Drug Delivery Principles and Engineering
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Lecture: 19
Nano and Micro particles – II

Hello everyone! welcome to another lecture for Drug Delivery Engineering and Principles. We are now talking about Micro and Nano Particles.

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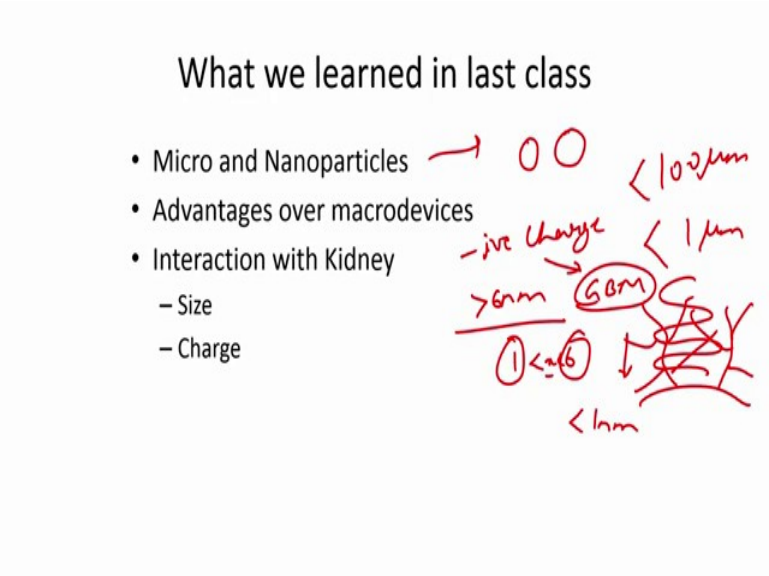

So, just a quick recap of what we did in the last class. In the last class we introduced what are micro nano particles. So, these are essentially the miniaturized versions of your matrix devices. So, fairly small, anything less than 100 micron, its micro particle and if you are talking about nano particle we are saying even less than 1 micron.

We talked about what are the advantages over macro devices. So, several of them we talked about first is of course, that you do not have to do any surgery now, you can just directly inject it, you can get more targeting, you can get more leverage with them. So, if you want to inject it in a sensitive organs, you can find the space, and won't have to do that surgery. Patient compliance is very high and then several other things we talked about.

And then we discussed how they interact with kidneys. So, kidneys again when on the major organ that decides what is the residence time of these particles and so, first we looked at what size range is these particles flow through kidney and which don't. So, first thing we talk about size and what we found that anything which is greater than 6 nanometer, will not be filtered just because the GBM, the glomerular membrane, the basement membrane is not going to let it pass through.

Then anything between 1 to 6 nanometer will pass through quite rapidly because the basement membrane is fairly permeable and 1nm is going to pass faster through the 6nm and then there is really no interaction. But once you go below one nanometer what happens is the glycocalyx of the cells in the surrounding will start interacting with this and eventually this will prevent the faster clearance, they will start interacting and we will have a much more tortuous route through this glycocalyx. So, these were some of the things we discuss in respect to kidney.

Then we talked about charge, so this again this GBM is negatively charged and so that means that if there is a positively charge particle it is strongly attracted towards it and so, the positive charge particles get cleared faster and the more dynamic the particle the less is that clearance through the kidney.

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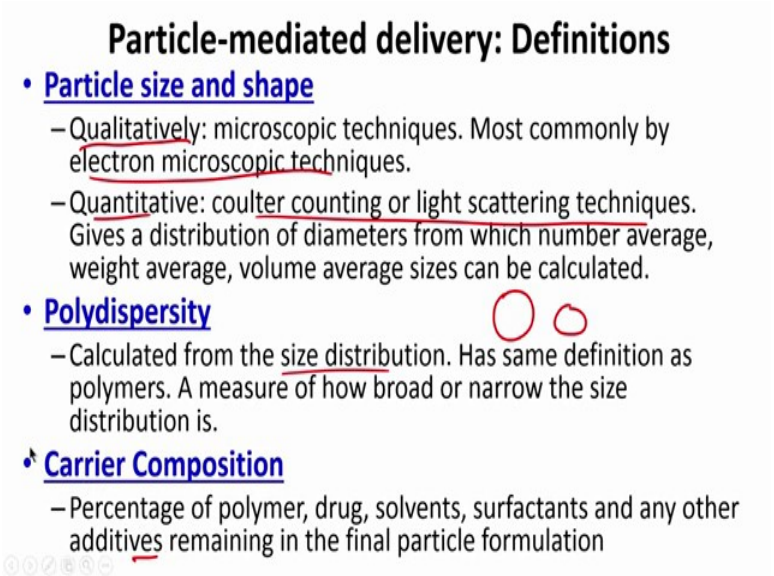

So, let us continue and let us define some terms for particle mediated delivery. And so, the first thing is particle, size and shape - these are really nothing but defining what is

the size in the shape of them; so how big they are, what sort of morphology they are, whether they are spherical, whether they are rod shaped, whether the disc shapes and whatever shape they might be. And of course, there are several techniques that you can use to sort of get an estimate of this. So, you can either get a some sort of a qualitative estimate - you can use microscopy we have now very powerful microscopes that can image even 10 nanometers and lower.

So, and you can get down to even electron microscopes that can now even go down to single digit size ranges. And then you can do this quantitatively, so there are several techniques - coulter counting or light scattering techniques and that will scatter light and you can get an estimate of what is the size that is scattering it. You can get a distribution of what sort of diameters are present in the whole population, maybe they are not all particles are the similarly sized and all of these can be used to get an estimate of the particle, size and shape.

Another is polydispersity, so it links to this average distribution. And what it is, is unlike these macro devices where you have quite a bit of control as to what is the size of the device you are making - if making 1 millimeter you typically get it within plus minus 0.1 millimeter. But with these micro devices and nano devices that is not the case because- and we will discuss about why this polydispersity- but then there is quite a bit of polydispersity that you may find in different samples.

And so to define that we have this term where polydispersity that basically calculates the size distribution. So, whether how many particles are there with, let us say, 1 micron, how many particles are there with 500 nanometer and all this will define what polydispersity is. And essentially it is just a measure of how broad or narrow the size distribution is.

And then the carrier composition. So, first of all what is the polymer that you were using, what amount of polymer is that you were using. So, how much percentage is polymer, how much percentage is drug, how much percentage is other components like solvents, and surfactants (if they are there) if there is any other additives that you are adding. So, all of that is present in that case.

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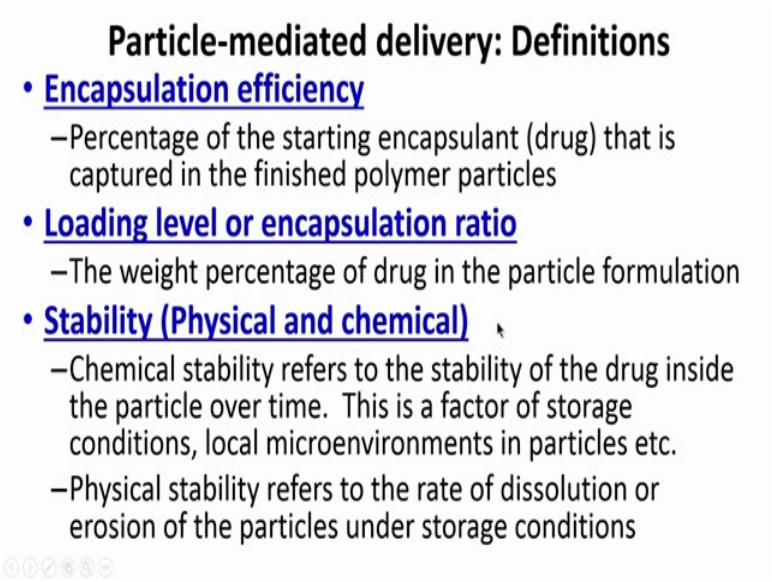

So, some more definitions. Next thing is the encapsulation efficiency and that comes in when we are sort of talking about drug itself. So, what is it is how much of the drug that I started with I was able to encapsulate in my polymer. So, let us say if I want to make particles and I want 1 milligram of drug to be encapsulated in these particles. So, I make these particles and we will discuss about various synthesis methods.

So, let us say the particle synthesis is done and at the end of it I end up with 100 milligram particles. Of course, this 100 milligram is essentially and the weight of the polymer plus the drug and then what I do is I then dissolve this whole 100 milligram of particle and see how much drug was actually there and I found that instead of one milligram I could only get about let us say 800 micrograms drug in the 100 milligram, so; that means, I have lost about 200 milligram of drug during the synthesis process.

So, my encapsulation efficiency is going to be 0.8 milligram divided by the initial drug which is 1 milligram multiplied by 100 to get a percentage and that is nothing but essentially 80 percent. So, in this case my encapsulation efficiency is 80 percent, but this is how it is going to be typically defined.

What about loading level encapsulation ratio? So, this is another way to define to get an estimate of how much drug is present. And what it is just the weight percentage of the drug in the particle formulation. If I just take the last example and I said I had 100 milligram polymer (particle) and that was containing about 800 microgram of drug. So,

in this case now loading level is nothing, but 0.8 milligram divided by 100 milligram and so, my loading level is actually less than 1 percent in this case. So, that is how it is sort of defined. If I multiply this by 100, I am going to get 80 by 100, so my loading level is only 0.8 percent. So, that is how it is typically defined.

And then we have stability. So, whether how stable the particle is, whether I can store it for longer durations or not I mean this may had nothing to do with the drug itself or it is a combination of drug in the polymer stability, but maybe my polymer itself does not really remain stable. So, the drug will obviously, come out.

So, essentially chemical stability refers to as disabled the drugs inside the particle over time. So, what environment you are storing it in, what are the different conditions and the physical stability is what if the particles are degrading, they are absorbing water or moisture from the air and then causing them to degrade and erode over time even before its put in the body. So, that becomes important in terms of determining the shelf life and efficacy of these particles.

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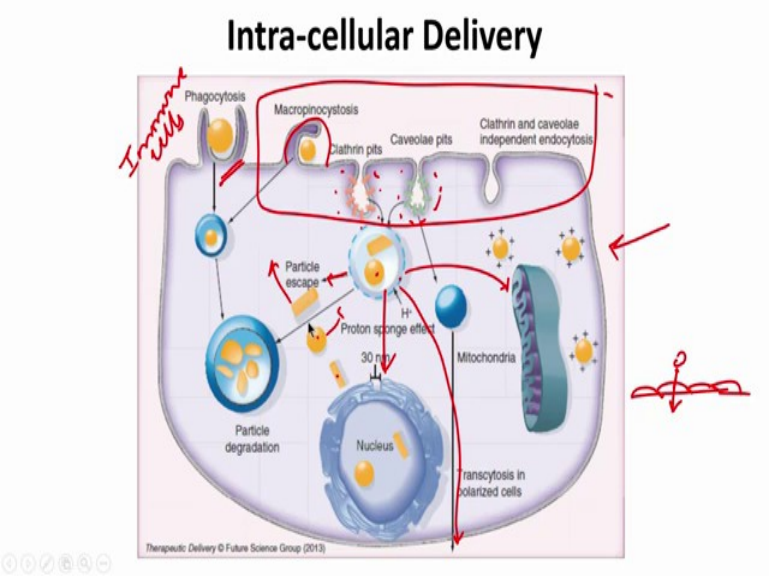

So, briefly I had mentioned in the previous class that these particles can also be used for intracellular delivery. And so, what do we mean by that? So, let us say this is a cell that we have a picture of. And so, cells itself have sort of evolved different pathways through which they can take up a external material- this could be glucose or any sort of energy that they need or could be food in some pathogenic sort of cells. So, the several of the

ways through which they take large molecules like particles and this could be phagocytosis, mostly present in immune cells.

And then best of the other other pathways that are defined here these are shown by nearly all types of cells. So, this could be macropinocytosis which is nothing but the membrane ruffling, so the membrane will ruffle and just sort of eat up whatever is in the surrounding, it could be is a mediated by some sort of proteins present on the cell membrane. So, this could be a clathrin protein and so, they form small pits all this could be caveolae proteins they also form small pits, but they're different proteins. And then there are some other pathways that are not really well known and they are being sort of clubbed into clathrin and caveolae independent endocytosis.

So, all of this will result in some sort of a vesicle being formed, containing particles that are membrane bound and depending on what sort of particles you are using, what cells it is, what stage this is being administered, these particles can escape through these vessels and cause intracellular delivery especially in the cytoplasm. Or these vesicles themselves can then be targeted different organs, they can be targeted to mitochondria, they can be targeted to nucleus, they can be targeted some other organ or they might just be transcytosed. So, let us say if I want to cross a barrier with cells on it and if the particles do transcytosis, it means, that this cell is going to take up this particle and essentially just throw it out on the other side. So, all of this is fairly feasible.

So, now, if these particles are degradable and are carrying a drug that is extremely hydrophilic and would not have been able to diffuse through the cell membrane, now this drug can actually do that. Because, now this drug is in these particles which get taken up through these specialized uptake pathways and now it is in the cell where it can get released. So, this is what we meant by intracellular delivery.

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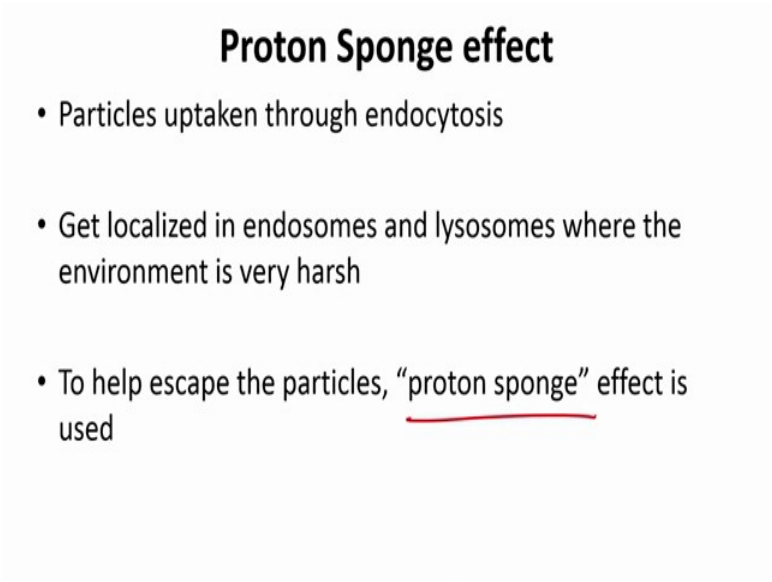

And then, since I mentioned here that these particles can actually escape from these vesicles. What is the mechanism through which they can escape from these vesicles? So, that is called a proton sponge effect - at least one of the ways that we can enhance this is using a proton sponge effect and which is when particles that are taken through endocytosis or phagocytosis.

They typically get localized and are entrapped in these endosomes and lysosomes which is a machinery for the cell to degrade any kind of external particles or nutrients that it has taken up, and these environments are actually very harsh, they have very low pH and lots and lots of degradative enzymes are there.

So, if your drug is getting released there, unless you want to target those locations, you don't want the drug to come out because these are not conducive for the drug action and it may it may even destroy the drug. So, what is done is to help the particle escape effect which is called proton sponge effect is used. And so, what is proton sponge effect?

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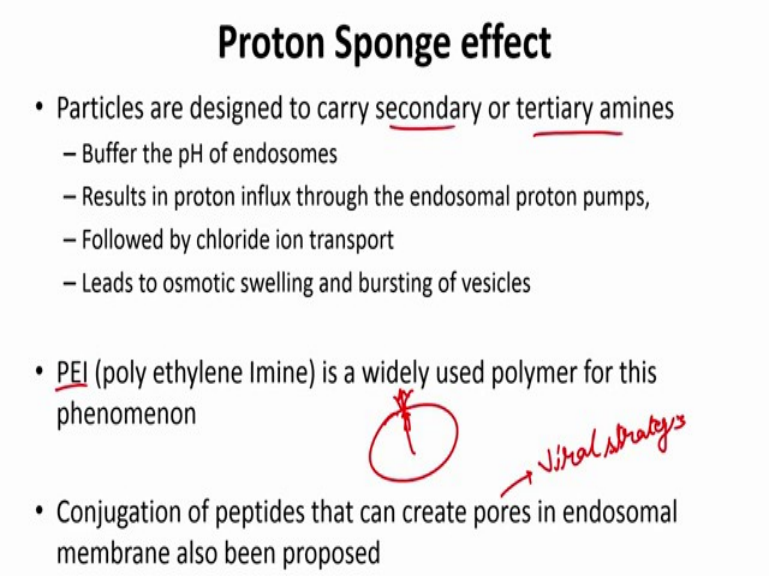

So, these particles are designed such that they carry lots of secondary and tertiary amines. So, let us say if I have a polymer that carries lots of primary, secondary and tertiary amines. What is going to happen is now these amines have quite a lot of capacity to take up H plus ions (protons) and as I just mentioned that there are endosomes and phagosome. So, this is a cell and here is my endosome.

So, the pH outside and the inside the cell is close to around 7, but the pH of this is now maintained at around 5 and it further decreases at it as it goes. So, these are early endosomes and when these mature, they turn into lysosomes, where the pH can drop down to 2 to 3. So, for the facilitation of this process to happen from here the pH is 7 goes to 5 goes to 2 to 3 the way the cell does this is it has lots of proton pumps.

So, what it does? It takes up H plus ions or protons and pumps it in to the system. And obviously, there has to be some sort of osmotic balance otherwise is these vesicles would not be able to last, more and more water will also go in. So, now what is happening is now I have put this polymer. If I zoom into one of these endosomes, so I have lots and lots of primary and tertiary amines that are present, they are taking up this H plus ion. So, they are taking up this H plus ions and not letting the pH drop. So, the pH is still let us say 6 at this place.

Now, the cell does not like that, so it is pumping more H plus ions into it and this continues till either you hit the saturation of your tertiary and primary amines or tertiary

second amines and if we assume that there are so many of them that they will not hit that that quickly. Then what will happen is this H plus will continue to pump in. Because of this now there is too much ions in your vesicles then there are outside. So, ions in here are greater than the ions outside and so, that causes an osmotic imbalance.

So, now there is an osmotic imbalance, as a result of which the water from the surrounding starts to go in and starts to maintain this osmotic balance and as it goes in there is a some sort of capacity to which it can absorb the water, but eventually the pressure inside becomes so high that these vesicles just burst. So, once they burst in whatever particles are residing here, they come out and they are now in the cytoplasm do not have to go through this harsh environment of 2 to 3 pH and that is essentially it is a proton sponge effect. So, I hope this is clear.

So, H plus is going in to maintain the charge, chloride ion also goes in. Now, you have lots and lots of H plus and chloride ions that are present in your system and because of that there are lot more ions in your vesicles containing these particles and which then release which then causes the imbalance in the osmosis, and the water goes in to maintain that balance, and these vesicles swell, and eventually they burst after a certain pressure is achieved.

So, one of the polymer is very widely used is polyethylene amine. It is a highly positively charged polymer just because it has lots of tertiary and primary amines and again very widely used. And then there are other mechanisms you can conjugate some peptides, this is mostly adopted from a viral strategy. So, some of the viruses what they do, is they have peptides that go and poke holes into the membrane.

So, these peptides will go and sort of make a hole through which your things can escape when these vesicles may burst up. So, these are different mechanisms you can use to sort of utilize this proton sponge effect or these pore creating peptides to come out from your endosomal membrane.

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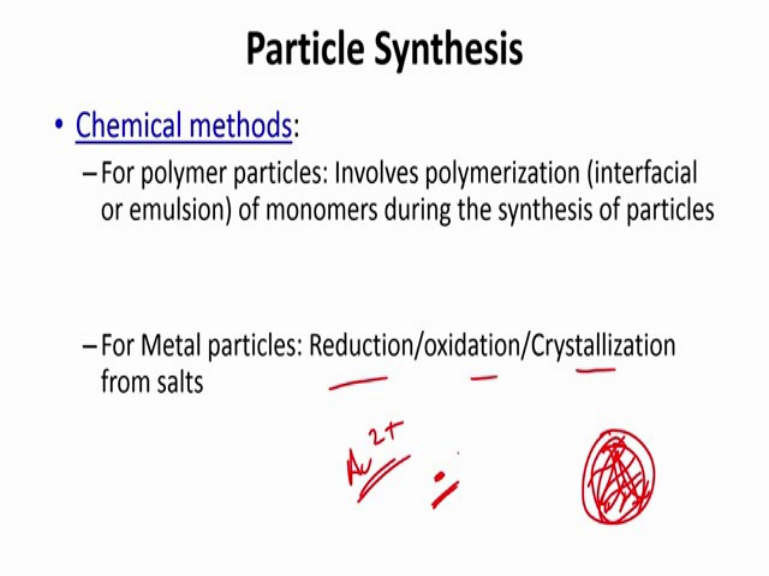

Let us talk about some particle synthesis methods. So, first we are going to talk about chemical methods. These, for polymeric particles, involve some kind of a polymerization. So, you can take polymer in confined states, and start this chemical reaction at the as the chemical reaction will proceed more and more of them will cross link and by the time that action finishes is the end of forming a particle.

But for metal particles reduction, oxidation, crystallization, from salts is used quite a lot, so you can get you can let us say have gold salt. So, you can have a gold salt and then you can reduce it or oxidize it to basically return it to unit of state where they will start to sort of interact with the other gold ions and form a particle in depending on what concentration of the salt you have used you can vary the size of these particles.

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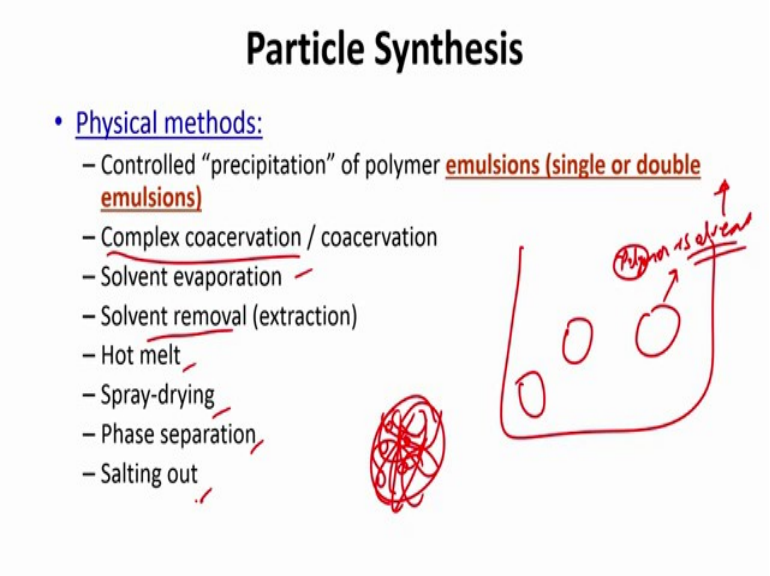

And then there are lots and lots of physical methods much more widely used these could be controlled precipitation of the polymer. So, basically you can have an emulsion process and we will describe this in a little more detail as we go along, but you can have an emulsion process and you, let us say, form these emulsion droplets with polymer plus solvent. But this solvent is volatile, so it is it tends to just dry off and once it does this polymer concentration is going to start increasing as well as this thing is going to shrink and eventually all this polymer will just precipitate and forms a physical cross linked network, which will then leads to particle and whatever drug that might be dissolved in here it just gets entrapped.

And again, as I said we will describe this process in more detail. So, solvent evaporation is again very similar method here. And there could be other methods that people are using, you can have a complex coacervation. So, we discussed briefly about this during ionic hydrogels the same mechanism can also happen at nano scale depending on the concentrations and ratios or different things that are using. You can actually actively remove the solvent rather than just relying on it evaporating out their process for hot melt, spray drying, you can phase separate things out and that may result in formation of nano and micro particles or you can use salt to sort of induce this sort of separation or precipitation of the polymers.

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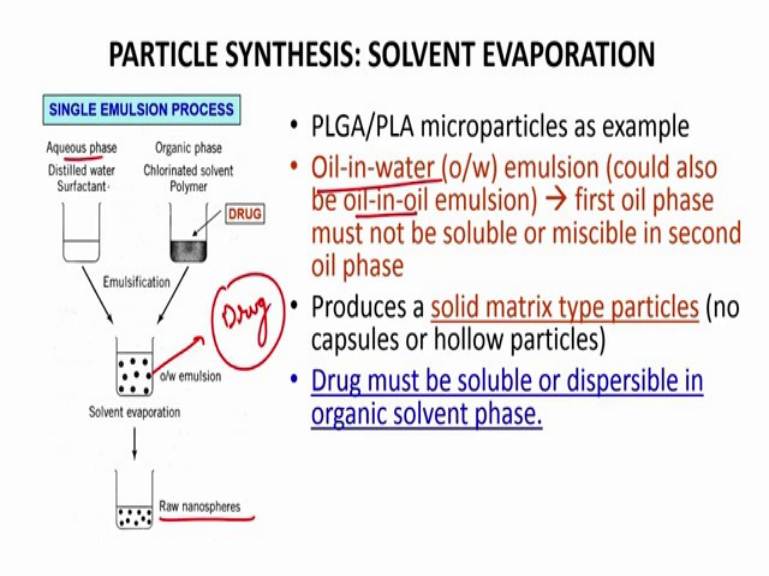

So, let us talk about the solvent evaporation method one of the very widely used method. So, first we are going to talk about single emulsion process. And so, what is typically done is this contains two phases you have equal phase and organic phase, and in equal phase you can have a distilled water with surfactant, in the organic phase you can have some chlorinated solvents or maybe something else which also contains a polymer, depending on whether your drug is hydrophilic or hydrophobic you choose these methods in this case it is mostly used for drug that is hydrophobic and I will describe why.

So, you add your drug into your organic phase because if it is a hydrophobic drug it is only going to get solubilized into the organic phase and then you emulsify it and emulsify just means you mix them and give it some energy. So, when you give that this organic phase does not want to interact with the aqueous phase at all. So, what did we do it will first if you do not give any energy, you will have them phase separate like this.

Where this is your equal phase or organic phase or this could be vice versa depending on which one is heavier, and they will just separate out. But when I constantly give it energy and force it to mix, they will mix, but they will mix very reluctantly. So, what will happen is even though they have mixed these two phases will not want to interact with each other.

So, what will happen is depending on which amount is more that will act as a bulk layer and rest of it just is going to make these micro and nano phase separation, which is to basically prevent bulk of the organics solvent from interacting with the aqueous solvent. And so, the more energy I give, the smaller these droplet us will become, and that is how they will sort of phase separate out.

And now let us say this organic solvent is volatile or as evaporates at a very rapid pace. So, once this evaporates, what will happen is first of all these droplet us will shrink and then eventually whatever the polymer is there will exceed the solubility limit because the solvent is constantly evaporating out and that will eventually cause this physical precipitation of these polymer molecules to result in nano or the micro spheres.

So, let us see, so you one of the examples that is very widely used for this type of process is PLGA or PLA micro particles they are again fairly hydrophobic. So, if you are using PLGA it has to go into the organic phase, one of the organic phase that is very used either chloroform or DCM (dichloromethane).

So, this process is again referred to as oil-in-water and the reason for the oil in water is because if the oil is in lower amount than this water, so essentially this oil droplets are in water. So, it is very famously known as oil-in-water or oil-in-water emulsion. This could also be oil-in-oil depending on what external phase you are using. So, you may decide to use instead of aqueous phase you may decide to use another oil, but this oil is immiscible with the other oil. So, in that case should be oil-oil, but again it is typically not used for the applications related to the body because we always want whatever particles that are made be able to interact with the water and so, for that one of the phase is typically aqueous.

So, as I said, if I zoom into these small droplet us that are evaporating solvent what you are essentially having is these polymer chains, when the size is decreased, these polymer chains are coming closer and closer and then eventually they are just, there is no solvent, all you have is this polymer chains and so, these will essentially represent a solid matrix. There is no sort of capsule or hollow particles here, these are all solid matrix that are formed. So, this process will result in a matrix type particle, not a hollow capsule.

The drug must be soluble and dispersible in the organic solvent phase that is why I said it is used for hydrophobic drugs. If the drug is not soluble here then it cannot really go in, only the drug is soluble here, it will also be present in these droplet us that are here.

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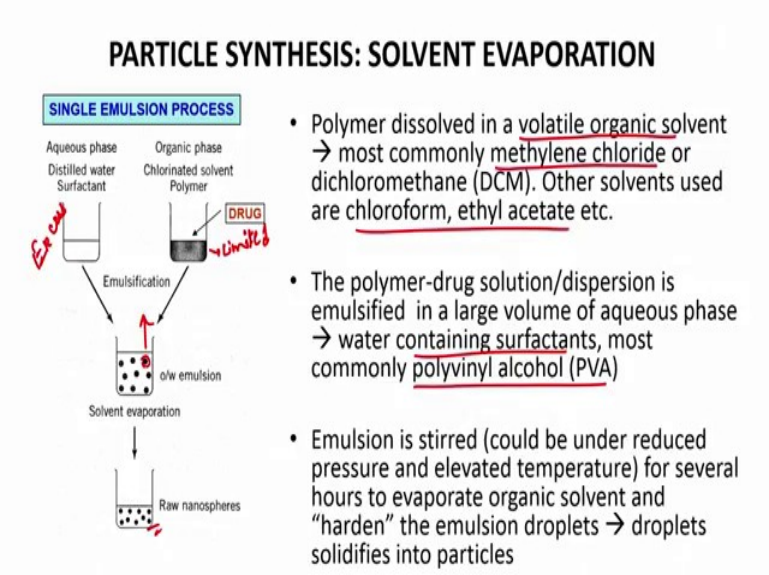

And then the polymer is typically dissolved in some volatile organic solvent. So, as I said one of the most commonly uses a methylene chloride or DCM. Other solvents like chloroform and the ethyl acetate are also used, but they have to be volatile. I mean if they are not volatile, then this process will take forever for the to evaporate and they had to be more volatile than water because you do not want the water to evaporate first.

The polymer drug solution dispersion is emulsified again in large volume. So, as I said this is going to be in excess whereas, this is going to be limited and then typically some sort of a surfactant is also added. So, what the surfactant does is it just localizes itself at the edge of these particles because these are surfactants have domains that want to interact with the aqueous phase they have domains which want to interact with the organic phase.

So, they sort of stabilize once these particles are formed. So, you do not have to continuously give the energy during the evaporation phase. You can just give the energy once for a certain amount of time and when these droplet have stabilized due to the presence of these surfactants like PVA, you can then leave it and do not have to continue to give energy to this.

And then this can be stirred under reduced pressure and elevated temperature, if you want to increase this evaporation rate or you can do it at the room temperature and normal pressure as well to let these particles harden. So, we will stop here and we will continue more in the next class.

Thank you.