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Measurements of Platinum Electrode Potential in Blood and Blood Plasma and Serum

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Abstract—The method of electrochemical pretreatment of platinum electrode with the goal of standardizing the initial state of electrode surface and its open-circuit potential (OCP) in the blood and other biological media is proposed. The platinum electrode potential is measured in 0.14 M Na_2SO_4 aqueous solution, in the blood and blood plasma and serum. By the examples of OCP measured in the blood serum of patients with acute poisoning, acute cerebral pathology and patients treated by the method of hyperbaric oxygenation, it was found that the values of blood serum OCP were different for studied pathological states and healthy people.

Key words: platinum electrode, open-circuit potential, biological liquids

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INTRODUCTION

In the normally functioning human organism, the prooxidants, i.e. active oxygen species (AOS), which are yielded by some physicochemical processes in the organism, and the components of antioxidant protection system, are at balance. The breakdown of the balance in the cases of acute pathological states of various etiology can lead to the oxidative stresses or to the retardation of radical processes, i.e. to the disturbance of organism purification processes. Oxygen, which comes into the organism in the molecular form, commonly does not enter uncontrolled chemical reactions inside the organism and does not impose a hazard upon organic cell macromolecules.

The main active oxygen forms are superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl (free) radicals ($HO^\bullet$, $HO_2^\bullet$), singlet forms of oxygen ($^1O_2$), and HO_2^- ions [1]. The main mechanisms of formation of AOS in the organism are commonly associated with disturbed functioning of electron-transport chains of mitochondrions or microsomes. The normal process of AOS formation by phagocytes in the course of stimulating nonspecific organism protection is a special case. The AOS play a significant part also in various processes in the protective immune mechanisms of organism. The most important ferments like superoxide dismutase, catalase, and peroxidase are significant elements of antioxidant protection of organism. Classical antioxidants (vitamins E and A, carotinoids) are active with respect to almost all of AOS; however, the contribution of above vitamins and carotinoids to the total antioxidant activity of organism is not too considerable [2].

In electrochemical terms, the reactions, which are typical of both prooxidant and antioxidant systems, can be described by a series of redox processes, which are in equilibrium in the normally functioning organism. A redox potential of blood and blood plasma or serum can serve as an integral reflection of the redox processes proceeding in the organism. The measurements of redox potential in various media are widely applied [3].

In the medicine, many attempts were made to estimate the redox properties of blood, other biological liquids, and tissues by measuring the redox potentials [4]. In the medical, biological, and ecological literature, a potential of platinum electrode placed into a test medium against a certain reference electrode is considered as the redox potential of the medium. In the electrochemical and analytical chemistry literature, the "redox potential" term is applied only to the reversible redox systems. Therewith, the redox poten-

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tials are independent of the metal nature and electrode surface state; the redox potentials are related to the ratio between the concentrations of oxidized and reduced forms of substance by the Nernst equation.

In the biological systems, in general case, several irreversible oxidation and reduction reactions, which determine the value of measured potential, proceed on the metal electrode (they depend on the composition of test solution). Therefore, this potential would be more correctly termed the open-circuit potential (OCP).

The adsorption of components of biological system and dissolved oxygen, which is present in the system, is an important potential-determining factor [3]. In the solutions containing water, adsorbed and phase layers of metal oxide form on the electrode surface. Thereby, the oxygen-exchange processes can proceed between the oxide and water molecules and affect the electrode potential. Nonhomogeneity of platinum surface can also be an important factor: the areas with substantially different activity with respect to the solution components can coexist on the electrode surface [5]. Under these conditions, the measured OCP of electrode can strongly depend not only on the metal nature, but also on its surface state. This frequently leads to poor reproducibility of measured results. In practice, it is also important that the OCP varies in the time. This is associated, in particular, with the retardation of adsorption processes on the electrode and the competition between different adsorbed species. As a rule, after a time, the OCP reaches a constant value. This period of time depends on the catalytic activity of electrode material in addition to other factors. Therefore (and also due to their high corrosion resistance and chemical passivity), gold electrodes [6–8] or, more frequently, platinum electrodes [9, 10] are used in the biology and medicine. It should be emphasized that, due to complex composition of biological systems, the period of reaching virtually constant potential value can be rather long. Therefore, it is important to monitor potential for rather long time. This frequently does not receive sufficient attention in the medicine [9, 11–13].

In spite of some difficulties in realizing reliable measurements of OCP, its application as a reflection of pathological states of organism is promising, because many homeostasis processes are electrochemical [14]. The OCP measurements are performed in various biological media [7, 15–17]. The development of the study supposes that, based on the measured values of OCP in the cases of various pathologic states, one can reveal certain potential ranges, which are typical for healthy people, follow the variations in the patient status and the quality of medical treatment using the OCP monitoring, and to elaborate the criteria, which enables one to determine the therapeutic approach.

In the electrochemical works, where platinum is one of basic electrode materials, much attention has been given to the development of the procedures of electrode surface pretreatment in order to obtain reproducible measured results. The anodic–cathodic activation of smooth platinum electrode is among these methods [18–21]; the detailed procedure depends on the test system. For example, the authors of [20] proposed to perform potential cycling with potential scan rates of 1 to 10 V/min in the potential range from –0.3 to 1.5 V (NCE) in the 1 M H_2SO_4 or 0.1 M $HClO_4$ solution. It is known that the desorption of impurities from the electrode surface is the main effect of anodic–cathodic activation of platinum [22].

The aim of this work is to develop the procedure of measuring OCP of platinum electrode in the biological media, to search for a method of platinum electrode pretreatment in order to standardize its initial potential, to study the dynamics of variation in the OCP with the time, and to measure OCP in the blood and blood plasma and serum using the procedure developed.

EXPERIMENTAL PROCEDURE

The platinum electrode potentials were measured in the aqueous solutions and biological media on smooth polycrystalline platinum electrode 3.3×10^{-2} cm^2 in area; a silver–chloride electrode served as the reference electrode. The potentials were measured and the time dependences of potential were recorded with an IPC-compact potentiostat (Research-Production Association "Volta"). The volume of liquid samples for investigations was 2.0 ml. The blood and blood plasma and serum of 50 apparently healthy 19–40 years old volunteers (36 men and 14 women) were used as the biological media. The blood sampling was performed by the venipuncture. Heparin (20 units/ml of blood) was used as the anticoagulant. The blood plasma was separated from the blood corpuscles by centrifuging in a CR 3.12 (Jouan) centrifuge at 1500 g for 20 min. To obtain the blood serum, the blood free of anticoagulant was incubated at 37°C for 40 min and, then, centrifuged at 1500 g for 20 min.

RESULTS AND DISCUSSION

To eliminate the effect of dissolved oxygen on the OCP, the deaerated test solutions are commonly used. However, this approach is not advantageous in studying biological subjects. Actually, in the human organism or other aerobic organisms, oxygen is a necessary participant of all oxidation processes. Human blood and its components are adapted to the functioning in the presence of oxygen. Therefore, the absence of oxygen in the system can distort the values of OCP corre-

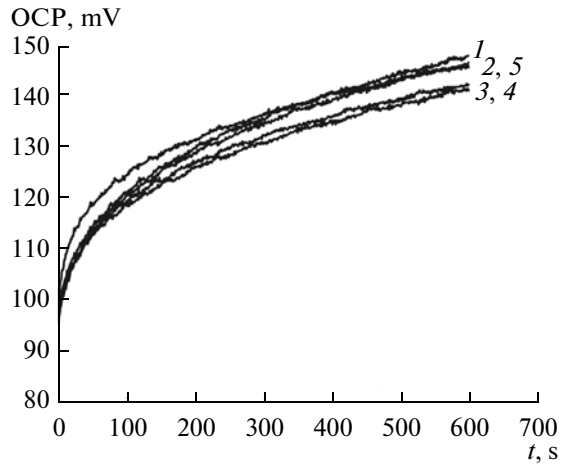

Fig. 1. The effect of platinum electrode pretreatment on the reproducibility of results obtained in 0.14 M Na_2SO_4 aqueous solution.

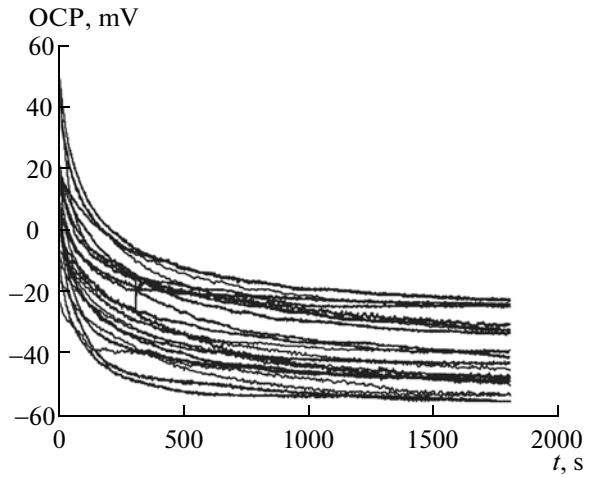

Fig. 2. The determination of statistically probable OCP range in the blood serum of healthy people (the data for 23 volunteers).

sponding to the normal physiological status of blood or another biological liquid.

In the case of platinum, in addition to the oxygen adsorption, it should be taken into consideration that proteins of blood plasma and serum are also not indifferent to platinum, because they can be adsorbed on the metal surface [23]. Large protein molecules adsorbed on platinum can shift platinum electrode potential [24]. The adsorption of proteins can lead to partial or complete denaturation of proteins on the platinum surface. Therefore, obviously, adsorbed proteins, as well as oxygen adsorbed during the experiment, should be removed from the platinum surface after each experiment.

For cleaning platinum surface after the completion of an experiment and before the beginning of the next one in order to standardize the platinum electrode surface state, a procedure of electrochemical treatment of platinum was developed. Many modes of treatment were tested, and, as a result, the following procedure was chosen. An electrode was placed into the deaerated solution of inorganic salt and treated by cycling with a potential scan rate of 500 mV/s, first, in the potential range from −600 to +600 mV, 50 cycles; then, in the range from +100 to +200 mV, 10 cycles. The cycling was finished at a potential of +100 mV. As a result of the treatment, the working electrode potential in the deaerated 0.14 M Na_2SO_4 aqueous solution reached a constant value of 140 ± 5 mV (Fig. 1). After the pretreatment of platinum electrode, the standard deviation of OCP was not more than ±3%. The pretreatment was performed prior to each experiment, and the initial value of OCP prior to the measurements in the test solutions was controlled. After reaching a constant OCP in the control solution, in 15 min, Na_2SO_4 solution was poured out of the cell through a stopcook, and the cell was filled with test biological medium.

Then, the OCP was measured in the human biological liquids: in the blood, blood plasma and serum.

First, the OCP was measured in the blood of fifteen healthy people. It was found that the OCP fell in the range of 68 to −12 mV. However, the blood is incorrect object for OCP measurements: the membranes of blood cells are negatively charged, which can affect the measured value due to adsorption or other interactions of cells with the working electrode [25]. In addition, their content in the blood and the sedimentation rate fluctuate; this also has an effect on the shape of the potential vs. time curve and the measured OCP. The presence of anticoagulant (for example, heparin or sodium citrate) in the blood can additionally distort the measured OCP.

Figure 2 gives the measured values of OCP for the blood serum of 23 healthy people. It is seen that the initial potentials in the potential vs. time curves vary from −23 to +57 mV, whereas, in 30 min, the OCP of blood serum lies in the range from −56 to −23 mV.

To check the effect of heparin on the OCP, the blood plasma and serum of one donor were examined. It was found that the time dependences of potential for serum containing heparin and free of it differ substantially (Fig. 3). The OCP for serum free of heparin was −32.7 mV, and after an addition of heparin, it was +6.9 mV. The OCP for the blood plasma of the same donor was +50.4 mV. Thus, an addition of heparin to the blood has a pronounced effect on the measured OCP of blood serum and plasma. Similar results were obtained for the effect of other stabilizers on the OCP of blood and plasma (sodium citrate and EDTA were examined). It was shown that the blood serum is the most appropriate object for measuring OCP. It was

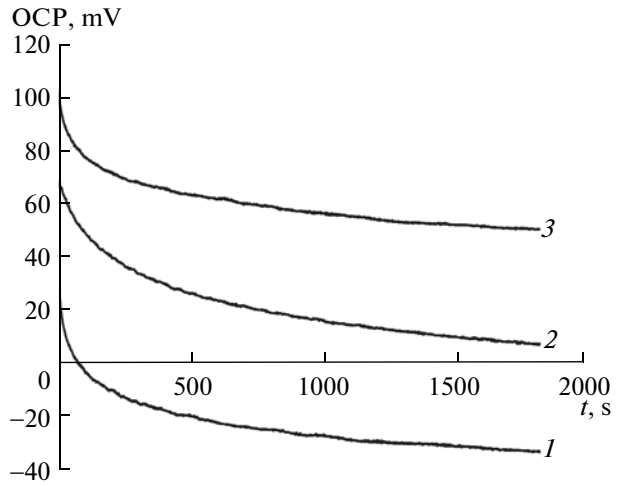

Fig. 3. The effect of heparin on the OCP of platinum electrode in the blood plasma and serum: (*1*) blood serum, (*2*) blood serum with a heparin additive, and (*3*) blood plasma.

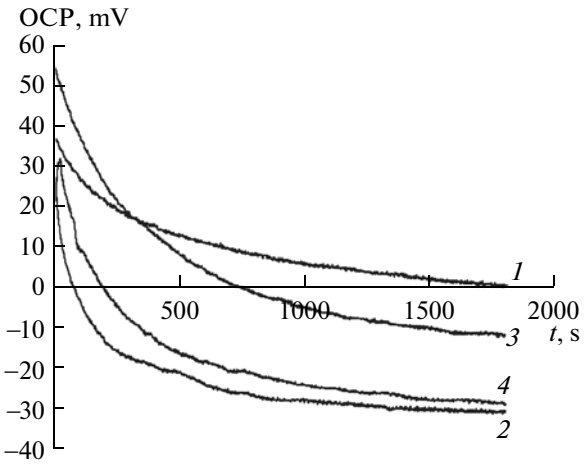

Fig. 5. The effect of hyperbaric oxygenation on the OCP of platinum electrode in the blood serum of patient: (*1*) before the first procedure, (*2*) after the first procedure, (*3*) before the second procedure, and (*4*) after the second procedure.

found that the concentration of stabilizer has an effect on the OCP. Therefore, to measure OCP in blood or plasma, it is necessary to standardize the concentration of stabilizer in order to take into account the contribution of stabilizer additive to the measured potential.

Further investigations showed that the method of OCP measurement with the use of proposed procedure of electrochemical pretreatment of platinum electrode can be used to reveal the pathologic state. Figure 4 gives the OCP data measured in the blood serum of apparently healthy man (a donor) (curve *1*), neurosurgical patient (curve *2*), and a patient with a diagnosis of hypoglycemic coma (curve *3*). It is seen that the values of OCP of these people are significantly different.

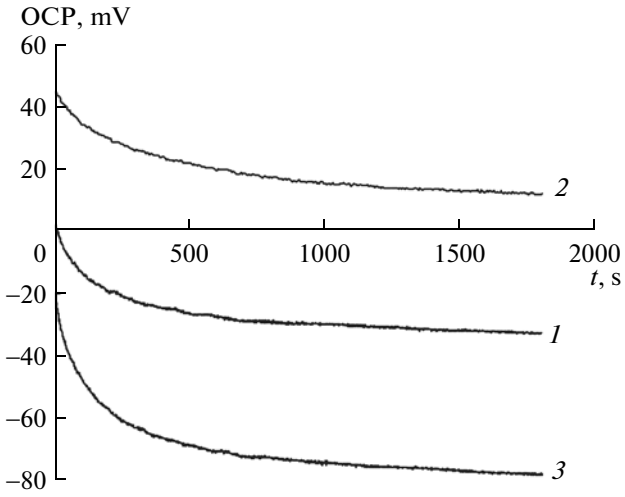

Fig. 4. The OCP measured in the blood serum of (*1*) apparently healthy man (a donor), (*2*) neurosurgical patient, and (*3*) a patient with a diagnosis of hypoglycemic coma.

Figure 5 shows the effect of medical treatment (a procedure of hyperbaric oxygenation for 1 h) of a patient with a cerebral pathology (craniocerebral trauma) on the OCP of blood serum. The medical treatment has a pronounced effect on the value of blood OCP and the time dependence of OCP.

The above examples show that the method of blood serum OCP measurements can give some information on the equilibrium between pro- and antioxidant activity of an organism.

CONCLUSIONS

A method of electrochemical pretreatment of platinum electrode is developed. The method enables one to clean the electrode surface and obtain reproducible data on the OCP in the biological liquids (blood, plasma, and blood serum).

It is shown that the measurements of OCP of platinum electrode after the electrochemical pretreatment enable one to determine the OCP range, which is typical for apparently healthy people, and the potentials, which are observed for the patients. The variations of the OCP in the course of medical treatment were indicated.

Based on these data, systematic studies can be performed in order to characterize the pro- and antioxidant activity of organism by the measured values of OCP of platinum electrode in the blood serum.

REFERENCES

1. Osipov, A.N., Azizova, O.A., and Vladimirov, Yu.V., *Usp. Biol. Khim.*, 1990, vol. 31, p. 180.
2. Karlsson, J., *Antioxidants and exercise*, Illinois: Human Kinetics, 1997.
3. Nikol'skii, B.P., Pal'chevskii, V.V., Pendin, A.A., and Yakubov, Kh.M., *Oksredmetriya* (Redoxmetry), Leningrad: Khimiya, 1975.
4. Polak, J., *Advances in Tissue Engineering*, London: Imperial College, 2008.
5. Povarov, Yu.M. and Lukovtsev, P.D., *Electrochim. Acta*, 1973, vol. 18, p. 13.
6. Ziegler, E., *The Redox Potential of the Blood in Vivo and in Vitro*, Springfield, Illinois: Charles C. *Thomas Publ.*, 1965.
7. Kuznetsova, I.N. and Pendin, A.A., *Biofizika*, 1976, vol. 31, p. 867.
8. Marmasae, C. and Grosz, H.J., *Nature*, 1964, vol. 202, p. 15.
9. Jellinek, M., Chandel, B., Abdulla, R., Shapiro, M.J., and Baue, A.E., *Cellular and Molecular Life Sciences*, 1992, vol. 48, p. 980.
10. Grosz, H.J. and Farmer, B.B., *Nature*, 1967, vol. 213, p. 1717.
11. Kuznetsov, V.A. and Leonov, A.N., *Patol. Fiziol. Eksp. Ter.*, 1988, no. 2, p. 18.
12. Landa, V.A., Tyurina, E.V., and Khudaidatov, I.S., *Ortopediya, Travmatologiya i Protezirovanie*, 1989, vol. 3, p. 17.
13. Yoshimura, N., Komada, K., and Yoshitake, J., *Brit. J Anaesthesia*, 1971, vol. 43, p. 1022.
14. Opritov, V.A., *Sorosov. Obrazov. Zh.,* 1996, no. 9, p. 40.
15. Azad, A.K., Ishikawa, K., and Islam, N., *Acta Horticulturae (ISHS)*, 2003, vol. 609, p. 487.
16. Kim, H., Kwon, S., Han, S., Yu, M., Kim, J., Gong, S., and Colosimo, M.F., *Water Sci. Technol.*, 2006, vol. 53, p. 145.
17. Rae, S.K., Palazzo, R.S., Metz, H.N., Wilson, D.W., Nikolic, S.D., Graver, M., and Rao, P.S., *J. Pharmacol. Toxicol. Methods*, 1997, vol. 38, p. 151.
18. Kandler, L., Knorr, C.A., and Schwitzer, C., *Z. Phys. Chem. (A)*, 1937, vol. 180, p. 281.
19. Frumkin, A.N, in *Advances in Electrochemistry and Electrochemical Engineering*, Delahay, P., Ed., Interscience, 1963, vol. 3, p. 287.
20. Untereker, D.F. and Bruckenstein, S., *J. Electrochem. Soc.*, 1974, vol. 121, p. 360.
21. Petukhova, R.P., Podlovchenko, B.I., and Gas'kov, A.M., *Elektrokhimiya,* 1985, vol. 21, p. 1414.
22. Petrii, O.A. and Shchigorev, I.G., *Elektrokhimiya*, 1968, vol. 3, p. 370.
23. Anzai, J., Guo, B., and Osa, T., *J. Colloid Interface Sci.*, 2003, vol. 262, p. 107.
24. Bernabeu, P. and Caprani, F., *Biomaterials*, 1990, vol. 11, p. 258.
25. Kharamonenko, S.S. and Rakityanskaya, A.A., *Elektroforez kletok v norme i patologii* (Electrophoresis of Normal and Pathological Cells), Minsk: Belarus, 1974.