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Metal Ion-Selective Adsorbent Prepared by Surface-Imprinting Polymerization

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We propose a metal ion-imprinted microsphere as a new metal ion-selective adsorbent. Imprinted microspheres were prepared by seeded emulsion polymerization of divinylbenzene, styrene, butyl acrylate, and methacrylic acid. The imprinted structure was introduced on the carboxylated microsphere by surface-imprinting, in which the carboxyl groups were reorganized through complexation with metal ions on the surface and then fixed by crosslinking polymerization in their specific orientation. The Cu(II), Ni(II), and Co(II)-imprinted microspheres were obtained as submicron particles with average diameters of 0.55—0.60 μm. They were immediately used as a metal ion-selective adsorbent without any further treatment, such as grinding and sieving. The adsorption behaviors of metal ions (Cu(II), Ni(II), and Co(II)) were examined, and the imprinted effects were verified on Cu(II)- and Ni(II)-imprinted microspheres. The imprinted microspheres adsorbed the corresponding guest ion more effectively than did unimprinted ones.

A molecular imprinting technique[1)] has attracted much attention as a method to prepare highly-selective host polymers. This method has been demonstrated to be effective for metal ions as well as for organic molecules. Nishide et al. have reported the first example of metal ion-imprinted resins,[2,3)] which could adsorb the guest metal ion selectively from a weakly acidic solution. This feature is in remarkable contrast with that of conventional metal-chelating resins, which are usually designed for use in more acidic solutions.[4)] Since their successful work, various metal ions have been examined as imprinting guests, which include Cu(II), Zn(II), Ni(II), Co(II), Cd(II), Fe(III), Hg(II), and Ca(II).

The metal ion-imprinted resins reported so far may be classified into the following two types in terms of their preparation procedures (Scheme 1). 1) Linear chain polymers carrying metal-binding groups are crosslinked with bifunctional reagent in the presence of metal ions. 2) Metal ion-complexing monomers are prepared, and the complexes are polymerized with matrix-forming monomers. As a typical example of method 1, Nishide et al. crosslinked poly (4-vinylpyridine) with 1,4-dibromobutane in the presence of metal ions.[2—4)] Kabanov et al. crosslinked a copolymer of diethyl vinylphosphonate and acrylic acid with *N*,*N*′-methylenediacrylamide in the presence of metal ions.[5,6)] Ohga et al. crosslinked chitosan with epichlorohydrin to give an imprinted resin.[7)] The obtained resin was utilized in column chromatography for separating metal ions.

The first example for method 2 was also reported by Nishide et al., who polymerized a metal complex of 1-vinylimidazole with 1-vinyl-2-pyrrolidone and divinylbenzene (DVB).[8)] Gupta et al. polymerized DVB and a metal complex of 4-vinyl-4′-methyl-2,2′-bipyridine in methanol.[9)] Rosatzin et al. polymerized *N*,*N*′-dimethyl-*N*,*N*′-bis(4-vinylphenyl)-3-oxapentanediamide (metal ionophore), DVB, and styrene in a chloroform solution in the presence of metal salts.[10)] In these resin preparations, polymerization was carried out without previously isolating the relevant monomer complexes.

As a more sophisticated way of carrying out method 2, specific metal complexes were prepared from ligands having vinyl groups and polymerized with matrix-forming monomers. For example, Kuchen et al. synthesized and isolated a metal ion-acrylic acid complex, and polymerized it with ethylene glycol dimethacrylate in a benzene/methanol solution.[11)] Fujii et al. prepared a metal-ion complex of 2-acetyl-5-(*p*-vinylbenzyloxy)phenol, and polymerized it with acrylamide and ethylene glycol dimethacrylate.[12)] Isobe et al. used Amberlite XAD-2 as a matrix resin and a 4-vinylpyridine–metal ion complex was introduced on the resin through polymerization.[13)]

Although all of these imprinting techniques are certainly promising ways to prepare metal ion-selective adsorbent, they have some fundamental problems in common: The imprinted structures specific to the guest metal ions are usually formed in the bulk resin. Therefore, the resin has to be ground and sieved before use. In addition, if one considers an extension of these imprinting techniques to a variety of guests, a difficulty arises when handling water-soluble substances in general, particularly those organic components of biological origin; the imprinting procedures described above require a guest molecule (or a guest-host monomer complex) to be soluble in matrix monomers, which are quite often hydrophobic. A drastic improvement in the imprinting technique is thus demanded for solving these problems.

We now propose a third category of template preparation. Imprinting is now conducted on a microsphere by surface-imprinting (Scheme 2).[14)] Scheme 2 shows a schematic illustration of the technique with which the imprinted structure is introduced onto the surface of microspheres, especially from the viewpoint of preparative reactions. The material relies on a surface-carboxylated

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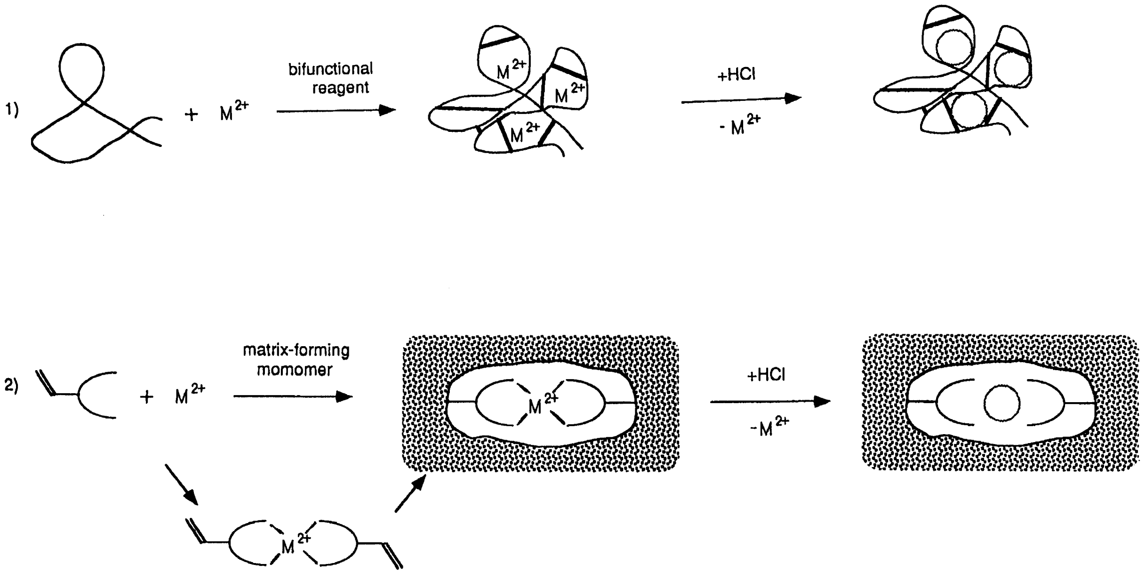
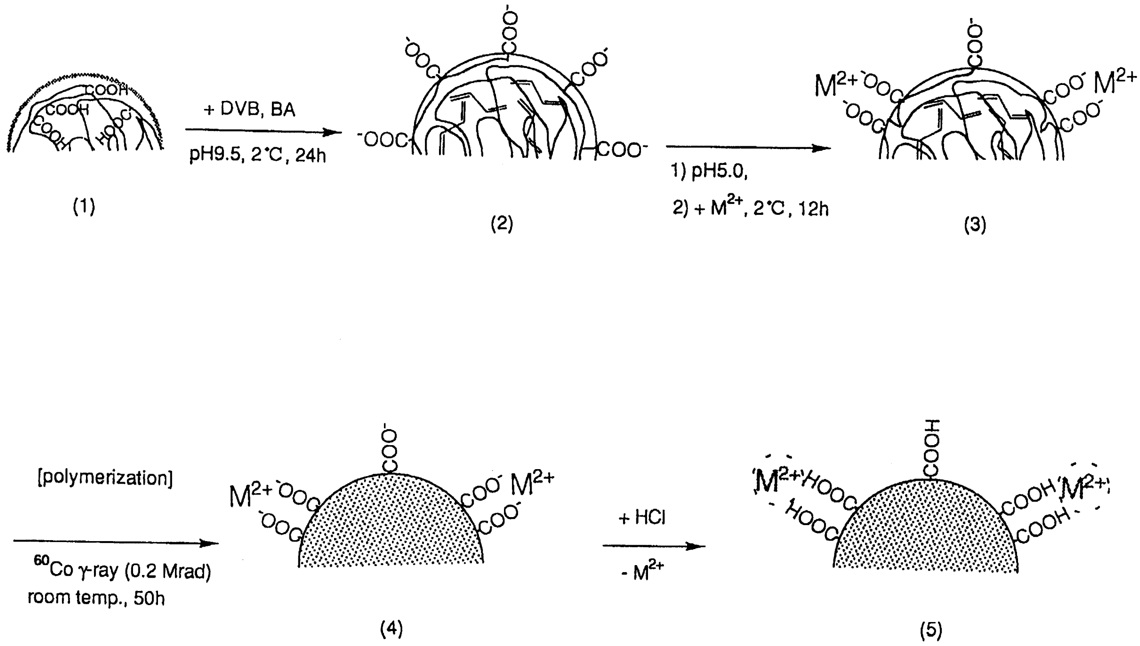


Scheme 1. Conventional methods for a metal-imprinted resin.



Scheme 2. Surface-imprinting technique.

microsphere developed by Okubo et al.[15] In the first step, a guest ion is introduced into a water suspension of the carboxylate-type microsphere. Since the carboxyl groups on the surface are mobile to some extent, a guest ion which interacts with carboxylate ions would induce a reoganization of the groups on the surface, resulting in some optimal conformation and configuration of the carboxylate-carrying polymer chains for complexing the guest. The structure is then immobilized by the second-step (seeded) polymerization by developing an interpenetrating network using a crosslinker. The obtained microsphere, which is spherically well defined and uniform, can be used immediately without either griding or seiving.

In this study, Cu(II), Ni(II), and Co(II)-imprinted microspheres were prepared as metal ion-selective adsorbents by the surface-imprinting technique proposed above. The obtained microspheres were submicron particles with a neat spherical shape. The adsorption of metal ions onto the microsphere was examined and the imprinting effect was characterized.

Experimental

Reagents and Apparatus. Divinylbenzene (DVB) (a gift from Sankyo Chemical Industries, Ltd.), styrene (S), methacrylic acid (MAA), and butyl acrylate (BA) (Tokyo

Kasei Kogyo Co., Ltd.) were used without further purification. Pure water obtained by means of a MILLI-Q Water Purification System (Nippon Millipore Ltd.) was used for metal adsorption tests, and ordinary ion-exchanged water was used for other experiments. A metal-ion standard solution (Wako Pure Chemical Industries Ltd., for atomic absorption analysis) was used for atomic absorption analysis. The other reagents used were of commercially available special grade.

A particle grading analysis and scanning electron microscopy were made by Microtrac CM-type (Nikkiso) and ABT-32 type (Akaishi Beam Technology), respectively. The metal-ion concentration was determined by SAS 760 Atomic Absorption Spectrophotometry (Seiko Instruments Inc.)

Preparation of Microsphere. The preparation procedure of metal ion-imprinted microspheres is summarized in Table 1. S, BA, MAA, and water (H_2O) in a reaction flask were polymerized under a nitrogen atmosphere at 70°C and pH 2.2 for 7 h by using potassium peroxodisulfate ($K_2S_2O_8$) as an initiator ("Emulsion polymerization" in Table 1). The seed emulsion solution was then brought to room temperature. DVB, BA, and H_2O were further added and the emulsion was left at 2°C and at pH 9.5 for 24 h ("Transfer of carboxyl groups" in Table 1). The pH of the emulsion was then lowered to 5.0 with hydrochloric acid, and a portion (8 cm^3) of the emulsion was combined with a metal-ion solution (40 cm^3 of 0.01 $mol\,dm^{-3}$ Cu(II), Ni(II), or Co(II); chlorides) to achieve complexation between the metal ion and the carboxyl group on the surface. The mixture (emulsion) was then polymerized by the use of ^{60}Co γ-rays (0.2 Mrad, 50 h) at room temperature ("Imprinting polymerization" in Table 1). The microsphere was separated from the medium by centrifugation (10000 rpm, 1 h), and was then washed with a hydrochloric acid solution (0.1 $mol\,dm^{-3}$, 150 cm^3) in order to exchange the bound metal ion with a proton. The obtained microsphere (on which the nature of the specific metal ion was imprinted) was dried under vacuum. Unimprinted microspheres as a reference were synthesized similarly (pH being adjusted at 5.0 when polymerized with γ-rays), but without any metal ion.

Measurement. The adsorption equilibrium of the metal ion onto the microsphere was examined in the following way. The microspheres (0.05 g) were placed in a plastic tube (1.5 cm^3 volume), to which was added a 5.0×10^{-4} $mol\,dm^{-3}$ metal-ion solution (1.0 cm^3). The pH was adjusted at 1.0—5.6 with hydrochloric acid and potassium hydroxide. This metal-ion concentration was adopted by referring to the conditions used in characterizing metal-imprinted resins,[2—13] where metal-ion concentrations of 4.0×10^{-5}—5.0×10^{-3} $mol\,dm^{-3}$ were used. The mixture was shaken at 20 °C for a specified period of time. The equilibrated mixture was centrifuged (10000 rpm, 1 h), and the supernatant solution was analyzed for the metal ion by atomic absorption spectrophotometry. The adsorption percentage (percentage of the amount of metal ion adsorbed to that initially added) was calculated from the data.

Results and Discussion

Concept of Surface-Imprinting and Preparation of Metal Ion-Imprinted Microsphere. The proposed concept of surface-imprinting is illustrated in Scheme 2, taking a surface-carboxylated microsphere as an example. A seed emulsion was first prepared from styrene (S), butyl acrylate (BA), and methacrylic acid (MAA) using $K_2S_2O_8$ as an initiator ((1) in Scheme 2). BA and divinylbenzene (DVB) were then added into the S/BA/MAA-made seed emulsion; the mixture was left at 2°C and pH 9.5 for 24 h. With this treatment, the seed microsphere becomes swollen (plasticized) and the polymer chains in the microsphere bulk are provided with substantial mobility. Since the carboxylic acid residue is converted into the anionic carboxylate form at the microsphere surface, most of the carboxyl groups in the bulk eventually migrate out onto the aqueous-organic interface and do not go back into the inside bulk of the microsphere ((2) in Scheme 2).

Now, heavy metal ions (0.01 $mol\,dm^{-3}$) were introduced into the swollen seed emulsion so as to induce a rearrangement of the surface-carboxylato groups by complexation with metals ((3) in Scheme 2). Here, Cu(II), Ni(II), Co(II), and Zn(II) were adopted as guest ions with reference to an Irving–Williams series of stability. However, Zn(II) could not be used because of the formation of metal hydroxide precipitates. Also, the concentrations of 0.10 and 0.05 $mol\,dm^{-3}$ of Cu(II), Ni(II), and Co(II) salts could not be used, since the addition of metal ions at these concentrations caused a destruction (coagulation) of the emulsion; complexation between the metal ion and the carboxylato group would reduce the surface charge of the microsphere and unstabilized the dispersion of particles in the emulsion solution. A reorganization of the semimobile, coordinating carboxylato groups should contribute to the formation of an imprinted structure.

It should be pointed out here that the metal imprinting and reorganization of the carboxylato groups were carried out at pH 5.0, rather than at pH 9.5, where carboxyl group migration to the microsphere surface was induced. This was done in order to avoid the formation of metal hydroxide precipitates. However, under such lowered pH conditions, a considerable fraction of the carboxylato groups are re-converted into the free carboxyl group. The free carboxylic acids are soluble in an organic medium; it is quite possible that such a free-acid portion of the carboxyl groups again submerges back into the organic bulk phase of the microsphere. This can lead to a diminished number of available carboxyl groups per unit weight (reduced capacity) of the resultant resin in conventional ion-exchange processes.

The DVB-containing emulsion was irradiated by ^{60}Co γ-rays at room temperature in order to initiate radical polymerization in the monomer-swollen microsphere ((4) in Scheme 2). Polymerizations at low temperatures are expected to produce a good imprinting effect.[16,17] Thus, the entire structural feature is preserved by the interpenetrating networks provided by polymerized DVB. The obtained imprinted microspheres were washed out with hydrochloric acid to remove the im-

Table 1. Recipes of Seeded Emulsion Polymerization

Materials	Treatment		
	Emulsion polymerization[a]	Transfer of carboxyl groups[b]	Imprinting polymerization[c]
Seeded emulsion solution (cm^3)	—	100	8[d]
Butyl acrylate (g)	2.71	2.9	—
Styrene (g)	20.55	—	—
Methacrylic acid (g)	1.64	—	—
Divinylbenzene (g)	—	29.86	—
Water (g)	75	95	—
Metal ion solution (cm^3)[e]	—	—	40
Potassium persulfate (mg)	125	—	—

a) The polymerization was conducted at 70°C and pH 2 for 7 h under nitrogen atmosphere. The pH was adjusted with hydrochloric acid. b) The emulsion solution was left at 2°C and pH 9.5 for 24 h in order to transfer carboxyl groups onto the surface from the inside of the seed particle. The pH was adjusted with potassium hydroxide. c) The pH of the emulsion solution was adjusted to 5.0 by careful addition of hydrochloric acid. A portion (8 cm^3) of the emulsion was taken and combined with an aqueous metal ion solution (40 cm^3). The mixed emulsion solution was polymerized at room temperature for 50 h by use of ^{60}Co γ-rays (0.2 Mrad). d) The figure stands for the amount of the emulsion solution after transfering carboxyl groups. e) 0.01 mol dm^{-3} metal ion adjusted at pH 5.0 with hydrochloric acid.

printed metal ions ((5) in Scheme 2).

Physicochemical Characterization of Microspere. Microspheres were obtained in 85—90% yield. The analysis was made for their chemical composition and particle size distributions. Some results are summarized in Table 2. The elemetal analyses are in good correspondence to the chemical composition in the feed mixture. A small lowering of the carbon content and a small increase of the hydrogen content seem to indicate that the microspheres are somewhat hygroscopic. Ash (inorganic component) was not detected in the imprinted microspheres, suggesting that the metal ion used as the template was throughly removed from the microspheres.

The particle grading analysis of Cu(II)-imprinted microsphere is shown in Fig. 1. Other microspheres indicated similar particle distributions. The graph in Fig. 1 reveals a somewhat broader particle size distribution in the present preparation, when compared with that of the S/BA/MAA-made surface-carboxylated microsphere prepared by the recipe of Okubo et al.[18] This may be ascribed to a small difference in the two preparation procedures. Figure 2 shows a typical view of Cu(II)-imprinted microspheres by scanning electron microscopy. Similar particle form and distribution patterns were observed with other microspheres. These confirm that the obtained microspheres have dimensions of submicron and assume a neat spherical shape. The results are well-consistent with those of the particle grading analyses.

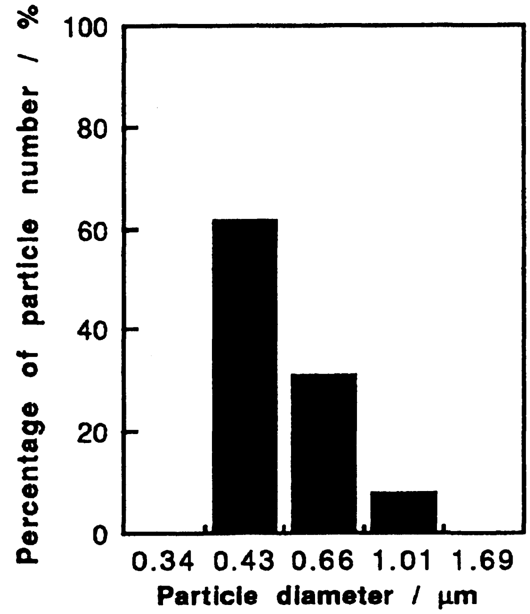


Fig. 1. Particle distribution of Cu(II)-imprinted microspheres.

Metal Adsorption Behavior of Microspheres. The metal adsorption behavior of the unimprinted and Cu(II)-, Ni(II)-, and Co(II)-imprinted microspheres were examined at pH 5.6 (Table 3). The adsorption percentage was calculated as an average value from 3—5 measurements, since each measurement showed some (small) deviation (±2%). The adsorption is conveniently expressed by the percentage of metal ions adsorbed on the resin to the total amount of metal ions originally added to the solution. Under the particular conditions given in Table 3, 100% adsorption corresponds to 1.0×10^{-2} mmol metal ion/g-resin on the dry-resin basis.

First, Cu(II) adsorption is considered in Table 3. If a comparison is made among the types of microspheres, Cu(II) is most effectively adsorbed by Cu(II)-imprinted microsphere, followed by unimprinted, Ni(II)-imprinted, and Co(II)-imprinted microspheres (in

Table 2. Analytical Data of Unimprinted and Metal Ion-Imprinted Microspheres

Microsphere	Yield%	Elemental analysis[a]			Average diameter/μm
		C%	H%	Ash content	
Unimprinted	85	87.85	7.91	N.D.	0.56
Cu(II)-imprinted	90	87.98	7.97	N.D.	0.55
Ni(II)-imprinted	89	87.83	7.94	N.D.	0.60
Co(II)-imprinted	86	87.83	7.91	N.D.	0.56

a) Theoretical feed value: C 88.62, H 7.89%.

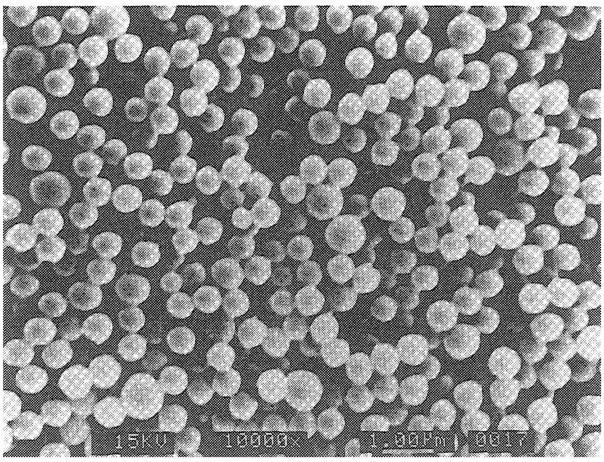


Fig. 2. Scanning electron micrograph of Cu(II)-imprinted microspheres.

Table 3. Adsorption Percentage of Metal Ion onto Unimprinted and Metal Ion-Imprinted Microspheres

Imprinted ion	Adsorption percentage/%		
	Cu(II)	Ni(II)	Co(II)
None	24	0	0
Cu(II)	40	7	0
Ni(II)	14	17	0
Co(II)	5	0	0

Conditions: pH 5.6, 0.05 g microsphere, 1.0 cm^3 metal ion solution (5.0×10^{-4} mol dm^{-3} metal), and 18 h shaking time.

this order). This sequence clearly verifies the effect of Cu(II) imprinting; the carboxylato groups and the polymer chains that carry them are placed on the Cu(II)-imprinted microsphere surface in such a way that they match the square planar coordination around the Cu(II) ion when they interact with the Cu(II) ion. However, a diminished affinity of Ni(II)- and Co(II)-imprinted microspheres, as compared to an unimprinted microsphere, should also deserve mention. In an unimprinted microsphere, carboxyl groups are distributed on the surface in some energetically optimal placements, which are determined by such solution conditions as the pH and counter ion (K^+) concentration. Although this distribution of carboxyl groups happens to favor the accomodation of the Cu(II) ion to a considerable extent (24% adsorption as compared to 40% by Cu(II)-imprinted microsphere), the Ni(II)- and Co(II)-imprinting conditions destroy such a distribution of the carboxyl functional groups. As a result, Cu(II) adsorption by Ni(II)- and Co(II)-imprinted microspheres decreases to 14% and 5%, respectively. This clearly reveals a negative imprinting effect to nontargeted metal ions.

As to for Ni(II) adsorption, only Cu(II)- and Ni(II)-imprinted microspheres exhibited a binding affinity under the present adsorption conditions; the adsorption is higher for Ni(II)-imprinted microspheres (17%) than for Cu(II)-imprinted microspheres (7%). This, again, clearly indicates the effect of imprinting.

It is important to note that whereas unimprinted microsphere do not show any adsorption affinity to the Ni(II) ion, the Ni(II)-imprinted microsphere now indicates considerable affinity to Ni(II); obviously, the Ni(II)-imprinting procedure altered the nature of the carboxylato group dispositions on the microsphere so as to accomodate otherwise inaccessible Ni(II) ions.

A small comment may be made regarding the fact that the Cu(II)-imprinted microsphere indicates the appreciable adsorption ability to Ni(II). Ideally, the surface structure of the Cu(II)-imprinted microsphere should have been constructed to accept specifically Cu(II). However, Ni(II) is a rather strongly complexing metal ion which is only proceeded by the Cu(II) ion in the Irving–Williams complexation stability series. In addition, although Cu(II) complexes usually assume a square planar configuration, a weak octahedral configuration around Cu(II) is also possible. It is therefore possible that some complexing sites on the Cu(II)-imprinted microsphere are so constructed that they are sufficiently flexible to adapt to octahedral coordination around the incoming metal ions, such as Ni(II), which is typically octahedrally coordinating.

Co(II) binding was not observed for all the microspheres under the present adsorption measurement conditions. This is probably due to the fact that the complexation tendency of the Co(II) ions is rather low, as seen in the Irving–Williams series.

The Ni(II)-imprinted microsphere is characterized by a Ni(II) binding affinity which exceedes the affinity to the Cu(II) ion. This is in contrary to the Irving–Williams series. In order to confirm this surprising result, we prepared a Ni(II)-imprinted microsphere in duplicate, and found that the two preparations gave almost identical results; preparation 1, 14% for Cu(II) and 17% for Ni(II); preparation 2, 13% for Cu(II) and 18% for Ni(II). It is emphasized that the results all came from the difference in the spatial disposition of the carboxylato groups which are fixed on the microsphere surface by crosslinked DVB. On the other hand, the Co(II)-imprinted microsphere is unique in that the resin does not seemingly show any characteristic preference to particular metal ions. We presume that the high metal concentration (8.3×10^{-3} mol dm^{-3}) in the imprinting step gave the Co(II)-imprinted microsphere some characteristic changes in the surface structure. Although this was suggested by the negative imprinting effect (as discussed above), the assessing technique presently adopted has so far failed to disclose them in detail.

Figure 3 shows the pH dependence of the metal-ion adsorption onto Cu(II)-and Ni(II)-imprinted microspheres and an unimprinted microsphere. The pH was adjusted at pH 1.0—5.6 with hydrochloric acid and potassium hydroxide. Imprinted microspheres adsorbed the corresponding imprinted metal ions more effectively than do unimprinted microspheres over the entire pH range where metal adsorption took place. The adsorption percentages increased with increasing pH on every microsphere. This means that the carboxyl groups on the microsphere surface participate in the metal-ion binding in their ionized carboxylate forms. Although the data given in Table 3 and Fig. 3 were collected with an 18 h equilibration reaction, the equilibria were confirmed to be attained practically within 1 h.

Preparation Conditions of Microspheres and Metal Adsorption Behavior. The present authors previously synthesized surface-carboxylated microspheres according to the report of Okubo et al., and examined its metal adsorption behavior.[18] If one compares the Cu(II) adsorption on that (standard) surface-carboxylated microsphere with that on the unimprinted microspheres prepared in the present study, the former indicates a larger adsorption percentage than do the latter. In other words, the standard microsphere exhibits a larger Cu(II)-binding capacity under the same pH conditions. This can be explained (as suggested before) by considering the effect of lowered pH conditions under which the sencond (seeded, imprinting)polymerization was carried out. The unimprinted microsphere in the present study was prepared at pH 5.0, while the standard microsphere was prepared at pH 9.5.

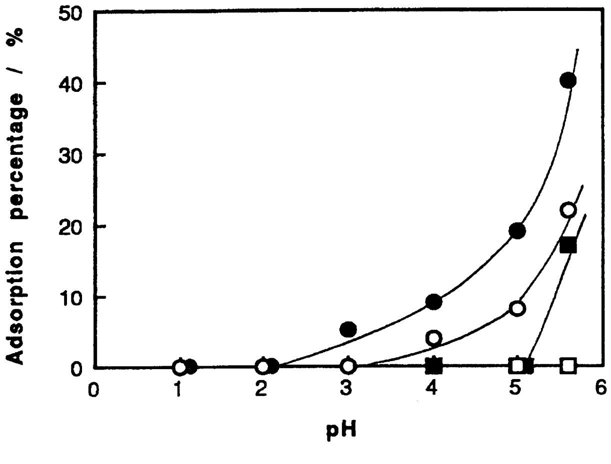


Fig. 3. Metal ion adsorption onto unimprinted and metal ion-imprinted microspheres. Effect of the pH. ●: Adsorption of Cu(II) onto Cu(II)-imprinted microsphere, ○: Adsorption of Cu(II) onto unimprinted microsphere, ■: Adsorption of Ni(II) onto Ni(II)-imprinted microsphere, and □: Adsorption of Ni(II) onto unimprinted microsphere. Conditions: 0.05 g microsphere, 1.0 cm^3 metal ion solution (5.0×10^{-4} mol dm^{-3} metal), and 12—18 h shaking time.

Concerning the reproducibility of the preparation of microspheres, some puzzling observations were made when the effect of lowering the pH was studied in relation to the treatment in stage (3) of Scheme 2. When the pH-adjusted (pH 5.0) emulsion was mixed with an imprinting Cu(II) solution and the mixed emulsion was left without stirring at 2°C for 12 h before polymerization with γ-rays (without stirring during the irradiation), the obtained Cu(II)-imprinted microsphere showed a Cu(II) adsorption percentage of 40%, as indicated in Table 3. Similarly, the pH-adjusted emulsion was left unstirred before polymerization with γ-rays, and the resultant unimprinted microspheres showed a Cu(II) adsorption percentage of 24%, as also indicated in Table 3. However, when the pH-adjusted (pH 5.0) emulsion was well-stirred at 2°C for 12 h before polymerization, the microspheres obtained proved to be characterized by a quite different Cu(II) binding behavior. Thus, when the pH-adjusted (pH 5.0) emulsion was stirred magnetically at 2°C for 12 h and then polymerized by γ-rays (without stirring during the irradiation), the obtained Cu(II)-imprinted microspheres showed Cu(II) adsorption of only 18% under the same adsorption measurement conditions to that given in Table 3. In the same manner, the unimprinted microspheres obtained with a stirring treatment showed Cu(II) adsorption of only 5%. The origin of this phenomena (discrepancy in surface structure) is still not clear. It is quite probable that this is associated with the rate process in the reorganization (relaxation) of polymer chains, which follows after changing the pH of the aqueous medium. However, as to why and how a hydrodynamic process (stirring) can affect the relaxation process of the polymer chain in the microsphere, the authors are not yet ready to present any rationalizing idea. It is obviously a complexing factor, in the preparative aspect, in the present surface imprinting polymerization.

In conclution, metal ion-imprinted microspheres as metal ion-selective adsorbent were synthesized by surface-imprinting. The imprinted structure was constructed on the surface with the aid of the interaction between the metal ion and the surface-mobile carboxyl group. The structure was then immobilized by seeded emulsion polymerization using a crosslinker. The imprinted microsphere, thus obtained, adsorbed the corresponding guest metal ion more effectively than did the unimprinted microsphere. The microsphere features rapid equilibration in adsorbing the metal ion. The uniform and neat spherical beads do not require any pretreatment, such as grinding or sieving, before use. The surface-imprinted microsphere is, therefore, expected to be a useful material for the separation and concentration of metal ions.

The "surface-imprinting" technique should be applicable to various kinds of guest molecules, such as biological components, which are soluble in water but not in oil. In addition, this technique would improve the complexation behaviors, including the capacity, reaction rate, selectivity, and so on, based on the unique features of submicron spheres.

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