Iranian Journal of Analytical Chemistry
Volume 3, Issue 1, March 2016 (27-37)

مجله ایرانی شیمی تجزیه
دوره ۳، شماره ۱، فروردین ماه ۱۳۹۵ (۳۷-۲۷)

مطالعه تجربی و تئوری استامینوفن با استفاده از الکترود خمیری کربن اصلاح‌شده با نانولوله‌های کربن چند دیواره

آرش محمدی نژاد[۱]، محمود ابراهیمی[۱*]، علی مرسلی[۱]، زرین اسحاقی[۲]، حامد چگینی[۱]، عبدالحسین ابراهیمی طلب[۱]
۱. گروه شیمی، دانشگاه آزاد اسلامی، واحد مشهد، ایران
۲. گروه شیمی، دانشگاه پیام نور، صندوق پستی ۳۶۹۷- ۱۹۳۹۵، تهران، ایران
تاریخ دریافت: ۱ مرداد ۱۳۹٤ تاریخ پذیرش: ۲۹ شهریور ۱۳۹٤

Experimental and Theoretical Study on Acetaminophen Using Multiwall Carbon Nanotubes Modified Carbon Paste Electrode

Arash Mohammadinejad[1], Mahmoud Ebrahimi[1,*], Ali Morsali[1], Zarrin Es'haghi[2],Hamed Chegini[1], Abdolhossein Ebrahimitalab[1]
1. Department of Chemistry, Mashhad Branch, Islamic Azad University, Mashhad, Iran
2. Department of Chemistry, Faculty of Sciences, Payame Noor University (PNU), P.O. Box 19395-3697, Tehran, Iran
Received: 23 July 2015 **Accepted:** 20 September 2015

چکیده
در این کار الکترود خمیری کربن اصلاح‌شده با نانولوله‌های کربن چند دیواره برای اندازه‌گیری به روش ولتاژسنجی داروی استامینوفن در نمونه‌های حقیقی بیولوژیکی و دارویی مورد استفاده قرارگرفت. رفتار الکتروشیمیایی استامینوفن با به‌کارگیری ولتاژسنجی چرخه‌ای مورد بررسی قرار گرفت. پتانسیل الکترود استاندارد نیم‌واکنش اکسایش/کاهش استامینوفن ۰/۸۹۸ ولت بدست آمد تحت شرایط بهینه جریان پیک اکسیداسیون با به‌کارگیری ولتاژسنجی پالسی تفاضلی برای استامینوفن در محدوده‌ی ۰/۱۲ تا ۹۹ میکرو مولار خطی بود و حد تشخیص ۰/۰۶ میکرومولار بدست آمد. محاسبات DFT-B3LYP/6-31G (d, p) و HF/6-31G (d, p) برای فرم‌های اکسیدشده و کاهیده‌شده استامینوفن انجام شد. پتانسیل الکترود استاندارد محاسبه شده نسبتا" با پتانسیل الکترود استاندارد تجربی مطابقت داشت. این الکترود برای اندازه‌گیری استامینوفن در نمونه‌های فاضلاب بیمارستان، مو، خون و قرص مورد استفاده قرار گرفت و حساسیت بالا، حد تشخیص مناسب، تکرارپذیری خوب و عدم وجود مزاحمت، آن را تبدیل به یک حسگر مناسب برای اندازگیری استامینوفن کرده است.

واژه‌های کلیدی
استامینوفن؛ ولتامتری پالس تفاضلی؛ ولتامتری چرخه‌ای؛ نانولوله‌های کربنی چنددیواره.

Abstract
In this work, a carbon paste electrode modified with multiwall carbon nanotubes (MWCNTPE) was used for the sensitive voltammetric determination of acetaminophen (AC) in biological and pharmaceutical samples. The electrochemical behavior of acetaminophen was investigated employing cyclic voltammetry. It was revealed that the standard electrode potential of half reaction for AC(O), H+/AC(R) was 0.898 V. under the optimized experimental conditions, the oxidation peak current for acetaminophen was found to vary linearly with concentration range of 0.12 to 99 µM with detection limit of 0.06 µM using differential pulse voltammetry. DFT-B3LYP/6-31G (d,p) and HF/6-31G (d,p) calculations were performed for deoxidized acetaminophen (AC(R)) and its oxidized form (AC(O)). The calculated standard electrode potentials are relatively in agreement with experimental data. This electrode was employed for determination of acetaminophen in hospital waste water, hair, blood and pharmaceutical samples considering its high sensitivity, low detection limit, good reproducibility and its non-existent interference at trace levels in clinical and quality control laboratories.

Keywords
Acetaminophen; Differential Pulse Voltammetry; Cyclic Voltammetry; Multiwall Carbon Nanotubes.

1. INTRODUCTION

Acetaminophen (N-acetyl-p-aminophenol or Paracetamol) is a long-established substance being one of the most extensively used drugs in the world. It is an antipyretic and analgesic drug commonly used against mild to moderate pain or

*Corresponding Author: m.ebrahimi@mshdiau.ac.ir

for reduction of fever. It is also non-carcinogenic and an effective substitute for aspirin for patients who are sensitive to aspirin, and also safe up to therapeutic doses. AC is metabolized predominantly in the liver, where it generates toxic metabolites. The large scale therapeutic use of this drug generates the need for the development of fast, simple and accurate methodologies for the detection of AC; for quality control analysis (in pharmaceutical formulations) and for medical control (in biological fluids such as urine, blood and plasma) [1]. Several methods have been reported for the determination of acetaminophen biological samples including HPLC-MS/MS [2], uv-vis spectrophotometry [3] and microanalytical technique [4]. Among these methods, electrochemical methods maybe the most widely applied because of the high sensitivity, simplicity and reproducibility of this approach [1].

Voltammetric methods enable the sensitive and selective measurement of organic compounds which are based on their specific electrochemical behavior at the working electrode surface [5-6]. Electrochemical methods are advantageous if the achieved selectivity eliminates the need for separation procedures, which simplifies procedures, enables short analysis times and lowers cost of analysis by using a relatively cheaper instrument and fewer consumables [7].

The carbon past electrode (CPE) was introduced by Adams in 1958 [8]. The ease and speed of preparation, the acquisition a new producible surface and the low cost of carbon paste are advantages of CPEs over other electrodes [9]. Carbon pastes undoubtedly represent one of the most convenient materials for the preparation of modified electrode [10]. Preparation of chemically modified carbon paste electrode (CMCPE) is very simple and fast [11].

Multiwall carbon nanotubes (MWCNTs) are, now, used extensively in the fabrication of novel nanostructure electrochemical sensors. MWCNT-modified electrodes have many advantages over other forms of carbon electrodes due to their small size, high electrical and thermal conductivity, high chemical stability, high mechanical strength and high specific surface area which make them very promising candidates in a wide range of applications [12]. In this work, we employed differential pulse voltammetry (DPV) for the quantitative determination of acetaminophen in hospital wastewater, biological samples and tablets. It was a simple, precise and sensitive voltammetric method utilizing carbon paste electrode modified with multiwall carbon nanotubes (MWCNTPE) vs. Ag/AgCl/KCl (3.0 M). Also, cyclic voltammetry (CV) was used for the study of the electrochemical properties of acetaminophen MWCNTPE and CPE.

Theoretical study on the electrochemical behavior of acetaminophen was performed according to the methods of Song [13]. As is known, standard electrode potential (E°) is a significant parameter which is widely used in analytical chemistry, organic chemistry, biochemistry and etc. Density functional theory (DFT) and ab initio method (HF) have predicted the standard electrode potential. We selected B3LYP and HF method applying 6-31G (d, p) basis sets to investigate the geometries of $AC_{(O)}$ and $AC_{(R)}$ and the standard electrode potential. The quantitative determination of AC and development of relationship between experimental and theoretical studies on electrochemical behavior of acetaminophen were the main purposes of this work and the obtained results revealed there was approximately a close relationship between experimental and theoretical studies.

2. EXPERIMENTAL

2.1. Regent and chemical

All chemicals were analytical-reagent grade and purchased from Merck (Darmstadt, Germany). Acetaminophen was purchased from Sigma-Aldrich (Helsinki, Finland). All aqueous solutions were prepared diluting in demineralized water and the experiments were carried out in room temperature. Acetaminophen stock solution (6.62×10^{-3} M) was prepared by dissolving 0.10 g of acetaminophen in 100 mL of demineralized water and was kept in laboratory refrigerator at 4°C in dark. A 6.62×10^{-4} M AC working solution was prepared daily by diluting 1.5 mL stock solution in 100 mL demineralized water. High viscosity paraffin ($d = 0.88$ kg L^{-1}) was used from Merck as a pasting liquid for the preparation of the carbon paste electrodes. Spectrally pure graphite powder (particle size 50 μm) was purchased from Merck and multiwall carbon nanotubes (purity > 95%, od< 8nm, length: 30 nm) were supplied from neutrino noavaranenano (made in Iran) as an effective modifier for the preparation of the electrodes.

2.2. Apparatus

All voltammetric measurements were performed using Metrohm 797 VA Computrace (Herisau, Switzerland) linked to a personal computer. The data were baseline corrected using GPES software. Three electrodes assembly cell consisted of Ag/AgCl in 3 M KCl (Metrohm) as a reference electrode, MWCNTPE as working electrode (4 mm diameter) and platinum wire (Metrohm) as an auxiliary electrode. The measurements of pH were carried out with a

Metrohm 780 pH meter (Metrohm Ltd., Switzerland) using a combined glass electrode. Also, we applied a centrifuge (Dynamicagmbh, model no: velocity 18r) for preparation of hospital wastewater and hemolyzed erythrocyte. The electrode modified with carbon nanotubes was characterized by scanning electron microscopy (SEM) Axford Co., S 360.

2.3. Preparation of the electrode

The MWCNTPE was prepared by hand-mixing 0.9 g graphite powder and 0.1 g plus paraffin at a ratio of 70/30 % (w/w) [1, 12, 14] and mixed well with a mortar and pestle for 30 min until a uniformly wetted paste was obtained. The obtained uniformly-wetted paste was packed into cavity of the home-made electrode and surface of electrode was polished with a smooth piece of paper.

2.4. Preparation of real samples

2.4.1. Preparation of hair matrix

For determination of AC in hair matrix, we prepared matrix according to Z. Es'haghi et al [15]. A hair bulk, which was necessary for method development and validation, was obtained from a barber shop in Mashhad, Iran. Hair samples were cut using round-point scissors from the vertex posterior region of the scalp about 5 mm in diameter. We selected samples which had a length of 2–4 cm, for the next stage. The hair was washed by following solvents on a hierarchy basis because fat and other surface contaminations on the hair needed to be removed. Washing was performed by 20 mL dichloromethane, 20 mL acetone and 15 mL methanol, respectively at room temperature, then they were dried, the washed and dried hair was cut into approximately 1 mm pieces, then digested by the following procedure: 2.0 mL methanol as an extracting solvent was added to 50 mg of hair in a 10 mL screw-cap tube lab and the pH was regulated to 7.4 by phosphate buffer solution. The samples were incubated at 55◦C for 5 h by a heater. Then remaining solid hair matrix was filtered and rinsed with 0.5 mL ethanol. The remaining was finally diluted with appropriate deionized water.

2.4.2. Preparation of hospital waste water

For separation of contaminant suspended particles, waste water provided by hospital was firstly centrifuged, then filtered through a filter paper before analysis. Also, a small amount of methanol was added for preventing the growth of micro-organisms.

2.4.3. Preparation of hemolyzed erythrocyte

Human whole blood was obtained from a clinical laboratory in Mashhad, Iran and erythrocytes were separated from whole blood by removing the plasma. Human whole blood (2.0 mL) was, firstly, centrifuged for 10 min at 3000 rpm. Next, the supernatant (plasma) was discarded and the rest was mixed with 5 mL of 0.9% NaCl solution. Then, the solution was centrifuged for another 10 min at 4000 rpm and the supernatant (diluted plasma) was again discarded. The washing procedure with NaCl solution was repeated three times in order to remove the plasma almost completely. The erythrocyte pellets were hemolyzed with water (1:1, v/v). As for protein precipitation, the hemolysate was mixed with 5-sulfosalicylic acid (10%, w/v) in the ratio of 2:1 (v/v). This mixture was centrifuged in the same condition described above [16]. Then, the supernatant was separated and diluted with small amounts of deionized water.

2.4.4. Preparation of tablets

The efficiency of MWCNTPE for determination of AC in pharmaceutical formulations was tested by measuring the AC dose in five commercial acetaminophen tablets. The tablets were, firstly, weighed and completely powdered, then dissolved in distilled water, filtered and diluted until the concentration of AC was located in the working range.

2.5. Procedure

2.5.1. Determination of acetaminophen

A certain volume of AC working solution was transferred into 15 mL glass cell containing 4.08 $\times 10^{-3}$ M $NaClO_4$ plus 0.06 M HCl as the supporting electrolyte, then differential pulse voltammetry was performed after 300 s purging by simultaneously stirring solution (200 rpm), and then 20 s equilibration time in the potential range of 500−1000 mV and the oxidation peak current at 659 mV was recorded. The cyclic voltammogramms were obtained by scanning the potential from 500 to 1000 mV at a scan rate 20 mV s^{-1}. All the measurements were carried out at room temperature. The differential pulse voltammetry (DPV) parameters were pulse amplitude = 110 mV, scan rate = 20 mVs^{-1}, pulse time =10 ms. For removing memory effect, before each measurement, consecutive scans from blank solution were recorded and background current was significantly reduced.

2.5.2. Calculation methods

All of the present calculations were performed with the B3LYP hybrid density functional level

using the Gaussian 03 package. The 6-31g (d,p) basis sets were employed. The solvent has an important role in chemical reactions. One group of approaches to study the solute-solvent interactions is referred to as self-consistent reaction field (SCRF) methods (Implicit solvent effects). In this work, polarized continuum model (PCM) was used. In the PCM method, the molecular cavity is comprised of the union of interlocking atomic spheres.

Firstly, all degrees of freedom for AC(O) and AC(R) were optimized. The geometries obtained were confirmed to have no imaginary frequency of the Hessian. The energies of solvation is calculated at geometries of AC(O) and AC(R) optimized at DFT- B3LYP/6-31G (d,p) and HF/6-31G (d,p) level, respectively. The energies of solvation of AC(O) and AC(R) at 298.15K and 1 atm were calculated by the Polarized Continuum model (PCM).

3. RESULTS AND DISCUSSION

3.1. SEM Characterization

As can be seen at a surface of electrode, the layer of irregularly flakes of graphite powder was present and MWCNTs were distributed with special three-dimensional structure (Fig.1), indicating that the MWCNTs were successfully modified on the MWCNTPE.

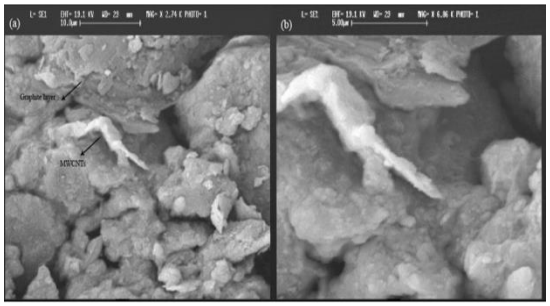

Fig. 1. (a) SEM image of MWCNTPE (b) Magnifying of (a).

3.2. Optimization of effective parameter

3.2.1. Selection of a supporting electrolyte

In electrochemical studies, selection of a supporting electrolyte (or medium) is very significant since the electrochemical responses of AC are very variable in different supporting electrolytes. In this work, the peak currents of AC in a variety of determining supporting electrolytes, such as pH 2–7 HAc-NaAc buffer, pH 4.0–8.0 phosphate buffer (each 0.1 M), pH 2–9 Britton-Robinson (BR) buffer, $NaClO_4$ (4.08×10^{-3} M) plus HCl or NaOH (each 0–0.5 M), Hg (OAc)2 (1.56×10^{-3}) plus HCl or NaOH (each 0–0.5 M), KCl (6.7×10^{-3} M) plus NaOH or HCl (each 0–0.5 M) were investigated. The results determined that the oxidation peak current improved in $NaClO_4$ + HCl .On the other hand, background current was low and the peak current was well-shaped. Thus, $NaClO_4$ + HCl was used as supporting electrolyte for analysis. Fig.2a shows oxidation peak current of AC improved under acidic condition ($NaClO_4$ + HCl) in comparison to basic condition ($NaClO_4$ + NaOH).

3.2.2. Effect of the HCl concentration

In this work $NaClO_4$ pluseHCl were used as supporting electrolyte. The concentration of $NaClO_4$ was fixed at 4.08×10^{-3} M and effect of different concentrations of HCl from 0 to 0.5 M on oxidation peak current was investigated by DPV. Also, electrochemical behaviors of acetaminophen in different concentrations of HCl for cyclic voltammetry studies were considered. The relation between the oxidation peak current of acetaminophen and the concentration of HCl is shown in Fig. 2b. As concentration of HCl increases gradually from 0 to 0.06 M, the oxidation peak current of AC improves gradually as well; when further increasing the HCl concentration to 0.2 M, the oxidation peak current changes slightly. However, when the concentration of HCl is higher than 0.2 M, acetaminophen molecules decompose and the oxidation peak current of acetaminophen conversely decreases. As a result, the concentration of HCl was selected as 0.06 M in this work. The electrochemical behavior of acetaminophen was also favorable in this concentration of HCl for cyclic voltammetry studies.

3.2.3. Effect of the electrode rotation rate

A home-made working electrode body was designed in which a steel cylindrical piece was used instead of wire to connect with paste through its bottom. The electrode was screwed to voltammetry system (in cell). The home-made working electrode body is shown in Fig. 3. The oxidation peak currents of 6.62×10^{-5} M AC in the presence of 4.08×10^{-3} M $NaClO_4$ plus 0.06 M HCl under different rotation rates from 0 to 1000 rpm (with simultaneously purging solution by N_2) were measured by DPV. The transfer of analyte toward modified electrode increased From 0 to 200 rpm; therefore, oxidation peak current of AC was increased too. When further increasing the rotation rate to 1000 rpm the oxidation peak current decreased. This is possibly due to the collisions of molecules and turbulence of surrounding environment of working electrode in solution and prevention of transfer of analyte toward modified electrode. It is necessary to mention that the differential pulse

voltammograms were recorded 20 s after equilibration of solution. Electrode rotation rate was chosen as 200 rpm in this work. Results are shown in Fig. 2c.

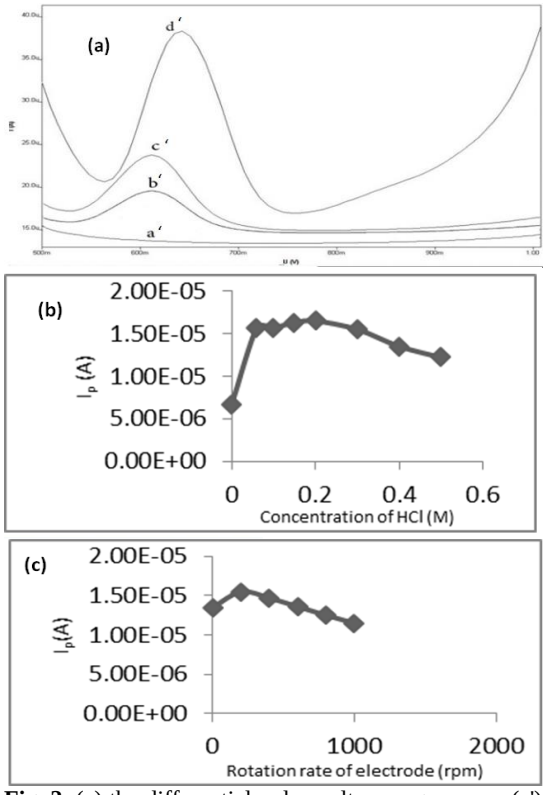

Fig. 2. (a) the differential pulse voltammogramms: (a') Background current in 4.08 $\times 10^{-3}$ M $NaClO_4$ plus 0.06 MHCl. Oxidation peak current of 6.62×10^{-5} M AC in (b') 4.08 $\times 10^{-3}$ M $NaClO_4$ plus 0.06 M NaOH; c') In 4.08 $\times 10^{-3}$ M $NaClO_4$; (d') in 4.08 $\times 10^{-3}$ M $NaClO_4$ plus 0.06 MHCl. (b) Effect of the HCl concentration on the oxidation peak current. (c) Effect of electrode rotation rate on the oxidation peak current. The conditions of determination: pulse time: 10 ms, pulse amplitude: 40 mV, scan rate: 20 mVs^{-1}

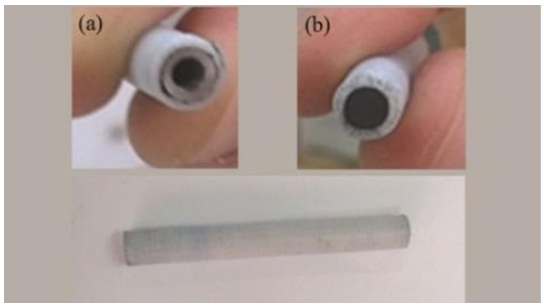

Fig. 3. Various views of the home-made working electrode. (a) Steel cylindrical piece. (b) Surface of electrode.

3.2.4 Effect of the pulse amplitude

The effect of the pulse amplitude on the oxidation peak current was examined over the 10 to 130 mV. According to theoretical equation[17]:

$$i_p = \frac{\left\{n^2F^2AC_a\Delta E_{pl}\,D^{\frac{1}{2}}\right\}}{4RT\left(\pi t_{pl}\right)^{\frac{1}{2}}}$$

where ΔEplis the pulse amplitude and tpl is pulse time, n is the number of electrons, F is the faraday constant, A is the electrode surface area, Cais the concentration and D is the diffusion coefficient. There is a direct link between peak current and pulse amplitude. The obtained experimental results were in agreement with this theoretical equation. From 10 to 110 mV, as pulse amplitude increases, the peak current increases proportionately. When further increasing pulse amplitude to 130 mV, the oxidation peak current increases slightly, indicating that the limiting value of the amount of acetaminophen at MWCNTPE surface was achieved. Fig. 4, inset a shows a better exhibition of peak current at 110 mV. Fig. 4, inset b indicates, the anodic peak potential (Epa) shifts towards less positive potentials by increasing pulse amplitude from 10 to 130 mV.

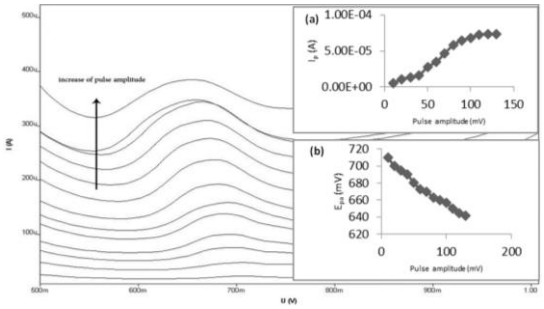

Fig. 4. Differential pulse voltammogramms of 6.62×10^{-5} MAC obtained at the MWCNTPE at different values of pulse amplitude (10–130 mV). Inset: variation of (a) peak current vs. pulse amplitude; (b) peak potential vs. pulse amplitude.

3.2.5 Effect of the pulse time

The influence of pulse time on the oxidation peak current of AC with various values from 10 to 90 ms was studied by DPV. According to the mentioned theoretical equation, there was a vice-versa relation between peak current and of tpl. It can be seen that, as pulse time increases gradually from 10 to 90 ms, the oxidation peak current decreases gradually (Fig. 5). The maximum oxidation peak current appeared at 10 ms.

3.3. Cyclic voltammetry studies

The electrochemical responses of MWCNTPE and CPE were determined by cyclic voltammetry (CV) in the blank solution. No redox peak appears for CPE and the MWCNTPE (Fig. 6). The nano-grade size and larger surface area of the nanoparticles caused the background current of the MWCNTPE in comparison to CPE increased

slowly (Fig. 6, curve a,b). The AC signal in the MWCNTPEincreasescomparatively to CPE about twice. Behavior of acetaminophen at the CPE and MWCNTPE was studied (Fig. 6, curve a', b'). As can be seen, AC exposes only a small anodic peak at 654 mV at the CPE but a pair of redox peak appears at 612 mV (Epc) and 652 mV (Epa) at the MWCNTPE. This means the electrochemical behavior of AC was improved using this modified electrode. All cyclic voltammogramms were carried out at a temperature of 25℃.

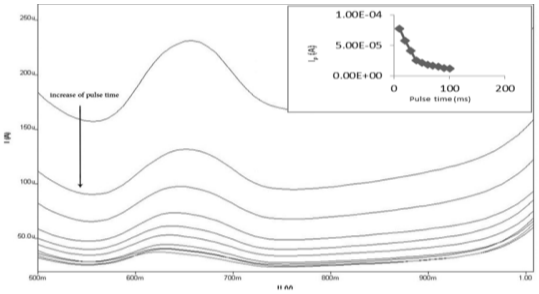

Fig. 5. Differential pulse voltammogramms of 6.62×10^{-5} M AC obtained at the MWCNTPE at different values of pulse time (10–90 ms).Inset shows the variation of peak current vs. pulse time.

3.3. Cyclic voltammetry studies

The electrochemical responses of MWCNTPE and CPE were determined by cyclic voltammetry (CV) in the blank solution. No redox peak appears for CPE and the MWCNTPE (Fig. 6). The nano-grade size and larger surface area of the nanoparticles caused the background current of the MWCNTPE in comparison to CPE increased slowly (Fig. 6, curve a,b). The AC signal in the MWCNTPEincreasescomparatively to CPE about twice. Behavior of acetaminophen at the CPE and MWCNTPE was studied (Fig. 6, curve a', b'). As can be seen, AC exposes only a small anodic peak at 654 mV at the CPE but a pair of redox peak appears at 612 mV (Epc) and 652 mV (Epa) at the MWCNTPE. This means the electrochemical behavior of AC was improved using this modified electrode. All cyclic voltammogramms were carried out at a temperature of 25℃.

The standard electrode potential (E◦) of half reaction for a reversible redox couple (Fig. 7) with cyclic voltammetry is calculated as [13]:

$$E^{\circ} = \frac{E_{pa} + E_{pc}}{2}$$

where Epaindicates the potential of oxidized peak and Epcindicates the potential of deoxidized peak. The conditionalpotential of AC(O), H+/AC(R) obtained from the average value of anodic cathodic peak potentials (Epa + Epc)/2 was 0. 632V versus Ag/AgCl for the MWCNTPE. AC undergoes two-electron oxidation at the modified electrode.

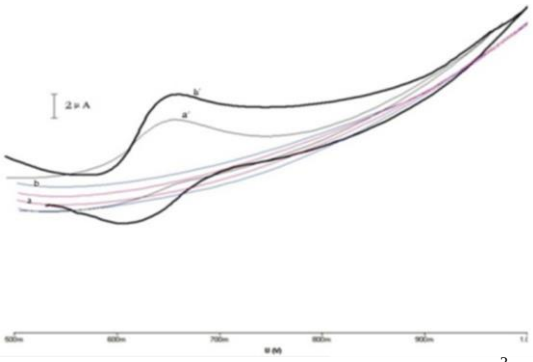

Fig. 6. The cyclic voltammogramms in 4.08×10^{-3} M $NaClO_4$ 0.1 MHCl. The absence of acetaminophen at the (a) CPE (b) MWCNTPE .presence of 6.62×10^{-5} M acetaminophen at the (a') CPE (b') MWCNPE.

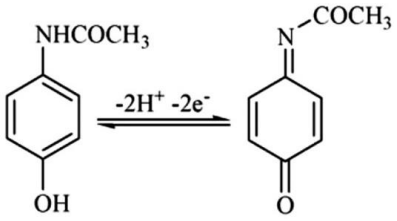

Fig. 7. Reaction scheme for the electrochemical oxidation of acetaminophen.

Thus, standard electrode potential E°1versus Ag/AgCl is calculated as:

$$E = E^{\circ}_{1} + 0.0591_{g}\,[H^{+}]\ (25^{\circ}C)$$

Then $[H^+]$ = 0.06 M, E°_{1}=0.704 V (1 atm, 25°C) at MWCNTPE. The standard electrode potential E°_{2} versus a normal hydrogen electrode (NHE) is given as [13]:

$E^{\circ}_{2} = E^{\circ}_{1} + E_{Ag/AgCl}$(3M KCl),

$E_{Ag/AgCl\ (3M\ KCl)} = E^{\circ}_{AgCl/Ag} - 0.0591_{g}\,[Cl^{-}]$

$=0.222V - 0.0591_{g}\,[3] = 0.194$ V (1 atm, 25°C)

where $E^{\circ}_{AgCl/Ag}$ indicates standard electrode potential for Ag/AgCl, therefore, E°_{2} is obtained as:

E°_{2} = 0.704V + 0.194V= 0.898 V (1 atm, 25°C) at MWCNTPE.

3.4. Theoretical studies

3.4.1. General description of method

The electrochemical behavior of the reaction at electrode is shown as: $O_1 + R_2 = O_2 + R_1$, which can be broken up two half reactions as:

$O_1 + ne^- + mH^+ = R_1$ (1)

$O_2 + ne^- = R_2$(2)

The reaction (1) indicates a reaction of redox couple and reaction (2) indicates a reaction of a reference electrode. The reference electrode potential is a constant at 298.15 K and 1 atm. Hence, we only compute the standard electrode potential of reaction (1), which represents the electrochemical property.

The theoretical calculation of standard electrode potential for acetaminophen versus a normal

hydrogen electrode (NHE) can be shown as:

$$AC_{(R),g} - 2e^- - 2H^+g = AC_{(O),g} \quad (3)$$

$$2H^+_g + 2e^- = H_{2,g} \quad (4)$$

where $AC_{(O),g}$, $AC_{(R),g}$, H^+_g, $H_{2,g}$ indicate oxidized AC, deoxidized AC, hydrogen ions, and hydrogen in gas state, respectively. Then

$$AC_{(O),g} + H_{2,g} = AC_{(R),g} \quad (5)$$

The transformed Gibbs energy of (5) is written as:

ΔrG°_1 (298.15K, 1 atm) $= G^\circ_{AC(R),g} - G^\circ_{AC(O),g} - G^\circ_{H2,g}$ $= (\varepsilon_0 + G^\circ_{corr})_{AC(R),g} - (\varepsilon_0 + G^\circ_{corr})AC_{(O),g} - (\varepsilon_0 + G^\circ_{corr})H_{2,g}$

where $(\varepsilon_0 + G^\circ_{corr})$ indicates corrected Gibbs release energies (G°) at 298.15 K and 1 atm calculated from Gaussian 03 package.

The redox reaction in solution is shown as (Fig. 8).Where ΔrG°_2 and ΔrG°_3 are energies of solvation of $AC_{(O)}$ and $AC_{(R)}$ in water at 298.15 K and 1 atm, respectively.

Thus, the standard transformed Gibbs energy of reaction in solution is calculated as:

ΔrG° (298.15K, 1 atm) $= \Delta rG^\circ_1 - \Delta rG^\circ_2 + \Delta rG^\circ_3$.

The standard electrode potential (E°) of half reaction for $AC_{(O)}$, $H^+/AC_{(R)}$ is computed as:

ΔrG° (298.15K, 1 atm) $= -nFE^\circ$,

Where F is Faraday constant = 96.485Cmol^{-1} and n is number of electrons transferred.

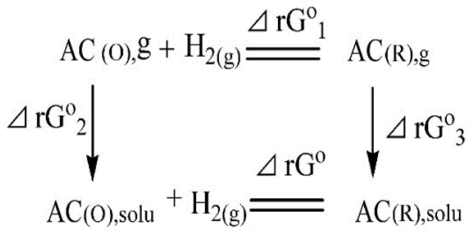

Fig. 8. Design of redox reaction of $AC_{(O)}$ and $AC_{(R)}$ in solution, g and solu denote gas and solution, respectively

3.4.2. Theoretical Calculation of the standard electrode potential (E◦)

The geometries of atoms in both AC(R) and AC(O) are shown in Fig. 9. Calculated thermochemistry values for reaction (5) are represented in Table 1. The Gibbs energies of reaction (5) and standard electrode potential calculated at B3LYP/6-31G (d,p) level represented in Table 1 consistent with those calculated at HF/6-31G (d,p) level. The calculated standard electrode potentials of 0.74 V at B3LYP/6-31G (d,p)-PCM level and 0.78V at HF/6-31G (d,p)-PCM level are relatively in agreement with our experimental values of 0.898 V.

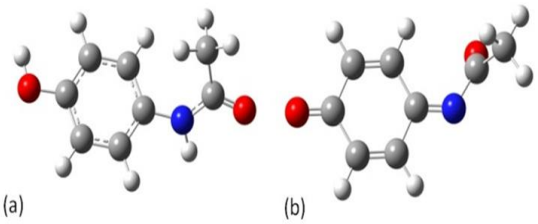

Fig. 9. Optimized geometries at B3LYP/6-31G (d,p) level: a) $AC_{(R)}$ b) $AC_{(o)}$

3.5. Stability and reproducibility

The repeatability and stability of MWCNTPE were investigated by cyclic voltammetric measurements of 6.62×10^{-5} M. Five MWCNTPE fabricated independently, were used and the relative standard deviation was 3.7%, showing an excellent repeatability of the electrode preparation procedure. Under similar conditions RSD for five successive assays was 2.5%. During 12 h, 12 cycles were performed at an interval of 1 h. No significant decrease was discovered in electroactivity. The modified electrode was stored in the atmosphere and the electrochemical response of the AC on the MWCNTPE decreased less than 13.5 % in one week after use.

3.6. Figures of merit

The relation between the oxidation peak current and the concentration ofacetaminophen was evaluated using DPV after 300 s purging with stirring solution and 20 s equilibration (Fig. 10). The concentration of AC was proportional to the oxidation peak current in the concentration range of AC from 0.12 µM to 99 µM. The regression equation in this range was obtained ipa=1.3736 c−6×10^{-6} (R^2 = 0.9995, c in M and ipain A). The excellent detection limit ($3SD_{blank}$/slope) was estimated 0.06 µM .The linear range and LOD in this work were compared to reported values in other studies and results are shown in Table 2.

Table 1. Calculated thermochemistry values.

Compound method	$AC_{(R)}$		$AC_{(O)}$		H_2	
	DFT	HF	DFT	HF	DFT	HF
$\varepsilon_0 + \Delta G^0_{corr}(Hartree)$	-515.372183	-512.260563	-514.140236	-511.073594	-1.179854	-1.132235
ΔG^0_1 (KJ mol^{-1}) PCM	-136.63	-143.56				
ΔG^0_2 (KJ mol^{-1}) PCM			-28.41		-35.27	
ΔG^0_3 (KJ mol^{-1}) PCM	-33.84	-42.04				
ΔG^0 (KJ mol^{-1}) PCM	-142.06	-150.3				
E^0 (V) PCM	0.74	0.78				

Table 2. Comparison of electroanalytical data for acetaminophen determination obtained using the present method to that reported in the literature

Electrode	Modifier	Detection limit (μM)	Linear Range (μM)	Ref.
CPE	Thionineimmobilized on multi-walled carbon nanotube	0.05	0.1– 100.0	[17]
CPE	In situ surfactant-modified multi-walled carbon nanotube	0.0258	0.291–62.7	[18]
CPE	Multi-wall carbon nanotubes	0.043	0.15 –126	[19]
CPE	Gold nanoparticle	0.33	0.66–530	[19]
CPE	Gold nanoparticle	0.026	0.05–100.0	[20]
CPE [a]	Poly(Patton and Reeder's reagent)	0.53	0.7–100.0	[21]
CPE	Carbon nanotubes and Poly(3-Aminophenol)	1.1	10 –100.0	[22]
CPE	Ethynylferrocene–NiO/MWCNT nanocomposite	0.5	0.8–600.0	[16]
CPE	N-(3,4-dihydroxyphenethyl)-3,5-dinitrobenzamide	10.0	15.0–270	[14]
CPE [b]	Multi-wallcarbon nanotube	0.8	2.0–400.0	[12]
GCE	F-MWCNT	0.6	3.0–300.0	[23]
GCE	Nafion/ROPCME	1.2	5.0–250.0	[24]
GCE	C_{60}	50	50.0–1500	[25]
GCE	SWCNTs–CHIT–RTIL	0.11	2.0 –200	[26]
GCE	Carbon-coated nickel magnetic nanoparticles	2.3	7.8–110.0	[27]
CPE	Multi-wall carbon nanotube	0.06	0.12–99.0	This work

[a]Electropolymerization of Patton and Reeder's reagent at modified carbon paste electrode;
[b] Modified electrode in presence of a mediator.

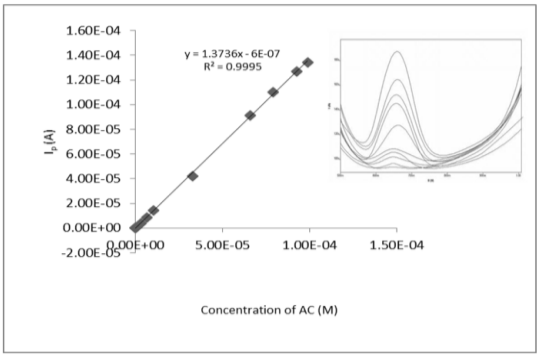

Fig. 10. The calibration curves for various concentrations of AC from 1.2×10^{-7}– 9.9×10^{-5} M Inset shows DPV curves of AC at the MWCNTPE.

Table 2 Comparison of electroanalytical data for acetaminophen determination obtained using the present method to that reported in the literature

3.7. Real samples

In order to illustrate the ability of the MWCNTPE to determine AC in real samples, this compound was determined in hospital waste water, hemolyzed erythrocyte, hair matrix and tablet using the DPV method.The results are shown in Table 3 and Table 4. The value of AC that was experimentally determined in various commercial tablets (made in Iran) compared to the reported amounts (Table 4). Clearly, MWCNTPE was capable of voltammetric determination of acetaminophen with remarkable selectivity and good reproducibility.

Table 3. Measurement of AC in real samples (n=3).

Sample	AC added (μM)	Found (μM)	Recovery (%)	RSD (%)
Hospital	3.32	3.29	99.09	1.8
waste	6.62	6.58	99.39	1.3
water	10.6	10.2	96.23	1.1
Hair matrix	3.32	3.26	98.19	1.4
	6.62	6.55	98.94	1.6
	10.6	10.7	100.94	1.9
Hemolyzed	3.32	3.19	96.08	2.1
erythrocyte	6.62	6.44	97.28	1.5
	10.6	10.4	98.11	1.7

Table 4. Results of commercial tablets analysis using DPV at the MWCNTPE.

Tablet name/labeled (mg)	Expected (μM)	Found [a] (μM)	Found in tablet (mg)	Error [b] (%)
Arya/325	66.2	65.9 ±0.72 [c]	324	-0.45
Hakim/325	66.2	65.3 ±0.77	321	- 1.36
Alborz/325	66.2	65.7 ±0.70	322	- 0.75
Tehran darou/325	66.2	66.5 ±0.74	326	+ 0.45
SOHA/325	66.2	66.8 ±0.71	327	+ 0.90

[a] Each values is the mean of 3 replicate measurements;
[b]Error percent is obtained from comparison of found and expected concentration;
[c]Standard deviations are reported.

3.8. Interference studies

Under optimized conditions, selectivity of the MWCNTPE in the presence of different organic compounds and metal ions were investigated. Anodic peak current (ipa) of 66.2 µM AC at the MWCNTPE was examined in the presence of 3 concentration levels (10, 100 and 1000fold) of interfering substances such as aspirin, ciprofloxacin, metronidazole, ascorbic acid, citric acid, Mg^{2+}, Ca^{2+}, K^{+} and Na^{+} using DPV. Percentage change in oxidation peak current for AC was evaluated and results are shown in Table 5. According to the results obtained, citric acid and metal ions have no effect on ipa of AC. In other cases, there was no substantial change in the current response until a 100-fold excess, except for ciprofloxacin which suppresses current response in 3 concentration levels.

Table 5. Effect of interferents on anodic peak current for 66.2 µM AC at MWCNTPE.

Interferents	Concentration levels (Interferent : Acetaminophen)	Percent change in anodic peak current for 66.2 µM Acetaminophen (%)
Aspirin	10:1	+ 5.7
	100:1	+7.4
	1000:1	+20.6
Ciprofloxacin	10:1	- 10.1
	100:1	- 22.4
	1000:1	-40.0
Metronidazole	10:1	+4.4
	100:1	+7.2
	1000:1	+27.1
Ascorbic acid	10:1	- 5.3
	100:1	-8.0
	1000:1	- 17.2
Citric acid	10:1	+1.0
	100:1	+ 3.1
	1000:1	+ 5.5
Mg^{2+}	10:1	+1.1
	100:1	+1.7
	1000:1	+ 3.1
Ca^{2+}	10:1	-1.3
	100:1	-2.4
	1000:1	-3.5
K^{+}	10:1	+ 1.7
	100:1	+ 1.9
	1000:1	+ 2.4
Na^{+}	10:1	+2.1
	100:1	+2.8
	1000:1	+ 3.2

4. CONCLUSIONS

This work indicates the successfull application of modified carbon paste electrode with MWCNTs in sensitive and selective determination of acetaminophen in biological and pharmaceutical samples. By applying DPV, a linear dynamic range for acetaminophen determination was obtained between 0.12 – 99 µM with detection limit of 0.06 µM. The electrochemical behavior of AC was investigated using cyclic voltammetry (CV). DFT and HF calculations were made for deoxidized acetaminophen (AC(R)) as well as the oxidized one (AC(O)). The calculated standard electrode potentials are relatively in agreement with experimental data. This method can be employed for determination of AC in hospital waste water, hair, blood and pharmaceutical sampleswith its high sensitivity, low detection limit, good reproducibility and its non-existent interference at trace levels in clinical and quality control laboratories.

Acknowledgments

The authors wish to thank, Mashhad Branch, Islamic Azad University for their support.

REFERENCES

[1] T. Tavana, M.A. Khalilzadeh, H. Karimi-Maleh, A.A. Ensafi, H. Beitollahi and D. Zareyee, Sensitive voltammetric determination of epinephrine in the presence of acetaminophen at a novel ionic liquid modified carbon nanotubes paste electrode, *J. Mol. Liq.* 168 (2012) 69-74.

[2] M. Barfield, N. Spooner, R. Lad, S. Parry and S. Fowles, Application of dried blood spots combined with HPLC-MS/MS for the quantification of acetaminophen in toxicokinetic studies, *J. Chromatogr. B* 870 (2008) 32-37.

[3] A. Afkhami, N. Sarlak and A.R. Zarei, Spectrophotometric Determination of Salicylamide and Paracetamol in Biological Samples and Pharmaceutical Formulations by a Differential Kinetic Method, *Acta Chim. Slov.* 53 (2006) 995-1003.

[4] E.J. Oliveira, D.G. Watson and N.S. Morton, A simple microanalytical technique for the determination of paracetamol and its main metabolites in blood spots, *J. Pharm. Biomed. Anal.* 29 (2002) 803-809.

[5] L.M. de Carvalho, M. Martini, A.P.L. Moreira, A.P.S. de Lima, D. Correia, T. Falcão, S.C. Garcia, A.V. deBairros, P.C. do Nascimento and D. Bohrer, Presence of synthetic pharmaceuticals as adulterants in slimming phytotherapeutic formulations and their analytical determination, *Forensic Sci. Int.* 204 (2011) 6-12.

[6] L.M.D. Carvalho, P.C.D. Nascimento, D. Bohrer, D. Correia, A.V.D. Bairros, V.J.

Pomblum and S.G. Pomblum, Voltammetric behavior of amfepramone (diethylpropion) at the hanging mercury drop electrode and its analytical determination in pharmaceutical formulations, *J. Braz. Chem. Soc.* 18 (2007) 789-796.

[7] K. Noh, K. Bae, B. Min, E. Kim, K.I. Kwon, T. Jeong and W. Kang, Enantioselective pharmacokinetics of sibutramine in rat, *Arch. Pharmacal. Res.* 33 (2010) 267-273.

[8] A. Safavi, N. Maleki, O. Moradlou and F. Tajabadi, Simultaneous determination of dopamine, ascorbic acid, and uric acid using carbon ionic liquid electrode, *Anal. Biochem*. 359 (2006) 224-229.

[9] H.R. Zare, N. Nasirizadeh, M. Mazloum-Ardakani and M. Namazian, Electrochemical properties and electrocatalytic activity of hematoxylin modified carbonpaste electrode toward the oxidation of reduced nicotinamide adenine dinucleotide (NADH), *Sens. Actuators B Chem.* 120 (2006) 288-294.

[10] H.R. Zare, N. Nasirizadeh, S.M. Golabi, M. Namazian, M. Mazloum-Ardakani and D. Nematollahi, Electrochemical evaluation of coumestan modified carbon paste electrode: Study on its application as a NADH biosensor in presence of uric acid, *Sens. Actuators B Chem.* 114 (2006) 610-617.

[11] M.M. Ardakani, Z. Akrami, H. Kazemian and H.R. Zare, Electrocatalytic characteristics ofuric acid oxidation at graphite–zeolite-modified electrode doped with iron (III), *J. Electroanal. Chem.* 586 (2006) 31-38.

[12] M. Keyvanfard, R. Shakeri, H. Karimi-Maleh and K. Alizad, Highly selective and sensitive voltammetric sensor based on modified multiwall carbon nanotube paste electrode for simultaneous determination of ascorbic acid, acetaminophen and tryptophan, *Mater. Sci. Eng. C* 33 (2013) 811-816.

[13] Y. Song, Theoretical study on the electrochemical behavior of norepinephrine at Nafion multi-walled carbon nanotubes modified pyrolytic graphite electrode, Spectrochim. *Acta. A Mol. Biomol. Spectrosc.* 67 (2007) 1169-1177.

[14] A.A. Ensafi, H. Karimi-Maleh, S. Mallakpour and M. Hatami, Simultaneous determination of N-acetylcysteine and acetaminophen byvoltammetric method using N-(3,4-dihydroxyphenethyl)-3,5-dinitrobenz-amide modified multiwall carbon nanotubes paste electrode, *Sens. Actuators B Chem.* 155 (2011) 464-472.

[15] Z. Es'haghi, M. Ebrahimi and M.S. Hosseini, Optimization of a novel method for determination of benzene, toluene, ethylbenzene, and xylenes in hair and waste water samples by carbon nanotubes reinforced sol–gel based hollow fiber solid phase microextraction and gas chromatography using factorial experimental design, *J. Chromatogr. A* 1218 (2011) 3400-3406.

[16] M.R. Shahmiri, A. Bahari, H. Karimi-Maleh, R. Hosseinzadeh and N. Mirnia, Ethynyl-ferrocene–NiO/MWCNT nanocomposite modified carbon paste electrode as a novel voltammetric sensor for simultaneous determination of glutathione and acetaminophen, *Sens. Actuators B Chem*. 177 (2013) 70-77.

[17] S. Shahrokhian and E. Asadian, Simultaneous voltammetric determination of ascorbic acid, acetaminophen and isoniazid using thionine immobilized multi-walled carbon nanotube modified carbon paste electrode, *Electrochim. Acta* 55 (2010) 666-672.

[18] B.J. Sanghavi and A.K. Srivastava, Simultaneous voltammetric determination of acetaminophen, aspirin and caffeine using an in situ surfactant-modified multiwalled carbon nanotube paste electrode, *Electrochim. Acta* 55 (2010) 8638-8648.

[19] Z. Xu, Q. Yue, Z. Zhuang and D. Xiao, Flow injection amperometric determination of acetaminophen at a gold nanoparticle modified carbon paste electrode, *Microchim. Acta* 164 (2009) 387-393.

[20] S.M. Ghoreishi, M. Behpour, S. Sadeghzadeh and M. Golestaneh, Electrochemical determination of acetaminophen in different pharmaceutical forms with gold nanoparticles carbon paste electrode, *Acta Chim. Slov.* 58 (2011) 69-74.

[21] T. Thomas, R.J. Mascarenhas, F. Cotta, K.S. Guha, B.E.K. Swamy, P. Martis and Z. Mekhalif, Poly(Patton and Reeder's reagent) modified carbon paste electrode for the

sensitive detection of acetaminophen in biological fluid and pharmaceutical formulations, *Colloids Surf. B* 101 (2013) 91-96.

[22] I. Noviandri and R. Rakhmana, Carbon paste electrode modified with carbon nanotubes and poly (3-aminophenol) for voltammetric determination of paracetamol, *Int. J. Electrochem. Sci* 7 (2012) 4479-4487.

[23] Z.A. Alothman, N. Bukhari, S.M. Wabaidur and S. Haider, Simultaneous electrochemical determination of dopamine and acetaminophen using multiwall carbon nanotubes modified glassy carbon electrode, *Sens. Actuators B Chem*. 146 (2010) 314-320.

[24] J.M. Zen and Y.S. Ting, Simultaneous determination of caffeine and acetaminophen in drug formulations by square-wave voltammetry using a chemically modified electrode, *Anal. Chim. Acta* 342 (1997) 175-180.

[25] R.N. Goyal and S.P. Singh, Voltammetric determination of paracetamol at C60-modified glassy carbon electrode, *Electrochim. Acta* 51 (2006) 3008-3012.

[26] M. Afrasiabi, S. Kianipour, A. Babaei, A.A. Nasimi and M. Shabanian, A new sensor based on glassy carbon electrode modified with nanocomposite for simultaneous determination of acetaminophen, ascorbic acid and uric acid, *J. Saudi Chem. Soc*. (2013) 545-552.

[27] S.F. Wang, F. Xie, R.-F. Hu, Carbon-coated nickel magnetic nanoparticles modified electrodes as a sensor for determination of acetaminophen, *Sens. Actuators B Chem*. 123 (2007) 495-500.