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A novel strategy to improve the sensitivity of antibiotics determination based on bioelectrocatalysis at molecularly imprinted polymer film electrodes

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ABSTRACT

A new strategy for the sensitive detection of kanamycin (KA) and other antibiotics based on molecularly imprinted polymer (MIP) and bioelectrocatalysis was developed in the present study. The KA-polypyrrole MIP films were electropolymerized on the surface of pyrolytic graphite (PG) electrodes, with pyrrole (PY) serving as the monomer and KA as the template. Because KA is electro-inactive, electroactive $K_3[Fe(CN)_6]$ was used as the probe in the cyclic voltammetric (CV) measurements. The difference of the CV reduction peaks of $K_3[Fe(CN)_6]$ at electrodes between the MIP films after KA removal and KA-rebinding MIP films could thus be used to determine KA quantitatively. When horseradish peroxidase (HRP) and H_2O_2 were added into the testing solution, the detection sensitivity of the system was greatly amplified because the electrochemical reduction of H_2O_2 could be catalyzed by HRP and mediated by $K_3[Fe(CN)_6]$. With the bioelectrocatalysis amplification, the limit of detection (LOD) for KA fell as low as 28 nM, approximately two orders of magnitude lower than that for the MIP films in the absence of enzymatic catalysis. The strategy demonstrated the generality. Not only KA but also other antibiotics, such as oxytetracycline (OTC), could be determined by this method. More significantly, in addition to the $K_3[Fe(CN)_6]$–HRP–H_2O_2 system, other bioelectrocatalysis systems, such as $Fc(COOH)_2$–GOD–glucose (Fc$(COOH)_2$=ferrocenedicarboxylic acid, GOD=glucose oxidase), could also be used to amplify the CV signal and realize the sensitive detection of KA for the MIP film system, thereby illustrating the great potential and prospects of the strategy.

1. Introduction

In recent years, molecularly imprinted polymer (MIP) has received considerable attention in analytical chemistry, primarily because specific recognition sites are formed in the MIP matrix, and excellent selectivity toward the analyte is realized (Ding and Heiden, 2014; Haupt and Mosbach, 2000). Electrochemical sensing demonstrates some advantages over other analytical methods, such as good sensitivity, low cost, simple procedure with the possibility of easy miniaturization and automation in analysis. Thus, the combination of MIP with electrochemical measurement has become a promising method for the detection of various substances (Alegret, 2002; Blanco-López et al., 2004; Piletsky and Turner, 2002; Suryanarayanan et al., 2010). Particularly, the electrochemical polymerization of MIP films on the surface of electrodes shows remarkable merits, such as the simple and tunable preparation procedure and the formation of very thin films that are beneficial to rapid response (Sharma et al., 2012, 2013).

Among different electrochemical sensing methods, voltammetry and amperometry are the most commonly used, and electroactive species are thus necessary. For MIP sensing in this field, if the template or analyte is electroactive, its detection is straightforward and simple. Taking cyclic voltammetry (CV) as an example, after the template molecule is removed from the MIP films on the electrode surface by washing, the rebinding of the analyte from the testing solution to the vacant MIP films leads to the direct observation of the CV response of the analyte (Hu et al., 2012; Li et al., 2012a; Marx et al., 2004; Shoji et al., 2003). However, because the majority of analytes are electro-inactive, an electroactive probe such as $K_3[Fe(CN)_6]$ has to be added to the testing solution. After the template is removed from the MIP films, the vacant films become more permeable to the probe, resulting in the easy diffusion of the probe through the films and the corresponding large CV response at the film electrode. When template-free MIP films are incubated in the analyte solution for rebinding, the analyte-rebinding MIP films become less porous, leading to the difficulty

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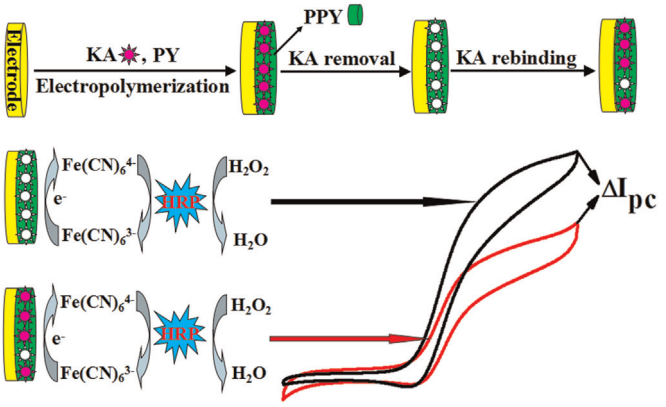
for the probe to pass through the films and a relatively small CV response in the testing solution. The decrease of the CV response could thus be used to indirectly detect the analyte quantitatively. This has been the general strategy for voltammetric and amperometric determination of electro-inactive molecules by MIP film electrodes (Karimian et al., 2013; Liu et al., 2011; Wang et al., 2014).

However, the determination sensitivity of this strategy is often unsatisfactory due to the limited amount of imprinting cavities in the vacant MIP films after template removal. To improve the sensitivity, nanomaterials have been introduced to MIP films in recent years (Guan et al., 2008; Han et al., 2014; Irshad et al., 2013; Xue et al., 2014; Yu et al., 2014). The introduction of nanoparticles not only increases the effective surface area and the corresponding amount of specific recognition sites in MIP films, but also improves the permeability of the films, leading to a higher CV response of the probe at the template-free MIP film electrodes and better detection sensitivity for the analyte. However, this strategy requires the synthesis or assembly of nanomaterials, making the preparation procedure of MIP films more complicated and time-consuming.

In the present work, a novel strategy for CV determination of kanamycin (KA) was developed using MIP film electrodes coupled with bioelectrocatalysis. As an aminoglycoside antibiotic, KA exhibits high activity against various Gram-positive and Gram-negative bacteria (Hardman and Limbird, 2001; Wang et al., 2013), and herein was chosen as the model target analyte. Pyrrole (PY), a widely used functional monomer in MIP fabrication (Kan et al., 2012; Silva et al., 2014; Turco et al., 2015), was electropolymerized into polypyrrole (PPY) films with KA as the template molecule on the surface of pyrolytic graphite (PG) electrodes. $K_3[Fe(CN)_6]$ was used as the electroactive probe in the testing solution, and the CV response of the probe was used to detect KA at the MIP film electrodes. To improve the detection sensitivity, enzymatic reactions were introduced. For the present system, the CV reduction peak current of $K_3[Fe(CN)_6]$ was significantly increased by the addition of HRP and H_2O_2 in the testing solution because the electrochemical reduction of H_2O_2 could be catalyzed by HRP and mediated by $K_3[Fe(CN)_6]$ (Li et al., 2008; Lu et al., 1996; Yao and Hu, 2009). The difference in the CV electrocatalytic reduction peak currents of the system at the electrodes between KA-free MIP films and KA-rebinding MIP films (ΔI_{pc}) was used to determine the KA concentration in the rebinding solution. The procedure and strategy for the CV measurement of KA by the MIP film system coupled with bioelectrocatalysis is illustrated in Scheme 1. While the enzymatic reaction was introduced in the voltammetric determination of some analytes at MIP film electrodes by Li's group to improve the detection sensitivity, the "isolation–incubation–competition" procedure was quite complicated and time-consuming (Li et al., 2010, 2012b). Thus, our novel strategy for the direct amplification of CV response of the electroactive probe by bioelectrocatalysis in the testing solution at MIP film electrodes shows obvious advantages. More importantly, the strategy could be easily extended to other antibiotics and other bioelectrocatalysis systems, indicating its generality and great potential in application.



Scheme 1. Schematic representation of the fabrication process of the MIP films and the simplified strategy of KA determination.

2. Experimental section

2.1. Chemicals

Horseradish peroxidase (HRP, E.C. 1.11.1.7, type II, MW ≈ 44,000, 250,000 units g^{-1}), glucose oxidase (GOD, E.C. 1.1.3.4, type VII, MW ≈ 160,000, 192 units g^{-1}) and 1,1′-ferrocenedicarboxylic acid ($Fc(COOH)_2$) were purchased from Sigma-Aldrich. Kanamycin sulfate (KA), oxytetracycline (OTC), pyrrole (PY), streptomycin sulfate, chloramphenicol and metronidazole were obtained from Aladdin. Hydrogen peroxide (H_2O_2, 30%), potassium ferricyanide ($K_3[Fe(CN)_6]$) and potassium ferrocyanide ($K_4[Fe(CN)_6]$) were obtained from Beijing Chemical Engineering. Glucose was obtained from Beijing Yili Fine Chemicals. All other reagents were of analytical grade and were used without further purification. Solutions were prepared with ultrapure water from a Millipore water purification system (18.2 MΩ cm).

2.2. Apparatus

All electrochemical measurements were performed with a CHI 660A electrochemical workstation (CH Instruments). A three-electrode system was used, where a platinum flake and a saturated calomel electrode (SCE) were used as the counter and reference electrodes, respectively, and a modified basal plane pyrolytic graphite (PG, Momentive Performance Materials) disk electrode (0.16 cm^2) was used as the working electrode. Electrochemical impedance spectroscopy (EIS) experiments were performed over a frequency range from 0.1 to 10^5 Hz in 5 mM $Fe(CN)_6^{4-/3-}$ (1:1, containing 0.1 M NaCl) solution at 0.17 V.

The scanning electron microscopic (SEM) characterization of the MIP films electrode was performed using an S-4800 scanning electron microscope (Hitachi). For SEM samples, the MIP films were first prepared on PG electrode with the same method as for the electrochemical experiments, and then the thin piece of PG with MIP films was cut off by a razor blade before tests. Thin platinum films were coated on the sample surface with an E-1045 sputtering coater (Hitachi) before SEM measurements.

Fourier transform infrared spectra (FTIR) were obtained using a Nicolet 380 FTIR spectrophotometer (Nicolet) at a resolution of 4 cm^{-1}.

2.3. Preparation of MIP film electrodes and KA measurement procedure

The MIP films were electropolymerized on the surface of the PG electrodes. Prior to deposition, the PG electrode was polished with 320 grit metallographic sandpaper and then ultrasonicated in water for 30 s. According to the previous method (Lian et al., 2012) with some modification, CV was performed at the PG electrodes in pH 6.8 phosphate buffer solutions containing 10 mM KA and 30 mM PY between −0.4 and 1.5 V at 0.05 V s^{-1} for 5 cycles after optimization. The PPY films with the entrapped template KA were formed on the surface of the PG electrodes and were designated as MIP or KA-PPY MIP films.

The MIP film electrode was dried at room temperature and