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Molecularly imprinted polymers microsphere prepared by precipitation polymerization for hydroquinone recognition

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Abstract

A one-step precipitation polymerization synthesis was adopted for the preparation of molecularly imprinted polymers (MIPs) by using hydroquinone as a template molecule. The transmission electron microscopy (TEM) exhibited that the polymers were uniform spheres with the diameter of about 700 nm. The results of adsorption experiments showed that the microspherical imprinted polymers possessed fast adsorption dynamics. Compared to the structurally similar compounds, catechol and resorcinol, the MIPs exhibited a high recognizable capacity to hydroquinone. And the electrochemical sensor fabricated by modifying the prepared MIPs microsphere on the glassy carbon electrode surface was used to detect the hydroquinone concentration. The current response was proportional to the concentration of hydroquinone in the range of 2.0×10^{-6} to 1.0×10^{-4} mol/L with the detection limit of 1.0×10^{-6} mol/L.

Keywords: Molecularly imprinted polymers microsphere; Precipitation polymerization; Hydroquinone; Recognition; Electrochemical detect

1. Introduction

Molecular imprinting of synthetic polymers is an approach where functional monomer and cross-linker are copolymerized in the presence of template molecule [1]. Removal of the template molecule from the obtained polymer by simple solvent extraction reveals the complementary binding sites that can recognize the template molecule from its structurally similar compounds. Owing to their mechanical and chemical stability, low cost of preparation, ease of mass production and fitting for wide range of operating conditions, molecularly imprinted polymers (MIPs) have been developed in wide fields, such as solid-phase extraction [2], chromatographic separation [3], catalysis [4] and biosensor [5].

Conventional MIPs have been prepared in the form of bulk monolith. The copolymers are then ground and sieved to obtain appropriate size particles with irregularly shape for further use [6–8]. This procedure is time-consuming and yields only moderate amounts of "useful" product [9]. And the obtained copolymer particles have low capacity and poor site accessibility for the template molecules because the grinding process may be detrimental to some of the binding sites. In order to overcome above limits, recently efforts have been made to prepare MIPs with desired shape and achieve MIPs materials for wide applications [10,11]. The ideal shape of MIPs for many applications may be the microsphere with regular size and shape [12], which can be prepared by precipitation polymerization [13], emulsion polymerization [14], suspension polymerization [15] and seed polymerization [16]. Compared with the latter three methods, precipitation polymerization might be the easiest method to prepare microspherical MIPs because this procedure is easy, and there is no need to add emulsifier or suspending reagent to reaction system.

Since it was first reported by Ye et al. [17], precipitation polymerization for the preparation of spherical MIPs has been developed rapidly and applied to recognize and determine various compounds, which mostly are biomolecules, such as amino acids [18], antibiotics [19] and herbicides [20]. Likewise, a little attention has been devoted for analytes of environmental interest, such as toxic compounds. Hydroquinone, a potentially carcinogenic substance, causes severe effects on the central nervous

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system. Several methods have been used for the determination of hydroquinone, including gas chromatography [21], chemiluminescence [22] and flow injection analysis [23]. These methods exhibited high sensitivity, but all needed relatively expensive instrument and long time to complete the determination process.

In the present work, a simple and efficient synthesis method, precipitation polymerization, was employed to prepare the MIPs microsphere by using hydroquinone as the template molecule. Ultraviolet–visible (UV–vis) spectrophotometry was used to evaluate the adsorption kinetics, special rebinding and selective recognition capability of the MIPs. At the same time, an electrochemical sensor prepared by modifying the MIPs microsphere on the glassy carbon electrode surface was used to detect the concentration of hydroquinone.

2. Experimental

2.1. Chemicals

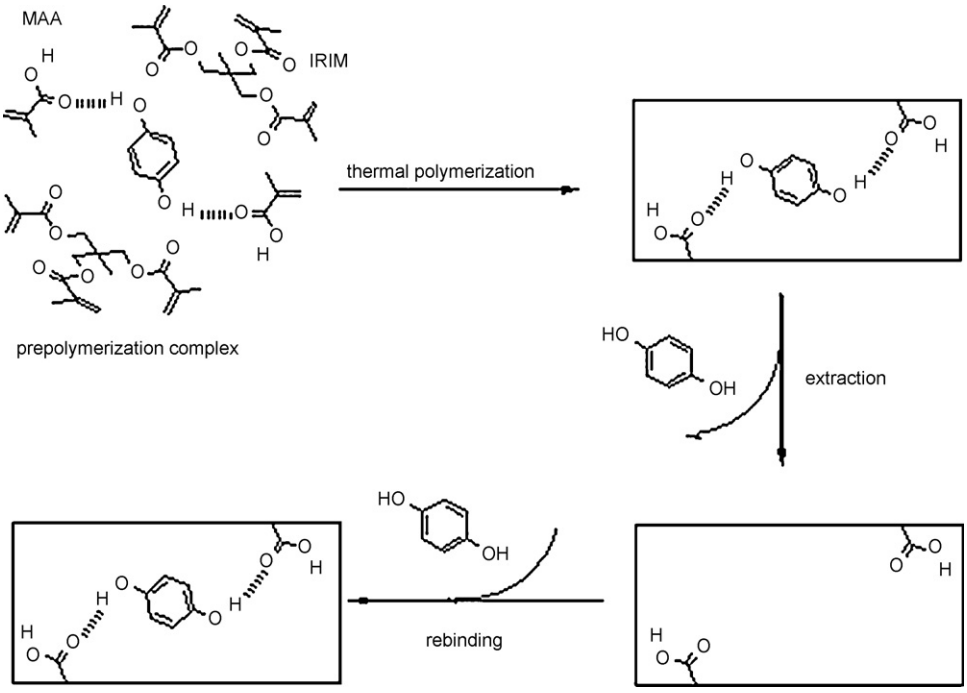
The cross-linker, trimethylolpropane trimethacrylate (TRIM), was purchased from Sigma. The functional monomer, methacrylic acid (MAA) was purified by distillation in vacuum to remove the polymerization inhibitor. All other reagents were of analytical grade and were used without further purification. 0.1 mol/L phosphate buffer solution (PBS) prepared in mixed solvent of doubly distilled water and methanol (9:1, v/v) at pH 7.0 was used as supporting electrolyte.

2.2. Apparatus and procedure

The morphology of MIPs microsphere was characterized by transmission electron microscopy (TEM, JEOL IEM-200CX). Ultraviolet–visible absorption spectra of hydroquinone were recorded by a UV-2401PC spectrometer. Electrochemical measurements were performed with a CHI 660B electrochemical workstation (Shanghai Chenhua Instrument) in a glass vial containing 10 mL of electrolyte at the room temperature. Chronoamperometry experiments were carried out in a typical three-electrode system with a platinum wire used as an auxiliary electrode, a saturated calomel electrode (SCE) as a reference electrode, and the MIPs microsphere modified glassy carbon electrode as a working electrode.

2.3. Preparation of MIPs microsphere

The hydroquinone MIPs microsphere were prepared by precipitation polymerization using hydroquinone, MAA, TRIM and 2, 2′-azobisisobutyronitrile (AIBN) as template molecule, functional monomer, cross linker and initiator, respectively. The procedure of preparing the MIPs was described in Scheme 1. One millimole template molecule and 8 mmol functional monomer were dissolved in a mixed solvent of acetonitrile and toluene. The mixture was sonicated to facilitate the combination between template molecule and functional monomer. Then 10 mmol cross-linker and 50 mg initiator were added to above mixture and stirred with magnetic stirrer. The temperature was increased from room temperature to 70 °C within 2 h under N_2 gas, and then kept at 70 °C for 24 h. After polymerization process, the resulting polymers were collected by centrifugation of 9000 rpm for 10 min. Then the polymers were eluted by the mixture solvent of methanol and acetic acid (9:1, v/v) for several times to extract the template molecules until there was no hydroquinone that could be detected by UV spectrometer in the eluent. The obtained polymers were finally rinsed with ethanol for



Scheme 1. Schematic illustration of the molecular imprinting procedure.

one time to remove the remaining acetic acid and then dried in the vacuum desiccators for 24 h before used. As a control, the non-molecularly imprinted polymer (NIPs) microspheres were prepared and treated in the same way, except that the template molecule was omitted from the polymerization process.

2.4. Binding experiments

Twenty milligrams microspherical MIPs or NIPs were added into 5 mL tubes, and mixed with 2.0 mL of hydroquinone–acetonitrile solutions with specific initial concentrations ranging from 0 to 5.0 mmol/L. After the samples were shaken at 25 °C for 4 h, the solution was centrifuged at 12,000 rpm for 5 min. The concentration of free hydroquinone in the supernate was measured by UV spectrophotometry at 289 nm. The amount of hydroquinone bound to the MIPs was calculated by subtracting the amount of free hydroquinone from the amount of hydroquinone initially added. Meanwhile, the adsorption dynamics of the MIPs was performed by measured the free hydroquinone concentration in the supernate at the different adsorption time intervals. The selectivity of the MIPs was investigated using catechol and resorcinol as the structurally related compounds.

2.5. Electrochemical detection of hydroquinone

Twenty milligrams MIPs were dispersed in 1 mL methanol with ultrasonic for 20 min. Then 10 μL of the suspension of MIPs was dropped on the clean glassy carbon electrode surface and dry at room temperature. Then 10 μL of 1% (v/v) agarose aqueous solution was overlapped on the above electrode surface till the accomplishment of gelling process of agarose. The prepared MIPs modified electrode was used as the work electrode to detect the concentration of hydroquinone by chronoamperometry.

3. Results and discussion

3.1. Preparation of MIPs microsphere

In the precipitation polymerization, the polymer was synthesized in the present of a larger amount of solution than that used in the traditional polymerization. The growing polymer chains do not overlap or coalesce but continue to grow individually by capturing newly formed oligomers and monomers in this diluted reaction system, and then separate from the solution with microspherical morphologies [24]. Molecular imprinting using a covalent approach was reported to be more efficient than the non-covalent approach. Nevertheless, imprinting using a non-covalent approach presents the advantage that guest binding and release are very fast [25]. Therefore, the present imprinted polymers were synthesized using a non-covalent approach.

MIPs microsphere was prepared using MAA as a functional monomer, TRIM as a cross-linker in a large volume mixed solvent of acetonitrile and toluene in presence of hydroquinone *via* thermal polymerization. Generally speaking, the most commonly used functional monomer is MAA, which can form hydrogen bond with template molecule prior to polymerization. The resulting specific and positioned interactions would contribute to the MIP's selectivity. As a cross-linker, TRIM, with three allyl groups, can much more favorably form the porous structure of polymers than ethylene glycol dimethacrylate (EMDA) with two allyl groups. And it has been shown that imprinted polymers prepared using this trifunctional cross-linker had higher load capacity [26]. In addition, both of MAA and TRIM are much more hydrophilic than vinylpyridine or divinylbenzene, which make the poly (MAA-*co*-TRIM) can be used in aqueous solution for the further detection of hydroquinone [27]. A mixture solvent of acetonitrile and toluene (11:1, v/v) was chosen as reactive medium and porogen to minimize the interference of a polar solvent like acetone with hydrogen bonding, as well as to increase the porosity of the polymers microsphere.

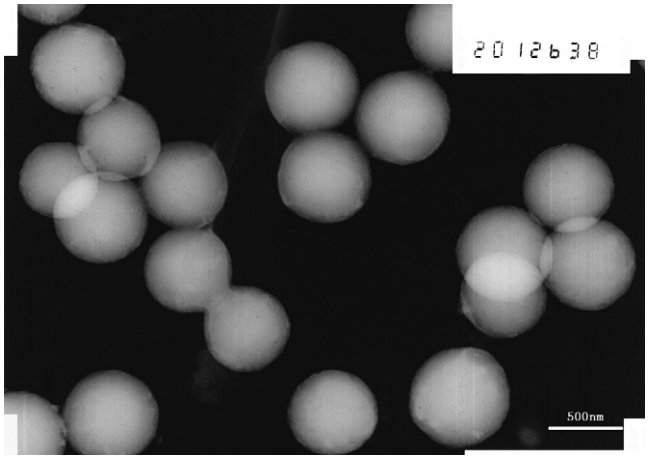


Fig. 1. TEM image of MIPs microsphere.

The TEM image as shown in Fig. 1 indicated the resulting polymers was uniform spherical morphology with about 700 nm in diameter.

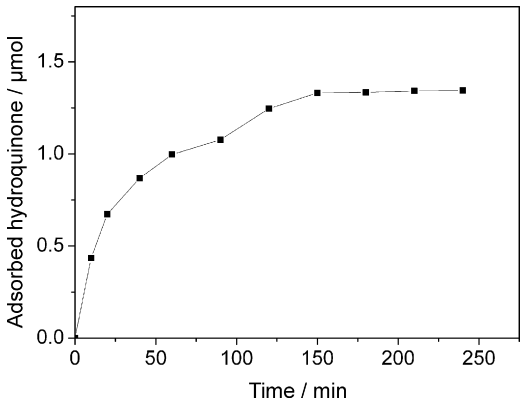


Fig. 2. Adsorption dynamics of MIPs towards hydroquinone in acetonitrile. Initial concentration of hydroquinone: 2.5 mmol/L, amount of MIPs: 20 mg, volume: 2.0 mL.

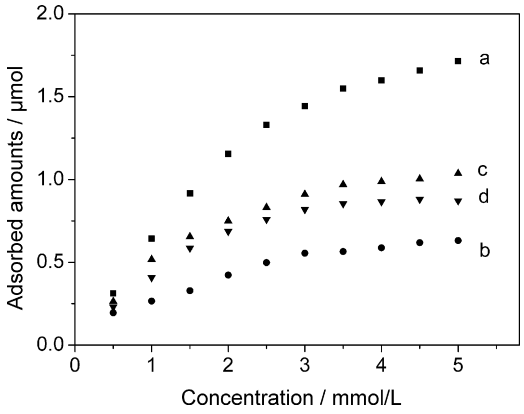


Fig. 3. Adsorption isotherms of (a) hydroquinone to MIPs, (b) hydroquinone to NIPs, (c) resorcinol to MIPs and (d) catechol to MIPs. Amount of polymers: 20 mg, volume: 2.0 mL, binding time: 4 h.

3.2. Adsorption characterizations of MIPs microsphere

3.2.1. Adsorption kinetics of MIPs microsphere

The adsorption kinetics of the MIPs for template molecule was carried out by adding 20 mg MIPs into 2 mL acetonitrile solution with template molecule concentration of 2.5 mmol/L. The curve of the adsorption dynamics was shown in Fig. 2. It can be seen that the adsorption amounts of hydroquinone increased with the increase of adsorption time. In the early 90 min, the adsorption rate increased quickly, while after 150 min, the adsorption almost reached equilibrium, which indicated that the imprinted cavities were saturated with the template molecules. At the early time, hydroquinone molecules were easy to reach the surface imprinting cavities of the MIPs microspheres and the adsorption rate increased very quickly. With the saturation of the surface imprinting cavities, hydroquinone began to diffuse towards the deep cavities, because the diffusion of hydroquinone met great resistance and led to the decrease of the adsorption rate. Compared with other spherical MIPs with the diameter about several microns or bulk MIPs prepared by traditional method, the present MIPs microspheres showed a faster binding kinetics [12,28]. This difference might be attributed to the small and uniform size of MIPs microsphere, which made the recognition sites of MIPs be easy accessible for the template molecules.

3.2.2. Binding property and selectively of MIPs microsphere

The binding properties of MIPs were measured with initial concentrations of hydroquinone ranging from 0 to 5.0 mmol/L. Curves (a) and (b) in Fig. 3 showed the adsorption isotherms of hydroquinone on MIPs and NIPs, respectively. It is obvious that the binding amount of template molecules to MIPs were much higher than that to NIPs. The high adsorption amount of hydroquinone to MIPs might result from the shape, size, and chemical functionality in imprinted cavities produced by the hydroquinone in the polymerization process.

In addition, curves (c) and (d) in Fig. 3 showed the adsorption isotherms of resorcinol and catechol to MIPs, respectively. The binding ability of MIPs to these compounds is speculated on the same interaction, two hydrogen bonds between the phenolic hydroxyl groups linked with aromatic ring of the phenolic compound and the carboxyl groups of MAA. When comparing curves (c) and (d) with curve (a), we found that the binding amounts of resorcinol and catechol to MIPs are much lower than that of hydroquinone. With the uniform molecular weight, the chemical structures of those three compounds are almost the same except for the different replacement site of phenolic hydroxyl groups. Since the structures of these two analogs are not complementary to the imprinted cavities in the polymers produced by the hydroquinone molecular imprinting, the ability of resorcinol or catechol interaction with the binding sites was weaker than that of hydroquinone. Therefore, the selective recognition for hydroquinone of MIPs may be attributed to shape selective fitting of hydroquinone into complementary cavities created into the MIPs during the imprinting procedure.

3.3. Electrochemical detection of hydroquinone

The electrochemical determination of hydroquinone in PBS was carried out by chronoamperometry. Fig. 4A represented the amperometric response obtained on the MIPs modified elec-

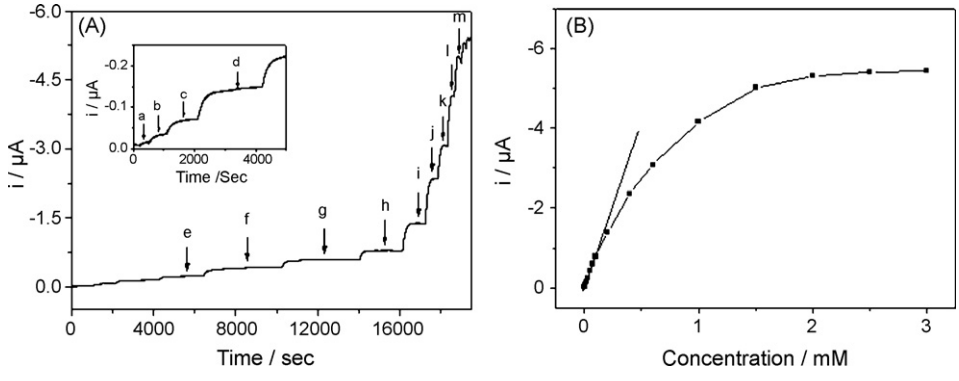


Fig. 4. (A) Typical current response curve at MIPs modified electrode on the addition of increasing concentration of hydroquinone in 0.1 M phosphate buffer pH 7.0. The electrode was polarized at 300 mV. (B) Calibration curve for hydroquinone obtained by *i–t* curve. Concentration of hydroquinone (a) 2.0×10^{-6} M, (b) 5.0×10^{-6} M, (c) 1.0×10^{-5} M, (d) 2.0×10^{-5} M, (e) 3.0×10^{-5} M, (f) 5.0×10^{-5} M, (g) 7.0×10^{-5} M, (h) 1.0×10^{-4} M, (i) 2.0×10^{-4} M, (j) 4.0×10^{-4} M, (k) 6.0×10^{-4} M, (l) 1.0×10^{-3} M, (m) 1.5×10^{-3} M.

trode. 0.3 V was chosen as the operating potential and aliquots of hydroquinone was injected into a stirred PBS solution. Stable response was obtained upon the repeated injection of hydroquinone into supporting electrolyte solution. Fig. 4B illustrates the corresponding plot showing a linear relationship between current response and hydroquinone concentration in the range of 2.0×10^{-6} to 1.0×10^{-4} mol/L with a correlation coefficient $r = 0.9983$. And the detection limit was found to be 1.0×10^{-6} mol/L. It was also found that the current response of the modified electrode leveled off at high concentration of hydroquinone, indicating the adsorption saturation of template molecule to the MIPs modified electrode.

4. Conclusion

MIPs composed of poly (MAA-*co*-TRIM) has been prepared by a one-step precipitation polymerization using non-covalent bond in the presence of template molecule, hydroquinone. The polymer particles showed excellent uniform microsphere and the precipitation polymerization was proved to be a feasible method for preparation spherical MIPs. Because of their small diameters, the MIPs possessed the fast adsorption kinetics, special adsorption capacity. Compared to the structurally similar compounds, catechol and resorcinol, the MIPs exhibited a high recognizable capacity to hydroquinone. An electrochemical sensor fabricated by modified MIPs on the glassy carbon electrode surface was used to detect the concentration of hydroquinone. A linear range between current response and the concentration of hydroquinone was obtained from 2.0×10^{-6} to 1.0×10^{-4} mol/L with a detection limit of 1.0×10^{-6} mol/L. And the present method may be a useful approach for the combination of MIPs microspheres and modified electrode to form a novel electrochemical sensor for detection of template molecule concentration.

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