

Cyclic voltammetry as a method of determining antioxidant activity of selected low-molecular compounds

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Please cite as: CHEMIK 2011, 65, 10, 1059-1062

Introduction

To maintain life functions, aerobic organisms need oxygen. Without food man can live for several weeks, without water – a few days, but without oxygen an organism dies within a few minutes. Oxygen is transferred from lungs to blood and is distributed to all cells of the human body to participate in a series of biochemical processes in which it is converted into energy necessary to fulfil the requirements of other metabolic reactions. However, oxygen is a double-edged sword. On the one hand it lets cells absorb more energy through respiration, but on the other it reacts with numerous one-electron compounds and forms Reactive Oxygen Species [1].

The Reactive Oxygen Species (ROS) are chemical entities which take part in biochemical and physiological processes that take place in living organisms. In a healthy organism there is an equilibrium between the generation and elimination of free radicals. There are, however, many factors which disturb that equilibrium and promote formation of free radicals and other reactive oxygen species. These include: environmental pollution, ionising radiation, exposure to UV radiation, smoking, food products that contain pesticides, herbicides and preserving agents, heavy metals, cosmetics and drugs that contain substances harmful to health (e.g.: aluminium in antiperspirant cosmetics), inflammatory state of the organism, and stress. Increased concentration of reactive oxygen species in the organism caused by the factors mentioned can lead to an oxidative stress [3, 6]. ROS, if present at sufficient levels, damage cells, and can even cause the death thereof [1].

Living cells defend themselves against the excess of ROS using compounds classified as low-molecular antioxidants. These are substances present at low concentrations, as compared to the substances subject to oxidation, which inhibit or prevent such oxidation by entering into redox reactions with the oxidising agents [3, 4]. The simple structure of low-molecular antioxidants makes them convenient models for the study of the processes of ROS neutralisation. By neutralising reactive oxygen species antioxidants mitigate damage to cells and biomolecules, protect against damage to blood vessels, against carcinogens, reduce the risk of coronary heart disease and of Alzheimer's disease. A rich source of antioxidants may be a proper diet comprising fruit, vegetables, cereals, fish. Antioxidants can also be supplied to the organism in the form of dietary supplements. They are widely used in the food, cosmetic and pharmaceutical industries – they prevent the oxidation of unstable ingredients [2, 3, 5].

The goal of the studies carried out was to determine the antioxidant activity of a group of selected low-molecular antioxidants and to compare their antioxidant power.

Experimental

Electrochemical tests were conducted using BAS 100B/W electrochemical analyser from Bio-analytical Systems (USA).

Electrochemical processes were carried out in a three-electrode electrolytic cell. The working electrode used was a glassy carbon (GC) electrode from Mineral (Poland), the auxiliary electrode was a platinum wire, the reference electrode was a silver chloride electrode filled with 3 M NaCl solution. The GC electrode was cleaned before each

measurement by polishing it with alumina suspension (Al_2O_3 0.05 μm) on polishing felt. The electrode was then washed with distilled water and dried. The tests were conducted in a phosphate buffer solution of constant pH (6.5–6.6) under air and pure argon (from Linde).

The tests were carried out within the potential range of 1.0 to -1.1 V, at scan rates of 0.05 to 1 V/s; most voltammograms were recorded at scan rate of 0.1 V/s. Prior to proper measurement the buffer solution was flushed with argon in the electrolytic cell and the background signal was registered. After measurement under argon the system was saturated with air. Then a solution of the measured compound in the buffer was added portion wise. The concentration of the added compound was 0.1 or 0.5 mM. Each time the concentration of the measured compound was increased, a voltammogram was plotted.

The following analytically pure compounds were used in the tests: ascorbic acid, caffeic acid, resveratrol. Other substances studied included: vitamin C200 (coated tablet) (Teva), green tea (Yunan), Nescafe Classic (Nestle) and red wine Pinot Noir 2007 (St. Andrea, Hungary). In the case of ascorbic acid, caffeic acid, resveratrol and vitamin C200 the solutions prepared had a concentration of 0.5 mM. The amount taken of vitamin C200 was such, as to attain 0.5 mM concentration of the active ingredient upon dissolution. Green tea and coffee solutions were prepared from 1 g of the substance dissolved in 40 cm^3 distilled water at 80°C (green tea) and 100°C (coffee). After 4 minutes the infusions were filtered. Samples of red wine were taken without diluting.

After plotting voltammograms for resveratrol, green tea, red wine and coffee, the working electrode was cleaned with polishing felt to remove polyphenolic compounds adsorbed on electrode surface.

Discussion of results

In nature the main source of ROS is oxygen, and aerobic organisms defend themselves against their excess by means of various antioxidant compounds. In connection with that electrochemical tests were conducted, the results of which are presented below.

Figure 1 presents a voltammogram plotted for the process of oxygen reduction (1). Voltammogram (2) was plotted for a system under argon.

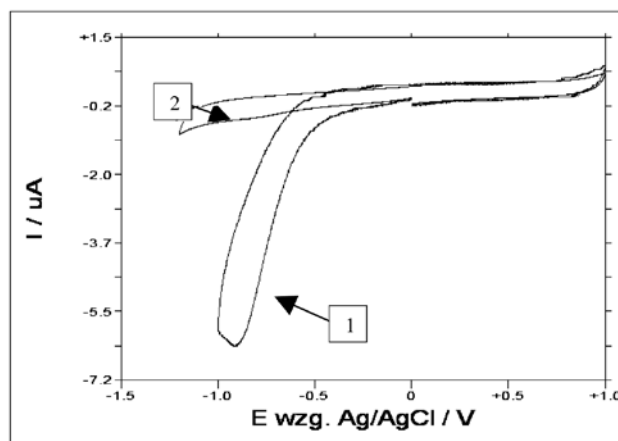


Fig. 1. Cyclic voltammogram (CV) of dioxygen reduction. CV registered on glassy carbon electrode (GCE) vs. Ag/AgCl reference at 0.1 V/s scan rate

It was observed that oxygen reduction commenced at the potential of -330 mV, measured versus silver chloride electrode. The peak maximum is reached at -910 mV. This agrees with the results of A. Cournet *et al.* [7]. Such wave is characteristic of electrochemical two-electron reduction of oxygen leading to formation of hydrogen peroxide ($O_2 + 2H^+ + 2e^- \rightarrow H_2O_2$).

As shown in Figure 2, with increasing antioxidant concentration the peak of the oxidation wave is growing.

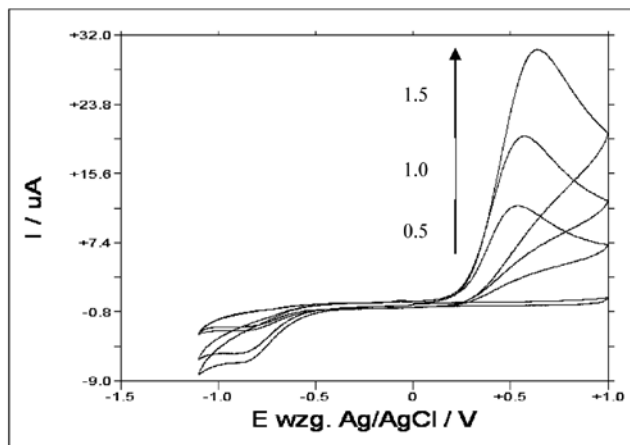


Fig. 2. Cyclic voltammogram (CV) of dioxygen reduction prior to (curve 1) and after adding ascorbic acid at following concentrations of the acid: 0.5, 1.0, 1.5 mM. CV registered on glassy carbon electrode (GCE) vs. Ag/AgCl reference at 0.1 V/s scan rate

The process of acid oxidation commences at the potential of +142 mV, irrespective of the concentration. The reduction peak, however, has not been registered near the oxidation potential, which would indicate that the process is reversible. The process was conducted within a broader range of potentials, covering the potential of oxygen reduction. It was observed that with increasing concentration of ascorbic acid in the solution, the reduction current decreased, indicating the participation of reduced oxygen in the process of ascorbic acid oxidation.

Figure 3 presents the process of ascorbic acid oxidation at one concentration of the acid ($c=0.5$ mM), at various scan rates. It was observed that with increasing scan rate, the current of the oxidation peak increased. There was also a slight shift of the potential value towards anodic potentials.

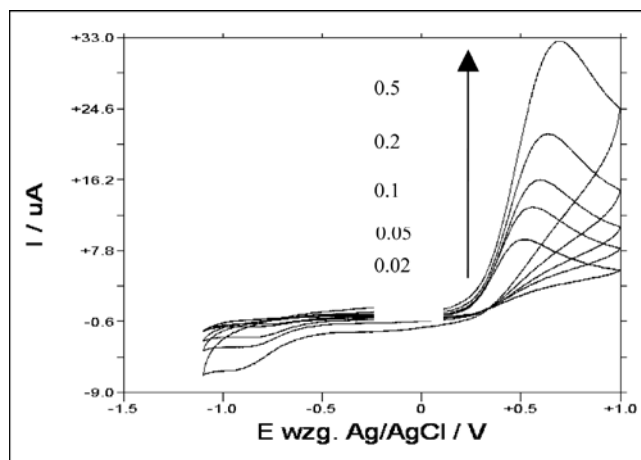


Fig. 3. Cyclic voltammogram (CV) of dioxygen reduction in the presence of 0.1 mM ascorbic acid. CV recorded on glassy carbon electrode (GCE) vs. Ag/AgCl reference at: 0.02, 0.05, 0.1, 0.2, 0.5 V/s scan rate

Figure 4 presents voltammograms for pure ascorbic acid and vitamin C tablet.

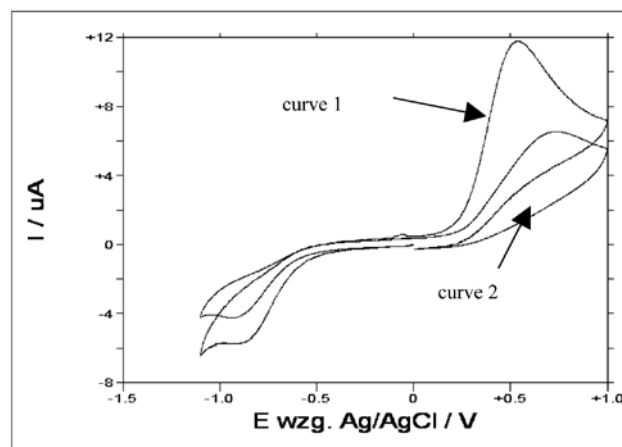


Fig. 4. Cyclic voltammogram (CV) of dioxygen reduction in the presence of ascorbic acid (curve 1) and after vitamin C200 addition (curve 2). Concentrations of active substances were equal at 0.5 mM. CV registered on glassy carbon electrode (GCE) vs. Ag/AgCl reference at 0.1 V/s scan rate

In both cases the concentration of ascorbic acid was comparable ($c=0.5$ mM). As can be seen, the oxidation peak current is much smaller in the case of tablet than in the case of acid. There was also a shift of the wave potential towards anodic potential and a distinct broadening of the peak. This is presumably associated with the presence of excipients in the tablet, which may attenuate the effect of ascorbic acid action.

Figure 5 presents voltammograms recorded for various concentrations of a) resveratrol and b) red wine. Comparison of these voltammograms is by all means justified, as resveratrol is present in grapes and many types of wines.

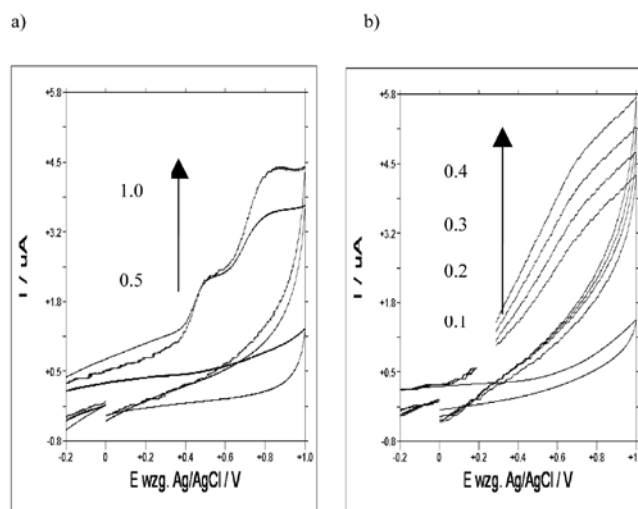


Fig. 5. CV recorded for increasing concentrations of: a) resveratrol at: 0.5, 1.0 mM, b) red wine at volumes of: 0.1, 0.2, 0.3, 0.4 ml. CV registered on glassy carbon electrode (GCE) vs. Ag/AgCl reference at 0.1 V/s scan rate

In both cases it can be seen (as was the case with previous compounds) that the current of the oxidation wave peak increased with increasing concentration of the tested compound. The second oxidation peak, present in voltammograms that demonstrate oxidation of resveratrol, has not appeared in voltammograms recorded after addition of wine. In this case there was a larger increase of the second peak (at more anodic potential). In the case of red wine, the anodic wave is not sharp and well defined (which is due to the synergetic effect between resveratrol and other antioxidant compounds contained in wine), although the oxidation process commences at the potential of +45 mV.

Figure 6 presents voltammograms of the oxidation of antioxidants present in green tea.

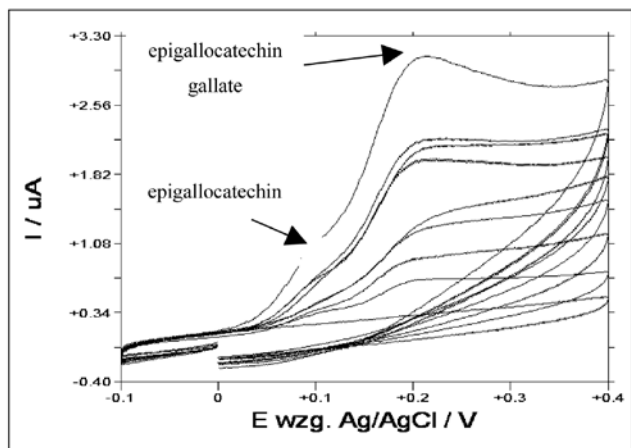


Fig. 6. Cyclic voltammogram in the presence of green tea. CV registered on glassy carbon electrode (GCE) vs. Ag/AgCl reference at 0.1 V/s scan rate

The process of oxidation commences at the low potential of +25 mV. Two anodic peaks were formed. The analysis results can be compared to those obtained by P. Kilmarin *et al.* [8]. Such comparison confirms the presence of epigallocatechin and epigallocatechin gallate in green tea infusion. The tests were carried out within the potential range of -100 to +400 mV.

Figure 7 presents a set of voltammograms of all the tested antioxidants.

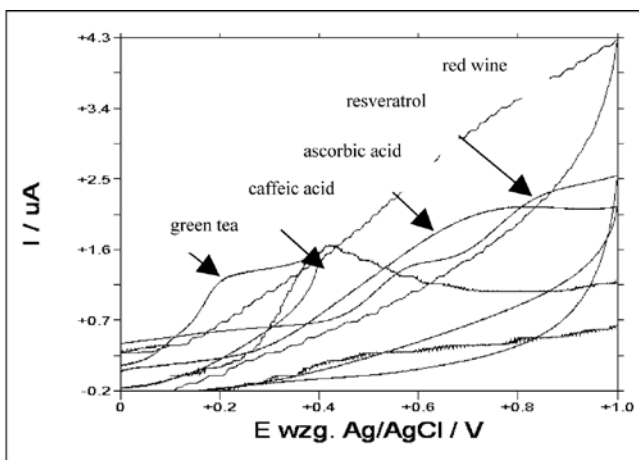


Fig. 7. Cyclic voltammogram in the presence of: ascorbic acid; caffeic acid; resveratrol; red wine; green tea. CV registered on glassy carbon electrode (GCE) vs. Ag/AgCl reference at 0.1 V/s scan rate

The voltammograms were recorded at equal 0.1 mM concentrations of: ascorbic acid, caffeic acid, resveratrol. In the case of red wine and green tea only the volume of the substance added was known. The values of recorded anodic wave currents were comparable for all compounds, which may indicate that 0.1 mL of green tea as well as of red wine contains about 0.1 mM of antioxidants.

The oxidation process commences at the earliest stage in the case of green tea (+25 mV), followed by resveratrol (+30 mV) and red wine (+45 mV). +140 mV is the potential at which ascorbic acid oxidation commences, while +175 mV corresponds to the start of caffeic acid oxidation. The sequence was different for potentials at which current maximums were observed.

Table I lists the potentials and current intensities for the tested compounds.

Table I

Values of potential and current intensity for the wave of oxidation of compounds tested

Name	Scan rate, V/s	Potential at peak 1, mV	Current at peak 1, A	Potential at peak 2, mV	Current at peak 2, A
Ascorbic acid	0.1	765.5	2.1430e ⁻⁵	-	-
Caffeic acid	0.1	398.8	2.0500e ⁻⁶	-	-
Resveratrol	0.1	584.4	1.3870e ⁻⁶	857.7	2.295e ⁻⁶
Green tea	0.1	96.5	0.4175e ⁻⁶	203.5	1.206e ⁻⁶
Wine	0.1	704	3.1090e ⁻⁶	-	-

Analysis of the data above indicates that the strongest antioxidant is the compound present in green tea – epigallocatechin gallate, followed by caffeic acid, resveratrol, red wine and ascorbic acid.

Of all the compounds tested, reduction peak in the positive potential range appears only in the case of caffeic acid. This is the result of quinone reduction to caffeic acid. However, as shown in Figure 8, this process is not reversible.

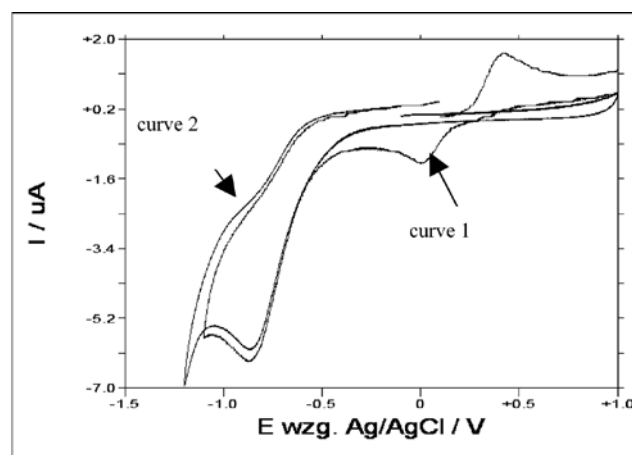


Fig. 8. Cyclic voltammogram (CV) of dioxygen reduction prior to (curve 1) and after adding 0.1 mM caffeic acid solution. CV registered on glassy carbon electrode (GCE) vs. Ag/AgCl reference at 0.1 V/s scan rate

The difference between oxidation and reduction potentials is $\Delta E = 417$ mV, and the ratio of anodic current to cathodic current I_a/I_c is 1.4, that is more than 1. The reduction wave in the reverse scan causes additional lowering of the oxygen reduction wave. The value by which it is increased is associated with the diffusion current.

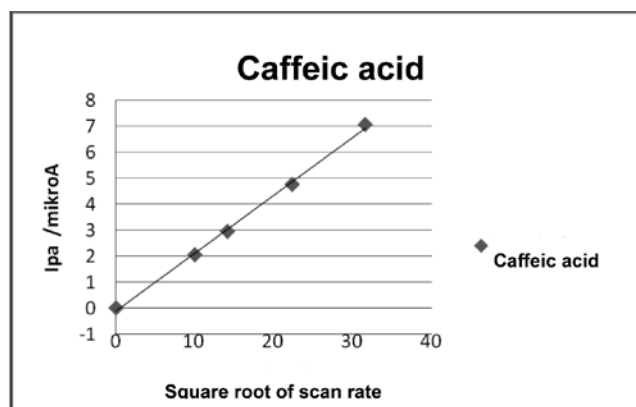


Fig. 9. The dependence of I_{pa} on the square root of scan rate

Processes of antioxidant oxidation are controlled by diffusion. Figure 9 presents the relationship between the current of caffeic acid oxidation peak and square root of scan rate.

As can be seen, the relationship is linear and the straight line goes the origin of coordinates, which indicates that the process is controlled by diffusion.

Cyclic voltammetry is a method which enables precise determination of oxidation potentials and of antioxidant concentration. However, in the case of phenolic compounds, the problem was the adsorption thereof on the surface of the electrode [9]. After recording each voltammogram the electrode had to be cleaned because the layer of adsorbed polyphenols made further measurements impossible.

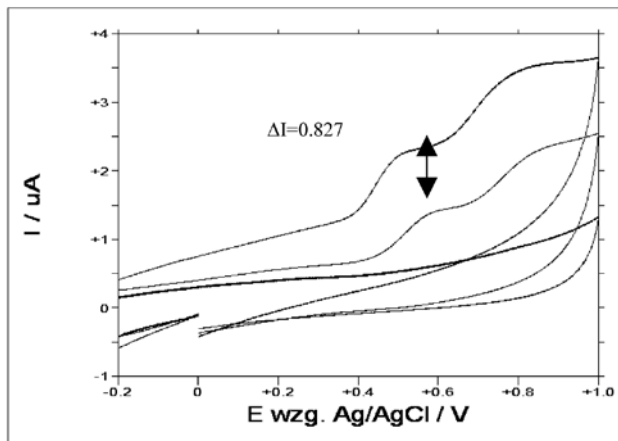


Fig. 10. Cyclic voltammogram prior to (curve 1) and after adding resveratrol; curve (2) obtained with a clean electrode; curve (3) obtained without electrode cleaning. CV registered on glassy carbon electrode (GCE) vs. Ag/AgCl reference at 0.1 V/s scan rate

When several scans were made without cleaning the electrode, the current at the peak decreased (Fig. 10) and the oxidation potential shifted to more anodic values. This caused a severe difficulty in making measurements. In order to obtain repeatable results, the surface of the electrode had to be cleaned, more than a dozen times on some occasions.

Summary and Conclusions

Cyclic voltammetry can successfully be applied to determine activity of antioxidants. It is an accurate and sensitive method for determining oxidation potentials. The determined potentials may serve as indicators of antioxidant activity.

On the basis of tests performed and analyses of voltammograms, the strongest antioxidant properties were detected in the compounds present in green tea, the weakest in ascorbic acid.

In the case of measurements of caffeic acid, green tea, resveratrol and red wine, the active substances tended to adsorb on the surface of the electrode, interfering with the proper course of analysis. The working electrode required thorough cleaning before each measurement.

The analysis of the current vs. square root of scan rate graph led to the conclusion that the process of antioxidant oxidation is controlled by diffusion.

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A Chemist's Guide to Chemical Engineering

Most chemical engineering courses aimed at chemists are actually developed and presented by chemical engineers. As a result they tend to be mathematically based and unsuitable or uninteresting for R&D chemists. This course is different; it has been developed and is presented by a chemist, for chemists. It does not dwell on the derivation of a mathematical formula or equations. Instead, it focuses on what chemists need to know about the information and data that engineers require for design and scale-up. Chemical engineering without mathematics is impossible and so this course does contain mathematics, but at a level that should not trouble degree-level chemists. Chemists do not need to know how to design plants or equipment and, since most chemists are not interested, this course does not attempt to teach them. A chemist does need to know what information is needed for design, scale-up and commercial plant operation and how the experimental data and information are used.

(<http://www.chemistry2011.org/participate/events.6.09.2011>)