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Review

Capillary electrochromatography on silica columns: factors influencing performance

K.D. Bartle*, R.A. Carney, A. Cavazza, M.G. Cikalo, P. Myers, M.M. Robson, S.C.P. Roulin, K. Sealey

School of Chemistry, University of Leeds, Leeds LS2 9JT, UK

Abstract

Much capillary electrochromatography (CEC) work is carried out on bonded silica packings which offer many advantages: the number of such packings which are available; the fact that the chemistry of bonding and the separation process are fairly well understood; and the possibility of the transfer to CEC of existing HPLC methods. Packing methods for the preparation of CEC columns have been investigated. The problems inherent in the use of burned-in frits remains an obstacle, but can be at least partially overcome by minimising the length and by silanisation. The influence of a variety of mobile phase variables on aspects of CEC is in agreement with theory for: ionic strength, organic content (including isoeluotropy), and pH. Temperature can be used as a variable to change column selectivity in CEC. The influence of pH on electroosmotic flow (EOF) by changing the degree of ionisation of residual silanol groups is similar for a wide range of neutral bonded groups, but is much less marked for bonded sulphonic acid groups. The EOF may be reversed for bonded groups containing nitrogen.

Keywords: Electrochromatography; Silica columns; Reviews; Capillary columns; Stationary phases, electrochromatography

Contents

*Corresponding author. Tel.: +44-113-233-6490; fax: +44-113-233-6565.
E-mail address: K.D.Bartle@chem.leeds.ac.uk (K.D. Bartle).

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1. Introduction

Capillary electrochromatography (CEC) is a recently developed hybrid of high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE). The separation mechanism is, therefore, a combination of differential electrical migration of charged species, and partition between mobile and stationary phases. The mobile phase is driven through a small (50–100 μm) I.D. capillary, containing the stationary phase, by an electric field rather than by an applied pressure [1,2]. Application of a large voltage across the column generates electroosmotic flow as positive ions of an added electrolyte accumulate in an electrical double layer, usually at the surface of silica particles, and move towards the cathode taking the liquid phase with them. A number of advantages arise from electrodrive, especially in increased column efficiency. Superposition of electromigration on partition should also lead to versatile separation methods.

A very wide variety of approaches is currently being made to the fabrication and technology of columns for CEC. Continuous polymer bed, or ''monolithic'' columns manufactured by in situ polymerisation within the capillary have been produced, filled with polyacrylamide gels [3,4]. Such columns have been used in numerous application areas, and have been shown to be highly efficient. In a second approach, a sol–gel process is employed to form a silica xerogel [5] within the capillary, followed by bonding of the stationary phase group; alternatively, the separation medium itself may be polymerised in situ. Open-tubular columns in which the wall is coated with the stationary phase, either physically or by bonding through chemical derivatisation, have attracted attention [6,7]. Column wall etching [8] is commonly employed to increase the capillary internal surface area, and hence the phase ratio.

Clearly, the above approaches are all likely to lead to highly significant advances in CEC, and its applications, and may subvert the problems inherent in the use of silica particles and frits. At Leeds, however, we have chosen to work with bonded silica columns, at least in what are still the early stages of progress of a new technique, in which understanding of many of the basic processes is still sought. Our reasons are as follows: a wide range of silica particles are available, because of intense activity in the development of stationary phases for HPLC where silica based materials dominate; the chemistry of the HPLC separation process is increasingly understood, and the direct transfer of HPLC methods to CEC versions with improved resolution is, therefore, most likely to employ silica based columns; and, if ''customised'' stationary phases are required, these may be synthesised through well-established procedures, in which, most usually, either chloro- or alkoxylsilane derivatives are reacted with surface silanol groups [9].

The purpose of this synoptic paper is to review progress made in understanding some of the factors which influence the performance of silica based columns in CEC.

2. Discussion

2.1. Packing columns with silica particles

A number of procedures have been suggested for the packing of silica particles into capillaries to produce columns for CEC. Electrokinetic [10] and centrifugal [11] packing have been proposed, although the use of liquid slurries with ultrasonication has been most common. Our previous work on the preparation of columns for micro-HPLC suggested [12] that packing with a supercritical fluid carrier and ultrasonication produced columns with very high efficiencies. Accordingly, 50 and 100 μm I.D. columns for our early CEC work [13] were prepared in an analogous fashion and were also found to be highly efficient, with plate numbers up to 250 000 per meter.

A study was then carried out to determine if the supercritical packing process offers real advantages over the more usual liquid slurries [14]. A large number of 50 μm I.D. fused-silica capillaries were packed with 3 μm octadecylsilane bonded silica, from the same batch, by four methods: supercritical fluid carbon dioxide or liquid carrier, each with and without the use of an ultrasonic probe. The resulting columns were evaluated by measuring the electroosmotic flow (EOF), migration time, column efficiency and retention factors for constituents of a

Table 1
Properties of CEC columns packed by different methods; values are means for six columns

Packing method	Migration times (min)			EOF (mm s^{-1})			Efficiencies (plates m^{-1})		
	Mean	SD	RSD (%)	Mean	SD	RSD (%)	Mean	SD	RSD (%)
CO_2	4.17	0.42	10.1	1.65	0.14	8.4	208 130	28 430	13.6
CO_2+ probe	3.73	0.38	10.3	1.74	0.09	5.0	199 210	28 485	14.3
Slurry	3.99	0.87	21.8	1.61	0.22	14.0	219 860	58 680	26.6
Slurry+probe	3.69	0.20	5.4	1.69	0.12	7.3	213 880	26 987	12.5

neutral test mixture with identical conditions of buffer and voltage. The results (Table 1) of this careful comparison revealed that there were no significant differences between the properties of columns packed by different methods or in the success rate of preparation.

Differences in column performance were found to originate in random variations between replicate columns, and not between packing methods, although there was least variation between columns packed when applying ultrasonication with a liquid slurry. Although reproducibility is clearly a problem during column preparation, CEC is highly repeatable (Table 2) on an individual column.

The presence of silanol groups, and hence an electrical double layer, on the fused-silica capillary wall of a packed CEC column could also contribute to the EOF. In an attempt to determine the role of the packed-bed length, the column was considered as two resistors in series, corresponding to the packed and open sections [15]. The currents flowing during CEC were measured for fully and partially (25–100%) packed capillaries, and hence the electrical field strengths in both sections and individual contributions to the total EOF were derived. The results showed that at extremes of mobile phase pH (2.9 and 10.5), field strengths were greater in the packed section, but at intermediate (7.5) pH, field strengths were similar in both sections. It follows that, at pH extremes, the EOF linear velocity decreases with the length of the packed bed, while at pH values most commonly employed in CEC, EOF velocity changes little with packed length. Substantial contributions to the EOF from the capillary wall are evident.

2.2. Problems with frits

Bubble formation in CEC has been addressed in a number of ways. The original suspicion, that bubble formation arose from Ohmic heating, was discounted by Knox [16] who showed that such an effect would arise only for columns with I.D. >200 μm with buffer concentration >0.01 *M* and an applied voltage of >50 kV. In fact, if heating effects are excluded, bubble formation almost always arises at the interface between packed and unpacked sections of the column, where a change in zeta potential, and hence EOF can result in a change of mobile phase velocity, and hence expansion. Fig. 1 shows how the length of the frit can have an important influence on the formation of bubbles.

A number of approaches to bubble prevention

Table 2
Repeatability of CEC on same column, %RSD

		EOF	Benzophenone peak		
			Retention time	Efficiency	Retention factor
Six consecutive runs	Operator A	0.2, 0.2 0.3, 0.4	0.2, 0.8	8.1	0.5
	Operator B	0.3	0.5	1.1	0.6
30 consecutive runs		2.0	1.7	9.1	2.5

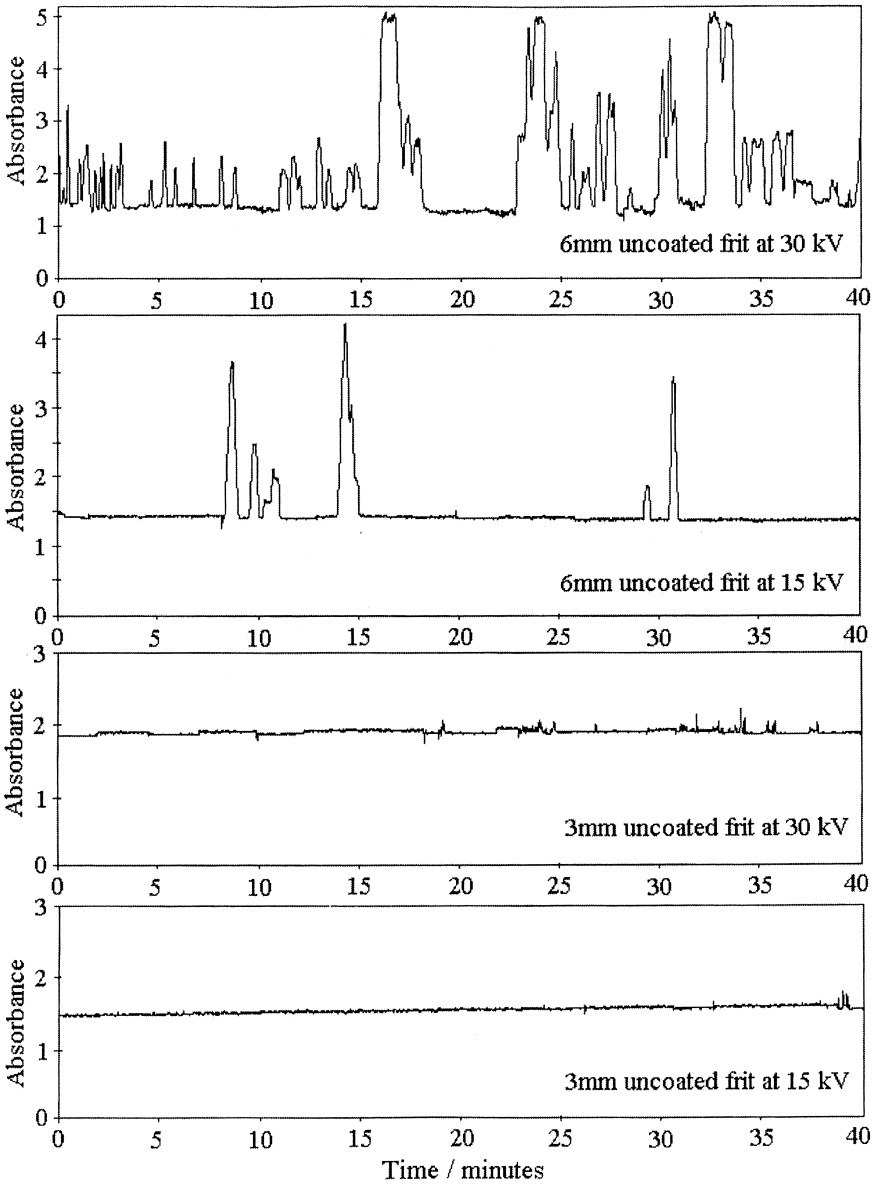

Fig. 1. Electropherograms showing how the formation of bubbles is affected by the length of the frit and the applied voltage.

have been made, including pressurisation, degassing, decreasing buffer concentration, and adding surfactant to the mobile phase. However, the problem can be tackled at source by: (a) cutting back the fit to a minimum length, and butt-connecting with a PTFE sleeve a piece of empty capillary with a length of polyimide removed to form a detector window; and (b) re-bonding stationary phase groups onto the frit surface, to overcome the effects of removal of carbon containing groups which results from the high temperatures during frit formation by means of a hot wire filament. The bonded groups are easily restored to the silica frit by treatment with, for example, chlorodimethyloctadecylsilane, thus minimising the

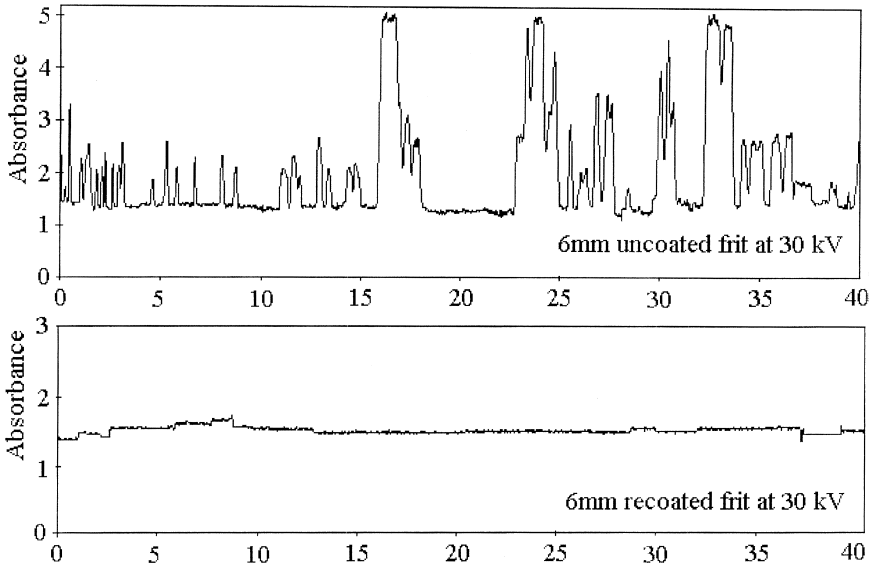

Fig. 2. Electropherograms showing how the recoating of stationary phase after formation of a frit can reduce the formation of bubbles.

change in zeta potential and markedly reducing bubble formation (Fig. 2).

Attempts have been reported [17] to avoid the necessity for a sintered frit by the use of a tapered glass tube into which the capillary is fitted. In our approach [18] tubes approximately 40 mm in length comprised a 375 μm I.D. inlet which tapered down to 100 μm at the center and back out to 375 μm at the outlet. The two 375 μm access holes formed a tight fitting sleeve for fused-silica capillaries. A single 120 μm silica bead was inserted into the tapered glass tube prior to the fitting of a packed and an empty section of capillary. The bead was trapped in the taper as the I.D. dropped below 120 μm, and when the packed capillary was then flushed with mobile phase, the packing material moved from the capillary to fill the tapered section of the glass tube until the blocking bead was reached (Fig. 3).

A problem with this method was that the internal diameter of the tapered glass tube alters greatly and such fluctuations resulted in a large degree of band broadening. Such a dramatic reduction in capillary diameter can be avoided, however, by locating a single 120 μm diameter silica particle between two

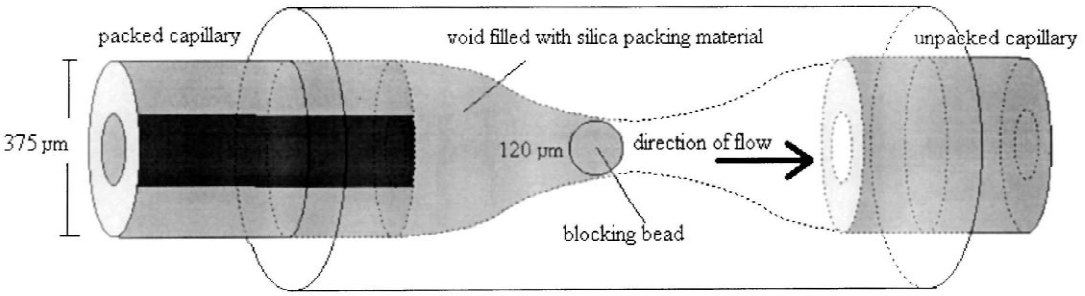

Fig. 3. Schematic showing how the capillary, bead and glass taper were combined to make a frit.

sections of capillary butted up to each other inside a PTFE tube. If a packed section of capillary of I.D. greater than 120 μm is butted to an unpacked capillary of I.D. less than 120 μm, then the bead becomes lodged and acts as a frit (Fig. 4).

This method results in an improved column efficiency, although not yet as consistently high as that seen for capillaries containing burnt-in frits.

2.3. Influence of properties of the silica based stationary phase on CEC

The majority of CEC separations so far reported have been reversed-phase experiments carried out on stationary phases for HPLC. Table 3 compares results from the literature for the (isocratic) CEC separation of polycyclic aromatic hydrocarbons (PAHs), on (nominal) 3 μm ODS packings.

Much greater differences are evident than would be expected for corresponding HPLC analyses, and the packing process is again probably important; although all the packings are listed as 3 μm diameter, the particle size distribution is unlikely to be uniform, and this distribution will vary with the measurement procedure. Number, area or volume distribution may be used to characterise particles for column packing, and this is seldom stipulated by manufacturers. If number distributions are compared, fine material below 2 μm is evident in virtually all nominal 3 μm and 5 μm materials, and this is both likely to cause difficulties with packing and to be difficult to remove via the usual air classification used by manufacturers to produce different particle sizes.

Table 3
Efficiencies obtained for isocratic CEC of PAH using HPLC stationary phases

Stationary phase	Range of efficiencies (plates m^{-1})	Ref.
3 μm Spherisorb ODSl	200 000–240 000	[19]
3 μm Nucleosil 100 C_{18}	91 000–147 000	[20]
3 μm Spherisorb C_{18} PAH	Up to 260 000	[13]
3 μm Synchrom	102 000–138 000	[21]
3 μm Vydac C_{18}	More than 160 000	[22]
3 μm CEC Hypersil	240 000–280 000	[23]

Very recently, Knox [24] has shown that substantial improvement in column packing is all important and that significant problems are involved in packing smaller particles as well as larger ones. Better particle fractionation, or the use of monodisperse particles is required for the production of narrow bore columns for both HPLC and CEC. Knox has also pointed out [24] that reduced plate heights, h, below unity can be obtained in HPLC for larger particles, whereas the practical minimum for 3 and 5 μm particles is nearer 2. Our CEC results on ODS 1 bonded silica showed that, while h was approximately 1.0 for 10 μm particles, it was significantly increased for 5 μm and 3 μm particles, presumably because of less uniform packing. These conclusions may have a significant influence on the results of CEC experiments with small-diameter particles. The early theoretical work of Knox and Grant showed how very highly efficient CEC should be attainable

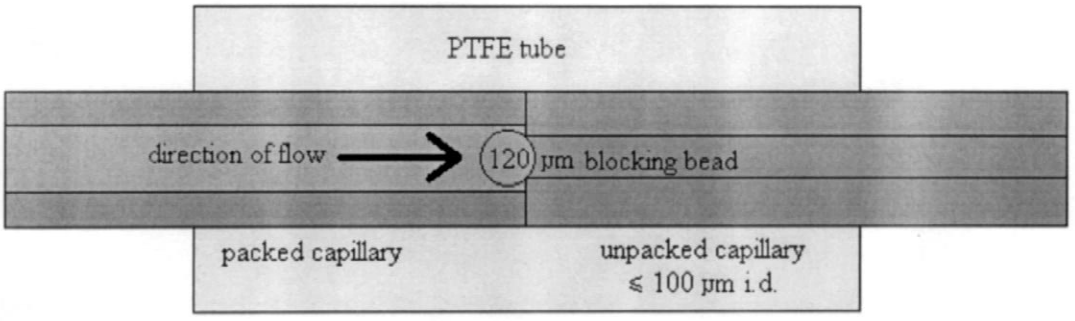

Fig. 4. Schematic showing the construction of a PTFE coupled bead fit.

on sub-micron particles, but there have been conflicting reports on the practical applicability of small non-monodisperse (<2 μm) particles in CEC. In our hands, the preparation of columns packed with such materials was largely unsuccessful, in spite of the use of ultrasonication. Agglomeration of packing material and discontinuities in the packed bed were evident, leading to poor column durability and performance, in stark contrast to results with 3 μm particles. Monodisperse stationary phases in the range 0.2–0.3 μm have been successfully packed into columns for CEC by Adam et al. [31]

As has been observed [25–27] in previous HPLC work on packed capillary columns, better efficiencies were observed for CEC on very small internal diameter columns. For example, 3 μm ODS1 particles packed into 30 μm I.D. fused-silica tubing gave columns with reduced plate height below 1. It is not yet clear, however, whether previous explanations [27] based on a ''wall effect'' are relevant in the case of plug flow.

2.4. Factors influencing EOF in silica CEC columns

2.4.1. Mobile phase parameters

The dependence of EOF mobility, μ_{EOF}, on mobile phase properties has been well explored theoretically, and confirmed in a series of experiments in our laboratory [28] on CEC columns packed with silica and ODS1 bonded silica. Comparisons were also made with the results of corresponding experiments in open-tubular (CE) columns under similar conditions. The well-known fall-off of μ_{EOF} with decreasing pH consequent on the decrease in silanol group ionisation was observed in both CEC and CE. Nonetheless, μ_{EOF} was sufficient even at pH 2.5 to permit CEC analysis of mixtures of salicylic and acetylsalicylic acid within 6 min (Fig. 5).

The effect of ionic strength, I, on μ_{EOF} was investigated [28] over the range 1–20 m*M* phosphate in acetonitrile–water for both open-tubular and ODS1 packed columns. From theory, the double-layer thickness and hence zeta potential and μ_{EOF} are proportional to $I^{-0.5}$, and this was confirmed for both CE and CEC. The increase of μ_{EOF} with $I^{-0.5}$ was similar for both CE and CEC at higher ionic strengths, but below 5 m*M* the CEC mobility levelled off and began to drop for silica packings with pore size 8 nm while continuing to rise in CE. This difference is in accord with double-layer overlap within the pores of packing material, which should occur at I≥2.5 m*M* where the double layer thickness is of the order of 4 nm.

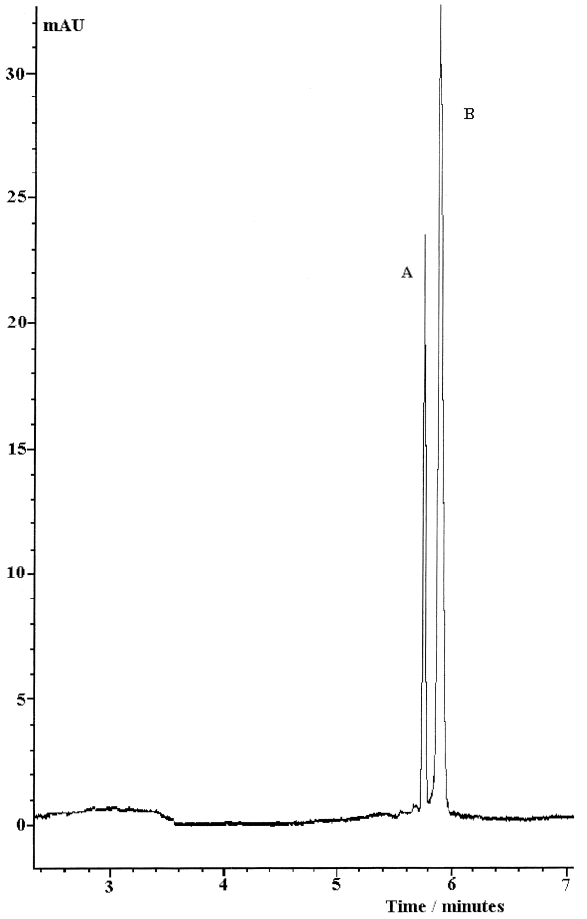

Fig. 5. Separation of acetyl salicylic acid (A) and salicylic acid (B) with a mobile phase of acetonitrile–(40 m*M* NaH_2PO_4, pH 2.5) (60:40).

The type and proportion of organic solvent in the mobile phase is predicted to influence μ_{EOF} through the ratio of permittivity to viscosity, $\in/\eta$, and this was borne out for binary mixtures of acetone, acetonitrile, methanol and 2-propanol with water; similar trends were observed in both CE and CEC [28]. Tests were carried out [29] to determine if the well-known HPLC principal of isoeluotropy (the reproduction of retention behaviour for mobile

phases of similar eluotropic strength) holds in CEC on ODS bonded silica. While, for example, retention times of neutral solutes are much longer for methanol–buffer (80:20) mobile phase than for acetonitrile–water (70:30) because of differing values of $\in/\eta$, retention factors are much more similar (Fig. 6). Deviations from the unit gradient line may arise from the choice of thiourea as retention marker.

As the percentage of acetonitrile in the mobile phase is increased, there was a linear fall in the logarithm of the retention factor [29,30], so that well-established theories used in HPLC method development should be equally applicable to the separation of neutral molecules in CEC.

2.4.2. Particle size and porosity

A reduction in mean particle diameter from 5 to 3 μm for ODS1 silica greatly reduced (by 60%) the mobile phase velocity for the same voltage and buffer concentration (50 m*M* phosphate) [29]. In fact, the velocity should be independent of particle size, as has been demonstrated [31] for monodisperse packings, and this result may arise from double layer overlap in a bed with smaller interstitial channels consequent on the presence of more very small particles in the nominal 3 μm packing. Pore size (8 nm compared to 30 nm) however had no significant affect on EOF velocity for 3 μm ODS1 packings [29].

2.4.3. Nature of the silica-bonded stationary phase

The most commonly used stationary phases for capillary electrochromatography (CEC) are silica particles with bonded groups, most usually alkyl groups, with octadecylsilyl groups pre-eminent; CEC separations on a number of other silica-bonded phases, e.g. phenyl have also been reported [32]; as discussed above, the flow of mobile phase towards the anode arises by virtue of the electrical double layer which originates from the accumulation of positive ions from the buffer next to the unbonded $Si–O^-$ groups of the particles and column wall. Attempts have also been made to increase the magnitude of the EOF by bonding negatively charged groups to the silica, especially sulphonic acid groups, in either strong cation-exchange (SCX) or so-called mixed-mode phases [33] which incorporate both sulphonic acid and alkyl groups.

On aminopropyl bonded silica [29], the EOF is reversed with changing pH, offering the possibility by changing the buffer of augmenting a separation based on partition by an electromigration either with or against the EOF. A strong anion-exchange (SAX)

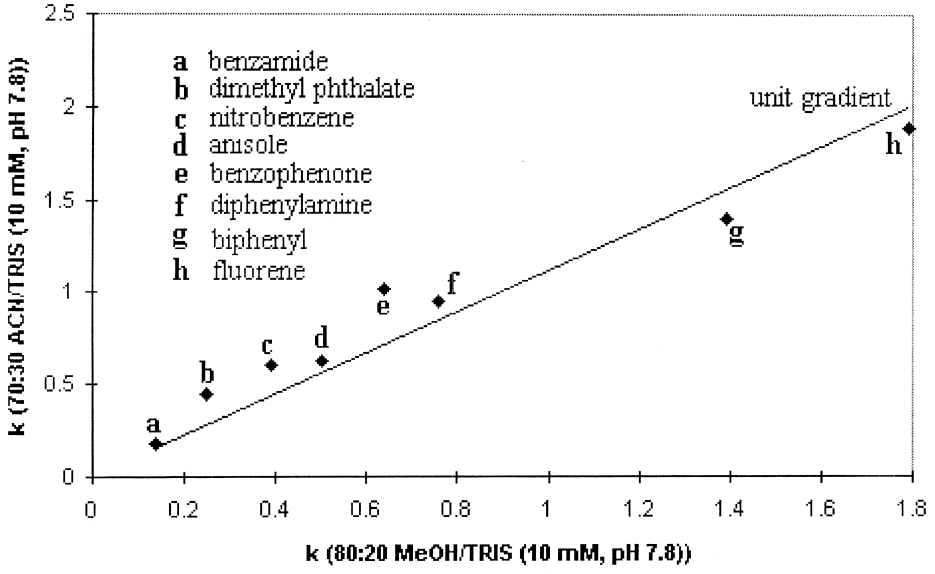

Fig. 6. CEC retention factors for mobile phases isoeluotropic in HPLC.

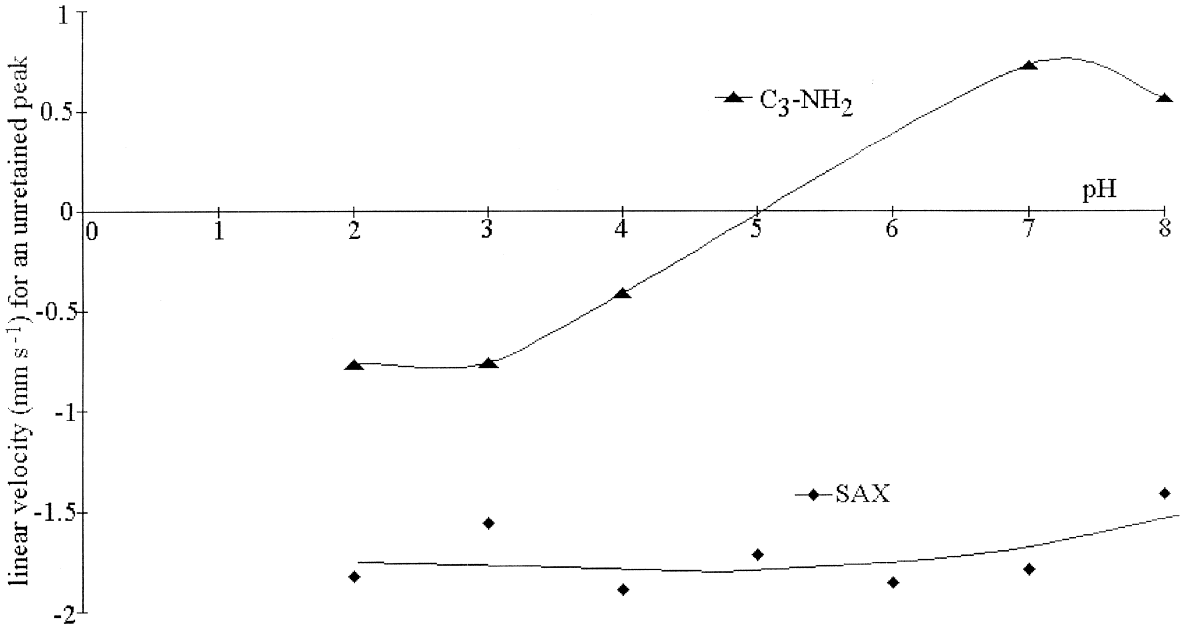

Fig. 7. Variation with pH for two amino-type stationary phases, acetonitrile–(50 m*M* phosphate buffer) (70:30).

packing, however, has reversed EOF which is virtually independent of pH (Fig. 7).

2.5. Influence of temperature on CEC on silica bonded columns

Column temperature is easily changed in CEC, and increased temperature reduces the mobile phase viscosity and hence increases μ_{EOF} so that more rapid analysis is possible for a given voltage [34] (Fig. 8). Retention factors, *k*, are also influenced by temperature and Van't Hoff graphs of ln *k* versus reciprocal of temperature are linear. The slopes of such lines may differ for different related compounds, making selectivity changes possible by changing the temperature [34]. For example, Fig. 8 shows the electrochromatograms of diuretics on ODS bonded silica at temperatures between 15 and 65°C. The resolution of chlorothiazide and hydrochlorothiazide increases with decreasing temperature, and the relative retention of chlorothalidone and hydroflumethizide is reversed with increasing temperature.

An increase in column temperature from 15 to 65°C saw an approximate 10% fall in plate number (and a 50% rise in current). The effect of more rapid mass transfer at the higher temperature is apparently exceeded by greater band broadening from longitudinal diffusion [29].

2.6. Practical CEC separations on silica based columns

The great majority of applications of CEC so far reported have involved high-resolution reversed-phase separations on bonded 3 μm or 5 μm particles. Acetonitrile–water mixtures have generally been employed as mobile phases because of the favourable $\in/\eta$ ratio discussed above. Separations of neutral molecules have predominated in the field of environmental and pharmaceutical analysis, and these have been reviewed [1,2,30].

Separations by CEC of closely-related neutral compounds on alkyl-bonded silica packings using non-aqueous mobile phases is also extremely promising. A peak capacity of over 200 is possible on a 50 cm long column packed with 3 μm particles, making such methods particularly attractive for the analysis of, for example triglycerides [35] and carotenoids [36]. Other mixtures of compounds of plant origin include separations of oxygen heterocyclic com-

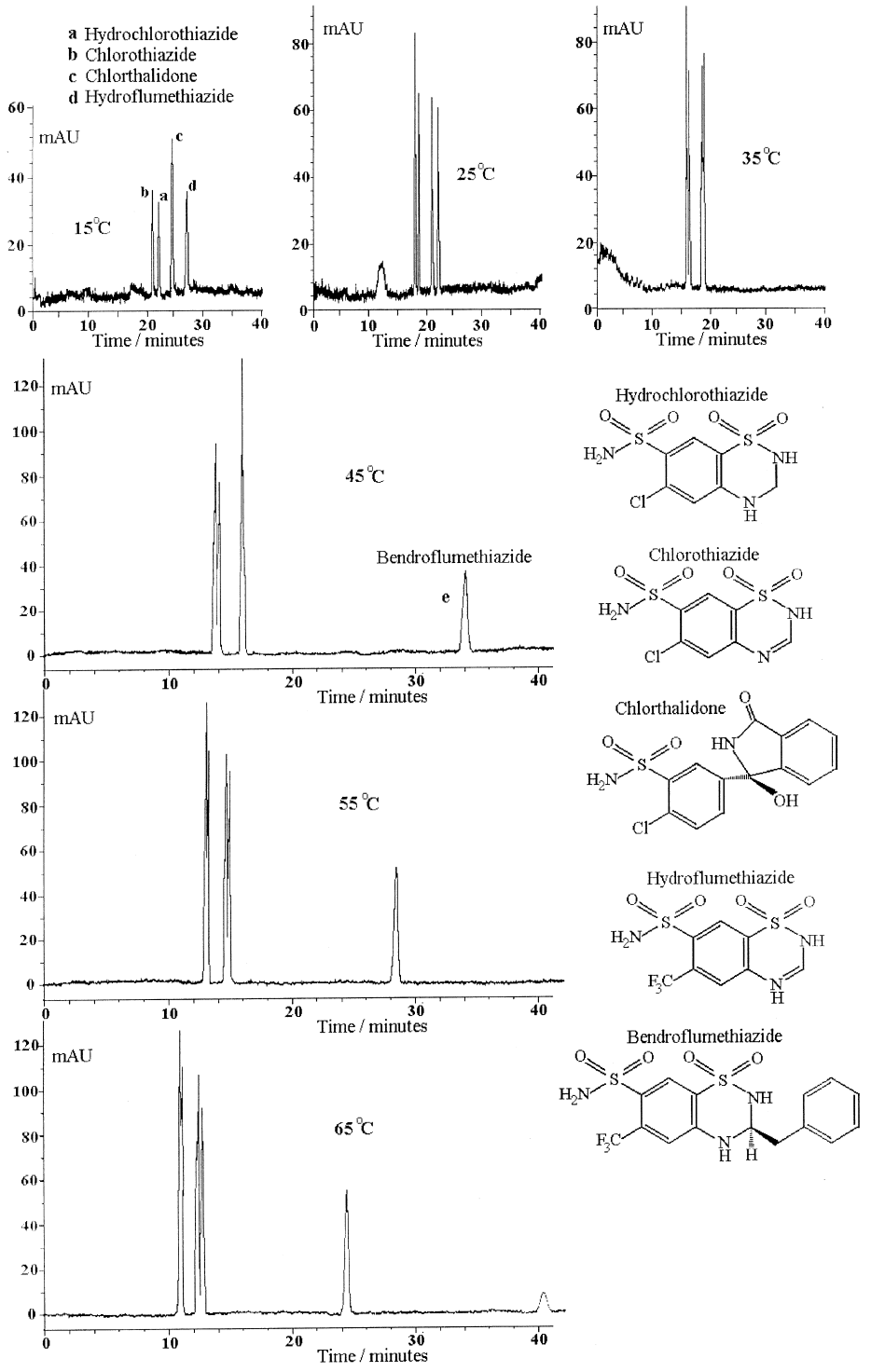

Fig. 8. Temperature effects on the separation of diuretics by CEC. Acetonitrile–water–(50 m*M* phosphate buffer, pH 2.5) (40:40:20).

pounds in citrus oils by both reversed-phase and non-aqueous CEC. More rapid analysis than by HPLC was achieved [37] by both methods.

Work has also been reported on the use of silica based packings for CEC with pore sizes up to 400 nm. These materials should be capable of supporting through-particle electroosmosis, and hence significant increases in column efficiency. A second consequence of the presence of large pores is electrically driven non-aqueous size-exclusion chromatography. Efficient separation of a series of narrow molecular mass (M_r) distribution polystyrene fractions on 30 nm pore size 3 μm silica particles was achieved with dimethylformamide as mobile phase; elution was in sequence of decreasing number-average M_r (M_n). The retention times showed the well-known logarithmic dependence on M_n (Fig. 9).

2.7. Column lifetime robustness

Over 300 consecutive successful injections of neutral test mixtures were possible at 30 kV on 25 cm×50 μm I.D. columns packed using supercritical CO_2 with 3 μm ODS1, with buffer vials changed every 10 runs and sample vials every 50 runs. In tests under the above conditions the EOF velocity and retention factors showed very small increases (<2% and 1.5–3.0% respectively) after 30 min, with small changes in efficiency and peak symmetry [34].

Column life times were, however, much shorter in tests in which a ''real'' sample of natural product origin was used. A supercritical carbon dioxide extract of red peppers was analysed by CEC for its content of capsacain and capsacain derivatives. Approximately 30 consecutive injections were made into a column similar to that above and operated under the same conditions [34]. Beyond this number there was substantial degradation of column performance. Partial restoration of EOF velocity and efficiency was achieved by flushing the column with buffer for 2 h, but it is clear that the small mass of stationary phase in CEC columns imposes stringent sample clean-up requirements if analytical capability is to be maintained.

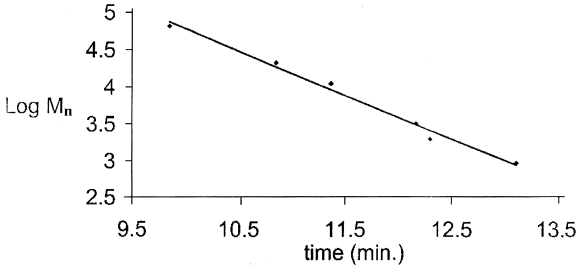

Fig. 9. Relation between log M_n and retention time for polystyrenes on 30 nm pore size silica by CEC-SEC.

3. Conclusions

Columns packed with bonded silica particles are currently commonly used in CEC. An evaluation of different packing procedures revealed that differences in column performance originate in random variations between individual columns. Measurements of currents during CEC suggest that the fused-silica column wall contributes substantially to the EOF. The problems arising from burned-in frits in CEC columns can be minimised by reducing the frit length and re-bonding stationary phase groups lost during frit manufacture. A single 120 μm silica bead in a tapered length of the column may provide a suitable frit replacement. The use of monodisperse particles may be necessary to allow the full resolution potential of CEC columns to be realised, especially for small diameter particles.

The CEC mobile phase variables of pH, ionic strength and organic content influence the EOF according to theory. The principle of isoeluotropy operates in CEC for measurements of the retention factor, and along with the linear variation of ln k with organic content should aid the transfer of HPLC methods to CEC. The pH influences the EOF mobility in a similar way for many neutral groups bonded to silica, but the effect is less marked for bonded sulphonic groups. Bonded nitrogen-containing groups reverse the direction of the EOF and should permit novel separations. Electrically driven size-exclusion separations are possible with non-aqueous mobile phases on wide pore diameter silica particles.

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