

Synthesis and Separating Properties of Tris(Merrifield Peptide Resin)

Dong Won Kim^a, Chang Suk Kim^b, Nam-Soo Lee^a, Haiil Ryu^c, Jong Seung Kim^d, and Young Hun Jang^a

^a Department of Chemistry, Physical Chemistry Laboratory, College of Natural Sciences, Chungbuk National University, Cheongju 361–763, Korea

^b Department of Chemical Education, College of Education, Chungbuk National University, Cheongju 361–763, Korea

^c Department of Chemistry Education, Kongju National University, Kongju 314–701, Korea

^d Department of Chemistry, Konyang University, Nonsan 320–711, Korea

Reprint requests to Dr. D. W. Kim. Fax: 82-043-267-2279.

E-mail: chem131@trut.chungbuk.ac.kr

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A triazacrown ion exchanger, 1,13,16-trioxa-4,7,10-triazacyclooctadecane (TOTA)-4,7,10-tris(Merrifield peptide resin) was synthesized. This resin has a capacity of 0.1 meq/g dry resin. The resin was used for the elution chromatographic separation of lithium isotopes. Upon column chromatography [0.2 cm I. D. × 35 cm height] using 3.0 M NH₄Cl solution as an eluent, the single stage separation factor of 1.027 was obtained. The heavier isotope, ⁷Li⁺, was enriched in the resin phase, while the lighter isotope, ⁶Li⁺, was enriched in the solution phase.

It was reported that the complex formation of macrocyclic polyethers and their analogues with cations has been investigated and crown ethers may also be applicable to the separation of isotopes. Pederson reported in 1967 that crown ethers can form complex with alkali, alkaline earth, and other cations [1–3]. He showed that the complex formation of the crown compounds could be correlated with the cavity diameter of the macrocyclic polyethers and the relative sizes of the cations. Cox *et al.* [4] studied the solvent dependence of the stability of cryptates, and the characteristics of ion exchangers with azacrown ether and cryptands to alkali and alkaline earth metal ion separation have also been studied [5]. The complex between crown ether and an inorganic salt is formed by ion-dipole interaction between the cation and the oxygen atoms on the polyether ring. Alkali and alkaline earth metal ions can form complexes with 15-, 18-, 21-, and 24-crown ethers [6]. The advantage of the use of a triazacrown ion exchanger to separate isotopes is to form a complex with the lithium ion. When the lithium-triazacrown (18-crown-6) complex is formed, a steric configuration is formed such that each oxygen donor atom is located the shortest distance from the cation, or occasionally a 1:2 complex [6]. Lehn *et al.* [7–8] first synthesized cryptands, which are capable of

binding, especially, alkali and alkaline earth metal ions. The first use of ion exchangers for lithium isotope separation is found in a publication of Taylor and Urey [9] in 1937. Glueckauf *et al.* [10] first applied synthetic organic ion exchangers to lithium isotope separation. Jepson and De Witt [11] attempted to separate ⁴⁰Ca²⁺ and ⁴⁴Ca²⁺ by the ion exchange reaction using dibenzo-18-crown-6 and dicyclohexyl-18-crown-6. In the liquid-liquid extraction of calcium chloride using chloroform or dichloroethane and dicyclohexyl-18-crown-6, the one step separation coefficient of the isotopes was 1.0010 ± 0.0002 . They found that the heavier calcium isotope ⁴⁴Ca²⁺ was concentrated in the aqueous phase, while the lighter isotope ⁴⁰Ca²⁺ was enriched in the organic phase. Nishizawa *et al.* [12] have determined separation factors of 1.042 for lithium isotope separation using benzo-15-crown-5 as an extractant, and of 1.047 with a cryptand [2_B.2.1] polymer. Jepson and Carins [13] first reported the large separation factors in the range of 1.026 to 1.041 by using cryptand[2.2.1] for two phase chemical exchange systems composed of an aqueous solution of a lithium salt and a chloroform solution. Ooi *et al.* [14] have determined separation factors for lithium isotope in an aqueous ion exchange system, using titanium phosphate ion exchangers granulated with polyvinyl chloride or

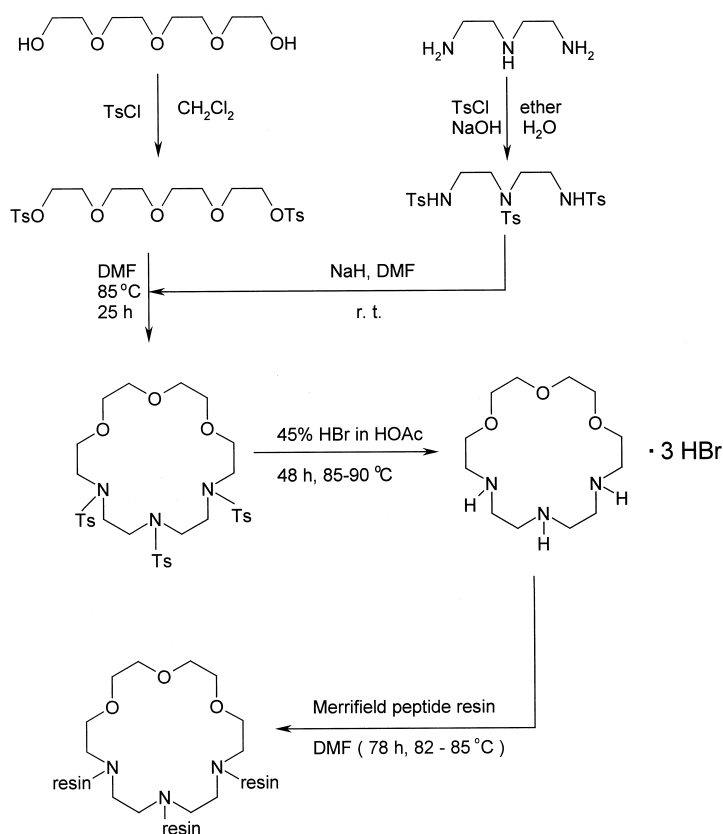
an inorganic binder. They reported that the separation factor of lithium isotopes was roughly 1.007, and the lighter isotope ^6Li was preferentially fractionated into the ion exchanger phase. Oi *et al.* [15] investigated the applicability of granular cubic antimonite acid as column packing material in chromatographic separation of lithium isotopes. They found that the lithium isotope separation effect was about ten times larger than that achieved with an organic ion exchanger. Kim *et al.* [16] reported a separation factor of 1.053 using styrene-divinyl benzene copolymer with monobenzo-15-crown-5. Fujine *et al.* [17] reported the separation factor of 1.014 at 40 °C using a cation exchanger, a [2.2.1] cryptand resin. Kim *et al.* [18] obtained the separation factors of 1.017–1.034, and 1.018–1.035 using dibenzo pyridino diamide azacrown (DBPDA) and reduced dibenzo pyridino diamide azacrown (RDBPDA) as anchor groups, respectively. The theoretical maximum value of the ele-

mentary separation factor for fractionation of lithium isotopes by molecular distillation is 1.080 ($\text{mass of } ^7\text{Li}/\text{mass of } ^6\text{Li}$)^{1/2} [19]. In this work, lithium isotope separation using a tris(Merrifield peptide resin) was examined.

Experimental Section

Synthesis of 1,13,16-Trioxa-4,7,10-triazacyclooctadecane-4,7,10-tris(Merrifield Peptide Resin)

The tris(Merrifield peptide resin) was prepared (Scheme 1) by the reaction of the corresponding 1,13,16-trioxa-4,7,10-triazacyclooctadecane trihydrobromide (TOTA·3HBr) with Merrifield peptide resin in dimethylformamide [20–23]. In a 100 ml round bottom flask, TOTA·3HBr, 1.008 g (2 mmol) was suspended in 20 ml of absolute ethanol. To this, 0.5 g (85%, 8 mmol) of KOH was added, and this mixture was stirred at 20 °C for 2 h. The KBr was removed by filtration, and the solvent was removed by vacuum distillation. It



Scheme 1. Synthesis of triazacrown tris(Merrifield peptide resin).

gave an oily residue. In to a 500 ml three-necked round-bottom flask, equipped with a condenser, addition funnels, and moisture protector, were placed dry dimethylformamide (200 ml) and triethylamine (2 ml). The oily residue, dissolved in dimethyl formamide (50 ml), was added slowly. After the mixture was stirred for 72 h at 88–90° C, the residue was washed with water and methanol. It gave a yellow powder. The C-Cl stretching vibration of the Merrifield peptide resin was found at 690 cm^{-1} in an IR spectrum. In the IR spectrum of the yellow product, the C-Cl absorption (KBr, 690 cm^{-1}) peak gave a lower intensity. This indicates a N-C bond formation between a nitrogen atom of the azacrown and the Merrifield peptide resin. In a TGA thermogram, the degradation of the triazacrown polymer began at 288° C and ended at 487° C, and T_{max} was 380° C as shown in Fig. 1. The degradation of the Merrifield resin began at 290° C and ended at 405° C, and T_{max} was 360° C, yield: 77%. – ^1H NMR (300 MHz, CDCl_3): δ = 3.87 (t, J = 4.8 Hz, 4H, O- $\text{CH}_2\text{CH}_2\text{-O}$), 3.65 (t, J = 6.63 Hz, 4H, O- $\text{CH}_2\text{CH}_2\text{-N}$), 3.42 (t, J = 5.18 Hz, 4H, O- $\text{CH}_2\text{CH}_2\text{-N}$), 3.11 (t, J = 6.14 Hz, 4H, N- $\text{CH}_2\text{CH}_2\text{-N}$). – ^{13}C NMR (300 MHz, CDCl_3): δ = 49.68, 52.58, 65.41, 72.12. – IR (KBr): ν = 3350 (s, =NH), 2580 (s, = N^+H_2), 1324 (s, - $\text{CH}_2\text{-O-CH}_2\text{-}$), 1160 (s, - $\text{CH}_2\text{-O-CH}_2\text{-}$) cm^{-1} . – $\text{C}_{12}\text{H}_{30}\text{N}_3\text{O}_3\text{Br}_3$ (503.7): calcd. C 28.59, H 5.99 N 8.34 ; found C 29.11, H 6.13, N 8.66.

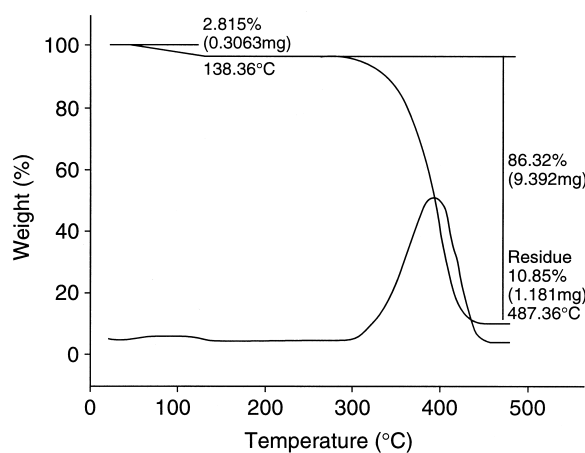


Fig. 1. TGA thermogram of triazacrown tris(Merrifield peptide resin).

Reagents and apparatus

Lithium chloride and ammonium chloride were purchased from Sigma Chemical Co., USA. An atomic absorption spectrophotometer (AAS, Hi-

tachi Z-8000) was used to determine the lithium ion concentration in the solution. The lithium isotope ratio was determined by using a thermal ionization mass spectrometer (Finnigan MAT 262) with a rhenium double filament. An amount of 1.0–2.0 μg of lithium was loaded on an evaporation filament. Ionization was then performed by passing a heating electric current through the ionization filament. After the ion beam intensities of $^6\text{Li}^+$ and $^7\text{Li}^+$ became sufficiently high, the $^6\text{Li}^+$ and $^7\text{Li}^+$ mass peaks were repeatedly recorded. The mass scanning was repeated several times in a block, and several blocks were recorded as one measurement. The mole fraction of $^6\text{Li}^+$ and $^7\text{Li}^+$ of each feed solution was an average of three times in this measurement.

Ion exchange capacity

For the determination of the capacity, the titration method was employed [24]. Each portion of 1.0 g of ion exchange resin (200 mesh, H-form) was weighed accurately, and transferred into a 100 ml polyethylene vial equipped with a polyethylene screw top. A 0.1 N NaOH solution (50 ml) containing NaCl (5%) was added, and the polyethylene vials were stoppered and shaken in a mechanical shaking device at 20° C for 24 h. The reaction mixture was centrifuged for 5 min at 5000 rpm, and then 20 ml of the supernatant were titrated with 0.1 N HCl solution. The capacity of the ion exchanger was calculated as

$$\text{Capacity (m eq/g)} = \frac{\{(V_{\text{NaOH}} \times N_{\text{NaOH}}) - (V_{\text{HCl}} \times N_{\text{HCl}}) \times 2.5\} \times 100}{m(100 - \% \text{H}_2\text{O})} \quad (1)$$

where V_{NaOH} is the volume of an aliquot taken for titration, V_{HCl} the equivalent volume of HCl in ml for the titration, N_{NaOH} and N_{HCl} are the normal concentration of NaOH and HCl, respectively, and m is the mass of air-dried resins in g, and % H_2O the weight percent of water in the resin. The capacity of the ion exchange resin is expressed in milliequivalents per g of dry (water-free) resin. The factor of 2.5 in eq. (1) indicates that the titrated volume of reaction solution is 2.5 times smaller than that of total volume.

Measurement of distribution coefficients

For the determination of the distribution coefficients, a batch method was employed [16]. Each portion of 1.0 g of the TOTA bonded Merrifield peptide resin (200 mesh) which has been dried to constant weight at 60 °C, was weighed accurately and transferred into a 100 ml polyethylene vial with a polyethylene screw cap. Then 1.0 ml of 0.01 M lithium chloride solution was added, followed by 49 ml of ammonium chloride solution of the desired concentration to give a final volume of 50 ml. The reaction mixture was subjected to reciprocal shaking at 100 strokes/min for 24 h, and then centrifuged for 5 min at 5000 rpm. The concentration of lithium ions in the supernatant was determined by AAS. The distribution coefficient K_d was calculated by equation (2):

$$K_d = \frac{(C_{st} - C_{eq})}{C_{eq}} \times \frac{V}{m} \quad (2)$$

where C_{st} is the metal ion concentration of the standard solution, C_{eq} the metal ion concentration after equilibrium, V the total volume in ml of the solution, and m the mass in g of dry resin.

Separation of lithium isotopes

The triazacrown ion exchanger, TOTATris(Merrifield peptide resin) was slurried in 3.0 M NH_4Cl solution. The slurried resin was packed in a water jacketed glass column (0.2 cm I. D. \times 35 cm height). The temperature was maintained at 20 °C with a water circulator (HAAKE A-80). Lithium ion (850 ppm) in distilled water was loaded on the top of the resin bed. 3.0 M molar ammonium chloride solution ($K_d = 72$) was used as an eluent for the separation. Lithium ion containing solution was then eluted through the column. The flow rate was controlled by a fine stopcock to be 0.3 ml/h. The effluent composed of a fraction of 0.1 ml was collected with an automatic fraction collector (Pharmacia LKB FRAC-100).

Results and Discussion

The 1,13,16-trioxa-4,7,10-triazacyclooctadecane-4,7,10-tris(Merrifield peptide resin) has a capacity of 0.1 meq/g dry resin. This value is less than those of SE 23 (cellulose cation exchanger, 0.2–0.3 meq/g) [25], ECTEOLA cellulose (anion exchanger, 0.35 meq/g) [25], and CAS cellulose (cation exchanger, 0.5 eq/L) [26]. Distribution coefficients of lithium ions on the tris(Merrifield peptide resin)

were determined by changing the concentration of NH_4Cl solution from 1.0×10^{-3} M to 6.0 M in the batch method and were calculated by eq. (2). As shown in Fig. 2, the values of $\log K_d$ increased in a non-linear manner with increasing concentration of NH_4Cl solution ranging from 1.0×10^{-3} M to 6.0 M. The chromatogram was obtained from the column operation with 3.0 M NH_4Cl solution ($K_d = 72$) as an eluent at 20 °C. The number of theoretical plates, N , in the column was calculated from the chromatogram by the following equation [27]

$$N = 8 \cdot \left(\frac{V_{max}}{\beta} \right)^2 \quad (3)$$

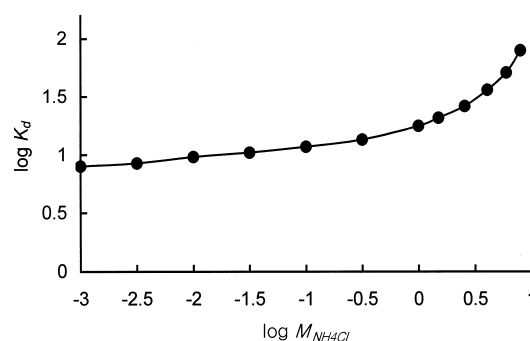


Fig. 2. Plot of $\log K_d$ for lithium ions on triazacrown tris(Merrifield peptide resin) as a function of NH_4Cl concentration.

where V_{max} is the retention volume, β the band width at the concentration $C = C_{max}/e = 0.368 C_{max}$, and C_{max} the maximum peak height. The separation factor of the lithium isotopes was calculated by the Glueckauf method [28].

The elution time increased with increasing the K_d due to the high adsorption of ion on the resin phase in the column. For this reason, eluents with K_d ranging from 30 to 300 were used to separate isotopes in our laboratory. From the elution curve and isotopic assay data, the single stage separation factor, $({}^6\text{Li}^+ / {}^7\text{Li}^+)_{\text{resin}} / ({}^6\text{Li}^+ / {}^7\text{Li}^+)_{\text{solution}}$ was determined by the Glueckauf method [28]. The local enrichment factor for a fraction is denoted by $R = (C_1/C_2)(C_2^0/C_1^0)$, where C_1 and C_2 are the relative abundances of the light and heavy isotopes, in addition, C_1^0 and C_2^0 the natural abundances of the light and heavy lithium isotopes. A plot of $\log R$ against $\Delta m/m$ gave a

straight line the slope of which corresponds to $\varepsilon N^{1/2}$, and separation factor, α , is defined as $1+\varepsilon$. The separation factor, α , was determined from the slope of a least squares line drawn as shown in Fig. 3. In this experiment, the separation factor was found to be 1.027 at 20 °C. This value is larger than obtained by

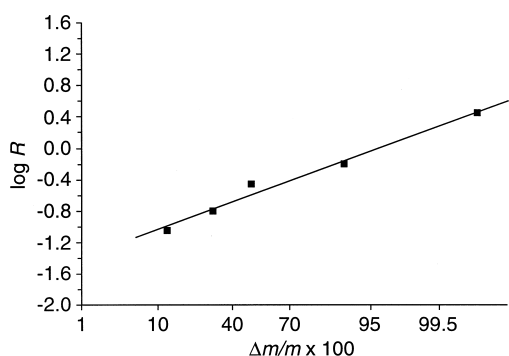
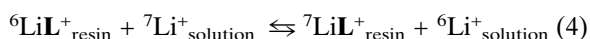


Fig. 3. Enrichment of lithium isotopes by cation exchange elution chromatography.

Ooi *et al.* [14] and Takeuchi *et al.* [29]. Takeuchi *et al.* also evaluated that the values of the ^7Li -to- ^6Li isotope separation factor are between 1.0040 and 1.0092 at 20° C. In this experiment, the heavier isotope, ^7Li , was enriched in the resin phase, while the lighter isotope, ^6Li , was enriched in the solution phase. This can be explained that the complexing ability of $^7\text{Li}^+$ with TOTA ligand in the resin phase is larger than that of $^6\text{Li}^+$. The basis of the enrichment process of isotopes is the chemical exchange reaction, which proceeds according to eq. (4) (L = polymer bound 18-crown-6 ligand).



Oi *et al.* [30] and Klinskii *et al.* [31] stated that the heavier isotopes of lithium were preferentially concentrated in the resin phase. These phenomena agree well with our work. In contrast, Oi *et al.* [32] and Kondoh *et al.* [33] stated that the heavier isotopes were preferentially enriched in the solution phase rather than in the resin phase. Gupta [34] has discussed the effect of anions for the isotope effects, and showed that halide ions are considered to be structure breakers, except for the

fluoride ion, and as the partial dehydrations of the lithium ions in the resin phase remains the same, this overall increase in the solvation of the lithium ions in the solution in the presence of the structure breaker increases the difference in hydration numbers for the ion in solution and the resin phase, and hence the isotope effect. Lee [35] showed that the lithium isotope separation factor increased as the degree of hydration of the eluting cation increased. The heat of hydration of eluting NH_4^+ ion is relatively small [36], but the counter ion Cl^- is the significant structure breaker [36–38], and this may be contributed to the isotope effect [34, 36–38] in this experiment. It was reported that the metal ion species in the resin phase is less hydrated than the metal ion species in the solution phase. This contributes to a difference in bonding and subsequent enrichment of the lighter isotopes in the resin phase [39], since the complexation effect of the heavier isotope with ligand is stronger than that of hydration in this system. In the resin phase, where the water molality is reduced and the water structure is disrupted by the presence of the organic matrix, lithium ions are less strongly hydrated, and hence, less strongly bonded, than in the dilute exterior solution surrounding the resin [40–41]. The heavier, smaller isotope, which tends to concentrate preferentially in the more strongly bonded species [41–44], thus favours the aqueous phase, and the lighter isotope is displaced into the resin phase. However, these phenomena are in contrast with our system. Lithium isotope separations using an azacrown ion exchange resin have an advantage due to the remarkable property of complexation with cations, especially, alkali and alkaline earth metal ions [1–8]. In the ion exchange elution chromatography, the separation factor is the ratio between the distribution coefficients of solutes $^6\text{Li}^+$ and $^7\text{Li}^+$ in a tris(Merrifield peptide resin) at a specified temperature.

Acknowledgements

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