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Surface modification of nonporous glass beads with chitosan and their adsorption property for transition metal ions

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Abstract

A new hybrid material that adsorbs transition metal ions was prepared by immobilizing chitosan on the surface of nonporous glass beads. The glass beads, prepared by etching in aqueous NaOH at 100°C, were first reacted with γ-aminopropyltriethoxysilane (APES) to introduce amino groups on the surface. Subsequently, the resulting aminated beads were treated with glutaraldehyde at 25°C to change the amino groups into aldehyde groups. Finally, chitosan of average molecular weight 40,000 was introduced via the aldehyde groups through a Schiff's reaction. After complete acid-hydrolysis of the immobilized chitosan, the Svennerholm method for glucosamine analysis showed that 0.3% (w/w) chitosan had been successfully introduced on the glass beads. Atomic absorption spectroscopic analysis of eluants of a column of the chitosan-modified glass beads showed that metal ions such as Cu^{2+}, Ag^{+}, Pb^{2+}, Fe^{3+}, and Cd^{2+} were more than 90% entrapped on a column of beads prepared in this manner.

Keywords: Chitosan; Immobilization; Glass beads; Heavy metal ions

1. Introduction

Chitosan is a β-(1,4)-linked polysaccharide of D-glucosamine derived from chitin, a major component of the shells of crustacean organisms and the second most abundant biopolymer next to cellulose (Muzzarelli, Jeuniaux & Gooday, 1986). The possibility of extending the use of chitosan to immobilize biologically active species or to remove metal ions from wastewater has been regarded as an area worthy of further investigation. Because of its coarse porous structure, low toxicity, and the presence of free amino groups, chitosan has been considered an excellent candidate as a support for such purposes. The amine groups on the chitosan chain have already been shown to serve as a selective chelating site for transition metal ions (Carol & Matthew, 1999; Inoue, Baba, Yoshizuka, Noguchi & Yoshizaki, 1988; Kawamura, Mitsuhashi, Tanibe & Yoshida, 1993; Rorrer, Hsien & Way, 1993).

An efficient and well-known method for synthesizing insoluble chitosan is to cross-link the amine groups on chitosan with a dialdehyde such as glutaraldehyde using a Schiff's reaction (Hsien & Rorrer, 1995; Hsien & Rorrer, 1997). Although highly porous chitosan beads have been produced by this method and used for engineering and biotechnological applications, it seems that the resulting beads lack mechanical strength. Glass beads, on the other hand, because of their arbitrarily controllable and narrow size dispersion, mechanical strength, and low cost have recently received attention as a preferred supporting material for these applications. Indeed, a number of new materials have been successfully prepared from the glass beads by employing surface modifications (Nemeth et al., 1997; Ottenbrite, Zengin & Siddiqui, 1998a,b; Tsubokawa, Ichioka & Satoh, 1998; Yin, Ottenbrite & Siddiqui, 1997), and these achievements prompted us to experiment with immobilizing chitosan on the surface of glass beads.

Based on the success of employing Schiff reactions to synthesize various chitosan derivatives, we planned to introduce an aldehyde that could couple chitosan to the surface of glass beads. In contrast to porous silica, which has sufficient silanol groups on the surface and a large total surface area to enable adequate coupling of chitosan, nonporous glass beads have a very low density of silanol groups and a small surface area (Yin et al., 1997). For this reason we first etched surface of the glass beads with aqueous NaOH to increase density of the silanol, and then, by using a silane-coupling agent, aminopropyltriethoxysilane (APES), introduced amino groups to the beads. The amino groups were then reacted with glutaraldehyde and subsequently grafted

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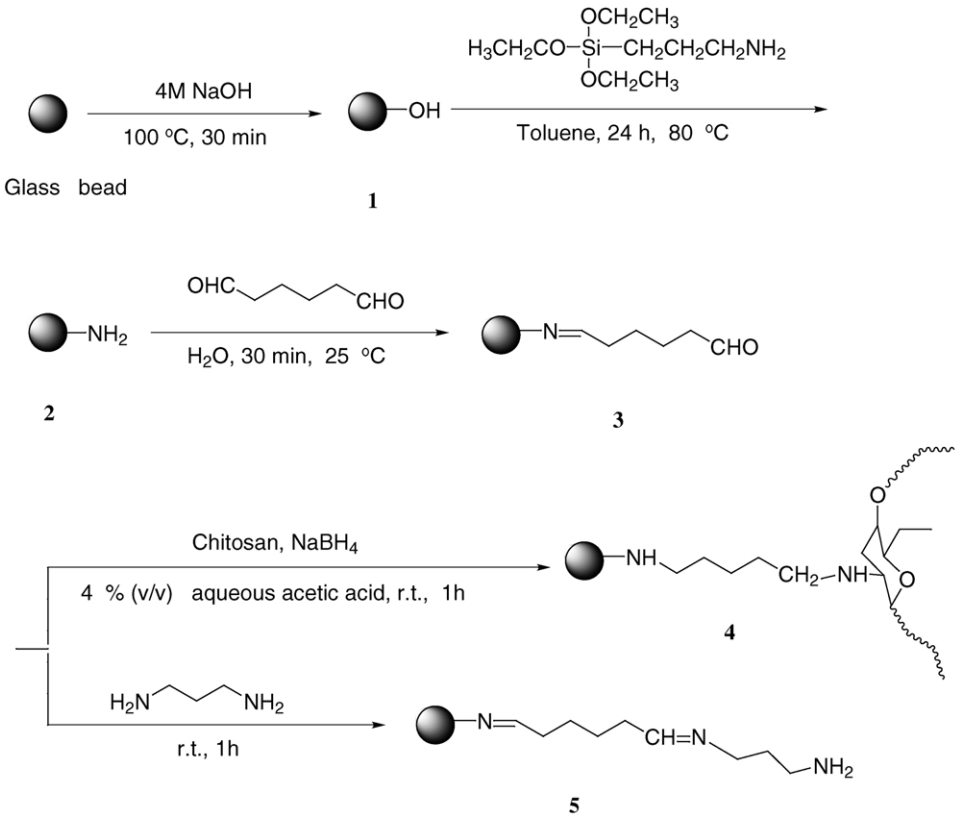

Scheme 1.

to the chitosan through a Schiff reaction. The results of our preliminary examination of the chitosan-modified glass beads thus obtained are presented below.

2. Materials and methods

2.1. General methods

Chitosan with an *N*-deacetylation degree of 0.80 and with average molecular weight of 40,000 and glass beads were purchased from Kyowa Techos Co., Ltd (Chiba, Japan) and Wako Pure Chemical Industries, Co., Ltd (Osaka, Japan), respectively. Aminopropyltriethoxysilane (APES) was obtained from Shin-Etsu Chemical Industries, Co., Ltd (Tokyo, Japan) and used as received. Toluene was distillated from sodium before use. All other chemicals used for the following investigations were of analytical grade.

Infrared spectra were recorded on a HORIBA FT-210 spectrophotometer using a potassium bromide pellet. Photographs of scanning electron microscopy (SEM) were taken on a Hitachi S-2400 instrument operating at 20–25 kV after spattering with gold. UV–Vis spectra were recorded with a HITACHI U-2000A spectrometer.

Fluorescence microscopic observation was performed on an OLYMPUS IX-FLA fluorescence microscope. The glass beads for this analysis were immersed in solution of 2′,7′-difluoro fluorescein in 0.05 M phosphate buffer (pH 6.5) at room temperature to introduce the fluorescent dye and then were washed several times by 0.05 M phosphate buffer (pH 9.0).

2.2. Analysis of the amount of the amino group on the glass beads

An aqueous saturated solution of methyl orange (10 ml) was added to an acetate buffer composed of sodium acetate trihydrate (0.14 g), glacial acetic acid (0.6 ml) and distilled water (40 ml). The glass beads (0.15 g) were immersed in this solution and stirred for 10 min, washed five times with distilled water (50 ml), and filtered. The resulting glass beads were shaken with a mixture of 1 M hydrochloric acid (1 ml) and 0.5 M KCl solution (1 ml) for 30 min, and then the absorbance of the solution was measured at 430 nm.

2.3. Immobilization of chitosan on the glass beads surface

Nonporous glass beads (50 g) with average diameters of 60 and 600 μm were dispersed in 4 M aqueous solution of NaOH (200 ml) with stirring. The mixture was gradually heated to reflux temperature for 15 min and kept the temperature with stirring for 15 min. The suspension was poured into deionized water (800 ml) at room temperature, and the glass beads were filtered, washed with deionized

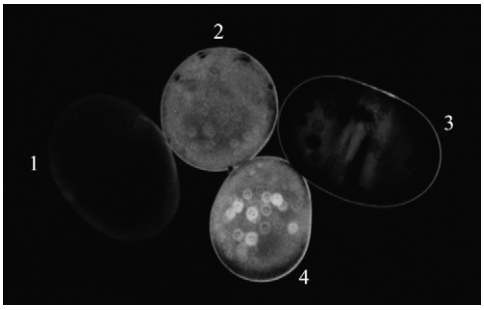

Fig. 1. Fluorescence microscopy of resulting beads with average diameter of 600 μm. (1) NaOH treated glass bead **1**; (2) APES (silane coupling reagent) treated glass bead **2**; (3) aldehyde-decorated glass bead **3**; (4) chitosan-modified glass bead **4**.

water to neutral, and dried in a vacuum oven at 120°C for 24 h. These activated glass beads **1** were transferred to a 300 ml flask containing anhydrous toluene (100 ml) and nitrogen was bubbled through the mixture. APES (30 ml) was added to the slurry and the mixture was stirred at 60 rpm at 80°C overnight under nitrogen atmosphere. The glass beads was filtered, washed successively with toluene (200 ml), dichloromethane (200 ml), and acetone (200 ml), and air-dried for 24 h, giving APES-treated glass beads **2**.

The resulting glass beads **2** (30 g) were suspended in aqueous 25% glutaraldehyde (100 ml), stirred at 25°C at 60 rpm for 1 h, and filtered. The residue was washed with methanol (200 ml), and dried under vacuum at 80°C for 24 h to give the glutaraldehyde-treated glass beads **3**.

The glass beads **3** (20 g) and $NaBH_4$ (0.5 g) were successively added to a solution of 8% (w/w) chitosan in 4% aqueous acetic acid. The suspension was stirred at 60 rpm at 25°C for 1 h, filtered, and washed with deionized water until neutral. Finally the glass beads were dried under vacuum at 100°C for 24 h, giving the chitosan-treated glass beads **4**.

2.4. Measurement of chitosan-content on the glass beads

The chitosan-treated glass beads **4** (0.2 g) were immersed with 4 M hydrochloric acid (0.5 ml) in a hermetic test tube, and heated at 100°C for 16 h. After cooling to room temperature, the amount of glucosamine in this mixture was determined by a modification from the Svennerholm method (Svennerholm, 1956) as follows. After a filtration of the mixture, the solution was neutralized with a 4 M aqueous NaOH (0.5 ml), titrated back to neutral with 1 M aqueous hydrochloric acid, and added a solution (0.5 ml) freshly prepared with acetylacetone (0.2 ml) in 1.25 M aqueous sodium hydrogen carbonate solution (4.8 ml), then the mixture was heated at 90°C for 1 h, cooled to room temperature, and diluted with ethanol (4 ml), and incubated at room temperature for 30 min. The mixture was added of a DMAB reagent (0.5 ml), made of (4-(*N*,*N*-dimethylamino)benzaldehyde) (DMAB, 0.4 g), 96% ethanol (7.5 ml) and concentrated hydrochloric acid

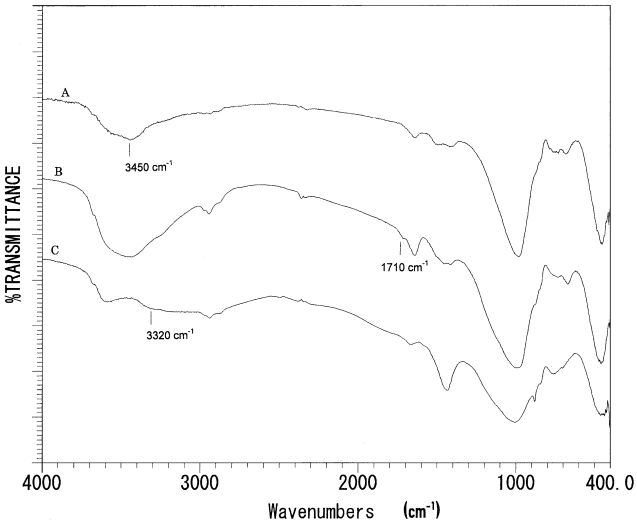

Fig. 2. Infrared spectra of beads with average diameter of 60 μm. (A) Silane coupling reagent (APES) treated glass bead **2**; (B) aldehyde-decorated glass bead **3**; (C) chitosan-modified glass bead **4**.

(7.5 ml), stirred at room temperature for 1 h, measured the absorbance at 530 nm.

2.5. Measurement of metal ion adsorption

Metal ion solutions of 20 ppm concentration were prepared by dissolving $CuSO_4{\cdot}5H_2O$, $ZnSO_4{\cdot}7H_2O$, $AgNO_3$, $PbCl_2$, $FeCl_3{\cdot}6H_2O$, $CrCl_3{\cdot}6H_2O$, $MnCl_2$, $SnCl_4{\cdot}5H_2O$, $CdCl_2$, $CoSO_4{\cdot}6H_2O$, $CaCl_2{\cdot}6H_2O$ and $MgSO_4$ in distilled water. The chitosan-modified glass beads **4** (2.0 g) were stuffed in a Pasteur pipette, and each metal ion solution (10 ml) was passed through the column. The eluate was collected in 5 ml fractions, and the concentrations of the metal ion were measured by atomic absorption spectrometry with SHMADZU SPCA-626D. The results are summarized in Fig. 5.

3. Results and discussions

3.1. Preparation of the chitosan-modified glass beads

Immobilization of chitosan on the nonporous glass beads, average diameter of 60 and 600 μm was carried out in four steps, as summarized in Scheme 1. In the first step, the nonporous glass beads were etched by aqueous sodium hydroxide to activate the surface. According to a procedure reported recently (Ottenbrite et al., 1998a,b), the glass beads were treated with aqueous sodium hydroxide (4 M) under reflux for 4 h. Because a large number of collapses were observed by SEM supervision (data not supplied), we examined the etching time. As a result, we found that the etching of the glass beads was completed within 30 min without any morphological change on the surface.

The activated beads **1** were subsequently treated with a silane coupling reagent, as reported recently (Nemeth et al.,

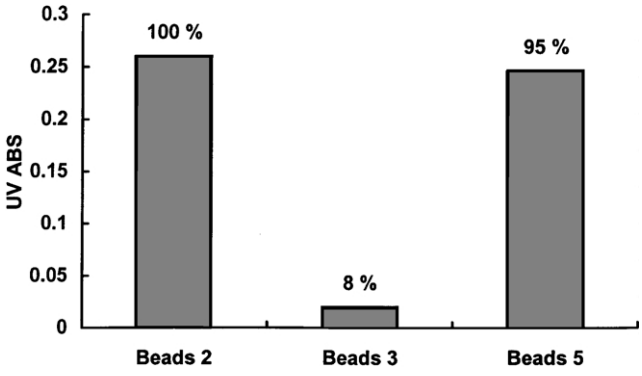

Fig. 3. Absorbances at 430 nm of the solution from beads with average diameter of 60 μm treated with methyl orange. (1) Silane-coupled beads **2**; (2) aldehyde-decorated glass bead **3**; and (3) beads transformed from aldehyde-decorated glass bead by treatment with ethylenediamine **5**.

1997; Ottenbrite et al., 1998a,b; Yin et al., 1997). Thus, a mixture of **1** and γ-aminopropyltriethoxysilane (APES) in anhydrous toluene under nitrogen give the APES-treated beads **2**. To confirm the presence of the amino groups on the surface, the resulting glass beads **2** were examined by fluorescence microscopy. The sample was prepared by treating the beads **2** (average diameter of 600 μm) with 2′,7′-difluoro fluorescein, a probe of amino group in phosphate buffer.

As shown in Fig. 1, the NaOH treated beads **1** showed almost no fluorescence, in contrast to the strong fluorescence observed on the surface of the silane-coupled bead **2**. These results suggested that amino groups were successfully introduced on the surface of **2**. Furthermore, an IR spectrum of the APES-treated beads **2** of average diameter of 60 μm, showed a broad peak at 3450 cm^{-1} due to –NH stretching, which was split into two peaks at 3650 and 3120 cm^{-1} after treatment with hydrochloric acid (Fig. 2A).

To introduce a spacer between the APES-treated beads **2** and chitosan, we treated the beads **2** with an aqueous solution of glutaraldehyde at 25°C. This modification was also monitored by fluorescent microscopy, which showed no fluorescence on the surface of the bead **3**, suggested that almost all amino groups on the glass bead **2** were modified to aldehyde groups. In comparison with the glass bead **2**, the IR spectrum (Fig. 2B) of glass beads **3** showed a new and distinct peak at 1710 cm^{-1}, attributable to C=O stretching of the aldehyde group.

To provide us with a rough estimate of the reactivity of

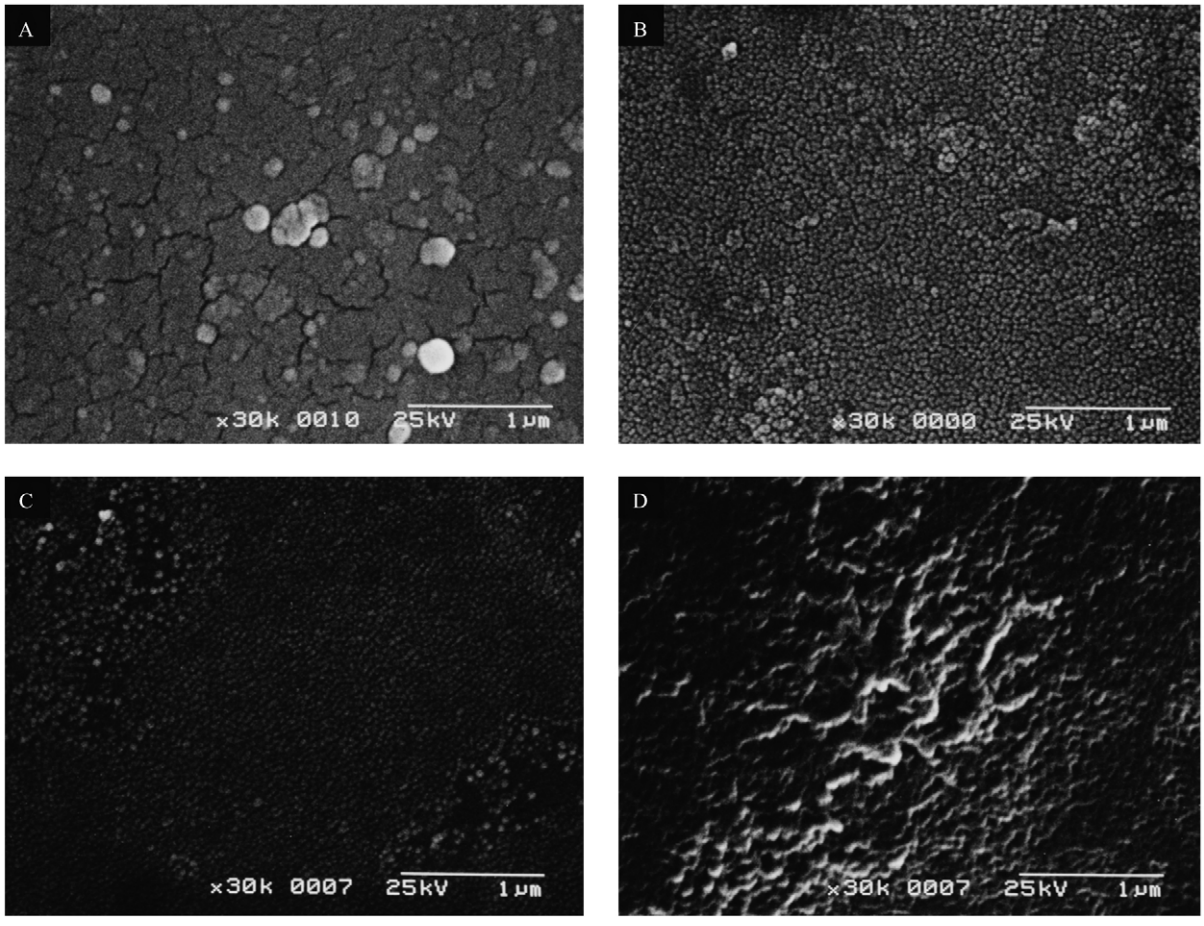

Fig. 4. SEM micrographs of beads with average diameter of 60 μm: (A) original glass bead; (B) silane coupling reagent (APES) treated glass bead **2**; (C) aldehyde-decorated glass bead **3**; (D) chitosan-modified glass bead **4**.

Table 1
The metal ion adsorption of the chitosan-modified glass beads with average diameter of 60 μm (adsorption was carried out by passing original solutions of metal ions through the column of chitosan-modified glass beads at room temperature)

Metal ions[a]	Original concentration		Eluate concentration	
	ppm[b]	mmol/l	ppm[b]	mmol/l
Cu^{2+} ($CuSO_4$)	20.40	0.321	0.14	0.002
Zn^{2+} ($ZnSO_4{\cdot}7H_2O$)	20.42	0.312	3.45	0.052
Ag^{+} ($AgNO_3$)	18.65	0.173	0.60	0.005
Pb^{2+} ($PbCl_2$)	20.01	0.097	0	0
Fe^{3+} ($FeCl_3{\cdot}6H_2O$)	21.27	0.381	1.80	0.032
Cr^{3+} ($CrCl_3{\cdot}6H_2O$)	20.51	0.394	6.84	0.131
Mn^{2+} ($MnCl_2$)	17.65	0.321	5.32	0.097
Sn^{4+} ($SnCl_4{\cdot}5H_2O$)	20.12	0.169	3.62	0.030
Cd^{2+} ($CdCl_2$)	18.80	0.167	0	0
Co^{2+} ($CoSO_4{\cdot}6H_2O$)	20.05	0.340	3.25	0.055
Ca^{2+} ($CaCl_2{\cdot}6H_2O$)	17.82	0.446	15.21	0.380
Mg^{2+} ($MgSO_4$)	16.36	0.673	15.48	0.637

[a] The original metal ion solutions were prepared from reagents in the parentheses.
[b] Concentrations of original metal ion solution and the eluate were measured with atomic adsorption spectrometry in ppm.

aldehyde group toward amino compound, the glass beads **3** were treated with a solution of methyl orange in acetate buffer, a simple procedure for the determination of amino groups (Silverstein, 1963). As a model reaction, the aldehyde-decorated bead **3** was treated with ethylenediamine at room temperature in water to give the bead **5**. The silane-coupled beads **2**, aldehyde-decorated beads **3** and the beads **5** were subjected to methyl orange analysis. The results are shown in Fig. 3, which indicates that the beads **3** had almost no amino groups and that amine groups were recovered after treatment with ethylenediamine.

On the basis of these findings, we proceeded to synthesize chitosan-linked beads by treating the aldehyde-decorated glass beads **3** with a solution of chitosan in aqueous acetic acid in the presence of a reducing agent, $NaBH_4$. After washing with distilled water, the beads were lyophilized. This modified glass bead **4** was then examined by difluoro-fluoroscein treatment and fluorescence microscopy. As we expected, the chitosan-modified bead **4** showed strong fluorescence on the surface (Fig. 1 (4)). In the IR spectrum (Fig. 2C), a peak characteristic of –NH stretching was visible at 3425 cm^{-1}, whereas the C=O characteristic peak observed at 1710 cm^{-1} in Fig. 2B disappeared.

The chitosan content of the resulting beads **4** with average diameter of 60 μm was determined by glucosamine analysis after complete acid-hydrolysis of the introduced chtosan. The beads **4** were treated with 4 M hydrochloric acid at 100°C in a sealed tube, and the resultant solution was subjected to the Svennerholm method (Svennerholm, 1956). Using this method, the amount of chitosan introduced on the glass beads **4** was calculated to be 0.3% (w/w). This chitosan content could, in theory, be readily increased simply by employing the same series of reactions but using glass beads finer than 60 μm large specific surface area. Moreover, SEM analysis of the surface morphology of the resulting beads **4** revealed more wrinkles and a complete lack of cracks (Fig. 4D) compared to other beads (Fig. 4A, B and C).

3.2. Metal ion adsorption by chitosan-modified glass beads

As mentioned in Section 1, it is well known that chitosan selectively adsorbs transition metal ions (Carol & Matthew, 1999; Inoue et al., 1988; Kawamura et al., 1993; Rorrer et al., 1993) through chelation by the amino group on glucosamine. Therefore, we sought to ascertain whether chitosan immobilized on the surface of the glass beads **4** possesses the same property of metal ion adsorption. Using column chromatography, we next examined the metal ion adsorption property to the chitosan-modified glass bead **4**.

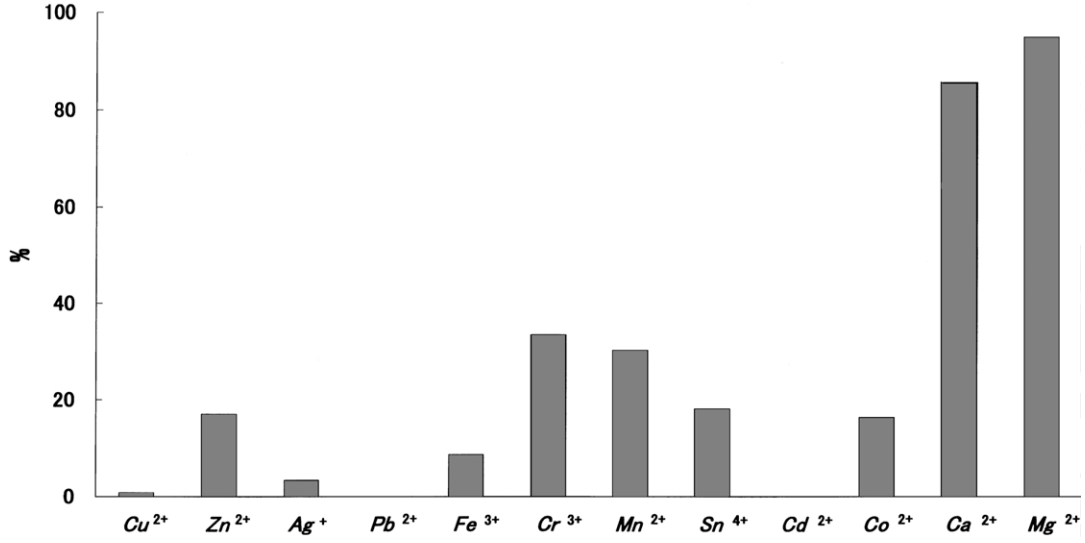

Fig. 5. The metal ion adsorption rates of the chitosan-modified glass beads with average diameter of 60 μm.

Various metal ion solutions with a concentration of approximately 20 ppm were passed through the column, and the amount of the metal ions in the eluants were measured by atomic absorption spectrometry. The results are summarized in Table 1 and the adsorption ratios are shown in Fig. 5. The data in Fig. 5 showed that transition metal ions such as Cu^{2+}, Ag^{+}, Pb^{2+}, Fe^{3+}, and Cd^{2+} were adsorbed more than 90%, whereas other ions, Zn^{2+}, Cr^{3+}, Mn^{2+}, Sn^{4+}, Co^{2+}, were adsorbed over 60%. However, the beads **4** were found to have almost no adsorption abilities against alkaline earth metals ions such as Ca^{2+} and Mg^{2+} (less than 15%). These results suggested that this chitosan-modified beads have strong affinity towards transition metal ions through coordination effect, rather than by a simple ion exchange effect.

4. Conclusion

Chitosan has been immobilized to the surface of nonporous glass beads through a series of reactions including surface activation with aqueous NaOH and successive treatment with aminopropyltriethoxysilane (APES) in toluene, aqueous glutaraldehyde and a chitosan solution in aqueous acetic acid. These modifications could be monitored by SEM, fluorescence microscopy, and FT-IR spectroscopy. The chitosan content of the glass beads with average diameter of 60 μm was about 0.3% (w/w). The chitosan-modified glass beads retain chitosan's adsorption affinity for various transition metal ions. In particular, column chromatography on the resulting glass beads revealed that they have strong affinities to Cu^{2+}, Ag^{+}, Pb^{2+}, Fe^{3+}, and Cd^{2+}.

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