

Computer Simulation of Porous Electrodes with Immobilized Enzymes: The Percolation Properties of Multicomponent Structures

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Received November 2, 2001

Abstract—The percolation characteristics of porous electrodes with immobilized enzymes are calculated. It is presumed that active centers of the enzymes undergo direct oxidation or reduction on the electrode. Two versions of a three-dimensional electrode structure consisting of particles of identical size are studied. In one, the frame of the porous electrode (PE) comprises only those parts of the support that conduct electrons. In the other, the electrode is a two-component structure capable of ensuring the supply of two participants of the electrochemical process to the enzymes. Calculated are: the fraction of the support parts that conduct electrons (taken as a whole, they form an “electron cluster”); the electron cluster’s transparency; and the number of exits the electron cluster has onto the rear surface of PE. The character of distribution of enzymes that are in contact with one or two macroscopic clusters, which comprise “conductive” particles, over the PE thickness is established. In doing so, it is assumed that the two clusters can be supplied either from one side (“parallel” clusters) or from two opposite sides (“collision” clusters) of PE. The electron cluster surface accessible to contact with enzymes and the number of enzymes in contact with such an electron cluster are determined. Two possibilities of the PE functioning are examined. In one, the electrochemical process proceeds at any contact of the enzyme with the support particles. In the other, a certain type of enzyme immobilization on the support is required. The region of optimum concentrations of components that make PE is established. Within this region the electrochemical activity may reach a maximum.

FORMULATION OF THE PROBLEM

The term “immobilized enzymes” was suggested three decades ago at the I International conference on engineering enzymology (1971, United States). Since that time a plethora of techniques of enzyme immobilization on supports have been tested [1]. These include the adsorption on insoluble supports; the incorporation of enzymes into gel, hollow fibers, and liposomes; the microencapsulation; and the cross-linkage of enzymes by bi- or polyfunctional reactants. The incorporation of enzymes into porous electrodes (PE) is a case apart. In the first place, the support in this case must be a conductor of electrons. Secondly, the support particles (SP) must be highly-dispersed in the porous space of the electrode. Only then an mixture of SP and enzymes may ensure considerable current densities in a unit volume of a porous electrode with immobilized enzymes (PIE).

The feasibility of realizing reversible electrochemical reactions with the participation of immobilized enzymes was shown in a number of works. The organization of transport of electrons between an active center of an enzyme and an electrode may be realized by two fundamentally different techniques: (1) by means of low-molecular-weight carriers or mediators and (2) by direct oxidation or reduction of active centers of the

enzymes. The second version is more alluring as it gives one a chance to eliminate an intermediate link, which is connected with a mediated electron transport from enzymes onto SP. For the first time the mediatorless technique of electron exchange between SP and enzymes was realized in [2]. In this particular communication we presume that there takes place a direct oxidation or reduction of active centers of enzymes on the electrode.

One of the absolutely new problems associated with use of enzymes in PE is the necessity to know in more detail the structure of the protein part of enzyme molecules. Compared with traditional catalysts that have atomic size, the volume of enzymes is considerable and the view that a biocatalyst is simply “smeared” over the support surface is erroneous. Hence it is necessary to have information about the degree of localization of active center in the ternary structure of enzymes, about the nature of electron transport over the protein part of enzymes, and about desirable position of an enzyme relative to particles in contact with it.

Another problem is the necessity to know more about specific features of the adsorption or chemical interaction of enzymes with aggregated SP. Earlier studies dealt with enzymes immobilized on electrodes whose size was much greater than that of the enzyme.

The enzyme size in PIE, may be commensurate with that of SP and even exceed it. Hence, it is necessary to find out the degree of contact the enzyme makes with SP, and the nature of ensembles formed by SP and enzymes.

And thirdly, it is necessary to have more information about the porous structure of the “filled” electrodes, into which one is going to implant enzymes by one means or another. It is necessary to study percolation properties of active layer that comprise an aggregate of SP and other particles that are required for the organization of electrochemical process in PIE.

At present the solution of the first two involved problems seems out of reach. Therefore, we will focus on the third point. Our aim will be to find conditions that would ensure an optimum degree of functioning of enzymes in PIE. We will construct computer models for PIE, examine their percolation properties, and compute a dependence of the electrochemical activity of PIE on their composition.

An example of the way one can conduct computer simulation of the structure of PIE, as well as the basic notions and terms that emerge in doing so—all this has been demonstrated in sufficient detail in [3]. In that study we used two-dimensional lattices as a simplest example that admitted a vivid presentation. We showed how one must perform calculations of percolation characteristics of PIE. In the present work we explore three-dimensional structures that are closer to reality.

We will carry out our theoretical research using three simplifying assumptions. Firstly, we will represent a PIE as a cube with a volume of $N \times N \times N$ arbitrary sizes (sizes of particles that make the electrode). All the particles that make the electrode are assumed to have an absolutely identical size, be shaped as “small cubes,” and be randomly scattered over the points of a cubic lattice that occupies the space of our model electrode. Thus, the electrode’s capacity is exactly N^3 particles. There are strong grounds for believing that this electrode is “porous.” This belief is based on the algorithms of percolation calculations remaining the same if we pass to studying more realistic components of a PE—spherical particles. Secondly, we assume that the principal parameter in a model of PIE is the concentration of SP in a unit volume and denote this parameter as g . And thirdly, the shape and size of the enzyme will be identical to those of all the other particles that make a PE.

Thus, from the viewpoint of percolation theory [4–12], we will consider a 3D model of percolation of a wetting fluid (say, electrons, over trains of SP) in a porous body with a random configuration of particles in a volume of $N \times N \times N$ arbitrary sizes. If we restrict our consideration for the time being to the case of a single-component structure, then every point of such a lattice will be an elementary cubic cell, which exists in two possible “phase states,” specifically, either as SP or as void into which an enzyme molecule may, in principle, be placed. Moreover, we assume that one of the six

faces of this “lattice of sites” (porous electrode) is in contact with a liquid phase (source of electrons, current lead).

In order to obtain high overall current densities in a PIE or produce a sufficient amount of a target product, it is necessary to supply a reactant, ions (supplied or removed), and an electric current (supplied or removed with the aid of SP) into a certain region of the porous structure. This is the region where enzymes are located and where the electrochemical process mainly occurs (we call this region “region of electrochemical conversions”). As for the reactant, it may be, for example, oxygen or hydrogen, if we speak of a biofuel cell with gaseous reactants. Thus, in a PIE, it is necessary to ensure conditions for a meeting of all the participants in electrochemical conversions in a maximum volume possible. Moreover, it is necessary to secure the occurrence of the following symbolic reaction:



where R stands for the reactant, I represents the supplied or removed ions, e means electrons, E denotes the enzyme, and P is the target product. Naturally, in doing so, the central moment in the construction of the structure of a PIE must be the organization of a developed surface of contact of SP with the enzyme. It is exploration of brand new opportunities that unfold here that we will busy ourselves with in the first place.

ONE CONDUCTING CLUSTER

We commence constructing a support of the “filling” type for an enzyme. We scatter SP in a random fashion over points of a cubic lattice in a volume that is isolated for constructing a model electrode. Each SP possesses electron conduction. However, for a SP to be a real supplier of electrons for the enzyme immobilized on the support, the particle must be connected with an electron source. A current lead situated on a PE face is such a supplier of electrons.

At small concentrations of SP in a unit volume, on average SP happen to be disconnected. That is why the enzymes cannot take part in the electrochemical process. As sufficiently large concentrations of SP, however, the enzymes begin forming linked (common face of neighboring SP) systems of particles called clusters. The properties of such clusters are the subject studied by a special branch of mathematics called the percolation theory. Its main conclusion is that, upon reaching a certain threshold value (called percolation threshold) of the concentration of “conductive” elements (in our case, SP) in infinite lattice-like structures, there emerges an infinitely large conductive cluster. It is quite understandable that the existence of such practically infinite (macroscopic) clusters is absolutely necessary for the PE functioning. The point here is that diameters and thicknesses of PE usually exceed the size of structural units that make the PE by a few orders of magnitude. That is why only a practically infinitely large con-

Table 1. Dependence of principal parameters of a PIE on the fraction of SP; single-component system, volume $100 \times 100 \times 100$

g	g_e	S_{\max}	S	ρ	n
0.05	5.5×10^{-4}	3.31×10^3	2.6×10^3	0.786	0
0.1	1.2×10^{-3}	7.25×10^3	5.39×10^3	0.744	0
0.2	3.3×10^{-3}	1.99×10^4	1.34×10^4	0.676	0
0.3	1.7×10^{-2}	1.02×10^5	6.28×10^4	0.617	0
0.315	3.11×10^{-2}	1.87×10^5	1.14×10^5	0.612	0
0.317	7.63×10^{-2}	4.58×10^5	2.8×10^5	0.613	160
0.4	0.357	2.14×10^6	1.22×10^6	0.57	3×10^3
0.5	0.488	2.93×10^6	1.44×10^6	0.491	4.7×10^3
0.6	0.597	3.58×10^6	1.43×10^6	0.399	5.9×10^3
0.7	0.699	4.2×10^6	1.27×10^6	0.303	7×10^3
0.8	0.8	4.8×10^6	9.82×10^5	0.205	8×10^3
0.9	0.9	5.4×10^6	5.71×10^5	0.106	9×10^3
0.999	0.999	5.99×10^6	4.59×10^4	7.65×10^{-3}	1×10^4

ductive cluster is capable to supply electrons to a large number of enzymes linked with this cluster or, in other words, to create acceptable for practice overall current densities in PIE. We will call such a macroscopic cluster an “electron cluster.”

An electron cluster is bound to perform two functions in a PIE. In the first place, an electron cluster is one common support for all enzymes attached to it. Therefore, it is important for an electron cluster to have free surface on which to adsorb enzymes. We introduced this property of an electron cluster in [3] and called it “transparency.” The transparency of an electron cluster is the ratio of the number of its faces open for the adsorption of enzymes to the number of all the faces constituting the electron cluster. It stands to reason that the transparency is maximum when parameter g tends to zero (when SP are disconnected) and then it steadily decays. We will obtain an example of an absolutely non-transparent cluster at $g = 1$. In this case all the cells of a porous space are occupied by SP; therefore, all the SP kind of stick to one another, and there is absolutely no chance for the cluster to adsorb enzymes. The transparency of such a cluster is rigorously equal to zero. If, of course, we ignore the PE external surfaces.

Another function of an electron cluster in PIE (we have already dwelt on this problem somewhat) is to ensure the supply or removal of electrons from a current lead situated on one of the electrode sides to all the enzymes sitting on the electron cluster. This property of an electron cluster comes into force only after parameter g reaches a certain threshold value, which we denote as g_{cr} . At smaller values of parameter g , support particles an electron cluster still do not form. Thus, in the interval $g_{\text{cr}} \leq g \leq 1$, there must exist a region of optimum values of parameter g at which the number of enzymes sitting on an electron cluster and supplied by electrons reaches a maximum. Naturally, we assume that any

conceivable limitations (ohmic, diffusion) on the current generation in PIE are either absent or simply ignored, for the time being.

Let us note also that the character of occupation of our model electrode by an electron cluster must, naturally, be essentially dependent on the PE size. According to preliminary calculations, beginning with the size $100 \times 100 \times 100$, percolation characteristics of PE undergo no substantial variations any longer. This calculation testifies that PE whose size is equal to or greater than $100 \times 100 \times 100$ could be called macroscopic electrodes or electrodes of an infinitely large size.

Having finished with this terse prolegomenon we turn our attention to expounding on the results of a computer simulation of the process of formation of an electron cluster in an electrode space and to calculating the number of enzymes that are in contact with it and make their contribution to the magnitude of the electrochemical activity of a PIE.

The major characteristics of a single-component electrode, which comprises only SP, appear in Tables 1 and 2. With parameter g tending to zero, the only SP that can take part in electron exchange are those in direct contact with a current lead. That is why, here, the fraction of SP that conduct electrons is infinitesimally small. We denote this fraction as g_e . However, with the density of SP in an electrode increasing, g_e also begins increasing. Should the electrode size have been infinite, the percolation threshold, i.e. the instant when there begins an explosive growth of g_e , would have begun in a cubic lattice of sites at the value

$$g = g_{\text{cr}} = [(1 + 2^{1/2})^{1/3} + (1 - 2^{1/2})^{1/3}] / 2 = 0.298, \quad (2)$$

as was demonstrated in [13]. In reality, however, an electrode, although being macroscopic, has non-infinite dimensions. Therefore, some of the conducting paths

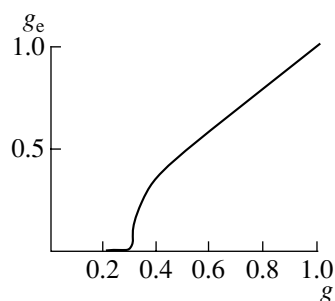


Fig. 1. The fraction of SP that conduct electrons vs. the fraction of SP in single-component PIE of volume $100 \times 100 \times 100$.

that do exist in an infinite space happen to be “axed off” in a finite electrode (we discuss the possibility of such percolation effects in [14–17]). That is why the percolation threshold is reached not at $g_{cr} = 0.298 \approx 0.3$, but at a greater value of parameter g . As we see from a comparison of data presented in the first and sixth columns of Table 1, an electron cluster emerges in an electrode at $g = 0.317$. This is the only value of parameter g at which SP supplied with electrons emerge on the electrode side opposite to the current lead, because in the last column of Table 1 we present values of quantity n , which is the number of exits an electron cluster has onto the PE rear surface.

Comparing the data presented in the first and second columns of Table 1 shows that there exist three characteristic ranges for parameter g . In the range $0 \leq g \leq g_{cr} = 0.317$, the growth of the number of SP that conduct electrons g_e heavily lags behind the growth of the number of all SP. Once parameter g reached the threshold value g_{cr} , there begins the range of a rapid growth of g_e , in which g_e tends to take over g . And finally, in the range of large values of g we can assume that g_e is at last approximately equal to g . This implies that practically all SP enter an electron cluster. Vividly, the run of a g_e vs. g dependence in all the three ranges is presented in Fig. 1. Clearly distinguishable are the range of practically vanishing values of quantity g_e ; the range of a rapid growth of g_e , which approximately terminates at $g = 0.5$ (see also the data we present in Table 1); and the range where g_e approximately equals g .

In the third column of Table 1 we present values of the surface area of all SP that conduct electrons, S_{max} . In the fourth column of Table 1 there appear values the surface area of electron-conducting SP that is ready to adsorb enzymes, S . Values of the ratio $\rho = S/S_{max}$, which defines the transparency of an electron cluster, appear in the fifth column of Table 1. As we have already mentioned in the foregoing, the transparency steadily decays with increasing parameter g . The transparency ρ reaches maximum value $\rho = \rho_{max} = 5/6 = 0.833$ at parameter g tending to zero. Then to conduct electrons are capable only SP that are in direct contact with a current lead. In this case, obviously, five out of six faces of

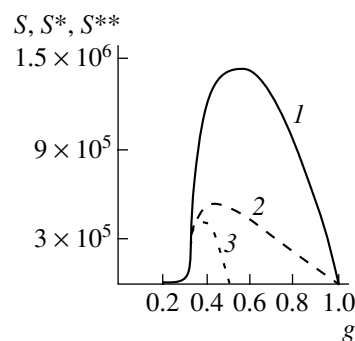


Fig. 2. Dependences of (1, 3) true and (2) effective surface areas ready to adsorb enzymes on the SP fraction in single-component PIE of volume $100 \times 100 \times 100$.

a cubic lattice “straddling” the current lead happen to be ready to adsorb enzymes. That is where the value $\rho_{max} = 5/6$ stems from. The run of an S vs. g dependence is illustrated by curve 2 in Fig. 1. The dependences of the transparency and the number of exits of the electron cluster onto the rear surface of PE on parameter g appear in Fig. 3 (curves 1 and 3, respectively).

We will address now an important point of our investigation, which is to establish the number of immobilized and accessible to bioelectrocatalysis enzymes and thus estimate the magnitude of the current generated in a PIE. These data are collected in Table 2.

Another distinguishing feature of enzymes as biocatalysts, which makes them different from metallic catalysts, is that in the course of an enzyme immobilization in a PE it is important that a certain part of

Table 2. The number of enzyme particles in contact with SP and reacting enzyme particles as a function of the fraction of SP in a PIE; single-component system, volume $100 \times 100 \times 100$

g	N_e	$N_c/10^6$	$N_r/10^6$	ρ^*
0.05	9.5×10^5	2.38×10^{-3}	4.22×10^{-4}	0.719
0.1	9×10^5	4.72×10^{-3}	8.92×10^{-4}	0.651
0.2	8×10^5	1.01×10^{-2}	2.22×10^{-3}	0.553
0.3	7×10^5	3.8×10^{-2}	1.04×10^{-2}	0.373
0.31	6.9×10^5	5.25×10^{-2}	1.47×10^{-2}	0.362
0.32	6.8×10^5	0.249	7.15×10^{-2}	0.354
0.33	6.7×10^5	0.37	0.11	0.336
0.35	6.5×10^5	0.474	0.152	0.308
0.4	6×10^5	0.535	0.201	0.25
0.5	5×10^5	0.488	0.237	0.167
0.6	4×10^5	0.398	0.235	0.111
0.7	3×10^5	0.3	0.207	0.071
0.8	2×10^5	0.2	0.158	0.042
0.9	1×10^5	0.1	8.92×10^{-2}	0.019
0.999	1×10^3	1×10^{-3}	9.87×10^{-4}	0.00017

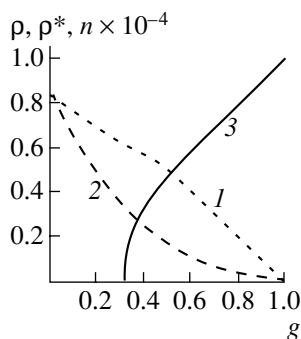


Fig. 3. Dependences of (1) true and (2) effective transparency and (3) the number of exits of an electron cluster onto the PIE rear surface on the SP fraction; single-component PIE of volume $100 \times 100 \times 100$.

enzymes should made contact with the SP that conduct electrons. We call it a “contact” center. Let us try and allow for this circumstance in calculations. Let us assume, for simplicity, that the contact center of an enzyme is located on one of its six faces (it will be remembered that we conditionally represent both enzymes and SP as small cubes of the same size). Then, it is obvious that an enzyme surrounded by six neighboring SP that conduct electrons may take part in bioelectrocatalysis with probability $\omega_6 = 1$. Similar probabilities ω_5 through ω_1 for enzymes surrounded by five to one electron-conducting SP equal $5/6$, $2/3$, $1/2$, $1/3$, and $1/6$. Thus, knowing how many enzymes in a PE have as their neighbors s SP that conduct electrons, and denoting these quantities as N_s , where s runs values from 1 to 6, we can find the number of enzymes, N_r , that take part in a bioelectrocatalysis process with the formula

$$N_r = N_1\omega_1 + N_2\omega_2 + N_3\omega_3 + N_4\omega_4 + N_5\omega_5 + N_6\omega_6. \quad (3)$$

This formula is valid under the assumptions that we adopted in the foregoing. Specifically, the contact center of an enzyme, which is localized at one of the six faces of a cubic enzyme, must be in direct contact with SP that conduct electrons.

The second column of Table 2 lists values of the number of maximum permissible enzymes that are capable of occupying a PE. This quantity was calculated with the obvious formula

$$N_c = N^3(1 - g) = 10^6(1 - g), \quad (4)$$

Here, g represents the fraction of SP in a PE. The third and fourth columns of Table 2 contain the fraction of enzymes in contact with an electron cluster, $N_c/10^6$, and the fraction of reacting enzymes, $N_r/10^6$, under the condition that a certain type of fixation of the enzyme on a SP (see relationship (3)) is required for taking part in the electrochemical process. To calculate quantities N_c and N_r we used methods presented in [3].

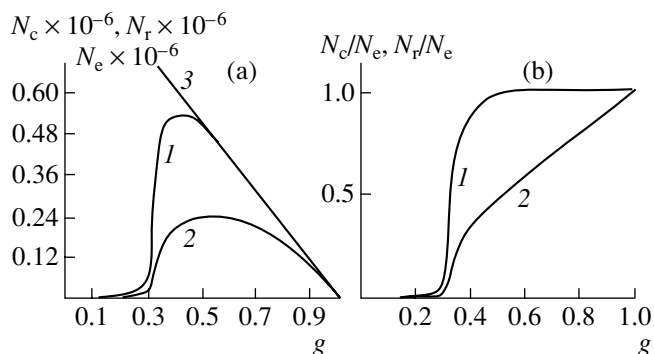


Fig. 4. Dependences of (a) absolute and (b) relative number of enzymes in contact with electron cluster on the SP fraction for a single-component PIE of volume $100 \times 100 \times 100$; see text for explanations.

In the last column of Table 2 we present values of the effective transparency of an electron cluster. The meaning of this quantity is as follows. The surface area of an electron cluster, which is ready to adsorb enzymes, S , is the number of faces that make an electron cluster of SP, which may, in principle, adsorb enzymes. If the size of enzymes is smaller than the size of SP, all these faces may adsorb the enzymes. The situation alters when the shape and size of enzymes and SP are identical. We see from a comparison of the data presented in the fourth column of Table 1 with the data presented in the third column of Table 2 that in this case the number of free faces of an electron cluster is greater than the number of enzymes sitting on the electron cluster, i.e. $S > N_c$. For every enzyme may be in contact with one, two, and so on free faces of an electron cluster. That is precisely why we have introduced the notion of an effective transparency of an electron cluster, which is defined by the expression $\rho^* = N_c/S_{\max}$. The effective surface area of an electron cluster, $S^* = N_c$, as a function of the fraction of SP in the PE bulk is indicated by curve 2 in Fig. 2. And the run of a ρ^* vs. g dependence is pointed out in Fig. 3 by curve 2.

Figures 4a and 4b show dependences of, respectively, absolute and relative numbers of enzymes in contact with an electron cluster on the fraction of SP. The curves that are marked in Fig. 4 with number “1” presume that an enzyme takes part in the electrochemical process no matter the way it contacts the SP. The curves that we marked with number “2” in Fig. 4 presume that an enzyme takes part in the electrochemical process under the condition that a certain type of fixation of the enzyme on a SP (see relationship (3)) is required. Curve 3 in Fig. 4a points out a maximum possible fraction of enzymes that may be contained in a PE. Exact numerical values of quantities N_c , N_r , and N_e appear in relevant columns of Table 2.

At $g \rightarrow 1$, $N_c = N_r$ (Table 2, Fig. 4), because at high g practically all enzymes are surrounded by electron-conducting SP. Hence, a bioelectrocatalysis act occurs the position of the enzyme and its contact center not-

Table 3. The number of enzyme particles in contact with SP and reacting enzyme particles as a function of the fraction of SP in a PIE; two-component system, two collision clusters, volume $100 \times 100 \times 100$

g	$N_c/10^6$	$N_r/10^6$	S^{**}
1×10^{-4}	0	0	5
1×10^{-3}	0	0	50
0.01	0	0	498
0.1	0	0	4.62×10^3
0.2	0	0	1.03×10^4
0.3	0	0	3.48×10^4
0.311	0	0	4.5×10^4
0.312	3.47×10^{-3}	8.92×10^{-4}	8.28×10^4
0.32	4.68×10^{-2}	1.23×10^{-2}	2.35×10^5
0.33	9.86×10^{-2}	2.72×10^{-2}	3.39×10^5
0.35	0.157	4.72×10^{-2}	4.31×10^5
0.37	0.174	5.63×10^{-2}	4.49×10^5
0.4	0.161	5.76×10^{-2}	4.17×10^5
0.45	9.21×10^{-2}	3.82×10^{-2}	2.62×10^5
0.47	5.68×10^{-2}	2.48×10^{-2}	1.74×10^5
0.499	1.95×10^{-3}	9.27×10^{-4}	2.45×10^4

Table 4. The number of enzyme particles in contact with SP and reacting enzyme particles as a function of the fraction of SP in a PIE; two-component system, two parallel clusters, volume $100 \times 100 \times 100$

g	$N_c/10^6$	$N_r/10^6$
1×10^{-4}	0	0
1×10^{-3}	0	0
0.01	2.1×10^{-5}	2.33×10^{-6}
0.1	2.38×10^{-3}	2.97×10^{-4}
0.2	6.8×10^{-3}	1.05×10^{-3}
0.3	1.29×10^{-2}	2.78×10^{-3}
0.32	5.28×10^{-2}	1.35×10^{-2}
0.33	0.105	2.87×10^{-2}
0.35	0.16	4.78×10^{-2}
0.37	0.176	5.65×10^{-2}
0.4	0.162	5.76×10^{-2}
0.45	9.21×10^{-2}	3.82×10^{-2}
0.47	5.68×10^{-2}	2.48×10^{-2}
0.499	1.95×10^{-3}	9.29×10^{-4}

withstanding: formally, terms N_s in (3) tend to zero at $s < 6$. Regrettably, at large g , the overall number of enzymes and the current generated by them tend to zero.

As follows from curve 1 in Fig. 4b, with the exception of a small region near a point of breakdown, practically every enzyme in a single-component PE is in contact with electron-conducting SP: $N_c/N_e \cong 1$ in the interval $0.5 < g \leq 1.0$. However, as follows from curve 2 in Fig. 4b, not every enzyme in contact with electron-conducting SP is capable of taking part in the electrochemical reaction, under the assumptions we adopted in the foregoing (see relationship (3)).

Let us also note that we can evaluate the maximum possible electrochemical activity of a PIE, I , under the conditions where a certain type of fixation of the enzyme on a SP is required. The evaluation can be done with the aid of a simple formula on the basis of the data presented in Fig. 4 and in the fourth column of Table 2, provided the contribution of each particular enzyme to the generation of current i is known. Specifically, we can assume that

$$I = iN_r. \quad (5)$$

Note that the current computed with the aid of formula (5) gives maximum of what we can hope for when dealing with PIE. For formula (5) fails to take into account a multitude of possible constraints that may take place (see general symbolic formula (1)) in the course of functioning of systems that complex. Possible are

outer- and inner-diffusion limitations, ohmic (electronic, ionic) losses, etc.

TWO CONDUCTING CLUSTERS

In the foregoing we considered only conditions that ensured enzymes' contact with SP (with an electron cluster) supplied with electrons. However, in accordance with formula (1), to successfully conduct an electrochemical process in a PIE, it might become necessary to guarantee also a continuous supply (or removal) of other participants in the electrochemical reaction to the enzymes. Let us examine one more simplest case, say, a two-component system that is a mixture of SP with particles that facilitate a continuous penetration of one more participant of the electrochemical reaction into the region of electrochemical conversions. In principle, SP could be mixed with particles of a solid polymer electrolyte, agglomerates of polytetrafluoroethylene particles, and so on. We will conditionally call the second, besides the SP, component "component two" (CT).

Consider now a SP with an enzyme immobilized on it, which is present somewhere at an arbitrary point of a PE. This SP must be connected through continuous "paths" both with current leads (via trains of SP) and with the source of the other (besides electrons) participant of the electrochemical process (via trains of CT). The source is localized on one of the PE faces. Under these conditions, for a full-fledged functioning of a PIE,

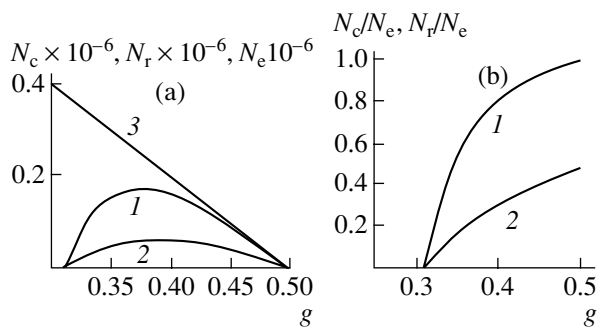


Fig. 5. Same as in Fig. 4, for a two-component PIE of volume $100 \times 100 \times 100$; see text for explanations.

in addition to the electron cluster, one more macroscopic cluster must be present in the electrode bulk.

As before, we assume that the principal parameter of a model PIE is the concentration of SP in a unit volume, i.e. parameter g . To simplify our analysis, we also assume that the concentration of CT (we denote it as g^*) was equal to g . That is why the maximum number of permissible enzymes that are capable of positioning themselves in a two-component PE is

$$N_e = N^3(1 - 2g) = 10^6(1 - 2g), \quad (6)$$

and now parameter g may vary only in the interval $0 \leq g \leq 0.5$.

There exist two possibilities for supplying a cluster of SP by electrons and a cluster of particles of CT by some substance (ions, gas, etc.). In one, both clusters are supplied and “grow” from one of the PE sides. This is the case of what we call “parallel” clusters. In the other, both clusters are supplied and “grow” from the opposite sides of PE. This is the case of what we call “collision” clusters. The results of modeling these two cases on a computer and a calculation of percolation characteristics appear in Tables 3 and 4.

We begin our analysis of calculation results with the case of collision clusters. The second column of Table 3 lists the fraction of enzymes that contact simultaneously two clusters. The third column represents the fraction of reacting enzymes. When computing this quantity we were once again guided by formula (3). The fourth column is the surface area, S^{**} , of SP that conduct electrons and is open for enzymes. The quantity S^{**} must be smaller than the quantity S determined previously in Table 1 for a single-component system. The point is that, with CT present in a PIE, some of the faces of the electron cluster that were previously open for enzymes now can be in contact with particles of component two and is no longer available for adsorbing enzymes. Apparently, it is irrelevant for quantity S^{**} how the supply of clusters occurs: from one side or from both sides. That is why Table 4 contains no fourth column. The way quantity S^{**} depends on parameter g is illustrated by curve 2 in Fig. 3. We see that the presence of CT considerably reduces the number of

enzymes capable of positioning themselves on the electron cluster.

We continue with our analysis of a two-component system by considering the case of collision clusters. With increasing g , these “grow” and “reach out” for each other from the opposite sides of PE. As a result, at small values of parameter g , the principal “body” of clusters is still concentrated next to the relevant sides. Consequently, the number of enzymes, which could have been in simultaneous contact with two clusters, rigorously equals zero. That is precisely what the data in Table 3 demonstrate (zeros in the second and third columns). And only at $g = 0.312$ the clusters become capable for the first time to touch one another, and quantities N_c and N_r become other than zero.

Note also that, as follows from the data of Table 1, an electron cluster in a single-component PE reaches the rear side of the electrode at a larger value of parameter g , specifically, at $g = 0.317$ (it is only here that the quantity n in the last column becomes other than zero). The difference between values $g = 0.312$ in Table 3 and $g = 0.317$ in Table 1 is explained by the fact that an electron cluster in a single-component PE must work its way through a path that is twice that it must overcome in a two-component PE with collision clusters, which are on the whole absolutely symmetrical.

We are coming now to the case of a parallel cluster. Consider the data we present in Table 4. Here both clusters grow on the same side of PIE. As we see, with the exception of a very narrow range of values of parameter g , at which isolated, accidental particles of two sorts “sit” on the PIE surface and still have no chance to simultaneously touch the enzyme (we still believe that all the particles, which make PE, and the enzyme have an identical shape and size), values of quantities N_c and N_r presented in Table 4, as opposed to those in Table 3, rather rapidly turn other than zero.

Interestingly enough, in the region of large values of parameter g (starting with $g = 0.4$), values of quantities N_c and N_r presented in Tables 3 and 4 become practically identical (apart from small fluctuations that emerge if clusters of different realizations are calculated). In the transition region of values of parameter g ($0 \leq g \leq 0.4$), values of quantities N_c and N_r for the case of parallel clusters marginally exceed those for the case of collision clusters.

The data compiled in Tables 3 and 4 give us a chance to construct curves that show how quantities N_c and N_r depend on parameter g (Fig. 5). Curve 1 shows the fraction of enzymes that take part in the electrochemical process no matter in what manner they contact the SP. Curve 2 shows the fraction of enzymes that take part in the electrochemical process under the condition that a certain type of fixation of the enzymes on a SP (according to relationship (3)) is required. Curve 3 is the maximum possible fraction of enzymes that can be contained in a PE.

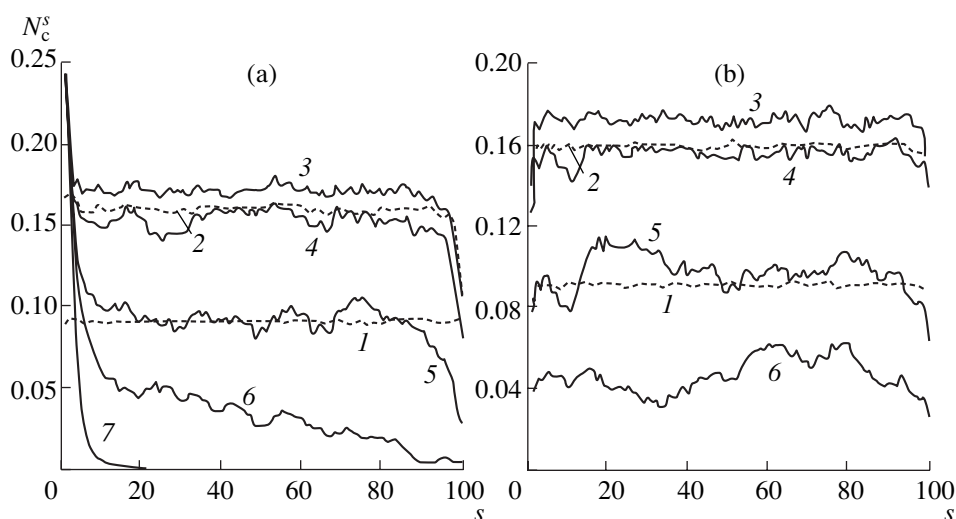


Fig. 6. The distribution of the density of enzymes that simultaneously make contact with two clusters of conducting particles, over the PIE thickness; see text for explanations.

Figure 5a shows quantities N_c and N_f normalized to the maximum possible number of particles in the PE bulk, i.e. to $100 \times 100 \times 100 = 10^6$. Figure 5b presents the same quantities normalized to the number of enzymes permissible for a given value of parameter g . As we see (here it is useful to compare Figs. 4a and 4b, i.e. single-component PE with two-component PE), at $g = 0.5$, almost every enzyme is capable of making contact with two clusters (curve 1, Fig. 5b) and only approximately half the enzymes are capable of taking part in the electrochemical reaction under the condition that a certain type of fixation of the enzymes on a SP is required.

The difference between the cases of parallel and collision clusters becomes still more pronounced when we examine the distribution of enzymes, which make simultaneous contact with two clusters of conducting particles of two sorts, over the PE thickness. The shape of these distributions is clear from Fig. 6. In Fig. 6a, the two clusters are supplied from one of the PE sides (parallel clusters). In Fig. 6b, the two clusters are supplied from the opposite sides of PE (collision clusters). The curves that appear in Fig. 6 were calculated for the following values of parameter g : (1) 0.45, (2) 0.4, (3) 0.37, (4) 0.35, (5) 0.33, (6) 0.32, and (7) 0.29.

In Fig. 6 we see assemblies of fluctuating curves. This is connected with the fact that even in the neighboring layers both the number of enzymes and the number of particles of two sorts that make a PE may heavily fluctuate. The distribution of the density of N_c^s over the thickness and the amplitude of fluctuations discernibly level out at large values of parameter g (dashed curves 1 and 2). Obviously, by summing values of N_c^s over all the layers we will obtain the quantity N_c .

Comparing Figs. 6a and 6b we comprehend that the curves in these two figures behave differently. In the case of parallel clusters (Fig. 6a), even in the pre-breakdown range of values of parameter g there is a chance for the existence of enzymes that are capable of making contact with both clusters (curve 7). No such “tails” exist in the case of collision clusters (Fig. 6b). Here, all the distributions of quantity N_c^s in the pre-breakdown range of values of parameter g vanish. Besides, in the case of collision clusters, all the distributions of quantity N_c^s slightly decay near the front and rear surfaces of PE. In the case of parallel clusters, a rather considerable decay of curves for N_c^s occurs only near the rear side (at large values of parameter s), while near the front surface there is a narrow region of increased values of N_c^s . However, on the whole, in their rather extended central portions, curves for N_c^s in the cases of parallel and collision clusters by absolute values happen to be close to each other. And the larger the quantity g the more considerable this similarity (dashed curves 1 and 2 in Figs. 6a and 6b).

CONCLUSIONS

The general conclusion that follows from this exploration is: with increasing number of components that make a PIE the number of enzymes that take part in the electrochemical process and, consequently, the overall current densities substantially decrease. A comparison of Figs. 4a and 5a shows that, upon going from a single-component PE to a two-component system, the currents drop by approximately 2.5 times.

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