M.Sc - 2nd Sem

Paper - 4

Unit - 4

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Michaelis-Menten Equation

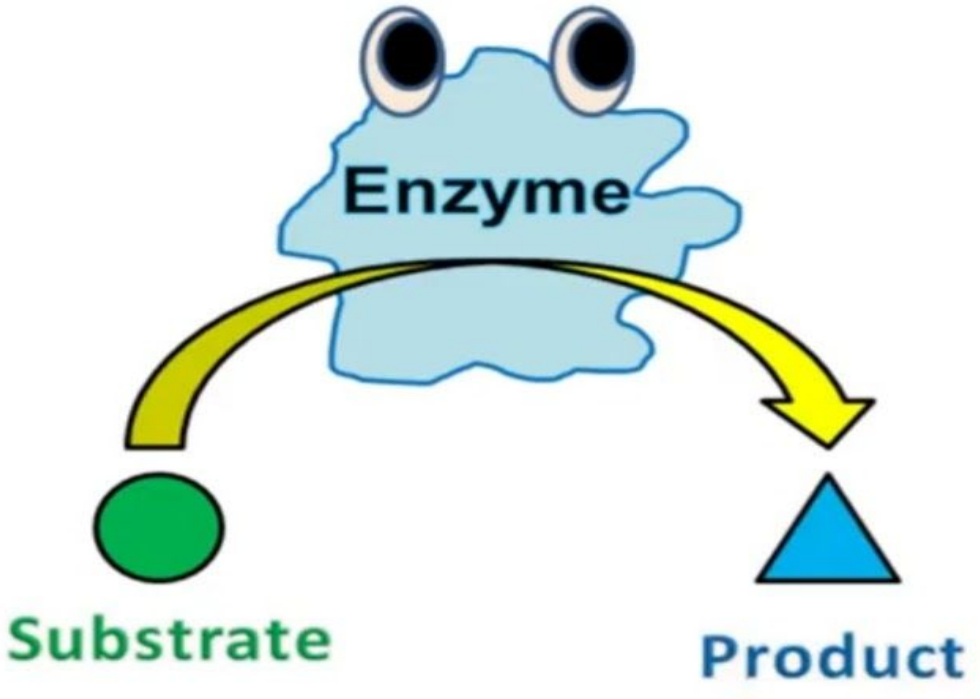
Introduction

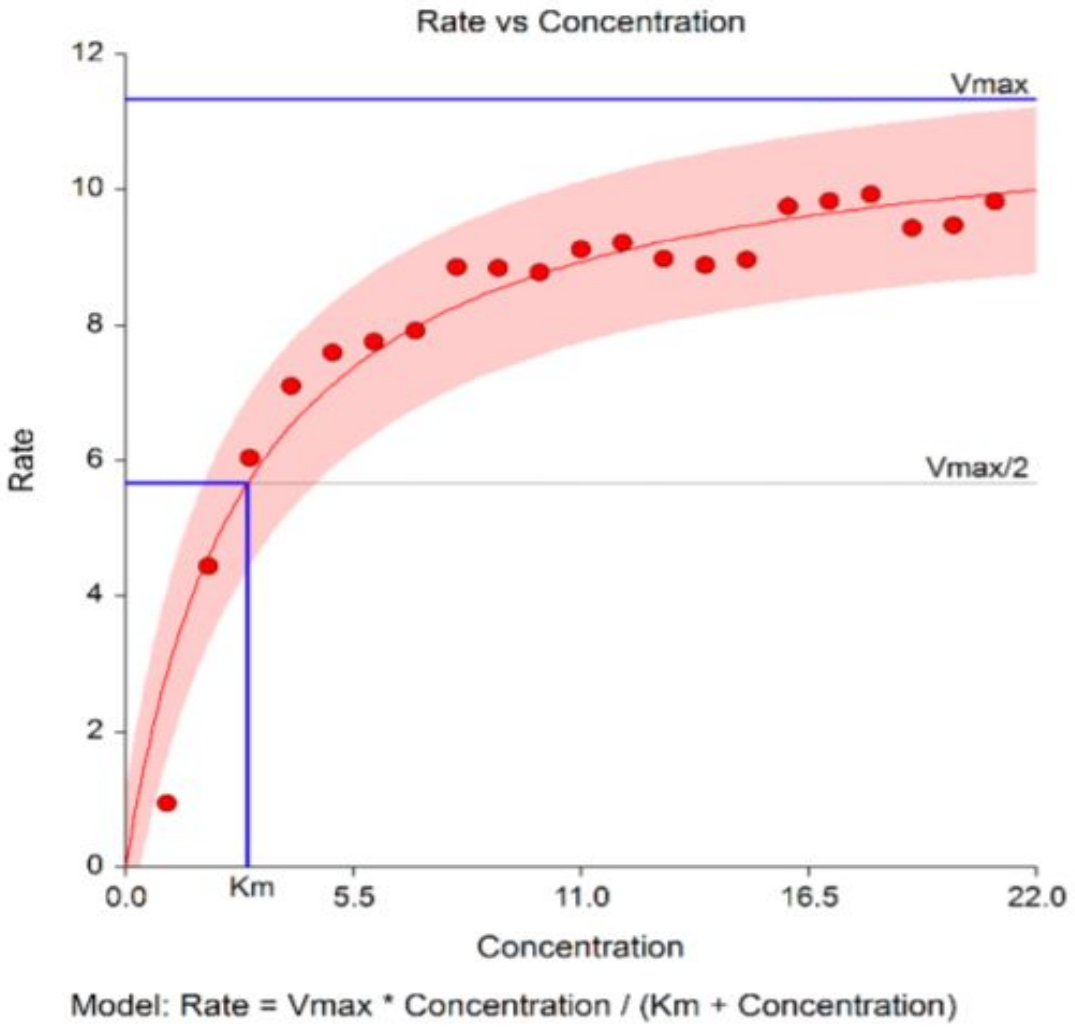
The Michaelis-Menten equation is a well-known model used in enzyme kinetics. It is a special arrangement of a two-parameter rectangular hyperbola. The mathematical model is

$$VV = \frac{C(Vmax)}{C + Km}$$

where V is the dependent variable, C is the independent variable, and Vmax and Km are parameters to be estimated. In enzyme kinetics, V is the velocity (rate) of an enzyme reaction and C is the substrate concentration. Vmax and Km have simple physical interpretations. Vmax is the maximum velocity and serves as a horizontal asymptote. Km, the Michaelis constant or ED50, is the value of C the results a velocity of Vmax/2.This provides new technologies for fitting and testing the parameters of the Michaelis-Mentenequation that have not been easily available. First, it can fit several batches of data simultaneously. Second, it compares fitted models across batches using both graphics and numerical tests such as an approximate F-test for curve coincidence and a computer-intensive randomization test that compares curve coincidence and individual parameter values. Third,

it fits both a maximum-likelihood and a nonlinear regression model. Fourth, it computes bootstrap confidence intervals for parameter values, predicted means, and predicted values using the latest computer-intensive bootstrapping technology.





Michaelis-Menten equation. An equation for evaluating enzyme kinetics in a system: v = VS/Km + S, where v = Initial velocity of reaction; V = Maximum (or limiting) velocity; S = Substrate concentration; and Km = Michaelis constant.

Many enzymatic reactions in biochemistry are far more complex than the celebrated Michaelis−Menten scheme, but the observed turnover rate often obeys the hyperbolic dependence on the substrate concentration, a relation

established almost a century ago for the simple Michaelis−Menten mechanism. To resolve the longstanding puzzle, we apply the flux balance method to predict the functional form of the substrate dependence in the mean turnover time of complex enzymatic reactions and identify detailed balance (i.e., the lack of unbalanced conformtional current) as a sufficient condition for the Michaelis−Menten equation to describe the substrate concentration dependence of the turnover rate in an enzymatic network. This prediction can be verified in single-molecule event-averaged measurements using the recently proposed signatures of detailed balance violations. The finding helps analyze recent single-molecule studies of enzymatic networks and can be applied to other external variables, such as force-dependence and voltage-dependence.

Estimation

Nonlinear regression is the algorithm used in NCSS to fit various nonlinear model. The nonlinear regression model associated with the Michaelis-Menten equation is

VV = C(Vmax)
C + Km + $\varepsilon\varepsilon$

where ε represents normally distributed errors with zero mean and constant variance $\sigma\sigma$2. It provides estimates, confidence intervals, and statistical hypothesis tests based on this assumption. The method is documented in the chapter entitled Introduction to Curve Fitting. We refer you to that chapter for details.

Modified Residuals
Davison and Hinkley (1999) page 279 recommend the use of a special rescaling of the residuals when bootstrapping to keep results unbiased. Because of the high amount of computing involved in bootstrapping, these modified residuals are calculated using

$$e_j^* = \frac{e_j}{\sqrt{1-\frac{1}{N}}} - \bar{e}$$

where

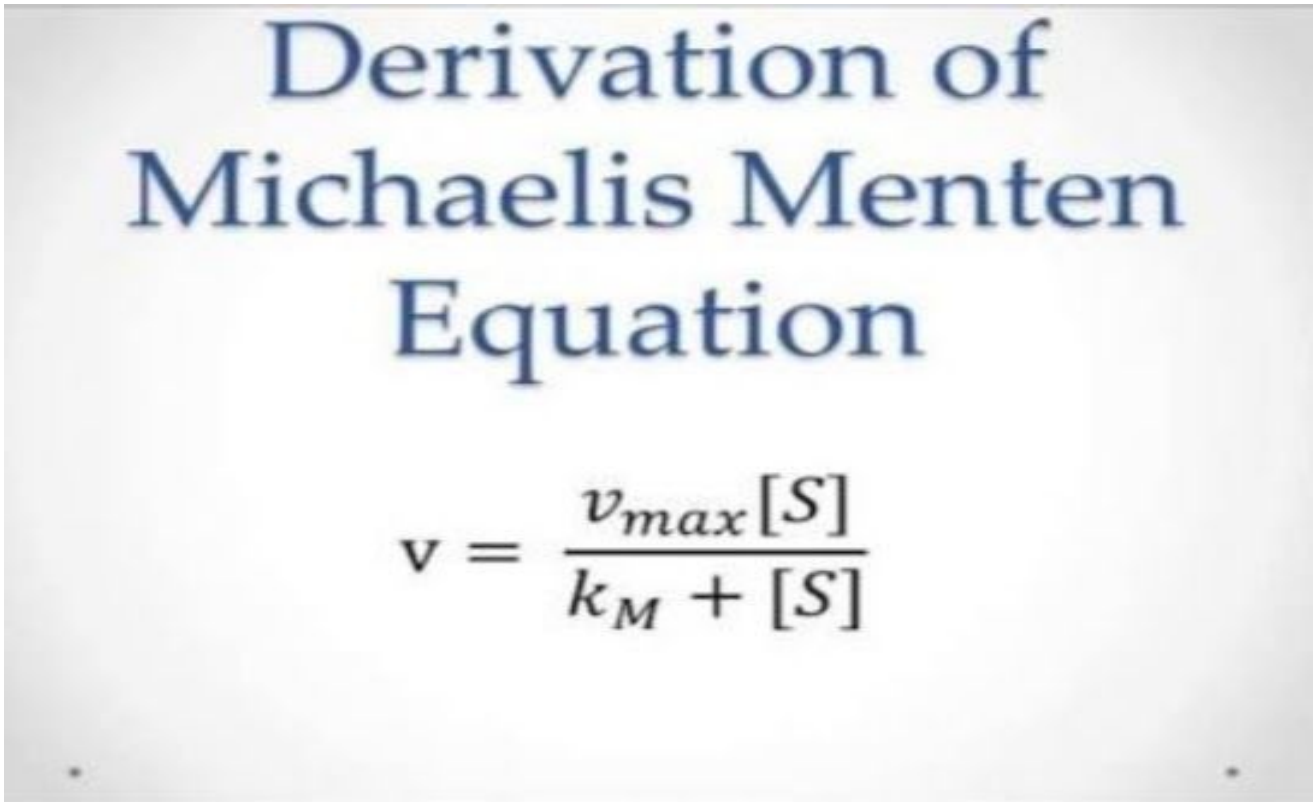
$$\bar{e} = \frac{\sum_{j=1}^{N} e_j}{N}$$

Note that there is a different rescaling than Davison and Hinkley recommended. We have used this rescaling because it is much quicker to calculate.

Hypothesis Testing

When curves are fit to two or more groups, it is often of interest to test whether certain regression parameters are equal and whether the fitted curves coincide. Although some approximate results have been obtained using indicator variables, these are asymptotic results and little

is known about their appropriateness in sample samples. We provide a test of the hypothesis that all group curves coincide using an F-test that compares the residual sum of squares obtained when the grouping is ignored with the total of the residual sum of squares obtained for each group. This test is routinely used in the analysis of variance associated with linear models and its application to nonlinear models has occasionally been suggested. However, it is based on naive assumptions that seldom occur.



Michaelis– Menten equation in term of *Vmax*

Vmax =maximum velocity.

This is the velocity at which all the enzyme molecules are in complex form. That is-

i.e., $[E]_o - [ES]$.

$V_o - Vm$

From eqn 1

$[E]_o - Vm/k_2$

Now from eqn (5)

$$v_o = \frac{vm[S]}{[S] + Km} \quad \text{.....6}$$

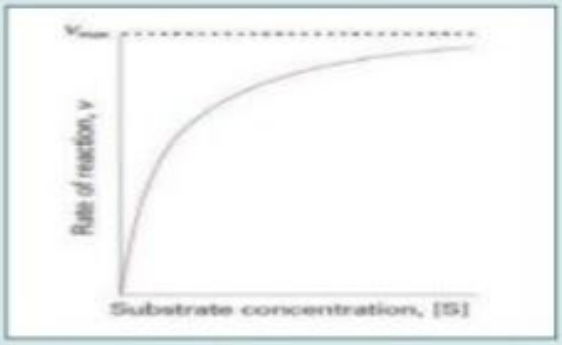


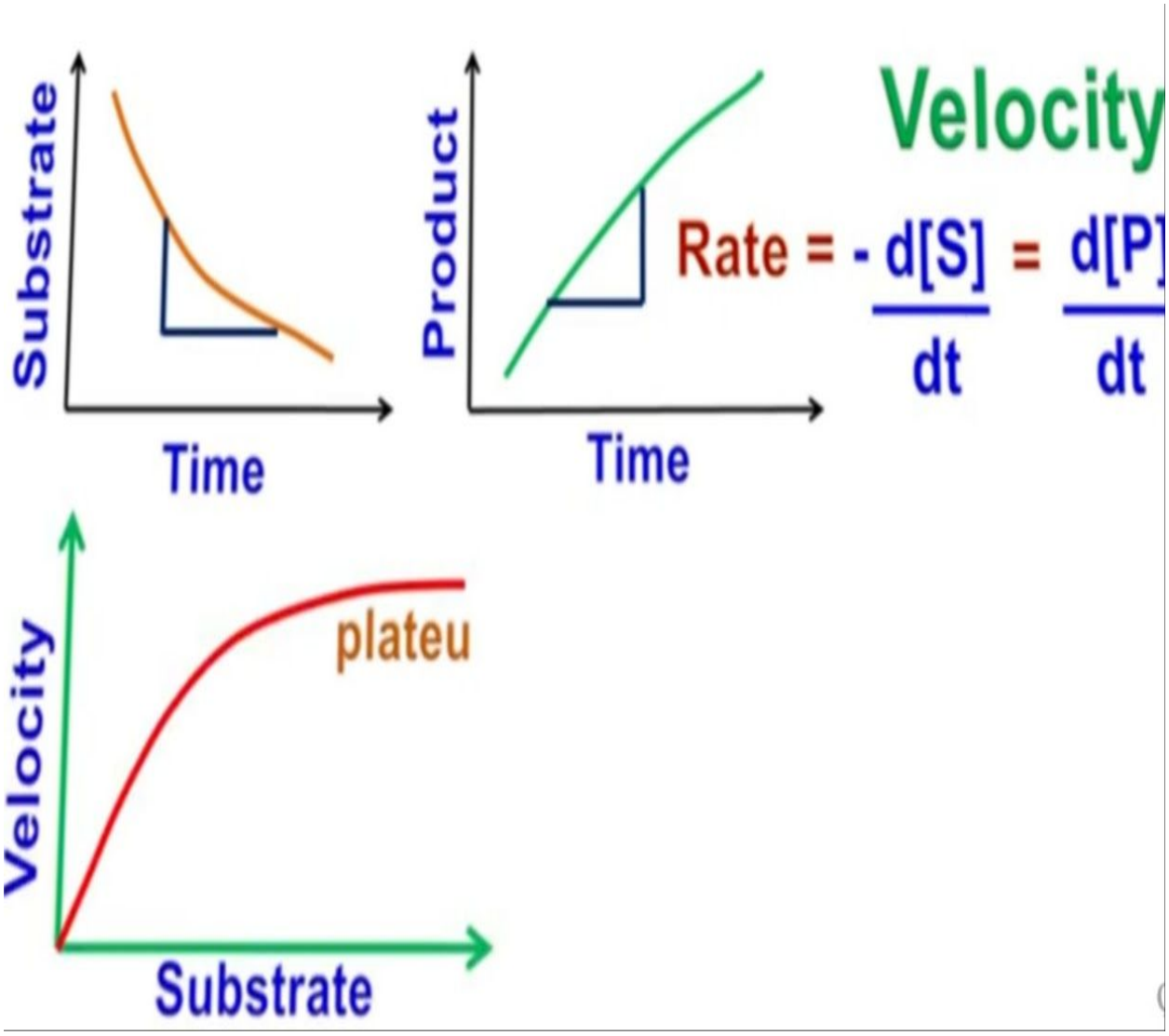
Fig 1: *The variation of the rate of an enzyme-catalysed reaction with substrate Concentration. The approach to maximum rate, Vmax, for large [S] is explained by the Michaelis–Menten mechanism.*

Confidence Intervals

Two methods are used to calculate confidence intervals of the regression parameters and predicted values. The first method is based on the usual normality and constant variance of residuals assumption. When the data follow these assumptions, standard expressions for the confidence intervals are used based on the Student's t distribution. Unfortunately, nonlinear regression dataset rarely follow these assumptions.

The second method is called the bootstrap method. This is a modern, computer-intensive method that has only

become available in recent years as extensive computer power has become available.



[ES] formation = [ES] Breakdown

$$K_F [E][S] = K_R [ES] + K_{cat}[ES]$$

$$K_F [E][S] = [ES] (K_R + K_{cat})$$

$$\frac{[E][S]}{[ES]} = \frac{K_R + K_{cat}}{K_F}$$

Briggs – Haldane

Pseudo-steady-state hypothesis

[ES] formation = [ES] Breakdown

$$[ES] \text{ formation} = K_F [E][S]$$

$$[ES] \text{ Breakdown} = K_R [ES] + K_{cat}[ES]$$

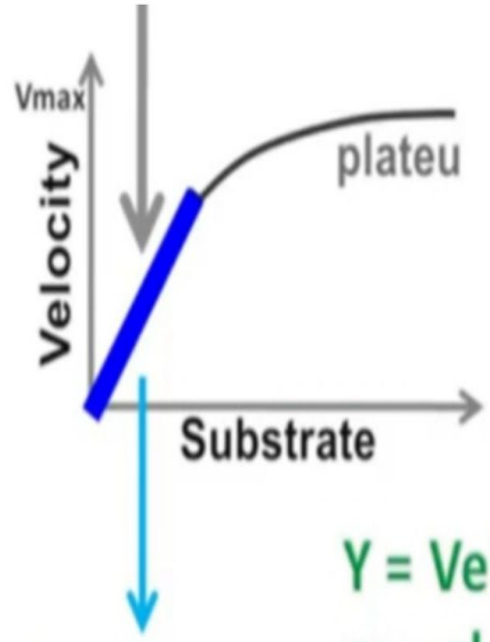
Enzyme and Substrate	K_M (mM)
Catalase	
H_2O_2	25
Hexokinase	
Glucose	0.15
Fructose	1.5
Chymotrypsin	
N-Benzoyltyrosinamide	2.5
N-Formyltyrosinamide	12.0
N-Acetyltyrosinamide	32
Glycyltyrosinamide	122
Carbonic anhydrase	
HCO_3^-	9.0
Glutamate dehydrogenase	
Glutamate	0.12
α-Ketoglutarate	2.0
NH_4^+	57
NAD_{ox}	0.025
NAD_{red}	0.018

$$E + S \underset{K_R}{\overset{K_F}{\rightleftharpoons}} ES$$

Equilibrium assumption

$$K_F\,[E][S] = K_R\,[ES]$$

$$\frac{[E][S]}{[ES]} = \frac{K_R}{K_F} = K_d$$



No Change in velocity with increase in substrate concentration

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0^{th} order kinetics

$Y = mx + c$

Y = Velocity

m = slope

X = substrate

C = intercept

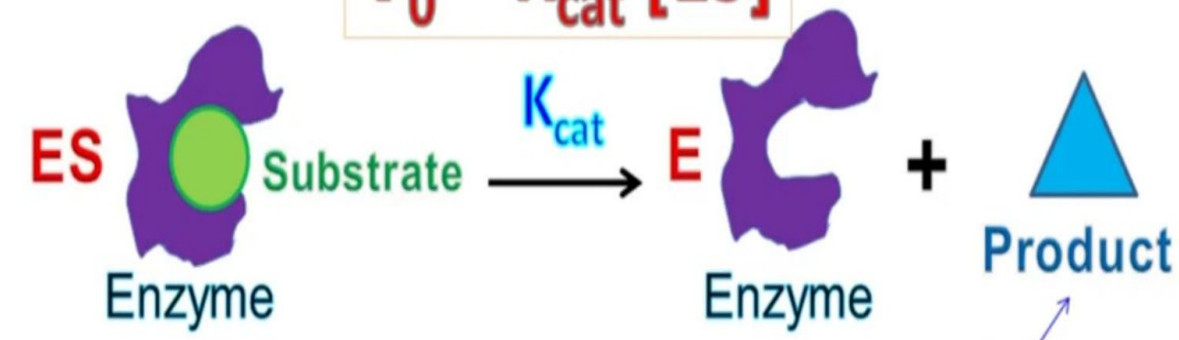
Aim :- Mathematical relation between

V_0, V_{max} and K_M

$$\frac{[E][S]}{[ES]} = K_M$$

$$E + S \underset{K_R}{\overset{K_F}{\rightleftharpoons}} ES \xrightarrow{K_{cat}} E + P$$

$$V_0 = K_{cat}\,[ES]$$



Velocity V_0 depends on break down of ES complex

Velocity $V_0 = \dfrac{d[P]}{dt}$

QBB
YouTube

$$E = E_0 - ES$$

$$V_0 = K_{cat}[ES]$$

$$V_{max} = K_{cat}[E_0]$$

$$K_M = \frac{[E][S]}{[ES]} = \frac{[E_0 - ES][S]}{[ES]}$$

Reference:-

1. **Quick biochemistry basics**
2. **Google**
3. **Cell physiology by - Arthur C. Giese**
4. **Lehninger Biochemistry .**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HOME
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