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The Effect of Hydrogen Ions on the Steady State Multiplicity of Substrate-Inhibited Enzymatic Reactions

III. Asymmetrical Steady States in Enzyme Membranes

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ABSTRACT

The present investigation concentrates on the study of the complex phenomenon of multiplicity in membranes carrying substrate-inhibited, hydrogen ion-sensitive enzyme. The investigation takes into consideration both symmetrical and asymmetrical steady states. The number of steady states (symmetrical and asymmetrical) found in this case is quite large, giving rise to multiple hysteresis loops and multiple "isolas."

Index Entries: Hydrogen ions, effects on multiplicity of substrate-inhibited enzymatic reactions; multiplicity of enzymatic reactions, effect of H^+ ions on; enzymatic reactions, effects of H^+ ions on multiplicity of; membranes, asymmetrical steady states in; isolas; hysteresis loops.

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INTRODUCTION

The classical theory of steady-state enzyme kinetics (*1*) describes the kinetics of enzymes in stirred solutions. This theory has been abundantly confirmed by experiments on purified enzymes removed from their cellular milieu and placed in well stirred solutions. In cells and tissues, however, enzymes are compartmentalized by the cell surface membrane as well as by intracellular organelles. It is likely, therefore, that diffusion modulates the movement of substrates and products within and between cells, leading to the development of concentration gradients.

To deal with this situation, the theory of enzyme kinetics must be extended to incorporate the role of diffusion. Although this topic has been studied extensively in the chemical engineering literature (*2*), recent studies (*3*) have shown that membrane-bound arrays of enzymes can differ markedly in their kinetic behavior from homogeneous solutions of the same enzyme.

The kinetics of enzyme reactions may be simple Michaelis-Menten kinetics or they may be inhibited or activated by substrates, products, or hydrogen ions.

The multiplicity phenomenon results from the coupling between a number of processes taking place within the system. At least one of these processes must be a nonmonotonic function of the state of the system. For substrate-inhibited kinetics, the dependence of the rate of reaction upon substrate concentration is a nonmonotonic function of state, and multiplicity of the steady state for such a reaction when it takes place in an open system (CSTR, perfectly mixed cell, . . . etc.) is known to occur (*4–9*). When the kinetics of the reaction obey the Michaelis-Menten expression, then multiplicity does not occur because the rate of reaction is a monotonic function of the state of the system. However, multiplicity is possible with Michaelis-Menten kinetics when the reaction produces hydrogen ions (e.g., the hydrolysis of ester by an enzyme such as chymotrypsin) (*10, 11*). This dependence of enzyme activity upon hydrogen ion concentration is a non-monotonic function of the state of the system (i.e., hydrogen ion concentration). When the enzyme is inhibited by excess substrate and the enzyme reaction produces hydrogen ions simultaneously, then the multiplicity phenomenon becomes more complex. This complexity arises because the rate of reaction depends upon two of the state variables of the system (i.e., hydrogen ion concentration and substrate concentration) and the dependence of the rate of reaction on each of these variables (with the other kept constant) is a nonmonotonic function (an example of such enzyme is "acetylcholinesterase").

Such double-effect reactions have been studied for the perfectly mixed case (i.e., negligible intracellular resistances) (*12*), and in addition to the classical hysteresis curve, the phenomenon of the "Isola" has been observed for certain combination of parameters.

The purpose of this work is to investigate the complex multiplicity phenomenon for this last case, but the system considered is a membrane separating two compartments with the enzyme activity concentrated on the surface of the membrane.

Both the symmetrical and the asymmetrical steady-state cases are considered for both symmetrical and asymmetrical external field.

FORMULATION OF THE MODEL

Consider a membrane of thickness L placed between two solutions with substrate concentration $[S]_{B_0}$, $[S]_{B_1}$, respectively. The enzyme (E) is attached to both external faces of the membrane, the activities of both faces are equal, and the enzyme catalyzes the reaction

$$S \xrightarrow{E} P_1 + P^- + H^+$$

One possible rate expression for such a case is

$$r(S,H) = \frac{V_m[S]}{[S] + [S]^2/K_i + K_s(K_n + [H] + [H]^2/K_h')/[H]} \quad (1)$$

where [S] is the substrate concentration, [H] is the hydrogen ion concentration, V_m is the maximum reaction rate per unit mass of enzyme, and K_s, K_i, K_h, and K_h' are constants characteristic of the enzyme (*13*).

We assume that the acidic product is fully ionized, so that a mole of hydrogen ions is produced for each mole of substrate reacted. Further assumptions are as follows:

1. The external mass transfer resistance can be lumped into a thin film.
2. The diffusion within the membrane can be adequately discribed by Fick's law.
3. The solution compartments are very large, so that the concentration in each compartment remains constant.
4. The reaction $H^+ + OH \rightleftharpoons H_2O$ is assumed to be in equilibrium at all points of the system. Hence the equilibrium constant is given by $K_w = [H^+][OH^-]$.
5. The diffusivities of hydrogen ions, substrate, and hydroxyl ions are assumed to be constant.

Mass balances for substrate (S), hydrogen ions (H), and hydroxyl ions (OH) expressing intramembrane diffusion and boundary conditions at the two active surfaces (0,L), as shown in Fig. 1, give

(a) Intramembrane diffusion:

$$\text{Substrate: } D_S \, d^2[S]/d\mathcal{L}^2 = 0 \quad (2)$$

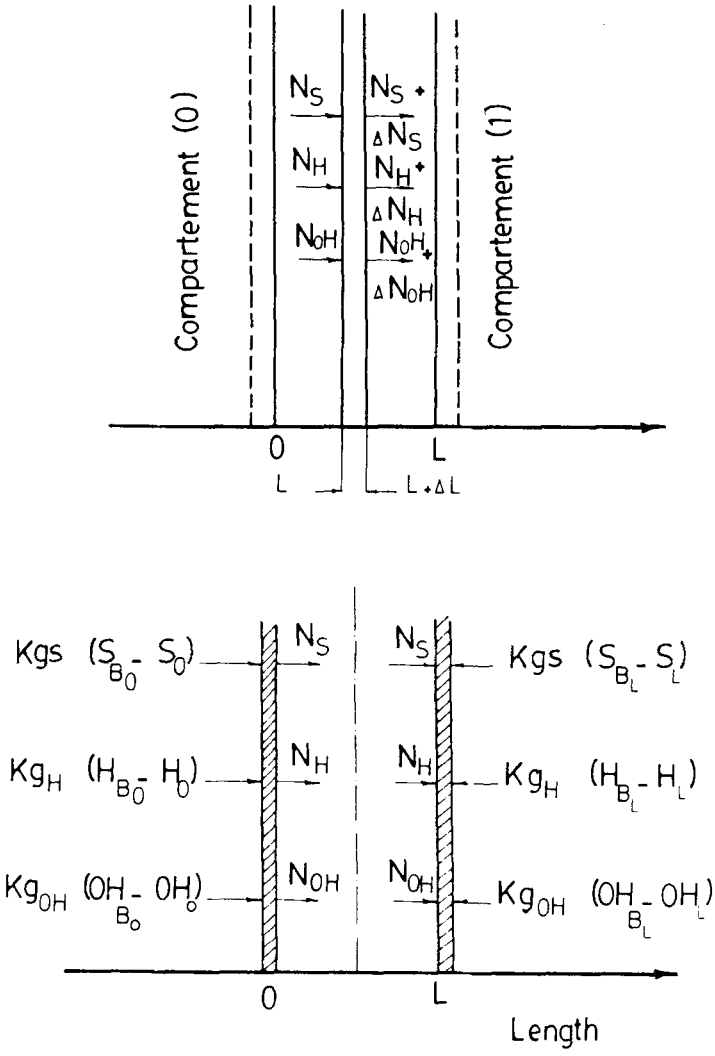

Fig. 1. Schematic representation of the membrane model.

$$\text{Hydrogen ions: } D_H \, d^2[H]/d\mathcal{L}^2 = r_w \tag{3}$$

$$\text{Hydroxyl ions: } D_{OH} \, d^2[OH]/d\mathcal{L}^2 = r_w \tag{4}$$

where r_w is the rate of water formation.

(b) Boundary conditions at surface (0):

$$\text{Substrate: } K_{gs}([S]_{Bo} - [S]_o) + D_s \left.\frac{d[S]}{d\mathcal{L}^2}\right|_{(o)} = r(S_o, H_o) \tag{5}$$

$$\text{Hydrogen ions: } K_{g_H}([H]_{Bo} - [H]_o) + D_H \left.\frac{d[H]}{d\mathcal{L}^2}\right|_{(o)} = r_w - r(S_o, H_o) \tag{6}$$

$$\text{Hydroxyl ions: } K_{g_{oH}}([OH]_{Bo} - [OH]_o) + D_{OH}\left.\frac{d[OH]}{d\mathcal{L}^2}\right|_{(o)} = r_w - r\,(S_o,H_o) \tag{7}$$

(c) Boundary conditions at surface (L):

$$\text{Substrate: } K_{gs}([S]_{BL} - [S]_L) - D_s\left.\frac{d[S]}{d\mathcal{L}^2}\right|_{(L)} = r(S_L,H_L) \tag{8}$$

$$\text{Hydrogen ions: } K_{g_H}([H]_{BL} - [H]_L) - D_H\left.\frac{d[H]}{d\mathcal{L}}\right|_{(L)} = r_w - r(S_L,H_L) \tag{9}$$

$$\text{Hydroxyl ions: } K_{g_{OH}}([OH]_{BL} - [OH]_L) - D_{OH}\left.\frac{d[OH]}{d\mathcal{L}}\right|_{(L)} = r_w - r(S_L,H_L) \tag{10}$$

Eliminating the hydroxyl ion concentration using assumption (4) ($[OH] = K_w/[H]$), and eliminating r_w, then introducing the following dimensionless groups:

$$\begin{aligned}
\gamma &= (D_{OH}K_w)/(D_HK_h^2)\\
w &= \mathcal{L}/L\\
Sh_s &= LK_{gs}/D_S\\
Sh_H &= LK_{gH}/D_H\\
B_s &= V_m/(K_sK_{gs})\\
B_H &= V_m/(K_{gH}K_h)\\
\theta &= (K_{gOH}K_w/(K_{gH}K_h^2)\\
S &= [S]/K_s\\
h &= [H]/K_h\\
\alpha_I &= K_s/K_i\\
\delta &= K_h/K_h'
\end{aligned}$$

The mathematical model equations become,

$$d^2S/dw^2 = 0 \tag{11}$$

$$d^2h/dw^2 - [2\gamma/(h^3 + \gamma h)](dh/dw)^2 = 0 \tag{12}$$

with the boundary conditions:

At surface (o)

$$(S_{B_o} - S_o) + (1/Sh_s)(dS/dw)\,|_{(o)} - B_sR(S_o,h_o) = 0 \tag{13}$$

and

$$(h_{B_o} - h_o) + B_H R(S_o,h_o) - \theta(1/h_{B_o} - 1/h_o) + (1 + \gamma/h^2)(1/Sh_H)(dh/dw)\,|_{(o)} = 0 \quad (14)$$

At surface (1)

$$(S_{B_1} - S_1) - (1/Sh_s)(dS/dw)\,|_{(1)} - B_s R\,(S_1,h_1) = 0 \quad (15)$$

and

$$(h_{B_1} - h_1) + B_H R(S_1,h_1) - \theta(1/h_{B_1} - 1/h_1) - (1 + \gamma/h^2)\,(1/Sh_H)(dh/dw)\,|_{(1)} = 0 \quad (16)$$

where

$$R(S,h) = S\{S + \alpha_I S^2 + [(h^2\delta + h + 1)/h]\}$$

ANALYTICAL MANIPULATION OF EQUATIONS

The analytical solutions of Eq. (11) and (12) in terms of the concentration of substrate and hydrogen ions at the two surface (0, 1) gives:

$$S(w) = (S_1 - S_o)w + S_o \quad (17)$$

$$h(w) - \gamma/h(w) = [(h_1 - \gamma/h_1) - (h_o - \gamma/h_0)]w + (h_o - \gamma/h_o) \quad (18)$$

Substituting the derivatives of Eqs. (17) and (18) into Eqs. (13)–(16), the problem is then reduced to the solution of algebraic equations:

$$(S_{B_o} - S_o) - B_s R(S_o,h_o) + (1/Sh_s)(S_1 - S_o) = 0 \quad (19)$$

$$(S_{B_1} - S_1) - B_s R(S_1,h_1) - (1/Sh_s)(S_1 - S_o) = 0 \quad (20)$$

$$(h_{Bo} - h_o) + B_H R(S_o,h_o) - \theta(1/h_{B_o} - 1/h_o) + (1/Sh_H)[(h_1 - \gamma/h_1) - (h_o - \gamma/h_o] = 0 \quad (21)$$

$$(h_{B_1} - h_1) + B_H R(S_1,h_1) - \theta(1/h_{B_1} - 1/h_1) - (1/Sh_H)[(h_1 - \gamma/h_1) - (h_o - \gamma/h_o)] = 0 \quad (22)$$

The four Eqs. (19)–(22) can be reduced to two equations as follows: Multiplying Eqs. (19) and (20) by B_H/B_s, the results are:

$$(B_H/B_s)(S_{B_o} - S_o) - B_H R(S_o,h_o) + (B_H/B_s Sh_s)(S_1 - S_o) = 0 \quad (23)$$

$$(B_H/B_s)(S_{B_o} - S_1) - B_H R(S_1,h_1) - (B_H/B_s Sh_s)(S_1 - S_o) = 0 \quad (24)$$

Summing Eqs. (21) and (23), and then Eqs. (22) and (24), the results are:

$$(h_{B_o} - h_o) - \theta\,(1/h_{B_o} - 1/h_o) + (1/Sh_H)[(h_1 - \gamma/h_1) - (h_o - \gamma/h_o)] + (B_H/B_s)(S_{B_o} - S_o) + (B_H/B_s Sh_s)(S_1 - S_o) = 0 \quad (25)$$

and

$$(h_{B_1} - h_1) - \theta(1/h_{B_1} - 1/h_1) - (1/\mathrm{Sh_H})[(h_1 - \gamma/h_1) - (h_o - \gamma/h_o)] + (B_H/B_s)(S_{B_1} - S_1) - (B_H/B_s\mathrm{Sh_s})(S_1 - S_o) = 0 \quad (26)$$

By summing Eqs. (25) and (26) and separating the variable S_o, the result is,

$$S_o = (B_s/B_H)(h_{B_1} - h_1) - \theta(1/h_{B_1} - 1/h_1) + (h_{B_o} - h_o) - \theta(1/h_{B_o} - 1/h_o) + S_{B_1} + S_{B_o} - S_1 \quad (27)$$

Substituting in Eq. (26), then separating the variable S_1, the result is

$$\frac{B_H}{B_s}\left(1 + \frac{2}{\mathrm{Sh_s}}\right) S_1 = \left(\frac{1}{\mathrm{Sh_H}} - \frac{1}{\mathrm{Sh_s}}\right) h_o - \left(1 + \frac{1}{\mathrm{Sh_H}} + \frac{1}{\mathrm{Sh_s}}\right) h_1 + \left(\frac{\theta}{\mathrm{Sh_s}} - \frac{\gamma}{\mathrm{Sh_H}}\right)\frac{1}{h_o} + \left(\theta + \frac{\theta}{\mathrm{Sh_s}} + \frac{\gamma}{\mathrm{Sh_s}}\right)\frac{1}{h_1} + \left[h_{B_1} - \frac{\theta}{h_{B_1}} + \frac{h_{B_1}}{\mathrm{Sh_s}} - \frac{\theta}{\mathrm{Sh_s}h_{B_1}} + \frac{B_H S_{B_1}}{B_s}\left(1 + \frac{1}{\mathrm{Sh_s}}\right) + \frac{B_H}{B_s} + \frac{S_{B_o}}{\mathrm{Sh_s}} + \frac{h_{B_o}}{\mathrm{Sh_s}} - \frac{\theta}{\mathrm{Sh_s}h_{Bo}}\right] \quad (28)$$

For easy manipulation of Eq. (28), we define,

$$a_o = \frac{B_H}{B_s}\left(1 + \frac{2}{\mathrm{Sh_s}}\right) \quad a_1 = \left(\frac{1}{\mathrm{Sh_H}} - \frac{1}{\mathrm{Sh_s}}\right)/a_o \quad a_2 = \left(1 + \frac{1}{\mathrm{Sh_H}} + \frac{1}{\mathrm{Sh_s}}\right)/a_o$$

$$a_3 = \left(\frac{\theta}{\mathrm{Sh_s}} - \frac{\gamma}{\mathrm{Sh_H}}\right)/a_o \quad a_4 = \left(\theta + \frac{\theta}{\mathrm{Sh_s}} + \frac{\gamma}{\mathrm{Sh_H}}\right)/a_o$$

$$a_5 = \left[h_{B_1} - \frac{\theta}{h_{B_1}} + \frac{h_{B_1}}{\mathrm{Sh_s}} - \frac{\theta}{\mathrm{Sh_s}h_{B_1}} + \frac{B_H S_{B_1}}{B_s}\left(1 + \frac{1}{\mathrm{Sh_s}}\right) + \frac{B_H S_{B_o}}{B_s\mathrm{Sh_s}} + \frac{hB_o}{\mathrm{Sh_s}} - \frac{0}{\mathrm{Sh_s} - h_{B_o}}\right]/a_o$$

Substiting the last definitions a_o, a_1, a_3, a_4, a_5 in Eq. (28) the result is

$$S_1 = a_1 h_o - a_2 h_1 + a_3/h_o + a_4/h_1 + a_5 \quad (29)$$

Now we have the variable S_1 as a function of h_o, h_1, i.e., $S_1 = f_1(h_o,h_1)$ Substituting in Eq. (27), the result is

$$S_o = (B_s/B_H)(h_{B_1} - h_1) - \theta[(1/h_{B_1}) - (1/h_1)] + (h_{B_o} - h_o) - \theta[(1/h_{B_o}) - (1/h_o)] + S_{B_1} + S_{B_o} - f_1(h_o,h_1) \quad (30)$$

i.e., $S_o = f_o(h_o,h_1)$

By substituting the values of S_o, S_1 from Eqs. (29) and (30) in the two Eqs. (19) and (20), the final result is two nonlinear algebraic equations,

$$S_{B_1} - f_1(h_o,h_1) - B_s R[f_1(h_o,h_1), h_1] - (1/\mathrm{Sh_s})[f_1(h_o,h_1) - f_o(h_o,h_1)] = 0 \quad (31)$$

and

$$S_{B_o} - f_o(h_o,h_1) - B_sR[f_o(h_o, h_1),h_o] + (1/Sh_s)[f_1(h_o,h) - f_o(h_o,h_1)] = 0 \quad (32)$$

The Special Case of Symmetrical Steady States

The symmetrical case means that both of hydrogen ion and substrate concentrations on both surfaces of the membrane are equal (i.e., $h = h_o = h_1$ and $S = S_o = S_1$). As a result of these equalities the bulk concentration for both hydrogen ions and substrate are equal (i.e., $S_B = S_{B_o} = S_{B_1}$ and $h_B = h_{B1} = h_{B_1}$).

Substituting in Eqs. (31) and (32), the result is

$$(S_B - S) = B_sR(s,h) \quad (33)$$

$$(h_B - h) = \theta[(1/h_B) - (1/h)] - B_HR(S,h) \quad (34)$$

Equations (33) and (34) can be reduced to one nonlinear algebraic equation as follows:

$$S_B - f(h) = B_s[f(h),h] \quad (35)$$

where

$$f(h) = S = (B_s/B_H)(h_B - h) - (\theta B_s/B_H)[(1/h_B) - (1/h)]$$

Methods of Solution

For the symmetrical case, a simple numerical method (the bisectional method) was used for solving Eq. (35).

For the asymmetrical case, the algorithm used to solve Eqs. (31) and (32) is:

1. Equation (31) is solved numerically for different values of h_o, the result is a relation between h_o, h_1. The relation between S_o, S_1 is obtained by substituting in Eqs. (29) and (30).
2. Equation (32) is solved numerically for different values of h_1, the result is a relation between h_o, h_1. The relation between S_o, S_1 is obtained by substituting in Eqs. (29) and (30).
3. The relations between S_o, S_1 obtained from 1 and 2 are plotted in the $(S_o - S_1)$ diagram.
4. The points of intersections are the required steady-state solution.

RESULTS AND DISCUSSION

Symmetrical Case

Single Effect Inhibition

In this case, the dimensionless rate of reaction term for substrate inhibition reduces to

$$r(s) = S/(1 + S + \alpha_I S^2) \tag{36}$$

and the mass balance equation is

$$(1/B_s)(S_B - s) = r(s)$$

It is useful for our study to show the effect of B_s and α_I, S_o. Figure 2 represents the solutions of the mass balance equation for three different values of B_s. For the smaller value that corresponds to high slope, the multiplicity region is small and shifted to lower values of S_B. For the medium value that corresponds to medium slope, the multiplicity region increases and shifts to higher values of S_B. For the larger value of B_s, which corresponds to lower slope, the multiplicity region increases and shifts to higher values of S_B.

To explain the effect of the parameter α_I on the multiplicity phenomenon, the maximum of rate Eq. (36) is given by,

$$dr(S)/dS = (1 - \alpha_I S^2)/(1 + S + \alpha_I S^2)^2 = 0$$

hence

$$S_{max} = \sqrt{1/\alpha_I},\ r_{max} = 1/(1 + 2\sqrt{\alpha_I})$$

i.e., as α_I increases, the value of the maximum conversion decreases, and the position of the maximum of the rate curve shifts to lower values of

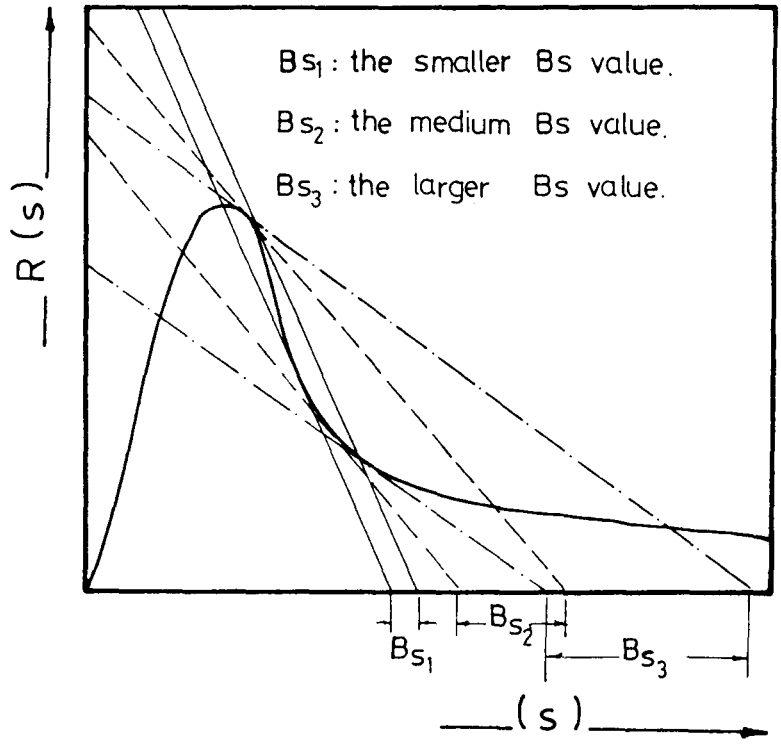

Fig. 2. Solutions of the mass balance equations for three different values of B_s.

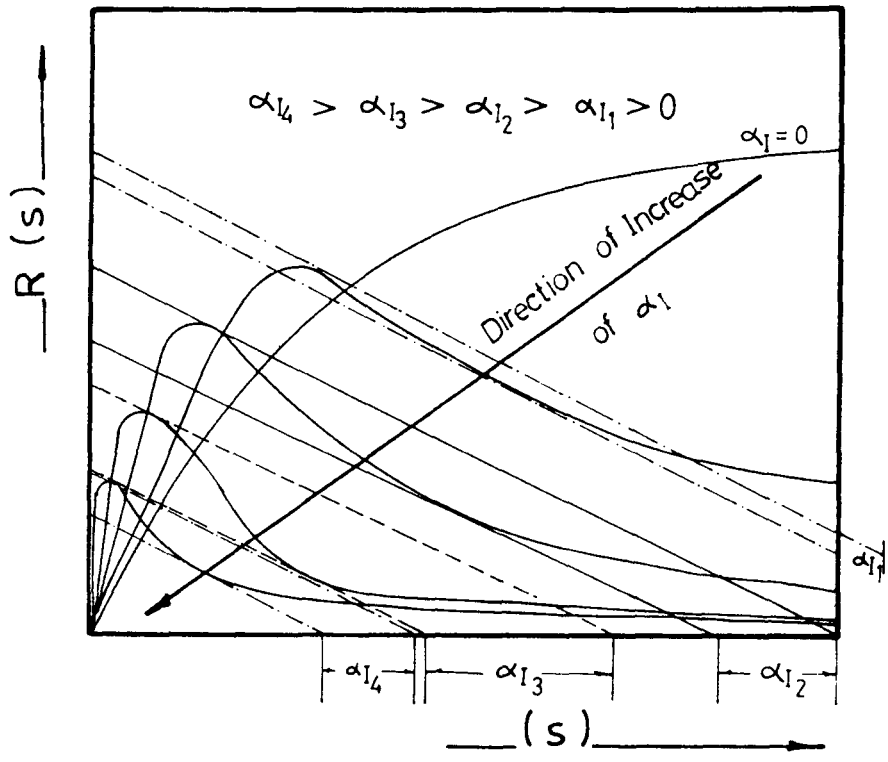

Fig. 3. Simple diagram to show the effect of α_I on the hysteresis loops.

(S). Figure 3 shows a simple diagram that illustrate the effect of increasing α_I from $\alpha_I = 0$ up to a value of $\alpha_I = 1.0$ (which is the realistic range) on the multiplicity phenomenon at certain value of B_s.

Double Effect Inhibition

In this case both the hydrogen ion inhibition and the substrate inhibition are taken into consideration, the rate expression in this case is,

$$r(S,h) = S/[S + \alpha_I S^2 + (1 + h + \delta h^2)/h]$$

In this general case the phenomenon of multiplicity becomes more complicated because of the appearance of the "isolas," which are disconnected closed curves on the multiplicity diagram ($S - S_B$ diagram). The phenomenon of "isolas," in this case, occurs because the rate of reaction depends upon two state variables simultaneously, i.e., substrate concentrations and hydrogen ions concentration, and the dependence of the rate of reaction upon each of these variables is nonmonotonic.

To explain the formation of "isolas," it is important to notice that as S decreases, h increases because h is a product of the reaction, whereas S is a reactant. Thus the rate of reaction may decrease or increase with the change of S_B depending upon the local conditions inside the membrane (S,h) and their combined effect on the rate of reaction.

Thus it is possible for the different branches of the multiplicity curves to

bend in opposite directions with the change of S_B or any of the other parameters, such as α_I, B_s, B_H, h_B, thus approaching each other, causing maxima and minima in the branches, and ultimately meeting to form an isola. This behavior is shown schematically in Figs. 4a, b. A typical example is given in Fig. 5, where it is shown how the decrease in $B(B = B_s = B_H)$ causes the lower and middle branches of multiplicity diagram to move in opposite directions till an isola is formed for $B(= B_s = B_H) = 40$. Also it has been found that for cases with two regions of multiplicity, the isola can be formed at certain critical values of the parameters B_s, B_H, h_B, α_I,. . ., through the movement of the two multiplicity regions toward each other. Such a case is shown in Figs. 6 and 7, where the increase of α_I from 0.25 to 0.55 causes the two multiplicity regions to approach each other (Fig. 6) and when α_I is increased further to $\alpha_I = 0.7$ an isola is formed (Fig. 7) and further increase in α_I cause the isola to shrink.

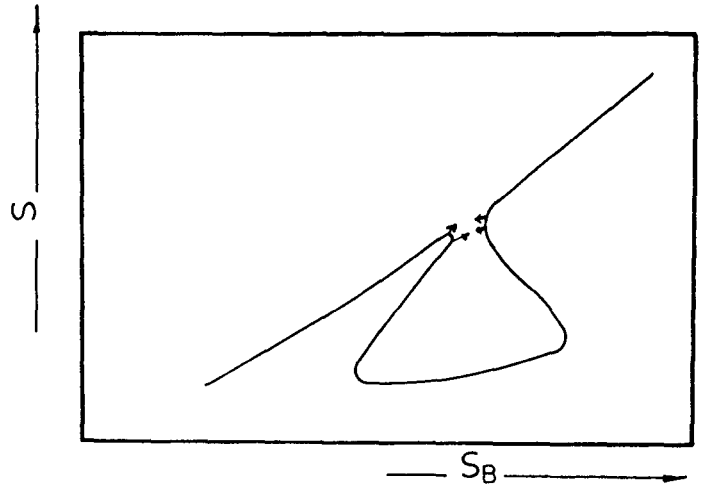

Fig. 4a. Hysteresis loops before the formation of an isola.

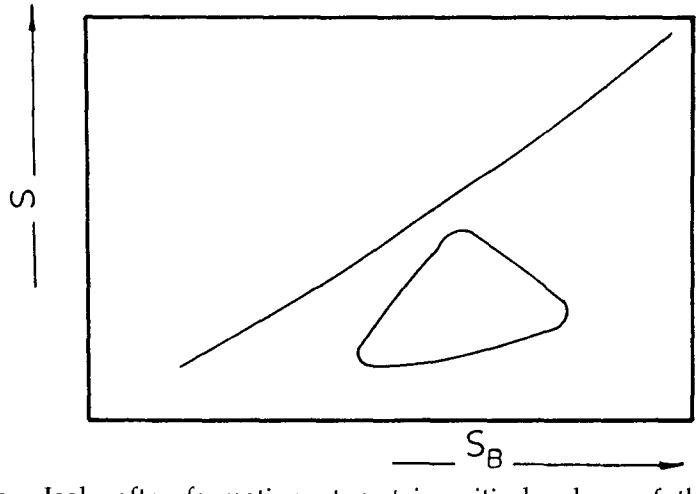

Fig. 4b. Isola after formation at certain critical values of the system parameters.

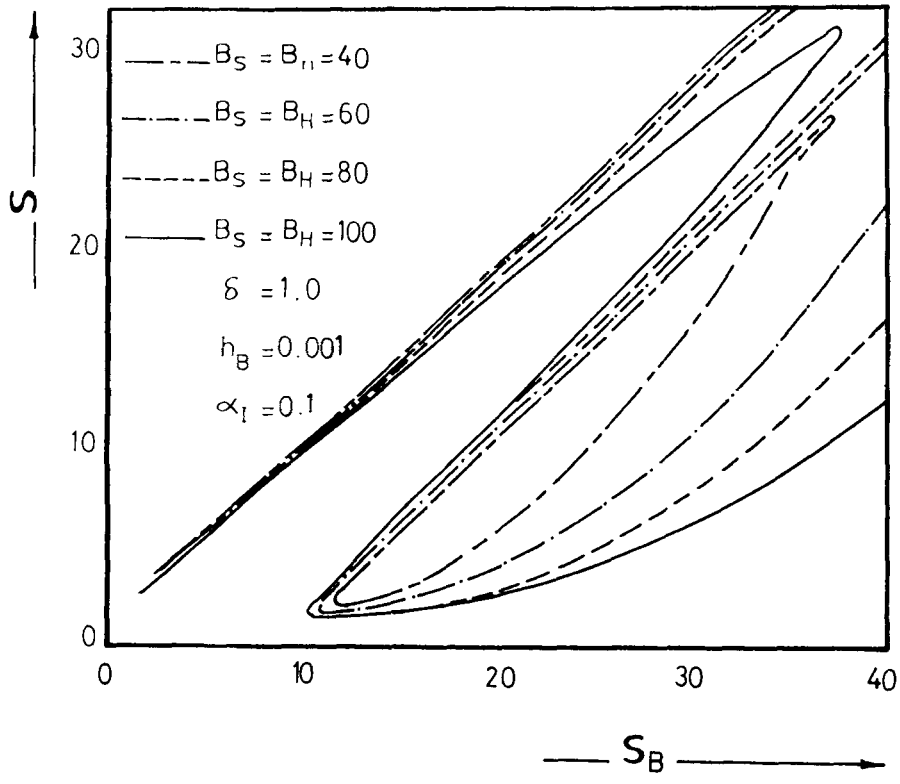

Fig. 5. Effect of $B = B_s = B_H$ on the hysteresis loops.

Asymmetrical Case

When the symmetrical assumption is relaxed the problem becomes more complicated.

Asymmetrical Case with Symmetrical External Field Concentration

For this case the external bulk concentrations for the substrate are equal in both compartments (i.e., $S_{B_1} = S_{B_0}$) also the external bulk concentrations for the hydrogen ions are equal (i.e., $h_{B_0} = h_{B_1}$). Figure 8 shows the hysteresis loops for a certain range of parameters. A maximum of nine steady states are observed in this case, and no isolas are observed. We notice also, that for the closely located points on the hysteresis loop, different concentration profiles are obtained. To illustrate this fact, Fig. 9 shows the asymmetrical profiles at $S_B = 18$. Figure 10 shows the more complex multiplicity phenomenon for the system under consideration, the value of the parameters in this case are the same as in the case shown in Fig. 8, except that h_B changes from 0.0025 in Fig. 8 to 0.002 in Fig. 10. In this case, multiple isolas are observed (five) and the maximum number of steady states reaches 25. Figure 11 represents the solu-

tion of the system of equations at $S_B = 17.8$, the number of steady states obtained in this case are 25 steady states, five of these are symmetrical (1.78, 5.22, 9, 13.17, and 16.13), and ten are mirror images to the other ten asymmetrical steady states. Figure 12 illustrates the concentration profiles of this case.

Asymmetrical Case with Asymmetrical External Field Concentration

This is the most general case, where the bulk substrate concentrations in the two compartments are not equal. Figure 13 shows the solution of the system of equations for the system parameters $B_H = B_s = 50$, $\gamma = \theta = 0.01$, $Sh_s = 20$, $Sh_H = 200$, $\alpha_I = 0.5$, $\delta = 0.1$, $h_{B_1} = h_{B_0} = 0.0025$, $S_{B_0} = 12$, $S_{B_1} = 5$, we notice that the system has nine steady states. No symmetrical profiles are obtained, i.e., all the nine profiles are asymmetrical profiles.

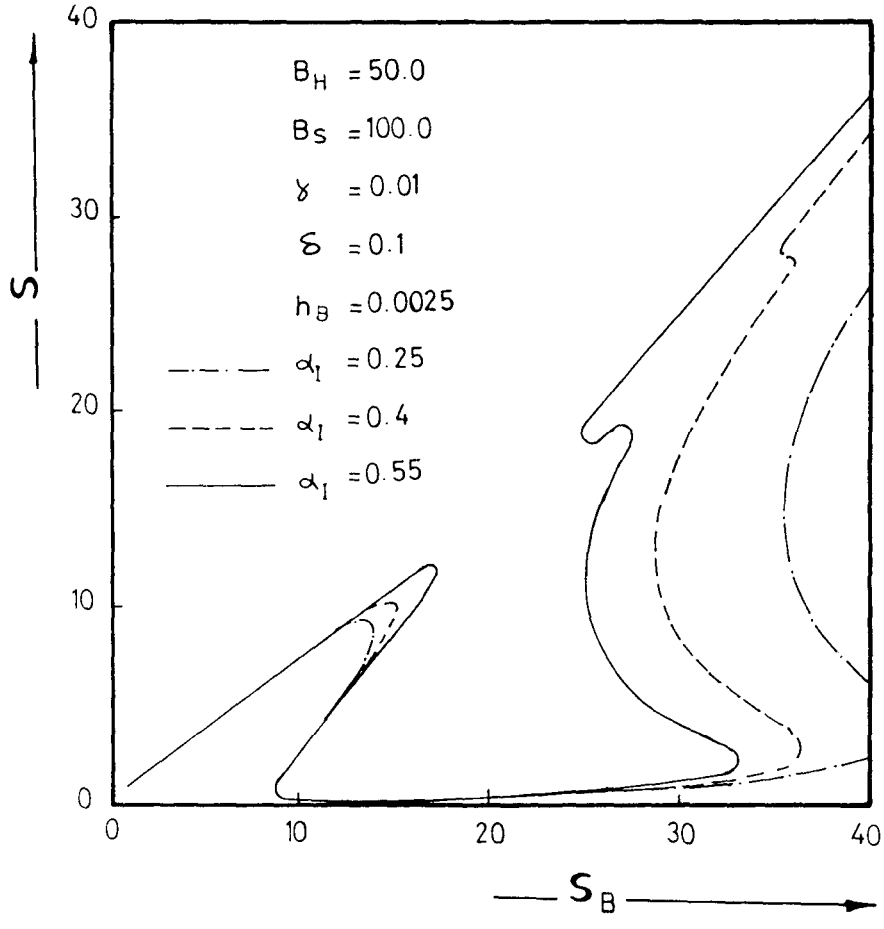

Fig. 6. Effect of α_I on the hysteresis loops before the formation of an isola.

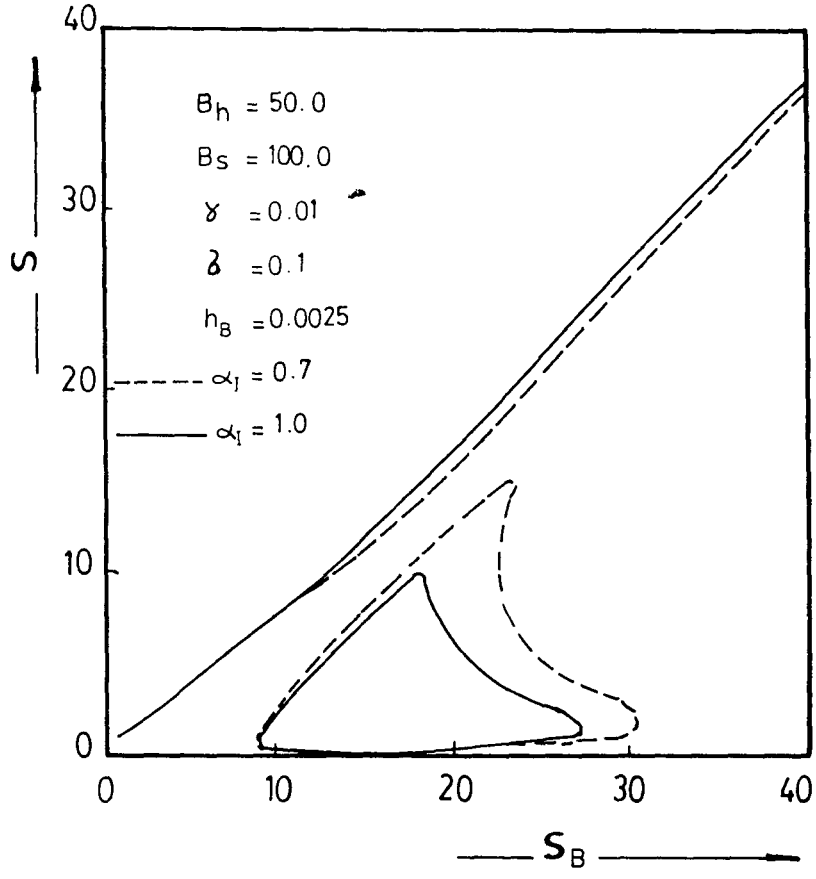

Fig. 7. Effect of α_I on the isolas.

CONCLUSIONS

For the active artificial membrane studied, important phenomena have been observed. For the symmetrical case (i.e., $S_o = S_1$), it was found that regions of multiple steady states are possible for the case of substrate inhibition without pH effect as well as for the case of pH effect without substrate inhibition.

For the case of the combined effect of substrate inhibition and pH, complex multiplicity phenomenon formed of classical hysteresis curves and isolas is observed.

The formation of the isolas is explained through the recognition of the dependence of the rate of reaction upon the two state variables (i.e., hydrogen ion concentration and substrate concentration), where the de-

pendence of the rate of reaction upon each of them is a nonmonotonic function.

When the assumption of symmetrical steady states is relaxed (i.e., $S_o \neq S_1$), a larger number of steady states and complex multiple isolas are obtained, this complex behavior has not been found previously and may have important implications regarding the understanding of the behavior of active membranes.

Up to 25 steady states have been discovered for certain ranges of parameters. Some of these steady states appear to be close to each other on the hysteresis curve, but investigation of the intramembrane concentration profiles show that they give rise to widely different intramembrane concentration profiles.

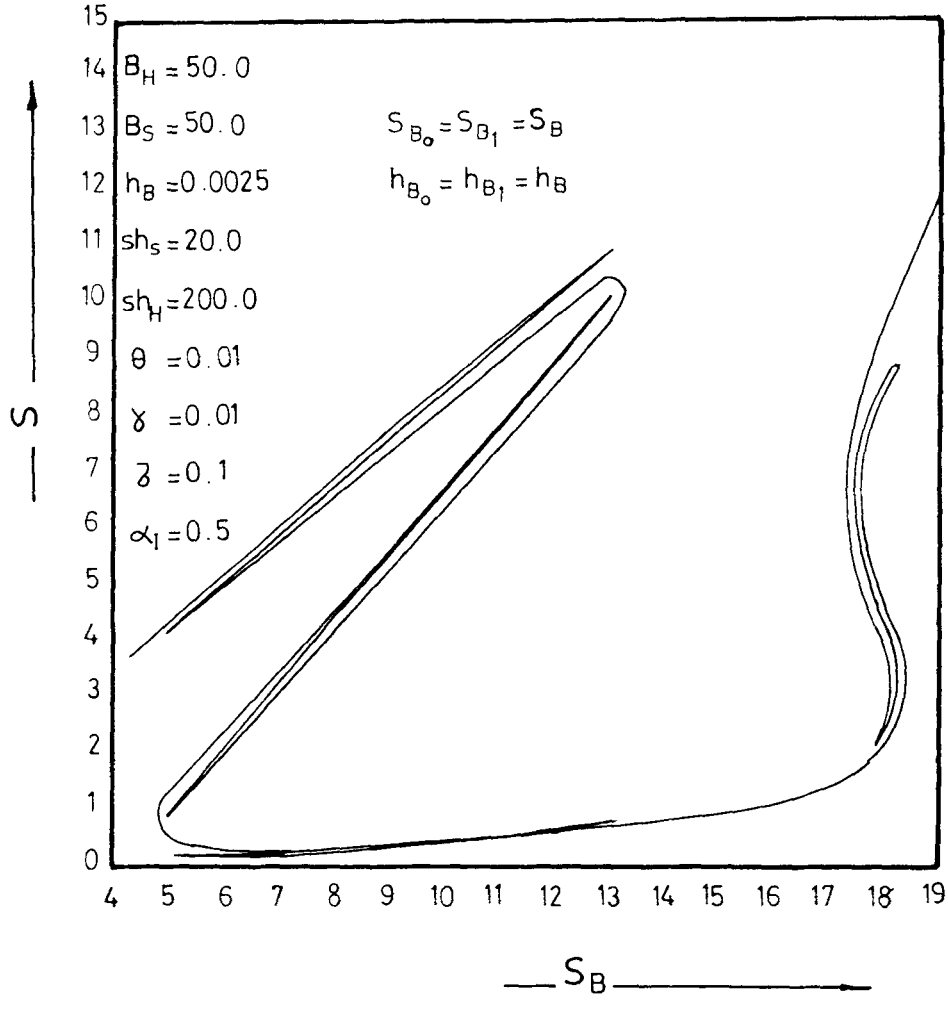

Fig. 8. The hysteresis loops before the appearance of multiple isolas.

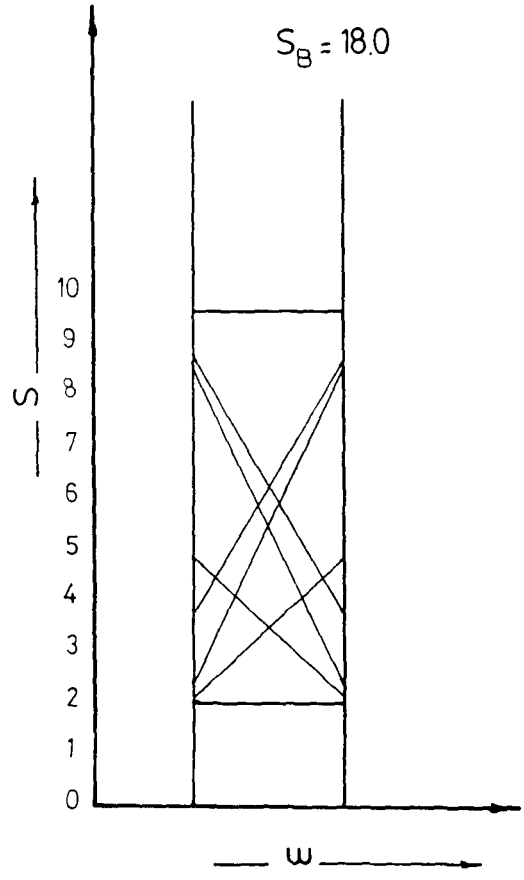

Fig. 9. Concentration profiles at $S_B = 18$.

LIST OF SYMBOLS

B_H	=	$V_m/Kg_H K h$
B_s	=	$V_m/Kg_s K_s$
D_H	=	Diffusion coefficient for hydrogen ions ($cm^2\ s^{-1}$)
D_{OH}	=	Diffusion coefficient for hydroxyl ions ($cm^2\ s^{-1}$)
D_s	=	Diffusion coefficient for substrate ($cm^2\ s^{-1}$)
E	=	Enzyme
H	=	Hydrogen ions
h	=	Dimensionless concentration of hydrogen ions inside the membrane
K_s, K_i, K_h, K_h'	=	Constants characteristic the enzyme
K_w	=	Equilibrium constant of water ($mol^2\ cm^{-6}$)
Kg_s	=	Mass-transfer coefficient for substrate ($cm\ s^{-1}$)
Kg_H	=	Mass-transfer coefficient for hydrogen ions ($cm\ s^{-1}$)
Kg_{OH}	=	Mass-transfer coefficient for hydroxyl ions (cm^{-1})
$\mathscr{L}$	=	Depth across the membrane (cm)

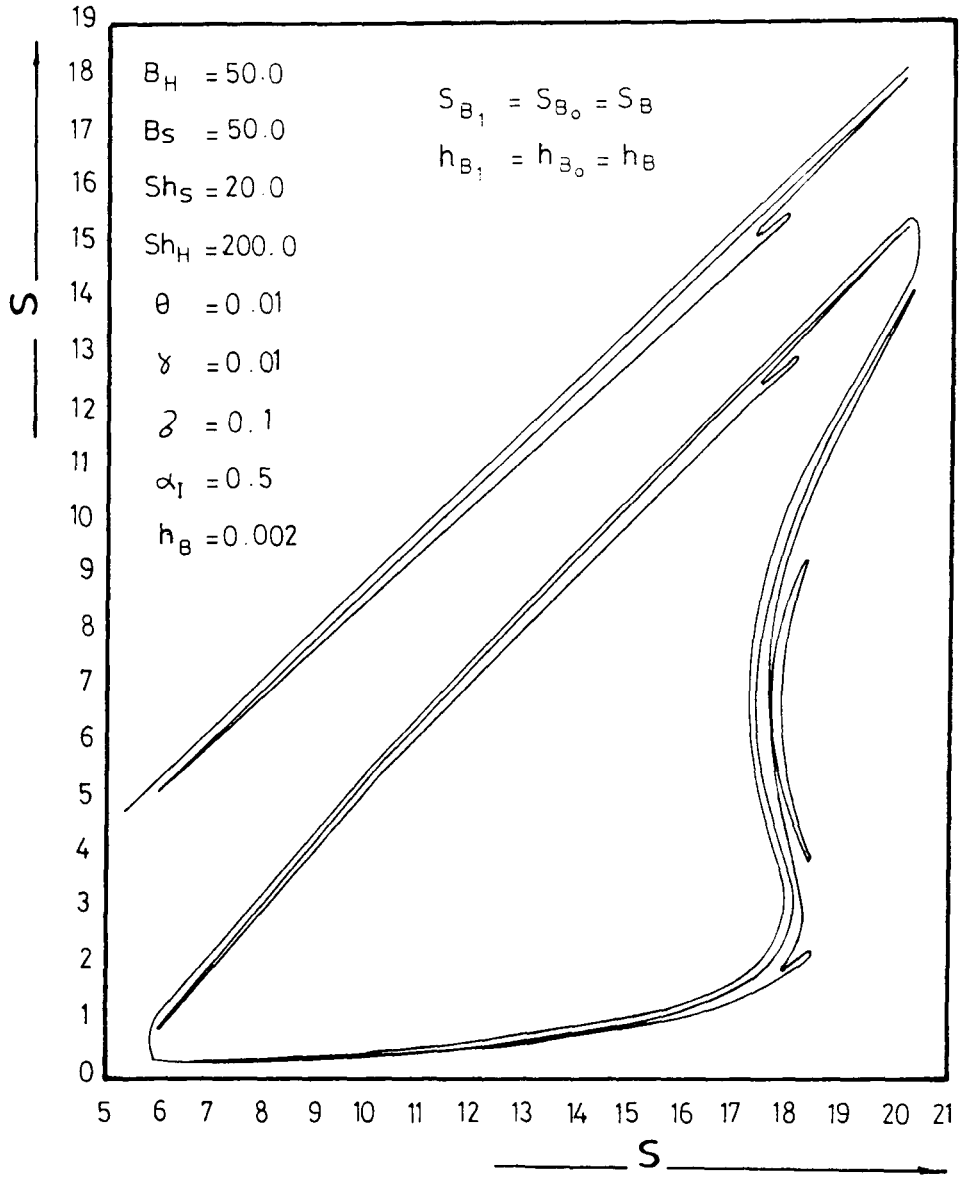

Fig. 10. The hysteresis loops after the appearance of the multiple isolas.

L	=	Membrane thickness (cm)
OH	=	Hydroxyl ions
P^-	=	Fully ionized product
P_1	=	Product
R	=	Dimensionless reaction rate
r	=	Rate of reaction (moles per gram enzyme per second)
r_w	=	Rate of water formation
S	=	Substrate
S	=	Dimensionless substrate concentration inside the membrane

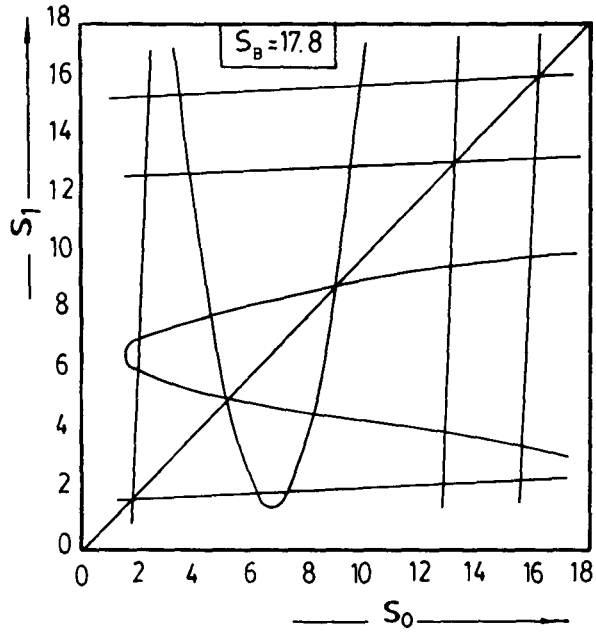

Fig. 11. Solution of the system of equations for the asymmetrical case [$S_B = S_{B_0} = S_{B_1} = 17.8$].

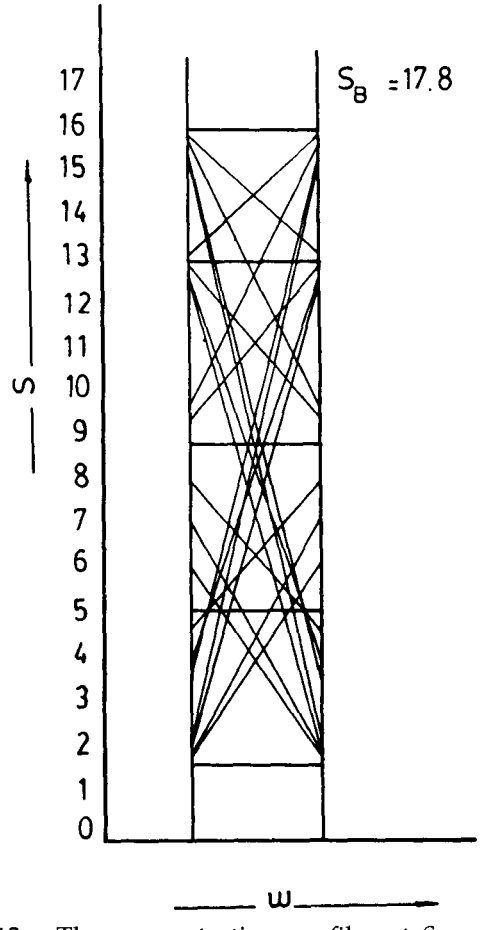

Fig. 12. The concentration profiles at $S_B = 17.8$.

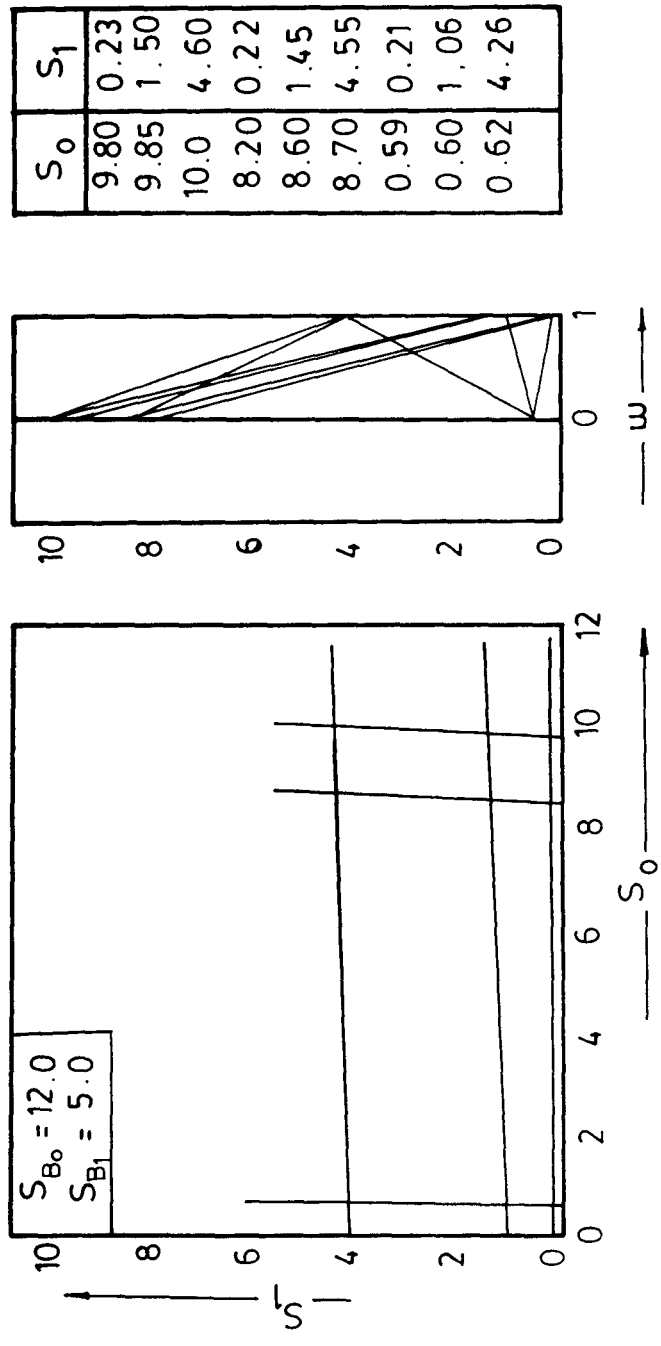

Fig. 13. Solution of the system of equations for the asymmetrical case.

S_{B_o} = Dimensionless substrate concentration in the compartment (o)
S_{B_1} = Dimensionless substrate concentration in compartment (1)
S_o = Dimensionless substrate concentration at surface (o)
S_1 = Dimensionless substrate concentration at surface (1)
w = Dimensionless length = $\mathcal{L}$/L
Sh_H = L Kg_H/D_H, Sherwood number
Sh_s = L Kg_s/D_s, Sherwood number
V_m = Maximum reaction rate (moles per gram of enzyme per second)
γ = $D_{OH}K_w/D_HK_h^2$
α_I = K_s/K_i
δ = K_h/K_h'
θ = $Kg_{OH}K_w/Kg_HK_h^2$

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