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Pore flow effects in electrically driven size-exclusion chromatography

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Abstract

The applicability of electrically driven (ED) size-exclusion chromatography for the separation of synthetic polymers was investigated in capillary columns packed with 5 μm particles with different pore sizes using dimethylformamide as the mobile phase. It was found that under ED conditions a significant intraparticle pore flow was generated. The relative intraparticle velocity with respect to the average interparticle velocity increased with the pore size and ionic strength. It was also observed that with increasing pore flow the plate height of polymers decreased considerably. On the other hand, the intraparticle velocity impaired the selectivity of the separation. In some cases the loss in selectivity was so large that hardly any separation of polymers was achieved. The effect of pore flow on the retention of polymers in porous particles could be described well with a simple theory. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

In the last two decades electrically (ED) driven separation methods have become one of the most intensively investigated separation techniques [1–6]. In the beginning only open capillaries (coated and uncoated) were used but there is an increasing interest to apply capillary electrochromatography (CEC). In this technique a fused silica capillary is filled with packing particles and the mobile phase is propelled electroosmotically by applying a voltage across both ends of the capillary. It has been found

that the efficiencies and resolutions in CEC are often superior to those in high-performance liquid chromatography (HPLC) due to the pluglike flow profile [7]. CEC is not yet fully developed and problems are still remaining such as reproducibility, detection and packings.

One mode of CEC has so far hardly been investigated, viz. electrically driven size-exclusion chromatography (SEC). Recently, the use of rigid polymer monolithic capillary columns for the separation of polystyrenes in CEC was reported [8]. However, the reported chromatogram shows an extremely low selectivity and only polymers with a very large difference in molecular mass could be separated on these columns.

In a previous paper, the feasibility of ED-SEC on

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capillaries packed with porous silica particles [9] was demonstrated. A clear improvement of the efficiency was found compared to that obtained for standard pressure driven SEC analysis of the same solutes. Unfortunately with ED-SEC the retention window is smaller than under pressure conditions and moreover appeared to depend strongly on the ionic strength of the mobile phase. This phenomenon was attributed to the occurrence of pore flow.

Giddings [10] already addressed the effect of pore flow on the analysis of polymers in conventional pressure driven SEC. The purpose of the present article is to provide insight in these phenomena with the aid of a theoretical backbone dealing with the flow in pores and the effects on the retention of polymers in ED-SEC.

For this purpose, the retention of polymers was measured in capillary columns packed with particles with pores radius ranging from 100 Å up to 1000 Å. Also the ionic strength of the solvent was varied. From the obtained data the ratio between the flow outside and inside the pores was calculated and compared with theoretical calculations.

2. Theory

2.1. Retention behaviour

In a previous report [9] it was observed that the retention window in ED-SEC can be largely influenced by the ionic strength of the solvent used. At higher ionic strengths the retention window decreases and eventually disappears. This effect was attributed to the flow in the pores of the packing material. With increasing ionic strength, the electric double layer thickness decreases and therefore, the flow inside increases. When the flow inside the pores becomes equal to the flow outside the pores no size separation can be observed anymore. This effect on the retention curve could be very well described using an empirical equation originally proposed by Giddings [10] in which a factor (ω) depicting the relative solvent flow inside the pores was incorporated:

$$\frac{t_{\mathrm{r}}}{t_{\mathrm{mark}}} = \frac{V_{\mathrm{R}}}{V_{\mathrm{M}} + V_{\mathrm{S}}} = \frac{V_{\mathrm{M}} + KV_{\mathrm{S}} + \omega(1 - K)V_{\mathrm{S}}}{V_{\mathrm{M}} + V_{\mathrm{S}}} \qquad (1)$$

Here, t_{r} is the retention time of the solute, t_{mark} is the retention time of a small marker solute, e.g. toluene, V_{R} is the retention volume of a solute with a specific distribution coefficient K, V_{M} is the interstitial volume of the liquid outside the particles, V_{S} is the volume inside the particles and ω is the ratio between the solvent velocity inside and outside the particles. For ω equal to 0, i.e., pore flow is absent, the normal SEC behaviour is described. If ω equals 1 all solutes move at the same speed through the SEC column and no separation is achieved. In Giddings' article [10] it was stated that for selective SEC the pore flow has to be suppressed despite the fact that pore flow can have a positive effect on the efficiency of the column. Other authors [11,12], also showed that a large pore flow is not desirable in SEC.

In pressure driven SEC this flow will only be of significant importance if the ratio between the interstitial channels between the packing and the pore radius smaller than 25 [10]. In most commercial packings, this is never the case.

The value of K in Eq. 1 depends on the ratio between the size of the polymer molecule (r_{pol}) and the size of the pores inside the particle (r_{pore}) and is called the aspect ratio $\lambda_{\mathrm{SEC}} = r_{\mathrm{pol}}/r_{\mathrm{pore}}$.

The relation between λ_{SEC} and K, needed to calculate t_{r}, can be described with the random sphere model (RSM) [13].

In Fig. 1 the relationship between λ_{SEC} and t_{r} is presented for different ω values. As was mentioned, for high ω values hardly any separation window is left because the average velocity outside and inside the particle become almost equal. Therefore it is necessary to use systems in ED-SEC with a relatively small ω value, as has been stated in our earlier work [9].

The actual value of ω can be estimated in a straightforward manner. To a first approximation, the column is represented as a bundle of capillaries with two different inner diameters. One radius equals to the intraparticle pore radius (r_{pore}), the other radius can be modelled by the effective radius, R_{eff}, of the interstitial channels formed by the packing.

R_{eff} is related to the hydraulic radius, R_{H}, used in the characterisation of flow in packed beds in technological sciences. The effective radius in packed beds is given by [14,15]:

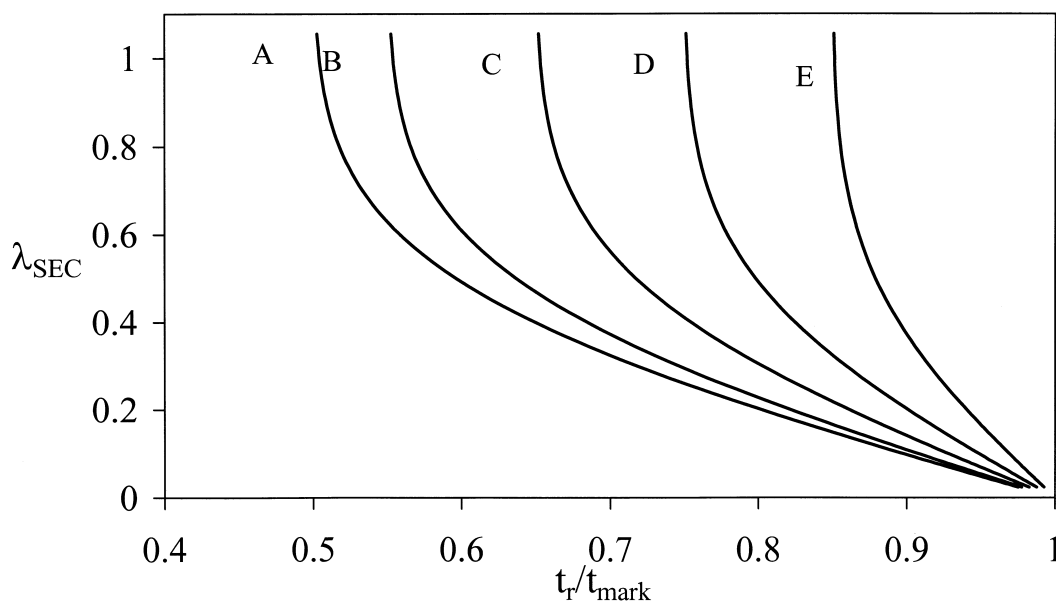$$R_{\mathrm{eff}} = 2R_{\mathrm{H}} = \frac{d_{\mathrm{p}}}{3} \frac{\epsilon}{1 - \epsilon} \qquad (2)$$

Fig. 1. The effect of ω on retention curves in SEC. (A) $\omega = 0$; (B) $\omega = 0.1$; (C) $\omega = 0.3$; (D) $\omega = 0.5$; (E) $\omega = 0.7$.

in which d_p is the particle diameter and ϵ is the interstitial porosity of the column, usually having a value close to 0.4.

The mean solvent velocity, u, in tubular channels with radius R_C under electroosmotic flow can be estimated with the theory derived by Rice and Whitehead [16]:

$$u(R_C) = -\frac{\epsilon_r \epsilon_0 \psi E}{4\pi\eta} \left[\frac{1 - 2I_1(\kappa R_C)}{\kappa R_C I_0(\kappa R_C)} \right] \tag{3}$$

in which ϵ_r is the relative permittivity of the solvent, ϵ_0 the permittivity of vacuum, ψ is the potential of the wall, η is the viscosity of the solvent, I_0, I_1 are the zero- and first-order modified Bessel functions and κ is the inverse double layer thickness.

Because, ω in Eq. (1) is the ratio between the solvent velocity inside the pores and the velocity outside the particles, ω can be calculated using Eq. (3):

$$\omega = \frac{u(r_{pore})}{u(R_{eff})} \tag{4}$$

Both velocities in Eq. (4) are calculated using Eq. (3) with r_{pore} and R_{eff}, respectively.

For a given packing material the effect of the ionic

strength on ω can be calculated when the electric double layer thickness, δ, is known. The latter can be calculated by [7]:

$$\delta = 1/\kappa = \sqrt{\frac{kT\epsilon_r\epsilon_0}{2ne^2z^2}} \tag{5}$$

Here k is the Boltzmann constant ($1.38 \cdot 10^{-23}$ J K^{-1}), T the absolute temperature, n the number of salt molecules per volume, e is the electrical charge of the electron ($1.6 \cdot 10^{-19}$ C), z is the valency of the ion, ϵ_0 is the permittivity of a vacuum ($8.85 \cdot 10^{-12}$ C^2 J^{-1} m^{-1}) and ϵ_r is the dielectric constant of the solvent.

Fig. 2 depicts the value of ω as a function of the ionic strength for a univalent salt in a capillary packed with 5 μm particles and different pore sizes.

Hence, for obtaining the required low values for ω, the ionic strength must be kept very small in order to insure a sufficiently large retention window. Furthermore, only solvents with high dielectric constants are suited to obtain large double layers. For example, if tetrahydrofuran (THF) is used, which has about a ten-times smaller dielectric constant than dimethylformamide (DMF), the relative pore flow in a given packing would be even higher.
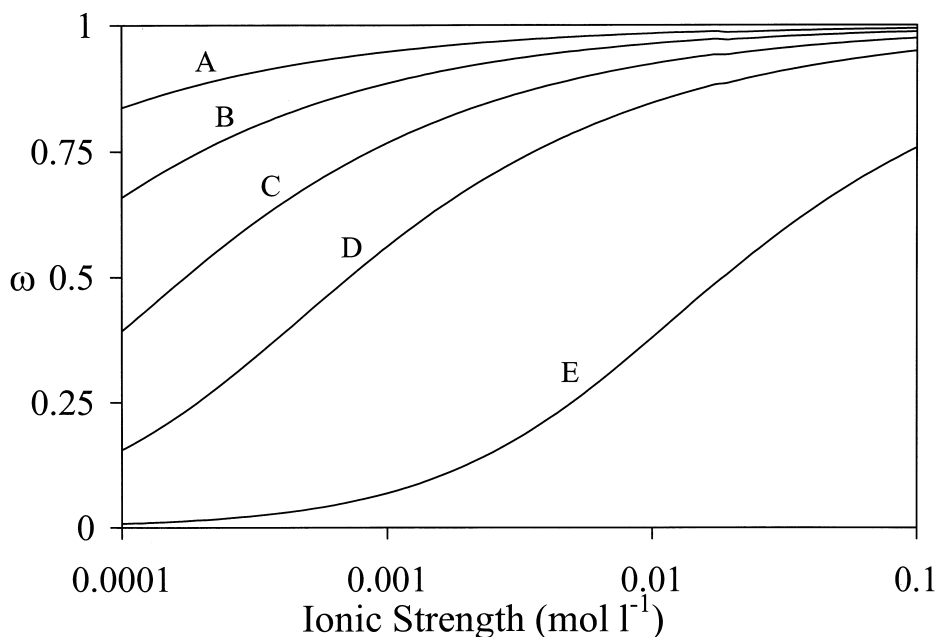
Fig. 2. Theoretical effect of the ionic strength on ω. Particle diameter: 5 μm; solvent: DMF; pore radius: (A) 2000 Å; (B) 1000 Å; (C) 500 Å; (D) 250 Å; (E) 50 Å.

2.2. Resolution

In a previous paper [9], it has been shown that the efficiency of SEC increases when a small pore flow is present. As a consequence, in ED-SEC two counteracting effects on resolution occur, both depending on the ionic strength. Firstly, the efficiency increases at higher salt concentrations due to the increased pore flow resulting in a faster mass transfer. This effect is counteracted by the decrease in resolution at higher ionic strengths due to the increase of ω and the associated decrease in retention window. Due to these effects, in practice three scenarios can occur depending on the pore radius of the particle used and the ionic strength of the buffer. Firstly, the most appreciated scenario occurs when the increased efficiency dominates the separation power of the system in which case ED-SEC will be superior to conventional pressure driven SEC. Secondly, the decrease in retention window is so large that the increased efficiency cannot balance this effect and a smaller separation power will be found. And finally the most unlikely scenario is that

both effects more or less compensate each other, resulting for ED-SEC having the same separation power as conventional pressure driven SEC.

The theoretical prediction of the effect of pore flow on the plate height in ED-SEC is still under development. However, it is possible to calculate the needed increase in theoretical plates in order to obtain the same resolution in ED-SEC systems with different ω values.

The relation between the number of plates and the resolution can be given by [10,17]:

$$N \sim \frac{R_s^2}{S^2} \tag{6}$$

in which R_s is the desired resolution and S is the mass selectivity of the chromatographic system and can be calculated using [17,18]:

$$S = \left| \frac{d \ln(R)}{d \ln(M)} \right| = \left| \frac{d \ln(R)}{d \ln(\lambda_{SEC})} \right| \cdot \left| \frac{d \ln(\lambda_{SEC})}{d \ln(M)} \right|$$

$$= b \left| \frac{\lambda_{SEC} d(R)}{R d(\lambda_{SEC})} \right| \tag{7}$$

Here, M is the mass of the analyte molecule, R is the retention ratio, b is a constant found in the Mark–Houwink description of the radius of a polymer as function of its molecular mass and often has a value between 0.5 and 0.6 for random coil molecules. λ_{SEC}, as before, represents the aspect ratio between the polymer radius and the pore radius of the packing.

The retention ratio R can be calculated using Eq. (1):

$$R = \frac{V_M}{V_M + KV_S + \omega(1-K)V_S} \tag{8}$$

Using the random sphere model for the intraparticle pore geometry, the value of K can be estimated by [13]:

$$K = \varphi^{(1+a\lambda_{SEC})^3-1}, \quad a = \frac{-2(1.32-\varphi)}{3\ln\varphi} \tag{9}$$

where φ stands for the intraparticle porosity of the porous material ($V_S/V_{particle}$) and often has a value between 0.6 an 0.7.

Now, S can be mathematically expressed as:

$$\frac{S}{b} = \frac{3a\lambda_{SEC}(1+a\lambda_{SEC})^2 KV_S\,(\omega-1)\ln(\varphi)}{V_M(1+\omega)+V_S K(1-\omega)} \tag{10}$$

This results in an extension of an expression by Giddings ([10] Eq. (27)).

The needed increase in plates in order to obtain the same resolution in ED-SEC with pore flow as in conventional SEC with no pore flow can now be calculated by:

$$\frac{N^{\omega\neq0}}{N^{\omega=0}} = \left(\frac{S^{\omega=0}}{S^{\omega\neq0}}\right)^2 \tag{11}$$

where $S^{\omega=0}$ represents the selectivity obtained in a system in which no pore flow occurs. In Fig. 3, the increase in plate number needed to compensate for the reduced retention window is displayed as a function of ω.

In CEC, compared to pressure driven chromatography for the same particles, a maximum increase in the obtained number of plates of about 3 can be expected. If this increase in efficiency also holds for ED-SEC, it can be concluded that ED-SEC works better than conventional SEC when ω has a value between 0 and 0.4. Higher values will possibly lead
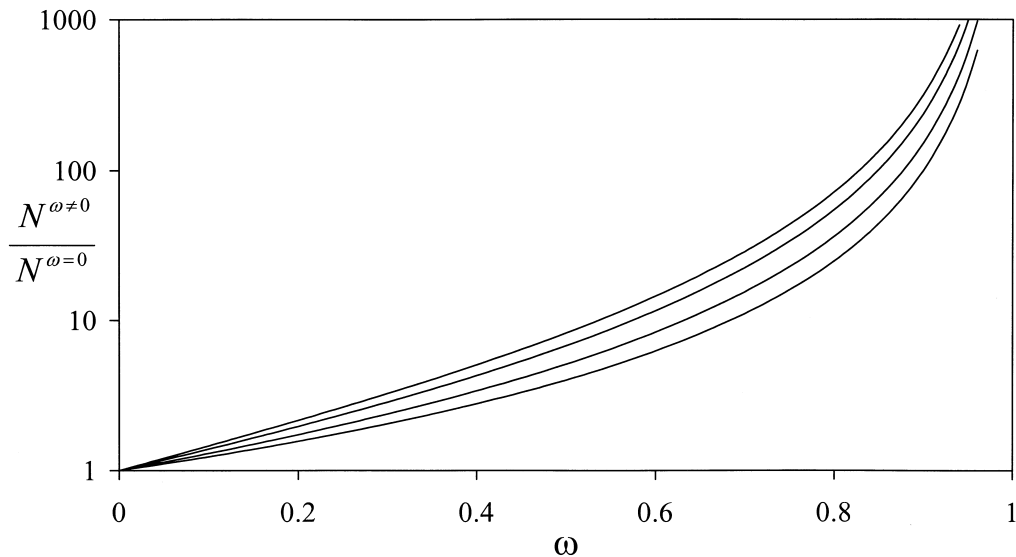

Fig. 3. Needed increase in plate number for constant resolution, $a=1.07$, $\varphi=2/3$. According to Eq. (11): top line: $\lambda_{SEC}=0.3$; second line: $\lambda_{SEC}=0.6$; third line: $\lambda_{SEC}=0.9$; lowest line: according to Giddings [10].

to higher efficiencies but the resolution may be lower due to the decreased retention window.

3. Experimental

3.1. Apparatus

The set-up for the CEC experiments consisted of a high-voltage supply (FUG, HCN 35-35000, Rosenheim, Germany), a laboratory-made injection timer, a UV detector (Linear Instruments, Reno, NV, USA) and two platinum electrodes to connect the power supply to the glass vials. The fused silica capillaries were 100 μm I.D.$\times$375 μm O.D. (Polymicro Technologies, Phoenix, AZ, USA) and had a total length of about 50 cm, of which 31 cm was filled with the particles. The whole set-up was placed in a plexiglass safety box. Samples were introduced electrokinetically at the anodic side (10 kV, 3 s). The chromatograms were digitally recorded using a 16 bits A/D board at 2.1 Hz (ADC-16, Keithley Metrabyte, Taunton, MA, USA). This frequency was chosen to avoid introduction of noise from the power grid. Before digitising, the detector signal was multiplied 100 times using a laboratory-made amplifier.

For the measurement of the capillary columns under pressure the same set-up was used but the high-voltage supply was replaced by a HPLC pump (Spectroflow 400, ABI, Ramsey, NJ, USA) and an injector (7010, Rheodyne, Cotati, CA, USA) with a 1.23-μl loop. After the injector a split (1:1000) was mounted to reduce the flow and injection volume to a nanoliter level.

3.2. Materials and chemicals

DMF was obtained from Acros (Geel, Belgium).

Lithium-bromide (LiBr) was obtained from Aldrich (Bornem, The Netherlands).

The polystyrene (PS) standards (with specified polydispersities ranging from 1.01 to 1.05) were obtained from different commercial sources and were dissolved in DMF at concentrations of 1 mg/ml. The standard solutions were prepared weekly and in most experiments a small molecular mass marker (toluene) was added to these polystyrene solutions.

All measurements were performed in triplicate and the average value found was used in the graphs and in the calculations.

The normal-phase packings used were Nucleosil 5 μm (Macherey-Nagel, Düren, Germany), with specified 1000, 500, 300 and 100 Å pores. Prior to use all particles were characterised using mercury porosimetry. Table 1 shows the obtained results.

As can be seen from Table 1 there are sometimes large differences between the specified and the measured pore radii. One has to keep in mind that the measured pore radius has to be multiplied by two, to find the corresponding pore diameter in Å. Especially the particles with 300 and 1000 Å pores show the largest deviations between the measured and the stated value. The first has even a bimodal pore distribution with a mean pore radius of 190 Å. The latter has a pore radius which is twice as large as stated by the supplier. In all further calculations the measured values were used.

3.3. Column preparation

On a 75 cm$\times$100 μm I.D. fused silica capillary, first a detection window was made by burning off of the protective polyamide layer by means of a heating filament. Next the capillary was connected to a 0.5 μm in-line solvent filter (Swagelock, Solon, OH, USA) and the other side to a slurry reservoir (a

Table 1
Measured data of the silica particles used

Sample (specified particle size, pore diameter)	Specific area (m^2/g)	Pore volume (m^3/g)	Pore radius (Å)
5 μm, 100 Å	348	0.982	50
5 μm, 300 Å	106	0.766	80 and 300
5 μm, 500 Å	77	0.794	300
5 μm, 1000 Å	14	0.693	1000

200×1 mm I.D. stainless steel tube). The slurry was made by suspending 40 mg silica in 1 ml methanol and this mixture was sonicated for 20 min. The slurry was pumped into the capillary using water as displacer solvent. During packing the pressure was increased to 400 bar in 2 min. While pumping, the capillary was placed in an ultrasonic waterbath for 1 min, leading to some bed compression. Next the column was flushed for another 30 min. After switching off of the pump, the pressure was allowed to release in 5 h. Next the slurry reservoir was removed and the column was flushed with a water–acetonitrile (80:20) mixture containing 5 mmol l^{-1} Na_2CO_3 at 360 bar for 30 min. While flushing the column with this mixture, the frits at the injection and detection side were made by heating the column locally over a length of 2 mm for 10–15 seconds at about 450°C. At the injection side, a frit of 6 mm was made in order to prevent dislodging of the frit. After preparation of the frits, the redundant piece of the capillary at the injection side was removed by cutting, whereas the remaining particles at the detection side were flushed away.

After flushing the capillary with DMF, the capillary was installed in the CEC system.

3.4. Retention behaviour of PS solutes

To investigate the effect of the pore size and the ionic strength on the retention of solutes in ED-SEC, PS standards were injected together with a small marker molecule (toluene) and the observed retention times were converted to τ values by dividing the retention time of the polymer with the retention time of the small marker.

In order to calculate the magnitude of pore flow under ED conditions, the polymer retention in the capillaries under pressure drive was also measured.

4. Results and discussion

4.1. Polymer retention in columns packed with 5 μm particles.

From previous results [9] there was evidence that a significant pore flow occurs under ED conditions which results in an increasing intraparticle mass transfer in SEC and higher efficiencies. Because the slow intraparticle mass transfer is a major contributor to peak broadening when high molecular masses are analysed, as a result of their low molecular diffusion coefficients, the highest improvement in efficiency can be expected with packings with large pore sizes.

For that reason, first a column packed with 5 μm, 1000 Å particles was tested in the ED-SEC system. With this packing under ED conditions the polymers were hardly separated due to a very small retention window, in contrast to pressure driven SEC where a wide retention window is found as can be seen in Fig. 4. This observation indicates that under ED conditions a large flow through the pores is generated. The flow through the pores is affected by the ionic strength which determines the double layer thickness.

Decreasing the ionic strength from 1 mmol l^{-1} LiBr to 0.1 mmol l^{-1} resulted in a slightly larger retention window. Unfortunately, even at this low salt concentration, the retention window appears to be rather small ranging from 0.88 to 1, whereas under pressure driven conditions the window ranges from 0.64 to 1. Diminishing further the salt concentration was not successful. The retention of polymers became irreproducible and the peak shape deteriorated and high mass polymers were not even eluted from the column.

Although the observed phenomenon ruins the advantages of ED-SEC the behaviour is in line with the proposed retention behaviour of solutes as given in the theoretical section. There, it was argued that using wider pore packing material and higher ionic strengths, the retention window should diminish. It remains to be seen what the optimal maximum pore size and ionic strength will be to make ED-SEC an attractive alternative for conventional SEC. In order to determine this, the retention behaviour of some packings with smaller pore sizes was investigated.

Fig. 5 shows the behaviour on a 500 Å packing. As can be seen, the retention window under ED conditions on this packing is larger than with the 1000 Å packing. Also, as expected, the retention window is again affected by the ionic strengths.

Only with the lowest ionic strength, the retention window is large enough to be applicable in practice for the separation of polymers as is illustrated in Fig. 6. From Fig. 5 it can be concluded that the ED-SEC
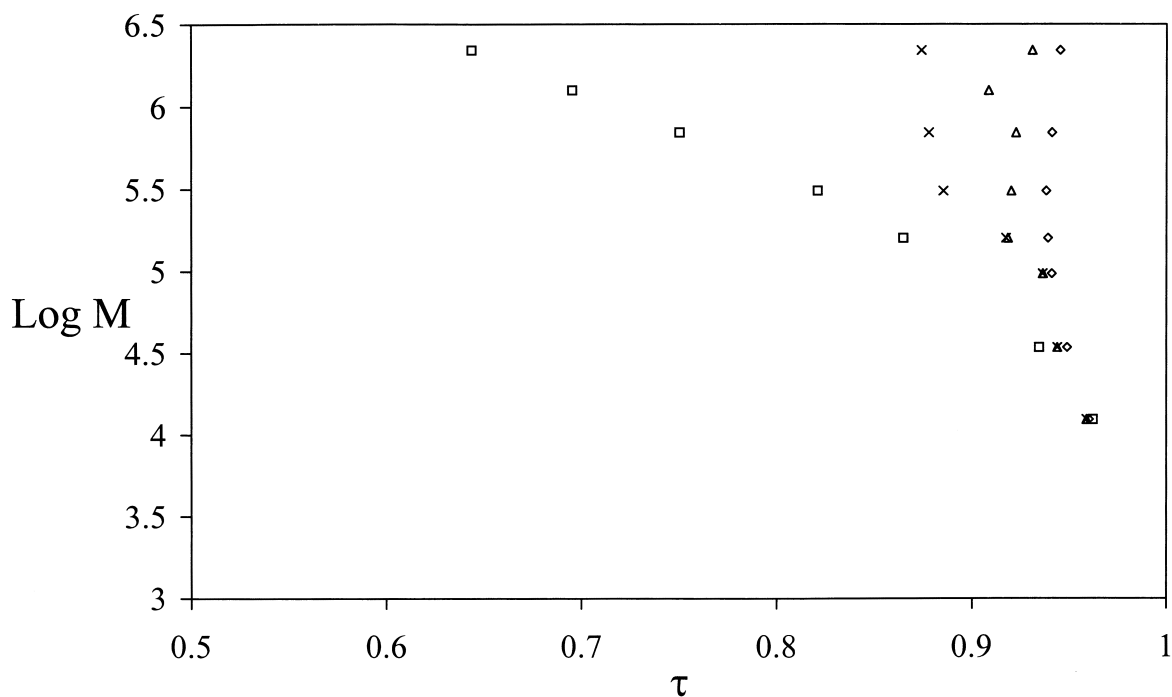
Fig. 4. Polymer retention with Nucleosil 5 μm, 1000 Å. □: Pressure drive; ×: electro drive, 0.1 mmol l^{-1} LiBr; △: electro drive, 0.5 mmol l^{-1} LiBr; ◊: electro drive, 1 mmol l^{-1} LiBr; field strength: 10 kV.

technique is applicable up to molecular masses of about $2 \cdot 10^6$.

Fig. 7 shows the retention behaviour on a 100 Å packing. The retention windows are significantly larger than for the wide pore packings.

As can be seen there is a stronger dependence of the retention window on the ionic strength. The reason for this is that the electric double layer at low ionic strength is relatively thick, compared to the pore radius. This causes a reduction of the pore flow and consequently leads to a larger retention window. Even at high ion concentrations (20 mmol l^{-1} LiBr), the window is large enough to be used for polymer separations, as is clearly demonstrated by the chromatogram shown in Fig. 8.

Comparing the chromatograms in Figs. 6 and 8, shows that the peak width of toluene is significantly larger in Fig. 6. This can be attributed to the difference in extra-column peak broadening in the injection frits. It was observed that the frits, all produced in the same manner, gave different amounts of extra-column peak broadening. The

porosity of the frit depended strongly on the type of packing material used. In one case the efficiency of the column could be doubled by removing 3 mm of the injection frit. Unfortunately, this frit quickly dislodged.

Fig. 9 shows the effect of the ionic strength on the efficiency on the 100 Å packing. As can be seen, the ionic strength has a strong effect on the efficiency of the solutes. For toluene, the plate height decreases about a factor of 3 when increasing the ionic strength. This is not caused by the occurrence of pore flow but by the fact that at the low ionic strength the retention time was two times as large. The observed dispersion of toluene can therefore be attributed partly to longitudinal diffusion. For polymers, the plate heights are significantly larger at low ionic strengths. At low salt concentration hardly any pore flow occurs due to the double layer overlap. This results in a slow mass transfer and consequently in very large plate heights. With increasing ionic strength, the pore flow increases which leads to a faster mass transfer and higher efficiencies. As can
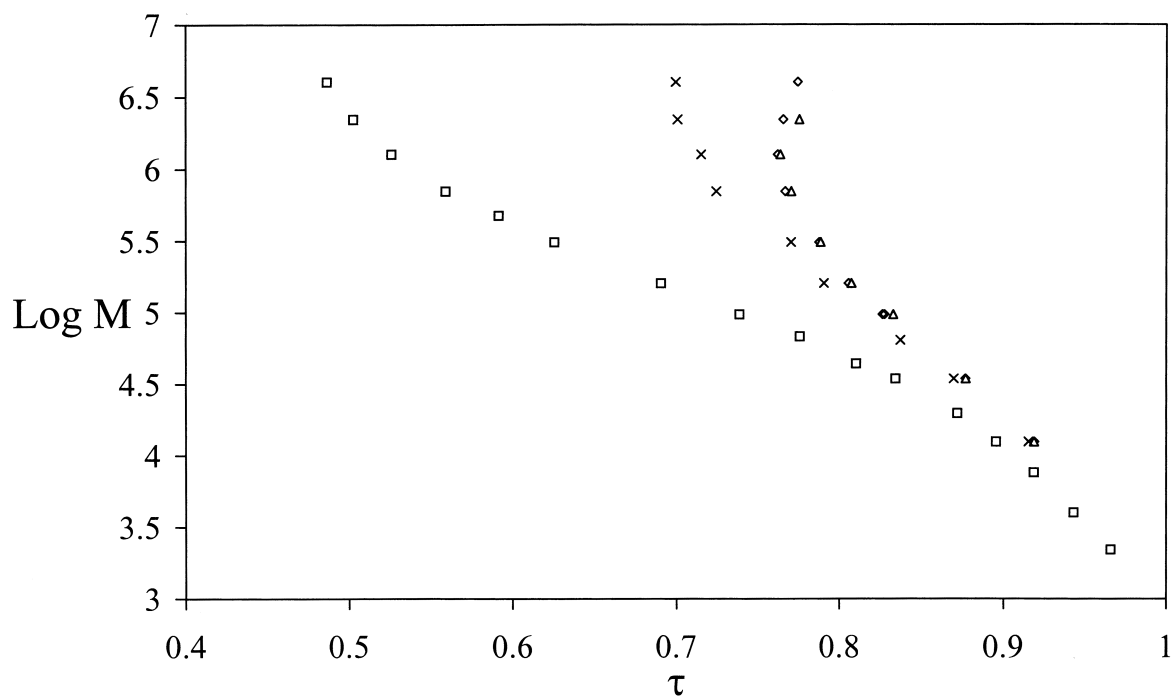
Fig. 5. Polymer retention with Nucleosil 5 μm, 500 Å. □: Pressure drive; ×: electro drive, 0.1 mmol l^{-1} LiBr; △: electro drive, 0.5 mmol l^{-1} LiBr; ◊: electro drive, 1 mmol l^{-1} LiBr; field strength: 10 kV.
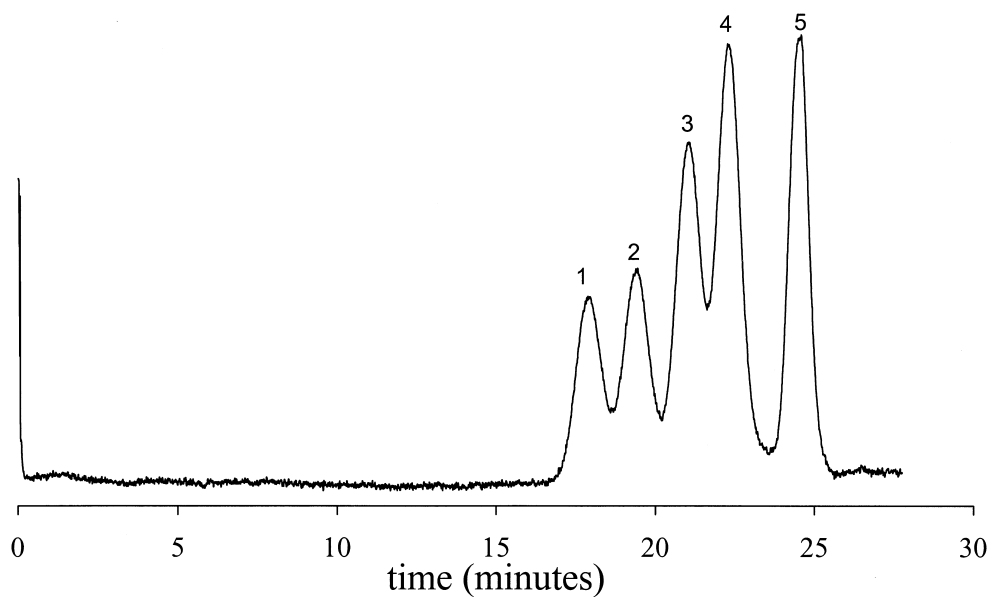

Fig. 6. Chromatogram of a test mixture of PS using Nucleosil 5 μm, 500 Å, 0.1 mmol l^{-1} LiBr. 1=PS 310·10^3; 2=PS 97.2·10^3; 3=PS 34.5·10^3; 4=PS 12.5·10^3; 5=toluene; field strength: 10 kV.
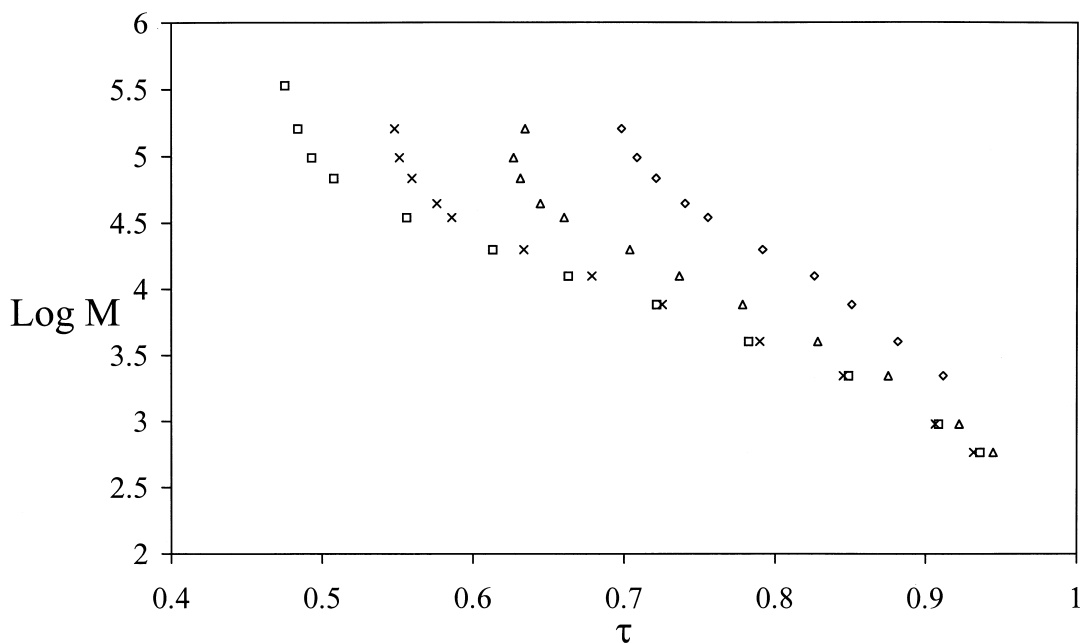
Fig. 7. Polymer retention with Nucleosil 5 μm, 100 Å. □: Pressure drive; ×: electro drive, 1 mmol l^{-1} LiBr; △: electro drive, 10 mmol l^{-1} LiBr; ◊: electro drive, 20 mmol l^{-1} LiBr; field strength: 10 kV.

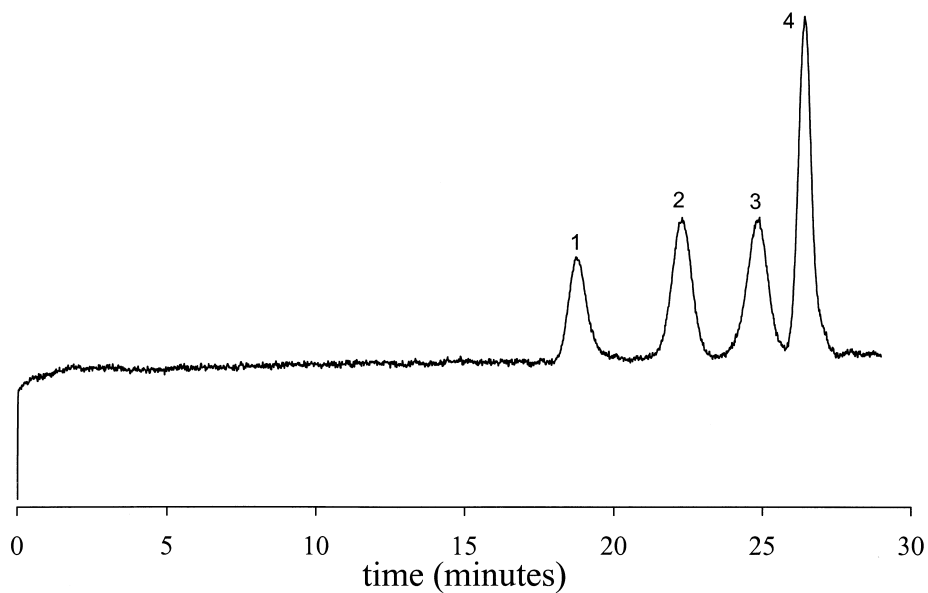Fig. 8. Chromatogram of a test mixture of PS using Nucleosil 5 μm, 100 Å, 20 mmol l^{-1} LiBr. 1=PS 68·10^3; 2=PS 7.6·10^3; 3=PS 950; 4=toluene; field strength: 10 kV.
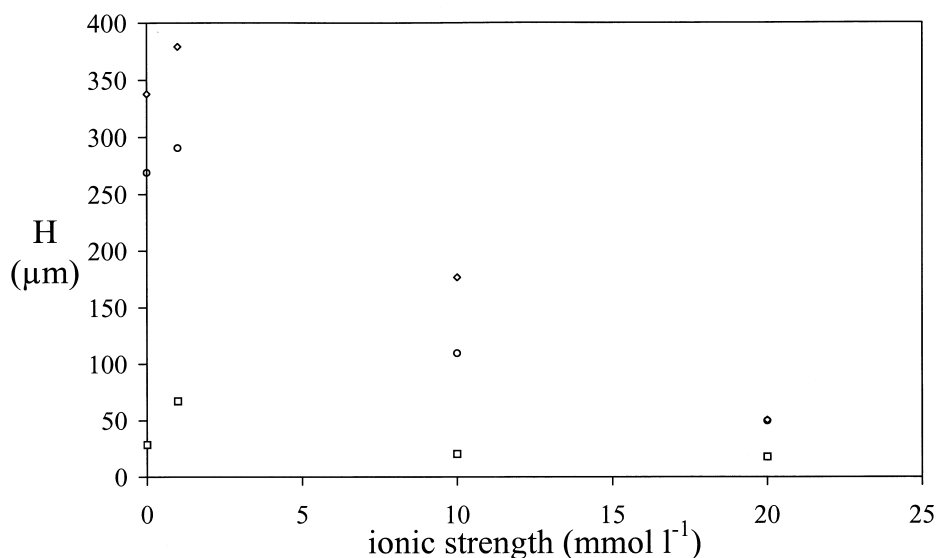
Fig. 9. Plate height versus ionic strength in Nucleosil 5 μm, 100 Å, field Strength: 10 kV. □: Toluene; ◊: PS 950; ○: PS 43.9·10³. At an ionic strength of zero, the *H* values under pressure driven conditions are given at comparable velocities; field strength: 10 kV.

be seen from Fig. 9 the plate height decreases by a factor of 8 when increasing the ionic strength from 1 mmol l^{-1} LiBr to 20 mmol l^{-1} LiBr. The obtained results correspond well with the expected behaviour outlined in the theoretical section which predicts a decrease of the plate height with increasing salt concentration due to increased mass transfer by pore flow.

4.2. Comparison of experimental and theoretical results

The results obtained from the retention experiments were used to calculate the ω. This was done by first fitting the data from the pressure driven system according to Eq. (1) with a $\omega = 0$ and abstracting the apparent values of V_S and V_M. These values were then used to fit the data under electro drive to calculate ω.

This procedure might fail when under pressure drive the retention of the polymers is not governed solely by SEC but also by hydrodynamic effects (HDC). This effect occurs when the molecules become too large to fit in the pores. The SEC theory predicts then that they will all co-elute. However, in practice this is often not found and an additional separation occurs induced by the flow profile in the

interparticle space. For geometrical reasons large solutes are excluded from the slow streamlines close to the wall and thus migrate faster than smaller solutes which can approach the wall closer. This hydrodynamic effect causes that the migration rate is the result of a mixed mechanism viz. SEC and HDC [19–22]. The HDC effect is small when the aspect ratio (the ratio of the radius of the analyte and the interstitial channel) is small but will become significant for large solutes. Thus, this HDC effect widens the elution window. Under electro drive the flow profile is assumed to be flat and no additional separation of solutes in the interparticle space by HDC is expected as long as the electric double layer thickness is small in comparison with the channel radius. Using 5 μm packing particles, this will always be the case. This means that totally excluded solutes migrate with the same velocity.

The occurrence of a hydrodynamic effect makes it more difficult to estimate V_S and V_M for packings with large pores. If the HDC effect is not recognised, this will lead to an overestimation of V_S because the apparent retention window is larger than it should be. This problem could be solved by determining the molecular mass position at which the retention under ED conditions no longer changed with increasing mass. This maximum molecular mass can then be

used to define the real exclusion limit in pressure driven SEC without HDC effects and accurate values for V_S and V_M can be determined.

Table 2 shows the values of ω, estimated from the measured retention curves and the theoretical values (in parentheses) according to Eq. (4).

As can be seen, most of the estimated ω values are somewhat lower than expected theoretically. However, the general trend, that ω increases with increasing ionic strength and pore size, supports the theory rather well.

The found deviation between theory and practice can be attributed to several reasons: (a) the pore size is a distribution instead of a fixed number. Therefore, there will be pores with higher and lower flows. However, this should lead to even higher ω values because larger pores contribute more to the overall mean pore flow than the smaller ones. (b) Small inaccuracies in the pore size measurements will lead to large errors in ω. (c) Not all the pores may be regarded as open capillaries. Some might be obstructed, with the result that a smaller portion of the total pore volume will have solvent flow under ED conditions. This will result in lower ω values. (d) Another cause might be the fact that the salt is not dissociated completely. In organic solvents with low dielectric constants, most salts do not dissociate completely. The ionic strength is that case lower than expected and this leads to lower ω values.

From the presented results with the 5 µm particles it can be concluded that ED-SEC will be limited to molecular masses below $2 \cdot 10^6$. However, it can be expected that highly efficient separations can be obtained when small particles (<2 µm) with pores up to 100 Å are used. It can be noted that the occurrence of pore flow, which hampers the general use of ED-SEC is very beneficiary for electrically driven liquid chromatography. For this separation method, the pore flow will considerably speed up the mass transfer of solutes between the mobile phase and the stationary phase inside the pores and will result in larger efficiencies as was demonstrated recently by Li et al. [23].

The ED-SEC experiments described in this work may be helpful to determine the pore flow in such chromatographic systems.

5. Conclusions

The presented results show that in ED-SEC, pore flow has two counteracting effects. It increases the efficiency due to faster mass-transfer but decreases the retention window. Only in those cases where the pore flow is small, which occurs when the ionic strength and the pores are small, ED-SEC can be an alternative method for standard SEC. However, the application is limited to the separation of polymers with masses below $2 \cdot 10^6$. The proposed theoretical description of polymer retention and pore flow is in good qualitative agreement with the experimental results and can also be used to calculate the amount of pore flow in other CEC systems. It was found that with increasing ionic strength, the column efficiency of polymers increased considerably due to the higher pore flow. This finding suggests that the observed increase in efficiency in CEC with porous particles cannot be attributed only to the plug flow profiles but also to the increased intraparticle mass transfer.

Table 2
Estimated and theoretical ω values

Packing type	V_S/V_M (Å)[b]	R_{pore}	ω				
			0.1 mmol l^{-1}	0.5 mmol l^{-1}	1 mmol l^{-1}	10 mmol l^{-1}	20 mmol l^{-1}
5 µm, 100 Å	1.0	50			0.1 (0.07)	0.25 (0.37)	0.4 (0.50)
5 µm, 300 Å[a]	0.67	190	0.12 (0.10)		0.4 (0.46)	1 (0.80)	
5 µm, 500 Å	0.68	300	0.25 (0.21)	0.40 (0.50)	0.41 (0.62)		
5 µm, 1000 Å	0.54	1000	0.50 (0.65)	0.70 (0.84)	0.80 (0.88)		

[a] Data from the previous paper [8].
[b] R_{pore} = Pore radius measured by mercury intrusion.

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