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A Novel Screen-Printed Electrode Modified by graphene Nanocomposite for Detecting Clozapine

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Present study aims to use Go/Fe_3O_4/SiO_2 nanocomposite modified screen printed electrode for electrochemical determination of clozapine. To that end, scanning electron microscopy (SEM) technique was used to study the surface morphology of the Go/Fe_3O_4/SiO_2 nanocomposite. The findings of the study showed that modified electrode has an excellent electrocatalytic performance toward oxidation of clozapine. A linear increase in differential pulse voltammetric peaks current of clozapine was observed with their concentrations in the range of 0.1-700.0 μM. The detection limit obtained for clozapine was 0.03 μM. Some advantages of using proposed sensor, among the others, were fast response time, high sensitivity, signal stability, low cost, ease of preparation and the lack of

need for using any specific electron-transfer mediator or specific reagent. Accordingly, modified electrode can be used without any need for separation or pretreatment steps to detect clozapine in real samples.

Keywords*:* Clozapine, Go/Fe_3O_4/SiO_2nanocomposite, Voltammetry, Screen printed electrode, Modified electrode.

1. INTRODUCTION

Schizophrenia is a serious chronic debilitating disease. The disease leads to both positive symptoms, such as hallucinations and delusions, and negative symptoms, such as cognitive deficits, socially withdrawn behavior and flat emotional expression [1,2]. Clozapine is an atypical antipsychotic drug. It was synthesized in Switzerland in 1958 and subsequently identified in 1959 [3,4]. Currently, it is the most efficacious medication available for patients unresponsive to other antipsychotics. It is also the only antipsychotic drug available for treatment-resistant schizophrenia which is approved by the Food and Drug Administration (FDA). Clozapine generally is used in people who are cannot eat other antipsychotics. Clozapine consumption binds several types of central nervous system receptors that leads to displaying a unique pharmacological profile [5-8].Given the importance of this issue, numerous analytical methods have been used to study it, including capillary zone electrophoresis [9,10], spectrophotometry [11,12], chromatography [13, 14] and mass spectrometry [15].

Although these methods show high sensitivity and selectivity, their high costs, complicated sample pretreatment and timely analysis has turned them to challenging methods [16,17]. On the contrary, the electrochemical methods have received much attention in analytical performances due to their simple, rapid detection, high sensitivity, portability, low-cost instrumentation and easy automation [18-31]. To modify electrode surface can have significant advantages in the electrochemical responses. Taking advantage of nanomaterials, containing catalysis, the large specific surface area and more adsorption sites to modify the electrodes will have numerous properties [32-34].

Todays, using nanotechnology-based approaches to detect biological compounds have attracted researchers' attention. Enormous research with myriad methodologies has been conducted on different types of nanomaterials, including nanoparticles, nanotubes and nanocomposites. Nanomaterials are widely used in sensor fabrication for the identification of biological compounds in diverse matrices [35-47].

Todays, graphene, the 2D carbon material has received much attentions because of its substantial electronic and electrocatalytic properties. Some advantages of graphene-based materials, among the others, are mass production, low cost, high conductivity, large surface area, good mechanical strength, high mobility of charge carriers and wide potential window. Accordingly, the application of them in the future is very promising [48-52].

However, improving graphene versatility through integrating it into more complex assemblies can reveal many of its unique properties. Currently, graphene/metaloxides have been widely reported. Metal oxides, including SiO_2, RuO_2 and SnO_2, are active and durable electro catalysts used for electrochemical study of the sensors. Silica oxide, due to its characteristic such as thermal and

chemical resistance, high surface area, pores with nanometer diameters, and the presence of reactive silanol groups (Si–OH) is widely used as to develop electrochemical sensors. Thus, the process of mass transfer due to its nanometer structure, providing electrochemical sensors with high sensitivity and low limits of detection has attracted substantial attention [53- 55].

In addition, Fe_3O_4 magnetic nanoparticles have also attracted the attentions in biotechnology and medicine. Specifications like strong superparamagnetic property, good biocompatibility, low toxicity, high adsorption ability, and easy preparation have motivated the researchers to use nano-Fe_3O_4 as electrode modified material in producing sensors and biosensors [56, 57].

Over the past few years, printing technology has used miniaturized and mass-produced sensors in field measurement due to their cost-effective and customized use. In screen printing, specific designs will be printed by pushing the ink through a mesh stencil onto a substrate. This printing technique is relatively cheap in compare to microfabrication. The technique also acts well from prototype to production. Therefore, it is used for mass production of low-cost sensors [58-63].

Herein, we designed a novel electrochemical sensor based on a screen-printed electrode modified with Go/Fe_3O_4/SiO_2nanocomposite. The electrochemical oxidation behavior of clozapine was also examined. An excellent electrocatalytic activity was observed regarding clozapine oxidation with the increased oxidation peak current. The clozapine contents in real samples were also determined to further confirm the feasibility of practical application.

2. EXPERIMENTAL

2.1. Chemicals and Apparatus

Auto-lab potentiostat/galvanostat (PGSTAT 302N; Eco Chemie:the Netherlands) was employed for electrochemical characterizations; the reaction system was monitored with a common electrochemical software. SPE (DropSens; DRP-110: Spain) is constructed with 3 conventional electrodes: graphite counter electrode; unmodified graphite working electrode; silver pseudo-reference electrode. A Metrohm 710 pH meter was used for measuring pH values.

Clozapine and other reagents with analytical grade were obtained from Merck (Darmstadt:Germany). The pH values were arranged with ortho-phosphoric acid and its salts to be in a range of 2.0 to 9.0. Go/Fe_3O_4/SiO_2nanocomposite was synthesized in our laboratory as reported previously [64]. The surface morphologies of (a) GO–COOH (b) Go/Fe_3O_4 and (c) Go/Fe_3O_4/SiO_2were investigated by SEM and depicted in Figure 1.

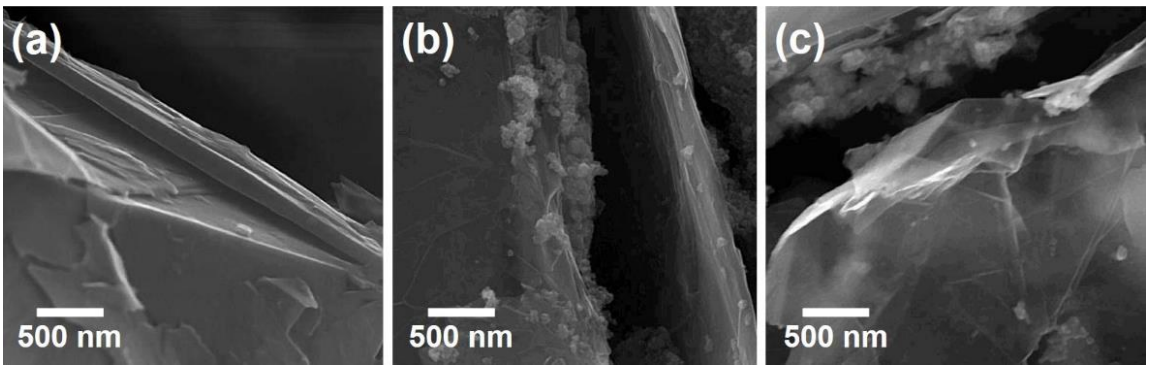

Figure 1. SEM image for (A) GO–COOH, (B) Go/Fe_3O_4 and (C) Go/Fe_3O_4/SiO_2.

2.2. Preparing Electrode

The bare screen-printed electrode was coated with the as-synthesized Go/Fe_3O_4/SiO_2 nanocomposite. Distributing 1 mg of Go/Fe_3O_4/SiO_2 nanocomposite through 30-min ultrasonication to prepare the Go/Fe_3O_4/SiO_2 nanocomposite solution, 2 µl of Go/Fe_3O_4/SiO_2 nanocomposite aliquots was casted on a carbon working electrode. Next, the solvent was evaporated at room temperature.

2.3. Real Samples Preparation

Five 100-mg clozapine pill (Tehran Chemie Pharmaceutical Co., Iran) were powdered to prepare a solution by dissolving the powder (500 mg) in water (25 mL) in exposure to ultrasonication. Then, different dilutions were poured in 25 mL volumetric flasks and reached final volume with PBS at pH of 7.0. The measurement of clozapine concentrations was achieved via a standard addition method.

Centrifugation was conducted at 2000 rpm for 15 mins for a 10 ml of refrigerated urine sample; the formed supernatant was filtered using a 0.45-µm filter. Solutions with different volumes were distributed into a 25 mL of volumetric flask, followed by diluting with PBS (pH = 7.0). Clozapine with diverse doses introduced for anaesthetization was to determine the clozapine concentrations.

3. RESULTS AND DISCUSSION

3.1. Electrochemical Profile of the clozapine on the Go/Fe_3O_4/SiO_2/SPE

The pH depending electrochemical behaviours of clozapine was analyzed using the modified electrode with a pH value of 2.0 to 9.0. As a result, reasonable outputs are observed for the electrooxidation of clozapine at pH equal to 7.0. Cyclic voltammograms were scanned using both Go/Fe_3O_4/SiO_2/SPE (Curve a) and bare SPE (Curve b) in the presence of 500.0-µM clozapine. The oxidation of clozapine occurred at 360 mV for the Go/Fe_3O_4/SiO_2/SPE that was ~100 mV more negative than that of the bare SPE.

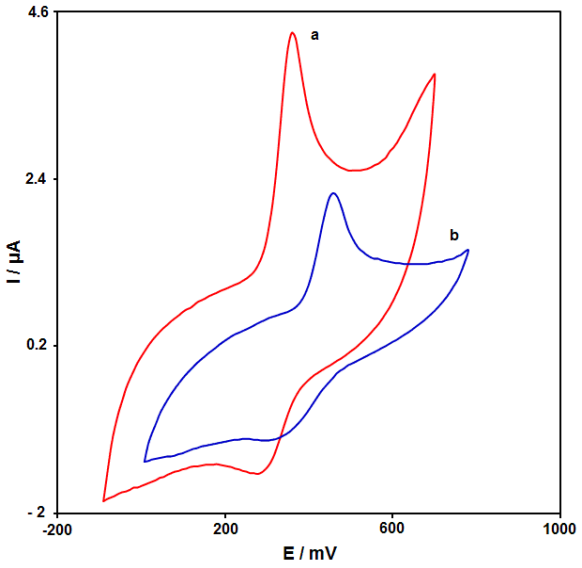

Figure 2. CV curves of (a) Go/Fe_3O_4/SiO_2/SPE, (b) bare SPE in 0.1 M PBS at pH equal to 7.0 in the presence of 500.0 µM of clozapine under a 50 mV s^{-1} scan rate.

3.2. Effects of Scan Rates

The current response increase with increasing the scan rates (Figure 3). It reveals linear relationship between the I_p and the square root of the potential scan rate ($v^{1/2}$), suggesting a diffusion-controlled clozapine oxidation process [65-70].

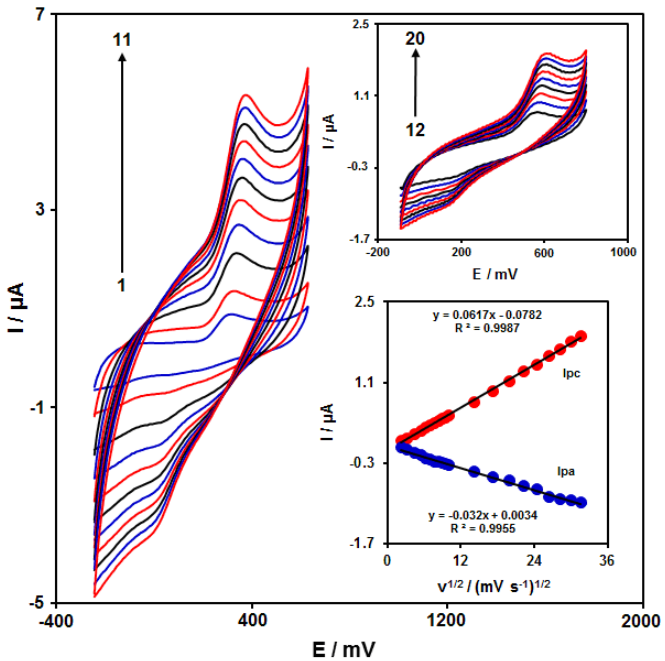

Figure 3. CV curves of the Go/Fe_3O_4/SiO_2/SPE in 0.1 M of PBS consisting of 400.0 µM of clozapine at pH equal to 7.0 under different scan rates; 1-20 corresponds to 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, and 1000 mV s^{-1}. Inset: variations in anodic peak currents.

3.3. Chronoamperometric Analyses

The Go/Fe_3O_4/SiO_2/SPE was used for the analysis of chronoamperometry of clozapine at 0.41 V. Chronoamperometric outputs of the clozapine sample in PBS with diverse concentrations at pH equal to 7.0 is provided in Figure 4. Cottrell equation for the chronoamperometric analyses of electroactive moiety was employed on the basis of the mass transfer restricted conditions [78]:

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

where D implies the diffusion coefficient ($cm^2\ s^{-1}$); C_b is the exerted bulk concentration (mol cm^{-3}). Figure 4A exhibits results of I vs. $t^{-1/2}$, suggesting the best fit of clozapine with distinct concentrations. Afterwards, final slopes in Figure 4A have been drawn vs. clozapine concentrations (Figure 4B). Therefore, the mean-value of D equaled to 1.14×10^{-6} cm^2/s with regard to Cottrell equation and resultant slopes.

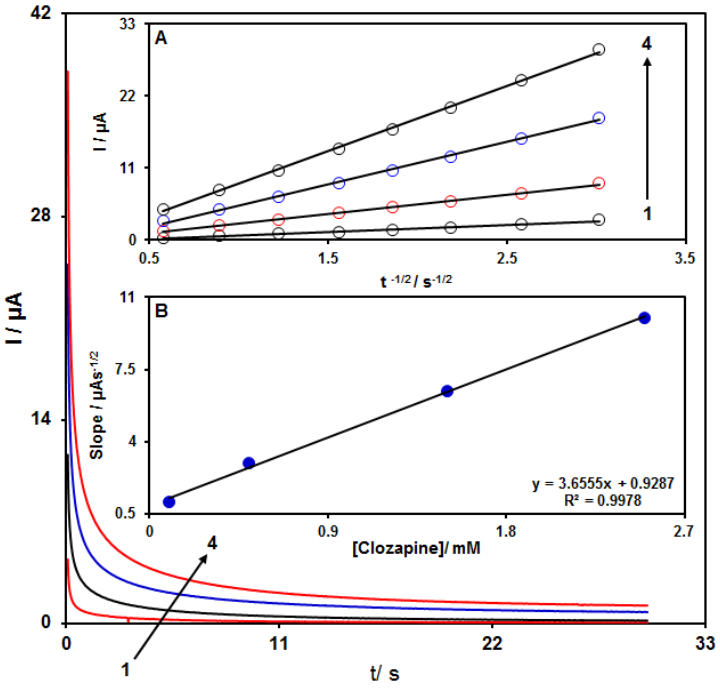

Figure 4. Chronoamperograms obtained over Go/Fe_3O_4/SiO_2/SPE in a 0.1 M of PBS solution at pH equal to 7.0 for clozapine with different concentrations. 1–4 corresponds to 1, 0.5, 1.5, and 5.5 mM of clozapine. Inset A: I plot vs. $t^{-1/2}$. B: slope plot of the straight lines vs. the concentration of clozapine.

3.4. Calibration Curve

Clozapine was quantitatively analysed in a water solution for evaluating the final peak currents of clozapine using the Go/Fe_3O_4/SiO_2/SPE (Figure 5). Therefore, we used the modified electrode, Go/Fe_3O_4/SiO_2/SPE, as a working electrode in 0.1 M of PBS in DPV with clozapine dissolved inside due to the DPV merits. A linear relationship was observed between the peak currents and the clozapine concentrations with a concentration range of 0.1-700.0 µM; the correlation coefficient is equal to 0.9996, LOD equal to 30.0 nM.

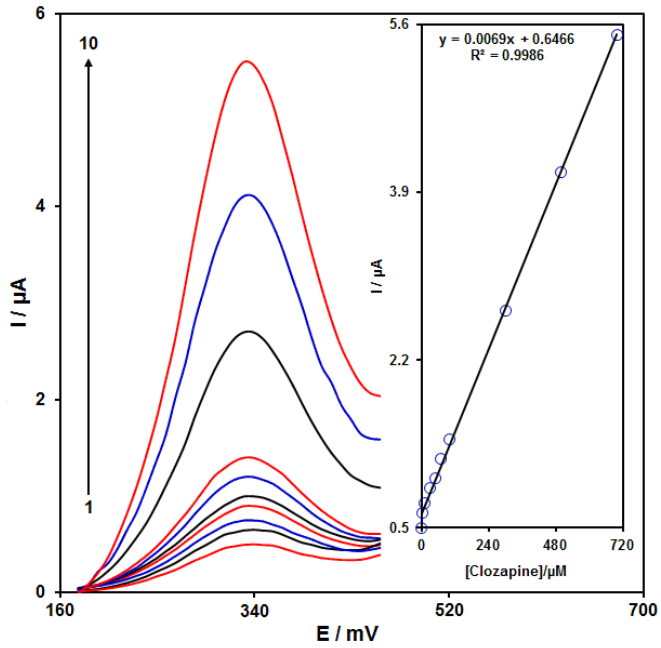

Figure 5. DPVs of Go/Fe_3O_4/SiO_2/SPE in 0.1 M of PBS with pH equal to 7.0. 1–10 corresponds to 0.1, 5.0, 10.0, 30.0, 50.0, 70.0, 100.0, 300.0, 500.0, and 700.0 µM of clozapine. Inset shows a linear relationship between the peak currents and the clozapine concentration in the range of 0.1-700.0 µM.

Table 1 shows a comparison of analytical properties for the detection of clozapine at the prepared electrode in this work and some other works.

Table 1. Comparison the determination of clozapine between Go/Fe_3O_4/SiO_2 /SPE and modified electrodes reported in the literature.

Electrode	Modifier	Analytical methods	Linear range (µM)	LOD	Ref
Gold	16-mercaptohexadecanoic acid	DPV	1×10^{-6}-5×10^{-5} M	7×10^{-9} M	71
carbon paste	Ruthenium doped titanium dioxide nanoparticles	SWV	9.0×10^{-7} - 4.0×10^{-5} M	0.43 nM	7
Glassy carbon	Thin film ofmultiwalled carbon nanotubes/new coccine doped polypyrrole	LSV	0.01–5.0 µM	3.0nM	72

Glassy carbon	composite of multiwall carbon nanotubes and WO_3 nanoparticles hydride	SWV	0.1-150.0 μM	30.0 nM	73
carbon ionic liquid	sodium dodecylsulfate (SDS)	AdSDPV	1×10^{-9}– 1×10^{-7} M	2.08×10^{-10} M	1
Screen printed	$Go/Fe_3O_4/SiO_2$ nanocomposite	DPV	0.1-700.0 μM	0.03 μM	This Work

3.5. Characterization using Real Samples

The usability of the prepared electrodes was characterized in the real samples; this new technique was used to detect clozapine in clozapine pills and urine samples. Next, the outputs are exhibited in Table 2. Reasonable recovery and reproducible outcomes are obtained.

Table 2. The application of $Go/Fe_3O_4/SiO_2/SPE$ for determination of clozapine in clozapine tablet and urinesamples(n=5). All concentrations are in μM.

Sample	Spiked	Found	Recovery (%)	R.S.D. (%)
Clozapine tablet	0	5.0	-	3.5
	2.5	7.6	101.3	1.7
	5.0	9.8	98.0	2.6
	7.5	12.4	99.2	2.1
	10.0	15.5	103.3	2.4
Urine	0	-	-	-
	5.0	4.9	98.0	3.4
	10.0	10.3	103.0	1.9
	15.0	14.9	99.3	2.8
	20.0	20.5	102.5	2.3

4. CONCLUSION

A new modified screen-printed electrode based on $Go/Fe_3O_4/SiO_2$nanocomposite determine clozapine was fabricated. To study the electrocatalytic performance of synthesized modified electrode regarding clozapine oxidation a voltammetry method was used. By determining the concentration of clozapine in real samples, the practical use of the modified electrode was assessed. The results indicated that the $Go/Fe_3O_4/SiO_2/SPE$ enjoys an excellent accuracy, a very good limit and great sensitivity in analytical determination of clozapine.

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