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Development of a biomimetic chitosan film-coated gold electrode for determination of dopamine in the presence of ascorbic acid and uric acid

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ABSTRACT

A gold electrode surface was modified using a dinuclear copper complex $[Cu^{II}_2$ (Ldtb)(μ-OCH_3)](BPh_4) and then coated with a chitosan film. This biomimetic polymer film-coated electrode was employed to eliminate the interference from ascorbic acid and uric acid in the sensitive and selective determination of dopamine. The optimized conditions obtained for the biomimetic electrode were 0.1 M phosphate buffer solution (pH 8.0), complex concentration of 2.0×10^{-4} M, 0.1% of chitosan and 0.25% of glyoxal. Under the optimum conditions, the calibration curve was linear in the concentration range of 4.99×10^{-7} to 1.92×10^{-5} M, and detection and quantification limits were 3.57×10^{-7} M and 1.07×10^{-6} M, respectively. The recovery study gave values of 95.2–102.6%. The lifetime of this biomimetic sensor showed apparent loss of activity after 70 determinations. The results obtained with the modified electrode for dopamine quantification in the injection solution matrix were in good agreement with those of the pharmacopoeia method.

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1. Introduction

Dopamine (DA) is one of the most important natural catecholamine neurotransmitters, playing an important role in the functioning of the central nervous, renal and hormonal systems [1]. Dysregulation of dopaminergic neurotransmission is associated with attention deficit hyperactivity disorder, mood disorders and schizophrenia [2].

Determination of DA is a subject of great importance and finding selective methods for its quantification in the presence of high levels of ascorbic acid (AA) and uric acid (UA) in body fluids is relatively complicated [3]. Common instrumental techniques like electrophoresis [4], chromatography [5], high performance liquid chromatography [6–8] and spectrophotometry [9–11] have been widely used for the determination of DA. However, such methods of detection are often sophisticated and very expensive [12].

As DA shows very strong electrochemical activity, electrochemical techniques appear to be strong candidates for its detection, offering advantages such as simplicity, fast response and ease of application. Most solid electrodes are associated with an overlapped signal, since the interferents may have close oxidation potentials, and thus different strategies have been used to modify the electrode surface, resulting in not only good sensitivity but also a high degree of selectivity [13–16]. One such strategy is to employ a polymer-modified electrode that minimizes the access of interferents through size exclusion, ion exchange and hydrophobic interactions [17].

Chitosan is considered to be a promising material for modification of the electrode surface due to its attractive properties, e.g., excellent film-forming ability, good stability, high permeability toward water, strong adherence to the electrode surface, biocompatibility, no toxicity, high mechanical strength and susceptibility to chemical modifications due to the presence of reactive amino and hydroxyl functional groups [13,18].

Mimetic complexes have been used as simple models of natural biochemical catalysts, mimicking their active site and their function as synthetic analogs, facilitating the electron transfer [19,20]. Additionally, they have the advantage of being more stable than the natural enzyme under conditions of changes in temperature, pH and solution composition, which often denature or inactivate the natural biocatalysts [21].

Several groups have synthesized and characterized complexes, which mimic the active site of enzymes [22–24], but very few use them as biomimetic sensors for the oxidation of phenol substrates [19–21,25–31]. Our group has studied a diversity of biomimetic complexes developed by Neves and co-workers and which have been successfully used to construct sensors [19,21,28–31]. These include heterodinuclear $Fe^{III}Zn^{II}$ [28] and $Fe^{III}Fe^{II}$ complexes [29], which mimic the active site of purple acid phosphatase, a

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homodinuclear $Mn^{III}Mn^{II}$ complex of manganese peroxidase [19] and tetranuclear [21] and dinuclear [30,31] $copper^{II}$ complexes, that mimic the enzyme catechol oxidase. The dicopper complex is one of a group of complexes with type 3 copper centers where the metal ions are responsible for the catalysis of the oxidation of *o*-diphenols to their corresponding *o*-quinones [32,33]. Dopamine, which is a catechol-like phenolic compound, can be detected using a catecholase-based system [34].

The aim of this study is to report an electroanalytical methodology that combines a dinuclear complex $[Cu^{II}_2(Ldtb)(\mu\text{-}OCH_3)](BPh_4)$, which mimics the active site of catechol oxidase, with a chitosan film coating for the fabrication of a novel biomimetic sensor. This sensor was constructed, optimized and applied for determination of DA in pharmaceutical samples in the presence of AA and UA. The results obtained with the proposed biomimetic sensor compared favorably with those obtained using the official method.

2. Experimental

2.1. Reagents

All reagents were of analytical grade and all solutions were prepared with a Millipore (Bedford, MA, USA) Milli-Q system (model UV Plus Ultra-Low Organic Water). The gold electrode was cleaned with alumina powder (0.3–0.05 μm) obtained from Aratec. Ascorbic acid, uric acid, dopamine, sulfuric acid, hydrochloric acid, ethyl alcohol, sodium acetate, sodium phosphate dibasic, sodium phosphate monobasic, acetic acid, glyoxal, chitosan and tris(hydroxymethyl)aminomethane (tris) were obtained from Sigma–Aldrich. Hydrogen peroxide, triethylamine (Et_3N), sodium tetraphenylboride ($NaBPh_4$) and dichloromethane were purchased from Merck. Inotropisa, labeled as containing 5.0 mg mL^{-1} dopamine, was purchased from a local pharmacy.

2.2. Apparatus

An ultrasonic bath (Unique, 1400A) with distilled water was used to clean the electrode surface. The pH measurements were taken with a Micronal B475 pH-meter using a combined glass electrode.

Elemental analysis was performed with a Carlo Erba E1110 analyzer. IR spectra were obtained in the range of 4000–400 cm^{-1} with KBr pellets, using a Perkin-Elmer 781 spectrometer. ^1H NMR analysis of the ligand was carried out with a Bruker 200 MHz spectrometer in $CDCl_3$ chloroform, at 25 °C. Chemical shifts were referenced to tetramethylsilane.

The cyclic voltammetry behavior of the complex was investigated in dichloromethane solution with a Princeton Applied Research (PARC) model 273 potentiostat/galvanostat. The electrochemical cell employed was of a standard three-electrode configuration: platinum working electrode, platinum wire counter electrode and Ag/AgCl reference electrode.

Voltammetric measurements for the dopamine determination were performed with an Autolab PGSTAT12 potentiostat/galvanostat (Eco Chemie, Utrecht, The Netherlands) driven by data processing software (GPES, software version 4.9.006, Eco Chemie). A conventional three-electrode cell was used, comprising Ag/AgCl (3.0 M KCl) as the reference electrode, a platinum wire as the counter electrode and a biomimetic modified gold sensor as the working electrode.

2.3. Synthesis of the ligand (H_2Ldtb) and the complex $[Cu^{II}_2(Ldtb)(\mu\text{-}OCH_3)](BPh_4)$

Peralta and co-workers have reported the synthesis of $[Cu^{II}_2(Ldtb)(\mu\text{-}OCH_3)](BPh_4)$ [33]. Firstly, the ligand H_2Ldtb ({2-[*N*,*N*-Bis(2-pyridylmethyl) aminomethyl]-6-[*N*′,*N*′-(3,5-di-*tert*-butylbenzyl-2-hydroxy)(2-pyridylmethyl)] aminomethyl}-4-methylphenol) was prepared by mixing 2-[bis-(2-pyridylmethyl) aminomethyl]-6-(2-pyridylmethyl)aminomethyl-4-methylphenol (3.4 g, 8 mmol) with triethylamine (1.21 g, 12 mmol) and 2-chloromethyl-4,6-di-*tert*-butylphenol (3.02 g, 12 mmol) with dichloromethane. The ^1H NMR ($CDCl_3$; δ ppm) data obtained for this product were: 8.54, 7.61, 7.53, 7.15, 6.97, 6.84 (16H, aromatic H), 3.92, 3.85, 3.84, 3.80 (s, 12H, CH_2), 2.23 (s, CH_3), 1.42 and 1.25 (s, *t*-butyl).

The complex $[Cu^{II}_2(Ldtb)(\mu\text{-}OCH_3)](BPh_4)$ was obtained by adding 1.0 mmol of $Cu(OAc)_2{\cdot}H_2O$ and 1.0 mmol of $NaBPh_4$ and Et_3N, to a methanolic solution of 0.5 mmol of the ligand H_2Ldtb, under magnetic stirring, at 40 °C for 20 min. The crystal structures were isolated for X-ray analysis, giving a product yield of 36%. Anal. Calc. for $Cu_2C_{67}H_{72}BN_5O_3$, M.W. = 1133.19 g mol^{-1}: C, 71.0; H, 6.4; N, 6.2. Found: C, 70.4; H, 6.0; N, 6.5%.

2.4. Pretreatment of the gold electrode

In the first instance, a pretreatment procedure was applied to the gold electrode surface (≥99.99% Au, 2 mm diameter, mounted in a Teflon rod). Initially, the surface was polished mechanically with alumina powder slurry on a polishing cloth for 2 min and rinsed with deionized water. To remove the residual alumina particles, the electrode was then ultrasonically cleaned in water and then ethanol for 2 min in each case. The electrode was further treated by soaking in a "piranha" solution (H_2O_2:H_2SO_4 = 1:3, v/v) for 10 min in order to remove organic contaminants and subsequently washed with deionized water. Finally, cyclic voltammograms were obtained in 0.5 M sulfuric acid solution at between 0.0 and +1.7 V at 100 mV s^{-1}, until reproducible voltammograms were obtained.

2.5. Preparation of the working electrode

The biomimetic sensor was prepared for film coating by deposition of 5 μL of the $[Cu^{II}_2(Ldtb)(\mu\text{-}OCH_3)](BPh_4)$ complex solution (2.0×10^{-4} M) in dichloromethane and then left for solvent evaporation at room temperature. Later, a chitosan film was obtained by mixing 10 μL of 0.1% (m/m) chitosan solution with 3% acetic acid solution together with another 10 μL of 0.25% (v/v) glyoxal solution. With a micropipette, 10 μL of this mixture was placed directly onto the gold electrode surface containing the complex and left to dry overnight. The complex electrode was stored at room temperature when not in use.

2.6. Electrochemical measurements

Volumes of standard or sample solutions were added by micropipette to the voltammetric cell containing 5.0 mL of the supporting electrolyte (0.1 M phosphate buffer, pH 8.0) together with ascorbic and uric acids when necessary. Square wave voltammetry (SWV) was performed in the range of +0.4 to −0.1 V vs Ag/AgCl (3.0 M KCl) after 60 s of homogenization by stirring, applying a frequency of 60.0 Hz, pulse amplitude of 100.0 mV and a step potential of 7.0 mV. Samples were analyzed by the standard addition method using the biomimetic gold electrode.

3. Results and discussion

3.1. Characterization of the dinuclear copper complex

The ligand {2-[*N*,*N*-Bis(2-pyridylmethyl)aminomethyl]-6-[*N*′,*N*′-(3,5-di-*tert*-butylbenzyl-2-hydroxy)(2-pyridylmethyl)] aminomethyl}-4-methylphenol (H_2Ldtb) was prepared in good yields following the synthetic procedures described in Section

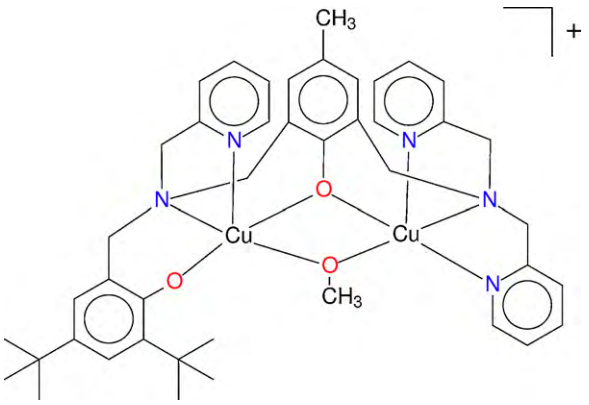


Fig. 1. Molecular structure of the complex cation $[Cu^{II}_2(Ldtb)(\mu\text{-}OCH_3)]^+$.

2. The complex $[Cu^{II}_2(Ldtb)(\mu\text{-}OCH_3)](BPh_4)$ was prepared in methanolic solution by adding $Cu(OAc)_2{\cdot}H_2O$ with $NaBPh_4$ and Et_3N to the ligand H_2Ldtb, yielding a dark red crystal. A schematic representation of the dinuclear copper complex is shown in Fig. 1, showing a pentacoordinated environment for both copper centers, but with a significant distortion in the coordination environment around Cu1, leading to an intermediate geometry between square pyramidal and trigonal bipyramidal for Cu1, while Cu2 maintains a square pyramidal geometry.

The cyclic voltammogram of $[Cu^{II}_2(Ldtb)(\mu\text{-}OCH_3)]^+$ in dichloromethane (Fig. 2) solution shows only one irreversible wave at −1.07 V vs Ag/AgCl as a consequence of the electronic density of the deprotonated phenolate group. In addition to the metal centered process, a quasi-reversible wave is apparent at $E_{1/2}$ = +0.27 V vs Ag/AgCl, attributed to a phenoxyl radical of the ligand.

Peralta et al. [33] have reported other methods for the characterization of this complex by UV–vis spectroscopy, magnetic susceptibility, electron paramagnetic resonance and potentiometric titration.

3.2. Behavior of the modified electrode

To evaluate the contribution of the biomimetic complex and the effect of the chitosan film on the electrode surface, different sensors were constructed and compared using the cyclic voltammetry technique. Fig. 3 shows their electrochemical performance in the analysis of a 9.0×10^{-4} M dopamine solution in phosphate buffer

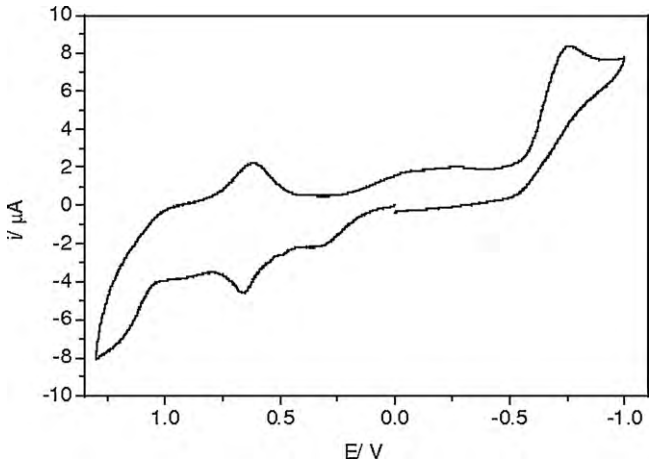


Fig. 2. Cyclic voltammogram for 1.0×10^{-3} M of the dinuclear copper complex in dichloromethane and 0.1 M tetrabutylammonium hexafluorophosphate at a scan rate of 100 mV s^{-1}.

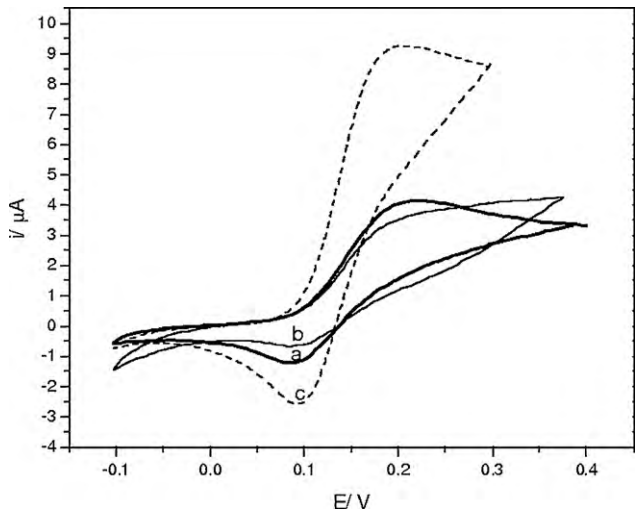


Fig. 3. Comparative cyclic voltammograms using (a) bare gold electrode, (b) chitosan film-coated electrode, and (c) biomimetic chitosan film-coated electrode, in a 9.0×10^{-4} M dopamine solution in phosphate buffer (0.1 M; pH 8.0).

(0.1 M; pH 8.0): (a) with the bare gold electrode; (b) chitosan film coated onto the gold surface and (c) the proposed biomimetic chitosan film-coated electrode, in the potential range of +0.4 to −0.1 V vs Ag/AgCl with a scan rate of 100 mV s^{-1}.

Compared to the bare gold electrode, the current peak decreased after coating the chitosan layer on the electrode surface, since it acts as a mass transfer blocking layer, inhibiting the diffusion of the dopamine towards the electrode surface. Despite this significant decreased in the current response, the chitosan layer seems to be important to eliminate the response of the concomitants for dopamine determination. When the electrode surface is previously prepared with the biomimetic complex, a greater response to the dopamine current was obtained. This indicates that the presence of the complex substantially improved the electron transfer on the electrode surface. Catechol oxidases are responsible for the oxidation of a variety of phenolic compounds to their corresponding *o*-quinones with the simultaneous reduction of the oxygen to water molecules. The $[Cu^{II}_2(Ldtb)(\mu\text{-}OCH_3)](BPh_4)$ complex mimics the action of catechol oxidase enzymes, oxidizing the dopamine molecule to its corresponding *o*-quinone, which is electrochemically reduced at the electrode surface at a potential of +0.12 V, as shown in Fig. 4.

3.3. Optimization conditions of the biomimetic sensor

For dopamine determination using the biomimetic chitosan film-coated electrode, several experimental conditions were evaluated: complex concentration, proportions of chitosan and glyoxal, and pH, along with the frequency, pulse amplitude and scan increment used in the SWV. The response of the biomimetic sensor was based on the resultant peak current for a 5.36×10^{-4} M dopamine solution in 0.1 M phosphate buffer solution at pH 8.0 using SWV.

Initially, the effect of varying the copper complex concentration from 2.0×10^{-3} to 2.0×10^{-5} M was investigated. The analytical response of the modified sensor in 2.0×10^{-4} M $Cu^{II}Cu^{II}$ complex showed the highest resultant current. This complex concentration was then used for further development of the biomimetic sensor.

Chitosan crosslinked with glyoxal was used to fix the complex on the gold electrode surface and also to act as a selective polymer modifier. Firstly, the glyoxal concentration was maintained constant at 0.25% (v/v) and the chitosan concentration was varied between 0.1, 0.5 and 1.0% (w/w) in 3.0% acetic acid solution. The electrode containing 0.1% (w/w) of chitosan gave the high-

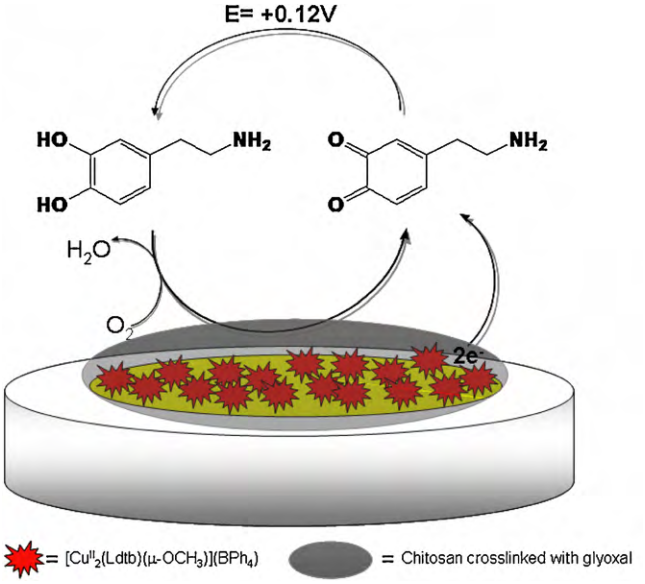


Fig. 4. Schematic representation of a reaction involving dopamine on the surface of the biomimetic sensor.

est response and was subsequently used in the construction of the electrodes.

The percentage of chitosan was then maintained constant at 0.1% (w/w) and the glyoxal percentage was varied from 0.025 to 2.5% (v/v). The electrode containing 0.25% (v/v) offered a higher response and this concentration was thus used to construct further electrodes.

The effect of the different supporting electrolytes (acetate, phosphate and tris buffer solutions) and pH (5.0–9.0) on the sensor response was investigated. The best voltammetric response was obtained in phosphate buffer solution at pH 8.0. Therefore, these conditions were used in further experiments.

The SWV parameters were optimized using the best response of the biomimetic chitosan film-coated sensor to 5.36×10^{-4} M dopamine solutions. Values of frequency in the range of 10–100 Hz, pulse amplitude between 10 and 100 mV and scan increment from 1.0 to 12.0 mV were evaluated. The best resultant peak currents were obtained using values for frequency, pulse amplitude and scan increment of 60.0 Hz, 100.0 mV and 7.0 mV, respectively.

3.4. Selectivity and recovery study

It is well known that, using a bare gold electrode, the oxidation peak potentials for ascorbic acid (AA), uric acid (UA) and dopamine (DA) are very close to each other and thus it is difficult to separate these compounds due to their overlapping signals [35,36]. This problem can be eliminated by electrostatic attraction, since a chitosan film ($pK_a = 6.5$), which is in its anionic form at the working electrode surface at pH 8.0, could attract DA molecules, which are mainly in their cationic form with a pK_a of 8.9. Also, AA and UA undergo an electrostatic repulsion by the film since their pK_a values are 4.2 and 5.4, respectively, being in anionic form, as is the chitosan. Table 1 shows a comparison between the potential and resultant peak currents for a 1.99×10^{-6} M dopamine solution alone and in the presence of different concentrations of AA and UA. On analyzing these values it can be concluded that the presence of AA and UA did not interfere in the dopamine determination.

Also, different standard dopamine concentrations (0.77, 1.53 and 2.30 mg mL^{-1}) were added to the samples followed by the calculation of the percentage recovery to investigate the influence of AA and UA when 1.99×10^{-4} M of these substances was added to injection samples. The recoveries obtained for these samples were 95.2–102.6%, as shown in Table 2. It can be clearly observed from the recovery results that AA and UA did not present interference in the determination of DA.

Table 2
Result for recovery of dopamine standard solution from pharmaceutical injection formulation using the proposed biomimetic chitosan film-coated electrode.

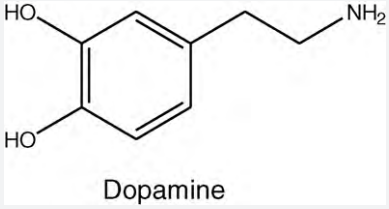
Dopamine (mg mL^{-1})			
Sample	Added	Found[a]	Recovery (%)
A	0.77	0.79 ± 0.07	102.6
	1.53	1.50 ± 0.07	98.0
	2.30	2.22 ± 0.09	96.5
B	0.77	0.77 ± 0.08	100.0
	1.53	1.52 ± 0.06	99.4
	2.30	2.30 ± 0.08	100.0
C	0.77	0.74 ± 0.1	96.1
	1.53	1.47 ± 0.08	96.1
	2.30	2.19 ± 0.1	95.2

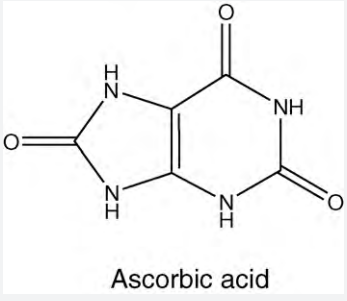
A–C, dopamine injection.
[a] Standard deviation for three replicates.

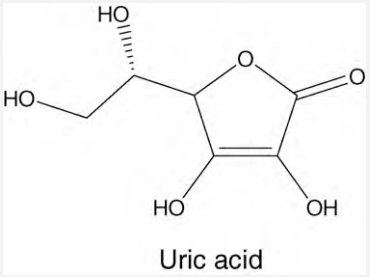
3.5. Calibration curve obtained in the electrochemical analysis of dopamine

Under the optimum conditions, the SWV currents were proportional to the dopamine concentrations over the range of 4.99×10^{-7} to 1.92×10^{-5} M, giving a regression equation of $-\Delta i = 0.024(\pm 0.006) + 6.032(\pm 0.071) \times 10^4$ [Dopamine]; where

Table 1
Comparison of potential and resultant peak currents for 1.99×10^{-6} M dopamine in the presence of different concentrations of AA and UA.







			E (V)	$-\Delta i$ (μA)
1.99×10^{-6} M	–	–	+0.12	1.570×10^{-7}
1.99×10^{-6} M	1.99×10^{-6} M	1.99×10^{-6} M	+0.12	1.568×10^{-7}
1.99×10^{-6} M	1.99×10^{-5} M	1.99×10^{-5} M	+0.12	1.568×10^{-7}
1.99×10^{-6} M	1.99×10^{-4} M	1.99×10^{-4} M	+0.12	1.569×10^{-7}

Table 3
Analytical response using modified gold electrodes in dopamine determination.

Modification on gold electrode	Linearity (M)	Detection limit (M)	Reference
Corrole and self-assembled monolayers	3.20×10^{-12}–3.20×10^{-10}	1.50×10^{-12}	[12]
Dopamine polymer film	1.00×10^{-6}–6.00×10^{-4}	2.00×10^{-7}	[36]
Multi-walled carbon nanotubes	5.00×10^{-7}–4.00×10^{-4}	2.00×10^{-7}	[37]
Cysteamine self-assembled monolayers	6.00×10^{-6}–3.84×10^{-4} and 3.36×10^{-4}–9.50×10^{-3}	2.31×10^{-6}	[38]
Self-assembled gold nanoparticle films	2.00×10^{-4}–1.20×10^{-3}	9.00×10^{-5}	[39]
Fullerene-C60	1.00×10^{-6}–5.00×10^{-3}	2.60×10^{-10}	[40]
Penicillamine self-assembled monolayer	8.06×10^{-6}–1.06×10^{-3}	4.56×10^{-7}	[41]
3-Amino-5-mercapto-1,2,4-triazole self-assembled monolayers	1.50×10^{-6}–1.00×10^{-4}	5.00×10^{-7}	[42]
N-acetylcysteine self-assembled monolayer.	1.00×10^{-6}–2.00×10^{-4}	8.00×10^{-7}	[43]
DL-Homocysteine self-assembled monolayers	5.00×10^{-6}–5.00×10^{-4}	5.00×10^{-7}	[44]
Thiolactic acid self-assembled monolayer	4.00×10^{-5}–8.00×10^{-4}	3.00×10^{-6}	[45]
L-Cysteine self-assembled monolayer with peroxidase from beans sprouts	2.37×10^{-6}–3.76×10^{-5}	2.95×10^{-6}	[46]
Biomimetic chitosan film-coated electrode	4.99×10^{-7}–1.92×10^{-5}	3.57×10^{-7}	This study

Table 4
Determination of dopamine ($mg\,mL^{-1}$) in pharmaceutical formulation using the chitosan film-coated electrode.

Samples	Label value	Official method[a]	Proposed electrode[a]	Er_1 (%)	Er_1 (%)
A	5.00	5.00 ± 0.1	4.95 ± 0.1	−1.0	−1.0
B	5.00	4.60 ± 0.2	4.87 ± 0.08	−2.7	+5.5
C	5.00	4.79 ± 0.07	4.92 ± 0.1	−1.6	+2.6

A–C, dopamine injection; Er_1, proposed electrode vs label value; Er_2, proposed electrode vs official method.
[a] Standard deviation for three replicates.

$-\Delta i$ is the resultant peak current in μA and [Dopamine] is the dopamine concentration in M with a correlation coefficient of 0.9997. Fig. 5 shows these voltammograms and the analytical curve can be seen in the inset. The detection limit (three times the blank signal/slope) and quantification limit (10 times the blank signal/slope) were found to be 3.57×10^{-7} M and 1.07×10^{-6} M, respectively. We can find a number of modified gold electrodes for dopamine detection in the literature. Table 3 compares the performance of the sensor proposed in this study with that of other electrodes, verifying that the biomimetic chitosan film-coated electrode exhibits a linear range and limit of detection comparable to those described in the literature, with the exception of two cases.

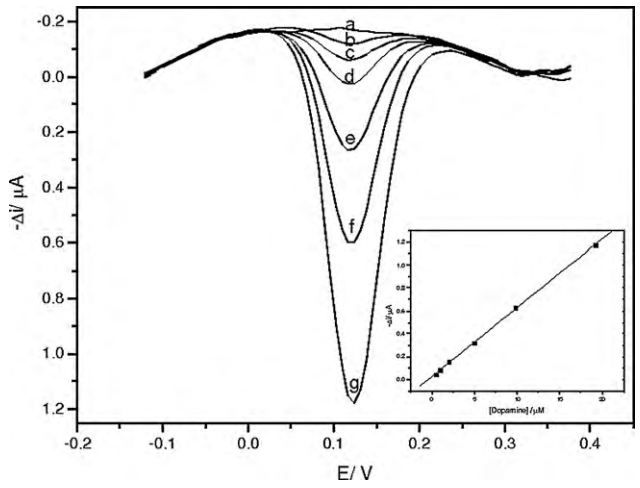


Fig. 5. Square wave voltammograms obtained using the biomimetic sensor for (a) blank in phosphate buffer solution (pH 8.0), and dopamine solutions at the following concentrations: (b) 4.99×10^{-7} M; (c) 9.98×10^{-7} M; (d) 1.99×10^{-6} M; (e) 4.95×10^{-6} M; (f) 9.80×10^{-6} M and (g) 1.92×10^{-5} M in 0.1 M phosphate buffer solution (pH 8.0).

3.6. Determination of dopamine using the proposed sensor

In order to evaluate the applicability of the proposed biomimetic chitosan film-coated sensor, dopamine concentrations of pharmaceutical samples were determined. Prior to the measurements, the dopamine injection samples were diluted in a 1:10 ratio with phosphate buffer solution (pH 8.0) and then used without previous treatment. The results of the analysis were compared with those obtained with the official USP method [47] as shown in Table 4, and showed agreement at the 95% confidence level, within an acceptable range of error. It can thus be concluded that this sensor is suitable for application in the determination of dopamine with the concomitant interferents.

3.7. Reproducibility, repeatability and stability of the proposed sensor

The reproducibility for five biomimetic chitosan film-coated electrodes was around 5.3% using a dopamine concentration of 1.99×10^{-6} M. The biomimetic sensor described herein offers a highly reliable current signal for the repeatability of 8 measurements for the same electrode with a relative standard deviation (R.S.D.) of 3.7%. The long-term stability was investigated daily with 1.99×10^{-6} M dopamine solutions where the sensor was reused for approximately 70 determinations showing 80% of activity loss after one week. The stability of the proposed sensor can be ascribed to the excellent immobilization of the complex in the chitosan covalently crosslinked with glyoxal, which provides a microenvironment favorable for the entrapment of the biomimetic complex. After 70 determinations the sensor response decreases probably due to leaching of the complex as the sensor surface loses its chitosan film.

4. Conclusions

In this paper we report a simple, sensitive and selective electroanalytical method using a biomimetic sensor based on a dinuclear

$Cu^{II}Cu^{II}$ complex coated with chitosan film for the electrochemical determination of dopamine. This complex improved the oxidation catalysis of dopamine enhancing the sensitivity of the biomimetic sensor significantly. Also, the chitosan polymer layer was suitable for the immobilization of this complex and was successfully employed as a good modifier for the selective determination of DA. Hence, at a working pH of 8.0, the chitosan attracts DA (which has a positive charge) and repels AA and UA (which have negative charges). The biomimetic chitosan film-coated electrode offers an excellent linear calibration range, low detection limit, good reproducibility and repeatability and facility of construction of the sensor. The results demonstrated that dopamine was effectively determined in the selected pharmaceutical samples using this biomimetic sensor, when compared with the official method.

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