V.I. Vernadskii Institute
of General and Inorganic Chemistry
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MEMBRANE AND SORPTION MATERIALS AND TECHNOLOGIES: PRESENT AND FUTURE

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CHAPTER 3

ADSORPTION MECHANISMS OF SELF-ORGANIZATION OF INORGANIC CARRIER – PREPARATION OF HETEROSTRUCTURES OF NATIVE ENZYMES

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Abstract: The influence of heterostructures (core-shell) formed by the native enzyme preparation extracted from black radish and white cabbage, immobilized on inorganic carriers with different surface nature on electrochemical properties of obtained materials is shown. Depending on the nature of support the mechanism for the binding of enzymes and their activity vary by the rate of activation. Based on the voltammetry of immobilized enzyme drugs on inorganic supports, the possibility of using these heterostructures as a basis for the devices for energy storage is shown.
Keywords: Native enzyme, immobilization, inorganic carrier, graphene, capacitor.

Introduction. Currently, the priority of electrochemical technology is the generation of energy from biomass [1-4]. Most studies are devoted to the creation of nanocomposite systems for the conversion of energy, components of which are electrically conductive "hierarchical" carbon materials, obtained by pyrolysis of natural raw materials [3-5]. Thus, currents of the order of 1 A/cm^2 of supercapacitor electrode based on products of white cabbage leaves pyrolysis [4], electrodes for lithium batteries based on rice husks [5] were obtained. The biofuel element [6], in which the cathode is zinc, and the anode is a laccase enzyme based on mushroom mildew is developed.

Enzymes, mainly peroxidase, are widely used in the biosensorics [7]. To enhance the biosensor signal, the adsorption of the enzyme to the quantum dot of metals, graphene, carbon nanotubes is used. In the biosensorics, the preparation of pure enzymes is needed. Inorganic materials, predominantly silicates, are investigated as carriers in a much smaller volume. Native enzyme preparations (extracts from plants) have not yet been investigated.

Experimental. We have studied a new class of materials – native enzyme drugs (NEDs), adsorbed on inorganic carriers with various acid-basic characteristics of the surface. As carriers, inorganic materials with a layered or gel structure were used, namely, aluminosilicates: bentonite and its acidic (phosphate) form, kaolin, and pyrogenic silica aerosol 300. NEDs were adsorbed from root extract of radish black and white cabbage leaves.

According to the basic idea of the work, adsorption and subsequent crosslinking of the inorganic component with the enzyme drug creates heterostructures capable of spatial distribution of charges, changes the mechanism of chemical and electrochemical reactions, and opens new areas of application of the obtained materials.

For the study of heterostructures in the system of inorganic carrier-NED the following physico-chemical and electrochemical methods were used: atomic absorption spectrophotometry (AAS), spectrophotometry, scanning electron microscopy (SEM), cyclic voltammetry (CVA), electrochemical impedance spectroscopy (EIS) [8-12].

On the basis of heterostructures such as inorganic carrier - NED - graphene, electrodes are synthesized, their electrochemical properties and possible applications are investigated.

Results and discussion. According to the data of atomic absorption analysis, in native and immobilized enzyme drugs the metal ions of variable valence (Fe^{3+}, Cu^{2+}, Ni^{2+}, Mn^{2+}, Zn^{2+}) that are capable of redox transitions and the formation of surface complexes with charge transfer on the surface of inorganic carriers has been established (Table 3.1).

Obviously, the content of metal ions in the samples is significantly different. This indicates the differences in the composition and structure of the catalytic and adsorption centers of the protein molecule, which affects the electrochemical properties of the heterostructures being formed.

Table 3.1. The content of metal ions in the native / immobilized on inorganic carriers enzyme drug based on extract from radish black/white cabbage

System	Metal ions content, %				
	$Fe^{3+}\times10^{-3}$	$Cu^{2+}\times10^{-4}$	$Ni^{2+}\times10^{-5}$	$Zn^{2+}\times10^{-4}$	$Mn^{2+}\times10^{-4}$
NED	1.85/0.63	1.20/0.10	6.72/7.40	5.38/3.46	– /0.64
Bentonite + NED	2.55/3.15	0.32/0.06	6.50/7.40	4.62/3.40	–/0.64
Mod. bent. + NED	1.40/1.35	0.17/0.03	6.70/4.70	4.30/23.00	–/0.64
Aerosil 300+ NED	0.30/1.74	0.012/ 0.016	2.40/1.20	1.50/0.99	–/0.06
Kaolin + NED	0.77/1.25	0.12/0.06	5.01/7.40	2.90/3.47	–/0.48

The analysis of irreversible binding of metal ions has established that natural bentonite selectively binds Cu^{2+} /Cu^{2+}, Ni^{2+}, Mn^{2+}, Zn^{2+} ions, modified with phosphate-ions bentonite – Fe^{3+}, Cu^{2+}, Zn^{2+}/ Cu^{2+}, Ni^{2+} ions, kaolin – ions Fe^{3+}, Cu^{2+}, Ni^{2+}, Zn^{2+}/ Fe^{3+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, and aerosol 300 – all metal ions (Fe^{3+},

Cu^{2+}, Ni^{2+}, Zn^{2+}/ Fe^{3+}, Cu^{2+}, Ni^{2+}, Mn^{2+}, Zn^{2+}). This dependence (Table 3.2) is in accordance with the structure of the carrier and the structure formed by the ion in the original enzyme. The presence of all metal ions indicates the presence of three classes of enzymes: peroxidase, superoxide dismutase, and catalase.

Table 3.2. Equivalent ratios of immobilized metal ions on the surface of inorganic supports

Support	**Equivalent ratios**	
	Black radish extract	White cabbage extract
Bentonite + NED	Cu_1	Cu_1: $Ni_{1.6}$: $Zn_{6.8}$: $Mn_{1.8}$
Mod.bent. + NED	$Fe_{11.7}$: $Cu_{5.5}$: Zn_1	Cu_1 : $Ni_{1.67}$
Kaolin + NED	$Fe_{89.8}$: $Cu_{9.8}$: Ni_1 : Zn_{19}	$Cu_{1.2}$: $Ni_{1.8}$: Zn_7 : Mn_1
Aerosil 300+ NED	$Fe_{40.1}$: $Cu_{3.1}$: Ni_1 : $Zn_{9.6}$	$Fe_{33.5}$: $Cu_{2.5}$: Ni_2 : Zn_{16} : Mn_1

Scanning electron microscopy of the obtained materials [11] showed that the adsorption of the native enzyme preparation on the surface of the carriers is accompanied by denaturation of the polysaccharide portion of the enzymes and the formation of a surface heterostructure. Namely in this heterostructure the spatial divide of charges of metal-containing fragments of enzymes occurs. This phenomenon is determined by the acidic - basic properties of the support surface.

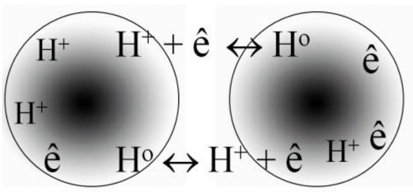


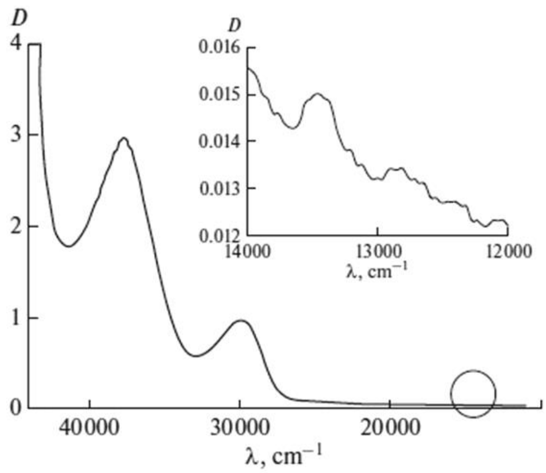
Fig. 3.1. *Scheme of charge exchange in the particles of heterostructures.*

The impedance measurements of suspensions established proton conductivity, as well as the divide into two groups [10]. In one group, the charge transfer was similar to that of imperfect electrolytes [13]. It includes all the systems of inorganic carrier – NED of white cabbage and NED of black radish adsorbed on bentonite and its acidic form. The second group (the systems of carrier and NED of black radish adsorbed on kaolin and aerosol) included systems with charge transfer according to two mechanisms: along a matrix and a shell (Figures 3.1, 3.2).

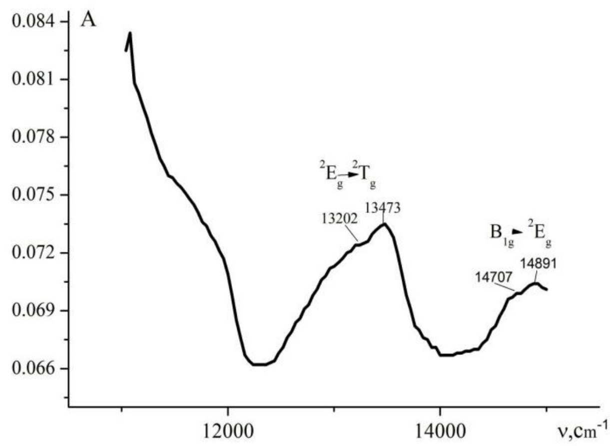
Fig. 3.2. *Equivalent schemes of suspensions based on aerosol 300-NED of black radish (a) and white cabbage (b).*

To find out the nature of the results obtained, the electronic spectra of 20% NED solutions obtained from different extracts were studied. They confirmed the formation of structures with charge transfer on the carrier surface. This indicates the mechanism of "crosslinking" the enzyme with the carrier.

Two peaks are revealed on the electronic spectra obtained from the NED of black radish (Figure 3.3a). The peak of 29920 cm^{-1} corresponds to Fe^{3+} ions with the d^5 configuration and the coordination number of 6 (octahedral symmetry group). This corresponds to a low spin octahedron with a of $T_{2g} \rightarrow {}^2A_{2g}$, ${}^2T_{1g}$. The triplet in the region of 30960 cm^{-1} confirms this assumption. A Gauss spectral deconvolution revealed a Cu^{2+} band with a maximum of 13459 cm^{-1}, which corresponds to a distorted hexagonal bipyramid with a coordination number of 5 ($A_{1g} \rightarrow T_{1g}$ transfer).



a



b

Fig. 3.3. *Electron absorption spectra of the NED ($C_{e.p.}$ = 20%, λ = 5000 - 11000 cm^{-1}, l=1 cm): extracted from radish black (a), white cabbage (b).*

In electron spectra obtained from NED of white cabbage (Figure 3.3b), the peaks correspond to Cu^{2+} ion (distorted octahedron) with transfers of ${}^2E_g \rightarrow {}^2T_g$ (13202 and 13473 cm^{-1}) and $B_{1g} \rightarrow {}^2E_g$ (14707 and 14891 cm^{-1}). No other interactions were detected.

An enzyme drug, which was prepared from radish black, contains two interacting enzymes: peroxidase and superoxide dismutase in approximately equal ratios [14]. The spectrum of NED of white cabbage extract indicates only the presence of Cu^{2+} ion.

It has been established by CVA [12] that the heterostructures based on aerosol 300-NED of the radish black root behave as a supercapacitor (Figure 3.4a) [1]. Somewhat lower values of the current and voltage data have been obtained for the white cabbage extract (Figure 3.4b).

At different rates of potential sweep ($\tau_2 - \tau_1$), the values of the ΔE difference of the initial and final sweep potentials E_1 to E_2, the mass of the sample (m), the module of the specific current (I) and the data of impedance spectroscopy on the initial capacitance.

Table 3.3. Characteristics of charge capacities of heterostructures

System	$C_{in}\cdot10^{-3}$, F/g	Black radish	White cabbage
		C_{ef}, F/g	$C_{ef}\cdot10^{-3}$, F/g
Bentonite + NED	0.62	1.6	26.3
Mod.bent. + NED	1.14	0.068	1.53
Kaolin + NED	1.74	51.3	0.39
Aerosil-300+ NED	0.38	95.7	3.56

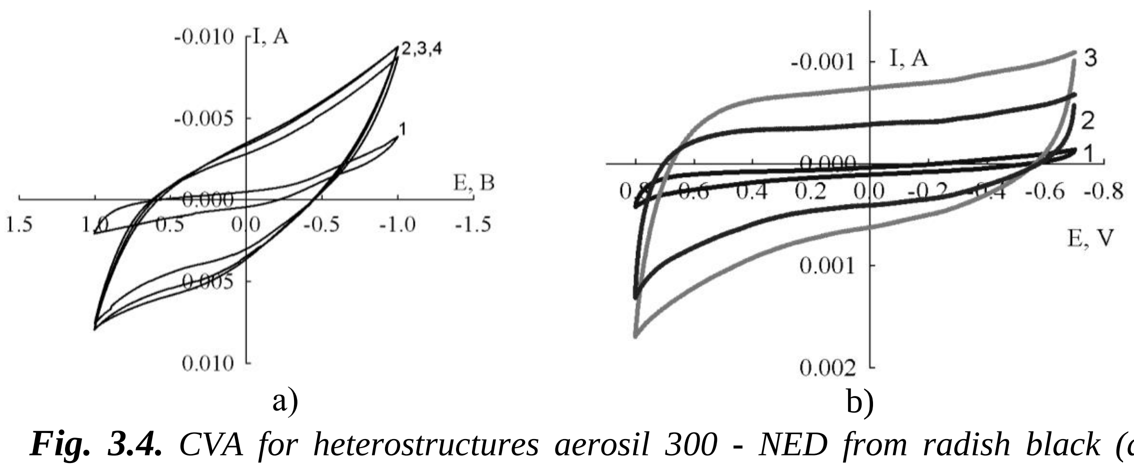


Fig. 3.4. *CVA for heterostructures aerosil 300 - NED from radish black (a), white cabbage (b) on the potential sweep rate (mV/s): 1- 5, 2-50, 3- 100. Electrolyte is a saturated solution of KCl, S_{el} = 1.5 cm², sample of heterostructure - 3 mg/cm².*

The capacitance of the studied materials (Table 3.3) are calculated according to the known ratio:

$$C = \frac{I \cdot (\tau_2 - \tau_1)}{(E_1 - E_2) \cdot m} \quad (3.1)$$

It has been established that the systems based on the carrier - NED (white cabbage) are characterized by low current values relative to materials based on radish black. This means the inexpediency of the use of these materials in electrochemical devices.

Different behavior can be seen in equivalent schemes obtained by impedance spectroscopy (see Figure 2). Regarding radish extract, an equivalent circuit shows the possibility of transfer of charge through two mechanisms, and hence the possibility of energy conservation. For cabbage, the scheme is an electrolyte.

Conclusions. The comparative analysis of the physicochemical properties of synthesized heterostructures, which contain an enzyme drug of black radish and white cabbage, shows different behavior of heterostructures depending on support. This is caused by different mechanisms of binding of an enzyme drug with an inorganic carrier and the possible formation of core-shell structures.

The formation of heterostructures, which are able to accumulate charge in inorganic systems of aerosol 300-enzyme-graphene, makes it possible to apply them to redox transformations. Such heterostructures can be promising materials in energy conversion devices (supercapacitors) up to 95 F/g, having commercial values. They can be also used for cheap and biocompatible current sources.

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АДСОРБЦІЙНІ МЕХАНІЗМИ САМООРГАНІЗАЦІЇ ГЕТЕРОСТРУКТУР НЕОРГАНІЧНИЙ НОСІЙ - НАТИВНИЙ ФЕРМЕНТНИЙ ПРЕПАРАТ

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Встановлено вплив складу композиційного матеріалу (core-shell структури), утвореного нативним ферментним препаратом – витяжкою з редьки чорної або капусти, іммобілізованим на неорганічних носіях з різною природою поверхні на їх активність і електрохімічні характеристики. Показано, що в залежності від природи носія механізм іммобілізації ферментів, їх активність і швидкість інактивації змінюються. На основі аналізу вольтамперометрії іммобілизованого ферментного препарату на неорганічних носіях показано можливість використання таких композитних матеріалів для створення пристроїв для накопичення енергії.

Ключові слова: *нативний фермент, іммобілізація, неорганічний носій, графен, ємність.*