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Book review

ENZYME KINETICS
A Modern Approach

Alejandro G. Marangoni
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"Structure is the ultimate expression of the complexity of nature."
Alejandro G. Marangoni

Enzymes are catalysts for the greater part of chemical reactions occurring in the body but at the same time these biocatalysts have proven to be one of the most useful tools for developing clean technologies and solving other problems related to a clean environment. The great interest of the chemists on such subjects of research and/or industrial applications gives reasons for many publications covering subjects like as enzymes sources, enzymes extraction, enzymes purification, enzymes kinetics, enzymes applications etc.

In this book, *Enzyme Kinetics, A Modern Approach*, the author presents new ways of looking at an old subject, namely, which are the kinetic laws governing the enzyme behavior, in other words how do enzymes work? The author's attention is focused on the mathematical model describing the catalytic function of the enzymes either in their soluble form or immobilized form. The treatment of enzyme kinetics in this book is different from the traditionally ones. The author emphasises the understanding of how researchers arrive at models, what are the models limitations, and how they can be used in practical ways to analyze enzyme kinetic data.

The enzyme kinetics is discussed in 229 pages including 15 chapters, a selective bibliography and an index.

The first chapter, *Tools and Techniques of Kinetic Analysis*, makes a survey on the two main approaches explaining the chemical reactivity: thermodynamic and kinetic. There are covered here the elementary rate laws, the dependence of the reactions rates on temperature, the principles of acid – base catalysis, the theory of reaction rates. The ending part of this chapter is dedicated to the complex reaction pathways.

How do Enzymes Work? – it is a short chapter of which content symbolizes the book quintessence. Here, based on key – equations, the author gives a concise description of enzyme catalysis pointing out the relation between biocatalyst and the activation energy.

Chapter 3, *Characterization of Enzyme Activity*, starts with a brief description of reaction rate based on the progress curve of substrate and products. Then, the author has considered being his duty to give some enzymatic reaction models at equilibrium and steady state and at the end, a typical analysis of reaction rate versus substrate concentration data set is described.

The inhibition is one of the significant parts of enzyme catalysis, the behaviour of a particular enzyme being changed in the presence of an inhibitor molecule. The 4th chapter, *Reversible Enzyme Inhibition*, is dedicated to reversible enzyme inhibition considering the four aspects: competitive, uncompetitive, non-competitive and linear mixed type. Some applications included in this chapter show the importance of the inhibition in the catalysis by enzymes.

The irreversible inhibition is pointed out in the chapter 5, *Irreversible Enzyme Inhibition*. In fact, here the author wants to emphasize the differences between the effects of reversible and irreversible inhibitions because sometimes irreversible inhibition could be considered as non-competitive reversible inhibition. Some simple models illustrate the irreversible inhibition.

Enzymes are proteins constituted by amino acids, which mean that positive and negative charges are well represented in the macromolecules. From another point of view, these "building pieces" are varying as both, disposal and weight in a protein – enzyme. As consequence, each enzyme works at a certain pH value. *pH Dependence of Enzyme – Catalyzed Reactions* makes the issue of the 6th chapter. The chapter starts with a model of pointing out the understanding the effects of pH on enzyme catalyzed reactions. In fact the author refers to both, pH dependence of the catalytically active functional groups in the enzyme and any ionisable groups in the substrate. Afterwards, the pH dependence of the catalytic parameters, and a new method of determining pK values of catalytically relevant functional groups are presented.

So far, the basic biocatalysis equations described are referred to the enzymatic reactions with one single substrate but there are enzymes catalyzing the transformations of two or more substrate and the mathematical models become more and more complicated. Chapter 7, *Two - Substrates Reactions*, includes considerations regarding the Bi Bi mechanism which presents three hypostases, namely: random – sequential Bi Bi mechanism, ordered – sequential Bi Bi mechanism, and respectively, Ping – Pong Bi Bi mechanism. Apparently, all the three models are very ease to mistake one with other and it is the reason for the last part of the chapter, where the author mentions how it could differentiate between them.

Multisite and Cooperative Enzymes is the chapter that cover the oligomer enzymes area. Clarifying information about allosteric and cooperative enzymes is done at the beginning of this chapter. The author develops kinetic models fitting the behaviour of these biocatalysts. The chapter ends with an illustrative analysis of the initial velocity for a cooperative enzyme using the Hill model (the simplest model describing the kinetic behaviour of cooperative enzymes) and respectively, CT model (concerted transition or symmetry model, accounted for

allosterism but it could not explain anticooperativity).

Immobilized enzymes that make the subject of many books and articles, is briefly reviewed in the chapter 9th. In fact, the author underlined only one feature of enzyme immobilization with respect of the book topic, namely the reactors with immobilized enzymes. The basic equations of the main types of immobilized reactors used (batch, plug – flow, and respectively, continuous – stirring) are done.

Interfacial enzymes, the subject of the 10th chapter, operate at an organized interface such as lipid aggregates in contact with the aqueous phase. Phospholipases and lipases are the main representative examples of this class. Succinct description of theses enzymes is given at the beginning of the chapter after that a model referring to interfacial binding and interfacial catalysis, respectively is done. Determination of interfacial area per unit volume is needed to complete the kinetic study of interfacial enzymes and it is clearly showing by the author. As usual, the theory is exemplified by simulations of the effects of changing of some variables such as dissociation constant of the interfacial enzyme, interfacial enzyme coverage and respectively, effective saturation surface concentration of interfacial enzyme on initial velocity. The determination of saturation interfacial enzyme reporting ends this chapter.

Transient phases of enzymatic reactions, chapter 11, stresses the methods required for the determination of individual rate constants of an enzymatic reaction, namely rapid – reactions techniques and relaxation techniques. The reaction mechanisms mutually accompanied by early and respectively late stages of the reaction providing valuable information for these mechanisms are done, as well.

Enzyme stability is a parameter required of many studies on enzymes behaviour. As consequence, the author does not overlook this feature and he describes it in the 12th chapter, *Characterization of enzyme stability*. Both kinetic and thermodynamic aspects characterize the enzyme stability. The kinetic treatment consists of a model, half – life, decimal reduction time, activation energy, Z value, the last one referring to the temperature dependence of the decimal reduction time, *D*. The thermodynamic characterization of enzyme stability gravities around the denaturation process considered as one – step, reversible transition between the native and denatured states. Once again the theory is illustrated by practical examples.

In the 13th chapter, *Mechanism based inhibition*, this kind of inhibition is discussed in its broadest sense, where an inhibitor is transformed by the enzyme catalytic mechanism to form an enzyme – inhibitor complex. In the literature this mechanism is also known as *suicide inhibitors, suicide substrate inhibitors, alternate substrate, substrate inhibitors*, and *enzyme inactivators*, as well as *irreversible, catalytic*, or k_{cat} *inhibitors*. The terms *alternate substrate inhibition* and *suicide inhibition* are used in this chapter to describe the two major subclasses of mechanism – based inhibition. 4H-3,1-benzoxazin-4-ones (inhibitors of serine – proteases) and a series of ynenol lactones (inhibitors of human leukocyte elastase) as inhibitors examples for *alternative substrate*

inhibition and respectively, *suicide inhibition* are done.

Putting kinetic principles into practice is a chapter of which each subchapter is in fact a question. The purpose of this chapter is to illustrate how the application of simple kinetic principles and the relationships are critical to analyzing and reaching appropriate conclusions for experimental observations on enzyme kinetic properties. The subchapters – questions such as "Were initial velocities measured?", or "Does the Michaelis – Menten model fit?", or "Is there consistency working within the context of a kinetic model?" have in their power to support the idea that enzymatic catalysis is more than a simply putt in practice of some theoretical models. For a particular enzyme, the model could suffer changes that could be greater than before, depending on the enzyme, or more specific the catalytic site surroundings.

Enzymes are proteins and each protein has a particular structure. The relationship between structure and function of a given protein is a key – element of the protein function. This relationship makes the subject of the ending chapter in this book, *Use of enzyme kinetic data in the study of structure – function relationships of the proteins*. In fact, here the author examines how enzyme kinetic data, by posing various questions, can be used in protein structure – function studies based on molecular biological techniques. It is pointed out that the knowledge of the kinetic and structural information will open ways not only to better understand enzyme catalytic mechanism at the molecular level but also to design enzymes for specific end uses.

Being treating radically different from the traditional ways, short and concise but complete, the subject of the presented book attest to be a practical instrument of work for students, technicians, young researchers or even experimented researchers because as the author said "time is today's most precious commodity" and why not, for any person interested in understanding of how nature works. Enzyme Kinetics is a helpful, innovative resource for practicing researchers in the chemical, pharmaceutical, and food science industries.

About the Author

A. G. MARANGONI, PhD, is Professor and Canada Research Chair in Food and Soft Materials in the Department of Food Science at the University of Guelph, Ontario, Canada. Since 1989 when he get his PhD in food chemistry, Dr. Marangoni's lab work concentrated on three major areas: 1) soft materials science, particularly the physical properties of plastic disperse systems in the form of edible fats and oils, 2) chemical and enzymatic lipid modification strategies for the production of nutritionally functional fats and oils, and 3) lipid and carbohydrate metabolism in plants and plant parts exposed to low temperatures.

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