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Noninvasive electrochemical antioxidant activity estimation: saliva analysis

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ABSTRACT

Monitoring of the oxidant/antioxidant state of the biological objects plays an important role in the detection of serious diseases in the early stages, the choice of therapy and evaluation of its effectiveness. This parameter is very important for understanding the processes occurring at the interfaces (biointerfaces) in living organisms, in particular, lipid bilayers of cell membranes and cellular organelles (mitochondria), etc. Under normal conditions and in physiological concentrations, active oxygen/nitrogen forms (ROS / ANA) are involved in the growth, differentiation, development, and death of cells, as well as in a number of other bioregulation processes, as natural signaling molecules. With an excessive generation of ROS / ANA oxidative stress develops, the result of which is the processes of oxidative destruction, occurring mainly on the biointerface and leading to apoptosis of cells and the appearance of pathological conditions. Disbalance between reactive oxygen/nitrogen (ROS / RNS) generation and activity of the antioxidant defense system is the cause of oxidative stress (OS) appearing. Antioxidant activity (AOA) of biological objects, as an integral parameter, is an indicator of the OS intensity. The paper presents a hybrid version of electrochemical methods for the determination of AOA, including a chemical signal-forming reaction that precedes or proceeds simultaneously with the recording of an analytical signal. The source of information on AOA (analytical signal) is: - the electrode potential shift that is observed when the sample is introduced into the mediator system-the mixture of 0.01M $K_3[Fe(CN)_6]$ +0.0001M $K_4[Fe(CN)_6]$ (potentiometric variant); - the increment of $K_4[Fe(CN)_6]$ oxidation current, resulting from the interaction of the AO, containing in the sample, with the oxidized form of the mediator system-10^{-2}M $K_3[Fe(CN)_6]$ previously introduced into the solution (chronoamperometric version). The method is applied to the analysis of saliva as a noninvasive alternative to invasive sampling blood fractions investigation. The existence of a relationship between oral diseases and the internal state of the body gives reason to believe that saliva's AOA can serve as a source of information on common health problems. Recovery of antioxidant standard addition introduced into analyzed sample is not less than 85.0% in chronoamperometry and 85.3% in chronopotentiometry. Reproducibility (Sr≤8.87%). The values of Student's and Fisher's criteria (texp <ttheor and Fexp <Ftheor) indicate an insignificant difference in the results obtained by chronopotentiometric and chronopotentiometric methods.

Keywords: *saliva; oxidants; antioxidants; chronoamperometry; chronopotentiometry.*

1. INTRODUCTION

Monitoring of the oxidant/antioxidant state of the biological matrices requires the development of a number of areas of science and technology. It plays an important role in the detection of serious diseases in the early stages, the choice of therapy and evaluation of its effectiveness. This parameter is very important for understanding the processes occurring at the interfaces (biointerfaces) in living organisms, in particular, lipid bilayers of cell membranes and cellular organelles (mitochondria), etc. Oxidative reactions play the leading role in providing cells of the human body with energy. They involve molecular oxygen as the main electron acceptor in the mitochondrial respiratory chain during the synthesis of adenosine triphosphate (ATP) up to the water molecule. In addition, a chain of chemical reactions of one-three-electron oxygen reduction is possible, occurring mainly in the I and III complexes of the mitochondrial respiratory chain [1,2,3], which underlie consecutive generation in the cells of various compounds of a radical nature.

Under normal conditions and in physiological concentrations, active oxygen forms (ROS) are involved in the growth, differentiation, development, and death of cells, as well as in a number of other bioregulation processes, as natural signaling molecules, intracellular messengers and potential factors of intercellular communication [4], regulation of ion channels, enzymes, transcription factors [5].

Excessive ROS generation is accompanied by oxidative stress developing. The latter leads to the oxidative destruction of DNA, proteins, lipids, biomembranes, cells, tissues and pathology development [6,7]. It is shown that ROS interact with the cell membrane, first oxidizing the head groups, followed by the lipid tails, cause intracellular oxidative damage leading to cell death [8]. Oxidative stress is one of the universal mechanisms for implementing typical (common) pathological processes. It plays an important role in the development and progression of a large number of diseases, such as cardiovascular, cancer, neurodegenerative, infertility [9-11], etc.

Disbalance between reactive oxygen/nitrogen active species (ROS/RNS) generation and activity of antioxidant defense system is the reason for oxidative stress (OS) appearing.

The antioxidant activity (AOA) of a biological object, being an integral parameter, can serve as an indicator of the degree of the OS severity [12]. Its value indicates the possibility and probability of the course of oxidation-reduction reactions on the biointerface (membranes, lipid bilayers), and also reflects the

oxidant/antioxidant status of the organism as a whole or its individual system.

The problems of OA/AOA monitoring (oxidant/antioxidant activity) are determined by:

- the complexity of the biological matrix and the rapid change of its composition after sampling;
- variety of compounds of different chemical nature with oxidative and antioxidant properties;
- short of radical compounds life-time;
- the absence of a single term and comparable units of expression of concentration and oxidant/antioxidant properties of compounds or complex of compounds, which does not allow to compare the obtained data with each other.

The general principles of biological objects AOA determination are based on oxidation-reduction reactions. The speed or completeness of the reaction is measured. The most common are two approaches, differing in the use of free radicals as reagents, incl. specially generated [13,14], and/or non-radical chemical reagents that change the optical, electrochemical and other properties of the medium when it interacts with the antioxidants of the test sample [15,16]. The main methods of detection are optical (chemiluminescent, photometric, fluorescent) and electrochemical ones.

From our point of view, the priority should be given to electrochemical methods of AOA assessment. In addition to the advantages associated with availability, low cost of equipment and reagents, the ease of automation of measurements, they allow us to directly assess the properties that determine of investigated system antioxidant state and to obtain direct information on AO concentration, which is expressed in the accepted units (mM-eq).

The authors of [15,17] proposed a hybrid version of electrochemical methods for AOA determination, which includes chemical (signal-forming) reaction that precedes or proceeds simultaneously with the recording of the analytical signal.

The reliability of the results obtained by the potentiometric version of the method is proved by comparative studies with the independent certified TAS Randox spectrophotometric method [18]. The method has been successfully applied to the analysis of blood and its fractions [18], seminal fluid [19], food products, food raw materials and nutrients [15,20]. A chronoamperometric method for determining AOA of blood plasma was also proposed [17]. The hybrid electrochemical method is implemented as well in a non-invasive version [21, 22] for skin AOA study.

Taking into account the information about the status of a living organism and the feasibility of creating methods, in particular for screening studies, it is important to develop newly non-invasive option for new biological samples. Saliva can serve as the object of analysis in this case. Saliva is an interesting new alternative diagnostic body fluid with several specific advantages over blood. These include noninvasive and easy collection. One of the problems of using of saliva for diagnostics and monitoring of non-oral diseases is its composition, which depends on oral status. However, this makes saliva useful parameter for investigation of oral diseases. Periodontitis, caries, oral precancerosis, and other pathologies are associated with oxidative stress. [23-28].

There is a correlation between a decreased level of saliva's AOA and acute coronary syndrome [29], dementia [30], diabetes mellitus [31] and sickle cell anemia [32]. The relationship between saliva's AOA and oral diseases and the internal state of the body leads to a conclusion that saliva's AOA can serve as a source of information on common health problems.

The most common methods for determining saliva's AOA are optical ones [25,33,34].

A method for determining the total value of saliva's AOA is proposed. It is based on the ability of antioxidants to inhibit the oxidation of ABTS to ABTS +. As a standard, a water-soluble synthetic analog of vitamin E - Trolox [35-38] is used. The method FRAP (Ferric reducing antioxidant power assay) is used as well [39]. The methods are time-consuming and multistage. Results obtained by different methods are expressed in non-comparable units.

The aims of this paper are: (i) to develop a hybrid electrochemical method for the determination of saliva's AOA as one step procedure, (ii) to find the conditions for the analysis implementation.

2. EXPERIMENTAL SECTION

2.1 Methods and calculations

2.1.1. Hybrid version of electrochemical methods for the determination of AOA. The methods include a chemical signal-forming reaction that precedes or proceeds simultaneously with the recording of an analytical signal. Two different sources of information on AOA (analytical signals) were used in chronopotentiometric and chronoamperometric versions. Oxidation of determined antioxidants by the oxidized form of mediator system ($K_3[Fe(CN)_6]$) served as signal forming reaction (eq.1):

$Fe(III) + \cdot AO \rightarrow \cdot Fe(II) + \cdot AOOx$ (1)

where AO – an antioxidant; AOOx – the oxidation product of the antioxidant; Fe (III) – potassium hexacyanoferrate (III); Fe (II) – potassium hexacyanoferrate (II).

2.1.2. Chronopotentiometric measurements. The information source with regard to AOA is the electrode potential shift in the mediator system $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ which is observed when antioxidants (the sample) are introduced into the medium. This shift results from changes in the concentration of mediator oxidized/reduced forms during the reaction (1). The equilibrium potential of the mediator system is described by Equation 2:

$$E = E_0 + b \cdot \lg \frac{f_{ox} C_{Ox}}{f_{red} C_{\mathrm{Red}}} \quad (2),$$

where $K_3[Fe(CN)_6]$

$b = 2.3RT/nF$;

R – universal gas constant, J mol^{-1} K^{-1};

T – temperature, K^o;

n – number of electrons involved in the process;

F – Faraday constant, C mol^{-1};

f – activity coefficients, in further calculations ΔE *f* disappears as ionic strength does not change during measurements;

E – potential of the system in its initial state, V;

E_0 – standard electrode potential of the mediator system, V;

C_{Ox} – concentration of $K_3[Fe(CN)_6]$ – oxidized form of the mediator system, M;

C_{Red} – concentration of $K_4[Fe(CN)_6]$– reduced form of the mediator system, M.

After the introduction of an AO containing sample into the solution, the potential of the mediator system is changed and expressed as follows (Eq. 3):

$$E_1 = E_0 + b \cdot \lg \frac{f_{ox}(C_{Ox} - X)}{f_{red}(C_{Red} + X)} \quad (3),$$

where

E1 – measured the potential of the mediator system after the introduction of the analyzed sample, V;

X – AO concentration in the solution after the introduction of the analyzed sample, M-eq.

$\Delta E = E_1 - E$ served as an analytical signal.

AOA of the solution in the electrochemical cell after introduction of the sample is calculated using the equations (4) and (5):

$$X = AOA = \frac{C_{Ox} - \alpha C_{Red}}{1 + \alpha} \quad (4)$$

$$\alpha = (C_{Ox} / C_{Red}) \cdot 10^{\Delta E/b} \quad (5)$$

AOA of the analyzed sample is calculated with the use of Equations (6) and (7):

$$X = AOA = \frac{C_{Ox} - \alpha C_{Red}}{1 + \alpha} \cdot h \quad (6)$$

$$h = (V_0 + V_{al}) / V_{al} \quad (7),$$

where

h –dilution factor;

V_0 - the volume of an initial solution in the electrochemical cell (mixture of a buffer solution and a mediator system), ml;

V_{al} - volume of aliquot of the sample (saliva) introduced into the cell, ml.

2.1.3 Chronoamperometric measurements. The increment of $K_4[Fe(CN)_6]$ oxidation current, resulting from the interaction of the AO, containing in the sample, with the oxidized form of the mediator system(10^{-2}M $K_3[Fe(CN)_6]$) previously introduced into the solution served as source of information (analytical signal).

Reaction (8) appears to be signal forming, that is described by the equation:

$$Fe(II) = Fe(III) + e \quad (8)$$

AOA is calculated with the use of eq. (9):

$$AOA = C_{addition} \cdot \left(\frac{\Delta I_{sample}}{\Delta I_{addition}}\right) \cdot \left(\frac{V_{addition}}{V_{sample}}\right) \quad (9),$$

AOA – antioxidant activity of the analysed sample, M-eq;

$C_{addition}$ – standard addition ($K_4[Fe(CN)_6]$) concentration, M;

ΔI_{sample} – the increment of the oxidation current of $K_4[Fe(CN)_6]$, obtained as a result of the interaction of $K_3[Fe(CN)_6]$ with AO (reaction 1);

$\Delta I_{addition}$ – increment of oxidation current of $K_4[Fe(CN)_6]$, obtained after the introduction to the cell the standard addition ($K_4[Fe(CN)_6]$);

V_{sample} – volume of the sample aliqoute;

$V_{addition}$ –volume of the standard addition $K_4[Fe(CN)_6]$introduced into the cell.

2.2. Experimental conditions

2.2.1 Instruments. Chronopotentiometric measurements were conducted using a pH-meter TA-ION (Tomanalit, Russia). Chronoamperometric measurements were conducted using a stripping voltammetry analyzer IVA-5 (IVA, Russia).

Platinum screen printed electrode ("IVA", Yekaterinburg, Russia) served as an indicator (working) electrode. A silver–silver chloride electrode EVL–1M3.1-Ag/AgCl/3.5M KCl (Gomel Plant of Measuring Equipment, Belarus) was applied as a reference electrode and Glassy carbon rod (R&D Institute Graphite, Russia) served as an auxiliary electrode.

2.2. Materials. Reagents:

- $K_3[Fe(CN)_6]$ analytical grade, $K_4[Fe(CN)_6]\cdot 3H_2O$ pure, ("Reachem", Moscow, Russia);
- KH_2PO_4 chemically pure ("NevaReaktiv", St Petersburg, Russia);
- $Na_2HPO_4\cdot 12H_2O$ analytical grade ("AlphaHim", St Petersburg, Russia);
- ascorbic acid (Fluka, Germany).

2.2.3. Saliva. Saliva was collected in the morning from 8:00 to 11:00. Respondents to refrain from eating, drinking, smoking, or brushing your teeth after midnight on the day of sampling. Saliva was collected for 5 minutes.

3. RESULTS SECTION

3.1. Chronopotentiometric measurements. Dependence of the electrode potential on time (chronopotentiograms) observed with solutions containing 0.01M $K_3[Fe(CN)_6]$ + 0.0001M $K_4[Fe(CN)_6]$ and ascorbic acid (1) or saliva (2) are presented in Fig.1. The character of the curves is one-type. The difference consists of changing the value of the potential accompanying the introduction of antioxidant (ascorbic acid) or saliva into the solution, which is to be expected. The composition of the mediator system was selected in accordance with the data we obtained earlier in the study of seminal fluid, whole blood and erythrocytic mass [18,19] and AOA saliva (from 0.5 to 2.5 mM-eq) given in the literature [29,40]. It corresponds to a range of 17-44 mV potential shifts. When using the selected mediator system, the potential is established quickly enough and stable over time.

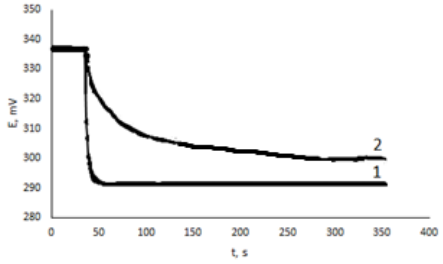

Fig. 1. Dependence of the electrode potential on time, recorded in the study of a solution containing a mediator system (10^{-2} M $K_3[Fe(CN)_6]$ + 10^{-4}M $K_4[Fe(CN)_6]$) and ascorbic acid (1), or saliva (2).

Influence of the degree of dilution of saliva on the results of analysis illustrates by data presented on Fig.2. They show the dependence of the saliva found AOA values on an aliquot of the sample introduced into 1 ml of a mediator system solution.

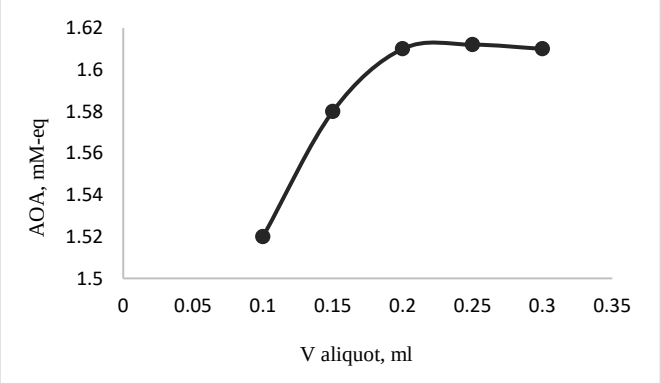

Fig. 2. Dependence of found AOA on saliva aliquot, introduced into 1 ml of the mediator system solution.

The degree of dilution is evidently limited on the one side by the detection limit and on the other by the concentration of the oxidized form of the mediator system in the solution, which should be sufficient for the reaction (1) to proceed. In the case under consideration, an aliquot should not be less than 0.2 ml.

The conditions for determining the antioxidant activity of saliva are given in Table 1.

Table 1. Conditions for analysis of saliva.

Object	Mediator system* C_{Ox}/C_{Red}, M/M	Buffer solution, (pH=7.4), volume, ml	Aliquot of the sample, ml	Final dilution of the sample
Saliva	$10^{-2}/10^{-4}$	1	0,2	6

* $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$

Table 2 shows the results of the determination of AOA of native saliva and a fraction isolated by centrifugation at 1500 rpm for 15 minutes.

Table 2. Results of AOA determination of native saliva and fraction isolated by centrifugation at 1500 rpm for 15 min (n = 3, P = 0.95).

№	AOA, mM-eq			
	Native saliva	S_r, %	Fraction isolated by centrifugation	S_r, %
1	0,87±0,03	3,04	0,81±0,03	3,80
2	1,56±0,03	1,69	1,45±0,06	4,22
3	1,13±0,06	4,60	1,06±0,07	6,53
4	1,33±0,04	2,85	1,21±0,04	2,89
5	0,59±0,04	6,78	0,44±0,04	8,87

The found AOA of saliva before centrifugation is almost identical to or slightly greater than the AOA of the sample obtained by centrifugation. In the future, native saliva without sample preparation was used as the object of analysis.

Influence of samples storage conditions on an analysis results demonstrates the data given in Table 3.

Table 3. Results of AOA determination of native saliva before and after storage for 7 days at -18^{0}C (n = 3, P = 0.95).

№	AOA, mM-eq			
	Before freezing	S_r, %	After freezing	S_r, %
1	1,15±0,05	4,39	1,12±0,05	4,09
2	0,95±0,08	8,51	0,94±0,04	3,83
3	1,29±0,05	3,96	1,28±0,02	2,06

It can be seen from the table that the values of the found antioxidant activity of fresh and stored saliva samples practically coincide. The accuracy of the results is illustrated by a sufficiently high value of Recovery of ascorbic acid, found after its introduction into the sample analyzed (Table 4).

Table 4. Results of AOA saliva determination by chronopotentiometric method, before and after injection of ascorbic acid (n=3, P=0.95).

Sample	Sample AOA, mM-eq	Introduced of Ascorbic acid (AA), mM-eq	AOA (mM-eq), found in sample, containing addition of AA	Found ascorbic acid AOA, mM-eq	Recovery, %
1	1,73±0,06	1,00	2,58±0,05	0,85±0,05	85,0
2	0,69±0,04	1,50	1,98±0,05	1,29±0,05	86,0
3	1,28±0,04	2,00	3,08±0,04	1,80±0,04	90

3.2. Chronoamperometric measurements. Figure 3 shows a cyclic voltamperogram recorded using a screen-printed platinum electrode immersed in a solution containing a 0.15mM equimolar mixture of $K_3[Fe(CN)_6]$ and $K_4[Fe(CN)_6]$ without (1) and with the addition of saliva (2).

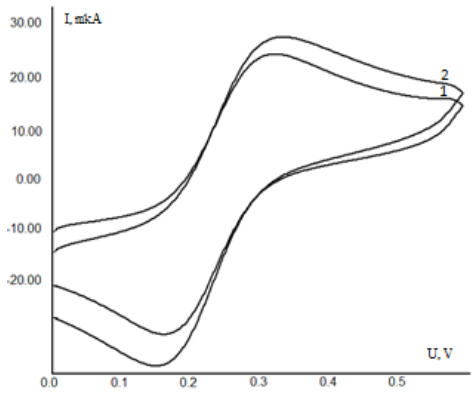

Fig. 3. A cyclic voltammogram recorded using a screen-printed platinum electrode immersed in a solution containing a 0.15 mM equimolar mixture of $K_3[Fe(CN)_6]$ and $K_4[Fe(CN)_6]$ without (1) and with the addition of saliva (2). Scan rate is 1 V/s.

It is easy to see that at potentials more positive than 0.35V occurs oxidation of $K_4[Fe(CN)_6]$. The process of reduction of $K_3[Fe(CN)_6]$ manifests itself in the cathodic branch of the curve at potentials more negative than 0.3V. Saliva does not affect the shape and position of the cycles relative to the potential axis and the difference between the half-peaks of the anodic and cathodic currents. This indicates that saliva has little or no effect on the mechanism of the electrode process.

Figure 4 shows chronoamperograms recorded at different preset potentials of the electrode in a phosphate buffer solution containing 10^{-4}M $K_4[Fe(CN)_6]$.

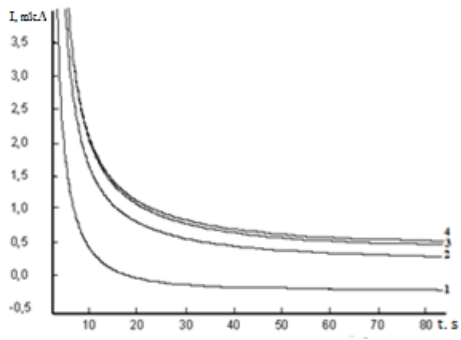

Fig. 4. Chronoamperograms recorded at different preset potentials of the electrode in a phosphate buffer solution containing 10^{-4}M $K_4[Fe(CN)_6]$. Potentials are the following: 0.2 (1), 0.3 (2), 0.4 (3), 0.5 (4) V.

The current that occurs when the potential is 0.3V and above is due to the oxidation of $K_4[Fe(CN)_6]$. It increases with an increase in the concentration of the reagent in the solution (Fig.5). Further, the oxidation current of $K_4[Fe(CN)_6]$ was recorded at a potential of 0.45V.

From the data in Fig. 4, it follows that the current ceases to change in time 70 seconds after the start of the measurement. Further, the current value was fixed after 80 seconds after the start of registration.

Figure 5 shows oxidation chronoamperograms of $K_4[Fe(CN)_6]$, formed in the reaction of $K_3[Fe(CN)_6]$ with antioxidants of saliva, which was introduced into the solution in different amounts. The introduction of saliva and $K_4[Fe(CN)_6]$ into the solution is accompanied by an increase in current. The latter served as a standard addition.

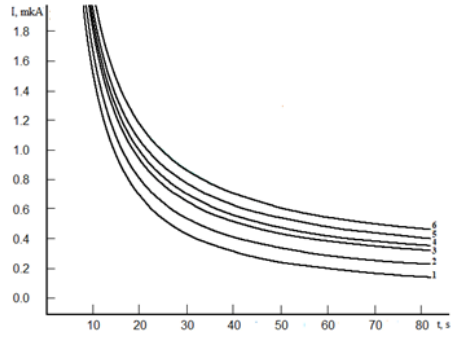

Fig. 5. Oxidation chronoamperogramm of $K_4[Fe(CN)_6]$ formed in the reaction of $K_3[Fe(CN)_6]$ with antioxidants of saliva. Solution contained: 10^{-2} M $K_3[Fe(CN)_6]$ (1), 0,1 ml (2), 0,2 ml (3) of saliva and $5\cdot10^{-4}$M (4), $1.5\cdot10^{-3}$M (5), $3\cdot10^{-3}$M (6) $K_4[Fe(CN)_6]$.

Figure 6 shows the dependence of the found value of AOA, from an aliquot of saliva introduced into 5 ml of a solution containing 0.01M $K_3[Fe(CN)_6]$.

Apparently, with a small amount of saliva introduced into the test saline, the antioxidants contained in it are insufficient to form a correct analytical signal. Further, the aliquot volume of the saliva analyzed was 0.1 ml.

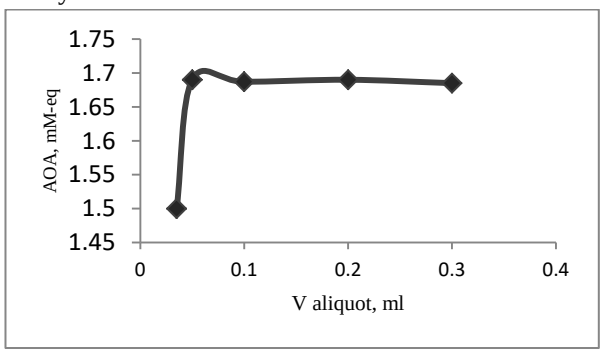

Fig. 6. Dependence of the found value of AOA saliva on the volume of an aliquot introduced into 5 ml of a solution containing 0.01M $K_3[Fe(CN)_6]$.

The found value of AOA increases with increasing aliquot volume.

The concentration of the reagent ($K_3[Fe(CN)_6]$) was chosen in such a way that its amounts were sufficient to oxidize the antioxidants, taking into account that the AOA of the saliva lies in the range of 0.5-2.5 mM-eq [29,40]. As a result, a solution containing $2\cdot10^{-4}$M $K_3[Fe(CN)_6]$ was used.

Table 5 shows the conditions for determining the antioxidant activity of saliva by the chronoamperometric method.

The accuracy of results is illustrated in Table 6. There is a good correspondence between the introduced and found values of AOA ascorbic acid and a sufficiently high degree of recovery of ascorbic acid, found after its introduction into the sample analyzed (Table 6.).

Table 5. Conditions for analysis of saliva.

Object	Concentration of $K_3[Fe(CN)_6]$, M	Preset potential, V	The measurement moment	Buffer solution volume, ml	Volume of the sample introduced into the cell, ml	Dilution of the sample
Saliva	$2\cdot10^{-4}$	0,4	80	5	0,1	51

Table 6. Results of AOA saliva determination by chronoamperometric method, before and after injection of ascorbic acid (n = 3, P = 0.95). Background: Phosphate buffer pH = 7.4.

Sample	Sample AOA, mM-eq	Ascorbic acid (AOA) introduced, mM-eq	AOA of the sample, containing addition, mM-eq	Ascorbic acid (AOA) found, mM-eq	Recovery, %
1	1,43±0,07	1,00	2,31±0,06	0,88±0,06	88,0
2	0,82±0,11	1,50	2,10±0,05	1,28±0,05	85,3
3	1,21±0,08	2,00	3,02±0,03	1,81±0,03	90,5

3.3. Comparison of the results of AOA determination of saliva by chronoamperometric and chronopotentiometric methods. Results of the determination of AOA of saliva samples by the methods of chronopotentiometry and chronoamperometry are given on Fig.7.

There is a high correlation of the results obtained ($R^2 = 0.92$).

The values of Student's and Fisher's criteria (texp = 0.32 and Fexp = 1.03) do not exceed the theoretical values (tteor = 2.31 and Fteor = 3.44 for significance level 0.05 for n = 3), which indicates an insignificant difference the results obtained by chronopotentiometric and chronoamperometric methods.

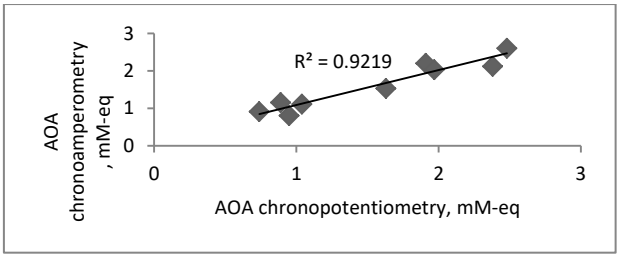

Figure 7. The results of the determination of saliva AOA by chronopotentiometric and chronoamperometric methods (n=3, P=0.95).

4. CONCLUSIONS

The hybrid versions of electrochemical methods for the determination of AOA, including a chemical signal-forming reaction that precedes or proceeds simultaneously with the recording of an analytical signal are described. AOA (analytical signal) information sources are:

- the electrode potential shift, observed when the sample is introduced into the mediator system- a mixture of 0.01M $K_3[Fe(CN)_6]$+0.0001M $K_4[Fe(CN)_6]$ (potentiometric variant);
- the increment of $K_4[Fe(CN)_6]$ oxidation current, resulting from the interaction of the AO, containing in the sample, with the oxidized form of the mediator system - 10^{-2}M $K_3[Fe(CN)_6]$ previously introduced into the solution (chronoamperometric version).

Here these versions are developed and used for saliva analysis. The methods are applied to the analysis of saliva as a noninvasive alternative to invasive sampling blood fractions investigation. The

existence of a relationship between oral diseases and the internal state of the body allows us to conclude that AOA of saliva can serve as a source of information on common health problems. Whereas that viability and apoptosis of cells depend on OS / AOA, it is logical to assume that the information on AOA of various biological fluids and tissues will be useful for understanding the physical-chemical processes in cells and on their interfaces.

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