Electrochemical determination of theophylline on a glassy carbon electrode modified with reduced graphene oxide—sodium dodecyl sulfate—Nafion composite film

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An electrochemical sensor based on glassy carbon electrode modified with reduced graphene oxide—sodium dodecyl sulfate—Nafion* composite film was constructed and used to determine theophylline. The synthesized graphene oxide and surface morphology of the reduced graphene oxide—sodium dodecyl sulfate—Nafion film were characterized using IR spectroscopy, X-ray diffraction studies, and electrochemical impedance spectroscopy. The electrochemical behavior of the theophylline on the modified electrode was investigated using cyclic voltammetry and adsorptive differential pulse voltammetry. Under optimized analytical conditions, the electrode showed a linear response at concentrations of theophylline of 0.01—0.10, 0.1—1.0, and 1.0—40 μmol L^{-1} with the detection limit of 5 nmol L^{-1}.

Key words: theophylline, reduced graphene oxide, sodium dodecyl sulfate, Nafion, electrochemical sensors.

Theophylline (TP, 1,3-dimethyl-7*H*-purine-2,6-dione, **1**) can be found in pharmaceutical formulations and foodstuffs. It is also used as bronchodilator and respiratory stimulant for the treatment of asthma and lung diseases.[1] However, high doses of TP may lead to insomnia, anorexia, and heartburn tachycardia.[2] Therefore, there is a need for development of a low-cost, simple, sensitive and rapid method for determination of TP in pharmaceutical and foodstuff samples. Some methods have been reported for determination of TP, such as spectrophotometry,[3] gas and liquid chromatography-mass spectrometry,[4,5] molecularly imprinted solid phase extraction,[6] high performance liquid chromatography,[7] and electrochemical methods.[1,8—14] In comparison to the other options, electroanalytical methods have the advantages of simplicity, fast results, and high sensitivity. Characteristics of some known electroanalytical sensors for the TP determination and developed herein an electrochemical sensor based on glassy carbon electrode (GCE) modified with reduced graphene oxide—sodium dodecyl sulfate—Nafion* composite film are summarized in Table 1.

1 (theophylline)
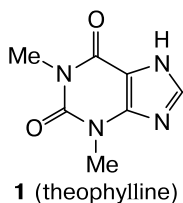
Reduced graphene oxide (RGO) is one of the most important derivatives of graphene, which has a large surface area, low cost, strong mechanical strength, and excellent conductivity. Therefore, it can be used for developing novel electronic devices, such as chemical sensors and biosensors. Graphene oxide- (GO) and RGO-based electrochemical sensors have been developed for the sensitive determination of ascorbic acid, dopamine, and uric acid[15] and pyridoxine.[16]

Surface active agents (surfactants) are amphiphilic molecules with a polar head at one end and a long hydrophobic tail at another. Surfactants are often applied in electroanalytical chemistry to enhance and alter the properties of the electrode/solution interface.[17] Modification of the electrode surface with a surfactant increases the electron transfer rate between the analyte and the electrode surface and improves the detection limits of some molecules.[18—20] In the present work, we used readily available sodium dodecyl sulfate (SDS) as an anionic surfactant for the GCE modification.

Nafion is a perfluorinated sulfonated cation exchanger with excellent antifouling capacity, chemical inertness, and high permeability to cations. Nafion has been extensively used as an electrode modifier.[20—26]

In the present study, the RGO—SDS—Nafion film-modified glassy GCE was fabricated and applied for the electrochemical determination of TP by differential pulse voltammetry (DPV).

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* Nafion is a sulfonated tetrafluoroethylene-based fluoropolymer.

Table 1. The comparison of electrochemical sensors for determination of TP

Electrode	Detection limit /mol L^{-1}	Linear range of the calibration curve/mol L^{-1}	Ref.
Nafion/MWCNT/GCE	$2.0 \cdot 10^{-8}$	$8.0 \cdot 10^{-8} - 6.0 \cdot 10^{-5}$	8
RGO/GCE	$1.0 \cdot 10^{-7}$	$8.0 \cdot 10^{-7} - 6.0 \cdot 10^{-5}$	12
Au-NP/MIP/GCE	$1.0 \cdot 10^{-7}$	$4.0 \cdot 10^{-7} - 1.5 \cdot 10^{-5}$, $2.4 \cdot 10^{-4} - 3.4 \cdot 10^{-3}$	11
Cysteic acid/GCE	$1.2 \cdot 10^{-6}$	$2.5 \cdot 10^{-6} - 6.8 \cdot 10^{-5}$	13
Graphene/Nafion/GCE	$6.0 \cdot 10^{-9}$	$1.0 \cdot 10^{-8} - 1.0 \cdot 10^{-6}$, $3.0 \cdot 10^{-6} - 3.0 \cdot 10^{-5}$	1
MWCNT /Pt-NP/[omim][PF_6]/GCE	$8.0 \cdot 10^{-9}$	$1.0 \cdot 10^{-8} - 1.0 \cdot 10^{-5}$	10
RNA aptamer-based biosensor	$2.0 \cdot 10^{-7}$	$2.0 \cdot 10^{-7} - 1.0 \cdot 10^{-5}$	9
Theophylline oxidase-modified electrodes	$2.0 \cdot 10^{-5}$	$2.0 \cdot 10^{-5} - 6.0 \cdot 10^{-4}$	14
RGO—SDS—Nafion/GCE	$5.0 \cdot 10^{-9}$	$1.0 \cdot 10^{-8} - 1.0 \cdot 10^{-7}$, $1.0 \cdot 10^{-7} - 1.0 \cdot 10^{-6}$, $1.0 \cdot 10^{-6} - 4.0 \cdot 10^{-5}$	Present work

Notes. MWCNT is multi-walled carbon nanotubes; Au-NP and Pt-NP are gold and platinum nanoparticles, respectively; MIP is molecular imprinted polymer.

Experimental

Voltammetric and impedance experiments were performed using a PAR (Princeton Applied Research) electrochemical analyzer model 394 and an Autolab electrochemical system with PGSTAT 12 (EcoChemie, Netherlands), respectively. The Autolab system was run on a PC using FRA 4.9 software. Three-electrode system consisted of an RGO—SDS—Nafion/GCE as a working electrode, Ag/AgCl/KCl (saturated) as a reference electrode, and a platinum wire as an auxiliary electrode. For the impedance measurements, frequency range of 0.10 Hz to 100 kHz was employed. The alternating current voltage amplitude used was 5 mV. FT-IR spectra were recorded on a Perkin Elmer RX I FT-IR spectrometer in KBr pellets. X-ray diffraction (XRD) analysis was carried out on powder samples using a Bruker D8 Advance X-ray diffractometer at room temperature (CuKα radiation, the 2θ range from 10 to 70°, voltage 30 kV, current 25 mA).

Theophylline and Nafion (5%) were purchased from Sigma-Aldrich, SDS was provided by Merck. Graphite oxide was prepared from graphite powder by the Hummers method.[27] The stock 2.0 mM TP solution was prepared by dissolving the required amount of TP in water. All other reagents were of analytical grade and used as received. All solutions were prepared with redistilled water.

Preparation of the RGO—SDS—Nafion composite modified electrode. To a mixture of 0.5% aqueous solution of Nafion (1 mL) and 0.2 M aqueous SDS (0.2 mL), graphite oxide (13.0 mg) was added. The mixture was sonicated for 30 min to obtain a uniform modifier suspension. The GCE was polished with 0.3 and 0.05 μm alumina powders using a polishing cloth with water as the lubricant. Then the electrode was sonicated successively in diluted HNO_3, ethanol, and distilled water for 10 min in each solvent and dried. The GO—SDS—Nafion suspension (4 μL) was applied onto the GCE and dried in air at room temperature. The GO—SDS—Nafion-modified GCE was immersed in 0.1 M phosphate buffer solution (pH 7.0), and graphene oxide was reduced electrochemically by cyclic sweeping from 0 to −1.5 V at 60 mV s^{-1} at five circles.

Theophylline determination. The sample solution, containing 0.15 M H_2SO_4 (1.0 mL) and a specified volume of a TP solution was diluted to the mark with water in a 10-mL volumetric flask, and the resulting solution was transferred into a voltammetric cell. Accumulation was performed under the open circuit conditions for 220 s under constant stirring. Stirring was stopped and after 5 s the cyclic voltammogram was recorded by applying a positive-going differential pulse scan from 1 to 1.4 V at a scan rate of 33.3 mV s^{-1}. After each measurement, the modified electrode was thoroughly rinsed with 1 mM HCl and water to remove any residue of adsorbate. A blank solution (without TP) was used to obtain the blank peak current.

Results and Discussion

Characterization of graphite oxide using XRD analysis and FT-IR spectroscopy. Initially synthesized GO graphite oxide was characterized by XRD studies and FT-IR spectroscopy (Fig. 1). The sharp diffraction peak observed at 2θ = 12° corresponds to graphite oxide[27] (Fig. 1, *a*). In the FT-IR spectrum, the broad peak at 3000—3700 cm^{-1} (Fig. 1, *b*) corresponds to the stretching and bending vibration of the OH groups, while the two absorption peaks observed in the medium frequency region at 1640 and 1500 cm^{-1} can be attributed to the stretching vibration of the graphite C=C bonds and the C=O bonds of the carboxyl groups present at the edges of graphite oxide. Finally, the absorption peak at 1110 cm^{-1} corresponds to the stretching vibration of the C—OH bonds in alcohols (Fig. 1, *b*). The presence of these oxygen containing groups confirms oxidation of graphite. The polar groups, especially the surface hydroxy groups, result in hydrogen bonding between graphite oxide and water molecules; this further explains the hydrophilic nature of graphene oxide.[28,29]
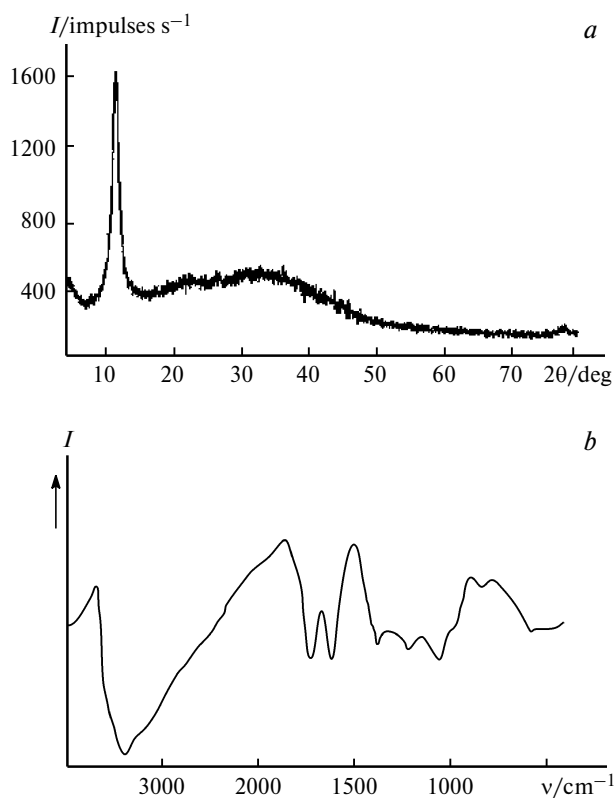
Fig. 1. XRD pattern (*a*) and FT-IR spectrum (*b*) of graphite oxide.

Characterization of electrochemical impedance of the modifier films. Conducting polymers, surfactants, nano-materials, and semiconducting material on the surface of an electrode can alter its double-layer capacitance and interfacial electron transfer resistance. Electrochemical impedance spectroscopy (EIS) can be applied to probe the changes in the surface of the modified electrodes.[30] Figure 2 shows Nyquist diagrams of bare GCE, Nafion/ GCE, and RGO—SDS—Nafion/GCE in 0.1 M KCl in the presence of 5 mM solutions of $K_3[Fe(CN)_6]$ and $K_4[Fe(CN)_6]$. A small well-defined semi-circle was obtained at high frequencies for bare GCE that indicated low interface impedance. When GCE was coated with Nafion, impedance dramatically increased. This could be attributed to the resistance introduced by the Nafion film into the electrode/solution system. In the other words, Nafion acted as a blocking layer that hindered the diffusion of ferricyanide ions toward the electrode surface. In contrast, the interface electron resistance decreased after modification of the electrode with RGO—SDS—Nafion composite. This is the result of low electron transfer resistance caused by the high electrical conductivity of RGO. This results also demonstrated that RGO—SDS—Nafion film was successfully immobilized on the GCE surface.

Electrochemical behavior of TP on different electrodes. Electrochemical oxidation of 40 μM TP in 0.015 M H$_2$SO$_4$
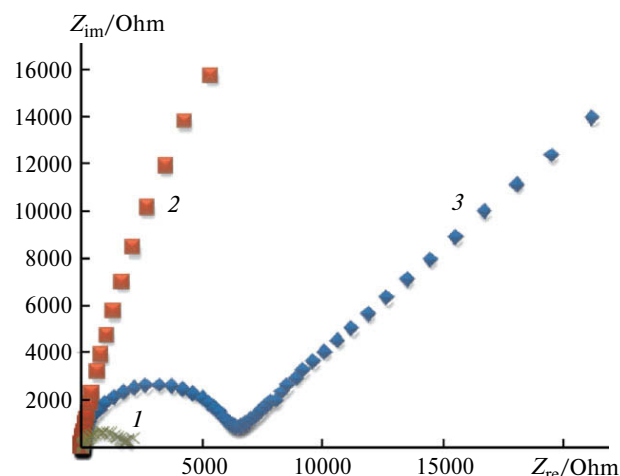

Fig. 2. The Nyquist diagrams of bare GCE, Nafion/GCE, and RGO—SDS—Nafion/GCE in 0.1 M KCl in the presence of 5 mM solutions of $K_3[Fe(CN)_6]$ and $K_4[Fe(CN)_6]$. Applied alternating current voltage of 5 mV, frequency range from 0.1 Hz to 100 kHz. Z_{re} and Z_{im} are the real and imaginary impedances, respectively.

on bare GCE and modified GCE (Nafion/GCE, RGO—SDS/GCE, and RGO—SDS—Nafion/GCE) was studied by DPV (Fig. 3). The most intense oxidation peak of TP at about 1.25 V (with respect to Ag/AgCl) was observed on RGO—SDS—Nafion/GCE. On the RGO—SDS—Nafion/GCE, the current was more than four times higher than that on the base GCE. Sodium dodecyl sulfate as an anionic surfactant facilitates accumulation of TP, especially of the cationic form of TP. Nafion being a cation exchanger with a high cation-exchange capacity attracts TP from the bulk solution to the electrode surface. We suggest that lowering of the detection limit for RGO—
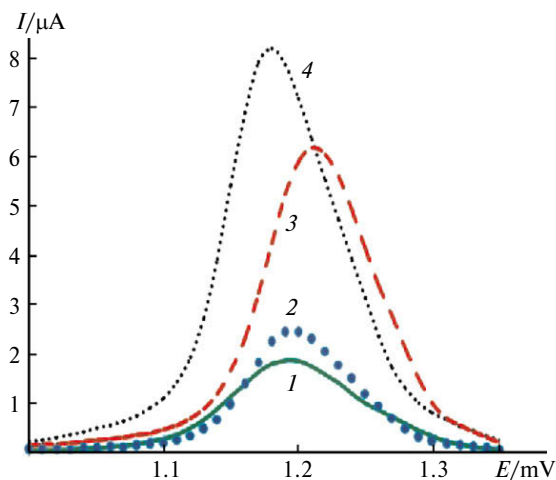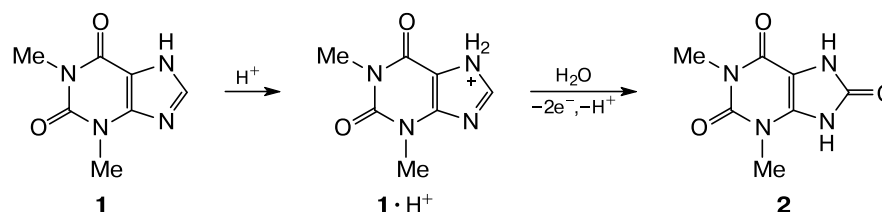

Fig. 3. Differential pulse voltammograms of 40 μM TP on bare GCE, Nafion/GCE, RGO—SDS/GCE, and RGO—SDS—Nafion/GCE recorded in 15 mM H$_2$SO$_4$ and at scan rate of 33.3 mV s^{-1} and accumulation time of 220 s. The curves are net response currents (after subtracting the blank current).

Scheme 1

SDS—Nafion/GCE is a result of the synergistic effect of both SDS and Nafion and the high conductivity of RGO.

The effect of pH on the detection of 20 μM TP was investigated in the phosphate buffer solution with pH ranges from 2.0 to 6.0. The results showed that the acidic media are appropriate for electrochemical determination of TP. The acidic supporting electrolytes (HNO_3, H_2SO_4, $HClO_4$, HCl, AcOH, and H_3PO_4) were tested to estimate the current response for TP. It was found that the highest peak current and the best peak shape for TP was obtained in 15 m*M* H_2SO_4; thus, it was selected as the appropriate medium for further studies. Since the dissociation constant (pK_a) of TP is 8.77,[8] it exists in 15 m*M* H_2SO_4 as the protonated form $1 \cdot H^+$ (Scheme 1). In fact, $1 \cdot H^+$ is more effectively adsorbed and accumulated on the electrode surface due to ion exchange and electrostatic attraction with Nafion and anionic SDS, and after that oxidized to trione **2** (see Scheme 1).[1]

The dependence of the peak potential *versus* pH was also investigated. The results showed that in range of pH 2—6 there is a linear dependence between the peak potential (E_p) and the pH value that was fitted to the regression equation E_p (mV) $= -28.9$pH $+ 1281.2$. The slope of -28.9 confirms the mechanism shown in Scheme 1.

Effect of the scan rate on the electrooxidation of TP was studied by cyclic and differential pulse voltammetry. Cyclic voltammetry showed that the anodic peak current increased linearly with increasing the scan rate (v) from 20 to 100 mV s^{-1} (Fig. 4). This is indicated the adsorption-controlled oxidation of TP on the surface of the modified electrode. The linear regression equation was I_p^a (μA) $= 0.084v$ (mV s^{-1}) $+ 4.11$ ($R^2 = 0.9998$).

The DPV results also showed an increased in the current with increasing the scan rate in the 6.6—33.3 mV s^{-1} range. Therefore, the scan rate of 33.3 mV s^{-1} (maximum obtainable scan rate for the instrument) was selected for further DPV measurements.

Effect of accumulation conditions on the oxidation peak current of TP was investigated by DPV. The accumulation time of 100 n*M* TP ranged from 0 to 240 s, the accumulation was carried out under the open circuit conditions as well as applying potentials from —0.4 to 0.4 V. The oxidation peak current first increased with an increase in the accumulation time from 0 to 200 s and then remained constant. Thus, the accumulation time of 220 s was se-

lected for the adsorption time for TP. The best response was obtained under the open circuit conditions. These conditions were chosen for further studies.

Effect of the pulse height was investigated at the 10—80 mV range. The results showed that the current increases with an increase in the pulse height to 70 mV and then leveled off. Therefore, the pulse height of 70 mV was selected as an optimum for the subsequent experiments.

Calibration curve for quantitative analysis of the TP concentration on RGO—SDS—Nafion/GCE was obtained using DPV under optimized experimental conditions. Three linear regions corresponding to the TP concentration ranges of 0.01—0.10, 0.1—0.5, and 0.5—40 μmol L^{-1} are shown in Fig. 5; the related differential pulse voltammograms are shown in Fig. 6. The detection limit (C_{LOD}) was 0.005 μmol L^{-1} ($C_{LOD} = 3S_b/m$, where S_b is the standard deviation of 10 replicates of blank and m is the slope of the calibration curve). In the present study, the RGO—SDS—Nafion/GCE showed a much better detection limit and a linear response over a wider concentration range than some previously studied electrodes (see Table 1). In addition, the fabrication process for this electrode is simple and fast.

Reproducibility and stability of the electrode. The relative standard deviations (RSD) obtained from five successive DPV measurements of 1.0 and 20 μ*M* TP were 1.8% and 1.7%, respectively. Reproducibility was also estimated using five different electrodes; the RSD for determination of 30 μ*M* TP was 2.3%, which indicated good reproduc-
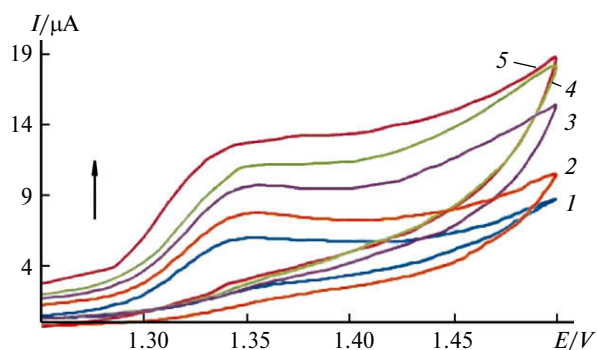
Fig. 4. Cyclic voltammograms of TP at scan rates of 20 (*1*), 40 (*2*), 60 (*3*), 80 (*4*), and 100 mV s^{-1} (*5*). Electrode potentials (*E*) were measured against Ag/AgCl/(saturated KCl) reference electrode.
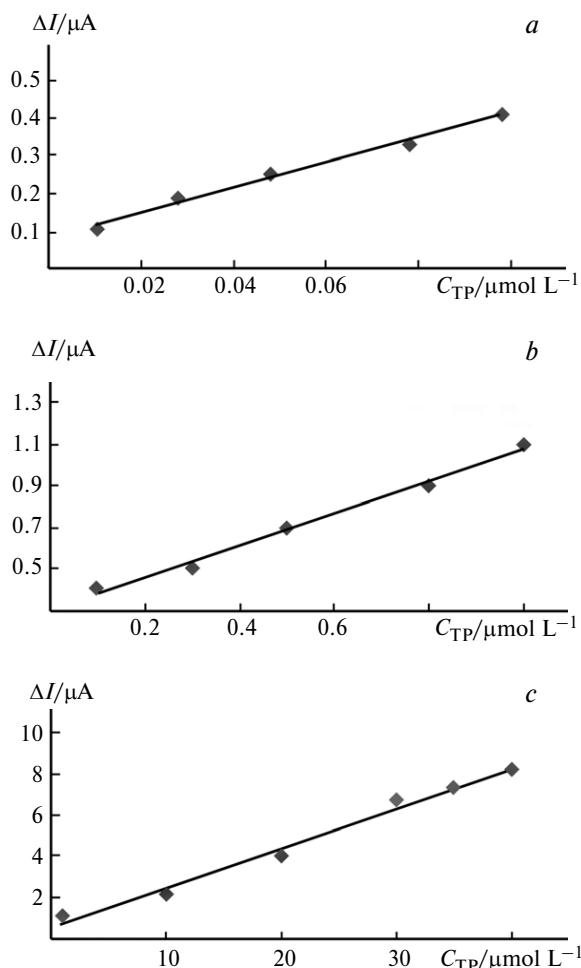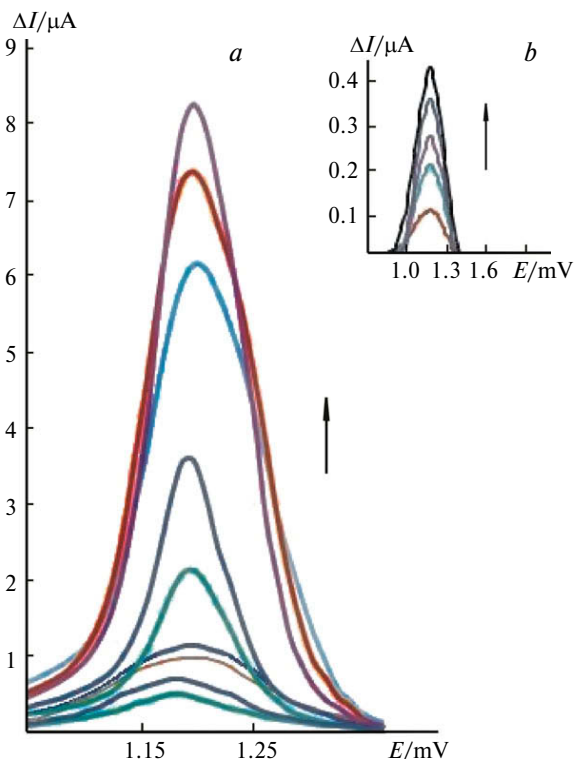
Fig. 5. Plots of ΔI *versus* the TP concentrations within the TP concentration ranges of 0.01—0.1 (*a*), 0.1—1.0 (*b*), and 1.0—40 µmol L^{-1} (*c*). The straight lines is described by the following equations: $y = 0.671x + 0.073$ ($R^2 = 0.992$) (*a*), $y = 0.154x + 0.304$ ($R^2 = 0.99$) (*b*), and $y = 0.038x + 0.0574$ ($R^2 = 0.99$) (*c*).

Fig. 6. Changes in stripping differential pulse voltammograms for the TP solutions with concentrations increasing from 0.01 to 0.1 µmol L^{-1} (*a*) and from 0.3 to 40 µmol L^{-1} (*b*) recorded in 15 mM H$_2$SO$_4$. The curves are net response currents (after subtracting the blank current).

ibility of the modified electrode. The stability of the electrode was also evaluated by measuring the electrode response to 30 µM TP every two days. The current response decreased to 88% of the initial value after seven days and to 79% after 15 days.

Interference studies. The effects of more than 20 species on the determination of 20 µM TP were studied under the optimized conditions described above (Table 2). The tolerance limit was defined as the maximum concentration of the foreign ion causing a relative error smaller than ±3.0% for the determination of TP. It was found that many species do not interfere with determination of TP.

Application of the electrode. To evaluate the applicability of the proposed method, the RGO—SDS—Nafion/GCE was applied successfully for the detection of TP in tablet samples (tablets with the TP content of 200 mg per 1 tablet were purchased from Dr Abidi and Darupaksh Pharmaceutical Co., both Iran). The tablets were ground

into powders, dissolved in water, filtered and then the filtrate was diluted so that the expected TP concentration will fall in the range of the calibration plot. This study was carried out by standard addition method. The data given in Table 3 shows the satisfactory results for assay of TP in drug samples. The statistical *t*-test indicated agreement between the spiked and obtained results.

In summary, we successfully fabricated a voltammetric sensor based on RGO—SDS—Nafion/GCE for deter-

Table 2. Tolerance limit of the foreign substances in determination of TP on the RGO—SDS—Nafion/GCE

Interferents	C/mg L^{-1}
Na$^+$, Ca^{2+}, Ba^{2+}, K$^+$, Al^{3+}, NH$_4^+$, Cl$^-$, NO$_3^-$, BrO$_3^-$, SCN$^-$, Mg^{2+}, Cd^{2+}, IO$_3^-$, Fe^{2+}, F$^-$, C$_2$O$_4^{2-}$, Zn^{2+}, Cu^{2+}	100*
Gabapentine acetaminophen ascorbic acid, phenobarbital, glucose	100*
Phenylefrine	50

* Maximum tested concentration.

Table 3. Determination of TP in the tablets fabricated by Dr. Abidi Co. (I) and Darupaksh Co. (II)

Sample	TP (mg)[a]		Recovery (%)
	Added	Found[b,c]	
I	200	195±7.2	97.5
II	200	209±6.5	104.5

[a] Per 1 tablet.

[b] Each sample was assayed in triplicate ($n = 3$).

[c] Mean ± standard deviation.

mination of TP. The proposed method is based on the preconcentration of the positively-charged TP molecules on the negatively-charged SDS—Nafion composite film. The modified electrode exhibited high activity and good selectivity toward the oxidation of TP. The sensitivity of the RGO—SDS—Nafion/GCE was much higher than that of some known electrochemical methods for determination of TP. The fabricated sensor was successfully applied to detect TP in the pharmaceutical samples.

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