Talanta, Vol. 41, No. 1, pp. 81–87, 1994
Printed in Great Britain.

0039-9140/94 $6.00 + 0.00

NONEXTRACTIVE SPECTROFLUORIMETRIC DETERMINATION OF ALUMINIUM IN REAL, ENVIRONMENTAL AND BIOLOGICAL SAMPLES USING CHROMOTROPIC ACID

KALYAN BAKSI* and BIJOLI KANTI PAL†
Analytical Chemistry Division, Chemistry Department, Jadavpur University, Calcutta 700 032, India

(*Received* 16 *April* 1992. *Revised* 2 *June* 1993. *Accepted* 3 *June* 1993)

Summary—An ultra-sensitive and highly selective nonextractive fluorimetric method is presented for the rapid determination of aluminium at nano-trace levels using chromotropic acid as a fluorimetric reagent [λ_{ex} = 360 nm and λ_{em} = 390 nm] in the pH range of 4.1–4.7. The fluorescence intensity of the metal chelate (2:3 complex) reaches a constant value within 1/2 hr and remains unchanged for over 48 hr. The fluorescence intensity aluminium concentration calibration curve is collinear between 1 and 300 ng/ml of Al. A constant fluorescence intensity is obtained over a wide range (1:50–1:1500) of Al:reagent molar concentrations. Large excesses of over 60 cations, anions and complexing agents (like tartrate, oxalate, phosphate, thio-urea, SCN^-, *etc.*) do not interfere in the Al determination. The developed method was successfully used in assaying aluminium in several standard reference materials (Al-bronze, brass, stainless steel) as well as in some environmental and biological samples. The method is very precise and accurate (S.D = ±0.001 on 10 ng/ml; 11 determinations).

The use of chromotropic acid as a metallo-fluorescing reagent was recently reported by us for beryllium determination.[1] The present paper records its use for the ultratrace analysis of aluminium.

Aluminium has long been considered as virtually non-toxic and non-absorbable from the gastrointestinal tract. More recent studies on humans, however, expose its acute toxicity,[2] including impaired memory, convulsions, characteristic EEG changes, uremia, Shaver's disease[3] (lung), Alzheimer's disease (brain)[4] and also increased risks of cancer[5] in lung, pancreas and leukaemia. It is estimated that out of 1.2–2 million patients in the U.S.A., 10,000 people die every year from Alzheimer's disease alone.[4] Aluminium at trace and sub-trace levels in the water used for dialysis can cause brain derangement.[6] All these findings cause alarming concern in public health, demanding accurate determination of this metal ion at trace and subtrace levels.

Compared to even some recently published fluorimetric methods[7–11] for the metal ion, the method prescribed here offers several distinct advantages, viz., higher sensitivity and selectivity, greater accuracy and precision, increased fluorescence stability, wider range of metal determinations and ease of operation.

*To whom correspondence should be addressed.
†Present address: Department of Chemistry, K.N. College, Berhampore, Murshidabad, West Bengal, India.

EXPERIMENTAL

Apparatus

A Perkin-Elmer (Model MPF-44B) spectrofluorimeter and an Electronic Corporation of India (Model pH 5651) digital pH meter were used for measurements of fluorescence intensity and pH, respectively.

Reagents

A $1 \times 10^{-2}M$ reagent, chromotropic acid salt solution, was prepared by dissolving a known weight of the disodium salt dihydrate (Merck p.a.) in deionized water and diluting to the required volume. This solution was diluted further as required. A stock standard solution of aluminium ($1.4 \times 10^{-2}M$, *i.e.* 378 ppm) was prepared by dissolving $AlK(SO_4)_2$, $12H_2O$ (Merck analytical-reagent grade) in water, adding 1.0 ml of 1 + 1 sulphuric acid and diluting to 100 ml. More dilute solutions were prepared by appropriate dilution of the aliquots from the stock solution with deionized water. Buffer solutions (100 ml, pH 4.3) were prepared by mixing

70 ml acetic acid (0.2M) and 30 ml sodium-acetate (0.2M) and adjusting to the desired pH. A large number of solutions of inorganic ions and complexing agents were prepared from their Analar grade or equivalent grade water soluble salts. In the case of insoluble substances, special methods were adopted.[12] Double distilled deionized water, nonfluorescent under ultraviolet radiation, was used throughout.

Glass vessels were cleaned by soaking with acidified solutions of $KMnO_4$ or of $K_2Cr_2O_7$ followed by washing with concentrated HNO_3 and using several times with deionized water. Stock solutions and environmental water samples were kept in polypropylene bottles containing 1 ml conc. HNO_3.

Procedure

To 1.0 ml of slightly acidic solution containing 0.01–0.1 μg or 0.1–3.0 μg aluminium in a 10-ml volumetric flask were added, respectively 0.07 or 0.7 ml of $3.702 \times 10^{-3}M$ chromotropic acid salt solution and 1.5 ml sodium acetate–acetic acid buffer (pH 4.3). The solution was then diluted to 10 ml with deionized water and allowed to stand for 30 min; then the fluorescence intensity of the complex was measured against a corresponding reagent blank at 390 nm, keeping the excitation wavelength maximum at 360 nm. The aluminium content in unknown sample was determined with the help of a concurrently prepared calibration graph.

RESULTS AND DISCUSSION

Spectral characteristics

The uncorrected excitation and emission spectra of the fluorescent system (Al + chromotropic acid) at pH 4.3 were recorded with the spectrofluorimeter (Fig. 1). Of the three wavelength peaks of excitation (emission wavelength 390 nm) occurring at 327, 342 and 360 nm, the region of 360 nm is preferred because of the highest fluorescent intensity value after blank correction. Again of the two emission peaks recorded at 373 and 390 nm (excitation wavelength 360 nm), 390 nm was preferred, as this emission wavelength yields the maximum fluorescence intensity. Whilst the reagent blank was found to exhibit a broad fluorescence maxima between 410 and 440 nm wavelength regions with much lower intensities, the different excitation peak regions of the reagent blank occur in the wavelength regions 327, 342 and 350 nm. At the excitation wavelength maxima (360 nm), the reagent blank exhibits fluorescence of much lower intensity, hence this peak region (360 nm) is preferred for fluorescence intensity measurement of the complex in subsequent studies.

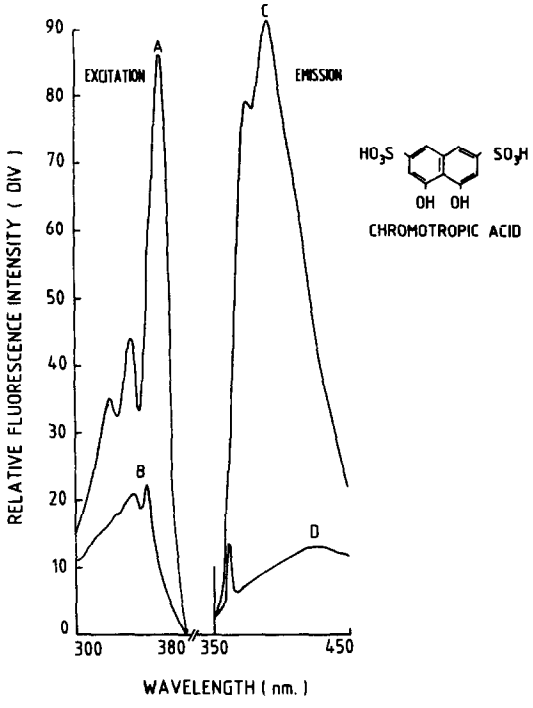

Fig. 1. Uncorrected spectra. A and B, excitation spectra of complex (0.3 ppm of Al) and reagent (0.3 ml of $3.702 \times 10^{-3}M$ chromotropic acid), respectively (emission wavelength = 390 nm): C and D corresponding emission spectra (excitation wavelength = 360 nm).

Effect of pH and buffer

The effect of pH on fluorescent intensities was studied for a wide range (3.5–5.5). The constant maximum fluorescence intensities, corrected against corresponding reagent blank, were found within the pH range of 4.1–4.7 (Fig. 2) at room temperature (25 ± 5°C). Sodium acetate–acetic

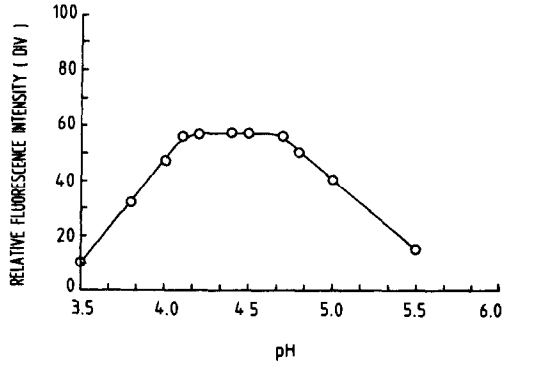

Fig. 2. Effect of pH on the fluorescence intensity of the Al–reagent complex.

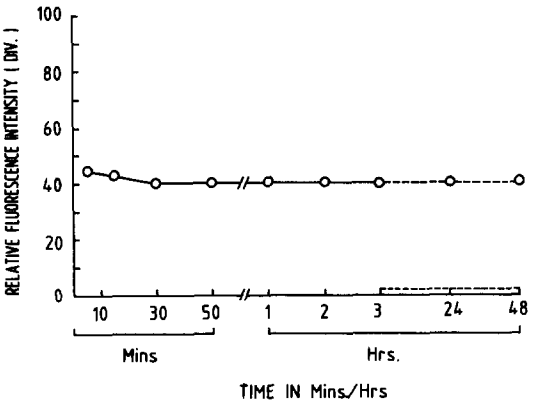

Fig. 3. Effect of time on the fluorescence intensity of Al–reagent system.

acid buffer (pH 4.3) up to 2 ml for every 10 ml total solution are without any adverse effect.

Effect of time

The constant fluorescence intensity reached after 30 min and remained unaltered even after 48 hr of measurements. Longer periods were not studied (Fig. 3).

Effect of reagent concentration

Different molar fold excess of chromotropic acid was added to fixed metal ion concentration and fluorescent intensities were measured according to the standard procedure. It was observed that at the 10 ppb Al, the metal:reagent molar ratios of 1:50 and 1:1500 produce constant fluorescence intensity of the Al-chelate. Greater excess of reagent was not studied. At higher metal ion concentration (0.1 ppm) however, the optimum Al:R molar ratio range lies between 1:10 and 1:75 (Fig. 4), because at higher reagent concentrations, the solution becomes yellow coloured and concentrational quenching is caused.

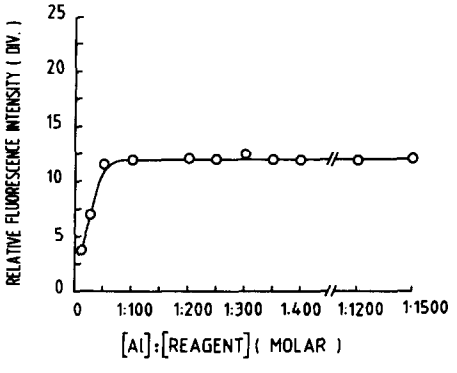

Fig. 4. Effect of reagent on the fluorescence intensity of Al–reagent system [0.01 ppm Al].

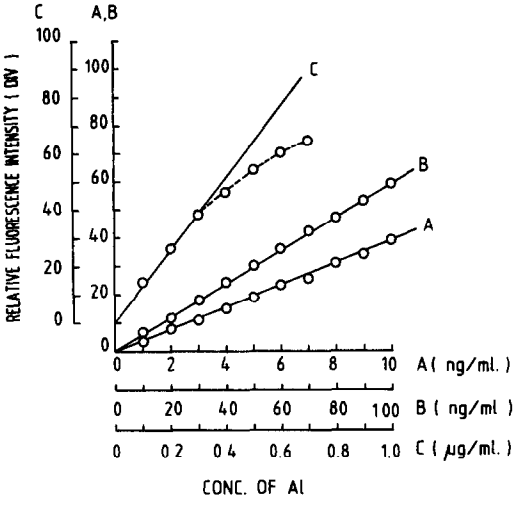

Fig. 5. Calibration graphs (A) 1–10 ng/ml Al at signal gain 10/max; (B) 10–100 ng/ml Al at signal gain 10/min; (C) 0.1–0.7 µg/ml Al at signal gain 10/min.

Calibration graph

The effect of metal concentration was studied over a wide overall range of 0.1–700 ng/ml distributed in four different sets (0.1–1.0 ng/ml; 1.0–10 ng/ml; 10–100 ng/ml; 0.1–0.7 µg/ml) of aluminium concentrations for convenience of measurements to have the readings within the fixed scale of the x–y recorder. The fluorescence intensities maintained a linear relationship in the range of 1.0–300 ng/ml of aluminium (Fig. 5). The standard deviation of the method on 10 ng/ml (11 determinations) was found to be ± 0.001 ng/ml.

Effect of foreign ions

Over 60 cations and anions and some complexing agents were studied individually to investigate their adverse effects, if any, on the determination of 0.01 µg/ml of aluminium according to the procedure described. In case of any precipitation, the centrifuged solution was used for fluorescence measurement. No interference was encountered from 10,000 fold molar excess of alkali metals, sulphate, chloride, phosphate, ascorbic acid; 1000 fold molar excess SCN^-, ClO_4^-, NO_3^-, Br^-, I^-, $S_2O_3^{2-}$, $S_2O_5^{2-}$, thio-urea, oxalate; 500 fold molar excess of Cd; 200 fold molar excess of Li, Mg, Ca, Pb, Sr, Ba, Zn, Ag(I), Se(VI), Sn(IV), Fe(III); 100 fold molar excess of La, Co(II), U(VI), Mn(II), Cu(II), Mo(VI), As(III), Cr(III), Te(VI), tartrate, BiO_3^{2-}; 50 fold molar excess Rh, Ta, Ni(II), W(VI), NO_2^-, $B_4O_7^{2-}$; 25 fold molar excess of Th, Zr, Hg(II), V(V), Ce(IV), Tl(III), Sb (in presence of 50 ppm thiourea), Fe(II) (in presence of

2000 ppm ascorbic acid), Ti (in presence of 200 ppm SCN^-). The quantities of these diverse ions mentioned were the actual amounts added and not the tolerance limits. Negative interference was encountered from EDTA, citrate, Ce(III), F^- and positive interference was caused by Be and Ga. Negative interference from F^- was avoided by acidifying the solution with sulphuric acid and heating, thereby fluoride was evaporated prior to the addition of the reagent. Interference from Ga was removed by simple one-step diethyl ether extraction from a 6*N* HCl. Interference due to Ce(III) (studied up to 25 fold) is removed by oxidation of Ce(III) to Ce(IV) by few drops of conc. HNO_3. However interference due to Be cannot be removed.

Composition of the fluorescent complex

Job's method of continuous variation and the molar-ratio method were applied to ascertain the stoichiometric composition of the fluorescent complex. A 2:3 (Al:Reagent) complex was suggested by both the methods (Fig. 6).

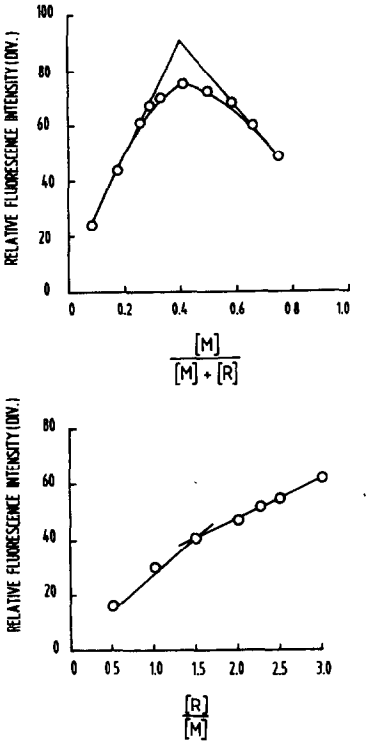

Fig. 6. Stoichiometric composition (a) Job's continuous variation graph; (b) molar ratio graph.

Application

The present method was successfully applied to the determination of aluminium contents in a series of synthetic mixtures of various compositions (Table 1) and also in number of real samples, *e.g.* several standard alloys and steel (Table 2). The method was also extended to the determination of aluminium in a number of environmental and biological samples. In view of the unknown compositions of environmental water samples and of biological samples, *e.g.* human gall-stone, the same aliquot portions of each such sample was analysed for the aluminium content, in both the 'spiked' (added to the samples before the mineralization or dissolution treatment) and the 'unspiked' conditions. The results of analysis in various types

Table 1. Analysis of synthetic mixtures

	Composition of mixtures ($\mu g/ml$)	Al taken (%)	Al found* (%)	Error (%)	Standard deviation
A	Al (0.01) + Cu (0.5) + Mg (1.0) + Mn (II) (0.5)	0.497	0.497	0.000	±0.0000
B	As in A + Ni (0.5) + Cd (5.0)	0.133	0.133	0.000	±0.0007
C	As in B + Co (1.0) + Se (1.0)	0.105	0.106	0.001	±0.0007
D	As in C + Ti (0.1) + Fe (III) (1.0) + SCN^- (5.0)	0.064	0.063	0.001	±0.0000
E	As in D + Ca (1.0) + Zn (1.0) + oxalate (5.0) + Li (1.0)	0.042	0.040	0.002	±0.0007
F	As in E + W (VI) (0.25) + Rh (0.25) + Ag (1.0) + La (0.5) + HPO_4^{2-} (2.0) + V (V) (0.1)	0.036	0.035	0.001	±0.001

*Average of three determinations using 0.07 ml of $3.702 \times 10^{-3}M$ chromotropic acid salt solution in each case.

Table 2. Determination of aluminium in standard bronze, brass and steel sample solutions

Ref. sample and composition (%)	Al spiked		
	Added (*ng/ml*)	Found* (*ng/ml*)	Recovery (%)
(i) BAS–32a, Al–Bronze alloy	0.0	8.7‡	99 ± 0.1†
Cu = 85.9			
Zn = 0.94			
Mn = 0.27			
Fe = 2.67			
Ni = 1.16			
Al = 8.8			
(ii) Brass–5f			
Cu = 70.8	10	10.2	102 ± 0.3
Zn = 24.2			
Sn = 1.85	20	20.2	101 ± 0.3
Pb = 2.52			
Fe = 0.31	30	29.8	99 ± 0.3
P = 0.06; Mn = 0.12,			
Ni = 0.17			
(iii) BCS–261 Straight			
Nb '18/12' stainless steel			
C = 0.083	10	10.0	100 ± 0.0
Si = 0.39			
Cr = 17.20	20	19.8	99 ± 0.4
Nb + Ta = 0.71			
Ni = 13.08	30	30.0	100 ± 0.0
Mn = 0.66			

*Values given represent the average of triplicate determinations.
†The measure of precision is the standard deviation.
‡In terms of sample composition 8.7%.

of samples are in good agreement with the amounts spiked which are summarized in Tables 3 and 4.

Determination of aluminium in alloy and steel

A 0.1 gm sample of alloy or steel was accurately weighed into a 50-ml Erlenmeyer flask. To it 10 ml of 20% (v/v) sulphuric acid was added, carefully covering with a watchglass until brisk reaction subsided. The solution was heated and simmered gently after addition of 1 ml of conc. HNO_3 until all the carbides were decomposed. Then 2 ml of 1:1 (v/v) H_2SO_4 was added and the solution was evaporated carefully to dense white fumes to drive off the oxides of nitrogen and cooled down to room temperature (25–30°C). After suitable dilution with water, the contents of the Erlenmeyer flask were warmed to dissolve the soluble salts. The resulting solution was filtered, if necessary, through a Whatman no. 40 filter paper into a 100-ml volumetric flask. The residue was washed with a small volume of hot (1:99) H_2SO_4 followed by water and the volume was made up to the mark with deionized water.

A suitable aliquot of the above solution was taken in a 10-ml volumetric flask and 1.5 ml of $1 \times 10^{-3}M$ reagent and 1.5 ml NaOAc–HOAc buffer (pH 4.3) were added; the volume was made up to the mark. The fluorescence intensity was measured at 390 nm against the corresponding reagent blank, keeping the excitation wavelength at 360 nm.

Determination of aluminium in environmental water samples

To 25 ml (filtered) environmental water sample (river, tube-well, tap, pond and drain water) contained in a 100-ml Pyrex beaker, 2 ml of conc. $H_2SO_4 + HNO_3$ (1 + 1) mixture was added in a fume cupboard and heated on a hot plate until the white fumes of sulphur trioxide evolved, and was then cooled to room temperature. The residue was then heated with 10 ml deionized water so as to dissolve the salts. The contents of the beaker were then quantitatively transferred into a 25-ml volumetric flask and made up to the mark with water.

A 1 ml aliquot of environmental water sample was pipetted into a 50-ml separatory funnel. To it 10 ml 6*N* HCL was added and Ga was removed by extraction as $GaCl_4^-$ by 10 ml diethyl ether. The volume of the aluminium solution in the aqueous phase was reduced by boiling and it was transferred quantitatively into a 10-ml volumetric flask. A 0.4 ml portion of $3.702 \times 10^{-3}M$ chromotgropic acid solution, 0.5 ml of 0.01% ascorbic acid for masking iron and 1.5 ml of sodium acetate–acetic acid buffer (pH 4.3) were added and diluted up to the mark with deionized water. The fluorescence intensity, corrected against corresponding reagent blank at 390 nm, keeping excitation wavelength at 360 nm, was measured after about 30 min. The concentration of aluminium was determined in terms of ng/ml with the help of a concurrently prepared standard calibration graph under the same instrumental setting. The final values were shown in Table 3, after correction for the volume of dilution.

The abnormally high value for tap water is probably due to leakage and/or excess addition of alum that it used as a flocculant in the water treatment plant. Occurrence of such high values of aluminium content are also reported in tap water of some developed countries.[10] The Ga found in the environmental water samples are expressed in brackets against the following samples: tap water (78.0 ng/ml); tube-well water (below the limit of detection); Ganges' water

Table 3. Determination of aluminium in some environmental water samples

Sample	Al added (*ng/ml*)	Al found* (*ng/ml*)	Recovery (%)	Coefficient of variation (C.V) (%)	Total conc. of Al in original sample solution‡ (*ng/ml*)
Tap water	0	51.0	±0.2†	0.41	510
	1	51.6	99 ± 0.1	0.31	
	10	61.0	100 ± 0.2	0.35	
	50	99.3	98 ± 0.2	0.24	
	100	152.8	101 ± 0.1	0.08	
	150	201.0	100 ± 0.2	0.09	
	200	253.5	101 ± 0.4	0.16	
	250	301.6	100 ± 0.5	0.16	
	300	333.0	95 ± 0.7	0.21	
Drain water (Ordinance factory)	0	34.0	±0.3	0.80	340
	1	35.0	100 ± 0.1	0.35	
	10	43.8	99 ± 0.2	0.47	
	50	84.1	100 ± 0.2	0.22	
	100	134.0	100 ± 0.4	0.29	
	150	183.6	99 ± 0.2	0.13	
	200	231.0	98.05	0.24	
	250	286.6	101 ± 0.3	0.09	
	300	334.8	100 ± 0.3	0.10	
Ganges' water	0	20.0			200
	10	30.0	100 ± 0.02	0.07	
	20	41.0	102 ± 0.10	0.24	
	40	60.0	100 ± 0.05	0.08	
Tube-well water	0	34.0			340
	10	44.0	100 ± 0.00	0.00	
	20	52.0	96 ± 0.01	0.19	
	40	74.0	100 ± 0.20	0.27	
Pond water	0	4.0			40
	10	14.0	100 ± 0.00	0.00	
	20	23.0	96 ± 0.01	0.04	
	40	45.0	102 ± 0.00	0.00	

*The average of five determinations.
†The measure of precision is the standard deviation.
‡Dilution factor = 10.

Table 4. Determination of aluminium in human gall-stone

Volume of gall-stone solution taken (*ml*)	Al added (*ng/ml*)	Al found* (*ng/ml*)	Recovery (%)	Total amount of Al in 50 *ml* of original gall-stone stock soln. (*μg*)	Al content in gall-stone (*μg/g*†)	Mean Al content in gall-stone (*μg/g*)
0.1	0	5.0	—	25.0	170	
	10	15.0	100.0			
	20	25.8	103.3			
	40	44.2	98.2			
0.2	0	10.0	—	25.0	170	172
	10	20.0	100.0			
	20	29.2	97.2			
	40	49.6	99.2			
0.3	0	15.4	—	25.7	175	
	10	25.4	100.0			
	20	35.8	101.2			
	40	55.4	100.0			

*Values given represent the average of five determinations.
†Allowing for dilution factor.

(15.0 ng/ml); drain water (45 ng/ml) and pond water (18 ng/ml). The abnormal high value of Ga in tap-water is probably due to the fact that commercial aluminium salts used as flocculants contained Ga. A similarly abnormal high value of Ga-content is also reported in tap-waters of other countries.[13]

Determination of aluminium in human gall-stone

The aluminium content in various spiked and unspiked gall-stone solutions was determined according to a special procedure.

A 0.1470 gm sample, taken in a 100 ml corning beaker, was digested with conc. HNO_3 till the solution became colourless. The solution was then treated with 2 ml (1:1) H_2SO_4, evaporated carefully to dense white fumes and cooled to room temperature. The residue was extracted with deionized water and transferred quantitatively into a 50 ml volumetric flask and the volume was made up to the mark with deionized water.

Different aliquots of this solution (0.1–0.3 ml) were taken in a 10 ml volumetric flask followed by the addition of 0.4 ml of $3.702 \times 10^{-3}M$ reagent and 1.5 ml NaOAc–HOAc buffer (pH 4.3) and the volume was made up to the mark. The fluorescence intensity, corrected against corresponding reagent blank at 390 nm, keeping excitation wavelength at 360 nm, was measured after about 30 min. The results are shown in Table 4.

Acknowledgements—One of the authors (K.B.) gratefully acknowledges a UGC Teacher-Fellowship, the laboratory facilities provided by the authority of Jadavpur University and also the leave sanctioned by the K. N. College authority. The authors express their gratitude to Dr A. K. Majundar, Prof. Emeritus, Department of Chemistry, Jadavpur University, for his encouragement and keen interest.

REFERENCES

1. B. K. Pal and K. Baksi, *Mikrochim. Acta*, 1992, **108**, 275.
2. C. G. Elinder and B. Sjogren, *Aluminium in 'Handbook on the Toxicology of Metals'*, L. Friberg, G. F. Nordberg and V. Vouk (Eds), 2nd ed., Vol. 2, p. 1, Chapter 1. Elsevier, Amsterdam, 1986.
3. B. Venugopal and T. D. Luckey, *Aluminium in 'Metal Toxicity in Mammals* 2', p. 111. Plenum Press, New York, 1979.
4. R. K. Iyer and S. G. Jadhav, *Science Today*, 1990, **24**, 9.
5. C. G. Elinder and B. Sjogren, *Aluminium in 'Handbook on the Toxicology of Metals'*, L. Friberg, G. F. Nordberg and V. Vouk (Eds), 2nd ed., Vol. 2, p. 20, Chapter 1. Elsevier, Amsterdam, 1986.
6. R. K. Iyer and S. G. Jadhav, *Science Today*, 1990, **24**, 10.
7. Izuo Aoki, Toshiaki Matsunaga and Kunihiro Watanabe, *Bull. Chem. Soc. Jpn.*, 1989, **62**, 2369.
8. Y. Suzuki, S. Imai and T. Kamiki, *Analyst*, 1989, **114**, 839.
9. I. G. Perkov, Yu. V. Podpruzhnikov and A. V. Drozd, *Vysokochist Veshchestva*, 1989, **1**, 189.
10. Nabuo Uehara, Makoto Kanbayashi, Hitoshi Hosino and Takao Yotsuyanagi, *Talanta*, 1989, **36**, 1031.
11. Nianqin Jie, Chaunhong Zhang and Shufu Jiang, *Yejin Fenxi*, 1988, **8**, 18.
12. B. K. Pal and B. Chowdhury, *Mikrochim Acta*, 1984, **II**, 121.
13. F. Capitan, A. Navalon, J. L. Vilchez and L. F. Capitan-Vallvey, *Talanta*, 1990, **37**, 193.