Quantitative Methods in Chemistry

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Lecture – 50
Course Revision (Week 06 and 07)

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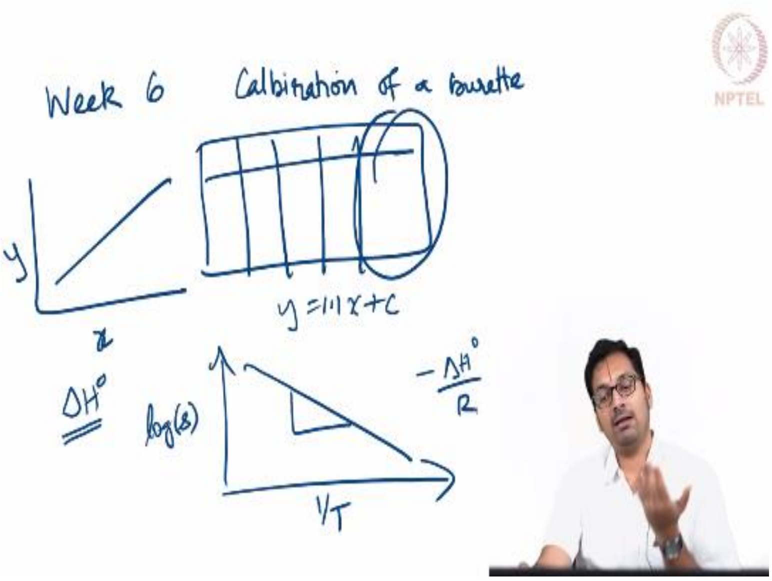

Thank you Aasheesh. So now that Aasheesh has completed his fourth and fifth week. In the sixth week what we started to do is that okay so we are talking so much about data, so much about understanding what data means and how to check the validity of the data with these very sophisticated different models. So in week 6, I started by trying to teach you guys with a very simple example of calibration of a burette.

We once again opened and enter your experimental protocol where we were checking where all systematic error would come from. After having done that we had a nice tabular column where one ends up calculating different variables and finally understanding what error comes up in the final measurement. So we ended up dealing doing that as an example to understand how very basic linear curves could be fit for something like y = mx + c type curve.

And following this week we were able to even take it forward where we wanted to understand linear (()) (01:36) by determining the heat of solution of Benzoic acid that means if you are adding a certain amount of Benzoic acid how much of that gets resolved at a certain temperature then we employed the (()) (01:47) of equation where we had the solubility the log of solubility as a function of 1 over temperature.

Then we get a linear curve which we fit to get the slope where the slope was able to give as the heat of solution. So in these 2 experiments we once again spent a lot of time understanding where all systematic errors could come up and how to avoid these carefully.

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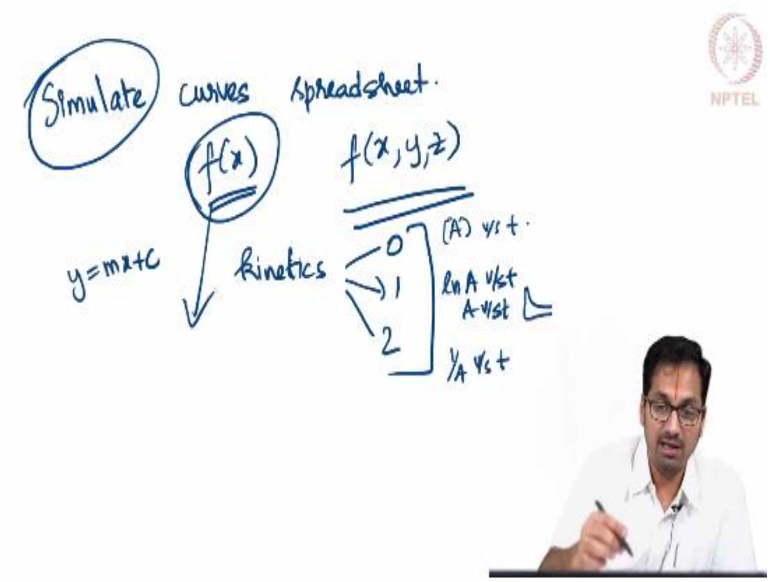

After having done these 2 examples the next step that we embarked on was to learn how to simulate curves using spreadsheet program. This was important because many times students end up having a phobia against equations and the main reason why we would like to simulate curves is that when you have a certain function f of x we would like to see what is the dependence of this as a function of x.

And more importantly when you have a function that is of multiple variables you like to understand where all it is maximum and minimum by plotting this as a function of x, y and z. So this was basically done in this week where we once again took very simple examples of y = mx + c and following this we took up examples of kinetics where you had zero order kinetics, first order kinetics and second order kinetics.

We simulated each one of this and after having simulated them we fit them to the curves of where we did concentration of A as a function of time then you had first order rate kinetics where you have lawn of A as a function of time or even A as a function of time which will be exponential function that you end up getting. On the other hand, second order was 1 over A as a function of time in order to understand what kind of (()) (03:30) ended up coming.

So using spreadsheet software which is freely available nowadays using cloud platform we were able to understand how to simulate curves and this forms an important aspect of understanding science because not always one is able to understand the functions that he or she is dealing with. So therefore if you end up plotting you will have an idea of how it ends up behaving.

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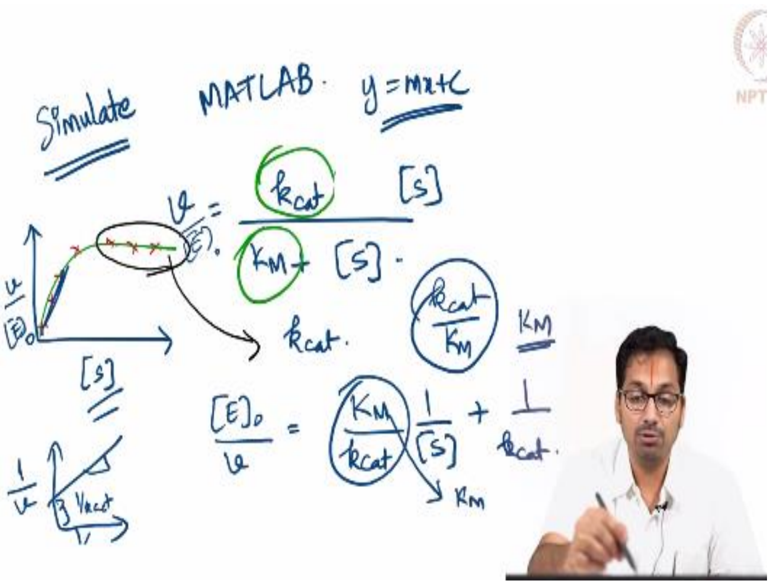

And in order to go a little faster we started to simulate more curves where we used this software called MATLAB. Using the MATLAB, we were able to understand once again how to simulate linear curves first which goes y = mx + c. After having done that we embarked on functions that are even more complicated than that. During this time, I introduced you to the Michaelis-Menten equation where you have velocity of the reaction that goes as kcat times E0 divided by kM E0 times s kM + s.

So basically this is one of the equations that we are able to understand by plotting and simulating where you had a plot of velocity of the reaction as a function of substrate concentration and then of course we end up fitting up in order to get the 2 parameters that are of paramount interest and here we spend a lot of time to understand our analytical thinking and how quantitation could vary for the same exact plot.

Meaning that there are different ways of doing it if one ends up taking the final points let us say we are able to plot v divided by E0 as a function of s. So what will end up happening the E0 will come here. So using this we were able to understand if you are estimating v / E0 at large concentrations of substrate you realize that you will be able to estimate what is kcat. Once you are able to estimate kcat if you have the initial slope which will give you something like kcat / kM one will be able to estimate what is kM.

And this is important because in this way you will be able to determine the 2 factors that help you characterize an enzyme. On the other hand, other way of doing it is by taking the lawn plots meaning that or reciprocal of this not the lawn plot. Due to one over of this equation so you are going to get something like when you take the reciprocal of this equation and then let us say you plot 1 over v as a function of 1 over s.

Then you get a linear line where the intercept gives you what is 1 over kcat from where kcat can be estimated and using the slope one will be able to estimate kM. So you have already been exposed to do different ways of getting the same number.

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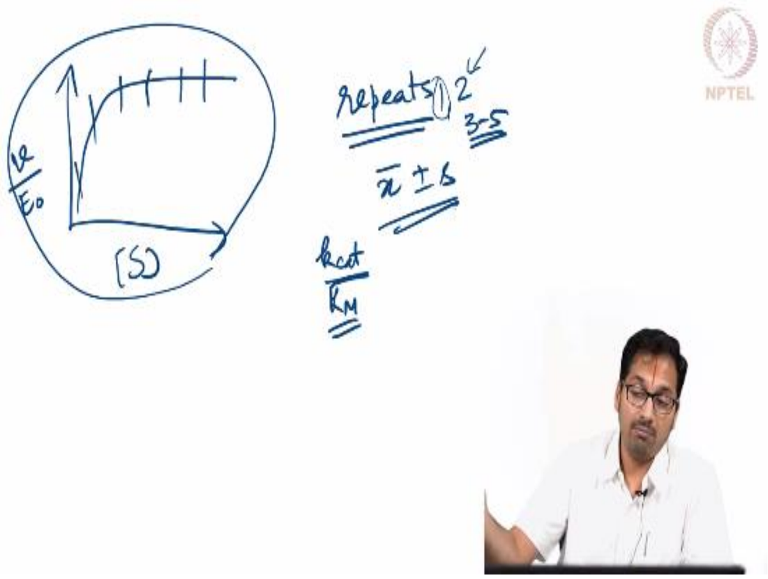

However, the exact way of doing this would actually be plotting v over E0 as a function of s where you get this curve and are able to fit it using MATLAB. This was one of the important things to understand where we realize this kind of a fitting protocol gave you the minimum error although you just simulated all curves with (()) (06:56) standard deviation or error you realize that when you followed these 2 protocols you ended up estimating very different values.

So this was an important piece of understanding that one ends up getting where when you are able to simulate the curve and refit it back you help yourself in understanding how the function behaves where are the functions very weak and where are they robust enough for you to fit and get the data back. So this was a very important piece of understanding how quantitation goes in any part of chemistry.

And therefore simulation ends up being an important aspect in it. Finally, we were able to understand how repeats help in getting what is an average and standard deviation. Once you get this you are able to plot each curve with a certain amount of error that goes in and therefore the kcat and kM that one ends up measuring also comes out with such an error and we realize that performing only one or two experiments to get average and standard deviation with one you cannot get an average and standard deviation.

But 2 you would be able to get it, but that is not a good way and if you are able to perform an experiment at least as a triplicate or by about 5 times it helps, but not always this is feasible depending upon the conditions of the experiments it becomes very difficult. It is just not restricted to Michaelis-Menten equation almost any aspect of practical aspect or even computational aspect.

One should be able to repeat the experiment several times to understand what is that we are measuring and how broad is the distribution that we are trying to look at. So these 2 things made us understand how simulating a curve and fitting it back in many, many different ways would give different values. So one has to be very careful and the way one fits and remember the aspect that we were able to understand also using the simulations.

That once you take a reciprocal the error that ends up coming in v is not is actually being is mitigated meaning that it is being reduced significantly. We have a large error and v measurement one over will be a very precise measurement which ends up giving very good fits, but the error on kM and kcat are significant. With this knowledge in hand the next few weeks Aasheesh gave you lecture on trying to understand how different separation techniques could be used.

And how mathematical frameworks would help you understand how these chemicals interacted to one another for purification and with this my lecture series came to an end and I hope you learnt quite a bit on understanding how to simulate and how to quantitate and what do numbers and data mean. I wish you all the very best and please follow up in the next few minutes so that Aasheesh’s (()) (09:31) for a eight to 11th week gets completed. Thank you.