A C T A U N I V E R S I T A T I S L O D Z I E N S I S
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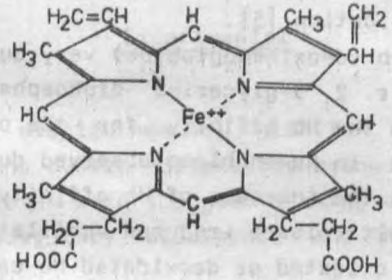
Bogusław Grzelak*, Wiesław Augustyniak**,
Jan M. Rogoziński***, Stanisław Romanowski*

SOME ATTEMPTS OF ELECTROCHEMICAL OXIDATION OF HUMAN BLOOD HEMOGLOBINE ON THE GOLD ELECTRODE****

Cyclic chronovoltamperometric method was applied in attempts of oxidation of deoxyhemoglobin human blood solutions in different anticoagulants on the gold electrode. On the basis of obtained results it was assumed that the oxidation of hemoglobin in 3.8% sodium citrate solution and also in 1% NaCl and 0.5% NaF mixtures is possible.

Hemoglobine (Hb) - the main coloured constituent of erythrocytes plays extremely important role in human organism as the oxygen and carbon dioxide carrier. It is built up of four hem molecules with two pairs of polypeptid chains:



Molecule of hem

* Department of General and Inorganic Chemistry, Institute of Chemistry, University of Łódź.
** Station of Blood Donors, MSW Hospital, Department of Internal Medicine Military Medical Academy, Łódź.
*** Clinic of Diabetology, Academy of Medicine, Łódź.
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Bivalent iron in the porphyrin complex is able to fix the oxygen without changing the valence in molar ratio 1 : 1. Oxygen addition to Hb called oxidation is not a completely reversible process. In the oxidated complex very slow chemical oxidation of the bivalent iron takes place the effect of which is the creation of methemoglobine (MetHb) consisted of porphyrin complexes Fe(III) called hemines, hence the another name of methemoglobine - hemiglobine [1, 33].

Enzymes reducing MetHb to Hb included in blood cells oppose this process but the content of MetHb in the oxidated blood approximates 2%. Greater ammount of MetHb causes the state of sickness mainly because of the reduced ventilation property of human blood [1-5, 30, 31].

The specific character of Hb ability of fixing the oxygen and also its very slow change into MetHb can be observed in the experiments with the oxygen oxidation in the synthetic hemo-similar compounds. Porphyrin complexes Fe(II) with protein chains are oxygen oxidated 10^8 times faster than natural Hb [6]. Piridinian solutions of synthetic porphyrin complexes with Fe(II) neither present properties of connecting with oxygen nor they oxidate to complexes with Fe(III). Only the addition of water to the solution causes quick auto-oxidation [5].

The deoxidated Hb (deoxihemoglobine) very quickly attaches the oxygen from the air. 2, 3-glycerine diphosphate (2, 3-DPG) has a great influence on the Hb affinity for the oxygen. The decrease of 2, 3-DPG content in human blood observed during long blood preservation influences the increase of Hb affinity for the oxygen and its more difficult release (reduced ventilation) [30, 31].

The presence of oxidated or deoxidated Hb can be easily stated by comparing adsorption spectra of Hb solutions. The oxidated Hb gives two clear absorption bands at 578 nm and 540 nm in the visible part of absorption spectrum, while the deoxidated Hb gives only one at 555 nm [1, 4].

The oxidation of Hb to MetHb can be easily carried out through the potentiometric ferricyanide titration. The normal Hb/MetHb potential in these conditions depends on pH and it changes from 0.175 V (SHE) at pH = 6 to 0.040 V (SHE) at pH = 9; pH of human blood is about 7.4. This reaction is not 4-electron as it should have been suspected but n value includes itself within the limits from

1.1 to 2.7 in dependence of pH [1-4]. It is probably connected with the change both in the hem structure and in the whole hemoglobine.

In the recent years the bioelectrochemical experiments concerning the biologically active metallocomplexes have been of great interest. In these experiments the iron complexes play the important role. In many works [7-29] the objects of experiments are synthetically obtained hemo-similar complexes. One can state, however, lack the experiments of Hb oxidation to MetHb on the solid electrodes and on the mercury. The great Hb molecule (mole about 65 000) is supposed to be an important obstacle in this type of measurements. The experiments of the kinetics of Hb oxidation reaction on solid electrodes without oxidizers or the influence of certain compounds (e.g. medicines) on the reaction rate would be a precious information mainly for the institutes directly connected with health service.

The starting of the attempts of electrochemical research of Hb solutions has been motivated by the importance of the problem, by great interest of the authors representing different scientific branches and by the obtained electrochemical research potential.

Experimental

The attempts of electrochemical oxidation of Hb solutions were carried out using the cyclic voltammetry method (CVM) in a standard three-electrode system with the gold working electrode, the cylindrical platinum auxiliary electrode, and the saturated calomel reference electrode.

The working electrode in form of wire of diameter $\emptyset$ = 1.0 mm and the surface A = 0.52 cm^2 was purified in the concentrated H_2SO_4 (p.a.) and electrochemically by means of cyclic polarization in the potential range from -1.1 V to +1.4 V with the sweep polarization potential rate 0.2 $V.s^{-1}$ up to the time of obtaining the typical CVM curve in supporting electrolyte (0.5 M H_2SO_4).

Equipment set constructed in the Department of General and Inorganic Chemistry of the University of Łódź (potentiostat, linear sweep potential generator, digital control meters) enabled to obtain CVM curves on the BAK-ST register. The sweep polarization

a small but clear hump that could suggest Hb oxidation peak was registered.

It has been decided then to use the oxidated Hb for the measurements. For it may be assumed that the oxygen added to Hb molecule, can, in an important way, "screen" hem molecules including Fe^{++} and not to let them to the electrode surface.

Moreover, as the structural experiments have proved, the oxygen in Hb molecule causes its deformation through the protein chains [32] shift. Deoxidation of Hb solution in vacuum conditions proved to be of a small use because of the long time of deoxidation, strong foaming of the solution and its distinct concentration.

Some positive results have been obtained during deoxidation through the transmitting the purified argon through the solution. Each time argon was transmitted through the solution in 20 minutes time. Gas flowed out from a capillary (with diameter inside Ø = 0.2 mm) under a pressure of 50 Atm. The addition of $NaBH_4$ in this technics appeared to be needless.

CVM curves of the deoxidized Hb in the citrate solution were clearly different from CVM curves of the solutions without Hb. The supposed Hb oxidation peak lies too near of the citrate oxidation peak and it is of the shape of a hump on the rising part of the curve (Figure 2).

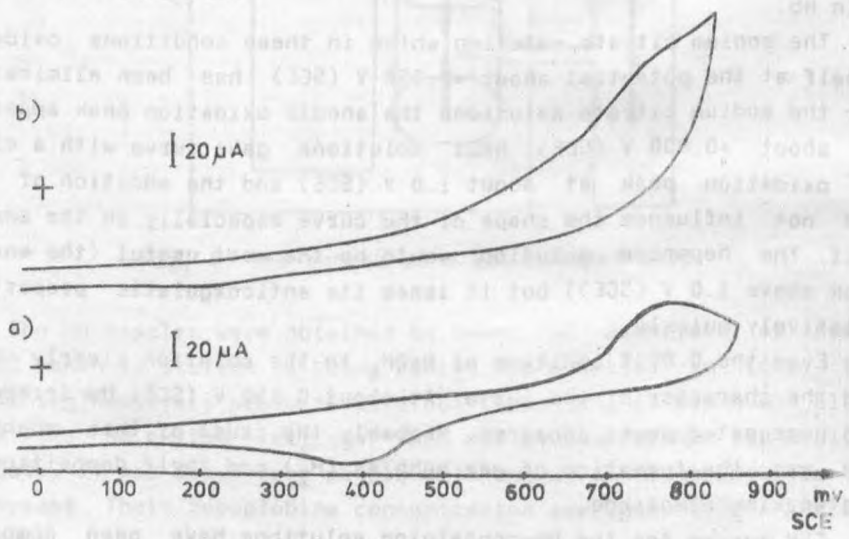

Fig. 2. CVM curves for the 3.8% sodium citrate solution: a) not containg Hb; b) containg Hb. Sweep potential rate v = 0.02 $V.s^{-1}$

A more clear peak have been suspected in the solutions of sodium chloride and sodium fluoride. As 1% NaCl solution chosen as a physiologic humour occuring in human organism does not posses anti-coagulant properties, the mixture of 1% NaCl and 0.5% NaF of different composition has been used as a basic solution. In these solutions the presence of Hb also caused the formation of humps on the rising part of the curve (Figure 3). The potential at which humps occured was higher in comparison with the potential of the citrate solutions curves.

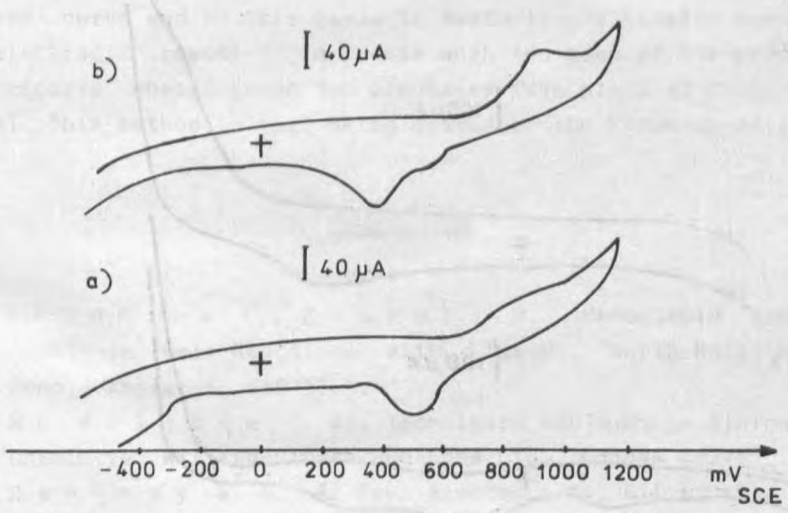

Fig. 3. CVM curves for the sodium chloride and sodium fluoride solution (0.5% NaF and 1% NaCl in voluminal ratio 100 : 1): a) not containing Hb; a) containing Hb. Sweep potential rate v = 0.02 $V.s^{-1}$

If we assume that Hb molecule devoid of molecular oxygen is able to such deformation in the electric field that iron ions Fe^{++} of this molecule could done an electron transfer with the electrode, we should expect a great overvoltage of this process. Thus it is not strange that instead of peak potential equal about 0.200 V (SCE) (near to E^{o} at ferricyanide titration) it is found at about 0.750 V (SCE) (in the citrate solution) or even at about 0.950 V (SCE) (in NaCl + NaF solutions).

The distinct character of the curves in the catodic part also suggests the electrodic oxidation of Hb. Peaks appearing on the catodic part of the curve reffer to the desorption or reduction of oxidation products of the basic solution (Cl^-, F^-).

In the solutions containing Hb they occur at different potentials and they have distinctly different character (Figure 4).

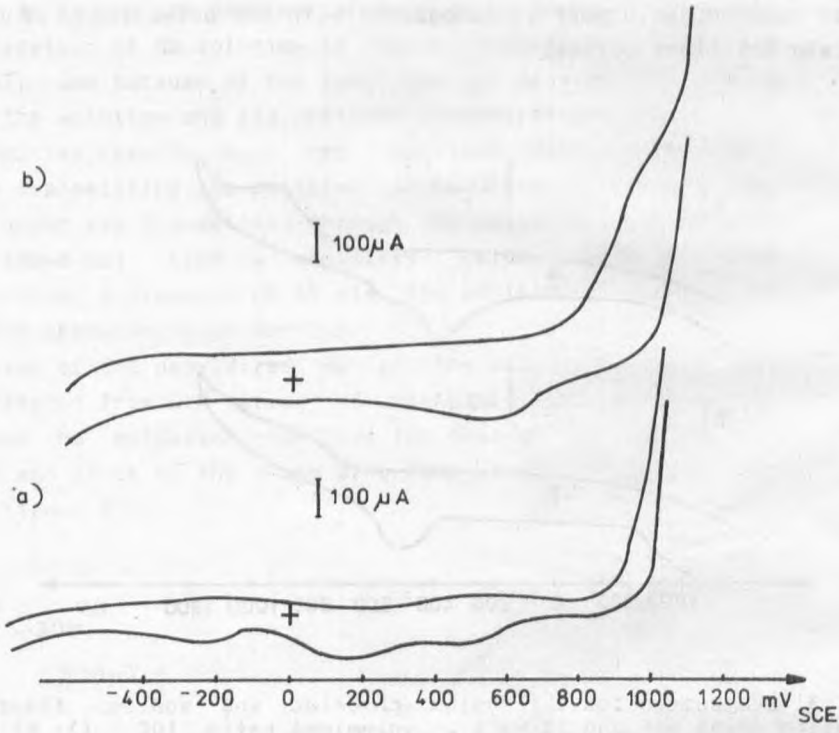

Fig. 4. CVM curves for the sodium chloride and sodium fluoride solution (0.5% NaF and 1% NaCl in voluminal ratio 1:100): a) not containing Hb; b) containing Hb. Sweep potential rate v = 0.05 $V.s^{-1}$

There is obviously a possibility of the electrodic oxidation of the groups occuring both in hem and Hb protein chains[1]. In the paper [19] describing the reduction of -S-S- group in protein chains of many substances biologically active, it has been stated that Hb in which only -SH groups occur does not give any polaro-

[1] No literature on this subject.

graphic wave in the examined potential range. However it is little probable that any group in the protein chain would oxidated more easily than Fe^{++}. On the basis of the obtained results it was assumed that the oxidation of the deoxidized Hb is possible. The experiment, however, meets a lot of technical troubles.

More distinct effects are expected from the measurements of the solutions of greater Hb concentration (up to this time impossible because of the spectrophotometer sensitivity). The quantity interpretation of the obtained Hb oxidation humps will be possible when the numerical methods of elaborating the results of CVM measurements are used. They enable to calculate the characteristic points of the curve and on this basis to evaluate the kinetic parameters of electrodic reaction in a case when the peak of the process is not clearly shaped (when two processes take place at close potentials). This method is just being tested on the known objects.

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Bogusław Grzelak, Wiesław Augustyniak,
Jan M. Rogoziński, Stanisław Romanowski

PRÓBY ELEKTROCHEMICZNEGO UTLENIENIA HEMOGLOBINY KRWI LUDZKIEJ NA ELEKTRODZIE ZŁOTEJ

Metodą chronowoltamperometrii cyklicznej wykonano próby utlenienia roztworów odtlenowanej hemoglobiny w różnych antykoagulantach na elektrodzie złotej. Na podstawie osiągniętych wyników sugeruje się, że jest możliwe utlenienie hemoglobiny w 3,8% roztworze cytrynianu sodu i w mieszaninach 1% NaCl z 0,5% NaF.