

## Electroanalytical Determination of Imipramine in Reconstituted Serum with a Graphite-Polyurethane Composite Electrode

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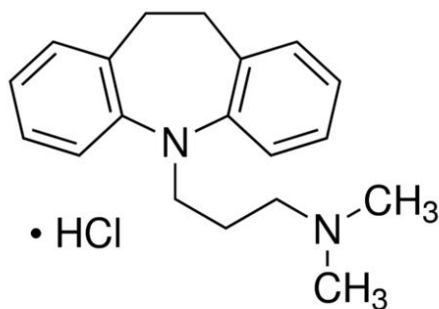
An electroanalytical procedure for the imipramine quantitation in commercial formulation and reconstituted serum was developed in Britton Robinson buffer solution, as supporting electrolyte (0.1 mol L<sup>-1</sup>, pH 7.0), with a GPU (graphite-polyurethane composite) electrode and the square wave voltammetry (SWV) technique. The obtained detection limit (LD) and the lower level of quantitation (LLOQ) were 58.3 ng L<sup>-1</sup> and 84 µg L<sup>-1</sup>, respectively. The procedure is simple, rapid, and precise for the analysis of imipramine in pharmaceutical formulation (98.9±0.13%, 24.7±0.4 mg) without the interference of the excipients. The proposal of direct analysis of imipramine in synthetic serum sample is satisfactory (90.3±3.6%, 22.6±0.9 mg, LD 26.4 µg L<sup>-1</sup>, LLOQ 100 µg L<sup>-1</sup>), considering the analysis was taken without prior extraction or other sample treatment, which can save costs and reduces the possibility of contamination caused by sample manipulation.

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**Keywords:** Imipramine, Reconstituted serum, GPU electrode, Square wave voltammetry

### 1. INTRODUCTION

Imipramine (Fig. 1) is a tricyclic antidepressant commonly used to treat both endogenous and reactive depression [1]. Spectrophotometry, atomic adsorption spectrometry, and electroanalytical methods have been commonly used to determine imipramine in pharmaceutical formulations [2-5].



**Figure 1.** Imipramine hydrochloride chemical structure.

The requirement of high selectivity and sensitivity to monitor imipramine in serum samples resulted in the utilization of chromatographic procedures with ultraviolet [6,7] and electrochemical detections [8]. Even though good sensitivity can be obtained with chromatography, the analysis is time-consuming due to the necessity of sample cleanup procedures. Therefore, electroanalytical methods are attractive when combining modified electrodes and pulse techniques because of their equivalent sensitivity with chromatography [9,10]. Additionally, the possibility of working directly with complex samples, such as biological ones, without the necessity of further purifications steps, make them an appealing choice for routine analysis.

The graphite polyurethane composite electrode (GPU) already presented good sensitivity and selectivity to quantify pharmaceutical compounds, thus becoming a potential electrode to be used in the analysis of biological and environmental relevance compounds [11,12].

This work intended to develop an electroanalytical procedure for the determination of imipramine in pharmaceutical formulation using the GPU electrode with the SWV technique. An imipramine assay in reconstituted serum sample was performed in the presence of its metabolite desipramine to evaluate the application of the electroanalytical procedure in biological samples without previous treatment.

## 2. MATERIALS AND METHODS

### 2.1. Chemicals

Imipramine and desipramine were obtained from Sigma ( $\geq 99\%$ ). All the other reagents were of analytical grade and were used as received. The  $0.1 \text{ mol L}^{-1}$  Britton-Robinson (BR) buffer solution was prepared as follows: boric acid ( $4.0 \times 10^{-2} \text{ mol L}^{-1}$ ), acetic glacial acid ( $4.0 \times 10^{-2} \text{ mol L}^{-1}$ ), phosphoric acid ( $4.0 \times 10^{-2} \text{ mol L}^{-1}$ ) and sodium perchlorate ( $0.1 \text{ mol L}^{-1}$ ). Sodium hydroxide ( $0.1 \text{ mol L}^{-1}$ ) was used to adjust the solutions pH. Milli-Q system from Millipore® was used to prepare all the solutions.

Acetonitrile and ammonium acetate (all HPLC grade) were obtained from Mallinckrodt Baker. Stock solutions of imipramine and desipramine were prepared in acetonitrile and were protected from light.

## 2.2. Preparation of reconstituted serum sample

The reconstituted serum sample was prepared in accordance with Parham & Zargar [13], by mixing alanine ( $4.1 \times 10^{-4} \text{ mol L}^{-1}$ ), arginine ( $2.1 \times 10^{-4} \text{ mol L}^{-1}$ ), aspartic acid ( $8.8 \times 10^{-4} \text{ mol L}^{-1}$ ), cysteine ( $5.1 \times 10^{-5} \text{ mol L}^{-1}$ ), glycine ( $1.4 \times 10^{-4} \text{ mol L}^{-1}$ ), histidine ( $1.2 \times 10^{-4} \text{ mol L}^{-1}$ ), lysine ( $2.0 \times 10^{-4} \text{ mol L}^{-1}$ ), phenylalaline ( $1.6 \times 10^{-4} \text{ mol L}^{-1}$ ), serine ( $1.2 \times 10^{-4} \text{ mol L}^{-1}$ ), tyrosine ( $8.1 \times 10^{-5} \text{ mol L}^{-1}$ ), tryptophan ( $6.9 \times 10^{-5} \text{ mol L}^{-1}$ ),  $\text{NaHCO}_3$  ( $7.9 \times 10^{-3} \text{ mol L}^{-1}$ ), and  $\text{NaCl}$  ( $8.7 \times 10^{-2} \text{ mol L}^{-1}$ ). All the concentrations used to prepare the reconstituted serum sample were selected according to their level in the human serum [13].

## 2.3. Preparation of pharmaceutical

One tablet of pharmaceutical (25 mg) was triturated in an agate mortar, pounded and dissolved in 25 mL of anhydrous ethanol ( $3.2 \times 10^{-5} \text{ mol L}^{-1}$  of imipramine hydrochloride). The flask was sonicated (iSonicl®) for 10 min for the complete imipramine dissolution.

All concentrations used for imipramine recovery in pharmaceutical formulations were adjusted to the analytical curve intervals.

## 2.4. Instrumentation

The electroanalytical measurements were done utilizing a Polarography analyzer PAR model 174 A under the optimized conditions of frequency ( $f = 100 \text{ s}^{-1}$ ), pulse amplitude ( $a = 50 \text{ mV}$ ), and scan increment ( $\Delta E_s = 2 \text{ mV}$ ).

The electrodes (GPU, the reference,  $\text{Ag}/\text{AgCl}_{(s)} - 3.0 \text{ mol L}^{-1} \text{ KCl}$ , and the platinum foil,  $A = 1.0 \text{ cm}^2$ ) were inserted into a 10 mL Pyrex® glass cell. The GPU electrode was fabricated by the mixture of graphite and polyurethane resin (60:40, w/w) and the details of its preparation can be found elsewhere [14].

The HPLC measurements were performed on a Shimadzu HPLC system (model CL-10 AVP) with a spectrophotometric UV/Vis detection (SPD-10Avp) and an oven CTO-10Asvp. The column used was a Zorbax Eclipse XDBC18 (2.1x150 mm; I.D., 5  $\mu\text{m}$ ). Imipramine was analyzed using isocratic conditions. The mobile phase consisted of acetonitrile/0.10 mol L<sup>-1</sup> ammonium acetate buffer (70:30, v/v, pH 5.0), which was filtered before using a vacuum system equipped with a 0.2  $\mu\text{m}$  filter membrane (Sartorius AG – 37070). The flow rate and injection loop were 1.0 mL min<sup>-1</sup> and 20  $\mu\text{L}$ , respectively. The column was operated at 40°C and the detection was monitored for UV absorption at 220 nm.

## 2.5. Analytical applications

The electroanalytical methodology to determine imipramine was developed by the optimization of SWV instrumental parameters (frequency, amplitude, and scan increment) using the reversible dimer peak ( $E_p = 0.04 \text{ V vs. } E_{\text{Ag}/\text{AgCl}}$ ) formed after the irreversible oxidation of imipramine ( $E_p = 0.83$

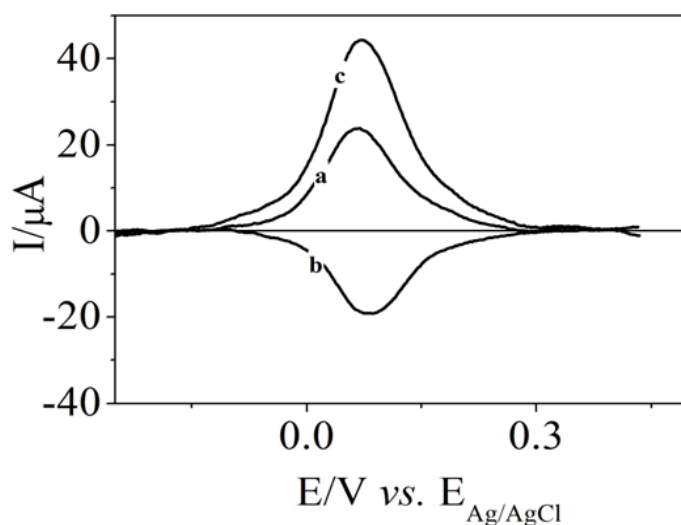
V vs.  $E_{\text{Ag}/\text{AgCl}}$ ). The dimer peak was selected due to the higher sensitivity combined with the lower overvoltage (0.79 V vs.  $E_{\text{Ag}/\text{AgCl}}$ ) for the imipramine electroanalysis. The voltamograms were obtained by scanning the potentials from -0.5 V to 1.1 V and the intensity of the current peaks was monitored at 0.04 V vs.  $E_{\text{Ag}/\text{AgCl}}$ .

The analytical curves were obtained by the standard addition method by spiking 100  $\mu\text{L}$  of a  $3.0 \times 10^{-5} \text{ mol L}^{-1}$  standard solution of imipramine into a 10 mL volume of BR buffer (pH 7.0). The detection limit (DL) was determined from experiments parameters by the equation  $\text{DL} = 3\sigma/\theta$ , as  $\sigma$  the standard deviation of y-intercepts and  $\theta$  the slope of the analytical curves. The lower level of quantitation (LLOQ) was the lowest standard concentration level from the analytical curves [15]. The precision was calculated by the relative standard deviation (RSD). It was checked on different days ( $n = 10$ , intraday) and between days ( $d = 6$ , interday) for  $3.6 \times 10^{-7} \text{ mol L}^{-1}$  imipramine solution. The accuracy and reproducibility was checked by the pharmaceutical formulation recovery experiments. An investigation about the interference of ions or organics compounds commonly found in serum fluid was also carried out.

The analytical reference method chosen to evaluate the validity of the developed electroanalytical methodology was HPLC.

### 3. RESULTS AND DISCUSSION

The oxidation reaction mechanism for imipramine was recently confirmed by Sanghavi & Srivastava [5]. Imipramine is irreversible oxidized ( $E_p = 0.83 \text{ V vs. } E_{\text{Ag}/\text{AgCl}}$ ) and the process is controlled by reagent adsorption with the formation of a dimeric molecule ( $E_p = 0.04 \text{ V vs. } E_{\text{Ag}/\text{AgCl}}$ ) in a chemical step after the loss of 2 electrons and 1 proton per imipramine molecule.



**Figure 2.** SWV curves for the dimer process ( $2.3 \times 10^{-5} \text{ mol L}^{-1}$  imipramine) in BR buffer solution (pH 7.0,  $0.10 \text{ mol L}^{-1}$ ) at GPU electrode. (a) Forward current; (b) Backward current; (c) Net current.  $f = 100 \text{ s}^{-1}$ ,  $a = 50 \text{ mV}$  and  $\Delta E_s = 2 \text{ mV}$ .

Fig. 2 shows the forward (a), backward (b), and (c) net currents for the dimer process. The current in SWV is measured twice at the end of each half cycle, which makes SWV the most sensitive electroanalytical technique. The dimer process is characterized as a reversible system and the net current was used for the development of the electroanalytical procedure due to its high sensitivity (additional contribution of the backward current component) and low overpotential comparing to the imipramine oxidation peak. The details about imipramine oxidation and dimer formation can be found elsewhere [16].

### 3.1. Optimization of experimental parameters

The effect of pH on the oxidation peak of dimer was investigated from 2 to 10. The maximum analytical signal was obtained at pH 7.0 (data not shown), which was chosen for the development of the analytical methodology.

The SWV frequency ( $f$ ) for the dimer process was evaluated from 10 to 100 s<sup>-1</sup>. The graph  $I_{pa}$  and  $f^{1/2}$  presented good linearity ( $R = 0.999$ ), described by the linear equation  $I_{pa}(\mu A) = (-4.4 \pm 0.3) + (3.9 \pm 0.1) \times f^{1/2}$ , proves the reversible nature of the process as well as the species adsorption on the electrode surface [17]. Since the voltammograms present deformity (data not shown) when frequencies were higher than 100 s<sup>-1</sup>, a value of 100 s<sup>-1</sup> was chosen.

Pulse amplitude ( $a$ ) and scan increment ( $\Delta E_s$ ) were also optimized the best values were 50 mV and 2 mV, respectively.

### 3.2. Development of the electroanalytical procedure

The imipramine determination was performed using the optimized conditions (pH 7.0,  $f = 100$  s<sup>-1</sup>,  $a = 50$  mV and  $\Delta E_s = 2$  mV) in the interval of  $3.0 \times 10^{-7}$  to  $22.8 \times 10^{-7}$  mol L<sup>-1</sup>. The analytical curve linear regression equation was  $I_p (1 \times 10^{-6} A) = (1.7 \times 10^{-6} \pm 1.2 \times 10^{-9}) + (17.2 \pm 0.1) \times C_{IMI} (1.0 \times 10^{-7} \text{ mol L}^{-1})$ ,  $R = 0.999$ . A detection limit (DL) of  $2.1 \times 10^{-10}$  mol L<sup>-1</sup> (58.7 ng L<sup>-1</sup>) and the lower level of quantitation (LLOQ) of  $3.0 \times 10^{-7}$  mol L<sup>-1</sup> (84  $\mu\text{g L}^{-1}$ ) was obtained. The precision ( $n = 10$ ) was determined in one concentration level ( $3.6 \times 10^{-7}$  mol L<sup>-1</sup>) and for repeatability (intraday), the relative standard deviation (RSD) was 2.1%. For intermediate precision (interday), the RSD was 2.7%.

The recovery of imipramine in pharmaceutical tablets ( $98.9 \pm 1.3\%$ ,  $24.7 \pm 0.4$  mg) was done by the standard addition method ( $n = 5$ ) and was in conformity with the manufacturer information (25 mg). The accuracy was checked by the relative error (Bias = 1.8%) in recovery experiments and was within acceptable limits.

To find out the developed electroanalytical methodology can be employed for the routine analysis of imipramine, the HPLC technique was used as the standard procedure. The analytical curves were also constructed by standard the addition method in the equivalent concentration interval used for the SWV ( $2.9 \times 10^{-7}$  to  $41.4 \times 10^{-7}$  mol L<sup>-1</sup>). The DL ( $2.2 \times 10^{-8}$  mol L<sup>-1</sup> or 6.2  $\mu\text{g L}^{-1}$ ) was also calculated using the same equation described above and the LLOQ ( $2.9 \times 10^{-7}$  mol L<sup>-1</sup> or 81.3  $\mu\text{g L}^{-1}$ ) was the lowest standard concentration in the analytical curve. The repeatability (intraday) and

intermediate precision (interday) were 2.4% and 4.6%, respectively, for the same concentration level used in the electroanalytical methodology.

The mean recovery was  $95.1 \pm 2.9\%$  ( $23.7 \pm 0.7$  mg), with Bias of 2.3%. These results indicate both methods have adequate precision/accuracy and the electroanalytical procedure can consequently be used for the imipramine quantitation in pharmaceutical tablets. The analytical characteristics of both methods are summarized in Table 1.

**Table 1.** Parameters of the SWV/GPU and the HPLC methodologies

Parameters	GPU/SWV	HPLC
Linear concentration range ( $10^{-7}$ mol L <sup>-1</sup> )	3.0-22.8	2.9-41.4
Correlation coefficient (R)	0.999	0.999
DL ( $\mu\text{g L}^{-1}$ )	$5.8 \times 10^{-2}$	6.2
LLOQ ( $\mu\text{g L}^{-1}$ )	84	81.3
Repeatability (RSD)	2.1%	2.7%
Intermediate precision (RSD)	2.4%	4.6%
Mean recovery	$98.9 \pm 1.3\%$	$95.1 \pm 2.9\%$
Bias	1.8%	2.3%

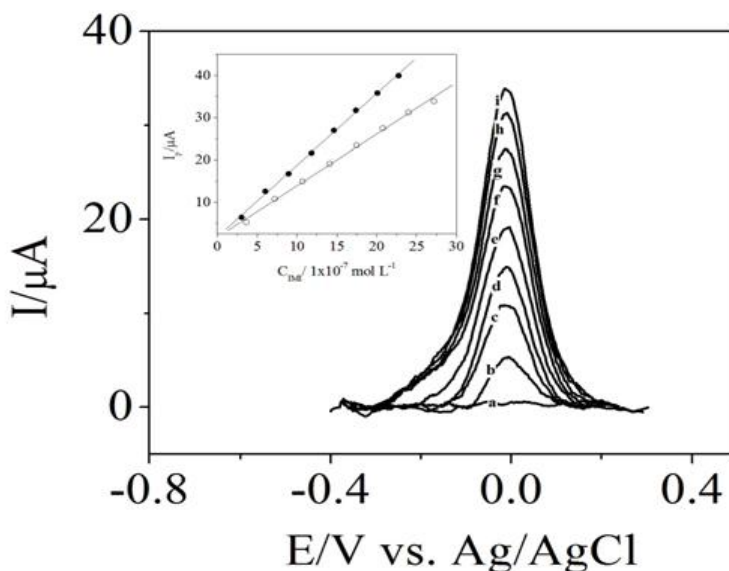
### 3.3. Imipramine assay in reconstituted serum sample

The electroanalytical methodology was also used to quantify imipramine in biological samples without previous treatment. No interference was observed for glycine (214), alanine (282), arginine (321), histidine (189), lysine (317), phenylalanine (290), serine (345), tyrosine (123) and tryptophan (180) (the number in parenthesis is the molar ratio of the compound presented in the serum sample in relation to  $6.5 \times 10^{-7}$  mol L<sup>-1</sup> of imipramine). Aspartic acid (71) and cysteine (60) probable interact with GPU surface by blocking the electrode surface active sites.

The analytical curves for imipramine were obtained in the range of  $3.6 \times 10^{-7}$  mol L<sup>-1</sup> to  $27.1 \times 10^{-7}$  mol L<sup>-1</sup> (Fig. 3). The linear regression equation was  $I_p$  ( $1 \times 10^{-6}$  A) =  $(1.9 \times 10^{-6} \pm 3.8 \times 10^{-7}) + (12.1 \pm 0.3) \times C_{\text{IMI}}$  ( $1.0 \times 10^{-7}$  mol L<sup>-1</sup>),  $R = 0.998$ .

As shown in the slopes of the analytical curve in pure electrolyte (16.9) and in serum sample (12.2), there is a loss in the sensitivity of the method applied to the reconstituted biological sample caused by the adsorption of some serum constituents with negative charge density (aspartic acid and cysteine). The DL ( $26.4 \mu\text{g L}^{-1}$ ) and the LLOQ ( $100 \mu\text{g L}^{-1}$ ) were also calculated. The QL achieved in

this matrix is still in the interval of the plasmatic therapeutically range of imipramine (75 to 250  $\mu\text{g L}^{-1}$ ) found in human serum [18].



**Figure 3.** SWV curves for different imipramine concentrations in reconstituted serum. (a) blank, (b) 3.6, (c) 7.1, (d) 10.2, (e) 14.1, (f) 17.4, (g) 20.7, (h) 23.9 and (i) 27.1  $\times 10^{-7}$  mol L<sup>-1</sup>;  $f = 100$  s<sup>-1</sup>,  $a = 50$  mV and  $\Delta E_s = 2$  mV. Insert: analytical curves: (●) 0.1 mol L<sup>-1</sup> BR buffer solution (pH 7.0, R = 0.999) and (□) reconstituted serum (pH 7.6, R = 0.998).

According to Kirchherr and Kühn-Velten, the therapeutic monitoring of antidepressants is necessary to avoid medical complications, intoxication, and noncompliance [19]. The developed electroanalytical methodology could be an alternative to the monitoring of imipramine in serum samples due its simplicity and the no requirement of sample pretreatment.

The recoveries of imipramine in serum samples were carried out ( $n = 5$ ) within the usual drug therapeutically concentrations range. The mean recovery ( $n = 5$ ) is  $90.3 \pm 3.6\%$  ( $22.6 \pm 0.9$  mg), with a Bias of 4.1%, which was acceptable when considering that the analysis was taken without prior extraction or other sample treatment.

Repeatability and intermediate precision ( $n = 10$ ) RSD were 1.9% and 3.2%, respectively, for a  $6.2 \times 10^{-7}$  mol L<sup>-1</sup> imipramine solution.

**Table 2** Comparison between the proposed electroanalytical methodology (GPU/SWV) and other ones already published in the literature for the quantitation of imipramine in serum samples.

Electrode	Technique	pH	Linear interval ( $\mu\text{mol L}^{-1}$ )	LD ( $\mu\text{g L}^{-1}$ )	Recovery (%)
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Nanoclay composite					
carbon ionic liquid electrode [20]	CV	7.2	0.1-2	$1.0 \times 10^{-2}$	94% (tablet) 104% (blood serum)
XAD2-TPN-GCPE [5]	AdSDPV	6.0	0.001-6.2	0.1	98.8±0.3 (tablet) 99±0.8 (urine) 99.1±0.2 (blood serum)
Glassy carbon electrode [21]	DPV	8.2	0.02-0.3	2.6	68±8.9 (blood serum)
Graphite- polyurethane composite electrode (this work)	SWV	7	0.3-2.3	$5.8 \times 10^{-2}$	97.6±0.9 (tablet) 90.3±3.6 (synthetic blood serum)

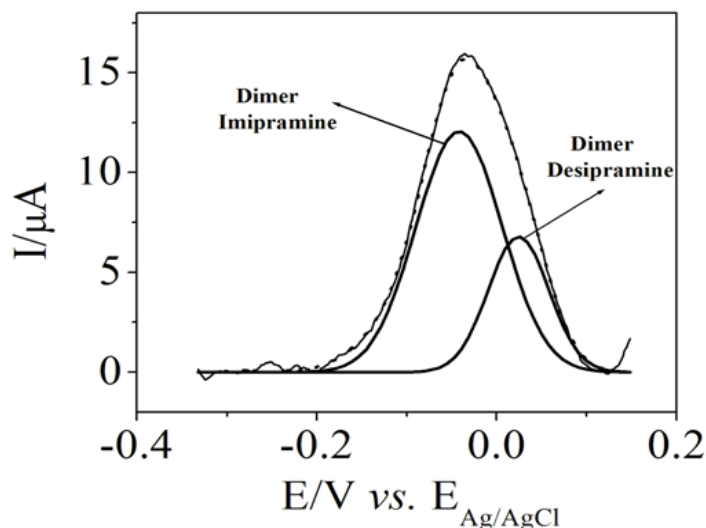
Table 2 shows the comparison between the proposed electroanalytical methodology (GPU/SWV) and other ones already published for the quantitation of imipramine in serum samples [5,20,21]. The detection limit of the GPU/SWV methodology is within the levels found by Eslami and coworkers [21] and also by Sanghavi and Srivastava [5]. This methodology can be successfully used in routine analysis to quantify imipramine in tablets. In addition, the developed methodology can be also used in synthetic serum sample without serious interference from other components of the reconstituted fluid. This procedure has the advantage to work without any fractionally pretreatment or necessity of reagent addition, which can save costs and reduces the possibility of contamination caused by sample manipulation.

### 3.4. Proposal of simultaneous analysis of imipramine/desipramine

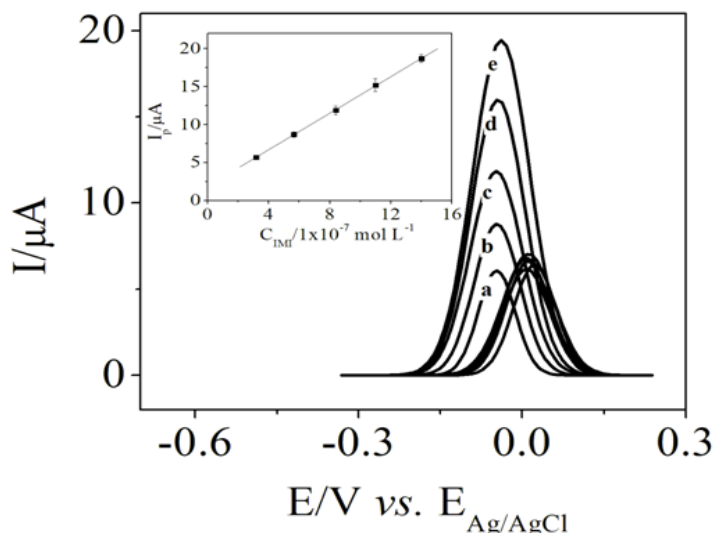
Imipramine was analyzed simultaneously with the metabolite desipramine in reconstituted serum sample with the expected therapeutic concentrations for both substances in human serum ( $75$  to  $250 \mu\text{g L}^{-1}$  for imipramine and  $175 \mu\text{g L}^{-1}$  for desipramine) [18] to study the selectivity of the GPU electrode. Fig. 4 shows the square wave voltammogram for a mixture of imipramine ( $175 \mu\text{g L}^{-1}$ ) and desipramine ( $170 \mu\text{g L}^{-1}$ ).

The voltammogram of the mixture showed only one peak with a broad profile, revealing the oxidation peaks of the two compounds are overlapped. A loss of selectivity was expected, since imipramine and desipramine have similar chemical structures and the same electroactive site (nitrogen ring).





**Figure 4.** SWV curves for a mixture of imipramine ( $175 \mu\text{g L}^{-1}$ ) and desipramine ( $170 \mu\text{g L}^{-1}$ ) in reconstituted serum (pH 7.6). (—) Experimental curve; (---) Curve obtained after deconvolution and the deconvoluted peaks for imipramine and desipramine dimers.  $f = 100 \text{ s}^{-1}$ ,  $a = 50 \text{ mV}$  and  $\Delta E_s = 2 \text{ mV}$ .



**Figure 5.** Deconvoluted SWV curves for different imipramine concentrations in reconstituted serum in the presence of desipramine ( $5.2 \times 10^{-7} \text{ mol L}^{-1}$ ). (a) 3.8, (b) 5.7, (c) 8.4, (d) 11.1, and (e)  $15.6 \times 10^{-7} \text{ mol L}^{-1}$ ;  $f = 100 \text{ s}^{-1}$ ,  $a = 50 \text{ mV}$  and  $\Delta E_s = 2 \text{ mV}$ . Insert: analytical curve: (■) reconstituted serum (pH 7.6,  $R = 0.998$ ).

The oxidation peaks were separated by the deconvolution process using Origin® software 6.0 curve-fitting method. The resolution of overlapped peaks is crucial for voltammetry methods when compounds with close properties are analysed [22,23]. The mathematical treatment was evaluated for the determination of imipramine in the presence of desipramine. The peak deconvolution procedure separated imipramine dimer oxidation peak ( $E_p = -41 \text{ mV}$ ) from desipramine one ( $E_p = 23 \text{ mV}$ )

effectively and accurately. The imipramine analytical curves ( $n = 3$ ) were constructed in the interval of  $3.8 \times 10^{-7} \text{ mol L}^{-1}$  to  $15.9 \times 10^{-7} \text{ mol L}^{-1}$  in reconstituted serum (Fig. 5).

The linear regression equation was  $I_p (1 \times 10^{-6} \text{ A}) = (1.8 \times 10^{-6} \pm 3.9 \times 10^{-7}) + (12.0 \pm 0.1) \times C_{\text{IMI}}$  ( $1.0 \times 10^{-7} \text{ mol L}^{-1}$ ),  $R = 0.999$ . The DL ( $27.3 \mu\text{g L}^{-1}$ ) and the LLOQ ( $106 \mu\text{g L}^{-1}$ ) were also calculated. The direct electroanalysis of imipramine in reconstituted serum sample with its metabolite desipramine was possible without serious interference of matrix constituents. The developed electroanalytical method can be an alternative procedure for the routine analysis of plasmatic therapeutically concentrations of imipramine.

#### 4. CONCLUSIONS

The obtained results show the above-described electroanalytical procedure is useful for the imipramine determination in pharmaceutical formulations. The principal advantage is the possibility to work without any pre-treatment, since there was no interference from excipients of the pharmaceutical preparation. This procedure is simple, rapid, precise, and easy to use for the direct quantitation of imipramine. The proposal of direct analysis of imipramine in reconstituted serum sample is satisfactory, since there is no serious interference from other components of the reconstituted fluid.

The selectivity of the methodology should be improved for the analysis of imipramine and desipramine. Studies are in course to develop a preparative column for the separation of both compounds and the subsequent detection using SWV/GPU methodology.

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#### References

1. M. Bourin and G. B. Baker, *Biomed. Pharmacother.*, 50 (1996) 7
2. E. M. Elnemma, F. M. El Zawawy and S. A. M Hassan, *Microchim. Acta*, 110 (1993) 79
3. I. Biryol, B. Uslu and Z. Küçükyavuz, *Z. J. Pharm. Biomed. Anal.*, 15 (1996) 371
4. G. M. Greenway and S. J. L. Dolman, *Analyst*, 124 (1999) 759
5. B. J. Sanghavi and A. K. Srivastava, *Analyst*, 138 (2013) 1395
6. C. Frahnert, M. L. Rao and K. Grasmader, *J. Chromatogr. B*, 794 (2003) 35
7. R. Theurillat and W. Thormann, *J. Pharm. Biomed. Anal.*, 18 (1998) 751
8. A. Chmielewska, L. Konieczna, A. Plenis and H. Lamparczy, *J. Chromatogr. B*, 839 (2006) 102
9. M. Zidan, R. M. Zawawi, M. Erhayem and A. Salhin, *Int. J. Electrochem. Sci.*, 9 (2014) 7605
10. S. M. Wang, W. Y. Su and S. H. Cheng, *Int. J. Electrochem. Sci.*, 5 (2010) 1649
11. G. B. Soares, W. T. L. Da Silva and C. M. P. Vaz, *Sensor Lett.*, 9 (2011) 1786
12. F. S. Semaan, E. M. Pinto, E. T. G. Cavalheiro and C. M. A. Brett, *Electroanalysis*, 20 (2008) 2287.
13. H. Parham and B. Zargar, *Talanta*, 55 (2001) 255

14. R. K. Mendes, S. Claro Neto and E. T. G. Cavalheiro, *Talanta*, 257 (2002) 909
15. US Environmental Protection Agency,  
<http://www.epa.gov/osa/fem/pdfs/MthDetQuant-guide-ref-final-October2010.pdf> Accessed on 21 May 2015
16. R. A. de Toledo, M. C. Santos, K. M. Honório, A. B. F. da Silva, E. T. G. Cavalheiro and L. H. Mazo, *Anal. Lett.*, 39 (2006) 507
17. M. Lovric and S. Komorsky-Lovric, *J. Electroanal. Chem.*, 248 (1988) 239
18. M. D. Cantú, S. Hillebrand, M. E. C. Queiroz, F. M. Lanças and E. Carrilho, *J. Chromatogr. B*, 799 (2004) 127
19. H. Kirchherr and W. N. Kühn-Velten, *J. Chromatogr. B*, 843 (2006) 100
20. T. Galeano-Díaz, M. I. Acedo Valenzuela, N. Mora-Diez and A. Silva Rodríguez, *Electroanalysis*, 23 (2011) 449
21. E. Eslami, F. Farjami, P. Aberoomand Azar and M. Saber Tehrani, *Electroanalysis*, 26 (2014) 424
22. S.V. Romanenko, A.G. Stromberg, E.V. Selivanova, E.S. Romanenko, *Chemometr. Intell. Lab. Syst*, 73 (2004) 7
23. V.A. Pedrosa, S.A.S. Machado, L.A. Avaca, *Anal. Lett.*, 39 (2007) 1955

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