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Core–Shell Molecularly Imprinted Polymer Nanospheres for the Recognition and Determination of Hydroquinone

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Core–shell structural molecularly imprinted polymer (Cs-MIP) has been successfully prepared by polymerization of MIP on the silica nanospheres surface. The vinyl groups modified on the surface of silica nanospheres have directed the copolymerization of functional monomers and cross linkers in the presence of template molecule, hydroquinone, onto the silica nanospheres surface. The prepared Cs-MIP exhibited fast binding kinetics because the rebinding sites situated at the surface and approximately to the surface of the outer MIP layer. And the Cs-MIP showed higher recognition capacity to hydroquinone than to analogous compounds, such as catechol and resorcinol. An electrochemical sensor fabricated by modifying Cs-MIP on the glassy carbon electrode surface was employed to detect the concentration of hydroquinone with a linear range from 2.0×10^{-6} to 1.0×10^{-4} mol/L.

Keywords: Core–Shell Molecularly Imprinted Polymer, Hydroquinone, Molecular Recognition, Electrochemical Detection.

1. INTRODUCTION

Molecularly imprinted technology has raised more and more attentions in the wide range of artificial receptors materials and the preparation method of molecularly imprinted polymers (MIP) has also been rapidly developed.[1–6] In molecularly imprinted technique, the template molecule is allowed to form a covalent or non covalent complex with functional monomers. Then the structure of the formed complex is fixed via polymerization in the presence of an excess amount of cross linkers. Removal of the template molecule from the polymer by simple solvent extraction leaves the binding sites with a shape and functional group complementary to the template molecule, resulting in the MIP with the capabilities of specific rebinding and recognition of template molecule. Traditional imprinted method prepared the MIP with the excessive cross linkers and a small quantity of solvent, followed by grinding, sieving and washing processes.[7] The obtained MIP is often the irregular microparticle with many imprinted sites embedded inside of the polymer and the poor mass-transfer,[8–11] which would restrict its applications. Therefore, some new methods for the synthesis of MIP have been developed to overcome the above limitations. Core–shell structural MIP has been reported to improve accessibility for the imprinted molecule to the MIP because the rebinding sites in the outer shell make the template molecules have rapid diffusion.[12, 13]

Generally, it needs a two-stage process for the preparation of the core–shell structural MIP. First, the supporting seeds prepared are often monodisperse with the certain mechanical intensity. Second, with the mixture of seeds, template molecules, functional monomers and cross linkers, the imprinted polymer is polymerized on the surface of the seeds.[14, 15] Among various kinds of seeds, silica microspheres are employed widely due to their excellent mechanical intensity.[16] In order to synthesize the imprinted polymers on the silica microspheres surface, the surface was often covalently bound by the initiator with several synthetical steps before the polymerization of the monomers and cross linkers. Initiator modified on the seeds surface can effectively direct polymerization only on the seed surface, but it is not very stable in the storage process.

In this paper, we described a simple, facile method to prepare the core–shell structural MIP (Cs-MIP) for recognition of hydroquinone by using silica nanospheres as the core, which were modified with vinyl groups on

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their surface by simple disposal. The prepared Cs-MIP nanospheres have a small dimension with high surface-to-volume ratio, so that most of the imprinted cavities are situated at the MIP surface or in the proximity of the surface, which have improved the adsorption dynamics toward template molecules. Also, special adsorption and selective recognition capacities of the Cs-MIP were evaluated. An electrochemical sensor constructed by modifying the Cs-MIP on glassy carbon electrode surface was used to detect the concentration of hydroquinone.

2. MATERIALS AND METHODS

2.1. Chemicals

Trimethylolpropane trimethacrylate (TRIM) was purchased from Sigma. Methacrylic acid (MAA) distilled under reduced pressure to remove inhibitor. γ-methacryloxypropyltrimethoxy silane (γ-MPS) were purchased from Jintan Eastchina Coupling Agent Factory, China. All other reagents used were of analytical grade and used as received 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 the supporting electrolyte.

The morphology of the Cs-MIP was observed by transmission electron microscopy (TEM, JEOL IEM-200CX). All Fourier transform infrared (FTIR) spectroscopic measurements were performed on a Bruker model VECTOR22 Fourier transform spectrometer using KBr pressed disks. Ultraviolet visible (UV-vis) absorption spectra of hydroquinone or analogs 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 experiment was carried out in a typical three-electrode system with a platinum wire using as an auxiliary electrode, a saturated calomel electrode (SCE) using as a reference electrode, and the Cs-MIP modified glassy carbon electrode using as a working electrode.

2.2. Preparation of the Cs-MIP

2.2.1. Preparation of Vinyl Groups Modified Silica Nanospheres

Typically, 12 mL tetraethoxysilane (TEOS) and 18 mL H_2O were mixed in 180 mL anhydrous ethanol for 5 min, followed by adding 8 mL ammonia. The mixture was stirred for 1 h at the room temperature. Then, the mixture of 10 mL TEOS and 6 mL γ-MPS was dropped in the above solution with the stirring for another 1 h. The obtained milky particles were collected by centrifugation and washed by ethanol for several times to remove the reagents.

2.2.2. Immobilization of the MIP on the Vinyl Groups Modified Silica Nanospheres

3.75 mmol hydroquinone and 15 mmol MAA were dissolved in a mixed solvent of 240 mL acetonitrile and 80 mL toluene, followed by adding 1 g as-prepared silica nanospheres. The mixture was sonicated to disperse the silica particles and facilitate the formation of the complex between hydroquinone and MAA. Then 40 mmol TRIM and 0.15 g 2, 2'-azobisisobutyronitrile (AIBN) were added to above mixture with magnetic stirring under N_2 gas. The temperature was increased from room temperature to 70 °C within 2 h, and then kept at 70 °C for 24 h. After polymerization process, the resulting polymers were collected by centrifugation. Then the polymers were eluted by the mixture solvent with methanol and acetic acid (9:1, v/v) for several times to extract the template molecules until no hydroquinone could be detected by UV spectra (wavelength is at 294 nm) in the eluent. The obtained polymers were finally rinsed with ethanol to remove the remaining acetic acid and then dried for 24 h before used. The core–shell structural non-molecularly imprinted polymer (Cs-NIP) was prepared under identical condition except that no template molecule was used in the polymerization process.

2.3. Binding Experiments

20 mg Cs-MIP was added into 2.0 mL of hydroquinone-acetonitrile solution with specific initial concentrations ranging from 0 to 5.0 mmol/L. After the samples were shaken at 25 °C for 3 h, the solution was centrifuged at 12000 rpm for 5 min. The concentration of free hydroquinone in supernate was measured by UV spectrometry at 294 nm. The amounts of hydroquinone bound to the MIPs were calculated by subtracting the amount of free hydroquinone in supernate from the amount of hydroquinone initially added. To investigate the adsorption kinetics of the Cs-MIP, the binding amounts of hydroquinone, at concentration of 2.5 mmol/L to the polymer were measured at the regular adsorption time intervals. The selectivity was investigated by using catechol and resorcinol as the structurally related compounds.

2.4. Electrochemical Detection of Hydroquinone

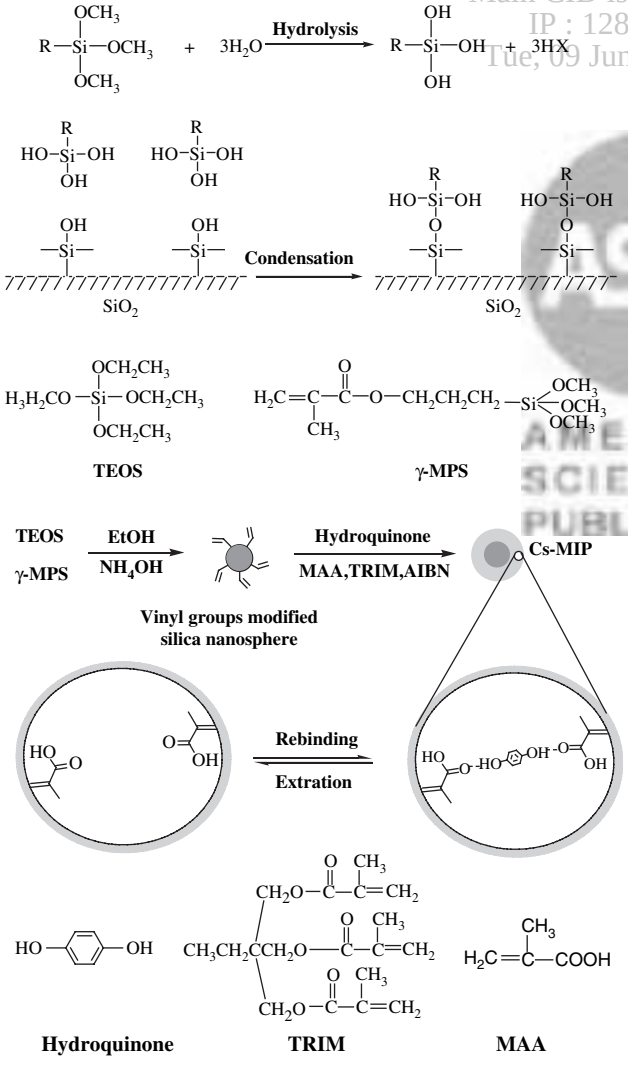
20 mg Cs-MIP was dispersed in 1 mL methanol with ultrasonic for 20 min. 5 μL of above suspension was dropped on the clean glassy carbon electrode surface and dry at room temperature. After the evaporation of methanol, 10 μL of 1% (v/v) agarose aqueous solution was overlapped on the above electrode surface till the accomplishment of gelling process of agarose. The prepared Cs-MIP modified electrode was used as working electrode to detect the concentration of hydroquinone by chronoamperometry.

3. RESULTS AND DISCUSSION

3.1. Characterization of Cs-MIP

Silica nanospheres were prepared by hydrolyzing TEOS and catalyzing with ammonia. In order to prepare the core–shell structure molecularly imprinted polymer, γ-MPS, a silane coupling agent with terminal double bond, was used to modify the silica nanospheres surface, which could improve the adherence between the MIP shell and silica core.[17, 18] The synthetic route of double bond modified silica nanospheres and further Cs-MIP was illustrated in Scheme 1.

FTIR studies were carried out to characterize the vinyl groups modified silica nanospheres, and their further graft with imprinted polymer. Figure 1 showed the FTIR spectra of pure silica nanospheres (a), vinyl groups modified silica nanospheres (b), pure imprinted polymer (c), and Cs-MIP (d). The main absorption bands of silica



Scheme 1. Synthetic route of preparation of vinyl groups modified silica nanospheres and the Cs-MIP.

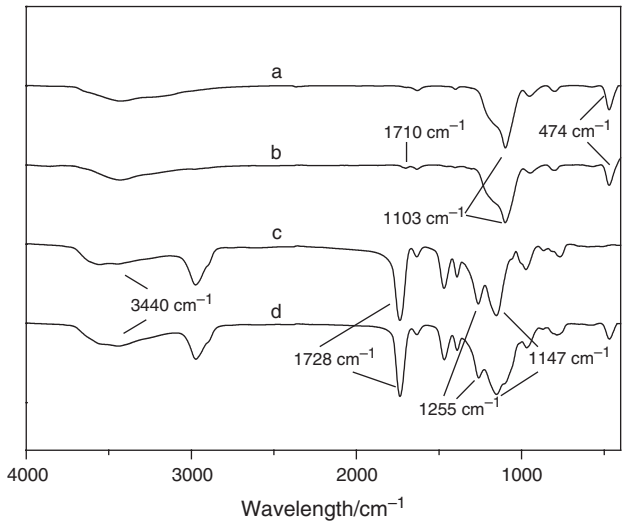


Fig. 1. FTIR spectra of silica nanospheres (a), vinyl group modified silica nanospheres (b), crude imprinted polymer (c) and Cs-MIP (d).

situated at 1103 cm^{-1} and 474 cm^{-1} could be referred to the Si-O-Si and Si-O stretching vibrations, respectively.[19] Compared with pure silica nanospheres, the FTIR spectrum of vinyl groups modified silica nanospheres appeared a new absorption bands situated at 1710 cm^{-1}, corresponding to the C═O stretching vibration of γ-MPS. Its appearance indicated the successful modification of γ-MPS on the silica surface. Therefore, the terminal double bond of γ-MPS was introduced onto the surface of silica nanospheres. The main absorption bands in pure MIP situated at 3440 cm^{-1} and 1728 cm^{-1}, 1255 cm^{-1} and 1147 cm^{-1} were assigned to the following vibrations: O-H stretching and C═O stretching vibration of carboxylic group, C-O stretching vibration of symmetric and asymmetric ester, respectively, as shown in Figure 1(c).[20] After polymerization of imprinted polymer shell on the silica nanospheres, the characteristic stretching vibrations of silica and pure MIP appeared in the FTIR spectrum of Cs-MIP (Fig. 1(d)). Therefore, we can conclude that successful grafting of the imprinted polymer onto silica nanospheres was achieved.

The core–shell structure of imprinted polymer could also be confirmed by TEM images. Figure 2(a) shows that the silica nanospheres were coated with imprinted polymers. In this image, the dark core was the silica nanosphere with the diameter about 150 nm and the grayer shell was the MIP layer with the average thickness about 120 nm. It was found that a few Cs-MIP coated onto two silica particles, which was possibly due to the incompletely dispersedness of silica nanospheres before the polymerization process. Figure 2(b) showed TEM image of the polymer prepared by polymerizing the imprinted polymer on amide groups modified silica nanospheres. It was obvious that none of the imprinted polymer was grafted onto the silica surface, but underwent a secondary nucleation to form new particles. The amide groups modified silica

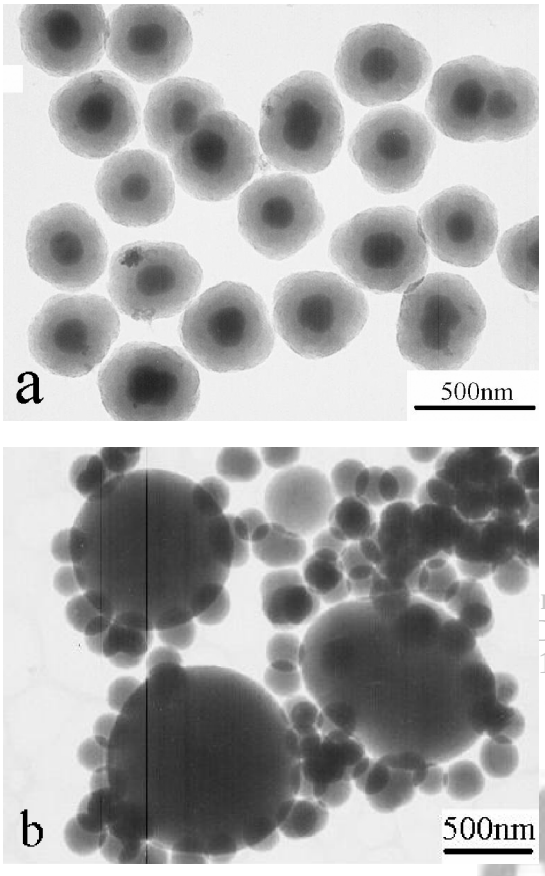


Fig. 2. Transmission electron microscopy of Cs-MIP (a) and MIP prepared with amine group silica nanospheres as core (b).

nanospheres (the smaller spheres) were arranged around the imprinted polymer microspheres. This also implied that the vinyl groups modified on the surface of silica nanospheres played an important role for the formation of Cs-MIP.

3.2. Adsorption Characterizations of Cs-MIP

3.2.1. Adsorption Kinetics of the Cs-MIP

Rapid quantification is an important requirement for an assay and separation process, so 20 mg Cs-MIP into acetonitrile solution with template molecule concentration of 2.5 mmol/L was shaken to measure the adsorption dynamics of the Cs-MIP. The curves of the adsorption dynamics were shown in Figure 3. It can be seen that the adsorption amounts of hydroquinone increased with the increase of adsorption time. In the early 30 min, the adsorptive velocity increased quickly because the template molecules were easy to reach the surface imprinting cavities. With the saturation of the surface imprinting cavities, hydroquinone began to diffuse toward the deeper imprinted cavities with great resistance and led to the decrease of the adsorption velocity. After 60 min, the adsorption almost reached equilibrium, which indicated that the imprinted

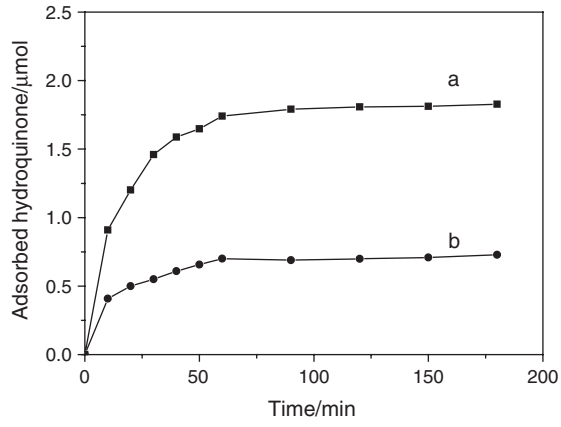


Fig. 3. Curves of adsorption dynamics of hydroquinone to Cs-MIP (a), and hydroquinone to Cs-NIP (b). Amount of polymers: 20 mg, volume: 2.0 mL, initial concentration of hydroquinone: 2.5 mmol/L.

sites were saturated with the template molecules. Compared with the MIP prepared by the traditional method[21] or MIP microspheres,[22] the Cs-MIP showed a faster binding kinetics. This difference must be attributed to most of the imprinted cavities situated at the surface and the proximity of the outer MIP shell, which enables the recognition cavities be accessible for the template molecules.

3.2.2. Selectively of Cs-MIP

Binding experiments were performed to evaluate the binding capacity of both Cs-MIP and Cs-NIP toward hydroquinone. 20 mg Cs-MIP or Cs-NIP was incubated in acetonitrile solution with concentrations of hydroquinone ranging from 0 to 5.0 mmol/L. Curve (a) and (b) of Figure 4 show the adsorption isotherms of hydroquinone on Cs-MIP and Cs-NIP, respectively. It is obvious that the binding amount of template molecule to Cs-MIP was much higher than that to Cs-NIP. This indicated that the Cs-MIP

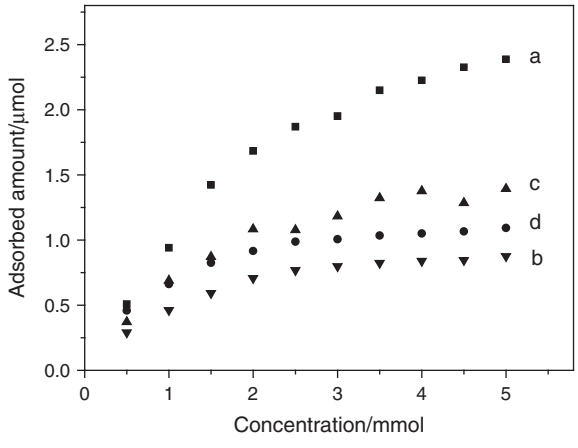


Fig. 4. Adsorption isotherms of hydroquinone on Cs-MIP (a), hydroquinone on Cs-NIP (b), resorcinol on Cs-MIP (c), and catechol on Cs-MIP (d). Amount of polymers: 20 mg, volume: 2.0 mL, binding time: 3 h.

has specifically binding capacity for template molecule, which could be explained by imprinting effect of hydroquinone with Cs-MIP. While the Cs-NIP does not possess the complementary imprinting recognition cavities to the template molecule since no template molecule was added during the polymerization process. The adsorption of hydroquinone to Cs-NIP is non-specific, which mainly is the interaction between the hydroxyl groups of template molecule and the carboxyl groups situated at surface of the polymer.

The selectivity experiments were carried out by using resorcinol and catechol as the similar compounds of hydroquinone in the binding process. 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. Curve (c) and (d) in Figure 4 show the binding isotherms of resorcinol and catechol to Cs-MIP, respectively. The binding capacities of Cs-MIP toward catechol and resorcinol were at about two times lower than that toward hydroquinone. The result confirmed that the Cs-MIP had a good selective recognition toward hydroquinone, which was attributed to shape selective fitting of hydroquinone into complementary cavities created into the Cs-MIP during the imprinting procedure.

3.2.3. Eelectrochemical Detection of Hydroquinone

The chronoamperometry measurement was performed in 0.1 M PBS solution to examine the current response of Cs-MIP modified electrode to hydroquinone. Figure 5(A) shows current change recorded during the successive addition of aliquots hydroquinone under stirred conditions, where the potential was kept at +0.3 V. It is obvious that the current increased with the increase of incubation time at the beginning when modified electrode exposed to each concentration of hydroquinone, which could be explained that hydroquinone was constantly preconcentrated onto the modified electrode surface and then occurred redox reaction.[23] With the addition of hydroquinone in lower concentration, the modified electrode exhibits a fast current response due to rapid adsorption of a small quantity of hydroquinone to the Cs-MIP modified electrode. Then the current response rate is slower with the increasing the concentration of template molecules.[24] With the gradually saturated by the template molecule, the modified electrode shows a faster current response again in higher concentration with the disproportion between the current response and hydroquinone concentration. A linear relationship between current and hydroquinone concentration was obtained in a range of $2 \times 10^{-6} \sim 4 \times 10^{-4}$ mol/L with a correlation coefficient of $r = 0.998$, as shown in the insert of Figure 5(B). The detection limit has been determined to be 1×10^{-6} mol/L.

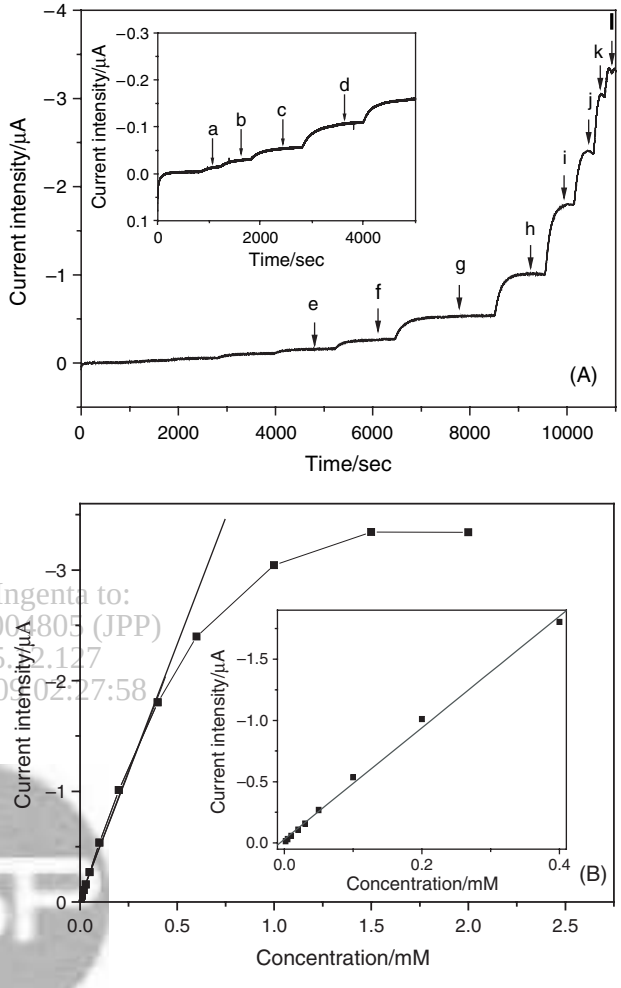


Fig. 5. Typical current response curve at Cs-MIP modified glassy carbon electrode with addition of increasing concentration of hydroquinone in 0.1 mol/L phosphate buffer solution (pH 7.0). The electrode was polarized at +0.3 V (A) and Calibration curve for hydroquinone obtained by i-t curve (B). Concentration of hydroquinone (a): 2.0×10^{-6}, (b): 3.0×10^{-6}, (c): 5.0×10^{-6}, (d): 2.0×10^{-5}, (e): 3.0×10^{-5}M, (f): 5.0×10^{-5}, (g): 1.0×10^{-5}, (h): 2.0×10^{-4}, (i): 4.0×10^{-4}, (j): 6.0×10^{-4}, (k): 1.0×10^{-4}, (l): 1.5×10^{-3} mol/L.

4. CONCLUSION

In this paper, Cs-MIP was prepared by copolymerizing of MAA and TRIM in the presence of hydroquinone on the terminal double bond functionalized silica nanospheres surface. Imprinted sites situated at the surface and approximately to surface of the outer MIP shell for the easy diffusion of template molecules into recognition cavities led to the rapid adsorption dynamics of Cs-MIP to hydroquinone. And the Cs-MIP also possessed high special adsorption and selective recognition to hydroquinone. The modified electrode fabricated by modifying the Cs-MIP on the glassy carbon electrode surface was used to detect the concentration of hydroquinone. The linear relationship between current response and the concentration of hydroquinone could be found in the range of $2 \times 10^{-6} \sim 4 \times 10^{-4}$ mol/L with the detection limit of 1×10^{-6} mol/L.

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