

# Electrochemical Sensor Based on Carbon Paste Modified by Cobalt(II) Porphyrin for Ascorbic Acid Determination

## *Sensor Eletroquímico à Base de Pasta de Carbono Modificada com Porfirina de Cobalto(II) para a Determinação de Ácido Ascórbico*

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Ascorbic acid (AA) is widely used in the food, cosmetics and pharmaceutical industries. Therefore, the development of sensitive, fast and cost-effective analytical method for AA determination is very relevant in these areas. In this work, an electrochemical sensor was developed based on carbon paste modified by cobalt(II) porphyrin complex immobilized on silica (SiO<sub>2</sub>/ TiO<sub>2</sub>) obtained by the sol-gel processing method, for AA detection. The modifying material, SiTiPPCo, was characterized by Scanning Electron Microscopy (SEM) and Fourier Transform Infrared Spectroscopy (FT-IR). The modified electrode (CPE-SiTiPPCo) results have shown excellent performance in the determination of AA. The method proposed presented linear range from 7.0 μmol L<sup>-1</sup> to 70.0 μmol L<sup>-1</sup> (r = 0.9986), with detection and quantification limits of 1.9 μmol L<sup>-1</sup> and 6.4 μmol L<sup>-1</sup>, respectively. CPE-SiTiPPCo was successfully applied to determine AA in samples of pharmaceutical formulation (Energil C<sup>®</sup>) with a relative standard deviation (RSD) lower than 4.08%.

**Keywords:** Electrochemical sensor; ascorbic acid; carbon paste electrode, cobalt(II) porphyrin complex

## 1. Introduction

Ascorbic acid (AA), also known as vitamin C, is a hydrosoluble vitamin that exists as two enantiomers. The L isomer is the one most often found.<sup>1</sup>

AA play an essential role in human metabolisms such as enzyme cofactor, chemical reductant and antioxidant.<sup>2</sup> AA is widely used in the food, cosmetics and pharmaceutical industries due of its antioxidant properties.<sup>3</sup>

The deficiency of AA is associated with causes various diseases such as scurvy, deterioration of collagen, cardiovascular disease, common cold, mental illness, infertility and lowering of body resistance from infections. By contrast, excessive amounts of AA are harmful for health and may cause gastric irritation and renal disorders.<sup>4</sup> Thus, the development of a sensitive, fast and cost-effective analytical method for the determination of AA is important for healthcare and quality control of pharmaceuticals.

Several analytical methods have been described in the literature for AA determination such as Colorimetry,<sup>5</sup> Spectrophotometry,<sup>6,7</sup> Chromatography,<sup>8,9</sup> Capillary Electrophoresis<sup>10</sup> and Fluorescence Techniques.<sup>11</sup> The electrochemical determination of AA it has also been reported since AA is an electroactive compound.<sup>1,12-14</sup>

Sensors based on Electrochemical Techniques are widely used due their advantages such as easy preparation, high sensitivity and low cost.<sup>15</sup> Another advantage is that the sensitivity and selectivity of the electroanalytical response can be improved with the use of chemically modified electrodes.<sup>16</sup>

Metalloporphyrins adsorbed on porous solid substrates, such as silica, have been used as modifying materials in the development of sensors.<sup>17</sup> Porphyrin derivatives are use as electron mediators due to electrocatalytic activity.<sup>18</sup>

Wang et al. described a DNA sensor based on nanocomposite of reduced graphene oxide (RGO) and a modified manganese (III) tetraphenylporphyrin (MnTPP). Porphyrin were used because they have a versatility that allows functionalization with reactive groups and due to the ability to incorporate many redox metals. The proposed method presented a detection limit of 6 × 10<sup>-14</sup> mol L<sup>-1</sup>.<sup>19</sup>

Therefore, the main objective of this study was to develop an electrochemical sensor based on carbon paste modified by cobalt(II) porphyrin complex immobilized on SiO<sub>2</sub>/ TiO<sub>2</sub>

obtained by the sol-gel processing method for ascorbic acid determination in pharmaceutical formulation samples.

## 2. Experimental

### 2.1. Reagents and materials

L-ascorbic acid and graphite powder were purchased from Synth<sup>®</sup> (Diadema, SP, Brazil). Anhydrous monobasic sodium phosphate and dibasic sodium phosphate were obtained from Vetec<sup>®</sup> (Rio de Janeiro, RJ, Brazil). Mineral oil, piperazine-1,4-bis[2-ethanesulfonic acid] (PIPES), Tris[hydroxymethyl]aminomethane (Trizma), 4-[2-hydroxyethyl] piperazine-1-ethanesulfonic acid (HEPES) and sodium hydroxide were acquired from Sigma-Aldrich<sup>®</sup> (St. Louis, MO, USA). Cobalt(II) chloride hexahydrate was obtained from Dinâmica<sup>®</sup> (Diadema, SP, Brazil). All solutions were prepared with distilled and deionized water (resistivity: >18 MΩ.cm<sup>1</sup>, 25°C; Millipore<sup>®</sup> Milli-Q<sup>®</sup> purification system, Bedford, MA, USA).

The modified silica (SiO<sub>2</sub>/TiO<sub>2</sub>) were synthesized by sol-gel process, after that the protoporphyrin-IX(H<sub>2</sub>PP) was immobilized on silica surface (denominated SiTiPP), according to reported procedure in the literature.<sup>20</sup>

Finally, the protoporphyrin metalation process was carried out. 0.3 g of SiTiPP were dispersed in 7.5 mL of an ethanoic solution of CoCl<sub>2</sub>.6H<sub>2</sub>O (0.2 mol L<sup>-1</sup>) and this system was constantly stirred for 19 hours followed by filtration and centrifuged. The material obtained was washed with water and ethanol and dried at 60°C for 1 hour. The material was denominated as SiTiPPCo.

### 2.2. Preparation of CPE-SiTiPPCo

The carbon paste electrode was prepared by mixing 15.00 mg graphite powder, 15.00 mg SiTiPPCo and 8.00 μL mineral oil. The materials were mixed until complete homogenization, and the paste formed was introduced into the bottom cavity of a glass tube. This cavity has a platinum plate, which is connected to a nickel/chromium wire responsible for electrical contact of electrode.

### 2.3. Apparatus

The electrochemical analysis was obtained in a potentiostat/galvanostat (PGSTAT 204). Three electrodes were used, CPE-SiTiPPCo as working electrode; an Ag/AgCl/KCl (3.0 mol L<sup>-1</sup>) electrode as reference electrode and a platinum wire as counter electrode. The materials were characterized by SEM and FT-IR. SEM images were obtained from a microscopy JEOL model JSM 300. The FT-IR spectra were recorded on Spectrum GX Perkin Elmer, by the KBr pellet technique.

### 2.4. Method validation

The validation of the developed analytical method was performed according to ANVISA standards.<sup>21</sup> Therefore, linearity, detection limit (LOD), quantification limit (LOQ), precision (repeatability and intermediate precision) and accuracy were evaluated. Analyzes were performed in triplicate by Square Wave Voltammetry (SWV) technique.

### 2.5. Preparation of pharmaceutical formulation

AA tablets (Energil C<sup>®</sup> 1.00 g) were purchased commercially. The samples were prepared from the maceration of these tablets. Then, solutions containing nominal concentrations of 15.00 μmol L<sup>-1</sup> and 45.00 μmol L<sup>-1</sup> of AA were analyzed.

## 3. Results and Discussion

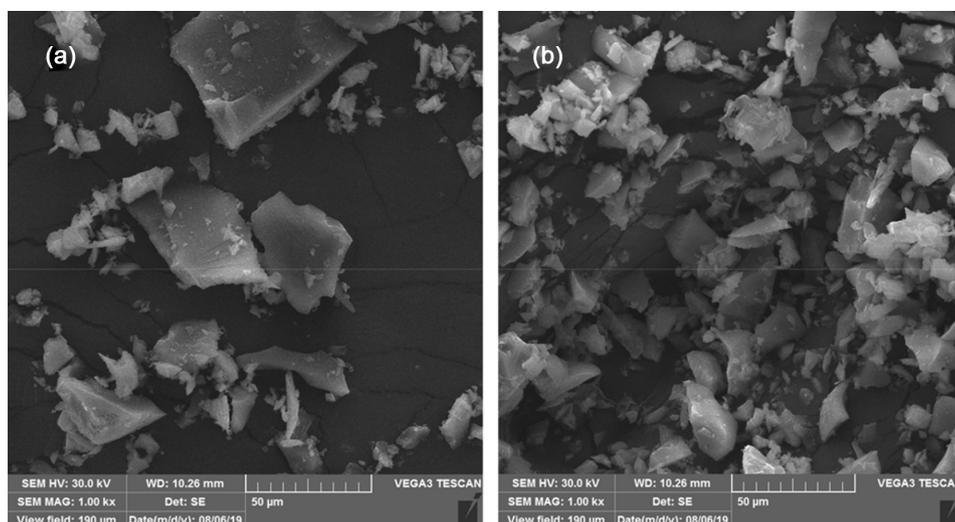
### 3.1. Characterization of materials

Figure 1 shows SEM images of the SiTiPP and SiTiPPCo with magnification 1000 times. It was possible to observe that the materials are aggregated, it may be due to the formation of hydrogen interaction between the silane groups.<sup>22</sup> The insertion of the metal in the immobilized protoporphyrin in the silica matrix does not alter the material morphology (Figure 1b).

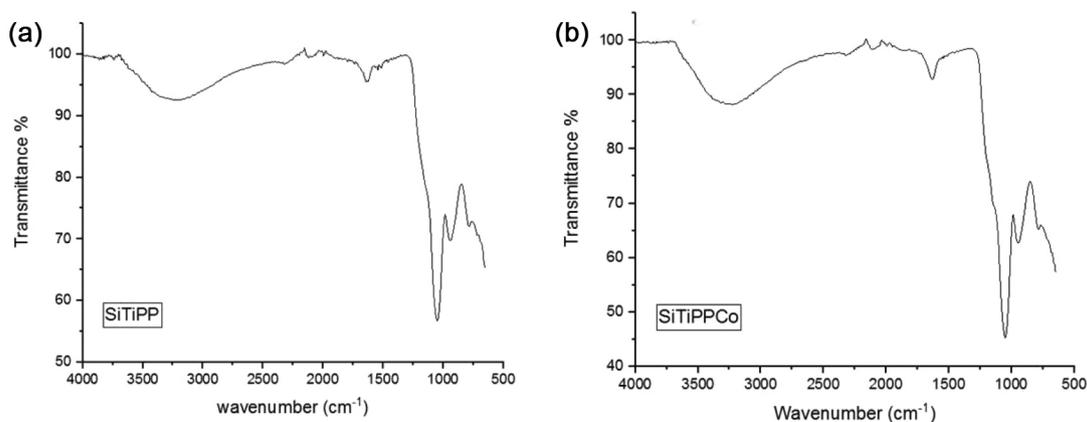
Figures 2 shows the infrared absorption spectrums of the silica samples. With the insertion of cobalt in the material, no new bands were observed. A broad band close to 3254 cm<sup>-1</sup> is observed in both spectra, characteristic of the OH bond vibrations, which may be due to the presence of silanol groups (Si-OH) and the presence of water in the sample. For both samples, an intense band close to 1049 cm<sup>-1</sup> was observed, attributed to the Si-O-Si group. Table 1 presents the observed bands.<sup>23</sup>

### 3.2. Study of working electrode configuration

Different electrode configurations were studied in the determination of AA. Figure 3 shows the cyclic voltammograms of the CPE, CPE-SiTiPP and CPE-SiTiPPCo. It was observed an irreversible oxidation peak for AA that was oxidated to dehydroascorbic acid.<sup>1</sup> The modified electrode (CPE-SiTiPPCo) showed an anodic peak current increase of 77.41% and a 0.17 V reduction in oxidation potential when compared to the bare electrode. These results may be associated with electrocatalytic activity of the SiTiPPCo. Probably occurs a metalloporphyrin interaction with AA. The modified electrode without the metal (Figure 3b) presents low intensity of oxidation current, thus showing the importance of the presence of the metal. Dinatale et al. argue that the metal is important to determine the properties such as sensitivity and selectivity of the



**Figure 1.** SEM images of (a) SiTiPP, (b) SiTiPPCo with magnification 1000 times



**Figure 2.** FT-IR spectrums of (a) SiTiPP; (b) SiTiPPCo

**Table 1.** Assignment of the bands observed in the SiTiPP and SiTiPPCo spectrums

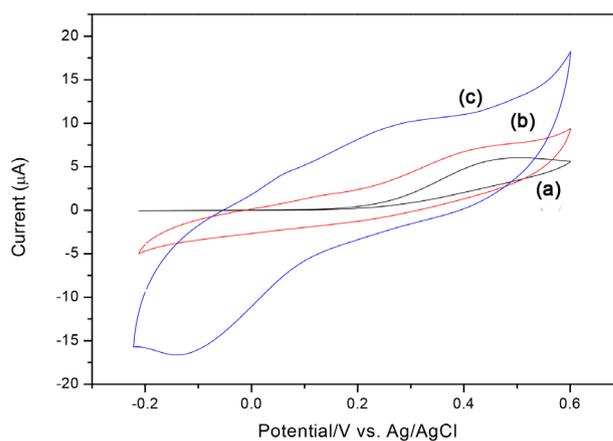
SiTiPP (cm <sup>-1</sup> )	SiTiPPCo (cm <sup>-1</sup> )	Assignment
3253	3254	OH
1641	1629	(C=C) sp <sup>2</sup>
1045	1049	Si-O-Si
930	920	Si-OH
780	778	Si-C

molecule this because coordination interactions occur at the metallic center in metalloporphyrin complexes.<sup>24</sup>

Scan rate also influences the redox process of electrode surface. A higher scan rate usually shift the peak potential to more positive regions. Thus, the scan rate of 20 mV s<sup>-1</sup> was chosen.

### 3.3. Influence of SiTiPPCo amount

Figure 4 presents the result of the effect of SiTiPPCo amount on the proposed sensor. The amount of 15.00 mg of silica was chosen as optimum due to the increase of AA oxidation current. There was a decrease in the analytical

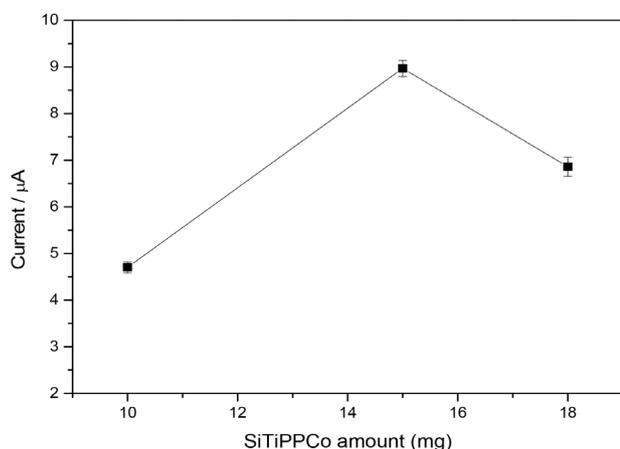


**Figure 3.** Cyclic voltammograms of 30 μmol L<sup>-1</sup> AA in 0.1 mol L<sup>-1</sup> phosphate buffer (PBS) at pH 7.0 (a) CPE, (b) CPE-SiTiPP and (c) CPE-SiTiPPCo, Scan rate: 20 mV s<sup>-1</sup>

signal with a larger amount of the modifier, this decrease is associated with a higher electrode resistivity.

### 3.4. Influence of pH

The performance of the proposed electrode was

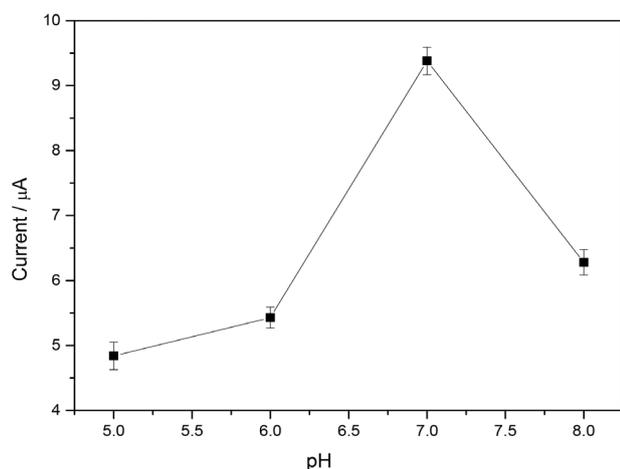


**Figure 4.** Influence of SiTiPPCo amount (mg) on anodic peak current at  $30 \mu\text{mol L}^{-1}$  AA in  $0.1 \text{ mol L}^{-1}$  PBS pH 7.0. Scan rate:  $20\text{mV s}^{-1}$

evaluated at different pH values, from 5 to 8 in phosphate buffer (PBS)  $0.1 \text{ mol L}^{-1}$ . The Figure 5 presents an increase of oxidation current with increasing pH. The pH 7 showed a higher oxidation current. Huang et al. describe in their work for determination of ascorbic acid the highest current also at pH 7.<sup>25</sup>

There was a decrease in the analytical signal at pH 8, the basic medium can affect the acid sites present in the silica (SiTiPPCo) causing chemical instability.<sup>26</sup>

Thus, the results show that for the proposed sensor the oxidation of AA is favored at pH 7, as shown previously in the literature.<sup>14, 25</sup>



**Figure 5.** Influence of the pH on potential anodic peak at  $30 \mu\text{mol L}^{-1}$  AA in  $0.1 \text{ mol L}^{-1}$  PBS Scan rate:  $20\text{mV s}^{-1}$

### 3.5. Influence of buffer solution type

The influence of different buffer solutions on the electrochemical response of the electrode was studied. The buffer solutions evaluated were phosphate, Pipes, Trizma and Hepes (pH 7,  $0.1 \text{ mol L}^{-1}$ ). Table 2 shows that the highest oxidation current was obtained in phosphate buffer. The other buffering systems possibly showed AA affinity, which led to a lower response of the analytical system.

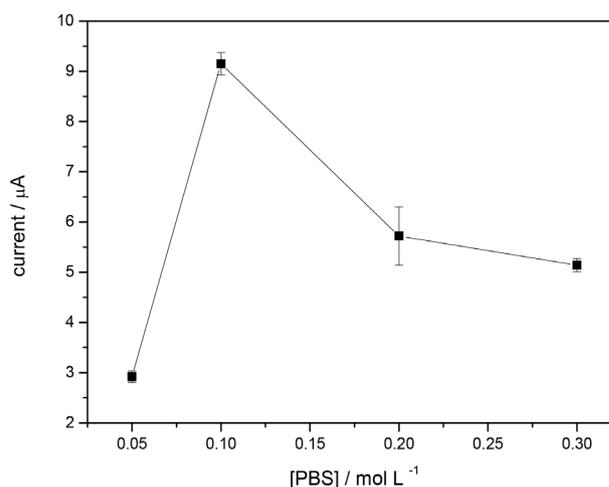
**Table 2.** Influence of different buffer solutions on the anodic peak current at  $30 \text{ mol L}^{-1}$  AA in  $0.1 \text{ mol L}^{-1}$  pH 7.0. Scan rate:  $20\text{mV s}^{-1}$ , (n=3)<sup>[a]</sup>

Buffer solution	$I_{\text{ap}} / \mu\text{A} \pm \text{RSD}$
PBS	$8.56 \pm 0.23$
Pipes	$4.52 \pm 0.17$
Trizma	$3.15 \pm 0.19$
Hepes	$1.12 \pm 0.28$

[a] n= number of determinations

### 3.6. Influence of ionic strength

Figure 6 shows the effect of different concentrations of PBS. The concentration of  $0.1 \text{ mol L}^{-1}$  presented the highest current in the oxidation of AA. At higher concentrations there was a decrease in analytical signal possibly because the excess of ions made it difficult for the analyte to access the electrode surface.



**Figure 6.** Influence of ionic strength of PBS pH 7.0 on anodic peak current at  $30 \mu\text{mol L}^{-1}$  AA. Scan rate:  $20\text{mV s}^{-1}$

### 3.7. Influence of electrochemical technique on the proposed sensor

Optimization of the electroanalytical technique is essential to obtain lower values of detection limit, quantification limit and higher sensitivity. Square Wave Voltammetry (SWV) (Amplitude 50 mV, Frequency 30 Hz) and Differential Pulse Voltammetry (DPV) (Amplitude 60 mV, Scan rate  $20 \text{ mV s}^{-1}$ ) were evaluated. As can be seen in Table 3, SWV presented higher sensitivity in determination of AA, so it was used in subsequent studies.

### 3.8. Method Validation

After the optimization of the experimental and operational parameters, it was possible to construct the analytical curve for AA as shown in Figure 7.

The Table 4 presents the analytical parameters were

**Table 3.** Influence of techniques on sensor response for AA determination. Conditions (0.1 mol L<sup>-1</sup> PBS pH 7.0), (n=3)

Electrochemical technique	Sensibility / $\mu\text{A L mol}^{-1}$
Square Wave Voltammetry (SWV)	0.20
Differential Pulse Voltammetry (DPV)	0.09

evaluated: linearity, LOD, LOQ, precision and accuracy. The relative standard deviation (%) and relative error (%) were lower than 5% as recommended.<sup>21</sup>

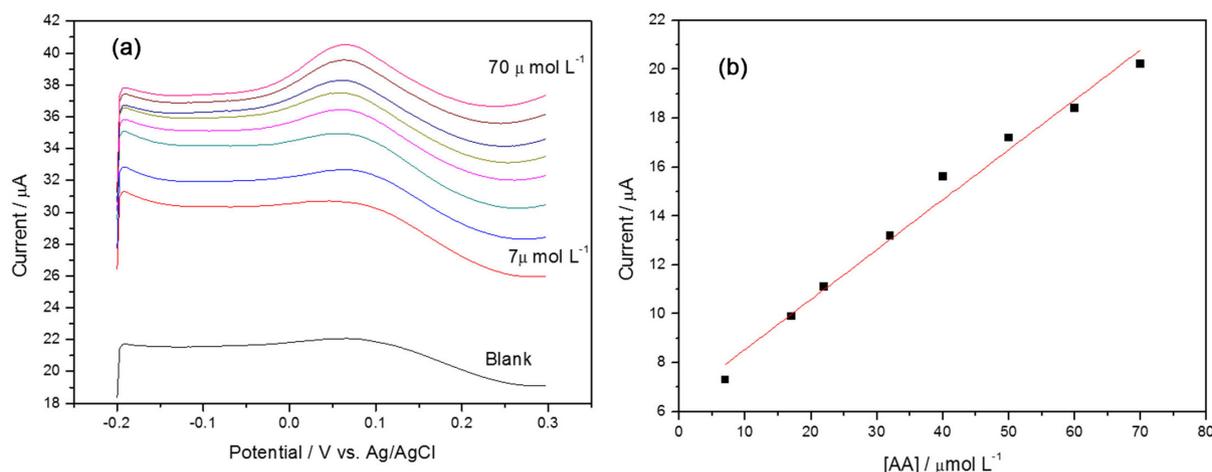
The intra-day and inter-day precision and accuracy values for AA determination using the proposed sensor were evaluated by three concentrations (low, medium and high), corresponding to 15.00  $\mu\text{mol L}^{-1}$ , 30.00  $\mu\text{mol L}^{-1}$  and 45.00  $\mu\text{mol L}^{-1}$ , respectively. The values obtained are shown in Table 5. The RSD value was below the recommended maximum limit of 5%.

**Table 4.** Parameters evaluated for the validation of the analytical method (n=3)

Parameters	
Linear Equation	$I_{\text{ap}}(\mu\text{A}) = 0,20[\text{AA}] + 6.5 \times 10^{-6}$
Coefficient correlation	0.9986
Linear range ( $\mu\text{mol L}^{-1}$ )	7.0-70.00
LOD ( $\mu\text{mol L}^{-1}$ )	1.9
LOQ ( $\mu\text{mol L}^{-1}$ )	6.4

### 3.9. Selectivity of the method

The selectivity of the developed sensor was evaluated in the presence of excipients contained in the analyzed pharmaceutical sample. The results are presented in Table 6, where it can be seen that the excipients do not interfere in the determination of AA.

**Figure 7.** (a) Voltammogram for the determination of AA concentrations obtained for 7 to 70  $\mu\text{mol L}^{-1}$ . (b) Analytical curve constructed from voltammogram (Figure 7(a)). Conditions: PBS 0.10 mol L<sup>-1</sup>, pH 7.0**Table 5.** Evaluation of the precision and accuracy of the analytical method

Parameters	Day	Concentrations ( $\mu\text{mol L}^{-1}$ )		
Nominal concentration		15.00	30.00	45.00
Within day (n <sup>[a]</sup> = 3)				
Analyzed concentration	1	15.40	30.45	45.55
	2	15.33	30.48	45.74
Precision (RSD, %) <sup>[b]</sup>	1	0.21	0.27	0.38
	2	0.19	0.24	0.35
Accuracy (RE, %) <sup>[c]</sup>	1	2.47	1.53	1.42
	2	1.17	1.48	1.26
Between day (n <sup>[d]</sup> = 2)				
Analyzed concentration		15.67	30.82	45.94
Precision (RSD, %) <sup>[b]</sup>		0.59	0.47	0.63
Accuracy (RE, %) <sup>[c]</sup>		1.55	1.48	1.39

[a] n=Number of determinations; [b] RSD (%), precision expressed as relative standard deviation percentage; [c] RE (%), accuracy expressed as relative error percentage; [d] n=Number of days.

**Table 6.** Study of the selectivity of the proposed sensor with the pharmaceutical sample excipients

Excipients	[AA] added ( $\mu\text{mol L}^{-1}$ )	[AA] found ( $\mu\text{mol L}^{-1} \pm \text{RSD}$ )	Recovery (%)
Sorbitol	30.00	29.42 ( $\pm 0.31$ )	98.07
Aspartame	30.00	30.27 ( $\pm 0.44$ )	100.90
Citric acid	30.00	30.58 ( $\pm 0.45$ )	101.93

**Table 7.** Comparison of the proposed sensor performance with other sensors from the literature

Electrode	Technique	Linear range ( $\mu\text{mol L}^{-1}$ )	LOD ( $\mu\text{mol L}^{-1}$ )	Ref.
PPy hydrogel/GCE <sup>[a]</sup>	SWV	2.5 - 1500	1.28	[13]
G-30 <sup>[b]</sup>	SWV	5.0 - 1000	17.80	[14]
poly-Trypan modified GCE <sup>[c]</sup>	DPV	1.0 - 630	0.10	[16]
sG/Pd/GCE <sup>[d]</sup>	DPV	300 - 1300	22.00	[27]
SMT/MWCNTs/PGE <sup>[e]</sup>	DPV	0.319 - 60	0.096	[28]
GPtNPs-GCE <sup>[f]</sup>	CV <sup>[g]</sup>	20.889 - 300	300	[29]
<b>CPE-SiTiPPCo</b>	<b>SWV</b>	<b>7 - 70</b>	<b>1.9</b>	<b>This work</b>

[a] modified glassy carbon electrode with polypyrrole hydrogel; [b] graphene ink coated glass; [c] modified glassy carbon electrode with poly-Trypan Blue; [d] modified glassy carbon electrode with reduced graphene oxide and palladium; [e] graphite electrode coated on sodium nanosmectite (SMT) mineral and MWCNTs (multi-walled carbon nanotubes); [f] modified glassy carbon electrode with platinum nanoparticles decorated graphene (GPtNPs) nanocomposite; [g] cyclic voltammetry.

CPE-SiTiPPCo sensor performance was compared with other sensors reported in the literature. According to Table 7, the proposed sensor presents a satisfactory performance, with a low detection limit associated with a simple preparation method and with the advantage of electrode surface renewal, which extends the use of the sensor.

### 3.10. Determination of AA in real sample

In the determination of AA, which acts as an active principle in various pharmaceutical formulations, were used Energil C<sup>®</sup> tablets. Samples containing known AA concentrations were analyzed and the results found showed high recovery percentages (Table 8), showing that the EPC-SiTiPPCo was effective in determining AA for this type of sample.

**Table 8.** Determination of AA in pharmaceutical formulation samples (n=3)

[AA] added ( $\mu\text{mol L}^{-1}$ )	[AA] found ( $\mu\text{mol L}^{-1} \pm \text{RSD}$ )	Recovery (%)
15.00	15.88 ( $\pm 0.55$ )	105.87
45.00	47.11 ( $\pm 1.12$ )	104.69

## 4. Conclusions

The proposed electrode presented excellent performance in the AA determination. The optimization of experimental and operational parameters contributed to the increase of the sensitivity.

The sensor also presented attractive features such as easy preparation, the possibility of renewal of the electrode surface and relatively low cost. The use of the sensor in the AA analysis in samples of pharmaceutical formulation without interference from other species and with high recovery rates attested to the effectiveness of the proposed method.

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