Xylitol Separation from a Polyol Mixture Using Lanthanide Ion-loaded Resins

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Abstract

Xylitol separation from a polyol mixture of the byproducts from bioethanol production processes was performed by liquid chromatography using short columns packed with lanthanide ion-loaded ion-exchange resins. Xylitol was successfully separated with sufficiently high resolution using the adsorbents with medium rare-earth metal ions such as Nd$^{3+}$ and Sm$^{3+}$. The adsorbents' specific nature is explained by the so-called “gadolinium break,” which is known as a discontinuous behavior of thermodynamic parameters in the complexation of lanthanide series. From the observed behavior, the optimum lanthanide ions could be chosen to prepare the appropriate adsorbents for ligand-exchange chromatography of given polyol mixtures.

**Keywords:** monosaccharide, polyol, lanthanide, rare-earth metal, ligand exchange, bioethanol, gadolinium break, liquid chromatography, ion-exchange resin, AG 50W-X4
Introduction

Bioethanol fuel is one of the petrol substitutes produced from agricultural crops by the sugar fermentation process.\(^1\) This fuel would be one of the solutions for the greenhouse effect and depletion of fossil fuels; however, the large-scale production of bioethanol from cereal crops such as corn and wheat could cause water shortages and adverse effects for the food supply. The use of nonfood plants, waste materials, such as used straw and willow, sawdust, and even municipal solid wastes are therefore ongoing research targets as a hopeful feedstock for bioethanol.\(^2\)–\(^4\)

Bamboo invasion is a serious environmental issue in many Japanese cities. The disordered proliferation of moso bamboo (\textit{Phyllostachys edulis}, non-native species) due to the lack of control rapidly converts the deciduous and broadleaf forests into bamboo forests.\(^5\)–\(^7\) Prompt action against bamboo invasion is also necessary in Kumamoto, where the effective use of fallen bamboo trees has been explored. We have taken part in the local project of bioethanol production from bamboo. Ethanol can be produced from bamboo through hydrolysis and sugar fermentation processes.\(^8\),\(^9\) From our optimized processes using \textit{Candida tropicalis}, three polyols, xylose, xylitol, and glycerol, are obtained as byproducts. Among them, xylitol is a high-value chemical due to its use as a sweetener.\(^10\)–\(^14\) However, a small amount of glycerol (b.p.: 290 °C) interferes with the crystallization of xylitol because of its highly hygroscopic nature.

In the present study, we tried to separate the polyols by liquid chromatography. As the separation mode, here we chose ligand exchange on the trivalent lanthanide ions (Ln\(^{3+}\)) loaded on cation-exchange resins. A strong-acid cation exchanger quantitatively captures Ln\(^{3+}\) and, in chromatography, the residual coordination room on the Ln\(^{3+}\) sphere on the other side of the resin could be used to retain the molecules with Lewis base atoms (Fig. 1). There are some merits of the ligand-exchange mode for separation of biomolecules.\(^15\)–\(^21\) Aqueous solutions (no organic solvent) of neutral pH are used as eluent, which is ecofriendly. Appropriate metal ions could be
chosen for each of the given mixtures of analytes on demand. Especially for Ln$^{3+}$, while they show similarity in various chemical properties, the size of the ions decreases from La$^{3+}$ to Lu$^{3+}$, which is known as lanthanide contraction. We can prepare a series of Ln$^{3+}$-loaded resins that could be subjected to systematic study for separation of analyte mixtures. Hydroxyl groups in polyols are the typical hard Lewis base. Therefore, the nature of Ln$^{3+}$ as a hard Lewis acid should make the Ln$^{3+}$-loaded resins excellent flexible adsorbents for polyols.22–24 In ligand-exchange chromatography, the Ln$^{3+}$-loaded adsorbents are expected to retain the polyols depending on their structural parameters, such as the number of hydroxyl groups, stereochemistry, and flexibility of molecular backbones (Fig. 1).

[Fig. 1]

Experimental

Reagents and chemicals

All materials and chemicals were of analytical grade and used without further purification. A strong-acid cation-exchange resin AG 50W-X4 (hydrogen ion form, crosslinkage: 4%, particle size: 0.075–0.150 mm, total cation exchange capacity: 1.1 meq/mL) was purchased from Bio-Rad Laboratories (Hercules, CA, USA). LaCl$_3$$\cdot$6H$_2$O, CeCl$_3$$\cdot$6H$_2$O, SmCl$_3$$\cdot$6H$_2$O, Gd(NO$_3$)$_3$$\cdot$6H$_2$O, HoCl$_3$$\cdot$6H$_2$O, YbCl$_3$$\cdot$6H$_2$O were purchased from Wako Pure Chemical Industries (Osaka, Japan). Nd(NO$_3$)$_3$$\cdot$6H$_2$O was purchased from Junsei Chemical (Tokyo, Japan). D-$(\pm)$-Xylose, xylitol, glycerol and all other chemicals used in this study were purchased from Wako Pure Chemical Industries. Ultra-pure water (Milli-Q, Integral MT5L; Nippon Millipore, Tokyo, Japan) was used for all experimental work.
Preparation of Ln$^{3+}$-loaded resin

Twenty milliliters of swollen AG 50W-X4 was washed by decantation with water several times, packed into the glass column (inner diameter: 12 mm, length: 20 cm), and then the column was subjected to Milli-Q water conditioning with a flow rate of 20 mL/h for 3 h. 100 mL (5.0 BV [Bed volumes]) of aqueous LaCl$_3$ solution (0.36 mol/L) was fed to the column at a flow rate of 20 mL/h. After washing the column with 100 mL of Milli-Q water, La$^{3+}$-loaded adsorbent was subjected to the experiments for the measurement of ion-exchange rate and polylol separation. Other adsorbents loaded with Ce$^{3+}$, Nd$^{3+}$, Sm$^{3+}$, Gd$^{3+}$, Ho$^{3+}$, and Yb$^{3+}$ were prepared similarly.

Measurement of ion exchange rate

The swollen volume of Ln$^{3+}$-loaded resins (ca. 1.0 mL) was measured with a measuring cylinder after overnight settling. The resins were completely dried in a vacuum desiccator and their dry weights were measured. Certain amounts of the dried resins were completely liquefied with 4 mL of conc. HNO$_3$ using a microwave digestion oven (Speedwave 4; Berghof Products Instruments, Berghof, Germany). Three samples were prepared for each of the adsorbents for duplicate measurements.

The digested solutions of each of the adsorbents were appropriately diluted to be around 8 ppm for the concentration of Ln$^{3+}$ and subjected to concentration measurement by ICP-AES (inductively coupled plasma-atomic emission spectroscopy, Nippon Jarrell Ash, Kyoto, Japan). The ion-exchange capacities ($IX_{obs}$ [meq/mL]) of the resins for each of Ln$^{3+}$ were estimated using respective standard curves. The ion-exchange rates ($RIX$ [%]) for each of Ln$^{3+}$ were calculated as a relative value with the theoretical ion-exchange capacities ($IX_{max}$ [meq/mL], the maximum Ln$^{3+}$ capacity loaded on the ion-exchange resin with 1.1 meq/mL acid capacity) estimated by following equation.
The shrinkage factor of the volume of resin when it fully loads Ln$^{3+}$.

**Polyols separation**

Ten milliliters of each of the Ln$^{3+}$-loaded resins were packed into a glass column (inner diameter: 12 mm, length: 20 cm), and the columns were subjected to Milli-Q water conditioning with a flow rate of 20 mL/h for 3 h. 3 mL (0.3 BV) of the mixture of xylose, xylitol, and glycerol (20 g/L for each) was loaded to the columns with a flow rate of 20 mL/h. Milli-Q water was fed to the columns as an eluent at a flow rate of 20 mL/h. All effluents were collected on a fraction collector by 1.5 mL (0.15 BV) for each fraction, which was analyzed with HPLC (Sugar Analyzer SU-300; DKK-TOA, Tokyo, Japan) after suitable dilution. The resolution ($R$) of the two components was calculated from a chromatogram. The $R$ value was defined as $2(V_{R2} - V_{R1})/(w_{b1} + w_{b2})$, where $V_{R1}$ and $V_{R2}$ are the retention volumes of polyols 1 and 2, and $w_{b1}$ and $w_{b2}$ are the peak widths (baseline) of polyols 1 and 2, respectively.

**Results and Discussion**

**Ion-exchange rate for the series of lanthanide ions**

Enormous knowledge of cation binding on various solid supports was accumulated in a long history of the studies on ion-exchange resins to date. Now we can choose the suitable resin appropriate for our research purpose considering several requirements regarding, for example, the types of cations (hard or soft) and how to use the resin (reversible or irreversible binding).25-28 Here we chose AG 50W-X4, which is a strong-acid cation-exchange resin with sulfonic acid groups. Seven Ln$^{3+}$ from lanthanide series, La$^{3+}$, Ce$^{3+}$, Nd$^{3+}$, Sm$^{3+}$, Gd$^{3+}$, Ho$^{3+}$, and Yb$^{3+}$, were loaded on the resin using the column method. Table 1 shows some properties of the Ln$^{3+}$-loaded...
resin regarding its ion-exchange capacities. The AG 50W-X4 resin significantly shrank after loading of Ln$^{3+}$. We observed ca. 30% SF throughout a series of Ln$^{3+}$. Trivalent cations would reduce the electrostatic repulsion between the sulfonate groups or, even more directly, convergently draw the distal two or three points on the polymer up to themselves by bridging the sulfonate groups.$^{29}$ Apparent particular trends such as lanthanide contraction in the SF were not observed. $RIX$ was calculated from $IX_{\text{max}}$ and $IX_{\text{obs}}$ as the value of ion-exchange rates for each of Ln$^{3+}$ after correcting the effect of resin shrinking. The $RIX$ values almost monotonously decreased from light (La$^{3+}$) to heavy (Yb$^{3+}$) lanthanide ions. It seems that the shorter the Ln$^{3+}$ radius is, the more difficult the effective bridge (or neutralization) forms between fixed anionic charges on the reticular polymer matrix.

[Table 1]

Polyols separation

The mixture of the main byproducts in bioethanol production processes from the bamboo tree, xylose, xylitol, and glycerol, was applied to chromatographic separation by ligand-exchange mode using a series of Ln$^{3+}$-loaded adsorbents. The chromatograms for the polyol separation using La$^{3+}$-loaded resins are shown in Fig. 2. Retention of xylitol was most strong and followed by glycerol and xylose in this order. Compared with glycerol having three hydroxyl groups, a five-hydroxyl groups sequence on the linear flexible backbone of xylitol seem to effectively (at least transiently) coordinate to the immobilized La$^{3+}$. $^{30}$ However, it is apparent that four hydroxyl groups in xylose seem not to coordinate to La$^{3+}$ effectively, because xylose retention was weaker than glycerol. In the rigid pyranose structure of xylose, any three neighboring hydroxyl groups are not allowed to direct the same side of the structural backbone, making it difficult to form a stable complex with La$^{3+}$. $^{31,32}$ Actually, the Morel group showed the significant difference in the thermodynamic parameters in the interaction of a pair of the
epimers, ribose and arabinose, with Ln\(^{3+}\).\(^{33,34}\) The results observed here show that not only the number, but also the relative directions (stereochemistry) of hydroxy groups are critical for the formation of stable La\(^{3+}\)/sugar complexes.

The \(R\) values between the two components were calculated to be 1.00, 0.80, and 0.39 for xylitol/xylose, xylitol/glycerol, and glycerol/xylose, respectively. That is, xylitol was nearly completely isolated from xylose and glycerol by almost baseline separation using the short column. The results coincide with those reported by the research groups of Gaset\(^{22}\) and Stankovic.\(^{30}\) They reported the chromatographic separation of a series of carbohydrates and alditols by ligand-exchange mode using La\(^{3+}\)-loaded resins, respectively. Sugar alcohols such as galactitol, xylitol, and arabinitol with linear backbone showed strong retention on the resin.

The adsorbents loaded with six other lanthanide ions were subjected to ligand-exchange chromatography for the polyol mixture in the same way. The obtained chromatograms are shown in Fig. 3. Reproducibility was observed for all of the adsorbents, showing negligible leakage of Ln\(^{3+}\). Interestingly, each of the Ln\(^{3+}\)-loaded resins provided quite different elution profiles, especially for the retention volume (\(V_k\)) of xylitol. The change in \(V_k\) or \(R\) does not apparently follow the trend of lanthanide contraction. The \(R\) values for the two components are plotted toward the atomic weight of lanthanide series in Fig. 4. The results show the maximum at medium lanthanide-loaded resins, such as of Nd\(^{3+}\) and Sm\(^{3+}\), and no significant retention was observed for the resins loaded with heavier Ln\(^{3+}\). Aside from separation of the byproducts from bioethanol production, it would be worth for La\(^{3+}\)-loaded resins to be subjected to the systematic studies of polyol separation as general adsorbents with tunable retention.

It is known that the thermodynamic parameters of lanthanide complexation with various ligands shows a nonlinear pattern as the function of atomic numbers. Many factors should be
considered to completely explain this unique behavior. One of the important ones would be desolvation. Complexation is the process of displacement of the hydrated water molecules on the metal ion by hydroxyl groups of analytes. The studies of X-ray diffraction, neutron scattering, and Raman spectra showed that the inner-sphere hydration number changes within the lanthanide ion series. The coordination number of the lanthanides is nine for the lighter ones (La$^{3+}$–Nd$^{3+}$) and eight for the heavier ones (Tb$^{3+}$–Lu$^{3+}$). This would be a main factor explaining the irregularity in complexation at or around Gd$^{3+}$, which is known as so-called “gadolinium break.”

The bell-shaped profile of $R$ value shown in Fig. 4 looks similar to the stability constant profile already reported for the complexation of ribose with a series of lanthanide ions in aqueous solution. It shows that the gadolinium break is also true in ligand-exchange chromatography for the separation of polyols. That is, although a part of the coordination sphere of Ln$^{3+}$ is tightly occupied by sulfonates on the resin, the unique character of lanthanide series is still preserved in the residual room for coordination. This would be important knowledge for the design of the adsorbents for the separation of carbohydrates.

Conclusions

As far as we know, this is the first report of the gadolinium break observed in the systematic studies of ligand-exchange chromatography for biomolecules separation. While the ion-exchange rates of each of Ln$^{3+}$ slightly depend on its ionic radius (Table 1), the methods for adsorbent preparation are identical for all Ln$^{3+}$. Leakage of metal ions during chromatography was negligible for all Ln$^{3+}$ used in this study. Under these well-specified or -arranged conditions, we can tune the retention of the analytes by choosing the appropriate Ln$^{3+}$ for given mixtures. Therefore, Ln$^{3+}$-loaded resins would be an effective and flexible adsorbent for separation of the biomolecules especially with the functional groups of hard Lewis bases. The
results obtained in this study would be a useful guideline for designing the systems of ligand-exchange chromatography for biomolecules in water.

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References

1. A. Singh and G. P. Rangaiah, *Ind. Eng. Chem. Res.*, **2017**, 56, 5147.

2. H. B. Aditiya, T. M. I. Mahlia, W. T. Chong, Hadi Nur, and A. H. Sebayang, *Renew. Sust. Energ. Rev.*, **2016**, 66, 631.

3. L. Rocha-Meneses, M. Raud, K. Orupold, and T. Kikas, *Agnon. Res.*, **2017**, 15, 830.

4. K. Robak and M. Balcerek, *Food Technol. Biotechnol.*, **2018**, 56, 174.

5. Q. -F. Xu, C. -F. Liang, J. -H. Chen. Y. -C. Li. H. Qin, and J. J. Fuhrmann, *Glob. Energ. Conserv.*, **2019**, 21, e00787.

6. S. Suzuki, *Plant Species Biol.*, **2015**, 30, 63.

7. K. Okutomi, S. Shinoda, and H. Fukuda, *J. Veg. Sci.*, **1996**, 7, 723.

8. Z. Y. Sun, Y. Q. Tang, T. Iwanaga, T. Sho, and K. Kida, *Bioresour. Technol.*, **2011**, 102, 10929.

9. Z. Y. Sun, Y. Q. Tang, S. Morimura, and K. Kida, *Bioresour. Technol.*, **2013**, 128, 87.

10. M. Miura, I. Watanabe, Y. Shimotori, M. Aoyama, Y. Kojima, and Y. Kato, *Wood Sci. Technol.*, **2013**, 47, 515.

11. W. G. Morais Junior, T. F. Pacheco, D. Trichez, J. R. M. Almeida, and S. B. Goncalves, *Yeast*, **2019**, 36, 349.

12. D. Camargo, L. Sene, D. I. L. S. Variz, and M. D. S. de Almeida Felipe, *Appl. Biochem. Biotechnol.*, **2015**, 175, 3628.

13. M. Miura, Y. Shimotori, H. Nakatani, A. Harada, and M. Aoyama, *Appl. Biochem. Biotechnol.*, **2015**, 176, 947.

14. L. Xu, L. Liu, S. Li, W. Zheng, Y. Cui, R. Liu, and W. Sun, *Sugar Tech*, **2019**, 21, 341.

15. X. Zhu and A. Jyo, *Sep. Sci. Technol.*, **2001**, 36, 3175.

16. X. Zhu and A. Jyo, *Water Res.*, **2005**, 39, 2301.

17. Md. R. Awual, A. Jyo, M. Tamada, and A. Katakai, *J. Ion Exchange*, **2007**, 18, 422.
18. Md. R. Awual, M. A. Shenashen, T. Yaita, H. Shiwaku, and A. Jyo, Water Res., 2012, 46, 5541.
19. Md. R. Awual, A. Jyo, T. Ihara, N. Seko, M. Tamada, and K. T. Lim, Water Res., 2011, 45, 4592.
20. Md. R. Awual, S. A. El-Safty, and A. Jyo, J. Environ. Sci., 2011, 23, 1947.
21. T. Ihara, T. Mitsuru, Y. Kitamura, Y. Chikaura, and A. Jyo., Anal. Sci., 2001, 17, i1229.
22. H. Caruel, L. Rigal, and A. Gaset, J. Chromatgr., 1991, 558, 89.
23. M. Stefansson and D. Westerlund, J. Chromatogr. A, 1996, 720, 127.
24. H. -T. Feng, X. -Y. Huang, C. -P. Luo, and M. M. Lee, J. Liq. Chrom. Rel. Technol., 2009, 32, 210.
25. R. Konradi and J. Rühe, Macromolecules, 2005, 38, 4345.
26. R. Kawamura, M. Satou, T. Yonesaka, and A. Yuchi, Anal. Sci., 2019, 35, 141.
27. S. Yoshii, M. Mori, D. Kozaki, T. Hosokawa, and H. Itabashi, Anal. Sci., 2019, 35, 1117.
28. I. Suzuki, Y. Kumai, M. Kitagawa, Y. Kishimoto, K. Umegaki, T. Chiba, and J. Takebayashi, Anal. Sci., 2019, 35, 1269.
29. S. Kagaya, R. Ikeda, T. Kajiwara, M. Gemmei-Ide, and Y. Inoue, Anal. Sci., 2019, 35, 413.
30. L. Petrus, V. Bilik, L. Kuniak, and L. Stankovic, Chem. Zvesti., 1980, 34, 530.
31. S. J. Angyal, Aust. J. Chem., 2000, 53, 567.
32. D. Goto, K. Ouchi, M. Shibukawa, and S. Saito, Anal. Sci., 2015, 31, 1143.
33. N. Morel-Derosiers and J. -P. Morel, J. Chem. Soc., Faraday Trans., 1989, 85, 3461.
34. N. Morel-Derosiers, C. Lhermet, and J. -P. Morel, J. Chem. Soc., Faraday Trans., 1993, 89, 1223.
35. A. Habenschuss and F. H. Spedding, J. Chem. Phys., 1980, 73, 442.
36. C. Cossy, A. C. Barnes, J. E. Enderby, and A. E. Merbach, J. Chem. Phys., 1989, 90, 3254.
37. H. Kanno, J. Phys. Chem., 1988, 92, 4232.
38. G. Schwarzenbach and P. Gut, *Helv. Chim. Acta*, **1956**, *39*, 1589.
Table 1  Ion-exchange parameters of Ln$^{3+}$-type resin (AG 50W-X4; Bio-Rad)

| Ln$^{3+}$ | La  | Ce  | Nd  | Sm  | Gd  | Ho  | Yb  |
|-----------|-----|-----|-----|-----|-----|-----|-----|
| Shrinkage ($S_F$), % | 30.1 | 31.2 | 29.9 | 32.1 | 30.6 | 31.1 | 26.7 |
| Ion-exchange capacity, meq/mL | $I_{X_{\text{max}}}$ | 0.53 | 0.53 | 0.52 | 0.54 | 0.53 | 0.53 | 0.50 |
| $I_{X_{\text{obs}}}$ | 0.535 | 0.520 | 0.494 | 0.513 | 0.471 | 0.473 | 0.411 |
| Ion-exchange rate ($R_{IX}$), % | ~100 | 98  | 95  | 95  | 91  | 89  | 84  |
**Figure Captions**

**Fig. 1** Chemical structures of ion-exchange resin and analytes used for the study of polyol separation by ligand-exchange mode using Ln$^{3+}$-loaded resin.

**Fig. 2** Polyols separation by ligand-exchange chromatography using La$^{3+}$-loaded resin. Feed volumes of eluent were plotted as relative value to column bed volume, (eluent volume)/(column bed volume). Column: 10 mL of La$^{3+}$-form wet resin (AG 50W-4X; Bio-Rad); column length: 8.8 cm; sample: 3 mL of 20 g/L for xylose, xylitol, and glycerol; eluent: water; flow rate: 20 mL/h; temperature: 25 °C.

**Fig. 3** Polyols separation by ligand-exchange chromatography using Ln$^{3+}$-loaded resins. All chromatographic conditions are the same as those shown in Fig. 2.

**Fig. 4** Resolution ($R$) of the two polyols by ligand-exchange chromatography using the adsorbents loaded a series of lanthanide ions. closed circle: xylitol/xylose, open circle: xylitol/glycerol, x: glycerol/xylose.
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Fig. 4 Resolution ($R$) of the two polyols by ligand-exchange chromatography using the adsorbents loaded a series of lanthanide ions. closed circle: xylitol/xylose, open circle: xylitol/glycerol, x: glycerol/xylose.
Graphical Index

La Ce...Sm.Gd......Lu

Gd break

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