# Investigation of antioxidant properties of metal ascorbates and their mixtures by voltammetry

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**Abstract**
The paper describes modern ways for selection of anti-radical substances. Molding of such components with a carbon-based material decreases the rate of its oxidative destruction. Addition of such a component to a carbon-based material decreases the rate of its oxidative destruction. The purpose of this study is to determine the antioxidant activity of ascorbates metals (Ca, Mg, Li, Co, Fe), used in the practice of medicine, as well as mixtures based on them together with well-known antioxidants. In this article we examine the effect of metals on the process of ascorbate oxygen electroreduction.
From these ascorbates lithium and magnesium ascorbate showed the greatest activity toward cathode oxygen reduction process.
Also mixtures with well-known examined antioxidants ascorbate (glucose, dihydroquercetin) were investigated at different concentrations of components. It is shown that the multicomponent mixtures exhibit lower activity than the individual drugs. Recommended the creation of drugs on the basis of ascorbate Mg and Li with not more than 3 number of components.

## 1. Introduction

Nowadays some calcium, lithium, iron, magnesium preparations, such as calcium chloride, lithium carbonate, lithium chloride and others are wildly used in clinical practice of psychoactive diseases [1, 2]. Calcium and magnesium play an important role in various physiological processes of an organism. In the fact, calcium ions are necessary for the process of transmission of nerve impulses and the activity of certain enzymes [3–6]. Magnesium plays a key role in the energy, plastic and electrolyte exchange, acts as a regulator of cellar growth, and is important component at all stages of the synthesis of protein molecules. In particular, the proper functioning of ribosomes and binding them with messenger RNA (a key mechanism of protein biosynthesis) depend on a sufficient amount of magnesium in the body. Besides, magnesium takes part in sharing phosphorus, ATP synthesis, regulation of glycolysis, the building of bone, etc. [7, 8]. Iron is an important component of a plenty processes of life. In the body's cells iron is consumed on the synthesis of gemo-containing enzymes and ferritin — the main protein containing iron stores. Iron is essential in the synthesis of hemoglobin, as well as in increasing of erythrocyte production. Lithium actively influences neurochemical processes occurring in the brain, that underlies its therapeutic activity in mental illness. It is found that lithium has the ability to stop acute manic excitement and to prevent affective attacks. However it is known that these drugs have toxic side effects on the organism [8].

Therefore creating new forms of drugs based on salts of these metals, as well as studying their bioactivity, including antioxidant properties, are current interests.

The aim of the paper is to investigate the influence of complexes of magnesium, lithium, cobalt, iron, calcium, containing ascorbic acid as bioactive ligand on the electrochemical behavior of oxygen in the aqueous medium in a semi-infinite linear diffusion.

## 2. Methods and Materials

A simple designed in hardware, express and highly sensitive method of voltammetry (VA) was used in the work.

Investigations were carried out on a computerized voltammetric analyzer TA-2 producted by LLC Research and Production Enterprise RPE «Tomanalyt» (Tomsk).

Voltammetric curves were recorded at room temperature in a three-electrode electrochemical cell connecting to the analyzer. A working mercury film electrode (MFE), a silver-silver chloride electrodes with KCl saturated ($Ag|AgCl|KCl_{sat}$), as reference and counter electrodes, were used. An open type cell was used in this investigation.

As a supporting electrolyte, phosphate buffer 0.025 $mol \cdot l^{-1}$ (equimolar mixtures of $Na_2HPO_4$ and $KH_2PO_4$, pH 6.86) was used.

Constantly cathode-current mode VA was used; speed of potential sweep (W) was 40 mV/s, working potential range 0 to -1 V. The substances were mixed by vibration of the electrodes.

Oxygen reduction reaction was used as a model reaction. Records were in following order: record of first wave cathodic reduction of oxygen dissolved in a supporting electrolyte in the specified potential range. After substance addition, the solution was stirred about 20s. After the stirring is stopped, the potential was scanned negatively, causing oxygen reduction, giving a current first wave electroreduction of oxygen (ER $O_2$). Its value was proportional to the amount of oxygen in the bulk of the solution. Oxygen concentration was monitored by oxygen analyzer. Based on the ammetric measurements the concentration of oxygen in phosphate buffer at 25.0 ± 0.5°C was $2.56 \pm 0.05 \cdot 10^{-4}$ $mol \cdot l^{-1}$.

Oxygen concentration in the electrolyte solutions was monitored by potentiometric oxygen sensor № 5972 produced by the center of computing automation and measuring "MERA-ELVRO", with automatic temperature compensation in the range of 0 to 40 C. Weigh sample material was carried out on laboratory analytical balance VL 210 firm "Gosmetr" with a weighing error of ± 0.0002 g. Nanopure water was used for making solutions.

## 3. Experimental

The list of methods of determining the antioxidant activity (AA) is wide enough: the chemiluminescent method, gas phase chromatography, and electrochemical methods, fluorimetric, etc. [9–14]. The method of cathode voltammetry (VA) in determining AA of metal ascorbates was used in the work [15].

The method is based on a process of ER $O_2$, that has a mechanism similar to the restoration of oxygen in the cells and body tissues (1-3). At the same time active forms of oxygen are generated on the electrode: superoxide anion of oxygen $O_2^{\cdot -}$ and hydroperoxide $HO_2^{\cdot}$.

$$O_2 + e^- \underset{}{\overset{k}{\rightleftharpoons}} O_2^{\cdot -} \quad (1)$$

$$O_2^{\cdot -} + H^+ \rightleftharpoons HO_2^{\cdot} \quad (2)$$

$$HO_2 + H^+ + e^- \rightleftharpoons H_2O_2 \quad (3)$$

The antioxidant activity was determined by a relative decrease in the current ER of $O_2$ in the presence of the test components in solutions.

This paper considers the influence of complexes of magnesium, lithium, calcium, cobalt, iron, that contain ascorbic as bioactive ligand (Figure 1), on the electrochemical behavior of oxygen in the aqueous medium in a semi-infinite linear diffusion. Also authors made a comparative evaluation of the activity of the new complex of magnesium with previously synthesized metal complexes and ascorbic.

The influence of the test substances on the electrochemical behavior of oxygen is considered in a supporting electrolyte – phosphate buffer with pH 6.86.

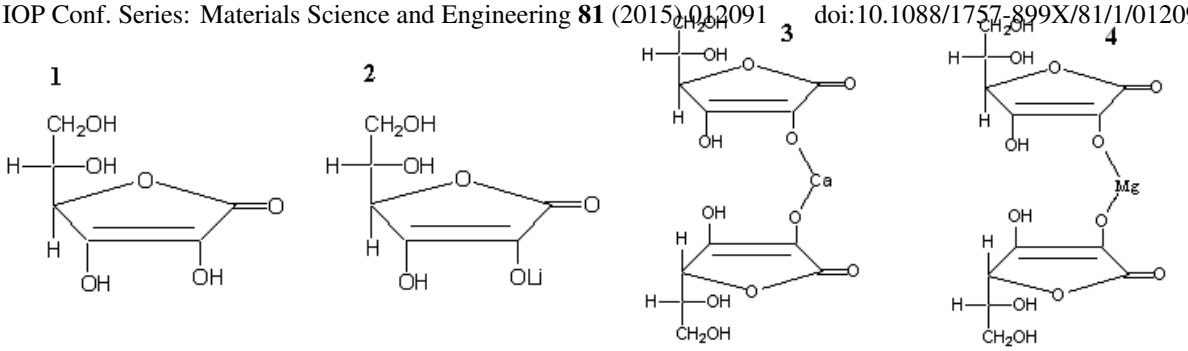


**Figure 1.** Structural formulas of the test substances: ascorbic (1); lithium ascorbate (2); calcium ascorbate (3); magnesium ascorbate (4)

For the study of AA of drugs voltammograms of first current wave ER $O_2$ in the absence and presence of investigated ascorbates were recorded. Studies showed a decrease in the limiting current ER $O_2$ in the presence of examined ascorbates, with the exception of iron ascorbate.

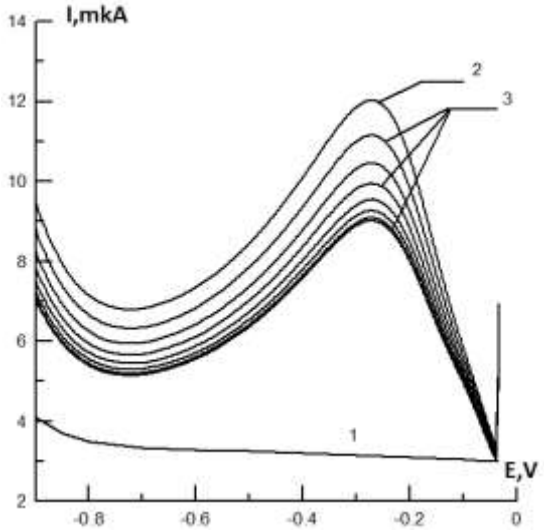


**Figure 2.** Voltammograms of the ER $O_2$ current in phosphate buffer (0.025M, pH 6.86) on the MFE without (2) and with $10^{-4}$ g/ml Mg ascorbate in solution (3), the residual current of supporting electrolyte in the absence of $O_2$ and substances in the solution (1)

Lines (3) in Figure 2 show the reduction of oxygen current in depending on the time of reaction between the active forms of oxygen and magnesium ascorbate solution. Similar dependences were obtained for all the studied ascorbates.

According to the results of obtained voltammograms graphs of dependency of relative decrease of current ER $O_2$ on the time of the process in the presence of the test drug were built.

The antioxidant activity of the investigated drugs was estimated by kinetic criteria of antioxidant activity – K (μmol $l^{-1}min^{-1}$), which reflects the efficiency of interaction of the sample with active forms of oxygen [14]:

$$K = (1 - \frac{I}{I_o})\frac{C^0}{t}$$

where $C^0$ [μmol·$l^{-1}$] is the oxygen concentration at the electrode in absence antioxidant, $I$ [A] is the ER $O_2$ current with the investigated substance addition in the solution, $I_o$ [A] is the limiting ER $O_2$ current without substance in the solution, $t$ [min] is time of the interaction between reactive oxygen species (ROS) and antioxidant at the MFE.

## 4. Discussion of Results

Reducing the current of first wave of ER $O_2$ is connected with the reaction between investigated metals ascorbates and products of oxygen reduction. The most active in relation to the process of the ER $O_2$ is Mg ascorbate, while Fe ascorbate is completely neutral and do not show antioxidant properties.

**Table 1.** AA of the samples ($c = 10^{-4}$ g/ml) in relation to the process of ER $O_2$ (p=0.95, n=5).

| Substance name | $K$, μmol $l^{-1}min^{-1}$ |
|---|---|
| Asc Mg | 2.125 ± 0.053 |
| Asc Li | 1.714 ± 0.024 |
| Asc Ca | 1.550 ± 0.042 |
| Asc Co | 0.879 ± 0.027 |
| Asc Fe | – |
| Ascorbic acid | 1.165 ± 0.053 |
| Glucose | 0.169 ± 0.037 |
| Dihydroquercetin | 0.650 ± 0.040 |

Most of the metal complexes and ascorbic acted more actively, then ascorbic itself, which is andoubtly antioxidant and synergist in the process of interrupting the chain radical reactions.

As AA is depends on both time of interaction of antioxidant with active forms of oxygen and concentration of antioxidant, the influence of Asc Mg on a process of ER $O_2$ (Figure 3) was considered. It is shown that with the increasing of concentration of Asc Mg in solution the relative change in the limiting current ER $O_2$ is growing, that means that the test substance is more responsive to the oxygen radicals.

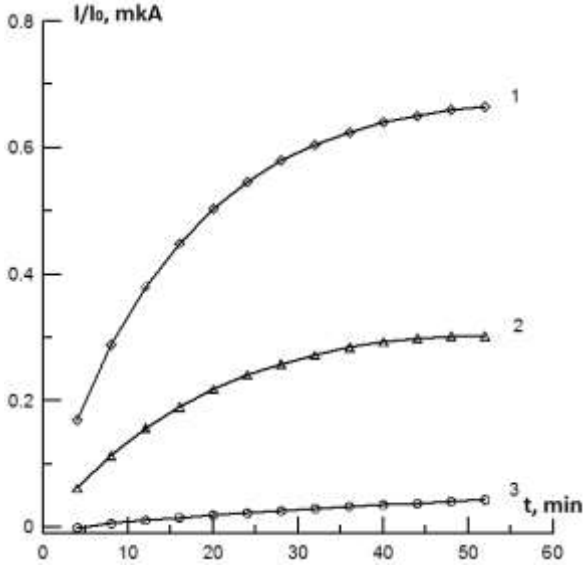


**Figure 3.** The dependence of the relative change of the current ER $O_2$ from the time of the process in the presence of $2 \cdot 10^{-4}$ (1), $1 \cdot 10^{-4}$ (2), $1 \cdot 10^{-5}$ g/ml (3) Asc Mg in the solution

**Table 2.** AA of the samples in relation to the process of ER $O_2$ in dependence on concentration (p=0.95, n=5).

| Concentrarion Asc Mg, g/ml | $K$, μmol $l^{-1}min^{-1}$ | Storage time Asc Mg |
|---|---|---|
| $2 \cdot 10^{-4}$ | 5.374 ± 0.082 | fresh |
| $2 \cdot 10^{-4}$ | 2.711 ± 0.023 | after 2 months |
| $1 \cdot 10^{-4}$ | 2.125 ± 0.053 | fresh |
| $1 \cdot 10^{-5}$ | 0.251 ± 0.004 | fresh |

Therefore with the growth of storage time Asc Mg becomes less active in relation to the process of ER $O_2$.

In order to establish the existence of synergies the activity of mixtures of known antioxidants and ascorbates was determined. The results are shown in Table 3.

**Table 3**. Antioxidant activity of samples in relation to the process of ER $O_2$ in dependence on concentration (p=0.95, n=5).

| The mixture of antioxidants | *K*, μmol $l^{-1}min^{-1}$ | The mixture of antioxidants | *K*, μmol $l^{-1}min^{-1}$ |
|---|---|---|---|
| Asc Mg, dihydroquercetin (1:1) | 2.727 ± 0.131 | Asc Li, glucose (1:1) | 0.338 ± 0.094 |
| Asc Mg, dihydroquercetin (1:3) | 1.111 ± 0.128 | Asc Li, glucose, dihydroquercetin (1:1:1) | 0.453 ± 0.023 |
| Asc Mg, glucose (1:1) | 0.383 ± 0.019 | Asc Li, glucose, dihydroquercetin (1:2:1) | 0.642 ± 0.068 |

Due to the data it is proper to say that multicomponent mixtures are less active in relation to the process of cathode oxygen reduction than two-components mixtures. It should be noted that in the case of binary mixtures the effect of synergy can be observed, i.e. strengthening of active action of each component in the presence of another one. The same behavior of components of Mg and Li ascorbates' mixtures wasn't noted. In the mixtures with the number of components more than 3 total activity in relation to the process of ER $O_2$ was decreasing arithmetically. Perhaps this is because of spherical difficulties of organic residues that prevent the cathodic reduction of $O_2$ in the MFE. Due to the obtained data it is fair to say that for more effective influence creating of new drugs based on Mg and Li ascorbates with a number of components no more that 3 is really necessary.

Toxicity studies were also carried out on the basis of the compositions of Mg and Li ascorbates 25 mice of BALB/c with intraperitoneal administration of an aqueous solution of the drug in a wide range of doses

Also researches of toxicity of mixtures based on Mg and Li ascorbates were held on 25 mice of BALB/c line with intra-abdominal impact of aqueous solution of preparation in wide dose range (from 100 to 1000 mg/kg weight). The observations were made within 30 days. During this time no toxic side effects or death of animals were observed. Perhaps reducing toxic effects of metals is connected with their binding with ascorbic and the creation of mixtures with widely known antioxidants such as dihydroquercetin, glucose and others.

## 5. Conclusion

Thus, studies of antioxidant properties of ascorbate metals showed significant activity of Asc Mg and Asc Li. It is shown that with the increasing of concentration of Asc Mg its activity in relation to the process of ER $O_2$ increases. It was also noted that there is a synergistic effect in mixtures of dihydroquercetin with Asc Mg with the number of components no more than 2. Based on these data it is possible to recommend the establishment of pharmacological drugs on the basis of magnesium and lithium ascorbates in mixtures with small amounts of components for greater impact on the body.

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