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Differential Pulse Voltammetry Determination of Anti-Hypertensive Drug Hydrochlorothiazide in Pharmaceuticals Using Glassy-Carbon Electrode Modified by Electropolymerization with L- and D- Glutamic Acids

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A simple and sensitive electrochemical sensor based on glassy carbon electrode modified by electropolymerization with both poly-glutamic acid enantiomers L- and D (GC-PGA) was developed to detect the anti-hypertensive drug hydrochlorotiazide (6-chloro-3,4-di-hydro-2H-1, 2,4-benzo-thiadiazine-7-sulfonamide-1,1 dioxide, HCTZ) in pharmaceuticals samples. Raman spectroscopy and electrochemical impedance spectroscopy were carried out to characterize L-PGA and D-PGA film. These procedures confirm the production of a polymer through an amide bond and the formation of a film resistant to charge transference in both cases. Also, an increase in the oxidation peak current for HCTZ in Buffer Britton Robinson solution 0,1 M pH 2 was obtained using the modified electrodes. With GC/D-PGA and GC/L-PGA, the peak current increased 2 times compared to unmodified GC, when using differential pulse voltammetry. A novel electroanalytical method for the determination of HCTZ was developed with both modified electrodes, showing a less limit of determination: 0.03186 and 0.01829 mM to GC/L-PGA and GC/D-PGA, respectively, and a less limit of quantification with respect to the GC electrode. The methodology developed was applied in the determination of HCTZ from pharmaceutical forms.

Keywords: Glutamic Acid, Hydrochlorotiazide, electropolymerization, differential pulse voltammetry, glassy carbon electrode.

1. INTRODUCTION

Globally, one of the major diseases that affect adult population is hypertension (HTA), which had a prevalence of 26.4% in 2000. It is projected that by 2025, 29.2% of the world population will be hypertensive [1]. In Chile, according to the latest national health survey published in 2003, 33.7% of the total population has hypertension [2] .The Chilean public health system has prescribed hydrochlorothiazide as the first priority anti-hypertensive drug to treat this disease.

Hydrochlorothiazide (HCTZ, figure 1), 6-chloro-3,4-di-hydro-2H-1, 2,4-benzo-thiadiazine-7-sulfonamide-1,1 dioxide, is a diuretic benzothiazide type which acts directly on the kidney to level distal nephron channels, inhibiting the re-absorption of Na^+/Cl^- ion water and less K^+ ions and resulting in a decrease in blood pressure. In addition, HCTZ is used in the treatment of edema, diabetes insipidus, renal tubular acidosis and prevention of kidney stones [3].

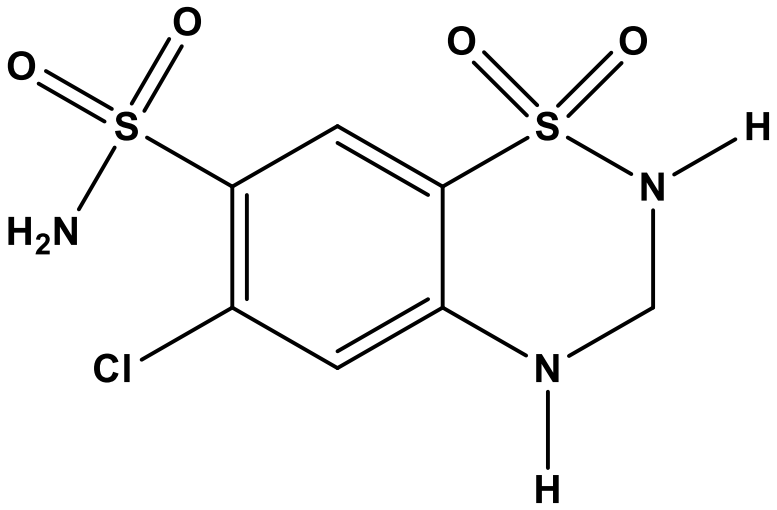

Figure 1. Hydrochlorothiazide

Currently, several analytical methods are used for determining HCTZ in different matrices and high performance liquid chromatography (HPLC) is the analytical tool recommended by the United States Pharmacopeia [4]. Moreover, in the literature it is possible to find other techniques applied in the determination of HCTZ such as chemiluminescence [5], capillary electrophoresis [6], conductimetry [7], spectrophotometry [8], among others. The main disadvantages of these methods are time-consuming analysis, pre-treatment of samples, excess reagent consumption, high economic cost in their implementation, in addition to low detection limits. Therefore, new procedures with faster measurement, lower economic cost, extensive detection and quantification limit, and higher sensitivity are required to identify and quantify HCTZ. In this sense, numerous electrochemical methods have been developed for the determination of this drug [9,10,11].

The modification of electrodes by electropolymerization is one of the electrochemical techniques of greatest interest for the development of new electroanalytical methods to determine different species [12,13,14 ,15], because the immobilization of monomeric species on the electrode

surface is quite simple and, together with the electrogenerated polymeric film, enables studying the kinetics of charge transfer at the electrode interface [16].

Glutamic acid (GA) is one of the 20 most common amino acids and can be easily electropolymerized on the electrode surface, producing a film of poly-glutamic acid (PGA). PGA has repeating units of glutamate and deprotonated carboxylic species (pKa = 4.45), linked through an amino bond between α-amino and β-carboxylic acid groups [17,18]. Also, due to the chirality of the second carbon structure glutamic acid, L- and D have (Figure 2) [19] enantiomers. The literature provides several examples of electrochemical sensors applications based on immobilization of poly-glutamic acid on electrode surface, where different analytes such as ascorbic acid [20,21], hydrazine [22], caffeic acid [23] were determined, showing higher limits of determination and quantification.

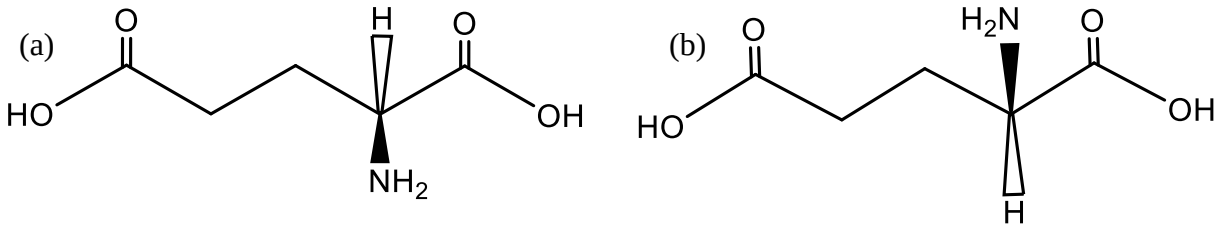

Figure 2. (a) L- glutamic acid, (b) D- glutamic acid.

The aim of this work was to develop a new electroanalytical methodology for the determination of hydrochlorothiazide antihypertensive drug using modified glassy carbon electrodes by electropolymerization with L- and D- glutamic acid monomers. With the modified electrodes, an increase in the voltammetric response was obtained in unmodified electrodes, thus developing a new methodology with greater sensitivity and wider determination and quantification limits.

2. EXPERIMENTAL

2.1 Reagents

(Pure) Hydrochlorothiazide was supplied by Sigma-Aldrich® (Lab Chile®, Santiago, Chile). Commercial capsules of HCTZ from Lab Chile® and ITF-Labomed® (both capsules declared 50 mg hydrochlorothiazide per tablet) were obtained commercially.

All reagents were analytical grade, L- and D- glutamic acids were Sigma Aldrich (≥ 99%), phosphoric, acetic, hydrochloric and boric acids, acetonitrile, phosphate disodium, phosphate monosodium and sodium hydroxide were supplied by Merk. Deionized water was obtained in the laboratory, using ionic interchange columns (Milli-Q).

2.2 Solution Preparation

2.2.1 Glutamic Acid Solution

0.02 mol L^{-1} of L- or D- glutamic acid in 0.04 mol L^{-1} HCl solutions was used to modify GCE by electropolymerization.

2.2.2 Buffer Solutions

0.1 mol L^{-1} Britton Robinson buffer (acetic acid, boric acid, phosphoric acid) was used for voltammetric experiments. 0.1 mol L^{-1} phosphate buffer (phosphate disodium and phosphate monosodium) was used for HPLC determination, and pH was adjusted with NaOH solutions.

2.2.3 Stock drug solution

2.9 mg of hydrochlorothiazide were dissolved in 10 mL 0.1 mol L^{-1} Britton-Robinson at pH 2.0 to obtain a final concentration around 1.0 mM HCTZ.

2.2.4 Work Solution

Different aliquot volumes of the stock solution were taken and then diluted in a Britton-Robinson solution for electrochemical, spectrophotometric and chromatographic analyses.

2.3 Apparatus

2.3.1 Voltammetric Analyzer

Cyclic voltammetry (CV) was performed to modify electrodes in a PalmSens® EmStat3 multichannel potentiostat. Differential pulse voltammetry (DPV) was used for hydrochlorothiazide quantification experiments and it was performed in an Autolab® PGSTAT204 potentiostat and the acquisition and treatment of date was made with the Autolab NOVA® 2.0 software.

A traditional three-electrode cell system was used in all electro-chemical experiments. The GC electrode obtained from CH-Instruments was used as working electrode with a geometric area of 12.57 mm^2. An $Ag/AgCl_{(sat)}$ in saturated KCl ($Ag/AgCl/KCl_{sat}$, CH-Instruments) electrode was used in aqueous media as reference electrode, while platinum wire was used as counter electrode.

2.3.2 Electrochemical Impedance Spectroscopy (EIS)

EIS was performed with a CH-Instrument 604C bi-potentiostat. Data from the impedance measurements were obtained by fitting with Z view® software purchased from Scribner Inc. A one-compartment cell was employed with three electrodes: Working Electrode: glassy carbon; Reference

Electrode: $Ag/AgCl_{(sat)}$; Auxiliary Electrode: platinum, E_{DC} = +0.2 V applied vs Ag/AgCl, Amplitude Pulse E_{AC} = 5 mV, Frequency Range = 0.01 Hz-100 kHz.

2.3.3 HPLC

Measurements were carried out using a Shimadzu LC-20AD with photodiode array detector. The acquisition and treatment of data were conducted with Lab Solutions software. A 150x4.5 mm Purospher® RP-18 5 μm column was used. The volume injection was 10 μLand the absorbance to HCTZ was followed at 273 nm.

A mixture of 0.1 M phosphate buffer pH 7 and acetonitrile with a ratio 70/30 v/v, respectively, was used as mobile phase. The flow was 1.0 mL min^{-1} and the working temperature was kept constant at 30±1 °C. These chromatographic conditions were based on USP assay for HCTZ [24**Error! Bookmark not defined.**].

2.3.4 Spectrophotometry

Measurements were carried out using an Agilent® 8453spectrophotometer. The acquisition and treatment of data were made with the UV.exe software. The absorbance of HCTZ was followed at 272 nm.

2.3.5 Raman Spectroscopy

Raman spectra were registered with a Renishaw micro-Raman system (RM1000) equipped with 785, 633 and 514 nm laser lines, a Leica microscope and an electrically cooled charge couple device (CCD) camera. The laser power on the samples was less than 2 mW. Acquisition time was set between 10 and 20s per accumulation; and the average of accumulations was 5 with spectral resolution of 4 cm^{-1}. Spectra were scanned in the 150-1800 cm^{-1} region. Spectral recording conditions and the choice of the laser line used were selected to prevent sample degradation; to this end, the 785 nm line was used.

Raman spectra of modified GC, GC modified with L-GA (GC/L-PGA) and GC modified with D-GA (GC/D-PGA) were acquired in-situ using 50x objective and without any sample preparation.

2.4 Preparation of the Modified Glutamic Acid Glassy Carbon Electrode (GC/PGA)

First, GC electrode were polished in 0.05 μm alumine and then rinsed with ultrapure water Mili-Q® and dried in an inert atmosphere. The GCE were immersed in 0.02 M AG, prepared in 0.04 M hydrochloric acid solution. The modification was made through electropolymerization, applying 15 voltammetric cycles between -200 and +2500 mV, at a scan rate of 100 mVs^{-1}. The modified GCE were rinsed with ultrapure water and dried under N_2 atmosphere.

2.4. Analytical Procedure

2.4.1. Calibration Curve Preparation

2.4.1.1. Voltammetry and HPLC

10 working solutions ranging between $1x10^{-5}$ and $1x10^{-3}$ were prepared for the calibration curve by dilution of hydrochlorothiazide stock solution with 0.1 M Britton-Robinson buffer pH 2. For the DPV analysis, the experimental conditions were: pulse amplitude 0.0050 V, pulse width 0.0050 s, scan rate 5 mVs^{-1}, between 600 and 1800 mV *vs* $Ag/AgCl_{(sat)}$. In HPLC, the solutions were injected using the above working procedure.

2.4.1.2. Assay for Hydrochlorothiazide Tablets

According to the Razak protocol [25], 5 tablets of both laboratories were separately crushed and homogenized in a ceramic mortar, and the amount equivalent to one tablet was weighed and was dissolved in 50 ml NaOH 0.02M. Then, the solution was sonicated for 10 minutes. 200 μL were taken and diluted in 50 mL of Britton-Robinson buffer 0.1 M pH 2. The resulting solutions were used for Voltammetric and HPLC analysis. Also, all excipients contained in pharmaceutical of both laboratories (LAB-Chile® and ITF-LABOMED): corn starch, lactose monohydrate, povidone, sodium starch glycolate, and magnesium stearate, were dissolved in 0.1 M Britton-Robinson buffer pH 2, to study the interference of excipients.

3. RESULTS AND DISCUSSION

3.1 Preparation of the Modified Glutamic Acid Glassy Carbon Electrode (GC/PAG)

Fig. 3(a) and (b) show continuous cyclic voltammograms (CVs) recorded during the electropolymerization of (a) L- glutamic acid and (b) D-glutamic acid on the surface of GCE in 0.04 M HCl solution. As it may be seen, in the first sweep, an irreversible oxidation peak was observed at about 2089 and 2140 mV, for L- and D- glutamic acid, respectively. Also, we can see that D-GA showed a higher oxidation current, corresponding to 1.01 uA, 104 times greater than that obtained for L-GA (7.166×10^{-3} uA). In both cases, the peak current decreased in the second sweep, and the changes in peak current were lower in the following cycles at these potentials. This phenomenon produces the formation of the poly-acid glutamic (PGA), which also indicates that the highest electropolymerized monomer concentration occurs in the first voltammetric cycle [26]. In the same figure, it may be observed that the terminal oxidation current (at 2500 mv) increased with the number of scans, indicating that polymer film was formed on the GC electrode.

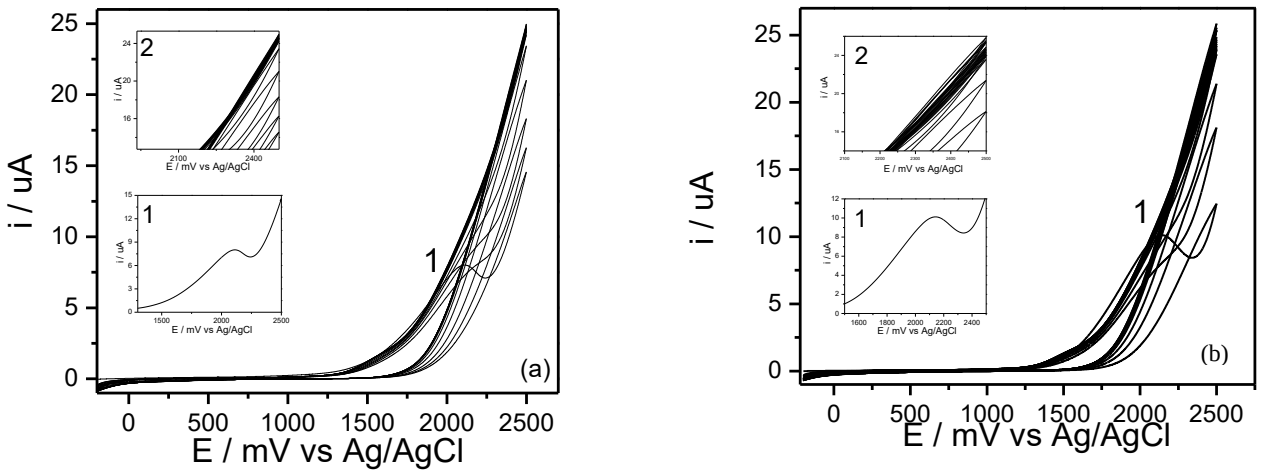

Figure 3. Cyclic Voltammetry of GCE of 0,02 mol L^{-1} (a) L-AG, (b) D-AG, Britton-Robinson Buffer 0,1 M pH 2.

3.2 . Characterization of the GCE/PGA

3.2.1 Electrochemistry Impedance Spectroscopy

Figure 4 shows Nyquist plots for every system studied in the presence of 2.0 mM $[Fe(CN)_6]^{3-}$ /$[Fe(CN)_6]^{4-}$. It can be observed that glassy carbon showed a small semicircle region at frequencies higher than 6.6 Hz, which is associated with the resistance to electron transfer of the redox couple and a linear zone at frequencies lower than 6.60 Hz and, in turn, with resistance to mass transfer for $[Fe(CN)_6]^{3-}$/$[Fe(CN)_6]^{4-}$. When L- and D-glutamic acid were electropolimerized on the glassy carbon surface, the semicircle zone was more predominant in the spectra, with a notorious increase in resistance to electron transfer compared to glassy carbon at higher frequencies than 0.55 Hz and 0.17 Hz, respectively. On the other hand, a small linear zone associated with diffusion of the redox couple is observed at frequencies lower than 0.55 Hz and 0.17 Hz. These results give an account of the fact that the modified surface is blocking the electron transfer of the redox couple. This could be explained by the electrostatic repulsion between $[Fe(CN)_6]^{3-}$/$[Fe(CN)_6]^{4-}$ and carboxyl groups present in poly L-glutamic and poly D-glutamic acid. A typical Randles circuit was used to fit experimental curves to obtain the electrical properties of the process, as observed in Diagram 1. The χ-square of all the fits is lower than 0.009, confirming the good accuracy of the equivalent circuitry proposed. A constant phase element (*CPE*), instead of an ideal capacitor for double-layer capacitance, was used because this type of impedance element is useful when the interface is not flat, as it occurs with the polymers studied in this work. $Z_{CPE}=1/(T_{(i\omega)})^P$, where the value of the *P* exponent changes from 0 to 1. Depending on this value, *T* can take resistance (~0) or capacitance (~1) units, showing the more resistive or capacitive behavior of the system [27].

Table 1 shows that electron transfer resistance is higher for GC/L-PAG and GC/D-PAG compared to the glassy carbon electrode, and also that poly D-glutamic acid is slightly higher than poly L-glutamic ~ 274 Ω. Similarly, the relaxation time ($\tau = R_{ct}C_{dl}$) for the electrochemical process is

slower after the generation of the films compared to glassy carbon electrode, and similar between both poly glutamic acid systems. An apparent surface coverage can be determined from the R_{ct} values, according to the following equation [28]:

$$\theta_{app} = \left(1 - \left(\frac{R_{ct\,GC}}{R_{ct\,GC/Poly\,G}}\right)\right) x100$$

The θ_{app} for poly L-glutamic and poly D-glutamic is very similar between them, with 90.1% and 90.7%, respectively; however, these values corroborate an almost complete modification of glassy carbon with the aforementioned polymers.

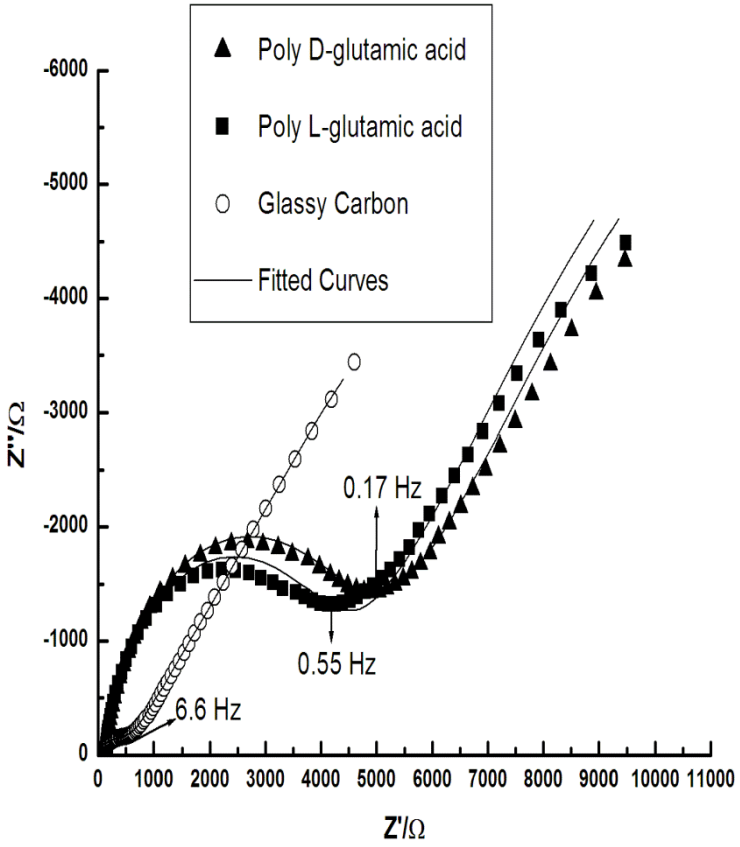

Figure 4. Nyquist plots from electrochemical impedance spectra of different bilayers onto ITO surfaces. 2 mM $Fe(CN)_6^{4-}/Fe(CN)_6^{3-}$, 10 mM Tris, 0.1 M KCl. Potential of +0.2 V applied *vs* Ag/AgCl. Amplitude Pulse Potential = 5 mV. Frequency Range = 0.01 Hz-100 kHz.

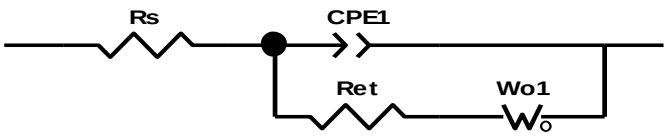

Diagram 1. Equivalent circuit employed where R_s= solution resistance, CPE_{dl} = constant phase element for electric double layer. R_{et}= resistance to electron transfer, W_{o1}= Warburg diffusion impedance.

Table 1. Electrochemical impedance data obtained by fitting GC/L-PGA and GC/D-PGA in the presence of 2 mM $Fe(CN)_6^{4-}/Fe(CN)_6^{3-}$, 0.1 M KCl. Potential of +0.2 V applied vs Ag/AgCl. Amplitude Pulse Potential = 5 mV. Frequency Range = 0.01 Hz-100 kHz

System	R_s/Ω	R_{et}/Ω	CPE_{dl} (P)	CPE (T)/μF	τ/ms	ω_{max}/s^{-1}
GC	106.8	405.2	0.7124	15.0	6.01	26.1
GC/L-PGA	121.5	4096.0	0.8520	11.5	47.1	3.38
GC/D-PGA	122.7	4370.0	0.8612	11.6	50.7	3.14

3.2.2 Raman Spectroscopy.

Raman spectra of L-glutamic acid and D-glutamic acid were registered in powder form, as shown in Figure 5(a). The spectra of both compounds are dominated by strong bands at 1415 cm^{-1} (stretching -COOH), 1357 cm^{-1} (bending -CH), 1316 cm^{-1} (bending -OH), 923 cm^{-1} (stretching C-C), 868 cm^{-1} (bending -COOH), 804 cm^{-1} (stretching C-C), 536 cm^{-1} (out-of-plane bending –OCC) and 386 cm^{-1} (skeletal deformation) [29]. The spectral differences are shown in the blue rectangle, where the change in band intensity, appearance of new bands, and disappearance of others is a consequence of two different conformations. The band at 1666 cm^{-1} in both spectra with a medium intensity is assigned to a C=O symmetric stretching mode.

Raman spectra of GC and modified GC are displayed in Figure 5(b). The contribution of D- and L- form of unpolymerized glutamic acid on modified electrodes is discarded mainly due to the absence of the band attributed to Amide I (-NHCO) deformation mode observed at 1680 cm^{-1} [30]. This band is extremely sensitive to its environment and used to study the presence of peptide. In GC/D-PGA these bands appear at 1690 cm^{-1} and in GC/D-PGA at 1680 cm^{-1}. This shift in both results can be explained based on two different conformations of the formed polymers. The same band is absent in the spectrum of GC, as seen in Figure 5b.

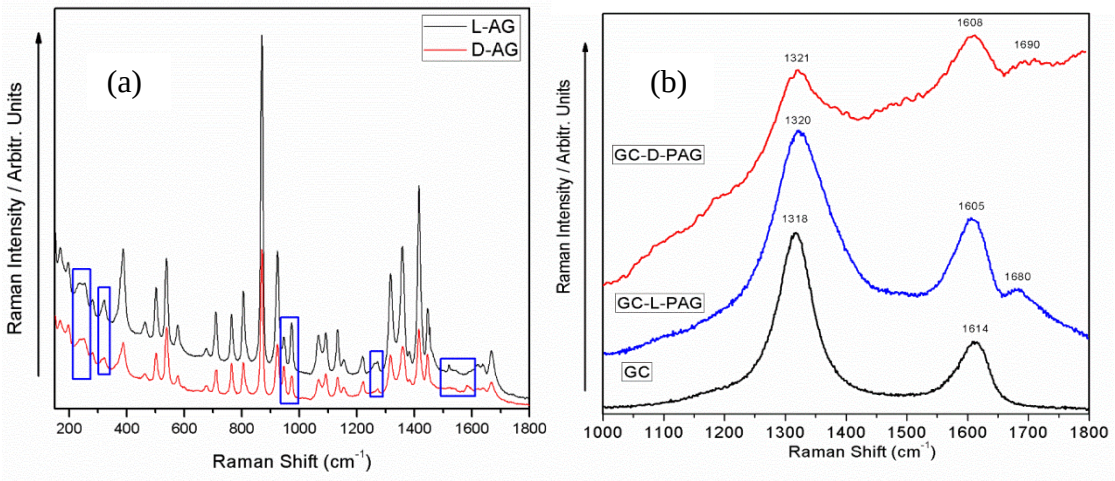

Figure 5. Raman spectra GC, GC/L-PGA y GC/D-PGA. Laser line785nm.

3.3 Electrochemical Oxidation of HCTZ on GC/L-PGA and GC/D-PGA

Differential Pulse Voltammetry was used to investigate the electrochemical oxidation of hydrochlorothiazide at GC/L-PGA and GC/D-PGA. The DPVs using GC, GC/L-PGA and GC/D-PGA in 0.1 M Buffer Britton-Robinson solution (pH 2) in the presence and absence of 1 mM HCTZ are presented in Figure 6. In the blank Britton-Robinson solution, the DPVs of GC (curve a), GC/L-PGA (curve b) and GC/D-PGA (curve c) did not show any oxidation peak. In the same figure we can see that the three systems in study showed a peak current oxidation in the presence of 1 mM HCTZ; nevertheless, GC/L-PGA and GC/D-PGA showed a higher current in comparison with GC unmodified. For GC/L-PGA the current is 2.0 times (8.6094 μA) GC and for GC/D-PGA is 2.5 times (9.456 μA) GC (5.1544 μA) under similar conditions. These results evidence that the GC/L-PGA and GC/D-PGA electrodes have a performance that is superior to that of unmodified GC. The registered DPVs show a resolute and symmetrical signal to 1 mM HCTZ under our experimental conditions. On the other hand, we can observe a slightly shift to more positive values potential at GC/D-PGA in comparison with GC/L-PGA and GC. This phenomenon could be explained by a possible hydrogen-bonding formation between the PGA on the surface electrode and HCTZ, which diffuses towards the modified electrode surface, displacing the oxidation potential towards more anodic values. This behavior is characteristic of an electrode surface modified with amino acids[12].

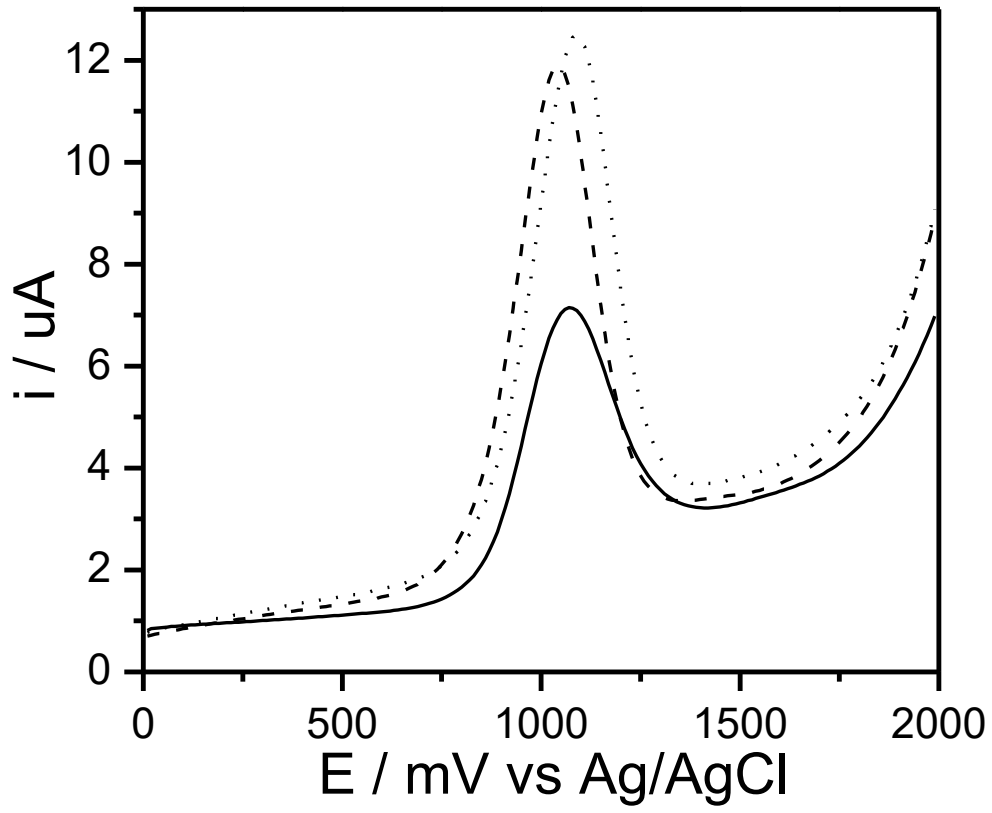

Figure 6. VDP 1 mM [HCTZ] in 0.1 M Buffer Britton-Robinson solution pH 2.( )GC, (---) GC/L-PAG, (•••) GC/D-PAG

3.3.2 Calibration curves of HCTZ

Calibration curve for standard additions of HCTZ corresponding to concentrations between $1x10^{-5}$ and $1x10^{-3}$ M, which were obtained using GC, GC/L-PGA and GC/D-PGA (Figure 8). The HCTZ detection limit (LOD) was determined from the equation $\frac{3SD}{sensitivity}$, where SD is the standard deviation of the response, and the sensitivity is the slope of the calibration plot. The quantification limit (LOQ), the lowest concentration that can be quantified with acceptable precision and accuracy, is given by LOQ $\frac{10SD}{sensitivity}$SD [31]. The electroanalytical determination of HCTZ was carried out by DPV and measured the total oxidation peak current of the drug with the three electrode systems. In figure 8 we can observe that the three analytical curves showed linearity in the studied concentration range, with a linear regression of 0.993, 0.994, and 0.993 for GC, GC/L-PGA and GC/D-PGA, respectively. The analytical parameters are provided in Table 2. According to these results, GC/L-PGA and GC/D-PGA present greater sensitivity in HCTZ determination than GC unmodified. Additionally, we can observe that GC/L-PGA and GC/D-PGA showed the minor LOD and LOQ to determine HCTZ in aqueous solution in comparison with GC unmodified. Nevertheless, GC/D-PGA presented lower LOD and LOQ than GC/L-PGA. These results are in accordance with the DPV response shown in figure 6, where a higher current peak oxidation for the HCTZ 1 mM solution, was observed GC/D-PGA. In this sense, the higher current observed for the oxidation of HCTZ could be due to a superficial difference between GC and GC with film of L- or D- glutamic acid. As can be seen in the Raman images (figure 5(b)), the difference is probably a consequence of the spatial arrangement of the -COOH and $-NH_2$ and the chiral carbon in the different AG enantiomer molecule configurations, which produces specific electrostatic forces and π-π interactions [32].

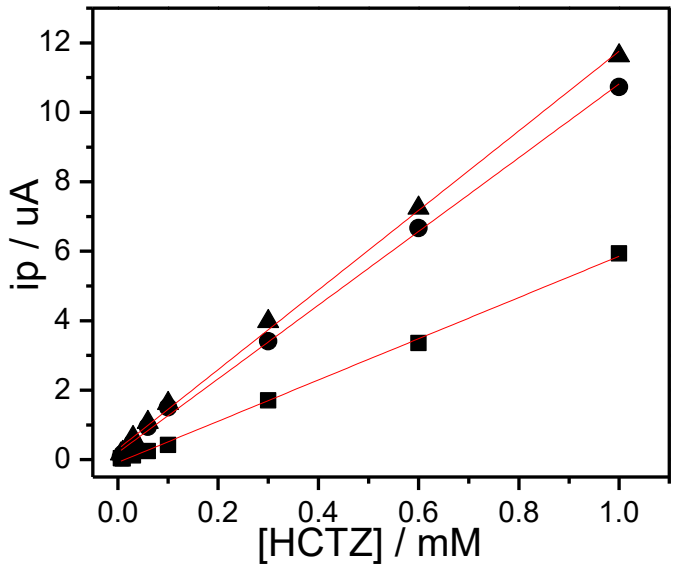

Figure 8. Plots calibration from 0.06 mM to 1 mM [HCTZ] in buffer Britton-Robinson solution pH 2, (■)GC, (●)GC/L-PAG, (▲)GC/D-PAG

Table 2. Analytical performance

	GC	***GC/L-PGA***	***GC/D-PGA***
LOD (mM)	*2.14 x10^{-2}*	*1.96 x10^{-2}*	*1.90 x10^{-2}*
LOQ (mM)	*7.15 x10^{-2}*	*6.50 x10^{-2}*	*6.33 x10^{-2}*
Sensitivity (μA mM^{-1})	*5.94*	*10.5*	*11.0*
r^2	*0.993*	*0.994*	*0.993*
% reproducibility	*1.26*	*3.61*	*2.15*
% repeatability	*8.60*	*10.7*	*8.60*

A comparison between the analytical parameters of the present method and some previous methods reported in literature for the HCTZ determination are showed in Table 3. Literature shows that chromatographic techniques exhibits better performance for HCTZ determination that others. However, electrochemical techniques require lower time and less solvents use in the total analysis. The spectrophotometric technique showed a highest LOD and LOQ that our method, but this technique has a low specificity in the general analysis. On the other hand, from these data, it can be seen that the LOD value obtained in this work for determination of HCTZ using our method is lower than those obtained by another electrochemical techniques, except using squarewave voltammetric (SWV).

Table 3. Comparison of the analytical performance of the proposed method with some previously reported methods for the determination of HCTZ.

Method	Lineal range (mM)	LOD (mM)	LOQ (mM)	[REF]
Spectrophotometric Dual Wavelength method	4.30 x10^{-3} -4.3.0 x10^{-2}	1.10 x10^{-2}	4.30 x10^{-3}	33
RP-HPLC	4.30 x10^{-2} - 4.30 x10^{-1}	3.70 x10^{-3}	1.10 x10^{-2}	34
LC-ESI-MS/MS	3.36 x10^{-6} - 3.36 x10^{-3}	1.68 x10^{-6}	3.36 x10^{-6}	35
LC/MS/MS	2.60 x10^{-6} - 6.70 x10^{-5}	1.30 x10^{-6}	2.60 x10^{-6}	36
SWV	5.00 x10^{-5} -0.20	2.00 x10^{-5}	5.00 x10^{-5}	37
SWV	1.97 x10^{-3} -8.81 x10^{-2}	6.39 x10^{-4}	1.97 x10^{-3}	38
Cyclic voltammetric	0.22 - 5.82	2.12 x10^{-2}	7.06 x10^{-2}	39
Cyclic voltammetric	0.01 - 0.60	5.00 x10^{-2}	1.00 x10^{-2}	40
DPV GC	7.15 x10^{-2} - 1.00	2.14 x10^{-2}	7.15 x10^{-2}	This work
DPV GC/L-PAG	6.50 x10^{-2}- 1.00	1.96 x10^{-2}	6.50 x10^{-2}	This work
DPV GC/D-PAG	6.33 x10^{-2}- 1.00	1.90 x10^{-2}	6.33 x10^{-2}	This work

RP-HPLC: Reverse Phase- High performance liquid chromatographic.
LC-ESI-MS/MS: Liquid Chromatography-Electrospray Ionization-tandem Mass Spectrometry/Mass Spectrometry.
LC/MS/MS Liquid Chromatography / Mass Spectrometry / Mass Spectrometry
SWV: Square-Wave voltammetric
DPV: Differential Pulse voltammetric

3.3.3 Analytical application, pharmaceutical HCTZ determination

The presence of various substances as compounds potentially interfering in HCTZ was studied under the described experimental conditions. The elements potentially interfering with HCTZ

determination in pharmaceuticals are the excipients. Figure 9 shows the DPV for synthetic tablet prepared with all the excipients present in the commercial tablet (LAB-CHILE® and ITF-LABOMED®) according to the experimental section. In the DPV, we can see that GC/L-PGA and GC/D-PGA do not exhibit any peak current attributable to any species present in the tablet. Thus, it can be established that both modified electrodes are selective in the presence of excipients.

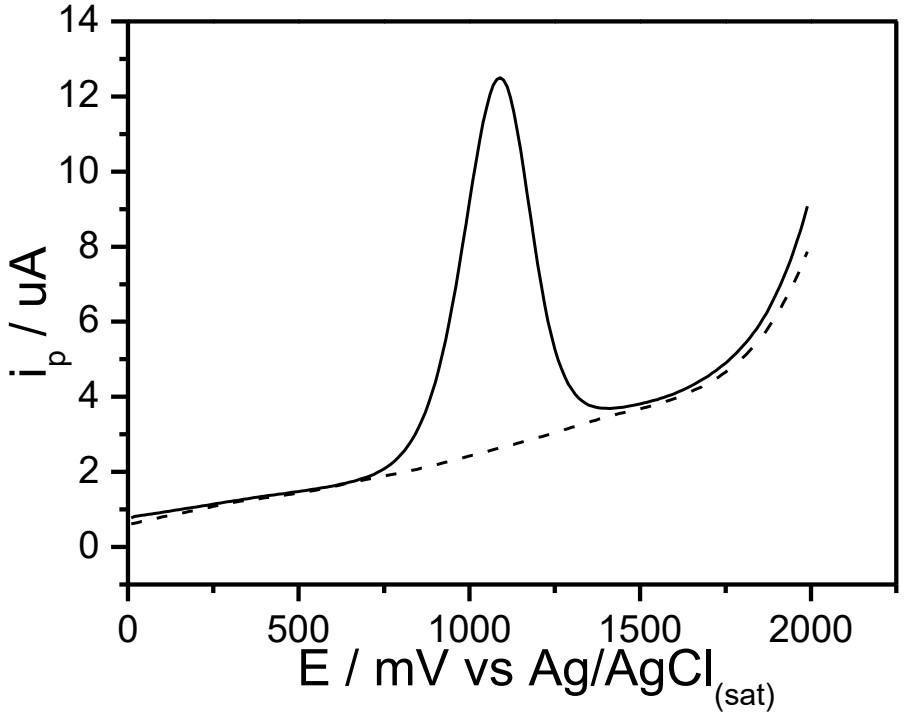

Figure 9. DPV (---) synthetic tablet without HCTZ, (—) HCTZ LAB-Chile® tablet in buffer Britton-Robinson 0,1 M pH 2.

Considering that pharmaceuticals excipients did not show an electroanalytical signal, the developed methodology was applied to determine HCTZ in a real sample, such as the commercial tablets from two laboratories, namely LAB-CHILE® and ITF-LABOMED®. The HCTZ declared concentration in both pharmaceuticals were 50 mg equivalent to 1.67×10^{-4} mol. The sample preparation was performed according to the Razak protocol [**Error! Bookmark not defined.**]; the determination was conducted by standard addition measured by DPV using modified electrodes, and these results were compared with the HPLC analysis, as shown in Table 4. For the determination of HCTZ in the LAB-CHILE® tablet, 95.93%, 102.0%, 98.24% were obtained for GC/L-PGA, GC/D-PGA and HPLC, respectively. On the other hand, 95.72%, 101.1% and 98.40% were respectively found for GC/L-PGA, GC/D-PGA and HPLC in the case of the determination in ITF-LABOMED tablet. These percentages corresponds to the label claims (50 mg/tablet) and fall within the percentages accepted by the United Sates Pharmacopoeia (95.0-105%). In addition, it is important to note that the content for all assayed tablets in the individual tablet assay is within the range of 85-115% of the label claim and no unit is outside the 75.0-125.0% range claimed in the label. Therefore, they fulfill the

Pharmacopoeia uniformity content requirement for tablets [41]. The results obtained with each method (DPV and HPLC analysis) were compared by applying Snedecor F-test (variance proportion), showing that there are no significant differences between them [42]

Table 4. Individual tablet assay of hydrochlorothiazide[a]

Tablet	DPV								HPLC			
	GC/L-PAG				GC/D-PAG							
	LAB-Chile®		ITF-LABOMED®		LAB-Chile®		ITF-LABOMED®		LAB-Chile®		ITF-LABOMED®	
	Found (mg)	Found (%)	Found (mg)	Found (%)	Found (mg)	Found (%)	Found (mg)	Found (%)	Found (mg)	Found (%)	Found (mg)	Found (%)
1	47.51	95.02	48.63	97.26	51.68	103.9	50.68	101.36	49.36	98.72	49.21	98.42
2	48.63	97.26	47.32	95.26	49.32	98.64	49.32	98.64	49.03	98.06	48.95	97.90
3	47.76	95.52	47.64	95.28	52.01	104.02	51.63	103.3	48.98	97.96	49.45	98.90
average	47.96	95,93	47.86	95.72	51.00	102.0	50.54	101.1	49.12	98.24	49.20	98.40
S.D.	0.48		0,55		1.19		0.94		0.17		0.13	
V.C. %	1.00		1.15		2.33		1.85		0.34		0.28	

[a] Declared amount/tablet: 50 mg HCTZ

4. CONCLUSIONS

A new modified glassy carbon electrode based on the electropolimerization of L-GA and D-GA for the determination of HCTZ in water was developed. Moreover, with both modified electrodes, GC/L-PGA and GC/D-PGA, the presence of hydrochlorothiazide in pharmaceuticals forms was determined by differential pulse voltammetry, in accordance with the values accepted for USP. Thus, this modified electrode constitutes an alternative to HCTZ determination in aqueous solution and pharmaceuticals. The LOD and LOQ achieved for HCTZ determination with both GCE modified are better than those obtained with GCE unmodified. In this sense, surface modification increases method sensibility. The development of this method is not time-consuming and less expensive than the methods used for HPLC.

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