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Full Length Research Paper

Sensitive determination of paracetamol using a graphene-modified carbon- paste electrode

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The electrochemical behavior of paracetamol was investigated at a graphene - modified carbon paste electrode by cyclic voltammetry in an ammonium buffer solution (pH 8.5). The modified electrode showed excellent electrocatalytic activity towards the oxidation and reduction of paracetamol resulting in a remarkable lowering of the peak potentials and considerable improvement of the peak currents as compared to the bare electrode. Despite the irreversible behavior at the surface of a carbon paste electrode, a quasi-reversible redox process at the graphene modified electrode was observed for paracetamol with a peak separation of 66 mV at a scan rate of 50 mV s^{-1}. The advantages are related to the unique properties of graphene such as large surface area, and increased electron transfer abilities compared to graphite. Square wave voltammetry was applied to the quantitative determination of paracetamol using the graphene modified carbon paste electrode after an accumulation time of 2 min. The calibration graph was linear in the concentration range of paracetamol of 2.5 to 143 µM with a sensitivity of 0.282 µA/µM and the detection limit was about 0.6 µM (S/N=3). The method was used in the determination of paracetamol in pharmaceutical preparations and urine samples successfully.

Key words: Paracetamol, graphene, carbon paste electrode.

INTRODUCTION

Graphene is a two-dimensional, planar sheet of sp^2 - bonded carbon atoms that are densely packed into a honeycomb lattice structure, and is a very large polyaromatic hydrocarbon (Pumera, 2009; Geim and Novoselov, 2007). It has received considerable attention from both the experimental and theoretical scientific communities because of its unique electric, thermal and mechanical properties. Among all properties, the unique electronic properties are assumed to be the most intriguing aspect of graphene (Gunlycke et al., 2007). It has also a large theoretical specific surface area (2630 m^2g^{-1}), high intrinsic mobility (Bolotin et al., 2008) and has attracted increasing attention for optoelectronic devices (Wang et al., 2008), electrochemical super-capacitors (Vivekchand et al., 2008), and ultrasensitive chemical sensors such as pH sensors (Ang et al., 2008), gas sensors (Schedin et al., 2007), and biosensors (Shan et al., 2009). Graphene has been used as an electrode material for sensing a range of biologically important molecules such as dopamine (Kim et al., 2010), NADH (Guoa et al., 2011), caffeine (Sun et al., 2011), hydrogen peroxide (Zhou et al., 2010) and glucose (Liu et al., 2010).

The use and performance of graphene paste electrodes prepared by mixing graphene and mineral oil has been reported in the literature (Parvin, 2011). The combination of the aforementioned advantages of graphene with the properties of carbon paste electrodes such as simple and inexpensive fabrication, biocom-patibility, and simple renewing of the electrode surface results in obtaining efficient working electrodes in sensitive and selective analyses.

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Paracetamol (N-acetyl-*p*-aminophenol, acetaminophen) is a popular analgesic and antipyretic agent. Due to the critical roles of paracetamol in the pharmaceutical industry, its determination is important in biological fluids and drug formulations. Many methods have been reported for the determination of paracetamol such as spectrophotometry (Bloomfield, 2002), liquid chromatography (Marin et al., 2002), high-performance liquid chromatography (Qil et al., 2002), chemiluminescence (Easwaramoorthy et al., 2001) and electrochemical techniques (Gutes et al., 2005; Baranowska and Koper, 2009; Saraswathyamma et al., 2008). Some of the modified electrodes used in electrochemical detection of paracetamol are C_{60} and carbon nanotube based electrodes which show high sensitivity and low detection limit (Goyal and Singh, 2006; Li et al., 2006), nanogold-modified indium tin oxide electrodes (Goyal et al., 2005), glassy carbon electrodes modified with carbon-coated nickel magnetic nanoparticles (Wang et al., 2007), and a glassy carbon electrode modified with graphene (Kanga et al., 2010).

In this work, we investigated the electrochemical behavior of paracetamol at the graphene paste electrode. The fabricated bulk-modified electrode showed excellent electrocatalytic activity towards paracetamol. It can be used for sensitive determination of paracetamol in pharmaceutical products as well as biological fluids using square-wave voltammetry.

EXPERIMENTAL

Chemicals and reagents

Graphite powder (natural) (99.9%, 325 mesh) from Serva, and paraffin oil from Merck (DC 350, density=0.88 g cm^{-3}) was used as binding agent for the graphite pastes. Paracetamol, $NH_3{\cdot}H_2O$, and NH_4Cl were purchased from Sigma. Hydrazine (50 to 60%) was from Aldrich and ammonium solution (25%) from Merck. A stock solution of paracetamol (0.01 M) was prepared by direct dissolution of paracetamol in doubly distilled water and kept in refrigerator (below 4°C).

All other chemicals were of analytical grade and used without further purification. All of the solutions were freshly prepared using doubly distilled water.

Apparatus

Electrochemical measurements were performed with a µ-Autolab type III, potentiostat/galvanostat instrument linked to a personal computer (Pentium IV, 1200 MHz) and the cell linked to the µ-Autolab software. The electrochemical data acquisition was performed using software NOVA 1.6.

A three-electrode system consisting of a saturated calomel electrode (SCE) as the reference, a platinum wire as the counter electrode, and a graphene modified carbon paste electrode (GRPE) as the working electrode were used in all voltammetric experiments. A pH meter (Jenway, Model 140) with a combined glass electrode was used to measure the pH of the solutions.

Preparation of graphene

Graphite oxide was synthesized from graphite through oxidization by using an improved method as reported (Marcano et al., 2010). The obtained graphite oxide (0.2 g) dispersed in 500 ml of deionized water and was exfoliated to graphene oxide under ultrasonic treatment for 1 h. The resulting homogeneous dispersion was mixed with 0.2 ml of hydrazine solution and 0.5 ml of ammonia solution (Guoa et al., 2011),. After being vigorously stirred for 30 min, a water bath (90°C) was launched for 3 h. The resulting mixture was filtered and washed to get the final graphene.

Electrode preparation

Graphite powder (0.900 g) was dissolved in ethanol (20 ml) and hand mixed with 0.100 g graphene using a mortar and pestle. The solvent was evaporated by stirring. A syringe was used to add paraffin to the mixture, which was mixed well for 40 min until a uniformly wetted paste, was obtained. The paste was then packed into a glass tube. Electrical contact was made by pushing a copper wire down the glass tube into the back of the mixture. When necessary, a new surface was obtained by pushing an excess of the paste out of the tube and polishing it on a weighing paper.

Sample preparation

For the analysis of paracetamol in tablets, ten tablets were accurately weighed and powdered. An amount of powder equivalent to the weight of one tablet was dissolved in doubly distilled water and then diluted with NH_3–NH_4Cl buffer (0.1 M, pH 8.5) to produce a solution of paracetamol with a concentration of 5 mM. Square wave voltammetry (SWV), in conjunction with standard addition technique was used for the determination of the paracetamol content of the sample.

Urine samples were diluted 10 times and spiked with different amounts of paracetamol. Recovery of the drug was obtained just like tablet sample.

Procedure

Voltammetric measurements were conducted at room temperature. GRPE, auxiliary and reference electrodes were immersed in 5.0 ml of NH_3–NH_4Cl (0.1 M, pH 8.5). A certain amount of paracetamol was added to the supporting electrolyte and after 2.0 min stirring for accumulation of the drug on the surface of the working electrode, the voltammetric method was applied to the quiescent solution at the appropriate potential interval.

RESULTS AND DISCUSSION

Electrochemical behavior of paracetamol on GRPE

The electrochemical behavior of paracetamol was investigated by cyclic voltammetry in an ammonium buffer solution (pH 8.5). As can be seen in Figure 1a and c, the

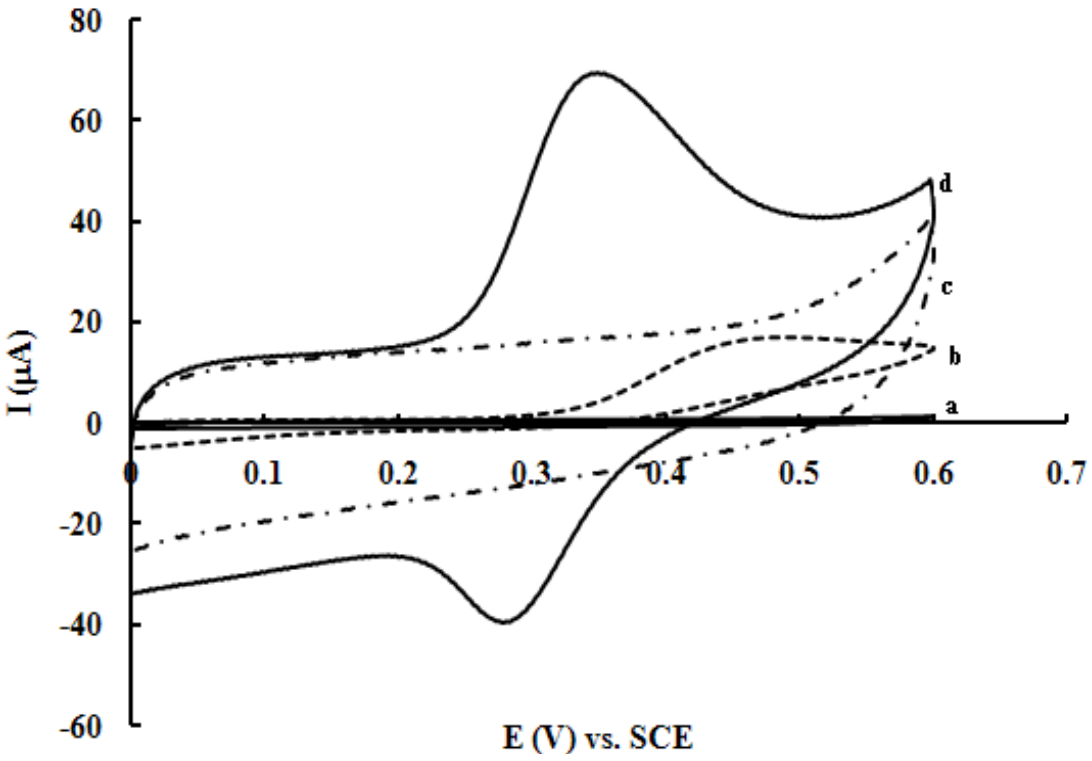


Figure 1. CVs at a bare CPE; (a) In the buffer of 0.1 M NH_3-NH_4Cl, pH 8.5; (b) a + 0.5 mM paracetamol; and at a GRPE (c) solution in (a); (d): solution in (b). Scan rate: 50 mVs^{-1}; accumulation time: 120 s.

background current of GRPE was larger than that of CPE, which can be attributed to the large specific area and good conductivity of graphene and a large capacitive current as a result (Zhu et al., 2010). On the CPE (Figure 1b), paracetamol shows irreversible behavior with relatively weak redox current peaks at 0.495 and -0.093 V and a peak-to-peak separation (ΔE_P) of 588 mV. In contrast, on the GRPE (Figure 1d), the anodic and cathodic peak currents are significantly increased with peak potentials located at 0.344 and 0.278 V, respectively, and ΔE_P about 0.066 V. Compared to the bare CPE, the redox peak currents obtained at GRPE were much larger and ΔE_P was much lower, which are clear evidences of electrocatalytic activity of graphene in the paste towards paracetamol.

Effect of pH

The electrochemical behavior of paracetamol at graphene paste electrode in different buffer solutions of pH 9.0 (0.05 M of BR, phosphate, NH_3–NH_4Cl buffers) was investigated by cyclic voltammetry (Figure 2). In the buffer system of NH_3–NH_4Cl, paracetamol shows a pair of well-defined redox waves due to a quasi-reversible process at the lowest anodic peak potential in comparison to other buffers. Therefore, NH_3–NH_4Cl (0.1 M) buffer solution was used in further studies.

The effect of solution pH on the electrochemical response of paracetamol at GRPE was investigated in the pH range from 7.0 to 10.5 by cyclic voltammetry. As shown in Figure 3, the pH of the solution obviously influenced the potential and the currents of both cathodic and anodic peaks of paracetamol. As was previously reported, the electrochemical behavior of paracetamol is pH dependent (Kanga et al., 2010). Figure 3A shows that both oxidation (E_p^a) and reduction (E_p^c) peak potentials shifted negatively with increasing pH. Figure 3B shows the linear relationship between the shift of oxidation potentials with increasing pH.

Using the relation $dE_p/dpH = 2.303\ mRT/\alpha nF$ (m is the number of protons involved in the electrochemical reaction; n is the number of electrons, α is the charge transfer coefficient, and the other constants have their usual meaning), m/nα was calculated to be 1.03 and 0.80 for the oxidation and reduction processes, respectively. Using an approximate value of 0.5 for α, m/n is nearly 0.5 in accordance with previous report (Kanga et al., 2010)

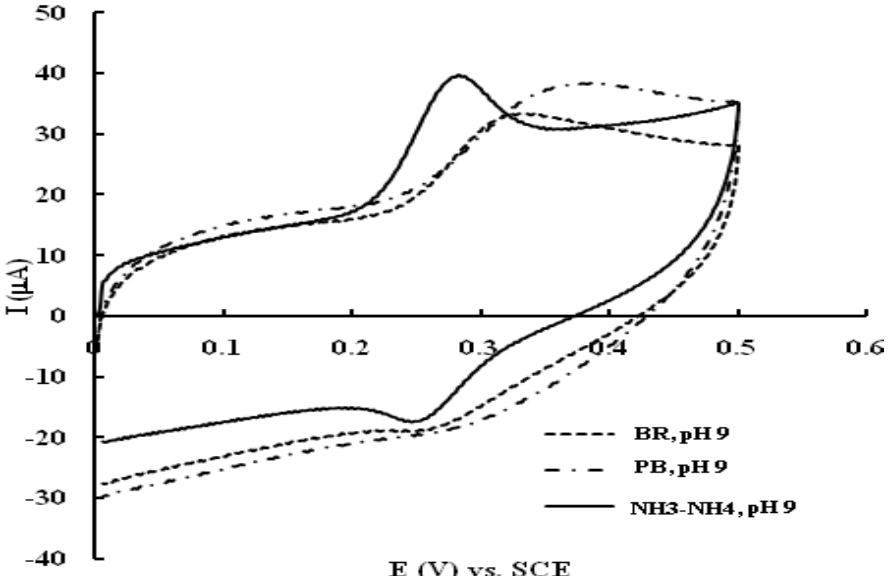


Figure 2. CVs obtained at GRPE with 0.5 mM paracetamol in different buffer solutions of pH 9.0.

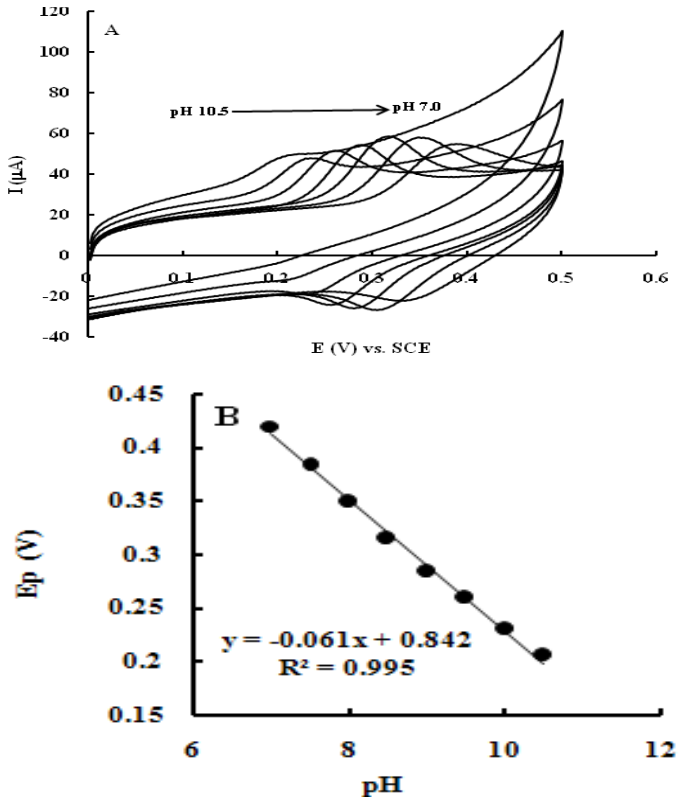
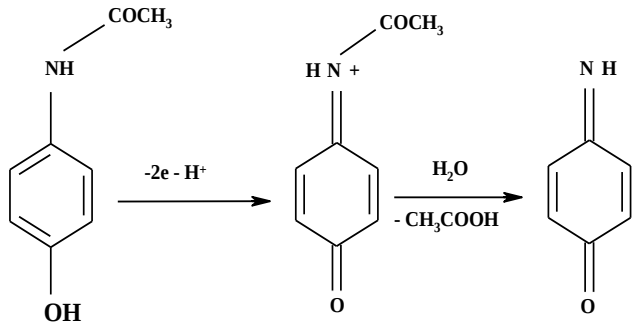


Figure 3. A: CVs obtained at GRPE with 0.2 mM paracetamol in 0.1 M NH_3-NH_4Cl buffers with pH values of 7.5, 8.0, 8.5, 9.0, 9.5, 10.0 and 10.5; B: Plot of oxidation peak potentials versus pH values of the solution. Scan rate: 50 mVs^{-1}.



Scheme 1. Mechanism of oxidation of paracetamol.

which confirmed that the redox processes of paracetamol involve the contribution of one proton and two electrons as is shown in Scheme 1.

Effect of scan rate

The effect of scan rate was studied on the electrochemical behavior of paracetamol on a GRPE in the range of 10 to 400 mVs^{-1} (Figure 4A). Linear relationships were observed between anodic (or cathodic) currents and scan rate (4B) in the range of 10 to 100 mVs^{-1} which is an indication of surface confined electron transfer process and adsorption of paracetamol on the GRPE surface.

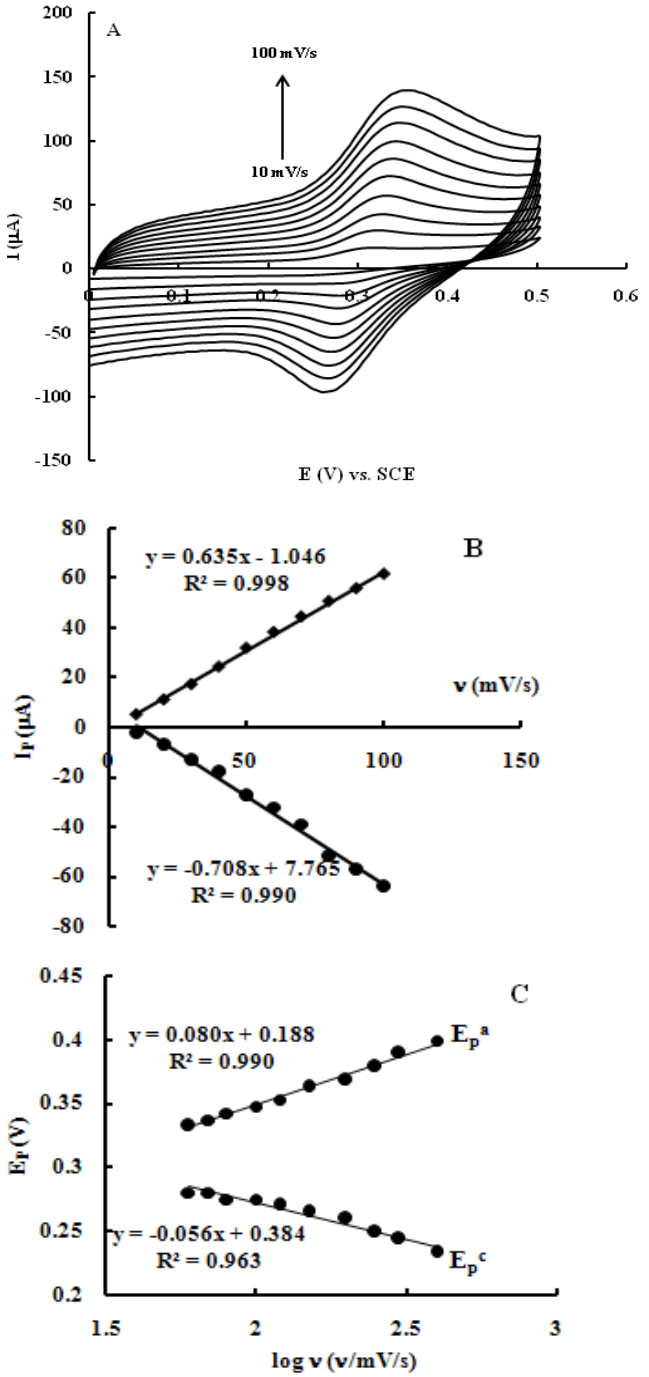


Figure 4. CVs acquired on GPE with 0.2 mM paracetamol in the buffer of NH_3·H_2O-NH_4Cl (0.1 M pH 8.5) at different scan rates from 10 to 100 mVs^{-1}. Inset is the plot of the peak current of paracetamol versus scan rate.

Based on Laviron theory (Laviron, 1979), the electron transfer rate constant (k_s) and charge transfer coefficient (α) can be determined by measuring the variation of peak potential with scan rate. In the scan rates ranging from 60 to 400 mVs^{-1}, the separation between anodic and cathodic peaks increased due to kinetics limitations in electron transfer at such short times.

Linear dependence of peak separations against the logarithm of scan rates was observed (4C) which gave an α value of 0.58 according to the following equation (n=2):

$$E_p = K - 2.303\ (RT/\alpha nF)\ \log\ (\nu) \quad (1)$$

Introducing this α value in the following equation, the electron transfer rate constant, ks, was estimated to be 1.76 s^{-1} (Laviron, 1974):

—— – ———— (2)

Effect of accumulation time

The influence of preconcentration time was investigated at GRPE in a 0.2 mM solution of paracetamol. The accumulation time had a remarkable effect on peak current, which increased greatly with time and reached a maximum at 30 min. At longer times, the peaks became progressively broader and a tendency to form a plateau was observed. A waiting time of 2 min was used in all subsequent experiments.

Calibration plot

In order to meet the needs for the determination of low paracetamol concentrations, a more sensitive electro-analytical technique, square wave voltammetry (SWV), was used at a scan rate of 10 mVs^{-1}. The dependence of the oxidation peak current (I_p^a) of paracetamol on its concentration was investigated in NH_3–NH_4Cl (0.1 M) buffer solution by SWV. The obtained SWVs at different concentrations of paracetamol are shown in Figure 5A. Under optimum conditions, the variation of SWV peak current with paracetamol concentration was linear for concentrations ranging from 2.5 to 143 µM (5B). The sensitivity of the method was 0.282 µA/µM and the detection limit was about 0.6 µM based on the signal-to-noise ratio of 3, which could be reduced by using a longer accumulation time.

Determination of paracetamol in pharmaceutical preparations and biological fluids

Commercial pharmaceutical samples (tablets) containing

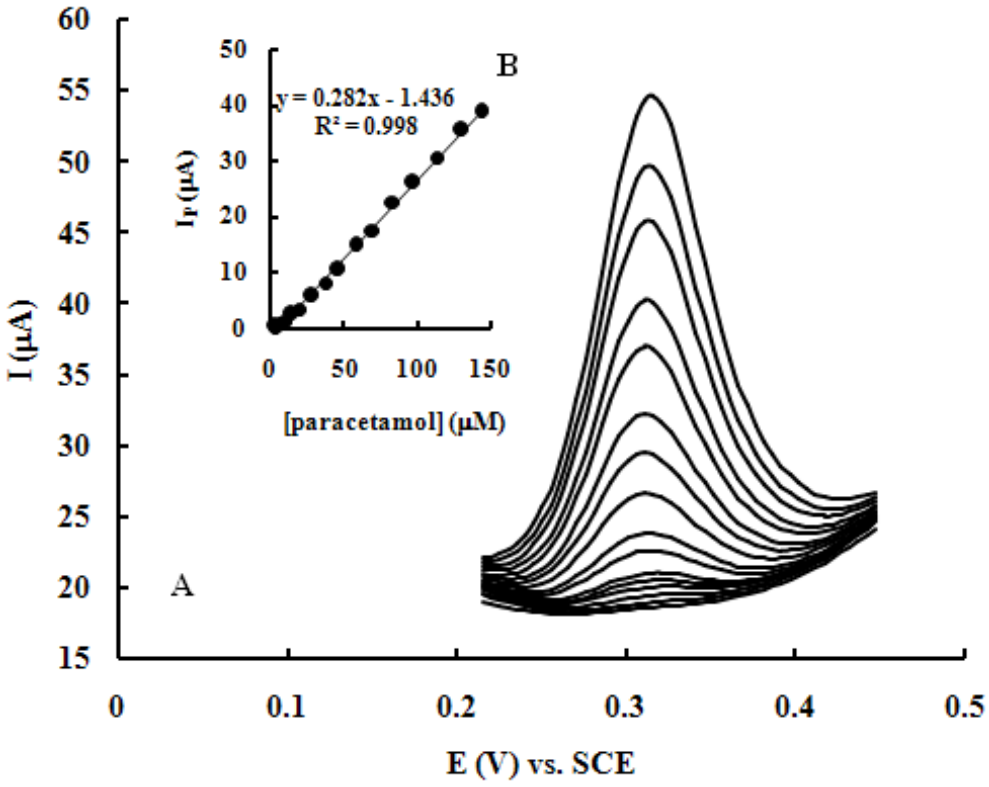


Figure 5. A: SWVs on GRPE for different paracetamol concentrations; from bottom: 0.0, 2.5, 3.5, 5.0, 7.4, 9.8, 14.6, 19.2, 28.3, 37, 45.5, 57.5, 69, 83.3, 96.8, 112, 130, and 143 μM in 0.1 M NH_3–NH_4Cl. B: Calibration plot.

Table 1. Results of real sample analysis using SWV at the GRPE.

Sample	Added (μM)	Expected (μM)	Found[a] (μM)	Recovery (%)	RSD (%)
	0	30	29.09 ± 0.09[c]	96.95	3.18
Tablet[b]	5	35	34.39 ± 0.08	98.25	2.21
	10	45	44.42 ± 0.07	99.24	1.51
	0	20	20.40 ± 0.04	102.02	1.78
Urine[d]	10	30	31.16 ± 0.07	103.88	2.16
	15	45	45.82 ± 0.05	101.83	0.80

[a], Average of three replicate measurements; [b], 325 mg tablet, Razi Company, Iran;[c], standard deviations are reported;[d], diluted 10 times.

paracetamol were analyzed to evaluate the validity of the method. Solutions obtained by dissolution of paracetamol tablets (325 mg paracetamol per tablet) were diluted so that paracetamol concentration lies in the range of the calibration plot. SWVs were recorded under optimum conditions, using the standard addition technique. As is shown in Table 1, an average recovery of 98.15% was obtained. The precision of the method was determined by calculating the relative standard deviation (%R.S.D.) which was less than 4.0%. The recovery study indicates that the GRPE can be effectively used for the selective determination of paracetamol in pharmaceutical samples.

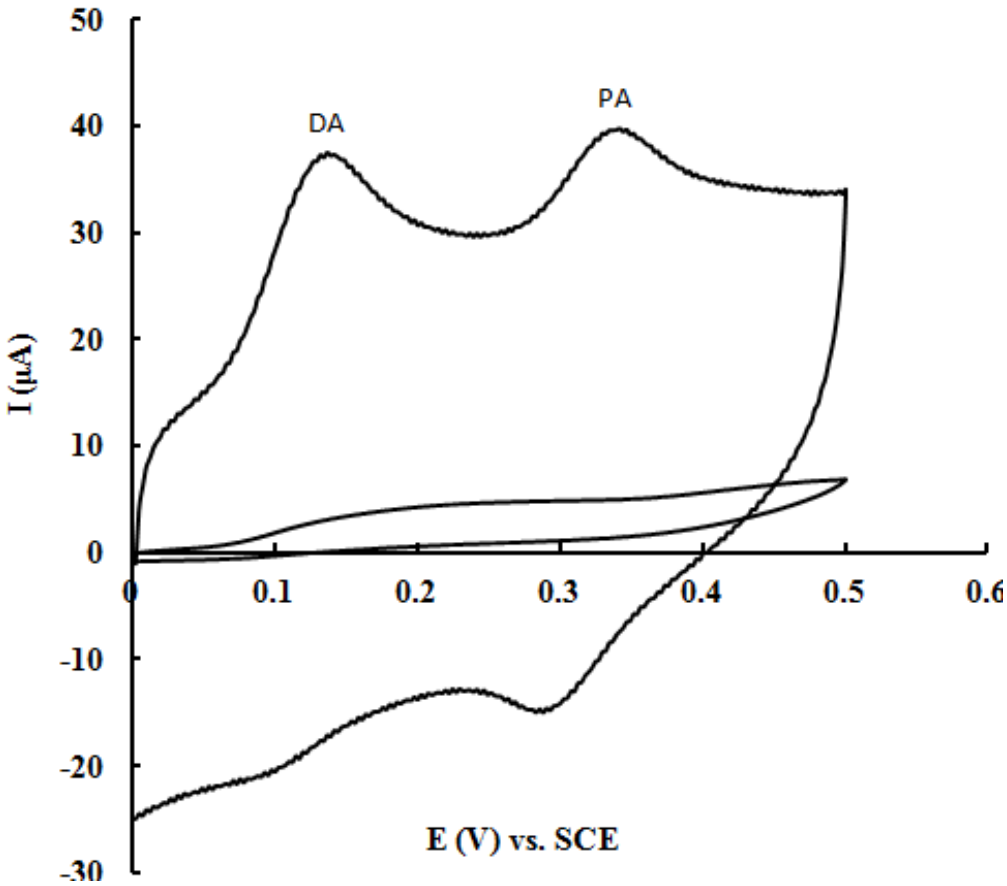


Figure 6. Cyclic voltammograms for a mixture of paracetamol (0.1 mM) and dopamine (0.1 mM) on a bare carbon paste electrode (lower) and a GRPE (upper).

Application of the method to the analysis of urine samples spiked with paracetamol resulted in acceptable validity of the procedure in complex matrices.

The interference of dopamine was examined on both bare carbon paste and GRPE on the determination of paracetamol. As is shown in Figure 6, two completely resolved peaks are observed on GRPE for these two species.

Conclusions

This work demonstrates the successful application of a carbon paste electrode modified with graphene in sensitive and selective determination of paracetamol. Cyclic voltammetry showed a great improvement in electrochemistry of paracetamol on a bulk modified carbon paste electrode with graphene. By application of square wave voltammetry, a linear dynamic range for paracetamol determination was obtained between 2.5×10^{-6} and 1.43×10^{-4} M. The limit of detection was 6.0×10^{-7} M, and the sensitivity of the calibration plot was 0.282 µA/µM which could be improved by using longer accumulation times before analysis. The real sample analyses revealed that the modified electrode is suitable for the selective determination of paracetamol in pharmaceutical as well as urine samples.

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