Organic Chemistry In Biology And Drug Development

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Lecture - 42
Fundamental Principles of Drug Development Process

Welcome back. In the last session, we have given a brief introduction to the drug discovery processes and we have seen that there are several stages in a drug discovery process. Now, even a drug can fail and there is a high failure rate. And that is because whatever you see in the test tube that is not reflected in the *in-vivo* studies. And whatever you see in the preclinical studies may not be reflected in the human subjects, so that is how the drugs can fail.

Now, suppose an intended drug molecule crosses the initial stages which includes target identification, target validation followed by drug *in-silico* studies. And then from *in-silico* studies, you get some molecules and then you synthesize that.

And then you study the actual interaction by a bioassay technique and then you try to find out what are called hits; that means some preliminary effect you need and then you tinker around the structure. And finally you get some lead and the lead has to be optimized through what is called structure activity relationship. First you make a large library of compounds, and then you try to find out a structure activity relationship.

That means, suppose there is a phenyl group at some point and then you replace the phenyl group with electron donating aromatic ring and an electron withdrawing aromatic ring and you see what is happening to the biological effect. Sometimes the electron donor may be helpful, sometimes the electron withdrawing may be helpful, that is what will give you the structure activity relationship. That means how the activity of the molecule depends on the structure at different segments in the molecule, so that will help you to identify a lead.

And then the lead has to be taken into the preclinical trials. And preclinical trials are basically done on animals. And in animals, you study the toxicological effect. Some people say that it is better to first study the toxicological effect. Because even if you observe good ADME properties good PD properties (pharmacodynamics), by then you

have already invested money on these and then you see the toxicological effect and you find that it is very toxic, then the whole money that you have put is gone.

So, some medicinal chemists say that better study the toxicological property first and then you proceed to study and invest money on other type of effects.

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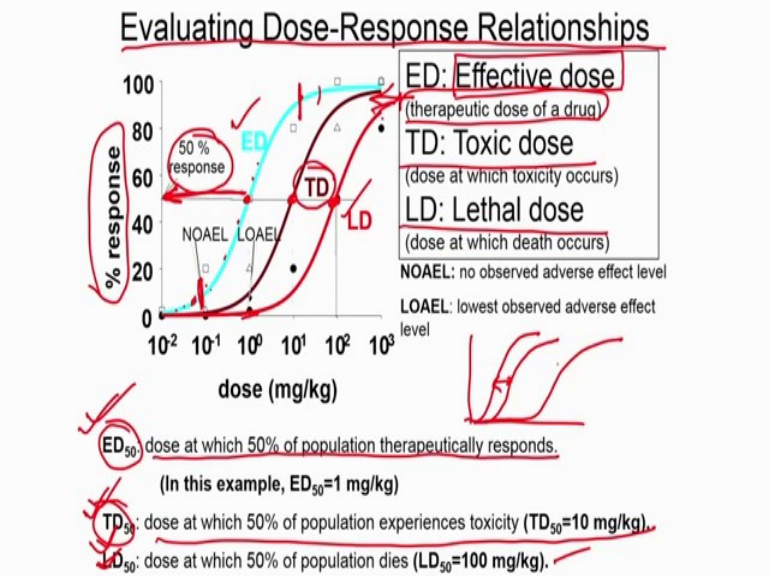

I already said that all drugs are ultimately toxic, but that they are toxic at a certain concentration. At lower concentration, as it is a drug, we can assure that it is having some good activity. But as you slowly increase the concentration, you might see that toxic effect developing. And in many occasions, it may also cross the lethal dose that means it can kill the subject.

Now, here in this graph, I have 3-sigmoidal curves. The first is basically when you are giving a certain lower concentration of a drug and you are measuring the response. Suppose if it is in the animal stage, so you have different animals, 50 animals or 100 animals and you are giving this drug to these animals at different concentration. And so you will see the effect, this is the average effect on the animals. This effect is what we call response.

Now, so basically at lower concentration, there will be very less effect. So, it actually picks up in this region. And the middle point of this curve corresponds to the ED_{50}, ED_{50}

is what is called effective dose, effective dose. The effective dose is somewhere in between this point and that point, that means, here it is the 50 percent.

That means, if you have say 100 animals and you are slowly increasing the concentration of the drug, if you see the desired effect in in 50 animals then that means, the concentration needed to have the effect shown by 50 percent of the subjects that is what is called effective dose.

If it is animal, then it is effective dose in animals. If it is in the clinical level, then it is effective dose on human. So, there is the term called effective dose or this is also called the therapeutic dose of a drug. Now, as you increase the concentration, you start seeing the toxicity. The toxicity will be definitely shown at a higher concentration, but it also follows a sigmoidal curve.

And there is also another terminology or another parameter which is called TD that is called the toxic dose. The TD is called toxic dose, ED is called the effective dose and LD is a lethal dose that means if you cross the toxic dose then you have a lethal does. So, the subject will be killed. Now, we have ED_{50}, as I told you this effective dose, that means, the dose which gives you the actual effect that you wanted is between here and there.

And here it is the 50 percent of the population is showing the effective response. So, according to that, we have ED_{50}, we have TD_{50} and we have LD_{50}. ED_{50} is the dose at which 50 percent of the population therapeutically responds because we know that the aspirin causes acidity to a person, but may not cause acidity to another person, may have very well an analgesic effect.

So, you have 100 persons and then I give this aspirin to several of these people and then if 50 percent of the people are saying that yes I am having this good effect (analgesic effect), so that dose what is called the effective dose. Similarly, you have TD_{50} TD_{50} is a dose that at which 50 percent of the population experiences toxicity and then you have lethal dose.

Dose at which 50 percent of the population dies is the LD_{50}. If you cross this then all the subjects will die; if you cross this point, that means, this concentration, but in between these two concentrations, in the middle of that sigmoidal curve that gives where 50 percent of the population dies. So, these three things are measured usually animals are,

animals are sacrificed. So, you can go to the LD_{50}, that means, you can see what is the dose required in which the 50 percent of the animals die.

So, for animals you can measure ED_{50}, and you can measure LD_{50}. Now, this ratio is very important this LD_{50} by ED_{50}, if the ratio is very high, that means, you have a very safe margin, you have a safe margin. That means, suppose are talking about this type of graph; suppose this is your effective dose curve and if the if your lethal dose curve is also very similar, so there is not much therapeutic safety level that you have.

If this goes here, then you have a better window that means, there is a less chance of crossing the lethal dose in that. So, this ratio that means, LD_{50} by ED_{50} is what is the therapeutic index, initially I told when I begin this. I told you about this.

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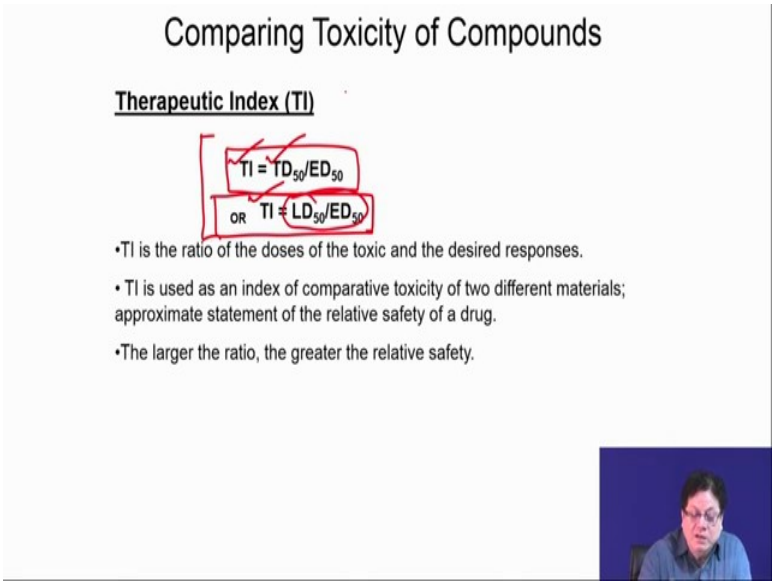

So, therapeutic index is, there are two ways of doing this for human you cannot kill the human. So, for human you have you just measure the toxic effect that when you slowly increase the dose level. So, some point you will see toxic level appearing. So, you see that what is the dose required to have 50 percent of the toxic level; in 50 percent of the population that you have taken and you are studying. So, TD_{50} by ED_{50} is called therapeutic index.

So, also LD_{50} by ED_{50}, is usually done for animals because where you can scarifies the animals. But for human it is usually the TD_{50} by ED_{50}, but any way this is what is called

the therapeutic index, and the larger this therapeutic index the safer is the drug that is most important.

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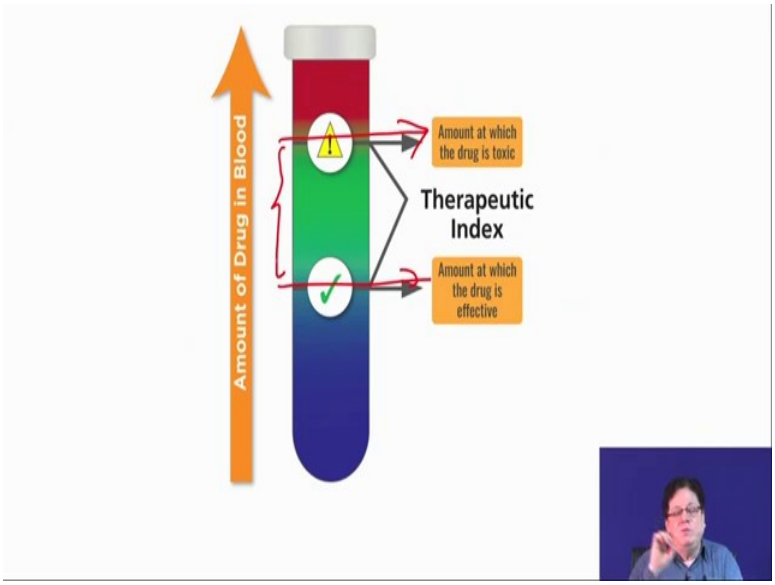

So, suppose this is the scale and this is the amount at which drug is effective and this is the amount the drug becomes toxic, that means, you have a safety margin of this. This is what is basically the therapeutic index but it is expressed as a ratio of TD_{50}, that means, the toxic dose TD_{50} divided by ED_{50} or LD_{50} divided by ED_{50}.

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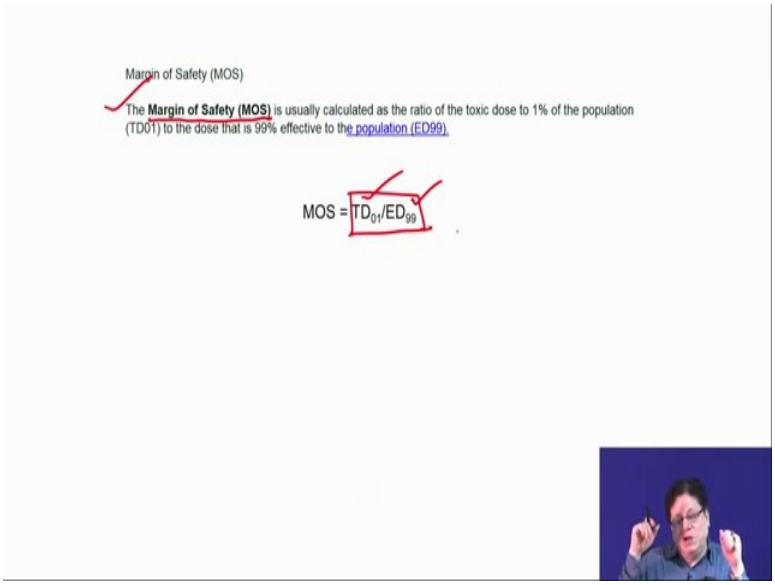

Today, also there is another, the margin of safety you can even be very sure that a person does not cross or he is not given a dose which is the toxic dose. So, to do that, there is something called margin of safety (MOS) which is calculated by TD_{01}, that means, 1 percent of the population is having a toxic dose and ED_{99}, that means, 99 percent of the population is having the effective showing that responds effective response.

So, that gives you a better even a safer margin, because you do not want even 1 percent of the population to be affected by the toxic dose. So, rather than going up to 50 percent of the population, basically you increase the effective dose for the 99 of the population and you reduce the toxic dose for only 1 percent of the population, so that gives what is called the margin of safety.

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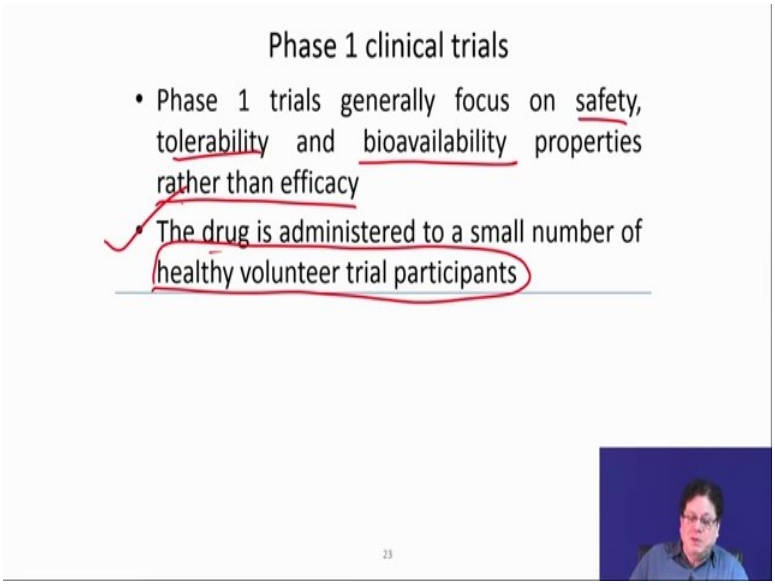

In clinical trials, basically there are phases, phase 1 is safety, tolerability, and there are different targets for this phase studies rather than efficacy. Firstly, as I said, they try tolerability; that means, what is the tolerance limit and then the bioavailability, that means, the ADME properties that whether the drug is absorbed and then shows effect.

Usually healthy volunteers are used for the trial period, because you are only checking the safety level, you are only checking the tolerability, and you are only checking the bioavailability. You are not focusing on the disease yet. So, healthy volunteers are required.

In Johns Hopkins University, I know people are really paid for acting as healthy volunteers to participate as trial participants. But this is safe, because they are given very minimum amount of the intended drug.

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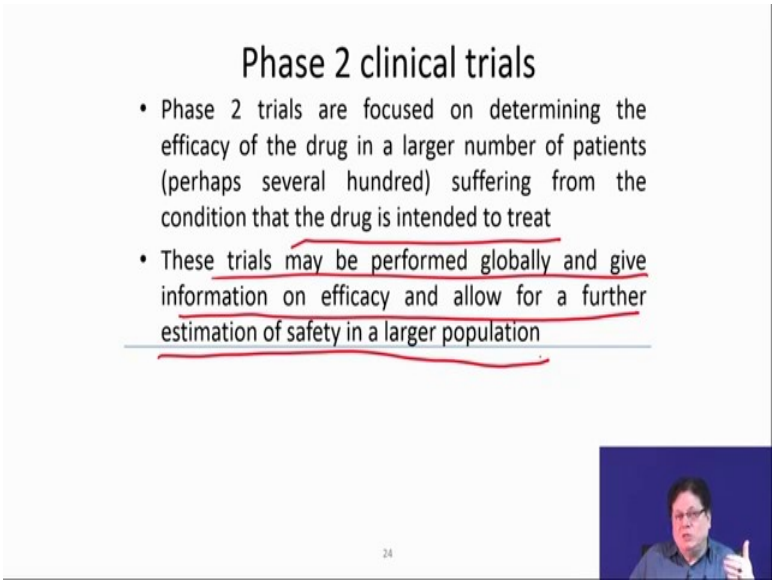

So, once these are through, that it has got a very good safety margin, then you go to phase two clinical trials. In phase 2 clinical trials, you actually take the patients now, several hundred suffering from the condition that the drug is intended to target. And then you try to you get information about the efficacy. And also you estimate the safety in a larger population rather than the healthy subjects earlier in the phase 1 clinical trial.

So, basically phase 1 is on healthy volunteers, so it measures the safety, it actually concentrates on determining the toxicological effects and the ADME effects that means, the absorption distribution metabolism and excretion those type of properties, but no PD that means, we are not targeting the efficacy of the molecule. Efficacy of the molecule is done on patients, but in a limited way not thousands of patients. And then try to find out the minimum amount required to get the efficacy, the dose that is required to cure the disease.

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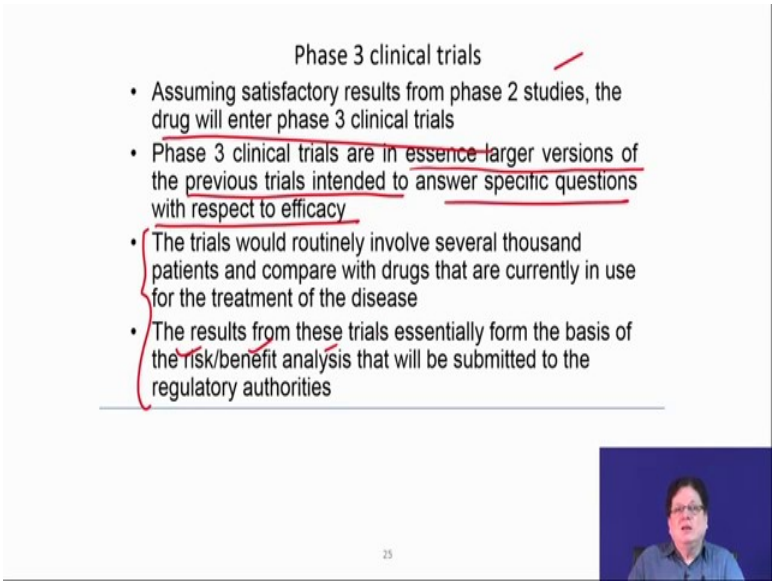

If satisfactory results from phase 2 trials are obtained, the drug will enter phase 3 clinical trials. These are larger versions of the previous trials that means, now it is a broad approach. You take the patients and you give the drugs which cross the phase 2 trials, and then you actually go to large number of patients and give these drugs, and then see the risk and the benefit analysis.

Risk and benefit that means, the toxic effect versus the benefit as I said every drug has a toxic effect. But the beneficial effect is that you have to compare the cost of this risk and benefit; which is better; which is more important to you. Like some people are taking anti-hypertensive drugs and many of these anti-hypertensive drugs in the long run is going to affect the kidney.

But question is that whether you want to live 20 years from now on by taking drugs which will reduce the blood pressure or you take the risk that you can die anytime if you do not take the drug. So, this benefit and risk you have to calculate and then finally, the regulatory authority approves the drug.

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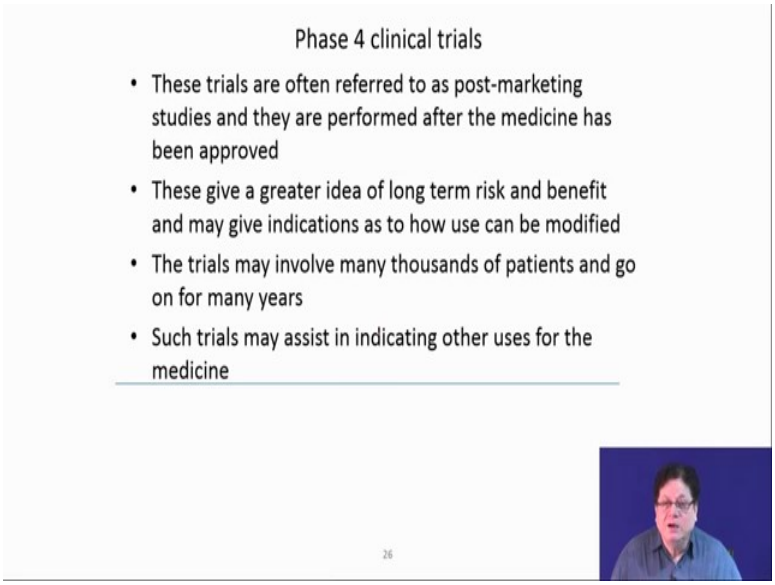

There is something which is called phase 4 clinical trials which is done after this trial phase 3. Once the drug is available in the market, still many pharmaceutical companies study a wider variety of people what they had their patients, and see that in the next 5 years, 10 years, what is the patient's report, how are they feeling, whether there are reporting something else which was over looked at that time; that is called the phase 4 clinical trials.

In many cases, some drugs have been approved, it entered the market and later it has to be withdrawn because they are having some other effects which may be detrimental.

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So, this is the drug developmental process: validation, assay development, high throughput screening, hit to lead and then lead optimization, pre-clinical and then clinical drug development.

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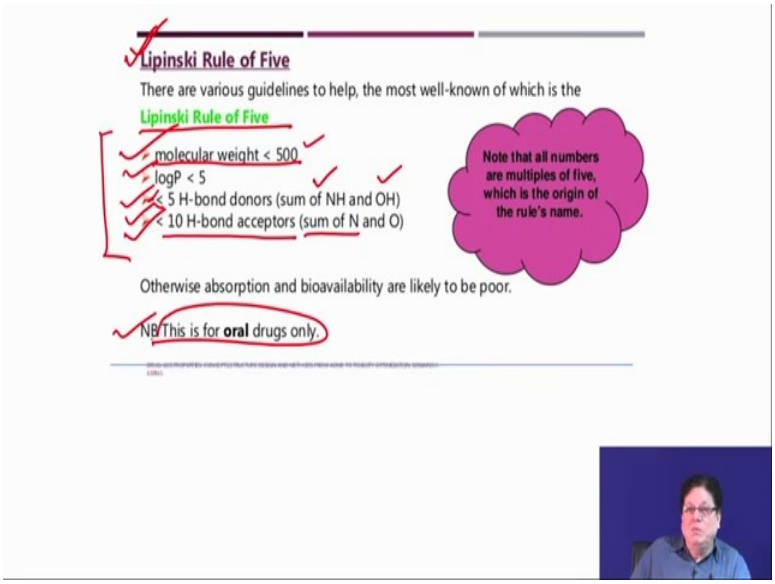

There are some empirical rules when you design certain molecules for *in-silico* screening; it is not that you write randomly whatever would like to do. You have to follow certain things like time and again I am saying the drugs are low molecular weight compounds. But what is the optimum molecular weight? That you should not cross or what is the threshold like lethal dose which you should not cross to have a lethal dose. Just like molecular weight, there is some restriction on the lipophilicity, which means, how soluble it is in a lipophilic solvent like organic solvents.

And then for interacting with the binding site, these interactions are electrostatics or could be hydrogen bonding; hydrogen bonding plays a very dominant role in this interactions. So, how many hydrogen bonds you need in the molecule to be there. Lipinski made some rule which are called Lipinski rule of five. Rule of five does not mean that there are five rules. Rule of five means that whatever numbers are shown here, they are either five or multiples of five.

So, Lipinski's rule of five says that whenever you select a molecule, you are thinking that it may be a possible drug, first check whether the molecular weight is greater than 500? If so, then discard that; it should be preferably below 500; though it is not a

sacrosanct that it has to be less than 500 always, there may be some molecules which are having 550 or 600, but this is a general guideline that a molecule is drug like when it has molecular weight of less than 500.

It has a logP value of less than 5. What is logP value? It is the logarithm of the partition coefficient between octanol and water, why octanol? Because octanol mimics the membrane through which the drugs crosses. Membrane is actually lipophilic this is large chains, this fatty acyl glycerol chains with a polar head group, but a majority portion in a membrane is lipophilic.

So, what happens? The drug has to cross this lipophilic membrane and then go inside, so that means, it should have some balanced lipophilicity or hydrophobicity. So, here this logP is the lipophilicity that means, the logarithm of partition coefficient between octanol and water. And octanol was found to resemble the biological membranes.

And then it should have less than or equal to 5 hydrogen bond donors, that means, the sum of hydrogen bonds donors which are usually OH and NH. So, sum of NH and OH in the molecule should not cross 5, ok, it could be 4, but the threshold value is 5.

And then the 10 hydrogen bond acceptors; that means, what are hydrogen bond acceptors? Nitrogen lone pair in free nitrogen, not NH and oxygen lone pair. So, oxygen is the hydrogen bond acceptor, OH is the donor, NH is the donor and N, a tertiary nitrogen, is an acceptor. So, this is what is called Lipinski rule.

So, now, all pharmaceutical companies initially screen the molecule based on the Lipinski rule and reject those molecules which do not satisfy these type of parameters. Remember this is only true for oral drugs, because we are talking about logP value; we are talking about this molecular weight, we are talking about this hydrogen bond, all these things. This is for oral drugs only.

And majority of the drugs we have are orally taken, only many of the life-saving drugs are usually given intravenously because of their poor bioavailability and absorption.

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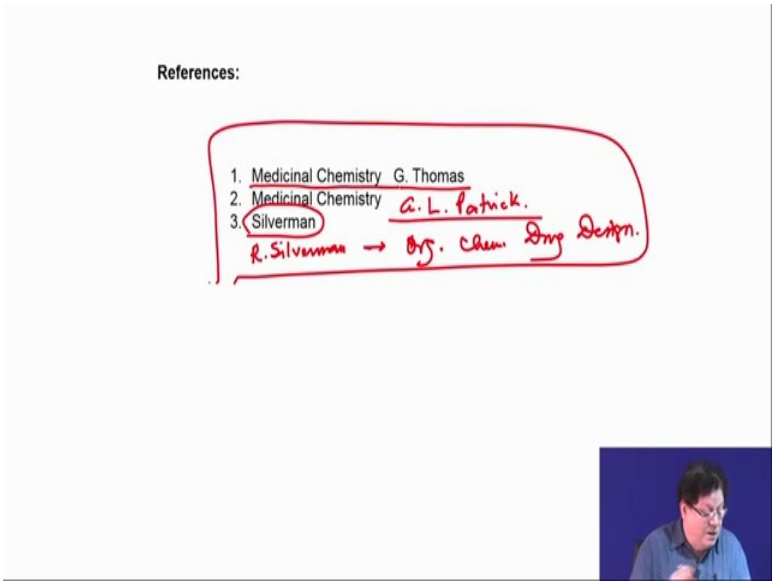

Now, let us go into some of this. Before that I think I should tell you about some of the references. There is a medicinal chemistry book that real name is Medicinal Chemistry: An Introduction, which is by Gareth Thomas. Then Introduction to Medicinal Chemistry, just the introduction to medicinal chemistry by G. L. Patrick that is a good book and finally, R. Silverman's book on Organic Chemistry of Drug Design, organic chemistry of drug design. These are very good books on the topic that I will be covering.

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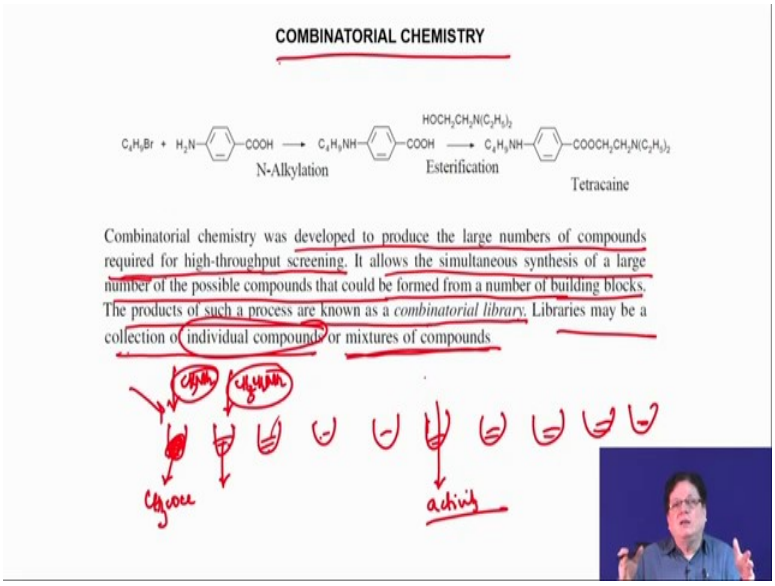

Once the target is known and validated, then you have to ultimately molecules, you have to synthesize molecules that is where the organic chemists are required. So, they have to make the molecules. And one thing is very clear, greater the number of molecules one makes, more is the chance of striking a hit. Suppose you have made five molecules only. These five molecules may not produce anything because you have a very small pool of molecules. So, larger the number of molecules, larger the molecular library, greater are the chances of finding a hit. If you are looking for a book and if the library has only 100 books, the chance that you will find that book is very less.

If the library has 1 million books, then the chances that the book you are talking about is there, is much more. Similarly, in drug discovery process, the more number of molecules that you make, the greater is the chance of success, there is no doubt about that. Because as I said whatever you do, computational studies can assist you in making the library a little bit smaller, but ultimately you have to make large number of molecules.

Now, initially what happened, organic chemistry including synthetic chemistry developed in a rapid stride in the 40s, 50s, and 60s. Starting from Robert Robinson, and then it went to Woodward and then many of the stalwarts in organic chemistry; they have now established the art of synthesizing molecules. So, organic molecules can be made. But how many molecules you can make per day? Usually you do one reaction or two reactions per day. So, one person can make one or two molecules per day.

What happened after the 1980s, there has been an enormous development in biology. And earlier what happened was that suppose some biologist has isolated an enzyme. And the amount of enzyme he or she has is very little. And then he or she once wants to check what is the interaction of different molecules with this new enzyme.

Now, the chemists supply one compound and the actual system was such that it takes about few days to ultimately come up to a conclusion whether the molecule has really interacted, how much effective was the interactions. So, it is taking long time. But after the 1980s something came which is called high-throughput screening, that means, you can screen several hundred molecules within a very short time, and that means, now gone are those days where the organic chemist synthesizes one molecule and gives it to the biologist and 1 month later get the result that nothing has happened.

Then you make another molecule. So, things were very slow, but then after the high throughput screening that every day you can screen large number of molecules, so that immediately you can put pressure on the organic chemist to come up with methods which can produce large molecules per day, so that can be supplied to the biologists. Now these issues have given rise what is called combinatorial chemistry.

Combinatorial chemistry was developed to produce large number of compounds required for a throughput screening. It allows the simultaneous synthesis of a large number of possible compounds that could be formed from a number of building blocks. The products of such process are known as combinatorial library. Libraries may be collection of individual compounds or mixture of compounds.

This is very interesting. Libraries may be collection of individual compounds that means, suppose you have 10 test tubes, each test tube is numbered as 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10. Now, suppose I take CH_3COCl in all these test tubes suppose (the same acid chloride) and then I add different amines, suppose this is methylamine I add.

And here I add ethylamine $CH_3CH_2NH_2$, then propyl amine and so on. Since I am doing in parallel fashion, so I will get the amide. In this 1^{st} case, it will be amide or methylamine and acetyl chloride, in this 2^{nd} case ethyl amide, in this 3^{rd} case, propyl amide or propionyl amide. So, you will get all these different amides, if you do the reaction in this fashion.

So, here basically each test tube has only collection of individual compounds, which means each test tube contains only one pure compound. And the other is mixture of compounds; that means, basically what you are doing, you are adding both methylamine and ethylamine (something like that) into each test tube. So, you get two compounds; one is the amide of the methyl another is an amide of the ethyl.

Now, you do the testing. Now, chances of getting more success if you have more number of molecules in a particular test tube, earlier case I said that 10 molecules in 10 test tubes, but there are techniques by which you can make 10 molecules in a particular test tube, so that you have hundred 10 multiplied with 10, that means, the 100 molecules in these 10 test tubes and then you test through high throughput screening.

But the question is suppose this test tube shows the activity; that means, there are 10 molecules out of which may be one is showing activity. Now, you can go back and then individually synthesize those 10 molecules and then see which one is active. But this is much better option because out of 100, there is more chance of getting a hit; and then you know which test tube is giving some activity and you know what are the ingredients that you have added and you try to separate them and then separately study those 10. So, it is much better option.

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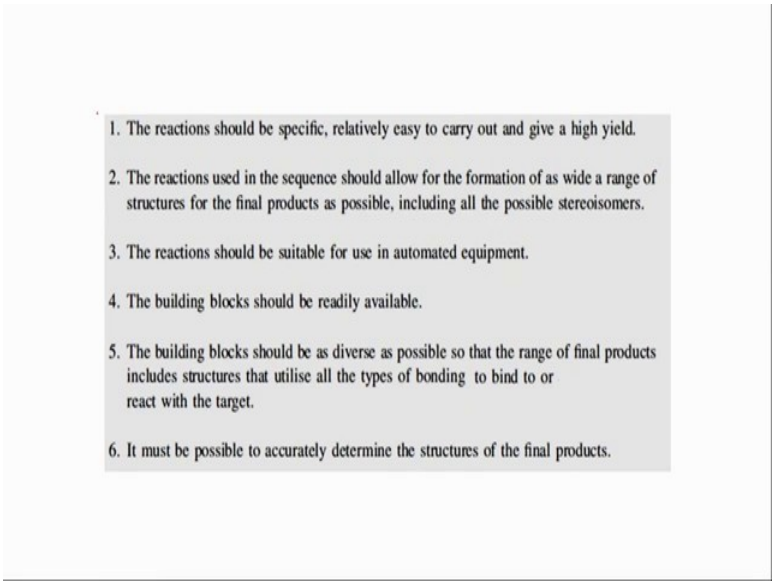

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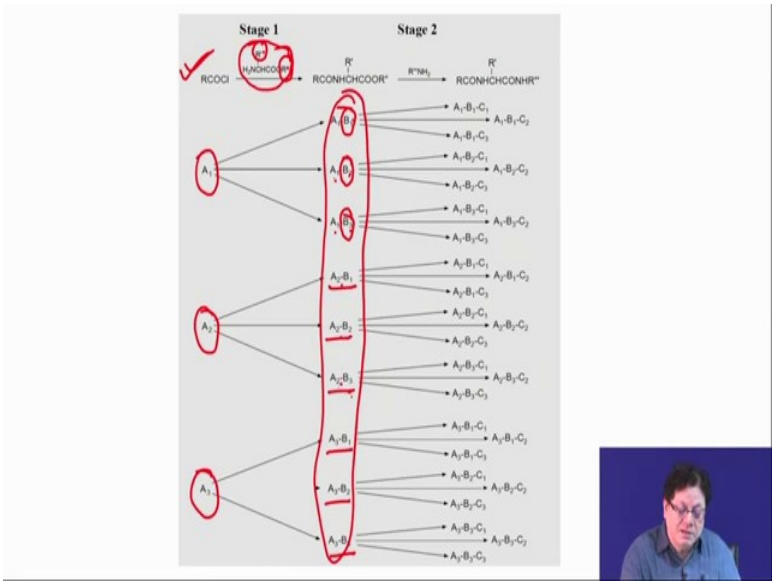

Now, this combinatorial chemistry was developed by using solid phase synthesis, because ultimately it was realized that this solution phase chemistry you have to do chromatography to purify the molecules.

Whereas, if the reaction is done on a solid phase, as we have seen in solid phase peptide synthesis, your purification is very easy; you just take the solid beads and then wash them after each reaction and then you detach it from the solid phase which ultimately gives the compounds. So, all these combinatorial chemistry are usually done on solid surfaces, like utilizing Merrifield's resin.

Now, I will give you an example of this combinatorial synthesis. Suppose you take RCOCl. Now, different types of R which are exemplified by or denoted by A_1, A_2, A_3, that means, suppose A_1 is methyl, that means, acetyl chloride, this is propionyl chloride and this is say butyryl chloride. And then you are adding an amino acid NH_2 with R' and then amino acid ester.

Now, for this ester, this R can be varied; you can take different amino acids, you can have 20 amino acids here depending on this R. These are your variables R R' and R''. So, you can vary this R' and you can vary these R'' also. And so suppose I say that B_1. So, basically if R_1 is something say methyl; that means alanine and this is $COOR'$ and this R' is suppose methyl, so that is exemplified by B_1.

So, similarly varying this R' and R'' I can have another entity called B_2, and then I can have a third entity called B_3. So, when I take A_1 in one test tube I add this B_1 that means, this is methyl and that is methyl. So, I will get A_1B_1. And in another test tube, I will get A_1B_2 and then A_1B_3 and if A_2 is the starting point, you will get A_2B_1 you are adding the same components B_1, B_2, B_3, A_2B_2, A_2B_3.

And then with A_3, you will get A_3B_1, A_3B_2 and A_3B_3. I am not actually proceeding any further, but you see that the same reaction is operated on three different acid chlorides and you get 9 compounds.

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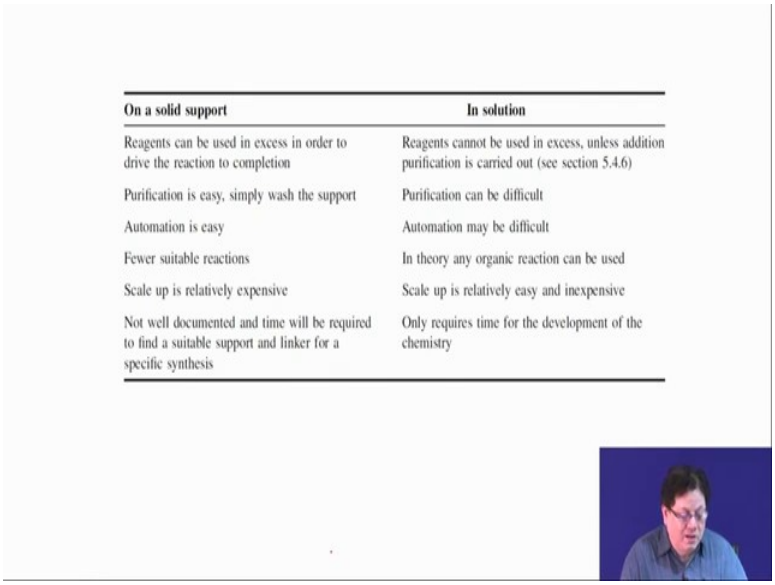

We know what the advantage of the solid support is.

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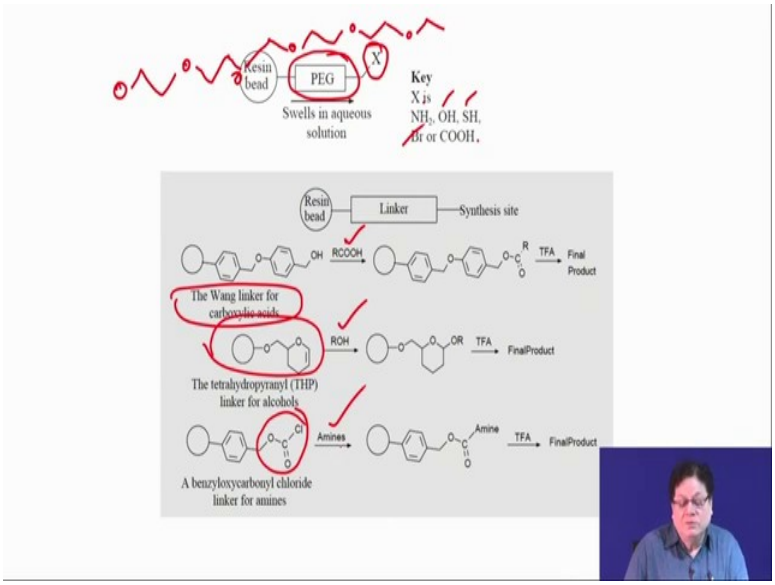

These are some of the resin beads in the Merrifield resin; we know that it is a polystyrene which is chloromethylated. Then there is polyethylene glycol chain, which means, you have this type of moiety. The beauty of this type of moiety is that it swells in water. So, you have the resin bead, then polyethylene glycol chain and then a reactive X.

As you put it in aqua solution, this swelling is important because that allows percolation of the other entity that is reacting with it. X is NH_2, OH, SH all these reactive

functionalities. There are other resins also; this is called one resin for carboxylic acid. This is the THP, THP ether for alcohols and then you have this chloroformate type of resin for amines.

These resins which are attached to a chloroformate resin, which are attached to a tetrahydropyranyl moiety. This is the tetrahydropyranyl linker. And this is the one resin which is the benzylic alcohol. These are the resin that you will be using depending on you are starting entities.

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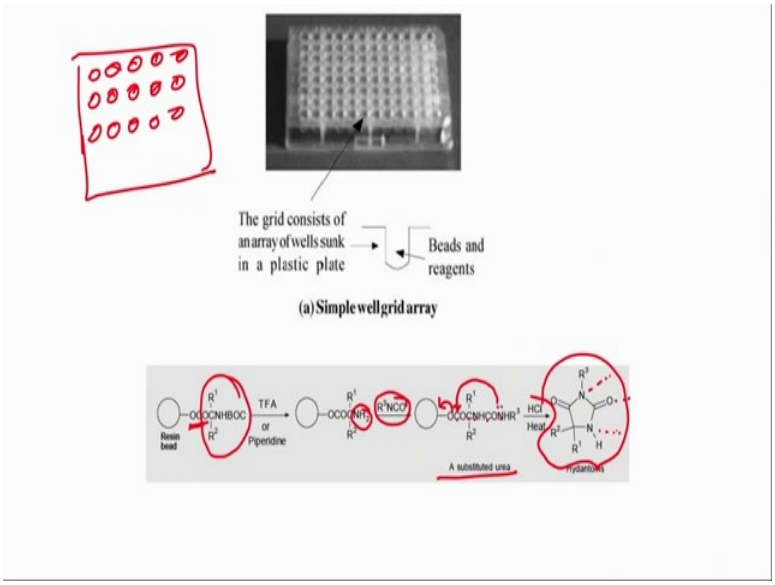

Now, this is how this combinatorial chemistry is done. Basically you take a plate. And there are wells in the plate; wells in the plate that means grooves. Now, suppose you are doing this type of reaction that this resin bead is attached to the where the ester linkage to NBoc amino acid with the R^1 and R^2 as shown here.

This is very similar to the peptide chemistry that we have done. And this amine is a free amine which can react with an isocyanate and that will give you a substituted urea. And it is known that if it is heated with HCl, then a reaction that takes place is this, attacks the carbonyl and the resin is released, so that makes a five membered ring that is called a hydantoins.

Now, hydantoins are privileged skeleton for drug development because it satisfies The Lipinski's rule of five.

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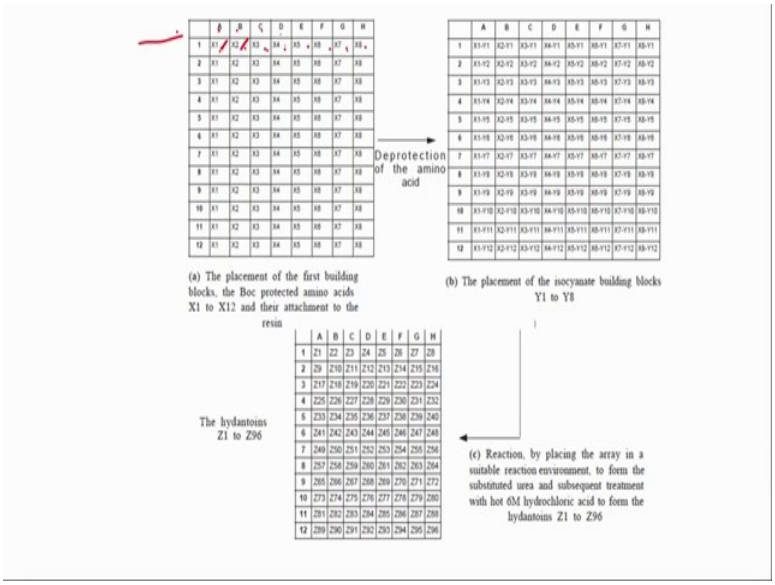

See these are my wells, all these grooves. So, I say that this is A, B, C, D, E, F, G, H on this row and on the column I said 1, 2, 3, 4 up to 12. So, what I do, I add the first component, the first component is this bead with the NHBoc. So, I add the bead with different amino acids with 8 different amino acids.

So, X1, X2, X3, X4, X5, X6, X7, and X8. So, these are resin beads which are attached to this X1. I added the beads which are having different amino acid attachments. On this side, I add the same bead with same amino acid attachment.

So, different beads are attached to different amino acids. And in this direction, that means, the column I have, the same bead attached to the same amino acid, but these are different as in the rows we have different ones because these are X2s, this is X1 rows, X3, X4 like that. On this side, it is X1, X2, X3, X4, and X5.

Now, you deprotect the amino acid Boc and then you add the next amino acid and couple. You have 12 grooves on this side on the in the column you have to select 12 amino acids and then couple with. So, Y1, now earlier your X1 X2, that means, different beads are having different amino acids here and on this side beads are having the same amino acid.

Now, what you do, you add the second amino acid, the same amino acid on this side on the row. So, you add the same amino on this row. So, what you will get X1Y1, X2Y1,

and X3Y1. So, Y1 will be attached to all the beads via the X. And in this you add, so basically what you are doing in the second one you are adding only Y2. So, you get X1Y2, X2Y2 these. Remember these are different.

So, then you add the Y3. So, in this case if you add all these, you get a library of compounds. How many compounds? Usually there are commercial plates available, usually having 96 plates. So, 96 compounds you can prepare in one go.

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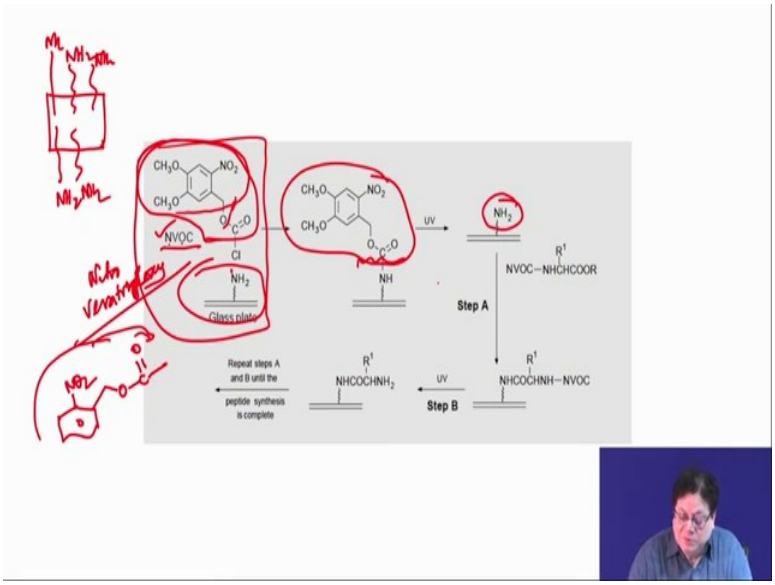

Another way you can do is this is very good technique that you have a glass plate. Suppose, you have a glass plate and the glass plates are made of silica. So, using the silica OH groups, you can hook up some organic species ending up with a NH_2; glass plates are available today that has lot of NH_2s attached to the glass surface, not directly attached, it is attached through the that OH and then an alkyl chain and that ends up with the NH_2.

This NH_2 can be used for further reaction and then that means, the plate is the solid phase, it has got this NH_2, then you are adding a compound which reacts with the NH_2. So, the compound will now stick to the NH_2. There is one medicinal chemist who developed this technique. What he did was that on a glass plate with NH_2, you react with a molecule which is called *N*-veratryl oxycarbonyl.

So, these group is what is called the nitro veratryl oxycarbonyl. The beauty of this is that first of all it is a chloroformate. So, this is going to react. So, you form the urethane or the carbamate. And then as you shine UV, this is the protecting group which is labile under UV. So, it cleaves. The NH_2 is again liberated.

So, this is a group which is called nitro veratryl oxy carbonyl; there is another group which is only nitro and a benzyl. The similar group: *ortho* nitro benzyl oxycarbonyl, also cleavable under light, but this is a better one because the wave length of the light that is used for removal of nitro veratryl oxy is around 365 nm. This is the photo labile group. So, you can again re generate your NH_2.

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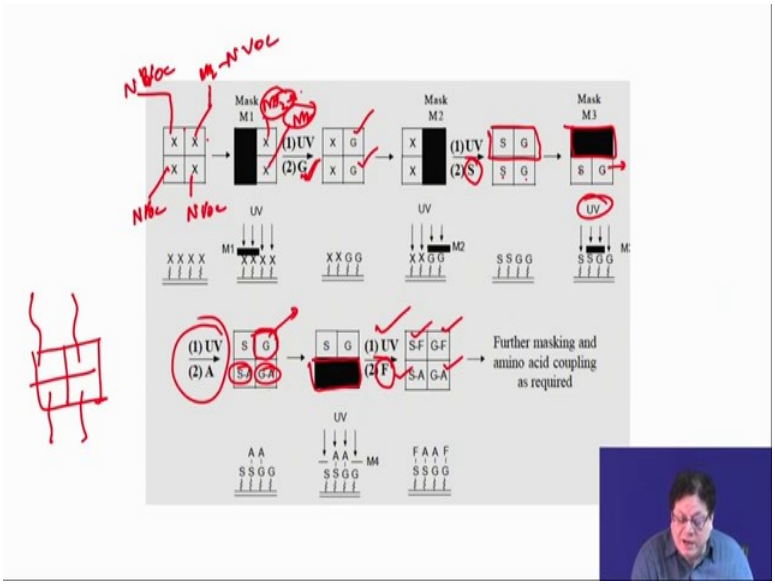

Now, let us see how you can utilize this technique. So, you take a glass plate like this, and see ultimate objective is to make library of compounds by a simple technique and then also a simple assay and you can check the bio activity. So, suppose this is a glass plate. So, first you cover one side of the glass plate with a opaque material, so that light cannot go through and fall on the glass surface.

You have made four quadrants and then two of these are covered by a by a opaque s screen which covers this part. Now, this X is actually the NH_2 attached to veratryl oxy, this NH_2 attached to veratryl group. So, all are attached to NVOC.

So, now, you have covered this part and shine light. So, what will happen? This will generate the NH_2 because you are adding light. So, now, you are adding light. So, this is going to form the free NH_2 and you add the first element whatever it is amino acid suppose. So, you get G and G on this side via the NH_2 you couple the amino acid.

Now, you cover this part and shine light on this side what was earlier covered by the opaque screen. Then this will form the NH_2 now and add S (another second component). So, now the plate will have G G S S. Now, S and G is covered, and you shine the light, because whatever amino acid you have attached to the amine, that is also protected as NVOC. So, you shine the light. So, G will now have free NH_2 after shining of UV and so there is a G and there is a S.

Now, you add the third component and suppose that is A. So, you have a G-A molecule, you have a S-A molecule which are linked on the surface of the glass plate. So, the next step is you cover this part where S-A and G-A are produced.

First UV and then you add the F. So, you have S-F, G-F, S-A, G-A. So, you keep on doing this. You mask this part now you shine the light. So, F will have NH_2, A will have NH_2. You add the fourth component, so in that way you can make these different compounds which are growing on the surface of the plate.

So, if you have different plates, you can do it in series and then generate molecules on the glass plate. And on the glass plate also you can see the activity that is what high throughput screening is. Even if there are four different compounds on the glass plate, you can check the activity of these four entities by high throughput screening.

So, this is a very good demonstration of combinatorial chemistry. However, whatever we have done is called parallel synthesis which means that after all these reactions, each quadrant here is basically homogeneous; each quadrant will either have a G-A or S-A or G-F or S-F. It is not that every quadrant has mixture of compounds.

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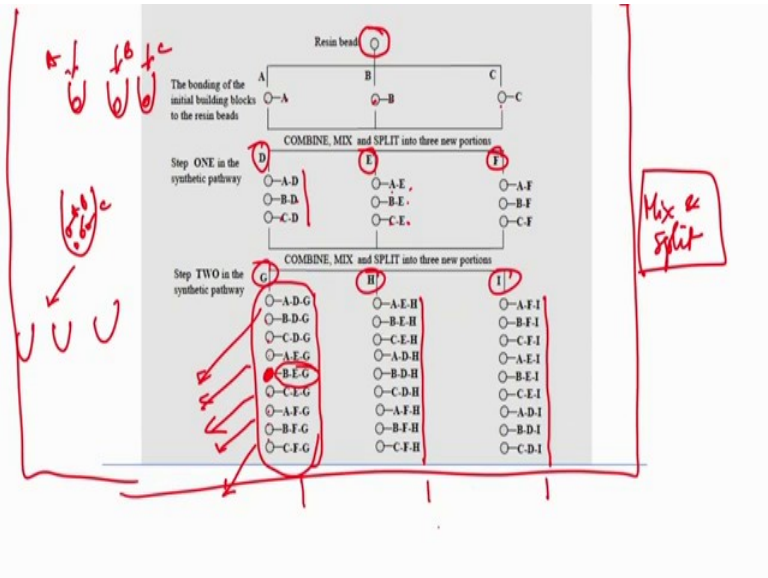

So, now there is another technique which is called mix and split technique by which you make larger library of compounds. So, how is it done? You take the resin bead and you take suppose three test tubes. So, in these three test tubes, you have this resin bead. Now, you add A here, you add B here, and you add C here. So, this will be attached to A; this will be attached to B; this will be attached to C. Now, you mix the contents of all the three test tubes.

Put all in a bigger test tube, everything is there, that means, some beads are attached to A, some beads are attached to B, and some beads are attached to C. So, you mix and then you split it, you split again into three. So, when you split into three. So, every test tube will have bead to attach to A, attach to B, and attach to C. So, you split into three test tubes and you add now D and here it is E and here it is F.

So, what will happen now, here you will get A-D, B-D, C-D, here are you will get A-E, B-E, C-E and here you will get A-F, B-F, C-F; then again you mix and then split. So, each bead here will have A-D, B-D, C-D, A-E, B-E, C-E, because you are mixing and then splitting. So, every test tube has this characteristic of these earlier three test tubes. So, that means, there are nine compounds.

Now, you add G here, you add H here, you had I here. So, what you have now A-D-G, this all end up with G, this all ends up with H, and these all will end up with I. But how many compounds after three steps? After three steps you are having 27 compounds. So,

you have a larger library. However, things are not that easy. So, what will happen now these beads which are present in one test tube, you separate these beads individually because these are like marbles.

So, you separate those beads and then see whether the bead has given some activity or not; so that means, you separate it to an individual bead and then check the activity. Suppose, one of these beads gives the activity. But the problem is how do you know what is actually attached here, because it could be A-G-G, it could be B-D-G, it could be B-E-G, C-E-G, all these things, so that is problem.

So, you have to finally, find out what is there attached to the bead. So, for that you have to detach it and then see what is there. I think in the next lecture, we will start from this point, this is called the mix and split method for combinatorial chemistry. And that gives a rapid growth because if you further increase this you know that it will be 81 molecules you are handling.

So, this is much better; only problem is later on you have to find out the identity of the compound; because every test tube has different collection. But you can isolate those beads and then see what the effect is and then finally, try to find out what is the compound that is attached here. So, we will discuss that in the next lecture.

So, thank you for today.