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Comparison of aqueous and non-aqueous capillary electrochromatography for the separation of basic solutes

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Abstract

The analysis of basic compounds by capillary electrochromatography (CEC) on silica-based materials using conventional HPLC stationary phases has failed to address the problem of severe peak tailing and non-reproducible chromatography. Several new generation stationary phases were evaluated using aqueous and non-aqueous mobile phases. The best results were obtained in the aqueous mode using Waters Symmetry Shield RP-8, a material in which the residual silanol groups were shielded by an octylcarbamate function. For comparison, experiments were carried out using unmodified silica.

Keywords: Stationary phases, CEC; Electrochromatography; Mobile phase composition; Silanol groups, residual

1. Introduction

Capillary electrochromatography (CEC) is a technique that has gained considerably in popularity over the last 5 years and has been demonstrated to give extremely high efficiency separations for a wide range of mostly neutral pharmaceutical compounds [1–31]. However, a large number of pharmaceutical products contain basic nitrogen functions, and it is well known that these compounds cause considerable problems when analysed by high-performance liquid chromatography (HPLC) [32–34]. This is because of interaction between the basic nitrogen and the residual silanol groups of the packing materials. These problems have largely been addressed by the introduction of a wide range of supports where the silanol groups have either been deactivated or shielded. Many of these phases have also been manufactured using very pure silica, thus removing a source of reactive acidic silanol groups which are also responsible for peak tailing with basic compounds. As a result though, such stationary phases yield a low or no electroosmotic flow (EOF), which is a mandatory condition to execute CEC. Very little research has been carried out into the use of non-aqueous CEC [7,22,23] and this paper compares the performance of stationary phases with basic compounds in the aqueous and non-aqueous modes.

2. Experimental

All experiments were conducted on a $HP^{3D}CE$ instrument (Hewlett-Packard, Waldbronn, Germany). This instrument is fully configured to run under pressurised conditions and also has a 48-position autosampler.

Capillaries were either packed in the laboratory or supplied by Innovatech (Stevenage, UK) Fused-silica

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capillaries, 50 μm I.D. were purchased from Composite Metals (Worcester, UK). Capillaries were packed according to the method described in a previous paper [2]. The 3.5 μm Waters Symmetry Shield RP-8 was a gift from Professor P. Myers of Phase Separations. HPLC-grade methanol and acetonitrile were purchased from BDH (Poole, UK). The components of the neutral test mixture, thiourea, dimethyl phthalate, anisole and naphthalene were also purchased from BDH. These were dissolved in acetonitrile–water (50:50) at a concentration of 1 mg ml^{-1}. The components of the basic test mixture, amitriptyline, clomipramine, imipramine and nortriptyline were purchased from Sigma (Poole, UK), whilst the compounds carbovir, ranitidine and ondansetron were supplied by GlaxoWellcome Research and Development (Stevenage, UK) and were dissolved in the mobile phase also at a concentration of 1 mg ml^{-1}.

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.

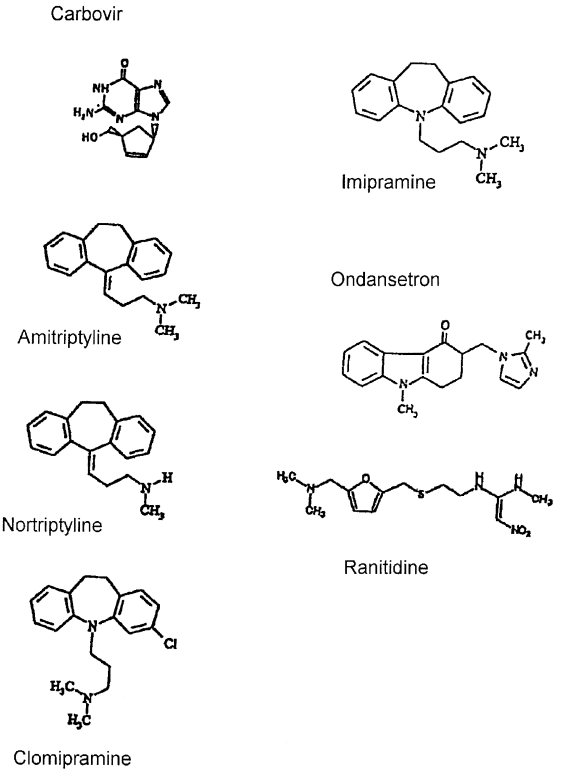

Fig. 1. Contents of basic test mixture.

3. Results and discussion

The structures of the components of the basic test mixture are shown in Fig. 1.

When the neutral test mixture is analysed using a 50 μm I.D. capillary packed with 3 μm Spherisorb ODS-1 stationary phase with an aqueous buffer–organic mobile phase, a highly efficient separation is achieved, as shown in Fig. 2. The plate number for peak 3 (anisole) is 36 240 on-column, which equates to 147 859 per metre. The buffer used for this separation was 0.01 *M* Na_2HPO_4, unadjusted pH 8.4.

When this test mixture is analysed on the same stationary phase using 0.01 *M* Tris in acetonitrile–methanol (1:1) as the mobile phase, then only three of the four components are resolved as shown in Fig. 3A. When these same compounds were analysed using unmodified silica with the same buffered non-aqueous mobile phase, then these neutral test compounds were actually better resolved than on the ODS-1 system as illustrated by Fig. 3B. It is interesting to note that this separation, because it is carried out under non-aqueous conditions, is characteristic of normal-phase chromatography, with the more polar components now the most retained. Also, as demonstrated later with the Develosil ODS UG-5 stationary phase, the addition of buffer to the mobile phase acted to reduce the EOF due to the effect on the zeta potential, however the separation remained identical.

Tricyclic antidepressants analysed on 50 μm I.D. capillary packed with 3 μm Spherisorb ODS-1 using the same conditions as for Fig. 3A and B produced very poor chromatography, which is not unexpected given the high concentration of accessible residual silanol groups on this material, and this is illustrated in Fig. 4A.

However, analysis of these basic compounds on a silica phase under the same conditions showed no improvement and this highlights the fact that the residual silanol groups on the ODS-1 phase are responsible for the poor chromatography with these class of compounds (Fig. 4B).

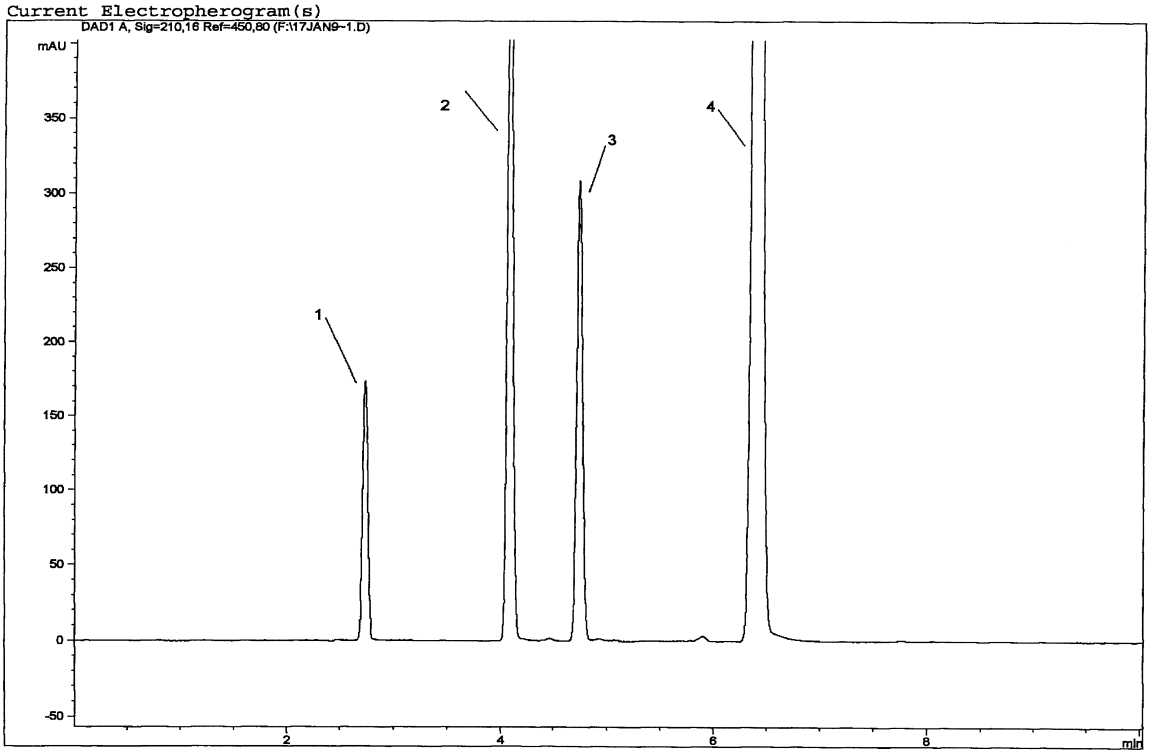

Fig. 2. Instrument: HP[3D]. Capillary: Innovatech 3 μm Spherisorb ODS-1, 33 cm (packed length 24.5 cm)×50 μm I.D. Mobile phase: acetonitrile–0.01 *M* Na_2HPO_4, unadjusted pH 8.4 (70:30). Voltage: 30 kV. Temperature: 30°C. Injection: 10 kV/10 s detection at 210 nm. 1=Thiourea, 2=dimethyl phthalate, 3=anisole, 4=naphthalene.

The new breed of HPLC stationary phases designed to eliminate analyte–silanol interactions, have either been chemically deactivated or modified in such a way as to shield unwanted silanol groups. Usually they are manufactured from high purity silica, thus eliminating another source of band broadening, i.e., silanol acidity due to the presence of metals in the silica matrix. An example of the effectiveness of these phases is shown in Fig. 5 where highly basic compounds are successfully chromatographed by HPLC on a 15 cm×4.6 mm I.D. column containing Develosil ODS UG-5, a new stationary phase developed by the Nomura Chemical Company of Japan. This material is a fully end-capped monomeric phase with an 18% carbon loading. It is 5 μm in diameter and has a pore diameter of 130 Å. This material has been shown to be highly stable under both acidic and basic conditions [35]. It is claimed by the manufacturers that this material provides excellent peak shapes for chelating, acidic as well as basic compounds. The increase in stability of these materials is attributed to a procedure that optimises the bonding of the alkylsilane followed by a dense endcapping procedure. Further details can be found in Ref. [35].

When the neutral test mixture is analysed on this phase by CEC using Tris buffer at pH 9.0, the highly efficient separation of all components is achieved in 12 min (Fig. 6A).

Our experience has shown that these materials are quite difficult to ''wet'' and therefore require high concentrations of organic solvent in order to elute analytes, typically >70%. If this same experiment is repeated using a non-aqueous mobile phase in the absence of a buffer, then the neutral test mixture is less retained than under the aqueous conditions, although the EOFs are very similar (1.33 mm s^{-1} for the aqueous and 1.46 mm s^{-1} for the non-aqueous systems, respectively). The overall reduction in analysis time is therefore assumed to be a result of

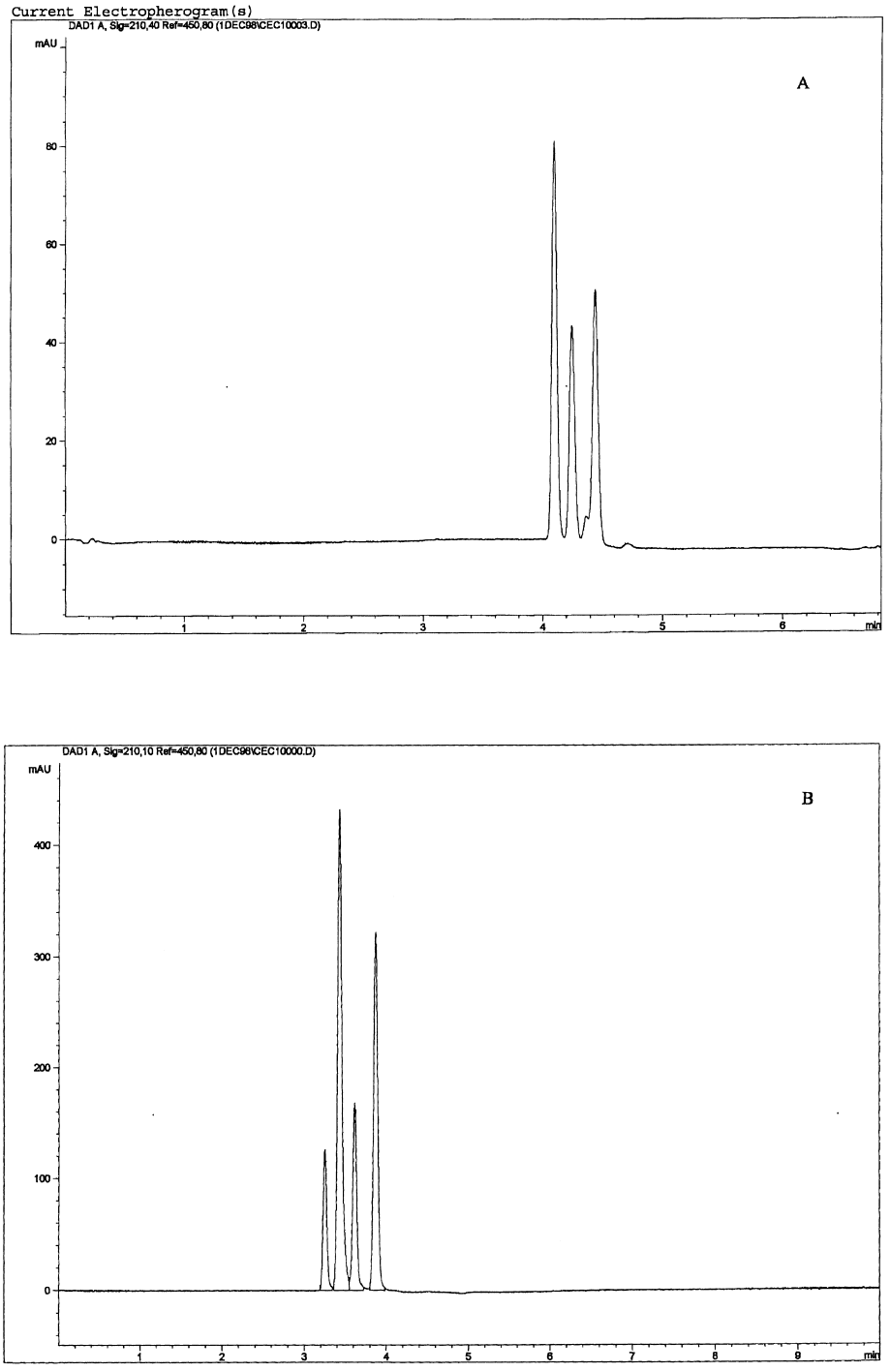

Fig. 3. (A) Instrument: HP[3D]. Capillary: Innovatech 3 μm Spherisorb ODS-1, 33 cm (packed length 24.5 cm)×50 μm I.D. Mobile phase: 0.01 *M* Tris in acetonitrile–methanol (1:1). Voltage: 30 kV. Temperature: 30°C. Injection: 10 kV/10 s detection at 210 nm. 1&2=Thiourea/dimethyl phthalate, 3=anisole, 4=naphthalene. (B) Instrument: HP[3D]. Capillary: Innovatech 3 μm Spherisorb Silica, 33 cm (packed length 24.5 cm)×50 μm I.D. Mobile phase: 0.01 *M* Tris in acetonitrile–methanol (1:1). Voltage: 20 kV. Temperature: 30°C. Injection: 5 kV/5 s detection at 210 nm. 1=Naphthalene, 2=anisole, 3=dimethyl phthalate, 4=thiourea.

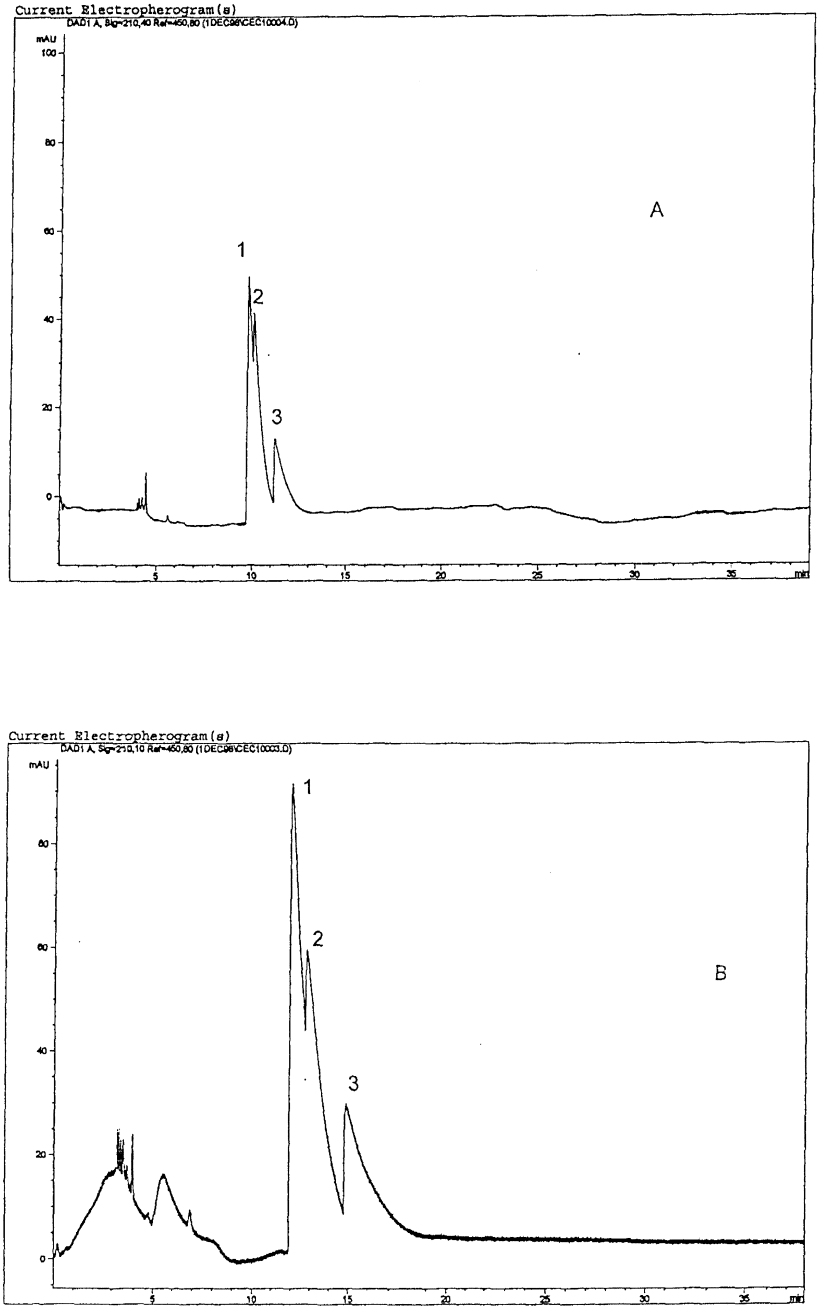

Fig. 4. (A) Instrument: HP[3D]. Capillary: Innovatech 3 μm Spherisorb ODS-1, 33 cm (packed length 24.5 cm)×50 μm I.D. Mobile phase: 0.01 *M* Tris in acetonitrile–methanol (1:1). Voltage: 30 kV. Temperature: 30°C. Injection: 10 kV/10 s detection at 210 nm. 1=Imipramine, 2=amitriptyline, 3=clomipramine. (B) Instrument: HP[3D]. Capillary: Innovatech 3 μm Spherisorb Silica, 33 cm (packed length 24.5 cm)×50 μm I.D. Mobile phase: 0.01 *M* Tris in acetonitrile–methanol (1:1). Voltage: 30 kV. Temperature: 30°C. Injection: 10 kV/10 s detection at 210 nm. 1=Imipramine, 2=amitriptyline, 3=clomipramine.

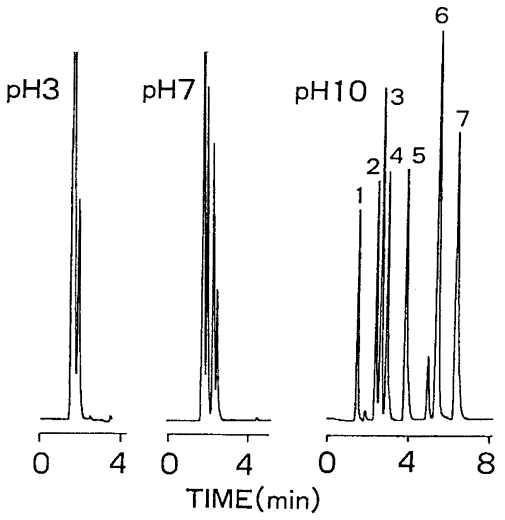

Fig. 5. HPLC separation of bases on Develosil ODS UG-5, 15 cm×4.6 mm I.D. Mobile phase: acetonitrile–0.02 *M* phosphate buffer (40:60, v/v). Flow-rate: 1.0 ml min^{-1}. Temperature: 30°C at 254 nm. 1=Pridoxine, 2=pyridine, 3=procainamide, 4=benzylamine, 5=*N*-methylbenzylamine, 6=metoclopramide, 7=*N*,*N*-dimethylbenzylamine.

the higher elution strength of this mobile phase (see Fig. 6B).

The addition of a buffer to this non-aqueous mobile phase has very little effect on the separation, although there was the expected increase in analysis time as a result of a significant reduction in the EOF, due to the effect of the buffer concentration on the zeta potential and consequently the linear velocity. The EOF in the non-buffered system, 1.46 mm s^{-1} reduces to 0.64 mm s^{-1} in the buffered system, thereby almost doubling the analysis time.

Attempts to analyse the basic compounds on the Develosil ODS UG-5 CEC column using an aqueous acetonitrile–Tris mobile phase produced extremely poor chromatography, as did the use of an unbuffered non-aqueous mobile phase. This was also observed when the same basic compounds were analysed using an unbuffered non-aqueous mobile phase on a Symmetry Shield RP-8 CEC capillary. When 0.01 *M* Tris was added to the non-aqueous mobile phase, a dramatic improvement was seen in the chromatography with very little evidence of peak tailing as illustrated in Fig. 7. The addition of a buffer to the mobile phase is believed to deactivate the silica surface silanol groups, as observed in the analysis of basic compounds by HPLC. It was apparent from the chromatography of this test mixture that decomposition was taking place, and separate experiments showed that nortriptyline gave a Gaussian peak at ~12 min when injected fresh, but appeared to decompose into many different components with the main degradation product eluting at ~7.0 min. Other components of the test mixture were much more stable under the operating conditions. Thiourea run under these conditions eluted with a retention time of 10.4 min giving an EOF of approximately 0.64 mm s^{-1}.

Symmetry Shield RP-8 is a 3.5 μm diameter stationary phase designed to ''shield'' highly polar and basic compounds from silanol activity [36]. This protection is accomplished by bonding an octylcarbamate ligand directly onto the silica surface and the protection results from three possible mechanisms. These are hydrogen bonding between the analyte and the polar carbonyl group of the carbamate ligand, hydrogen bonding between the carbonyl group of the carbamate with neighbouring silanol groups, and finally stabilisation of water at the silica surface by the embedded polar group. This results in an increased water concentration at the silica surface thus reducing silanol activity. A schematic diagram illustrating these three mechanisms is illustrated in Fig. 8.

We noticed that the EOF of this material was much more dependent on pH than conventional phases, dropping off considerably below pH 5.0. When the neutral test mixture is analysed on a 50 μm I.D. capillary packed with Symmetry Shield RP-8 material using a buffered acetonitrile mobile phase, good separation is achieved with highly Gaussian peaks in approximately 5 min (Fig. 9). The plate number for the anisole peak is 33 780 on-column, or 137 822 per metre.

When repeated in a acetonitrile–methanol (1:1) mobile phase, there appears to be a significant drop in EOF, measured assuming thiourea to be an EOF marker (2.1 and 1.0 mm s^{-1}, respectively). However, there is only a slight increase in the overall analysis time due to the solvent strength of the non-aqueous solvent, Fig. 10A. The addition of Tris buffer to the mobile phase has very little effect on the separation or the analysis time as shown by Fig. 10B.

This in contrast to our findings with the Develosil

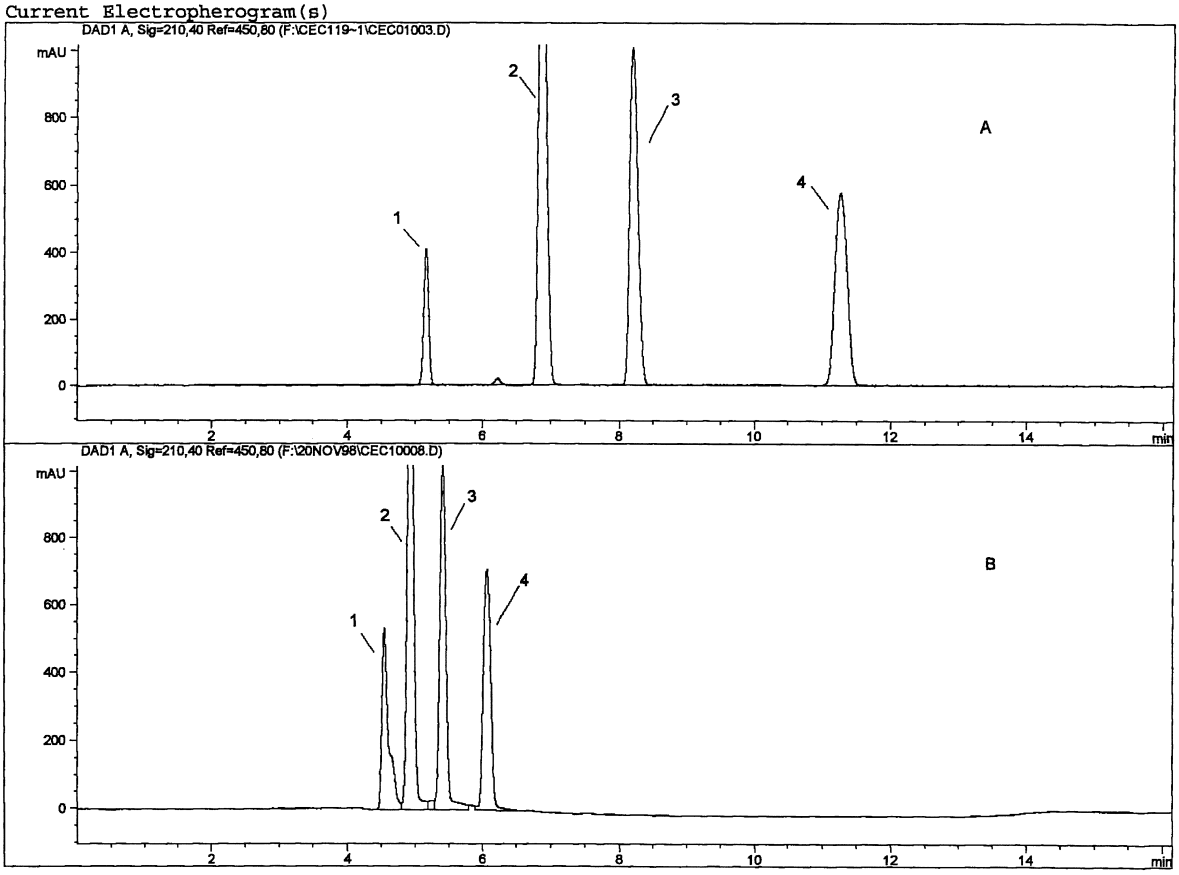

Fig. 6. (A) Instrument: HP[3D]. Capillary: Develosil ODS UG-5, 48.5 cm (packed length 40 cm)×50μm I.D. Mobile phase: acetonitrile–20 m*M* Tris, pH 9.0 (80:20). Voltage: 30 kV. Temperature: 30°C. Injection: 10 kV/10 s detection at 210 nm. 1=Thiourea, 2=dimethyl phthalate, 3=anisole, 4=naphthalene. (B) Instrument: HP[3D]. Capillary: Develosil ODS UG-5, 48.5 cm (packed length 40 cm)×50 μm I.D. Mobile phase: acetonitrile–methanol (1:1). Voltage: 30 kV. Temperature: 30°C. Injection: 10 kV/10 s detection at 210 nm. 1=Thiourea, 2=dimethyl phthalate, 3=anisole, 4=naphthalene.

ODS UG-5 material where the addition of Tris buffer to the non-aqueous mobile phase lead to a marked reduction in the EOF. The reduction of EOF with Develosil ODS UG-5 material on the addition of a buffer is in line with theory which predicts that the electrical double layer will be reduced, leading to a lower EOF. The fact that there is little change in the EOF on adding Tris to the acetonitrile–methanol (1:1) mobile phase on the Symmetry Shield RP-8 column could be due to the way in which the surface charge is ''shielded''.

Tricyclic antidepressants however, produce disastrous chromatography with the unbuffered mobile phase, whereas the addition of 0.01 *M* Tris to the mobile phase produces a significant improvement in performance as illustrated by Fig. 11A. Almost baseline resolution of imipramine from amitriptyline and clomipramine is achieved in less than 8 min with little evidence for peak tailing. However, the best overall results for the analysis of basic compounds including tricyclic antidepressants, was with a 50 μm I.D. capillary packed with Symmetry Shield RP-8 in the aqueous mode using acetonitrile–30% 0.1 *M* Tris buffer at pH 9.0 (70:30). It was possible to achieve the baseline separation of 6 basic compounds in under 6 min under these conditions, with all components giving acceptable peak symmetry. An example of this separation is shown in Fig. 11B.

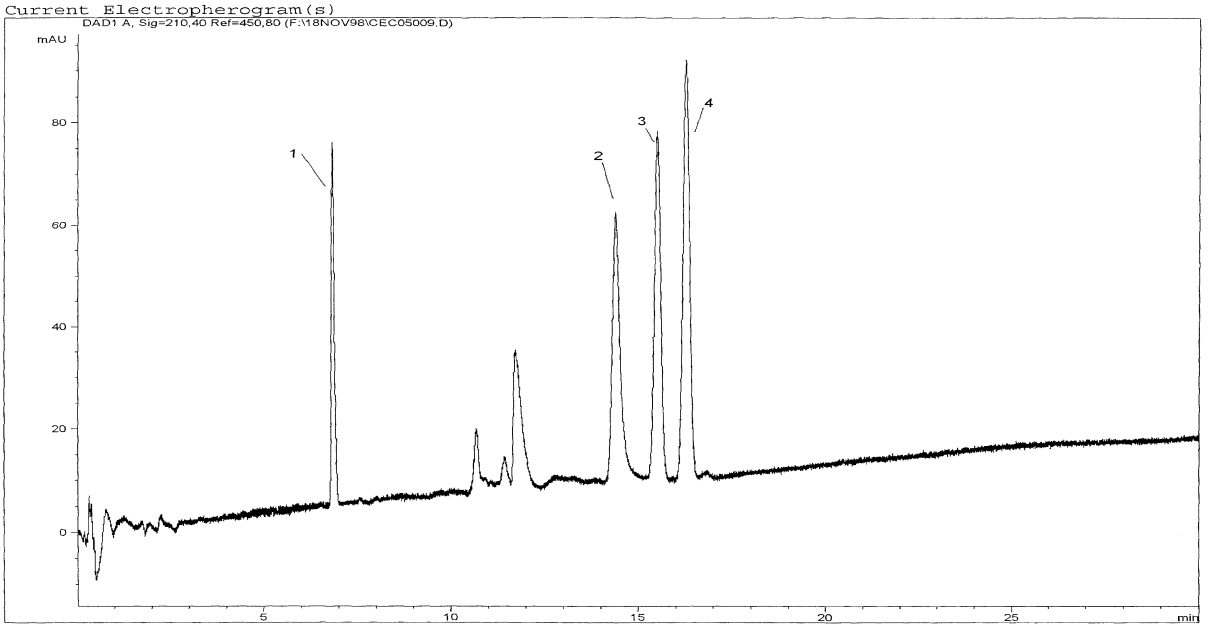

Fig. 7. Instrument: HP[3D]. Capillary: Develosil ODS UG-5, 48.5 cm (packed length 40 cm)×50 μm I.D. Mobile phase: 0.01 *M* Tris in acetonitrile–methanol (1:1). Voltage: 30 kV. Temperature: 30°C. Injection: 10 kV/10 s detection at 210 nm. 1=Decomposition product, 2=imipramine, 3=amitriptyline, 4=clomipramine.

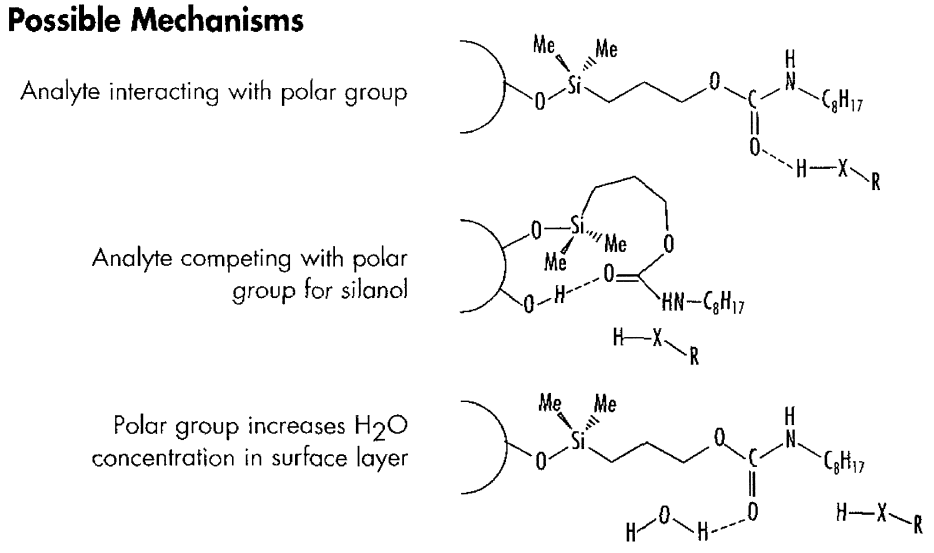

Fig. 8. Schematic of Symmetry Shield RP-8 mechanisms.

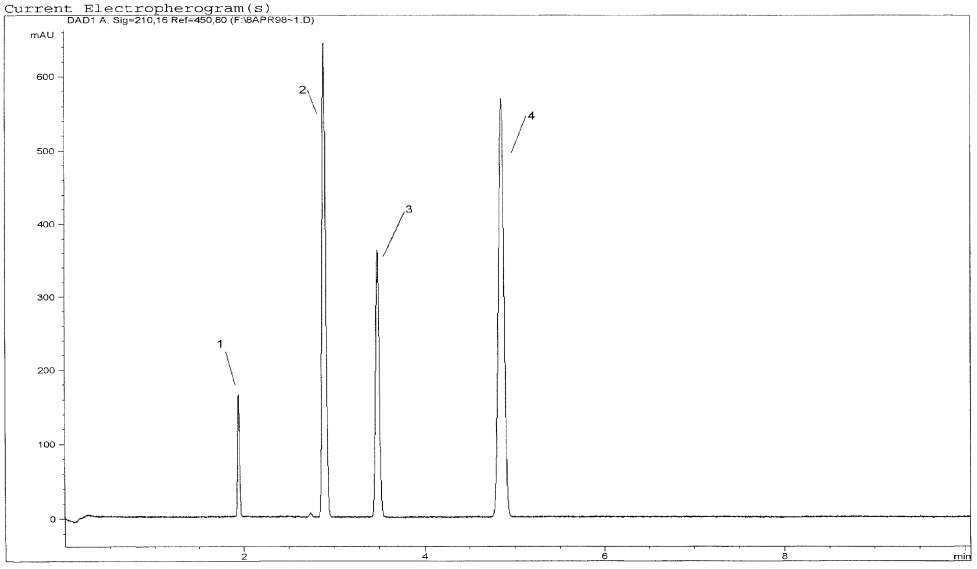

Fig. 9. Instrument: HP[3D]. Capillary: Innovatech 3.5 μm Waters Symmetry Shield RP-8, 33 cm (packed length 24.5 cm)×50 μm I.D. Mobile phase: acetonitrile–20 m*M* Tris, pH 9.0 (70:30). Voltage: 30 kV. Temperature: 30°C. Injection: 10 kV/10 s detection at 210 nm. 1=Thiourea, 2=dimethyl phthalate, 3=anisole, 4=naphthalene.

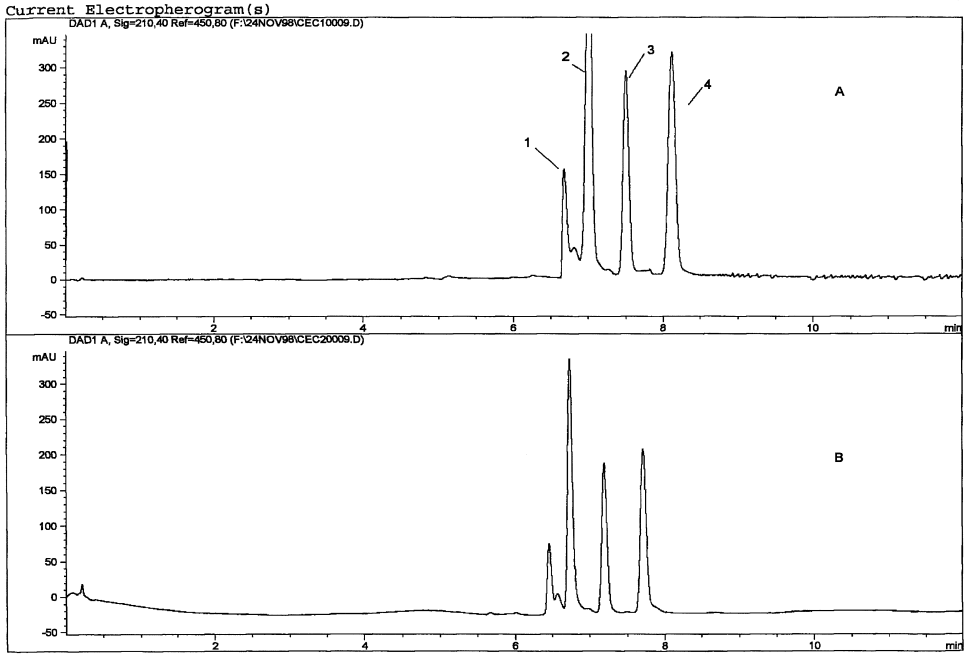

Fig. 10. (A) Instrument: HP[3D]. Capillary: Innovatech 3.5 μm Waters Symmetry Shield RP-8, 48.5 cm (packed length 40 cm)×50 μm I.D. Mobile phase: acetonitrile–methanol (1:1). Voltage: 30 kV. Temperature: 30°C. Injection: 10 kV/10 s detection at 210 nm. 1=Thiourea, 2=dimethyl phthalate, 3=anisole, 4=naphthalene. (B) Instrument: HP[3D]. Capillary: Innovatech 3.5 μm Waters Symmetry Shield RP-8, 48.5 cm (packed length 40 cm)×50 μm I.D. Mobile phase: 0.01 *M* Tris in acetonitrile–methanol (1:1). Voltage: 30 kV. Temperature: 30°C. Injection: 10 kV/10 s detection at 210 nm. 1=Thiourea, 2=dimethyl phthalate, 3=anisole, 4=naphthalene.

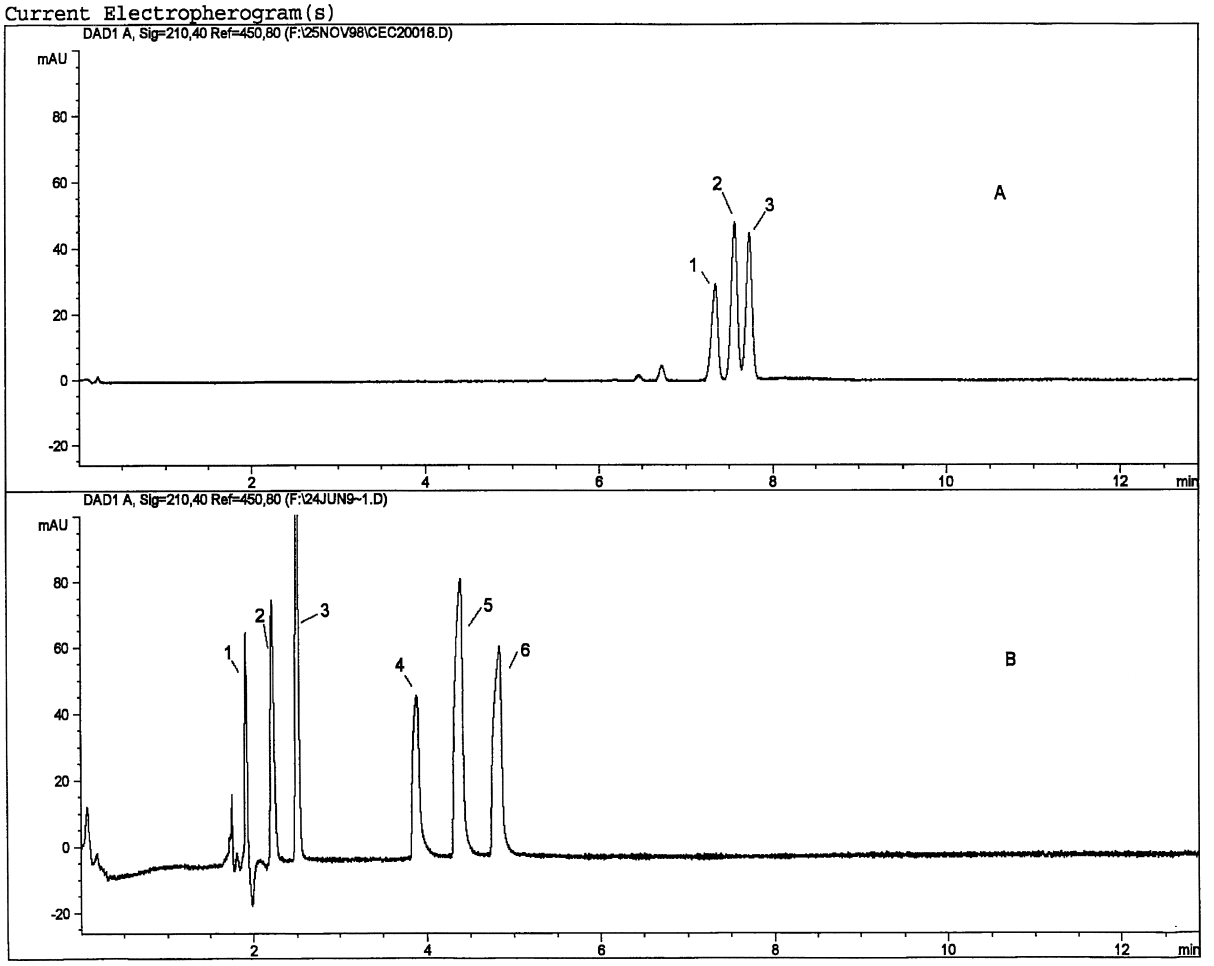

Fig. 11. (A) Instrument: HP[3D]. Capillary: Innovatech 3.5 μm Waters Symmetry Shield RP-8, 48.5 cm (packed length 40 cm)×50 μm I.D. Mobile phase: 0.01 *M* Tris in acetonitrile–methanol (1:1). Voltage: 30 kV. Temperature: 30°C. Injection: 10 kV/10 s detection at 210 nm. 1=Imipramine, 2=amitriptyline, 3=clomipramine. (B) Instrument: HP[3D]. Capillary: Innovatech 3.5 μm Waters Symmetry Shield RP-8, 33 cm (packed length 24.5 cm)×50 μm I.D. Mobile phase: acetonitrile–100 m*M* Tris, pH 9.0 (70:30). Voltage: 30 kV. Temperature: 40°C. Injection: 5 kV/5 s detection at 210 nm. 1=Carbovir, 2=ranitidine, 3=ondansetron, 4=imipramine, 5=amitriptyline, 6=clomipramine.

The EOF in the acetonitrile–Tris, pH 9.0 mobile phase was 1.9 mm s^{-1} and 1.0 mm s^{-1} in the 0.01 *M* Tris in acetonitrile–methanol (1:1) mobile phase. The higher EOF with the aqueous mobile phase could be due to the presence of a higher concentration of surface silanol groups at pH 9.0.

Because of time constraints it was not possible to evaluate the ability to analyse neutral as well as basic compounds using the Symmetry Shield material in the non-aqueous mode in the presence of a buffer, but obviously this is important if this material is to have a future as a CEC stationary phase. Similarly, very little solvent optimisation was carried out. Although it was found that most separations were best performed at high pH, no attempts to optimise the non-aqueous conditions were made.

4. Conclusions

Non-aqueous CEC in an unbuffered mode has been shown to give good chromatography with a

variety of stationary phases including underivatised silica, when analysing neutral compounds. However, it has been shown that under these conditions, the analysis of basic compounds fails spectacularly. The addition of an electrolyte to the non-aqueous mobile phase produces a significant improvement in chromatography. Of the phases studied, Waters Symmetry Shield RP-8 used in an aqueous mode was shown to give the best overall performance for neutral and basic compounds. However, there is scope for further optimisation of the conditions used in the buffered non-aqueous mode, including the type and composition of organic solvent and buffer.

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