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CONDUCTIVITY-MEDIATED REGULATION IN A COMPARTMENTAL ENZYME SYSTEM

SUPERACTIVATION AND CONDUCTANCE BISTABILITY

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This paper deals with the 'conductivity effect' which results from the action of an electric current in a compartmental system involving a chemical reaction producing ionic species. This effect, due to the difference between internal and external conductivities, leads to the accumulation of ionic species inside the reactive compartment. Different results are obtained depending on the considered kinetics: amplification of reaction rate increase, and chemical and conductance bistabilities.

1. Introduction

Immobilized enzyme systems have been widely studied both theoretically and experimentally because of their importance in biotechnologies [1] and because they can be used to model certain biological systems [2,3]. Enzymic diffusion-reaction models allowed a better understanding of the effects of diffusion limitations [2,4] and the optimization of the yield of immobilized enzyme systems.

Electric fields have been proposed to lower diffusion limitations in enzyme membranes or compartmented systems, and to increase indirectly enzyme activities when ionic species are involved in the modulation of these activities (proton, ionic substrate, activators, inhibitors, etc.) [5–7]. Theoretical studies have been confirmed by experiments [7–9].

In addition, when a low ionic strength and an ionic species-producing reaction are considered in a membrane system, a new effect of electric fields appears: the 'conductivity effect' [5,7], which leads to the accumulation of ionic species inside the membrane; it is even possible to accumulate a consumed species above its boundary concentration, hence providing new possibilities for activation [5–7]. The conductivity effect may be compared to the effects observed with polyelectrolyte membranes, for which the high internal conductivity is not functional, but structural [10].

In order to understand the conductivity effect and its limitations more fully, we have considered here a compartmental system; the modelling of this type of system is simpler than that of a membrane, due to the separation in space of transport and reaction. We considered two different kinetics: Michaelis kinetics and substrate excess inhibition kinetics. The latter kinetics, because it may induce bistabilities in structured media [5,11], has been very useful in studying the combination of the conductivity effect with a potential bistability.

2. The model

The system considered comprises a reactive compartment of thickness L, in which an enzyme is solubilized and trapped. The reactive compartment (II) is separated from two concentration reservoirs by identical membranes (I and III) of thickness e (fig. 1); these membranes are chemically inert and electrically neutral; all the chemical species (except the enzyme) and the solvent can move freely through the membranes. Both the reservoirs and the compartment are assumed to be perfectly stirred, so the reactive phenomena observed will be space independent.

An electric current may be imposed by two electrodes placed in the reservoirs; for experimental approaches it is necessary to consider a more sophisticated system, in which the electrodes are separated from the reservoirs by two supplementary membranes to avoid the back-diffusion of any species produced electrochemically at the electrodes. The electric field is imposed along the direction $X'X$, perpendicular to the membrane surfaces.

The following reaction scheme was considered:

$$S^- \rightarrow 2P^- + Y^+ \qquad (1)$$

Remarks

(i) This reaction scheme leads potentially to a conductivity increase.

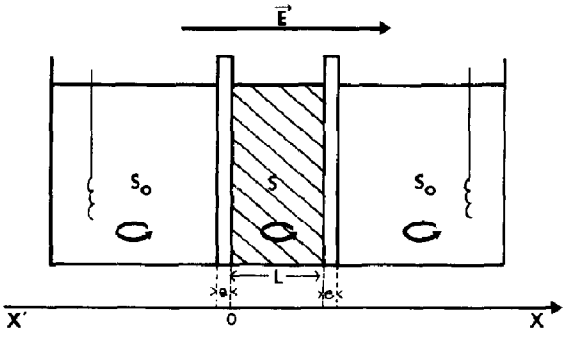

Fig. 1. Structure of the compartmental system: the system comprises a reactive compartment (thickness L) in which an enzyme is solubilized; this compartment is separated from two infinite reservoirs by two identical membranes (thickness e); an electric current may be imposed by two electrodes placed in the reservoirs.

(ii) Since the substrate is ionic, the conductivity effect will apply to it.

(iii) S^-, P^- and Y^+ are the only ionic species involved in the system (proton and hydroxyl concentrations are neglected; this assumption is not necessarily satisfied when the conductivity effect takes place).

(iv) The concentration of P^- in the reservoirs (P_0) is assumed to be zero (infinite reservoirs).

(v) For S^-, P^- and Y^+ identical diffusion coefficients D and mobilities were considered.

The only species present in the reservoirs are S^- and Y^+, and for reasons of electroneutrality their boundary concentrations S_0 and Y_0 satisfy the equality:

$$S_0 = Y_0 \qquad (2)$$

In the reactive compartment and in the membranes S^-, P^- and Y^+ are present and have to satisfy the electroneutrality equations:

$$\begin{aligned} S(X)^{I} + P(X)^{I} &= Y(X)^{I} \\ S^{II} + P^{II} &= Y^{II} \\ S(X)^{III} + P(X)^{III} &= Y(X)^{III} \end{aligned} \qquad (3)$$

Two different kinetics were associated with the reaction scheme of eq. 1: either Michaelis kinetics, or substrate excess inhibition kinetics, characterized by the respective reaction rate expressions:

$$v = V_m S/(K_m + S) \qquad (4)$$

$$v = V_m S/(K_m + S + K_i S^2) \qquad (5)$$

To obtain analytical solutions, zero order was assumed for Michaelis kinetics; general solutions for global Michaelis kinetics and for substrate excess inhibition kinetics were obtained by computations.

3. Theoretical

Transport processes by diffusion and electromigration are considered only in the membranes, since the solution in the reactive compartment is homogenized by adequate stirring. The enzyme reaction takes place only in the compartment, since the enzyme is trapped within it.

The transport of ionic species in the membranes is described by the Nernst-Planck relation; for a general species i:

$$J_i = -D_i \partial C_i/\partial x + z_i D_i F C_i E/RT \tag{6}$$

where C_i is the concentration of species i, z_i its valence, E the electric field intensity, F the Faraday constant, R the gas constant and T the absolute temperature.

Combining the implicit electroneutrality relation,

$$\mathscr{J}/F = \sum_i z_i J_i \tag{7}$$

in which $\mathscr{J}$ is the current density, with eq. 6 leads to the expression of the electric field as a function of the imposed current density and of the ionic species concentrations:

$$E = RT\left(\mathscr{J}/F + \sum_i z_i D_i \partial C_i/\partial x\right)/\left(F\sum_i z_i^2 D_i C_i\right) \tag{8}$$

Because of the assumption of equal diffusion coefficients for all species, eq. 8 reduces to:

$$E = \mathscr{J}RT/(2DF^2 Y(X)) \tag{9}$$

This relation indicates that Ohm's law is verified for our reactive system (because of identical diffusion coefficients), that the values of the electric field inside the system (both the compartment and the membranes) are inversely proportional to Y concentrations, and consequently that the conductivity values are proportional to Y concentrations.

For any species i, the steady-state mass balance in the membranes is:

$$\mathrm{d}^2 C_i/\mathrm{d}X^2 - z_i F(C_i\, \mathrm{d}E/\mathrm{d}X + E\, \mathrm{d}C_i/\mathrm{d}X)/RT = 0 \tag{10}$$

Writing eq. 10 for Y leads, because of the expression of eq. 9, to the very simple diffusion-like equation:

$$\mathrm{d}^2 Y/\mathrm{d}X^2 = 0 \tag{11}$$

The solving of this equation leads to the expression of the profiles in membranes I and III:

$$Y(\mathrm{X})^{\mathrm{I}} = (Y^{\mathrm{II}} - Y_0)X/e + Y^{\mathrm{II}} \tag{12}$$

$$Y(\mathrm{X})^{\mathrm{III}} = -(Y^{\mathrm{II}} - Y_0)X/e + (Y^{\mathrm{II}}(L+e) - Y_0 L)/e \tag{13}$$

The mass balance for Y in the reactive compartment is:

$$V_{\mathrm{m}} L f(S^{\mathrm{II}}) + J_{\mathrm{Y}}^1 - J_{\mathrm{Y}}^2 = 0 \tag{14}$$

In this expression, $f(S)$ represents any of the considered kinetics (Michaelis or substrate excess inhibition), and J_{Y}^1 and J_{Y}^2 are the fluxes of Y at the two boundaries of the reactive compartment.

The expression of the fluxes, using eqs. 6, 12 and 13, substituted into eq. 14 leads to the expression for Y^{II}:

$$Y^{\mathrm{II}} = Y_0 + V_{\mathrm{m}} e L f(S)/2D \tag{15}$$

Introducing the dimensionless concentration $y = Y/K_{\mathrm{m}}$ (a lower case letter will be the dimensionless concentration corresponding to the dimensional concentration denoted by the capital letter) and the dimensionless diffusion-reaction parameter σ [2] (a Thiele-type parameter) defined by:

$$\sigma = V_{\mathrm{m}} e L/(K_{\mathrm{m}} D) \tag{16}$$

gives:

$$y^{\mathrm{II}} = y_0 + \sigma f(s)/2 = s_0 + \sigma f(s)/2 \tag{17}$$

Now, S profiles have to be calculated in the two membranes. This is done by considering the general expression, eq. 10, in which the expression of the electric field is calculated taking into account eqs. 12 and 13 of Y for membranes I and III, respectively. By introducing in each of the two cases the new variables:

$$x = Y^{\mathrm{I}}(\mathrm{X}) \text{ and } x' = Y^{\mathrm{III}}(\mathrm{X}) \tag{18}$$

it is possible to obtain two equidimensional linear differential equations (eqs. 19 and 20 for layers I and III, respectively):

$$x^2\, \mathrm{d}^2 S/\mathrm{d}x^2 + ax\, \mathrm{d}S/\mathrm{d}x - aS = 0 \tag{19}$$

$$x'^2\, \mathrm{d}^2 S/\mathrm{d}x'^2 - ax'\, \mathrm{d}S/\mathrm{d}x' + aS = 0 \tag{20}$$

In these equations coefficient a is:

$$a = \mathscr{J}e/(2DF(Y^{\mathrm{II}} - Y_0)) \tag{21}$$

The solutions to eqs. 19 and 20 are:

$$S^{I}(x) = \lambda x + \mu x^{a} \tag{22}$$

$$S^{III}(x') = \lambda' x' + \mu' x'^{a} \tag{23}$$

In these equations, coefficients λ, μ, λ' and μ', determined by the boundary conditions, have the following expressions:

$$\lambda = -\left(\beta^{-(1+a)} + S^{II}/Y^{II}\right)/\left(1-\beta^{-(1+a)}\right) \tag{24}$$

$$\mu = \left(\left(1 - S^{II}/Y^{II}\right)Y^{II(1+a)}\right)/\left(\beta^{(1+a)} - 1\right) \tag{25}$$

$$\lambda' = \left(S^{II}/Y^{II} - \beta^{a-1}\right)/\left(1-\beta^{a-1}\right) \tag{26}$$

$$\mu' = \left(\left(1 - S^{II}/Y^{II}\right)Y^{II^{1-a}}\right)/\left(\beta^{1-a} - 1\right) \tag{27}$$

In these expressions β is defined as the ratio Y^{II}/S_0. Then the mass balance for S is expressed in order to calculate S:

$$-V_m L f(S^{II}) + J_S^1 - J_S^2 = 0 \tag{28}$$

The fluxes calculated from the expressions of S(X) are substituted into eq. 28. It is possible to obtain analytical expressions of S^{II} for a Michaelis kinetics with zero order; numerical solutions were computed for the global Michaelis kinetics and for the substrate excess inhibition kinetics.

3.1. Michaelis kinetics; zero order

In this case the intra-compartment Y expression reduces to:

$$y^{II} = s_0 + \sigma/2 \tag{29}$$

We may note that y^{II} is no longer dependent on the current density: this is in agreement with the following statements: (i) under zero-order conditions the reaction rate is constant, (ii) the transport of Y in the membranes is independent of the current density (eq. 11).

The expression of the dimensionless substrate concentration in the reactive compartment is:

$$s^{II} = y^{II}\left((a+3)\beta^{1-a} + (3-a)\beta^{1+a} - 2\beta^2 - 4\right)/$$
$$\left(2\beta^2 + 2a - (1+a)\left(\beta^{1+a} + \beta^{1-a}\right)\right) \tag{30}$$

p^{II} can be calculated by inserting the expressions of s^{II} and y^{II} into the electroneutrality equation (eq. 3).

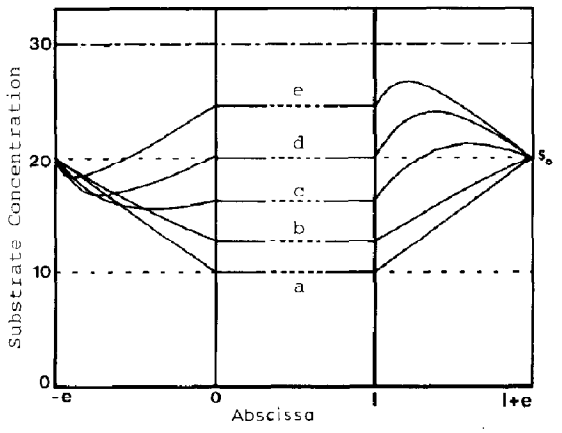

Fig. 2. Substrate concentration profiles through the system obtained for $s_0 = 20$ (Michaelis kinetics, zero order), $\sigma = 20$, with different values of the current density (mA/cm^2): (a) $\mathcal{J} = 0$, (b) $\mathcal{J} = 0.01$, (c) $\mathcal{J} = 0.1$, (d) $\mathcal{J} = 0.2$, (e) $\mathcal{J} = 0.4$; for sufficient current density values, substrate may accumulate inside the reactive compartment above its boundary concentration level.

s profiles through the whole system, obtained by combining eqs. 22, 23 and 30, are illustrated in fig. 2 for $s_0 = 20$, $\sigma = 20$, with different values of the current density. The evolution of s profiles with $\mathcal{J}$ shows clearly that inside the system an

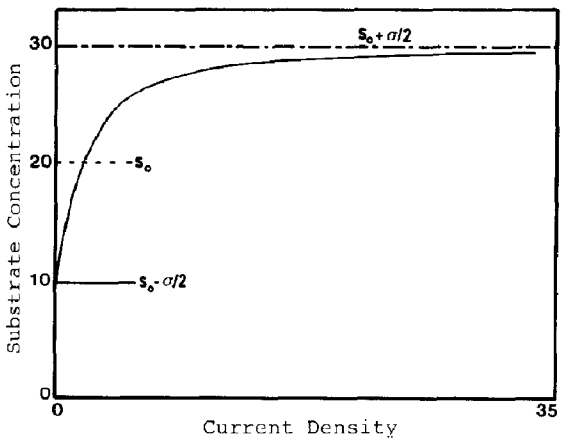

Fig. 3. Variations of substrate concentration inside the reactive compartment in terms of current density (mA/cm^2) (Michaelis kinetics, zero order). Substrate concentration starts from diffusion-reaction values $s_0 - \sigma/2$ and for high current densities tends toward the limit value $s_0 + \sigma/2$.

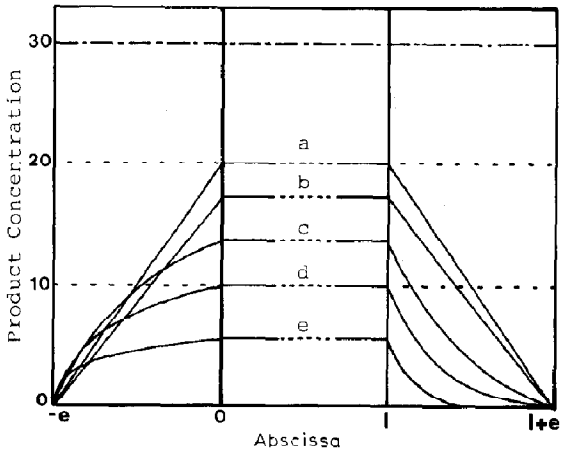

Fig. 4. Product concentration profiles through the system obtained for $s_0 = 20$ (Michaelis kinetics, zero order), $\sigma = 20$, with different values of the current density (mA/cm^2): (a) $\mathcal{J} = 0$, (b) $\mathcal{J} = 0.01$, (c) $\mathcal{J} = 0.1$, (d) $\mathcal{J} = 0.2$, (e) $\mathcal{J} = 0.4$. With increasing current densities, P concentration tends toward 0.

ionic substrate can be accumulated above its boundary value, in spite of being consumed by the enzyme reaction. The variations of s inside the reactive compartment are presented in fig. 3 for $s_0 = 20$ and $\sigma = 20$. It is easy to demonstrate that the initial diffusion-reaction value of s is $s_0 - \sigma/2$ and that the limiting value for theoretical infinite currents is $s_0 + \sigma/2$ (the value of y in the reactive compartment for any current).

If we characterize the accumulation of substrate by the difference $s^{II}(\mathcal{J}) - s^{II}(\mathcal{J} = 0)$, it is clear that the maximum accumulation is directly proportional to σ.

Since we are considering here a zero-order kinetics, this accumulation of substrate does not correspond to an increase in the enzyme activity.

p profiles (fig. 4) exhibit a quite different evolution, since the value of p^{II} decreases continuously with increasing current densities; this is due to the fact that the boundary value of p is zero. For diffusion-reaction regimes, $p^{II} = \sigma$; for infinite currents, p^{II} tends toward zero.

For the electrical variables χ (the conductivity) and E, we may note that the conductivity is proportional to y (eq. 9) which is independent of the current density (eq. 29). Thus, the conductivity in the reactive compartment is independent of $\mathcal{J}$ and depends only on σ: the higher the σ value, the higher the internal conductivity:

$$\chi^{II}/\chi_0 = (s_0 + \sigma/2)/s_0 \tag{31}$$

The electric field is proportional to $\mathcal{J}$ (Ohm's law), but the ratio E_0/E^{II} is independent of $\mathcal{J}$. Conversely, this ratio is dependent on σ and the higher the σ value, the higher the ratio.

3.2. Global Michaelis kinetics

Taking into account the global Michaelis kinetics, it is possible to demonstrate that s^{II} is the solution to an equation similar to eq. 30 but in which y^{II}, a and β are now functions of s^{II} itself, and it is no longer possible to obtain analytical solutions.

Computations reveal the same qualitative results as those obtained for zero order. It was possible to show that for very high intensities the following relation holds:

$$s^{II} = \left(\sigma/2 + s_0 + 1 + \sqrt{(\sigma/2 + s_0 + 1)^2 + 4s_0}\right)/2 \tag{32}$$

and in this case also, the accumulation of s increases with σ (fig. 5A).

These results also show that the higher the σ value, the higher the current necessary to reach the boundary value s_0 (fig. 5B).

For low values of s_0 (e.g., < 1), the accumulations of substrate appearing with increasing electric currents lead to increasing enzyme activities. With the conductivity effect it is possible to obtain higher activations than those obtained for the same system with a supporting electrolyte (fig. 5C) since in the latter case, the substrate concentration is limited by its boundary value.

3.3. Substrate excess inhibition kinetics: Conductance bistability

The approach is similar to that for the global Michaelis kinetics, and no analytical solution was obtained. However, it is possible to show that for very high current intensities the following relation

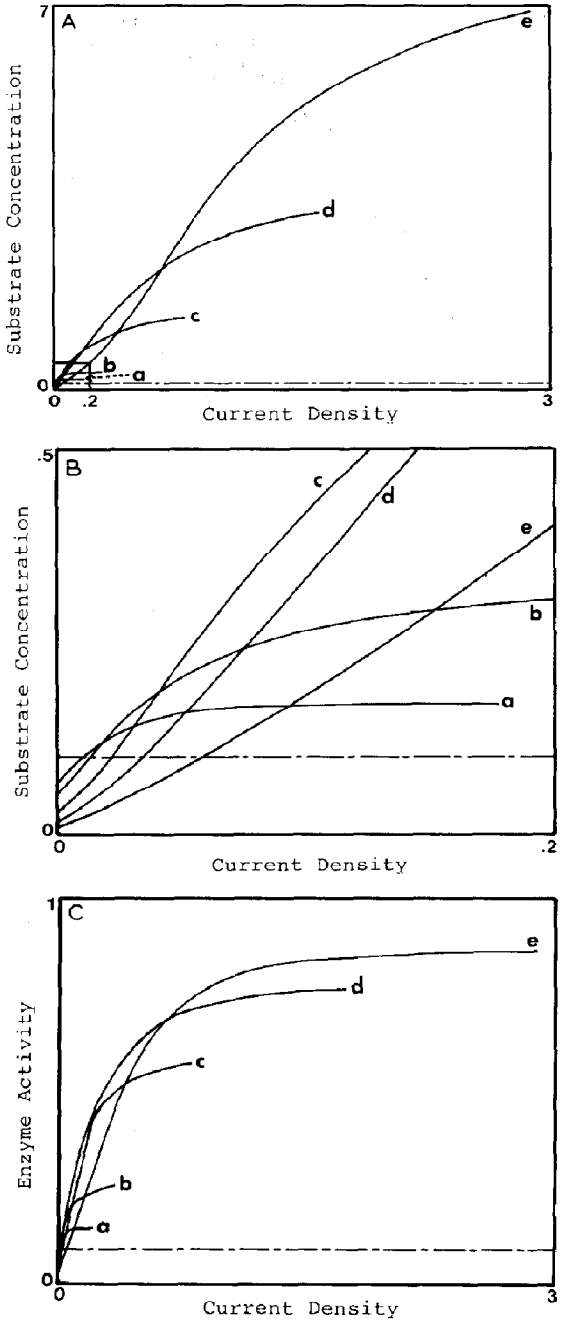

Fig. 5. (A) Variations of substrate concentration in terms of current density (mA/cm^2) for different values of the dimensionless diffusion-reaction parameter σ: (a) $\sigma = 1$, (b) $\sigma = 2$, (c) $\sigma = 5$, (d) $\sigma = 10$, (e) $\sigma = 20$; Michaelis kinetics, first order; $s_0 = 0.1$. (B) The part of panel A enclosed in the small rectangle is enlarged here; it is possible to see on this enlargement that the higher the σ value, the higher the current necessary to reach the boundary value s_0. (C) Corresponding enzyme activities in terms of current densities for the different values of σ.

holds:

$$s^{II} = \left((k_i s_0 - 1)/k_i + \sqrt{(k_i s_0 - 1)^2/k_i^2 + 4(s_0 + \sigma/2 - 1)/k_i}\right)/2 \quad (33)$$

We know that for a substrate excess inhibition kinetics, a functional bistability may be obtained, depending on the values of σ, s_0 and k_i. Moreover, we showed that specific electric signals are able to make this type of system commute from one state to the other, when a 'buffered' conductivity is considered (high level of conductivity, which cannot be modulated by the ionic species produced by the enzyme reaction). Experiments were performed successfully with uricase [5,7,12].

Here, the conductivity level is minimum; however, it is still possible to obtain a bistability leading to discrete changes in substrate concentration and enzyme activity when adequate electric signals are imposed (fig. 6A and B).

In addition, due to the conductivity effect a new possibility arises: it is now possible to obtain a conductance bistability (fig. 6C) which corresponds to the existence, in a given domain of current intensities, of two possible values of conductivity: a high value and a lower one; so two branches are defined in the $\chi(\mathcal{J})$ variations. It is possible to go from the higher to the lower branch by the action of a current density greater than a threshold value (here $\mathcal{J} > 1.4$). However, it is not possible to go from the lower to the higher branch by an electrical action, since this one always tends to decrease the diffusion limitations in this system. The introduction of asymmetrical boundary conditions might be used to allow the two-way commutation [5,12].

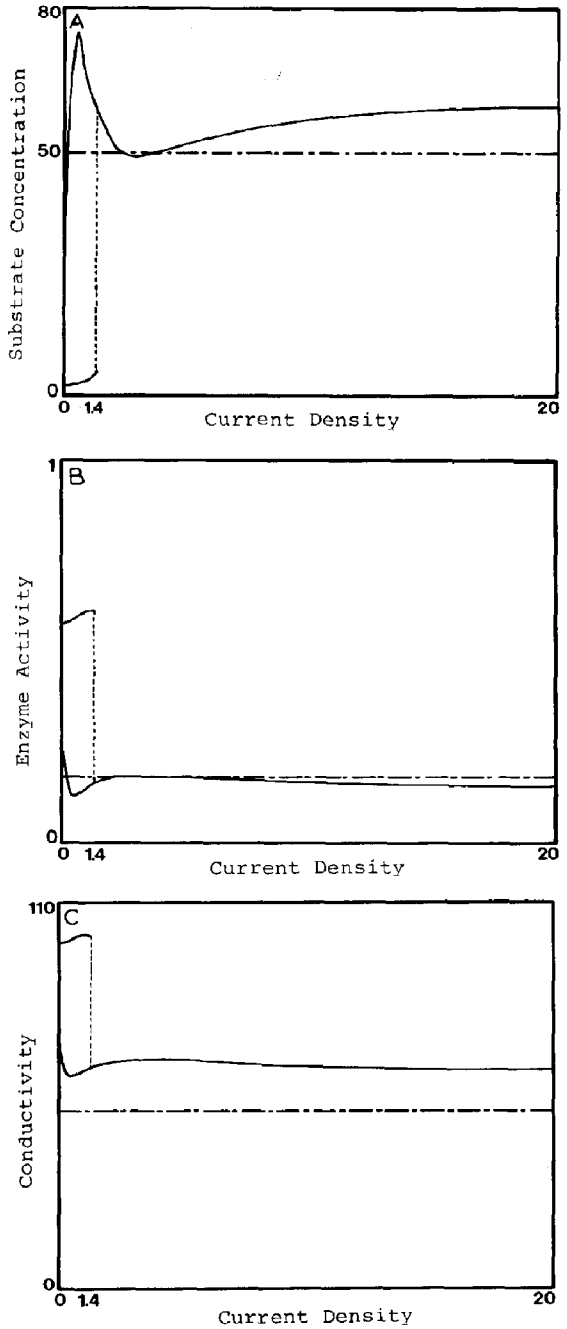

Fig. 6. Bistability phenomenon obtained with substrate excess inhibition kinetics in the domain $0 < \mathscr{J} < 1.4$ (mA/cm^2); $\sigma = 170$; dimensionless inhibition constant $k_i = 0.1$ ($k_i = K_i/K_m$), $s_0 = 50$. (A) Substrate concentration bistability corresponding to two possible levels of concentration. (B) Enzyme activity bistability (in relation to the substrate concentration bistability). (C) Conductance bistability corresponding to two possible levels of conduction.

4. Conclusion

We have considered here a very simple model to study the conductivity effect; a compartmental homogeneous system allowed us to consider space-independent phenomena (in the reactive part of the system) and when only three chemical species associated with simple kinetics were considered, a mainly analytical approach was possible.

The conductivity effect appears when an electric current is applied in a system involving a reaction producing ionic species, when the conductivity level is low at the boundaries, and when a limiting diffusion is considered. The effect due to the difference in conductance between the inside and outside of the system appears when an electric current is imposed. It corresponds to an accumulation inside the system of any ionic species which has a boundary concentration different from zero. It is possible, in particular, to accumulate an ionic substrate inside the system in spite of its consumption by the enzymic reaction. The maximum level of accumulation (virtually infinite electrical current) is proportional to the diffusion-reaction parameter value.

When a Michaelis kinetics is considered this accumulation of substrate corresponds to a large increase in enzyme activity.

When a substrate excess inhibition kinetics is considered, with adequate values of the diffusion-reaction parameter and of the boundary concentrations, it is possible to obtain a bistability of substrate concentration as was observed theoretically and experimentally with the same type of system but with a supporting electrolyte; now, in addition, a conductance bistability can be obtained, corresponding to the existence of a high and a low level of conductance in the same domain of intensities.

Experiments related to these theoretical results are now underway.

The results described here might be applied either to the improvement of the yield of chemical

or biochemical reactions in industrial processes (results associated with the Michaelian kinetics in particular), or to the development of biosensors or information-processing systems (results associated with the substrate excess inhibition kinetics) [12,13]. This latter aspect has been discussed during recent seminars [12,13].

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