Molecularly Imprinted Polymer Functionalized on Reduced Graphene Oxide Electrochemical Sensor for Detection of Ciprofloxacin

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Abstract. Ciprofloxacin (CIP) is widely utilised to treat bacterial infections. Currently, CIP is present in water sources at higher concentrations, thus necessitating close monitoring. This study developed electrochemical nano-sensors based on molecularly imprinted polymer (MIP) and reduced graphene oxide (rGO) composites to detect CIP. rGO served as the loading platform for MIP immobilisation on a glassy carbon electrode (GCE). A copolymer thin film, comprised of polyaniline copolymerized with o-phenylenediamine (PAni-co-PDA) was obtained by electropolymerisation utilizing cyclic voltammetry (CV) under suitable conditions. The performance of the modified GCE was examined utilizing CV mode in a hexacyanoferrate electrolyte as an electrochemical probe. The PAni-co-PDA/rGO-modified GCE exhibited enhanced improvement and efficient electrocatalytic behaviour in the oxidation of CIP with relatively high sensitivity and stability. The sensor was operated in differential pulse voltammetry (DPV) mode. Our best results revealed good linearity response to CIP in the range of 0.001–10.0 μM with an R-squared of 0.949, a detection limit of 0.09 μM (3.3 SD/S), and the calibration plot of ΔI minus the logarithm of the CIP concentration exhibited a sensitivity of −1.521. The sensor demonstrated a conductive polymer-based device that can be utilised for rapid CIP determination in pharmaceutical samples and biological fluids.

1. Introduction

Ciprofloxacin (CIP) is a second-generation fluoroquinolone antibacterial agent that is relatively active against a broad spectrum of both gram-positive and gram-negative aerobic pathogens. It is widely administered for antibiotic purposes in humans and livestock [1]. CIP has been polluted in surface waters because of hospital and domestic wastewater effluents. A handful of electrochemical sensors were demonstrated for ciprofloxacin determination [2]; however, most sensors suffered from signal...
interference by biological substances, resulting in false positive measurements. However, conventional techniques, such as spectrophotometry, high-performance liquid chromatography (HPLC) and capillary electrophoresis [3], were costly, time-consuming, and relied on complicated procedures and trained personals. A sensor device that can detect CIP in water at a sufficient level of sensitivity was required.

The molecularly imprinted technique (MIT) has exhibited great promise as an analytical technique for the development of an electrochemical sensor [4]. This technique creates recognition cavities that target analyses based on molecular geometry and interactions. It allows the target analyte to adsorb and interact with recognition sites, and yields the possibility for analyte desorption. MIP-based sensors prepared by electro-polymerisation are an effective method for polymer film deposition at a controllable thickness [5]. Conductive polymers, which are conjugated polymers with semiconductive characteristics are often utilised as templates for MIPs. The polymer can be electropolymerized from an aqueous solution in which the target analyte dissolves in creating the polymer film with the analyte in the matrix. The analyte can later be removed, leaving behind the recognition cavities on the polymer. Regarding conductive polymers, the outer layer of the film is often over-oxidized or exposed to charge transfer. This phenomenon renders the outer layer non-conductive or poorly electroactive. To cope with the over-oxidation of conductive polymers, carbon nanostructures, such as carbon nanotubes (CNTs) and graphene, can be utilised as signal amplifiers. In particular, graphene has been explored as an outstanding semiconductor for electrochemical sensing applications. Graphene also has a large specific surface area, high conductivity, and high chemical and mechanical stability [6]. Integrating graphene and MIP as recognition and sensitive elements on an electrochemical sensor with unique abilities from the two materials.

This study developed a simple, rapid, and low-cost electrochemical sensor based on MIP, synthesized by the electro-polymerisation of copolymer aniline-co-o-phenylenediamine integrated with chemically exfoliated reduced graphene oxide (rGO) for the determination of ciprofloxacin. The interaction between the sensor and CIP was investigated utilizing a redox couple ferro/ferricyanide \([\text{FeCN}_6^{3/4}]\) solution in cyclic voltammetry (CV) and differential pulse stripping voltammetry (DPV) modes.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals were of AR grade and were utilised as received. Reduced graphene oxide (rGO) was synthesised using a chemical exfoliation path [7]. Ciprofloxacin (CIP) (≥98%, HPLC), aniline (ANI), o-phenylenediamine (PDA), potassium hexacyanoferrate (III) (K₃Fe(CN)₆), potassium hexacyanoferrate (II) trihydrate (K₃Fe(CN)₆·3H₂O), and potassium chloride (KCl) were purchased from Sigma–Aldrich (Singapore). Hydrochloric acid (HCl) was obtained from Merck (Germany). All solutions were prepared with ultrapure water of resistivity ≥ 18 MΩ cm⁻¹, provided by a Milli-Q system (Millipore®). A stock solution of 100.0 mM ciprofloxacin was prepared in-house by dissolving the CIP powder in a hydrochloric acid solution approximately 0.1 N.

2.2. Fabrication of the rGO-film-modified GCE

A glassy carbon electrode (GCE) was cleaned on two polishing pads, utilizing two sizes of polishing powder, i.e., 0.3, and 0.05 μm alumina powder (Al₂O₃) (CHI Instruments). The GCE was first polished on the 1st pad with 0.3 μm alumina powder and rinsed with ethyl alcohol and DI water. It was polished for the 2nd time using 0.05 μm alumina powder. To fabricate the electrode, 5 μl of a 0.5 mg mL⁻¹ rGO suspension was cast on the GCE and dried in an oven (Memmert, Germany) at 60 °C for 15 min. Several coats were subjected to a mass loading of 0.71 μg mm⁻².

2.3 Preparation of MIP and non-molecular imprinted polymer (NIP) modified electrodes

The rGO/GCE was immersed in 10.0 mL hydrochloric acid solution (pH = 2.5) containing 1.0 mM aniline, 1.0 mM PDA and 5.0 mM ciprofloxacin as a template. The MIP was fabricated via CV mode using the potential window of 0.0 to 0.7 V at a scan rate of 50 mV s⁻¹ for 20 cycles. The modified electrode was eluted in a 50% ethanol aqueous solution (v/v = 1:1) for 3 min to form imprinted cavities.
The MIP/rGO/GC electrode was rinsed with DI water for 10 s, washed with excess hydrochloric acid, and dried at room temperature. For comparison, a non-imprinted polymer electrode, NIP/rGO/GCE, was prepared following a similar procedure with no ciprofloxacin during electro-polymerisation.

2.4 Electrochemical characterization and measurements
All electrochemical experiments were performed utilizing a PalmSens® potentiostat/galvanostat instrument (PalmSens BV, Utrecht, the Netherlands), controlled with PSTrace 5.8 software. Either the MIP/rGO/GCE or NIP/rGO/GCE was employed as the working electrode, along with the Ag/AgCl reference and the platinum mesh counter electrode. The sensor was operated in CV or DPV modes to observe the electrochemical activities. In the CV mode, the modified GCE was positioned in a solution of 0.1 mM [Fe(CN)₆]³⁻/⁴⁻ (1:1) and 0.1 M KCl, while potentials were scanned in the −0.1 to 0.6 V window at a scan rate of 50 mV s⁻¹. DPV measurements were performed with E_step = 5.00 mV, E_pulse = 60.00 mV, t_pulse = 0.02 s, and a scan rate of 50 mV s⁻¹. All the solutions were purged with high-purity nitrogen gas for 10 min prior to the electrochemical experiments.

3. Results and discussion
3.1 Characterization of MIP/rGO/GCE
The electrochemical activities of the modified electrodes were characterised by the CV mode. The voltammograms of the bare GCE (black line), rGO/GCE (green line), NIP/rGO/GCE (blue line), and MIP/rGO/GCE (red line) are illustrated in Figure 1. The response current from the bare GCE was much lower than that of the rGO/GCE because of the low surface area, which led to a poor electrochemical signal. The results indicated that rGO can promote the electrochemical activities of the electrode. For the NIP/rGO/GCE, the voltammogram exhibited stronger electroactivity owing to the affinity between the polymer and the aqueous electrolyte. The MIP/rGO/GCE exhibited the highest electroactivity, which was attributed to the cavities on the polymer film that enhanced the surface activity of the electrode.

![Figure 1. CV of the modified electrodes: bare GCE (black line), rGO/GCE (green line), NIP/rGO/GCE (blue line), and MIP/rGO/GCE (red line).](image-url)

3.2. Optimization of conditions for MIP/rGO/GCE preparation

3.2.1. Effect of the volume and thickness of rGO suspension. To achieve an optimal analytical performance of the MIP/rGO/GCE sensor, the selected experimental parameters were investigated. First, the effect of rGO coatings on GCE was examined utilizing different drop sizes for the rGO suspension (2.5 – 10.0 µL). A total rGO loading of 0.71 µg mm⁻² (n = 5) was maintained. The drop size of the rGO suspension affected the porosity of the rGO film and film adhesion to the GCE [8]. The electrodes were tested in CV mode, in which the current at the cathodic peak was collected. The rGO/GCE fabricated with 10.0 µL of rGO suspension drop size displayed a response current of 5.2 µA. The current increased to 6.0 µA as the drop size reduced to 5.0 µL. However, as the drop size decreased to 2.5 and 2.0 µL, the current deteriorated to 5.0 and 4.7 µA, respectively. Therefore, 5 µL of rGO was optimised in this study.
Figure 2. Effect of volumes of rGO suspension on responding current.

Figure 3. Influence of pH values of the electro-polymerisation on the current.

3.2.2. Optimization of pH. The pH of the solution had a significant influence on the stability of the electrochemical sensor. The pH of the HCl solution on the electrochemical performance of the MIP sensor was examined, as illustrated in Figure 3. The copolymerisation was performed in a 10 mL HCl solution containing 1.0 mM ANI, PDA, and CIP. The main interactions between monomers and the template involved hydrogen bonding and electrostatic forces, depending on the pH of the solution. pH affected the electrostatic interactions between the active monomers. PANi was relatively more conductive than PDA, and mainly constituted the matrix of the copolymer film. A lower pH enhanced the electropolymerization rate for both polymers, leading to variation in the ANI: PDA content and, eventually, the conductivity of the copolymer film [9]. The maximum response current was observed at pH 2.5, and the pH 2.5 solution was chosen for the CIP sensing operations.

3.2.3. Optimization of template concentration. To investigate the effect of template concentration on the MIP/rGR/GCE, the electrodes were electropolymerized at different template concentrations in the range of 1.0 to 10.0 mM, while the monomer molar ratio of ANI:PDA was fixed at 1:1. The highest peak current of [Fe(CN)₆]³⁻/⁴⁻ was observed at 5.0 mM of template concentration (Figure 4). Ideally, CIP could only be entrapped in the outer layer of the copolymer film and later removed, leaving cavities on the film. However, CIP was nonconductive and caused surface passivation when left in the film matrix, decreasing the electroactivity of the film.

Figure 4. Effect of template concentration on the response currents of the MIP sensors.

Figure 5. Effect of ANI and PDA ratio on peak current of the MIP sensor.

3.2.4. Optimization of monomers ratio. The initial concentration of ANI and PDA monomers affected the composition of the film and, clearly, the characteristics of the copolymer film [10]. At an ANI: PDA molar ratio of 2:1 (Figure 5), a responding current of 10.3 μA was obtained. As the ratio decreased to 1:1, the current peaked at 11.5 μA. The responding current decreased from 9.2 μA to 8.9 and 8.8 μA, as
the ratio decreased from 1:2 to 1:3 and 1:4, respectively. Increasing the PDA ratio reduced the capture of CIP during electropolymerization and a suitable mainstay conformation for the formation of imprinted sites.

3.2.5. Optimization of cycles for electropolymerization. The thickness of the MIP film mainly depended on the number of cycles utilised in electropolymerization [11]. For the MIP/rGO/GCE, electropolymerization was performed in CV mode at 5.0 a CIP template concentration and 1:1 of ANI:PDA ratio. As shown in Figure 6, the number of CV cycles increased from 5 to 10, 15, and 20, the peak current rose from 10.1 to 10.8, 12.0 and 12.6 μA, respectively. The maximum current was obtained from MIP/rGO/GCE after 20 cycles of CV electro-polymerisation. However, the current dropped from 10.3 μA to 10.2 μA as the CV cycles increased from 25 to 30 which led to thicker films with poor accessibility and loss of sensor sensitivity.

3.2.6. Optimization of template removal. The elution conditions of the template molecules were very important for releasing the imprinted sites in the polymer matrix. To investigate the effect of elution time, the MIP/rGO/GCE was immersed in 50% (v/v) ethanol in water. The immersion time varied from 30 s to 300 s, and the results are illustrated in Figure 7. As the extraction time increased from 30 s to 120 s, the responding current decreased from 8.7 μA to 7.7 μA. The highest peak current of MIP/rGR/GCE was observed when the elution time was at 10.2 μA of 180 s.

3.2.7. Determination of ciprofloxacin in pharmaceutical samples. The MIP/rGO/GCE with 20 CV electropolymerization cycles, 180 s extraction time, 5.0 mM CIP template, and 1:1 ANI: PDA copolymer ratio, was utilised for CIP detection (Figure 8). The sensor was operated in DPV mode in the pH 6.5 solution of 0.1 mM [Fe(CN)6]3-/4- and 0.1 M KCl. The sensing signal was obtained from the current shift (ΔI), where

\[ \Delta I = I_0 - I_c \]  

where \( I_0 \) is the original current and \( I_c \) denotes the current response of the MIP/rGO/GCE incubated in a solution of a particular concentration. The sensor was employed in the pH 6.5 background electrolyte. The DPV curves revealed a reduction in the responding currents at the peak position as the CIP concentration increased. This reduction was attributed to the nonconductive passivation of CIP on the MIP/rGO/GCE electrode, which hindered charge transfer between the sensor and electrolyte. The calibration plot (Figure 8, inset) of \( \Delta I \) minus the logarithm of the CIP concentration exhibited a sensitivity of −1.521 with the linear equation of \( \Delta I (\mu A) = -1.521 \log C_{\text{CIP}}(\text{M}) + 16.404 \). R² = 0.949. Based on the linear regression equation, the detection limit was determined as 0.09 μM (29.25 ppb) at the response time of 50 s. The LOD was calculated using the 3.3 SD/S, where SD is the standard deviation of the intercept and S is the slope of the calibration plot [12]. Compared with various reports
from MWCNT/GCE [13], Cd(II) on CdS QDs/GCE [14], and CMK-3 Nafion/GE [15], the optimised MIP/rGO/GCE in this study indicated a lower LOD of CIP than most sensors.

4. Conclusion

In this study, a novel MIP/rGO/GCE for the determination of CIP was obtained by electro-polymerisation of aniline and o-phenylenediamine monomers on a surface modified with reduced graphene oxide (rGO). The modified electrode exhibited ideal sensitivity, low detection limits, high stability, and good applicability for the detection of CIP. Despite the known advantages, the device had a few limitations and challenges to be implemented in real and routine analyses. Accordingly, this procedure was an interesting tool, and is expected to be utilized for the development of various pharmaceutical molecular imprinting-based sensors for biological and environmental samples.

Acknowledgements

This research was supported by the National Research Council of Thailand (NRCT) under the Royal Golden Jubilee (RGJ) Ph.D. Program scholarship of the Thailand Research Fund (PHD/0051/2561), and On-site Laboratory Initiative of the Graduate School of Global Environmental Studies, Kyoto University.

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