Drug Delivery Principles and Engineering
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Lecture – 08
Polymer Drug Conjugates – II

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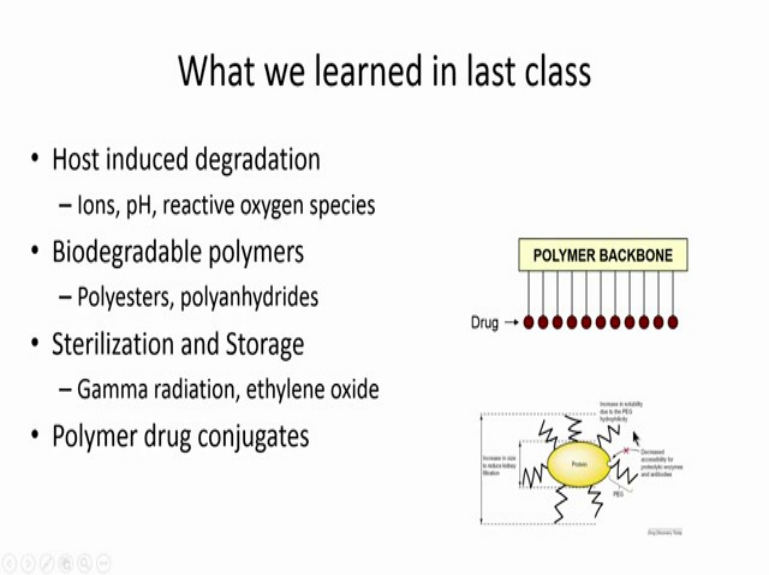

Hello everyone, welcome to another lecture of our course Drug Delivery Principles and Engineering, just a quick recap or what we learned in the last class. In the last class we talked about some host induced degradation mechanisms for, how the polymers can degrade, how the device can degrade in the body. Some of them were ion mediated, they could be changes in the pH, it may change the degradation rate, they could be oxidizing species present the site due to various reasons and modulate the degradation rate.

We then talked about some of the biodegradable polymers very widely used that includes polyesters, polyanhydrides, some of the polyesters are like PGA, PLA, PLGA, very widely used and then polyanhydrides also we discussed and we also talked about some other polymers that are very widely used.

Another thing we discussed is sterilization and storage. So, how to store them, you want to store them in an environment which does not have too much moisture because that can cause hydrolytic degradation to occur; and sterilization again we talked about gamma

radiation, ethylene oxide especially in cases where the heat will not work. So, those become important when you get to these modified and innovative systems.

And then at the end of the lecture we talked about Polymer Drug Conjugates, there can be various types of volumetric conjugates, it could be a large polymer backbone with drug attached to it or it could be a big drug molecule with some polymer attached to the drug itself. So, we are going to continue our polymer drug conjugates discussion in this class.

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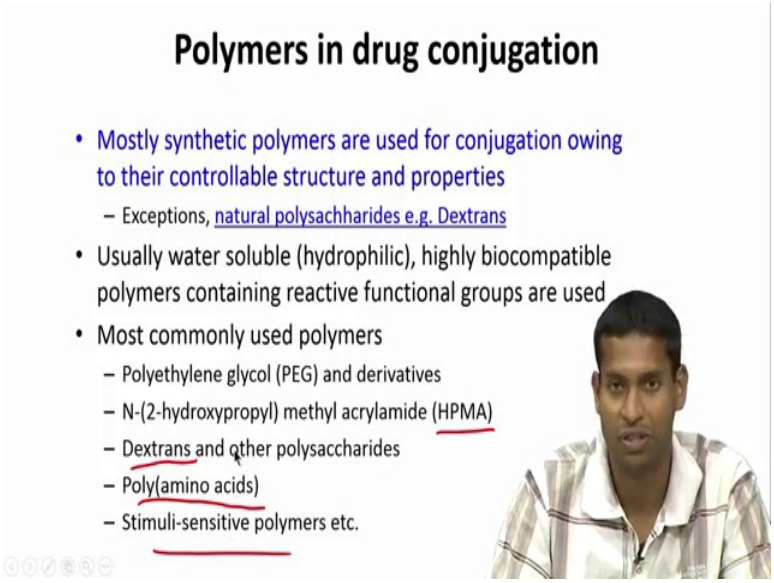

So, some of the polymers that are widely used for drug conjugation, most of them are synthetic and the reason for that is they allow some control, much more control over properties, very controllable; however, there are exceptions some natural polysaccharides its dextrans are also very widely used. Typically, when you are trying to attach something you still want it to remain soluble and so most of the time you are either trying to increase the solubility or at least maintain the solubility. And so, you are using some water soluble hydrophilic polymers and those have also been shown to have a much higher circulation in the body then let us say a hydrophobic polymer.

So, and they are highly biocompatible and they contain some kind of reactive functional group through which you attach them to your drug molecule. So, some of the most commonly used polymers are polythene glycol by far is very widely used polymer and some of its derivatives other polymers such as HPMA. The dextrans I just mentioned

earlier as a natural polymer, you have poly amino acids like your proteins and you can also have some stimuli sensitive polymers, so all of these are very widely used. PEG of course, is by far the most abundant polymer for this particular application.

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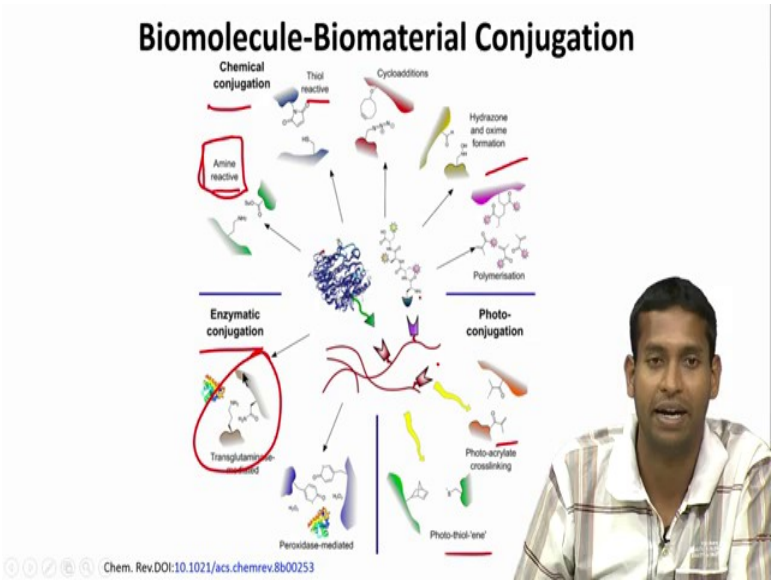

And so, how would you combine them again this is lots and lots of biochemical reactions are there to combine your material to your biomolecule. You can have some kind of a photoconjugation which basically means that in presence of light you have some moieties that is going to attach to your drug molecule and so some of them are acrylate and some thiol ene chemistry. Another one that is very widely used is using some kind of a hydrozone or oxime formation.

So, you use these kinds of chemistry, then there is chemical conjugation. So, there can be thiol reactive or amine reactive, this one again is the most widely used one typically EDC NHS is coupling or in certain examples some in enzymatic conjugation is also used where some enzyme will mediate this and kind of attach your particular drug to your polymer molecule.

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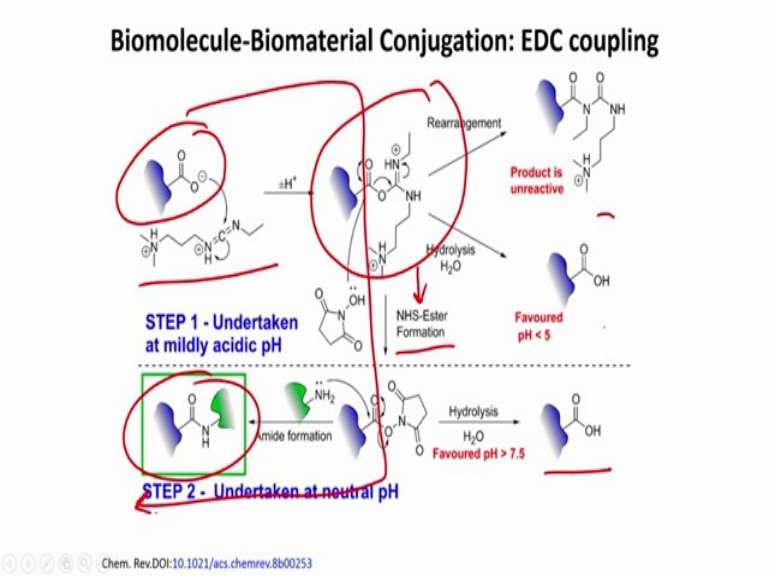

So, just going into a lot more detail of some of the commonly used one; so, the one of the most common one is the EDC coupling and what it is essentially, its a two step reaction. So, in the first step you have your molecule it could be drug or it could be polymeric molecule that contains a carboxyl and you come up with a reagent called EDC at a certain mild acidic pH and; it basically then forms a product which is highly reactive.

Now, this product can go and do another kind of recombination reactions and may go back down to what is his original state was. So, you have to be careful in terms of the time as well as the conditions at which you are doing this reaction. So, once you have this you go to the next step and you essentially have this product, then react with your amine to form the conjugation that you want.

It could again at this point become hydrolytically cleavable and go back to its original state. So, you have to be careful as to what are you doing, but essentially you want this to go in this direction and there is another catalyst that is used which is NHS and that helps in this reaction proceeding in this direction for your successful coupling.

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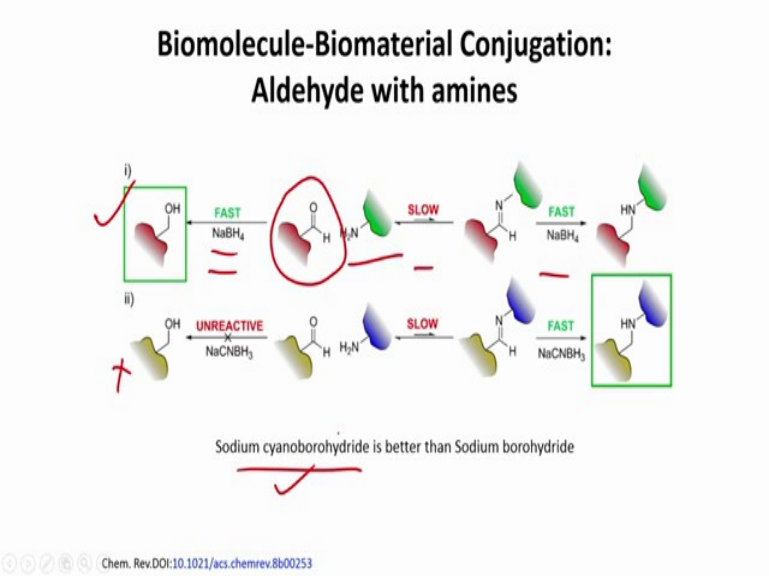

Then another one is to react aldehyde with amines. So, in this case you have an aldehyde group which is a COH bond and your amine group this might be on your drug molecule, most of your drug molecules, atleast the protein based drug molecules will all contain amines and then you can have in presence of certain catalysts, you can have different kinds of reactions that take place.

So, and then you can do it with two different kinds of polymers, you can do it with two different kinds of catalysts, you can do it with NaBH4 which is the sodium borohydride or you can do it with the sodium cyanoborohydride which is slightly better just because you do not have some kind of side reactions that are occurring with the sodium borohydride. So, that is why the cyanoborohydride is better in certain cases.

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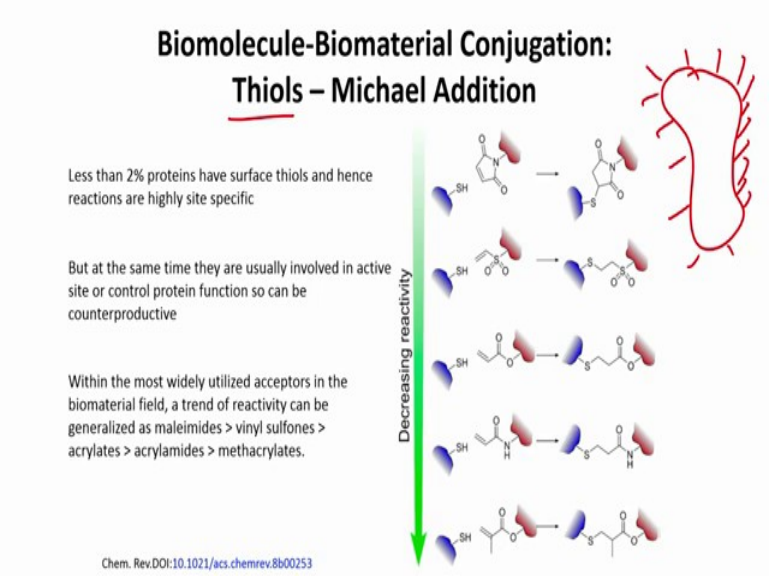

Then you have thiols and Michael addition, these are click chemistries, very widely used, so and the thing here is they use thiols instead of amines. So, proteins have lot more amines and then they have thiols and the thiols can be much more site specific because if you have a larger protein and if you do a reaction with amine the chances are that its going to react at a lot of places, including the site which are active sites.

So, it may block your active site; however, if you are using thiol the chances are that you know whether thiols are and whether they are present in the active site or not. So, those reactions are lot more specific and lot more efficient in that regards.

But caveat to that is typically thiols are mostly used at the active site, so you got to be very careful in choosing this. So, you need to be sure that the thiol that you are using for your chemical conjugation is not being used as an active site molecule.

And then of course, it has different rates of reactivity, so this thiol maleimide is a faster reaction then say the thiol methacrylates. So, these are some things that you will have to consider when you do these reactions.

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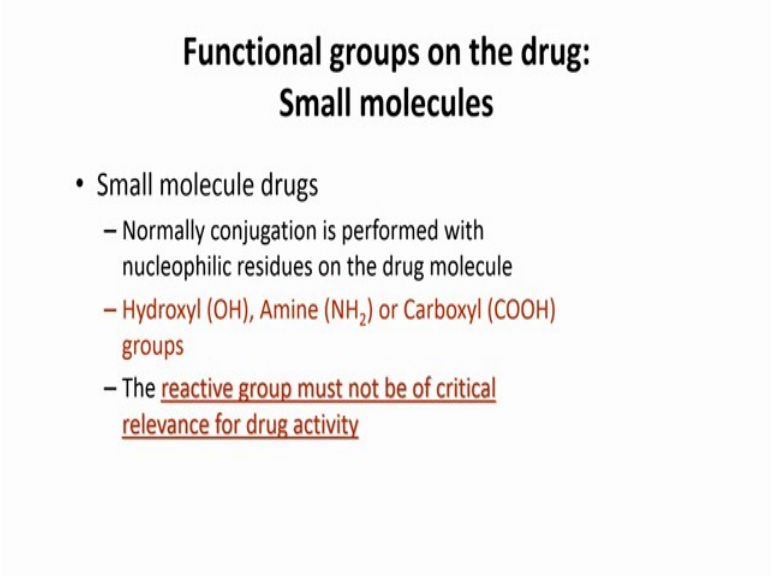

So, let us talk about using the functional groups on the small molecule drugs. So, typically the small molecule drugs, normally conjugation is performed using a nucleophilic residues. So, they have hydroxyl, amine, carboxyl, again these are widely present on most molecules and we have to make sure that these are not involved in the activity of the drug itself. Since they are small molecule they do not have many functional groups the chances are that some of them might be involved. So, in that case you cannot really use this particular strategy.

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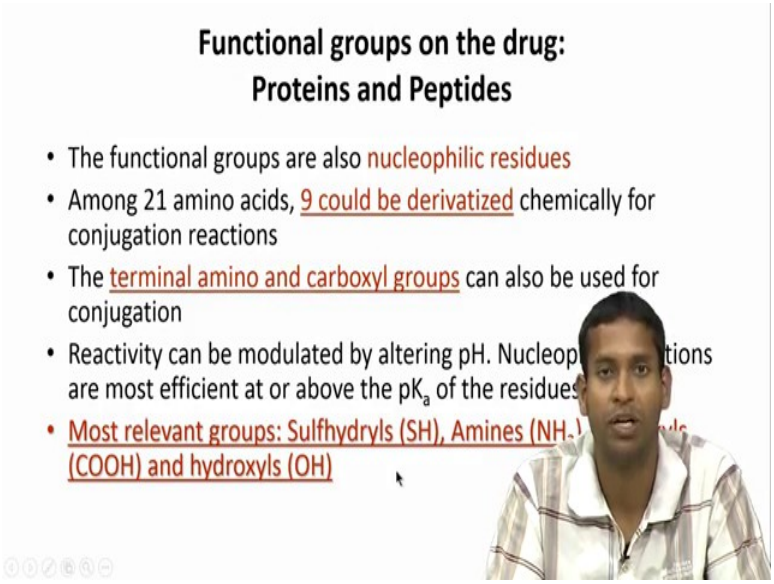

And then the other way is you have some proteins and peptides, those have lots and lots of functional groups just because we have about 9 of the amino acids, it could be derivatized and so you can perform chemical conjugations on them. And there is also a terminal amino and a carboxyl group that is available that you can use and depending on what pH you are using some of these terminal amino acids might be different from the one that are internal in the protein backbone and so that way you can kind of tailor at which site you are getting this reaction. So, some of these are again sulfhydryls, amines, carboxyl and hydroxyls that are widely present on your protein or peptide molecules.

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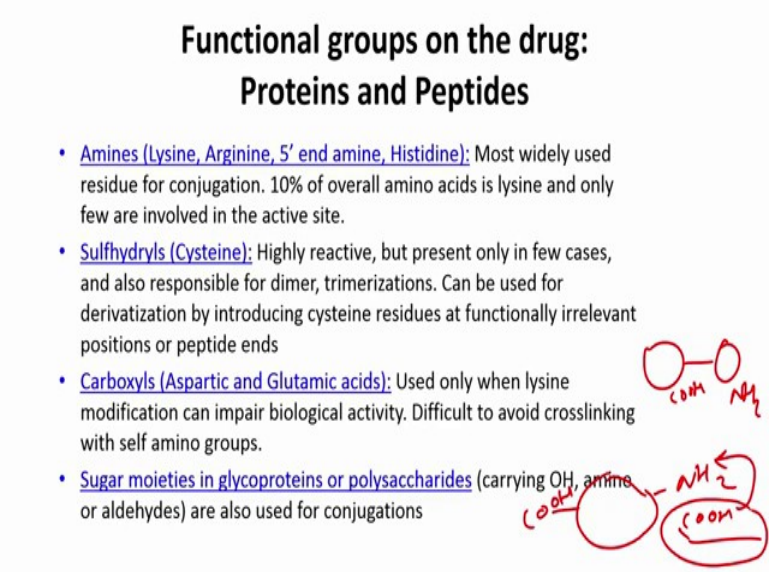

So, let us talk about functional groups specifically, so when we say amines they are present on lysine, arginine and there is a 5 prime end as well as histidine this is very widely used, almost 10 percent of all amino acids in protein is lysine and very few are involved in the active site. So, it is a safe molecule to attach to without worrying about active site kind of being blocked by your polymer.

Then again sulfhydryls has we discussed, contain cysteine which is a thiol group has thiol group is highly reactive, the problem is not all proteins will contain cysteine and these cysteine are also typically involved in dimerization and trimerization which is related to the activity of the protein. So, you have to be careful in terms of when you are reacting with this you are not really causing the activity to go down by a large amount.

Then we have amino acids this is aspartic acid and glutamic acids, they also have carboxyl present on them as well as this and the c terminal thiol on the protein will also have a free carboxyl. So, you can use them, but typically you only use them if lysine modification is kind of having some issues because maybe it is causing the decrease in the activity of the protein then you can go to the carboxyls.

So, because the problem is we know that most proteins will contain your both amine and carboxyls. So, we use carboxyl as one of the thing it is very easy to cross link the protein. So, you will have one protein molecule cross linking COOH one protein molecule cross linking to another amine. This typically does not happen if you do EDC reaction with only amines on the proteins because in the other case you may have your protein molecules with amines and your polymer molecule with carboxyl which does not contain amine.

So, you first activate the polymer molecule and then reacted with the amine, so that the carboxyl on the protein is not involved in the reaction. So, that is why it is important to choose first amines if you are using the EDC NHS coupling and only then go to the carboxyl if that is not really feasible.

And then of course, there are sugar moieties on proteins, the glycoproteins and there is always some kind of a post translational modification of these proteins. So, they carry hydroxyl, amines and aldehydes which can then also be used for conjugations and typically a safe target, in most proteins you will find that they not involved in the active site.

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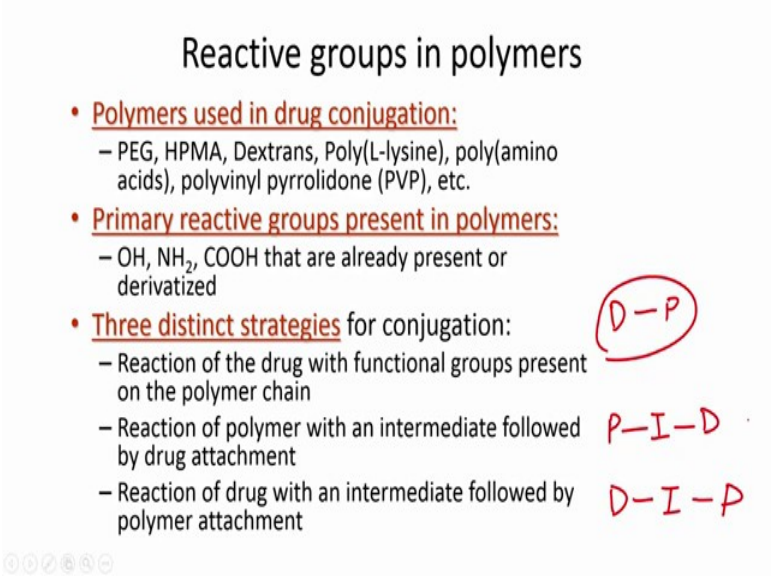

Reactive group in the polymers of course, we are designing the polymers we are choosing the polymers is a whole lot of a library to choose from all these polymers that we have they have different functional groups and you can deriverize them further if need be. The primary active groups are again the same the hydroxyls, the amines, the carboxyls you unless they are already present you can further derivitize them.

And then typically the three distinct strategies are used, so you can either react the drug with the functional groups that are present in the polymer chain, you can first react the polymer to form an intermediate which then you use to put the drug reaction or you can react the drug with an intermediate first and then attach it to your polymer. So, I hope this clear, so essentially what we are talking about here is you have a drug molecule D which directly goes and binds to P, so that is the first case.

In the second case you can have a polymer P which then binds to an intermediate I, which then binds to the drug molecule D and this could be because of several reasons maybe we want this to be very specific or certain distance from the polymer or the drug cannot directly interact with the polymer, the side groups are not compatible, so you use an intermediate. Or the other case could be you take that drug you reacted with the intermediate and then you reacted with the polymer, similar case here, but the sequence is different.

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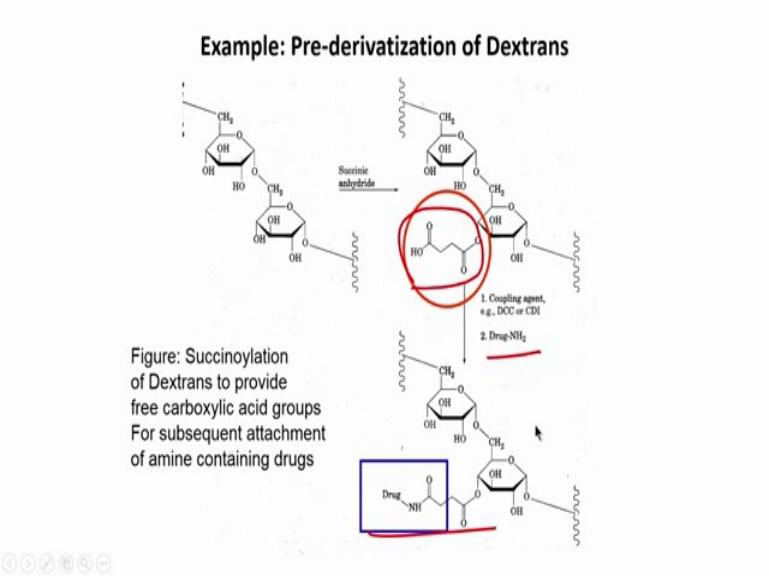

So, some examples of pre derivatization, so here you have a big sugar molecule here you first derivatize it using a succinic anhydride because of that you have now added a succinic group on this polymer and then you use that to then attach your drug molecule to come here.

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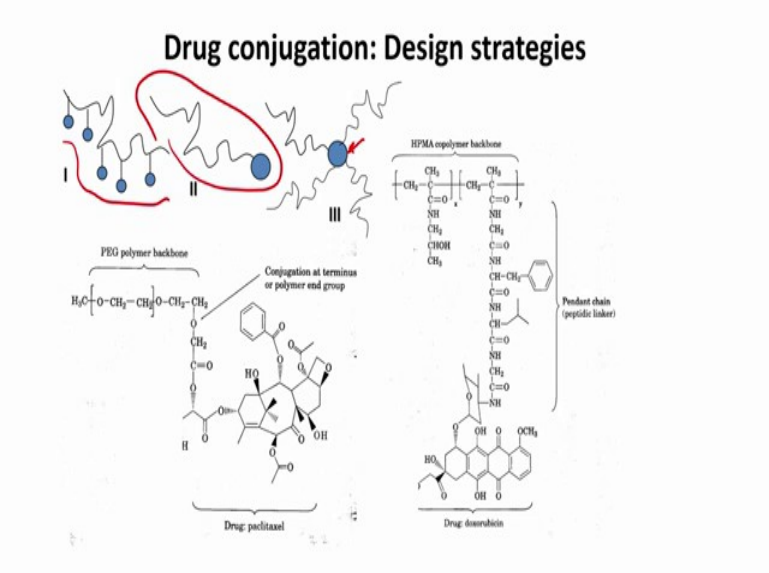

And then similarly there are other design strategies, in this case you can have as I said, you can have drug dangling on the polymer surface you can only one drug molecule for

one polymer or you can have one drug molecule for several polymers. So, all of this possible, here is just another example of that the first case in this case.

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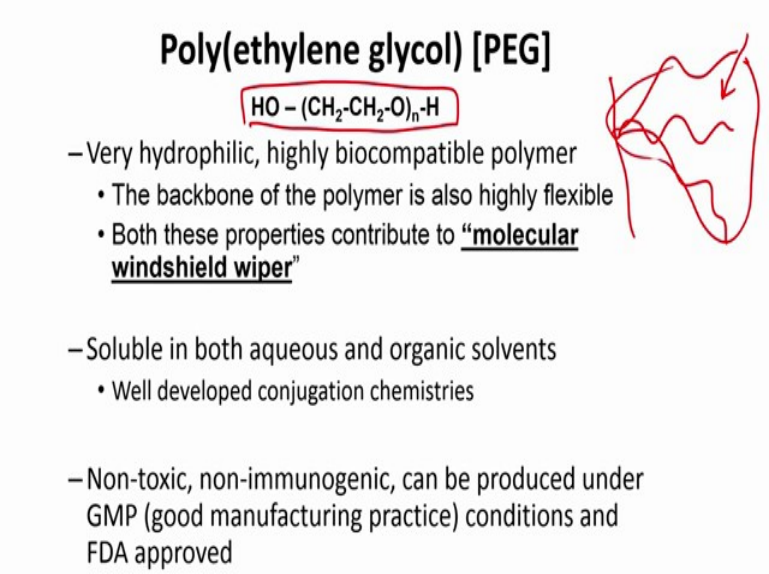

So, let us talk about PEG which is again, as I said is one of the most widely used polymer for drug polymer conjugates. So, this is the simple structure of the PEG it is an ether group. So, it is very hydrophilic and it is shown to be very compatible with the body, the backbone of the polymer is also very flexible. So, it is just in liquid it just keeps on moving around, so that essentially makes it a kind of a molecular wiper.

So, if I have a surface on which the PEG is attached, you will have let us say a protein is coming because the this is acting like a wiper, all of this space is kind of prevented by the PEG molecule, so that none of the other molecules can come into the space because it is kind of just shooing them away.

Another advantage its actually soluble in both aqueous and organic solvents, so that is very useful. So, you can put it on both hydrophilic and hydrophobic drugs as well as there are lot more chemistries that are now available because some chemistries are only specific to aqueous solvent, some are only specific to organic.

So, you can do all kinds of chemistries on it, so that is another advantage here. And of course, its non toxic non immunogenic very very important can be produced and there are a good manufacturing practices and is FDA approved.

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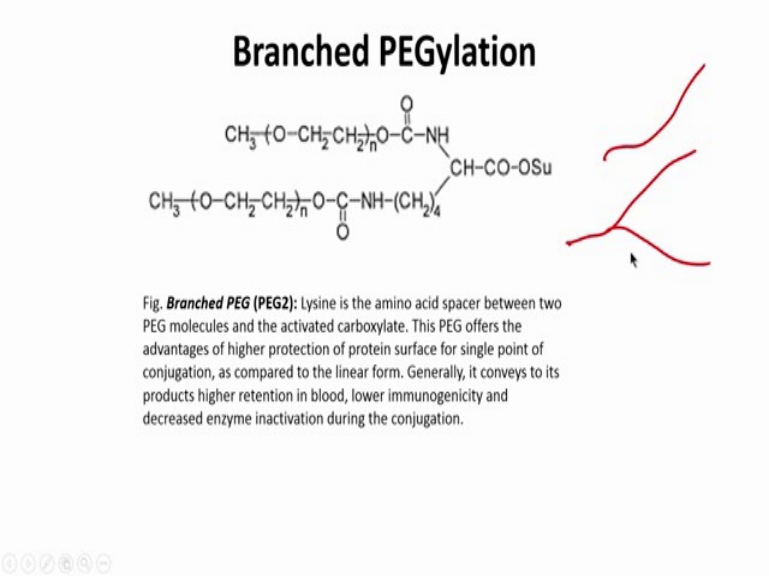

Another variation of the PEG is a branched PEG, so instead of having a single PEG chain like this you can have a PEG which is essentially like this. So, now that windshield wiper effect is much more effective because now it is going to wave around from two different chains in a single conjugation, so its a lot more effective in that case. So, you can have, in this case this is a 2 PEG chain you can have multiple back chains all of that is feasible and so typically it is found in the literature that this branched PEG is much more effective than the single PEG chain in terms of polymeric drug conjugates.

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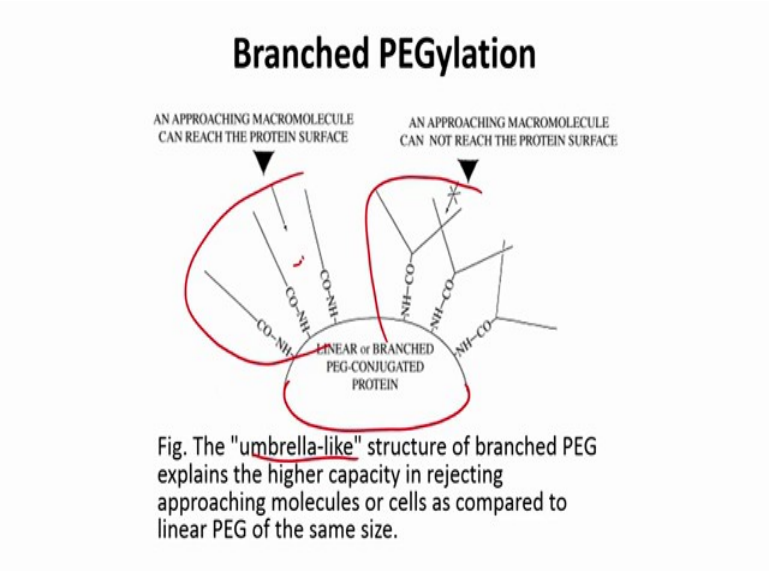

So, here is some example for that let us say you have a big molecule, protein molecule that you have now conjugated to either a single chain PEG or a branch chain PEG just because branching is a lot more coverage it will not allow molecules to go in between the polymer chain even if its sparsely distributed. So, that is why it becomes a lot more umbrella like structure which then is more effective in terms of shielding your drug molecule.

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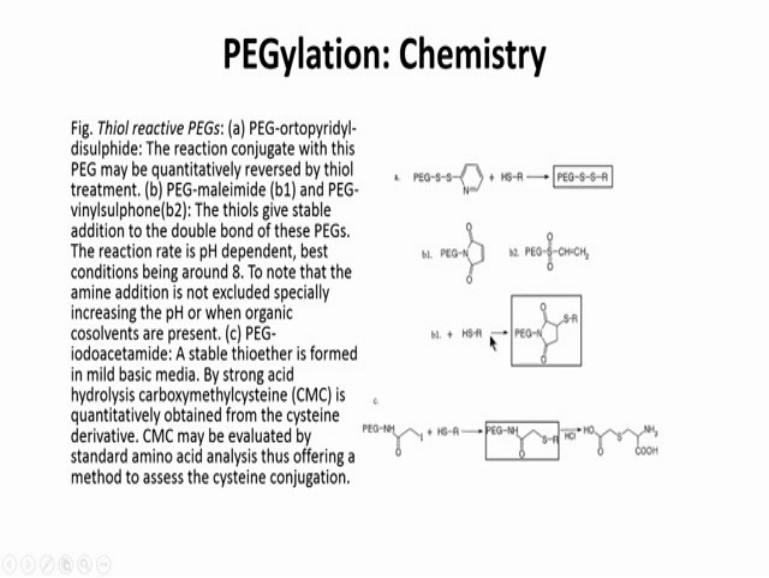

So, again some of the chemistry that are being used, there is a thiol reactive pegs are there, all kinds of PEG molecules, all kinds of derivatization this is a maleimide, so there is an aldehyde, there is an acrylate all kinds of things are used. So, all of these are now commercially available, you can just buy them off the shelf from some company and then use it for your drug molecule.

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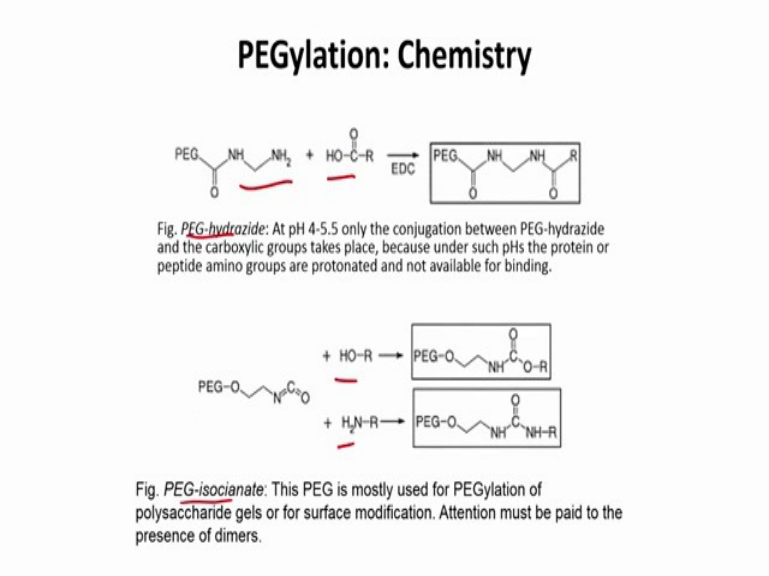

So, there is an another example here is a PEG hydrozide, this reacts with the carboxyl group on the drug. So, essentially something very similar to the EDC reaction, the PEG has the mean your drug may contain the carboxyl group and it will eventually form a bond with that and you can have PEG isocianate that is being used for the action with both hydroxyls and amines. So, all kinds of chemistries are available, depending on the drug and the application that you are looking for.

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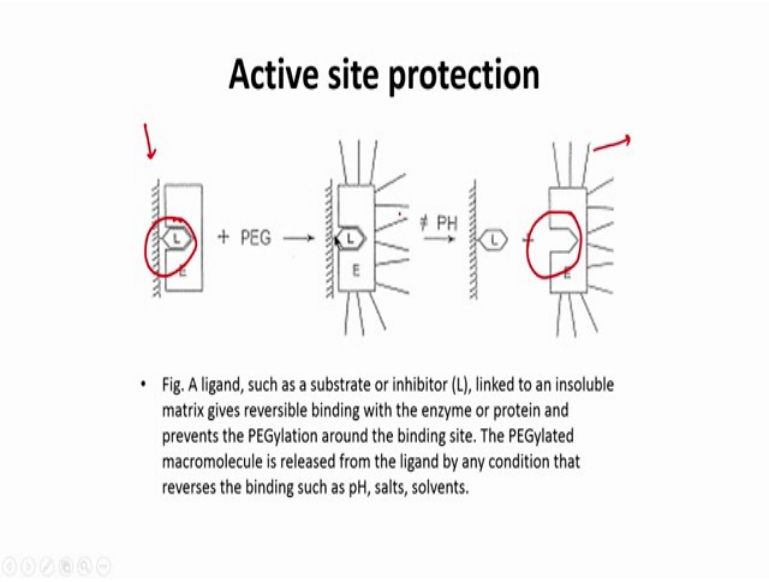

So, as I talked before how would you prevent an active site from basically get damaged through this reaction. So, what you can do is, you can pre bind your ligand at the active site. So, what that will mean, it will immobilize it on a surface and then if you do the reaction on the surface, so what will mean is now this surface is not accessible to your polymer chains.

So, this surface is now protected, so when you release the ligand from the enzyme you ensure that the active site is still available and it is not getting steric hindrance by any of these polymers. So, that is just one strategy, there can be several of the strategies you can used to prevent active site from not being able to access it is a original target.

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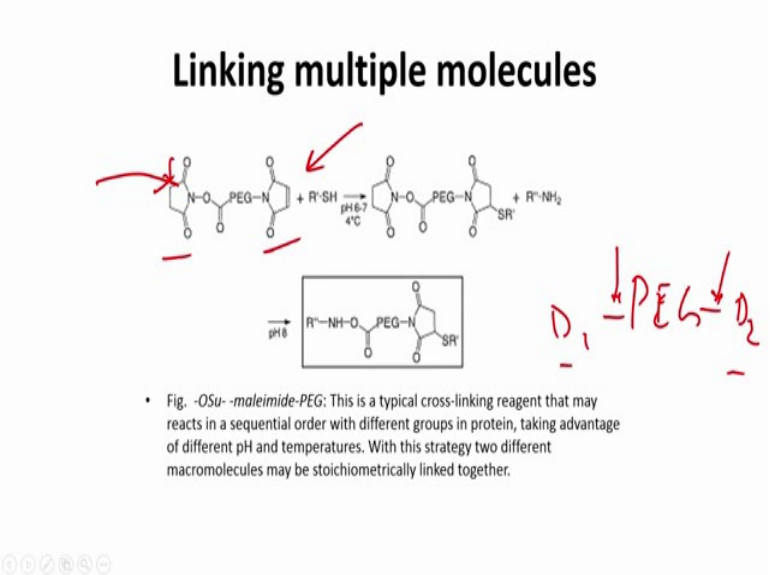

- Fig. *-OSu- -maleimide-PEG*: This is a typical cross-linking reagent that may reacts in a sequential order with different groups in protein, taking advantage of different pH and temperatures. With this strategy two different macromolecules may be stoichiometrically linked together.

You can link multiple molecules, so there are PEG's available which are bifunctional or trifunctional, so what you can do is, you can link one sort of molecule on one side through one chemistry and then another set of molecule on another side. So, you can have a structure such as PEG, drug 1 and drug 2 and these bonds are also different, so they will have different degradation rate, they can be same as well. So, that way you can get a lot more control now with a single system you can get two drugs released at different rates.

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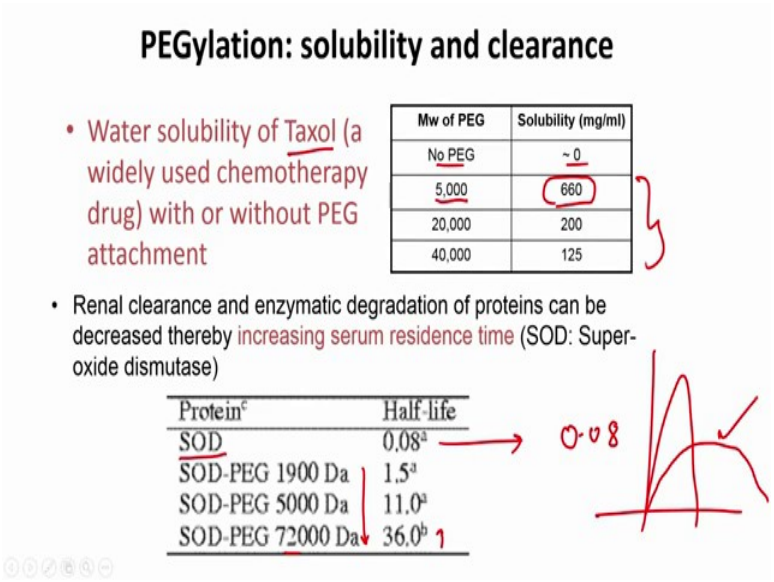

So, here are some more example, so let us say taxol which is one of the very widely used chemotherapeutic drug; however, taxol is fairly hydrophobic, so the solubility without any PEG molecule is almost 0. However, you put a 5000 Dalton PEG on it, the solubility is increased quite dramatically to 660 mg per ml and as you go further up the solubility start to decrease. So, again, but all of these are still soluble at these concentrations, so now the drug that was first of all not really feasible to use is now can be used for this application.

The renal clearance is changed, so if you have; if you have only the let us say a drug molecule called SOD, the super oxide dismutase, the half life in the body is only 0.08 the unit is not listed here, but it has to be hours. But you can attach different PEG of different lengths, so then the bigger the PEG you are attaching and the higher is the half life.

So, now instead of getting released in about 0.8 hours its getting cleared in 36 hours. So, of course, now instead of getting a graph in the body like this you are essentially achieving concentrations like this is of course, always better.

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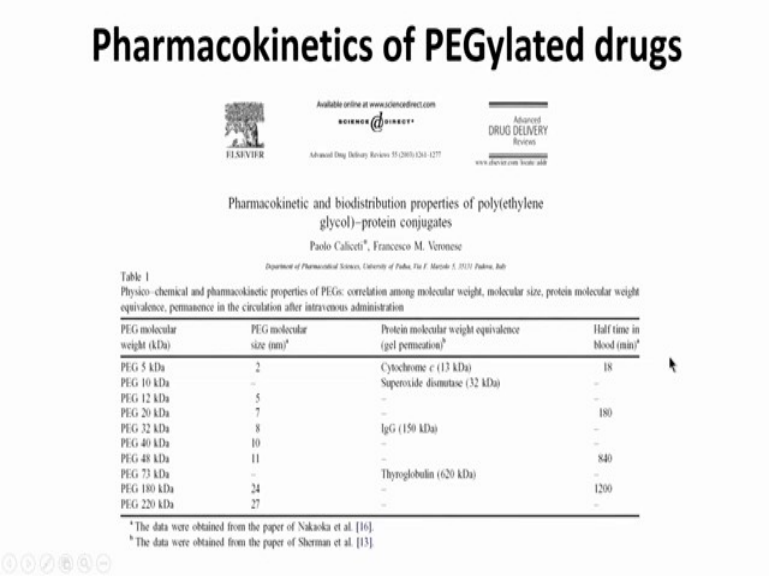

Here, some more pharmacokinetics for different drugs as I said before you can significantly increase the half life depending on what PEG you are using and that way you will have a lot more control release and sustained lease in the body.

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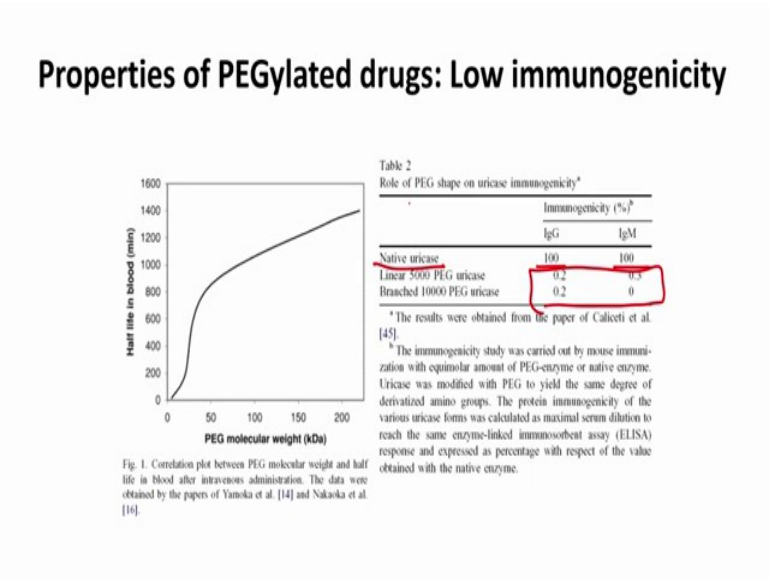

Some of the properties of the PEGylated drugs, they also have lower immunogenicity. So, let us say if I have a native protein in this case uricase and let us say whatever the antibodies I am getting, IgG or IgM I say that those are 100 percent antibodies. Once I

conjugate linear or branched polymer, I see dramatic decrease in the antibody is present for that particular protein.

So, now not only am I increasing the circulation time, what I am also doing is I am decreasing the amount of antibodies that the body is generating. So, the immune response is lowered, the patient is much happier, the half life is increasing quite a lot in the blood.

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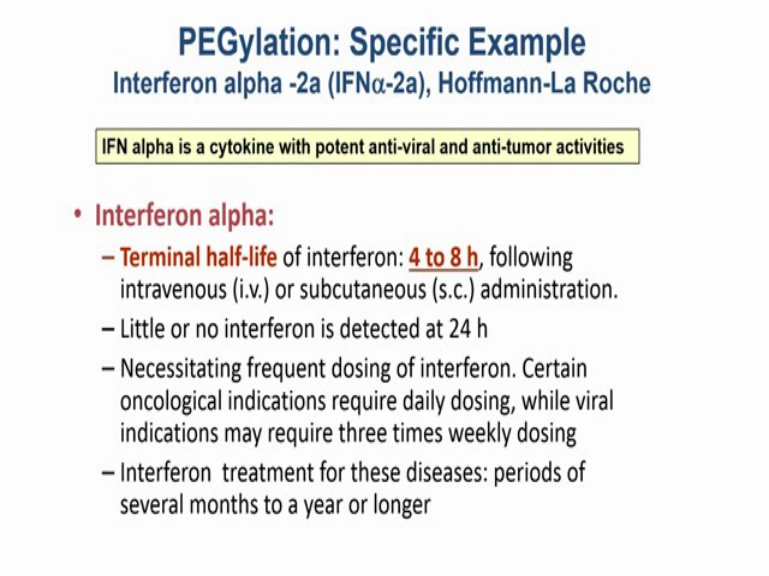

So, let us take a specific example. So, if we have interferon alpha this is a drug which is a very potent cytokine and it is just an antiviral and anti-tumor activities. However, when the drug is injected in the body its half life is only about 4 to 8 hours, once you give it in terminus or subcutaneous.

So, really after 24 hours of the injection you do not really detected at all in the blood and that basically means if the patient has to take it every 12 to 24 hours for it to have any kind of therapeutic benefits and the treatment is very long. I mean this can last several months or more than few years. So, obviously, the patients are not happy, compliance is very low the quality of life is very low. So, something that is being done here is PEGylation.

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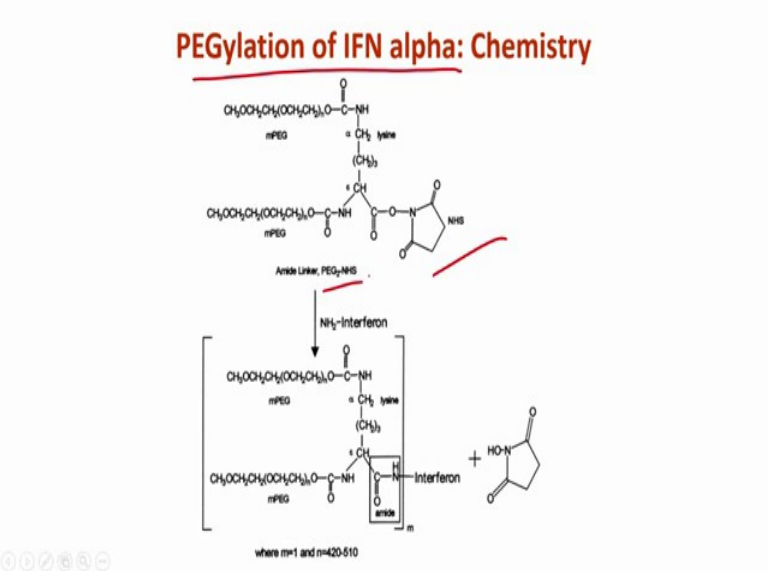

So, what people have done is they PEGylated this IFN alpha, here is the chemistry that they have used. So, in this case they have used the NHS chemistry, they have a di- PEG and or essentially a bi functional PEG.

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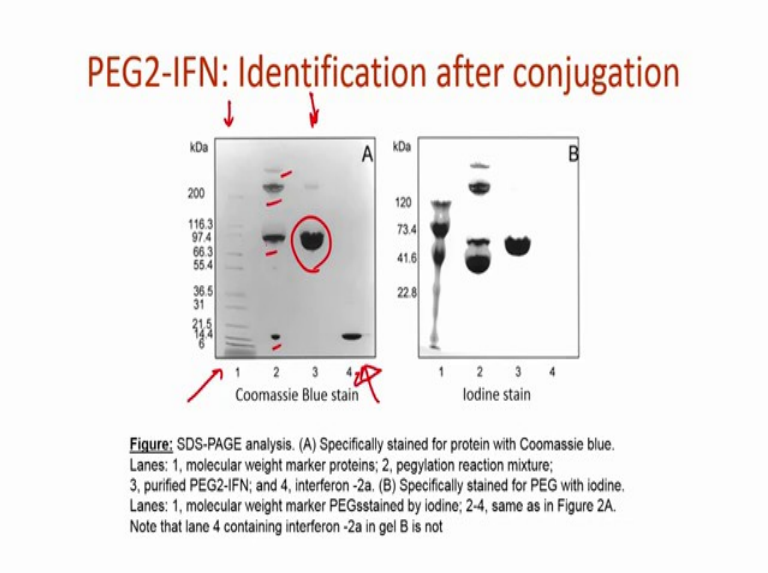

Figure: SDS-PAGE analysis. (A) Specifically stained for protein with Coomassie blue. Lanes: 1, molecular weight marker proteins; 2, pegylation reaction mixture; 3, purified PEG2-IFN; and 4, interferon -2a. (B) Specifically stained for PEG with iodine. Lanes: 1, molecular weight marker PEGsstained by iodine; 2-4, same as in Figure 2A. Note that lane 4 containing interferon -2a in gel B is not

And then they do that and then now this is verification of whether the PEG is attached or not. So, what you have is a SDS PAGE gel, which essentially stains for proteins and so let us focus on this graph first and so this lane is a marker lane essentially with a

different molecular weight to show what molecular weight your bands are lying. And this particular lane 4, is essentially just the lane that contains free protein.

So, a free protein is lying somewhere around 15 kilo Daltons, which is what is expected you in lane 2 what you have done is you have reacted with PEG. So, now, you see that the protein is appearing in lots and lots of different places one protein is here, one protein is here, another band is here, another band is here. So; that means, that quite a lot of it is reacted and has increased this molecular weight and then you can further purify it based on the size and now you get a very nice big single band of the protein.

So, now you have increased the molecular weight from 15 kilo Dalton to about 97 kilo Dalton and this is just an iodine stain which stains for the PEG. So, now, in this case only the PEG is showing up and not the protein as was expected, these bands correspond to the same ones here.

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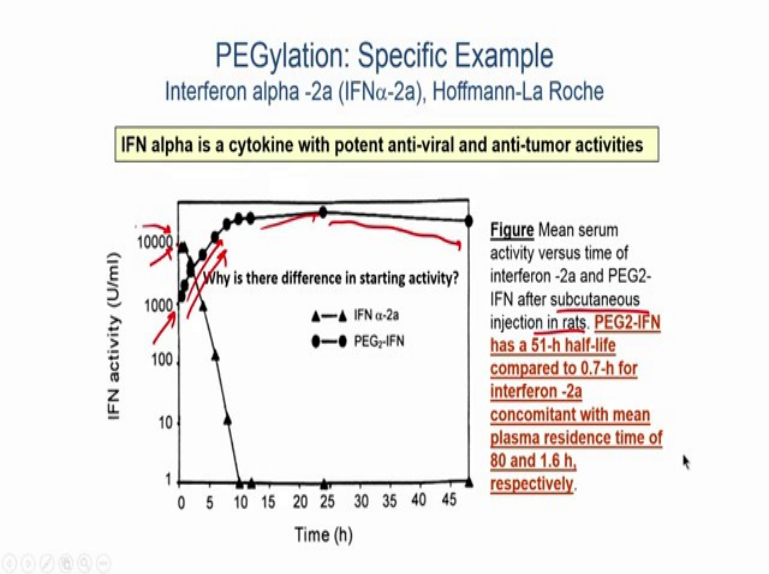

So, and now when you inject it into the body, you see that the half life is significantly different. So, now, you have a half life of 51 hours; that means, now the patient will only have to take injection after let us say 3 days, instead of taking every day. And then of course, the residence time in the plasma is 80 hours compared to 1.6 hours earlier.

So, if you look here closely, this is the free drug that was injected most of it gets cleared out at least in this graph in 10 hours whereas, this is the PEGylated drug this was injected

and you find that even after 48 hours, it is still pretty high in the body. And notice how they both start from the different points in here and then for the free drag it actually goes down whereas, for the PEGylated drug it goes up, can you guess think of a reason why this is the case? This is subcutaneously injected in rats I will give you a moment to think over it.

So, the answer to that is when you inject subcutaneously, the free drug is very small and it diffuses very quickly into the circulation and because its so small it gets cleared out very rapidly. So, you see a profile like this whereas, when you have PEGylated drug it takes time to go into the circulation.

So, the first few hours its actually building up the concentration into the circulation and because it is not getting cleared very quickly, this concentration is actually increasing. Only when at certain point the maximum concentration is achieved by diffusion into the blood, only then now its starting to decrease and over time its going to go down. So, this has kind of become a sustained release. So, we already talked about why there is a different starting activity.

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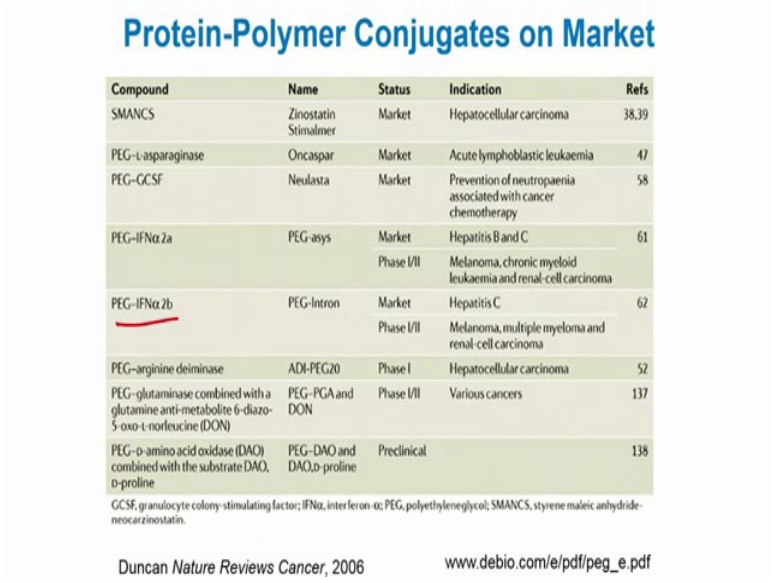

So, some of the protein polymer conjugates on market, these is already being used in humans this PEG ifn-alpha we already just talked about, but then there are several others that are being used for different applications. So, all of these either there in market or there under some kind of clinical trials, phase I, phase II. So, this has been a very

successful strategy. So, we will stop right here, thank you for your attention we will talk further about other drug delivery type systems in the next course, in the next class.

Thank you.