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ARTICLES

Direct Determination of Hypoxen and Its Analogs by Galvanostatic Coulometry

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Abstract—A method is developed for determining microgram amounts of antioxidants, i.e., hypoxen and its analogs in model solutions by coulometric titration with electrogenerated halogens using the biamperometric indication of the titration endpoint. The relative standard deviation of the determination varies from 1 to 5%.

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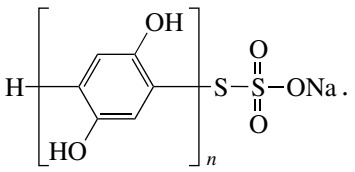
Biophysicits and physiologists were the first who expressed interest in studying compounds that prevent free-radical oxidation in cells. Chemists embarked on the investigation of these problems later. Their fruitful cooperation gave a great body of information about antioxidants and their functions in the human body. Synthetic chemists considerably widened the spectrum of compounds of this type.

Antioxidants are substances differing in chemical nature that can inhibit or eliminate the free-radical oxidation of organic compounds with different forms of oxygen. They suppress free-radical oxidation, thus, controlling its effect on the majority of metabolic processes. The resulting effect of antioxidants is the creation of the optimum conditions for the metabolism and normal growth of cells and tissues [1, 2].

Antihypoxants belong to a large group of preparations with antioxidant properties that improve the utilization of oxygen circulating in the human body and reduce the oxygen demand of organs and tissues.

Hypoxen, olifen, and mitofen, the registered commercial preparations, are a sodium salt of (poly-(2,5-dihydroxy-phenylene))-4-sulfonic acid



Hypoxen bearing a polymerized phenol fragment with an attached thiosulfate group exhibit a high antiradical activity. Being an antihypoxant, hypoxen stimulates the destruction of the products of lipid peroxide oxidation and protects cell and mitochondrion membranes against the destructive action of free radicals formed in the peroxide oxidation of lipids [3].

The polyhydroxyphenylene structure of the main nuclear of hypoxen explains its antioxidant properties. The hydroxyl groups of hypoxen easily donate hydrogen atoms to active radicals to form peroxides. The hypoxen molecule can contain up to 12 hydroxyl groups capable of binding free radicals either in one stage or successively. The hydroquinoid units of the oligomer are converted to quinines, which are regenerated into the initial structure in the presence of DT-diaphorase [4].

It should be emphasized that the energy of the H–O bond in the oligomeric structure is low, which facilitates the binding of free radicals.

In the presence of hypoxen, the oxygen demand of cells is reduced; and the regeneration of the active forms of oxygen, whose avalanche-like accumulation was inevitable during hypoxia, was inhibited [5].

Thus, the study of pharmaceuticals with an antioxidant effect, widely used in clinical medicine, is of high priority. In addition, the advent of new pharmaceuticals makes the development of simple and rapid procedures for the direct determination of hypoxen and its analogs urgent.

EXPERIMENTAL

Halogens were electrogenerated at a constant current intensity of 5.0 μA from aqueous 0.2 M KCl and KBr solutions in a 0.1 M H_2SO_4 solution and from a 0.1 M KI solution in a tartrate buffer solution with pH 3.56 using a P-5827 M potentiostat. The end-point of titration was determined amperometrically using two polarized platinum electrodes (ΔE = 300 mV). A smooth platinum plate with a surface area of 1 cm^2 served as the working electrode, and a platinum helix separated from the anode compartment of the cell with a semipermeable diaphragm was the auxiliary electrode.

Table 1. Numbers of electrons involved in the reactions of hypoxen and its analogs with electrogenerated halogens ($n = 5$, $P = 0.95$)

Compound		Molar mass, g/mol	Number of electrons involved in the reaction with Cl_2	Number of electrons involved in the reaction with Br_2
Hypoxen		362	3	2
Olifen		395	4	2
Mitofen	7.1	255	3	1
	73–77	521	6	4
	111	312	3	2

Coulometric titration. A 20.0-mL portion of the supporting electrolyte and 0.1–5.0 mL of a test antioxidant solution were placed in a 50.0-mL coulometric cell containing the working, auxiliary, and indicator electrodes. Such aliquot portions were taken to spend no longer than 5 min for titration.

The change of current with time was recorded, and the end-point of titration was found from the inflection of the titration curve. The substance mass was calculated using the following equation:

$$m = ItM/nF,$$

where I is current intensity, A; t is the time of titration, s; M is the molar mass of a substance, g/mol; n is the number of electrons involved in the reaction; and F is the Faraday constant equal to 96 500 C/mol.

Standard solutions of hypoxen, olifen, and mitofen were prepared by dissolving accurately weighed samples (0.1 g) in 50.0 mL of distilled water.

Molar masses of hypoxen and its analogs were found from their matrix-assisted laser desorption–ionization mass spectra, which were recorded on a DYNAMO MALDI TOF time-of-flight mass spectrometer (Thermo Bioanalysis Finnigan, United States). A pulse UV laser with a wavelength of 337 nm was used for laser desorption. Dihydroxybenzoic acid served as the matrix. The samples were prepared by the method of a dry drop: a mixture of a 1 wt % ethanolic solution of the matrix and a 0.1 wt % acetone solution of a test substance were applied onto a substrate and dried at 40°C.

RESULTS AND DISCUSSION

Hypoxen, olifen, and mitofen quickly and quantitatively reacted with electrogenerated chlorine and bromine. Their reactions with iodine proceeded in time, which was indicative of the kinetic inertness of the thiosulfate group in this oligomer.

To determine the stoichiometry of reactions, we titrated aqueous standard solutions of compounds (Table 1).

Table 2. Coulometric determination of hypoxen and its analogs in model solutions ($n = 5$, $P = 0.95$)

Compound		Titrant	Added, μg	Found, μg	RSD, %
Hypoxen		Cl_2	181	178 ± 7	3
			362	346 ± 8	2
			1086	1089 ± 21	2
		Br_2	181	182 ± 9	4
			362	363 ± 10	2
			1086	1048 ± 36	4
Olifen		Cl_2	395	397 ± 12	2
			790	782 ± 28	3
			1580	1568 ± 15	1
		Br_2	395	389 ± 15	3
			790	804 ± 29	3
			1580	1591 ± 10	1
Mitofen	7.1	Cl_2	255	254 ± 10	3
			510	503 ± 18	3
			1020	1042 ± 29	2
		Br_2	255	254 ± 15	5
			510	506 ± 8	1
			1020	994 ± 46	3
	73–77	Cl_2	52.1	51.4 ± 0.8	2
			130	128.9 ± 0.7	1
			260	257 ± 2	1
		Br_2	260.5	257 ± 7	2
			521	515 ± 19	3
			1042	1037 ± 10	1
	111	Cl_2	312	307 ± 5	2
			624	615 ± 19	2
			1248	1250 ± 36	2
		Br_2	312	309 ± 6	1
			624	615 ± 25	3
			1248	1243 ± 33	2

Because hypoxen is an oligomer, it is difficult to propose the mechanism of its reactions with halogens. Evidently, phenolic fragments are oxidized to give quinoid structures.

The accuracy of determining hypoxen, olifen, and mitofen in model solutions was verified by the added–found method (Table 2).

Based on our data, we proposed a procedure for the rapid coulometric determination of antioxidants (antihypoxants).

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