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Research Article

Voltammetric determination of hydrochlorothiazide at SnO_2-NiO nanocomposite/ionic liquid modified carbon paste electrode

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ABSTRACT

Herein, SnO_2-NiO nanocomposite was prepared via a hydrothermal method and characterized by various techniques consisting X-ray diffraction (XRD), field emission scanning electron microscopy (FESEM) and energy-dispersive X-ray spectroscopy (EDS). The FESEM image showed particles with the size at about 20–130 nm in diameters. The electrochemical oxidation of hydrochlorothiazide (HCTZ) at a carbon paste electrode modified with SnO_2-NiO nanocomposite and an ionic liquid (1-butyl-3-methylimidazolium hexafluorophosphate (BMIM-PF_6)) (SnO_2-NiO/IL/CPE) was evaluated in phosphate buffer (PB) pH 8, by using cyclic voltammetry, linear sweep voltammetry, differential pulse voltammetry and chronoamperometry. The SnO_2-NiO/IL/CPE indicated good electrocatalytic behaviour towards to oxidation of hydrochlorothiazide. The anodic peak currents were increased with the HCTZ concentration and indicated two linear dynamic range from 0.01 to 0.1 µM and 0.1 to 100 µM and a detection limit of 0.002 µM (S/N = 5) under the optimized conditions. The introduced electrochemical sensor was successfully utilized for the determination of HCTZ in the pharmaceutical formulations and biological sample.

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1. Introduction

Hypertension can led to risk of serious problem such as stroke, heart disease, aneurysm and sometimes death. Most of the people with hypertension need treatment with antihypertensive drugs such as losartan (LOS), valsartan (VAL), amlodipine (AML) and HCTZ. HCTZ, (6-chloro-3, 4-dihydro-2H-1, 2, 4-benzo-thiadiazine-7-sulfonamide 1, 1 dioxide) has been used with or without other medication to treat high blood pressure [1, 2]

HCTZ is a type of diuretic that helps regulate the level of fluids in the body. It is effective in treating high blood pressure by controlling blood pressure and risk of stroke and heart attack is reduced [3, 4]. It may also be used to reduce fluid accumulation in the body due to heart failure, liver disease or kidney disease [3]. Different analytical techniques including chromatography [5], liquid chromatography tandem mass spectrometry (LC/MS) [6], high-performance liquid chromatography (HPLC) [7] and spectrophotometry [8] have been applied to determine HCTZ in pharmaceutical drugs and biological samples. The HPLC technique recommended as an official method for the individual determination of HCTZ in the United States pharmacopeia [9].

The chromatographic based methods need the use of organic solvents, time-consuming derivitization steps and extensive preliminary sample pretreatment. Electrochemical methods, have gained including easy to operate, rapid, economical, sensitive, short analysis time and inexpensive production. Various electrochemical sensors have been reported for HCTZ sensing in the pharmaceutical compounds and biological samples such as tantalum electrode coated with graphene nanowalls [10], benzoyl ferrocene/carbon nanotubes paste electrode [11], boron-doped diamond electrode [12], copper hydroxide nanoparticles modified carbon ionic liquid electrode [13], 2-chlorobenzoyl ferrocene modified graphene oxide sheet paste electrode [14] and Trypan Blue modified pencil graphite reference platinum electrode [15].

Ionic liquids (ILs) due to good chemical and thermal stability, low vapor pressure and high ionic conductivity have been used to make various modified electrodes [16, 17]. They could decrease the overpotential and enhance the electrochemical signal of analytes. Metal oxide nanostructures have received much attention in many fields because of their large surface area, catalytic effect and chemical stability [18-21]. Among them, SnO_2 based nanostructures and nanocomposites have been attracted considerable attention in the recent years. SnO_2 as a good catalyst and one of the n-type metal oxide semiconductors have been applied in chemical and electrochemical sensors [22-25], gas sensor [26], supercapacitors [27] and batteries [28].

Here, the advantage of ionic conductivity of ionic liquid (BMIM.PF_6) was combined with large surface area and catalytic effect of SnO_2-NiO nanocomposite and used to develop a CP modified electrode (SnO_2-NiO/IL/CPE) for the determination of HTCZ in pharmaceutical formulation, human urine and blood plasma samples.

2. Experimental

2.1. Materials

Graphite powder (extra pure, particle size < 50 µm), hydrochloric acid, sodium hydroxide, nickel (II) nitrate hexahydrate ($Ni(NO_3)_2.6H_2O$), sodium dodecyl sulphate (SDS), tin (IV) chloride tetrahydrate ($SnCl_4.4H_2O$), ethanol, heptane and mineral oil were purchased from Merck.. Hydrochlorothiazide (purity: 98.7%) was obtained from DarouPakhsh pharmaceutical Co Iran. The commercial tablets labeled Hydrochlorothiazide 25 mg and Hydrochlorothiazide tablets labeled 50 mg were purchased from local drugstore.

2.2. Apparatus

All voltammetric measurements were recorded using an Autolab potentiostat/galvanostat type 302N (Metrohm, The Switzerland) controlled by NOVA software version 2.1.2. A conventional three-electrode system consisted of a bare or modified CPE as working electrode, an Ag/AgCl and a Pt wire as the reference and auxiliary electrode, respectively. A Metrohm-481 pH-meter (Switzerland) was used for pH adjustments. The morphology of the synthesized nanocomposite was analyzed with scanning electron microscope (FESEM), (Zeiss Co., Sigma, Germany) equipped with an energy-dispersive X-ray spectrometer (EDX) (Zeiss Co., Ultra Plus, Germany). Gold coating of thickness (approximately) 8 nm at 10 mA for 60 s was carried out on sample using sputter coater before FESEM imaging. The X-ray diffraction (XRD) pattern was recorded by an X-pertpro, Panalytical (Netherlands) and operated at 40 KV and 40 mA with λ= 1.5406 Å in the 2θ range of 10°-90°.

2.3. Synthesis of the SnO_2-NiO Nanocomposite

SnO_2-NiO nanocomposite was prepared by the hydrothermal method. First, 0.169 g of $Ni(NO_3)_2.6H_2O$ and 1.55 g of SDS were dissolved in a solution containing 10 ml of heptane and 3.5 ml of ethanol. Then, 0.35 g of $SnCl_4.4H_2O$ and 1.2 g of sodium hydroxide were added to above solution and stirred for 15 min. In continue, the prepared solution was transferred into Teflon-lined autoclave and heated to 200 °C for 17 h. The green precipitate was formed. The prepared green precipitate was washed with ethanol and distilled water by centrifugation. The precipitate was filtered and dried in an oven at 60 °C for 24 hours. Then, the dried precipitate was calcined in a muffle furnace at 500 °C for 2 h.

2.4. Preparation of Modified Electrodes

The SnO_2-NiO/IL/CPE was fabricated by mixing the 0.37 g of graphite powder, 0.03 g of SnO_2-NiO, 30 µL of BMIM.PF_6 and 0.1 g of mineral oil. The fabricated paste was packed inside a poly-amide tube (inner diameter 2.5 mm) and the electrical contact with a copper wire was provided. Before use, the surface of SnO_2-NiO/IL/CPE was smoothed on a piece of polishing paper. The conventional CPE was fabricated by mixing of 0.4 g of graphite powder and 0.1 g of mineral oil in a mortar and pestle. The IL/CPE modified electrode was fabricated by mixing of 0.4 g of graphite powder, 30 µl of BMIM.PF_6 and 0.1 g of mineral oil and the SnO_2-NiO/CPE modified electrode was prepared by mixing the 0.37 g of graphite powder, 0.03 g of SnO_2-NiO and 0.1 g of mineral oil.

2.5. Electrochemical Measurements

A 1 mM stock solution of HCTZ was prepared in methanol. The 0.1 M PB was used as supporting electrolyte and prepared by mixing 0.2 M $Na_2HPO_4.2H_2O$ and 0.2 M $NaH_2PO_4.H_2O$. The pH of PB was adjusted using 0.2 M NaOH and 0.1 M HCl. HCTZ working solution was daily prepared by dilution of the stock solution with PB.

The electrochemical properties of the CPE and modified electrodes were investigated in 5 mM $K_4[Fe(CN)_6]$ and 0.1 M KCl solution by cyclic voltammetry (CV). The CVs were recorded between 0 to 0.8 V at a scan rate range from 5-100 mV s^{-1}. Electrochemical behaviours and determination of HCTZ were studied by differential pulse voltammetry (DPV), linear sweep voltammetry (LSV) and chronoamperometric (CA) methods. DPV was used at a potential range from 0.45 to 0.75 V and chronoamperometric responses were recorded at a potential step 0.7 V. The linear sweep voltammograms were recorded between 0 to 0.9 V at a scan rate range from 50 mV s^{-1}. All measurements were carried out in triplicate for each concentration.

2.6. Analysis of Real Sample

HCTZ is available in tablet form for oral administration containing the equivalent to 25 and 50 mg. Ten tablets (Hydrochlorothiazide 25 and 50 from DarouPakhsh Pharmaceutical Co.) were accurately weighed and powdered in a mortar. An adequate amount of the finely powdered tablets was dissolved in 5 ml methanol and sonicated for 5 min. Then, the solution was filtered and transferred into a 5 ml volumetric flask and diluted to mark by methanol to obtain a final solution containing 0.0167 and 0.0336 M of HCTZ. Required volumes to prepare each concentration were transferred into a 10 ml volumetric flask and diluted with 0.1 M PB. The prepared transferred to the electrochemical cell just prior to electrochemical analysis.

10 ml of a human urine sample was stored in the refrigerator. 5 ml of urine was centrifuged (10 min at 1500 rpm) and diluted with PB pH 8 in a ratio of 1:2,. Human blood plasma was prepared from Iranian Blood Transfusion Organization and stored in the centrifuged. The sample was centrifuged (2 min at 2000 rpm) and 4 ml of methanol added to precipitate the proteins and clear supernatant layer was filtrated. The free protein human blood plasma diluted with PB pH 8 in a 10 ml volumetric flask. The urine and blood plasma were used for HCTZ determination by standard addition method.

3. Results and Discussion

3.1. Microstructure Characterization

The FESEM was utilized to evaluate morphology of the SnO_2-NiO nanocomposite (Fig. 1a). As seen, the cluster of SnO_2 was mixed with NiO [29] and particles size distribution around 20-130 nm with some agglomeration. The composition of the sample is evaluated by EDS analysis (Fig. 1b) which shows Sn, Ni and O elements in composition without any appreciable impurities. The observed Au peaks in the EDS spectrum is due to gold coating of the sample to avoid charging in FESEM. The XRD pattern of the SnO_2-NiO nanocomposite is displayed in Fig. 1c. In this Figure, the presence of SnO_2 and NiO were clearly revealed by the characteristic diffraction peaks. The diffraction peaks at 26.41°, 33.85°, 47.38°, 49.58°, 52.01°, 57.09°, 64.40° and 65.69° are related to the SnO_2 tetragonal rutile and has agreement with the standard card (JCPDS no. 01-077-0449). Also, the other diffraction peaks at 43.12°, 47.38°, 61.28°, 75.09° and 78.89° are related to the cubic structure of NiO that are in agreement with standard card of JCPDS no. 01-073-1523. The average crystalline size of the SnO_2-NiO nanocomposite was calculated to be 43 nm by the Debye-Scherrer equation Eq. (1):

$$D = \frac{k\,\lambda}{\beta \cos\theta} \qquad (1)$$

where D is the crystallite size (nm), k is a constant (0.94 for spherical particles), λ is the wavelength of the x-ray radiation (Cu-Kα =0.1541 nm), β is the full width at half maximum (FWHM) of the intense and broad peaks and θ is the Bragg's or diffraction angle.

3.2. Electrochemical Properties of Working Electrodes

The CVs of the CPE, SnO_2-NiO/CPE, IL/CPE and SnO_2-NiO/IL/CPE, in the 5 mM $K_4Fe(CN)_6$ and 0.1 M KCl at the scan rate of 50 mV s^{-1} are investigated. The peak separations (ΔE_p) are obtained 0.37, 0.24, 0.30 and 0.23 V for CPE, SnO_2-NiO/CPE, IL/CPE and SnO_2-NiO/IL/CPE respectively. The obtained results indicate a good improvement in the electron transfer kinetic at the surface of the SnO_2-NiO/IL/CPE.

The effective surface area (A) was calculated for the electrodes by the Randles-Sevcik [30] equation Eq. (2):

$$I_{pa} = (2.69 \times 10^5)\, n^{2/3}\, C\, A\, D^{1/2}\, \upsilon^{1/2} \qquad (2)$$

Where I_{pa} is the peak current, A is the surface area of the electrode, n is the electron number, υ is the scan rate, D and C are the diffusion coefficient and concentration of $K_4Fe(CN)_6$. The effective surface area values found for the electrodes were: 0.08 cm^2 (CPE), 0.23 cm^2 (SnO_2-NiO/IL/CPE). This is mainly attributed to much higher redox currents and improved sensitivity of SnO_2-NiO/IL/CPE.

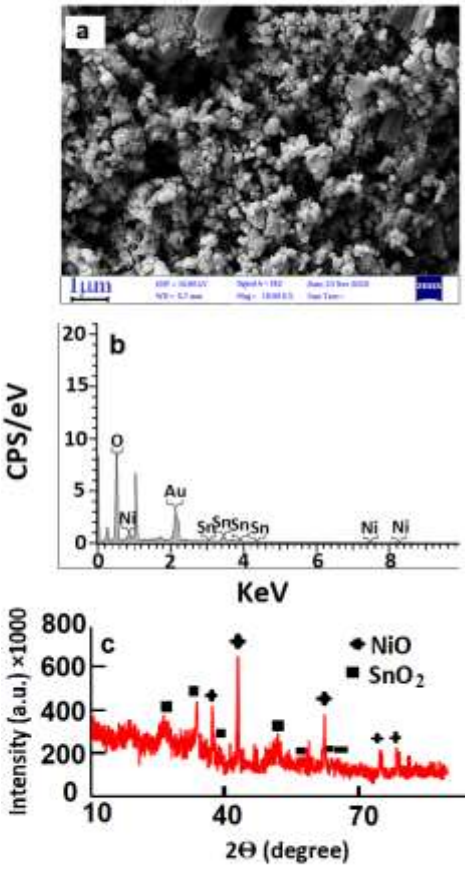

Fig. 1. (a) FESEM image (b) EDS spectra and (c) XRD patterns of SnO_2-NiO nanocomposite.

3.3. Electrochemical Behaviour of HCTZ

The electrochemical behaviour of HCTZ was investigated at surface of bare and modified CPEs in 0.1 M PB and scan rate of 50 mV s^{-1} (Fig. 2). In this Figure, the scans a, b, c and d show the cyclic voltammograms of CPE, SnO_2-NiO/CPE, IL/CPE and SnO_2-NiO/IL/CPE respectively. It can be seen that over a potential range from 0.0 to 0.85 V, the cyclic voltammograms of all electrodes show a single irreversible oxidation with a peak potential of nearly 0.65 V (vs. Ag/AgCl as reference electrode). The SnO_2-NiO/IL/CPE shows a significant oxidation current starting at about 0.25 V *vs* Ag/AgCl reference electrode. This result shows low redox activity at the bare CPE over the same potential range and HCTZ oxidation current was started at about 0.6 V. A considerable negative shift of the onset potential for electrooxidation of HCTZ and a large enhancement of HCTZ peak current indicate the significant catalytic ability of SnO_2-NiO/IL toward HCTZ oxidation.

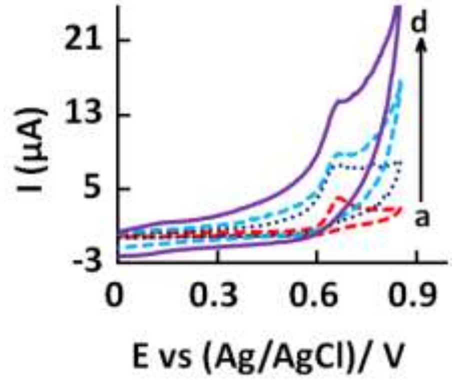

Fig. 2. CVs of (a) CPE (b) SnO_2-NiO/CPE (c) IL/CPE (d) SnO_2-NiO/IL/CPE in 0.1 M PB at pH 8 containing 100 µM HCTZ at scan rate of 50 mV/s

3.4. Effect of pH

The effect of pH on oxidation of HCTZ at 100 µM concentration was investigated in the range of pH 4.0-8.0 by LSV (Fig. 3A). The oxidation peak potentials (E_p) are shifted to less positive values with increasing of pH solution and the oxidation peak current are increased. The E_p has linear relationship with pH from 4.0 to 8.0 with a slope of 55 mV pH (Fig. 3B).

The slope value (55 mV/pH) is reasonably close to the theoretical Nerstian value of 59 mV for a two-electron and two-proton process [31]. On the other hand, the oxidation peak current is increased by the increasing of pH solution from 4.0 to 8.0 and it achieves a maximum at about pH 8 (Fig. 3C). After pH 8, a decrease in the peak current was observed along with the peak broadening (not shown) properly due to hydrolysis of HCTZ. Therefore, PB with pH 8 was utilized as optimum pH in all voltammetric measurements.

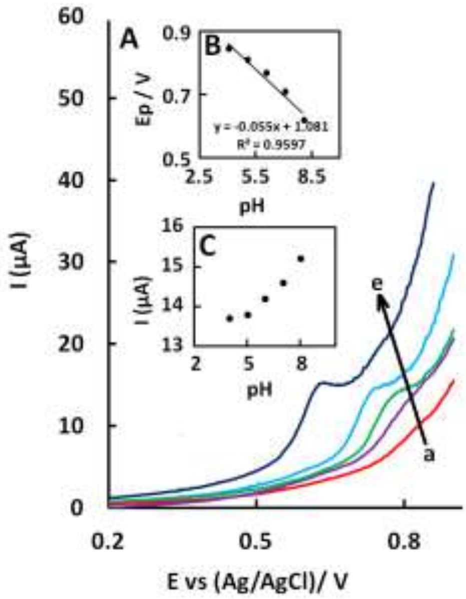

Fig. 3. (A) LSVs of 100 µM HCTZ on SnO_2-NiO/IL/CPE at different pH values, a to e are 4, 5, 6, 7 and 8 respectively and the scan rate was 50 mV/s. (B) The relationship between the peak potential (E_p) and pH. (C) The relationship between the oxidation peak current (I_{pa}) and pH

3.5. Effect of Scan Rate

The influence of scan rate (υ) on the oxidation peak current of 100 µM HCTZ at surface of SnO_2-NiO/IL/CPE was studied by CV in the range of 5 to 100 mV s^{-1}. As shown in Fig. 4A, the oxidation currents increased gradually with the increase of the scan rate. The oxidation peak currents showed a linear relationship with the square root of scan rate (I_{pa} (µA) = 2.637 $υ^{1/2}$ mV s^{-1} - 3.797 with a correlation of $R^2 = 0.9945$) (Fig. 4B), demonstrating a diffusion-controlled process. Also, the number of electrons in the overall reaction can be obtained from the slope of this Figure (2.637). It is estimated that the total number of electrons involved in the anodic oxidation of HCTZ is $n = 2.11$ (~ =2).

Tafel plot was drawn from points of the tafel region of the voltammogram at scan rate of 5 mV s^{-1} to obtain information on the rate determining step. The tafel slope of 0.1238 V obtained from Fig. 4C agrees with the involvement of one electron in the rate-determining step for the electrooxidation of HCTZ, assuming a charge transfer coefficient of α= 0.52. Based on the obtained results, the electrochemical oxidation mechanism of HCTZ at SnO_2-NiO/IL/CPE can be occurring by releasing two electrons and two protons according to Fig. 5.

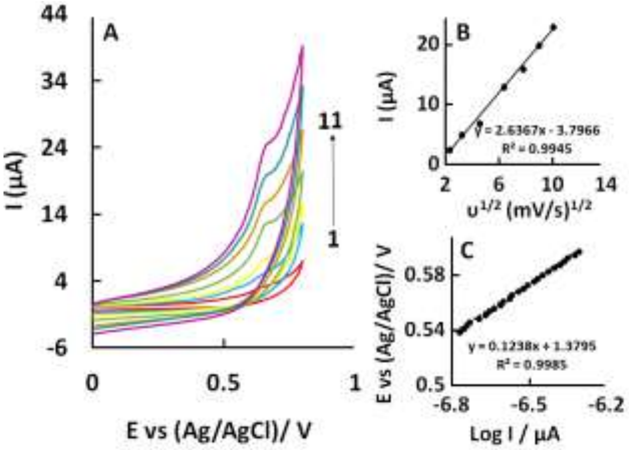

Fig. 4. (A) Cyclic voltammograms of 100 µM HCTZ on SnO_2-NiO/IL/CPE in 0.1 M PB at pH 8 with different scan rates (υ): 1) 5, 2) 10, 3) 20, 4) 40, 5) 60, 6) 80 and 11) 100 mV/s. (B) linear dependence of I_p versus square root of the scan rate. (C) The tafel plot derived from the CV at scan rate of 5 mV/s.

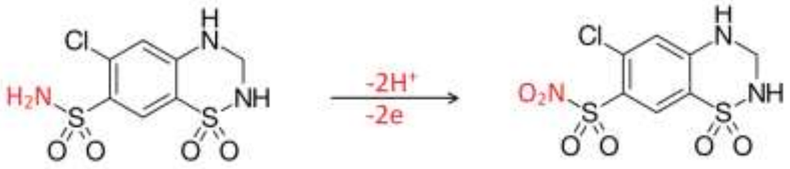

Fig. 5. Electrooxidation mechanism of HCTZ

3.7. Chronoamperometry Measurement

The catalytic oxidation of HCTZ by SnO_2-NiO/IL/CPE is evaluated by choronoamperometry (CA). Fig. 6 indicates the chronoamperogram of the HCTZ 100 µM recorded at the potential step of 0.7 V. Under diffusion control and according to Cottrell equation [30] Eq. (3), a plot of I vs. $t^{-1/2}$ gives a straight line which the slope is $nFAD^{1/2}C/\pi^{1/2}$ as shown in inset of Fig 6.

$$I_{pa} = n F A C D^{1/2} \pi^{-1/2} t^{-1/2} \qquad (3)$$

Where I_{pa} is the current, F is the Faraday constant, n is number of electrons (n=2 for HCTZ electrooxidation), D is the diffusion coefficient, C is the analyte concentration, t is the time and A geometrical area (0.23 cm^{-2}). The diffusion coefficient (D) was obtained to be 1.52×10^{-6} for HCTZ.

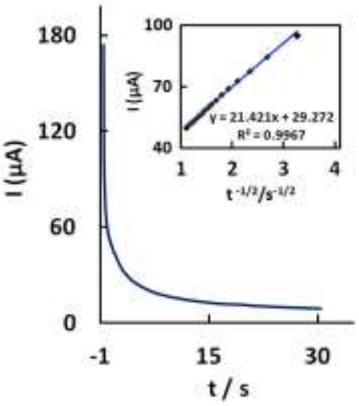

Fig. 6. Chronoamperometric response of the SnO_2-NiO/IL/CPE in 0.1 M PB (pH 8) containing 100 µM HCTZ at a potential step of 0.7 V vs Ag/AgCl reference electrode. Inset is the plots of I *vs* $t^{1/2}$.

3.8. Determination of HCTZ

Owing to the high sensitivity and selectivity DPV was applied to determine HCTZ. Differential pulse voltammograms of various concentrations of the HCTZ were shown in Fig. 7A. As can be seen, the oxidation peak currents increased linearly with increasing HCTZ concentration in the range from 0.01 to 100 µM. Fig. 7B shows that the plot of peak currents versus the HCTZ concentration consists of two linear ranges with different slopes. The linear equations are I_{pa} (µA) = 6.0246 C (µmol L^{-1}) + 1.7087 and Ipa (µA) = 0.0225 C (µmol L^{-1}) + 2.5373. From the analysis of these data, the limit of detection (LOD) was calculated to be 0.002 µM based on the signal-to-noise ratio (S/N) of 5 by the equation Eq. (4). Some characteristics of the SnO_2-NiO/IL/CPE compared with other reported voltammetric methods are shown in Table 1.

$$\text{LOD} = \frac{3\,S_b}{m} \qquad (4)$$

where S_b and m are the standard deviation of the blank and the slope of the calibration graph, respectively.

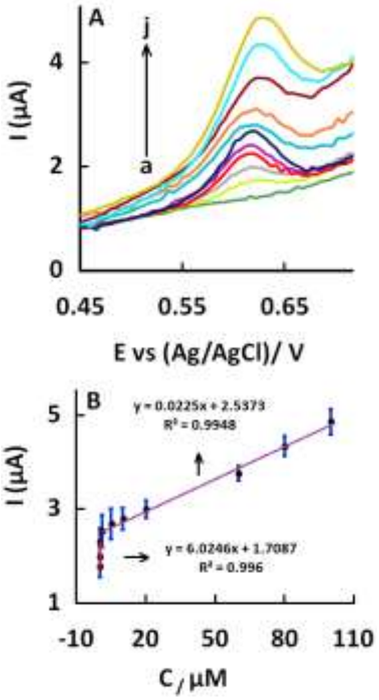

Fig. 7. (A) DPVs of SnO_2-NiO/IL/CPE in the solution containing HCTZ at different concentrations: a) 0, b) 0.01, c) 0.05, d) 1, e) 5, f) 10, g) 20, h) 60, i) 80 and j) 100 µM, respectively). (B) The relationship of the anodic peak current with the HCTZ concentration.

Table 1. The comparison of different methods for HCTZ determination

Electrode	Technique	Linear range	LOD	Ref.
graphene/Ta[a]	DPV	3–500 µM	0.4 µM	[10]
BFCN/CPE[b]	SWV	6.0×10^{-7} –3.0×10^{-4} M	90 nM	[11]
Boron-doped diamond electrode	SWV	1.97-88.1 µM	0.639 µM	[12]
$Cu(OH)_2$NP/CILE[c]	SWV	3.0–600 µM	60 nM	[13]
2CBFGPE[d]	SWV	5.0×10^{-8}- 2.0×10^{-4} M	20.0 nM	[14]
TyB/GGG[e]	DPV	0.5–7.0 µM	0.133 µM	[15]
SnO_2-NiO/IL/CPE	DPV	0.01-0.1 µM 0.1-100 µM	2.0 nM	This work

[a] Tantalum electrode coated with graphene nanowalls
[b] Benzoyl ferrocene/carbon nanotubes paste electrode
[c] Copper hydroxide nanoparticles modified carbon ionic liquid electrode
[d] 2-chlorobenzoyl ferrocene modified graphene oxide sheet paste electrode
[e] Trypan Blue modified combined pencil graphite electrode

3.9. Interference Study

The selectivity of the SnO_2-NiO/IL/CPE for HCTZ with some different substances as potential interfering substances was investigated. The interference study was examined by DPV in the solution mixture containing 100 µM HCTZ at PB pH 8. Ascorbic acid (AA) is the one of the main metabolites existing in the human urine. The hydrochlorothiazide (HCTZ) and losartan (LOS) are used for treatment of hypertension separately or together in a combined pharmaceutical formulation. Fig. 8 shows the DPV of a solution containing 1 mM AA, 100 µM HCTZ and 30 µM LOS at surface of SnO_2-NiO/IL/CPE. The result indicated that oxidation of AA and LOS is occurred in potential peaks of 0.12 V and 1.2 V respectively and no interference on oxidation of HCTZ.

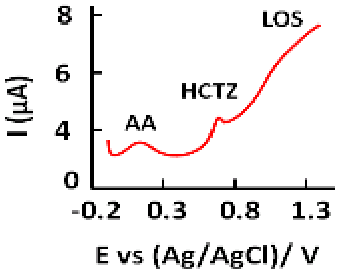

Fig. 8. DPVs of SnO_2-NiO/IL/CPE in a solution of containing 1mM of AA and 100µM of HCTZ and 30 µM LOS in 0.1 M PB/pH 8.

3.10. Precision, Stability and Reproducibility

The precision of the method was estimated in terms of relative standard deviation (RSD) for five repeated measurements of 100 µM HCTZ on the same day (within-day precision) and over 7 days (day-to-day precision). The RSD values from intra-day and inter-day analysis were found to be (0.51 %) and (1.1 %) respectively. The long term stability of the SnO_2-NiO/IL/CPE was evaluated by DPV_S in 0.1 M PB at pH 8 solution containing 100 µM HCTZ during two mounts. The result showed the response of electrode didn't considerable change. However, renewing of the electrode surface is necessary before each analysis due to poisoning of the electrode surface by adsorption of intermediates or product of electrooxidation. The three different SnO_2-NiO/IL/CPE_S were prepared at the same conditions the electrode to electrode reproducibility showed a satisfactory RSD value of 4.6% for the determination of 100 µM HCTZ. According to this result, the modified electrodes have suitable reproducibility in manufacture process and response. A summary of analytical parameters in determination of HCTZ by SnO_2-NiO/IL/CPE is given in Table 2. The repeatability for 3 replicate determinations of HCTZ 100 µM with the proposed sensor was 1.64 %.

Table 2. Analytical parameters of suggested electrode for determination of HCTZ

parameter	amount
Linearity range (µM)	0.01-0.1 and 0.1-100
Sensitivity (µA/µM)	6.0246
LOD (µM)	0.002
LOQ (µM)	0.006
within-day precision (RSD %**)** n = 5	0.51 %
Day to day precision (RSD %) n = 5	1.1 %
Reproducibility (RSD %)	4.6
Mean recovery for real samples (%)	100.1
Stability (day)	60
Bias %	0.94
Repeatability (RSD %) n = 3	1.64

3.11. Real Sample Analysis

The standard addition method was performed for determination of HCTZ in real sample. The results show a good recovery for drug determinations as well as the drug declared amounts in the used drug preparations (Table 3). This clearly indicates any interference of the other ingredients and the excipients which are present in the formulations.

The recovery results of the electrochemical sensor were in the range from 98.5 to 102.0 % by the Eq. (5):

$$Recovery = \frac{Detected}{Added} \times 100 \qquad (5)$$

The RSD of three sequential measurements of the response currents HCTZ was found to be about 0.89 % by the Eq. (6):

$$RSD = \frac{Sd}{x} \times 100 \qquad (6)$$

4. Conclusion

This work indicates that SnO_2-NiO/IL/CPE can be employed to form a novel CP modified electrode for determination of HCTZ with enhance the electroanalytical performance. The SnO_2-NiO/IL/CPE demonstrated a low detection limit of 0.002 µM with two linear range of the HCTZ concentration from 0.01 to 0.1 µM and 0.1 to 100 µM and high surface area. The proposed modified electrode exhibited several advantages, including, good mechanical stability, simple preparation high sensitivity and low cost. These attractive features proved that this proposed electrode is promising for other drug determination.

Table 3. Recovery test of HCTZ determination in real sample.

Sample	Standard added (µM)	Total concentration (µM)	Found (µM)	Recovery (%)	RSD (%)	Bias %
Hydrochlorothiazide 25 mg	0	100.0	99.4	99.4	0.6	0.6
	5	105.0	103.5	98.5	0.8	1.5
	10	110.0	109.7	99.7	0.5	0.3
Hydrochlorothiazide 50 mg	0	100.0	99.7	99.7	1.0	0.3
	5	105.0	104.9	99.9	0.7	0.1
	10	110.0	110.2	100.1	1.3	0.1
Human blood plasma	0	0	0	0	-	-
	5	5.0	4.9	98.8	0.9	1.2
	10	10.0	10.2	102.0	0.4	2
Urine	0	0	0	0	-	-
	5	5.0	5.1	102.0	1.6	2
	10	10.0	10.13	101.3	1.1	1.3

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Conflicts of Interest

The author declares that there is no conflict of interest regarding the publication of this manuscript. In addition, the authors have entirely observed the ethical issues; including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy.

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