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Properties and Application of Macroreticular Polymer Beads as Support in Extraction Chromatography

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Extraction and elution behavior of Zn(II), Cd(II) and Hg(II) was investigated by using dithizone-dibutyl phthalate solution retained on macroreticular type polymer beads such as styrene-divinylbenzene and ethylstyrene-divinylbenzene copolymers. The behavior of the metal ions was affected by the degree of cross-linking of polymers used as the support.

Keywords——extraction chromatography; dithizone; polymer beads; separation of Zn(II), Cd(II) and Hg(II); dibutyl phthalate; tracer method

Many inorganic and organic substances have been used for the supports in the extraction chromatography. Macroreticular (MR) type styrene-divinylbenzene(DVB) copolymer is one of the interesting supports in this chromatography owing to the outstanding physical properties such as large internal surfaces and porosity. In addition spherical polymer beads having various physical properties are easily prepared.

Beranova and Novak[2] and Spevackova and Krivanek[3] have reported on the separation of some radionuclides by using a column packed with a porous styrene-DVB copolymer retaining organic solvent. However, in these works the role of polymer support has not been described. On the other hand, low cross-linkage hydrophobic gel media swollen with solvent have been reported by several investigators.[4]

In the present study, an attempt was made to elucidate the effect of polymer supports on the extraction and elution behavior of metal ions such as Zn(II), Cd(II) and Hg(II) by using dithizone solution in dibutyl phthalate(DBP) as a chelating agent.

Experimental

Reagent——Commercial styrene monomer and DVB solution (nominally about 50% weight of DVB isomers, the remainder being ethylstyrene) were washed with dilute sodium hydroxide solution to remove phenolic inhibitors, washed with water, and then dried over calcium chloride. The suspension stabilizer was "Gohsenol NH-26," a poly (vinyl alcohol) preparation (Nihon Gohsei Co. Ltd., Osaka), and reagent grade of benzoyl peroxide was used as initiator. Dithizone (analytical reagent grade) was purified by the usual method,[5] and DBP (reagent grade) was purified by distillation. The radioisotopes, ^{65}Zn, ^{115m}Cd and ^{203}Hg as chloride in a hydrochloric acid solution, were supplied by the New England Nuclear Corp. and the Radiochemical Center, and were used as the tracer. The purity of each radionuclide was checked from its γ-spectrum. All other materials were of analytical reagent grade.

Polymers——Spherical beads of MR type styrene-DVB copolymers (7.5, 15, 30% DVB) and ethylstyrene-DVB copolymer (50% DVB) were prepared by the method of Egawa and Sugahara.[6] The appropri-

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[2] H. Beranova and M. Novak, *Collect. Czech. Chem. Commun.*, 30, 1073 (1965) [*C.A.*, 63, 2590*a* (1965)].

[3] V. Spevackova and M. Krivanek, *Radiochem. Radioanal. Letters*, 3, 63 (1970).

[4] H. Small, *J. Inorg. Nucl. Chem.*, **18**, 232 (1961); A.L. Clingman and J.R. Parrish, *J. Appl. Chem.*, **13**, 193 (1963); Y. Sekizuka, T. Kojima, T. Yano, and K. Ueno, *Talanta*, **20**, 979 (1973); T. Yano, S. Ide, Y. Tobeta, H. Kobayashi, and K. Ueno, *ibid.*, **23**, 457 (1976).

[5] G.H. Morrison and H. Freiser, "Solvent Extraction in Analytical Chemistry," John Wiley and Sons, Inc., New York, 1957, p. 173.

[6] H. Egawa and K. Sugahara, *Kogyo Kagaku Zasshi*, **74**, 1026 (1971).

ate mixture of styrene, 50% DVB solution and isooctane (equal volume of the monomers) containing benzoyl peroxide (1.5% of the monomers) was suspended in 0.2% Gohsenol NH-26 aqueous solution (five times of organic phase) and maintained at 70° with stirring for 6 hr at a controlled rate, and then the reaction temperature was raised to 80° for 2 hr to aid completion of the reaction. The polymer beads were collected by filtration, and transferred into a beaker with water, and boiled for 4 hr. Finally, the beads were filtered off, washed with hot water until they were free from adhering stabilizer, and dried. Gel type styrene-DVB copolymer (1% DVB) was prepared in the usual way. A fraction of 35—60 mesh was collected, washed with benzene in order to remove the small amount of residual reaction initiator and dried *in vacuo*. Structurally such copolymers may be regarded as being an interlacing net work of polystyrene chains cross-linked by DVB units. In general, the nominal DVB content in the polymerizing mixture is used to indicate the degree of cross-linking and in this paper they are abbreviated as MR-7.5, 15, 30, 50 and Gel-1, respectively.

Preparation of Stationary Phase——Ten mg of dithizone was dissolved in 100 ml of ethyl acetate and then 10 ml of DBP was added. Polymer beads (20 g) were immersed in the dithizone solution for 1 hr, and ethyl acetate was evaporated *in vacuo* (about 20 mmHg) by means of a rotary evaporator at 30—40° of bath temperature. The polymer thus obtained (*ca.* 30 g) was green but turned to a light brown on standing for a few days, therefore it is preferable to use immediately after the preparation. For a column operation to separate metal ions each other, following ratio of reagents and polymer were employed: dithizone 10 mg, DBP 2 ml, ethyl acetate 30 ml and polymer beads 4 g. The beads treated with the dithizone solution are abbreviated as Gel-1-D, MR-7.5-D, MR-15-D, MR-30-D and MR-50-D, respectively.

Batch Operation——Six hundred mg of the polymer beads treated with dithizone solution was weighed in a glass-stoppered tube, to which 10 ml of 1×10^{-6} M metal ion solution in various pH was added. After a given period of agitation at 20±1°, the γ-activity of the filtrate through glass wool was counted.

Column Operation——The stationary phase (6 g) deaerated with suction in 20% ethanol was packed into a column (1 cm in diameter). The column was conditioned with 50 ml of 1 M $HClO_4$ and 100 ml of acetate buffer (0.2 M CH_3COOH-0.2 M CH_3COONa, pH 5.0), then 0.5 ml of 1×10^{-5} M metal ion solution (Zn(II) or Cd(II)) containing ^{65}Zn or ^{115m}Cd was loaded and the column was washed with 30 ml of the buffer solution at a rate of 1 ml/min. Then the metal ion was eluted with 0.01 M HCl at a rate of 1 ml/min. In the case of separation of the metal ions, the column was conditioned with 50 ml of 1 M $HClO_4$ and enough water to give a neutral. Then 2 ml of sample solution (mixture of equal volume of 1×10^{-3} M Zn(II), Cd(II) and Hg(II)) containing each radioisotope was passed through the column at a rate of 1 ml/min. After washing of the column with 50 ml of water, the metal ions were eluted with buffer or acid solutions.

Characterization of Polymer——Solvent regain was essentially carried out by the method of Pepper *et al.*,⁷⁾ but the resins were equilibrated at 30° for 72 hr. Adsorption of iodine was determined from the gain in weight of resin samples treated with iodine at 50° (30 mmHg) for 2 hr. Surface area was measured by BET method with a model P-600 apparatus (Shibata Chemical Apparatus Co.) and pore volume was measured with a mercury porosimeter, Carlo Elba Series 1500.

Results and Discussion

The physical properties of spherical polymer beads used are listed in Table I. Solvent regain, amount of iodine adsorption, surface area and pore volume increased with increasing of the degree of cross-linking.

In the extraction chromatography by using dithizone as chelating agent, usually carbon tetrachloride, chloroform and chlorobenzene have been employed for the solvents. In this experiment DBP was chosen as the solvent because of its lower degree of swelling for the styrene-DVB copolymer.⁸⁾ Therefore it was supposed that polymer retaining the solvent kept characteristic physical properties of the copolymer to some extent. In addition, the polymer beads retaining DBP solution of dithizone could be easily prepared by evaporation of ethyl acetate under reduced pressure after they had been treated with a mixed solvent of DBP and ethyl acetate containing dithizone. Thus treated MR type polymers were available in a dry state apparently, but those of gel type were sticky. Extraction behaviors of Zn(II), Cd(II) and Hg(II) by Gel-1-D, MR-7.5-D and MR-50-D are shown in Fig. 1—3. In the pH range used, percentages of the extraction of Zn(II) and Cd(II) varied with the pH values, while Hg(II) was independent of pH. Hg(II) which showed the highest adsorption rate attained an equibrium within one min in the case of MR-50-D. In general, Gel-1-D

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7) K.W. Pepper, D. Reichenberg, and D.K. Hale, *J. Chem. Soc.*, **1952**, 3129.
8) R. Boundy and R.F. Boyer, "Styrene," Hafner Publishing Co., Inc., Conn., 1952, p. 427.

Table I. Physical Properties of Polymer Beads

Beads	Solvent regain (ml/g) Hexane	Solvent regain (ml/g) Dibutyl phthalate	Iodine adsorption (mg/g)	Surface area (m^2/g)	Pore volume (ml/g)
MR-7.5	0.76	1.02	123	19	0.43
15	1.40	1.53	198	61	1.22
30	1.49	1.62	233	149	1.32
50	1.46	1.55	374	378	1.44

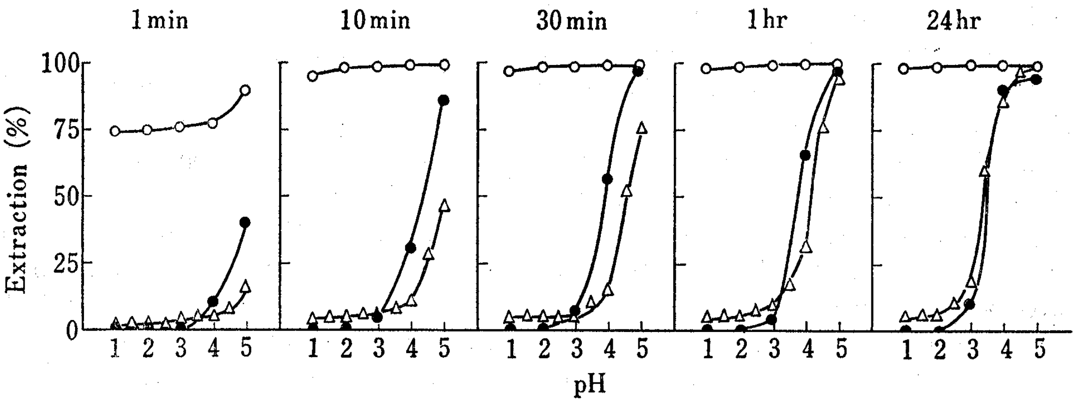

Fig. 1. Relationships between Shaking Time and pH on Extraction of Metal Ions with Gel-1-D

—○—: Hg(II), —△—: Zn(II), —●—: Cd(II).

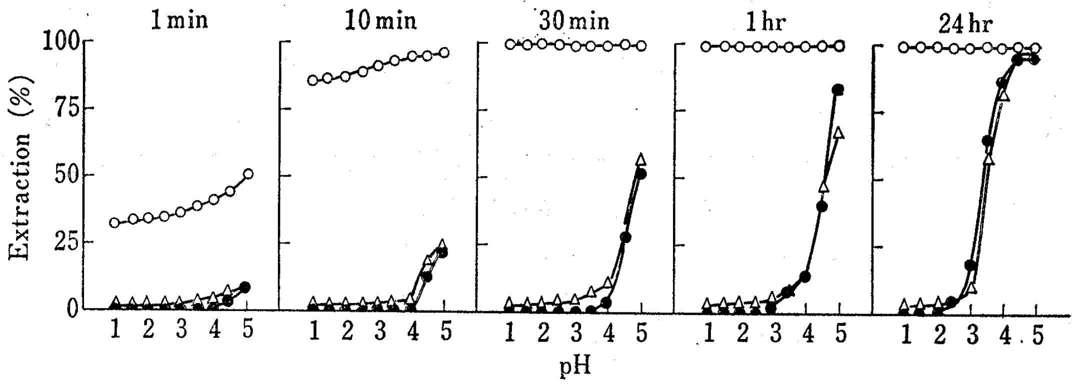

Fig. 2. Relationships between Shaking Time and pH on Extraction of Metal Ions with MR-7.5-D

—○—: Hg(II), —△—: Zn(II), —●—: Cd(II).

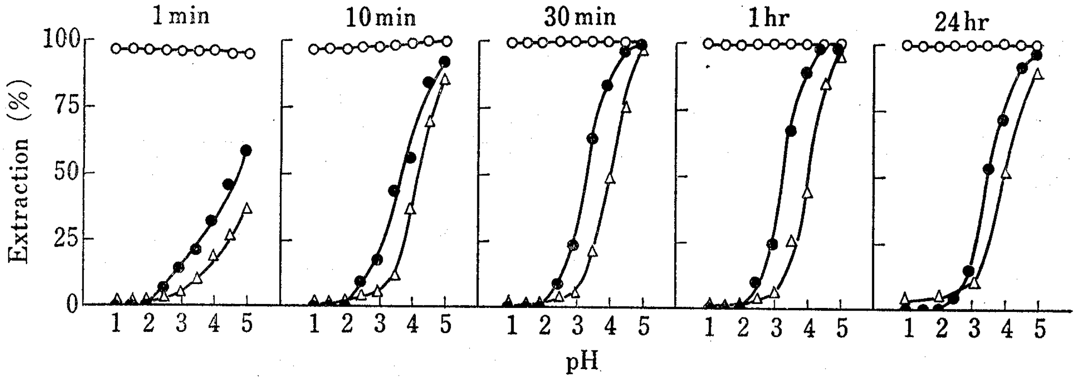

Fig. 3. Relationships between Shaking Time and pH on Extraction of Metal Ions with MR-50-D

—○—: Hg(II), —△—: Zn(II), —●—: Cd(II).

and MR-50-D showed the similar extraction behavior. Although Gel-1 has small surface area and has no apparent porosity, it swells even in DBP because of its lower cross-linking. Consequently, the metal ions may react easily with the dithizone retaining on the polymer. On the other hand, it is suspected that the large surface area of MR-50 contributes to the fast extraction of the metal ions.

In the separation of metal ions by column operation, it is of interest to investigate the effect of the supports on the extraction and elution of metal ions. Chromatographic behavior of Zn(II) was examined by using Gel-1-D, MR-7.5-D, MR-15-D, MR-30-D and MR-50-D. The extraction and elution data of Zn(II) are shown in Fig. 4 and Table II. In the case of Gel-1-D, good result was obtained in the extraction, but the elution was only about 50% and tailing was appreciable. This tailing seems to be the slower elution of Zn(II) out of the inner part of the beads. In the case of MR type series, the percentages of extraction and elution of Zn(II) were increased with increasing of the degree of cross-linking and the peaks in the elution curves became sharper and tailing was suppressed. A chromatogram obtained with MR-50-D was symmetrical and did not show tailing. This was explained on the basis of the large surface area of polymer support(MR-50) retaining DBP with dithizone, and hence elution of metal ion was fast. In addition, it could be assumed that the dithizone on MR-50 coordinated weakly with metal ion compared to that on gel and lower cross-linked MR type resins. When Cd(II) was used in above experiment, a similar chromatographic behavior was observed. From these facts, it was concluded that MR-50-D has the supreme property for the chromatography of metal ions in the polymers tested.

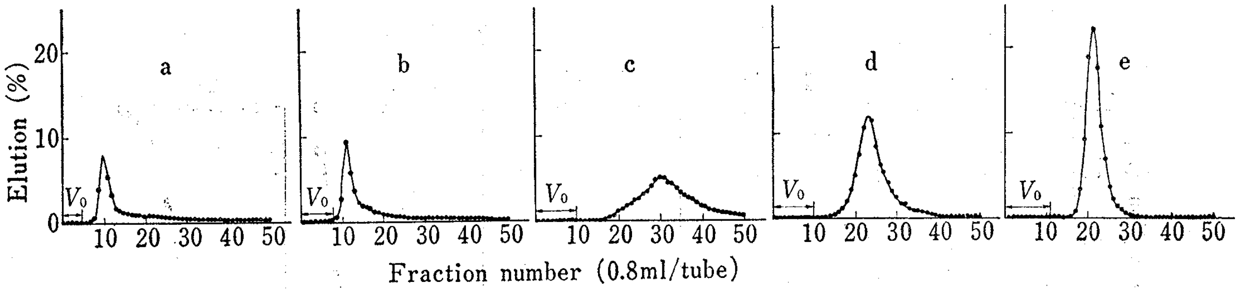

Fig. 4. Elution Curves of Zn (II) by Column Operation

Metal ion : 1×10^{-5}M, 0.5 ml, eluent: 1×10^{-2}M HCl.
Flow rate: 1 ml/min, V_0: void volume.
a: Gel-1-D, 1×14.5 cm b: MR-7.5-D, 1×13.5 cm c: MR-15-D, 1×19.5 cm.
d: MR-30-D, 1×20.0 cm e: MR-50-D, 1×20.0 cm.

Table II. Per Cent Extraction and Elution of Zn (II)

Adsorbents	Extraction (%)	Elution (%)
Gel-1-D	97.3	45.3
MR-7.5-D	22.2	50.9
MR-15-D	85.2	80.6
MR-30-D	98.5	96.0
MR-50-D	100.0	100.0

Conditions: the same as described in Fig. 4.

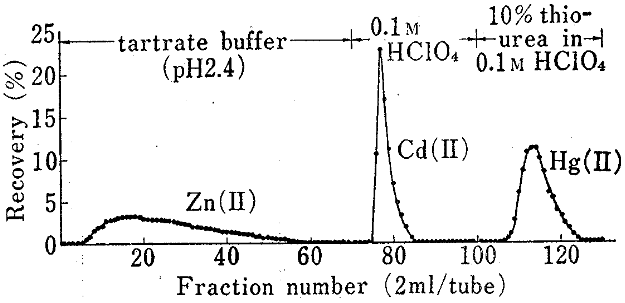

Fig. 5. Separation of Zn (II), Cd (II) and Hg (II) by Column Operation

Column: MR-50-D 6 g, 1.0×20.0 cm, flow rate: 1 ml/min.

A typical chromatogram of Zn(II), Cd(II) and Hg(II) by using a column of MR-50-D is shown in Fig. 5. Separation of these ions was achieved by stepwise elution with 0.05M tartrate buffer(pH 2.4), 0.1M perchloric acid and 10% thiourea in 0.1M perchloric acid. The recovery of metal ions was almost quantitative.

Important features of the proposed chromatographic system by using MR-50-D are as follows: (1) Chelating agent dissolved in DBP is retained on the large surface area of the MR type polymer beads . (2) Deformation or expansion of spherical polymer beads by treating with DBP solution of dithizone is scarcely observed. (3) The flow rate in column operation is easily controlled under the usual chromatographic conditions at atmospheric pressure. Although the disadvantages of the system are low capacity and short life, the polymer beads may be used repeatedly by washing out the DBP solution with ethyl acetate. In this study, relatively oxidizable dithizone was used as a chelating agent, however a modified method employing more stable chelating agent is promisingly considered.

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