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Original scientific paper

Analysis of sulfamethoxazole by square wave voltammetry using new carbon paste electrode

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Abstract

In this work a new model of carbon paste electrode was employed to determine sulfamethoxazole (SMX), an antibiotic used to treat infections in human and veterinary medicine, by the square wave voltammetric modality (SWV). More specifically, the electrochemical behavior of SMX was investigated by cyclic voltammetry (CV), and the quantitative analysis of SMX was provided by SWV. The analytical curve was obtained with a linear correlation coefficient (r) of 0.985 and standard deviation (SD) of 0.005 μA. Limits of detection and quantification were found as 2.3×10^{-6} and 7.7×10^{-6} mol L^{-1}, respectively. According to the obtained results, the new carbon paste prototype electrode can successfully be employed in this kind of electroanalytical applications.

Keywords

Carbon paste electrode; Electroanalysis of pharmaceutical compounds; electrochemical sensors; voltammetric analysis

Introduction

Concerning a drug analysis, electroanalytical methods offer several advantages: they are versatile, fast, sensitive, inexpensive, and environmentally friendly due to the limited use of chemicals [1]. Recently developed electrochemical devices efficiently monitor pollutants through direct or indirect reactions between the contaminant and the electrode surface, what makes them potentially applicable *in situ* [2-4]. The large-scale use of this technology relies on the scientific knowledge

and nowadays, scientists have investigated many electrodes for this purpose [5]. Gold electrode, glassy carbon electrode, platinum disc electrode, graphite electrode, chemically modified electrodes, carbon nanotube and carbon paste electrodes (CPEs) are some of the examples already reported in the literature [1,5].

Ralph Norman Adams introduced CPE in 1958 [6-9]. This electrode consists of a mixture of carbon powder and a non-electroactive liquid binder and offers a broad potential window, low residual current (background), unique surface characteristics, low cost, and versatile preparation [6-16]. For this reason, CPE has been widely employed to determine the sulfamethoxazole (SMX) by the voltammetric analysis. SMX is chemically known as 4-amino-N-(5-methylisoxazol-3-yl)-benzene sulfonamide and its chemical structure is shown in Figure 1. SMX constitutes a sulfonamide that helps to treat infections in human and veterinarian medicine [17,18]. Obviously large consumption of this antibiotic agent, however, can lead to environmental and public health problems.

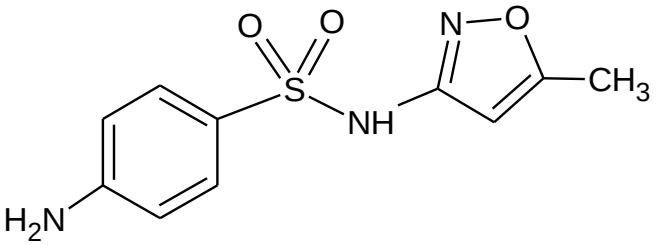


Figure 1. *Chemical structure of sulfamethoxazole.*

Several methods for sulfamethoxazole determination have already been described in the literature, including chromatographic methods coupled with different detectors [18,22,24], capillary electrophoresis [25,26], spectrophotometry [27,28], and electroanalytical methods [29-39]. It is possible to observe in the literature, however, that non-renewable surface devices for CPE application were applied. Conventional CPEs must usually be refilled with the carbon paste, increasing thus the consumption of material and time analysis. In this context, we developed a new model of CPE and applied it for the sulfamethoxazole analysis using the square-wave voltammetry.

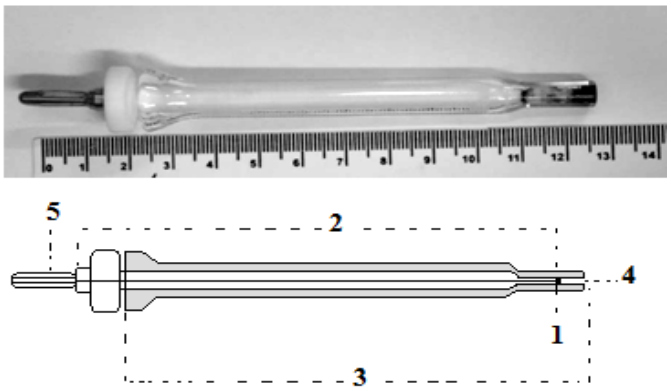
Experimental

Materials and reagents

A stock solution of 0.005 mol L^{-1} SMX (Sigma Aldrich) was prepared in the methanol (Merck). The following electrolyte solutions were used in this research: 0.1 mol L^{-1} potassium chloride (Synth), 0.1 mol L^{-1} sodium nitrate containing 0.01 mol L^{-1} potassium ferricyanide, 0.1 mol L^{-1} sodium perchlorate; 0.05 mol L^{-1} sulfuric acid (Merck)/methanol in 70:30 % (v/v) ratio and 0.04 mol L^{-1} Britton–Robinson (BR) buffer, pH 2.18, prepared by mixing 0.04 mol L^{-1} boric acid (Carlo Erba), acetic acid and orthophosforic acid (Merck). Mixtures of graphite powder (particle size = 19.2-168.5 µm, Analítica) and mineral oil (Nujol, União Química) were used to prepare the carbon paste [14,16].

Carbon paste electrode construction

The carbon paste electrode was prepared by mixing 2.25 g of graphite powder with 0.75 g of mineral oil. This mixture was homogenized by magnetic stirring in a 25 mL beaker containing 10 mL of chloroform (Merck). The paste was obtained after evaporation of the solvent. The carbon paste was packed into a versatile electrode body fabricated in our laboratory. It consisted of a glass cylindrical tube in the form of a syringe (o.d. 7 mm, i.d. 3 mm) and contained a platinum rod to establish the electric contact (Figure 2).



1 - Electric contact (platinum) **2 - poston (Teflon)**
3 - cylindrical glass tube **4 - carbon paste**
5 - electric contact (stainless steel)

Figure 2. *Carbon paste electrode developed in our research group.*

Instrumentation

The voltammetric experiments were conducted using a potentiostat model μAUTOLAB III (Eco Chemie) connected to a personal computer. The experiments were carried out in a three-electrode system consisting of a carbon paste working electrode, platinum spiral wire auxiliary electrode and Ag/AgCl, 3.0 mol L^{-1} KCl, reference electrode, all arranged in a 5-mL electrochemical cell (Figure 3). The solutions were deoxygenated with nitrogen for 15 min prior measurements.

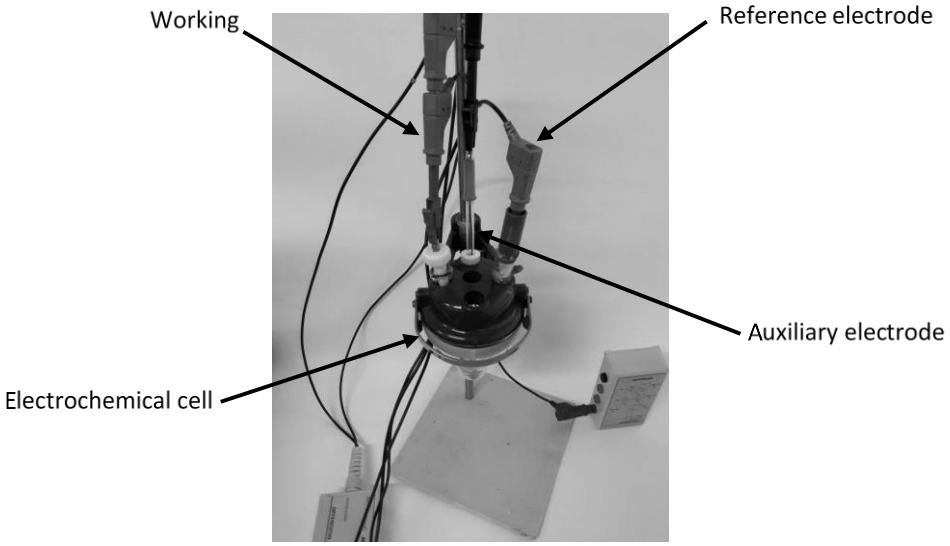


Figure 3. *Electrode arrangement in electrochemical cell.*

Voltammetric analysis

The voltammetric behavior of the carbon paste electrode was initially investigated by CV measurements performed in different solutions, such as 0.1 mol L^{-1} KCl, 0.1 mol L^{-1} $NaNO_3$ containing 0.01 mol L^{-1} $K_3Fe(CN)_6$, 0.04 mol L^{-1} BR–buffer (pH 2.18) and 0.1 mol L^{-1} $NaClO_4$. CV analysis was carried out at potentials ranging from –0.1 V up to 1.8 V and scan rates from 10 to 100 mV s^{-1}. Subsequently, the quantitative analysis of SMX was conducted by SWV with previously optimized experimental parameters (frequency and pulse amplitude) and a constant step potential of 50 mV. Potential window ranged from –0.1 to 1.8 V *vs.* Ag/AgCl and 0.04 mol L^{-1} BR–buffer (pH 2.18) was used as the supporting electrolyte. The voltammetric parameters investigated for the SMX assay were: frequencies of 8, 12, 18, 20, and 24 Hz and pulse amplitude ranging from 10 to 100 mV. SMX was

analyzed at different concentrations by the standard addition method, *i.e.* the analytical curve was constructed by adding aliquots of the SMX stock solution to the electrochemical cell. A linear curve was achieved for SMX concentrations ranging from 7.9 to 24.0 μmol L^{-1}.

Results and discussion

Electrochemical properties of CPE in different electrolyte solutions

The CPE was initially tested in various aqueous systems, in order to provide information about its basic response and stability in different electrolytes. Figure 4(A-D) illustrates the cyclic voltammograms of CPE measured at different scan rates in 0.1 mol L^{-1} KCl, 0.1 mol L^{-1} $NaNO_3$ containing 0.01 mol L^{-1} $K_3Fe(CN)_6$, 0.01 mol L^{-1} $NaClO_4$, and 0.04 mol L^{-1} BR–buffer (pH 2.18). Among almost all presented CVs suggesting stability of CPE with not specific response in the specific electrolyte and given potential region, Figure 4B presents a typical cyclic voltammogram for the $K_3Fe(CN)_6^{4-/3-}$ redox couple. This particular reaction usually served as the test for the redox activity of an electrode. Peaks at 0.36 V (E_{pa}) and 0.19 V (E_{pc}) suggest reversible behavior of redox couple at the CPE in the potential range from –0.1 V to 0.7 V *vs.* Ag/AgCl, with scan rate between 10 to 100 mV s^{-1} [12].

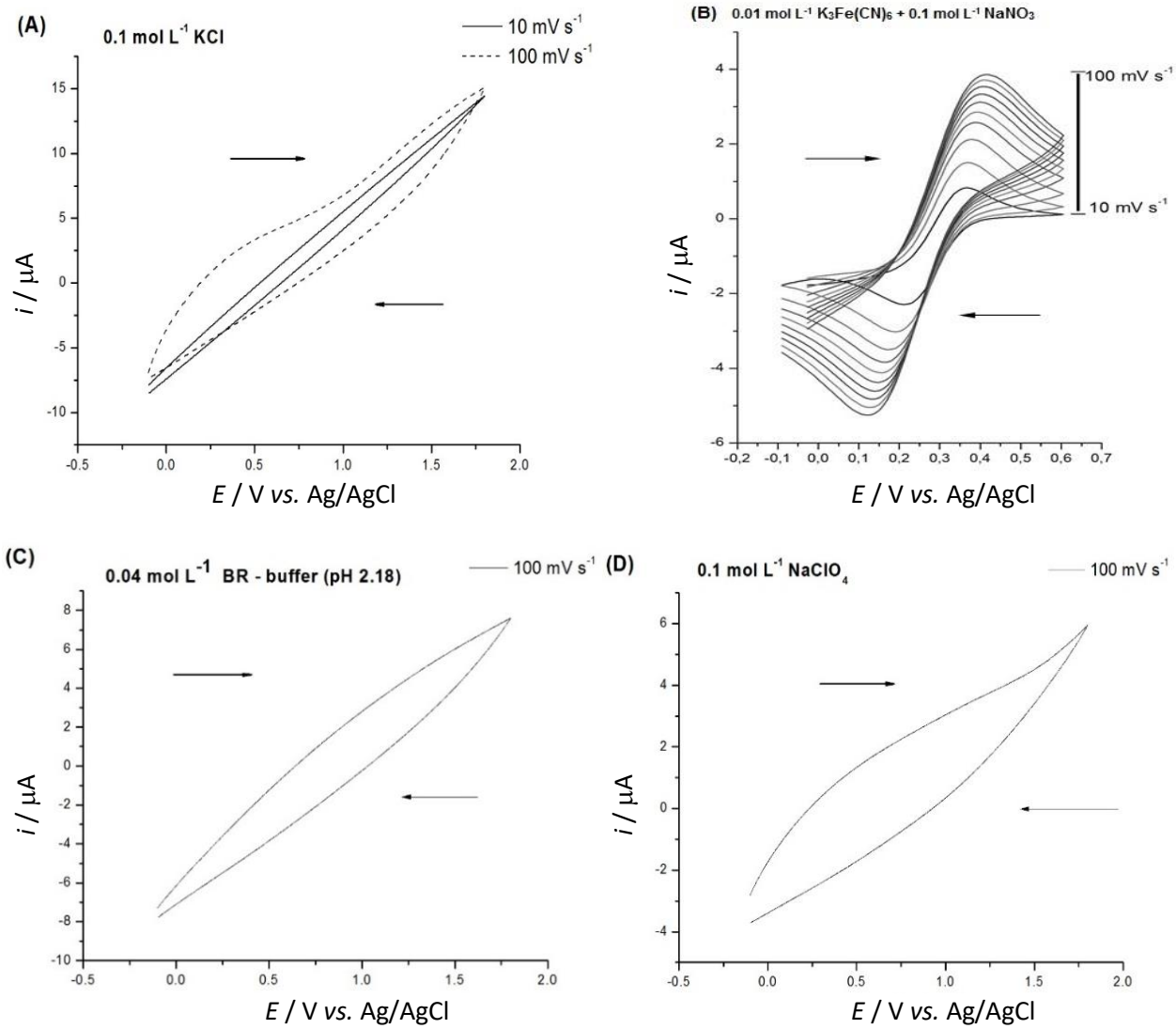


Figure 4. *Cyclic voltammograms of the CPE in (A) 0.1 mol L^{-1} KCl (100 mV s^{-1}), (B) 0.1 mol L^{-1} $NaNO_3$ + 0.01 mol L^{-1} $K_3Fe(CN)_6$, (C) 0.04 mol L^{-1} BR–buffer (pH 2.18) (100 mV s^{-1}) and (D) 0.1 mol L^{-1} $NaClO_4$, at denoted scan rates and potential range from –0.1 to 1.8 V* vs. *Ag/AgCl.*

Literature reveals that the supporting electrolyte plays an essential role in the voltammetric signal of SMX [29,40-42]. Besides, the sulfa drugs have two dissociation constants (pK_a), which in the case of the SMX correspond to the amino functional group with p*K*a value of 1.8 and amide functional group with a pK_a value of 5.6 [29,33,37,40,44]. Therefore the BR–buffer solution (0.04 mol L^{-1} in acetic, phosphoric and boric acids) (pH 2.18), with CV shown in Figure 4C was chosen as the experimental medium in the voltammetric studies of SMX.

Figure 5(A-B) displays representative CVs of 0.005 mol L^{-1} SMX together with the corresponding background currents recorded for the proposed CPE and a commercial glassy carbon electrode, respectively. Figure 5A shows CV profiles of CPE in a blank solution of 0.04 mol L^{-1} BR–buffer (pH 2.18) and in the same solution containing 0.005 mol L^{-1} SMX, whereas Figure 5B shows CV profiles of glassy carbon in a blank solution of 0.05 mol L^{-1} s acid/methanol 70:3 (v/v) (pH 1.38) and in the same solution containing 0.005 mol L^{-1} SMX. An irreversible two-electron oxidation voltammetric peak appeared in both cases when 0.005 mol L^{-1} SMX was present in the solution [29,40]. Also, much lower background current that was obtained for the CPE as compared with the solid glassy carbon, suggests that the proposed CPE electrode could be more sensitive for SMX oxidation [6-16]. The higher background current observed for the glassy carbon electrode in Figure 5B stems from the oxygen evolution [43].

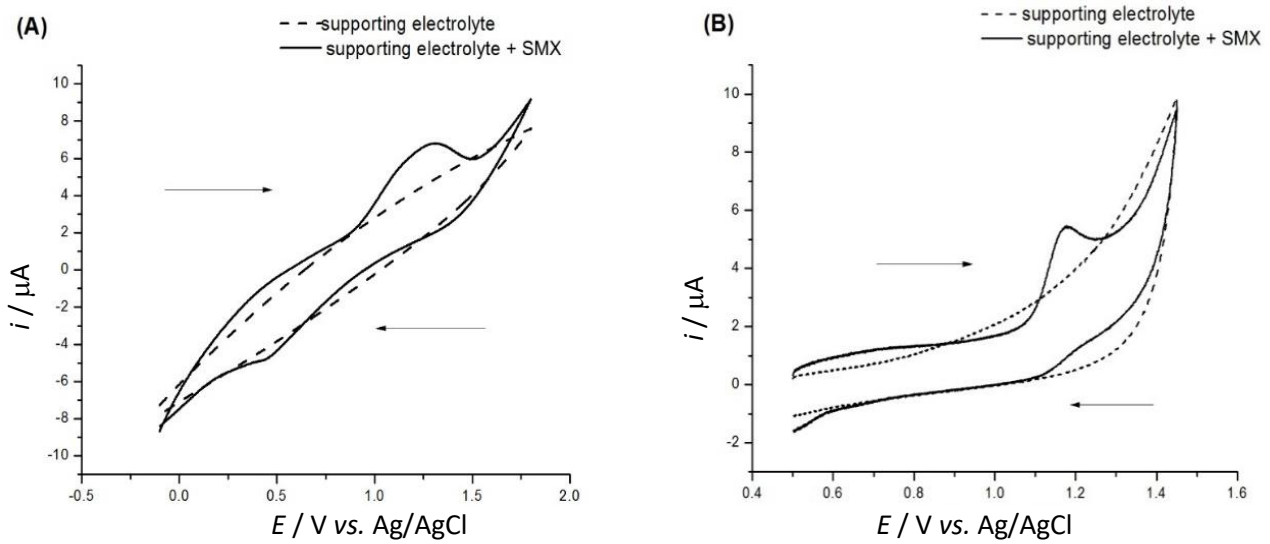


Figure 5. *Cyclic voltammograms at 100 mV s^{-1} of 5 mmol L^{-1} SMX for (A) CPE in 0.04 mol L^{-1} BR–buffer (pH 2.18), potential range = –0.1 V to 1.8 V* vs. *Ag/AgCl and (**B**) glassy carbon electrode in 50 mmol L^{-1} sulfuric acid/methanol 70:3 (v/v) (pH 1.38), potential range = 0.5 V to 1.5 V* vs. *Ag/AgC).*

CPE electrochemical response toward SMX

The optimized experimental parameters that pointed out the best results for SMX determination using SWV technique were obtained by variations of pulse amplitude and frequency. The pulse amplitude was varied in the range of 10–100 mV, at the constant frequency of 12 Hz. In this case, the optimized result was defined as the parameter value that produced increase of the peak current without shifting the peak potential or making any significant increase in the peak width. Hence, 100 mV was chosen as the square-wave pulse amplitude value. Afterwards, the effect of frequency was evaluated in the range 8–24 Hz, keeping constant the pulse amplitude of 100 mV. The best result was achieved at *f* = 12 Hz.

According to previous literature investigations, sulfonamide oxidation results in a formation of the corresponding iminobenzoquinone intermediate, shown as peak (1) in Figure 6. The SWV

responses presented in Figure 6 showed that the oxidation current peaks increased with increase of the frequency. Hence the frequency of 12 Hz was chosen for further analysis because of the best resolution of the voltammetric peak (Figure 6, peak (2)).

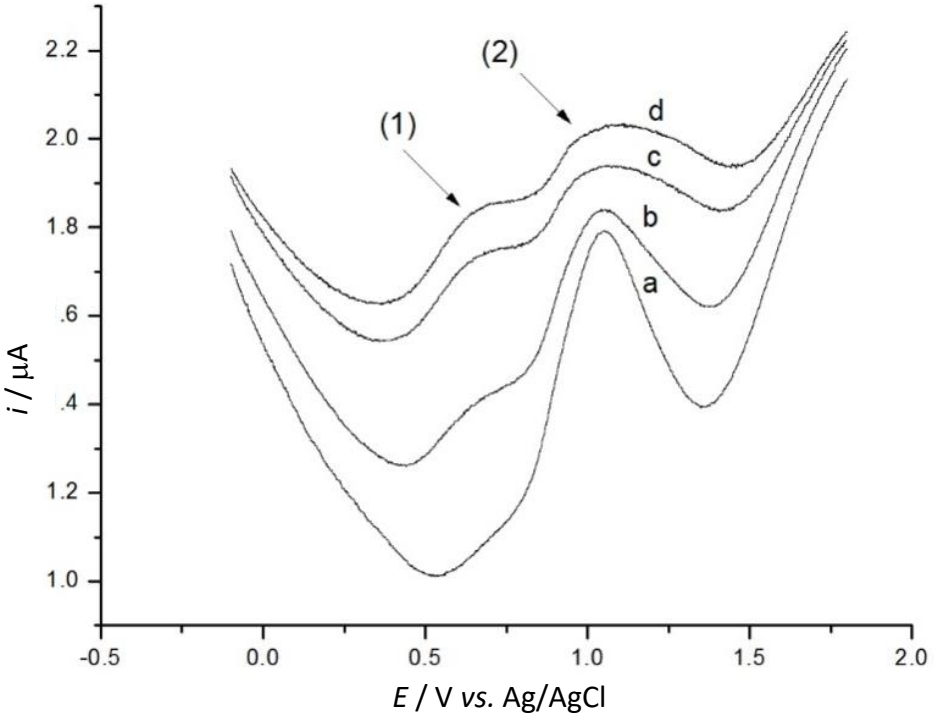


Figure 6. *Effect of the frequency parameter on the SWV response of the CPE in 0.04 mol L^{-1} BR–buffer (pH 2.18) containing 24.0 µmol L^{-1} SMX: (a) 12 Hz, (b) 18 Hz, (c) 20 Hz, (d) 24 Hz. Potential range = 0.5 to 1.6 V* vs. *Ag/ACl, pulse amplitude = 100 mV, scan increment = 2 mV.*

According to the literature, the SMX electrochemical oxidation occurs at the primary amino groups (-NH_2) [29,40]. Figure 7 illustrates the mechanism of SMX oxidation that as a two-electron and pH dependent reaction, possibly takes place in an acid medium.

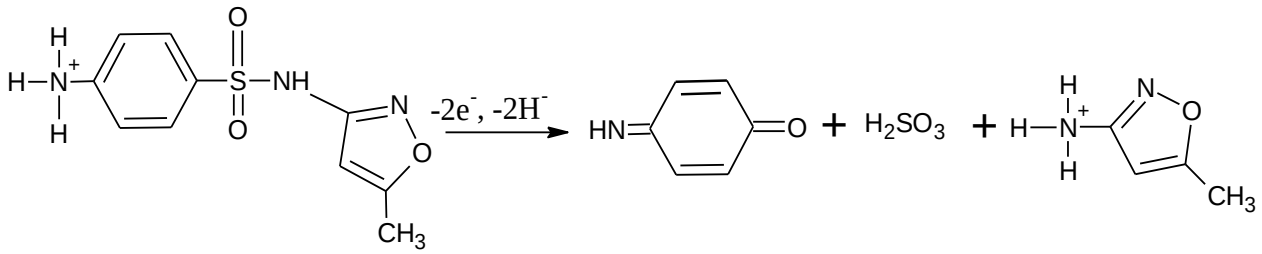


Figure 7. *SMX oxidation at carbon paste electrode in acid medium*

The electrochemical behavior of SMX in different concentrations was assessed by successive additions of this drug in concentrations ranging from 7.9 to 24.0 µmol L^{-1} to the electrochemical cell. As seen in Figure 8(A), the anodic peak current at 1.07 V *vs.* Ag/AgCl (irreversible oxidation peak) increased upon rising of the SMX concentration. The analytical curve drawn in Figure 8(B) shows a linear correlation coefficient r = 0.985 with a standard deviation SD = ±0.005 µA. The corresponding linear equation was adjusted as i_{pa} = 0.24 µA + 6.5×10^3 µA /mol L^{-1} [SMX]. The limit of detection calculated according to the criterion 3SD/m ratio, where m is the slope of the analytical curve, gave 2.3×10^{-6} mol L^{-1}, while the limit of quantification based on the criterion of 10SD/m ratio, was adjusted as 7.7×10^{-6} mol L^{-1}.

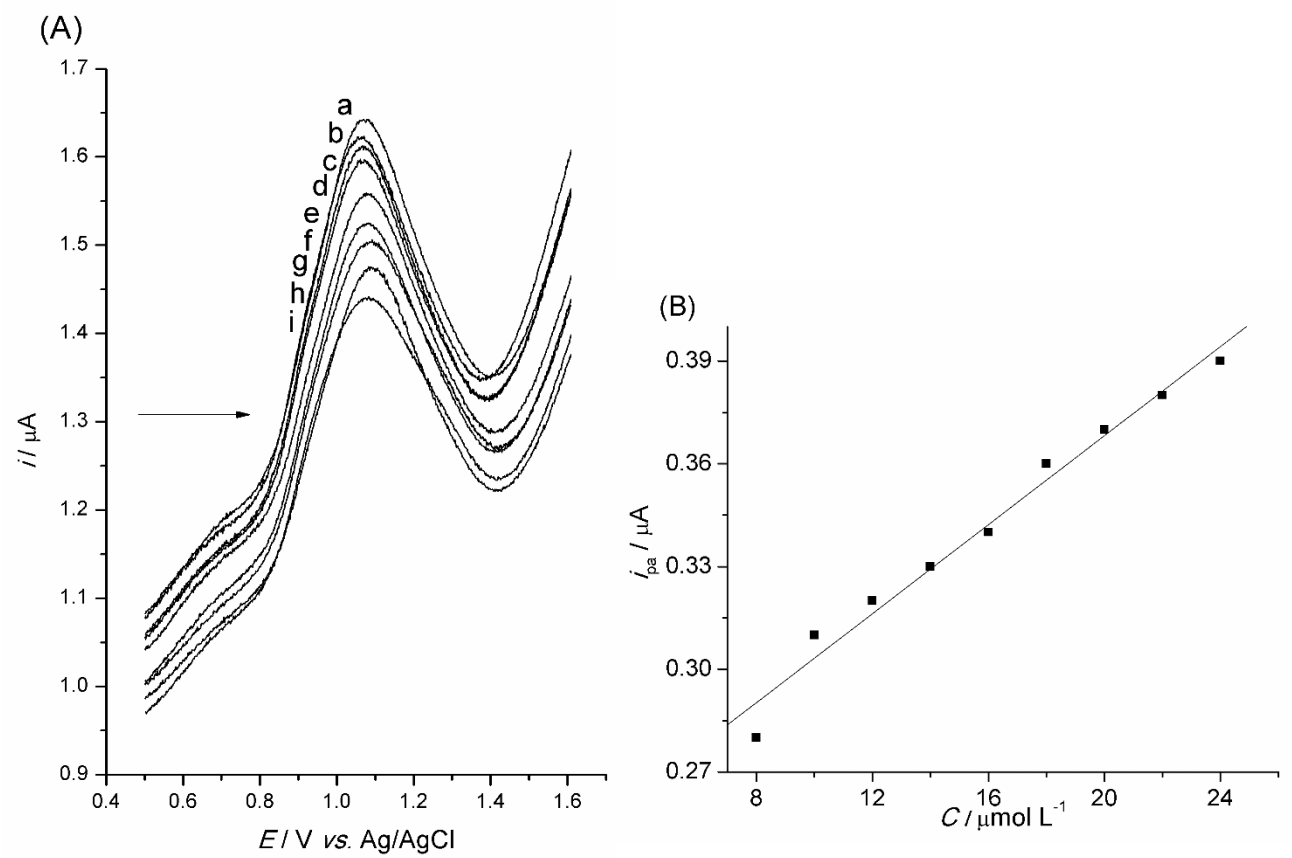


Figure 8. *(A) Influence of SMX concentration in 0.04 mol L^{-1} Britton–Robinson buffer (pH 2.18) solution on the voltammetric response of CPE: (a) 24.0 µmol L^{-1}, (b) 21.9 µmol L^{-1}, (c) 20.1 µmol L^{-1}, (d) 18.0 µmol L^{-1}, (e) 15.9 µmol L^{-1}, (f) 14.0 µmol L^{-1}, (g) 12.1 µmol L^{-1}, (h) 10.0 µmol L^{-1}, and (i) 7.9 µmol L^{-1}. Potential range = 0.5 to 1.6 V* vs. *(Ag/ACl), frequency = 12 Hz, pulse amplitude = 100 mV, scan increment = 2 mV. (B) Analytical curve of the peak current, µA* vs. *SMX concentration, µmol L^{-}).*

Conclusions

The novel and efficient support for the carbon paste substrate is developed allowing determination of sulfamethoxazole at the µmol L^{-1} level. The developed CPE showed an excellent stability in different electrolyte media and excellent voltammetric response for the $K_3Fe(CN)_6^{4-/3-}$ redox couple probe. Oxidation of SMX at the CPE occurring at about 1.07 V *vs.* Ag/AgCl was found to be an irreversible 2-electron and pH dependent process. An electrocatalytic effect was observed in comparison with glassy carbon electrode. Another peak occurring at about 0.49 V *vs.* Ag/AgCl was observed when frequency values higher than 12 Hz were applied and ascribed to the formation of the corresponding iminobenzoquinone intermediate. The developed CPE is an inexpensive and versatile electrode, having high potential for application as a transducer in a device serving for determination of sulfamethoxazole.

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