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# Zwitterionic ion chromatography using a dynamically coated column and mobile phase recycling

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## Abstract

An investigation into the use of zwitterionic ion chromatography for the determination of inorganic anions in water samples was carried out. When using an ODS stationary phase precoated with Zwittergent 3–14 and a pure water mobile phase, the stability of the adsorbed coating was insufficient for quantitative work. Recycling of the water mobile phase was used to stabilise the zwitterionic coating, and resulted in improved retention time precision (15.2% RSD down to 2.4% RSD for nitrate). Post-detection cation- and anion-exchange columns in acid and hydroxide form removed sample ions from the recycling mobile phase, with the desorbed Zwittergent 3–14 passing through unretained and passing back through the pump to the analytical column. A 200-ml volume of mobile phase was recycled over a 3-week period with retention times for sulphate, chloride and nitrate standards injected at the start and end of the period varying less than 2.5%. The same system was then used with a mobile phase containing 2 mM Zwittergent 3–14. This resulted in further improvements in retention time (0.2–0.5% RSD,  $n=10$ ) and peak area precision (2.6–6.0% RSD, 1 mM standards) and improved peak efficiencies (2421–4047  $N$ ). The developed method was applied to water samples, and results compared to those obtained using anion-exchange chromatography. All sample cations were exchanged to sodium using an off-line cation-exchange procedure prior to injection.

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## 1. Introduction

Zwitterionic ion chromatography (ZIC), also termed electrostatic ion chromatography (EIC), is a mode of ion chromatography (IC), first proposed by Hu et al. [1], which utilises a zwitterionic stationary phase in place of a cation or anion exchanger. This

novel phase contains both positive and negative charges simultaneously and so analyte ions experience both attractive and repulsive forces as they migrate through the stationary phase. What results can be visualised as an extremely weak ion exchanger, where an eluting ion (as required in standard ion exchange) is no longer necessary to facilitate elution of the analyte ion. Therefore, pure water can be used as the mobile phase, with retention and selectivity being dependent upon the relative locality of the charged groups on the zwitterionic function-

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ality and the nature of the analyte ions and their counter ions. An exact model describing this process when using a water mobile phase has been proposed by Hu and co-workers [2,3], with a second model later developed to describe retention in ZIC when using more traditional ion chromatographic eluents incorporating suitable electrolytes [4–6].

Early work into the development of ZIC focused on the use of water only mobile phases for the separation of inorganic anions and cations. Much of this was based on the use of Zwittergent 3–14 coated ODS columns for anion separations, although many alternative zwitterionic reagents were also studied, several of which exhibited sufficient selectivity for cation separations. A short overview of some of these early results was compiled by Hu and Haddad, which highlights some of the principles and problems associated with the technique [7].

Two significant problems identified with ZIC were specifically related to the use of a pure water mobile phase. These being (a) the instability of the zwitterionic coating when using a water mobile phase and (b) the formation of multiple peaks due to analyte ions having multiple counter ions instead of just one counter ion, as would be the case if an electrolyte were used as the mobile phase. Both these problems were significant obstacles in the development of the technique and eventually led to the focus of research being directed away from water as a mobile phase and back to the use of more traditional ion chromatographic eluents, whereupon the above problems were no longer so significant [8,9]. However, the use of a water mobile phase has more advantages than simply reduced reagent costs and preparation times. A water only mobile phase also means the technique can easily be placed on-line in a monitoring environment and also facilitates simple methods of mobile phase recycling. Therefore, as these latter advantages were of interest to our research group, ZIC with a water mobile phase was revisited. In particular, the following study investigated the use of post-detector cation and anion-exchange columns for the removal of sample ions and thus enable the continuous recycling of the water eluent. This then led to a study into the inclusion of the zwitterionic reagent into the mobile phase, where simple mobile phase recycling was still possible due to the reagent passing unretained through the two

post-detector ion-exchanger cartridges. This led to the stabilisation of the zwitterionic coating and improved system reproducibility. The following paper details these results and other analytical performance parameters of the above methods, together with applications to natural and treated water samples.

## 2. Experimental

### 2.1. Instrumentation

A Dionex DX-100 chromatograph (Dionex, Surrey, UK) provided the pump and the conductivity detector used throughout. In addition, a UV detector (LDC monitor III, model 1204A) monitoring at 210 nm was placed in series after the conductivity detector. Both detectors were connected to a PC via an ADC-16 high-resolution data logger (Pico Technology, Cambridgeshire, UK). Integration and retention time analysis was carried out using Microsoft EXCEL and ORIGIN software packages. For the eluent recycling work the two detectors were followed by two ion exchangers, one for the removal of analyte cations and one for analyte anions. The cation exchanger used was an Alltech anion-exchange suppressor cartridge (10×0.46 cm I.D.) and holder containing a strong cation-exchange resin (Alltech, Lancashire, UK). The anion exchanger comprised of a similar cartridge and holder, with the cartridge containing Amberlite IRA 400 resin, (BDH, Poole, UK). The cation-exchange resin (sold by Alltech for use within an anion suppressor column) was coloured yellow when in the acid form and pink when it contained metal cations. In this way, it was possible to tell when the cartridge was spent and needed regeneration, although as the exchangers were only removing sample ions, they did not require regeneration during the length of this study. The separating column was a Waters Spherisorb ODS 2, 5 µm analytical column (250×4.6 mm, Supelco–Sigma–Aldrich, Tallaght, Dublin, Ireland) coated with Zwittergent 3–14 surfactant using a coating method proposed by Hu et al. [1] and later by Umemura et al. [10]. Supelclean LC-SCX 1 ml cation-exchange cartridge tubes (Supelco–Sigma–Aldrich) were used for the off-line cation exchange

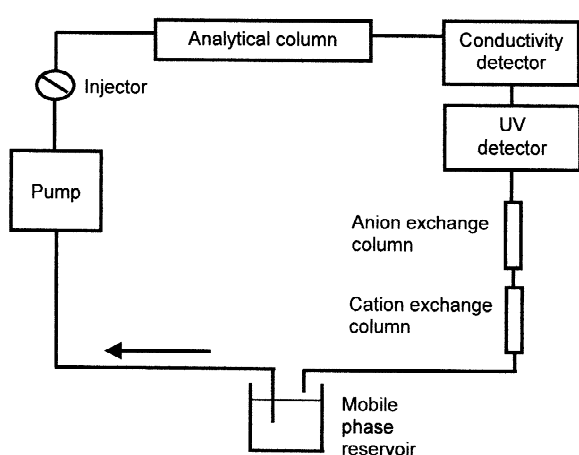


Fig. 1. Schematic diagram of instrumentation used for mobile phase recycling.

of water samples. A schematic diagram of the instrumentation used for the recycling work is shown as Fig. 1.

For comparative sample analysis (Section 3.3) an AS17 25 cm analytical column and an AG17 guard column (Dionex) were used with a 45 mM NaOH eluent (Aldrich, Sigma–Aldrich). Detection was again obtained using direct UV at 210 nm.

## 2.2. Reagents

All chemicals used (analytes, solvents and Zwittergent 3–14) were analytical reagent grade and were supplied by Sigma–Aldrich. All reagent and standard solutions were prepared using deionised water from a Milli-Q<sup>50</sup> cartridge type water purification system, (Millipore, Walford, UK).

## 3. Results and discussion

### 3.1. Off-line cation exchange

As mentioned in the Introduction, a problem with the use of mobile phases in ionchromatographic methods that do not contain an electrolyte is that the ions of interest (these being either anions or cations) may have more than one type of counter ion when present in the sample. For example, in a river water sample containing nitrate there may also be present

sodium, potassium, calcium, magnesium etc. If this sample is injected into a mobile phase of pure water, nitrate will travel through the column ‘weakly associated’ with any one or all of the above metal ions and elute as a number of peaks, which are often rather broad and in the case of real samples totally irreproducible. The only way to solve this problem without switching to an electrolyte mobile phase is to pretreat the sample to ensure all the analyte ions are associated with a single type of counter ion. This has been achieved either by passing the sample through an ion-exchange column in a suitable form prior to the separation column [11,12], or by introducing what is termed a ‘decoy analyte’ into the sample at a high enough concentration such that the analyte ions of interest become associated with introduced decoy counter ions [13]. Both these approaches have merit, although the latter can be time consuming and result in large introduced peaks in the resultant chromatogram. The ion-exchange approach is rather simpler and can be incorporated into the chromatographic system and therefore automated. In this study, the ion-exchange approach was used in the form of cation-exchange cartridges (1 ml) in the sodium form, with samples and standards being injected directly through the cation-exchange cartridge into the injection port of the ion chromatograph. A water only mobile phase was used with a freshly coated zwitterionic analytical column. The cation-exchange cartridges used could be discarded after each sample or easily regenerated using a solution of NaCl and a wash with deionised water. To test for quantitative exchange of all sample cations for sodium, linearity studies were carried out with anion standards prepared from sodium salts and similar anion standards prepared from divalent metal ion salts, which were subjected to the cation-exchange procedure. For standards containing monovalent anions and divalent cations, such as calcium nitrate,  $\text{Ca}(\text{NO}_3)_2$ , or calcium chloride,  $\text{CaCl}_2$ , passage of a 0.1 mM solution of these salts through the sodium cation-exchange cartridge, results in the elution of a 0.2 mM solution of the sodium form of the anion. However, this is not the case for a divalent anion such as sulphate, where the passage of a 0.1 mM standard prepared from a divalent metal sulphate salt (e.g.  $\text{ZnSO}_4$ ) through the sodium exchange column would only result in the elution of a 0.1 mM solution of  $\text{Na}_2\text{SO}_4$ . Therefore,

if quantitative cation exchange takes place, the slopes obtained from calibration graphs constructed using standards prepared from the above salts after passage through the sodium exchange column should reflect the above, with slopes obtained using  $\text{Ca}(\text{NO}_3)_2$  and  $\text{CaCl}_2$  standards being twice those obtained using standards prepared from  $\text{NaNO}_3$  and  $\text{NaCl}$ . For divalent anions such as sulphate, the slopes of resultant calibration graphs should be the same regardless of whether the standard was prepared from a monovalent or divalent cation salt, but again only if quantitative exchange takes place. Table 1 shows the results obtained from this study. It is clear from the calibration slopes obtained that quantitative exchange was indeed being achieved using the 1-ml cation-exchange cartridges and that detector linearity was not affected by the procedure. Fig. 2 shows the pre- and post-cation-exchange separations of sulphate, chloride and nitrate, prepared from sodium salts of sulphate and chloride and a mixture of sodium, calcium and cerium salts of nitrate. As can be seen from the chromatograms shown, all divalent and trivalent metal ions are exchanged quantitatively for sodium, resulting in a single large sharp peak replacing three smaller peaks for nitrate. Additionally, sulphate and chloride peaks are also slightly improved as any calcium and cerium forms of these ions, which would have inevitably been present due to ion redistribution within in the

Table 1

Method linearity for anion standard solutions prepared from sodium salts and anion solutions prepared from divalent metal salts and converted to sodium form using cation-exchange procedure

Standards prepared	Range (mM)	<i>n</i>	<i>r</i> <sup>2</sup>	Slope
Sodium nitrate	0.1–1	6	0.9944	9.37
Calcium nitrate (exchanged <sup>a</sup> )	0.1–1	6	0.9996	18.84
Sodium chloride	0.1–1	6	0.9875	12.14
Calcium chloride (exchanged <sup>a</sup> )	0.1–1	6	0.9915	24.12
Sodium sulphate	0.1–1	6	0.9926	9.08
Zinc sulphate (exchanged <sup>a</sup> )	0.1–1	6	0.9902	9.03

<sup>a</sup> Standard solutions passed through cation-exchange cartridge in sodium form upon injection.

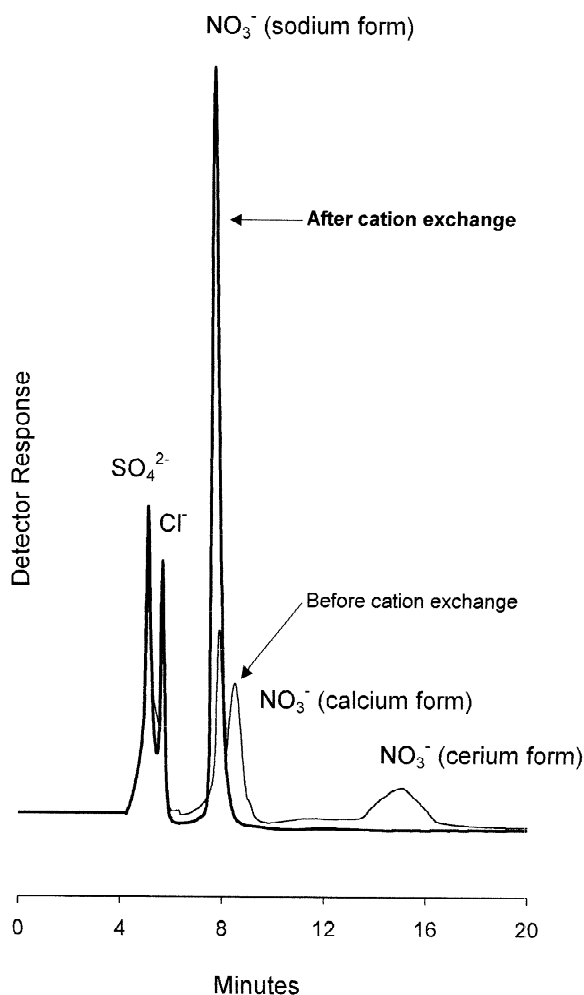


Fig. 2. Chromatograms showing the elimination of ion redistribution in ZIC using the off-line cation-exchange procedure. Aqueous solution containing 1.0 mM  $\text{NaCl}$ ,  $\text{Na}_2\text{SO}_4$ ,  $\text{NaNO}_3$ ,  $\text{Ca}(\text{NO}_3)_2$ , and  $\text{Ce}(\text{NO}_3)_3$  before and after cation exchange. Pure water as the mobile phase. Conductivity detection.

mixed standard solution were also converted back to sodium.

### 3.2. Eluent recycling

#### 3.2.1. Water only mobile phase

Two obvious advantages of using water as a mobile phase are the ability to (a) generate the mobile phase automatically by connecting the chromatograph to a continuous source of deionised

water, or (b) regenerate (and recycle) the mobile phase by carrying out post-detector deionisation of the mobile phase. The second approach is the least complicated as it simply involves the inclusion of post-detector ion-exchange columns to remove analyte ions from the system. If these ‘in-line’ cation and anion exchangers are preconditioned in the acid and hydroxide form, then all sample ions are removed and the purity of the water mobile phase restored.

However, here the recycling approach also has the added advantage that when used with precoated columns it helps to stabilise the zwitterionic coating and thus capacity of the column. This is because the zwitterionic reagent used to coat the ODS column is continuously bleeding from the column, which in nonrecycling mode means the column has to be periodically recoated if capacity is to be maintained. This is evident from the chromatogram shown in Fig. 2, which shows a retention time of almost 8 min for nitrate (in sodium form) on a freshly coated column. Typically, this retention time would gradually reduce to just over 4 min after approximately 2 days continuous usage with a water only mobile phase. However, due to the zwitterionic nature of the coating reagent, in recycled mode, the reagent continually being lost from the column passed unretained through both post-detector ion-exchange cartridges and back onto the analytical column. This resulted in the mobile phase no longer being pure water but instead containing a small background level of Zwittergent 3–14, sufficient to maintain the

column coating at a consistent level. To test if this was indeed the case a standard solution containing 1 mM sulphate, chloride and nitrate was injected ten times over a 2-day period with a water mobile phase in the nonrecycled mode and then the same sample was repeatedly injected over a similar time period using the recycling mode. Before each set of injections the column was freshly coated and equilibrated with the mobile phase for >1 h. The results of the above can be seen in the left section of Table 2. It is quite clear that the recycling of the mobile phase has led to a drastic improvement in retention time reproducibility by stabilising the column coating and thus capacity. Interestingly, retention times with the recycled system were slightly lower than those obtained with a freshly coated column, which shows how some of the Zwittergent 3–14 is quite rapidly lost to the mobile phase and how this mobile phase concentration then remains fairly constant. To illustrate this excellent reproducibility further, a 200-ml volume of pure water mobile phase was left to recycle within the system over a 3-week period. The volume was set at 200 ml due to the size of the mobile phase container, although it is clear smaller volumes could be used. Injections of the same sample mix were carried out over regular intervals throughout this period and the baseline conductivity and absorbance monitored. No increase in either baseline was observed over this entire period indicating the recycling system was operating successfully. Fig. 3 shows overlaid chromatograms of (a) a single 1 mM sulphate standard and (b) a mixed 1 mM

Table 2  
Comparison of retention time ( $t_R$ ) and peak area precision, efficiency ( $N$ ) and resolution ( $R_s$ ) under differing mobile phase conditions

Analyte anion	Pure water mobile phase (nonrecycled mode)		Pure water mobile phase (recycled mode)					Zwittergent 3–14 mobile phase (recycled mode)				
	$t_R^a$	$t_R^b$ RSD (%) ( $n=10$ )	$t_R$	$t_R^b$ RSD (%) ( $n=10$ )	Peak area <sup>b</sup> RSD (%) ( $n=10$ )	$N$	$R_s$	$t_R$	$t_R^b$ RSD (%) ( $n=13$ )	Peak area <sup>b</sup> RSD (%) ( $n=13$ )	$N$	$R_s$
Sulphate	2.7–4.2	11.3	4.0	1.6	9.1	1300	–	3.0	0.5	3.3	2400	–
Chloride	3.1–4.8	13.9	4.5	1.4	12.6	1950	1.3	3.4	0.5	6.0	2700	1.9
Nitrite	3.5–5.7	–	5.1	–	–	2200	1.4	4.4	0.2	2.6	3600	2.3
Nitrate	4.4–7.2	15.2	6.3	2.4	11.1	3100	2.9	4.9	0.3	3.4	4050	4.4

<sup>a</sup> Range of retention times ( $t_R$ ) dependent upon time since coating of column. Data shown based upon injection of 1 mM standard solutions.

<sup>b</sup> Injections carried out over 2-day period.

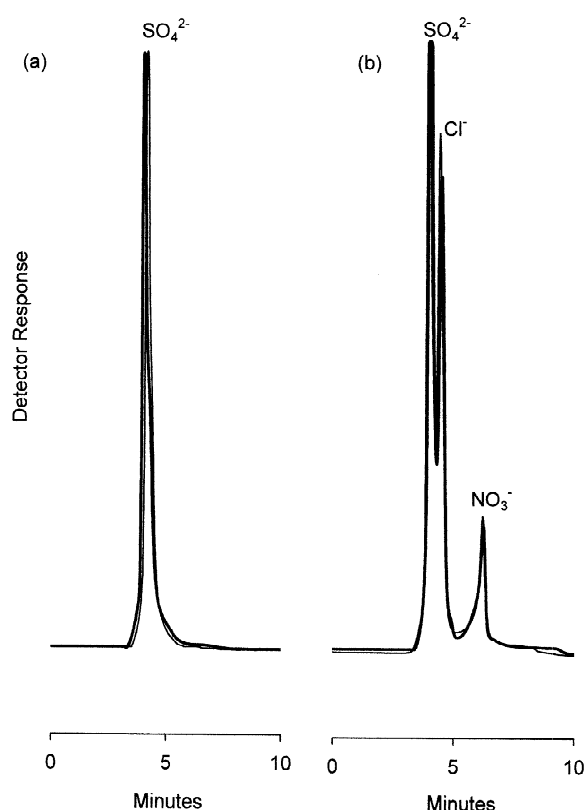


Fig. 3. Overlaid chromatograms showing the reproducibility of the recycling system. 1 mM standards injected at start and end of 3-week recycling period. Pure water as the mobile phase. Conductivity detection.

sulphate, chloride and nitrate standard, injected at the beginning and end of the 3-week period. The chromatograms show that remarkable long term reproducibility was obtained.

However, as can be seen from Table 2, the peak area precision for the recycled water mobile phase was still rather poor, being between 9 and 13% RSD. This represented an unacceptable level of error for quantitative work. The poor precision was mainly due to problems with integrating partially resolved peaks and peaks which were either tailed or fronted (see nitrate peak in Fig. 3).

### 3.2.2. Zwitterionic mobile phase

In an attempt to improve peak shapes and further resolve the standard anions, and thus improve the above poor peak area precision, it was decided to

investigate the effect of adding additional Zwittergent 3–14 to the mobile phase (it should be noted here that in previous work by Hu and co-workers, Zwittergent 3–14 was often added to the water mobile phase to improve reproducibility, although this is only specifically mentioned in Ref. [14]). This would have no effect upon the ability to recycle the mobile phase but would introduce a mobile phase parameter that could perhaps be manipulated to affect retention, selectivity and peak shapes. The effect of adding between 0 and 5 mM Zwittergent 3–14 into the mobile phase, and using these mobile phases with a precoated column was investigated. It was found that between 0 and 2 mM, there was a mixed effect upon analyte retention times, with early eluting anions, such as sulphate, chloride and nitrate showing a slight decrease in retention, and later eluting anions, such as iodide and thiocyanate showing a slight increase. When the mobile phase concentration was increased further to 5 mM Zwittergent 3–14, there was a significant increase in retention for all anions tested. However, a more significant result was that the inclusion of additional reagent into the mobile phase did indeed result in a general improvement in peak shapes, through an improvement in peak symmetry. Fig. 4 shows chromatograms of a sulphate, chloride, nitrite and nitrate standard solution obtained using (a) a pure water mobile phase and (b) a 2 mM Zwittergent 3–14 mobile phase, both in recycled mode. As can be seen for the four test anions, overall resolution was slightly improved with the zwitterionic mobile phase despite the reduced overall run time, and with only sulphate exhibiting peak fronting. To evaluate the effect of the above addition on retention time and peak area precision, a series of 13 repeat standard injections were again carried out over a 2-day period, the results of which are also shown in Table 2. It was clear that the addition of Zwittergent 3–14 to the mobile phase not only further improved retention time precision to <1% RSD for all four anions but also resulted in much improved peak area precision. Therefore, for the remainder of this study, 2 mM Zwittergent 3–14 was used as the mobile phase.

### 3.3. Analysis of water samples

A number of water samples were collected of

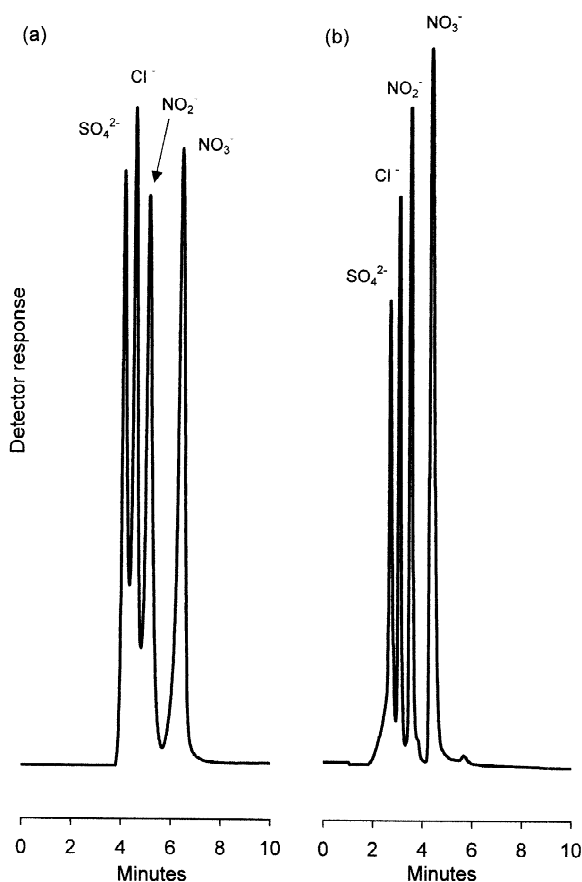


Fig. 4. Chromatograms of standard test mixtures of sulphate, chloride, nitrite and nitrate. Concentrations 0.5, 1.0, 1.5 and 2.5 mM, respectively. (a) Pure water mobile phase, (b) 2 mM Zwittergent 3–14 mobile phase. Conductivity detection.

varying complexity, namely river water samples, tap water samples and bottled mineral water samples. The river and tap water samples were known to have relatively high sulphate levels, whilst the mineral water was known to be high in chloride. To evaluate the accuracy of the ZIC method the samples were analysed for their sulphate, chloride and nitrate concentrations, using both a standard calibration method and using standard addition calibration. For the determination of nitrate levels in the above samples, both conductivity and direct UV absorbance detectors were used in-line. For the standard calibration curves correlation coefficients ranging from  $R^2=0.983$  ( $\text{NO}_3^-$ , cond.) to  $R^2=0.996$  ( $\text{NO}_3^-$ , UV) were obtained, with each standard injected in dupli-

cate, ranging from 0 to 1.0 mM ( $n=6$ ). The standard additions resulted in correlation coefficients ranging from  $R^2=0.971$  ( $\text{Cl}^-$ , cond.) to  $R^2=0.998$  ( $\text{NO}_3^-$ , UV), again with each addition analysed in duplicate and over the same concentration range ( $n=6$ ). The relative standard deviation obtained resulted from random error and no systematic curvature to the calibration was seen.

In addition to the above, the determination of chloride and nitrate in the mineral water sample was also carried out using a standard IC method (AS17 column, 45 mM NaOH eluent, detection at 210 nm) to obtain comparative values. Here for the standard calibration curves, typical correlation coefficients of  $R^2=0.979$  were obtained ( $\text{NO}_3^-$ , UV), with each standard injected in duplicate, ranging from 0 to 1.0 mM ( $n=6$ ).

The results of these analyses are shown in Table 3. The results obtained show that the developed method is not only precise but also accurate, with in most cases <1% variation between the results obtained for the mineral water sample using ZIC and conventional IC. The chromatograms shown in Fig. 5 were obtained during the analysis of the river water sample. Fig. 5a shows the chromatogram obtained using the conductivity detector. As can be seen the sample contains an excess of sulphate and chloride compared to a lower concentration of nitrate and trace nitrite, with the latter being poorly resolved from the chloride peak. The peak for nitrate, present in the sample at 0.16 mM, is particularly sharp and clearly resolved from other anions present, including carbonate. When using the UV detector (Fig. 5b) the sulphate peak is no longer seen and the chloride peak is greatly reduced, showing the clear presence of both nitrite and nitrate in the sample. In the river water sample analysed a small irregular shaped peak could be seen with the UV detector that co-eluted with the nitrate. It was assumed this was likely to be some organic species as it was not detected using conductivity. Fig. 5c shows the necessity of the cation-exchange procedure described in Section 3.1. The chromatogram was obtained for the same water sample when analysed without using the sodium exchange cartridge and shows the presence of multiple peaks for nitrate. This indicated the presence of divalent cations in the sample, likely to be calcium and magnesium.

Table 3  
Results obtained for the analysis of water samples using ZIC and standard IC

Analyte anion	Conc. found using ZIC, mM ( $\pm$ SD)			Standard addition <sup>b</sup>	Quoted conc. <sup>c</sup> mM	Conc. found using standard IC (std. calibration <sup>d</sup> ) mM
	Standard calibration <sup>a</sup>	Tap water	River water			
Sulphate	1.38 (0.05)	2.41 (0.08)	0.11 (<0.01)	Mineral water	0.11	–
Chloride	0.46 (0.03)	0.68 (0.04)	1.18 (0.07)	Mineral water	1.40	1.17
Nitrate (Cond.)	0.10 (<0.01)	0.16 (0.01)	0.18 (0.01)	Mineral water	0.23	–
Nitrate (UV)	0.10 (<0.01)	0.15 (0.01)	0.18 (0.01)	Mineral water	0.17 (0.01)	0.18

<sup>a</sup>  $n=6$ , 0–2 mM.

<sup>b</sup>  $n=6$ , 0–2 mM.

<sup>c</sup> Finches mineral water.

<sup>d</sup>  $n=5$ , 0–2 mM.

### 3.4. Trace analysis

A significant advantage of ZIC using either the pure water mobile phase or the zwitterionic mobile phase is that they both have a low background conductance and background absorbance. In terms of conductivity detection this allows sensitive detection without the need for suppression. Here detection

limits were investigated using the 2 mM Zwittergent 3–14 mobile phase. Using a 50- $\mu$ l injection loop and conductivity detection, detection limits ( $3\times$  baseline noise) of 0.09, 0.42 and 0.18  $\mu$ M (11.5, 14.9 and 11.2  $\mu$ g/l) were calculated for sulphate, chloride and nitrate, respectively. For nitrite and nitrate the UV detector exhibited similar sensitivity, with detection limits of 0.39 and 0.27  $\mu$ M (17.9 and 16.7  $\mu$ g/l).

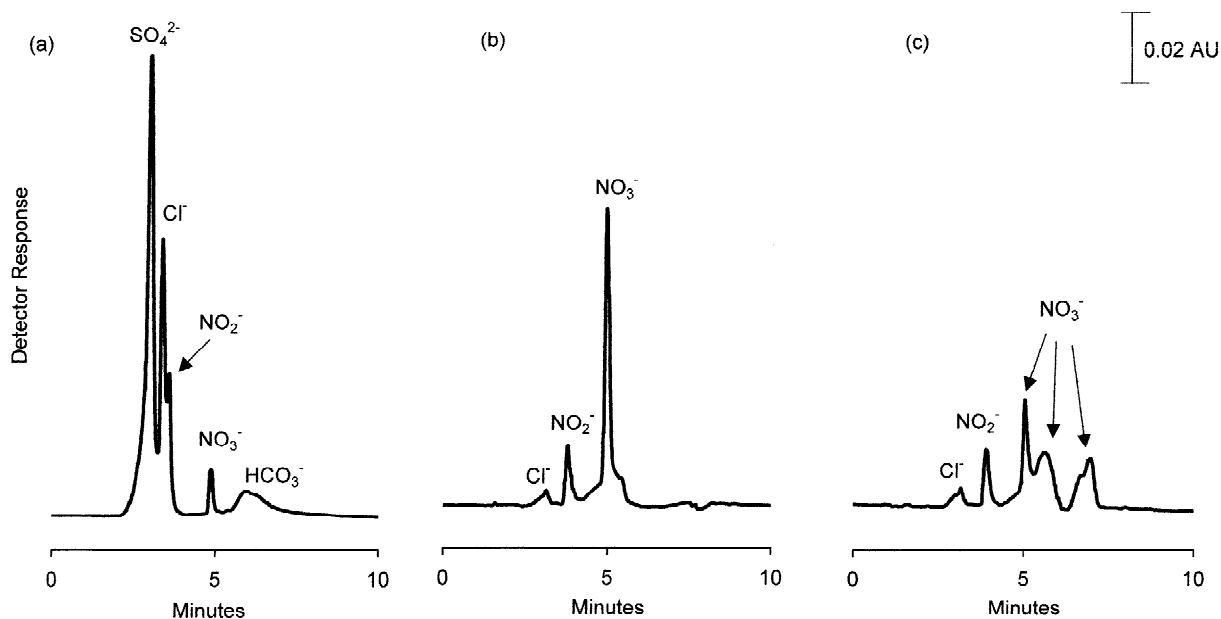


Fig. 5. Chromatograms of a river water sample (a) after passage through sodium exchange cartridge, 2 mM Zwittergent 3–14 as the mobile phase, with conductivity detection, (b) as (a) with UV detection, (c) chromatogram of a river water sample without passage through exchange cartridge with UV detection.



These detection limits are approximately 10 times higher than those that can be obtained using suppressed IC.

However, during the study into detection limits an interesting aspect of the recycling system was noted. It was found that the use of the post-detection ion-exchange cartridges effectively acted to not only remove analyte ions but also trace levels of ionic impurities present in the prepared mobile phase. In this study it was found that traces of chloride and to a lesser degree sulphate and nitrate were present in the Milli-Q deionised water used to prepare the mobile phase. Therefore, when this source of deionised water was injected as a blank solution, these impurities could be seen, even though the same water had been used as the mobile phase. This rather interesting effect meant that if a fresh mobile phase were prepared and a blank injected, no impurity peaks would be seen. However, as the mobile phase began to pass through the system and back into the mobile phase reservoir, having been effectively 'cleaned-up', these impurity peaks began to appear and continuously increased in size until effectively all of the impurities had been removed from the mobile phase. The chromatograms shown in Fig. 6 show the levels of the above anionic impurities in the water supply. The major contaminant, namely chlo-

ride, was found to be originating from a disinfection procedure used with the Milli-Q system. The approximate concentrations of the anions shown were found to be  $<0.10 \mu\text{M}$  sulphate,  $2.82 \mu\text{M}$  chloride and  $0.29 \mu\text{M}$  nitrate.

The chromatograms shown in Fig. 6 also illustrate how when using either a water only or zwitterionic mobile phase, retention times in ZIC are related to analyte concentration, with reduced retention being seen when the analyte ions are at trace concentrations (in this case leading to the co-elution of sulphate and chloride). The effect of analyte concentration upon retention times has been noted previously [2], although no detailed studies on this phenomenon have been published. However, this effect is not a significant problem if peak areas are always used for quantification calculations, although spiking of real samples with the ions of interest is required to assign peaks.

### 3.5. Analysis of saline samples

In a number of recent articles by Hu and co-workers [15–17] ZIC has been applied to the analysis of saline samples. Due to the unique retention mechanism involved in ZIC and the unusual selectivity exhibited by the zwitterionic stationary phase (particularly the low retention of chloride), the direct determination of UV absorbing anions in seawater has been shown. Using a Zwittergent 3–14 coated ODS column and a mobile phase consisting of a 20-fold dilution of an artificial seawater solution, bromide, nitrate and iodide could be determined directly in seawater samples. However, the retention time for iodide was rather excessive at 30 min. In later work this retention time was reduced to only six min using a  $0.2 \text{ mM}$  perchlorate– $0.3 \text{ mM}$  Zwittergent 3–14 mobile phase. In this work we decided to investigate if such samples could be analysed with our recycling system, without the addition of an electrolyte to the mobile phase and without excessive run times.

The determination of iodide in iodised table salt was used to illustrate the capacity of the developed method for saline samples. With the  $2 \text{ mM}$  Zwittergent 3–14 mobile phase iodide eluted at approximately 10 min, well resolved from other common anions and less common UV absorbing

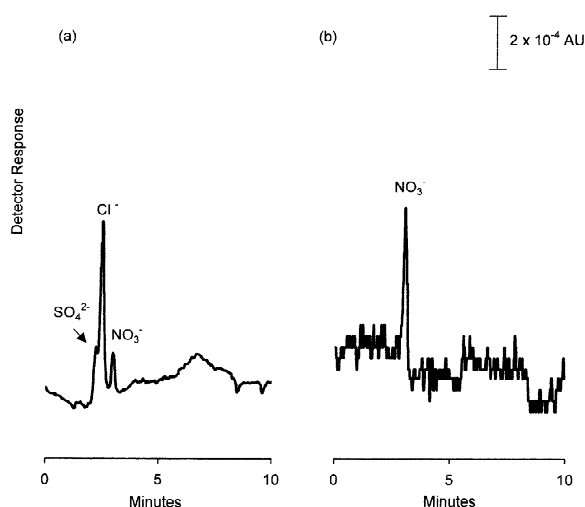


Fig. 6. Chromatograms showing the presence of anionic impurities in Milli-Q deionised water sample.  $2 \text{ mM}$  Zwittergent 3–14 as mobile phase, (a) conductivity detection, (b) UV detection.

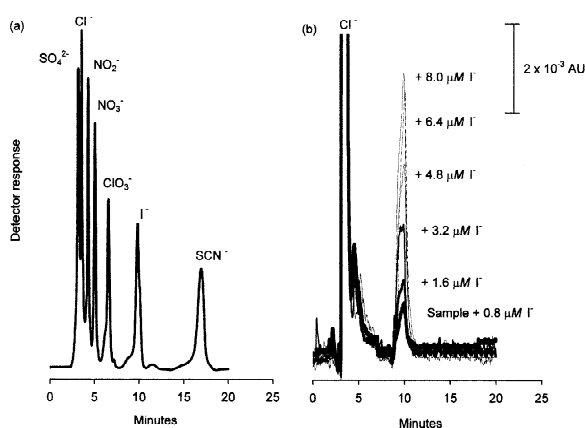


Fig. 7. Chromatogram showing (a) the separation of sulphate, chloride, nitrite, nitrate, chlorate, iodide and thiocyanate (sodium form, concentrations between 0.15 and 0.5 mM), 2 mM Zwittergent 3–14 as mobile phase with conductivity detection, (b) 20 g/l NaCl sample spiked with between 0.8 and 8.0  $\mu\text{M}$  iodide, 2 mM Zwittergent 3–14 as mobile phase with UV detection.

anions, such as thiocyanate. Fig. 7 shows the chromatograms obtained from (a) a standard mixture of anions (conductivity detection) and (b) a 20 g/l solution of iodised table salt spiked with 0.8 to 8  $\mu\text{M}$  of iodide (UV detection). The standards used were prepared from their respective sodium salts and as the sample was NaCl based, there was no need in this case to apply the cation-exchange procedure described in Section 3.1. The chromatograms in Fig. 7 show how the retention time of iodide was remarkably unaffected by the high salinity sample matrix. Indeed unlike the results discussed in Section 3.4, Fig. 7b shows that the retention time for iodide at low levels in a high ionic strength sample is the same as that obtained for a relatively high concentration of iodide in a low ionic strength standard (Fig. 7a). This indicates that the high concentration of matrix ions in the sample facilitate in some way an increased interaction of the analyte ions with the zwitterionic stationary phase, in what could be described as the reverse of what is termed 'self elution' in conventional IC. To determine if this was the case a sample solution was prepared containing 100 g/l of the iodised salt and analysed using the developed method. It was found that the retention time of iodide (which was calculated using standard addition to be present at a concentration of 3.07  $\mu\text{M}$

in the 100 g/l solution,  $R^2=0.9987$ ,  $n=6$ ), did indeed increase further to approximately 12 min. Even though the peak shape for iodide was relatively broad (2 min), it was well resolved from the matrix chloride peak, and there are few other LC methods that would produce a quantifiable peak for this concentration of analyte from the direct injection of a sample containing 10% NaCl.

#### 4. Conclusions

The use of water only or zwitterionic mobile phases in ZIC allow simple eluent recycling procedures to be used, such as the post-detector ion exchangers employed in this study. The results obtained here show that for a selected number of analyte anions, this approach can be used to obtain both precise and accurate results in various types of water samples. The system described has obvious potential for long term monitoring applications, with on-going studies already showing how the system can be left recycling and analysing samples for up to 3 months on as little as 200 ml (or less) of mobile phase.

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