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Surface Imprinting. Characterization of a Latex Resin and the Origin of the Imprinting Effect

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In accordance with the concept of Surface Imprinting, as proposed by the present authors, a Cu(II)-imprinted microsphere was studied in detail in order to obtain information about the origin of the imprinting effect. Unimprinted and Cu(II)-imprinted microspheres were prepared under several pH (4.0, 5.0, 5.6, or 6.0) conditions in second-step polymerization. The obtained microspheres were characterized regarding particle form, size-distribution pattern, average diameter, Cu(II) adsorption behavior, and so on. During preparation at pH 4.0—5.6 in the second-step polymerization, imprinted microspheres adsorbed Cu(II) more effectively than did unimprinted microspheres in this pH range, indicating a clear metal-imprinting effect. On the other hand, the imprinting effect was not observed during preparation at pH 6.0, because of the formation of copper(II) hydroxide precipitates. The maximum imprinting effect was provided by microspheres obtained at pH 5.6. Spectroscopic studies were made on Cu(II)-loaded microspheres. The FT-IR spectra suggested that carboxyl groups on the microsphere surface participated in the Cu(II) binding in their ionized carboxylate forms. An FE-SEM (ångstrom SEM) observation of the microsphere indicated a smooth surface structure at the 10 nm level. The collected data indicate that the origin of the imprinting effect on a Cu(II)-imprinted microsphere is the interaction between Cu(II) and the carboxylate group at the aqueous-organic interface, supporting the concept of Surface Imprinting.

A molecular imprinting technique has attracted much attention as a method to prepare support materials for molecular separation and concentration, easily, rapidly, inexpensively, and on a large scale.[1)] This technique has been demonstrated to be effective for metal ions,[2,3)] saccharides,[4,5)] amino acid derivatives,[6,7)] organic compounds of low molecular weight,[8,9)] and so on. Since the preparations are all based on solution polymerization or bulk-polymerization techniques, the imprinted structures are usually formed in the resultant bulk resins. This raises some fundamental problems, such as: 1) grinding and sieving necessary before use, 2) partial destruction of the imprinted structures, which is inevitable in the grinding process, 3) a residual guest in the resin possible, and 4) difficulty in handling the water-soluble organic molecules of biological importance as a guest molecule.

We have proposed a new imprinting technique called "Surface Imprinting" to solve these problems.[10,11)] In this technique, an aqueous–organic interface in an emulsified resin suspension (latex) is used as a guest-recognition field in the imprinting polymerization. The concept of this technique is shown in Scheme 1. First, a seed microsphere emulsion is prepared, which contains linear-chain polymers carrying the functional groups, which can interact with the guest molecule (I). Divinyl-type monomers are then added to this emulsion. The seed microspheres become swollen and the polymer chains are provided with substantial mobility. When guest molecules are introduced into the emulsion, the functional groups interact with the guest molecules at the aqueous–organic interface to form complexes (II). The thus-obtained structures are immobilized by subsequent cross-linking polymerization of the divinyl-type monomers (III). The guest molecules are removed from the microsphere to give an imprinted host structure.

In previous papers,[11,12)] we reported on the preparation of Cu(II)-, Ni(II)-, and Co(II)-imprinted microspheres, which were spherically well defined and uniform, and could be used immediately as a metal adsorbent without either grinding or sieving. However, the previous papers dealt with only the verification of an

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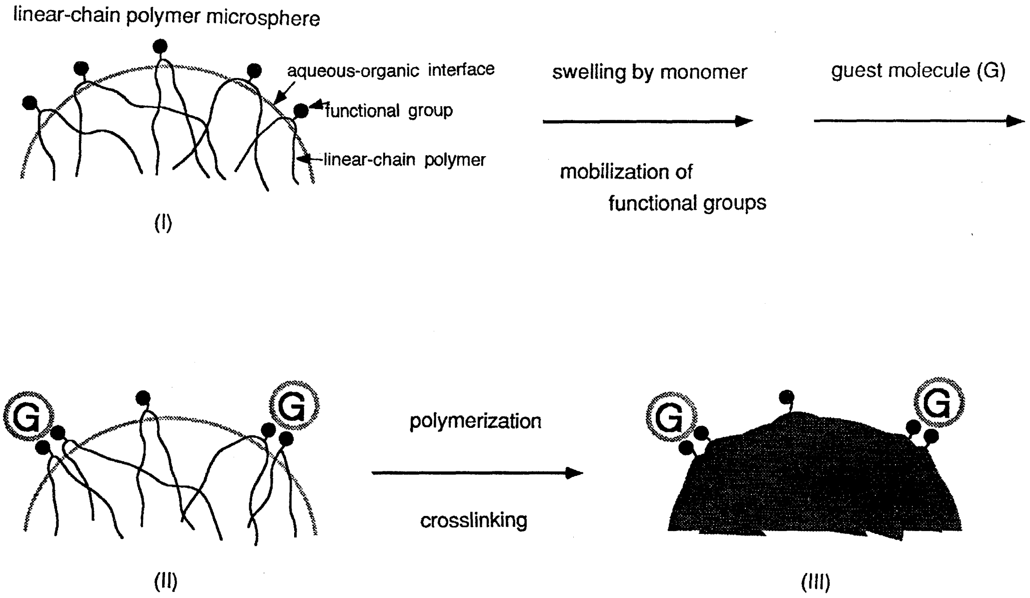


Scheme 1. Concept of surface imprinting.

imprinting effect, as estimated by a metal adsorption test. In the present paper, a Cu(II)-imprinted microsphere was studied in detail in order to obtain information about the origin of the imprinting effect in this technique.

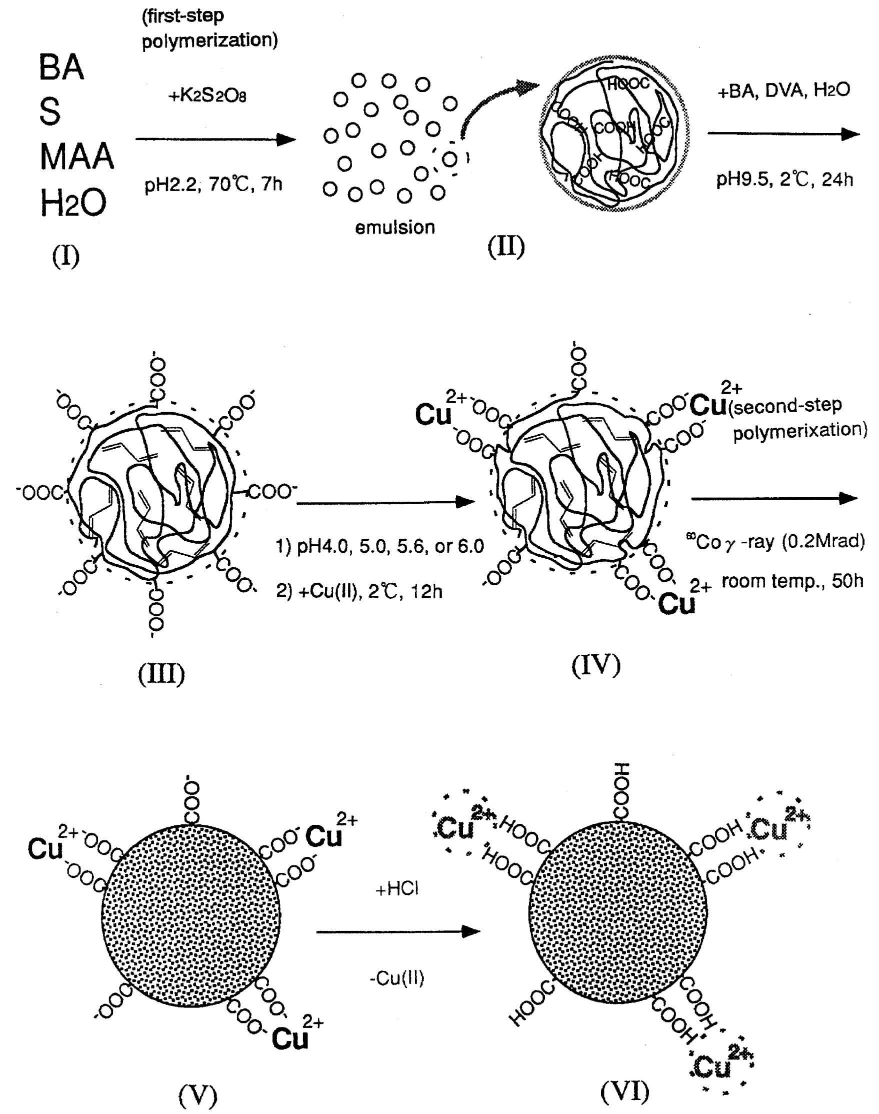
Complex formation between guest molecules (Cu(II)) and functional groups (carboxylate) in Scheme 1 (II), should depend on the pH. In this connection, the effect of the pH in the imprinting (the second-step polymerization) step on the imprinting effect was examined. Spectrophotometry can give important information about metal-complex formation. Nishide et al.[2] examined their metal-imprinted resins based on the electronic and ESR spectra. Kabanov et al.[3] studied their resin using the IR and NMR spectra, and Isobe et al.[13] using the ESR spectra. In the present study, both FT-IR and ESR spectra measurements were made. The structure of the surface of microsphere was also studied by FE-SEM. Furthermore, an equilibrium consideration was made concerning the chemical species formed at the surface of the swollen pre-polymerization microsphere in order to obtain a clue about the formation of the template structure in the present imprinting technique. The origin of the imprinting effect is discussed based on these data.

Experimental

Reagents and Apparatus. Divinylbenzene (DVB) was a gift from Sankyo Chemical Industries, Ltd. Styrene (S), methacrylic acid (MAA), and butyl acrylate (BA) were obtained from Tokyo Kasei Kogyo Co., Ltd. and were used as received. 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. Metal-ion standard solutions for atomic-absorption analyses were obtained from Wako Pure Chemical Industries Ltd. All other reagents were of commercially available special grade.

Particle grading analysis and FT-IR spectra measurements were made by Microtrac CM-type (Nikkiso) and 510M-type (Nicolet), respectively. Scanning electron microscopy (SEM) was made on an ABT-32 type (Akaishi Beam Technology) and S-900 type (FE-SEM; Hitachi). ESR spectra were obtained by JES-FE1XG (JEOL). The metal-ion concentration was determined by SAS 760 Atomic Absorption Spectrophotometry (Seiko Instruments Inc.).

Preparation of Microspheres. Cu(II)-imprinted microspheres were prepared in accordance with the procedure described in the previous paper[11] (Scheme 2). S, BA, MAA, and water (H_2O) in a reaction flask were polymerized under a nitrogen atmosphere at 70 °C and at pH 2.2 for 7 h using potassium peroxodisulfate ($K_2S_2O_8$) as an initiator (first-step polymerization) ((I) in Scheme 2). The obtained seed emulsion solution was then brought to room temperature. DVB, BA, and H_2O were further added and the emulsion was kept at 2 °C and pH 9.5 for 24 h. With this treatment, the seed microsphere becomes swollen or plasticized and the polymer chains in the microsphere bulk are provided with substantial mobility. Since the carboxylic acid residue is converted into an anionic carboxylate form at the microsphere surface, a substantial portion of the carboxyl groups in the bulk eventually migrate out onto the aqueous-organic interface and stay there ((III) in Scheme 2). The pH value of the emulsion was then lowered to 4.0, 5.0, 5.6, or 6.0 with hydrochloric acid. A portion (8 cm^3) of the emulsion was taken and combined with a Cu(II) solution (chloride, 0.01 $\mathrm{mol\,dm}^{-3}$, 40 cm^3). The complexation between the metal ions and the carboxyl groups on the microsphere surface should result in a reorganization of the carboxylate groups to form a metal-imprinted structure on the microsphere surface ((IV) in Scheme 2). The mixture (emulsion) was then polymerized by the use of $^{60}\mathrm{Co}$ γ-rays[14,15] (0.2 Mrad, 50 h) at room temperature (second-step polymerization) ((V) in Scheme 2). With this procedure, the imprinted structure was fixed or immobilized through an interpenetrating net-



Scheme 2. Synthetic procedure for Cu(II)-imprinted microsphere.

work formation by a crosslinker, DVB. The microsphere was separated from the medium by centrifugation (10000 rpm, 1 h), and then washed with a hydrochloric acid solution (0.1 mol dm^{-3}, 150 cm^{3}) to exchange the bound metal ions with protons ((VI) in Scheme 2). The obtained microsphere (on which the nature of the specific metal ion was imprinted) was dried under a vacuum. An unimprinted microsphere as a reference was synthesized similarly (pH being adjusted at 4.0, 5.0, 5.6, or 6.0 when polymerized with γ-rays), but without metal ions.

Cu(II) Adsorption Measurement. The adsorption equilibrium of Cu(II) 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} Cu(II) solution (chloride, 1.0 cm^{3}). The pH was adjusted to 5.6 with hydrochloric acid and potassium hydroxide. 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 metal ions by atomic absorption spectrophotometry. The adsorption percentage (the percentage of the amount of metal ions adsorbed to that initially added) was calculated from the data.

Preparation of Cu(II)-Loaded Microspheres for FT-IR and ESR Studies. In order to examine the origin of the imprinting effect through FT-IR and ESR, three types of Cu(II)-loaded microspheres were prepared, and named Samples 1, 2, and 3 in the following way. A Cu(II)-imprinted microsphere (VI in Scheme 2) as well as an unimprinted one (0.15 g, respectively), which were prepared at pH 5.6 in the second-step polymerization, were added to a 5.0×10^{-4} mol dm^{-3} Cu(II) solution (3.0 cm^{3}). The pH of the solution was adjusted to 5.6 with hydrochloric acid and potassium hydroxide. The mixture was shaken at 20 °C for 18 h. The equilibrated mixture was centrifuged (10000 rpm, 1 h), and the separated Cu(II)-loaded microspheres were dried under a vacuum. The amount of Cu(II) adsorbed on the microsphere was determined by analyzing the remainder of the metal ions in the supernatant solution after centrifugation. The Cu(II)-loaded unimprinted and Cu(II)-loaded imprinted microspheres were named Samples 1 and 2, respectively. On the other hand, the microsphere V in Scheme 2 was separated from the second-step polymerization mixture by simple centrifugation. The obtained Cu(II)-loaded microsphere was dried under vacuum and named Sample 3.

Results and Discussion

Chemical Composition of Microsphere. Unimprinted and Cu(II)-imprinted microspheres, which were prepared at pH 4.0, 5.0, 5.6, and 6.0 in second-step polymerization, were obtained in 85—90% isolated yield. The elemental analyses (C; 87.84—87.98%, H; 7.91—7.97%) were in good agreement with the chemical composition of the feed mixture (C; 87.89%, H; 7.96%). The FT-IR spectra for these microspheres (KBr disk) indicated the absorption bands for carboxylate ester (C=O; stretching vibration) at 1730 cm^{-1}, dimeric carboxyl group (C=O stretch, 1705 cm^{-1}), and other functional groups essential to the expected chemical structure.

Particle-Size Distribution Pattern and Average Diameter of a Microsphere. The particle form, size-distribution pattern, and average diameter were examined for unimprinted and Cu(II)-imprinted microspheres. Copper(II) hydroxide precipitates were observed only when a Cu(II)-imprinted microsphere was prepared at a relatively high-pH region (pH 6.0).

The particle-distribution pattern became sharp with increasing preparation pH in the second-step polymerization for both unimprinted and Cu(II)-imprinted microspheres, except for the Cu(II)-imprinted microspheres obtained at pH 6.0 (partial precipitation of copper(II) hydroxide) (Fig. 1). Meanwhile, the average diameters decreased with increasing preparation pH, except, again, for Cu(II)-imprinted microspheres obtained at pH 6.0 (Fig. 2). There was no difference in either the distribution pattern or the average diameter between unimprinted and Cu(II)-imprinted microspheres prepared at pH 4.0—5.6. The observations discussed above by a Microtrac CM instrument were also con-

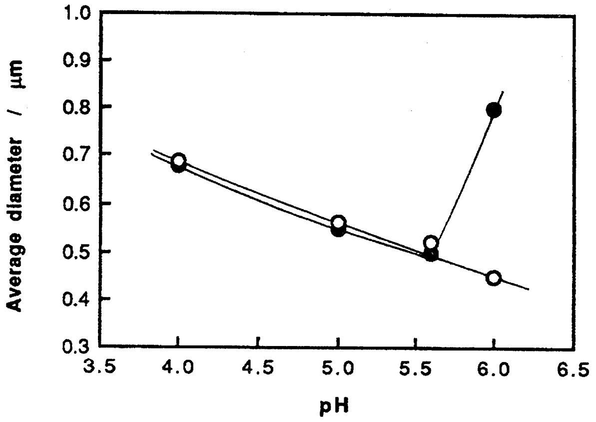
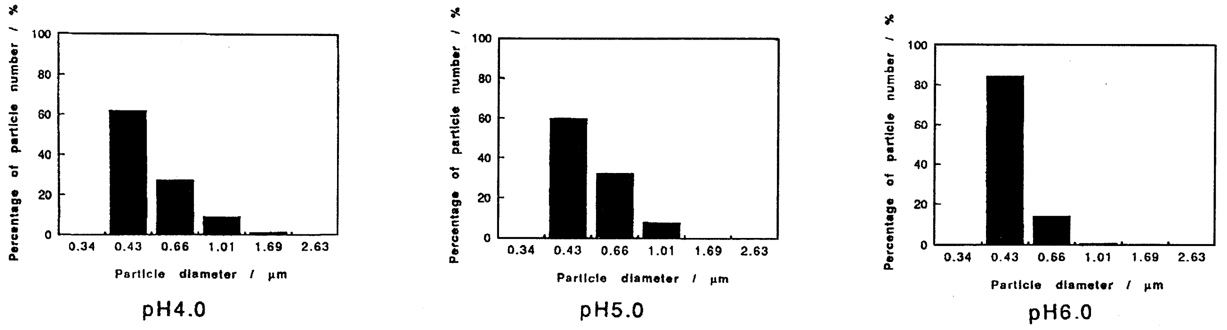
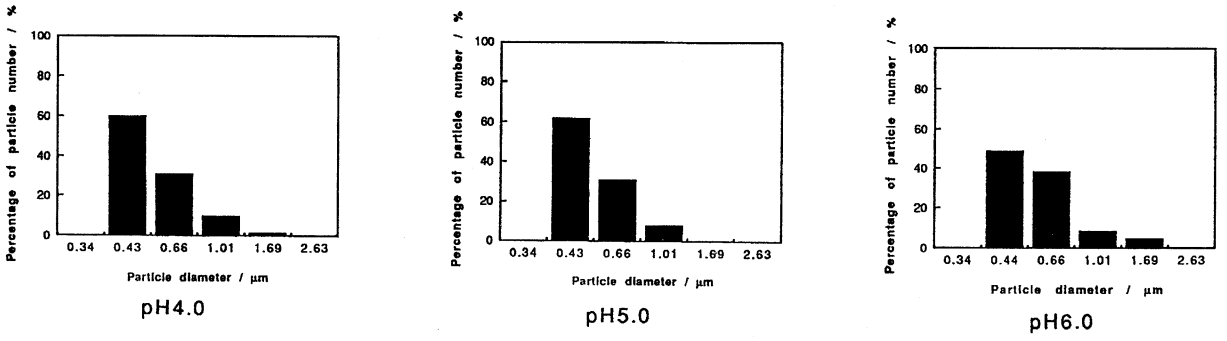


Fig. 2. pH regulation in preparation[1)] and average diameters of resultant microspheres. ○, unimprinted microsphere; ●, Cu(II)-imprinted microsphere. 1) The pH values are for treatment (III)—(IV) in Scheme 2.



(a) Unimprinted microsphere.



(b) Cu(II)-imprinted microsphere.

Fig. 1. pH regulation in preparation[1)] and particle size distribution of resultant microspheres. 1) The pH values are for treatment (III)—(IV) in Scheme 2.

firmed by means of SEM. However, the cause-and-effect relationship for such a size distribution is not yet clear, and is to be disclosed from now on, especially from the view point of the polymerization mechanism.

In general, the average diameter of the microsphere is expected to be closely related to the cation adsorption (or cation exchange) capacity of the microsphere; the capacity per unit mass of the microsphere should increase with decreasing average diameter if the carboxylate groups of the same chemical characteristics are to be placed on the microsphere with the same surface density. However, experimentally there was found no apparent relationship between the average diameter and the Cu(II) adsorption behavior under the conditions of our study, as mentioned in the next section. Therefore, the differences in the average diameters of our microsphere preparations are not given any particular attention for the moment when discussing the origin of the imprinting effect.

Cu(II) Adsorption Test. The adsorption equilibrium of Cu(II) onto the microspheres was examined (Fig. 3). Cu(II)-imprinted microspheres adsorbed Cu(II) more effectively than did unimprinted microspheres over the preparation pH range of 4.0—5.6 in the second-step polymerization. The results clearly verified the imprinting effect. On the other hand, an imprinting effect could not be observed for those microspheres prepared at pH 6.0. The formation of copper(II) hydroxide precipitates in the polymerization mixture should have disturbed the introduction of an imprinted structure on the microsphere surface.

The Cu(II) adsorption behavior did not change over preparation pH range 5.0—6.0 (pH values in the second polymerization step) for unimprinted microspheres. This means that the amount of carboxyl groups available for Cu(II) adsorption is the same among the microspheres prepared in this pH region. This is rather unexpected because in the second polymerization step one can anticipate that a higher pH places more carboxylate groups on the surface of the microsphere, which should lead to a higher Cu(II)-binding capacity. On the other hand, for Cu(II)-imprinted microspheres, the adsorption percentage increased with increasing preparation pH from pH 4.0 to pH 5.6. An imprinting effect (increased Cu(II) adsorption over the unimprinted microsphere) was clearly observed all over this pH range. This should be equivalent to the increase in the number of the available carboxylate sites for Cu(II) binding.

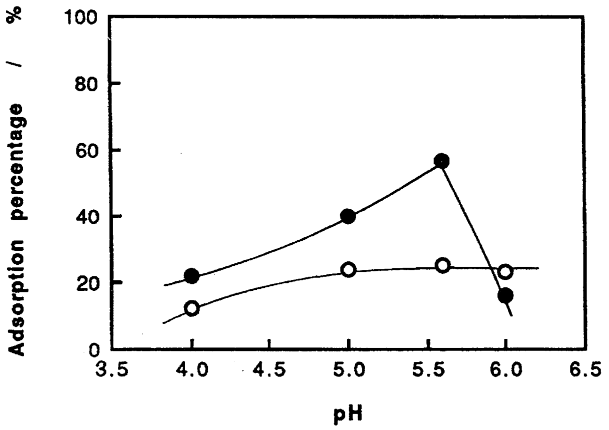


Fig. 3. pH regulation in preparation$^{1)}$ and Cu(II)-adsorption behavior of resultant microspheres. ○, unimprinted microsphere; ●, Cu(II)-imprinted microsphere. 1) The pH values are for treatment (III)—(IV) in Scheme 2. Conditions: 0.05 g microsphere, 1.0 cm^3 Cu(II) solution (5.0×10^{-4} mol dm^{-3}, pH 5.6), and 12 h shaking time.

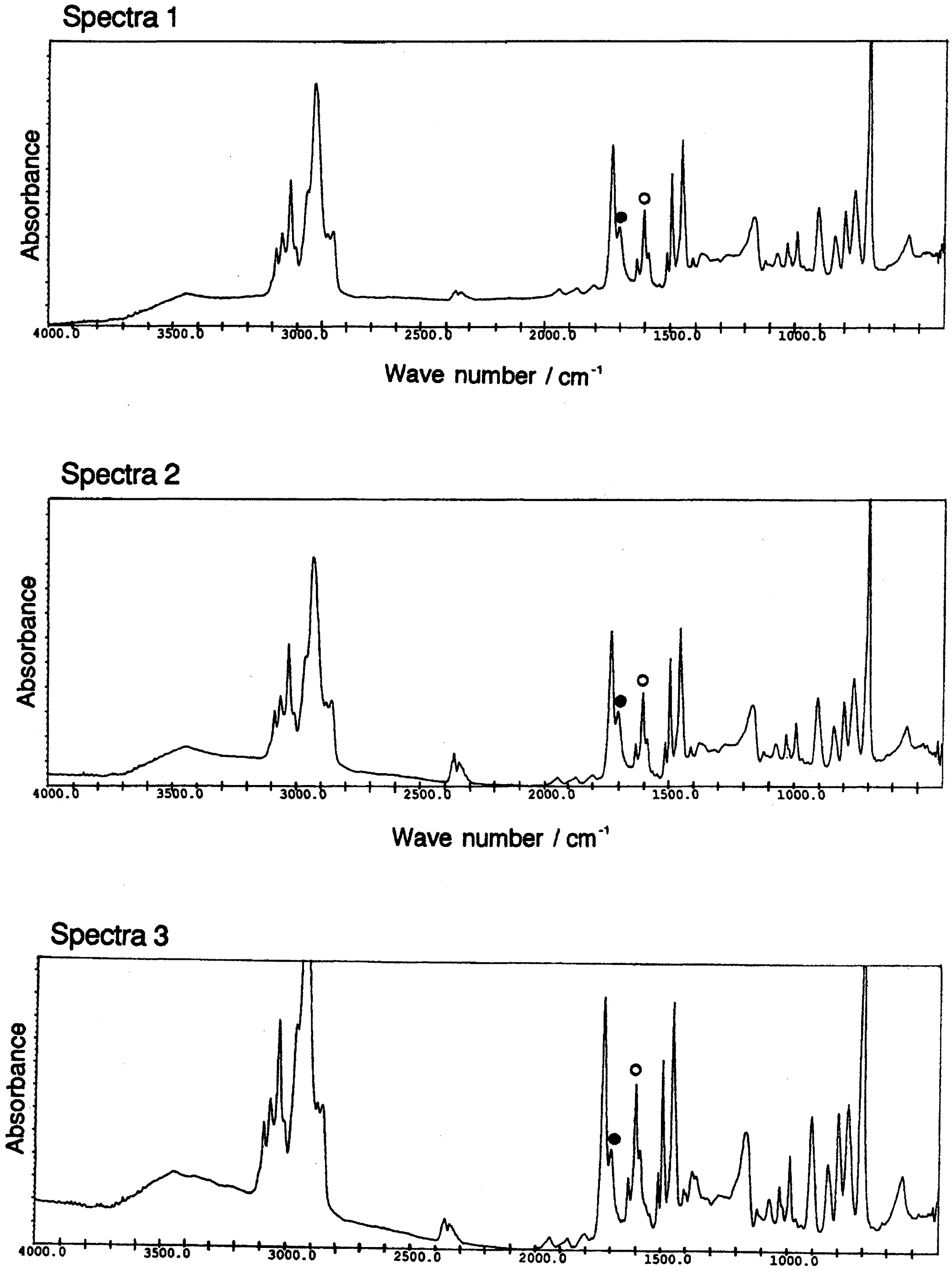
The complex formation between Cu(II) and carboxylic acid does not proceed under very low pH conditions. The increase in pH causes deprotonation of carboxylic acid, which then leads to the formation of carboxylato copper(II) complex; on further raising the pH, the complexation process is finally hampered by copper(II) hydroxide precipitation, the complexation of Cu(II) with hydroxide ion. The features of these complexation process is clearly reflected in the Cu(II) binding profile in Fig. 3. This emphasizes that the origin of the imprinting effect is based on the interaction of Cu(II) and carboxylate group at the aqueous–organic interface of microsphere during the second-step polymerization.

Among many template polymerization practices described to date, the present surface imprinting procedure is the only technique which is carried out in aqueous solution and allows pH adjustment in a substantially wide range. This feature should hopefully find application when water-soluble organic molecules or biological substances are concerned as template guests.

FT-IR and ESR Study on Cu(II)-Loaded Microspheres. First, the amount of Cu(II) carried by the microsphere samples was estimated for Sample 1—3. This was made by measuring the concentration of free Cu(II) in aqueous solution at adsorption equilibrium: Sample 1, 1.5×10^{-6} mol g^{-1}; Sample 2, 5.7×10^{-6} mol g^{-1}; and Sample 3, 1.4×10^{-4} mol g^{-1}. The difference between the amount of Cu(II) on Sample 1 and that on Sample 2 clearly indicated the imprinting effect under the condition of the present adsorption test, 5.0×10^{-4} mol dm^{-3} Cu(II) concentration. On the other hand, the amount of Cu(II) on Sample 3 meant, as described below, that most of carboxyl groups on the surface of Sample 3 interact with Cu(II) in the imprinting step (treatment III—IV in Scheme 2) under the condition of 8.3×10^{-3} mol dm^{-3} Cu(II) concentration.

Okubo et al.$^{16)}$ reported that the amount of carboxyl groups on the microsphere surface and inside the microsphere were 2.2×10^{-4} mol g^{-1} and 1.5×10^{-4} mol g^{-1}, respectively, for the surface-carboxylated microspheres developed by them. The preparation of our present microspheres relied on the recipe by Okubo.$^{16)}$ Therefore, the amount of carboxyl groups in the present microspheres is expected to be similar to that of Okubo's microsphere. We now make here an assumption that the total amount of surface carboxyl groups in our microspheres is 2.2×10^{-4} mol g^{-1} as in the case of Okubo and that each Cu(II) is bound by two carboxylate groups. Then, the mole fraction of carboxyl groups bound to Cu(II) in the total surface carboxyl groups in the Cu(II)-loaded microspheres is to be as follows: Sample 1, 1/70; Sample 2, 1/20; Sample 3, 1/1. It is to be emphasized that all the carboxyl groups on the surface of Sample 3 seem to be complexed with Cu(II).

FT-IR spectra, Spectra 1—3, which correspond to Samples 1—3, are shown in Fig. 4. When the absorption band intensity of acrylic butyl ester at 1730 cm^{-1} was taken as standard (normalized intensity, 1.0), then the absorption band intensities for dimeric carboxyl group at 1705 cm^{-1} and carboxylate anion at 1601 cm^{-1} were, respectively, found to be as follows: Sample 1, 0.41 and 0.52; Sample 2, 0.41 and 0.54; Sam-


Spectra 1

Spectra 2

Spectra 3

Fig. 4. FT-IR spectra of Cu(II)-loaded microsphere. Mark ○ is for absorption by carboxylate group, and mark ● for dimeric carboxyl group. Spectra 1, 2, and 3 are for Sample 1, 2, and 3, respectively. Sample 1, 2, and 3 carry 1.5×10^{-6}, 5.7×10^{-6}, and 1.4×10^{-4} mol g-resin^{-1}, respectively, of Cu(II).

ple 3, 0.31 and 0.62. The values for Samples 1 and 2 were very similar to those of microspheres not loaded with Cu(II) (0.41 and 0.52). In fact, for Samples 1 and 2, the fraction of Cu(II)-bound carboxylate groups to the total carboxyl groups in the microsphere (2.2×10^{-4} mol g^{-1}+1.5×10^{-4} mol g^{-1}) was calculated to be about 1/120 and 1/30, respectively. This small value should be the reason why the change in absorption intensity at 1705 and 1601 cm^{-1} was not observed for Samples 1 and 2. On the other hand, almost all of the surface carboxyl groups in Sample 3 were suggested to be used for Cu(II) adsorption; the band intensity for dimeric carboxyl decreased and that of carboxylate increased as shown in Fig. 4. This means that carboxyl groups on the microsphere surface participated in the Cu(II) binding in their ionized carboxylate forms. That the band intensity for dimeric carboxyl group did not decrease beyond a certain definite limit is in accord with the fact that some fraction of carboxyl groups are placed deep in the inside of microsphere, which can not interact with Cu(II) in aqueous solution. It should be also noted in the passing that the 1601 cm^{-1} absorption did not decrease beyond a certain level (0.51) for all the microsphere preparations tested in the present study. This is ascribed to the overrapping of the band with the absorption due to skeletal vibration of benzene ring.

ESR spectra were examined for Samples 1—3. As these spectra suffer a considerable noise due to small sample volume of Cu(II), g'' and A'' values could not be determined clearly. However, all spectra indicated a peculiar pattern[17,18] for square-planar Cu(II) complex carrying four coordinating oxgen atoms ($g^1=g''$, g^2, $g^3=g_\perp$, axis symmetrical spectra; comparatively large values of g'' and A''; four splits patters of hyperfine splitting).

Cu(II) spectral intensities for Samples 1—3 were in the order of Sample 3>Sample 2>Sample 1. Although ESR spectrometry in general is semi-quantitative in nature, the order of spectral intensity is consistent with the amount of Cu(II) on the microsphere as determined by Cu(II) adsorption study. The imprinting effect or an enhanced Cu(II) adsorption behavior as indicated by metal adsorption test is in a sense indirect because it only studies the decrease in Cu(II) concentration in a test solution. Meanwhile, the present ESR spectra directly indicated such an enhanced Cu(II) adsorption and complex-chemical structure of the adsorbed Cu(II) species in the Cu(II)-imprinted microsphere.

FE-SEM. Sample 3 was studied by means of FE-SEM at magnifications of 10000, 50000, 250000, and 600000 (Fig. 5). The particles were neatly spherical and the surfaces were uniform. Quite similar results were obtained with Samples 1 and 2. The view at 250000 and 600000 magnifications clearly showed the coating patterns by platinum mini-particles. A fine structure of the microsphere surface was not detected at the level of distribution of platinum mini-particles. Judging from these views, microspheres do not have any particular surface structure but just a plane surface at 10—50 nm level.

Complex Formation Equilibria and the Imprinting Effect in the Interface Domain of Microsphere. The pH dependence of imprinting effect shown in Fig. 3 indicates the participation of carboxylate groups in imprinting. IR and ESR spectra also support the interaction of Cu(II) with carboxylate group. Sample 3 suggested in the previous section that Cu(II) is bound to microsphere with a Cu(II) : carboxyl group molar ratio of roughly 1 : 2. In addition, in the related system in our previous Cu(II) imprinting study,[19] it was also suggested that the formation of 1 : 2 Cu(II) carboxylate complex is a key step for the introduction of imprinted structure on the microsphere surface.

However, a supposition that such 1 : 2 complexes are formed in the imprinting reaction mixture may not be simply acceptable if one looks at the concentrations of Cu(II) and the carboxyl group practically used in the second-step polymerization. In fact, the total Cu(II) concentration was 8.3×10^{-3} mol dm^{-3} and that of the carboxyl group on the microsphere was 7.5×10^{-3} mol dm^{-3} if one assumes a hypothetical homogeneous solution for this polymerization mixture. Under such a Cu(II) to carboxyl group concentration ratio, 1 : 1 complex may be formed but 1 : 2 complex is totally not. Therefore, one has to focus on to some restricted domain at the aqueous solution–microsphere interface where all the complexation reactions are taking place. In this domain, we can assume a highly concentrated aqueous solution of carboxyl group (from the polymer matrix) and a certain concentration of free Cu(II) (aqua copper(II) complex) which is similar to the concentration in the bulk aqueous solution.

In order to symplify the system, a model calculation was made for Cu(II) complexation with carboxylate anion (RCOO$^-$), taking the complex stability constant data[20] from copper(II)–acetate system. In Fig. 6, the percentage of the concentration of Cu(II)-containing species in the total Cu(II) concentration is plotted against carboxylate concentration [RCOO$^-$] on the assumption of ordinary homogeneous complex formation reaction. We then consider below the reactions taking place in the interface domain referring to this model calculation.

After the second-step polymerization and centrifugation, the supernatant solution was analyzed for free Cu(II) concentration. The percentage of free Cu(II) to the total Cu(II) concentration in the polymerization mixture was estimated to be 19%. Since Cu(II) is obviously heavily concentrated in the interface domain, the percentage of free Cu(II) in the total Cu(II) concentration in this region should be far much lower than 19% ("19%" is an averaged estimation for polymerization mixture as a whole). Figure 6 tells that at 19% of free

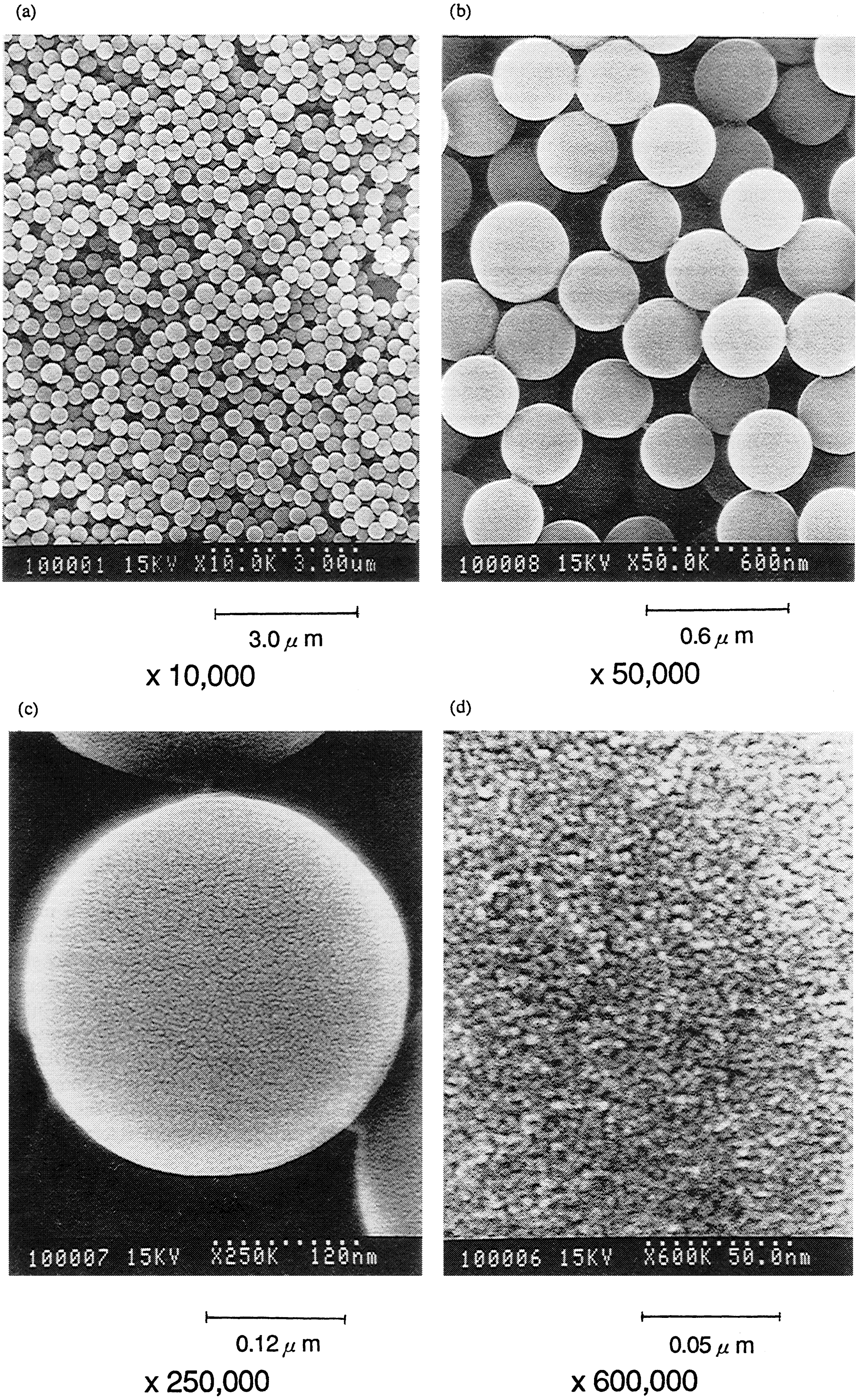


Fig. 5. Field emmition scanning electron microgragh of Sample 3.

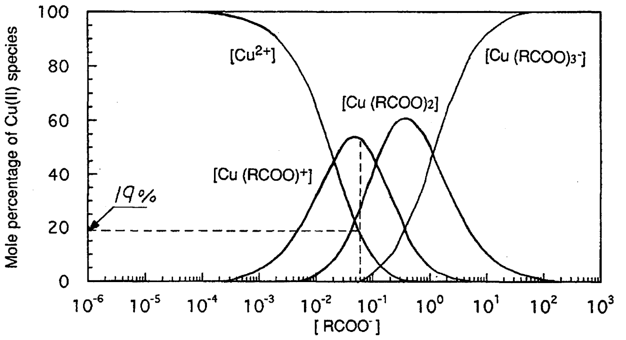


Fig. 6. Relationship between $[RCOO^-]$ at equilibrium and percentage of Cu(II)-containing species.

Cu(II) the rest of the Cu(II) species is accounted for by 53% of $[Cu(RCOO)^+]$, 26% of $[Cu(RCOO)_2]$, and 2% of $[Cu(RCOO)_3^-]$. In fact, since 19% for free Cu(II) is a too high estimation, the fraction of Cu(II) carbxylate species in the real interface domain should be much higher than these estimations. It may also be pointed out that Fig. 6 tells at the same time that at 19% of free Cu(II), $[RCOO^-]$ is 6×10^{-2} mol dm^{-3}. This implies a high carboxylate anion concentration in the interface domain. These model considerations above support the formation of substantial fraction of 1 : 2 complex in the interface domain in the course of second-step polymerization (i.e., imprinting structure-forming step).

A Cu(II)-imprinted structure formation actually takes place through an interaction of Cu(II) with the carboxylate groups in the linear polymer chain and through a subsequent chain immobilization by cross-linking. Describing the imprinting process by model consideration on acetatocopper(II) complex in aqueous solution is obviously an over simplification. However, it no doubt gives a clue in understanding the chemical behaviors of imprinted microspheres and the operational parameters in their preparation such as pH and imprinting metal ion concentration.

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