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Porous Electrodes with Immobilized Enzymes: The Problem of Development of Nanocomposites with High Concentrations of Molecules of Active Enzymes

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 currents that are generated in a porous electrode with an immobilized enzyme increase with increasing concentration of molecules of an electrochemically active enzyme. However, a finely divided composite, which is manufactured from colloidal particles of a support that have nanodimensions and molecules of the enzyme with the aid of methods of colloid chemistry, has a peculiar structure: it consists of a set of fractal clusters, which are capable of adsorbing only a limited number of enzyme molecules. The paper is devoted to computer simulation of all the stages of immobilization of the enzyme, specifically, producing random fractal clusters of required dimensions and deploying molecules of the enzyme on them. An analysis of the link of the concentration of molecules of an active enzyme with the structure and characteristics of a porous composite makes it possible to give an interpretation to experimental facts obtained by other authors for an oxygen electrode consisting of finely divided colloidal graphite and laccase.

Key words: porous electrodes, immobilized enzyme, nanocomposite materials, finely divided colloidal graphite, laccase, coagulation and heterocoagulation, computer simulation, fractal clusters, diffusion limited aggregation

AN INTRODUCTION TO THE PROBLEM

In this work we continue the investigations that were begun by the authors of [1]. The study we have cited was concerned with parameters of the active layer (AL) of an oxygen electrode, which had been manufactured on the basis of a nanocomposite material comprising a finely divided carbonaceous support (of interest to us in what follows will be the finely divided colloidal graphite, FDCG) and an enzyme (laccase). It had been assumed that the tiny particles of FDCG (of diameter $d_s \sim 3$–4 nm) in the solution would "stick" around larger molecules (of diameter $d_e \sim 5$ nm) of the enzyme and convert them into immobilized molecules and there would be reached an ideal structure of AL and would emerge a finely divided support with active molecules of the immobilized enzyme "disseminated" in it, which would supply electrons to molecules of the enzyme. It had been expected that, in these conditions, it would be possible to manufacture an electrode that would have high discharge characteristics. However, the currents observed in actual experiments happened to be not high enough. It could have been explained, in particular, by that the bulk concentration of immobilized molecules of the enzyme happened to be not high enough.

Let us perform estimation of the concentration of molecules of laccase in the solution and in the composite. Out of 130 pmol of laccase, which were deployed on each milligram of FDCG, only 25 pmol (1.5×10^{13} molecules) underwent adsorption, while the rest of the molecules were desorbed into the bulk solution. Consequently, according to conditions of experiment, the initial concentration of laccase in 100 μl (0.1 cm^3) of solution c_e^* happened to be equal to $2\ mg \times 1.5 \times 10^{13}$ molecules $mg^{-1}/0.1\ cm^3 = 3 \times 10^{14}$ molecules/cm^{-3}. As the composite that forms in the solution can occupy only a portion of the initial volume (not 0.1 cm^3, but smaller), we find it difficult to determine more correctly the concentration of molecules of immobilized laccase in the volume occupied by the composite. However, we deem it possible to claim, with a certain degree of definiteness, that the initial concentration of laccase was very low. The maximum number of molecules that can be deployed in a unit volume of space is equal to $1/d_e^3$ molecules (in the case of a cubic placement of molecules of laccase it is presumed that every molecule of laccase is rolled into a ball-globule). As a result, for molecules of laccase it is possible to reach the density $c_e = 8 \times 10^{18}$ molecules/cm^3. This value is greater than

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

the value 3×10^{14} molecules/cm^3 by more than four orders of magnitude.

We will make one more estimation, it will be of use to us later on. Specifically, we will determine the number of particles of FDCG that are contained in 1 mg of this substance. We will presume that all the particles of graphite are spherical, with the average diameter d_s = 3.5 nm. Then the sought-for number of particles of FDCG $c_s = 10^{-3}/(\pi\rho d_s^3/6)$. The carbon density is ρ = 2 g/cm^3. Thus, 1 mg of FDCG contains $10^{-3}/[3.14 \times 2 \times (3.5 \times 10^{-7})^3/6] = 2.23 \times 10^{15}$ particles/cm^3. That is why for each molecule of laccase in the composite there were $2.23 \times 10^{15}/1.5 \times 10^{13} = 149$ particles of FDCG.

The problem of the development of porous electrodes that would possess high discharge characteristics for fuel cells has had a sufficiently long history. Further on, for definiteness sake we will speak only of hydrogen–oxygen fuel cells. The electrochemical activity I, A/cm^2 of an electrode, obviously, must be proportional to the magnitude of the current that is generated in a unit of volume of the active layer $\hat{\imath}$, A/cm^3. In porous electrodes of the first and second generations (hydrophilic biporous electrodes with a blocking layer and hydrophobized porous electrodes [2]), the magnitude of $\hat{\imath}$ could have been estimated with the aid of the formula

$$\hat{\imath} = jS, \tag{1}$$

where S, cm^2/cm^3 is the specific surface area of the catalyst/solution of electrolyte interface, it is inversely proportional to the radius of particles (or of their grains) r, out of which the catalyst is manufactured

$$S \sim 1/r, \tag{2}$$

and j, A/cm^2 is the density of the current, which is generated on such a surface.

The development of the specific surface area and the transition to smaller particles (grains) invariably gave positive result. In porous electrodes of the first-generation fuel cells (hydrophilic, with a blocking layer) there were used catalyst grains with $r \sim 1$ μm and the currents were $I \sim 0.1$ A/cm^2 at the electrode thickness $\Delta \sim 1$ mm. In porous electrodes of the second-generation fuel cells (hydrophobized electrodes where the catalyst was mixed with the hydrophobizing agent, polytetrafluoroethylene), the grains of platinum black had the size $r \sim 0.1$ μm, in so doing, it proved possible to reach currents of the magnitude $I \sim 1$ A/cm^2 at the thickness $\Delta \sim 100$ μm. In the last two decades great expectations were linked with the third-generation fuel cells—with a solid polymer electrolyte (usually, it is Nafion) [3]. The porous electrodes in these cells are capable of generating the currents $I \sim 1$ A/cm^2 at the thickness of the active layer equal to a mere $\Delta \sim 10$ μm. The characteristics that high are the result of the transition, according to formulas (1) and (2), to yet smaller particles. The microscopic grains of platinum black, which have dimension r_c on the order of a few nanometers, are now deployed onto grains of the support (agglomerates of carbonaceous particles) with dimensions r_s on the order of a few tens of nanometers. Thus, the usual inequality

$$r_s \gg r_c, \tag{3}$$

is fulfilled here as well, which means that the support grains are much larger than the grains of the catalyst.

And now we are going to turn our attention to the object we are going to investigate, that is to fuel cells of, conditionally speaking, the fourth generation, i.e. to biofuel cells. As formulas (1) and (2) are inapplicable in this case, according to [4],

$$\hat{\imath} = JN_A, \tag{4}$$

where N_A, cm^{-3} is the concentration of molecules of an active enzyme of that portion of molecules of the enzyme to which the access of a gas reactant, ions, and, in the first place, electrons is ensured, i.e. of all that makes an enzyme molecule a participant of the electrochemical process, and J is the current that is generated by one molecule of the enzyme. The way one should perform calculation of concentration N_A, the difficulties that emerge here have comprehensively been discussed in a series of papers [4] where we examined multicomponent (with equidimensional particles) electrodes with enzyme laccase.

The necessity of employing formula (4) instead of formulas (1) and (2) when performing estimates of the electrochemical activity of porous electrodes upon going to biofuel cells is their first peculiarity. Another substantial distinction of porous electrodes with an immobilized enzyme—for these electrodes the notion of a macroscopic porous support, in which characteristic dimensions of pores discernibly exceeded the size of the catalyst particles and whose specific surface area it was desirable to develop by all means possible, may acquire another meaning. As the example of work [1] shows, particles of a support, which provide for the bringing of electrons towards molecules of an enzyme, as molecules of the enzyme, may have nanometer dimensions. Moreover, the case where (by the example of FDCG and laccase) criterion (3) is replaced by the inequality

$$r_s < r_e, \tag{5}$$

which means that the dimensions of molecules of the enzyme exceed the dimensions of particles of the carrier (support), it quite possible. That is why we can speak now not of a finely divided support (relationships (1) and (2)) with molecules of an enzyme adsorbed on its surface, but of some composite comprising particles of the support and molecules of the enzyme, which has a complicated internal structure.

Of course, for biofuel cells, too, it could probably have been possible to manufacture, out of nanosized particles, a macroscopic porous support, but then there would have emerged considerable difficulties with the insertion into its pores, and these must be small, of molecules of an enzyme and with their immobilization on

the internal side of such a support [4]. If, on the other hand, one would try and prepare a porous support out of large particles of the support, purposely attempting to create broad pores in it, thus making it easier to land molecules of an enzyme onto its internal surface, then, if criterion (3) is fulfilled, it would also be difficult to hope to reach high values of concentration N_A, because the major part of the volume of space occupied by the composite would be given to particles of the support.

FORMULATION OF THE PROBLEM

The third, of fundamental importance for this particular investigation, specific feature of porous electrodes with an immobilized enzyme is connected with the technique of their manufacture. An electrochemical active nanocomposite comprising particles of the support and molecules of an enzyme is obtained from colloidal solutions [5]. Processes of coagulation and heterocoagulation go on in these. Taking part in the Brownian movement, particles of two sorts join to form peculiar structures, which are random fractal clusters. It is precisely these random fractal clusters that define all the major properties of a would-be AL. For example, the presence or, on the other hand, the absence in it of an "electron cluster" [4], which is a system of macroscopic dimensions consisting of particles of the support. Such a random fractal cluster connects the front and the rear sides of AL of the electrode and provides for the passing of an electric current through the porous electrode. The presence of random fractal clusters serves also as the reason for the division of molecules of the enzyme in AL into active, which are adjacent to an electron cluster, and inactive, which are isolated from an electron cluster.

Processes of coagulation have been investigated well enough both theoretically and experimentally [6–9]. An enormous number of works, which have been performed by the present time, are devoted to investigation of aggregates of colloidal particles in the solutions. Obtained were aggregates of finely divided particles of iron [10], gold [11, 12], hematite [13, 14], silica [15], poly(styrene) [16], and carbon [17]. And yet, the basis of our notions on these objects proved to be possible to acquire in the last two decades mostly with the aid of computer-aided methods. It is precisely with the aid of these methods that it was established that all these aggregates of colloids, which have loose ramified structure, are in fact fractal clusters.

There exists extensive bibliography of works dealing with coagulation, but processes of heterocoagulation, which are met far more frequently in real practice (suspensions of sols of various substances, their mutual gluing), have been investigated so far not enough [18]. The development of theoretical notions concerning the process of heterocoagulation was begun in the works by B.V. Deryagin [8, 19, 20]. However, it is precisely with the heterocoagulation (particles of FDCG + molecules of laccase) that the authors of work [1] had to do. We are bound to add that only particles of FDCG in the solution represented a real colloid, while molecules of the enzyme, which is a high-molecular-weight substance, were a pseudocolloid, and here there additionally emerge many perplexing instances.

The problem of the obtaining, in colloidal solutions, of nanocomposites comprising particles of FDCG and molecules of laccase and having a perfect structure is very involved. Here, three processes go on in parallel, specifically, coagulation of lyophobic particles of the support, coagulation of molecules of the enzyme (it is known that, in aqueous solutions, molecules of proteins tend to coalesce into agglomerates), and heterocoagulation of particles of the support with molecules of the enzyme. At this juncture we find it difficult to judge upon which particular technologies will be proposed in order to reach a more intensive heterocoagulation of the FDCG particles and molecules of laccase in order to neutralize the natural tendency of molecules of an enzyme to glue together to form agglomerates (in this work we do not aim at giving any recommendations that would make it possible to raise the concentration of enzyme molecules in a composite). Luckily, the arsenal of means offered by colloid chemistry, which would permit to influence the coagulation processes, is great enough. One can alter the electrolyte concentration, resort to SAOS, and charge one sol positively and another negatively: then the oppositely charged particles would attract one another. A positive effect is promised here also by hydrophobization. Modern information on the structure of biomembranes shows that native enzymes in them form nonpolar and polar contacts lipid–protein. In view of this, the authors of [21] recommended carriers that were modified with monolayers of lipids.

Established was that adding a hydrophilic sol to a hydrophobic sol gives rise to a phenomenon called a "protective action" [22]. To reduce the free energy, it is profitable to a system to lower the overall hydrophobicity, which is achieved by that the hydrophilic particles gather around the hydrophobic particles. It is possible that was what was observed in [1]: molecules of laccase mixed in the solution with particles of carbon black AD 100. That must have been why the amount of laccase molecules strongly adsorbed on FDCG (hydrophilic material) happened to be 4.5 times as small as that adsorbed on acetylene black (partially hydrophobic material). However, we are aware of the existence of yet other facts, which would be capable of explaining the fact that the enzyme adsorption on electrodes of carbon black is practically irreversible [23]. A significant role in the laccase adsorption may be played by the existence, at the surface of carbon black, of functional groups of the quinoid series, which are analogues of natural substrates of laccase.

Processes of heterocoagulation of the support particles with molecules of the enzyme must also be very sensitive to mutual dimensions and shape of these par-

ticles and to properties of the protein-enzyme. Some information about the structure of laccase and examples of practical applications of this enzyme immobilized on a variety of supports can be found in [24, 25] and in review [26]. We are still lacking reliable knowledge of the nature of the forces connecting molecules of a protein-enzyme with particles of a support. There is still much to do in order to make processes of heterocoagulation more transparent, in order to create with more assurance nanocomposites comprising particles of a support and molecules of an enzyme and having an ideal structure. All this is the subject of some future investigations. As for now, the problem facing us is much simpler. We will try to use the means supplied by computer simulation in order to establish connection between the structure of a composite, whose carcass comprises fractal clusters of particles of the support, with the concentration of molecules of an enzyme immobilized on such clusters for the system FDCG/laccase (dimensions of particles of the support are smaller than dimensions of molecules of the enzyme) that was studied by the authors of [1] and to show that the concentration of molecules of the enzyme deployed on fractal clusters cannot be high and that a finely divided composite, which consists of colloidal particles of the support having nanodimensions, are capable of adsorbing only a limited number of molecules of the enzyme.

The following two circumstances will help manage this problem. One of these is the assumption that the FDCG particles, being particles that are smaller than molecules of laccase, are likely to more energetically participate in the Brownian movement. According to the formula of A. Einstein [27], the speed v of movement of a colloidal particle in the solution

$$v = 1.5[kT/(\pi\rho R^3)]^{1/2}, \quad (6)$$

where k is Boltzmann's constant, T is the absolute temperature, ρ is the density of the material of the particle, and R is its radius, decreases with increasing dimensions of particles. Besides, to our minds, the interaction of the solvent molecules with a long enzyme molecule, which is rolled up in a ball, more likely than not makes the latter perform elastic oscillations around a certain center, rather than move as a whole, as is the case with monolithic particles of FDCG.

The other circumstance is the noncommensurateness of concentration in the solution of the carbonaceous material and the enzyme. The concentration of FDCG in the solution was much higher than the concentration of laccase. According to [1], 1 cm^3 of solution contained 20 mg of the support and 3×10^{14} molecules of the enzyme. That is why the bulk concentration of particles of the finely divided colloidal graphite was 2×10^{-2} g/2 g cm^{-3}/1 cm^3 = 0.01. It is a trivial matter to try and estimate the bulk concentration of laccase as well, is equal to 3×10^{14} molecules $\times 4\pi/3 \times d_e^3$ /8 cm^3/molecule/1 cm^3. The diameter of a molecule of laccase d_e = 5 nm; therefore, finally we have for the concentration of laccase the value 2×10^{-5} molecules of the enzyme in cm^3. Thus, we will obtain that the concentration of the enzyme in the solution is three orders of magnitude as small as the concentration of the carbonaceous component.

The result of the performed estimations is of great importance. It seems reasonable enough to presume that molecules of the enzyme, in view of their exceedingly small concentration, did not react between one another, while on the other hand particles of FDCG, by virtue of their numerous amount and higher mobility, largely had enough time to react with each other, gathering in random fractal clusters. Then, molecules of the enzyme somehow attached themselves to this loose structure, which formed in the solution. That is why the major volume of space happened to be filled, after the aliquot was dried up, by porous agglomerates of the FDCG particles.

COMPUTER SIMULATION

The sequence of our actions in what follows will be as follows. In the first place it is necessary to construct a fractal cluster out of particles of the support and then to "land" molecules of the enzyme onto it. The principal characteristic of a fractal cluster is the fractal dimension D [28], it is by no means equal to $d = 2$ or $d = 3$, which are the dimensionalities of two-dimensional or three-dimensional Euclidean space. The fractal dimension is fractional and always satisfies the inequality $D < d$. The noncoincidence of D with d testifies to "defectness" of plane or volumetric structure of a fractal and to the degree of deviation of its porous structure from monolithic crystalline bodies. For a plane cluster of a fractal structure, the number of particles in it (its power) n_s is connected with the fractal radius R by means of the relationship

$$n_s = \xi(R/a)^D, \quad (7)$$

where a is the diameter of particles, and ξ is a proportionality coefficient (which is also called a prefactor). The rarefied nature (looseness) of a plane fractal cluster is caused by that $1 < D < 2$ in expression (7).

Let us pass to expounding on the specific features pertaining to computer simulation. For the sake of simplicity and vividness, we will investigate two-dimensional, rather than three-dimensional, objects. We will assume that an AL is broken into square cells of conditional size $L \times L$ and fractal clusters are deployed in these cells. There exist a large number of computer models for the growth of fractal clusters of lyophobic colloids [29]. All these models differ by four points. In the first place, they differ in the structural organization of space in which there occurs the growth of a fractal cluster: the movement of particles proceeds either via the points of the lattice or via the free space, without any limitations. The second difference is in the character of the movement of particles or clusters in the solution: this is either the Brownian movement or a linear

"ballistic" movement. The third point involves the type of aggregation of particles: here we have two versions, specifically, a version "particle–cluster," if the fractal grows at the expense of successive attachment of individual particles to the cluster, and a version "cluster–cluster," if there occurs the unification of ever increasing clusters. And finally, the fourth distinction is in the magnitude of the probability of the gluing ($0 \le p \le 1$) of colliding objects: there is possible a model of "kinetically controlled aggregation," in accordance with which particles or clusters, before undergoing the gluing, must experience a sufficiently large number of collisions, which is specified in the simulation by a small value of probability p.

Out of this arsenal of model we have selected the simplest and most frequently applied technique, which was proposed by the authors of [30], namely, diffusion limited aggregation (DLA). We will presume that the movement of particles proceeds via the points of a square lattice of points, that the gluing probability $p = 1$ (for two objects in the solution to join, suffice it that they touch one another only once), and that all the particles take part in the Brownian movement. Under these conditions the limiting stage of the process obviously becomes the diffusion transport of particles toward a cluster (hence the name of the model we have selected).

The essence of the DLA procedure consists of the following. The first step consists of the positioning the center of a future random fractal cluster, an inoculation particle is deployed here. Then, a particle is released out of the perimeter of a very large circumference with its center in the inoculation cell. The particle moves in a random fashion (emulation of the Brownian movement) until it reaches the perimeter of the inoculation cell and docks with it (act of coagulation). Then a second particle is released and the process is repeated, but this time this particle has a chance to somehow settle on an agglomerate formed by two particles. In this manner there emerges a cluster that contains now three particles. Such a procedure is then repeated many thousands of times, until there forms a random fractal cluster of large dimensions.

The next stage of the computer simulation we performed consisted of that, out of the initial fractal cluster of large dimensions, there were cut squares of a small size $L \times L$. This was one of the cells of the inoculation layer under investigation (the inoculation particle, naturally, is positioned at the center of the cell). Then molecules of the enzyme were deployed on the fractal cluster of the given dimensions $L \times L$. Every molecule of the enzyme was launched to the fractal cluster from a boundary whose points were removed away from the center of the fractal cluster to a distance that exceeded its size L twofold. The Brownian movement of molecules of the enzyme is easy to follow by the position of any cell out of those that make it up (every molecule is a square that consists of $n \times n$ cells where $n > 1$; when performing movement, this square may move by one point upward, downward, to the right, or to the left).

Before each next step of a molecule of the enzyme in a randomly selected direction, there was verified the presence of contact of the sides of the molecule with at least one cell out of those that make up a fractal cluster. If, on the other hand, a molecule of the enzyme approached any boundary that arrested its movement, it was elastically rebounded. Once an enzyme molecule touched a fractal cluster, it then slid over its surface. If, at some instant, the character of the contact between an enzyme molecule and a fractal cluster satisfied certain conditions, its movement was arrested and the position of the molecule was fixed (the points of a region that were occupied by the molecule became inaccessible for the movement of subsequent molecules of the enzyme) and it was transferred into the family of molecules of the immobilized enzyme. After fixing an enzyme molecule on the fractal cluster, a fresh molecule of the enzyme was released out of a point randomly selected on the square perimeter (out of the points of square $2L \times 2L$) that embraces the fractal cluster. The process of the search, by a molecule of the enzyme, of a place on the fractal cluster continued until this particular molecule of the enzyme, after traversing the entire perimeter, returned to the point of its first encounter with it. That meant that all the places that were accessible for the landing of the molecule of the enzyme had been exhausted.

The number of molecules of the enzyme, which in principle could be deployed additionally on a fractal cluster was determined in the following fashion. A wave is launched from the center of a fractal cluster along its branches, the wave conditionally altering the color of every particles of the support. The propagation of the wavefront occurs in accordance with Huygens' principle—every cell of the fractal, which altered its color when the wavefront was passing through it, turns a source of a secondary wave. Once the cell altered its color, the free vicinity adjacent to it is analyzed and the possibility of deploying molecules of the enzyme here is checked.

And finally, when solving the question of whether an immobilization act was accomplished upon contact of a molecule of the enzyme with a fractal cluster or not, we were guided by the following simple considerations. In the first place, we had assumed that the conditional dimensions of particles of the support were 1×1, while the dimensions of molecules of the enzyme must exceed the dimensions of particles of the support (they will have the dimensions 2×2, 3×3, and so forth). Secondly, it seemed only natural to presume that, in view of the weakness of the connecting forces between a molecule of the enzyme and particles of the support, following the contact, a molecule of the enzyme would "land" on a fractal cluster (an immobilization act would occur) only in the case where the protein-enzyme would have a sufficiently large area of

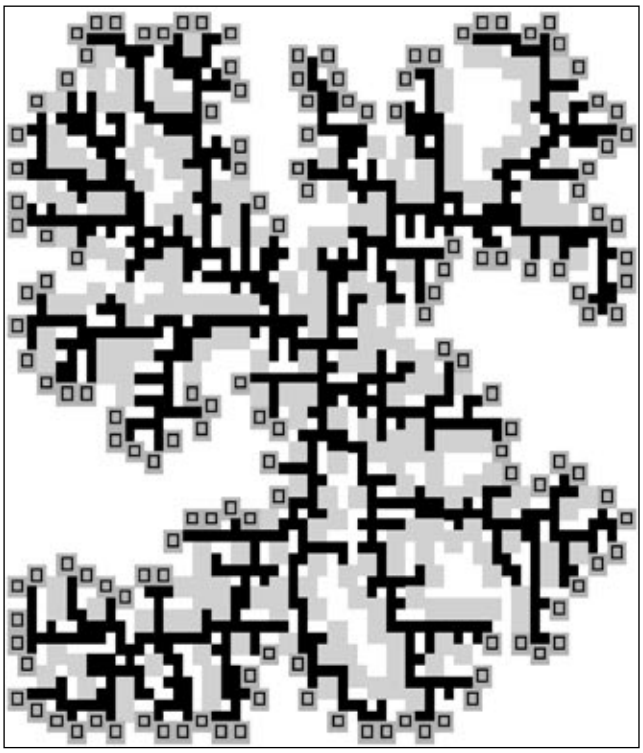

Fig. 1. The distribution of molecules of the enzyme (marked with gray squares with a black frame inside) in a random fractal cluster consisting of particles of the support (marked with black) of size 61×61. Gray color marks all the places at the cluster perimeter, which could, in principle, be filled by molecules of the enzyme. The number of particles of the support is equal to 908, the number of molecules of the enzyme immobilized on the cluster is equal to 146, the number of all the places at the cluster perimeter that are accessible to the landing of molecules of the enzyme is equal to 374. The cluster has the fractal dimension $D = 1.6$.

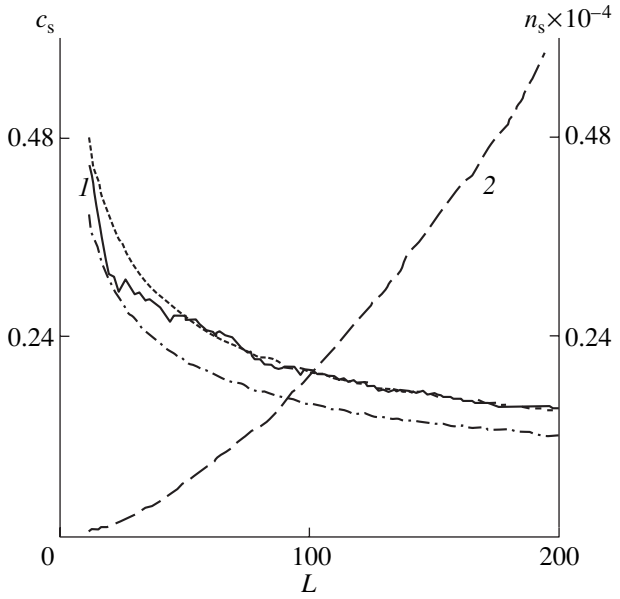

Fig. 2. Dependences of (*1*) the average density of particles of the support and (*2*) the number (power) of particles of the support on the dimension of a random fractal cluster consisting of particles of the support. Other explanations are in the text of the paper.

contact with the support particles that make up the fractal cluster. Thus, there are two parameters, specifically, the relative dimensions of molecules of the enzyme and the minimum area of contact that is required for a molecule of the enzyme to "land" on a random fractal cluster.

DISCUSSION OF THE RESULTS OF CALCULATIONS

In Fig. 1 we present, as an example, a random fractal cluster of size 61×61 with the fractal dimension $D = 1.6$. The particles of the support that are constituting the cluster are marked with black, each particle is a square of conditional dimensions 1×1, and the overall number of particles of the support in the cluster is equal to 908. Each molecule of the enzyme is also a square of conditional dimensions 2×2, and a molecule of the enzyme occupies an area that if four times larger than a particle of the support. In order to be capable of identifying the immobilized molecules of the enzyme, they are singled out with the aid of a black square frame. Altogether, 146 molecules of the enzyme are immobilized on the periphery of the fractal cluster. An analysis of their arrangement shows that the molecules that were the first to "land," frequently give no chance to other molecules of the enzyme to penetrate into the inner regions of the fractal cluster. That is why not all places that are present at the perimeter of the fractal cluster are accessible to molecules of the enzyme. All places, which are present at the perimeter of the fractal cluster and which in principle could have been occupied by molecules of the enzyme, are marked with gray color; the overall number of such places is equal to 374. This number is far smaller than the overall number of particles of the support in the fractal cluster, which is equal to 908, because molecules of the enzyme have larger dimensions than particles of the support.

Should we begin now varying dimensions of a fractal cluster, then, to estimate the average density of the number of particles of the support in the cluster, we would do it with the aid of formula (7), after substituting L in it for R/a. The average density of the number of particles of the support in the cluster, which was calculated with the aid of a computer, is equal to $c_s = n_s/L^2 = \xi L^{D-2}$, where n_s is the power of a cluster of size $L \times L$. This average density of the number of particles of the support in the cluster is presented in Fig. 2 by a broken solid line (curve *1*). The dot-and-dash line, which is lying below, represents a rough approximation of a c_s,L curve ($c_s \sim L^{D-2}$), and the exact approximation is given by the dashed line, which was obtained at the value $\xi = \exp 0.22$.

In the model that we have investigated, the power of a fractal cluster n_s grows faster than its dimension L. As is shown in Table 1, following an increase in L from 21 to 201, the number of particles of the support in the cluster rises from 128 to 6481. The complete form of an n_s,L dependence is represented by curve *2* in Fig. 2.

Table 1. Relations between the power of a random fractal cluster and its dimension

L	21	51	81	111	141	171	201
n_s	128	680	1359	2311	3476	4718	6481

We will now vary dimensions of molecules of the enzyme and the conditions, under which, when these are satisfied, an enzyme molecule, upon touching a fractal cluster, can be considered immobilized. In Figs. 3a and 3b, dimensions of molecules of the enzyme are 2×2, and in Figs. 3c and 3d, the dimensions are 3×3. Figures 3a and 3c assume that, for the immobilization to occur, it is necessary that one of the sides of a molecule of the enzyme should touch 2 (Fig. 3a) or 3 (Fig. 3c) particles of the support. If, on the other hand, as was the case in Fig. 1, a molecule of the enzyme is in contact with only one particle of the support, then such a contact must not lead to the fixation of the molecule of the enzyme on a fractal cluster. In contradistinction to the situation presented in Fig. 1, where, for the enzyme to become immobilized, it was enough for it to touch at least one of the particles of the support that enter the composition of the fractal cluster, Figs. 3b and 3d assume that, for the connection between a molecule of

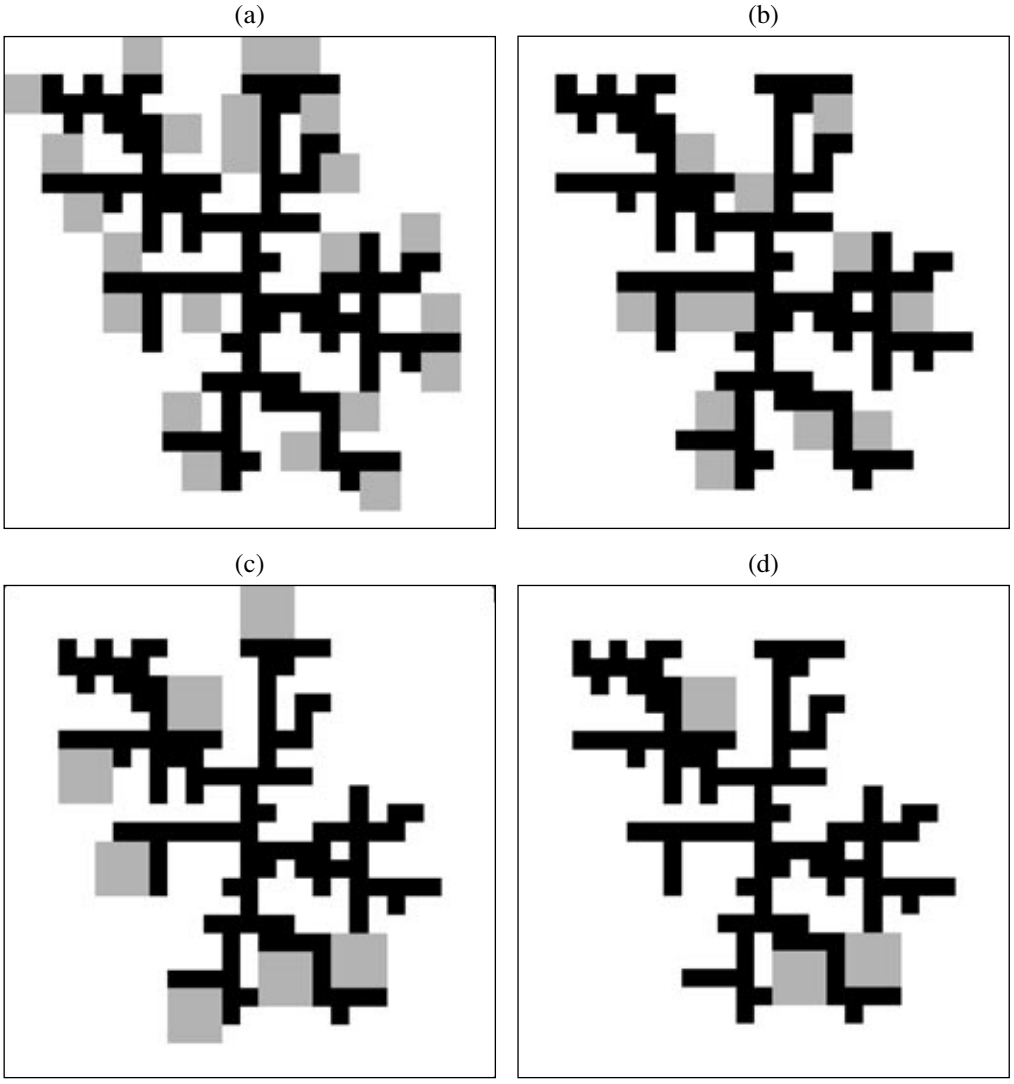

Fig. 3. Demonstration of how the number of immobilized molecules of the enzyme (these are marked with gray color) on a random fractal cluster consisting of particles of the support (of size 21×21, the cluster is marked with black color, it consists of particles of conditional size 1×1) varies as a function of the dimension of molecules of the enzyme ($p \times p$) and the conditions of their "fixation" to the perimeter of the cluster (version 1—by one side of a molecule of the enzyme, version 2—by two adjoining sides of a molecule of the enzyme): (a) $p = 2$, version 1; (b) $p = 2$, version 2; (c) $p = 3$, version 1; and (d) $p = 3$, version 2.

Table 2. Main characteristics of enzyme molecules immobilized on a random fractal cluster

L	m	n_e	n_e^*	c_e
21	1	23	31	0.052
	2	7	8	0.016
	3	3	4	6.8×10^{-3}
51	1	128	186	0.049
	2	41	63	0.016
	3	7	12	2.69×10^{-3}
81	1	255	387	0.039
	2	80	125	0.012
	3	16	26	2.44×10^{-3}
111	1	424	642	0.034
	2	136	205	0.011
	3	20	34	1.62×10^{-3}
141	1	650	963	0.033
	2	204	305	0.01
	3	32	49	1.61×10^{-3}
171	1	946	1333	0.032
	2	298	421	0.01
	3	42	65	1.44×10^{-3}
201	1	1296	1814	0.032
	2	402	560	9.95×10^{-3}
	3	66	98	1.63×10^{-3}

the enzyme and the fractal cluster to be strong, something more is necessary. In this case it is necessary that two out of the adjoining sides of a molecule of the enzyme should be in contact, by their entire surface with, correspondingly, 4 (Fig. 3b) or 6 (Fig. 3d) particles of the support. Comparing all Figs. 3 between one another, we see how on the same random fractal cluster of size 21×21, depending on the gradually stiffer conditions of the landing of molecules of the enzyme (their dimensions increase from 2×2 to 3×3 and the degree of the required connection between a molecule of the enzyme and particles of the support increases as well), the number of molecules of the enzyme that settled on the fractal cluster correspondingly drops. The overall number of immobilized molecules in Figs. 3a through 3d is equal to 23, 12, 7, and 3.

We will now select three versions out of the four versions we presented in Fig. 3, specifically, versions a, c, and d. In what follows these will appear under corresponding numbers m from 1 to 3. And, we will perform calculation of the dependence of major parameters, which characterize the process of immobilization of molecules of the enzyme, on the dimension of the fractal cluster L. The parameters in question are the number of immobilized molecules of the enzyme n_e; the number of places at the perimeter of the fractal cluster, which in principle could be occupied by molecules of the enzyme n_e^*; and the density of the number of immobilized molecules of the enzyme on the fractal cluster c_e. All these data are gathered in Table 2. Quantities n_e and c_e are presented more fully in Figs. 4 and 5.

We see in Fig. 4 that the number of immobilized molecules of the enzyme positioned on the fractal cluster rapidly diminishes with the conditions of the landing of molecules of the enzyme steadily becoming stiffer (an increase in their dimensions and the demand for a larger area of contact when getting in contact). At $L = 201$ the number of immobilized enzymes drops from 1296 to 66 (data of the 3rd column and lower rows in Table 2). Very revealing here are also numbers that are presented in the 4th column of Table 2—the number of places at the perimeter of the fractal cluster, which in principle could be filled by molecules of the enzyme. An increase in the dimensions of molecules of the enzyme (see Figs. 3a and 3c) leads to a decrease in the number of molecules of the immobilized enzyme by approximately three times, and the strengthening of the demands to an act of immobilization (see Figs. 3c and 3d) leads to a decrease in the number of molecules of the immobilized enzyme by approximately another five times. In view of this, the number of particles of the support per immobilized molecule of the enzyme, as Fig. 6 shows, increases to a hundred. In the 5th column of Table 2 and in Fig. 5 we present values of the density of the number of the immobilized molecules of the enzyme. With the stiffening of conditions of the landing of molecules of the enzyme onto the fractal cluster this quantity drops to a few thousandth.

In conclusion we will again focus our attention on the attempt at a more comprehensive explanation of the results that had been obtained by the authors of [1]. We have already stated in the foregoing that the bulk concentration of the FDCG particles in the solution had been small, specifically, 0.01. According to curve *1* in Fig. 2 this fact implies that the dimensions of the random fractal clusters that form in the solution must be large. We will conditionally assume that, in a two-dimensional analogue of phenomena that are observed in reality, the dimension of a fractal cluster $L = 201$. We will also adopt the hardest conditions for the landing of molecules of the enzyme, specifically, we will assume that $m = 3$. Then, the number of molecules of our conditional laccase, which is capable of settling on a fractal cluster, is very small (see for example, Fig. 3d). By the way, let us mention the following important fact. Possibly, the forces of connection of molecules of laccase with colloidal particles of graphite in a calculate are infinitesimal. And molecules of laccase happen to be connected with a fractal cluster by virtue of that they simple "got lost" in the "curls" of the fractal cluster: they happened in the same (two bottom molecules of the enzyme in Fig. 3d), where they got stuck virtually for evermore. Such a connection of an enzyme with a support can be called a "gel effect."

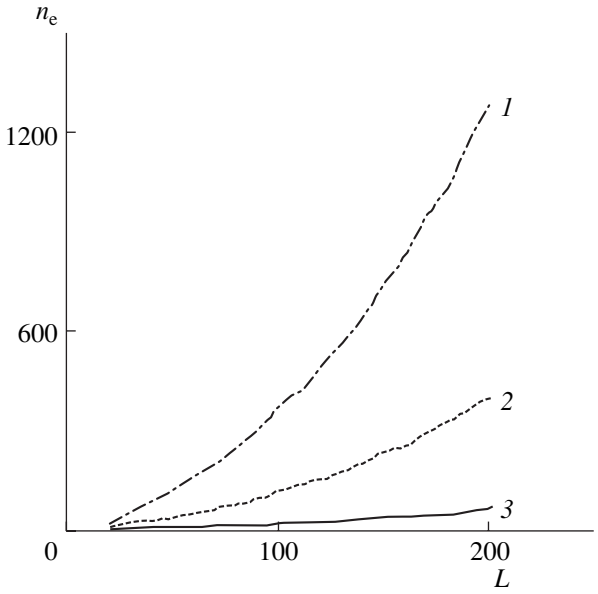

Fig. 4. Dependences of the number of molecules of the enzyme immobilized on a random fractal cluster consisting of particles of the support on the dimensions of the cluster. Versions of *m*: (*1*) 1, (*2*) 2, and (*3*) 3 (explanations in text).

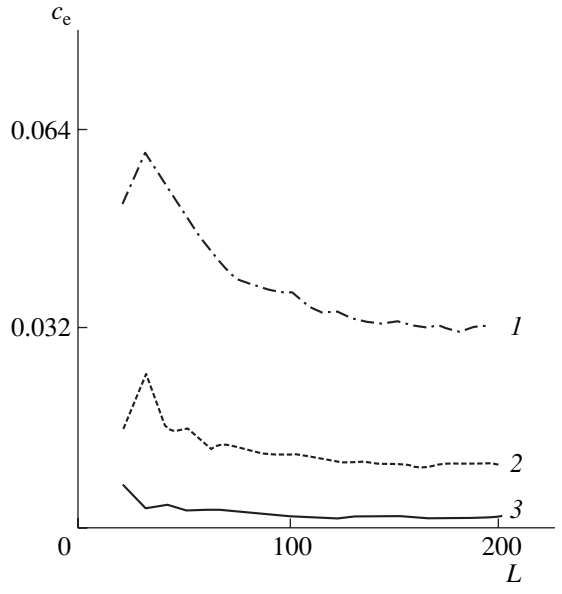

Fig. 5. Dependences of the density of the number of molecules of the enzyme immobilized on a random fractal cluster on the cluster dimensions. Versions of *m*: (*1*) 1, (*2*) 2, and (*3*) 3.

Thus, we will select parameters $L = 201$ and $m = 3$ and evaluate quantities that are most interesting for an interpretation of experimental data. The number of molecules of the enzyme deployed at a site of size $201 \times 201 = 40401$ is equal to 66 (Table 2); thus, the concentration of molecules of the enzyme is equal to $66/40401 = 0.0016$. In the case of maximum stacking, the number of molecules of the enzyme that are capable of settling at the same site is equal to $(201/3)^2 = 67 \times 67 = 4489$ and that is why the maximum concentration of molecules of the enzyme is equal to $4489/40401 = 0.11$. Consequently, the real concentration of molecules of the enzyme is significantly lower than the maximum concentration and their ratio is equal to $66/4489 = 0.015$. This conclusion is in good agreement with what had been observed in the experiments performed by the authors of [1], where the concentration of active molecules of laccase was a few orders of magnitude as small as the maximum possible concentration.

And now we will estimate the number of particles of the support corresponding to one immobilized molecule of the enzyme. As follows from the data we presented in Tables 1 and 2 and in curve *3* in Fig. 6, this ratio is equal to $6481/66 = 98$. The obtained is also in good agreement with the conclusion that (see estimations that were presented in the first section of this paper) for active molecule of laccase there were 149 particles of finely divided colloidal graphite in the experiments performed by the authors of [1].

When performing computer simulation, we restricted our consideration to plane models, but it could have been possible to extend the modeling approaches that we used to a three-dimensional case as well, although this would have required a more strenuous effort and a larger program running time. Unfortunately, we are still much in ignorance about the way the heterocoagulation of laccase molecules at the FDCG particles collected in random fractal clusters can pro-

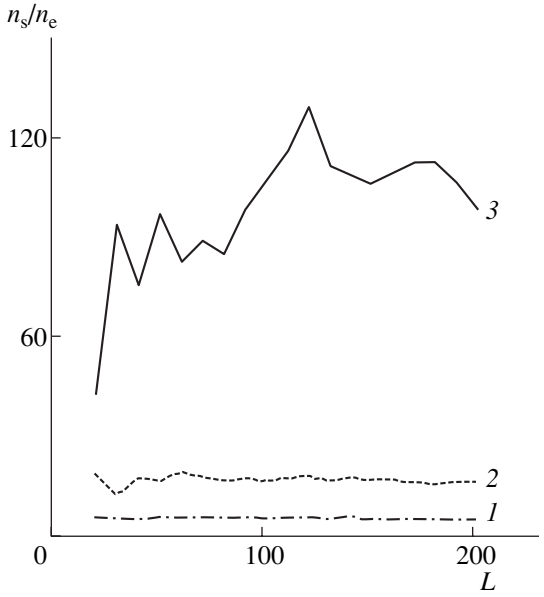

Fig. 6. Dependences of the number of particles of the support in a random fractal cluster per molecule of the enzyme immobilized on the cluster on the dimensions of a random fractal cluster. Versions of *m*: (*1*) 1, (*2*) 2, and (*3*) 3.

ceed in real conditions and the shape and dimensions of laccase molecules, as we are in the dark about many other things. That is why it was difficult for us to select, out of the wide collection of means of computer simulation, a model that would have really been adequate for describing the system under investigation (FDCG + laccase). And that is why we had decided at this juncture to restrict our consideration to studying a two-dimensional model.

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