Synthesis of Salicylideneamino-2-thiophenol Immobilized Glass Beads and Their Application to a Preconcentration of Trace Aluminum¹⁾

Jun KOBAYASHI* and Motoichi MIYAZAKI

Faculty of Pharmaceutical Sciences, Kanazawa University, 13-1, Takara-machi, Kanazawa 920, Japan.

Received October 30, 1992

Chelating glass beads (SAB) containing salicylideneamino-2-thiophenol (SATP) as a functional group were synthesized for the selective adsorption and separation of ionic aluminum (Al^{3+}). SAB was prepared by reacting amino-propyl-CPG (controlled pore glass) with SATP, shielded from light in toluene at 110 °C for 12 h. The exchange capacity of SAB for Al^{3+} was about 1.1 mmol/g (support). When SAB was packed into a column, only Al^{3+} was retained out of all the metal ions examined, at pH 5.5 with hydroxylamine hydrochloride present. Aluminum ion was then eluted quantitatively with nitric acid. Aluminum samples below 1 ppm were concentrated by a factor of 100. This column method was applied to the preconcentration of Al^{3+} in drinking water.

Keywords preconcentration; aluminum ion; drinking water; salicylideneamino-2-thiophenol

Aluminum is widely present in the environment, and the concentration levels differ greatly between soil-, water-, and the atmosphere. Hitherto aluminum, with its reputed low toxicity, has been used to produce food, tableware and various industrial products. But in recent years, it has come to light that high intake causes gastrointestinal damage and phosphate deficiency (rickets and so on).^{2–4)} Moreover, aluminum is also thought to play a role in dialysis encephalopathy, renal osteodystrophy and Alzheimer's diseases,^{2,4–6)} although detailed mechanisms are as yet unknown. In exploring the causes of these illnesses, it is important to analyze not only the aluminum concentrations of biological samples but also those of environmental samples, such as rain-, river-, and drinking water. Drinking water, particularly, needs to be analyzed, as it would seem to be one of the main sources in human intake. Although aluminum concentration levels in the environment are very low, enrichment by biological magnification *in vivo* produces toxicity.

There are many analytical methods for aluminum (ion) determination nowadays, including atomic absorption spectrophotometry (AAS), Eriochrome Cyanine R spectrophotometry and aluminum spectrofluorimetry.^{7,8)} However, when these methods are used in the determination of environmental samples like drinking water, difficulties with sensitivity often arise due to interference by coexisting compounds.

Various separation/enrichment techniques have been used to solve the above problems such as solvent extraction and ion-exchange, but they have proven to be less than optimum in separating our aluminum ion (Al^{3+}) and/or time-consuming.

Therefore, we decided that by preparing an Al^{3+} selective chelating agent and immobilizing it on a support material, we would have a quick and selective preconcentration method for Al^{3+} . Accordingly, the purpose of this study was to develop a preconcentration method for trace aluminum in water samples. We report the immobilization of salicylideneamino-2-thiophenol (SATP) on a glass bead support, and the use of this support to selectively chelate and preconcentrate Al^{3+} .

Experimental

Materials SATP immobilized glass beads (SAB) were synthesized by

employing glass beads (CPG-10, CPG INC.) and 3-aminopropyltriethoxysilane (Tokyo Kasei Kogyo) as starting materials. Typical features of the glass beads are as follows: pore diameter, $82 \pm 6.519 \text{ \AA}$; pore volume, 0.37 ml/g; surface area, 224.5 m²/g; particle size, 80–120 mesh. Glass beads were used after heating with 5% nitric acid to remove metals. SATP (specially prepared analytical reagent) was purchased from Dojindo Laboratories and Tokyo Kasei Kogyo, and used without any further recrystallization. Aqueous aluminum stock solution (atomic absorption spectrometric grade, 1000 ppm) was purchased from Wako Pure Chemical Industries and diluted appropriately with water before use. Other aqueous metal solutions were prepared by dissolving the corresponding salts (special grade, chlorides or nitrates; Nacalai Tesque) in water. All acid solutions used were heavy metal analysis grade reagents (concentrated, Wako Pure Chemical Industries). All water used was distilled and deionized. Organic solvents were distilled before use. All other reagents used were of special grade.

Apparatus All metal concentrations were determined using a polarized Zeeman atomic absorption spectrometer (Z-8000, Hitachi). Determination of aluminum was carried out *via* a graphite furnace method, and other metals *via* an air-acetylene flame method. All determinations followed standard instrumental conditions.

All glass and polyethylene utensils were soaked overnight in dilute nitric acid, and then rinsed with water.

Drinking Water Samples We obtained tap water (DW (drinking water) 1–3, Kanazawa city water supply) and groundwater (DW 4, Hakui city) samples for SAB preconcentration/AAS determination. However, it should be noted that DW 1 contained a mixture of mains and groundwater, while DW 2, 3 contained only mains water.

Synthesis of SAB. (1) Synthesis of Amino-Propyl-Glass Beads⁹⁾ Glass beads (3.0 g) were added to a 10 ml solution of 3-aminopropyltriethoxysilane (50%, adjusted to pH 3.45 by hydrochloric acid), and the mixture allowed to stand at 75 °C for 2 h with stirring every 15 min. The excess reagent was decanted off. The reaction mixture was filtered under reduced pressure and washed with water, then dried at room temperature.

(2) Immobilization of SATP on Amino-Propyl-Glass Beads Amino-propyl-glass beads (1.0 g) were added to a solution of SATP (0.1 g) dissolved in toluene, and the mixture refluxed, shading it with foil at 110 °C for 12 h.¹⁰⁾ The excess SATP was then decanted off. The reaction mixture was heated in toluene and ethanol several times, and the supernatant was decanted to completely remove excess reagent. The beads were filtered under reduced pressure, and dried at room temperature. The product consisted of light-yellow beads (referred to hereafter as SAB). Elemental analysis of the resulting SAB showed an increased nitrogen and carbon content (%) after the synthesis reaction, and thus we assumed that SATP immobilized on the glass bead support *via* an amino functional group. The exchange capacity of SAB for Al^{3+} , determined by a batch method, was about 1.1 mmol/g (support).

Batch Method Examination of Aluminum Retention Batch analysis was carried out as follows. SAB (0.05 g), solution of Al^{3+} , buffer and reducing agent were added together in a polyethylene tube and the mixture was shaken mechanically (BT-21, water bath incubator, Yamato) at room temperature for 12 h. A constant volume of supernatant was then taken,

and the amount of retained Al^{3+} determined by AAS.

Column Method Examination of Aluminum Preconcentration/Elution Aluminum solutions including drinking sample, buffer and reducing agent were all mixed in a polyethylene bottle and delivered by peristaltic pump (SJ-1211, Mitsumi Science) and polytetrafluoroethylene (Teflon) and Tygon (R3603, Norton) tubing through a glass column packed with SAB (30 × 4.0 mm i.d., about 0.25 g) at 5.0 ml/min. A constant volume of effluent was collected by fraction collector (SF-100G, Toyo Kagaku Sangyo), and the amount of aluminum in each fraction determined by AAS.

Results and Discussion

Strategy of This Study We selected a chelating agent according to the following: a) degree to which it could react with Al^{3+} , b) whether it could be immobilized covalently on the support, c) whether chelating groups coordinating to Al^{3+} differed from the moieties covalently bonding to the support. SATP was selected, a reagent known to be tridentate.^{11,12} It coordinates *via* the nitrogen atom, thiol and hydroxyl groups with copper,^{11,13} nickel¹² and tin,^{10,14} but not to any great extent with aluminum. However, if immobilized covalently on the support *via* the thiol group, we thought that SATP immobilized support (SAB) would selectively coordinated with Al^{3+} , in a bidentate fashion, similar to salicylaldehyde (Fig. 1).

Batch Method Examination of Aluminum Retention We examined whether Al^{3+} was retained on SAB using an acetate buffer and found that it was quantitatively retained but that neither copper nor nickel was. We then determined the conditions required for the quantitative retention of Al^{3+} on SAB. As shown in Fig. 2, Al^{3+} was retained

Fig. 1. Synthesis and Chelation of SAB

Fig. 2. Effect of pH on Aluminum Retention

Sample: SAB, 0.05 g; Al^{3+} , 1 μg ; 0.05 M acetate (pH 5.5) added. Shaking time: 12 h.

quantitatively on SAB at pH 5.0–7.0, and we adjusted all Al^{3+} solutions to pH 5.5 in the following experiments. It can be seen from Table I that $\geq 95\%$ of Al^{3+} was retained in all the examined buffer solutions at this pH. In general, buffering action is the strongest at a pH near the pK_a of the buffer; also, chelation of Al^{3+} by the buffer prevents retention on SAB. We selected a succinate buffer solution for the following experiments because it has a pK_a near pH 5.5 and shows no chelation of Al^{3+} .^{15,16} Buffer concentration was set at 0.01 M in order to adjust the samples to pH 5.5. In the following experiments, we used a 1.0 M succinate buffer solution (pH 5.5) to adjust the pH of the samples.

Column Method Examination of Al Preconcentration/Elution We examined whether or not Al^{3+} was retained on a column packed with SAB, and it was clear that Al^{3+} was quantitatively retained by this column method in a similar fashion to the batch method. Examination of the Al^{3+} sample solution flow rate is summarized in Fig. 3; in all cases (0.5–5.0 ml/min), Al^{3+} was clearly retained. We then decided on a sample solution flow rate of 5.0 ml/min except when fractionating the column effluent (1.0 ml/min). Upon examining the applicable Al^{3+} concentration range, we found that Al concentrations above 1 ppm were not completely retained by the column, probably due to the interference in chelate formation caused by aluminum hydroxide production and/or flocculation. When Al^{3+} below 1 ppm was preconcentrated, however, no leakage was observed. Thus, we decided that sufficient low level preconcentration of Al^{3+} was achievable.

Effect of Interfering Ions on Aluminum Retention The effect of coexisting metal ions on Al^{3+} retention on SAB was examined using a mixture of 14 metal ions (Fe^{2+} , Fe^{3+} , Mg^{2+} , Ca^{2+} , Mn^{2+} , Pb^{2+} : 0.2 mM; Zn^{2+} , Cd^{2+} : 0.1 mM; Cr^{3+} , Cr^{6+} , Ni^{2+} , Cu^{2+} , Pt^{2+} : 0.4 mM; Al^{3+} : 1.5 μM). The

TABLE I. Effect of Buffer Species on Aluminum Retention

Buffers ^{a)}	pK_a	Retention (%) ^{b)}
Succinic acid/NaOH	5.64	98.8 ± 0.7
Acetic acid/NaOH	4.76	96.3 ± 1.1
Propionic acid/NaOH	4.86	95.7 ± 1.7
<i>n</i> -Butyric acid/NaOH	4.83	100.0 ± 1.1
Piperazine/ HNO_3	5.55	95.4 ± 2.1
Imidazole/NaOH	6.95	96.2 ± 0.9
HEPES/NaOH	7.55	100.0 ± 0.9
MES/NaOH	6.15	100.0 ± 0.6

a) All buffers have the same pH value (5.5) and concentration (0.05 M). b) Obtained from 3 determinations (mean ± S.D.).

Fig. 3. Effect of Flow Rate on Aluminum Retention

Sample: 100 ml of Al^{3+} (10 ppb); 0.01 M acetate (pH 5.5) added.

results showed that Fe^{3+} , Cr^{3+} , Pt^{2+} were partly retained as well as Al^{3+} . Assuming that environmental samples are analyzed, the effect of Pt^{2+} will be negligible but coexisting Fe^{3+} and/or Cr^{3+} may possibly interfere with Al^{3+} retention. Accordingly, we carried out the following examination in order to remove the effects of these metal ions. We first tried to find a buffer (Table I) that would selectively retain Al^{3+} over Fe^{3+} and Cr^{3+} on SAB. Among the buffering agents examined, succinate was the most effective; Cr^{3+} was not retained at all but about 30% of the Fe^{3+} present was still retained. Secondly, we investigated the addition of a reducing agent to the sample solution, given that Fe and Cr are transition metals with lower ionic valencies than 3. The reducing agents used were sodium pyrophosphate, sodium sulfite, sodium nitrite, sodium thiocyanate, formic acid, L-ascorbic acid and hydroxylamine hydrochloride (all 0.1 M). The reducing strength, precipitate formation, coloring, AAS response and interference in Al retention of these agents was examined. Hydroxylamine hydrochloride was found to inhibit Fe^{3+} and Cr^{3+} retention completely (Fig. 4). Various amounts of the reducing agent were used according to the Fe^{3+} and Cr^{3+} amounts expected in the sample solution in the following experiments.

Aluminum Eluting Conditions Considering their speedy

Fig. 4. Separation of Aluminum Ion from Iron and Chromium Ions by Reducing Agent

Sample: 50 ml mixture of Al^{3+} (20 ppb, ●), Cr^{3+} (20 ppm, □) and Fe^{3+} (20 ppm, △); 1.0% hydroxylamine hydrochloride added. Flow rate: 1.0 ml/min. Fraction volume: 5.0 ml. Fraction: No. 1—10, sample; No. 11—20, water; No. 21—30, 0.1 M nitric acid.

TABLE II. Determination of Aluminum in Drinking Water

Sample volume (ml)	Al added (ppb)	Al found (ppb)	Al recovery ^{e)} (%)
DW 1 ^{a)}			
1000	— ^{d)}	1.71 ± 0.02	—
500	0.5	2.22 ± 0.03	100.6
	1.0	2.73 ± 0.03	101.6
1000	0.5	2.24 ± 0.02	101.8
	1.0	2.70 ± 0.06	99.4
DW 2 ^{b)}			
100	—	20.31 ± 0.36	—
500	—	20.31 ± 0.36	—
1000	—	20.30 ± 0.24	—
DW 3 ^{b)}			
100	—	17.09 ± 0.08	—
1000	—	17.24 ± 0.24	—
DW 4 ^{c)}			
500	—	0.67 ± 0.01	—

a) Contained a mixture of mains water and groundwater. b) Contained only mains water at different locations in Kanazawa. c) Contained only groundwater in Hakui. d) No additive aluminum. e) Obtained from 3 determinations (mean $\pm$ S.D.).

elution of Al^{3+} , compatibility with a wide range of measurement methods, and the adverse effect of buffers on Al^{3+} retention, we chose acid solutions as eluents. We examined species and concentration of possible acid eluents. Batch method analysis showed that both nitric and sulfuric acid would elute Al^{3+} quantitatively. Batch and column method examining of acid concentration showed that nitric acid above 0.01 M eluted Al^{3+} quantitatively from SAB, but that nitric acid above 0.1 M led to contamination from the system and Tygon tubing. Despite obtaining aluminum recoveries of over 100%, we decided to elute Al^{3+} using 0.1 M nitric acid. As sulfuric acid eluted Al^{3+} quantitatively at a much higher concentration, we did not examine it any further.

Analysis of Aluminum in Drinking Water One application of SAB preconcentration to real samples is the analysis of Al^{3+} in drinking water. Drinking water is considered to contain Al^{3+} at very low levels which are not often determinable by graphite furnace-AAS. However, Al^{3+} was selectively enriched and recovered from tap water (DW 1—3) and groundwater (DW 4) samples, despite the high concentration of Zn^{2+} (0.1 ppm) present in one sample (DW 1), and the results are shown in Table II. As stated previously, Zn^{2+} is not retained at all by the SAB column. Aluminum added to DW 1 was completely recovered by SAB preconcentration and showed a 100-fold increase, which is sufficient preconcentration for the trace analysis of Al^{3+} . DW 1 consisted of a mixture of ground and mains water, while DW 2—3 were solely mains water. In order of decreased aluminum concentration: $\text{DW } 2 \geq \text{DW } 3 > \text{DW } 1 > \text{DW } 4$. We considered these differences to be due to the aluminum compounds purgation treatment of mains water to remove suspended solids.^{2b)}

In conclusion, it is possible to concentrate Al^{3+} using SAB, simply and without interference by coexisting matter, under the conditions outlined here. In this study, Al^{3+} concentrations obtained from tap water (mains water, DW 2—3) were the same as the reference data (10—6330 ppb).^{2b)} Recently, it has been reported that *in vivo* aluminum obtained from drinking water, including tap water, is retained by the body longer than that from medicines and food, because of the difference in water solubility and abnormally acidic medium.^{3,4)} In the European Community, the concentration of aluminum residue in drinking water is controlled, and we therefore believed that the aluminum concentration in tap water should not be ignored. In this study, we measured metal concentrations including aluminum by AAS. However if the pH is modified to retain Al^{3+} , both spectrophotometric and spectrofluorimetric methods (although inferior in sensitivity) can be used, as no chelate compounds such as a buffer agent, were used to elute the Al^{3+} . The SAB column showed good stability and could be used continuously over a period of at least 1 month. In future work, we are going to apply this method to samples with a more complicated matrix such as sea water.

References and Notes

- 1) A portion of this study was presented at the 112nd annual meeting of the Pharmaceutical Society of Japan. J. Kobayashi and M. Miyazaki, Abstracts of Papers, 112nd Annual Meeting of Pharmaceutical Society of Japan, Fukuoka, March 1992, No. 3, p. 163.
- 2) a) The Chemical Society of Japan (ed.), "Kankyo-Bousai Library

- Biryogenso: Eiyō To Dokusei," Maruzen, Tokyo, 1975; b) O. Wada, "Kinzoku To Hito: Ecotoxicology To Rinsho," Asakura-shoten, Tokyo, 1985, p. 218.
- 3) J. B. Eastwood, G. E. Levin, M. Pazianas, A. P. Taylor, J. Denton and A. J. Freemont, *Lancet*, **336**, 462 (1990).
 - 4) S. Morita, *Seikatsu Eisei*, **34**, 199 (1990).
 - 5) E. Kaneko, H. Hoshino, T. Yotsuyanagi, N. Gunji, M. Sato, T. Kikuta and M. Yuasa, *Anal. Chem.*, **63**, 2219 (1991).
 - 6) D. P. Perl and A. R. Brody, *Science*, **208**, 297 (1980).
 - 7) American Public Health Association, American Water Works Association, Water Pollution Control Federation (ed.), "Standard Methods for the Examination of Water and Wastewater," 14th Edition, Washington D.C., 1975, p. 171.
 - 8) T. Shigematsu *et al.*, "Mukioyohishokubunseki," Vol. 1, Kyoritsu, Tokyo, 1980, p. 35.
 - 9) M. Masoon and A. Townshend, *Anal. Chim. Acta*, **166**, 111 (1984).
 - 10) G. R. E. C. Gregory and P. G. Jeffery, *Analyst*, **92**, 293 (1967).
 - 11) K. Isagai and K. Koguchi, *Nippon Kagaku Zasshi*, **86**, 213 (1965).
 - 12) H. Ishii and H. Einaga, *Nippon Kagaku Zasshi*, **90**, 175 (1969).
 - 13) H. Ishii and H. Einaga, *Nippon Kagaku Zasshi*, **91**, 734 (1970).
 - 14) S. Maekawa and K. Kato, *Japan Analyst*, **20**, 474 (1971).
 - 15) A. E. Martell and R. M. Smith, "Critical Stability Constants," Plenum Publishing Corp., New York, 1974.
 - 16) D. D. Perrin, "Stability Constants of Metal-Ion Complexes," IUPAC Chemical Data Series No. 22, Pergamon Press, Oxford, 1979.