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Life-time studies with capillary electrochromatography columns operated under different conditions

Karin Walhagen[a], Klaus K. Unger[a], Milton T.W. Hearn[b,*]

[a]*Institut fur Anorganische Chemie und Analytische Chemie, Johannes Gutenberg-Universität, Duesbergweg 10-14, D-55128 Mainz, Germany*

[b]*Centre for Bioprocess Technology, Department of Biochemistry and Molecular Biology, Monash University, Clayton, Victoria 3168, Australia*

Abstract

A test system has been established to permit the monitoring of the life-time performance of several reversed- phase capillary electrochromatography (CEC) columns. The retention factors, k_{cec}, peak symmetry coefficients, λ_{sym}, and column efficiencies, *N*, of three neutral *n*-alkylbenzene analytes, namely ethyl-, *n*-butyl- and *n*-pentylbenzenes, were determined for Hypersil 3 μm *n*-octylsilica and *n*-octadecylsilica packed into CEC capillary columns of 100 μm I.D., with a packed length of 250 mm, and a total length of 335 mm. The performances of these CEC capillary columns were examined for a variety of eluents with pH values ranging between pH 2.0 – 8.0, similar to those employed to study the retention behaviour of peptides that we have previously reported. The relative standard deviation (RSD) of the retention factors (k_{cec} values) of these *n*-alkylbenzenes, acquired with an eluent of (25 m*M* Tris–HCl, pH 8.0,)–acetonitrile (1:4, v/v), when the CEC capillary columns were used for the first time (virgin values), were 4% (based on data acquired with 4 CEC capillary columns) for the *n*-octyl bonded silica capillary columns, and 6% (based on 8 columns) for *n*-octadecyl bonded silica capillary columns. The RSD values of the k_{cec} values of the *n*-alkylbenzenes for one set of replicates ($n=6$) with one CEC capillary column was $<0.5\%$. The theoretical plate numbers, *N*, for the virgin CEC capillary columns were ca. 60 000, whilst the observed *N* values for all new CEC capillary columns were $\geq$40 000 for *n*-octyl bonded silica capillary columns and $\geq$50 000 for *n*-octadecyl bonded silica capillary columns. The peak symmetry coefficients, λ_{sym}, of the *n*-alkylbenzenes for virgin CEC capillary columns and for CEC capillary columns used for more than 1000 injections were always in the range 0.95–1.05. The experimental results clearly document that the life-time performance of the CEC capillary columns depends on the eluent composition, as well as the nature of the analytes to which the CEC capillary columns are exposed. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Capillary columns; Electrochromatography; Retention factors; Peak symmetry; Efficiency; Alkylbenzenes

1. Introduction

Capillary electrochromatography (CEC) can be considered to be a hybrid of high-performance capillary zone electrophoresis (HPCZE) and high-performance liquid chromatography (HPLC). The high column efficiency in CEC is mainly due to the plug flow profile that is generated in the channels between the sorbent particles and through the pores of porous particles of the CEC capillary column [1,2]. When silica-based sorbents are used as packing materials, the magnitude of the electroosmotic flow

*Corresponding author. fax: +61-3-9905-5882.

E-mail address: milton.hearn@med.monash.edu.au (M.T.W. Hearn).

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(EOF) is determined by the zeta-potential (ζ) at the surface of shear between the charged silica surface and the electrolyte solution when an electric field is applied [3–6]. The EOF is directly proportional to ζ and thus to the applied electric field strength, E, but is inversely proportional to the viscosity, η, of the eluent. At pH≥8, with silica-based particles the surface silanols are fully ionised and a high EOF is thus obtained. In contrast, with decreasing pH the charge density of the silanol groups and the zeta-potential diminishes, giving rise to a corresponding decline in the value of the EOF. Since the iso-electric point of silica particles is between pH 2 and 3, the EOF becomes negligibly small at low pH values. The choice of electrolyte solution and the ionic strength can have a large impact on the thickness of the electric double layer at the solid-liquid interface when a potential is applied across the CEC capillary column. Consequently, the buffer choice will affect the zeta-potential and thereby the EOF as well.

With CEC capillary columns packed with *n*-alkylsilica reversed-phase particles, neutral analytes are retained by partitioning between the *n*-alkyl chains immobilised onto the silica surface and the eluent. The retention mechanisms for charged analytes are more complex, since both electrophoretic migration as well as chromatographic retention can occur [7–9]. Dittmann et al. [10] have studied the performance of a neutral test mixture with two Hypersil CEC capillary columns for more than 300 repetitive runs at pH 6 and pH 8, respectively. No deterioration in the chromatographic performance (as measured from the theoretical plate number and peak symmetry) was observed over the test period. These investigators also pointed out the need to frequently replenishing the eluent to avoid fluctuation in retention times of the analytes due to buffer depletion [6]. Dulay et al. [11] have also shown good reproducibility at pH 6.5 for hundreds of consecutive runs with CEC capillary columns packed with 3 μm *n*-octadecyl silica particles.

The different CEC capillary columns reported in this paper have been used as part of our fundamental studies into the retention mechanism of basic and acidic peptides, including investigations into the origin of selectivity effects arising from the interactions between the peptides and the *n*-alkyl chains or the silica surface [7,12,13]. However, very little is known about the life-time durability of commercial CEC capillary columns, when they are used for extensive periods of time with different electrolyte solutions as used for separation of charged analytes, such as peptides. The purpose of the present investigation test was to continuously monitor the effects of various operating conditions, such as eluents of different compositions and pH values, on the performance of CEC capillary columns using a test system, whilst other data were concurrently acquired on the retention mechanism of peptides in these CEC systems. In these studies, we have examined the life-time characteristics of a number of Hypersil CEC capillary columns, packed with *n*-octylsilica (OS) and *n*-octadecylsilica (ODS) sorbents. The Hypersil *n*-octyl bonded and *n*-octadecyl bonded silica materials, respectively, originated from the same batches of *n*-alkyl bonded silica. The performance of these CEC capillary columns were repeatedly monitored with a test mixture composed of uracil as the EOF marker and three *n*-alkylbenzenes, namely ethyl-, *n*-butyl- and *n*-pentylbenzenes. The retention factors, k_{cec} values, symmetry coefficients, λ_{sym} values, and the number of theoretical plates per column, N's, of these neutral analytes were then used as parameters to establish the life-time of the CEC capillary columns.

2. Experimental

2.1. Chemicals

Acetonitrile of HPLC ultra pure gradient grade was purchased from J.T. Baker, (Deventer, Netherlands). Tris(hydroxymethyl)aminomethane (Tris) and hydrochloric acid 32%, both analytical-reagent grade were obtained from Merck (Darmstadt, Germany). Deionised water was obtained with a Milli-Q water purification system (Millipore, Eschborn, Germany). The Tris buffer was prepared by adding 8 ml of acetonitrile to 2 ml of 25 m*M* Tris pH 8.0. The eluents were filtered through a 0.2 μm PTFE filter, Nalgene, Labotec (Wiesbaden, Germany).

2.2. Samples

Uracil (minimum 99%) was purchased from Sigma (Deisenhofen, Germany) and used as the EOF

marker. Ethylbenzene and *n*-butylbenzene were purchased from Aldrich (Deisenhofen, Germany) and *n*-pentylbenzene was purchased from Merck. The samples were injected electrokinetically at 5 kV for 6 s.

2.3. Instrumentation

An instrument, HP^{3D}CE capillary electrophoresis system, from Agilent Technologies (Waldbronn, Germany) was used for the CEC experiments. UV detection was performed at 214 nm and both the inlet and outlet capillary ends were pressurised at 10 bar during analysis. The temperature of the cassette was set to 20°C and the applied voltage over the CEC capillary columns was 25 kV with a ramping time of 0.5 min.

2.4. CEC capillary columns

The CEC capillary columns (100 μm I.D., packed with 3 μm Hypersil *n*-octyl bonded or *n*-octadecyl bonded silica) were supplied by Agilent Technologies. The total length of the capillaries was 335 mm and the packed length was 250 mm. The *n*-octylsilica and *n*-octadecylsilica packing materials respectively in the capillary columns originated from the same batch of *n*-alkyl bonded silica. The virgin CEC capillary columns were conditioned in (25 m*M* Tris–HCl) pH 8.0–acetonitrile (1:4, v/v) according to the standard procedure recommended by the manufacturer.

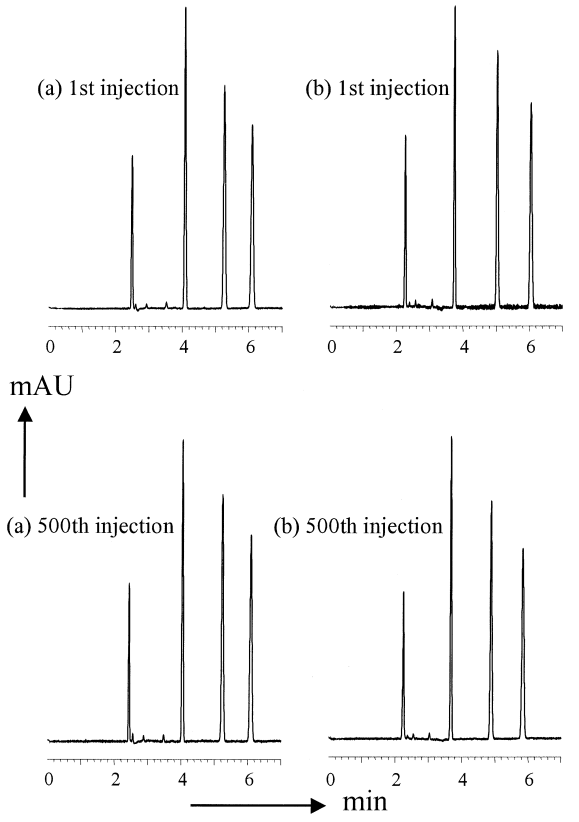

Fig. 1. Chromatograms of the test mixture on virgin CEC capillary columns and on used CEC capillary columns packed with *n*-octyl bonded and *n*-octadecyl bonded silica. The elution sequence is: uracil, ethylbenzene, *n*-butylbenzene and *n*-pentylbenzene. (a) Hypersil *n*-octyl bonded silica CEC capillary column; (b) Hypersil *n*-octadecyl bonded silica CEC capillary column.

3. Results and discussion

3.1. Characterisation of the test system

In order to monitor the performance of different CEC capillary columns during the course of our investigations into the retention behaviour of different peptides in CEC systems, a neutral test mixture comprising uracil and three *n*-alkylbenzenes; ethyl-, *n*-butyl- and *n*-pentylbenzenes, was repeatedly analysed with a freshly prepared eluent containing (25 m*M* Tris–HCl pH 8.0)–acetonitrile (1:4, v/v) at 20°C applying a potential of 25 kV (Fig. 1). The (k_{cec}), (λ_{sym}) and (N) values of each analyte were calculated as a function of the number of injections on each capillary column. Only experiments with a stable current throughout the whole analysis time were used for further calculations. If current instability arose, as occasionally occurred at the beginning of an analysis, we found one way to avoid these fluctuations in current was to use a ramping time of 30 s as the electric field was raised from 0 to 25 kV.

The test system described in this paper has been used for monitoring the life-time and performance of commercial Hypersil CEC capillary columns for almost one year. Initially, when this test system was used only five to six replicates were performed with the test mixture. As some data had to be discarded due to the current fluctuation mentioned earlier, this resulted in only three to four replicate sets of data being available for statistical evaluation. In sub-

sequent studies, we increased the total number of analyses to ten replicates, with six consecutive runs used for the calculations of the k_{cec}, λ_{sym} and N values for each analyte. Thus, the calculated N values shown in Figs. 2 and 3 were derived with the experimental data ($n=6$) derived with ODS-CEC capillary column number 4, 7 and 8 and are representative of the results obtained with the other *n*-octylsilica and *n*-octadecylsilica CEC capillary columns. Good laboratory 'house-keeping' practices with the CEC capillary columns was essential for long term maintenance of the high performance, with the capillary ends always stored in water and never in the eluents after an experiment to prevent the inlet and outlet ends from drying and also to avoid stretching of the polyimide layer on the fused capillary due to the presence of acetonitrile in the eluent [10].

The different batches of commercial Hypersil CEC capillary columns described in Table 1 have been simultaneously used in our associated studies into the fundamentals of peptide retention in these CEC systems. As part of these peptide-based studies, the CEC capillary columns were exposed to an array of different experimental conditions, using a variety of eluents and pH values, temperatures, applied potentials and injection modes, i.e. electrokinetic vs. pressurised injection or a combination of both injection modes. The different eluents used for these peptide studies [7,12,13] were (i) triethylamine–phosphoric acid, pH 2.0–3.0; (ii) sodium dihydrogen–phosphate–phosphoric acid, pH 2.0–3.0; (iii) eluents containing ion-pairing reagents such as trifluoroacetic acid (TFA), perfluoropropionic acid (PFPA) and perfluorobutyric acid (PFBA); (iv) ammonium acetate–acetic acid, pH 3.5–5.5; (v) Tris–HCl, pH 8.0; and (vi) morpholinoethyl–sulfonic acid (MES), pH 6.0. The organic modifier employed as a co-eluent was typically acetonitrile, but in some cases mixtures of acetonitrile and methanol were used for these peptide studies. The temperature was normally kept at 20°C, but for some experiments the temperature was raised up to 60°C [13].

In this paper, we describe the life-time data acquired for the CEC capillary columns under the conditions previously described in the experimental part. This information is representative of the overall trends observed when the test mixture of *n*-alkylbenzenes

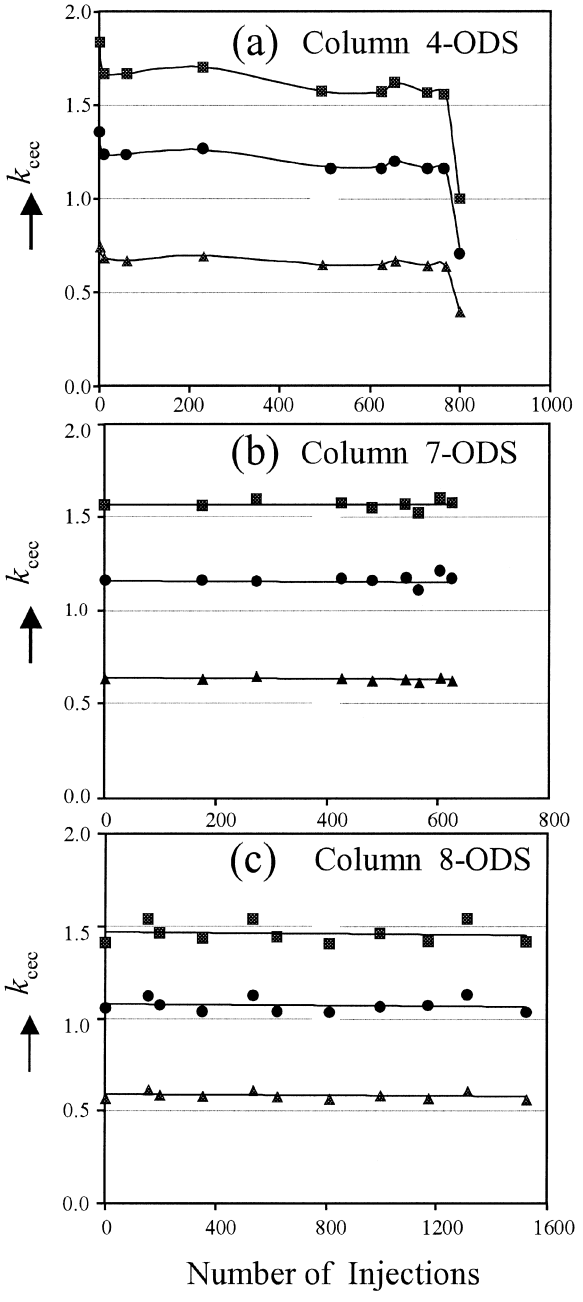

Fig. 2. The retention factors, k_{cec} values, for the three *n*-alkylbenzenes versus the number of injections on three *n*-octadecyl bonded silica CEC capillary columns are illustrated. (a) CEC 4-ODS capillary column; (b) CEC 7-ODS capillary column; and (c) CEC 8-ODS capillary column. Ethylbenzene (– ▲ –), *n*-butylbenzene (– ● –), *n*-pentylbenzene (– ■ –).

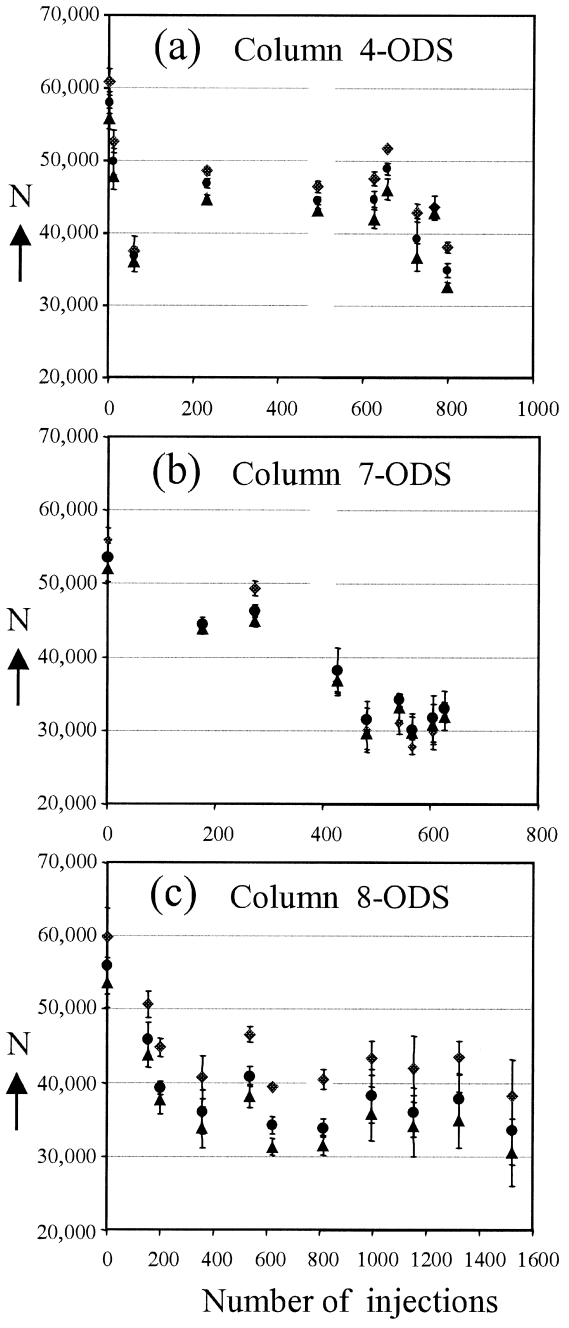

Fig. 3. Theoretical plate numbers, *N* values, for the three *n*-alkylbenzenes as a function of the number of injections for three *n*-octadecyl bonded silica CEC capillary columns are illustrated. (a) CEC 4-ODS capillary column; (b) CEC 7-ODS capillary column; and (c) CEC 8-ODS capillary column. Ethylbenzene (– ♦ –), *n*-butylbenzene (– ● –), *n*-pentylbenzene (– ▲ –).

benzenes was con-jointly run with the peptide samples under other conditions. To facilitate data acquisition, the test mixture was, moreover, used in these life-time stability studies between peptide experiments to permit the life-time performance of the packing material in the CEC capillary columns to be monitor under actual operational conditions, rather than after a pre-determined number of injections. Thus, in some cases the tests were performed after 50 injections, whereas in other cases the tests were carried out after 200 injections with peptide samples had been performed.

3.2. Life-time characteristics of the commercial hypersil CEC capillary columns

The life-time of the CEC capillary columns used in this study was found to depend on the composition of the electrolyte solutions, with respect to molarity and pH value, the temperature, the applied potential and the type of injection mode used, i.e. whether electrokinetic or pressurised or a combination of both. The purity of the injected samples was also crucial for a long life-time to be achieved for the packing material. Consequently, for our peptide studies these Hypersil CEC capillary columns were only exposed to pure peptide samples and care was taken to ensure that the *n*-alkyl bonded silica surface was not irreversible modified by long-term exposure to some basic peptides. With CEC capillary columns, manufacturers currently take considerable precautions to ensure that the quality of the packing of the CEC columns as well as the quality of the detector window and frits are high. In contrast, the responsibility rests with the user to follow good laboratory maintenance practices to ensure that the inlet and outlet ends of the capillary are kept free of polyimide coating debris at all time, and that care is also taken during the handling the capillary columns when they are placed into the capillary cassette and later into the instrument.

Our experience indicates that the life-time performance of the current generation of CEC columns is very much dependent on the history of what eluents have been used with the CEC packing material. With CEC capillary columns, eluents of pH 2.0 appear to represent very harsh conditions, frequently resulting in disruption of the current as the

Table 1
Details of the types of CEC capillary columns packed either with *n*-octylsilica (OS) or *n*-octadecylsilica (ODS) used in the present investigations, together with the compositions of the different eluents employed in the associated peptide resolution studies

CEC column number	Eluent
1-OS	KH_2PO_4–H_3PO_4 pH 2–3; TFA pH 2
2-OS	KH_2PO_4–H_3PO_4 pH 2–3
3-OS	Triethylamine–H_3PO_4 pH 2–3
4-OS	NH_4OAc–AcOH pH 4–5
1-ODS	Triethylamine–H_3PO_4 pH 2–3; MES pH 6
2-ODS	KH_2PO_4–H_3PO_4 pH 2–3
3-ODS	KH_2PO_4–H_3PO_4 pH 2–3
4-ODS	KH_2PO_4–H_3PO_4 pH 2–3; TFA; PFPA; PFBA
5-ODS	Triethylamine–H_3PO_4 pH 2–3
6-ODS	NH_4OAc–AcOH pH 4–5
7-ODS	NaH_2PO_4–H_3PO_4 pH 2.8; NH_4OAc–AcOH pH 4–5
8-ODS	NH_4OAc–AcOH pH 4–5
9-ODS	Triethylamine–H_3PO_4 pH 2–3; TFA; NH_4OAc–AcOH pH 4–5

capillary aged due to air-bubble formation, making continuation of the measurements impossible. Increasing the buffer pH to pH 3.0 improved the stability of the current and thereby the reproducibility of the experiments. The reason for this effect is probably due to an increase in the surface density of charged silanol groups at pH 3.0 compared to pH 2.0. Illustrative of these conclusions are the results obtained for the CEC 8-ODS column, which was used with ammonium acetate buffers of pH values between pH 3.8 and pH 5.4 and the buffer shown for the test system, Tris–HCl pH 8.0. Despite the fact that this CEC column had also been used extensively in temperature studies up to 60°C with peptides, its performance remained acceptable for more than 1500 injections as shown in Figs. 2c and 3c. Based on these and associated observations, the k_{cec} values of the *n*-alkylbenzenes and the theoretical plate numbers (*N* values) appear to be good indicators of the life-time of the CEC capillary columns. Severe changes of the *n*-alkyl bonded silica surface due to peptide adsorption or other modification of the surface resulted in significant changes of the k_{cec} values, as well as the *N* values for the neutral test mixture. As the symmetry coefficients, λ_{sym}-values, of the *n*-alkylbenzenes were always between 0.95 and 1.05, this parameter clearly was a less useful diagnostic measure of capillary column life-time. Typically, the *N* values per column for the *n*-alkylbenzenes decreased after about 600 injections to $\leq$40 000. In terms of useful life-time of the Hypersil CEC capillary columns containing the chemically modified silica reversed-phase packing materials, performance was maintained for at least 500 and in most cases for over 1000 injections. Thus, for some Hypersil *n*-octadecyl bonded silica CEC capillary columns >1000 injections could be performed with different eluents resulting in separation parameters of excellent reproducibility based on the results obtained with the test mixture at pH 8. Similarly, the CEC 8-ODS capillary column could be used for more than 1500 injections with excellent resolution and performance maintained, as assessed with the test mixture of the *n*-alkylbenzenes.

3.3. Retention factors of the neutral analytes

The retention factors of neutral analytes in CEC are by definition the same as the capacity factors, k' values, in HPLC [14,15]. In these studies, the k_{cec} values were calculated using uracil as EOF marker in the test mixture. The relative standard deviation (RSD) of the k_{cec} values were<6% for a total of 50 sets of analyses based each on six injections of the neutral sample for all Hypersil *n*-octadecylsilica CEC capillary columns. The RSD values of the k_{cec} values, based on one experiment with $n=6$ were <0.5% for the commercial Hypersil CEC capillary columns. The k_{cec} values, measured after different numbers of injections for three *n*-octadecyl bonded silica CEC capillary columns (column numbers 4-ODS, 7-ODS and 8-ODS) are illustrated in Fig. 2

Table 2
Comparative ranges of the calculated k_{cec} values of the *n*-alkylbenzenes determined during the life-time studies of three *n*-octadecyl bonded silica CEC capillary columns as illustrated in Fig. 2

CEC column number	Ethylbenzene ($n=6$)	*n*-Butylbenzene ($n=6$)	*n*-Pentylbenzene ($n=6$)
4-ODS	0.64–0.75	1.16–1.36	1.56–1.84
7-ODS	0.61–0.65	1.12–1.17	1.52–1.60
8-ODS	0.56–0.61	1.00–1.13	1.36–1.54

and the obtained ranges of the k_{cec} values are listed in Table 2 for further comparison. The CEC 4-ODS capillary column was used for about 800 injections after which the k_{cec} values for ethylbenzene decreased to 0.40; *n*-butylbenzene to 0.73 and *n*-pentylbenzene to 1.00 and the capillary column was discarded; whereas the CEC 7-ODS capillary column only gave consistent k_{cec} values through to about 620 injections after which the column efficiency dropped below 30 000 theoretical plate numbers and the column was not used for any further studies. In contrast, the CEC 8-ODS capillary column was used for about 1500 injections with little change in the k_{cec} values of the *n*-alkylbenzenes, whilst the *N* values were still >30 000.

The average k_{cec} values for virgin CEC capillary columns obtained for the three analytes with different CEC capillary columns, but originating from the same batch of *n*-alkyl bonded silica material, are reported in Table 3. The RSD for the k_{cec} values for the different virgin CEC capillary columns were 4% and 6% for the Hypersil *n*-octylsilica and *n*-octadecylsilica CEC capillary columns, respectively. The initial range of k_{cec} values for the Hypersil *n*-octadecylsilica CEC capillary columns based on eight capillary columns was between 0.58 and 0.66 for ethylbenzene, between 1.06 and 1.20 for *n*-butylbenzene, and between 1.45 and 1.64 for *n*-pentylbenzene with a 99.7% confidence interval. When only four Hypersil *n*-octylsilica CEC capillary columns were used for this test study, the range in the k_{cec} values with the same 99.7% confidence interval were between 0.61 and 0.67 for ethylbenzene, between 1.07 and 1.17 for *n*-butylbenzene, and between 1.39 and 1.56 for *n*-pentylbenzene. There was no significant change in the selectivity of the three analytes with the two different *n*-alkyl bonded silica phases.

3.4. Changes in the theoretical plate numbers of the CEC capillary columns

The column efficiencies [expressed in terms of the theoretical plate numbers for the capillary columns (N) were calculated by the HP software according to the half-width method (i.e. $N=5.54(T_r/W_{50})^2$] and the results are listed in Table 4. The initial *N* values per column were about 50 000 for the *n*-octadecylsilica CEC capillary columns and about 40 000 for the *n*-octylsilica CEC capillary columns, which can be compared with about 60 000 theoretical plate numbers for a virgin CEC capillary column. The initial average *N* values per column and the RSD values based on eight Hypersil *n*-octadecylsilica CEC capillary columns were for the three *n*-alkylbenzenes respectively: $N=53\,000$ with 12% RSD for

Table 3
Calculated k_{cec} values for the different *n*-alkylbenzenes with nine different virgin *n*-octadecylsilica (ODS) CEC capillary columns and four different virgin *n*-octylsilica (OS) CEC capillary columns

CEC column number	k_{cec}		
	Ethylbenzene	*n*-Butylbenzene	*n*-Pentylbenzene
1-ODS	0.65	1.19	1.60
2-ODS	0.60	1.09	1.48
3-ODS	0.58	1.06	1.45
4-ODS	0.69	1.24	1.67
5-ODS	0.65	1.18	1.60
6-ODS[a]	0.34	0.62	0.84
7-ODS	0.63	1.15	1.56
8-ODS	0.57	1.04	1.41
9-ODS	0.62	1.12	1.52
1-OS	0.61	1.13	1.54
2-OS	0.64	1.09	1.42
3-OS	0.64	1.11	1.44
4-OS	0.66	1.16	1.52

[a] As the CEC 6-ODS capillary column gave divergent k_{cec} values, the data were not used for further calculations.

Table 4
Calculated theoretical plate numbers per column length, N values, for the different *n*-alkylbenzenes with nine different virgin *n*-octadecylsilica (ODS) CEC capillary columns and four different virgin *n*-octylsilica (OS) CEC capillary columns

CEC column number	N		
	Ethylbenzene	*n*-Butylbenzene	*n*-Pentylbenzene
1-ODS	45 000	43 000	40 000
2-ODS[a]	28 000	28 000	28 000
3-ODS	45 000	43 000	43 000
4-ODS	61 000	58 000	56 000
5-ODS	51 000	48 000	46 000
6-ODS	49 000	46 000	41 000
7-ODS	56 000	54 000	52 000
8-ODS	60 000	56 000	54 000
9-ODS	57 000	58 000	57 000
1-OS	34 000	28 000	28 000
2-OS	39 000	38 000	37 000
3-OS	42 000	40 000	40 000
4-OS	49 000	49 000	42 000

[a] As the CEC 2-ODS capillary columns gave divergent N values, the data were not used for further calculations.

ethylbenzene, $N=50\,000$ with 13% RSD for *n*-butylbenzene, and $N=49\,000$ with 14% RSD for *n*-pentylbenzene. The corresponding data for four virgin CEC capillary columns packed with *n*-octylsilica were $N=41\,000$ with 16% RSD for ethylbenzene; $N=39\,000$ with 21% RSD for *n*-butylbenzene; and $N=38\,000$ with 21% RSD for *n*-pentylbenzene. Although the RSD'S of the N values were typically $<3\%$, in some cases the RSD'S for the N values could reach as high as 10% in one set of replicates ($n=6$) with a specific CEC capillary column respectively. In these cases, the change in the RSD for the N values appeared to be a consequence of the nature of the analytes used in the preceding runs with these same CEC capillary columns, i.e. with analyte mixtures containing highly basic or acidic peptides, which caused subtle changes in the solute-surface interaction. This effect was remedied on the subsequent run or alternatively by a blank injection with fresh buffer. The average N values per column for the Hypersil CEC capillary columns that had been used for extensive times are illustrated in Fig. 3. It is evident from this plot that the N values decrease with increasing number of injections. When the N values of $<30\,000$ occurred, then the CEC capillary column was discarded. Whether this limit is relevant to practical applications in CEC usage, rather than being solely based on the work practices of this laboratory with CEC capillary columns packed with particles derived from the same batch of reversed-phase sorbent, has to be determined for a larger set of data based on a wider array of CEC capillary columns.

3.5. Effects on symmetry coefficients

The symmetry coefficients (λ_{sym} values) of the three analytes calculated by the HP Chem-station software were typically in the range of 0.95–1.05 for replicated analyses performed with Hypersil CEC capillary columns. There was no significant change in the λ_{sym} values that correlated with changes in capillary column life-time performance with the symmetry coefficients of the *n*-alkylbenzenes peaks consistently falling within this range even after the capillary columns had been extensively used with a variety of peptides and the N values approached about 30 000.

4. Conclusions

A life-time test mixture consisting of three *n*-alkylbenzenes has been investigated with nine *n*-octadecylsilica and four *n*-octylsilica Hypersil CEC capillary columns. The data for the k_{cec}, N and λ_{sym} values indicate that excellent life-time performances with peptides can be achieved with the Hypersil CEC capillary columns from 500 to 1000 injections, depending on the composition of the eluent used for the analytical separation. The life-time of these CEC capillary columns, as assessed with these neutral *n*-alkylbenzenes, was also found to depend on the purity and properties of the peptide analytes themselves, with non-specific adsorption to the silica surface can occur evident with some basic peptides. With the availability of additional data with more CEC capillary columns, packed with reversed-phase sorbents of different selectivity and surface chemistries, the limits of acceptable performance can be established before the CEC capillary column is discarded. However, the results described in this paper provide a basis to validate the life-time of CEC capillary columns, with the results leading to the encouraging observation that about 1000 repetitive

analyses can be reproducibly run on the same CEC capillary column, provided appropriate experimental care is taken.

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