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PORTABLE AMPEROMETRIC SENSOR FOR MEASURING THE TOTAL ANTIOXIDANT ACTIVITY OF SUBSTANCES

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ABSTRACT

Understanding the role of antioxidants as substances interrupting free radical reactions intensifies the search for new natural and synthetic substances with antioxidant properties. Along with this, intensive research is underway to create methods for assessing the antioxidant (AO) properties of various substances and devices for determining antioxidant activity (AOA). This article proposes an amperometric method and a sensor for measuring the total antioxidant activity of substances. The method is based on the process of oxygen electroreduction in the absence and presence of antioxidants of various origins in the solution. The measurement of the analytical signal before and after the introduction of the antioxidant substance into the electrochemical cell is carried out using a portable two-electrode sensor, "pencil" type, with a silver cathode, on which a potential of -500 mV is applied from a DC source (batteries). As an antioxidant standard, a 1% solution of ascorbic acid in a phosphate buffer with a pH of 6.86 was conventionally adopted. The sensor is connected to the measuring and driving device through a microcircuit - an operational amplifier. The measured signal in arbitrary units is displayed on the liquid crystal screen of the setting and measuring device and characterizes the maximum depolarization current of the indicator electrode. Using the resistors of the measuring device, a range of 0 - 100 units is set, for the analyte in the absence of an antioxidant and in the presence of a standard - 1% ascorbic acid solution, conventionally taken as 100%, respectively. Thus, the antioxidant activity of substances can be measured as a percentage relative to ascorbic acid (or other antioxidant). The results of determining the total antioxidant activity of some drinks and aqueous-alcoholic extracts of medicinal plants are presented.

KEYWORDS: Voltammetry, Oxygen electroreduction, Amperometric sensor, Antioxidants.

1. INTRODUCTION

Intensive studies of recent years on the creation of methods for assessing the antioxidant (AO) properties of various substances indicate that the problem of developing new, express, universal and affordable methods for their determination remains relevant [1-3].

The existing methods for monitoring AO are based on the use of chromatographic [4-8], spectrophotometric [9,10] and chemiluminescent [11-13] methods, which are characterized as laborious, time-consuming and expensive. The research results are often incomparable, since they were obtained using various model systems [14]. The question remains: what should be the criterion for assessing the antioxidant activity of substances? What should be taken as the benchmark for antioxidant activity (AOA)? To answer the questions posed, an adequate system for assessing antioxidant activity is needed, which allows one to correctly interpret the results obtained and compare them with different model systems. Thus, the problem of determining AOA lies not so much in the methodological and instrumental approach as in the methodological one.

Almost all researchers dealing with the problems of determining the antioxidant activity of natural and synthetic antioxidants note that the main property of AO is their ability to easily oxidize and take part in radical and redox reactions, which is accompanied by electron transfer. Therefore, a certain perspective is presented by methods for

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determining the antioxidant activity of substances using electrochemical methods, which are characterized by low cost, high sensitivity, the ability to analyze both aqueous and non-aqueous media.

Currently, among the methods for determining the antioxidant activity of substances using electrochemical methods of analysis can be distinguished: coulometric [15,16], potentiometric [18-21], amperometric [5,17,22], voltamperometric [23,24].

All of these methods have their own advantages, but they are also not without disadvantages. In these methods, the total antioxidant activity is determined by signal inhibition in the presence of antioxidants in a model or natural mediator system.

Considering that the voltammetric method, like the antioxidants themselves, is very sensitive to the presence of dissolved oxygen and its active radicals in the medium, Tomsk scientists Yu.A. Karbainov, E.I. Korotkova et al. Proposed a new approach to determining the total antioxidant activity of objects, using the process of oxygen electroreduction in the absence and presence of antioxidants of various origins in the solution as a model mediator system [24-26].

The established regularities of the process of electrochemical reduction of O_2 in the presence of antioxidants make it possible to use this process more optimally for analytical purposes.

The aim of this study is to develop the voltammetric principle of electrochemical determination of the total antioxidant activity of substances and to develop new algorithms for its determination using the developed amperometric sensor.

2. MATERIALS AND METHODS

We used a PU-1 universal polarograph connected to a computer [27]. Voltammograms were recorded in a glass cell using three- and two-electrode connection schemes. The working electrode was a silvered or amalgamated copper wire. The auxiliary electrode is a graphite rod. In the three-electrode scheme, all measurements were carried out relative to a saturated EVL-1M3 silver chloride electrode.

Silvering of the working electrode was carried out by lowering a copper wire 1 mm in diameter, purified with concentrated nitric acid and then washed with distilled water, in a 0.1 M $AgNO_3$ solution for 5 minutes. Then the electrode was dried at 110 ^{0}C, polished with filter paper, and again immersed in a solution of silver nitrate. The operation was carried out 3-4 times until a uniform silver coating was formed on the surface.
Amalgamation was carried out in a similar manner, by immersing the cleaned copper wire in a 0.1 M $Hg(NO_3)_2$ solution.

We also used an I-130 M ionomer (ZIP, Gomel, Belarus) operating in the pH meter mode, with an ESL-63G glass electrode and a Tecnomiers 5221 oxygen meter (Poland).

As test solutions, solutions of ascorbic acid of known concentration were used, 0.2 ml of which were introduced into a cell with a supporting electrolyte (0.05 M phosphate buffer, pH 6.86 and 0.05 M tetraborate buffer with pH 9.18).

Research on antioxidant activity was carried out on substances of plant and animal origin, as well as some drinks.

3. RESULTS AND DISCUSSION

It is known that the electroreduction of oxygen at the electrode proceeds in several stages with the generation of reactive oxygen species on the electrode surface [28]:

$$O_2 + e^- \rightleftarrows O_2^{*-} \quad E^0 = +0.012\ V \qquad (1)$$

$$O_2^{*-} + H^+ \rightleftarrows HO_2^* \qquad (2)$$

$$HO_2^* + H^+ + e^- \rightarrow H_2O_2\ ;\ E^0 = +0.682\ V \qquad (3)$$

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$$H_2O_2 + 2H^+ + 2e^- \underset{\leftarrow}{\overset{\leftarrow}{\rightarrow}} 2H_2O \; ; E^0 = +1.770 \text{ V} \qquad (4)$$

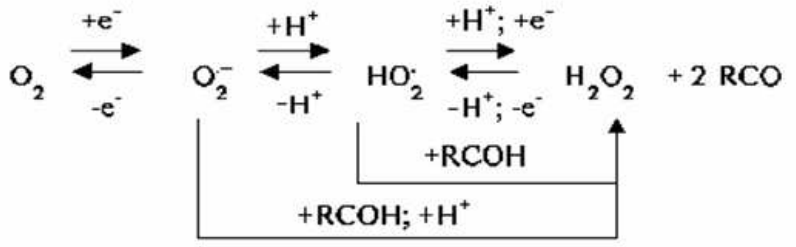
To determine the activity of antioxidants, the authors of [28, 29] proposed to use the first wave of O_2 electroreduction corresponding to stages (1) - (3), when active oxygen radicals and hydrogen peroxide are formed on the surface of the indicator electrode as the final product. However, when assessing the effect of the nature of antioxidants on the process of oxygen electroreduction, it was noted that they have different effects on this process, while they themselves are not electrochemically active in this potential range (E = 0… –1.0 V). Therefore, all known antioxidants were divided into three groups (Table 1.) [29].

Table 1 Groups of substances differing in the nature of their influence on the process of oxygen electroreduction (ER)

Group number	Name of substances	Influence on ER O_2	Prospective electrode mechanism
1.	Metal complexes: catalase, phthalocyanines, metal porphyrins, humic acids	Increase in the ER O_2 current, potential shift towards the negative region	EC mechanism with partial regeneration of molecular oxygen
2.	Phenolic compounds, vitamins A, E, C, B, flavonoids, ubiquinones, glucose	Decrease in the ER current of O_2, potential shift to the positive region	EC mechanism
3.	N, S, Se-containing compounds, amines, amino acids, active aldehydes	Decrease in the ER O_2 current, potential shift towards the negative region	CEC mechanism

Note: E - electrode stage of the process, C - chemical reaction

Of these groups, the second group of substances is the most numerous, since it is these substances that are classified as classical AO. Substances of this group decreased the current of O_2 electroreduction, shifting the potential to the positive region and exhibiting the EC mechanism with subsequent chemical reactions of interaction between AO and active oxygen radicals. It is assumed that the substances of the third group interact mainly according to the CE_mechanism with molecular oxygen dissolved in the electrolyte, or the CEC_mechanism with the preceding and subsequent chemical reactions of the interaction of AO with O_2 and the products of its reduction. A possible model of this process can be represented by the following diagram: [26,29].



where RCOH and RCO are the reduced and oxidized forms of the antioxidant substance, respectively.
It follows from the proposed model that the ER O_2 is a complex, multi-stage process, and the implementation of each stage, its reversibility, kinetic parameters depend on a number of factors. Therefore, in this case, it is advisable to consider only the delayed stage of the cathodic reduction of oxygen, which greatly simplifies the scheme of the electrode process of ER O_2 for medium pH values: 3 <pH <10 in aqueous media:

$$O_2 + e^- \underset{}{\overset{k_o}{\rightleftharpoons}} O_2^{\bullet -} + \text{R-OH} + H^+ \underset{}{\overset{k_1^*}{\rightleftharpoons}} H_2O_2 + \text{R=O}$$

Based on this scheme, we investigated the electrochemical process of oxygen electroreduction on electrodes made of various materials, as well as the adequacy of the obtained analytical signal for the presence of antioxidants in the solution.

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Figure 1 shows the volt-ampere dependences of the ER O_2 on silvered and amalgamated electrodes in a background electrolyte without and in the presence of an antioxidant (ascorbic acid) against the background of phosphate and tetraborate buffer solutions with pH 6.86 and 9.18, respectively.

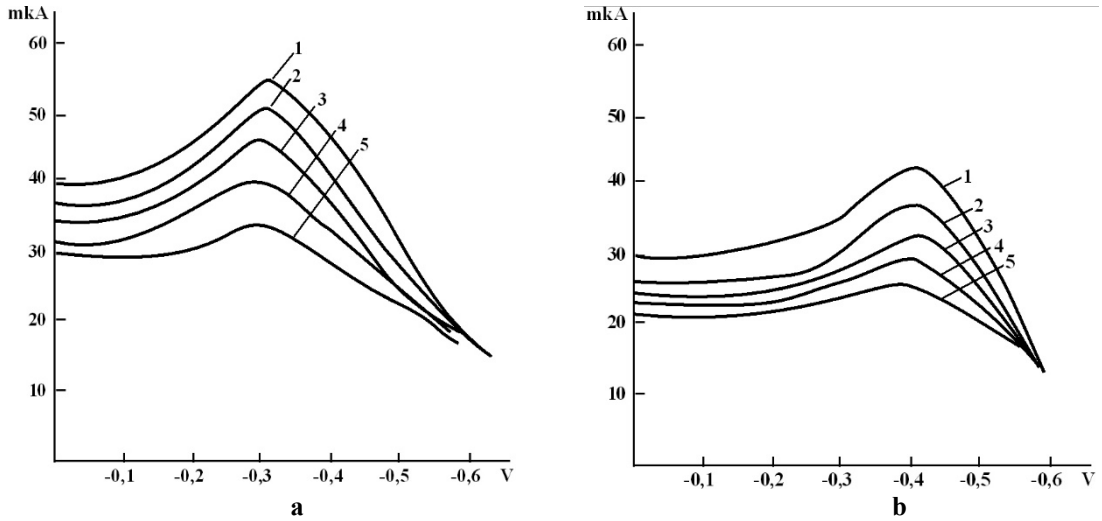

Fig. 1. Polarization curve of ER O_2 on silvered (a) and amalgamated (b) electrodes in phosphate-buffered saline with pH 6.86 containing various concentrations of ascorbic acid.

1 - background; 2 - 0.25% ascorbic acid; 3 - 0.50% ascorbic acid; 4 - 0.75% ascorbic acid;
5 - 1.0% ascorbic acid.

As can be seen from the figures, the polarization potential of the first wave of electroreduction of dissolved oxygen for the amalgamated electrode is shifted to the region of more negative values, compared to those for the silver electrode. For a silver electrode, the limiting current values are also higher than for an amalgamated one.

Figure 2 shows the polarization curves for the same electrodes against the background of a tetraborate buffer with pH 9.18.

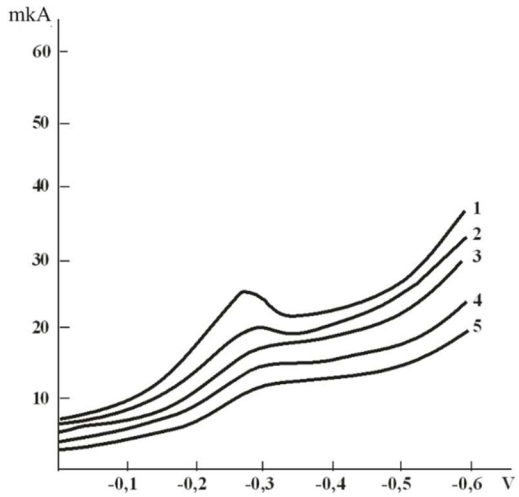

Fig 2. Polarization curve of ER O_2 on a silvered electrode. (pH 9.18)

1 - background
2 - 0.25% ascorbic acid
3 - 0.50% ascorbic acid

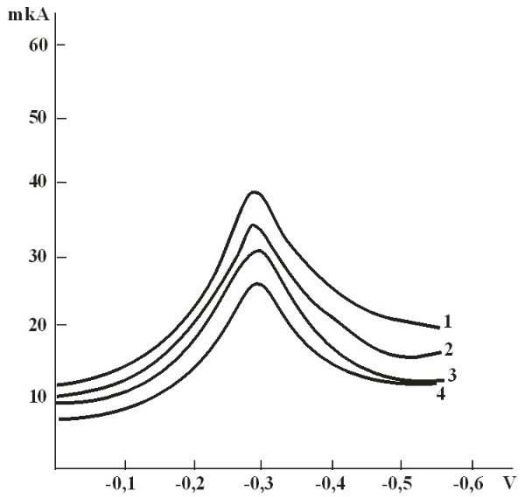

Fig. 3. Polarization curve of ER O_2 on an amalgamated electrode. (pH 9.18)

1 - background
2 - infusion of green tea. Dilution 1: 4
3 - infusion of green tea. Dilution 1: 2

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4 - 0.75% ascorbic acid
5 - 1.0% ascorbic acid

4 - infusion of green tea original concentration

The limiting currents of oxygen electroreduction in the presence of an antioxidant are lower than the values obtained against the background of a phosphate buffer with a pH of 6.86, and the polarization potential values are shifted to a less negative region. The polarization curves of the ER of oxygen on the amalgamated electrode at pH 9.18, obtained in the presence of tea antioxidants, show a clear dependence of the depolarization current of the indicator electrode on the concentration of the antioxidant substance (Fig 3).

In fig. 4 shows the dependences of the limiting current on the concentration of ascorbic acid solution for silvered (1) and amalgamated (2) electrodes.

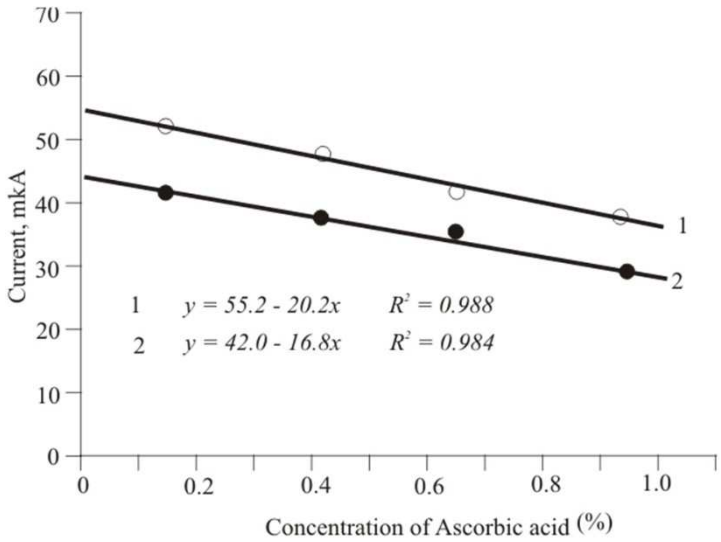

Fig. 4. Concentration dependences of the limiting current on the concentration of ascorbic acid for silvered (1) and amalgamated (2) electrodes.

The regression coefficients R^2 for these dependences are 0.988 and 0.984, respectively, which, in principle, is equivalent. In the first case, the tangents of the slope angles of the calibration graph are slightly larger: tg α_1 = 0.3838 and tg α_2 = 0.3057, respectively.

Thus, the studies carried out have shown that the silver and amalgamated electrodes give an adequate response to the presence of an antioxidant substance in the solution. At the same time, the silver-silver electrode meets the requirements of ecological analysis to a greater extent. The results of these studies served as the basis for the development of a portable amperometric sensor for measuring the total antioxidant activity of substances [30]. The method is based on the measurement of the depolarization current of the working electrode during the cathodic electroreduction (ER) of oxygen dissolved in the buffer medium before and after the introduction of an antioxidant substance into the electrochemical cell.

Figure 5 shows a schematic diagram (a), a general view (b) and a method of using an amperometric sensor to determine the total AOA (c).

The sensor includes a working electrode - a cathode made of silver copper wire 1 mm in diameter and an auxiliary electrode made of copper wire or carbon-graphite rod with a diameter of 1-2 mm. A reference voltage of -500 mV from a DC source (battery) is applied to the cathode. This potential corresponds to the first wave of the ER of oxygen in weakly protonated solutions.

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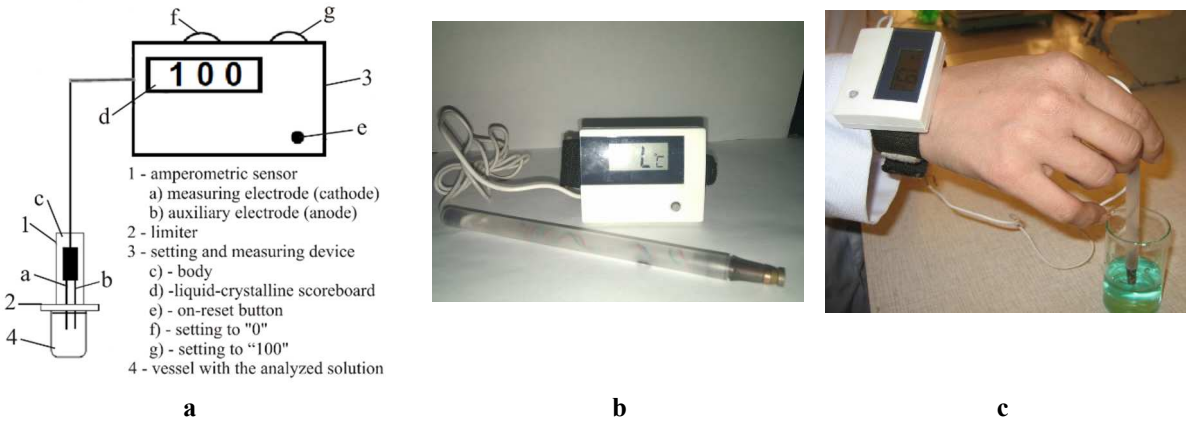

a **b** **c**

Fig. 5. Schematic diagram (a), general view (b) and method of using (c) an amperometric sensor to determine the total AOA.

The sensor is connected to the measuring and driving device through a microcircuit - an operational amplifier, which provides indication of the maximum depolarization current, as well as the ability to set the scale range. Before starting work, the sensor is calibrated by immersing it in a buffer solution (0.5 M phosphate buffer, pH = 6.86). The readings on the digital display are set to "0" using a resistor on the device case. Then a dosed volume of a solution of a substance - an antioxidant, for example, ascorbic acid, of a certain concentration is introduced into the buffer solution, and the readings of the device with the help of the second resistor are set to "100". A calibrated device is used to measure the AOA of other biological products. Thus, the AOA of solutions of substances can be measured as a percentage relative to ascorbic acid (or other antioxidant).

The small dimensions of the setting and measuring device make it possible to place it on the wrist of the operator's right hand using a “burdock” strap. The performance of any analytical device will be characterized by reproducibility of measurement results. In our case, this parameter is directly related to the characteristics of the working electrode. In this regard, the dynamics of the change in the analytical signal of the sensor with a silver working electrode in time was studied, which makes it possible to establish the optimal service life of the electrode without additional regeneration. A series of measurements of the stability of the sensor readings were carried out for 10 measurements in each series. For the time limiting the service of the working electrode, a 10% decrease in the signal was taken.

The observation results are shown in Figure 6.

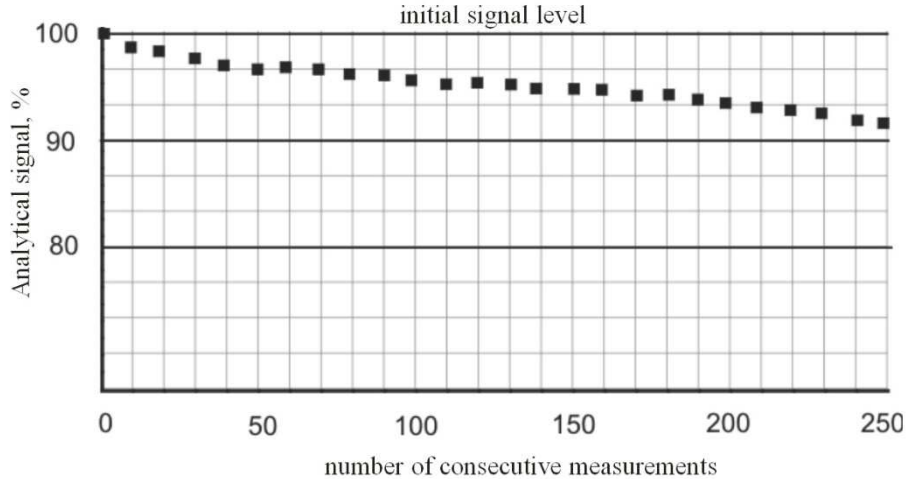

Fig. 6. The dynamics of changes in the analytical signal of the working electrode (silvered copper wire) in time.

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As follows from this diagram, the service life of the indicator electrode corresponds to at least 250 measurements. After that, another regeneration should be carried out, which consists in cleaning the electrode surface with a solution of nitric acid and applying a layer of silver on it. Thus, during laboratory operation of the sensor, its service life can be 7 - 10 days.

This is quite acceptable, given the fact that regeneration of the working electrode surface is not a laborious and complicated operation. In the course of laboratory studies, the antioxidant activity of aqueous-alcoholic solutions of substances of plant and animal origin, as well as some drinks (red wine), was measured.

The activity value of 1% ascorbic acid solution was taken as 100% AOA. Infusions of green tea, tannin, sage, chamomile, brewer's yeast, and wine were studied. In all cases, except for the measurement of yeast AOA, 1% solutions of these substances were used. Green tea, chamomile, and sage (1 g dry weight) were infused in distilled water at t = 550 C for 30 minutes, followed by cooling to room temperature. Yeast in the amount of 100 mg was diluted in 10 ml of distilled water. We have already noted that the antioxidant activity of substances is due to their ability to bind molecular oxygen and its radicals in electrolyte solutions. In order to establish the adequacy of the obtained analytical signal of the sensor precisely with the presence of an antioxidant substance in the solution, we compared the results of measurements using the Clarke electrode and the developed amperometric sensor. The measurement results are summarized in Table 2.

Table 2 The results of evaluating the antioxidant activity of some

Name of the drug with AOA	Dilution of the stock solution		
	1:10	**1:4**	**1:2**
Green tea	45	55	65
Tannin	18	24	35
Sage	25	35	45
Chamomile	35	45	55
Yeast	55	70	85
Red wine	64	95	110

For this purpose, the Clarke electrode, connected to the corresponding connector of the Tecnomiers 5221 oxygen meter, was immersed in a cell — a glass with 20 ml of distilled water. The sensitivity "50" of the oxygen meter measuring scale was set to the maximum value (in our case it corresponded to "90"). Then, using a micropipette, a solution of an antioxidant substance was introduced into the cell with a step of 0.1 ml. Oxygen meter scale readings were recorded after each addition. In parallel, measurements were carried out using an amperometric sensor. The measurement results are presented by correlation dependences in Figure 7.

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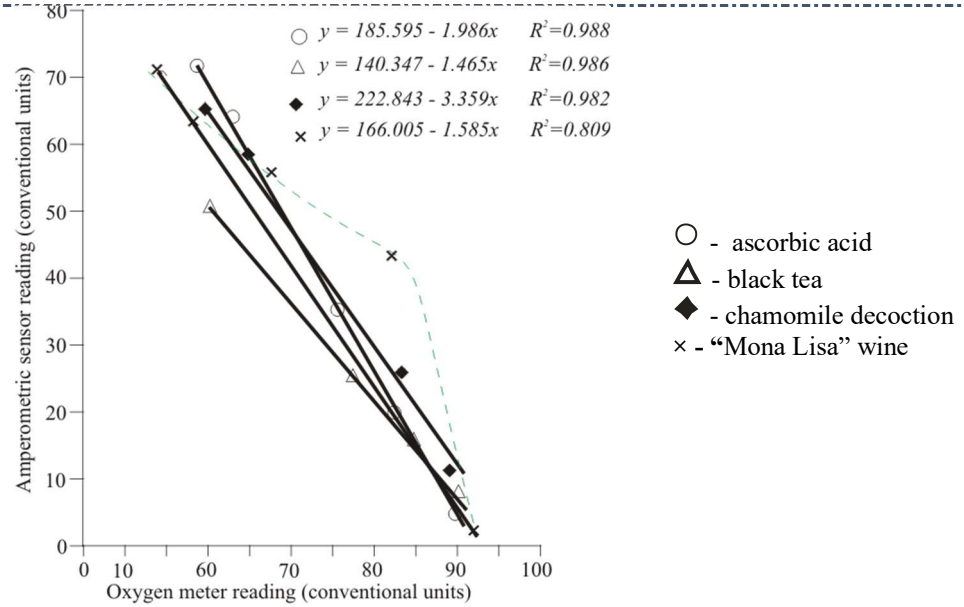

Fig. 7. Correlation of the analytical signal obtained by different methods for solutions of some antioxidants.

As can be seen from the correlation dependences, the analytical signal obtained using the developed amperometric sensor is in good agreement with measurements of the dissolved oxygen concentration. The correlation coefficient, excluding Mona Lisa wine, is 0.98. The relatively low correlation $R^2 = 0.8$ for the red dessert wine "Mona Lisa" can be explained by the fact that in addition to antioxidants of a phenolic nature, the wine contains antioxidants belonging to the 3rd group (Table 1), namely N, S, Se-containing compounds, amines, amino acids, active aldehydes.

The developed amperometric sensor was used to assess the relative antioxidant activity of aqueous-alcoholic plant extracts and pharmaceutical preparations. As a comparison, we used the analytical signal of the sensor in a 1% aqueous solution of ascorbic acid, which was taken as 1 (100%). The research results are presented by the diagram in Figure 8.

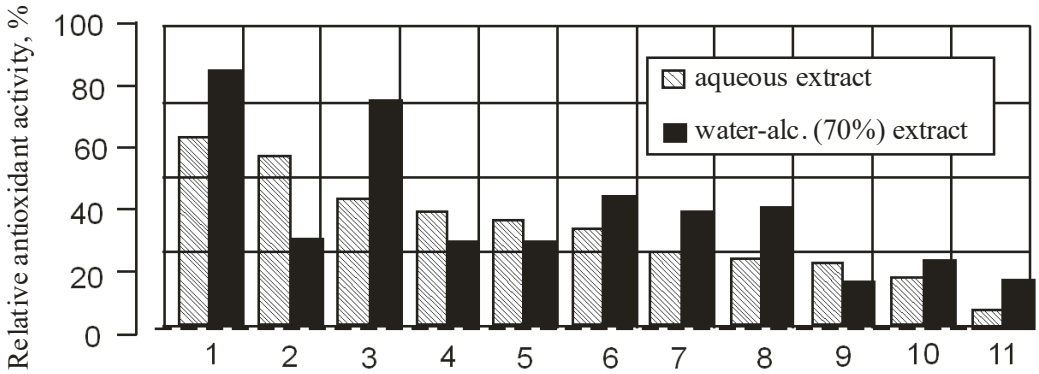

Fig. 8. Relative antioxidant activity of aqueous-alcoholic solutions of some plants and pharmacy fees (standard 1% solution of ascorbic acid):

1- aloe juice, 2-rhodiola rosea, 3-sea buckthorn, 4-currant leaf, 5-oak bark, 6-plantain leaves, 7- wormwood roots, 8 - corn stigmas, 9 - yarrow flowers, 10-St. John's wort, 11- flowers of common dandelion.

As can be seen from the figure, the studied plant extracts have different antioxidant activity. In this case, alcohol solutions, as a rule, have large values of the total AOA. The results obtained indicate that the proposed method for assessing the AOA of drugs can be used for screening antioxidant substances of natural and synthetic origin.

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4. CONCLUSION

During the research, a portable amperometric sensor was developed to determine the total antioxidant activity of substances relative to ascorbic acid, which was selected as a control antioxidant.

The sensor is made in the shape of a pencil and contains two electrodes: an annular cathode made of silver-plated copper and an anode made of a carbon rod . A potential of -0.5 V from a direct current source (battery) is applied to the cathode. The analytical signal measured in a phosphate buffer with a pH of 6.86 is compared with the signal after the introduction of the analyte. The measurement principle is based on the cathodic reduction of dissolved oxygen in the analyzed liquid. The measured signal in conventional units is displayed on the liquid crystal screen of the measuring device and characterizes the maximum depolarization current of the indicator electrode. The sensor allows for multiple measurements. After 150-200 measurements, the surface of the electrodes is updated by depositing a silver film on the copper surface of the cathode. The sensor shows an adequate response to the presence of dissolved oxygen in the analyzed solution. Testing of the portable sensor in measuring the total antioxidant activity of a number of beverages, plant extracts showed the possibility of its use for routine determination of the presence of antioxidant activity of substances.

5. ACKNOWLEDGEMENTS

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6. CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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