ELSEVIER

Journal of Chromatography A, 945 (2002) 231–238

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Effect of ionic strength on perfusive flow in capillary electrochromatography columns packed with wide-pore stationary phases

H.S. Dearie[a], N.W. Smith[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]*Zeneca Agrochemicals, Jealott's Hill Research Station, Bracknell, Berkshire RG42 6ET, UK*

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

[d]*Avecia Research Centre, P.O. Box 42, Hexagon House, Blackley, Manchester M9 8ZS, UK*

Received 11 May 2001; received in revised form 31 October 2001; accepted 7 November 2001

Abstract

The use of wide-pore stationary phases in capillary electrochromatography has shown exceptional increases in separation efficiency in conjunction with high electroosmotic flow. These effects are due to the perfusive flow mechanism which is primarily controlled by the ionic strength of the mobile phase. Good correlation between calculated values of electrochemical double-layer thickness and efficiency data have also been obtained. Reduced plate height values of <0.5 have been observed with pore sizes of 4000 Å. In addition, electroosmotic flow mobility twice that of 3 μm Spherisorb ODS-1 has been obtained.

Keywords: Ionic strength; Perfusive flow; Stationary phases, electrochromatography

1. Introduction

It is well known that capillary electrochromatography (CEC) offers a significant reduction in plate height over high-performance liquid chromatography (HPLC) [1]. In the first instance, it is the characteristic plug-flow profile of the electroosmotic flow (EOF) generated in electro-driven systems that provide the higher efficiencies over pressure-driven parabolic flow profiles [2].

Secondly, because the flow is driven electrically stationary phase support material down to 0.2 μm in diameter can be employed to achieve very high numbers of theoretical plates [3–11]. Ludtke et al. [11] used columns packed with 0.5, 1 and 3 μm particles to demonstrate the contribution of eddy diffusion (A), axial (longitudinal) diffusion (B) and mass transfer effects (C) to plate height in CEC. They confirmed the opinion of Knox and Grant [3] that plate height is solely determined by the axial diffusion especially when submicron size particles are employed. However, they did emphasise that particle sizes <1 μm operated at moderate EOF velocities of about 3 mm s^{-1} hardly affect the plate height. To exploit the performance benefits of smaller diameter particles (0.5 μm) it is necessary to

*Corresponding author. Tel.: +44-207-5945-841; fax: +44-207-5945-859/833.

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

0021-9673/02/$ – see front matter
PII: S0021-9673(01)01484-4

generate higher EOF velocities by reducing the total capillary length and/or by the application of higher voltages. There is however, an inherent disadvantage in using submicron particles and that is the difficulties reported in column production. Bartle et al. [12] have observed agglomeration of the packing material and thus discontinuities in the packed bed.

The use of wide pore packing materials is an alternative to employing submicron particles to yield similar results in terms of efficiency. Li and Remcho [13] indicated that the use of these wide pore materials in CEC offer notable increases in efficiency provided that the pores are large enough to preclude overlap of the electrochemical double layer and hence support perfusive ''through-pore'' flow. This perfusive electroosmotic transport serves to minimise plate height by eliminating stagnant pools of mobile phase into which analytes can diffuse. This in turn reduces the contribution of slow mass transfer to plate height.

The existence of pore flow was also assumed by Venema et al. [14,15] who investigated the applicability of electrically driven size-exclusion chromatography (ED-SEC) using wide pore native silica gel. They also observed that plate heights for a polymer standard decreased considerably with increased pore flow. Similar observations have been reported by Stol et al. [16] and Santalla-Garcia et al. [17] for columns packed with ODS gel with a mean pore diameter of 4000 Å. The latter obtained reduced plate heights as low as 0.26 for retained solutes.

The magnitude of through-pore flow will be primarily determined by the pore size and thickness of the electrical double-layer and consequently controlled by the ionic strength of the mobile phase. Studies have shown that increasing the ionic strength of the buffer at a fixed pH compresses the electrical double-layer [18]. This phenomenon has the effect of decreasing the electroosmotic velocity. Using wide-pore materials, a number of groups have investigated the ionic strength of the mobile phase to induce perfusive flow [15,16,19–21]. Stol et al. [19] compared the migration behaviour of narrow polystyrene standards with calculated values of pore flow derived from the Rice and Whitehead model [2]. They concluded that the pore flow velocity could be adequately predicted by the model provided that the silica particles possessed narrow pores ($<$100 Å) which were uniformly distributed throughout the packed bed. As expected, the presence of this through-pore flow mechanism was evident in the generation of higher EOF velocities and higher separation efficiencies. Similarly, Vallano and Remcho [20] developed a model to estimate the extent of perfusive flow in CEC columns packed with macroporous ($>$500 Å) particles by employing the Rice and Whitehead relationship [2].

More recently further studies by Stol et al. [21] have investigated an additional parameter to control perfusive flow: the application of pressure to electro-driven flow. This elution mode has been previously described by Knox [22] as pressurised capillary size-exclusion electrochromatography (PCSEEC). Stol et al. [21] showed that through employing PCSEEC for columns packed with 100 and 500 Å pore size particles, efficiencies were obtained that approach those seen for pure ED-SEC. It was however apparent that enhancements in mass transfer processes occurred at low pore flow velocities under the application of pressure. But, as yet the effects of PCSEEC at high flow velocity ($>$2 mm s^{-1}) are to be studied.

Other methods have been documented to describe the magnitude of perfusive flow. Grimes et al. [23] devised a pore network model based on mathematical [24] and experimental [25–27] data to describe and determine the convective flow of solute through the intraparticle and interparticle pathways of a packed capillary column. They concluded that intraparticle EOF can contribute significantly to efficiency and resolution by (i) decreasing the intraparticle mass transfer resistance, (ii) decreasing the dispersive mass transfer effects, and (iii) increasing the intraparticle mass transfer rates. Vallano and Remcho [28] used the conductivity of CEC columns packed with wide pore material ($>$100 Å) to estimate the intraparticle flow. They based their premise on work reported by Wan [29] who suggested the column conductivity to be an indication of the quality of the packed bed. Conductive properties of the columns tested arose primarily from ion transport around the particles and not from the particles themselves, hence in the interstitial region. Therefore Vallano and Remcho suggested that the extent of ion transport would increase with increasing pore size. Their results showed that this was true for columns

packed with silica particles possessing pore sizes >1000 Å and therefore greater intraparticle flow.

This report discusses the effect of changing the ionic strength of the mobile phase on the EOF and efficiency in wide-pore stationary phases. In an attempt to confirm the presence or absence of perfusive flow, double layer thickness values were calculated and compared with efficiency data obtained for each pore size phase.

2. Experimental

2.1. Chemicals

Thiourea and naphthalene were purchased from Aldrich (Gillingham, UK). Tris(hydroxymethyl)-aminomethane (Tris) was of analytical grade and purchased from BDH (Poole, UK). Acetonitrile and acetone were of HPLC grade and also purchased from BDH.

Buffer solutions were prepared using distilled and deionized water from an Elga Maxima waterpurifier. The mobile phase used to investigate the effect of ionic strength were produced as follows:

(1) 6 ml acetonitrile plus 4 ml 6.3 m*M* Tris buffer (overall ionic strength 2.5 m*M*).

(2) 6 ml acetonitrile plus 4 ml 12.5 m*M* Tris buffer (overall ionic strength 5 m*M*).

(3) 6 ml acetonitrile plus 4 ml 25 m*M* Tris buffer (overall ionic strength 10 m*M*).

(4) 6 ml acetonitrile plus 4 ml 50 m*M* Tris buffer (overall ionic strength 20 m*M*).

(5) 6 ml acetonitrile plus 4 ml 100 m*M* Tris buffer (overall ionic strength 40 m*M*).

Sample solutions were prepared in acetonitrile–water (50:50, v/v) to give an overall concentration of 1 mg ml^{-1}.

2.2. Columns

Fused-silica capillaries (100 μm I.D.×375 μm O.D.) were supplied by Composite Metals (Worcester, UK). They were packed using an ultrasonic packing reservoir manufactured by the GlaxoWellcome Bioengineering Department (Stevenage, UK) as previously described by Boughtflower et al. [30]. The column packing pump was obtained from Shandon Instruments (Runcorn, UK). Frits and windows were fabricated using an Innovatech, ACF electrical burner (Stevenage, UK).

The stationary phases employed in this study, Nucleosil 120-5 C_{18}, Nucleosil 300-7 C_{18}, Nucleosil 500-7 C_{18}, Nucleosil 1000-7 C_{18} and Nucleosil 4000-7 C_{18} were a gift from Macherey–Nagel (Düren, Germany). In order to effectively evaluate pore flow mechanisms, all experiments were compared with 3 μm Spherisorb ODS-1 (80 Å) purchased from Phase Separations (Watford, UK), and operated under identical conditions.

As an alternative to producing a sintered silica end frit to retain the stationary phase material, a micro HPLC end fitting (Microtech Scientific, Sunnyvale, CA, USA) was connected to the capillary prior to packing. This fitting contained a 2 μm porosity retaining mesh. The other end of the capillary was connected to the ultrasonic packing reservoir which contained the slurry (0.1 g ml^{-1} in acetone). It was necessary at this point to ensure that the packing pressure applied did not cause the wide pore particles to crush. This essentially involved a ''trial and error'' process, by periodically observing the particles of stationary phase entering the column under the application of different pressures, using a microscope. At the first signs of particle degradation, evident by dark areas in the packed bed, the packing process was stopped and subsequently instigated at a lower pressure. Table 1 gives the recommended packing pressure necessary to produce a bed of uncrushed, wide pore particles. After packing, the capillary columns were flushed with water for at least 12 h prior to fabrication of the window and inlet and outlet frits.

The columns were then preconditioned in the CEC instrumentation by applying an incremental voltage from 0 to 30 kV over 1 h. During this precondition-

Table 1
Recommended packing pressure for wide pore material

Pore size (Å)	Packing pressure (p.s.i.)
4000	4000
1000	5000
500	6000
300	6500
120	10 000

1 p.s.i. = 6894.76 Pa.

ing stage, it was evident that the packed particles resettled to give a more condensed bed, as voids appeared towards the outlet end of the column. This was particularly evident with the 4000, 1000 and 500 Å phases. Subsequently, the column was reconnected to a pressure pump and a new inlet frit prepared further into the packed bed. As a result of this observation it became common practice, when fabricating columns with wide pore particles, to prepare a column bed approximately 2 cm longer than the usual specification of the instrument. This allowed the column to be re-fritted on evidence of bed settling, without becoming too short for re-assembly in the required instrument.

2.3. Instrumentation

Experiments were performed on a Unicam Lauerlabs Prince CE instrument (Emmen, The Netherlands), modified in the laboratory to allow pressurization of both the inlet and outlet vials. This instrument was equipped with a UV variable-wavelength detector operated at 214 nm.

Samples were injected electrokinetically at 10 kV for 5 s. The column was thermostated at 25 °C throughout all experiments.

3. Results and discussion

The effect of changing the ionic strength of the mobile phase on the EOF was investigated for all Nucleosil C_{18} pore sizes by injecting thiourea, a neutral unretained component, onto each column. Experiments were performed in the range of 2.5–40 m*M*. The effect of ionic strength on the electroosmotic flow in wide pore materials was determined and compared with Spherisorb ODS-1. In order to determine the presence or absence of perfusive flow, double layer thickness values were calculated and compared with efficiency data obtained for each phase using naphthalene.

3.1. The effect of the ionic strength on the EOF

Theoretical considerations concerning the effect of ionic strength of the electrolyte in CEC indicate that in the absence of thermal [31,32] and double layer overlap [2,3,33–35] effects, the electroosmotic mobility should decrease steadily when increasing the electrolyte concentration [33]. Cahours et al. [36] observed a 58% decrease in EOF mobility when the ionic strength increased from 2.5 to 25 m*M* using capillaries packed with 3 μm phenyl particles. Similar results were found by Choudhary and Horváth [37] and Dittmann and Rozing [38].

As illustrated in Fig. 1 the data obtained for Spherisorb ODS-1 and the Nucleosil 120 Å pore size agrees with the theory, i.e., there is a gradual decrease in EOF with increasing ionic strength. However, for larger pore sizes i.e., 4000 and 500 Å, it appears that EOF increases with increasing ionic strength. These observations are reflected in the trend of the lines. It is clear that as the pore size increases the effect of ionic strength on the EOF changes. This may be due to the mechanism of pore flow. A larger proportion of the total flow may be through the pores, particularly exhibited in the 4000 Å and to a lesser extent in the 500 Å material. The use of high ionic strength buffers may therefore result in an increase in pore flow, due to a reduction in the double layer thickness, thus giving faster EOF at higher concentrations.

It is also apparent from Fig. 1 that the larger pore size materials exhibit the fastest EOF. This is most

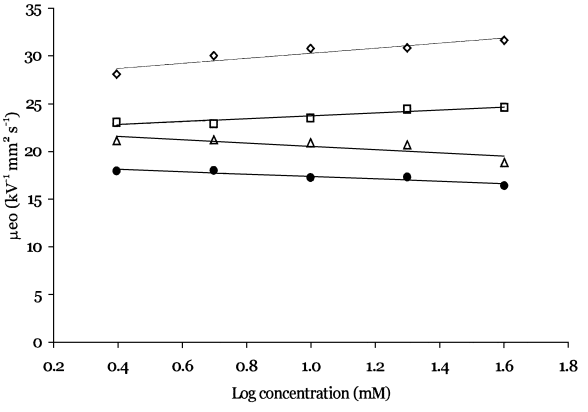

Fig. 1. The effect of increasing the ionic strength on the EOF mobility of Nucleosil C_{18} wide pore phases. Conditions; column: 55 cm (40 cm packed length)×100 μm I.D. Eluent: acetonitrile–Tris (pH 9.0) (60:40, v/v) ionic strength variable. Temperature: 25 °C. Detection: 214 nm. Voltage: 30 kV. (◇) 7 μm Nucleosil 4000 Å, (□) 7 μm Nucleosil 500 Å, (△) 5 μm Nucleosil 120 Å and (●) 3 μm Spherisorb ODS-1, 80 Å.

likely due to the presence of perfusive flow within the pores and therefore less resistance to flow. This flow mechanism will essentially serve to minimize the formation of stagnant mobile phase in the pores into which the separating analytes can diffuse. As the pore size decreases, this through pore flow will begin to diminish therefore resulting in slower EOF.

Fig. 2 shows the effect of increasing the ionic strength on the EOF for pore sizes ranging from 120 to 4000 Å. It is clear that an increase in ionic strength has the least effect on the flow velocity in 500 Å pores. The mobility in this material changes only slightly when the ionic strength increases from 2.5 to 40 m*M*. This may be due to an equilibrium of perfusive–non-perfusive flow being attained. It is from this point in Fig. 2 that the opposing effects of ionic strength are clearly exhibited. For the wider pore materials i.e., 4000 and 1000 Å, further increases in EOF mobility with increasing ionic strength may be due to the distribution of pore diameters. Stol et al. [16] measured the actual pore diameter of a number of wide pore phases. They found that the particles with a higher nominal pore diameter have a larger actual pore diameter. Because there is such a large pore size distribution range for these phases, especially for the wider pore materials, double layer overlap will still be present within a defined portion of the phase dependent on the ionic strength employed. This is reflected by further increases in EOF mobility with ionic strength.

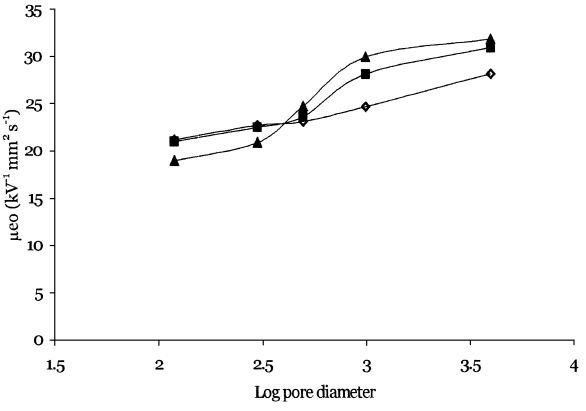

Fig. 2. EOF mobility versus log of the pore diameter for Nucleosil C_{18} phases at different mobile phase ionic strengths. Conditions; column: 55 cm (40 cm packed length)×100 μm I.D. Eluent: acetonitrile–Tris (pH 9.0) (60:40, v/v) ionic strength variable. Temperature: 25 °C. Detection: 214 nm. Voltage: 30 kV. (▲) 40 m*M*, (■) 10 m*M* and (◇) 2.5 m*M*.

In order to truly determine when perfusive flow is present it is necessary to calculate the expected double-layer thickness. By doing so it is therefore possible to estimate the presence or absence of pore flow, depending on the actual size of the pore.

3.2. Comparison of calculated double-layer thickness values

By calculating the double layer thickness (δ) present (Eq. (1)), using a controlled system it is possible to estimate when double layer overlap is likely to occur in the pores:

$$\delta = \left(\frac{\epsilon_0 \epsilon_r RT}{2cF^2} \right)^{1/2} \quad (1)$$

where ϵ_0 is the permittivity of a vacuum, ϵ_r is the dielectric constant, R is the universal gas constant, T is the absolute temperature, c is the electrolyte concentration and F is the Faraday constant. These values can then be applied to different pore sizes in order to determine the presence or absence of perfusive flow. For a mobile phase containing acetonitrile–Tris buffer (60:40, v/v) with an overall ionic strength of 1 m*M*, the double layer thickness was calculated to be 7.54 nm; where $\epsilon_0 = 8.85 \cdot 10^{-12}$ C^2 N^{-1} m^{-2}, $\epsilon_r = 50.77$, $R = 8.3145$ J K^{-1} mol^{-1}, $T = 293$ K, $c = 0.001$ M and $F = 96\,500$ C mol^{-1}. It must be noted that the values for dielectric constant are for an acetonitrile–water mixture which have been taken from the literature [39,40].

Table 2 shows the calculated values of double layer thickness at varying ionic strengths. As a general rule, if the pore diameter:double layer thickness ratio (values shown in heavy boarder section in Table 2) is <10, double layer overlap is present within the pores thus inhibiting perfusive transport. This rule has been adopted from Pretorius et al. [41] which dictates that the flow in an open capillary is independent of capillary diameter provided that the bore is $>10\delta$. The shaded area with the values in bold text therefore denotes the absence of through pore flow, with the shaded area (non-bold text) being most likely to exhibiting borderline interparticle:intraparticle flow.

Table 2
Calculated values of double layer thickness at varying ionic strength

	Ionic strength (mM)					
	1	2.5	5	10	20	40
	Double-layer thickness,δ (nm)					
Pore size (nm)	7.54	4.77	3.37	2.38	1.68	1.19
400	53.1	83.9	118.7	167.9	237.4	335.7
100	13.3	21.0	29.7	42.0	59.4	83.9
50	**6.6**	10.5	14.8	21.0	29.7	42.0
30	**4.0**	**6.3**	**8.9**	12.6	17.8	25.1
12	**1.6**	**2.5**	**3.6**	**5.0**	**7.1**	10.1

The presence of perfusive flow should be apparent in the separation efficiency of a column. Therefore by determining the reduced plate height it would be evident when through pore flow was occurring. It must be noted that this table merely serves as a guide to estimate the presence or absence of perfusive flow, as it has been proven in the past [16] that the nominal pore diameter is not accurate.

3.3. The effect of ionic strength on reduced plate height

The separation efficiency was studied using mobile phases with different ionic strengths. The reduced plate height was determined for each buffer system for all pore size phases using naphthalene, a retained component. It was expected that the larger pore diameters would show the highest separation efficiency over the widest range of ionic strength as they were most likely to support through pore flow. This transport mechanism serves to reduce the resistance to mass transfer in the mobile phase yielding increases in efficiency. Fig. 3 shows the effect of increasing ionic strength on efficiency. The reduced plate height was calculated using the following equation:

$$\text{Reduced plate height} = \frac{H}{d_p} \quad (2)$$

where H is the plate height (Eq. (3)) and d_p is the particle diameter:

$$H = \frac{L}{N} \quad (3)$$

where L is the column length and N is the number of theoretical plates. It is evident from Fig. 3 that the use of higher ionic strength buffers gives the biggest improvement in efficiency on the smaller pore size phases. Taking into consideration the values calcu-

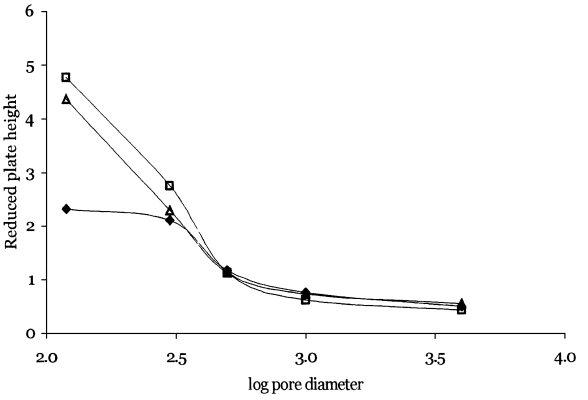

Fig. 3. Effect of increasing the ionic strength on the reduced plate height of Nucleosil C_{18} phases. Conditions; column: 55 cm (40 cm packed length)×100 μm I.D. Eluent: acetonitrile–Tris (pH 9.0) (60:40, v/v) ionic strength variable. Temperature: 25 °C. Detection: 214 nm. Voltage: 30 kV. (□) 2.5 m*M*, (△) 10 m*M* and (♦) 40 m*M*.

lated in Table 2 this would suggest that perfusive flow is occurring at high ionic strength. Several other authors also found an increase in separation efficiency when the ionic strength was increased [42–44], while using stationary phases containing pores of much smaller diameters. The wider pore materials (1000 and 4000 Å) are expected to exhibit perfusive flow throughout a good proportion of the ionic strength range investigated. It is these phases that show exceptionally high efficiencies (reduced plate height <1), independent of buffer concentration in the range measured (see Fig. 3).

In order to see the real benefit of these wide pore materials on efficiency, it is necessary to compare the values with a standard phase of 3 μm Spherisorb ODS-1. Fig. 4 shows this comparison.

It is evident that the selection of ionic strength in order to achieve high efficiency and fast EOF depends upon the pore size used. In terms of the smaller pores (120 Å) high efficiencies will only be attained at high ionic strength. However, with these small pore phases, higher ionic strengths have been shown to result in lower EOF. Increases in EOF may be exhibited in these small pore phases at ionic strengths higher than those measured in this study, when perfusive flow becomes the dominant flow mechanism. High efficiencies and fast EOF however, can be maintained using large pore phases (4000 and 1000 Å) essentially independent of ionic strength.

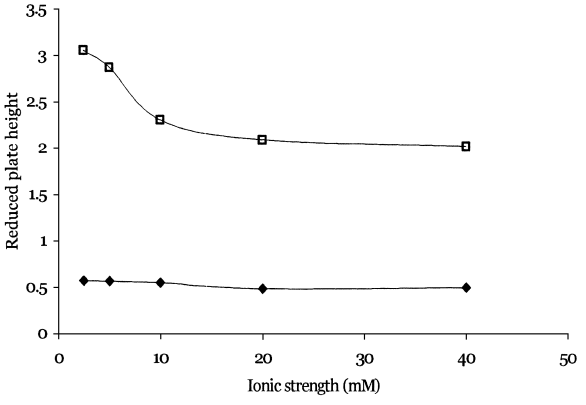

Fig. 4. A comparison of reduced plate height for 7 μm Nucleosil 4000 Å C_{18} and 3 μm Spherisorb ODS-1. Conditions; column: 55 cm (40 cm packed length)×100 μm I.D. Eluent: acetonitrile–Tris (pH 9.0) (60:40, v/v) ionic strength variable. Temperature: 25 °C. Detection: 214 nm. Voltage: 30 kV. (□) 3 μm Spherisorb ODS-1 and (♦) 7 μm Nucleosil 4000 Å C_{18}.

4. Conclusions

The use of wide-pore stationary phases in CEC has shown exceptional increases in efficiency in comparison with a standard phase of 3 μm Spherisorb ODS-1. The presence of perfusive flow yields reduced plate height values <0.5, which is four times lower than those seen for ODS-1. These observed increases in efficiency are due to the reduced resistance to mass transfer effects in the mobile phase, which occur in the presence of through-pore flow. In addition this flow mechanism exhibits EOF mobility that is twice as fast compared to a 3 μm Spherisorb ODS-1 phase.

The primary factor that determines the presence or absence of perfusive flow is ionic strength. For smaller pore size materials (120 and 300 Å), the employment of high ionic strength buffers is necessary to achieve through-pore flow. A high ionic strength mobile phase has also shown increases in the EOF, particularly with wider pore materials (1000 and 4000 Å). In both cases, double-layer overlap has been eliminated to yield such results, and this has been corroborated by comparison with calculated values of double-layer thickness. It is expected that further increases in ionic strength concentration would yield higher flow-rates than those previously observed.

Acknowledgements

The authors would like to thank Macherey–Nagel for the kind donation of the wide-pore stationary phases. We would also like to acknowledge Zeneca and SmithKline Beecham for funding the analytical centre at Imperial College. This work was supported by a Ph.D. scholarship from AstraZeneca.

References

[1] M.M. Dittmann, K. Wienand, F. Bek, G.P. Rozing, LC–GC 13 (1995) 800.

[2] C.L. Rice, R. Whitehead, J. Phys. Chem. 69 (1965) 4017.
[3] J.H. Knox, I.H. Grant, Chromatographia 32 (1991) 317.
[4] B. Behnke, E. Grom, E. Bayer, J. Chromatogr. A 716 (1995) 207.
[5] R.M. Seifar, W.Th. Kok, J.C. Kraak, H. Poppe, Chromatographia 46 (1997) 131.
[6] S. Ludtke, T. Adams, K.K. Unger, J. Chromatogr. A 786 (1997) 229.
[7] H. Engelhardt, S. Lamotte, F.T. Hafner, Am. Lab. 30 (1998) 40.
[8] R. Dadoo, R.N. Zare, C. Yan, D.S. Anex, Anal. Chem. 70 (1998) 4787.
[9] C.G. Bailey, C. Yan, Anal. Chem. 70 (1998) 3275.
[10] T. Adams, S. Ludtke, K.K. Unger, Chromatographia 49 (1999) 549.
[11] S. Ludtke, T. Adams, N. von Doehren, K.K. Unger, J. Chromatogr. A 887 (2000) 339.
[12] K.D. Bartle, R.A. Carney, A. Cavazzo, M.G. Cikalo, P. Myers, M.M. Robson, S.C.P. Roulin, K. Sealey, J. Chromatogr. A 892 (2000) 279.
[13] D.M. Li, V.T. Remcho, J. Microcol. Sep. 9 (1997) 389.
[14] E. Venema, J.C. Kraak, H. Poppe, R. Tijssen, Chromatographia 48 (1998) 347.
[15] E. Venema, J.C. Kraak, H. Poppe, R. Tijssen, J. Chromatogr. A 837 (1999) 3.
[16] R. Stol, W.Th. Kok, H. Poppe, J. Chromatogr. A 853 (1999) 45.
[17] T. Santalla-Garcia, L. de Ribera-Martin, R. Stol, W.Th. Kok, H. Poppe, presented at the 23rd International Symposium on High Performance Liquid Phase Separations and Related Techniques, Granada, May 1999, poster.
[18] M.G. Cikalo, K.D. Bartle, P. Myers, J. Chromatogr. A 836 (1999) 35.
[19] R. Stol, H. Poppe, W.Th. Kok, J. Chromatogr. A 887 (2000) 199.
[20] P.T. Vallano, V.T. Remcho, Anal. Chem. 72 (2000) 4255.
[21] R. Stol, W.Th. Kok, H. Poppe, J. Chromatogr. A 914 (2001) 201.
[22] J.H. Knox, J. Chromatogr. A 680 (1994) 3.
[23] B.A. Grimes, J.J. Meyers, A.I. Liapis, J. Chromatogr. A 890 (2000) 61.
[24] A.I. Liapis, B.A. Grimes, J. Chromatogr. A 877 (2000) 181.
[25] J.J. Meyers, A.I. Liapis, J. Chromatogr. A 827 (1998) 197.
[26] J.J. Meyers, A.I. Liapis, J. Chromatogr. A 852 (1999) 3.
[27] A.I. Liapis, J.J. Meyers, O.K. Crosser, J. Chromatogr. A 865 (1999) 13.
[28] P.T. Vallano, V.T. Remcho, J. Phys. Chem. B 105 (2001) 3223.
[29] Q.H. Wan, J. Phys. Chem. B 101 (1997) 8449.
[30] R.J. Boughtflower, T. Underwood, J. Maddin, Chromatographia 41 (1995) 398.
[31] J.H. Knox, J. Chromatogr. A 26 (1988) 329.
[32] E. Grushka, R.M. McCormick, J.J. Kirkland, Anal. Chem. 61 (1989) 241.
[33] J.H. Knox, I.H. Grant, Chromatographia 24 (1987) 135.
[34] T.S. Stevens, H.J. Cortes, Anal. Chem. 55 (1983) 1365.
[35] Q.H. Wan, Anal. Chem. 69 (1997) 361.
[36] X. Cahours, Ph. Morin, M. Dreux, J. Chromatogr. A 845 (1999) 203.
[37] C. Choudhary, Cs. Horváth, J. Chromatogr. A 781 (1997) 161.
[38] M.M. Dittmann, G.P. Rozing, J. Microcol. Sep. 9 (1997) 399.
[39] H. Wode, W. Seidel, Phys. Chem. 9 (1994) 927.
[40] C. Moreau, G. Douhéret, J. Chem. Thermodynamics 8 (1976) 403.
[41] V. Pretorius, B.J. Hopkins, J.D. Schieke, J. Chromatogr. 99 (1974) 23.
[42] Q.H. Wan, J. Chromatogr. A 782 (1997) 181.
[43] A.E. Rodriques, J.C. Lopes, Z.P. Lu, J.M. Loureiro, M.M. Dias, J. Chromatogr. 590 (1992) 93.
[44] S.E. van den Bosch, S. Heemstra, J.C. Kraak, H. Poppe, J. Chromatogr. A 755 (1996) 165.