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Computer Simulation of the Structure of Porous Electrodes with an Immobilized Enzyme and Nanosized Support Particles

Yu. G. Chirkov[a,z] and V. I. Rostokin[b]

[a] *Frumkin Institute of Electrochemistry, Russian Academy of Sciences, Leninskii pr. 31, Moscow, 119071 Russia*
[b] *Moscow Institute of Engineering Physics, Kashirskoe sh. 31, Moscow, 117409 Russia*

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Abstract—The efficiency of the operation of a porous electrode with an immobilized enzyme is defined, in particular, by a lucky structure of its active layer, which can contain nanosized particles of the support. The composites of such a kind are prepared with the aid of methods of colloidal chemistry. The aim of this particular investigation is to perform a computer simulation of processes of coagulation of particles of the support and their possible heterocoagulation with molecules of the enzyme. Algorithms of the formation of nanocomposite structures in solution are suggested. Calculations show that the concentration of the enzyme molecules in the nanocomposite structures cannot exceed a certain critical value. On the other hand, at a fixed value of the concentration of the enzyme molecules, the concentration of the support particles must not fall below a certain threshold quantity, which provides for the passing of current through the active layer. In order for all the enzyme molecules, rather than for a fraction of these, in the composite to take part in the process of bioelectrocatalysis, the concentration of support particles must be increased even higher, to an optimum value.

Key words: porous electrode, immobilized enzyme, nanoparticles, fractals, percolation, coagulation, heterocoagulation, computer simulation, cluster–cluster aggregation

FORMULATION OF THE PROBLEM

The central problem in porous electrodes with an immobilized enzyme and nanosized particles of the support is the creation of an active layer, which is a composite comprising the support particles and enzyme molecules. For a composite to function efficiently, a connected macroscopic-size structure comprising support particles must be formed in the composite i.e. there must emerge a percolation cluster [1], which is labeled here an electron cluster [2]. This connected macroscopic-size structure connects the front and the rear surfaces of the porous electrode. The task of this structure is to conduct current and supply electrons to the enzyme molecules. It is obvious that only with the emergence of an electron cluster upon reaching a certain minimum concentration (percolation threshold) of the support particles in the composite the electrochemical activity of a porous electrode with an immobilized enzyme becomes other than zero. And now all the enzyme molecules that enter the composition of the composite happen to be divided into two classes. One class comprises molecules that are intimately linked to an electron cluster, having been supplied with electrons, these molecules are capable of realizing bioelectrocatalysis. We had labeled such enzyme molecules "active" [2]. The other class consists of the rest of the enzyme molecules; these are not intimately linked to an electron cluster, not supplied with electrons, and exhibit no electrochemical activity.

In porous electrodes of fuel cells the setting of a function of electrocatalysis (bioelectrocatalysis—special case) apart from a function of transport of electrons required for performing an electrochemical process occurred not right away. In the first generation of hydrogen–oxygen fuel cells [3] (in what follows we will bear in mind only hydrogen–oxygen systems) a porous metallic matrix (it was prepared from skeleton catalysts) performed two functions simultaneously: it was both an electrocatalyst and a carrier of electrons. In so doing, any locus of such a matrix could have taken part in electrocatalysis. The porous electrodes for the second generation of fuel cells were prepared by mixing and subsequent caking of agglomerates of particles of a catalyst (usually it was platinum black) and agglomerates of particles of a hydrophobizing agent (polytetrafluoroethylene). The mechanism of the action of hydrophobized porous electrodes was dismantled for example in [4]. In contradistinction to porous electrodes functioning in fuel cells of the first generation, now the entire amount of platinum black could not have been provided for with electrons, only its connected portion that formed an electron cluster could. However, just as before, functions of electrocatalyst and conduc-

[z] Corresponding author, e-mail: olgayuri@mtu-net.ru

tor of electrons were blended in one material, which was the platinum black.

In porous electrodes for the third generation of fuel cells a solid polymer electrolyte was used (usually, Nafion). Such systems could have been three-component (electrolyzers of water [5]) and consisted of a mixture of particles of a catalyst (usually, platinum black), particles of a solid polymer electrolyte, and particles of a hydrophobizing agent (usually, polytetrafluoroethylene, it facilitated the penetration into an active layer of a gas reactant), but could also have consisted of four components, provided particles of platinum black were precipitated on particles of an electroconducting support that consisted of agglomerates of carbonaceous particles (see for example work [6]). Here there already took place a partial setting-apart of functions: the platinum black chiefly played the role of a catalyst and the connected system of grains of the support provided for the supply of electrons. The mechanism of the functioning of porous electrodes in fuel cells of the third generation has not been established to the end yet. We studied the problem of optimization of weights of platinum in such systems in [7].

The porous electrodes with an immobilized enzyme and nanosized particles of the support can be conditionally labeled porous electrodes of the fourth generation. Here there was made the ultimate step in the direction of the setting-apart of functions. The enzyme molecules, as opposed to platinum black (the third generation of porous electrodes), do not conduct any current, for their main assignment is to ensure the realization of bioelectrocatalysis. And as far as the supply of electrons toward the enzyme molecules is concerned, this is the task of the support particles.

At present the porous electrodes with an immobilized enzyme and nanosized particles of the support are prepared by means of methods of colloidal chemistry [8]. The enzyme molecules and the support particles are deployed in a solution, thoroughly mixed, and left alone for a time. Then an aliquot of the mixture is applied onto a support and dried up. Thus forms a composite, from which the active layer of an electrode is then prepared. All the properties of the composite thus produced are defined by the selection of the enzyme and the support, as well as by processes of coagulation and heterocoagulation proceeding in the solution.

There is a large number of experimental works devoted to studying aggregates of colloidal particles in solutions. Obtained were aggregates of finely divided particles of iron, gold, hematite, silica, poly(styrene), and carbon. The coagulation processes have been developed theoretically well enough (theory of DLFO [9–12]). Established was that aggregates of colloids with a loose and branched structure were fractal clusters [13]. The groundwork of our notions concerning these objects in the last two decades happened to be possible to obtain largely with the aid of computer methods [14]. Processes of coagulation have been studied well enough, far lesser attention has been paid to studies of processes of heterocoagulation [15] and coagulation in solution of particles of various sols (in our cases these are the support particles and the enzyme molecules). The creation of a theory for these phenomena had been begun by B.V. Deryagin [16–18] but even today the number of works devoted to computer simulation of heterocoagulation of particles of two sorts is very small (see for example publications [19, 20]).

The goal of this research is to perform computer simulation of processes of formation in solutions containing mixtures of nanoparticles of fractal structures. Further on we will assume that there takes place not only coagulation between themselves of the support particles that are smaller than the enzyme molecules, the gathering of these into fractal clusters, but also, in parallel, there may also proceed heterocoagulation of these particles. There may also proceed coagulation of the enzyme molecules, it is known that molecules of enzymes-proteins are prone to coagulation.

The research we have undertaken has not only a theoretical value but a practical value as well. A composite fractal structure that forms out of nanoparticles of two sorts must possess contradictory properties. In order to obtain a large current, it is desirable that the concentration of the enzyme molecules in a composite be as large as possible. However, an increase in the concentration of the enzyme molecules is obviously restricted from above, there must exist a certain maximum concentration of the enzyme molecules c_f^*. For, with increasing concentration c_f, sooner or later there must come a moment when the concentration of the support particles, which, obviously, must drop with increasing concentration of the enzyme molecules, having diminished to a certain critical value c_b^*, would shed properties of connectedness (will stop being an electron cluster). There and then all the enzyme molecules will not be active any longer (will not be provided for by electrons), for an electric current will not be capable of passing through the composite. But, even at any fixed concentration of the enzyme molecules that is smaller than a critical value, at $c_f < c_f^*$ it is necessary to also be capable of finding optimum values for two mutually related parameters, which are the concentration of the enzyme molecules c_f and the concentration of the support particles c_b. The determination of these quantities by an experimental means is a process that is arduous and can be attained not directly but indirectly, through the facilitation of something different, the optimumness of parameters c_f and c_b will probably be possible to judge upon only from the magnitude of current generated in a porous electrode. Computer simulation, on the other hand, not only permits a sufficiently fast obtaining of results but also makes the process of the obtaining of the results transparent and descriptive, accessible for a detailed analysis.

ALGORITHMS FOR THE FORMATION OF NANOCOMPOSITE STRUCTURES

When performing computer simulation of composites for the porous electrodes with an immobilized enzyme and nanosized particles of the support, we will, for simplicity and descriptiveness, investigate not three-dimensional but two-dimensional objects. Fractal clusters consisting of the particles of a support and the molecules of an enzyme will be constructed in the following manner. There exist a large number of computer models for gathering particles into random fractal clusters [21]. One of the expedients resorted to most frequently (it was suggested in [22]) is the diffusion limited aggregation with (hereafter abbreviated to DLA). The essence of this procedure is as follows. The first step consists of deploying the center of a would-be random fractal cluster on a plane, at one of the points of a square lattice. Then, an inoculation particle is positioned at this center. Then, a particle that possesses the form of a square of conditional size 1 by 1 is released out of the perimeter of a very large circumference whose center is located in an inoculation cell. The particle in question travels in a random fashion (emulation of Brownian movement, each time the particle can shift to one of the four neighboring points) until it reaches the perimeter of the inoculation cell and docks it (an act of coagulation). Then a second particle is released, the process is repeated, but now the fresh particle has a chance to settle on an agglomerate comprising two particles. Thus emerges a cluster of now three particles. The procedure is then repeated again and again until a random cluster of large dimensions forms (grows).

The DLA procedure apart, other computer algorithms are also used when investigating fractal structures. The first here was a trick known as the Eden model [23–25]. In this model, a curved trajectory of the movement of a colloidal particle (Brownian movement) is replaced by movement along a straight line (that is why this type of models is also called "ballistic"). Also known is a model of a reaction-limited cluster aggregation (RLCA) [26, 27]. Its distinguishing feature is that particles, when bumping into one another in solution, do not glue to one another with a probability equal to unity, but rather with some low probability specified beforehand. What this means is that in, say, a DLA procedure, a particle that touched a flat cluster does not become copulate with it right away, rather it is still capable of gliding along its perimeter for a time.

The DLA procedure was used by us in [28, 29]. An attempt was undertaken to supply a theoretical explanation to experimental facts that were obtained in [30], where a study of an oxygen electrode was performed. The electrode was based on a nanocomposite material consisting of a disperse carbonaceous support (in particular, taken was finely divided colloidal graphite with particles of diameter d_b = 3–4 nm) and a laccase. Molecules of this enzyme, when wound up into a ball in solution, have diameter d_f = 5 nm. The DLA procedure was used by us in [28, 29] because concentrations of two components in solution were very small. Estimates showed that particles of finely divided colloidal graphite occupied a one hundredth part of the solution volume, and laccase molecules occupied an even smaller portion, specifically, 2×10^{-4} of the solution volume. That is why it was reasonable to assume that graphite particles, in view of their numerousness mainly had enough time to coagulate with one another, gathering in random fractal clusters. And only thereafter, laccase molecules attached themselves to such clusters by the same DLA method.

In this work we will assume that the concentration of the support particles and the enzyme molecules in solution may be not small. This means is that the probabilities of coagulation of the support particles and heterocoagulation of the support particles with the enzyme molecules may be commensurate. These two processes are realized simultaneously. It seems to us that it would more natural, this time, not to employ the DLA procedure for computer simulation. From the weaponry of computer simulation models we selected yet another approach, which is called a model of cluster–cluster aggregation (CCA), aggregation cluster–cluster [31, 32]. The essence of this approach is as follows. In the course of coagulation and heterocoagulation, individual particles in solution unite themselves first into clusters of small dimensions and then, out of these, by means of unification of these small clusters, there emerge clusters of even larger dimensions, and, in the long run, all the particles in solution gather themselves into a unified cluster of large dimensions.

And now we will embark on the rendering of an algorithm for the formation of fractal structures. The support particles will be presented in the form of identical square cells whose sides are set equal to a conditional unit of length. The flat model electrode we are going to investigate will have the form of a square. The area of the square, which is defined by the formula

$$S = L \times L \tag{1}$$

will, at the same time, define the maximum number of the support particles $n_{b,\,max}$, which may be deployed on the electrode surface. At a specified value of the density of the support particles ρ_b (the share of the surface of the model electrode per the support), the number of the support particles in the electrode is described by the formula

$$n_b = \rho_b \times L^2. \tag{2}$$

In so doing, all the support particles will be deployed over the surface of the model electrode in a random fashion. To continue, we will assume that the enzyme molecules are larger than the support particles and look like square cells with a rib length equal to h_f conditional units. In the case where the enzyme molecules take part in the formation of fractal structures, we will select the length L of a rib of the model electrode in such a manner that an integer number of the enzyme molecules

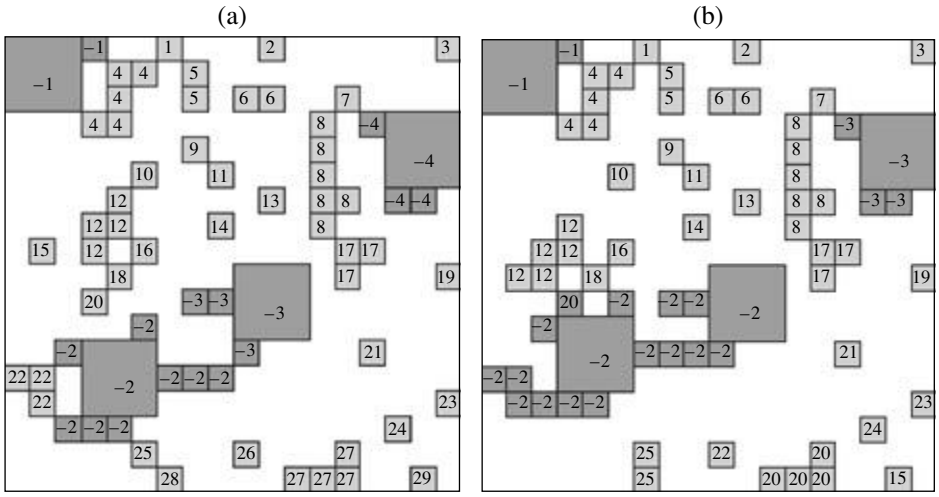

Fig. 1. To an explanation of specific features that are characterizing the process of the formation of a nanosized active layer in a porous electrode with an immobilized enzyme out of 65 particles of a support and 4 molecules of the enzyme. The support particles are marked with light gray color, their size is 1 by 1, clusters of these particles are enumerated with the aid of positive numbers. The enzyme molecules are marked with dark gray color, their size is 3 by 3, together with the support particles that are in contact with them, they are enumerated with the aid of negative numbers: (a) the beginning of the process of coagulation and heterocoagulation; (b) the very moment when two out of four enzyme molecules joined to form a unified electron cluster. The rest of the explanations can be found in the text of the paper.

should arrange themselves in the area occupied by the electrode. As a result,

$$L = h_f \times N. \quad (3)$$

Here, N is an arbitrary whole number. Thus, the model electrode is broken into N by N square cells, each having an area that is equal to $h_f \times h_f$. The maximum possible number of the enzyme molecules $n_{f,\max}$ is, obviously, described by the formula

$$n_{f,\max} = N \times N, \quad (4)$$

and the number n_f of the enzyme molecules that are scattered over the electrode surface in a random fashion at a given value of the enzyme density ρ_f (the share of the surface of the model electrode per the enzyme molecules) is described by the formula

$$n_f = \rho_f \times N^2. \quad (5)$$

At first we will scatter the enzyme molecules over an area, and only thereafter we will scatter the support particles in the places that remained unoccupied. The algorithm of our subsequent actions we will explain by resorting to a particular example. Let us consider Fig. 1. Over an area of size $S = 18 \times 18$ ($L = 18$, $n_{b,\max} = 18 \times 18 = 324$) there are scattered the enzyme molecules of size $(h_f)^2 = 3 \times 3$ ($n_{f,\max} = N^2 = 6 \times 6 = 36$), their number is $n_f = 4$ ($\rho_f = 4/36 = 1/9$), and 65 particles of the support ($\rho_b = 65/324 \cong 0.2$). Now we will renumber the support particles with positive numbers and, separately, we will renumber the enzyme molecules with negative numbers, in order to subsequently distinguish these from the support particles. The support particles can gather themselves in clusters that would comprise 2, 3, and so on particles. Two particles of the support enter a cluster if they have a common side. All the support particles that enter a cluster receive the same number (we view an individual cell as a cluster, too). The support particles that are in touch with an enzyme molecule are given the negative number that belongs to this molecule. The meaning of this operation will be explained later in more detail.

The smaller the size of a particle that experiences the Brownian movement, the greater its speed [33]. Our models take into account this circumstance as well. In the final analysis we have in Fig. 1a four clusters consisting of the enzyme molecules, together with the support particles that settled on their perimeter, these are painted by dark gray color, and 29 (instead of 65 particles initially randomly scattered over an area) clusters that are formed by the support particles, these are painted by light gray color. All the clusters we have just enumerated are characterized by their weight. The weight of the enzyme cluster numbered "–1" is equal to $3 \times 3 + 1 = 10$, the weight of the enzyme cluster numbered "–2" is equal to $3 \times 3 + 8 = 17$, and so on. The cluster consisting of the support particles, which has number "8," has weight 6; cluster "6," weight 2; cluster (particle) "29," weight 1; and so on.

When emulating chaotic movement of clusters, we will take into account the fact that the movement speed depends on the weight. Calculated is the weight $M_{\max}$ of the bulkiest. This cluster will be granted permission to make a single step only, specifically, it will be allowed to shift as a unified wholeness by one cell upward, downward, to the left, or to the right, under the condition that this movement will not be restricted in any manner. Then, for a particle of the weight m, the num-

ber of allowable steps, which are successively performed in random directions (emulation of Brownian movement), will be determined by the relationship

$$N_{step} = M_{max}/m. \quad (6)$$

The process of the movement of particles and the mechanism of the gradual formation of a "colloidal" fractal cluster out of the enzyme particles and particles of the support is analyzed in the following sequence. Out of 29 + 4 = 33 clusters depicted in Fig. 1a we select in a random fashion some cluster. Suppose that the selected cluster is a cluster comprising the support particles with number 6, it has a chance to make one step in any direction. Then its weight is calculated, in our example, $m = 2$, and the number of steps permitted to this cluster is determine with the aid of formula (6). As M_{max} in Fig. 1a is equal to 17 (the molecule of the enzyme whose number is "–2" and with the support particles that are adjacent to it), the cluster "6" may make maximally 17/2 = 8.5 or, if we take the whole portion of the ratio, 8 steps. If, for example, cluster "6" makes its first step to the left, it will blend with cluster "5" to form a fresh cluster whose weight is equal to 4. And this will be the end of this cycle of movements of cluster "6." All the cells in cluster "6" will be bestowed with number "5," while the numbers of all clusters, beginning with cluster "7" (a single particle of the support), will diminish their numbers by unity, and now a mere 28 clusters comprising the support particles will be present in the area. On the other hand, if cluster "6" shifts by one cell downward (Fig. 1a), it will be capable of going on with its movements, until exhausting the limit (eight allowable steps).

Whatever, before each next step made by a cluster selected randomly, a random direction (up, down, left, right) of supposed movement by one cell is determined. Thereafter there is determined the possibility of such a shift by means of looking through cells bordering with the cluster. If the cluster comes butts against a "wall" (borders of our model electrode), then we undertake another attempt to displace the cluster in other directions. If no hindrances occur, all the cells of the cluster as a whole are displaced by one step in a selected direction. After making each step, we check the possibility of "collision" between the cluster on the move and other clusters. Should such a collision occur, the movement of this cluster interrupts. The collision may result in two occurrences.

Occurrence one: if the particle that was performing the movement is a cluster that consisted of the support particles, then, upon getting in touch with some other cluster consisting of the support particles, it will attach to itself this cluster. On the other hand, if the contact occurred with an enzyme cluster, it joins it. In so doing, the cluster consisting of the support particles, which is being attached, is excluded from the relevant family, and the overall number of the family of clusters diminishes by unity.

Occurrence two: if the particle that was performing the movement is an enzyme cluster, then, in the course of a collision, it will attach to itself all clusters, which consist of the support particles, or clusters, which consist of the enzyme molecules together with the support particles connected with the enzyme molecules, which it encountered on its way. In so doing, the enzyme cluster being attached is excluded from its family, and the number of the family of enzyme clusters diminishes by unity.

In Fig. 1b we present the moment of unification of two enzyme clusters after their "collision" (touch). Cluster "–3" (see Fig. 1a) joined cluster "–2," after which they receive common number "–2," and under mark "–3" now is meant the enzyme cluster that before this carried mark "–4." From Figs. 1a and 1b we can also notice that the moment of collision of enzyme clusters was preceded by the unification of some clusters of the support, the result of which appeared to be a diminishment of the overall number of clusters consisting of the support particles from 29 (Fig. 1a) to 25 (Fig. 1b). Upon attaining the situation we portrayed in Fig. 1b, there is calculated a new value for M_{max} (now $M_{max} = 33$) and then, out of all the 3 + 25 = 28 clusters of two sorts, there is again selected, in a random way, some cluster or another, and a calculation is subsequently performed of the maximally possible number of steps-dislocations allotted to it with regard to weight, and the process is led further on, in accordance with the scheme prescribed in the foregoing. The entire procedure of the unification of clusters is continued until there forms a unified fractal cluster, in which all the enzyme molecules are chained between themselves by links comprising the support particles or exist in an intimate contact with one another.

THE RESULTS OF COMPUTER-AIDED CALCULATIONS

The final products of model calculations will be rendered in two stages. In the first stage, we will explore the coagulation of the support particles. No enzyme will be present in solution. Figure 2 depicts a flat analogue of a "solution" of the support particles. The dimensions of an area are 42 by 42. This is enough to accommodate maximum 1764 particles. We will set the concentration of the support particles $c_b = 0.1$. These 176 particles are scattered over the area in a random fashion (Fig. 2a). In some places the particles are already from the very beginning gathered in small clusters containing two–three particles. As a result of the Brownian movement and the aggregation of clusters in accordance with the algorithms that were formulated in the previous section, all the particles gather themselves in one fractal cluster. This fractal cluster is presented in Fig. 2b.

Let us now perform an analysis of how an increase in the concentration of the support particles in the course of the process of coagulation alters the character

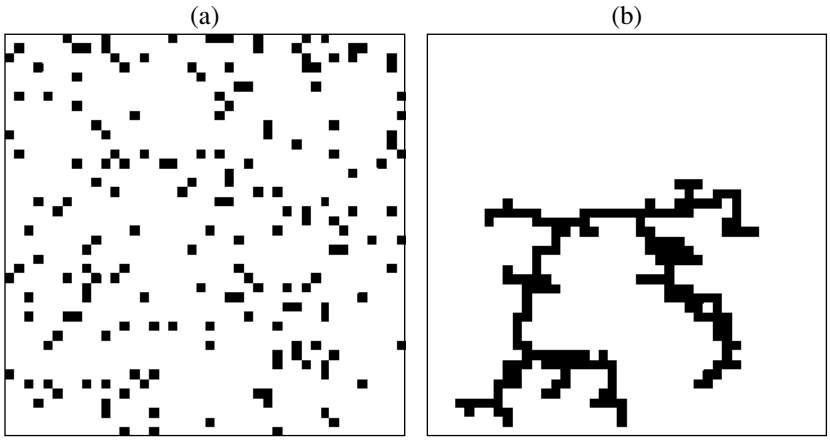

Fig. 2. (a) Initial (particles are scattered over the surface in a random fashion) and (b) final (particles gathered to form a random fractal cluster) steps of the process of coagulation of 176 particles of the support that were scattered in a random fashion over an area of size 42 by 42. The concentration of the support particles (the share of the surface occupied by the support particles) is equal to 0.1. The maximum possible number of particles that are capable of occupying the area is equal to 1764.

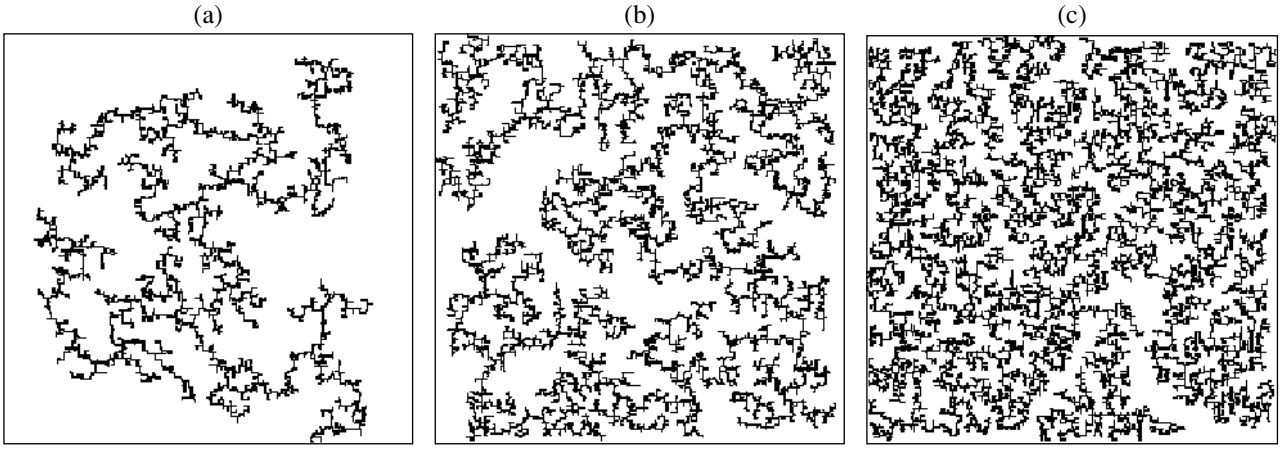

Fig. 3. Terminal stages in the process of coagulation of the support particles that were scattered in a random fashion over an area of size 198 by 198. Particles gather themselves in random fractal structures. The surface concentration of the support particles: (a) 0.1 (cluster consists of 3920 particles), (b) 0.2 (cluster consists of 7841 particles), and (c) 0.3 (cluster consists of 11761 particles). The maximum possible number of particles that are capable of occupying the area is equal to 39 204.

of the aggregation of particles and tells on the shape of the arising finite cluster. In Fig. 3 clusters were "engendered" on an area with the dimensions 198 by 198. Here we can deploy 39204 particles. We see that, with increasing the concentration of the support particles c_b from 0.1 (3920 randomly scattered particles, Fig. 3a) to 0.2 (7841 particles, Fig. 3b) to 0.3 (11761 particles, Fig. 3c), the random fractal cluster gradually converts into gel [34].

Let us also note the following. Even without performing special numerical evaluations (the technique for a calculation of an effective electroconductance for fractal structures is rendered in [35]) it can be deduced that the effective electroconductance of fractal clusters presented in Fig. 3 is small despite a sufficiently large concentration of conducting elements. Therefore, probably, with the aid of the modeling of the aggregation of the support particles into random fractal clusters with the aid of a CCA model also can be given an explanation to the "fractal–percolation effect" discovered in experiments of work [30] and quantitatively unraveled in [28] when performing the modeling of the aggregation of the support particles in accordance with the DLA procedure.

The data we presented in Fig. 3 allow us to draw yet one more conclusion. To create an electrochemically active structure out of the enzyme molecules and the support particles we can utilize two techniques. In the first version, at first, by one technique or another, we will form a finely divided support and then, by one means or another, we will supply the enzyme molecules to its internal surface via a porous space. Figure 3 shows how not easy it would have been to supply large molecules of the enzyme (their dimensions can consid-

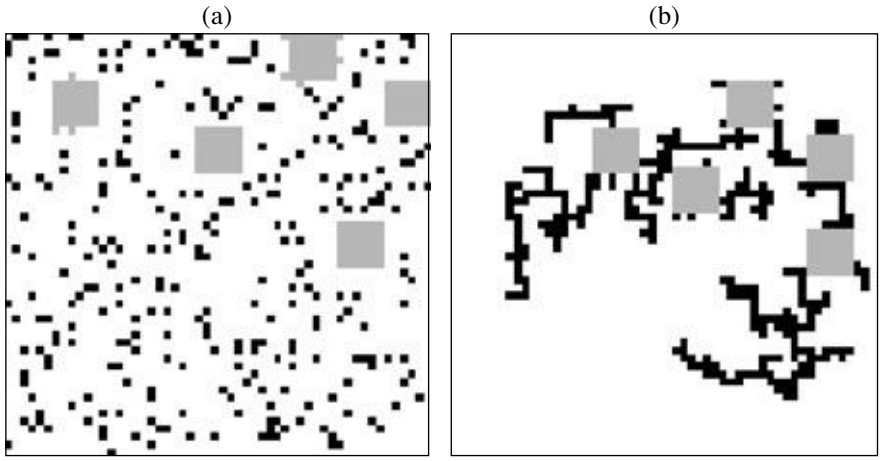

Fig. 4. (a) Initial and (b) terminal stages in the process of coagulation and heterocoagulation of 5 molecules of the enzyme and 291 particles of the support, which are scattered in a random fashion over an area of size 54 by 54. The concentration of the enzyme molecules is equal to 0.06 and the concentration of the support particles is equal to 0.1. Conditional dimensions of the enzyme molecules are equal to 6 by 6 and those of the support particles are equal to 1 by 1. The maximum possible number of the enzyme molecules that are capable of occupying the area in question is equal to $9 \times 9 = 81$, and the maximum possible number of the support particles is equal to 2916.

erably exceed the dimensions of the support particles) via pores that are narrow, tortuous, of a complicated geometry, and corrugated, how difficult it would have been to uniformly distribute the enzyme molecules over the support. It would be much simpler to make use of the second technique for the construction of an active layer, trying to achieve the unification of the enzyme molecules with the support particles with the aid of processes of coagulation and heterocoagulation in solution. It is the results of a modeling of such a process that we will now pass to.

In Fig. 4 we present a model electrode of size 54 by 54. The maximum number of the enzyme molecules (each of the enzyme molecules possesses dimensions 6 by 6) we can deploy in an area is equal to 9 times 9 makes 81, together with 54 times 54 makes 2916 support particles. The values of the concentration of components that will be selected by us are as follows: for the enzyme $c_f = 0.06$ (5 molecules) and for the support $c_b = 0.1$ (291 particles). Figure 4a presents the initial state of the system and Fig. 4b, the final state of the system. While gathering into a unified fractal cluster, do their position alter not only tiny particles of the support (these are marked with black color, molecules of the enzyme–with gray) but also the larger molecules (by area–by 36 times) of the enzyme. As we distinguish from Fig. 4a, some individual particles of the support an initial time instant may, when scattered in a random fashion, find themselves at the perimeter of individual molecules of the enzyme. It was our intention to specifically mark these particles with a gray color, in order to underline that a complex that comprises an enzyme molecule and the support particles that are in touch with it possesses a greater weight than does the enzyme molecule itself and, as a result, must move now somewhat slower. The unhurriedness of the transposition of the enzyme molecules is manifested also in that ton configuration of the enzyme molecules in the initial and final states (Fig. 4) approximately persists, whereas the support particles carbon monoxide very strongly withdraw themselves from their initial location.

We will now pass to discussing results that now possess a fundamental value. In Fig. 5, in an area of macroscopic dimensions 102 by 102, we distributed, in a random fashion, a considerable number of the enzyme molecules with the concentration $c_f = 0.1$ (29 molecules, whereas their maximum number is 17 times 17 makes 289). The enzyme molecules may well be descried in Fig. 5a: in this figure these are marked off with dark gray color (squares of conditional dimensions 6 by 6). In order for these molecules to turn active, it is necessary to lead electrons to them. We will vary the number of electron-conducting particles of the support: their concentration c_b in Fig. 5a is equal to 0.3475 (3615 particles), in Fig. 5b it is equal to 0.528 (5493 particles), and in Fig. 5c the concentration of electron-conducting particles of the support is equal to 0.58 (6034 particles). The maximum possible number of the support particles that are capable of settling in the area is equal to 10 404. Having gathered at these concentrations of the support particles into a unified bifractal cluster, a fraction of the support particles may form an electron cluster as well, which is capable of tying the top and bottom boundaries of the area (these boundaries conditionally emulate the front and rear surfaces of the active layer). This is the sole case where an electric current will be capable of flowing through our model active layer and it is only in this case that some fraction of the enzyme molecules in the electrode or other pass into an active state and will be capable of taking participation in an electrochemical process.

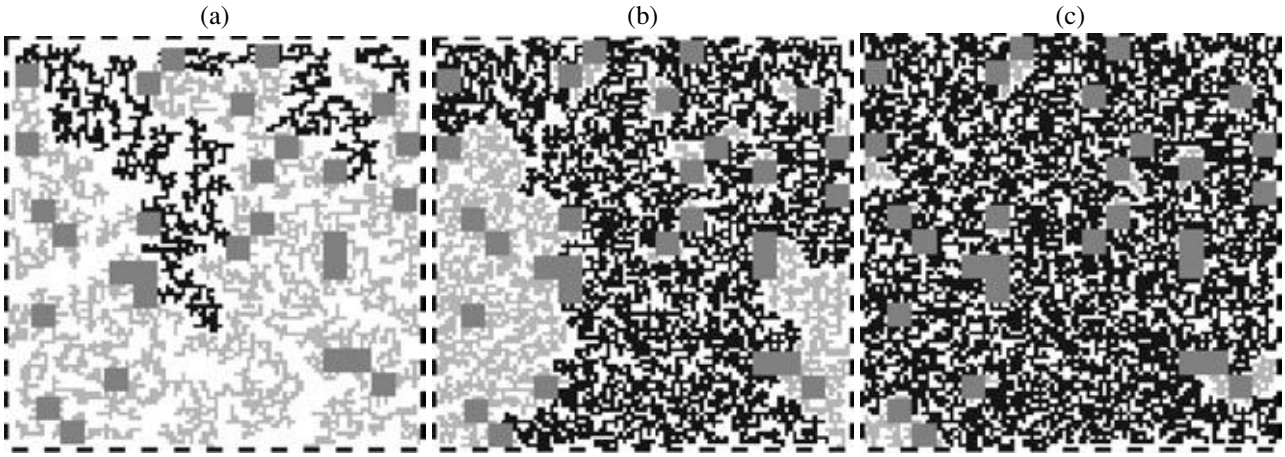

Fig. 5. The major stages in the formation, on an area of size 102 by 102, of a fractal cluster that comprises nanoparticles of two sorts, specifically, molecules of an enzyme of conditional dimensions 6 by 6 and particles of a support of conditional dimensions 1 by 1. The concentration of the enzyme molecules, these are marked with dark gray color, is equal to 0.1 (the overall number of molecules is equal to 29) and the concentration of the support particles, these are marked with light gray color is as follows: (a) 0.3475 (3615 particles), (b) 0.528 (5493 particles), and (c) 0.58 (6034 particles). The number of support particles that are bringing electrons to the enzymes is as follows: (a) 0 (percolation threshold is not reached yet), (b) 3978, and (c) 5834. The number of active molecules of the enzymes is as follows: (a) 0, (b) 23, and (c) 29. The maximum possible number of the enzyme molecules that are capable of occupying the area in question is equal to $17 \times 17 = 289$, and the maximum possible number of the support particles is equal to 10 404. The rest of the explanations can be found in the text of the paper.

Figure 5a demonstrates that the concentration c_f of the support particles in a composite at a fixed value of the concentration c_f of the enzyme molecules ($c_b = 0.1$) must exceed a certain threshold value. Whatever the case, Fig. 5a demonstrates that the concentration of the support particles must be higher than $c_b = 0.3475$. Because the clustered "electron tongues" (the overall number of particles making them up is equal to 1057, while the overall number of the support particles scattered over the area is equal to 3615), which are marked with black color in Fig. 5a and which are tied to the upper surface of the area (conditional source of electrons), do not yet stretch enough to reach the opposite side of our model electrode. Thus, no "electron cluster" has been realized in the area yet and no current flows through the area and, as a consequence, all the 29 enzyme molecules that are present in our model electrode cannot function and the amount of active enzyme is rigorously equal to zero.

And now we will consider Fig. 5b. The concentration of the support particles increased to the quantity $c_b = 0.528$. This time, we clearly distinguish the presence of an electron cluster in the area (the support particles that are conducting current are marked with black color and their overall number is equal to 3978; the insulated, not conducting current, particles of the support are marked with gray color and their overall number is equal to 1515). The number of the enzyme molecules that are in contact with an electron cluster and converted into active enzyme molecules in the area is now greater, and, if we express ourselves more specifically, this number is equal to 23 out of 29. But in order to attain full operation of all the enzyme molecules in the area, it is necessary to even grater increase the concentration of the support particles. Figure 5c demonstrates that, at the concentration of the support particles $c_b = 0.58$, the situation is such that now all the 29 enzyme molecules came in contact with an electron cluster and passed into the category of active molecules of the enzyme. And now it is already virtually all the support particles in the area that take participation in the conducting of the current, and the number of particles of the support that remained outside this process is equal to a meager 200.

The results of calculations we have just enumerated have a practical value. A computer simulation gives one a chance to determine, at a given concentration of the enzyme molecules in solution, the values of the concentration of the support particles that are capable of providing for a partial (upon reaching a percolation threshold, a critical value of the concentration of the support particles c_b^*, Fig. 5b) or full functioning of the enzyme molecules in the active layer (attainment of a second critical concentration for the support particles c_b^{**}, Fig. 5c). When the amount of the support in solution is varied, the effective specific electroconductivity of the composite varies as well, as does its effective specific ionic conductivity. That is why, when performing a calculation of the electrochemical activity and other characteristics of the porous electrodes with an immobilized enzyme and nanosized particles of the support, it is necessary to perform the selection of optimum concentrations of both the enzyme molecules c_f and the support particles c_b even more finer than we have just done (see Fig. 5).

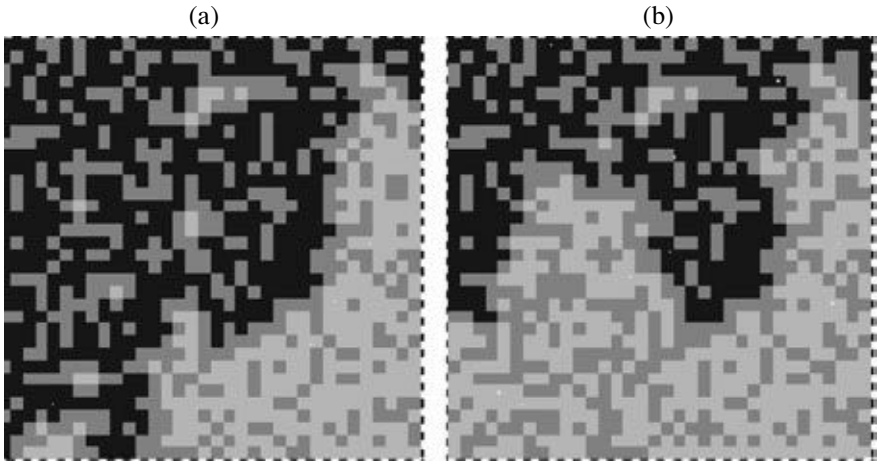

Fig. 6. To an estimation of the magnitude of the maximum possible concentration of molecules of an enzyme in the active layer of a porous electrode with an immobilized enzyme. The size of the model electrode is 204 by 204. The enzyme molecules are marked with gray color, the rest of the cells are fully filled with particles of the support, these are marked with light gray color and their fraction that is tied to the top side of the area a mixture of a percolation technique is marked with black color. The concentration of the enzyme molecules is equal to (a) 0.36 and (b) 0.38. The concentration of the support particles is equal to (a) 0.64 and (b) 0.62. The rest of the explanations can be found in the text of the paper.

And eventually we turn our attention to yet another circumstance of importance. When manufacturing a nanocomposite, the concentration of the enzyme molecules in solution cannot be selected in a random fashion, that is quite understandable. It is obvious that at $c_f = 1$ there will be no space left for the electron-supplying particles of the support. To approximately estimate the upper boundary for c_f and establish the critical quantity c_f^* would be possible when being guided by simple considerations. Let us gradually increase the concentration c_f while covering to an extent of the entire 100 percent with the support particles all the space in the selected area that remained unoccupied by the enzyme molecules. Let the dimensions of this area we selected be 204 by 204 (it is shown in Fig. 6). In this manner we each time provide for maximally salubrious conditions for the flowing of a current through the area. The enzyme molecules in Fig. 6 are marked with gray color and the support particles are marked with light gray color. And we perceive, in Fig. 6a, that at $c_f = 0.36$, respectively, at $c_b = 1 - c_f = 0.64$, there is an electron-conducting electron cluster in the area (the support particles that enter it are marked with black color), which ties the opposite sides of our model electrode. Therefore, a certain fraction of the enzyme molecules present in the area may generate current. But, at a further increase in the concentration of the enzyme molecules, suppose, at $c_f = 0.38$ and at, respectively, the fraction of the support particles reduced to $c_b = 0.62$, the electron cluster "splits" (Fig. 6b) and is no longer capable to "reach" the bottom boundary of the model electrode. What this means is that, now, there is not a single molecule of the enzyme in our model active layer that would be capable of generating current. Thus, we have a value for the critical concentration of the enzyme c_f^*, which is approximately equal to 0.37.

The magnitude of c_f^* is likely to increase upon going from two-dimensional structures to real, three-dimensional, structures. Because an electron cluster in this case forms at much smaller concentrations of the support particles (the percolation threshold in a simple cubic lattice of points in the absence of molecules of an enzyme is reached at value of c_b as small as 0.31 [1]).

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