ENZYME SCIENCE AND ENGINEERING

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LECTURE – 7

**KINETICS OF ENZYME CATALYSED REACTIONS**
(CONTD.)

So in the last lecture we had discussed Michaelis Menten kinetics as applicable to enzyme catalyzed reactions and also the proposed modifications by Briggs and Haldane where by he confirmed the formation of enzyme substrate complex and the steady state hypothesis with respect to the formation of ES complex and that the enzyme substrate complex is in steady state soon after the reaction starts.

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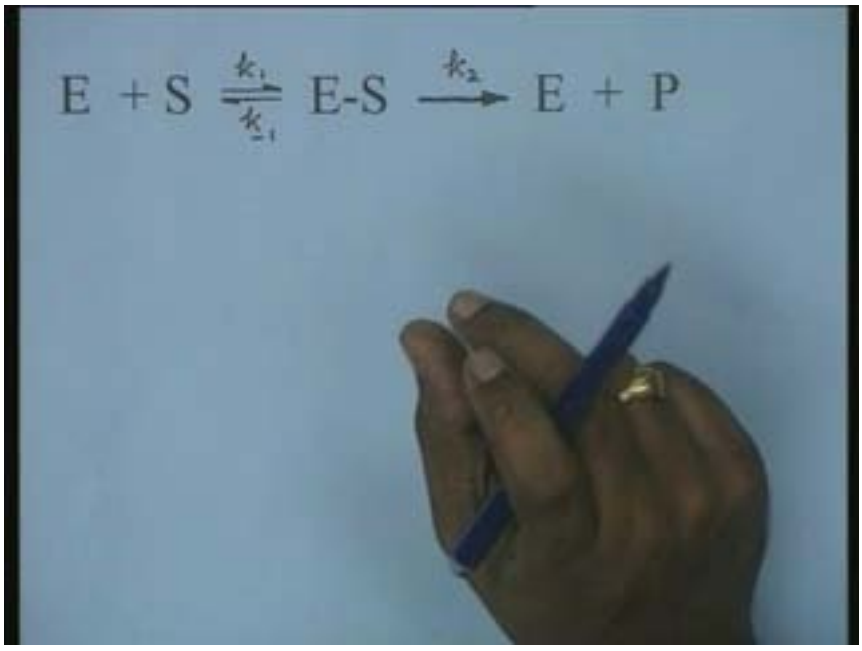


After a very brief induction period the concentration of enzyme substrate complex comes to a steady state. That was initial hypothesis and based on this hypothesis we had also arrived at a rate expression

$$v_o = k_2 E_0 S/K_m + S$$

I am just writing here only the capital letters for the concentration terms without putting the parenthesis and

$$k_m = k_{-1}+k_2/k_1; \quad V_m = k_2E_0$$

These two parameters that is Michaelis Menten constant and the maximum reaction velocity can be used to characterize an enzyme. I also pointed out that $V_m$ is not a constant; it's a function of enzyme concentration and we must take a corresponding kinetic parameter as $k_2$ which is often called as turn over number.

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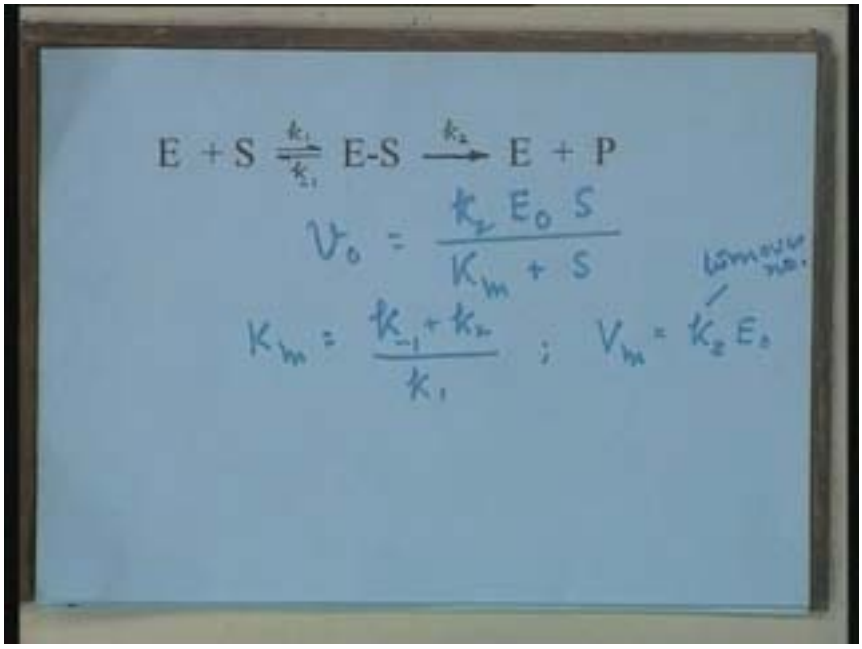


One of the most important features of these kinetics which I like to reiterate are the assumptions that are involved in arriving at these rate expressions and I would like you to clearly consider them while applying this kinetics to any experimental situation. As we also mentioned right in the beginning, that any kinetic analysis will involve the match between the hypothesis, the rate expression and the experimental data. Therefore whenever we look at any experimental data you must ensure that under certain set of conditions the assumptions that are built in, in the Michaelis Menten kinetics are true and they are met.

Just to reiterate the assumptions, the major assumption which were made here are formation of E-S complex. We have assumed that the enzyme has a single substrate binding site and on this the substrate molecule binds and E-S complex is formed. This also limits to a single substrate reaction. The kinetics is basically developed on single substrate enzyme catalyzed reactions. It doesn't directly interpret the multi substrate reactions which are so common in the case of biochemical systems. The third assumption is it talks of initial reaction rate and when I say initial reaction rate the emphasis is that

the product concentration is almost zero. That means we are talking at the rate when there is no product formation. So inhibition by the product or the effect of product concentration on the reaction rate has been negligible. Fourthly we also assumed that the substrate concentration is much, much larger than enzyme concentration which is in most cases a very true assumption and the enzyme concentration being a catalyst is very, very small compared to the reactant as is also the case in most of the catalytic reactions.

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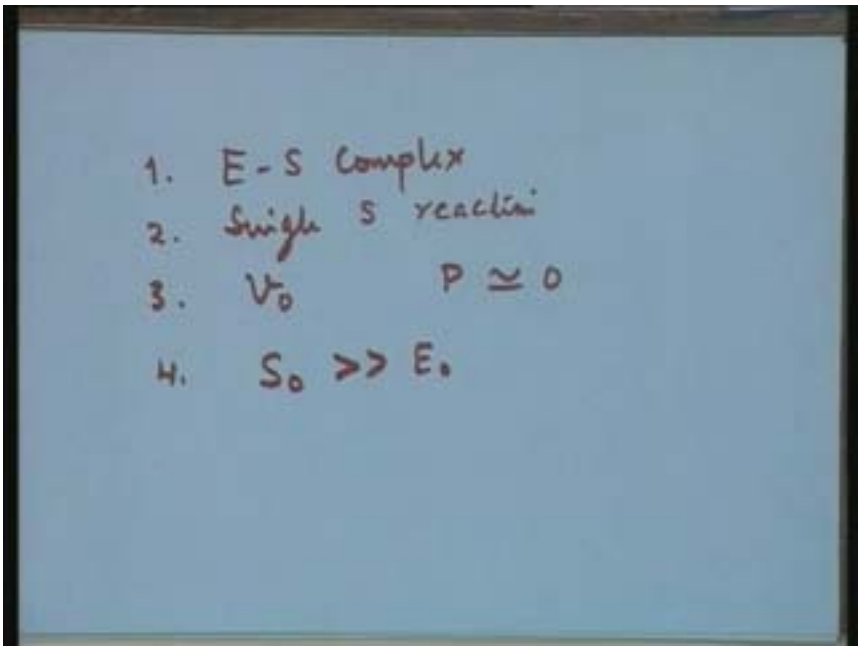


The implications of this assumption are the fact that when we monitor the reaction rates we can safely assume that $S_0$ is approximately equal to S. That means substrate concentration at any given time because not much of product is formed and therefore such an assumption can be held true.

Briggs and Haldane hypothesis was regarding the steady state with reference to E-S complex. But this is a very loaded statement in the sense particularly a deviation from the original Michaelis Menten hypothesis which states of equilibrium of E-S.

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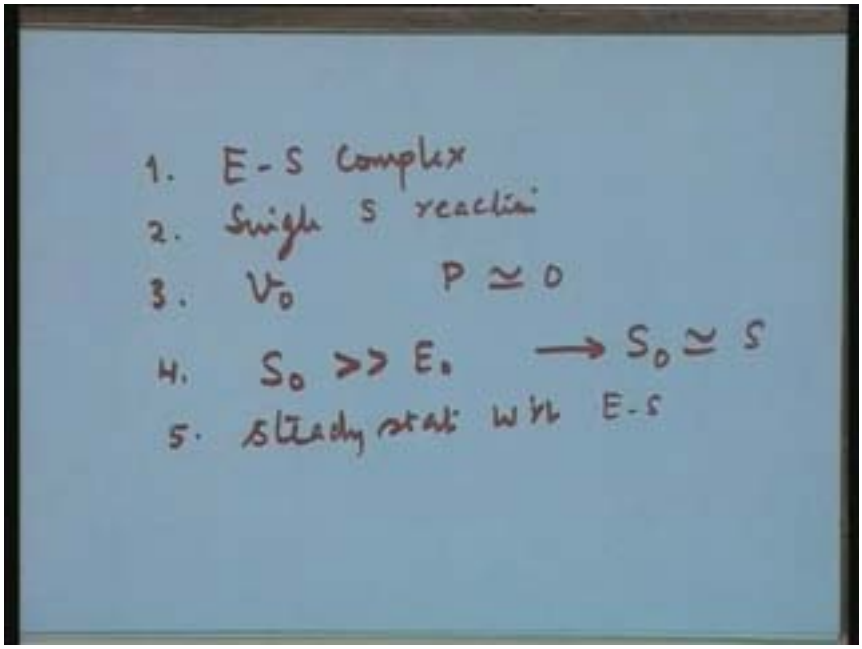


Here you must appreciate that when we talk of equilibrium of enzyme substrate complex with enzyme and substrate molecules it means that the break down of enzyme substrate complex into product is a very, very slow process and it does not disturb the equilibrium process of the enzyme substrate binding. That was the original concept of Michaelis Menten.

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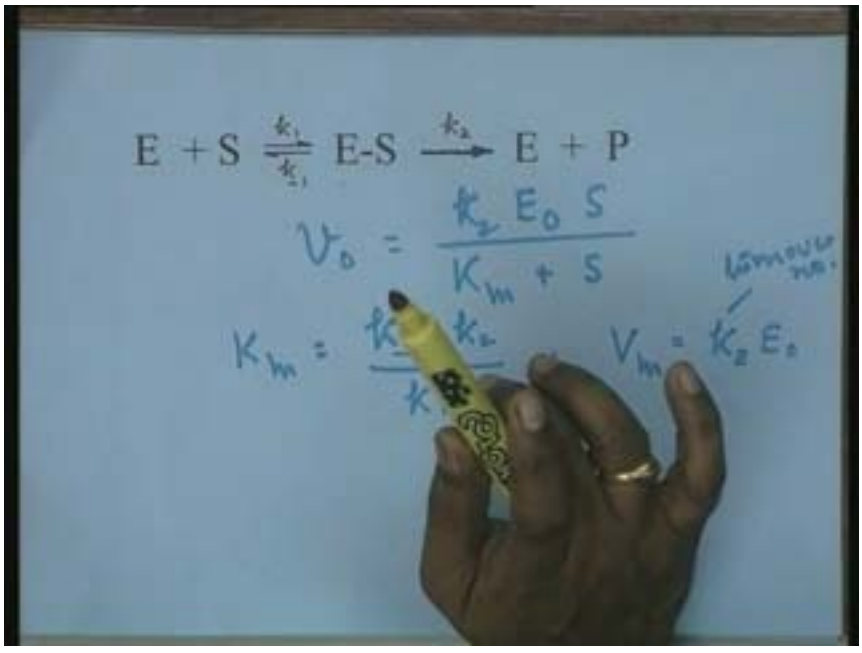


But this was disputed by Briggs and Haldane particularly observing certain reactions which were known to be relatively fast. Then you cannot say that the break down of E-S

into product is a very slow step and a reasonable rate of break down will disturb the equilibrium. Therefore he mentioned that the rate of break down of E-S and rate of formation of E-S are probably the same and E-S remains under steady state. So the rate of change of concentration of E-S is zero and the steady state assumption. That is a very major understanding which we must appreciate.

Finally very simplistic assumption is that we always monitor or study or consider these kinetics in terms of constant pH, temperature, ionic strength and all other environmental parameters we try to keep constant.

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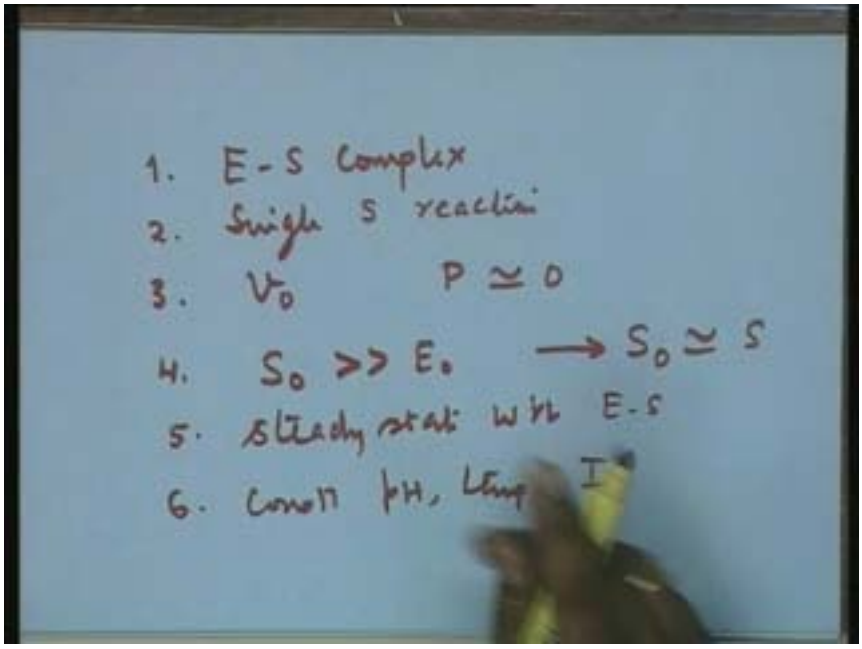


May be some times water activity and all those parameters are maintained constant. Any kinetic analysis should be made keeping in view the mechanistic hypothesis, the rate expression and the experimental data.

For understanding or for probably analyzing the experimental data for any given enzyme catalyzed reactions we can simply use Michaelis Menten hypothesis directly. But the direct use of Michaelis Menten hypothesis or Michaelis Menten equation has a limitation because it represents a rectangular hyperbola where the maximum reaction velocity is an asymptotic situation and you can never experimentally reach that. Therefore it often gives problem in determining the various reaction parameters particularly the $V_m$ and the $k_m$ values. So therefore this simplistic way as we were also talking yesterday was to linearize the Michaelis Menten equation and one of the simplest ways to linearize was double reciprocal plot. That means you take reciprocals on both the sides of the expression.

$$1/v_0 = 1/v_m + k_m/v_m.1/S$$

Such an equation which is double reciprocal on both the sides of the Michaelis Menten equation represents a straight line and the experimental data can be easily analyzed based on 1/s vs 1/v profile. The expression will represent a straight line with a slope of $k_m/V_m$ and intercept on the y-axis as $1/v_m$ and intercept on x axis as $-1/k_m$.

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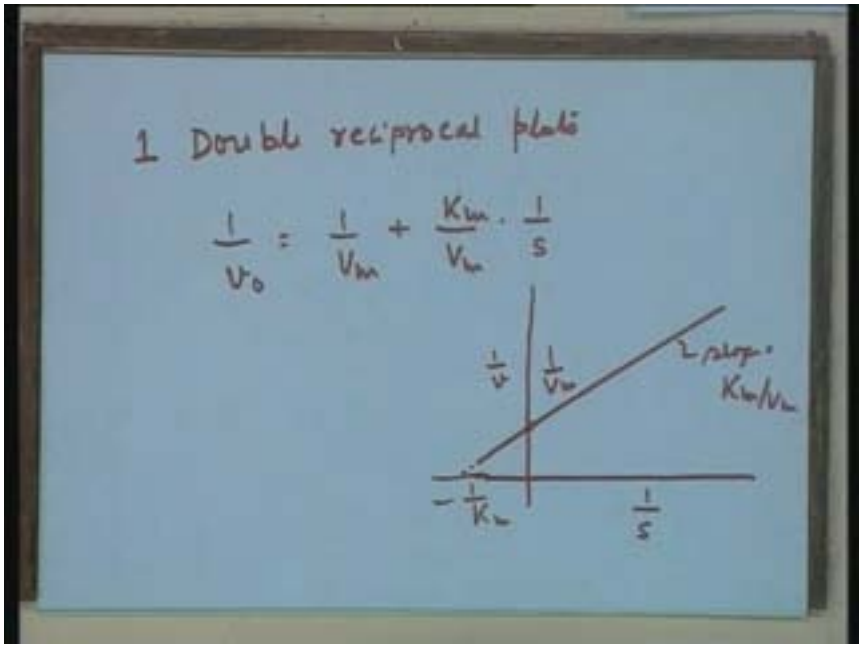


This was one of the earliest analyses for the experimental data in the light of Michaelis Menten hypothesis. It was noted to have very serious limitations and the serious limitations are that if you look at any set of data for enzyme catalyzed reaction that means data on the substrate concentration versus reaction velocity you will notice that the experimental data gave very low weightage to the measurements at low substrate concentrations. Most the data points will be rather concentrated near the origin where as weightage at a low substrate concentration will be very, very low. Under weightage at the low substrate concentrations and at high substrate concentrations near the origin the data points will be heavily concentrated.

If you consider substrate concentration in millimolar quantity and the reaction velocity in some arbitrary units, you see at 5, 1/S will be 0.2, 10-0.1, 15-0.66, 20-0.05, 30-0.033, 40-.025 and so on. You see that when you increase the substrate concentration the weightage to 1/S concentration is so low that ultimately you end up in a scattering, a cluster of data near the origin and therefore getting the realistic value of $k_m$ and $v_m$ often tends to be difficult even after making a statistical analysis.

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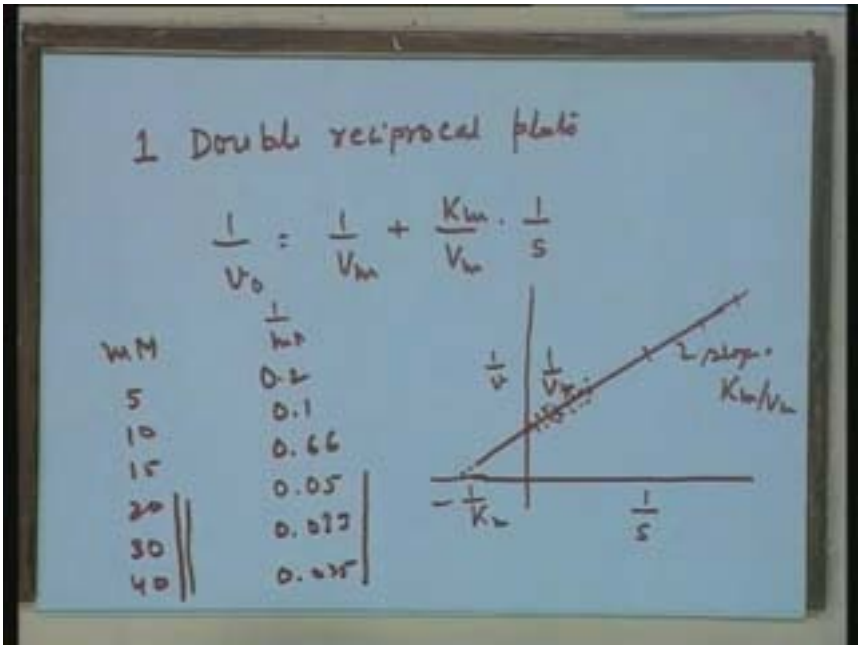


Therefore a number of other linear plots were thought of and the other linear plots are Hanes plot which is nothing else but the modification of original equation. That means if you multiply the Lineweaver Burk equation simply by substrate concentration on both the sides you arrive at

$$S/v_0 = k_m/v_m + S/vm$$

This also represents a straight line and if you see a graphical representation of such a plot of substrate concentration verses $S/v_0$, you will again end up in a straight line position with a slope of $1/v_m$ and intercept of $k_m/v_m$, the intercept on the X-axis as $-k_m$. Here you have little better situations in the sense that the data are usually not so clustered. They are scattered because you have substrate concentration on both the axis and therefore you get a much better fit.

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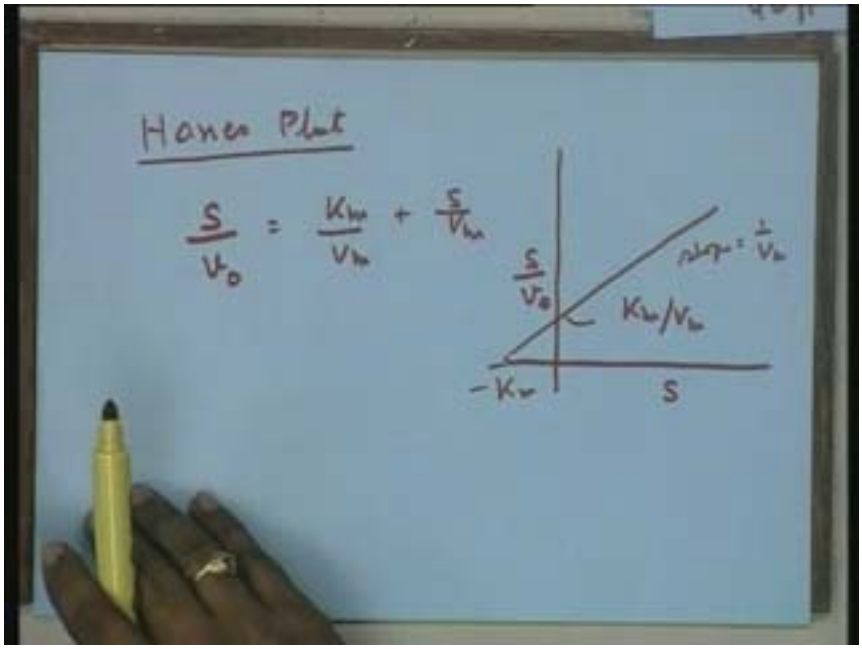


Similar profile an alternative profile is Eadie Hofste plot. Another version of the linear form of Michaelis Menten equation where you can arrive at

$$v_0 = v_m - k_m/s.v_0$$

If you plot such an equation and that you can obtain by multiplying the Lineweaver equation by $v_0.v_m$ on both the sides and then you get a straight line with negative slope where slope is $-k_m$. The axis here is $v_0/s$ versus $v_0$. You get a slope of $-k_m$ and the intercept on the y axis is $v_m$ itself. This also has the advantage of almost well scattered or data points that can be easily used.

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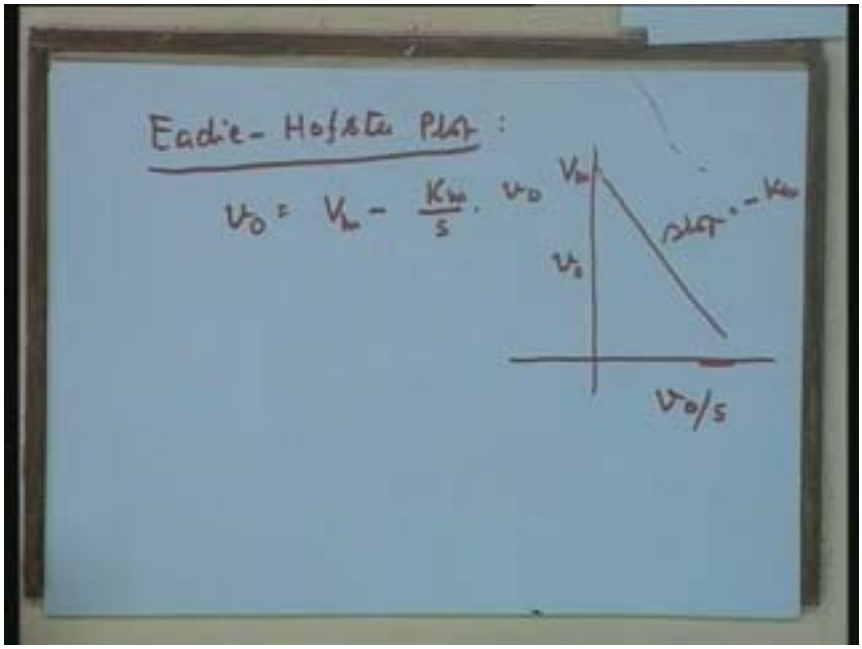


Now in all the three linear plots which I have discussed that is Lineweaver plot, Eadie Hofste plot as well as the Hanes plot again there is one limitation which has been noticed. We have no means to look at the adequacy or the effectiveness of our analytical procedure which we have used. After all the accuracy of our determination or analysis of experimental data will largely depend upon the accuracy of our experimental methods and whatever data point you get we can always make a statistical fit to form a straight line and get the values. We don't get any insight into the correctness of the data. To take care of that another important plot was proposed in 1974 by Cornish and Bowden.

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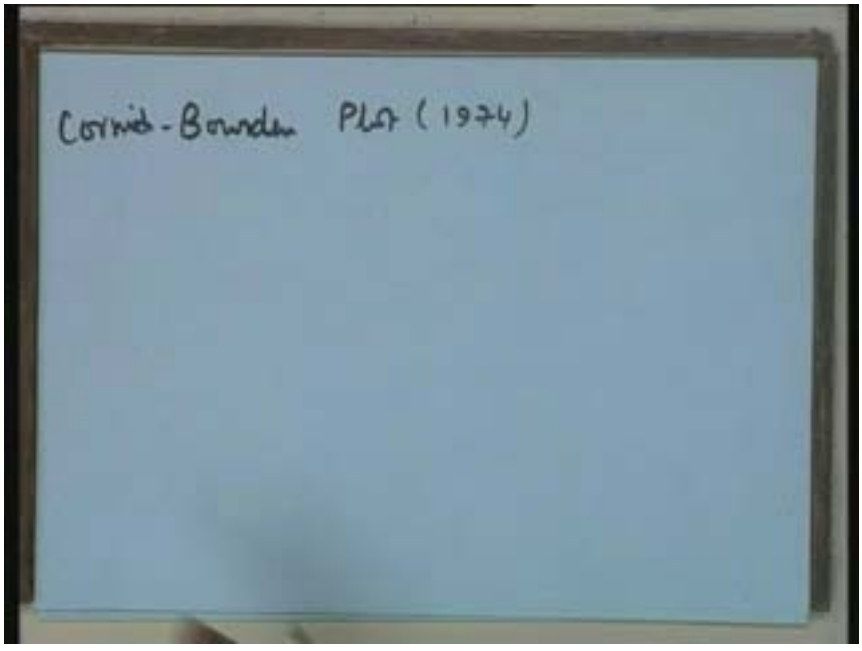

This had the characteristic feature in the sense that if you write the Michaelis Menten equation assuming the substrate concentration and the initial reaction velocity as constants and $k_m$ and $v_m$ as variables although it is little unusual and unrealistic but just for the sake of a mathematical analysis if we write that, we can write for example

$$1/v_0 = k_m+S/v_m.S$$

If you just take a reciprocal

$$v_m/v_0 = k_m+S/S$$

$$v_m = v_0(1+k_m/s)$$

We have just written the equation in the form of $v_m$ and $k_m$ as variables. $v_m$ and $k_m$ also represent a straight line with a slope of 1/s and intercept of $v_0$.

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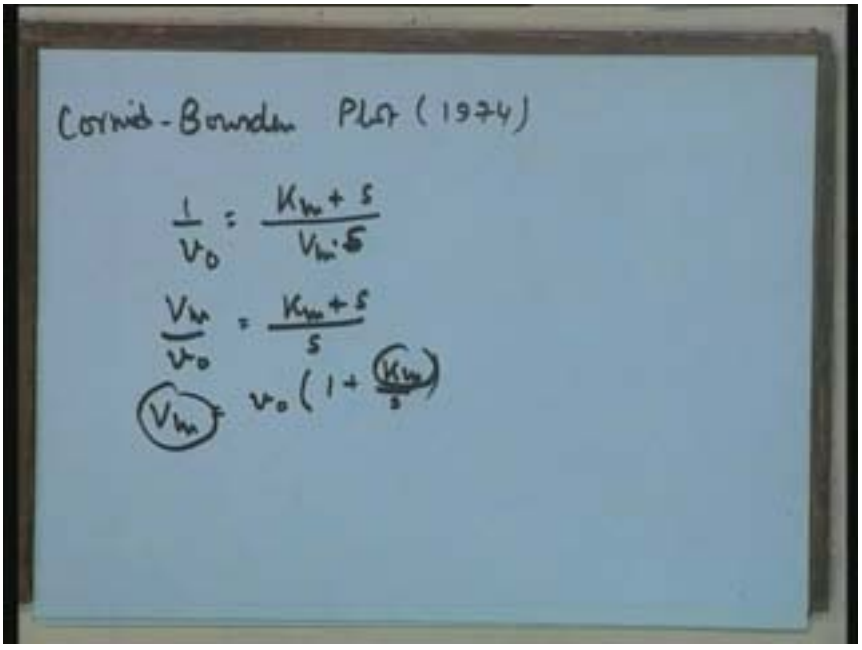


It may look little unorthodox and because $k_m$ and $v_m$ we know are constants. At a constant enzyme concentration $v_m$ is also constant. If we consider a single data point that means at any given point of $S_1$, we consider $v_1$. Another set of conditions $S_2$ we consider $v_2$. For each of these two data points a straight line relationship between $k_m$ and $v_m$ must be valid at least from the mathematical point of view.

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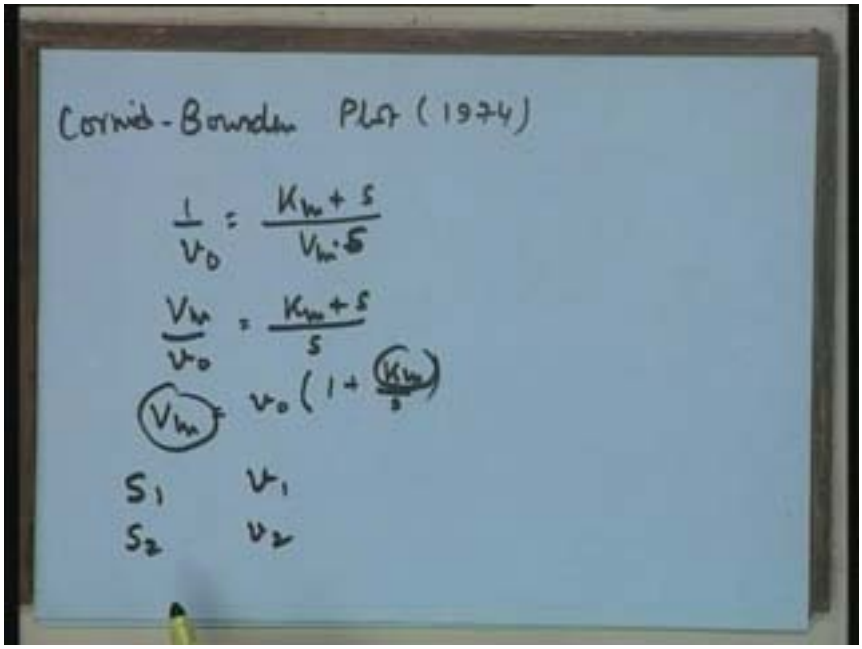


So what we do is in this case therefore for each set of data point between S and v we develop a straight line. That means we simulate the value of v. We just assumed the hypothetical value of $k_m$, corresponding $v_m$ is calculated in a table and we draw a straight line with reference to let us say $S_1V_1$. Similar exercise we do for the second data point we also develop another expression, another straight line and this gives you let us say for $S_2$ $v_2$. Third point we also develop $S_3$ $v_3$. So for each data point we can develop a straight line.

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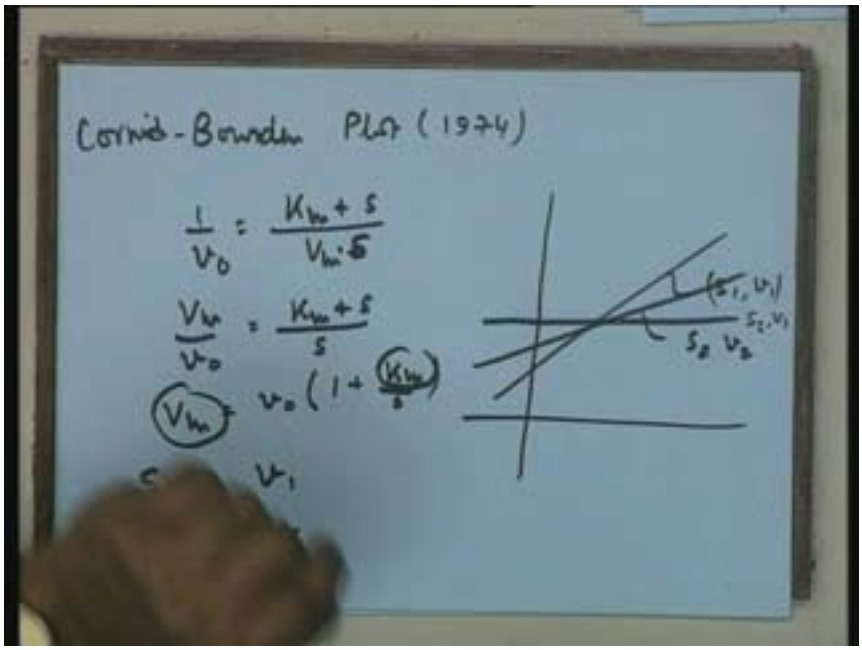

In the enzyme system the reaction will have only one set of $k_m$ and $v_m$ and that will be the one which is obtained by intersection of these straight line relations and that gives you a perfect check on your experimental data. Very often you notice that in experimental data you may not get a perfect point of intersection. The point may let us say go something like this. Then you are sure in one thing that your $k_m$ and $v_m$ values are somewhere in this range.

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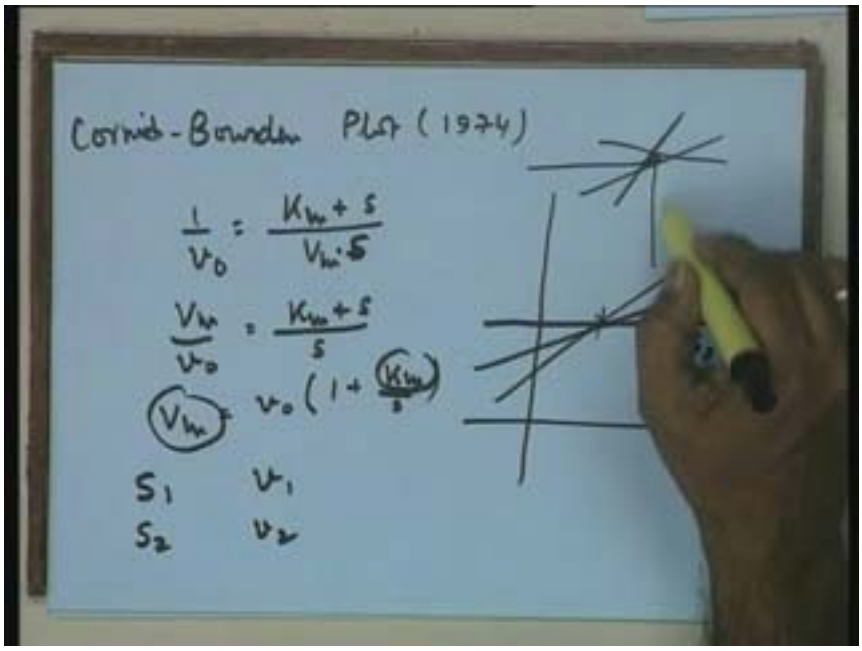


You know the range of $k_m$ and $v_m$ values not the absolute value and you also know the limitations of your experimental methods that you are following. There is some where something flaw because theoretically if all your data points or measurements or substrate concentration or reaction velocity are perfect they must intersect at each other assuming that Michaelis Menten kinetics is valid.

Cornish Bowden plot gives you a possibility not only to get the range of $k_m$ and $v_m$ values but also makes a check on the validity or accuracy of your experimental data and which is probably one of the very basic features in the case of enzyme kinetics that your experimental data must be pretty accurate to be able to analyze the kinetics of enzyme catalyzed reaction. Using either of these plots, personally I recommend you Cornish Bowden plot, for any analyses. We must be able to analyze or get the kinetic features or characteristics of any enzyme catalyzed reaction broadly in terms of the value of $k_2$ that is what we call as turn over number and $v_m$ and km value.

There is another very interesting feature as far as the Michaelis Menten equation is concerned. When we mentioned our assumptions of Michaelis Menten equation, you will notice that these assumptions will largely not fit into most of the actual enzyme catalyzed reactions.

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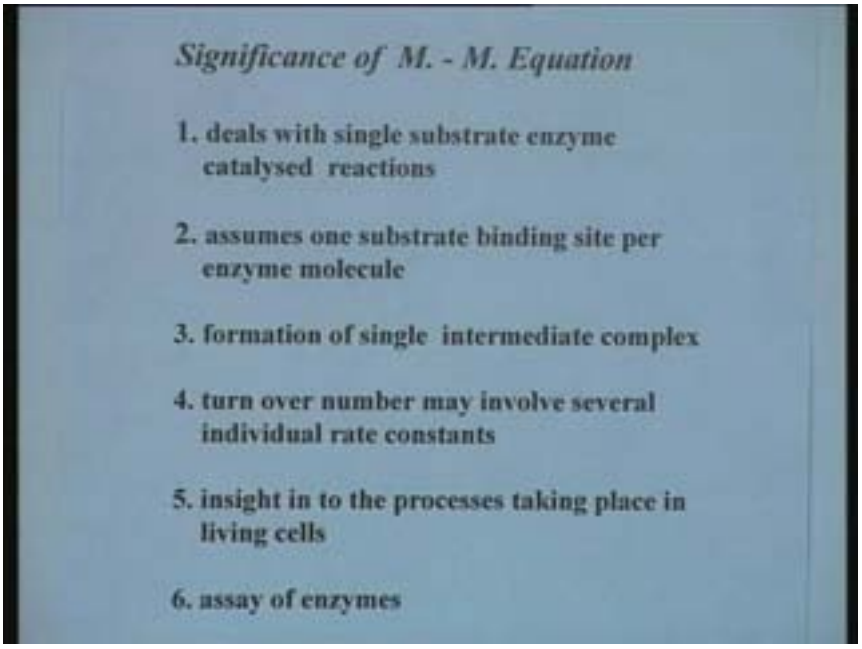


But Michaelis Menten equation is still considered to be the universal model for analyzing the enzyme catalyzed reactions. There is some where some compromise. If you look at each of the points where we have paradoxes in terms of the actual enzyme catalyzed reaction and those which can be done by Michaelis Menten kinetics, the first one that the Michaelis Menten kinetics deals with is the single substrate enzyme catalyzed reaction.

With the background in the biochemical metabolics you will appreciate that most of the enzyme catalyzed reactions in practice in the living cells are not single substrate reactions. They are multisubtrate. At least two substrate reactions and if not any other directly converting substrate water is a molecule which participates in the reaction.

Now in such cases the applicability of Michaelis Menten equation is still valid under conditions that one of the substrate is held at a constant concentration which is in excess and one substrate which is limiting. The Michaelis Menten kinetics should be applied to a limiting substrate and the other substrate must be in excess and therefore its concentration can be considered to be constant. Even after it is consumed partly the concentration drop is not very large and actually if we look into most of the hydrolytic reactions the water which is almost more than fifteen molar concentration in the reaction mixture, even after participation of water in the hydrolytic reaction, the concentration doesn't drop to significant level and therefore Michaelis Menten kinetics is still valid and gives you on analysis practical results.

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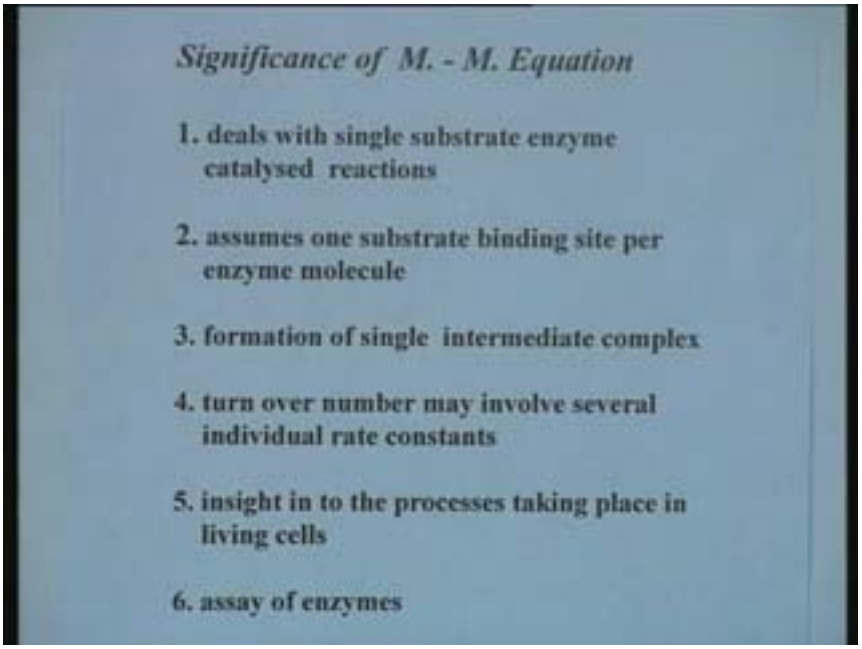


The significance of Michaelis Menten equation is that it assumes one substrate binding site per enzyme molecule. When we wrote mechanistic expression for enzyme substrate binding, we are taking one substrate molecule which binds towards enzyme molecule thereby assuming that the enzyme possesses only one substrate binding site which is also not true in many cases.

Most of the enzymes, particularly the ones which have quaternary structure for their function, they will have more than one binding site on the enzyme molecule. But such a fallacy can be taken care in the light of Michaelis Menten kinetics. If the binding sites even if they are more than one, if they are not interactive that means they are independent of each other and do not interfere in binding of each other, then Michaelis Menten kinetics will be valid. The only difference will be that we must take in account multiple binding sites in terms of concentration of enzyme. That means concentration of enzyme will reflect the concentration of active sites. The third is we have talked about the formation of single intermediate complex. When we say enzyme substrate complex is formed we are talking of only one enzyme substrate complex that is formed. In many cases even which are now practically understood very clearly this is not true. Very often we end up with kind of interactions as

$$E+S \rightleftharpoons ES \rightleftharpoons ET \longrightarrow E + P$$

There are a variety of intermediate complexes that are formed not only one complex. This is very true very often in the case of reversible reactions and in such cases the Michaelis Menten kinetics will also be valid provided the interconversions of the intermediate complexes are the rate limiting step. We just simply consider these the irreversible process. This is $k_1$, $k_{-1}$, $k_2$ and $k_3$. You must identify the rate limiting step and put the rate constant $k_2$ that appears in Michaelis Menten kinetics as one which is the rate limiting step and then Michaelis Menten kinetics will apply to it.

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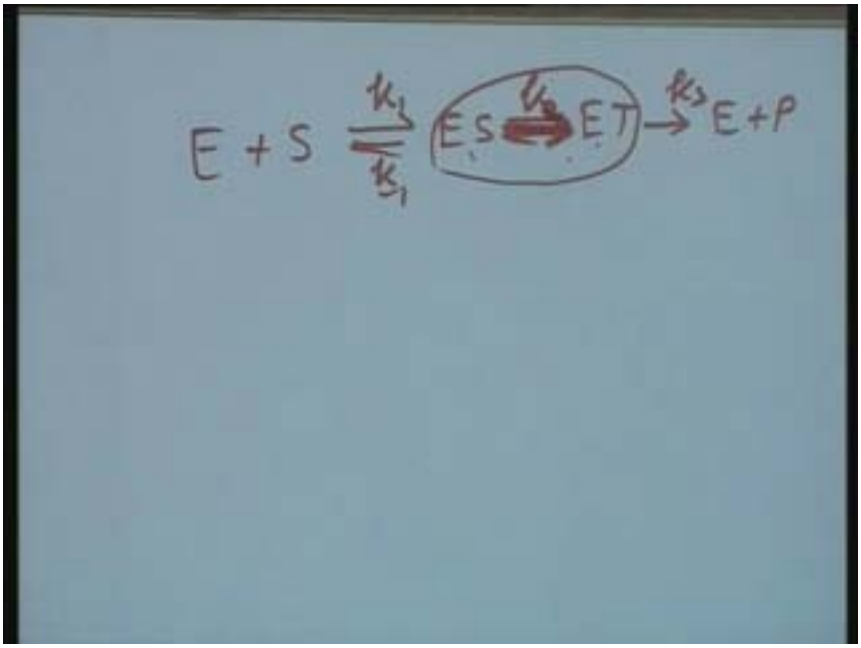


Most of the enzyme catalyzed reactions involve more than one intermediate complex. Now the Michaelis Menten kinetics can still apply to those reactions provided the inter conversion of the complexes is assumed to be the rate limiting step. Or in other words in place of turn over number $k_2$ that appear in the case of Michaelis Menten kinetics you put the rate limiting rate constant in this sequence of interaction in the enzyme substrate or in the enzyme kinetics and that will still be valid.

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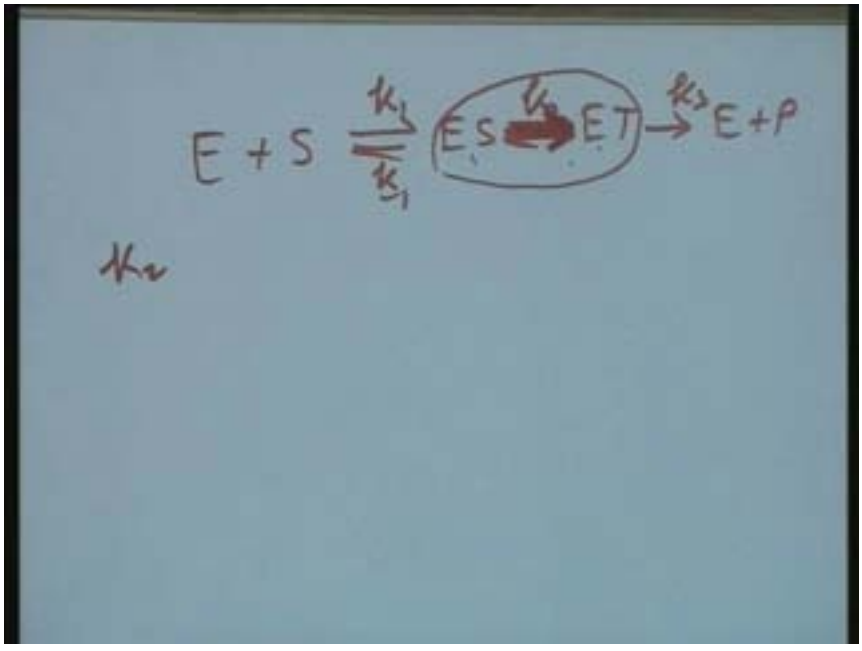

Related to the same issue of multiple intermediate complexes is turn over number which may involve several individual rate constant.

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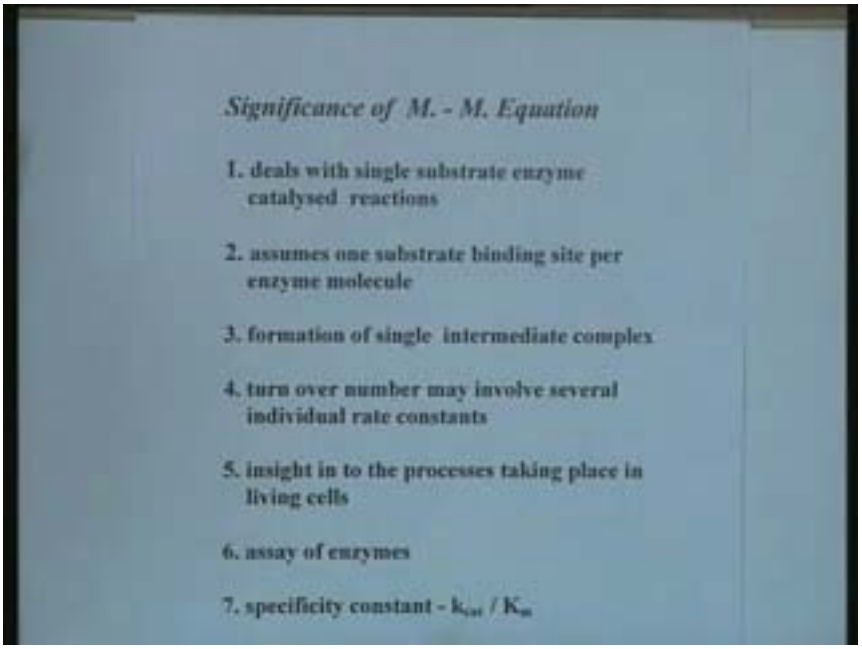


We often define our turn over number

$$v_m = k_2 [E]_0$$

and this $k_2$ is defined as the turn over number.

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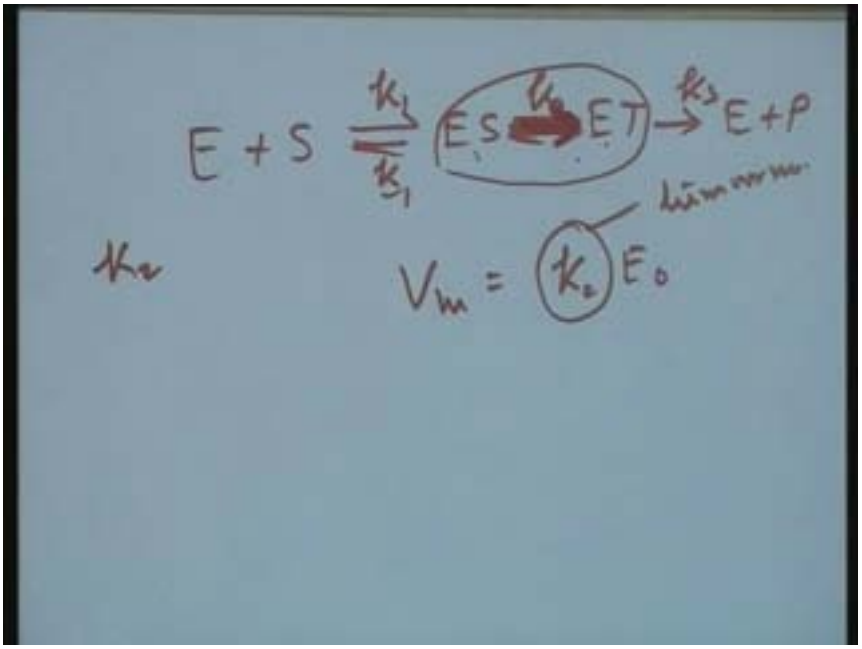


In most of the enzyme catalyzed reactions which involve multiple intermediates, a single rate constant may not able to define the turn over number. Turn over number may work out to be a lump parameter consisting of more than one rate constant. Just for example you consider a very practical case which has been studied extensively like ..... (27.48) catalyzed hydrolysis of esters and amides and if we look at reaction sequence which it follows today

$$E + S \rightleftharpoons ES \rightarrow EAc \rightarrow E + P$$

This is $k_1$, $k_{-1}$, $k_2$. Another product is formed. This is $P_2$. This is $P_1$. There are two products formed on hydrolysis, acid as well as alcohol and this is your $k_3$.

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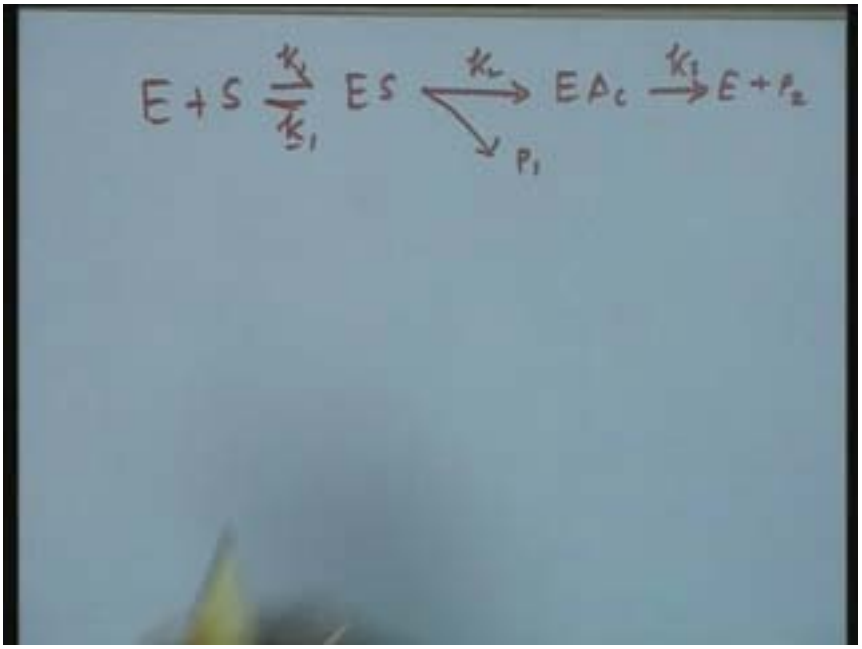


If you just analyze this steady state hypothesis with respect EAc which is acylated enzyme complex you get the reaction velocity as

$$v_0 = E_0.S.\ k_2\ k_3 / k_2 + k_3$$

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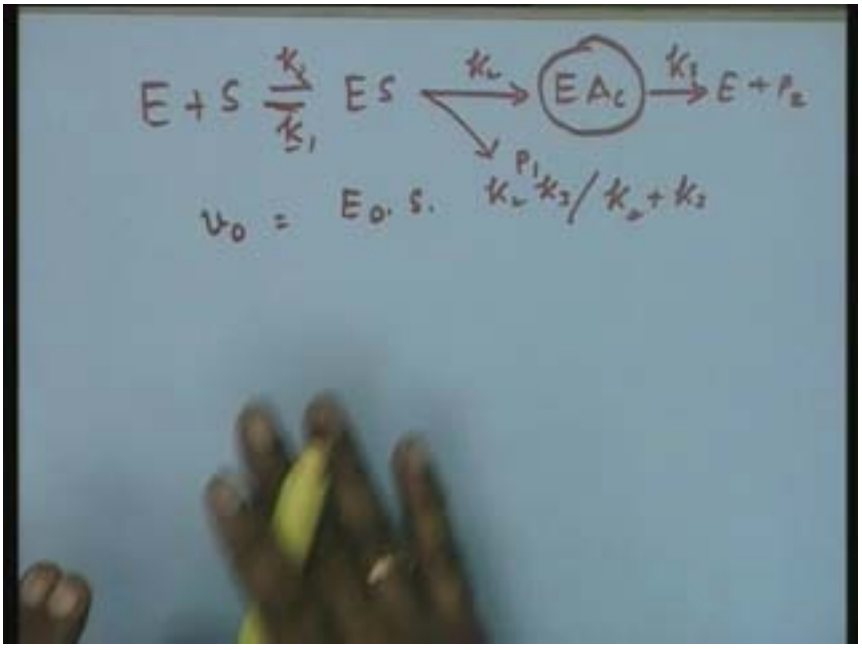


In fact just assume when we will proceed further we will develop a system by which we can write the expression for more complicated kinetics because when we write for a single enzyme substrate complex developing the steady state rate expression is very

simple. As you reach more complexity in terms of more enzyme substrate complexes the mathematical analysis becomes more and more complex and there are routes by which one can write straight away by observation the rate expression and we will discuss that in the subsequence classes.

$$v_0 = \frac{E_0.S.\ k_2\ k_3 / k_2 + k_3}{\{(k_{-1}/k_1)/k_2+k_3\}+ S}$$

Here in such a case k catalytic in analogy to the original Michaelis Menten hypothesis will be equivalent to

$$K_{cat} = k_2k_3/k_2 + k_3$$

Not a simple $k_2$, the turn over number will depend upon both the parameters $k_2$ as well as $k_3$ and that is another fallacy which is built in the Michaelis Menten kinetics. It doesn't take care of that.

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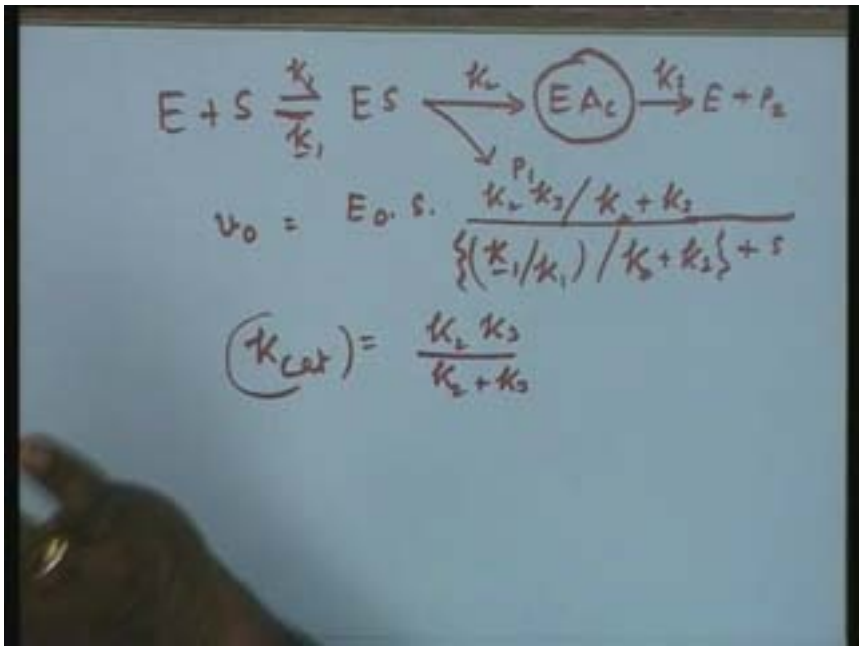


So the Michaelis Menten kinetics need to be extended to develop a rate expression which takes into account steady state with respect to one of the intermediates and analyze it in analogy to develop a lump parameter for defining the turn over number. But turn over number for most of the enzyme catalyzed reaction varies in the order from 10-$10^4$ per second. That is the order which it goes and it is a fairly large range. So that also dictates that there are certain reactions which are slow there are certain reactions which are very fast.

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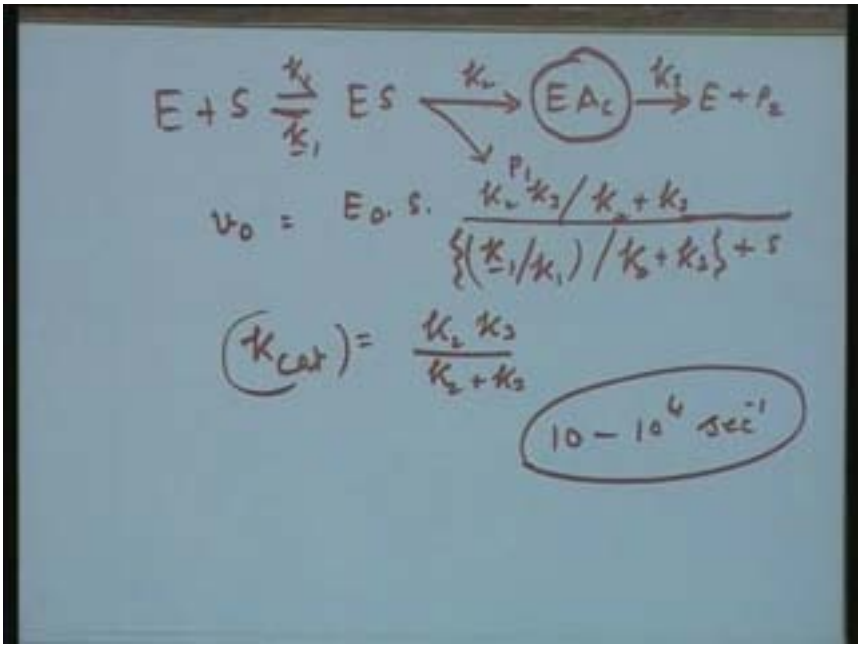


A reaction with a turn over number of $10^4$per second means so many numbers of molecules of substrate is converted to product per mole of the enzyme which is a very large turn over number as against a turn over number of ten. The next and very significant advantage of Michaelis Menten kinetics and particularly the significance of the $k_m$, Michaelis Menten constant, is very often we get an insight into the functioning of enzymes in the living cell.

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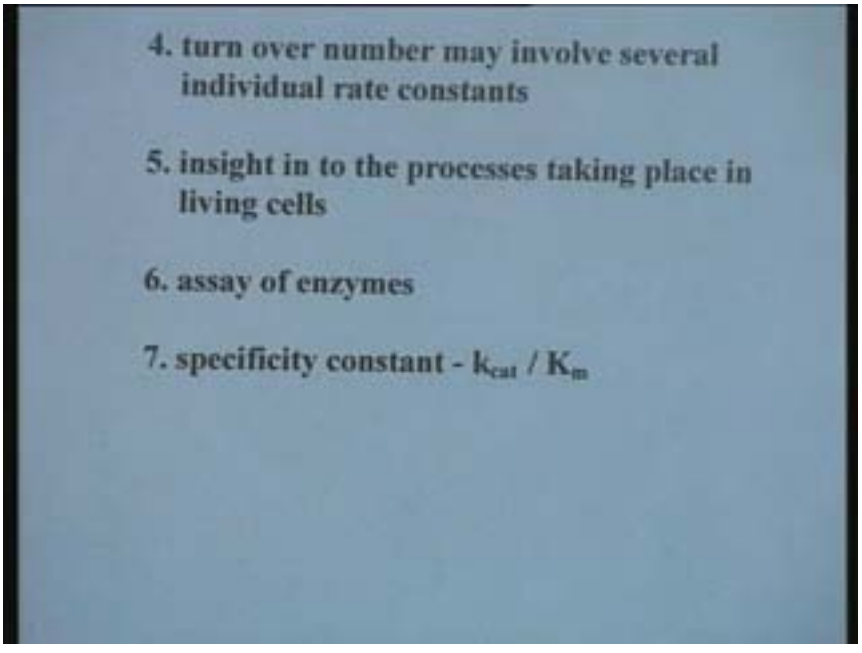

Take the simplest example of glucose-1-phosphate. Glucose is the starting point when we go into the metabolics and its first step is phosporylation of glucose and you get a series of products glucose-1-phosphate, glucose-6-phosphate or fructose-1-phosphate and so on. These molecules can go further either into the glycogen synthesis as a storage molecule or further to fructose biphosphate.

The route which these molecules will follow will depend on the relative $k_m$ value of the two enzymes involved. The $k_m$ value for the fructose biphosphate forming enzyme is much, much smaller at least thousand folds smaller than the glycogen synthesizing route. Therefore as long as the concentration of these molecules, phosporylated glucose, is not very high it will always tend to go to the fructose biphosphate route and release the energy. Only when the concentration of this phosporylated glucose becomes quiet high, a part of it, depending upon the ratio of the $k_m$ value will go to glycogen synthesis. So that is one simple example but at many points where the substrate has more than one routes to follow in the biochemical metabolics its preference or the likely physiological route can be decided on the basis of $k_m$ value and that has been possible based on the understanding of Michaelis Menten kinetics.

Another important significance of the Michaelis Menten kinetics is its application to assay of the enzymes.

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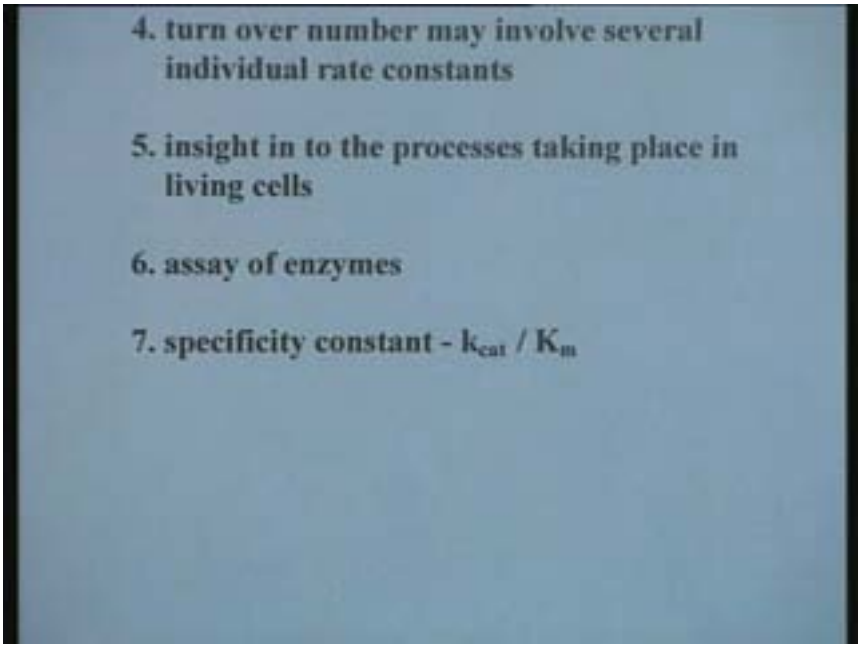


One of the analytical advantage is very often we are required to estimate the activity of an enzyme sample. These enzyme sample activity must depend upon the effect of enzyme concentration on the reaction velocity and we use such a parameter particularly when we plot the concentration of enzyme on the reaction velocity. We get a profile. Initially it is a straight line which is linear and then it tapers off towards the end when the enzyme

concentration becomes high. Normally we assay the enzyme concentration in the linear range.

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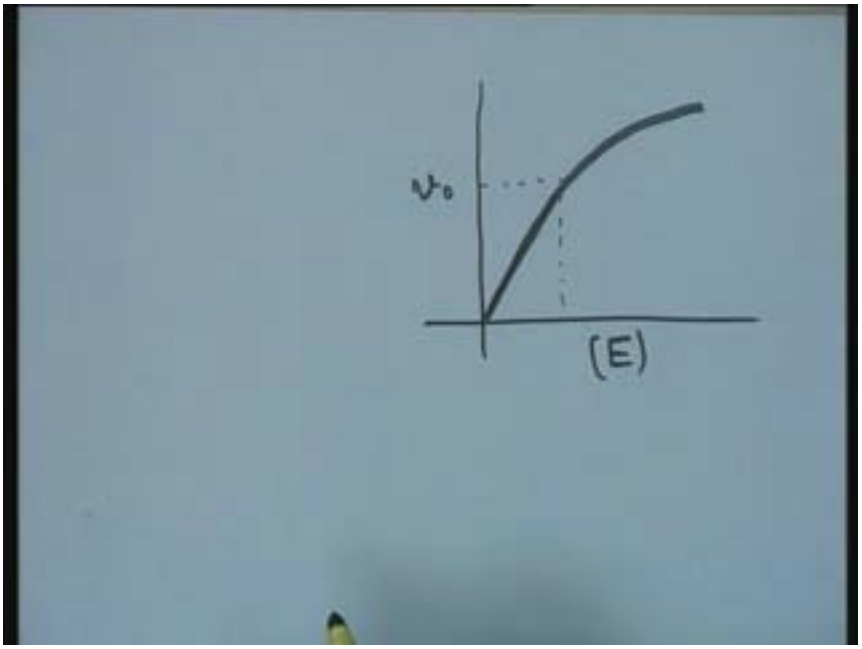


There are two parameters, two constraints which have to be strictly ….. when we talk of assay of the enzyme. There are three constraints. The first constraint is the detection of the product. You must have a method for the detection of the product formed of the reaction. The minimum quantity of product you can detect will dictate the time of the reaction. The time required for the reaction to be carried out will be considered on the basis of the detection method of the product so that you can accurately determine the product.

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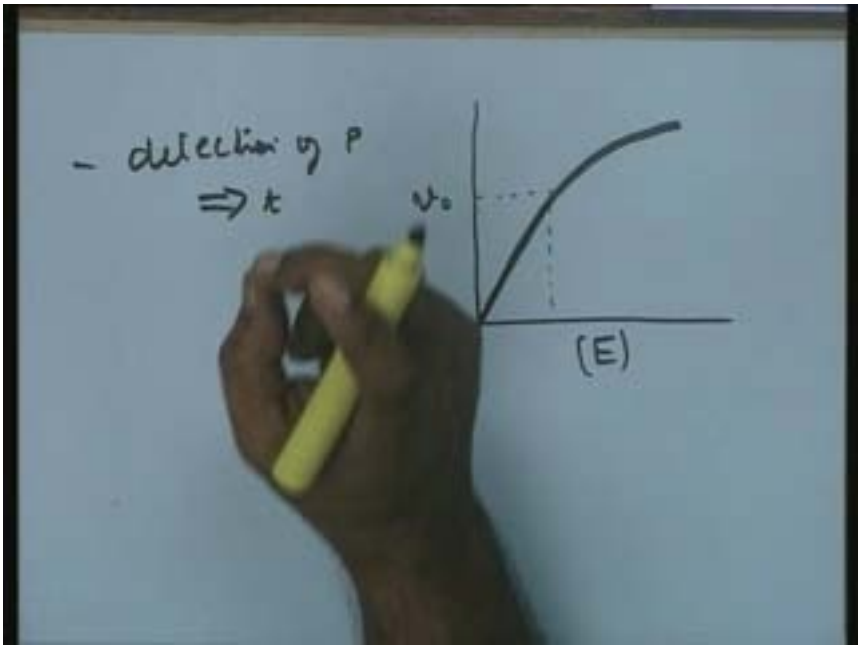


The second limitation is that you must follow the linear range of enzyme reaction velocity profile. The third parameter is one must ensure the substrate concentration for assay of the enzyme. And for all analytical purposes, the substrate concentration that is taken is much, much larger than enzyme concentration. For all practical purposes the reaction velocity what we measure is

$$v_0 \longrightarrow v_{max}$$

That means the substrate concentration is very, very large and as I mentioned that we can never reach effectively to $v_m$. We can only approach to $v_m$ and for all practical purposes the substrate concentration that is chosen is roughly of order of about greater or equal to hundred $k_m$ values. So the magnitude of $k_m$, the magnitude of Michaelis Menten constant, also gives you a parameter for assay of the enzyme so that you can choose your substrate concentration for carrying out the experiments.

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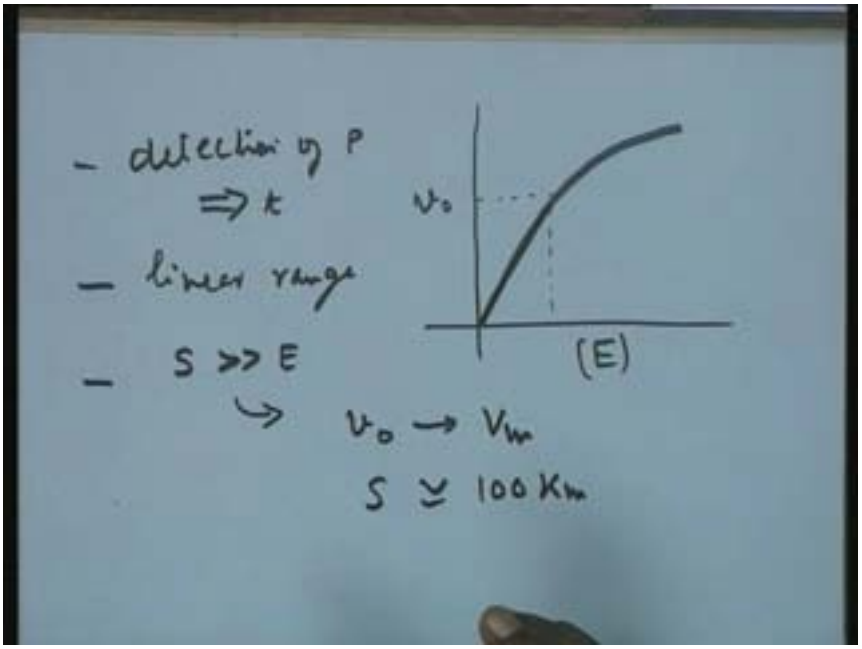


That is another major significance of the Michaelis Menten kinetics. Another important feature of Michaelis Menten kinetics is to define specificity constant.

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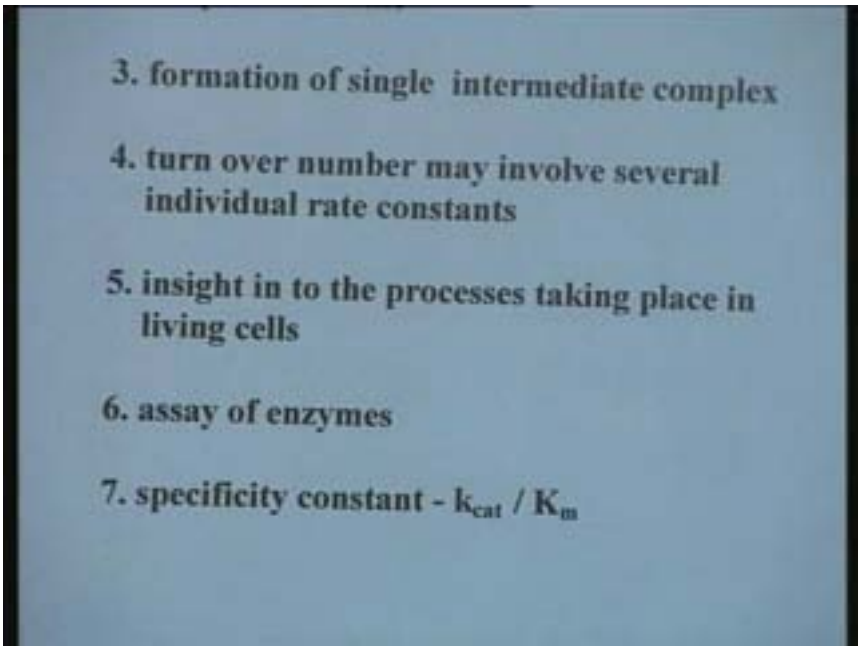


If suppose we have an enzyme acting on more than one substrate. How to define its specificity or compare specificity of the enzyme with respect to different substrates? The kinetic parameter available from Michaelis Menten kinetics can give you some insight into this.

At a very low substrate concentration, when substrate concentration is much, much smaller than $k_m$ then your initial reaction velocity will be equal to

$$v_0 = k_2 E_0 S / k_m$$

a first order kinetics, with $k_2 E_0 / k_m$ as a pseudo first order rate constant $k_f$. If you consider $E_0$ as a variable then $k_2/k_m$ is a constant which is considered as specificity constant. Sometimes some people call it as pseudo second order rate constant considering enzyme also as one of the reactant which is not really a second order rate constant. $k_2/k_m$ considered as a specificity constant will determine the relative specificity of an enzyme to different substrate. One can determine these values from the kinetic analysis and one can get the picture of specificity.

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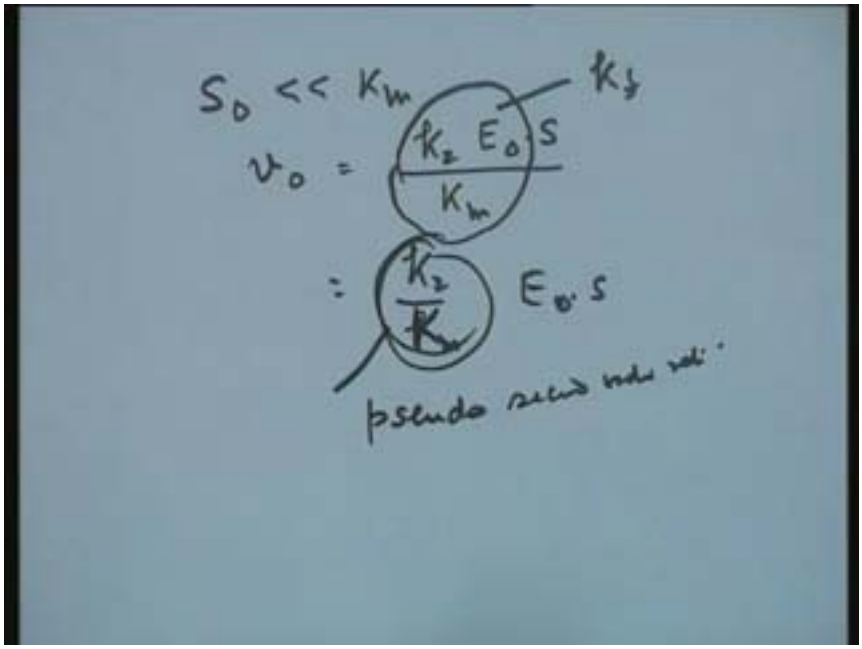


Under this first order design or under condition of $S_0 << k_m$ there is another feature which we must look at. At this feature $E_0$ is approximately equal to E, the initial enzyme concentration.

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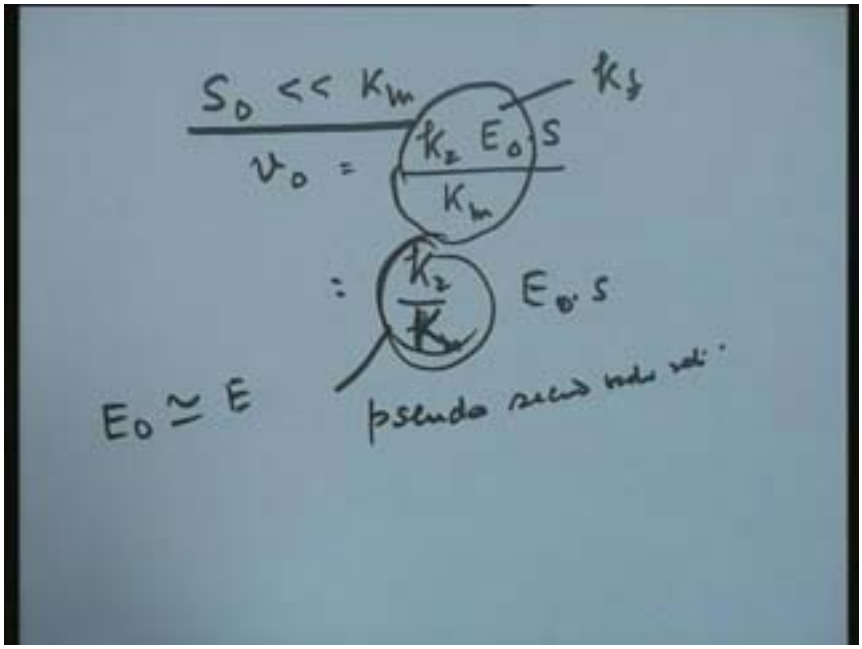


We are assuming that the substrate concentration is very low. We can also go to a level that not all the enzyme is saturated; all the enzyme is not bound, bulk of the enzyme is still available in the free form. Therefore the specificity constant relates initial reaction velocity to the concentration of free enzyme rather than the total enzyme. So these are some of the practical implications of the significance of Michaelis Menten kinetics which applies to most of the kinetic analysis. One of the most important features is to test the validity of steady state assumption. We have been always assuming that Briggs and Haldane gave it.

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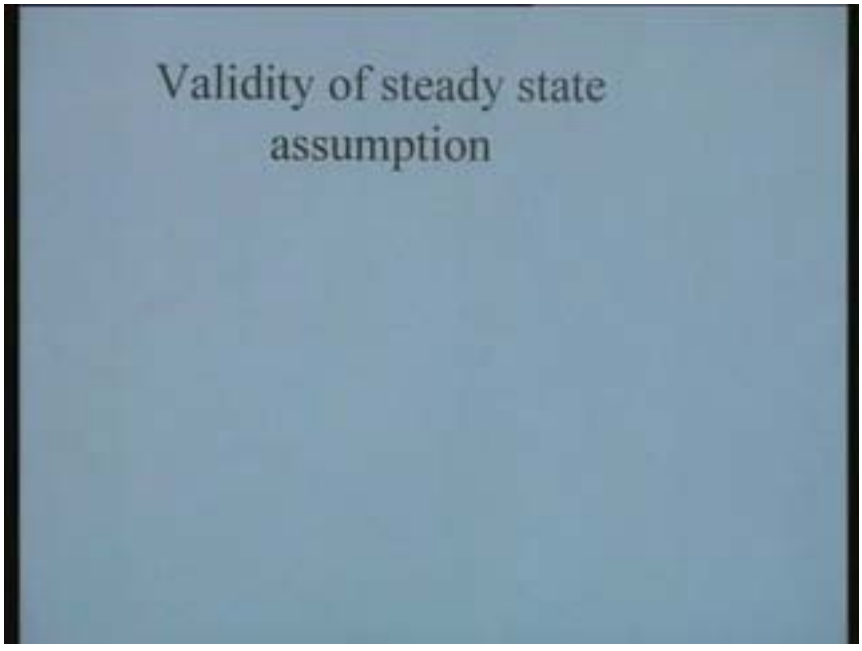

The steady state hypothesis have undergone very severe tests both analytical as well as experimental and it has proved those tests and that's why it has become so much universally valid today. How to analyze or what are the experimental constraints within which the steady state hypothesis must be considered valid? That you can very easily arrive at, if you write down the rate expression for rate of change of enzyme substrate complex for example if you look at your original equation that we discussed

$$E + S \rightleftharpoons X \longrightarrow E + P$$

If we consider the original equation that we mentioned earlier, we wrote the rate of change of concentration of X. I am again not writing the parenthesis but the capital letters denote the concentration terms. They can be written as $k_1$, $k_{-1}$, $k_2$.

$$dx/dt = k_1 E\, S - k_{-1}x - k_2 x$$

$$= k_1[E_0 - X]S - k_{-1}x - k_2 x$$

Now you can separate the variables x and t in this equation and ultimately therefore you can write

$$\frac{\int dx}{k_1 E_0\, S - (k_1 S + k_{-1} + k_2)\, X} = \int dt$$

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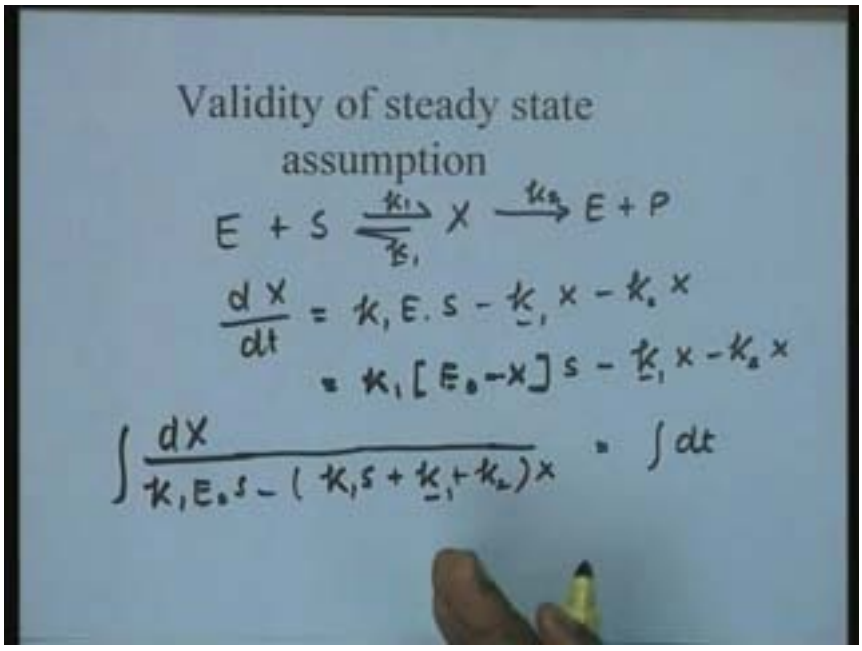


If you just solve it you will get a logarithmic term and the solution will be

$$\frac{\ln [k_1E_0S-(k_1S+k_{-1}+k_2)x]}{-(k_1S + k_{-1} + k_2)} = t + \alpha$$

Let us say alpha is the constant for integration. This alpha can be easily determined at t=0and x = 0. At initial time there will be no enzyme substrate complex and that can give you simply the value of alpha as

$$\alpha = \ln (k_1E_0S)/(-k_1S + k_{-1}+k_2)$$

So now you substitute the value of alpha and there by get your final solution to the two integrals and if you take exponential on both the sides and simplify you will come out with an expression

$$v = k_2 x$$

and v can be developed and with just two three iterations in between you will arrive at

$$v= k_2x =v_mS \{1- \exp [-(k_1S +k_{-1} + k_2)t]/ k_m + S$$

In this exponential term if we consider that t is very small that means right from the beginning of the reaction the steady state hypothesis is not valid. If the reaction has to progress to an extent that the t gets a magnitude and the exponential term tend to go towards zero.

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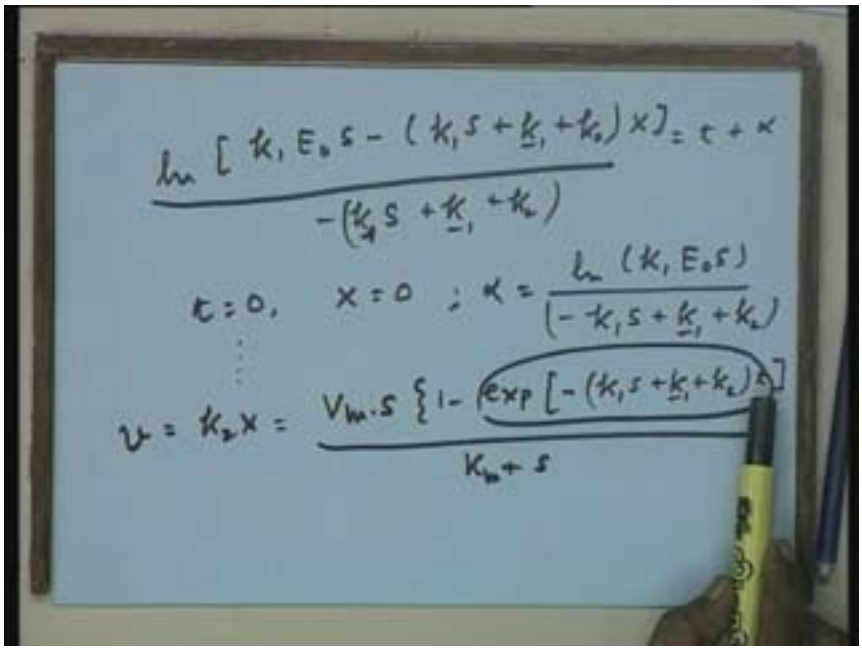

If the t becomes large now the required size of the t will depend upon the magnitude of $(k_1S + k_{-1} + k_2)$. The magnitude of this parameter dictates what should be the size of the t. Or in other words this t is nothing else but the induction phase after which the reaction measurements should be made so that steady state hypothesis is valid. For all practical purposes by rapid mixing techniques people have been able to analyze and determine the value of $k_{-1}$, $k_1$ and $k_2$ a reasonable value.

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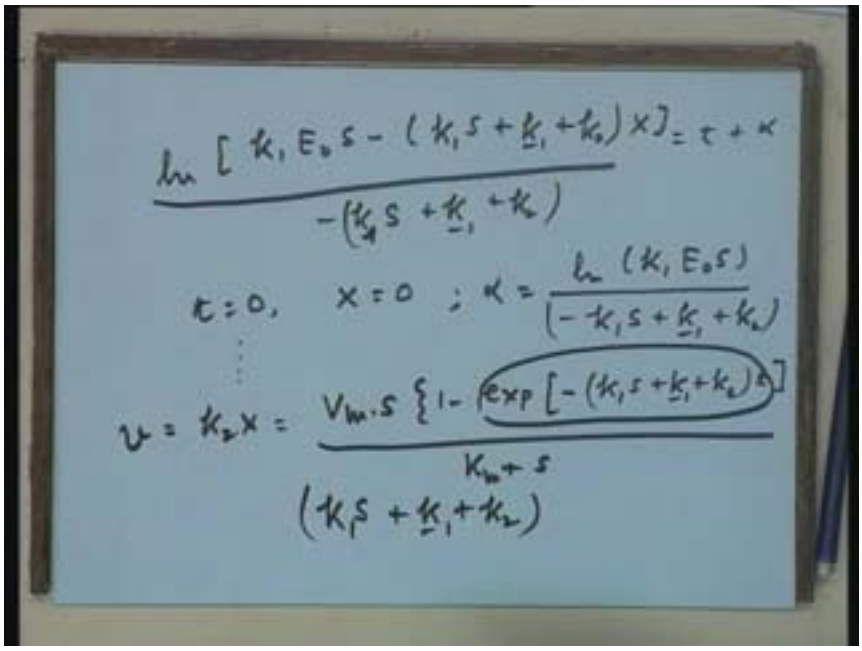


Particularly if you assume $k_2$ as the turn over number and we mentioned that the value ranges from $10\text{-}10^4$. A reasonable magnitude of this will be let us say about thousand per second and even at this thousand per second the exponential of course at time equal to let us say five minutes or three hundred seconds the magnitude of this exponential will be less than .01. A very, very small quantity and then the rate expression will turn up into the Michaelis Menten kinetics.

$$v_0 = v_m S / k_m + S$$

That means the magnitude of this exponential is negligible. This magnitude will depend on two factors one is turn over number and as we already know that turn over number of these reactions is pretty high, orders of magnitude of few thousand. Only then it depends on the magnitude of time.

If you take a reasonable time then the multiplication of turn over number or rather not exactly turn over number but sum total of $(k_1S + k_{-1} + k_2)$, out of which only one factor is turn over number, that magnitude if it becomes large enough so that the exponential of it's negative is approaching zero, our system will turn up into a Michaelis Menten expression.

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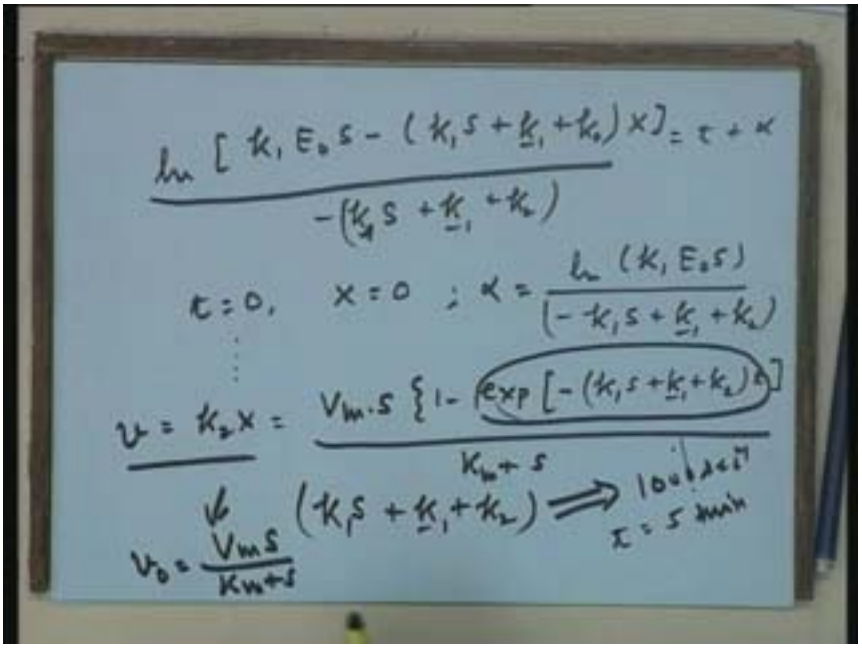


So the constraints for the applicability of Michaelis Menten hypothesis particularly steady state hypothesis will be valid in case when we take a reasonable time period and also the turn over number is high. In most of the practical cases where the reactions have been studied the two parameters have been found to be valid and that gives validity to the steady state hypothesis for all practical purposes. Also in many of the critical situations where the assumptions of the enzyme catalyzed reactions were not valid strictly under certain set of condition the Michaelis Menten kinetics could be considered to be applicable.