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Comparison of the electroosmotic flow profiles and selectivity of stationary phases used in capillary electrochromatography

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Abstract

Over 95% of all papers published to the present time on capillary electrochromatography (CEC) have reported on the use of C_{18} and to a much lesser extent, C_8 stationary phases. The relationship between the electroosmotic flow and pH is of considerable importance in CEC because it allows the analyst to develop methods under optimum conditions, especially with respect to analysis times. Although the electroosmotic flow dependence on pH for C_{18} phases has been reported, there have been very few comparable studies carried out on other phases, and for this reason a systematic study of the performance of a wide range of stationary phases was initiated. The phases studied were Waters 3 μm Spherisorb ODS-1, propyl SCX, phenyl SCX, Symmetry SCX and a C_6/SCX mixed-mode. Important new knowledge regarding the performance of CEC columns has been realised through this study, the first results of which are now reported.

Keywords: Electroosmotic flow; Stationary phases, LC; Electrochromatography; Selectivity; pH effects

1. Introduction

Capillary electrochromatography (CEC) is a hybrid technique that brings together the advantages of both capillary electrophoresis and high-performance liquid chromatography (HPLC). It is of no surprise, since by far the most popular stationary phase used in HPLC is still C_{18}, that chromatographers wishing to enter into the area of CEC choose this phase for their studies.

Although Pretorius et al. [1] first described the electrical pumping of solvents through a packed column, it was Jorgenson and Lukacs in 1981 [2] who reported the separation of 9-methylanthracene from perylene on a 170 μm I.D. capillary packed with a 10 μm reversed-phase packing material. The solvent used to electroosmotically drive the liquid through the capillary was acetonitrile. With a packed bed of 58 cm, a plate number of 31 000 was achieved for 9-methylanthracene. However, it was the work of Knox and Grant [3,4] that extensively detailed much of both the theoretical and practical implications of CEC, providing the breakthrough needed to demonstrate that CEC could be a serious analytical technique which could rival HPLC in terms of selectivity and efficiency.

The relationship between the linear flow through a packed capillary and the applied electric field is

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described by the Helmholtz–Smoluchowski equation as follows:

$$u = \frac{\epsilon_0 \epsilon_r E \zeta}{\eta} \quad (1)$$

where ϵ_0 and ϵ_r are the relative and vacuum permitivities, respectively and: u = linear velocity, E = applied electric field, ζ = zeta potential and η = viscosity of the solvent.

Clearly it follows that in CEC, stationary phase particle size does not influence the electroosmotic flow (EOF) through the capillary. Therefore it should be possible to use very small particles and still maintain a good flow through the column in contrast to HPLC. The plate height equation shown below describes the theoretical limitations of the performance expected of electrically- and pressure-driven chromatography systems.

$$H = Ad_p(ud_p/D_M)^{1/3} + B/u + Cud_p^2/D_M \quad (2)$$

where the A term refers to the contribution from eddy diffusion to the overall plate height, the B term the contribution from axial diffusion, and the C term the contribution from the resistance to mass transfer, and: H = the height equivalent to a theoretical plate (HETP), u = linear velocity, d_p = particle diameter and D_M = diffusion coefficient.

It is clear from Eq. (2) that as the particle diameter decreases then both the A and C terms reduce, particularly the C term. If the particle diameter is reduced to 0.5 μm, then the contribution to the plate height from the A term (eddy diffusion) can be shown to be ~0.5 μm, and from the C term 0.025 μm. Thus the major contribution to plate height (2 μm) would be from axial molecular diffusion i.e., the B term in Eq. (2), which is analogous to the expression used in capillary zone electrophoresis (CZE).

$$H = \frac{2D_M}{u} \quad (3)$$

where H = the height equivalent to a theoretical plate, D_M = the diffusion coefficient of the solute and u = the linear velocity.

A detailed analysis of the individual contributions to the overall plate height in CEC has been given by Dittmann et al. [5]. They showed that eddy diffusion (the A term) is greatly reduced in CEC giving rise to lower minima in the van Deemter plot and consequently smaller values of HETP. Although theory predicts that very small particles can be used in an electrically driven system since there is no pressure drop across the capillary, most reports on the use of CEC to date have been on 3–10 μm materials such as those commonly used in HPLC [6–30]. This is mainly as a result of a lack of packing materials specifically designed for CEC and difficulty in packing small particles into capillaries typically in the order of 50–100 μm in diameter. Although smaller diameter materials are available as research materials, we have had difficulty packing reasonable lengths of such materials, and it is our view that modified packing techniques are needed once the diameter of the packing material is <1.0 μm. In a previous paper [9] we described a method for the reliable packing of capillaries with 3.0 μm materials, subsequently modified by Boughtflower et al. [17].

The linear velocity in a packed capillary can also be expressed as follows:

$$u = \frac{1}{\kappa} \cdot \frac{\sigma E}{\eta} \quad (4)$$

where $1/\kappa$ = the thickness of the double layer, σ = charge density, E = applied electric field and η = the viscosity of the solvent.

It is apparent from Eq. (4) that the EOF depends upon the surface charge density, the field strength, the thickness of the electrical double layer, and the viscosity of the separation medium which in turn is dependent upon the temperature.

The EOF properties of stationary phases therefore depend upon the number of silanol groups present on the packing, their degree of ionisation, the surface area of the particles and the nature of the bonded ligand. Because different manufacturers use different silicas and processes for producing stationary phases, it is not unreasonable to expect variations in EOF profiles between such materials. Most of the stationary phases used for CEC studies have been materials primarily synthesised for HPLC, e.g., C_{18} and in most instances separations performed on these phases are readily transformed into CEC analyses. This is usually the case for neutral molecules, but when charged species are analysed problems can

arise if, during the optimisation of separation, the manipulation of pH is required. This is because most reversed-phase packing materials are silica-based and the EOF is pH dependent, dropping off considerably for pH values below 5-6, depending on the source of packing material. Of considerable interest when developing CEC methods is the degree to which the EOF of a particular phase varies with pH, and equally important, the resolving power of the material over that same pH range. This forms the basis of the present study.

2. Experimental

Experiments were conducted on three different instruments. Two of these were modified by the Bioengineering Department of GlaxoWellcome, Greenford, UK, to operate under pressure, details of which have been reported in a previous article [10].

The first of these was a Perkin-Elmer Applied Biosystems Instrument Model 270A (Foster City, CA, USA), and the second an ATI Unicam Prince Instrument, (Cambridge, UK). In order to carry out the modification, it was necessary to remove the autosamplers from these instruments. A modification that retained use of the autosamplers was considered but the associated engineering difficulties precluded this option. The third instrument used for this study was a Hewlett-Packard (HP) 3D capillary electrophoresis system (Hewlett-Packard, Waldbronn, Germany). This instrument is fully configured to run under pressurised conditions and also has a 48-position autosampler.

All capillaries were supplied by Innovatech (Stevenage, UK) and were packed with Waters Spherisorb stationary phases supplied by Phase Separations (Clwyd, UK) These phases were prepared specifically for CEC by Professor P. Myers of Phase Separations. Thiourea was obtained from BDH, Poole, UK. GR57888X, GR57794X, fluticasone propionate and its desfluoro analogue were supplied by GlaxoWellcome, Stevenage, UK. The test mixture used for these studies, test mixture 1, (see Fig. 1) contained thiourea (which is frequently used as an EOF marker), GR57888X which being a diol is reasonably polar, and the non-polar dibenzyl analogue, GR57794X. These two compounds therefore have quite widely differing polarities. The steroid fluticasone propionate and its desfluoro compound are of intermediate polarities and being very closely related, test the resolving power of the phases. All components of the test mixture are neutral under the experimental conditions studied. The concentration of the components of test mixture 1 is as follows: thiourea: 30 mg/ml in acetonitrile–water (50:50); GR57888X: 60 mg/ml in acetonitrile–water (50:50); GR57994X: 10 mg/ml in acetonitrile–water (50:50); fluticasone propionate: 10 mg/ml in acetonitrile–water (50:50); desfluoro analogue: 10 mg/ml in acetonitrile–water (50:50).

The tricyclic antidepressants amitriptyline, nortriptyline, imipramine, clomipramine and methdilazine (Fig. 2) were gifts from Dr. Bob Flanagan of the Poisons Unit at NewCross Hospital, London, UK. These were dissolved in the mobile phase at a concentration of 1 mg/ml.

If no pH adjustment was necessary, mobile phases were prepared simply by mixing the appropriate volumes of organic solvent and buffer. When pH adjustment was required, the pH of the buffers was altered before mixing with organic solvent. The running buffers used to measure EOF were prepared by mixing 70 ml of HPLC-grade acetonitrile with 30 ml of 20 m*M* NaH_2PO_4, pH 2.3; 20 m*M* sodium acetate, pH 4 and 5; 20 m*M* Na_2HPO_4, pH 6.5 and 7.5; and 20 m*M* Tris, pH 9.0.

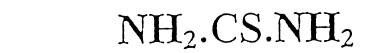
3. Results and discussion

Five phases were evaluated in this particular study, namely: 3 μm Waters Spherisorb ODS-1; 3 μm Waters Spherisorb propyl SCX; 3 μm Waters Spherisorb phenyl SCX; 3 μm Waters Symmetry SCX and 3 μm Waters Spherisorb mixed mode. All were supplied by Phase Separations Ltd.

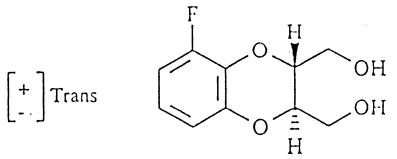
3.1. The origin of EOF in a packed capillary

In CZE, EOF results from the presence of a zeta potential at the surface of the fused-silica capillary. It was therefore expected that in a packed capillary under conditions that promote EOF, both the fused-silica surface and the packing material would contribute to this phenomenon. In order to test this

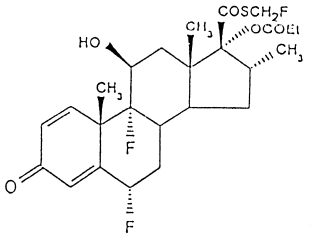
1. Thiourea



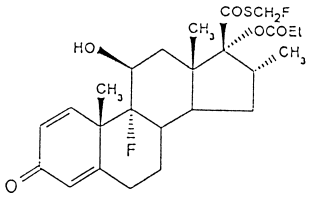
2. GR57888X



3. Fluticasone propionate



4. Des-fluoro analogue



5. GR57794X

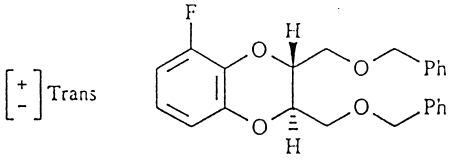
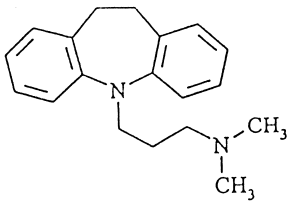
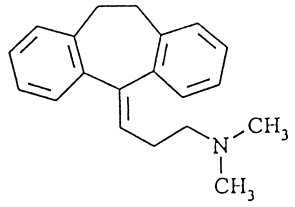


Fig. 1. Components of test mixture 1.

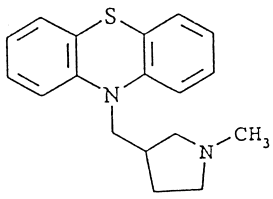
theory, the EOF in a capillary that had been coated with polyvinyl alcohol (PVA) was compared with that of an uncoated capillary in the CZE mode. Thiourea was used as the EOF marker. The capillary lengths were identical thereby ensuring constant field strengths for the experiment. The capillary coated with PVA not surprisingly had a very low EOF across the whole pH range. Next, these same two capillaries were packed with 3 μm Waters Spherisorb ODS-1, and once again for ease of comparison, the total lengths and packed lengths were identical, although shorter than the capillaries used in the CZE experiments (the result of introducing frits into the packed capillaries). The results of this experiment show clearly that there appears to be very little difference in the EOF between pH 2.8 and pH 9.0 for the two packed capillaries even though the surface of one (PVA) has been deliberately coated in order to reduce the EOF of the fused-silica surface. In order to assess the overall effect of these experiments, the mobilities (i.e., the linear velocity at unit field strength) were plotted against pH for the packed and unpacked capillaries and the data is shown in Fig. 3. The clear observation is that in CZE the PVA coating



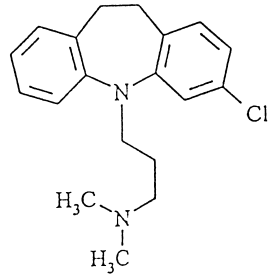
Imipramine



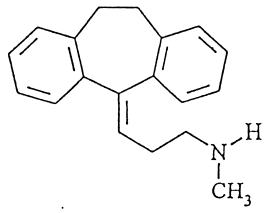
Amitriptyline



Methdilazine



Clomipramine



Nortriptyline

Fig. 2. Structures of tricyclic antidepressants.

suppresses EOF, and yet in a packed capillary, the mobility of analytes appears to be independent of the fused-silica capillary surface. It is also apparent that as predicted by Knox and Grant [4], the EOF in a packed capillary is significantly less than in an open tube of the same material. These results confirm the findings of Dittmann [31]. The conclusion drawn therefore is that in packed CEC the packing material contributes predominantly to the EOF. This is presumably due in part to the very large surface area of the packing material and the lack of double layer overlap under the conditions employed. However the probability is that at the surface of the capillary, double layer overlap occurs because of contact with the packing material, thereby eliminating EOF.

3.2. 3 μm Waters Spherisorb ODS

When test mixture 1 was run on 3 μm Waters Spherisorb ODS-1, the chromatogram shown in Fig. 4 was obtained, and the results confirm that thiourea gives a reasonable measure of t_0 in line with the findings of other researchers [11,12,17,18,24,28]. Not surprisingly compounds GR57888X and GR57794X are easily resolved eluting in exactly the same order as would be expected for HPLC. Also, the des-6α-fluoro impurity was readily separated from the parent steroid.

The electrophoretic mobility for this phase was measured between pH 2.3 and 9.0 using thiourea. Firstly, the capillary was equilibrated with the initial

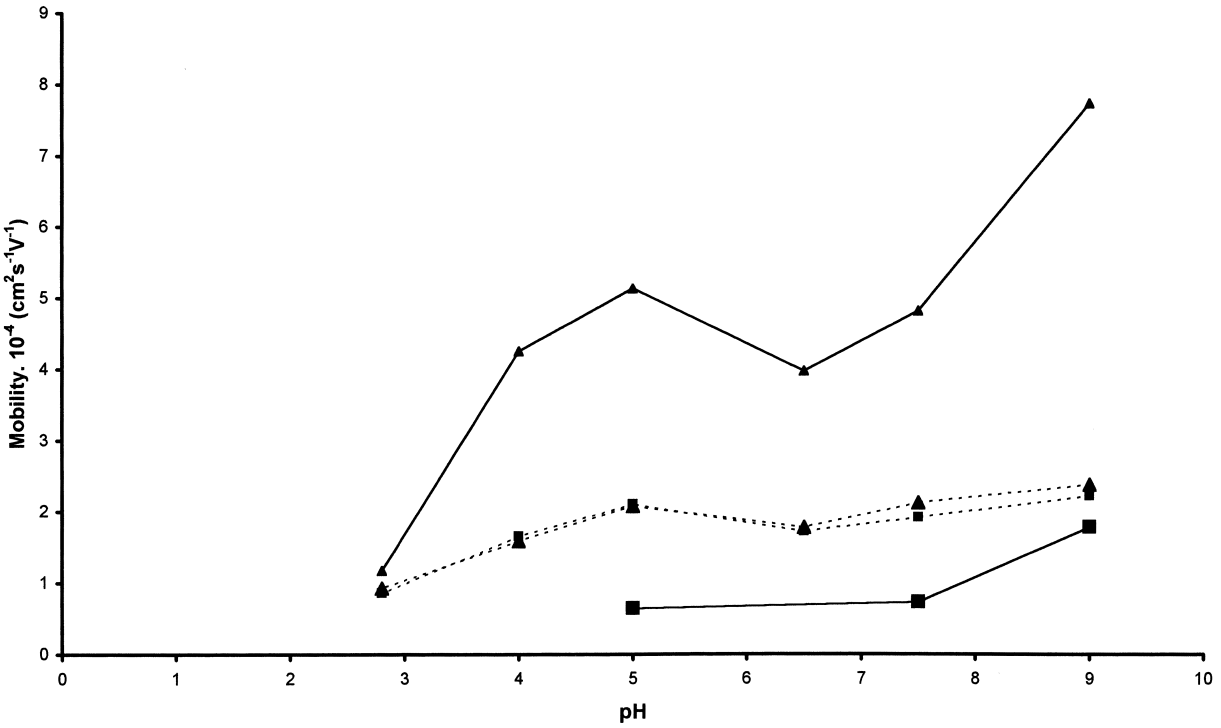


Fig. 3. Electrophoretic mobilities in coated and uncoated capillaries in CZE and CEC mode. —— ▲ —— Uncoated-CZE, – – ▲ – – uncoated-packed, – – ■ – – PVA coated-packed, —— ■ —— PVA coated-CZE.

solvent at pH 9.0. A sequence control programme was written for the HP 3D instrument that allowed for equilibration between the other pH adjusted mobile phases. This was accomplished by running blanks during equilibration under the same conditions as for the analysis, which means that each new solvent would have 60 min in which to equilibrate before the next experiments were started. The overall profile (as depicted in Fig. 5) is very much in-line with data reported by other workers, although the decrease in retention of about 50% over the range pH 2.3–9.0 is lower than reported on other phases. This is probably due to the fact that Waters Spherisorb silica is more acidic than many other manufacturer's materials, giving rise to a significant EOF even at pH 2.3.

3.3. 3 μm Waters Spherisorb Propyl SCX

With the ODS-1 phase the EOF would be expected to drop off at low pH as a result of the residual silanol groups on the packing becoming protonated. However because the SCX phase contains a sulphonic acid function the contribution from the stationary phase should remain virtually constant across the whole pH range. At high pH, both the $-SO_3H$ ligand and residual silanol groups on the packing material would be expected to contribute to the overall EOF. However, at low pH, because the strongly acidic $-SO_3H$ groups attached to the silica particles are ionised even in acidic solutions, their contribution to the EOF would remain unaltered. It was therefore expected that SCX phases would be capable of resolving neutral compounds at low as well as at high pH.

The use of this material for the highly efficient analyses of tricyclic antidepressants has been reported previously and widely discussed [16]. However when first reported, no study of the EOF profile was carried out. Fig. 6 shows the result obtained using test mixture 1 under the same conditions used for the ODS-1 phase, except for the use of a longer

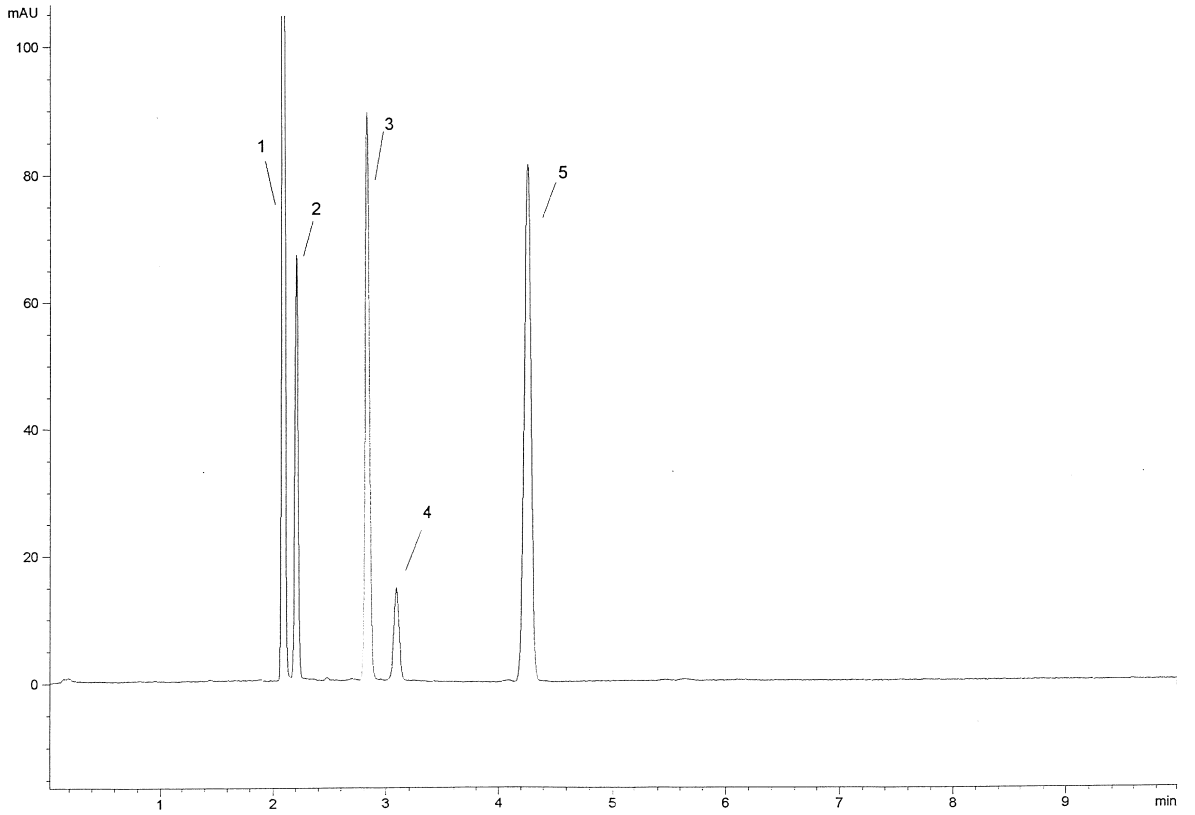


Fig. 4. Separation of test mixture 1 on an ODS-1 stationary phase. Instrument: HP 3D CEC system. Packing: 3 μm Waters Spherisorb ODS-1. Capillary packed length 24.5 cm, total length 33 cm. Mobile phase: acetonitrile–0.01 *M* Na_2HPO_4 (70:30), the buffer is used unadjusted, pH~8.2. Applied voltage: 30 kV, temperature: 30°C, injection: 10 kV for 10 s, detection: 210 nm. 1=Thiourea, 2=GR57888X, 3=fluticasone propionate, 4=desfluoro analogue, 5=GR57794X.

capillary and injection at 10 kV for 5 s rather than for 10 s.

Several differences are apparent in this chromatogram when compared to that obtained on the ODS-1 phase. Firstly, because no attempt has been made to optimise the conditions, it can be seen that many peaks co-elute. What is interesting is the fact that there appears to have been a reversal in the elution order with thiourea now eluting last, with the steroid fluticasone propionate and GR57794X co-eluting first.

The plot of electrophoretic mobility vs. pH for this material (see Fig. 5) is obtained by assuming that GR57794X is a good t_0 indicator, and although we have not conducted exhaustive experiments, we have found no compound that elutes faster on this phase. The results show that between pH 4.0 and 9.0 there is practically no decrease in EOF, but once again this drops by about 50% between pH 4.0 and 2.3. The elution order on this phase is more typical of normal-phase chromatography, and surprisingly it is now the dibenzyl compound that is the best EOF marker, whilst thiourea appears to be retained by the stationary phase.

The capacity factors of three of the constituents of test mixture 1 obtained on the ODS-1 and propyl SCX phases under the conditions used in Fig. 1 are given in Table 1.

Not surprisingly, the propyl SCX phase lacks the ability to resolve the neutral components of these mixtures due to a reduction in the hydrophobic selectivity, and this was to prove the case with all of the SCX phases evaluated, severely restricting their usefulness as CEC stationary phases.

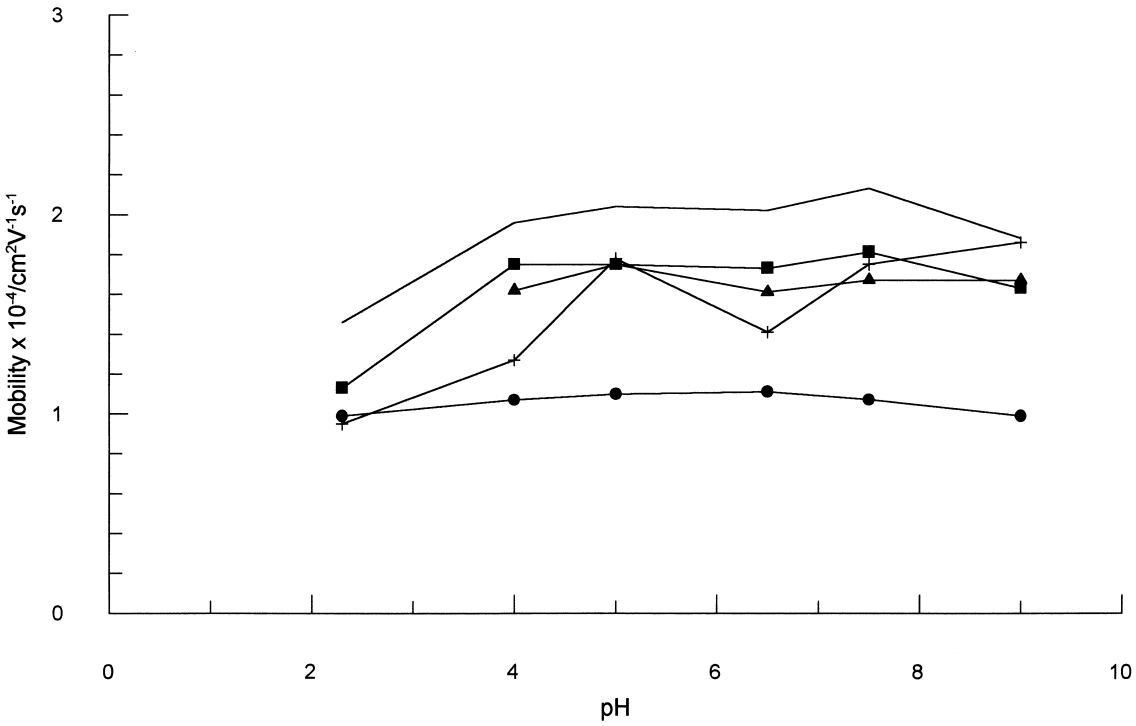


Fig. 5. Plot of electrophoretic mobility vs. pH. ——— Phenyl SCX, – + – Spherisorb ODS-1, – ▲ – Symmetry SCX, – ■ – Propyl SCX, – ● – C_6/SCX.

3.4. 3 μm Waters Spherisorb Phenyl SCX

In this case the $-SO_3H$ group is attached to the silica surface via an alkylaryl group in contrast to the previous SCX phase where the link was a propyl group. Of interest is the fact that this material also shows the amazing focusing effect for the same highly basic tricyclic antidepressants that we reported when using the propyl SCX [16].

An example of this is shown in Fig. 7 where the highly focused separation of methdilazine from clomipramine and imipramine is illustrated using a mobile phase at pH 2.3, whereby the highly basic analytes are resolved as their cations. It is worth noting that there is a reversal of the elution order for these antidepressants on this phase, since with the propyl SCX packing, methdilazine eluted last whereas now it elutes first.

In order to demonstrate the ability to perform CEC at low pH, test mixture 1 was run at pH 2.3 and the resulting chromatogram is illustrated in Fig. 8. As expected with this phase, there is once again a reversal in the order in which the components elute, with several peaks once again being unresolved demonstrating the not unexpected poor hydrophobic character of these materials. There is however a consistency with the elution order for the neutral test compounds on this phase because once again the dibenzyl compound runs fastest and thiourea slowest, which is as if this material was also behaving in a normal-phase mode.

The plot of electrophoretic mobility vs. pH for the phenyl SCX phase was constructed once again assuming that GR57794X gives a reasonable measure of t_0 as shown in Fig. 5. The decrease in EOF observed with the propyl SCX below pH 4 is much less evident with the phenyl SCX phase. This could be as a result of better surface coverage in the case of the phenyl SCX, leading to a higher proportion of $-SO_3^-$ groups, and effectively less SiO^-. The latter

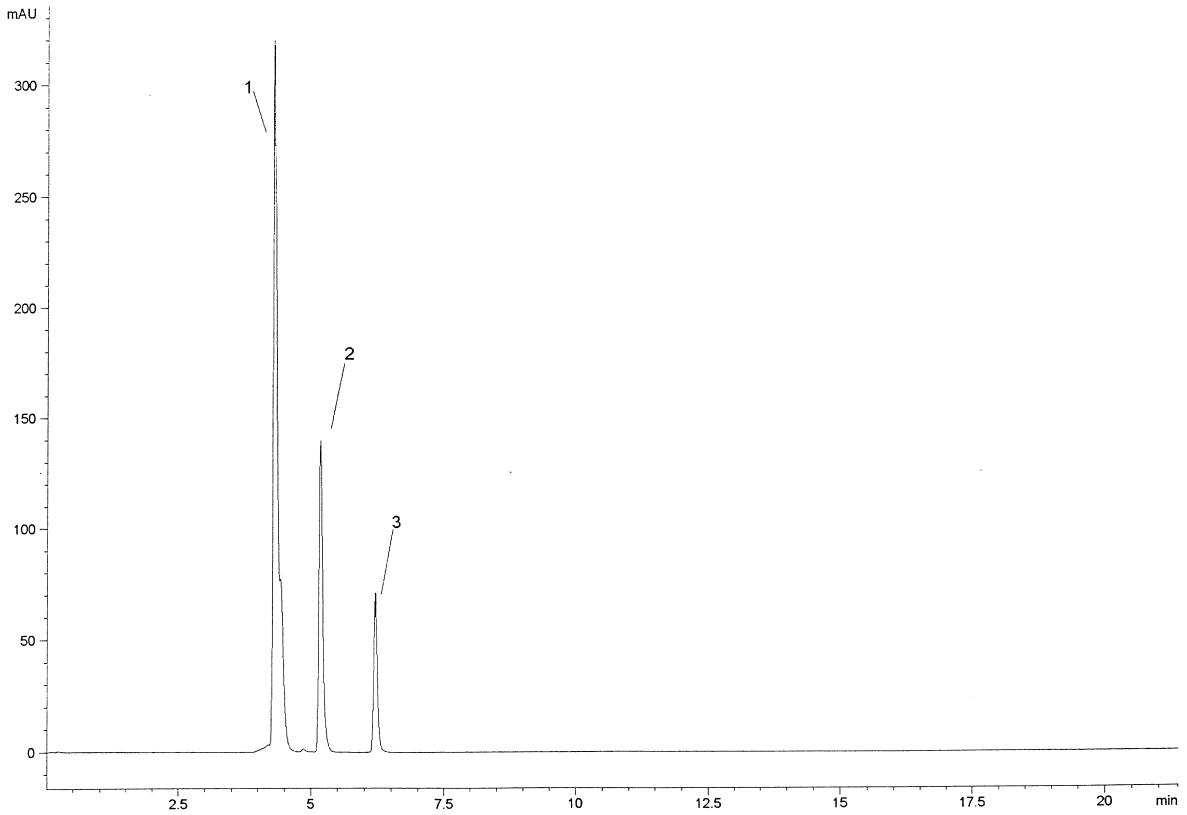


Fig. 6. Separation of test mixture 1 on a propyl SCX stationary phase. Instrument: HP 3D CEC system. Packing: 3 μm Waters Spherisorb propyl SCX. Capillary packed length 40 cm, total length 48.5 cm. Mobile phase: acetonitrile–0.01 *M* Na_2HPO_4 (70:30), the buffer is used unadjusted, pH~8.2. Applied voltage: 30 kV, temperature: 30°C, injection: 10 kV for 5 s, detection: 210 nm. 1=Fluticasone propionate/GR57794X, 2=GR57888X, 3=thiourea.

would be expected to be protonated at low pH leading to a reduced EOF. There is however a consistency with the elution order for the neutral test compounds on this phase because as with the other SCX phases, the dibenzyl compound runs fastest and thiourea slowest, which is once again as if this material was behaving in a normal-phase mode.

3.5. 3 μm Waters Symmetry SCX

This material differs from other Waters Spherisorb phases in that the silica used to produce this material is of a much higher quality, having none of the high metal content of the previous examples. Due to the small amount of material supplied, only a limited

Table 1
Capacity factors on an ODS-1 and SCX phase

	Capacity factor κ'			
Stationary phase	GR57888X	CCI 18781	GR57794X	Thiourea
ODS-1	0.056	0.354	1.038	
Propyl SCX	0.202	0.026	Unretained	0.44

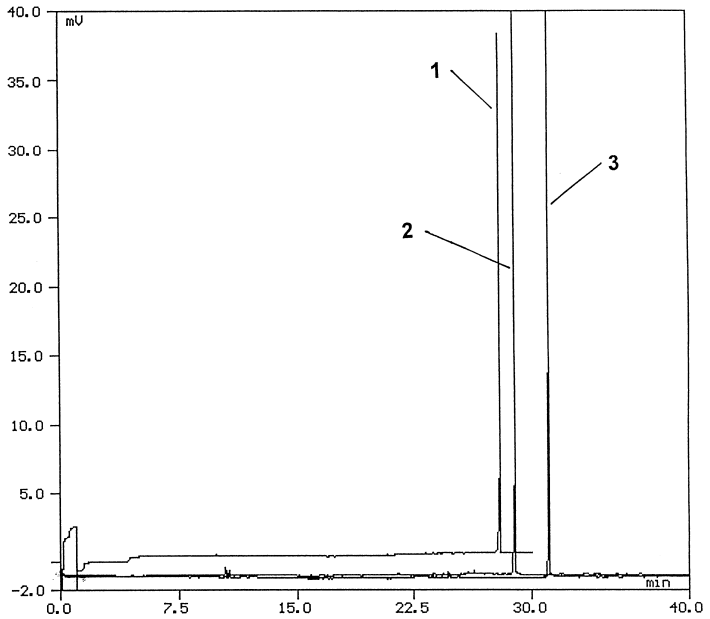


Fig. 7. Separation of antidepressants on a phenyl SCX phase. Instrument: Unicam Prince. Packing: 3 μm Waters Spherisorb phenyl SCX. Capillary packed length 40 cm, total length 56 cm. Mobile phase: acetonitrile–0.05 *M* Na_2HPO_4, pH 2.3 (70:30). Applied voltage: 30 kV, temperature: ambient (~25°C), injection: 10 kV for 24 s, detection: 210 nm, 0.02 AUFS. 1=Methdilazine, 2=clomipramine, 3=imipramine.

amount of data has been generated, nevertheless, the results are sufficiently interesting to revisit when more material becomes available. The electrophoretic mobility plot for this material falls exactly in line with all the other SCX phases studied so far (see Fig. 5). The results for this material show a remarkable linearity between pH 4.0 and 9.0 (no experiments were carried out below pH 4.0) with practically no deterioration in the EOF within this range.

Because this capillary was packed before we began investigating test mixture 1, there is no equivalent data to that produced for other SCX phases. However, a mixture consisting of thiourea, GR57794X and GR57888X only, confirmed that the elution order for these three components was the same as for the other SCX phases. The dibenzyl compound once again elutes first and thiourea last. A mixture of amitriptyline and nortriptyline was analysed on this phase at high pH and the result is shown in Fig. 9. The focusing effect reported for other SCX phases normally occurred at low pH, with relatively poor chromatography at high pH. Symmetry SCX appeared to yield good chromatography for the mixture of antidepressants although it is not clear whether the peaks are focused. However, for this class of compound the efficiencies are very good, as are the peak shapes.

The results obtained for the SCX phases are consistent with our suggestion that ligands with terminal sulphonic acid groups lead to enhanced EOF, particularly at low pH. On the other hand, they lack the selectivity to resolve compounds of similar hydrophobicity.

3.6. 3 μm Waters Spherisorb Mixed Mode

The work on the SCX phases adequately demonstrated that it was possible to enhance EOF at low pH by the introduction of $-SO_3H$ groups. However, it is apparent that although it is possible to resolve a range of compounds with this type of phase, their resolving power is limited by a lack of hydrophobic

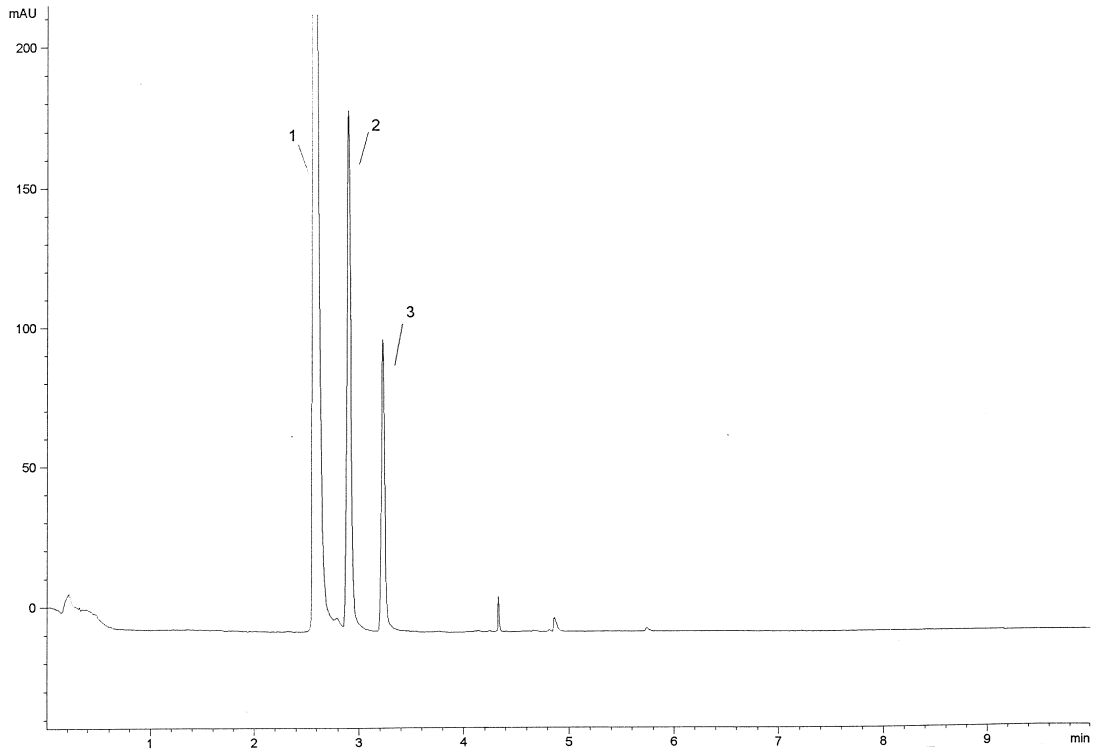


Fig. 8. Separation of test mixture 1 on a phenyl SCX phase at low pH. Instrument: HP 3D CEC system. Packing: 3 μm Waters Spherisorb phenyl SCX. Capillary packed length 24.5 cm, total length 33 cm. Mobile phase: acetonitrile–0.05 *M* Na_2HPO_4, pH 2.3 (70:30). Applied voltage: 30 kV, temperature: 30°C, injection: 5 kV for 10 s, detection: 210 nm. 1=Fluticasone propionate/GR57794X, 2=GR57888X, 3=thiourea.

character. In order to solve this problem it was decided to investigate phases combining ODS and SCX functionalities.

A mixed mode material that possessed both $-SO_3H$ groups along with hydrophobic alkyl ligands was manufactured by Professor P. Myers of Phase Separations to test the feasibility of such a phase. A comparison of the EOF profile of the material using thiourea demonstrated that it was capable of maintaining a substantial EOF between pH 2.3 and 9.0, which we consider to be essential if the phase is to have widespread application. For comparison, the plot of electrophoretic mobility vs. pH for this material is also illustrated in Fig. 5.

The elution order of this phase mimicked the ODS-1 material with thiourea giving a good indication of the EOF, with the non-polar dibenzyl compound once again eluting last. The highly efficient separation of the constituents of the standard test mixture was achieved even at pH 3.5 with all components baseline resolved in <4 min, demonstrating both a high EOF and a high degree of hydrophobicity. A sample chromatogram of the separation of the test components on the mixed mode phase at pH 3.5 is shown in Fig. 10.

The capacity factors of the constituents of the test mixture at high pH (pH~8.2) and pH 3.5 on the mixed mode phase are given in Table 2.

It is interesting to note that the EOF at pH 3.5 is very similar to that at pH 8.2 resulting in fast analysis even at low pH. Unlike the SCX phases, this mixed-mode phase shows both a remarkable EOF

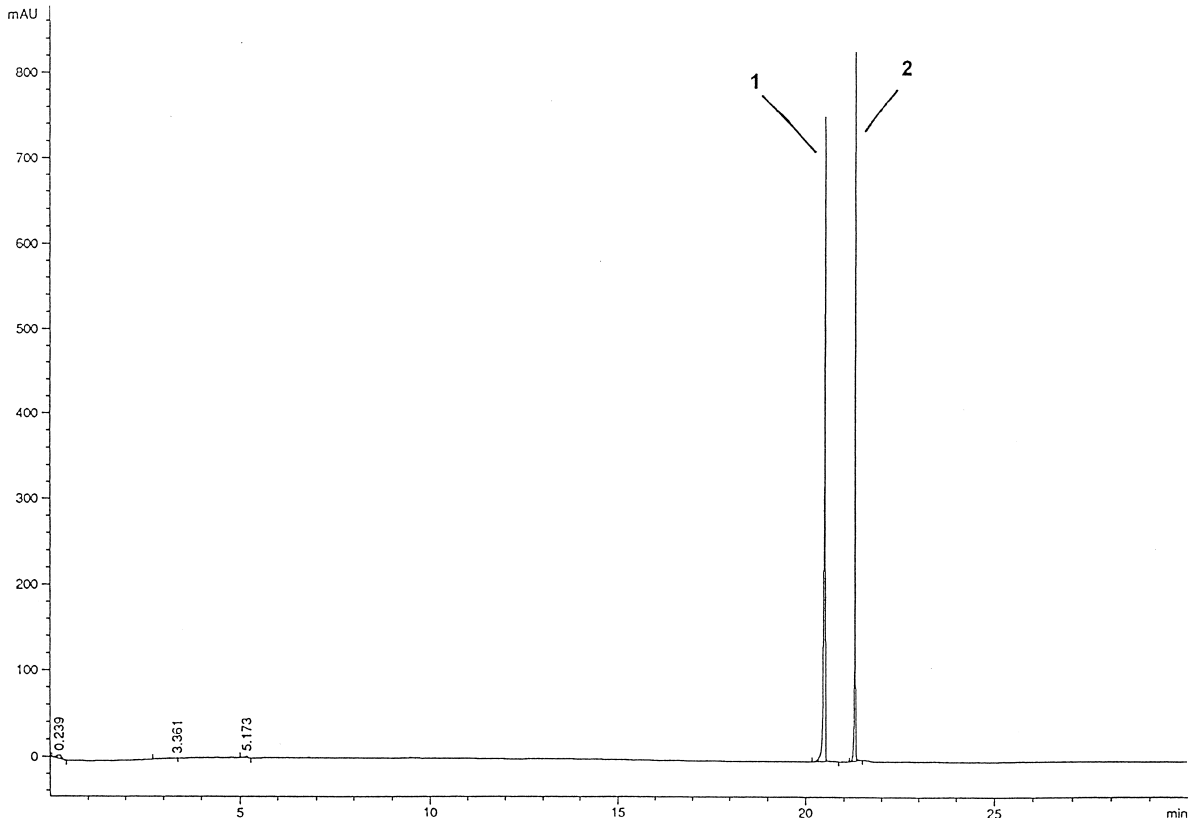


Fig. 9. Separation of nortriptyline from amitriptyline on a Symmetry SCX phase. Instrument: HP 3D CEC system. Packing: 3 μm Waters Symmetry SCX. Capillary packed length 40 cm, total length 48 cm. Mobile phase: acetonitrile—20 m*M* borate, pH 9.0 (70:30). Applied voltage: 30 kV, temperature: 30°C, injection: 5 kV for 5 s, detection: 210 nm. 1 = Amitriptyline, 2 = nortriptyline.

across a wide pH range and also good hydrophobic characteristics enabling all the components of the test mixture to be resolved at both pH~8.2 and pH 3.5.

4. Conclusions

CEC is a technique that can be of tremendous benefit to the analytical chemist, offering highly efficient separations, fast analysis and the advantage of having a dual separation mechanism, which could make method development easier. However, most CEC separations up to the present time have been performed on stationary phases designed for HPLC. While much of the chromatography reported has been highly efficient, because of the nature of the materials the studies have invariably been carried out at high pH. In our study we have looked at the EOF profiles and separation characteristics of a range of stationary phases that have been especially synthesised for CEC, and used the results to determine their ability to act as suitable CEC stationary phases. The hydrophobic ODS-1 and mixed-mode phases, not surprisingly, were found to have very similar selectivities as illustrated by the capacity factors in Tables 1 and 2. However, all of the SCX phases studied had quite different selectivities compared to the hydrophobic phases, resulting in a reversal of the elution of the test analytes, analogous to a normal-phase mechanism. These SCX phases also exhibited poor selectivity resulting in the inability to resolve compounds of similar hydrophobicity.

This work forms part of an ongoing study which should lead to the design of more suitable stationary phases and in turn extend the scope of this highly promising technique.

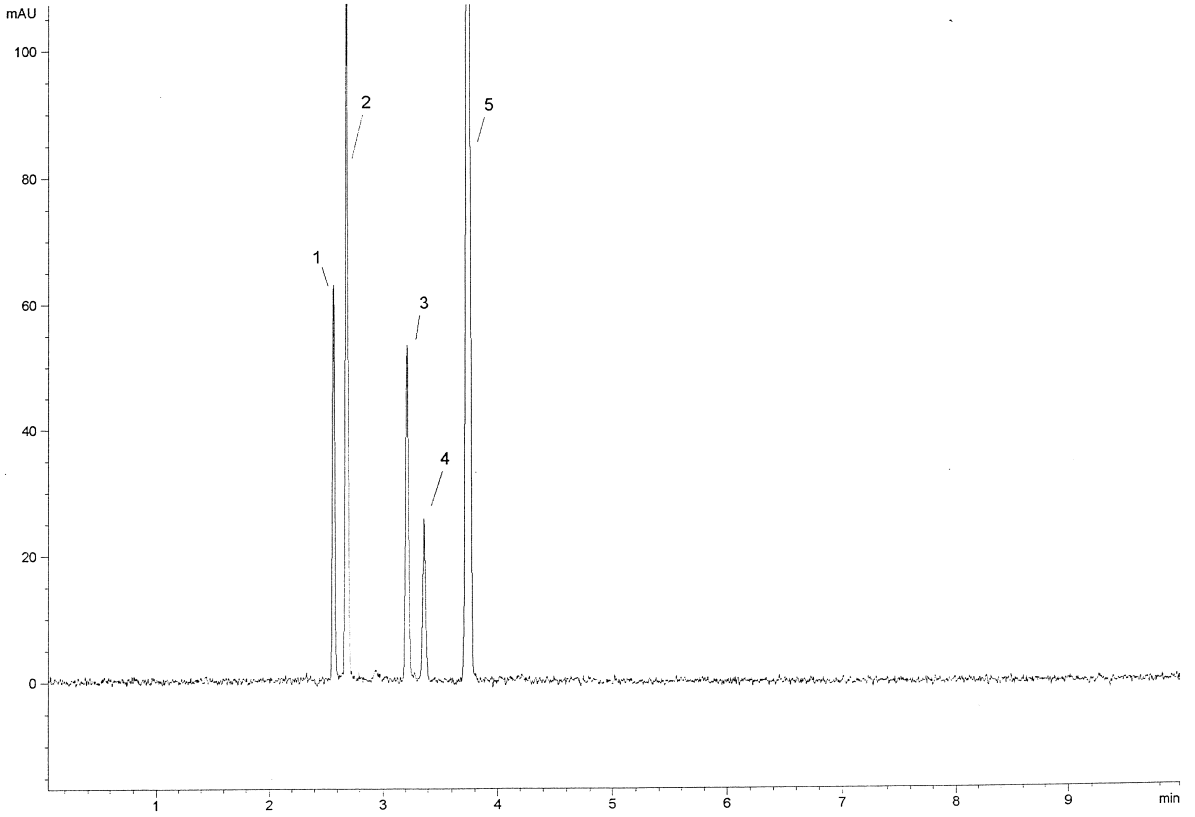


Fig. 10. Separation of test mixture 1 on a mixed mode phase at pH 3.5. Instrument: HP 3D CEC system. Packing: 3 μm Waters Spherisorb mixed mode. Capillary packed length 24.5 cm, total length 33 cm. Mobile phase: acetonitrile–30% 0.05 *M* Na_2HPO_4, pH 3.5 (70:30). Applied voltage: 30 kV, temperature: 30°C, injection: 10 kV for 5 s, detection: 210 nm. 1=Thiourea, 2=GR57888X, 3=fluticasone propionate, 4=desfluoro analogue, 5=GR57794X.

Table 2
Capacity factors on a mixed mode phase at pH~8.2 and pH 3.5

	Capacity factor κ'		
pH	GR57888X	CCI 18781	GR57794X
(~8.2)	0.048	0.25	0.47
3.5	0.045	0.25	0.46

References

[1] V. Pretorius, B.J. Hopkins, J.D. Schieke, J. Chromatogr. 99 (1974) 23.
[2] J.W. Jorgenson, K.D. Lukacs, J. Chromatogr. 218 (1981) 209.
[3] J.H. Knox, I.H. Grant, Chromatographia 24 (1987) 135.
[4] J.H. Knox, I.H. Grant, Chromatographia 32 (1991) 317.
[5] M.M. Dittmann, K. Wienand, F. Bek, G.P. Rozing, LC·GC 13 (1995) 800.
[6] T. Tsuda, K. Nomura, G. Nakagawa, J. Chromatogr. 248 (1982) 241.
[7] H. Yamamoto, J. Baumann, F. Erni, J. Chromatogr. 593 (1992) 313.
[8] S. Li, D.K. Lloyd, Anal. Chem. 65 (1993) 3684.
[9] N.W. Smith, M.B. Evans, Chromatographia 38 (1994) 649.
[10] S. Li, D.K. Lloyd, J. Chromatogr. A 666 (1994) 321.
[11] H. Rebscher, U. Pyell, Chromatographia 38 (1994) 737.
[12] C. Yan, D. Schaufelberger, F. Erni, J. Chromatogr. A 670 (1994) 15.
[13] B. Behnke, E. Bayer, J. Chromatogr. A 680 (1994) 93.
[14] S. Kitagawa, T. Tsuda, J. Microcol. Sep. 6 (1994) 91.
[15] S. Kitagawa, T. Tsuda, J. Microcol. Sep. 7 (1995) 59.
[16] N.W. Smith, M.B. Evans, Chromatographia 41 (1995) 197.
[17] R.J. Boughtflower, T. Underwood, C.J. Paterson, Chromatographia 40 (1995) 329.
[18] R.J. Boughtflower, T. Underwood, J. Maddin, Chromatographia 41 (1995) 398.
[19] C. Yan, R. Dadoo, H. Zhao, R.N. Zare, Anal. Chem. 67 (1995) 2026.

[20] G.A. Lord, D.B. Gordon, L.W. Tetler, C.M. Carr, J. Chromatogr. A 700 (1995) 27.
[21] B. Behnke, E. Grom, E. Bayer, J. Chromatogr. A 716 (1995) 207.
[22] C. Yan, R. Dadoo, R.N. Zare, D.J. Rakestraw, D.S. Anex, Anal. Chem. 68 (1996) 2726.
[23] S.E. Van den Bosch, S. Heemstra, J.C. Kraak, H. Poppe, J. Chromatogr. A 755 (1996) 165.
[24] M.T. Dulay, C. Yan, D.J. Rakestraw, R.J. Zare, J. Chromatogr. A 725 (1996) 361.
[25] E. Bayer, GIT Special, Chromatogr. Int. 96 (1996) 22.
[26] J.H. Knox, GIT Special, Chromatogr. Int. 96 (1996) 39.
[27] H. Rebscher, U. Pyell, Chromatographia 42 (1996) 171.
[28] T.M. Zimina, R.M. Smith, P. Myers, J. Chromatogr. A 758 (1997) 191.
[29] Q.-H. Wan, Anal. Chem. 69 (1997) 361.
[30] J. Ding, P. Vouros, Anal. Chem. 69 (1997) 379.
[31] M.M. Dittmann, personal communication.