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Metronidazole determination with an extremely sensitive and selective electrochemical sensor based on graphene nanoplatelets and molecularly imprinted polymers on graphene quantum dots

Ali A. Ensafi*, Parisa Nasr-Esfahani, B. Rezaei

Department of Chemistry, Isfahan University of Technology, Isfahan 84156-83111, Iran

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ABSTRACT

An extremely sensitive and selective electrochemical sensor based on a modified glassy carbon electrode (GCE) for metronidazole (MNZ) determination was developed. At first, molecularly imprinted polymers (MIPs) on the surface of graphene quantum dots (GQDs) was synthesized via sol-gel method. Then, it was dropped on the surface of GCE that modified with graphene nanoplatelets (GNPs). The excellent synergistic effect of GNPs and MIPs shows significantly enhanced electrocatalytic activity for MNZ. Electrochemical behavior of the imprinted electrochemical sensor was studied by cyclic voltammetry (CV), differential pulse voltammetry (DPV) and electrochemical impedance spectroscopy (EIS). Several effective parameters on the sensor response were investigated and optimized. The proposed imprinted electrochemical sensor, under the optimized conditions, showed two linear dynamic ranges from 0.005 to 0.75 μmol L^{-1} and 0.75–10.0 μmol L^{-1} with a low detection limit of 0.52 nmol L^{-1} for MNZ determination. Finally, the proposed sensor was used for MNZ analysis in human blood plasma samples and provided acceptable results.

1. Introduction

Metronidazole (MNZ) (2-(2-methyl-5-nitroimidazole-1-yl) ethanol) is an antibiotic of the nitroimidazole group with the ability to treat infectious diseases caused by susceptible organisms, especially anaerobic bacteria and protozoans [1]. Also, it has been used as an additive to animal feed for promoting growth. If MNZ does is increased than a certain amount in the human body, it will be caused seizures, peripheral neuropathy, and ataxia. According to research, MNZ has been shown to cause cancer in animals, but there is not enough evidence for carcinogenicity of MNZ for humans [2]. Therefore, determination of MNZ with a selective and sensitive method is very important in human health and food security. So far, different methods such as gas chromatography [3], high-performance liquid chromatography [4], capillary electrophoresis [5], spectrophotometry [6] and electrochemical techniques [7] have been used for determination of MNZ. Recently, electrochemical techniques for determination of MNZ have attracted much attention because of the nitro group in the structure of MNZ can act as a redox-active center.

Graphene is a two-dimensional structure of carbon atoms that because of its nontoxicity, chemical and thermal stability, electric conductivity and mechanical hardness has attracted enormous interest [8]. Graphene nanoplatelets (GNPs, also named graphene sheets) are one of the graphene derivatives that consist of small stacks of graphene that are 5–10 nm thick with diameters in varying sizes up to 50 μm. These nanoparticles due to their unique features such as high conductivity, large surface area, low cost and electrocatalytic activity are widely used as a modifier for electrode surface in electrochemical sensors [9].

Molecularly imprinted polymers (MIPs) have specific recognition sites that exhibit high selectivity for target molecules. In recent years, surface imprinting technique has attracted much attention due to the fact that made MIPs with the conventional methods have some disadvantages, such as incomplete template removal, low affinity binding, and slow mass transfer. Surface MIPs have significant advantages such as enhanced mass transfer, rebinding percentage and high selectivity [10]. Different materials have been reported as supporters for preparing surface MIPs such as silica nanoparticles [11], polymer nanoparticles [12], magnetic nanoparticles [13], carbon nanotubes [14] and quantum dots (QDs) [15].

* Corresponding author.
E-mail address: Ensafi@cc.iut.ac.ir (A.A. Ensafi).

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Among these materials, QDs have received extensive attention because of their very small size and high surface-to-volume ratio. Semiconductor QDs and their applications are limited due to the toxicity of heavy metals. Therefore, replacing of semiconductor QDs with the metal-free system is favorable. Graphene quantum dots (GQDs) are graphene sheets with size less than 10 nm. GQDs have attracted much consideration due to their unique properties such as low toxicity, high water solubility, large surface area and significant fluorescent activity [16].

Various electrochemical sensors based MIPs have been developed for the determination of MNZ. For example, Gu et al. [17] reported an electrochemical MNZ sensor by combining the concept of MIPs using mimetic enzyme. Li et al. [18] presented fabrication of an electrochemical nanosensor based on nanoporous gold leaf and MIPs for MNZ determination. Liu et al. [7] developed MIPs decorated GCE into an electrochemical sensing platform for detection of MNZ. Also, Liu et al. [19] reported an electrochemical sensor for determining of MNZ based on a composite structure of MIPs and multiwall carbon nanotubes. In all of these reports, electropolymerization method was used to prepare MIPs films directly on the electrode surfaces. Generally, the performance of an imprinted sensor depends on the number of specific recognition sites. Therefore, it is very important to prepare MIPs on the surfaces of nanoscale materials, which have high surface-to-surface ratios, such as QDs. Due to the available surface for the formation of the MIPs increases, the number of formed specific recognition sites increase, that improves the accumulation of MNZ on the surface of electrode.

In this paper, we reported an electrochemical sensor based on GCE modified with GNPs and MIPs on the surface of GQDs (GQDs-MIPs) for MNZ determination. GQDs were synthesized with hydrothermal method using glucose, ethylene diamine and hydrochloric acid as source materials. In the synthesis of MIPs, 3-aminopropyltriethoxysilane (APTES), tetraethoxysilane (TEOS) and MNZ were used as a functional monomer, a cross-linker and the template molecule, respectively. Sol-gel method was used for the synthesis of GQDs-MIPs. To improve the sensitivity of the electrochemical sensor, GNPs were dropped on the GCE before dropping of GQDs-MIPs. The combination of GNPs and MIPs led to increased sensitivity and selectivity of this electrochemical sensor. Electrochemical behavior of the sensor was studied by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). Finally, the prepared sensor was successfully used for the MNZ detection in real samples by differential pulse voltammetry (DPV).

2. Experimental

2.1. Chemicals

MNZ was purchased from Zhonghan Tianhin Company. Graphite powder was obtained from Merck. D-(+)-glucose, ethylene diamine and hydrochloric acid (HCl) were purchased from Sigma Aldrich. Triton X-100, TEOS, APTES, ammonium hydroxide (25–28% v/v), cyclohexane, acetone, ethanol, acetonitrile and tricholoroacetic acid were obtained from Aldrich. Doubly distilled water was used for the preparation of all solutions. 0.10 mol L^{-1} H_3PO_4 and NaOH solution were used for preparing phosphate solutions with various pHs. A stock solution of MNZ (0.010 mol L^{-1}) was prepared by dissolving 0.0171 g of MNZ in 10 mL of 50% ethanol solution.

2.2. Apparatus

All electrochemical studies were performed using an Auto lab potentiostat/galvanostat (model PGSTAT 302N) equipped with a three-electrode cell. An Ag/AgCl saturated KCl electrode, a platinum electrode and a modified GCE were used as the reference, the counter, and the working electrodes, respectively. UV–vis absorption spectrum was obtained with a double beam Jasco V–570 UV/Vis/NIR spectrophotometer (Tokyo, Japan). A Jasco FP–750 spectrofluorometers (Tokyo, Japan) was used for fluorescence measurements. Fourier transform infrared (FT-IR) spectra were recorded on a Jasco 680-plus spectrophotometer (Tokyo, Japan). Transmission electron microscopy (TEM) analysis was done on a Philips CM30 300 kV TEM operating at 300 kV. The GNPs characterization was done with field emission scanning electron microscopy (FE-SEM), HITACHI (S-4160). X-ray diffraction (XRD) was done with a Bruker D8/Advance X-ray diffractometer (Germany). Dynamic light scattering (DLS) instrument (Malvern ZEN 3600, UK) was used for obtaining particle size distribution of the GQDs. pH-meter (Corning, Model 140) was used for pH determination of the solutions.

2.3. Synthesis of GNPs

GNPs was synthesized according to the literature as the following steps [20]: At first, chemical oxidation of natural graphite was done in the presence of concentrated sulfuric acid. Then, sulfuric acid intercalated graphite was heated in a microwave environment. The worm- or accordion-like expanded structure of graphite intercalated compounds was exfoliated up to about 500 times in its initial volume by rapid heating in a microwave environment. Pulverization, using an ultrasonic processor, is employed to break down the worm-like structure and to reduce its size.

2.4. Synthesis of GQDs

Synthesis of GQDs was performed according to the Gu et al. method [21]. For this synthesis, 1.0 g glucose, 400 μL ethylene diamine and 200 μL HCl (37%, w/w) were dissolved in 15 mL water and transferred into an autoclave. Then the autoclave was heated at 200 °C for 6 h. After that, the autoclave was cooled to room temperature. The brown product of the reaction was centrifuged at 10000 rpm for 20 min to remove large particles. In this synthesis, surface modification was not required.

2.5. Synthesis of GQDs-MIPs

The GQDs-MIPs were synthesized according to our previous method [22]. Briefly, a mixture contains 1.8 mL Triton X–100, 5.0 mg MNZ, 50 μL TEOS, 20 μL APTES and 7.5 mL cyclohexane were prepared. The mixture was placed in an ultrasonic bath for 30 min. Then, 500 μL GQDs and 100 μL of ammonium hydroxide (25–28% v/v) were added to the above mixture. Next, the reaction system was sealed for 12 h at room temperature under continuous stirring. During this time GQDs-MIPs nanocomposites were obtained. Finally, acetone (20 mL) was used as a microemulsion broker agent. Then, the nanocomposites were collected by centrifugation (10000 rpm for 5 min) and washed with a mixture solvent of ethanol/acetonitrile (8:2 v/v) to removal the template until no MNZ was detected by UV–vis spectrometry. In the end, GQDs-MIPs composite was dried in the oven at 80° C for 60 min.

The non-imprinted polymers (NIPs) on the surface of GQDs (GQDs-NIPs) were synthesized via the above procedure without the addition of MNZ.

2.6. Sensor fabrication

Before fabricating the sensor, the bare GCE was polished with 0.05 μm alumina powder. Then the electrode was washed with the mixture of ethanol/water (1/1, v/v) in a sonication bath and dried at room temperature. 3.0 mg of the GNPs were dispersed in 1.0 mL of