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A novel electrochemical sensor for assaying of antipsychotic drug quetiapine

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ABSTRACT

A novel electrochemical sensor based on poly(2-hydroxy-5-[(4-sulfophenyl)azo]benzoic acid) film modified glassy carbon electrode for fast and simple quantification of trace amount of quetiapine fumarate (QF) was developed. It exhibits excellent enhancement effects on the electrooxidation of QF facilitating preconcentration of drug molecules on the electrode surface. Based on its strong adsorptive activity, the concentration of QF in pharmaceutical formulations and biological fluids was determined directly by voltammetry with excellent sensitivity and high selectivity. The introduction of carboxylated and sulfonated functionalities in polymer film improves the uniform selectivity for positively charged target QF molecules. The calibration curve is linear in QF concentration range of 8.0×10^{-8} to 7.5×10^{-6} M with detection limit 1.9×10^{-8} and sensitivity 8.96×10^{5} μA M^{-1}. The presented sensor has long term stability and good reproducibility with benefits of fast response time, ease of preparation and regeneration of the surface that makes the proposed method useful in the determination of QF in real samples.

1. Introduction

Quetiapine fumarate (QF) is a relatively new atypical antipsychotic drug with a dibenzoethiazepine structure (Fig. 1). It is used for the treatment of schizophrenia or manic episodes associated with bipolar disorder [1]. QF acts as an antagonist of multiple neurotransmitter receptors in the brain including serotonin 5-HT_{1A} and 5-HT_2, dopamine D_1 and D_2, histamine H_1 and adrenergic α_1 and α_2 receptors. Drug molecule has no appreciable affinity at cholinergic muscarinic and benzodiazepine receptors. QF is rapidly absorbed after oral administration, reaching peak plasma concentrations in 1.5 h. Elimination of drug is mainly via hepatic metabolism with a mean terminal half-life of about 6 h within the proposed clinical dose range (between 150 and 750 mg/day). The major metabolic pathways are sulfoxidation to the sulfoxide metabolite and oxidation to the parent acid metabolite. Both metabolites are pharmacologically inactive and only 1% of unchanged drug is excreted in the urine [2].

QF is one of the top 15 best selling drugs and, therefore, the development of an accurate and sensitive analytical method to evaluate the quality of its pharmaceutical product or to determine drug concentration in biological fluids is highly required. At present, several analytical methods have been employed for the determination of QF in biological samples or pharmaceutical preparations, such as HPLC combined with mass spectrometry [3–6] or UV detection [7–13], UPLC with tandem MS [14,15], HPTLC [16], spectrophotometry [17], chemiluminescence [18] and capillary zone electrophoresis [17,19]. However, most of these methods are time-consuming, solvent-usage intensive, expensive and involved tedious sample preparations or, on the other hand, suffer from poor selectivity. An official method for quantification of this drug in bulk form and pharmaceutical formulations has not been approved in any pharmacopoeia.

In addition to described methods, electrochemical approaches are favourable owing to the high sensitivity, rapid response and simple operations. Apart from its relatively inexpensive instrumentation, electroanalysis also offers practically unlimited possibilities for new sensor platform designs, measuring in untreated samples, on-site testing and miniaturization of sensing elements. In spite of that, only one method has been reported in literature on the electrochemical determination of quetiapine [20]. It is based on the oxidation of drug molecule in a pH 3.5 acetate buffer solution at a glassy carbon electrode (GCE). However, carbon-based electrodes suffer from fouling and their obvious disadvantages arise from the difficulty of controlling their surfaces in a reproducible manner by tedious mechanical polishing.

Electrodes coated with conducting polymer films have received considerable interest due to their unique physical and chemical properties. Polymer modified electrodes are known to exhibit an enhanced response for the determination of various biological and clinical species as well as an attractive properties by improving the analytical selectivity. Electro-polymerization is a very convenient way to immobilize polymers on the electrode surface [21]. The thickness, permeability and charge transport characteristics of the polymer film can be controlled by the potential applied. Polymer modified electrodes prepared by electro-polymerization

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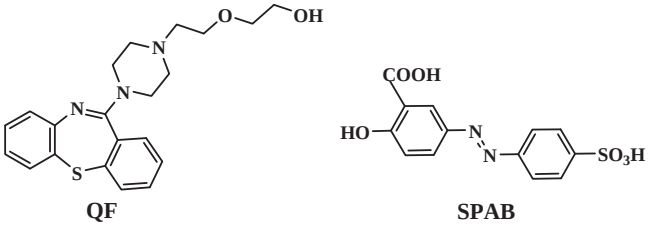

Fig. 1. Chemical structures of quetiapine fumarate (QF) and 2-hydroxy-5-[(4-sulfophenyl)azo]benzoic acid (SPAB).

have received extensive interest in the detection of analytes, not only due to theirs high sensitivity and selectivity, but also because of the film homogeneity attained in electrochemical deposition, its chemical stability and strong adherence to the electrode surface. Selectivity of polymer modified electrodes can be attained by different mechanisms such as ion exchange, hydrophobic interaction and size exclusion.

Dye molecules have been widely used as mediators to study the electrochemical response of pharmacologically important compounds [22,23]. The polymeric film formed via bonding dye molecules to aromatic rings covalently could improve the adsorptive ability of modified electrode. Although the electrooxidation of quetiapine has been reported, the adsorption characteristic of drug molecule has not yet been investigated. Hence, finding a new electrode material for determination of active molecule quetiapine using adsorptive stripping voltammetry is of great interest.

Aromatic azo molecules are important substances in many industrial processes aiming at the production of dyes or drugs. Presently, two azosalicylic acids, sulfasalazine and olsalazine are employed in clinical praxis as precursor of 5-aminosalicylic acid which is identified as an active component in the therapy of inflammatory bowel disease. 2-Hydroxy-5-[(4-sulfophenyl)azo]benzoic acid (SPAB) belongs to this class of compounds (Fig. 1). It is also described as a colon-specific prodrug of active 5-aminosalicylic acid formed after reductive azo bond cleavage by digestive enzymes [24]. On the other hand, disodium salt of SPAB (known as Mordant yellow 10) is widely used as textile dye. The electrochemical properties of SPAB were investigated at GCE and hanging mercury drop electrode in our previously reported papers [25–28].

The aim of present work is to develop a novel sensor for fast and simple quantification of trace amount of QF with high selectivity. The electrochemical sensor based on a poly(2-hydroxy-5-[(4-sulfophenyl)azo]benzoic acid) film is demonstrated for the first time to the determination of analytes by voltammetry. This sensor was fabricated by electro-polymerization of SPAB on the surface of GCE in a Britton–Robinson buffer solution. The SPAB monomer has an azo bond, an amine group, a carboxyl and a sulfonate groups. Because of the presence of the hydrophilic charged carboxyl and sulfonate groups the polymer formed is promising to adsorb positively charged QP molecule by electrostatic interaction, while the aromatic rings could enable adsorption of dibenzothiazepine moiety by hydrophobic interaction. The introduction of sulfonated functionalities in proton exchange polymer film improves the uniform selectivity for cationic target analytes similar like Nafion, however fabrication of poly(SPAB) film based sensor provides an attractive means of overcoming the problems caused by the solvent evaporation method used in preparation of Nafion coated GCE. The experimental results show that a novel sensor exhibits excellent enhancement effects on the electrooxidation of QP facilitating its preconcentration on the electrode surface. By using this phenomenon a simple and inexpensive analytical method for quantification of QP was developed on a poly(2-hydroxy-5-[(4-sulfophenyl)azo]benzoic acid) film modified GCE (SPAB/GCE). The practical application of electrochemical sensor designed was demonstrated to the determination of QF in commercial pharmaceutical product and biological fluids by square-wave adsorptive stripping voltammetry.

2. Experimental

2.1. Apparatus

Voltammetric measurements were performed using a μ-Autolab potentiostat (Eco Chemie, Utrecht, The Netherlands) controlled by GPES 4.9 software. A conventional three-electrode system was employed, comprising a bare GCE (3-mm diameter, Metrohm, Switzerland) or polymer modified GCE as working electrode, a platinum wire as counter electrode and an Ag/AgCl/3 M KCl (Metrohm) as the reference electrode. All electrochemical experiments were carried out at room temperature ($23 \pm 1\,^{\circ}C$).

High-performance liquid chromatographic experiments were carried out using an Agilent 1100 Series LC system equipped with a diode array detector (Agilent Technologies, Waldbronn, Germany).

2.2. Chemicals

QF kindly donated by Pliva (Zagreb, Croatia) was used as received without any further purification. Loquen® (Pliva) enteric-coated tablets, containing 230.26 mg of QF equivalent to 200 mg quetiapine, were supplied from local pharmacy. Loquen® contains the following excipients: lactose, microcrystalline cellulose, hydroxypropylcellulose, hypromellose, calcium hydrogen phosphate, sodium starch glycolate, talc, silicium dioxide, magnesium stearate, macrogol 4000 and titanium dioxide. SPAB was prepared according to the procedure described previously [24]. Valproic acid was obtained from Acros Organics (Geel, Belgium). Diazepam was purchased from Sigma–Aldrich (Steinheim, Germany). All other chemicals were of analytical grade quality. Britton–Robinson buffer solutions (0.04 M in each of acetic, phosphoric and boric acids) adjusted to the desired pH with additions of a 0.2 M solution of sodium hydroxide were used as a supporting electrolyte. Ultra pure water used for the preparation of standard solutions and buffers was obtained by a Milli-Q system (Millipore, Bradford, USA).

2.3. Preparation of the electrode

Prior to modification, the GCE was polished with aqueous slurry of 0.05 μm alumina powder on a smooth polishing cloth, thoroughly rinsed with water and then ultrasonically cleaned in water for 30 s. Finally, the electrode was washed with purified water and dried. The cleaned GCE was then immersed in a Britton–Robinson buffer solution (pH 4) containing 1×10^{-3} M SPAB and electrochemically polymerized by cyclic voltammetry sweeping from the potential −0.1 to 1.6 V at a scan rate of 100 mV s^{-1} for ten scans to form the SPAB/GCE. After electro-polymerization, the modified electrode was carefully rinsed with purified water to remove any physically adsorbed material.

2.4. Electrochemical measurement procedures

Stock solutions of QP (1×10^{-3} M) were prepared in a purified water and stored under refrigeration. The working standard solutions of QF were obtained by serial dilution of stock solutions with a supporting electrolyte just before voltammetric measurements. The oxidative behaviour of QF was investigated by cyclic voltammetry in the scan range from 0.0 V to 1.5 V. Square-wave voltammetry (SWV) was used for the determination procedure. The sensor was immersed in the sample solution and preconcentration of QF was carried out at open circuit conditions for a predefined time period. After 5 s equilibrium period, the voltammogram was recorded in

the SWV mode (frequency, 15 Hz; potential step, 4 mV; amplitude, 25 mV) from 0.5 to 1.4 V. Then, the electrode was cleaned from traces of remaining target molecules in solution of supporting electrolyte by applying single positive-going SWV potential scan from 0.5 to 1.4 V.

2.5. Pharmaceutical dosage form assay procedure

To prepare the solutions of QF commercial pharmaceutical product, ten tablets were weighted and crushed to a fine powder. An accurately weighted powdered sample of drug formulation equivalent to 8.83 mg of active ingredient was transferred into a 10.0 mL calibrated flask and dispersed in water. The tablet solution was sonicated for 15 min to provide complete dissolution. After the sonification, the sample was filtered through 0.45 μm Acrodisc GHP filters (Gelman, Ann Arbor, USA). An aliquot of filtrate was then transferred into a calibrated flask and diluted with water to yield a final drug concentration of 1.0×10^{-4} M. A series of dilutions were made with supporting electrolyte solution to cover the working concentration range. The content of QF in the pharmaceutical preparations was determined by standard addition method. For recovery studies, aliquots of the QF standard solutions were added to real samples prepared from tablets.

2.6. Determination of QF in biological samples

Human serum and urine samples were obtained from healthy volunteer abstained from any medications during the week preceding the study. Serum samples were stored frozen until assay. An aliquot of serum sample containing the drug was mixed with acetonitrile (1:1) to remove serum protein effectively. After vortexing for 60 s, the mixture was then centrifuged for 6 min at 6000 rpm. Appropriate volume of this supernatant were transferred into the volumetric flask and diluted up with Britton–Robinson buffer solution (pH 4) and analyzed in the voltammetric cell. It should be noted that the serum sample was fortified with QF standard solution to achieve a concentration (5.0×10^{-7} M) found in serum coming from patient who had ingested only 50 mg quetiapine dose. It was considered to be a relatively low dose given because the therapeutic range is between 150 and 750 mg/day [18,29]. Urine samples were fortified with appropriate aliquot volume of QF standard solution to achieve a final drug concentration (3.0×10^{-6} M) that is found in urine after treatment with the therapeutic daily dose of 50 mg [18]. Prior to voltammetric analysis, urine samples were filtered through a 0.45 μm Acrodisc GHP filters and diluted with supporting electrolyte so that the concentration of drug was in the working range (3.0×10^{-7} M). Quantitations in both biological fluids were performed by means of the standard addition method by adding three successive aliquots of drug standard solution.

3. Results and discussion

3.1. Electrochemical properties of poly(SPAB) film

The chemical modified electrode based on azosalicylic acid polymer could be a promising sensor in electroanalysis of QF. A strategy for cation selectivity and adsorption of positively charged QF molecule has been to incorporate polyanions in conducting polymer film deposited onto GCE surface. This kind of organic polymer layer was easily generated by electrooxidation of SPAB monomer. The electro-polymerization procedure was performed directly in aqueous media. Fig. 2 presents the cyclic voltammetric curves during scanning. The peak currents at potential of 1.1 V assigned to the oxidation of SPAB solution were decreased on the subsequent scans, while a reduction peak at potential of 0.16 V in the reverse reduction scan and a new oxidation peak at 0.48 V in the following anodic scan were appeared, indicating the accumulation of poly(SPAB) film deposit on the electrode surface. With the growth of polymer film on the electrode surface, both new peak currents increased gradually with continuous scans increasing. The thickness of polymer film formed was controlled by the cyclic number of voltammetric scans. The choice of potential window had also an effect on preparation of poly(SPAB) film. The efficiency of the film growth, expressed in terms of the height of peak currents, was the highest when electrode was cycled in the modification solution between −0.1 and 1.6 V at a scan rate of 100 mV s^{-1}. In order to find optimum preparative conditions for electro-polymerization procedure, the pH value of Britton–Robinson buffer solution containing 1×10^{-3} M SPAB was varied between 2 and 7. The use of buffer solution pH 4 helped to enhance the film growth linearly with scan cycles. Hence, the film deposited by using 10-times cyclic potential sweeps in the above mentioned electrolyte was described as optimum. Polymer film formation process was characterized by recording the cyclic voltammetric response of poly(SPAB) film in pure supporting electrolyte (Britton–Robinson buffer pH 3) at various scan rates between −0.1 and 0.7 V (inset of Fig. 2). The film formed on the GCE surface had two reversible redox couple (E_{pa1} = 0.23 and E_{pa2} = 0.49 V) in comparison to the response of a bare GCE and the peak currents increased with increasing the cyclic voltammetric scan rate. The couple of redox waves observed may be resulted for the oxidation/reduction of some functional groups inside the poly(SPAB) film. Separation of the peak potentials, ΔE_p, was 29 for first and 32 mV for second reversible couple, therefore the number of electrons involved in both redox reactions was 2 ($n_1 \approx 2.03$ and $n_2 \approx 1.84$). The effect of various pH values of the supporting electrolyte on the electrochemical behaviour of the poly(SPAB) film deposited was also studied. The anodic and cathodic peak potentials of both redox reactions were shifted to a less positive value with increasing pH of the contacting solution. The plot of the anodic peak potentials versus pH shows linearity in the pH range of 3–7, with a slope of −68.8 and −65.3 mV/pH for first and second reversible peak, respectively. This implies that the ratio of the participated protons to the transferred electrons through the poly(SPAB) film is 1:1 in both redox reactions. The results obtained clearly indicate that the redox processes were confined to the polymer modified surface of the electrode, confirming the successful

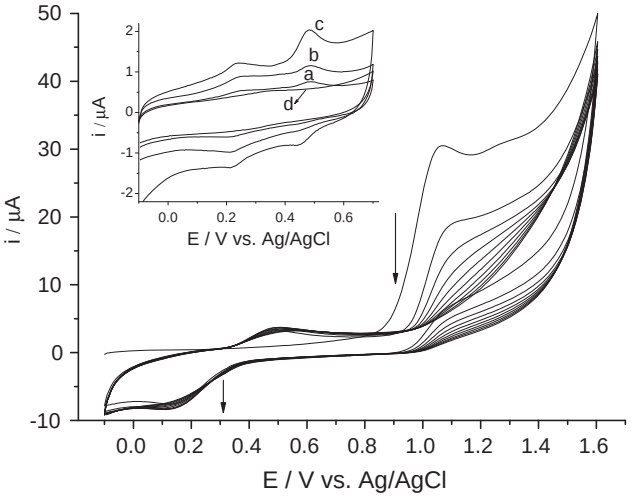

Fig. 2. Electrochemical polymerization of 2-hydroxy-5-[(4-sulfophenyl)azo]benzoic acid on GCE surface for ten cycles in Britton–Robinson buffer solution (pH 4) containing 1×10^{-3} M SPAB (scan rate 100 mV s^{-1}). Inset shows cyclic voltammetric response of the SBAP/GCE in pure supporting electrolyte (Britton–Robinson buffer pH 3) at various scan rates: (a) 50, (b) 75 and (c) 100 mV s^{-1} (d; response of a bare GCE electrode).

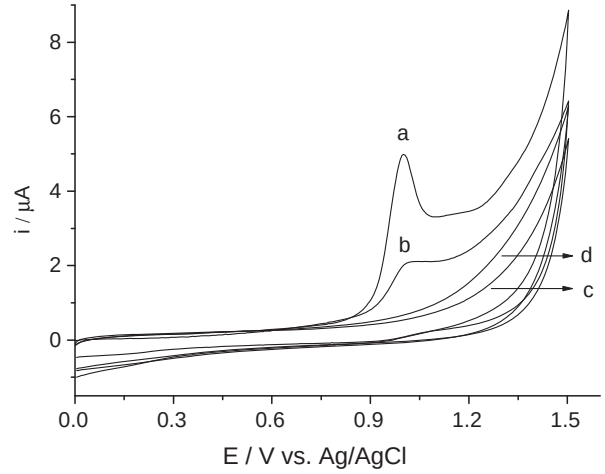

Fig. 3. Cyclic voltammograms of quetiapine fumarate obtained at the SBAP/GCE (a) and at a bare GCE (b) together with corresponding background recordings at the SBAP/GCE (c) and a bare GCE (d) and in Britton–Robinson buffer pH 3; [QF] = 1×10^{-5} M, scan rate 50 mV s^{-1}.

polymerization and immobilized state of the poly(SPAB) film on the electrode surface.

3.2. Electrochemical behaviour of QF on modified electrode

In order to elucidate the potential of electrochemical sensor designed in the determination of QF, the electrochemical behaviour of drug molecule at a bare GCE and SPAB/GCE were compared by cyclic voltammetry, and the results are shown in Fig. 3. In cyclic voltammogram of the polymer modified electrode, a well-shaped irreversible oxidation peak at 0.99 V was observed. The voltammetric signal of QF was improved considerably at SPAB/GCE, and the anodic peak current was intensively increased. Compared with a bare GCE, the oxidation peak potential of QF was shifted to less positive values by 50 mV, indicating a faster electron transfer reaction at the electrode surface and enhanced electrocatalytic properties of film immobilized. Therefore, the poly(SPAB) film had a remarkable enhancement effect to the electrochemical response of QF. In order to choose the best pH value of supporting electrolyte for the electroanalytical measurements, the effect of pH on the voltammetric response of QF was firstly investigated. The variation of peak intensity and peak potential with pH for 1×10^{-5} M QF solution was studied by square-wave voltammetry between pH 2 and 9 (Fig. 4). At pH values greater than 6 the oxidation peak was split into two peaks. At $2 \leq \text{pH} \leq 7$, the oxidation peak potentials were shifted to less positive values with an increase of pH and observed linear relationship (inset of Fig. 4) was expressed by equation $E_p = 1.26 - 0.0577$ pH ($r = 0.9994$), suggesting that the number of protons participated in the oxidation reaction of QF was equal to the number of electron transferred. The results also indicate that the electroactive group responsible for the oxidation process is in acid-base equilibrium with pK_a of about 7. This is in agreement with previous findings which exhibited that the piperazine ring in drug molecule underwent oxidation. A maximum peak current was achieved at pH 3 Britton–Robinson buffer solution and, therefore, this solution was selected as suitable supporting electrolyte for the drug determination. Quetiapine is a weak base with 3.3 and 6.8 pK_a, and in buffer solution at pH 3 the piperazine moiety is almost completely protonated on both nitrogen. Hence, the higher current values could be attributed to increased adsorption of positively charged drug molecule which was attracted by carboxyl and sulfonate functionalities in the polymer formed on the electrode surface.

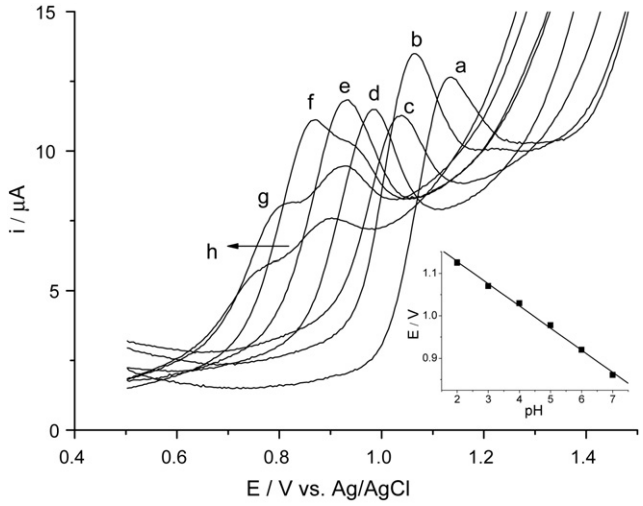

Fig. 4. Square-wave voltammograms of quetiapine fumarate obtained at the SBAP/GCE in Britton–Robinson buffer solutions with pH 2 (a), 3 (b), 4 (c), 5 (d), 6 (e), 7 (f), 8 (g) and 9 (h); [QF] = 1×10^{-5} M, SWV settings: frequency of 15 Hz, amplitude of 25 mV and potential step of 4 mV. Inset shows a dependence of peak potential upon the pH of measurements solution.

3.3. Adsorption characteristics of QF on modified electrode

The influence of the scan rate on the electrochemical response of drug molecule in Britton–Robinson buffer pH 3 was investigated by cyclic voltammetry in order to obtain information regarding the adsorptive behaviour of QF at the SBAP/GCE. The oxidation peak current of QF increased with scan rate increasing and a linear relationship was observed in the range 10–500 mV s^{-1}. The oxidation peak current is significantly suppressed in second cyclic scan. Those results indicated that the electrode process of QF at the electrochemical sensor developed is controlled by an adsorption step. The plot of $\log i_p$ measured with preconcentration versus $\log \nu$ was a straight line following the relation: $\log i_p$ (μA) = 0.89 $\log \nu$ (mV s^{-1}) + 0.11. Its slope value was close to the theoretical value of 1.0 that expected for an ideal electrode reaction of surface species. Fig. 5 shows square-wave voltammograms for 5×10^{-6} M solutions of QF without accumulation and after a 120 s accumulation

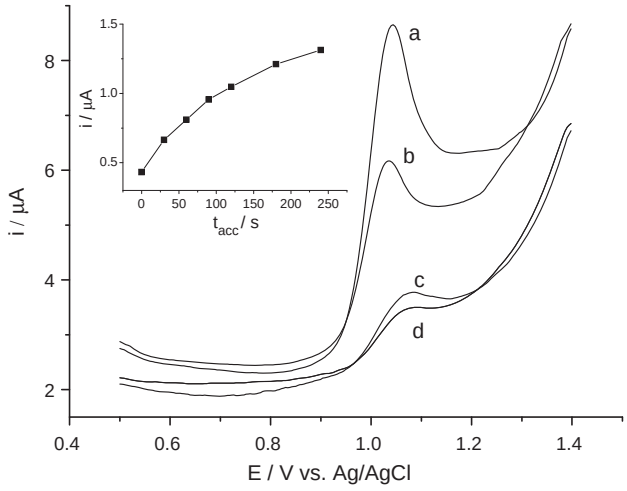

Fig. 5. Square-wave voltammograms of quetiapine fumarate obtained at the SBAP/GCE (a and b) and a bare GCE (c and d) in Britton–Robinson buffer solution pH 3 without accumulation (b and d) and after accumulation of 120 s (a and c) in open circuit condition; [QF] = 5×10^{-6} M, SWV settings same as in Fig. 4. Inset shows relationship between oxidation peak current ([QF] = 1×10^{-6} M) and the accumulation time.

Table 1
Validation data for quetiapine fumarate determination by square-wave adsorptive stripping voltammetry at the SBAP/GCE.

Analytical parameter	
Linearity range (M)	8.0×10^{-8}–7.5×10^{-6}
Slope ($\mu A\,M^{-1}$)	8.96×10^{5}
Intercept (μA)	0.17
SE of slope ($\mu A\,M^{-1}$)	1.91×10^{3}
SE of intercept (μA)	0.0058
Limit of detection (M)	1.9×10^{-8}
Limit of quantitation (M)	6.5×10^{-8}
Repeatability of peak current (RSD%)	1.3
Repeatability of peak potential (RSD%)	0.8
Reproducibility of peak current (RSD%)	1.9
Reproducibility of peak potential (RSD%)	1.1

step at a bare GCE and polymer modified electrode. This finding revealed that the adsorption of QF at SBAP/GCE can be used as an effective preconcentration step prior to the voltammetric quantification of the drug. To achieve the maximum sensitivity and the optimum conditions for the maximum adsorption, the effect of accumulation potential on the stripping peak current was evaluated from 0.0 to 0.6 V. The oxidation peak current of 1×10^{-6} M drug solution remained almost unchanged in potential range studied. However, a maximum enhancement of the peak current was observed at open circuit accumulation, so this deposition condition was selected in all subsequent studies. The accumulation time significantly affected the voltammetric response of QF at the SBAP/GCE. The oxidation peak current enhanced greatly with the increase of the accumulation time within first 120 s (inset of Fig. 5). The linear relationship between the voltammetric response and the deposition time pointed out a constant adsorption of positive-charged drug molecules attracted on the surface of the SBAP/GCE. Further increment of accumulation time period showed a deviation of the peak current from linearity indicating surface adsorption saturation. Therefore, the value of 120 s was considered as optimum because it provided the largest peak current in acceptable analysis time.

3.4. Determination of QF

The voltammetric response of different drug concentration was recorded in a Britton–Robinson buffer solution pH 3 using a novel electrochemical sensor after 120-s accumulation time in open circuit condition. The oxidation peak showed a linear response at the SBAP/GCE in the concentration range of 8.0×10^{-8}–7.5×10^{-6} M. The calibration plot is described by the following regression curve: i_{pa} (μA) = $8.96 \times 10^{5} c$ (M) + 0.17, r = 0.999. The analytical characteristics for the calibration graph and the related validation parameters are given in Table 1. The detection limit (LOD) and the quantification limit (LOQ) estimated from the calibration curve as LOD = $3s/b$ and LOQ = $10s/b$, where s is the standard deviation of the intercept and b is the slope of the calibration curve [30], were calculated to be 1.9×10^{-8} M for LOD and 6.5×10^{-8} M for LOQ.

For comparison, the proposed new electrochemical sensor provides much higher sensitivity than literature reported (e.g. slope of the calibration plot for GCE was 4.50×10^{4} $\mu A\,M^{-1}$) [20]. Both, the linearity and the detection limit obtained at the SBAP/GCE for drug examined were in a lower concentration range than at a bare GCE reported earlier. However, the most important advantage of electrochemical sensor developed is its remarkable improvement in the reproducibility, with no requirement for mechanical maintenance in order to obtain reproducible voltammetric response by time consuming polishing the substrate surface before each measurement as in the case of GCE. Compared with other methods, the detection limit of QF obtained at novel sensor is of the same order as for HPLC with UV detection [9–12] and is better than that obtained in capillary electrophoresis and spectrophotometry [17,19], but it is lower than LODs reported for LC–MS methods [5,6,14]. However, the proposed voltammetric method using the SBAP/GCE offers several advantages over chromatographic techniques applied only to the quantification of drug, including short analysis time, simplicity of operation and lower running cost.

3.5. Stability and reproducibility of the modified electrode

The stability of the SBAP/GCE was evaluated by measuring the current response of 1×10^{-6} M drug solution over a period of three weeks. The SBAP/GCE was used daily and stored in the air at room temperature. The experimental results indicated that the SBAP/GCE could retain 97.8% of its initial response after two weeks, revealing that novel sensor fabricated possesses long-term stability. Thereafter, the voltammetric signal started to decrease indicating the need for re-depositing of a new polymer film.

The reproducibility of SBAP/GCE was evaluated by five replicate measurements of 1×10^{-6} M drug solutions over three days using freshly prepared QF solutions, yielding relative standard deviations of 1.9%. To evaluate the repeatability of the electrode response, the drug solution at a fixed QF concentration of 2.5×10^{-6} M was examined by performing six experiments on the same day using the same analyte standard solution. The RSD values of the peak current (mean i_p = 2.54 μA) and the recorded oxidation peak potential (mean E_p = 1.05 V) were no greater than 1.3% and 0.8%, respectively. Additionally, a series of five sensors prepared repeatedly in the same manner were also tested and the RSD observed was only 1.4% indicating good fabrication reproducibility. These experiments confirmed that the SBAP/GCE prepared by electro-polymerization has uniform and stabile film on the electrode and seemed to be acceptable for most practical application.

3.6. Interference

Under optimized experimental conditions described previously, the effect of some foreign species on the determination of QF at 1×10^{-6} M level were evaluated in detail. The tolerance limit was taken as the concentration of foreign substances which gave an error less than ±5% in the determination of the drug. The presence of K^+, Na^+, Ca^{2+}, Cl^-, NO^{3-} (1000-fold), Zn^{2+}, Mg^{2+}, $HPO_4{}^{2-}$, $SO_4{}^{2-}$ (500-fold), L-alanine, citric acid (400-fold), lactose (300-fold) and L-cystine (200-fold), had no influence on the peak current of QF. Possible interferences for QF detection were investigated by addition of ascorbic acid, glucose and uric acid to the buffer solution in the presence of 1×10^{-6} M drug. The experiments displayed that the voltammetric response of QF at the SBAP/GCE did not change after adding 400-fold concentration of ascorbic acid, and 300-fold of glucose and 200-fold of uric acid indicating that the selectivity of method could be satisfied for the quantification of QF in biological fluids where these endogenous substances are always present. Selectivity of the developed procedure for the assay of QF using SBAP/GCE was investigated by observing any interference encountered from co-administrated drugs such as diazepam, valproic acid and lithium carbonate. The results showed that the equal concentration (2.5×10^{-6} M) of these drugs had no influence on the QF currents, with deviations below 5%.

3.7. Applications of novel electrochemical sensor

In order to evaluate practical utility of novel electrochemical sensor, QF was analyzed in commercial tablets. There is no official method reported in any pharmacopoeias so far for the determination of QF in its dosage form. In order to eliminate matrix effects, the standard addition method was used for the determination of

Table 2
Application of the proposed method to the determination of quetiapine fumarate in delayed-release tablets.

	QF
Labelled claim (mg)	230.26
Amount found (mg)[a]	227.27
RSD%	1.51
Bias%	−1.30
Added 10^7 (mol L^{-1})	5.00
Found 10^7 (mol L^{-1})[a]	4.96
Recovery %	99.28
RSD%	1.59
Bias%	−0.72

[a] Each value is the mean of five experiments.

QF in commercial delayed-release tablets. The results obtained using proposed sensor are in good agreement with the claimed amount. The results of these analyses are summarized in Table 2. The analysis of QF in its pharmaceutical formulation exhibited the mean recovery of 98.7% with the relative standard deviation of 1.5% indicating the adequate accuracy and precision of the SBAP/GCE. The effect of excipients upon the voltammetric response for the examined drug was studied by adding different known amounts of standard to the formulation solution samples. The mean recovery of 99.3% indicated that excipients did not interfere with the assay avoiding a separation step, and thus corroborating the suitability of the proposed sensor for this purpose. The results obtained with the proposed method were compared with those obtained by reported HPLC method [11]. The mean recovery obtained after five repeated experiments was calculated as 99.6% with RSD of 0.9%. The results of the Student *t*-test (0.20) and variance ratio *F*-test (0.74) show that there are no significant differences between the techniques with regard to accuracy and precision. Theoretical values for *t* and *F* at a significance level of 0.05 are 2.31 and 6.39, respectively.

Finally, the SBAP/GCE in combination with the proposed voltammetric protocol was applied for direct measurement of the drug in biological samples (Fig. 6). The method sensitivity obtained complies with the expected serum concentration level after treatment with the therapeutic relatively low daily dose (50 mg). Quantification of QF in human serum and urine samples at the therapeutic concentration range could not be feasible without preconcentration of the positively charged drug molecule on the SBAP/GCE surface. The pretreatment for the samples preparation, such as time-consuming extraction and evaporation steps prior to the analysis of drug in biological samples, was not required. The recovery rates of the spiked serum samples were determined and ranged from 97.3% to 99.1% with RSD of 1.6%. The well-defined anodic peak (E_p = +0.56 V) was noticed in urine samples along with peak of QF. The less positive peak in the square-wave voltammograms was identified as uric acid via the addition of a known standard of uric acid which is fairly readily oxidizable. The peak observed was differentiated from that of QF and did not increase with increasing the accumulation time. Uric acid (pK_a = 5.75) are completely in non-ionized forms in experimental conditions under which voltammetric measurements were performed. Poly(SBAP) film contains negatively charged sulfonate and carboxyl groups and such surface layer allow facile accumulation of the positively charged drug molecule, while prevent accumulation of uric acid onto the electrode surface, as well as other negatively charged oxidizable substances found in biological fluids, so the selectivity of the proposed method is sufficient for direct quantification of QF in human urine samples. The mean recovery of 96.9 ± 2.1% was achieved from this type of matrix. From the experimental results, it is obvious that the novel sensor has great potentials for practical analysis of biological samples.

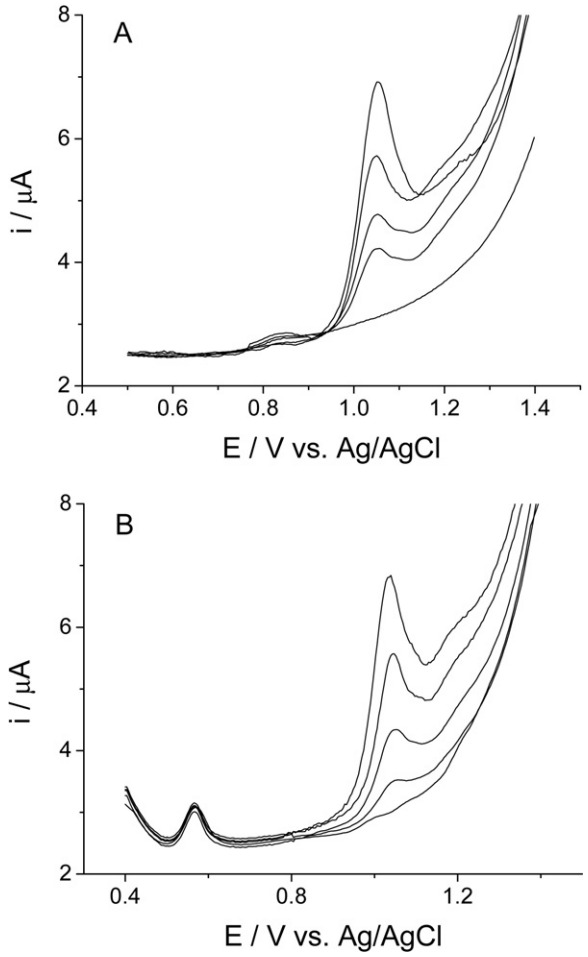

Fig. 6. Square-wave voltammograms of quetiapine fumarate for increasing drug concentrations at the SBAP/GCE recorded in (A) spiked human serum (5×10^{-7}–3×10^{-6} M) and (B) urine samples (3×10^{-7}–1.8×10^{-6} M) together with corresponding background recordings; SWV settings and accumulation conditions same as in Fig. 5.

4. Conclusions

In this work, a simply designed and promising sensor for fast quantification of trace amount of QF was developed. A novel electrochemical sensor displayed a strong adsorptive activity. Based on this property, the concentration of QF in pharmaceutical formulations and biological fluids could be determined directly by voltammetry with excellent sensitivity and high selectivity. The proposed sensor has long term stability and good reproducibility with benefits of fast response time, ease of preparation and regeneration of the surface that makes the proposed method useful in the determination of QF in real samples. The paper described, for the first time, the interfacial adsorptive character of QF molecule.

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