

Voltammetric detection of synthetic water-soluble phenolic antioxidants using carbon nanotube based electrodes

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Abstract Glassy carbon electrodes (GCE) modified with carbon nanotubes (CNT) have been created for detection of phenolic compounds—one of the important group of antioxidants in life sciences. The surface of electrode has been characterized by atomic force microscopy. The presence of CNT leads to an at least 20-fold increase in the surface roughness of the electrode. The CNT layer displays closely intertwined vermicular structures with high degree of homogeneity at CNT suspension concentration of 0.2–0.5 mg L⁻¹. Synthetic water-soluble antioxidants (hydroquinone, catechol, pyrogallol, and their derivatives) are electrochemically active on bare GCE and CNT-modified GCE in phosphate buffer solution pH 7.4. Effect of substitutes in molecular structure of phenolic antioxidants has been evaluated. In several cases, oxidation at CNT-modified GCE

occurs at potentials that are less positive by 100–200 mV in comparison to bare GCE. The electrodes were studied with respect to their capability of phenols voltammetric sensing. CNT-modified GCE display an enlarged linear range in the calibration graphs and lower detection limits. Voltammetric method for determination of hydroquinone, catechol, pyrogallol, and their derivatives has been developed.

Keywords Carbon nanotubes · Chemically modified electrodes · Voltammetry · Synthetic antioxidants · Phenols

Introduction

The degradation of carbon-based materials including polymers, fuel, lubricating oils, foodstuff is usually caused by peroxy radical (ROO[•]) initiation followed by a chemical reaction with molecular oxygen to generate more peroxy radicals. This process, known as autoxidation, can propagate and generate more peroxide and free radicals [1]. In order to avoid or decrease the intensity of radical oxidation, the special additives—stabilizers in particular antioxidants are usually applied.

Phenolic antioxidants form an important class of compounds which serve to inhibit the oxidation of materials of both commercial and biological importance by trapping of peroxy radicals [2]. The phenol donates a hydrogen atom and terminates the propagation of further radical reactions as outlined in Scheme 1.



Scheme 1 Termination of chain radical reactions by phenols

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So far, as reactions of phenols with free radicals include the electron transfer, electrochemical methods can be applied for their investigation. Methods of electroanalysis, in particular voltammetry, are highly sensitive, express, relatively inexpensive and in combination with possibility of miniaturization very attractive for the decision of such kind problems. Moreover, it is caused by development of chemically modified electrodes increasing selectivity and sensitivity of analyte determination as well as decreasing of detection limits.

Mesoporous Al-doped silica (Al/SiO₂) carbon paste electrode has been created for the determination of catechol in tea [3]. The linear range is $5.0 \times 10^{-7} \div 5.0 \times 10^{-5}$ M with a correlation coefficient of 0.998. The limit of detection is as low as 1.0×10^{-7} M.

A carbon ionic liquid electrode based on the use of pyridinium-*n*-octylpyridinium hexafluorophosphate as a binder has been applied for the investigation of the electrochemical oxidation of phenolic compounds in acidic media by cyclic voltammetry, chronoamperometry, and square wave voltammetry [4].

A number of electrochemical biosensors [5, 6] have been developed for the monitoring of phenols in waste products like composting systems, waters. Laccase [7, 8], catechol oxidase [9], and tyrosinase [10, 11] have been used as biosensitive part of sensors in combination with other modifiers like carbon nanotubes (CNT) [12], magnetic core-shell (Fe₃O₄-SiO₂) nanoparticles [13], and polypyrrole [14]. This approach leads to improvement of determination analytical characteristics.

Another important area of phenols investigation is possibility of simultaneous determination of hydroquinone and catechol. This problem is usually solved using chemically modified electrodes. It should be noted that carbon nanomaterials in particular CNT are widely used as modifiers of electrode surface in electroanalytical chemistry due to their excellent performance of enhancing the electrochemical reactivity, promoting the electron transfer reactions and alleviating surface fouling [15, 16]. Carbon atom wires [17], multiwall carbon nanotubes in multielectrode array system [18], nanostructured mesoporous platinum film electrochemically deposited from the hexagonal liquid crystalline template of polyethylene(10) cetyler surfactant [19] and bare indium tin oxide electrodes with employing catechol redox cycling by hydrazine [20] have been successfully used for detection of dihydroxybenzene isomers.

In summary, it should be noted that electrochemical methods are widely used for the analysis of phenolic compounds. Application of modified electrodes based on enzymes and nanomaterials gives future trends in this field of science.

Synthetic water-soluble antioxidants (hydroquinone, catechol, and pyrogallol derivatives) have shown antioxidant properties [21, 22] that permitted to use these compounds as antioxidant additives for prevention of oxidative degradation in different materials. Therefore, methods for their determination are of interest. The present work is focused on development of novel voltammetric approach for detection of new synthetic water-soluble phenolic antioxidants using CNT-modified glassy carbon electrode.

Material and methods

Reagents

CNT were synthesized by catalytic pyrolysis of ethanol vapor on nickel catalyst. Then catalyst was deleted by acid treatment. CNT were washed twice with deionized water, dried, and bolted (CNT(I)) [23]. Then CNT(II) were got by further anneal at 700 °C for 2–3 h in argon [24]. The homogeneous suspension of both CNT types was got by ultrasonic dispersion in 2.5 mL of dimethylformamide.

Hydroquinone, catechol and pyrogallol of 99% purity were purchased from Sigma-Aldrich (Germany). Their water-soluble derivatives were synthesized in Organic Chemistry Department of Ural Federal University. Stock solutions of phenols under investigation (0.01 M) were prepared by dissolving of exact amount of the appropriate substance in 25.0 mL of water. More dilute solutions were prepared every day by exact dilution of the stock solutions.

All other chemicals were analytical reagent grade purity and used as received. Double-distilled water was used for the measurements. The experiments were carried out at laboratory temperature (20–23 °C). All solutions were kept in glass vessels in the dark at laboratory temperature excepting phenols solution stored at –4 °C.

Procedures

Electrode preparation

The glassy carbon electrode (GCE) was carefully polished with alumina on polishing cloth. Then it was rinsed with acetone and double-distilled water before use. Modification of GCE was performed by formation of homogeneous layer of CNT on the electrode surface after evaporation to dryness of 2 μL CNT suspension.

Cyclic voltammetry

Voltammetric measurements were performed using voltammetric analyzer “Ecotest-VA” (Russian Federa-

tion). The electrochemical cell ($V=50$ ml) consisted of working glassy carbon electrode (6.07 mm² geometric surface area), a silver-silver chloride saturated KCl reference electrode and a counter electrode (platinum wire). Phosphate buffer solution pH 7.4 was chosen as supporting electrolyte. After adding 15.0 ml of supporting electrolyte and aliquot portion of analyte test solution, cyclic voltammograms were recorded at potential scan rate of 100 mV s⁻¹ and potential range from -0.4 to 1.0 V.

Atomic force microscopy

Atomic force microscopy (AFM) of the electrode surfaces was performed using atomic force microscope NTegra Prima (NT-MDT, Russia) and operated at room temperature in ambient conditions. Silicon cantilever NSG03 (NT-MDT, Russia) with resonance frequency of 80 kHz was used for the scanning in semi-contact mode. Radius of curvature for cantilever tip was near 10 nm. The 2 μ L of CNT suspension was dropped on the GC surface and allowed to evaporate to dryness. Then the 5×5 μ m AFM image of the surface was scanned.

Statistical analysis

All the measurements were performed in five replications. Statistical evaluation was performed at significance level of 5%. All data are expressed as the $X \pm \Delta X$ with X as average value and ΔX as confidence interval.

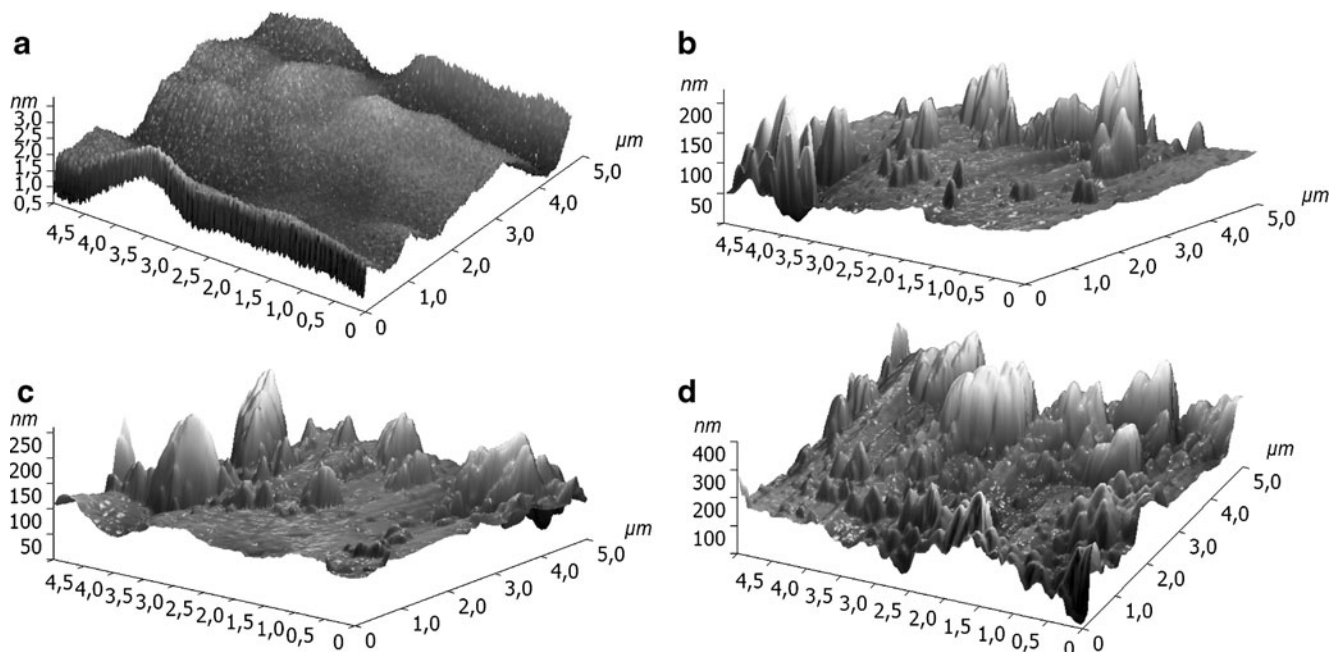


Fig. 1 AFM image of electrode surface morphology: **a** bare GCE, **b** CNT(I)-GCE, $C_{\text{CNT(I)}}=0.2$ mg mL⁻¹, **c** CNT(I)-GCE, $C_{\text{CNT(I)}}=0.5$ mg mL⁻¹, **d** CNT(I)-GCE, $C_{\text{CNT(I)}}=0.7$ mg mL⁻¹

Results and discussion

AFM characterization of electrode surface

In order to find the working conditions of electrode modification and characterize the electrodes created, the surface of electrodes with different amounts of CNT(I) and (II) as modifier was studied using AFM. Figures 1 and 2 represent the morphology of electrode surface based on the AFM measurements. As one can see, bare GCE possesses unstructured amorphous surface. CNT layer coverage leads to significant increase of roughness and structuring of electrode surface.

Independently of the concentration, CNT(I) layer represents big aggregates sizes of which increase with concentration of nanomaterial. The electrode coverage is highly heterogeneous that is seen from Fig. 1. Separate aggregates are very high up to 230 nm.

CNT(II) layer at concentrations of 0.2 and 0.5 mg mL⁻¹ is vermiform structures with height of 20 – 70 nm which is closely interlaced with each other that complicates determination of their length. It averages 135 – 400 nm for some single tubes. For CNT(II) concentration of 0.7 mg mL⁻¹, large aggregates of 130 nm height were observed and suspension was unstable and unsuitable for electrode modification.

Main characteristics of electrode surface are shown in Table 1. Due to the formation of aggregates in the case of CNT(I), it was impossible to determine their length.

So, electrode modification leads to significant growth of surface roughness (minimum to 20 times) with increase of CNT concentration till 0.5 mg mL⁻¹. However, average

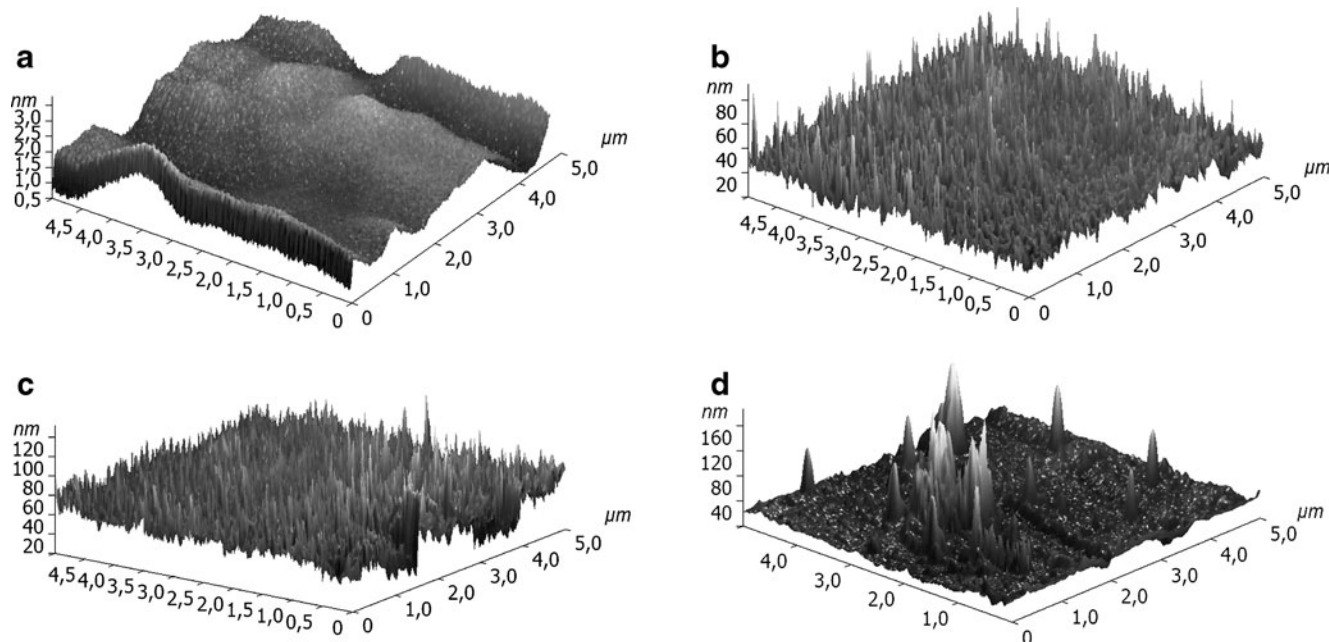


Fig. 2 AFM image of electrode surface morphology: **a** bare GCE, **b** CNT(II)-GCE, $C_{\text{CNT(II)}}=0.2 \text{ mg mL}^{-1}$, **c** CNT(II)-GCE, $C_{\text{CNT(II)}}=0.5 \text{ mg mL}^{-1}$, **d** CNT(II)-GCE, $C_{\text{CNT(II)}}=0.7 \text{ mg mL}^{-1}$

roughness decreases at 0.7 mg mL^{-1} of CNT that is caused by heterogeneity of modifier layer on the electrode surface.

CNT selection for the detection of phenolic compounds

CNT under investigation were tested for the detection of phenolic compounds in phosphate buffer solution pH 7.4. Reversible oxidation step was observed on the cyclic voltammograms on CNT(I)-GCE in supporting electrolyte (Fig. 3) that corresponds to oxidation of hydroquinone fragments on the electrode surface. These results are confirmed by comparison with oxidation/reduction potentials of hydroquinone standard solution on the GCE at the same conditions. Moreover, the increase in oxidation/reduction currents at corresponding potentials was obtained with addition of hydroquinone into the electrochemical cell. Appearance of hydroquinone fragments on CNT(I) is caused by acid treatment of CNT on the catalyst cleaning stage during their production.

Table 1 Electrode surface characteristics based on AFM data

Electrode	CNT type	C_{CNT} (mg mL^{-1})	R_a (nm)	R_q (nm)	h (nm)	l (nm)	d (nm)
GC		0	0.39	0.47	2–3	–	–
CNT-GC	I	0.2	13.1	23.2	30–80	–	300–400
		0.5	15.0	24.8	60–170	–	300–600
		0.7	40.0	55.6	100–230	–	500–1,000
	II	0.2	7.9	10.7	20–35	135–187	32–69
		0.5	9.1	11.8	50–70	170–200 300–400	29–51
		0.7	7.9	14.3	100–130	–	250–355

R_a the average roughness, R_q the root mean square roughness

So, CNT(I) cannot be used for the detection of phenolic compounds which are electrochemically active at the same anodic potentials.

As for the CNT(II), additional treatment by anneal in argon leads to removal of hydroxyl groups and electrochemical inertness of CNT(II) under conditions of the experiment. Therefore, CNT(II) can be applied as modifier of electrode surface for further detection of phenolic antioxidants.

Cyclic voltammetry of water-soluble phenolic antioxidants

Voltammetric response of hydroquinone, catechol, pyrogallol, and their water-soluble derivatives on GCE and CNT-GCE has been studied. The structures of compounds under investigation are shown in Fig. 4.

Effect of potential scan rate on the voltammetric characteristics of phenols oxidation on GCE has been investigated (Fig. 5a). Hydroquinone was chosen as a model compound. Its oxidation current increases at higher scan rates and maximum

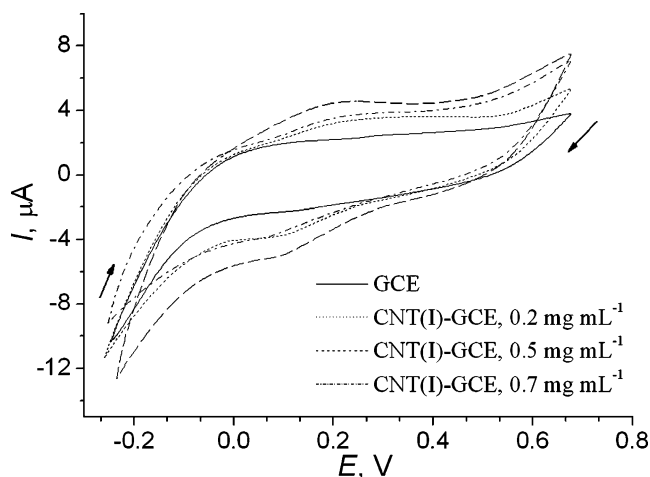


Fig. 3 Cyclic voltammograms of phosphate buffer solution pH 7.4 on different electrodes. Potential scan rate is 100 mV s^{-1}

current was observed at 100 mV s^{-1} . There is a good linear relationship between peak current and $v^{1/2}$ (Fig. 5b) that indicates diffusion control of hydroquinone oxidation on the electrode surface.

CNT concentration effect on the hydroquinone oxidation has been evaluated. The corresponding cyclic voltammograms are shown in Fig. 6. The best form and characteristics of analytical response were got for 0.2 mg mL^{-1} of CNT suspension.

Water-soluble phenolic antioxidants under investigation are electrochemically active on stationary GCE and CNT-GCE in phosphate buffer solution pH 7.4. Voltammetric characteristics are presented in Table 2. The results obtained for hydroquinone (I) and catechol (III) on the CNT-GCE are better than reported earlier [18] using multiwalled carbon nanotubes modified electrode.

It should be noted that analytes are easily oxidized on both electrodes excluding compounds II and VI. Their oxidation potentials are shifted to more positive area that is caused by presence of thiosulfate group. Catechol derivatives IV and V are oxidized at the same potentials as catechol. Pyrogallol derivatives (compounds VIII–XIII) oxidation potentials are more positive in comparison with pyrogallol because of the sterical hindrances in their structures. Decrease of overpotential on 100–200 mV and increase of oxidation current were observed for great part of phenols under investigation using CNT-GCE. It can be explained by increase in the electron transfer rate on CNT-GCE caused by high real area of the electrode surface.

Oxidation of hydroquinone, catechol, and pyrogallol derivatives goes irreversibly that confirms by absence of cathodic steps on the corresponding cyclic voltammograms (Fig. 7).

As known [25, 26], hydroquinone and catechol are oxidized with formation of *p*- and *o*-benzoquinones, respectively. The first step on pyrogallol voltammograms corresponds to oxidation of galloyl group (Eq. 3) [27–29].

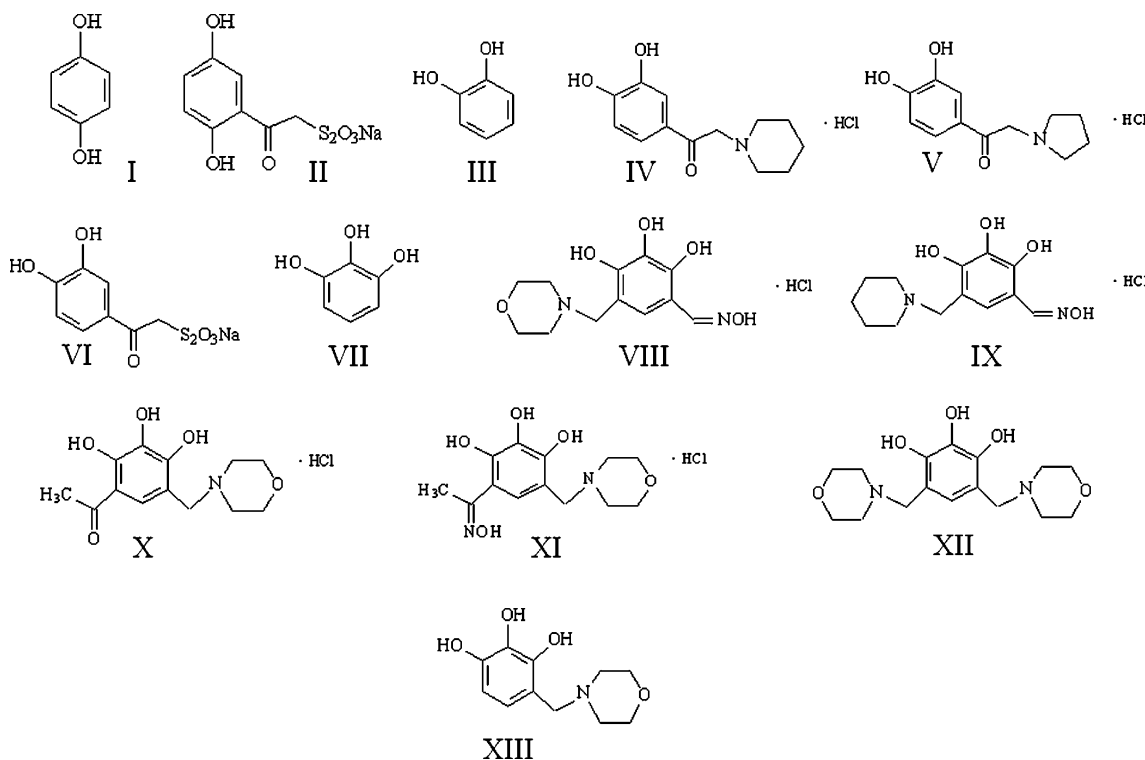


Fig. 4 Structure of synthetic water-soluble phenolic antioxidants under investigation

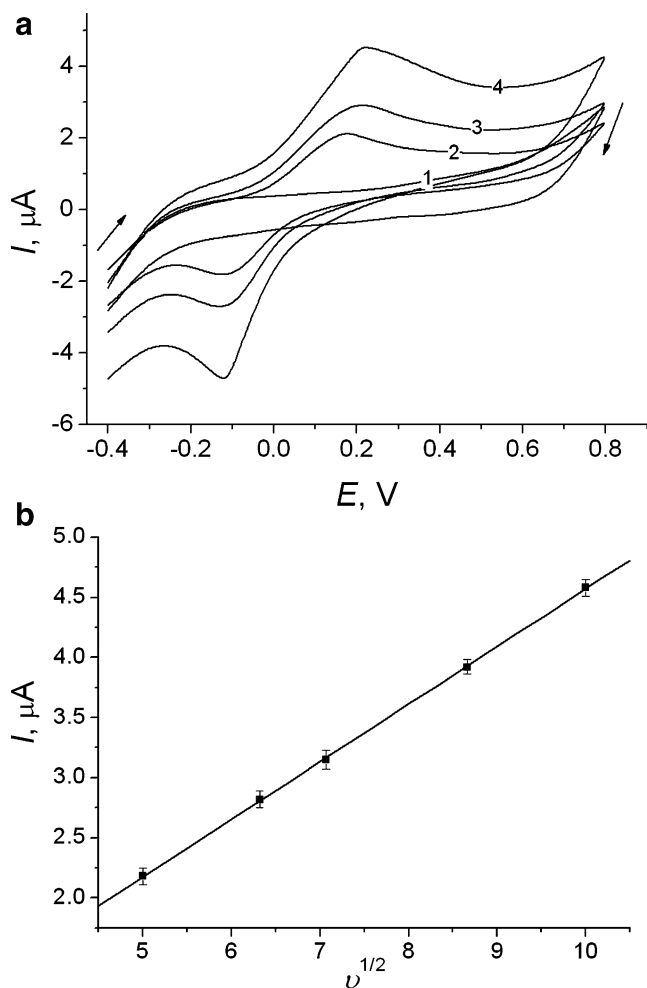


Fig. 5 **a** Cyclic voltammetry on GCE in phosphate buffer solution pH 7.4 in the absence (1) and in the presence of 2.3×10^{-4} M hydroquinone at the following scan rates: 25 mV s^{-1} (2), 50 mV s^{-1} (3), and 100 mV s^{-1} (4). **b** Relationship between hydroquinone oxidation current and $v^{1/2}$

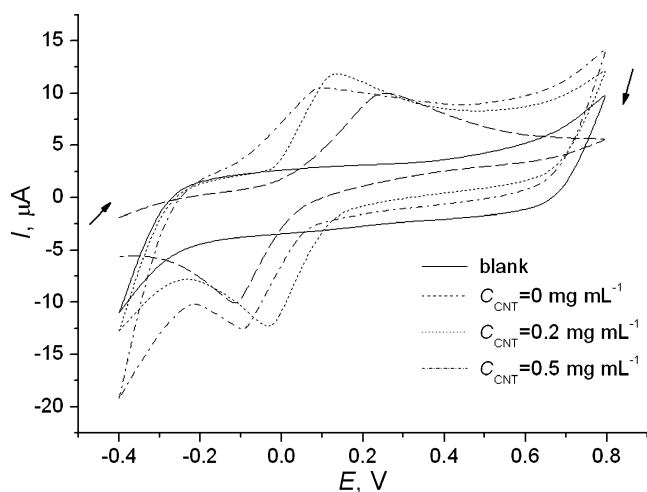
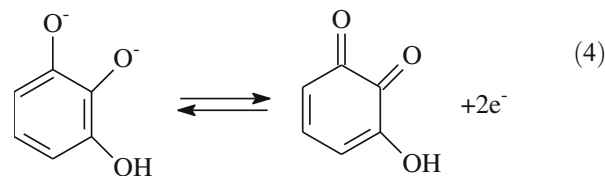
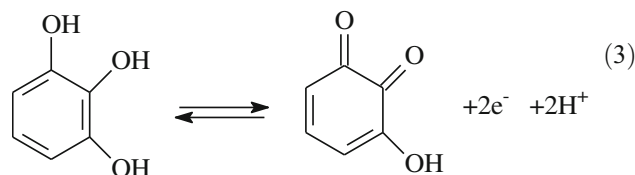


Fig. 6 Cyclic voltammograms of 3.74×10^{-4} M hydroquinone on CNT-GCE for different concentration of CNT (mg mL^{-1}). Potential scan rate is 100 mV s^{-1} . Supporting electrolyte—phosphate buffer solution pH 7.4

Table 2 Voltammetric characteristics of hydroquinone, catechol, pyrogallol, and their derivatives oxidation in phosphate buffer solution pH 7.4

No	E_{max}, V		$\frac{I_{\text{mod}}}{I}$
	GCE	CNT-GCE	
I	+0.25	+0.12	2
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
V	+0.23, +0.45	+0.23, +0.46	1.3
VI	+0.64	+0.53	1
VII	+0.03, +0.27, +0.75	0, +0.17, +0.69	3
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
XII	+0.18, +0.6	+0.1, +0.54	1.6
XIII	+0.29, +0.63	+0.08, +0.56	1.4

The second peak is most probably caused by the oxidation of dissociated pyrogallol group [30] (Eq. 4).



Oxidation of this compounds derivatives on the first step is caused by reaction of hydroxyl groups that confirms by oxidation potentials.

There are linear relationship between oxidation current and analyte concentration for all compounds under investigation with correlation coefficient in the range of 0.9972–0.9999. Analytical characteristics of phenols oxidation are shown in Table 3. Usage of CNT-GCE leads to analytical range enlargement and detection limit decrease for determination of phenolic compounds under investigation.

Quantitative determination of phenolic antioxidants in model solutions using CNT-modified GCE was carried out.

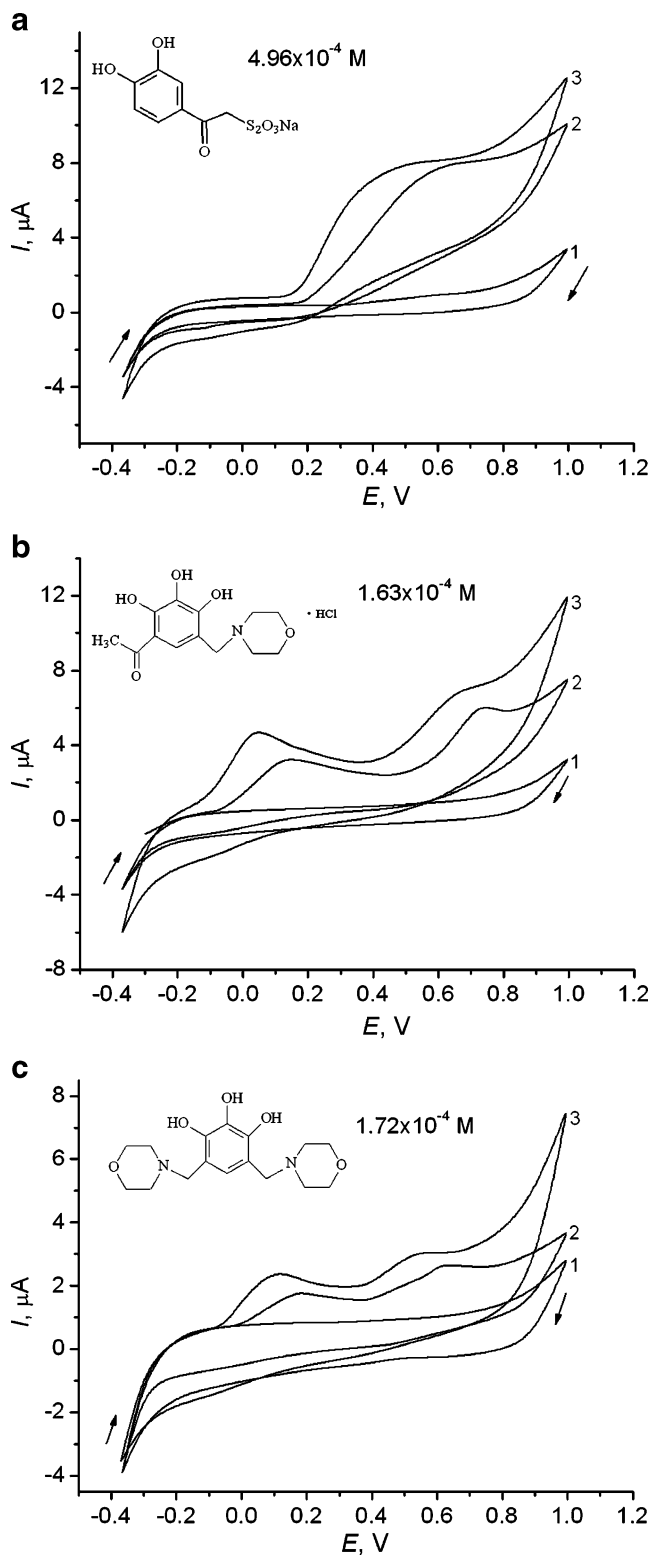


Fig. 7 Cyclic voltammograms of phenolic antioxidants on GCE (2) and CNT-modified (3) GCE. 1 Blank line from phosphate buffer solution pH 7.4. Potential scan rate is 100 mV s⁻¹

Table 3 Analytical characteristics of voltammetric determination of phenolic antioxidants

Compound	Limit of detection (μM)		Analytical range (μM)	
	GCE	CNT-GCE	GCE	CNT-GCE
I	3.9	2.9	3.9÷1,360	2.9÷1,430
II	14.4	10.1	28.7÷193	11.5÷722
III	7.33	1.47	14.7÷688	3.67÷1,290
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÷1,320	2.12÷1,320
VII	33.2	20.0	66÷440	66÷1,660
VIII	3.8	1.9	3.8÷1,900	1.9÷671
IX	1.44	0.73	1.44÷1,830	1.83÷1,290
X	3.36	0.67	3.36÷1,680	1.68÷1,680
XI	3.36	0.67	3.36÷630	0.67÷1,680
XII	1.43	1.78	1.43÷1,780	1.78÷1,780
XIII	1.61	0.81	1.61÷2,020	2.02÷756

Table 4 Voltammetric determination of water-soluble phenolic antioxidants in model solutions using CNT-GCE in phosphate buffer solution pH 7.4 (n=5; P=0.95)

Analyte	Added (μg)	Found (μg)	RSD (%)
I	58	56±2	3.1
	347	372±16	4.3
	867	880±41	2.2
II	61	60.5±0.5	0.83
	605	605±5	0.83
III	23	23±1	5.0
	923	910±25	3.4
IV	14	14.0±0.4	3.0
	560	555±15	3.1
V	23	23.1±0.7	3.0
	6,810	6,770±340	5.0
VI	126	122±17	6.1
	3,780	3,790±50	5.0
VII	26	26.4±0.7	3.1
	6,940	6,943±45	0.9
VIII	832	835±21	2.5
	6,660	6,567±47	0.7
IX	767	778±11	1.4
	6,130	6,273±38	0.6
X	81	84±4	4.5
	6,430	6,127±47	0.8
XI	173	170±4	2.4
	6,930	6,850±122	1.8
XII	68	67±3	4.4
	2,720	2,700±30	1.1

The accuracy of results obtained was evaluated by added-found method (Table 4).

Conclusion

CNT-based electrodes were created for the detection of synthetic water-soluble phenolic antioxidants using cyclic voltammetry. Hydroquinone, catechol, pyrogallol, and their derivatives are easily oxidized on the electrode surface. The effect of substituents in phenol structure on its voltammetric characteristics has been studied. The addition of a substituent in phenol structure leads to different electrochemical behavior of compounds, i.e., oxidation potential and current. One could see a difference between electrochemical characteristics for bare GCE and CNT-GCE that could have an analytical application. The knowledge of oxidation potential will help to investigate the antioxidant efficacy of phenols under investigation. The application of a modified electrode allows enlarging the analytical range and decreasing the detection limits for hydroquinone, catechol, pyrogallol, and their derivatives. Voltammetric approach developed is express, reproducible and reliable and can be used for their determination. The investigations carried out contribute to the development of organic voltammetry in general.

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