**Synthesis of nickel nanowrinkles and its application for the electrocatalytic
oxidation and sensitive detection of hydrochlorothiazide**

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Abstract

Nickel nanowrinkles were synthesized by a simple hydrothermal method in one step in the presence of fluoride ions in ethylene glycol. The nanowrinkles were then employed as the modifier of a carbon paste electrode. The kinetics of the charge transfer across the modified electrode/solution interface was studied and the modified electrode was employed to fabricate an amperometric sensor of hydrochlorothiazide. The mechanism and kinetics of the electrocatalytic oxidation of hydrochlorothiazide on the modified electrode surface were studied by cyclic voltammetry and chronoamperometry. An amperometric procedure was developed for determination of hydrochlorothiazide with a sensitivity of $13.50 \text{ mA mol}^{-1} \text{ dm}^3 \text{ cm}^{-2}$ and a limit of detection of $21.2 \text{ } \mu\text{mol dm}^{-3}$. The method was used for the direct assay of hydrochlorothiazide in human serum samples and hydrochlorothiazide tablets. The sensor had the advantages of sensitivity (guaranteed by a Nafion layer on the sensor surface), high electrocatalytic activity and long-term stability toward hydrochlorothiazide.

Keywords: Nickel; Nanowrinkles; Hydrochlorothiazide; Hydrothermal synthesis; Electrocatalysis

1. Introduction

Analysis of drugs and pharmaceuticals has an extensive impact on public health. Amperometry and voltammetry can be employed as convenient alternatives to the routine methods of analysis. Electroanalytical methods permit direct, simple and rapid determination without, in most instances, derivatization, requiring a minimum volume of sample, having been used for determination of a wide range of drugs [1]. In addition, knowledge of redox properties of drugs can provide insight into their metabolic fate, their in vivo processes, and their pharmacological activity [2-5].

Hydrochlorothiazide (6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide, HCT, Scheme 1) is a benzothiadiazine diuretic drug which reduces active sodium reabsorption and peripheral vascular resistance [6]. It treats fluid retention in congestive heart failure, cirrhosis of the liver, or kidney disorders, and may also be used to treat high blood pressure.

Several methods have been proposed for the individual or simultaneous determination of hydrochlorothiazide with other pharmaceutical compounds including chemometry [7], spectroscopy [8-11], capillary electrophoresis [12, 13], high performance liquid chromatography (HPLC) [14-17], time resolved chemiluminescence [18], liquid chromatography/tandem mass spectrometry [19, 20], chemometry [21], and electrochemistry [22-26].

In the past decades, there have been considerable attentions to the synthesis of metal nanostructures due to their different physical and chemical properties related to their similar large-scale ones, and potential applications in optical, electronic, catalytic and magnetic devices [27-29]. Among the metal nanostructures, nickel nanostructures have attracted much attention due to their applications in fabrication of sensors, biosensors, catalysts and conducting and magnetic materials [30-37].

In the present study, nickel nanowrinkles were synthesized through one-pot hydrothermal synthesis in the presence of fluoride ions. The nanowrinkles were then characterized and applied to modify carbon paste electrodes for the electrocatalytic oxidation and sensitive determination of hydrochlorothiazide. The nanowrinkles-modified electrode was then applied for quantitation of hydrochlorothiazide in bulk form, spiked human blood serum and tablets.

2. Experimental section

2.1. Materials

Graphite fine powder extra pure with a particle size of $<50\ \mu\text{m}$ was obtained from Merck (Catalogue Number 1042062500). Hydrochlorothiazide ($>99\%$) was received from Darou Pakhsh Pharmaceutical Inc., Tehran, Iran. The hydrochlorothiazide tablets were purchased from a local drugstore. All other chemicals used in the experiments were of analytical grade from Merck or Sigma and used without further purification. All solutions were prepared with the doubly distilled water.

2.2. Synthesis of nickel nanowrinkles

Nickel nanowrinkles were synthesized by a simple, one-pot, hydrothermal method. In a typical procedure, 0.50 g $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ and 0.15 g NaF were firstly dissolved in 56 mL ethylene glycol. The obtained blue solution was transferred into a Teflon-lined autoclave of 80 mL capacity with a stainless steel shell, followed by hydrothermal treatment at $190\ ^\circ\text{C}$ for 50 h. Then, the autoclave was allowed to reach the room temperature. The final product of the reaction as a black precipitate was filtered and washed several times with distilled water and then dried at ambient temperature.

2.3. Working electrodes preparation

Unmodified carbon paste electrode (UCPE) was prepared by careful hand-mixing graphite fine powder and mineral oil with a ratio of 80/20% (w/w). The paste was packed firmly into a cavity (2.2 mm diameter) at the end of a Teflon tube. Electrical contact was established by a copper wire.

Modified carbon paste electrodes with nickel nanowrinkles were prepared with different ratios of the modifier. Based on the results, the optimum modified electrode (NCPE) was obtained by mixing graphite fine powder, mineral oil, and nickel nanowrinkles with weight ratios of 75:20:5 for about 10 min. To cover the NCPE surface with a Nafion layer and provide the selectivity of the electrode, 10 μL of a 2% w/v low aliphatic alcohols Nafion solution was dropped on the electrode surface and placed under an IR lamp to dry.

Before performing any experiment, NCPE was transferred to a 100 mmol dm^{-3} NaOH solution (the supporting electrolyte) and 45 potential cycles were applied to the electrode in the potential range of 0 to 750 mV at a potential sweep rate of 50 mV s^{-1} . This procedure caused transformation of nickel nanowrinkles to the corresponding hydroxide.

2.4. General procedures

Standard stock solutions containing hydrochlorothiazide were prepared by dissolving an accurate mass of pure drug in an appropriate volume of doubly distilled water. All stock solutions were kept in the dark at 4°C. Additional diluted solutions were prepared daily by accurate dilution just before use. The drug solutions were stable, and their concentrations did not change with time.

Aiming at analysis of hydrochlorothiazide in serum samples, we obtained drugs-free serum samples from healthy male volunteers and stored frozen until assay. The serum samples were diluted with 100 mmol dm⁻³ NaOH solution (final dilution of 2:3 with 100 mmol dm⁻³ NaOH solution). The spiked serum solutions were directly analyzed by calibration method, according to the proposed procedure without any pretreatment or extraction steps. The calibration curves for the drug in human blood serum samples were measured using an amperometric method.

In order to analyze the hydrochlorothiazide tablets, we measured the average mass of ten tablets from each sample. Tablets were then finely powdered and homogenized in a mortar and an accurately weighed amount of this sample was dissolved in an appropriate volume of doubly distilled water. The sample solution was sonicated in an ultrasound bath for 30 min and filtered to remove the undissolved excipients.

2.5. Equipment and methods

Electrochemical studies were performed in a conventional three-electrode cell powered by a μ -Autolab potentiostat/galvanostat, type III, FRA2 (The Netherlands) run by a computer through GPES 4.9 commercial software. An Ag/AgCl, 3 mol dm⁻³ KCl, and a platinum rod were used as the reference and counter electrodes, respectively. A 100 mmol dm⁻³ NaOH solution was used as the supporting electrolyte.

Field emission scanning electron microscopy (FESEM) was performed using a Zeiss, Sigma-IGMA/VP (Germany). Before FESEM, each sample was recovered with a 2-5 nm thin film of gold.

The calibration curves for the drug were measured with an amperometric technique in a magnet-stirring solution and the solution was rapidly homogenized after injection of each drug solution. Working potential of 500 mV (vs. Ag/AgCl, 3

mol dm⁻³) was applied in amperometric measurements in which the transient currents were allowed to decay to steady-state values. All the studies were carried out at room temperature.

3. Results and discussion

3.1. Characterization of nickel nanowrinkles

Fig. 1 represents SEM images (A-D) of nickel nanowrinkles with different magnifications, and an EDS spectrum (E). The low-magnified image shows that the nickel sample comprises a network of intertwined skeins. At low magnifications, the skeins comprise small wrinkled flakes of ≈ 20 nm thick. This morphology provides a large real surface area and ease of permeability of the electrolyte species into the nanowrinkles. EDS spectrum of the sample confirms the purity of the sample.

Typical cyclic voltammogram of NCPE recorded in 100 mmol dm⁻³ NaOH solution is shown in Fig. 2. A well-defined pair of peaks appears in the voltammogram and its pattern is comparable with those reported previously [33, 34, 38, 39]. The formal potential of the redox process in the voltammogram was obtained as the average mid peaks of cyclic voltammograms recorded at slow potential sweep rates of ≤ 10 mV s⁻¹ as ≈ 400 mV and the ratio of anodic-to-cathodic peak currents (I_{pa}/I_{pc}) was almost equal to unity. The redox reaction occurred producing the peaks in the voltammogram including solid state transition of Ni(II)/Ni(III) at the NCPE/solution interface, according to the following reaction [33, 34, 38, 39]:

In the voltammogram of NCPE presented in Fig. 2, the peak potential separation is about 215 mV. This value is greater than that of immobilized redox transitions

(zero), and it increases with increment in the potential sweep rate (vide infra). This is due to the limitation in the rate of charge transfer process arising from limitations in the electronic conductivity of the carbon paste, electrolyte ions-immobilized redox sites interactions, lateral interactions between redox sites and/or diffusion and coupled migration of ions into solid state nickel hydroxide. In addition, the average value of full width at half height of anodic peak in the voltammogram is >110 mV which is greater than the theoretical value of 90 mV, for non-interacting one-electron surface redox sites [40]. This indicates the domination of repulsions between the redox active sites [41, 42].

Cyclic voltammograms of modified carbon paste electrodes with different amounts of nickel nanowrinkles were recorded (data not shown), and the anodic peak current and peak potential separation were measured and shown in Fig. 2, inset. These values were selected as criteria for reversibility of the kinetics of Reaction (1). It was observed that the highest peak current and lowest peak potential separation were attained at 5 wt% of nickel nanowrinkles as the electrode modifier. It seems that higher amount of modifier caused to decrement in the electronic conductivity of the paste. Therefore, 5 wt% of nickel nanowrinkles was selected as the optimum amount of the modifier in the following studies.

Cyclic voltammograms of NCPE recorded at different potential sweep rates of 1 to 250 mV s^{-1} are shown in Fig. 3A. In the voltammograms, the peak currents increased with the potential sweep rate increment and the ratio of the anodic-to-cathodic charges (Q_{pa}/Q_{pc}) remained almost constant and near unity. The anodic and cathodic peak currents depended linearly on the potential sweep rate, at low values of the potential sweep rate of $\leq 20 \text{ mV s}^{-1}$, (Fig. 3B). This behavior is related to the

immobilized surface redox reaction of nickel species. Using the average values of the slopes of this linear dependency, and using the following equation [43]:

$$I_p = (n^2 F^2 / 4RT) v A \Gamma^* \quad (2)$$

where I_p is the peak current, n is the number of exchanged electrons, A is the geometric surface area of the electrode, and Γ^* is the surface coverage of the redox species, the mean value for the surface concentrations of redox species of Ni(II)/Ni(III) was obtained as $1.76 \times 10^{-8} \text{ mol cm}^{-2}$. The anodic and cathodic peak currents depended linearly on the square root of the potential sweep rate at high values of the potential sweep rate of $\geq 100 \text{ mV s}^{-1}$ (Fig. 3C). This indicates that at high potential sweep rates, the charge transfer process is controlled by a diffusion process. This diffusional behavior was also observed for other nickel-based electrodes [33, 34, 38, 39], due to diffusion of proton in the bulk of nickel oxide [44].

To obtain the kinetic parameters of the redox transition of nickel species in the NCPE, general equations for the voltammetric responses were employed [45]. For a peak-to-peak potential separation (ΔE_p) of $> 200/n \text{ mV}$, these equations are:

$$E_{p,a} = E^0 + X \ln [(1-\alpha_s)/Z] \quad (3)$$

$$E_{p,c} = E^0 + Y \ln [\alpha_s/Z] \quad (4)$$

$$\ln k_s = \alpha_s \ln(1-\alpha_s) + (1-\alpha_s) \ln \alpha_s - \ln(RT/nFv) - \alpha_s(1-\alpha_s)nF\Delta E_p/RT \quad (5)$$

where

$$X = RT/(1-\alpha_s)nF \quad (6)$$

$$Y = RT/\alpha_s nF \quad (7)$$

$$Z = (RT/F)(k_s/nv) \quad (8)$$

In these equations, k_s is the apparent rate constant for the electron transfer across the NCPE/solution interface, $E_{p,a}$ and $E_{p,c}$ are anodic and cathodic peak potentials, respectively, and α_s is the electron transfer coefficient for the redox process of nickel

species. Fig. 3D shows the dependency of the anodic and cathodic peak potentials on the natural logarithm of the potential sweep rate. At $v \geq 500 \text{ mV s}^{-1}$, the values of E_p are linearly dependent on $\ln v$ and the values of α_s and k_s were obtained as 0.52 and 2.96 s^{-1} , respectively. The value of k_s for nickel nanowrinkles is higher than lichen-like nickel oxide nanostructure [38], nickel hydroxide nanopetals [39], nickel hydroxide nanoparticles [46] and flower-like nickel oxide nanostructure [47].

3.2. Electrocatalytic oxidation of hydrochlorothiazide on NCPE

Fig. 4 shows cyclic voltammograms of UCPE and NCPE in the absence and presence of 5.0 mmol dm^{-3} hydrochlorothiazide. In the voltammogram of UCPE, a negligible anodic current related to the electrooxidation of hydrochlorothiazide was observed, while NCPE oxidized the drug. In the voltammograms of NCPE, it was observed that compared to the voltammograms recorded in the absence of the drug, the anodic peak current increased, while the cathodic one decreased in the presence of hydrochlorothiazide. Therefore, hydrochlorothiazide was oxidized on the NCPE surfaces via a surface mediation electron transfer process. The redox transition of the nickel species,

is followed by the chemical oxidation of hydrochlorothiazide via the high-valence nickel species:

Regarding the electrooxidation product of hydrochlorothiazide, it bears one primary and two secondary amine functional groups (Scheme 1). Due to the lack of α -hydrogen(s), the primary amine group cannot be oxidized; its oxidation needs

dehydrogenation of α -carbon (atom) to produce imine and/or nitrile [48-51]. It should be noted that for the electrooxidation of primary amines, formation of cation-radical, followed by deamination and production of cation can occur [52] (Scheme 2). However, sulfur atom has higher negativity (than carbon atom), and is bound to two oxygen atoms, and the assumed resultant sulfo-cation is highly unstable. Therefore, deamination of hydrochlorothiazide (and production of $\text{NH}_2^\bullet$) during its electrooxidation is not possible. On the other hand, secondary amine groups can undergo electrooxidation to form the corresponding imines (Scheme 3). The imines can further undergo hydrolysis and produce imides.

Fig. 5 presents cyclic voltammograms of NCPE recorded in the presence of 5.0 mmol dm⁻³ hydrochlorothiazide at different potential sweep rates of 2 to 200 mV s⁻¹. Based on these results, the linear dependency of the anodic peak potential on the natural logarithm of the potential sweep rate is shown in Fig. 5B. Using this plot and the following equation [53],

$$E_{\text{pa}} = (RT/2\alpha_{\text{HCT}}F) \ln v + \text{constant} \quad (11)$$

where E_{pa} and α_{HCT} are the anodic peak potential and the electron transfer coefficient for the electrooxidation of hydrochlorothiazide, respectively, α_{HCT} was obtained as 0.41. In addition, the linear dependency of the anodic peak current on the square root of the potential sweep rate is presented in Fig. 5C. This indicates that the electrooxidation of hydrochlorothiazide is controlled by its diffusion in the bulk of the solution. Based on the following equation [43]:

$$I_{\text{pa}} = (2.99 \times 10^5) \alpha_{\text{HCT}}^{1/2} n^{3/2} A C_{\text{HCT}} D_{\text{HCT}}^{1/2} v^{1/2} \quad (12)$$

where I_{pa} is the anodic peak current, A is the electrode surface area, D_{HCT} is the hydrochlorothiazide diffusion coefficient, and C_{HCT} is the drug concentration, the diffusion coefficient of hydrochlorothiazide was obtained as $4.55 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$.

Moreover, the dependency of the current function (peak current divided by the square root of the potential sweep rate) on the square root of the potential sweep rate is shown in Fig. 5D. The current function decayed with the square root of the potential sweep rate. This confirms the electrocatalytic nature of hydrochlorothiazide electrooxidation reaction.

To obtain the kinetic parameters of the hydrochlorothiazide electrocatalytic oxidation by the Ni(III) species, we recorded chronoamperograms using NCPE in the absence and presence of different hydrochlorothiazide concentrations, as shown in Fig. 6A. The transient current decayed in a Cottrellian manner (Fig. 6B) confirms the fact that the electrooxidation process was controlled by diffusion. The diffusion coefficient of hydrochlorothiazide was obtained based on Cottrell's equation [43]:

$$I = nFAD_{\text{HCT}}^{1/2}C_{\text{HCT}}\pi^{-1/2}t^{-1/2} \quad (13)$$

as $3.63 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$. This value is in agreement with that found using cyclic voltammetry. Moreover, the catalytic rate constant of hydrochlorothiazide electrooxidation is also obtained using the following equation [43]:

$$I_{\text{cat}}/I_{\text{d}} = \gamma^{1/2}[\pi^{1/2}\text{erf}(\gamma^{1/2}) + \exp(-\gamma)/\gamma^{1/2}] \quad (14)$$

where I_{cat} and I_{d} are the currents in the presence and absence of hydrochlorothiazide, respectively, $\gamma = k_{\text{cat}}C_{\text{HCT}}t$ is the argument of the error function, k_{cat} is the catalytic rate constant, and t is elapsed time. When $\gamma > 1.5$, $\text{erf}(\gamma^{1/2})$ is almost equal to unity, and Equation (14) is reduced to:

$$I_{\text{cat}}/I_{\text{d}} = \gamma^{1/2} \pi^{1/2} = \pi^{1/2} (k_{\text{cat}}C_{\text{HCT}}t)^{1/2} \quad (15)$$

Using the plot of $I_{\text{cat}}/I_{\text{d}}$ versus $t^{1/2}$ (Fig. 6C), k_{cat} is obtained as $8.50 \times 10^3 \text{ cm}^3 \text{ mol}^{-1} \text{ s}^{-1}$.

3.3. Determination of hydrochlorothiazide

In order to determine hydrochlorothiazide using NCPE, amperometry technique was employed. Typical amperometric signals of NCPE toward the successive increments of hydrochlorothiazide concentrations and the corresponding calibration plot are shown in Fig. 7. The limits of detection (LOD) and quantitation (LOQ) of the procedure were calculated using the $3SD/b$ and $10SD/b$ criteria, respectively, where SD and b are the standard deviation of the intercept and the slope of the calibration curve, respectively [54]. The determined parameters for the calibration curve of hydrochlorothiazide using NCPE are presented in Table 1.

In order to develop an electrochemical procedure for determination of hydrochlorothiazide in biological fluids, amperometry method was attempted for the analysis of the drug in human serum blood, and the obtained results are also displayed in Table 1.

The applicability of the proposed amperometric method for the sample dosage form was examined by analyzing the hydrochlorothiazide tablets. It was found that the drug amounts determined using this method are in the same range of nominal values, and the results are reported in Table 2.

3.4. Stability and selectivity of NCPE

To inspect the stability of NCPE, consecutive cyclic voltammograms using the electrode were recorded. The change in the peak currents was <5% after 100 cycles. In addition, the electrode was stored in 100 mmol dm^{-3} NaOH solution when not in use and its electrochemical reactivity was retained for at least for 25 days.

Selectivity of the NCPE electrode for the assay of hydrochlorothiazide was examined by analyzing hydroxypropylmethylcellulose, microcrystalline cellulose, titanium dioxide, colloidal silicon dioxide, talc, magnesium stearate, starch, gelatin,

D-glucose, L-ascorbic acid, dopamine and uric acid, when a Nafion membrane was applied to the NCPE surface. Under the experimental conditions applied in this work, no chemical interference was observed for all these compounds. The former six compounds are insoluble in the running electrolyte and/or don't bear electroreactive group. The later compounds have a net negative charge in the running electrolyte, and the Nafion membrane rejects the diffusion of these anionic compounds. It should also be added that hydrochlorothiazide is neutral in the electrolyte, and the Nafion membrane does not affect the electrooxidation of hydrochlorothiazide. Therefore, the electrode is able to hydrochlorothiazide the assay without interfering effects, and can be considered selective.

Conclusion

Nickel nanowrinkles were synthesized using a one-pot green hydrothermal synthesis. The synthesis method may be extendable to the synthesis of other transition metal nanostructures, or synthesis of diverse shape of nickel nanostructures using other organic or inorganic additives. Nickel nanowrinkles represent an enhanced electrocatalytic activity towards the oxidation of hydrochlorothiazide. Voltammetric and amperometric measurements showed that the current generated from the oxidation of amine functional group was used for quantification of hydrochlorothiazide and the electrode can act as a sensor with a low detection limit and high selectivity.

Acknowledgments

We would like to thank the Research Council of Shiraz University of Medical Sciences (10300) and also the Iran National Science Foundation (INSF) for supporting this research.

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Figure legends:

Fig. 1: SEM images (A-D) of nickel nanowrinkles with different magnifications, and an EDS spectrum (E).

Fig. 2: Typical cyclic voltammogram of NCPE recorded in 100 mmol dm⁻³ NaOH solution. The potential sweep rate was 25 mV s⁻¹.

Inset: Dependencies of the anodic peak current and peak potential separation on the amount of modifier.

Fig. 3:

- (A) Cyclic voltammograms of NCPE recorded at different potential sweep rates of 1, 2, 5, 7.5, 10, 15, 20, 25, 30, 35, 40, 50, 60, 75, 90, 100, 125, 150, 175, 200, 250, 300, 350, 400, 500, 600, 750, 1000, 1250, 1500, 1750 and 2000 mV s⁻¹.
- (B) Dependency of anodic and cathodic peak currents on the potential sweep rate at low values of ≤ 20 mV s⁻¹.

- (C) Dependency of anodic and cathodic peak currents on the square root of the potential sweep rate at high values of $\geq 100 \text{ mV s}^{-1}$.
- (D) Variation of the anodic and cathodic peak potentials with the natural logarithm of the potential sweep rate.

Fig. 4: Cyclic voltammograms of UCPE and NCPE in the absence and presence of 5.0 mmol dm^{-3} hydrochlorothiazide. The potential sweep rate was 25 mV s^{-1} .

Fig. 5:

- (A) Cyclic voltammograms of NCPE recorded in the presence of 5.0 mmol dm^{-3} hydrochlorothiazide at different potential sweep rates of 2, 3, 5, 7.5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 75, 90, 100, 125, 150, 175 and 200 mV s^{-1} .
- (B) Dependency of the anodic peak potential on the natural logarithm of the potential sweep rate.
- (C) Dependency of the anodic peak current on the square root of the potential sweep rate.
- (D) Dependency of the current function (peak current divided by the square root of the potential sweep rate) on the square root of the potential sweep rate.

Fig. 6:

- (A) Chronoamperograms recorded using NCPE in the absence (a) and presence of different hydrochlorothiazide concentrations of $10 \text{ } \mu\text{mol dm}^{-3}$ (b), $50 \text{ } \mu\text{mol dm}^{-3}$ (c), $100 \text{ } \mu\text{mol dm}^{-3}$ (d), $280 \text{ } \mu\text{mol dm}^{-3}$ (e), $500 \text{ } \mu\text{mol dm}^{-3}$ (f), 1.5 mmol dm^{-3} (g), and 5.0 mmol dm^{-3} (h). The applied potential step was 500 mV .
- (B) The Cottrell plot for curve (h) in (A).
- (C) Dependency of $I_{\text{cat}}/I_{\text{d}}$ on the square root of time for curve (h) in (A).

Fig. 7: Typical amperometric signals obtained during successive increments of hydrochlorothiazide using NCPE. The working potential was 500 mV .

Inset: The calibration curve for the amperogram.

Figure 1

10 μm

(A)

1 μm

(B)

200 nm

(C)

200 nm

(D)

(E)

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7

Scheme 1

Scheme 1: Chemical structure of hydrochlorothiazide.

Scheme 2

Scheme 2: An impossible mechanism for the electrooxidation of hydrochlorothiazide.

Scheme 3

Scheme 3: A possible mechanism for the electrooxidation of hydrochlorothiazide, and its further hydrolysis.

Table 1: The determined parameters for the calibration curve of hydrochlorothiazide in the supporting electrolyte and human serum blood using NCPE.

Medium	Supporting electrolyte	Serum blood
Linear range / mmol dm ⁻³	0.22-5.82	0.22-5.82
Sensitivity (Slope) / mA mol ⁻¹ dm cm ⁻²	13.50	11.38
Intercept / μA cm ⁻²	-0.48	-016
R ²	0.9994	0.9988
Standard error of slope (P=0.005)	2.59×10 ⁻³	2.85×10 ⁻³
Standard error of intercept (P=0.005)	8.96×10 ⁻³	1.81×10 ⁻²
LOD / μmol dm ⁻³	21.2	28.8
LOQ / μmol dm ⁻³	70.6	96.0
RSD%	3.21	3.63

Table 2: Determination of hydrochlorothiazide in commercial tablets using NCPE.

Tablet	Amount labeled / mg	Amount found / mg	Recovery%	Bias%
A	50	51.5	103.0	3.0
B	50	47.8	95.6	-4.4
C	50	48.1	96.2	-3.8