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Portable potentiometric device for determining the antioxidant capacity

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Abstract

At present, the development of portable devices for the express assessment of the content of biologically active objects, such as antioxidants, is one of the relevant technological problems of modern chemistry, medicine, and engineering. The main advantages of such devices are the simplicity and rapidity of analysis, small volumes of analyte, as well as miniaturization of equipment, making it possible to carry out the on-site analysis and, thus, to take a step towards the personalized medicine. The potentiometric method using the $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ system, which in the laboratory-scale version proved to be the most accurate, reproducible, and express, was the basis for the developed prototypes of portable devices. In this study, two versions of prototypes of the portable device are proposed, namely, the open microcell with the 0.2 ml volume and the microfluidic device with flow control. The correctness of the antioxidant capacity (AOC) determination in both systems was confirmed by comparing the results of the "introduced-found" method on model solutions of antioxidants and their mixtures with the AOC results obtained in a standard laboratory electrochemical cell. The relative standard deviation did not exceed 10%. The AOC of some beverage industry was determined using the microfluidic device. The correlation coefficient of the results, obtained in the microfluidic device and the laboratory cell, was 0.90, which indicates good data convergence and the possibility of using the potentiometric method implemented in the microfluidic device to assess the AOC of multicomponent objects.

Keywords

portable device
antioxidant
potentiometry
microcell
microfluidic device

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Key findings

- The open microcell and the microfluidic device were developed for the determination of the antioxidants capacity of various objects.
- The potentiometric method implemented in the devices allows estimating the AOC of model and multicomponent objects.
- The relative standard deviation of the AOC in the devices did not exceed 10%.

1. Introduction

It is known that the oxidative stress is one of the factors causing the occurrence of many pathological conditions in the human body, on the one hand, and accompanying the course of diseases and causing complications in a large number of diseases, on the other. The risk of the oxidative stress increases in the conditions of the technogenic development of society, the environmental degradation, the growth of pathological conditions of the population. As a consequence the assessment of the antioxidants content in various objects, such as food, pharmacy, biological objects, is becoming more and more demanded [1–3].

One of the relevant technological tasks of modern chemistry, engineering and medicine is the transition to the miniaturization of equipment and the creation of portable devices for personal use. Their undoubted advantages are the simplicity and rapidity of analysis, and small volumes of the analyte, which will make it possible to take a

step towards personalized medicine. From this point of view, the purpose of this work was the creation of a portable device for the express determination of the antioxidants content in various objects.

Currently, a large number of methods have been developed for determining the antioxidant content. Based on the three main mechanisms of antioxidant action in the human body, the existing methods can be classified into the methods based on the reaction of electron transfer from an antioxidant to a reagent (ET-mechanism), the reaction of a hydrogen atom transfer from an antioxidant to a reagent (HAT-mechanism), and the complex formation reactions of an antioxidant with metal ions of variable valence [4].

The developed methods for determining the antioxidant content are quite accurate and informative, but they have disadvantages and limitations, which mainly include the complexity of the methodology and equipment, and the high cost of instruments and reagents. The main disadvantage of optical methods for determining the antioxidant content, which include such well-known approaches as TEAC (Trolox equivalent antioxidant capacity) [5, 6], TRAP (Total radical trapping parameter) [7, 8], ORAC (Oxygen radical absorbance capacity) [9], FRAP (Ferric reducing antioxidant power) [10], CUPRAC (Cupric Reducing Antioxidant Capacity) [11], is the difficulty in studying turbid and colored samples. Many methods for assessing antioxidant properties use strong oxidizing agents, or the analysis is carried out in an acidic solution, which, again, makes it difficult to use them for the analysis of objects containing proteins and amino acids.

The literature describes works on the creation of devices for the transition to miniaturization for some of the methods for determining the antioxidants content: FRAP and CUPRAC, implemented in the form of test strips [12–14], sensors based on nanoparticles of transition metal oxides (CeO_2, Fe_2O_3, SiO_2, etc.) [15–17]. Despite the high reproducibility and accuracy of the analysis results, the devices have disadvantages associated with the difficulty in manufacturing and using them on-site, as well as the use of expensive materials and reagents.

For more objective information about the antioxidant properties of objects, it is advisable to use the integrated approach that allows one to evaluate the antioxidant effect by three main oxidation mechanisms [18]. Numerous studies [3, 19] show that, despite the variety of mechanisms of antioxidant action in the human body, the determination of antioxidant content using the ET-mechanism is quite informative and has a high correlation degree with known methods of analysis [18]. In addition, methods based on the ET-mechanism are simple to implement, which has been confirmed by numerous works.

Electrochemical methods are among the most promising methods of analysis, characterized by the simplicity of techniques and equipment, and the low cost of devices. The potentiometric method, in contrast with the other electrochemical methods, is more rapid and sensitive, allows studying turbid and colored samples, and the instrumentation can be implemented in a portable format [20–23].

In this work, the potentiometric method using the $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ system, which in the laboratory-scale version proved to be the most accurate, reproducible, and express, was the basis for the developed prototypes of portable devices [24]. Our proposed approach for creating the analytical platform for determining the antioxidant content includes the transition from a standard laboratory electrochemical cell to a microcell for the purpose of conducting studies in small volumes, and then creating a prototype of a microfluidic device.

2. Experimental

2.1. Reagents and objects

The following reagents were used in this work: potassium hexacyanoferrate (III) $K_3[Fe(CN)_6]$ (Sigma-Aldrich, USA), puriss. grade; potassium hexacyanoferrate (II) $K_4[Fe(CN)_6]$ (Reachim, Russia), puriss. grade; KCl (Sigma-Aldrich), puriss. grade; L- ascorbic acid (Panreac), puriss. grade; L-cysteine (Panreac), puriss. grade.; Glutathione (Panreac), puriss. grade.; Pyrogallol (Panreac), puriss. grade.; Caffeic acid (Panreac), puriss. grade.; Rutin (Panreac), puriss. grade.; Quercetin (Panreac), puriss. grade.; Luteolin (Panreac), puriss. grade.; Dihydromyricetin (Panreac), puriss. grade.; Phloroglucinol (Panreac), puriss. grade.; Catechol (Panreac), puriss. grade.

As objects, mass-produced drinks were investigated: Rich Orange, Sady Pridonia Apple-Cherry, Lyubimiy Peach, Dobry Cherry, Lyubimiy Multifruit, Dobry Apple-Pear, Lipton Green Iced Tea, and Coca-Cola Lime.

2.2. Apparatus

Potentiometric measurements were carried out using the pH-meter Expert-pH (OOO Econics-Expert, Moscow).

EPV-1 redox platinum electrode (Gomelsky ZIP, Gomel, Belarus) and EVL-1M silver-silver chloride electrode (Ag/AgCl/3M KCl) (Gomelsky ZIP, Gomel, Belarus) were used as electrodes when working with the standard laboratory electrochemical cell, and platinum wire and silver wire coated with an insoluble silver salt – for operation in the microcell and microfluidic device.

The design of the microcell and microfluidic device was carried out in Tinkercad for 3D modeling. The devices were printed on the Longer Orange 10 3D printer (Longer, China) using SLA technology, which is characterized by high accuracy, good surface quality, no visible polymer layers, and a wide selection of consumables. The photopolymer with increased strength characteristics was used as a material.

The selection criteria for this polymer resin were:

– high speed, accuracy and reproducibility of printing;

– the ability to create complex models (including systems of microchannels with thin walls);

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– high level of detail;
– resistance to reagents of the system;
– easy post-processing.

The reagents and samples were introduced into the microfluidic device using the Syringe Pump Model No 1000 (Syringe Pump, NY, USA).

2.3. The potentiometric method of determining AOC

The potentiometric method of determining the antioxidant capacity was used with the $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ oxidizing agent to measure the potential of the platinum electrode of the $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ system and after the chemical reaction between the sample antioxidants and the oxidizing agent [24]. The potential change occurs as a result of a chemical reaction in solution (1):

$$n[Fe(CN)_6]^{3-} + AO = n[Fe(CN)_6]^{4-} + AO_{Ox}, \quad (1)$$

where AO – an antioxidant, AO_{Ox} – an oxidation product of antioxidant.

The dependence of the potential change on time is shown in Figure 1.

Antioxidant capacity (AOC) is calculated as (2–3):

$$AOC = \frac{C_{Ox} - \alpha \cdot C_{Red}}{1 - \alpha} \cdot n, \quad (2)$$

$$\alpha = \left({}^{C_{Ox}}/_{C_{Red}}\right) \cdot 10^{(E_2 - E_1)\cdot \frac{2.3 \cdot R \cdot T}{n \cdot F}}, \quad (3)$$

where C_{Ox} is the $K_3[Fe(CN)_6]$ concentration, mol/dm³; C_{Red} is the $K_4[Fe(CN)_6]$ concentration, mol/dm³; E_1 is the potential measured before the introduction of a test sample, V; E_2 is the potential measured after the addition of the test sample, V, n is the dilution degree.

Since potassium hexacyanoferrate (III) satisfies the requirements for oxidizing agents that can be used to determine antioxidant properties [25], as previously established, AOC is an integral parameter equal to the total effective equivalent concentration of potassium hexacyanoferrate (III) that entered in reaction with antioxidants of the analyzed sample.

The half-life period ($t_{1/2}$, s) of the interaction of the studied compound with potassium hexacyanoferrate (III) was calculated from the kinetic curves of the concentration change of the reduced form of iron on time during reaction (1). The half-life period corresponds to the time at which 50% AOC is recorded ($AOC_{1/2}$ = AOC/2 mol/l) (Figure 2).

The experimentally obtained stoichiometric coefficient is calculated as the ratio of AOC to the introduced antioxidant concentration (n = AOC/C_{AO}).

2.4. Data treatment

The measurements were replicated five times. Statistical evaluation was performed at the significance level of 5%. All data were expressed as $X \pm \Delta X$, where X is the average value and ΔX is the expanded standard uncertainty. The relationship between the results obtained in the microcells and in the standard laboratory cell was calculated using the Pearson correlation.

The convergence of the analysis results in micro- and macrocells was assessed using the t-test to compare mean values and the F-test to compare dispersions.

2.5. Quantitation limit

The quantitation limit C_{lim} was calculated by a probabilistic method [26]. The dependence of the relative standard deviation σ on the average values, in this case, the antioxidant capacity (AOC), determined by the potentiometric method in the microfluidic device, for the entire concentration range, was built for this purpose.

The quantitation limit was estimated from the plot of $\sigma = f(C_i)$ dependence. The C_{lim} value corresponded to the minimum content of the sample component determined by this approach with the relative standard deviation σ = 0.33.

3. Results and Discussion

In this work, the determination of the antioxidant capacity in the standard laboratory electrochemical cell and the microsystems was carried out by the potentiometric method using the $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ system.

The following requirements guided the choice of the oxidizer model [25]:

– the reaction of electron transfer from AO to the oxidant molecule must be thermodynamically possible;

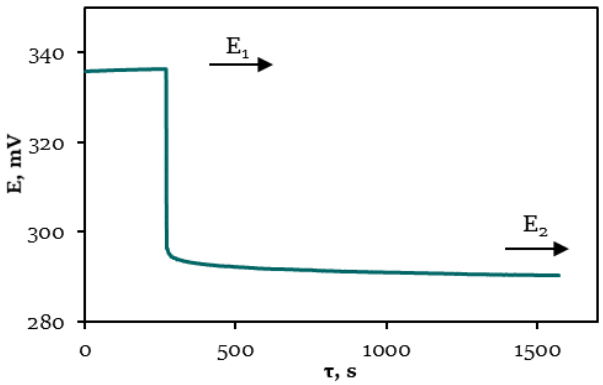

Figure 1 Time-dependent potential change when the pyrogallol (C = 0.1 mM) is introduced into the solution of the $K_3[Fe(CN)_6]$ (0.01 M)/$K_4[Fe(CN)_6]$ (0.1 mM) system.

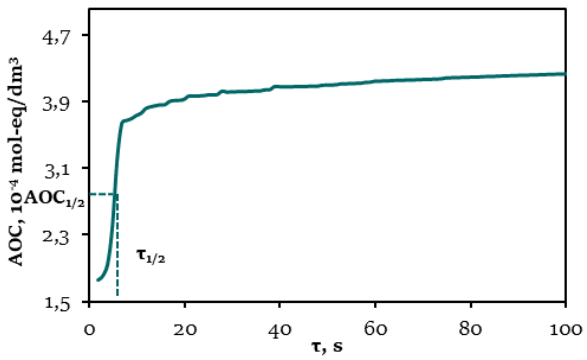

Figure 2 AOC change with time during the interaction of the pyrogallol with the $K_3[Fe(CN)_6]$ ($K_3[Fe(CN)_6]$ (0.01 M)/$K_4[Fe(CN)_6]$ (0.1 mM)).

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– the redox potential of the oxidizing agent under the analysis conditions should be between the potentials of active oxygen metabolites and AO, but there should be a certain difference between them;

– the reaction rate between the oxidizing agent and the antioxidant must be sufficiently high.

In this case, the choice of $K_3[Fe(CN)_6]$ as a model of an oxidizing agent is substantiated theoretically and experimentally as an optimal model for studying the antioxidant properties of compounds according to the electron transfer mechanism [27–28].

This method makes it possible to evaluate the redox characteristics of the studied compounds and the thermodynamic possibility of their interaction with active oxygen metabolites, which is a rather important parameter in the study of antioxidant properties. The method for evaluating antioxidant properties using potassium hexacyanoferrate (III) as a model of an oxidizing agent was tested on a large number of objects and proved to be accurate, informative, simple, and express [24, 25, 28].

At the first stage, to move towards microvolumes, the open microcell with the 0.2 ml volume, structurally repeating the form of a standard laboratory electrochemical cell (Figure 3), was developed. The volume and form of the microcell were selected empirically. The potential was recorded using the electrode pair – the platinum wire and the silver wire, electrochemically coated with an insoluble silver salt. The background electrolyte was the 0.1 M KCl solution.

The platinum wire was used as a working electrode. The potentials of platinum wire relative to silver wire and silver wire with electrochemically deposited silver chloride were recorded at different ratios of the components of the oxidized and reduced forms of the $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ system in the 0.1 M KCl solution to select the reference electrode.

High reproducibility, as well as potential stability, was achieved using silver wire coated with electrochemically deposited silver chloride. Thus, all further studies were carried out with respect to this reference electrode. The slope of the dependence of the potential on the logarithm of the $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ concentration ratio in the microcell relative to the silver wire coated with electrochemically deposited silver chloride was 57±1 mV/decade (Figure 4).

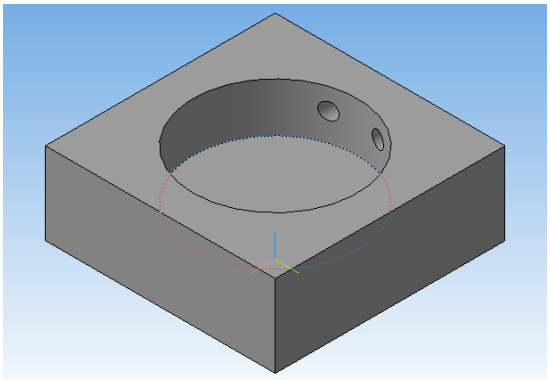


Figure 3 Prototype of the open microcell.

Model solutions of antioxidants and their mixtures with known mechanisms of oxidation by the electron transfer mechanism were chosen as analysis objects [29–33]. It is known that one of the mechanisms of oxidation of ascorbic acid and pyrogallol consists in the transfer of two electrons and two hydrogen atoms (Scheme 1–2). Thiol compounds such as the cysteine and glutathione are oxidized to form dimers. In this case, one electron and one hydrogen atom are transferred from one antioxidant molecule (Scheme 3–4).

Table 1 presents the AOC values of model solutions of antioxidants and their mixtures in different ratios obtained in the microcell. The correctness of the AOC determination was confirmed by the "introduced-found" method, taking into account stoichiometric coefficients, as well as in comparison with the data obtained in the standard laboratory electrochemical cell (in macrocell).

It follows from the data in the Table 1 that the interaction of ascorbic acid (AA) and cysteine (Cys) occurs according to equations (4–5), which correspond to the mechanisms of oxidation of these AOs described in the literature.

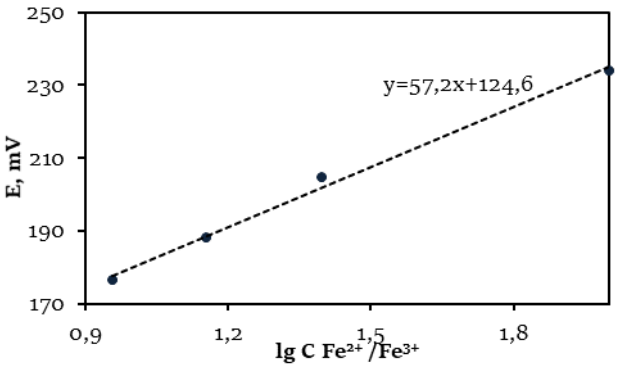
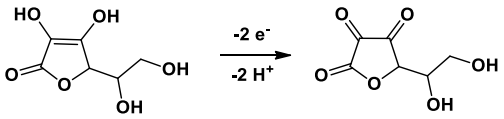
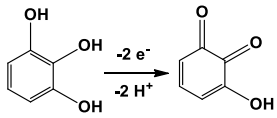


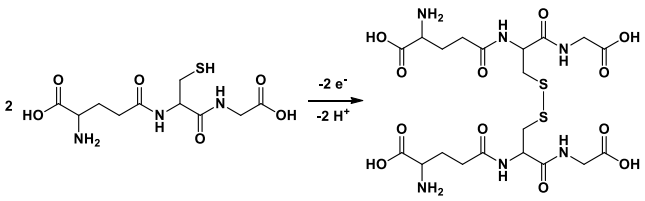
Figure 4 Dependence of the potential on the logarithm of the $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ ratio, obtained using a microcell.



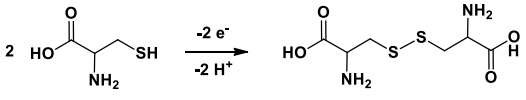
Scheme 1 Ascorbic acid oxidation scheme.



Scheme 2 Pyrogallol oxidation scheme.



Scheme 3 Glutathione oxidation scheme.



Scheme 4 Cysteine oxidation scheme.

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$$2K_3[Fe(CN)_6]+AA=2K_4[Fe(CN)_6]+AA_{OX} \quad \overset{-2e,\ -2H^+}{\curvearrowright} \quad (4)$$

$$K_3[Fe(CN)_6]+Cys=K_4[Fe(CN)_6]+Cys_{OX} \quad \overset{-1e,\ -1H^+}{\curvearrowright} \quad (5)$$

In case of pyrogallol, rather high values of stoichiometric coefficients were obtained in the reaction with potassium hexacyanoferrate (III). Such values are associated not only with the electron transfer mechanism, but also with the possible mechanism of complex formation with iron ions [34–35] as a result of a competing reaction. It is known that polyphenolic compounds having gallic structures are able to form fairly stable complexes with metals of variable valence and inhibit radical processes at the stage of chain branching (Scheme 5).

Thus, the AOC values of the model solutions of antioxidants obtained in the microcell are confirmed by the "introduced-found" method, taking into account stoichiometric coefficients, and these values are similar to those obtained in the standard electrochemical cell. The calculated t-test and F-test these values are range from 0.5 to 1.7 and 0.4 to 1.1, respectively, which are significantly below the critical values at a 95% confidence level (t_{krit} = 2.57, F_{krit} = 5.05). This shows that the variances of the two populations are homogeneous. The relative standard deviation does not exceed 8%.

Since the AOC data obtained using the microcell are quite reproducible and similar to the results obtained in the laboratory cell, the microcell design can be used to determine the content of antioxidants. However, the open design of the microcell is difficult to use on-site.

The design of the second model in the form of the microfluidic device was developed to eliminate this drawback.

Figure 5 shows the scheme of preparation and analysis on the microfluidic device.

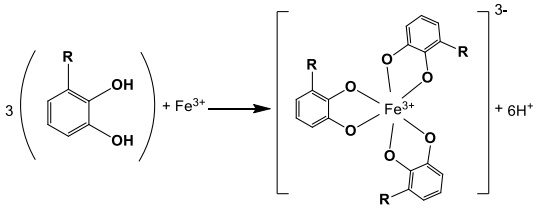
The scheme of the microfluidic device is shown in Figure 6.

For analysis, the purified platinum wire and the silver wire coated with an insoluble silver salt are placed in the electrode holes of the microfluidic device (stage I). The next step is to wash the microfluidic device with water and the solution of the $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ system (stage II). The systems of potassium hexacyanoferrates (1) and analyte (2) are injected using the syringe pump. In this case, the $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ system is pumped continuously at a constant flow rate (stage III). The antioxidant solution is injected after equilibrium is established on the platinum electrode (stage IV). The system solution, flowing through the microfluidic channels (3) to the near-electrode space (4), is mixed with the antioxidant. Then, the potential change is recorded. All studies were carried out in 0.1 M KCl.

The kinetic characteristics, namely, the half-reaction period of the interaction of the antioxidant with $K_3[Fe(CN)_6]$, for a number of natural antioxidants, which are most often found in food and pharmaceutical objects, were analyzed to select the length and shape of microfluidic channels (Table 2).

Table 1 AOC of model solutions of antioxidants (C_{AO}=0.1 mmol/dm³, n = 5, P = 0.95) and their mixtures.

Name	C_{AO}, 10^{-4} mol/dm³	AOC, 10^{-4} mol-eq/dm³ (in micro-cell)	AOC, 10^{-4} mol-eq/dm³ (in macro-cell)	n
Ascorbic acid	1	1.83±0.05	1.98±0.02	1.83
Cysteine	1	1.10±0.04	1.01±0.01	1.10
Pyrogallol	1	5.43±0.11	5.17±0.06	5.43
Mixtures of antioxidant solutions				
Ascorbic acid : Cysteine	1:1	3.05±0.09	2.94±0.03	
	2:1	5.01±0.05	4.96±0.06	
	1:2	4.13±0.17	4.05±0.04	



Scheme 5 Scheme of the formation of pyrogallol complexes with iron ions.

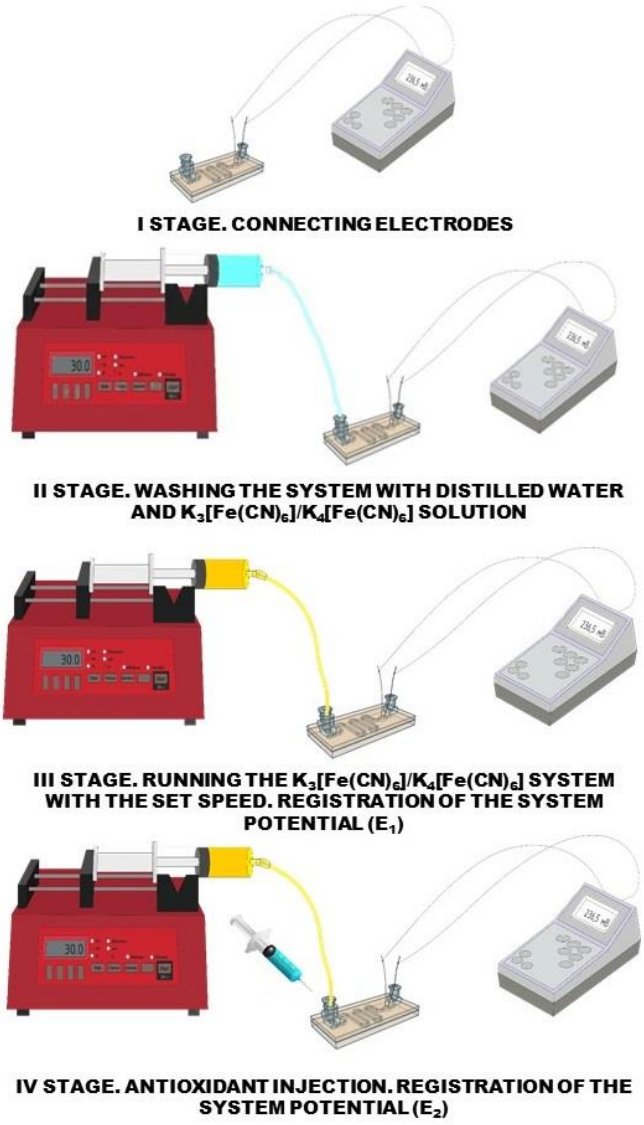


Figure 5 Scheme of analysis using the microfluidic device.

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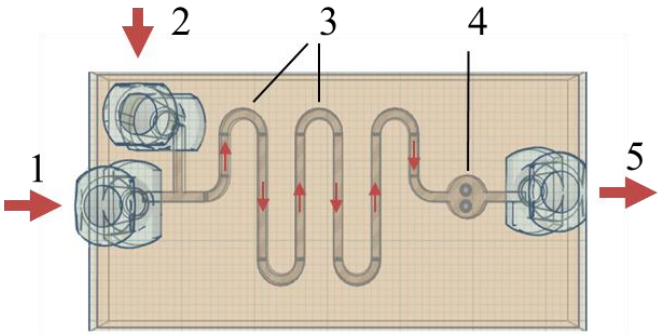

Figure 6 The scheme of the microfluidic device.

Table 2 Half-reaction periods of the interaction of antioxidants with $K_3[Fe(CN)_6]$ (C_{AO} = 0.1 mmol/dm³, n = 5, P = 0.95).

Name	$\tau_{1/2}$, sec	RSD (%)
Ascorbic acid	3	5
Cysteine	7	5
Pyrogallol	5	3
Caffeic acid	3	5
Rutin	3	5
Quercetin	10	4
Luteolin	4	3
Dihydromyricetin	6	1
Phloroglucinol	133	3
Glutathione	155	6
Catechol	376	5

The optimal reaction time was chosen based on the data in Table 2, which was 10 minutes. The overall dimensions of the microfluidic device were 60×30 mm, the channel diameter was d = 1 mm, the channel length was l = 100 mm, the flow rate was V = 50 ml/hour.

The antioxidant capacity of some solutions of model antioxidants and their mixtures was determined using the microfluidic device (Table 3). The correctness of the obtained results, similarly to the AOC obtained in the microcell, was determined by the "introduced-found" method, taking into account stoichiometric coefficients. The data obtained in the microfluidic device and in the standard laboratory electrochemical cell agree with each other (calculated t-test and F-test range from 0.3 to 1.5 and 0.2 to 1.3, respectively), and are consistent with the literature data. The relative standard deviation does not exceed 10%.

The quantitation limit of AOC by the potentiometric method using the microfluidic device was calculated. The dependence of the calculated values of the relative standard deviation from the average values of AOC at different concentrations of ascorbic acid was plotted to determine the quantitation limit (Figure 7). According to the 3σ criterion, it was $5.20 \cdot 10^{-6}$ mol-eq/dm³, which is sufficient for studying objects with a low content of antioxidants. Thus, working range of the developed device was ($5.2 \cdot 10^{-6}$–$9.9 \cdot 10^{-3}$) mol-eq/dm³.

According to IUPAC, the response time of electrodes is defined as the time required to reach 95% of the equilibrium potential for each tenfold change in concentration [36].

Table 3 AOC of model solutions of antioxidants (C_{AO} = 0.1 mmol/dm³, n = 5, P = 0.95) and their mixtures.

Name	C_{AO}, 10^{-4} mol/dm³	AOC, 10^{-4} mol-eq/dm³ (in micro-cell)	AOC, 10^{-4} mol-eq/dm³ (in macro-cell)	n
Ascorbic acid	1	2.16±0.07	1.98±0.02	2.16
Cysteine	1	1.28±0.06	1.01±0.01	1.28
Glutathione	1	1.22±0.09	0.98±0.01	1.22
Pyrogallol	1	4.48±0.27	5.17±0.06	4.48
Mixtures of antioxidant solutions				
Ascorbic acid : Cysteine	1:1	3.32±0.17	2.94±0.03	
Ascorbic acid : Glutathione	1:1	3.41±0,16	3.12±0.09	

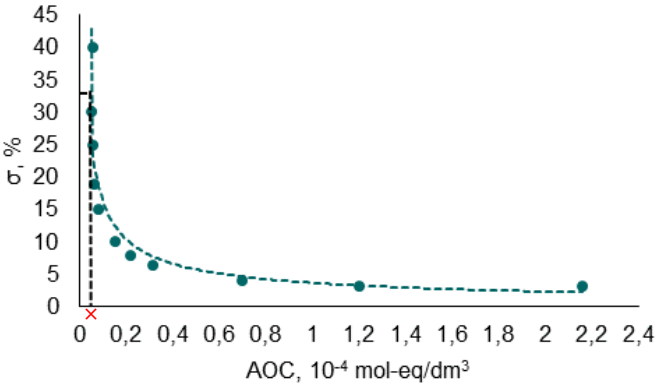

Figure 7 Dependence of relative standard deviation on AOC at different concentrations of ascorbic acid.

In this study, the response time was investigated in the concentration range of the $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ system from 0.01 M/0.1 mM to 0.01 M/1 mM. As a result, the response time of the developed device was found to be 60 s.

AOC of multicomponent objects, which are mass-produced drinks (Table 4), is determined. The composition of mass-produced drinks includes freshly squeezed juice and pulp of fruits and berries containing polyphenolic compounds, vitamins A, C and E – natural antioxidants [29]. The selected objects of analysis were not subjected to additional sample preparation.

Thus, beverages such as Lyubimiy Peach and Dobry Cherry juices, as well as Coca-Cola Lime, did not show antioxidant properties. Perhaps, this is due to the quality and storage conditions of raw materials and finished products.

Rich Orange, Sady Pridonya Apple-Cherry, Lyubimiy Multifruit, Dobry Apple-Pear, and Lipton Green Iced Tea exhibit antioxidant properties. The relative standard deviation of the AOC results obtained in the microfluidic device did not exceed 10%. The highest content of antioxidants was found in Rich Orange juice.

The correlation coefficient of the AOC results obtained in the microfluidic device and in the laboratory cell was 0.90 (r_{krit} = 0.80), which indicates a good convergence of the data and the possibility of using the potentiometric method implemented in the microfluidic device to assess the antioxidant capacity of multicomponent objects.

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Table 4 AOC of industrial drinks (n = 5, P = 0.95).

Name	AOC, 10^{-2} mol-eq/dm^3 (in microcell)	AOC, 10^{-2} mol-eq/dm^3 (in macrocell)
Rich Orange	5.60±0.29	6.20±0.07
Sady Pridonia Apple-Cherry	0.09±0.02	0.19±0.01
Lipton Green Iced Tea	2.14±0.11	2.62±0.04
Lyubimiy Multifruit	3.24±0.94	2.57±0.02
Dobry Apple-Pear	0.43±0.06	0.75±0.04

4. Limitations

In this work, the silver wire, coated with an insoluble silver salt, was used as a reference electrode. This electrode is quite common. However, the use of silver wire has some limitations associated with damage to the upper layer of silver chloride during repeated use and the need for its redeposition. In order to increase the capability of using these devices multiple times, it will be necessary to conduct a study on the choice of material and design of the reference electrode.

5. Conclusions

In this work, two prototypes of portable devices were developed – the open microcell and the flow microfluidic device, based on the potentiometric method using the $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ system. The high reproducibility of the AOC results and the similarity with the data obtained in the standard laboratory electrochemical cell suggest the possibility of using the developed designs of portable devices for the express assessment of the antioxidant content in complex multicomponent objects.

Thus, the potentiometric method for determining the antioxidant content, due to the simplicity of hardware design, the possibility of miniaturization of the measurement cell and express analysis, is quite promising from the point of view of implementation in the portable device. Further research will be aimed at improving the design of the prototypes in order to develop the analytical platform for the determination of antioxidants in various objects.

• Supplementary materials

No supplementary materials are available.

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• Conflict of interest

The authors declare no conflict of interest.

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