Flow Injection Analysis for Trace Aluminum with On-Line Preconcentration and Spectrophotometric Detection

Jun KOBAYASHI, Miyako BABA and Motoichi MIYAZAKI

*Faculty of Pharmaceutical Sciences, Kanazawa University,
Takara-machi, Kanazawa 920, Japan*

We developed a flow injection analysis method for the determination of trace aluminum in water coupled with on-line preconcentration on salicylideneamino-2-thiophenol immobilized glass beads (SAB) and spectrophotometric detection using Pyrocatechol Violet (PV). The FIA system consisted of two pumps, a 6-port switching valve, a concentrator column, a UV/VIS spectrophotometric detector and a recorder. A sample solution was combined with a succinic acid/NaOH buffer (pH 6.0) and hydroxylammonium chloride and this mixture was loaded on the concentrator column. Aluminum retained on the concentrator column was eluted with 0.1 mol dm⁻³ nitric acid and mixed with PV. A colored PV-aluminum complex was spectrophotometrically monitored at 576 nm. A linear relationship was observed between the peak height and aluminum concentration at a constant sample volume. A correlation coefficient of 0.9997 and determination limit of 3.7 nmol dm⁻³ were obtained for a sample volume of 10 ml. Relative standard deviations were below 2% at nmol levels. Aluminum traces in some Kanazawa City tap water samples were determined by this method.

Keywords Aluminum, trace analysis, preconcentration, flow injection, spectrophotometry, tap water

Aluminum intake has recently been recognized as a causative agent for dialysis encephalopathy, renal osteodystrophy and Alzheimer's disease.¹⁻⁶ For many years aluminum was thought to be innocuous and to pass through the gastrointestinal tract largely unabsorbed. However, acute toxicity (lethargy, anorexia, periorbital bleeding and death) after oral ingestion was documented in 1972.^{5,7} This has led to the development of a number of analytical methods for the determination of aluminum in drinking and environmental water samples of clinical and hygienic interest. The most commonly used analytical technique is graphite furnace atomic absorption spectrophotometry (AAS).⁸ Other techniques which have been employed include flow injection analysis with spectrophotometric detection⁹, spectrofluorometry¹⁰ and inductively coupled plasma emission spectrometry.¹¹ However, direct determination is often limited due to interferences from the sample matrix and insufficient precision. Various separation/enrichment techniques have been used to solve these problems.^{12,13}

We prepared salicylideneamino-2-thiophenol immobilized glass beads (hereafter, referred to as SAB; the beads, however, do not contain the mercapto group), and used them to develop a preconcentration method which retained aluminum ion selectively.⁸ In this paper, a FIA method for the determination of trace aluminum (ng ml⁻¹ level) in water samples coupled with on-line preconcentration by SAB and spectrophotometric detection using Pyrocatechol Violet as the chromogenic

reagent is reported.

Experimental

Reagents

SAB was synthesized as stated before⁸, using glass beads (CPG-10, 200–400 mesh, CPG, Inc.) and salicylideneamino-2-thiophenol (SATP, Dojin Laboratories) as starting materials. An aqueous aluminum stock solution (atomic absorption spectrometric grade, 1000 ppm, 1 mol dm⁻³ hydrochloric acid) was purchased from Wako Pure Chemical Industries and diluted appropriately with water before use. A 1.0 mol dm⁻³ succinic acid/NaOH buffer (pH 6.0) was prepared by dissolving 4.7 g of succinic acid monosodium salt (Nacalai Tesque) and 48.0 g of disodium salt (Nacalai Tesque) in 200 ml of water and filtering the mixture through a membrane filter (pore size: 0.45 µm, FM-45, Fuji), before suitable dilution. A Pyrocatechol Violet stock solution (0.1%) was prepared by dissolving 0.1 g of Pyrocatechol Violet (PV, Dojin; without purification) in 100 ml of water and diluting 10-times with the 0.5 mol dm⁻³ succinate buffer before use. The hydroxylammonium chloride and nitric acid used were heavy metal analysis grade reagents (Wako Pure Chemical Industries). All water used was distilled and deionized with a Milli-Q II system (Millipore). All other reagents used were of special grade.

Preparation of aluminum sample solutions

Sample solutions were made up using 0.01 mol dm⁻³ succinic acid/NaOH buffer (pH 6.0) and 0.1% hydroxylammonium chloride immediately before use.

Apparatus

Aluminum used in preliminary tests and all other metals were determined by a polarized Zeeman atomic absorption spectrometer (Z-8000, Hitachi). The pH readings and conductivity were obtained using a pH meter (PH82, Yokogawa) and a conductivity meter (DS-14, Horiba), respectively. All glasswares were prepared by immersion for 24 h in dilute nitric acid, followed by rinsing in water.

Instrumentation

A systematic diagram of the on-line analysis system used this study and the basic procedure are shown in Fig. 1 and Table 1. This FIA system consisted of two pumps (JASCO 880-PU), a 6-port switching valve (JASCO Column Selection Unit 892-01), a plastic column packed with SAB (50×4.6 mm i.d.; SAB, 0.47 g), a UV/VIS spectrophotometric detector (JASCO 870-UV; 576 nm), a column oven (JASCO 860-CO; 40°C), a reaction coil (5 m×0.5 mm i.d.; PTFE tubing) and a recorder (Shimadzu C-R1B). PTFE and polypropylene

Fig. 1 Schematic diagram of on-line analysis system for Al³⁺. P₁, P₂: pumps; R₁: reservoir 1 (sample or 0.1 mol dm⁻³ nitric acid); V: 6-port switching valve; C: concentrator column (50×4.6 mm i.d.; packed with SAB (0.47 g)); S: short-circuit line; R₂: reservoir 2 (Pyrocatechol Violet in 0.5 mol dm⁻³ succinate (pH 6.0)); RC: reaction coil (5 m×0.5 mm i.d., 40°C); D: UV-VIS spectrophotometric detector (576 nm); I: recorder.

tubing were used as much as possible, and 0.1 mol dm⁻³ nitric acid and water were passed through before use.

Tap water samples

We obtained some tap water samples for FIA. Conductivity and pH of sample solutions were measured immediately after sampling. Particulate matter was removed using a membrane filter (0.45 µm, FM-45, Fuji) and 0.1 mol dm⁻³ nitric acid was added to the filtrate before storing in a refrigerator (5°C). The filtrate was

Table 2 Characteristics of tap water

Sample number	pH	EC/ µS cm ⁻¹	Concentrations of several metals
1	7.94	234.5	Fe, 15.6 ppb; Mn, 9.3 ppb; Pb, <2 ppb; Cu, 0.9 ppb; Ni, <1 ppb; Mg, 3.4 ppm; Ca, 12.5 ppm; Na, 26.2 ppm
2	7.58	73.7	Fe, 28.8 ppb; Mn, 0.5 ppb; Pb, 9 ppb; Cu, 9.0 ppb; Ni, <1 ppb; Mg, 1.5 ppm; Ca, 6.3 ppm; Na, 13.7 ppm
3	7.50	85.7	Fe, 36.5 ppb; Mn, 0.9 ppb; Pb, <2 ppb; Cu, 3.5 ppb; Ni, <1 ppb; Mg, 1.4 ppm; Ca, 4.4 ppm; Na, 20.0 ppm
4	7.34	82.6	Fe, 37.8 ppb; Mn, 1.9 ppb; Pb, 8 ppb; Cu, 12.5 ppb; Ni, <1 ppb; Mg, 1.3 ppm; Ca, 4.3 ppm; Na, 19.2 ppm
5	7.59	84.2	Fe, 9.7 ppb; Mn, 0.8 ppb; Pb, 3 ppb; Cu, 7.9 ppb; Ni, <1 ppb; Mg, 1.6 ppm; Ca, 6.2 ppm; Na, 15.8 ppm
6	7.43	78.8	Fe, 15.0 ppb; Mn, 1.1 ppb; Pb, <2 ppb; Cu, 5.7 ppb; Ni, 2.0 ppb; Mg, 1.6 ppm; Ca, 6.1 ppm; Na, 13.7 ppm
7	7.65	97.7	Fe, 5.7 ppb; Mn, <0.5 ppb; Pb, <2 ppb; Cu, 7.8 ppb; Ni, <1 ppb; Mg, 1.6 ppm; Ca, 8.1 ppm; Na, 14.5 ppm

EC: conductivity. Sample numbers are the same as in Fig. 7. Among the detected metals, Mn, Pb, Cu, Ni and Mg give positive errors in spectrophotometric determination.

Table 1 Basic procedure of Al³⁺ determination

Step	Time/min	Pump 1 ^a	Valve position ^a	Purpose of step
1	0 - t	sample ^b (5.0 ml min ⁻¹)	solid	load Al ³⁺ onto concentrator column
2	t - t+10	water (1.0 ml min ⁻¹)	solid	flush the column and tubing of non-retained aluminum
3	t+10 - t+18	eluent (1.0 ml min ⁻¹)	dotted	back-flush Al ³⁺ from the column, clean up, and start recorder
4	t+18 - t+28	water (1.0 ml min ⁻¹)	dotted	stop recorder, rinse with nitric acid to stand by for next sample

t: aluminum sample loading time. Eluent: 0.1 mol dm⁻³ nitric acid. a. System diagrams (pump 1 and valve) were the same as Fig. 1. Pump 2 was left for delivering color agent (contained buffer and PV) at 1.0 ml min⁻¹. b. This sample was already treated with a buffer and a reducing agent.

Fig. 2 Effect of flow rate on retention of Al^{3+} . Sample: 100 ml of Al (10 ppb); 0.01 M succinate (pH 6.0) and hydroxylammonium chloride (0.5 g) added. Data were averaged from 3 determinations.

Fig. 3 Effect of pH on absorbance. Sample: 5 ppm Al (0.5 ml)+0.1 M acetate (5 ml)+0.05% PV (0.5 ml). Reaction temperature: r.t. Reaction time: 10 min. Blank: reagent only.

divided in order to determine concentrations of several metals by AAS. The results are shown in Table 2. The residue was made weakly acidic by the addition of NaOH and treated as stated in "Preparation of aluminum sample solutions" just before analysis.

Results and Discussion

Effects of several conditions on the retention/elution of aluminum

The effects of several system conditions on retention and elution of aluminum from SAB were investigated. Examination of the Al^{3+} sample solution flow rate in the preconcentration stage is summarized in Fig. 2; Al^{3+} was almost completely retained in the 0.5 to 5.0 ml min⁻¹ region. A sample solution flow rate of 5.0 ml min⁻¹ in the preconcentration stage was chosen for the analytical procedure. A 0.1 mol dm⁻³ nitric acid was selected as the column eluent for the following reasons: 1) it would not prevent colorimetric determination of aluminum; 2) rapid elution of Al^{3+} from the SAB column; and 3) lower contamination from the system.

Color reagent

Selection of an appropriate color reagent and investigation of optimum detection conditions were performed. Since aluminum is selectively concentrated by SAB, the reagent does not have to have a specific color development for aluminum. A sensitive color reagent, Pyrocatechol Violet was chosen for the following reasons: 1) it is water soluble; 2) it has a rapid complexing rate with aluminum; 3) its absorption spectrum is different from that of the aluminum complex; and 4) it has a large molar absorption coefficient at the detection wavelength. PV-aluminum complexes have an absorption maximum at 576 nm and ϵ (molecular extinction coefficient) of 5.5×10^4 .

To minimize aluminum contamination from the laboratory environment and to shorten the analytical procedure, postcolumn reaction was performed after

Table 3 Effect of buffer species on absorbance

Buffer species	Absorbance at 576 nm		
	Sample	Blank	Sample-Blank
Succinic acid/NaOH	1.035	0.067	0.968
Propionic acid/NaOH	1.005	0.041	0.964
Acetic acid/ NH_3	0.938	0.036	0.902
Pyridine/ HNO_3	0.937	0.045	0.892
Acetic acid/NaOH	0.816	0.037	0.779
Piperazine/ HNO_3	0.850	0.082	0.768
Isobutyric acid/NaOH	0.614	0.036	0.578
HEPES/NaOH	0.040	0.023	0.017
Imidazole/ HNO_3	0.036	0.028	0.008
Citric acid/NaOH	0.065	0.061	0.004

Sample: 5 ppm Al (0.5 ml)+0.1 M buffer (pH 6.0, 5.0 ml)+0.05% PV (0.5 ml). Reaction temperature: r.t. Reaction time: 10 min. Blanks were measured at the same time, without Al. HEPES: 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid.

aluminum was eluted from the column. The post-column solution consisted of a PV stock solution and buffer, this simplified FIA manipulation. Absorbance was measured at 576 nm for aluminum against a reagent blank.

As shown in Fig. 3 and Table 3, a succinic acid/NaOH (pH 6.0) postcolumn buffer gave a maximum absorbance value. Pyrocatechol Violet concentration was decided to be 0.005% (final) by ϵ value. The PV reagent was added in excess. The composition of the complex formed under analytical conditions was estimated by the molar ratio method to be 1:2 (metal: reagent). When the PV solution was delivered at the same flow rate as the eluent, buffer concentration above 0.5 mol dm⁻³ was needed to maintain pH 6.0. When more concentrated buffer than 0.5 mol dm⁻³ was used, the linearity of calibration curve was lost. On the other hand, when more alkaline and dilute buffers were used, a reduction in absorbance was observed, which seems to be due to the decomposition of PV.¹⁴ As a result, 0.5 mol dm⁻³ succinic acid/NaOH (pH 6.0) was used.

Fig. 4 Effect of temperature on absorbance. Sample: 15 ml of Al (10 ppb); 0.01 M succinate (pH 6.0) and 0.1% hydroxylammonium chloride added. Data were averaged from 3 determinations.

Fig. 5 Effect of flow rate on peak height. Sample: 15 ml of Al (10 ppb); 0.01 M succinate (pH 6.0) and 0.1% hydroxylammonium chloride added. Data were averaged from 3 determinations.

Postcolumn reaction conditions

The reaction of aluminum ion with Pyrocatechol Violet was investigated with regard to reaction temperature and time. The reaction time was varied by changing the dimensions of the reaction coil and the flow rate of the pumps. Under a fixed conditions (10 m×0.25 mm i.d. reaction coil and 1.0 ml min⁻¹ flow rate), a reaction temperature of 40°C gave better reproducibility, as shown in Fig. 4. On the other hand, pump flow rates and reaction coil features were also investigated at a fixed temperature (40°C), as shown in Fig. 5: maximum peak height was obtained at 0.2 ml min⁻¹ but reproducibility was bad. The noise level hardly changed at any flow rate. At a fixed flow rate of 1.0 ml min⁻¹, 10 m×0.25 mm i.d., 5 m×0.5 mm i.d. and 2.5 m×0.5 mm i.d. reaction coils were tried, with the second coil proving to be the best. This was thought to be due to a greater reaction time and mixing efficiency. No further investigation of the reaction coil was carried out.

Introduction of a 6-port valve

Under all the experimental conditions studied above, a linear relationship exists between aluminum concentra-

Fig. 6 Typical FIA peak of Al³⁺-PV chelate. Sample: 50 ml of Al; 0.01 M succinate (pH 6.0) and 0.1% hydroxylammonium chloride added. Al concentration: (A) and (B), 0 ppb; (C), 10 ppb. (B) and (C) were stored in 0.1 mol dm⁻³ nitric acid and adjusted to a near neutral pH using sodium hydroxide just before analysis. (A) was prepared just before analysis and did not contain nitric acid and sodium hydroxide.

Table 4 Effect of sample volume on peak height

Sample volume/ml	Slope/AU μg ⁻¹	Intercept/AU
10	1.08	0.069
20	1.09	0.080
50	1.08	0.117
100	1.09	0.155

AU: absorbance unit. All slopes were calculated by peak heights (AU)/Al loaded (μg), and intercepts were calculated by peak heights (AU), where Al concentration=0. All intercepts are approximated by the following equation: 0.062+0.001×sample volume (ml).

tion and peak area. However, no such relationship exists between aluminum concentration and peak height. Since this linear relationship applies to various loading volumes, the peaks broadened at high loading volume. We thought this was a problem not to detectable few aluminum. We assumed that this is due to retention of aluminum ions occurring only at the front of the SAB column, thereby causing the retention volume (peak width) to change when they diffuse down the column at elution and/or retention steps. Back-flushing can solve this problem, so we introduced a 6-port valve into the FIA system to permit back-flushing. The 6-port valve was tubed with a short-circuit line and the system was arranged so as to allow carrier flow to continually pass through the detector, as shown in Fig. 1.

Analytical characteristics

Typical FIA peaks were shown in Fig. 6. A linear

Fig. 7 Determination of Al in tap water. Sample volume: 20–30 ml (this study) and 20 μ l (AAS). Data were averaged from 3 determinations. Standard deviations are shown as x (this study)- and y (AAS)-bars. Sample numbers are the same as in Table 2.

relationship was observed between peak height and the amount of aluminum in the range 0–500 ng. The correlation coefficient was 0.9997 and the determination limit ($S/N=3$) 3.7 nmol dm⁻³ (0.1 ppb), for a 10 ml sample volume. Relative standard deviation ($n=3$) were below 2% at the nmol level (0.9% at 25 ppb; 1.5% at 50 ppb). This linearity was also achieved at other sample loading volumes for these experimental conditions and each calibration curve was linear. The blank consisting of the same volume of reagent was determined on the same day. Several samples containing the same amount of aluminum were compared at different loading volumes. Calibration curves had the same slopes, but not the same intercepts (Table 4).

Water analysis

Aluminum in some Kanazawa City tap water samples was determined by the present FIA method. Generally, tap water is considered to contain aluminum at very low levels and to contain many interfering substances. As shown in Fig. 7, aluminum concentrations at the ppb level were determined, despite the presence of many other

ions. Determined values (1, 2.28 ± 0.01 ; 2, 4.84 ± 0.04 ; 3, 9.29 ± 0.12 ; 4, 10.58 ± 0.07 ; 5, 11.98 ± 0.06 ; 6, 14.31 ± 0.08 ; 7, 16.42 ± 0.05 ppb as shown in Fig. 7) were in good agreement with direct graphite furnace-AAS analysis. In future work, we are going to apply this method to samples with a more complicated matrix, such as seawater.

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