



INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

New Modification of Michaelis-Menten Equation & its Graphical Approach

Narayan G Hebsur & B.K Prabhakar

Associate Professor

Department of Chemistry, SSAS Government First Grade College, Hospet-583201, Karnataka, India

ABSTRACT

Physical constants are either absolute or conditional. The conditional constants are correlated a new with the physicochemical properties. This new correlation is verified experimentally by considering enzyme kinetic studies. The Henri-Michaelis-Menten equation has been transformed into a generalised expression to suit the concept. Using established methods, the proteolytic and nonproteolytic enzyme activities have been plotted against the corresponding Michaelis constants K_m . Linear graphs as anticipated are obtained in justification of the new correlation. Possible extensions to other fields are proposed.

INTRODUCTION

Physical constants in science are not only inevitable, but also invaluable. They are actually of two types. One of them absolute. It neither changes with the system nor the environmental conditions. The other is conditional it not only varies from system to system but also with the environmental conditions. Thus, we have (1) absolute and (2) conditional or variable constants.

The conditional constants with their subtle variation have been providing an insight to the STATE OF ART of the system. Hammett with his famous Linear Free Energy Relations (LEER) and Bronsted with his acidity relations have opened an invaluable concept which today is a legend. Taft, Charton and others have extended this concept which is now being immensely used in Quantitative Structure Activity Relation (QSAR) and biological correlation studies. These correlations are based on the study of substituent effects. They are restricted to a type of compounds (aliphatic, aromatic etc).

It is also possible to explore a more generalised correlation encompassing all types of systems. Therefore, we propose that:

"Conditional constants which do vary with the system and/or environment, have definite relation with one and/or more variable properties, of a series of such system "

In this report we place the experimental observations in support of the above relation.

Several fields involving physical constants can be considered. However, we chose the enzyme kinetic studies in view of its significance.

The profound utility of enzyme kinetic studies is well known. Several attempts have been made to describe the catalytic activity of enzyme in exact mathematical terms. Henri and Brown put forth a mathematical expression to explain the effect of substrate concentration on the velocity of reaction. Michaelis and Menten have extended the Henri's equation, based on simple chemical equilibrium principles. Briggs and Haldane have introduced the steady state concept, while several others (4) have provided alternate models. In this report, we are merely concerned with the famous Henri—Michaelis Menten equation (HMM). The Michaelis constant K_m is the chosen conditional constant and the activity of enzyme, the variable physicochemical property.

The Concept

The concept envisages the correlation between a conditional constant of the system and one of the physicochemical properties involved in the system. This results in generalising the straight line equation as:

$$Y_i = m_i X + c_i$$

where y_i is a common property and m_i is conditional constant of the i th system and c_i is fixed or variable intercept. X which was a variable for one of the property now made as affixed property.

To suit the straight line equation (I) the H.M.M. equation which was modified by Lineweaver and Burk has been transformed by us. The details are given in results and discussion.

Materials and Methods

The materials available and the methods permissible in this laboratory are used. The results presented thereby are accurate to the limit of constraints which if necessary could be improved with better facilities.

Double distilled water whenever used was boiled to free it from carbon dioxide and cooled.

All the glasswares used were pyrex or corning. They have been washed thoroughly and calibrated wherever necessary. The buffers (acetate and phosphate) were prepared as per standard procedures.

The chemicals used were all high quality grade. Starch, maltose, casein, tyrosine and 3,5-dinitrosalicylic acid were BDE make. alpha and beeta amylase (both saliva and barley) were Sigma Chemicals. Trypsin, chymotrypsin, protease and papain were from Sisco Chem. All the necessary reagents DNS, Folin-Ciocalteu, alkali copper reagent, TCA were prepared as per known procedures. In all experiments 1% substrate solution was used by dissolving 5 g of substrate in 50 ml of the corresponding buffer solution.

The maltose and tyrosine calibration curves were constructed as per known methods. The K_m and V_{max} values were obtained primarily by Lineweaver-Burk plots. They were verified wherever necessary by other standard plots.

The units of enzyme activity is such that one unit is the amount of enzyme that catalyses the conversion of 1 mg of substrate per min under defined conditions.

Digital spectrophotometer (Elico model CL-27) and pH meter (Elico model Li-122) were used for all the measurements.

Results and Discussion

The aim is to test the validity of the proposed relation involving conditional constants. This cannot be construed as being achieved unless the experimental results are found to be authentic. Experiments, therefore on the activity studies of both proteolytic and non-proteolytic enzyme systems as shown were carried out.

Enzyme	Source	Substrate	Product
NonProteolytic			
Alpha Amylase	Porcine Pancrease & Saliva	Starch	Maltose
Beta Amylase	Barley	Starch	Maltose
Proteolytic			
Trypsin	Porcine Pancrease	Casein	Tyrosine
Chymotrypsin			
Protease			

Papain			
--------	--	--	--

The activity with respect to different substrate concentrations has been measured for all the system at room temperature (25 °C) . From these, the values of Vmax and

Km have been found by established procedures. They are shown in Table—I and 2. The Vmax and K values are in the Order as expected .

In order to verify the new correlation, the H.M.M. equation is transformed as follows:

The HMM equation for a single enzyme—substrate system is,

$$v = \frac{V_{max}[S]}{K_m + [S]}$$

where the parameters represent the initial velocity, V max—maximal velocity, [S] = substrate concentration.

The above equation is generalised as follows for all systems.

$$\frac{V_{max}(i)}{vi} = \frac{K_m}{[S]} + 1$$

This equation is similar to equation for Straight line.

[S] fixed for all the systems. The graph should be linear with the slope = 1/[S] with an intercept. using the data in Table—I and 2, new graphs on the lines of proposed relation have been plotted in Fig. 1 and 2 for non—proteolytic and proteolytic enzymes respectively.

This has been done by considering the activities of different enzymes on same substrate corresponding to same concentration— Vmax (i) ions by keeping the other factors constant. The Vmax/v Vs K plots show a linear relation. The intercept =1 as anticipated. Fig. 1 and 2

It is also obvious that the same trend occurs for all the other substrate concentration held constant for the series. For each selected substrate concentration for different series, the slope remains the same. For different selected

substrate concentrations, the slopes are different. It is very interesting and welcome to observe that each slope corresponds to the reciprocal substrate concentration chosen.

The intercept remains $1/V_m$ for all the substrate concentrations.

Thus, it is a positive proof of series of enzyme kinetic studies providing a linear relation in accordance with the new concept. It is to be noticed that the proteolytic and non-proteolytic enzyme systems have been shown on two different graphs. This is obviously due to the wide concentration ranges of application •

This definite relation between the conditional maximum content K_m and variable physico-chemical property μ ,

in accordance with the new concept, leads to a new graphical approach in enzyme kinetics encompassing all the systems in general. Even though, several modifications of the M.

equation and the resulting plots are reported in the literature, they all are confined to a single system. No correlation between the conditional constant and the associated physico-chemical properties entangling a series of systems is found.

In view of the experimental justification, it is $V_{max}(i)$ now possible to construct V vs K graphs theoretically, because the intercept which is always unity and which can be chosen and hence, the reciprocal of the slope are preknown. This helps in producing the V values for any K_m value. It may also be noticed that there is no need of least square fittings. If experimental values of any system do not fit into this graph, then they may simply does not obey the H.M.M. mechanism. The accuracy with which the slopes provide the substrate concentration needs to be explored towards the plausible analytical applications of this new graphical method. validity of this relation is sure to hold good when other environmental conditions like, pH, temperature etc. are varied.

Application to Other Physico-Chemical Properties

In view of the results obtained in justification of the new correlation, it is now possible to extend its application to other systems.

1. Acid-Base Equilibria is one fertile field wherein the ionisation constants are the conditional constants and pH, a very important physico-chemical property. Here also the proposed correlation should be valid. In fact in this laboratory the work by Prabhakar and Srinivas Rao has yielded positive results. Further work is in progress.

2. The Michaelis—Menten type of approach has also been incorporated in adsorption studies. The expression for Langmuir isotherm is akin to the H.M.M. expression. This expression when suitably modified for verification of the proposed correlation may turn out to be of immense potential in predicting the surface properties for better absorption.

Physical constants which vary from system to system as well as environmental conditions for the same system may

as well be subjected to this type of study in any field of science.

We believe that approach may generate new vistas if it withstands the test of time and attracts a score of researchers.

NOTE: We have presented the experimental results in this report. The well established LFERs have their own unique approaches and utilities,

