

Short Communication

Synthesis of Lanthanum-doped ZnO Nanoflowers: Supported on Graphite Screen Printed Electrode for Selective and Sensitive Detection of Hydrochlorothiazide

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Received: 18 March 2018 / Accepted: 9 July 2018 / Published: 5 January 2019

In this paper, we report a novel electrochemical sensor for the sensitive detection of hydrochlorothiazide based on La³⁺/ZnO nanoflower supported on graphite screen printed Electrode. Firstly we reported the synthesis and characterization of La³⁺/ZnO nanoflower. The as-synthesized nanostructures. La³⁺/ZnO nanoflower was employed to modify the screen printed electrode and demonstrated its excellent electrocatalytic activities towards hydrochlorothiazide (HCT). Voltammetric measurements were utilized to assess the electrochemical properties towards the hydrochlorothiazide detection. The resulting sensor exhibits excellent sensitivity. Finally, this modified electrode was effectively applied to determine hydrochlorothiazide in real samples with good recoveries.

Keywords: Hydrochlorothiazide, Voltammetric sensor, Screen printed electrode, Nanoflowers

1. INTRODUCTION

Hypertension is another name for high blood pressure. Long-term high blood pressure, is a major risk factor for stroke, heart attacks and kidney failure [1,2]. These problems may be less likely to occur if blood pressure is controlled. First line medications for hypertension contain thiazide-diuretics, calcium channel blockers, angiotensin converting enzyme inhibitors and angiotensin receptor blockers [3]. Hydrochlorothiazide (HCT), 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide, is a thiazide diuretic [4]. Hydrochlorothiazide, is a drug widely used around the world for

hypertension treatment since the advent of chlorothiazide in 1957 and that acts directly on the kidney and blocks the renal sodium chloride channel and increases the excretion of sodium chloride and water and, to a lesser extent, that of potassium ion [5]. Hydrochlorothiazide is used for the treatment of heart failure, cirrhosis of the liver, or kidney disorders [6]. The half-life of hydrochlorothiazide varies between 6 to 15 h and nearly 50–60% of the orally administered drug excreted through urine [7]. An overdose of hydrochlorothiazide the patient loss fluid and electrolytes and the symptoms observed are dizziness, sedation/impairment of consciousness, hypotension and muscle cramps [8]. Therefore, the development of convenient, rapid, selective and sensitive analytical methods for the determination of hydrochlorothiazide, in pharmaceutical formulations and biological samples has gained great importance. For the quantification of hydrochlorothiazide, various analytical methods have been proposed such as chemiluminescence [9], spectrophotometric [10], capillary zone electrophoresis [11], high performance liquid chromatography [12], conductimetry [13], derivative spectroscopy [14], chemometry [15], and electrochemistry [16]. However, most of the above techniques suffer from some disadvantages like low sensitivity and selectivity, expensive instrumental setup, long analysis time, large amounts of sample requirement and hectic pre cleaning steps which make them unsuitable for routine analysis [17-24]. In this regard, electrochemical methods especially voltammetry with respect to their advantages such as simplicity, sensitivity, accuracy, low cost and selective determination of electro-active analytes, have received considerable attention in recent years [25-31].

Because hydrochlorothiazide is an electrochemical active compound, developing and employing of electrochemical methods for hydrochlorothiazide detection are attractive for the researchers.

A screen-printed electrode (SPE) is an attractive alternative choice due to their miniaturized size, inexpensive, easy to fabricate, rapid responses and disposable, which makes them especially suitable for on-site analysis [32-36]. However, the signal to noise of bare electrode is insufficient to determine the trace level of hydrochlorothiazide. Thus, many efforts have been done by modifying the surface of bare electrode using various types of nanomaterials. nanosized materials have high surface area-to-volume ratio, provide a decrease of the overpotential of many analytes that occur at unmodified electrodes, increase the magnitude of the voltammetric response, and result in faster electron transfer between the electrode and analyte [37-52].

Metal oxide based nanocomposites have wide applications in photocatalysis, gas-sensors, piezoelectric materials, and solar cells due to its features such as photosensitivity, non-toxic and low cost. Among metal oxides, ZnO nanostructures, a n-type semiconductor with wide band gap (3.37 eV), large excitation binding energy (60 eV), non-toxicity, near ultraviolet (UV) emission, lower cost, ease of availability, piezoelectricity and high electron communication features is preferred for the fabrication of efficient sensors. The combination metals with metal oxide nanocomposites have a significant effect on the catalytic activity due to the interactions and surface reactivity. Due to their large specific surface area, excellent electro conductivity of lanthanum metal has also been used as modifier in voltammetry for the detection of various species [53, 54].

Therefore, in this work, the preparation and electrochemical characterization of a $\text{La}^{3+}/\text{ZnO}$ nanoflowers modified screen printed electrode, as well as, its behavior as electrocatalyst toward the oxidation and sensitive determination of hydrochlorothiazide were investigated.

2. EXPERIMENTAL

2.1. Chemicals and Apparatus

An Autolab potentiostat/galvanostat (PGSTAT 302N, Eco Chemie, the Netherlands) was employed to perform the electrochemical experiments and the system was controlled using a general purpose electrochemical system software.

The screen-printed electrode (DropSens, DRP-110, Spain) consists of three conventional electrodes: graphite counter electrode, a silver pseudo-reference electrode and an unmodified graphite working electrode. pH was measured by a Metrohm 710 pH meter.

Hydrochlorothiazide and all other reagents were analytical grade, and were purchased from Merck (Darmstadt, Germany). For the preparation of buffers, the orthophosphoric acid and its salts were used to provide the pH range of 2.0–9.0.

2.2. Preparation of La^{3+} -doped ZnO nanoflowers

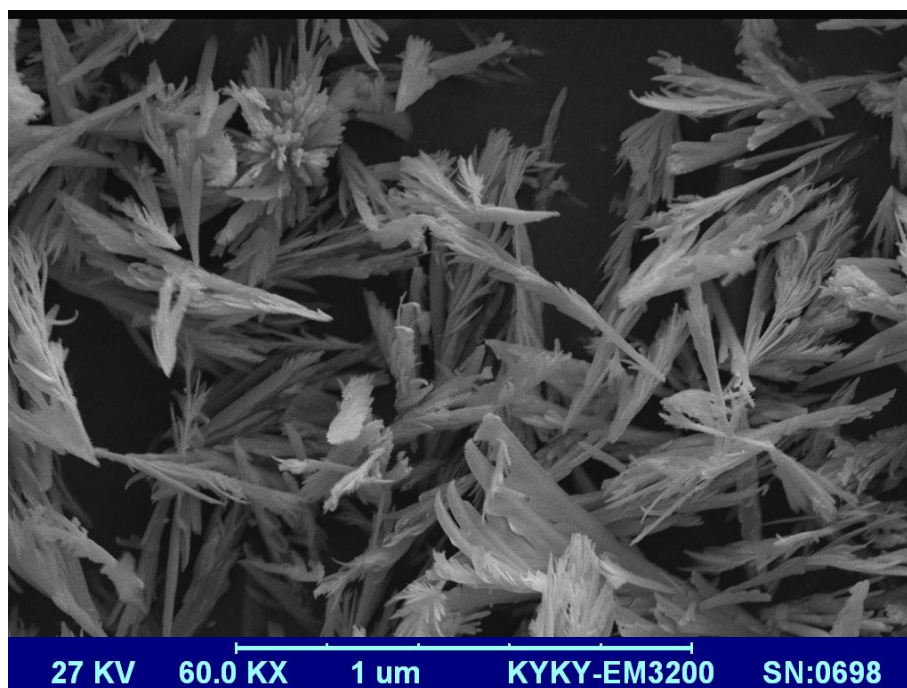


Figure 1. SEM images of La^{3+} -doped ZnO nanoflowers.

All the chemicals used for the preparation of the nano-powders, namely zinc acetate ($\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$), lanthanum nitrate ($\text{La}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$), thiourea ($(\text{NH}_2)_2\text{CS}$) and ammonia (25% NH_3), were of analytical grade. All the precursors were dissolved in deionized water. During the preparation of the nano-powders, ammonia was used as a complexing agent. The ZnO nanostructures were prepared by dissolving 0.46 mol of zinc acetate in 80 mL of deionized water, 0.0046 mol of lanthanum nitrate in 80 mL of deionized water, 0.18 mol of thiourea in 80 mL of deionised water and lastly by adding 19.76 mL of ammonia in 80 mL of deionised water. The amount of solutions of zinc acetate, thiourea and ammonia was held constant at a ratio of 1:1:1. Then the zinc acetate solution was

added in a beaker in the reaction bath, followed by adding thiourea and lanthanum nitrate solution in the same reaction bath and the mixture was stirred for a few seconds. Lastly ammonia solution was added slowly into the mixture, while continuing stirring for 5 min. The temperature of the bath was then allowed to increase up to 80 °C. After that the precipitates were formed and were left overnight and filtered thereafter. The precipitates were then washed with ethanol. The obtained powders were dried at ambient conditions for several days. A typical SEM for synthesized La³⁺-doped ZnO nanoflowers is shown in Fig. 1.

2.3. Preparation of the electrode

The bare graphite screen printed electrode was coated with La³⁺/ZnO nanoflower according to the following simple procedure. 1 mg La³⁺/ZnO nanoflower was dispersed in 1 mL aqueous solution within 45 min ultrasonication. Then, 5 µl of the prepared suspension was dropped on the surface of carbon working electrodes. It remains at room temperature until becomes dry.

2.4. Preparation of real samples

Ten hydrochlorothiazide tablets (labeled 50 mg per tablet, *Irandarou* Company, Iran) were grinding. Then, the tablet solution was prepared by dissolving 50 mg of the powder in 25 mL water by ultrasonication. Then, different volume of the diluted solution was transferred into a 25 mL volumetric flask and diluted to the mark with PBS (pH 7.0). The hydrochlorothiazide content was analyzed by the proposed method using the standard addition method.

Urine samples were stored in a refrigerator immediately after collection. Ten milliliters of the sample was centrifuged for 15 min at 2000 rpm. The supernatant was filtered out using a 0.45 µm filter. Then, different volume of the solution was transferred into a 25 mL volumetric flask and diluted to the mark with PBS (pH 7.0). The diluted urine sample was spiked with different amounts of hydrochlorothiazide.

3. RESULT AND DISCUSSION

3.1. Electrochemical profile of the hydrochlorothiazide on the La³⁺/ZnO/SPE

To study the electrochemical behaviour of hydrochlorothiazide which is pH-dependent, it is necessary to obtain the optimized pH value in order to achieve the accurate results. By performing the experiments by use of modified electrodes at various pH values ranging from 2.0–9.0, it was revealed that the best results for electro-oxidation of hydrochlorothiazide occur at pH=7.0. The obtained cyclic voltammograms in the presence of 400.0 µM hydrochlorothiazide using La³⁺/ZnO/SPE the (Curve a) and bare SPE (Curve b) are shown in Fig. 2. According to CV results the maximum oxidation of hydrochlorothiazide on the La³⁺/ZnO/SPE occurs at 750 mV which is about 100 mV more negative compared with unmodified SPE.

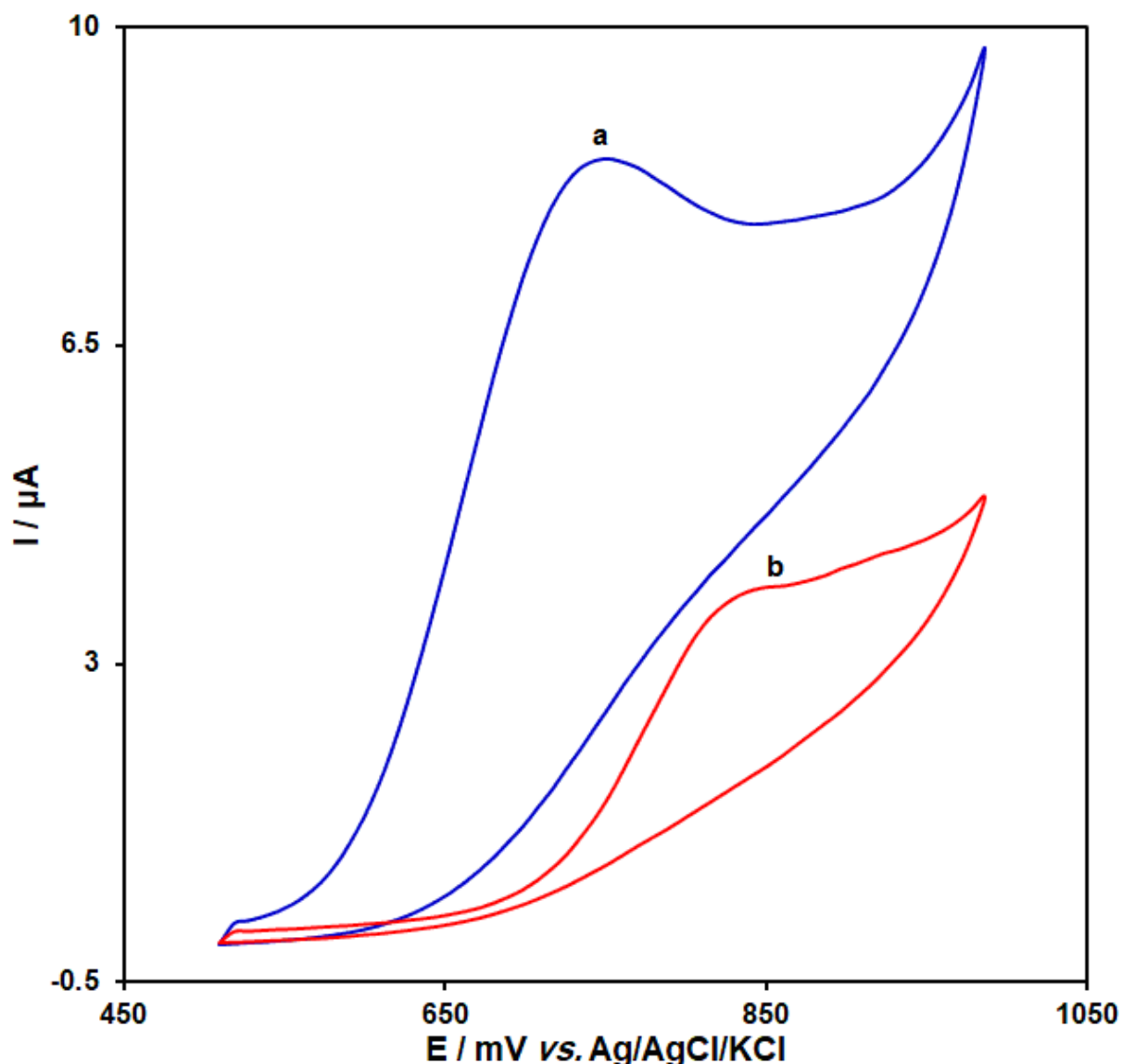


Figure 2 Cyclic voltammograms of (a) $\text{La}^{3+}/\text{ZnO}/\text{SPE}$ and (b) bare SPE in 0.1 M PBS (pH 7.0) in the presence of 400.0 μM hydrochlorothiazide at the scan rate 50 mVs^{-1} .

3.2. Effect of scan rate on the results

Increasing in scan rate leads to enhanced oxidation peak current according to the obtained results from the study of the effect of potential scan rates on the oxidation currents of hydrochlorothiazide, Fig. 3. In addition, there is a linear relationship between I_p and the square root of the potential scan rate ($v^{1/2}$) that demonstrates that the oxidation procedure of analyst is in control of diffusion.

Fig. 4 show a Tafel plot that was drawn from points of the Tafel region of the CV. The Tafel slope of 0.0966 V obtained in this case, assuming a charge transfer coefficient of $\alpha=0.64$ [55].

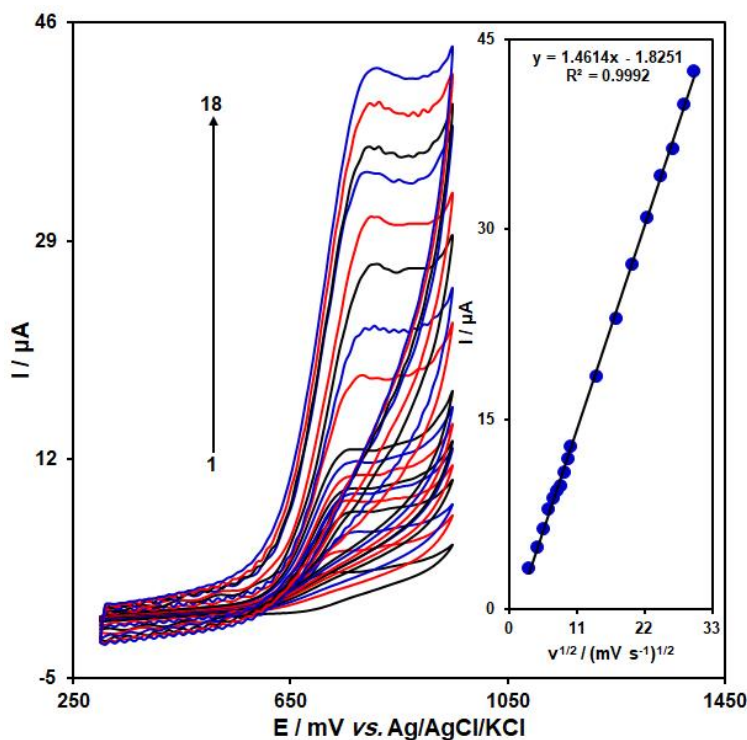


Figure 3. Cyclic voltammograms of La³⁺/ZnO/SPE in 0.1 M PBS (pH 7.0) containing 400.0 μM hydrochlorothiazide at various scan rates; numbers 1-18 correspond to 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800 and 900 mV s⁻¹, respectively. Inset: variation of anodic peak current vs. v^{1/2}.

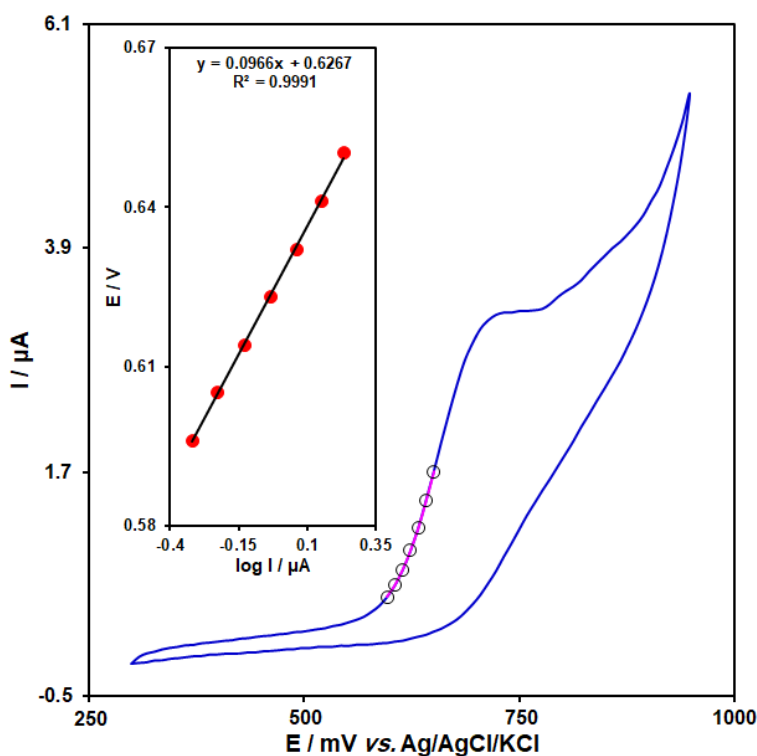


Figure 4. Cyclic voltammogram (at 10 mV s⁻¹) of La³⁺/ZnO/SPE in 0.1 M PBS (pH 7.0) containing 400.0 μM hydrochlorothiazide. The points are the data used in the Tafel plot. The inset shows the Tafel plot derived from the CV.

3.3. Chronoamperometric analysis

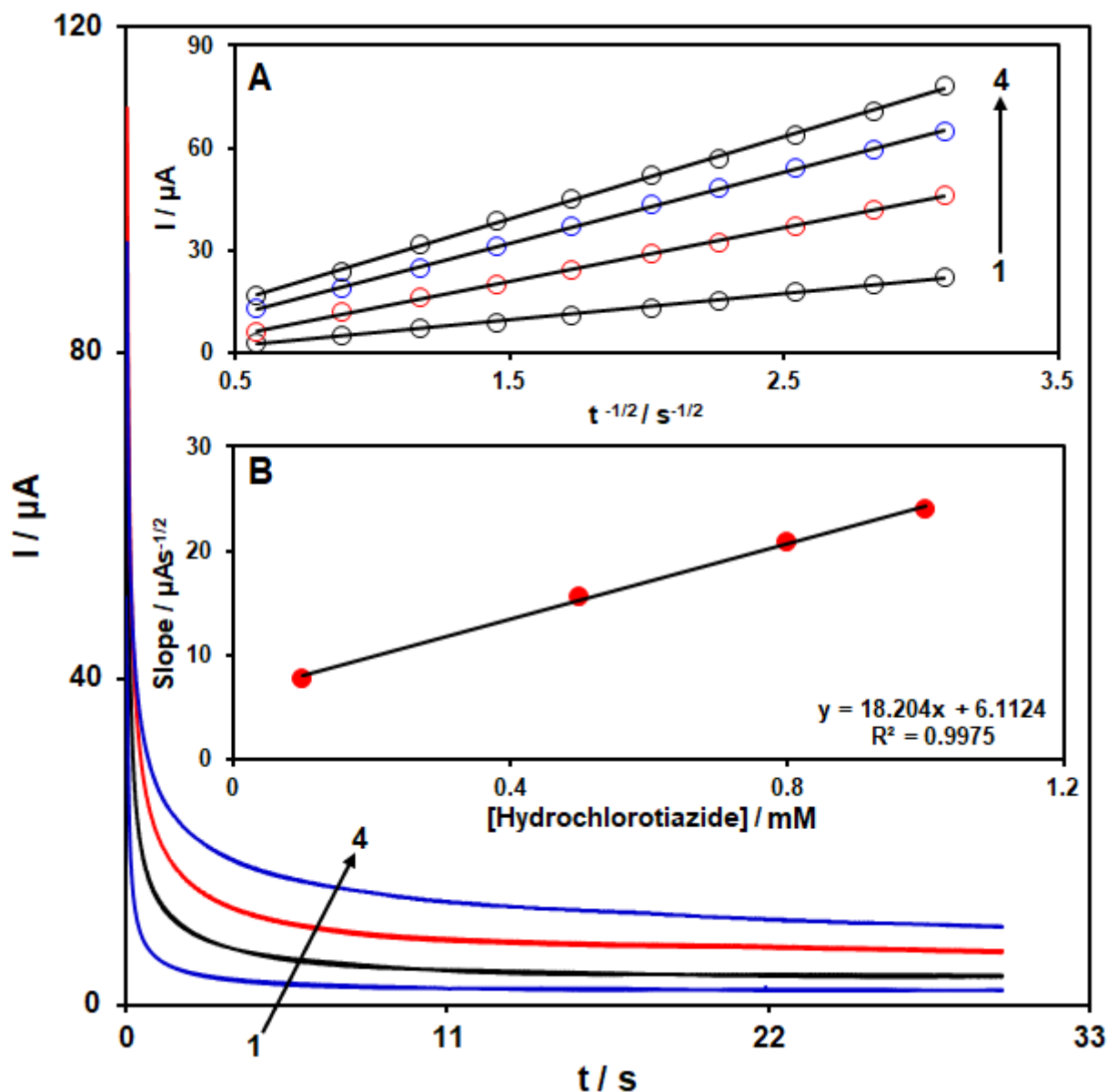


Figure 5. Chronoamperograms obtained at $\text{La}^{3+}/\text{ZnO}/\text{SPE}$ in 0.1 M PBS (pH 7.0) for different concentration of hydrochlorothiazide. The numbers 1–4 correspond to 0.1, 0.5, 0.8, and 1.0 mM of hydrochlorothiazide. Insets: (A) Plots of I vs. $t^{-1/2}$ obtained from chronoamperograms 1–4. (B) Plot of the slope of the straight lines against hydrochlorothiazide concentration.

The analysis of chronoamperometry for hydrochlorothiazide samples was performed by use of $\text{La}^{3+}/\text{ZnO}/\text{SPE}$ vs. $\text{Ag}/\text{AgCl}/\text{KCl}$ (3.0 M) at 0.8 V. The Chronoamperometric results of different concentration of hydrochlorothiazide sample in PBS (pH 7.0) are demonstrated in Fig. 5. The Cottrell equation for chronoamperometric analysis of electroactive moieties under mass transfer limited conditions is as follow [55]:

$$I = nFAD^{1/2}C_b\pi^{-1/2}t^{-1/2}$$

Where D represents the diffusion coefficient ($\text{cm}^2 \text{s}^{-1}$), and C_b is the applied bulk concentration (mol cm^{-3}). Experimental results of I vs. $t^{-1/2}$ were plotted in Fig. 5A, with the best fits for different concentrations of hydrochlorothiazide. The resulted slopes corresponding to straight lines in Fig. 5A, were then plotted against the concentration of hydrochlorothiazide (Fig. 5

B). The mean value of D was determined to be $1.3 \times 10^{-5} \text{ cm}^2/\text{s}$ according to the resulting slope and Cottrell equation.

3.4. Calibration curves

Based on the resulting peak currents of hydrochlorothiazide by use of $\text{La}^{3+}/\text{ZnO}/\text{SPE}$, the quantitative analysis of target was done in water solutions. The modified electrode ($\text{La}^{3+}/\text{ZnO}/\text{SPE}$) as working electrode in the range of hydrochlorothiazide concentration in 0.1 M PBS was used in differential pulse voltammetry (DPV) due to the advantages of DPV including the improved sensitivity and better performance in analytical applications. According to the results, a linear relationship exists between the peak currents and concentrations of hydrochlorothiazide within the concentration range of 1.0 to 600.0 μM with the correlation coefficient of 0.9996 (Fig. 6). The detection limit was obtained 0.6 μM

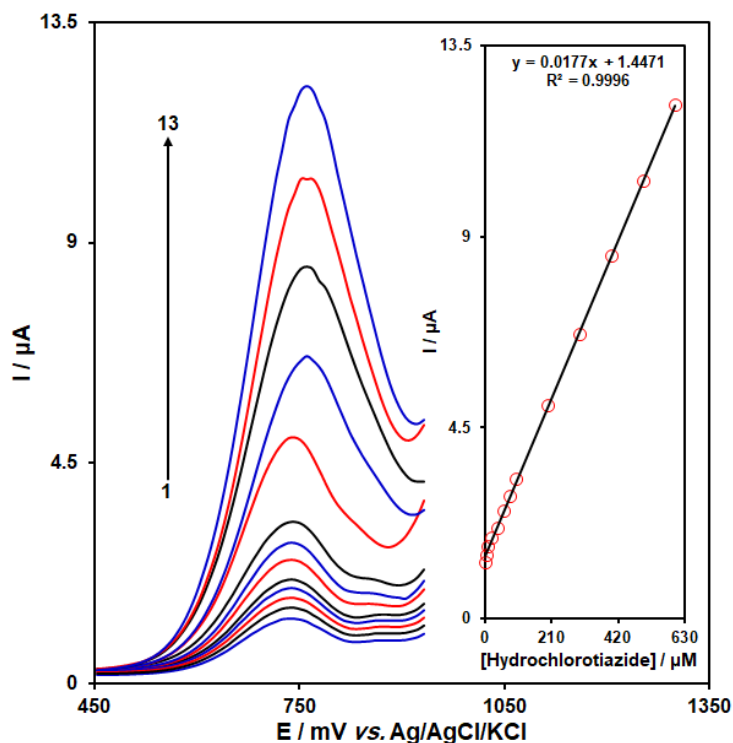


Figure 6. DPVs of $\text{La}^{3+}/\text{ZnO}/\text{SPE}$ in 0.1 M (pH 7.0) containing different concentrations of hydrochlorothiazide. Numbers 1–13 correspond to 1.0, 5.0, 10.0, 20.0, 40.0, 60.0, 80.0, 100.0, 200.0, 300.0, 400.0, 500.0 and 600.0 μM of hydrochlorothiazide. Inset: plot of the electrocatalytic peak current as a function of hydrochlorothiazide concentration in the range of 1.0-600.0 μM .

3.5. Analysis of real samples

In order to evaluate the analytical applicability of the proposed method, also it was applied to the determination of hydrochlorothiazide in hydrochlorothiazide tablet and urine samples. The results for determination of the hydrochlorothiazide in real samples are given in Table 1. Satisfactory recovery of the experimental results was found for hydrochlorothiazide. The reproducibility of the method was demonstrated by the mean relative standard deviation (R.S.D.).

Table 1. The application of $\text{La}^{3+}/\text{ZnO}/\text{SPE}$ for determination of hydrochlorothiazide in hydrochlorothiazide tablet and urine samples (n=5).

Sample	Spiked (μM)	Found (μM)	Recovery (%)	R.S.D. (%)
Hydrochlorothiazide tablet	0	5.0	-	3.1
	2.5	7.4	98.6	2.5
	7.5	12.3	98.4	1.8
	12.5	17.9	102.3	2.6
	17.5	22.3	99.1	2.9
Urine	0	-	-	-
	5.0	5.1	102.0	1.9
	10.0	9.9	99.0	2.1
	15.0	15.2	101.3	3.2
	20.0	20.6	103.0	2.8

4. CONCLUSIONS

In summary, $\text{La}^{3+}/\text{ZnO}$ nanoflower were synthesized. Finally, a screen printed electrode was fabricated using $\text{La}^{3+}/\text{ZnO}$ nanoflower to provide a sensitive electrochemical device in hydrochlorothiazide sensing. The detection limit of the method for hydrochlorothiazide was $0.6 \mu\text{M}$ ($S/N = 3$) and the response was found to be linear in the concentration range of 1.0 to $600.0 \mu\text{M}$. The modified electrode was used for the detection of hydrochlorothiazide in real samples and found to produce satisfactory results.

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