

Ferrocenedicarboxylic Acid Modified Carbon Paste Electrode: A Sensor for Electrocatalytic Determination of Hydrochlorothiazide

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A oxidação eletrolítica de hidroclorotiazida foi estudada usando-se eletrodo de pasta de carbono modificado com ácido ferrocenedicarboxílico. Voltametria cíclica e cronoamperometria foram usadas para investigar a possibilidade de aplicação do ácido ferrocenedicarboxílico como mediador na oxidação eletrocatalítica de hidroclorotiazida em solução aquosa. A oxidação de hidroclorotiazida ocorre em um potencial, aproximadamente, 300 mV menos positivo do que com o eletrodo de pasta de carbono não modificado, em pH 9,0. A constante de velocidade da reação catalítica, k_h , foi calculada ($3,38 \times 10^2 \text{ cm}^3 \text{ mol}^{-1} \text{ s}^{-1}$) usando-se cronoamperometria. Em condições ótimas, a corrente de pico da oxidação eletrocatalítica da hidroclorotiazida mostrou dois intervalos dinâmicos lineares com limite de detecção de $0,037 \mu\text{mol L}^{-1}$ de hidroclorotiazida. Os intervalos de calibração lineares ocorreram entre $0,08\text{-}5,8 \mu\text{mol L}^{-1}$ e $5,8\text{-}500,0 \mu\text{mol L}^{-1}$ de hidroclorotiazida, usando-se voltametria de onda quadrada. Finalmente, o método proposto foi também examinado quanto à seletividade, simplicidade e precisão na determinação de hidroclorotiazida em amostras reais como droga e urina.

The electrocatalytic oxidation of hydrochlorothiazide has been studied by ferrocenedicarboxylic acid modified carbon paste electrode. Cyclic voltammetry and chronoamperometry were used to investigate the suitability of ferrocenedicarboxylic acid as a mediator for the electrocatalytic oxidation of hydrochlorothiazide in aqueous solution. The oxidation of hydrochlorothiazide occurs at a potential about 300 mV less positive than with the unmodified carbon paste electrode at pH 9.0. The catalytic reaction rate constant, k_h was calculated ($3.38 \times 10^2 \text{ cm}^3 \text{ mol}^{-1} \text{ s}^{-1}$) using chronoamperometry. Under the optimized conditions, the electrocatalytic oxidation peak current of hydrochlorothiazide showed two linear dynamic ranges with a detection limit of $0.037 \mu\text{mol L}^{-1}$ hydrochlorothiazide. The linear calibration ranges were between $0.08\text{-}5.8 \mu\text{mol L}^{-1}$ and $5.8\text{-}500.0 \mu\text{mol L}^{-1}$ hydrochlorothiazide using square wave voltammetric method. Finally, the proposed method was also examined as a selective, simple and precise electrochemical sensor for the determination of hydrochlorothiazide in real samples such as drug and urine.

Keywords: ferrocenedicarboxylic acid, hydrochlorothiazide, electrocatalysis, square wave voltammetry

Introduction

Hydrochlorothiazide (HCTZ) (6-chloro-7-sulfamoyl-3,4-dihydro-1,2,4-benzothiazine 1,1-dioxide) has gained attention because it is a benzothiazide diuretic that acts directly on the kidney by increasing the excretion of sodium chloride and water and, to a lesser extent, that of potassium ions. HCTZ 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 HCTZ is well absorbed, is shortly bound to plasma proteins, and mainly excreted unchanged in the urine.² Owing to the wide variety of analogous products developed in the following years, the clinical, forensic, and antidoping analysts require informative and unambiguous data about administered pharmaceuticals.

Several methods have been proposed for the determination of HCTZ including high-performance liquid chromatography,³⁻⁷ capillary electrophoresis,^{8,9} spectrophotometry,¹⁰⁻¹⁴ chemiluminescence,¹⁵ LC-MS/MS¹⁶ and electrochemical methods.^{17,18} Many of which are not sufficiently sensitive^{10-14,17,18} and/or are time consuming

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and/or use expensive instrumentations.¹⁶ The comparison of the proposed method for HCTZ determination with other papers published in electrochemical field is given in Table 1.

It is well known that the oxidation of HCTZ at ordinary carbon electrode has a very poor electrochemical response, and has a large oxidation overpotential.¹⁵ As far as we know, electrocatalytic determination of HCTZ using other electrodes has not been reported in literature. Therefore, in this study the application of ferrocenedicarboxylic acid was discussed as a suitable mediator for the determination of HCTZ in aqueous media using voltammetric method. In addition, the suitability of the ferrocenedicarboxylic acid modified carbon paste electrode (FDCMCPE) in the electrocatalysis and the determination of HCTZ are discussed by cyclic voltammetry (CV), double potential step chronoamperometry (DPCA) and square wave voltammetry (SWV).

Experimental

Chemicals

All chemicals were of analytical reagent grade and were purchased from Merck (Darmstadt, Germany) unless otherwise stated. Doubly distilled water was used throughout.

Universal buffer (boric acid, phosphoric acid, acetic acid and sodium hydroxide, 0.1 mol L⁻¹) solutions with different pH values were used.

High viscosity paraffin ($d = 0.88 \text{ g cm}^{-3}$) from Fluka was used as the pasting liquid for the preparation of carbon paste electrode. Graphite powder (particle diameter = 0.1 mm) from Merck (Darmstadt, Germany) was used as a substrate for the preparation of the working electrode (WE).

Ferrocenedicarboxylic acid from Fluka and hydrochlorothiazide from Sigma were used as received.

Apparatus

All the cyclic voltammetric measurements were performed using a Micro-Autolab, potentiostat/galvanostat connected to a three electrode cell, Metrohm Model 663 VA stand, linked with a computer (Pentium IV, 1200 MHz) and with micro-Autolab software. A platinum wire was used as an auxiliary electrode, FDCMCPE and Ag/AgCl (KCl_{sat}) were used as the working and reference electrodes, respectively.

A pH-meter (Corning, Model 140) with a double junction glass electrode was used to check the pH of the solutions.

Preparation of the sensor

A 1.0% (w/w) ferrocenedicarboxylic acid spiked carbon powder was made by dissolving the given quantity of ferrocenedicarboxylic acid in diethyl ether and by hand mixing with 99.0 times its weight of graphite powder with a mortar and pestle. The solvent was evaporated by stirring. A mixture of 1.0% (w/w) ferrocenedicarboxylic acid spiked carbon powder plus paraffin oil was blended by hand-mixing. The resulting paste was inserted in the bottom of a glass tube (with an internal radius of 3.0 mm). The electrical connection was implemented with a copper wire fitted into a glass tube. A carbon paste electrode without ferrocenedicarboxylic acid was prepared as the above procedure without addition of ferrocenedicarboxylic acid being added.

Preparation of real samples

Ten tablets of hydrochlorothiazide (labeled 50 mg *per* tablet, 10 tablets) were completely ground and homogenized. Then, 100 mg of the powders were accurately weighed and dissolved with ultrasonication in 100 mL of ethanol-water (1:1) solution. The resultant solution was diluted 10-times, and then 4 mL of the solution plus 10 mL of 0.10 mol L⁻¹ buffer (pH 9.0) were used for the analysis.

The urine samples used for measurements were centrifuged and diluted twice with water without any further pretreatment. The standard addition method was used for the determination of hydrochlorothiazide in the real samples.

Results and Discussion

Electrochemistry of the mediator

Ferrocenedicarboxylic acid, a well known mediator, has been used for the determination of some organic compounds such as cysteine,¹⁹ ascorbic acid and uric acid,²⁰ Here we have used this mediator for the determination of HCTZ. The electrochemical properties of FDCMCPE were studied by cyclic voltammetry in a buffer solution (pH 7.0). Figure 1A shows the cyclic voltammograms of FDCMCPE at various scan rates, ($v = 10 - 250 \text{ mV s}^{-1}$). The experimental results show well-defined and reproducible anodic and cathodic peaks related to Fc/Fc⁺ redox couple with quasi-reversible behavior,^{19,20} with a peak separation potential of $\Delta E_p = 100 \text{ mV}$ ($E_{pa} - E_{pc}$). These cyclic voltammograms were used to examine the variation of the peak currents *vs.* potential scan rates. The plots of the cathodic and anodic peak currents were linearly dependent on $v^{1/2}$ with

a correlation coefficient of 0.9764 and 0.9832 at all scan rates (Figure 1B). This behavior indicates that the nature of the redox process is diffusion controlled.

Double step potential chronoamperometry was employed to investigate the electrochemical processes of FDCMCPE. The results show very symmetrical chronoamperograms with an equal charge consumed for the oxidation and reduction of the redox couple in the CPE. The plot of net electrolysis current *vs.* $t^{1/2}$ shows a straight line, which extrapolates close to the origin. Therefore, this type of near-Cottrellian behavior is not due to a linear semi-infinite diffusion process, but may be caused by finite diffusion in a thin film, where the near-Cottrell equation behavior can be approximated over a short time.

Consequently, we can assume diffusion controlled behavior for charge transfer at FDCMCPE and use potential-step chronoamperometric experiments to estimate the diffusion coefficient of ferrocenedicarboxylic acid into the paraffin oil used for paste preparation. The slope of the linear region of the $I-t^{1/2}$ plot in the short time region produces the apparent diffusion coefficient (D_{app}) of the spiked ferrocenedicarboxylic acid into FDCMCPE using Cottrell equation:²¹

$$I = nFA_g D_{app}^{1/2} C \pi^{1/2} t^{-1/2} \quad (1)$$

Where C is the known concentration, D_{app} is the apparent diffusion coefficient of spiked ferrocenedicarboxylic acid in paraffin oil and A_g is the geometric area of this electrode (the diameter (d) of the FDCMCPE was measured and then the geometric area of the FDCMCPE was calculated according to $\pi(d/2)^2$. Therefore, we calculated the apparent diffusion coefficient for ferrocenedicarboxylic acid in carbon paste matrix. It was found that $D = 1.81 \times 10^{-10} \text{ cm}^2 \text{ s}^{-1}$, while in the above equation $n = 1$, $F = 96485 \text{ C mol}^{-1}$, $A_g = 0.091 \text{ cm}^2$.

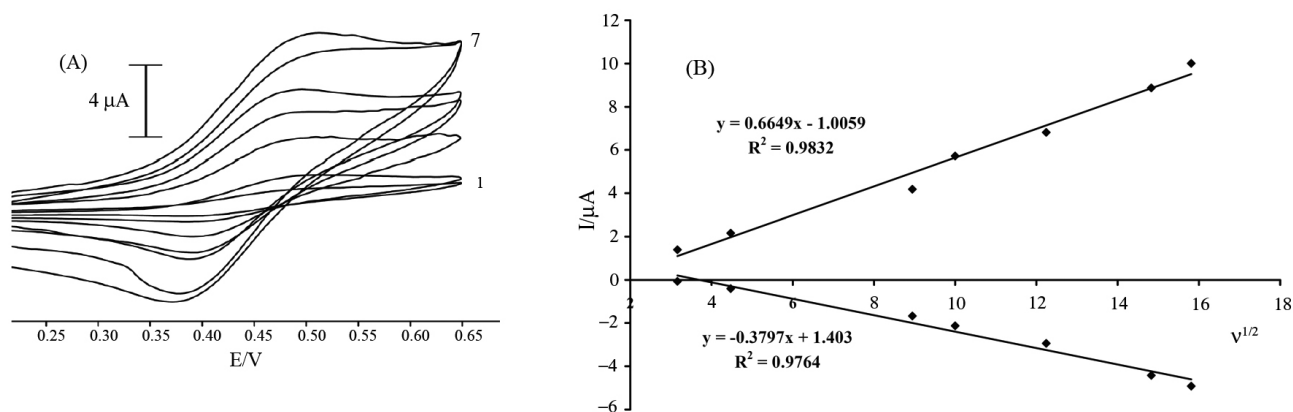


Figure 1. (A) Cyclic voltammograms of FDCMCPE in 0.1 mol L⁻¹ universal buffer at pH 7.0 at various scan rates: 1) 10; 2) 20; 3) 80; 4) 100; 5) 150; 6) 220; 7) 250 mV s⁻¹. (B) Plots of the anodic and cathodic peak currents of FDCMCPE *vs.* $v^{1/2}$ from cyclic voltammograms of A.

Comparative electrochemical impedimetric response

Electrochemical impedance spectroscopy (EIS) is a powerful technique to probe charge transfer properties at the electrode-solution interface.^{22,23} We have used electrochemical impedance spectroscopy to study FDCMCPE to evaluate its diffusion and/or charge transfer control. Figure 2 indicates the Nyquist plot of the FDCMCPE recorded in the universal buffer (pH 9.0) at the oxidation peak potential of 0.40 V. The electrical equivalent circuit compatible with the impedance spectra is shown in scheme 1 which incorporates $R_s = 343 \Omega$ (the solution/electrolyte resistance), $R_{ct} = 1014 \Omega$ (charge-transfer resistance), $Z_w = 0.1136 \times 10^{-3}$ (Warburg impedance) related to the semi-infinite linear diffusion, and Q ($Y_0 = 0.418 \times 10^{-5}$, $n = 0.7273$) (constant phase element). As shown in Figure 2, the first part showed small charge transfer limitation, whereas the second part (linear part) emphasized its diffusion control of FDCMCPE. The results showed that the electrode system has both diffusion and charge transfer limitation.

Electrooxidation of HCTZ at the surface of FDCMCPE

To test the electrocatalytic behavior of the ferrocenedicarboxylic acid, cyclic voltammograms were recorded at 10 mV s⁻¹ in a universal buffer solution (0.1 mol L⁻¹, pH 9.0) in the absence and presence of HCTZ, using bare carbon paste electrode and FDCMCPE (Figure 3). The oxidation of HCTZ took place only to a small extent at highly positive overpotential at the surface of bare carbon paste electrode. However, with FDCMCPE, a significant enhancement in the anodic peak current of HCTZ is achieved at a potential close to the formal potential of Fc/Fc⁺ redox coupled with a decrease in the cathodic current. Therefore, ferrocenedicarboxylic acid is a suitable

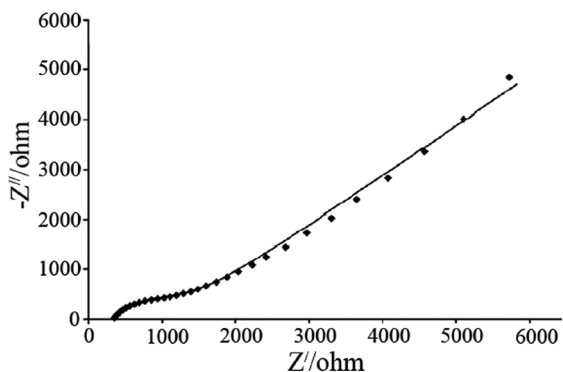
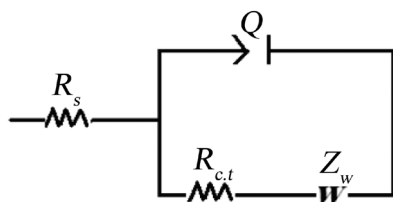


Figure 2. Impedance plot for the FDCMCPE system in a pure buffer (pH 9.0) solution with E_p of +0.40 V vs. Ag/AgCl reference electrode.



Scheme 1. Equivalent circuit for the system.

mediator for electrocatalytic oxidation of HCTZ. This process corresponds to an EC' mechanism (Diagram 1). This means that the electrochemically formed Fc^+ reacts chemically with HCTZ that is diffused into the electrode surface, while the simultaneous oxidation of regenerated Fc causes an increase in the anodic current. For the same reason, the cathodic current of the modified electrode is smaller in the presence of HCTZ.

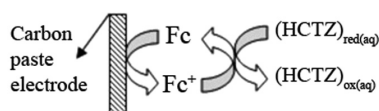


Diagram 1

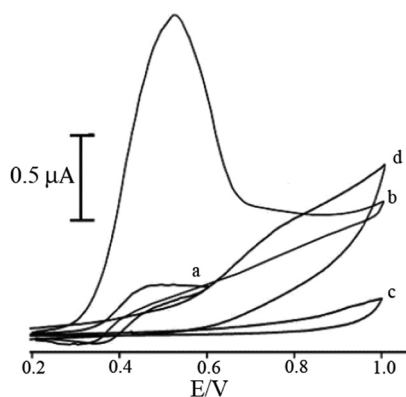


Figure 3. Cyclic voltammograms of FDCMCPE with scan rate of 10 mV s^{-1} in the buffer solution (pH 9.0). (a) In the absence and (b) in the presence of $150.0 \mu\text{mol L}^{-1}$ HCTZ; (c) as (a) and (d) as (b) for unmodified carbon paste electrode, respectively.

In order to get the information about the rate determining step, a Tafel plot was developed for FDCMCPE using the data derived from the raising part of the current-voltage curve (Figure 4). The slope of the Tafel plot is equal to $n(1-\alpha)F/2 \cdot 3RT$ and equal to $7.224 \text{ V decade}^{-1}$. We can calculate $n\alpha = 0.57$. If assuming $n = 1$, then $\alpha = 0.57$.

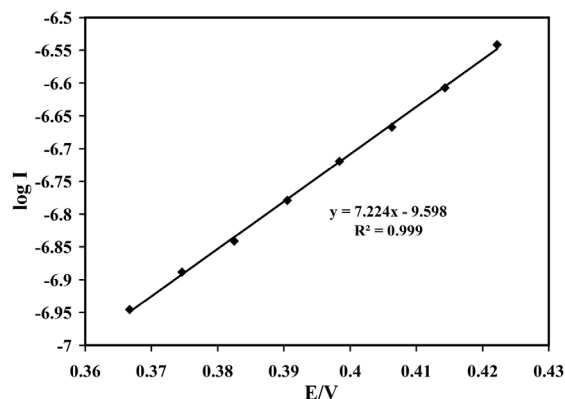


Figure 4. Tafel plot for FDCMCPE.

The following equation has been used to calculate transfer coefficient as:²⁴

$$E_p = b/2 \log(v) + \text{constant} \quad (2)$$

Based on equation 2, the slope of E_p vs. $\log(v)$ is $b/2$. The slope of E_p vs. $\log(v)$ was found to be 0.0691 V . Thus, $b = 2 \times 0.0691$ and is equal to 0.1382 V . According to one electron transfer in Fc/Fc^+ process, the transfer coefficient (α) is equal to 0.57 . In addition, the values of αn_α (n_α is the number of electrons involved in the rate determining step) was calculated for the oxidation of HCTZ at pH 9.0 with both modified and unmodified carbon paste electrodes according to the following equation:²⁵

$$\alpha n_\alpha = 0.048/(E_p - E_{p/2}) \quad (3)$$

where $E_{p/2}$ is the potential corresponding to $I_{p/2}$. The values for αn_α were found to be 0.565 and 0.230 at the surface of FDCMCPE and unmodified carbon paste electrode, respectively. Those values show that the overpotential of HCTZ oxidation is reduced at the surface of FDCMCPE, and also the rate of electron transfer process is greatly enhanced. This phenomenon is thus confirmed by the larger I_{pa} values recorded during cyclic voltammetry at FDCMCPE.

Chronoamperometry study

Double potential step chronoamperometry was used with FDCMCPE to determine the diffusion coefficient

of HCTZ. Figure 5A shows the current-time curves of FDCMCPE by setting the electrode potential at 700 mV (first step) and 200 mV (second step) vs. $\text{Ag}|\text{AgCl}|\text{KCl}_{\text{sat}}$ for different concentrations of HCTZ. As can be seen, there is not a net anodic current corresponding to the oxidation of the mediator in the presence of HCTZ. On the other hand, the forward and backward potential step chronoamperometry for the mediator in the absence of HCTZ shows a symmetrical chronoamperogram with an equal charge consumed for the reduction and oxidation of the ferrocenedicarboxylic acid at the surface of unmodified CPE (Figure 5B, a'). But, in the presence of HCTZ, the charge value associated with forward chronoamperometry is significantly greater than that observed for backward chronoamperometry (Figure 5B, b'-d'). The linearity of the electrocatalytic current vs. $t^{1/2}$ shows that the current is controlled by diffusion of HCTZ from the bulk solution towards the surface of the electrode that causes near Cottrellian behavior. Therefore, the slope of linear region of Cottrell's plot can be used to estimate the diffusion coefficient of HCTZ. A plot of I vs. $t^{1/2}$ for a FDCMCPE in the presence of HCTZ gives a straight line. The slope can be used to estimate the diffusion coefficient of HCTZ (D) in the ranges of 100 to 200 $\mu\text{mol L}^{-1}$. The mean value of the D for HCTZ was found to be $1.1 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$.

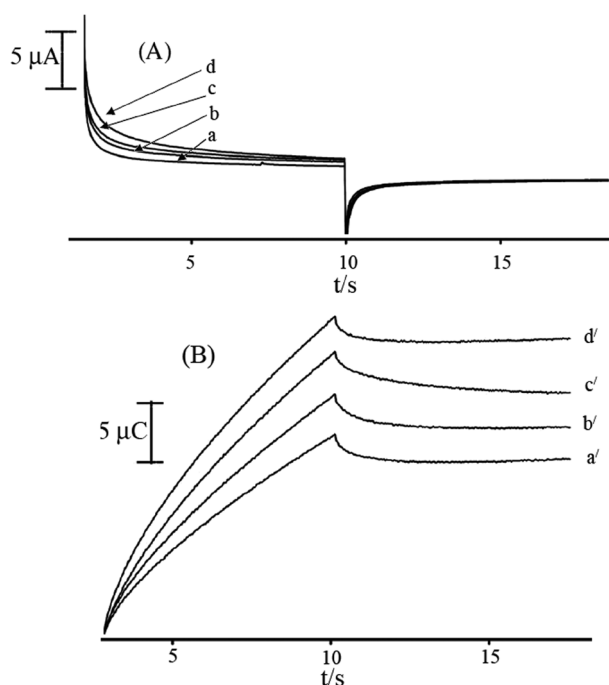


Figure 5. A) Chronoamperograms obtained at the FDCMCPE in the (a) absence and presence of (b) 100.0, (c) 150.0 and (d) 200.0 $\mu\text{mol L}^{-1}$ of HCTZ in the buffer solution (pH 9.0). First and second potential steps correspond to 0.70 and 0.20 V. vs. Ag/AgCl . B) The charge-time curves: (a') for curve a, (b') for curve b, (c') for curve c and (d') for curve d.

In addition, chronoamperometry can be performed to evaluate the rate of electrocatalyzed oxidation. At intermediate times ($t = 0.2$ -1.1 s) the catalytic current I_{cat} is dominated by the rate of electrocatalyzed oxidation of HCTZ. The rate constant for the chemical reaction between HCTZ and redox sites of the ferrocenedicarboxylic acid can be evaluated according to the method of Galus:²⁶

$$I_c/I_L = \gamma^{1/2} [\pi^{1/2} \text{erf}(\gamma^{1/2}) + \exp(-\gamma)/\gamma^{1/2}] \quad (4)$$

where I_c is the catalytic current of FDCMCPE in the presence of HCTZ and I_L is the limited current in the absence of HCTZ and $\gamma = k_h C_b t$ (C_b is the bulk concentration of HCTZ, mol cm^{-3}) is the argument of error function. In cases where γ exceeds 2, the error function is almost equal to 1 and the above equation can be reduced to:

$$I_c / I_L = \pi^{1/2} \gamma^{1/2} = \pi^{1/2} (k_h C_b t)^{1/2} \quad (5)$$

where k_h and t are the catalytic rate constant ($\text{cm}^3 \text{ mol}^{-1} \text{ s}^{-1}$) and time elapsed (s), respectively. Equation 5 can be used to calculate the rate constant of the catalytic process, k_h . From the slope of I_c/I_L vs. $t^{1/2}$ plot the value of k_h it can be simply calculated for a given concentration of substrate (Figure 6). The calculated value of k_h is equal to $3.38 \times 10^2 \text{ cm}^3 \text{ mol}^{-1} \text{ s}^{-1}$. This value of k_h explains the sharp feature of the catalytic peak observed for catalytic oxidation of HCTZ at the surface of FDCMCPE as well. This method has been used for estimation of k_h in some compounds.²⁷⁻³⁰

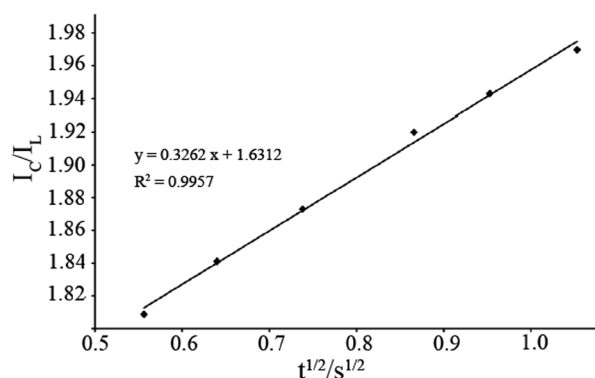


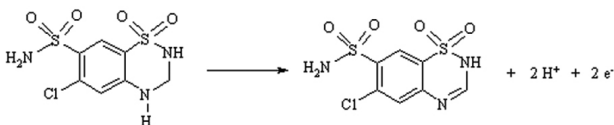
Figure 6. Dependence of I_c/I_L on the $t^{1/2}$ driven from the chronoamperogram data.

Influence of pH

In order to optimize the electrocatalytic response of the sensor to HCTZ oxidation, we investigated the effect of pH on the electrocatalytic oxidation of HCTZ in 0.1 mol L^{-1} universal buffer solutions with various pH ($4.0 < \text{pH} < 11.0$) at the surface of FDCMCPE using cyclic voltammetry. The

influence of pH on both peak currents and peak potentials was assessed through examining the electrode response in the buffer solutions.

It is well known that the electrochemical behavior of HCTZ is dependent on pH value of the aqueous solution, as shown in scheme 2, whereas the electrochemical property of Fc/Fc⁺ redox couple is independent of the solution pH. The variation of I_{pa} vs. pH is shown in Figure 7. The results showed that the maximum electrocatalytic current was obtained at pH 9.0. Therefore, pH 9.0 was chosen as the optimum pH for the determination of HCTZ at FDCMCPE.



Scheme 2. Oxidation reaction of HCTZ.

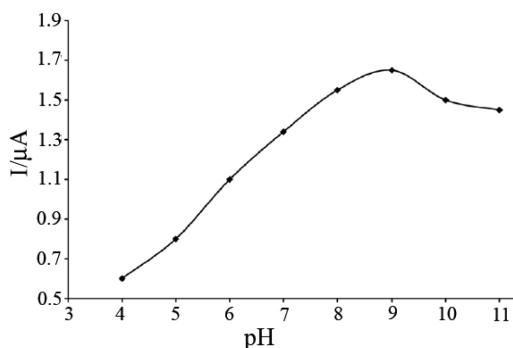


Figure 7. Current-pH curves for electro-oxidation of 150.0 $\mu\text{mol L}^{-1}$ HCTZ; the buffer solution with various pH values at the surface of FDCMCPE with a scan rate 10 mV s^{-1} .

Prior to using FDCMCPE for the electrocatalytic oxidation of HCTZ, the rate of loss of electrochemical activity for the electrode was investigated. This rate was evaluated by measuring any decrease in the anodic charge, q_a , in consecutive potential scan cycles. The results showed a decrease in the anodic and cathodic peak currents of ferrocenedicarboxylic acid/ferriceniumdicarboxylic acid couple. This means that the electrochemical activity of FDCMCPE during successive scans is reduced without any change in the half-wave potential, $E_{1/2}$. This is due to the dissolution of ferriceniumdicarboxylic acid ion generated at the surface of the electrode into the aqueous solution. Therefore, the surface regeneration of FDCMCPE before each experiment is necessary.

Electrocatalytic determination of HCTZ

Since square wave voltammetry (SWV) has a much higher current sensitivity than cyclic voltammetry,

it was used to determine of HCTZ. SWV was used under the optimum pH 9.0, with increment potential of 6 mV, amplitude potential of 10 mV and frequency of 20 Hz, (Figure 8A). The results showed two linear segments with different slopes for HCTZ concentration: for 0.08-5.8 $\mu\text{mol L}^{-1}$ HCTZ, the regression equation was $I_p(\mu\text{A}) = 133.32C_{\text{HCTZ}} + 1.599$ ($r^2 = 0.9849$, $n = 10$) (Figure 8B) and for 5.8-500 $\mu\text{mol L}^{-1}$ HCTZ, the regression equation was $I_p(\mu\text{A}) = 2.612C_{\text{HCTZ}} + 2.731$ ($r^2 = 0.9733$, $n=6$) (Figure 8C), where C_{HCTZ} is mmol L^{-1} of HCTZ.

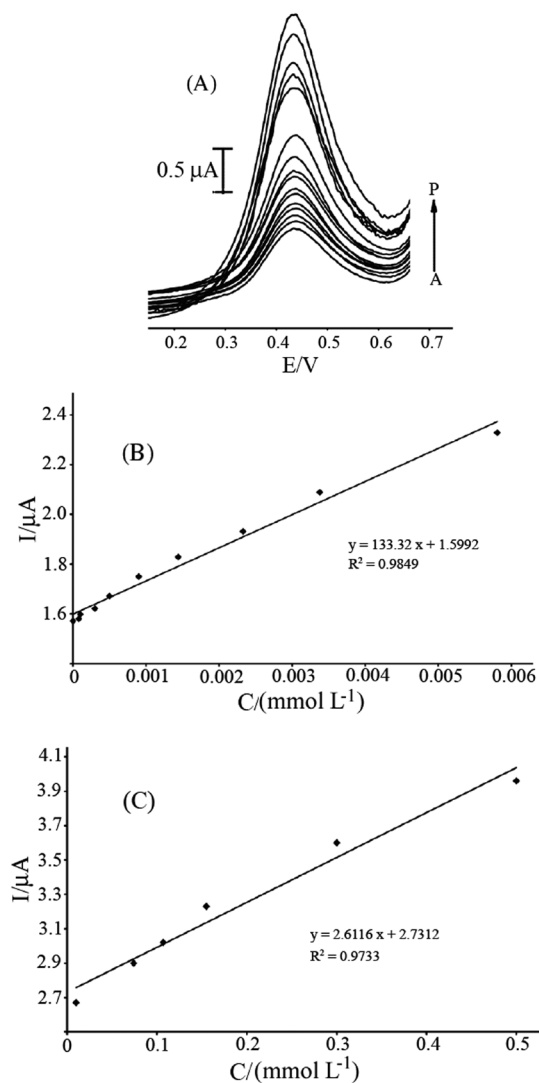


Figure 8. A) Square wave voltammograms of FDCMCPE in the buffer solution (pH 9.0) containing different concentrations of HCTZ. The A-P correspond to 0, 0.08, 0.10, 0.30, 0.50, 0.90, 1.40, 2.30, 3.30, 5.80, 10.0, 74.0, 100.0, 150.0, 300.0 and 500.0 $\mu\text{mol L}^{-1}$ HCTZ. Plots of electrocatalytic peak current as a function of HCTZ concentration in the range of B) 0.08-5.8 $\mu\text{mol L}^{-1}$ and C) 5.8-500.0 $\mu\text{mol L}^{-1}$.

The detection limit was obtained as 0.037 $\mu\text{mol L}^{-1}$ HCTZ according to the definition of $Y_{\text{LOD}} = Y_{\text{B}} + 3\sigma$.³¹ The detection limit, linear dynamic range and the sensitivity

for HCTZ with FDCMCPE are comparable and even better than those obtained by using several modified electrodes (Table 1).

Table 1. Comparison of the efficiency of some methods in the determination of HCTZ

Methods	Detector	LOD / ($\mu\text{mol L}^{-1}$)	LOQ / ($\mu\text{mol L}^{-1}$)	Ref.
LC-MS/MS	MS/MS	0.013	0.013-3.35	16
Electrochemical	GCE	0.0167	0.08-1.074	17
CZE ^a	Amperometry	0.5	2-100	32
DDS ^b	Spectrophotometer	0.75	6.7-47.02	11
SWV ^c	CPME ^d	0.037	0.08-500	This work

^aCapillary zone electrophoresis; ^bDerivative-differential spectrophotometry;

^csquare wave voltammetry; ^dCarbon paste modified electrode. LOD: Limit of detection; LOQ: Limit of qualification.

Because the surface of FDCMCPE should be regenerated before each experiment therefore, the reproducibility of the sensor was evaluated using the precision of the electrode to measure $0.50 \mu\text{mol L}^{-1}$ HCTZ in ten replicate analysis. The results showed that the relative standard deviation was 1.1% (n=10).

Interference studies

The influence of various substances as potential interference compounds on the determination of HCTZ under the optimum conditions with $3.35 \mu\text{mol L}^{-1}$ HCTZ at pH 9.0 was studied. The tolerance limit was defined as the maximum concentration of the interfering substance that caused an error less than 3% for the determination of HCTZ. The results are given in Table 2 and show that the peak current of HCTZ is not significantly affected by all conventional cations, anions, and organic substances.

Determination of HCTZ in real sample

To investigate the applicability of the proposed sensor for the electrocatalytic determination of HCTZ in real

Table 2. Interference study for the determination of $5.0 \mu\text{mol L}^{-1}$ HCTZ under the optimized conditions

Species	Tolerance limits (m/m)
Glucose, Sucrose, Lactose, Fructose, Citric acid,	
Methanol, Ethanol, K^+ , Na^+ , Cl^- , Ca^{2+} , Mg^{2+} , SO_4^{2-} , Fe^{+2} , Fe^{+3} , F^- , NO_3^-	800*
Starch	Saturated
Urea	300
Uric acid, Ascorbic acid	200

*Maximum concentration of species tested.

samples, we have selected urine and tablet samples for the analysis of their HCTZ contents. HCTZ contents in the samples were measured after sample preparation using standard addition method. The results are given in Table 3. These results demonstrate the ability of FDCMCPE for voltammetric determination of HCTZ in real samples with good recoveries of the spiked HCTZ and good reproducibility.

Table 3. Determination of HCTZ in drug and urine samples

Sample No.	Added / ($\mu\text{mol L}^{-1}$)	Expected / ($\mu\text{mol L}^{-1}$)	Founded / ($\mu\text{mol L}^{-1}$) ^a	RSD / (%)
Drug				
1	0	10.0	9.73 ± 0.25	1.87
2	20.0	30.0	29.85 ± 0.42	1.23
3	40.0	50.0	50.46 ± 0.36	1.10
4	50.0	60.0	60.01 ± 0.12	0.85
Urine				
5	0	---	< detection limit	---
6	10.0	10.0	10.13 ± 0.43	1.72
7	20.0	20.0	20.08 ± 0.58	2.80
8	40.0	40.0	40.22 ± 0.46	0.97

^aAverage of four replicate measurements.

Conclusions

This work demonstrates the construction of a chemically modified carbon paste electrode by incorporating of ferrocenedicarboxylic acid as a suitable electrochemical sensor for HCTZ determination at trace level. The new voltammetric method for the determination of HCTZ is very rapid (less than 1 min *per* sample solution), reproducible, selective and sensitive, and can be used for real sample analysis. The results show that the oxidation of HCTZ is catalyzed at pH 9.0, whereas the peak potential of HCTZ is shifted by 300 mV to a less positive potential at the surface of the FDCMCPE. The proposed method is a selective, simple and precise method for voltammetric determination of HCTZ in real samples such as drug and urine, as low as $0.037 \mu\text{mol L}^{-1}$ HCTZ. In addition, the kinetic parameters of the system have been calculated from the experimental results.

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