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Self-sustained pH oscillations in a compartmentalized enzyme reactor system

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Abstract

This work represents our continued effort toward fulfilling the need to discover a model system for experimental investigations of temporal oscillations in an enzyme–membrane system. In this paper, the regions in the parameter space where self-sustained pH oscillations can be induced for a compartmentalized enzyme reactor system, which consists of a well-stirred reactor, a reservoir and a membrane containing no enzyme, were determined via numerical simulation with two proteolytic enzymes: papain (EC 3.4.22.2) and α-chymotrypsin (EC 3.4.21.1). The sizes of the regions were qualitatively compared with those associated with enzymic membrane system. As a result, we found that the possibility of experimentally observing self-sustained oscillations in the compartmentalized papain reactor system, as well as in the papain–membrane system, is high. However, self-sustained pH oscillations are less likely in the compartmentalized α-chymotrypsin reactor system than in the α-chymotrypsin–membrane system. © 1997 Elsevier Science B.V.

Keywords: Self-sustained oscillations; pH oscillations; Compartmentalized enzyme reactor; Proteolytic enzyme; Enzyme kinetics

1. Introduction

There is an urgent need for the researchers in the areas of biotechnology and non-linear dynamics to have some simple, robust and easily reproducible enzyme–membrane systems for investigating the dynamics of non-linear biochemical reaction–diffusion systems. The study on the dynamics may lead to a better understanding of the biological regulations of living cells, and may also contribute to the development of biomimetic devices. The goal of this work is to establish an optimum enzyme–membrane system that could lead to a definite experimental confirmation of the existence of limit-cycle type self-sustained oscillations (SSO) in such a system and provide a basis for further experimental investigation of its dynamic behavior. In a previous paper [1], we investigated SSO in pH resulting from the coupling of a proton-producing, pH-dependent hydrolysis reaction with diffusion in an enzymic membrane system. The regions in the parameter space where the SSO might be induced were determined via numerical simulation for five enzyme–substrate combinations involving four proteolytic enzymes. The study

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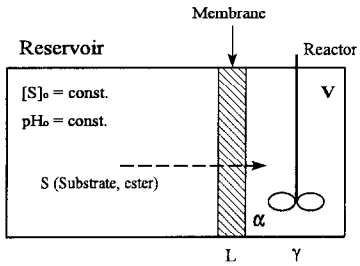

Fig. 1. Compartmentalized enzyme reactor system.

led to the conclusion that the possibility of experimentally observing SSO is high for the system of α-chymotrypsin-*N*-acetyl-L-tryptophane ethyl ester (ATEE), in addition to the well-known papain system [2–6].

However, various factors such as inhomogeneity of membrane and deactivation of enzyme are known to prevent us from experimentally observing SSO in an enzymic membrane system [7]. In fact, no definite experimental confirmation of SSO has been achieved in any enzymic membrane systems, though Naparstek et al. [3] and Ohmori and Yang [7], respectively have experimentally observed autonomous pH oscillations in a papain gel system.

Another type of enzyme–membrane system, a compartmentalized enzyme reactor, was used by Hervagault and Thomas to observe experimentally oscillations associated with phosphofructokinase [8]. In this system, an inert membrane containing no enzymes separates an outside reservoir and a well-stirred reactor (Fig. 1). While the reactor is filled with enzyme solution, a fixed concentration of substrate solution with a given pH is contained in the reservoir. The membrane is permeable to the substrate and the products, but not to the enzyme involved. As the substrate diffuses from the reservoir into the reactor via the membrane, it is catalyzed by the enzyme and hydrogen ion is liberated in the reactor. Because of the bell-shaped dependence of the reaction rate on pH, autocatalysis is induced when the pH is larger than the optimal pH of the enzyme reaction. Thus, under appropriate conditions, SSO occur as a result of coupled diffusion and autocatalysis similar to that in the enzymic membrane system. This system has been regarded as a 'zero-dimensional' approximation to the one-dimensional enzymic membrane system [4,9,10].

In this compartmentalized enzyme reactor system, membrane inhomogeneity is not an important factor in inhibiting the induction of SSO because no reactions occur inside the membrane. In addition, higher enzyme concentration than that allowed in an enzyme gel may be used in this system. Therefore, in principle, it would be easier to observe SSO experimentally in this system than in the corresponding enzymic membrane system.

In this paper, we have found that SSO can also occur in the compartmentalized reactor system involving two enzymes, papain (EC 3.4.22.2) and α-chymotrypsin (EC 3.4.21.1). For each enzyme, the region of self-sustained oscillations (ROSSO) in a parameter plane has been determined, and the sizes of the ROSSOs for both the compartmentalized reactor system and the enzymic membrane system are qualitatively compared to discuss the possibility of experimentally observing SSO in these systems.

2. Enzyme systems and reaction kinetics

Among a variety of proteolytic enzyme–ester substrate combinations, we have chosen papain–*N*-α-benzoyl-L-arginine ethyl ester (BAEE) and α-chymotrypsin–ATEE for this study, after taking into consideration the results of our previous investigations [1,7], namely the observation of autonomous pH oscillations in the former and the high possibility of observing SSO in the latter. The hydrolysis reactions of both BAEE and ATEE in the presence of papain and α-chymotrypsin, respectively, proceed via a three-step reaction mechanism as follows [11–13]:

$$E + S \overset{K_s}{\rightleftharpoons} ES \overset{k_2}{\rightarrow} ES' + P_1 \overset{k_3}{\rightarrow} E + P_2 \qquad (1)$$

where E represents enzyme, S substrate, ES enzyme–substrate complex, ES′ acyl–enzyme intermediate, and P_1 and P_2 are, respectively, alcohol and acid produced by splitting of the ester substrate. The rate expression based on Eq. (1) is as follows:

$$R = \frac{k_2 k_3 [E]}{k_2 + k_3 + k_3 K_S [S]^{-1}} \qquad (2)$$

$$k_2 = \frac{k_{2,\max}}{C_2} \tag{3}$$

$$k_3 = \frac{k_{3,\max}}{C_3} \tag{4}$$

where

$$C_2 = 1 + 10^{a}[H^+] + 10^{-b}[H^+]^{-1} \tag{5}$$

$$C_3 = 1 + 10^{c}[H^+] \tag{6}$$

In these equations, enzyme, substrate and hydrogen ion concentrations are in mol/m^3. For the papain–BAEE system, $K_S = 54.5$ mol/m^3, $k_{2,\max} = 64.9$ s^{-1}, $k_{3,\max} = 20.2$ s^{-1}, $a = 1.29$, $b = 5.49$, $c = 0.92$, which have been experimentally determined in range of pH = 3.6–9.56 by Whitaker and Bender [14].

For the α-chymotrypsin–ATEE system, however, the experimental data available in the literature [16,17] are not sufficient to determine all the parameters in the above rate expression, and hence the following two-step reaction mechanism [15] with the same parameters determined in the previous paper [1] was adopted:

$$R = \frac{k_{cat}[E][S]}{K_m C_4 + [S]C_5} \tag{7}$$

where

$$C_4 = 1 + 10^{d}[H^+] + \frac{10^{-e}}{[H^+]} \tag{8}$$

$$C_5 = 1 + 10^{f}[H^+] + \frac{10^{-g}}{[H^+]} \tag{9}$$

and the enzyme, substrate and hydrogen ion concentrations are also in mol/m^3. The values of the parameters used, $k_{cat} = 44.0$ s^{-1}, $K_m = 0.11$ mol/m^3, $d = 3.80$, $10^{-e} = 0$, $f = 3.65$, $g = 6.26$, which are only applicable within pH = 5.04–11.22, were determined in [1]. Both rate expressions, Eqs. (2)–(6) and Eqs. (7)–(9), which exhibit similar bell-shaped dependence of the reaction rate on pH [1,7], are derived from the Michaelis-Menten kinetics. Therefore, those equations can be used only if the substrate concentration is much higher than the enzyme concentration.

3. Mathematical model and numerical simulation

The compartmentalized enzyme reactor system being simulated is illustrated schematically in Fig. 1. It is assumed that the membrane involved is homogeneous with its thickness L being small compared to the other dimensions of the membrane, and thus transport in the membrane is effectively one-dimensional. Since the inert membrane chosen would not allow penetration of the enzyme involved, the diffusive fluxes of substrate, hydrogen ion and hydroxyl ion remain the same throughout the membrane. Hence, the following ordinary differential equations are adequate to simulate the time courses of pH and substrate concentration in the reactor:

$$\upsilon\frac{d[S]}{dt} = \frac{\alpha D_S([S]_o - [S])}{L} - \upsilon R \tag{10}$$

$$\upsilon\frac{dA}{dt} = \frac{\alpha D_H(A_o - A)}{L} + \upsilon R \tag{11}$$

where

$$A = [H^+] - [OH^-] \tag{12}$$

Here it is assumed that the effective diffusion coefficients of the hydroxyl ion and the hydrogen ion in the membrane are the same, and that electrical effect is negligible. The accompanying initial conditions are:

$$[S] = 0,\ A = A_o \text{ at } t = 0 \tag{13}$$

Introducing the following dimensionless variables: $U = [S]/[S]_o$, $V = A/[S]_o$, $\theta = \alpha D_H t/(\upsilon L)$, $\psi = k_{2,\max}\upsilon L/(\alpha D_H)$ for papain or $\psi = k_{cat}\upsilon L/(\alpha D_H)$ for α-chymotrypsin, Eqs. (10) and (11), together with the initial conditions, Eq. (13), are transformed, respectively, into

$$\frac{dU}{d\theta} = \frac{D_S}{D_H}(1 - U) - R' \tag{14}$$

$$\frac{dV}{d\theta} = \frac{A_o}{[S]_o} - V + R' \tag{15}$$

$$U = 0,\ V = \frac{A_o}{[S]_o} \text{ at } \theta = 0 \tag{16}$$

where

$$R' = \frac{\psi[\mathrm{E}]U}{C_2\{K_S + (1 + k_2 k_3^{-1})U[\mathrm{S}]_o\}} \quad (17)$$

or

$$R' = \frac{\psi[\mathrm{E}]U}{K_m C_4 + U[\mathrm{S}]_o C_5} \quad (18)$$

R' is dimensionless reaction rate, and Eqs. (17) and (18) were used, respectively, for papain–BAEE and α-chymotrypsin–ATEE. From the above equations, it is clear that four parameters, i.e. $\psi[\mathrm{E}]$, D_S/D_H, $[\mathrm{S}]_o$ and $A_o([\mathrm{H}^+]_o$ or $\mathrm{pH}_o)$, completely govern the dynamic behavior of the system, similar to the enzymic membrane system studied before [1]. However, the definition of ψ here is a little different from that of ϕ ($= k_{cat} L_r^2/D_H$, L_r being thickness of the reactive membrane) associated with the enzymic membrane system.

Eqs. (14)–(16) with Eq. (17) or Eq. (18) were solved numerically via a backward differentiation formula method [18] for a large number of different parameter sets to obtain the time courses of pH and substrate concentration in the reactor. A value of 0.1 was used for D_S/D_H [7] throughout the simulations unless specified otherwise.

4. Results and discussion

For each system investigated, the ROSSO corresponding to a given pH in a reservoir, pH_o, is expressed as a closed and shaded region in the two-dimensional parameter space of $[\mathrm{S}]_o$ vs $\psi[\mathrm{E}]$. The rest of the parameter planes represent the region where other types of transient behavior, such as damped oscillations or non-oscillatory behavior, may take place. Only SSO with an amplitude of more than one pH unit are included in the ROSSO. Hence, the sizes of the ROSSOs shown in this paper are in general on the conservative side.

Fig. 2 shows the ROSSOs corresponding to four different pH_o values for the compartmentalized papain reactor system using BAEE. The values of the pH_o at which the ROSSOs were obtained are all within the pH range where the kinetic measurements were carried out. Furthermore, the ranges of $[\mathrm{S}]_o$ and

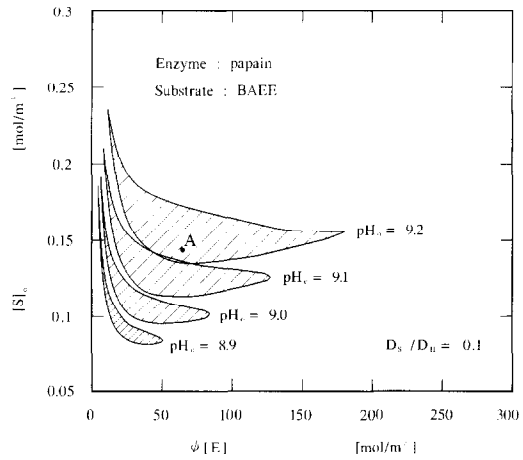

Fig. 2. Region of self-sustained oscillations for compartmentalized enzyme reactor system (papain–*N*-α-benzoyl-L-arginine ethyl ester).

$\psi[\mathrm{E}]$ are also in the experimentally realizable region. Hence, it appears that SSO can be induced in this system under experimentally realizable operating conditions. It is noted, however, that the size of the ROSSO becomes smaller as the values of pH_o decreases.

The oscillations with various values of the periods and the amplitudes are induced even within one ROSSO. The period and the amplitude depend on the position in the ROSSO. Shown in Fig. 3a are examples of the SSO in pH and the substrate concentration in the reactor. The same oscillations are presented in a phase-plane plot of pH versus substrate concentration in Fig. 3b, which exhibits a limit cycle, confirming the achievement of the SSO. The parameters associated with the oscillations shown in Fig. 3 correspond to the spot A in Fig. 2. It is noticeable from Fig. 3a that the periods of the oscillations of pH and the substrate concentration are the same, but the phases are different. Further, the assumption of $[\mathrm{S}] \gg [\mathrm{E}]$ mentioned in Section 2 is satisfied since [E] is equal to 1×10^{-4} mol/m^3 if we choose a reactor with $vL/\alpha = 1 \times 10^{-5}$ m^2 and a value of $D_H = 1 \times 10^{-9}$ m^2/s.

Shown in Fig. 4 are the ROSSOs corresponding to the same four pH_o values but for the papain gel system. The system and the model used for simulation are the same as in the previous works [1,7]. Comparison of Figs. 2 and 4 indicates that although

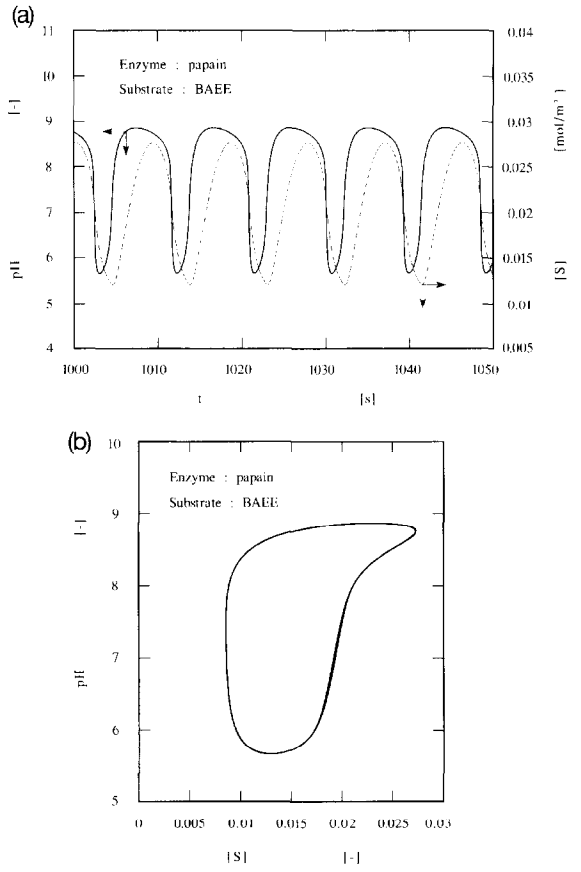

Fig. 3. Simulated self-sustained oscillations for compartmentalized enzyme reactor system (papain–*N*-α-benzoyl-L-arginine ethyl ester). (a) Time courses of pH and substrate concentration. (b) Phase-plane plot. $[S]_0 = 0.144$ mol/m^3, $\psi[E] = 64.9$ mol/m^3, $pH_0 = 9.2$, $D_S/D_H = 0.1$.

their abscissas, $\psi[E]$ and $\phi[E]$, are different, the shapes of the ROSSOs corresponding to the same pH_0 values are quite similar, their sizes are qualitatively of comparable magnitude, and the dependence of pH_0 on the size of the ROSSO is nearly the same. The value of $\psi[E]$ may be adjusted to the same value of $\phi[E]$ by choosing appropriate values of the design parameters, such as the 'reactor width', γ ($= v/\alpha$, Fig. 1), membrane thickness, L, effective diffusion coefficient of the hydrogen ion, D_H, and enzyme concentration, [E]. Hence, direct comparison between the ROSSOs of the compartmentalized reactor system and the enzymic membrane system may provide us with a qualitative criterion as to which system has higher possibility of experimentally observing SSO. For papain–BAEE, the possibilities appear to be nearly the same for both systems.

Fig. 5 shows the ROSSOs of the α-chymotrypsin–ATEE system for the compartmentalized reactor and the enzymic membrane. The ROSSOs ((6) to (8) in Fig. 5) for the latter system are the same as those reported in the previous paper [1]. The ROSSOs ((1) to (5) in Fig. 5) for the compartmentalized reactor system are located in experimentally realizable parameters regions. Hence, it appears that SSO can also be induced in this system although the sizes of the ROSSOs are quite small. Since the same consideration about the difference of the abscissa mentioned above is also valid, we may directly compare the ROSSOs for both systems. It is obvious that for the same pH_0 values the ROSSOs for the compartmentalized reactor system are much narrower than those for the enzymic membrane system. While the size of the ROSSO, especially in $\phi[E]$, for the enzymic membrane system becomes larger, as the values of pH_0 increases, the sizes of the ROSSOs for the compartmentalized reactor system are almost the same for the different values of pH_0.

To further investigate the effect of the other parameter, D_S/D_H, on the ROSSO for the compartmentalized reactor system, simulations were carried out with smaller values of D_S/D_H and $pH_0 = 10.4$, neglecting the fact that a membrane with smaller values of D_S/D_H may be less available. The results are shown as a semi-log plot in Fig. 6. The ROSSO

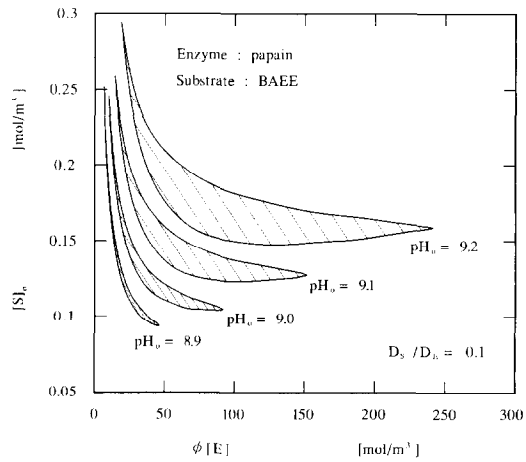

Fig. 4. Region of self-sustained oscillations for enzymic membrane system (papain–*N*-α-benzoyl-L-arginine ethyl ester).

for $D_S/D_H = 0.1$ shown as (1) in Fig. 6 is the same as that for $pH_o = 10.4$ shown as (3) in Fig. 5. As the values of D_S/D_H decreases, the size of the ROSSO in $[S]_o$ becomes larger, but the size in $\psi[E]$ is nearly the same. Hence, the range of $\psi[E]$ where SSO can be induced may not be extended using larger value of pH_o or smaller value of D_S/D_H. In comparison with the enzymic membrane system, it is concluded that SSO are less likely in the compartmentalized α-chymotrypsin reactor system.

The significant difference of the ROSSOs between the systems of the compartmentalized reactor and the enzymic membrane using α-chymotrypsin–ATEE is contrary to the case of papain–BAEE. This is obviously a direct reflection of the fact that the rate expression with its associated kinetic parameters is uniquely fixed for each enzyme–substrate combination, while all the other parts of the models are the same. However, we believe this discrepancy is not due to the fact that the rate expression based on the two-step reaction mechanism was used for papain–BAEE while that based on the two-step reaction mechanism was used for α-chymotrypsin–ATEE. Though not shown here, simulation for the compartmentalized reactor system using ficin, which is the same cysteine proteinase as papain, with a rate expression based on the two-step mechanism has produced almost the same results as those for the papain reactor system described above. Further investigations to explain the difference between the papain and the α-chymotrypsin systems are in progress in our laboratory.

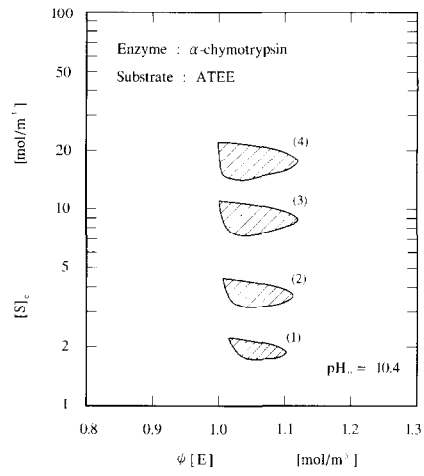

Fig. 6. Region of self-sustained oscillations for compartmentalized enzyme reactor system (α-chymotrypsin-*N*-acetyl-L-tryptophane ethyl ester). (1)–(4): $D_S/D_H = 0.1$, 0.05, 0.02 and 0.01, respectively, with $pH_o = 10.4$.

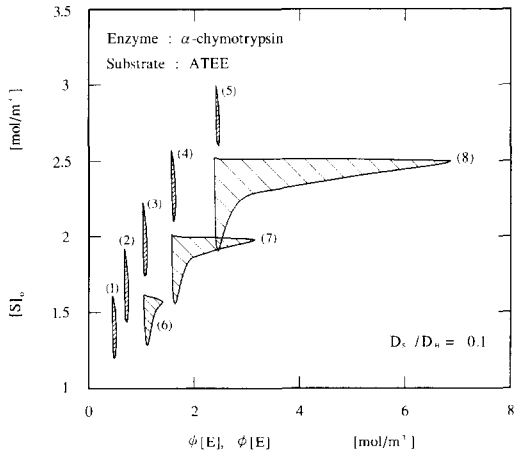

Fig. 5. Region of self-sustained oscillations (α-chymotrypsin-*N*-acetyl-L-tryptophane ethyl ester). (1)–(5): Compartmentalized enzyme reactor system (abscissa: $\psi[E]$), (6)–(8): enzymic membrane system (abscissa: $\phi[E]$). (1) and (6): $pH_o = 10.2$; (2) and (7): $pH_o = 10.3$; (3) and (8): $pH_o = 10.4$; (4): $pH_o = 10.5$; (5): $pH_o = 10.6$.

5. Conclusions

An extensive computational investigation has been carried out to determine the regions in parameter space where self-sustained oscillations in pH can be induced for two compartmentalized proteolytic enzyme reactor systems. The results show that self-sustained pH oscillations may occur in both papain and α-chymotrypsin systems, though the sizes of the ROSSOs for α-chymotrypsin–ATEE are significantly smaller. The sizes of the ROSSOs for the compartmentalized reactor and the enzymic membrane have been qualitatively compared. As a result, we have concluded that, for papain–BAEE, the possibilities of experimentally observing SSO are nearly the same in both systems, while for α-chymotrypsin–ATEE, the possibility is much lower for the compartmentalized reactor system than for the enzymic membrane system.

6. Nomenclature

A	$[H^+] - [OH^-]$, as defined in Eq. (12) (mol/m^3)
a, b, c, d, e, f, g	kinetic parameters in Eq. (5), Eq. (6), Eq. (8) and Eq. (9)
D	effective diffusion coefficient (m^2/s)
[E]	concentration of enzyme (mol/m^3)
$[H^+]$	concentration of hydrogen ion (mol/m^3)
K_m	Michaelis constant (mol/m^3)
K_S	dissociation constant (mol/m^3)
k_2, k_3, k_{cat}	reaction rate constant (1/s)
L	membrane thickness (m)
$[OH^-]$	concentration of hydroxyl ion (mol/m^3)
R	reaction rate (mol/(m^{3}s))
[S]	concentration of substrate (mol/m^3)
t	time (s)
v	reactor volume (m^3)
α	area (one side) of membrane (m^2)
γ	'reactor width' ($=v/\alpha$), as shown in Fig. 1.
ϕ	$k_{2,max} L_r^2/D_H$ for papain or $k_{cat} L_r^2/D_H$ for α-chymotrypsin (enzymic membrane system)
ψ	$k_{2,max} vL/(\alpha D_H)$ for papain or $k_{cat} vL/(\alpha D_H)$ for α-chymotrypsin (compartmentalized reactor system)

6.1. Subscripts

H	hydrogen ion
max	maximum value
o	solution in reservoir
r	reactive (immobilized) membrane
S	substrate

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