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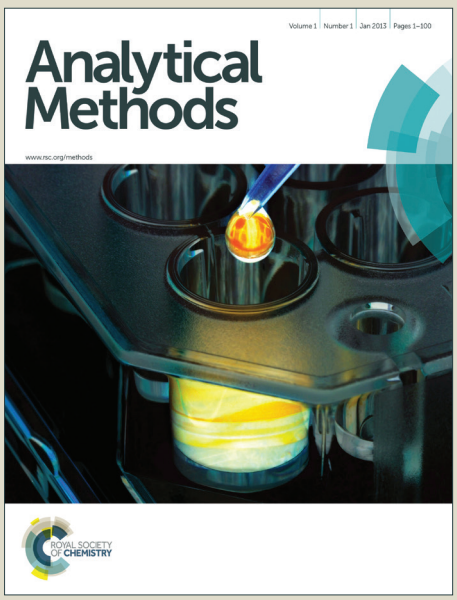

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Do voltammetry electrodes modified with MIPs really work? The role of large molecules: folic acid as a probe

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ABSTRACT

There are many uncertainties concerning the use of molecularly imprinted polymers (MIPs) as electrode modifiers. In order to contribute to clarification of this issue, a large target molecule was used to prepare an MIP-modified composite electrode. The MIP was synthesized using folic acid (FA) as a template, and employed in the modification of composite electrodes based on graphite and polyurethane (GPU), varying its percentage from 2.5 to 10%. Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were used to compare the performance of modified and unmodified electrodes. After optimization of the electrode composition, pH, and the electrolytic medium, in which the best results were obtained using 2.5% MIP, pH 4.5 and acetate buffer, respectively, differential pulse voltammograms were used to obtain analytical curves for electrodes modified with the 2.5% MIP or 2.5% non-imprinted polymer (NIP), as well as for the unmodified GPU electrode. Although the sensitivities were similar in all cases, the electrode modified with 2.5% MIP presented a lower limit of detection (LOD) of 0.17 μmol L^{-1} and 0.034 μmol L^{-1} for the catodic peaks in -0.52 and -0.58 V *vs.* SCE, respectively, under specific conditions, allowing the determination of FA in commercial pharmaceutical formulations with results that were in agreement with the official HPLC method. Finally, evaluation was made of the interference of a structurally similar molecule, the methotrexate, as well as small molecules containing functional groups similar to those present in the structure of FA, such as uric acid, ascorbic acid and dopamine. The 2.5% MIP electrode was more selective than 2.5% NIP electrode.

Keywords: DPV, graphite-polyurethane composite electrode, MIP, folic acid.

INTRODUCTION

The molecular imprinting approach is claimed to have several advantages over other procedures used to prepare synthetic receptors[1], with the resulting material showing chemical, thermal, and mechanical stability, robustness, and affinity and selectivity for the imprinted analyte similar to that of natural receptors[2,3].

The concept of molecular imprinting originated with Pauling's theory of antibody formation, where an antigen molecule is used as a template for shaping the antibody polypeptide chain[4]. From this concept emerged the idea of developing three-dimensional rigid structures, produced using a polymer around an analyte as the template molecule, capable of performing molecular recognition during analytical procedures. These biomimetic materials are known as molecularly imprinted polymers (MIPs)[5,6].

MIPs are widely used in analytical chemistry and can be applied to a wide range of analytes, such as small organic molecules including drugs, pesticides, amino acids, and sugars. However, the selective application of MIPs to larger organic compounds stills remains a challenge in chemical analysis[7].

The use of molecular imprinting on the surface of a substrate offers a solution to problems of accessibility and mass transfer of large molecules. In addition, surface molecular imprinting could provide better control of the orientation and density of binding sites, resulting in greater speed and accuracy of analyte detection. Meanwhile, a generic protocol for molecular imprinting has not yet been established, and adaptation of protocols is required for each specific analyte.

Applications of MIPs include their use in association with analytical techniques such as HPLC[8], surface plasmon resonance spectroscopy (SPR)[9], fluorescence[10], solid phase extraction (SPE)[11], and solid phase microextraction[12]. MIPs are also employed to prepare sensors used in voltammetry[13-15] and amperometry[16,17].

Folic acid (FA) is an example of a relatively large compound that is of pharmaceutical interest (Figure 1). This vitamin is used in the treatment of macrocytic and megaloblastic anemia, where it stimulates the production of red and white blood cells and platelets. FA is essential for the synthesis of nucleoprotein and is converted to the metabolite tetrahydrofolic acid in the presence of ascorbic acid[18].

FIGURE 1

A range of techniques can be used to determine FA, including ion chromatography with electrochemical detection[19], microemulsion electrokinetic chromatography[20], HPLC[21,22], flow injection analysis[23], LC-MS-MS[24], and capillary electrophoresis[25] with chemiluminescence detection[26].

Regarding voltammetric determination of FA one can find the reports of carbon paste electrodes modified with calixarene[27], palmitic and stearic acids[28], 2,2′-[1,2-ethanediylbis(nitriloethylidyne)]-bis-hydroquinone double-wall carbon nanotube[29]; glassy carbon with single-wall carbon nanotube film[30], single-walled carbon nanotube-ionic liquid[31], lead film[32], phosphomolybdic-polypyrrole film[33] as well as 2-mercaptobenzothiazol self assembled monolayer[34] and multi-walled carbon nanotubes[35] modified gold electrodes that had also been used. Mercury[36,37] and HMDE[38] electrodes were applied in such determination, but after pre-concentration steps.

However, only two studies have been described for voltammetric measurement of FA using MIP-modified electrodes, both in differential pulse cathodic stripping voltammetry. In the first one, Prasad *et. al* determined FA using a molecular imprinted polymer immobilized sol-gel-modified pencil graphite electrode[39] and in the second work, an imprinted polymer-carbon consolidated composite fiber sensor[40] was used.

The present study describes the preparation of a MIP using a polymethacrylate matrix containing FA, and its use in the bulk modification of a graphite/polyurethane composite electrode (GPU). The modified electrode was then used for the determination of FA in pharmaceutical formulations.

The objectives were to demonstrate that MIP modified electrodes are valuable for quantitative voltammetric determinations, in the present case for a large molecule as FA. In addition we would like to demonstrate that selectivity of such devices is strongly dependent on the size of the template molecule regarding the number, nature and disposition of functional groups present in its structure.

EXPERIMENTAL

Apparatus

The voltammetric measurements were performed using a μAUTOLAB type III potentiostat/galvanostat (Ecochemie) coupled to a microcomputer and controlled by GPES 4.9 (Ecochemie) software. All measurements were performed in a glass cell with a total capacity of 25 mL.

Reagents and solutions

All reagents were analytical grade and were used without further purification. The reagents used in the synthesis of the MIP were acrylic acid (AA, Aldrich), hydroquinone (HQ, Vetec), benzoyl chloride (BC, Aldrich), melamine (MEL, Aldrich), acryloyl chloride (AC), and 2,4,6-trisacrylamide-1,3,5-triazine (TAT) monomer, synthesized as described below.

The other reagents used were dimethylformamide (DMF, Vetec), azobisisobutyronitrile (AIBN, Acros Organics), dimethylsulfoxide (DMSO, Vetec), ethyleneglycol dimethacrylate (EGDMA, Polysciencis), triethanolamine (TEA, Vetec), and acetonitrile (ACN, Vetec).

The FA solution (Sigma-Aldrich) was prepared daily by dissolving the chemical in phosphate and acetate buffer solutions at different pHs. Commercial products containing folic acid, Afopic® (Teuto Brasileiro S/A), Folifolin® (EMS), and Folacin® (Arese) were purchased in local pharmacies.

Synthesis and characterization of the molecularly imprinted (and non-impreted) polymer

It was first necessary to synthesize AC, used in the synthesis of the TAT monomer (a precursor required to obtain the MIP). This was performed following the methodology described by Stempel[41]. The TAT monomer was then prepared according to the procedure proposed by Prasad *et al*.[40], after which the MIP was synthesized as described by Prasad *et al*.[39], using the conventional bulk polymerization approach. A polymer without molecular imprinting (NIP) was also synthesized in the same manner as the MIP, but without addition of the template.

Briefly, 0.50 mmol of TAT monomer and 20 mmol of crosslinking agent (EGDMA) were added to a glass ampoule, followed by 0.16 mmol of FA analyte and 0.45 mmol of AIBN initiator, and the mixture was dissolved in 24 mL of DMSO. The ampoule was sealed with a rubber septum and purged with nitrogen for 30 minutes using a hypodermic needle. The ampoule was then left in a thermostatically-controlled water bath (Model MA-184, Marconi, Brazil) at 50 °C for 24 hours.

After synthesis of the MIP, the FA was removed by washing the material with ACN/TEA (4:1, *v/v*) in a sintered plate funnel. Figure 2 represents the synthesis and FA removal procedure. The resulting polymer was ground in a mortar and sieved to obtain an MIP with a particle size of 150 μm, which was used in fabrication of the electrodes.[15]

FIGURE 2

The resulting MIP and NIP samples were analyzed by Scanning Electron Microscopy (SEM) in order to evaluate eventual changes in the morphologic features promoted by the FA imprinting using a Le0 440 Scanning electron. Particles were recovered with a gold layer.

Changes in surface area for MIP and NIP had also been evaluated using BET measurements performed from physical N_2 adsorption in a Micromeritics ASAP 2020 V3 equipment.

Preparation of the modified graphite-polyurethane composite electrode

The best composition of the GPU composite electrode was previously found to be 60% graphite and 40% polyurethane[42], and similar proportions of graphite and polyurethane were used here to fabricate electrodes modified with 2.5, 5.0, 7.5, and 10% (w/w) of MIP[15]. The mixtures were homogenized for 5 minutes in a glass mortar, pressed in a manual press, extruded as 3 mm diameter rods, and allowed to cure for 24 hours at room temperature, after which the rods were cut into 1.0 cm sections. The sections were then connected to copper wires with silver epoxy (EPO-TEK 410E, Epoxy Technology). After 24 hours, the composite/copper wire assemblies were inserted into glass tubes (6 mm diameter, 9 cm length), which were filled with epoxy resin (Silaex SQ 3024) and allowed to cure for 24 hours. Mechanical abrasion with 500 grit sandpaper was used to remove excess epoxy resin from the surface and expose the modified composite. Finally, the electrode was sonicated in isopropanol for 5 minutes and then in water for 5 minutes before each measurement.

Procedures

The best electrode composition using 2.5, 5.0, 7.5, or 10% (w/w) of MIP was first determined by CV and DPV under the operating conditions reported by Prasad *et al.*[39,40] for measurement of folic acid (10 mV s^{-1}, pulse amplitude 50 mV, and FA concentrations from 0.1 to 10 μmol L^{-1} in acetate buffer at pH 4.5). The best response in terms of resolution and current intensity was obtained for the electrode containing 2.5% (w/w) of MIP. Comparative analytical curves were then obtained using this electrode, the 2.5% (w/w) NIP electrode, and the unmodified GPU electrode.

The 2.5% MIP electrode was also used to determine FA in pharmaceutical formulations by the standard additions method. FA was chosen as a test compound because it is a fairly large molecule with a variety of functional groups in its structure. In accordance

with the Brazilian Pharmacopeia[43], 20 tablets each of Afopic®, Folifolin®, and Folacin® were accurately weighed out and ground. Portions of each powdered formulation equivalent to 5 mg of FA (based on the labeled contents) were dissolved in 100.0 mL volumes of 0.10 mol L^{-1} NaOH (pH 13) in order to obtain solutions containing 100.0 μmol L^{-1} of FA. The solutions were sonicated for 20 minutes to ensure complete dissolution.

The comparative method employed was high performance liquid chromatography (HPLC), in accordance with U.S. Pharmacopeia recommendations[44]. Chromatograms were obtained using a Shimadzu LC-10AD UP chromatograph equipped with an SPD-10A UV-UP detector and an LC-6AD (610) pump, controlled using Class-VP software. A C-18 column (15 cm x 4.6 cm x 5 μm) was maintained at room temperature, and the mobile phase was a mixture of sodium perchlorate, monobasic potassium phosphate, potassium hydroxide (1.0 mol L^{-1}) and methanol, pumped at a flow rate of 0.8 mL min^{-1}. The detector wavelength was set at 225 nm.

An interference study was conducted using methotrexate (MTX), whose structure is quite similar to that of FA, to check the selectivity of the 2.5% MIP electrode. The interferences of ascorbic acid (AA), dopamine (DA), and uric acid (UA) were also evaluated, considering the biological relevance of these substances and the presence in their structures of functional groups similar to those present in FA. The 2.5% NIP electrode was used for comparison. Voltammograms were obtained using DPV for solutions containing 0.90 μmol L^{-1} FA in acetate solution (pH 4.5), in the presence of 0.60, 0.90, and 1.2 mol L^{-1} of each interferent.

RESULTS AND DISCUSSION

MIP and NIP characterization

SEM images of both MIP and NIP samples are presented in Figures 3.a/3.c and 3.b/3.d, respectively. From such images it is possible to observe that MIP particles are relatively smaller (Fig. 3.a and 3.b) and present a more porous and rougher surface (Fig. 3.c and 3.d) than the NIP ones. On the MIP particle surface small structures can be observed under higher magnification while largest agglomerates are present on the NIP.

FIGURE 3

BET analysis agreed with the observed in SEM images once the MIP surface area (279.7 m^2 g^{-1}) is much higher than that for NIP (153.2 m^2 g^{-1}).

Such morphological and surface differences could be provoked by the presence of the template molecule on the polymer.

Determination of the best electrode composition

The behavior of FA at the unmodified electrode and electrodes modified with different percentages of MIP was initially evaluated using cyclic voltammetry to identify the best electrode composition, under the conditions described previously.

According to El-Maali *et al*.[28], during the anodic sweep with a carbon paste electrode modified with palmitic acid and stearic acid, an oxidation peak appears at around +0.8 V (*vs*. SCE), related to the loss of two electrons and two protons. In the cathodic sweep, two reduction peaks are observed. The first reduction peak, at -0.4 V (*vs*. SCE), is due to the gain of two electrons and two protons, followed by a tautomerization of the molecule, and the second peak, at around -0.8 V (*vs*. SCE), is due to an irreversible reduction[38].

In a first approach we had obtained cyclic voltammograms in phosphate buffer pH 7.8 (not presented) following the Prasad's *et al* work[40]. However it was noticed that better results could be obtained in acetate buffer pH 4.5 as suggested by Gall *et al*.[38] regarding the better peak definition and lower potential. In the second case the peaks were more intense and well defined than those in phosphate buffer. In addition these reduction peaks were not so close of the supporting electrolyte discharge, as occurred in the oxidation, presenting a flatter base line for extrapolation (Fig. 4). Thus reduction process was focused for further investigation.

Figure 4 presents the cyclic voltammograms obtained for the GPU composite electrodes with different MIP contents in acetate buffer pH 4.5. In these voltammograms it is possible to observe a low intensity anodic peak at +0.69 V (*vs*. SCE) related to FA oxidation and two cathodic peaks at -0.41 and -0.65 V (*vs*. SCE). These reduction signals have correspondent very low intensity oxidation peaks at -0.49 and 0.33V (*vs*. SCE), already discussed by Luo[45] as a two step irreversible electrochemical process at a HDME, in sulfuric acid.

Very small intensity reversible signals at *c.a.* +0.43/0.40 V (*vs*. SCE) had been attributed to functional groups in the polyurethane matrix electrode as previously reported[42].

FIGURE 4

With the objective of being able to quantitatively determine FA, experiments were performed using DPV and the electrodes containing 2.5, 5.0, 7.5, and 10% (w/w) MIP, with a cathodic sweep from 0.0 to -1.2 V. The results are presented in Figure 5.

FIGURE 5

In this case, similar DPV profiles were observed for MIP contents of between 2.5 and 7.5% (w/w), while the resolution was lost and the peak was distorted when the MIP content of the modified electrode was increased to 10%. In addition, the peak current was higher at lower MIP contents (Table 1). The 2.5% MIP electrode was therefore selected for use in the subsequent experiments.

TABLE 1

Choosing the supporting electrolyte for DPV

Since the reduction of FA is pH-dependent, the effect of the pH of the medium on the voltammetric response of the 2.5% MIP electrode was investigated using 10 μmol L^{-1} of FA in phosphate solution at pH values of 2.2, 4.2, 6.0, and 8.6. The measurements were performed with a = 25 mV and ν = 10 mV s^{-1}, as used previously by Prasad *et al.*[40] These experiments were performed in phosphate medium, which enables the use of a wide pH range.

The voltammograms (Figure 6) showed that the reduction process split into two peaks when the pH was changed from pH 2.2 to 6.0, and became a single event again at pH higher than 6.0. This could be explained by the reduction of acidic and basic forms of FA, since the pK_a of FA is 4.7[46].

FIGURE 6

The effect of the nature of the supporting electrolyte was also evaluated by comparing DPV voltammograms obtained using phosphate (pH 4.2) and acetate (pH 4.5) buffer solutions, with measurement of the currents at the highest peak potentials. The results (Table 2) revealed better peak definition and higher current intensity using the acetate medium, compared to the phosphate solution. The pH 4.5 acetate buffer was therefore employed in subsequent experiments.

TABLE 2

Analytical evaluation

Once the best working conditions had been identified, analytical curves were constructed for the unmodified electrode and the electrodes modified with 2.5% MIP and NIP, in order to determine the effect of the presence of the MIP on the sensitivity of the analytical response.

As mentioned above, two reduction peaks, at around -0.52 (peak a) and -0.58 V (peak b) (*vs.* SCE), were observed at this pH. The currents were therefore taken at the potentials of these two peaks in order to evaluate the changes in sensitivity due to the presence of MIP and NIP in the modified electrodes, compared with the unmodified GPU electrode. The voltammograms obtained (Figure 7) showed that in all cases, both faradaic and capacitive currents increased with FA concentration.

FIGURE 7

Figures 8(a) and 8(b) present overlays of the analytical curves obtained for the 2.5% NIP, 2.5% MIP, and unmodified electrodes, using FA concentrations up to 10.0 μmol L^{-1}, at -0.52 and -0.58 V (*vs.* SCE), respectively. Above 2.0 μmol L^{-1}, there was a trend towards saturation of the active sites, as indicated by the constancy of the peak current despite the increasing FA concentration. The insets in Figures 8(a) and 8(b) show the linear regions at lower concentrations.

FIGURE 8

Table 3 summarizes the analytical data for the response to FA obtained with the modified and unmodified electrodes at -0.52 and -0.58 V (*vs.* SCE).

TABLE 3

For the peak currents obtained at -0.52 V, it can be seen that although the sensitivity was slightly higher for the unmodified and NIP-modified electrodes, the linear range was wider for the MIP-modified electrode. The LOD was calculated as three times the standard deviation of the blank, divided by the slope[47], and was similar for all electrodes.

In the case of the peak currents at -0.58 V, despite the higher sensitivities of the NIP-modified and unmodified electrodes, the linear ranges were the same for all electrodes and the LOD was lowest for the MIP-modified electrode, indicating that this electrode should be able

to provide better repeatability and reduced dispersion (as confirmed by the correlation coefficients). Under the conditions used here, the 2.5% MIP electrode therefore only showed an advantage in terms of LOD at -0.58 V.

At modified carbon electrodes LOD ranging from 10^{-6} to 10^{-10} mol L^{-1} had been reported as presented in the Introduction section. Despite these low limits, those works involve pre-concentration steps, that result in poor reproducible results[48], use mercury electrodes bring together all the environmental and health issues related to this metal[36-38] and the growing of films on the surface of a glassy carbon[30], that use to be lost during surface renewing. The present electrode, although presenting a relatively higher LOD is simple of performing, once having the MIP prepared.

Determination of FA in pharmaceutical formulations

Folic acid was determined in the Afopic®, Folifolin®, and Folacin® commercial pharmaceutical samples using DPV with standard additions and the 2.5% MIP electrode. Successive aliquots of FA standard solution were added to samples containing 0.9 μmol L^{-1} FA (according to the label) in acetate solution (at pH 4.5), in order to obtain additions of 0.6, 0.9, and 1.2 μmol L^{-1}. Three voltammograms were recorded for each sample, after each addition. No significant interferences from the excipients present in the commercial samples (Table 4) were observed during the FA analyses.

TABLE 4

Table 5 presents the FA concentrations obtained by DPV with the 2.5% MIP electrode, together with the results using the recommended HPLC procedure[44]. The DPV results were in agreement with those obtained using HPLC (within a 95% confidence interval, according to the Student's t-test), which confirmed the efficiency and satisfactory performance of the 2.5% MIP electrode, when compared with the official procedure for the analysis of FA in commercial pharmaceutical samples. The recoveries achieved were 97±7, 97±9, and 102±8% for Afopic®, Folacin®, and Folifolin®, respectively (analyzed in triplicate).

TABLE 5

One single GPU composite electrode modified with 2.5% MIP was used in all the measurements described in this work, suggesting that the device presents a long lasting useful life.

Interference tests

The results described above demonstrated that the MIP-modified electrode could be successfully used for the quantitative determination of FA in commercial pharmaceutical formulations, without interference from the excipients present in these matrices, with a slight gain in sensitivity at low concentrations when compared to the unmodified electrode and the electrode modified with NIP.

However, the main reason to use an MIP as an electrode modifier is the possibility of improving selectivity. In the present case, FA, a relatively large molecule containing many functional groups in its structure, was used as a probe to evaluate the selectivity of the MIP-modified electrode. Interference tests were performed using MTX, a substance with a relatively large molecular size and a chemical structure very similar to that of FA, as well as smaller molecules containing functional groups also present in the structure of FA, which were chosen based on their biological relevance. The structures of FA and the interferents used in the present study are illustrated in Figure 8.

Table 6 summarizes the interference results for MTX in FA solutions. It could be concluded that interference from MTX occurred in all cases, for both the 2.5% MIP and the 2.5% NIP electrodes. However, it is interesting to note that although the expected interference should be 100% (a current signal equal to twice the signal for FA alone) when equal concentrations of FA and MTX were present, the current increases were only 69.6 and 73.4% for the MIP-modified and NIP-modified electrodes, respectively. The expected signal increase for a 1:2 ratio (FA:MTX, mol/mol) in solution should be 200% (three times the signal for FA alone), but the measured increases were 108 and 123% for the MIP-modified and NIP-modified electrodes, respectively. Similar results were obtained using a ratio of 1:1.5 (FA:MTX, mol/mol).

TABLE 6

These results suggest that although the interference was relatively large, the MIP was able to provide some discrimination between the analyte and the interferent, despite the close

structural similarity between FA and MTX, so that the observed interference was lower than expected, and also lower than found for the electrode modified with NIP.

The FA and the MTX have very close structures, including similar functional groups. Such functional groups surely interact with those in the methacrylate matrix once it responds to the both analytes no matter if there is or not being imprinted with FA (Tab. 3). When the same concentration of FA and MTX is present in solution it seems that one still have cavities available for pre-concentration of both, thus resulting in non recognition of NIP and MIP and a severe interference appears. When the MTX concentration is raised up a competition for the cavities is established being the FA favored in the MIP while in the NIP there is any discrimination between FA and MTX, once one have no cavities to discriminate between them, resulting in a higher interference.

The results obtained for the interference of the biologically relevant substances ascorbic acid (AA), dopamine (DA), and uric acid (UA) in the FA signal obtained using the MIP-modified electrode are shown in Table 7. Interference was observed for all the species evaluated, especially AA and DA. The lower interference from UA may have been due to its compact structure, in which the heteroatoms are fixed in the rings, which could have hindered its complexation with the coordination sites in the cavity of the MIP, despite its similarity with the region of the FA molecule in which heterogeneous rings containing nitrogen heteroatoms are present. On the other hand, AA and DA possess functional groups in aliphatic chains, as well as pendant groups bonded to rings, which could have facilitated coordination to the active sites in the cavities, resulting in greater interference.

TABLE 7

The interference therefore seemed to result from the MIP preparation process in which the template was inserted in the polymeric chain by interaction with the monomers, initiators, and crosslinking agents during the polymerization process. This interaction occurred by coordination between the functional groups in the template structure and those in the precursors. Removal of the template created a cavity in the polymer structure in which pendant functional groups were able to coordinate the template molecule during the analysis. The use of a large template molecule with many different functional groups in its structure created a large cavity full of coordinating sites, with the conformation of the template.

There are two possible sources of interference when a large molecule is used as a template. The first is that a molecule with similar conformation and functional groups can fit

inside the cavity as well as coordinate with the pendant coordination groups of the polymer. In the present case, this effect was illustrated by the interference of MTX. The second interference resulting from the use of a large template is that small molecules containing similar functional groups can coordinate with the coordination sites of the cavity, as demonstrated by the effects of AA and DA, and to a lesser extent by the effect of UA.

When the template is a small molecule, only the conformational effect seems to play an important role in selectivity, because few functional groups are present. Examples include the use of the templates paracetamol[15], pyridoxine and epinephrine (unpublished results), and other molecules[47,49].

CONCLUSIONS

Composite electrodes modified with different percentages of MIP exhibited satisfactory voltammetric responses in the determination of folic acid, with a small gain in sensitivity when compared to an unmodified GPU composite electrode. The LOD achieved for the electrode modified with 2.5% MIP was lower than for the other modified electrodes, but sensitivity was similar to that of the unmodified electrode.

The application of this electrode for the determination of folic acid in pharmaceutical samples resulted in good levels of recovery, showing the efficiency of the proposed method, with a long lasting electrode life. Interference tests showed that the electrode modified with 2.5% MIP was more selective towards folic acid, compared to an electrode modified with NIP.

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Table 1 - Peak currents obtained from DPV voltammograms, attributed to the reduction of FA

Electrodes	GPU*	2.5%	5.0%	7.5%	10%
$I_p/10^{-6}$ A	1.76	2.48	2.25	2.45	1.61

*Unmodified electrode

Table 2 - Currents from DPV voltammograms for FA in phosphate (pH 4.2) and acetate (pH 4.5) buffer solutions

Electrolytic medium	I_p (10^{-6} A) at MIP % (w/w)				
	GPU*	2.5	5.0	7.5	10
Phosphate	1.76	2.48	2.25	2.45	1.61
Acetate	2.38	4.48	2.83	3.76	3.23

*Unmodified electrode

Table 3 – Figures of merit corresponding to the peaks at -0.52 and -0.58 V

Peak potential (V) *vs.* SCE	Electrodes	Linear range (µmol L^{-1})	Correlation coefficient	Slope (µA µmol L^{-1})	LOD (µmol L^{-1})
-0.52	MIP	0.6-2	0.992	0.69	0.17
	NIP	0.6-1.5	0.992	0.88	0.16
	GPU	0.6-1.5	0.992	0.88	0.16
-0.58	MIP	0.6-2	0.999	0.56	0.034
	NIP	0.6-2	0.975	0.65	0.303
	GPU	0.6-2	0.975	0.66	0.303

Table 4 – Excipients in the pharmaceutical formulations used in this study

Formulation	Excipients
Folacin®	Lactose, starch, sodium gluconate, magnesium stearate, talc, titanium dioxide, polyethylene glycol, triethylcitrate, polysorbate, methacrylic acid copolymer, acetone, simethicone, distilled water, coloring agent
Afopic®	Magnesium stearate, microcrystalline cellulose, sodium croscarmellose
Folifolin®	Lactose monohydrate, talc, magnesium stearate, microcrystalline cellulose, sodium croscarmellose, sodium lauryl sulfate, povidone

Table 5 - Determination of FA in pharmaceutical formulations using the 2.5% MIP electrode, and comparison with the HPLC method

Samples	Labeled (mg)	DPV (mg)	E_1^a (%)	HPLC (mg)	E_2^b (%)	E_3^c (%)
Afopic®	5.00	4.71	- 5.78	4.82	- 3.62	- 2.31
Folifolin®	5.00	4.64	- 7.14	4.76	- 4.82	- 2.44
Folacin®	5.00	5.21	+ 4.25	5.09	+ 1.79	+ 2.43

a = DPV *vs*. Labeled: ((DPV-Labeled)/Labeled) x100%
b = HPLC *vs*. Labeled: ((HPLC-Labeled)/Labeled) x100%
c = DPV *vs*. HPLC: ((DPV-HPLC)/HPLC) x100%

Table 6 - Interference of MTX in the signal obtained for 0.6 mmol L^{-1} FA

	MIP 2.5%		Expected	NIP 2.5%	
FA:MTX C (μmol L^{-1})	I_p (μA)	Interference (%)	Interference (%)	I_p (μA)	Interference (%)
0.6:0.0	1.76	-	-	0.97	-
0.6:0.6	2.99	69.6	100	1.68	73.4
0.6:0.9	3.28	86.0	150	2.07	114
0.6:1.2	3.67	108	200	2.16	123

Table 7 - Interference of ascorbic acid (AA), dopamine (DA), and uric acid (UA) in the signal obtained for 0.6 mmol L^{-1} folic acid (FA) using the MIP-modified electrode

	Expected	AA		UA		DA	
FA:Interferent C (μmol L^{-1})		I_p (μA)	Interference (%)	I_p (μA)	Interference (%)	I_p (μA)	Interference (%)
0.6:0.0	-	0.63	-	0.78	-	0.58	-
0.6:0.6	100	0.94	48.1	0.88	12.2	0.70	21
0.6:0.9	150	1.28	102	0.93	19.2	1.40	143
0.6:1.2	200	1.52	140	1.19	52.3	1.48	157

Legend Figures

Figure 1 – Structures of (a) folic acid, (b) MTX, (c) ascorbic acid, (d) dopamine, and (e) uric acid.

Figure 2 – Schematic representation of FA molecular imprinting, subsequent removal of FA, and inclusion of the MIP in the GPU composite electrode.

Figure 3 – SEM of MIP (a/c) and NIP (b/d) particles. Magnification 10000x (3.a/b) and 50000 x (3.c/d)

Figure 4 – Cyclic voltammograms obtained for 1.0 mmol L^{-1} FA in acetate buffer (pH 4.5), with ν = 25 mV s^{-1}, using the unmodified GPU electrode and the electrodes modified with 2.5, 5.0 and 7.5% of MIP.

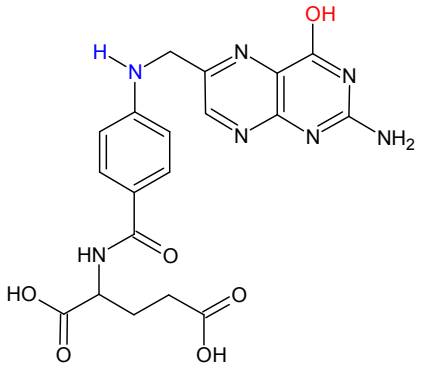
Figure 5 – Differential pulse voltammograms obtained for 10 µmol L^{-1} FA in phosphate buffer (pH 4.2), with ν = 10 mV s^{-1} and a = 25 mV, using the unmodified GPU electrode and the electrodes modified with 2.5, 5.0, 7.5, and 10% of MIP.

Figure 6 - Differential pulse voltammograms obtained for 10 µmol L^{-1} of FA in phosphate buffer, using the 2.5% MIP electrode (a = 25 mV, ν = 10 mV s^{-1}).

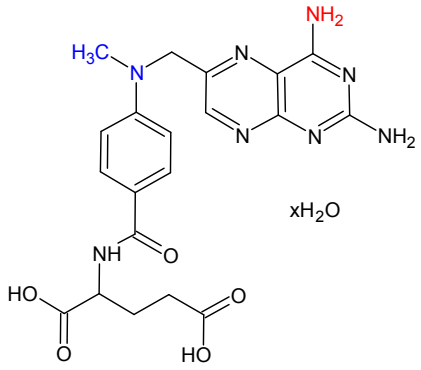
Figure 7 - DPV voltammograms for FA concentrations (C_{FA}) of between 0.1 and 10 µmol L^{-1} in acetate buffer solution (pH 4.5), using a = 50 mV and ν = 10mV s^{-1}: (a) unmodified electrode, (b) 2.5% MIP electrode, and (c) 2.5% NIP electrode.

Figure 8 - Analytical curves for the unmodified electrode, 2.5% MIP electrode, and 2.5% NIP electrode, using the peak at (a) -0.52 V (*vs*. SCE) and (b) -0.58 V (*vs*. SCE).

Figure 1 – Pereira *et.al*



(a)



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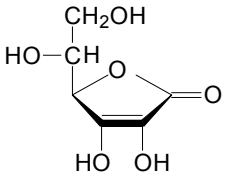

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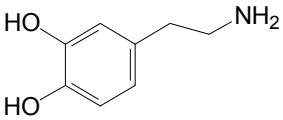

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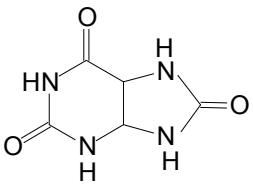


(e)

Figure 2 – Pereira *et.al*

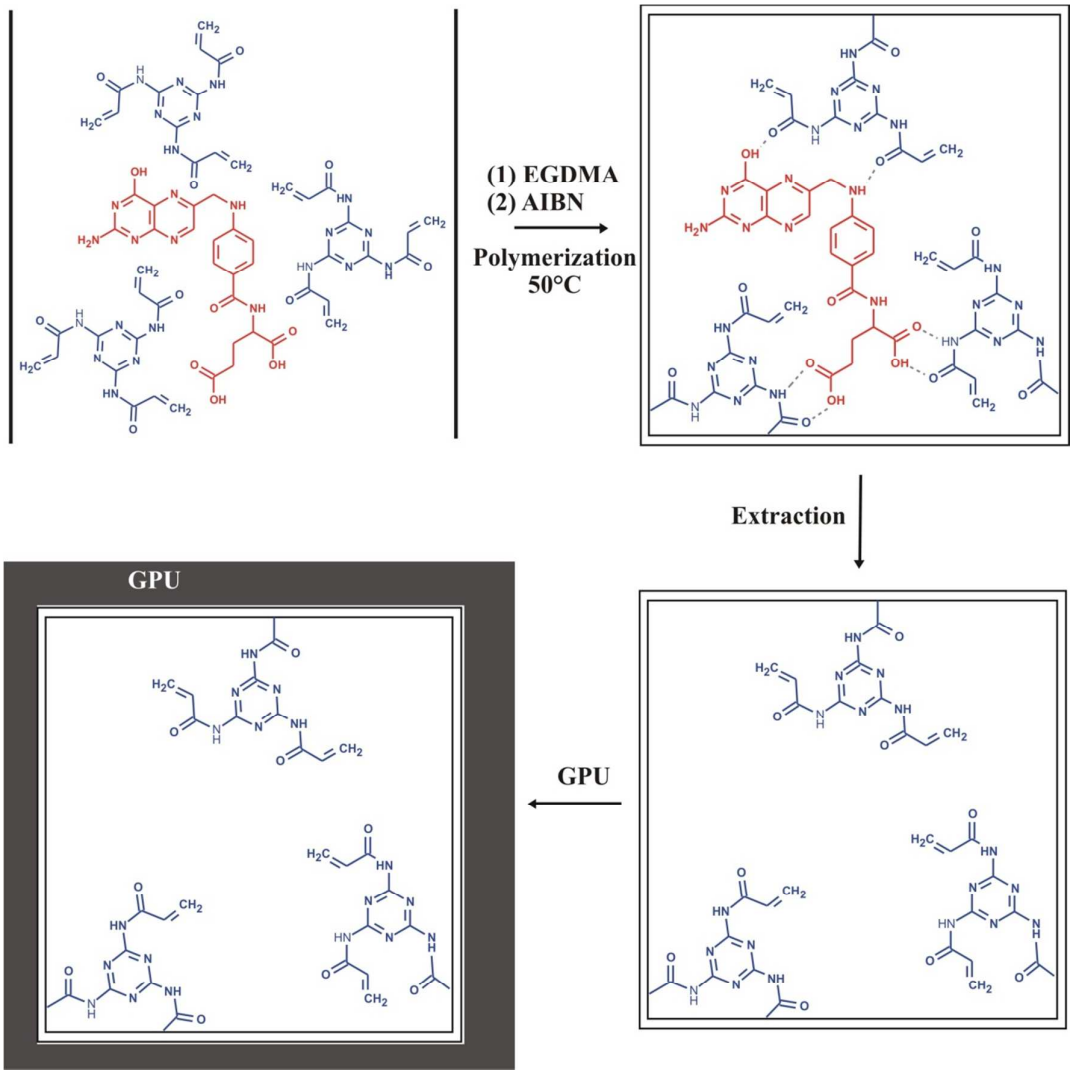
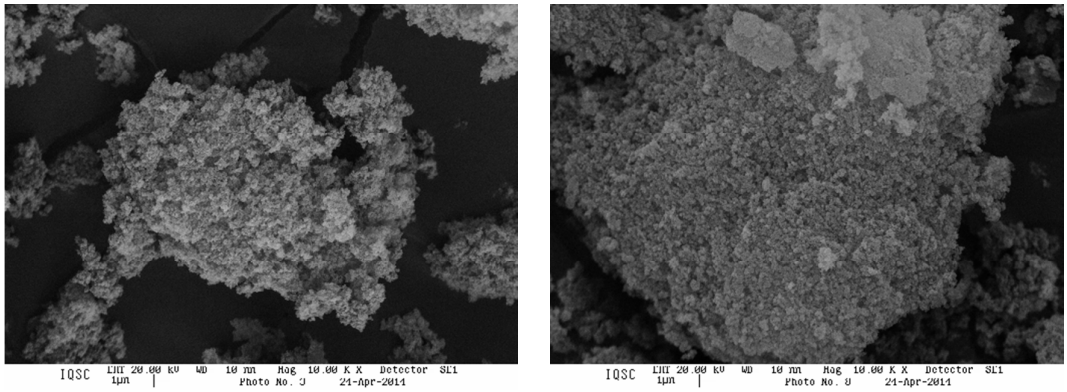


Figure 3 – Pereira *et. al*



(a) (b)

(c) (d)

Figure 4 – Pereira *et.al*

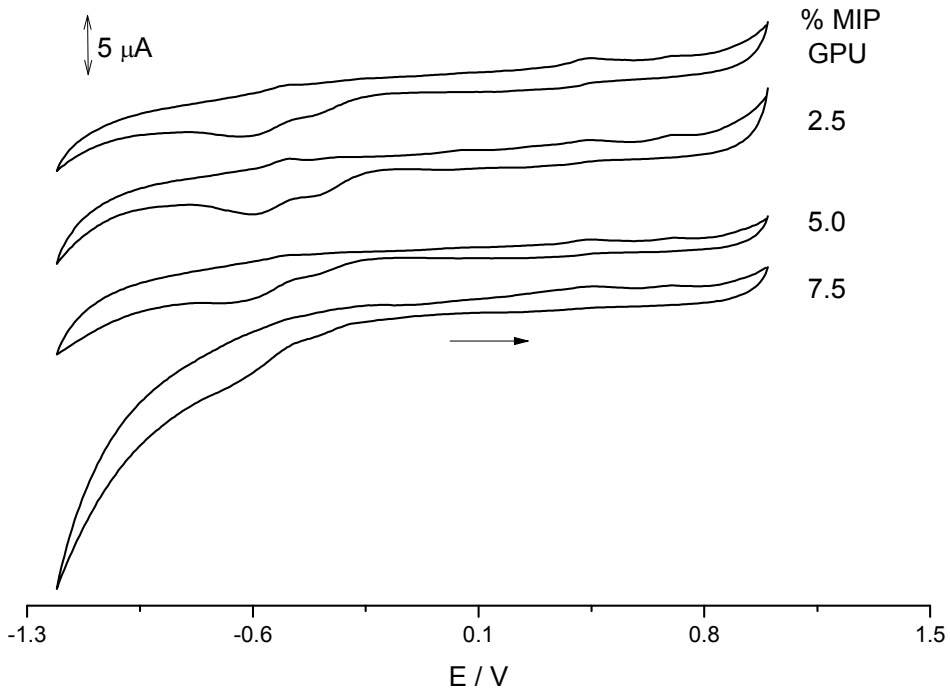


Figure 5 – Pereira *et.al*

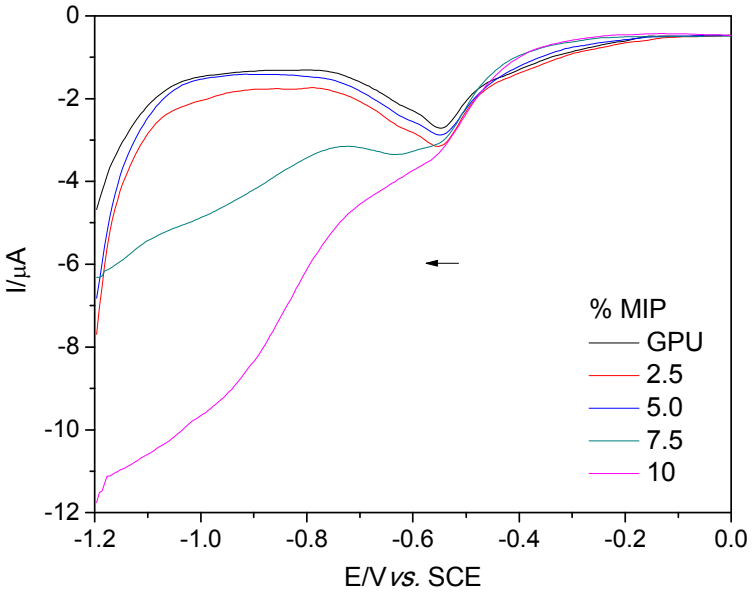


Figure 6 – Pereira *et.al*

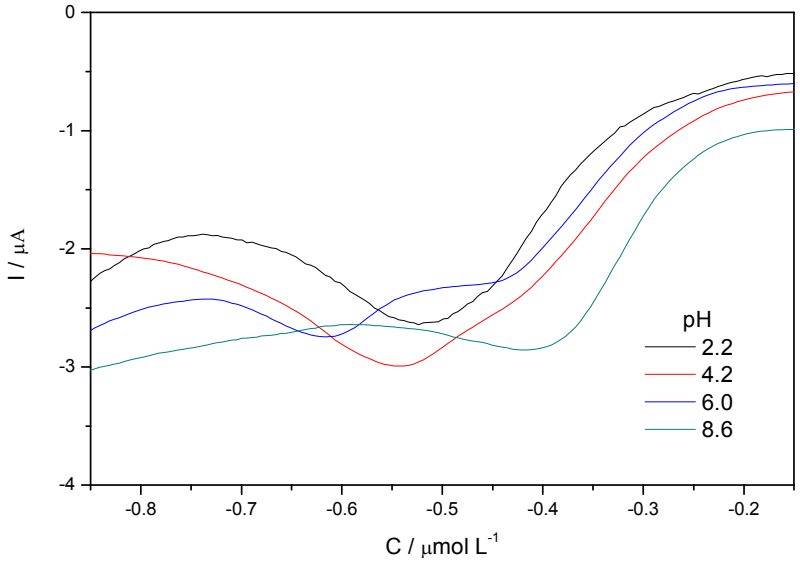
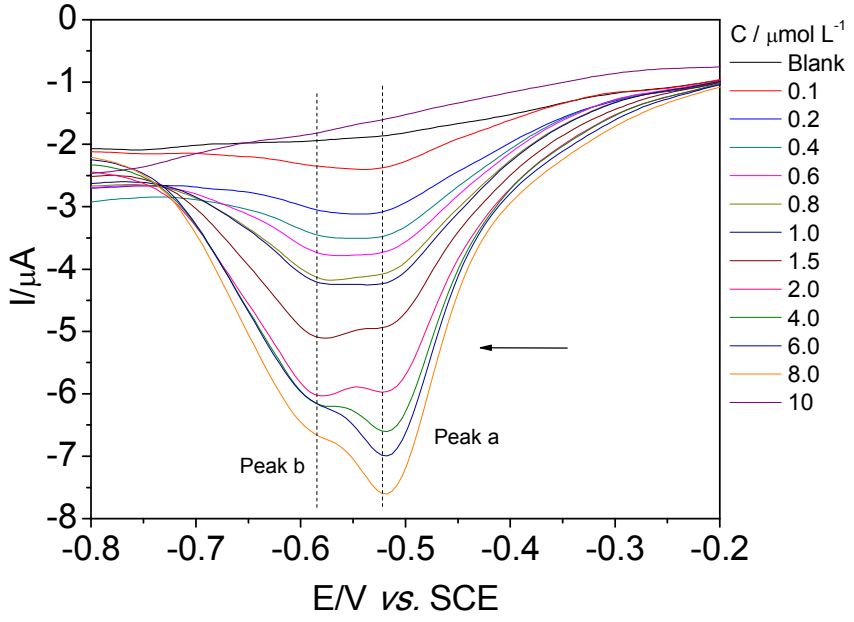
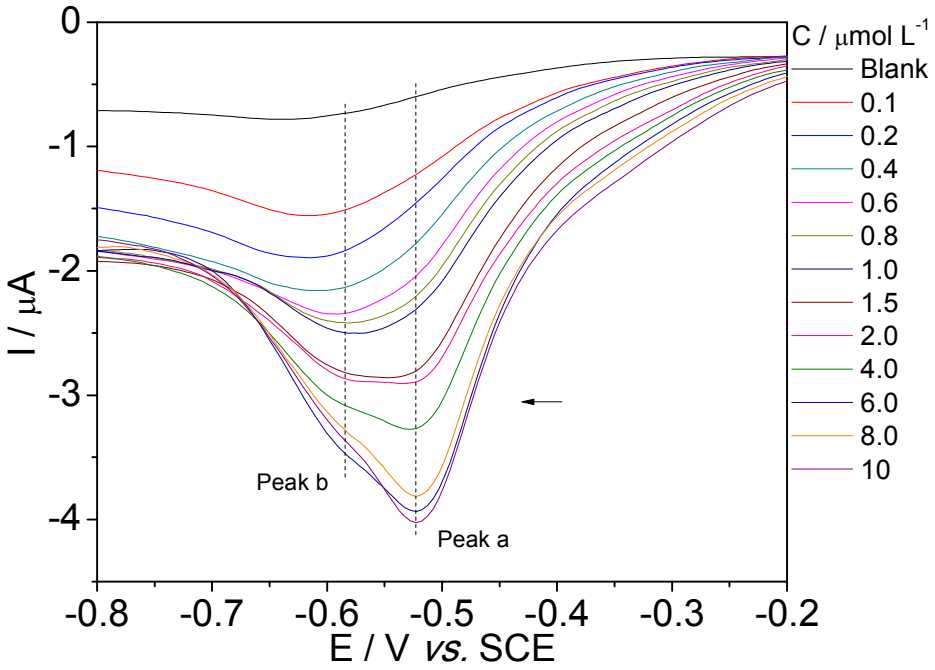


Figure 7a – Pereira *et.al*



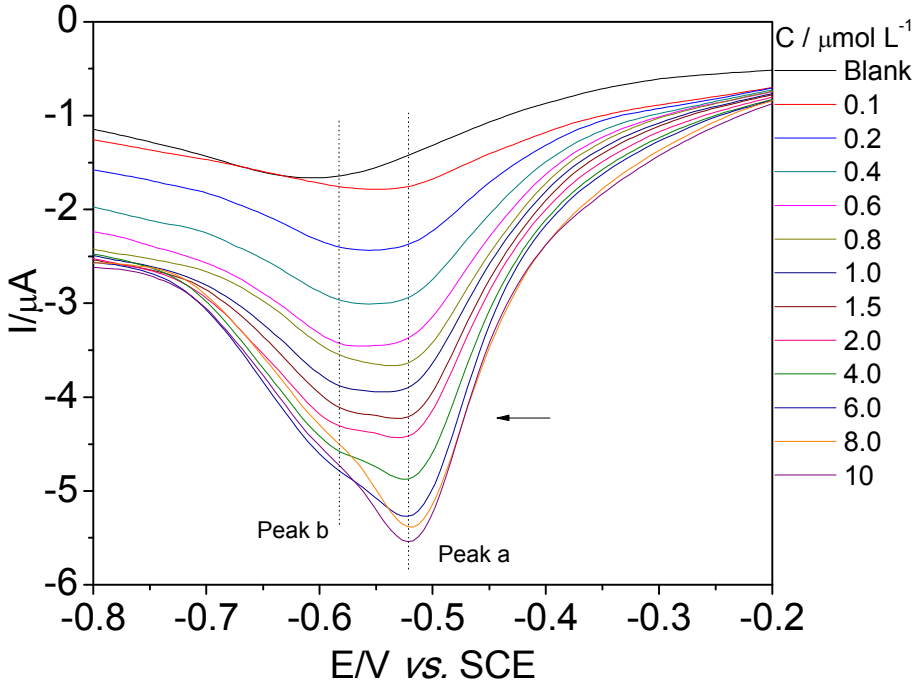
(a)

Figure 7b – Pereira *et.al*



(b)

Figure 7c – Pereira *et.al*



(c)

Figure 8a – Pereira *et.al*

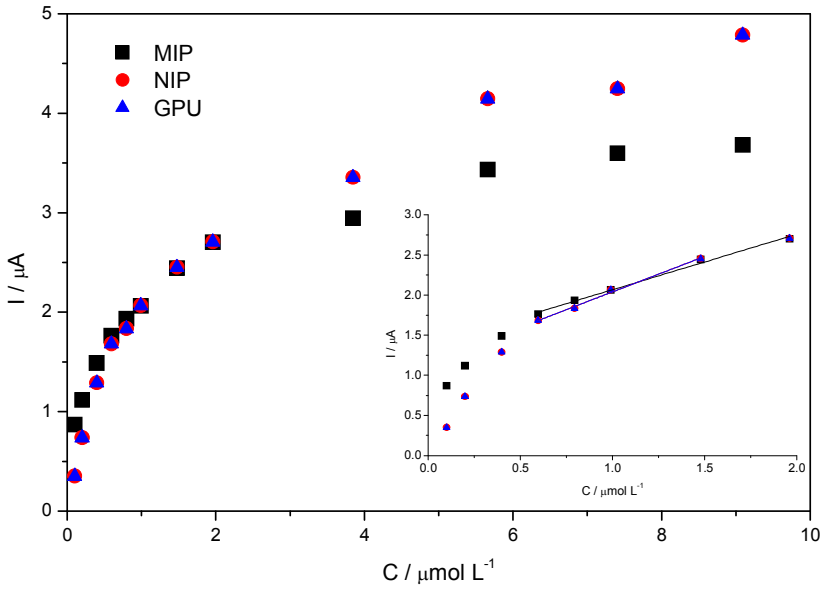


Figure 8b – Pereira *et.al*