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Determination of hydrochlorothiazide in pharmaceutical formulations using activated carbon paste electrode by cyclic voltammetry

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Abstract : A voltammetric study of hydrochlorothiazide (HTZ) at activated carbon paste electrode was carried out. The drug in phosphate buffer (0.2 mol/l, pH 7.7) is oxidized at +800 mV using 100 mV s^{-1} scanning rate, giving rise to a well-defined anodic peak. Cyclic voltammetric study indicates that the oxidation process is irreversible and diffusion-controlled. A sensitive, simple and time-saving anodic cyclic voltammetric procedure has been developed. The procedure has been applied for the drug determination in pure form and in commercial tablets with no prior extraction. HTZ showed a significant current response at activated carbon paste electrode under the optimum conditions with two linear dynamic ranges (experimental detection limit of the standard solution was 2×10^{-6} mol/l). The linear calibration ranges were between 6.5×10^{-6} - 1×10^{-4} mol/l and 1×10^{-4} - 3×10^{-3} mol/l HTZ.

Keywords : Hydrochlorothiazide; Cyclic voltammetry; Activated carbon paste electrode; Commercial tablets.

INTRODUCTION

Hydrochlorothiazide (HTZ) (6-chloro-3,4-dihydro-2H-1,2,4-benzo-thiadiazine-7-sulfonamide 1,1-dioxide) (Figure 1) has gained attention because it is a benzothiazide diuretic drug that acts directly on the kidney by increasing the excretion of sodium chloride and water and, to a lesser extent, that of potassium ions. HTZ is an antihypertensive substance and improves the action of other hypotensive substances, allowing a decrease in the dose of those below the level where these substances present secondary effects. Novello and Sprague observed the diuretic effect of thiazides with chlorothiazide in 1957[1]. HTZ is well absorbed, shortly bound to plasma proteins, and mainly excreted unchanged in the urine[2]. It is indicated for the treatment of edema, control of essential hypertension and management of diabetes insipidus[3].

Several methods have been reported for the determination of HTZ. Among these methods are high-performance liquid chromatography[4-9], capillary electrophoresis[10-12], spectrophotometry[13-16], chemilumines-

ORIGINAL ARTICLE

cence[17,18], conductimetric[19], first derivative differential pulse polarography[20,21], LC–MS/MS[22,23], square wave voltammetry[24,25], differential pulse voltammetry[26-30] and adsorptive stripping voltammetry[31].

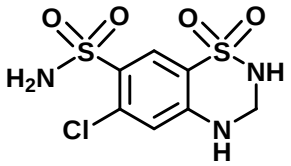

Figure 1 : The molecular structure of hydrochlorothiazide.

Electrochemical sensors[32] owing to their inherent specificity, rapid response, sensitivity and simplicity of preparation were considered intensively for the determination of various pharmaceutical products in biological fluids and in pharmaceutical preparations. One of the electrochemical techniques is cyclic voltammetry which allows direct and rapid measurements, its sensitivity is sufficient to allow the determination of HTZ in pure form and pharmaceutical preparations.

The oxidation of HTZ at ordinary carbon electrode has a very poor electrochemical response, and has large oxidation overpotential[18], therefore, in this study the application of electrochemically activated carbon paste electrode (ACPE) was discussed as a suitable electrode for the determination of HTZ in aqueous media using cyclic voltammetry, where activated electrodes were used for the electrochemical determination of many other compounds[33-35].

EXPERIMENTAL

Materials and reagents

Pure HTZ in powdered form was obtained from SmithKline Beecham Egypt Co., Cairo, Egypt and used as received. Its pharmaceutical preparations (capozide, monozide and aldactazide tablets) were obtained from local drug stores. Paraffin oil (IR grade) and graphite powder (particle size <50 micron) were used as the pasting liquid and the working electrode substrate, respectively. All other reagents used were of analytical grade (purchased from Merck (Darmstadt, Germany)) and their solutions were prepared with doubly distilled water.

Concentrated stock solution of HTZ (1.0×10^{-2} mol/l) was prepared in 0.06 mol/l NaOH and kept in dark vials in the refrigerator. Working solutions of lower concentrations were prepared daily by appropriate dilution with the selected supporting electrolyte.

Apparatus

All the voltammetric measurements were performed using a VoltaLab 06 (PST 006 & Voltamaster 4) Potentiostat. Ag|AgCl|3M KCl electrode models Metrohm 6.0733.100 and platinum wire were used as the reference and the auxiliary electrodes, respectively. Carbon paste electrode (CPE) (see 2.3) was used as the working electrode.

pH measurements were carried out using Hanna pH-millvoltmeter model 8519. All electrochemical experiments were carried out in a one-compartment 20 ml voltammetric cell at the ambient temperature.

Preparation of the carbon paste electrode (CPE)

A teflon holder (12 cm length) with a hole at one end (3 mm diameter, 3.5 mm depth) for the carbon paste filling served as the electrode body. Electrical contact was made with a stainless steel rod through the center of the holder. This rod can move up and down by screw movement to press the paste down when renewal of the electrode surface is needed. The paste of each electrode was prepared by mixing 150 mg of high purity graphite with 136 µl paraffin oil. Very intimate homogenization is then achieved by careful mixing with glass rod in agate mortar and afterwards rubbed by intensive pressing with a pestle. The ready-prepared paste is then packed into the hole of the electrode body. The carbon paste was smoothed onto paper until it has a shiny appearance. Then it was rinsed gently with bi-distilled water, and used directly for voltammetric measurements.

Construction of calibration graph

The electrode surface was renewed when needed using the screw movement to press paste down. Before each voltammogram was run, the electrode was resurfaced by smoothing onto paper. The CPE was first activated in phosphate buffer (0.2 M, pH 7.7) by cyclic voltammetric sweeps from +0.5 to +2 V until a stable cyclic voltammogram was obtained. Then, the electrode was transferred into another cell containing aliquots of the prepared standard solution of HTZ.

ORIGINAL ARTICLE

The accumulation step was carried out under open-circuit potential with 1.5 min. stirred solution, then the cyclic voltammograms from +550 to +1000 mV were recorded with a scan rate of 100 mV s^{-1} after 15 s quiet time, and finally the peak current at +800 mV was measured. All measurements were carried out in four replicates for each concentration. The oxidation peak current obtained was plotted as a function of HTZ concentration to construct the calibration graph. The concentration of HTZ was calculated using standard addition method.

Analysis of pharmaceutical preparations

TABLE 1 includes the analyzed pharmaceutical preparations. Ten tablets were weighed and powdered. A portion of the powder equivalent to the average weight of one tablet was transferred into a proper volumetric flask using 0.01 M NaOH solution (all prepared solutions were 0.5 mg ml^{-1}). The mixture was sonicated for 5 min then completed to the mark with the same solvent. The resulted suspension was allowed to settle and aliquots from the supernatant solution were diluted to 20 ml using phosphate buffer and subjected to voltammetric measurement mentioned previously.

RESULTS AND DISCUSSION

Electrocatalytic activation and the electrode performance enhancement

Cyclic voltammetric technique was applied as a diagnostic tool to get information about the mechanism of the redox reaction of HTZ at the activated CPE. It was found that the drug in phosphate buffer pH 7.7 gave anodic peak at +800 mV and no peaks were observed in the cathodic scan (Figure 2b); pointing to the irreversibility of the oxidation process.

Figure 2a shows that the oxidation peak is wide and covers the potential range between +0.75 and +1.00 V (without activation), which makes difficult to measure within this potential range. The electrochemical activation of the working electrode surface is a specific type of modification to enhance sensitivity and selectivity in voltammetric analysis of organic compounds[36-39]. In our case it was observed that two successive cyclic voltammetric sweeps from +0.5 to +2 V are sufficient to obtain a stable and reproducible response of the working electrode.

It was also observed that the oxidation peak current of HTZ increased significantly after pretreatment (Figure 2b). The peak current enhancement may be attributed to the electrolytic activation (anodic-cathodic cycling via intensive electrode oxidation/reduction at high potential), where a partial oxidation of the surface of graphite particles exposed to the solution. During their activation, various oxygen-containing functional groups are formed and instantaneously protonated. Owing to these fragments the electrode surface become markedly hydrophilic and repels hydrophobic molecules of the binder. So, this activation leads to a removal of the lipophilic layer of pasting liquid and results in the principal changes of surface conditions at CPEs. Their surface becomes hydrophilic and behaves, more or less, like that of solid graphites. The enhancement of the current and the decrease of E_p of the activated electrode may be attributed also to surface roughness causing a higher effective area for electron transfer than the geometric area. To prove this assumption, scanning electron microscopy (SEM) was applied.

Scanning electron microscopy (SEM) of untreated and activated CPEs

The structures of both electrodes were studied by SEM. There are appreciable differences in morphology of SEM of untreated and ACPE (Figure 3). After activation, the surface became rougher. The roughness of the electrode surface could provide more sites for the accumulation of HTZ, and could improve the sensitivity of the electrode.

Effect of accumulation conditions

The interfacial accumulation of HTZ on ACPE surface is indicated from the cyclic voltammograms

TABLE 1 : Pharmaceutical formulations analyzed

Trade name	Ingredients per tablet	Company
Aldactazide	25 mg HTZ and 25 mg spironolactone	Kahira Pharm. & Chem. Ind. Co. Egypt
Capozide	25 mg HTZ and 50 mg captopril	SmithKline Beecham Egypt LLC.
Monozide	12.5 mg HTZ and 10 mg fosinopril sodium	SmithKline Beecham Egypt LLC.

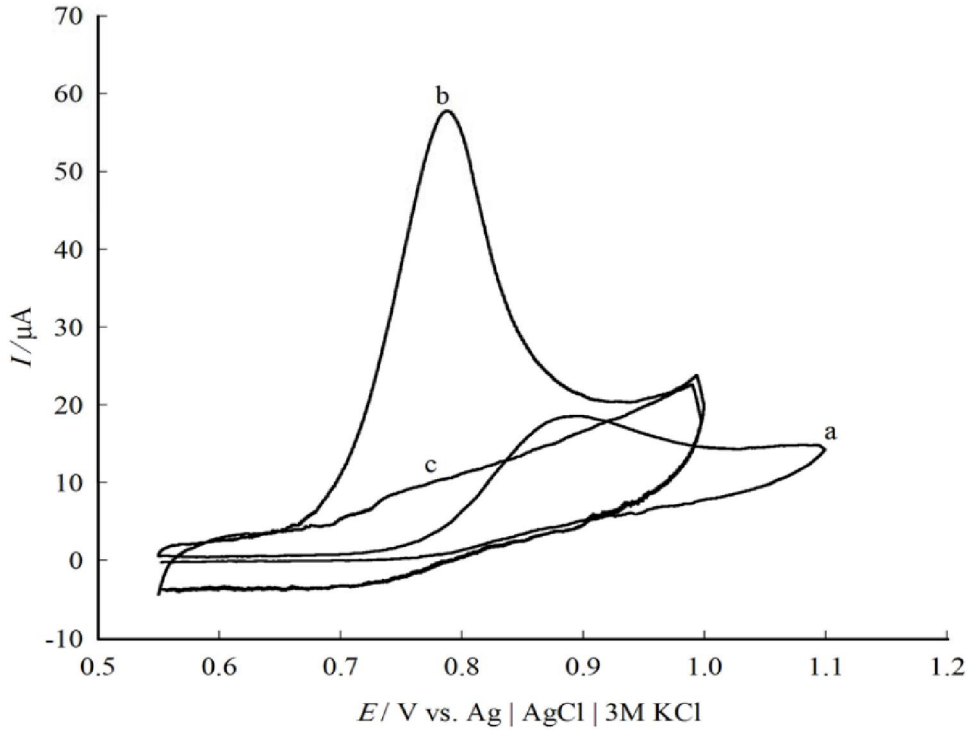

Figure 2 : Cyclic voltammograms of (a) untreated, (b) activated carbon paste electrodes in solutions having 2.5 × 10^{-4} mol/l HTZ and (c) an analogous voltammogram of blank phosphate buffer (0.2 M, pH 7.7) using activated CPE, with scan rate 100 mV s^{-1}.

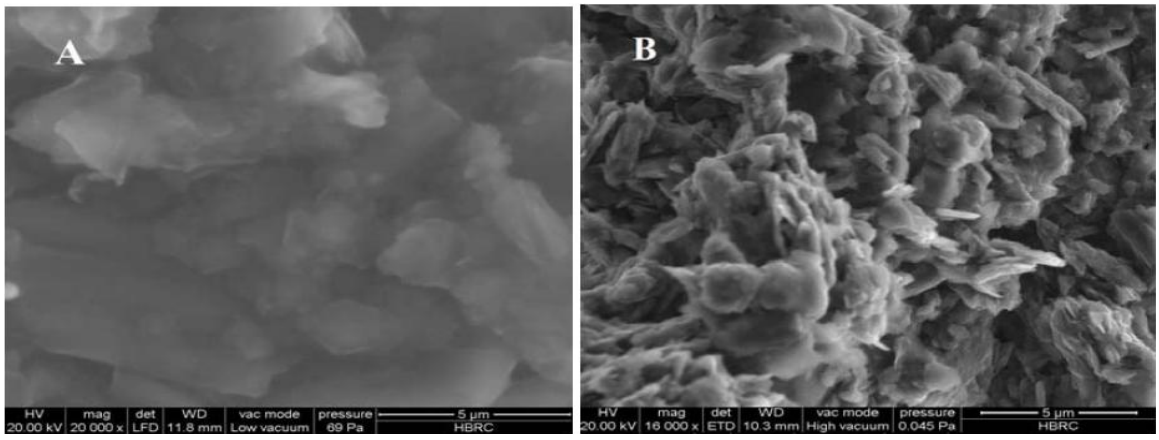

Figure 3 : SEM images of surface films of (A) untreated electrode and (B) ACPE.

recorded before and after stirring the solution. The deposition of the analyzed drug on the surface of the ACPE is one of the essential conditions for highly sensitive voltammetric determinations. The dependence of the peak current developed in phosphate buffer solution (pH 7.7) on the accumulation time (from 0-180 s) was studied at HTZ concentration of 5×10^{-5} mol/l (Figure 4). The effect of accumulation time ($t_{acc.}$) on the amount of drug accumulated on the electrode surface increased till 90 s. However, with further increasing of accumulation time beyond 90 s, the peak current tends to be almost stable. Therefore, optimal accumulation time of 90 s was employed in further experiments.

The effect of the accumulation potential as a function of the peak current of investigated drug was evaluated over a range of +0.2 to +1.3 V. The peak current was independent on accumulation potential; thus the adsorption stage was carried out at an open-circuit potential.

Effect of supporting electrolyte and pH value

The electrochemical oxidation of 2.5×10^{-5} mol/l

ORIGINAL ARTICLE

HTZ in presence of phosphate buffer (PB) (0.2 mol/l, Na_2HPO_4 and KH_2PO_4, pH 5.0 ~ 8.0), Britton-Robinson buffer (B-R) (0.08 mol/l boric, phosphoric and acetic acids adjusted with varying amounts of sodium hydroxide to pH values of 4 ~ 8) and McIlvaine buffer (different mixtures of 0.2 mol/l Na_2HPO_4 and 0.1 mol/l citric acid, pH 3 ~ 8) were investigated in details and the results were summarized in TABLE 2. It was found that the highest oxidation peak current of HTZ was obtained in 0.2 M, pH 7.7 phosphate buffer. Both of peak potential and peak current intensity depend on the pH of the supporting electrolyte (Figure 5). This pH-dependence indicates the involvement of protons in the electrode reaction and that the proton-transfer reaction precedes the electrode process properly. The slope value (Figure 5b) of -77 mV per pH reveals that the same number of protons and electrons are involved in the oxidation process, which is in agreement with the mechanism proposed by O. A. Razak[30]. Thus, 0.2 mol/l phosphate buffer (pH 7.7) was used as the supporting electrolyte for the cyclic voltammetric determination of HTZ.

Effect of scan rate

The effect of scan rate (v) on the peak potential (E_p) and on the peak current (I_p) was examined from 10 to 400 mV s^{-1} using 9×10^{-4} mol/l HTZ (Figure 6). The peak potential was variable with the scan rate (81

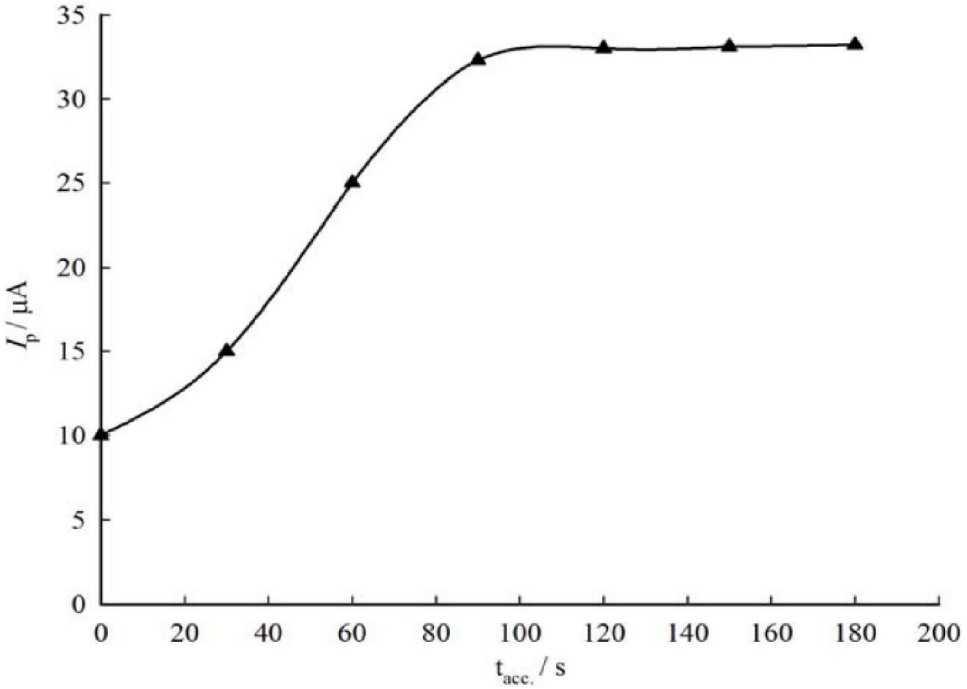

Figure 4 : Influence of accumulation time on the oxidation peak current of 5.0×10^{-5} mol/l HTZ.

TABLE 2 : Supporting electrolyte and optimum pH

Britton-Robinson buffer (B-R)			McIlvaine buffer			Phosphate buffer (PB)		
pH	I_p / μA	E_p / mV	pH	I_p / μA	E_p / mV	pH	I_p / μA	E_p / mV
4	4.5310	1031	3	11.768	1216	5.29	20.87	966
5	7.2040	968.0	4	12.494	1146	5.91	20.58	910
6	7.2770	892.0	5	15.158	1079	6.47	20.28	867
7	8.3180	823.5	6	15.645	1022	6.81	22.06	840
7.7	8.6820	772.5	7	15.858	934.0	7.17	24.57	813
8	6.9570	752.5	7.7	16.502	890.0	7.38	25.39	798
			8	16.833	870.0	7.7*	26.57	773
						8.04	24.80	753

Note: *The best pH value and buffer (supporting electrolyte).

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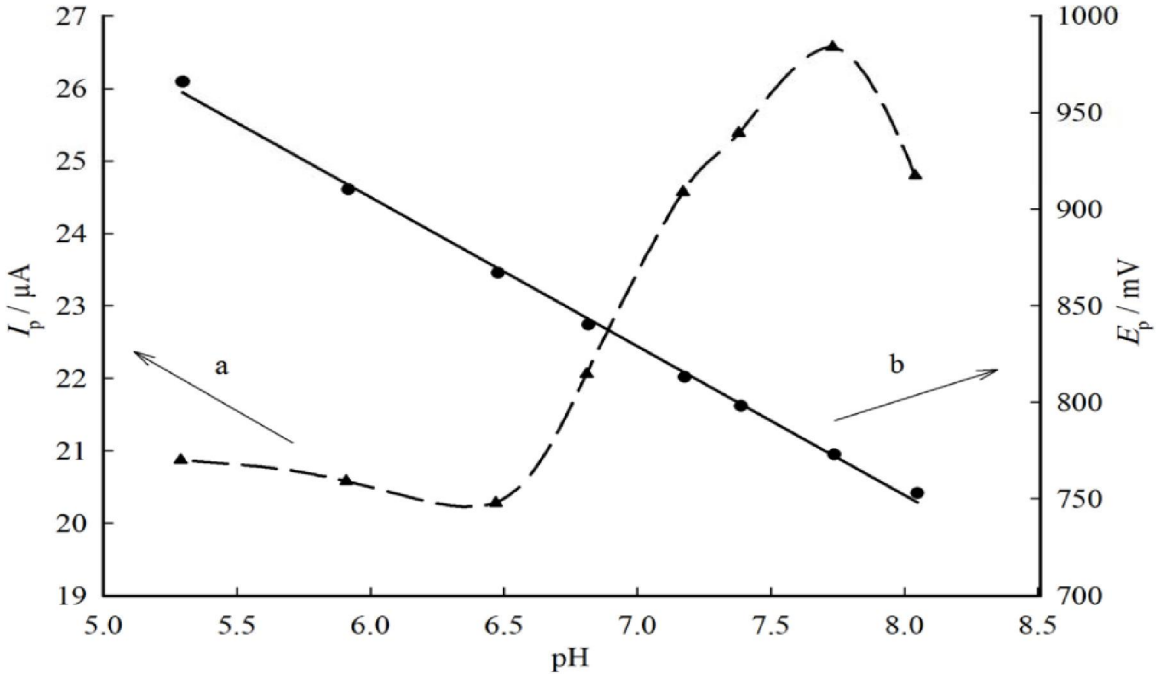

Figure 5 : Effect of pH of phosphate buffer on (a) oxidation peak current, (b) oxidation peak potential (E_p = -77.1923 pH +1368, r^2 = 0.9977), each containing 2.5×10^{-5} mol/ l HTZ, scan rate 100 mV s^{-1}.

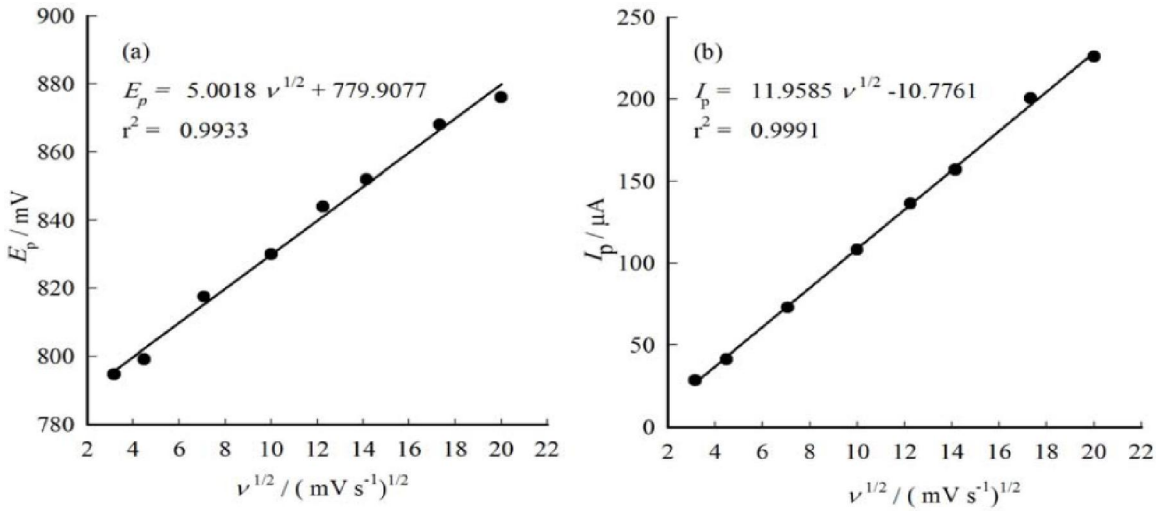

Figure 6 : (a) Dependence of oxidation peak potential on square root of the scan rate, (b) Dependence of oxidation peak current on the square root of scan rate at activated CPE in solution having 9 $\times10^{-4}$ mol/ l HTZ and PB (0.2 M, pH 7.7) with an accumulation time of 90 s at open-circuit potential.

mV positive shift in the peak potential on increasing the scan rate) confirming the irreversibility of the oxidation process of HTZ at the ACPE[40].

A linear Randles-Seveik plot (plot of I_p against $\nu^{1/2}$) (correlation coefficient = 0.9991) was obtained indicating that diffusion is the main contributing process of mass transport[41]. In this study, 100 mV s^{-1} was chosen as the scan rate for cyclic voltammetric measurements.

Linear range, limit of detection and precision

According to the obtained results, we recommended the application of cyclic voltammetric technique using ACPE for the quantitative analysis of HTZ in the pure form and in the pharmaceutical preparations. The phosphate buffer solution of pH 7.7 was selected as the supporting electrolyte for the quantification of HTZ as it gave maximum peak current with scan rate of 100 mV s^{-1} and accumulation time 90 s at open circuit po-

ORIGINAL ARTICLE

tential. The peak current increased linearly with increasing HTZ concentration (Figure 7). Applying the optimum conditions described above, the results showed linearity over two concentration ranges with different slopes: for 6.5×10^{-6} - 1×10^{-4} mol/l HTZ [the regression equation was $I_{p,}$ μA = 303.1856 C_{HTZ} + 17.4911 (r^2 = 0.998) (Figure 7a)] and for 1×10^{-4} - 3×10^{-3} mol/l HTZ [the regression equation was I_p, μA = 70.3287 C_{HTZ} + 43.2225 (r^2 = 0.9954) (Figure 7b)], where C_{HTZ} is the concentration of HTZ in mmol/l. Deviation from linearity was observed for more concentrated solutions, due to the adsorption of HTZ or its oxidative product on the electrode surface.

The detection limit for the standard solution was obtained experimentally as 2×10^{-6} mol/l HTZ.

In order to study the reproducibility of the electrode preparation procedure, a 4.0×10^{-4} mol/l HTZ solution was measured with the same electrode (renewed every time) for every several hours within a day, the RSD of the peak current was 3.82% (n = 7). The

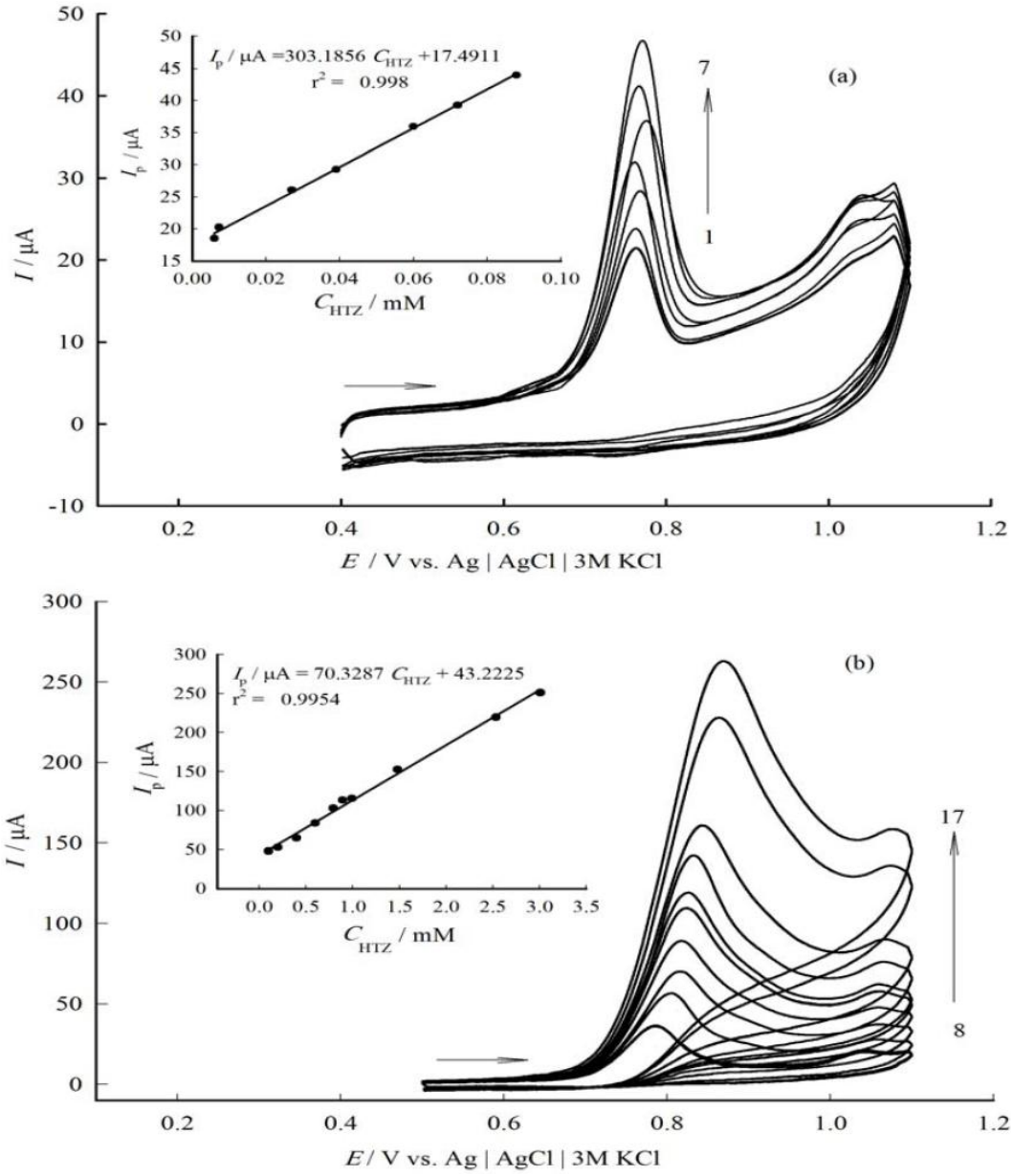

Figure 7 : Cyclic voltammograms of ACPE in the buffer solution (pH 7.7) containing: (a) curves (1-7) corresponding to 6, 7.3, 27, 39, 60, 72 and 88 mmol/ l HTZ, (b) Curves (8-17) corresponding to 0.1, 0.2, 0.4, 0.6, 0.8, 0.9, 1, 1.5, 2.5 and 3 mmol/ l HTZ. Inset: calibration plots between HTZ concentration and oxidation peak current. Scan rate 100 mV s^{-1} and accumulation time 90 s at open-circuit potential.

ORIGINAL ARTICLE

between days reproducibility was similar to that of within a day if the temperature was kept almost unchanged. Owing to the adsorption of oxidative product of HTZ onto the electrode surface, the current response of the activated electrode would decrease after successive use. In this case, the electrode should be activated again (see section 2.4).

The proposed method is selective for HTZ in presence of its related substances, chlorothiazide and salamide (4-amino-6-chlorobenzene-1,3-disulphonamide)[42] as well as its hydrolytic and photodegradation product (5-chloro-2, 4- disulfamoylaniline)[43]; as they lack the presence of the two hydrogens in the 3,4-positions, which are oxidized (dehydrogenated) at the ACPE under the recommended conditions.

A comparison between the analytical parameters of the present method and some previous methods reported in the literature for the determination of HTZ is given in TABLE 3. From these data, it can be seen that the DL and selectivity of the proposed method are comparable with the other methods in addition to its simplicity.

TABLE 3 : Comparison of the analytical parameters obtained using different electrodes and/or methods for the determination of HTZ

Electrode	Methods	pH	Concentration range (mol/l)	Detection limits (mol/l)	Reference
GCE	DPV	3.3	8.1×10^{-8} - 1.1×10^{-6}	1.7×10^{-8}	[30]
GCE/ MWCNTs	AdSCV	7	2.0×10^{-9} - 2.0×10^{-8} and 2.0×10^{-7} - 1.0×10^{-4}	8.0×10^{-10}	[31]
CPE/ FDC	SWV	9	8.0×10^{-8} - 5.8×10^{-6} and 5.8×10^{-6} - 5.0×10^{-4}	3.7×10^{-8}	[25]
MWCNT/ SR	DPV	7	5.0×10^{-6} - 7.0×10^{-5}	2.6×10^{-6}	[29]
BDDE	DPV	9.5	3.0×10^{-6} - 7.4×10^{-5}	1.2×10^{-6}	[27]
GR/ Fc/ CP	DPV	7	5.0×10^{-7}- 3.9×10^{-4}	3.8×10^{-7}	[28]
MIPs/ MWCNTs/ PGE	DPV	2.67	9.0×10^{-10} -1.0×10^{-5} and 1.0×10^{-5} - 1.0×10^{-2}	1.0×10^{-10}	[26]
ACPE	CV	7.7	6.5×10^{-6} - $1.0\times10^{?4}$ and 1.0×10^{-4} - 3.0×10^{-3}	2.0×10^{-6}	Present work

Note: AdSCV is the adsorptive stripping cyclic voltammetry; FDC is the ferrocenedicarboxylic acid; MWCNT/ SR are the multiwall carbon nanotube/silicone rubber; BDDE is the boron-doped diamond electrode; GR/Fc/CP is the graphene/ferrocene composite carbon paste electrode; MIPs is the molecularly imprinted polymers.

Interferences

Hydrochlorothiazide is formulated as a single component tablets and in multi-ingradient preparations. Interference studies were carried out in order to investigate the effect of the co-formulated drugs (captopril, fosinopril and spironolactone) and some common excipients used in pharmaceutical preparations on the anodic voltammetric determination of HTZ. The voltammograms of the rest of drugs solutions in phosphate buffer pH 7.7 were recorded from +500 to +1000 mV by analyzing sample solutions containing fixed amount of 4×10^{-4} mol/l HTZ solution spiked with various excess amounts (up to 50-folds) under the same experimental conditions. The tolerance limit was taken as the maximum concentration of the foreign substances which caused an approximately ±5% relative error in the determination. As can be seen from TABLE 4, there was no serious interference occurred from the tested materials rather than glycine and ascorbic acid that had apparent influence on the voltammetric signal of HTZ.

TABLE 4 : Influence of potential interferents on the voltammetric response of 4.0×10^{-4} mol /l HTZ

Interferents	Concentration (mol/ l)	Signal change (%)
Captopril	2.0×10^{-2}	2.50
Spironolactone	2.0×10^{-2}	1.70
Fosinopril	2.0×10^{-2}	1.25
Glucose	2.0×10^{-2}	- 0.81
Lactose	2.0×10^{-2}	0.87
Starch	2.0×10^{-2}	- 3.14
Magnesium stearate	2.0×10^{-2}	1.56
Citric acid	2.0×10^{-2}	-3.45
Glycin	2.0×10^{-2}	5.50
Ascorbic acid	2.0×10^{-2}	7.50

Analytical applications

In order to evaluate the applicability of the proposed method for the real sample analysis, it was used to detect HTZ in tablets. The procedure for the tablet analysis was followed as described in section 2.5. The results are in good agreement with the content marked

ORIGINAL ARTICLE

in the label. Recovery studies were carried out after the addition of known amounts of the drug to various preanalyzed formulations of HTZ. The results are listed in TABLE 5. The recoveries in different samples were found to lie in the range of 98.21 - 104.35%, with RSD of 1.36 - 4%.

TABLE 5 : Determination of HTZ in pure solution and in drugs

Drug	Taken (mol/ l)	Found* (mol/ l)	Recovery %	RSD %
Pure solution	1.68×10^{-4}	1.591×10^{-4}	94.702	2.08
	2.52×10^{-4}	2.440×10^{-4}	96.825	2.21
	3.36×10^{-4}	3.344×10^{-4}	99.524	1.70
	4.20×10^{-4}	4.112×10^{-4}	97.905	1.87
Aldactazide	1.68×10^{-4}	1.660×10^{-4}	98.81	3.38
	2.52×10^{-4}	2.508×10^{-4}	99.52	3.14
	3.36×10^{-4}	3.506×10^{-4}	104.35	2.23
	4.20×10^{-4}	4.032×10^{-4}	96.00	2.78
Capozide	1.68×10^{-4}	1.740×10^{-4}	103.57	2.80
	2.52×10^{-4}	2.475×10^{-4}	98.21	1.91
	3.36×10^{-4}	3.315×10^{-4}	98.66	2.49
	4.20×10^{-4}	4.308×10^{-4}	102.57	1.24
Monozide	1.68×10^{-4}	1.716×10^{-4}	102.14	2.16
	2.52×10^{-4}	2.495×10^{-4}	99.01	4.00
	3.36×10^{-4}	3.325×10^{-4}	98.96	1.36
	4.20×10^{-4}	4.215×10^{-4}	100.36	3.70

Note: * Average of four replicate measurements.

CONCLUSION

The results obtained in the determination of HTZ allowed concluding that the activated carbon paste can be used as an electrode material, presenting advantages in relation to the analogue composites prepared with graphite, verified by good sensitivity and low DL obtained with activated carbon paste electrode. The main advantages in relation to the other papers described in the literature are the possibility of rapid determination without the necessity of sample pretreatments or time-consuming extraction or overlapped data analysis, with satisfactory results. The effects of potential interfering materials were studied, and it was found that the proposed procedure is free from interference from most common interfering organic compounds. The simple fabrication procedure, high speed, reproducibility, high stability, wide linear dynamic range, low detection limit, high sensitivity and a distinct advantage of polishing in the event of surface fouling, suggest that the proposed sensor is an attractive candidate for practical applications. Furthermore, the present method could possibly be adopted for quality control laboratories.

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