# Potentiometry for Determination of Oxidant Activity

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PAPER

# Potentiometry for Determination of Oxidant Activity

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This paper aims to describe a new approach to using potentiometry for determining oxidants in liquids, ozonized and chlorinated water, in particular. The source of information is the electrode potential shift of the mediator system observed when an analyzed sample, containing oxidant, is inserted into an electrochemical cell. Criteria for the selection of the mediator system for oxidants determination is proposed. Potassium ferri/ferrocyanides serve as the mediator system in the proposed method. $K_3[Fe(CN)_6]$ is chosen as model oxidant. Special attention focus on the role of state of an indicator electrode surface and ways of its regeneration in generating an analytical signal. The information is essential for obtaining correct results in different areas of electrochemical analysis. As a result, a simple, fast, sensitive, and reliable method that does not require the use of reference solution is proposed, which ensures its advantages over other methods. Accuracy and reliability of the data obtained are confirmed by the analysis results received by using the standard method.

**Key words:** Reactive oxygen species, potentiometry, mediator system, chlorine, ozone, oxidant activity (OA) of water, Au and Pt electrodes.

## Introduction

Monitoring of oxidants is important for human health assessment, oxidative stress in particular. Oxidative stress (OS) is an imbalance between generation of reactive oxygen species (ROS) and the capacity of the antioxidant defense system to maintain their concentration at a physiological level[1]. OS may be caused by excess generation of free radicals as a result of an impact of adverse endogenous (various pathologies) and exogenous factors (environmental pollution, UV and ionizing radiation, the consumption of water containing excess chlorine or ozone used for disinfection). The causes and effects are numerous, including socially significant diseases[2]. In this connection, the issue of monitoring antioxidant/oxidant status of the human body, the content of antioxidants (AO) and oxidants in food, beverages, medicines, and water becomes essential.

The methods for determining antioxidant (AO) are widely described in the literature[3-8]. The most common are two approaches, which differ in using free radicals as reagents, including specially generated[9], or using non-radical chemicals that change optical, electrochemical, and other properties of the medium when contacting with antioxidants of the test sample[7-11].

Monitoring of oxidants (OA) is discussed less extensively. The most objective direct method for detection and identification of free radicals is electron paramagnetic resonance (EPR) technique[12], and chemoluminescence[13]. Other methods are also described: detection of superoxide anion radicals by spectrophotometric and electrochemical methods[14], hydroxyl radical quantification by UV spectrophotometry[15], and chromatography[16]. Widely used is the method of determining the overall impact of oxidants on the human body by concentration of products of lipid peroxidation, malondialdehyde, in particular[17]. The main shortcomings of these methods as applied to oxidant activity monitoring are that they provide information about individual oxidants or/and their impact after the damage has already been done.

As antioxidant/oxidant activity (AOA/OA) reflects the properties of the overall system and is determined by the complex of compounds, then it is advisable to use methods for measuring AOA/OA as an integral parameter for its characteristics.

From our point of view the most suitable and rapid methods of AO/OA measurements are electrochemical methods. In addition to the advantages related to availability and low cost of instruments and reagents; and easy automation procedure of measurements, electrochemical methods enable direct evaluation of electron-donor-acceptor properties of the investigated system, i.e. the properties which determine its antioxidant/oxidative status[18]. The approach to detecting radicals and reactive oxygen species using an electrochemical procedure, in which they destroy a self-assembled layer on an electrode, and an electrochemical signal (cyclic voltammogram) of a dissolved hexammine ruthenium(III) complex is used as source of information, was described earlier[19]. Turyan et al.[20] have modified our method for AO[21] determination and used it to monitor oxidation of proteins.

Ozone and chlorine are challenging objects of study in terms of their oxidative properties and the use of chlorination and ozonation for disinfection of drinking water as well as water for swimming pools, and toxicity of residual chlorine and ozone[22]. On the other hand, ozone is considered a safe and environmentally

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friendly tool for the seafood industry, which is used to improve fresh seafood safety[23], and its monitoring is also important here.

Moreover, determination of the concentration of dissolved ozone draws more attention due to growing interest in ozone therapy[24], where saline, containing dissolved ozone[25-26], or ozone-enriched gas mixtures[27] are active substances. All this leads to a change in the oxidant/antioxidant status of the body and, of course, requires monitoring of treatment. The absence of simple and reliable methods for measuring oxidants (oxidative activity), ozone in particular, in treatment media is attributed to a variety of physicians' opinions regarding pros and cons of ozone therapy.

The general trend toward lower allowable levels of ozone and chlorine in water claims for designing new methods for the range of a few tenth of ppbw. One of the problem of the known methods is to prepare accurate reference solutions for measuring dissolved ozone and chlorine concentration[28-29]. The potentiometric method[7] requires neither preliminary plotting of calibration curves nor the use of the method of standard additions. In this case, reference solution is not needed, which gives potentiometry a distinct advantage over other methods.

The objectives of this study are (i) to develop a new approach to using potentiometry for determining oxidants in liquids, ozonized and chlorinated water, in particular,

(ii) to investigate the role of an indicator electrode surface state, the ways of its regeneration and to formulate evaluation criteria to estimate its quality and measurement suitability.

## Methods and calculations

Equation 1 describes equilibrium between the potential and the components of the mediator system:

$$E = E_0 + b \cdot \lg \frac{f_{ox} C_{Ox}}{f_{red} C_{\mathrm{Re}d}} \qquad (1)$$

where

b=2,3·RT/nF;

$f$ – activity coefficients; with further calculation of ΔE, $f$ disappears from calculations;

E – system potential in the initial state, V;

$C_{Ox}$ – concentration of an oxidized form of the mediator system, M;

$C_{Red}$ – concentration of a reduced form of the mediator system, M.

Introduction of antioxidant or oxidant into the system leads to the shift of the equilibrium concentration of components and, respectively, to the shift of the potential. This shift serves as an analytical signal.

After a sample containing **antioxidants** is introduced into the solution, the potential of the mediator system can be expressed by the following equation:

$$E_1 = E_0 + b \cdot \lg \frac{f_{ox}(C_{Ox} - X)}{f_{red}(C_{\mathrm{Red}} + X)} \qquad (2)$$

where

$E_1$– system potential after the introduction of the test sample into the solution, V;

X – concentration of antioxidants in the solution after the introduction of the test sample, M-eq.

After a sample containing **oxidants** is introduced into the solution, the potential of the mediator system can be expressed by the following equation:

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

where

$E_1$– system potential after the introduction of the test sample into the solution, V;

X – concentration of oxidants in the solution after the introduction of the test sample, M-eq.

Taking $\Delta E = E_1 - E$, it is easy to calculate

$$X = AOA = \frac{C_{Ox} - \alpha C_{\mathrm{Re}d}}{1+\alpha} \qquad (4)$$

$$X = OA = \frac{\alpha C_{\mathrm{Re}d} - C_{Ox}}{1+\alpha} \qquad (5)$$

or

$$X = \pm \frac{C_{Ox} - \alpha C_{\mathrm{Re}\,d}}{1+\alpha} \qquad (6)$$

$$\alpha = (C_{Ox} / C_{\mathrm{Re}\,d}) \cdot 10^{(E_1 - E)nF\,/\,2.3RT} \qquad (7)$$

Therefore, in determination of oxidants, it is the redox reaction between the determined component (oxidizer, oxidant) and the reduced form of the mediator system that generates a signal. A shift of the initial potential as a result of reaction between oxidants and the reduced form of the mediator system serves as a source of information about the total concentration of oxidants in the cell (and eventually in the sample).

### Mediator system selection

The mediator system should meet the following requirements:

1. An electrochemical reaction between the reduced form of the mediator system and most oxidants should be possible thermodynamically.

2. The redox potential of the determined oxidant should be higher than the system potential.

3. The reaction between the reduced form of the mediator system and the oxidant should occur stechiometrically in accordance with a number of functional groups exhibiting oxidative properties.

4. Results should be obtained in universal units of measurement (for example, in M-eq).

5. Any objects could be analyzed including coloured ones;

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6. The rate of the redox reaction between oxidant and the reduced form of the mediator system should be sufficient to ensure that measurements are taken within a reasonable timeframe.

Whereas our earlier studies focused on determination of antioxidant activity of various objects[21, 30-34], it is natural to assume that the system that meets the aforementioned requirements and the task set is the system containing a mixture of potassium hexacyanoferrite (III) and hexacyanoferrate (II), at least in regard to items 1,2,4,5. Thus, the standard redox potential of $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ relative to the standard hydrogen electrode at 25 °C is +0.356 V. The redox potential of the mediator system is considerably lower than the potentials of half-reactions of the determined substances:

$$Cl_2\uparrow + 2e = 2Cl^- \ (E_0 = +1.359 \text{ V}) \qquad (8)$$

and

$$O_3\uparrow + 2H^+ + 2e = O_2\uparrow + H_2O \ (E_0 = +2.07 \text{ V}) \qquad (9)$$

This offers thermodynamic opportunity for chemical reactions between substances (chlorine and ozone) and potassium ferrocyanide. The latter has served as the basis for developing the proposed potentiometric option as an example and illustration of the capabilities of the method as well as the solution of a practical task: analysis of ozonized and chlorinated water.

A signal-generating reaction in this case is:

$$K_4[Fe(CN)_6] + OA \rightarrow K_3[Fe(CN)_6] + OA_{Red} \qquad (10)$$

where OA – oxidant (in this case, $O_3$ or $Cl_2$), $OA_{red}$ – reduced form of oxidant. Then oxidative activity of the studied object (water, in this case) can be calculated by Equation (5) and expressed in M-eq, i.e., in universal units of measurement, which enables to compare results obtained by different methods.

## Experiments and Discussion

### Instruments

IVA-5, a stripping voltammetric analyzer ("IVA", Yekaterinburg, Russia) was used to register cyclic curves.

Potentiometric measurements were performed on TA-ION ionometer ("Tomanalyt", Tomsk, Russia). A two electrode electrochemical cell (ECC), 3 ml volume, with magnetic stirrer, was used. Anode and cathode chambers of the two-electrode ECC were separated by salt bridge filled with 3.5 M KCl solution.

TA-ION ionometer and reference solutions were used to measure pH of solutions.

Chlorine for chlorination of water was obtained in the usual way by the following reaction:

$$2KMnO_4 + 16HCl = 2KCl + 2MnCl_2 + 5Cl_2\uparrow + 8H_2O \qquad (11)$$

A multifunctional ozonizer (GL-188) ("Rados", Krasnoyarsk, Russia) served as ozone generator in laboratory conditions.

### Objects of the study

Chlorinated and ozonized water served as objects of the study.

### Reagents

The following reagents were used:

- $K_3[Fe(CN)_6)]$ analytical grade, $K_4[Fe(CN)_6]\cdot 3H_2O$ pure, (Ltd "JSC Reachem", Moscow, Russia);
- $KH_2PO_4$ chemically pure (Ltd "NevaReaktiv", St Petersburg, Russia);
- $Na_2HPO_4\cdot 12H_2O$ analytical grade (Ltd "Alfachem plus", St Petersburg, Russia);
- $H_2SO_4$ chemically pure (Ltd "JSC Reachem", Moscow, Russia);
- KCl chemically pure (Ltd "TH Lake Raspberry", Barnaul, Russia);
- Buffer solutions: pH industry standards (JSC "Ural Plant of Chemical Reagents", Verkhnaya Pieshma, Russia);
- $KMnO_4$ analytical grade (JSC "Reactiv", St Petersburg, Russia);
- Soluble starch for iodometry, analytical grade (Ltd "JSC Reachem", Moscow, Russia);
- $Na_2S_2O_3\cdot 5H_2O$ analytical grade (JSC "Reactiv", St Petersburg, Russia).

### Electrodes

Platinum or gold electrodes and screen printed electrodes on ceramic substrate (Ltd "Research and Production Innovation Enterprise IVA", Yekaterinburg, Russia) served as the indicator (working) electrodes. Ethanol solution of Cementit Universal glue (Swiss, Niederwangen) was used to isolate the working area of the screen printed electrode ($S \le 25$ mm$^2$). Bulk Pt and Au electrodes (Ltd "Metrohm", Switzerland) were utilized as control electrodes. Double-junction silver/silver chloride electrodes (Ltd "Metrohm", Switzerland) were used for measurements and quality control of the reference electrode EVL–1M3 (PC "Gomel Plant of Measuring Equipment", Gomel, Republic of Belarus). The latter one as a rule was used in measurements.

### Studies of electrodes

### Indicator electrode

An indicator electrode should be adsorptive, chemically and electrochemically stable in the working potential range of the mediator system, and should ensure a rapid exchange of electrons between its oxidized and reduced forms. The following resources were used as a source of information about these processes:

- cyclic voltammograms recorded using 1M KCl as a background;
- cyclic voltammograms recorded using 1M KCl as a background in the presence of the mediator system components;
- rate of stabilizing the electrode potential in the mediator system.

Figure 1 shows cyclic voltammograms illustrating the processes at Pt and Au screen-printed and control electrodes in 1 M KCl. Cyclic voltammograms observed in the presence of the mediator system are given in Figure 2. It is apparent from Figures 1 and 2 that in the selected range of potentials the electrodes are chemically and electrochemically inactive. The shape of the voltammogram for $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ system is characteristic for a quasi-reversible process, i.e., it ensures quite rapid exchange of electrons between

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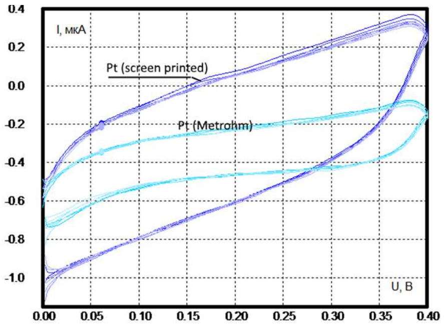
the oxidized and reduced forms of the mediator system. The potential of the platinum and gold working electrodes is stabilizes quite fast - from 120 to 300 seconds. In this case the potential dependence on the logarithm of the concentration ratio of $K_3[Fe(CN)_6]$ and $K_4[Fe(CN)_6]$ satisfies the Nernst equation (Fig. 3). These data indicate that the used electrodes meet the requirements listed above, which is the main condition for obtaining correct analysis results.



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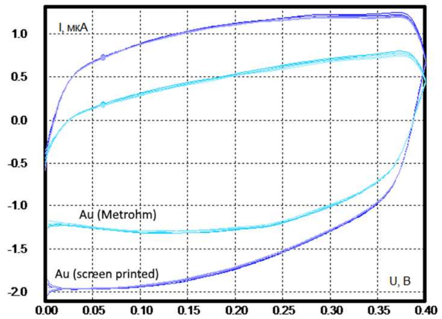
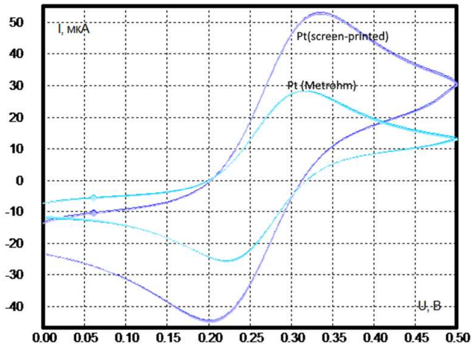


B

Figure1. Cyclic voltammograms recorded at Pt (A) and Au (B) screen-printed and control electrodes, (v) 0.1 V/sec. Background solution: 1M KCl



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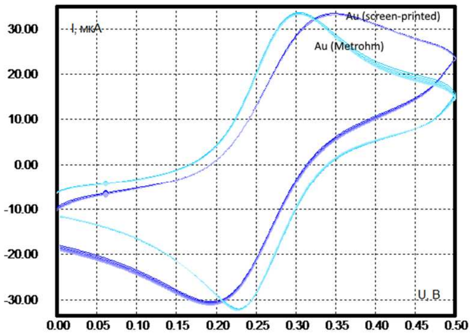

B

Figure 2. Cyclic voltammograms recorded at Pt (A) and Au (B) screen-printed and control electrodes, (v) 0.1 V/sec. Background solution: 1M KCl containing $1·10^{-3}$ M $K_3[Fe(CN)_6]$ + $1·10^{-3}$ M $K_4[Fe(CN)_6]$

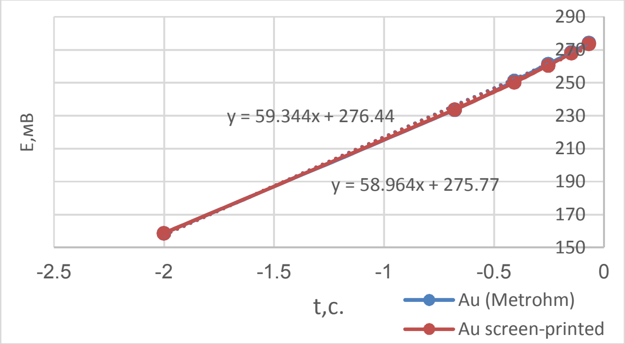

Figure 3. Experimentally obtained dependence of Au (Metrohm) and Au screen-printed electrode potentials set in $K_3[Fe(CN)_6]$ / $K_4[Fe(CN)_6]$ on the logarithm of the concentration ration in the range $10^{-3}/10^{-5}$ M/l in 1M KCl

Unfortunately, this is not always the case as the surface of the electrode changes during experiments. These changes are visible in the cyclic curves: the difference between the currents of the cathodic and anodic branches grows; different cathodic or anodic peaks appear; reproducibility of cyclic voltammograms in the mediator system worsens (Fig.4). Moreover, the rate of the electrode potential stabilization in the mediator system reduces. Sometimes the potential does not stabilize at all.

In this case, it is practically impossible to obtain correct analysis results. The electrode should be regenerated, which can be done as described below.

*Gold screen printed electrode*

- Careful polishing of the electrode surface with finely divided powder $Al_2O_3$, applied to a piece of cloth that has been dampened with deionized water
- Cyclic polarization of the electrode (10 cycles) in 0.2 M $H_2SO_4$ solution in the potential range from +0.5 to +1.5 V, with the scan rate 0.1 V/s[35],

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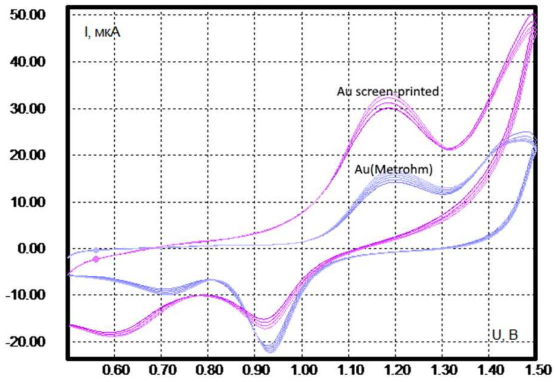

Figure 4. Cyclic voltammograms recorded at control and gold screen-printed electrodes after they had been used in measurements, (v) 0.1 V/s. Background solution: 0.2 M $H_2SO_4$

*Platinum screen printed electrode*

- Careful polishing of the electrode surface with finely divided powder $Al_2O_3$, applied to a piece of cloth that is dampened with deionized water
- Cyclic polarization of the electrode (10 cycles) in 0.5 M $H_2SO_4$ solution in the potential range from +0.04 to +1.5 V, with the scan rate 0.1 V/s[36],

The criterion for suitability of the electrodes for further measurements is a cyclic voltammograms similar to the ones shown in Figure 2 and quick (no more than 2-3 minutes) stabilization of the potential in time.

Thus, the accepted view that in potentiometry the role of the electrode material (in case of noble metals) and the preparation of the surface before measurements is not so significant as, say, in voltammetry, is not quite true. For example, a platinum electrode is used as working electrode when measuring redox potentials in different media, without considering whether the range of measurements is in the double-layer area of the electrode and whether analyte (e.g., hydrogen peroxide) contributes to the formation of oxide layers on the electrode surface. Apparently, the latter process may worsen the platinum electrode performance during determination of oxidative activity. A gold electrode seems to be more resistant to oxidation occurring in the range of measurements.

**Reference electrode**

In the presence of the mediator system, the potential of the silver/silver chloride electrode changes as a result of formation of insoluble silver ferri/ferrocyanides on the surface. This problem was further solved by using (i) a cell with separated spaces, and/or (ii) double-junction silver/silver chloride electrode (Metrohm). The criteria for correct operation of the silver/ silver/silver chloride electrode are:

- the potential of the silver/silver chloride electrode (EVL) immersed in 3.5 M KCl solution does not change or changes slightly in time with respect to the Metrohm reference electrode (the potential oscillations for 15-30 minutes do not exceed 0.5 mV),
- the potential difference measured between the above-mentioned electrodes should not exceed the value range ±5 mV.

Replacing the intermediate 3.5 M KCl solution with a fresh one before each series of measurements contributes to stability of the electrode potential.

**Conditions for determining OA**

**pH selection**

It is known that properties of the used mediator system depend on medium acidity. Thus, $K_3[Fe(CN)_6]$ and $K_4[Fe(CN)_6]$ undergo the protonation reaction, accompanied by the formation of $H_3[Fe(CN)_6]$ and $H_4[Fe(CN)_6]$. $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ system is stable in the pH range of 5-8. Within these limits, the system satisfies the Nernst equation, which makes it possible to use the system in these media for determining OA.

**Mediator system composition selection**

$K_3[Fe(CN)_6]$ was used as the compound that models oxidants.

The maximum possible potential shift, occurring when analyte is introduced into the system, and sufficient stability of the system served as the criteria for selecting concentration of the mediator system components and their ratio in the mixture. Table 1 presents the calculated values of the potential change of the electrode inserted in mediator systems with different compositions, when $K_3[Fe(CN)_6]$ oxidant is added in the solution, at concentrations ranging from $1{\cdot}10^{-7}$ to $9{\cdot}10^{-4}$.

Table 1. Calculated values of the potential shift of the electrode inserted in mediator systems with different compositions (when $K_3[Fe(CN)_6]$ is added in the solution)

| Concentration of components in mediator system, $C_{Ox}/_{Red}$, M/M. | Concentration of introduced oxidizer, M | ΔE, mV |
|---|---|---|
| $10^{-6}/10^{-4}$ | $1{\cdot}10^{-7}$ - $9{\cdot}10^{-5}$ | 3-175 |
| $10^{-5}/10^{-5}$ | $5{\cdot}10^{-7}$ - $9{\cdot}10^{-6}$ | 3-75 |
| $5{\cdot}10^{-6}/5{\cdot}10^{-4}$ | $5{\cdot}10^{-7}$ - $4{\cdot}10^{-4}$ | 2-153 |
| $10^{-5}/10^{-3}$ | $5{\cdot}10^{-7}$ - $9{\cdot}10^{-4}$ | 1-175 |
| $10^{-4}/10^{-3}$ | $5{\cdot}10^{-6}$ - $9{\cdot}10^{-4}$ | 1-118 |
| $10^{-4}/10^{-4}$ | $1{\cdot}10^{-5}$ - $9{\cdot}10^{-5}$ | 5-75 |

Table 1 shows that in order to determine oxidants at concentrations ranging from $1{\cdot}10^{-7}$ to $9{\cdot}10^{-5}$ M-eq, the first system is optimal. In order to determine oxidants at concentrations ranging from $5{\cdot}10^{-7}$ to $9{\cdot}10^{-4}$ M-eq, the fourth system is optimal. Unilike determination of antioxidants, in our case the measured potential shift is mostly determined by concentration of the oxidized form of the system. As it is stable, the system containing low concentrations can be used for analysis. This enables to lower the detection limit of oxidants in comparison with AO. The ratio of $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ in the mediator system ($C_{ox}$: $C_{Red}$ = 1:100) allows us to obtain the maximum potential shift.

*Criteria for selecting analysis conditions:*

- the Nernst equation should be satisfied in a predetermined concentration range,
- deviation of the determined concentration from the input concentration should not exceed 20%.

Table 2. Results of determining concentration of $K_3[Fe(CN)_6]$ in phosphate buffer solution (pH 7.2) (n=3)

| Indicator electrode | System composition $C_{Ox}$/ $C_{Red}$ | $K_3[Fe(CN)_6]$ introduced, M-eq | ΔE, mV | | $K_3[Fe(CN)_6]$ found | |
|---|---|---|---|---|---|---|
| | | | theory | experiment | M-eq | Recovery |
| Pt | $10^{-4}/10^{-2}$ | $2.0{\cdot}10^{-4}$ | 29 | 26 | $1.7{\cdot}10^{-4}$ | 85 |
| | | $3.8{\cdot}10^{-4}$ | 41 | 39 | $3.3{\cdot}10^{-4}$ | 88 |
| | | $5.5{\cdot}10^{-4}$ | 49 | 47 | $4.9{\cdot}10^{-4}$ | 89 |
| | | $7.0{\cdot}10^{-4}$ | 55 | 53 | $6.3{\cdot}10^{-4}$ | 90 |
| | | $8.5{\cdot}10^{-4}$ | 60 | 58 | $7.7{\cdot}10^{-4}$ | 91 |
| | $10^{-5}/10^{-3}$ | $2.0{\cdot}10^{-5}$ | 29 | 23 | $1.5{\cdot}10^{-5}$ | 77 |
| | | $3.8{\cdot}10^{-5}$ | 41 | 35 | $3.1{\cdot}10^{-5}$ | 81 |
| | | $5.5{\cdot}10^{-5}$ | 49 | 44 | $4.7{\cdot}10^{-5}$ | 85 |
| | | $7.0{\cdot}10^{-5}$ | 55 | 50 | $6.2{\cdot}10^{-5}$ | 89 |
| | | $8.4{\cdot}10^{-5}$ | 60 | 55 | $7.7{\cdot}10^{-5}$ | 92 |
| Au | $10^{-5}/10^{-3}$ | $2.0{\cdot}10^{-5}$ | 29 | 25 | $1.6{\cdot}10^{-5}$ | 79 |
| | | $3.8{\cdot}10^{-5}$ | 41 | 37 | $3.2{\cdot}10^{-5}$ | 83 |
| | | $5.5{\cdot}10^{-5}$ | 49 | 45 | $4.7{\cdot}10^{-5}$ | 86 |
| | | $7.0{\cdot}10^{-5}$ | 55 | 52 | $6.3{\cdot}10^{-5}$ | 90 |
| | | $8.4{\cdot}10^{-5}$ | 60 | 56 | $7.7{\cdot}10^{-5}$ | 92 |
| | $10^{-6}/10^{-4}$ | $2.0{\cdot}10^{-6}$ | 29 | 22 | $1.4{\cdot}10^{-6}$ | 69 |
| | | $3.8{\cdot}10^{-6}$ | 41 | 35 | $2.9{\cdot}10^{-6}$ | 77 |
| | | $5.5{\cdot}10^{-6}$ | 49 | 44 | $4.4{\cdot}10^{-6}$ | 80 |
| | | $7.0{\cdot}10^{-6}$ | 55 | 50 | $5.9{\cdot}10^{-6}$ | 84 |
| | | $8.4{\cdot}10^{-6}$ | 60 | 55 | $7.4{\cdot}10^{-6}$ | 87 |

Table 2 reveals that under these conditions and with the use of Pt-electrode, an acceptable value of recovery is observed at concentrations above $1.6{\cdot}10^{-5}$ M-eq, but the system potential does not stabilize in time. When Au-electrode is used, an acceptable value of recovery is observed at concentrations above $4.4{\cdot}10^{-6}$ M-eq. Unfortunately, these values exceed the maximum permissible concentration of chlorine (0.3 - 0.5 mg/l = 4 - $7{\cdot}10^{-6}$ M-eq) and ozone (0.3 mg/l = $6.3{\cdot}10^{-6}$ M-eq)[28]. Replacement of the background solution, as it is evident from the data given in Table 3, allows to reduce the detection limit to $2.0{\cdot}10^{-7}$ M-eq and, thus, to solve the problem.

Table 3. Results of determining concentration of $K_3[Fe(CN)_6]$ in 1M KCl solution (pH 6.0), Au-indicator electrode (n=3)

| System composition $C_{Ox}$/ $C_{Red}$ | $K_3[Fe(CN)_6]$ input, M-eq | ΔE, mV | | $K_3[Fe(CN)_6]$ found | |
|---|---|---|---|---|---|
| | | calculated | observed | M-eq | Recovery (%) |
| $10^{-6}/10^{-4}$ | $2.0{\cdot}10^{-6}$ | 29 | 25 | $1.9{\cdot}10^{-6}$ | 96 |
| | $3.8{\cdot}10^{-6}$ | 41 | 36 | $3.3{\cdot}10^{-6}$ | 87 |
| | $5.5{\cdot}10^{-6}$ | 49 | 44 | $4.9{\cdot}10^{-6}$ | 89 |
| | $7.0{\cdot}10^{-6}$ | 55 | 50 | $6.2{\cdot}10^{-6}$ | 88 |
| | $8.5{\cdot}10^{-6}$ | 60 | 56 | $7.7{\cdot}10^{-6}$ | 91 |
| | $2.0{\cdot}10^{-7}$ | 5 | 5 | $2{\cdot}10^{-7}$ | 100 |
| | $3.8{\cdot}10^{-7}$ | 8 | 8 | $3.8{\cdot}10^{-7}$ | 100 |
| | $5.5{\cdot}10^{-7}$ | 11 | 11 | $5.0{\cdot}10^{-7}$ | 100 |
| | $7.0{\cdot}10^{-7}$ | 14 | 13 | $6.7{\cdot}10^{-7}$ | 95 |
| | $8.5{\cdot}10^{-7}$ | 16 | 16 | $8.5{\cdot}10^{-7}$ | 100 |

It may be concluded from the aforementioned data that potential measurements of Au electrode immersed in a solution containing 1M KCl (pH 6.0) and the mediator system ($10^{-6}$M $K_3[Fe(CN)_6]$ +$10^{-4}$M $K_4[Fe(CN)_6]$ ), ensures correct results, provides low detection limit.

**Analysis algorithm**

1. Place the silver/silver chloride electrodes (EVL- ordinary used measurements and control) in a cell filled with a saturated solution of potassium chloride. It shall not exceed ±5 mV. The fluctuations in the values of the potential for 10 minutes shall not exceed 0.5 mV. Otherwise, replace the ordinar used EVL electrode.
2. Insert Au indicator (screen printed or Metrohm) electrode in the solution containing 1M KCl (pH 6.0) +$10^{-3}$M $K_3[Fe(CN)_6]$+$10^{-3}$M $K_4[Fe(CN)_6]$. Record a cyclic voltammogram in the potential range 0-0.5 V, with the scan rate 0.1 V/s (its shape should match the curve shown in Fig. 2), or record the potential shift within 10 minutes (It might take 2-3 minutes for a potential value to stabilize). Otherwise, regenerate the electrode as described above (cycling in 0.2 M $H_2SO_4$ solution in the potential range from +0.5 to +1.5 V (20 cycles, for Metrohm electrode and 10 cycles for screen printed electrode), with the scan rate 0.1 V/s

   The need for repetition of the described procedure depends on the object of study. One of the indicators that the procedure should be repeated is slow stabilization of a potential during a measurement process.
3. If the range of the determined concentrations in the sample is in the range from $2{\cdot}10^{-7}$ to $9{\cdot}10^{-5}$ M-eq, place a solution containing 1M KCl (pH 6.0) + $10^{-6}$ M $K_3[Fe(CN)_6]$ +$10^{-4}$ M $K_4[Fe(CN)_6]$ in a measuring electrochemical cell. For other concentrations, select the composition of the mediator systems according to the data in Table 1.
4. Measure the potential in time.
5. Add an aliquot of the sample into the cell.
6. Register the potential in time.

7. Calculate oxidant activity of the solution in the cell following Equation 5.
8. Calculate oxidant activity of the sample following Equation 15:

$$X_s = X\cdot(V_{cell} + V_{sample}) / V_{sample} \qquad (12)$$

where: $X_s$=OA of the sample, X=OA of the solution in the cell,
$V_{cell}$ - volume of the solution in the cell, $V_{sample}$ - volume of the added aliquot of the sample.

The results of the analysis of ozonized water by standard and proposed methods are given in Table.4.

Table 4. Results of ozonized water analysis

| Measurement number | Titrimetry (iodomentry)[37] mg/l | $10^{-5}$M-eq | Proposed method OA, $10^{-5}$ M-eq |
|---|---|---|---|
| 1 | not found | | 0.06±0.021 |
| 2 | 0.24 | 0.51 | 0.48±0.015 |
| 3 | 0.30 | 0.63 | 0.65±0.015 |
| 4 | 0.43 | 0.9 | 0.89±0.015 |
| 5 | 0.85 | 1.8 | 1.9±0.01 |
| Analysis conditions | 10 ml 0.12M KJ and 20 ml 0.5M $H_2SO_4$ are added to the sample (250 ml). The solution is titrated with a mixture containing 0.005 M $Na_2S_2O_3$·+ 0.002 M $Na_2CO_3$, until it is coloured straw-yellow. Then 2 ml 0.5% starch solution are added, and titration is continued until blue colour disappears. | | 0.6 ml of the sample are added to 1.8 ml solution containing 1M KCl (pH 6.0) +2·$10^{-6}$M $K_3[Fe(CN)_6]$, +2·$10^{-4}$M $K_4[Fe(CN)_6]$. Then, the potential is recorded in time. |

From Table 4 it can be seen that the results correlate with each other: the correlation coefficient is 0.99. Obtained $F_{empiric}$ = 1.075. Value of F-criteria do not exceed critical one ($F_{critical}$ =4.11). Those values allow to state that iodometry and proposed method are equally accurate. Obtained Student criteria ($t_{empiric} < t_{critical}$ =1.03 < 2.776) confirm that the difference between the results is not significant statistically and that there is no bias error in the methods.

The proposed method enables to determine the content of ozone in water at the maximum permissible concentration (the Russian Standard[37]). The detection limit of the proposed method is 2·$10^{-7}$ M-eq, which is much lower than the detection limit offered by titrimetry (1·$10^{-6}$ M)[37].

Table 5. Results of chlorinated water analysis

| Measurement number | Titrimetry (iodomentry)[38] mg/l | $10^{-5}$M-eq | Proposed method OA, $10^{-5}$M-eq |
|---|---|---|---|
| 1 | 0.15 | 0.21 | 0.17±0.017 |
| 2 | 0.33 | 0.47 | 0.43±0.015 |
| 3 | 1.33 | 1.9 | 2.0±0.015 |
| 4 | 2.57 | 3.6 | 3.8±0.011 |
| 5 | 5.31 | 7.5 | 6.8±0.01 |
| Analysis conditions | 5 ml 2M KJ and 1 ml 0.5% starch solution are added to the sample (250 ml). The solution is titrated with 0.01 M $Na_2S_2O_3$ until blue colour disappears. | | 0.6 ml of the sample are added to 1.8 ml solution containing 1M KCl (pH 6.0) +2·$10^{-6}$M $K_3[Fe(CN)_6]$, +2·$10^{-4}$M $K_4[Fe(CN)_6]$. Then, the potential is recorded in time. |

From Table 5 it can be seen that the results correlate with each other: the correlation coefficient is 0.99. Obtained $F_{empiric}$ = 1.089. Value of F-criteria do not exceed critical one ($F_{critical}$ =4.11). Those values allow to state that iodometry and proposed method are equally accurate. Obtained Student criteria ($t_{empiric} < t_{critical}$ =-3.04 < 2.776) confirm that the difference between the results is not significant statistically and that there is no bias error in the methods.

The proposed method enables to determine the content of chlorine in water at the maximum, permissible concentration (the Russian[38] Standard). The detection limit of the proposed method is 2·$10^{-7}$ M-eq, which is much lower than the detection limit offered by titrimetry (1·$10^{-6}$ M)[38].

## Conclusion

Accurate determination of oxidants including dissolved ozone and chlorine is considerably important because of their strong oxidative activity and, consequently, their high toxicity for living organisms. The existing methods, suitable for solving these problems are not numerous. One of the challenging issues is to prepare right reference solutions that can be used for measuring, for example, dissolved ozone and chlorine concentration The reason is instability of solutions, containing analyts or its complex composition. One of the advantages of the proposed potentiometric method over other methods is that it does not require the use of reference solutions.

In the present version of the method, a signal is generated as a result of the redox reaction between the determined component (oxidizer, oxidant) and the reduced form of the mediator system. The potential shift caused by the reaction between oxidants and the reduced form of the mediator system serves as a source of information about the total concentration of oxidants in the sample. Meanwhile the correctness of analysis results is determined by accurate measurements of the potential value and choice of the mediator system. In this regard, special attention was drawn to characterization of an indicator electrode. It was shown that cyclic voltammorgams recorded by using 1M KCl as the background in the presence of the mediator system can be taken as criteria for suitability of the electrode for taking measurements.

Requirements for the mediator system and its selection for solving the analytical problem are also described.

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We expect that this approach being applied in food and water analysis, biology and medicine will be useful for monitoring of oxidant activity, the latter being an important criterion for human health evaluation

In future, it is advised that the proposed method can be applied for monitoring oxidant activity in foodstuff, biological and medical objects and water. The application of the method could be useful for evaluating oxidative stress

The problem of oxidative stress evaluation is attributed to lack of universal standardized methods, techniques, and parameters, which makes this evaluation subjective and its outcomes incomparable. Potentiometric methods may significantly contribute to the solution of this problem.

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