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Review

Capillary electrochromatography in the size-exclusion mode

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Abstract

In this review the literature on the application of capillary electrochromatography (CEC) for size-based separations of macromolecules is summarized. Opportunities and limitations of CEC specially related to the size-exclusion mode (SEEC) are indicated. Applications with synthetic polymer samples as well as with biomacromolecules (polysaccharides, proteins) are shown. The prospects for a further development and application of SEEC are discussed.

Keywords: Size-exclusion chromatography; Size-exclusion electrochromatography; Electrochromatography; Polymers; Polysaccharides; Proteins

Contents

1. Introduction

In the last decade hundreds of papers have been published every year on the technical and methodological development of capillary electrochromatography (CEC) and on its application in chemical and biochemical analysis. CEC is a separation technique that combines elements of electrophoresis and liquid chromatography (LC). As in chromatography, differences in partition of analytes between two phases are the basis for separations, and, as in electrophoresis, an electric field is used as the driving force for such separations. CEC can be realized in different formats. The separations can be performed in (usually fused-silica) capillaries with a bulk stationary phase that can be present either as a packed bed of solid particles or as a monolithic phase synthesized in situ. Alternatively, in the so-called open-tubular variant, the retaining phase is applied as a layer on the capillary wall only (OT-CEC). Instead of cylindrical capillaries channels created by micromachining techniques in various substrates (glass, silica, polymeric materials) can be utilized. Although it may be a matter of debate whether this is to be called CEC, such formats will also be discussed in this review.

The various phase systems developed for conventional, pressure-driven liquid chromatography (HPLC) can also be utilized in CEC separations. Inspection of the recent literature shows that in the majority of the applications of CEC a reversed-phase system is applied [1,2]. In most studies a capillary is used packed with silica particles modified with hydrophobic groups (C_8, C_{18}). When monolithic stationary phases are used, these are also usually more hydrophobic than the mobile phase, and a reversed-phase mechanism determines the separation. Ion-exchange materials are also used, sometimes mixed with reversed-phase particles. However, the role of the ion-exchange stationary phase material is often just to generate enough electroosmotic flow (EOF),

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and (neutral) analytes are still separated in a reversed-phase order. When ion-exchange materials are used in CEC for ionic analytes, the separation mechanism will be a combination of partitioning and electrophoresis.

Affinity CEC is also popular. Many ligands have been developed showing a specific interaction with a variety of compounds of biochemical or biological interest. Such materials are often fairly easily attached to a capillary wall, opening the possibility for tailor-made CEC separations. Enantiomeric separations with chiral phases, attached to the capillary wall or to a particulate support, are relatively easily obtained using CEC [3].

In contrast to the abundance of publications on reversed-phase and affinity CEC, the number of research papers on CEC in the size-exclusion mode (size-exclusion electrochromatography, SEEC) is very limited. Within pressure-driven chromatography the size-exclusion mode is a common variant used for the molecular-size based separation of a variety of synthetic and natural macromolecular compound classes. As will be shown below, the potential advantages of CEC over pressure-driven LC may be especially relevant for the macromolecules typically separated by size-exclusion mechanisms. In 1998 two research groups independently reported on size-exclusion electrochromatography [4,5]. Still, since then only few other groups have explored the possibilities of this mode of CEC. In this short review, the experimental experiences with SEEC will be summarized and future prospects will be discussed.

2. Selectivity and efficiency

For the development of CEC and its implementation in practice several arguments have been given. With CEC higher efficiencies are expected than with the alternative separation technique, pressure-driven LC. First, the velocity of an electrokinetic liquid flow is in first approximation independent of the characteristic dimension (width) of the flow channels. Therefore, the downscaling of the particle or channel size in CEC does not stop at a pressure limitation, as it would in pressure-driven LC. Indeed, with CEC plate numbers have been generated that are only possible in conventional LC under extreme experimental conditions. However, in most CEC experiments in practice sizes are used that could also have been applied in LC. In such cases the main argument for CEC is that it gives a higher separation efficiency at a higher speed than HPLC with the same column. These advantages of CEC over LC are amply documented in the literature (see, e.g., [6,7] and references cited therein).

The higher efficiency of CEC is brought about by several factors. The channel-size-independent velocity of the electrokinetic flow makes imperfections of the packing structure of the column less noticeable. This leads to a lower A-term contribution to the plate height in CEC. More important, however, is the decrease of the C-term obtained with

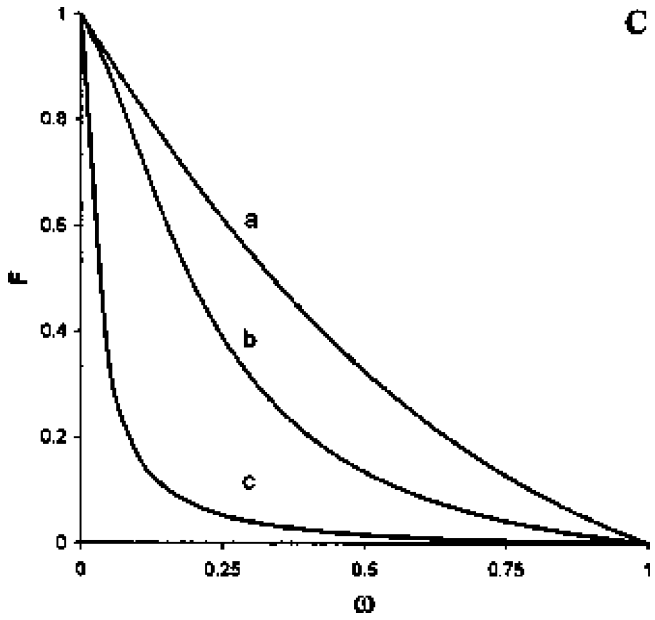

Fig. 1. Effect of the relative pore flow velocity ω on the mass-transfer contribution to the plate height for an analyte with a diffusion coefficient of (a) $5 \times 10^{-10}\,m^2\,s^{-1}$, (b) $1 \times 10^{-10}\,m^2\,s^{-1}$, and (c) $1 \times 10^{-11}\,m^2\,s^{-1}$. From ref. [11], with permission.

an electrically driven mobile phase, especially when porous particles are used as stationary phase. This improvement of the separation efficiency and speed is strongly related to the perfusive electrokinetic flow through the pores of the separation particles. The existence and importance of the (perfusive) pore flow in CEC with porous particles has been substantiated experimentally by inverse SEC experiments [8], by NMR [9] and recently it could be visualized with confocal laser scanning microscopy [10]. This improvement of the C-term (mass-transfer) contribution to dispersion would be of special interest for the compounds typically separated by a size-exclusion mechanism, i.e., for slowly diffusing macromolecules. In a discussion on the effect of pore flow on efficiency, the dependency of the C-term improvement on the diffusion coefficient of the analyte has been modeled [11]. In Fig. 1 the "C-term improvement factor" F is shown as a function of the relative pore flow velocity ω for compounds with different diffusion coefficients (D). Curve a is for a typical low-molecular mass compound with $D = 5 \times 10^{-10}\,m^2\,s^{-1}$. Curve b ($D = 10^{-10}\,m^2\,s^{-1}$) could be for a M_r 30 000 polystyrene (PS) in tetrahydrofuran (THF) or a M_r 20 000 protein, and curve c for an ultra-high-molecular mass compound ($D = 10^{-11}\,m^2\,s^{-1}$). Clearly, for the separation of high-M_r compounds electrochromatography would give the largest gain in separation performance.

However, the pore flow typical for electrochromatography does not only have positive effects on the separation. Size-exclusion separations are based on differences in fluid velocity in different volume fractions of the mobile phase in the separation channel. In conventional pressure-driven SEC, the liquid flow through the pores inside the stationary

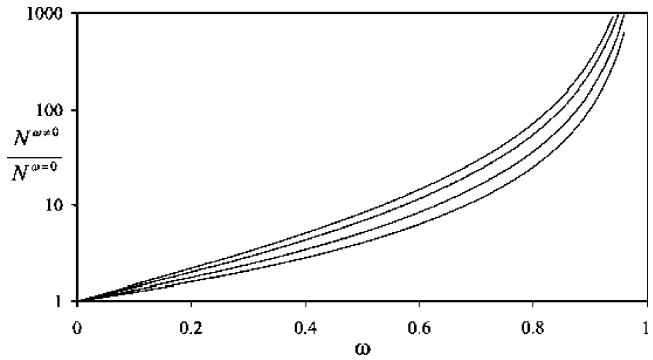

Fig. 2. Required increase of the plate number to obtain the same resolution in SEEC with a relative pore flow velocity ω as in pressure-driven SEC ($\omega = 0$). The different lines are for different relative retention values. From ref. [12], with permission.

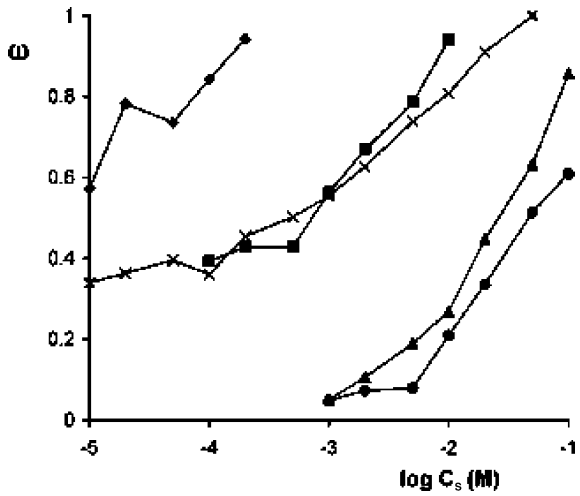

Fig. 3. Relative pore flow velocity as a function of the ionic strength of the mobile phase, for particles with a nominal pore size of (●) 5 nm, (▲) 10 nm, (×) 30 nm, (■) 50 nm, or (▼) 100 nm. From ref. [14], with permission.

phase particles is virtually zero. This leads to differences in the average flow velocity between molecules of different size that can enter a different fraction of the total pore volume. A key factor in the selectivity that can be obtained in SEC is the retention window, the retention time difference between molecules that are totally excluded from the pores and the solvent (measured with the help of a low-M_r marker). In pressure-driven SEC, the relative retention time (realtive to the low-M_r marker) for a totally excluded compound τ_{excl} is a function of the interstitial (ε_{out}) and intraparticle (ε_{in}) porsosities:

$$\tau_{excl} = \frac{\varepsilon_{out}}{\varepsilon_{in} + \varepsilon_{out}} \quad (1)$$

When a significant pore flow exists through the particles in a packed bed, these flow velocity differences will be decreased. This will lead to a smaller retention window and a decreased separation selectivity, as was already discussed by Venema et al. [12]. When the flow velocity through the pores is (on average) a fraction ω of the velocity between the particles, the retention window will be decreased to:

$$\tau_{excl} = \frac{\omega\varepsilon_{in} + \varepsilon_{out}}{\varepsilon_{in} + \varepsilon_{out}} \quad (2)$$

The improved separation efficiency obtained when the mobile phase is driven by an electric field instead of a pressure over the column, will be needed to compensate for the decreased selectivity (see Fig. 2). Apparently, to realize the inherent possibilities of SEEC a careful tuning of the pore-to-interstitial flowrate ratio ω will be required [13].

A key factor for ω is the ionic strength of the mobile phase, that determines the extent of double-layer overlap. Stol et al. [14] have measured the influence of the ionic strength of the dimethylformamide (DMF) mobile phase on the pore flow velocity in silica particles with different average pore diameters. Fig. 3 shows the results obtained in this study. A conclusion derived from this work could be that it is more difficult to suppress pore flow than to generate it, especially with particles with relatively wide pores. For ultra-high-M_r compounds, that require large pore sizes, SEEC will be less suited.

In a study described in a recent paper [15], size-exclusion effects appear to play a role in non-aqueous capillary gel electrophoresis (CGE). Of course, size-based separations by CGE are common practice, but usually only for charged analytes. For such compounds, the sieving effect of the gel leads to a decrease of the migration velocity with increasing molecular mass (M_r) of compounds with fixed charge-to-size ratio. In the study by Li et al., neutral synthetic polymers were made to migrate through a replaceable polyethylene gel by the interaction with cationic surfactants added to the non-aqueous mobile phase. It was found that the migration velocity of the polymers tested (polystyrenes, poly(methylmethacrylates) (PMMA)) increased with their M_r. Although definite conclusions could not be drawn yet, size-exclusion effects within the gel are a possible explanation.

3. Stationary and mobile phases

In our group we have used mainly capillary columns packed with standard bare silica particles for SEEC [5,8,11–14]. Such particles are well characterized and are available with various well-defined particle and (average) pore sizes. In capillary columns packed with these particles the interstitial and intraparticle mobile phase volume fractions are approximately equal. In the SEEC mode, retention windows from approximately 0.6 to 1 could be obtained. With synthetic and natural polymers in the 10 000–100 000 molecular-mass range the best results were obtained using 5 μm particles with 10 nm (100 Å) nominal pore diameter. For macromolecules larger than M_r 100 000 silica particles with 30 nm pores were used.

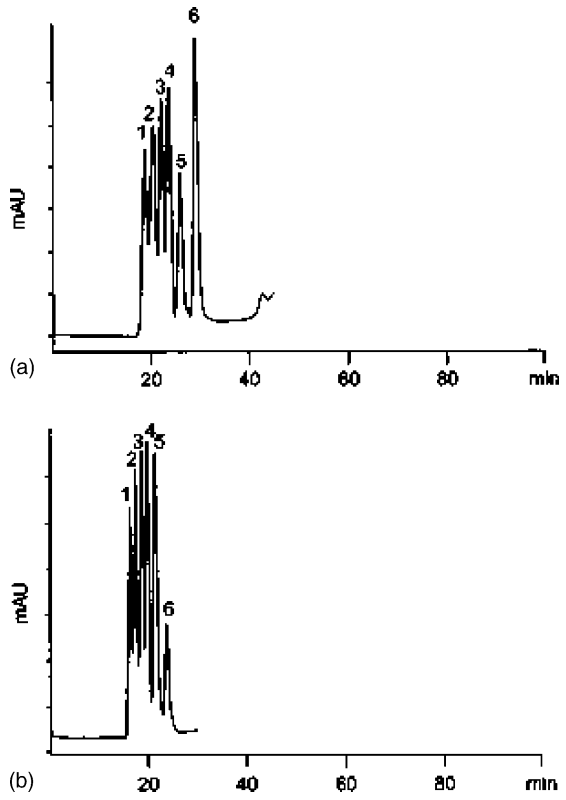

Fig. 4. Effect of the composition of a mixture of cation-exchange and SEC particles used as stationary phase on the speed and efficiency of SEEC. Particle ratio: (a) 15:85 and (b) 40:60. Sample: PS standards. From ref. [16], with permission.

Mistry et al. tested different polymeric HPLC stationary phase particles for use in SEEC [16]. With sulfonated polystyrene–divinylbenzene (PSD–VB) particles with a high ion-exchange capacity (PRP x400 particles) a substantial electro-osmotic flow was found, but the retention window was narrow. When non-sulfonated PSD–VB particles that were designed for SEC separations (PLGel mixed C) were packed in a capillary column, a suitable retention window was obtained, but a low EOF velocity. The solution to this dilemma was to pack columns with a mixture of both particle types. The best results were obtained with a 40:60 mixture of x400 and PLGel C particles. Fig. 4 shows the SEEC separation of a number of polystyrene standards obtained with such a column, using THF with 2% water as the mobile phase.

In the pioneering work of Peters et al. [4] a methacrylate-based monolithic column was used for the SEEC separation of polystyrenes. Using THF–2% water as the mobile phase, a suitable EOF velocity was obtained. The retention window, however, was fairly narrow with this column material (see Fig. 5). It should be noted that the monolith material was not specially designed for size-exclusion separations. In fact, the optimization strategy in the synthesis of monolithic columns is often directed on producing a material with a narrow pore-size distribution (see, e.g., [17]). In other separation modes (e.g., in reversed-phase or affinity chromatography) a narrow pore-size distribution is expected to be beneficial for the efficiency of the separation. In SEEC on the other hand, a broad pore-size distribution is essential for the separation. When polymeric monoliths are to be used in SEEC, the stationary phase material should be designed for this purpose, with preferentially a clear distinction between macropores and mesopores.

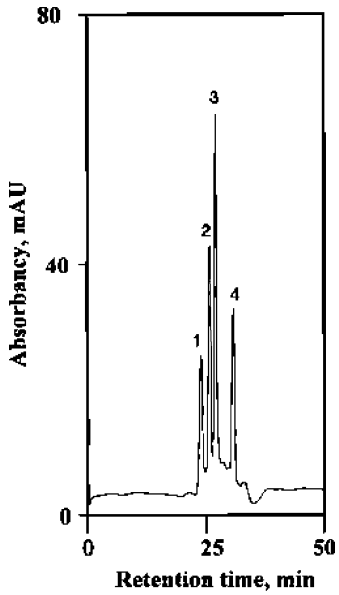

Fig. 5. SEEC separation of polystyrene standards on a methacrylate monolithic column material. Mobile phase: THF–2% water. From ref. [4], with permission.

Monoliths based on silica, produced by many different routes and procedures, have also been applied extensively in CEC [18,19]. Although so far no attempts have been made to use this material for SEEC, its structure can be very suitable for the size-exclusion principle. A macroporous structure is formed during the original production process and afterwards mesopores can be tailor-made by, e.g., heat-treatment [20]. Experiments with inverse SEC showed that commercially available silica monoliths contain a relatively large volume of mesopores with a proper size (2–50 nm) for SEC and SEEC [21].

Many of the synthetic and natural polymers for which SEEC would be an appropriate separation technique are not soluble in water. Therefore, in most of the published work on SEEC organic solvents have been used for the mobile phase. The electrokinetic flow required in SEEC relies on the presence of a surface charge on the stationary phase. Therefore, solvents with a low permittivity (dielectric constant) are not suitable. In solvents such as hydrocarbons or chlorinated hydrocarbons the ionization of the stationary phase surface is negligible. Suitable organic solvents include *N*-methylformamide (NMF), dimethylformamide, acetonitrile, acetone, and hexafluoroisopropanol (HFIP). With pure THF as the mobile phase no electro-osmotic flow has been found. However, an appreciable flow could be obtained when

a small amount (2–3%) of water was added to the THF, both with a monolithic column material [4] as with a particulate stationary phase [16]. Noteworthy in these experiments is that the mobile phase did not contain a buffer or other electrolyte. For the generation of an EOF bulk conduction of the liquid is not a prerequisite. Still, in most modes of electrokinetic separations a salt has to be added to the liquid-phase to suppress electromigration dispersion or concentration overloading. For the neutral macromolecular compounds to be separated by SEEC such overloading phenomena do not play a role. In the experiments with columns packed with silica particles suitable salts (lithium or quaternary ammonium salts) in concentrations of 0.1–1 mM were added to the organic solvents used. The role of the ionic strength of the mobile phase in the regulation of the pore-to-interstitial flow ratio, and with that of the retention window, has been discussed above.

4. Separations of synthetic polymers

In the first stage of the development of SEEC most experiments have been carried out with polystyrenes. PS can be readily detected by UV absorbance detection, and narrow molecular-mass standards are available. Polystyrene samples could be characterized by SEEC with bare silica particles as the stationary phase and DMF containing 0.1 mM LiCl as the mobile phase [22], and on polymeric particles with THF–2% water as the mobile phase [16]. The retention window was slightly wider in the first study, with an exclusion limit at a relative retention of 0.62 against 0.65 for the second study, but in both cases the exclusion limit was at approximately M_r 1 $\times 10^6$ for PS. In both studies the characteristic parameters [number-average molecular mass (M_n), weight-average molecular mass (M_w) and the polydispersity P] of different technical samples as obtained by SEEC were compared with the values obtained by conventional SEC, and in both studies a fair agreement was found.

Ding et al. studied the repeatability of the SEEC system. The run-to-run and day-to-day repeatability for the retention times of PS standards within the calibration range was in the order of 1–2%; for the relative retention (using toluene as a marker) these repeatabilities were 0.1%. The column-to-column variation was larger, with R.S.D. values for the retention time in the order of 8% and for the relative retention of 2%. Apparently, a separate calibration curve has to be constructed for every column. For polycarbonates [poly(bisphenol-A)] the same separation system could be used. Again, the characteristic values obtained for the two samples matched very well with the SEC data.

Mistry et al. characterized a number of other polymeric materials with the phase system developed for PS: polycarbonates, a styrene–acrylonitrile copolymer and thermoplastic polyurethane. Some typical chromatograms obtained with their system are shown as Fig. 6.

Ding et al. [22] studied the use of HFIP as the mobile phase solvent. HFIP is a effective solvent for a variety of synthetic polymers. The use of HFIP as solvent in conventional SEC is restricted because of its toxicity and high cost. On the volume scale of SEEC these disadvantages are less important. When using bare silica particles as the stationary phase, only a very low EOF could be generated. Better results were obtained with silica particles chemically modified with sulfonic acid groups (a strong cation-exchange material). Although the EOF velocity obtained with this stationary phase was still relatively low, and its repeatability not very satisfactory, the feasibility of using HFIP in SEEC could be demonstrated. Some pilot separations were shown of samples of poly(methylmethacrylate) standards (see Fig. 7), poly(ethyleneterephthalate) (PET) and of poly(caprolactam) (nylon-6).

5. Biomacromolecules

Mistry et al. [23] showed that the mixed polymeric stationary phase (PRP ×400–PLGel C) that had been developed before for non-aqueous SEEC of synthetic polymers, can also be used in combination with aqueous solvents [Tris buffer–acetonitrile (ACN)]. They used this system for the separation of pullulan, a natural polysaccharide. Pullulan molecular-weight standards that are commercially available were used for calibration. For detection of the (underivatized) polysaccharides an indirect UV method was used. To the separation buffer 1% dimethyl sulfoxide (DMSO) was added, that was monitored at 220 nm. The elution of polysaccharides from the column could be detected as a decrease in UV absorption. Since the displacement of DMSO was merely the result of the bulk hydrodynamic volume of the analytes, the sensitivity was low.

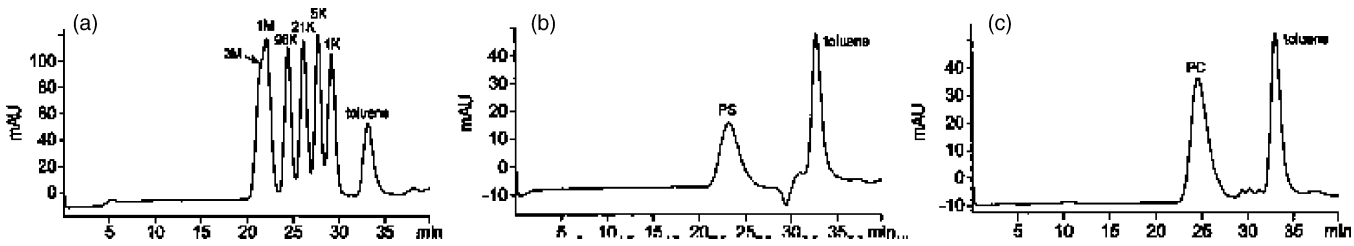

Fig. 6. SEEC separation of (a) polystyrene standards, (b) a polydisperse PS sample, and (c) a polycarbonate sample on a column packed with polymeric particles. Mobile phase: THF–2% water. From ref. [16], with permission.

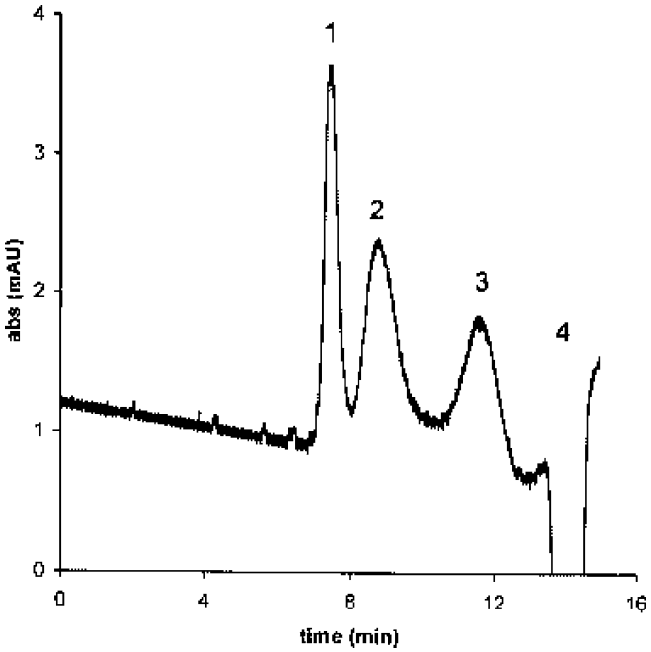

Fig. 7. Separation of PMMA standards with HFIP as the mobile phase. From ref. [22], with permission.

Better results (in terms of sensitivity and quantitation possibilities) were obtained with polysaccharides after derivatization with phenyl isocyanate (PIC). After derivatization the analytes were hydrophobic enough to use the previously studied non-aqueous system [16]. A linear calibration curve (relative retention versus $\log M_r$) was obtained with derivatized pullulan standards from M_r 738 to 404 000. The run-to-run repeatability of the relative retention was well below 0.1%. The method developed could be applied for the M_r distribution determination of polydisperse pullulan samples, but also of different amyloses. Typical chromatograms are shown in Fig. 8.

In an earlier study Stol et al. [24] used the PIC derivatization approach for celluloses. The derivatized compounds were separated by SEEC using a column packed with silica particles. Several organic solvents were considered for use as mobile phase. The best results were obtained with acetone with 0.1 mM of a quaternary ammonium salt. Since the polysaccharide standards available for calibration were not optimal, the method of universal calibration was applied with a number of different polymer types. The method developed was applied for the characterization of celluloses from paper of different origin, and to study the influence of (artificial) aging of paper. An additional advantage of the proposed method over conventional SEC in the present application field (studying works of art) was the small sample amounts required. Multiple analyses could be performed on a single paper fibre.

An interesting application for SEEC would be in protein separations. Still, in only few publications this topic has been addressed. Tellez and Cole [25] have shown that the size-exclusion effect can play a role in electrokinetic separations of proteins. They studied the preparative separation of whey proteins and used cm-scale columns packed with partly hydrolyzed dextran or agarose particles for this. The mobile phase, an aqueous buffer solution, was driven by a pressure difference and an applied electric field simultaneously. The main separation mechanism was electrophoresis with “concentration polarization” on the beads. However, a clear difference in behaviour was observed between proteins that could enter the pores of the stationary phase and those that could not. This implies that the separation was at least partially based on size-exclusion phenomena.

The combination of pressure-induced and electrokinetic flow was also applied by Stahl et al. [26] for the separation of proteins. This so-called pressure-assisted electrochromatography (pCEC) method was used with capillary columns packed with porous silica particles. With pCEC shorter analysis times and narrower peaks were found than with capillary HPLC. Linear relations were obtained when the elution times of model proteins were plotted against $\log M_r$.

In a symposium abstract Jemere et al. [27] report the separation of proteins by SEEC on a microchip. The authors produced very short (2 mm) columns in a glass substrate and packed these with 5 μm SEC beads. Using various surfactant containing buffer systems, fluorescein isothiocyanate (FITC) labelled IgG (M_r 150 000) could be separated from FITC-insulin (M_r 5800) in less than 30 s, with applied voltages from 300 to 600 V. For the insulin peak approximately 120 plates were obtained, which corresponds

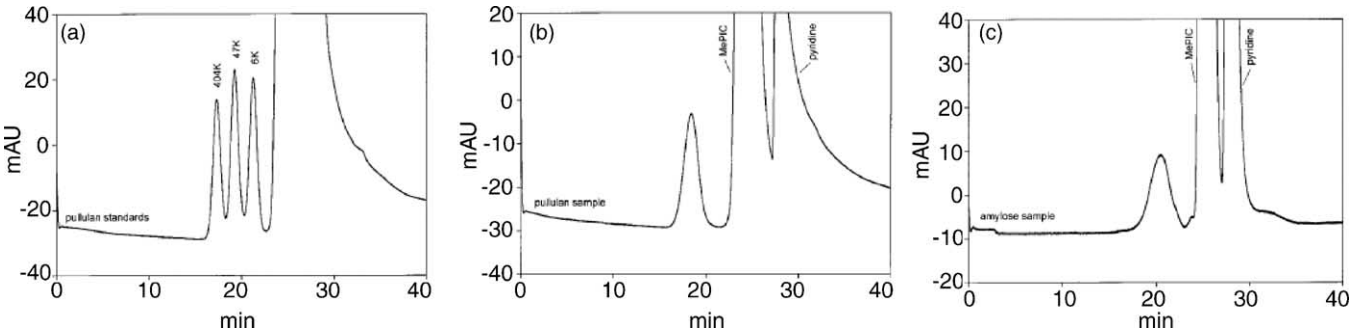

Fig. 8. Separations of PIC-derivatized polysaccharides by SEEC on a column packed with polymeric particles: (a) pullulan standards, (b) a polydisperse pullulan, and (c) amylose. From ref. [23], with permission.

to 60 000 plates/m. The electropherograms were recorded using fluorescence detection.

6. Prospects

Although the practical experience with SEEC is still limited, it has already been shown clearly that it can provide higher separation efficiencies than pressure-driven SEC. However, in the industrial use of a separation method for synthetic or natural polymers, the repeatability and reproducibility of the results obtained are often a more important aspect than the separation efficiency. In respect to precision, SEEC is still inferior to conventional pressure-driven SEC. In a study on the predictive properties of the two methods for molecular weight determinations, it was found that the prediction errors of SEEC are two to three times larger than those with conventional SEC [28]. Before the method will be accepted in routine laboratories, an improvement in this respect will be necessary.

An advantage of SEEC that might be appreciated more is the increased speed with which separations can be carried out. This would not only be of value in high-throughput applications (e.g., in proteomics) but certainly also in two-dimensional liquid-phase separation systems. Because of the inherent complexity of many macromolecular samples, two-dimensional separations are becoming increasingly popular. Unless when the second-dimension separation can be carried out in a multiplexed way, the second-dimension separation should be really fast to keep the total analysis time within limits. A fast SEEC separation, that has by principle a fixed end time, would be perfectly suited for this.

In the last decade a huge research effort has been directed on the development of separation and analysis systems created by micromachining methods (or nanotechnology) on suitable substrates, the lab-on-a-chip approach. The potential of this technology is enormous; however, its practical realization and application is still not trivial. What has become clear is that for liquid-phase separations it is much easier to direct and control flows by electrical fields than by pressure gradients. For separations on the chip format CEC is therefore much more popular than for separations where an “ordinary” capillary is used. For the same practical reasons SEEC might become the prevalent technique for molecular-size based separations of macromolecular samples in the chip format. It has already been shown that monolith materials can be used for SEEC. Creating monolithic stationary phases with a suitable pore-size distribution in micromachined channels may be the next challenge.

References

[1] G. Vanhoenacker, T. Van den Bosch, G.P. Rozing, P. Sandra, Electrophoresis 22 (2001) 4064.
[2] S. Eeltink, G.P. Rozing, W.Th. Kok, Electrophoresis 24 (2003) 3935.
[3] Y. Tanaka, S. Terabe, J. Biochem. Biophys. Methods 48 (2001) 103.
[4] E.C. Peters, M. Petro, F. Svec, J.M.J. Frechet, Anal. Chem. 70 (1998) 2296.
[5] E. Venema, J.C. Kraak, R. Tijssen, H. Poppe, Chromatographia 58 (1998) 347.
[6] L.A. Colon, G. Burgos, T.D. Maloney, J.M. Cintron, R.L. Rodriguez, Electrophoresis 21 (2000) 3965.
[7] K. Mistry, I. Krull, N. Grinberg, J. Sep. Sci. 25 (2002) 935.
[8] R. Stol, H. Poppe, W.Th. Kok, J. Chromatogr. A 887 (2000) 199.
[9] U. Tallarek, E. Rapp, H. van As, E. Bayer, Angew. Chem. Int. Ed. Eng1. 40 (2001) 1874.
[10] U. Tallarek, M. Paces, E. Rapp, Electrophoresis 24 (2003) 4241.
[11] R. Stol, H. Poppe, W.Th. Kok, Anal. Chem. 73 (2001) 3332.
[12] E. Venema, J.C. Kraak, H. Poppe, R. Tijssen, J. Chromatogr. A 837 (1999) 3.
[13] R. Stol, W.Th. Kok, H. Poppe, J. Chromatogr. A 914 (2001) 201.
[14] R. Stol, H. Poppe, W.Th. Kok, Anal. Chem. 75 (2003) 5246.
[15] G.D. Li, X.J. Zhou, Y.H. Wang, I.S. Krull, K. Mistry, N. Grinberg, H. Cortes, J. Liq. Chromatogr. 27 (2004) 939.
[16] K. Mistry, H. Cortes, D. Meunier, C. Schmidt, B. Feibush, N. Grinberg, I. Krull, Anal. Chem. 74 (2002) 617.
[17] C. Yu, M. Xu, F. Svec, J.M.J. Frechet, J. Polym. Sci. A 40 (2002) 755.
[18] N. Tanaka, M. Motokawa, H. Kobayashi, K. Hosoya, T. Ikegami, J. Chromatogr. Libr. 67 (2003) 173.
[19] A. Darrin, Z. El Rassi, Electrophoresis 24 (2003) 3962.
[20] N. Kazuki, S. Hiroko, I. Norio, K. Norihito, S. Naohiro, J. High Resolut. Chromatogr. 23 (2000) 106.
[21] M. Al-Bokari, D. Cherak, G. Guiochon, J. Chromatogr. A 975 (2002) 275.
[22] F. Ding, R. Stol, W.Th. Kok, H. Poppe, J. Chromatogr. A 924 (2001) 239.
[23] K. Mistry, I. Krull, N. Grinberg, Electrophoresis 24 (2003) 1753.
[24] R. Stol, J.L. Pedersoli Jr., H. Poppe, W.Th. Kok, Anal. Chem 74 (2002) 2314.
[25] C.M. Tellez, K.D. Cole, Electrophoresis 21 (2000) 1001.
[26] M. Stahl, A. Jacob, A. von Brocke, G. Nicholson, E. Bayer, Electrophoresis 23 (2002) 2949.
[27] A.B. Jemere, R.D. Oleschuk, D.J. Harrison, in: Y. Baba, S. Shoji, A. van den Berg (Eds.), Proceedings of the 6th μTAS Symposium, 2000, Nara, Japan, Kluwer Academic Publishers, Dordrecht, Netherlands, 2002.
[28] Y. Vander Heyden, S.T. Popovic, P.J. Schoenmakers, J. Chromatogr. A 957 (2002) 127.