Fast direct determination of strontium in seawater using high-performance chelation ion chromatography

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ABSTRACT

A new method for the fast direct chromatographic determination of strontium in seawater has been developed. Two coupled silica monolithic columns (total dimensions 200×4.6 mm I.D.) chemically modified with iminodiacetic acid (IDA) functional groups were used for baseline separation of all alkaline-earth metals in seawater in less than 4 min at flow rate of 4.0 mL min^{-1}, using an eluent containing 0.25 M sodium chloride and 2 mM glycolic acid, pH 5.11. Several colour-forming ligands, including o-cresolphthalein complexone (o-CPC), methylthymol blue (MTB), xylenol orange (XO), 4-(2-pyridylazo)resorcinol (PAR) and ZnEDTA–PAR were evaluated as reagents for post-column reaction (PCR) based spectrophotometric detection. Sensitivity of the developed method for strontium was at the low mg L^{-1} level in undiluted seawater samples, in the presence of excessive amounts of calcium and magnesium, using post-column reaction detection with o-CPC at 570 nm. The method allowed quantitative determination of strontium in seawater samples (LOD 3.23 ± 0.15 mg L^{-1}, $s_r = 4.6\%$), with direct injection of just 4 μL of seawater onto the chromatographic column.

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1. Introduction

Determination of alkaline-earth metal cations in seawater is of great importance for marine science and various environmental studies. For example, there is considerable demand for methods enabling the monitoring of Mg^{2+}, Ca^{2+} and Sr^{2+} ion concentrations in seawater as a part of coral skeleton growth and calcification studies [1] following the effects of seawater acidity with variations in ocean temperatures. However, seawater has been recognised as one of the most difficult matrices for direct analysis of metal ions due to its high salinity and incommensurable levels of analyte and matrix ions. At present, it is a routine practice in marine science and, especially in research on coral skeleton growth, to determine changes in Mg^{2+}/Ca^{2+} [2] and Sr^{2+}/Ca^{2+} concentration ratios [3] rather than to measure accurately the absolute concentrations of these elements. Having said this, it is clear that determination of the exact concentration levels of these elements in seawater is an important goal, providing accurate data for the correct interpretation and modelling of numerous biochemical processes in seawater.

The number of suitable methods for the direct (undiluted) determination of strontium in seawater is limited, most providing rather low precision and accuracy, as a result of the complexity and high ionic strength of seawater as a sample matrix. The majority of current methods, including atomic absorption spectroscopy (AAS) [4], flame

photometry [5], and neutron activation analysis (NAA) [6] were developed in the later half of last century, and have changed little since that time. The suitability of these methods for the analysis of large numbers of seawater samples is also questionable, due to issues related to salt build-up and precipitation [4,7,8]. For example, the officially recommended AAS protocol "ASTM D3352-08a Standard Test Method for Determination of Strontium Ion in Brackish Water, Seawater, and Brines", covers the concentration range of 5–2100 mg L^{-1} strontium [9]. According to this method, the procedure requires some sample preparation including the dilution of seawater samples and the addition of the releasing agent lanthanum, to minimise the chemical interferences from the other components of seawater, such as sulphate and phosphate.

It should be noted that suitability of inductively coupled plasma mass spectrometry (ICP-MS) to the determination of strontium in seawater is also limited, in the first instance by a strong interference of calcium dimer ions, and secondly by analysis cost. Strontium has four naturally occurring stable isotopes, ^{84}Sr, ^{86}Sr, ^{87}Sr, and ^{88}Sr, with abundances of 0.56, 9.87, 7.04, and 82.53 at.%, respectively, and calcium in nature is presented by six isotopes, ^{40}Ca, ^{42}Ca, ^{43}Ca, ^{44}Ca, ^{46}Ca, and ^{48}Ca, with abundances of 96.95, 0.65, 0.14, 2.086, 0.004, and 0.19 at.%, respectively. So, the signal from Ca-dimer ions ($^{40}Ca^{44}Ca^+$, $^{42}Ca_2^+$, $^{40}Ca^{46}Ca^+$, $^{42}Ca^{44}Ca^+$, $^{43}Ca_2^+$, $^{40}Ca^{48}Ca^+$, $^{42}Ca^{46}Ca^+$, $^{44}Ca_2^+$) results in isobaric interferences on Sr ($^{84}Sr^+$, $^{86}Sr^+$ and $^{88}Sr^+$) signals. The isobaric interference of molecular ions or Ca-argides ($^{36}Ar^{48}Ca^+$, $^{38}Ar^{46}Ca^+$, $^{40}Ar^{44}Ca^+$, $^{38}Ar^{48}Ca^+$, $^{40}Ar^{46}Ca^+$, $^{40}Ar^{48}Ca^+$) should be also taken into consideration for the ICP-MS determination of strontium

and calcium [10]. Rubidium has two naturally occurring stable isotopes [85]Rb and [87]Rb, with abundances of 72.17 and 27.83 at.%, respectively. The concentration of Rb in seawater is about 0.12 mg L^{-1} and there is isobaric interference of the signal from [87]Rb^{+} on the [87]Sr signal. Interference correction both in multi-collector ICP-MS and in thermal ionisation mass spectrometry (TI-MS) modes requires chromatographic separation of strontium from calcium and rubidium. For this purpose, the application of cation-exchange chromatography or extraction chromatography with a resin coated by solution of (4,4′(5′)-di-t-butylcyclohexano-18-crown-6) in octanol-1 (Sr-resin, TrisKem International, France) is required [11,12]. However, the complete ion-exchange separation of strontium from other metals is not possible without high efficiency ion chromatographic separation after dilution of seawater samples (see discussion later in Introduction). Only 60% recovery of strontium was found for the use of extraction chromatography with Sr-resin [13].

Unsurprisingly, in many cases using the above instrumental methods, time consuming sample preparation or substantial dilution of seawater samples is recommended to improve analytical performance.

Ideally, accurate determination of mg L^{-1} concentration levels of strontium in seawater benefits from its separation from other alkaline earth metals, which are present at much higher concentration levels (the ratio of Sr^{2+}/Mg^{2+} is ~1/140 and Sr^{2+}/Ca^{2+} is ~1/65), and also from other major ionic components, most notably the ~0.52 mol L^{-1} concentration of NaCl. Ion chromatography (IC) has long been an obvious choice for this task [14]. However, ion-exchange separation of brines and seawater, which contain excessive amounts of alkali to alkaline-earth metals, using common ion-exchange resins, is subject to problems of serious overloading of ion-exchange sites, large chromatographic peaks of matrix ions masking peaks of minor components, and serious baseline disturbances. Additionally, the use of conductivity detection in traditional IC means analysis becomes nearly impossible without substantial sample dilution (approx. 130–500 times), which can lead to serious errors in final quantitation [15–19]. The alternative method is spectrophotometric detection. However, in this case post-column reaction with colour forming reagents is required [20].

Other analytical separation techniques, which have been reported for the determination of strontium in seawater, also have serious limitations, not least complexity and robustness, such as those reported combining capillary isotachophoresis (ITP) with cationic ion chromatography [21] or with capillary zone electrophoresis (CZE) [22]. Indeed, given the above complexity of the sample and analysis, remarkably to-date no chromatographic or electrophoretic methods for the direct (undiluted) determination of strontium in seawater have been reported.

In the last decade high-performance chelation ion chromatography (HPCIC) has received recognition as a simple and reliable method for the analysis of complex samples, including brines, seawater, and various industrial high ionic strength solutions [23]. The separation mechanism in HPCIC is based on the formation of labile complexes of metal ions with immobilised chelating ion-exchange groups, so the retention of metal ions (commonly alkaline earth and transition metal ions) is significantly less sensitive to the ionic strength of the samples, which translates to minimal sample pretreatment, and the possibility of the analysis of filtered undiluted seawater samples by direct injection onto the chromatographic column.

HPCIC has been used in the past for the separation and determination of strontium in saline samples, namely Antarctic lake samples [24]. The reported method used a Hamilton PRP-1 (150 × 4.1 mm I.D.) column packed with 5 micron neutral poly(styrene-divinylbenzene) (PS-DVB) spherical particles, which had been dynamically coated with the chelating ligand o-cresolphthalein complexone (o-CPC). However, prior to analysis the sample was diluted 1:15 with deionised water to minimise the effects of excessive amounts of Mg^{2+}. Also, to stabilise the column coating o-CPC was added to the mobile phase, complicating the system, which also required gradient elution to obtain the

separation, resulting in substantial disturbances of the baseline. Additionally, the separation efficiency of the coated column was unsatisfactory, resulting in poor peak shapes even under gradient elution conditions.

Recently, the chromatographic performance of columns, and typical peak efficiencies obtained using HPCIC have substantially improved. Monolithic columns have shown particular potential for use in HPCIC, most notably for fast HPCIC based methods, as a result of their lower operational backpressures and thus the possibility of operation at higher linear velocities whilst maintaining good peak efficiencies, as compared to particles packed beds [25]. To-date publications on the application of monolithic columns in HPCIC have utilised commercially available silica-based monoliths, additionally modified with iminodiacetic acid IDA or lysine functional groups [26–28].

The aim of the present work was to develop a fast, accurate and robust chromatographic method for the direct determination of strontium in undiluted seawater samples. The following paper describes a developed HPCIC method based upon the use of IDA bonded monolithic silica columns, which provide the efficiency and selectivity required for the separation and quantitation of the strontium peak. The effects of mobile phase composition and flow rate on the separation were investigated, as were various post-column reagents, which were compared to provide optimum responses to alkaline-earth metal cations, and particularly to deliver sensitive detection of strontium.

2. Experimental

2.1. Chemicals and reagents

The reagents used for column modification, namely 3-glycidoxy-propyltrimethoxysilane and iminodiacetic acid (IDA), were both purchased from Sigma-Aldrich (Gillingham, UK or Sydney, Australia). Methylthymol blue (MTB) or 3,3′-bis[N,N-di(carboxymethyl)aminomethyl]thymol-sulfonephthalein (99% dye content), and o-cresolphthalein complexone (o-CPC) or 3,3′-bis[N,N-di(carboxymethyl)-aminomethyl]-o-cresolphthalein (99% dye content), were obtained from Fluka (Buchs, Switzerland). Xylenol orange (XO) or 3,3′-bis [N,N-bis(carboxymethyl)-aminomethyl]-o-cresolsulfonephthalein tetrasodium salt (90% dye content), and 4-(2-pyridylazo) resorcinol (PAR) (96% dye content) were obtained from Sigma-Aldrich (Sydney, Australia). All chelating dyes were used without further purification.

Hydrochloric acid, nitric acid, acetic acid, ammonia, glycolic acid, sodium hydroxide, and sodium chloride were supplied by Sigma-Aldrich (Sydney, Australia). Boric acid and Spectrosol atomic absorption standard solutions of Ca^{2+}, Sr^{2+}, Mg^{2+}, Ba^{2+}, Mn^{2+}, Co^{2+}, Cd^{2+} and Zn^{2+}, with concentrations of 1.00 g L^{-1} were purchased from BDH Chemicals (Poole, UK). An International Association for the Physical Sciences of the Oceans (IAPSO) normal standard seawater sample, batch P149 (5/10/2007), salinity 34.994 was supplied by OSIL (Havant, UK). Deionised water was supplied from a Milli-Q system (Millipore, Bedford, USA).

2.2. Instrumentation

For the chromatographic studies and sample analysis two chromatographic systems were used. The first was a Metrohm ion chromatography system, comprising model 844 compact IC with built in peristaltic reagent pump and post-column reactor, 830 IC interface (Metrohm, Herisau, Switzerland) and a Shimadzu UV/Vis detector, model SPD-10AV (Shimadzu, Kyoto, Japan). ICNet 2.3 SR4 software (Metrohm, Herisau, Switzerland) was used for data acquisition and processing of chromatograms. The second chromatographic system used was based upon a Waters 2695 separation module, a dual wavelength absorbance detector model 2487 (Waters, Milford, MA, USA), and an Eldex (Eldex Laboratories, Inc., Napa, CA, USA) PEEK-lined pump for delivery of the post-column reagents. Empower 3 software

(Waters, Milford, MA, USA) was used for data acquisition with this chromatographic system. Inductively coupled plasma mass spectrometry using an ELEMENT 2 ICP-MS system (Thermo Fisher Scientific, MA USA) was used as a reference method for the determination of Sr^{2+} in the seawater sample. A PEEK 100×4.0 mm I.D. column packed with 5 μm spherical IDA bonded Nucleosil-100 silica particles was purchased from JPP Chromatography (Plymouth, UK).

2.3. Modification of silica monoliths

Two PEEK housed bare monolithic silica columns (Onyx Monolithic Si) of 100×4.6 mm I.D. were purchased from Phenomenex (Cheshire, UK). According to the manufacturer, the silica monolith had a surface area of 300 m² g⁻¹ with a bimodal porous structure (porosity 81%), comprising macropores of 2 μm diameter, and mesopores of 13 nm diameter. The functionalisation of silica monoliths was performed as described by Sugrue et al. [27] with slight modification of the procedure. The structure of bonded layer in the column is shown in Fig. 1. To achieve this 60 mL of reagent mixture consisting of the reaction product of 3.8 mL (17.4 mmol) of 3-glycidoxypropyltrimethoxysilane and 8.0 g (60 mmol) of IDA was pumped in recycling mode through the column at a flow rate of 0.5 mL min⁻¹ for 8 h in the forward direction, and for 8 h in the reversed direction at 60 °C. The amounts of 3-glycidoxypropyltrimethoxysilane (Mw 236.34 and density 1.078 g cm⁻³) and IDA (Mw 133.1) used for the modification were estimated by calculation of the total amount of the reactive silanol groups in monolithic silica column as described in monograph [29]. The total volume of empty column of size 100×4.6 mm I.D. is about 1.66 cm³, so by using values of column porosity (81%) and density of glass 2.5 g cm⁻³ the mass of unmodified silica in the column is about $1.66 \times (1.0–0.81) \times 2.5 = 0.789$ g. According to the manufacturer, the monolithic silica porous rods have a specific surface area of 300 m² g⁻¹, so the total surface area of silica in the column is about 236 m². There are 4.6 silanols groups per 1 nm² at the surface of silica dioxides [30], and only half of them can react with bulky trimethoxy-anchor groups of silanes because of sterical hindrance, so the total number of reactive silanol groups in the monolithic column is $236 \times 2.3 \cdot 10^{18} = 5.42 \cdot 10^{20}$, which is equivalent to 902 μmol. So, approximately a 20-fold excess of 3-glycidoxypropyltrimethoxysilane and 60-fold excess of IDA relatively available silanols were used.

2.4. Preparation of post-column reagents

Where post-column reaction–spectrophotometric detection was required, and the evaluation of various post-column reagents for the detection of alkaline earth metal cations was performed, the following reagents were used: 0.4 mmol·L⁻¹ o-cresolphthalein complexone (o-CPC), 0.25 mol·L⁻¹ boric acid adjusted to pH 10.5 using NaOH; 0.05 mmol·L⁻¹ PAR, 0.4 mol·L⁻¹ NH₄OH, adjusted to pH 10.48 with nitric acid; 0.33 mmol·L⁻¹ xylenol orange in 0.2 mol·L⁻¹ ammonium acetate; 0.4 mmol·L⁻¹ MTB in 0.2 mol·L⁻¹ ammonium acetate; 0.2 mmol·L⁻¹ ZnEDTA and 0.12 mmol·L⁻¹ PAR in 2 mol·L⁻¹ NH₄OH, adjusted to pH 11.7.

3. Results and discussion

Most of the chelating columns for HPCIC have very low affinity to alkali metal cations, so there is no problem to separate strontium from them. However, it is more difficult to find the column with required selectivity and to optimise conditions providing baseline separation of strontium from excessive amounts of magnesium and calcium in seawater. Technically, in the case of seawater analysis the ideal separation selectivity should provide:

i) elution of the major alkaline earth metal in seawater, magnesium, in column dead volume or as close as possible to it to prevent column overloading, which deteriorates the chromatographic performance and limits the volume of injected sample and sensitivity of determination;
ii) a gap between peaks of magnesium and the second major component, calcium, which should be big enough for baseline resolution of chromatographic peak strontium from other peaks;
iii) separation of strontium and barium, which is not too significant, as the concentration of barium in seawater is approximately 390 times less than the concentration of strontium.

One of the most important advantages of HPCIC is the possibility of a priori evaluation of the separation selectivity of chelating ion-exchanger for metals of interest [23,31,32]. The retention factor of a metal ion in HPCIC is proportional to the stability of its complex with chelating groups at the surface as follows:

$$k = \beta_1^{M}[R]\varphi$$

where [R] is the concentration of chelating groups and φ — phase ratio of chromatographic column. So, the selectivity ratio for the separation of metals M_2 and M_1 is equal to the ratio of the corresponding stability constants

$$\alpha = \beta_1^{M2}/\beta_1^{M1}.$$

Clearly, in many cases the structure of immobilised ligand is too complex, so, in the first approximation, the used values from a database (e.g. [33]) of stability constants for close analogues of immobilised ligands can provide reasonable accuracy of prediction. Table 1 contains the values of stability constants for IDA and its derivatives, which correspond to the structure of grafted molecules for the most common IDA functionalised chelating ion-exchangers. As can be concluded from these data, the bonding chemistry may provide very different selectivity of adsorbents. Obviously, the data in Table 1 shows that N-benzyliminodiacetic acid (see its structure in Fig. 1), which is an analogue of the functional groups in chelating resin Chelex 100 and other chelating ion-exchangers for HPCIC [34], and methyliminodiacetic acid (analogue of chelating ion-exchanger used by Yamazaki et.al [20]) can't provide favourable separation selectivity. At the same time, the presence of hydroxyl group at carbon in beta-position from nitrogen atom of IDA provides dramatic changes in selectivity of complexation as
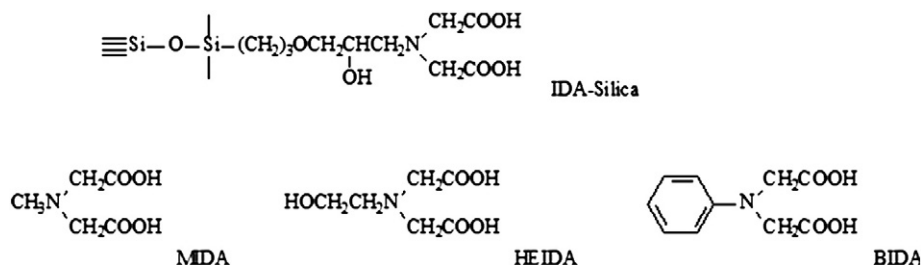

Fig. 1. Structures of bonded layer in monolithic column and some of IDA derivatives.

Table 1
The properties of substituted IDA derivatives as illustration of possible changes in the properties of IDA due to different chemistry of the immobilisation.

Ligand	pK_{a2} (COOH)	Stability constants for the complexes 1:1 (Me:L) at 25 °C and $I = 1$				
		Mg(II)	Ca(II)	Sr(II)	Ba(II)	Mn(II)
Iminodiacetic acid	2.62	2.98	2.60	2.23	1.67	4.72
N-Methyliminodiacetic acid (MIDA)	2.32	3.51	3.80	2.90	2.60	5.39
N-(2-Hydroxypropyl)-iminodiacetic acid (HEIDA)	2.20	3.41	4.69	3.75	3.38	5.46
N-Benzyliminodiacetic acid (BIDA)	2.21	2.65	3.17	2.34	2.23	6.60

compared with unsubstituted IDA and other IDA derivatives (Table 1). N-(2-hydroxypropyl)-iminodiacetic acid mimicking the structure of bonded layer in monolithic columns prepared for this investigation (Fig. 1) has unexpectedly high selectivity towards calcium and strontium as compared with other IDA derivatives (Fig. 2), and, hence, the prepared chelating monolithic column should fulfil to all selectivity requirements discussed earlier. The similar strong effect of bonding chemistry on complexing properties of chelating groups was observed recently for acetyliminodiacetic acid immobilised on the surface of polymethacrylate monolith [35].

3.1. Optimisation of the separation

The retention behaviour of alkaline earth metals on IDA bonded silica stationary phases in HPCIC has been thoroughly investigated previously [26,27,36,37]. The retention of alkaline earth metal cations on IDA-silica can occur either by ion-exchange, or via a chelation mechanism [31,32]. For chelating stationary phases, and IDA functionalised sorbents in particular, both eluent pH and ionic strength play a crucial role in controlling which mechanism is the dominant one. It has been shown that for non-complexing acidic eluents (diluted nitric or perchloric acids) with low ionic strength, the dominant mechanism for the retention of alkaline earth metals is ion-exchange, with complexation playing only a minor role [36,37]. If eluents with high ionic strength are utilised, ion-exchange effects are minimised and chelation becomes the dominant mechanism. Usually in HPCIC the ionic strength of eluents is regulated by the addition of nitrates, perchlorates or chlorides of alkali metals. In earlier works [27,37] it was shown that changing of mechanism from ion-exchange to chelation due to an increase of ionic strength of the eluent resulted in a reversal of the elution order of alkaline earth metals from $Mg^{2+} < Ca^{2+} \approx Sr^{2+} < Ba^{2+}$ to $Mg^{2+} < Ba^{2+} < Sr^{2+} < Ca^{2+}$. This latter elution order corresponds to the values of stability constants for an analogue of the bonded chelating groups, HEIDA (see Table 1), so this
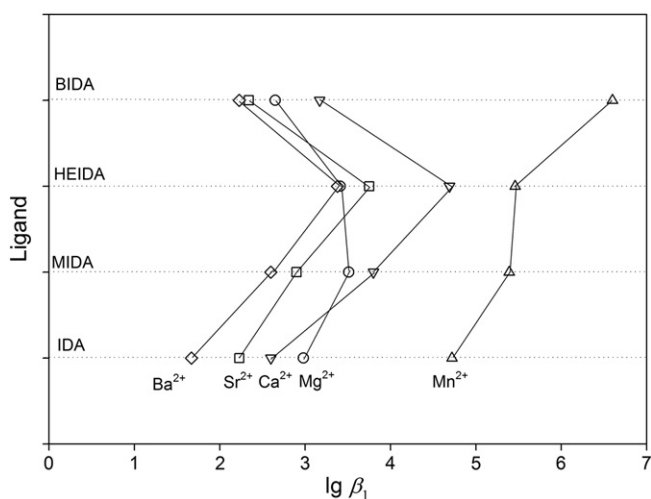

Fig. 2. The stability constants of complexes of alkaline metals with some IDA-derivatives illustrating possible changes in separation selectivity due to difference in bonding chemistry.

effect can be used for the attenuation of separation selectivity, allowing baseline separation of alkaline earth metal cations at different concentration ratios in samples of complex ionic nature.

Although the separation selectivity of IDA-silica stationary phases is well known, some basic optimisation of separation conditions was performed. In this case, the effects of pH at fixed ionic strength, and of ionic strength at fixed pH on retention of alkaline earth metal cations were evaluated. Sodium and chloride are two major components of seawater, so addition of sodium chloride to the eluent should minimise the probability of undesirable interactions, such as the formation of precipitates or other eluent induced chemical species. As expected, the retention for all alkaline earth metal cations decreased with an increase in eluent ionic strength (Fig. 3(a)). Increasing eluent pH at a constant ionic strength ($I = 0.5$ mol·L^{-1}) (Fig. 3(b), resulted in exponential increase in the retention of Ca^{2+}, while the increase in retention for Mg^{2+}, Sr^{2+} and Ba^{2+} was linear. From the above plots it was found that the optimal eluent pH was ~5.1 and the optimal ionic strength was 0.3. However, for the direct analysis of seawater a further decrease of ionic strength of the eluent to 0.25 was applied to increase the retention time of calcium. This provided a bigger time window between chromatographic peaks of magnesium and calcium and almost complete elution of massive peak of magnesium and more reliable resolution and integration of the small peak of strontium.

Taking into account the pH buffering capability of the carboxylic groups ($pK_{a1} = 1.85$; $pK_{a2} = 2.57$) within the silica bound IDA molecules, the eluent should also include some pH buffering capacity, for example, the inclusion of some weak acid species. However, when adding sodium acetate buffers to the eluent it was found that the shape of the chromatographic peak for Ca^{2+} was very poor. The peak was broad, tailed and split into two unresolved bands. Additionally, relatively small variations in eluent pH also changed peak shapes with this eluent system. For example, an eluent pH of 5.08 resulted in the chromatographic peak for calcium tailing, while increasing pH to 5.2 changed the peak shape to a fronted one. As peak shape indicates the type of isotherm of adsorption, namely a convex isotherm corresponds to tailed peaks and a concave isotherm corresponds to fronted peak, such changes in peak shape can be associated with changes in chemical equilibrium involving the Ca^{2+} ion. Hydrolysis or formation of hydrated ions may be responsible for this effect. To suppress these unwanted secondary interactions, the addition of glycolic acid to the eluent was investigated. This resulted in a significant improvement in peak shapes, with an optimal eluent composition being 0.25 mol L^{-1} NaCl and 2 mmol·L^{-1} glycolic acid, pH adjusted to 5.11.

3.2. Detection optimisation

Typical metallochromic indicators used as post-column reagents for the photometric detection of alkaline earth metals include PAR, ZnEDTA–PAR, and o-CPC [32]. However, to-date there has been no comparison published on their respective sensitivities for the detection of Sr^{2+} using PCR.

For the evaluation of Sr^{2+} detection sensitivity, a test mixture, containing 50 mg L^{-1} of each metal cation was separated using a single monolithic chromatographic column (100×4.6 mm I.D.) with an eluent of 0.25 mol·L^{-1} NaCl and 2 mmol·L^{-1} glycolic acid, and
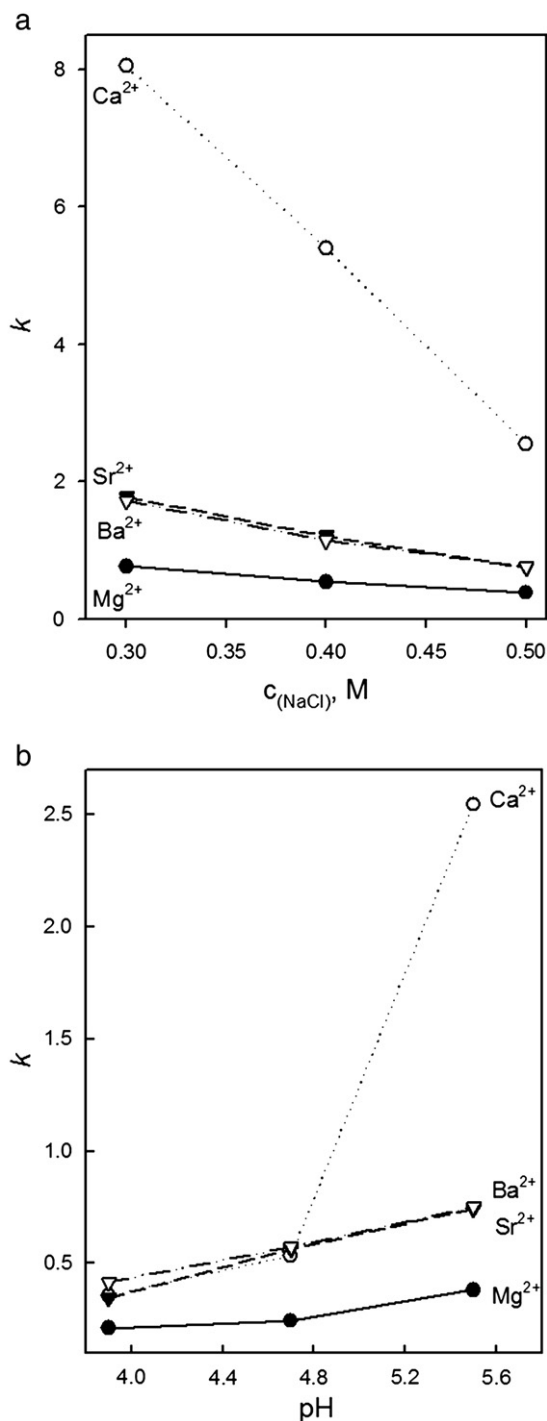
a

b

Fig. 3. The dependence of the retention of alkaline earth metal cations on (a) ionic strength of the eluent at constant pH 5.5, (b) pH of the eluent at constant ionic strength $I = 0.5$ mol L^{-1}.

Table 2
Comparison of various metalochromic indicators for post-column reaction and spectrophotometric detection of alkaline-earth metal ions.

Metal	Wavelength and photometric detector response[a], mV				
	o-CPC 570 nm	MTB 600 nm	XO 570 nm	ZnEDTA–PAR 520 nm	PAR 510 nm
Magnesium	182.9/159.5[b]	62.9	−88.2	58.3	39.0
Calcium	124.8/127.8	3.6	30.9	30.8	9.6
Strontium	37.3/92.7	0.7	23.8	24.5	1.2
Barium	8.91/25.3	No peak	10.3	12.9	0.2

[a] Average of two measurements for separate runs.
[b] The reagent mixture has pH 11.2.

overlapping of chromatographic peaks for seawater samples with large concentration of these metal ions. It should be noted that most organic reagents form more stable associates/complexes with Mg^{2+} and, hence, provides more intensive colour and high responses for this cation. Therefore one can expect significant interference for the detection of strontium from the large chromatographic peak of Mg^{2+}, which elutes first from the Onyx-IDA column. From Table 2 it can be seen that the reaction with o-CPC is overall the most sensitive to Sr^{2+}, and provides a 4-fold higher response for Mg^{2+} compared to Sr^{2+}. Post-column reaction with ZnEDTA–PAR is less sensitive for detection of Sr^{2+}, but it has more equal responses for all four alkaline earth metal cations, and thus can be used for their simultaneous determination. ZnEDTA–PAR reagent displayed an approximately 2.5 times higher response for Mg^{2+} than for Sr^{2+}. The serious limitation of using ZnEDTA–PAR reagent was its significant sensitivity/reactivity towards alkali metal cations. Alkali metal cations presenting at high
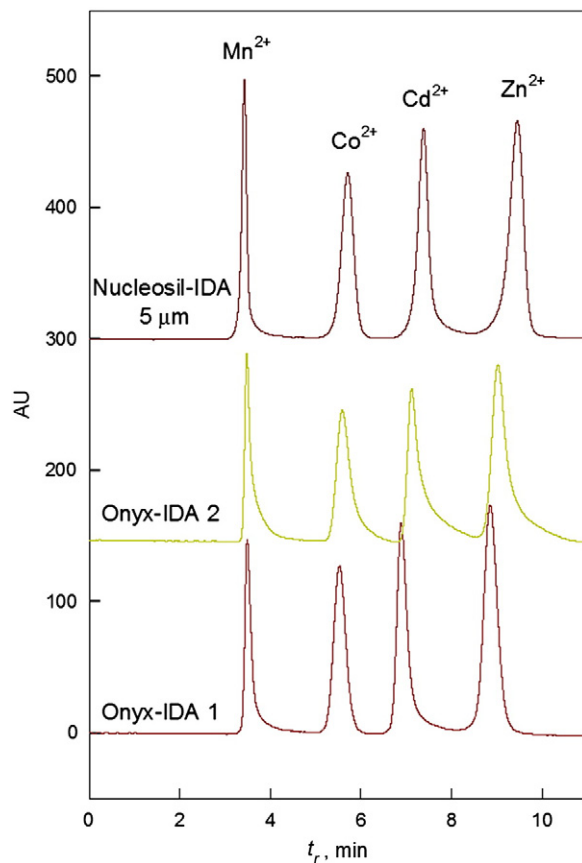

Fig. 4. Overlayed chromatograms of transition metals separation on two independently prepared columns Onyx-IDA (1 and 2) and on particle packed column Nucleosil-IDA (150×4.6 mm I.D., 5 μm). Eluent: 8 mmol L^{-1} M HNO_3, flow rate 1 mL min^{-1}, sample volume 20 μL, photometric detection at 510 nm after post-column reaction with PAR.

pH adjusted to 5.11. The flow rate of the eluent was 1.0 mL min^{-1} and each selected post-column reagent was delivered at a flow rate of 0.36 mL min^{-1}. The post-column reactor consisted of a plastic tee and 2.5 m long PTFE capillary reaction coil. Five metallochromic reagents including o-CPC, MTB, XO, PAR and ZnEDTA–PAR were tested, with special attention given to two detection characteristics. One was the sensitivity of detection for Sr^{2+}, which should be high enough to suit the concentration levels of this metal in seawater, namely 7.2–8.2 mg L^{-1} [4,7]. The second was a preferred low detection sensitivity for Mg^{2+} and Ca^{2+}, to prevent the possible

concentrations in seawater can bind EDTA from the reagent and deactivate the part of ZnEDTA–PAR reagent for reaction/detection with Sr^{2+}.

3.3. Reproducibility of the on-column modification of monolithic IDA columns

Preliminary experiments demonstrated that a single 100 mm long Onyx-IDA column cannot provide a complete separation of Ba^{2+} and Sr^{2+} under any eluent conditions investigated. To increase separation selectivity and resolution of large Mg^{2+} and the small Sr^{2+} peaks, it was necessary to use two Onyx-IDA columns connected in series. Two Onyx silica columns (from different batches), were independently subjected to modification (modification in separate laboratories), using the same method. To compare the chromatographic performance of the columns, a separation of the model mixture containing 0.25 mg L^{-1} Mn^{2+}, 0.5 mg L^{-1} each of Co^{2+} and Zn^{2+}, and 1 mg L^{-1} of Cd^{2+} was obtained on each of the prepared columns. The retention times, efficiency and peak asymmetry for each of the analytes were compared between monolithic columns and with a reference commercial particle packed Nucleosil-IDA column (100×4 mm I.D., 5 μm, 330 m^2 g^{-1}). It was found (Fig. 4) that variation of the retention factor for Zn^{2+}, the most retained analyte for two monolithic columns was within 1.1%. This proves identical ion-exchange capacity for the independently prepared columns and demonstrates excellent reproducibility for the on-column modification of the bare silica Onyx columns with IDA functional groups. However, for unknown reasons (although likely to be related

to different degrees of usage between the two columns), peak asymmetry for peaks on Onyx-IDA column 2 was higher than for Onyx-IDA column 1 (for 2.9 and 1.2, respectively, for Zn^{2+} peak), with higher efficiency obtained for Onyx-IDA column 1 (30,000 theoretical plates per metre for column 1 and 21,000 — for column 2 calculated for Zn^{2+} peak). Nevertheless, when coupled together the two columns delivered the overall capacity and selectivity for the desired application, without evidence of excessive peak tailing.

Fig. 5(a) shows the separation achieved of a model mixture of alkaline earth metal cations on the coupled monolithic columns, under optimised conditions (eluent composition, column length, detection). Baseline separation was achieved within 12 min at flow rate of just 1.0 ml min^{-1}. The high permeability of the monolithic columns allowed use of elevated flow rates to reduce the analysis time required for the separation shown [25], and so the effect of flow rate upon peak shape and selectivity was also evaluated here.

A separation of a standard mixture of alkaline earth metal cations was performed at flow rates up to 4 mL min^{-1} (Fig. 5(b)). It was possible to reduce the run time from 12 min at a flow rate of 1 mL min^{-1} to just 3.5 min at 4 mL min^{-1} without any noticeable loss of resolution of the four peaks. However, a reduction in peak areas and peak heights was noted with an increase of the eluent flow rate, reducing method sensitivity as a consequence of reduced analysis time. The reason for this was the difference in the mixing ratio of column effluent and post-column reagent flows. The peristaltic pump operating at constant flow rate 0.36 mL min^{-1} was used in this set of experiments, so all detector responses were reduced proportionally to
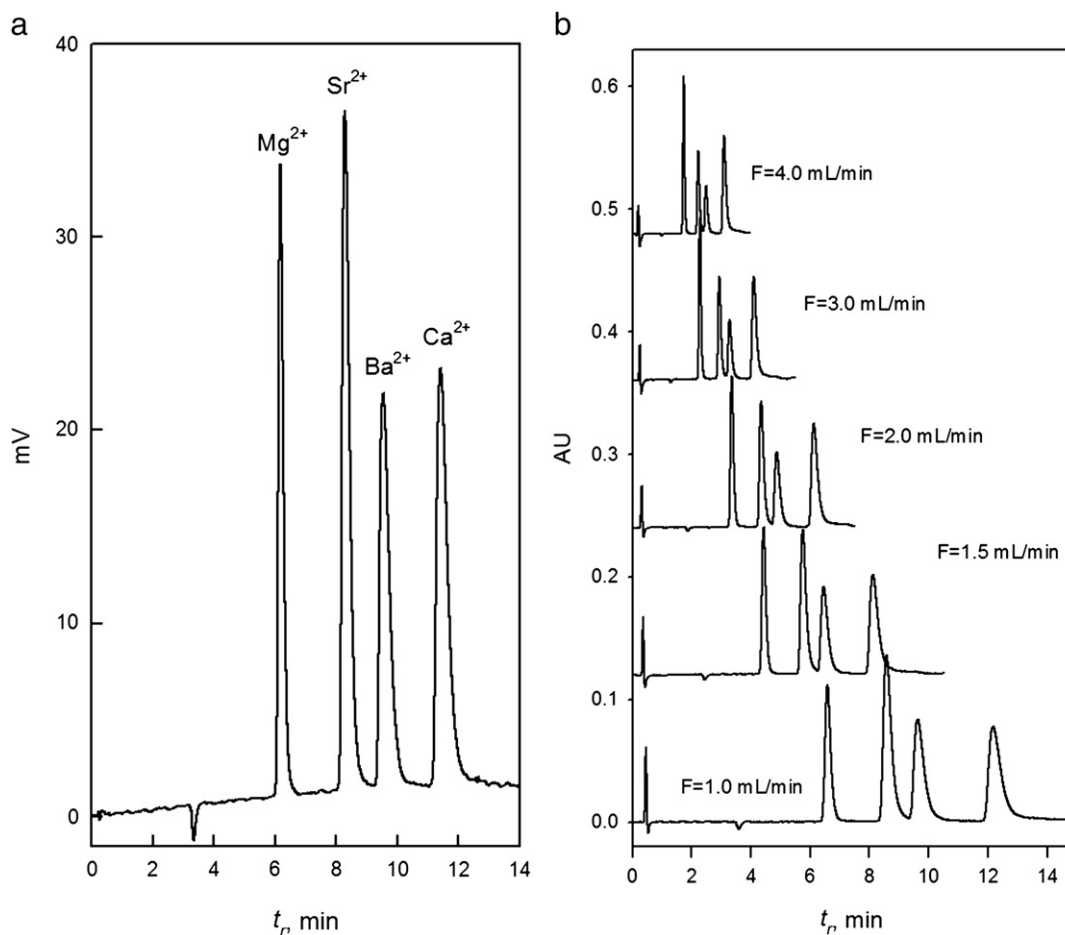

Fig. 5. Chromatograms of the test mixture of alkaline earth metal cations. Column: two Onyx-IDA columns connected in series ($2 \times 100 \times 4.6$ mm I.D.), eluent: 2 mmol L^{-1} glycolic acid, 0.25 mol L^{-1} NaCl, pH 5.11, injection volume 4 μL, flow rate (a) 1 mL min^{-1}, (b) varies from 1 to 4 mL min^{-1}. Photometric detection at 570 nm after post-column reaction with o-CPC. PCR reagent flow rate 0.36 mL min^{-1}.

reagent-to-eluent mixing ratio. Obviously, this problem can be solved either by using a reagent pump with variable flow rate or by an increase in concentration of the metallochromic ligands as the PCR reagents.

The responses of Sr^{2+} and Ba^{2+} were affected more than those of Mg^{2+} and Ca^{2+} by increase in the mobile phase flow rate. Obviously, this effect is connected with a decrease of pH of the reaction mixture after dilution of reagent composition with effluent. The study of the effect of pH of the reagent on response shows that o-CPC forms coloured complexes with Sr^{2+} and Ba^{2+} at higher pH values than with Mg^{2+} and Ca^{2+}, while detected absorbance for complex of strontium at pH 10.5 is only one-third of those at pH 11.2 (Table 2). These results are in a good agreement with data of Anderegg et al. [38], who reported the same behaviour of complexes with o-CPC. However, in the case of a more alkaline reagent mixture, the background absorbance and baseline noise were significantly higher and interfered with detection. So, pH and reagent concentration for the determination of alkaline earth metals at higher flow rates should be attuned for on-line detection at high flow rate of acidic eluents.

In the analysis of complex samples such as seawater, using a chelating ion-exchange chromatographic column, there is a high probability of contamination and saturation of the stationary phase with strongly retained multivalent cations following high numbers of samples. Usually, regeneration of such chromatographic columns requires intensive washing with more concentrated mineral acids or with a solution containing a strong complexing reagent, such as dipicolinic or oxalic acid. Therefore, here the stability of the developed chromatographic system was tested. For this purpose, more than 140 samples of seawater spiked with Mn^{2+} were injected in triplicate onto the Onyx-IDA 2 column. Obtained chromatograms contained three clear chromatographic peaks for Mg^{2+}, Ca^{2+} and Mn^{2+}. After 400 injections and 6000 column volumes of the mobile phase passed through the column, the retention time variation for each of these three analyte ions was within 0.85% for Mg^{2+}, 1.1% for Ca^{2+} and 1.5% for Mn^{2+} (Fig. 6). The step on the graph corresponds to a break in experimental work for 2 weeks. This remarkable stability, following such a large number of undiluted seawater sample injections, can only be connected with the small sample volumes injected with the developed method (4 μL), and represents a significant advantage to the current method over alternative approaches.

3.4. The analysis of the seawater sample

Finally, the method was applied to the determination or Sr^{2+} in seawater samples. A chromatogram for a certified reference seawater
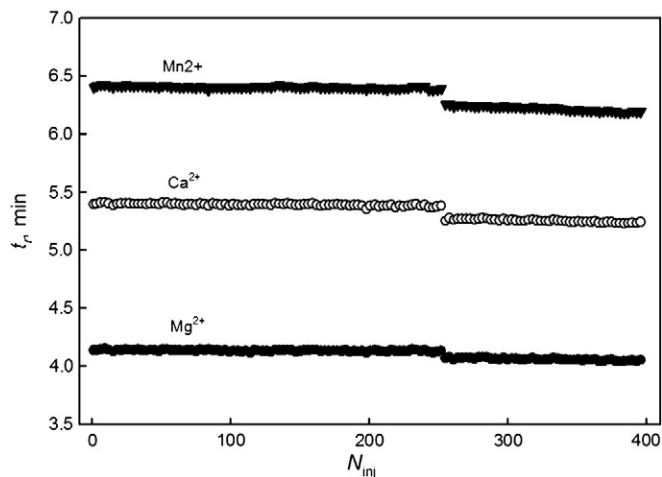

Fig. 6. The dependence of the retention times of selected analytes on the number of injections.
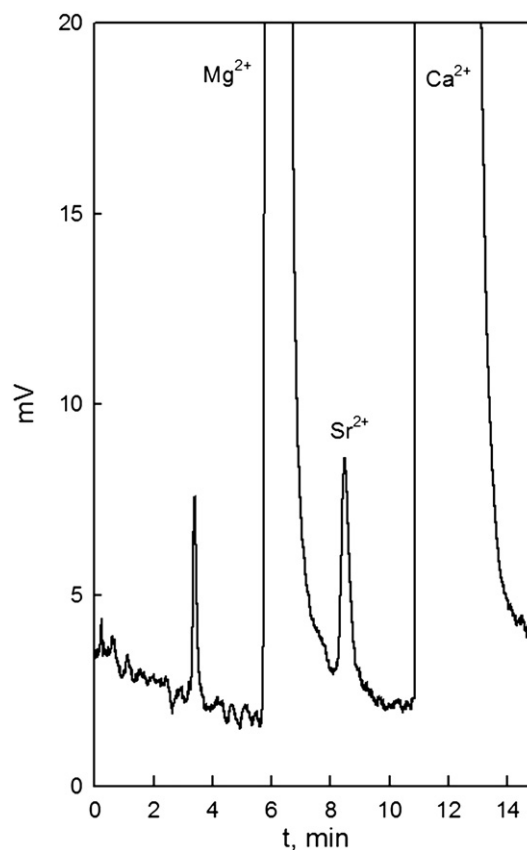

Fig. 7. The chromatogram of seawater sample (salinity standard IAPSO, batch P149). Separation conditions as in Fig. 5a.

sample (IAPSO seawater salinity standard), obtained under optimal conditions is shown in Fig. 7. Using a standard addition method, the concentration of Sr^{2+} in the sample was found to be 8.26 ± 0.19 mg L^{-1}, with $s_r = 2.3\%$ at $n = 6$. Slope and intercept for the calibration graph were 11.48 and 94.82 respectively, with $r^2 = 0.997$. This result was compared with that, obtained by an ICP-MS method, to confirm accuracy, with which the concentration of Sr^{2+} was found to be 8.20 mg L^{-1}, within 1% difference to the HPCIC value. However, the ICP-MS method required a 100 fold dilution of seawater before analysis and potential interference from excessive calcium ions was still possible at this dilution level.

From the calibration graph plotted for standard Sr^{2+} solutions with concentrations varied from 5 to 50 mg L^{-1}, LOD was found to be 3.23 ± 0.15 mg L^{-1} $s_r = 4.6\%$ (obtained without full optimisation of PCR conditions, such as reduction of PCR pump noise). The LOD for the developed method is lower than for the officially recommended AAS determination protocol "ASTM D3352-08a Standard Test Method for Determination of Strontium Ion in Brackish Water, Seawater, and Brines", where LOD is 5 mg L^{-1} strontium [9].

4. Conclusions

A simple HPCIC method allowing fast determination of Sr^{2+} in seawater has been developed. For the first time, the analytical determination of strontium in seawater does not require any sample pretreatment. The other special features of this chromatographic method include excellent robustness of chromatographic system, good reproducibility of the results and the possibility of ship-board analysis. In addition, as the results can be acquired in substantially shorter times, with less labour required for each analysis, the HPCIC method will prove an extremely useful analytical tool for various

marine and environmental studies, when hundreds of samples should be analysed for the production of concentration profiles and models.

Acknowledgement

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