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▶ **To cite this version:**

M. Boulkroune, Alizée Lemaire, A. Chibani, Florence Geneste. Monocopper complex based on N-tripodal ligand immobilized in a Nafion® film for biomimetic detection of catechols: Application to dopamine. Electrochimica Acta, 2016, 221, pp.80–85. 10.1016/j.electacta.2016.10.040 . hal-01416440

HAL Id: hal-01416440

https://univ-rennes.hal.science/hal-01416440

Submitted on 25 Aug 2017

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Monocopper complex based on *N*-tripodal ligand immobilized in a Nafion® film for biomimetic detection of catechols: application to dopamine

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Abstract

A complex based on bis-pyrazolyl *N*-tripodal ligand known to present a good catalytic activity for the oxidation of 3,5-di-*tert*-butylcatechol is used to prepare a novel biomimetic sensor for the determination of catechol derivatives. The modified electrode prepared by encapsulation of the complex in a Nafion® film leads to an electrochemical signal 4.4 times higher than with a glassy carbon electrode for dopamine detection in pH 7 medium. After optimization of the conditions used to immobilize the catalyst on the electrode surface, a calibration curve was obtained with linearity in the range of 30 to 320 μmol L^{-1} and a detection limit of 8 μmol L^{-1}. The sensor was not affected by ascorbic acid and uric acid in similar concentrations to dopamine and its electrochemical response was stable after 40 determinations. The biomimetic sensor can also be used for the detection of other catechol derivatives.

Keywords: biomimetic sensor, dopamine, catechol, monocopper complex, modified electrode

1. Introduction

The presence of phenol and its derivatives in the environment results from their important use in many industrial sectors such as chemicals, pharmaceuticals and textile industries. An important source of dissemination is the production, the use and the transformation of many pesticides including some herbicides such as 2,4-dichlorophenoxyacetic acid and biocides such as 4-chlorophenol and pentachlorophenol [1, 2]. Most of phenols are toxic since they easily penetrate the cell membrane, causing necrosis on skin and body organs [3]. They are also cytotoxic, mutagen and carcinogen [4-7]. For this reason, they are admitted at trace levels

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in water. French order dated 11 January 2007 on drinking water quality set the limit for phenol index between 1 and 100 μg L^{-1} according to water quality.

Thus, quantitative analysis of phenols in water requires very sensitive methods. It is usually carried out by gas chromatography/mass spectrometry (GCMS) or gas chromatography with flame ionization detection (GCFID). These analytical methods are reliable and allow phenol analysis at trace levels. However, they are expensive and require long analysis time, handling with the impossibility to perform field analyses. In view of these technical constraints, it seems to be interesting to develop on-site analytical methods to obtain a fast and real-time analysis. It would give a first indication of pollution before a complete study in laboratory. Electroanalytical methods can overcome this problem since they are sensitive, fast and inexpensive, and they can easily be miniaturized to achieve portable sensor.

Phenol and its derivatives are electroactive but often lead to passivation of the electrode due to polymerization processes. Moreover, since interferents can have a close oxidation potential, modified electrodes have been used to increase the selectivity of the electroanalytical method. Thus biosensors have been developed for phenol detection. Most of them use natural enzymes that have a catalytic activity toward the oxidation of phenols. For example, Tyrosinase, a copper enzyme that catalyzes in the presence of dioxygen the ortho-hydroxylation of monophenols and the oxidation of o-diphenol into o-quinone has been widely used [8-10]. The phenolic substrate diffuses in the film of Tyrosinase immobilized on the electrode surface where it is oxidized into quinone with dioxygen consumption. The resulting quinone is then electrochemically reduced giving a reduction current that is proportional to the concentration of phenol in solution. Since Tyrosinase has a specific catalytic activity toward the oxidation of phenol, a selective sensor to phenols can be obtained.

Whereas they have a very good selectivity towards phenol derivatives, biosensors suffer the drawback of low stability and quite high detection limits. An alternative consists in reducing the diffusion barrier and increasing the electronic transfer between the active site of the enzyme and the electrode surface thanks to biomimetic catalysts, avoiding the presence of the protein backbone used as a protecting shield around the active site. Moreover, biomimetic catalysts are often less expensive than enzymes and can lead to sensors that are more stable against time, pH and temperature variations. The term “enzyme-less biosensor” has been chosen as a general term to define this type of sensors [11-15].

We have previously developed a simple synthetic method to prepare mononuclear copper complexes based on *N*-tripodal ligands for biomimetic catalysis of catechol oxidase [16, 17].

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Despite the fact that they are drifted from modeling biological systems, these mononuclear complexes are interesting because their synthesis is easy and allows the modification of their structure in a systematic way, getting to a better understanding of the elements favoring their catecholase activity. Complexes based on bis-pyrazolyl *N*-tripodal ligands have shown a good catalytic activity for the oxidation of 3,5-di-*tert*-butylcatechol [18, 19]. In this work, we took advantage of this catalytic behavior to prepare a novel biomimetic sensor for the determination of catechol derivatives. Dopamine was chosen as an analyte target since it is an essential neurotransmitter that is implicated in neurological diseases of the brain such as Parkinson's and Alzheimer's diseases [20]. Therefore, the development of analytical methods to determine its concentration is particularly useful to make advances in the treatment of such diseases. The modified electrode was prepared by encapsulation of the complex into a Nafion® film and its performances towards dopamine detection have been studied.

2. Experimental part

2.1 Reagents and materials

4-(2-aminoethyl)benzene-1,2-diol (dopamine), (S)-2-amino-3-(3,4-dihydroxyphenyl)propanoic acid (L-dopa), uric acid and 3,4-dihydroxybenzoic acid were purchased from Alfa Aesar. L-ascorbic acid was from Fisher Scientific, 2,3-dihydroxynaphthalene, catechol, and sodium 4,5-dihydroxybenzene-1,3-disulfonate (Tiron) were from Fluka, and 4-nitrocatechol and 5% solution of Nafion® 117 from Aldrich. The $[Cu^{II}(L)Cl]Cl$ complex (L = 6-(Bis-pyrazol-1-ylmethyl-amino)-hexan-1-ol) was prepared as previously reported [17]. All solutions were prepared with ultrapure water (18.2 MΩ, Millipore Simplicity).

2.2 Instrumentation

Voltammetric experiments were carried out using an EDAQ potentiostat unit, with the EChem software package. The working electrode, a platinum wire auxiliary electrode, and a saturated calomel reference electrode were used in a standard three-electrode configuration. Cyclic voltammetry experiments were performed without degassing. Square wave voltammetry was performed from 250 mV to -300 mV at pulse height 100 mV, frequency 80 Hz and Step potential -5 mV in a pH=7.2 phosphate (0.1 M) buffer solution.

2.3 Modification of the glassy carbon electrodes

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The GC electrode was home-made and carefully polished before modification.
50 µL of a 5% solution of Nafion® were added to 100 µL of a DMF solution containing 1-5 g L^{-1} of solubilized [Cu^{II}(L)Cl]Cl complex (L = 6-(Bis-pyrazol-1-ylmethyl-amino)-hexan-1-ol). 50 µL of this solution were deposited on the surface of a glassy carbon electrode (disc with a diameter of 3 mm) and air-dried for 1 night. The modified electrode was dipped in phosphate buffer pH=7.2 (NaH_2PO_4 0.05 M; Na_2HPO_4 0.05 M) for 15 min before use.
Reproducibility was not easy to obtain and required to use the same GC electrode (same diameter of GC and of glass around it), a good polishing before modification and a good drying.

3. Results and discussion

3.1 Preparation of the modified electrode for dopamine detection

The [Cu^{II}(L)Cl]Cl complex (L = 6-(Bis-pyrazol-1-ylmethyl-amino)-hexan-1-ol) **1** [17] (Scheme 1) was immobilized on a Glassy Carbon electrode by incorporation into a Nafion® film.

Scheme 1

The Nafion® ion-exchange polymer was chosen for its mechanical stability along with its high cation conductivity well-adapted for the immobilization of cationic complexes [21]. To prepare the modified electrode, 50 µL of a Nafion® solution containing the complex (0.7-3.3 g L^{-1}) were deposited on the electrode surface. Since dopamine has to be detected in human fluids, all experiments were carried out at physiological pH. After drying, the electrode was analyzed by cyclic voltammetry in a pH=7.2 phosphate (0.1 M) buffer solution (Fig. 1a).

Figure 1

A reversible system corresponding to the $Cu^{II/I}$ couple was observed at 0.097 V_{SCE}. Since the copper complex **1** was not soluble in aqueous medium, it was analyzed by cyclic voltammetry in dichloromethane (Fig. 1b). A reversible system corresponding to $Cu^{II/I}$ couple was obtained but at more anodic potential, 0.34 V_{SCE}. This phenomenon can come from the coordination of the metal, which can be different in dichloromethane than when it is immobilized in the

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Nafion® film and dipped in an aqueous solution. Indeed, the nature of the fourth and fifth ligands of the pentacoordinated metal can varied. The surface concentration of the immobilized complex was roughly estimated (Eq. 1 and 2) by integration of the peak using the Faraday law and was around 1.8×10^{-8} mol cm^{-2}.

$$Q = nF = S/v \quad (1)$$

$$\tau_s = n/s \quad (2)$$

with Q the electric charge (C), n the number of moles of immobilized catalyst (mol), F the Faraday constant 96485 C mol^{-1}, S the surface area of the peak (A V), v the scan rate (V s^{-1}), s the electrode surface (cm^{-2}) and τ_s the surface concentration (mol cm^{-2}).

This estimated value seems coherent since it is close to those of other complexes immobilized in a Nafion® film [21].

Dopamine is a catechol that can be electrochemically oxidized in its corresponding quinone by a two electrons process (Scheme 2).

Scheme 2

The quinone is stable in acidic medium when the primary amino group is protonated but undergoes intramolecular cyclisation at higher pH, leading to 5,6-dihydroxyindoline. Thus, at neutral pH, the electrochemical system begins to be affected leading to a high oxidation peak compared with the reduction one (Fig. 2a). Moreover, the formation of 5,6-dihydroxyindoline leads to electrode passivation due to the formation of an aminochrome that polymerizes readily to melanin-like products [22, 23], as seen when multiple scans are performed without polishing (Fig. 2a). The second peak around -0.3 V_{SCE} previously attributed to the aminochrome reduction [24, 25] shows that the degradation mechanism already occurs at pH 7.

Figure 2

When dopamine was analyzed with the $[Cu^{II}(L)Cl]Cl$-modified GC electrode, an increase (× 4.2) of the cathodic peak at 0.13 V_{SCE} and a decrease of the peak at -0.3 V_{SCE} were clearly observed (Fig. 2b). Since Nafion® is a perfluorinated ion exchange polymer with sulfonic

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groups, it works like a cationic membrane leaving only positive ions such as protonated dopamine able to diffuse inside. The enhancement of the cathodic peak is probably due to the combination of two phenomenons: i) incorporation of dopamine into the Nafion® membrane [26] ii) the catalytic oxidation of dopamine into its quinone derivative by the copper complex. The proposed mechanism of quinone formation is given in Scheme 3.

Scheme 3

It involves the formation of a copper(II)catecholate complex [17]. The reaction of these species with dioxygen results in the two-electron reduction of O_2 and release of H_2O_2 and a quinone molecule. The quinone is reduced at the electrode, leading to an electrochemical signal. Catechol is then involved again in the catalytic process.
The effect of Cu complex concentration in the Nafion® film on the electrochemical signal of dopamine was studied (Table 1).

Table 1

Dopamine was analyzed with the different modified electrodes by Square Wave Voltammetry (SWV). The electrochemical signal increased with the Cu complex concentration, as expected since a higher ratio of catalyst over substrate increases the kinetic of the reaction. However, an optimum was found for a concentration of 2 g L^{-1}, showing that a saturation of the Nafion® film was reached. The frequency (from 15 to 80 Hz) and the step H (-4 and -5 mV) have been varied. Whereas the step H did not modified the electrochemical signal, the increase of the frequency significantly improved it (Figure 3). A step amplitude of 100 mV has been chosen since it increased the signal compared with another test at 70 mV.

Figure 3

These optimum conditions were used in the following experiments.

3.2 Calibration curve and detection limit

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The dependence of the electrochemical signal on the concentration of dopamine was estimated by SWV and is given in Figure 4.

Figure 4

The curve is nonlinear, as previously observed with a Bis(2,2'-bipyridil) copper(II) chloride-modified electrode [27]. The kinetics behavior of the catalyst that is of Michaelis-Menten type [17] can explain the shape of the calibration curve. As expected the reaction rate rapidly increased with the concentration of dopamine leading to an increase of the electrochemical signal and grows more slowly when the substrate/catalyst ratio is too high, limiting the formation of quinone in the vicinity of the electrode surface. The curve is linear in the range of 30 to 320 μmol L^{-1} with a correlation coefficient of 0.9991 and can be expressed according to the following equation (3):

$$\Delta I\ (\mu A) = -3.81\ (\pm\ 1.68) + 0.503\ (\pm\ 0.009)\ [\text{Dopamine}]\ (\mu\text{mol}\ L^{-1}) \quad (3)$$

The limit of detection (3 × the standard deviation of five blank determinations) was determined from equation 4 [28],

$$St\text{-}Sb \geq 3\sigma \quad (4)$$

where St is the gross analyte signal, Sb the field blank and σ the variability in the field blank.

St and Sb were the maximum current intensity of the corresponding peaks.

We found a limit of detection of 8.2 μmol L^{-1} with $Sb + 3\sigma = 0.348$ μA.

The value is close to the limits of detection measured by other biomimetic sensors ranging from 0.1 to 8 μmol L^{-1} [27, 29-31]. This result is encouraging meaning that it is possible to reach low limit of detection with a simple mononuclear *N*-tripodal complex.

3.3 Selectivity and stability

The selectivity of the sensor was tested in the presence of strong common interferents in human fluids, ascorbic acid and uric acid that are known to be oxidized at the same potential than dopamine [32]. Thus, the electrochemical response of a 105 μM dopamine solution was

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measured by square wave voltammetry in the presence of uric acid and ascorbic acid in different concentrations (Table 2).

Table 2

No significant interferences were observed when the tested compounds were in a small excess, showing that in these conditions, the amount of quinone formed in the vicinity of the electrode surface is significantly higher than the amount of uric and ascorbic acid. The electrochemical signal drastically decreased when the concentration of interferents was 12 times higher.

The sensor presents very good stability and can be used after several days kept at room temperature in air. As highlighted in Figure 5, the signal was not affected after 40 determinations with stirring of the solution after each measurement and a decrease of about 7% was observed after 110 determinations.

Figure 5

3.4 Effect of the nature of the catechol derivative

The sensor response for other catechol derivatives was studied (Table 3).

Table 3

Among the 7 tested molecules, only catechol derivatives containing an acid group gave no signal. Since the analyses were performed at pH 7, this can be due to a blocking effect of the carboxylate or sulfonate containing compounds by the perfluorinated anionic polyelectrolyte membrane which exhibits permselectivity only towards cations. The highest signal was obtained with dopamine and catechol compared with 4-nitrocatechol and 2,3-dihydroxynaphthalene. This could be explained by the fact that they are easier to oxidize compared with their analogues containing electrowithdrawing groups. These results show that the efficiency of the sensor is directly linked to the catalytic activity of the complex towards the oxidation of the catechol derivative and to its immobilization process.

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4. Conclusions

A biomimetic sensor was prepared by immobilization of a monocopper complex containing *N*-tripodal ligands into a Nafion® film. The catalytic activity of the complex towards catechol oxidation allowed the enhancement of the reduction peak of the quinone form of dopamine, allowing its determination in pH 7 medium. A calibration curve was obtained with a linearity in the range of 16 to 250 μmol L^{-1} and a good detection limit. The sensor presents a good selectivity in the presence of uric and ascorbic acid and a high stability. The effectiveness of the sensor towards the detection of other catechol derivatives was also underlined and depends on the oxidation ability of the substrate and on the immobilization process. These results show the possibility to prepare performant biomimetic sensors for catechols with *N*-tripodal monocopper complexes. The versatility of the synthesis of this family of catalysts would allow to establish a systematic correspondence between the catecholase activity of the immobilized complex and the performances of the sensor.

Acknowledgements

We thank the Ministère de l'Enseignement Supérieur et de la Recherche Scientifique of Algérie (MESRS) for financial support.

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Scheme and figure captions:

Scheme 1: structure of the $[Cu^{II}(L)Cl]Cl$ complex.

Scheme 2: electrochemical oxidation of dopamine in neutral medium

Scheme 3: mechanism of quinone formation at the $[Cu^{II}(L)Cl]Cl$-modified GC electrode.

Figure 1: Cyclic voltammograms of copper complex **1** a) freshly immobilized in a Nafion® film (2 g L^{-1}) in a pH=7.2 phosphate (0.1 M) buffer solution and b) saturated in dichloromethane solution + 0.1M Bu_4NPF_6. Scan rate 0.05 V s^{-1}.

Figure 2: Cyclic voltammograms of dopamine (5.3×10^{-4} mol L^{-1}) on a) a GC electrode ((—) 1^{st} scan, (….) 10^{th} scan) b) a $[Cu^{II}(L)Cl]Cl$-modified GC electrode in a pH=7.2 phosphate (0.1 M) buffer solution. The blank performed on a GC electrode without dopamine is given (----) Scan rate 0.05 V s^{-1}

Figure 3: Square wave voltammograms obtained using a $[Cu^{II}(L)Cl]Cl$-modified GC electrode for a concentration of dopamine of 45 μmol L^{-1} in a pH=7.2 phosphate (0.1 M) buffer solution at pulse height 100 mV and frequency 80 Hz, Step potential -5 mV (—); frequency 15 Hz; Step potential -4 mV (----); frequency 15 Hz; Step potential -5 mV (.....).

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Figure 4: a) Square wave voltammograms obtained using a [Cu^{II}(L)Cl]Cl-modified GC electrode for the concentrations a to j: a) 30 ; b) 45; c) 55; d) 120; e) 180; f) 250; g) 320; h) 440; i) 560; j) 680 μmol L^{-1} at pulse height 100 mV, frequency 80 Hz and Step potential -5 mV in a pH=7.2 phosphate (0.1 M) buffer solution b) Calibration curve of the biomimetic sensor and linear fit for a concentration range of 15 to 680 μmol L^{-1}. Error bars are based on electrochemical signal given by the same electrode modified 5 times.

Figure 5: Relative response (%) at a function of the number of determinations. Pulse height 100 mV, frequency 80 Hz and Step potential -5 mV in a pH=7.2 phosphate (0.1 M) buffer solution containing 105 μmol L^{-1} of dopamine. Error bars are based on 4 experiments.

Table 1: Effect of the concentration of Cu complex in the Nafion® film on the electrochemical signal of dopamine measured by SWV.

[Cu^{II}(L)Cl]Cl / g L^{-1}	Electrochemical response / %
0.7	44
2	100
3.3	90

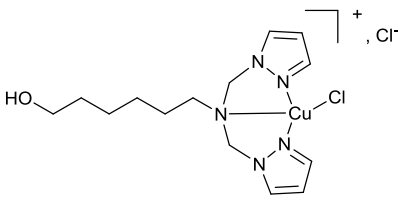
Table 2: Comparison of the electrochemical signal (SWV) of dopamine (105 μmol L^{-1}) in the presence of different concentrations of uric and ascorbic acids.

[Uric acid] (μM)	[Ascorbic acid] (μM)	Electrochemical signal (%)
130	130	100
600	600	90.5
1300	1300	19.3

Table 3 : Electrochemical response obtained with the [Cu^{II}(L)Cl]Cl-modified GC electrode for different catechols (130 μmol L^{-1})

Analyzed substrate	Electrochemical response (%)
Dopamine	100
Catechol	49.9
L-Dopa	0
3,4-Dihydroxybenzoic acid	0
4-Nitrocatechol	3.2
2,3-Dihydroxynaphthalene	3.9
Tiron	0

Scheme 1



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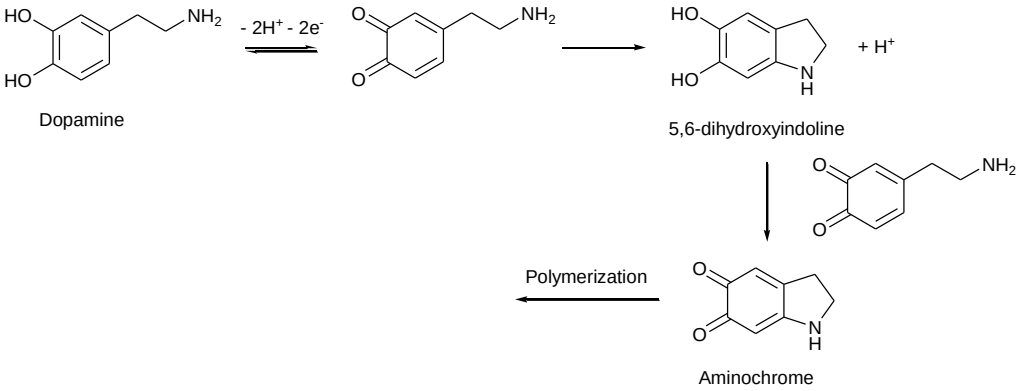
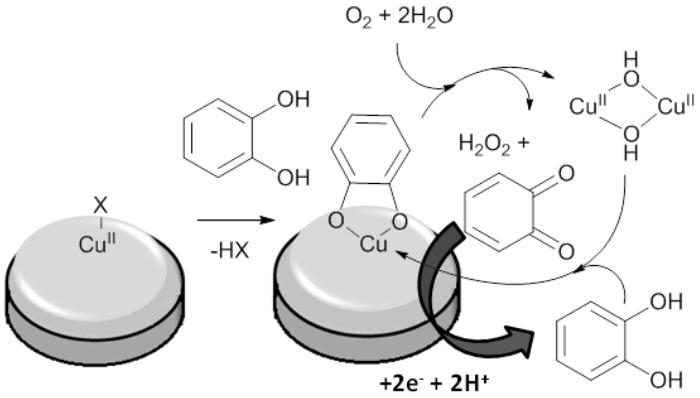
1

Scheme 2



Scheme 3



ACCEPTED MANUSCRIPT

Figure 1

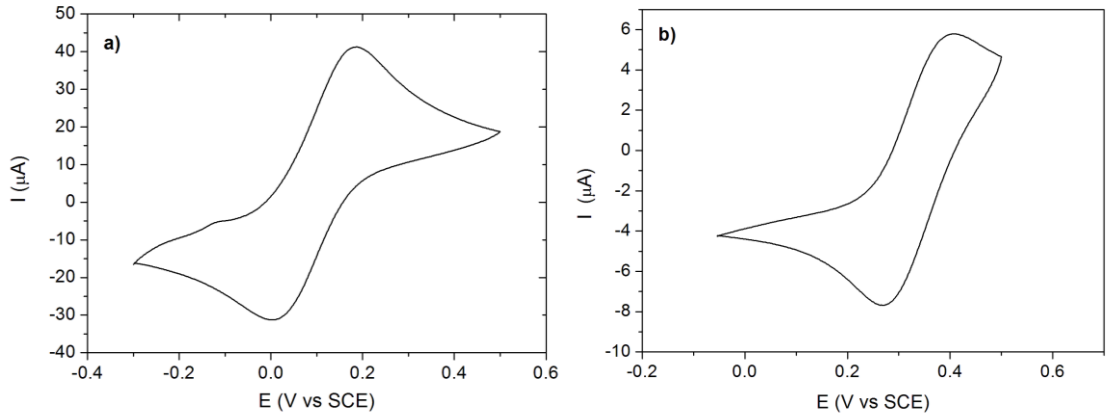


Figure 2

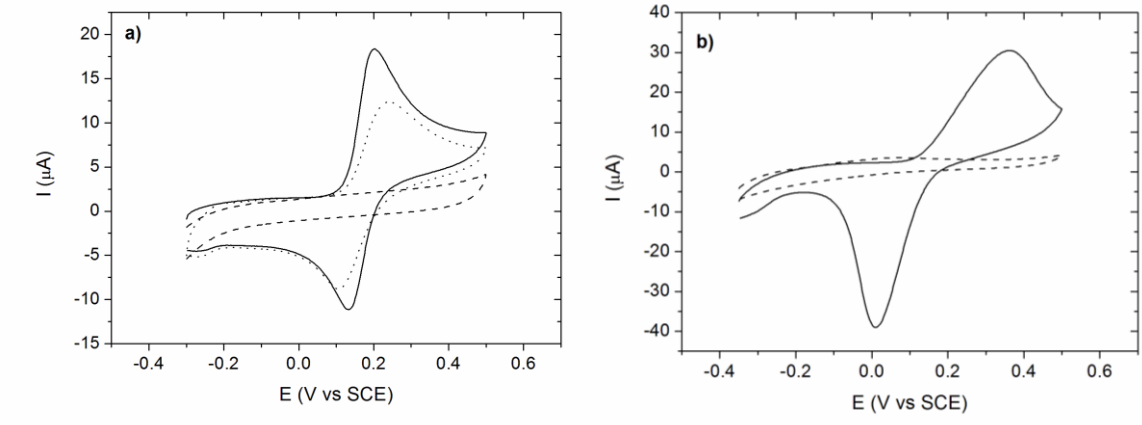
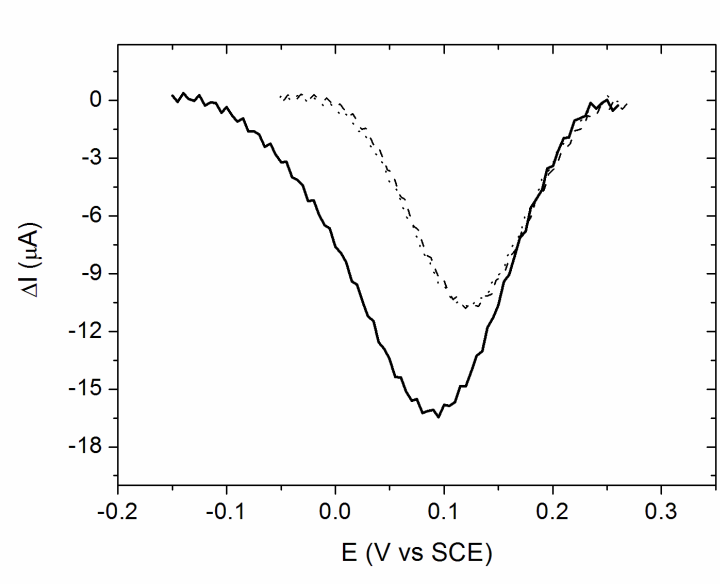
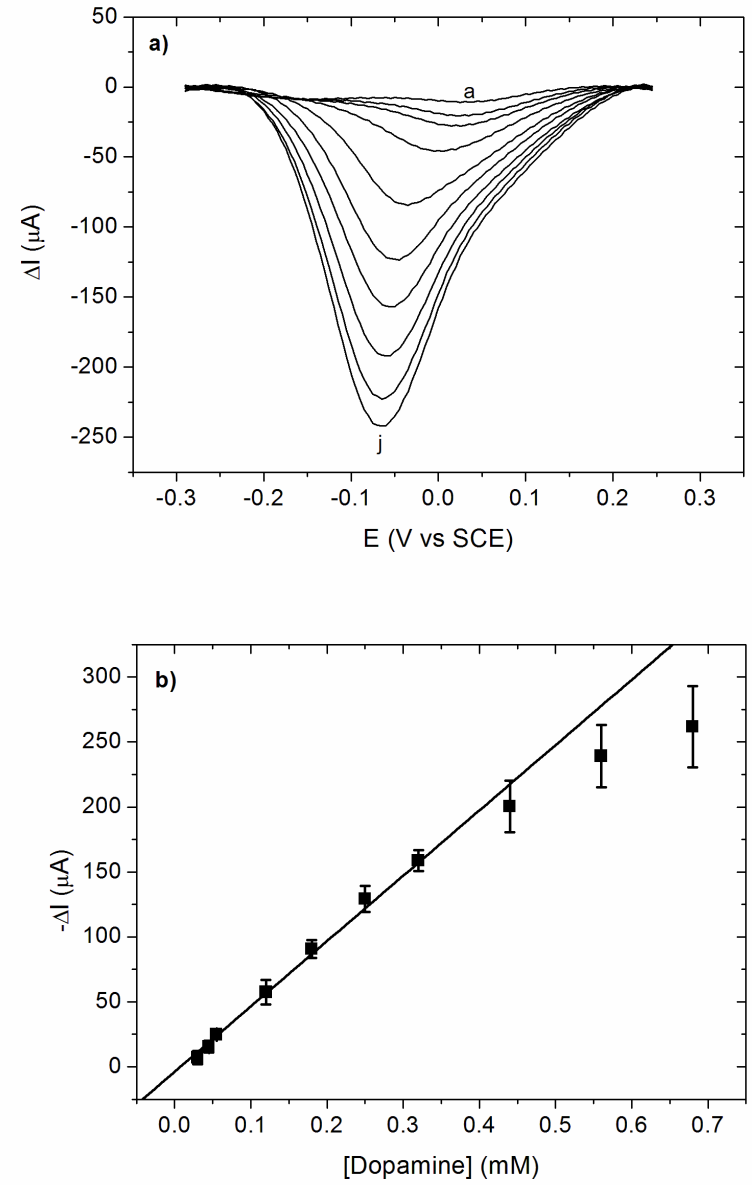


Figure 3



ACCEPTED MANUSCRIPT

Figure 4



ACCEPTED MANUSCRIPT

Figure 5