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Sensitive determination of metronidazole based on Graphene-TiO_2 modified glassy carbon electrode in human serum and urine samples

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ABSTRACT

In the present work, a new Graphene-TiO_2 (GR-TiO_2) modified glassy carbon electrode (GCE) is suggested for sensitive electrochemical determination of metronidazole (MTZ). Electrochemical studies revealed that GR-TiO_2 nanoparticles increased the efficiency of electron transfer kinetics by increasing the available surface area of the electrode and charge mobility between MTZ and GR-TiO_2 modified electrode. Compared to bare GCE, the modified electrode greatly enhanced the reduction signal of MTZ. The electrochemical behaviour of the modified electrode and the electrochemical reduction of MTZ were investigated with electrochemical impedance spectroscopy, cyclic voltammetry and differential pulse voltammetry techniques. The charge transfer coefficient (α) was calculated to be 0.694. Under optimized conditions, the linear concentration range and detection limit of MTZ were 5.0×10^{-7} to 2.5×10^{-5} M^{-1} and 5.4×10^{-8} (S/N = 3), respectively. Finally, this sensing method was successfully applied for the determination of MTZ in human blood serum and urine samples.

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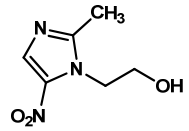
1. Introduction

The application of nanomaterials has been developed extensively in various fields of science such as electro-chemistry, pharmaceutical, and biomedical science [1-5]. These materials are being employed by virtue of their excellent mechanical, electrical, magnetic and adhesive properties [1,6]. Recently graphene (GR), which consists of two-dimensional sheets of sp^2 hybridized carbon atoms, has attracted enormous attention from scientists. Because of its large surface area, excellent thermal and electrical conductivity, high mobility of charge carriers, high mechanical strength and elasticity, GR is used as an excellent electrode modifier [7-9]. On the other hand nanocrystalline TiO_2 films and membranes have good structural and catalytic properties, such as high surface area and good biocompatibility [10-12]. Hence TiO_2 modified electrodes have been used extensively for the immobilization of biomolecules in electrochemistry and photochemistry [13-16]. Despite the good electrocatalytic activity of GR and Nano-TiO_2, the composites display unusual properties when immobilized with biochemical compounds [17-18].

Metronidazole (2-methyl-5-nitroimidazole-1-ethanol, MTZ) is a nitroimidazole antibiotic, which is used particularly for protozoal and an aerobic bacterial infections [9,19].Several methods have been stated for the measurement of metronidazole including spectrophotometry [20-24], high performance liquid chromatography (HPLC) [25-28] and gas chromatography [29,30]. MTZ can also be electrochemically investigated by the reduction of its nitro group (Scheme 1).



Chemical structure of MTZ

Scheme 1

Different electrodes such as activated GCE [31], MWCNTs/GCE [32], DNA-modified GCE [33,34], hanging mercury drop electrode (HDME) [35] and molecularly imprinted polymer-carbon paste electrode have been used inMTZ electrochemical assays [36,37].

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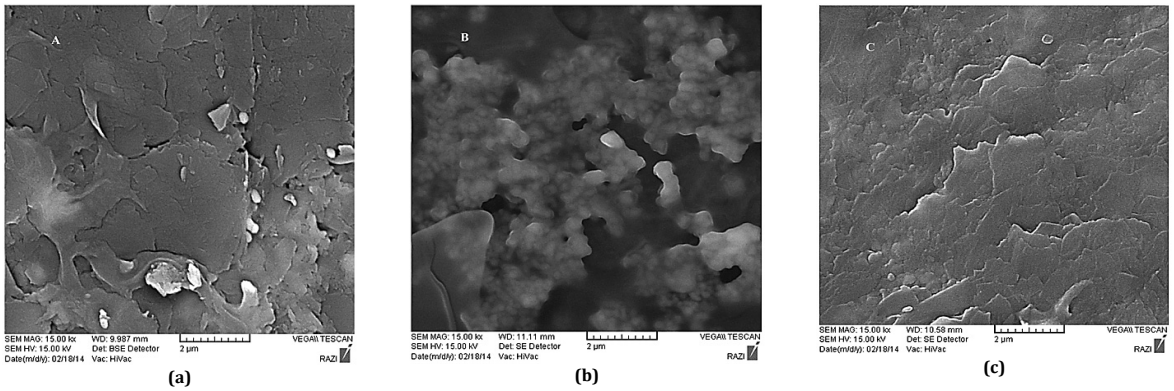

Figure 1. SEM image of GR in DMF solvent (a), TiO_2 nanoparticles (b) and GR-TiO_2/GCE (a).

In the present study, the electrochemical behaviour of MTZ and the effects of some experimental variables on the electro reduction of this drug have been investigated on a GR-TiO_2 modified GCE by cyclic voltammetry and differential pulse voltammetry techniques.

2. Experimental

2.1. Instrumentation

Electrochemical measurements were carried out with an Autolab PGSTAT30 Potentiostat/Galvanostat equipped with a frequency response analyzer (FRA 4.9) and controlled by GPES 4.9 software (Eco Chemie, Utrecht, Netherlands) with a conventional three electrode cell. A bare or GR-TiO_2 modified GCE was used as the working electrode, while a saturated calomel electrode (SCE) and a platinum wire were used as the reference and auxiliary electrodes, respectively. All the electrodes used were obtained from Azar Electrode Instruments (Urmia, Iran). The scanning electron microscope (SEM) images were obtained at Tehran University (Iran).MTZ was supplied by Alborz Darou Pharmaceutical Company (Iran) and used without prior purification. A stock solution of 1.0×10^{-3} M, MTZ was prepared using Britton-Robinson (B-R) buffer and then stored at 4 °C. Chitosan (Chit, medium molecular weight) was obtained from Merck. All other chemicals were of analytical grade and double distilled water was used in the experiments. GR was synthesized according to previous reports [38].

2.2. Preparation of TiO_2 nanoparticles

TiO_2 nanoparticles were prepared using a chemical process similar to that described by Kasuga *et al.* [39]. Briefly, 1 g TiO_2 white powder was placed into a 100 mL Teflon-lined autoclave. Then, the autoclave was filled using 80 mL NaOH 10 M, sealed and maintained at 200 °C for 24 h without shaking or stirring during the heating. After cooling down to room temperature, the sample was washed with an aqueous solution of dilute HCl, double distilled water and absolute ethanol for several times. In the end, the samples were dried at 80 °C for 7 h.

2.3. Preparation of GR-TiO_2 modified GCE

Before modification, the GCE was polished with 0.05 μm alumina slurry, and then sonicated in double distilled water. GR and HCl 0.1 M were added in a conical flask and after 15 min of ultrasonication, 5 mg Chitosan was added into the clear brown dispersion, followed by ultrasonication for 2 h to form a homogenous GR dispersion. Then 1 mg TiO_2 nanoparticles were added under vigorous stirring. 2.5 μL of this solution was applied onto the GCE surface. The modified GCE was then dried at 40 °C for 15 min before experiments.

2.4. Analytical procedure

The pH = 5.0 B-R buffer was used as the supporting electrolyte for MTZ. The cyclic voltammetry and DPV curves were recorded from -1.0 to 1.0 V and the reduction peak currents were measured at -0.59 V for MTZ.

3. Results and discussion

3.1. Characterization of modified GCE

Direct morphological observations of GR/GCE, TiO_2/GCE and GR-TiO_2/GCE prepared as above was obtained using SEM. Figure 1A shows the SEM image of GR film (in DMF solvent) on the surface of GCE, which disclose the typical crumpled and wrinkled GR sheet structure on the electrode. Figure 1B shows the SEM image of TiO_2 nanoparticles prepared; as can be seen the particle size is less than 100 nm (indicated with a circle). In addition, most of the particles are spherical in shape. In Figure 1C, it can be seen that TiO_2 nanoparticles are trapped between the highly faceted morphology of GR sheets. As shown in the SEM images, the GR-TiO_2/GCE exhibits significant edge plane defect structures. These edge plane defects have special role in high electron transfer kinetics and the electrocatalytic activity of GR. Also, they contribute significantly in the electrochemical property of the GR-TiO_2/GCE electrode.

3.2. Electrochemical properties of GR-TiO_2 modified electrodes

The cyclic voltammograms of MTZ on bare GCE, GR/GCE, TiO_2/GCE and GR-TiO_2/GCE in presence of 10^{-5} mol/L MTZ in B-R buffer solution (pH = 5) are shown in Figure 2. As can be seen, the electrochemical reaction of MTZ is an irreversible process with a reduction peak potential of -0.59 V *vs.* SCE. This is consistent with that reported in the literature [9]. The peak current value (Ip) of MTZ at GR-TiO_2 modified electrode is larger than that of other modified electrodes (Figure 2d). This suggests that that the enhancement observed may be due to the contributory effect of GR-TiO_2. The adsorption of MTZ from the solution onto the electrode surface is expedited through physical adsorption by modification of the nature and area of the used electrode. The data obtained clearly show that the combination of GR and TiO_2 nanoparticles in presence of chitosan improves the peak current of MTZ.

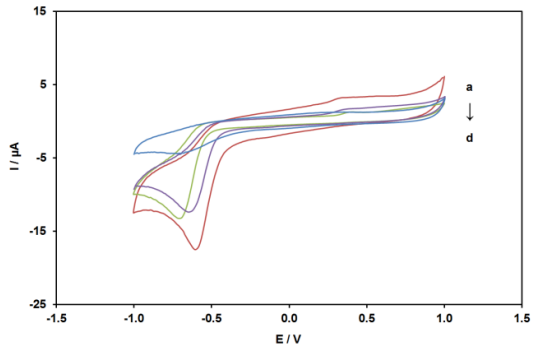

Figure 2. Voltammograms of bare GCE (a), TiO_2/GCE (b) GR/GCE (c) and GR-TiO_2/GCE(d) in the presence of 1×10^{-5} mol/L MTZ. Supporting electrolyte: B-R buffer solution (pH = 5.0).

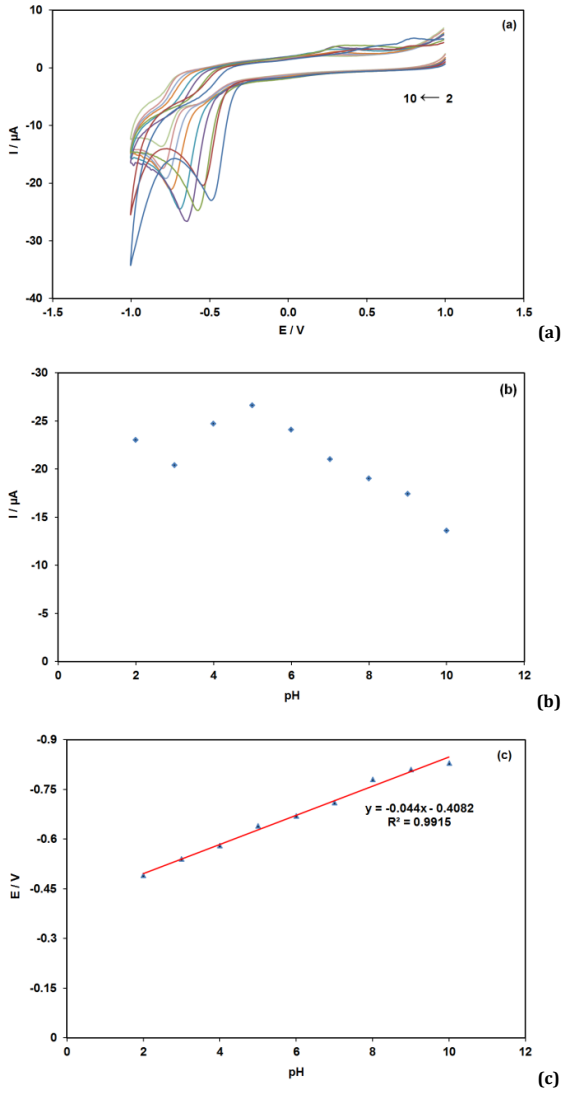

Figure 3. Cyclic voltammograms of 2.5×10^{-5} mol/L MTZ in B-R buffer solution at different pH (a). Influence of pH on the reduction peak current and peak potential of MTZ at the modified electrode (b, c).

3.3. Effect of pH

The influence of pH on the electrochemical behaviour of 2.5×10^{-5}mol/L MTZ onGR-TiO_2/GC electrode have been investigated within the pH range of 2-10 B-R buffer solution by cyclic voltammetry (Figure 3a). It can be seen from Figure 3b that the peak current increased with the increase the pH until pH of 5 at GR- TiO_2/GCE, and then decreased downto pH =10.

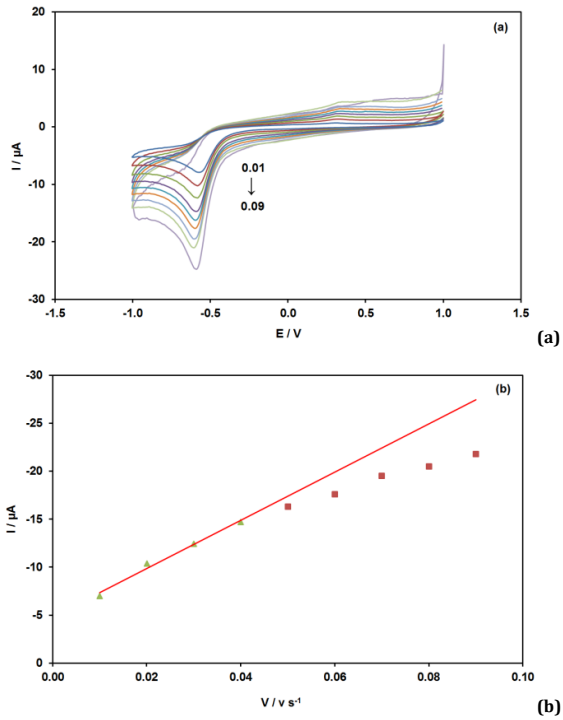

Figure 4. Cyclic voltammograms of 2×10^{-5} mol/L MTZ in B-R buffer solution with different scan rates (from 0.01 to 0.09 *Vs*$^{-1}$) (a), plot of I_p*vs* V (b).

This revealed that the pH of the supporting electrolyte has a significant influence on the electro reduction of MTZ. The best signals with respect to enhancement, shape and reproducibility were obtained with a B-R buffer of pH = 5.

This study reveals that the peak potential is closely dependent on the pH of the solution. It can be seen in Figure 3c that the peak potential values shifts towards more negative values up to pH = 10. This finding is in complete agreement with previous reports [9,32].

3.4. Effect of scan rate

Cyclic voltammetry as an electrochemical technique was used for the study of electrochemical behaviour of modified electrode. For this purpose, CV responses of 2×10^{-5} mol/L MTZ with different scan rates on GR-TiO_2/GCE surface were examined in B-R buffer as the supporting electrolyte (Figure 4a). As can be seen in Figure 4b, the voltammograms exhibit a cathodic peak with a peak potential of -0.59 V *vs.* SCE. The peak current of the voltammogram is linearly proportional to the scan rate up to 40 mV/s (Figure 4b). At higher scan rates, the cathodic peak potential shifts towards negative potentials, indicating the limitation arising from the charge transfer kinetics. For scan rates higher than 40 mV/s, the cathodic peak currents are linearly proportional to the square root of scan rate which is expected for a diffusion controlled electrode process.

The relationship between the potential (Ep) and scan rate (v) for a totally irreversible electrode process is expressed by the Laviron equation below [40]:

$$E_{pa} = E^\circ + (RT / \alpha nF) \ln (RTk^\circ / \alpha nF) + (RT / \alpha nF) \ln \nu \qquad (1)$$

where n is the number of electrons transferred, α is the cathodic electron transfer coefficient, and F, T, and R have their common meanings. According to Bard and Faulkner [38], for an irreversible reaction, Ep is a function of scan rate. For a reduction reaction Ep shifts in the negative direction by value of 1.15RT/αF (or 30/α mV at 25 °C) for each tenfold increase in scan rate. The α value for this reaction was calculated to be 0.694. This finding is consistent with the findings of previous research [9].

For thin layer voltammetry, the total amount of charge passed through the electrode surface (Q) for the reduction of MTZ was obtained by integration of CV peaks. Thus the surface coverage (Γ) of the modified electrode can be estimated to be 7.95×10^{-6} mol/cm^2 from the integration of the cathodic peak of the MTZ CVs according to Γ = Q/nFA. In this formula n is the number of electron transferred, Q is the charge involved in the reaction, F and A are Faraday constant and the electrode surface area respectively. This value for the modified electrode is much larger than that of a bare electrode.

3.5. Electrochemical impedance spectroscopy studies (EIS)

Electrochemical impedance spectroscopic measurement (EIS) was carried out to study the characteristics of the modified electrode. When a conducting substrates like $[Fe(CN)6]^{3-/4-}$ is used as a redox probe (solution concentration: 1.0×10^{-3} mol/L), the semicircular diameter in the ESI plot shows the electron transfer resistance (Rct). The value of Rct alters with different electrode surfaces. Figure 5 shows the Nyquist plots obtained using different modified GC electrodes. The Rct values measured on the bare GC and TiO_2 modified electrodes were high (1832, 1205 Ω), indicating a high electron transfer resistance. Whereas for GR/GCE and GR-TiO_2/GCE, the Rct values nearly equalled to zero, which reveals that the electrochemical probes to the substrate electrodes were accelerated. The conducting and porous nature of the GR-TiO_2 facilitates the charge transfer between the $[Fe(CN)6]^{3-/4-}$ probe and the electrode surface. Therefore, GR-TiO_2 modified electrode is a much more effective matrix than pure TiO_2 for the construction of an electrochemical sensor for the determination of MTZ, due to the larger pore size and good electrocatalytic properties of the modified electrode. Similar results have also been reported in the literature [9].

Table 1. Performance comparison of GR-TiO_2/GC electrode for MTZ with other electrodes.

Electrode	Technique	Linear range (mol/L)	LOD (mol/L)	RSD (%)	Ref.
Activated GCE	LSV [a]	2.0×10^{-6}–6.0×10^{-4}	1.1×10^{-6}	1.7	[31]
MWNT-GCE	DPV	2.5×10^{-8}–1.0×10^{-5}	6.0×10^{-9}	4.8	[32]
Carbon fibermicrodisk electrode	SWASV [b]	5.0×10^{-6}–1.6×10^{-5}	5.0×10^{-7}	3.7	[40]
SWCNTs-GCE	CV	1.0×10^{-7}–2.0×10^{-4}	6.3×10^{-8}		[41]
Gold electrode		5.0×10^{-7}–1.0×10^{-5}	1.5×10^{-7}	<5	[42]
HMDE [c]	DPV	2.3×10^{-7}–1.8×10^{-6}	3.6×10^{-8}		[35]
MIP-CP [d]	DPV	3.3×10^{-10}–1.5×10^{-8}	1.5×10^{-10}	4.5	[36]
GR-IL/GCE	DPV	1.0×10^{-7}–2.5×10^{-5}	4.7×10^{-8}	2.1	[9]
GR-TiO_2/GCE	DPV	5.0×10^{-7}–2.5×10^{-5}	5.4×10^{-8}	3.2	This work

[a] Linear sweep voltammetry.
[b] Square wave adsorptive stripping voltammetry.
[c] Hanging mercury drop electrode.
[d] Molecularly imprinted polymer-carbon paste electrode.

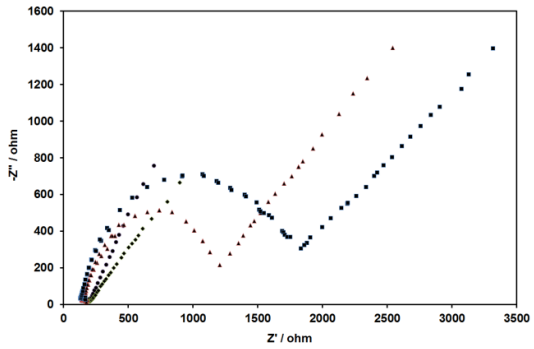

Figure 5. EIS measurement of GCE, TiO_2/GCE, GR/GCE and GR-TiO_2/GCE in a solution of $Fe(CN)_6^{3-/4-}$, 1.0×10^{-3} mol/L, the applied amplitude was 0.005, init E was 0.146 V, and the frequency swept from 10^5 to 1 Hz.

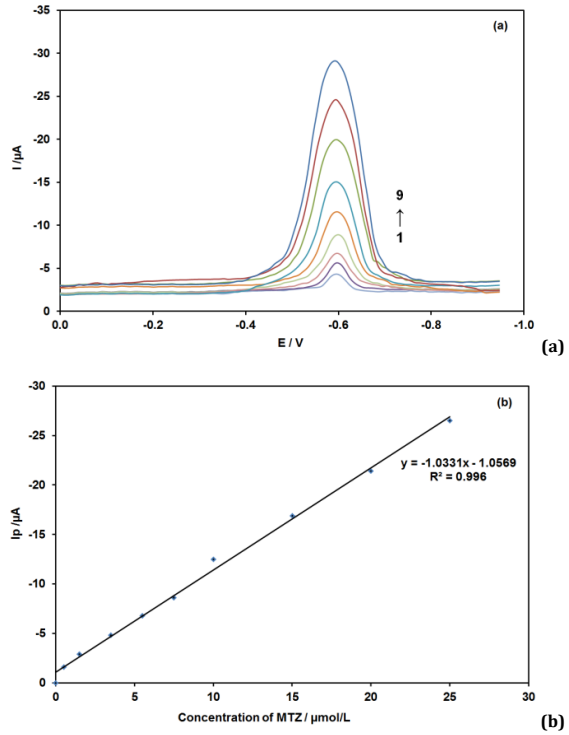

Figure 6. Differential pulse voltammograms of GR-TiO_2/GCE in different concentrations of MTZ solutions. Line 1-9: 0.5, 1.5, 3.5, 5.5, 7.5, 10, 15, 20 and 25 µmol/L (a), $I_p$vs MTZ concentration (b).

3.6. Calibration curve

DPV technique was used for the determination of MTZ, because of its higher sensitivity and excellent resolution. Since, the optimum peak current for MTZ was observed in B-R buffer of pH = 5, we have used this buffer as the supporting electrolyte. Figure 6a shows the DPVs obtained for the reduction of different amounts of MTZ at GR-TiO_2 modified

glassy carbon electrode. Under the optimized conditions, a linear relation between the peak current and the concentration of MTZ was observed in the range of 5×10^{-7} to 2.5×10^{-5} M^{-1} (Figure 6b). The value of LOD (S/N = 3) was calculated and found to be 5.4×10^{-8} M^{-1}. The low LOD value obtained, confirms the good sensitivity of the proposed method for the quantitative determination of MTZ.

The comparison of GR-TiO_2/GCE response towards the determination of MTZ with other electrodes is listed in Table 1. As can be seen, the GR-TiO_2/GC electrode offers a good linear range and lower detection limit compared to some previous reports for determination of MTZ.

For investigating the reproducibility of the fabricated electrode, a 1×10^{-6} M^{-1} of MTZ solution was measured by five modified electrodes and the RSD of the peak current was found to be 3.2%, which reveals good reproducibility.

3.7. Real sample analysis

The applicability of the proposed method for the MTZ determination in spiked human biological fluids was evaluated without any pre-treatment prior to the analysis. The recoveries from urine were measured by spiking drug free urine with known amounts of MTZ and recording the DPVs. The amount of MTZ in spiked urine and serum samples was evaluated from the calibration graph. Analysis of serum samples by DPV involved only precipitation of protein and centrifugation. The data obtained from the analysis are listed in Table 2. The average recovery values and the RSD values indicate accuracy and reproducibility of the results.

Table 2. Analysis of human blood serum and urine samples by proposed method.

Sample	MTZ added (M)	n	Amount found (M)	Average recovery (%)	RSD (%)
Urine	5×10^{-6}	3	4.76×10^{-6}	95.2	2.67
Serum	5×10^{-6}	3	4.68×10^{-6}	93.6	2.51

4. Conclusions

In the present work, we have prepared a modified GCE based on GR-TiO_2 nanoparticles for sensitive determination of MTZ for the first time. The electrochemical behaviour of MTZ was investigated using this modified electrode. The GR-TiO_2/GCE increased the cathodic peak currents by decreasing the charge transfer resistance value. GR-TiO_2/GCE sensor facilitated the electron transfer of MTZ and significantly enhanced its reduction signal. Based on this, a simple, rapid and sensitive electrochemical method has been developed for the determination of MTZ. The linear response of peak current was observed in the concentration range of 5×10^{-7} to 2.5×10^{-5} M^{-1}. The calculated LOD value was found to be 5.4×10^{-8} M^{-1}. The applicability of the proposed sensor was successfully demonstrated for the determination of MTZ in blood serum and urine samples. In comparison to other methods, the GR-TiO_2/GCE possesses preparative ease, rapid response and good sensitivity toward the determination of MTZ.

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