

# Zwitterionic ion chromatography with carboxybetaine surfactant-coated particle packed and monolithic type columns

Colmán Ó Ríordáin<sup>a</sup>, Pavel Nesterenko<sup>b</sup>, Brett Paull<sup>a,\*</sup>

<sup>a</sup> National Centre for Sensor Research, School of Chemical Sciences, Dublin City University, Glasnevin, Dublin 9, Ireland

<sup>b</sup> Department of Analytical Chemistry, Lomonosov Moscow State University, Moscow 119899, Russian Federation

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## Abstract

Both particle packed (25 cm × 0.46 cm I.D. SUPELCOSIL 5 μm C<sub>18</sub>) and monolithic type (10 cm × 0.46 cm I.D. Merck Chromolith Performance C<sub>18</sub>) reversed-phase substrates were dynamically coated with a carboxybetaine type zwitterionic surfactant ((dodecyldimethyl-amino) acetic acid) and investigated as stationary phases for use in zwitterionic ion chromatography (ZIC). Investigations into eluent concentration and pH were carried out using KCl eluents containing 0.2 mM of the carboxybetaine surfactant to stabilise the column coatings. It was found that eluent concentration decreased anion retention whilst simultaneously increasing peak efficiencies, which may be due to the dissociation of intra- and inter-molecular salts of the carboxybetaine surfactant under higher ionic strength conditions. The Effect of eluent pH was an increase in anion retention with decreased eluent pH due to the increased protonation of the weak acid terminal group of the carboxybetaine, causing both a relative increase in the positive charge of the stationary phase and less repulsion of the anions by the dissociated weak acid group. The carboxybetaine-coated monolithic phase was applied to rapid anion separations using elevated flow rates and flow rate gradients. © 2005 Elsevier B.V. All rights reserved.

**Keywords:** Zwitterionic ion chromatography; Carboxybetaine; Monolithic columns; Flow gradients

## 1. Introduction

Since the first publication on the application of zwitterionic stationary phases to the separation of small ions in 1991 [1], the development of new zwitterionic stationary phases for ion chromatography has received considerable attention [2–14]. This has effectively led to the establishment of a new mode of IC, initially termed ‘electrostatic ion chromatography (EIC)’ [3], but later more correctly named as ‘zwitterionic ion chromatography (ZIC)’ [4], and less commonly known as ‘hydrophilic interaction chromatography’ (HILIC) [5]. As the basis of ZIC is the presence of a zwitterionic stationary phase (commonly a zwitterionic surfactant-coated reversed-phase substrate), rather than a simple anionic or cationic exchange surface, well-documented retention theory based upon simple ion exchange does not directly apply.

Therefore, a number of groups have proposed general mechanisms for understanding the unusual selectivities exhibited for small anions and cations by zwitterionic phases [6,15–17]. However, if one considers the zwitterionic surfactant itself, there would appear to be four obvious parameters which influence selectivity in ZIC and investigations to evaluate their exact contribution toward selectivity are still required. The parameters involved are: (i) the length of the hydrophobic tail region (which will impact upon coating stability and column capacity), (ii) the spacing between the cationic and anionic groups within the immobilised zwitterion, (iii) the order of the two ionic groups within the molecule (which governs which ionic groups are ‘shielded’ from analyte ions by the oppositely charged sites, and (iv), the relative strength of the two ionic groups (e.g. strong acid and strong base, weak acid and weak base, strong acid and weak base or weak acid and strong base). In relation to the latter parameter, a large amount of work has been focussed on strong-strong type surfactants [3,4,6–8,15]. However, recently Hu et al. investigated

\* Corresponding author. Tel.: +353 1 7005060; fax: +353 1 7005503.  
E-mail address: [brett.paull@dcu.ie](mailto:brett.paull@dcu.ie) (B. Paull).

a carboxybetaine type zwitterionic reagent to coat a reversed-phase column and applied the resultant phase to anion separations [18]. The rather complex reagent used to coat the column, namely 3-(heptadecafluorooctylsulfonylamino)-*N,N*-dimethylpropanammonioethyl-carboxylate, ( $C_8F_{17}SO_2NHC_3H_6N^+(CH_3)_2-C_2H_4-COO^-$ ), contained a strong quarternary ammonium group and a weak carboxylic acid terminal group. It was found that when using this carboxybetaine type column, no retention of inorganic anions was seen with  $NaHCO_3$  eluents, pH  $\sim 8.5$  (from 1.0 to 30 mM). This was presumably due to strong repulsion effects of the terminal carboxylic acid group at this pH. When the authors switched to dilute strong acid eluents, such as 1 mM  $H_2SO_4$ , they recorded very strong retention of the inorganic anions investigated with a selectivity and retention order similar to that of standard anion exchange separations.

However, the above mentioned surfactant is not an ideal carboxybetaine for use in ZIC as it contains an additional polar functional sulfonylamido group which is affected by strong polarization from the heptadecafluorooctyl radical, that could affect upon the ion-exchange properties of the resulting substrate. In addition to this, the two methylene groups between the oppositely charged ammonium and carboxyl functionalities provides the molecule with some degree of unwanted flexibility and hence the possibility for undesirable inter- and intra-molecular interactions [10]. These drawbacks, coupled with the complexity and high cost of the above surfactant, prompted the following investigations into the use of a simple carboxybetaine type surfactant that could be used to produce a dynamically coated zwitterionic ion exchanger. It was envisaged that the resultant carboxybetaine-coated phase would exhibit retention characteristics of a strong anion exchanger under acidic conditions, whilst at the same time allowing the use of pH gradients to control effective capacity and retention. The surfactant investigated was (dodecyldimethyl-amino) acetic acid, which contains a single methylene group between the two oppositely charged ionic sites, unlike the surfactant used before [18].

It was hoped that the adjustable capacity zwitterionic phase produced in this study would be analogous to the adjustable-capacity cryptand anion-exchangers described by Lamb and Smith [19], and recently released to the market by Dionex Ltd. [20]. This would give an added variable to control column selectivity, whilst at the same time furthering the understanding of the exact retention mechanism on carboxybetaine type columns. Two types of reversed-phase column were investigated for coating with the above surfactant, these being a standard 25 cm  $5\ \mu m$   $C_{18}$  silica particle packed column, and a 10 cm  $C_{18}$  modified monolithic silica column. With the latter coated monolithic column, elevated flow rates and flow rate gradients were investigated for rapid anion separations. To illustrate a potential application the two carboxybetaine-coated columns were applied to the analysis of coastal seawater samples.

## 2. Experimental

### 2.1. Apparatus

The chromatographic system used consisted of a Dionex Model DX-100 ion chromatograph (Dionex, Sunnyvale, CA, USA), fitted with a 20  $\mu L$  injection loop and coupled to a Shimadzu SPD-6AV UV-Vis Spectrophotometric Detector (Kyoto, Japan), monitoring at 214 nm. In later work involving flow gradients, an Applied Biosystems 400 Solvent Delivery System (Foster City, CA 94404, USA) was used to deliver the eluent. The pH of eluents was measured using an Orion Model 420 pH meter (Thermo Orion, Beverly, MA, USA) with a glass electrode. Processing of chromatograms was performed using a PeakNet 6.30 chromatography workstation (Dionex). Peak efficiencies were calculated using  $N = 16 t_r^2/w^2$  and retention factors determined using  $t_0$  taken as the start of the injection dip.

### 2.2. Reagents

The carboxybetaine-type zwitterionic surfactant used to dynamically coat the stationary phase was (dodecyldimethyl-amino) acetic acid ( $C_{12}H_{25}N(CH_3)_2CH_2COOH$ ), and was synthesised in-house according to a procedure of Tanaka and Kato [21]. All other chemicals used were of analytical reagent grade, and were supplied by Sigma-Aldrich (Tallaght, Dublin, Ireland). All reagent and standard solutions were prepared using deionised water from a Millipore Milli-Q water purification system (Bedford, MA, USA). All eluents and standard solutions were filtered through a 0.45  $\mu m$  filter and degassed by sonication. Dilute solutions of NaOH and HCl were used to adjust the pH of the eluents.

### 2.3. Column preparation

The particle packed separation column was prepared by coating a SUPELCO SIL LC-18-DB reversed-phase  $C_{18}$  column (250 mm  $\times$  4.6 mm I.D.; 5  $\mu m$  spherical particle size, 100  $\text{\AA}$  pore size, surface area = 170  $m^2/g$ , pore volume = 0.6 mL/g, carbon load = 11%, obtained from Supelco, Belafonte, PA, USA) with the carboxybetaine surfactant, by passing 100 mL of a 20 mM aqueous solution of the surfactant through the column at a flow-rate of 1.0 mL/min for a period of >60 min. The monolithic type separation column was a Chromolith Performance RP-18e monolithic reversed-phase  $C_{18}$  column (100 mm  $\times$  4.6 mm I.D., macropore size = 2  $\mu m$ , mesopore size = 13 nm, surface area 300  $m^2/g$ , pore volume = 1 mL/g, total porosity > 80%, obtained from Merck, Darmstadt, Germany), and was also coated with the zwitterionic surfactant using the same method. Following initial coating the columns were then conditioned with eluents until a steady baseline was achieved. To stabilise and maintain a constant amount of the carboxybetaine surfactant on the surface of the stationary phase, all the eluents used always contained 0.2 mM of the surfactant. This maintained surface

coverage and stopped capacity drop-off due to column bleed, resulting in greatly improved day-to-day reproducibility of <5% RSD for peak retention times. Columns were run at room temperature for the duration of the study.

### 3. Results and discussion

#### 3.1. Carboxybetaine-coated 25 cm particle packed $C_{18}$ column

##### 3.1.1. Effect of electrolyte additives to the eluent

A simple inorganic salt (KCl) was used to evaluate the effect of electrolyte concentration upon anion retention and peak efficiency using the carboxybetaine column. The electrolyte concentration was varied from 10 to 600 mM KCl (including 0.2 mM carboxybetaine) and the retention times and peak efficiencies for nitrate, bromide, nitrite and iodide determined. A plot of  $\log k$  against  $\log C_{\text{KCl}}$  was found to be linear over this concentration range (shown as Fig. 1(a)), with slopes ranging from between  $-0.617$  for iodide and  $-0.681$  for nitrate. However, interestingly each slope was considerably smaller than the expected slope of  $-1$  for a simple ion exchange mechanism. It is proposed that such a retention dependence originates from two simultaneous effects, similar to those recently described for another zwitterionic ion exchanger, namely poly(aspartic acid) modified silica [22]. First, the addition of small amounts of KCl to the eluent cause some increase of ion-exchange capacity of the zwitterionic ion-exchanger due to the destruction of intra- and inter-molecular salt structures, present at low ion strength between oppositely charged groups. At the same time, increasing electrolyte concentration causes the expected decrease in retention in agreement with simple exchange theory. Combination of these two processes will result in the effect of electrolyte concentration upon retention being less than that expected for simple ion-exchange, as shown here.

In addition to the above effect upon retention, increases in eluent KCl concentration also affected the observed separation efficiency for zwitterionic stationary phase. A plot of the effect of KCl concentration upon peak efficiency showed considerable improvements in peak shapes could be obtained over the KCl concentration range 10–100 mM (shown as Fig. 2(a)).

It is expected that these improvements are once again related to structural form of the carboxybetaine molecule on the surface of the  $C_{18}$  phase. It is probable that the low eluent concentrations carboxybetaine molecules arrange themselves as: (a) simple intra-molecular salts (least favoured due to single methylene group), (b) simple inter-molecular salts, or (c) micelles. In practice the surface may contain a mixture of each of the above at low ionic strength, which may result in poor ion exchange kinetics. Increasing the eluent concentration would eliminate these molecular salt forms/arrangements of the molecule on the surface and this may be the cause of the observed increases in exchange efficiencies.

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##### 3.1.2. Effect of eluent pH

It was shown that with a carboxybetaine type ion exchanger, the strong affinity of the weak acid group for  $\text{H}^+$

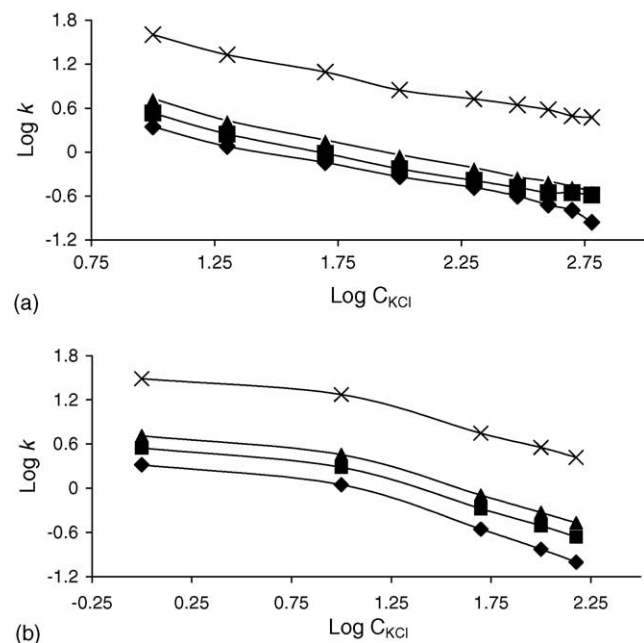


Fig. 1. Effect of eluent KCl concentration upon retention factor ( $k$ ) for carboxybetaine-coated: (a) 25 cm particle packed reversed-phase column; flow rate = 0.5 mL/min. (b) 10 cm monolithic reversed-phase column; flow rate = 2.0 mL/min. Other eluent conditions: 0.2 mM carboxybetaine, pH 6. ( $\times$ )  $\text{I}^-$ , ( $\blacktriangle$ )  $\text{NO}_3^-$ , ( $\blacksquare$ )  $\text{Br}^-$ , ( $\blacklozenge$ )  $\text{NO}_2^-$ .

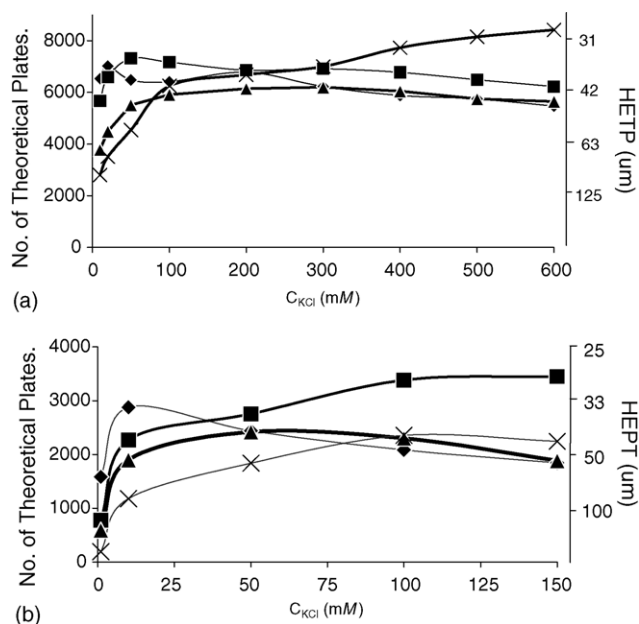


Fig. 2. Effect of eluent KCl concentration upon efficiency for carboxybetaine-coated: (a) 25 cm particle packed reversed-phase column; (b) 10 cm monolithic reversed-phase column. Other conditions: as Fig. 1. ( $\times$ )  $\text{I}^-$ , ( $\blacktriangle$ )  $\text{NO}_3^-$ , ( $\blacksquare$ )  $\text{Br}^-$ , ( $\blacklozenge$ )  $\text{NO}_2^-$ .

means eluent pH can be used to affect anion retention, as this governs the degree of protonation of the weak carboxylic acid group [18]. A reduction in the number of dissociated carboxylate groups would mean the ‘repulsion’ effect experienced by anionic species at the terminal site on the surfactant would be reduced, hence, the relative attraction to the strong anion exchange site would be increased. However, in this work the surfactant was included in the eluent as well as being pre-coated onto the stationary phase, in order to stabilise the coating. This meant that an increase in retention resulting from a decrease in eluent pH could be partially offset by an increase in the attraction between the analyte anions and the protonated carboxybetaine in the eluent. To determine if this was the case, the pH of a 150 mM KCl eluent, containing 0.2 mM of the carboxybetaine surfactant, was systematically varied over the pH range 3.5–6.5 ( $n = 7$ ), and the retention of nitrite, nitrate, bromide and iodide determined under each condition. Fig. 3 shows the results obtained.

A number of observations can be made from the results obtained. Firstly, it is clear from the data shown that the effect of eluent pH upon the retention of nitrate, bromide and iodide is similar, and there is indeed the expected reduction in retention with increasing eluent pH. However, this effect is considerably smaller than that shown by other investigators, who reported slopes of close to  $-0.83$  for  $\log k$  versus pH (pH range from 5.0 to 5.6) for nitrite, nitrate, bromide, iodide and thiocyanate (using a 10 mM acetate eluent) [18]. In our work, over a much larger pH range (pH 3.5–6.5), slopes of between  $-0.042$  and  $-0.053$  ( $\log k$  versus pH) were recorded for nitrate, bromide and iodide (with iodide being most affected). At pH values  $<6.5$ , thiocyanate was exceptionally strongly retained on the column ( $>60$  min) and could only be eluted at higher pHs. This large difference in the pH effect would indicate that, under the conditions used, the effect of the carboxybetaine in the eluent is relatively large compared to a system where the zwitterions are only present in the stationary phase. The second point of significant interest is the non-linear behaviour of nitrite. From the graph it can be seen that the slope for the retention of nitrite was similar to the

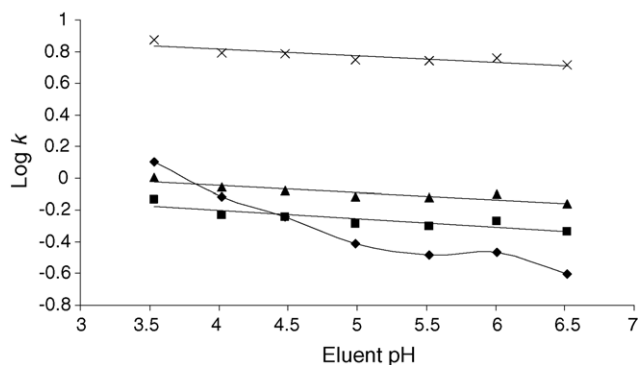


Fig. 3. Effect of eluent pH upon retention factor ( $k$ ) for a carboxybetaine-coated 25 cm particle packed reversed-phase column. Other eluent conditions: 150 mM KCl, 0.2 mM carboxybetaine. Other conditions: as Fig. 1. ( $\times$ )  $I^-$ , ( $\blacktriangle$ )  $NO_3^-$ , ( $\blacksquare$ )  $Br^-$ , ( $\blacklozenge$ )  $NO_2^-$ .

above anions only above pH  $\sim 5$ , and that below this pH retention began to increase more rapidly, clearly due to the low dissociation constant of  $HNO_2$ . This effect is potentially very useful, as is shown in Section 3.3.

During the investigation into the effect of pH upon retention it was noted that, as with eluent concentration, pH also had a significant affect upon peak efficiency, as shown in Fig. 4.

The exact reason for such an effect is not totally understood, although it would be expected that for an adsorbed zwitterion, which contains a weak acid site, the protonation and deprotonation of that site would again result in a physical change in the orientation of the adsorbed molecule. However, as the maximum efficiency was seen between pH 5 and 6 (above expected  $pK_a$  of carboxylic acid group), and the eluent used contained 150 mM KCl (limiting the formation of inner salts), an exact reason for this observed behaviour is unclear and the subject of further investigation.

### 3.1.3. Separation achieved under optimal conditions/pH gradient

The ability to use pH to selectively alter retention offers some interesting possibilities [23], and so here the use of a pH gradient separation was investigated. It was clear from the pH study above that the use of such a gradient should not only allow the complete separation of all the UV absorbing anions investigated, namely bromide, nitrate, nitrite, iodide and thiocyanate, in a much reduced overall run time, but would also result in a change in elution order for the nitrite, bromide and nitrate. Fig. 5(a) shows the optimal separation of nitrite, bromide, nitrate and iodide obtained using a 150 mM KCl, 0.2 mM carboxybetaine eluent adjusted to pH 6.0, at a flow rate of 0.5 mL/min (thiocyanate completely retained).

An attempt to facilitate the elution of thiocyanate, by simply increasing the pH of the eluent to pH 7 or 8, led to the reduction in the resolution of the early eluting peaks of nitrite, bromide and nitrate. Therefore, a pH gradient from pH 3 to 8 over the first 2.5 min was investigated (using the same eluent, except adjusted to either pH 3 or 8 using the addition of 10 mM phosphate buffer). This resulted in the expected

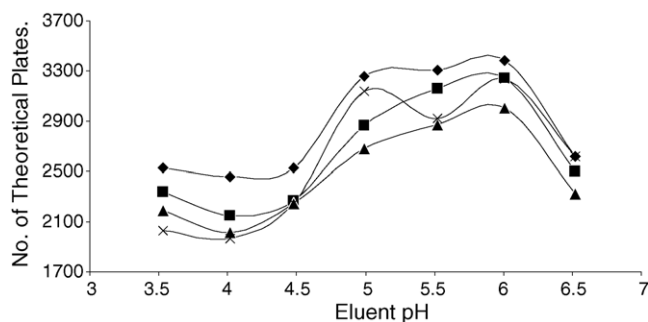


Fig. 4. Effect of eluent pH upon efficiency for a carboxybetaine-coated 25 cm particle packed reversed-phase column. Other eluent conditions: 150 mM KCl, 0.2 mM carboxybetaine. Other conditions: as Fig. 1. ( $\times$ )  $I^-$ , ( $\blacktriangle$ )  $NO_3^-$ , ( $\blacksquare$ )  $Br^-$ , ( $\blacklozenge$ )  $NO_2^-$ .

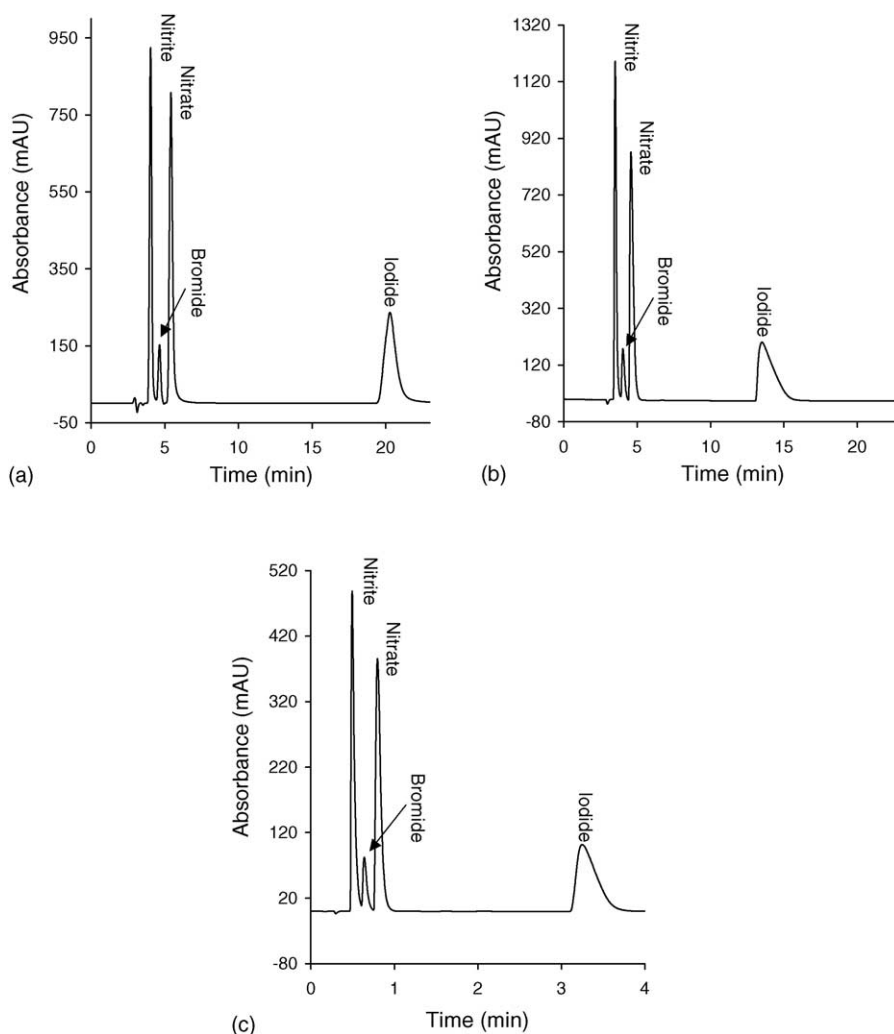


Fig. 5. Separation of a 1 mM mixture of nitrite, bromide, nitrate and iodide on: (a) a carboxybetaine-coated 25 cm particle packed reversed-phase column. Eluent = 150 mM KCl, 0.2 mM carboxybetaine, pH 6, flow rate = 0.5 mL/min; (b) a 10 cm carboxybetaine-coated monolithic reversed-phase column. Eluent = 10 mM KCl, 0.2 mM carboxybetaine, pH 6, flow rate = 0.5 mL/min, (c) a 10 cm carboxybetaine-coated monolithic reversed-phase column. Eluent = as (b), flow rate = 4.5 mL/min.

change in elution order for the first three peaks, with nitrite now eluting last, and also resulted in a slight increase in the retention of iodide, which was due to the low starting pH. However, the gradient did produce the desired elution of thiocyanate, although at a flow rate of 0.5 mL/min the retention time of thiocyanate was still  $\sim 100$  min. Increasing the eluent flow rate to 2.0 mL/min saw this reduced to only 24 min, and produced the separation of all five analytes shown as Fig. 6(b). Fig. 6(a) shows the separation at pH 6 only for comparison. The peak shapes for the iodide and thiocyanate peaks are particularly improved through using the pH gradient, although a system peak does result prior to but separated from iodide.

### 3.2. Carboxybetaine-coated 10 cm monolithic $C_{18}$ column

Given the long retention of iodide and excessive retention of thiocyanate on the above particle packed column (even

with the pH gradient), the use of a short monolithic silica type reversed-phase column was investigated, which could be used at higher flow rates and so reduce runs times further. A 10-cm monolithic column was initially coated with the carboxybetaine in the same way as the particle packed column, and a small amount (0.2 mM) of the surfactant was again added to all the eluents used. Initial studies on pH effects and eluent concentration showed similar trends to those observed with the particle packed column, although the relative capacity of the coated monolith was considerably lower. A study into the effect of electrolyte concentration (KCl) over the range 1 to 150 mM was carried out and the resultant effects upon retention and peak efficiency can be seen in comparison to the particle packed column in Figs. 1(b) and 2(b) respectively. These results matched reasonably well with those seen on the particle packed phase. However, due to the lower capacity of the monolithic phase, the investigation into the effect of electrolyte concentration was carried out over a lower con-

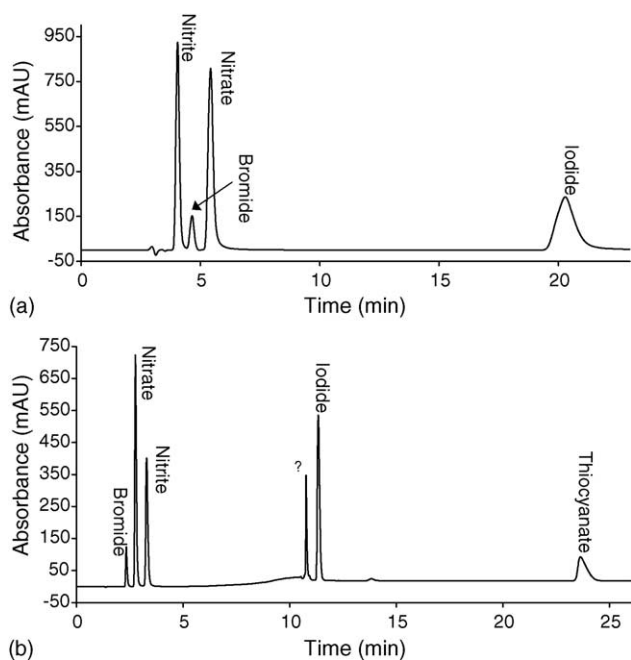


Fig. 6. Separation of inorganic anions using: (a) a carboxybetaine-coated 25 cm particle packed reversed-phase column. Eluent = 150 mM KCl, 0.2 mM carboxybetaine, pH 6, flow rate = 0.5 mL/min; (b) as (a) with eluent pH increased from 3 to 8 between  $t = 0$  and 2.5 min (using 10 mM phosphate buffer), flow rate = 2.0 mL/min.

centration range with interesting results. These being that, as shown in Fig. 1(b), the previously mentioned counter-acting effect of an increase in retention due to intra- and inter-salt destruction (Section 3.1.1), is clearly more evident, with a deviation from linearity below 10 mM KCl.

It was found that an eluent of 10 mM KCl produced acceptable resolution and peak shapes for the nitrite, bromide, nitrate and iodide peaks, and as with the particle packed column, the optimum pH was also  $\sim 6$ . Using these eluent conditions the separation shown as Fig. 5(b) was obtained with the monolithic column at a flow rate of 0.5 mL/min. However, the advantage of the monolithic phase is the ability to run elevated flow rates and so the chromatogram shown as Fig. 5(c) was obtained at an eluent flow rate of 4.5 mL/min. Under these conditions all four analytes are still baseline resolved, removed from the eluent dip and all eluted in under 4 min.

### 3.2.1. Use of flow gradients

A further option made possible through the use of monolithic type stationary phases is that of using flow gradients to affect peak elution times. This option is particularly useful to speed the elution of isolated late eluting peaks, whilst leaving eluting peaks unaffected both in terms of resolution and peak area (peak areas are reduced at elevated flow rates). Until now the use of flow gradients have been only rarely used in LC [24] and IC [25], and then only at low to moderate flow rates (between 0.2 and 1.0 mL/min). Here, a mixed standard containing nitrite, bromide, nitrate and iodide was run un-

der isofluentic and gradient flow conditions and the resultant chromatograms compared. The isofluentic run was carried out at 4.5 mL/min. The gradient flow run began at 2 mL/min and increased to 5 mL/min between  $t = 2$  and 3 min. The resultant chromatograms are shown as Fig. 7.

The effect of maintaining a standard flow rate upon peak area for the early eluting peaks can be clearly seen, as can an improvement in the peak efficiency of the latter eluting iodide peak. Using  $N = 16t_r/w^2$  the value obtained for the iodide peak at a constant flow rate of 4.5 mL/min is  $N = 332$ . Using the flow gradient up to 5.0 mL/min, this improved to  $N = 566$ .

Although here the test mixture of anions is rather simple, the results clearly illustrate an advantage in the use of the monolithic phases with flow gradients for reducing run times. This can be achieved without the requirement for multiple solutions as in traditional eluent gradient separations, and can also improve efficiency significantly.

### 3.2.2. Combined pH and flow gradients

Clearly with the combination of the monolithic column and the carboxybetaine coating, an opportunity exists for the use of a combined flow and pH gradient.

As with the particle coated column increasing the eluent pH above 6 decreased the retention of thiocyanate markedly. Therefore, a pH gradient from pH 6 to 8 (from  $t = 0$  to 5 min)

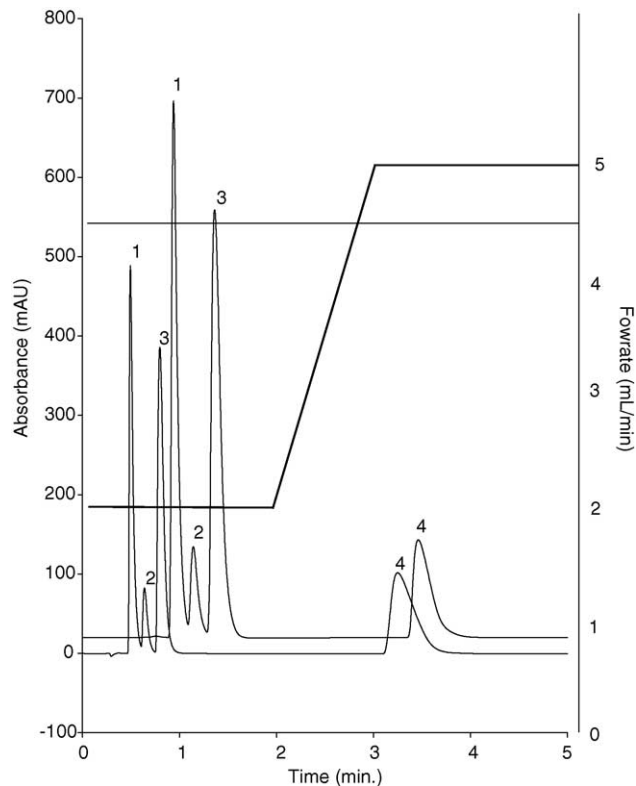


Fig. 7. Separation of nitrite (1), bromide (2), nitrate (3) and iodide (4) on a 10 cm carboxybetaine-coated monolithic reversed-phase column under isofluentic conditions at 4.5 mL/min (light trace), and flow gradient conditions beginning at 2 mL/min and increased to 5 mL/min between  $t = 2$  and 3 min (bold trace). Other conditions: as Fig. 5(b).

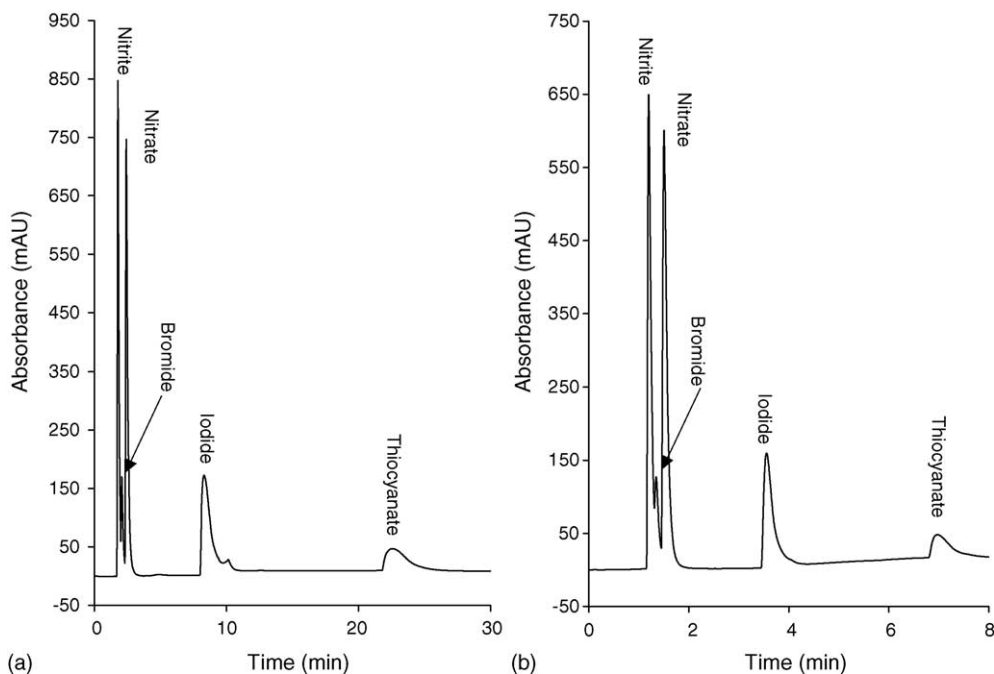


Fig. 8. Separation of inorganic anions on a 10 cm carboxybetaine-coated monolithic reversed-phase column using: (a) a pH gradient from pH 6 to 8 between  $t = 0$  and 5 min (using 10 mM phosphate buffer). Other eluent conditions as Fig. 5(b). Flow rate = 4.5 mL/min; (b) as (a) with flow gradient from 1.0 to 5.0 mL/min over  $t = 0$ –5 min.

was first investigated. At an eluent flow rate of 2.0 mL/min, this resulted in the chromatogram shown as Fig. 8(a).

An increasing eluent pH resulted saw a slight reduction in the resolution of the early peaks, as expected, but facilitated the elution of the otherwise totally retained thiocyanate. Next, whilst maintaining the same pH gradient, a flow gradient was introduced from 1.0 up to 5.0 mL/min over the first 5 min. The resultant chromatogram is shown overlaid within Fig. 8 (b). Under these conditions all five anions are eluted under 8 min. Significantly, once again an improvement in the efficiency was noticeable ( $N = 463$  for thiocyanate at 2.0 mL/min isofluent conditions,  $N = 1314$  for thiocyanate at 5.0 mL/min gradient flow).

### 3.3. Application to the analysis of nutrients in seawater samples

Considerable interest in zwitterionic stationary phases has arisen due to their compatibility with certain complex matrices, most significant of which are saline samples [26]. Here, we applied to the two types of carboxybetaine-coated column to the analysis of nitrate and nitrite in saline estuarine and coastal seawater samples. Neither pH nor flow gradients were used in this application as the peaks of interest eluted in under 10 min on both columns under the isocratic eluent condition used. A number of seawater and saline estuarine samples were collected from within the north coastal region of Dublin Port, Ireland. With the particle packed carboxybetaine column these samples were injected directly using the eluent detailed in Section 3.1.3 (namely using a 150 mM

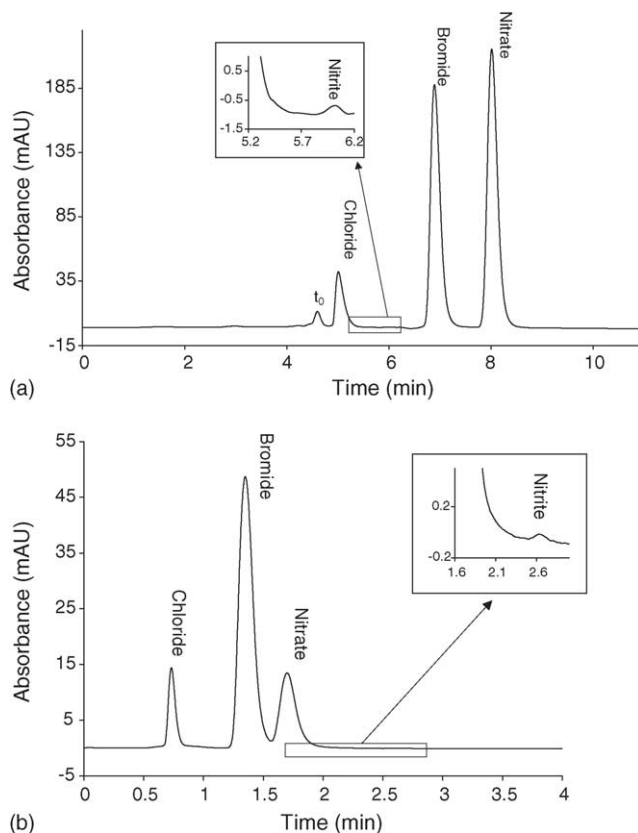


Fig. 9. Inorganic UV absorbing anions in seawater samples analysed using: (a) a carboxybetaine-coated 25 cm particle packed reversed-phase column. Eluent conditions: as Fig. 5(a); (b) a carboxybetaine-coated 10 cm monolithic reversed-phase column. Eluent = 40 mM KCl, 0.2 mM carboxybetaine, pH 3.5, flow rate = 2.0 mL/min.

KCl, 0.2 mM carboxybetaine eluent adjusted to pH 6.0, at a flow rate of 0.5 mL/min). With the lower capacity monolithic phase, using the 10 mM KCl, 0.2 mM carboxybetaine eluent (pH 6.0), it was found that the saline matrix (0.52 M NaCl) caused unacceptable peak broadening and poor resolution of the nitrite, bromide and nitrate peaks. To remedy this the eluent KCl concentration was increased to 40 mM to limit peak broadening and the eluent pH was reduced to 3.5 to increase analyte retention. The combination of the effects allowed the direct injection of the seawater sample with excellent resolution of the analyte peaks. As expected the differing eluent pH values resulted in different elution orders for the analyte peaks on the two columns, although this affect gave the added advantage of verifying the presence of trace nitrite in the seawater samples tested. Fig. 9 shows the chromatograms obtained from the injection of (a) a coastal seawater/estuary sample (Portmarknock Estuary, Dublin), using the particle packed carboxybetaine column, and (b) a coastal seawater sample (North Bull Island, Dublin) using the monolithic carboxybetaine column.

The concentration of nitrate found in the estuarine sample was 16.8 mg/L (a) and 2.0 mg/L in seawater sample (b). Nitrite was found to be present in both samples, as shown, but below the quantifiable limit. With the monolithic carboxybetaine column, nitrate, bromide and nitrite levels could be determined in undiluted seawater samples in under 3 min.

#### 4. Conclusions

Carboxybetaine-coated C<sub>18</sub> columns show unique anion exchange selectivity, which differs to that seen previously with alternative zwitterionic surfactant-coated phases. Both capacity and efficiency are dependent upon eluent pH and concentration, giving new options for manipulation of analyte retention. With the use of a monolithic type C<sub>18</sub> phase, new

approaches to reduce run times and control selectivity can also be used, such as individual and combined flow and pH gradients.

#### References

- [1] P.N. Nesterenko, J. High. Resolut. Chromatogr. 14 (1991) 767.
- [2] P.N. Nesterenko, J. Chromatogr. 605 (1992) 192.
- [3] W. Hu, T. Takeuchi, H. Haraguchi, Anal. Chem. 65 (1993) 2204.
- [4] H.A. Cook, G.W. Dicoski, P.R. Haddad, J. Chromatogr. A 997 (2003) 13.
- [5] T. Jonsson, P. Appelblad, LC-GC Europe 17 (2004) 40.
- [6] W. Hu, P.R. Haddad, K. Hasebe, K. Tanaka, P. Tong, C. Khoo, Anal. Chem. 71 (1999) 1617.
- [7] T. Umemura, S. Kamiya, A. Itoh, K. Chiba, H. Haraguchi, Anal. Chim. Acta 349 (1997) 231.
- [8] E. Twohill, B. Paull, J. Chromatogr. A 973 (2002) 103.
- [9] W. Jiang, K. Irgum, Anal. Chem. 71 (1999) 333.
- [10] P.N. Nesterenko, P.R. Haddad, Anal. Sci. 16 (2000) 565.
- [11] W. Jiang, K. Irgum, Anal. Chem. 73 (2001) 1993.
- [12] P.N. Nesterenko, A.I. Elefterov, D.A. Tarasenko, O.A. Shpigun, J. Chromatogr. A 706 (1995) 59.
- [13] W. Jiang, K. Irgum, Anal. Chem. 74 (2002) 4682.
- [14] M.G. Kiseleva, P.A. Kebets, P.N. Nesterenko, Analyst 126 (2001) 2119.
- [15] H.A. Cook, W. Hu, J.S. Fritz, P.R. Haddad, Anal. Chem. 73 (2001) 3022.
- [16] W. Hu, Langmuir 15 (1999) 6241.
- [17] J.M. Patil, T. Okada, Anal. Comm. 36 (1999) 9.
- [18] W. Hu, P.R. Haddad, K. Tanaka, K. Hasebe, Anal. Bioanal. Chem. 375 (2003) 259.
- [19] J.D. Lamb, R.G. Smith, Talanta 39 (1992) 923.
- [20] A. Woodruff, C.A. Pohl, A. Bordunov, N. Avdalovic, J. Chromatogr. A 956 (2002) 35.
- [21] F. Tanaka, Y. Kato, J. Pharm. Soc. Jpn. 63 (1943) 592.
- [22] P.A. Kebets, E.P. Nesterenko, P.N. Nesterenko, A.J. Alpert, Mikrochim. Acta 146 (2004) 103.
- [23] A.V. Ivanov, P.N. Nesterenko, Russ. J. Anal. Chem. 54 (1999) 566.
- [24] A. Ramaiya, C. March, H. Thomas Karnes, J. Pharm. Biomed. Anal. 15 (1997) 729.
- [25] K. Deguchi, K. Kohda, M. Ito, J. Chromatogr. A 845 (1999) 165.
- [26] B. Paull, P. Nesterenko, Analyst 130 (2005) 134.