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

Lecture – 20
Nano- and Micro-particles-III

Hello everyone, welcome to another lecture for Drug Delivery- Engineering and Principles. We have been talking about now nano and micro particles and we defined some of the things and we were talking about the synthesis methods.

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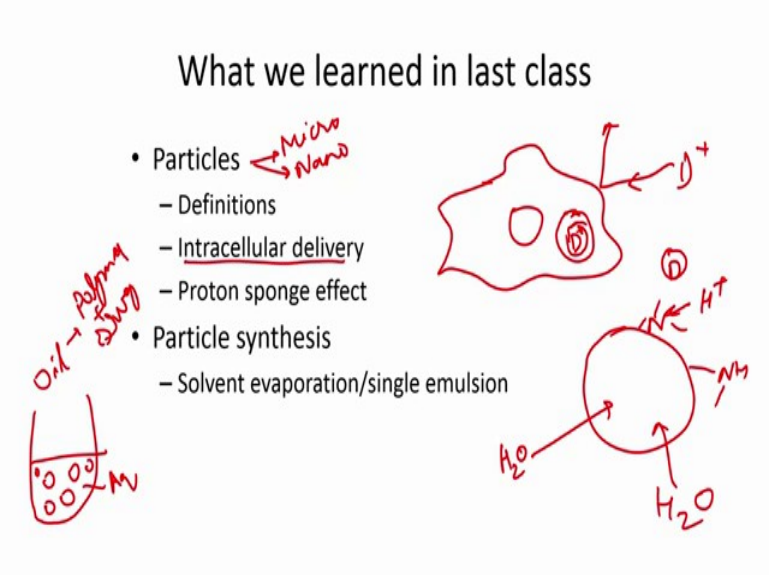

So, let us get started; so, just a quick recap of what we learned in the last class itself. So, as I said we talked about particles, both micro and nano. So, the definitions as to what are micro, what are nano, what are the size limits and things like that. And, then we talked about that these particles have advantages over, let's say, an implant system where these can be used for delivery to intracellular regions.

So, you can have a cell- usually if a drug is hydrophilic or charged the drug is not able to go through, if let us say the drug is charged and is hydrophilic. However, once you package it into a particle and encapsulate this drug inside, these cells have endocytosis mechanisms through which they can take up these particles with drug and that is how you can deliver things intracellularly as well. Then we talked about proton sponge effect. So, what was proton in sponge effect?

So, again zooming into this if we have let us say a vesicle containing particles, these vesicles will have to burst for the particles to come out into the intracellular environment .Because, we do not really want to deliver most of the drugs to these endosomes and lysosomes which are very sort of toxic to these drugs. So, for that to happen we use something called a proton sponge effect and in this you make your polymer with lots of tertiary and secondary amines. And so, they keep on absorbing H plus ions that are being pumped into these endosomes and does not let the pH to drop.

And, because of that the cell keeps on pumping more and more H plus and other ions into these vesicles causing an osmotic pressure and the water to start moving in because of this is osmotic pressure and ultimately the vesicle then bursts. Then, talked about few of the particle synthesis methods, both chemical and physical, one last thing that we were talking about before we left in the last class the solvent evaporation, single emulsion method. And, what we do in this is you have, let us say, your polymer being dissolved in let us say some oil phase and which contains your polymer plus drug.

And, then what you essentially do is you add that to an aqueous phase and give some energy that results in emulsion main form which is essentially is the separation of this oil from the aqueous phase. And, you let the oil slowly evaporate out maybe its volatile and that causes the precipitation of these polymers will be present in the oil phase to form particles encapsulating the drug. So, we are going to continue further today in a particle synthesis methods.

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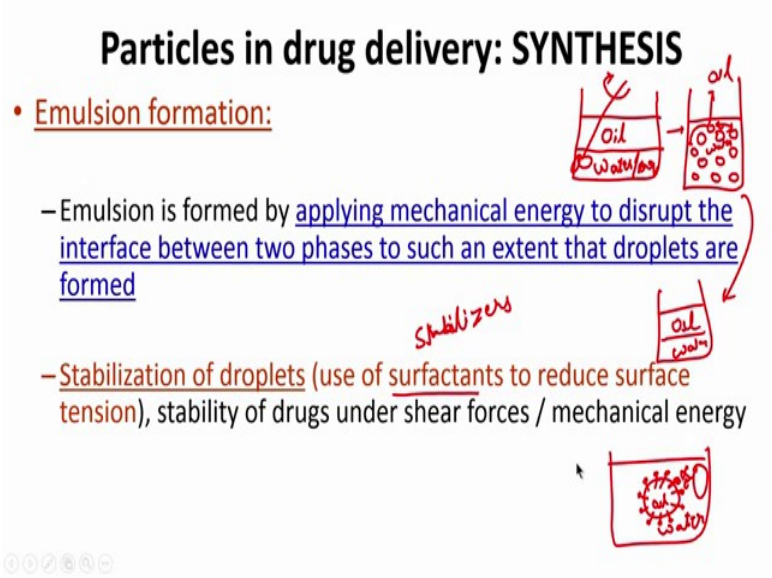

So, we talked about it briefly in the last class too, but emulsion formation is when the key steps which leads to the synthesis of these particles. So, what is emulsion? Emulsion is nothing, but if you apply some mechanical energy to disrupt the interface between two phases it causes the droplets to form. So, let us say, if I have this beaker and if I put both the oil and the water phase. So, what will happen they will phase separate out, they do not really want to interact with each other. So, you will have water or aqueous phase separating from the oil phase again depending on which one is lighter. So, typically oil is lighter than water, so it will float above water.

However, what happens if now I come and give some energy to it? So, what will happen because of this energy and like forcing this oil and water to mix, but the oil and water does not really want to mix. So, what will eventually happen is instead of mixing, there will be a single phase, depending on which one is the higher amount. If water is in excess or oil in excess and the other phase, the oil or the water will tend to form these droplets. And, these droplets are being formed because of this energy we are sort of breaking this interface again and again.

So, the more energy will give the smaller these droplets will be and these droplets will then tend to form. So, this process is nothing, but it is an emulsion. But, let us say I stop this process, what will happen is because these droplets are continuously moving around and they do not really want to interact with the water interface, let us say this is oil and

this is water. So, these do not really want to interact, they want to minimize the contact with the water. So, what will they will do is they will start to coalesce, so they will mix and essentially go back to the initial separation. But, this is something that we do not want because ultimately all of these individual particles that we had initially is what is going to give us the particles.

So, to prevent that we add these surfactants or sometimes they are also called as stabilizers to these mixtures. And so, it stabilizes- if I now zoom into one of these droplets. So, this surfactant and what a surfactant is nothing, but an amphiphilic molecule which has parts which are both hydrophilic and hydrophobic. So, what will happen if let us say this is oil, this is water. So, what the surfactant will do is the hydrophilic part will try to interact with water and the hydrophobic part will try to interact with the oil. So, it will form this barrier between the oil and the water.

And so, what this does is it sort of stabilizes this droplet because now technically speaking the oil is not in direct contact with water and neither is the water in contact with oil. So, in that way these droplets are much more stable and they do not tend to mix with another droplet. So, this will not happen once the stabilizer is present.

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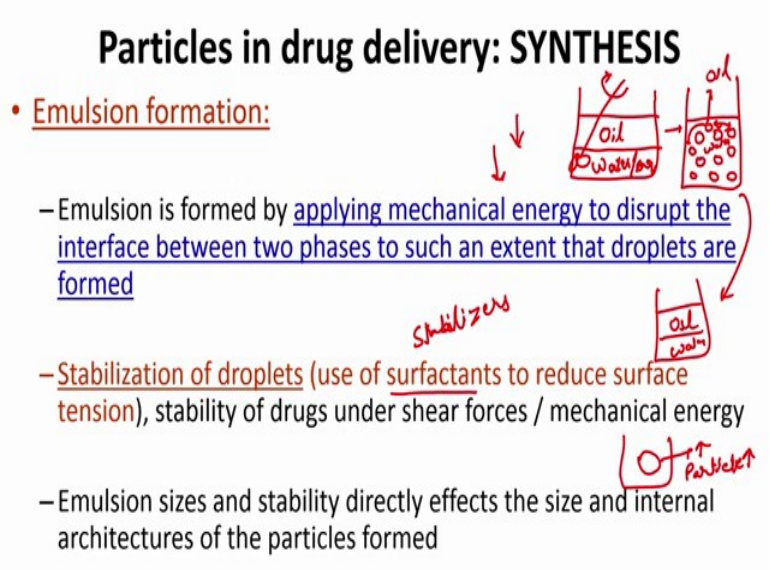

And, once we have that, the emulsion size and the stability will directly affect the size and internal architecture of the particle form. So, bigger is these droplets that we have, the bigger is the particle, if this is higher then now eventually the particle will also be

bigger. And, that is because whatever polymer is there will essentially collapse and they will be more polymer in the bigger droplet. So, that is how you will determine the sizes of your particle.

So, if I want bigger particles what I will do is this mechanical energy supplied will decrease. Because, if it is decrease then you get bigger sized droplets and those will eventually result in bigger sized particles. And, if I want smaller and smaller particles I will continue to increase this mechanical energy till I achieve that size range that I am desiring.

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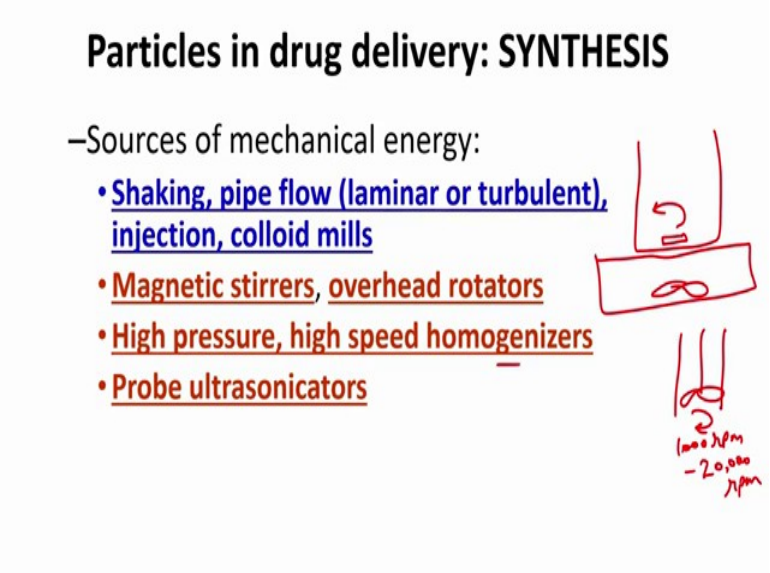

So, what can be the sources of these mechanical energy? So, there can be several of them; so, it could be just simply shaking - you this hold the beaker in your hand and just keep on rotating it, you can give it a lot more energy, you can put a magnetic stirrer bead in that. So, this is very commonly seen. So, you have a magnetic heat plate, it has some kind of a magnetic rotation that is happening and you keep a magnetic bead here. So, this magnetic bead will also rotate giving energy in the system.

So, these are some somewhat some low energy based things that we talked about, then you can have high speed homogenizers. Homogenizers that can then have a propeller in them and these things can spin it anywhere between 1000 rpm to 20,000, 30,000 rpm. And, that can give a lot more energy to get smaller sizes or you can give something like an ultrasonicator which will then send out these very strong magnetical forces that will

result in very small droplets. So, all of these methods you can then use to sort of vary the size range that you are looking for. So, we talked about the single emulsion already.

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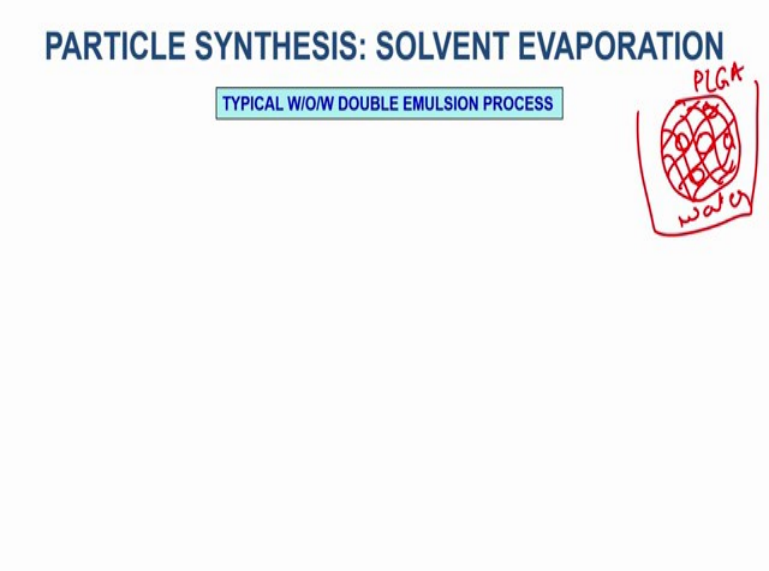

We are going to now take this forward and talk about double emulsion. So, the problem of the single emulsion is that you can only get a hydrophobic drugs in there, because let us say if this was this is my particle that is form of a single emulsion. This particle is completely covered with polymer. And then this polymer let us say in case of PLGA, this polymer is fairly hydrophobic which essentially means that the drug that is going to stay in here has to be hydrophobic.

If it is a hydrophilic drug then it will not tend to stay in, but it will never actually go in the oil phase, it will always be remaining in the water phase which is outside and you will never get that drug encapsulated. So, this double emulsion is sort of a modification to the single emulsion process which allows you to encapsulate both hydrophilic and hydrophobic drugs and let us talk about how we actually do that.

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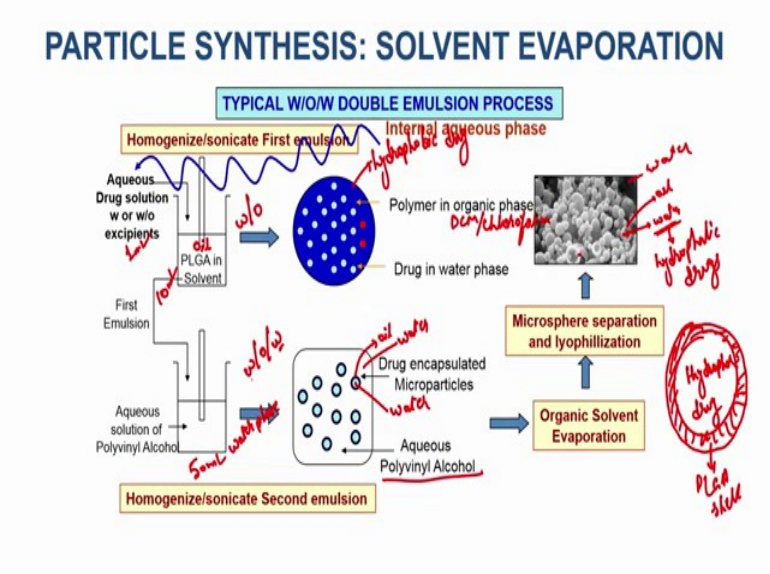

So, to do that what we have is we have aqueous drug solution which is typically water or you can have water in oil. So, in this case you have this is an oil phase, you have aqueous drug that you add a little bit of it let us say this was some 10 ml, then you added let us say 1 ml to that and then you homogenize it. So, what will happen you will get a very similar thing that happened in the previous case so, you will get an emulsion. Single emulsion in this case and the single emulsion is the other way round. So, in the previous case we had oil in water, in this case now we have since oil is in excess and water is in limitation.

So, let us say this was 10 ml and this was 1 ml. So, what you will have is you will have predominantly small-small water droplets in the oil phase which is the PLGA phase. And so, that is single emulsion there which is called water in oil, then what you do is; so, this is essentially just a zoomed an image. So, you have drug in the aqueous water core, you have this the polymer in the organic phase, this could be DCM, this could be chloroform.

And so, that is how you stabilize the first sort of your emulsion process and then you take this whole first emulsion and let us say you dump it in 50 ml water phase. So now, what is happening and now we have now increased the water content in the whole mixture and now if you give energy to this. So, now you are basically taking that whole thing and giving it energy. So, what will happen is these initial droplets have already stabilized.

So, what will happen now is these will result in a double emulsion. So, earlier we were talking about we have water in oil. Now, we have water in oil in water; now this water is in excess, but this water is stabilized within this oil. So, you will get something like this where you have this is an aqueous. So, in this case we have used polyvinyl alcohol which is a stabilizer or say a factor. You have an inner aqueous phase which is the same as this guy and then you have these blue oil phase has been pinched off into smaller smaller droplets.

So, essentially you have this is oil, this is water and this is water as well. So now, what you will get is you will get a hollow particle, so instead of getting a solid particle in the single emulsion case now you are getting a hollow particle. So, then all you have to do is let this oil phase to evaporate. So, DCM or chloroform both have very with a very volatile and they will evaporate fairly quickly. And then you will get microspheres which you can then use centrifugation by pelleting and then lyophilize them to dry them off and that is how they will typically look.

So, if you notice here there is a sort of a shell, so this is in an SEM image one of the particle or two of the particles have broken down. So, what you can see is that there is a shell and then inside its just hollow. So, this is the inner water phase this was what was the oil phase and then of course, the outside is all water which of course, pelleted here. So, that is how you will get a hollow particle and why is that advantageous because, now since this is initially water phase you can have hydrophilic drugs getting encapsulated.

So, here is sort of how this is going to look, so that you will have a PLGA shell which is surrounding a hydrophilic drug. The PLGA shell can still be used to encapsulate hydrophobic drugs because, any drug that I have which is here can also be hydrophobic. So, that way you can have both hydrophobic drug as well as hydrophilic drug being present in the same particle and so just some more terminologies. So, the internal aqueous phase is what you had added initially.

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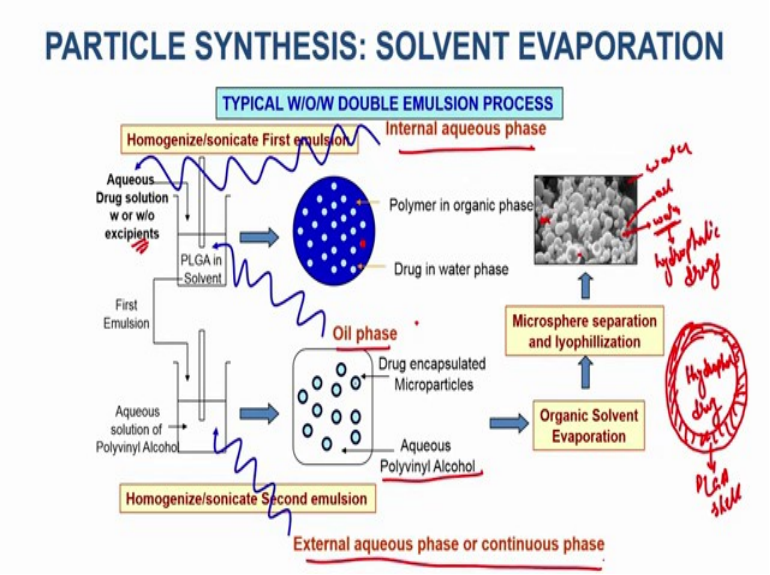

So, whatever was here - this is called internal aqueous space, whatever is here it is called the oil phase (there is only one oil phase in this case). And, whatever was in the final larger water volume is called the external aqueous space or the continuous phase.

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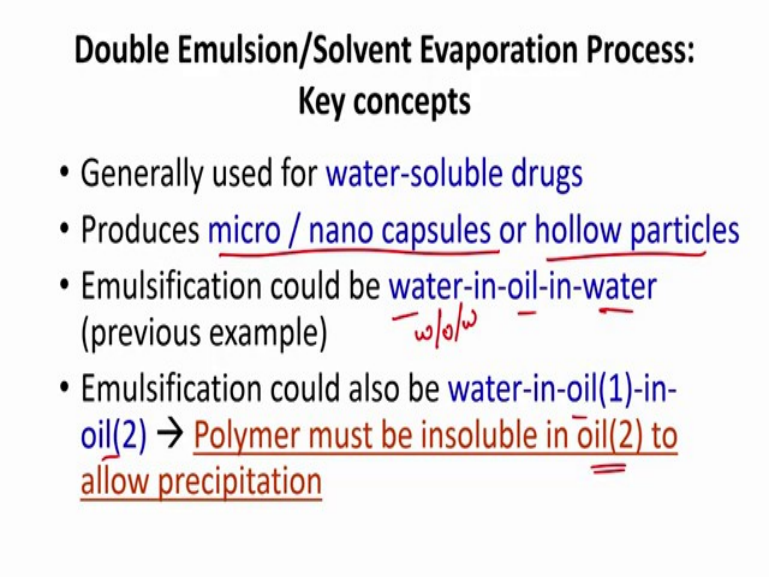

So, let us talk about some of the key concepts from this double emulsion process. So, again as I said it is generally used if you want to encapsulate with water soluble drugs. So, if you are looking for drugs that are only going to be hydrophobic then the single emulsion is the best way to go about it its simpler as well as you get a lot more area or

volume in which you can encapsulate drug. But, if you want a water soluble drug then you want to create some sort of a cavity where the water phase can reside and that is where your drugs get will get encapsulated. So, these produces micro and nano capsules. So, this is some sort of a reservoir system or hollow particles that we are talking about.

So, unlike your single emulsion where the particles will be completely uniform inside, this is going to be more of a capsule sort of scenario, where this is a small shell surrounding your empty cavity. So, as I said, this emulsion could be called as water in oil in water. So, typically you will find this written as w / o / w and again this is not really limited to this emulsification could also be water in oil in oil . I mean it does not have to be the external phase has to be water, it is just that you just have to ensure that they are between the two oil phases the polymer is only soluble in one of the oil phase.

So, that way you can also make sure although this is not really used in any of the biological sort of scenarios because, the particles that you want has to be able to survive in water, has to be able to go and be stable in water. So, typically the external phase is also usually water, but you technically can have a two immiscible oils being used here as well.

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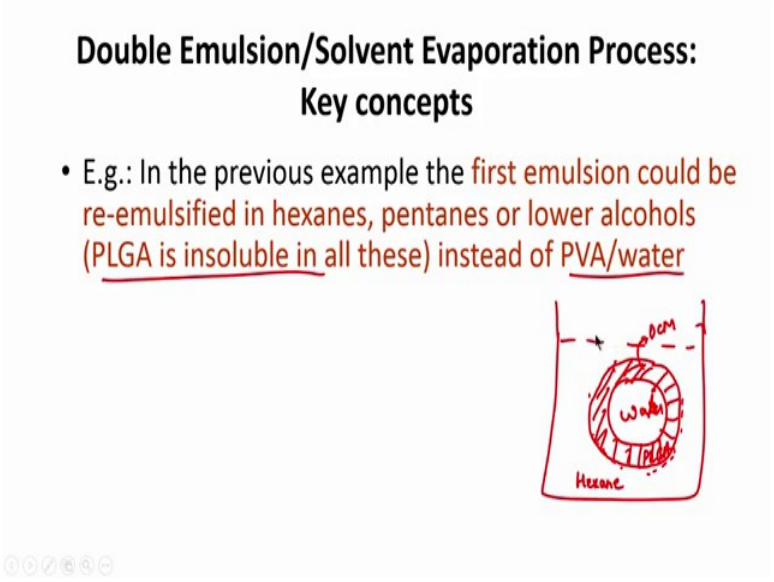

So, one example to that is in the first emulsion could be re-emulsified in hexanes or pentanes and the PLGA is insoluble in all of these. So, if you essentially what we are talking about here is you will have water, you will have a shell of oil after the first

emulsion, after the second emulsion and let us say this oil is DCM. Now, if I know that the DCM and hexane are immiscible that is they are not going to mix; then what I can do is I can add this to a solution of hexane which will not solubilized my PLGA and which is not going to mix with DCM.

So, this can technically still result in a emulsion as well as particles, the only problem is these particles will tend to agglomerate in water because these are stable in hexanes. But once you put them in water they may not want to interact with water whereas, when we had PVA in the water, the PVA had quoted these particles and had kind of stabilized these particles, but this may not happen in case of hexane.

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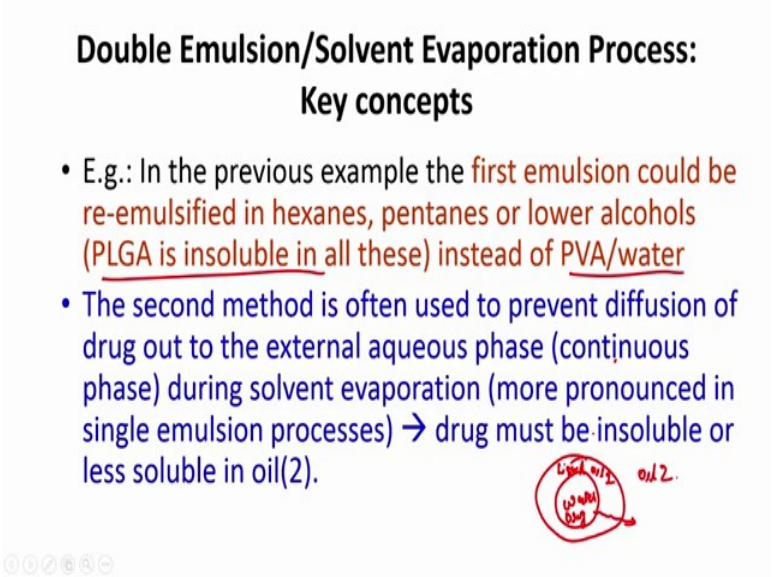

And, then the second method is often used to prevent diffusion on the drug out of the external aqueous phase. So, so this is going to result in a more pronounced in single emulsion process. So, the drug has to be insoluble or less soluble in the oil 2 also because, initially when we are talking about these emulsion process this is still liquid. This is still liquid oil 1 and then oil 2 and then water, the drug. So, then let us say the drug is soluble and oil 2 then the drug will tend to slowly diffuse out into the oil 2 and the drug is insoluble or it will not really tend to go there. And so, this will still you have to make sure also that whatever drug you are encapsulating is insoluble in oil 2.

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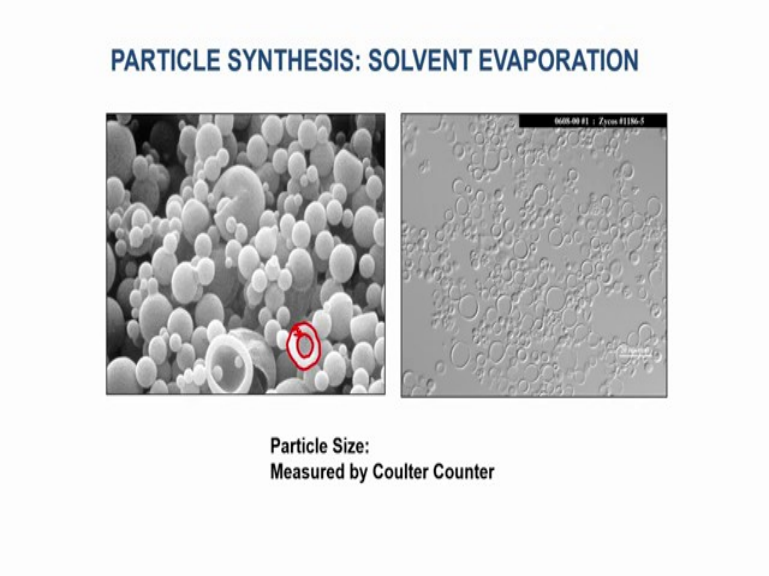

So, a little bit more about the solvent evaporation process. So, again as I said this is what you get you have these hollow particles that you will get with a polymeric shell surrounding it. And then external is of course, in biological applications will be water and then you can use various kinds of techniques. So, this is an SEM image you can use other techniques, you can determine the particle size by dynamic light scattering, using coulter counter or other similar instruments. And, you can get some sort of an idea as to what is the size; in this case since this scale bar is about 20 microns. We can say that the average size here might be about 5 microns, but you can then again vary that by changing the energy that you are providing to the system.

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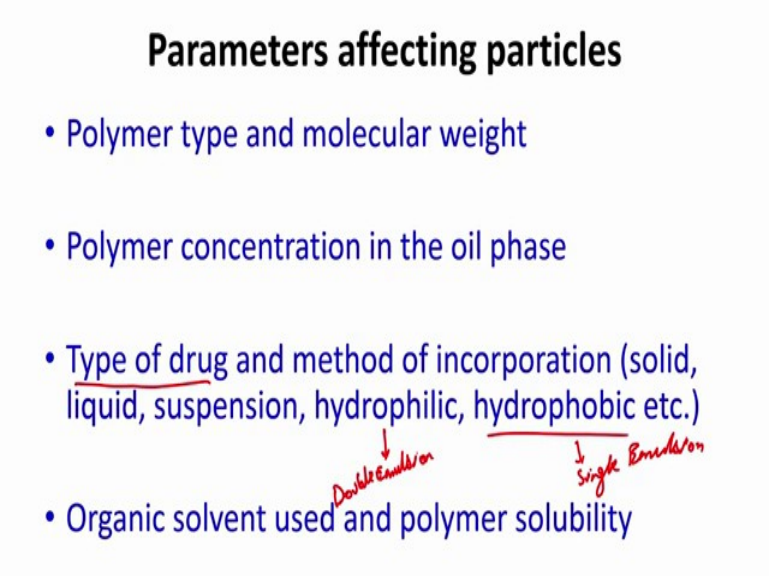

So, what are the different parameters that are going to affect these particles? So, of course, the first thing is the what polymer you are using and what is the molecular weight. So, that is going to have a profound effect on first of all whether it is hydrophilic, hydrophobic and then also what is the thickness of the shell, how stable it is, how fast it degrades all of that will depend on the polymer you are using. Then of course, the polymer concentration in the oil phase. So, the more concentration you have the more closely we will pack. So, all of that will determine what sort of particles you get the type of drug.

So, that is of course, very important because that will determine what method to use. So, you can whether its hydrophilic, hydrophobic whether its liquid or some suspension, depending on that. So, if its hydrophobic you only will go with single emulsion this is of course, in case of PLGA if its hydrophilic then you will have to go with double emulsion. So, all of these are important criteria that you have to consider and then of course, what organic solvent you are using and what is the polymer solubility that will determine how much polymer concentration you can get in that particular solvent. So, all of these are important parameters.

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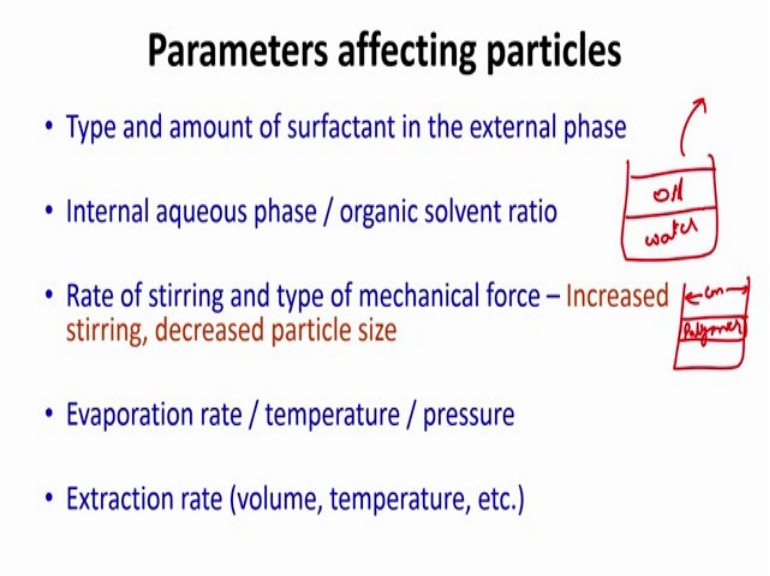

What about the type of amount and surfactants? So, of course, you would want to add some surfactant to make sure that these particles are stable and are not very poly dispersed. So, and then how much the amount of its the most surfactants will find in literature is also toxic. So, if you add too much of that and you are not able to wash it off then new particles may not be compatible with your biological assess. So, all of that needs to be sort of optimized and you need to use surfactants which is a fairly biocompatible as well as their amount is also limited. So, amount should be enough so, that these particles are stable, but not too much that they become toxic.

Then what is the ratio of your internal aqueous phase to organic solvent? So, that will determine what is the size of your particles as well, how much energy you need to give; again energy by far is the most important criteria in terms of determining the size. So, if you have very high amount of energy being given it you will have a decreased amount of particle size as a result.

Whereas, if your energy is lower then you will get a bigger sized particle and that is very easy to see, if you do not give any energy you get a huge block of PLGA; I mean if I do not give any energy and I have these water and oil phase separate out and if I let it evaporate. Then eventually what I will end up with? Eventually, I will end up with a block of the polymer.

So, this is going to be a huge block, this we are talking about centimeters and the more energy will give the smaller this will become, so it is easy to remember. And then at what rate we are evaporating it out, what temperature we are evaporating out, so when we say evaporation this is we are talking about the oil phase itself.

So, the oil phase will have different evaporation rate at different temperatures and pressure. So, depending on all that you will have a different amount of precipitation of your polymer happening. So, that will also affect the particle size. So, again you know how much volume is there, what is the temperature at the time of evaporation.

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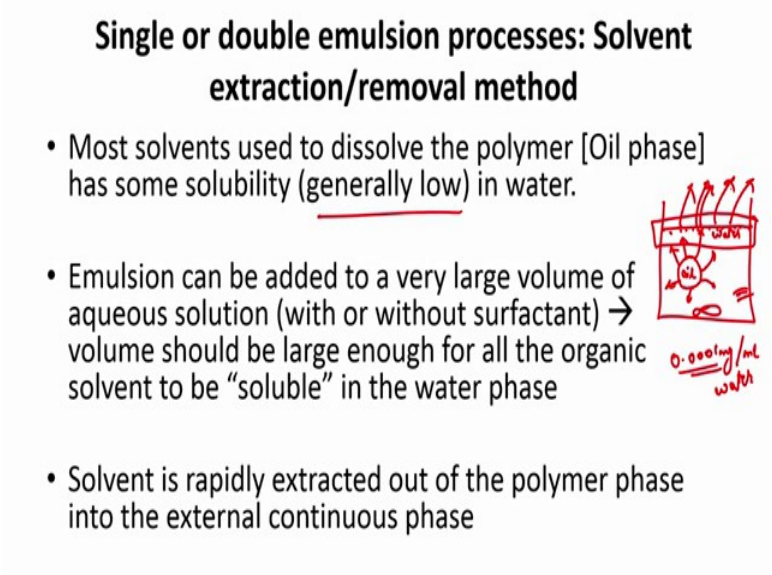

So, a little bit more on the solvent extraction or the removal method. So, most solvents that are used to dissolve the polymer have some solubility in water. So, I actually how does it happen, how does these things able to evaporate through the water? So, that can only happen if they have certain solubility in the water. And so, what do you mean by that? So for that to happen the emulsion has to get into very large amount of a aqueous solution with or without surfactant and the value should be large enough. So, that the organic solvent is actually soluble in the water phase. So now, what I am saying is initially if you look at the system after the emulsion has been done, what we are saying is let us say for a single emulsion this is your oil droplet.

And then of course, there is some shaking going on so, it is been continuously moving around, but to be very the evaporation can only happen from the surface. So, but the

surface is here is water. So, for this oil to evaporate there has to be some oil present on the surface and so, for that to happen what happens is the oil will have some solubility in water. So, let us say the solubility is very low, let us say it is only about 0.0001 milligram per ml of water. And, then as more and more oil is going to evaporate more and more oil is going to come out from here and dissolve in water and this process is going to continue.

So, if you want to evaporate everything, you want to make sure you have a very high surface area at this interface; so, that more and more oil is getting evaporated. So, that is what we mean by the solubility of the oil in water- it is low, but then it is there hence highly volatile, so it is going to continue to facilitate that process. So, again the solvent is rapidly extracted out of the polymer phase into the external continuous phase. So, this is the external continuous phase. So, because it has had the solubility and the solubility decreases or the amount decreases as its evaporating. So, to compensate for that more and more oil comes and gets dissolved in the external aqueous phase.

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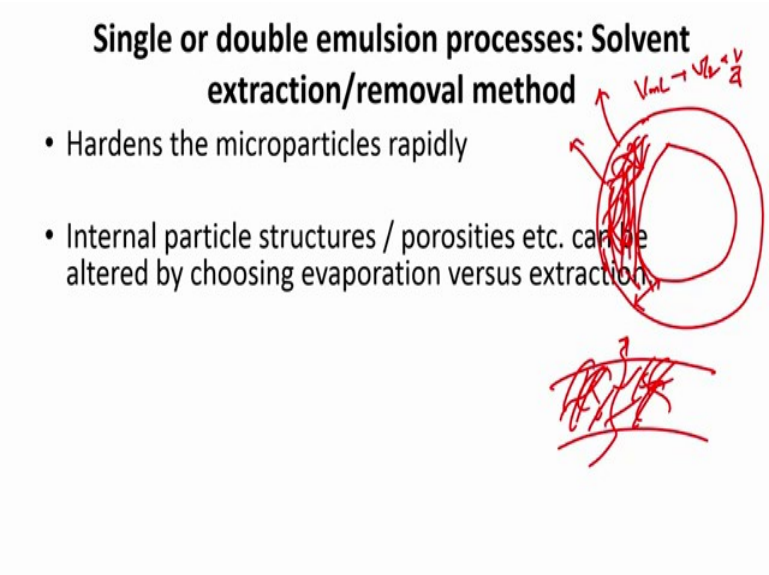

And so, what this does is eventually let us say you had; so, if I focus now only on the on the particle. So, this is oil, so slowly and slowly; so, let us say this is a certain volume V ml of oil in here. So, what is happening slowly and slowly this V is now decreasing it is becoming V by 2, it is becoming V by 4 and further so and so forth. But, the amount of polymer that is there in this amount is actually constant that cannot evaporate.

So, that is now condensing more and more; more and more chains are coming closer together and eventually it starts to form this thick particle. So, the thickness of the shell will be determined on what? Will be essentially determined on the polymer itself. So, how much polymer, what is the molecular weight all of this will determine the thickness of the shell. The internal particle structure porosity etc. can be altered.

So, if I if I do it very slowly, I will get a very very hard particle, but if I let us say evaporate this oil phase very very quickly these polymer chains may not have time to move around and sort of make a very condensed structure. So, in that case what will happen is instead of getting a very condensed structure you may have lots of polymer in one phase, lots of polymer another phase and then very little polymer in this. So, you may get like these pores and sort of these holes into these polymer structure.

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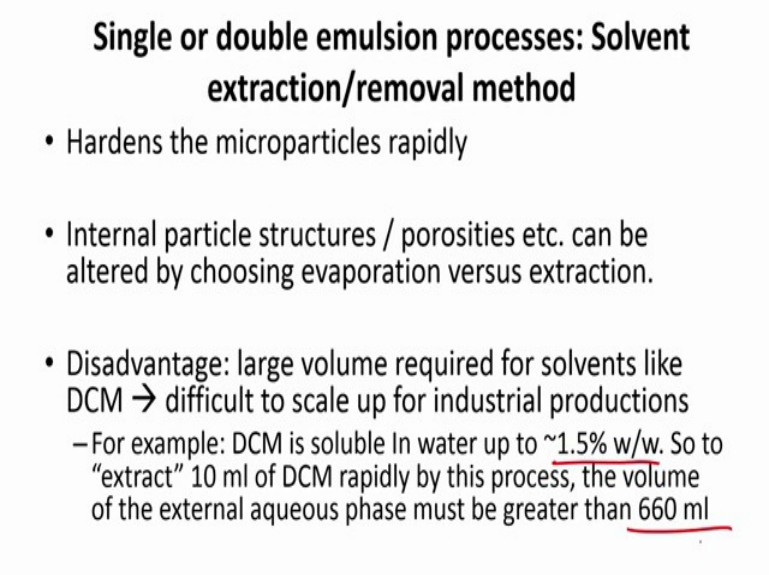

And then finally, one of the disadvantages of this system as it requires very very large volumes. So, the reason for that is if you want to evaporate out and especially at a reasonable time frame, you need to make sure that it has a lot of surface area through which the oil is evaporating. So, just to give you an example DCM has a solubility of about 1.5 and percent weight by weight. So, to extract 10 ml of DCM rapidly by this process the volume of external phase will come out to be greater than 660 ml and that is extremely large volume.

So now, you are talking about very high volume that you need to now precipitate or sort of centrifuge to collect the particles and you need very large reactors and all that. So, that sort of poses quite a lot of limitations on to what you can do ok. So, we will stop right here for this lecture and we will continue the rest in the next class.

Thank you.