Bull. Environm. Contam. Toxicol. 23,509-516 (1979)

Spectrophotometric Method Free of Matrix Effects for the Sensitive Quantitation of Aluminum

Shane S. Que Hee, James Boyle, and Vincent N. Finelli

Department of Environmental Health, Kettering Laboratory, University of Cincinnati Medical Center, 3223 Eden Avenue, Cincinnati, OH 45267

It is commonly thought that aluminum is a comparatively non-toxic element in its substances. Though it is ubiquitous, most of its naturally occurring compounds e.g. the silicates and oxides, are relatively inert, chemically as well as physically. However, water soluble compounds such as the chloride and nitrate have rat oral LD_{50} values of 3.7 (STECHER 1960) and 4.3 g/kg (SMYTH et al. 1969), and excess aluminum hydroxide has been shown to induce phosphorus deficiency (STECHER 1960). Moreover, inhalation of aluminum sulfate produced drastic alterations in the viability, morphology and lysing of rat lung macrophages as well as in the permeability of the alveolar wall to serum proteins (FINELLI et al. 1978). We have also observed that aluminum is a major metallic constituent in the stack emissions of coal-burning power plants and since SO_2 and NO_2 are also produced in abundance by the combustion of fossil fuels, the formation of water soluble sulfates and nitrates in the aerosol phase is likely. Unfortunately, few speciation studies have been reported for these emissions, although some workers have found high levels of Al III, SO_4^{2-}, NO_3^- and Cl^- in acid rains (LILJESTRAND and MORGAN 1978) and in water from soils leached with acid rains (CRONAN and SCHOFIELD 1979). SCHOFIELD (1977) has observed significant reductions in brook trout growth at Al III levels of $\sim 10^{-5}$M. Aluminum in its ionic form is therefore quite bioavailable. Thus, the quantitation of aluminum has assumed a new importance.

Currently, the most used analytical method for aluminum is atomic absorption (AA). Flame AA, though specific and reproducible, has low sensitivity, $\sim$ 0.2 ppm (TIKHONOV 1973); flameless AA is sensitive to the 0.001 ppm (ppb) levels (JULSHAMN et al. 1978, GORSKY and DIETZ 1978) but tends to be irreproducible, and requires skilled personnel. Matrix effects pose problems for both variants. Aluminum compounds also tend to form refractory oxides at high temperatures. Colorimetry is advantageous because it can be sensitive and precise, and utilizes accessible, inexpensive instrumentation. However, although many reagents have been used, there are no unique colorimetric reagents for aluminum since Fe III and other metals usually interfere (TIKHONOV 1973). In this paper, we present a liquid/liquid extraction scheme to extract aluminum specifically from a mixture of metal ions and a subsequent colorimetric determination sensitive to 6 ppb Al.

0007-4861/79/0023-0509 $01.60

MATERIALS AND METHODS

An aqueous metal ion mixture ("soup") with 995 ppm Ca II ($CaCl_2$), 0.831 ppm Cr III ($Cr(NO_3)_3$), 8.31 ppm Cu II ($CuSO_4 \cdot 5\ H_2O$), 10.3 ppm Fe III ($FeCl_3 \cdot 6\ H_2O$), 323 ppm Mg II ($MgCl_2$), 2.05 ppm Pb II ($PbCl_2$) and 103 ppm Zn II ($ZnCl_2$) was prepared from the indicated reagent grade chemicals (Fisher) and deionized organic-free distilled water. These concentrations are about one order of magnitude greater than the usual values encountered in biological fluids and tissues (ALTMAN and DITTMER 1974), and these metal ions constitute the most common likely inorganic interferences in biological tissues to aluminum analysis (TIKHONOV 1973). To extract aluminum specifically, we modified the 8-hydroxyquinoline extraction method of CLAASSEN et al. (1954) for separation of Al from metals in stainless steel. The method allows extraction of aluminum from such interferences as As III, As V, Ba II, Be II, BO_3^{3-}, Br^-, Cd II, Ca II, Ce III, Cs I, Cr III, Co II, Co III, Cu II, F^-, GeO_4^-, Au III, I^-, La III, Pb II, Li I, Mg II, Mn II, Hg I, Hg II, Mo III, Mo VI, Ni II, Ni III, PO_4^{3-}, Pt IV, Se IV, Se VI, Si IV, Ag I, Sc III, Sr II, Ta V, Te IV, Te VI, Th IV, Sn IV, W VI, V (V) Zn II, Fe III and Fe II. Only F^- in excess quantities interferes.

All glassware was rinsed consecutively with distilled water, concentrated ammonia, distilled water and then allowed to soak for at least 16 hours in $\geq$80% nitric acid. The cleaned glassware was rinsed, then soaked in deionized water for half an hour, rinsed several times and finally air-dried in a clean, dustless hood. All chemicals were reagent grade.

Specific Separation of Aluminum:

Fifty µL aliquots of Al III ($AlCl_3,6\ H_2O$) of concentrations 0, 1, 2, 5, 10, 20, 40, 80, and 160 ppm were each placed in 25 mL graduated centrifuge tubes containing 0.5 mL 2N HNO_3, 0.5 mL 2N HCl, and 1.95 mL of the metal "soup"; the volumes were then adjusted to 5 mL with water. In some runs, the 0.5 mL 2 N HCl was replaced by 0.5 mL of 2N HNO_3, and in others, no "soup" was used.

Ten µL of 0.1% phenol red was added, the tubes shaken, and the pH adjusted to just alkaline (10% NaOH); 2N HCl(0.5 mL) was added, the samples shaken, and ammonium bisulfite (200 µL) (CLAASSEN et al. 1954) added and the solutions heated at 90°C for 10 minutes (stoppers on) to reduce Fe III to Fe II; after cooling, 10% disodium EDTA (1 mL) was added to complex the divalent ions, and the pH was adjusted (concentrated NH_4OH) until it was just alkaline; 30% sodium cyanide (1 mL) was then added, the solutions shaken and then heated for 10 minutes at 90°C (stoppers on) to form the ferrocyanide; after cooling, 5% 8-hydroxyquinoline in ethanol solvent (200 µL) was added, the solutions shaken and allowed to stand at room temperature for one hour to allow the aluminum quinolate to be nucleated to completion. The solutions were then extracted with chloroform (3 x 15 mL). Each extraction was accomplished by shaking the solutions vigorously for

three minutes and allowing these solutions to stand for twenty minutes before transfer of the chloroform layers to 50 mL pyrex volumetric flasks. The combined chloroform extracts were washed with freshly prepared distilled deionized water (2 x 5 mL) or until no pink or yellow color was observed in the water layer. The final volumes were adjusted to 50 mL with chloroform. The well mixed solutions were then read immediately at 390 nm on a spectrophotometer (1 cm cell) against a chloroform blank. UV-visible spectra were also run. Flame AA of the quinolate solutions at 396.1 nm utilized a nitrous oxide/acetylene (15.2 L/min :: 9.0 L/min) flame (KRISHNAN et al. 1972). The standards for both forms of analysis were prepared by mixing 50 µL aliquots of the aluminum solutions utilized above with 5% 8-hydroxyquinoline (200 µL); chloroform was added to a final volume of 50 mL, and the solutions shaken until they cleared. The efficiency of the extraction procedure was calculated for the aluminum samples in the presence and absence of metal "soup" by both methods.

Quantitation of Samples Containing Al below 0.1 ppm

Known volumes of the chloroform solutions containing the aluminum quinolate were then evaporated to dryness in pyrex beakers (25 mL) at 60°C on a hot plate in a fume hood; 1:1 (v/v) concentrated perchloric/nitric acid (2 mL) added, and the samples hydrolyzed for two hours at 90°C and evaporated as shown by the first absence of white fumes evolved on holding the beaker just outside the hood but in its air entry-stream. A solution of 2N NaOH (1 mL) was added drop by drop to irrigate the warm walls from lip to bottom of the insides of the beakers. All of the wall must be carefully wetted. The lower walls were wetted by gentle sideways rotation of the beakers. Water (1 mL) and 2N HCl (1 mL) were added down the walls in the same manner. The solutions were then chilled briefly (ice bath) and brought to room temperature; 1 M ascorbic acid (10 µL) was added to reduce the Fe III known to be in the reagents, the solutions shaken and allowed to stand for five minutes, and 0.1% 1,10-phenanthroline in 10% ethanol/water (70 µL) added with shaking, and the solutions again allowed to stand for five minutes to complex the Fe II; 200 µL of 0.1% Chrome Azurol S(CAS), the sodium salt of 2,6-dichlorodimethylsulfoxyfuchsone dicarboxylic acid (Eastman), λ_{max} 430 nm, was added and then 1 mL of 4 M pH 6.10 acetate buffer. The solutions were transferred into narrow, calibrated 10 mL measuring cylinders with ground glass joints, and the beakers rinsed copiously with distilled water so that the final volume of the solution was ca 5 mL. At this stage the pH of one sample should be checked. If the pH is not between 5.8 to 6.2, adjustment must be made with known amounts of 2N NaOH or 2N HCl. This aliquot can be added to all the samples. This procedure is generally satisfactory and avoids contamination of all the samples with metal ions from the pH electrodes before quantitation. The pH adjusted samples were then made up to the same volume, vigorously shaken, and allowed to stand 20 minutes before reading at 630 nm against a water blank. Known

aluminum ion concentrations in triplicate were also subjected to the above procedure from the step dealing with the addition of ascorbic acid. The efficiencies of the hydrolysis of the aluminum quinolate samples derived from the standards and those in the presence and absence of metal "soup" were calculated relative to the response of the corresponding Al ion concentrations.

Optimization of the Chrome Azurol S Quantitation

The optimal conditions utilized above were found in the following manner: the pH of the acetate buffer was varied by adding excess glacial acetic acid or sodium hydroxide to the pH 6.1 buffer and following the color development at room temperature over an hour at pH 4·2, 4·8, 5·1, 5·6, 5·8, 6·0, 6·2; the final ionic strength of the buffer at the optimum pH and incubation time was varied (0·1, 0·2, 0·3, 0·4, 0·5, 0·6, 0·7, 0·8, 0·9, 1·0 M); the effects of strong acids, bases and heat on color development were also noted; the amount of masking agent, 1,10-phenanthroline, added after the ascorbic acid was varied (5, 10, 30, 50, 70, 100 and 120 µL) as was the incubation time before addition of CAS.

RESULTS AND DISCUSSION

Table I presents the efficiencies, sensitivities and errors characterizing the specific extraction of Al III in the presence and absence of interfering ions by the CAS and 8-hydroxyquinoline spectrophotometric methods and by flame AA. The latter two methods are more precise (ca 3% standard error in the slope of the Beer's Law plots) than the CAS assay (8-9% relative errors) but are much less sensitive. Reliable AA was possible only above 0·2 ppm. For 8-hydroxyquinoline spectrophotometry, it was possible to detect 60 ppb of Al with $\sim$ 5% error. Although one chloroform extraction was $\sim$ 80% efficient, three are necessary for optimal extraction. Fluorescence assay of the chloroform solutions of the quinolates (λ excitation 400 nm; λ emission, 520 nm) was sensitive to ppb levels but the fluorescence emission intensity was a complex function of the concentration of the aluminum concentration in the cleaned-up samples. UV visible spectra of the chloroform solutions of the standards and cleaned-up samples were superimposable. The CAS assay permits reproducible detection of aluminum levels as low as 6 ppb (Figure 1). The relatively large error is caused by losses incurred in the evaporation after the acid oxidation step. The latter was necessary because of the high stability of the aluminum quinolate with respect to CAS. Hydrolysis combinations investigated included: concentrated nitric, acetic, nitric/acetic, perchloric/nitric, hydrochloric, nitric/hydrochloric and nitric/perchloric/sulfuric acids. Nitric acid hydrolysis produced high blanks and produced less precision than the perchloric/nitric combination. One disadvantage is that the heating must be at low temperature otherwise much Al is lost, and the washing of the beaker walls must be carefully performed.

TABLE I

Extraction of Aluminum III Solutions in the Presence and Absence of Interfering Ions (see Text) by Various Methods

(r^2 is the correlation coefficient)

Method	Parameter	Al Standards	Al in Absence of Interferences	Al in Presence of Interferences
8-hydroxy-quinoline (100 to 4000 ppb)	% Efficiency	100	88 ± 2	87 ± 2
	slope ± S.E.M. (absorb units/ppb)	$(2.44 \pm 0.05) \times 10^{-4}$ ($r^2 = 0.998$)	$(2.44 \pm 0.06) \times 10^{-4}$ ($r^2 = 0.999$)	$(2.41 \pm 0.06) \times 10^{-4}$ ($r^2 = 0.999$)
Flame Atomic Absorption (200 to 4000 ppb)	% Efficiency	100%	90 ± 2	89 ± 2
Chrome Azurol S (6 to 160 ppb)	% Efficiency	100	92 ± 8	92 ± 8
	slope ± S.E.M. (absorb units/ppb)	$(1.55 \pm 0.03) \times 10^{-3}$ ($r^2 = 0.996$)	$(1.43 \pm 0.12) \times 10^{-3}$ ($r^2 = 0.954$)	$(1.43 \pm 0.12) \times 10^{-3}$ ($r^2 = 0.954$)

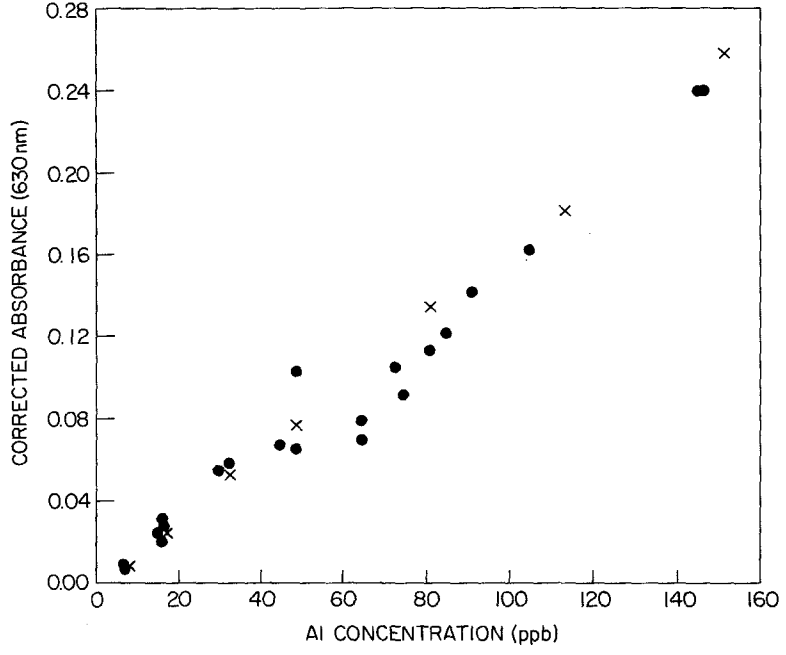

Figure 1. Correlation of absorbance versus Al concentration for standards (x) and for hydrolysed samples of aluminum quinolate (•). Each point is the mean of a set of triplicates.

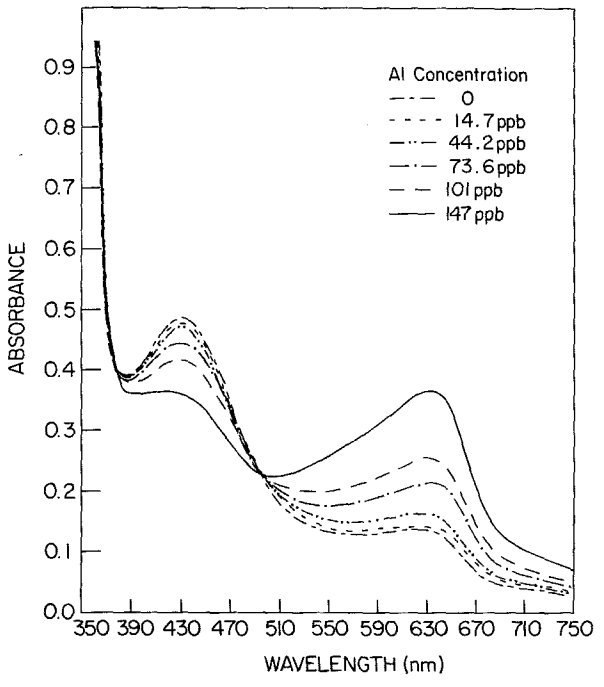

Figure 2. Absorption spectra of the aluminum/Chrome Azurol S complex in pH 6.1 acetate buffer (4 M) in the presence of ascorbic acid and 1,10-phenanthroline after hydrolysis of aluminum quinolate with 1:1 nitric/perchloric acid. The blank is water.

It was found that the intensity of the CAS color was maximal and independent of pH in the 5·8 to 6·2 pH range; the optimal final ionic strength of the acetate buffer was 0·7 to 0·8 M; the lowest final Chrome Azurol S concentration commensurate with linearity, sensitivity and low blank valves was 0·004%; the color was stable after 15 minutes for at least one hour; boiling the dye or exposure to very basic or acidic conditions caused high blanks. The presence of masking agents was essential since the reagents contained significant amounts of Fe III. Final concentrations of 0·0006 to 0.002% 1,10-phenanthroline allowed constant, optimal color development.

Figure 2 demonstrates how the λ_{max} for Chrome Azurol S at 430 nm relative to a water blank decreases while the peak at 630 nm due to the Al-CAS complex increases as Al III concentration increases. The concentration dependence of the 430 and 630 nm peaks on Al III concentration implied a Al/CAS stoichiometry of 1:2 (TIKHONOV, 1973). The approximate ε_M of the Al/CAS complex was $\sim$ 57,400 L mol^{-1} cm^{-1} at 630 nm and 25°C with respect to a water blank.

The method presented here has many advantages. Wet-ashed biological samples may be analyzed independent of the matrix effect that so often plagues aluminum analysis. Interferences in the reagents are masked. Both flame AA and 8-hydroxyquinoline spectrophotometry should agree quantitatively above Al III levels of 0.2 ppm. If more sensitivity is required, the CAS method allows quantitation of Al between 6 and 160 ppb. The latter should agree with the levels estimated using graphite rod AA analysis of the aluminum quinolate solutions or by inductively coupled plasma, neutron activation or X-ray fluorescence if these expensive pieces of instrumentation are available. Experiments are proceeding to optimize the wet-ashing of biological samples.

The procedure has also been applied to water samples containing Al III. A further step is required in the procedure, however. The final chloroform extract was evaporated under vacuum to ca. 3 mL, and then extracted with equal volumes of 2N HCl (3X; 99.5% efficient); 0.1% phenol red (10 µL) was added to the combined acidic extracts, the solutions made alkaline (concentrated NH_4OH) and the alkaline solution extracted with equal volumes of chloroform (3X). Analysis as outlined above can then be done. The extra step is necessary to exclude any chloroform soluble organics. The efficiency of the entire procedure is unaffected. Air particulate samples could be treated in a similar manner after acid hydrolysis.

ACKNOWLEDGMENT

This study was funded by U.S. EPA No. 68-03-2601 and U.S. PHS Grant ES-00159. Thanks go to Jim Boiano for technical help on the AA.

REFERENCES

ALTMAN, P. L. and D. S. DITTMER: Biology Data Book, 2nd Ed., Vol. III, 1974, pp 1751-54.

CLAASSEN, A., J. BASTINGS and J. VISSER: Anal. Chim. Acta. 10, 373 (1954).

CRONAN, C. S. and C. L. SCHOFIELD: Science 204, 304 (1979).

FINELLI, V. N., S. D. LEE, R. M. DANNER, J. BOIANO, L. McMILLAN and G. P. COOPER: Inhalation of Sulfate Particulates II: Pulmonary Biochemical Effects, Society of Toxicology, San Francisco, California, 17th Annual Meeting, Toxicol. Appl. Pharmacol. 45, 246 (1978).

GORSKY, J. E. and A. A. DIETZ: Clin. Chem. 24, 1485 (1978).

JULSHAMN, K., K. J. ANDERSEN, Y. WILLASEN and O. R. BRAEKKAN: Anal. Biochem. 88, 552 (1978).

KRISHNAN, M. S., K. A. GILLESPIE and D. R. CRAPPER: Anal. Chem. 44, 1469 (1972).

LILJESTRAND, H. M. and J. J. MORGAN: Environ. Sci. Technol. 12, 1271 (1978).

SCHOFIELD, C. L., Research Project Technical Completion Report A-072-NY, Office of Water Research and Technology, Department of the Interior, Washington, D. C., 1977.

SMYTH, H. F., C. P. CARPENTER, C. S. WEIL, U. C. POZZANI, J. A. STRIEGEL and J. S. WYCOM: Amer. Hyg. Assoc. J. 30, 470 (1969).

STECHER, P. G.: The Merck Index, 7th Ed., Merck, 1960.

TIKHONOV, V. N.: The Analytical Chemistry of Aluminum, John Wiley, New York, 1973.