Study on the Preparation and Application of Chitosan/Silica Cu(II) Imprinted Microspheres

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Abstract. Molecularly imprinted polymers are functional polymers for selective recognition of imprinted molecules, which may have a wide range of applications in separation and purification, environmental monitoring, etc. Chitosan has excellent chelation for metal ions, being widely used in food, environmental protection, etc. In this paper, chitosan/silica Cu(II) imprinted microspheres were prepared by using natural product chitosan as functional monomer, silane-doped, Cu(II) as template ion and glutaraldehyde as crosslinking agent. The adsorption properties of the chitosan/silica Cu(II) imprinted microspheres for Cu(II) were studied by flame atomic absorption spectrometry. The experimental results showed that the adsorptivity of Cu(II) on the chitosan/silica Cu(II) imprinted microspheres was better than that of chitosan/silica non imprinted microspheres. The results of orthogonal test showed that the order of influence on the adsorption capacity of the chitosan/silica Cu(II) imprinted microspheres was chitosan, glutaraldehyde, Cu(II) and silane. Under the optimum conditions, the adsorption capacity of the chitosan/silica Cu(II) imprinted microspheres for Cu(II) could reach 33.38mg/g. Scanning electron microscopy showed that the diameter of the chitosan/silica Cu(II) imprinted microspheres was lower than 3μm. Chitosan/silica Cu(II) imprinted microspheres were obviously harder than chitosan Cu(II) imprinted microspheres.

1. Introduction
Molecularly imprinted polymers are important molecular recognition materials, which match the spatial structures and binding sites of template molecules exactly[1]. In recent years, molecularly imprinted polymers have received much attention due to their broad application prospects, such as chromatographic separation[2], membrane separation[3], solid phase extraction, metal ion enrichment [4], selective catalysis[5], biomimetic sensing[6], etc. Molecularly imprinted polymers can be divided into synthetic polymers and natural polymers. Compared with the synthetic polymers, the natural polymers have many advantages of rich resources, renewability, easy modification, excellent biocompatibility and biodegradability[7].

Chitosan, an N-deacetylated derivative of chitin which can be obtained from crustaceans, insects, fungi, etc., is well known to be the most abundant natural amino-polysaccharide[8]. Because of a large number of amino groups and hydroxyl groups in chitosan, which are beneficial to structural modifications and preparation of molecularly imprinted polymers, chitosan has been proved to be effective in molecular imprinting[9-11].

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The aim of this work was to enhance the application performance of chitosan imprinted materials, and silica[12] was taken into consideration. Silica can be obtained by hydrolytic condensation of 3-aminopropyl triethoxy silane. The presence of amino groups in 3-aminopropyl triethoxy silane might contribute to crosslinking and heavy metal adsorption of chitosan. So we tried to synthesize a novel chitosan/silica Cu(II) imprinted microspheres by using chitosan as functional monomer, 3-aminopropyl triethoxy silane-doped in order to improve the strength and capacity of microspheres, Cu(II) as the template ion and glutaraldehyde as the cross-linking agent. The adsorption performances of chitosan/silica Cu(II) imprinted microspheres were studied. The resulting product was characterized by means of scanning electron microscopy (SEM).

2. Materials and Methods

2.1. Materials

Chitosan (MW 40kDa, 95% deacetylated) was purchased from AK Biotech Ltd. (Shandong, China). CuSO₄·5H₂O, acetic acid, ethanol, sodium hydroxide, epichlorohydrin, glutaraldehyde (50%) and liquid paraffin were all purchased from Tianjin Damao Chemical Reagent Factory (Tianjin, China). Span 80 and 3-aminopropyl triethoxy silane were from Sinopharm (Shanghai, China). Petroleum ether (60-90°C) was supplied from Tianjin Fuyu Fine Chemical Co., Ltd. (Tianjin, China).

2.2. Methods

Preparation of chitosan/silica Cu(II) imprinted microspheres. The preparation process of the aqueous phase was as follows: Chitosan (0.2-0.3g) was dissolved in 10mL acetic acid solution (2.5% w/w) with stirring on a magnetic stirrer. Then CuSO₄·5H₂O (0.01-0.02g) and a certain amount of 3-aminopropyl triethoxy silane acetic acid solution were successively added to this homogeneous solution under stirring for 1h. Meanwhile, the oil phase was prepared by adding Span 80 to the liquid paraffin of 50mL, and stirred for 1h. Then the aqueous phase was slowly dropped into the oil phase under stirring constantly for nearly 1.5h until uniform emulsion appeared at room temperature. The crosslinking reaction was continued under stirring for 3.5h by adding a certain amount of glutaraldehyde at room temperature, while the color of the emulsion gradually deepened. The microspheres were obtained after statoration for 3h. The upper liquid was removed, and the microspheres were poured into the centrifugal tube. Petroleum ether and ethanol were used to repeatedly wash the microspheres until the oil phase was removed, and every time the upper liquid was poured out after centrifugal separation. The microspheres were soaked in 1mol/L hydrochloric acid for 8h in order to remove Cu(II), then filtered, washed with excess distilled water and treated with 0.05mol/L NaOH for regenerating the binding sites of amino groups in chitosan. The microspheres were repeatedly rinsed with distilled water and ethanol, and chitosan/silica Cu(II) imprinted microspheres were obtained after drying at 50°C. The preparation method of non imprinted chitosan/silica microspheres was similar to the above process, but in the absence of Cu(II). And chitosan Cu(II) imprinted microspheres was prepared by the same way without 3-aminopropyl triethoxy silane-doped. Besides, epichlorohydrin was used as a cross-linking agent to prepare chitosan Cu(II) imprinted microspheres so as to compare with glutaraldehyde. Scheme of preparation of chitosan/silica Cu(II) imprinted microspheres was shown in Figure 1.

2.2.1. Instruments. The concentration of Cu(II) was determined by flame atomic absorption spectrometry (WFX-1D, Beijing Ruili, China). The surface morphology of the chitosan/silica Cu(II) imprinted microspheres was investigated by scanning electron microscopy (CP62-JSM-6390LV, JEOL, Japan).

Batch Adsorption Experiments. Adsorption experiments were performed by adding 100mg dried chitosan/silica Cu(II) imprinted microspheres into 100mL of 85mg/L Cu(II) solution. The mixtures were stirred for 3.5h at room temperature, then allowed to settle down for 0.5h. The adsorption capacity of Cu(II) was calculated as follows:
\[ Q = \frac{(C_0 - C_e)}{V} \frac{V}{m} \]  \hspace{1cm} (1)

Where \( Q \) is the amount absorbed (mg/g), \( C_0 \), \( C_e \) is the initial and residual concentration (mg/L), respectively; \( V \) is the volume of Cu(II) solution (L), \( m \) is the mass of the chitosan/silica Cu(II) imprinted microspheres(g).

![Scheme of preparation of chitosan / silica Cu(II) imprinted microspheres.](image)

Figure 1. Scheme of preparation of chitosan / silica Cu(II) imprinted microspheres.

3. Results and discussion

3.1. SEM Images of the Microspheres

Figure 2 showed that most of the microspheres were smooth and approached sphere, especially the non imprinted microspheres (Figure 2, a). The sizes of the microspheres crosslinked by glutaraldehyde...
were not uniform, with diameters ranging from 500nm to 3μm. Compared with the non imprinted chitosan/silica microspheres, both of the diameters of the chitosan/silica Cu(II) imprinted microspheres (Figure 2, b) and the chitosan Cu(II) imprinted microspheres (Figure 2, c) were slightly larger. Besides, chitosan/silica Cu(II) imprinted microspheres were obviously harder than chitosan Cu(II) imprinted microspheres due to the presence of silica. The chitosan Cu(II) imprinted microspheres crosslinked by epichlorohydrin (Figure 2, d) were obviously larger than chitosan Cu(II) imprinted microspheres crosslinked by glutaraldehyde, so we used glutaraldehyde as the cross-linking agent.

Figure 2. SEM images of non imprinted chitosan/silica microspheres(a), chitosan/silica Cu(II) imprinted microspheres(b), chitosan Cu(II) imprinted microspheres(c), chitosan Cu(II) imprinted microspheres crosslinked by epichlorohydrin(d)

3.2. Optimization of Reaction Condition
In order to improve imprinting capacities of chitosan/silica Cu(II) imprinted microspheres, an orthogonal array was designed to investigate the suitable reaction condition. Four factors were studied, including the dosage of chitosan, glutaraldehyde, silane dosage and Cu(II). The design matrix and experimental data were listed in Table 1. The results showed that the chitosan dosage for preparing chitosan/silica Cu(II) imprinted microspheres was the most notable influence on the adsorption capacity of Cu(II). Glutaraldehyde dosage and Cu(II) dosage were the relatively remarkable factors, comparing with silane dosage. When 0.3g, 0.2mL, 0.015g and 0.3mL were selected for chitosan dosage, glutaraldehyde dosage and silane dosage, respectively, 33.38mg/g of adsorption capacity of Cu(II) was achieved under the optimized condition. Under the same condition, adsorption capacity of Cu(II) of chitosan/silica non imprinted microspheres was only 10.65 mg/g. This suggested that the imprinted cavities of template Cu(II) had been bound within imprinted microspheres. However, the non imprinted microspheres lacked imprinted cavities. Besides, adsorption capacity of Cu(II) of
chitosan/silica imprinted microspheres was nearly 5mg/g higher than that of chitosan imprinted microspheres.

Table 1. Design matrix and experimental results for the L₉ (4³) orthogonal array

| Experiment | Chitosan dosage (g) | Glutaraldehyde dosage (mL) | Silane dosage (mL) | Cu(II) dosage (g) | Adsorption capacity of Cu(II) (mg/g) |
|------------|---------------------|---------------------------|--------------------|-------------------|-----------------------------------|
| 1          | 0.20                | 0.2                       | 0.1                | 0.01              | 23.53                             |
| 2          | 0.20                | 0.4                       | 0.2                | 0.015             | 14.53                             |
| 3          | 0.20                | 0.6                       | 0.3                | 0.02              | 16.35                             |
| 4          | 0.25                | 0.2                       | 0.2                | 0.02              | 15.74                             |
| 5          | 0.25                | 0.4                       | 0.3                | 0.01              | 19.50                             |
| 6          | 0.25                | 0.6                       | 0.1                | 0.015             | 14.02                             |
| 7          | 0.30                | 0.2                       | 0.3                | 0.015             | 33.38                             |
| 8          | 0.30                | 0.4                       | 0.1                | 0.02              | 19.10                             |
| 9          | 0.30                | 0.6                       | 0.2                | 0.01              | 24.77                             |
| K1         | 54.41               | 72.65                     | 56.65              | 67.8              |                                   |
| K2         | 49.26               | 53.13                     | 55.04              | 61.93             |                                   |
| K3         | 77.25               | 55.14                     | 69.23              | 51.19             |                                   |
| R          | 27.99               | 19.52                     | 14.19              | 16.61             |                                   |

4. Conclusions
Chitosan/silica Cu(II) imprinted microspheres were successfully synthesized by using chitosan as functional monomer, 3-aminopropyl triethoxy silane-doped, Cu(II) as the template ion and glutaraldehyde as crosslinking agent. Chitosan/silica Cu(II) imprinted microspheres were obviously harder than chitosan Cu(II) imprinted microspheres due to the presence of silica. The results of orthogonal test showed that the order of influence on the adsorption capacity of Cu(II) of the chitosan/silica Cu(II) imprinted microspheres was chitosan, glutaraldehyde, Cu(II) and 3-aminopropyl triethoxy silane. Under the optimum conditions, the adsorption capacity of the chitosan/silica Cu(II) imprinted microspheres for Cu(II) could reach 33.38mg/g. SEM images showed that the sizes of Chitosan/silica Cu(II) imprinted microspheres ranged from 0.5μm to 3μm. As far as the particle sizes of imprinted microspheres were concerned, it was better to use glutaraldehyde as crosslinker rather than epichlorohydrin.

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