ELSEVIER

Journal of Chromatography A, 927 (2001) 169–177

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Influence of the unpacked section on the chromatographic performance of duplex strong anion-exchange columns in capillary electrochromatography☆

C.D. Byrne[a], N.W. Smith[a,*], H.S. Dearie[a], F. Moffatt[b], S.A.C. Wren[c], K.P. Evans[d]

[a]*Zeneca/SmithKline Beecham Centre for Analytical Sciences, Department of Chemistry, Imperial college of Science, Technology and Medicine, South Kensington, London SW7 2AY, UK*

[b]*Syngenta Agrochemicals, Jealott's Hill Research Station, Bracknell, Berkshire RG42 6ET, UK*

[c]*AstraZeneca Pharmaceuticals, Macclesfield, Cheshire SK10 2NA, UK*

[d]*Avecia Ltd., Hexagon House, Blackley, Manchester M9 8ZS, UK*

Received 9 January 2001; received in revised form 12 June 2001; accepted 22 June 2001

Abstract

This work describes initial investigations of strong anion-exchange (SAX) packing materials for capillary electrochromatography (CEC). The use of SAX phases in CEC is theoretically appealing for the analysis of negatively charged species. The reversed direction of the electroosmotic flow (EOF) generated by SAX phases (in comparison to reversed phases and strong cation-exchange phases) means that negative species can migrate with the EOF, not against it, hence the analysis times, of such species should be decreased and efficiencies improved. Duplex CEC columns (the standard for instruments using UV detection) consist of a packed and an unpacked section. Using common reversed-phase packing materials the direction of the EOF in both sections is co-linear, however when normal fused-silica capillaries are packed with SAX material the direction of the EOF in the two sections oppose one another. It has been shown, using conventional duplex CEC columns and fully packed CEC–MS columns that the opposing direction of EOF causes a massive degradation in column performance. Consequentially, it is demonstrated that if the EOF in the open section of the duplex SAX column can be controlled via pH or capillary derivatisation then good, reproducible CEC can be performed on anionic species using SAX packed CEC columns.

Keywords: Electroosmotic flow; Duplex columns; Stationary phases, CEC; Anion exchangers; Anionic analytes

1. Introduction

Capillary electrochromatography (CEC) is, in many ways analogous to high-performance liquid chromatography (HPLC). However CEC is theoretically able to provide better separations, due to the inherently high efficiencies associated with the ''plug like'' shape of the electroosmotic flow (EOF) and by the use of small diameter column packing particles. The majority of CEC columns, including commercially available ones, are based on silica as the chromatographic support. These include reversed-

☆Presented at the 4th International Symposium on Capillary Electrochromatography, San Francisco, CA, 12–14 August 2000.

*Corresponding author. Tel.: +44-207-5945-841; fax: +44-207-5945-859.

E-mail address: n.w.smith@ic.ac.uk (N.W. Smith).

0021-9673/01/$ – see front matter
PII: S0021-9673(01)01061-5

phase (RP), strong cation-exchange (SCX) and mixed mode (RP/SCX) packing materials. As a consequence of the negatively charged residual silanol groups and/or sulfonic acid groups, the EOF in such columns flows towards the cathode. Under these conditions, cations will migrate in the same direction as the EOF under the application of voltage, undergoing similar separation effects to liquid chromatography [1–5]. However, anions will tend to migrate against the EOF, which can result in long analysis times. In addition, the negative charge on these packing materials will tend to repel the negatively charged analytes, causing minimal chromatographic retention and poor efficiencies [6,7]. Because of this, the majority of CEC publications and applications to date have concentrated on the analysis of neutral and cationic species [1,3,4,8]. However, it is quickly becoming obvious, that if CEC is to gain widespread acceptance as a viable separation technique, alternative methods must be devised that allow for fast and efficient analysis of anionic species.

Currently, the most successful acid analyses have been achieved using ion-suppressed CEC with reversed-phase packing materials [5]. When CEC is operated in ion-suppressed mode, the pH of the mobile phase is adjusted so that it is well below the pK_a value of the acidic analytes [9–11]. Under these conditions the compounds become uncharged and consequently the applied voltage does not influence their electrophoretic mobility. In addition, the removal of the negative charge on the analyte allows for greater chromatographic interaction with the hydrophobic stationary phase. However at low pH the EOF generated in standard reversed-phase columns can be very low, again resulting in long analysis times. This problem has been addressed by increasing the applied field strength through the use of higher voltages and short packed column lengths [10]. In addition, the small EOF at low pH can to some extent be addressed by using a strong cation-exchange (SCX) stationary phase [6,12–20]. The majority of SCX stationary phases used contain sulfonic acid functional groups bonded to a silica support and therefore are negatively charged over a wider pH range than unmodified silica. Columns packed with SCX material will therefore generate an acceptable EOF at low pH and so are more effective when used for ion-suppressed CEC. In particular ''mixed mode'' phases containing both cation-exchange functions as well as hydrophobic alkyl groups have proved useful in many applications. These mixed-mode phases would appear to offer a wide working pH range combined with reversed-phase chromatographic properties, and as such the possibility of faster and more efficient separations of acids at low pH [5,21].

Alternatively, strong anion-exchange (SAX) phases, which are typically quaternary amines bonded to silica, are positively charged at all pH levels. This results in the reversal of the direction of the generated EOF compared to conventional reversed-phase and SCX packing materials. This allows anions to migrate in the same direction as the EOF. In addition the positively charged packed bed promotes ion-exchange chromatography, theoretically increasing the selectivity of the phase. Indeed anion-exchange liquid chromatography has been successfully used to separate acidic mixtures [19,22–31]. However, to date, there has been relatively little interest in the use of SAX phases in CEC. The work in this paper covers the initial findings on the use of SAX phases and the results give an indication of the potential power of SAX material for the analysis of anionic species using CEC. This study also demonstrated the dramatic effect the open section of a duplex column has on the performance of SAX-CEC analyses. Accordingly methods are devised to reduce these effects and produce more reliable SAX separations using CEC.

2. Experimental

2.1. Chemicals

Thiourea, uracil, α-, β-, γ- resorcylic acids, salicylic acid, *m*-hydroxybenzoic acid and gentisic acid were purchased from Aldrich (Gillingham, UK). γ-(Trimethoxysilyl)propyl methacrylate, 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH), 3-(methacryloylamino)propyltrimethylammonium chloride and potassium persulfate ($K_2S_2O_8$) were also purchased from Aldrich. Anthraquinone-1-, anthraquinone-2-, anthraquinone-1,5- and anthraquinone-1,8-sulfonic acids were a gift from Ken Evans (Avecia,

UK). Ammonia, ammonium acetate, acetic acid, dimethylformamide (DMF), disodium hydrogenorthophosphate (Na_2HPO_4), phosphoric acid (H_3PO_4), sodium acetate (NaAc), sodium dihydrogenorthophosphate (NaH_2PO_4), sodium hydroxide and tris(hydroxymethyl)aminomethane (Tris) were of analytical grade and purchased from BDH (Poole, UK). Acetonitrile, methanol and methylene chloride were of HPLC grade and also purchased from BDH. Buffer solutions were prepared using deionized water from an Elga Maxima water purifier. The mobile phase used to investigate the effect of pH were produced as follows:

Five ml acetonitrile plus 5 ml 20 m*M* Tris buffer adjusted to pH 9.0 with 0.1 *M* H_3PO_4.

Five ml acetonitrile plus 5 ml 20 m*M* Na_2HPO_4 buffer adjusted to pH 7.5 with 0.1 *M* H_3PO_4.

Five ml acetonitrile plus 5 ml 20 m*M* Na_2HPO_4 buffer adjusted to pH 6.5 with 0.1 *M* H_3PO_4.

Five ml acetonitrile plus 5 ml 20 m*M* NaAc buffer adjusted to pH 5.0 with 0.1 *M* acetic acid.

Five ml acetonitrile plus 5 ml 20 m*M* NaAc buffer adjusted to pH 4.0 with 0.1 *M* acetic acid.

Five ml acetonitrile plus 5 ml 20 m*M* NaH_2PO_4 buffer adjusted to pH 2.8 with 0.1 *M* H_3PO_4.

Five ml acetonitrile plus 5 ml 20 m*M* NaH_2PO_4 buffer adjusted to pH 2.2 with 0.1 *M* H_3PO_4.

The mobile phase used for the CEC–MS investigations were prepared using 5 ml acetonitrile plus 5 ml, 20 m*M* ammonium acetate and adjusted to the appropriate pH using 0.1 *M* acetic acid or ammonia.

The structures of the substituted benzoic acids and the sulfonic acids are shown in Figs. 1 and 2, respectively. All analytes were dissolved in acetonitrile–water (50:50, v/v) to give a concentration of 1 mg/ml.

2.2. Columns

CEC columns were prepared using a method previously detailed by Carter-Finch and Smith [32]. Capillaries were supplied by Composite Metals (Worcs., UK) and the column packer obtained from Shandon Instruments (Runcorn, UK). Windows and frits were fabricated using an Innovatech (Stevenage, UK) ACF electrical burner (Stevenage, UK). The 3 μm Waters Spherisorb SAX material was a gift from Professor Peter Myers, at the time with Phase Separations (Deeside, UK). This material contained a quaternary ammonium group attached to 3 μm silica but no ion-exchange capacity data is available for this material.

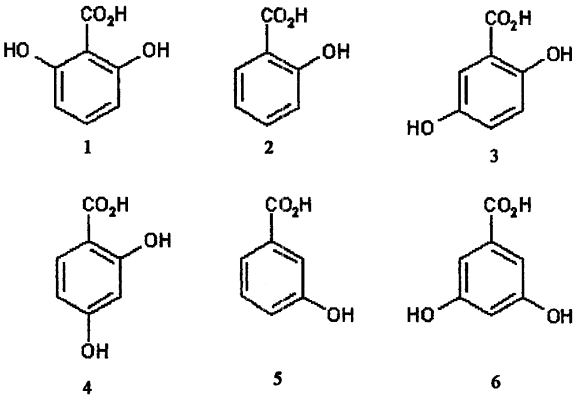

Fig. 1. Structures of benzoic acid derivatives. (1) γ-Resorcylic, (2) salicylic, (3) gentisic, (4) β-resorcylic, (5) *m*-hydroxybenzoic and (6) α-resorcylic acids.

2.3. Instrumentation

To allow a complete understanding of the behaviour of the SAX phase and the effect of the open and unpacked sections of the duplex columns, various capillary configurations were investigated, ranging from fully packed to fully open. This necessitated the use of a variety of instruments. Table 1

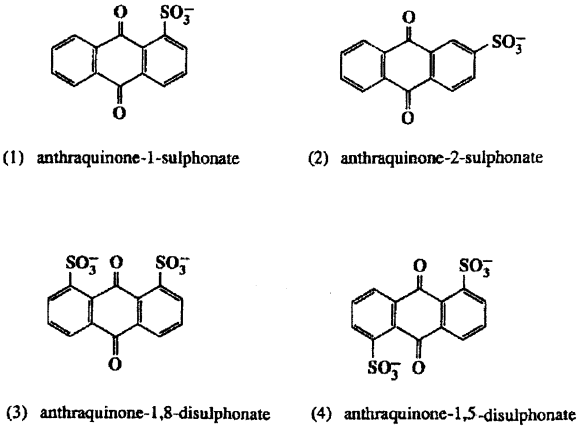

Fig. 2. Structures of sulfonic acid compounds. (1) Anthraquinone-1-sulfonate, (2) anthraquinone-2-sulfonate, (3) anthraquinone-1,8-disulfonate, (4) anthraquinone-1,5-disulfonate.

Table 1
Possible column configuration available for CEC

Instrument	Detection/ wavelength (nm)	Capillary dimensions	
		Open length (mm)	Packed length (mm)
Hewlett-Packard ^{3D}CE	UV diode array/214 nm	85	>200
Beckman MDQ CE	UV/214 nm	100	>200
Modified Applied Biosystems 270A	UV/214 nm	250–500	>250
CEC–MS[a]	MS	0–200	>150

For all instruments using UV detection the applied voltage can be reversed, allowing the length of the packed and opens sections to be reversed.

[a] Innovatech CEC–MS interface coupled to Applied Biosystems/MDS Sciex MS API150.

shows the list of instruments used in this work and the possible column configurations that they offer. All column configurations were produced to allow detection immediately after the outlet frit.

The fully packed column experiments were performed by CEC–MS on a Sciex API 150MCA single quadruple mass spectrometer (Applied BioSystems/MDS Sciex, Warrington, UK), using a micro-electrospray CEC–MS interface. Injections were performed with an automated injection system (Innovatech) [33–35]. The spray voltage was set at −4.5 kV. The sheath flow composition was methanol–water+0.1% acetic acid (70:30, v/v) and was supplied at a flow-rate of 2.0 μl/min by a Harvard syringe pump. Injections and separations were performed using a high-voltage power supply unit (Spellman, Hauppauge, USA). The separation voltage applied was −20 kV in all the experiments (−15.5 kV overall). Injections were performed at −10 kV for 5 s.

3. Results

Initial investigations of SAX packing materials in CEC were performed on a Hewlett-Packard (HP) ^{3D}CE instrument. The design of the HP capillary cartridge dictates that a duplex column must be used. In the standard format the packed length of the capillary (L_p) was approximately 220 mm and the unpacked length (L_o) was approximately 85 mm. A 100 μm I.D. untreated fused-silica capillary was packed with 3 μm Spherisorb SAX material. In order to achieve a stable current, the columns were flushed with mobile phase for 1 h using a HPLC pump, prior to mounting in the cartridge. Additional conditioning at −10 kV for ~5 h was also necessary. All experiments were run using reversed polarity.

Fig. 3 illustrates the linear velocity in the SAX column as a function of pH, measured using uracil and thiourea as t_0 markers. Under the conditions tested both uracil and thiourea gave equivalent t_0 times. As the pH of the mobile phase is increased the EOF in the SAX packed duplex capillary was dramatically reduced. This observation is counter intuitive, as one would expect only a minor decrease in the EOF over the pH range measured, since the charge on the quaternary ammonium groups would

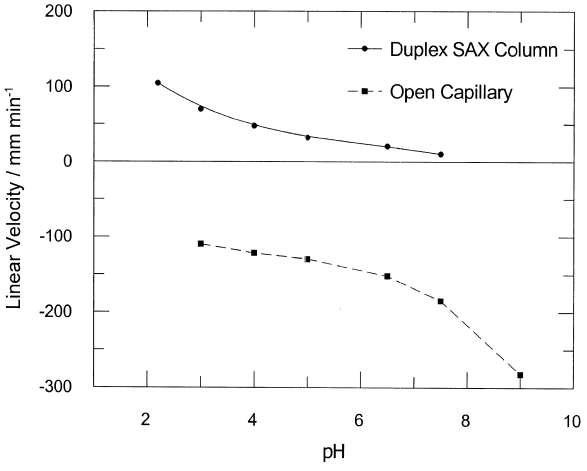

Fig. 3. The effect of the buffer pH on the linear velocity in packed and open capillaries, measured using thiourea and uracil. Conditions: mobile phase: 20 m*M* buffer (pH variable)–acetonitrile (50:50, v/v). Applied voltage: −20 kV. Column dimensions: Duplex column (●) 220 mm×100 μm I.D. fused-silica capillary packed with 3 μm Waters Spherisorb SAX, total column length 305 mm. Open fused-silica capillary (■) 220 mm×100 μm I.D., total column length 305 mm. Instrument: HP ^{3D}CE. Wavelength: 214 nm.

be expected to remain essentially unchanged at all pH values between 2.5 and 9. In essence, it is the increase in anodic (positive to negative) EOF due to deprotonation of the silanol groups on the surface of the capillary wall that accounts for the decrease in overall EOF with increasing pH. This effect is likely to be most pronounced in the open section. Additionally, underivatised silanol groups on the silica particles will be a contributing factor to these observations. Perhaps more importantly, as the pH of the mobile phase was increased the stability of the column deteriorated, i.e., the current became erratic and reproducibility was poor. For comparison the mean linear velocity in an untreated open fused-silica capillary as a function of pH is also illustrated in Fig. 3 (voltage reversed, hence the linear velocity is plotted on a negative axis). As expected, under identical conditions the direction of the EOF in the open column is reversed in comparison to the direction of the EOF generated in the SAX duplex column. In the untreated fused-silica capillary the greatest EOF was achieved at high pH and dropped to almost zero at a pH lower than 2.8. This was a consequence of the surface silanol groups becoming protonated at low pH.

In an attempt to understand the results given in Fig. 3 the duplex SAX column can essentially be considered as a hybrid of a packed SAX section and open fused-silica capillary. Under the application of voltage the direction of generated EOF is different in each section, or it would be if the two sections were not coupled. As the overall EOF can only be in one direction, in this configuration the equilibrium between the opposing flows will determine the direction and magnitude of the EOF. It is possible that the equilibrium of the EOF was adversely affecting the column performance, since a large flow profile discontinuity would have been established at the interface between the two sections. This will be especially true at high pH, when the EOF in the open section is greatest.

Considering these findings it is important that we try to understand the process occurring in the packed section of the duplex column. CEC–MS removes the need for the open section that is present in the duplex column, hence CEC–MS studies were performed to allow the electrokinetic characteristics of the packed bed to be measured. Again 100 μm I.D. untreated fused-silica capillary was packed with 3 μm SAX particles. Initially the packed length was 220 mm. In this configuration, with no unpacked section, equilibration using only voltage was achieved in approximately 30 min, a dramatic improvement over the duplex column. Fig. 4 illustrates the effect of pH on the mean linear velocity in the fully packed column. The phosphate buffers used in the previous studies were incompatible with MS, hence they were replaced by corresponding ammonium buffers (20 m*M*). Unless otherwise stated all other conditions were identical to the previous experiment.

Fig. 4 illustrates that the linear velocity generated in the packed section of a SAX column is unaffected by pH, up to approximately pH 7.0, and drops only slightly at higher pH values. This observation is in line with our understanding of the chemistry of strong anion-exchange packing materials. The results also indicate that it was the open section of the duplex column that was adversely affecting its performance. The flexibility of the CEC–MS interface also allows the length of the open section of a

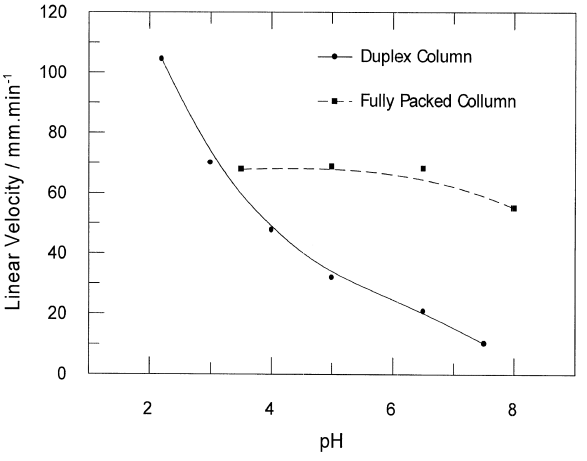

Fig. 4. The effect of buffer pH on the linear velocity of the EOF in 3 μm Waters Spherisorb SAX packed columns, measured using thiourea and uracil. Conditions: Duplex column (●). Mobile phase: 20 m*M* buffer (pH variable)–acetonitrile (50:50, v/v). Applied voltage: −20 kV. Column dimensions: 220 mm×100 μm I.D. (total column length 305 mm). Instrument: HP ^{3D}CE. Wavelength: 214 nm. Fully packed column (■). Mobile phase: 20 m*M* ammonium acetate buffer (pH variable)–acetonitrile (50:50, v/v). Applied voltage: −20 kV. Column dimensions: 220 mm× 100 μm I.D. (total column length 220 mm). Instrument: CEC–MS.

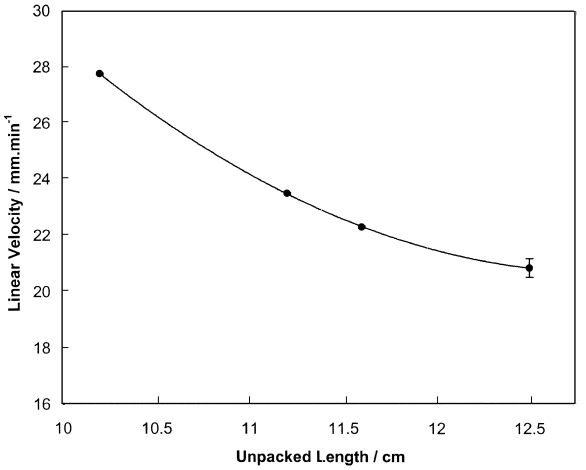

Fig. 5. The effect of the length of the open section of a duplex SAX column on the EOF velocity and column stability. Conditions: mobile phase: 20 m*M* ammonium acetate buffer (pH 5.5)–acetonitrile (50:50, v/v). Applied voltage: −20 kV. Column dimensions: 240 mm×100 μm I.D. (total column length variable). Instrument: CEC–MS.

duplex column to be varied, something that is difficult to achieve with the commercial instruments. Fig. 5 shows the effect of the open length of a duplex column on the EOF using a capillary with packed length=240 mm at pH 5.5 (other conditions as in Fig. 4). Varying field strengths in the different length columns account for the change in observed velocity. Data points are the average of at least 25 injections, and standard deviations also shown as error bars.

The results in Fig. 5 demonstrate that as the length of the open section was decreased the mobility was seen to increase, and this was almost certainly a result of a reduction in the negative EOF contribution from the open section. Above an open length of 125 mm, uracil and thiourea failed to elute, demonstrating the significant contribution from the open section, even at pH 5.5. In addition as the open length is increased the standard deviation of the measured mobility increased, i.e., the increased negative EOF contribution from the open section was making the column less stable. These observations are in agreement with the observations made in the previous section and provide conclusive evidence that the unpacked section in the duplex columns is adversely affecting the performance of the SAX columns when used in the conventional UV instruments.

The investigations using the duplex and the fully packed SAX-CEC columns has demonstrated that at low pH (<2.5) the EOF generated in the open section of the duplex column is eliminated, or at worst can be considered negligible. Under these conditions it was shown that reproducible chromatography could be performed on acidic mixtures. Initially, a simple six acid test mixture containing some benzoic acid derivatives was devised to test the effectiveness of the phases. Fig. 6 shows the separation of the six acid test mixture was possible in less than 6 min, with theoretical plate numbers of over 100 000/m. It is probable that at these low pH values, when the acids are only partially ionised, that the minimal retention was due to weak ion-exchange mechanisms, with the separation being achieved by both ion-exchange and electrophoretic processes. As the pH was increase the separation became very slow, until at a pH greater than 4.0 none of the acids eluted, again demonstrating the considerable effect of the open section.

In our laboratory, very strong acids have presented

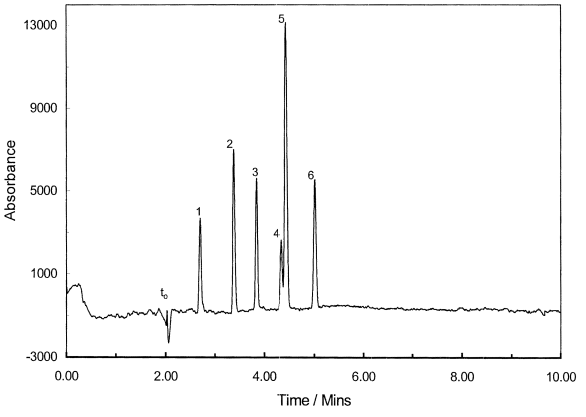

Fig. 6. Chromatogram showing the separation of six test acids using a 3 μm Waters Spherisorb SAX column. Conditions: mobile phase: 20 m*M* sodium dihydrogenorthophosphate (pH 2.5)–acetonitrile. Applied voltage: −25 kV. Injection: −5 kV for 5 s. Column dimensions: 220 mm×100 μm I.D. (total column length 320 mm). Instrument: MDQ. Wavelength: 214 nm. Peaks: (1) γ-resorcylic, (2) salicylic, (3) gentisic, (4) β-resorcylic, (5) *m*-hydroxybenzoic and (6) α-resorcylic acids.

significant analysis problems for CEC using RP/SCX and can sometimes be very difficult to even introduce onto the column because of their strong negative charges. Sulfonic acids fall into this category and have proved very difficult to analyse using CEC. Fig. 7 shows the low pH separation of four sulfonic acids on a duplex SAX-CEC column. This separation was performed on an ABI instrument where the packed and unpacked sections of the column are of equal length (~250 mm). This long overall column length results in a low total field strength, leading to a reduced EOF (EOF is measured in mm/s). However the slower analysis times were also probably due to the greater degree of sample ionisation causing a greater amount of ion-exchange retention. Again as the pH was increased to over 3, none of the acids eluted as the effects of the open section became dominant.

These results demonstrated that if reproducible chromatography is to be performed using SAX stationary phases then the effect of the open section of the duplex column must be eliminated. However it may not always be desirable to do this by lowering the pH. Indeed if chromatography can be performed above the pK_a values of the acids then they will migrate in the same direction as the EOF, offering the possibility of faster analysis and greater ion-exchange selectivity.

It is possible to chemically modify the internal surface of the fused-silica capillary in order to control the direction and the strength of the EOF. By using an amine coating, the direction of the EOF is reversed in comparison to standard fused-silica capillaries. Under these conditions the EOF generated in the open section will be in the same direction as the EOF generated in the SAX packed section. Pretreatment and silanization of the fused-silica capillary inner wall was performed using procedures detailed by Zhang et al. [29]. The amine coating was achieved using a method published by Huang and Horváth [36] as follows. The silanized capillary was filled with 10% (v/v), 3-(methacryloylamino)propyltrimethylammonium chloride plus 0.1% (w/v) $K_2S_2O_8$ and the ends sealed. The capillary was then heated at 90°C for 6 h after which it was cooled to room temperature, flushed with deionised water and methanol and blown dry with nitrogen. Fig. 8 compares the liner velocity of the EOF in a SAX packed amine coated capillary and a SAX packed

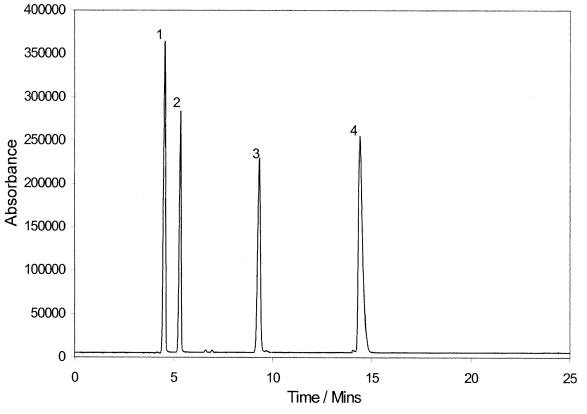

Fig. 7. Chromatograms for the separation of the four test sulfonic acids using a 3 μm Waters Spherisorb SAX column. Conditions: mobile phase: 20 m*M* sodium dihydrogenorthophosphate (pH 2.5)–acetonitrile (50:50, v/v). Applied voltage: −25 kV. Injection: −5 kV for 5 s. Column dimensions: 250 mm×100 μm I.D. (total column length 500 mm). Instrument: ABI. Wavelength: 214 nm. Peaks: (1) anthraquinone-1-sulfonate, (2) anthraquinone-2-sulfonate, (3) anthraquinone-1,8-disulfonate, (4) anthraquinone-1,5-disulfonate.

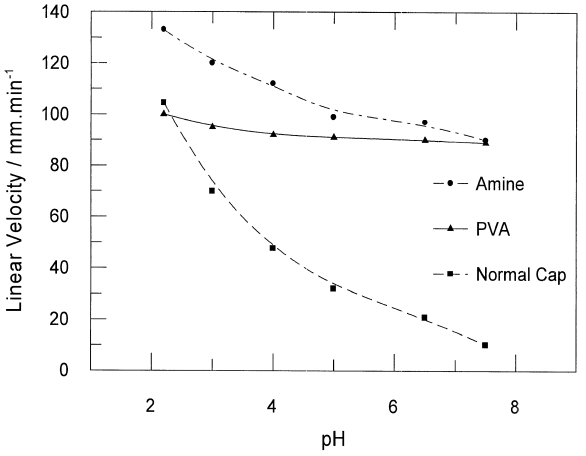

Fig. 8. The effect of pH on the linear velocity in untreated fused-silica, PVA and amine coated capillary packed with 3 μm Waters Spherisorb SAX material. Conditions: mobile phase: 20 m*M* sodium dihydrogenorthophosphate (pH 2.5)–acetonitrile (50:50, v/v). Applied voltage: −25 kV. Injection: −5 kV for 5 s. Column dimensions: 220 mm×100 μm I.D. (total column length 320 mm). Instrument: MDQ. Wavelength: 214 nm.

uncoated capillary. In both cases, L_p=220 mm and L_o=120 mm. It is also possible to prevent any EOF being generated in the capillaries so that the open section of the capillary will have no effect on the column performance. Protein separations using CE are strongly affected by adsorption onto the capillary wall. Poly(vinyl alcohol) (PVA) has a great tendency to be adsorbed onto polar surfaces, suppressing the contribution of silanol groups and preventing the adsorption of proteins [37]. The PVA coating suppresses the silanol groups and consequently neutralises the EOF in fused-silica capillaries. Capillaries were permanently PVA coated using the following procedure.

A 10% (w/w) solution of PVA in water is forced through a 100 μm I.D. capillary using a syringe. After completely filling, the coating solution was slowly discharged from the capillary using dried nitrogen, leaving a thin polymer film over the entire length of the capillary. Finally the PVA coating was immobilised by heating the capillary to 140°C under nitrogen for several hours. The coated capillary is then flushed with copious volumes of water before being packed with the SAX phase using the conventional procedure. The behaviour of the EOF of the PVA coated SAX capillaries as a function of pH are illustrated in Fig. 8.

Fig. 8 shows that in an amine coated SAX packed capillary, the velocity of the EOF is greater at all pH values than in both an untreated and PVA coated capillary. In this situation (amine coated) the EOF generated in the open section of the column is increasing the EOF measured through the packed section. The removal of the negative effect of the open section also means that the EOF is less effected by the pH of the mobile phase. It was also observed that the capillary was more stable over the pH range, and was less prone to bubble formation. However, because the EOF is still generated in the open section there will still be a flow discontinuity at the interface between the open and packed sections. In the SAX packed PVA coated capillary, this flow discontinuity would be enhanced as no EOF will be generated in the open section.

Fig. 8 also shows that over the entire pH range the velocity of the EOF in the PVA coated SAX packed capillary was less that that of the amine coated SAX packed capillary, but greater than the fused-silica packed open capillary. It is also important to note that over the entire pH range the velocity of EOF in the PVA coated capillary was almost unchanged. This observation is in agreement with the CEC–MS measurements, and shows that by suppressing the EOF in the open section of the capillary the observed mobility is generated almost exclusively by the packed bed. In addition the duplex SAX columns packed in the PVA coated capillaries were extremely stable, in comparison to the columns packed using the fused-silica and amine coated capillaries. It must also be noted that the EOF has yet to be measured in the open PVA and amine coated capillaries. The extent to which these coatings affect the EOF should give a greater insight into the overall velocity of the EOF in a packed duplex SAX CEC column.

4. Conclusions

The results presented in this work indicate that if care is taken, CEC using SAX packed columns is capable of the efficient and fast separation of acidic compounds. The negative EOF generated by the SAX packing material and the selectivity of anion-exchange chromatography, make the technique ideal for the analysis on anionic species.

However, the results presented also demonstrate another interesting property of CEC. That is the dramatic effect that the open section of a duplex CEC column has on its performance. Although this work has concentrated on the effects in SAX packed capillaries, the findings have implications on all forms of CEC employing duplex columns. Work conducted in our laboratory previously has shown when using conventional reversed-phase packing materials, that the EOF generated in a fully packed fused-silica column is slower than in a duplex column, all other conditions being equal. It should therefore come as no surprise that when the directions of the flow in the two sections are opposing, problems arise.

The previously made assumption that the wall has no effect in CEC [16], can still be considered true, but only in the packed section, where its contribution can be considered negligible. In the open section the wall has a significant effect on the columns performance. In fact it has been shown that under certain condition the EOF generated in the open section is, in effect, a pump pulling the mobile phase

through the packed bed [38]. This would allow uncharged phases to be packed into normal capillaries and an EOF would still be generated.

Another concern in CEC over the past years has been the durability of the technique, since CEC columns that perform well one day may be useless the next. It may be that the discontinuities in the flow velocities at the interface between the packed and unpacked sections (normally in the region of the UV detection window) causes pressure variations and turbulence, which at best degrades the performance of the column and at worst causes outgasssing of the solvent and bubble formation. It is possible that neutralized capillaries, which generate no EOF, may remove this problem, allowing the properties of only the packed bed to influence the columns performance.

Now that we are able to produce stable, reliable SAX-CEC columns, their chromatographic and physical properties must be investigated further. It is possible that proteins are a potential application area for this stationary phase now that we have a better understanding of its characteristics.

Acknowledgements

The authors would like to thank Zeneca SRF for funding, Hewlett-Packard, Beckman Instruments and Applied Biosystems/MDS Sciex for kind provision of equipment and Val Spikmans for useful discussions and assistance with experimental work.

References

[1] A.L. Crego, A. Gonzalez, M.L. Marina, Crit. Rev. Anal. Chem. 26 (1996) 261.
[2] L.A. Colon, K.J. Reynolds, R. Alicea Maldonado, A.M. Fermier, Electrophoresis 18 (1997) 2162.
[3] L.A. Colon, Y. Guo, A. Fermier, Anal. Chem. 69 (1997) A461.
[4] K.D. Altria, N.W. Smith, C.H. Turnbull, Chromatographia 46 (1997) 664.
[5] K.D. Altria, N.W. Smith, C.H. Turnbull, J. Chromatogr. B 717 (1998) 341.
[6] G. Choudhary, Cs. Horváth, J. Chromatogr. A 781 (1997) 161.
[7] F. Moffatt, P.A. Cooper, K.M. Jessop, Anal. Chem. 71 (1999) 1119.
[8] M.M. Robson, M.G. Cikalo, P. Myers, M.R. Euerby, K.D. Bartle, J. Microcol. Sep. 9 (1997) 357.
[9] M.R. Euerby, D. Gilligan, C.M. Johnson, K.D. Bartle, Analyst 122 (1997) 1087.
[10] M.R. Euerby, C.M. Johnson, M. Cikalo, K.D. Bartle, Chromatographia 47 (1998) 135.
[11] R. Dadoo, C. Yan, R.N. Zare, D.S. Anex, D.J. Rakestraw, G.A. Hux, LC–GC Int. 15 (1997) 630.
[12] M.L. Ye, H.F. Zou, Z. Lui, J.Y. Ni, J. Chromatogr. A 869 (2000) 385.
[13] N.W. Smith, M.B. Evans, Chromatographia 41 (1995) 197.
[14] I.S. Lurie, T.S. Conver, V.L. Ford, Anal. Chem. 70 (1998) 4563.
[15] T. Adam, S. Ludtke, K.K. Unger, Chromatographia 49 (1999) S49.
[16] N.W. Smith, M.B. Evans, J. Chromatogr. A 832 (1999) 41.
[17] K. Walhagen, K.K. Unger, A.M. Olsson, M.T.W. Hearn, J. Chromatogr. A 853 (1999) 263.
[18] M.Q. Zhang, Z. El Rassi, Electrophoresis 19 (1998) 2068.
[19] P.Q. Huang, X.Y. Jin, Y.J. Chen, J.R. Srinivasan, D.M. Lubman, Anal. Chem. 71 (1999) 1786.
[20] T. Adam, M. Kramer, Chromatographia 49 (1999) S35.
[21] M. Ye, H. Zou, Z. Lui, J.Y. Ni, Y.K. Zhang, Anal. Chem. 72 (2000) 616.
[22] G.B. Cox, M.J. Loscombe, M.J. Slucutt, J.A. Sugden, J. Upfield, J. Chromatogr. 117 (1976) 269.
[23] R.D. Rocklin, C.A. Pohl, J.A. Schibler, J. Chromatogr. 411 (1987) 107.
[24] D. Li, H.H. Knobel, V.T. Remcho, J. Chromatogr. B 695 (1997) 169.
[25] S. Kitagawa, A. Tsuji, H. Watanabe, T. Nakashima, T. Tsuda, J. Microcol. Sep. 9 (1997) 347.
[26] M. Ye, H. Zou, Z. Liu, J. Ni, J. Chromatogr. A 887 (2000) 223.
[27] E.F. Hilder, C.W. Klampfl, M. Macka, P.R. Haddad, P. Myers, Analyst 125 (2000) 1.
[28] C.W. Klampfl, E.F. Hilder, P.R. Haddad, J. Chromatogr. A 888 (2000) 267.
[29] J. Zhang, X. Huang, S. Zhang, Cs. Horváth, Anal. Chem. 72 (2000) 3022.
[30] M. Ye, H. Zou, Z. Lei, R. Wu, Z. Lui, J. Ni, Chromatographia 53 (2001) 425.
[31] M. Ye, H. Zou, Z. Lei, R. Wu, Z. Lui, J. Ni, Electrophoresis 22 (2001) 518.
[32] A.S. CarterFinch, N.W. Smith, J. Chromatogr. A 848 (1999) 375.
[33] S.J. Lane, R. Boughtflower, C. Paterson, T. Underwood, Rapid Commun. Mass Spectrom. 9 (1995) 1283.
[34] S.J. Lane, R. Boughtflower, C. Paterson, M. Morris, Rapid Commun. Mass Spectrom. 10 (1996) 733.
[35] S.J. Lane, M.G. Tucker, Rapid Commun. Mass Spectrom. 12 (1998) 947.
[36] X. Huang, Cs. Horváth, J. Chromatogr. A 788 (1997) 155.
[37] M. Gilges, M.H. Kleemiss, G. Schomburg, Anal. Chem. 66 (1994) 2038.
[38] H.S. Dearie, V. Spikmans, N.W. Smith, F. Moffatt, S.A.C. Wren, K.P. Evans, J. Chromatogr. A, in press.