QUANTITATIVE INORGANIC CHROMATOGRAPHY

XI. THE CHROMATOGRAPHIC SEPARATION AND AUTOMATIC FLAME SPECTROPHOTOMETRIC DETERMINATION OF THE ALKALINE EARTH METALS USING α-HYDROXYISOBUTYRIC ACID

F. H. POLLARD, G. NICKLESS AND D. SPINCER

*Department of Chemistry,
The University, Bristol (Great Britain)*

A previous communication[1] described the chromatographic separation and automatic flame spectrophotometric determination of the alkaline earth metals using ammonium lactate as eluting agent in the ion exchange procedure. The disadvantage of this method was that the separation took twelve hours, and the present separation was studied to give a more rapid determination. The technique has been developed so that magnesium, calcium, strontium, and barium may be separated and determined in under five hours.

WISH[2] found that calcium, strontium and barium could be separated by cation exchange chromatography, eluting with α-hydroxyisobutyric acid. As this separation did not include the separation of magnesium, a slightly modified procedure has been devised to separate all four alkaline earth metals.

EXPERIMENTAL

This paper describes the recommended procedure for the quantitative separation and estimation of sub-milligram quantities of all four alkaline earth metals using conventional cation exchange chromatography and automatic flame spectrophotometric detection. The separation was achieved by batch elution technique with ammonium α-hydroxyisobutyrate and the estimation was achieved by direct aspiration of the eluate into the flame after constant dilution. The area of the elution peaks so obtained was a measure of the total alkaline earth metal loaded on to the column. The accuracy of the method was found to be within 4 % at sub milligram levels.

Apparatus and general technique

The cation exchange material used for the separation was Dowex 50 W resin with 8 % divinylbenzene cross-linking, 100–200 mesh. The resin bed was 9 cm long and 0.4 cm diameter. A glass wool plug was placed at the top of the resin so that in loading samples on to the column, the upper layers of the resin remained undisturbed. A 250 ml dropping funnel was fitted to the top of the column so that there was an approx. 10 cm pressure head of eluting agent above the column bed. The dropping rate of the column was 0.65 ml/min.

In the initial investigations of the separation, a Shandon automatic fraction collector (balance type) was used to collect 5 ml samples of eluate to be analysed.

For the automatic procedure, the constant volume delivery burette, previously described[1], was used to enable the column effluent to drop into a constant volume of water. This was achieved by balancing the aspiration rate of the flame with the total flow from the column and the constant volume delivery burette.

Eluting agents

The magnesium was eluted with 0.8 M α-hydroxyisobutyric acid pH 4.15. Calcium and strontium were eluted with 1 M α-hydroxyisobutyric acid pH 5.00 and barium with 2 M solution pH 6.20.

The solutions were prepared as follows:

0.8 M solution was prepared by dissolving 83.3 g of the acid in water and adding 0.880 ammonia (AnalaR) until the solution reached pH 4.15, and water was added to 1 l.

1 M solution required 104.1 g acid dissolved in water, ammonia was added until pH 5.0 and made up to 1 l.

2 M solution required 208.2 g acid in water, ammonia was added until pH 6.20, and made up to 1 l.

Most of the α-hydroxyisobutyric acid used was prepared from acetone and potassium cyanide to form acetone cyanohydrin as suggested by WELCH AND CLEMO[3], and this was hydrolysed and extracted to give the α-hydroxyisobutyric acid as done by RULE AND HARROWER[4]. The acid was recrystallised from 60°–80° petroleum ether.

Purity of water and reagents

All water used was deionised by passing distilled water from an all-glass apparatus, through a mixed Zeocarb resin column. The water was stored in hard glass or plastic aspirators, and the alkaline earth content of the water was below the detection limit of the flame spectrophotometer. All alkaline earth metal solutions were prepared from AnalaR reagents.

The alkaline earth metal content of the α-hydroxyisobutyric acid was found to be negligible, whether commercially or laboratory prepared. The laboratory prepared acid had a slight sodium impurity, but this did not affect results obtained, and so no further purification was deemed necessary. The effect of organic matter in the eluant gave slight enhancement of emission of the alkaline earths, and also an increase in background emission during automatic detection. Thus when determining fraction concentrations, prior to the automatic runs, standard solutions of the alkaline earth metals were prepared containing a similar quantity of α-hydroxyisobutyric acid to that in the sample fraction being examined.

Solutions

(a) For chromatography: Standard barium solution: AnalaR barium chloride $BaCl_2 \cdot 2H_2O$ in water containing 1 mg/ml of barium.

Standard calcium solution: AnalaR calcium carbonate $CaCO_3$ in 0.01 N hydrochloric acid containing 1 mg/ml calcium.

Standard magnesium solution: AnalaR magnesium carbonate $MgCO_3$ in 0.01 N hydrochloric acid containing 1 mg/ml magnesium.

Standard strontium solution: AnalaR strontium carbonate $SrCO_3$ in $0.01 N$ hydrochloric acid containing 1 mg/ml strontium.

The column was loaded with 0.25 ml, 0.5 ml, 1.0 ml or 1.5 ml of each solution for standard runs.

(b) *For flame spectrophotometric analysis of fractions.* The above solutions were diluted for comparison standards when initial runs were carried out, by collecting fractions and analysing them. Each solution contained the appropriate quantity of α-hydroxyisobutyric acid solution for exact comparison with fraction samples. The working solutions used were:

Calcium and strontium: 0–2 μg/ml of alkaline earth metal.

Barium and magnesium: 0–5 μg/ml of alkaline earth metal.

The quantitative chromatogram

The resin was generated in the ammonium form by the passage of $3 M$ ammonium chloride down the column followed by copious washing with water. The column was loaded with the alkaline earth metal bearing solution, followed by further washing with water. The dropping funnel was then filled with $0.8 M$ α-hydroxyisobutyric acid which was passed down the column for 13 fractions when using the fraction collector or until the magnesium had been eluted when using automatic detection. This solution was then replaced by $1 M$ acid in the funnel. The passage of $1 M$ α-hydroxyisobutyric acid was continued until fraction 28 when collecting fractions, or until both the calcium and strontium were removed automatically. The elution was then continued replacing the $1 M$ acid by $2 M$ until fraction 35 or barium had been eluted.

The column was washed with water and regenerated with ammonium chloride. If quantities of alkaline earth metals much greater than 1–2 mg are used for separation, then a longer column will be necessary.

The detection of the alkaline earth metals

(a) *Using a fraction collector.* 1 ml aliquots of the 5 ml fraction were diluted until the concentration of the alkaline earth metal in the sample solution was within the standard range. The quantity of the alkaline earth metal in each fraction was then determined by comparison with standard calibrations in the normal manner.

(b) *For automatic detection.* The column effluent was run into 10 ml of water which was continuously stirred. The resulting solution was aspirated directly into the flame of the instrument. As the flame aspiration rate was greater than the flow rate of the column, a constant volume delivery burette was required to keep the volume of water, into which the effluent fell constant. The use of this technique has previously been described in detail[1]. The flame spectrophotometer wavelength settings were the same as used for the ammonium lactate procedure, as these wavelengths gave good peaks. The concentration of alkaline earth metal in the 10 ml of water was found to be proportional to the concentration of the ion in the column effluent, and so the elution curve for each metal was directly recorded by coupling the output of the flame spectrophotometer's photomultiplier to a potentiometric recorder with a chart speed of 2 in. per h.

In order to overcome day to day variations in conditions, it was found most practicable to fix the gain settings of the instrument and vary the pressure of acetylene daily. This was done by passing a 1 μg/ml solution of calcium with standard

instrument settings, and vary the acetylene pressure until the scanned line gave the same deflection as on previous days.

Calibration curves were obtained using synthetic mixtures containing 0–1.5 mg amounts of individual alkaline earth metals by plotting area of elution curve against quantity of alkaline earth loaded on to the column. Using these calibration curves, mineral ore and biological samples were successfully analysed.

Flame spectrophotometry

The Unicam SP 900 was used operating under the following conditions:

B.O.G. cylinder gases with approximately 3 in. of acetylene indicated on the dibutyl phthalate manometer, and 30 lb./sq. in. of compressed air.

(*a*) *With fraction collector*. Samples and standards were aspirated under the conditions shown in Table I.

TABLE I

CONDITIONS FOR ANALYSIS OF FRACTIONS

Electrical bandwidth 4. Photomultiplier Mazda 27M3 Sunvic RSP 2 high speed potentiometric recorder. Chart speed 1 in./min.

	Magnesium	*Calcium*	*Strontium*	*Barium*
Slit (mm)	0.08	0.04	0.04	0.06
Wavelength (mμ)	284.5–286.0	420–427	457–467	554–561.0
Amplifier gain	4.3	4.5	4.3	4.3
Recorder f.s.d. (mV.)	1	10	10	2.5

The amplifier gain is only approximate in these cases, and varies slightly from day to day. Peak heights recorded in these conditions are measured. Barium and magnesium were determined by allowing the recorder to "settle" after scanning as described earlier[5]. The elution pattern for separation with a fraction collector is shown in Fig. 1.

(*b*) *Automatic detection*. In this procedure, the gain settings were kept constant, day to day variations were minimised by adjustment of acetylene pressure. Previously
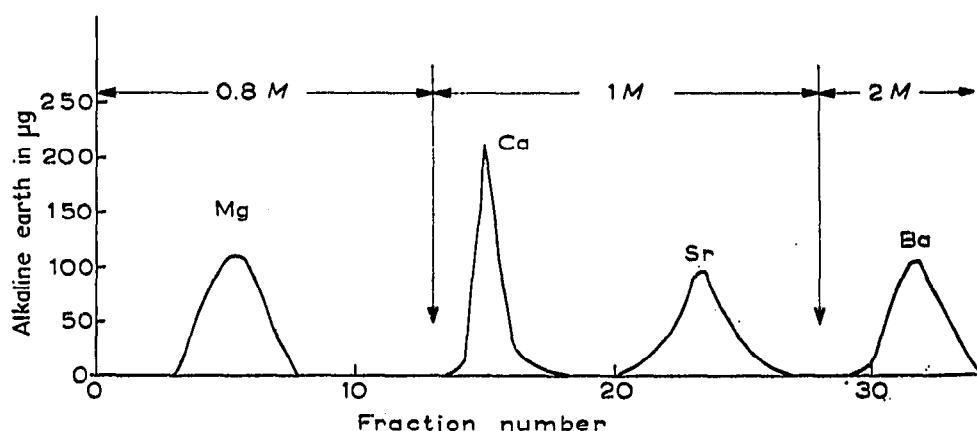

Fig. 1. Elution curve of alkaline earth metals eluted with α-hydroxyisobutyric acid using a fraction collector and analysing fractions. 5 ml fractions collected.

the most critical factor affecting determinations was found to be the wavelength setting[1]. The wavelengths used for the previous ammonium lactate process gave good results with this present separation, and no further investigation of wavelength settings was deemed necessary.

The settings used for analysis by automatic flame spectrophotometry are shown in Table II.

A constant volume of 10 ml of solution was used.

TABLE II

CONDITIONS FOR AUTOMATIC ANALYSIS

Electrical bandwidth 4. Sunvic RSP 2 high speed potentiometric recorder 10 mV f.s.d. and chart speed 2 in./h. Photomultiplier Mazda 27 M 3.

	Magnesium	*Calcium*	*Strontium*	*Barium*
Slit (mm)	0.08	0.04	0.04	0.06
Wavelength (mμ)	285.5	423.0	461.0	560.0
Amplifier gain	4.7	2.5	2.5	4.5

The shapes of the elution curves recorded automatically are shown in Fig. 2.

Magnesium was found to give a slightly flatter shaped elution curve compared with the other alkaline earth metals, and also a lower sensitivity as was found in the previous ammonium lactate procedure[1]. Also the calibration curve for magnesium is non-linear, unlike the other metals, and these differences appear to be due to the phenomenon reported by DEAN[6] that the wavelength of maximum intensity for magnesium varies with concentration of magnesium in solution. Before the actual peaks of magnesium and barium, a raising of background level of emission appears,
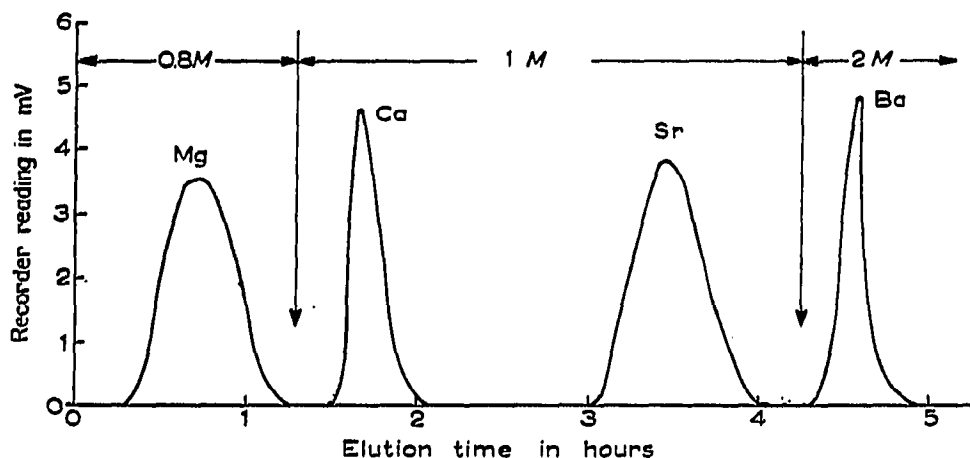

Fig. 2. Elution curves obtained automatically for alkaline earth metals eluted with α-hydroxyisobutyric acid.

which does not disappear after elution of the metal. This phenomenon was noticed with elution of barium with ethylenediaminetetraacetic acid (EDTA)[1] and the explanation would seem to be the comparatively sudden increase of organic matter

reaching the flame causing a rise in flame temperature, and hence of flame background. All areas of peaks drawn were measured with a planimeter.

Analysis of synthetic mixtures

(a) During initial investigations of the separation, the use of a fraction collector was employed to demonstrate the quantitative nature of the chromatography. The figures given in Table III illustrate this and serve to show that this method may be used where a flame spectrophotometer cannot be used to record directly.

TABLE III
ANALYSIS OF SYNTHETIC SAMPLES IN FRACTIONS

Loaded on to the column (µg)				Found by analysis of fractions (µg)			
Magnesium	Calcium	Strontium	Barium	Magnesium	Calcium	Strontium	Barium
300	300	300	300	309	294	282	293
300	500	500	500	311	505	504	502

These results showed that the separation was indeed quantitative and all further results were recorded automatically.

(b) Results of automatic separation of standard synthetic mixtures for calibration purposes are given in Table IV.

These results were used to obtain the calibration curves shown in Fig. 3.

TABLE IV
AUTOMATIC ANALYSIS OF SYNTHETIC SAMPLES FOR CALIBRATION CURVES

	Magnesium			Calcium			Strontium			Barium		
Quantity of metal (mg)	0.5	1.0	1.5	0.25	0.5	1.0	0.25	0.5	1.0	0.25	0.5	1.0
Peak area	0.70	1.41	1.62	1.46	2.97	6.16	0.71	1.55	3.09	0.83	1.58	3.20
(sq. in.)	0.71	1.36	1.60	1.47	2.94	6.25	0.73	1.53	3.17	0.85	1.62	3.10
	0.70	1.34	1.59	1.52	3.00	6.20	0.73	1.56	3.19	0.80	1.65	3.10
	0.73	1.32	1.61	1.46	3.12	6.13	0.72	1.55	3.06	0.85	1.56	3.30
						6.30						
Mean area	0.71	1.36	1.61	1.48	3.01	6.21	0.72	1.55	3.12	0.83	1.60	3.17

Analysis of mineral ores by automatic detection

Samples of mineral ores were taken up into solution and aliquots of these solutions loaded on to the column and analysed for their alkaline earth metal content. The area of the elution peaks for each sample was measured, and the amount of alkaline earth metal in each sample determined from the calibration graph in Fig. 3.
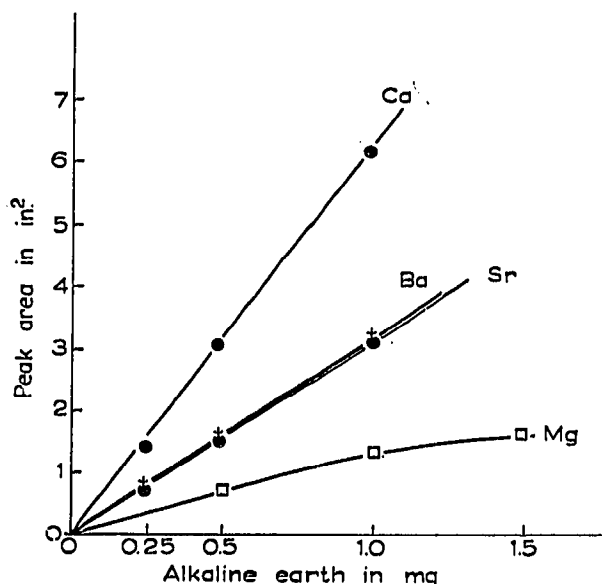
The results obtained are given in Table V.

Fig. 3. Calibration curves for alkaline earth metals determined automatically when eluted with α-hydroxyisobutyric acid.

TABLE V

RESULTS OF AUTOMATIC ANALYSIS OF MINERAL ORE SAMPLES

Ore	Metal	% found	% found by previous automatic analysis[1]	% by chemical analysis
Dolomite	Magnesium	5.99	5.80	5.78
	Calcium	31.2	30.3	31.1
Celestine	Strontium	46.9	47.3	45.6
Barytes	Calcium	16.5	16.7	16.6
	Barium	33.3	34.7	35.4

[1] All chromatographic analyses of these barytes have given low results between 32.2 %–34.7 %.

Analysis of biological samples

Samples of blood serum and bones were obtained from a rat and ashed with concentrated nitric and sulphuric acids in the ratio of 10 : 1 respectively. The blood sample was diluted to a standard volume as the ash remained in solution in even a very small volume of remaining sulphuric acid after evaporating to almost dryness. The bone ash was fused with sodium carbonate, leached with water and the resultant residue dissolved in dilute hydrochloric acid and made up to a standard volume.

These solutions were analysed chemically and chromatographically. The chemical calcium analysis was carried out according to the CLARK-COLLIP method[7] by precipitating it as oxalate and, after acidification, titrating with standard potassium permanganate.

The magnesium analysis was carried out colorimetrically using Eriochrome Black T as directed by VOGEL[8].

The results by automatic chromatographic and chemical analyses are given in Table VI.

TABLE VI
RESULTS OF AUTOMATIC ANALYSIS OF BIOLOGICAL SAMPLES

	Found chromatographically %	Found chemically %
Calcium in blood	0.0053	0.0055
Magnesium in bone	0.034	0.032
Calcium in bone	17.6	17.1

Thus this cation exchange process with automatic flame spectrophotometric detection has been used successfully for synthetic, mineral ore and biological samples containing alkaline earth metals.

SUMMARY

An ion exchange separation of submilligram quantities of alkaline earth metals has been developed using α-hydroxyisobutyric acid as eluting agent and automatic flame spectrophotometric determination.

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