Quantitative Methods in Chemistry
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Module No # 06
Lecture No # 28
Curve Fitting and Simulating with Variance for the Michaelis Menten Kinetics using MATLAB

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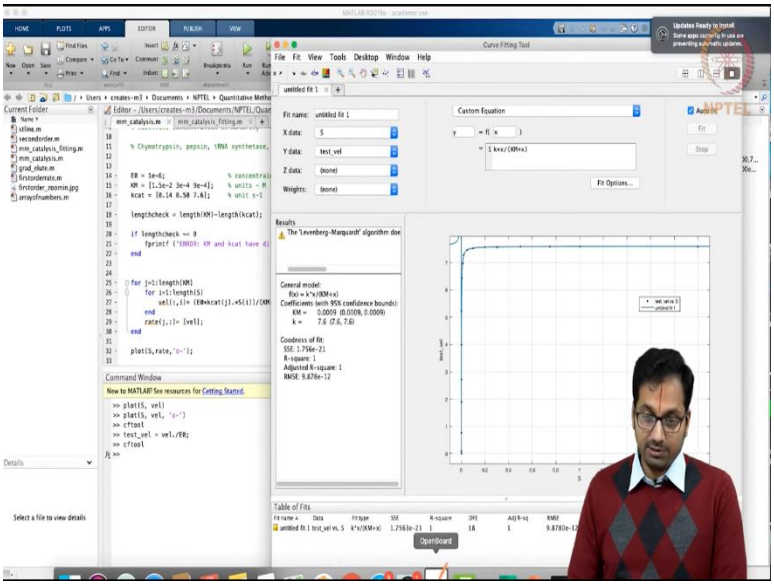

Welcome to the next lecture in this series quantitative methods in chemistry. In the last class we were able to see how using MATLAB we could simulate curves that are just not linear curve but also something slightly complicated by the Michaelis Menten curve. And we also realize that once the curve has been simulated, we have a better way of guessing whether we are sampling the right concentrations for a given kM and kcat.

This helps because if you are trying to mutate for a protein for a certain function let say the function is not supposed to get altered then one can carefully make sure this concentration of the substrates are made in such a way that this can be done. So now going to the next step once you have collected the data we have to fit it to in order to re-determine what is kM and k cat. Let us repeat the simulation that we did in the last class where I have a bunch of substrate

concentrations and this were simulated with three different kM's and kcat's for chymotrypsin, pepsin and tRNA synthetase respectively.

And what did we see let re simulated we see 3 different color here one for chymotrypsin in blue, pepsin in red, and in orange the tRNA synthetase. Now that we have obtained this, this is just a simulation let us now forget the fact we have simulated this data. Let us try to take the final one the tRNA synthetase data. Let us try to plot it plot S and then we can actually say velocity so you get this curve that we have just simulated few minutes back right.

Now remember the line here is not a fit it is just a joining the dots the data points that have been acquired. Now if one has to determine what is the kM and kcat this has to be fit to the Michaelis Menten equation. And as we saw in the last class it is very tempting to invert this that is take 1 over the velocity and plot it 1 over the substrate concentration which helps us get the kM over kcat from the slope and intercept and therefore one can determine what is kM and kcat.

This is not a good way of doing it as I have mentioned earlier because the error that comes in the velocity of the reaction is not going to be same when you going to invert it and plot in extract the information out of it. We will still doing just for the sake of completeness so that we understand how initial guesses for a certain fit can be used in order for this purpose ok.

So now that we see these are the data points they will be starting a tool called curve fit cftol gives you this graphical user interface which can help us plot any type of curve and fit it to the equation of our interest. So the x data here will be the substrate concentration and the y data here is the velocity of the reaction basically the rate of the reaction. And what you are able to see here is that the black dots indicate the data points and the blue is the fit.

Of course it is fitting with the equation that is polynomial with the first degree basically a linear equation as seen here. And as you are able to adjust the R square is extremely poor and more importantly you are able to see none of the data points pass through the fitted curve. So this indicates that it is a poor fit and we need to find something better. And since we know this is not going to be a simple polynomial equation we can go to custom equation and rewrite the equation.

That equation that we need to write is k times x where x is the substrate concentration divided by KM + S X right. Why I am not writing S here? Because the variable here is x you can always change it to S and write the same thing. And what you are able to see here is that this is having a problem largely because the y axis is having a 10 power - 6 term and it is unable to fit this. If you recollect the equation that we are trying to use we have velocity = kcat times E naught times the substrate concentration the whole divided by KM + S.

And we know for a fact that we are take the substrate concentration as a known amount in this case we used for 10 micromolar concentration of the enzyme. And then we simulated this curves. And important thing one has to understand is that this k here has that information that goes in. So one thing that a person can do is to actually change the velocity. So let us call it a new variable so we can call it as test underscore velocity = velocity / E naught.

So now that we have gotten it we can actually recall curve fitting tool and now you get an equation that takes care of enzyme concentration. This is important because if the same enzyme is prepared in multiple batches again and again and when somebody wants to determine what is their KM and kcat the fits here would be independent of the concentration of the enzyme implied. So this help in a compare in things across. So here once again you are seeing a problem that comes up where it is not able to determine the proper fit alright.

So now when I change the fit option so fit option will give you what is the start point of the fit? What is the lower level and what is the higher-level upper level of the fit? And you can always set the lower level to 0 because you are not going to have KM or kcat as is negative. So this help the fitting algorithm go faster. And for some extremely weird reason it is also trying to fit for substrate concentrations that are negative which do not make sense physically so that for lets choose to ignore the part of fitting that comes for concentration that are negative.

And what you are able to nicely see here is that fitting that is in the blue curve is nicely passing through every data point. And since this is a third simulation that was done for tRNA synthetase we are able to realize that the KM that was used which is 9 e power 9 e - 4 that is 10 e power - 4 is exactly what it is fits out. You are able to see that the fit is 0.0009 and the value of kcat which is given as K here is nicely coming as 7.6.

And as you might remember from previous lectures you also would like to know not just the average value and also that the standard deviations that would go with these measurements and you are able to see the coefficients are predicted with a 95% confidence bounds as shown in the number of this bracket. It is not surprising that these numbers are exactly that of number that comes up as an average because we are simulated this curve with no error that goes with each any of these measurement.

The same is the case is for kcat where kcat is the reliably determine. Previously when you did not divide the velocity of the reaction in the enzyme concentration the problem was the fit was trying to get the product of E naught to kcat and what ends up happening is that you are able to realize is that kcat is like 7.6 units but E naught is something like 10 power - 6 units right. So this could actually have a problem here one has to be careful to check whether we are doing all the fits that are right way I have divided by E naught not by 10 power 6 so that make sense.

So, one has to be careful when you are having a fit that is the product of 2 different numbers that you are trying to figure out. And if one of the number is already know it is a smart choice for us to actually divide the rate with the parameter this case that is the enzyme concentration right. Now that we have seen this we will try to see how the one over velocity went plotted with one over substrate concentration might help us to get this. In order to do that let us recollect ourselves what is the equation that is involved.

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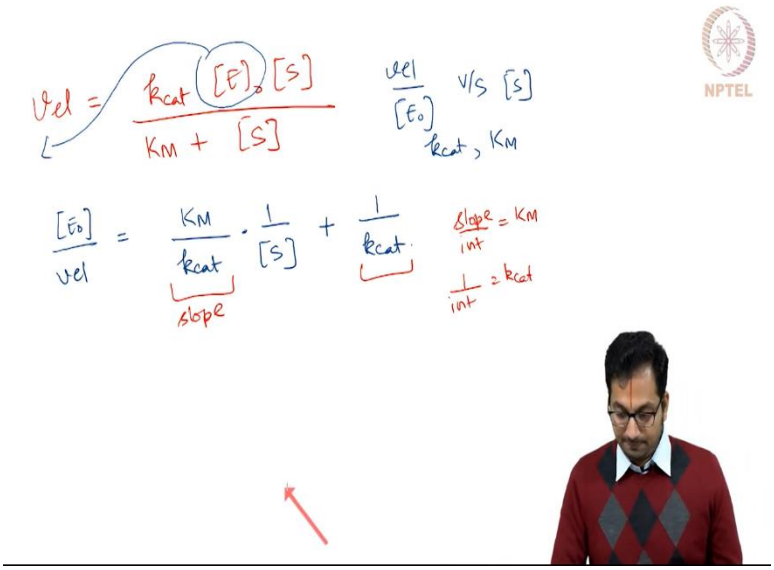

So we have velocity = kcat times E naught times substrates concentration divided by KM + S this is the Michaelis Menten equation. Of course what we have done here is to take the E naught to the denominator so that makes it velocity divided by E naught as a function of S which was plotted to get kcat and KM. So now what we are trying to say is that let say we take the reciprocal of this it is going to be E naught / Vel that is going to be equal to KM over kcat times 1 over S the substrate concentration + 1 over kcat.

So what we are able to realize is that we will get the slope of this fit as KM over kcat well the intercept will be 1 over kcat. So if we are able to take if we are able to divide the slope over intercept one will get KM slope by intercept will give you KM. On the other hand you will also get 1 over intercept as kcat right. So why do not we take a look at how this can be done.

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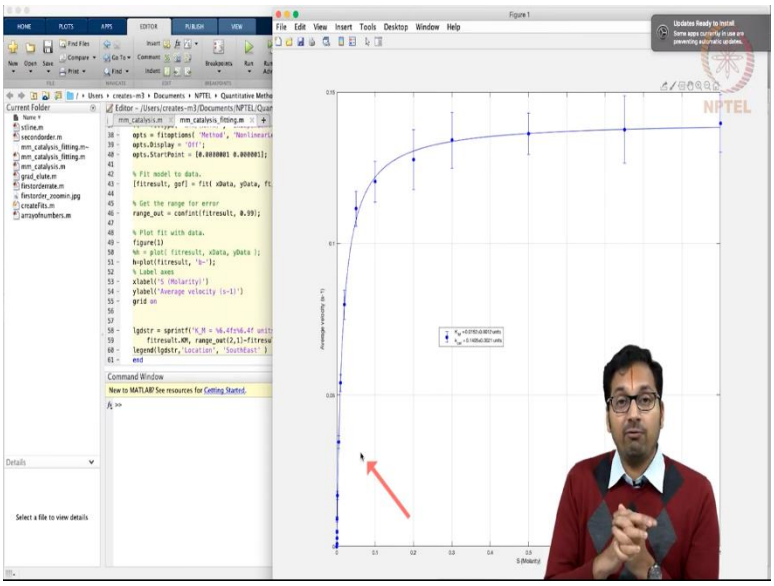

So we can say test inverse = 1 over text vel ok and we also need to have S inverse so we are going to say S inverse = 1 over dot S and lets go back to curve fitting tool and then here we can actually add another fit which will be S inverse that is in the x axis and the y axis you want text underscore vel underscore inverse. So now you do this what you are able to see already is that once again MATLAB is in a hurry it is taking all the data points you are able to see multiple data points here and here. And it is fitting with the polynomial with a first-degree polynomial well let us not do that.

What we can do is to write of our own custom equation such that it is m star x + c and then you say fit of course the fit options here might not be that required because it is able to nicely fit R square comes as 1 because we have simulated this data. And what you are able to see here that the intercept is 0.1316. So what is 1 over the intercept you are able to see that it comes of a 7. 5988. It is a 7.6 that you have simulated.

Then if you would like to know what is the other one the way you do it is that you take the intercept which is 0.0001184 divided by 0.1316. And you are able to realize you are able to get 9 e power - 4 which is what you ended up using. And the important this one has to note is that altogether the values come up similar the moment errors starts to play a role you realize that these could end up being different. That is what will be the focus of this current class. So let us go back here so I have written another code to perform the same.

So let us take similar type of concentration that we would like to work with for a sub enzyme. And this time we are taking the chymotrypsin an example so what is going to end up happening is that we are able to understand and in the initial portion of the lecture also we were telling that the repeats a matter. One has to repeat the experiment number of times we will understand many different aspects by this stimulation how repeats help you understand what kind of values come. And how do they help in determining the precision better.

As always, we started clearing the screen clearing any variable that might have existed and clearing closing all the figures that might be present. And these are the set of values that we end up using for concentration of the substrate. And then we are going to repeat this experiment 10 times let us start with 2 time reputation. And here one again we are using enzyme concentration as 1 e power -6 that is 1 micro molar and molarity is the concentration that we are trying to use.

If you might remember the initial lectures where we were trying to understand the Gaussian distribution basically the normal distribution we use this kind of function called makelist. We are trying to do the same thing where this time we are trying to noise corrupt the Michaelis Menten constant that is KM and kcat with their average values. We are still using the same average value that has been used for chymotrypsin earlier. Let us see what was used 0.15 0.015 sorry 0.015 and 0.14.

So we are still using the same value but what we are trying to say is that we are trying to say for KM the third decimal has an error or uncertainty associated and it is simulated as a normal distribution. And the other hand for kcat also we are assumed the same value but we are assuming the second decimal here has an error. So what you are able to realize is that the error propagation is nicely taken care by this where we will be assuming random error for each of this variable to basically both KM and kcat vary randomly and are not correlated with each other.

We can also make them correlated but let us not worry about such a case right now. We can assume that purely KM and kcat can be randomly varied. And then we are repeating the stimulation the same way just that here J goes from 1 to the number of repeats. Let say you are repeating this experiments 2 times now this will be this protocol will be repeated twice where the rate of the reaction is calculated as E naught star random value of kcat. So basically what it does

is that now let us try to understand what is this random distribution? Let say clear any variables that exist let say x = makelist and then we can say normal that means it is Gaussian distribution.

Let us keep it at 0 and we can say 0.1 is the standard deviation associated with it. So then we say random x what MATLAB does is to randomly pick a value with an average of 0 and a standard deviation of 0.1 across many such numbers. So you can keep repeating this random x and you are able to realize that the value is coming quite difference each of the time you are stimulating. So this is nice way to stimulate a normal distribution of error for a given average value of the variable that you are trying to look at.

And So whenever I call random x it is picking a certain value. So if you are able to realize here the random value of kcat is picked and the random value of KM is picked. And these 2 are very different processes and when you repeat the same random x multiple times you do not even see the multiple numbers coming up. Similar if you repeat the n number if you take the average in the standard deviation you will finally converges the value that we have given in this case which is 0 and 0.1.

So similarly this is the case for KM and kcat where you have certain average in a standard deviation that has been given and it is simulated with a normal or a Gaussian distribution. So what ends up happening now the velocity is now stimulated for very substrate concentration and the enzymes concentration is kept constant you can also introduce an error there that could also be done. And the random value for each KM and kcat will simulate a certain specific value of rate.

And as the concentration of S is increased this kcat is also randomize so as KM. So therefore we going to randomly determine the rate and then of course as we did the last time we are also trying to get the rate saved for all this repeats. So that we have a memory of how many repeats when through and that goes finally in to the value rate. We can always change this rate into cumulative. So that we understand what is variable is? And next step what I am trying to do is to divide the cumulative rate with the enzyme concentration.

So as to normalize the plots that might end up coming so that finally when you fit up you just determine KM and kcat and you would not have the problem of fitting the concentration of the

enzyme as well. Then the next step what I am trying to do is that for the length of S meaning that how many ever times repeats that happen I am trying to get the average of the cumulative rate. Basically this cumulative rate right now will be 2 / 11 matrix because we are having 2 repeats and 11 concentration of substrates maybe even more since I added a few.

And now it will find the average for these two repeats. When you are doing 10 times this will be 10 / n where n is the number of substrate concentration it will be 10 / matrix and now it can determine what is the average and the standard deviation each of this separate test. And then what I am trying to do is to plot the error bar. Let me not fit this curve for now so that we understand how we can fit ourselves using the curve fit tool so all I am doing is to plot this figure.

So let us see how it looks. So there you go. So what you are able to realize is that as of function of substrate concentration you have measured the rate normalize to the concentration of the enzymes. And you are nicely able to see the every data point has its own random error that comes. This is not a constant error or a propagating error whereas as you keep increasing the concentration the error increases that is not happening here because the highest of concentration has lowest error and something in the middle has a lesser error that are one that is previous and the next point.

So this indicates that there is no correlation between the error that comes because we have randomly simulated KM and kcat. So now let us go to the next step and try to use the curve fit tool you can erase any of the previous fits or we can still continue to have them. So in this case S is the substrate concentration and you want something like the average rate of fit. So what you are able to see you do not see the error bars here. All you are able to see is only the average value.

So we go back here and then say custom equation. So the custom equation what do we end up writing k star x / KM + x alright. So now what you are able to see in comparison to what ever fits we got in previous time where there are no error involved. In this case you are able to realize that the fit almost passes through all the data points but some of the data points are out of above and below.

Of course the moment you start introducing the error into this you will be able to understand yes the fit does satisfy the data better. We will be fitting this and showing the plot simultaneously in a moment but let us take look what values ended up coming. So in this case what is happening is that for 2 repeats that you perform the KM although you simulated KM with 0.015 as its average value. The average value end up coming as 0.0127 which is less than the value you simulated and the 95% confidence interval that is within 2 standard deviation on either side goes from 0.01065 to 0.01475.

So what you are able to realize is that this value is slightly far away from what you simulated and that comes up because of the error that came up in KM and kcat. Let us take a look at what was KM? KM we was provided the 0.14 and we are getting something like 0.1364. And the range between 0.1324 to 0.1404 and this is quite interesting because you nicely gave an average in standard deviation here.

So one would assume that or one would anticipate that the average of KM and kcat come very close to wherever you anticipated and the error nicely represented. This is not happening largely because the number of repeats that you have done is extremely low. Why do not we repeat the same thing and instead of doing it for just 2 times so why do not we do 10 times? So now you are able to see nicely the error and all the other values that also coming into play.

So let us go ahead and fitted again alright so now what ends up happening is that for 10 repeats to ended up doing you are able to realize that the KM is coming very close to the KM that was provided. And probably the error does end up happening in that decimal. Basically it goes from 0.0146 to 0.0169. So you are able to realize that the lack of confidence comes in the third decimal and in this case which is what you ended up simulating.

If you have even more number of simulation this might get better. We will take a look at that in the moment and nicely the kcat average comes up to the value ended up giving and in fact the error probably is in that decimal that you have provide in the second decimal. If you realize 0.1385 is 0.3 0.139 and 0.142 is 0.142 so you are able to realize the error comes in the third decimal.

So this once again illustrates the fact that the more number of repeats that you are able to perform you would be able to get an analysis of the error that goes nicer. And one important fact one has to realize is that in this case the fit almost passes through every data point that one is able to get. Only a few data points are further away. In order to exemplify this why do not we repeat this again. So we are repeating with 2 sets of repeats and then let us get average rate again.

And you are able to realize that the some of the deviation that come even the R square might be nice and indicative of the same but you are able to see visually how many points go further. So now wanted for the sake of understanding this better we could stimulate this, so many number of times that now the fit will also come up beautiful. See you are able to realize as the number of repeat start to get better the fit nicely goes through the average values.

Almost only this data point is slightly off but that is not big deal and you are able to realize that the confidence intervals the range of values that comes after fitting reduces significantly same is the case with kcat. And of course your R square increasing but be a little careful about the R square we will take a look in a moment what happens there. Why do not we repeat the same process that we did earlier of doing the 1 over S plot.

So how would we do it we will say S inv = 1 over dot S and then we have a cumulative rate actually we have we have a average rate that we would like to do. So average rate inverse = 1 over do we take it 1 over average so now let us go back and fit it. This the third fit, remember we had 100 such data points which going to look very reasonable and you are able to realize that the intercept which is p2 comes up as we can actually go back and write our own equation that is a good practice.

Write a custom equation of m star x + c this is y = mx + c the standard plots that we have. And the intercept is a 7.898 so we have taken the inverse of that so what is 17.898 that is 0.1266 so you are able to realize that there is the difference that comes in kcat. You wanted kcat of 0.14 the error in this slop or the intercept is significant. You are able to see the error in the slope goes from 6.943 why do not we invert and see that 6.943 that is 0.144 and 1 over 9.303.

So you are able to realize all though you did 100’s simulation when you are inverting the plot to get the fit although the fit is fantastic we are able to realize your kcat goes from 0.10 to 0. 144.

So this indicates the fact that although your fit is great although it is easy to fit linear curves you are able to realize that when you take a reciprocal of the model the noise seems to be corrupting the data when you are getting it back.

So this is an important inference that one has to have in mind. Why do not we take a look that what happens for KM? So KM is determine by getting 0.1051 / 7.898 and what do we get it comes up as 0.133 and of course remember you also have a range that goes here and other question comes are you going to be dividing this with this value, this with that value. So you once one starts to understand the error that goes in the slope and they intercept it goes in to propagate and result in more error in the KM determination.

So I leave it to you guys to determine how this can be propagated and measured what goes on when you are mixing and matching different values. So one has to be very careful when data's is obtained and one fits. And this case we are able to realize that even with 100 repeats this is the problem. Why do not we repeat the same linear plot it is just 2 repeats. We can generate this in same way and then let us try to fit this in average rate.

And here you are able to realize that it starts to go much further away. And the range of this intercept that we got previously we got somewhere between 6 and 9 and now we are getting 4 and 13 so it starts to get extremely poor. Even m the slope was better determined the previous time with 100 repeat now it is getting worse. So I hope this make you understand it is not a great idea if you know the model precisely it is not a great idea to invert it to linearize the plot and get your final values and it is better to use them as it is to fine the actual errors that propagate.

Now that we have determine this why do not we take a look at how a simulation can be done in a single way and the fitted values have come up in a single plot. Once again I am showing it in the value of simulation hopefully the later week we can try to do an experiment get an experimental data and fit it and go forward with that fitting in MATLAB itself. So now what we can do is to have same 2 repeat and the code that I have hired ended up muting right now I will erased the if statement the same thing that we ended up doing in the curve fit tool can be done in terms of command prompt as well.

So I like to show how MATLAB is used for even right codes although we are not knowledgeable in generating the codes. For instance let us say you have this code that you ended up fitting the average rate what one could do is that one can actually go to this file menu up here and then say generate code. The moment you click this what MATLAB does nicely for you is that it is nicely generates the code of how this fit has been performed with the different variable that you ended up providing.

If you remember what did we do? We took x data as a substrate concentration and y data as the text underscore velocity which we ended up simulating. And once you get it and we fitting this equation with the fit type of k star x / KM + x but the independent variable is x and dependent variable is y. This is where I said if you like to use independent variable of S you can do the same thing you can remove the x here and make it X where you determine what is x.

And the algorithm that is used and the fit option that one ends up using can also be mentioned here and if you realize all this were present in the fit options here. You can change it in the graphical user interface but when you are having a large set of dataset to handle let say the same Michaelis Menten curve is done for 10 different enzymes at multiple different concentration at very different temperatures to determine other thermodynamic parameters then one might start to actually write script such that the data is read from the excel sheet as I had shown earlier and then MATLAB used towards such fitting.

So this same thing we ended up using here is in fact can be written as a code so if you see here the method that is used here is non-linear least squares fit and the algorithm that is used here can be changed to Levenberg Marquardt. These are different algorithms are not going to go into the details of it I am sure if you research a little bit you will able to understand what is the difference between trust region algorithm versus Levenberg Marquardt.

And then whether to display the data points and I mean what are the lower limit so basically we can end up changing the lower limit so that the fits can start wherever it is. Now coming back to the fact where could the linearized fit could be useful basically where can the 1 over s and 1 over velocity fits could be useful is that you can give reliable static points. If you many times this kind

of fits here we have only fitting 2 variables but you could be dealing with equations that are multiple variables that go into them.

So in such a case what end up happening is that it would be a good idea to have a reasonable guess of where you are starting point should be as you are giving here ok. So the starting point would help you end up converging the fits faster. Remember although all these are done by computers these are indeed memory intensive. So at times, where if you are able to bias it carefully then one can give the starting points and the lower and upper limits within the safe constraints.

For instance in this case you know for a fact that the KM and kcat cannot have values that are less than 0. So in which case what one could do is to say set the lower limit to certain number. Let say that you mutated the chymotrypsin change the kinetic and if you know for a fact that you not going to have reaction rate that is much higher than that of the natural enzymes then you can put a higher bounds also to whatever is already known in the literature.

Of course you can go let us say 10 times more but any value less that that is what is going to be probe. So what you are going to realize is for 2 variables you need to set multiple parameters such as the start point the lower limit and the upper limit. All this could be educated guesses so as to make sure your convergence goes much faster. And these are the fit parameters that you ended up providing in the script and you what you are trying to say is that go ahead fitted for me and give me something called a goodness of the fit.

So basically the 95% confidence intervals 98% confidence interval are all stored in this variable called goodness of it. And then what it is trying to it is trying to say ok after you have done that plot the fit result to the x and y dataset that we have done. So if you run this of course you need to save the fit it is having an error I am not sure ok that is because we have rewritten the test underscore velocity.

But we will be taking a look at it in a moment where you will able to see that the plot of the fit to the x and y data that we have provided is nicely done. And the legend is provided and where should the legend be kept. So basically, it is kept in northeast top right corner of this and what is

the x label or what is y label where it will keep the grid on and how to prepare the script also. So all this comes up so as to it actually generated the fit for all the 4 fits.

So what will that happening is that since we have 4 fits here it ended up creating it ended up creating the script for all of them. So what one to do is that instead of doing this after you prepare the fit for one of them you could generate the code understand how the code goes and you can also write the loops such that if you have 10 dataset it can loop through the number of dataset make the fits and finally give you the values.

So that is what you are going to take a look at and this is the script that I have given. So what I am trying to do we are once again using the same equation keeping the same substrate concentrations. So we can have any number of repeats let us say we have 10 repeats and then enzyme concentration is kept at 1 micro molar. We are keeping the same values that we have used for chymotrypsin KM and kcat.

And we are repeating it several times so that velocity gets determine 10 times the cumulated rate is saved within that. And the average in the standard deviation from the cumulated rate is used towards showing in error bar fit of concentration of enzyme to the average and the standard deviation. After having done that the next thing that we do is to prepare the curve of S and S in the x axis the average rate in the y axis and then fit it with the Michaelis Menten curve as I had shown earlier with the start point and all that.

You can actually keep the start point as 00 it his case. I have also generated the code from the MATLAB so picked up from something like that. And then the goodness of fit is given here and now I am also determining what is the range of answers I got? And I am saving it in this variable called range underscore out ok. And then I am plotting the figure with fit result if you remember I had already plotted it with the error bar and then now we will be plotting it with the fitted result.

So let us once again keep this portion of the fitting off so that we can see what is that we end up getting let us run this. So what you are able to realize is that the average and the standard deviation are nicely provided. Just to exemplify the point if you only do 2 repeats you will

realize that the error is quite haphazard. You will able to realize that the error occurs different measurements is quite variable because you just take 2 measurements.

Remember the concept that we introduce you that the sample and the population. This sample is a very small subset of the population you might not properly represent the average mean average of the population with the mean of the sample. So it is always a good idea to have a good number of repeats. So we get something like this as you increase the number of repeats the error bar will slightly look very reasonable across all the points that we get.

And remember once again we are simulating here and reality you would be performing the experiments to get the error values. Ok now that we have seen this why do not we see how the fit also works out ok. So now what we end up doing is to run this where the starting point is slightly changed instead of 0, 0 which results in an error. I just put it to a very small value you can add a more zeros so that you ensure that you will get the right KM and Kcat and there is no biased.

And what ends up happening is that the same thing I ended up writing in the script it is able to plot the data points. So let us take a look at it. It is able to plot the average velocity as a function of a substrate concentration and it is showing the fit in the blue line with the datapoints with the blue circle and the error that goes with each of them. So one has to understand here the fit that comes up has to pass through most of the data points. And if you remembered when I showed you the cftool you realize that the fit did not pass through each of the point but was very close to it.

And here one is able to understand that the error that is associated with each of the measurement. You see this data point what ends up happening is that let me zoom in a bit so you can see it better. What ends up happening although the fitted curve does not pass through the data point. You are able to realize within 2 standard deviation gets very close. Within 3 standard deviation it is going to fall. Once again this helps you to understand what is precision mean?

Apart from that data point if you are able to take a close look all of them fall very nicely within the fitted curve. We can repeat it again and again. You realize that now when we repeated the second time unlike the last time this time all the data points and the errors nicely falls. So this clearly illustrates the effect last time this is the data point that is the problematic. So this clearly

illustrates the effect that the when the person repeats the experiments multiple time the true value does come out in the average standard deviation.

And that is why it repeats a matter a lot okay. So now that we have understood this the same way the fitting can actually be changed to other parameters as well. So if you want very high 3 standard deviations you would be able to get this values also. And one thing that I am able to do here is that if you pay close attention to the legend that I end up plotting and I am able to get the KM and kcat values as well the average and the standard deviation.

So one can actually fit curves at a much faster rate by doing this than by using a graphical user interface. So let us recollect and think about what has happened so far. Remember I actually did not go through the step of how the initial rate is determined for each of this substrate concentration. So one of the examples that we could try working out as an assignment for this week could be where the rate is given for every substrate concentration for a given amount of enzyme and we can get the initial rate.

After you get the initial rate you can try to plot the velocity normalized to the enzyme concentration of the function as a substrate concentration fit kcat and KM and get these values. Therefore you finally end up seeking the true value that you would like to measure. So I hope these set of lectures this week have made you understand that using computational tool like MATLAB would help you simulate some data simulate functions that you might not be very comfortable with to start.

But then as you start simulating the more and more as you start repeat them you understand which variable is sensitive to the parameter that you end up measuring. And at the same time you get a complete hold of which are variables can vary and which would end up determining the final fate of what you end up fitting. So all this I hope has given you an understanding and also basic introduction towards how data can be simulated and fitted using a software like MATLAB. Thank you very much.