CYCLIC VOLTAMMETRY OF NATURAL FLAVONOIDS ON MWNT-MODIFIED ELECTRODE AND THEIR DETERMINATION IN PHARMACEUTICALS

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Paper submitted for a special issue of the Collection of Czechoslovak Chemical Communications devoted to "distinguished followers of the J. Heyrovský School of Polarography". This work is dedicated to Professor Robert Kalvoda to commemorate his 85th birthday.

The determination of rutin, quercetin and taxifolin in pharmaceutical dosage forms using cyclic voltammetry on multi-walled carbon nanotube modified glassy carbon electrode (MWNT-GCE) has been developed. The surface of the electrode created has been characterized by atomic force microscopy. Electrode modification with MWNT increases the surface average roughness (190-fold) and structures it. There are two oxidation steps at 0.22 and 0.80, 0.23 and 0.80, 0.26 and 0.86 V on cyclic voltammograms of taxifolin, quercetin and rutin, respectively, in phosphate buffer solution of pH 7.4. The linear dynamic range is 1.4–28 and 28–210, 2.0–220 and 0.52–210 μM with detection limits of 0.71, 1.0 and 0.26 μM for rutin, quercetin and taxifolin, respectively. The relative standard deviation of flavonoids determination in pharmaceuticals does not exceed of 7%. The data obtained are in good agreement with coulometric determination.

Keywords: Carbon nanotubes; Chemically modified electrodes; Cyclic voltammetry; Flavonoids; Pharmaceutical analysis.

Flavonoids (rutin, quercetin and taxifolin (Fig. 1)) are important biologically active compounds with a wide variety of physiological effects such as anti-inflammatory, antitumor, antiallergic, antibacterial and antiviral properties[1–3]. They also have ability to scavenge free radical of particular reactive

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oxygen species[4,5] and thus show antioxidant properties[6]. Because of these properties flavonoids are used in medicine as acting substances in different pharmaceutical dosage forms. Therefore development of new reliable, simple and express methods for flavonoids determination is important area of investigations.

Flavonoids like all phenolic compounds are easy oxidized that allows to use electrochemical methods for their determination. In particular, voltammetry is characterized by simplicity, sensitivity, cost-efficiency, precision, accuracy and speed and useful for the investigation of electron transfer reactions.

The mechanism of electrochemical oxidation of rutin on a glassy carbon electrode (GCE) has been studied at different pH by using several electrochemical techniques (cyclic, linear sweep, differential pulse and square-wave voltammetry). Rutin oxidation process is reversible, pH dependent[7] and includes the transfer of 2 electrons and 2 H^+. Electrochemical behavior of quercetin has been studied on the procaine and aminophenyl modified electrodes in non-aqueous media[8] and graphite-wax electrode by *in situ* spectroelectrochemical method[9]. The mechanism of taxifolin electrooxidation has been investigated by different electrochemical techniques at different pH values[10].

Platinum and carbon-based electrodes have been used for the determination of flavonoids in biological fluids[11] and pharmaceuticals[12–14].

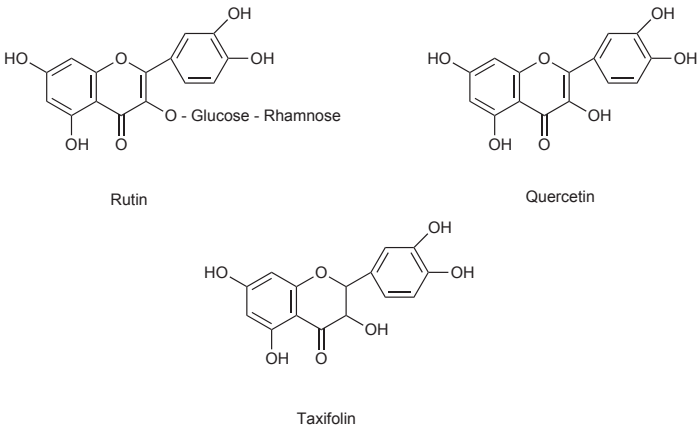

FIG. 1
Structure of flavonoids under investigation

Active development in the field of chemically modified electrodes opens new possibilities to improve the analytical characteristics of flavonoids determination. GCE modified with acetylene black nanoparticles[15], lead film-modified GCE [16], ionic liquid[17,18], mesoporous silica- modified[19] and poly(vinylpyrrolidone)-modified[20] carbon paste electrodes has been reported for the detection of rutin. Different types of carbon nanomaterials has been used as modifiers of the electrode surface and applied for the flavonoids determination[21–24]. GCE coated with graphene nanosheets, chitosan and a poly(amidoamine) dendrimer has been applied for the voltammetric determination of rutin in pharmaceutical preparations, spiked human serum, and traditional Chinese medicines[25] with recoveries between 97.2 and 104.7%.

Amperometric detection on boron-doped diamond electrode for quantification of flavonoids in tea samples using a flow injection system has been described[26]. The calibration graph is linear in the range of 10–250 μM rutin and the detection limit is 7.7 μM.

A number of amperometric biosensors based on laccase[27], tyrosinase[28] and polyphenol oxidase[29] for the determination of polyphenols are described. Bi-immobilization of laccase and tyrosinase phenoloxidase enzymes and the sonogel–carbon as electrochemical transducer have been developed for estimation of the total polyphenol index in beer samples[30].

The aim of this article is to develop new voltammetric method for the detection of rutin, quercetin and taxifolin using MWNT-based electrodes and apply this approach for the analysis of pharmaceutical dosage forms.

EXPERIMENTAL

Reagents

Rutin trihydrate of 95% purity and taxifolin of analytical standard purity were purchased from Fluka (Germany), quercetin dihydrate of 98% purity from Sigma (Germany). Their stock solutions (0.01 M) were prepared by dissolving a definite amount of the substance in 25.0 ml of ethanol. More dilute solutions were prepared every day by exact dilution of the stock solutions and used as model.

MWNTs (OD 40–60 nm, ID 5–10 nm, length 0.5–500 μm) were obtained from Aldrich (Germany). The homogeneous suspension of MWNTs with final concentration of 0.5 mg ml^{-1} was got by ultrasonic dispersion for 18 min in 1% sodium dodecyl sulfate (Panreac, Spain).

All other chemicals were analytical reagent grade purity and used as received. Double distilled water was used for the measurements. All solutions were kept in glass vessels in the dark at laboratory temperature excepting flavonoids solution stored at –4 °C.

Apparatus

Voltammetric measurements were performed using voltammetric analyzer "Ecotest-VA" (Econix-Expert Ltd., Russia). The electrochemical cell (V = 15 ml) consisted of working glassy carbon electrode (6.07 mm^2 geometric surface area), silver|silver chloride saturated KCl reference electrode and counter electrode (platinum wire).

Atomic force microscopy (AFM) of the electrode surfaces was performed using atomic force microscope NTegra Prima (NT-MDT, Russia) with silicon cantilever NSG03 (NT-MDT, Russia).

Coulometric measurements were carried out using P-5827 M potentiostat (Russia) with four-electrode two-compartment electrochemical cell. A bare platinum foil with the surface area of 1 cm^2 was used as the working electrode, and a platinum wire as the auxiliary electrode. A pair of polarized platinum electrodes was used for detection of the titration endpoint.

"Expert-001" pH meter (Econix-Expert Ltd., Russia) equipped with the glass electrode was used for pH measurements.

Procedures

Electrode preparation. The GCE was carefully polished with alumina (0.05 μm) 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 MWNTs on the electrode surface after evaporation to dryness of 5 μl MWNTs suspension.

Cyclic voltammetry. 0.1 M Phosphate buffer solution pH 7.4 was chosen as supporting electrolyte. After adding 10.0 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 –0.1 to 1.0 V.

Atomic force microscopy. AFM images of the electrode surfaces were performed at room temperature in ambient conditions. Silicon cantilever with resonance frequency of 160 kHz was used for the scanning in semi-contact mode. Radius of curvature for cantilever tip was near to 10 nm. The 5 μl of MWNT suspension was dropped on the GCE surface and allowed to evaporate to dryness. Then the 5 × 5 μm AFM-image of the surface was scanned.

Constant-current coulometry. Coulometric titration with hexacyanoferrate(III) ions was carried out in accordance to previous report[31].

All experiments were carried out at laboratory temperature (20–23 °C).

Sample Preparation

Pharmaceuticals "Capilar" and "Oculist TM" from "Diod" Co Ltd. (Moscow, Russia), "Antistax" (Pharmaton SA, Lugano-Bioggio, Switzerland), "Ascorutin" from JSC "Altaivitaminy" (Biysk, Russia) and Public Corp. "Marbiopharm" (Yoshkar-Ola, Russia) from local drugstores were investigated. "Capilar" contains taxifolin and sorbitol. "Oculist TM" consists of taxifolin, freeze-dried bilberry fruits, β-carotene and seleksen (the source of selenium). The active substances of "Ascorutin" are rutin and ascorbic acid. "Antistax" is based on natural extract of red vine leaves.

Ten tablets (capsule content) were weighted separately and average weight of one tablet (capsule content) was measured. Then 10 tablets (capsule content) were grinded and exact amount (0.25 g) of powder was dissolved in ethanol in 10.0 ml volumetric flask. Then

0.1–0.6 ml of solution was inserted in electrochemical cell containing 9.9–9.4 ml of supporting electrolyte. Total volume in the electrochemical cell was 10.0 ml. Then cyclic voltammograms were recorded in the range from –0.1 to 1.0 V with scan rate of 100 mV s^{-1}.

Statistical Analysis

All 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.

RESULTS AND DISCUSSION

Characterization of the Electrode Surface by AFM

The electrodes surface has been investigated using AFM. Figure 2 represents the morphology of surfaces for bare GCE and MWNT-GCE based on the AFM measurements.

As one can see bare GCE has unstructured amorphous surface. MWNT coverage gives the significant changes in surface shape and its structure. MWNTs are aligned as rows with average width of 0.8–1.0 μm and alternating hills of 586 nm heights. The main characteristics of electrodes surface are shown in Table I. As it can be seen from the data in Table I modification of electrode leads to substantial increase (in 190 times) of its roughness.

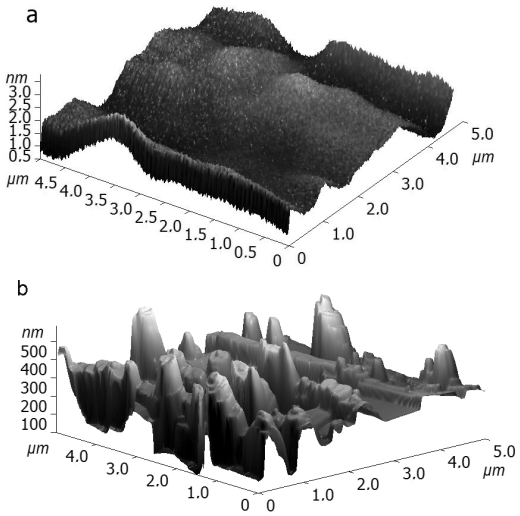

FIG. 2
AFM image of electrode surface morphology: bare GCE (a); MWNT-GCE (b)

TABLE I
Characteristics of electrode surfaces based on AFM measurements

Electrode	R_a, nm[a]	R_q, nm[b]	h, nm	w, μm
GCE	0.39	0.47	2–3	–
MWNT-GCE	73.1	92.0	200–586	06–1.0

[a] R_a average roughness; [b] R_q root mean-square roughness.

Determination of Natural Flavonoids by Cyclic Voltammetry

Voltammetric behavior of natural flavonoids on GCE and MWNT-GCE has been studied. Rutin, quercetin and taxifolin are easily oxidized on stationary GCE and MWNT-GCE in phosphate buffer solution pH 7.4. There are two steps on cyclic voltammograms at 0.22 and 0.80 V for taxifolin, 0.23 and 0.80 V for quercetin and 0.26 and 0.86 V for rutin (Fig. 3). The first step has form of a reversible peak.

As one can see from the Fig. 3 there were no any changes in flavonoids overpotential on MWNT-GCE. However significant increase (2–3-fold) in

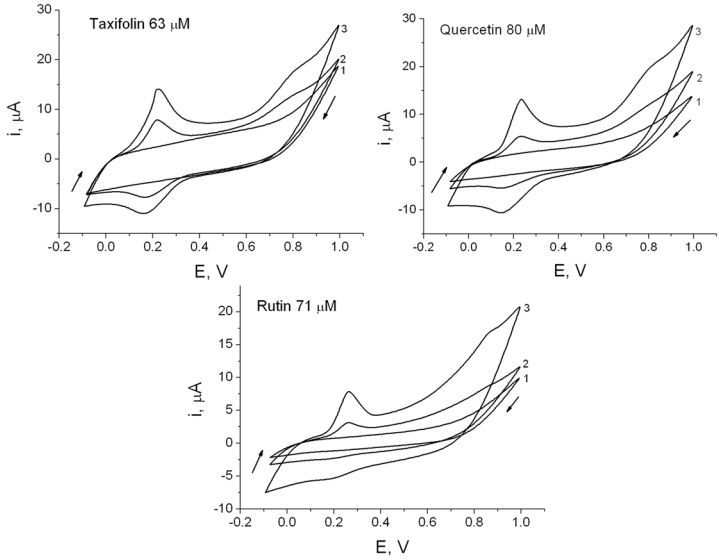

FIG. 3
Cyclic voltammograms of various flavonoids on GCE (*2*) and MWNT-GCE (*3*) in phosphate buffer solution pH 7.4 (*1*). Potential scan rate 100 mV s^{-1}

their oxidation current was observed. Modification of the electrode surface with MWNTs increases the effective surface area that leads to growth of oxidation currents that corresponds well with literature data[32–34].

All flavonoids under investigation are oxidized at closely related potentials. The difference in oxidation potential for rutin is probably caused by presence of glycoside residue in its structure that slightly complicates the oxidation process. The oxidation potential values observed confirm the similar mechanism of oxidation reaction for rutin, quercetin and taxifolin in spite of their structural differences.

As known, hydroxy groups of B-ring participate in flavonoids oxidation with formation of corresponding quinoid derivatives[35] in accordance to Fig. 4.

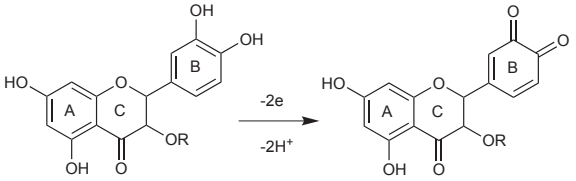

FIG. 4
Scheme of flavonoids electrooxidation

The effect of potential scan rate on the voltammetric characteristics of flavonoids has been evaluated on the example of taxifolin (Fig. 5a). Electrochemical oxidation of taxifolin is controlled by the diffusion of electroactive species as the slowest step in the electrochemical process. The diffusion nature of the taxifolin voltammetric peak is confirmed on the basis of linear dependence[36] of peak current on the square root of scan rate $v^{1/2}$ (Fig. 5b) with $R = 0.9999$.

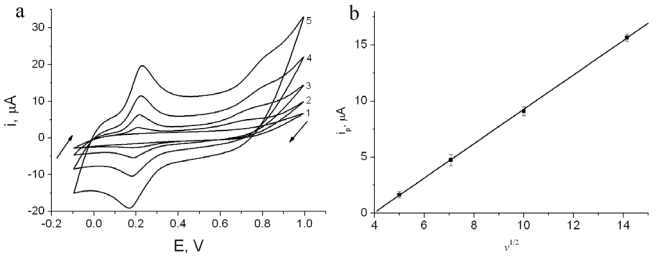

FIG. 5
a Cyclic voltammetry on MWNT-GCE in phosphate buffer solution pH 7.4 in the absence (*1*) and in the presence of 52 μM taxifolin at the following scan rates (in mV s^{-1}): 25 (*2*), 50 (*3*), 100 (*4*) and 200 (*5*). b Relationship between taxifolin oxidation current and $v^{1/2}$

The oxidation current increases at higher scan rates but at 200 mV s^{-1} the background current is too high that partially distorts the voltammogram form on initial area. Therefore scan rate 100 mV s^{-1} has been chosen for further measurements. There are linear relationship between oxidation current and analyte concentration for compounds under investigation. Analytical characteristics of flavonoids oxidation are shown in Table II. Application of MWNT-modified electrode leads to analytical range enlargement and decrease of detection limit for determination of rutin, quercetin and taxifolin.

TABLE II
Analytical characteristics of voltammetric determination of flavonoids in phosphate buffer solution pH 7.4

Compound	Electrode	Detection limit, μM	Analytical range, μM	Linear regression equation $y = a + bx$		R
				a μA	b × 10^{-4} μA l mol^{-1}	
Quercetin	GCE	10	20–80	0.227±0.083	5.03±0.16	0.9995
	MWNT-GCE	1.0	2.0–220	2.25±0.25	9.48±0.23	0.9995
Taxifolin	GCE	5.2	21–306	5.03±0.32	3.44±0.18	0.9959
	MWNT-GCE	0.26	0.52–210	2.992±0.096	8.53±0.13	0.9989
Rutin	GCE	5.4	7.1–280	0.460±0.058	21.07±0.43	0.9988
	MWNT-GCE	0.71	1.4–28	1.347±0.033	9.19±0.25	0.9985
			28–210	2.51±0.14	5.20±0.13	0.9988

Quantitative determination of flavonoids in model solutions using MWNT-modified GCE was carried out. The accuracy of results obtained was evaluated by added-found method (Table III). Based on the results obtained, voltammetric method for flavonoids determination in real samples has been developed. Pharmaceutical dosage forms have been chosen as objects of analysis. As one can see from the Fig. 6, there are well-defined signal of flavonoid on the cyclic voltammograms of pharmaceutical dosage forms. The oxidation potentials in mono-component forms correspond to oxidation of rutin, quercetin or taxifolin, respectively.

For the multi-component pharmaceuticals ("Antistax", "Oculist TM" and "Ascorutin"), the signal-forming substance has been checked by standard addition method (Fig. 7). Addition of flavonoid standard solution leads to increase of analytical signal at the same potential. Positive shift in the oxi-

dation potential by 50 mV was observed for the "Antistax" in comparison to quercetin signal. The effect observed is probably caused by presence of other constituents in dosage forms. Nevertheless, the peak corresponds to quercetin that was confirmed by addition of its standard solution.

In order to check the determination accuracy, a known amount of flavonoid standard solution was spiked in the sample solution and the recovery was tested. The results of flavonoids determination are presented in

TABLE III
Voltammetric determination of flavonoids in model solutions (n = 5; P = 0.95)

Analyte	Added, μg	Found, μg	RSD, %
Quercetin	24.2	23.9 ± 1.9	6.4
	242	241.9 ± 1.6	0.5
	725	724.7 ± 1.6	0.2
Taxifolin	19.2	18.58 ± 0.84	4.3
	64	63.96 ± 0.91	1.2
	639	638.5 ± 1.9	0.2
Rutin	17.5	17.36 ± 0.76	3.5
	175	174.74 ± 0.73	0.3
	1310	1309.2 ± 3.8	0.2

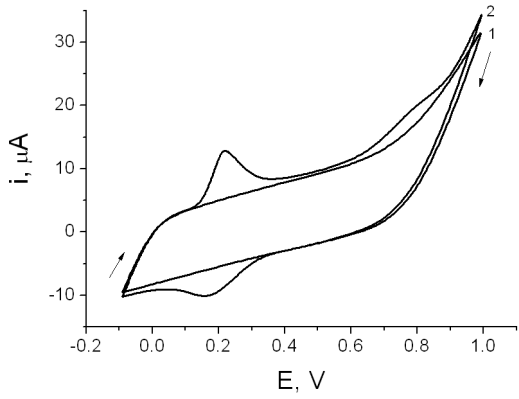

FIG. 6
Cyclic voltammogram of 0.1 ml "Capilar" (*2*) on MWNT-GCE in phosphate buffer solution pH 7.4 (*1*). Potential scan rate 100 mV s^{-1}

Table IV. The value of recovery is in the range from 99.6 to 100% indicating an absence of matrix effects in these determinations.

TABLE IV
Voltammetric determination of flavonoids in solutions of multi-somponent dosage forms (n = 5; P = 0.95)

Sample	Analyte	Spiked, μg	Expected, μg	Found, μg	Recovery, %
Oculist™	Taxifolin	0		170 ± 1.5	
		32	202	202.0 ± 1.8	100
Antistax	Quercetin	0		353.2 ± 2.0	
		121	474	473.2 ± 2.1	99.8
Ascorutin	Rutin	0		247.8 ± 6.5	
		218		464.4 ± 4.7	99.6

The results of flavonoids determination in pharmaceutical dosage forms are presented in Table V. The content of ground substance in tablets corresponds to the labeled amount. The relative standard deviation of the measurements does not exceed of 7%.

The results obtained are in good agreement with the data based on coulometric titration by electrogenerated hexacyanoferrate(III) ions. It was im-

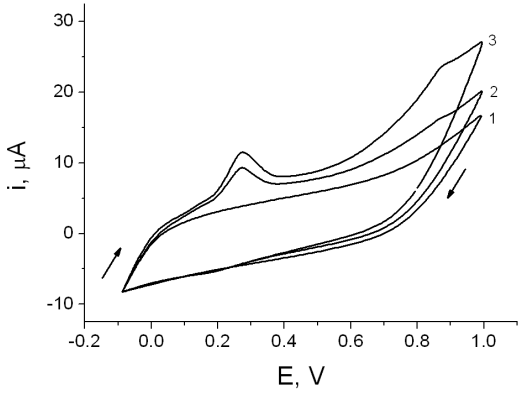

FIG. 7
Cyclic voltammograms of "Ascorutin" on MWNT-GCE in phosphate buffer solution pH 7.4: blank (*1*), "Ascorutin" (*2*), "Ascorutin" + 218 μg of rutin (*3*). Potential scan rate 100 mV s^{-1}

possible to perform coulometric measurements of flavonoids in "Antistax" and "Oculist TM" that is caused by composition of these pharmaceuticals. They contain extracts from red vine leaves and bilberry, respectively, which presented by different polyphenols reacting with titrant under conditions of coulometry.

The calculated *F*-test is less than *F*-table for the one-sample test (F_{tab} = 6.4 ($df_1 = df_2 = 4$, where df is degree of freedom)). This allows to conclude (with 95% confidence) that variances of two populations are homogeneous and there are no significant differences in precision of voltammetry and coulometry.

In conclusion it should be noted, that application of MWNT-modified electrodes improves the analytical characteristics of rutin, quercetin and taxifolin determination leading to decrease the detection limits and enlarge the analytical range that contributes in development of voltammetry of flavonoids. The voltammetric method developed is express, reproducible and reliable and can be used in laboratories of pharmaceutical industry as well as centers for pharmaceuticals quality control for the determination of flavonoids as acting substances in pharmaceutical dosage forms.

TABLE V
Rutin, quercetin and taxifolin content in pharmaceutical dosage forms using cyclic voltammetry (n = 5; P = 0.95)

Sample	Analyte	Labeled amount mg	Found by voltammetry mg	RSD %	Found by coulometry[37] mg	RSD %	*F*-test
Rutin tablets	Rutin	20	20.1 ± 1.1	5.2	20 ± 2	6.8	5.5
Ascorutin tablets	Rutin	50	50.0 ± 1.0[a] 50.84 ± 0.82[b]	2.1 1.3	50 ± 3[a] 51 ± 2[b]	4.9 2.4	5.6 3.5
Quercetin tablets	Quercetin	20	18.63 ± 0.92	4.8	16.6 ± 0.4	2.5	1.3
Antistax capsules	Quercetin	–	10.59 ± 0.61	5.5	–	–	–
Capilar tablets	Taxifolin	10	10.02 ± 0.43	3.8	9.7 ± 0.4	3.4	2.6
Oculist™ capsules	Taxifolin	15	14.71 ± 0.82	5.1	–	–	–

[a] Producer 1; [b] producer 2.

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