Full Paper

A Sensitive Modified Electrode based on Reduced Graphene Oxide Composite with Cu@SnS/SnO$_2$ for Determination of Benserazide

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Abstract- Benserazide (BZ), a dopadecarboxylase inhibitor, is the most widely prescribed drug in treatment of Parkinson's disease. Therefore, it is urgent to develop a sensitive and selective BZ sensor. We used tin oxide nanocomposite (Cu@SnS/SnO$_2$) for developing a new electrochemical sensor for determination of BZ in human urine and pharmaceutical tablets. Morphology of the synthesized Cu@SnS/SnO$_2$ was investigated by scanning electron microscopy (SEM). The modified electrode was characterized by electrochemical impedance spectroscopy (EIS) and voltammetric techniques. The sensitivity and limit of detection of RGO/Cu@SnS/SnO$_2$ electrode for BZ are 0.275 µAµM^{-1} in the range of 1-100 µM and 0.58 µM. Moreover, the proposed electrode shows good selectivity, reproducibility and stability. The applicability of the modified electrode was demonstrated by determination of BZ in human urine and pharmaceutical formulation.

1. INTRODUCTION

Nanosized structures with special behaviors usually exhibit fascinating features in their compound, shape and dimension [1-3]. Currently, nanotechnology is able to develop and fabricate nanomaterials with specific size and configuration. This is why the usage of nanomaterials with developed application is alarmingly growing. Via novel and advanced procedures, nowadays, scientists can fully understand different fields such as chemistry as well as electrochemistry. Hence, such breakthroughs have led to use nanostructures in our daily routine [4,5].

Parkinson's disease occurs when level of a neurotransmitter called dopamine is reduced. This disease causes some adverse effects such as tremors and muscle stiffness. Due to dopamine disability to cross the blood–brain barrier, can't be used directly in treatment [6]. Benserazide (BZ) (D, L-serine-2-[2,3,4(trihydroxyphenyl)methyl] hydrazide) is a drug used concomitantly with levodopa (LD) in the unique tablet for treatment of Parkinson's disease. BZ is an irreversible inhibitor of peripheral aromatic L-amino acid decarboxylase. This drug absorbs rapidly after oral administration and causes most amounts LD is converted into dopamine in the brain. Thus, BZ impedes some side effects such as nausea, palpitations and vomiting by preventing the conversion of LD to dopamine in peripheral nervous system. Hence determination of BZ is important [7-9]. Different analytical methods, such as electrochemical analysis [10-13], liquid chromatography [14] and spectrophotometry [15], have been used for the determination of BZ along with other drugs is reported.

In recent years, various nanomaterials have been used to improve sensitivity of the electrochemical sensors. Among this tin oxide nanostructures have been a most important n-type semiconductor with a lot of properties such as catalytic activity, good chemical stability, non-toxicity and inexpensiveness [16-19]. Nowadays, the doped tin oxides and its composites with some materials such as Ni [20], Cu [21], Fe [22], graphene [23], reduced graphene Oxide [24], Co_3O_4 [25] have been reported for various application.

Reduced graphene oxide as a single - layer carbon nanosheet with two dimensional structure of a honeycomb, has attracted much attention due to exceptional properties such as excellent mechanical resistance, higher electrical conductivity, high surface and thermal and chemical stability made it good ideas in order to use in sensing different combinations such as drug, ions, metals and supramolecular organic and inorganic [26,27].

In this research, we used tin oxide nanocomposite ($Cu@SnS/SnO_2$) for developing a new electrochemical sensor for determination of BZ in human urine and pharmaceutical tablets (Madopar®125 mg). The results show the proposed modified electrode is a useful device for this purpose.

2. EXPRIMENTAL

2.1. Chemicals and apparatus

Tin(II) chloride (SnCl$_2$, 2H$_2$O), Tin(IV) chloride (SnCl$_4$), thiourea (NH$_3$CSNH$_2$), copper acetate Cu(CH$_3$COO)$_2$ and ammonium acetate (NH$_4$CH$_3$COO) were purchased from Scharlau Company. Phosphate salt, sodium hydroxide, solvents and reagents was purchased from Merck Company. Benzerazide ≥99.5% was purchased from Zhejiang Nexchem Pharmaceutical Co. All chemicals were used as received without further purification. Madopar®125 mg tablets, containing active ingredients: levodopa (100 mg)+benserazide (25 mg) was obtained from Razan Pharmed Iranian Co.

Cyclic voltammetry measurements were performed by palm sense EmStat^{3+} and PSTrace software for Windows. Three electrode systems were used, Ag/AgCl/KCl (3 M) as reference electrode, a platinum wire as auxiliary electrode and bare and modified carbon paste as working electrodes. All measurements were performed at 25±1 °C.

2.2. Preparation of Cu@SnS/SnO$_2$ nanoparticles

The Cu@SnS/SnO$_2$ nanoparticles were synthesized according to the procedure explained in the literature [28]. Briefly, 16.0 ml of 0.5 M Tin(II) chloride dehydrate (SnCl$_2$,2H$_2$O) solution was mixed with 4.0 ml of 1.0 M ammonium acetate (NH$_4$(CH$_3$COO)) solution, Then, 30 ml of 8% (w/v) Tin (IV) chloride solution, 4.0 ml of 1.0 M thiourea (NH$_2$CSNH$_2$) solution and 2.0 ml 1.0 M NaOH solution were added to the initial mixed solution. In the next step, de-ionized water was added to the prepared mixed solution to make a total volume of 100 ml. In the following, 50 ml of the prepared reaction solution in a baker was transferred to an oven with temperature of 50 °C for 24 h. After 24 h, 48 ml of 0.002 M copper acetate solution was added to the reaction solution drop by drop to obtain the Cu@SnS/SnO$_2$ nanoparticles. The obtained Cu@SnS/SnO$_2$ nanoparticles were then dispersed in 150 ml of de-ionized water in an Erlenmeyer flask to make an insoluble suspension.

2.3. Characterization methods

The structure and surface morphology of nano materials was investigated by scanning electron microscopy (FE-SEM-EDX, MIRA3, TESCAN) and Transmission electron microscopy (TEM JEM-2010 transmission electron microscope operating at 200 kV). The crystallization degree of the nanomaterials was specified by X-ray diffractometer (XRD, PHILIPS-PW1730)

2.4. Preparation of working electrodes

To determine the electrochemical behavior of nanomaterials, an appropriate carbon paste electrode (CPE) was prepared by hand-mixing of 0.9 g reduced graphene oxide fine powder,

30 mg mineral oil, and 70 mg from Cu@SnS/SnO$_2$ nanoparticles with a mortar and pestle at 30 min for complete uniformity. The carbon paste packed into one a tube (internal diameter of 2.1 mm) with a copper wire to obtain an electrical contact.

2.5. Real sample analysis

2.5.1. Urine sample

The urine sample was collected a healthy man in a fresh test tube and stored at 4 $^{\circ}$C in a refrigerator. Then obtained sample was centrifuged for 1 h at 5000 rpm. Finally, sample was filtered with a 0.45 μm filter. Urine sample after pre-treatment was diluted tenfold with 0.1 M PBS.

2.5.2. Tablet sample

For tablet analysis, ten Madopar tablets (one tablet contains 100 mg levodopa and 25 mg benserazide) were weighed and powdered using a mortar and pestle. Next, the adequate mass of tablets was transferred into a 100 ml of flask and dissolved into 20 ml of 0.1 M PBS and sonicated for 30 min. Finally, the solution was filtered with a 0.45 μm filter and diluted with deionized water. Replicate samples were prepared by taken the appropriate amount of this solution completed to 10 ml with 0.1 M PBS (pH 5.0) electrolyte [29].
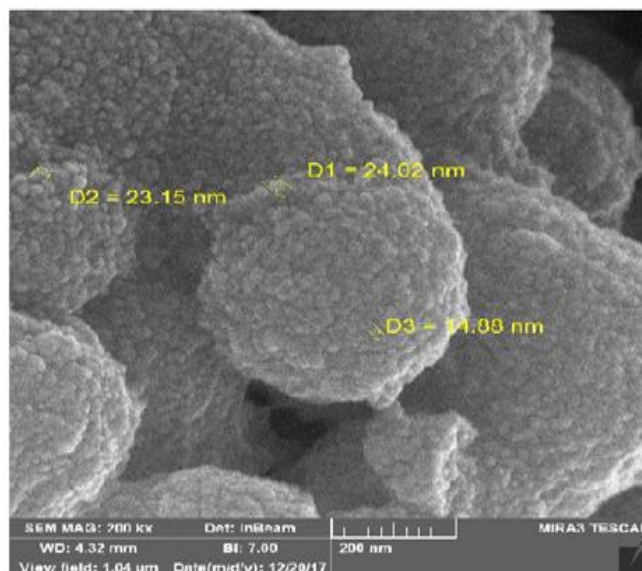
Fig. 1. SEM images and structures of Cu@SnS/SnO$_2$ nanocomposite

3. RESULT AND DISCUSSION

3.1. Characterization of the Cu@SnS/SnO₂

Morphology of the synthesized Cu@SnS/SnO$_2$ was investigated by scanning electron microscopy (SEM) method. As shown in Figure 1 the presence of uniform small spherical particles in nanoscale size with 14 to 24 nm diameter confirms the synthesis of Cu@SnS/SnO$_2$.

3.2. The electrochemical behavior of RGO/Cu@SnS/SnO₂

In the first step, we investigate the electrochemical behavior of different modified electrodes using cyclic voltammetry in solution containing 0.1 mmol L^{-1} of Fe(CN)$_6^{-3}$ in the 0.1 mmol L^{-1} supporting electrolyte (see Fig. 2)

As can be seen in Fig 2A, redox picks with different intensity and peak to peak potential separation were obtained for the investigated electrodes. The ΔE$_p$ value were 145, 130, 125 and 210 mV for the modified with graphene, Cu@SnS/SnO$_2$, RGO/Cu@SnS/SnO$_2$ and unmodified electrode, respectively. The obtained results show the highest increase in electron transfer rate for the RGO/Cu@SnS/SnO$_2$ modified electrode.
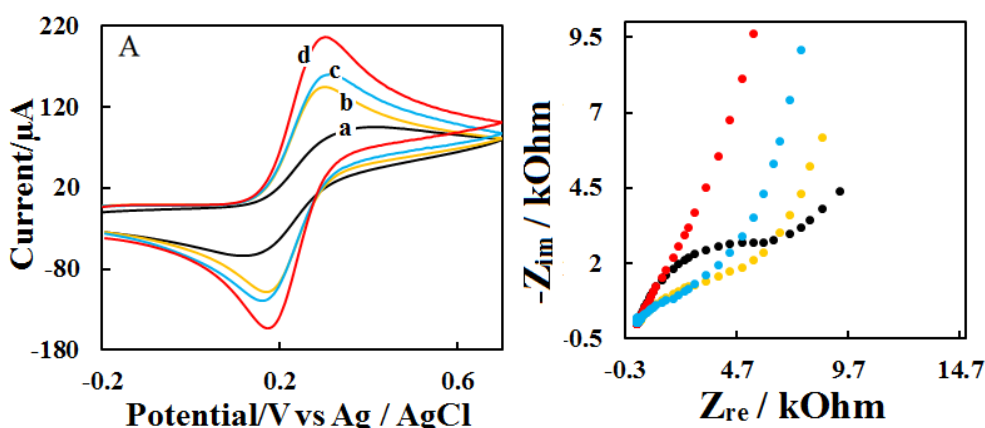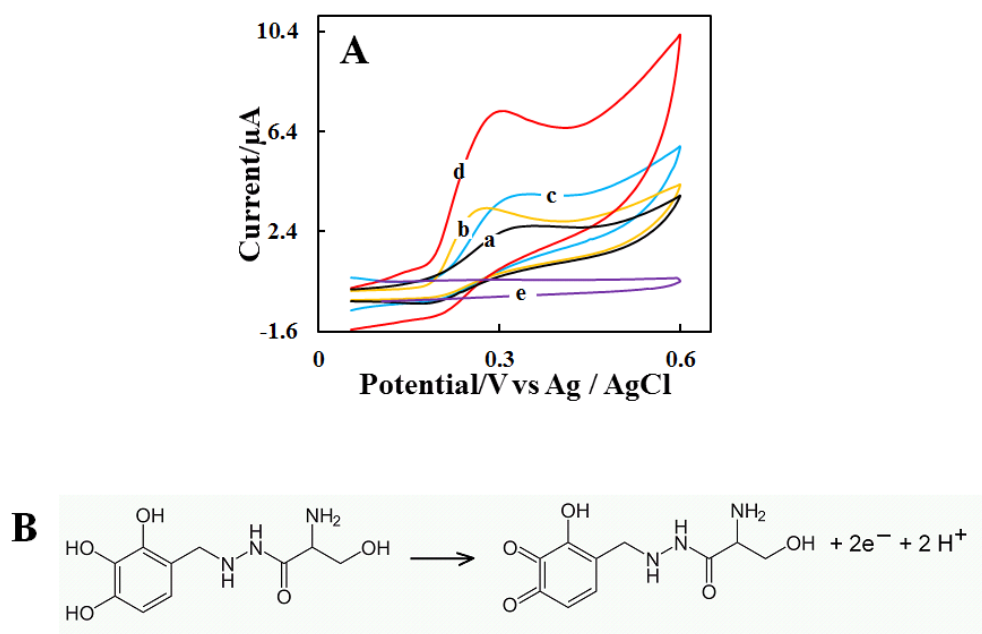

Fig. 2. A: CVs of 5.0 mM [Fe(CN)$_6$]$^{-3/-4}$ in 0.1 M KCl obtained at (a) bare CPE, (b) Cu@SnS/SnO$_2$, (c) CPE/ RGO and (d) RGO/Cu@SnS/SnO$_2$ scan rate, 0.05 Vs^{-1}; B: Nyquist plots of (a) bare CPE, (b) Cu@SnS/SnO$_2$, (c) CPE/ RGO and (d) RGO/Cu@SnS/SnO$_2$ in a 5.0 mM [Fe(CN)$_6$]$^{-3/-4}$ in 0.1 M KCl

Furthermore, the electroactive surface area of the modified and unmodified electrodes can be determined on the basis of the Randels-servick equation (1) [29] by CV at different scan rates using 5 mM K$_3$Fe(CN)$_6$ containing 1 M KCl. The electroactive surface area of modified with graphene, Cu@SnS/SnO$_2$, RGO/Cu@ SnS/SnO$_2$ and unmodified electrode was calculated to be 0.81, 0.80, 0.87, 0.65 cm^2, respectively.

Figure 2B demonstrates the Nyquist plots of the modified and unmodified electrodes which were obtained from the electrochemical impedance spectroscopy (EIS) measurements in 5 mM $K_3Fe(CN)_6$ (1:1) solution in 0.1 M KCl. According to the Nyquist plots, the unmodified electrode has a large semicircle diameter compared to the modified electrodes. These results demonstrates that the charge transfer resistance in modified electrodes is lower than the unmodified electrodes. It means that the existence of graphene, $Cu@SnS/SnO_2$, RGO/Cu@ SnS/SnO_2 in modified electrode improves the electron transfer process significantly. The electrochemical behavior of BZ was investigated at the surface of the prepared electrodes.

Figure 3A shows the voltammograms of 50 µM BZ in 0.1 M buffer phosphate solution (pH =4) at modified and unmodified electrodes. As can be seen in figure 3, the presence of BZ oxidation peak in all of the electrodes indicates the oxidation of the proposed analyte at the surface of the surface of the modified and unmodified electrodes but the value of current intensity obtained from the oxidation of BZ at the surface of modified CPE with RGO/Cu@ SnS/SnO_2 has a significant increase compared to other modified electrodes. Moreover, the synergic effect between graphene and $Cu@SnS/SnO_2$ in RGO/Cu@SnS/SnO_2$ composite is obvious. The value of the electrochemical response for RGO/Cu@ SnS/SnO_2 electrode was 2.5 times higher than unmodified electrode, which could be attributed to the increase in the surface area due to the presence of nanocomposites.

Fig. 3. A) CVs of 0.5 mM BZ in PBS of pH 5 obtained at (a) bare CPE, (b) $Cu@SnS/SnO_2$ (c) CPE/CPE/RGO and (d) RGO/ $Cu@SnS/SnO_2$; scan rate, 0.05 Vs^{-1} and CVs of (e) RGO/ $Cu@SnS/SnO_2$ in PBS of pH 5; B) The electrooxidation reaction of BZ at a surface of RGO/Cu@ SnS/SnO_2 modified electrode

As can be seen in Fig. 3A the BZ oxidation potential at the modified with RGO//Cu@ SnS/SnO$_2$ electrode and unmodified electrode were 280 and 340 mV, respectively. According to the Fig. 3, the RGO/Cu@ SnS/SnO$_2$ modified electrode does not have any electro catalytic peak but it shows an electrocatalytic property. According to the obtained results, the RGO/Cu@ SnS/SnO$_2$ modified electrode was used as a sensor for BZ measurements. The electrooxidation reaction of BZ at a surface of RGO/Cu@ SnS/SnO$_2$ modified electrode is presented in Fig. 3B.

3.2.1. Effect of pH solution

The influence of pH on the electrochemical response of 50 µM BZ at a surface of RGO/Cu@SnS/SnO$_2$ modified electrode was studied using the CV in the PBS buffer solution within the pH range of 2-7. The dependence of peak current and peak potential on pH is shown in Fig. 4.

As can be seen in this figure, the maximum current intensity is observed at pH 5. So, this pH was chosen as an optimum condition for BZ determination. Fig. 4 shows the dependent oxidation potential on the solution pH. As can be seen in Fig. 4 the oxidation peak potential is shifted to more negative values with increasing the pH value, indicating that the protons have took part in the electrochemical reaction there was a good linear relationship between the E$_P$ and pH, which was obtained as following: E$_P$ (V)$=-0.0724$ pH$+0.6434$, R^2=0.99.

This linear ship indicates that the slop of 0.0724V/pH which is close to Nernstian value of 59 mV, suggesting that the number of protons and electrons involved.
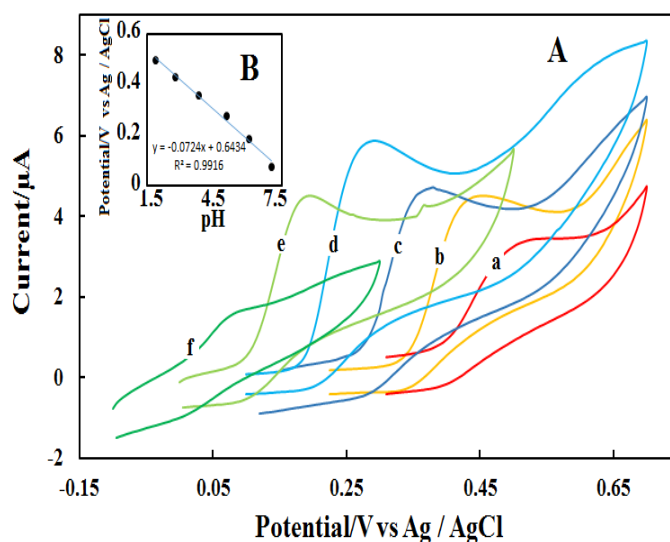

Fig. 4. (A) CV of 0.5 mM BZ on the surface of RGO/TMU-22PE at different pH values (PBS): (a) 2.0, (b) 3.0, (c) 4.0, (d) 5.0, (e) 6.0 and (f) 7.0. Scan rate 0.05 Vs^{-1}; (B) Effect of pH on E$_P$

3.2.2. Effect of scan rate

The effect of scan rate on the oxidation peak current of 0.05 mM BZ in 0.1 M PBS (pH 5) at the surface of RGO/Cu@SnS/SnO$_2$ modified electrode was evaluated (see Fig. 5).

In the scan rate between 10 and 200 mV/s, response signal shows a linear relationship with *v*, which indicates diffusion process for reaction. As can be seen in figure 5, it is clear that the cathodic peak appears with high intensity whenever the scan rate increases. This behavior put emphasis on the presence of a chemical reaction after an electrochemical reaction. It means that there is an EC mechanism on the surface of the electrode.
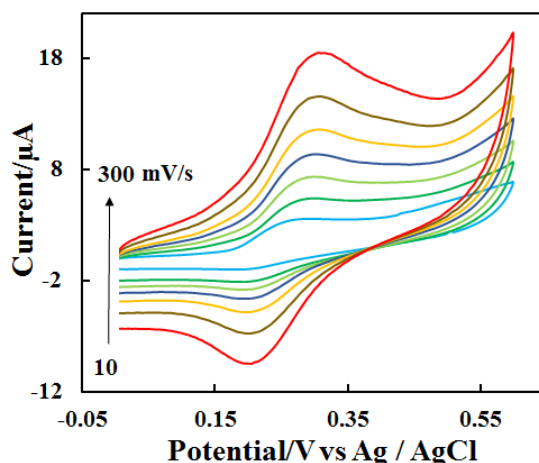
Fig. 5. (A) CVs of 0.05 mM BZ on surface of RGO/Cu@SnS/SnO$_2$ at various scan rates; 10, 30, 50, 75, 100, 150 and 200 mVs^{-1} in PBS of pH 5
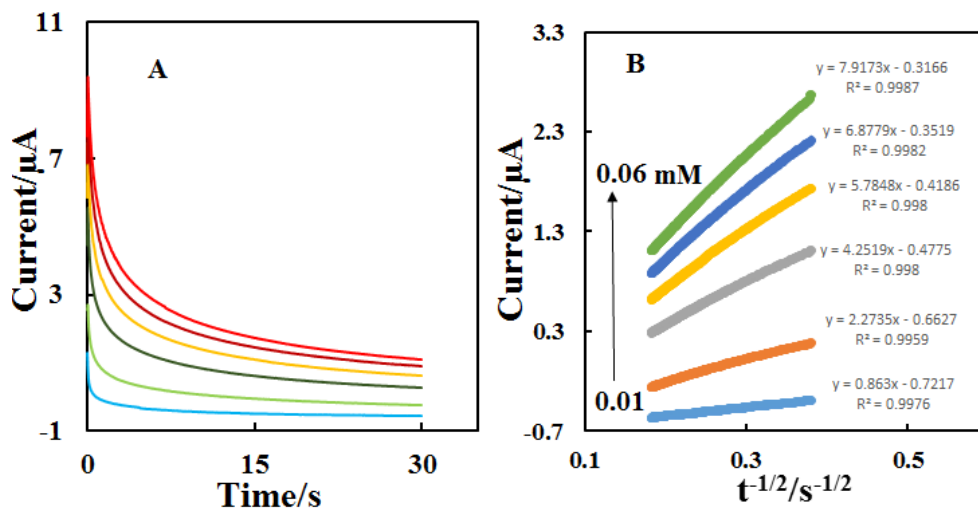
Fig. 6. (A) Chronoamperograms obtained at RGO/Cu@SnS/SnO$_2$ in 0.1 M PBS (pH 5) for different concentrations of BZ. The numbers 1–5 correspond to 0.01, 0.02, 0.03, 0.04, 0.05 and 0.6 mM of BZ; (B) Insets: plots of I *vs.* t$^{-1/2}$ obtained from chronoamperograms

3.2.3. Chronoamperometric measurements

Chronoamperometric investigation of BZ in PBS 0.1 M with pH 5 for the various concentration at the surface of CPE modified with RGO/Cu@SnS/SnO$_2$ electrode was carried out by setting potential 400 mV. According to the obtained data, the measured Cottrell current increases with the concentration enhancement. Figure 6 shows the plot of I *vs.* $t^{-1/2}$ and slope of them which can be used for the calculation of diffusion coefficient (D) value by the following equation: $I=nFACD^{1/2}\pi^{-1/2}t^{-1/2}$ The diffusion co efficient value was 1.74×10^{-5} cm^{2}s^{-1}.

3.3. differential pulse voltammetric determination of LD

The main purpose of this work is to present a suitable and advanced method for BZ measurement by CPE modified with RGO/Cu@SnS/SnO$_2$ electrode. As the CV studies showed the BZ oxidation peak at the surface of modified electrode, so the differential pulse voltammetry was used as sensitive method for the drawing of the calibration curve and BZ measurement at real samples. Figure 7 shows the obtained differential pulse voltammograms of BZ oxidation for various concentrations at the surface of the CPE modified electrode with RGO/Cu@SnS/SnO$_2$. In an optimized situation, the range of 1 and 100 µM. the slope of the calibration curve, which shows the method's sensitivity, was calculated to be 0.275 µA/µM.
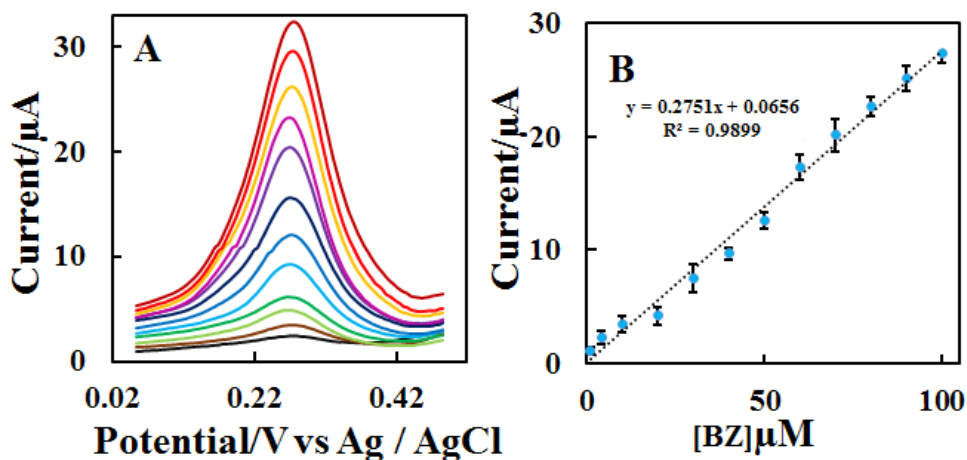
Fig. 8. (A) DPV voltammograms of BZ at RGO/Cu@SnS/SnO$_2$ in PBS at pH 5; (B) Calibration plot of Ip (µA) versus [BZ] in PBS of pH 5

Table1. Comparison of the efficiency the fabricated electrode with some modified electrodes for

Electrode	Method	Linearity rang [M]	Detection limit [M]	Ref.
ZnO/ILCPE	SWV	0.1×10^{-6}–430×10^{-6}	0.06×10^{-6}	[7]
GCE	DPVs	2.5×10^{-5}–2.2×10^{-4}	7.12×10^{-6}	[9]
IL–TiO$_2$/CPE	SWV	1×10^{-6}–600×10^{-6}	0.4×10^{-6}	[12]
FCCNPE	SWV	8×10^{-7}–7×10^{-4}	0.1×10^{-6}	[13]
poly[3-methylthiophene] /MWCNT	DPVs	4×10^{-6}–10×10^{-6}	32.5×10^{-6}	[24]
PAR-film /GCE	DPVs	0.01×10^{-3}–0.2×10^{-3}	0.002×10^{-3}	[29]
RGO/Cu@SnS/SnO$_2$	DPVs	1×10^{-6}–100×10^{-6}	0.58×10^{-6}	This work

Also, the detection limit (S/N=3) was 0.580 µM. The obtained analytical parameters of this investigation were compared to other reported electrochemical sensors for BZ measurement and the results were summarized in Table 1.

It is obvious that the sensitivity and detection limit of the suggested method are comparable to other reported works. The linear range of this method is also extensive or even comparable to other electrodes. The obtained results show that the CPE modified electrode with RGO/Cu@SnS/SnO$_2$ electrode which was used in this investigation has suitable properties to BZ quantitative measurement. The repeatability, reproducibility and stability of the CPE modified with RGO/Cu@SnS/SnO$_2$ electrode at the optimized condition was studied by differential pulse voltammetry.

3.4. Reproducibility, repeatability and stability of the RGO/Cu@SnS/SnO$_2$

The prepared electrode was used to 6 times measurements of BZ with the concentration of 25µM and the relative standard deviation was less than 5% which indicated the suitable repeatability for the prepared electrode. Reproducibility is the ability of electrodes construction with nip and tuck response between multiple prepared electrodes. The relative standard deviation for the reproducibility was approximately 2.2% for 5 CPE modified with RGO/Cu@SnS/SnO$_2$ electrodes. To investigate the stability of the electrode, the prepared electrode was kept in at room temperature and the BZ measurement was done certain intervals between 1 to 4 weeks. From the electrochemical response investigation the deviation response was 4.1% which demonstrates that the electrode has good stability for one month.

3.5. Interference studies

As you know, the texture of the actual samples is different from the standard samples. So, we investigated the interference effects of some substance on the accurate determination of BZ in real samples the obtained results showed that coexistence of over 100 fold excess of some ions like Na^+, K^+, Ca^{2+}, NO_3^-, SO_4^{2-} and some of the biologically compounds such as glucose, uric acid, ascorbic acid, paraamipiksol and acetaminophen for BZ determination, showed a low degree of interference with signal deviation for BZ less than 8%.
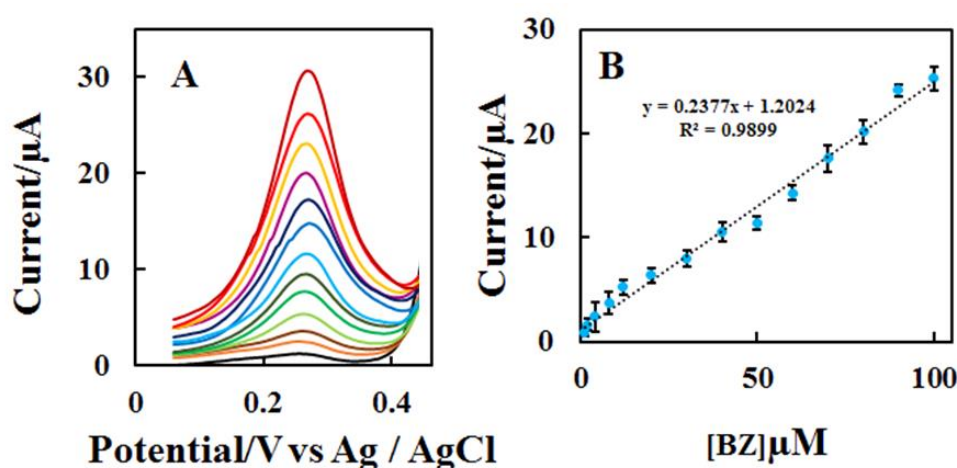
3.6. Determination of BZ in real samples

To investigate the applicability of the CPE modified with $RGO/Cu@SnS/SnO_2$ electrode in real samples was studied by analysis and measurement of BZ according to the DPV method under the optimized condition in tablets and human urine samples. For this purpose, we prepared the real samples for analysis as following:

1-Healthy human urine samples were diluted with PBS after adding an appropriate amount of standard BZ solution.

2-Different concentration of Madopar tablets were diluted by PBS 0.1 M with pH 5.

Figure 9 shows the linear enhancement of signal with concentration ranging from 1 to 100 µM in urine samples. The detection limit of BZ in urine samples was found to be 0.55 µM. As can be seen in Figure 9, there is not significant effect from the excipients in human urine samples on the response signal. Also, the uric acid separated oxidation peak was appeared across the BZ oxidation peak.

Fig. 9. (A) DPV voltammograms for determination of BZ spiked in human urine samples at $RGO/Cu@SnS/SnO_2$; (B) Calibration plot of I_p (µA) versus [BZ] in PBS of pH 5

According to the satisfactory results, the CPE modified with $RGO/Cu@SnS/SnO_2$ electrode was found to be suitable for BZ analysis and measurement in human urine

environment. For pharmaceutical samples, the calibration plot was demonstrated in Fig. 10 ranging from 1 to 100 µM. The detection limit (S/N=3) of BZ in urine sample was calculated to be 0.68 µM.
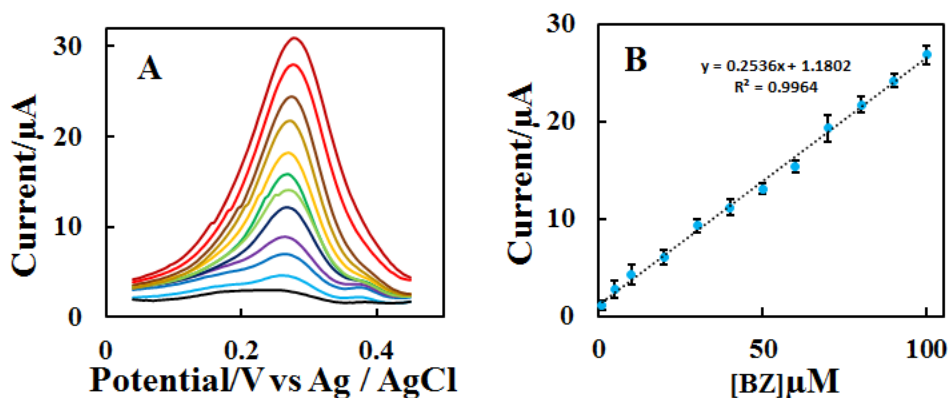
Fig. 10. (A) DPV voltammograms for the determination of BZ in Madopar tablet solution at RGO/Cu@SnS/SnO$_2$; (B) Calibration plot of Ip (µA) versus [BZ] in PBS of pH 5

As can be seen in Fig. 10, the determination and measurement of BZ value was performed without any interference from the excipients in tablets on response signal. So, the proposed electrode can be used for BZ analysis and measurement in pharmaceutical samples.

The data in Table 2 shows the good and comparable ability of the proposed electrode for analysis and measurement of BZ in real samples by standard addition method compared to other reported literatures.

Table 2. Recovery test of BZ determination in its real samples

Sample	Added (mg)	Found[a] (mg)	Recovery (%)
	0	26.13±0.81	104.52
Madopar® tablet 125 mg	5	27.17±1.10	90.57
(25 mg BZ + 100 mg LD)	10	32.63±0.91	93.23
	15	38.40±0.82	96.00
	25	23.00 ±1.21	92.08
	35	31.99±1.74	91.40
Urine	45	42.75±0.92	95.00
	50	47.17±1.11	94.34

4. CONCLUSION

In this work, a novel modified CPE based on RGO and Cu@SnS/SnO$_2$ nanoparticles for determination of BZ was introduced. The construction of sensor is simple. The sensor showed

a selectivity, wide dynamic linear range, low detection limit, good reproducibility and repeatability and long life storage. The obtained results confirm that the method can be used for the determination of BZ in pharmaceutical formulation and human urine samples.

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