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Electrochemical behaviour of epinephrine and uric acid at a Sonogel–Carbon L-Cysteine modified electrode

H. El Bouhouti [a,b], I. Naranjo-Rodríguez [a,*], J.L. Hidalgo-Hidalgo de Cisneros [a], M. ElKaoutit [a], K.R. Temsamani [b], D. Bouchta [b], L.M. Cubillana Aguilera [a]

[a] Departamento de Química Analítica, Facultad de Ciencias, Universidad de Cádiz, 11510 Puerto Real, Cádiz, Spain
[b] University Abdelmalek Essaâdi, Department of Chemistry, Faculty of Sciences of Tétouan, Equipe de Recherche Electrochimie et Systèmes Interfaciaux (ERESI), M'Hannech II, B.P. 2121, 93002, Tétouan, Morocco

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ABSTRACT

The Sonogel–Carbon electrode is a special class of sol–gel electrode that exhibits favourable mechanic and electric properties to be used as electrochemical sensor. In this study, Sonogel–Carbon modified with L-Cysteine was used to prepare a novel electrochemical sensor. The objective of this novel electrode modification was to seek new electrochemical performances for detection of epinephrine in the presence of uric acid. The response of catalytic current with epinephrine concentration shows a linear relation in the range from 1×10^{-7} to 5×10^{-4} M with a correlation coefficient of 0.998, and a detection limit of 8.7×10^{-8} M. The modified electrode had also been applied to the determination of epinephrine and uric acid in biological samples with satisfactory results. A surface characterisation of this modified electrode was carried out helped by scanning electron microscopy (SEM) and X-Ray energy dispersive spectroscopy (EDS).

1. Introduction

Epinephrine (EP) is an important neurotransmitters in mammalian central nervous systems [1,2], and it exits in the nervous tissue and body fluid in the form of large organic cations. The changes of its concentration may result in many diseases [3]. Thus, a quantitative determination of EP concentration is significant for developing nerve physiology, pharmacological research and life science.

There are some methods applied for the determination of EP, such as high performance liquid chromatography (HPLC) [4,5], capillary electrophoresis [6,7], flow injection [8,9], chemiluminescence [10,11], fluorimetry [12] and spectrophotometry [13,14]. As an electroactive device, it can also be studied via electrochemical techniques. Some reports showed the electrochemical response of EP on different kinds of electrodes, such as electrochemically pretreated glassy carbon electrode [15], carbon fiber microelectrode [16], polymer film modified glassy carbon electrode [17,18] and self-assembled monolayer modified electrode [19,20].

Uric acid (2,6,8-trihydroxypurine, UA) is the primary product of purine metabolism [21]. Physiological UA serum levels range from 41 to 88 mg mL^{-1} and urinary excretion is typically 250–750 mg per day [22]. Its abnormal concentration level in a human body may be symptoms of several diseases, such as gout, hyperuricaemia, and Lesch-Nyhan syndrome. Leukemia, pneumonia, and so on are also associated with enhanced urate levels [23,24]. So it is desirable to have a simple and direct method for monitoring the concentration of UA in biological fluids. UA and EP are coexistent in biological fluids of human, so the simultaneous detection of UA and EP in a mixture is quite attractive to biological and chemical researches. Individual determination of UA or EP has been reported by many researches; however, simultaneous determination of them is rarely presented.

It nowadays exists a great interest in the development and application of sol–gel-derived carbon-based electrodes for electrochemical applications. The sol–gel process is a chemical synthesis technique that enables the possibility of preparing a wide variety of oxide compounds at far lower temperatures than conventional methods. Recently, some of us have developed a new type of graphite-based sol–gel electrode, the Sonogel–Carbon electrode, which is obtained using high-energy ultrasounds. Classical procedures for the synthesis of acid catalysed sol–gel-based electrode materials include the addition of an alcoholic solvent to the initial precursor mixture to make it homogeneous and the employment of an ultrasound bath for several minutes to promote the hydrolysis. On the contrary, by means of sonocatalysis, high-energy ultrasounds are applied directly to the precursors, and ultrasonic cavitation is achieved so that hydrol-

* Corresponding author. Tel.: +34 956 01 63 53; fax: + 34 956 01 4 60.
E-mail address: ignacio.naranjo@uca.es (I. Naranjo-Rodríguez).

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ysis with acidic water is promoted in only a few seconds and in the absence of any additional solvent. Thanks to the phenomenon of ultrasonic cavitation, sol–gel reactions occur in a unique environment, leading to gels with special characteristics. These so-called sonogels are mainly of high density, with a fine texture and homogeneous structure. The mix of sonogel with spectroscopic grade graphite leads to the Sonogel–Carbon electrode [25,26]. The Sonogel–Carbon electrodes show the general good properties of the other CCE's (Ceramic Carbon Electrodes). Besides, in comparison with other carbon electrodes, they exhibit especially favourable electrochemical properties, such as broad operational range of voltage and very low values of observed charging capacity (C_{obs}). These electrodes show very favourable electroanalytical properties for their use as amperometric sensors and, furthermore, they can easily permit the incorporation of numerous receptor molecules at the Sonogel–Carbon materials, and the deliberate chemical modification of the electrode surface with a suitable reagent results in the control of the rates and selectivities of electrochemical reactions at the solid/liquid interface [27–31].

In the present paper, we propose a new application of Sonogel–Carbon electrodes based on the incorporation of L-Cysteine and the response of the new modified electrode for EP and UA.

2. Experimental

2.1. Reagents and materials

Methyltrimethoxysilane (MTMOS) was from Merk (Darmstad, Germany), Hydrochloric acid (HCl) and sulfuric acid (H_2SO_4) were from Panreac (Barcelona, Spain). L-Cysteine (>99%) was obtained from Fluka Chemical Company (Switzerland). UA (99%) was purchased from Sigma (Barcelona, Spain), EP was purchased from Aldrich (Milwaukee, USA), and used as received. KH_2PO_4 and K_2HPO_4 for phosphate buffer were from Fluka. All reagents were of analytical grade or higher and used as received without further purification. Graphite powder (spectroscopic grade RBW) was from SGL Carbon (Ringsdorff, Germany). Nanopure water was obtained by passing twice-distilled water through a Milli-Q system (18 MΩ cm, Millipore, Bedford, MA).

Glassy capillary tubes, i.d. 1.15 mm, were used as the bodies for the composite electrodes.

2.2. Instrumentation

All electrochemical measurements were performed with an Autolab PGSTAT20 (Ecochemie, Utrecht, The Netherlands). The experiments were carried out in a three-electrode cell at room temperature (25 ± 1 °C), the counter electrode was a platinum wire and a Ag/AgCl, 3 M KCl electrode was used as the reference, the composite-filled capillary tubes were used as working electrode. Differential Pulse Voltammetry (DPV) and Cyclic Voltammetry (CV) were the electrochemical techniques applied to study the behaviour of the Sonogel–Carbon electrodes. Measurements were carried out under N_2 atmosphere when required.

The synthesis of the Sonogels-Carbon was carried out sonicating with a high-power ultrasonic generator, SONICATOR 3000, from MISONIX (MISONIX Inc., Farmingdale, NY, USA) (equipped with a 13-mm titanium tip), that provides a maximum power of 600 W.

Scanning electron microscopy (SEM) studies were carried out on a QUANTA 200 (FEI Company, Hillsboro, Oregon, USA) operating at 20 keV and equipped with a Microanalyzer to perform X-Ray energy dispersive spectroscopy (EDS).

2.3. Preparation of the Sonogel electrode

To prepare the Sonosol, the general procedure was as follows: 500 μl of MTMOS and 100 μl of 0.2 M HCl were mixed and then insonated during 5 s with the high-power ultrasonic processor; in this way the mixture is subjected to the phenomenon of ultrasonic cavitation, by which the sol–gel process begins, avoiding the use of alcoholic solvent and reducing drastically the time needed to get an unique phase. In the next step, the adequate amounts of L-Cysteine and graphite powder were added and homogenously dispersed in the obtained Sonosol. After several minutes, the resulting material starts to acquire enough consistency thus it could fill easily the glass capillaries leaving a little extra mixture sticking out of the glass tube to facilitate the subsequent polishing step. After 24 h, the Sonogel–Carbon L-Cysteine composite electrode becomes hardened and, therefore, structured. Adherence between the developed material and the glass was excellent. Before use, the electrodes were polished with No. 1200 emery paper to remove extra composite material and wiped gently with weighing paper. Electrical contact was established by inserting a cooper wire into the capillary.

3. Results and discussion

3.1. SEM and EDS studies

Four different samples of Sonogel–Carbon L-Cysteine electrodes were analyzed by SEM: used Sonogel–Carbon electrode with 2.5% of L-Cysteine; used Sonogel–Carbon electrode with 5% of L-Cysteine; used Sonogel–Carbon electrode with 7.5% of L-Cysteine and used Sonogel–Carbon electrode with 10% of L-Cysteine. A sample of used Sonogel–Carbon electrode without modification was analyzed too.

The used electrodes that contained 2.5% and 5% of modifier show a minimum erosion after use, and a light separation among the material and the glass capillary that serves as body for the electrode. The electrodes with a higher modifier percentage show a higher number of holes, fissures and fractures on their surfaces, besides a much more marked separation between the glass capillary and the Sonogel–Carbon material. This would explain the worst electrochemical behaviour of the modified electrodes with 7.5% and 10% with respect to the Sonogel–Carbon material modified with 5%; this percentage shows the better electrochemical behaviour compared with the other modifier percentages tested.

In the micrographs, great differences are not observed among the electrodes modified with 2.5% and 5%. The difference of electrochemical behaviour between these two types of electrodes can be due fundamentally to the higher modifier proportion that implies a greater presence of the modifier on the active surface of the electrode in the case of the electrodes modified with 5% of L-Cysteine.

In the micrographs of Fig. 1, an image of a Sonogel–Carbon electrode without modifier, together with its EDS spectrum (a); and an image of a Sonogel–Carbon electrode modified with 5% of L-Cysteine, together with its spectrum of EDS (b) are shown. It can be seen that in both cases the separation between the material and the capillary body is minimum. The main difference between both images is the presence of particles of L-Cysteine on the surface of the material that is proven by the presence of sulphur in the elementary composition of the modified material, as it can be observed in the EDS spectra. In Fig. 2, an amplification of a part of the micrograph 1.b is collected; a bright particle is observed and identified as L-Cysteine thanks to the presence of sulphur in the EDS spectrum.

3.2. Cyclic voltammetry

Since uric acid and epinephrine have similar oxidation potential at most solid electrodes and many modified electrodes [32,33], overlapped signals are usually obtained. Separated sig-

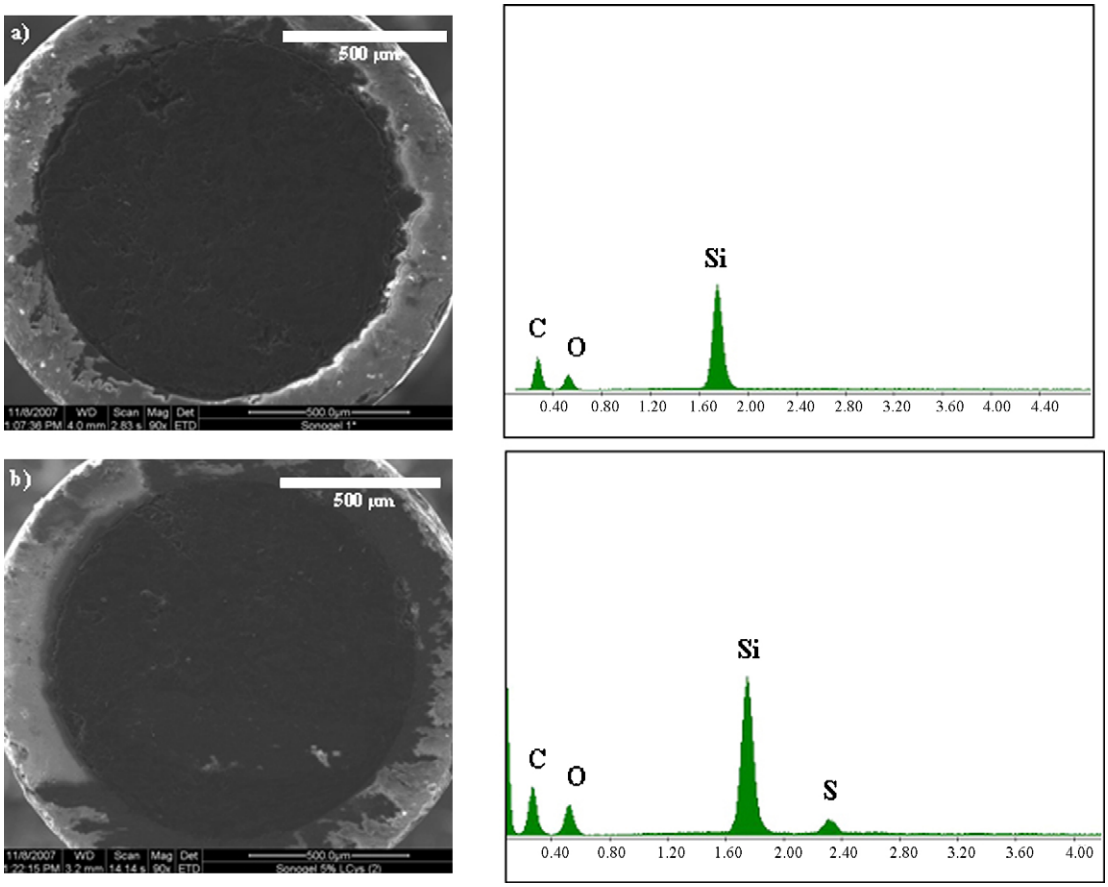

Fig. 1. (a) SEM micrograph and EDS spectrum of a modified Sonogel–Carbon electrode; (b) SEM micrograph and EDS spectrum of a 5% L-Cysteine Sonogel–Carbon modified electrode.

nals can be obtained for UA and EP using a Sonogel–Carbon electrode modified with L-Cysteine. To evaluate the sensitivity and selectivity of this sensor for simultaneous analysis, cyclic voltammograms of mixtures of these species are recorded at 5% L-Cysteine Sonogel–Carbon modified electrode, and compared with an unmodified Sonogel–Carbon electrode (Fig. 3). It can be observed that the peak potentials for EP and UA are indistinguishable at an unmodified Sonogel–Carbon electrode. However, for a 5% L-Cysteine Sonogel–Carbon modified electrode the overlapped voltammetric peaks are resolved into well defined peaks (Fig. 3b) at about 0.38 V and 0.532 V corresponding to the oxidation of EP and UA, respectively.

3.3. Analysis of epinephrine

In bibliography, different detection limits are reported depending of the electrode used and the purpose of the sensing activity. DPV seems to be suitable pulse technique to achieve good result, since it favours the measurement of the faradic over the non-faradic current, which improves sensitivity during the measurements. In this work, the anodic differential pulse peak was used for epinephrine analysis in 0.05 M phosphate buffer solution (pH 6). The calibration curve provided linear relationships for EP: $i = 0.4344 + 0.0521C$, $R^2 = 0.998$, in the range from 10^{-7} to 5×10^{-4} M. The detection limit for EP (S/N = 3) was 8.7×10^{-8} M.

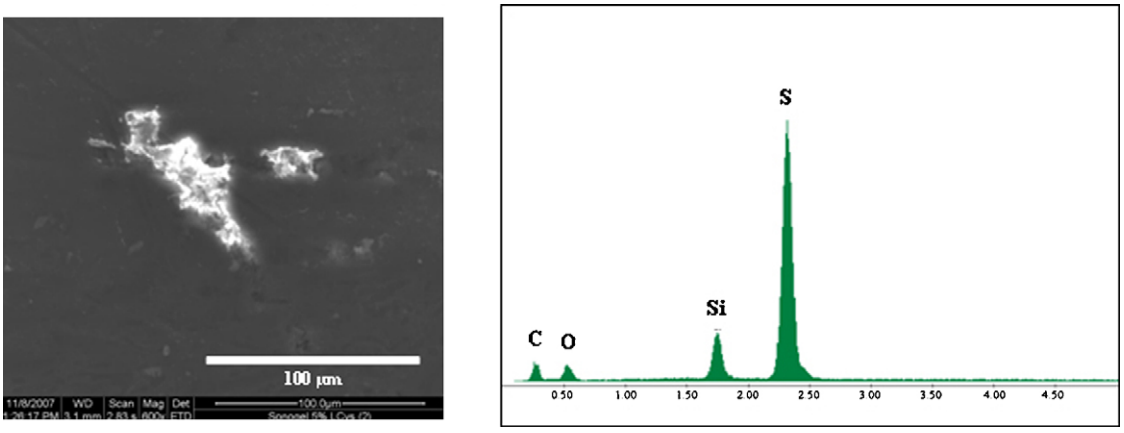

Fig. 2. SEM micrograph detail and EDS spectrum of a L-Cysteine particle in the surface of a modified electrode.

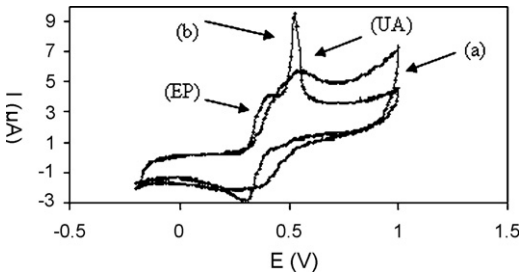

Fig. 3. Cyclic voltammograms for mixture of 10^{-6} M EP and 10^{-4} M UA in 0.05 M phosphate buffer pH 6 (a) unmodified electrode, (b) 5% L-Cysteine Sonogel–Carbon modified electrode. Scan rate 50 mV s^{-1} and $T = 20\,^{\circ}C$.

This value is much lower than values previously obtained [34,35], and compares well with the best values reported [19].

According to SEM studies, the used electrodes containing 5% of modifier exhibit minimum erosion after use; this fact, and the presence of L-Cysteine on the electrode surface, allow us to assume that the properties of the electrodes remain after use. The repeatability of the electrode was estimated by ten repetitive measurements in the same day using the same electrode; relative standard deviation (RSD) of 3.3% was obtained. The useful lifetime is at least one month after repetitive measurements every day. These results show clearly the good performances of our novel sensor.

3.4. Simultaneous determination of EP and UA

Under the optimum condition, the electro-oxidation processes of EP and UA in the mixture were investigated when the concentration of one species changed while the other species was kept constant. The results show that the peak current for EP increased with the increase of EP concentration while the concentration of UA was kept constant. Although the charge current was slightly enhanced after EP oxidation, the anodic peak current for UA did no change. In a similar way, keeping the concentration of EP constant, the oxidation peak current of UA was positively proportional to its concentration, while that of EP did not change. From the experimental results described previously, it was known that in the mixture containing EP and UA the oxidation peak potentials of the two compounds were clearly separated from each other.

The current responses of EP and UA have been investigated to validate the performance of the 5% L-Cysteine Sonogel–Carbon modified electrode for the simultaneous analysis of the two species by changing the concentration of EP and UA in the mixture at the same time. Fig. 4 shows the DPV response at the 5% L-Cysteine Sonogel–Carbon modified electrode simultaneously varying the

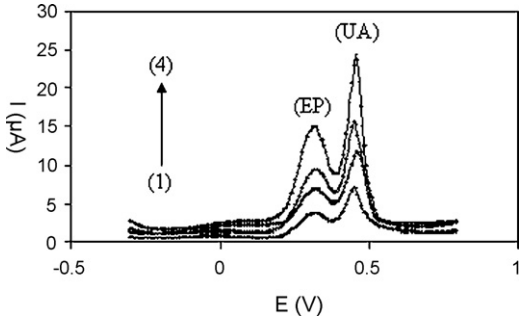

Fig. 4. Differential Pulse Voltmammograms of EP and UA mixtures at a 5% L-Cysteine Sonogel–Carbon modified electrode in phosphate buffer (pH 6). EP contents from 1 to 4 are 1×10^{-6}, 2×10^{-6}, 3×10^{-6} and 6×10^{-6} M, respectively. UA contents from 1 to 4 are 1×10^{-4}, 2×10^{-4}, 3×10^{-4} and 6×10^{-4} M, respectively. Scan rate: 50 mV s^{-1}. Pulse amplitude: 25 mV.

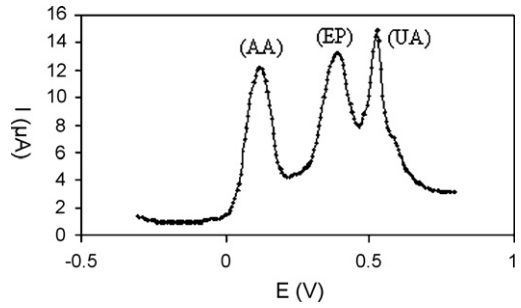

Fig. 5. Differential Pulse Voltammogram of 5×10^{-4} M AA, 5×10^{-6} M EP and 5×10^{-4} M UA mixtures at a 5% L-Cysteine Sonogel–Carbon modified electrode in 0.05 M phosphate buffer (pH 6). Scan rate: 50 mV s^{-1}. Pulse amplitude: 25 mV.

concentration of both EP and UA; the peak current values were proportional to the concentration of EP and UA in the mixture.

3.5. Interference study

It is well known that UA coexists with EP in the extracellular fluid of the central nervous system and its concentration is much higher than that of EP. Hence, UA and AA are important interfering substances for the electrochemical analysis of EP, and the interference from AA and UA was investigated. Fig. 5 shows the DPV of 5×10^{-4} M AA + 5×10^{-6} M EP + 5×10^{-4} M UA in phosphate buffer, pH 6. Well defined anodic peaks at −0.002, 0.277 and 0.438 mV for the oxidation of AA, EP and UA, respectively, were obtained at the 5% L-Cysteine Sonogel–Carbon modified electrode. However, the presence of AA and UA does not modify significantly the signal for EP. The interference studies were also performed with other compounds; for 5×10^{-4} M EP, no interference could be observed for glucose (1:300), glutamic acid (1:300), citric acid (1:200), NaCl (1:700) and KCl (1:700), where the data in brackets were the EP:interferent con-

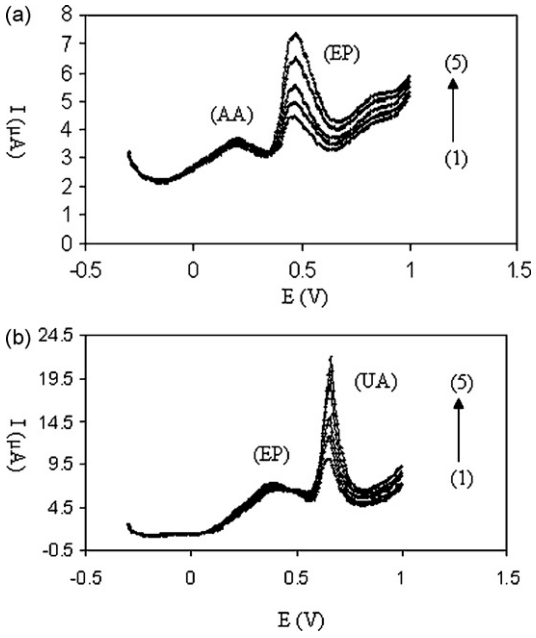

Fig. 6. (a) Differential Pulse Voltammograms of 10^{-4} M AA at a 5% L-Cysteine Sonogel–Carbon modified electrode in urine sample diluted 50 times with phosphate buffer (pH 6) in different concentration of EP (1–5): 2×10^{-6}, 4×10^{-6}, 6×10^{-6}, 8×10^{-6} and 1×10^{-5} M. Scan rate: 50 mV s^{-1}. Pulse amplitude: 25 mV. (b) Differential Pulse Voltammograms of 8×10^{-6} M EP at 5% L-Cysteine Sonogel–Carbon modified electrode in urine sample diluted 50 times with phosphate buffer (pH 6) in different concentration of AU (a–e: 1×10^{-4}, 2×10^{-4}, 4×10^{-4}, 6×10^{-4} and 8×10^{-4} M). Scan rate: 50 mV s^{-1}. Pulse amplitude: 25 mV.

centration ratios. Theses results show a no significant influence of many interferents for EP electrochemical signal at a 5% L-Cysteine Sonogel–Carbon modified electrode.

3.6. Analytical performance in urine sample

The applicability of our Sonogel–Carbon L-Cysteine modified electrode was tested in urine sample. The sample was diluted 50 times with phosphate buffer solution (pH 6) before the measurements to prevent the matrix effect. The DPV recorded for urine sample are shown in Fig. 6. Examination of Fig. 6a shows that peak current of EP increased with an increase in EP concentration, while the concentration of AA was kept constant. As shown in Fig. 6b, keeping the concentration of EP constant, the oxidation peak current of UA was positively proportional to its concentration, while that of EP did not change. These results indicate that selective analysis of AA, EP and UA is possible at a 5% L-Cysteine Sonogel–Carbon modified electrode in urine sample.

4. Conclusion

In this paper, we have prepared a new modified Sonogel–Carbon electrode based on the incorporation of L-Cysteine, and the simultaneous electrochemical analysis of EP and UA in phosphate buffer and urine sample has been tested.

The novel L-Cysteine Sonogel–Carbon electrode shows good behaviour and electrochemical response; thanks to the adequate resolution of EP signal in presence of interferents, the developed electrode is a useful tool for the selective analysis of EP, and for simultaneous electrochemical analysis. The detection limit for EP (8.7×10^{-8} M) is much lower, or compares well with other values reported in literature. The application of the modified Sonogel–Carbon electrode has been also tested in urine sample, with good results.

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