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A highly sensitive poly-arginine based MIP as an electrochemical sensor for selective detection of dimetridazole

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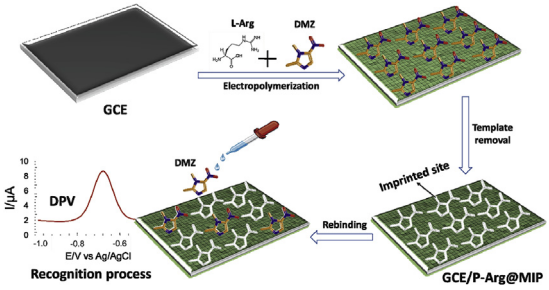
HIGHLIGHTS

- Highly effective electrochemical MIP sensor developed for ultrasensitive detection of dimetridazole.
- GCE/P-Arg@MIP electrode was fabricated via electrochemical deposition technique.
- The proposed sensor exhibited a wide linear detection range with an LOD of 0.1 nM.
- The GCE/P-Arg@MIP sensor was applied to detect analyte in egg, milk and honey.

GRAPHICAL ABSTRACT



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ABSTRACTS

In this experiment, a highly effective electrochemical sensor based on a molecularly imprinted polymer has been developed for ultrasensitive detection of dimetridazole. The sensor was made by incorporating of dimetridazole as a template molecule during the electropolymerization of poly-arginine on a glassy carbon electrode. The modified electrode GCE/P-Arg@MIP was characterized by voltammetric and microscopic techniques. Differential pulse voltammetry method was used to detect target analyte under the optimum condition. The DPV response to dimetridazole was linear at 0.1×10^{-9} to 10×10^{-6} mol L^{-1} ($R^2 = 0.996$), with a method detection limit (S/N = 3) of 0.1×10^{-9} mol L^{-1}. Moreover, the proposed sensor shows satisfactory recovery ranges for the determination dimetridazole in commercially available egg, milk and honey samples.

1. Introduction

Dimetridazole (DMZ, 1,2-dimethyl-5-nitroimidazole) is a part of the antibacterial and anti-protozoal veterinary drug that is widely used in the treatment of anaerobic and protozoal infections like *Amoebic Dysentery and Trichomonas Vaginalis* [1]. It has been found to be used in animal feed to increase the growth of animals [2]. Harmful side-effects on human beings, like genotoxicity, carcinogenicity, and mutagenicity can be caused by these drug residues due to its strong carcinogenic teratogenicity ring structures [3–5]. DMZ residues are strictly monitored in different countries namely the USA, European Union, China and Canada and they have not

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allowed its use as feed additives [6–8]. Therefore, the determination of DMZ residue is very necessary to protect public health.

Several analytical methods including high-performance liquid chromatography-mass spectrometry [7,8], liquid chromatography [9], gas chromatography [10,11], capillary electrophoresis [12,13], immunoassay [14], adsorptive stripping voltammetry [15], polarography [16], have been established to detect DMZ residues. But these methods have some drawbacks like slow, costly, unsatisfied selectivity and sensitivity. Therefore, the improvement of a simple, sensitive and selective method is very important for DMZ determination. At present, electrochemical sensors play a significant role in DMZ determination. For instance, developed an electrochemical sensor for DMZ detection using molecularly imprinted polymer (MIP) which exhibited poor sensitivity [17]. Hence, until now it is not easy to improve highly sensitive and selective electrochemical sensors for DMZ residue determination.

In comparison with conventional techniques, the electrochemical technique caused a revolution in the analytical detection field because it is a high sensitivity, low cost and high selectivity. The development of molecular imprinting technique has been observed for electrochemical determination of various analyte due to high desired selectivity and high affinity on a pre-defined aim molecule [18]. As a key component of a sensor, molecularly imprinted polymers (MIPs) can effectively exceed the interfaces of nearly connected compounds as well as rebind the target analyte and can provide specific recognition [17]. Usually, polymerization of functional monomers is used to synthesize MIPs in the presence of a template molecule [19]. These MIPs have some distinctive amenities such as significant structures stability, toughness, inexpensive and MIPs have elevated affinity to the template [20]. The advantages of electrochemical methods and MIPs can attach by molecularly imprinted electrochemical sensing systems [21]. For the development of novel sensors, these MIPs make the targeted molecule a very enchanting postulant [22]. During last decade, MIPs technology have received increasing interest and been widely used for quantitative discrimination of target molecules in the presence of closely related chemical species for selective recognize and bind the template structures [23–32].

In the field of an electrochemical sensor, polymerization of amino acids has devoted considerable attention during the past few years [33]. Many researchers have used poly amino acid modified electrodes due to their excellent electrocatalytic properties [34]. It was indicated that poly(L-arginine) promotes the electroactivity of the analytes and the stability of the electrodes [35]. For the determination of several pharmaceutical drugs, the poly amino acid modified electrodes were employed thoroughly [36].

In the present study, a cost-effective molecularly imprinted poly(L-arginine) based electrochemical sensor was proposed for the selective and sensitive determination of DMZ at a very low concentration level. The electrocatalytic activity of the different modified electrodes was also evaluated. Moreover, the potential application of the proposed sensor for detecting a target analyte in different commercial real samples was also evaluated.

2. Experimental

2.1. Reagent and chemicals

All purchased chemicals were used at their highest purity. Dimetridazole, L-Arginine was purchased from Aladdin's Reagents, Shanghai China. Phosphate buffer saline (PBS) was purchased from Sigma Aldrich, China. Sodium hydroxide and other common reagents were purchased from Merck, India. The electrolyte solution for all electrochemical measurements was 0.1 mol L^{-1} PBS solution (pH 7.4). Ultrapure water (Evoqua Type-I, Germany; resistivity < 18.2 MΩ) was used for all preparations. The electrolyte solutions were deoxygenated with nitrogen (N_2) gas for at least 5 min before each measurement.

2.2. Apparatus

An electrochemical workstation (Corrtest CS300, Wuhan, China) with three electrodes systems was used for all electrochemical measurements. In the system, a glassy carbon electrode (3.00 mm), silver/silver chloride (Ag/AgCl) electrode, platinum wire electrode was used as a working electrode, reference electrode and counter electrode respectively. The surface morphology of modified electrodes was performed with a scanning electron microscope (SEM) using a ZEISS GeminiSEM500 model.

2.3. Fabrications of MIP and NIP sensor

Before each modification, an aqueous slurry of Aluminum of 0.05 μm was used to polish the GCE surface on a micro cloth. Later, the polished electrode was rinsed by ultrapure water and washed stepwise in an ultrasonic bath for 5 min with dilute nitric acid, ethanol deionized water (DI) respectively and dried on air. After each experiment, the cleanliness of GCE electrodes was tested electrochemically. For the purpose of fabrication of MIP, finely clean electrodes were immersed in PBS (0.1 mol L^{-1}, pH 7.4) solution containing 0.05 mol L^{-1} L-arginine and 1×10^{-3} mol L^{-1} DMZ. The electrodeposition technique was used to fabricate the MIP film on the surface of GCE by CV method for 12 cycles at a scan rate of 100 mVs^{-1}. The potential range of electro-deposition was −2.0 to 2.2V vs. Ag/AgCl. After that, alkaline treatment was done to remove the drug template. Different concentration of alkaline solution and different incubation time was investigated to extract DMZ template molecule from the film and finally the most appropriate template removal incubation process was optimized. Moreover, the rebinding efficiencies after the different treatment conditions were compared Finally, the MIP electrode was immersed in 0.25 mol L^{-1} NaOH for 5 min approximately and the modified electrode was named as GCE/P-Arg@MIP. Without adding DMZ, the GCE/P-Arg@NIP electrode was fabricated in the same procedure. Finally, both electrodes were rinsed with ultrapure water and refrigerated at 4 °C.

2.4. Electrochemical measurement

During electrochemical measurements, the GCE/P-Arg@MIP electrodes were immersed in a solution of different concentrations of DMZ in 0.1 mol L^{-1} PBS. The Differential Pulse Voltammetry (DPV) analyses were carried out from −1.2 to 0V vs. Ag/AgCl with amplitude, pulse width and pulse period of 50 mV, 50 ms, and 0.5s, respectively. All DPV measurements were performed at room temperature under a nitrogen atmosphere.

3. Results and discussion

3.1. Electropolymerization of GCE/P-Arg@MIP sensor

The cyclic voltammetry (CV) method was used to electropolymerized L-Arginine (−2.2 to 2.0 V at 100 mV/s for twelve cycles) in 0.1 mol L^{-1} PBS containing 2.5×10^{-3} mol L^{-1} L-Arginine solution at pH 7.4. The continuous cyclic voltammograms of P-Arg electrochemical polymerization on the GCE surface is shown in Fig. 1a. A significant increase in the anodic oxidation peak during the deposition cycles confirms the formation of a non-conductive film polymer film on the bare GCE electrode surface which supports our previous report [27]. Fig. 1b represents the CV of P-Arg

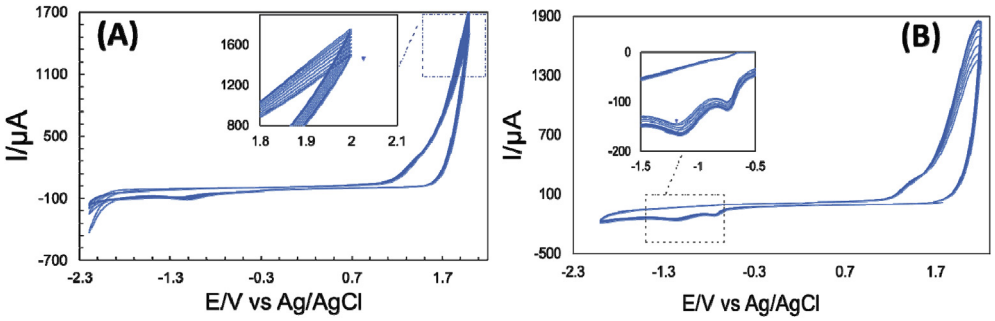

Fig. 1. Cyclic voltammograms for the electrochemical polymerization of 2.5×10^{-3} mol L^{-1} P-Arg on GCE electrode (A) and CV of P-Arg electropolymerization with 1×10^{-3} mol L^{-1} DMZ during the modification of GCE/P-Arg@MIP sensor (B). Deposition condition: 0.1 mol L^{-1} PBS solution (pH 7.4) at the scan rate 100 mV/s for 12 cycles).

electropolymerization with 1.0×10^{-3} mol L^{-1} DMZ during the modification of the GCE/P-Arg@MIP sensor. The incorporation of the DMZ template on polymer film was confirmed by the observed extra reduction peak at 0.7 mV during cyclic voltammograms in presence of a drug.

3.2. Surface topographical characterization of MIP sensor

Scanning electron microscopy (SEM) was used to investigate the surface topography of the non-imprinted and imprinted electrodes. Fig. 2a represents the surface of the GCE/P-Arg electrode after electrochemical deposition of the L-Arginine layer. The observed regular and patterned interconnected net-like structure clearly indicated the successful deposition of the P-Arg layer which adhered to the electrode surface as reported in our previous work [38]. The SEM image of the DMZ imprinted electrode (GCE/P-Arg@MIP) before and after the template removal is shown in Fig. 2b and c respectively. It can be revealed that MIP showed a rougher surface compared to relatively flat NIP surface due to the successful deposition and removal of the DMZ in/from the film.

3.3. Electrochemical characterization of modified electrodes

To understand the suitability of proposed modified different electrodes for sensor applications, electrochemical behaviors were studied using cyclic voltammetry in a 5.0×10^{-3} mol L^{-1} $[Fe(CN)_6]^{3-/4-}$ solution containing 0.1 mol L^{-1} KCl. A well-defined reversible redox peak observed for all modified electrodes as presented in Fig. 3a. The difference between oxidation and reduction peak current (ΔEp) for the bare GCE was calculated as 222 mV indicating a slow transfer of electron toward the electrode surface. In contrast, an additional increase in the intensity of redox peaks was observed ($\Delta Ep = 157$ mV) after P-Arg modification (GCE/P-Arg@NIP) which is attributed to the enhanced electron conduction [28]. On the other hand, the redox peak intensity decreases and peak to peak separation value increased ($\Delta Ep = 185$ mV) after removal of DMZ template (GCE/P-Arg@MIP) leads to decrease polymer conductivity [39–41].

The effect of the scan rate was analyzed in PBS (pH 7.4) solution containing 1×10^{-3} mol L^{-1} DMZ using the GCE/P-Arg@MIP modified electrode. Fig. 3b represents the CV current with a scan rate between 10 and 200 mVs^{-1}. It has been observed that as the scan rate increased, the current of the electrode also increased progressively and the scan rate values established proportional relationship with a sharp oxidation peak current of DMZ by the following equation,

$$I_p\ (\mu A) = 0.12(mVs^{-1}) + 1.5$$

This study indicating that the adsorption of DMZ regulates the behavior of the GCE/P-Arg@MIP electrode. Moreover, Randlessevcik equation was used to calculate the active surface area (A) of different electrodes. The calculated A value for the bare GCE, GCE/P-Arg@NIP, and GCE/P-Arg@MIP electrodes were 0.112 cm^2, 0.174 cm^2 and 0.198 cm^2 respectively.

3.4. Electrochemical detection of DMZ

Differential pulse voltammetry (DPV) was carried out for the detection of DMZ using different modified electrodes under optimized experimental conditions. The obtained DPV curves of 10×10^{-6} mol L^{-1} DMZ on these three different electrodes are shown in Fig. 4a. The voltammetry response of DMZ on the surface of the GCE/P-Arg@MIP electrode shows a remarkable peak current in the oxidation peak potential at 0.71 V, comparing with other electrodes. Fig. 4b represents the analytical plot of the anodic peak current of DMZ vs. their various concentration. It can be observed that the anodic current increased with the increase of analyte concentrations. The calibration curve for DMZ exhibits a linear range from 0.1×10^{-9} mol L^{-1} to 100×10^{-6} mol L^{-1} ($R^2 = 0.985$)

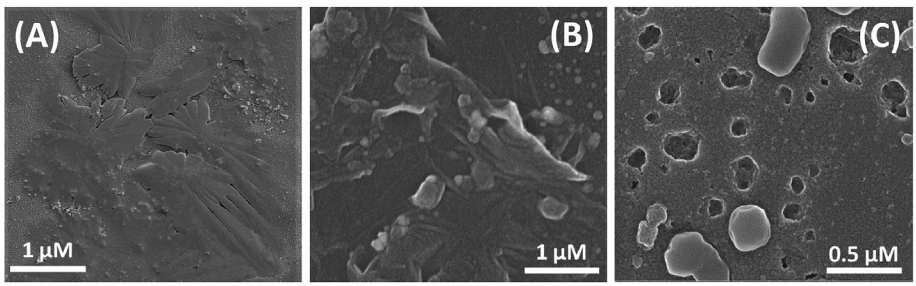

Fig. 2. SEM images of GCE/P-Arg electrode (A), GCE/P-Arg@MIP electrode before (B), and after (C) removal of DMZ template.

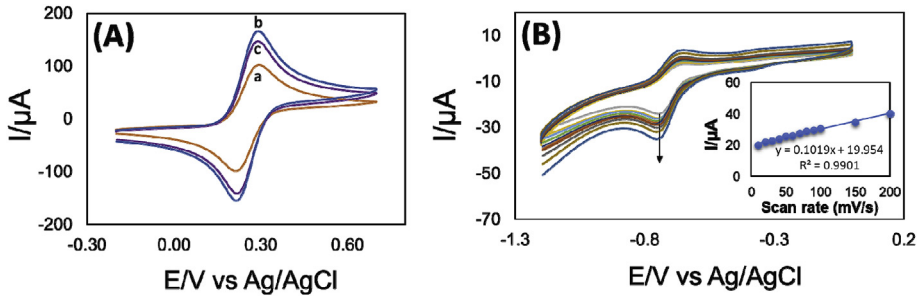

Fig. 3. Cyclic voltammograms (A) of the different electrodes measured in 0.1 mol L^{-1} KCl including 5.0 × 10^{-3} mol L^{-1} $Fe(CN)_6^{3-/4-}$: (a) bare GCE, (b) GCE/P-Arg@NIP, and (c) GCE/P-Arg@MIP. CV response obtained for GCE/P-Arg/MIP sensor different scan rates (from inner to outer): 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150 and 200 mV/s in 0.1 mol L^{-1} KCl including 5.0 × 10^{-3} mol L^{-1} $Fe(CN)_6^{3-/4-}$ solution (B). All potentials are given vs. Ag/AgCl. The inset shows the dependence of peak currents on the scan rates.

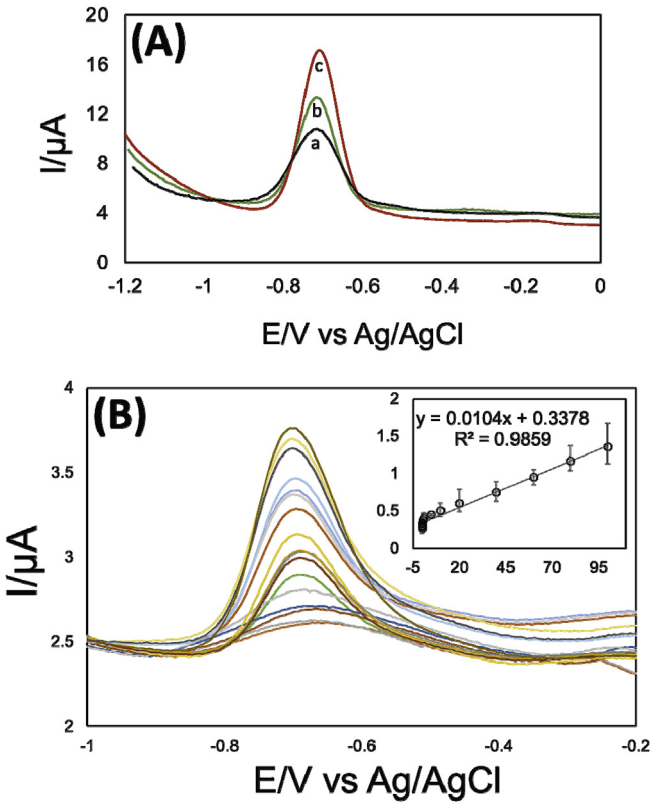

Fig. 4. (A) DPVs of 1 × 10^{-3} mol L^{-1} DMZ at bare GCE (a), GCE/P-Arg@NIP (b), and GCE/P-Arg@MIP electrode (c) in pH 7.4 phosphate buffer at a scan rate of 100 mV s^{-1}. (B) DPVs obtained for detection of DMZ using GCE/P-Arg@MIP sensor in pH 7.4 phosphate buffer at a scan rate of 100 mV s^{-1} with a wide range of concentrations from 0.1 × 10^{-9} mol L^{-1} to 100 × 10^{-6} mol L^{-1}. The inset shows the calibration curves for all concentrations.

with a method detection limit (S/N = 3) 0.1 × 10^{-9} mol L^{-1}. The comparison of present findings with other previous reports for electrochemical detection of DMZ is presented in Table 1. It can be concluded that the proposed sensor is more sensitive compared with other modified electrodes recently reported [17,42–44].

3.5. Reproducibility, and stability of the modified electrode

For evaluating the performance of the modified electrode, the reproducibility, selectivity, and stability are important parameters. To investigate the reproducibility of the sensor, a series of repetitive DPV measurements were carried out with the same electrode. No deviation in the current response (<1%) was found for different concentrations of the analyte sample. The selectivity of the modified electrode was studied with different compounds and metal ions such as Na^+, K^+, Ca^{2+}, Mg^{2+}, Fe^{2+}, Cu^{2+}, Zn^{2+}, Al^{3+}, Ascorbic acid and Uric acid. Some nitroimidazole analogs including 200-fold of Ornidazole, Metronidazole and Ronidazole were also performed for evaluating interference study. The oxidation signals of DMZ remain unchangeable in the presence of all interfering substances (<5%) which proves the selectivity of the proposed sensor.

The DPV curve of 10 × 10^{-6} mol L^{-1} of DMZ was recorded weekly to examine the stability of the proposed sensor after its preservation at 4 °C in the refrigerator. There was no deviation in peak response after two-week preservation. Therefore, our proposed sensor has excellent reproducibility, selectivity and stability for the detection of DMZ.

3.6. Real sample analysis

Recovery test measurement was done to study the feasibility of the proposed MIP sensor for practical application in commercially available real samples. The egg, milk, and honey samples were obtained from the local market and prepared according to the standard method as reported earlier [17]. In short, 1 gm of each real sample and 3 ml of 10% trichloroacetic acid was added into a 15 ml polypropylene centrifugal tube and vortexed the mixture for 1min. After 5min ultrasonication and 10 min centrifuging at 3500 rpm, the supernatant was filtrate through filter paper. Then the prepared real samples were diluted 100 times using 0.1 × 10^{-3} mol L^{-1} PBS (pH 7.4). The proposed MIP sensor exhibited recovery ranged from 94.2% to 101.8% suggests excellent feasibility for practical

Table 1
Comparison of the proposed sensor with other recently reported methods for the detection of DMZ.

Electrodes	Methods	Linear range (μmolL^{-1})	The detection limit (nmolL^{-1})	Reference
GCE/AuNPs@MIP	DPV	0.002–250	0.5	[32]
GCE/Chitosan	Amperometric	0.002–150	2.7	[33]
Cu_2O/ErGO	DPV	0.03–0.15	3.6	[34]
MIS-CPE	CV	1–10	3.6	[17]
GCE/P-Arg@MIP	DPV	0.0001–100	0.1	This work

Table 2
Detection of DMZ in egg, milk and honey samples after standard addition.

Sample	Added (μM)	Found (μM)	Relative recovery (%)
Egg	10	9.42	94.2
Milk	10	10.18	101.8
Honey	10	9.55	95.5

application in real sample analysis. Table 2 represents the recovery values obtained after the standard addition of the target analyte in real samples.

4. Conclusions

A novel molecularly imprinted polymer sensor was developed towards electrochemical detection of dimetridazole in the presence of interferences. The proposed modified electrode shows a linear relationship between the peak current and concentration for DMZ in the range of 0.1 nmolL^{-1} to 100 μmolL^{-1}with a method detection limit of 0.1 nmolL^{-1}, which is the lowest ever reported. Moreover, the developed sensor constitutes a promising low-cost approach with long-term stability, high selectivity, and reproducibility. In particular, the device obtained promising reliability for the detection of target analyte in real samples with high recovery data.

Declaration of competing interest

The authors declare there is no conflict of interest.

CRediT authorship contribution statement

M.R. Ali: Methodology, Validation, Writing - original draft. **M.S. Bacchu:** Conceptualization, Investigation, Writing - original draft. **M. Daizy:** Investigation, Methodology. **C. Tarafder:** Methodology, Investigation, Writing - original draft. **M.S. Hossain:** Methodology, Validation, Writing - original draft. **M.M. Rahman:** Writing - review & editing. **M.Z.H. Khan:** Supervision, Validation, Writing - review & editing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aca.2020.05.004.

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