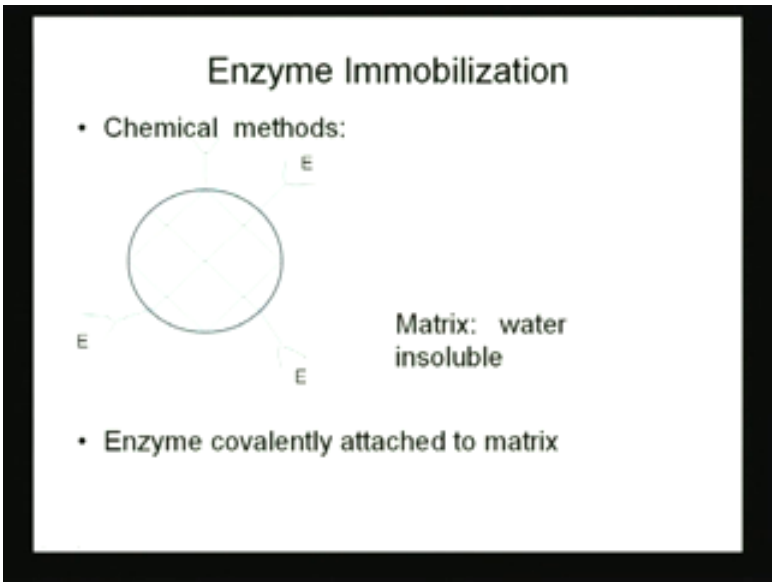
Biochemical Engineering
Prof. Dr. Rintu Banerjee
Department of Agricultural and Food Engineering
Asst. Prof. Dr. Saikat Chakraborty
Department of Chemical Engineering
Indian Institute of Technology, Kharagpur

Module No. # 01
Lecture No. # 13
Immobilized Enzymes

Welcome to the next lecture in the series of lectures in Biochemical Engineering, today we are going to start discussing Immobilized Enzyme, so the enzymes that we discussed in the previous classes are kind of enzymes, which are mobile you know, so that you can call them mobilized enzymes. So, the say, so these enzymes are in the liquid phase, this was something that we were discussing at the end of last class, so these enzymes are in the liquid phase substrate in the same phase and they they are you know swimming around sort of floating around and then reacting with a substrate.

So, today we are going to discuss immobilized enzymes which which is at the enzyme is immobilized in a certain phase and the substrate is most likely in another phase. Now, as a as a prelude to this, if you remember in the at the end of the last class, we started discussing, what is known as heterogeneous enzyme catalysis, so as a post homogeneous enzyme catalysis, which is what we did in the last 5 lecture. So, that that is where the spirit of immobilized enzyme lies, which is the case that the enzyme in the certain phase, solid phase or something immobilized and then the substrate is in another phase.

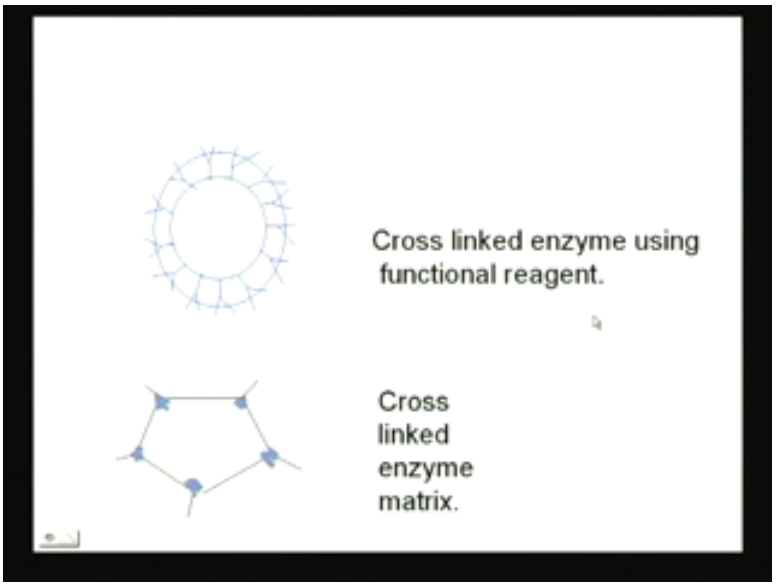
(Refer Slide Time: 01:23)



So, let me show you how immobilized enzymes look like, so this is, so this chapter that we are starting today is enzyme immobilization, and there are several methods in immobilization that, we will briefly discuss before we go on to the understanding of the process, so there are chemical methods as well as physical methods.

Now, see this is what you see on this screen is an example of a chemical method, where this enzyme that you that you see over here, given by E shown here, is covalently attached to the matrix, so this is the matrix and this matrix is typically water insoluble, why is it water insoluble, because because for the enzyme to remain in that state, the matrix should not dissolve into water; if it dissolves, then the immobilization would all be gone, so if you once the enzyme to remain immobilized this has to be water insoluble matrix. So, this is the chemical method, why is it chemical, because enzyme is actually covalently attached to the matrix, so that is why it is chemical method.

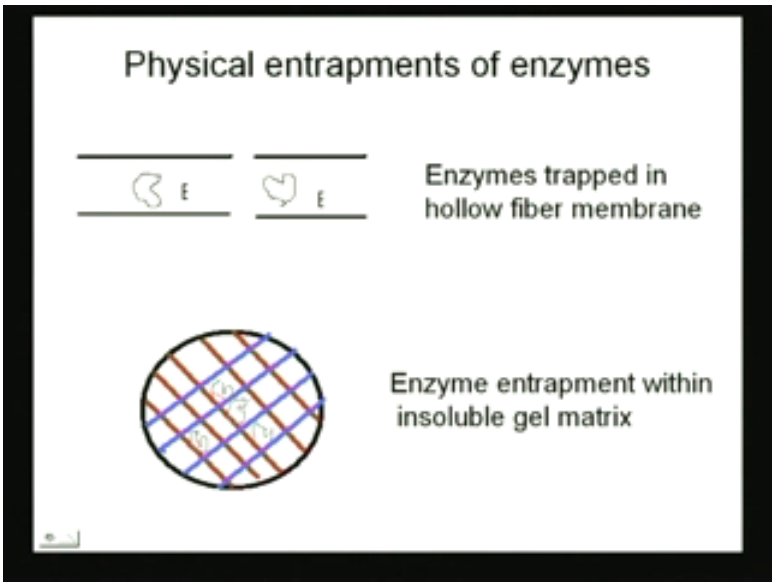
(Refer Slide Time: 02:21)



Other chemical methods of you know enzyme immobilization are cross linking of enzymes, this is lot more if you look at, if you compare with this one, this is the lot tighter arrangement in the sense that, the enzymes are cross linked as I as is more or less obviously from picture itself, you compare this with this, you see then the enzymes are cross linked with the functional reagent, which allows it cross linking to the these are there is the matrix itself is a functional reagent embedded in it, which will allow the matrix, will allow the enzyme to cross linked to it.

As a result it will be a much much more tight arrangement and then you can have a possibility where the enzymes look at this, the bottom one here, where the enzymes themselves cross link among each other from the matrix; so this is a difference between this and this here the enzyme is attached or or or or bound to a functional reagent and that that how the cross linking is attained while below the enzymes themselves cross link with each other, to form the cross linked matrix. So, these are few examples, and if you go to some of the books you will find plenty more examples that, just to give you a sense of this, how this chemical chemically bound enzymes look like.

(Refer Slide Time: 03:39)



Now, there are other kinds of entrapments or other kinds of enzyme immobilization which are known as physical immobilization, and one example of physical immobilization is that you you trap the enzymes in hollow fiber membranes. In this hollow fiber membranes are really very thin thin cylinder sort of, if you think of them they are like a bunch of cylinders like shell in tube heat exchanger except that the tube size is really really small, and so you entrap the enzymes, enzyme being small themselves you entrap the enzyme within those hollow fiber membrane.

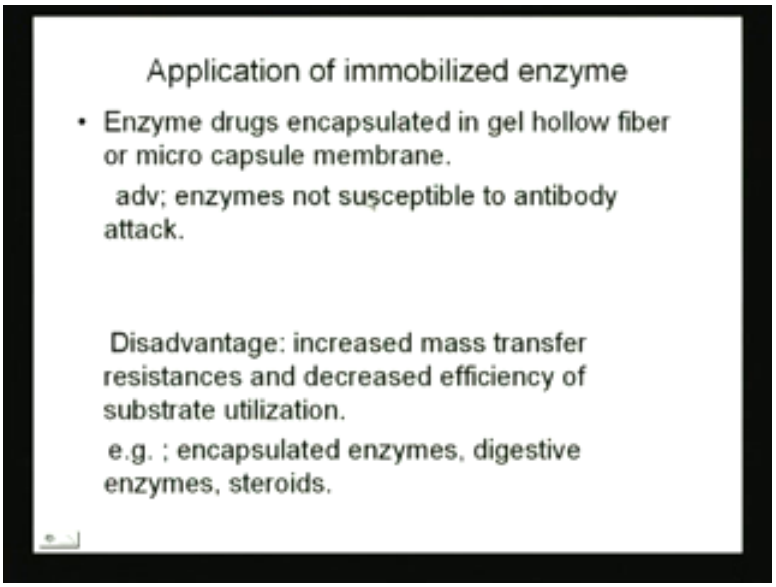
And then you are, you can have what is known as enzyme entrapment within the insoluble gel matrix, so you there is an insoluble gel matrix and you physical entrap there is no chemical bound bonding out here, and you physically entrap the enzyme and and so this is one other possible, these are these are some just a couple of examples, that I am showing you for each type. And then if you are interested in the (()) lists of the kind of chemical and physical entrapment that are possible you can always go back to the book Bailey and Olasin, is a good back book to go back to any time.

So, but the point is these are several there are several ways we can entrap an enzyme, but the question is that, why would we like to entrap an enzyme, why would we like to immobilize an enzyme, what what do you think.

(())

To prevent the law of enzyme (()), that is a good point

(Refer Slide Time: 04:59)



And what else, what what else could is a possibility to make it you know in a way it is it comes under, what you said to make it less susceptible to other kinds of reactions right, because you want to specific kind of reaction for example, and is if the enzyme is you know allowed to be attacked our through by other kinds of reagents, other kinds of substrate then your enzyme is sort of lost to reduced.

So, that that is a point and here what I am trying to say is the enzymes is a encapsulated then hollow fiber membranes or micro capsule, so that they are not susceptible to antibody attacks. So, this is specific point, that not susceptible to antibody attacks, but in general you can generalize in sense and say that, so that they are not susceptible to attack from other kinds of substrates or reagents.

What is a disadvantage, this is something that I really want you to try and understand the advantage fine you know, this is sort of easy, but what is the disadvantage of, what could be the disadvantage of this, entrapment or enzyme immobilization.

(())

Will be

(()) active sites (())

Active sites will be

(())

Well yeah not not active site would not be reduced as such directly, because number of active sites trill the fakes but but but, but what the accessibility to the active sites will be reduced?

(())

Yeah, so why is that, what what is the basic phenomena that, this the very basic chemical engineering thing that you are probably all aware of, what is the basic phenomena there.

(())

Yeah but, basically what will happen is that the adsorption is the part of what, mass transfer.

Yeah

So, essentially there will be all earlier the difference between this and the earlier things this is that, earlier we had simple reaction taking place, because it was dissolved in their their substrate was in excess and stuffs like that.

But now, what will happen is because, the enzymes say let us go back to the earlier picture see for example here, the bottom picture you see, so the enzyme is deep inside

the matrix, so for the substrate or whatever its going to react with to reach this there is a lot of mass transfer distance that it has to overcome.

Even if it is forget adsorption you know, even if adsorption is there forget adsorption first there is that diffusion resistance that has to be overcome, look at this here also if the substrate is in solution dissolved in the solution, the there is still a diffusion over resistance that is there for the solution to the substrate to overcome till it can reach the enzyme. Where as if, the enzyme was you know allowed to dissolve freely into the liquid then it could at any point of time be reacting with the substrate.

So, basically the basic difference is that there is a lot of mass transfer resistance that have to be overcome, so you you know what it says is increase in mass transfer resistance and decrease the efficiency of substrate utilizations of course, if there is increase in mass transfer resistance, there is going to be decreased efficiency of substrate in utilization.

And you know encapsulated enzymes, digestive enzymes and so on, so as I told you at the first example, I gave you on the first day itself I think that you know where are these enzymes used immobilized enzymes, the digestive pills for example, if you did not digest something well, there are two possibilities you can take a liquid enzyme, these are like carbozymes and so on, like lots of liquid enzymes are there.

And you can take a solid enzyme, which is like a tablet or a capsule, so that is the precisely the difference between a entrapped or immobilized enzyme in the in the solid form as opposed to the liquid form which is the mobile enzyme. You see the difference, so physically in terms of example is the difference, so if you take the same digestive medicine in liquid form then it is a it is a mobile enzyme, if you take it in the solid form it is a immobilized enzyme, so that is a basic difference.

So, what we will try and understand first is that, what are these resistances, so because once you, one one other thing that you understand is that, the basic kinetics of enzyme enzyme reaction still remains the Michaelis-Menten kinetics right, which with what we studied, basic kinetics still remains the Michaelis-Menten kinetics.

But, because of mass transfer resistances, the kinetics that you see now is going to the observed kinetics kinetics, that you see you know this is the very important word to remember, the observed kinetics is going to be different from the intrinsic kinetics, so a chemical reaction has two kinds of kinetics and probably you have studied this in your reaction engineering course, one is a intrinsic kinetics and the intrinsic reaction rate and the other is the observed reaction rate. So, why are these two different the intrinsic reaction rate and observed reaction rate, because of what kind of limitations.

Mass transfer

Because of mass transfer resistances, so the observed and the and the intrinsic kinetics are different, because of a mass transfer resistances.

So, what we have to figure out, so earlier the the the first chapter that we did the advantage was that there was only intrinsic resistance there kinetics, there was no observed kinetics I mean observed kinetics are in other words, where same as intrinsic kinetics right. So, the mass transfer resistances where not there here, because of mass transfer resistances observed kinetics is different from the intrinsic kinetics, and what matters to us is the observed kinetics, because the intrinsic kinetics kinetics intrinsic kinetics is a measure of the maximum possibility of reaction rate right.

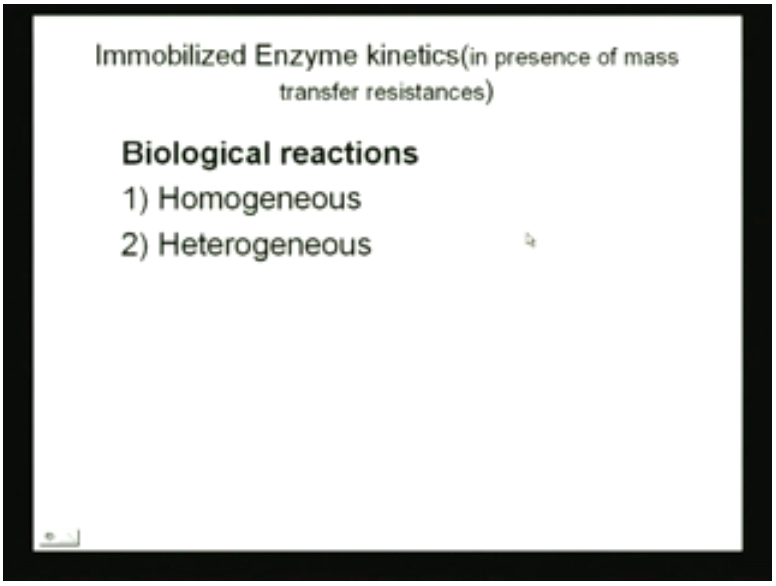
So but, that is not any use to us it is important, but it is not of any use to us, unless the reaction is actually happening and that rate is not of any use to us, what we want to figure out is that at what rate the reaction is actually occurring and that is observed reaction rate, so most of what we are going to do in this chapter is to figure out the observed reaction rate; and this reaction rate is reaction rate in the presence of mass transfer resistances, have you done this in your reaction during course this, the effective mass transfer on reaction, under graduate level.

So, anyway that is good, so it is a new thing that we will probably try and do over here, so is it is it concept clear, that what we are going to do here, how is that fundamentally different from what we had done in the previous chapter previous chapter, we had only looked at only kinetics and come up with pure kinetics here you we are aware of the

kinetics, we were aware that it is a mass Michaelis-Menten kinetics we are aware how to measure those rate constants, everything we are aware of, but the point is that it is not of any use to us, we have to figure out what the intrinsic the kinetics says and so we will make we will take the intrinsic kinetics and try to see, how mass transfer is influencing that, and how mass transfer is reducing the observed kinetics.

I use the word reducing, because the observed kinetics is always going to be lower than the intrinsic kinetics. The observed reaction rate is always going be lower than the intrinsic reaction rate, there could be one or two very very exceptional cases, I might want to talk about that later, but otherwise observed reaction rate is going to be less than the intrinsic reaction rate.

(Refer Slide Time: 12:31)



Now, before we try and do that lets understand that in the presence of mass transfer resistances, what are the kind of reactions that we have the biological reactions. So, the first kind of reaction that, we have is homogeneous reaction right, we studied that in the chapter before and then the end of last class we studied, what kind of reaction?

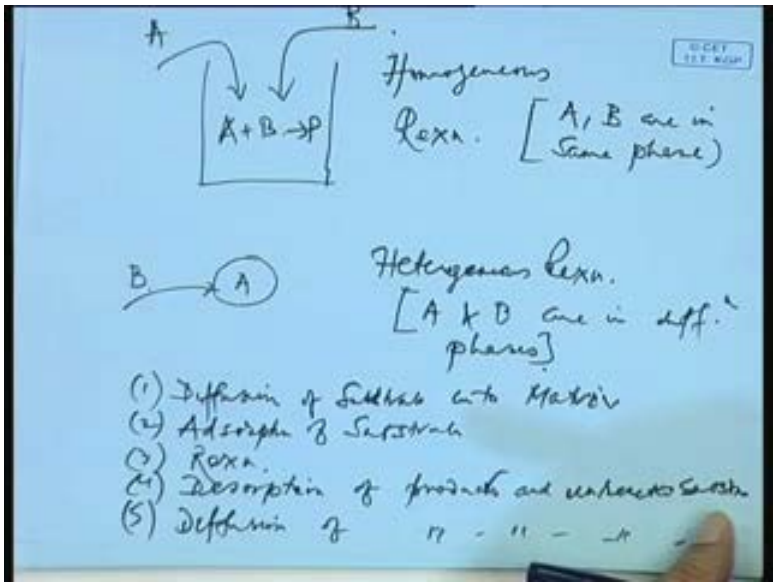
(())

What kind of reaction, did we study in the last end of last class?

Heterogeneous

Heterogeneous reaction, then what else could be there, so homogeneous reaction is the first kind of reaction that we studied in the first 4, 5 lectures, then the second thing is heterogeneous reaction is the end of last class we looked at it, and yeah we looked at you know what is the role that adsorption plays and we also tried to understand what is the role the diffusion plays and so on, a little bit. But, much of this chapter itself in the next 3 or 4 lectures we are going to do this, so that is heterogeneous and what other kinds of reactions are possible when you are talking of reaction model, reactor models, so what other kinds of reactions are possible.

(Refer Slide Time: 13:57)



So, number one is homogeneous reaction, so you have the A and the B going in and reaction is occurring, then the second one is say a catalyst it we took this heterogeneous reaction, so which is say A so homogeneous reaction the definition is that A and B are in same phase, this is A and B are in different phases. So, what are the processes here, the process here is essentially reaction, so may be some diffusion the process is here is diffusion of substrates into matrix followed by adsorption of substrates.

Followed by

Reaction

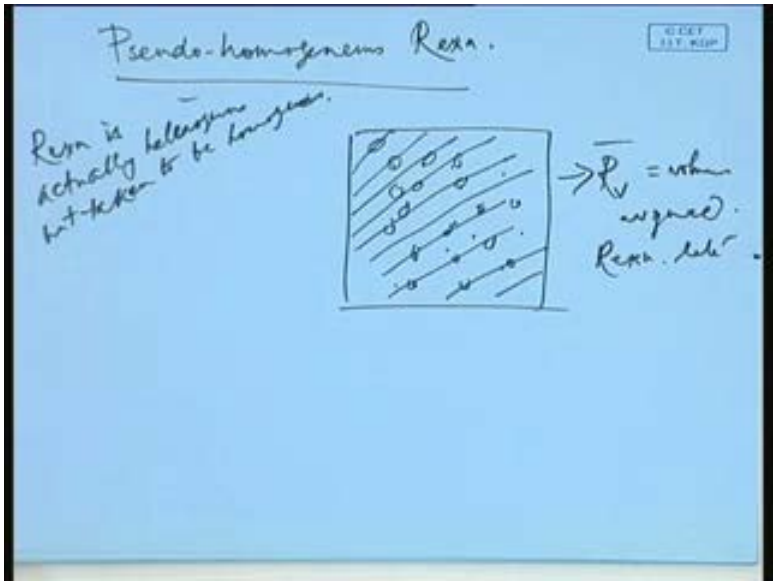
Reaction, followed by desorption of products products and

(())

And no, and desorption of products and unreacted substrates and then diffusion of products and unreacted substrates, so this is a homogeneous reaction and heterogeneous reactions it two major kinds of reaction.

Now, when we modulate we model a homogeneous reaction though you know as such in the CSTRA, you know how to do this you have been taught, how to do this, heterogeneous also may be you might have been able to taught so these are the processes that you have to describe or discuss or understand then quantify.

(Refer Slide Time: 16:25)



Now, there is another kind which is not really a reaction type, but sort of a model, which is known as pseudo homogeneous reaction, what is this pseudo-homogeneous reaction

does anybody have any idea, what what is this pseudo what is pseudo-homogeneous you know, it is cells not homogeneous, that pseudo homogeneous.

(( )) actually in (()), but it appears (())

Homogeneous right, reaction is not actually homogeneous, but it we take it to be homogeneous rather, so reaction is actually what, if it is not homogeneous, what is it?

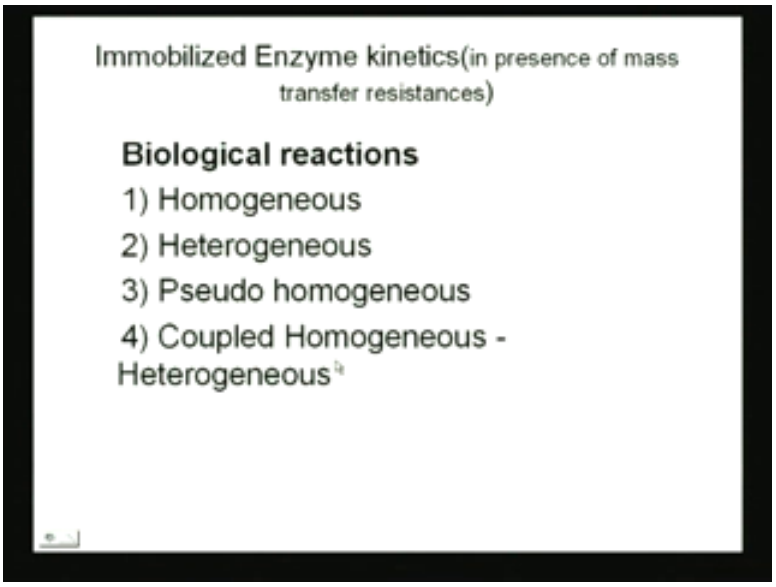
Heterogeneous

Yeah, so reaction is actually heterogeneous, but taken to be homogeneous, let us see you know for example, you have these say in a in a in a catalyst bed or something, so you have these different points in a, points of reaction, these are different catalyst pellet cellular. So, one way would be to look at it in a heterogeneous way, so take each catalyst, catalyst pellet and look at what is reaching this catalyst to diffusion reaction adsorption reaction, desorption, diffusion and so on. The other is you would say for example, you are lazy and you really do not want to quantify each catalyst pellet, there is thousands of catalyst pellets and you really do not want to quantify each catalyst pellet or you do not you do not want to quantify a single catalyst pellet.

So, what you do is, you come up with the pseudo homogeneous model where you average out the reaction rate, so this is average out the reaction rates, so at each point you have a certain reaction rate, but you figure out that what is my total reaction rate in the system, what is the average number of pellets. So, you have say 1000 or 2000 pellets in there, what is the reaction rate for each pellet, find out the total reaction rate, divided by the volume and you get the volume volume average reaction rate of the system. So, then you probably be able to say that, so this is my R v typically this is known as R v, which is R v bar sorry volume average reaction rate.

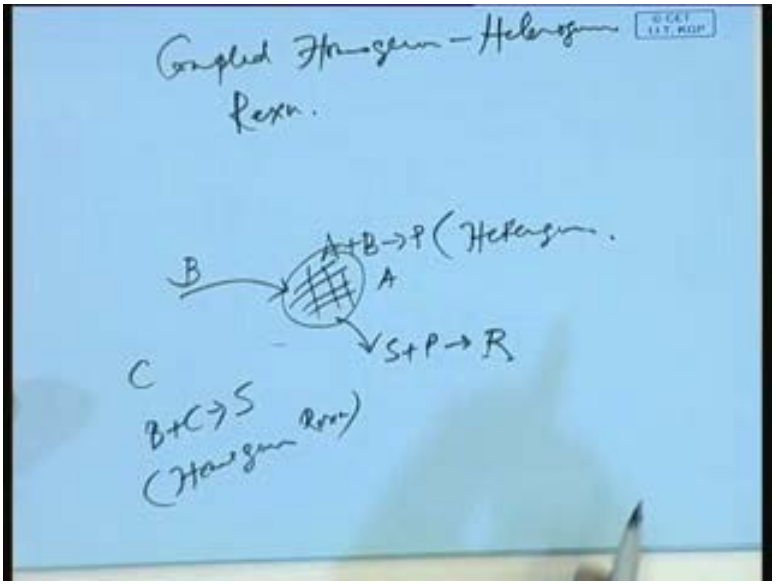
So, this is the pseudo homogeneous, so pseudo homogeneous is not really you know I want to be clear, pseudo homogeneous is not really a reaction but, it is essentially a way of description.

(Refer Slide Time: 19:20)



And the last thing that we will do you know that that, I will show you here is known as coupled homogeneous heterogeneous reaction, this is very straight forward, this is not form of description.

(Refer Slide Time: 19:32)



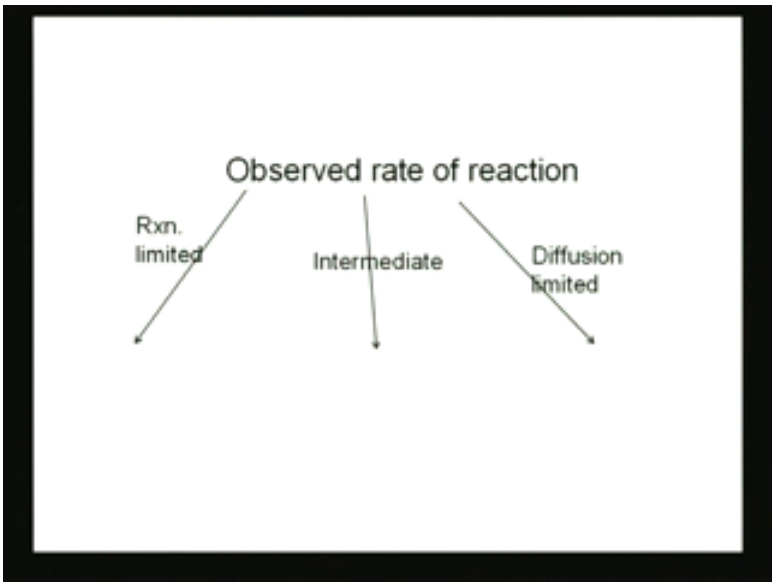
A coupled homogeneous heterogeneous reaction is (No audio from 19:32 to 19:43) a

coupled homogeneous heterogeneous reaction is for example, you have the catalyst pellet out here, so this is reacting, so this reaction A plus B is happening on the catalyst pellet right, so this is the heterogeneous reaction and then you have C in the bulk phase and another reaction B plus C giving as is happening and this is in the bulk phase and this is the homogeneous reaction is it clear.

Coupled homogeneous heterogeneous reaction, is a reaction in which some reactions of network of reactions in which some of the reactions are in the bulk phase or homogeneous phase, some of them are in the heterogeneous phase; and then you can have other kinds of things happening for example, you can have this and then you can have a reaction on the surface of this S plus p giving R.

So, this is the whole network of reactions and typically in real life most of the reactions are belongs to this category, where they will be some reactions which are homogeneous, so if you go and work in the industry and work with certain reactions you know chemical reactions in the catalyst beds stuffs like that, that is always something that will happen why, because may be your heterogeneous reactions are the ones that are desirable, the ones that you want, but there will be always side reactions that will happen in the bulk, so this is something that, that that is very likely, so this is the fourth kind of reaction the coupled homogeneous heterogeneous reaction. So, now let us try and understand what would be the reaction rates and so on, for some of these processes.

(Refer Slide Time: 21:23)



So, next what I want to discuss is one what is known as observed reaction rate another I said, that the observed reaction rate is different from the intrinsic reaction right, it could be equal to the intrinsic reaction rate in the certain cases, so what we do is when we talk about the observed reaction rate we divide it up into different regimes, we divide it up into different regimes, and two of the regimes are the reaction limited regime and these in names that you should remember, reaction limited regime and another one is called mass transfer diffusion limited regime, and there is something that is in between is called intermediate regime which falls between the reaction limited and the mass transfer limited or diffusion limited regime.

So, what is the reaction limited regime

(())

Which is the slowest

Reaction

Reaction is the slowest step, so you might have other kinds of, so basically it is mass

transfer and reaction, so whatever mass transfer could be convection diffusion whatever, so reaction is to is in this case reaction is the slowest step right, so that is called the reaction limited regime.

Now, mass transfer limited regime is the regime in which mass transfer is a slowest step and intermediate is that where reaction and mass transfer are equal you know in terms of their speed. So, in intermediate reaction, so how would we quantify these, the way we quantify these rates are through the through the, so this is these are the three regime that I discussed, reaction limited, intermediate and diffusion limited, so how do we quantify these how do we how do we figure out that, what is the slowest step, what is the fastest step, how do we figure over these out.

(()) a number (())

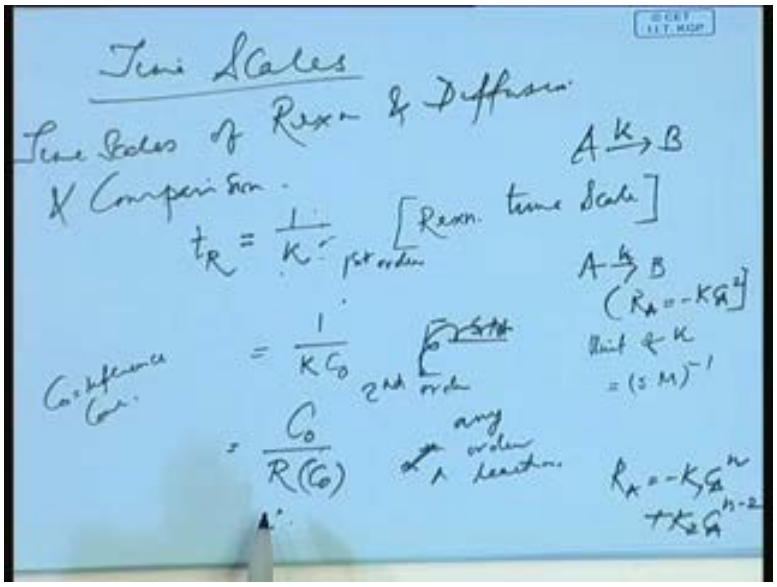
Yeah what on numbers or what

Damkohler numbers

Damkohler numbers

Well yeah Damkohler number and is not really, but phi square, the Thiele modulus is the one but, forget the numbers in a simple way through time scales, so time scales.

(Refer Slide Time: 23:45)



So, time scales of reaction and diffusion and comparison, so comparing the time scales of reaction and diffusion, and obtaining the time scales and comparing the time scales of reaction and diffusion. So, if I give you first order reaction for example, what would be the time scale the t R let us call it, t R is the time scale for reaction, what would be the what would be the time scale, how would you write it, so you have a reaction of A going to B k first or der, what is the unit of k first order reaction?

(()) time inverse

Time inverse, so what would be the time scale

1 by k

1 by k right, so that is my reaction time scale, and if it is a second order say, this is first order A going to B k R A equals minus K C s square. So, if it is a second order then then what is the unit of k in second order reaction, you to always look at this way you know once you figure out, what the unit is what is the unit of k? (No audio from 25:34 to 25.46)

(())

So, second times molar inversal that right, so basically so once the things here figured out is that, when you try to figure out what the reaction time is the rate constant should be there, should be a part of it. So, if this is k, if I write then there is a the unit is it becomes second times molar right, so you will have K C, if you divide it by molar to get time, so you put a K C naught what is naught.

(())

Yeah is some reference concentration, so C naught is the it could be the initial concentration typically, so again this is the unit of time. Now, if I for any units order reactions of this is first order, this is second order, n th order reaction, so R A equals minus K C A to the n, I do not care what is this any reaction let us call it, how do you write that, how do you generalize this.

(()) 1 by 1

1 by

K

Say, let us say any reaction, I do not even know what that, whether it is n th dot or its simple elementary n th order or the complicated form, if I have any reaction what would be my time scale for reaction.

The time is concentration to the power 1 minus A

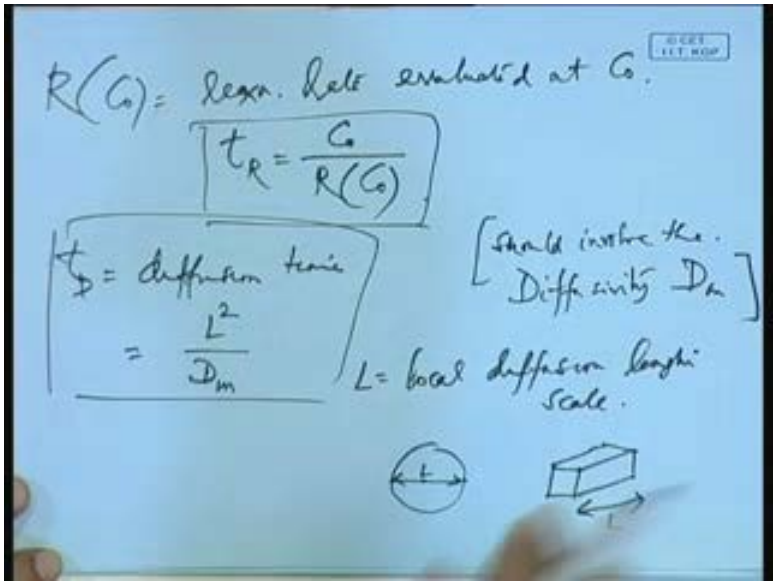
No, I am I am saying, I do not even know if this is, this if say this is not the form, so the form is k 2 C A to the power n minus 2, something like this, what would you do you know, if the form is something which I wrote to the corner here, if the form is not a simple elementary form it could be anything, so what would you do, this is what you do,

is it clear, see yeah it is a R th R C naught over R C naught, so match this with this, so in first order R C naught is K C naught and C naught in the numerator it gets canceled, so you have 1 over k, second order you had C naught over K C naught square, so 1 C naught gets cancel you have one over K C naught and in any order this is not even n th order any order, can I have C naught over R C naught is it clear. So, this is an important thing to remember say any reaction I give you, so what is R C naught mean, what does the R C naught mean?

(())

Reaction rate evaluated at concentration C naught

(Refer Slide Time: 29:08)



So, R C naught is (No audio from 29:08 to 29:23) fine, so this is a generalize expression for any reaction rate we got. Now, I have to figure out, what is the diffusion time, so just as reaction time involves the reaction rate, diffusion time should involve the, should involve involve the…

(())

Diffusion we are talking of, so it is specifically diffusion mass transfer could have convection effects and so on, but let us take the simple case of just diffusion.

(())

Should involve the diffusivity D m let us say molecular diffusivity, so do you have any idea what, any of you what the diffusion time is?

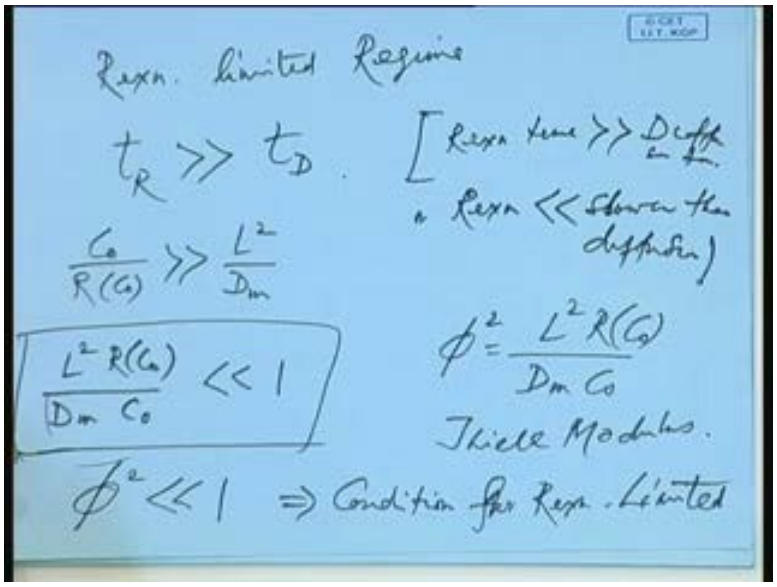
(())

Yeah that is correct

L square by diffusion

Diffusion, so L square let us say by D m L being the local diffusion length scale, so if you have catalyst pellet, a spherical catalyst pellet say L is the radius or the diameter whichever you can just use a diameter if you want, if you have a catalyst pellet which is like a slab then L is a distance length along which it diffuses, so this is now now, so you got this, you got this, so I say t R you got is this. Now, what what would you like to do, if you want to figure out if it is in the reaction limited regime.

(Refer Slide Time: 31:22)



So, if it is in the reaction limited regime, then what happens t R what is the relationship between t R and t D?

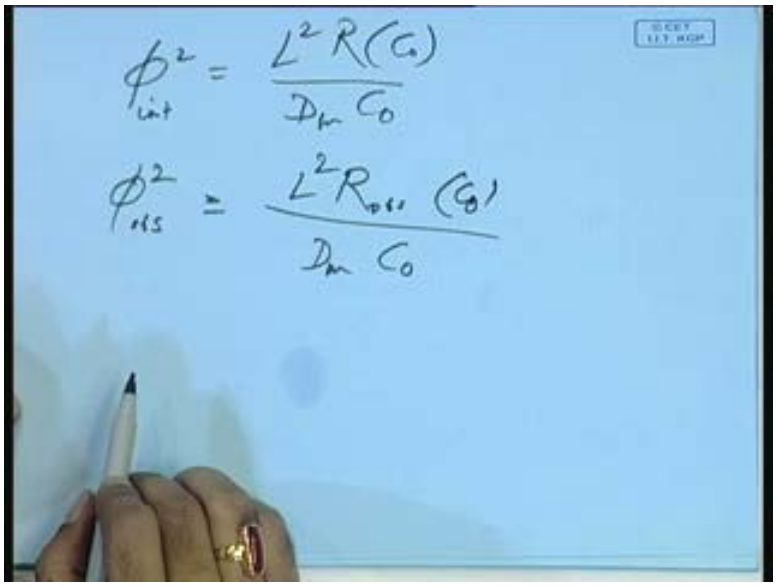
t R is (())

In the reaction limited regimes t R is much much greater than t D so the

Reaction

Reaction time is much much greater than diffusion time or reaction is much much slower than than diffusion; so if I put it over here, what I got what I got here, so C naught over R C naught much much greater than L square over D m fine. So, which means L square R C naught over D m C naught is much much less than 1 right, what is this do any of you know, what this what this is, this is phi square, phi square equals L square, this is Thiele modulus, named after a very famous reaction engineer, chemical engineer, so this is Thiele, so this is my condition for reaction limitation. So, later on in this chapter when we do, when we will talk about this that reaction limited regime, so what we need to measure is phi square the Thiele modulus.

(Refer Slide Time: 33:46)

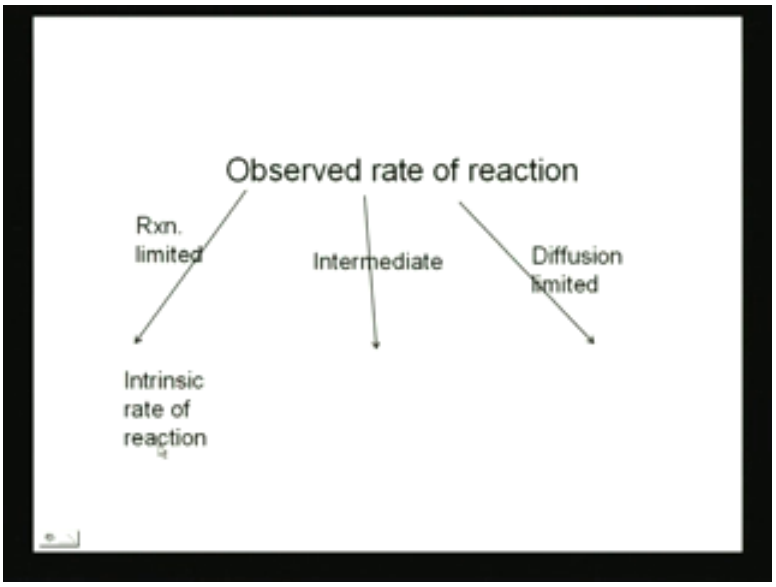


Now, there is I just at this point I will tell you that there is phi square is my and there is something called an intrinsic silly Thiele modulus, and there is something called a observed Thiele modulus, did you can you tell me what the, so this is the intrinsic Thiele modulus what the observed Thiele modulus is going to be. So, the Thiele modulus, that is the actual Thiele modulus of the of the particular reaction and material is different from the Thiele modulus that you observe, when you observe experimentally; if you can tell me you are, you can think in that direction why that happens, then you will be able to tell me that what the observed Thiele modulus is this, why does this happen, first of all why does observed Thiele modulus sometimes is different from the intrinsic Thiele modulus.

(())

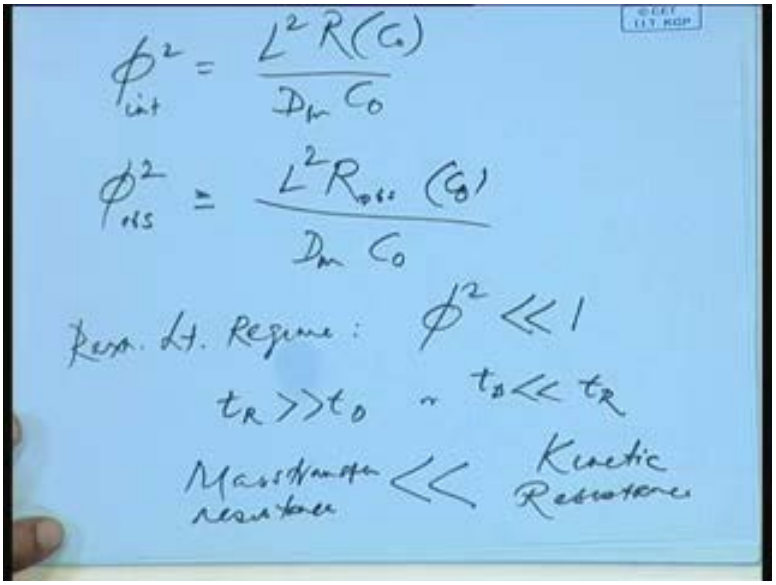
Right good, so the observed Thiele modulus is exactly the same thing except that, the rate here has to be the observed reaction rate just as I told you you know in on this screen now, if you look…

(Refer Slide Time: 34:56)



That the observed reaction rate, so the observed reaction rate is different from the intrinsic reaction rate and therefore, you have the observed Thiele modulus can be less than the third, it is typically less than the intrinsic reaction rate. So, in the reaction limited regime, the observed reaction in the reaction rate is is it same or different or larger than intrinsic reaction rate, so this is the reaction limited regime we are doing.

(Refer Slide Time: 35:28)



And phi square is, so reaction limited regime regime is phi square is much much less than 1 right, so which means that t R is much much greater than t D or t D is much much less than t R; so when t D is much much less than t R what does it mean that, mass transfer resistance and less put kinetic resistance on this side, what is the relationship between these two?

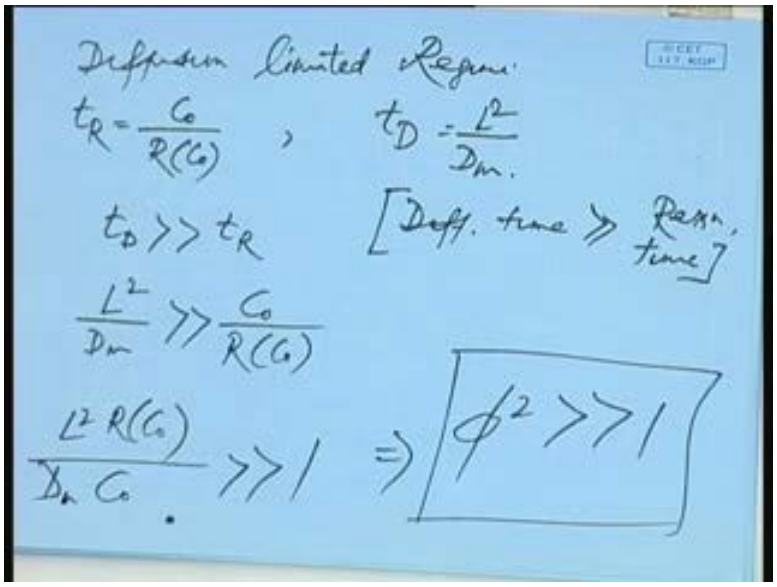
Kinetic resistance (())

Kinetic resistance is much higher, so mass transfer resistance is much lower why is that, because mass transfer time is very small, so if time is very small then the time is inversely proportional to resistance, say mass transfer time is very small, and then mass transfer resistance is much much lower. So, if mass transfer resistance is much much lower then what is the relationship between the observed reaction rate and the intrinsic reaction rate.

(())

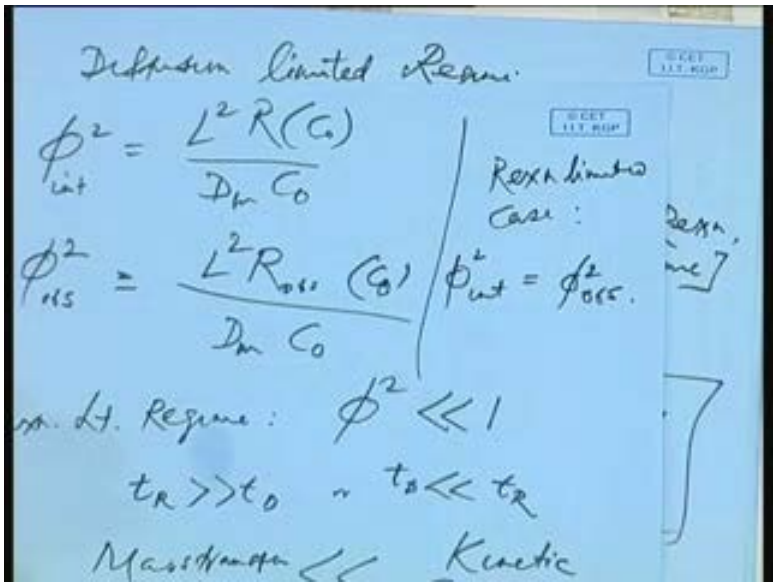
Both are the same right, so let us go back to the screen for the moment for a moment and you will see that observed reaction rate here for the reaction limited regime is same as a intrinsic reaction rate why, because I explained that observed reaction rate is less than intrinsic reaction rate, because of mass transfer limitation. Now, if there are mass transfer limitations are not present in the system then, the observed reaction rate and the intrinsic reaction rate are the same.

(Refer Slide Time: 37:14)



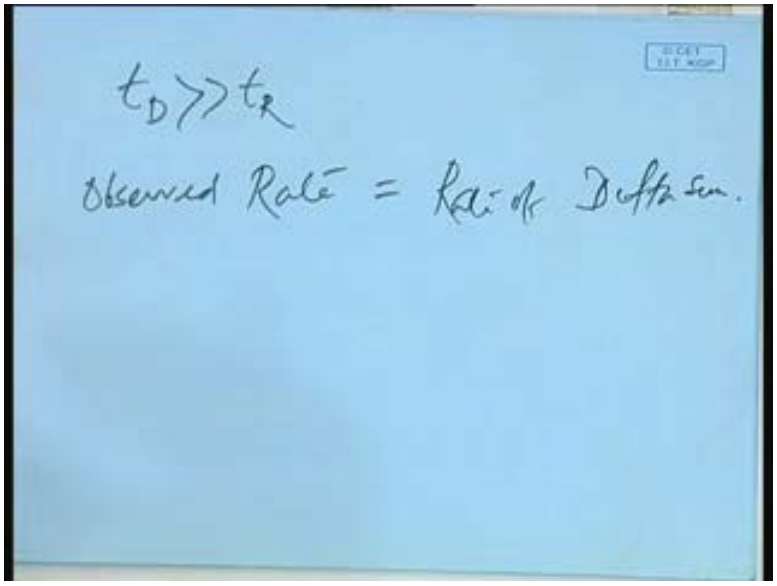
So, let us go to the next one, what I want to do is the last one, the diffusion limited regime and we have already done this, so you know it will take a few seconds to do. So, diffusion limited regime, again my t R is C naught over R C naught my t D L square over D m, in the diffusion limited regime what means diffusion is limiting right, which means that t D is much much greater than t R or diffusion time is much much greater than reaction time fine; so diffusion time is much much greater than reaction time, so L square over D m is much much greater than C naught over R C naught or L square R C naught over D m C naught is much much greater than 1, which implies phi square is greater than 1, so by the way this, if I for a minute just, do not want to confuse you.

(Refer Slide Time: 38:22)



So, in the reaction limited regime, limited case my phi square intrinsic is going to be equal to phi square observed right, because the reaction rate intrinsic and observed are the same, so in the diffusion limited regime this is my criteria.

(Refer Slide Time: 38:51)



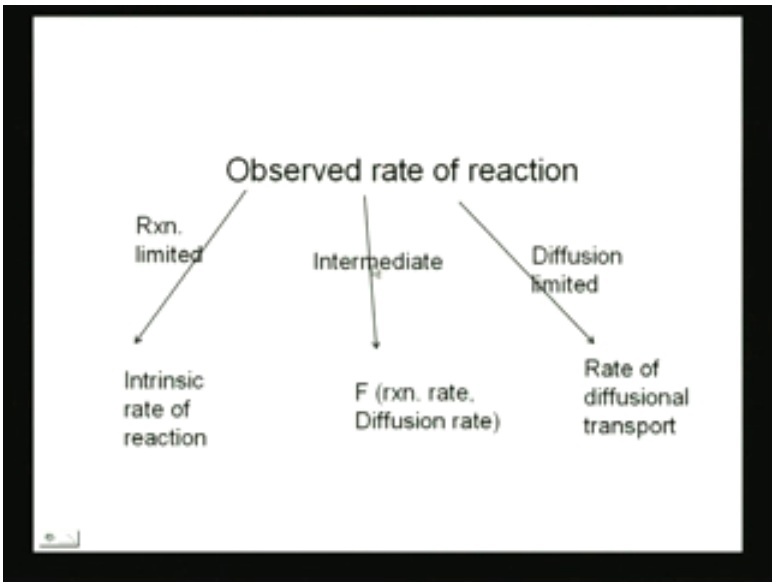
So, in case of so if, this is the case phi square is much much greater than 1 or in other

words t D is much much greater than t R, in the extreme limit of this case, what would be my rate of you know observed rate of reaction, observed rate equals. So, first case reaction limited regime we had that the observed rate equals the rate of intrinsic rate of reaction, the other extreme asymptote where the reaction is very very fast as compared to mass transfer. What would be my observed rate of reaction, what would be the rate limiting step, first case what was the rate limiting step?

(())

Reaction was rate limiting step, so in the other asymptote what is now the, so there are two things the diffusion and reaction, see if diffusion is much slower than reaction, then what is the rate limiting step of course, diffusion is the rate limiting step, so observed rate would be equal to the rate of diffusion in this case.

(Refer Slide Time: 40:00)

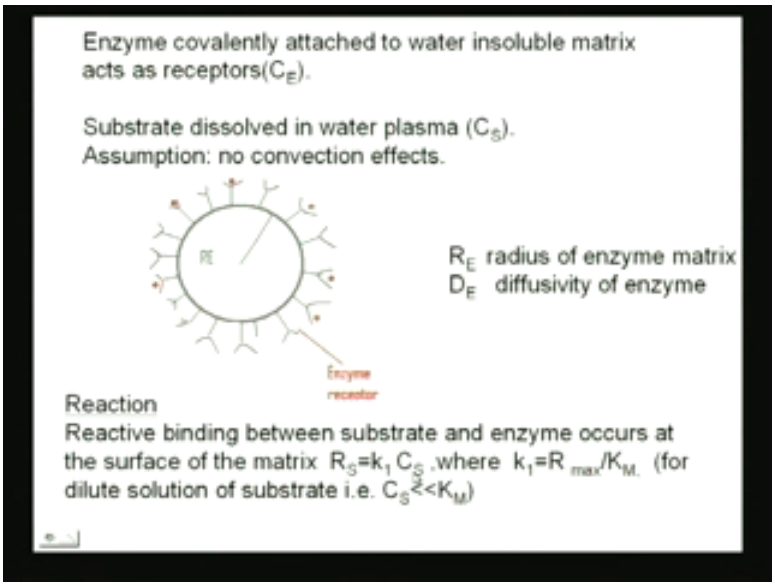


So, if I can go back to the screen, you will see that, so in the observed in the in the diffusion limited regime, the observed rate equals the rate of diffusion transfer and in the intermediate regime in the intermediate regime. The reaction rate is a function the observed reaction rate, is the function of the intrinsic reaction rate and the rate of diffusion, so if you see here, when I say reaction rate I mean the intrinsic reaction rate

over here in the middle; so the observed reaction rate is the function of the intrinsic reaction rate and the diffusion rate and these are the most difficults difficult things to actually calculate as compared to the other two. Because, the other two conceptually once you make a sense that it is going to be in the diffusion limited regime or the reaction limited regime you directly know the rate, but in the intermediate zone you have to calculate and as you know as things are life is always difficult, so you have to calculate, because most of the things that we deal with in real life or the intermediate zone between reaction and and diffusion limited regime.

So, what we will do is we will start now with a problem, that we want to do and the problem relates pertains to what I just showed you in the picture that I showed you this one, so this is a for example, enzyme which is bound to the matrix and how does this diffusion mechanism happen you know, how does the diffusion and reaction mechanism happen, so we have studied the in a mobile enzymes in the first in the first few lectures 4 or 5 lectures then, that was the pure reaction (Refer Slide Time: 41:14). But, now it is the diffusion plays the major role in that, we need to try and understand that how this happens, what we will do is in the in the next part of this lecture, is try and understand how this happens.

(Refer Slide Time: 41:43)



So, we consider enzyme which is covalently attached to a water insoluble matrix as receptors and acts as receptors and the substrate is dissolved in the water water or plasma water whatever it is, and their assumption is that there are no convection effects, this is this is water or plasma let us call it. So, this is how it looks like, so this is my matrix and these are the enzymes which are so this is the symbol for receptors by the way, so these are the enzymes and the the red ones are the enzymes and their attached to this matrix by being bound to receptors fine.

Now, so the substrate is dissolved in the in the surrounding C s, so what we are trying to figure out is that, what is the what is the reaction rate, observed reaction rate sort of say because, the reaction intrinsic reaction rate is something we know, but we have already understood that, because of mass transfer limitations observed reaction rate is going to be less than the intrinsic reaction rate, it is not going to be that.

So, the problem that we are going to face first of all is that any kind of you know in this chapter that is a that is the problem will always face is that, any kind of analytical solution or analytical quantifications that we try to do would be limited by the fact that the reaction, intrinsic reaction kinetics that the that is that governments interaction between the enzyme and substrates is what type it is a Michaelis-Menten kinetics right, Which is by default non-linear in nature right. So, any kind of analytical effort or analytical quantification that we try to do in this class is going to be limited by the fact that its Michaelis-Menten and therefore, non-linear and non-linear systems cannot be treated in non-linear O D E cannot be treated or P D E cannot be treated so easily.

So, what is the way out, what can we do about this, I mean we can leave it and not do it we can do it quantize it that is you can compute it, but when we compute it we do not get a sense of it you know, the advantage with analytical solutions you get you can solve it and you can get a sense of what is happening, so what would be my other possibility and you know we already did that if you remember something like this in the in the earlier lectures.

(()) extreme

Two extremes right, so you try and figure out what you can do at the two asymptotes of, of what, large concentration of the substrates and small concentrations of the substrates, and the limit of large concentrations of the substrates, the Michaelis-Menten kinetic kinetics tends to become….

0

0 with order in the limit of small concentration tends to behave with first order, so the actual order of the Michaelis-Menten kinetics is the fractional order between 0 and 1 right, so we get the two asymptotes and once we get the two asymptotes, so we figure out how it is going to behave in at first order, how its going to behave with 0th order and the real nature is going to be limited or it is going to be bounded by these two behavior. So, that is the strategy you will take that while we understand that we cannot accept for one little case later on, while we understand that we cannot do it for all for the non-linear kinetics; we will do it for the linear and the 0th order kinetics and then have our solution actual solution be bounded by these two solutions.

So, that is exactly what we will do we will split it up, so here what you see again going back to the picture, so this is my matrix and R E is the radius of the enzyme matrix, D E is the diffusivity of the matrix and the our receptors are out here which and your enzymes are the red dots out here, and the receptors are bound to it. And the substrate is in the plasma the water whatever it is surrounding typically say, let us say plasma, if it is in a biological system; so what would do you think, what would be the process here let us talk about the process first, before we try and do any calculations.

What do you think would be the process, how how our things going to be happen? So, the enzyme is sitting their tight right, so it is covalently attached to the matrix and it cannot move fine, why it cannot it move, it can move the matrix itself can move, but it is very hard for this a matrix is in a if you can say that, the matrix has compares to the water the density of a matrix is much higher than the density of water or the plasma that surrounds it. So, the possibilities at the matrix itself will move is very very little, the reason being that we have already neglected convection effects righ, we have already said that convection effects are neglected.

So, if that is the case then the possibilities the matrix will itself will move with very little, so what is other possibility, so they have to come together the enzyme and the substrate has to come together, so the enzyme cannot go to the substrate this substrate will come to the enzyme right right is it clear. So, the substrate will come to the enzyme before it reacts, so how will it come, it will come through what is the process simple diffusion, right so that is the mechanism, that we have to a try and model; so our mechanism is that the substrates reaches the enzyme through diffusion and once it reaches the enzyme (()) adsorption and all that stuff here, we once it once it reaches the enzyme it reacts with the enzyme, so it is a simple process, but it is a two step process, that you have to in kind of a model.

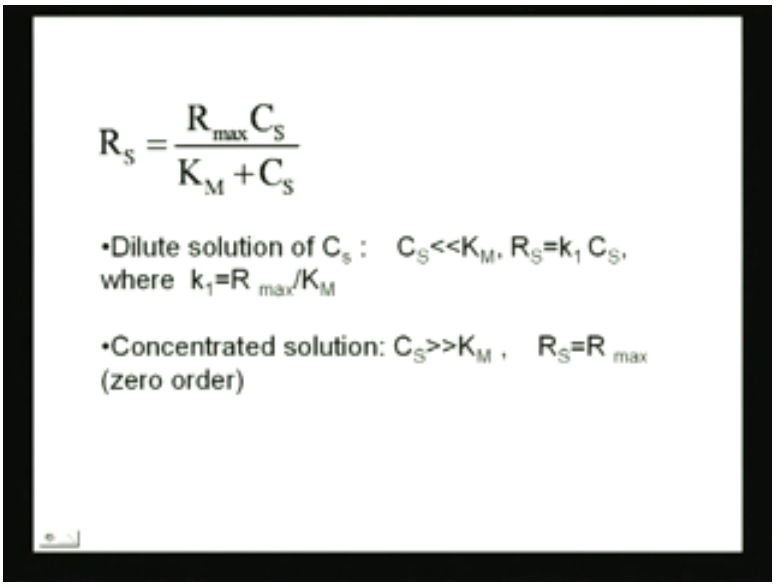
So, I am not sure if you have done this, have done this in any reaction or anything this probably not, so I will go slowly through it, so essentially this two step process one is diffusion of the substrate through the enzyme followed by reaction, where is the reaction going to take place you tell me now, let us the look at the picture and so this is this is matrix and you have the receptors bound on the surface, and the enzyme is this red dots on the receptor there is nothing no enzyme inside remember it is a enzyme on the surface you can have encapsulation this is not encapsulated enzymes right, these are immobilized enzymes (Refer Slide Time: 47:17). But, immobilize on the surface, so you can have two or three different kinds of things, we can have immobilization which is inside the matrix right you can have immobilization on the surface of the matrix and you can have immobilization, which is both on the surface and the inside encapsulation plus immobilization on the surface right.

So, this if you go back to the picture, so this is my mobilization on the surface, so where is the reaction going to occur.

(())

Out the surface right, so the reaction is you know the reactive binding between the substrate and enzyme occurs at the surface, and we will do as I said two extreme cases of large concentration of surface and surface sorry, large concentration of substrate and small concentration of substrate.

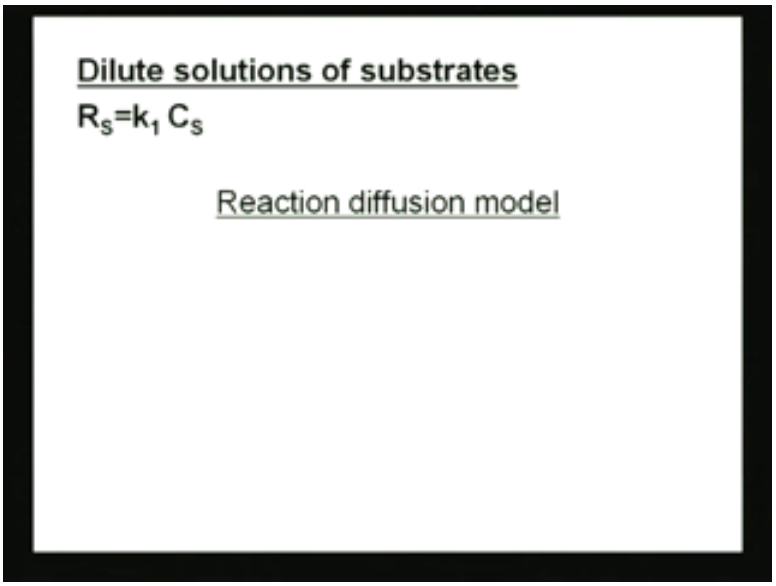
(Refer Slide Time: 48:24)



So, let us look at look at these two cases, so this is my Michaelis-Menten kinetics in the in the limit of dilute concentration solution of C s that is small concentration, C s is much much smaller than k K M and my R s is k 1 times C s, where k 1 is the first order come, looks like a first order rate constants its R max over K M clear.

So, I want you to ensure understand that though we will deal with first order and 0th order kinetics, but these are limits of the Michaelis-Menten, we are not randomly or arbitrarily working with first order 0th order kinetics, this is something that you need to remember. The second point is, that for the concentrated solution that is C s much larger than k M, the R s is the maximum it is a 0th order kinetics and R s is the maximum reaction rate right, so we will do both these cases I am not sure probably we would not finish today, but we will continue.

(Refer Slide Time: 49:14)



So, let us do the dilute solution case, which is the small you know low concentration of C s and what we take is the reaction diffusion model, so what kind of let us you know, let us let me ask you first, that what kind of the reaction diffusion model fine, but what kind excuse me, what kind of geometry are we considering here.

(())

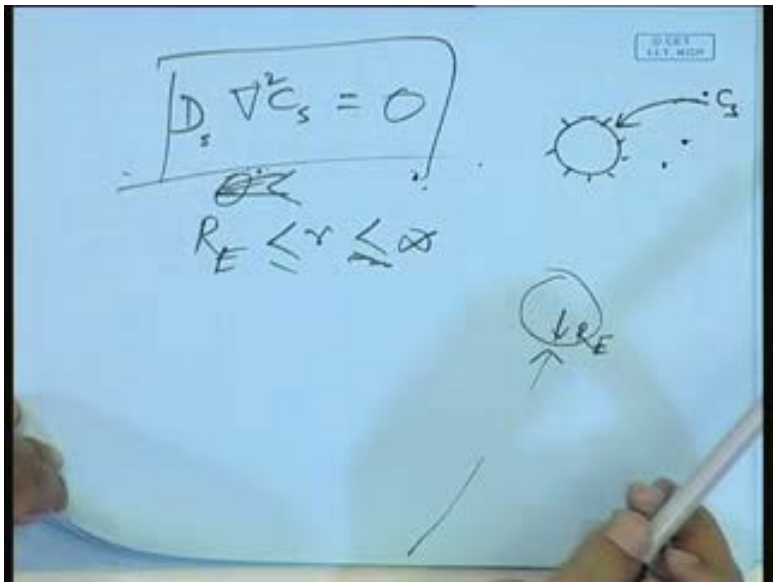
Yeah but, remember that you are not solving for the matrix itself matrix in, inside the matrix nothing is happening, whatever is happening is outside the matrix, that is something you need to remember, where you are solving you are solving outside in the in the plasma or in the water is that clear, and your matrix is spherical, so what kind of coordinator you are going to take, matrix is a sphere and you are solving outside the matrix.

So, what kind of coordinator would you take, its best to take a spherical coordinate why, even though you are not solving this is of something, the according to me I want you to understand that, even though you are not solving inside the matrix, you are solving outside the matrix still its better to take a spherical matrix, spherical coordinate why is that, because no because of the boundary conditions. See, the boundary conditions is on

the sphere right and you have to at the end of the day you have to implement the boundary conditions on the surface of this sphere, so the boundary condition is on this sphere, so in order to be able to implement it with maximum ease you better have a spherical coordinate.

So, when I write if I ask you to write this diffusion reaction equation, you probably do not remember the diffusion equation I am sure for each of this coordinates, you remember, no I think, so I will ask you to do that, but let me let me first….

(Refer Slide Time: 51:07)



So, this is diffusion into laplacian of, so this is for the substrate we are drawing for the substrate, so this is my matrix with the enzymes struck out here, and the surface is coming C s the substrate is coming from wherever to this, so we are drawing the you know we are writing the diffusion equation for the substrate. So, this is my laplacian equal something so remember I am writing my equation I am telling you again and again I am writing my equation outside this sphere, so what is my this is the diffusion part right diffusion times laplacian of C s, what would be my this side, right hand side.

(())

Everywhere you think it should be reaction

(())

This is not going to be reaction, it is going to 0, because I am writing that is what I am trying to tell you, that I am writing my I am trying to solve in the in the water or in the plasma outside and no reaction is occurring.

(())

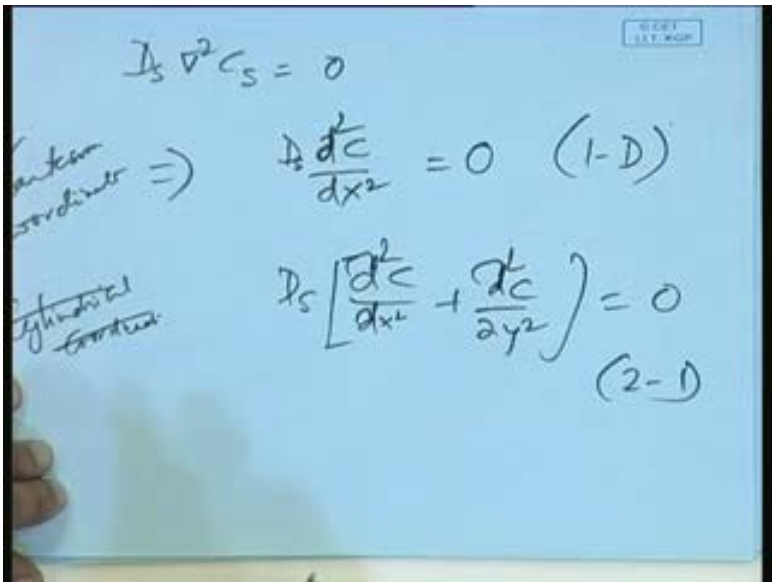
No no no, I am I am so wherever it is no not from approaching the surface, I am I am I am my domain is from anywhere to the surface, so my surface is R E and my domain of interest is 0 sorry R E less and equals r less less there is an infinity, so it is approaching from anywhere in the solution and the way in the in the in the solution to the surface, so D or that is my domain of interest.

So, on in during this area or in this domain of interest there is no reaction that is happening, all reaction that is what I tried to specify, before that all reactions are happening on the surface of the matrix is that clear to everybody is that clear. All reactions are happening on the surface, then when I am writing my equation the domain of interest there is no reaction, that is happening; so if I am to write this yes.

We will include the reaction in the boundary condition

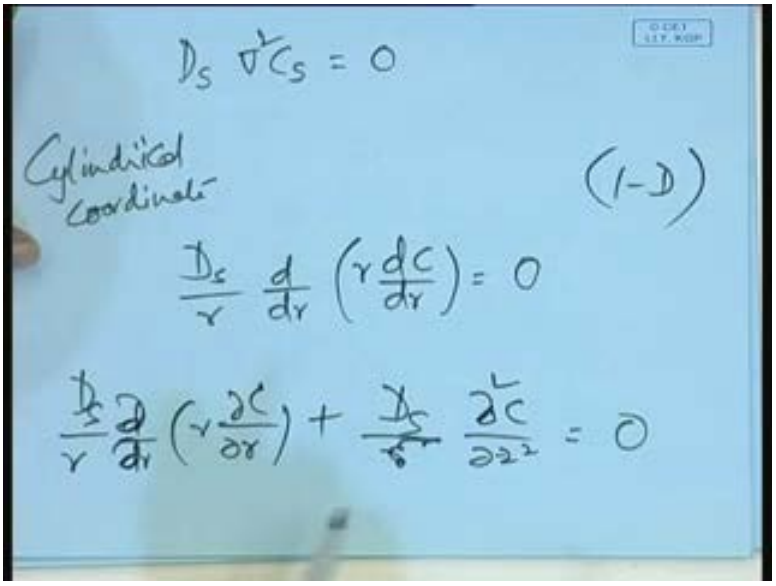
We will include the reaction and the boundary condition; I will come to that, but just going a little slow.

(Refer Slide Time: 53:11)



So, if I am, if this is what it is this, then in the Cartesian coordinate, how do you write this I am just writing it for sake of you in all coordinates, so one time, so that you remember this and I do not have to go through this again and again, so the D s into (Refer Slide Time: 53:31) right, in the cylindrical coordinate and if I am to do a 2 D, this is 1 D and a 2 D would be D x into (Refer Slide Time: 53:54).

(Refer Slide Time: 54:09)

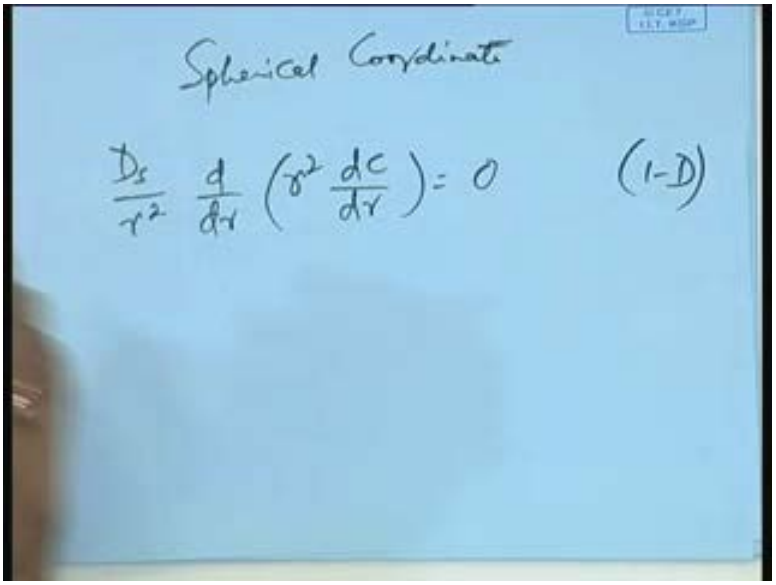


Now, let us write it in the cylindrical coordinate (No audio from 54:12 to 54:21), so one D would be in radial, only in radial if I am drawing writing do you know what it is you remember laplacian.

(()) r d c

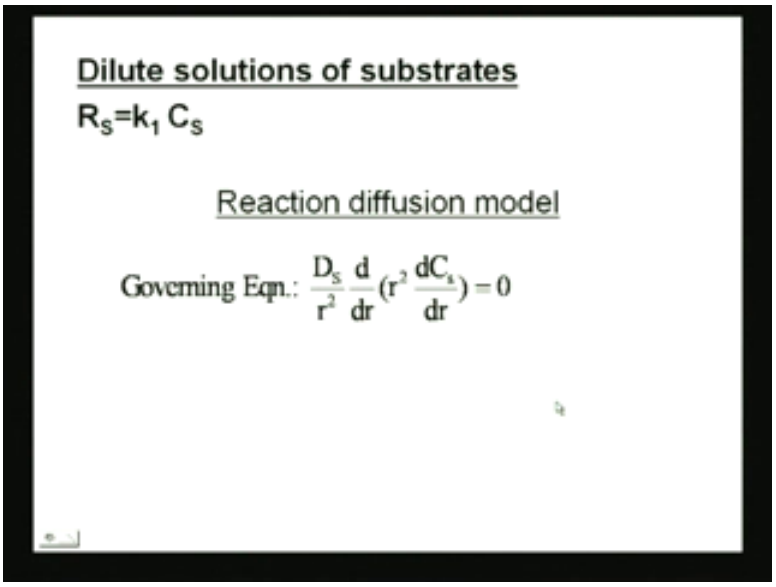
This is 1 D and then 2 D would be (No audio from 54:43 to 54:55) fine, so D d a D s times 1 over R del del r of r del C del r times D s then del 2 C del s square equal to 0 fine.

(Refer Slide Time: 55:13)



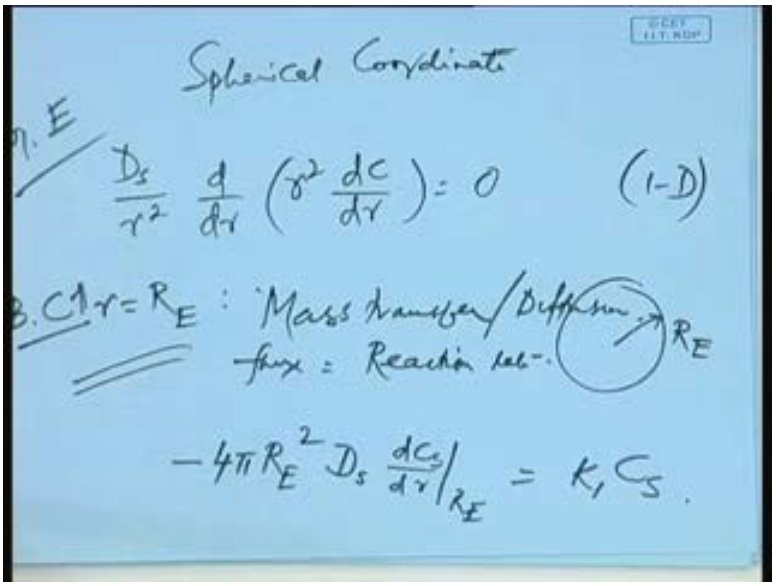
In the spherical coordinate spherical coordinate, we typically do not have 2 D we have either 1 D or 3 D right, because if it is asymmetric, it is asymmetric in both the (()) directions typically and if it is symmetric if you have only r is that clear. So, in the in the spherical coordinate if it is symmetric that you only have r and if it is asymmetric, then you have r theta and C all (()). So, in the spherical coordinate what is going to be, so 1 over r do you remember one of you, any of you, so this is my equation.

(Refer Slide Time: 56:03)



So, this is what I have if I go back to the screen and what I will do is that quickly I will you know we are probably running out of time today, so I will just give you the boundary condition, because of we are at it, so as we discussed that one of the boundary conditions is that, one of the boundary condition is that the reaction is occurring on the surface.

(Refer Slide Time: 56:27)



So, what you have, so r equals R E, so this is my this is my boundary, this is what did you have, just tell me conceptually may be, we will write write it a little later.

(()) mass transfer rate is equal to (())

Very good yes, mass transfer flux, mass transfer flux equals reaction rate, so what is your mass transfer diffusion flux, mass transfer flux is same as diffusion flux, so what is it that going to be minus 4 pi R E square if I am 2 add the surface area times very simple.

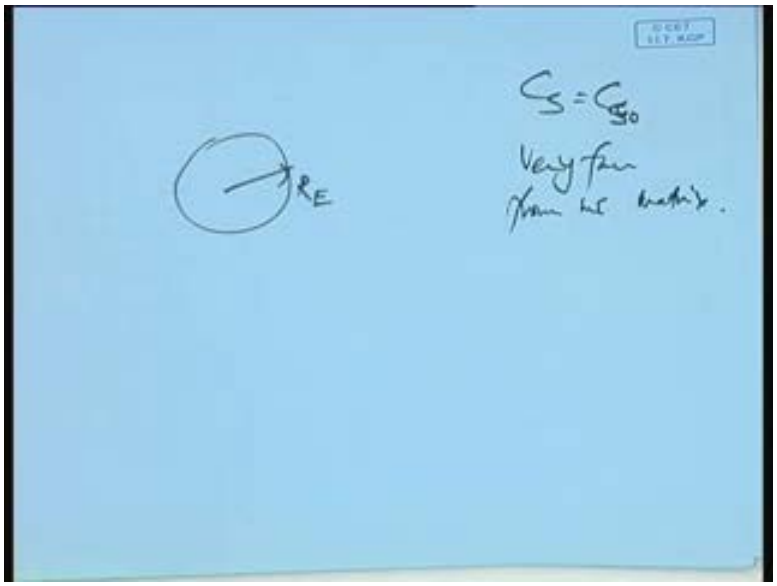
D s

D s times

(())

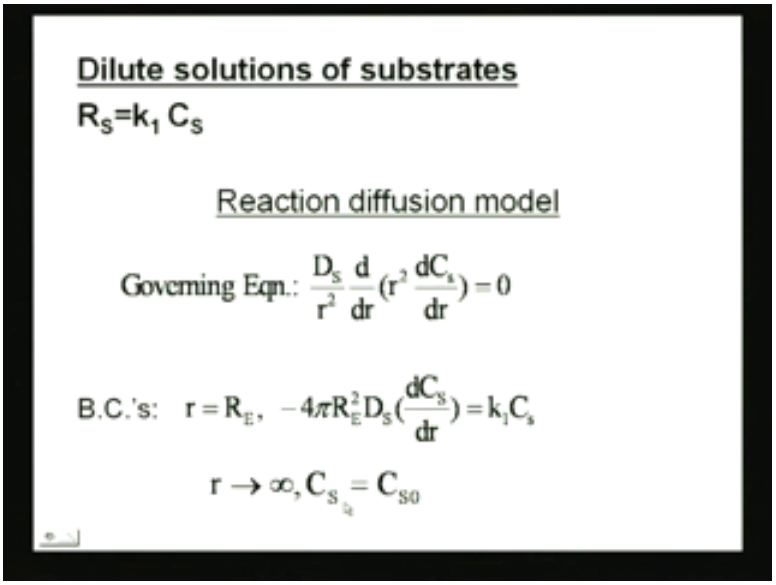
D C, let us say d C d C s D R and R E right, so this is my diffusional flux equal to reaction rate which is k 1 times C S is that clear everybody, so my mass transfer, mass transfer or diffusion flux, so this my boundary condition B C 1, so this is my governing equation and my last in, my last boundary condition is that….

(Refer Slide Time: 57:55)



So, this is my surface, let us say and this is R E my last boundary condition is very far away from this the concentration, is the constant, so C s equals C naught C s naught very far from the matrix, very far from the matrix is the constant.

(Refer Slide Time: 58:16)



And we will close today, because we have completely run out of time, so the boundary condition at r equals R E is given by this and the boundary, other boundary condition is very far that if R is going to infinity, the substrate concentration is a constant given by C s naught. So, we will start in the next class from this, let us and continue, so thank you for your time.