Quantitative Methods in Chemistry
Prof. Dr. Bharatwaj Sathyamoorthy
Department of Chemistry
Indian Institute of Science Education and Research – Bhopal

Module No # 06
Lecture No # 27
Simulating the Michaelis Menten Kinetics using MATLAB

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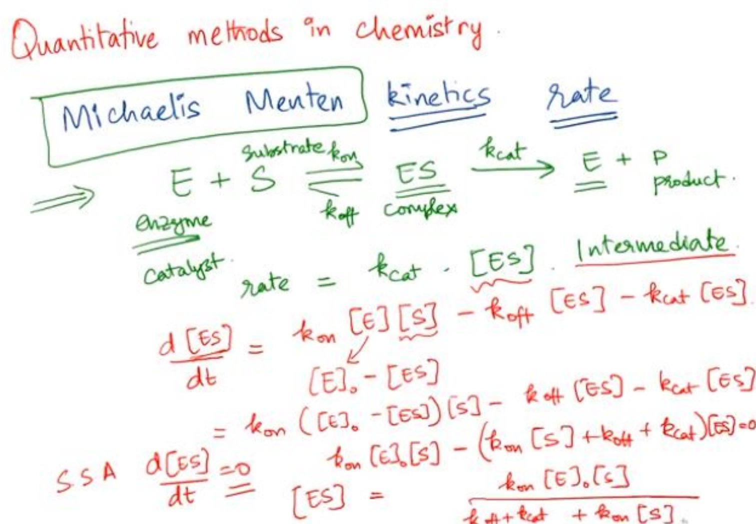

Welcome to the next lecture in the course quantitative methods and chemistry the last class we saw how the computation program MATLAB could be used towards numerically simulating different datasets. So as to help you set up your experiment or even understand how a setup works of how a equation works? In this lecture what we will be taking a look at would be take a similar example once again we will be dwelling on the rate kinetics chemical kinetics and we will be using it for Michaelis Menten kinetics.

As an example why did I pick this example because we have already seen how rate can be determined we saw in the previous week if you are able to get concentration as a function of time you would be able to discern what is the rate of a reaction? The same thing will be applied here and just that I will assume the rate can be determined by any technique that you end up using any analytical technique like spectroscopy or even basic titration that you might end up using that part will not be spoken about what is the specific enzyme that we are discussing.

But we will write the basic equation that well and help MATLAB or rather use MATLAB toward simulating this functions to understand how this experiment can be reliably setup across different enzymes. So to go over some of the basics Michaelis Menten catalysis is about how in a substrate interacts with an enzyme it forms an enzyme substrate complex that goes on to release the enzyme and make the product.

And the rate constant for the formation and dissociation of the enzyme substrate complex is given as K on and K off respectively and the step that results in the product formation is given as K cat where K is the rate constant for each of these step people who are afraid of bio-chemistry do not have to be worried we are going to be treating enzyme as a catalyst here that is about it. Enzymes can perform very specific reaction because of their 3 dimensional structure and therefore people where quite fascinated earlier on understand how enzyme functions and how do they deal with different substrates.

Like you can have the same reaction done by different catalyst same substrate can be converted to product also by different enzymes and this model of Michaelis Menten was to understand how these different enzymes can be compared with one another and in order to do that they came up with a proposal of a mechanism of this sort where the enzyme and substrate become a complex after they form a complex the product of this formed and the product is released where the enzyme is regenerated for further catalysis.

Is not the definition of a catalyst you have the catalyst regenerated such as it can be used again and again. So for this process what ends up happening when you want to have the rate of the velocity you are going to giving at as K cat times concentration of ES. One has to remember that this is a transition state many times or even an intermediate we cannot call it a transition state if you are writing it this then it is an intermediate and then something is the intermediate this cannot be isolated.

So therefore getting the concentration of the enzyme substrate complex is not trivial so what one does is to get what is the rate of change of the enzyme substrate complex as a function of time. So since it is formation it is going to be K on times the free enzyme concentration and the free

substrate concentration minus the off rate with the concentration of the enzymes substrate complex minus the K cat on the enzyme substrate concentration.

Remember here you are talking about the free enzyme and the free substrate you can always have a assumption that you have excess substrate where the concentration of the substrate can be assumed as whatever you gave in and however you cannot do the same assumption here. Here what might end up happening not all of the enzyme need to be interacting with the substrates. So what you would like to do is that you like to get at the zero concentration of the basically the initial concentration of the enzyme minus the enzyme substrate complex.

So what this ends up becoming is k on times E0 – ES times S – k off ES – k cat ES by applying a steady state approximation meaning that the rate of formation of enzyme substrate complex is the rate of dissociation of it meaning that it is a either at a constant concentration or ask ES as being formed it gets converted to product and the steady state approximation you will have rate of change of ES as 0.

If you do quick math what you are going to end up realizing is that k on E naught S - k on S as k off as k cat times ES = 0. So once we see this because we have just applied the steady state approximation enzymes substrate concentration would be given by k on E naught S k off + k cat + k on times S.

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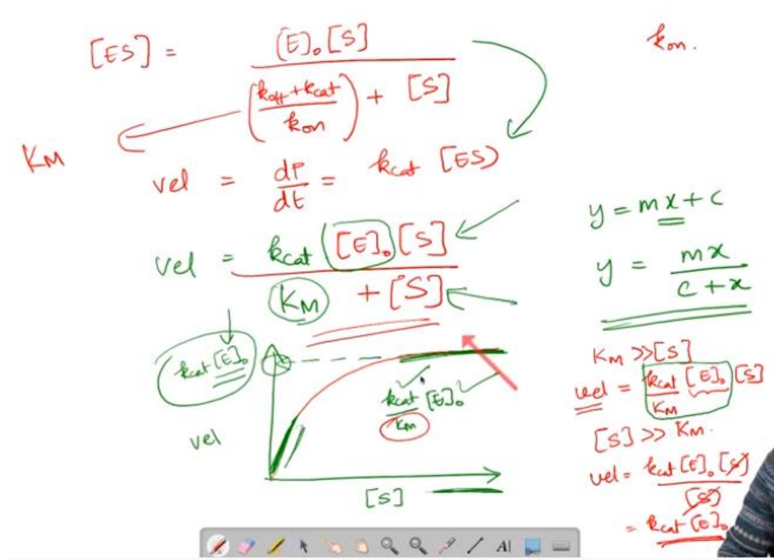

So one can immediately realize that ES is now given by S times k off + k cat divided by k on + S all that is been done divided by k on. And then what did we say the velocity of the rate of the reaction is dp/dt which is given as k cat times ES now we were just determined what is ES therefore so this is going to be equal to k cat multiplied by am I missing something? Yes I am missing the E naught let us add it E naught times S divided by so this is given an equilibrium constant called kM + S.

Let me write kM in green so that we understand which are all the things of interest so now what you are able to realize for this system kM is of interest because it helps you understand how much of the dissociation of ES. In this equation we are concerned about k cat and KM is form of equilibrium constant and what you are able to realize here is that how quickly the enzymes substrate complex comes off either due to product formation or going back to the reactants divided by the rate at which it gets formed.

So this is something that gives the strength of the enzyme substrate complex while it gets formed and k cat is the rate at which you will form the products. So this has an information of how quickly the enzymes are substrate complex goes towards the product formation. So for biochemist these 2 are the variables that are of prime interest and what you are able to realize is that the velocity of the reaction is based on the initial concentration of the enzyme the substrate concentration at any as you set up this kinetics.

Unlike the previous situation where add a simple y = mx+c where x was independent variable here you are able to realize you are having an equation of SAR y = mx divided by c+x of SAR. So what ends up happening is that it is difficult for you understand how this function will look so that is the basic purpose of what we will end up doing right now so let us once again switch back to MATLAB.

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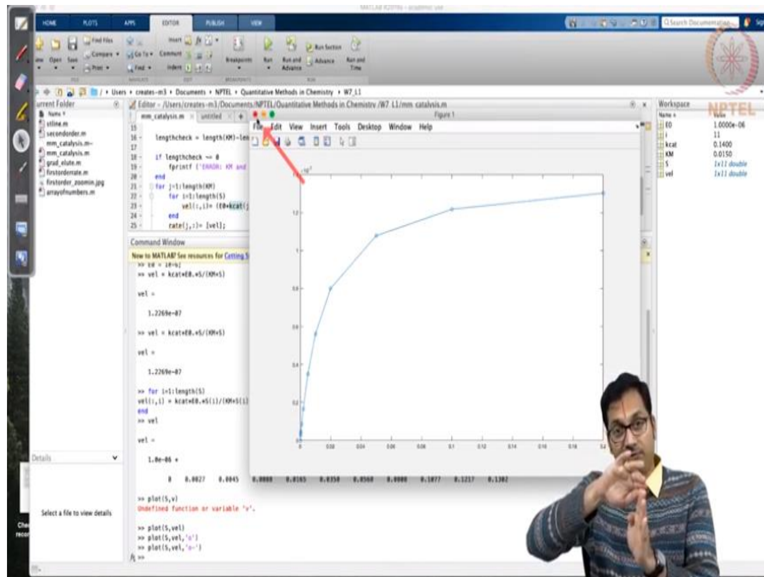

So as the first thing what we can try to do is to give a certain substrate concentration since I have already written the code I am going to copy a portion of it. So I am setting the concentration of substrate which is given as S in this form and the we can actually take some values for known enzymes KM we take it as 1.5 10 to the power of -2 and k cat can be taken as 0.14 just for the sake of simulation and these are all for the protein called chymotrypsin okay so protein E is chymotrypsin here the substrate is the one that is ends up catalyzing and at the KM the form of equilibrium constant is 1.5 10 power -2 and k catalysis is 0.14 units.

So now when you have something like this what we can try to do we can say velocity of the reaction is given by we also have to assume a certain concentration of the enzyme. So why do not we set that E0 is 1e power -6 so basically what am I trying to do is a setting the concentration of enzyme concentration in micro molar many times proteins are generally getting used or set up at micro molar concentrations.

So what we have to do here is that we have to set E0 as 1e power -6 okay now that we have done that one thing that I have to put in here which I forgot earlier is E0 star. So now what we can do velocity of the reaction is going to be equal to k cat star E0 star S you have to say dot S meaning that for every entity do this KM + S okay. So what as happen here is that when you put that when you put this formula in it has calculated for every value but just that it keeps on updating it.

So we can actually say for I = 1 into length of s velocity is given by basically the same way we have simulated data earlier okay. So now you have the velocity we can try to plot this plot of S is the function of velocity. Of course you always like to plot it with this. As you change the concentration of the substrate this is what ends up happening you are able to see a nice buildup curve and maybe it is saturated maybe it is not saturated.

So now you are able realize it is a function that goes something of this sort so when you are plotting velocity as a function of substrate concentration you get a curve that looks like this. This does makes sense because when KM is greater than the concentration of S what ends up happening velocity is going to be given by since much greater than S. So this is going to be k cat time E naught divided KM times S basically we are approximating the denominator to be KM since this is the constant we have said these 2 are constant velocity varies linearly with a substrate concentration.

You are able to see this part of the curve has a linear dependence on the concentration of the substrate but as the substrate concentration increase which is a next limit and S is much greater than KM what ends up happening is that you can approximate velocity is k cat times E naught times S divided by S. So the S cancels so this I going to be k cat times E naught so which is a constant which you are able to see as a concentration of substrate increases a lot this tends to saturate.

So let us say if you are able to extrapolate or if you are able to read this value off this will give you k cat times enzymes concentration and since enzyme concentration is known since you are put it in this is what this value will help you determine what is the k cat on the other hand if you are able to get the initial line which will have this as the slope right you already know the concentration of E naught.

So you have k cat / KM times E naught k cat can be determined at saturating concentrations while k cat / KM times E naught can be determined at initial concentrations since you know what is K cat and you know what is E naught you will be able to determine what is KM. So basically you are able to understand how to set this experimental let us go back to our simulation right now and try to see whether this has saturated.

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You are able to realize even at the substrate concentration that has been provided between 0 to 200 milli molar one is not able to see saturation. Let us make sure that the concentration of substrate is given properly so this is given at molar sorry yeah so basically between 0 to 200 milli molar you realize that it is increasing but it is probably not saturated. So one way of checking this further is by changing S so give more values and then you can redo this simulation then we can plot this again.

So what ends up happening all I just did with a simple simulation is for chymotrypsin we need to have larger concentrations of substrate. If you realize previously we stopped here and we are able to realize as you keep increasing oh from 0.2 to 0.3 also the value increases. Let us zoom in take trick take a look they will able to realize 0.2 to 0.3 also this changes and then between 0.3 to 0.5 it changes all by much lesser than what it changed from 0.2 to 0.3.

And of course as you go really high you will realize that the amount of change that is happening is much lesser. So what one is able to understand is that by doing very simple simulation one can discern what concentrations are required. Of course I have to also dwell a little bit on the initial concentrations if these are the points that one can actually take a slope out of so as to determine KM and at very high concentrations one can determine kcat from this assuming a given concentration of E naught.

Now that we have done this what we would like to do in terms of the numerical simulation is of course not be happy with one enzyme simulated for different enzymes. In this example what I have done is that I am although I have data for a lot more of them I am just doing it for 3 representative examples we are of course starting the code with similar things that we have seen before clear the screen we can also say close all and then you clear the screen you clear all the variables and you set up a certain concentration of substrate we just learnt we probably need more.

So why do not we provide it even we can give 2.0 right all this we have to write a comment as always substrate concentration in molar okay. So now that there has been done now you are able to realize this is the concentration once again in molarity concentration in molarity KM is in units of M which is molarity again. So what we have done here is that we have set the substrate concentration we have set the E naught we are setting KM which has the units of molarity equilibrium constant is unit less.

But then we give a concentration of the molarity meaning that to that the what base has the equilibrium constant being defined in this case this molarity. So as to keep everything constant and k cat is given the units of second inverse. So now you get all of that so now what I am trying to show here is that I am checking whether the length of KM and k cat are same if you are having 3 enzymes each enzyme as its own enzyme has its own k cat and I am trying to tell the program make sure the length of KM and k cat are similar by this line of code.

So what are we doing here we checking length check of course is the variable that we are defining we are saying length check is length of KM – length of k cat if length check is not equal to 0 then given an errors. So why do not try during that we wanted to reduce the length and see that there you know. It gives an errors saying that errors came k cat is not correct that I mention do not agree.

And then of course it as a problem as it goes further so let us go back and reintroduce when you run the program you are able to get this back up again. So what you are able to see here is that one can nicely write a program which also takes care of the variables whether they are consistent whether it is written properly or not. Okay now that we are done we will do the same thing that

that you have done so far meaning that we will use 2 for loops and in order to make sure we get our job done first loop for loop is for the KM that has been used and the second for loop is for the concentration of S that we have got okay.

So and then we calculate the velocity given by E naught times k cat times substrate concentration divided by the KM plus the substrate concentration this is what we derived in the Michaelis Menter equation. Finally what we are saying is the rate is given by each of the velocity and I am of course writing the output into another variable so that this variable can be reused. And then finally we are plotting rate as a function of substrate concentration you go here hit enter what you are able see is that a lot of different things are happening here.

You are able to realize is for one of the enzymes so let us say insert legend so you are able to say data 1 data 2 data 3. So data 3 is for the tRNA synthetase which has a huge different that comes up meaning that the initial rise so you are able to realize that the initial rise is quite steep for the tRNA synthetase well for the other enzymes it gets done very fast right. You already reach saturation probably for something like this this you have already reached saturation for the blue curve it is also trying to get to the saturation but what you are able to appreciate in this is that of all of these 3 different enzymes data 2 which corresponds to pepsin ends up reaching equilibrium the fastest right.

It gets saturated already while the blue and the orange curves are just starting to build up right so if one is able to get the initial substrate concentration if one is able to fit KM times E naught by k cat to this one which will give you a linear slope you would be able to determine what is a KM provided you get the k cat on the saturation curve. But you are able to appreciate the fact that right away that when I zoom in for the tRNA synthetase the initial concentration of substrate itself indicates the rate is not linear.

These lines are not falling in a straight we are able to see the lines that are joining them are not forming a straight line it is actually changing quite a bit. This is what comes up as a problem that one when one ends up taking different portions of the curve as detailed here to take this initial portion and the final portion and estimate KM and k cat you end up introducing systematic error. How many initial points are you going to collect maybe you do not have enough sample source

to collect a lot of points of initial curve which results in end up in ending up having a systematic bias to your analysis on the other hand.

If you are able to see some other concentration you are able to say okay this fairly is linear in this concentration regime and I can fit it and to get the ratio of k cat to KM. This depends on the system and you are able to realize by this very simple numerical simulation although for the concentration of the substrate used in this simulation between tRNA synthetase and chymotrypsin while chymotrypsin one can actually use this philosophy of initial concentration and saturation concentration to estimate KM and k cat it actually fails in this case of tRNA synthetase.

Let us try to take a look at what happens for the other enzyme interestingly enough in very few points you are very few points fitting this to a linear curve will be a grave mistake meaning that neither of the 3 points will be properly satisfied which will end up resulting in a problem well the KM will be crossly misrepresented by this dataset why am I not blaming the k cat is because k cat almost we can zoom in how much ever we want and we will see that they are really close to each other.

Remember since it is a simulation these numbers are different but what will end up happening in a real case condition is that your noise in your measurement that comes up due to different experimental artifact would be not helping you to distinguish these points. Basically the error bars and each would be so high maybe you will not be able to distinguish them. So what ends up happening is that one has to be very careful using this approximation methods such that you cannot just take the initial point and the final point and estimated.

A better way of doing it is to fit all of this data points so as to get whatever you want we will be taking a look at data fitting in a moment. But before going ahead what you are able to realize and appreciate the fact is that this simple simulation has helped you understand how much data points are required for instance let us try to see for this red curve since it is not in a linear regime we can actually add more data points in a earlier time or earlier substrate concentration.

So let us try to say 1 E-6 3E or 1E – Phi and try to say how this curves look alright so now let us zoom in. So now if you are able to zoom in the you are going to realize at least of few more data

points came into place right. If you are able to see the initial rate at least as a few points that tends to fall in the curve we cannot add even more points to this and see what ends up happening there you are.

Now you at least have 4 sets of data points which has still not linear but is able to give you something as linear as you could get on the other hand if you see for the same thing for this one it is so linear that you actually do not bother these points are not required for this enzyme right. So what we are once again able to realize is that one has to carefully iteration in the concentration because remember the enzyme could be expensive the substrate could be expensive performing the experiment may not be easy right

So there are lot of problems that come which one with a very simple simulation that comes up by having and understanding of the functional dependence of this given equation. But having blame so much of the initial rate and the final rate that one could get from this curve why do not we take a final look at how this data can be fitted using the curve fitting tool. We have already seen how simple rates can be fitted in order to get you final parameters in this case you would like to get KM and k cat. So what we will end up doing here is that we have velocity alright.

So we will plot S as function of velocity which is for the final one okay so I will just take a given example and taking the example of the tRNA synthetase of course one can add even more number of values to these curves let us plot figure 2 okay. So this is the figure 2 we were able to see that it exactly mimics what you got here. So now let us try to see how this can be fitted with the tools that we have here.

In the spreadsheet program you are able to see that very simple functions like linear function some polynomials could be fit if you are having a function that is neither linear or polynomial it becomes a little tricky it is quiet common in the field of science where people end up telling you that okay.

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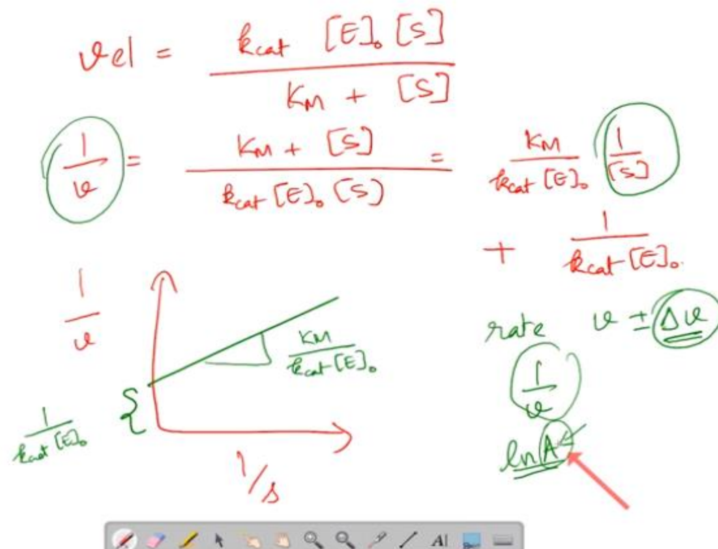

You have an equation that goes velocity is given by k cat time E naught times S divided by KM + S it is quiet tempting to take the inverse of it and reduce it to something like this 1 over S 1 over k cat times E naught. So what one might end up telling you is that plot 1 over v as function of 1 over s you are going to get a curve which as an intercept that goes as 1 over k cat times E naught and then the slope that comes up as KM over k cat times E naught.

So this is quite tempting to do because it is easier to fit linear curves but however there is a problem that comes up remember when you have a rate that you are measuring let us say V plus minus delta v taking the inverse of 1 over v how much of a small errors that you have in v will get blown over when you are doing 1 over v. So that is not a good idea right we have learnt what is error propagation and at the same time 1 over s could also have an error that is going to get unnecessarily amplified or in some cases mitigated.

For instance in the first order reaction kinetics we tend to lon of A where we take the logarithm of concentration in this case what ends up happening the measurement that you get from concentration is actually reduced in terms of the error when you take the lawn so therefore you are going to have better fit. So these are all caused a problem although these have been done quite a bit in the past that was done because the fitting algorithms were not excellent but right now we have on wonderful software to do all this for us.

Now that we have seen how to simulate Michaelis Menten curves this class will try to take a look at how these curves can be fitted in order to determine what is the KM and k cat in the next class thank you.