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Determination of barium and strontium by capillary zone electrophoresis using an electrolyte containing sulfonazo III

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Abstract

Capillary zone electrophoresis has been applied to the separation and detection of barium(II) and strontium(II) in the presence of excess levels of calcium and magnesium using an electrolyte containing sulfonazo III. The analytes were separated and detected as their anionic chelates, using direct spectrophotometric detection at 654 nm. Calibration curves for areas were linear in the range of 1.25 to 80 μM with correlation coefficients $r = 0.9996$ for both barium(II) and strontium(II). Precision (as %R.S.D.) was 0.8% and 0.4% for migration times and 1.2% and 2.9% for peak areas for barium(II) and strontium(II), respectively. Recovery was determined by the standard addition method and found to be 108% for barium(II) and 104% for strontium(II). Limits of detection (based on a signal-to-noise ratio of 2 and a noise level of 2.8 10^{-5} AU) of 0.35 μM (0.048 mg dm^{-3}) for barium(II) and 0.47 μM (0.041 mg dm^{-3}) for strontium(II) were possible in up to 100 000-fold excess levels of Ca^{2+} and Mg^{2+}. The method was applied to the determination of barium(II) and strontium(II) in a mineral water sample.

Keywords: Electrolytes; Barium; Strontium; Sulfonazo III

1. Introduction

There is increasing interest in the determination of trace levels of barium(II) and strontium(II) in certain environmental and biological samples. Due to a number of cardiovascular problems associated with the ingestion of soluble barium(II) salts, new safe limits are being established for the level of barium(II) allowed in potable water supplies, including a proposed EC limit of 0.7 mg dm^{-3} placed upon the level of barium(II) allowed in bottled mineral waters [1].

Ion chromatography (IC) has been applied to the determination of all the alkaline earth metals in a

wide variety of samples [2]. However, due to the retention order of the alkaline earth metals obtained when using IC, the determination of trace levels of barium(II) and strontium(II) becomes difficult when the samples contain higher levels of both calcium(II) and magnesium(II). These are eluted prior to barium(II) and strontium(II) and can cause total or partial masking of the analyte peaks. Dilution of the sample does not solve this problem when only trace levels of the analytes are present. One solution to this problem was reported by Jones et al. [3] who used a novel chelating exchange resin which allowed the elution of barium(II) and strontium(II) prior to calcium(II) and magnesium(II). This method was applied to the analysis of barium(II) and strontium(II) in mineral waters and gave detection limits

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of 0.03 mg dm^{-3} for both metals in samples containing up to 1000 times higher concentrations of calcium(II) and magnesium(II). However, the analysis time was over 25 min due to the need to elute the large concentration of calcium(II) and magnesium(II) from the analytical column prior to the next analysis.

The use of atomic spectroscopy for the determination of barium(II) and strontium(II) in a variety of sample types has been reported by a number of workers [4–6]. The presence of calcium(II) and magnesium(II) at much higher concentrations has been shown to cause severe interferences when using atomic absorption spectroscopy (AAS) [4] and also when using atomic emission spectroscopy [5]. Fagioli et al. [6] has reported the use of graphite furnace atomic absorption spectrometry for the determination of barium(II) in bottled drinking waters. No interferences were reported from high levels of calcium(II) and the technique was extremely sensitive.

A possible alternative to the above methods is to use capillary zone electrophoresis (CZE), which is rapidly becoming an accepted analytical technique for the analysis of small inorganic ions [7]. In HPLC, a variety of metallochromic ligands have been used both as post-column reagents and as constituents of the mobile phase [2], resulting in sensitive visible detection methods for a large number of metal ions. This approach is now being applied to CZE, with several workers using colour-forming metallochromic ligands contained within the electrolyte to facilitate the visible detection of metal ions [8–19] and has permitted both improved sensitivity and selectivity in the separation and detection of certain metal ions by CZE.

Sulfonazo III (SAIII), a derivative of arsenazo III in which the $-AsO_3H_2$ groups have been replaced by $-SO_3H$, is one of the most sensitive metallochromic reagents for barium(II), having an absorption maximum at 638 nm with $\varepsilon = 1.73 \cdot 10^4$ L mol^{-1} cm^{-1} [20]. Previously, SAIII has been mainly used as an indicator for sulfate precipitation titrations using barium chloride [20]. However, the use of SAIII as a colour-forming ligand for the direct spectrophotometric analysis of barium(II) and strontium(II) was shown by Manna et al. [21] to be impossible due to interferences caused by calcium(II) and mag-

nesium(II), even in a relatively simple sample matrix such as natural water. To eliminate these possible interferences it was necessary to employ a separation technique prior to the detection of these analytes.

In this paper we describe a study into the use of sulfonazo III, contained within the electrolyte, as a sensitive and selective colour-forming ligand for the detection of barium(II) and strontium(II) in the presence of highly excess levels of calcium(II) and magnesium(II). The analytes are separated and detected as their anionic chelates, allowing visible detection at 654 nm. The method is applied to the determination of barium(II) and strontium(II) in a mineral water sample, with the results being compared to those obtained using inductively coupled plasma mass spectrometry (ICP-MS).

2. Experimental

2.1. Reagents

Sulfonazo III [3,6-bis(2-sulfophenylazo)-4,5-dihydroxy-2,7-naphthalenedisulfonic acid, SAIII], Na$_x$ salt, was purchased from Aldrich (Milwaukee, WI, USA). The sodium content was determined by flame AAS, the result showed that the dye was supplied as the disodium salt. Tris(hydroxymethyl)-aminomethane (Tris) was purchased from Aldrich as "99.8+%", hexane sulfonic acid, sodium salt, (HSNa) was purchased from Sigma (St. Louis, MO, USA) at 98% purity and 2-morpholino-ethanesulfonic acid (MES) monohydrate was purchased from Fluka (Buchs, Switzerland) as ">99%". All other chemicals were of analytical grade. Deionised water was obtained using a Millipore (Bedford, MA, USA) Milli-Q water purification apparatus.

A MES–Tris background electrolyte (BGE) containing SAIII was prepared by the appropriate dilution of MES to give a final concentration of 20 mM and of Tris to give a final concentration of 10 mM, with SAIII added to give a concentration of 0.30 mM. The pH of the BGE was 6.2. Before use the BGE was degassed by vacuum and filtered with a MILLEX-HA 0.45-μm disc filter (Millipore). Unless stated otherwise, 2% (v/v) of a 1 M solution of

HSNa was added to each standard or sample solution, to give a final HSNa concentration of 20 mM.

The mineral water sample used for these experiments (Hartz Mineral Water, Hobart, Australia) contained was specified to contain the following approximate concentrations of ions: 70 mg dm^{-3} Cl^{-}, 26 mg dm^{-3} SO$_4^{2-}$, 86 mg dm^{-3} Ca^{2+}, 9 mg dm^{-3} Mg^{2+}, 35 mg dm^{-3} Na^{+} and 0.2 mg dm^{-3} K^{+}.

2.2. Instrumentation

A Polymicro Technologies (Phoenix, AZ, USA) fused-silica capillary, 0.60 m length (0.52 m to detector)×75 μm I.D., was used for these experiments. This capillary was initially flushed with 1 M NaOH for 1 h followed by flushing with the BGE. The instrument used was a Quanta 4000 (Waters, Milford, MA, USA) interfaced to a Maxima 820 data station (Waters). Instead of the original mercury lamp, a light-emitting diode (LED) using a 654 nm ultra-bright LED (RS 564-015, RS Components, VIC, Australia) was fitted to the instrument, as described elsewhere [22].

2.3. Procedures

Injection was performed hydrostatically by elevating the sample at 100 mm for 20 s at the anodic side of the capillary. At the cathodic (detector) side a buffer reservoir of 4 ml was used. Instead of the standard 20 ml buffer reservoir at the anodic side, a plastic sample vial of approximately 0.6 ml was installed inside of the standard 20 ml container. The running voltage was +30 kV.

The EOF was determined from a mesityloxide injection (0.1% solution in BGE) using detection at 254 nm and/or from the peak or dip of water injection with the detection at 654 nm (both methods generally giving the same values). The baseline noise was determined by statistical evaluation of the digital data, as described previously [23]. Two 0.2 min segments of the baseline adjacent to the barium(II) and strontium(II) peaks were examined and the noise expressed as five times the measured standard deviation of the examined data.

3. Results and discussion

In the system under consideration the metal ion is migrating as an equilibrium mixture of the free metal ion and its SAIII complex. Throughout this paper we will adopt the nomenclature of referring to the observed peaks as barium(II) and strontium(II), whilst recognising that varying proportions of the metal–SAIII complex are also present.

3.1. Selection of the ligand

A number of metallochromic ligands were investigated as on-capillary chelating agents. Several properties of the BGE, such as pH, concentration of metallochromic ligand, buffer type and buffer concentration, were found to exert major influences on the system. Two of the metallochromic ligands investigated, arsenazo I and o-cresolphtalein complexone (used in an alkaline BGE, pH approx. 8.5 to 11.5), offered comparable detection sensitivity for barium(II) and strontium(II) to that obtained using SAIII in weakly acidic or neutral BGEs (pH approx. 3 to 7), but gave asymmetrical and broad peaks. The ligand arsenazo III, which has been used both as a post-column reagent and mobile phase component in IC determinations of alkaline earth metal ions [2], also offered favourable detection properties (colour contrast of reaction) in neutral BGEs, but again resulted in poor peak shapes for the metals investigated. Although the reasons for the poor shapes were not investigated in detail here, such effects are commonly caused in CZE by either solute–wall interactions or by relatively slow kinetics of the equilibria involved. The best results were obtained with the SAIII as the ligand and this was used for the remainder of this study.

3.2. Description of the chosen separation system

There are two peaks that can be observed in all the electropherograms, even when only water is injected. These are the peaks due to electroosmotic flow (EOF, for measurement of EOF see Section 2.3) and a system peak S (see Fig. 1). Even though the nature of the system peak S is not fully clear, it migrated with a positive electrophoretic mobility ranging (from peak start to end) from +60·10^{-9} m^{2} V^{-1} s^{-1}
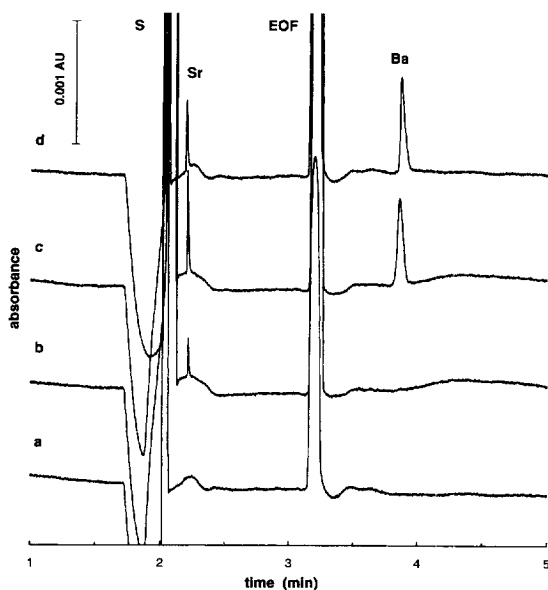
Fig. 1. Electropherogram of (a) blank, (b) sample of mineral water, (c) sample spiked with 5 μM strontium(II) and barium(II), and (d) standard of 5 μM strontium(II) and barium(II). All injected solutions contained an addition of 20 mM HSNa. BGE: 0.3 mM SAIII in 20 mM MES and 10 mM Tris (pH 6.2), separation voltage: +30 kV (15 μA), temperature: 25°C, detection: LED 654 nm, injection: hydrostatic (30 s), for other conditions see Section 2. S=system peak.

to $+27 \cdot 10^{-9}$ m^2 V^{-1} s^{-1} which indicates that its nature could be associated with the cationic component(s) of the BGE. Another indication that peak S is a system peak is that the calculated apparent mobilities (from peak start to end) range from $+116 \cdot 10^{-9}$ m^2 V^{-1} s^{-1} to $+83 \cdot 10^{-9}$ m^2 V^{-1} s^{-1}, which would be too high for an EOF at this pH.

SAIII has $pK_{a6}=2.8$ and $pK_{a7}=11.6$ [20], so that only small changes to the degree of dissociation of the ligand would be expected over the pH range 3.8–9.6. Experiments using various BGEs in acidic to neutral pH (pH approx. 3 to 7), showed that at SAIII concentrations in the range 0.1–0.6 mM, strontium(II) always migrated as a cation, indicating that over this pH range the majority of strontium(II) was present as the free metal ion, despite the fact that the degree of complexation with SAIII might have varied over this range. In the case of barium(II), the net charge ranged from positive to slightly negative with increasing SAIII concentration over the same range. The relatively low mobility of

barium(II) necessitated use of a CZE system with sufficient EOF to carry this analyte through the detection window, and in turn this requirement placed a limitation on the minimum pH of the BGE. At pH 6.2 and using 20 mM MES buffer, the migration time corresponding to electroosmosis was $t_{EOF}=3.1$ min (corresponds to $+56 \cdot 10^{-9}$ m^2 V^{-1} s^{-1}). The nature of different BGEs also had an effect on the system performance and several auxiliary complexing agents and buffers were evaluated. Best results in terms of selectivity, analysis time, peak shape, and reproducibility of results was obtained with a BGE composition of 0.3 mM SAIII, 20 mM MES and 10 mM Tris at pH 6.2. Electropherograms obtained under these conditions can be seen in Fig. 1 and show that the strontium(II) peak migrates ahead of the EOF, whilst the barium(II) peak migrates after the EOF due to its overall negative charge resulting from complexation with SAIII.

3.3. Detection system

Direct photometric detection was employed using a low noise LED-based detector, the design of which has been described earlier [22]. Two different LEDs were compared; one with maximum at 647 nm (close to the spectral maximum of the SAIII complexes) and another at 654 nm. The use of the 647 nm LED resulted in a 15% higher sensitivity compared to 654 nm LED. However, the noise of the 654 nm LED was two times lower than the 647 nm LED, resulting in a better signal-to-noise ratio. The 654 nm LED was therefore used for the remainder of this study.

3.4. Injection and sample stacking

Hydrostatic injection at 10 cm and electromigration injection at 5 kV were compared for the analysis of a mineral water sample and no significant sensitivity advantage using the electromigration injection method was obtained. Hydrostatic injection was therefore used throughout the study.

As sample stacking can result in significant decreases in limits of detection (LOD), stacking procedures were investigated. When injecting standard solutions of barium(II) and strontium(II) prepared in a BGE containing SAIII, hydrostatic injection for
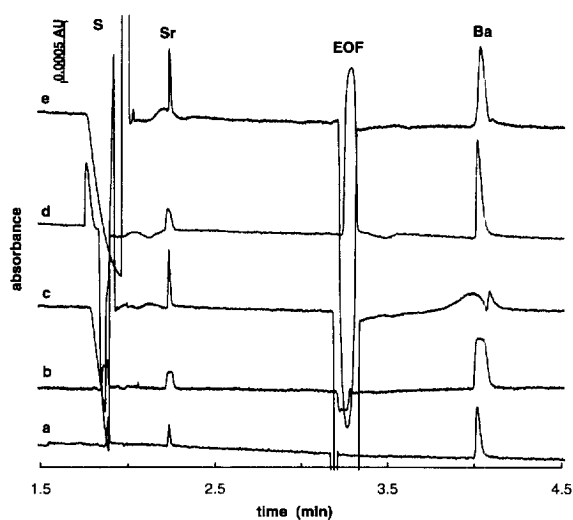
Fig. 2. Electropherogram of a standard of 5 µM strontium(II) and barium(II) dissolved in (a) BGE, 10 s injection; (b) BGE, 30 s injection; (c) 4 mM MES–2 mM Tris buffer (pH 6.2), 30 s injection; (d) BGE without SAIII, 30 s injection; and (e) 20 mM MES–10 mM NaOH buffer, 30 s injection. Other conditions as in Fig. 1.

periods longer than approximately 10 s resulted in square-shaped peaks for both analytes, indicating that no stacking had occurred (see Fig. 2a,b). Interestingly, injecting the sample from a diluted BGE, which is a common practice in CZE to achieve sample stacking, led to increased broadening of the barium(II) peak (see Fig. 2c). Another possible way of achieving stacking of metal ions is to use on-capillary complexation, i.e., injecting the sample from a solution of similar composition to the BGE, but without the ligand [13,24]. This approach is based upon the opposite mobilities of the injected metal cations and their anionic complexes formed after reaction with the ligand in the BGE. When investigated, this resulted in stacking for barium(II) but the strontium(II) peak remained broad (see Fig. 2d), which may be explained by the low degree of complexation of the strontium(II). However, both barium(II) and strontium(II) were stacked when the standard was injected from a MES–Na buffer instead of the MES–Tris buffer, as shown in Fig. 2e, or when the sample contained 10 mM of a sodium salt. This behaviour could be explained by the iso-tachophoretic stacking effect of the analyte cations in

the presence of excess levels of the much less mobile sodium cations [25,26]. Replacement of sodium ion in the sample with a more mobile cation, such as potassium (added as 10 mM KCl) resulted in a very broad barium(II) peak.

Using sodium as the cationic component of the injected solution, the influence on stacking of some common counter-ions was studied for standard solutions of barium(II) and strontium(II). The counterions investigated were MES, chloride, benzenesufonate and hexanesulfonate, the last of which gave the best peak shape for barium(II) (electropherograms obtained for MES and hexanesulfonate as counterions in the injected solution are shown in Fig. 2e Fig. 1d, respectively). Using these conditions the peak height to peak area ratio was investigated by varying the hydrostatic injection time from 10 s to 80 s. Broadening of the barium(II) peak was observed for injections over 30 s and this was therefore used as the maximum injection time in the final method.

3.5. Tolerance to Ca^{2+}, Mg^{2+} and ionic strength as NaCl

The tolerance to possible interfering ions such as Ca^{2+}, Mg^{2+} and NaCl in the sample was investigated. No identifiable effects on the determination of barium(II) and strontium(II) were observed at concentrations up to 40 mM Ca^{2+}, 40 mM Mg^{2+} and 50 mM NaCl. However, at these concentrations of the above metals the barium(II) peak was somewhat broadened and the strontium(II) peak migrated closer to the large frontal peak. Nevertheless, the highly selective nature of the SAIII ligand made it possible to separate and detect both barium(II) and strontium(II) in matrices containing up to a 100 000-fold excess of both calcium(II) and magnesium(II). This is illustrated in Fig. 3 which shows the separation of 5 mM barium(II) and strontium(II) from 40 mM Ca^{2+} in under 5 min. Since both barium(II) and strontium(II) are often found in the presence of such excess concentrations of these common metals in many sample types, the above technique has obvious advantages for their selective determination.

3.6. Analytical performance characteristics

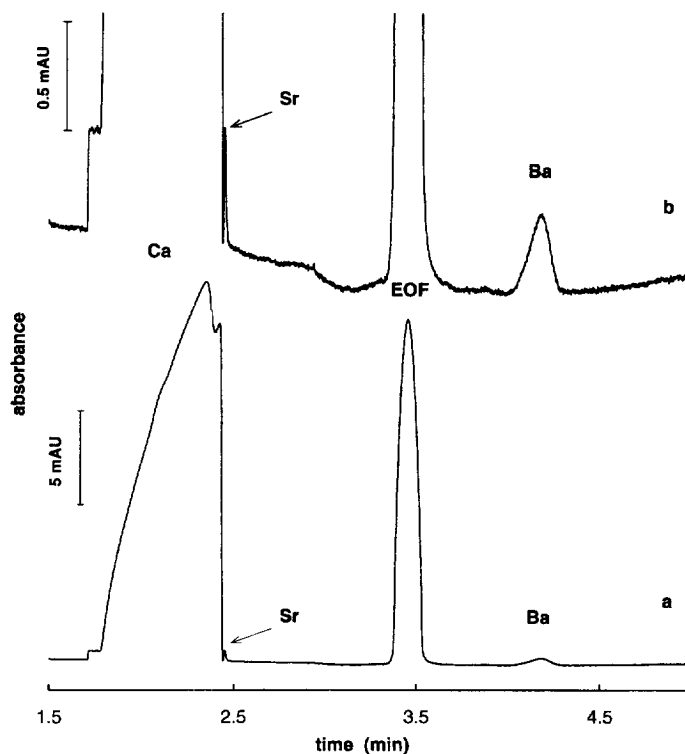Analytical performance characteristics for the

Fig. 3. Electropherogram of (a) a standard of 5 μM strontium(II) and barium(II) in 40 mM Ca(NO$_3$)$_2$ and (b) the same electropherogram scaled to show the barium(II) and strontium(II) peaks. The injected solution contained an addition of 20 mM HSNa. BGE: 0.3 mM SAIII in 20 mM MES and 10 mM Tris (pH 6.2), separation voltage: +30 kV (15 μA), temperature: 25°C, detection: LED 654 nm, injection: hydrostatic (30 s), for other conditions see Section 2.

method are summarised in Table 1. Linearity was determined using standard additions ($n=8$) to a mineral water sample over the concentration range likely to be found in commercial bottled mineral waters, the calibration graphs went through the origin. The reproducibility of both migration times and peak areas, determined as percentage relative standard deviation (% R.S.D.) for 9 consecutive

injections of the sample spiked with 5 μM barium(II) and strontium(II) (as in Fig. 1c) was particularly good for a CZE method, being less than 3% in each case. Recovery values determined by a standard addition method (as in Fig. 1c,d) were satisfactory for both metals. The favourable noise characteristics of the LED-based absorbance detector [22] meant the observed noise for the electropherog-

Table 1
Analytical performance characteristics

Analyte	Linearity range	Migration time R.S.D. (%)	Area R.S.D. (%)	Recovery st. addition (%)	LOD
Barium(II)	0.17 to 11.0 mg dm^{-3} (1.25 to 80 μM) $r=0.9996$	0.8	2.9	108	0.048 mg dm^{-3} (0.35 μM)
Strontium(II)	0.11 to 7.0 mg dm^{-3} (1.25 to 80 μM) $r=0.9996$	0.4	1.2	104	0.041 mg dm^{-3} (0.47 μM)

Conditions as in Fig. 1.

Table 2
Analysis results of the bottled mineral water by CE and ICP-MS

Analyte	CZE	ICP-MS
Barium(II)	<0.048 mg dm^{-3} (0.35 μM)	0.015 mg dm^{-3} (0.11 μM)
Strontium(II)	0.19 mg dm^{-3} (2.17 μM)	0.17 mg dm^{-3} (1.95 μM)

Conditions as in Fig. 1.

rams in Fig. 1 was in the range of $2.3 \cdot 10^{-5}$–$2.8 \cdot 10^{-5}$ absorbance units (the higher value was used for the LOD calculations). As the electropherograms exhibited generally only regular, short term noise, the LODs were calculated using a signal-to-noise ratio of 2. Although barium(II) is more strongly complexed and the resultant complex has a higher absorptivity than strontium(II), LODs for both metals were similar due to the fact that the strontium(II) peak is narrower than the barium(II) peak (226 000 and 64 000 theoretical plates, respectively).

3.7. Analysis of a mineral water sample

Results for the determination of barium(II) and strontium(II) in a mineral water sample obtained using the proposed method and by ICP-MS are shown in Table 2. A good agreement between the two methods of analysis was obtained for strontium(II), the level of barium(II) in the particular sample was less than the LOD for the proposed method. However, determination of barium(II) by CE and ICP-MS at the 5 μM level showed a variation of less than 2% between the two techniques. It can also be noted that the proposed method offers sufficient sensitivity to monitor barium(II) and strontium(II) at levels significantly less than the maximum permissible amounts of these ions in mineral water.

4. Conclusions

The use of metallochromic ligands within the electrolyte provides a sensitive method for the detection of barium(II) and strontium(II) by CZE and offers the advantage of being highly selective, allowing the detection of trace levels of the analytes

in matrices containing non-reacting metals in high excess. These principles have been illustrated successfully with the use of sufonazo III for the determination of barium(II) and strontium(II) in excess levels of Na^+, Ca^{2+} and Mg^{2+} and the proposed method was applied to the analysis of a mineral water sample. However, such systems are based upon complex solution equilibria which make the separation system complicated and can make it difficult to control peak shapes. Considerably more work in this area is required if this mode of detection is to become applied routinely.

Acknowledgments

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