

*Full Paper*

## **Electrochemical Sensors for Dopamine using Graphene-cobalt(II) Complex Modified Glassy Carbon Electrode by Adsorptive Voltammetry**

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**Abstract-** [Co(bdmpzm)<sub>2</sub>(NCS)<sub>2</sub>] (bdmpzm=bis(3,5-dimethylpyrazol-1-yl)methane) was dispersed in graphene oxide (GOx). Composite GOx-[Co(bdmpzm)<sub>2</sub>(NCS)<sub>2</sub>] was deposited on a glassy carbon electrode (GCE) and reduced at -1.0 by 60 s. The modified electrode rGOx-[Co(bdmpzm)<sub>2</sub>(NCS)<sub>2</sub>]/GCE allowed the oxidation for dopamine (DA) at 0.41 V. Our results showed reversible and sensitive process, ( $\Delta V$  0.09 V) compared with the electrode without modified in buffer phosphate solution pH 3.0. The detection limit ( $3\sigma/b$ ) for dopamine was 0.11  $\mu\text{mol L}^{-1}$  and the RSD was 3.0%. The presence of uric acid and ascorbic acid did not interfere with the signal for dopamine.

**Keywords-** Dopamine, Uric acid, Human urine, Cobalt(II) complex, Adsorptive voltammetry, Graphene oxide

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### **1. INTRODUCTION**

Dopamine (DA) is considered a neuromodulator which acts in the synapses from the central nervous system [1]. Diseases such as Alzheimer and Parkinson are increases with the

low production of dopamine in the dopaminergic neurons [2]. DA in biological fluids is present in low concentrations and significant amounts of uric acid (UA) and ascorbic acid (AA) may be present in higher concentrations. Moreover, AA and UA can be electro-oxidized at similar potential of the DA [3]. Importance of DA as neurotransmitter and the potential interference of AA and UA justify the detection of dopamine in the presence of AA and UA.

**Table 1.** Modified electrodes with graphene for DA

Electrode	Materials	Detection limit ( $\mu\text{mol L}^{-1}$ )	Application	Ref.
GCE	Mn-TPP	0.008	Pharmaceutical dosage, human urine, human blood	[15]
GCE	Au <sub>NP</sub>	0.095	Pharmaceutical dosage	[16]
GCE	Cu <sub>2</sub> O	0.050	Human urine, human blood	[17]
GCE	Pdop	1	Human urine, human blood	[18]
GCE	ZnO	1.08	Human urine, human blood	[19]
GCE	PVIPS/PPy	0.0173		[20]
GCE	PG	2		[21]
GCE	CS	5		[22]

GCE: glassy carbon electrode; CS: chitosan; Mn-TPP: manganese tetraphenylporphyrin; Au<sub>NP</sub>: gold nanoparticles; PVIPS/PPy: poly(3-(1-vinylimidazolium-3-yl) propane-1-sulfonate/polypyrrole); PG: pristine graphene; Pdop: polydopamine; ZnO: zinc oxide.

Some techniques that have been used for the determination of DA are: high performance liquid chromatography (HPLC) with fluorimetric detection [4] and mass spectrometry detection [5], capillary electrophoresis [6] and spectrophotometric [7]. These reports have shown to be sensitive, with limits of detection between 10.0 and 1.0 nmol L<sup>-1</sup> and selective for DA. Also, has a high operational cost and it is necessary to pre-treatment of the samples. Electroanalytical techniques have emerged in recent years as an important alternative for the detection of DA. Have proved to be sensitive, low cost and allow quantifying DA in the presence of UA and AA. The innovation in each report is based mainly on the modification of the working electrode to eliminate interference and improve the sensitivity. Electroanalytical techniques have been used also in the determination of diclofenac using

ionic liquids and carbon nanotubes [8], penicillamine, uric acid, tryptophan, ascorbic acid acetaminophen with N-(3,4-Dihydroxyphenethyl)-3,5-dinitrobenzamide using carbon nanotubes [9,10], L-cysteine, tryptophan with p-aminophenol using carbon nanotube [11], isoprenaline with ZnO and ionic liquids using carbon paste [12] and glutathione with NADH using carbon nanotubes [13]. Graphene oxide (GOx) was reported in 1840 first time by Schafhaeuti, and in recent years its use has grown in the modification of electrodes. With the objective of improving the conductivity of the material is necessary reduce the graphene oxide for generating a material called graphene oxide reduced (rGOx) this will eliminate the excesses of functional groups in the graphene oxide. [14] Some reports where it has been used graphene for DA determination are summarized in Table 1.

Cobalt compounds acts as redox mediators, being very convenient for construction of electrochemical sensors for dopamine. Among these cobalt compounds which have been used in the quantification electroanalytical of DA are cobalt-5-nitrosalophen on carbon paste [23], cobalt nanoparticles and cobalt-phenanthroline on carbon nanotubes and glassy carbon [24,25]. On the other hand, Detection of dopamine with graphene and Cobalt complex have been reported using cobalt oxide [26], cobalt tetraphenylporphyrin [27], Cobalt phthalocyanine [28] and cobalt hexacyanoferrate [29].

The aim of this study is detect and quantify DA using a GCE modified with graphene and  $[\text{Co}(\text{bdmpzm})_2(\text{NCS})_2]$  in presence de AA y AU.

## 2. EXPERIMENTAL PART

### 2.1. Apparatus

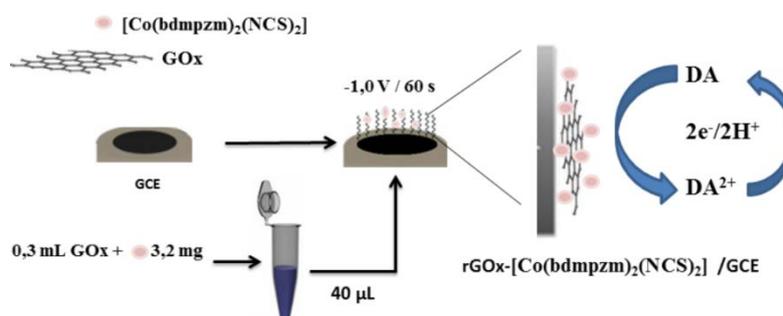
Potentiostat/galvanostat Versa Stat 3 was used to obtain the Nyquist plot by electrochemical impedance spectroscopy (EIS). Bipotentiostat/galvanostat Dropssens  $\mu\text{Stat}$  400 was used to obtain the linear sweep voltammograms and cyclic voltammograms. Glassy carbon electrode (3 mm) was obtained from Ch-instrument. The reference electrode was Ag/AgCl/KCl 3 mol L<sup>-1</sup> and the auxiliary electrode was platinum wire. The pH measurements were made with a Starter ST 3000-B equipped with a combined pH glass electrode.

### 2.2. Chemicals

$[\text{Co}(\text{bdmpzm})_2(\text{NCS})_2]$  was synthesized as described in the literature [30]. Water was obtained of Milli-Q system (18.2 M $\Omega$ .cm, Millipore, USA). Nitric acid, phosphoric acid, monosodium and disodium phosphate were analytical grade. Solutions of DA, AA and UA (Sigma-Aldrich) were freshly prepared (5.0 mmol L<sup>-1</sup>) each day.  $\text{K}_4\text{Fe}(\text{CN})_6$ , and graphene oxide (2 mg/mL) were obtained from Sigma-Aldrich. Phosphate buffer solutions (PBS) were

prepared between pH 2.0-6.0. All reagent-grade solvents were dried, distilled, and stored under a nitrogen atmosphere.

### 2.3. Preparation of modified (GCE) with graphene oxide and $[(\text{Co}(\text{bdmpzm})_2(\text{NCS})_2)]$



**Fig. 1.** Preparation of modified electrode

Before modification, GCE was clean using  $\text{Al}_2\text{O}_3$  0.3 and 0.05  $\mu\text{m}$  and sonicated with  $\text{HNO}_3$  0.3  $\text{mol L}^{-1}$  by 5 min. The modified  $\text{rGOx}-[\text{Co}(\text{bdmpzm})_2(\text{NCS})_2]/\text{GCE}$  was prepared with the dispersion of  $[(\text{Co}(\text{bdmpzm})_2(\text{NCS})_2)]$  (3.3 mg) in 0.36 mL of GOx. 30  $\mu\text{L}$  were incorporated on GCE surface, and drying the excess solvent with heat. The freshly modified electrode was treated by application of a potential of -1.0 V by 60 s in BPS pH 3.0 then cyclic voltammetry was used with 3 cycles of potential between -0.3 to 1.2 V ( $100 \text{ mV s}^{-1}$ ) to obtain a clean surface. Electrode preparation is showed in Fig. 1.

### 2.4. Sample preparation

Urine samples were obtained of students from Universidad de Ibagu e. Without any further treatment, immediately after collection between 4-9 mL of sample were deposited in the cell and diluted with 5 and 0.5 mL of water mili Q and 0.5 mL ( $0.01 \text{ mol L}^{-1}$ ) PBS pH 3.0 to be analyzed. Standard addition method was used. All the samples were diluted 3 times.

### 2.5. Measurement procedure

#### 2.5.1. Electrochemical impedance spectroscopy (EIS)

10 mL of  $\text{Fe}(\text{CN})_6^{3-}$  0.01  $\text{mol L}^{-1}$  in  $\text{K}^+$  0.01  $\text{mol L}^{-1}$  were deposited in the cell and Electrochemistry Impedance Spectrum (EIS) was done between 10.0 kHz and 1.0 Hz using  $\text{rGOx}-[\text{Co}(\text{bdmpzm})_2(\text{NCS})_2]/\text{GCE}$ .

### 2.5.2 Cyclic voltammograms

0.10 mL of DA, AA and UA solution ( $50.0 \text{ mmol L}^{-1}$ ) were pipetted in the cell. After the equilibration time of 3 s, cyclic voltammograms were recorded, while the potential was scanned from 0.0 to 0.8 V with scan rate of  $0.1 \text{ mV s}^{-1}$ . Each cyclic voltammograms were repeated three times.

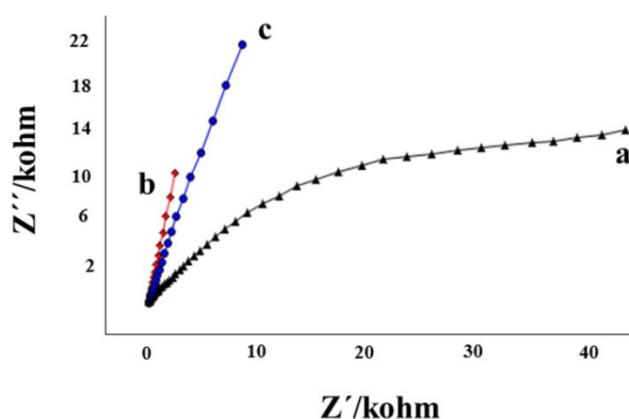
### 2.5.3 Adsorptive voltammograms

10.0 mL of water (or 4.0 mL of samples), 500  $\mu\text{L}$  of PBS pH 3.0 ( $0.01 \text{ mol L}^{-1}$ ), 10–80  $\mu\text{L}$  of DA ( $0.50 \text{ mmol L}^{-1}$ ) were added in the cell. Pre-concentration step was initiated at a stirring speed of 500 rpm. After the equilibration time (3 s), adsorptive scan voltammograms were made of 0.0 to 0.7 V scan line modulation with 10 mV step amplitude and scan rate  $0.11 \text{ mV s}^{-1}$ . Each voltammograms were repeated three times. Detection limited (DL) was calculated by  $y_{DL} = a + 3\sigma_{x/y} * b$ , where a, is the intercept,  $\sigma_{x/y}$ , is the random error in x and y, and b is the slope. In addition  $DL = x_{DL} = 3s_{x/y}/b$  [31]. Matrix effects are eliminated using standard addition method.

## 3. RESULTS AND DISCUSSION

### 3.1. rGOx-[Co(bdmpzm)<sub>2</sub>(NCS)<sub>2</sub>]/GCE Electrochemical characterization

With the aim of verifying the conductive properties of the modified electrode with GOx and cobalt complex, was studied the electrode surface using EIS.



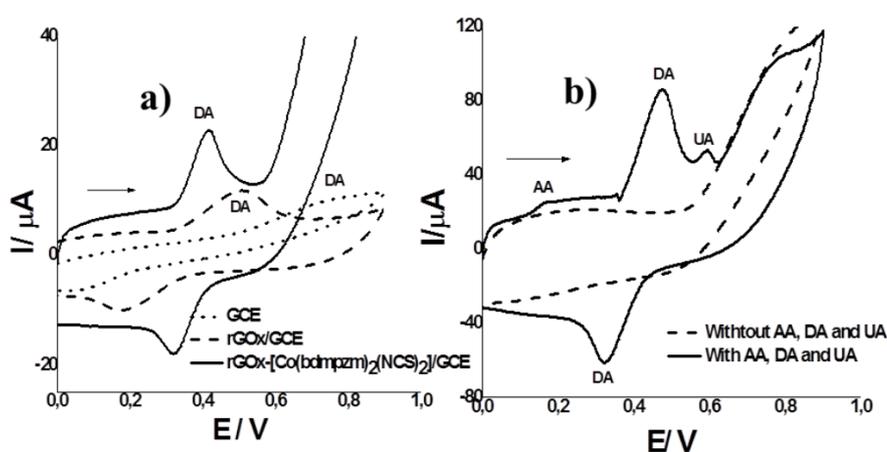
**Fig. 2.** Nyquist plot of  $0.01 \text{ mol L}^{-1} \text{Fe(CN)}_6^{-3}$  in  $0.01 \text{ mol L}^{-1} \text{K}^+$  a) unmodified GCE, b) rGOx-GCE and c) rGOx-[Co(bdmpzm)<sub>2</sub>(NCS)<sub>2</sub>]/GCE

These results normally are reported in a Nyquist plot, where real resistance ( $Z'$ ) is plotted vs imaginary resistance ( $Z''$ ) for an electrical circuit for high and low frequencies. High resistance values indicate a high impediment to the transfer of electrons [32]. Nyquist plot of

the EIS at GCE, GOx/GCE and GOx-[Co(bdmpzm)<sub>2</sub>(NCS)<sub>2</sub>]/GCE in a solution containing 0.01 mol L<sup>-1</sup> of Fe(CN)<sub>6</sub><sup>3-</sup> in K<sup>+</sup> 0.10 mol L<sup>-1</sup> are shown in Fig. 2. The results showed a Nyquist plot with a semi-circle (curve a), using GCE in high frequency part between 5-1 kHz, that suggests high impediment to the transfer of electrons. Using rGOx/GCE (curve b) and rGOx-[Co(bdmpzm)<sub>2</sub>(NCS)<sub>2</sub>]/GCE (curve c), the values of z' resistance decreased considerably. These results are evidence that conductivity and the transfer of electrons were improved when was deposited rGOx and cobalt complex in the surface of the electrode. Similar results have been reported using others cobalt complexes [25].

### 3.2. Electrochemical behavior of DA, UA and AA using different electrodes

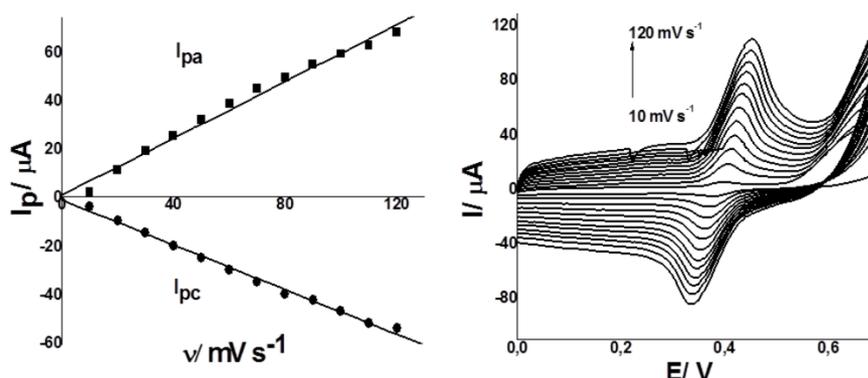
Dihydroxyphenethyl region is related with the redox activity for DA, UA and AA [19]. Redox behavior of DA was studied using GCE unmodified, rGOx/GCE and rGOx-[Co(bdmpzm)<sub>2</sub>(NCS)<sub>2</sub>]/GCE. Fig. 3a shows the CV's of DA (0.5 mmol L<sup>-1</sup>) at 100 mV s<sup>-1</sup> and pH 4.0 PBS, on GCE (dot line), rGOx/GCE (dash line) and rGOx-[Co(bdmpzm)<sub>2</sub>(NCS)<sub>2</sub>]/GCE (solid line). The results obtained showed with the unmodified GCE an oxidation peak to 0.71 V (1.04 μA anodic peak current) and a reduction peak to 0.078 V (-1.4 μA cathodic peak current) for DA. This result indicates a quasi-reversible process for DA. Using rGOx/GCE anodic peak appears to potential less positive (0.50 V) and cathodic peak appears to 0.18 V with ΔV 0.314 V. Moreover, was observed an increase in the anodic and cathodic peak currents (6.27 and -4.62 μA) almost 100 %. This result suggests that rGOx increase the surface area and DA oxidation is improved. Some researchers have suggested that π-π interaction between phenyl structure of DA and two-dimensional planar hexagonal carbon structure of graphene makes the electron transfer feasible [22].



**Fig. 3.** a) CV for DA 0.50 mmol L<sup>-1</sup> and b) for AA, DA and UA 0.50 mmol L<sup>-1</sup> on GCE (dot line), rGOx-GCE(dash line) and rGOx-[Co(bdmpzm)<sub>2</sub>(NCS)<sub>2</sub>]/GCE (solid line), in 0.01 mol L<sup>-1</sup> PBS (pH 3.0). Scan rate 100 mVs<sup>-1</sup>

Using rGOx-[Co(bdmpzm)<sub>2</sub>(NCS)<sub>2</sub>]/GCE, was observed a considerable increase in the anodic and cathodic peak currents (12.11 and -8.48  $\mu\text{A}$ ). Moreover, anodic peak appears to potential less positive (0.41 V) and cathodic peak appears to potential more positive (0.32 V) with  $\Delta V$  0.09 V. This result suggests that the presence of cobalt complex has a catalytic effect on the oxidation potential and on the oxidation current for DA. AA and UA coexist with DA in biological fluid. Moreover, concentrations of UA and AA are much greater. Therefore, the interference that can produce AA and UA was studied with cyclic voltammetry. Fig. 3b shows cycle voltammograms of AA, DA and UA (0.5 mmol L<sup>-1</sup>) in PBS pH 3.0, using rGOx-[Co(bdmpzm)<sub>2</sub>(NCS)<sub>2</sub>]/GCE. CV's obtained for AA, DA and UA showed that the electrode modified using rGOx and cobalt complex improves the anodic peak current for DA. We believe that AA and UA have a weak  $\pi$ - $\pi$  interaction with the graphene and therefore the peak currents are lower and that there is an interaction by affinity with the organic part of the cobalt complex more favorable for DA.

### 3.3. Influence of the scan rate for DA using rGOx-[Co(bdmpzm)<sub>2</sub>(NCS)<sub>2</sub>]/GCE

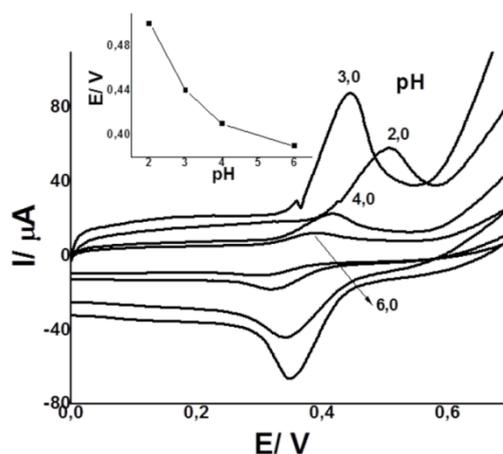


**Fig. 4.** CV's and dependence of peak currents on square root of scan rate for DA (0.5 mmol L<sup>-1</sup>) in pH 3.0 PBS using rGOx-[Co(bdmpzm)<sub>2</sub>(NCS)<sub>2</sub>]-GCE

With the aim of understanding the transport of mass on the surface of the electrode, the scan rate ( $v$ ) on anodic and cathodic peak currents for DA was studied. Regression equation for anodic peak currents was  $i_{pa}=1.32+0.58v$  (correlation coefficient  $r=0.9939$ ) and cathodic peak currents was  $i_{pc}=-0.60-0.461v$  (correlation coefficient  $r=-0.9970$ ). This result indicates that the peak currents increased linearly with the scan rate between 10 to 120  $\text{mV s}^{-1}$  (Fig. 4). Therefore, the reaction of DA is controlled by adsorption. Similar results were reported for DA using rGOx with Cu, where the number of electrons calculated by the Laviron's equation was  $1.8 \approx 2.0$  [17].

### 3.4. Effect of pH on the anodic peak currents of DA

The pH influence on the anodic peak currents for DA  $0.50 \text{ mmol L}^{-1}$  was studied at pH values of 2.0 to 6.0 using PBS ( $0.001 \text{ mol L}^{-1}$ ). Anodic peak potentials for DA shift toward less positive values with increasing pH (Fig. 5 insert). These results suggest that protons ( $\text{H}^+$ ) are involved in the reaction for DA. The maximum anodic peak current was obtained at pH 3.0 and was used for further experiments.



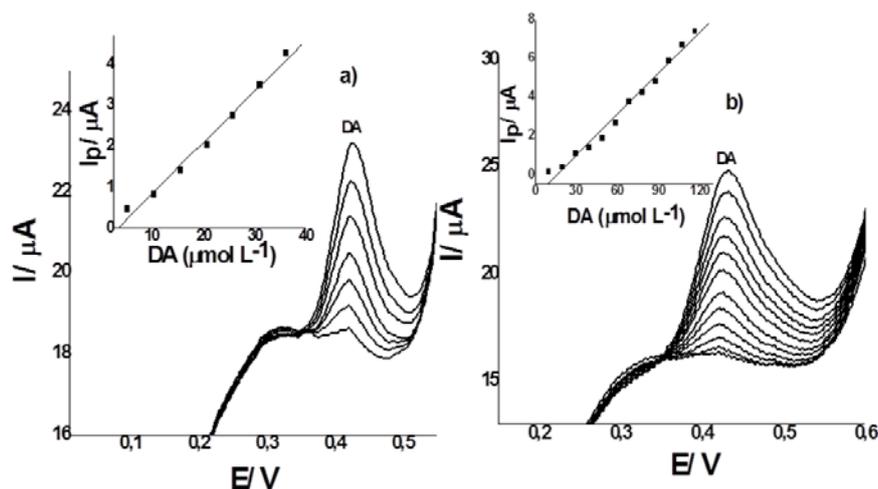
**Fig. 5.** Influence of pH on anodic peak currents for DA  $0.50 \text{ mmol L}^{-1}$ . (insert, pH vs anodic peak potential) Conditions:  $t_{\text{ads}} 60 \text{ s}$ ;  $E_{\text{ads}} 0.1 \text{ V}$  and scan rate  $100 \text{ mV S}^{-1}$

### 3.5. Effect of potential and time adsorption ( $E_{\text{ads}}$ , $t_{\text{ads}}$ ) on anodic peak currents of DA

Effect of adsorptive potential on anodic peak currents was studied in the range from 0.15 to  $-0.20 \text{ V}$  using scan line voltammetry for DA  $52.0 \mu\text{mol L}^{-1}$  (pH 3.0,  $t_{\text{ads}} 60 \text{ s}$ ). The results showed that the anodic peak currents for DA increases linearly with the accumulation potential in the range from 0.1 to  $-0.05 \text{ V}$ . To positive values anodic peak decreased. An accumulative potential of  $0.0 \text{ V}$  gives the best sensitivity for DA and was selected for further measurements. The effect of adsorption time was examined in the range 10–100 s. Anodic peak currents increased linearly with accumulation time until 60 s. With higher adsorption time, anodic peak currents decreased, probably due to the saturation of the electrode surface. With these results, we used an adsorptive time of 60 s for all measurements.

### 3.6. Influence of instrumental variables

The instrumental parameters studied were step amplitude and scan rate. Anodic peak currents for DA increased when step amplitude and scan rate were not higher than  $2.0 \text{ mV}$  and  $100 \text{ mV s}^{-1}$  respectively.



**Fig. 6.** Adsorptive voltammograms and calibration curve (insert) for DA 5.2 to 36.14  $\mu\text{mol L}^{-1}$  (a) and 9.8 to 116.2  $\mu\text{mol L}^{-1}$  (b). Conditions: pH 3.0; Eads 0.0 V and tads 60 s

### 3.8. Validation and analysis of human urine samples

**Table 2.** Results of the detected spiked DA and UA in human urine samples. (n=3)

Sample	substance	Detected ( $\mu\text{mol L}^{-1}$ )	Added ( $\mu\text{mol L}^{-1}$ )	Detected ( $\mu\text{mol L}^{-1}$ )	% relative error
1	DA	-	95.0	89	-6.13
	UA	-	-	-	-
	AA	-	-	-	-
2	DA	-	188.0	181	-3.70
	UA	163.0	20.0	180	-1.63
	AA	-	20.0	-	-
*3	DA	20.0	10.0	28.5	-5.0
	UA	500.0	20.0	517	-0.50
	AA	-	154.0	-	-
4	DA	-	92.0	70.0	-23.9
	UA	52.0	20.0	70.0	-2.85
	AA	-	300.0	-	-

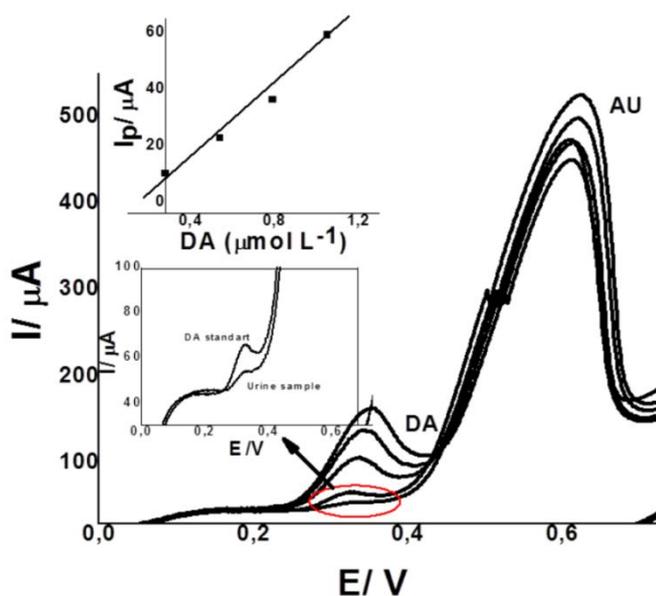
\* Fig.7 shows adsorptive voltammograms and calibration plot obtained for DA

### 3.7. Analytical parameters

Adsorptive voltammograms for DA were obtained under the optimized conditions: pH 3.0 (PBS 0.001 mol L<sup>-1</sup>), Eads 0.0 V, tads 60 s. Fig. 5 shows the adsorptive voltammograms for DA. The results showed an increases proportionally of the anodic peak currents when the

concentrations for DA were increased from 5.2 to 36.14  $\mu\text{mol L}^{-1}$  (Fig. 6a) and 9.8 to 116.2  $\mu\text{mol L}^{-1}$  (Fig. 6b) with detection limits ( $3\sigma/b$ ) of 0.13  $\mu\text{mol L}^{-1}$  (24.57  $\mu\text{g L}^{-1}$ ) and 0.11  $\mu\text{mol L}^{-1}$  (20.79  $\mu\text{g L}^{-1}$ ) respectively. Reproducibility for DA 10  $\mu\text{mol L}^{-1}$  was 3.0% ( $n=7$ ). With respect to the sensitivity for DA, using modified electrodes containing graphene oxide and cobalt complex, have been reported limits of detection between 0.03-3.0  $\mu\text{mol L}^{-1}$  [26,29]. In our measurements the sensibility for DA was almost equal

For validation and analysis of real samples, human urine was used. The values obtained are summarized in Table 2. Only in a sample was possible to detect dopamine (Fig. 7), possibly in the other samples was present below the limit of detection. It was also possible to detect UA because it was in high concentrations. AA no was detected. These results are similar to other reports where the detection of DA in human urine was not possible. [33].



**Fig. 7.** Adsorptive voltammograms and calibration curve (insert) for human urine sample (sample 3\*). Others conditions as Fig. 6

#### 4. CONCLUSION

The modified electrode with cobalt complex and graphene oxide showed sensitivity and selectivity for DA with detection limits of 0.11  $\mu\text{mol L}^{-1}$ . On the other hand, the detection of UA is possible to high concentrations. Presence of cobalt facilitated the transport of electrons and allowed a reversible system compared with the electrode unmodified. The analysis time was reduced to not apply treatment to real samples.

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