

Using of a Graphite-Polyurethane Composite Electrode Modified with a Schiff Base as a Bio-Inspired Sensor in the Dopamine Determination

Sidney X. dos Santos^{a,b} and Éder T. G. Cavalheiro^{*b}

^aInstituto de Ciências Exatas e Tecnológicas, Universidade Federal de Viçosa,
38810-000 Rio Paranaíba-MG, Brazil

^bDepartamento de Química e Física Molecular, Instituto de Química de São Carlos,
Universidade de São Paulo, 13560-970 São Carlos-SP, Brazil

Um eletrodo compósito sólido à base de grafite e resina poliuretana modificado com um complexo de base de Schiff do tipo tetradentada simétrica, *N,N'*-bis(salicilideno)etilenodiamino de cobre(II) ([Cu^{II}Salen]), foi avaliado como sensor bio-inspirado, usando dopamina (DA) como sonda eletroquímica. O comportamento voltamétrico do modificador [Cu^{II}Salen] foi avaliado em eletrodo de pasta de carbono modificado e um mecanismo redox foi proposto. As potencialidades analíticas do eletrodo compósito à base de grafite e resina poliuretana (GPU) modificado com [Cu^{II}Salen] (MGPU) foram avaliadas usando DA. O eletrodo MGPU apresentou melhores resultados quando comparado ao GPU sem modificação, exibindo efeito eletrocatalítico, o que o caracteriza como sensor bio-inspirado. O eletrodo MGPU foi aplicado na determinação de DA em formulações farmacêuticas utilizando DPV e o resultado foi comparado àquele obtido com o método oficial (cromatografia líquida de alta eficiência, HPLC), descrito na farmacopeia americana, concordando em um nível de 95% de confiança de acordo com o teste *t-student*.

A solid composite electrode based on graphite and polyurethane resin modified with a copper(II) complex of the symmetrical tetradentate Schiff's base type, *N,N'*-ethylenebis(salicylideneiminato) copper(II) ([Cu^{II}Salen]), was evaluated as a bio-inspired sensor using dopamine (DA) as a model analyte. The voltammetric behavior of the modifier [Cu^{II}Salen] was investigated at a modified carbon paste electrode and a redox mechanism was proposed. The analytical potentialities of the composite electrode based on graphite and polyurethane resin (GPU) modified with [Cu^{II}Salen] (MGPU) were evaluated using DA as probe. MGPU presents best results when compared with GPU without modification, with electrocatalytic effect, indicating that the electrode can be characterized as a bio-inspired sensor. The MGPU electrode was applied in the determination of DA in pharmaceutical formulation using DPV and the results were compared to those obtained with the official method (high performance liquid chromatography, HPLC) as described in US Pharmacopoeia, agreeing within 95% confidence level according to student's *t*-test.

Keywords: Schiff base, composite electrode, dopamine, bio-inspired sensor

Introduction

The modification of electrodes is an interesting proposal when the intent is to get higher selectivity and sensitivity for applications in electroanalysis. In most cases, the modifier is an electroactive specie, acting as a mediator in the electron transfer between the solution and the electrode substrate. In this context, it can be highlighted the bio-inspired sensors. This concept,

according to Zapp *et al.*,¹ comes from the observation of molecules and architecture inspired by biological models.

The development of modified electrochemical sensors can be extended to the preparation of solid composite electrodes in which the graphite is agglutinated by a polymer such as polyurethane resin. According to Tallman and Petersen,² a composite electrode is a material consisting of at least one conductor phase and at least one insulator phase. In this case, the polyurethane resin is the insulator phase acting also as an agglutinant,

*e-mail: cavalheiro@iqsc.usp.br

while the graphite acts as the conductor phase. The main advantages of solid composite electrodes are the easiness of preparation and surface renewal, low cost, robustness and possibility of use in a non-aqueous medium or flow applications.

A Schiff base is defined as compound with an imine group that contains a carbon-nitrogen double bond with the nitrogen atom connected to an aryl or alkyl group.³ Schiff bases form stable complexes with metals, which present biological relevance and serve as catalyst in several chemical reactions.^{3,4}

The metal complexes of Schiff bases have shown similar behavior to metalloporphyrins with respect to their catalytic activity, despite their structural differences.⁵ Schiff bases present advantages over the metalloporphyrins such as easiness of synthesizing, low cost of preparation, easy dissolution and formation of stable solutions.⁵

Schiff base complexes containing cobalt(II) or copper(II) were evaluated by Xie *et al.*⁶ as mimetic peroxidases for oxidation of phenolic species in the presence of H₂O₂ showing good catalytic activity and similar catalytic character to a natural enzyme.

Dopamine (DA), 3,4-dihydroxy-phenylethylamine, is a neurotransmitter of the catecholamine class.⁷ DA is a phenolic compound, whose electrochemical oxidation using unmodified electrodes occurs in a reversible process involving two protons and two electrons.

In this work a solid composite electrode based on graphite and polyurethane resin was modified with a copper(II) complex of a symmetrical tetradentate Schiff's base type, *N,N'*-ethylenebis(salicylideneiminato) copper(II) ([Cu^(II)Salen]), which was evaluated as a bio-inspired sensor, based on catechol oxidase behavior in the determination of DA, as a model analyte.

Experimental

Reagents and solutions

All reagents were analytical grade (p.a.) and used without previous purification. Graphite powder (1-2 μm), DA, salicylaldehyde and ethylenediamine were purchased from Aldrich®, and also used as received.

All solutions were prepared using water purified in a Barnstead™ EasyPure® RoDi (Thermoscientific, model D13321) system resistivity ≥ 18 MΩ cm.

The electrolytical media were Britton-Robinson (B-R) buffer, 0.1 mol L⁻¹ acetate buffer and 0.1 mol L⁻¹ phosphate buffer. The pH was adjusted to the desired value with 1.0 mol L⁻¹ HCl or NaOH solution.

Apparatus

Voltammetric experiments were performed using a potentiostat/galvanostat Autolab PGSTAT-30 (Ecochemie) coupled to a microcomputer and controlled by a GPES 4.9 software (Ecochemie). A glass cell with a total capacity of 25.0 mL, containing the working electrode (modified carbon paste electrode or modified GPU composite electrode), a platinum wire (1 cm long) as auxiliary electrode and the saturated calomel reference electrode (SCE) were used. All experiments were performed at 25 ± 1 °C.

The TGA/DTG (thermogravimetric and derivative thermogravimetric curves) curves were obtained in a TGA-DTA simultaneous SDT-Q600 module, controlled by a ThermoAdvantage® software (release 4.2.1) both from TA Instruments. The studies were conducted with sample mass of c.a. 6.0 mg in open α-alumina crucibles within a 25-1000 °C range at a heating rate of 10 °C min⁻¹ under a dynamic dry air atmosphere flowing at 100 mL min⁻¹.

The infrared spectra were recorded in a Bomem/MB-102 FTIR spectrophotometer in the 4000 and 400 cm⁻¹ range, using KBr pellets for the ligand and complex.

For analytical comparative method it was used a chromatographic procedure performed in a Shimadzu high performance liquid chromatographic (HPLC) system comprising a SLC-10A VP controller, a LC-6 AD pump and a SPD 10A VP UV-Vis detector, equipped with a Supelcosil LC-18 column (150 × 4.6 mm, 5 μmol L⁻¹), coupled to a microcomputer and controlled by CLASS-VP 6.1 software (Shimadzu).

Synthesis of ligand and complex

The synthesis of the ligand *N,N'*-ethylenebis(salicylimine) (Salen) was performed according to procedures previously described in the literature⁸⁻¹⁰ based on the 2:1 (mol:mol) direct condensation reaction between salicylaldehyde and primary amine ethylenediamine to obtain Salen.

The complex was synthesized by the 1:1 (mol:mol) reaction between the Salen previously prepared and copper(II) acetate. The resulting product was further purified using a Soxhlet extractor in CHCl₃.¹¹ The complex was dried in an oven under vacuum (60 °C) for 24 h and kept in a desiccator.

Preparation of modified carbon paste electrode

The modified carbon paste was prepared by mixing graphite powder, modifier [Cu^(II)Salen] and mineral oil in

the mass ratio: 65:10:25%, respectively. The first step was to mix graphite powder with the modifier, in a mortar for 10 min. Then the mineral oil was added and the mixture was homogenized in order to obtain the modified carbon paste.

The electrode was prepared by inserting the paste in a syringe from the lower end with a rod of copper placed on the other end for compression.

Preparation of modified graphite polyurethane composite electrode

The composite electrode based on graphite and polyurethane resin (GPU) modified with [Cu^(II)Salen] (MGPU) was prepared by mixing graphite powder, modifier and polyurethane resin in the mass ratio 55:10:35%, respectively.¹² The polyurethane resin (Poliquil) was prepared by mixing 0.8 parts of adhesive (B-471) and 1.0 part of the hardner (A-249), according to manufacturer instructions.

The mixture was homogenized in a glass mortar for 7 min, pressed in a hydraulic press (100 kgf cm⁻²) and extruded as 3.0 mm diameter rods. After 24 h curing time, the rods were cut into 1.0 cm sections. Electrical contacts were made by attaching the composite rods to copper wire with the help of a silver epoxy (Silver Conductive Epoxy kit, Electron Microscopy Sciences, USA).

After 24 h, this assembly was inserted into a glass tube (diameter × length, 0.5 × 7.0 cm) and sealed with a nonconducting epoxy resin (SQ 2004, Silaex Química, Brazil). The surfaces were polished using abrasive paper followed by 1.0 mm α-Al₂O₃ in a polishing wheel (Arotec, Brazil). After that the electrodes were sonicated in 2-propanol and water for 5 min in each solvent.

Procedure for real sample analysis

The standard addition method was used in the analysis of DA in parenteral pharmaceutical formulation. The solution of commercial sample was prepared by diluting 100 μL portion of the formulation in phosphate buffer pH 6.0 in order to obtain a stock sample solution of 1.05 × 10⁻⁴ mol L⁻¹ of DA according to the label.

For the analysis, 500 μL of stock sample solution were added to 15.0 mL of the 0.1 mol L⁻¹ phosphate buffer pH 6.0 into the voltammetric cell. To this solution, it was successively added four aliquots of 25 μL of standard solution at a concentration of 7.75 × 10⁻⁴ mol L⁻¹ of DA. The voltammograms were recorded for the sample and after each standard addition in triplicate, using the optimized conditions for DPV techniques.

Results and Discussion

Characterization of ligand and complex

The tetradentate symmetrical ligand and its copper(II) complex were characterized using TGA/DTG and FTIR spectroscopy techniques.

Thermal analytical data comprising mass loss, temperature ranges and a description of thermal events for both ligand and its copper(II) complex are summarized in Table 1.

According to the TGA/DTG results, the decomposition for the ligand occurred in two steps without no residue on the sample holder in agreement with literature data^{8,13} (TGA/DTG curves are presented in Figure S1 in the Supplementary Information (SI) section).

The decomposition of the [Cu^(II)Salen] complex resulted in CuO as a main residue at 550 °C after two decomposition steps. The value of residue obtained by TGA was 23.90% for [Cu^(II)Salen] and fully agreed with the expected one (calculated: 24.11%).

The infrared spectra of the free ligand and its copper(II) complex exhibited multiple bands in the region between 400-4000 cm⁻¹. The O–H stretching band of free ligand, expected in the region of 3300-3800 cm⁻¹ is shifted to the region between 2554-2603 cm⁻¹ as a result of intramolecular hydrogen bond (O–H...N=C).^{8,13} The FTIR spectra are showed in Figure S2 in the SI section, and the most relevant infrared peaks are listed in Table 2.

Table 1. TGA mass losses, temperature range, residues related to the thermal decomposition process of the ligand and copper complex under dynamic air atmosphere

Compound	Thermal event	Temperature range / °C	Mass loss or residue / %
Salen	1 st	148-326	84.36
	2 nd	356-497	16.27
[Cu ^(II) Salen]	1 st	244-335	23.5
	2 nd	353-500	51.94
	residue	–	23.90

Table 2. Infrared data for ligand and complex

Compound	Main IR signal / cm ⁻¹ ^a				
	ν C=N	ν C–N	ν C–O	Cu–N	Cu–O
Salen	1636	1370	1283	–	–
[Cu ^(II) Salen]	1649	1386	1302	569 616	441 466

^aν: axial deformation.

Intense bands near 1270 cm^{-1} were assigned to bending vibrations in the plane (O–H).^{14,15} The absence of these bands in the spectrum of the complex indicates the deprotonation of the O–H groups caused by the coordination of the ligand to copper.

The characteristic bands stretch C=N for Schiff base were observed between $1600\text{--}1636\text{ cm}^{-1}$. C–O stretches were observed in the region between $1270\text{--}1330\text{ cm}^{-1}$ for the free ligand and between $1305\text{--}1330\text{ cm}^{-1}$ for the complex, consistent with the literature.¹³

The displacement of the bands related to the C=N and the C–O stretchings of free ligand in the corresponding complex is an evidence of hydroxyl and imine group coordination.^{13,16,17} Further evidence was the appearance of bands in the lower frequency region of the spectrum at regions of $560\text{--}620$ and $440\text{--}540\text{ cm}^{-1}$ related to the Cu–N and Cu–O bonds, respectively.

Voltammetric studies with carbon paste electrode

After the characterization of synthesized ligand and the copper(II) complex, a carbon paste electrode was prepared containing the complex. The conditions for using such device were optimized as presented below. Initially, the carbon paste electrode modified with 10% (m:m) of [Cu^(II)Salen] had its voltammetric behavior evaluated using different supporting electrolytes: Britton-Robinson (B-R), acetate and phosphate buffers, at pH 5.0.

Similar results were observed in all cases, with the presence of anodic and cathodic peaks from oxidation and reduction of complexed and free copper. The first used supporting electrolyte was the B-R buffer, for which the potential range was limited in the positive potential at around 1.0 V (*vs.* SCE). The second supporting electrolyte was acetate buffer, that did not present the limitation observed in B-R buffer, but in which the reduction peaks did not present a well defined shape. The phosphate buffer was the one in which the best voltammetric profile could be observed when using the modified composite electrode. Thus the redox behavior of [Cu^(II)Salen] was studied in this electrolyte medium.

The voltammetric profile of [Cu^(II)Salen] modified carbon paste electrode, in the potential range between -1.0 and 1.2 V (*vs.* SCE), is presented in Figure 1. In the beginning of the voltammetric cycle, the -1.0 V potential is sufficiently negative to reduce both complexed and free copper in the equilibrium at the electrode/solution interface.

Thus the anodic peak I ($E = -0.3\text{ V}$, *vs.* SCE) was attributed to the $\text{Cu}^0_{\text{free}}/\text{Cu}^{1+}_{\text{free}}$ oxidation. The anodic peak II ($E = -0.04\text{ V}$, *vs.* SCE) was attributed to the complexed copper oxidation and the anodic peak III ($E = 0.05\text{ V}$, *vs.* SCE) refers to the $\text{Cu}^{1+}_{\text{free}}/\text{Cu}^{2+}_{\text{free}}$ oxidation.

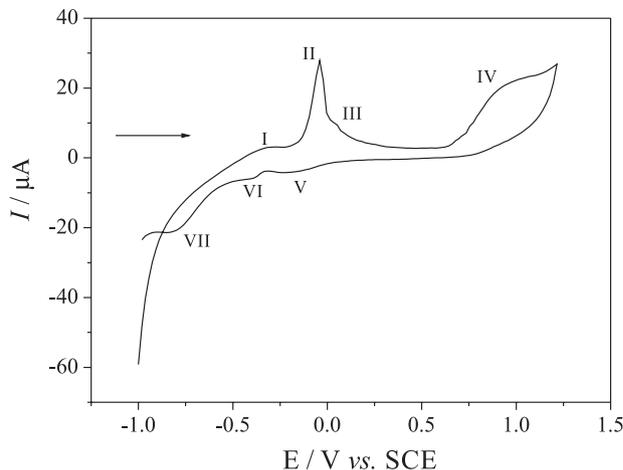


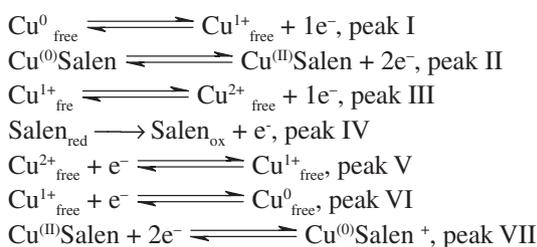
Figure 1. Cyclic voltammogram obtained with carbon paste electrode modified with [Cu^(II)Salen] in 0.1 mol L^{-1} phosphate buffer pH 5.0, $v = 50\text{ mV s}^{-1}$.

In relation to the reduction peaks, the cathodic peak V ($E = -0.2\text{ V}$, *vs.* SCE) refers to the $\text{Cu}^{2+}_{\text{free}}/\text{Cu}^{1+}_{\text{free}}$ reduction, the peak VI ($E = -0.4\text{ V}$, *vs.* SCE) corresponds to the $\text{Cu}^{1+}_{\text{free}}/\text{Cu}^0_{\text{free}}$ reduction and finally the peak VII ($E = -0.8\text{ V}$, *vs.* SCE) refers to the reduction of complexed copper. The anodic peak IV ($E = 0.9\text{ V}$, *vs.* SCE) was attributed to the irreversible oxidation of Salen ligand.^{3,17}

Some cyclic voltammetric experiments were carried out to corroborate the attribution of the observed peaks. When the potential range was limited at 0.2 V in the positive potential, there was a decrease in the peak V, which refers to the $\text{Cu}^{2+}_{\text{free}}/\text{Cu}^{1+}_{\text{free}}$ reduction. This decrease occurred because the potential was not sufficiently positive to oxidize all $\text{Cu}^{1+}_{\text{free}}$ to $\text{Cu}^{2+}_{\text{free}}$. When the potential scan was performed until 0.4 V , the peak V reappeared (Figures S3 and S4 in the SI section).

In another experiment, different voltammograms were obtained, in which the initial potential was varied between -1.0 and -0.5 V . It was observed a dependence between the oxidation peak (II) and reduction peak (VII). When the potential was not sufficiently negative to reduce species [Cu^(II)Salen] to [Cu⁽⁰⁾Salen], the peak II intensity was decreased (Figure S5 in the SI section).

From these considerations, it was possible proposing the following mechanism of oxidation/reduction, for such system based on the peaks in Figure 1:



Voltammetric studies with MGPU

After the studies using the modified carbon paste electrode, the GPU composite electrode also was modified with [Cu^(II)Salen] and its voltammetric behavior were also evaluated. The results were similar to those obtained at carbon paste, with a small shift on the peak positions to more positive potentials. After successive cycling, there was a decrease in the intensity of the peak currents as observed with the carbon paste electrode. Therefore, MGPU was submitted to an electrochemical treatment in order to reach stabilization of modifier response.

During treatment at high scan rates (100 cycles at 200 mV s⁻¹), there was a decrease in the intensity of the peak currents referring to [Cu⁽⁰⁾Salen]/[Cu^(II)Salen] oxidation, with overlapping of voltammograms from 80th cycle.

According to the literature, the surface modification of the electrode with metal-Salen complex should be directed to catalyze chemical reactions resulting in diminishing of peak potentials and increasing the peak currents. The electrocatalysis in modified electrodes is used to amplify the detection signal and consists in accelerated electronic transfer of target analyte producing well defined peaks, with the advantage of diminishing the potential peak separation (ΔE_p).³

Dopamine was chosen as a probe due to its well-known electrochemical behavior and reversibility. At conventional electrodes, the oxidation of DA to *o*-quinone form is a two-proton/two-electron process.¹⁸⁻²⁰

The MGPU electrode performance was evaluated in the determination of DA. Figure 2 shows the voltammograms of DA at the bare GPU and MGPU in 0.1 mol L⁻¹ phosphate buffer at pH 6.0. In such curves it can be observed the difference promoted by the modifier. At bare GPU electrode, the cyclic voltammogram of DA (dashed line) exhibits an oxidation peak at 0.240 V and a reduction peak at 0.123 V. Under the same conditions, MGPU presented peaks with higher current intensity at 0.206 V (anodic) and 0.146 V (cathodic). These peaks revealed to be dependent of DA concentration.

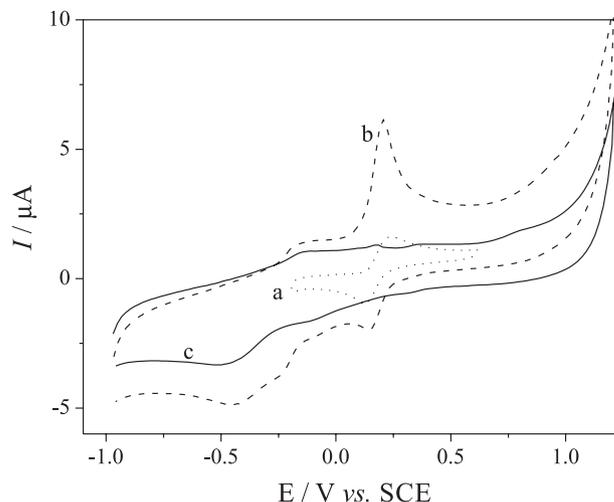


Figure 2. Cyclic voltammograms obtained at GPU (a) and MGPU (b) electrodes in 0.1 mol L⁻¹ phosphate buffer pH 6.0 containing 0.16 mmol L⁻¹ DA, $v = 25$ mV s⁻¹. The blank for MGPU (c) is also presented.

As can be seen in Figure 2, it is evident that with the presence of DA in the solution the peak current at MGPU is much higher than that observed at bare electrode. A small signal can be observed in the blank curve at ca. 0.2 V (Figure 2 curve c), being attributed to the modifier, close to the potential that rises up when DA is present in the solution (Figure 2 curve b). The electrocatalytic effect is evident from this signal enhancement associated to the presence of DA at 0.210 V as well as the diminishing on ΔE_p .

Table 3 resumes the results obtained for DA at MGPU in different pH values and compares to those obtained at GPU without modifier. MGPU presented higher peak currents and lower separation between the anodic and cathodic peaks potential. Higher peak currents were reached in pH 6.0 and 7.0. Thus pH 6.0 was chosen to the determination of DA using DPV.

Determination of dopamine

For the quantitative determination of DA using MGPU, it was necessary to optimize some experimental parameters of differential pulse voltammetry (DPV). This was performed in phosphate buffer pH 6.0 containing

Table 3. Results obtained with MGPU electrode in different pH values. The results for GPU without modifier are presented in parenthesis

pH	$I_{p,a} / \mu A$	$E_{p,a} / mV$	$I_{p,c} / \mu A$	$E_{p,c} / mV$	$\Delta E_p / mV$
4.0	1.96 (1.23)	349 (431)	-2.08 (-0.99)	229 (184)	120 (247)
5.0	2.81 (1.39)	262 (341)	-2.20 (-1.36)	193 (151)	69 (190)
6.0	4.41 (1.33)	207 (240)	-1.95 (-1.29)	146 (123)	61 (117)
7.0	4.16 (1.25)	140 (170)	-1.34 (-0.88)	99 (80)	41 (90)
8.0	3.30 (1.08)	93 (118)	-0.85 (-0.58)	58 (29)	58 (89)

$1.0 \times 10^{-5} \text{ mol L}^{-1}$ DA, using a GPU composite electrode modified with $\text{Cu}^{\text{(II)}}\text{Salen}$.

Thus, four experiments were performed using a multivariate calibration involving pulse amplitude (a) and scan rate (v). A 2^n factorial design, in which n is the number of variable (a = 10 and 50 mV; v = 5 and 25 mV s^{-1}). The optimized values were 50 mV and 25 mV s^{-1} for pulse amplitude and scan rate, respectively, once higher peak current and a better peak definition were obtained.

After this optimization, an analytical curve was obtained using voltammetric measurements carried out in 0.1 mol L^{-1} phosphate buffer pH 6.0 in different DA concentrations (0.7 to $17 \text{ } \mu\text{mol L}^{-1}$). The resulting voltammograms are shown in Figure 3 presenting a linear response in the 0.7 and $9.0 \text{ } \mu\text{mol L}^{-1}$ ($n = 6$) concentration range, obeying equation 1 (inset in Figure 3):

$$I_{p,a} = 0.11 \text{ } \mu\text{A} + 0.81 \text{ } \mu\text{A } \mu\text{mol}^{-1} \text{ L} \times C_{\text{DA}}, r^2 = 0.990 \quad (1)$$

in which $I_{p,a}$ is the anodic peak current (μA) and C_{DA} is the total concentration of dopamine ($\mu\text{mol L}^{-1}$). The analytical curve was obtained determining the peak current of three successive measures in DPV for each concentration.

From these data, a limit of detection (LOD) of $3.8 \times 10^{-7} \text{ mol L}^{-1}$ was determined for DA (LOD = $3 \times$ blank standard deviation slope $^{-1}$).²¹

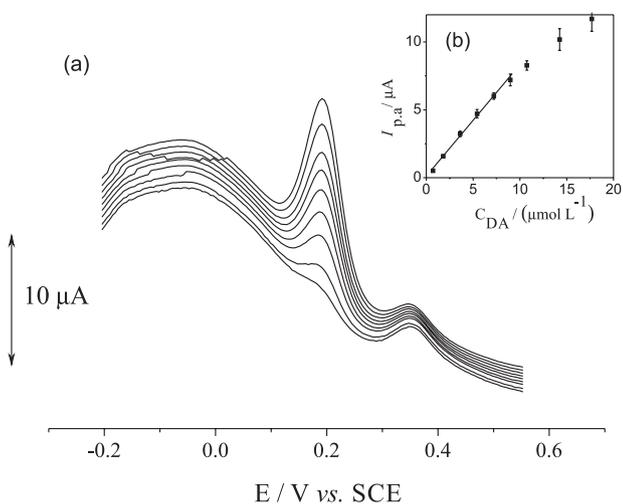


Figure 3. (a) Differential pulse voltammograms obtained with MGPU in 0.1 mol L^{-1} phosphate buffer pH 6.0, containing different DA concentrations, $v = 25 \text{ mV s}^{-1}$ and pulse amplitude = 50 mV, and (b) analytical curve.

Previous reports described LOD of $1.3 \text{ } \mu\text{mol L}^{-1}$, using DPV²² and $6.4 \times 10^{-8} \text{ mol L}^{-1}$ using SWV²³ at a bare GPU electrode. As DPV as used in the present work, one can expect better results in the present case for LOD, once SWV uses to be at least one order of magnitude more sensitive when compared to DPV.

Analysis of commercial sample

In order to evaluate the applicability of the proposed electrode, the standard addition approach was applied in the determination of DA in the commercial pharmaceutical formulation Revivan[®] using the optimized DPV procedure. The voltammograms and the standard addition curve are presented in Figure 4.

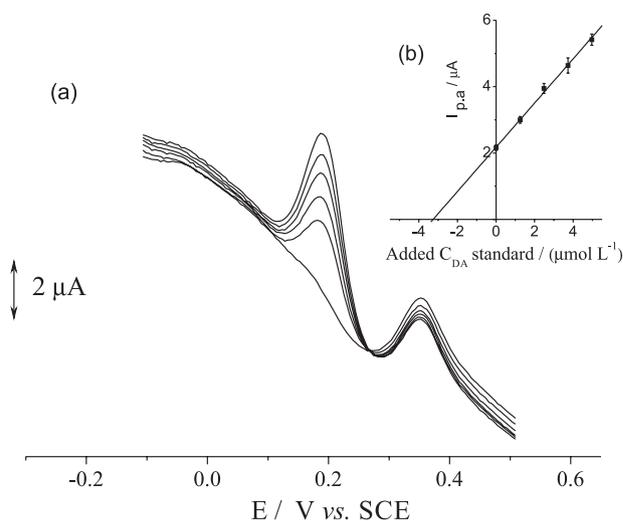


Figure 4. (a) DPV voltammograms obtained with MGPU composite electrode for the quantification of DA in the pharmaceutical formulation Revivan[®]; $v = 25 \text{ mV s}^{-1}$ and pulse amplitude = 50 mV, and (b) standard addition curve.

The result obtained by the proposed method was compared with that obtained by the official HPLC method, described in the United States Pharmacopoeia.²⁴ The results presented in Table 4 agreed within 95% confidence level according to the student t -test once $t_{\text{calculated}}$ (2.230) is smaller than t_{table} (2.776). Recovery tests pointed to recoveries of 98-106%.

Table 4. Determination of DA in the commercial pharmaceutical formulation Revivan[®] using DPV and HPLC

Sample	Dopamine / (mg mL^{-1})		Error / %	
	DPV	HPLC		
Revivan [®]	4.91 ± 0.03	4.80 ± 0.08	-1.8 ^a	+2.3 ^b

Labeled value: 5 mg mL^{-1} ; ^aerror obtained with DPV in relation to labeled value; ^berror obtained with DPV in relation to comparative HPLC method.

Conclusions

The Salen ligand and its copper(II) complex have been obtained and characterized by thermogravimetry and FTIR. The results obtained confirmed the synthesis of ligand and showed its coordination to the copper(II).

In the voltammetric studies, the modifier [Cu^{II} Salen] had its redox behavior evaluated at a carbon paste electrode and a redox mechanism was proposed. The analytical potentialities of MGPU electrode were evaluated using DA as a probe and showed better results when compared to the GPU electrode without modification. These results indicated that the MGPU electrode shows electrocatalytic effect and can be characterized as a bio-inspired sensor.

The MGPU electrode was applied in the determination of dopamine in pharmaceutical formulation using DPV and the result was compared to that obtained with the official method (HPLC) as described in US Pharmacopoeia (USP) agreeing on 95% confidence level according to student's *t*-test.

The electrode showed to be robust, with long useful life, once only one was used in all this work and any adsorption effect of the analyte or its oxidation products was adhered to the surface resulting in no need of surface renewing between measurements.

Supplementary Information

Supplementary data are available free of charge at <http://jbcs.sbc.org.br> as PDF file.

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