

Metallic Nanoparticles Modification of Epinephrine Biosensor Improves its Amperometric Response

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Abstract

Effect of metallic deposited nanoparticles on screen printed carbon with tetrathiafulvalene incorporated ink, was tested by three electrochemical procedures. First one electrodeposition at constant potential and two cyclic voltammetry procedures that applied changes in step potential, scan rate and delay time. X-rays Fluorescence (XRF), Scanning Electronic and Atomic Force Microscopy (SEM) and (AFM) respectively were performed and metallic nanoparticles deposits were characterized through their SEM images. XRF provide metal percentage deposited by electrochemical procedures. AFM parameters let to know electrodes modified surface characteristics. Nanoparticles electrochemically deposited electrodes were modified with superoxide dismutase enzyme and tested on epinephrine biosensor amperometric calibration curves. Platinum Nanoparticles (PtNPs) modified electrodes nature surpasses AFM surface parameters. There is correlation between low AFM parameters and high slope epinephrine calibration curve excepting PtNPs case. Nanoparticles of platinum deposited by cyclic voltammetry procedure 1 showed higher epinephrine slope calibration curve than the others nanoparticles deposited by constant potential and cyclic voltammetry procedure. Higher linearity and stability was showed for PtNPs performed cyclic voltammetry procedure; this one was selected to validation of biosensor. Developed biosensor showed reproducibility of 2.9% and detection limit of 22.8 μM . Biosensor analysis of epinephrine pharmaceutical injection was 103.1% with RSD 5.4%.

Keywords: Metallic Nanoparticles • Epinephrine Biosensor • Amperometric • Platinum nanoparticles

Introduction

Biosensors constructed with Screen Printed Carbon Electrodes (SPCEs) are advantageous in analytical work due their low cost, easy handling and disposability. They offer the possibility of performance parameters determination, showing low detection limits, repeatability, reproducibility and application to biocompatible matrices. Their behavior can be improved with the use of metallic Nanoparticles (NPs). NPSPCEs electrochemical modification is fast and allows easy assemblage of different metallic nanoparticles. Electrodes contamination is minimal compared with chemical modifications. Further, NPs have revealed excellent biocompatibility with enzymes and antibodies [1]. Metallic NPs intrinsic properties such as high chemical and thermal stability, mechanical power and small size that reached nanometric size allow them to offer SPCEs a stable and larger surface for immobilized enzymes, increasing reaction kinetics. Larger surface area provided by NPs, allows higher enzyme charging per mass unit of nanoparticles. Results display high sensitivity and enhanced mass transport [2].

NPs use combined with redox enzymes, increase electric conductivity and electron transfer between redox center and electrode surface. NPs of TiO_2 , black carbon, fullerol and polystyrene

have shown ability to catalyze Reactive Oxygen Species (ROS) on phagocytic cell lines causing structural organelles damage. Electrochemical sensors and biosensors extensively have incorporated NPs assembled from metal and metallic oxides, carbon nanotubes, graphene and graphene oxide and composite-metal NPs, in miniaturized systems, improving devices performance parameters. AuNPs are the most used in sensors and biosensors applications, due to their chemical characteristics, low cost and relatively ease of preparation. They can be synthesized by different chemical and electrochemical methods, a fact that favors its use. Moreover, synthesis of gold nanoparticles using different plant sources have been performed and biosynthesized gold nanoparticles have been characterized morphologically and spectroscopically [3].

AuNPs and reduced graphene oxide composite were applied to trace metal ions determination by differential pulse voltammetry. AuNPs were used in electro-chemical detection of As (III) in anodic potential range. AuNPs used in amperometric biosensors and electrochemical techniques enhances detection sensitivity. AuNPs were synthesized *in situ* and combined with monolayers were applied to malathion inhibitive determination. Due to their inherent low reactivity, platinum NPs (PtNPs) are the selected metal NPs for anodic current range and have been applied to As(III), formaldehyde,

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neurotransmitters, glucose oxidation and uric acid determination in Ascorbic Acid (AA) presence. PtNPs and PdNPs/Methylthiophene (PMT) sensors have been applied to Dopamine (DA) and AA determination. Bimetallic Nanoparticles (BNPs) incorporating Pt, have sought the attention of sensor engineers and there has been rapid increase in sensors incorporating BNPs in a short period of time [4].

Biofabrication of platinum nanoparticles has been achieved with *Fumariae* herba extract. RhNPs are catalysts in hydrogenation and silylformylation reactions; RhNPs were synthesized by means of the chemical reduction method and applied as precursor to steaming reforming of propane; catalysis and sensing of cytochrome c and modified with dendrimers and graphene for H_2O_2 sensing; as well as used in biosensor for α ketoglutarate [5]. RhNPs-modified screen-printed electrode was developed and applied to bromide anions determination. On the other hand, Epinephrine (EPI) is a neurotransmitter found in biological systems at very low concentration, performs fast irreversible autooxidation to insoluble products and blocks surface electrode. Also, EPI is found in biological systems accompanied by Ascorbic (AA) and Uric Acid (UA), which are oxidized at conventional electrodes in the same potential region and are important interferences. Traditionally EPI was determinate through electrochemical methods such as Cyclic Voltammetry (CV) and Pulse Differential Voltammetry (DPV).

Various electrochemical sensors using these techniques for EPI determination have been published. Electrodes used in first place are carbon, glassy carbon and carbon paste modified with molecularly imprinted polymer arrays of metallic oxides and AuNPs. Also are modified with metallic oxides and functionalized Carbon Nanotubes (CNTs). Electrodes modification also includes electropolymerization of macrocyclic nickel complex and polyalanine. Modified electrodes with alumina/graphene/Cu hybrid graphene oxide nano sheets and Ni-doped nano ion exchange resin have been applied to EPI determination in presence of acid ascorbic and uric; pharmaceutical compounds and dopamine. With the goal to avoid interferences; many modified electrodes have been proposed. Summarizing nano modifications of electrodes provide sensitivity and selectivity in neurotransmitter determinations [6].

Screen Printed Carbon Electrodes (SCPCEs) are versatile and are used in analytical determination of contaminants, drugs, heavy metals at very low concentrations. SCPCEs modified with metallic Nps (NPs/SCPCE) and enzymes offer sensitivity and specificity. This research work was conducted with the goal to compare the effect of NPs of Au, Pt and Rh deposited by distinct electrochemical procedures on the sensitivity of amperometric EPI calibration curves performed with SCPCEs modified with TTF as electrochemical mediator (SCPC_{TTF}E). TTF allows a rapid electron transfer between SOD and electrode surface that can be carried out at lower potential. EPI role and mechanism of SOD was established in previous work. The enzyme Superoxide Dismutase (SOD) was immobilized in modified NPs/SCPC_{TTF}E, namely SOD/NPs/SCPC_{TTF}E. Very few biosensors regarding EPI electrochemical determination have been found showing a research gap over this neurotransmitter. Moreover, complete characterization of metallic NPs used for electrodes modification not had been performed. NPs deposited on electrodes were characterized through XRF that provides metallic percentage on SCPC_{TTF}E. SEM allowed observes morphologic aspects of

electrode surface. AFM height and statistical parameters were useful to evaluate roughness electrodes surface and nanoparticles size distribution [7].

The novelty of this work lies in XRF, SEM and AFM characterization of electrode surface modified with NPs of Au, Rh, Pt deposited by electrochemical procedures. Modification showed effect on amperometric response of an EPI disposable superoxide dismutase biosensor. Also, it showed a correlation between lower AFM parameters and higher slope EPI calibration curves except in PtNPs case. Additionally the successful application of the sensor to EPI injection showed suitable recovery value (~100%). Biosensor results are agreed with labeled concentration [8].

Materials and Methods

Reagents

All solutions were prepared with purified water supplied by TKA purification system, inverse osmosis, with a UV lamp irradiation system with 0.055 μ S/cm. SOD enzyme (30 KU) and EPI were purchased from Sigma Steinheim, Germany, Bovine Serum Albumine (BSA), glutaraldehyde and hydrogen tetrachloroaurate (III) trihydrate ($HAuCl_4$) were obtained from Sigma-Aldrich (Sigma-Aldrich, Steinheim, Germany), it was used 0.10 mM. Solutions of platinum and rhodium 0.10 mM were prepared from rhodium and platinum standards for AAS.

Britton Robinson (BR) supporting electrolyte solutions were prepared as usual with boric, phosphoric and acetic acids, pH value was obtained adjusting with NaOH solution. Several inks were used in the fabrication of SPEs, namely electrodag PF-407 A (carbon ink), electrodag 6037 SS (silver/silver chloride ink) and electrodag 452 SS (dielectric ink) supplied by Acheson Colloiden. Inks were screen printed and cured accordingly fabricant instructions. The working electrode ink was prepared by thoroughly mixing carbon ink with TTF (TTF) 5% v/w and immediately screen printed [9].

Equipment

An electrochemical system Autolab PGSTAT Echo Chemie 128 N with GPS software was used to record electrochemical measurements. All pH values were adjusted with a pH meter. An equipment S-3700 Hitachi was used to perform Scanning Electronic Microscopy (SEM) of modified NPs/SCPC_{TTF}Es. An equipment IXRF Systems model 550i was used to obtain spectra of elements deposited on the SCPC_{TTF}Es. AFM parameters and images were obtained with NanoScopeQuadrex digital instruments Veeco Metrology Group [10].

Hand-made SCPC_{TTF}Es were produced on a DEK 248 printing machine using polyester screens with appropriate stencil designs mounted at 45° to the printer stroke. Three screen printed electrodes were deposited on polyethylene terephthalate. SCPC_{TTF}E used for nanoparticles modification is depicted in Figure 1 [11].

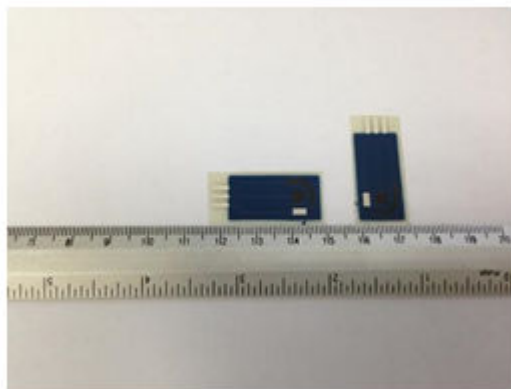


Figure 1. Dimensions of SCPC_{TTFE} used for nanoparticles modification.

Nanoparticles electrodeposition methods on SCPC_{TTFE}s

SCPC_{TTFE}s modification with nanoparticles (NPs/SCPC_{TTFE}s) was carried out by controlled potential and cyclic voltammetry scan methods.

- Controlled potential deposition was carried at two different potentials namely 0.3 and 0.18 V, in a quartz cell containing gold, platinum, rhodium solutions 0.10 mM in H₂SO₄ 0.5 M [12].
- Following electrodeposition process, the NPs/SCPC_{TTFE}s were removed from plating solution and rinsed with purified water.
- Cyclic voltammetry deposition was performed doing a set of seven successive voltammetric scans between 1.25 and -0.2 V in a quartz cell containing gold, platinum, rhodium solutions 0.10 mM in H₂SO₄ 0.5 M. Electrodes were prepared by setting two cyclic Voltammetric Conditions (VC) specifically VC₁ and VC₂. VC₁: Delay time 60 s; step potential 0.01495 V, scan rate 0.050

Variables level	Equilibrium time Pt, Pd, Rh	Scan rate (V/S) Pt, Pd, Rh	Step potential Pt, Pd, Rh
VC ₁	60	0.05	0.01495
VC ₂	120	0.1	0.025

Table 1. Parameters of cyclic voltammetry for SPC_{TTFE}s modified with NPs of Pt, Rh and Pd.

XRF percentage SCPC_{TTFE}s modified with Pt, Rh and Au by controlled potential and VC₁ and VC₂

Two different controlled potentials, +0.18 V and +0.3 V, were applied during 15 seconds to SCPC_{TTFE}s in order to deposit NPs of every metal. XRF spectra were obtained from surfaces of SCPC_{TTFE}s modified with AuNPs, PtNPs and RhNPs. In Figure 2 is showed XRF spectrum for Rh, Pt and Au for controlled potential electrodeposition and VC₁ and VC₂ methods (Figures 2-4).

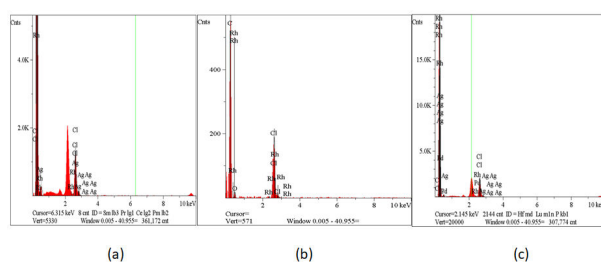


Figure 2. XRF: a) RhNPs/SCPC_{TTFE} deposited at 0.18 V; b) RhNPs/SCPC_{TTFE} deposited VC₁; c) RhNPs/SCPC_{TTFE} deposited VC₂.

V/s and VC₂: Delay time 120 s, step potential, 0.025 V, scan rate 0.1 V/s. After nanoparticles deposition, the electrode was rinsed with purified water and wiped carefully. SEM, RXF and AFM were performed on metallic nanoparticles modified SCPC_{TTFE}s [13].

SOD enzyme immobilization onto Nps/SCPC_{TTFE}s

After nanoparticles controlled potentials and cyclic voltammetry procedures were performed; SOD enzyme was immobilized on surface electrodes.

Enzyme was immobilized by polymerization with glutaraldehyde, on the surface of AuNPs/SCPC_{TTFE}s, PtNPs/SCPC_{TTFE}s and RhNPs/SCPC_{TTFE}s, namely SOD/Nps/SCPC_{TTFE}s. Immobilization procedure was developed previous work. Electrodes were stored at 40°C before use and between measurements. Modified electrodes were washed with purified water, before and after its use [14].

Results

Optimization of experimental electrodeposition parameters

From preliminary CVs for tested metals were chosen conditions where anodic current starts to increase. It was observed an increase of anodic current with scan rate, delay time and step potential, for the three metals tested. In order to obtain experimental conditions for nanoparticles deposition were changed three parameters for every tested metal namely; step potential from 0.01495 V to 0.03995 V; scan rate from 0.05 V/s to 0.20 V/s and delay time from 0 to 300 s. Two levels of these variables were chosen (Table 1). SEM, AFM and XRF electrodes were prepared setting VC₁ and VC₂ levels of variables and controlled potential condition [15].

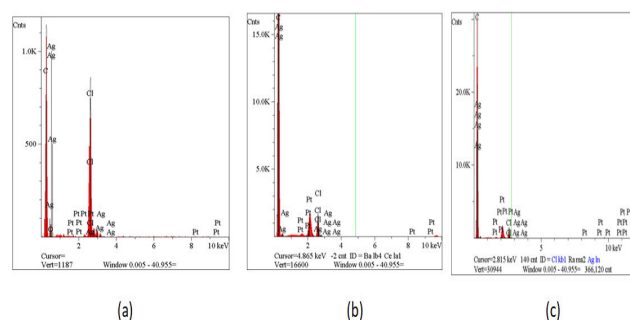


Figure 3. a) PtNPs/SCPC_{TTFE} deposited at 0.18 V; b) PtNPs/SCPC_{TTFE} deposited VC₁; c) PtNPs/SCPC_{TTFE} deposited VC₂.

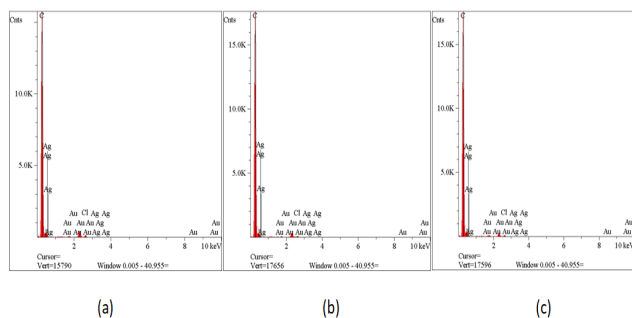


Figure 4. a) AuNPs/SCPC_{TTFE} deposited at 0.18 V; b) AuNPs/SCPC_{TTFE} deposited VC₁; c) AuNPs/SCPC_{TTFE} deposited VC₂.

Plating of metals at +0.18 V during 15 seconds, produced a higher percent of Au (1.42) and Rh (0.693) than Pt (0.223); at +0.30 V, Pt percent deposited was higher (1.48) than Rh (0.380) but the application of +0.3 V did not deposited Au, then conditions of Eap of +0.18 V and 15 seconds of controlled potential method were selected to deposit NPs.

XRF metallic percentages for SCPC_{TTFE}s modified with Nps of Pt, Rh and Au obtained for VC₁ were for Pt (2.74) for Rh (4.49) and Au(1.87) and for VC₂ were Pt (2.71), Rh (2.95) and Au (2.23) both by scanning voltammetry method.

SEM images of NPs/SCPC_{TTFE}s prepared by constant potential and VC₁, VC₂ conditions

SEM images of modified NPs/SCPC_{TTFE}s were obtained, in order to observe surface metallic modifications. SEM pictures of NPs/SCPC_{TTFE} are showed in Figure 5 for constant potential method and for VC₁ and VC₂.

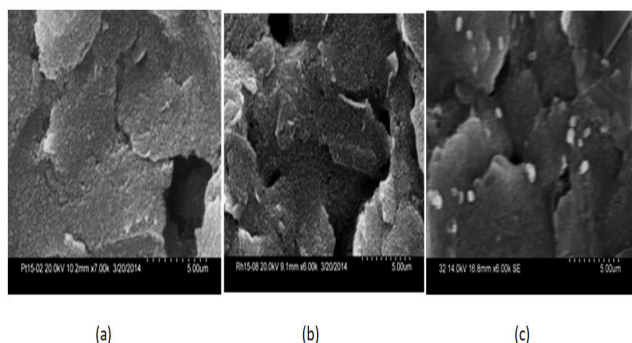


Figure 5. SEM images: a) PtNPs/SCPC_{TTFE}s; b) RhNPs/SCPC_{TTFE}s; c) AuNPs/SCPC_{TTFE}s prepared by constant potential electrodeposition at 0.18V.

NPs/SCPC_{TTFE}s were prepared using chosen controlled potential conditions and EPI calibration curves were recorded; it was found that the highest slope belongs to PtNPs/SCPC_{TTFE}, followed by RhNPs/SCPC_{TTFE} and AuNPs/SCPC_{TTFE}. NPs/SCPC_{TTFE}s prepared by direct electrodeposition displayed lower EPI calibration curves slopes than VC₁, VC₂; therefore AFM parameters were obtained only for NPs/SCPC_{TTFE}s prepared by VC₁, VC₂ conditions in order to correlate surface parameters with slopes of EPI calibration curves (Figures 6-8).

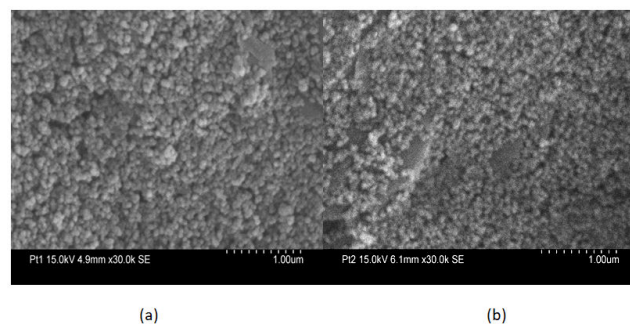


Figure 6. a) PtNPs/SCPC_{TTFE}s prepared by VC₁; b) of PtNPs/SCPC_{TTFE}s prepared by VC₂.

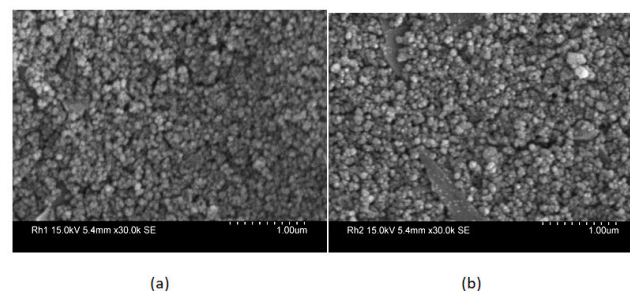


Figure 7. a) RhNPs/SCPC_{TTFE}s prepared by VC₁; b) RhNPs/SCPC_{TTFE}s prepared by VC₂.

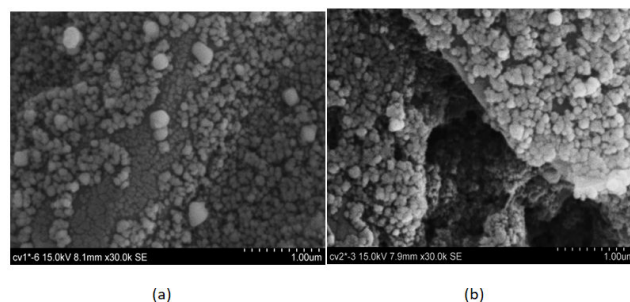


Figure 8. a) AuNPs/SCPC_{TTFE}s prepared VC₁; b) AuNPs/SCPC_{TTFE}s prepared by VC₂.

AFM parameters SPC_{TTFE}s modified with NPS of Pt, Au and Rh

The most important AFM height parameters Roughness Average (RA), Roughness maxima (Rmax) in bold and statistical parameters Roughness Kurtosis (RKu) and Roughness Skewness (RSk) coefficients are showed in Figures 9-11. AFM parameters analysis showed metallic deposits was nanometric size.

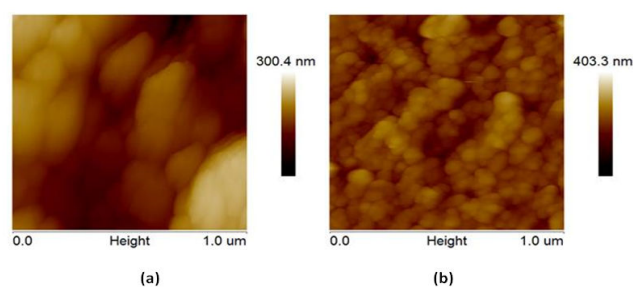


Figure 9. a) AuNPs/SCPC_{TTFE}s prepared by VC₁; RA 33.8, RKu 2.62, RSk 0.126; b) AuNPs/SCPC_{TTFE} prepared VC₂; RA 34.6, RKu 3.19, RSk 0.292.

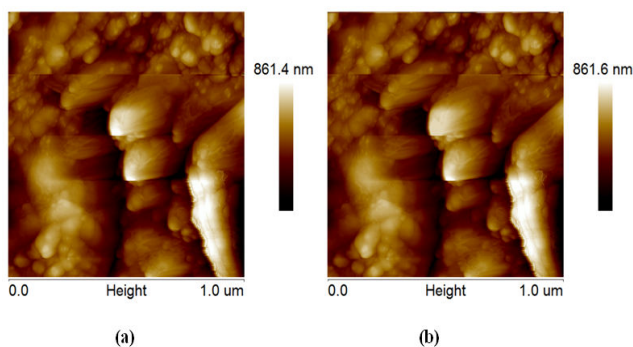


Figure 10. a) PtNPs/SCPC_{TTFE}s prepared by VC₁; RRA 106, RKu 3.60, RSk 0.141; b) PtNPs/SCPC_{TTFE}s prepared by VC₂.

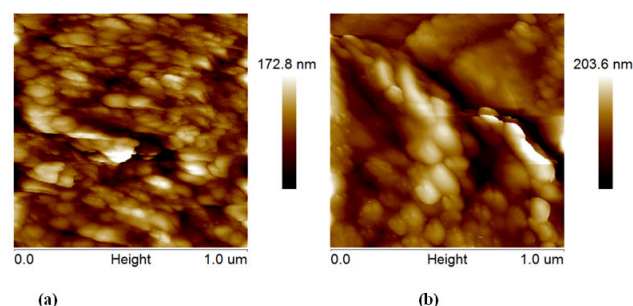


Figure 11. a) RhNPs/SCPC_{TTFE}s prepared by VC₁; RA 22.0, RKu 3.30, RSk 0.0733 ; b) RhNPs/SCPC_{TTFE}s prepared by VC₂; RA 25.6, RKu 3.65, RSk 0.189.

EPI calibration curves performed with SOD/NPs/SCPC_{TTFE}s prepared by controlled potential and VC₁, VC₂, conditions

Optimization of EPI amperometric current signal was achieved at 0.20 V and pH 5.02. EPI concentration additions produced a defined current increasing signal.

SOD/NPs/SCPC_{TTFE}s current was corrected subtracting residual current (*I*_{res}) from recorded current namely (*I*) for every EPI addition, namely (ΔI). This procedure was performed for direct electrodeposition VC₁, VC₂ methods in order to standardize the electrodes current prepared under different conditions. Calibration curves performed for SOD/NPs/SCPC_{TTFE}s prepared under constant potential are showed in Figure 12.

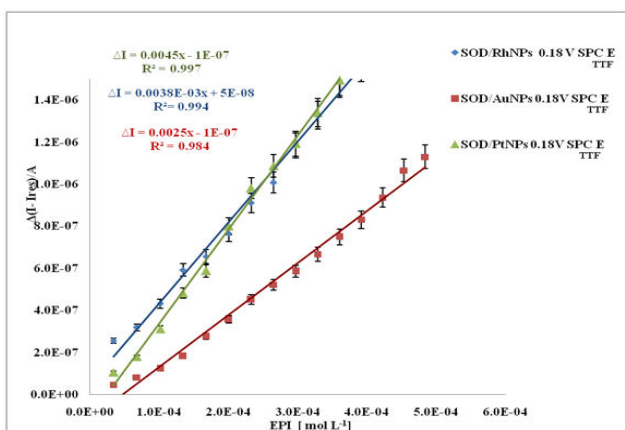


Figure 12. EPI calibration curves for SOD/NPs/SCPC_{TTFE}s, prepared constant potential, pH 5.0; +0.2 V vs. SPE Ag/AgCl. For successive additions of EPI from a stock solution 8.56×10^{-3} M. Concentration error bars 5%.

EPI calibration curves performed under VC₁ showed linearity and amperometric recordings current reached constant value after every EPI addition. The highest slope belongs to SOD/PtNPs/SCPC_{TTFE} prepared VC₁, followed by SOD/RhNPs/SCPC_{TTFE} and SOD/AuNPs/SCPC_{TTFE}, which are showed in Figure 13. EPI calibrations performed under VC₂ condition showed higher linearity and signal stability than VC₁. Slopes of SOD/NPs/SCPC_{TTFE}s modified with metallic nanoparticles deposited by VC₁ were higher than the slopes deposited by VC₂ and controlled potential methods. SOD/PtNPs/SCPC_{TTFE}s deposited by VC₁ showed highest slope of SOD/NPs/SCPC_{TTFE}s prepared by tested methods. Moreover SOD/AuNPs/SCPC_{TTFE}s prepared by VC₁ method showed higher slope than VC₂ method. SOD/RhNPs/SCPC_{TTFE}s prepared by VC₂ method showing similar behavior.

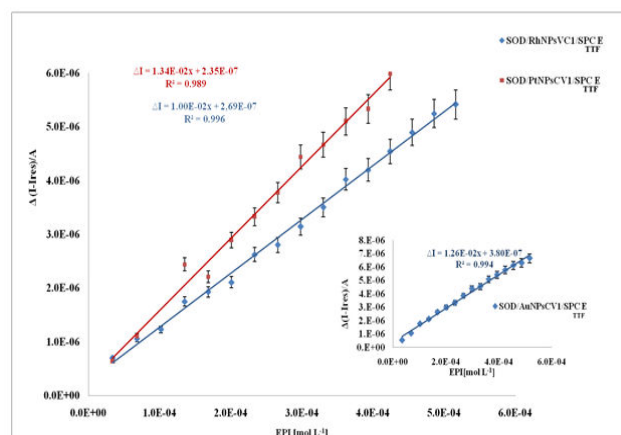


Figure 13. EPI calibration curves for SOD/NPs/SCPC_{TTFE}s, prepared VC₁ pH 5.0; +0.2 V vs. SPE Ag/AgCl. For successive additions of EPI from a stock solution 8.58×10^{-3} M. Inset calibration curve of AuNPs/SCPC_{TTFE}. Concentration error bars 5%.

Figure 10 depicts EPI calibration curves for SOD/NPs/SCPC_{TTFE}s modified by VC₂. Clearly it can be observed from Figure 14 that NPs expand the linear ambit of EPI calibration curves compared with no modified SCPC_{TTFE}.

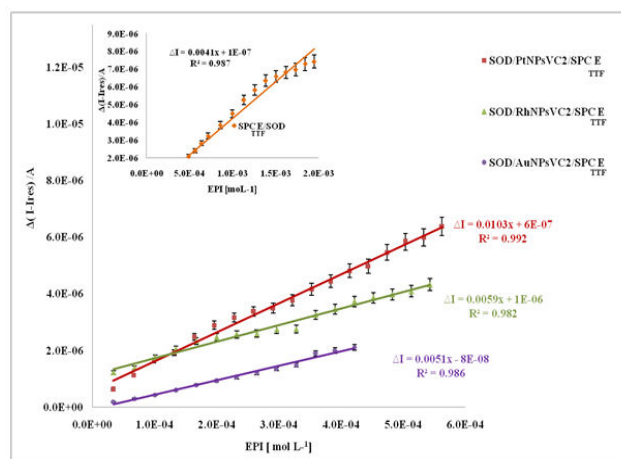


Figure 14. EPI calibration curves for SOD/NPs/SCPC_{TTFE}s, prepared VC₂ pH 5.0; +0.2 V vs. SPE Ag/AgCl. For successive additions of EPI from a stock solution 8.56×10^{-3} M. Inset calibration curve of SOD/SCPC_{TTFE}. Concentration error bars 5%.

Michaelis Menten behavior of SOD/NPsSC_{TTF}Es modified EPI biosensor

NPs/SC_{TTF}Es modified with SOD enzyme immobilized on their surfaces, respond to increasing EPI concentration added to cell enhancing amperometric current recordings. Linear regression is

obtained when amperometric current namely (*I*) corrected subtracting residual current of electrode namely (*I*_{res}) is plotted against EPI cell concentration. Michaelis Menten apparent constants (*K_m* app) were estimated for SOD/NPs/SC_{TTF}Es modified with AuNPs, RhNPs, PtNPs by controlled potential and CV₁ and CV₂ electrodeposition methods, by means of Lineweaver Burk plot; when 1/*I* corrected is plotted vs. 1/EPI cell concentration. Table 2 contains estimated *K_m* app values.

<i>K_m</i> app (M)	EPI/SOD/ AuNPsSC _{TTF} E	EPI/SOD/ RhNPsSC _{TTF} E	EPI/SOD/ PtNPsSC _{TTF} E
Electrodeposition 0.18 V	$(7.8 \pm 0.3) \times 10^{-4}$	$(3.3 \pm 0.2) \times 10^{-3}$	$(1.2 \pm 0.3) \times 10^{-4}$
VC ₁ conditions	$(3.0 \pm 1.0) \times 10^{-3}$	$(6.0 \pm 2.0) \times 10^{-3}$	$(2.5 \pm 0.9) \times 10^{-3}$
VC ₂ conditions	$(1.5 \pm 0.2) \times 10^{-3}$	$(1.2 \pm 0.1) \times 10^{-4}$	$5.8 \pm 0.3) \times 10^{-4}$

Table 2. *K_m* app values for EPI/SOD/NPsSC_{TTF}Es biosensors.

It can be seen for Table 2 that *K_m* app values for EPI/SOD/NPsSC_{TTF}Es biosensors showed increased *K_m* app values for biosensors modified with metallic nanoparticles deposited by VC₁ conditions, meaning lower affinity of SOD enzyme for EPI substrate. In contrast EPI/SOD/NPsSC_{TTF}Es biosensors prepared VC₂ conditions showed *K_m* app lower values than electrodes prepared by VC₁ conditions meaning higher affinity of SOD enzyme for EPI substrate.

Interferences and behavior of SOD/NPsSC_{TTF}Es modified EPI biosensor

Interference effect on EPI was tested by means of superoxide dismutase enzyme electrodes modified with nanoparticles of platinum deposited VC₂; this one was selected regarding analytical application due to higher linearity of calibration curves and *K_m* value. It was performed adding 100 μ L of EPI 8.53 mM, 100 μ L of ascorbic acid 0.10 M and 100 μ L of uric acid 0.96 mM in cell, recording cyclic voltammogram and peak potential of every addition. Voltammograms were performed between -0.250 V and 1.25 V (vs. Ag/AgCl) at 0.10 V s⁻¹. CV of EPI and interferences are showed in Figure 15.

Potential peak of EPI in presence of interferences showed a lower value than these ones, allowing avoid interferences of such species at 0.20 V, potential chosen for EPI determination.

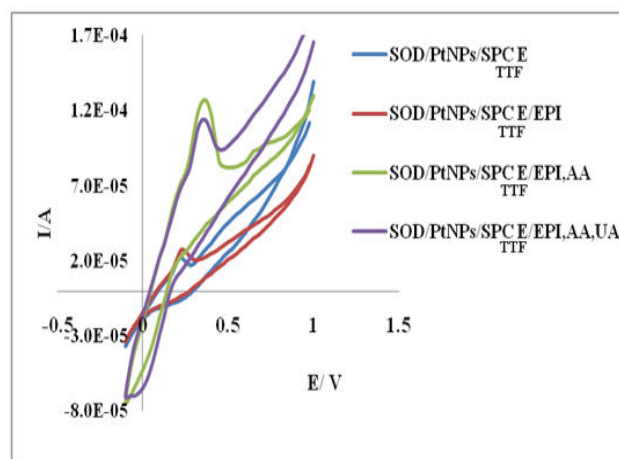


Figure 15. Cyclic voltammograms of AA and UA and EPI using SOD/PtNPs/SC_{TTF}E, pH 5.0 Britton Robinson buffer.

SOD/PtNPs/SC_{TTF}E biosensor characterization

SOD/PtNPs/SC_{TTF}E performed by VC₂ showed higher linearity of calibration curves and better signal stability than performed under VC₁, the former condition was selected for biosensor characterization. The precision of the proposed method was established in terms of reproducibility. Using the optimum voltammetric conditions and BR buffer pH 5.0 as supporting electrolyte, several EPI calibration curves with concentration ranging from 3.0×10^{-5} M to 6.0×10^{-4} M were performed.

Table 3 shows calibration curves parameters estimated for different SOD/PtNPs/SC_{TTF}E biosensors in order to estimate reproducibility SOD/PtNPs/SC_{TTF}Es biosensor slope.

Calibration	Slope (A/M)	Intercept (A)	Sy/x	R ²
I	0.01004	6.74×10^{-7}	1.3×10^{-7}	0.993
II	0.00947	3.36×10^{-7}	1.2×10^{-7}	0.999
III	0.0098	2.34×10^{-7}	8.2×10^{-8}	0.999
IV	0.01009	-3.22×10^{-7}	2.6×10^{-7}	0.992
Mean	0.00985	-	-	-
SD	0.00029	-	-	-
RSD	2.9	-	-	-

Table 3. Parameters of EPI calibration curves performed in order to estimate reproducibility for SOD/PtNPs/SC_{TTF}Es biosensor.

Biosensor reproducibility, determined as Residual Standard Deviation (RSD%) associated with calibration curves slopes, was 2.9%. Despite the disposable character of SOD/PtNPs/SC_{TTF}Es, repeatability studies were also performed using the same SOD/PtNPs/SC_{TTF}Es based biosensor and it was observed RSD slope increase after second replicate. Regarding disposable character of biosensor, reproducibility is a better estimate of precision. Limit of Detection (LOD) of 22.8 μ M and Quantification Limit (LOQ) of 76.2 μ M were estimated for SOD/PtNPs/SC_{TTF}Es based sensor, using

the LOD=3Sy/x, LOQ=10Sx/y criteria, where Sy/x corresponds with standard deviation of six calibration curves. Recovery study was performed using EPI pharmaceutical injection (1.0 mg/mL), diluted to concentration of developed method. Added concentration was evaluated by standard addition method. Mean recovery of 103.1% with RSD 5.4% (n=6) was achieved. Recovery figures are shown in Table 4. Experimental value obtained is accordingly with declared concentration regarding confidence limit. This fact confirms analytical performance of proposed biosensor.

EPI spiked (M)	EPI recovered (M)	g EPI/L	Recovery (%)
5.459×10^{-3}	5.360×10^{-3}	0.982	98.2
	6.071×10^{-3}	1.1122	111.2
	5.660×10^{-3}	1.037	103.7
	5.302×10^{-3}	0.9713	97.1
	5.888×10^{-3}	1.0788	107.9
	5.472×10^{-3}	1.0024	100.2
Mean	5.626×10^{-3}	1.031	103.1
SD	3.059×10^{-4}	0.056	5.6
RSD	5.4	5.4	5.4

Table 4. Recovery of EPI pharmaceutical injection spiked to buffer solution estimated with SOD/PtNPs/SC_{TTF}Es biosensor.

Discussion

EPI biosensor current showed effect of percentages of every metal deposited by tested methods: Controlled potential and two procedures of cyclic voltammetry scanning. Preliminary screening was performed because 0.18 V and 15 sec produced higher percentages of Au, Pt and Rh accordingly with XRF figures. EPI biosensor calibration curves done at controlled potential showed a slightly higher slope for PtNPs although with lower percentage than AuNPs and RhNPs. Catalytic effect of PtNPs is not determine for its amount deposited on electrode surface.

Consequently, NPs obtained by different methods influence biosensor sensitivity. It can be seen that amperometric slopes of tested metals are lower for controlled potential than for cyclic voltammetry scanning. Similarly, slopes obtained applying VC₁ are higher than VC₂. From SEM images can be observed, that NPsSC_{TTF}Es were modified for methodologies used to deposit each metal. Moreover, electro deposition method modifies electrode surface characteristics. AFM parameters determination is also an

adequate tool for evaluating electrodes nanoparticles surface modification. This modification affects particle size and catalytic activity as could be observed from slopes calibration curves. SOD/AuNPs/SC_{TTF}E prepared by VC₁ method show larger slope than electrode prepared by VC₂ although there is a little difference in height parameters RA and Rmax; these parameters are lower for VC₁. Catalytic effect for AuNPs is increased by VC₁. Although AFM parameters PtNPs/SC_{TTF}E by VC₁ and VC₂ are practically the same, PtNPs/SC_{TTF}E showed a larger EPI calibration slope for VC₁ and VC₂ and could be explained because PtNPs/SC_{TTF}E, XRF percentage deposited by VC₁ and VC₂ is larger than controlled potential method. On the other hand, RhNPs/SC_{TTF}E slopes EPI calibration curves VC₁ are significantly higher than VC₂ and there is little difference in their RA and Rmax values, which are lower for VC₁. RhNPs/SC_{TTF}E showed lower AFM parameters RA, RMS and Rmax than AuNPs/SC_{TTF}E; this fact allows higher slopes of the former than AuNPs/SC_{TTF}E.

Linearity and sensitivity were obtained for EPI calibration curves performed under VC₁ and VC₂ for PtNPs. A possible explanation lies

in statistical parameters such as R_{Ku} and Skewness R_{Sk}. When R_{Ku} is closer to 3, value distribution nanoparticles are near to normal distribution as in this case. R_{Sk} measures asymmetry profile surrounding mean line. When R_{Sk} is positive, it means the surface has more peaks than valleys and if R_{Sk} is negative, it means the opposite. PtNPs/SC_{TTF}E shows positive R_{Sk} coefficients for CV₂ (0.157) and for CV₁ (0.141), meaning more surface peaks that increase amperometric current on SOD/PtNPs/SC_{TTF}E as it is the case of CV₁ and making a surface more regular in CV₂ favoring signal stability. Higher slope for CV₁ could be assigned to a higher R_{Sk} than CV₂. Specifically, for SOD/PtNPs/SC_{TTF}E is more important the catalytic effect inherently linked to metallic properties than R_{max} value. Moreover, this fact is reinforced by their highest R_{max} (Figure 5). On other hand, RhNPs/SC_{TTF}E shows negative R_{Sk} coefficients meaning more valleys than peaks.

AuNPs/SC_{TTF}E shows positive R_{Sk} for VC₁ and negative for VC₂ meaning peaks for the first condition and valleys for the second, fact that could explain the sensitivity difference between slopes for AuNPs/SC_{TTF}Es. Enzymatic behavior is displayed in Lineaweaver Burke plots applied to NPs/SC_{TTF}Es, which showed increased K_m app values for VC₁ and lower values for VC₂, meaning higher affinity of EPI biosensor for this last condition.

Conclusion

EPI superoxide dismutase biosensor shows effect of electrodeposition method on amperometric response. Amperometric response of AuNPs/SC_{TTF}E, RhNPs/SC_{TTF}E and PtNPs/SC_{TTF}E biosensors increases from controlled potential to VC₂ and VC₁ in this order. XRF allowed to estimate metal percentage deposited on NPs/SC_{TTF}Es by each tested metal and different electrochemical methodologies. SEM images show surface modifications and AFM permitted to confirm these ones are at nanometric level. For AuNPs/SC_{TTF}E and RhNPs/SC_{TTF}E, AFM parameters support slopes values for EPI calibration curves. AFM allowed setting differences between electrochemical behavior of Au/NPs/SC_{TTF}Es with larger AFM parameters and lower slopes than RhNPs/SC_{TTF}Es. AA and UA do not represent interference for EPI determination performed at 0.2 V. Comparing slope of SOD/SC_{TTF}E with slopes of modified SOD/NPs/SC_{TTF}Es, these last ones showed larger slopes for both VC₁ and VC₂ conditions. Linearity and signal stability for VC₂ condition are higher than VC₁, conversely VC₁ shows higher slope sensibility than VC₂. SOD/PtNPs/SC_{TTF}Es electrochemical behaviors do not correlate with AFM parameters. Validation parameters of SOD/Pt/SC_{TTF}Es are adequate for EPI low concentration determination.

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Conflicts of Interest

The author declares no conflict of interest.

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