Superoxide Dismutase Based Biosensor for the Electrochemical Determination of Epinephrine

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Abstract

New amperometric biosensor for epinephrine was developed, using a screen printed carbon electrode with tetrathiafulvalene (5% v/w) incorporated into carbon ink ($SPC_{TTF}E$). Electrodic surface was modified with nanoparticles of Au, Pt, Pd and Rh deposited on $SPC_{TTF}E$ by cyclic voltammetry. When using Pd nanoparticles, slope of EPI calibration curves was higher than the performed with the others NPs. The electrodeposited nanoparticles were evaluated with atomic force microscopy and electrochemical impedance spectroscopy was used to characterize electrode process. The developed superoxide dismutase-based biosensor was characterized by: limit of detection of 5.3×10^{-6} M (n=4), limit of quantification of 17.5×10^{-6} M (n=4), reproducibility with RSD of 2.8% (n=5), repeatability with RSD of 0.97% (n=3), accuracy was 102.8% with RSD of 4.3% (n=5). Linearity was obtained from 17.0×10^{-5} M to 8.59×10^{-4} M.

Interference study performed adding ascorbic acid and uric acid exhibits that the peak potential of both species are higher than the chosen for epinephrine analysis. Therefore developed biosensor and described in this paper, has been successfully applied to the determination of epinephrine in human gamma globulin and pharmaceutical samples. Developed biosensor offers easy assembly, excellent linearity and good performance parameters.

Keywords: *Epinephrine; Superoxide dismutase enzyme; Screen-printed electrodes; Nanoparticles; Electrochemical impedance spectroscopy; Atomic force microscopy; Biosensor*

Introduction

Epinephrine (EPI), also known as adrenaline is an important neurotransmitter released under stress conditions. The determination and control of EPI levels, is very important since this molecule is an indicator of some neurological diseases. Specifically in Parkinson's patients, reduced levels of EPI are found when comparing with normal controls [1]. On the other hand, under stress conditions EPI is secreted from adrenal glands, increasing the normal level. Thus EPI determination may be important at low concentration, especially for biological fluids.

Several works regarding the electrochemical determination of EPI in biological and pharmaceutical samples use electrodes modified with nanoporous materials [2]; using the presence of interfering compounds [3]; or using a microbial biosensor [4]. Some electrode modifications, such as AuNPs [5], hydroxide film [6], gold and AuNPs [7] films and other metallic NPs [8,9], MWCNTs [10], and also PdNPs [11], had been successful used in EPI determination.

Screen printed carbon electrodes (SPCEs) are versatile and widely used in many analytical applications, such as in the determination of contaminants, drugs, and low concentrations metals [12,13]. The easy modification of SPCE with metallic nanoparticles improves the devices performance, while the enzymatic modification allows increasing selectivity, and diminishes the potential of amperometric determinations.

EPI is found with ascorbic (AA) and uric acid (UA), which are oxidized in the same potential region in conventional electrodes [2,14], and may be considered as analysis interferences. Since electrochemical behavior of EPI displays an irreversible autoxidation that blocks the electrode surface [14], and to overcome interferences; modified electrodes had been proposed [4,8,14-16]. Some of the electrode modifications are shown in TABLE 1 [17-33]. The preferable electroanalytical techniques used for EPI analysis are differential pulse voltammetry (DPV), followed by cyclic voltammetry (CV). Only a few use amperometric techniques [10,15,18,21,22], with only two based in SPEs [18,33]. Outstanding previous electrodes modifications are very complicated is proposed a new biosensor for EPI in which SPCE sensitivity and performance for EPI determination is achieved by using superoxide dismutase enzyme (SOD) immobilized onto a $SPC_{TTF}E$ modified with palladium NPs (PdNPs). The developed biosensor shows very good performance parameters and an easy assembly procedure. Its applicability was validated to EPI pharmaceutical injections. To our best knowledge this is the first screen printed SOD-based biosensor used to determine neurotransmitter EPI. The mechanism of EPI on $SOD/SPC_{TTF}E$ is described by reference [16].

Materials and Methods

Reagents

Purified water supplied by TKA Gen Pure, inverse osmosis, with a UV lamp irradiation system was used to prepare all solutions. SOD enzyme (30 KU), EPI, bovine serum albumine (BSA), glutaraldehyde (GA) and hydrogen tetrachloroaurate (III) trihydrate ($HAuCl_4$) $3H_2O$ were obtained from Sigma-Aldrich (Sigma-Aldrich), Steinheim, Germany). Solutions of platinum, rhodium and palladium 0.1 mM were prepared from ICP solutions of 1000 mg/L (Merck, Darmstad, Germany). Britton Robinson (BR) supporting electrolyte solutions were prepared as usual with boric, phosphoric and acetic acids

(Merck, Darmstadt, Germany), and the required pH was obtained by adjusting with NaOH solution (Suprapur, Merck, Darmstadt, Germany).

Several inks were used in the fabrication of SPEs, namely Electrodag PF-407 A (carbon ink), Electrodag 6037 SS (silver/silver chloride ink), Electrodag 452 SS (dielectric ink) supplied by Acheson Colloiden (Acheson Colloiden, Scheemda, Netherlands and Gold Polymer Paste C2041206D2 (gold ink) supplied by Gwent Group, Belgium. The working electrode ink was prepared by thoroughly mixing carbon ink with tetrathiafulvalene (C_{TTF}) 5%. TTF was obtained from Acros Organics (Acros Organics, Geel, Belgium)

Equipment

An electrochemical system Autolab PGSTAT Echo Chemie 128 N with GPS and FRA software was used to record electrochemical measurements (Echo Chemie, Utrech, Netherlands). All pH values were adjusted with a pH meter (Mettler Toledo, Schwerzenbach, Switzerland).

$SPC_{TTF}Es$ construction

$SPC_{TTF}Es$ were homemade built using a DEK 248 printing machine (DEK, Weymouth, UK) using polyester screens with appropriate stencil designs mounted at 45° to the printer stroke. These transducers consisted of three screen-printed electrodes deposited onto polyethylene terephthalate films (HiFi Industrial Film, Dardilly, France). The different inks were screen-printed and cured according to the manufacturer's specifications. The working electrode ink was prepared by thoroughly mixing carbon ink with TTF (5% *v/w*) and straightaway screen-printed. One electrode is shown in FIG. 1.

Nanoparticles electrodeposition method

$SPC_{TTF}Es$ modification with nanoparticles ($NPs/SPC_{TTF}Es$) was carried out by cyclic voltammetry scan methods. Cyclic voltammetry deposition was performed doing a set of seven successive voltammetric scans between +1.0 and −0.2 V in a quartz cell containing Au(III), Pt(IV), Rh(IV) or Pd(IV) (0.1 mM) in H_2SO_4 (0.5 M). It was performed under delay time of 120 s, step potential of 0.025 V and scan rate of 0.100 V/s [16]. After deposition, $NPs/SPC_{TTF}Es$ were rinsed, wiped and modified with enzyme SOD ($SOD/NPs/SPC_{TTF}E$). The $NPs/SPC_{TTF}Es$ were characterized through their AFM parameters.

SOD Enzyme immobilization onto $NPs/SPC_{TTF}Es$

SOD was immobilized by GA crosslinking on the working electrode surface, previously modified with $AuNPs/SPC_{TTF}Es$, $PtNPs/SPC_{TTF}Es$, $PdNPs/SPC_{TTF}Es$ and $RhNPs/SPC_{TTF}Es$. To perform the immobilization procedure, SOD enzyme solution was prepared by dissolving the enzyme in Britton Robinson buffer at pH 7.0. From the immobilization mixture, constituted by 20 μL of SOD (5.9 mg/mL), 10 μL of BSA (1.69% *w/v*) and 10 μL of GA (2.5% *v/v*), 10 μL were dropped onto the working electrode surface and stored at 4°C until use, and between the measurements. The biosensor was washed with purified water, before and after use. Scheme procedure used to electrodeposition, AFM characterization and

enzyme immobilization on electrodes surface is showed in FIG. 1, and effect of enzyme SOD on EPI response is showed in FIG. 2.

TABLE 1. **Modified electrodes applied to EPI analysis.**

Modified Electrode	Technique	pH	Potential	Linearity	LD	Sample	Reference
3D MIP array [1]	(DPV)	-	+0.34 V	1–10 μM 10–800 μM	-	EPI injections	[17]
SPCE MP [2]	Amperometry	PBS	-0.20V	0-500 ηM	100 ηM	Rabbit blood	[18]
PBCB/graphene/GCE [3]	CV	PBS	+0.04 V	1.0–10 μM	0.24 μM	EPI pharmaceutical sample	[5]
MWCNT [4] modified bppg	Amperometry	7.0	+0.25 V	0.1 μM–0.1 mM	-	EPI and AA	[15]
Caffeic Acid /GCE [7]	CV	7.4	+0.22 V	2.0–300 μM	0.60 μM	EPI injection	[8]
(FA)/AuNPs/GCE) [8]	DPV	3.0	+0.40 V	0.5–792.7 μM	0.01 μM	EPI pharmaceutical samples, urine	[3]
(Chit-fCNT) [9]	DPV	7.4	+0.20 V	0.05–10 μM	30 ηM	pharmaceutical sample	[19]
Au-MWCNT-PANI-TiO$_2$ [10] Au-MWCNT-PANI-RuO [10]	DPV	7.0	+0.20 V	4.9–76.9 mM.	0.16 μM 0.18 μM	EPI injection	[9]
Zn-Al LDH [11].	DPV	7.0	+0.35 V	0.5 μM–0.3 mM	0.13 μM	EPI injection/ urine	[6]
Au/Au-NPs/MPA/CA/Au-NPs [6]	CV	7.0	-060 V	0.1–800 μM	5 μM	EPI/ AA/ UA	[7]
GR/Au/GCE [12]	CV	7.0	+0.16 V	0.05–8.0 μM	7.0 ηM	EPI injection	[20]
poly-FA) (MWCNT) [13]	Amperometry	4.5	+0.20	73–1406 μM	22.2 μM	pharmaceutical sample	[21]
GC/Ni(II) complex film/PU-C [14]	Amperometry	7.4	+0.7 V	1-10 μM	0.01 μM	Human blood	[22]
Au/AAO [15]	LSV	7.0	+0.15 V	20–100 μM	2.42 μM	EPI injection	[23]
GCE (S-MCF/GCE) [16]	DPV	7.0	+0.20 V	0.1–12 μM	40 ηM.	EPI injection	[24]
(CPE) (MWCNTs) [17]	DPV	7.0	+0.20 V	0.05-10 μM 0.1-100 μM	2.9×10^{-8} M	EPI injection, blood serum	[25]
Au/Ag sponge [18]	DPV	7.0	+0.28 V	10–100 μM	5.05 μM	EPI and AA	[26]
Au/PILs/PPyNTs/ GCE [19]	DPV	7.4	+0.30 V	35–960 μM	298.9 ηM	EPI, glucose, D-fructose, sucrose, citric acid, UA ,AA.	[27]
(CFE) (MWCNTs) [20]	DPV	7.0	+0.10 V	1–100 μM	0.9 μM	EPI injection	[14]
TX-100 /BCE [21]	CV	7.0	+0.20 V	10-50 μM,	1 μM	human serum	[28]
TDPA/CA/AuNPs/ SAMs [22]	CV	7.0	-0.5 V	0.1-0.75 μM	0.082 μM	EPI, AA, AU	[29]
TiO$_2$-Au/MWCNTs/rGO [23]	DPS	6.0	+0.15 V	1.0–300 ηM	0.34 ηM	EPI injection, urine	[30]
SDS-WO$_3$/GCE [24]	DPS, CV	7.0		9 nM–1 mM.	1.8 ηM	human serum	[31]
MWNT-Nafion-Tyr [25]	Amperometry	7.0	0.0	10-40 μM,	-	phosphate buffer	[10]
Au TMBH [5]	CV	6.0	+0.20 V	1.7–24.9 μM 24.9–91.7 μM	0.19 μM	spiked human blood serum	[32]
(o-SWCNHs)/SPE [26]	DPV	7.4	+0.50V	2–2500 μM	0.1 μM	AA, AC, AU	[33]
SOD/PdNPs/SPC$_{TTF}$E	Amperometry	5.0	+0.20 V	17–859 μM	5.3 μM	EPI injection GG2	This article

Acronyms: [1] molecularly imprinted polymer arrays electrode, [2] screen printed mesoporous carbon electrode, [3] poly(brilliant cresyl blue glassy carbon electrode, [4] multi walled carbon nanotubes modified basal plane pyrolitic graphite electrode, [5] Au thiol Schiff base self-assembled monolayer modified electrode, [6] mercaptopropionic acid, gold nanoparticles and cystamine modified gold bare electrode, [7] caffeic acid and glassy carbon electrode, [8] poly-fuchsine acid film Au nanoparticles modified glassy carbon electrode, [9] carbon nanotube–chitosan biopolymer nanocomposite electrode, [10] multi walled carbon nanotubes/polyaniline doped with metal oxide (TiO$_2$, RuO$_2$) nanoparticles electrodes, [11] layered double Zn-Al hydroxide film modified glassy carbon electrode, [12] graphene Au nanocomposites, [13] poly ferulic on multi-walled carbon nanotubes modified glassy carbon electrode, [14] glassy carbon electrode with two inner polymer layers of macrocyclic nickel complex and outer of

polyurethane g-benzyl l-glutamate, [15] nanoporous thin Au films deposited on a highly ordered anodic aluminum oxide electrode, [16] mesoporous carbon foam dispersed in Salep solution modified glassy carbon electrode, [17] carbon paste electrode modified with multi-walled carbon nanotubes [18] Au modified Ag sponge electrode, [19] Au nanoparticles, poly(ionic liquids, polypyrrole nanotubes graphite carbon electrodes hybrids, [20] carbon film electrode multiwalled carbon nanotubes modified in a chitosan matrix, [21] TX-100 surfactant on bare carbon electrode, [22] thiodipropionoc acid, cysteamine and gold nanoparticles modified gold pure electrodes with self-assembled monolayers, [23] TiO_2-Au multi walled carbon nanotubes reduced graphene, graphite carbon composite electrode, [24] gamma irradiated sodium dodecyl sulfate, tungsten trioxide nanoparticles modified glassy carbon electrodes, [25] multiwalled carbon nanotube-Nafion tyrosinase electrode, [26] Oxidized Single-Wall Carbon Nanohorns o-SWCNHs)/SPE.
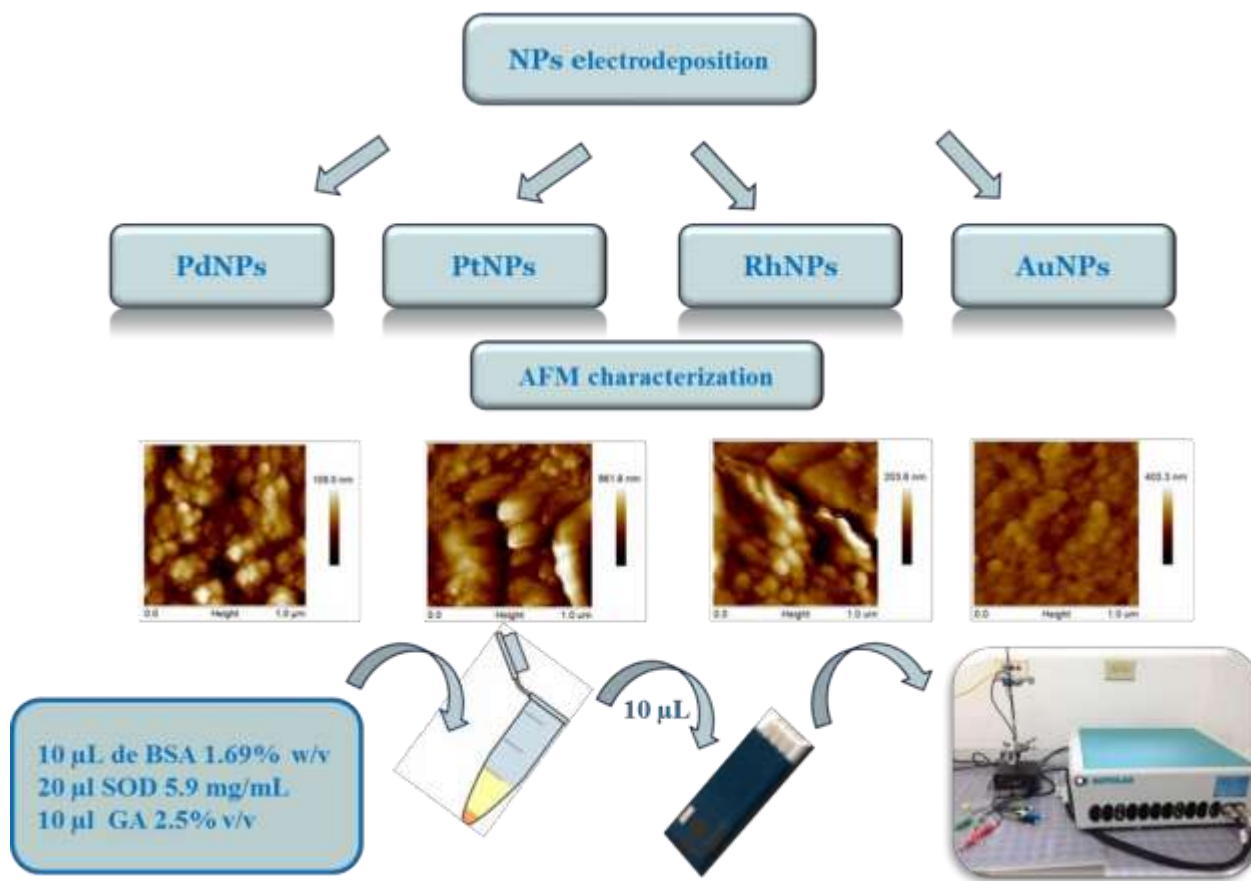

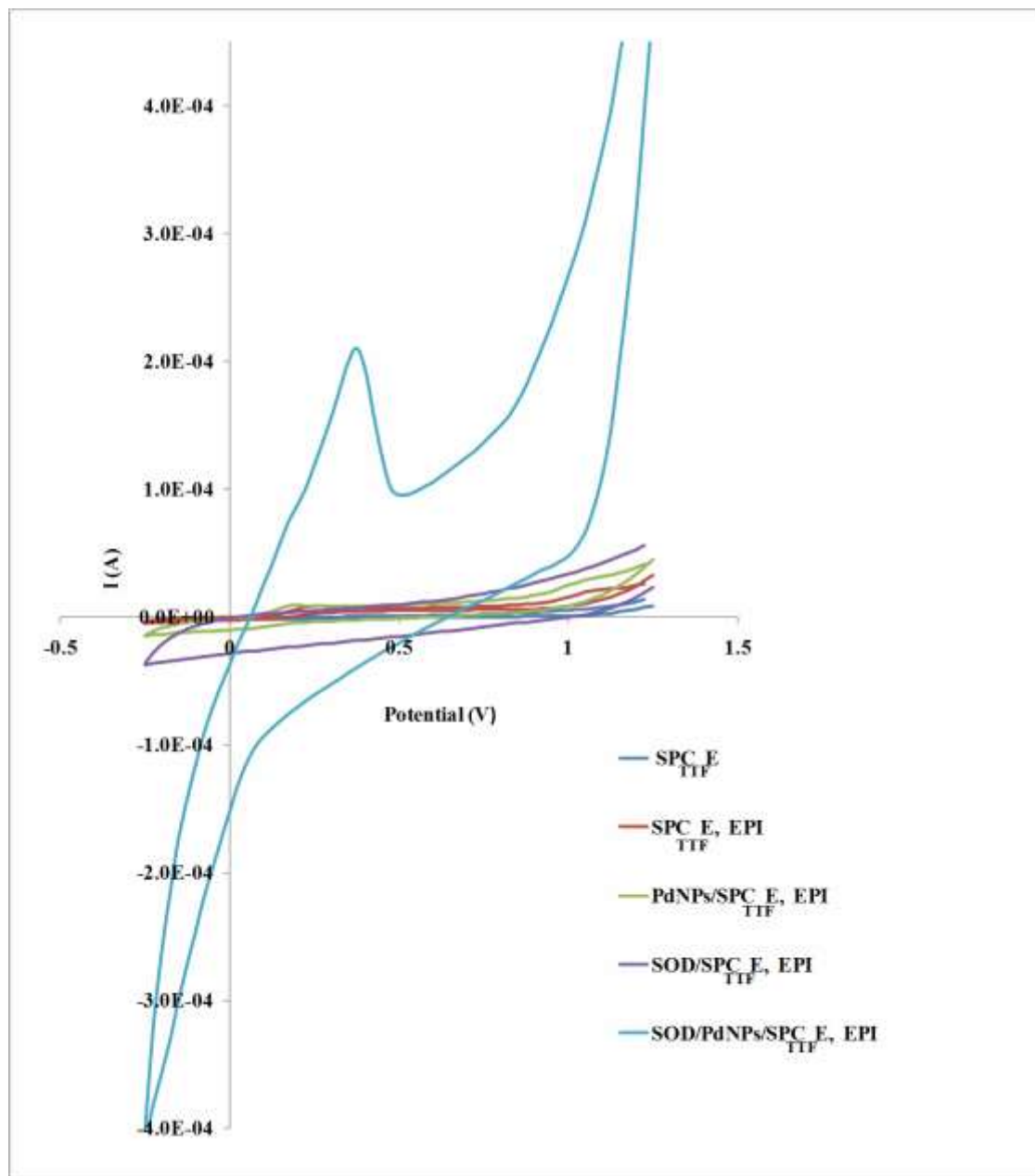FIG. 1. **NPs electrodeposition, AFM characterization and SOD immobilization on SPC$_{TTF}$E.**

FIG 2. **EPI response with SPC$_{TTF}$E, in presence of SOD enzyme, Britton Robinson buffer pH 5.0; EPI 1.69 $\times$ 10^{-4} M.**

Results

Optimization of experimental parameters of EPI biosensor

A linear dependence between current (I) and EPI concentrations is found, and study in order to optimize the amperometric response was carried out. The EPI response was evaluated between a potential range from +0.20 V and +0.60 V. Regarding the FIG. 3, a potential of 0.2 V was found as more suitable regarding signal stability, since greater selectivity for EPI can be achieved, avoiding interferences. Also, the influence of supporting electrolyte pH was evaluated (FIG. 4), by performing several EPI calibration curves at different pH values, and comparing the sensitivities obtained between the experiments. From the evaluated pH range, from 5.0 to 8.0, the higher slope was obtained at pH 5.0 and Eap of +0.2 V, being these conditions chosen to perform EPI calibration curves with SOD/PdNPs/SPC$_{TTF}$E biosensor.
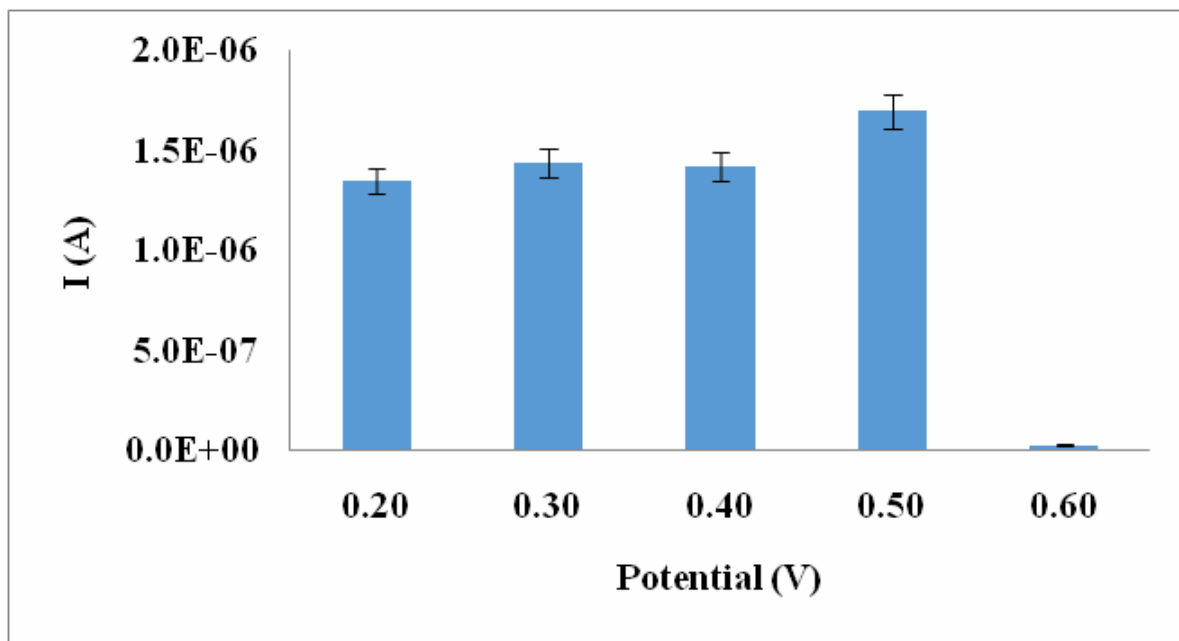

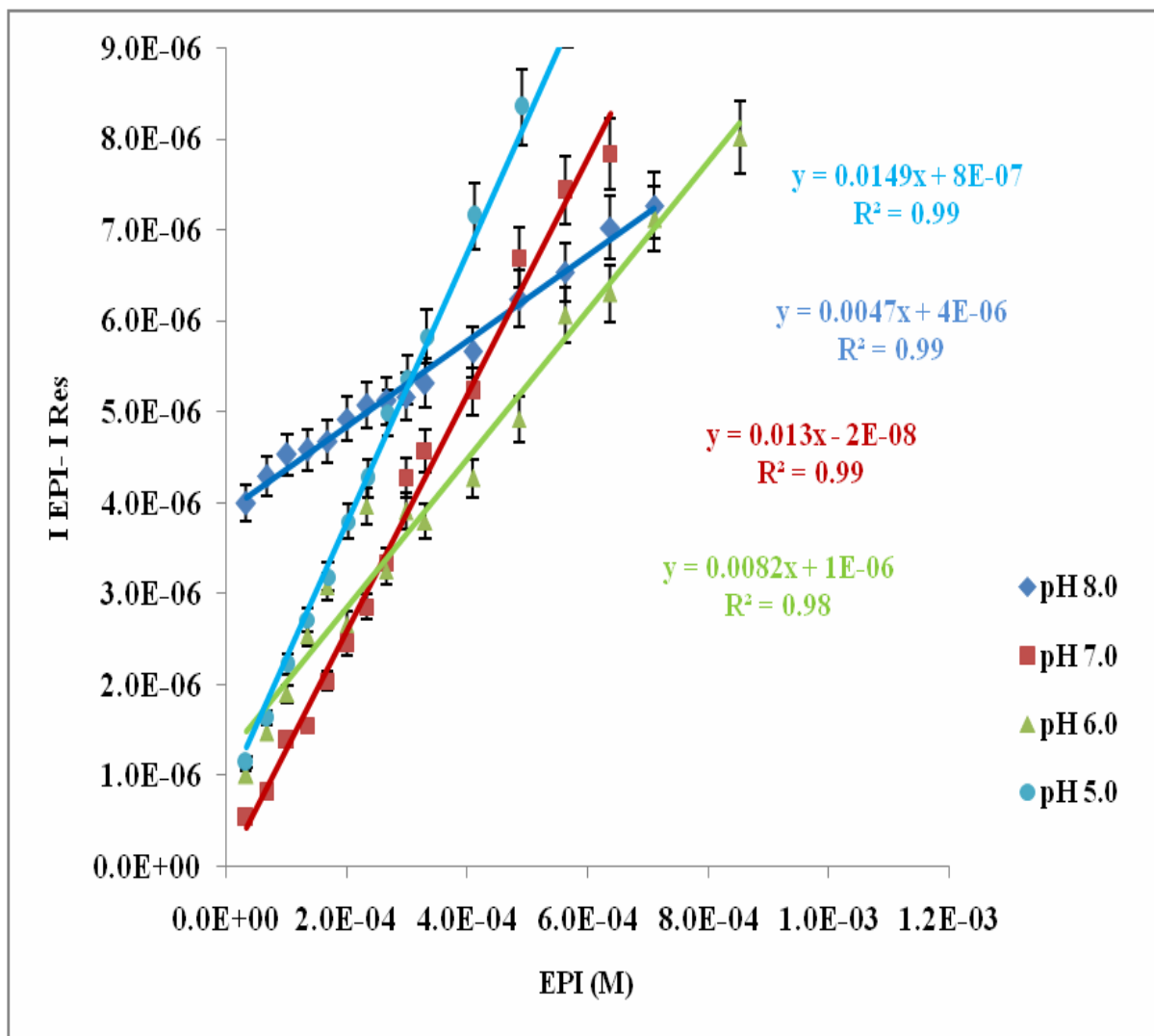FIG. 3. **Effect of potential on EPI current with SOD/PdNPs/SPC$_{TTF}$E.**

FIG. 4. **Effect of pH on EPI/SOD/PdNPs/SPC$_{TTF}$E; Eapp = +0.2 V** *vs.* **SPE Ag/AgCl.**

Characterization by AFM of modified nanoparticles electrodes

SPC$_{TTF}$Es were modified with nanoparticles by using the electrodeposition method described previously, AFM parameters were determinate and their values are showed in TABLE 2. PdNPs/SPC$_{TTF}$E showed RA, RMS and Rmax lowest values meaning a homogeneous and regular surface [16] and highest slope of EPI calibration curves accordingly with FIG. 5. Electrodes current with different Nps deposited was corrected subtracting residual current of every one.
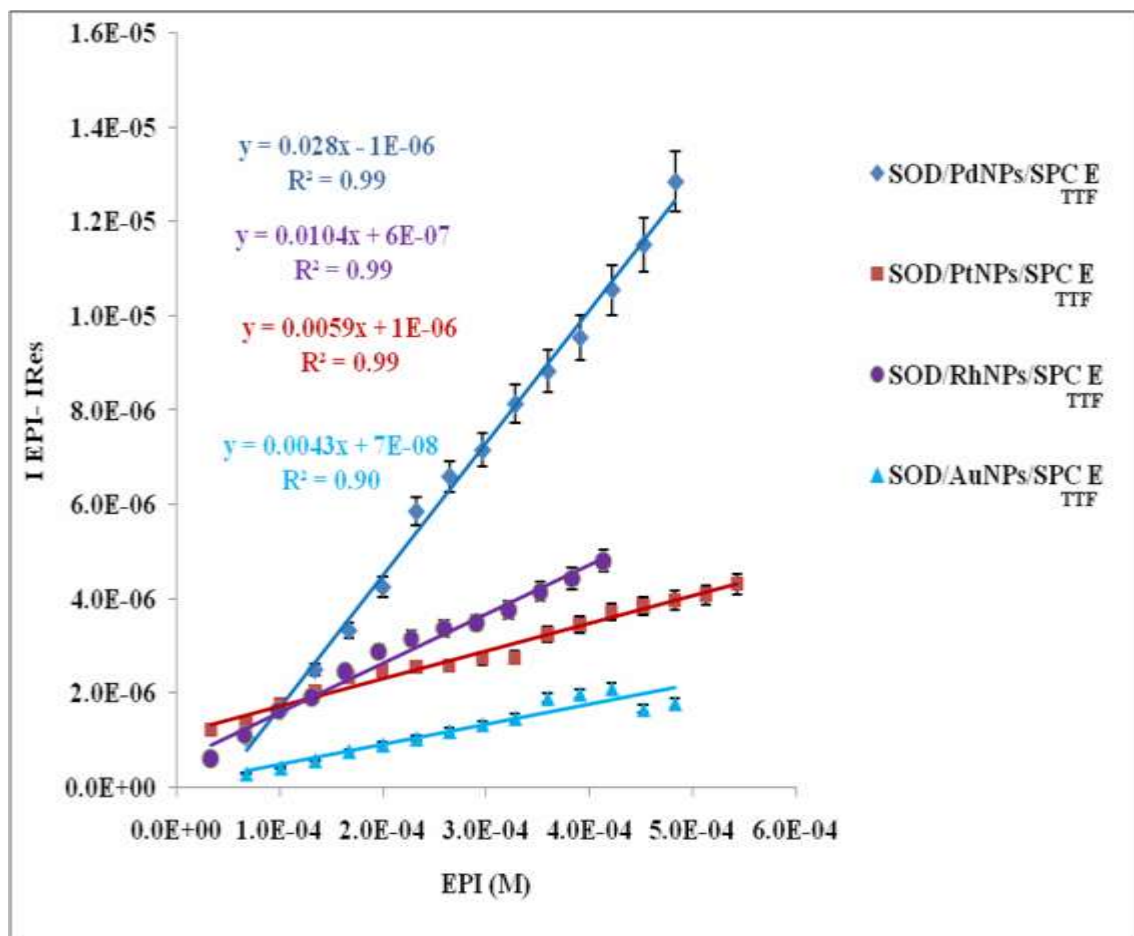
FIG. 5. **EPI calibration curves SOD/NPs/SPC$_{TTF}$Es, pH 5.0; +0.2 V *vs*. SPE Ag/AgCl. For successive additions of EPI from a stock solution 8.60 × 10^{-3} M**

TABLE 2. **AFM parameters of CV modified NPs/SPC$_{TTF}$Es**.

NPs/SPC$_{TTF}$E	RA (nm)	RMS (nm)	R max (nm)	Rku	RSk
AuNPs/SPC$_{TTF}$E	34.6	44.5	403	3.19	-0.0292
PdNPs/SPC$_{TTF}$E	14.7	18.2	106	2.69	0.0939
PtNPs/SPC$_{TTF}$E	106	140	864	3.60	0.141
RhNPs/SPC$_{TTF}$E	25.6	33.9	204	3.65	-0.189

In FIG. 6, it is depicted an amperogram performed under optimized conditions for the SOD/PdNPs/SPC$_{TTF}$E biosensor and its corresponding EPI calibration curve.
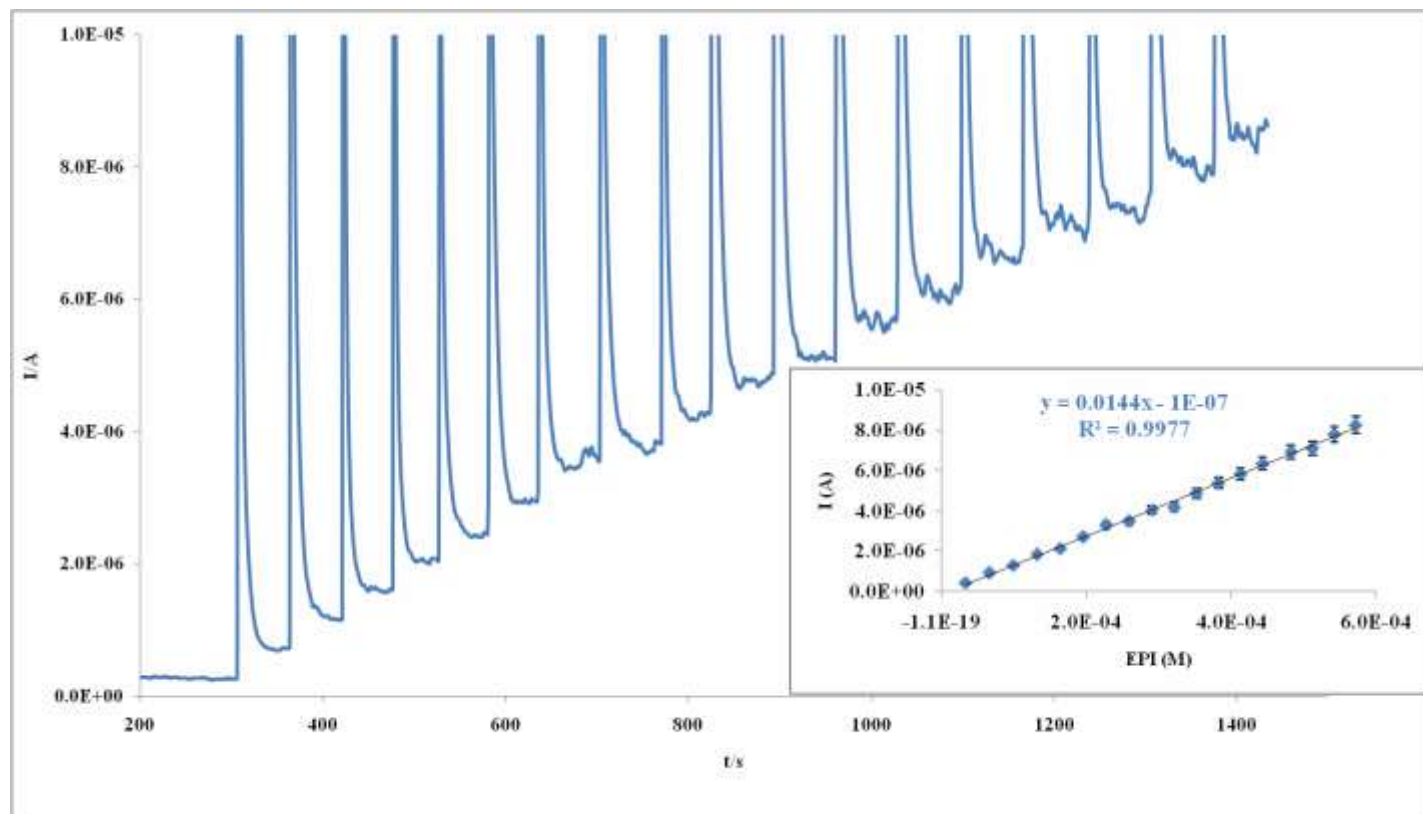

FIG. 6. **Amperogram performed under optimized conditions for the SOD/PdNPs/SPC$_{TTF}$E biosensor Inset corresponding EPI calibration curve obtained at pH 5.0; +0.2 V *vs*. SPE Ag/AgCl**.

EIS characterization of modified SOD/PdNPs/SPC$_{TTF}$E

The successive electrode modifications performed were characterized through an impedance study, evaluating the redox probe K$_4$(FeCN)$_6$ at 4.5×10^{-3} M, first were settled open circuit potential and later were obtained impedance parameters. First tested electrode was SPCE and corresponding Nyquist plot shows homogeneous layer driven by mass transfer and diffusion; N component of constant phase approaches ideal capacitor. Electrode modification with TTF showed similar behavior and N value of 0.939, Nyquist plot as semicircle is associated with charge transfer process and line with slope at 45^0 represents diffusion controlled process.

Palladium nanoparticles SPC$_{TTF}$E modification shows the effect of homogeneous layer and mass transfer conserved, also diffusion is present. Effect of enzyme immobilization on Pd/SPC$_{TTF}$E showed electrode process governed by charge transfer

10

and also homogeneous layer was settled due an observed serial circuit. Circuits of successive electrodes modifications are depicted in FIG. 7.

SPCE shows larger resistance of 121.7 than $SPC_{TTF}E$ that showed a decrease in electron transfer resistance with a value of 74.2. Likewise $PdNps/SPC_{TTF}E$ modification decrease electron transfer resistance with a value of 62.4, agree with AFM lower parameters of PdNps compared with the others metallic NPs, meaning improved conductivity of the electrode surface. With SOD enzyme glutaraldehyde immobilization an increase in electron transfer resistance happens with a value of 74.8 meaning that electron transfer is hindered with enzyme polymerization process of $SOD/PdNPs/SPC_{TTF}E$. This fact leaded to stable setting of amperometric measurements.

In FIG. 8 is showed simultaneous Nyquist plot for all described electrodes and their modifications.
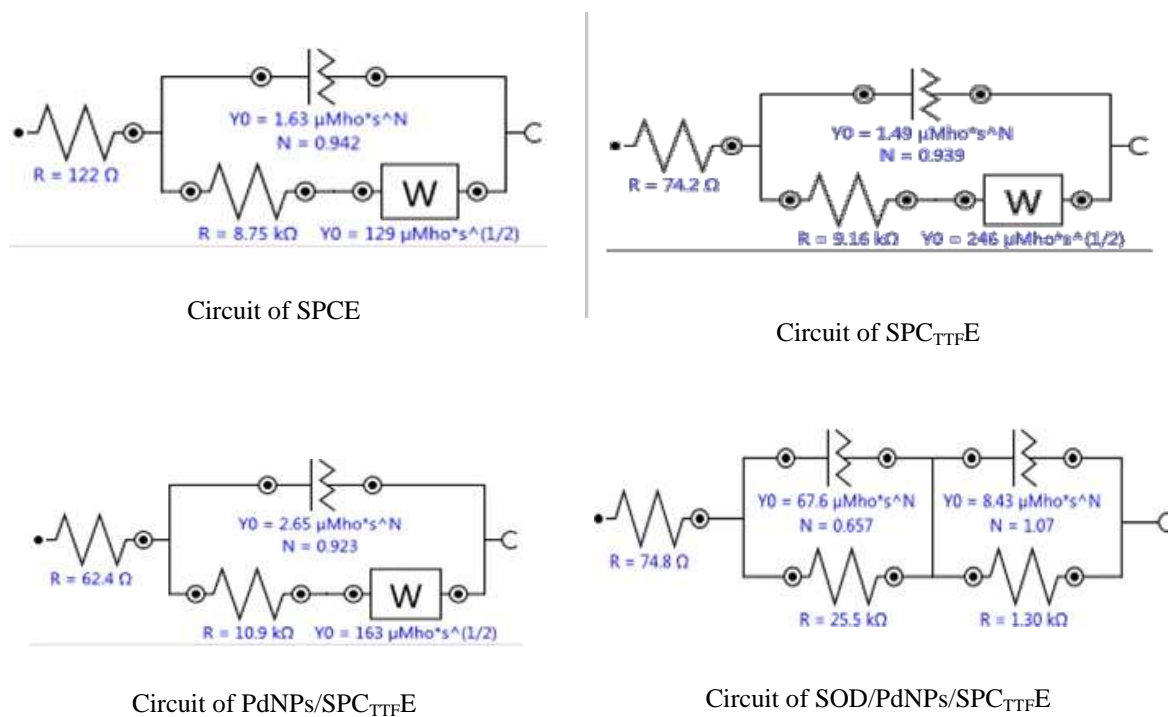

Circuit of SPCE

Circuit of $SPC_{TTF}E$

Circuit of $PdNPs/SPC_{TTF}E$

Circuit of $SOD/PdNPs/SPC_{TTF}E$

FIG. 7. **Circuits of electrodes modifications obtained in $K_4(FeCN)_6$ 4.5×10^{-3} M.**
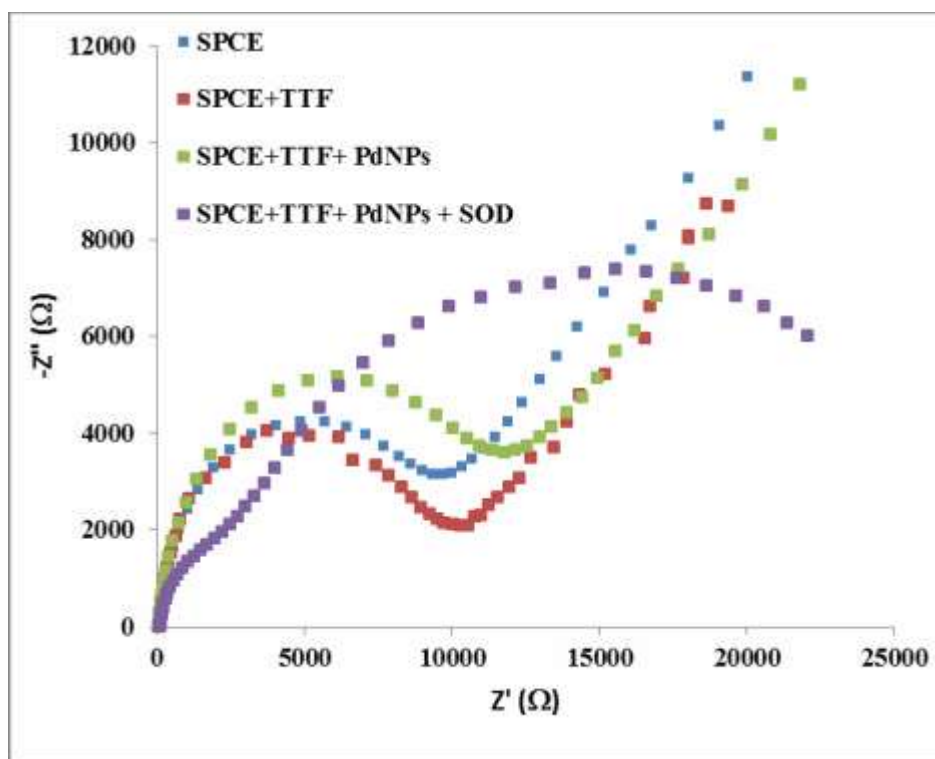
FIG. 8. **Recorded Nyquist plot of tested electrodes in $K_4(FeCN)_6$ 4.5×10^{-3} M**.

Validation of SOD/PdNps/SP$_{TTF}$E biosensor

Limit of detection: The limit of detection (LOD) of $5.2 \pm 0.3 \times 10^{-6}$ M was calculated as three standard deviation of four EPI replicates concentration (3s) evaluated from standard addition calibration curves (n=4) under the optimum working conditions. Similar to LOD, the limit of quantification (LOQ) estimated was calculated as ten standard deviation of four EPI replicates concentration (10s) obtained from standard addition calibration curves (n=4) [34,35] and its value was $17.5 \pm 0.9 \times 10^{-6}$ M

Precision: The developed biosensor was characterized by establishing its precision in terms of reproducibility and repeatability. The repeatability assay was assessed performing three successive EPI calibrations using the same SOD/PdNPs/SPC$_{TTF}$E (n=3) in CV conditions. The electrodes were conditioned in a Britton Robinson buffer solution, pH 5.0 stirring for 5 min between experiments, RSD of slopes obtained with the same electrode was 0.97%. Likewise, the reproducibility of the amperometric signal was checked using the slopes of five EPI regression lines carried out with (n=5) different electrode surfaces, RSD slope value estimated was 2.8%.

Accuracy: An EPI solution of known concentration was used to spike buffer solution and mean recovery obtained was 102.8% with a RSD of 4.3% (n=5) as it is shown in TABLE 3. Also human gamma globulin fraction 2 (GG2) 1% w/v was

12

spiked with EPI at two added concentrations specifically 1.608×10^{-4} M and 2.794×10^{-4} M analyzed by standard addition with a mean recovery of 103.1% with RSD of 3.5% (n=3) and 95.1% with RSD of 7.4% (n=3), as can be seen in TABLE 4.

TABLE 3. **Recovery of EPI solution spiked to buffer solution.**

EPI added	Recovered	Recovery
(M)	(M)	%
	5.354×10^{-4}	103.5
	5.435×10^{-4}	105.1
5.171×10^{-4}	5.498×10^{-4}	106.2
	5.379×10^{-4}	104.0
	4.922×10^{-4}	95.2
Mean	5.318×10^{-4}	102.8
SD		4.4
RSD		4.3

Analytical application: A pharmaceutical injection of EPI with a reported concentration of 1 mg/mL was evaluated using the SOD/PdNPs/SP$_{TTF}$Es biosensor. After a proper dilution, and using standard addition method analysis, an EPI mean recovery of 102.4 % was obtained, with a RSD of 7.9% (n=4) as it is shown in TABLE 5. Reported value of EPI injection is in agreement with mean experimental value considering confidence limit values. Recovered EPI concentration expressed as 1.02 ± 0.13 mg/mL contains reported injection concentration.

TABLE 4**. Recovery of EPI spiked to GG2 solution.**

EPI added	EPI recovered	Recovery
(M)	(M)	%
1.608×10^{-4}	1.6284×10^{-4}	101.3
	1.619×10^{-4}	100.7
	1.724×10^{-4}	107.3
Mean		103.1
SD		3.6
RSD		3.5
2.794×10^{-4}	3.007×10^{-4}	103.2
	2.298×10^{-4}	91.2
	2.539×10^{-4}	90.9
Mean		95.1
SD		7.0
RSD		7.4

TABLE 5**. Recovery of EPI pharmaceutical injection spiked to buffer solution**.

EPI added	EPI recovered	DF 5020/20	g EPI/L	Recovery
(M)	(M)			%
	2.396×10^{-5}	6.014×10^{-3}	1.1017	110.2
2.175×10^{-5}	2.106×10^{-5}	5.295×10^{-3}	0.9682	96.8
	2.048×10^{-5}	5.149×10^{-3}	0.9416	94.2
	2.357×10^{-5}	5.925×10^{-3}	1.0837	108.4
Mean			1.024	102.4
SD			0.081	8.1
RSD			7.9	7.9
DF: Dilution Factor 20 μl of sample diluted to 5020 μl with buffer				

Interferences

AA and UA are usually present in biological samples, and had been reported as interferences for EPI. As shown in FIG. 9, the effect of such species on SOD/PdNPs/SPC$_{TTF}$ E electrochemical signal for EPI, was evaluated by cyclic voltammograms recorded for EPI 1.69×10^{-4} M in presence of AA 1.90×10^{-3} M and UA 1.80×10^{-5} M. Cyclic voltammograms of AA, UA, and EPI using SOD/PdNPs/SPC$_{TTF}$E, in Britton Robinson buffer pH 5.0, have shown that AA and AU oxidation potential is higher than +0.2 V, allowing to avoid interference of such species, since this was the potential chosen for EPI determination using SOD/PdNPs/SPC$_{TTF}$Es.
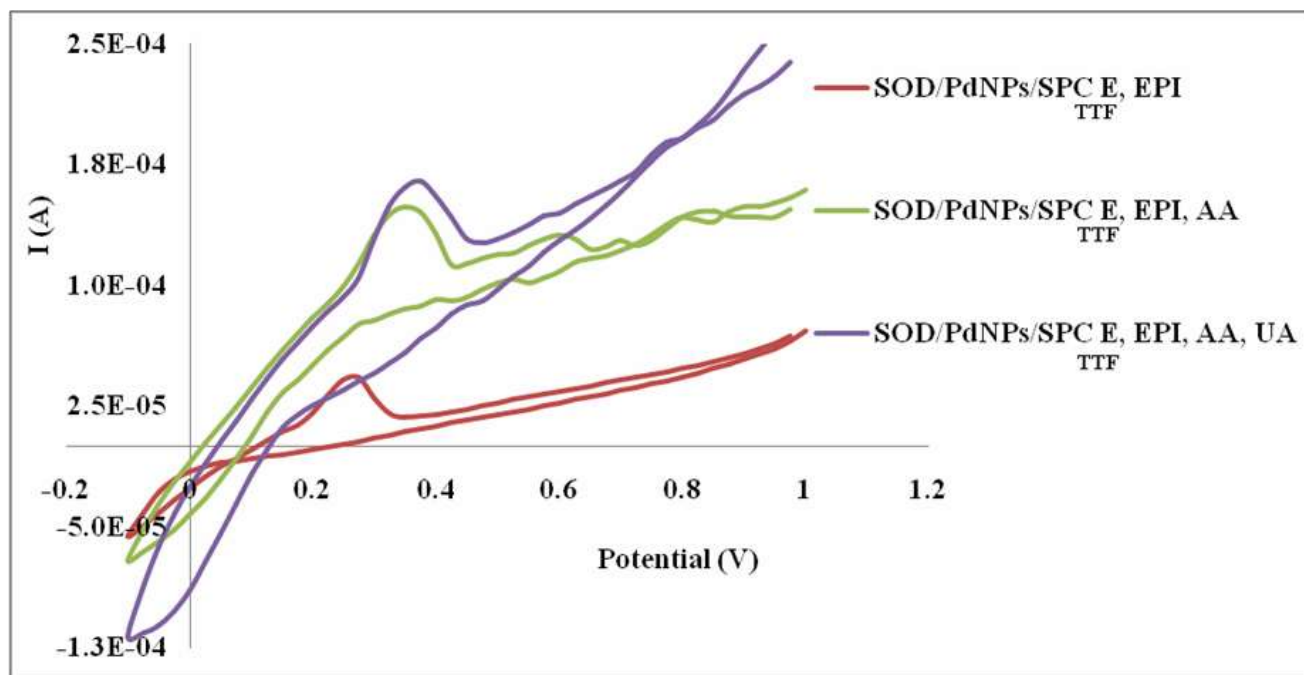
FIG. 9. **Cyclic voltammograms that shows peak potential for EPI 1.69 × 10^{-4} M and sequential additions of AA and UA using EPI/SOD/PdNPs/ SPC$_{TTF}$E, Britton Robinson buffer pH 5.0.**

Discussion

Cyclic voltammograms performed at SOD/NPs/SPC$_{TTF}$Es, were evaluated regarding the linearity and sensibility to EPI additons. PdNPs deposition shows improved results, resulting in highest slope of calibration curves, than the other metals used. Despite that, the biosensor sensitivity was found also improved for RhNPs, followed by PtNPs and AuNps.

AFM parameters of PdNPs showed lowest values of RA of 14.7, RMS of 18.2 and Rmax of 106, compared with the other metallic NPs deposited on SPC$_{TTF}$Es. Also, a more regular surface of PdNPs/SPC$_{TTF}$E is accordingly with the highest slope of EPI calibration curves performed with above mentioned electrode.

EIS study of modified SOD/PdNPs/SPC$_{TTF}$E allowed characterization of sequential modifications realized on SPCEs. The EIS experiments showed that the modifications decrease of R1 value, leading to an improved conductivity of modified SPCEs, with exception in the enzymatic crosslinking, which produces an R1 increase. Notwithstanding, the SOD immobilization produces stable amperometric measurements, confirmed in assays of biosensor characterization. All tested electrodes showed a good agree between proposed circuit and experimental values indicated by chi square values χ^2 lower than 0.1605. Regarding electrodes modification proposed for EPI determination, showed in TABLE 1, this is the only SPCE modified with SOD and PdNPs to be applied to EPI determination. Also, pH proves to be an important variable to control,

15

since different pH values results in different sensitivities of the SOD/NPs/SPC$_{TTF}$Es biosensor to EPI concentrations. The supporting electrolyte with pH value of 5.0, demonstrates to offer a higher slope of the calibration curves, improving the analytical response of the system. Furthermore, working potential of +0.2 V was selected in order to avoid insoluble EPI oxidation products, such as epinephrine quinone.

Compared with the other modified electrodes applied to EPI determinations that performed very complex methodologies with several successive steps to reach electrode surface modification, proposal modification procedure is easier, faster and performance parameters of developed SOD/PdNPs/SPC$_{TTF}$E shows low detection limit and very good precision described as repeatability with RSD of 0.97% and RSD reproducibility of 2.8%. Its applicability to pharmaceutical samples was verified with analysis of EPI injection, it was diluted to appropriate method concentration and recovered with 102.4% and reported value was included in experimental value with its RSD. Cyclic voltammetric experiments for EPI determination in presence of interfering species, such as the AA and AU, exhibited selectivity for the interest neurotransmitter, using a low working potential to perform the analysis.

Conclusions

NPs of Au, Pt, Pd and Rh influenced slopes of EPI calibration curves. NPs electrodeposited onto SPC$_{TTF}$E were characterized by AFM parameters and EPI amperometric calibration curves. PdNPs/SPC$_{TTF}$E showed lower AFM parameters than others NPs/SPC$_{TTF}$Es. In agreement with these data, EIS electrode process characterization proves that the biosensor is improved with PdNPs on CV conditions, achieving a good linearity and higher catalytic effect.

The LOD obtained for EPI concentrations in the SOD/PdNPs/SPC$_{TTF}$E biosensor, allowed to quantify low amounts of EPI at low working potentials, minimizing possible interferences that could be oxidized in real samples.

GG2, 1% *w/v* was spiked with EPI at two concentrations with a mean recovery of 103.1% with RSD of 3.5% (n=3) and 95.1% with RSD of 7.4% (n=3). An EPI injection was analyzed with developed SOD/PdNPs/SPC$_{TTF}$E biosensor, recovering 102.4% and presenting a RSD of 7.9% (n=4). EPI biosensor validation was performed under optimized conditions: pH 5.0, applied potential of +0.2 V. The recovery value obtained using GG2, supported the feasibility of SOD/PdNPs/SPC$_{TTF}$Es based biosensor for EPI determination. Moreover analysis of pharmaceutical sample enforces applicability of developed biosensor to this type of samples.

Acknowledgments

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