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ARTICLES

Electrochemical Determination of Unithiol and Lipoic Acid at Electrodes Modified with Carbon Nanotubes

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Abstract—Conditions are found for the voltammetric determination of lipoic acid and unithiol at a glassy-carbon electrode modified with multiwalled carbon nanotubes. Possible mechanisms for the oxidation of lipoic acid and unithiol are proposed. As compared to an unmodified electrode, the use of the modified electrode allows the analyst to reduce overvoltage ($\Delta E = 0.1$ V) and increase the oxidation current of lipoic acid. Unithiol is oxidized in the accessible range of potentials only at an electrode modified with carbon nanotubes. The determination limits for unithiol and lipoic acid are 4.1×10^{-5} and 1.9×10^{-5} M, respectively. Milligram amounts of these substances are determined in model solutions with RSD = 1–5%. Procedures for determining the active substances (lipoic acid and unithiol) in pharmaceuticals are proposed.

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Calcium disodium ethylenediaminetetraacetate, penycillamine, and sulfur-containing compounds (unithiol, dimercaprol, and dimercaptosuccinic acid) are used to treat poisoning with toxic metals.

Unithiol (sodium 2,3-dimercaptopropane sulfonate) was first proposed as an antidote to organic and inorganic compounds of arsenic and toxic metals (chromium, zinc, molybdenum, mercury, lead, cadmium, copper, etc.). The antidote properties of unithiol are due to its ability to form complexes with toxic metals and remove them from the body through the urine. Low toxicity, safety, and stability in aqueous solutions are the advantages of unithiol over other preparations. It has no effect on the metabolism of biometals, it does not redistribute toxic metals in the body of poisoned animals, and can be combined with other drugs [1].

Unithiol also exhibits antioxidant properties due to the presence of two thiol groups in its structure. On the one hand, it acts as a chelator of metals with variable valences [2], and, on the other hand, it counteracts the growth of lipid peroxide oxidation in different types of pathologies [3–5].

α-Lipoic acid (6,8-dithioctic acid) is also an important antioxidant in biological systems. In the body, α-lipoic acid works as a reserve system for the start-up of important antioxidants, and, in addition, it is an effective trap for radicals by itself. α-Lipoic acid and its reduced form are called "universal antioxidants"; they function in both membrane cells and the intercellular space [6].

The antioxidant effect of α-lipoic acid is due to the disulfide bond present in its molecule. It binds oxygen active forms (hydroxyl radicals and singlet oxygen) [7] and free iron in tissues, preventing their participation in peroxide oxidation of lipids. α-Lipoic acid oxidizes Fe(II) [8] and can also bind Fe(II) and Cu(II) and remove them from the body [9–11].

α-Lipoic acid was proved not only to possess its own antioxidant potential but also considerably assists other antioxidant processes in the body. In this case, its protective action is closely allied with homeostasis in the glutathione and ubiquinone system [12, 13].

Thus, the above-mentioned sulfur-containing antioxidants are high-priority subjects of investigations, and the development of procedures for determination is of interest to analysts and biochemists.

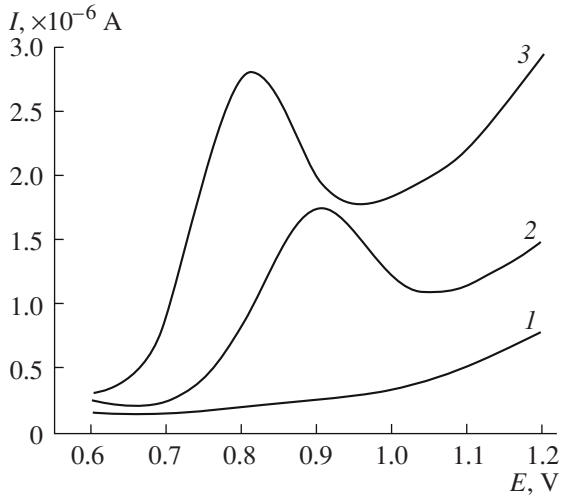
Voltammetry opens up wide possibilities for determining biologically active compounds of different nature, particularly, oxidizable ones [14]. The voltammetric procedure was developed for determining α-lipoic acid at a glassy–carbon electrode [15].

In the last decade, chemically modified electrodes have been widely used in the voltammetry of biologically active substances, including antioxidants. Carbon nanotubes (**CNs**), as a new form of carbon materials exhibiting high electrocatalytic activity and high rate of electron transfer, are extensively used to create electrochemical sensors [16].

The goal of this work was to evaluate the potentials of voltammetry at electrodes modified with multiwalled CNs for determining lipoic acid and unithiol and to develop a procedure for determining these substances in pharmaceuticals.

EXPERIMENTAL

Measurements were carried out using an Ekotest-VA voltammetric analyzer. A 25.0-mL portion of a 0.1 M H_2SO_4 solution and an aliquot of a test solution were placed in a 50.0-mL electrochemical cell. Work-



Anodic voltammograms of a 1.3×10^{-4} M lipoic acid solution in (*1*) 0.1 M H_2SO_4 solution at (*2*) GCE and (*3*) CNs-GCE (the rate of potential sweep was 25 mV/s).

ing, auxiliary, and saturated silver–silver chloride reference electrodes were immersed and voltammograms were recorded with a linear sweep of potential from 0 to 1.2 V at a rate of 25 mV/s.

Multiwalled CNs with an inner diameter of 1–3 nm, an outer diameter of 3–10 nm, and 0.1–10 μm in length from Sigma-Aldrich (Germany) were used.

A glassy-carbon electrode (**GCE**) with a surface area of 3.14 mm^2 was modified by applying 7 μL of the suspension of multiwalled CNs followed by evaporating the solvent in air to form a homogeneous layer of CNs on the electrode's working surface. To obtain the homogeneous suspension of CNs, the latter were previously oxidized with the mixture of nitric and sulfuric acids (3 : 1) and simultaneously ultrasonically dispersed and precipitated by centrifuging [17].

Standard solutions of lipoic acid and unithiol were prepared by dissolving samples in distilled water.

Procedure for determining lipoic acid in tablets. Ten tablets were weighed and triturated in a mortar. An accurately weighed sample (~0.02 g) of powdered tablets was dissolved in distilled water in a 50.0-mL volumetric flask. The solution was filtered. A 0.5-mL aliquot was placed in the electrochemical cell, and anodic voltammograms were recorded. The concentration of lipoic acid was found from the calibration graph.

The solution of unithiol for injections (0.5 mL) was analyzed without sample preparation.

RESULTS AND DISCUSSION

In a 0.1 M H_2SO_4 solution, lipoic acid was oxidized at both GCE (peak at 0.91 V) and GCE modified with multiwalled CNs (peak at 0.81 V). A clearly defined anodic peak was observed in voltammograms (see the figure). Unithiol was electrochemically inert at the GCE in the potential range from 0.0 to 1.3 V. The peak of unithiol was observed at 0.60 V at the electrode modified with CNs.

The modification of the GCE with CNs reduced overvoltage almost by 0.1 V and increased the oxidation current of lipoic acid by a factor of 1.6 as compared to the unmodified electrode.

The modification of the electrode surface with multiwalled CNs increased its effective area, which resulted in the growth of substrate oxidation currents. In addition, the electrocatalytic action of CNs is due to the presence of oxygen-containing functional groups resulting from the treatment of CNs with the mixture of acids during the preparation of CN suspension [18]. A decrease in the overvoltage of lipoic acid and unithiol pointed to an increase in the rate of electron transfer in oxidation reactions, which is in agreement with the published data [19].

Lipoic acid is oxidized with the rupture of the disulfide bond, as in the case of cystine [20]. Unithiol is oxidized to give disulfide by the reaction

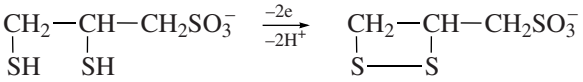


Table 1. Analytical characteristics of the voltammetric determination of unithiol and lipoic acid in a 0.1 M H_2SO_4 solution

	Electrode	Determination limit, M	Analytical range, M	Regression equation $y = a + bx$		R
				a	$b \times 10^{-3}$	
Unithiol	CNs-GCE	4.1×10^{-5}	1.8×10^{-4}–1.4×10^{-3}	–0.9 ± 0.7	20.9 ± 0.9	0.9953
			2.6×10^{-3}–6.9×10^{-3}	–77 ± 5	52.3 ± 0.9	0.9993
Lipoic acid	GCE	1.9×10^{-5}	3.7×10^{-5}–3.5×10^{-4}	–0.22 ± 0.04	12.4 ± 0.2	0.9991
			4.2×10^{-4}–7.8×10^{-4}	2.4 ± 0.1	5.3 ± 0.2	0.9973
	CNs-GCE	1.9×10^{-5}	2.6×10^{-5}–1.8×10^{-4}	0.29 ± 0.03	17.7 ± 0.3	0.9996
			2.1×10^{-4}–7.8×10^{-4}	1.84 ± 0.04	7.35 ± 0.07	0.9997

Table 2. Voltammetric determination of unithiol and lipoic acid in model solutions at CNs-GCE in a 0.1 M H_2SO_4 solution ($n = 5$, $P = 0.95$)

Analyte	Added, mg	Found, mg	RSD, %
Unithiol	3.0	2.9 ± 0.2	4
	5.0	4.9 ± 0.1	2
	8.0	7.9 ± 0.2	1
	25.0	25.7 ± 0.3	1
	40.0	39.9 ± 0.3	1
Lipoic acid	0.3	0.32 ± 0.04	5
	0.8	0.79 ± 0.03	3
	1.5	1.56 ± 0.04	2
	2.9	2.90 ± 0.02	1
	3.8	3.79 ± 0.03	1

Oxidation currents linearly grew as the analyte concentration in the solution was increased. The parameters of calibration graphs for the oxidation of unithiol and lipoic acid are presented in Table 1. The use of the GCE modified with CNs (CNs-GCE) extends the range of the linear dependence of oxidation current on the concentration of lipoic acid.

Table 2 summarizes the results of determining unithiol and lipoic acid in model solutions. The results were verified by the added–found method.

A procedure for determining the main (active) substances in pharmaceuticals was developed on the basis of these results. Table 3 demonstrates that the RSD value was at most 2%.

The procedure is simple, highly sensitive, and rapid. Compounds electrochemically active in the given range of potentials interfere with the determination of unithiol and lipoic acid. The procedure is suitable for the quality control of drugs based on these substances.

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Table 3. Voltammetric determination of unithiol and lipoic acid in pharmaceuticals ($n = 5$, $P = 0.95$)

Preparation	Producer	Concentration, mg	Found, mg	RSD, %
Unithiol, solution for injections	ZAO Bryntsalov-A, Moscow	250	247 ± 3	1
Tablets of lipoic acid	AO ICN Oktyabr', St. Petersburg	12	11.9 ± 0.2	2
	OAO Marbiofarm, Yoshkar-Ola	25	24.8 ± 0.6	2

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