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Short communication

Electrochemical study of hydrochlorothiazide and its determination in urine and tablets

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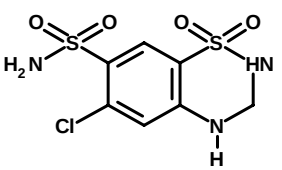
Abstract

A voltammetric study of hydrochlorothiazide (HCTZ) at glassy carbon electrode was carried out. The drug in Britton–Robinson buffer (pH 3.3) is oxidized at +1040 mV, giving rise to a well-defined peak. Cyclic voltammetric study indicates that the oxidation process is irreversible and diffusion-controlled.

A sensitive, simple and time-saving differential pulse anodic voltammetric procedure has been developed. The procedure has been applied for the drug determination in human urine with no prior extraction and in commercial tablets. The proposed method has been validated. The limit of detection for the standard solution was 5.0 ng ml^{-1} and for the drug in urine was 14.0 ng ml^{-1}. The results obtained from the analysis of commercial tablets were compared statistically with those obtained from the USP 26 high-performance liquid chromatographic (HPLC) method.

Keywords: Hydrochlorothiazide; Cyclic voltammetry; Differential pulse anodic voltammetry; Human urine; Commercial tablets

1. Introduction



Hydrochlorothiazide (HCTZ) (6-chloro-3,4-dihydro-2*H*-1,2,4-benzo-thiadiazine-7-sulfonamide 1,1-dioxide) is a widely prescribed diuretic. It is indicated for the treatment of edema, control of essential hypertension and management of diabetes insipidus [1]. Hydrochlorthiazide is official in the European pharmacopoeia 4 [2]; the drug and its tablets are official in the USP 26 which describes high-performance liquid chromatographic (HPLC) procedures for their quantitations [3].

Different methods have been applied for the determination of HCTZ in bulk and pharmaceutical preparations including capillary electrophoresis [4], chemiluminescence [5], HPLC [6–10], spectrophotometric [11], conductimetric [12] and first derivative differential pulse polarography [13]. A high-performance liquid chromatographic method was developed for the screening of 17 diuretics (in-

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cluding HCTZ) in urine after extraction with ethyl acetate [14]. The quantitation of HCTZ in urine was based on using different liquid chromatographic techniques such as narrow bore HPLC [15], fully automated procedure [16] and miceller LC [17]. The literature reveals that no voltammetric methods were developed from the analysis of HCTZ.

Due to the frequency in which HCTZ is prescribed and its abuse for reduction of body weight by some athletes (who are categorized in several body weight classes) [14], its analysis is of great importance. Urinary recovery is the preferred way for analyzing and estimating bioavailability of HCTZ in humans [18,19] because it is not metabolized and excreted unchanged in urine [1].

Owing to the high sensitivity and simplicity of the voltammetric techniques, this work was directed to study the voltammetric behavior of HCTZ and to find out simple, rapid, sensitive and selective voltammetric procedure for HCTZ determination in human urine and in pharmaceutical tablets.

2. Experimental

2.1. Apparatus

Voltammetric measurements were performed using Metrohm 693 VA stand. The three electrode system consisted of a glassy carbon working electrode (disk diameter = 2.0 ± 0.1 mm), Ag/AgCl (3 M KCl) reference electrode and a Pt auxiliary electrode.

High-performance liquid chromatographic experiments were carried out using an HPLC Shimadzu model C-R7A Plus chromatopac equipped with a UV detector SPD-10A.

All pH measurements were made with a Schott-Gerate pH meter Model CG 710 calibrated with standard buffers.

2.2. Materials and reagents

- Authentic samples of hydrochlorothiazine (HCTZ) amiloride, bisoprolol fumarate, captopril, enalapril, lisinopril, losartam potassium, methyldopa, metoprolol tartarate, propranolol, reserpine, spironolactone, and triamterene were kindly donated by the manufacturers and were used without any purification.
- Britton–Robinson (BR) buffer 0.08 M [20].
- Monozide® tablets (The Jordanian Pharmaceutical Manufacturing Co. Ltd.) Labeled to contain 25 mg HCTZ per tablet.
- Concentrated solution of HCTZ (1 mg ml^{-1}) was prepared in 0.02 M NaOH. A 0.1 ml from this solution was diluted to 250 ml using BR buffer pH 3.3 to obtain standard stock solution of 400 ng ml^{-1}. The stock solution was stable for at least 1 week when kept in the refrigerator.
- All solvents and chemicals used were of analytical grade.

2.3. Construction of calibration curve

Aliquots from the standard stock solution (0.3–4 ml) were transferred into a set of 5 ml volumetric flasks. The volumes were completed to mark with BR buffer pH 3.3 the content of each flask was transferred into the measuring vessel for measurement. Before each voltammogram was run the glassy carbon electrode was resurfaced by polishing for about 30 s with aqueous slurry of α-alumina using polishing cloth. The differential pulse scan from +800 to +1200 mV was conducted using the following parameters: pulse amplitude = 50 mV, time interval for voltage step = 0.1 s, measurement time = 20 ms, pulse time = 40 ms, and scan rate = 100 mV s^{-1}. The peak height at +1040 mV was evaluated automatically using linear type base line.

2.4. Application procedures

2.4.1. Analysis of hydrochlorothiazide in urine

Urine was centrifuged at $4000 \times g$. Into a set of 10 ml volumetric flasks, separate aliquots of urine (1 ml) were spiked with varying amounts of HCTZ (4,5, and 6 μg each in three replicates). The volumes were adjusted to 10 ml with BR buffer pH 3.3. A 1 ml aliquot from each solution was diluted to 5 ml with the same buffer and transferred into the measuring vessel. Voltammograms were recorded as under construction of calibration graph. The recovery was calculated using the standard addition method [21].

2.4.2. Analysis of monozide® tablets

Twenty tablets were weighed and powdered. A portion of the powder equivalent to the average weight of

one tablet was transferred into a 25 ml volumetric flask using about 15 ml of 0.02 M NaOH. The mixture was sonicated for 5 min then completed to volume with the same solvent. The resulted suspension was allowed to settle and 0.1 ml from the supernatent was diluted to 250 ml using BR buffer pH 3.3. Aliquots were diluted to 5 ml with the same buffer and subjected to voltammetric measurement as previously mentioned.

3. Results and discussion

Hydrochlorothiazide in BR pH 3.3 exhibits a well-defined anodic peak at +1040 mV (Fig. 1).

3.1. Mechanistic study of the oxidation of HCTZ

Cyclic voltammetric technique was applied as a diagnostic tool to get information about the mechanism of the redox reaction of HCTZ at the glassy carbon

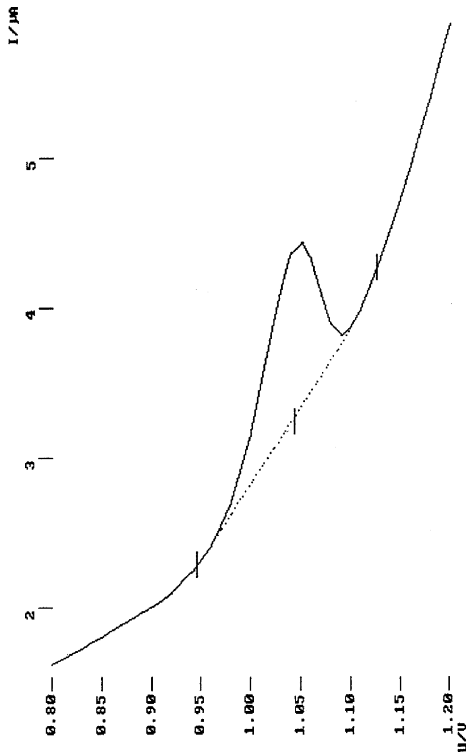


Fig. 1. Differential pulse voltammogram of hydrochlorothiazide (100 ng ml^{-1}) in BR buffer pH 3.3 (scan rate = 100 mV s^{-1}).

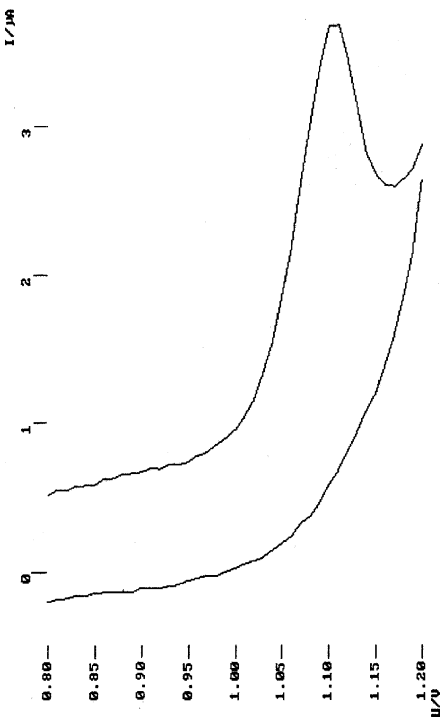


Fig. 2. Cyclic voltammogram of hydrochlorothiazide (20 μg ml^{-1}) in BR buffer pH 3.3 (scan rate = 100 mV s^{-1}).

electrode. It was found that the drug in BR buffer pH 3.3 give anodic peak and no peaks were observed in the cathodic scan (Fig. 2); pointing to the irreversibility of the oxidation process.

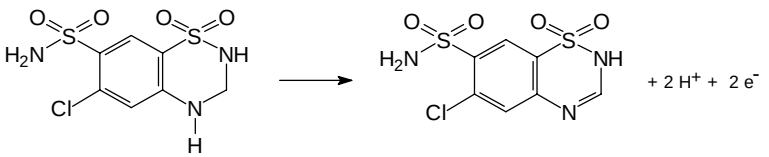
The effect of scan rate (v) on the peak potential (E_p) and peak current (I_p) was examined from 50 to 400 mV s^{-1}. The peak potential was variable with the scan rate (52 mV positive shift in the peak potential when increasing the scan rate) confirming the irreversibility of the oxidation process of HCTZ at the glassy carbon electrode [22].

A linear Randles-Seveik plot (plot of I_p against $\sqrt{v}$) (correlation coefficient = 0.9972) was obtained indicating that diffusion is the means of mass transport [23]. This finding was more confirmed by plotting $\log I_p$ against $\log v$; a straight line was obtained (correlation coefficient = 0.9973) with a slope of 0.572 (slopes of 0.50 and 1.0 are expected for ideal reactions of solution and surface species, respectively [24]).

Therefore, the oxidation of HCTZ at the glassy carbon electrode (in BR buffer pH 2.6–9) is an irreversible

diffusion-controlled process. It is worthwhile to mention that at pH 10 (higher than its pK_a), HCTZ is adsorbed at the electrode surface, but the resulted stripping peak was of poor sensitivity and can not be used for the quantitative determination of the drug.

It is reported that careful oxidation of 1,2,3,4-tetrahydroquinazolines forms the corresponding 3,4-dehydroquinazolines [25]. Based on this fact, we can assume that chlorothiazide is the product of electro-oxidation of HCTZ. For confirmation, a solution of HCTZ in BR buffer pH 3.3 (3 μg ml^{-1}) was subjected to a very large number of successive voltammetric runs (from +800 to +1200 mV). Comparing the UV spectra of the solution before and after electro-oxidation, it was found that the peak at 225 nm (characteristic for chlorothiazide in presence of HCTZ) increases by a small but reproducible value. Consequently, the electro-oxidation of HCTZ could be represented by the following equation.



3.2. Quantitative study

In order to quantitate the diffusion current resulting from the electro-oxidation of HCTZ at the glassy carbon electrode, the sensitive differential pulse and square-wave techniques have been tried. Both techniques gave comparable results; the more simple differential pulse technique has been chosen as it requires less parameters adjustment.

3.2.1. Factors affecting the peak current and peak potential

3.2.1.1. Supporting electrolyte and its pH. Various buffers (Mcllvaine, Teorell and Stenhagen, Britton–Robinson, and Sorensen [20]) were examined as supporting electrolyte. The best results with respect to sensitivity accompanied with sharper response was obtained with Britton–Robinson (0.08 M) buffer.

The effect of pH of BR buffer on peak current (I_p) and peak potential (E_p) was studied over the pH range 2.6–12. The peak current shows maximum value at pH 3.3 (Fig. 3a), which was used for the drug determination. The peak potential moves to less positive values with increasing pH. The relation is linear in the pH range of 2.6–12 (Fig. 3b). The slope $= -73.66$ (mV per pH) $= (60/\alpha) \times (m/n)$ (α: transfer coefficient, m and n: number of protons and electrons involved in the reaction, respectively). The transfer coefficient was found to be 0.815 (less than 1) confirming the irreversibility of the oxidation reaction.

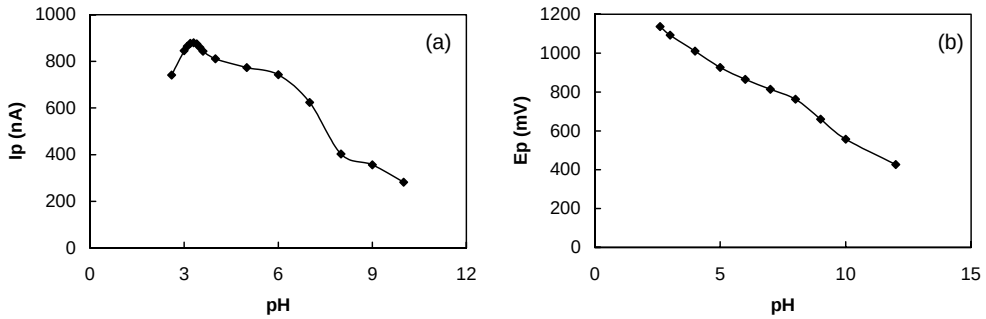


Fig. 3. Effect of pH of BR buffer on the differential pulse (a) peak current and (b) peak potential of hydrochlorothiazide (80 ng ml^{-1}) (scan rate = 100 mV s^{-1}).

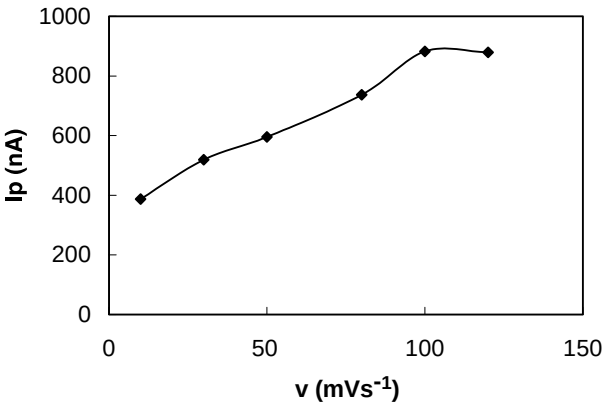


Fig. 4. Effect of scan rate on the differential pulse peak current and of hydrochlorothiazide (80 ng ml^{-1}) in BR buffer pH 3.3.

3.2.1.2. Scan rate. The magnitude of the peak current increases with increasing the scan rate of the sweep from 10 to 100 mV s^{-1} then remains nearly constant till 120 mV s^{-1} (the upper instrumental limit) (Fig. 4). Scan rate of 100 mV s^{-1} was chosen as the most suitable to obtain maximum sensitivity.

3.3. Validation of the proposed method

3.3.1. Linearity

Using the optimum conditions described in Section 2 for construction of calibration graph, a linear relation between peak current and HCTZ concentration in the range of 24–320 ng ml^{-1} was obtained.

$$I_p = 10.12C + 40.01$$

The good linearity is evident from the value of the correlation coefficient ($r = 0.9999$) (Table 1).

3.3.2. Accuracy

The accuracy of the proposed method has been determined by spiking commercial tablets powder and urine sample with three different concentrations of HCTZ each in three replicates. Good percentage recoveries was obtained (Table 1).

3.3.3. Repeatability

The within-day and between-day repeatability studies were performed (three concentrations and three replicates). The relative standard deviation is presented in Table 1.

3.3.4. Specificity

The proposed method is selective for HCTZ in the presence of its related substance and impurities, chlorothiazide and salamide (4-amino-6-chlorobenzene-1,3-disulphonamide) [2,3] as well as its hydrolytic and photodegradation product (5-chloro-2, 4-disulfamoylaniline) [26,11]; as they lack the presence of the two hydrogens in the 3,4-positions, which are oxidized (dehydrogenated) at the glassy carbon under the recommended conditions. The two other photodegradation products, methoxyhydrothiazide and hydroxyhydrothiazide can interfere.

3.3.5. Detection limit

The calculated detection limit (DL = $3.3S_a/b$, where S_a is the standard deviation of intercept and b the slope of the regression line) [27] for standard solution was 4.19 ng ml^{-1}, but when verified experimentally it was found to be equal to 5.0 ng ml^{-1}. In urine, the experimental limit of detection was 14.0 ng ml^{-1}.

3.3.6. Quantitation limit

The quantitation limit for standard solution was 16 ng ml^{-1} with a 2% relative standard deviation. For the determination of HCTZ in urine the QL was 24 ng ml^{-1} with a 10% relative standard deviation.

3.4. Interferences from co-formulated drugs

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 (amiloride hydrochloride, bisoprolol fumarate, captopril, enalapril maleate, lisinopril hydrochloride, losartam potassium, metoprolol tartarate, methyldopa propranolol hydrochloride, triametrene, reserpine and spironolactone) on the anodic voltammetric determination of HCTZ. The later two drugs are water insoluble. The voltammograms of the rest of drugs solutions in BR buffer pH 3.3 were recorded from +800 to +1200 mV under the instrumental parameters adjusted for HCTZ determination. Only timolol maleate exhibits small peak at +963 mV, which is far from the HCTZ peak and does not interfere. The determination of HCTZ (100 ng ml^{-1}) in the presence of each of the above mentioned drugs (the concentration of each drug is calculated according to its

Table 1
Validation data for the determination of HCTZ using the proposed differential pulse anodic voltammetric procedure

Range ($ng\,ml^{-1}$)	Linearity (regression equation)	Accuracy (% recovery ± S.D.)		Repeatability (% RSD)		Detection limit ($ng\,ml^{-1}$)		Quantitation limit ($ng\,ml^{-1}$)	
		Excipients	Urine	Within-day	Between-day	Standard solution	In urine	Standard solution	In urine
24–320	$a = 40.01$, $S_a = 12.85$, $b = 10.12$, $S_b = 0.08$, $r = 0.999$, $S_{y/x} = 21.79$	100.76 ± 0.96	99.19 ± 1.17	1.08	1.66	5.0[a] 4.19[b]	14.0[a]	16.0	24.0

[a] Experimentally.
[b] Calculated.

ratio to HCTZ in the pharmaceutical formulation) was evaluated. The recovery of HCTZ ranged from 98.80 to 100.18%. It could be concluded that using the proposed method with the specified conditions, the peak at +1040 mV could be used for the selective determination of HCTZ.

3.5. Analytical applications

3.5.1. Analysis of HCTZ spiked in urine

Urine was spiked with different quantities of HCTZ in the range of ±20% from the minimal drug concentration in urine at chronic drug use ($5\,\mu g\,ml^{-1}$) [14]. Under the optimum experimental conditions, blank urine exhibits anodic peak at +895 nm. This peak did not interfere as it is far from the analytical peak (Fig. 5). The interference from endogenous component of urine was due to its negative effect on the drug peak current. This has been nearly eliminated by dilution with buffer (50-folds). The recovery studies of HCTZ spiked in urine was performed using the standard addition method [21] to nullify any remaining interference. The results are presented in Table 2.

Table 2
Analytical results of HCTZ in urine using the proposed differential pulse anodic voltammetric procedure

Added[a] ($\mu g\,ml^{-1}$)	Found[b] ($\mu g\,ml^{-1}$)	Recovery (%) ± RSD
4	3.99	99.74 ± 1.53
5	5.00	100.54 ± 1.18
6	5.93	99.14 ± 0.82

[a] Each in three replicates.
[b] Mean of three determinations.

3.5.2. Determination of HCTZ in pharmaceutical tablets

The proposed voltammetric method was applied for the analysis of monozide® tablets. No need for filtration of tablets extract from undissolved excipients;

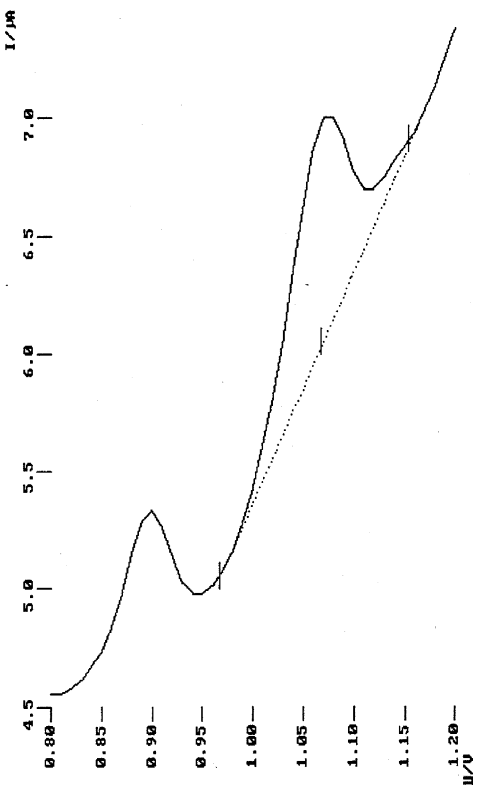


Fig. 5. Differential pulse voltammogram of hydrochlorothiazide in urine after 50-fold dilution with BR buffer pH 3.3 ($100\,ng\,ml^{-1}$) ($v = 100\,mV\,s^{-1}$).

Table 3
Assay results for the analysis of monozide® tablets

Labeled concentration	Recovery (%)[a] ± RSD	
	Proposed voltammetric method	USP 26 HPLC method
25 mg per tablet	102.41 ± 0.85	103.30 ± 0.68
t	1.83	
F	1.59	

Theoretical values for t and F at $P = 0.05$ are 2.31 and 6.39, respectively.
[a] Mean of five measurements.

just dilution of an aliquot from the supernatent layer with the supporting electrolyte (BR buffer pH 3.3) is required before measurement. The results obtained were compared statistically with those obtained from the USP 26 HPLC method using the Student *t*-test and variance ratio *F*-test (Table 3). The experimental values did not exceed the theoretical ones indicating the good agreement with the official method.

4. Comparison with the published HPLC methods

The principal advantages of the proposed voltammetric method is its rapidity and simplicity. Each voltammetric run takes few seconds. It involves no clean up procedures; just dilution of the urine with buffer nearly eliminates its potential interference. However most of the published HPLC methods requires lengthy and tedious extraction procedures, such as liquid–liquid extraction [14] and solid phase extraction [16]. For tablets analysis it requires no filtration from undissolved excipients. The proposed method is of comparable sensitivity as the reported HPLC. The method is specific as the chromatographic one, as peak potential is characteristic for the compound. Although the proposed method could be used for the selective determination of HCTZ in presence of its related substances (impurities and one degradation product), but the chromatographic procedures has the advantage of simultaneous quantitation of the drug and its related substances when the chromatographic conditions are properly selected.

5. Conclusion

The proposed method provides a sensitive and simple approach to the determination of HCTZ in urine. Its accuracy, reproducibility and simplicity suggest its application in quality control analysis of the drug.

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