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Polymer/Iron Oxide Nanoparticle Modified Glassy Carbon Electrodes for the Enhanced Detection of Epinephrine

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Abstract: Novel electrochemical sensors for epinephrine (EP) based on a glassy carbon electrode (GCE) modified with a redox polymer film and iron (III) oxide nanoparticles (Fe_2O_3NP) have been developed. Two redox polymers-poly(brilliant cresyl blue) (PBCB) and poly (Nile blue) (PNB), and two different architectures-polymer/Fe_2O_3/GCE and Fe_2O_3/polymer/GCE were investigated. The electrochemical oxidation of epinephrine at the modified electrodes was performed by differential pulse voltammetry (DPV), in pH 7 electrolyte, and the analytical parameters were determined. The results show enhanced performance, more sensitive responses and lower detection limits at the modified electrodes, compared to other electrochemical epinephrine sensors reported in the literature. The best voltammetric response with the lowest detection limit was obtained for the determination of epinephrine at PBCB/Fe_2O_3/GCE. The novel sensors are reusable, with good reproducibility and stability, and were successfully applied to the determination of epinephrine in commercial injectable adrenaline samples.

Keywords: electrochemical sensors · iron oxide nanoparticles · redox polymers · epinephrine.

1 Introduction

The physiological, medical and pharmacological importance of epinephrine (EP) has motivated significant efforts to develop reliable methods for its quantitative determination in both biological fluids and pharmaceutical preparations.

Epinephrine is a catecholamine neurotransmitter which plays a crucial role in the mammalian central nervous system [1,2]. Abnormal levels of epinephrine in the body are related to many diseases, such as cardiac pathologies, schizophrenia and Huntington's and Parkinson's diseases [3–7]. Medically, epinephrine has been used as a common emergency healthcare medicine [8,9]. The detection and quantification of this compound is thus of great interest for neurochemistry, medical diagnosis and treatment, as well as for the discovery of new drugs [10,11].

Various methods, including liquid chromatography [12], spectrophotometry [13], flow injection analysis [14], chemiluminescence [8], capillary electrophoresis [15], and photokinetics [16] have been reported for the determination of epinephrine. However, since epinephrine is easily oxidized, electrochemical methods constitute a quicker, cost-effective, simple and more sensitive alternative, and have been amongst the most appealing and suitable techniques employed [17–39]. Despite the advantages, the electrochemical determination of epinephrine can be a challenging task. Epinephrine electrochemical analysis at bare electrodes gives weak electrochemical responses due to slow electron transfer rate and adsorption on the electrode surface [31,40]. Additionally, epinephrine coexists in natural environments with other electroactive species such as ascorbic acid and uric acid, which oxidize at unmodified electrodes at almost the same potential, leading to poor selectivity and reproducibility [41,42]. To overcome these limitations, the use of modified electrodes for monitoring epinephrine has been proposed [17–39].

Amongst the modified electrodes developed for the determination of epinephrine, polymer modified GCE have been the most reported. Poly(caffeic acid) [43], poly (L-aspartic acid) [44], poly(indoleacetic acid) [17], poly(L-methionine) [18], poly(eriochrome black) [19] are a few examples of polymers that have been used to modify carbon electrodes. The combination of poly(phenazine) polymers with multi-walled carbon nanotubes (MWCNT) [20–23] or graphene [24] to prepare modified GCE with different architectures has also been frequently investigated. For instance, poly(methylene blue)/MWCNT/GCE [20], poly(brilliant cresyl blue)/MWCNT/GCE [21], poly (malachite green)/MWCNT/GCE [22], poly(neutral red)/MWCNT/GCE [23] and poly(brilliant cresyl blue)/graphene/GCE [24] have been successfully applied as EP sensors. Carbon nanotube modified electrodes have also been prepared and used for EP detection, in combination with other components such as cyclodextrin [25], Nafion [26], hematoxylin [27], cobalt phthalocyanine [28], tyrosinase [29], dopamine dithiocarbamate [30], chitosan [31], and iron oxide nanoparticles (Fe_3O_4) [45]. Gold [33–36], ITO [37] and graphene modified electrodes [38,39] are further attempts to the fabrication of EP electrochemical sensors.

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In this work, glassy carbon electrodes (GCE) were modified with the poly(phenazine) polymers poly(brilliant cresyl blue) (PBCB) and poly(Nile blue) (PNB) and Fe_2O_3NP to develop novel electrochemical sensors for the detection of epinephrine. Four polymer/Fe_2O_3NP sensor architectures-PBCB/Fe_2O_3/GCE, Fe_2O_3/PBCB/GCE, PNB/Fe_2O_3/GCE, Fe_2O_3/PNB/GCE were employed for epinephrine determination, in phosphate buffer pH 7 aqueous solution, using differential pulse voltammetry (DPV). The results showed that the new electrochemical sensors have good sensitivity and lower detection limits than those reported for other epinephrine sensors, well below the detection limits needed for medical and pharmaceutical applications. In addition, the sensors here proposed are innovative, reusable, cost-effective and easy-to-prepare. To the best of our knowledge, redox polymers have never been used together with iron oxide nanoparticles (IONP) to develop epinephrine sensors. The practical applicability of the modified GCEs was evaluated by performing recovery measurements in injectable adrenaline samples.

2 Experimental

2.1 Reagents and Solutions

Iron (III) oxide nanoparticles (Fe_2O_3NP) were acquired from NanoArc, Germany, with 20–40 nm diameter.

Brilliant cresyl blue and Nile blue were obtained from Fluka, Switzerland. Chitosan, epinephrine and L-ascorbic acid were purchased from Sigma-Aldrich, Germany. Acetic acid was from Carlo Erba Reagents, France, sodium dihydrogen phosphate was from Riedel-de Haën, Germany, and di-sodium hydrogen phosphate 2-hydrate was from Fluka, Switzerland. All chemicals were of high purity and analytical grade and were used without further purification.

Buffer solutions of 0.1 M phosphate buffer (PB), pH 7, were prepared with sodium dihydrogen phosphate and di-sodium hydrogen phosphate 2-dihydrate.

A stock solution of 1 % (m/v) chitosan in 1 % (v/v) acetic acid was prepared for the Fe_2O_3NP dispersion used for the electrode preparation. For BCB electropolymerization, a solution of 0.1 M PB + 0.1 M KNO_3, pH 7 and 1 mM BCB was employed [46], and for NB electropolymerization a solution of 0.1 M PB, pH 6 and 0.5 mM NB was used [46]. These solutions were all kept in the refrigerator.

Stock solutions of 10 mM, 1.5 mM and 25 μM epinephrine in 0.1 M PB pH 7 were prepared for epinephrine determination. The solutions were freshly prepared for each experiment.

Injectable adrenaline commercial solutions were from B. Braun Medical, Lda, with labelled concentration 1 mg ml^{-1}.

Millipore Milli-Q nanopure water (resistivity ≥ 18 MΩ cm) was used for the preparation of all solutions. All experiments were performed at room temperature (25 ± 1) °C.

2.2 Apparatus

All electrochemical measurements were performed using a computer-controlled potentiostat/galvanostat (Autolab PGSTAT30) with GPES v4.9 software (Metrohm-Autolab, Netherlands). A three-electrode electrochemical cell of 2.5 cm^3 volume was used, containing the modified glassy carbon electrode (BAS Inc, Japan, geometric area 0.785 mm^2) as working electrode, a platinum/titanium wire anode as counter electrode and a Ag/AgCl (3 M KCl) as reference electrode.

The pH-measurements were carried out with a CRISON 2001 micro pH-meter (Crison Instruments S.A., Spain) at room temperature.

2.3 Sensor Preparation

Before use, the GCE surface was polished using diamond spray (Kemet, UK) on a polishing cloth down to 1 μm particle size and then rinsed with Milli-Q nanopure water.

Commercial Fe_2O_3NP were dispersed in a 1 % (m/v) chitosan in 1 % (v/v) acetic acid solution, and sonicated for 30 min to ensure a homogeneous mixture. The dispersions prepared were 1 % (m/v) Fe_2O_3NP. PBCB and PNB films were formed by electropolymerization using potential cycling. Polymerization of BCB was carried out in a solution containing 1 mM BCB in 0.1 M PB + 0.1 M KNO_3, pH 7, sweeping the potential between −0.7 and +1.2 V at a scan rate of 50 mV s^{-1} during 15 cycles [46]. Polymerization of NB was carried out in a solution containing 0.5 mM NB in 0.1 M PB pH 6, sweeping the potential between −0.6 and +1.2 V at a scan rate of 50 mV s^{-1} during 5 cycles [46]. For each polymer, two architectures of the modified GCE were prepared. In one of them, the polymer film was first formed on the polished GCE, and allowed to dry at room temperature. Following this, the polymer/GCE was coated with 2 μL of the 1 % Fe_2O_3NP/Chit dispersion, using a micropipette and allowing to dry at room temperature. For the other architecture, the polished GCEs were first coated with 2 μL of the 1 % Fe_2O_3NP/Chit dispersion, using a micropipette and allowed to dry at room temperature. Electropolymerization of the monomers was then carried out at the 1 % Fe_2O_3/GCE, and the modified electrode allowed to dry at room temperature.

This procedure enabled the preparation of four sensor architectures, which are denoted by PBCB/Fe_2O_3/GCE, Fe_2O_3/PBCB/GCE, PNB/Fe_2O_3/GCE, Fe_2O_3/PNB/GCE.

3 Results and Discussion

3.1 Electrochemical Characterization of the Polymer/Fe_2O_3-Modified GCE

To evaluate the stability of the polymer/Fe_2O_3NP composite films, the modified GCE were characterized by cyclic voltammetry (CV), in 0.1 M PB pH 7 solution. Cyclic voltammograms recorded for two sensor architectures are given in Figure 1 as examples. The voltammograms obtained at different scan rates (10–200 mV s^{-1}) show that for all the modified GCE studied, the anodic and cathodic peak currents of the composite film increase with the scan rate. For PBCB-based GCE, oxidation and reduction peaks are detected at ~ +0.03 V and −0.15 V, with considerably higher currents than those observed for PNB-based sensors, for which the anodic and cathodic peaks appear at ~ +0.00 V and −0.12 V, respectively, with very low currents.

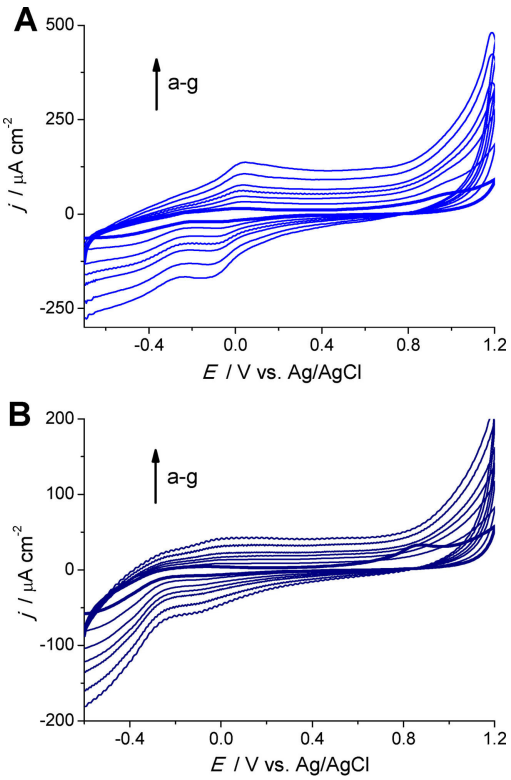


Fig. 1. Cyclic voltammograms at different scan rates (a-g: 10–200 mV s^{-1}) and step potential 5 mV at (A) PBCB/Fe_2O_3/GCE and (B) PNB/Fe_2O_3/GCE in 0.1 M PB pH 7 buffer solution. The CV for the lowest scan rate (10 mV s^{-1}) is shown by a thicker line.

3.2 Epinephrine Detection at the Polymer/Fe_2O_3-Modified GCE

The electrochemical oxidation of epinephrine at the polymer/Fe_2O_3NP-modified GCE was investigated using differential pulse voltammetry, in 0.1 M PB pH 7 solution.

First, the influence of DPV scan parameters on the response of EP at the modified GCE was studied. The experiments were performed for EP (c = 50 µM) in 0.1 M PB pH 7 buffer. The parameters were: pulse amplitude (50 mV), pulse time (25 and 50 ms), potential step (1, 2 and 4 mV) and scan rate (2, 4 and 10 mV s^{-1}). DP voltammograms at PBCB/Fe_2O_3/GCE with different DP parameters are shown in Figure 2A. The optimum values of pulse amplitude 50 mV, pulse time 50 ms, potential step 2 mV and scan rate 4 mV s^{-1} were chosen on the basis of the highest and best-shaped peak, within an acceptable experimental time.

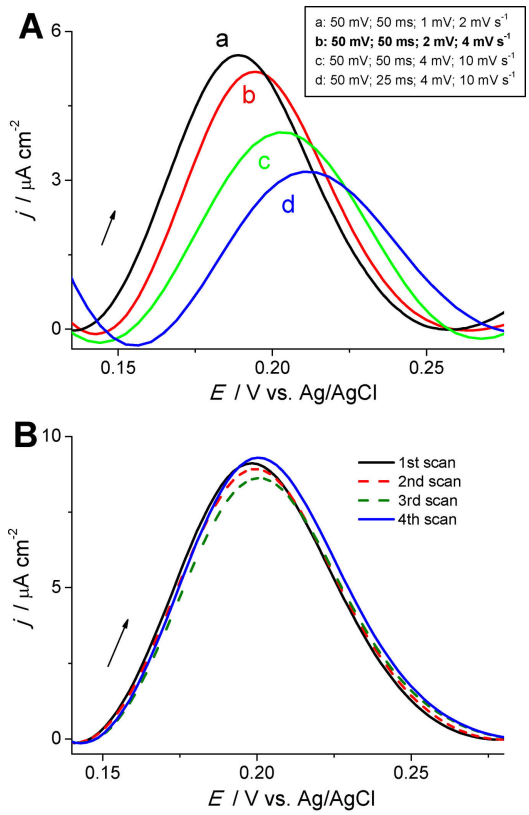


Fig. 2. DP voltammograms (baseline subtracted) of epinephrine at PBCB/Fe_2O_3/GCE in 0.1 M PB pH 7 buffer. (A) 50 µM EP, with different DP parameters. The chosen values (voltammogram b) are shown in bold. (B) 100 µM EP, successive DP voltammograms (pulse amplitude 50 mV, pulse time 50 ms, potential step 2 mV and scan rate 4 mV s^{-1}).

A preliminary study of the adsorption behavior of EP at the modified GCEs was also carried out. DP voltammograms obtained for EP (c = 100 µM) in 0.1 M PB pH 7 buffer at polymer/Fe_2O_3NP-modified GCEs do not present significant differences in the oxidation peak currents after successive scanning without cleaning the electrode surface (Figure 2B), showing that EP does not block the electrode surface.

The DP voltammograms obtained for the determination of epinephrine in 0.1 M PB pH 7 buffer at the PBCB/

Fe_2O_3NP-modified GCEs, in the 0.05–15 μM concentration range, are shown in Figure 3A, and the corresponding calibration plots are depicted in Figure 3B. Calibration plots were also constructed for bare GCE, PBCB/GCE, PNB/GCE and Fe_2O_3/GCE. Table 1 gives the analytical parameters from these plots.

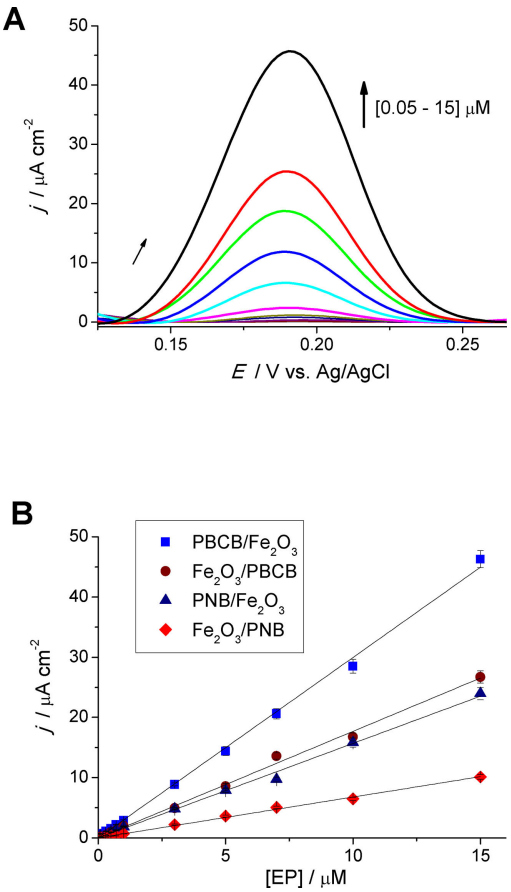
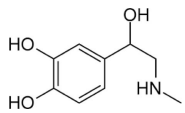


Fig. 3. (A) DP voltammograms (baseline subtracted) of increasing concentrations of epinephrine in 0.1 M PB pH 7 buffer solution at glassy carbon electrodes modified with PBCB/Fe_2O_3. (B) Calibration plots for the modified glassy carbon electrodes.

As can be observed in Table 1, all the polymer/Fe_2O_3 sensors presented an EP oxidation peak around +0.2 V vs Ag/AgCl. Globally, modification of the GCE by both polymer and Fe_2O_3NP leads to a substantial improvement of the analytical performances of the sensors, with a significant decrease of detection limit values, compared to those obtained at bare GCE and by just polymer- or Fe_2O_3-modified GCE. A very low detection limit of 0.31 μM and high sensitivity 23.5 nA mm^{-2} μM^{-1} were observed for PBCB/Fe_2O_3/GCE with linear range up to 0.05 μM. Both PNB-based sensors presented better analytical parameters than the bare GCE and the PNB-modified GCE. The PBCB/Fe_2O_3/GCE sensor architecture showed the best analytical performance, while the Fe_2O_3/PBCB/GCE was the least good.

The different influence of PNB and PBCB on the response to epinephrine may be ascribed to the fact that Nile blue monomer possesses an extra aromatic ring, which confers it with a more hydrophobic character than BCB. Epinephrine, see Scheme 1, has three hydroxyl groups and, at pH 7, the amine group exists in its cationic form (pK_a = 9.9). Therefore, the interaction of epinephrine is more favorable with a less hydrophobic surface such as that of the PBCB film, with possible electrostatic, π-π and dipole-dipole interactions. The location of the polymer film, on top of or beneath the IONP, also showed a significant effect on the performance of the sensors. The access of epinephrine to the polymer is hindered when it is covered with Fe_2O_3. Nevertheless, the presence of Fe_2O_3 provides higher electrical conductivity than bare GCE which benefits the analytical performance of the configuration with the polymer on top of the IONP.



Scheme 1. Structure of epinephrine.

The analytical characteristics reported for the sensing devices developed here are comparable to the values in the literature for other modified GCE (Table 2). Electrochemical sensors with incorporated iron oxide nanoparticles (Fe_3O_4), plus MWCNT and phthalocyanines [47] have higher limits of detection than the electrochemical sensors developed in this work. The best performing

Table 1. Analytical parameters from epinephrine calibration curves obtained by DP voltammetry at bare and modified GCE.

Electrode	Linear dynamic range/ μM	E_p/ V	Sensitivity/ nA mm^{-2} μM^{-1}	LOD/ μM	Correlation coefficient (R^2)
PBCB/Fe_2O_3/GCE	0.05–15	0.194	23.53 ± 0.37	0.310 ± 0.06	0.998
Fe_2O_3/PBCB/GCE	0.05–15	0.196	13.89 ± 0.27	1.64 ± 0.21	0.997
PNB/Fe_2O_3/GCE	0.10–15	0.189	12.31 ± 0.26	1.36 ± 0.30	0.997
Fe_2O_3/PNB/GCE	0.05–15	0.198	5.32 ± 0.09	0.96 ± 0.21	0.997
bare-GCE	0.05–15	0.184	29.49 ± 1.70	2.88 ± 0.61	0.971
PBCB/GCE	0.30–15	0.194	20.92 ± 0.09	1.33 ± 0.22	0.999
PNB/GCE	0.05–15	0.183	19.19 ± 0.87	2.31 ± 0.16	0.982
Fe_2O_3/GCE	0.05–15	0.161	14.09 ± 0.60	1.60 ± 0.86	0.984

Table 2. Analytical parameters from epinephrine calibration curves recorded at different modified GCE.

Modified electrode	Method[a]	Linear range/ μM	Sensitivity/ $\mu A\,cm^{-2}\,mM^{-1}$	LOD/ μM	Ref.
PMaIG/MWCNT/GCE	DPV 0.20 V	0.1–100	6700	0.08	[46]
PMB/MWCNT/GCE	CV 0.23 V	2.5–800	12	69.9	
PNR/f-MWCNT/GCE	CV 0.35 V	1500	20.0	–	
PNR/f-MWCNT/GCE	CV 0.35 V	1000	80.0	–	
PBCB/MWCNT/GCE	LSV	0.05–50	–	0.01	
MWCNT/Fe_3O_4/2,3-Nc/GCE	DPV 0.15 V	7.5–48	214	12.3	[47]
MWCNT/Fe_3O_4/29H,31H-Pc/GCE			48	4.6	
MWCNT/ZnO/2,3-Nc/GCE			–	7.6	
MWCNT/Fe_3O_4/29H,31H-Pc/GCE			461	6.5	
PBCB/graphene/GCE	CV	1.0–1000	–	0.24	[48]
PTh/AuNP/GCE	DPV 0.4 V	3.9–156.7	–	1.18	[49]
GCB/GCE	SWV	0.1–0.5	–	$3.56e^{-3}$	[50]
AuNP/cysteic acid/GCE	DPV	0.4–11.7	1263.1	$80e^{-3}$	[51]
P(L-Asp)/ERGO/GCE	SWV 0.335 V	0.1–110	–	0.025	[52]
CTAB-SnO_2/GCE	SWV −0.045 V	0.1–250	–	0.01	[53]
Au/ZnO/PPy/RGO/GCE	DPV 0.15 V	0.6–500	–	0.06	[54]
S-MCF/GCE	DPV 0.140 V	0.1–12	1310	0.040	[55]
MWCNT/GCE	DPV	22.5–547	68.7	3.92	[56]
ZnO-Pt/CNT/GCE	CV 0.220 V	0.5–250	–	0.1	[57]
EB-Ppy-BSA/GCE	CV 0.328 V	0.1–1000		0.0071	[58]

[a] vs. Ag/AgCl. PMaIG-poly(malachite green). PMB-poly(methylene blue). PNR-poly(neutral red). PBCB-poly(brilliant cresyl blue). 2,3-Nc-2,3-naphthalocyanine. 29H,31H–Pc-29H,31H-phthalocyanine. PTh-poly(thionine). AuNP-gold nanoparticles. GCB-graphene/chitosan/bismuth oxide nanocomposite. P(L-Asp)-poly(L-aspartic acid). ERGO-electrochemically reduced graphene oxide. CTAB-cetyltrimethylammonium bromide. PPy-polypyrrole. S-MCF-Salep solution of mesoporous carbon foam. EB-Ppy-BSA-electron beam irradiated polypyrrole nanospheres embedded over bovine serum albumin porous structure.

MWCNT/Fe_3O_4/phthalocyanine sensor presents a limit of detection that is higher by a factor of 3 than the highest value obtained in the current work. The electrochemical sensors which combine polymers and MWCNT [46] or graphene [48] have lower limits of detection than the sensing devices investigated in this work. Nevertheless, with the exception of PMaIG/MWCNT/GCE, the sensitivities reported here are higher, which represents an advantageous feature. The lowest limits of detection displayed in Table 2 were achieved for sensors constructed with either nanocomposites [50,58] or other compounds [51–55,57] with less appealing characteristics, both in practical and economic terms. Furthermore, they do not fulfill the main requirements for modified electrode sensors, which are high performance, robustness, low cost and easy preparation.

3.3 Electrochemical Response of Epinephrine in the Presence of Ascorbic Acid

As mentioned above, the coexistence of epinephrine with other biomolecules in biological fluids, with similar oxidation potentials at bare GCE, can be a major problem in the practical determination of epinephrine. Due to its crucial role in neurotransmission and thus neurological diseases, the main target sample for epinephrine detection and quantification is brain fluids. The principal co-existing electroactive compound present in the central nervous system is ascorbic acid (AA). To evaluate the selectivity of the sensors, EP detection was carried out in the presence of AA in 0.1 M PB solution, using DP voltammetry and a concentration of ascorbate of 20 μM, two times that of epinephrine. As can be seen in Figure 4, that illustrates the response at PBCB/Fe_2O_3/GCE, the AA oxidation peak is coincident with the polymer oxidation peak and the increase in current is small. Most importantly, the EP peak is separated from the polymer/AA peak by about 0.21 V, sufficient for avoiding any influence on the EP peak. The sensor recovery in the presence of AA for all types of modified electrode was 100%.

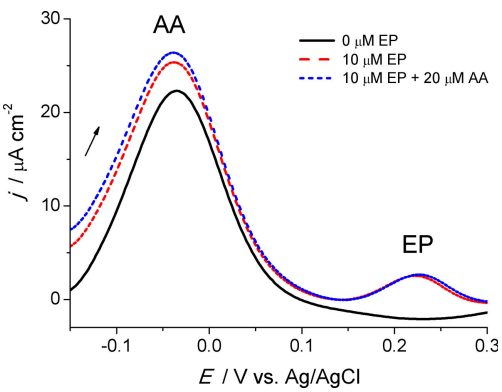


Fig. 4. DP voltammograms at PBCB/Fe_2O_3/GCE in 0.1 M PB pH 7 buffer solution (——), after addition of 10 μM EP (– – – –) and after further addition of 20 μM AA (----). DP experimental conditions as for Figure 2B.

3.4 Determination of Epinephrine in Adrenaline Injection Solutions

The potential practical application of the polymer/Fe_2O_3 electrochemical sensors was assessed by application to the quantification of epinephrine in adrenaline injection solution using DP voltammetry and the standard addition method.

DP voltammograms were recorded, first with the addition of the chosen amount of the adrenaline injection solution to 0.1 M PB pH 7 solution in the measuring cell, then plus aliquots of a standard 0.27 mM EP/0.1 M PB pH 7 solution. An example is shown in Figure 5 for the determination of EP at PBCB/Fe_2O_3/GCE. These experiments were repeated 3 times for each sample and modified electrode. For all the polymer/Fe_2O_3 sensors, the concentrations of epinephrine determined were in very good agreement with the labelled value (1 mg ml^{-1}), Table 3. Recoveries are 100 %, suggesting excellent applicability of the proposed sensors for the fast determination of epinephrine.

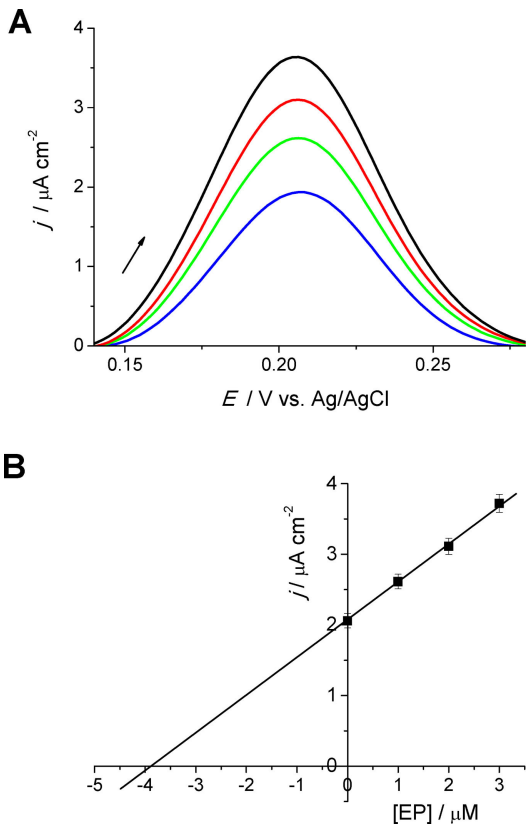


Fig. 5. Standard addition method used for the determination of epinephrine in adrenaline injection solutions at PBCB/Fe_2O_3/GCE, using DP voltammetry. (A) DP scans (B) corresponding standard addition plot. DP experimental conditions as for Figure 2B.

Table 3. Concentration of epinephrine in adrenaline injection solutions determined at the different modified GCE using the standard addition method, and the corresponding recoveries.

Electrode	$[EP]_{SAM}$/mg ml^{-1}	Recovery (%)
PBCB/Fe_2O_3/GCE	1.002 ± 0.028	100.2
Fe_2O_3/PBCB/GCE	0.998 ± 0.018	99.8
PNB/Fe_2O_3/GCE	0.997 ± 0.047	99.7
Fe_2O_3/PNB/GCE	0.997 ± 0.014	99.7

4 Conclusions

New redox polymer/Fe_2O_3NP modified GCE with different architectures have been developed and applied to the determination of epinephrine, using PBCB and PNB as polymer films.

Under the optimized DPV conditions, the sensors with both redox polymer film and iron oxide nanoparticles as modifiers revealed good sensitivities and lower detection limits than either just polymer- or Fe_2O_3-coated GCE. The analytical parameters and the range of epinephrine concentrations which can be measured are comparable to the best reports in the literature. Different sensor architectures showed different analytical performances. The best voltammetric response was observed at the PBCB/Fe_2O_3/GCE sensor, and the lowest at Fe_2O_3/PBCB/GCE.

Epinephrine detection at the polymer/Fe_2O_3 modified GCE was free of interferences from ascorbic acid and application to the analysis of adrenaline injection solutions was successfully performed.

The novel redox polymer/Fe_2O_3 sensors show good reproducibility, good stability and high selectivity. In addition, compared to other type of sensors, they are simple, easy-to-prepare and low cost, thus being promising for further application.

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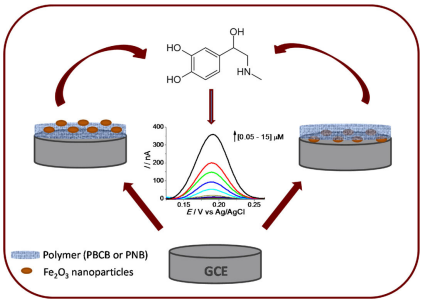

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Polymer/Iron Oxide Nanoparticle Modified Glassy Carbon Electrodes for the Enhanced Detection of Epinephrine