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NEW APPROACHES TO THE ELECTROCHEMICAL EVALUATION OF THE ANTIOXIDANT PROPERTIES OF FOODSTUFF AND PHARMACEUTICAL SAMPLES BASED ON THE ORGANIZED MEDIA AND NANOMATERIALS

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Abstract
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Scientific Secretary of the
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THE GENERAL CHARACTERIZATION OF THE WORK

The topic relevance. Antioxidants (**AO**) play a key role in antioxidant defense system of living organisms protecting cells from negative effect of free radicals. The main exogenous AO sources for human are foodstuff and pharmaceuticals. Their antioxidant properties are strongly depend on compounds structure, the chemical form and concentration as well as from the nature of the sample or medium in which AO are included. Therefore, the evaluation of foodstuff and pharmaceuticals antioxidant properties is important and also connected with their quality control. It requires the development of easy and express methods for reliable determination of samples antioxidant characteristics.

Reactions of AO with free radicals based on electrons transfer that allows to use electroanalytical methods for their determination, that are characterized with high sensitivity, rapidity of the procedure and relatively low costs, possibility of miniaturization making them very attractive to solve this kind of problems. Moreover, these methods can be combined with detection in different types of liquid chromatography and capillary electrophoresis.

The reactivity of AO is shown in two types of processes. These are the oxidation on the electrode surface under conditions of voltammetry and reactions in the solution with arising on the electrode oxidants in particular, coulometric titrants. Therefore, the approaches based on voltammetry and galvanostatic coulometry for the determination of individual AO in foodstuff and pharmaceutical samples and evaluation of their antioxidant properties via total parameters have to be doubtless considered as promising.

The application of one electron electrogenerated oxidant, in particular hexacyanoferrate(III) ions is of interest. In general case, this approach can exclude side and concurrent reactions affecting on the determination and can simulate reactions occurring *in vivo*, on the other hand. The great attention in organic voltammetry is paid lately to the chemically modified electrodes including that one based on carbon nanomaterials and their combination with surfactants. Moreover, surfactants provide solubilization of lipophilic compounds in water media that can be used in electroanalysis of liposoluble AO.

The aim of the work is the development of new electrochemical methods for the determination of individual AO in foodstuff and pharmaceutical samples and evaluation of their antioxidant properties by total characteristics using chemically modified electrodes based on carbon nanomaterials and organized media of surfactants.

The next tasks have been stated in correspondence to the aim of investigation:

- to develop the methods of voltammetric determination of individual AO of different classes in foodstuff and pharmaceuticals using electrodes modified with carbon nanotubes;
- to develop the methods of coulometric determination of natural and synthetic phenolic AO based on reactions with electrogenerated hexacyanoferrate(III) ions;
- to evaluate the possibility of use galvanostatic coulometry with electrogenerated bromine and hexacyanoferrate(III) ions for the investigation of interaction between natural phenolic AO and proteins;
- to develop the methods of electrochemical evaluation of foodstuff total antioxidant parameters (antioxidant capacity (**AOC**) and ferric reducing power (**FRP**));
- to consider the possibility of electrochemical generation of coulometric titrants in the presence of surfactants and to develop the methods of coulometric determination of lipophilic AO in pharmaceutical dosage forms;
- to find the characteristics of individual AO electrochemical oxidation in surfactant-containing media and to develop the methods of their extraction-voltammetric determination in foodstuff and pharmaceutical dosage forms;
- to create the electrodes modified with co-immobilized carbon nanomaterials and surfactants for the determination natural and synthetic phenolic AO in plant materials and foodstuff.

The scientific novelty of the work consists of the unified methodology of electrochemical methods application for the evaluation of foodstuff and pharmaceuticals antioxidant properties.

The parameters of electrochemical oxidation of natural and synthetic phenolic (di- and trihydroxybenzenes, flavonoids, gallic and hydroxycinnamic acids) and S-containing AO (aminoacids, unithiol and α-lipoic acid) as well as α-tocopherol and retinol on the electrodes modified with carbon nanotubes are found. The effect of nanomaterial type and way of production on the electrode electrocatalytic properties is shown.

The stoichiometric coefficients for natural and synthetic phenolic AO in the reactions with electrogenerated titrants are found and the corresponding schemes of interaction are proposed. The applicability of coulometric titration with electrogenerated bromine and hexacyanoferrate(III) ions for the evaluation of bioaccessibility of the phenolic AO in the

presence of proteins is shown. Milk proteins (casein, bovine serum albumin (**BSA**) and β-lactoglobulin) bond rutin, quercetin and taxifolin inactivating them. Rutin is less bonded in the raw of compound under consideration.

The method for the evaluation of beverages and spices FRP based on the reactions of their AO with electrogenerated and hexacyanoferrate(III) ions.

Oxidation characteristics of spices, cognacs, brandies and wines components on the carbon-based electrodes under conditons of cyclic (**CV**) and differential pulse voltammetry (**DPV**) have been obtained. The analytical signals of foodstuff have an integral nature and caused by oxidation of their phenolic AO (hydroxycinnamic acids of coffee, catechins and tannins of teas and wines and also hydroxybenzoic acids and aromatic aldehydes)

The working conditions of halogens and hexacyanoferrate(III) ions electrochemical generation in the presence of surfactants of different nature providing the quantitative current yield have been obtained. The 1 mM concentration of surfactants does not affect the generation of hexacyanoferrate(III) ions while only relatively low concentrations of surfactants allowable for the halogens.

The electrochemical activity of lipophilic AO (α-tocopherol, retinol, β-carotene, eugenol, sterically hindered phenols (**SHP**)) and menadione fulfilling redox mediator function in living systems has been shown in surfactant-containing media. The effect of surfactant nature and concentration on voltammetric response of analytes has been evaluated. The corresponding schemes of reactions have been proposed.

The conditions of lipophilic AO and menadione quantitative extraction form pharmaceutical dosage forms, plant materials and foodstuff have been found.

The new chemically modified electrodes based on co-immobilized carbon nanomaterials and surfactants have been created for the determination of natural (morin, vanillin and syringaldehyde) and synthetic (*tert*-butylhydroquinone and *tert*-butylhydroxyanisole) AO. The way of electrode surface modification, the nature and concentration of surfactant effect on the AO voltammetric characteristics; the corresponding reaction schemes are provided. The possibility of simultaneous determination of structurally related compounds on example of aromatic aldehydes and SHP is shown.

Theoretical and practical significance of the work. The developed methodology for the determination of individual AO and their total contents in real samples extends the application of electrochemical methods in organic analysis.

Voltammetric methods of determination of natural phenolic (rutin, quercetin and taxifolin), S-containing (methionine, α-lipoic acid and unithiol) AO, retinol and α-tocopherol in pharmaceutical dosage forms have been developed using carbon nanotube-based electrodes. The relative standard deviation values do not exceed 6%.

The coulometric approaches for the determination of AO in mono- and polycomponent pharmaceutical dosage forms based on their interaction with electrogenerated titrants including surfactant-containing media are developed.

The FRP of spices extracts, tea and coffee is evaluated. FRP of beverages is significantly decreased in the presence of milk due to the interaction of its proteins with phenolic AO of tea and coffee.

The voltammetric evaluation of spices, tea and coffee AOC based on their AO oxidation on glassy carbon (**GCE**) and multi-walled carbon nanotube-modified GCE (**MWNT/GCE**) under conditions of CV and DPV. The correlations with standard antioxidant parameters are found.

Galvanostatic coulometry with electrogenerated bromine and hexacyanoferrate(III) ions, DPV and chronoamperometry have been applied for the first time for the evaluation of antioxidant properties and quality of cognacs and brandies. The complex of approaches developed is characterized by simplicity, accessibility and reliability of the results obtained and can be applied for the brandy quality control allowing to identify their crude adulterations using rectified ethanol of foodstuff or technical grade ethanol and flavoring additives mimeting the brandy organolepric properties.

The chronocoulometric method for the evaluation of wine AOC based on oxidation of its phenolic components on MWNT/GCE is developed and characterized with simplicity and rapidity as well as allows to decrease significantly the sample volume required for the analysis.

The combined methods of extraction-voltammetric determination of lipophilic AO (α-tocopherol, retinol, β-carotene, eugenol and SHP) and menadione in pharmaceutical dosage forms, cosmetics and foodstuff using surfactant-containing media have been developed; the relative standard deviation values do not exceed 8.2%.

The amperometric sensors based on co-immobilized carbon nanomaterials and surfactants have been created for the determination of natural andsynthetic phenolic AO in plant materials and foodstuff. The possibility of simultaneous determination of structurally

related SHP as well as vanillin and syringaldehyde is evaluated. The sensors analytical characteristics obtained are much better in comparison with other existing approaches.

The usage of electrodes modified with carbon nanomaterils and surfactants-based organized media in AO electroanalysis significantly improves the analytical characteristics of their determination. The portion of organic solvent can be significantly reduced or fully substituted with water solutions in the case of surfactant-based media.

Methodology and research methods. Galvanostatic coulometry with electrogenerated titrants, CV and DPV, chronoampero-and chronocoulometry have used in the investigations performed. Scanning electron microscopy (**SEM**) and atomic force microscopy (**AFM**) have been applied for the electrode surface characterization; the spectrophotometry has been used as independent method for the results validation.

Propositions for the defense:

- voltammetric methods for the determination of AO of different nature on the electrodes modified with carbon nanomaterials and their application in real samples analysis;
- coulometric methods of natural and synthetic phenolic AO determination based on their reactions with electrogenerated halogens and hexacyanoferrate(III) ions;
- coulometric evaluation of interaction between natural phenolic AO and milk proteins;
- methods for electrochemical evaluation of antioxidant properties of foodstuff (spices, teas, coffee, cognacs and brandies, wines) using galvanostatic coulometry, voltammetry, chronoampero- and chronocoulometry; their correlation with standard methods and analytical application;
- the results of surfactant application in galvanostatic coulometry with electrogenerated titrants and methods of lipophilic AO determination in pharmaceutical dosage forms using surfactant-containing media;
- voltammetric characteristics of AO and menadione, methods of their extraction-voltammetric determination in real samples (pharmaceutical forms and foodstuff) in the presentce of surfactants;
- voltammetric methods of natural and synthetic phenolic AO determination using electrodes modified with carbon nanotubes and surfactants, their analytical and metrological characteristics.

The reliability and approval of the work. The reliability of the results obtained is confirmed by the high volume of experimental data obtained using modern sertified

equipment and their comparison with the results of independent standard methods and literature data.

The results of the work have been presented and discussed in many oral and poster presentations on national and international conferences: 10th Analytical Russian-German-Ukrainian Symposium “ARGUS’2007 – Nanoanalytics” (Saratov, 2007), Mendeleev Congress of General and Applied Chemistry (Moscow, 2007, Volgograd, 2011), National Conferences «Analytics of Russia» (Krasnodar, 2007, 2009), “Chemical Analysis” (Moscow, 2008), Conferences on Electrochemical Methods of Analysis with International Participation “EMA” (Ufa, 2008, 2012), International Conference on Electroanalysis “ESEAC” (Prague, 2008, Malmö, 2014), “Analytics of Siberia and Far East” (Tomsk, 2008, Krasnoyarsk, 2012), Scientific Conference of Young Scientists, PhD Students and Students of SEC KSU “Materials and Technologies of XXI Century” (Kazan, 2009, 2011, 2012), “Modern Methods of Analytical Control of Pharmaceutical Products” (Moscow, 2009), International Seminar on Modern Electrochemical Methods (Jetřichovice, 2010), Congress of Russian analysts (Moscow, 2010, 2013), Republican Scientific Conference on Analytical Chemistry with International Participation “Analytics of RB-2010” (Minsk, 2010), XI Medzinárodná konferencia "SÚČASNÝ STAV A PERSPEKTÍVY ANALYTICKEJ CHÉMIE V PRAXI" (Bratislava, 2010), Symposium with International Participation “Theory and Practice of Electroanalytical Chemistry” (Tomsk, 2010), International Conference “Extraction of organic compounds” (Voronezh, 2008, 2010), International Conference “Renewable Wood and Plant Resources: Chemistry, Technology, Pharmacology, Medicine” (St. Petersburg, 2011), International Congress on Organic Chemistry (Kazan, 2011), International Scientific Forum “Butlerov Heritage” (Казань, 2011, 2015), National Symposium “Separation and Concentration in Analytical and Radiochemistry” (Krasnodar, 2011, 2014), V National Conference “New achievements in chemistry and chemical technology of plant materials” (Barnaul, 2012), Heyrovsky Discussions (Brno, 2012, Třešť, 2014), 4th EuCheMS Chemistry Congress (Prague, 2012), Annual Meetings of the International Society of Electrochemistry (Prague, 2012, Lausanne, 2014), National Conference “Chemistry and Medicine” (Ufa-Abzakovo, 2013), International Scientific and Technical Conference “Nigmatullin’s Readings - 2013” (Kazan, 2013), European Conference on Analytical Chemistry “Euroanalysis” (Warsaw, 2013, Bordeaux, 2015) and 26th International Symposium on Pharmaceutical and Biomedical Analysis “PBA 2015” (Tbilisi, 2015).

Publications. 92 scientific works have been published including the chapter in the collective monograph, 37 articles in peer-reviewed scientific journals (19 in international journals), and 54 abstracts (the complete number of publications – 259 including 3 chapters in monographs, 124 articles (5 review-articles), 11 patents and 3 school-books).

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The personal contribution of the author is concluded in the formulation and solution of problems, experimental data acquisition and their discussion, interpretation and systematization of the research results.

Structure and volume of the thesis. The thesis consists of preface, introduction, six chapters, conclusion, references and appendix. It is presented on 380 pages and contains of 102 tables, 85 figures and reference list of 608 positions.

The topic relevance, the aims and tasks of the investigation, as well as scientific novelty and practical significance of the work are presented in the *introduction*.

The question of AO classification and properties including bioavailability and bioacessibility of phenolic AO in the presence of proteins are considered in the *literature review* (chapter 1). The critical review of the AO electroanalysis in particular the determination of individual AO and evaluation of total antioxidant parameters is performed. The advantages of chemically modified electrodes and flow methods are emphasized. The attention is also paid to the voltammetric determination of AO in the presence of surfactants.

The samples investigated, methods and equipment as well as experimental procedures are described in the *second chapter*.

EXPERIMENTAL

Bromine electrogeneration was carried out at a constant current of 5.0 mA from 0.2 M $(C_2H_5)_4NBr$ in 0.1 M $HClO_4$ in acetonitrile and 0.2 M KBr in 0.1 M H_2SO_4. Chlorine and iodine were generated from 0.2 M KCl in 0.1 M H_2SO_4 and 0.1 M KI in acetate buffer solution pH 3.56. The $[Fe(CN)_6]^{3-}$-ions were electrogenerated from 0.1 M $K_4[Fe(CN)_6]$ in 2 M NaOH. The titration end-point was detected biamperometrically (ΔE=200 mV).

Voltammetric measurements were performed on analyser "Ecotest-VA" и potentiostat/galvanostat μAutolab Type III. GCE and graphite electrode (GE) was used as working electrodes. Different types of carbon nanomaterials (MWNT (Aldrich, Germany), carboxylic acid functionalized single-walled carbon nanotubes (**SWNT-COOH**) (Sigma-Aldrich, Germany), carbon nanofibers (**CNF**) (Aldrich, Germany) and CNT synthesized by catalytic pyrolysis of ethanol vapor on nickel catalyst (IMT RAS, Chernogolovka[1])). Silver-silver chloride saturated KCl electrode was used as a reference one.

Different classes of individual AO of 50-99% purity (Fluka, Sigma, Aldrich, Germany; Reanal, Hungary) were used as standard compounds. Hydroquinone, catechol and propyl gallate derivatives were synthesized in Organic Chemistry Department of Ural Federal University (Yekaterinburg)[2]. All other chemicals were chemically pure grade and ethanol rectificate.

Surfactants of different nature (anionic sodium dodecyl sulfate (**SDS**), cationic N-dodecylpyridinium bromide (DDPB) and cetylpyridinium bromide (CPB), nonionic Brij® 35 and Triton X100 as well as nonionic high molecular weight PEG-4000) were used for the organized media formation and electrodes modification.

Spectrophotometric measurements were performed on spectrophotometer PE-5300 (NPO Ecros, Russia).

Brandy constituents were determined by gas chromatography on on Chromatec-Crystal 5000.2 (JSC SDO Chromatec, Yoshkar-Ola, Russia) with quartz capillary column Agilent J&W HP-FFAP (Agilent Technologies, Santa Clara, CA USA) and flame ionization[3].

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[1] *Granted by DSc in Chemistry, Professor S.S. Grazhulene and DSc in Physics and Mathematics, Professor A.N. Red'kin*

[2] *Granted by DSc in Chemistry, Professor Kh.Z. Brainina*

[3] *The results are given by M.V. Chibisova and N.N. Skorobogatova (Forensic Centre of The Ministry of Internal Affairs of the Russian Federation, Moscow)*

THE MAIN CONTENTS OF THE WORK

Voltammetry of individual antioxidants on the electrodes modified with carbon nanomaterials

Chemically modified electrodes based on carbon nanomaterials in particular CNT are recently applied in electroanalytical chemistry of AO. The variety of CNT and their properties can be used for the control of AO analytical signal selectivity and sensitivity.

The effect of CNT types and way of treatment on the morphology of modified electrode surface is evaluate using AFM data[1]. Consideration of CNT synthesized by catalytic pyrolysis of ethanol vapor on nickel catalyst (CNT I). CNT II are got by additional anneal of CNT I in argon. GCE possesses unstructured amorphous surface (Fig. 1A). The CNT coverage leads to the changes in the electrode surface structure.

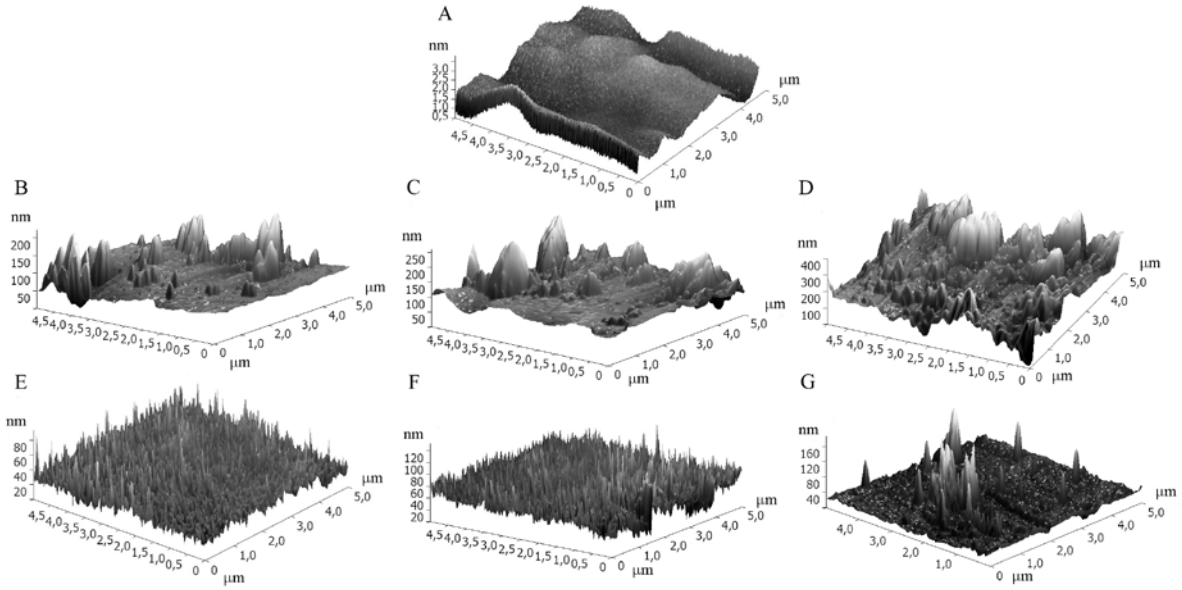


Fig.1 – Electrode surface morphology by AFM data. **A –** GCE; CNT I/GCE: **B –** 0.2; **C –** 0.5 and **D –** 0.7 mg mL^{-1}; CNT II/GCE: **E –** 0.2; **F –** 0.5 и **G –** 0.7 mg mL^{-1}.

Independently of the concentration, CNT I layer represents big aggregates sizes of which increase with concentration of nanomaterial (Fig. 1B-D). The height of the some aggregates reaches 230 nm. In the case of 0.2 and 0.5 mg mL^{-1} CNT II (Fig. 1E-G), the equally spaced needle-like torns of 20-70 nm consisted of closely interlaced nanotubes are observed. For the concentration of 0.7 mg mL^{-1}, the large aggregates up to 130 nm height are observed and suspension is unstable and unsuitable for electrode modification.

The main characteristics of electrode surface are presented in Table 1.

[1] *Acknowledgements to the PhD in Technics M.V. Morozov and DSc in Physics and Mathematics, Professor A.Kh. Gilmutdinov for the AFM investigations*

Table 1 – Electrodes surface characteristics based on AFM data

Electrode	c_{CNT}, mg mL^{-1}	R_a, nm	R_q, nm	h, nm	l, nm	d, nm
GCE	0	0.4	0.5	2-3		
CNT I/GCE	0.2	13	23	30-80	-	300-400
	0.5	15	25	60-170	-	300-600
	0.7	40	56	100-230	-	500-1000
CNT II/GCE	0.2	7.9	11	20-35	135-187	32-69
	0.5	9.1	12	50-70	170-200 300-400	29-51
	0.7	7.9	14	100-130	-	250-355

R_a – the average roughness; R_q– the root mean-square roughness

The structurization of the surface as a rows with the average width of 0.8-1.0 μm and alternating hills of MWNT aggregates of 50-586 nm heights is observed (Fig. 2B). The average roughness of MWNT/GCE surface is 190-fold increased in comparison to GCE (73 and 0.4 nm, respectively). In the case of GCE modified with oxidized MWNT (Fig. 2C), the $MWNT_{ox}$ layer consists of strongly interlaced vermicular structures with the average diameter of 25 nm. The length of some of them reaches 300-700 nm. The average roughness of 6.5 nm confirms the increase of electrode working area.

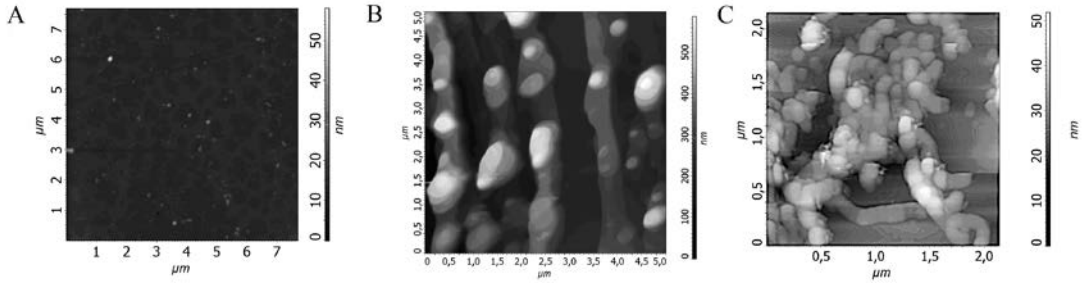


Fig. 2 – AFM images of the electrodes surface morphology: GCE (**A**), MWNT/GCE (**B**) and $MWNT_{ox}$/GCE (**B**).

GE shows unstructured surface with nodular features and randomly distributed single spines of 0.5-3.5 nm height (Fig. 3A). The average and root mean square roughness is 0.32 and 0.38 nm, respectively. The $MWNT_{ox}$-modified electrode is characterized by structured surface and significant (26-fold) increase of roughness. The surface consists of aggregates with “thorn-like" structure of high regularity. Their height is in the range of 10-35 nm (100-110 for some aggregates) and diameter 80-400 nm (Fig. 2B). The average roughness is 8.2 nm (the root mean square roughness – 11.5 nm).

Thus, all the modified electrodes under consideration show significant increase of surface roughness and changes in its structure. The surface of the electrode contains many reactive centers that can exhibit electrocatalytic properties towards AO oxidation.

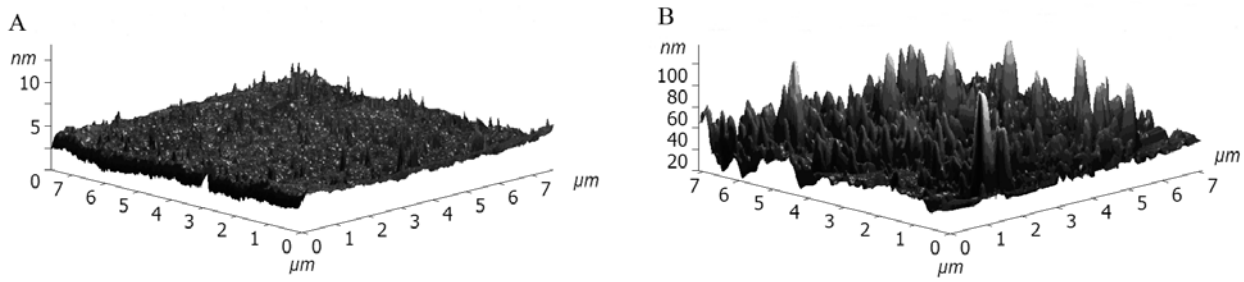


Fig. 3 – AFM image of GE (**A**) and $MWNT_{ox}$/GE (**B**) surface.

The electrooxidation of a wide range of different nature AO (phenolic, S-containing and liposoluble) is investigated on the electrodes created. The corresponding voltammetric characteristics are presented in Table 2.

Table 2 – The voltammetric characteristics of AO on bare and CNT-modified electrodes

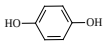
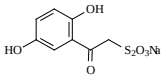
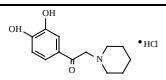
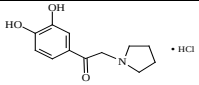
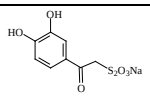
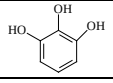
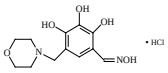
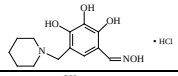
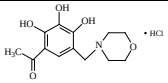
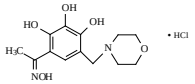
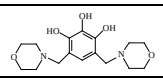
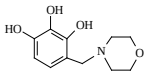
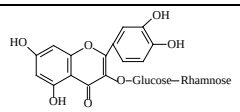
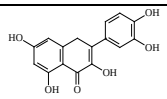
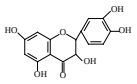
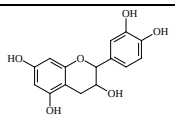
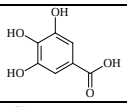
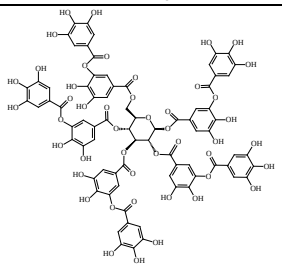
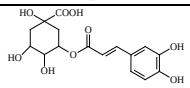
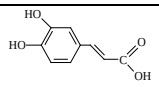
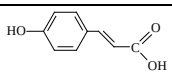
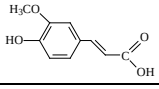
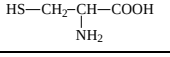
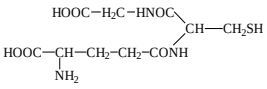
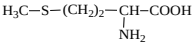
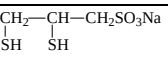
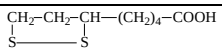
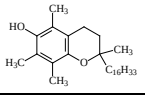
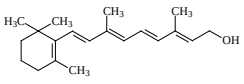
Compound	Structure	Supporting electrolyte	*E*, V		$I_{мод}/I$
			GCE or GE*	CNT/GCE[a] or $MWNT_{ox}$/GE*	
1	2	3	4	5	6
I		Phosphate buffer solution pH 7.4	+ 0.25	+ 0.12	2.0
II			+ 0.40; 0.66	+ 0.37; 0.70	1.4
III			+0.20	+0.15	1.6
IV			+ 0.24; 0.46	+ 0.23; 0.44	1.0
V			+ 0.23; 0.45	+ 0.23; 0.46	1.3
VI			+ 0.64	+ 0.53	1.0
VII			+ 0.03; 0.27; 0.75	0; 0.17; 0.69	3.0
VIII			+ 0.16; 0.50	+ 0.03; 0.37	1.6
IX			+ 0.11; 0.48	+ 0.11; 0.46	1.3
X			+ 0.13; 0.74	+ 0.04; 0.65	1.6
XI			+ 0.32; 0.58	+ 0.59; 0.78	4.0
XII			+ 0.18; 0.6	+ 0.1; 0.54	1.6
XIII			+ 0.29; 0.63	+ 0.08; 0.56	1.4

Table 2 (continued)

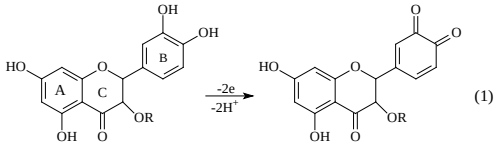
1	2	3	4	5	6
Rutin		Phosphate buffer solution pH 7.4	+0.26; 0.86	+0.26; 0.86	3.0
Quercetin			+0.23; 0.80	+0.23; 0.80	3.0
Taxifolin			+0.22; 0.80	+0.22; 0.80	2.0
Catechin			+0.23; 0.57	+0.21; 0.56	3.5
Gallic acid			+0.32; 0.66	+0.26; 0.63	3.5
Tannin			+0.24; 0.37	+0.22; 0.34	4.0
Chlorogenic acid			+0.49	+0.26	2.4
Caffeic acid			+0.59	+0.30	3.0
p-Coumaric acid			—	+0.53	—
Ferulic acid			+0.52	+0.42; 0.50	1.7
Cysteine		0.1 M H_2SO_4	+ 1.00	+ 0.80	5.0
Glutathione			—	+ 0.65	—
Methionine			—	+ 0.80	—
Unithiol			—	+0.60	—
α-Lipoic acid			+0.91	+0.81	1.6
α-Tocopherol		0.1 M $HClO_4$ in acetonitrile	+0.52; 0.91*	+0.32; 0.60*	2.0
Retinol			+0.87; 1.12*	+0.83; 1.10*	2.3

[a] CNT(II)/GCE for the compounds I-XIII, MWNT/GCE for the natural phenolic AO, $MWNT_{ox}$/GCE for the S-containing AO.

The AO under consideration are electrochemically active on GCE (or GE) and CNT-modified electrodes excluding *p*-coumaric acid and several S-containing AO that are

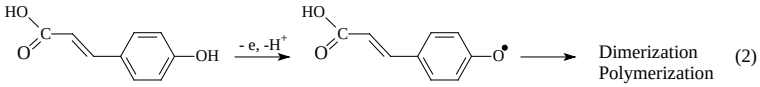
oxidized in available potential window on the modified electrodes only. The electrode surface modification leads to the improvement of the voltammogram forms and decrease of cathodic to anodic peak potential separation (on 470 mV for chlorogenic and 520 mV for caffeic acids, for example) indicating the increase of electrochemical systems reversibility. The overpotential decrease (on 20-290 mV) and significant increase of analytes oxidation currents are observed in comparison with bare electrodes caused by the increase of the electron transfer rate and effective surface area of the modified electrode, respectively. Moreover, the elecrtocatalytic effect of $MWNT_{ox}$ is due to the presence of oxygen-containing functional groups (carboxylic and hydroxyl) on the walls and ends of CNT formed via their acid treatment during preparation of CNT suspension.

Flavonoids undergo oxidation with participation of hydroxyl groups in the ring B forming corresponding *o*-quinones (Scheme 1)



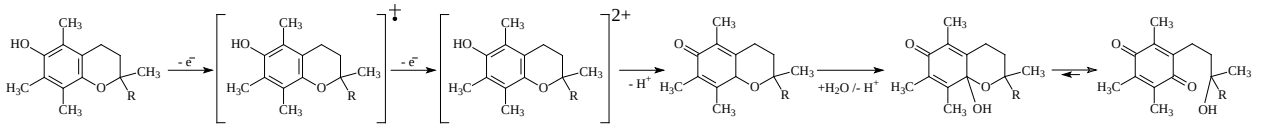
(1)

Gallic acid, hydroquinone, catechol and their derivatives, chlorogenic, caffeic and ferulic acids are oxidized by two-electron mechanism to the corresponding *o*-qionones. Oxidation of *p*-coumaric acid leads to the formation of phenoxyl radical that can undergo further dimerization and polymerization (Scheme 2).



(2)

Cysteine, glutathione and unithiol oxidation proceeds with disulfide formation, while the disulfide bond breakage takes place in the case of lipoic acid. Methionine is probably oxidized to the sulfoxide. α-Tocopherol is relatively easy oxidized via teo-electron mechanism to the *p*-tocopherylquinone (Scheme 3).



(3)

Two oxidation steps on the retinol volammograms correspond to the formation of retinal and retinoic acid that is indirectly confirmed by the approximately equal height of the oxidation steps.

The oxidation currents of AO are linearly depend on their concentration. The correlation coefficients of calibration graphs are in the range of 0.9938-0.9999. The analytical characteristics of AO determination are presented in Table 3. The application of

CNT-based modified electrodes allows to improve significantly the analytical characteristics of AO determination in particular to decrease the limits of detection (**LOD**) and enlarge the linear dynamic ranges.

Table 3 – The analytical characteristics of AO voltammetric determination using CNT-modified electrodes

AO class	Analyte	LOD, μM		Linear dynamic range, μM	
Synthetic phenolic AO		**GCE**	**CNT II/GCE**	**GCE**	**CNT II/GCE**
	I	3.90	2.90	3.90-1360	2.90-1430
	II	14.4	10.1	28.7-193	11.5-722
	III	7.33	1.47	14.7-688	3.67-1290
	IV	1.79	1.57	2.24-280	1.79-420
	V	5.81	0.29	5.06-100	0.29-182
	VI	10.6	2.12	10.6-1320	2.12-1320
	VII	33.2	20.0	66-440	66-1660
	VIII	3.8	1.90	3.8-1900	1.9-671
	IX	1.44	0.73	1.44-1830	1.83-1290
	X	3.36	0.67	3.36-1680	1.68-1680
	XI	3.36	0.67	3.36-630	0.67-1680
	XII	1.43	1.78	1.43-1780	1.78-1780
	XIII	1.61	0.81	1.61-2020	2.02-756
Natural phenolic AO		**GCE**	**MWNT/GCE**	**GCE**	**MWNT/GCE**
	Quercetin	10	1.0	20-80	2.0-220
	Taxifolin	5.2	0.26	21-306	0.52-210
	Rutin	5.4	0.71	7.1-280	1.4-28; 28-210
	Catechin	3.1	1.2	12-120	1.7-8.5; 8.7-170
	Gallic acid	20	6.0	39-290	9.9-99; 100-910
	Tannin	5.0	2.0	10-100	4-39; 40-390
S-containing AO		**GCE**	**$MWNT_{ox}$ /GCE**	**GCE**	**$MWNT_{ox}$ /GCE**
	Cysteine	107	220-1800	64	75-1900
	Glutathione	—	—	43	76-1800
	Methionine	—	—	270	360-2600; 3300-6900
	α-Lipoic acid	19	37-350; 420-780	19	26-180; 210-780
	Unithiol	—	—	41	180-1400; 2600-6900
Liposoluble AO		**GE**	**$MWNT_{ox}$ /GE**	**GE**	**$MWNT_{ox}$ /GE**
	α-Tocopherol	160	50	220-1340	65-2000
	Retinol	95	40	130-1200	50-1500

The approaches developed are tested on the model solutions of AO. The recovery of 100±2 % allows to used these methods for the real samples analysis.

The methods of AO voltammetric determination in pharmaceutical dosage forms are developed. The results are well reproduced and agreed well with the independent coulometric determination (Table 4).

Table 4 – AO determination in pharmaceutical dosage forms (n=5; P=0.95)

Sample	Analyte	Labeled amount, mg, *%	Found by voltammetry, mg, *%	s_r	Found by coulometry, mg, *%	s_r	F-test[a]	t-test[b]
Rutin, tablets	Rutin	20	20±1	0.052	20±2	0.068	1.99	0.844
Ascorutin, tablets	Rutin	50	50±1 50.8±0.8	0.021 0.013	50±3 51.0±2.0	0.049 0.024	2.30 3.49	0.652 0.263
Quercetin, tablets	Quercetin	20	18.6±0.9	0.048	18.5±0.4	0.021	5.49	0.204
Antistax, capsule	Quercetin	-	10.6±0.6	0.055	-	-	-	
Capilar, tablets	Taxifolin	10	10.0±0.4	0.038	9.7±0.4	0.034	1.01	1.20
Окулист ТМ, capsule	Taxifolin	15	14.7±0.8	0.051	-	-	-	
Unithiol, injection solution	Unithiol	250	247±3	0.012	249±5	0.019	2.78	0.773
Lipoic acid, tablets	α-Lipoic acid	12	11.9±0.2	0.016	12.0±0.4	0.032	4.00	0.532
		25	24.8±0.6	0.022	24.4±0.5	0.020	1.44	1.56
Methionine, tablets	Methionine	250	250±2	0.0082	252±9	0.031	12.6	0.486
			248±2	0.0079	246±9	0.034	10.1	0.670
			247±3	0.011	246±5	0.020	4.71	0.161
α-Tocopherol acetate oil solution	α-Tocopherol	10*	9.8±0.3*	0.026	10.0±0.5	0.048	5.56	0.820
		30*	29.5±0.5* 29.9±0.2*	0.014 0.0060	29±1* 29.5±0.9*	0.032 0.030	2.78 1.49	0.687 1.53
Retinol acetate oil solution	Retinol	3.34*	3.32±0.03*	0.0069	3.3±0.1*	0.028	11.1	0.433
Retinol palmitate oil solution	Retinol	5.55*	5.51±0.03*	0.0048	5.5±0.1*	0.016	5.62	0.291

[a] t_{crit}=2.31 at P=0.95 and df=8 [b] F_{crit}=6.39 at P=0.95 and df_1=4, df_2=4

The results obtained do not overstep the allowable limits. The s_r values do not exceed 6%. t-Test confirms the absence of systematic errors of the determination. The calculated F-test results are less than critical value showing the uniformly precision of the methods. The exclusions are methionine tablets and retinol acetate oil solution when voltammetry show a higher precision than coulometry. The approaches developed can be successfully used in pharmaceutical analysis as an alternative for the existing methods.

Galvanostatic coulometry of the individual antioxidants

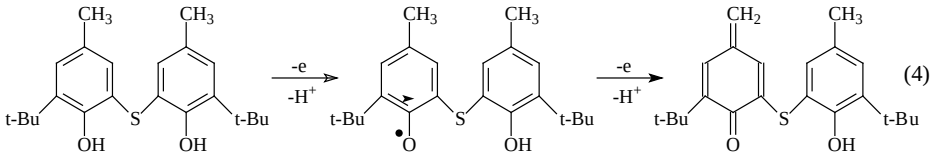
The stoichiometric coefficients for the reactions of synthetic and natural phenolic AO with electrogenerated titrants (halogens and $[Fe(CN)_6]^{3-}$-ions) under conditions of galvanostatic coulometry are found. Reactions of water-soluble AO (di- and trihydroxybenzenes) with electrogenerated halogens involve a multitude number of electrons (Table 5). The OH-groups oxidation as well as the electrophilic substitution reactions take place in this case. Moreover, the further reactions of the primary formed products can occur. OH-groups participate in the reactions with $[Fe(CN)_6]^{3-}$-ions

Table 5 – The number of electrons participating in the reactions of synthetic water-soluble phenolic AO with electrogenerated titrants

Compound	Number of electrons		
	Cl_2	Br_2	$[Fe(CN)_6]^{3-}$
I	2	2	2
II	12	7	4
III	2	2	2
IV	9	7	4
V	9	7	4
VI	12	7	4
VII	2	2	2
VIII	8	5	2
IX	8	5	2
X	6	3	2
XI	8	5	2
XII	2	2	2
XIII	4	4	2

Hydroquinone, catechol and pyrogallol are oxidized by $[Fe(CN)_6]^{3-}$-ions to *p*- and *o*-quinones, respectively. The number of electrons for the compounds II, IV, V, VI is more than can be assumed from the number of hydroxyl groups (4e instead of 2e) that can be explained by the presence of carbonyl and thiosulfate groups in their structure. Compounds VIII-XIII are oxidized with participation of 2 electrons similar to pyrogallol.

SHP (*tert*-butylhydroxytoluene, Irganox® 1081, 2,6-di-*tert*-butyl-4-β,β- diacetyl-ethylphenol, 2,6-di-*tert*-butyl-4-(dimethylaminomethyl)phenol, 2,6-di-*tert*-butyl-4-(N,N-bis-β-hydroxyethylamino)methylphenol and mediborol) excluding mediborol interact with $[Fe(CN)_6]^{3-}$-ions with participation of two electrons oxidizing to corresponding methylene quinones. The one phenolic fragment is oxidized in the case of Irganox® 1081 according to Scheme 4.



(4)

The total AOC of di-and trihydroxybenzenes based on the reaction with electrogenerated bromine is evaluated. The same total AOC for hydroquinone, catechol and pyrogallol has been obtained. Catechol and hydroquinone derivatives show highest AOC that is caused by the presence of thiosulfate residue as well as pyrrolydine and pyperidine fragments in their structures.

The reactions of electrogenerated bromine, iodine and $[Fe(CN)_6]^{3-}$-ions with natural phenolic AO are studied (Table 6). Electrogenerated iodine does not react with phenolic AO.

Table 6 – The number of electrons participating in the reactions of natural phenolic AO with electrogenerated titrants

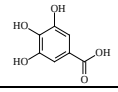
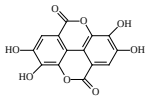
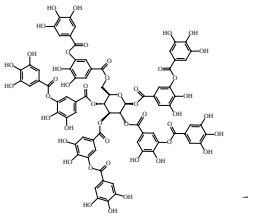
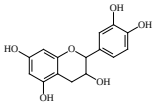
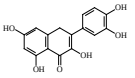
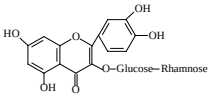
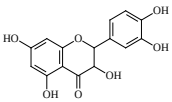
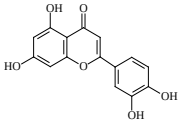
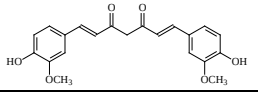
Compounds	Structure	Number of electrons	
		Br_2	$Fe(CN)_6^{3-}$
1	2	3	4
Gallic acid		4	4
Ellagic acid		4	4
Tannin		30	25
Catechin		4	4
Quercetin		4	4
Rutin		4	4
Taxifolin		6	4
Luteolin		8	4
Curcumin		10	5

Table 6 (continued)

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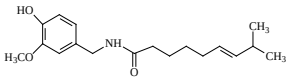
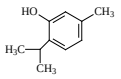
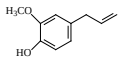
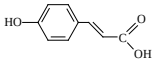
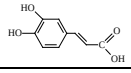
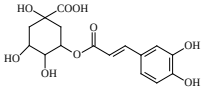
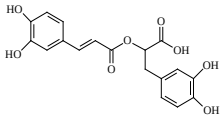
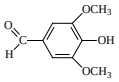
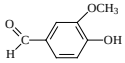
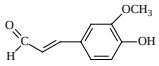
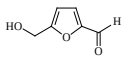
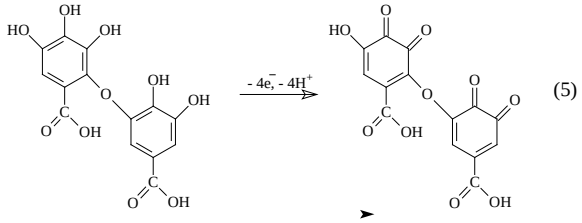
Capsaicin		6	1
Thymol		4	1
Eugenol		6	1
p-Coumaric acid		6	1
Caffeic acid		4	2
Chlorogenic acid		4	2
Rosmarinic acid		6	4
Syringaldehyde		2	-
Vanillin		2	-
Coniferaldehyde		5	-
Furfural		-	-
5-Hydroxymethyl-furfural		1	-

The electrogenerated bromine fast and quantitatively reacts with compounds under investigation excluding furfural. Bromine participates in the oxidation reactions as well as electrophilic addition to multiple bonds and substitution to aromatic ring that explains a big number of electrons participating in the reactions with AO under investigation. The oxidation of hydroxyl groups with formation of corresponding quinones probably occurs first of all. In the case of flavonoids (catechin, rutin, luteolin and quercetin), the oxidation of hydroxyl groups of ring B takes place initially. Gallic acid undergoes oxidation to *o*-quinone that is unstable and may undergo further reactions, for instance, condensation and dimerization reactions. Ellagic acid is oxidized to corresponding di-*o*-quinone. Five electrons participate in the reaction of coniferaldehyde with electrogenerated bromine. Oxidation of hydroxyl group to o-quinone as well as electrophilic addition to double bond and substitution to aromatic ring can occur in this case. 5-Hydroxymethylfurfural is probably involved in the reaction with bromine radicals forming relatively stable radical.

Reactions of $[Fe(CN)_6]^{3-}$-ions with AO involve their hydroxyl groups. In basic medium at pH>11 under the air oxygen action, gallic acid undergoes dimerization forming dehydrodigallic acid which is then oxidized to corresponding di-*o*-quinone (Scheme 5).



(5)

One electron is transferred in the reactions of monophenols (thymol, eugenol and *p*-coumaric acid) that corresponds to the formation of relatively stable phenoxyl radical. The number of electrons for tannin coincides with the number of OH-groups in its molecule. Flavonoids are oxidized with participation of OH-groups in aromatic rings. The glycoside moiety does not react with the titrant as has been shown on example of rutin (quercetin glicoside). Curcumin is unstable in basic medium and destroyed with the time to feruloylmethane and ferulic acid. Chlorogenic, caffeic and rosmarinic acids are oxidized with OH-groups participation to corresponding *o*- and di-*o*-quinones.

Taking into account the data obtained, $[Fe(CN)_6]^{3-}$-ions are of the most interest as a coulometric titrant being one-electron oxidants and showing high selectivity towards phenolic AO. Moreover, the one-electron reactions as a rule do not have concurrent steps complicating the process.

The accuracy of quantitative determination of natural phenolic AO has been evaluated by "added"– "found" method using titration of model solutions. The recovery is 97-102%, s_r value does not exceed 3% confirming the absence of random errors in the determinations.

The approach has been applied for the direct determination of phenolic AO in pharmaceutical dosage forms (Table 7). The auxiliary components of tablets do not show interference effect.

Table 7 – Coulometric determination of AO in pharmaceutical dosage forms (n=5; P=0.95)

Sample	Analyte	Labeled amount, mg	Found, mg	s_r
Rutin, tablets	Rutin	20	20±2	0.068
Quercetin, tablets	Quercetin	20	16.6±0.4	0.025
Ascorutin, tablets	Rutin	50	50±3 51±2	0.049 0.039
	Ascorbic acid	50	51±1 49.3±0.8	0.017 0.013
Capilar, tablets	Taxifolin	10	9.7±0.4	0.034

The variation of the titrants (iodine and $[Fe(CN)_6]^{3-}$-ions) allows to determine rutin in the presence of ascorbic acid. The method developed is characterized by high accuracy and simplicity that allows to recommend it as an alternative to the standard Pharmacopoeia methods based of volumetric titration.

As known, the natural phenolic AO interact with the proteins lading to the changes in the antioxidant properties of phenolic compounds. Therefore, the development of new approaches for the evaluation of phenolic AO – protein interactions is of interest. Taking into account the reactivity of phenolic AO towards electrogenerated titrants (bromine and $[Fe(CN)_6]^{3-}$-ions), the galvanostatic coulometry has been proposed for the evaluation of phenolic AO bioaccessibility expressed as a portion of free phenolic AO. The approach is tested on the systems phenolic AO – protein in particular mixtures of rutin, quercetin and taxifolin with milk proteins (casein, BSA and β-lactoglobulin). The increase of the protein portion in mixture leads to the decrease of free phenolic AO contents (Fig. 4) that indicates their transformation to the inactive forms. Rutin is less bonded by proteins than quercetin and taxifolin. It is probably caused by the presence of glycoside residue in position 3 of the ring C. β-Lactoglobulin binds phenolic AO less intensive than casein and BSA. The difference in the case of quercetin and taxifolin is statistically insignificant.

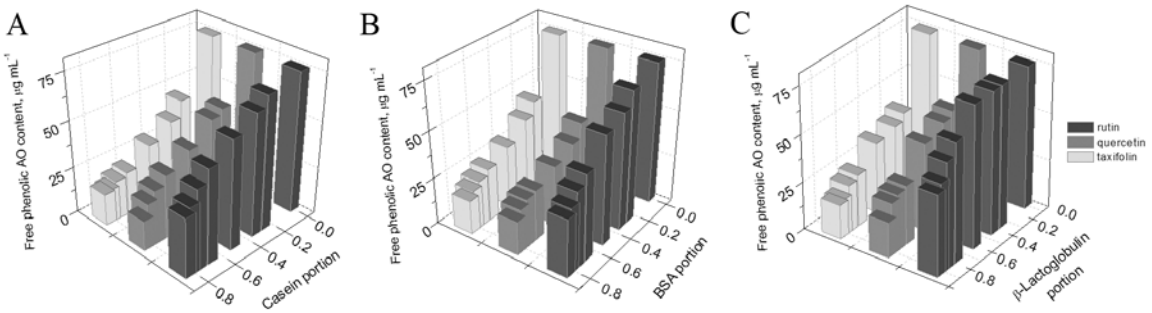


Fig. 4 – Free phenolic AO contents depending on portion of casein (**A**), BSA (**B**) and β-lactoglobulin (**B**) portion in the mixture. The titrant is electrogenerated $[Fe(CN)_6]^{3-}$-ions.

The results obtained using electrogenerated bromine and $[Fe(CN)_6]^{3-}$-ions are similar. The difference is not exceed the confidence intervals of the values. The binding is realized via hydrogen bonds, hydrophobic and electrostatic interactions. The contribution of the each type of interactions can be changed in dependence from the structure of phenolic AO and proteins nature. The interaction proceeds with high rate and does not change with the time that was shown on the mixtures of quercetin with casein.

Galvanostatic coulometry with electrogenerated titrants can be successfully for the evaluation phenolic AO bioaccessibility in the presence of proteins.

Electrochemical evaluation of foodstuff antioxidant capacity

A lot of attention is paid at present time to the so-called total parameters allowing to characterize the sample as the whole without determination of each component. This approach is especially attractive for the AO determination in foodstuff as far as the last one contain a wide range of compounds with antioxidant properties. The total parameters permit to take into account the possible synergetic or antagonistic effect of single compounds and, therefore, to predict the biological activity of the whole sample. The development of new methods for the electrochemical evaluation of foodstuff AOC is of interest.

Antioxidant capacity of spices. Taking into account the chemical composition of spices, it can be concluded that the major contribution among AO belongs to phenolic compounds which are oxidized in the reactions with the electrogenerated titrants in galvanostatic coulometry and on the electrode surface in voltammetry. The FRP and AOC were chosen as parameters characterizing spices antioxidant properties.

Liquid extraction with ethanol and methanol was used for the removal of AO from spices. Single extraction with ethanol for 10 min provided quantitative yield of the components. The raw material/extractant ratio giving the maximum recovery depended on the spice type. The recovery was controlled using coulometric titration with electrogenerated $[Fe(CN)_6]^{3-}$-ions. Methanolic extracts were obtained by extraction for 48 h at raw material/extractant ratio of 1:12 for oregano, basil and bay leaf and 1:6 for other spices[1].

FRP of spices extracts recalculated per 1 g of dry spice is evaluated (Table 8). Methanolic extracts are characterized with higher FRP values and the difference is statistically significant that is caused by extractive properties of methanol and the extraction time as well as the higher solubility of many AO in methanol than in ethanol. Moreover, methanol inhibits polyphenoloxydases preventing oxidation of phenolic AO in plant materials[2]. The maximum FRP is observed for the cinnamon, clove and rosemary independently of the extragent. The methanolic extracts were used for the further investigations.

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[1] Oya T., Osawa T., Kawakishi S. Spice constituents scavenging free radicals and inhibiting pentosidine formation in a model system // Biosci. Biotechnol. Biochem. – 1997. – V. 61. – No. 2. – P. 263.

[2] Paneerchelvan S., Lai H.-Y., Kailasapathy K. Antioxidant, antibacterial and tyrosinase inhibiting activities of extracts from *Myristica fragrans* Houtt. / // Eur. J. Med. Plants. – 2015. – V. 8. – № 1. – P. 39

Table 8 – FRP of spices (n=5; P=0.95)

Spice	Trademark	V(C_2H_5OH) per 1 g of spice, mL	FRP, C g^{-1}			
			Ethanolic extract	s_r	Methanolic extract	s_r
Cinnamon	“Appetita”	4	42.3±0.2	0.004	137±3	0.018
Clove	“Appetita”	2	13.3±0.2	0.013	64±2	0.026
Rosemary	“Appetita”	6	7.44±0.08	0.009	37±2	0.037
Curcuma	M&S	6	11.0±0.9	0.067	14.5±0.3	0.014
Cumin	“Magiya Vostoka”	2	4.5±0.2	0.032	8.8±0.2	0.019
Oregano	“Galeo”	6	3.69±0.08	0.017	18.1±0.5	0.022
Ginger	“Magic tree”	2	2.80±0.09	0.025	5.9±0.2	0.029
Juniper berries	“Appetita”	2	1.68±0.04	0.014	7.7±0.1	0.013
Red hot pepper	“Galeo”	6	1.55±0.03	0.017	1.8±0.1	0.057
Nutmeg	“Interjarek”	6	1.40±0.06	0.032	25±1	0.032
Red sweet pepper	“Magic tree”	2	0.94±0.01	0.012	2.02±0.08	0.031
Black pepper	“Galeo”	2	0.94±0.07	0.065	3.2±0.2	0.040
Basil	“Appetita”	6	0.79±0.04	0.036	9.4±0.3	0.022
Coriander	“Appetita”	4	0.54±0.03	0.044	2.0±0.1	0.044

There are oxidation steps and peaks on the cyclic voltammograms of spices methanolic extracts on GCE in 0.1 M $LiClO_4$ in ethanol. The potential and area of oxidation steps depend on the spices type (Fig. 5).

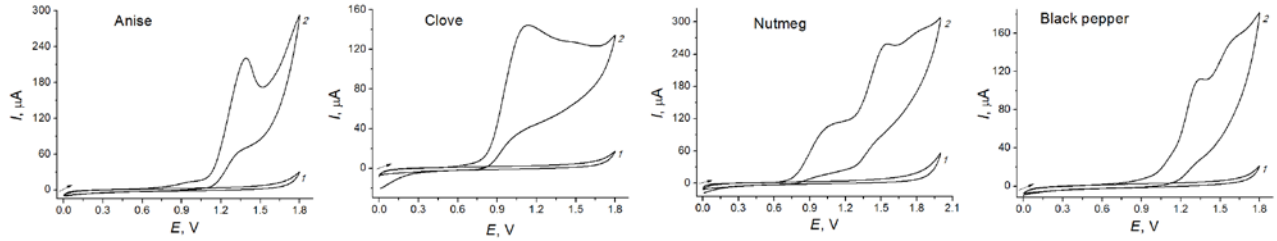


Fig. 5 – Cyclic voltammograms of spices methanolic extracts (curve *2*) on GCE in 0.1 M $LiClO_4$ in ethanol (curve *1*). υ = 0.75 V s^{-1}.

On the basis of the literature data of spices composition and oxidation potentials of their individual AO (gallic and rosmarinic acids, thymol, eugenol and capsaicin), their contributions in scpices analytical signal are evaluated using standard addition method. The proportional increase of the oxidation steps area is observed for the majority of scpices. The analytical signals of spices extracts have an integral natures that is confirmed by recovery values.

AOC of spices were evaluated via integral area of oxidation steps and expressed in gallic acid equivalents recalculated per 1 g of dry spice (Table 9). The AOC obtained are

caused by the presence of different classes of AO containing in spices. Just the major AO with the high contents show signals on the voltammograms.

Table 9 – AOC of spices based on CV data (n=5; P=0.95)

Spice	Trademark	AOC, mg gallic acid g^{-1}	s_r
Clove	“Appetita”	191.0±0.5	0.0011
Juniper berries	“Appetita”	82±3	0.031
Nutmeg	“Interjarek”	55±2	0.016
Cinnamon	“Appetita”	42±4	0.093
Rosemary	“Appetita”	30.2±0.4	0.0058
Anise	“Appetita”	20±1	0.020
Oregano	“Galeo”	19.5±0.8	0.016
Black pepper	“Magic tree”	15.5±0.3	0.0081
Ginger	“Magic tree”	13.8±3	0.079
Basil	“Appetita”	11.5±0.4	0.015
Curcuma	M&S	8.2±0.5	0.026
Red hot pepper	“Galeo”	6.2±0.2	0.016
Bay leaf	“Magiya Vostoka”	6.0±0.1	0.0067
Coriander	“Appetita”	5.7±0.2	0.011
Red sweet pepper	“Magic tree”	5.6±0.1	0.0094
Cumin	“Magiya Vostoka”	2.84±0.04	0.0054
Caraway	“Magic tree”	1.4±0.2	0.068

In general, the AOC row obtained are in agreement with the FRP data. The observed difference for cinnamon is due to the presence of eugenol that is “silent” on the extract voltammograms but contributes to FRP. AOC of spices is mainly caused by phenolic AO of different classes.

For the validation of the approach developed the AOC and FRP were compared with other antioxidant parameters of spices: total AOC based on the reaction with electrogenerated bromine, antioxidant activity (**AOA**) towards 2,2-diphenyl-1-picrylhydrazyl and total phenolics contents (**TPh**) by Folin-Ciocalteu method. The parameters under consideration show good correlation (r = 0.8886 - 0.9615 at r_{crit} = 0.482, P=0.95).

Total antioxidant capacity and ferric reducing power of tea and coffee. The antioxidant properties of tea and coffee based o the reactions with electrogenerated bromine and $[Fe(CN)_6]^{3-}$-ions (Tables 10 and 11) have been evaluated. Total AOC of tea id statistically significant higher than FRP that is explained by reactivity of the titrants used.

FRP is caused by phenolic AO reacting with $[Fe(CN)_6]^{3-}$-ions. Electrogenerated bromine is more active and strong oxidant that allows to cover a wide range of different nature AO including high-molecular weight AO, albumins and glutenins of tea, for example.

Таблица 10 – Total AOC and FRP of tea (n=5; P=0.95)

Sample	Type	Total AOC, C per 250 mL	s_r	FRP, C per 250 mL	s_r
Princess “Java”, Erl Grey	Leaves	645±9	0.011	277±5	0.013
English tea		315±17	0.043	136±2	0.012
“Greenfield Delicate”, Keemun	Tea bags	485±17	0.028	224±3	0.012
“Ahmad”, English Tea № 1		790±17	0.017	405±14	0.028
Princess “Noori” Earl Grey		625±11	0.014	273±3	0.009
Princess “Candy”		630±26	0.033	270±2	0.007
“Ahmad”, London Tea		740±47	0.051	321±4	0.011
“Ahmad”, Earl Grey		780±25	0.029	339±3	0.008
“Riston”, Premium English Tea		700±31	0.036	304±3	0.007
“Akbar”, green tea	Leaves	915±17	0.015	333±8	0.021
Princess “Java”, green tea		765±28	0.029	278±5	0.025
“Ahmad”, Jasmine Green Tea		1028±15	0.015	374±9	0.018
“Greenfield”, Green Mellissa	Tea bags	698±13	0.015	250±5	0.018
“Hyleys”, English green tea		818±14	0.014	299±2	0.006

Table 11 – Total AOC and FRP of coffee (n=5; P=0.95)

Sample	Type	Total AOC, C per 1250mL	s_r	FRP, C per 1250mL	s_r
Arabica “Tradition”	Beans	219±8	0.029	241±3	0.009
“Paulig” Vending Espresso Aroma		275±8	0.023	283±7	0.019
“Dallmayr” Promodo	Ground coffee	290±4	0.012	259±4	0.013
“Jockey” Traditional		345±7	0.016	331±5	0.013
“Grandos” Exclusive	Freeze-dried instant coffee	539±9	0.013	441±10	0.019
”Grandos” Egoiste Noir		525±5	0.008	488±16	0.026
“Nescafe” Cap Colombie		531±8	0.012	335±7	0.017
“Carte Noire”		334±7	0.017	337±6	0.013
“Vipcafe” Black Label		394±8	0.016	344±8	0.018
“Jockey” Triumph		398±9	0.018	344±8	0.018
“Jacobs” Monarch		404±7	0.014	455±7	0.011
“Tchibo” Exclusive		375±13	0.033	291±4	0.011
“Ambassador” Adora		435±7	0.013	336±3	0.008
“Moccona” Excellent	Spray-dried instant coffee	431±5	0.010	331±4	0.011
“Maxwell House”		375±11	0.024	294±3	0.008
“Nescafe” Classic		408±7	0.013	311±2	0.006
“Dancafe”		385±4	0.009	334±10	0.025

The difference in total AOC and FRP of coffee is statistically insignificant (parameters are almost coincide for some samples of coffe beans). This is caused by the AO of coffee in particular chlorogenic acids and melanoidins that react with the both of titrants in a similar way.

Both antioxidant parameters are increased in the row coffee beans < spray-dried instant coffee < freeze-dried instant coffee that is caused by technological process of instant

coffee production in particular the dehydration of coffee extract leading to the components concentration.

The positive correlations of the total AOC and FRP for tea and coffee are obtained (r = 0.8666 for tea (r_{crit}=0.532) and 0.8366 for coffee (r_{crit}=0.482) at P=0.95).

The FRP of tea and coffee is estimated in the presence of milk. In this case, FRP reflects the free phenolic AO contents in beverages and allows to perform an indirect evaluation their bioaccessibility. Beverages with the milk (ω_{fat}=2.5 %) were prepared by volume ratio (milk portion was 5, 20, 50 and 70 %). The own FRP of milk (taking into acoount the milk portion in the mixtures) is negligibly small in comparison to FRP of tea and coffee. Titration of tea and coffee mixtures with milk has shown the statistically significant decrease of FRP (Fig. 6).

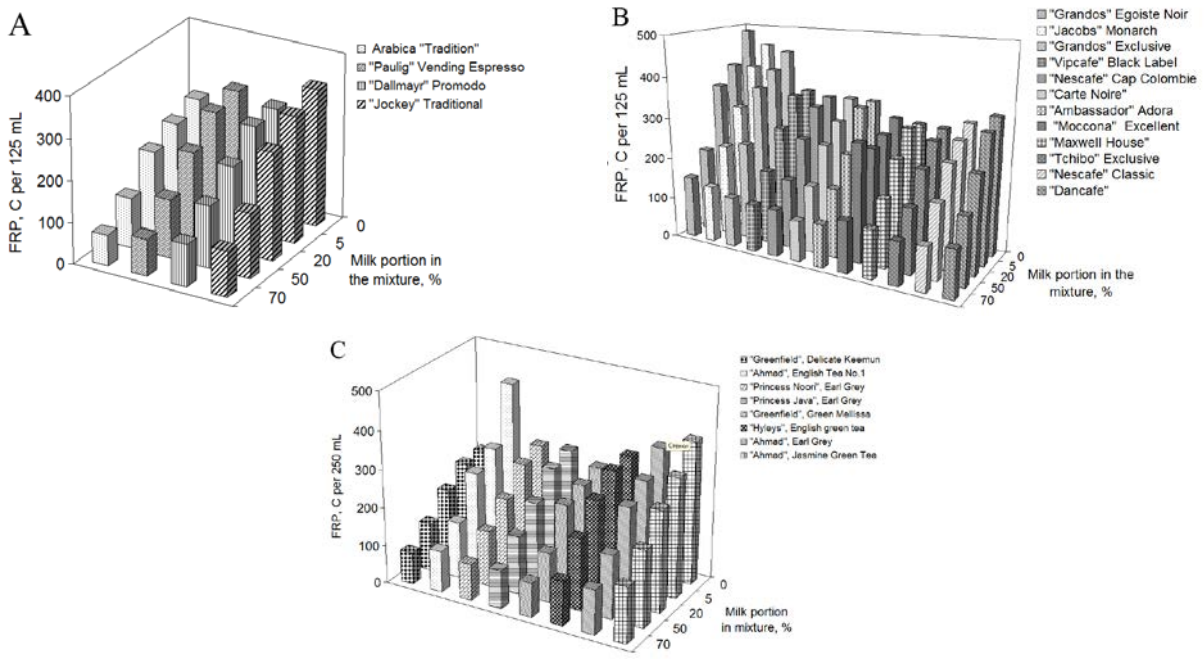


Fig. 6 – FRP of coffee beans (**A**), instant coffee (**B**) and tea (**C)** in the presence of milk.

Tea and coffee FRP is insignificantly changed at the relatively low contents of milk in the beverages (up to 20 %) excluding “Ahmad” English Tea No. 1”. The further increase of milk portion to 50% leads to approximately 2-fold decrease in FRP independently of the samples trademarks. It is especially clear seen in the case of coffee beans (Fig. 6A). These data confirm the role of phenolic AO and proteins concentration ratio in components binding.

The investigations carried out permit to conclude that galvanostatic coulometry with electrogenerated $[Fe(CN)_6]^{3-}$-ions can be used for the evaluation of tea and coffee antioxidant properties and their changes in the milk proteins presence.

Voltammetric evaluation of tea and coffee AOC. The knowledge of electrochemical behavior of phenolic AO on MWNT/GCE allowed to develop methods of tea and coffee AOC evaluation. Green, white, oolong and black teas of different trademarks are onvestigated. There are oxidation steps on the corresponding voltammograms which form, potential and area depend on the tea type (Fig. 7).

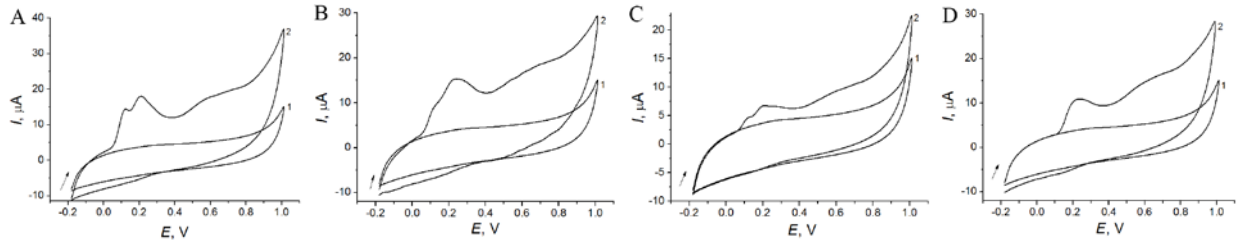


Fig. 7 – Cyclic voltammograms of tea (curve 2) on MWNT/GCE: **A** – green; **B** – white; **C** – oolong; **D** – black. Supporting electrolyte is 0.1 M phosphate buffer solution pH 7.4 (curve 1). υ = 100 мВ/с.

The components of green, white and oolong teas are oxidized on three steps at 0.13-0.17, 0.20-0.25 V and 0.6 V (Fig. 7A-C). The oxidation current of white and green tea are comparable while it is significantly lower for the oolong tea but the form of the voltammograms is the similar. The oxidation peak at 0.2-0.26 V and weekly pronounced step at 0.6 V are observed for the black tea (Fig. 7D). The absence of oxidation step at 0.15 V is caused by the oxidation and condensation of catechins during fermentation process. The first step corresponds to the catechins oxidation that can be concluded from the oxidation potentials. Oxidation potentials of rutin, quercetin and taxifolin confirm their contribution to the teas oxidation peak at 0.20-0.25 V. Oxidation step at 0.6 V for the all types of tea corresponds to the second oxidation step of catechin and gallic acid.

AOC has been chosen as parameter for the evaluation of tea antioxidant properties reflecting the total contents of AO in the sample. Taking into account the form of cyclic voltammograms, the total area of first and second steps of tea oxidation have been used for the AOC determination. AOC has been expressed in catechin equivalents recalculated per 250 mL of tea.

The AOC of different tea samples is investigated (Table 12). Green and white teas showed comparable AOC (725±100 and 638±28 mg of catechin per 250 mL, respectively, p>0.05) that is caused by withering and drying tea leaves leading to partial oxidation of catechins on 5-10% under the action of air oxygen. AOC of oolong tea (175±13 mg of catechin per 250 mL) is on 76% lower than that one for the green tea and statistically

insignificant higher than for the black tea (135±55 mg of catechin per 250 mL). Black tea AOC is lower on 81% than for the green tea (135±55 and 725±100 mg of catechin per 250 mL, $p<0.05$) that agrees well with the fermentation degree of 80-95 % for black tea. Herbal teas AOC is varied in wide ranges depending on their basic components. For the same type of samples, chamomile tea, for instance, the comparable AOC are obtained.

Table 12 – Voltammetric determination of tea AOC in catechin equivalents (n=5; P=0.95)

Tea type	Sample	AOC, mg of catechin per 250 mL	s_r
Green tea	“Monkey King” Jasmine tea	675±20	0.028
	Krasnodar tea “Unikal’nyi” T.G.F.O.P.	758±17	0.019
	“Greenfield” Green melissa	630±17	0.025
	“Beseda” with blackcurrant leaves	658±21	0.028
	“Ahmad” Jasmine green tea	900±22	0.021
White tea	“Nordqvist” White tea grapeberry	667±3	0.004
	“Lipton” White tea Rose violet	618±3	0.003
	“Lipton” White tea Pomegranate	603±3	0.004
	“Greenfield” Mango delight	660±12	0.014
	“Greenfield” White bloom	638±12	0.016
Oolong tea	“Greenfield” Highland oolong	180±3	0.016
	“Sang Min Hua” Oolong with vanilla	190±7	0.027
	“Tea Collection” Tie Guan Yin	180±8	0.039
	“Black Dragon” Ginseng oolong	173±5	0.029
	“Chinese famous tea” Tie Guan Yin oolong tea	155±3	0.015
Black tea	“Hyleys” English Aristocratic tea	138±10	0.054
	“Ahmad” Ceylon tea F.B.O.P.F.	240±10	0.032
	“Jumbo Brand tea” Ceylon tea	150±10	0.054
	“Lipton” Yellow label	43±5	0.040
	“Zolotaya Chasha” with blackcurrant leaves	50±2	0.030
	“Dilmah” Premium Ceylon	105±5	0.046
	“Ahmad” English breakfast	173±5	0.024
	“Greenfield” Spring melody	115±7	0.044
Herbal tea	Hibiscus tea	45±5	0.062
	“Tess” Light	133±5	0.032
	“Tess” Daisy	9.5±0.5	0.037
	“Greenfield” Rich Camomile	11.0±0.7	0.049

DPV has been used for coffee AOC estimation. The oxidation peaks at 0.2 and 0.43 V are characteristic for the coffee (Fig. 8) and the second peak is of low intensity especially for the instant coffee.

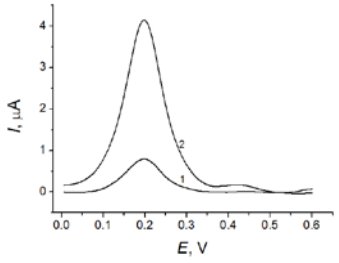


Fig. 8 – Baseline-corrected differential pulse voltammograms of coffee on MWNT/GCE: 1 – instant coffee; 2 – coffee beans. The supporting electrolyte is phosphate buffer solution pH 7.4. Potential scan rate is 10 mV s^{-1}.

Taking into account the oxidation potential of hydroxycinnamic acids on MWNT/GCE (P. 14, Table 2), the first peak corresponds to the oxidation of chlorogenic and caffeic acids that is confirmed by the standard addition method. Moreover, the caffeic acid contribution is higher than that one of chlorogenic acid that is explained by the presence of caffeic acid moiety in its structure being oxidized on the electrode. The second peak of coffee caused by ferulic acid oxidation.

AC of coffee has been expressed via the height of both oxidation peaks recalculated in chlorogenic acid equivalents being the main form of caffeic acid in coffee (Table 13). The AOC of coffee beans and ground coffee is statistically insignificant higher than for instant coffee (194±53 и 148±103 mg of chlorogenic acid per 100 mL, p>0.05). It is caused by a wide fluctuations of instant coffee AOC depending on a trademark and price category.

Table 13 – AOC of coffee based on DPV data on MWNT/GCE (n=5; P=0.95)

Coffee type	Sample	AOC, mg of chlorogenic acid per 100 mL	s_r
Ground coffee	“Starbucks” Guatemala Casl Cielo	162±9	0.047
	“Starbucks” Tribute Blend	215±2	0.007
	“Carte Noire” Arabica Exclusif	166±3	0.019
	“Melitta” Aroma	199±6	0.024
	“Melitta” Excellent	282±9	0.026
	“Grandos” Double Espresso	148±20	0.108
	“Sunny” Brazilian Coffee	269±12	0.036
	“Dallmayr” Prodomo	176±4	0.017
	“Auchan” Tradition café	128±7	0.042
Instant coffee	“Grandos” Cafe Gold Super	45±5	0.091
	“Moccona” Premium Selection	53±6	0.084
	“Vipcafe” Espresso	161±6	0.029
	“Carte Noire”	77±4	0.044
	“Jacobs” Monarch	162±9	0.045
	“Nescafe” Classic	338±21	0.050
	”Nescafe” Gold	201±10	0.042

v

Complex electrochemical evaluation of antioxidant properties and quality of cognacs and brandies. Ellagic and gallic acids, syringealdehyde, vanillin, coniferaldehyde, furfural and 5-hydroxymethylfurfural are the main AO of cognac according to Bureau National Interprofessionnel du Cognac. They are extracted from the oak barrels during distilled spirits aging. Their titration with electrogenerated bromine and $[Fe(CN)_6]^{3-}$-ions shows that bromine reacts with all the compounds mentioned above excluding furfural while $[Fe(CN)_6]^{3-}$-ions – with ellagic and gallic acids only. Therefore, the coulometric titration with electrogeberated oxidants can be proposed for the evaluation of cognac and brandy antioxidant properties. The difference in acids and aldehydes reactivity towards

electrogenerated bromine and $[Fe(CN)_6]^{3-}$-ions can be used for the evaluation of the contribution of these classes of AO to common parameters.

The total AOC and FRP of cognacs and brandies are evaluated (Table 14). Total AOC is statistically significantly higher than FRP for a great part of samples under investigations. Both parameters for cognac and brandy are increased with their age but the difference is statistically insignificant (p>0.05) that is caused by strongly different data for some brandy samples in the each denomination as well as for the “Hennessy”cognacs.

Table 14 – Antioxidant properties of cognacs and brandies based on the galavanostatic coulometry data (n=5; P=0.95)

Beverage	Commercial denomination	Sample	Country of origin	Total AOC, C per 100 mL	s_r	FRP, C per 100 mL	s_r
Cognac	VS	Hennessy	France	86±1	0.010	63±2	0.027
		Courvoisier		29±1	0.037	23±1	0.037
		Meukow		32.5±0.8	0.019	34±1	0.031
		Martell		49±2	0.034	44±3	0.048
	VSOP	Hennessy		112±4	0.027	84±3	0.027
		Courvoisier		41±3	0.053	36±3	0.066
		Martell		70±3	0.031	57±3	0.045
		Louis Royer		51±1	0.016	46.4±0.7	0.012
	XO	Hennessy		160±2	0.010	136±1	0.007
		Courvoisier		73±2	0.026	65±1	0.017
		Martell		94±2	0.020	91±2	0.022
Brandy	3-Star	Araks	Armenia	41±1	0.020	26±1	0.042
		Brandy “Kizlyarsky”	Russia	15.4±0.5	0.027	16±1	0.058
		Zoloto of Dagestan	Russia	29±1	0.037	36.0±0.9	0.020
		Father’s Old Barrel	Russia	41±1	0.028	36±2	0.047
		Tri zvezdochki	Russia	46±2	0.037	45.7±0.3	0.060
	4-Star	Armyansky cognac	Armenia	33±1	0.030	14.0±0.4	0.025
	5-Star	Armyanskoe zoloto	Armenia	43±2	0.018	28±2	0.059
		Babek	Azerbaijan	68±3	0.040	29±2	0.064
		Odessky	Ukraine	30±3	0.085	40±4	0.082
		Rossyisky cognac “Pyat’ zvezdochek”	Russia	60±2	0.026	49±2	0.032
		Pyat’ zvezdochek	Russia	24±3	0.091	20±3	0.10
	KV	Ararat “Ani”	Armenia	75±2	0.025	55±2	0.030
		Lezginka	Russia	30±1	0.015	25±1	0.040
	KS	Ararat “Ahtamar”	Armenia	88±2	0.015	58±2	0.026
		Kizlyar	Russia	63±2	0.023	55±3	0.035
		Jacques de la Croix Maron	Russia	66.5±0.6	0.008	61.2±0.9	0.012
	OS	Prazdnichny	Armenia	108±2	0.014	88±3	0.023

The oxidation of cognac and brandy on GCE and MWNT/GCE in phaosphate buffer solution is studied using DPV. The best characteristic are obtained at pH 3.0. The weekly pronounced oxidation step at 0.43 V and clear peak at 0.57 V (Fig. 9A) are observed on GCE. The second peak at 0.57 V is displayed for the VS cognacs and ordinary brandies. In

the case of MWNT/GCE, there are well-defined oxidation peaks at 0.44 and 0.59 V (Fig. 9B) for all the samples under investigation.

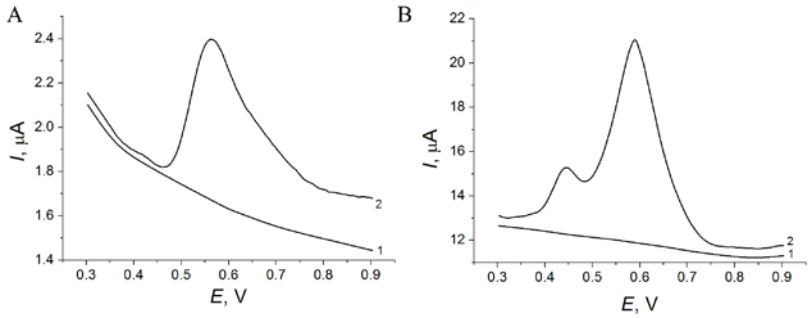


Fig. 9 – Typical differential pulse voltammograms of cognac (curve 2) in phosphate buffer solution pH 3.0 (curve 1): **A** – GCE; **B** – MWNT/GCE. Pulse amplitude is 50 mV, pulse width – 50 ms and υ =10 mV s^{-1}.

The oxidation potentials of cognac and brandy individual AO are found. Furfurals are electrochemically inactive in the range of potentials under investigation. Ellagic acid gives one oxidation peak at 0.59 V. Gallic acid is oxidized via two steps (0.44 and 0.75 V). The first oxidation steps potentials of gallic acid, syring- and coniferaldehydes are near (0.44, 0.46 and 0.41 V, respectively) thus these compounds can contribute to the first oxidation peak of cognacs and brandies. The second oxidation peak of cognacs and brandies coincides with ellagic acid oxidation potential. Ellagic and gallic acids contributions to cognacs and brandies oxidation currents are confirmed by the standard addition method. The recoveries of 96% for ellagic acid and 13.6% for galli acid are obtained. Thus, cognacs and brandies oxidation peak at 0.59 V is caused of ellagic acid oxidation. The low recovery of gallic acid confirms the integral nature of the first oxidation peak.

The voltammetric method for cognacs and brandies AOC evaluation is developed. The summarized oxidation current on two steps has been chosen as parameter characterizing antioxidant properties. So far as ellagic acid is the major AO of cognacs and brandies, AOC has been expressed in its equivalents recalculated per 100 mL of the beverage (Table 15).

Table 15 – AOE коньяков и бренди по данным ДИВ (n=5; P=0.95)

Sample	Commercial denomination	AOC, mg of ellagic acid per 100 mL	s_r
Hennessy	VS	3.6±0.2	0.023
Cognac 5 zvezd	5-Star	0.62±0.05	0.065
Lezginka	KV	1.29±0.06	0.020
Ararat “Ani”	KV	5.9±0.4	0.068
Kizlyar	KS	3.0±0.2	0.022
Ararat “Ahtamar”	KS	6.7±0.1	0.013
Prazdnichny	OS	10.0±0.2	0.018

On the basis of cognacs and brandy oxidation potentials, chronoamperometric method has been developed for their AOC evaluation. One-step chronoamperometry at 0.59 V for 100 s has been used (Fig. 10). The electrolysis time of 75 s is enough to achieve the steady-state. The sample volume for chronoamperometric detection is 4-fold lower than that one for DPV.

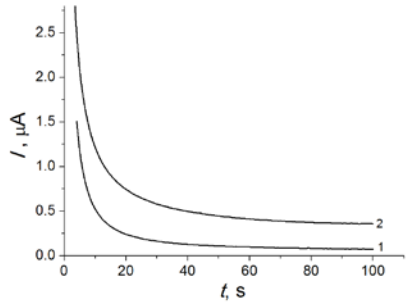


Fig. 10 – Typical one-step chronoamperogram of 0.5 mL cognac (curve 2) on MWNT/GCE in phosphate buffer solution pH 3.0 (curve 1) at 0.59 V.

The difference in currents for the sample and supporting electrolyte after 75 s of electrolysis has been used for the AOC evaluation. AOC was expressed in ellagic acid equivalents per 100 mL of cognac or brandy (Table 16)

Table 16 – Chronoamperometric evalution of cognac and brandy AOC (n=5; P=0.95)

Beverage	Commercial denomination	Sample	AOC, mg of ellagic acid per 100 mL	s_r
Cognac	VS	Hennessy VS	10.5±0.8	0.061
		Courvoisier VS	2.97±0.07	0.019
		Meukow VS	3.4±0.3	0.097
		Martell VS	9.6±0.2	0.013
	VSOP	Hennessy VSOP	13.8±0.1	0.066
		Courvoisier VSOP	5.0±0.3	0.055
		Martell VSOP	7.4±0.8	0.088
		Louis Royer VSOP	7.3±0.7	0.073
	XO	Hennessy XO	19.3±0.2	0.061
		Courvoisier XO	8.4±0.6	0.061
		Martell XO	11.6±0.2	0.014
Brandy	3-Star	Araks	4.3±0.4	0.077
	4-Star	Armyansky cognac	12±4	0.031
	5-Star	Babek	8.9±0.2	0.020
		Odessky	4.1±0.1	0.026
		Rossyisky cognac “Pyat’ zvezdochek”	2.8±0.1	0.029
		Armyanskoe zoloto	3.51±0.5	0.14
		Pyat’ zvezdochek	1.6±0.2	0.13
	KV	Lezginka	2.4±0.2	0.083
		Ararat “Ani”	9.8±0.4	0.031
	KS	Ararat “Ahtamar”	7.2±0.6	0.066
		Kizlyar	6.2±0.1	0.015
	OS	Prazdnichny	13.6±0.6	0.034

The positive correlations (r=0.8311-0.9847 at P=0.95) of the parameters characterizing antioxidant properties based on the coulometric, voltammetric and chronoamperometric data as well as standard parameters (AOA and TPh). It should be noted

that methods for AOA and TPh determination have several disadvantages that sometimes decrease the reliability of the determination.

The adulteration of alcoholic beverages is often occured on the alcoholic products market at present time. Therefore, the evaluation of alcoholic products quality is the actual problem having great social importance. The most crude and widespread way of cognac and brandy adulteration is the full or partial substitution of “eaux-de-vie” by rectified ethanol of foodstuff or technical grade. The applicability of the developed electrochemical methods for AOC determination has been tested for the estimation of alcoholic beverages quality. 10 samples of brandy have been investigated and 5 of them have been recognized as adulterations using gas chromatography. The changes in chromatographic profile by major compounds (normal lower alcohols, esters, for example) and the presence of untypical components peaks, *i.e.* synthetic flavoring additives like 1,2-propanediol (peak 22) and glycerol (peak 23) have been observed for the adulterated samples (Fig. 11). The adulterated samples contain very high concentration of 1,2-propanediol (45-1755 mg L^{-1}) and glycerol (40-381 mg L^{-1}). Three adulterated samples contained also toxic 1,2-ethanediol (3-18 mg L^{-1}) used as sweetener.

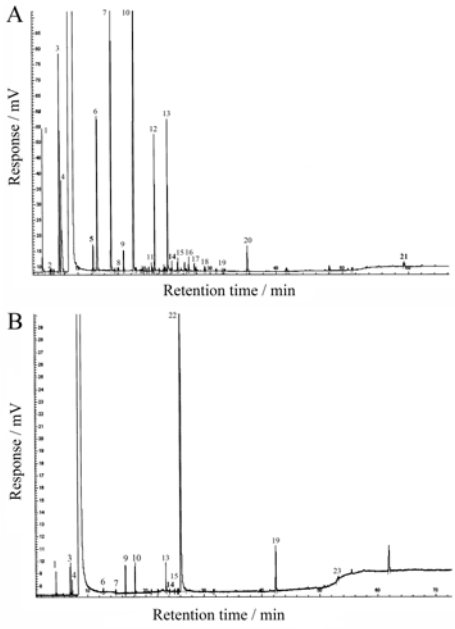


Fig. 11 – Typical chromatographic profiles of brandy (**A**) and adulteration (**B**). The main detected compounds are acetaldehyde (peak 1), methyl acetate (2), ethyl acetate (3), methanol (4), 2-butanol (5), 1-propanol (6), 2-methyl-1-propanol (7), 2-propene-1-ol (8), 1-butanol (9), 3-methyl-1-butanol (10), 1-hexanol (11), ethyl lactate (12), acetic acid (13), furfural (14), propionic acid (15), ethyl decanoate (16), isovaleric acid (17), valeric acid (18), ethyl laurate (19), 2-phenylethanol (20), 5-hydroxymethylfurfural (21), 1,2-propanediol (22) and glycerol (23).

The brandy samples are free of synthetic flavoring agents. Their chromatograms exhibit signals of furanic compounds (furfural (peak 14) and 5-hydroxymethylfurfural (peak 21)) formed as a consequence of the barrel heat treatment and partially come from caramel color that is legally added to brandy during production. Adulterations show furfural peak too but the intensity is much lower than that one for the brandy. The average contents of furfural in adulterations are in the range of 0-5.8 mg L^{-1} while for brandy it is 9.0-16.0 mg

L^{-1}. 5-Hydroxymethylfurfural has been detected in brandy only and the amount is significant (33-119 mg L^{-1}). It can be concluded, that all the adulterations investigated contain synthetic flavoring mixtures for imitation of brandy organoleptic properties.

A comparative analysis of brandy and adulterations antioxidants parameters has been performed using complex of electrochemical metods in particular, coulometric titration, DPV and chronoamperometry.

Both total AOC and FRP of brandy and their adulteration are significantly different (Table 17). In the case of adulteration, addition of flavoring mixtures containing compounds like vanillin, ascorbic acid and some other phenolic compounds provides total AOC and FRP of samples. Moreover, the adulterations can contain any other organic compounds that react with the titrants. Nevertheless, the FRP is more selective parameter. The total AOC and FRP values for the all adulterated samples are approximately the same (10±3 и 6±2 C per 100 mL for TAC and FRP, respectively) confirming the identical way of adulteration with addition of similar synthetic flavoring agents. These data correspond well with the chromatographic analysis results.

Table 17 – Total AOC and FRP of brandy and adulterations (n=5; P=0.95)

Category	Sample and denomination	Total AOC, C per 100 mL	s_r	FRP, C per 100 mL	s_r
Brandy	Pyat’ zvezdochek	24±3	0.091	20±3	0.10
	Lezginka, KV	30±1	0.015	25±1	0.040
	Ararat “Ani”, KV	75±2	0.025	55±2	0.030
	Kizlyar, KS	63±2	0.023	55±3	0.035
	Prazdnichny, OS	108±2	0.014	88±3	0.023
Adulteration	Lezginka, KV	10±1	0.085	5±2	0.16
	Kizlyar, KS	10±2	0.13	7±1	0.041
	Zukhra	15±2	0.11	5±1	0.18
	Staryi Kenigsberg, 4-Star	8±1	0.057	8±2	0.097
	Bol’shoi priz, 3-Star	8±2	0.22	4.3±0.9	0.18

The voltammetric behavior of brandy adulterations has been studied for the first time. Different form of DPVs has been obtained for adulterations (Fig. 12B-E) that are significantly vary form brandy (Fig. 12A). There are no characteristics oxidation steps at 0.44 and 0.59 V on voltammograms of adulterations while other oxidation peaks are presented. Moreover, the signals observed are of very low intensity (more than 10-fold lower) in comparison with signals for brandy. The standard addition methods has shown that one of the flavoring agent used for the improvement of the organoleptic properties of adulterations is vanillin. Voltammogram of adulteration “Bol’shoi priz, 3-Star” coincides with the supporting electrolyte curve indicating the absence of aging step in oak barrel

during production as well as the application of some flavouring agents that are electrochemically inactive in the potential window under investigation.

So, the DPV profile of brandy based on electrooxidation of its antioxidants extracted from oak barrels while aging can be applied for the evaluation of beverages quality.

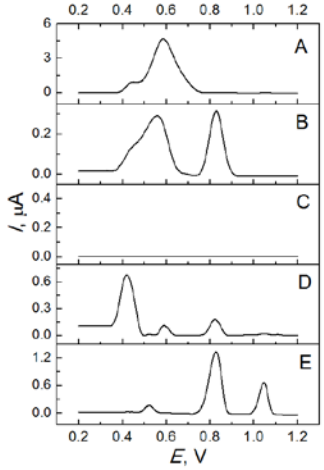


Fig. 12 – Typical base-line corrected DPVs of brandy (**A**) and different adulteration (**B**-**E**) on MWNT/GCE: **A** – Kizlyar, KS; **B** – Kizlyar, KS; **C** – Bol'shoi priz, 3-Star; **D** – Zukhra; **E** - Staryi Kenigsberg, 4-Star. Supporting electrolyte is 0.1 M phosphate buffer solution pH 3.0.

The chronoamperograms of adulteration at 0.59 V fully coincide with the curves of supporting electrolyte and significantly differ from the curves of brandy. These changes in chronoamperometric response allow to identify brandies and adulterations.

The AOC parameter can be used for the quantitative estimation. AOC of brandy and adulterations obtained using DPV and chronoamperometry are statistically different (Table 18). The data observed indicate the breach of brandy production process, in particular the aging step protocol. The possible addition of oak wood extract can takes place in the case of adulteration "Zukhra"

Table 18 – AOC of brandy and adulterations based on DPV and chronoamperometric data (n=5; P=0.95)

Category Brandy	Sample and denomination Pyat' zvezdochek	Voltammetry		Chronoamperometry	
		AOC, mg of ellagic acid per 100 mL	s_r	AOC, mg of ellagic acid per 100 mL	s_r
Adulteration	Lezginka, KV	0.86±0.05	0.056	1.6±0.1	0.058
	Ararat "Ani", KV	1.29±0.06	0.020	2.4±0.1	0.039
	Kizlyar, KS	5.9±0.4	0.068	9.8±0.4	0.031
	Prazdnichny, OS	3.0±0.2	0.022	6.2±0.1	0.015
	Lezginka, KV	10.0±0.2	0.018	13.6±0.6	0.034
Category	Kizlyar, KS	0.012±0.002	0.13	0	
	Zukhra	0.0093±0.0001	0.012	0	
	Staryi Kenigsberg, 4-Star	0.106±0.009	0.066	0.09±0.02	0.015
	Bol'shoi priz, 3-Star	0		0	
	Sample and denomination	0		0	

The investigations carried out have shown the applicability of the electrochemical approaches developed for the evaluation of cognac and brandy quality.

Chronocoulometric evaluation of wine antioxidant capacity. There are three oxidation peaks (0.39, 0.61 and 0.83 V for the red wines and 0.39, 0.80 and 1.18 V for the e dry wines) on the differential pulse voltammograms of dry wines on MWNT/GCE in phosphate buffer solution pH 4.0 (Fig. 13).

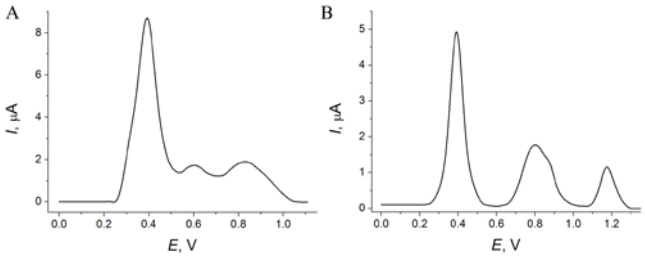


Fig. 13 – Typical baseline corrected differential pulse voltammograms of 1 mL red (**A**) and white (**B**) dry wine in 0.1 M phosphate buffer solution pH 4.0. υ = 10 mV s^{-1}.

The oxidation steps have an integral character that is confirmed by the standard addition of individual AO. The first peak at 0.39 V does not depend on the wine type, but is caused by the oxidation of flavonoids (catechins, quercetin and tannins) and hudroxycinnamic acids (caffeic and ferulic acids) on the first step. The second peak of the red wines is associated with the anthocyanins oxidation as well as the second oxidation steps of catechins and aldehydes. The white wine second and the red wine third peaks correspond to the oxidation of *p*-coumaric acid and flavonoids on the second and third steps. The white wine third peak at 1.18 V belongs to sulfites.

On the basis of the data obtained, a chronocoulometric method was developed for the evaluation of the antioxidant capacity of wine. A one-step chronocoulometry at 0.83 and 1.18 V was used for the red and white wines, respectively (Fig.14). The electrolysis time of 100 s was enough for the steady-state achievement.

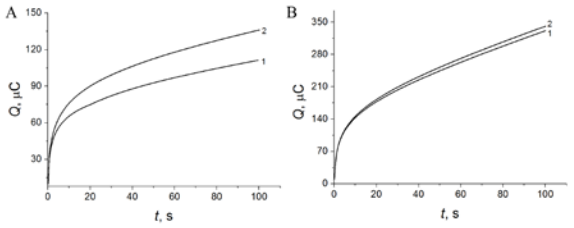


Fig. 14 – Chronocoulometric curves of red (**A**) and white (**B**) wine (curve 2) in 0.1 M phosphate buffer solution pH 4.0 (curve 1).

Gallic acid was used as a standard compound. The linear dynamic ranges of 0.52-388.5 at 0.83 V and 2.0-400 μM of gallic acid at 1.18 V with the LOD of 0.25 and 1.00 μM, respectively. The difference in the quantity of electricity for the sample and supporting electrolyte after 100 s was used for the quantitative evaluation of wine AOC. It was expressed in gallic acid equivalents recalculated per 1 L of wine (Table 19). Statistically significant differences in AOC for the red and white wines (1224±184 and 386±112 mg of

gallic acid per L, $p < 0.0001$) was obtained. A 20-fold decrease in sample volume (50 μL *vs.* 1 mL in DPV) required for the measurement was achieved using chronocoulometry.

Table 19 – Wine AOC based on chronocoulometric assay (n=5; P=0.95)

Wine type	Sample (year)	AOC, mg of gallic acid per L	s_r
Red wine	Barton&Guestier Réserve Merlot (2010)	1311±34	0.021
	Le clos du Manoir Montagne Saint-Emilion (2009)	1508±22	0.012
	Tour de Mandelotte Bordeaux(2010)	1073±53	0.040
	Chateau Garriga Saint Martin (2008)	1124±76	0.054
	Chianti Riserva “Tancia”(2007)	1104±32	0.023
White wine	Candidato Viura (2010)	318±27	0.069
	Biserni Rhine Riesling (2008)	422±27	0.051
	Le clos du Manoir Bordeaux sec Blanc (2010)	277±6	0.018
	Marquis d’Orleton Chardonnay (2011)	527±6	0.010

The results of chronocoulometric determination of wine AOC correlate with their total AOC ($r = 0.8957$ and 0.8986 for the red and white dry wines, respectively at r_{crit}=0.811 and 0.878 and P=0.95).

Electroanalysis of antioxidants in the presence of surfactants

A wide range of biologically active compounds including some AO are slightly soluble in water. Therefore, their determination is usually performed in organic media being toxic and volatile. At last decades, the “green chemistry” concept based on substitution of organic solvents on the less or non-toxic media is of interest. Surfactant-based media is one of the possibilities to realize this approach. They change the solubility of organic compounds in water medium and can also effect on the rate and direction of electrode reactions that allows to control the analytical signal and, thus, improve the sensitivity and selectivity of the response towards target analytes.

Galvanostatic coulometry of the antioxidants in surfactant containing media. The effectivity of halogens and $[Fe(CN)_6]^{3-}$- ions coulometric generation in the presence of 1μM-10 mM surfactants has been estimated. The maximum concentration limits of surfactant in electrochemical cell providing 100% current yield of the titrants are found (Table 20).

Surfactant-containing media can be applied for the AO coulometric determination as was shown on example or ascorbic acid, rutin and α-tocopherol. Anionic surfactants give overstated results for the titration with halogens while cationic surfactants – in the case of $[Fe(CN)_6]^{3-}$-ions that is caused by the pH of medium in which the titrants generation occurs as well as the chemical form of the analyte under these conditions.

Table 20 – Maximum concentration of surfactants providing 100% effectivity of titrants electrochemical generation

ПАВ	$C_{surfactant(max)}$, M			
	Cl_2	Br_2	I_2	$[Fe(CN)_6[^{3-}$
DDPB	1.0×10^{-3}	1.0×10^{-4}	1.0×10^{-5}	1.0×10^{-3}
CPB	1.0×10^{-3}	1.0×10^{-5}	2.0×10^{-6}	1.0×10^{-3}
Triton X100	0	1.0×10^{-5}	2.2×10^{-4}	1.0×10^{-3}
Brij® 35	0	1.0×10^{-5}	2.0×10^{-4}	1.0×10^{-3}
PEG 4000	1.0×10^{-4}	1.0×10^{-5}	4.0×10^{-4}	1.0×10^{-3}
SDS	5.0×10^{-3}	2.0×10^{-4}	6.0×10^{-4}	1.0×10^{-3}

The results obtained were used for the determination of AO in pharmaceutical dosage forms and their quality evaluation (Table 21).

Table 21 – AO determination in pharmaceutical dosage forms (n=5; P=0.95)

Sample	Analyte	Labeled amount, %, mg*	Galvanostatic coulometry				Voltammetry		F-test[b]	t-test[c]
			Titrant	Surfactant	Found, %, mg*	s_r	Found, %, mg*	s_r		
α-Tocopherol acetate oil solution	α-Tocopherol	30[a]	Br_2	0.1 mM DDPB	28±1	0.028	29.5±0.5[a]	0.014	1.96	1.34
		10[a]	Br_2		10.6±0.2	0.014	9.8±0.3[a]	0.026	1.22	2.22
Rutin	Rutin	20*	$Fe(CN)_6^{3-}$	0.22 mM Triton X100	20±1*	0.039	20.3±0.4*	0.016	5.64	1.64
Ascorutin	Rutin	50*	$Fe(CN)_6^{3-}$		50±1*	0.020	50±1*	0.021	1.37	1.12
	Ascorbic acid	50*	I_2		50±2*	0.033				

[a] – α-Tocopherol contents in α-tocopherol acetate equivalents

[b] F_{crit}=6.39 at P=0.95 and df_1=4, df_2=4 [c] t_{crit}=2.31 at P=0.95 and df=8

Voltammetry of antioxidants in the presence of surfactants has been used for the determination of different AO and redox mediator menadione on GCE. The effects of nature and concentration of surfactants on the voltammetric characteristics of lipophilic AO and menadione have been investigated. The surfactant-containing media application improves the form of the voltammograms and the electrode reactions reversibility. The AO electrooxidation parameters are found and the corresponding oxidation schemes are suggested. As menadione electroreduction shows, SDS micellar medium stabilizes the semiquinone anion-radical that is usually undergoes to the fast protonation. The application of surfactant-containing media allows to improve the analytical characteristics of analytes determination and to perform measurements in water media or significantly reduce the organic solvent portion (Table 22).

The developed voltammetric methods of AO and menadione determination in the presence of surfactants are tested on the real samples (foodstuff, pharmaceutical dosage formd and cosmetics). The conditions of analytes quantitative recovery using proper

extractants are found. The absence of the matrix interference effects on the AO and menadione voltammetric determination is confirmed. The determination of α-tocopherol and retinol in pharmaceutical forms using organized media is shown in Table 23.

Table 22 – The analytical characteristics of voltammetric determination of lipophilic AO and menadione in surfactant-containing media

Analyte	Supporting electrolyte	Surfactant	C_{surf}, mM	LOD, μM	Linear dynamic range, μM
α-Tocopherol	0.1 M $LiClO_4$ in acetonitrile/H_2O(6:4)	-	-	17.0	34.0-70.9
		DDPB	1.0	1.02	2-140
		Triton X100	1.0	1.02	2-100
		CPB	0.5	2.04	4.1-10
Retinol	0.1 M $LiClO_4$ in H_2O/ethanol (93:7)	-	-	30	49-392
		SDS	0.11	15	29.4-980
β-Carotene	0.1 M $LiClO_4$ in ethanol/CH_2Cl_2 (9:1)	Triton X100	10	2.5	10-380
Eugenol	0.1 M $LiClO_4$ in 10 % ethanol	Triton X100	100	10	20-10000
	0.1 M $LiClO_4$	Triton X100	100	3.8	15-1230
		Brij® 35	100	5.0	20-1380
Menadione	0.1 M H_3PO_4 in 10 % ethanol	SDS	9.0	1.66	7.0-560 600-2550
tert-Butylhydroquinone	Britton-Robinson buffer pH 3.0 – acetonitrile (9:1)	SDS	10	0.23	2.02-1010
tert-Butylhydroxyanisole				0.18	2.34-1170
tert-Butylhydroxytoluene				3.5	6.15-615

Table 23 – Voltammetric determination of α-tocopherol and retinol in pharmaceutical forms and cosmetics using organized media (n=5; P=0.95)

Sample	Producer	Analyte	Sufractant	ω, %	Found, %	s_r
α-Tocopherol acetate oil solution	OAO "Marbiopharm"	α-Tocopherol	1 mM DDPB	30	29±1	0.031
			1 mM Triton X100		28±2	0.060
α-Tocopherol acetate oil solution	OAO "Uralbiopharm"		1 mM DDPB	10	10.4±0.2	0.016
			1 mM Triton X100		10±1	0.070
Aqualia antiox	Vichy Laboratoires		1 mM Triton X100	-	4.22±0.02	0.004
Effaclar-M	La Roche-Posay		1 mM Triton X100	-	0.326±0.002	0.005
Retinol acetate oil solution	OAO "Marbiopharm"	Retinol	0.11 mM SDS	3.44	3.1±0.2	0.043
Retinol acetate oil solution (best before 2009)	OAO "Pharmatzevtiche skaya fabrika Sankt-Peterburga""			3.44	1.5±0.2	0.105
Retinol acetate oil solution	ZAO "Retinoidy"			5.50	5.43±0.08	0.011
Retinol acetate oil solution (best before 2008)	ZAO "Retinoidy"			5.50	3.06±0.02	0.005
Hyséac "Active Care with AHA"	Uriage Eau Thermale			-	0.159±0.002	0.001

The volatmmetric determination of AO in foodstuff and menadione in pharmaceutical "Aekol" are presented in Table 24. The results obtained are in a good agreement with independent spectrophotometric data. The methods with the several exclusions are uniformly precise (F-test < F_{crit}) and t-test data cofirm the absence of systematic errors of determination.

Table 24 – Determination of eugenol and β-carotene in foodstuff and menadion in "Aekol" using surfactant-containing media (n=5; P=0.95)

Sample	Analyte	Found, mg g^{-1}, *mg per 100 g, ** g				F-test[a]	t-test[б]
		Voltammetry	s_r	Spectrophtometry	s_r		
Clove essential oil	Eugenol	757±12	0.012	766±7	0.007	2.94	1.87
		731±12	0.013	743±16	0.017	1.78	1.64
Sweet basil essential oil		1.8±0.2	0.082	2.1±0.2	0.070	1.10	2.29
Clove		48±1	0.021	49±2	0.027	2.16	1.64
Cinnamon		12.3±0.7	0.048	12.7±0.5	0.032	1.93	1.58
Nutmeg		0.71±0.03	0.042	0.75±0.05	0.067	3.88	1.12
Bay leaf		0.40±0.02	0.050	0.38±0.03	0.079	1.03	0.832
Sweet basil		1.23±0.07	0.049	1.2±0.1	0.083	11.4	1.25
Currot	β-carotene	9.9±0.3*	0.025	9.8±0.4*	0.032	1.78	0.441
Pumpkin		2.94±0.05*	0.018	2.90±0.07*	0.025	1.96	1.04
Rowan berries		7.82±0.08*	0.010	7.9±0.1*	0.013	1.56	1.16
Rose hips		2.90±0.08*	0.028	2.8±0.1*	0.036	1.43	1.95
Parsley		5.5±0.2*	0.022	5.4±0.2*	0.031	1.01	0.912
Aekol	Menadione	0.050±0.002**	0.029	0.048±0.004**	0.036	1.52	1.59
		0.049±0.003**	0.049	0.050±0.004**	0.034	2.04	0.791

[a] t_{crit}=2.31 at P=0.95 and df=8 [b] F_{crit}=6.39 at P=0.95 and df_1=4, df_2=4

The possibility of extraction-voltammetric determination of retinol and *tert*-butylhydroxytoluene in foodstuff is shown (Table 25).

Tabke 25 – Voltammetric determination of retinol and *tert*-butylhydroxytoluene in foodstuff (n=5; P=0.95)

Analyte	Method, supporting electrolyte	Sample	Found, Mg per100 g; * mg L^{-1}	s_r
Retinol	CV 0.11 mM SDS in 0.1 M $LiClO_4$ inwater-ethanol (93:7)	Fresh carrot	-	-
		Boiled carrot	45.79±0.03	0.026
		Beef liver	61.16±0.02	0.016
		Linseed oil	14.18±0.02	0.015
			21.29±0.02	0.016
		Butter	< LOD	-
tert-butylhydroxy-toluene	DPV 10 mM SDS in acetonitrile-Britton-Robinson buffer pH 3.0 (1:9)	Linseed oil 100%	135±1	0.007
		Linseed oil with selenium	177.3±0.9	0.004
		Linseed oil with vitamin E	77±1	0.010
		Linseed oil unrefined	44.5±0.7	0.013
		Sea-buckthorn oil	124±2	0.015

Surfactant-modified electrodes for the antioxidants determination. Electrode surface modification with self-organized systems are used for the concentration of analytes and improvement of the analytical characteristics of their determination. The nature of the analyte and surfactant plays an important role in this case. Concentration occurs via electrostatic interaction of ionogenic groups of surfactants with corresponding functional groups of the analyte or via hydrophobic interactions of surfactant hydrocarbon fragments with hydrophobic molecules of analytes. The combination of carbon nanomaterials with surfactants as an electrode surface modifier is of interest. Surfactant-modified electrode are created for the determination of natural and synthetic phenolic AO (morin, vanillin, syringaldehyde, *tert*-butylhydroquinone and *tert*-butylhydroxyanisole). The effect of surfactant nature and concentration on AO voltammetric characteristics is studied. The best parameters for the natural phenolic AO are obtained on the electrodes modified with cationic CPB due to the structural similarity of the analytes and aromatic mojety of surfactant. Moreover, the electrostatic interaction is realized in the case of morin oxidation on CPB/SWNT-COOH/GCE.

The electrode surface is characterized by SEM (Fig. 15). GCE shows unstructured smooth surface. CNF are relatively homogeneously distributed as well-intertwined net of fibers indicating immobilization of nanomaterial on the electrode surface. CPB layer does not change the top view of the electrode.

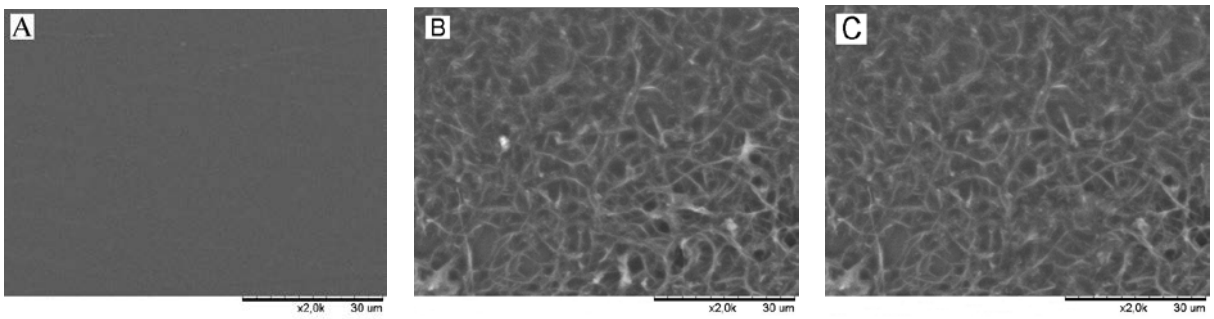
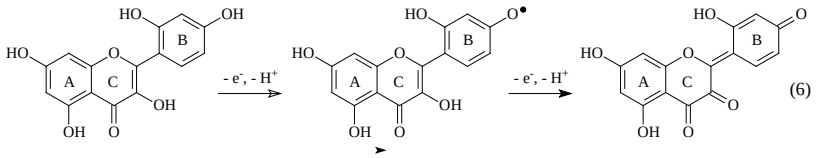


Fig. 15 – SEM-images of electrode surface morphology: **A** – GCE; **B** – CNF/GCE; **C** – CPB/CNF/GCE. C_{CNF} =0.5 mg mL^{-1}, c_{CPB} =0.5 mM.

The 1.3-1.8-fold increase of the oxidation currents are observed on surfactant/CNT/GCE in comparison to CNT/GCE. Electrooxidation parameters are found and the corresponding oxidation schemes are proposed. Morin oxidation on CPB/SWNT-COOH/GCE is irreversible two-step process with participation of one electron and one proton on each step acoording to Scheme 6. Vanillin and syringaldehyde are oxidized on two-electron mechanism to o-quinones.



Combination of surfactant-modified electrodes and micellar media providing solubilization of the analytes is developed for the SHP determination. MWNT suspensions in micellar media of SDS, CPB, Triton X100 and Brij® 35 have been investigated as potential modifiers of GCE surface. The best homogeneity and stability has been obtained for the suspension in Brij® 35 micellar media. The voltammetric behavior of SHP has been investigated on GCE and MWNT- Brij® 35/GCE in 0.1 M $LiClO_4$ in water containing 1 M of Brij® 35. The increase of the oxidation currents and the decrease of oxidation overpotentials on 130 and 40 mV has been observed for *tert*-butylhydroquinone and *tert*-butylhydroxyanisole, respectively. The oxidation involves two electrons and two with the formation of corresponding *p*-quinones.

DPV has been used for the quantitative determination. The analytical characteristics observed (Table 26) are better than reported earlier for other modified electrodes.

Table 26 – The analytical characteristics of AO voltammetric determination using surfactant-modified electrodes

Analyte	Electrode	LOD, μM	Linear dynamic range, μM	$I=a+bc$		R^2
				$a\pm\Delta a$, μA	$(b\pm\Delta b)\times10^{-2}$, μA M^{-1}	
Morin	CPB/SWNT-COOH/GCE	0.029	0.10-100 100-750	-0.011±0.008 0.9±0.2	208±2 104±4	0.9996 0.9954
Vanillin	CPB/CNF/GCE	0.14	0.50-75 75-750	-0.006±0.002 0.8±0.1	158.3±0.5 56±3	0.9999 0.9912
Syringaldehyde		0.19	0.75-10 10-1000	$(-8.4\pm0.4)\times10^{-3}$ 0.03±0.04	126.8±0.7 161±1	0.9910 0.9958
tert- Butyl-hydroquinone	MWNT- Brij® 35/GCE	0.26	1.0-1000	-0.13±0.04	76.9±0.9	0.9985
tert-Butyl-hydroxyanisole	MWNT- Brij® 35/GCE	0.15	0.50-7.50 10.0-750	-0.006±0.001 0.17±0.03	149±3 110.8±0.9	0.9980 0.9996

The achieved peaks potential separation of *tert*-butylhydroquinone and *tert*-butylhydroxyanisole on MWNT- Brij® 35/GCE (200 mV), vanillin and syringaldehyde on CPB/CNF/GCE (110 mV) as well as the form of analytical signals allows their simulataneous determination in a wide range of concentrations (2.5-30 μM for vanillin, 5.0-40 μM for syringaldehyde and 2.50-750 μM for *tert*-butylhydroquinone and *tert*-butylhydroxyanisole). The approach is tested on the example of *tert*-butylhydroquinone and *tert*-butylhydroxyanisole determination in linseed oil extracts. The possibility of their chronoamperometric determination in edible oils extracts is shown too.

The created amperometric sensors based on co-immobilized carbon nanomaterials and surfactants are highly selective and have been applied for morin and vanillin determination in foodstuff (Table 27).

Table 27 – Morin and vanillin determination in foodstuff (n=5; P=0.95)

Analyte	Sample	Found by voltammetry, mg g^{-1}	s_r	Found by standard method, mg g^{-1}	s_r	t-test[a]	F-test[b]
Morin	Mulberry leaves	1.59±0.02	0.096	1.61±0.05	0.012	1.94	1.71
		1.71±0.04	0.017	1.75±0.06	0.014	1.80	1.34
Vanillin	Vanilla sugar "Dr. Oetker"	2.86±0.06	0.016	2.9±0.3	0.042	0.422	0.71
	Sugar with natural vanilla "Dr. Oetker"	0.16±0.01	0.064	0.17±0.02	0.044	2.14	1.56
	Vanillin "Haas"	48±2	0.038	46±3	0.024	1.46	2.75
	Vanilla pods	4.4±0.2	0.030	4.3±0.1	0.0093	0.867	4.69
	Cream milk powder	1.99±0.04	0.015	2.1±0.1	0.043	1.54	4.00

[a] t_{crit}=2.45 at P=0.95 and df =6 [b] F_{crit}=6.94 at P=0.095 and df_1= 4, df_2=2

CONCLUSIONS

The investigation carried out for a wide range of AO of different structure and origin using electrochemical methods allowed to estimate the individual and group characteristics of this class of compounds as an objects of bioanalytical chemistry. The main conclusions on the on the work are presented below.

1. Voltammetric methods for the determination of natural and synthetic phenolic (di- and trihydroxybenzenes, flavonoids, gallic and hydrocinnamic acids), S-containing (cysteine, glutathione, methionine, unithiol and α-lipoic acid) AO as well as α-tocopherol and retinol in the pharmaceutical dosage forms on the electrodes modified with carbon nanotubes. The relative standard deviation does not exceed 6%. The results obtained agree well with the independent methods data.
2. The nanomaterial nature, way of its synthesis and treatment effect on the electrode properties. CNT act as an electron transfer mediator decreasing the analytes overpotential and increase their oxidation currents. Methionine, glutathione and unithiol are electrochemically active in available potential window on the modified electrode only. The application of carbon nanotube-based electrodes leads to improvement of AO determination in particular 2-5-fold decrease of LOD (up to 20 times for some cases) and 1-2 order enlargement of the linear dynamic ranges.

3. Approaches for voltammetric determination of lipophilic AO (α-tocopherol, retinol, β-carotene, eugenol, SHP) and menadione in foodstuff, pharmaceutical dosage forms and cosmetics in the presence of surfactants are developed. The effect of surfactant nature and concentration on analytes voltammetric characteristics are studied. The application of surfactant-containing media improves the form of voltammograms, increases the reversibility of the electrode reactions and, consequently, enhances the analytical and metrological parameters of analytes determination. As investigations carried out show, surfactant-containing media are a good alternative to organic solvents in the electroanalysis of lipophilic AO.
4. The amperometric sensors based on co-immobilized carbon nanomaterials and surfactants have been created for the determination of natural (morin, vanillin and syrungaldehyde) and synthetic (*tert*-butylhydroquinone and *tert*-butylhydroxyanisole) AO including simultaneous quantification of structurally related aldehydes and SHP mentioned above. The analytical characteristics of the sensors are significantly higher than that one for the existing electrochemical analogues. The LOD values on the nM level and the linear dynamic ranges of 3-4 order are achieved.
5. The stoichiometric coefficients for the reactions and natural and synthetic phenolic AO with electrogenerated halogens and hexacyanoferrate(III) ions are found. The choice of hexacyanoferrate(III) ions as a titrant for the determination of phenolic AO is justified. The possibility of titrants electrogeneration in surfactant-containing media is shown. The coulometric methods for the determination of α-tocopherol, flavonoids and ascorbic acid in pharmaceutical dosage forms with the relative standard deviation of 1.3-6.8% are developed.
6. Galvanostatic coulometry with electrogenerated bromine and hexacyanoferrate(III) ions allows to evaluate the interaction of flavonoids with milk proteins. Casein, bovine serum albumin and β-lactoglobulin bind rutin, quercetin and taxifolin via intermolecular interactions converting them into inactive chemical form.
7. Methods for the evaluation of total antioxidant parameters (FRP and AOC) of spices, tea and coffee using voltammetry and coulometric titration are developed. The individual AO contributing to AOC under conditions of voltammetry are found. AOC is expressed in the equivalent of gallic acid, catechin and chlorogenic acid for the spicesm tea and coffee, respectively. The AOC for the various types of tea and coffee are statistically significant different that allows to estimate the beverages quality. The

galvanostatic coulometry data show that FRP of tea and coffee significantly decreases in the presence of milk. The positive correlations of spices AOC with AOA and TPh (r = 0.8892-0.9479 at r_{crit}= 0.472) are obtained.

8. The applicability of galvanostatic coulometry, DPV and chronoamperometry for the evaluation of cognac and brandy antioxidant properties and quality is proved. The AO contributing to the beverages AOC is found. The correlations of the results obtained using methods developed and standard protocols are obtained (r = 0.8311-0.9847 at r_{crit}= 0.444). The approaches developed can be used for the beverages quality evaluation. AOC of brandy in 8-15-fold higher (depending on the age) than for the adulterations. As DPV and chronoamperometry show, the AOC of adulterations in most cases near or equal to zero confirming the absence of aging step in oak barrel, *i.e.* product adulteration.
9. Chronocoulometric method for wine AOC evaluation is developed and characterized with simplicity and low (40-fold less) sample consumption. AOC of red and white dry wines is statistically different (1224±184 и 386±112 mg of gallic acid per L, respectivelyo, p<0.0001).

The problems of AO bioanalytical chemistry resolving using electrochemical methods are discussed in the thesis. The tremendous reserve of thousands individual AO and the samples in which they occur give a broad are of investigations in the future. Taking into account the permanent interest to the AO problems in the world under the frames of biomedicine as well as the progress trends of the methods and approaches in analytical chemistry, the analysis of AO remains popular and perspective in the social aspect. The natural sciences picture of AO world is too multicolored that is caused by a great variety of biological targets which are defended by AO. It can be expected, that the efforts of researchers and developers working in the field of biomedicine will be focused on the creation of new pharmaceuticals with antioxidant effect.

The future trends in AO electroanalysis can be defined as:

- research and development of new chemically modified electrodes with high analytical and operational characteristics allowing simultaneous determination of several structurally related analytes including isomers and enentiomers;
- development of the amperometric sensors batteries for the evaluation of antioxidant “portreit” of the sample using chemometric approaches. The innovations can be expected in this area;

- systems miniaturization via creation of portable equipment based on the screen-printed electrodes for the fast screening of sample antioxidant properties;
- application of the new types of electrochemical detectors for the flow methods under conditions of chromatography and capillary electrophoresis.

The main contents of the work is presented in the next publications:

1. Ziyatdinova, G.K. The possibilities of electroanalytical chemistry in solution of pharmaceutical analysis problems / G.K. Ziyatdinova, H.C. Budnikov // Pharmaceutical analysis (Series “The problems of Analytical Chemistry”. V. 16). 6). – M.: ARGAMAK-MEDIA, 2013. – P. 230-268. (In Russian)
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