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Electrochemical Sensing of Thioridazine in Human Serum Samples Using Modified Glassy Carbon Electrode

Ali Shamsi, Fatemeh Ahour*

Nanotechnology Research Center, Faculty of Science, Urmia University, Urmia, Iran

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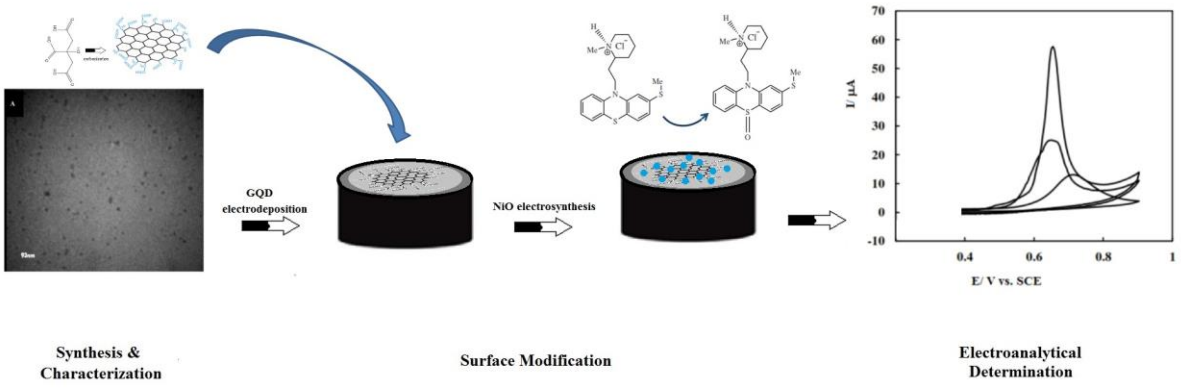
ABSTRACT

In this work, thioridazine (TR) as an important neuroleptic drug has been detected simply by an electrochemical approach using a glassy carbon electrode modified by nickel oxide nanoparticles decorated graphene quantum dot (NiO/GQD/GCE). The bare and modified electrodes were characterized using the scanning electron microscope (SEM) and electrochemical techniques. The cyclic voltammetric studies demonstrated that the NiO/GQD/GCE has remarkably enhanced electro-catalytic activity towards the oxidation of TR in neutral solutions. The results (significant increase in peak current and a negative shift in TR oxidation potential) are related to the increase in electrode surface area and electron transfer rate along with the modifier catalytic role. The NiO/GQD modified electrode used for sensitive determination of TR by differential pulse voltammetry (DPV) method. The effect of experimental parameters on the obtained results was studied and optimized. The NiO/GQD/GCE modified electrode revealed a linear response in the concentration range from 2×10^{-9} to 200×10^{-9} M with a limit of detection (LOD) equal to 0.05×10^{-9} M (S/N=3). The sensor was applied to determine TR in serum and pharmaceutical samples, which proves this sensor is an ideal device for TR determination.

GRAPHICAL ABSTRACT



* Corresponding author: Ahour, Fatemeh
✉ E-mail: f.ahour@urmia.ac.ir
☏ Tel number: +98 44-32752746
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Introduction

Electrochemical sensors and biosensors based on the application of nanostructured materials have attracted considerable attention due to their amazing characteristic different from bulk forms [1, 2]. Among the various nanostructured materials, carbon nanomaterials such as graphene (Gr), graphene oxide (GO), and graphene quantum dot (GQDs), have gained great notice. GQDs are composed of graphene sheets smaller than 100 nm and due to numerous unique physicochemical properties were applied in many fields and applications. GQDs with great surface area and quick electron transferability is one of the best electrode modifiers in electrochemical sensors [3-5].

Transition metals and metal oxides with high surface area, enhanced mass transport, and good biocompatibility are suitable candidates for electron-transfer processes [6-8]. Nickel oxide (NiO) is more attractive compared with other nanomaterials in electro-catalysis due to its low cost, good catalytic activity, and inimitable electrochemical properties and used by several groups as an electrode modifier for sensing purpose [9-18]. Improved electrocatalytic activity and enhanced specific surface area can be obtained with the decoration of the conductive GQD matrix with transition metal nanoparticles. Recently, magnetic nano-particles embedded GQDs composites applied for electrochemical sensing of amino acids and progesterone [19, 20]. The active substance thioridazine is a neuroleptic drug that has calming and antipsychotic properties. It can be used to treat schizophrenia, control of mania, and other mental illnesses. From a chemical point of view, it is one of the phenothiazine drugs and works by blockade of the serotonin and dopamine receptors within the central nervous system. This drug is only used if it was not possible to achieve a successful treatment with other drugs as it can cause dangerous cardiac arrhythmias. The development of a simple and sensitive method for the determination of TR in medical and clinical specimens is important given its clinical significance and adverse side effects .

Various methods have been used to determine the concentration of the phenothiazine drugs in real samples [21-27]. Electrochemical methods are among the useful methods for quantitative and qualitative analysis of the species in solutions, especially aqueous solutions. Due to advances in electrochemical systems and the electrochemical behavior of drugs and biomolecules, their application in the analysis is increasing rapidly. In previous studies, we have reported the use of GO and GQD as electrode modifiers for the manufacture of sensors and biosensors [28-31]. Recently, we used NiO decorated GQD modified GCE for the sensitive measurement of clozapine in real samples [32]. In current work, the electrochemical behavior of TR and its selective and sensitive measurement in clinical and pharmaceutical samples were studied using NiO/GQD/GCE.

The prepared electrode has increased sensitivity compared to the bare and GQD modified electrode indicating the synergistic effect of NiO and GQD in the composite material along with good reproducibility, stability, and wide-ranging linearity. The electrode has superior performance and a suitable LOD for TR electro-oxidation.

Experimental

Instrumentation and reagents

The AUTOLAB PGSTAT 30 electrochemical analysis system and the GPES 4.9 (Eco Chemie. Netherlands) software package were applied for performing electrochemical experiments.

The NiO/GQD modified GCE, Hg/Hg_2Cl_2, Cl^- (saturated), and a platinum wire were used as the working, reference, and auxiliary electrodes in the experiments, respectively. PH measurements were performed using a Metrohm

digital pH meter (pH Lab 827) and electrode surface cleansing and preparation of modifier suspensions were performed using an ultrasonic bath (KODO model JAC1002). Thioridazine hydrochloride (99% purity) and thioridazine hydrochloride tablets were bought from Minoo Pharmaceutical Company (Tehran, Iran). A phosphate buffer solution with the pH 3, was used to prepare the TR stock solution and the solutions were kept in the dark medium. Dilute solutions of TR with specific concentrations and pH were prepared using this stock solution. Other chemicals were of analytical grade and purchased from Merck. Serum samples were achieved from Motahhari Hospital, Urmia, Iran.

Double distilled water was used to prepare the solutions with appropriate concentration. To prepare GQD, 2 g of CA was poured into a 5 ml beaker and heated to 200 °C using a heating mantle. Citric acid first melted and then the resulting colorless liquid turned yellow and after about 20 minutes changed color to orange, indicating the formation of GQD. The resulting orange liquid was added drop wise to 100 mL of NaOH solution under vigorous stirring to obtain an aqueous solution of neutral GQDs as reported previously [33].

Working electrode fabrication

Firstly, the GCE electrode (diameter 2 mm) was polished on a polishing cloth with alumina slurry and washed carefully with distilled water. After 5 min of successive sonication in double-distilled water and drying at ambient conditions, the electrodes were immersed in GQD solution for electrochemical deposition of modifier by 60 repetitive cycles from 0.0 to 1.0 V using 100 mV s^{-1} as scan rate. After this step, GQD modified GCE (GQD/GCE) was dipped in 10 mL $Ni(NO_3)_2$ solution (10 mM, pH 4.0) and then under stirring conditions, a potential of −1.1 V was applied to the electrode for 210 s for the nucleation and growth of Ni nanoparticles. Then, the resulting electrode was immersed in a 0.1 M NaOH solution and scanned at a scanning speed of 0.1 V s^{-1} from 0.1 to 0.7 volts until repeatable cycles were obtained. At this point, Ni electro-dissolves and the NiO layer forms at the GQD modified electrode surface, in other words, NiO/GQD/GCE is constructed and applyed for electrochemical purposes.

Preparation of real samples

Human serum samples were diluted 1:100 with 0.1 mM phosphate buffer solution of pH 7 (PBS) after pretreatment (centrifugation and filtration). The diluted samples were contaminated with certain amounts of TR to evaluate the recovery of the method. Four tablets each having 25 mg TR were weighed, powdered, and used as a real sample. The exact amount of these fine particles equal to 37.05 mg TR was dissolved in 100 mL of PBS (pH 3) by ultrasound and then centrifuged. The resultant supernatant contains thioridazine at a concentration of 1 mM and used as a real sample. This sample was used to prepare dilute TR solutions with specific concentrations and pH. The deferential pulse voltammograms were recorded and then keeping the dilution factor in consideration, the concentration of TR in the pharmaceutical formulations was determined.

Results and Discussion

Characterization of NiO/GQD nanocomposite

The morphology and microscopic structure of the GCE before and after the modification were characterized using the SEM analysis. The results (Figure 1) proved the immobilization of GQD and the preparation of NiO nanoparticles at the electrode surface.

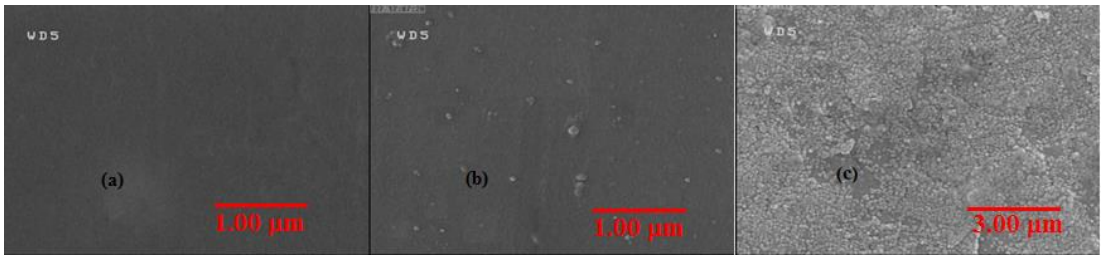


Figure 1. SEM image of (a) bare, (b) GQD, and (c) NiO/GQD modified GCE

Electrochemical behavior of TR at various electrodes

Figure 2 illustrates the cyclic voltammetric responses of bare, GQD and NiO/GQD modified GCEs in 6 µM TR solution. As can be seen, the small anode peak associated with TR oxidation at the bare GCE surface, after modification with GQD and NiO/GQD, increases and shifts to less positive potentials. These variations in electrochemical behavior indicate that the composite film has catalytic effect and accelerates the electrochemical reaction. Additionally, TR behaves totally irreversible and no cathodic peak was observed in experiments.

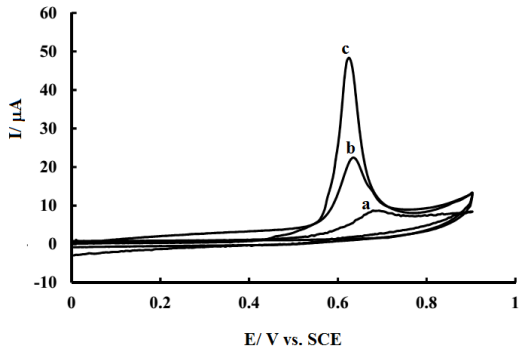


Figure 2. CVs of bare (a), GQD (b) and NiO/GQD (c) modified GCE after immersion in 5 µM TR in PBS pH 7. Accumulation condition: pH 7, time 400 s at -0.1 V; Scan rate: 50 mV s^{-1}

Effect of pH

The electrochemical behavior of 7 µM TR at the NiO/GQD/GCE was studied at various pH values (pH: 2-8) by cyclic voltammetry. As seen in Figure 3, the peak current increases remarkably with increasing solution pH, and the maximum value is obtained at pH 7. This proves that the electrode reaction accelerated by increasing pH up to 7, thus PBS with pH 7 was selected as the suitable electrolyte in the voltammetric measurements. Anodic peak current decrease at pH 8 may be related to the instability of TR at higher pHs. On the other hand, the potential of TR oxidation peak is constant in different pH values which means that TR does not exchange any protons in oxidation process as seen previously for promethazine derivatives [34].

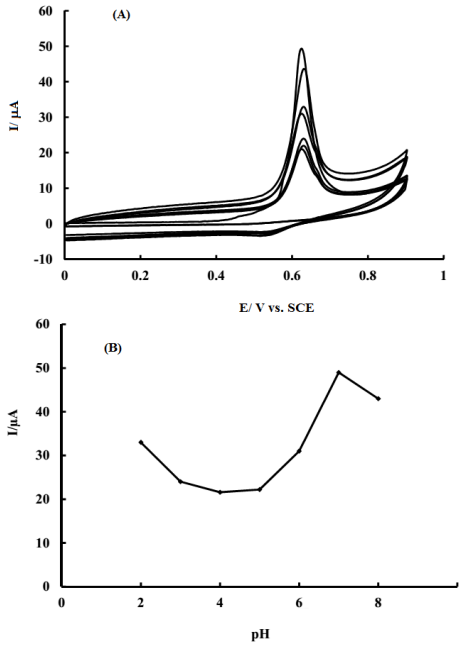


Figure 3. (A) CVs of 5 µM TR at the NiO/GQD/GCE in various pHs of buffer solution; (B) Dependence of the oxidation peak current (I_P) with the solution pH. Accumulation condition: time 400 s at -0.1 V; Scan rate: 50 mV s^{-1}

Effect of scan rate

Cyclic voltammetry was used to investigate the predominant type of material transfer and to obtain information about the electrochemical mechanism. Figure 4 demonstrates the voltammetric behavior of NiO/GQD modified electrode in 5μM TR at different scan rates from 5 to 250 mV s^{-1}. With the scan rate increasing, the anodic peak current (I_P) raised and a linear relationship was found between the peak currents and scan rate in the range of 10-250 mV s^{-1} which indicates that the TR oxidation reaction is a process controlled by adsorption. On the other hand, as the scan rate increases, the oxidation peak potential shifts to more positive values, which confirm the limitation of the electrochemical reaction kinetics. The linear relation between peak potential (E_P) and logarithm of scan rate can be expressed with the equation E (V) = 0.0647 log *v* + 0.602 (n = 10, r = 994). As for a totally irreversible electrode process, according to Laviron [35], the peak potential is a function of scan rate and defined by the following equation:

$$E_p = E^{0\prime} + \frac{2.3RT}{(1-\alpha)n_\alpha F}\log\left(\frac{RTk^0}{(1-\alpha)n_\alpha F}\right) + \frac{2.3RT}{(1-\alpha)n_\alpha F}\log(v)$$

where, E_P is the peak potential, $E^{0\prime}$ is the formal redox potential, α is the charge transfer coefficient, k^0 is the standard heterogeneous rate constant of the reaction, n_α is the number of electrons involved in the rate determining step, ν is the scan rate and other symbols have their usual meanings.

By using this equation, the value of (1- α) n_α found to be 0.92. By using this value and assuming the value of charge transfer coefficient (α) equal to 0.5, the number of electrons involved in the rate determining step of TR electrooxidation was obtained about 2.

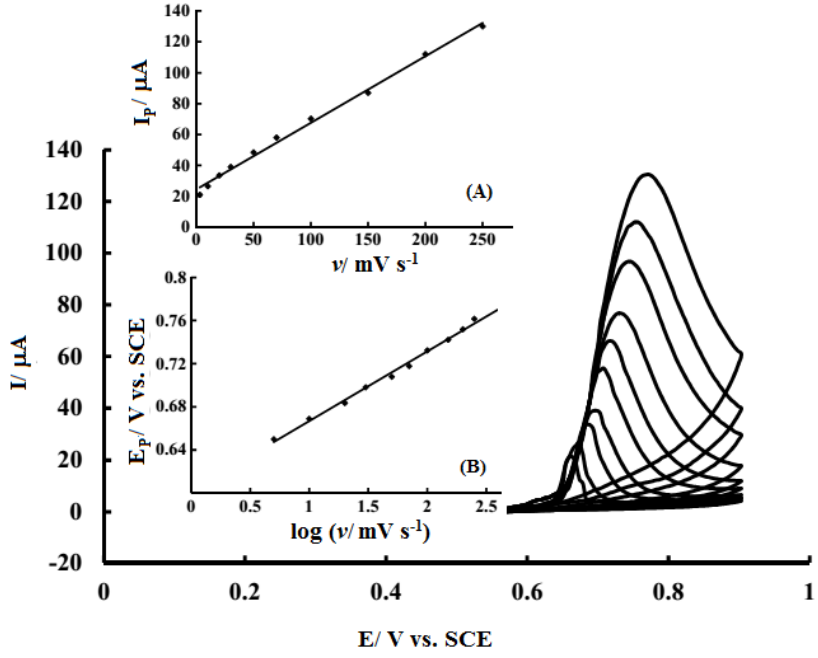
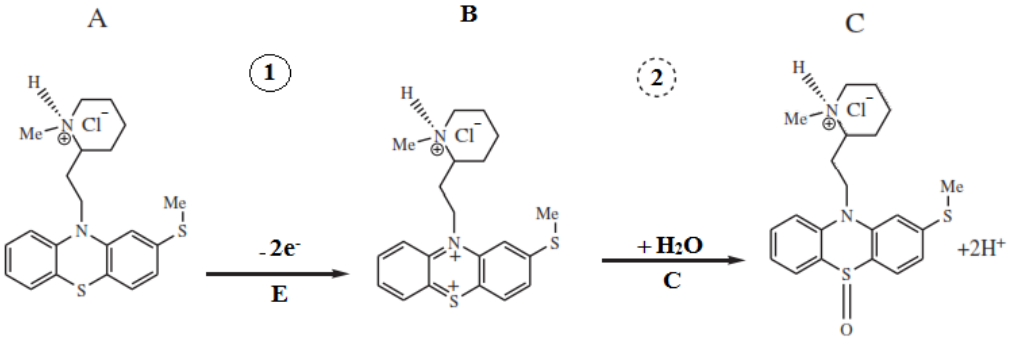


Figure 4. Cyclic voltammograms of NiO/GQD/GCE after immersion in 5 μM TR at various scan rates; Inset (A): Dependence of oxidation peak current versus scan rate; Inset (B) Variation of E_P versus log *v*. Accumulation condition: pH 7, time 400 s at -0.1 V

Based on the results obtained in "effect of pH" section, the irreversible anodic oxidation peak potential of TR was found to be pH-independent. In addition, CV studies performed at different

scan rates showed that there is no cathodic peak appeared in the reverse scan which is a confirmation of the catalytic mechanism of EC, in which an irreversible rapid chemical reaction occurs after Electron transfer. Based on this explanation and as described previously, a mechanism involving two electrons and not any protons (Scheme 1) could be optional for the TR electro-oxidation at the surface of NiO/GQD/GCE coupled by the irreversible hydrolysis of the product [36].



Scheme 1. Proposed mechanism for electrooxidation of thioridazine

Analytical measurements

To detect small amounts of thioridazine, the differential pulse voltammetry (DPV) method was used as a very sensitive and fast electrochemical technique. CV studies confirmed that TR adsorbs on the electrode surface. Therefore, the effect of experimental variables on the obtained results were examined.

The accumulation time and potential are the main factors affecting the sensitivity of adsorptive stripping methods. For this, TR (40 nM) was accumulated at the electrode surface applying various potential from -1.0 to 1 V for 400 s and the obtained results compared to each other (Fig. 5A). Decreasing accumulation potential up to -0.1V resulted in increased accumulation of TR and larger oxidation current, and then remained constant. Therefore, -0.1 V was chosen as the optimized voltage for analyte accumulation in other studies.

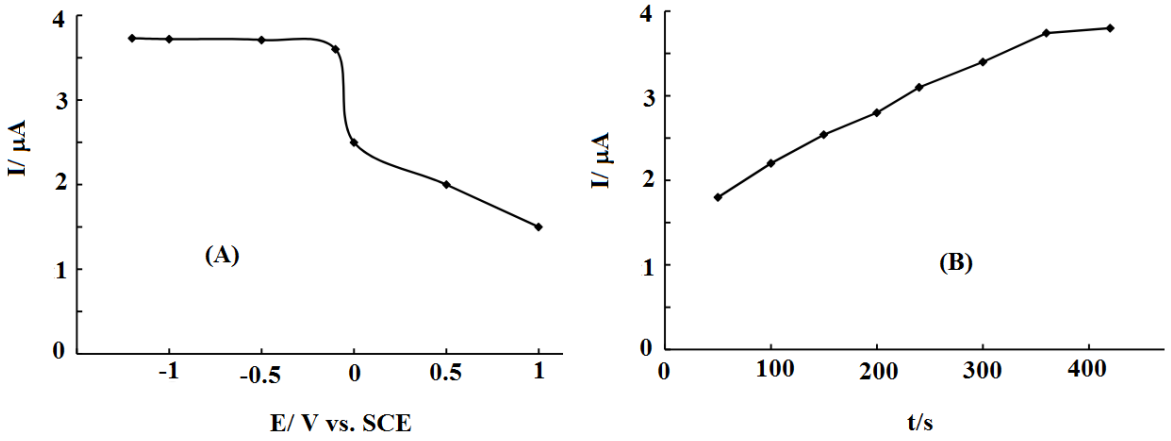


Figure 5. The effect of accumulation (A) potential and (B) time on the DPV response of NiO/GQD/GCE after immersion in 40 nM TR in PBS pH 7

In continue, -0.1 V was applied to the modified electrode for the various time duration (0 to 600 s), and the best time for experiments was selected. Based on the gained results (Fig. 5B) by increasing the accumulation time up to 360 s, the sensitivity of the sensor improves due to the more adsorption of the analyte at the electrode surface. Therefore, 360 s was selected as an optimized accumulation time for further experiments. Further studies showed that using negative accumulation potentials (eg. -1 V), the required time for obtaining the best signal decrease may be due to the positive charge of TR drug in pH values lower than pK_a (equal to 9.5) which result in fast adsorption of TR on the electrode surface due to the electrostatic attraction. But in this work to avoid interference from other compounds that can be adsorbed on the electrode surface by applying negative accumulation potentials, we used 360 s and -0.1 V for this accumulation step.

Analytical Parameters

Under optimum conditions, the prepared sensor showed a rapid and stable response to TR. Differential pulse voltammograms obtained with an increasing amount of TR were shown in Fig. 6.

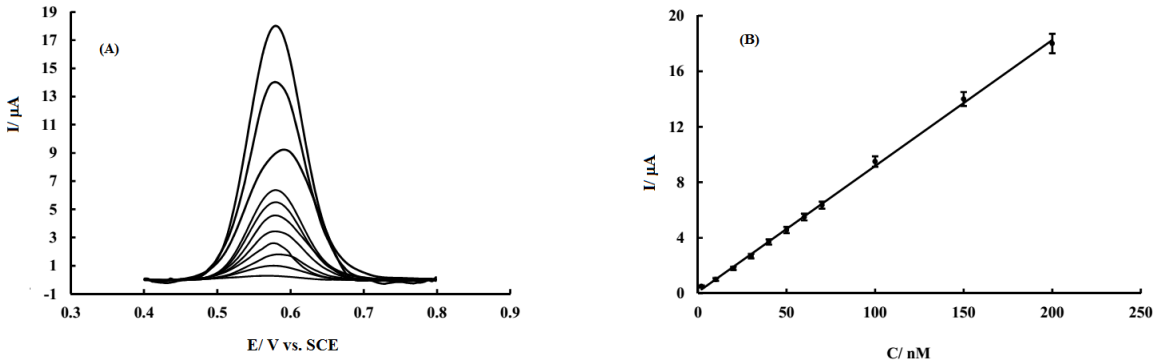


Figure 6. (A) DPVs of NiO/GQD/GCE after immersion in 2, 10, 20, 30, 40, 50, 60, 70, 100, 150 and 200 nM TR; inset: Corresponding linear calibration curve of Ip as a function of TR concentration

The results showed that increasing TR concentration results in a linear increase of peak current in the concentration range of 2–200 nM (Figure 6, the inset) with the related equation as follows:

I_p (µA)=(0.090±0.002) + (89.8±0.9) C (µM)

The LOD for TR determination was accounted to be 0.05 nM.

Table 1 reports and compares the linear range, sensitivity, and detection limit of the proposed TR sensor with the other modified electrodes.

Table 1. Comparison of the NiO/GQD modified GCE with other modified electrodes as TR sensor

Electrode	Modifier	Technique	Linear range	Detection limit	Ref
Nanodiamond-graphite	AgNP	CV	0.08–100 µM	0.01 µM	37
GCE		DPV, SWV	3.2–750 µM	750 nM	38
CPE	nickel (II) incorporated aluminophosphate (NiAlPO-5)	DPV	0.1–10 µM	90 nM	36
GCE	CoNP/MWCNT	DPV	0.5–100 µM	50 nM	5
CPE	ZnSNPs	DPV	0.1–36 µM	65 nM	6

It can be seen that the obtained analytical parameters of this electrode are better than previous reports.

Interference studies

The influence of various electroactive substances such as uric acid (UA), ascorbic acid (AA), clozapine (CLZ), glucose (GL), dopamine (DP) may be present in the analyte solution was examined by exposing the NiO/GQD/GCE in a solution containing 40 nM TR and gradual addition of the interfering compounds continued until a maximum of 5% change in the electrode response. The results indicated that the mentioned compounds had no significant effect on the obtained results until 100 fold concentrations may be due to the oxidation of these compounds occurs at different potential or lower concentrations of these compounds than the sensor's LOD. Therefore, it can be demonstrated that the sensing platform is relatively selective.

Reusability and repeatability of electrodes

The repeatability of the proposed sensor was evaluated by five analyses of 40 nM TR with the same electrode in the optimized condition. The resulted DPV signals are close to each other with a relative standard deviation (RSD) of 2.38 %, indicating the proposed sensor has good repeatability. To assess the long-term stability of the modified electrode, the TR oxidation signal measured one time every day. Results presented that the response was stable for 29 days when the sensor stored in the air at room temperature, which indicates long-term stability of the prepared sensor. In order to investigate the reproducibility of the electrode preparation procedure, five modified electrodes were prepared by the same fabrication procedure and used for the determination of 40 nM TR solution. The RSD for the obtained peak currents of these electrodes (3 determinations on each electrode) was calculated to be 4.21%. The results confirm the high reproducibility and repeatability of the sensor in both the preparation procedure and the voltammetric determinations.

Analysis of real samples

Analytical applicability of the proposed sensor in real sample analysis was assessed by the application of the NiO/GQD/GCE for the determination of TR in human serum samples and pharmaceutical tablets under the optimized condition. The obtained voltammograms in the prepared serum samples showed that there is no TR in the healthy human serum samples (Figure 7A).

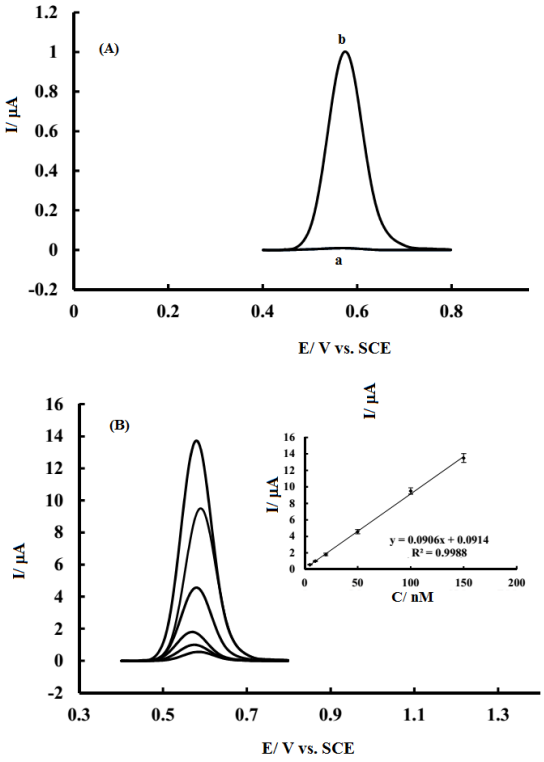


Figure 7. (A) DPVs of NiO/GQD/GCE after immersion in a: serum sample and b: tablet sample; (B) DPVs of NiO/GQD/GCE after addition 5, 10, 20, 50, 100 and 150 nM TR in serum sample containing 0.5 µM of UA, AA, DP, GL, CLZ; Inset: Dependence of oxidation peak current of the obtained voltammograms versus TR concentration.

In addition, the slope of calibration curve in serum sample containing 0.5 μM of UA, AA, DP, GL, and CLZ (Figure 7B) is equal to blank distilled water sample which prove interference free behavior and applicability of the proposed sensor for clinical real samples.

In continue, the serum samples were spiked with different amounts of TR and using the standard addition method the concentrations of TR were determined. The prepared electrode was also used for the determination of TR in pharmaceutical tablets. After the preparation of pharmaceutical samples as mentioned in experimental section, the concentration of a 10 nM solution of TR was determined using the standard addition method. Another measurement performed for the determination of TR in a tablet sample spiked with a known amounts of TR and the recovery percentages were calculated. The calculated recoveries and the relative standard deviations for serum samples and pharmaceutical tablets were reported in Table 2.

Table 2. Results of the determination of TR in human serum and pharmaceutical samples

Sample	TR added (nM)	TR found (nM)	Recovery (%)	RSD (%)
Serum samples	10	10.3	103	2.35
	20	19.8	99.0	1.86
pharmaceutical tablets	0	9.82		2.1
	5	14.9	101.6	1.91
	10	20.1	102.3	1.82

The high accuracy and selectivity of the proposed sensor in practical applications were proved by the obtained results which shows suitability of the prepared sensor for TR analysis.

Conclusion

In this research study, a simple and sensitive detection of thioridazine was achieved using NiO/GQD modified GCE prepared by simple electro-deposition. The oxidation peak current of TR increased at this electrode compared to the unmodified or GQD modified electrode which related to the synergistic action of GQD and NiO nano-particles resulted from large surface area and elctrocatalytic activity of GQD and NiO. The oxidation process follows EC mechanism and was independent from solution pH. The results indicated a linear relationship between the concentration and oxidation peak current of TR in CV and DPV. The DPV was successfully used as sensitive method to determine trace amount of TR in the human serum samples.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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