

Metal Sequestering by a Poly(ethylenimine)-Sephadex G-25 Conjugate Containing 2,2'-Dihydroxyazobenzene

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2,2'-Dihydroxyazobenzene (DHAB) was attached to poly(ethylenimine) (PEI) to obtain DHAB-PEI. Spectral titration revealed that uranyl, Fe(III), Cu(II), and Zn(II) ion form 1 : 1-type complexes with DHAB attached to PEI. Formation constants for the metal complexes formed by the DHAB moieties of DHAB-PEI were measured by using various competing ligands. The results indicated that the concentrations of uranyl, Fe(III), and Cu(II) ions can be reduced to 10^{-16} - 10^{-23} M at pH 8 with DHAB-PEI when the concentration of the DHAB moiety is 1 residue M. By using cyanuric chloride as the coupling reagent, DHAB-PEI was immobilized on Sephadex G-25 resin to obtain DHAB-PEI-Seph. Binding of uranyl, Fe(III), Cu(II), and Zn(II) ion by DHAB-PEI-Seph was characterized by using competing ligands. A new method has been developed for characterization of metal sequestering ability of a chelating resin. Formation constants and metal-binding capacity of two sets of binding sites on the resin were estimated for each metal ion. DHAB-PEI-Seph was applied to recovery of metals such as uranium, Fe, Cu, Zn, Pb, V, Mn, and W from seawater. The uranium recovery from seawater by DHAB-PEI-Seph does not meet the criterion for economical feasibility partly due to interference by Fe and Zn ions. The seawater used in the experiment was contaminated by Fe and Zn and, therefore, the efficiency of uranium extraction from seawater with DHAB-PEI-Seph could be improved if the experiment is carried out in a cleaner sea.

Introduction

Metal sequestering from water is important for environmental protection and recovery of resources. Chelating resins are much more effective than simple ion exchange resins in metal sequestering as they manifest higher selectivity and greater complexation constants toward guest metal ions.¹⁻³ Thus, chelating resins are useful in practical applications such as treatment of drinking or waste water as well as extraction of metals from seawater. For these purposes, it is necessary to attach chelating groups to solid supports. In addition, it is also desirable to modify surface of the solid supports to improve chemical and physical properties of the chelating resins.

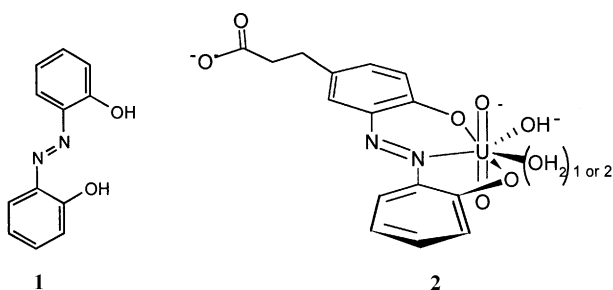
2,2'-Dihydroxyazobenzene (DHAB) (**1**) forms a 1 : 1-type complex with uranyl ion.⁴ Complexation of two molecules of **1** to one metal ion has been reported for some transition metal ions.⁵ A previous study on pH dependence of formation constants for the uranyl complex (**2**) of a DHAB derivative indicated that the ionization ($pK_1 = 8.19$, $pK_2 = 11.19$) of

the two phenol groups facilitate uranyl binding by DHAB.⁴

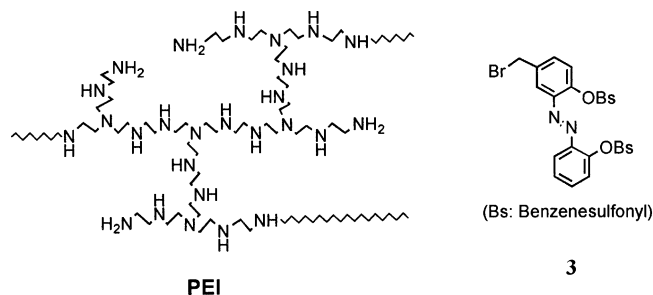
Metal complexing ability of DHAB is promoted by enhancing ionization of the phenol groups of DHAB. By providing DHAB with cationic microenvironment, ionization of the phenol groups can be facilitated. To improve the uranyl-binding ability of DHAB, for example, we have attempted to create cationic microenvironments around DHAB attached to soluble or insoluble synthetic polymers.

In the first study, partially cross-linked polystyrene was used as the insoluble backbone that holds DHAB.^{6,7} By attaching quaternary ammonium groups to the styryl moieties, cationic environment was introduced to the resin. The cationic character promoted adsorption of $UO_2(CO_3)_3^{4-}$ anion to the resin, ionization of phenol groups of DHAB attached to the resin, hydrophilicity of the resin, and, consequently, the complexation of uranyl ion by the resin. Uranium extraction from seawater was carried out with the DHAB attached to the polystyrenes.^{6,7} The amount of uranium extracted in an open sea was up to $150 \mu\text{g/g}$ resin.

In the second study, DHAB was attached to branched poly(ethylenimine) (PEI), a soluble polymer, in an attempt to create cationic microenvironment around DHAB with ammonium ions of PEI.⁸ PEI (M.W. 60000) contains ethylamine as the repeating unit. Among *ca.* 1400 amines of PEI, 25% are primary amines, 50% are secondary amines, and 25% are tertiary amines. The tertiary amines represent branching points on the polymer backbone, and, therefore, PEI is highly branched. Since various structural elements can be incorporated to PEI by modification of the amino groups, PEI has been used as the backbone of biomimetic catalysts



or biomimetic metal-sequestering agents.⁹⁻¹³ For the DHAB attached to PEI (DHAB-PEI), in which the content of DHAB was 1.1 residue mol % of PEI, log K_f of ca. 17 was obtained for uranyl binding at pH 8.0.⁸ Thus, K_f for uranyl binding by DHAB was improved by ca. 10^4 -fold through attachment of DHAB to PEI. This was mainly attributed to the enhanced ionization of the phenol groups of DHAB in the microenvironment of PEI.



For practical applications in extraction of metal ions from water, the sequestering agent should be immobilized. In this study, Sephadex G-25 resin (Seph) was chosen as the solid support and DHAB-PEI was attached to Seph to obtain DHAB-PEI-Seph. In this paper, preparation and metal-binding ability of the metal-sequestering agents are reported, together with results of metal-extraction from seawater.

Experimental Section

Materials.

PEI containing 2,2'-dihydroxyazobenzene (DHAB-PEI):

This was synthesized according to literature¹⁴ using 2-(2-benzenesulfonyloxy-5-bromomethylphenylazanyl)phenyl benzenesulfonate (**3**), except that dimethyl sulfoxide (DMSO) was used as solvent to improve the solubility of **3**. The content of DHAB in PEI-DHAB was 5.1 residue mol %, as determined by spectral titration⁸ with uranyl ion.

Seph-cyanuric chloride conjugate (Cya-Seph): Seph (5.0 g; purchased from Sigma; dry bead diameter 50-150 μm), cyanuric chloride (31 mg) and Na_2CO_3 (6.0 g) were added to toluene (100 mL). The heterogeneous mixture was shaken for 5 days at 50 °C. The resulting beads collected by filtration were washed with water (20 mL) 10 times and with acetone (20 mL) 10 times, and dried *in vacuo*. Based on the results (C, H, N) of elemental analysis, the content of cyanuric moiety in Cya-Seph was estimated as 0.10 residue mol %. This corresponds to incorporation of 20% of the added cyanuric chloride to Seph.

DHAB-PEI attached to Cya-Seph (DHAB-PEI-Seph):

To solutions of dried DHAB-PEI (0.10 g; 2.3 residue mmol of PEI) dissolved in DMSO (50 mL), Cya-Seph (1.0 g) was added. The heterogeneous mixture was shaken for 5 days at 50 °C. The resulting beads collected by filtration were washed with water (20 mL) 10 times, 1 N HCl (20 mL) 3 times, and acetone (20 mL) 10 times, and dried *in vacuo*. Based on the results (C, H, N) of elemental analysis, it was estimated that 0.019 g of PEI-DHAB was attached to 1 g of

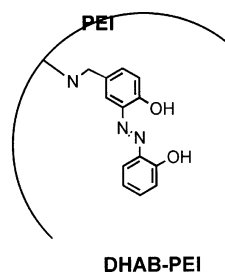
Cya-Seph. Thus, the content of DHAB moiety in DHAB-PEI-Seph is 1.8×10^{-5} mol per gram resin.

Other Materials. CuCl_2 and ZnCl_2 were prepared from CuO (99.999%, purchased from Aldrich) and ZnO (99.999+, purchased from Aldrich) according to the process reported¹⁵ previously for NiCl_2 . Solutions of FeCl_3 were prepared from reagent grade FeCl_3 and quantified by back titration with ethylenediaminetetraacetic acid (EDTA). $\text{UO}_2(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$ (99.0%, purchased from Merck) was used without further purification.

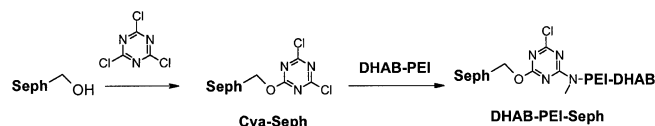
Measurements. Spectrophotometric measurement was performed with a Beckman DU68 UV/VIS spectrophotometer. Temperature was adjusted to 25 ± 0.1 °C with a Lauda/Brinkman Model RC3 circulator. Buffer solutions (0.1 M) were prepared with 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) at pH 8.02. pH measurement was carried out with a Dongwoo Medical DP-880 pH/ion meter. Inductively coupled plasma mass spectroscopic (ICP-MS) measurement was carried out with a Laptam 8410 plasmascan or a VG/PQ2 turbo ICP spectrometer. Experiments for uranium extraction from seawater were carried out in Aninjin-ri, Kangnung City, Kangwon Province on the east coast of Korean peninsula.

Results

Synthesis of DHAB-PEI-Seph. When **3** is added to PEI, the amino group of the polymer substitutes the bromo group of **3**, leading to alkylation of the polymer. Removal of the benzenesulfonyl groups from the alkylated polymer produces DHAB-PEI. The content of DHAB in DHAB-PEI was determined as 5.1 residue mol % by titration with uranyl ion as described in the next section.



Sephadex resins are manufactured by cross-linking cellulose with epichlorohydrin. Coupling of Seph and the DHAB-PEI was carried out as summarized in Scheme 1. The first chloro group of cyanuric chloride is readily substituted by hydroxyl groups. One of the chloro groups of the resulting cyanuric ether can be substituted by stronger nucleophiles such as amines.¹⁶ The other chloro group, however, resists further substitution once an amine is incorporated into the cyanuric moiety. Thus, treatment of cyanuric chloride with Seph would lead to single attachment of the cyanuric moiety to Seph. Substitution of one of the chloro groups in each cyanuric moiety of Cya-Seph with an amino group of a modified PEI leads to conjugation of the PEI derivative to the cellulose-based resin.



As indicated in the experimental section, the content of cyanuric moieties introduced to Cya-Sep is estimated as 0.10 residue mol %, the content of PEI in DHAB-PEI-Seph as 1.9 weight %, and the content of DHAB in DHAB-PEI-Seph as 1.8×10^{-5} mol per gram resin.

Spectral titration of DHAB-PEI with metal ions. Illustrated in Figure 1 is the absorbance change observed when uranyl ion was added to a solution containing a fixed concentration of DHAB-PEI. Here, the intersecting point represents the equivalence point for the titration of the DHAB moiety with uranyl ion. Since DHAB forms a 1 : 1-type complex with uranyl ion,⁴ the amount of DHAB moiety is calculated from the equivalence point. The content of DHAB moieties was thus estimated as 5.1 residue mol %.

Because PEI contains ethylenediamine moieties, PEI backbone can bind metal ions, although attachment of hydrophobic residues to PEI and cross-linkage of PEI reduces metal-binding ability of PEI considerably.¹⁷ Metal complexation to the PEI backbone does not produce appreciable spectral changes in the visible region. On the other hand, metal-binding by the DHAB moiety of DHAB-PEI is accompanied by considerable spectral changes in the visible region. The plot illustrated in Figure 1 demonstrates that uranyl ion is bound by the DHAB moiety when the total concentration of uranyl ion does not exceed that of the DHAB moieties.

For metal ions other than uranyl ion, two or more DHAB moieties may be bound by one metal ion.⁵ When DHAB is randomly attached to a PEI derivative, however, complexation of two or more DHAB moieties to one metal ion is not likely due to the conformational strain imposed by the poly-

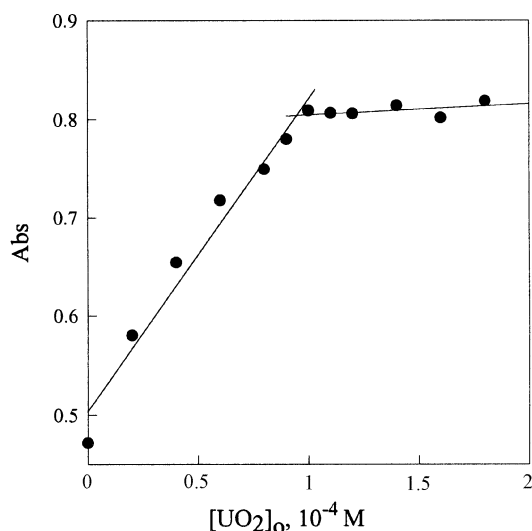


Figure 1. Absorbance change observed at 485 nm for DHAB-PEI (9.51×10^{-5} M in terms of DHAB concentration) with various amounts of uranyl ion added at pH 6.00.

mer backbone. To check the binding modes of other metal ions, absorbance changes in the visible region were measured by adding Fe(III), Cu(II), or Zn(II) ion to DHAB-PEI. The spectral titration data of Figures 2-4 reveal that Fe(III), Cu(II), or Zn(II) ion is complexed by one DHAB moiety and complexation by the PEI backbone alone is much weaker than that by the DHAB moiety. The possibility that Cu(II), Fe(III), Zn(II), or uranyl ion bound by the DHAB moiety is also complexed by amino groups of PEI backbone is not excluded by the data of Figures 1-4.

Formation constants for metal complexes of DHAB-

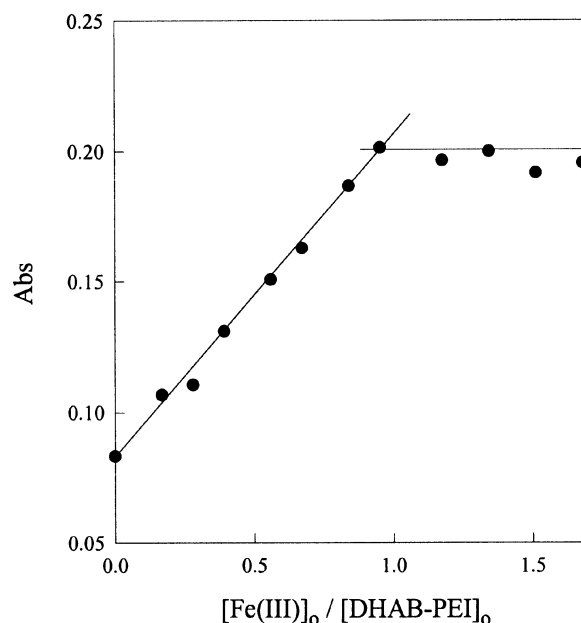


Figure 2. Absorbance change observed at 560 nm for DHAB-PEI (9.51×10^{-5} M in terms of DHAB concentration) with various amounts of Fe(III) ion added at pH 8.02.

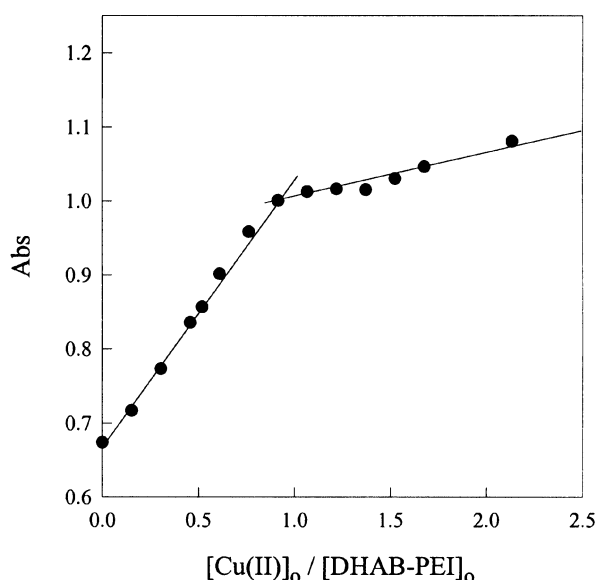


Figure 3. Absorbance change observed at 495 nm for DHAB-PEI (9.51×10^{-5} M in terms of DHAB concentration) with various amounts of Cu(II) ion added at pH 8.02.

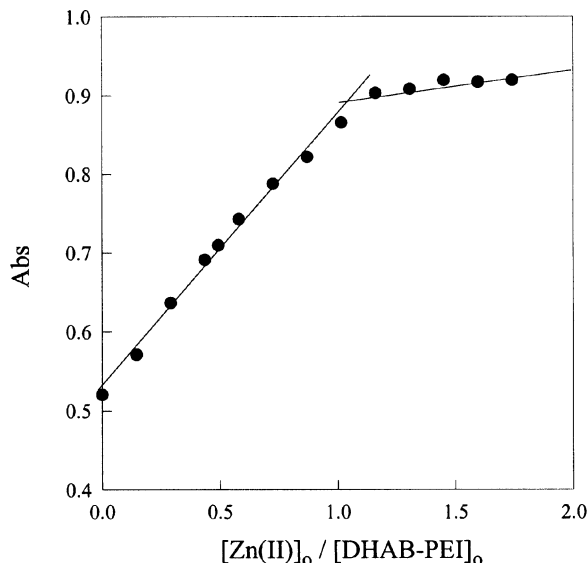


Figure 4. Absorbance change observed at 490 nm for DHAB-PEI (9.51×10^{-5} M in terms of DHAB concentration) with various amounts of Zn(II) ion added at pH 8.02.

PEI. Various types of small molecules including metal ions and organic molecules are complexed to PEI derivatives, and sometimes a large number of the small molecules are bound to each molecule of a PEI derivative. The binding of small molecules can be simplified, however, as independent complexation to individual binding sites. This approximation is valid as far as the complexation to a binding site does not affect the succeeding bindings appreciably and has been found to be valid for binding of several types of small molecules to PEI derivatives.^{8,12-14,18-22}

It was not possible to measure the formation constants for metal complexes of DHAB-PEI directly from absorbance change accompanying complexation of metal ions. Instead, the formation constants were estimated by using competing ligands^{8,20-22} according to Scheme 2. Here, DHAB^{PEI} represents DHAB attached to PEI. The formation constants for metal complex of DHAB-PEI can be defined as Eq. (1). Complexation of the competing ligand (CL) to the metal ion is expressed as Eq. (2). Literature values²³ of n and K_{CL} were used in the analysis of the experimental data obtained with both DHAB-PEI and DHAB-PEI-Seph. Equilibrium for exchange of the metal ion between the DHAB^{PEI} and CL is described by Eq. (3). In the equations, $[\text{DHAB}^{\text{PEI}}]$ or $[\text{CL}]$ stand for sum of concentrations of the DHAB moieties or CL, respectively, in various ionization states. The value of K_{ex} was estimated by analysis of data points, such as those illustrated in Figure 5, in terms of Scheme 2 with a computer

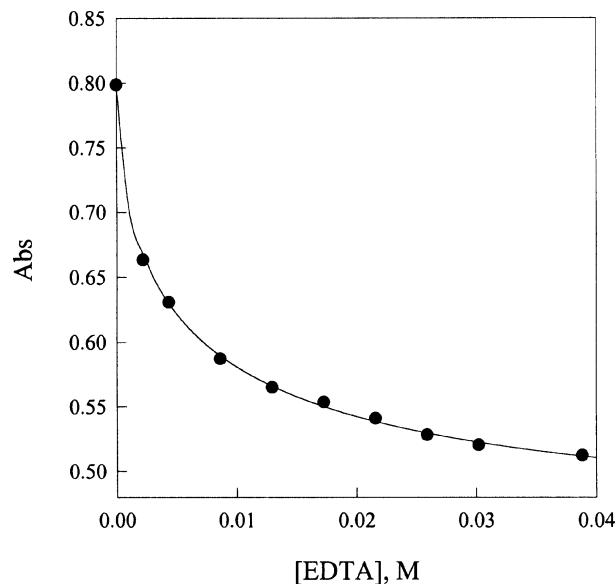
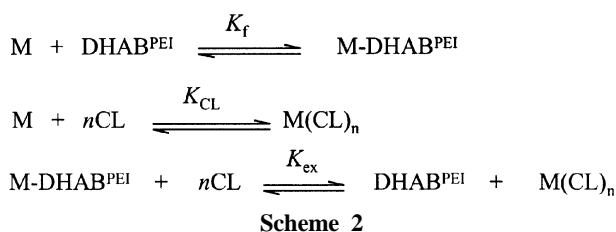


Figure 5. Absorbance change observed at 495 nm for the Cu(II) ion complex of DHAB-PEI with various amounts of EDTA added at pH 8.02. The curve was obtained by analysis of the data according to Scheme 2.

program for nonlinear regression. Then, K_f was calculated (Eq. (3)) from K_{ex} and K_{CL} . The values of K_f thus estimated for uranyl, Fe(III), Cu(II), or Zn(II) complexes of PEI-DHAB measured at pH 8.02 and 25 °C are summarized in Table 1.

$$K_f = [\text{M-DHAB}^{\text{PEI}}]/[\text{M}][\text{DHAB}^{\text{PEI}}] \quad (1)$$

$$K_{\text{CL}} = [\text{M}(\text{CL})_n]/[\text{M}][\text{CL}]^n \quad (2)$$

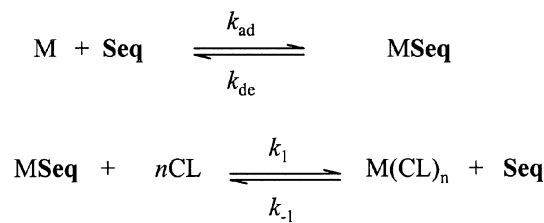
$$\begin{aligned}
 K_{\text{ex}} &= [\text{DHAB}^{\text{PEI}}][\text{M}(\text{CL})_n]/[\text{M-DHAB}^{\text{PEI}}][\text{CL}]^n \\
 &= K_{\text{CL}}/K_f \quad (3)
 \end{aligned}$$

Formation constants and sequestering capacity of DHAB-PEI-Seph. Complexation of UO_2^{2+} , Fe(III), Cu(II), or Zn(II) ion to the sequestering site (**Seq**) on DHAB-PEI-Seph was examined. In a typical experiment, DHAB-PEI-Seph (20 mg) was suspended in a solution (20 mL) containing a metal ion, a competing ligand (CL), and HEPES (0.1 M) at pH 8.02, and the mixture was shaken at the speed of 150 rpm for one day. For a given metal ion, the initially added concentration of the metal ion ($[\text{M}]_0$) was fixed at *ca.* 0.1 mM. The initially added concentration of CL ($[\text{CL}]_0$) was much greater than $[\text{M}]_0$. The resin separated by filtration was washed with water (10 mL) thrice, and was washed again with 1 N HNO_3 solution (10 mL). The amount of the metal ion released by the HNO_3 solution was measured by ICP-MS, from which the amount of the metal ion (**MSeq**)

Table 1. Formation constants for metal complexes of PEI-DHAB at pH 8.02 and 25 °C^a

metal	UO_2^{2+}	Fe(III)	Cu(II)	Zn(II)
log K_f	16.10 ± 0.26	23.17 ± 0.40	18.62 ± 0.03	8.45 ± 0.42

^aCompeting ligands used were sodium bicarbonate for UO_2^{2+} , 2-(hydroxyethyl)ethylenediaminetriacetic acid (HEDTA) for Fe(III), EDTA for Cu(II), and dipicolinic acid (DPA) for Zn(II).



Scheme 3

bound to **Seq** was calculated.

Data for complex formation between a guest metal ion and an insoluble metal-sequestering agent may be analyzed in terms of Scheme 3 by analogy with Langmuir isotherm²⁴ for gas adsorption to solid surfaces.^{6,7,25,26} It is assumed in Scheme 3 that metal complexation is independent of succeeding bindings. The formation constant (K_f) is represented by $k_{\text{ad}}/k_{\text{de}}$. When K_f is very large, it can be estimated indirectly by measuring the equilibrium constant ($K_{\text{ex}} = k_1/k_{-1}$) for the exchange of the bound metal ion with a competing ligand. For Scheme 3, Eqs. (4)-(6) are derived. In the equations, $[\text{Seq}]$, $[\text{Seq}]_0$, and $[\text{MSeq}]$ represent the concentrations of **Seq**, the initially added concentration of **Seq**, and the concentration of **MSeq**, respectively, obtainable when **Seq** and **MSeq** are assumed to be dissolved. By analyzing the dependence of $[\text{MSeq}]$ on $[\text{CL}]$ in terms of Eq. (6), the values of K_{ex} and $[\text{Seq}]_0$ can be estimated. Then, $K_f (= K_{\text{CL}}/K_{\text{ex}})$ can be calculated from K_{ex} and K_{CL} .

$$\begin{aligned}
 [\text{MSeq}]/[\text{Seq}] &= k_{\text{ad}}[\text{M}]/k_{\text{de}} = K_f[\text{M}] \\
 &= k_{-1}[\text{M}(\text{CL})_n]/k_1[\text{CL}]^n \\
 &= [\text{M}(\text{CL})_n]/[\text{CL}]^n K_{\text{ex}}
 \end{aligned} \quad (4)$$

$$\begin{aligned}
 K_{\text{ex}} &= [\text{Seq}][\text{M}(\text{CL})_n]/[\text{MSeq}][\text{CL}]^n \\
 &= ([\text{Seq}]_0 - [\text{MSeq}])([\text{M}]_0 - [\text{MSeq}])/[\text{MSeq}][\text{CL}]^n
 \end{aligned} \quad (5)$$

$$\begin{aligned}
 [\text{MSeq}] &= \{ \alpha - (\alpha^2 - 4[\text{M}]_0[\text{Seq}]_0)^{1/2} \} / 2 \\
 \text{where } \alpha &= [\text{Seq}]_0 + [\text{M}]_0 + K_{\text{ex}}[\text{CL}]^n
 \end{aligned} \quad (6)$$

For complexation of uranyl, Fe(III), Cu(II), or Zn(II) ion by DHAB-PEI-Seph, however, Scheme 3 alone does not account for the experimental data. As illustrated in Figure 6, the data for dependence of $[\text{MSeq}]$ on $[\text{CL}]$ is to be divide into two parts in order to fit the data with Eq. (6). Thus, the whole data set does not fit a single equation. Instead, the data were analyzed by assuming the presence of two sets of binding sites as indicated by Scheme 4. Here, two sets of binding sites are indicated as **Seq** and **Seq*** with formation constants $K_f (= k_{\text{ad}}/k_{\text{de}})$ and $K_f^* (= k_{\text{ad}}^*/k_{\text{de}}^*)$, respectively, and exchange constants $K_{\text{ex}} (= k_1/k_{-1})$ and $K_{\text{ex}}^* (= k_1^*/k_{-1}^*)$, respectively.

For Scheme 4, Eq. (7) is derived for the dependence of $[\text{MSeq}]_t (= [\text{MSeq}] + [\text{MSeq}^*])$ on $[\text{M}]$. Here, Seq_0 and Seq^*_0 represent the initially added concentration of **Seq** and **Seq***, respectively. The values of $[\text{M}]$ were calculated according to Eq. (2) from the experimentally measured values of $[\text{M}(\text{CL})_n]$ ($= M_0 - [\text{MSeq}]_t$) and $[\text{CL}]$. The dependence of $[\text{MSeq}]_t$ on $\log [\text{M}]$ is illustrated in Figures 7-10. The data points were analyzed in terms of Eq. (7) by putting $\log [\text{M}]$ as the independent variable and values of Seq_0 , Seq^*_0 , $\log K_f$, and \log

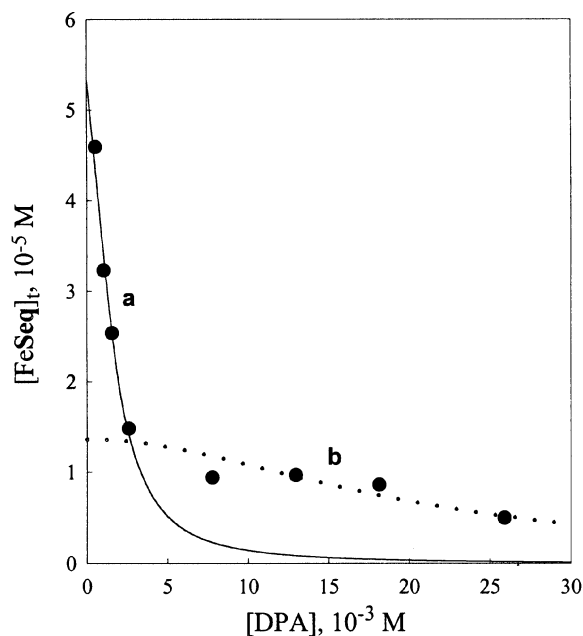
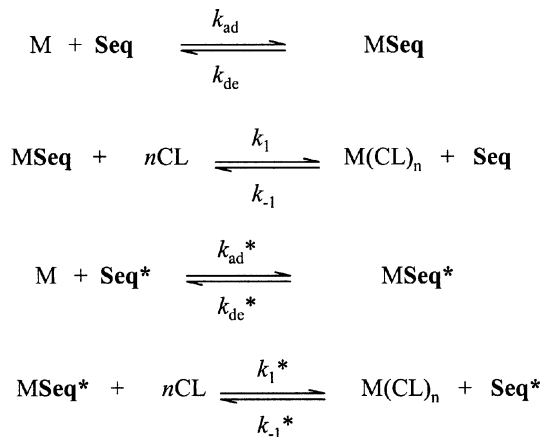


Figure 6. Plot of $[\text{FeSeq}]_t$ against $[\text{DPA}]$ for the exchange of Fe(III) ion ($[\text{Fe(III)}]_0 = 0.990 \times 10^{-4} \text{ M}$) between DHAB-PEI-Seph and DPA at pH 8.02. Curves a and b were obtained by analyzing the first 4 data points and the last 5 data points, respectively, according to Eq. (6).



Scheme 4

K_f^* were estimated by using a computer program for nonlinear regression. The parameter values thus estimated are summarized in Table 2.

$$[\text{MSeq}]_t = K_f[\text{M}]\text{Seq}_0 / (1 + K_f[\text{M}]) + K_f^*[\text{M}]\text{Seq}^*_0 / (1 + K_f^*[\text{M}]) \quad (7)$$

Metal extraction from seawater. Seawater was passed for 20 hours through DHAB-PEI-Seph (0.10 g) contained in an apparatus described⁶ previously. After rinsing the resins several times with distilled water, the resin was suspended in 1 N HNO_3 solution for 25-30 min and the amounts of various metal ions released by treatment with the acid were measured by ICP-MS and the results are summarized in Table 3. Concentrations of various metal ions present in the seawater used in this experiment and average concentrations of the

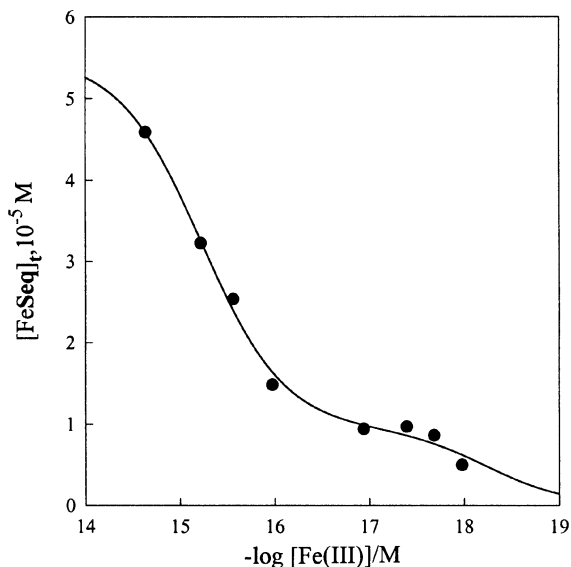


Figure 7. Plot of $[\text{FeSeq}]_t$ against $\log [\text{Fe(III)}]$ for the exchange of Fe(III) ion between DHAB-PEI-Seph and DPA at pH 8.02 with $[\text{Fe(III)}]_0 = 0.990 \times 10^{-4}$ M. The curve was obtained by fitting the data with Eq. (7).

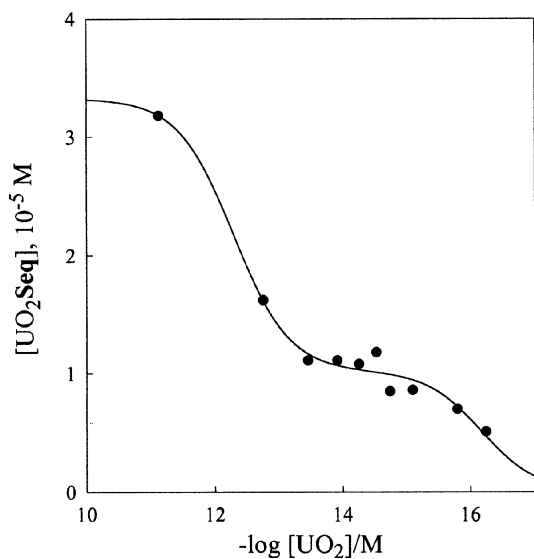


Figure 8. Plot of $[\text{UO}_2\text{Seq}]_t$ against $\log [\text{UO}_2]$ for the exchange of uranyl ion between DHAB-PEI-Seph and carbonate at pH 8.02 with $[\text{UO}_2]_0 = 1.90 \times 10^{-4}$ M. The curve was obtained by fitting the data with Eq. (7).

metal ions in various seawater reported in the literature²⁷ are also presented in Table 3.

Discussion

A molecule of DHAB-PEI contains 71 DHAB moieties and 1400 amino groups on the average. The results of Figures 1 and 2 indicate that uranyl, Fe(III), Cu(II), or Zn(II) ion is preferentially bound by the DHAB moiety when mixed with DHAB-PEI. Under the conditions of $[M]_0 < [\text{DHAB}^{\text{PEI}}]_0$, each metal ion is complexed by one DHAB moiety and possibly by some amino groups of PEI backbone. Formation

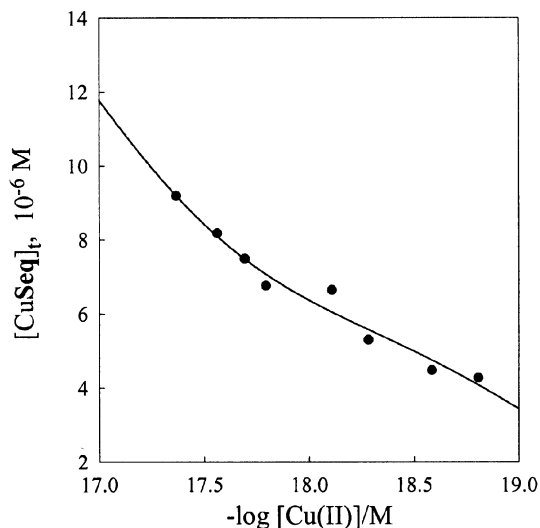


Figure 9. Plot of $[\text{CuSeq}]_t$ against $\log [\text{Cu(II)}]$ for the exchange of Cu(II) ion between DHAB-PEI-Seph and EDTA at pH 8.02 with $[\text{Cu(II)}]_0 = 1.44 \times 10^{-4}$ M. The curve was obtained by fitting the data with Eq. (7).

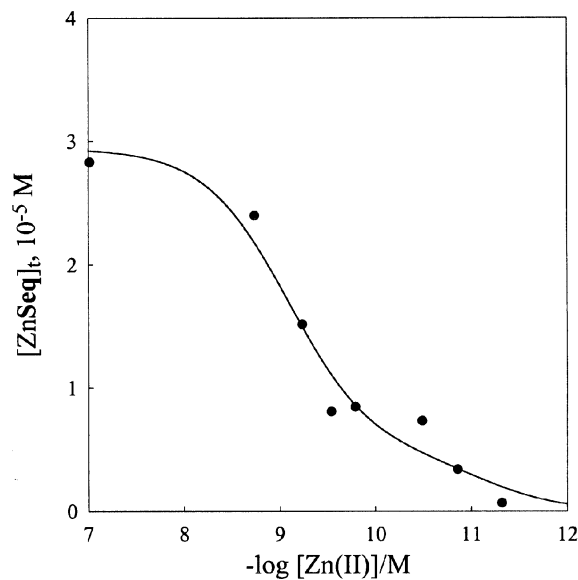


Figure 10. Plot of $[\text{ZnSeq}]_t$ against $\log [\text{Zn(II)}]$ for the exchange of Zn(II) ion between DHAB-PEI-Seph and DPA at pH 8.02 with $[\text{Zn(II)}]_0 = 1.37 \times 10^{-4}$ M. The curve was obtained by fitting the data with Eq. (7).

constants for such metal complexes measured at pH 8.02 and 25° C are summarized in Table 1.

In a previous study, $\log K_f$ of 17.01 ± 0.17 was estimated at pH 7.99 for the uranyl complex of DHAB attached to PEI.⁸ This is considerably greater than $\log K_f$ (16.10 ± 0.26 at pH 8.02; Table 1) measured for the uranyl complex of DHAB-PEI prepared in this study. The content of DHAB in DHAB-PEI prepared in the previous study was 1.1 residue mol % which is considerably smaller than that (5.1 residue mol %) prepared in the present study. The content of DHAB was raised in the present study to increase the capacity for metal sequestering.

Table 2. Formation constants and sequestering capacity for metal complexes of DHAB-PEI- Seph at pH 8.02, 25 °C^{a,b}

	log K_f	log K_f^*	Seq ₀	Seq* ₀
UO ₂ ²⁺	12.30 ± 0.13	16.18 ± 0.15	4.62 ± 0.26	2.04 ± 0.10
Fe ³⁺	15.23 ± 0.10	18.24 ± 0.37	6.17 ± 0.40	1.27 ± 0.20
Cu ²⁺	16.95 ± 1.27	19.15 ± 0.36	1.75 ± 0.33	0.77 ± 0.19
Zn ²⁺	9.08 ± 0.25	11.12 ± 1.17	3.33 ± 0.86	0.63 ± 0.08

^aCompeting ligands used were sodium bicarbonate for UO₂²⁺, DPA for Fe(III), EDTA for Cu(II), and DPA for Zn(II). ^bUnits of Seq₀ and Seq*₀ are 10⁻⁵ mol/g resin.

Table 3. Amounts of various metal ions extracted from seawater with DHAB-PEI-Seph^a

metal	amount extracted from seawater ^a (μg/g resin)	concn. in seawater (ppb)	
		this work ^b	lit. value ^c
U	18	2.9	3.3
Fe	1286	32.2	1.3
Cu	32	0.8	0.1
Zn	474	30.6	0.01
Pb	24	1.8	0.015
V	17	1.1	1.9
Mn	1	2.6	0.2
W	16	2.9	0.1

^aAmount of resin used; 0.1 g. Amount of seawater passed through; 500 L over 20 h (average flow rate; 0.35 km/h). Water temperature; 18-21 °C. The amount of metal ions extracted did not increase appreciably when seawater was passed through for 24-48 hours. ^bConcentration of the metal ions in the seawater used in this experiment. ^cAverage concentration of the metal ions in seawater reported in ref. 27.

When hydrophobic pendants are attached to PEI, they form clusters in water.^{13,17,28,29} When DHAB moieties attached to PEI forms hydrophobic clusters, ionization of the phenolic hydroxyl groups of DHAB should be suppressed due to the decreased polarity of the microenvironment. This would lower the ability of DHAB to chelate metal ions.

A previous study disclosed that the metal chelating ability of the amino groups of PEI is diminished when the content of hydrophobic pendants attached to PEI is raised.¹⁷ This has been explained in terms of strains imposed on the conformation of the PEI backbone by the cluster formation. An increase in the content of DHAB would lead to formation of a greater amount of hydrophobic clusters. If the amino groups of PEI backbone cooperate with DHAB moieties in uranyl complexation of DHAB-PEI, effectiveness of the amino groups can be diminished when the DHAB content is raised. The lower value of log K_f for uranyl complex of DHAB-PEI prepared in this study, therefore, can be ascribed to the suppressed ionization of the hydroxyl groups of DHAB moieties and/or the diminished participation of the amino groups in chelation.

The results summarized in Table 1 indicate that the concentrations of uranyl, Fe(III), and Cu(II) ion can be reduced to 10⁻¹⁶-10⁻²³ M at pH 8 with DHAB-PEI when the concentration of DHAB moiety is 1 residue M. In view of the high chelating ability of the DHAB moieties attached to PEI, DHAB-PEI was immobilized on Seph. Cyanuric chloride,

which is frequently used to activate polysaccharide carriers for the binding of proteins,³⁰ was employed in this study for conjugation of DHAB-PEI with Seph. The content of cyanuric moiety in Cya-Seph is ca. 0.10 residue mol %. Thus, one cyanuric group is introduced for 1000 glucose monomers of Seph. If a DHAB-PEI molecule is connected to every cyanuric moiety of Cya-Seph, the content of DHAB-PEI in the resulting DHAB-PEI-Seph can be ca. 30% if an excess DHAB-PEI is added. If DHAB-PEI used in the preparation of DHAB-PEI-Seph is attached to Cya-Seph quantitatively, the content of DHAB-PEI in DHAB-PEI-Seph prepared in the present study would be ca. 9%. The content is, however, estimated as 1.9%, as described in the experimental section. Seph has pores that accommodate molecules with M.W. smaller than 5000. Thus, DHAB-PEI would have no access to cyanuric moieties present on the walls of pores of Seph. Thus, only a part of the cyanuric moieties of Cya-Seph are exposed to attack by DHAB-PEI, accounting for the low content of DHAB-PEI in DHAB-PEI-Seph. The content of DHAB-PEI would be further reduced if DHAB-PEI is connected to Cya-Seph via multiple attachment.

With DHAB-PEI dissolved in water, it was possible to determine the concentration of the DHAB moiety and to ensure that added metal ions were complexed by the DHAB moieties. With DHAB-PEI-Seph, however, it was not possible to have metal ions complexed exclusively by the DHAB moieties. Instead, an excess of a guest metal ion was added to DHAB-PEI-Seph and thermodynamic behavior of the resulting complex was characterized.

The results summarized in Table 2 reveal that at least two kinds of binding sites are present on DHAB-PEI-Seph for the metal ions. The metal-binding abilities of the weaker and the stronger binding sites are represented by log K_f and log K_f^* , respectively. The contents of the weaker and the stronger binding sites are represented by Seq₀ and Seq*₀, respectively. The values of log K_f^* (Table 2) measured for DHAB-PEI-Seph are similar to those of log K_f (Table 1) measured for DHAB-PEI. Moreover, Seq*₀ (Table 2) measured for DHAB-PEI-Seph are comparable to the content (1.8 × 10⁻⁵ mole per gram resin) of DHAB in DHAB-PEI-Seph. It appears that the stronger binding sites of DHAB-PEI-Seph corresponds to the DHAB moieties. On the other hand, each weaker binding site may be composed of two or three ethylenediamine moieties of PEI backbone without involvement of the DHAB moiety.

It is noteworthy that log K_f^* for DHAB-PEI-Seph is somewhat higher than log K_f for DHAB-PEI in case of Cu(II) or Zn(II) binding and lower in case of Fe(III) binding. The Seq*₀ values are appreciably smaller than the DHAB content in case of Cu(II) or Zn(II) binding. These complications may be due to additional features of metal sequestering arising from attachment of DHAB-PEI to Cya-Seph. For example, conformation of the PEI backbone may be distorted by multiple attachment of the PEI to Seph and/or interaction of amino groups of PEI with hydroxyl groups of Seph, affecting the metal binding ability. It is also possible that hydroxyl groups of Seph cooperate with DHAB or amino groups of

PEI in metal binding giving rise to several new types of binding sites.

In the present study, a new method is presented for characterization of metal sequestering ability of a resin containing two different sets of chelating sites. If a metal ion bound to the resin affects the succeeding binding of a different kind of metal ion, simultaneous complexation of several different kinds of metal ions would be more difficult to analyze quantitatively.

Application of DHAB-PEI-Seph to recovery of several metal ions from seawater has been tested. The results summarized in Table 3 reveal that various metal ions are extracted from seawater by passing seawater through DHAB-PEI-Seph. The total amount of the metal ions extracted is comparable to the Seq^* , the content of the stronger binding sites on DHAB-PEI-Seph. The amount of a metal ion recovered by the resin would be greater if the metal ion has greater affinity ($\log K_i^*$) toward the sequestering sites, faster rates for complexation, and a larger concentration in seawater. In addition, the presence of 2.5 mM bicarbonate ion in seawater complicates the sequestering of various metal ions by the resin since bicarbonate ion behaves as a competing ligand for the metal ions.

It has been estimated that extraction of more than 500 μg of uranium is needed for 1 gram of sequestering agent per day to meet the economical feasibility of extraction of uranium from seawater.^{7,31} In addition, the sequestering agent must be recycled many times. To date, no uranyl sequestering agents meeting these criteria have been designed.³² The best results were obtained with amidoxime resins in terms of the amount (up to 600 μg per day per g resin) of uranium extracted.³³ The amidoxime resin, however, cannot be recycled for more than several times due to destruction during reactivation step.³⁴

The amount of uranium extracted per gram of DHAB-PEI-Seph does not meet the aforementioned criterion. The amount of uranium extracted from seawater with DHAB-PEI-Seph is low because most of the metal binding sites are occupied by Fe(III) and Zn(II) ions. The position of seawater uptake employed in this experiment was about 600 meters away from the shore and about 100 meters below the sea level. As summarized in Table 3, the seawater used in this experiment is heavily contaminated with Fe(III) and Zn(II) ion. This is attributed to river water flowing into the sea from mining and industrial areas in Kangwon Province of Korean peninsula. If tested with uncontaminated seawater in another sea, the amount of uranium extracted by DHAB-PEI-Seph might be significantly improved.³⁵

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References and Notes

- Schmuckler, G. *Talanta* **1965**, *12*, 281.
- Moyers, E. M.; Fritz, J. S. *J. Chromatogr.* **1976**, *176*, 189.
- Kantipuly, C.; Katragadda, S.; Chow, A.; Gesser, H. D. *Tal-*

- anta **1990**, *37*, 491.
- Lee, K.; Jang, B.-B.; Suh, J. *Bull. Korean Chem. Soc.* **1996**, *17*, 814.
- Dutta, S.; Basu, P.; Chakravorty, A. *Inorg. Chem.* **1991**, *30*, 4031.
- Jang, B.-B.; Lee, K.; Kwon, W. J.; Suh, J. *J. Polymer Sci. A: Polymer Chem.* **1998**, *37*, 3169.
- Lee, K.; Jang, B.-B.; Kwon, W. J.; Suh, J. *J. Polymer Sci. A: Polymer Chem.* **1999**, *37*, 4117.
- Noh, Y.-S.; Lee, K.; Suh, J. *J. Polymer Sci. A: Polymer Chem.* **1999**, *37*, 3936.
- Klotz, I. M. In *Enzyme Mechanisms*; Page, M. I., Williams, A. Eds.; Royal Society of Chemistry: London, 1987; Chapter 2.
- Suh, J. *Acc. Chem. Res.* **1992**, *25*, 273.
- Suh, J. In *Polymeric Materials Encyclopedia*; Salamone, J. C., Ed.; CRC Press: Boca Raton, 1996; Vol. 6, pp 4210-4218.
- Suh, J.; Hah, S. S. *J. Am. Chem. Soc.* **1998**, *120*, 10088.
- Suh, J.; Hong, S. H. *J. Am. Chem. Soc.* **1998**, *120*, 12545.
- Lee, K.; Suh, M. P.; Suh, J. *J. Polymer Sci. A: Polymer Chem.* **1997**, *35*, 1825.
- Suh, J.; Chung, S.; Lee, S. H. *Bioorg. Chem.* **1987**, *15*, 383.
- Dudley, J. R.; Thurston, J. T.; Schaefer, F. C.; Holm-Hansen, D.; Hull, C. J.; Adams, P. *J. Am. Chem. Soc.* **1951**, *73*, 2891.
- Suh, J.; Lee, S. H.; Kim, S. M.; Hah S. S. *Bioorg. Chem.* **1997**, *25*, 221.
- Suh, J.; Cho, Y.; Lee, K. J. *J. Am. Chem. Soc.* **1991**, *113*, 4198.
- Suh, J.; Lee, S. H.; Zoh, K. D. *J. Am. Chem. Soc.* **1992**, *114*, 7916.
- Suh, J.; Hong, S. H. *J. Polymer Sci. A: Polymer Chem.* **1997**, *35*, 533.
- Suh, J.; Park, H. S. *J. Polymer Sci. Polymer Chem.* **1997**, *35*, 1197.
- Suh, J.; Lee, S. H. *J. Org. Chem.* **1998**, *63*, 1519.
- Martell, A. E.; Smith, R. M. *Critical Stability Constants*; Plenum: New York, 1974-1982; Vol. 1-5.
- Atkins, P. W. *Physical Chemistry*, 4th ed.; Oxford University Press: Oxford, 1990; pp 885-888.
- Suh, J.; Park, H. S. *J. Polymer Sci. A: Polymer Chem.* **1997**, *35*, 1197.
- Jang, B.-B.; Lee, K. P.; Min, D. H.; Suh, J. *J. Am. Chem. Soc.* **1998**, *120*, 12008.
- Wilson, T. R. S. In *Chemical Oceanography*, 2nd ed.; Riley, J. P., Skirrow, G., Eds.; Academic Press: London, 1975; Vol.1, Chapter 6 and 7.
- Johnson, T. W.; Klotz, I. M. *Macromolecules* **1974**, *7*, 618.
- Suh, J.; Kim, M. J. *Bioorg. Chem.* **1992**, *20*, 366.
- Kay, G.; Crook, E. M. *Nature* **1967**, *216*, 514.
- Kobuke, Y.; Tabushi, I.; Aoki, T.; Kamaishi, T.; Hagiwara, I. *Ind. Eng. Chem. Res.* **1988**, *27*, 1461.
- Kabay, N.; Egawa, H. *Sep. Sci. Technol.* **1994**, *29*, 135.
- Kago, T.; Goto, A.; Kusakabe, K.; Morooka, S. *Ind. Eng. Chem. Res.* **1992**, *31*, 204.
- Egawa, H.; Kabay, N.; Shuto, T.; Jyo, A. *Ind. Eng. Chem. Res.* **1993**, *32*, 540.
- In previous studies, uranium extraction experiments with the DHAB-containing cross-linked polystyrenes were carried out in the same sea on the east coast of Korean peninsula.^{6,7} Major portions of the DHAB moieties of the polystyrene resins were also occupied by Fe and Zn ions. In view of heavy contamination of the sea by Fe and Zn ions, uranium extraction by the polystyrene resins would be also considerably improved if tested in a cleaner sea.