

Double gradient ion chromatography on a short carboxybetaine coated monolithic anion exchanger

Brett Paull,^{*a} Colmán Ó Riordáin^a and Pavel N. Nesterenko^b

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An ultra-short 1.0×0.46 cm carboxybetaine coated monolithic anion exchanger has been used with a new double gradient ion chromatography (DG-IC) technique. The novel monolithic column can be used with flow rate gradients giving excellent peak efficiencies, and retention times can be simultaneously reduced through combining flow gradients with an eluent pH gradient.

The recent introduction of porous monolithic columns to liquid chromatography has brought new possibilities to dramatically reduce run times in chromatographic separations. The high permeability of these columns allows increases in flow rates of 5 to 6 times that which could be used with traditional particle packed columns, without any dramatic loss in efficiency. This novel advantage of monolithic columns now provides the analyst with a new option to optimise separations of solutes having very different affinities to the stationary phase, through the use of continuous flow gradient separations.^{1,2} However, although the application of steep flow gradients can speed overall run times substantially, flow gradients do not affect selectivity and so alone their use is limited in situations where excessive retention volumes between weakly and strongly retained solutes exist. In an attempt to solve this problem, for the first time, a new double gradient chromatographic technique has been developed here.

A perfect example of the above situation can be seen in anion chromatography, where large polarizable anions like iodide and thiocyanate have a much stronger affinity to the anion-exchanger than small common anions like chloride, nitrite, bromide and nitrate, this leading to considerable differences in retention volumes. This was recently shown to be the case using a C_{12} carboxybetaine coated 10 cm monolithic Chromolith C_{18} column by Ó Riordáin *et al.*, where the retention of thiocyanate was in excess of 100 min compared to 15 min for iodide and 2–3 min for nitrate, nitrite and bromide.³

Here, in an attempt to reduce the excessive run time described above, an ultra-short carboxybetaine coated monolithic column was investigated under similar eluent conditions. A 1.0×0.46 cm reversed-phase monolithic silica based column ($2 \mu\text{m}$ flow through macropores and 13 nm mesopores, as supplied by Merck KgaA, Darmstadt, Germany) was modified as above⁴ with the zwitterionic carboxybetaine type surfactant (dodecyltrimethylamino)acetic acid ($C_{12}H_{25}N(CH_3)_2CH_2COOH$), which was synthesised in-house according to a procedure of Tanaka *et al.*⁵ Upon characterisation this short column resulted in a very similar separation of the above anions (0.05 mM mixed standard, 20 μL injection vol.), but in a

much reduced overall run time of 18 min (eluent delivered at a constant flow rate of 1 mL/min). This separation is shown here as Fig. 1(a–b), with 1(a) showing the separation of nitrite, bromide and nitrate on the 1 cm column, all removed from the eluent dip (t_0) (seen as eluting at 0.1 min). All three species were well resolved and elute completely in under 1 min, under constant flow (isofluentic) conditions.

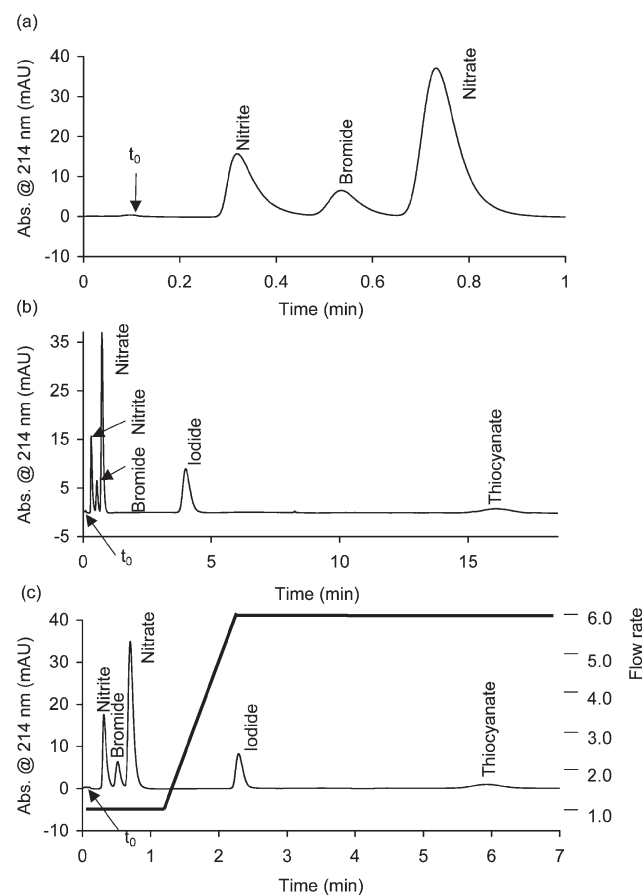


Fig. 1 Ion chromatograms obtained using a 1.0×0.46 cm carboxybetaine modified silica monolith. (a) The separation of 0.05 mM nitrite, bromide and nitrate in under 1 minute. (b) The separation of 0.05 mM nitrite, bromide, nitrate, iodide and thiocyanate in 18 minutes. (c) Separation in 6.5 min obtained using an eluent flow gradient. Eluent = 10 mM KCl, 10 mM phosphate buffer (pH 3), 0.2 mM carboxybetaine. (a–b) Eluent delivered at 1 mL/min. (c) Eluent delivered at 1 mL/min for first 1 min, increased to 6 mL/min between $t = 1$ and $t = 2$ min. Injection vol. = 20 μL .

*Brett.Paull@dcu.ie

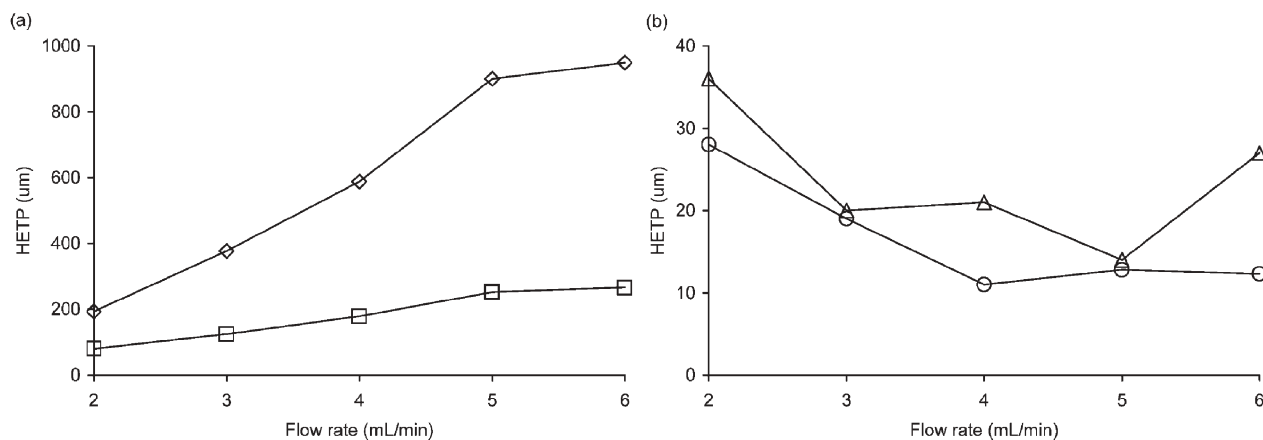


Fig. 2 Effect of eluent flow rate upon peak efficiencies using the carboxybetaine modified silica monolith. \diamond = nitrite, \square = nitrate, \triangle = iodide, \circ = thiocyanate. Eluent = 10 mM KCl, 0.2 mM carboxybetaine (pH 3).

As can be seen from Fig. 1(a–b), the peak efficiencies for a column of such dimensions were impressive (the numbers of theoretical plates for the iodide and thiocyanate peaks (calculated using $N = 16(t_r^2)/W^2$) were found to be 335 and 608, respectively. Given as N/m this corresponds to a remarkable 60,800 for thiocyanate). However the chromatogram is far from optimal, given that 14 min (80% of whole run time) were wasted waiting for the strongly retained thiocyanate peak to elute. Simply increasing the flow rate directly to reduce run times further in this situation is not possible as the baseline separation of the weakly retained nitrite, nitrate and bromide would be lost at elevated isofluent conditions.

A study of peak efficiency as a function of flow rate was carried out for both early and late eluting anions (shown above as Fig. 2). Interestingly, it was found that for the very early eluting species, such as nitrite and nitrate, increasing flow rate caused a continuous decrease in N . This was the opposite effect to that shown for the later eluting iodide and thiocyanate peaks, which appeared to show optimal efficiency at a flow rate of 4 to 5 mL/min. The reason for this contradictory behaviour is likely to be the extremely small interaction time for the early eluting species with the actual stationary phase. For example, at a flow rate of 6 mL/min, the retention time for nitrite is only 6 s.

With the above results in mind, and in an attempt to further decrease run times, whilst maintaining the limited resolution and efficiency of the early eluting species, a flow gradient program was established. Flow gradients have found very limited use in traditional liquid and ion chromatographic applications due to the pressure limitations of traditional length (10–25 cm) particulate packed columns, and where used have been limited to maximum flow rates below 1 mL/min.^{6,7} However, in a recent study by Nesterenko *et al.*¹ it was shown that continuous flow gradients used with polymeric monolithic discs could result in significant improvements in peak efficiencies. Here, the same approach was used with the short silica based zwitterion coated monolith. Fig. 1(c) shows the chromatogram resulting from a flow gradient of 1–6 mL/min, over $t = 1$ to $t = 2$ min. Now the separation of all five UV absorbing anions, including the strongly retained thiocyanate, can be achieved in under 7 min. Under these flow gradient conditions the peak shape for iodide has much improved, and the width of the thiocyanate peak has also reduced.

Due to the nature of the novel zwitterionic carboxybetaine coating used in this study, which contains an inner strong anion exchange site and a terminal weak cation exchange site, eluent pH can be used to adjust retention through control of protonation of the terminal weak acid site and hence increased interaction of anionic analyte ions with the inner strong anion exchange site. This has been shown clearly in the previous work of Ó Riordáin *et al.*³ Maximum retention is therefore seen at low eluent pH, and increasing eluent pH reduces retention. In experiments on a standard size 25 × 0.46 cm carboxybetaine coated reversed-phase column, this meant the retention of iodide and thiocyanate could be substantially reduced through increasing the eluent to maximum pH of 8.0.³ Therefore, here the combination of the flow gradient with an eluent pH gradient (double gradient IC) was investigated (pH of the eluent was controlled through the addition of a 10 mM phosphate buffer). The eluent (at pH 3.0) was delivered at 2 mL/min for first 1 min increased to 6 mL/min between 1 and 2 min (and simultaneously increased to pH 8.0). The results of combining a flow and pH gradient on the 1 cm

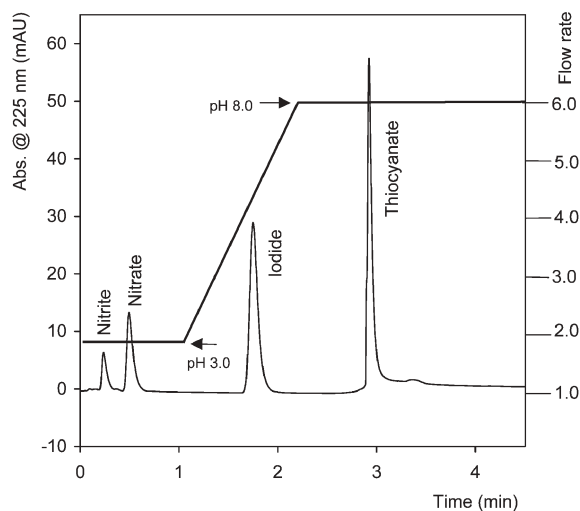


Fig. 3 Ion chromatogram obtained using a 1.0 × 0.46 cm carboxybetaine modified silica monolith with an eluent flow and pH gradient. Analyte conc. = 0.05 mM (thiocyanate = 0.25 mM). Injection vol. = 20 µL.

monolithic carboxybetaine column can be seen as Fig. 3. As can be seen from the chromatogram shown, under these conditions it is now possible to considerably reduce the retention of the late eluting thiocyanate, to a little over 3 min, whilst still separating early eluting peaks. In the chromatogram shown, peak shape and width for both iodide and thiocyanate have also been considerably improved, and as far as the authors are aware, these peaks are by far the most efficient shown for an IC column of such small dimensions.

The results presented herein illustrate the ability to produce high efficiency, rapid ion chromatographic separations on ultra-short monolithic type anion exchangers, which are compatible with flow gradient and double gradient separations. The double gradient separations achieved here have not only not been shown previously, but also challenge what was previously thought possible for columns of such short dimensions. Therefore the significance of these results cannot be overstated for the future developments in such areas as low pressure LC, miniature and portable LC and fast LC.

Brett Paull,^{*a} Colmán Ó Riordáin^a and Pavel N. Nesterenko^b
^a*National Centre for Sensor Research, School of Chemical Sciences, Dublin City University, Glasnevin, Dublin 9, Ireland.*
E-mail: Brett.Paull@dcu.ie; Fax: 00353 (0)1 7005503; Tel: 00353 (0)1 7005060
^b*Department of Analytical Chemistry, Moscow State University, Moscow, 119899, Russian Federation.*
E-mail: PavelN@analyt.chem.msu.ru; Fax: 007-(095)-939-46-75; Tel: 007-(095)-939-44-16

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