

Microspheres Sensor Based on Molecularly Imprinted Polymer Synthesized by Precipitation Polymerization

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A new biomimetic bulk acoustic wave sensor based on molecularly imprinted microspheres (MIM) technique was described. The sensing materials were synthesized by precipitation polymerization. By using the Scatchard analysis, the equilibrium dissociation constant K_D and the apparent maximum number Q_{max} of the binding sites were calculated to be $3.70 \text{ mmol} \cdot \text{L}^{-1}$ and $9.11 \mu\text{mol} \cdot \text{g}^{-1}$, respectively. The sensor exhibited a sensitive response to the template compound (dipyridamole) in liquid phase with a detection limit of $2 \times 10^{-9} \text{ mol} \cdot \text{L}^{-1}$. The recoveries of the sensor were 95.1%—105.4%. Studies presented in this paper show that the stability of this sensor is excellent. The sensor has been applied successfully to the determination of dipyridamole in human urine.

Keywords microspheres sensor, molecularly imprinted polymer, dipyridamole, precipitation polymerization

Introduction

Since the piezoelectric bulk acoustic wave (BAW) sensors were applied in liquid phase in the 1980s, many papers have been reported.^{1,2} However, the application of BAW sensors based on mass effect is limited because of lack of the special selectivity to the analyte. Various methods have been proposed to solve this problem, especially the application of the biomaterials.³ Unfortunately, the result was not so good as expected, due to the instinct disadvantage of the biomaterials, *e. g.*, poor stability, short life span, although possessing high selectivity. Therefore, it is essential to research for stable, facile and highly selective materials as the active sensing components for BAW sensors.

Imprinting polymer technique is a powerful tool for preparing recognizing materials. During the past two decades, rapid developments have been made in the field of preparing molecularly imprinted polymers (MIPs). Most methods for preparation of MIPs include three steps: firstly, mixing and interacting the functional monomers with the imprinted molecules; secondly, copolymerizing the cross-linkers with the functional monomers; thirdly, removing the template from the imprinted polymers. However, these methods for preparation of MIPs are time-consuming in the course of the grinding/sieving and the resulted MIPs might be configuration-ir-

regular. Recently, new methods for preparation of MIPs have been proposed, involving normal suspension polymerization,⁴ suspension polymerization in perfluorocarbon,⁵ seed polymerization, graft polymerization, polymerization of reactive surfactants,⁶ precipitation polymerization⁷, *etc.* Among these techniques, the precipitation polymerization is advantageous as it is easy to handle.

With the physical robustness, mechanical strength, durability to heat and pressure, high affinity and selectivity, molecularly imprinted polymers have been widely applied in the fields of solid-phase extraction,⁸ pharmaceutical analysis,⁹ separation in high-performance liquid (HPLC),¹⁰ capillary electrophoresis (CEC),¹¹ thin-layer chromatography (TLC),¹² capillary electrophoresis (CE)¹³ and chemical sensing.^{14,15} During the last few years, a big boost in the use of mass-sensitive acoustic transducers based on the MIPs has been observed, such as surface-acoustic wave (SAW) oscillator,¹⁶ the Love-wave oscillator¹⁷ and the quartz crystal microbalance (QCM).¹⁸ However, application MIM-BAW sensor in liquid phase has not been reported in the literature. In this paper, we attempted to fabricate a highly selective and sensitive BAW sensor based on molecularly imprinted microspheres (MIM). Dipyridamole, 2, 2', 2'', 2'''-(4-8-dipiperidinopyrimido [5,4-*d*] pyrimidine-2, 6-diylidinitrilo) tetraethanol, was used as template. The MIPs were characterized by using TEM and the Scatchard analysis. Influencing factors such as ethanol, pH value and the weight ratio of polyvinyl chloride (PVC) to MIM were investigated. The sensors, which were modified by PVC, were also successfully applied to the determination of dipyridamole in water and urine, and the results were satisfactory.

Experimental

Materials

Ethylene glycol dimethacrylate (EDMA) was purchased from Sigma (St. Louis, MO). Methacrylic acid (MAA),

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Received July 11, 2002; revised November 27, 2002; accepted January 1, 2003.

Project supported by the National Natural Science Foundation of China (Nos. 20075007 and 20275009).

azobisisobutyronitrile (AIBN), PVC powders, chloroform and tetrahydrofuran (THF) were obtained from Shanghai No. 2 Chemical Reagent Co. Dipyrindamole was purchased from Shanghai No. 6 Medical Reagent Co. All reagents were of analytical grade except for AIBN, which was of chemical purity grade. MAA and EDMA were purified by distillation prior to the use. Double-distilled water was used throughout the experiment.

Apparatus

A 9 MHz piezoelectric quartz crystal (12.5 mm in diameter) with silver electrodes (6 mm in diameter) on both sides was used in this experiment. The schematic diagram of the detection system employed was the same as previous reported.¹⁹ The quartz crystal was fixed to a silicone rubber tube with HZ-704 silicone adhesive. Only one side of the quartz crystal, with coated membrane, was allowed to contact the solution directly. The sensor was connected to a home-made oscillating circuit supplied by a JWY-30B dc voltage regulator that was set at 4 V. A model SS7200 universal frequency counter was employed to measure the frequency. The temperature of the sample solution was kept at 25 °C using a thermostat.

Synthesis of molecularly imprinted microspheres

The molecularly imprinted microspheres were prepared using precipitation polymerization technique. 0.25 mmol (0.125 g) of dipyrindamole was dissolved in 50 mL of chloroform containing 1 mmol (0.086 g) of MAA by sonication (SB5200, Shanghai Branson) for 10 min at 60 °C in a 100 mL of volumetric flask. Then 10 mmol (0.994 g) of EDMA and 0.1 mmol of AIBN were added. After being purged with nitrogen gas for 15 min, the flask was sealed under nitrogen gas and kept in thermostated water bath (60 °C) overnight. The polymer microspheres were obtained by centrifugation at 8000 r/min for 20 min. Then the template was removed from the polymer microspheres by repetitive rinsing with ethanol-acetic acid (9:1, V:V) for three times and then with ethanol until the template molecule could no longer be detected under UV ($\lambda_{\max} = 286 \text{ nm}$). Finally, the polymer microspheres were suspended in acetone and allowed to settle for 4 h. The fine polymer microspheres, obtained from the suspensions by centrifuging at 8000 r/min, were dried under vacuum.

Non-imprinted microspheres (NIM) were prepared in the same way as described above except that no template molecule was added in the polymerization stage.

Preparation of the BAW sensor

The surface modification of the piezoelectric quartz crystal (PQC) was carried out as follows: 25 mg of the fine polymer microspheres prepared by the aforementioned method were suspended in 20 mL of tetrahydrofuran which contained 10 mg of PVC powders. About 10 μL of this suspension was spread over the silver electrode surface of the PQC. After slow evap-

oration of tetrahydrofuran at room temperature, MIM were coated on the BAW sensor surface. This procedure was repeated 2 or 3 times to get a proper polymer coating on the sensor surface. Another reference sensor was fabricated in the same way with NIM. All the sensors were stored in a desiccator when they were not in use.

Procedure

Before the determination of dipyrindamole, the BAW sensor was immersed into 10 mL of double-distilled water at 25 °C and a steady resonant frequency (f_1 , frequency shift < 3 Hz in 5 min) was recorded. Then, a standard solution was added into it and the frequency (f_2) was recorded. The frequency shift for each solution was calculated as $\Delta f = f_2 - f_1$. After determination, dipyrindamole was removed from the coating with a series of sequential washes, including the three washes with ethanol-acetic acid (9:1, V:V), then two washes with ethanol, and finally three washes with water.

Scatchard analysis

The washed microspheres (10 mg) were placed in a 10 mL of conical flask and mixed with dipyrindamole standard solution. The concentrations of the standard solutions varied from 0 to 4.5 mmol·L⁻¹. After incubation for 10 h at 25 °C, the mixture was transferred into a centrifuge tube and centrifuged for 10 min at 6000 r/min. Then the supernatant was analyzed using a spectrophotometer at 286 nm. The amount of dipyrindamole bound to the microspheres was calculated by subtracting the concentration of free dipyrindamole from its initial concentration.

Results and discussion

Affinity characterization of microspheres

Figs. 1a and b show that the collected microspheres possess similar size and spherical appearance under the polymerization condition. The average diameter of the microspheres is about 0.2 μm . In order to estimate the binding parameters of dipyrindamole microspheres, Scatchard analysis was used.²⁸ Scatchard equation is as follows:

$$Q/[Dy] = (Q_{\max} - Q)/K_D$$

Where Q is the amount of dipyrindamole bound to the microspheres, Q_{\max} is the apparent maximum number of binding sites, $[Dy]$ is dipyrindamole concentration at equilibrium and K_D is the equilibrium dissociation constant. At first, microspheres-binding isotherm in the aqueous system was determined by UV-vis spectrophotometry in the range of 0–4.5 mmol·L⁻¹ initial concentration of dipyrindamole. The result is shown in Fig. 2a, where the amount of dipyrindamole bound to the imprinted polymer is plotted against varied initial concentration of dipyrindamole. Then, the obtained binding data were

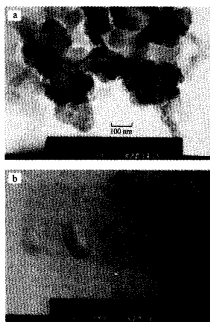


Fig. 1 TEM of the molecularly imprinted microspheres in clusters (a) and separated particles (b) under 100000 \times magnification.

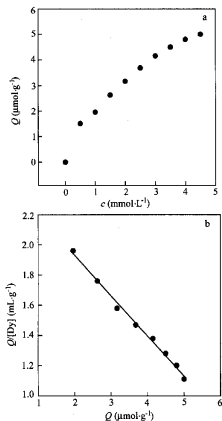


Fig. 2 Binding isotherm of the dipyrindamole (a) and Scatchard plot to estimate the affinity nature of the imprinted polymer (b).

plotted to estimate the dissociation constant and the apparent maximum number of binding sites according to the Scatchard equation. A straight line is shown in Fig. 2b. It indicates that the binding sites of MIM are homogeneous with respect to the affinity for dipyrindamole.²⁸ The Scatchard regression equation is:

$$Q/[Dy] = 2.46 - 0.27 \times Q \quad (r = 0.994)$$

In Fig. 2b, the slope and intercept are equal to $-1/K_D$ and Q_{max}/K_D , respectively. Hence, from the slope and intercept of Scatchard plot, the equilibrium dissociation constant K_D and the apparent maximum number Q_{max} of the binding sites can be calculated to be $3.70 \text{ mmol} \cdot \text{L}^{-1}$ and $9.11 \text{ } \mu\text{mol} \cdot \text{g}^{-1}$ of dry microspheres, respectively.

Sensor performance

In order to avoid the influence of the membrane thickness, the same sensor was used repeatedly throughout experiment. The frequency of this sensor remained stable after being immersed into double-distilled water over a stabilization period of about 20 min. A series of standard solution in an increasing concentration order was injected and frequency response of the sensor was recorded. The result is given in Fig. 3. To investigate the influence of the amount of the action MIM material on the response of sensor, different amounts of MIM were applied to the sensor surface, and the frequency shifts were recorded and used to represent the coating amounts. No significant difference in the sensor response was observed when the coating amount expressed by frequency change was in the range of 8.0–8.3 kHz (Fig. 4). The relations in the range of the frequency shift of the three representative sensors and the dipyrindamole concentration are also shown in Fig. 4. Linear calibration curves of $\log(-\Delta f_i)$ versus $\log c_i$ from $1 \times 10^{-8} \text{ mol} \cdot \text{L}^{-1}$ to $1 \times 10^{-4} \text{ mol} \cdot \text{L}^{-1}$ were established:

$$\text{Sensor 1: } \log(-\Delta f_i) = 3.13 + 0.18 \log c_i \quad (r = 0.994)$$

$$\text{Sensor 2: } \log(-\Delta f_i) = 3.20 + 0.19 \log c_i \quad (r = 0.988)$$

$$\text{Sensor 3: } \log(-\Delta f_i) = 3.15 + 0.18 \log c_i \quad (r = 0.994)$$

For all the three sensors, the detection limit is $2 \times 10^{-9} \text{ mol} \cdot \text{L}^{-1}$. It can also be seen from Fig. 4 that the response of the MIM sensor to dipyrindamole is much higher than that of the NIM sensor. Therefore, it was found that the MIM coating showed higher affinity for the dipyrindamole than the NIM coating as expected.

Effect of pH and the ratio of MIM to PVC

The binding ability of the MIM to dipyrindamole can be related to the hydrogen bond and ionic adsorption between carboxyl group and dipyrindamole. Therefore, the solution pH may affect the interaction between the analyte and the carboxyl group. To study the effect of solution pH, the sensor

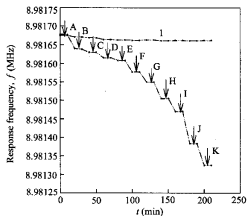


Fig. 3 Course of the observed frequency shift response of the sensor to each concentration of dipyrindamole. (1) In water; (2) in dipyrindamole solution. A, 0; B, 1×10^{-8} mol·L⁻¹; C, 5×10^{-8} mol·L⁻¹; D, 1×10^{-7} mol·L⁻¹; E, 2×10^{-7} mol·L⁻¹; F, 1×10^{-6} mol·L⁻¹; G, 3×10^{-6} mol·L⁻¹; H, 5×10^{-6} mol·L⁻¹; I, 1×10^{-5} mol·L⁻¹; J, 2×10^{-5} mol·L⁻¹; K, 1×10^{-4} mol·L⁻¹.

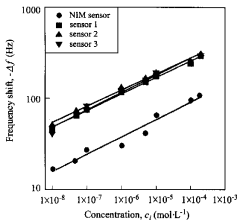


Fig. 4 Typical response of the PQC sensor. Sensor 1, with $-\Delta f_{\text{mod}} = 8120$ Hz; Sensor 2, with $-\Delta f_{\text{mod}} = 8072$ Hz; Sensor 3, with $-\Delta f_{\text{mod}} = 8240$ Hz.

was immersed in 1×10^{-5} mol·L⁻¹ dipyrindamole solution and the solution pH value was adjusted using 0.1 mol·L⁻¹ NaOH solution or 0.1 mol·L⁻¹ HCl solution. The results are shown in Fig. 5. When pH value is in the range of 5–9, the frequency responses of the sensor are not significant affected by solution pH. When pH > 9 or pH < 5, the frequency shift increases, which can be explained by enhancement of the ionic adsorption. In the following experiments, pH 7 was used.

PVC was selected as matrix in this study. It has been found that the weight ratio of MIM to PVC in the modified material affects the frequency response of the sensor to dipyrindamole (Fig. 6). With the increase of the weight ratio of

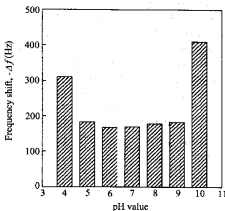


Fig. 5 Relation of frequency shifts vs. pH. 0.1 mol·L⁻¹ HCl solution or 0.1 mol·L⁻¹ NaOH solution was used to adjust pH value. The concentration of dipyrindamole in the matrix was 1×10^{-5} mol·L⁻¹.

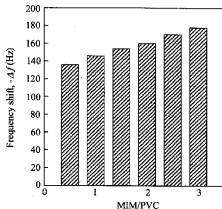


Fig. 6 Relation between frequency shift and the weight ratio of MIM/PVC in 1×10^{-5} mol·L⁻¹ dipyrindamole standard solution.

MIM to PVC, the frequency shift increases. However, when the ratio is over 3, the coating flakes off easily and the reproducibility is poor. Therefore, in this study, the weight ratio of 2.5 was employed.

Influence of ethanol and harsh treatments on sensor response

Dipyrindamole can not be dissolved easily in water, hence, ethanol was employed as solvent in the course of preparation of the standard solution so that dipyrindamole can be dissolved completely. The influence of ethanol concentration on the response of sensor was tested and the result is shown in Table 1. It can be seen that the amount of ethanol has no significant effect on the determination.

As a new sensor, it is important to test its stability and durability. In order to study the stability of the new sensing material, which was fabricated by precipitation polymerization

method, the sensor was treated in harsh environments, including heating (60 °C, 1 h), acidic solution (pH 4, 1 h) and basic solution (pH 9, 1 h). The sensor responses calculated as the frequency shift ratio of the treated sensor to the untreated one were 98.5%, 93.7% and 92.1%, respectively. The result exhibits that the harsh environment has no significant effect on the sensor response.

Table 1 Influence of ethanol

Ethanol concentration (mol·L ⁻¹)	Frequency of the sensor (MHz)	Frequency shift (Hz)
0	8.981578	0
2.2 × 10 ⁻⁶	8.981586	8
4.4 × 10 ⁻⁶	8.981582	4
1.1 × 10 ⁻⁵	8.981573	-5
2.2 × 10 ⁻⁵	8.981570	-8
4.4 × 10 ⁻⁵	8.981580	2
1.3 × 10 ⁻⁴	8.981576	-2

Selectivity of the sensor

The biomimetic MIM has good selectivity to template molecule. It is more important for dipyrindamole because it can not be used with many pharmaceuticals together in clinical practice. In the selectivity study of the sensor, the concentration of dipyrindamole and the interfering substance was kept at 1 × 10⁻⁵ mol·L⁻¹. $k_{is} = \Delta f_i / \Delta f_s$ is defined as the selectivity coefficient, where, Δf_i is the frequency shift of the MIM sensor to the interfering substance and Δf_s is the frequency shift response to dipyrindamole. The results in Table 2 exhibit that the investigated substances, including the various inorganic compounds and the organic compounds, cause no significant interference. It is reasonable to suppose that the

Table 2 Selectivity of the sensor

Interfering substance	$k_{is} = \Delta f_i / \Delta f_s$
Magnesium sulfate	No response
Sodium chloride	No response
Potassium chloride	No response
Ammonium sulfate	No response
Cane sugar	0.05
Lactose	0.04
Sodium DL-aspartate	0.07
DL- α -Alanine	0.06
Mannitol	0.06
D-Glucose	0.16
Resorcinol	No response
<i>o</i> -Aminophenol	No response
2-Naphthol	0.02
Tris	0.06
Sodium 2-hydroxy-3-naphthoate	0.05
8-Hydroxyquinoline	0.05
Sodium aridotriacetate	0.05
Vitamin C	0.07

sensor selectivity is related not only to the affinity of the functional monomers to the tested substance, but also to the compatibility of the cavity shape with the tested substance.

Application of the sensor

The sensor was applied to the determination of dipyrindamole in water and urine. Tests of the recovery efficiencies for known amounts of dipyrindamole in water and urine were operated. Series of dipyrindamole samples were prepared by adding known amount of a standard solution of dipyrindamole. After appropriate dilution, the sample was placed in the detection cell, and processed as mentioned in the experimental section. Table 3 shows that the results are satisfactory.

Table 3 Application of the sensor to the determination of dipyrindamole

Sample	Added (mg)	Found ^a (mg)	R ^b (%)	R. S. D. (%)
Urine	1.51	1.48 ± 0.022	98.0	
	2.02	1.95 ± 0.027	96.5	
	2.52	2.49 ± 0.031	98.8	0.99
	3.02	3.11 ± 0.018	103.9	
	4.54	4.37 ± 0.021	96.2	
	9.07	8.63 ± 0.034	95.1	
Water	1.51	1.49 ± 0.018	98.7	
	2.02	2.13 ± 0.031	105.4	
	2.52	2.51 ± 0.021	99.6	1.31
	3.02	2.96 ± 0.057	98.0	
	4.54	4.51 ± 0.033	99.3	
	9.07	8.78 ± 0.035	96.8	

^a Means ± standard deviations ($n = 3$); ^b recovery.

Comparison with other methods for the determination dipyrindamole

Comparison of the present method with some other detection techniques is summarized in Table 4. It is shown that the MIM by precipitation polymer method has some advantages with lower determination limit, easier fabrication and cheaper instrumentation than other methods. The spectrophotometric method is not so sensitive and its detection range is only down to 100–500 µg/mL level. Moreover, it is time-consuming in the extraction step. The detection limit of the polarographic method is at micromolar level, so its application is limited only for tablets. HPLC method requires tedious sample clean-up procedure, including the required preliminary extraction and purification.

Conclusion

In this work, MIM-BAW sensor, which was synthesized with precipitation polymerization, was developed. The study showed that the MIM-sensor has high selectivity and sensitivity for dipyrindamole in liquid phase. It has been applied successfully to the determination of dipyrindamole in human urine and water. As a new kind of method for detection of dipyrindamole, the MIM-sensor possesses advantages in easy fabrica-

Table 4 Comparison of the present method with other methods for determination of dipyrindamole

Method	Detection limit (mol·L ⁻¹)	Linear range (mol·L ⁻¹)	Recovery (%)	R. S. D. (%)	Ref.	Application
Fluorescence spectrometry	2 × 10 ⁻¹⁰	—	96.8—110	0.8—2.5	23	Compound
Voltammetry	—	9.9 × 10 ⁻⁷ —2 × 10 ⁻⁵	100.5—101.2	1.5—2.2	24	Tablet
FLA	4.3 × 10 ⁻⁸	9.9 × 10 ⁻⁸ —7.9 × 10 ⁻⁷	—	1.4—11.5	25	Pharmaceutical
Chemiluminescence	1.1 × 10 ⁻⁷	4 × 10 ⁻⁷ —1.6 × 10 ⁻⁷	—	1.2—1.4	26	Tablet and water
Liquid chromatography	3 × 10 ⁻¹⁰	10 ⁻⁶ —10 ⁻⁹	—	3.7	21	Drugs
Potentiometry	10 ⁻⁶	10 ⁻² —10 ⁻⁶	98.7	0.6	22	Pharmaceutical
Spectrophotometry	—	—	99.9	0.6	20	Drugs
MIM	2 × 10 ⁻⁹	10 ⁻⁴ —10 ⁻⁸	95.1—105.4	1.0—1.3		Water, urine

tion, cheap instrumentation, satisfactory recovery and low detection limit. Therefore, it is possible to apply the MIP-sensor widely in pharmaceutical, medical and biological fields.

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