Emodin Voltammetric Sensor Based on Molecularly Imprinted Polymer Membrane-Modified Electrode Using a Multiple Hydrogen Bonds Strategy

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ABSTRACT: Similar to those in complementary nucleotides' base pairs, we present a novel molecularly imprinted electrochemical sensor for emodin, constructed using a multiple hydrogen bonds strategy. We obtained the sensor by *in situ* photopolymerization, using allobarbital as a new functional monomer. We optimized the conditions of membrane imprinting and the composition of adsorption solvent. This artificial receptor exhibits high selectivity for the template in comparison with closely related analogs, aloin A and simetryne. The sensor was successfully applied in determination of emodin levels in one of the traditional Chinese medicines, the content of emodin in Sanhuang tablets detected using the voltammetric sensor and high performance liquid chromatography (HPLC) were 0.249 ± 0.009 (mg/tablet) and 0.246 ± 0.007 (mg/tablet), respectively. © 2012 Wiley Periodicals, Inc. J Appl Polym Sci 000: 000–000, 2012

Key words: emodin; electrochemistry; molecular imprinting; multiple hydrogen bonds; thin films

INTRODUCTION

Emodin is the main biologically active ingredient of some traditional Chinese medicines, such as herbal preparations of *Polygonum cuspidatum* and *Polygonum multiflorum*, and Sanhuang tablets (a Chinese patent medicine). It has attracted considerable interest because of its antimicrobial, immunosuppressive, laxative, anti-inflammatory, and antineoplastic activities.[1] Emodin is usually detected using thin-layer scanning method,[2] capillary electrophoresis with amperometric detection,[3] high performance thin layer chromatography[4,5] or near-infrared surface-enhanced Raman spectroscopic methods.[6] Most of these methods require pretreatment, several separation steps, and the use of elaborate instrumentation. Emodin can be chemically classified as an anthraquinone derivative with electroactivity and can be also investigated using electrochemical methods,[7,8] with their advantages of low-cost, speed, and simplicity. The electrode process dynamics of emodin has been studied by Ye and coworkers.[8] However, to our knowledge, there are no previous reports on electrochemical emodin sensors constructed using molecular imprinting technology (MIT).

Molecular imprinting is a general method for preparing artificial recognition elements. Molecularly imprinted polymers (MIPs) have a prearranged structure and specific molecular recognition ability.[9–11] They also exhibit very high thermal and chemical stability and can be used in aggressive media.[12] Moreover, their selectivity is as good as that of biological receptors.[13] These useful features of MIPs have attracted a lot of attention and make them ideal candidates for the recognition elements of biomimetic sensors.[14–17] Molecular recognition of MIPs depends mainly on the weak noncovalent interactions between host and guest; the functional monomer plays a crucial role in constructing MIPs with high affinity and specificity toward the template molecule.[18] Acrylamide, methacrylic acid, and 4-vinyl pyridine are most widely used as functional monomers in development of MIT. Wu et al.[19] reported molecularly imprinted microspheres (MIMs) based on a biologically inspired hydrogen-bond array were prepared using allobarbital as the novel functional monomer and simetryne as the template. In the work, authors evaluated the MIMs by using HPLC and solid-phase extraction; furthermore, they used the MIMs as a sorbent for selective extraction of simetryne from corn and soil samples by molecularly imprinted solid phase extraction.

In this study, we develop an electrochemical sensor for recognition of emodin by creating a membrane layer of MIPs on the surface of a glassy carbon electrode (GCE). Taking into account the chemical structure of emodin, we selected allobarbital as a

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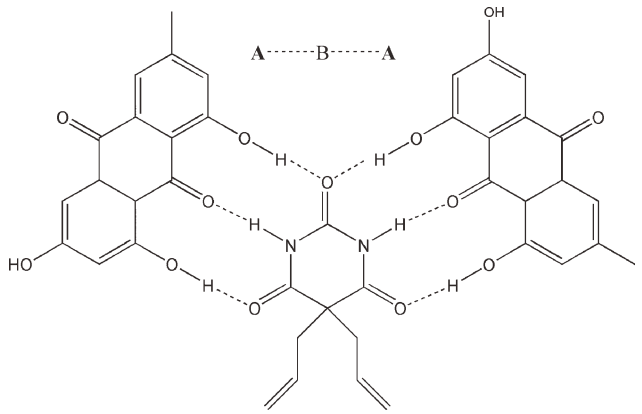


Figure 1 The prearrangement between emodin (A) and allylbarbital (B).

novel functional monomer. The multiple hydrogen bonds,[20,21] similar to those in complementary nucleotides' base pairs, are possibly formed between emodin and allobarbital (Fig. 1), giving an electrode with emodin-imprinted polymer membrane.

EXPERIMENTAL

Reagents

Emodin, rhein, and aloin A (purity > 98%) were purchased from Xi'an Acetar Bio-Tech (Shanxi, China). Simetryne was kindly provided by Bingzhou Pesticide Plant (Shandong, China). Ethylene glycol dimethacrylate (EDMA) was purchased from Shanghu (Shanghai, China) and was distilled before use to remove any stabilizers. 2,2′-Azobisisobutyronitrile (AIBN) came from Sihewei (Shanghai, China). Disodium hydrogen phosphate, monopotassium phosphate, sodium chloride, and ethanol were of analytical grade and came from Guangdong Guanghua Chemical (Guangdong, China). Chloroform and hydrochloric acid (AR) came from Guangzhou Chemical Reagent Factory (Guangdong, China). Sanhuang tablets were purchased from Jianmin Pharmaceutical (Shandong, China). We used redistilled water throughout.

The synthesis and characterization of allobarbital were performed according to Ref. 22. The product's characteristics were mp: 173–174°C; IR peak (KBr) (cm^{-1}): 3205, 3094, 2930, 2862, 1702; ^{1}H-NMR ($CDCl_3$) (ppm): δ 7.96 (s, 2H), 5.58–5.68 (m, 2H), 5.15–5.22 (m, 4H), 2.74 (d, 4H, 7.6 Hz,); ^{13}C-NMR (ppm): 42, 57, 121, 130, 148, 171.

Apparatus

The cyclic voltammetric (CV) and differential pulse voltammetric (DPV) experiments were performed using a CHI660 electrochemical workstation (Chenhua, Shanghai, China) coupled with a conventional three-electrode cell. The MIP-modified GCE (d = 3 mm) acted as the working electrode. A saturated calomel electrode and a platinum electrode were used as the reference and auxiliary electrode, respectively. The surface of the modified electrode was examined using a scanning electron microscope (SEM) (S-520, Hitachi, and Tokyo, Japan). We used the HPLC system using a Shimadzu LC-10AD pump; Shimadzu SPD-10A UV/vis detector was produced by Shimadzu Company (Kyoto, Japan).

Preparation of MIPs-modified electrode

Before modification, the surface of the GCE was polished with aqueous alumina slurries with gradually decreasing particle size (1–0.3 mm), and then immersed for 5 min in solution of nitric acid and water (1 : 1, v/v), ammonia and water (1 : 1, v/v), and ethanol and water (1 : 1, v/v). This procedure was followed by 10-min ultrasonic treatment in redistilled water.

A series of molecularly imprinted membranes (MIP1–MIP5) was prepared in porogen solvents, using the reagent amounts presented in Table I. The typical experimental procedure of *in situ* MIP polymerization on the electrode surface was previously reported.[24] Emodin and allobarbital were dissolved in 4 mL of chloroform–ethanol mixture (1 : 1, v/v). To ensure sufficient template-functional monomer

TABLE I
Results of Emodin Analysis in Real Samples

	Voltammetric sensor method[a]	HPLC method[b]	Reported method[23]
Detection result (mg/tablet)	0.249 ± 0.009	0.246 ± 0.007	0.231
Average recovery (%)	95.3%	–	–

[a] Voltammetric sensor method: the measurement was carried out in phosphoric buffer solution (pH 5.7) after the M-electrode was kept in the samples for 4 min. DPV potential was swept from −0.5 to 0.2 V.

[b] HPLC conditions: mobile phases: methanol and 1% phosphoric acid (8 : 2, v/v); flow-rate 1 mL/min. The amounts of analytes were determined at 254 nm; HPLC column: Hypersil ODS, 5 μm, 250 × 4.6 mm^2 I.D.; Injection volume: 5 μL. Three replicates were assayed.

interaction, the solution was shaken at room temperature for 4 h, after which 0.80 mmol of EDMA crosslinker was added. After degassing with N_2 for 5 min, AIBN (0.010 g) was added as an initiator and a 2-μL aliquot of the mixture was dropped onto the electrode surface. The electrode was left to undergo membrane polymerization for 48 h under N_2 atmosphere and UV radiation ($\lambda = 365$ nm), at 4°C. The control N-electrode (nonimprinted polymer, NIP) was prepared following the same procedure but without emodin template.

Measurements and evaluation

The template was extracted from the polymeric membrane by immersing the electrode in ethanol for 20 min. The complete extraction was verified by the disappearance of emodin redox signals recorded between −1.3 and 0 V; then the electrode was transferred to 1.0mL of 1×10^{-4} mol/L solution of analyte and left for 4 min. To remove the weakly adsorbed molecules, the modified electrode was washed carefully with redistilled water before transferring to the electrochemical cell. The measurements were carried out in aqueous phosphoric buffer (pH 5.7), at scan rate of 100 mV s^{-1}. The modified electrode could be reused after washing in ethanol. Imprinted factor was defined as: $IF = i_{M\text{-electrode}}/i_{N\text{-electrode}}$, $i_{M\text{-electrode}}$ and $i_{N\text{-electrode}}$ are M-electrode (MIP)and N-electrode emodin cathodic peak current (P_1) at −0.583 V, respectively. All measurements were replicated four times.

Sample preparation

The sample preparation process can be briefly described as follows: three Sanhuang tablets (1.4240 g) were crushed to obtain grains smaller than 80 μm. The sample powder (1.0 g) was extracted ultrasonically, with 20 mL of ethanol, for 20 min. The extraction procedure was repeated three times. The extracts were combined and filtered, then diluted to 250 mL with ethanol. Aliquots (0.000, 0.070, and 0.140 mg) of emodin were added to three volumetric flasks, each containing 25 mL of the extract solution, and then diluted to 50 mL with chloroform, for future examination. All measurements were performed three times.

RESULTS AND DISCUSSION

Electrochemical behavior of emodin on electrodes

The electrochemical behavior of different electrodes was investigated by cyclic voltammetry. A pair of quasi-reversible reduction–oxidation peaks (P_1 and P_2) of emodin were found (Fig. 2), with peak potentials of −0.594V and −0.536V for bare GCE, and an irreversible oxidation peak (P_3) at peak potential of −0.185V.

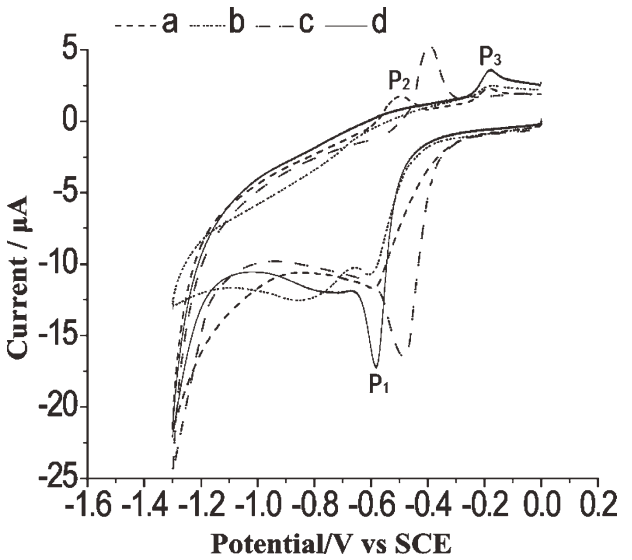


Figure 2 Cyclic voltammograms obtained after bare GCE (a) and N-electrode (b) and MIP2-electrode (d) were incubated in 1×10^{-4} mol/L of emodin in chloroform–ethanol mixture (1 : 1, v/v) ; MIP2-electrode (c) incubated in 1×10^{-4} mol/L of rhein in chloroform–ethanol mixture (1 : 1, v/v); CV potential sweep from −1.3 to 0 V.

For the MIP2 membrane-modified electrode (MIP2-electrode), the reductive peak (P_1) appeared at −0.583 V with the peak current of 1.48×10^{-5}A, about 2.9 times larger than that for bare GCE. The oxidative peak (P_3) was found at −0.179 V, with the peak current of 1.66×10^{-6} A, about 2.1 times larger than for unmodified GCE. It is clear that the electrochemical response to emodin on MIP2-electrode is much stronger than that for bare GCE and that its oxidative peak (P_2) disappears. These preliminary results demonstrate a significant imprinting effect on MIP2-electrode. P_1, with its strong emodin signal, was used in the subsequent study of the electrochemical performance of M-electrodes.

Optimization of the polymer composition

The highly crosslinked MIPs membrane grafted on the electrode surface is a nonconductive polymer containing numerous recognition sites. The analyte detection is possible if the pore size and pore density in MIPs membrane is appropriate for the continuous reaction with analyte diffusing to electrode surface. Moreover, the concentration of recognition sites is related to the molar ratios of template, crosslinker, and functional monomer on the MIPs membrane.[13]

To find the optimal required quantity of the template, we prepared several different M-electrodes. The adsorption current of the modified electrode

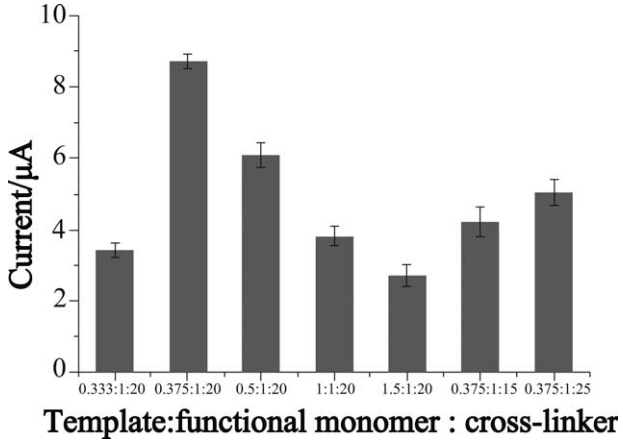


Figure 3 Adsorption current of modified electrodes in different molar ratio of template, functional monomer, and crosslinker; DPV was swept from −1.3 to −0.1 V.

was the highest when the molar ratio of emodin and allobarbital was 0.375 : 1 (Fig. 3). However, the shape, size, rigidity, and number of pores in MIPs membranes are related to the molar ratio of functional monomer and crosslinker; this can affect the specific adsorption of M-electrodes.[14] Therefore, we investigated the effect of the relative amounts of crosslinker and monomer. Several different molar ratios of functional monomer and crosslinker (1 : 15, 1 : 20, and 1 : 25) were chosen for testing. We found that with the increase of functional monomer to crosslinker ratios, the adsorption current of M-electrodes increased at first and then decreased (Fig. 3). The optimal molar ratio of template, functional monomer, and crosslinker was 0.375 : 1 : 20. MIP2-electrode, prepared using this ratio, was chosen for the subsequent experiments.

Surface characterization of MIP-electrode

SEM images of the surface of MIP2-electrode are shown in Figure 4. A great number of micropores were distributed evenly on the MIPs membrane of the electrode. Each large micropore was surrounded by a lot of smaller micropores; these were formed during polymerization in the presence of porogen. We inferred that these micropores were the channels connecting the electrode surface to the exterior environment, constructing a network of recognition sites in the membrane.

Optimization of the solvent for adsorption

Polymer morphology is related to the uptake of organic solvent diffusing into the polymer and causing it to swell. Nonoptimal uptake could result in deformed recognition cavities and weak hydrogen bonding between host and guest. The relationship between the adsorption currents of modified electrodes and the type of adsorption solvent is shown in Figure 5. The MIP2-electrode had high specific adsorption, and the highest imprinting factor (IF = 7.9) was obtained using chloroform and ethanol mixture (1 : 1, v/v) as solvent. Therefore, this mixture was chosen as adsorption solvent for further experiments. The results in Figure 5 demonstrate that the adsorption solvent chosen for recognition

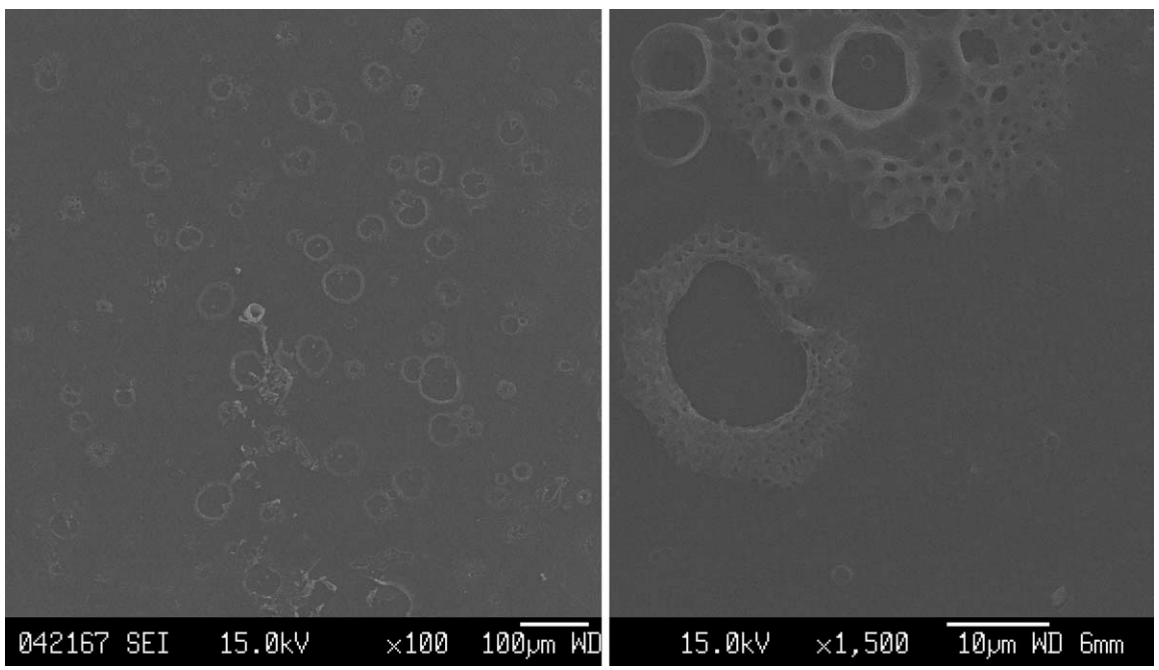


Figure 4 SEM images of the surfaces of modified electrode.

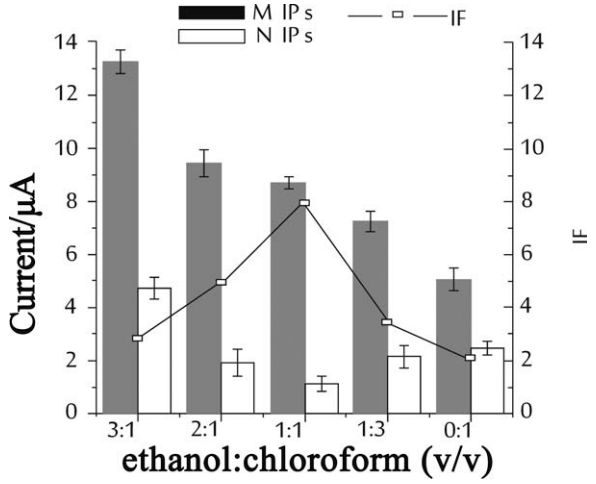


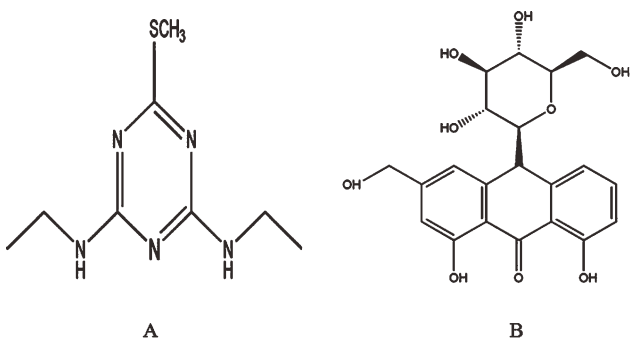
Figure 5 Adsorption current and IF of modified electrodes in different solvent mixtures; DPV potential was swept from −1.3 to −0.1 V.

procedure should be appropriate for the porogen, avoiding MIPs swelling and nonspecific adsorption.

The equilibrium time for emodin adsorption from the solution was evaluated by observing the changes in peak current until it was stable; 4-min period was sufficient for emodin to diffuse and bind to the MIP2 membrane.

Selectivity

The existing research shows clearly that as a result of the imprinting the template molecules pass freely through the polymer membrane of M-electrode. Both simetryne and aloin A are structurally similar to emodin (Fig. 6), and can form complexes with allobarbital, using multiple hydrogen bonds. Therefore, these molecules were chosen to investigate the selectivity of MIP2-electrode. We chose 0.1 mol/L HCl and 10 g/L NaCl (1 : 3, v/v) as the supporting electrolytes for measurements of aloin A. i_M, i_N, and i_O represent the peak currents of MIP2-electrode, N-electrode, and bare GCE, respectively. The selectivity factor ($\alpha_1 = i_{M\ \text{emodin}}/i_{M\ \text{aloin A}}$) was used for evaluating molecular recognition ability of MIP2-electrode



A B

Figure 6 The structure of simetryne (A) and aloin A (B).

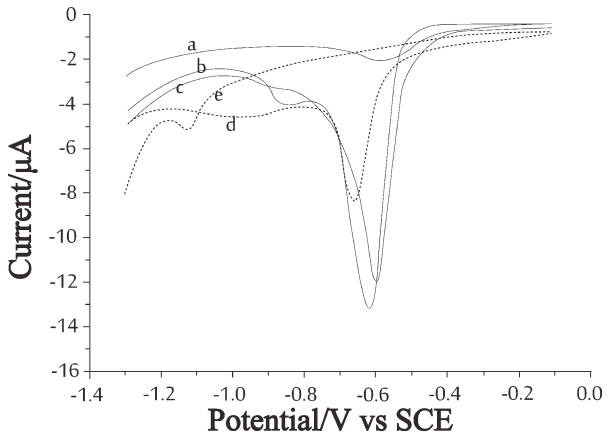


Figure 7 DPV voltammograms obtained after N-electrode (a) and MIP2-electrode (b) were incubated in 1 × 10^{-4} mol/L of emodin in chloroform–ethanol mixture (1 : 1, v/v), MIP2-electrode (c) incubated in 1 × 10^{-4} mol/L of emodin/simetryne of mixture in chloroform–ethanol mixture (1 : 1, v/v), measure media, phosphate buffer solution (pH 5.7) (B); MIP2-electrode incubated in (d) 1 × 10^{-4} mol/L of emodin in chloroform–ethanol mixture (1 : 1, v/v), and (e) 1 × 10^{-4} mol/L of aloin A in chloroform–ethanol mixture (1 : 1, v/v), measure media, 0.1 mol/L HCl and 10 g/L NaCl mixture (1 : 3, v/v) (A). DPV potential sweep from −1.3 to −0.1 V.

for these chemicals, where $i_{M\ \text{emodin}}$ and $i_{M\ \text{aloin A}}$ are the current responses of the electrode to emodin and aloin A, respectively. As there was no electrochemical response to simetryne on GCE, the competitive adsorption of emodin and simetryne on MIP2 membrane was used. The selectivity factor ($\alpha_2 = i_{M\ \text{emodin}}/(i_{M\ \text{emodin}} - i_{M\ \text{mixture}})$) was used, where $i_{M\ \text{mixture}}$ was the current responses of MIP2-electrode in emodin and simetryne mixture.

The current responses of the three electrodes (MIP2, N, and GCE) to various analytes are shown in Table II. The current response to emodin was the highest for MIP2 electrode (i_M), with lower values for GCE and N-electrode ($i_M > i_O > i_N$). The MIP2-electrode shows good affinity and enrichment ability. The highest response to aloin A was found for GCE (with $i_O > i_M > i_N$), which clearly indicates a size exclusion effect. The selectivity factor (α_1) was 5.8. Emodin was preferentially adsorbed onto MIP2-electrode from emodin and simetryne mixture; the selectivity factor (α_2) was 7.5. This demonstrates that the molecular structure of simetryne does not match the binding cavity. MIP2-electrode is the only one with high affinity to emodin.

Binding isotherms and reproducibility

The current intensities of MIP2-electrode and N-electrode were nonlinear in the range of 0.01–5 mmol/L (Fig. 8). The equilibrium dissociation constants [K_D, eq. (1)] for emodin binding to the electrodes were

TABLE II
Template and Monomer Ratio

	MIP1	MIP2	MIP3	MIP4	MIP5	NIP[a]
Emodin (mmol)	0.013	0.015	0.020	0.040	0.060	0.000
Allobarbital (mmol)	0.040	0.040	0.040	0.040	0.040	0.040
EDMA (mmol)	0.80	0.80	0.80	0.80	0.80	0.80

[a] The nonimprinted polymer (NIP) was obtained following the MIP procedure in the absence of the template.

estimated by Scatchard plot analysis of binding data.[25] In the Scatchard analysis, the experimental binding was plotted in B/F versus B format; *B* and *F* correspond to the concentration of bound and free emodin. In this case, the concentration of bound emodin, *B*, was directly proportional to the peak current *I*, and the concentration of free emodin was almost the same as the original concentration *C*. Thus, we can define:

$$B_{\text{bound}/C} = (B_{\max} - B_{\text{bound}})/K_D \quad (1)$$
$$B = K * I \text{ and } B_{\max} = K * I_{\max}.$$

The experimental binding isotherm can be replotted in *I/C* versus *C* format (Fig. 9).

$$I/C = -I/K_D + I_{\max}/K_D \quad (2)$$

These results indicate that the MIPs membrane contains only one homogeneous population of binding sites with specific adsorption for emodin. We speculated that this type of binding site might be related to the multiple hydrogen bonds between the host and guest. The K_D for MIP2-electrode was 0.09 mmol/L. For the N-electrode, the Scatchard plot also fell onto a straight line, but we believe that the only type of binding site in NIPs membrane is formed with the functional monomer distributed randomly, because of the absence of template molecule in the polymerization process. Thus, the membrane shows nonspecific, low adsorbability for emodin with a high K_D value (0.24 mmol/L).

The reproducibility and the stability of the sensor were studied by repeating the incubation-measure cycle with MIP2-electrode. The relative standard deviation of 2.4% for 1×10^{-4} mol/L emodin concentration ($n = 6$) demonstrates very good reproducibility. MIP2-electrode has been through an ongoing test for 7 days. Compared with the medium value of the first six times, the intensity of the current was dropped by about 5% after we reused this sensor about 60 times.

Analytical application of MIP-electrode

Under optimized conditions, the peak current shows linear dependence on emodin concentration in the range of 5×10^{-6} mol/L to 2×10^{-4} mol/L, with detection limit of 7.4×10^{-7} mol/L (S/N = 3). The linear regression equation is $Y = 0.684 + 26.73X$ and correlation coefficient $R = 0.998$.

Emodin, aloe-emodin, rhein, chrysophanol, and physcion, all of which possess 9,10-anthraquinone structure, are the major biologically active ingredients of Sanhuang tablets.[23] The anthraquinone ring of emodin has a 3-hydroxyl substituent. This is

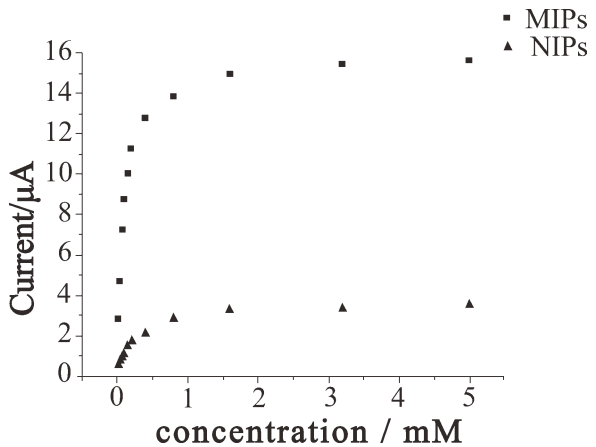


Figure 8 Adsorption isotherms of emodin on MIP2-electrode and N-electrode; DPV potential sweep from −1.3 to −0.1 V. The measurements were carried out in phosphate buffer (pH 5.7).

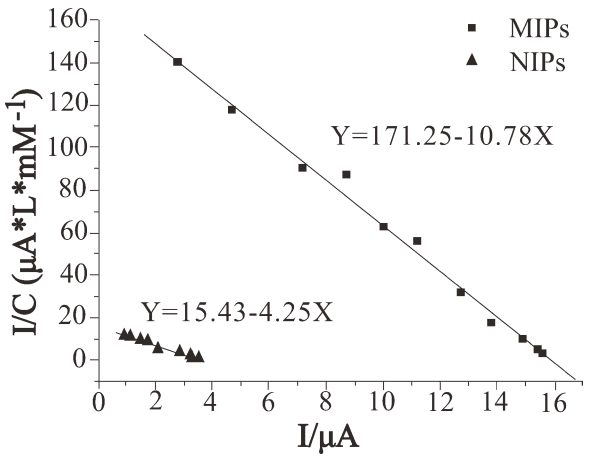


Figure 9 Scatchard plot for MIP2-electrode and N-electrode.

TABLE III
Comparison of Peak Currents Measured with Different Electrodes[a]

Analytes	i_M (μA)	i_N (μA)	i_O (μA)
Emodin (A)	5.13	0.61	2.43
Aloin A (A)	0.88	0.58	2.20
Emodin (B)	8.72	1.10	3.00
Emodin/simetryne of mixture (B)	7.56	0.99	2.91

i_M, response values for MIP2 electrode; i_N, for N-electrode; i_O, for BGE.

[a] The measurements were carried out in 0.1 mol/L HCl and 10 g/L NaCl mixture (1 : 3, v/v) (A) and phosphate buffer solution (pH 5.7) (B). Analyte concentration was 1×10^{-4} mol/L. DPV potential sweep from −1.3 to −0.1 V.

absent in the remaining chemicals of that group; they are not emodin's isomers. The CV curves of rhein and emodin are shown in Figure 2; emodin has the peak P_3, missing in the case of rhein. To avoid interference, the typical oxidation peak of emodin, P_3, was chosen for establishing analytical method for emodin.[8] The voltammetric sensor was used to determine the amount of emodin in Sanhuang tablet samples, and the results were compared to those obtained using the classical HPLC method. The results shown in Table III were obtained applying standard addition method. The concentrations of emodin detected using the voltammetric sensor and HPLC were 0.249 ± 0.009 (mg/tablet) and 0.246 ± 0.007 (mg/tablet), respectively. The enriching effect of the sensor made it exhibit good analytical feature in complex samples such as traditional Chinese medicine.

CONCLUSIONS

We developed a novel MIPs membrane-modified electrode using multiple hydrogen bonds strategy, with allobarbital as the new functional monomer and emodin as the template. We found only one type of binding site in the membrane, with high adsorbability and low K_D value. We believe this is related to the multiple hydrogen bonds between the template and functional monomer. The MIPs electrode (unlike the control devices, N, and bare GC-electrode) shows higher affinity and selectivity for the template molecule than for some closely related analogues. We used this new electrochemical sensor to determine the amount of emodin in complex samples (Sanhuang tablets). The results matched those obtained by HPLC method. The determination of emodin concentration using this electrode was accurate, sensitive, and reliable. Such MIPs membrane-modified electrodes are very promising as potential elements of new, highly selective analytical sensors.

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