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Separation and visible detection of alkaline earth metals on a polymeric reversed-phase column with a mobile phase containing a selective colour-forming chelating ligand

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Abstract

A polystyrene–divinylbenzene reversed-phase column has been used in conjunction with a mobile phase containing *o*-cresolphthalein complexone for the separation and visible detection of alkaline earth metals. Retention is achieved due to the formation of a dynamically coated chelating stationary phase. The retention order obtained for the alkaline earth metals is the reverse of that achieved with simple ion exchange, with barium(II) being eluted first, followed by strontium(II), calcium(II) and magnesium(II). Visible detection at 575 nm is possible without the need for addition of postcolumn reagents. Detection limits of 10 μM for barium(II) and strontium(II) were achieved in standards containing massive excesses of sodium, calcium(II) and magnesium(II). The method has been applied successfully to the determination of trace amounts of strontium(II) in a highly concentrated brine sample taken from an Antarctic lake. © 1998 Elsevier Science B.V.

Keywords: Mobile phase composition; Complexation; Water analysis; Environmental analysis; Alkaline earth metals; Metals; Cresolphthalein complexone

1. Introduction

The use of various metallochromic ligands to coat or ‘impregnate’ the surface of high-performance substrates, resulting in the formation of chelating stationary phases, has been carried out by a number of workers in recent years [1–6]. These chelating columns have been shown to exhibit unique selectivities and have been applied to metal separations in complex sample matrices, including seawaters [2,3,6] and concentrated brines [2,5]. However, in each of the above studies the ligand had been used to precoat the surface of the stationary phase and was not included as a component of the mobile phase. The incorporation of colour-forming ligands into the mobile phase can, under certain conditions, act both to dynamically coat the stationary phase surface and remove the need for postcolumn reaction detection, therefore simplifying the chromatographic technique. One example of this approach was reported by Dasgupta et al. [7]. Here a polymeric reversed-phase substrate was used with a mobile phase containing 8-hydroxyquinoline-5-sulphonic acid. While a number of metal separations were achieved with fluorescence detection, the separations shown were generally poor.

Inclusion of a colour-forming ligand within the mobile phase when using sulfonated cation-exchange columns has been investigated by a number of workers, including Zenki [8] and more recently by Toei and co-workers [9–14]. The most promising systems were those which used highly selective

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metallochromic ligands, suitable for the separation of a particular group of metals. One such ligand is *o*-cresolphthalein complexone (OCPC), which was used as a mobile phase component by Toei and co-workers [9–11] for the determination of alkaline earth metals in a range of samples.

In a recent preliminary communication [15] we reported the use of a porous graphitic carbon reversed-phase column with a mobile phase containing OCPC for the separation and visible detection of magnesium(II) and calcium(II). The ligand dynamically coated the surface of the substrate through typical reversed-phase hydrophobic interactions, resulting in a chelating stationary phase. The method was applied to the determination of magnesium(II) and calcium(II) in seawater. Further work [16] investigated the addition of NaCl to the mobile phase, which resulted in a salting-out effect upon the ligand and an increase in the dynamic capacity of the column, thereby increasing retention. A significant improvement in the resolution of the two metals was obtained with the addition of between 0.05 and 0.2 *M* NaCl to the mobile phase. This method was applied to the determination of magnesium(II) and calcium(II) in a range of water samples of differing salinity, including saturated natural brines, with the results comparing well to those achieved using a variety of alternative analytical methods.

The present paper details an investigation into the use of a polystyrene–divinylbenzene reversed-phase column with a mobile phase containing OCPC for the separation and visible detection of alkaline earth metals. The use of a polystyrene reversed-phase column instead of the porous graphitic carbon column used in the above studies arises from work carried out by Jones et al. [4,6] in which an OCPC coated polystyrene-based resin produced a retention order for the alkaline earth metals which was the reverse of that achieved with the above graphitic column. However, in the above study the ligand was present in the stationary phase only and was not included in the mobile phase, therefore detection of the eluted metals was carried out using postcolumn reaction.

The aim of the present work was to reproduce the selectivity and retention order obtained by Jones et al., using a dynamically coated column, and to determine the effects upon these parameters of adding the ligand to the mobile phase. The addition of the ligand to the mobile phase would also remove the need for postcolumn reaction detection.

2. Experimental

2.1. Instrumentation

A Waters Model 600 Programmable Pump (Waters, Milford, MA., USA) was used to deliver the mobile phase. Sample injection was via a Rheodyne Model 7125 syringe loading injector, (Rheodyne, Cotati, CA, USA) fitted with a 100-μl sample loop. The analytical column used was a Hamilton 5 μm PRP-1 (150×4.1 mm I.D.), (Hamilton, Reno, Nevada, USA). The detector used was a Shimadzu Model SPD-6AV UV–vis spectrophotometric detector (Shimadzu, Kyoto, Japan), set at 575 nm and interfaced to a Waters MAXIMA data station. For work requiring postcolumn reaction detection an additional reagent pump (Waters Model 6000A) was used to deliver the postcolumn reagent solution. The postcolumn reagent was mixed with the mobile phase using a zero dead volume T-piece, followed by a 1.0 m×0.3 mm I.D. polyether ether ketone (PEEK) reaction coil. The flow-rate of the postcolumn reagent was set at 1 ml min^{-1}.

2.2. Reagents

OCPC {3,3′-bis[N,N-bis(carboxymethyl)aminomethyl]-*o*-cresolphthalein} was obtained from Fluka (Buchs, Switzerland) and used without further purification (99% dye content). Methanol (HPLC grade) was supplied by Ajax (Sydney, Australia). Boric acid was supplied by Aldrich (Milwaukee, WI, USA). All other chemicals used were obtained from BDH (Kilsyth, Australia) and were of analytical reagent grade unless stated otherwise. Solutions were prepared using distilled and deionised water from a Milli-Q water purification system (Millipore, Bedford, MA, USA). Dilute sodium or lithium hydroxide (1 *M*) was used to adjust the pH of the mobile phase. The mobile phase was filtered using a 0.45-μm disc filter (Millipore) and degassed using an ultrasonic bath prior to use. Once prepared, the mobile phase remained stable for several days if stored away from

direct sunlight. Stock metal standards were prepared in 0.1 *M* HNO_3 from nitrate and carbonate salts and diluted as required. Samples were analysed untreated, except for dilution where necessary.

A postcolumn reagent was prepared using 0.4 m*M* OCPC dissolved in 40% methanol. This was buffered using 100 m*M* boric acid, adjusted to pH 10 using LiOH. Absorbance was monitored at 575 nm.

2.3. Column preparation

To coat the surface of the Hamilton PRP-1 column with OCPC, procedures detailed in previous studies were followed [6]. A concentrated solution (80 m*M*) of the ligand was prepared in 10% methanol and adjusted to pH 8.0. The solution was passed through the column overnight at a flow-rate of 1 ml min^{-1}. The column was then conditioned to remove the unstable portion of the adsorbed ligand with a series of dilute solutions (50 m*M*) of ammonia, nitric acid, acetic acid, and deionised water.

3. Results and discussion

3.1. Mobile phase without OCPC added

Recent studies by Jones et al. [4,6] have shown that a reverse retention order to that produced by cation-exchange chromatography for the alkaline earth metals, can be achieved with the use of a polystyrene–divinylbenzene reversed-phase substrate (PLRP-S 10-μm resin, Polymer Labs., Shropshire, UK), precoated with OCPC. This approach was investigated using a Hamilton PRP-1 column. The ligand itself is a triphenylmethane-based chelating dye, containing two iminodiacetic acid (IDA) functional groups. Therefore, retention of metal ions is based upon the conditional formation constants of the IDA–metal complexes. These are dependent upon the pH of the mobile phase, and thus retention of the alkaline earth metals can be manipulated through control of the above parameter. A solution of 0.5 *M* KNO_3 was used as the mobile phase, buffered with 20 m*M* borate to between pH 8.5 and 10.2, and detection was achieved using postcolumn reaction with a buffered solution of 0.4 m*M* OCPC. At pH 8.5 barium(II) and strontium(II) were eluted at the solvent front and calcium(II) and magnesium(II) were unresolved. Increasing the pH to 9.4 allowed the complete separation of calcium(II) and magnesium(II), which were both resolved from a coeluted barium(II) and strontium(II) peak at the solvent front. The peak for magnesium(II) tailed more severely than expected at the above pH, although this did not interfere with the separation. The reason for this tailing is likely to be due to competing secondary equilibria with the formation of hydroxo species at this pH. Fig. 1 shows the separation of 0.5 m*M* calcium(II) and magnesium(II) achieved under the above conditions.

To obtain a baseline separation of barium(II) and strontium(II) the pH of the mobile phase had to be increased to 10.2. At this pH calcium(II) was retained for over 25 min and magnesium(II) was completely retained. Fig. 2 shows the separation of 0.5 m*M* barium(II) and strontium(II) in under 3 min on the OCPC coated column.

The separation selectivity shown by the PRP-1 column was similar to that obtained by Jones et al., although the pH required to obtain retention and the separation of barium(II) and strontium(II) was higher than that used previously, indicating this was a slightly lower capacity column.

3.2. Effect upon metal retention with addition of OCPC to mobile phase

The effects on retention of adding OCPC to the mobile phase were determined using additions of 0.02 – 0.1 m*M* of the ligand to a 0.5 *M* KNO_3 mobile phase, adjusted to pH 9.5. The postcolumn reaction detection system was left in place so that the eluted metals could still be detected at the lower ligand concentrations. The column was equilibrated for 1 h with each mobile phase prior to injection. Fig. 3 shows the retention times of barium(II), strontium(II), calcium(II) and magnesium(II) as a function of OCPC concentration in the mobile phase. The retention order of the four metals remained the same but the retention times increased with the addition of OCPC over this range. This result indicated that the surface of the stationary phase was not saturated with OCPC during the coating and conditioning procedure detailed above, and that adding the ligand to the mobile phase resulted in an

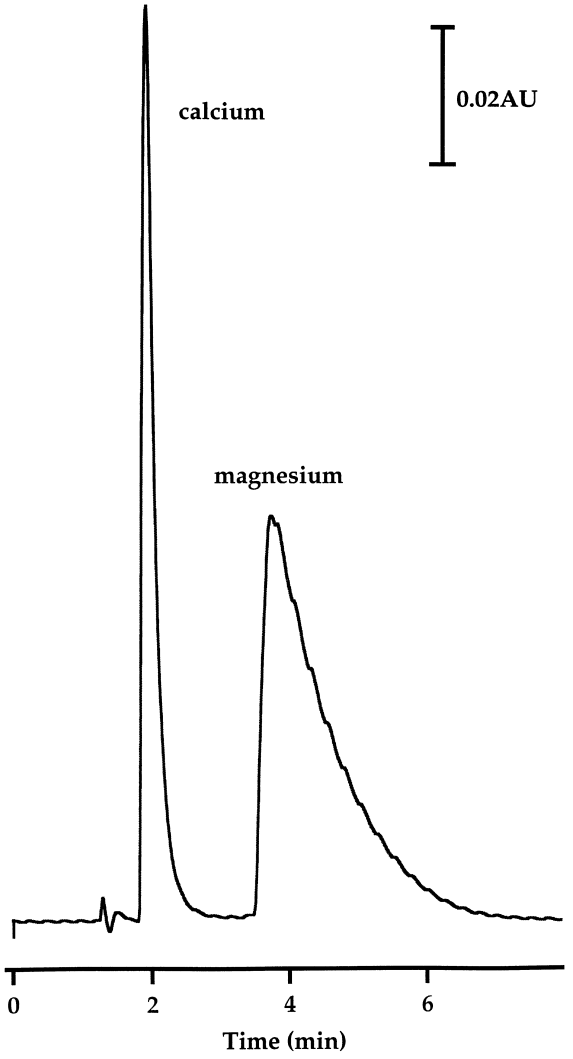

Fig. 1. Separation of calcium(II) and magnesium(II) on a precoated OCPC column. Conditions: 100-μl injection of 0.5 m*M* calcium(II) and magnesium(II). Mobile phase: 0.5 *M* KNO_3, 20 m*M* borate (pH 9.4). Flow-rate: 1.5 ml min^{-1}.

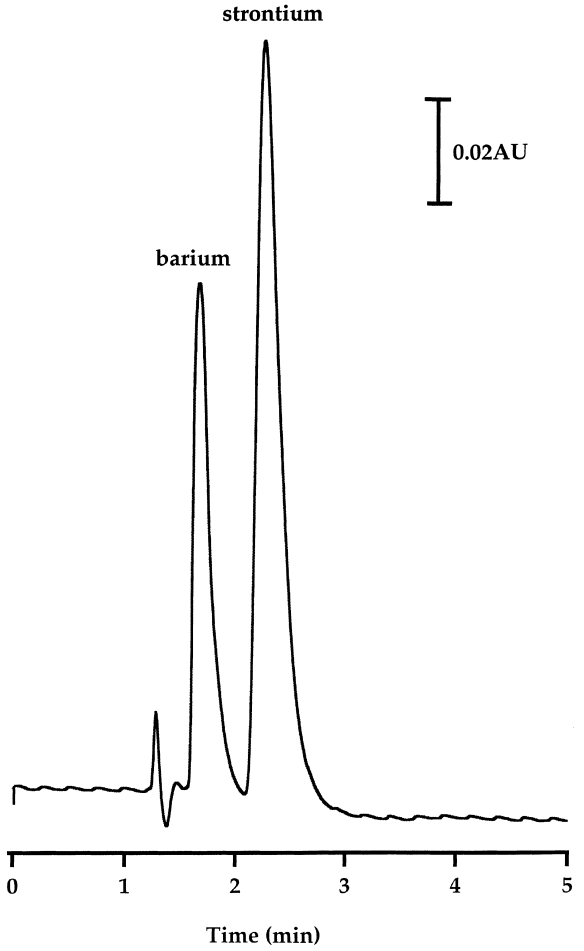

Fig. 2. Separation of barium(II) and strontium(II) on a precoated OCPC column. Conditions: 100-μl injection of 0.5 m*M* barium(II) and strontium(II). Mobile phase: as for Fig. 1 (pH 10.2). Flow-rate: 1.5 ml min^{-1}.

increased dynamic loading on the stationary phase, thereby increasing the column capacity and therefore retention. This result differs to that shown previously [15] using a porous graphitic carbon column, with which the concentration of OCPC in the mobile phase was varied between 0.2 and 0.8 m*M*, made up in 58% methanol. Increasing the concentration of OCPC over this range led to a decrease in the retention of magnesium(II) and calcium(II). The reason for this difference was that under the above conditions the surface of the highly hydrophobic graphitic carbon stationary phase was already saturated with OCPC at 0.2 m*M*, therefore it was the mobile phase concentration of OCPC which was actually being increased and this acted to increase elution of the two metals from the column.

3.3. Separations of alkaline earth metals

From Fig. 3 it can be seen that the addition of greater than 0.08 m*M* OCPC to the KNO_3 mobile phase led to the complete retention of magnesium(II)

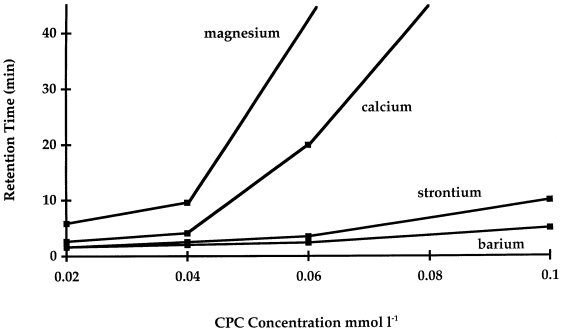

Fig. 3. Retention times of barium(II), strontium(II), calcium(II) and magnesium(II) as a function of OCPC concentration in the mobile phase. Other conditions: as for Fig. 1.

and calcium(II). In order to have sufficient OCPC present in the mobile phase to remove the need for postcolumn reaction detection, and also to be able to elute magnesium(II) and calcium(II) in a reasonable time, it was necessary to add methanol to the mobile phase. This decreased the dynamic capacity of the column by lowering the concentration of OCPC adsorbed onto the stationary phase. Under these conditions barium(II) and strontium(II) were eluted at the solvent front and so did not interfere with the separation. Fig. 4 shows the separation of magnesium(II) and calcium(II) achieved using a mobile phase consisting of 0.5 *M* KNO_3 and 0.4 m*M* OCPC with 25% methanol added (pH 10.0). It should be noted that the elution order achieved under these conditions for magnesium(II) and calcium(II) was the reverse of that shown in Fig. 1, which was obtained without the ligand added to the mobile phase. This was because under the above conditions (with the addition of methanol) the surface of the stationary phase was saturated with OCPC and that the OCPC in the mobile phase was again acting to elute the two metals. As OCPC forms a much stronger complex with magnesium(II) than calcium(II) (Ca log K_{MHL}=7.8, Mg log K_{MHL}=8.9 [17]), magnesium(II) was eluted first. The above separation is similar to that achieved in previous work using the graphitic carbon column, although here, due to differences in the hydrophobic character of the two base substrates, a substantially lower concentration of methanol was needed to achieve a separation in under 8 min.

As shown in Fig. 3, the retention of barium(II)

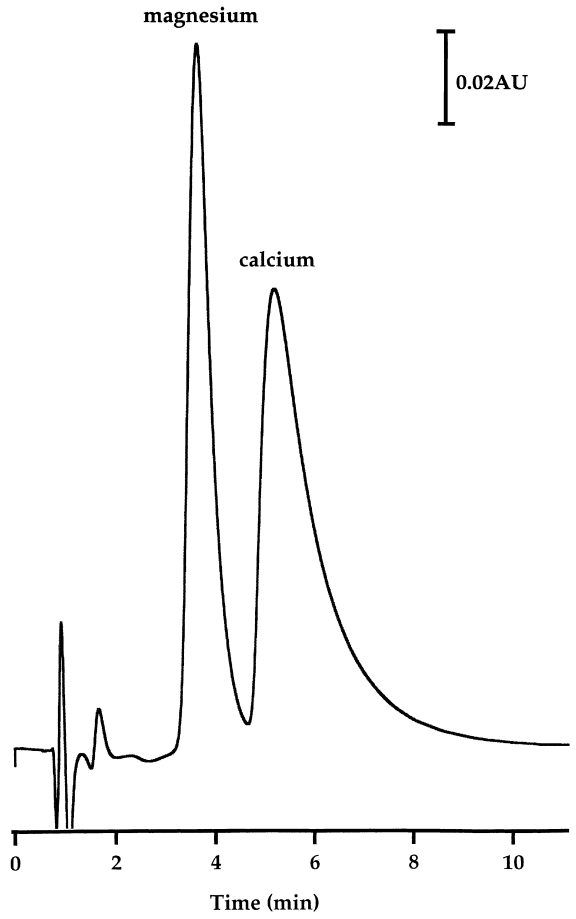

Fig. 4. Separation of magnesium(II) and calcium(II) with OCPC in the mobile phase. Conditions: 100-μl injection of 0.5 m*M* magnesium(II) and calcium(II). Mobile phase: 25% methanol, 0.5 *M* KNO_3, 0.4 m*M* OCPC, 20 m*M* borate (pH 10.0). Flow-rate: 1.5 ml min^{-1}.

and strontium(II) was significantly less than that of calcium(II) and magnesium(II) with the addition of up to 0.1 m*M* OCPC to the mobile phase. This concentration was sufficient to allow the separation and detection of the two metals without the need for postcolumn reaction, although this was later increased to 0.2 m*M* OCPC to increase the detector linear range. This further increase in mobile phase OCPC concentration increased the retention times of both barium(II) and strontium(II), although to a lesser extent than was expected, with both metals being eluted in under 14 min. Fig. 5 shows the separation of 1 m*M* of barium(II) and strontium(II)

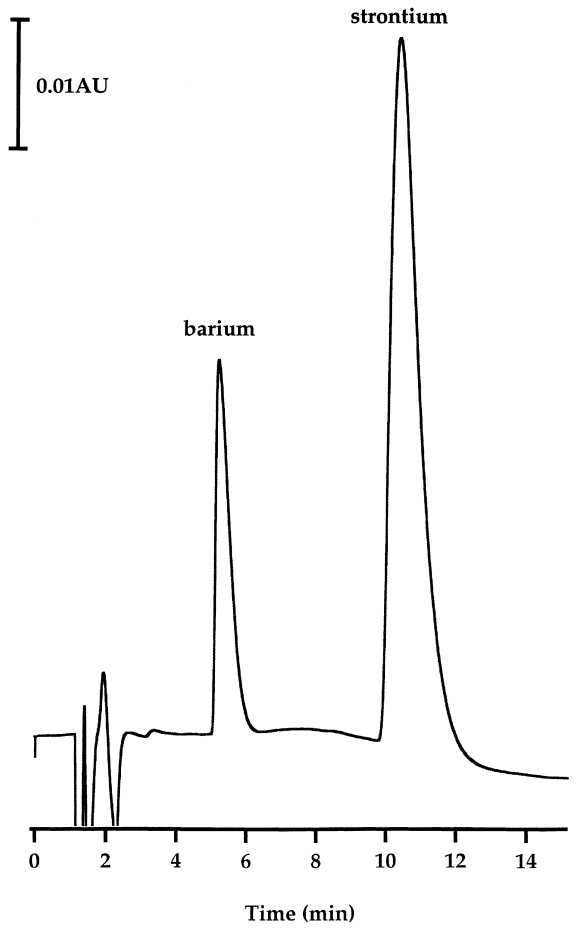

Fig. 5. Separation of barium(II) and strontium(II) with OCPC in the mobile phase. Conditions: 100-μl injection of 1 m*M* barium(II) and strontium(II) in 0.5 *M* sodium chloride. Mobile phase: 0.5 *M* KNO_3, 0.2 m*M* OCPC, 20 m*M* borate (pH 9.5). Flow-rate: 1.5 ml min^{-1}.

in a standard containing 0.5 *M* sodium chloride, using a 0.5 *M* KNO_3 mobile phase containing 0.2 m*M* OCPC (pH 9.5). As can be seen from the chromatogram the sodium chloride matrix caused no visible interference, with both metals being well separated from each other and from the solvent front. The advantage of including a colour-forming ligand within the mobile phase, rather than using post-column addition, can be clearly seen from a comparison of Figs. 1 and 2 with Figs. 4 and 5. The pulsing shown in the first two figures is typical of postcolumn reaction detection when a pump is used to deliver the reagent into the mobile phase. This does not occur when the reagent is included in the mobile phase itself.

The retention order shown above is the same as that found by Jones et al. [4,6], who used similar precoated chelating columns, but without including the ligand in the mobile phase. However, the retention order obtained here differs substantially from the previous work carried out on the graphitic reversed-phase column, where strontium(II) eluted after magnesium(II) and calcium(II), and no peak was observed for barium(II). The retention order found here is also the reverse of that found in all the previous studies using cation-exchange chromatography with colour-forming ligands in the mobile phase [8–14]. In these studies, as expected with simple ion exchange, strontium(II) and barium(II) were eluted after calcium(II) and magnesium(II).

A drawback to the proposed technique is that under conditions suitable for the separation and detection of barium(II) and strontium(II), both calcium(II) and magnesium(II) were totally retained on the column. Therefore, to determine barium(II) and strontium(II) in real samples [which in the majority of cases would be likely to contain higher concentrations of calcium(II) and magnesium(II)], these retained metals would need to be eluted from the column after a certain number of analyses. In order not to greatly affect the equilibrium of the system [which would be the case if the addition of methanol to the mobile phase or a reduction in OCPC concentration were used to elute calcium(II) and magnesium(II)], a pH step gradient was used to elute these two metals. A step down in mobile phase pH, from 9.5 to 8.0, following the elution of strontium(II), resulted in both calcium(II) and magnesium(II) being eluted from the column as separated and relatively well defined peaks. A typical chromatogram showing the separation of 1 m*M* barium(II) and strontium(II) from 2 m*M* calcium(II) and magnesium(II) using the above step gradient is shown in Fig. 6. The change in pH of the mobile phase resulted in a drop in the background absorbance, which can be seen in the chromatogram. At pH 8.0 the sensitivity of the method for calcium(II) and magnesium(II) was reduced significantly, although this could be an advantage in the analysis of barium(II) and strontium(II) in samples

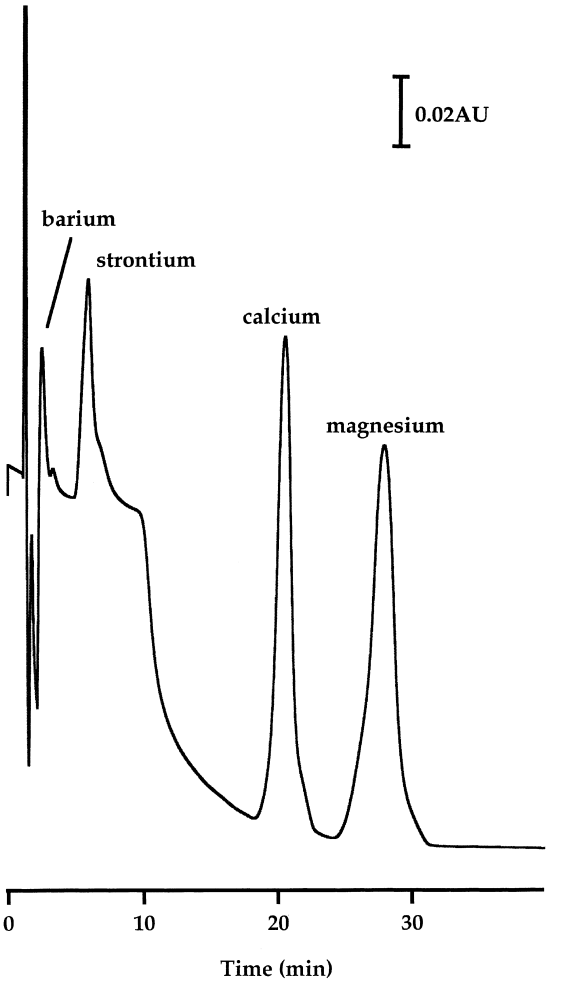

Fig. 6. Separation of barium(II), strontium(II), calcium(II) and magnesium(II) using a pH step gradient. Conditions: 100-μl injection of 1 m*M* barium(II) and strontium(II), and 2 m*M* calcium(II) and magnesium(II). Mobile phase: 0.5 *M* KNO_3, 0.2 m*M* OCPC, 20 m*M* borate (pH 9.5 stepped to 8.0 after 10 min). Flow-rate: 1.5 ml min^{-1}.

containing high levels of calcium(II) and magnesium(II). As can be seen in Fig. 6, the use of a pH step gradient resulted in calcium(II) being eluted before magnesium(II). The large reduction in pH greatly diminished the conditional stability constants in both the stationary and mobile phase, and unlike the separation of magnesium(II) and calcium(II) shown in Fig. 4, the effective concentration of ligand in the mobile phase was too low to cause a reversal in the retention order. Re-equilibration of the column to pH 9.5 following the step gradient was complete in under 5 min.

3.4. Analytical performance characteristics

The analytical performance characteristics of the above technique for the determination of barium(II) and strontium(II) were investigated. Linearity was determined under the conditions used in Fig. 5. For barium(II), over the range 0.01–1.0 m*M* ($n=6$), a correlation coefficient of $r^2=0.9991$ was achieved. Over the same concentration range of strontium(II) ($n=6$), this value was $r^2=0.9951$. Reproducibility of the method was investigated with five replicate injections of a 0.5 m*M* mixed metal standard. Using peak area, values of 5.1% R.S.D. for barium(II) and 6.6% R.S.D. for strontium(II) were obtained. The approximate detection limit for both barium(II) and strontium(II) under the above conditions, using a 100-μl sample loop, was determined to be 10 μ*M*. It was possible to further reduce this value by increasing the pH of the mobile phase, although this led to a slight increase in retention times. The tolerance of the system to ionic strength was investigated with the injection of standards prepared in up to 5 *M* sodium chloride. At this concentration the peak for strontium(II) showed some additional broadening but remained well separated from barium(II) and both were removed from the solvent front.

3.5. Strontium in an Antarctic brine sample

Few real applications have been published on metal ion determinations in brines using single column chelation ion chromatography. The majority of the present literature involves the use of a chelating column in series with an ion-exchange column to remove the sample matrix before separating the metals of interest. This approach has been generally applied to the determination of transition metals, selected heavy metals and the lanthanides [18–22]. For the determination of alkaline earth metals in brines this approach has also been shown [23], as has the use of ion chromatography using high capacity cation-exchange columns [although for calcium(II) and magnesium(II) only] [24]. The main advantages of the method proposed here, are firstly the simplicity of the instrumentation required, name-

ly a single column and a single HPLC pump, and secondly, the reversed elution order obtained for the alkaline earth metals, when compared to ion-exchange methods. With this in mind, a brief application of the above technique was carried out, with the determination of strontium(II) in an Antarctic lake brine sample (Deep Lake, 20-m depth). The sample matrix contained 3.13 *M* sodium, 0.101 *M* potassium, 0.573 *M* magnesium(II) and 0.054 *M* calcium(II). The sample required a dilution factor of 1:15 (w/w) with deionised water due to overloading of the column by the very high levels of calcium(II) and magnesium(II), causing strontium(II) to be eluted close to the solvent front. Fig. 7 shows a chromatogram obtained from an injection of the diluted Antarctic brine sample. The concentration of barium(II) was below the detection limit and a large negative peak was eluted close to the solvent front. Injection of standards prepared in artificial sodium chloride brines did not show this disturbance, but as the dip was well removed from the peak for strontium(II), it was not further investigated. A value of 0.86 m*M* strontium(II) (R.S.D. of 0.06 m*M*) was obtained, whilst analysis of the same sample using atomic absorption spectroscopy gave a value of 0.98 m*M*.

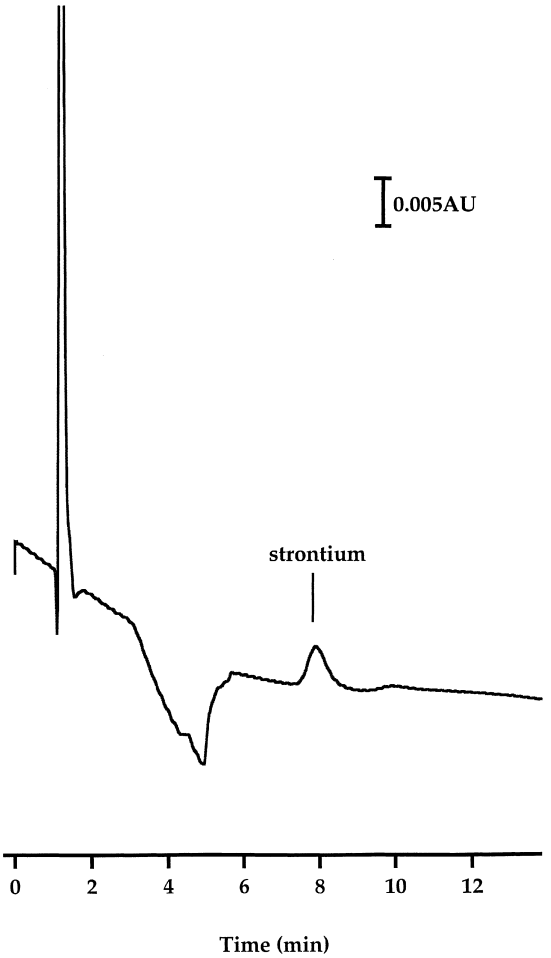

Fig. 7. Determination of strontium(II) in an Antarctic brine sample (Deep Lake, 20 m). Conditions: 100-μl injection. Mobile phase: as for Fig. 5. Flow-rate: 1.5 ml min^{-1}.

4. Conclusions

Chelating ion chromatography of metal ions is a technique which offers differing selectivities and increased sensitivity compared to simple ion-exchange chromatography. The technique can be applied to the analysis of samples of high ionic strength, and can be used for the determination of selected metals in the presence of very large excesses of matrix components. Use of a dynamically coated substrate is a simpler alternative to the chemical bonding of chelating groups to the supporting material. By using a colour-forming ligand to coat the substrate, and also including it in the mobile phase, metal ions can be separated and also detected without the need for postcolumn reaction.

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