these ones are multi lamellar vesicles. These are called MLV and this is called single or the unilamellar. So, SLV or ULV. So, that is how you get various sizes of liposomes as well as various drug encapsulated both in hydrophobic and hydrophilic domains.

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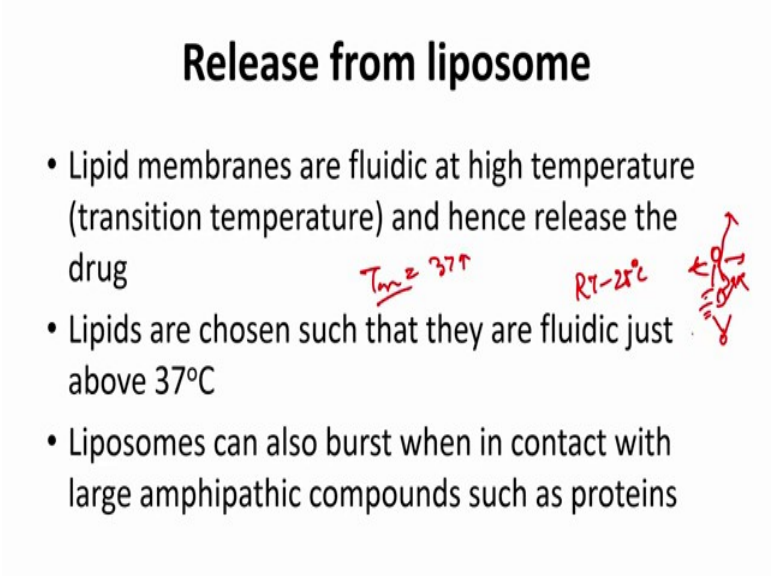

Now, once you have encapsulated you can either pellet them down or do some dialysis to remove any external drug and lipids that do not react it. So, how does the release happen? So, the lipid membranes clearly fluidic at high temperature; so, even though it looks like this very nicely packed, there is some movement with these lipid molecules and which increases as the temperature increases. So, the drugs can actually just diffuse out and depending on the solubility through this phase out from this structure. So, that is one way for the release to happen. So, for that to happen the lipids are chosen such that their fluidic just above 37.

So, at that lower temperature; let us say at room temperature, which is let us say 25 degree Celsius, they are fairly solid. So, their movement of the lipid in the sideways direction is very low. So, the drug which is then encapsulated between these lipid structures is not able to come out, but then, as you increase the temperature the

movement increases and the drug can slowly just diffuse out from here. So, you use lipids, which have a Tm of about let us say 37 or higher and then, what will happen is once you inject it in the body they will start to release the drug, and higher the Tm slower is the release the drug plus what can happen is liposomes can burst when they come to a amphiphilic molecules.

So, proteins also have both hydrophobic and hydrophilic domain. So, they can technically go and interact with these vesicles and start interacting with these inner domains and if the environment is favorable, these will open up and sort of just kind of burst and release whatever is inside these liposomes. So, these are two of the major mechanism through which the release of the liposome happens.

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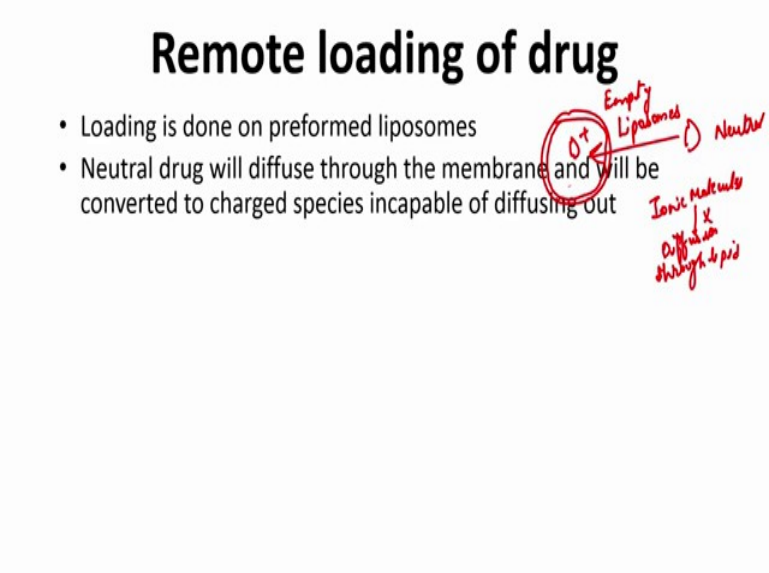

So what we talked about was for the passive loading; so, as I said if the drug is hydrophobic, it works very well most the drug goes into this lipid bilayer. If the drug is hydrophilic, it does not really work very well because there is a very little compartment that is there for the hydrophilic drug. And you just sort of relying on the diffusion or sort of entrapment of the drug when, these lipid vesicles were being formed. So, if you want to increase that efficiency there is another method called remote loading of the drug.

And so, what is remote loading? So, in this case what is done is a loading is done on a preformed liposomes. So, you already have liposomes that are formed, initially you have not put any drug in here. So, these are what you can call empty liposomes. What this

does is typically, what you will find is neutral drug will diffuse through the membrane. And once it goes inside the liposome the concept is that it will become charged.

So, what you are utilizing is we know that ionic molecules have very little diffusion through lipid bilayer, right. So, this diffusion is very low and if is neutral then the diffusion is high. So, that is what are utilizing. So, if we can somehow take a drug, which is neutral outside get it to go in the liposome and there it becomes D positive or D negative; then, it is going to get entrapped in here. And so that is the whole concept with the remote loading.

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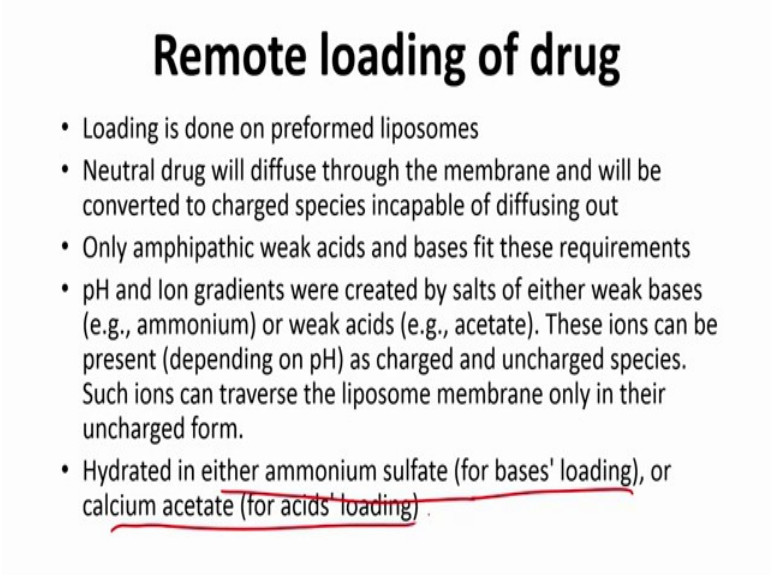

And so, what is typically done is only the drugs that are weakly acids or weekly bases can fit this requirement, because if the drugs is a strong acid or a strong base then, they will always be the charged or uncharged. So, only the ones that are slightly weak acids and weak bases fit these requirements. Then a pH and ion gradient is created. So, this could be done by using weak bases like ammonium or weak acids like acetate and depending on what the pH is inside of the outside they can be charged or uncharged and that is how they can then traverse through the liposome membrane only in their uncharged form. And we will explain how this actually happens.

And so, what is done is these empty liposomes are not really empty liposomes, but they carry these salts. These can carry either ammonium sulfate, if you are trying to load some weak bases or they can carry calcium acetate if you are trying to load some weak acids.

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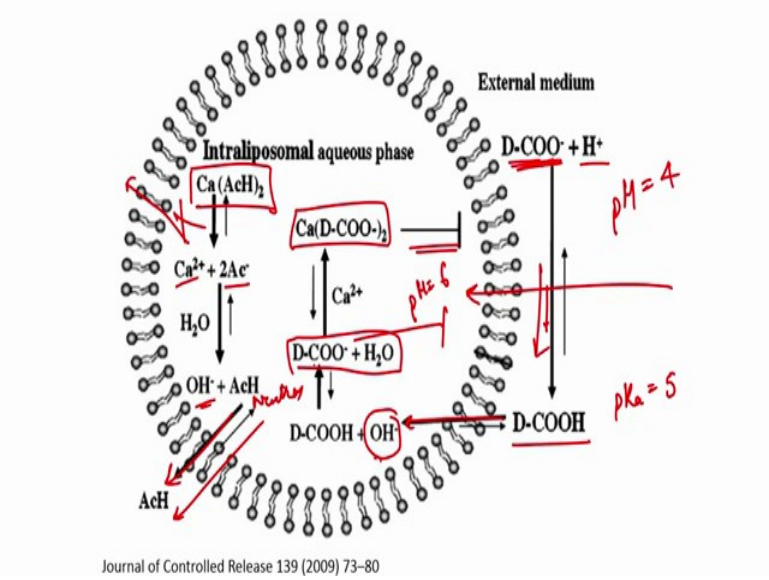

And so, let us look at pictorially as to what is happening. So, in this case is we have done is we have encapsulated calcium acetate into these liposomes and this can be done at the time of formation. Calcium acetate is a very cheap molecule and you can make a very high concentration and then, get a very high concentration in the liposome and that is present with a certain pH. Now, this calcium acetate inverter phase is going to break down into calcium and acetate which is not going to be able to diffuse out; I mean both of these are charged species; so, they cannot diffuse out.

And so, in this case let us say I have a drug, which is a weak acid; which is DCOOH right. So, this drug has a little bit of equilibrium to the D the anionic phase where it has lost its proton and this will depend on the pH of the external environment. So, let us say the pKa of this drug is 5. So, let us say the outside pH, I make it at 4. Then, what will happen? This will predominantly be going in this direction. There will be very little drug which will be actually charged, most of the drug will be uncharged. Now, because this drug is uncharged, it can diffuse through the membrane. Once it goes there let us say the pH inside is 6.

So, now once it goes there, it will take up a hydroxyl ion and become charged. Now, that the drug is charged it cannot go out. And, because there is calcium here, this will eventually bind with the calcium to form a calcium precipitate of this drug; as the concentration will increase- it is of course, not going to go out and the acetate ion will

give up the hydroxyl which is being used here and then, the acetate is coming back to the neutral form which can then diffuse out.

So, that way because of this equilibrium status of all these reactions, what will happen is the effective movement of the drug is going to be inside and the acetate ion is going to be outside. And slowing and slowly you can build up quite a lot of concentration of the drug in these liposomes, but as I said this only works if you have a weak acid or a weak base. If you have a strong acid or a completely neutral molecule this is not going to work.

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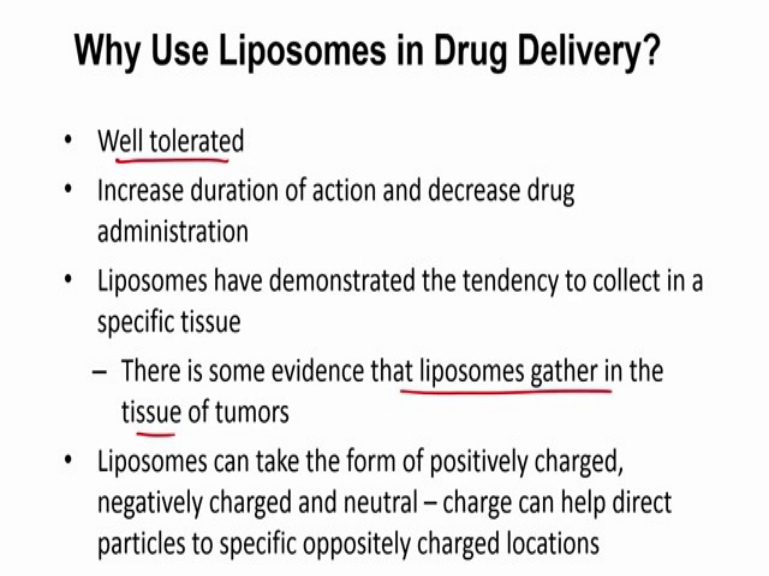

So, why use Liposomes in Drug Delivery? So, as I said first of all they are very well tolerated. I mean essentially, using the same components that are already present in the body in these cells, these phospholipids and all. You can increase the duration in action because of course, this is a controlled release.

So, now your drug is being slowly diffusing out. So, you can have a much more controlled release. You can also have these liposome design in such a way that they accumulate in a certain specific tissues because you can play around with the size you can make them 100 nanometer to whatever size you want. And there is also some evidence in the literature that they actually gather in the tumor tissues; so, very widely useful tumors.

So, these are some of the advantages and again many others they are fairly simple to use and they are; there is a lot of literature that is out there which you can then tap into to determine what sort of phospholipids to use, what drug to use, how to remote load and all of this is very well established. And then you can, take the positively charged or negatively charged lipids themselves to change the charge of the liposome and that can have again profound effects on their residence in the body as well as their motion through different tissues.

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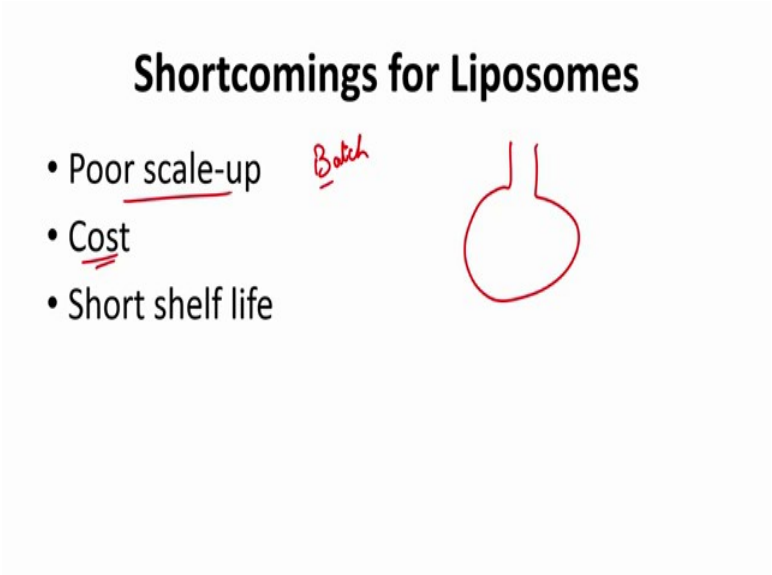

So, what are the shortcomings? Again, this is a batch process as we just said. So, scale up is fairly poor; you only have one round bottom flask at a time and because of that there is also variability in batch to batch then another issue is the cost is fairly high, these phospholipids and they are not cheap. So, the cost can be typically high compared to the polymers. Of course, which are much cheaper than; let us say these lipids and then, they have a very short shelf life; because we are talking about the drug is now diffusing out. This is constantly in some liquid phase, you are not really drying it off.

So, the shelf life is fairly short ; the drug will diffuse out and it will no longer be useful. So, these are some of the shortcomings for these liposomes.

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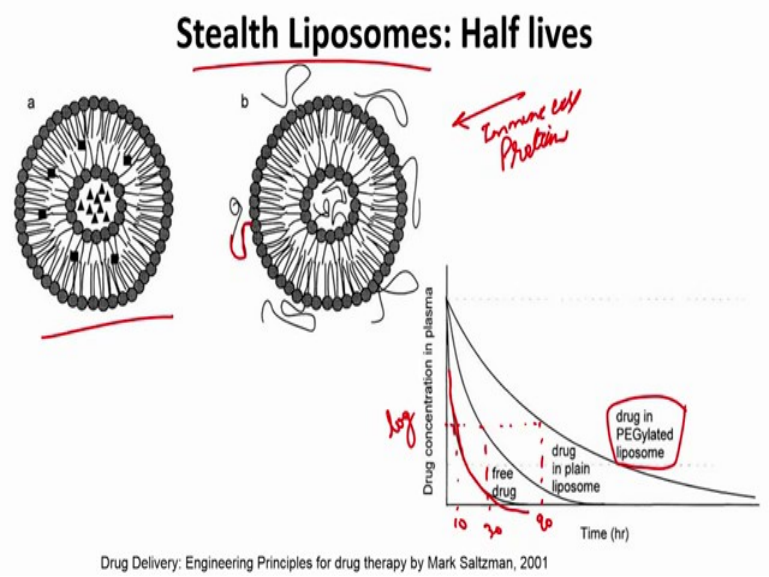

And then, like what we discussed in case of polymer drug conjugates. You can have for all the particles; I mean here, I am talking about liposome itself, but the stealth properties you can add to any particles, you can add to dendrimers, you can add to any of the polymeric particles that we prepared by emulsion processes and what it is essentially is? You can have particles by themselves or you can have particles which have been conjugated by PEG.

So, in this case what will happen is. So, here is just some model plasma concentration being shown over a period of time. So, the free drug gets quickly removed from the system. If you have drugs in some plain liposome it of course, increases this is on a log scale let us say. So, it increases the half life of the drug by quite a bit. Let us say, this is the half life; then and let us say this is 10 hours then this is almost tripled.

So, this is now become 30 hours, but what you can do is you can also PEGylate the liposome. And again, what this will do is this will act as a windshield wiper. If you remember what we talked about in a polymer class and that will prevent any kind of immune cell or proteins to interact with it and that is going to essentially increase the residence of these liposomes in the body and in the drug itself. And so, now you are talking about further enhancement - let us say to 90 hours or whatever.

By using these polymer based approaches of PEGylation. You can all increase the half life or any of the particles they will be looking about.

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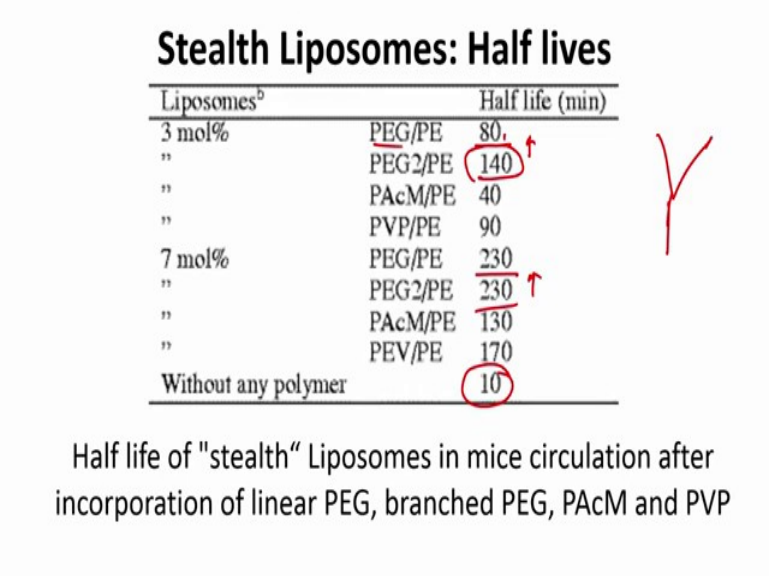

So, here is some more examples- So, these are some of the liposomal formulations that were used. So, if you used 3 percent PEG or 7 percent PEG. What do you find is the half life is greatly increased- it is about 80 minutes; without the polymer is only about 10 minutes, with the polymer for that certain liposome, it increased to 80 minutes, you can have a the branched PEG that we talked about and it further increases because more effective. And then, you can increase the PEG amount and then, these values also then further increased. As you can see it is got from 80 to 230 and the branched PEG did not really change much. So, all of these strategies can then, now be used to increase the half life of these.

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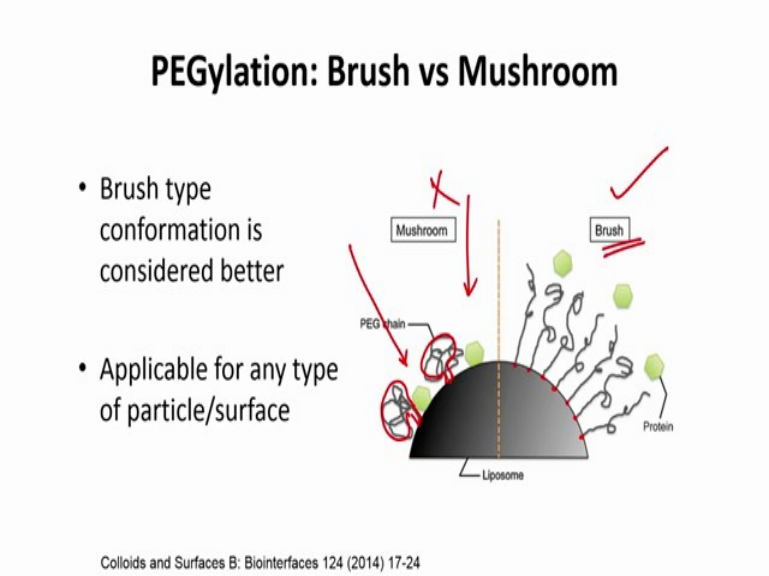

And then, PEGylation on a bigger particle like a liposome can be of a varying degree. You can have PEGylation which is being done very very close together at a very high density. And this results in sort of a linear structure of the PEG, which is called a brush conformation or you can have PEG very far away such that the PEG is then collapsed and sort of forms a mushroom like structure.

So, this is a mushroom configuration, this is a brush configuration. Of course, if it is a mushroom configuration as you can see from the picture. You have more and more sites that are open for access to the particle surface for the proteins and immune cells. So, this is not the ideal conformation, this is typically what you want if you want to prevent clearance from the body and so, that also becomes important when you are talking about a big particle and lot of PEGylation sites end up being present there. So, brush type conformation is considered better. And as I said this is applicable for any type of particle surface that you can think of.

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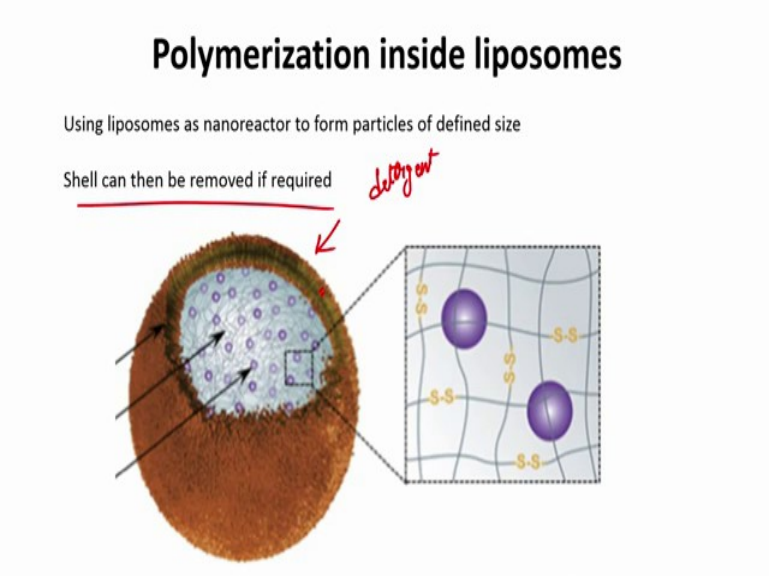

And then one other thing that we can talk about here is a special case where, you can actually use the liposomes to do polymerization. And so, how does that work is if I encapsulate- instead of encapsulating my drug only, I encapsulate some polymer precursors into my liposomes and that is now on nothing, but a nano reactor. Now, you have a 100 nanometer, diameter reactor that contains your polymer.

So, what is the advantage of that? First of all, you have precise control of the size and the second is as we said the liposomes are not very stable and they have to be always in liquid and they can continuously release drug. What you can do is you can do a polymerization inside the liposome essentially making a dense network where, then the drug becomes much more stable as well as the liposome.

And so, you can remove the shell or leave the shell whatever up to you these phospholipids. You can just put some detergent and that is going to take out all the lipid represent on the membrane or you can leave it there, it does not really matter that much, but these are essentially the nano reactors which have a defined size in which you can do the polymerization. And then, in this polymerization could be triggered by either time, the heating, the pH - anything that might cause this or a cross linker that may be able to diffuse through the membrane any of that could be used to yeah get this done. So, we will stop here and we will continue further in the next class.

Thank you.