Biochemical Engineering

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Module No. # 01

Lecture No. # 31

Effects of Ligand Depletion and Multiple Receptors on Binding Kinetics

Continuation of what we are doing on receptor Ligand binding in the last lecture. So, what we looked at in the last lecture was a case of receptor binding to a Ligand. So, I talked about the receptor being on the cell surface, a part of it inside the cell surface cell you know in the cytoplasm a part of it outside the cytoplasm the Ligand comes and swims in you know binds to the receptors. So, then we looked at the kinetics of receptor Ligand binding and we looked at the simplest possible kinetics which is the case of R plus R receptor binding to the Ligand directly R plus L giving R l. So, and then we talked about free free receptors and total number of receptors and free receptors in the complexes form.

So, today what we will do is, we will look at other kinds of kinetics. So, this is a simplified kinetics that may or may not occur all the time. But in a realistic situation you can have other kinds of kinetics coming in. Before we do that let us very quickly go through what we did in the last class because I hurried a little bit towards the end of the lecture.

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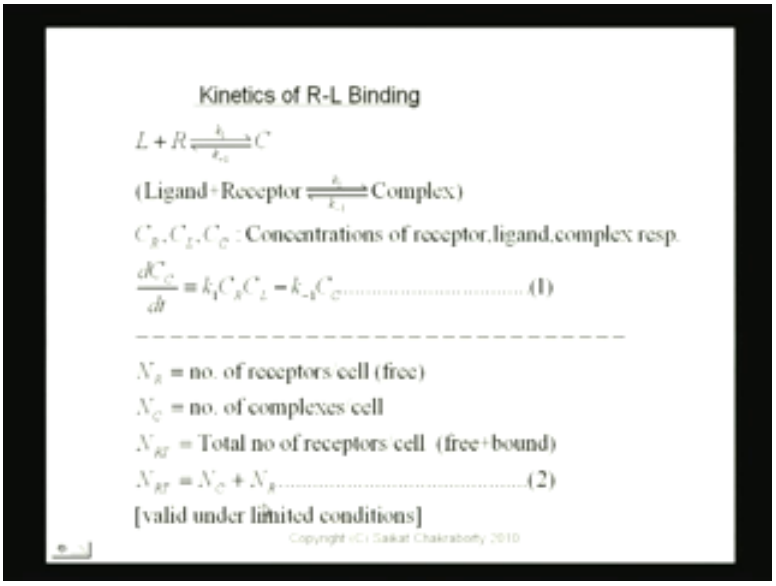

So, this is a kinetics that we looked at L plus R giving C complex and then the complex will then you know, that is that is what is the required over here and K 1 and K minus 1 being the forward and the backward grade constant for the system and this is how we wrote the kinetic equation, the basic balance equation for the complex D. So, d C dt of C equals a forward reaction K 1 times C R times C L minus K minus 1 C C if you remember it.

Now, what we said that we do not want to express this in terms of concentration. We want to express this in terms of numbers. So, N R being the number of receptors, N C being the number of complexes so, free receptors is N R, N C being the number of complexes and N R T is the total number of receptors which includes both the complex and the free receptors.

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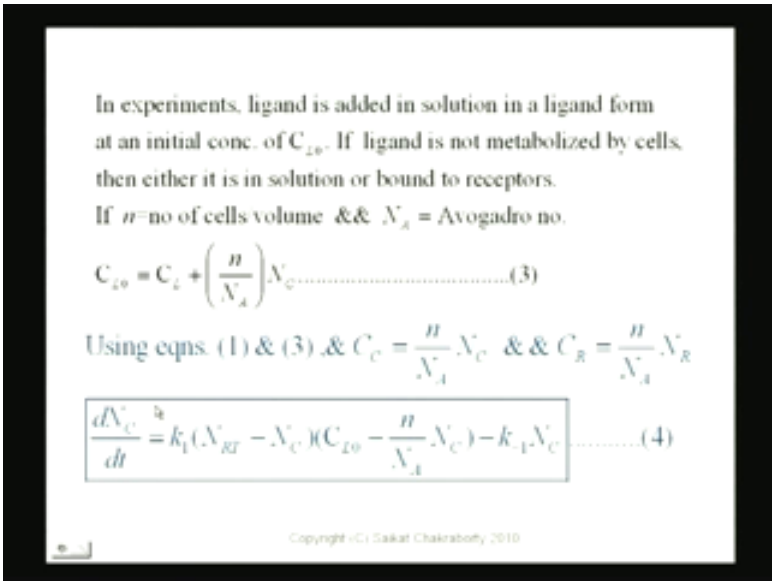

So, then we said that how do we convert these concentrations into numbers. So, Ligand is obviously, in a concentration because it is in the liquid form, but we found out a way of converting this to numbers because the total amount of Ligand C L naught equals the amount of Ligand that is present right now plus the amount of Ligand that is reacted with the receptor to form the complex.

So, that is given. The concentration of that is given by n times N C over N A. N being the number of cells per unit volume. So, if N C is the number of complexes per cell then N N and N is the number of cells per unit volume then N times N C would be the number of complexes per unit volume. That you divide by the Avogadro number to give the get the molar concentration of the complex that is formed. And so, therefore, that you. So, the C L that you have the concentration of the Ligand is initial concentration minus this value or in other words initial concentration is current value plus whatever current free Ligand plus whatever has formed complexes correct. So, then we can substitute it back.

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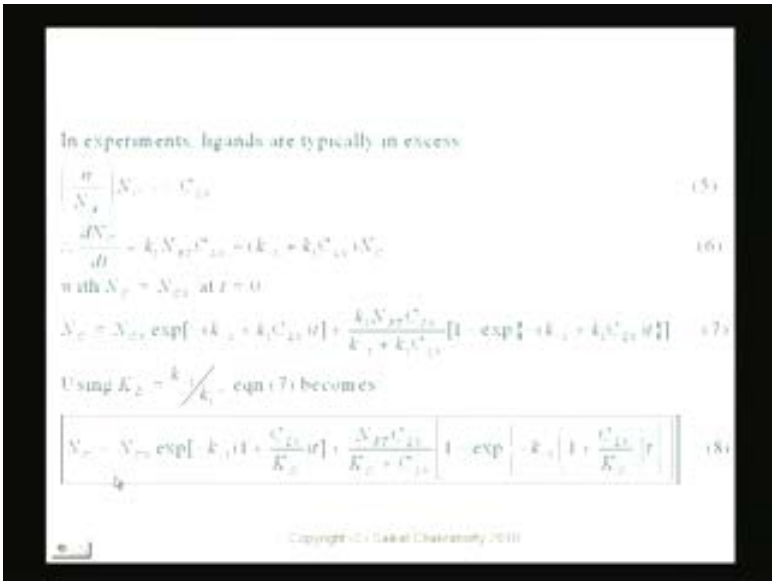

So, now, we can have this entire equation almost as a, in the form of a number right. And then we said that we can go ahead and solve it we can go ahead and solve this also as you as you can see over here this is a second order equation in N C. And this could be solved is in partial fractions, but we meant we wanted to wanted our life little simpler and we made a simplifying assumption which is that C L naught is much, much larger than this. So, this could be taken as a constant and it becomes a first order equation. So, we had this first order equation over here and then N C naught, we assumed N C naught to be 0. So, we had exponential part and then we figured out how to evaluate the half time and this is the plot and so on.

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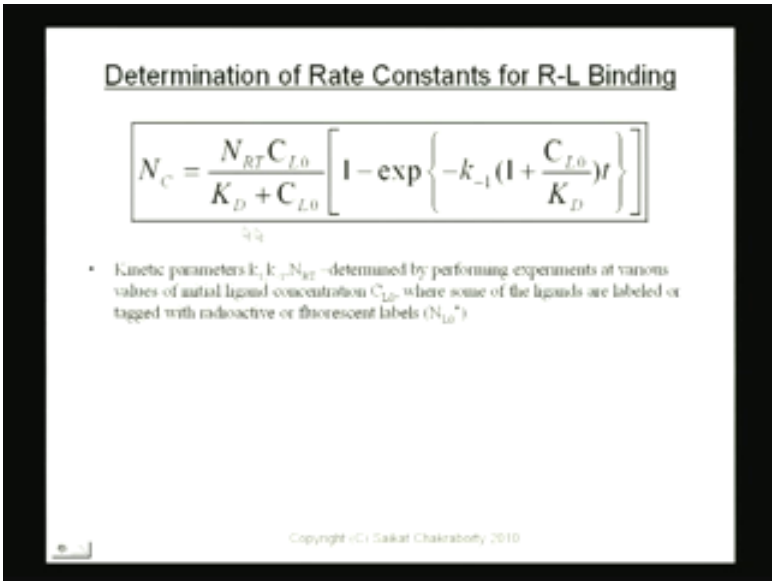

So, I just want to do the last part, bit yes I think this is where I was little fast. So, N C is the you know, so, if N C naught is 0 then you have the exponential variation and we figured out how to evaluate these constants.

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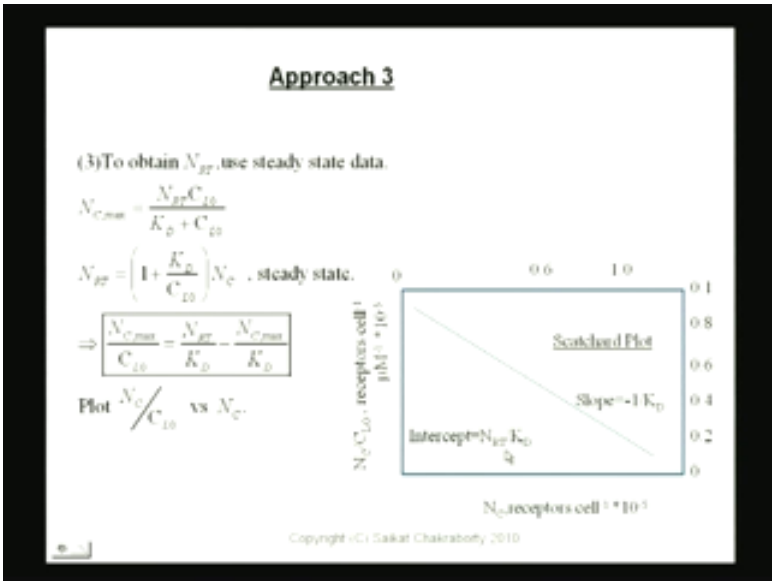

Now, the thing that I want to talk about is this one the last thing I did which is a Scatchard plot. So, scatchard plot is essentially a steady state diagram. So, for example, here when you are plotting this, this is this is your basic expression the one in the box.

Now, when you are plotting this over here you are doing an unsteady state plot, but this scatchard plot is the steady state version of it.

So, steady state means this expression with time going to infinity. So, the steady state would be N C would be N R T C L naught over K D plus C L naught. And that is a very important number for us or a important expression for us because it is easy to measure because you know, it is always harder to measure dynamic dynamics of a process because we have to keep measuring at every time instant at every time interval whereas, for steady state you can let it happen as far as long as you want and then you can measure it. So, this so, this steady state is easier to measure and that is why it is important in our calculations and you will see that this is what we used mostly. So, N C A steady state would be N R T C L naught K D plus C L naught clear? So, that is what we do when we plot in the do a scatchard plot.

So, this is what we start with. So, this is the N C max this is what we call N C max. Why? Because that is the maximum as you can see over the from the plot over here. That is the maximum value of N C that is attainable. So, the maximum value is attained at steady state. So, we call this steady N C max and that is given as N N R T C L naught over K D plus C L naught. This I can rearrange you know dividing the both sides by C L naught, I can rearrange this as N C max over C L naught equals N R T over K D minus N C max over K D. And this I can plot because easily because I can measure N C max. So, I can what I do is I vary in my C L naught. That is the Ligand concentration I use different Ligand concentrations. Let it happen for as long as it happens. Then take this steady state, measure my N C max. How will I measure? Because I told you the other day that we will label these Ligands. So, we label Ligands. We will limit fluorescence or something some kind of labeling if it is a fluorescence some kind of labeling then, we can measure the complexes straight away.

So, at steady state we measure how much complexes have been formed and we plot this N C over C L naught versus N C. We plot this and then we can get the rate constant. The rate constants K D as well as N R T fine from the slope and the intercept. So, the slope will give you minus 1 over K D. The intercept will give you N R T over K d. So, you can evaluate both N R T and K D fine. This is known as the scatchard plot and it is a very important plot because we will keep referring to this. What I want to mention to you is that scatchard plots can come in different ways you know. I think I gave you the

assignment also where you had to plot the scatchard plot. So, scatchard plot in come can come in various ways. The only thing that is common or unifying between these different ways is this scatchard plot appears appears a is the fact that these are steady state plots steady state plots of complex versus Ligand in some form.

So, you can keep varying the form of this scatchard plot, but it still remains a scatchard plot you know as long as the complex versus the Ligand is in one axis it is you have the complex and the other axis you have the Ligand fine. So, this is I think the problem that I gave you and I hope you did that. So, today we start something. So, this was all these analysis that we did was for the case where we assumed simple reaction kinetics R plus L giving a complex and we assumed that it is a second order in the forward direction of first order in the backward direction. Now, that may not be the case all the time. So, that is what we are going to look at. So, as you see on the screen what this this is called deviations from bimolecular kinetics.

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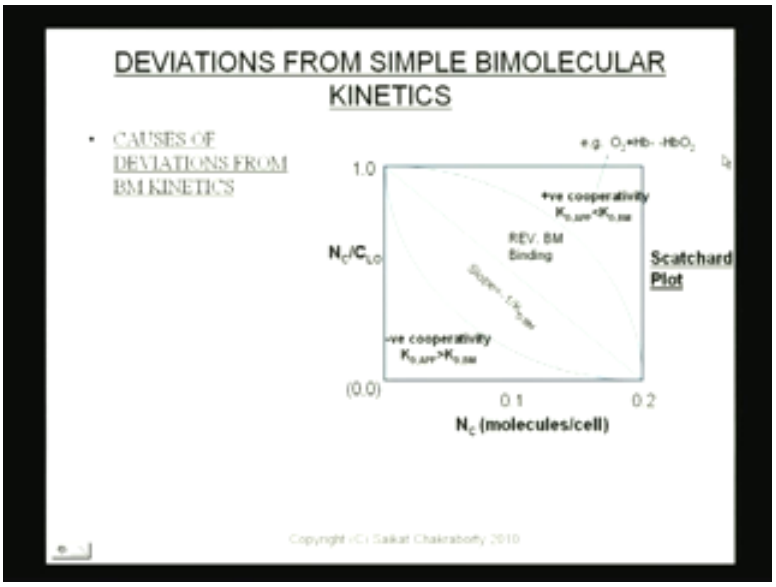

So, what we had looked at till now is bimolecular kinetics so, deviations from simple bimolecular kinetics. So, R plus L giving C is the biomolecular kinetics and this is if I am doing a scatchard plot again this is a scatchard plot. So, N C over C L naught versus N C. So, this is scatchard plot and this scatchard plot is something like linear. Just as you we plotted it here right this is this is the linear scatchard plot because N C over C L naught versus N c. So, this linear this line in the middle that you see corresponds to the

corresponds to the bimolecular kinetics. Now, this line below is this curved line and the line above this corresponds to deviation from the bimolecular kinetics. So, it does not have to be necessarily bimolecular and what we will do in today's lecture, we will study that why could it does it not have to be necessarily bimolecular? If it is not bimolecular what is the options how does it react? And try and understand this and this is known as cooperativity. Positive cooperativity and negative cooperativity and in earlier course you know we had studied this if you remember that oxygen plus hemoglobin you know.

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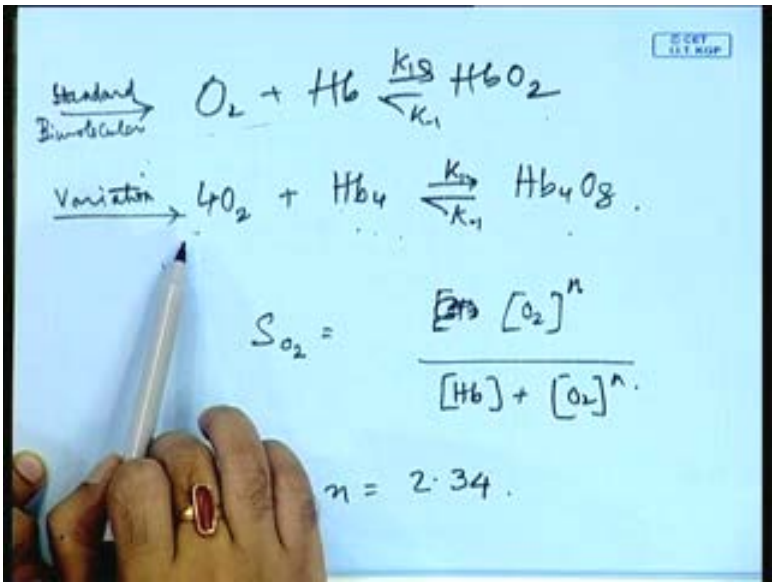

So, this was the reaction that we studied oxygen plus hemoglobin is H b O 2 in one of the earlier cases and what we figured is that 1 can assume it to be 4 O 2 plus H b 4 because hemoglobin comes as a molecular for H b 4 O 8 K 1 K minus 1. So, this is very important physiological reaction. As you all know the reaction between oxygen and hemoglobin and that is how oxygen is carried into our blood through oxyhemoglobin and this is the formation of the oxyhemoglobin. So, this is what is written standard. This is a variation on that. this is the variation on that. So, this is a standard bimolecular form. This is bimolecular. standard bimolecular form.

So, this is assumed that one molecule of oxygen reacts with one molecule of hemoglobin that that was the assumption. But then later it was found out that these hemoglobin molecules remain in cluster of four. So, therefore, one molecule of oxygen cannot react with the cluster of four. It has to be four molecules of oxygen reacting with the cluster of

four. So, the bimolecular form is no longer preserved over here. So, it is one molecule of haemoglobin reacting with four molecules of oxygen essentially.

But in reality, this is also a hypothesis. In reality what happens is that, when you write the oxygen hemoglobin equation you find that if I am to write it in a certain way say for example, S o 2 that is the saturation of oxygen it turns out it goes as O 2 to the power N divided by this O 2 to the power N something like this. So, where N equals 2 point 1 or 2 point 3 or 2 point 3 4 something like that which means that it is neither it is neither 4 molecules nor 1 molecule, but some fractional molecule reacting with hemoglobin which what does it mean? What does it implies that that the fractional molecule 2 point 3 4 molecules of oxygen are reacting with hemoglobin? Of course not. What it means is that, there is co-operativity between the molecules. Co-operativity means that the affinity towards.

So, first say for example, so, you have the hemoglobin which consists of four 4 4 of molecules and you have together cluster of it and you have one oxygen combining with the first hemoglobin molecule. What follows is after that, there is a certain change of affinity of that process of the of the system which means that the second oxygen molecule may not be attracted towards the second hemoglobin molecule as much as the first one was or may be more attractive. Whatever is the case. In most cases this is less attracted or more more attractive whatever is the case, but the point I am trying to make over here is that there is a change in the affinity.

So, for the first molecule, it reacts as if it is simple bimolecular. The second molecule in in the presence of the first reaction having occurred does not react any further as if it is simple biomolecular. There is an alteration in the thermodynamics of the process. There is the alteration in the basic which leads to the alteration in the basic kinetics of the process.

So, this is known as co-operativity that having one of them having reacted in the vicinity if you have gone to have the second reaction. The second reaction may be more favoured or less favoured. And they form, that you come up with the concept of the positive cooperativity or negative cooperativity. Positive cooperativity means there is a positive that is a more favoured effect. So, positive co-operation between the groups of molecule.

So, the presence of the first group for the first complex actually facilitates the formation of the second complex. Negative cooperativity means the presence of the first complex actually retards the formation of the second complex. So, this is the concept of co-operativity is that clear? I think for many of you it might be a new concept, but the point fact of the matter I am trying to you know trying to convey over here is simple. It is that you have for most cases we assume a simple bimolecular kinetics. But, when clusters of molecule react with each other then simple bimolecular kinetics is no longer preserved, not necessarily preserved. You can have the first reaction the first set of reactions favouring or hindering the second reactions.

Similarly, once two clusters, two 2 reactions have occurred for example, in the case of haemoglobin once two reactions have occurred, the second set, third set of reactions may be favoured or hindered. So, depending on that you can have positive cooperativity or negative cooperativity. And if I go back to this screen now you will see that this is the case. So, central line in the middle this is the scatchard plot again N C over C L naught versus N C. This is the case where there is just normal bimolecular b m represents bimolecular reversible R e v b m binding reversible bimolecular binding.

So, this is the case, but simple bimolecular binding is there. This is the case where positive cooperativity is there. K D K K D is the dissociation rate constant. So, if I go with I think K minus 1 let us see I think yeah K minus 1 over K 1 is a dissociation rate constant the dissociate not the rate constant the equilibrium rate constant, dissociation equilibrium rate constant.

So, positive cooperativity as you will, as you as I just as I showed you for the case of hemoglobin is what did I say that positive cooperativity means that the second reaction is more favoured than the first reaction. The third reaction is more favoured by then the second and so on. So, for earlier formations actually favour the later ones fine which means that the K D apparent the dissociation rate constant apparent dissociation rate constant is less than the bimolecular rate constant. Is that clear? Because if the dissociation dissociation dissociation is what backward or forward?

So, if the backward rate constant is actually lower than the than the previous case then, it means that dissociation is less favoured and association is favoured. That is formation is favoured you know binding is favoured. So, this reaction oxygen plus hemoglobin over

here is is pushed in the forward direction more for the second case. So, the third case it would be even more pushed in the even more forward direction. So, I gave you this example of hemoglobin to explain this. So, how these four molecules bind one after another is it clear. So, that is the concept of positive cooperativity.

So, positive cooperativity means that, the dissociation rate constant, apparent dissociation rate constant is less than the bimolecular dissociation rate constant. Similarly, you can have negative co-operativity and I will, we will have examples here itself where the apparent dissociation rate constant is greater than the bimolecular rate constant which or in other words it means that the dissociation is being favoured. Now, dissociation is being favoured, the association is being less favoured.

So, this is this is what we have the negative cooperativity. So, what we want to study is that as I said that what we did in the last class was a simplistic case, a simplistic case of simple bimolecular kinetics. And if you have a simple bimolecular kinetics as I showed that the slope of this is simply going to be minus 1 over K D and the intercept is going to be N R T over K D. And you can evaluate K D O and N R t, but if it is if it is positive cooperativity or negative cooperativity then what happens? These slopes are these are convex or concave in shape and then you cannot make such simplistic or straight forward calculations from the slope.

So, you have difficulty in figuring out what the apparent, even the apparent the K D apparent that you have is. So, what we will try to understand is how these what is the real chemistry behind this question of co-operativity, how does it happen? and and try and understand and quantify these phases.

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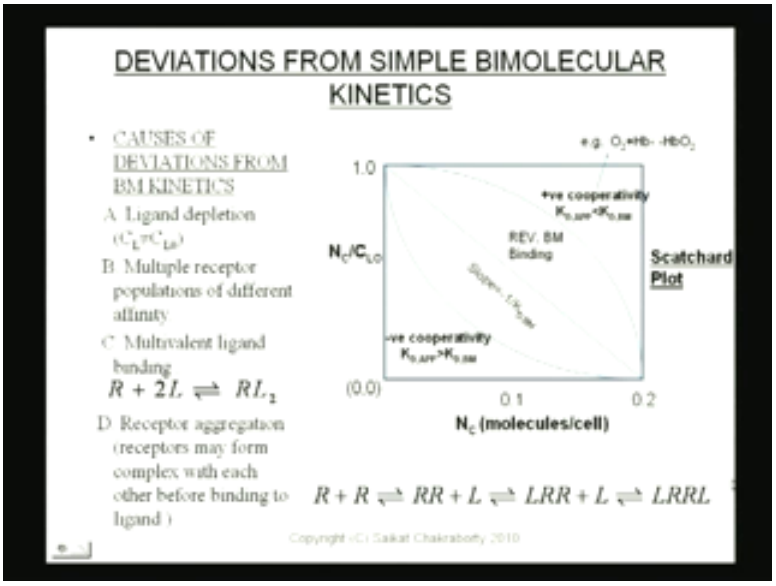

So, we will look at each of these cases slowly. So, the first case is Ligand depletion and I already talked about this in the previous class. What did we talk about in the Ligand depletion case? What is that? You assumed that the Ligand is present in present enough yeah that is present in excess, but that is not necessarily true. The Ligand is obviously, going to be depleted unless you have lot of Ligands. So, in real cases the Ligand depletion can occur. So, that is case A.

So, that can lead to this kind of deviations from simple bimolecular kinetics right. The second possibility is that the multiple receptor populations of different affinity. So, which means that, the same Ligand can bind to multiple receptor. You can have 2 kinds of receptors 2 populations of receptors; R 1 and R 2 and the same Ligand can bind to these multiple receptors. So, in which case there is; obviously, a deviation from the simple bimolecule still might be bimolecular, but each of these might be same bimolecular, but the simple one Ligand one receptor bimolecular kinetics is no longer there. The third case is multivalent Ligand binding which means that earlier we had one Ligand binding to one receptor one Ligand molecule you can have more than one Ligand molecule binding to one receptor molecule.

So, that is the third possibility and the fourth possibility is receptor aggregation. So, in receptor aggregation what you have is receptors themselves can aggregate in form of complex before they bind with the Ligand. So, receptors may form complex with each

other before binding to the Ligand. So, as you see over here an example is given. So, two receptors R and R they bind to form RR and then they react with the Ligand L to form LL LRRL and then they again react to find another Ligand L to form LRRL. So, what. So, this is exactly similar to the hemoglobin thing you know. So, hemoglobin hemoglobin what happens is essentially if I can write this or if I have to do this four 4 of them.

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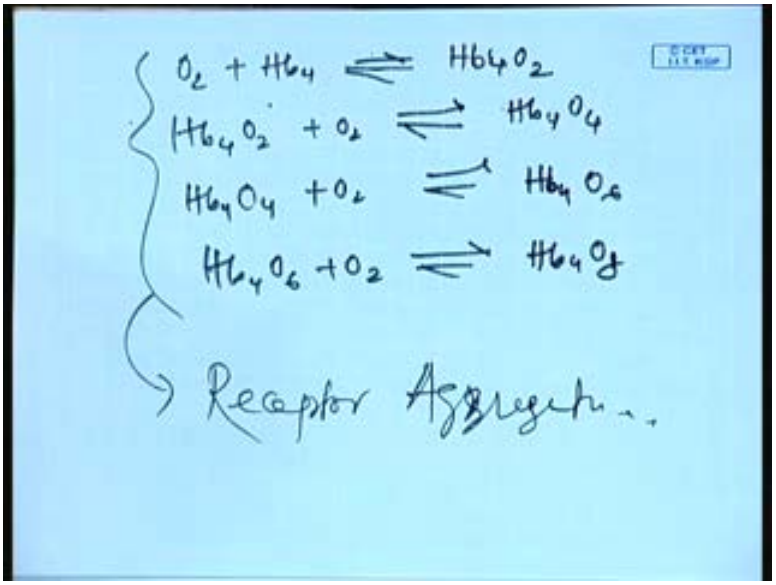

So, this is how it happens. H b 4 O 2 then, H b 4 O 2 reacts with O 2 again this is exactly that that thing. So, it is like a Ligand aggregation thing or receptor aggregation thing is O 4 then H b 4 O 4 plus O 2. So, this is how it happens. So, you have the, say let us say this is the receptor for your case. So, you have four of those receptors aggregated together and the Ligand come one by one and bind to them.

So, this is receptor aggregation. So, so go back to the screen. So, this is this is what you have. So, these receptors have aggregated and it does not have to be two. I gave you an example of two. It could be three receptors, four receptors here. For haemoglobin you can imagine the four receptors being aggregated. So, four receptors may be aggregated then the Ligand will come one by one and bind and finally, we will still have the one receptor for one Ligand.

So you will have R N L N that kind of molecule. So, the molecule formed over here is R N L N where N equals two, but you can have N equals 3 4 and so on. But the point that I

am trying to make is that when you have these kinds of in terms of molar ratio you can still have the one is to L ratio of R N L. In the final thing, but the kinetics changes whenever you do something like this. This kind of aggregation occurs the kinetics is no longer simple bimolecular kinetics. So, that is the thing that I am trying to convey you over here. So, let us look. So, what we will do is we will you had these four different cases.

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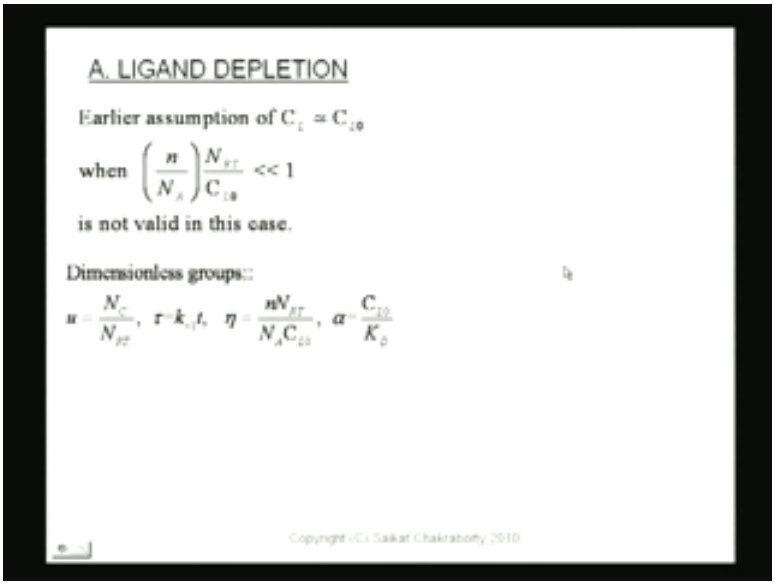

So, I will try to look 1 by 1 at all the cases and I might leave out some and which might give give an assignment. So, the first case I gave was, talked about was the Ligand depletion. So, let us assume that Ligand depletion does not occur we and does occur. So, we had an initially assumed that Ligand depletion does not occur or what how did it help us not assuming that Ligand depletion occurs.

Reduce the order of (( )).

Reduce the order of kinetics from second order to first order easier for us to solve now what will happen? It is very straight forward. We will simply have a second order kinetics if I go back to the screen. So, my earlier assumption was that C L equals C L naught because this term was very valid, very small and this is no longer valid. So, what I do over here is let us go back to the equation otherwise you will not remember here sorry here this is this is my equation after I had I had converted all the concentration into numbers.

So, this is the equation I have. So, what I want to do to this equation; I want you to you know do this on your copy, may be you write down this equation and unless you do this quick you would not be able to follow what I am trying to say. So, write this equation for and D d T of N C equals K 1 N R T minus N C times C L naught minus N over N a N C minus K minus 1 N C done.

So, So, this becomes the second second order if C L naught if I do not make any assumption. But before that I want to make want you to make this dimensionless and this is something that we need later. So, now, I want to make this dimensionless using these dimensionless groups which is, u equals N C over N R T. Tau equals K inverse T K K minus 1 T K minus 1 is first order rate constant. So, the tau is dimensionless. Eta is N R T over N A C N n N R T over N a C L naught or n N R T over N a because that has the units of concentration right. N N R T, N is number of receptors per unit sorry N R T is number of receptors per unit cell N is number of number of cells per unit volume. So, N times N R T would be number of receptors per unit volume that divided by Avogadro number would be molar, molar concentration.

So, that you divide by C L naught it is dimensionless and alpha is C L naught over K D. Is that dimensionless, C L naught over K D? Yes that is because K D has K is K minus 1 over K 1 K k minus 1 has units of inverse time and K 1 has units of inverse concentration inverse time. So, K D has units of concentration fine. So, what you what do you get if you put that? If you, so, what I want you to do now is, put these dimensionless constants in to your equation the equation four that you wrote.

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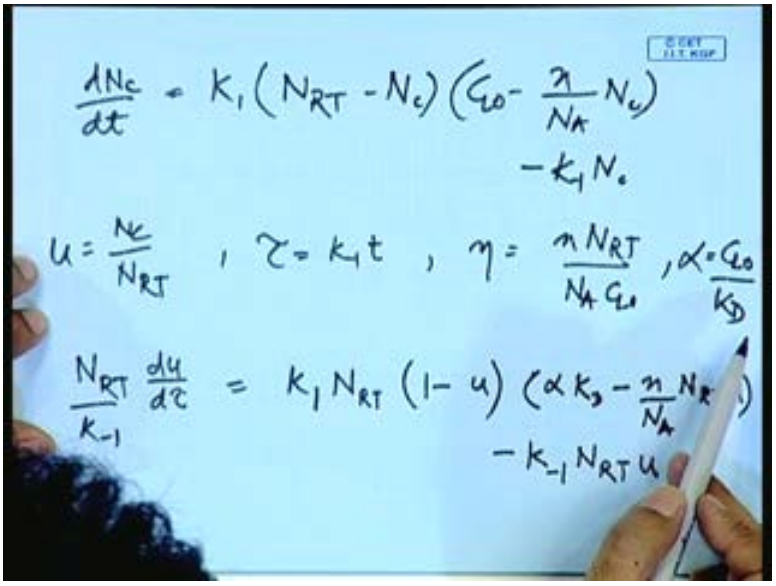

Equation 4 was so, then u equals (( )) naught over K d. So, this is N R T del u del tau N R T over K minus 1 equals K 1 N R T 1 minus u and C L naught minus K minus 1 times N R T times u. Then it should be alpha K naught K D. Then, if I cancel if I cancel N R T K minus 1 all through equation.

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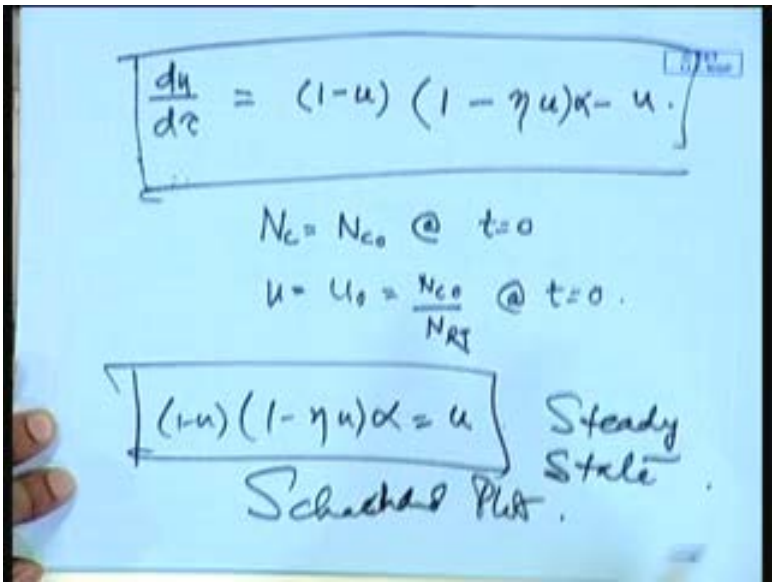

Then you will get del u del tau equals 1 minus u u (( )) alpha K. Alpha will come in the outside here if we checked it. So, you have K 1 N R T is this what I is this right or is there some term missing here?

(( )) will be K minus.

Which one? This one? This side.

Yes sir.

Into K minus 1. No yeah that is why it will come in the denominator then and then you'll have K D over here from that yeah into K minus 1 and this is correct right alpha alpha D over this. So, basically what I have to do is I have to actually it might be it might be better to just keep this as C L naught instead of alpha D and then divide the whole thing by C L naught yeah. So, so then I think you will get alpha yeah because. So, you divide the whole equation everything by both sides by K minus 1 N R T C L C L K minus 1 N R t. So, this side it will cancel out you'll get K minus 1 N R T over here. So, let me do that. So, you'll get del del u del tau equals K 1 K 1 over K minus 1 which is 1 over K D into 1 minus u and this is C L naught minus N n R T C L naught I can take out. So, this will have 1 minus eta times u right minus u fine.

So, and this will give me alpha. So, this is 1 minus u times 1 minus eta u times alpha minus u all right. So, then you will have. So, this is the basic equation and I now need to solve. So, what is going to be my initial boundary conditions and so, on initial or boundary condition whatever it is. So, initial my initial condition earlier was that N C equals N C naught at T equals 0. So, now, if I put back into my dimensionless variables, I will have u equals u naught equals N C naught over N R T at T equals zero fine. So, this is my equation that I now need to integrate. So, these are you need the steps. These are the steps. So, so these are the steps and. So, these are what I got and this is what I need to integrate now.

So, how do I do that? We discussed this already in the last class. You can use that use partial fractions and integrate it and because this is the second order and it is already in the form you can break this up and get one full partial fractions in u and then it would be two exponential fine and we will do that in a minute. Before we do that we are going to do something little above which is the steady state solution of this. So, this thing that you have over here we will look at the steady state. So, the steady state is simply 1 minus eta u alpha equals u steady state fine. So, why are we looking at the steady state? So, that we can do they do the scatchard plot (( )). So, let us now go to the screen yeah.

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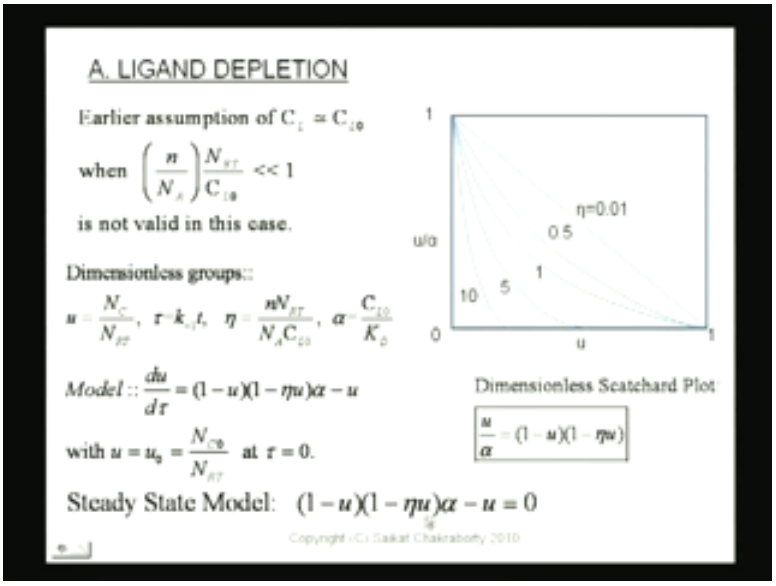

So, so what we have over here is the scatchard plot. So, this is u u over alpha equals 1 minus u times 1 minus eta u is the scatchard plot and this is how it varies. So, as you can see over here, what we have here is that two variables are here. So, one is alpha and the other one is eta. Two parameters sorry u is a variable and we have two parameters in the system. So, earlier we had how many parameters we had we had plenty of parameters actually we had the forward rate constant backward rate constant N n R T N a C L naught plenty of parameters. So, six parameters we had.

What we had been able to do through these dimensionless these dimensionless numbers and groups over here is that we have been able to reduce the number of unknown parameters from six to two because it is always hard to be able to evaluate these unknown parameters and now we have been able to reduce them from six to two.

Now, let us look at this expression carefully u over alpha equals 1 minus u times 1 minus eta. You got this expression one of these say steady state plus. Now what is alpha? Alpha is is. So, this is as I told you just a little while back that this is scatchard plot and there is no need that to assume that scatchard plot is simply going to be N C over C L naught versus C L naught. All steady state plots that involves the complex concentration and the C L naught that is a Ligand concentration and the complex concentration are scatchard plots. So, this is also a scatchard plot and there is no need to presume the scatchard plots are only going to be N C over C L naught versus C L naught. So, if you look at this plot

what is u over alpha look at the dimensionless variables given on the screen. So, u over alpha is N C over C L naught times K D over N R T.

So, it is essentially again N C over C L naught. So, the original scatchard plot had N C over C L naught versus N C. So, this is also N C over C L naught times some parameter and what is u? U is N C over N R t. So, simply it is the same kind of thing. Now, what is eta? That is something you have to pay a little attention to it. Eta is N n R T over N a over C L naught. So, what does this eta signify to you just look at the screen and tell me what does this eta signify to you? And the answer is right there in front of you on the screen. So, you just have to look and tell me. So, just look at these plots over here for different values of eta what does it signify?

It signifies about the (( )).

About what?

Concentration of Ligand.

Yes. So, essentially look at eta eta is N R T N a over C L naught. So, N R T N a N n R T N a over N a is fine, denominator a of C L naught which means that if C L naught is very large if the Ligand concentration is very large eta goes decreases and it goes to 0. So, as you see over here that is why I purposely drew this for eta going to 0 point 01 it goes back it collapses to the linear first order kinetics and for eta larger larger and larger more is a deviation from the first order kinetics. That that is all I wanted to say over here. That is the reason I drew this scatchard plot. As I just wanted to convey the fact that if you go away from eta from eta equals 0 your deviation from the simple linear kinetics is more and you have this kind of things.

So, that is the scatchard plot now can we this model that we over here yes here. sorry This model that we have over here we can we integrate it in time of course, we can as I said through partial fractions and we can get the temporal variation. Also, we are always more interested in steady state concentrations of scatchard plots. The reason I just mentioned this because they are easier to quantify.

Experimentally scatchard plots are easy to draw because you can just leave the system as far as long as you want in your, let let it I you know let let the experiments happen for

four hours, six hours, eight hours, ten hours. And at the end of which you can go and collect where as if you want to do a dynamic study you have to collect at every time point you know, every thirty minutes or twenty minutes you have to collect and it makes it difficult.

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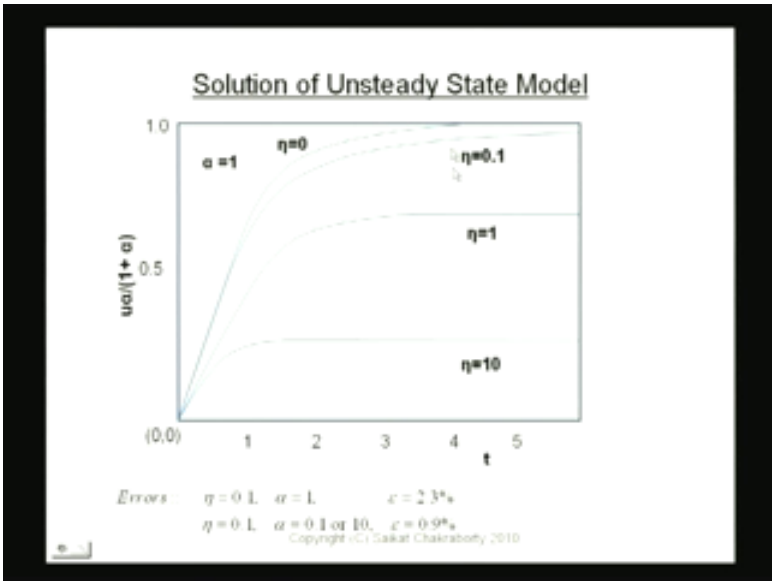

So, this, but again you know you can always do that. So, again this is this is this is the solution of the unsteady state model and what I have done over here is, this is the case for eta equals zero which means that simple linear kinetics and these are the deviations from that and as you see that as you keep increasing eta, the deviation keeps increasing more and more. And also, the deviations in dependent alpha two, but here I think this is small typo over here. excuse me errors are are the errors errors means basically deviation. These are not errors, these are deviations as you look on the screen at the bottom of the screen here I think this is typo.

So, for eta equals 0 point 1 the error is 1 percent and eta equals point 1 no I think it is sorry the typo is eta equals zero point zero 1 the the deviation is 1 percent and for eta equals point 1 the deviation is around 3 percent. So, these numbers and then as you keep increasing your eta is going to see, that the huge difference this is almost what forty percent difference and this is error is around 40 percent not error is essentially these are differences. So, this forty percent and this could be around seventy percent or so. So for so, what this is trying to show is that the that near eta equals 0. So, this I am sorry this

this 1 eta should be eta equals 0 point 01. So, for that the deviation is 1 percent for eta equals 1 and the deviation is 3 percent.

So, the next case we look at. So, if you go back and looked at the four, look at four different cases was. First one was Ligand depletion, second was the multiple receptor and third one is multivalent Ligand. So, the next case that we look at is the multiple receptor two or more receptor populations. Now what do you what does your intuition tell you you know. So, the first case we did was very straight forward where we had the equation and the model and all we needed to do is fiddle around with the C L a term. But this we do not have a model for for multiple receptors. So, if I am to if I have to ask you to write a model. So, what does your intuition tell you? How would you write a model for multiple receptors? You have one set of Ligands, but let us say two set of sets of receptors. How would how would you do that? One possibility and the simplest possibility is that the receptors would not interfere with each other where they bind which means that receptor one binds independently with Ligand and receptor two binds independently to Ligand. That is the that is a possibility.

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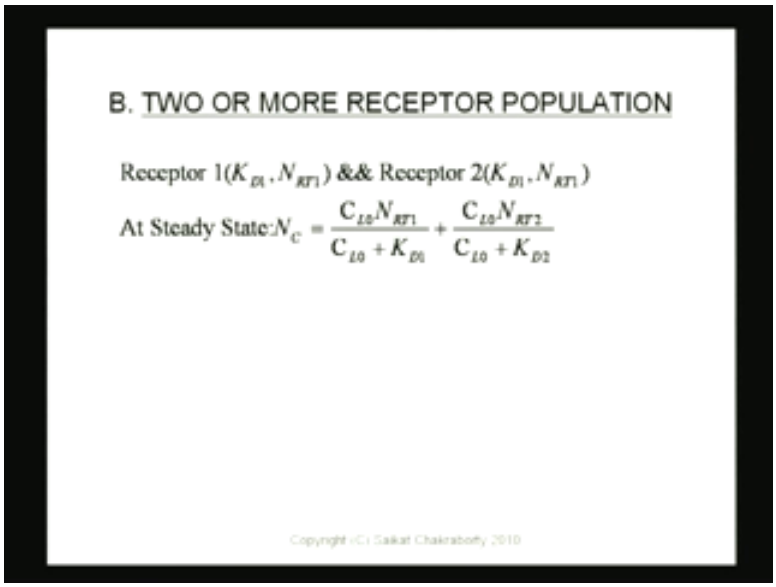

So, this is this is the possibility that we explore over here where receptor one and receptor two bind independently to each other. And if they bind independently, how do you, how do you model it? The model is still not there on the screen. So, how do you model it? Let us go back to the old model and then you can tell me how you are going to

model it. This is this is the old model I have right. Now I tell tell you that very very clearly that the receptors do not interfere with each other. So, what will happen?

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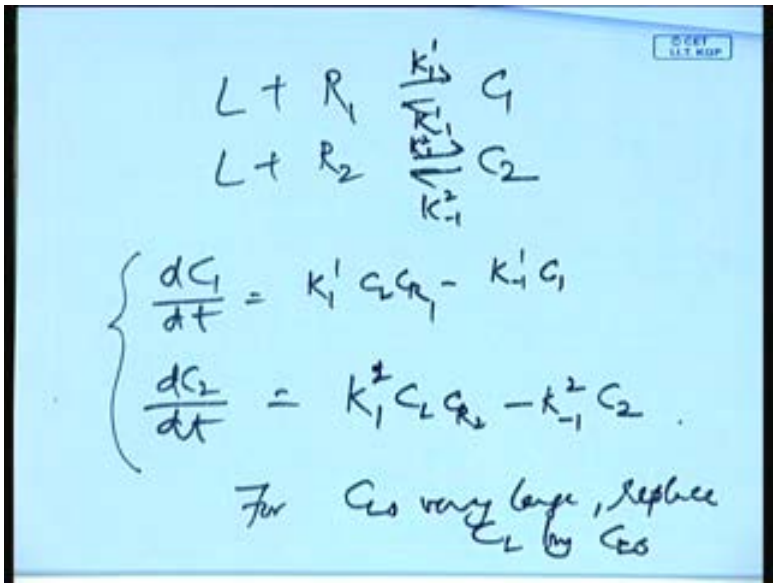

So, you will have these two sets of reactions L plus R 1 giving C 1 and L plus R 2 giving C 2 right. So, D C 1 D T would equal. Let us say this is K 1 1 and K 1 minus 1 and K 2 1 and K 2 minus 1 fine. So, then this will be my K 1 one C L C R minus K minus 1 1 C C 1 and D C 2 D T would be K 2 1 C L C have R 2 minus K minus 1 2 C 2 over.

Now, if the receptors so, from these set of systems are these two inter dependent on each other? This is a question you have to answer a and under what circumstances they are not really inter dependent? b Two questions. Are these two equations that I wrote in a curly brackets here are they dependent on each other? Yes they are because through C L what conditions does they not inter dependent?

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Large if there is no Ligand depletion if Ligand is in excess then C L each of these C L you can write it as C L naught over here. So, for C L not very large, replace C L by C L naught fine. We can replace C L by C L naught and then what happens is these two systems become decoupled. So, typically the Ligand concentration is more or less large if it is not large then what what you are suppose to do? You are suppose to solve these two coupled equations together. The two coupled equations that you have on you have

here you have to solve these two together that is the possibility. I might give you a question like that, but if the Ligand is large enough then, you can solve, decouple them you can solve them separately and what will happen you will get the same solution for each of them right. So, the maximum N C max that you that you got previously depended only on one Ligand concentration and one receptor concentration concentration.

Now, they are going to be two of them and you can add them up if these two are completely independent then what you see over here is not complete independent. But if the Ligand concentration is very large then, they become completely independent you allow each of them to react in their own way get the steady state value and you add them up. That is that is a possibility, but that is not the always the possibility that is one of the possibility that can happen. So, this is the case where each receptor acts independently.

So, as you see on the screen the first part C L naught. So, this is this is the case where everything is C L naught. So, there is no Ligand depletion at all and C L naught N R T 1 over C L naught plus K D 1. That is the first one and then C L naught N R T 2 over C L naught plus K D 2. Now, I I tell you one thing I give you an assignment you can do it in and submit it you are suppose to submit one assignment today.

No whatever. You submit the assignment you know, whatever you did you submit the assignment and I gave you this assignment for the, for next week. I I probably add in another problem tomorrow. So, this assignment is this is the case where we consider two or more receptor populations. Previous case what did we assume Ligand depletion. This case there what we have done here there is no Ligand depletion. What I want you to do is, couple these two cases a and b. That is Ligand this depletion plus two or more receptor population's fine. And then get the model for that and the solution and come up with the steady state. Is that clear? You can write, all of you down yes.

So, first case we did was Ligand depletion. Second case we we are doing now is two or more receptor population I want to, want you to couple these two and come up with the model. I model, I already wrote, but you solution of the model essentially and the steady state value of two or more receptors in the presence of Ligand depletion. Clear?

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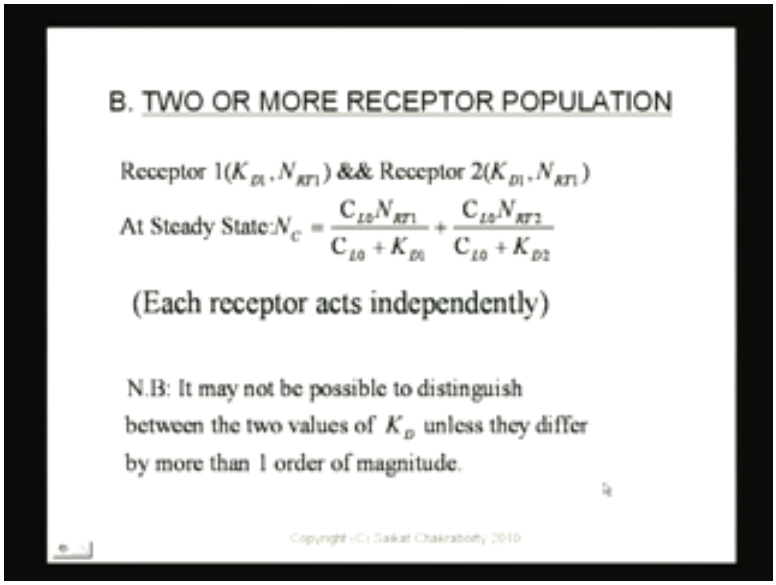

So, then the receptors cannot act completely independently like it does over here fine. So, even here when you have a steady state solution, steady steady state solution or steady state value for this as you see, given on the screen what happens you know, you were doing this whole experiment in a beaker with two sets of receptor, one set of receptor and let us say two sets of one, two sets of receptors and one set of Ligand at the end what you can you probably have? How do you how do you how do you measure? how do you measure how do you measure I mentioned this. By labeling the Ligands right. You label the Ligands with some sort of fluorescence or something and once the complex is formed you have the labeled complex right. So, you have one one set of Ligands and two sets of reactor receptors and you have labeled your Ligands for example.

Now, the complex is formed you can get the total amount of complex that is formed at steady state right. But it is going to be very hard to be able to separate out the kinetics of these two. Why is that? Because what you will see at the end of the day is a labeled labeled Ligand. Now, there is a way when you draw the scatchard plot you may be able to separate out these two the kinetics of these two reactions. That is reaction of Ligand with receptor one as against reaction of Ligand with receptor two. You may be able to separate it out through scatchard plot, but only in the case when these two are very dissimilar or the kinetics of these two are very dissimilar. What I mean by dissimilar is that for example, one of them for example, is very slow, the kinetics of one of them is

very slowest compare to the other then only can you can you separate them out and we will look at this now.

So, is as I said and it is the on screen that it may be may not be possible to distinguish between the two kinetic constants, the dissociation constants here unless they are very different from each other and by very different at least by one order of magnitudes. So, one has to be ten times larger than the other at least.

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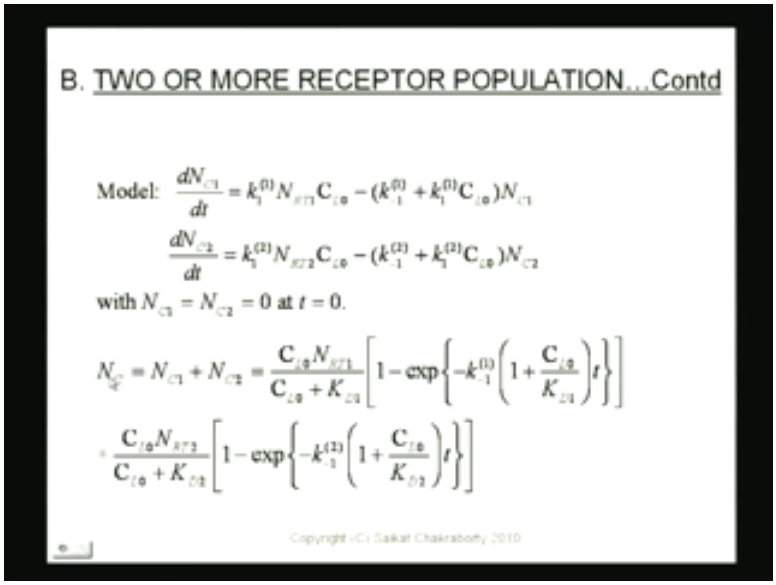

So, this is this is the this is a this is the thing you know, with the assumption that this is not what you are suppose to write the model. But this is the model with the assumption that Ligand depletion does not occur. So, then these two equations become independent with each, to each other and you can solve them and this is the solution you get. So, this is the I want you to get same kind of solution, but with these two coupled systems and yeah.

So, yeah I think these are the second order coupled equations. You probably have to use what do you think would you be able to solve this analytically the equation that I if I can go to this page over here? This this thing that I gave you this these two equations do you think you can solve it analytically? Here we could solve it analytically the one that we did. But do you think these these two we would be able to solve analytically? Just as a hint you know to help you with the process. Do you think you can solve this analytically? Or numerically of course, you can solve second order second order system,

but what about analytically can you solve this? Once you put you have to go back and put your N a and all that stuff you know, convert this into numbers like we did did all the process, but after that what if it is a single second ordered equation you can solve it analytically.

Now, I have coupled the system then what? I showed you last the single Ligand depletion case. Can you or can you not? Anyway, let us not debate over that you do that as an assignment, but for this case where Ligand depletion does not occur you can get an analytical solution. So, each of them becomes like what we got before and this is an analytical solution. Remember the initial condition is given as N C 1 equals 0 and N C 2 equals 0. So, that any other term that was say drop out and you have these two.

Now, so, but what I am, I was trying to tell you is experimentally you cannot separate out N C 1 from N C 2. Is that clear? When you are measuring you can only measure N C because you would be able to get the labeled Ligand. Now, how do you separate these two rate constants? Then, if you can only measure and see and how then what is the way to separate out these two rate constants? The way would be to look at the slopes and we will do that in in the next few minutes. So, look at the slopes and look at if there are differences in the in the slopes and what you will find is that, if if one of those dissociation constants is ten times other then there are differences in the slopes.

So, this is how it looks like the diagram that you see on the screen. So, this is the dynamics of the process. So, this is this equation the final model that I wrote over here that one solved. So, this is the case of a single receptor. This is the old solution solved sorry. This this one solved and and this this this one is the last one, the two points.

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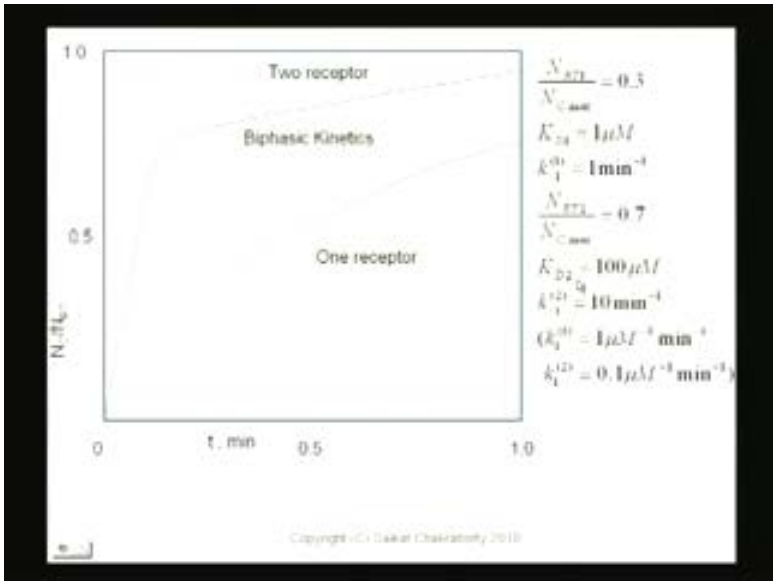

So, as you can see over here that with two receptor model what we have done over here is K D we have taken a system where there is a large difference in the dissociation constant. We have purposely taken this where there is a two orders of magnitude difference one is hundred times the other. As the result what happens you see a very distinct slope, difference in slope. Can you explain why this is happening? The and This curve is the x axis is time, the y axis is the total amount of complex that has been formed and x axis is time.

Now, you have to tell me. So, given the value the K D 1 and K D 2 values are given. So, you have to tell me that what is happening? May be we stick to the go back to the screen and look at the screen here. So, what is happening over here you know, these two slope being very different, why are the two slopes very different? And what can you infer from the difference of slopes? K D 1 is K D 2 is hundred times K D 1 what does that mean? you know Not that complicated.

(( )).

Which 1 you have to tell me specifically not one of the term. K D 2 is dissociation rate constant. So, if K D 2 is higher which means what? K D 2 is higher; much much higher than K D 1. Which means out of receptor one and two what is the process what is really happening here? Let us forget the, look at the curve because it is important to look at the curve.

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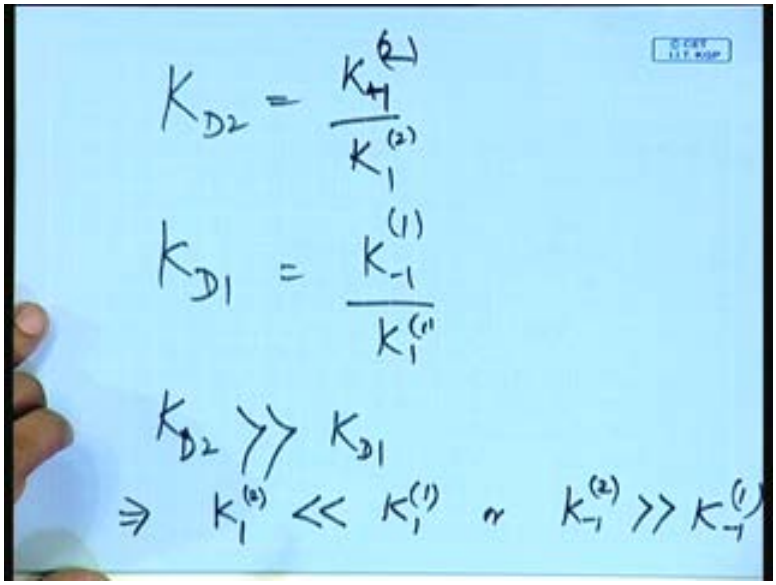

But from the curve, let us try and understand what is happening, what is the process that is happening here? Say K D 2 is much, much higher than K D 1. K D K 2 K minus 1 let us say 2 over K 1 and K D 1 is K minus 1 1 over K 1. So, if K D 2 is much, much higher than K D 1. It implies that K 1 2 let us call this and K 1 1.

So, one possibility is that K 1 2 is much, much lesser than K 1 1 or K minus 1 2 is much, much greater than K minus 1 1 whichever way. What does it imply? That what does it implies here that the second if second association rate constant for the second is much, much lower than the first. So, as soon as you put the whole stuff in there you know, into your beaker the receptors are there and you put your Ligands in there. What, what starts to happen is that receptor one starts to react. Receptor one starts to react with the Ligand and then if there is a large difference in magnitude. So, receptor two hardly reacts with the Ligand. So, receptor one starts to react dynamically. This is what happens receptor one starts to react with the Ligand and then it saturates out.

So, all the receptor ones receptor one that are there are being combined or reacting with the Ligand. They are being taken up and complexes are been formed still a point where no more receptor one is available is that clear? So, till now till a point where no more receptor one is available and then the receptor two starts to react with receptor one with Ligand. So, if there is a hundred to 1 ratio then if I go to the screen and we will see if there is a 100 to 1 ratio then what happens? When this this is reacting for the first

receptor one is reacting. So, ninety nine percent of the receptors that are reacting and are receptor one and only 1 percent are receptor two and that continues more or less till steady state is b.

So, this time over here, whatever the time is let us say 0 point 1 five minutes of a something one 0 point 1 minute whatever the time is, is a time when the first process that is receptor one combining with the Ligand has more or less reached the steady state and then the second process starts. So, ideally what would happen is, if there is a one receptor only receptor one would be there. This will saturate out over here, flatten out over here, but that does not happen because that is second receptor staying there, standing there and that starts to react and form this.

So, this difference in the slope can help you figure out what the constants are and as you can see over here look at this numbers N R T 1 over N C max is point 3 and N R T 2 over N C max is point seven. This is very intuitive. The reason being that N R T 1 plus N R N C max is what the maximum number of complex being can that can be formed. The maximum number of complex equals the total number of receptors that are there if there are, no other resistance is in the system. The total number of receptor one at the limit at best can what can happen is total number of receptor, all the receptor one can react which it does and also all the receptor two can also react which may or may not happen, but all receptor one will definitely react.

So, if that happens then N C max will be N R T 1 plus N R T 2. As a result N R T 1 over N C max is zero point 3 and this is point seven and the summation of these two would be one or in other words N C max would be N R T 1 plus N R T two. So, the total number maximum number of receptor complexes that can be formed is a sum total of the two kinds of receptor.

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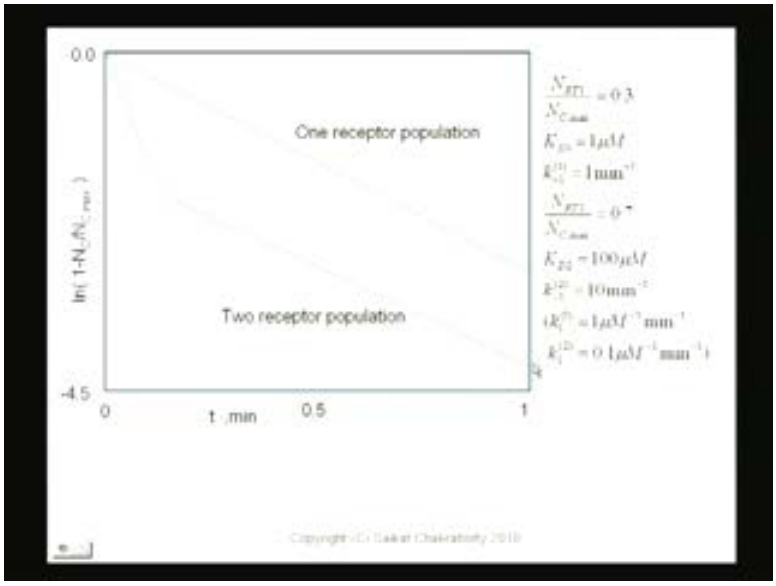

Now, the last thing we will do today is look at this Scatchard plot over here sorry not this scatchard plot. This is the still the unsteady state plot, but this is written done in terms of 1 minus N C over N C max why 1 minus N C over N C max because, if you if you look over here this there is this terms over here this constant terms over here and if you do the 1 minus N C minus N C max and what will happen is that you will get the exponential parts and you can take the log of that.

So, is the same kind of variation as you see exactly the same thing it is only that the plot has reversed itself because it is in the log scale and you know minus and all that. So, you have 2 diff, varying slopes and from these two varying slopes you could be able to you would be able to separate this thing out. Now what I want you to do is quickly of again we have a class 1 more class today.

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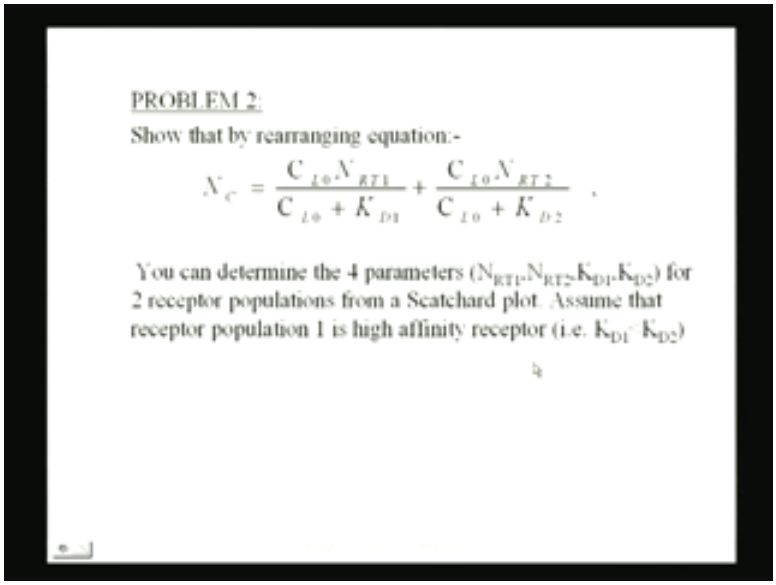

But write write down problems for now and this should be a second problem for assignment. Show by rearranging the equation N C equals C L naught N R T 1 over C L naught plus K D 1 plus C L naught N R T 2 over C L naught plus K D 2.

So, this is essentially N C max 1 plus N C max 2. Show that by rearranging this equation you can determine the four parameters N R T 1 N R T 2 K D 1 K D 2 for two receptor populations from the scatchard plot. Assume that one of them is matching. So, you can assume one to be hundred times I had in the other. So, what I did I showed you the scatchard plot and I all I am trying to tell you is that how can you exploit this this steady states steady state equation that is there to get how can you exploit this to get the four parameters N R T 1, N R T 2, K D 1 and K D two. So, we will stop here and we will continue later today.