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CRITERIA FOR MULTIPLICITY FOR COMPLEX BIOCHEMICAL REACTIONS

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INTRODUCTION

Many biochemical reactions involving immobilised or free enzymes as catalyst follow a complex kinetic scheme and do not obey simple Michaelis-Menten kinetics. Substrate inhibited and product inhibited reactions are common examples. The kinetics of substrate inhibited reactions can be represented as

$$r_A = \frac{kES}{K_m + S + K_i S^2} \tag{1}$$

These reactions can have multiple operating steady states as shown by earlier workers [Moo-Young and Kobayashi, 1972, Ramachandran, 1975, Wadiak and Carbonell, 1975]. The multiple steady states arise due to the fact that r_A attains a maximum at some intermediate finite value of the substrate concentration. The conditions under which these steady states can exist have been identified [McGrath and Yang, 1975, Kulkarni and Ramachandran 1980].

A kinetic scheme which exhibits similar behaviour can be represented as

$$r_A = \frac{kES + k'ES^2}{K_m + S + K_i S^2} \tag{2}$$

Such a scheme arises in a number of biochemical systems due to certain complex mechanisms, such as enzymes existing in two different forms, two substrate systems, enzymes with multiple subsites etc. A number of examples for these are given by Laidler [1973] [see also Reiner (1969)]. Under certain conditions the reaction rate given by Eq. (2) also attains a maximum at a certain intermediate value of the concentration, and therefore shows the possibility of existence of multiplicity. It is important to identify the values of the kinetic and operating parameters within which the multiplicity of states exists so that the rational analysis of the experimental data is possible. Such information is also useful in the design and operation of immobilised enzyme reactors. This paper attempts to delineate the conditions for the occurrence of multiplicity for the kinetic scheme given by Eq. (2). Such an analysis does not appear to have been published before. The earlier work on substrate inhibited kinetics [Eq. (1)] can be obtained as a particular case of the present analysis by letting $k' \rightarrow 0$.

THEORY

The conservation equation for the substrate following the kinetics given by Eq. (2) can be written for a fully backmixed reactor as

$$\frac{F}{V}[S_0 - S] = \frac{kES + k'ES^2}{K_m + S + K_iS^2} \tag{3}$$

where F is the flow rate, V is the volume of the enzyme in the reactor, S_0 and S represents the concentration at the reactor inlet and in the reactor.

Equation (3) can be written in dimensionless form as:

$$\alpha[1 - a] = \frac{a + \delta a^2}{1 + \beta a + \gamma a^2} \tag{4}$$

where,

$$a = \frac{S}{S_0} \tag{5}$$

$$\alpha = \frac{K_m F}{kEV}; \tag{6}$$

$$\delta = \frac{k'S_0}{k} \tag{7}$$

$$\beta = \frac{S_0}{K_m} \tag{8}$$

$$\gamma = \frac{K_i S_0^2}{K_m} \tag{9}$$

The formulation as given by Eq. (3) assumes that there are no mass transfer limitations. Also it may be noted here that although Eq. (3) has been written for a fully backmixed system it can also represent the other cases with α defined suitably. Thus for the case of single enzyme system where the rate is controlled by external film diffusion α would be defined as:

$$\alpha = \frac{3k_s K_m}{RkE}$$

Similarly for the case of intraparticle diffusion of substrate with reaction, α can be approximated as $(10.5/\phi^2)\ K_m/kE$, where ϕ represents the Thiele modulus [Ramachandran, 1975, Kulkarni and Ramachandran 1980]. Thus the formulation as given by Eq. (4) is generally applicable with α defined suitably.

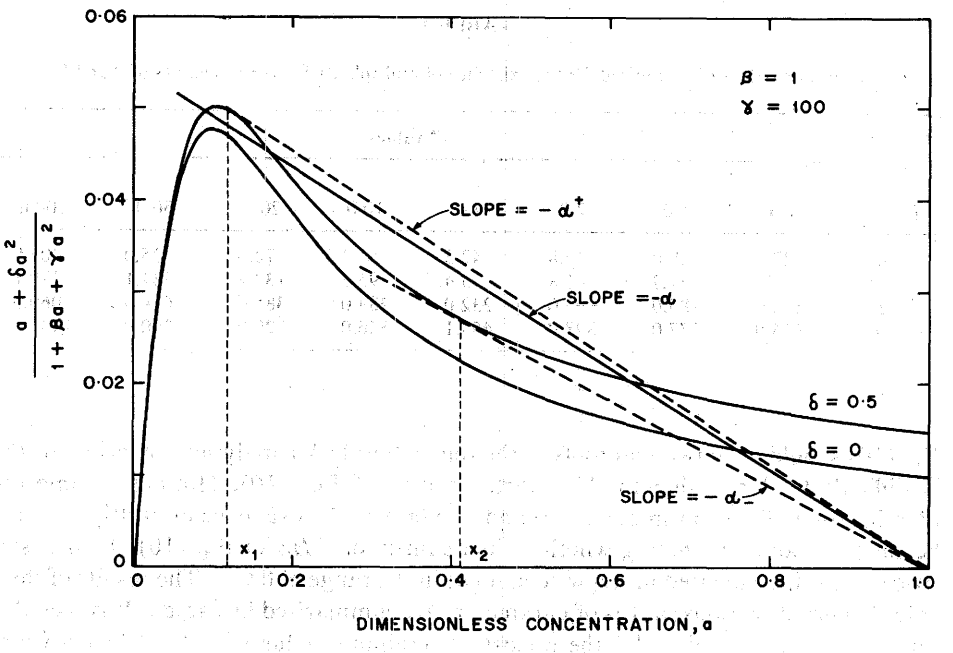

FIGURE 1. Illustration of the Region of Multiple Steady States.

In order to determine the region of multiplicity consider a graphical solution to Eq. (4). Figure 1 illustrates the procedure for $\beta = 1$, $\gamma = 100$ and $\delta = 0.5$ and $\delta = 0$. The possible solutions of Eq. (4) correspond to the points of intersection of the rate curve

$$y = \frac{a + \delta a^2}{1 + \beta a + \gamma a^2} \qquad [RHS \text{ of Equation (4)}]$$

with the straight line

$$y = \alpha[1 - a] \qquad [LHS \text{ of Equation (4)}]$$

The latter is a straight line passing through [1, 0] and having a slope of $-\alpha$. The existence of multiplicity depends on the value of α. [that is, the straight line should cut the rate curve at three points]. This implies that the slope $[-\alpha]$ should be between the slopes of the two dotted lines which are tangents to the rate curve from the point [1, 0]. The points at which the tangents touch the rate curve, x, [x_1 and x_2] can be obtained by equating the slope of the rate curve to the slope of the tangent. The resulting equation is

$$\delta x^4 + x^3 + \left(\frac{\beta\delta - \gamma + \beta - \delta}{\gamma}\right) x^2 + \frac{2}{\gamma}\delta x + \frac{1}{\gamma} = 0 \qquad (10)$$

Hence the necessary condition for the existence of multiplicity can be stated as that

TABLE I

Minimum values of γ required for the existence of multiplicity for various values of β and δ

δ \ β	γ^* values							
	0.1	1.0	2.0	5.0	10.0	20.0	50.0	100.0
0	27.4	30.0	32.8	40.3	51.7	72.0	125.0	205.0
1	52.7	57.2	61.8	75.4	95.5	132.2	231.1	379.1
5	172.0	183.0	195.0	232.0	283.0	385.0	654.0	1060.0
10	356.0	377.0	398.0	453.1	546.0	729.0	1200.0	1434.0

Eq. (10) should have two real roots in the region 0 to 1. An analytical criterion for this is difficult to derive due to the quartic nature of Eq. (10). However, numerical identification of the parameters β, γ and δ to ensure the existence of multiplicity can be easily obtained by noting whether the quantity on *LHS* of Eq. (10) changes sign twice as x is incremented in stepwise manner in the range of 0 to 1. The results of these calculations for various ranges of parameters are summarised in Table I. It is seen that for a given value of β and δ there exists a minimum value of γ [γ^*] below which multiplicity is absent. These values are given in Table I. The value of γ^* can also be expressed by an empirical equation

$$\gamma^* = 28.76 + 2.16\beta - 4.01 \times 10^{-3}\beta^2 + \delta[25.78 + 1.75\beta - 2.73 \times 10^{-3}\beta^2] \quad (11)$$

This correlation is purely empirical and merely represents the data in Table I; the only advantage of it being that it can be used readily to examine whether multiplicity exists for a given set of parameters. The correlation is fairly accurate upto $\delta = 1$ and can be used conservatively upto $\delta = 5$. These are the values which are likely to be in the region of practical interest.

For the limiting case of $\delta = 0$ Eq. (10) reduces to a cubic and the condition for the existence of multiplicity can now be obtained analytically as:

$$\frac{27}{\gamma} + \left(\frac{\beta - \gamma}{\gamma}\right)^3 \leq 0 \quad (12)$$

The situation corresponds to the case of substrate inhibition and has been analysed earlier [McGrath and Yang, 1975, Kulkarni and Ramachandran 1980].

The results of Table I represent only the necessary condition for the existence of multiplicity. In order to obtain a sufficiency criterion we note from Fig. 1 that the value of α must lie between [α^+] and [α_-] which correspond to the negative slopes of the two tangents [dotted lines] to the rate curve. Thus if x_1 and x_2 are the two roots to Eq. (10) in the region $0 < x < 1$ than α^+ and α_- are given as

$$\alpha^+ = \frac{x_1 + \delta x_1^2}{1 + \beta x_1 + \gamma x_1^2} \frac{1}{[1 - x_1]} \quad (13)$$

$$\alpha_- = \frac{x_2 + \delta x_2^2}{1 + \beta x_2 + \gamma x_2^2} \frac{1}{[1 - x_2]} \tag{14}$$

Thus the sufficiency condition can be written as:

$$\alpha_- < \alpha < \alpha^+ \tag{15}$$

As an illustration consider the parameters given in Fig. 1, [$\beta = 1$, $\delta = 0.5$, $\gamma = 100$]. The solution of Eq. (10) gives two real roots in the interval 0 to 1.

$$x_1 = 0.125 \text{ and } x_2 = 0.415$$

which can also be obtained from Fig. 1. Substituting these values in Eqs. (13) and (14) results in:

$$\alpha^+ = 0.056$$

$$\alpha_- = 0.045$$

Hence we note from Eq. (15) that the value of α should be between

$$0.045 < \alpha < 0.056$$

for the multiplicity to exist.

CONCLUSIONS

The necessary and sufficient conditions for the existence of the multiplicity have been obtained for a general biochemical reaction scheme. The parameter values for the existence of necessary condition of multiplicity have been tabulated and empirically correlated. Procedure for the calculation of the sufficiency condition is also illustrated where a simple graphical method can also be used. It is felt that the work would be useful in connection with the experimental studies on some of these complex reactions.

NOTATIONS

a	dimensionless concentration, S/S_0
D_e	effective diffusivity
E	enzyme concentration
F	flow rate of reactant
k,k'	enzymatic reaction rate constants as defined in Eqs. (1) and (2)
k_s	mass transfer coefficient across the film
K_i	substrate inhibition constant

K_m	Michaelis-Menten constant
r_A	rate of disappearance of substrate by reaction
R	radius of pellet
S,S_0	concentration of substrate in the reactor and incoming feed respectively
V	volume of the reactor
x,x_1,x_2	points at which the tangents touch the rate curve

Greek letters

α	defined as K_mF/kEV
α^+,α_-	defined by Eqs. (13) and (14) respectively
β	defined as S_0/K_m
γ	defined as $K_iS_0^2/K_m$
γ^*	minimum value of γ above which multiplicity would exist
δ	defined as $k'S_0/k$

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