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Electrochemical behavior of azithromycin at graphene and ionic liquid composite film modified electrode

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ABSTRACT

An electrochemical method has been successfully demonstrated for sensitive determination of azithromycin (Azi) with room temperature ionic liquid (IL) of 1-butyl-3-methylimidazolium hexafluorophosphate ($BMIMPF_6$) – graphene (Gr) composite modified glassy carbon electrode (GCE). The cyclic voltammetric results indicate that Gr/IL/GCE can remarkably enhance electrocatalytic activity towards the oxidation of Azi in neutral solutions. Azi produce an anodic peak at about 0.82 V at this electrode. The electrocatalytic behavior was further exploited as a sensitive detection scheme for the Azi determination by differential-pulse voltammetry (DPV). Under optimized conditions, the concentration range and detection limit were 0.49–28.57 $\mu g\,ml^{-1}$ and 0.19 $\mu g\,ml^{-1}$ (S/N = 3) respectively for Azi. The method was successfully applied assay of the drug in the pharmaceutical dosage forms.

1. Introduction

Azithromycin (Azi), (*N*-methyl-9a-aza-9-deoxo-9-dihydro-9a-homoerythromycin A) is a member of the family of 15-membered macrolide antibiotics called azalides. It is used as an antibiotic in the human medicine for the treatment or prophylaxis of a number of health problems such as pharyngitis, pneumonia, chronic bronchitis, bronchopneumonia, skin and soft tissue infections and some sexually transmitted diseases [1]. Azi can be determined and studied through several methods [2–14], such as bioassay [2], spectrophotometry [3–5], spectrofluorometry [6], high-performance liquid chromatography using electrochemical [7], amperometric [8], fluorescence [9,10], UV [11] and mass spectrometric [12–14] detection. These methods can be applied to the detection of Azi in various samples, but they are generally time-consuming and/or complicated. Azi can also be assayed and studied by using electrochemical methods. The oxidative behavior of Azi at different electrodes such as glassy carbon electrode, hand-make carbon paste electrode and gold electrode was used for the determination of Azi [15–17]. A polymeric membrane electrode has been developed for the determination of Azi with the limit of detection (LOD) 1.54 $\mu g\,ml^{-1}$ [18]. The FIA-amperometric method for the Azi determination was also developed based on the electrochemical oxidation of the Azi at 0.9 V in Britton–Robinson buffer solution (pH 8.0) with a detection limit (LOD) of 0.76 $\mu g\,ml^{-1}$ [19].

To enhance detection sensitivity, functional materials are generally used to modify electrodes. Owing to its extraordinary electronic transport properties and high electro catalytic activities [20,21], graphene (Gr) have been investigated as electrode materials in opto-electronic devices [22], electrochemical super-capacitors [23], fabricated field-effect transistors [24], and constructed ultrasensitive chemical sensors [25,26], such as pH sensors [27], gas sensors [28,29], and biosensors [30]. Due to its negligible vapor pressure, outstanding chemical and thermal stabilities, high conductivity, and low toxicity, ionic liquid (IL) has been widely applied in liquid–liquid extractions and synthesis [31]. And it has been attracted considerable attention in the fields of analytical chemistry and electrochemical sensors based on its wide electrochemical windows, high ionic conductivity and good solubility [32,33]. Furthermore, the Gr–IL composite material has been used as a modifier for the chemically modified electrode based on the specific interaction of Gr with IL and their special properties. For example, Guo's group [34] mixed 1-butyl-3-methylimidazolium hexafluorophosphate with Gr to form gels by simple grinding. Comparing with CNT–IL electrodes and graphite–IL paste electrodes, Gr–IL paste electrodes provide higher electro-active surface area and lower charger transfer resistance. And the Gr–IL paste electrode was used for TNT detection with low detection limits of 0.5 ppb. Guo et al. determined trinitrotoluene using Gr–IL as an advanced electrode material [35]. Shan and coworkers [36] used IL-functionalized Gr to modify a glassy carbon electrode for sensitive

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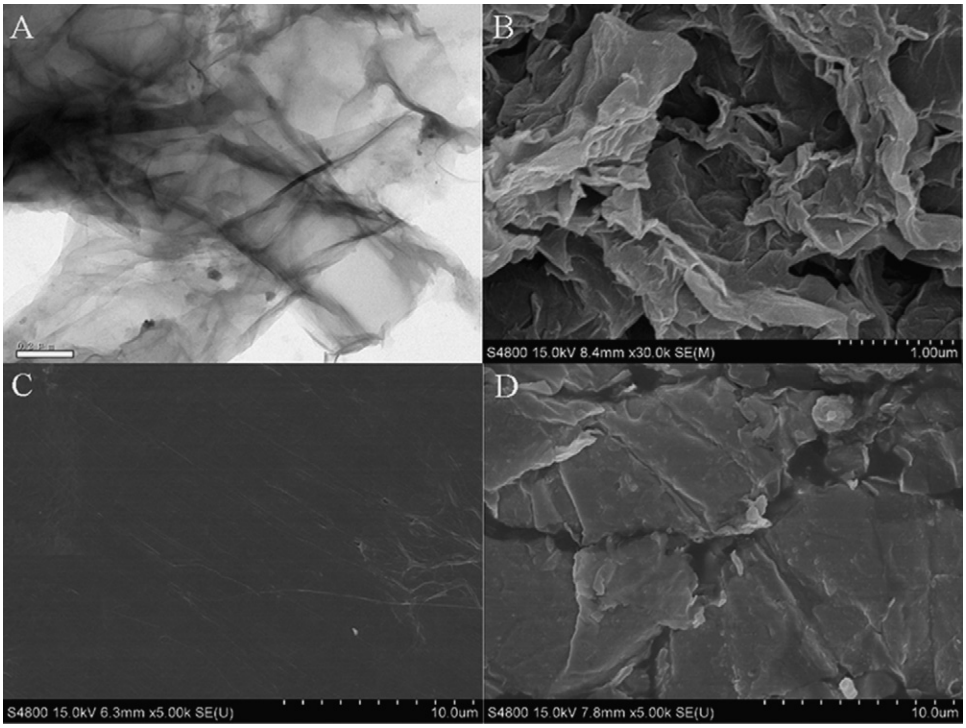


Fig. 1. TEM (A) of Gr in DMF solvent and SEM image of Gr/GCE (B), IL/GCE (C) and Gr/IL/GCE (D).

determination of NADH and ethanol. Li et al. determined ascorbic acid and dopamine simultaneously using glassy carbon electrode modified with Pt/IL/Gr nanocomposite [37]. However, to the best of our knowledge, very little work has been performed on the determination of Azi by the direct electrochemical measurement with the nanocomposites of Gr and IL electrode.

The purpose of this work is to study the voltammetric behavior of Azi at a Gr/IL/GCE. This modified electrode strongly enhanced the oxidation of Azi and leads to considerable improvement of its anodic peak current. This allows the development of a highly sensitive voltammetric sensor for the determination of Azi in pharmaceutical samples. Finally, this modified electrode was used for the analysis of Azi in commercial tablets from different manufactures with satisfactory results. The method is simple, stable and sensitive.

2. Experiments

2.1. Reagents and apparatus

Azi reference standard was from the National Institute for the Control of Pharmaceutical and Biological Products (NICPBP) (China), and its standard solution (1.09×10^{-3} mol l^{-1}) was prepared with absolute ethanol. The solutions are stable at least for 1 week if kept in the refrigerator at 4 °C, [38]. Graphite powder (spectroscopically pure reagent) was obtained from Qingdao Hensen Graphite Co. Ltd. (China). Gr was synthesized according to previous report [39]. The room temperature IL, 1-butyl-3-methylimidazolium hexafluorophosphate ([BMIM][PF_6]), was purchased from Aladdin-Reagent Company (China). Phosphate buffer solutions (PBS) were prepared by mixing the stock solutions of 0.2 mol l^{-1} Na_2HPO_4 and 0.3 mol l^{-1} NaH_2PO_4, and then adjusting the pH with 0.2 mol l^{-1} H_3PO_4 or 0.2 mol l^{-1} NaOH. Other reagents were of analytical grade and used as received. Aqueous solutions were prepared with double redistilled water.

The transmission electron microscope (TEM) images were obtained at JEOL-1200 EX TEM (Japan). The images of scanning electron microscope (SEM) were obtained at Hitachi S-4800 (Japan). Electrochemical experiments were performed with a CHI660D electrochemical workstation (Shanghai Chenhua Co., China) with a conventional three-electrode cell. A bare or grapheme modified GCE(φ = 3 mm) was used as working electrode. A saturated calomel electrode (SCE) and a platinum wire were used as reference electrode and auxiliary electrode, respectively. The pH measurements were carried out using a pHS-25 pH-meter (Shanghai Leici Instrument Plant, China) at room temperature.

2.2. Preparation of the Gr-modified electrode

Before modification, the GCE surface (Alda Co. Ltd., China) was polished with 0.05 μm alumina slurry, and sonicated in redistilled water to give a clean surface. For the preparation of Gr/IL/GCE, Gr (2 mg) and IL (20 μl) were dispersed in 2 ml N,N-dimethylformamide (DMF) to achieve a well-dispersed suspension. Then, 5 μl of the mixture was dropped on the surface of the pretreated GCE and dried under an infrared lamp. For comparison, Gr/GCE was also prepared by this simple drop-casting technique.

2.3. Procedure for capsules and tablets

Sanhe@ capsules were obtained from Changjiang Pharm (Jiangsu, China). Shimen@ tablets were obtained from Shijiazhuang No. 4 Pharm (Hebei, China). The amounts declared of Azi are both 250 mg per capsule and tablet. Twelve tablets were weighed and finely powdered. In the case of capsules, the contents of ten capsules were completely removed from shells and finely powdered. The accurately weighted quantities of these powders equivalent to 250 mg of Azi were dissolved in 25 ml absolute ethanol. After sonicating and shaking the mixture for 10 min, it was filtered and washed thoroughly with ethanol. The filtrate and washing were combined in a 50 ml volumetric flask, and diluted to mark with the same solvent.

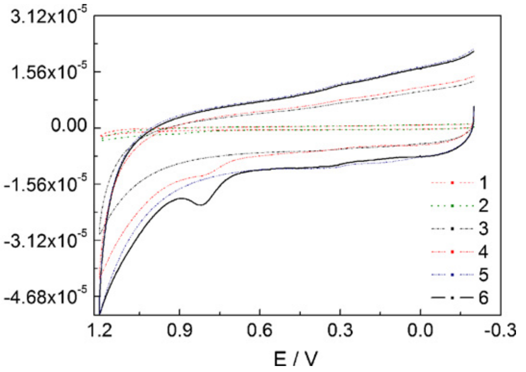


Fig. 2. Voltammograms of bare GCE (1 and 2), Gr/GCE (3 and 4) and Gr/IL/GCE (5 and 6) in solution without (1, 3 and 5) or with 16.3 μg ml-1 Azi (2, 4 and 6). Supporting electrolyte: 0.10 mol l^{-1}; phosphate buffer solution (pH = 6.0); scan rate: 50 mV s^{-1}.

2.4. Analytical procedure

The pH 5.8 PBS was used as the supporting electrolyte for Azi. After 30 s stirring, the differential pulse voltammetry curves were recorded from −0.2 to 1.2 V, and the oxidation peak currents at 0.81 V were measured for Azi. The pulse amplitude is 50 mV, the pulse width is 0.05 s, and the pulse period is 0.2 s.

3. Results and discussion

3.1. Characterization of Gr dispersed in DMF

Fig. 1A shows the TEM image of Gr sheets. The wrinkles on the Gr sheets are clearly observed. This wrinkled nature of Gr is highly beneficial in maintaining a high surface area on the electrode since the sheets cannot readily collapse back to a graphitic structure [40,41]. Fig. 1B shows the SEM image of Gr film on the surface of the GCE, revealing the typical crumpled and wrinkled Gr sheet structure on the rough surface of the film.

However, for GR/IL membrane (Fig. 1D), a totally different morphology was obtained. It seemed that the Gr/IL film was characterized with uniform and smooth surface. The degree of evenness was better that could be attributed to the binding and blanketing effect of viscous IL [34].

3.2. Electrochemical behaviors of Azi

The voltammograms of Azi at a bare GCE, Gr/GCE and Gr/IL/GCE in PBS (pH 6.0) were shown in Fig. 2. It showed that no reduction peak was observed in the reverse scan, suggesting that the electrochemical reaction was a totally irreversible process. As can be seen, the anodic peak potential for the oxidation of Azi at Gr/IL/GCE (curve 6) is about 0.82 V, while at the Gr/GCE (curve 4) and bare GCE (curve 2) peak potentials are about 0.80 and 0.91 V, respectively. The oxidation peak current value (I_p) of Azi was 8.453×10^{-6} A, which is 59 times larger than the one at the GCE, and 8 times larger than that of I_p at the Gr/GCE. The data obtained clearly show that the combination of Gr and IL definitely improve the characteristics of Azi oxidation. That might be related to the excellent property of Gr, such as high specific surface area and electrical conductivity. The role of IL was also important for promoting the electrochemical oxidation of Azi. So IL–Gr could be substituted for the oxidation of Azi.

3.3. Effect of pH value

The effect of solution pH on the electrochemical response of 8.15 μg ml^{-1} Azi at Gr/IL/GCE was investigated with PBS solution in the pH range from 5.0 to 8.0 by DPV. As can be seen in Fig. 3, the peak current decreased rapidly with the increase of pH. This can be attributed to the deprotonization of Azi. When Azi changed from cation to neutral molecule its electrostatic accumulation on the electrode surface decreased. Also, a series of supporting electrolytes were tested (phosphate–citrate buffer, sodium acetate–acetic acid buffer), and results showed that PBS solution gave the best response. Thus PBS solution of pH 5.8 was chosen for the subsequent analytical experiments.

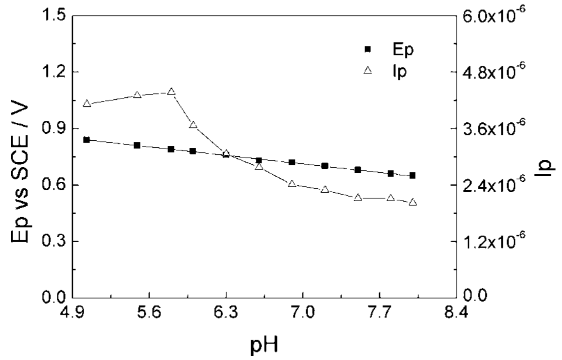


Fig. 3. Influence of pH on the oxidation peak current and potential of 8.15 μg ml^{-1} Azi.

The peak potential was closely dependent on the pH of the solution. It was found that the values of peak potential shifted to more negative values with the increase of pH (as shown in Fig. 3). The peak potential (E_p) moved in negative direction with pH rising and they showed such relation ship as: E_p (V) = 1.16–0.0646 pH (r = 0.998). The slope of −64.6 mV pH^{-1} demonstrated that the numbers of electron and proton transferred in the electrochemical reaction of Azi were equal.

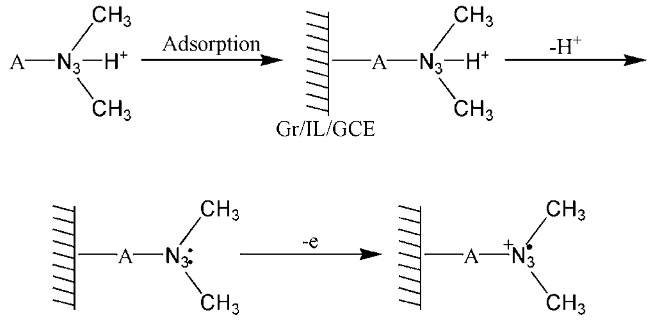
3.4. Effect of scan rate

Useful information involving electrochemical mechanism usually can be acquired from the relationship between peak current and scan rate. Therefore, the electrochemical behaviors of Azi at different scan rates were investigated on the surface of the Gr/IL/GCE by cyclic voltammetry. A linear relationship ($I_p = -1.001 \times 10^{-4} \upsilon - 1.454 \times 10^{-6}$, $n = 13$, $r = 0.9987$) was obtained between the peak current and the scan rate in the range of 0.02–0.3 V s^{-1}, which revealed that the oxidation of Azi was an adsorption-controlled step.

The peak potential shifted to more positive values with the increasing scan rates. The linear relation between peak potential and logarithm of scan rate can be expressed as E_p (V) = 0.0384 ln υ (V s^{-1}) + 0.9877 (n = 13, r = 9963). As for an irreversible electrode process, according to Laviron [42], E_p is defined by the following equation:

$$E_p = E^{\theta'} + \frac{RT}{(1-\alpha)nF} \ln \nu \qquad (1)$$

where E_p is the peak potential (V vs. SCE), $E^{\theta'}$ is the formal potential (V vs. SCE), R is the universal gas constant (8.314 J K^{-1} mol^{-1}), T is the temperature (K), α (alpha) is the charge transfer coefficient for the oxidation step, n is the number of electrons involved in the rate determining step, F is the Faraday constant (96,485 C mol^{-1}). According to Bard and Faulkner [43], for a totally irreversible wave, E_p is a function of scan rate, shifting (for an oxidation) in a positive direction by an amount $1.15RT/\alpha F$ (or $30/\alpha$ mV at 25 °C) for each ten fold increase in ν. So, from this we got the value of α to be 0.30. The number of electron (n) transferred in the electro-oxidation of Azi was calculated to be 0.97(approximately equal to 1). Azi is



Scheme 1. Electrochemical mechanism of Azi.

protonated at both N_9 and N_3 and is a very hydrophobic molecule at pH 5.8. Azi is adsorbed and oxidized on the surface of Gr/IL/GCE due to the hydrophobic of Gr. Among Azi's various functional groups, the amine group is most easily oxidized. Dialkylamines oxidize to form a radical cation by the loss of one electron [44]. Compared with N_3, N_9's lone pair electron(s) is not easily lost just as it is situated in the macrocyclic lactone ring. Thus, the observed oxidation peak is owing to one of the electrons in N_3's lone pair set. So the proposed mechanism on the Gr/IL/GCE may be expressed with the following equation, which involved a one electron and one proton oxidation process (Scheme 1). Similar results have been reported by Wu et al., they have reported N_3 is initially deprotonated with subsequent lose of one electron to form a radical cation when pH < pK_a [45].

3.5. Electrochemical impedance spectroscopy (EIS) of different electrodes

Electrochemical impedance spectroscopic measurement (EIS) was carried out in order to further understand the electron transfer on the modified electrode surface. The semicircle diameter of well conducting substrates equals to the electron transfer resistance (R_{ct}). The value of Ret varies when different substances are present on the electrode surface. By using 1.0×10^{-3} mol l^{-1} $Fe(CN)_6{}^{3-/4-}$ as redox probe, the EIS of different electrodes were recorded and the results were shown in Fig. 4. At the GCE, R_{ct} can be estimated to be 2000 Ω (curve a), indicating a big electron transfer resistance. Whereas, for the Gr/GCE and Gr/IL/GCE, the R_{ct} nearly equal to zero, which indicated that the electrochemical probe to the substrate electrodes were accelerated. The result showed that the Gr and Gr–IL composite film can act as effective electron conduction pathway between the electrode and electrolyte.

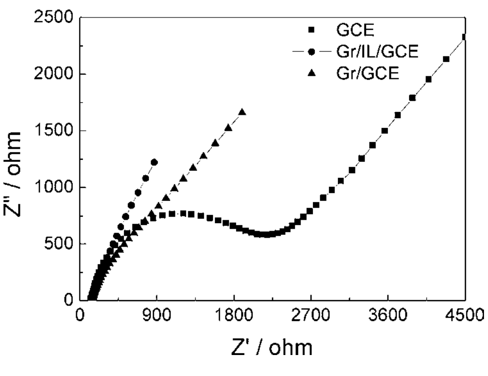


Fig. 4. Measured (symbols) EIS of (1) GCE, (2) Gr/GCE and (3) Gr/IL/GCE in a solution of 1.0×10^{-3} mol l^{-1} $K_3[Fe(CN)_6]$ + 1.0×10^{-3} mol l^{-1} $K_4[Fe(CN)_6]$ + 0.1 mol l^{-1} KCl attached by the corresponding fitted plots (lines). The applied perturbation amplitude was 0.005 V, init E was 0.236 V, the frequencies swept from 10^5 to 1 Hz, quiet time was 2 s.

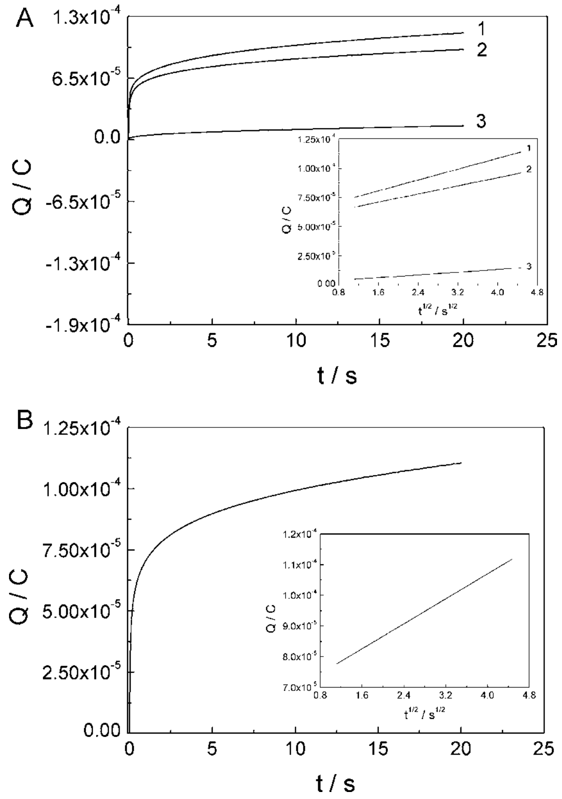


Fig. 5. (A) Plot of Q–t curve of GCE (3), Gr/IL/GCE (2) and Gr/GCE (1) in 1.0×10^{-4} mol l^{-1} $K_3[Fe(CN)_6]$ containing 1 mol l^{-1} KCl. Insert: plot of Q–$t^{1/2}$ curve on GCE (3), Gr/IL/GCE (2) and Gr/GCE (1). (B) Plot of Q–t curve of 8.12 μg ml^{-1} Azi at grapheme/IL/GCE after background subtraction. Insert: plot of Q–$t^{1/2}$ curve.

3.6. Chronocoulumetry

The electrochemically effective surface areas (A) of GCE and Gr/GCE were investigated by chronocoulometry using 1.0×10^{-4} mol l^{-1} $K_3[Fe(CN)_6]$ as model complex (the diffusion coefficient of $K_3[Fe(CN)_6]$ in 1 mol l^{-1} KCl is 7.6×10^{-6} cm^2 s^{-1} [46]) based on Anson equation [47]:

$$Q(t) = \frac{2nFAcD^{1/2}t^{1/2}}{\pi^{1/2}} + Q_{dl} + Q_{ads} \qquad (2)$$

where c is the substrate concentration, D is the diffusion coefficient, n is the electron transfer number, Q_{dl} is double layer charge which could be eliminated by background subtraction, Q_{ads} is Faradic charge. Other symbols have their usual meanings. Based on the slopes of the curves of Q versus $t^{1/2}$ (Fig. 5A), A was calculated to be 0.097 cm^2 (GCE), 0.406 cm^2 (Gr/GCE) and 0.309 cm^2 (Gr/IL/GCE). These results indicate Gr can significantly increase the reaction surface area of electrodes and enhance the current of the charge transfer reaction between the electrode and solution species for lower electrode polarization.

The diffusion coefficient (D) of Azi at grapheme/IL/GCE can also be determined by chronocoulometry based on Eq. (1). As can be seen in Fig. 5B, a linear relationship between Q and $t^{1/2}$ can be obtained after point-by-point background subtraction, and the regression can be expressed as $Q = 1.017 \times 10^{-5}\, t^{1/2} + 6.634 \times 10^{-5}$ (C s$^{1/2}$, r = 0.9944). Based on the slope of 1.017×10^{-5} C s$^{-1/2}$, it is calculated that $D = 4.8 \times 10^{-5}$ cm^2 s^{-1}.

3.7. Calibration curve

In order to develop a voltammetric method for determining the drug, we selected the differential-pulse voltammetric mode,

Table 1
Performance comparison of the fabricated electrode for Azi with other electrodes.

Electrode	Technique	Linear range (μg ml^{-1})	Correlation coefficient	LOD (μg ml^{-1})	Ref.
GCE	DPV	1–15	0.9982	0.7	[16]
CPE[a]	SWV[b]	4.71×10^{-4}–7.07×10^{-3}	0.9784	4.63×10^{-4}	[17]
Gold electrode	LSV[c]	235–588	0.9921		[18]
PME[d]	PA[e]	5.24–7489.80	0.9998	1.54	[19]
GCE	FIA-A[f]	1.0–10.0	0.9987	0.76	[20]
GCE	ADPV[g]	0.25–2	0.9992	0.11	[44]
Gr/IL/GCE	DPV	0.49–28.57	0.9962	0.19	Our work

[a] Carbon paste electrode.
[b] Square-wave voltammetric.
[c] Cyclic linear sweep voltammetry.
[d] Polymeric membrane electrode.
[e] Potentiometric analysis
[f] FIA-amperometric method.
[g] Adsorptive differential-pulse voltammograms.

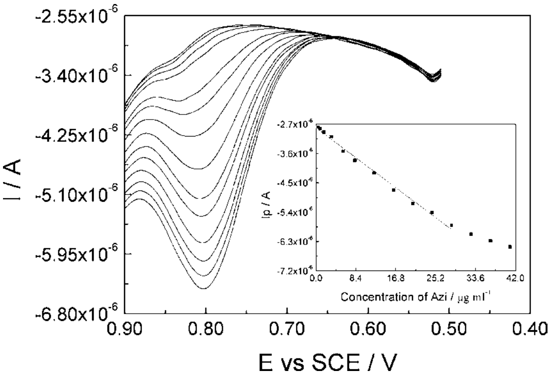


Fig. 6. Differential pulse voltammograms of Gr/IL/GCE in different concentrations of Azi solutions. Insert: calibration curve.

because the peaks are sharper and better defined at lower concentration of Azi than those obtained by cyclic voltammetry, with a lower background current, resulting in improved resolution. According to the obtained results, it was possible to apply this technique to the quantitative analysis of Azi. The phosphate buffer solution of pH 5.8 was selected as the supporting electrolyte for the quantification of Azi as it gave maximum peak current at pH 5.8. The peak at about 0.80 V was considered for the analysis. Differential-pulse voltammograms obtained with increasing amounts of Azi showed that the peak current increased linearly with increasing concentration, as shown in Fig. 6. Using the optimum conditions described above, linear calibration curves were obtained for Azi in the range of 0.49–28.57 μg ml^{-1}. The linear equation was I_p (A) = $-1.09 \times 10^{-7} c$ (μg ml^{-1}) $- 2.81 \times 10^{-6}$ ($r = 0.9962$). The detection limit was estimated to be 0.19 μg ml^{-1} (S/N = 3). The comparison of Gr/IL/GCE with other electrodes for the Azi determination was listed in Table 1. It can be seen that the Gr/IL/GCE offered reasonable linear range for Azi detection and the detection limit was lower than some of previous reports. These results indicated that Gr/IL/GCE is an appropriate platform for the determination of Azi.

3.8. Reproducibility, stability and interferences

For investigating the fabrication reproducibility, an 8.16 μg ml^{-1} Azi solution was measured by six modified electrodes prepared independently and the RSD of the peak current was 3.7%, revealing that the results are reproducible. After the electrode was stored for 7 days at 4 °C in humidity environment, it could retain 93.3% of its original response, suggesting that Gr/IL/GCE possess stability. In order to access the possible analytical applications of the proposed method, the effect of some common excipients used in pharmaceutical preparations were studied by analyzing sample solutions containing a fixed amount of Azi (8.16 μg ml^{-1}) spiked with various excess amounts of each excipient under the same experimental conditions. In this study, no interference (relative error <±5%) for lactose, sucrose, glucose (>250-fold in excess over analyte concentration), magnesium sulfate (>100-fold in excess), starch, hydroxypropyl cellulose (6-fold in excess), sodium dodecyl sulfate (3.5-fold in excess) were found. Since sample solution of Azi and each tested additive was prepared first in absolute ethanol then diluted to the desired concentration with distilled water, some common excipients as starch could not be tested due to insolubility in absolute ethanol and hence could not interfere. These results indicated that Gr/IL/GCE is not significantly affected by the presence of interferents, the method might be applied to determine Azi in the pharmaceutical dosage forms.

3.9. Analytical application

The applicability of the developed method was evaluated by analyzing two samples of commercial drugs containing Azi commercialized in the market. The results obtained by the proposed and reference (HPLC method [11] for the assay of Azi was adopted) procedures for pharmaceutical formulations are compared in Table 2 and are in good agreement.

Table 2
Analysis of pharmaceutical formulations by proposed procedures.

Pharmaceutical formulation[a]	Labeled values (mg/tablet)	Reference procedures[b] (mg/tablet)	Proposed procedures [c] (mg/tablet)	Add (mg/tablet)	Found (mg/tablet)	Recovery (%)
Capsules	250	242.3	244.7 ± 2.5	163.3	407.2	99.5
Tablets	250	246.5	246.8 ± 2.1	163.3	406.3	97.7

[a] Capsules: batch no. 20100502, expiry date: 04/2012, from Changjiang Pharm; tablets: batch no. 101158, expiry date: 11/2012, from Shijiazhuang No. 4 Pharm.
[b] HPLC method.
[c] Average of five replicate measurements ± SD.

4. Conclusions

Gr–IL can adsorb Azi and promote its electron-transfer, thus Azi can produce a more sensitive anodic peak at the Gr/IL/GCE. The Gr/IL/GCE provides higher electroactive surface area and lower charger transfer resistance compared with GCE. The electrochemical process is irreversible and presents the feature of an adsorption-controlled system. The peak current and Azi concentration show a linear relationship in a certain range. The electrode exhibits good repeatability and stability. This proposed method could be applied to the determination of Azi in medicines.

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