Capillary electrophoresis for the characterization of synthetic polymers
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Chapter 6

Capacitively coupled contactless conductivity detection of neutral synthetic polymers in non-aqueous size-exclusion electrokinetic chromatography

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Abstract

In this chapter it is shown that capacitively coupled contactless conductivity detection (C4D) can be used for the detection of synthetic polymers in size-exclusion electrokinetic chromatography (SEEC). Polystyrene standards that were used as model compounds were separated on a capillary column packed with porous 10 μm silica particles with an electrokinetically driven mobile phase, and detected by C4D and UV detection simultaneously. Detection limits with the C4D system were in the order of 5 g l⁻¹. Mass-calibration curves for polystyrene were constructed. Satisfactory results were obtained for the linearity, the run-to-run repeatability (< 0.2% for the relative retention and < 4% for the peak area) and the robustness of the detector.

One of the major issues in this preliminary study was to investigate the origin of the peaks observed for the polystyrene standards. The effect of the molar mass of the polystyrene standards on the sensitivity was small. Therefore, the signals obtained cannot be explained as the result of an increased viscosity and a decreased solution conductivity of the solute zone. An alternative hypothesis is suggested, and recommendations for further research are given.
Introduction

While in the last decade the use of miniaturized liquid chromatography (LC) systems in the (bio)analytical and pharmaceutical industries increased radically, in the field of polymer analysis only a limited number of research groups is working on miniaturized separation systems such as size-exclusion chromatography (SEC) with microbore or capillary columns [1-5] or size-exclusion electrochromatography (SEEC) (see e.g., ref. 6). One of the reasons to switch from a conventional system to a reduced scale set-up is the ability to analyse smaller sample volumes. In polymer analysis the smaller sample volume is an issue only in exceptional cases [7]. Hyphenation with mass spectrometry [2, 3] or with another separation technique in a multidimensional system [4] is a more important argument for miniaturization.

Detection options for (synthetic) macromolecules in micro-scale SEC systems are still limited. In most applications micro-LC systems are combined with an UV detector, which limits their use to specific polymers. Therefore, there is still a need for ‘micro detection techniques’ that are sensitive for synthetic macromolecules. A refractive index detector has been developed for small-scale SEC [8], but it is not (yet) commercially available. Another option is to use a miniaturised evaporative light scattering detector, as has been done in capillary reversed-phase chromatography [9].

Capacitively coupled contactless conductivity detection (C^4D) for capillary electrophoresis was introduced by Zemann et al. in 1998 [10]. A few months later Fracassi da Silva and do Lago [11] presented a similar detection system. They termed the new contactless conductivity mode oscillometric detection. Modifications of these designs have been proposed to provide a high sensitivity for the detection of various ionic species [12]. Limits of detection reported were at a low-ppb level for small ions. Recently, a miniaturized contactless conductivity detection cell was developed, which showed a similar sensitivity as the conventional cells [13]. This micro-cell offers the advantage that it can be placed at any point along the capillary in any commercial capillary cassette.

A C^4D set-up is basically composed of two electrode rings on the outside of the capillary that act as capacitors. To monitor the conductance of the solution in the capillary over the detection gap (the distance between the electrode rings) an capacitively (ac) voltage, generated by an oscillator, is applied on the inlet electrode causing a current through the background solution, which is picked up by the second electrode and amplified, rectified and recorded [14]. When an analyte zone with conductivity different from that of the background electrolyte passes the detection gap, a change in the signal over the amplification unit will be measured. The response of the detector is related to the
displacement of background-electrolyte ions by the solute ions, which is determined by their effective charge and mobility. In general, the highest sensitivity is obtained with the highest mobility difference between the sample and background ions. When neutral analytes are present in the solute zone, electrophoretic displacement of the background ions does not occur. However, it has been shown that aliphatic alcohols, separated by micellar electrokinetic chromatography (MEKC), can be detected with C4D [15]. The principle of the conductivity response was unclear. According to the authors, it might be based on the effect of the dielectric constant (ε) of the analytes, on a change of the micelle volume, or on the influence of the solution viscosity on the mobility of the background ions. The latter effect could be the basis for the use of C4D for the detection of synthetic polymers.

In this chapter, preliminary experiments on the application of C4D for the detection of neutral synthetic polymers are described. Size-based separations of polystyrene standards were performed by SEEC with simultaneous on-capillary UV and conductivity detection. Analytical performance parameters have been established, and the influence of the molar mass of the polymers on the detector response was studied.

Experimental

Chemicals and materials

Narrow polystyrene standards with molar masses (MMs) between 2,100 and 675,000 Da were purchased from different manufactures (Polymer Laboratories, Heerlen, The Netherlands; Machery-Nagel, Düren, Germany and Sigma-Aldrich, Steinheim, Germany). All standards had polydispersities < 1.1, as specified by the suppliers. All other chemicals used were of analytical-grade purity and obtained from certified suppliers. Sample solutions of polystyrenes were prepared in N,N-dimethylformamide (DMF) at concentrations of 5 – 50 g l⁻¹. Toluene used as a marker for the total eluent volume was added to the sample solutions at a concentration of 0.9% (v/v).

Fused-silica capillaries of 100 μm I.D. × 375 μm O.D. were purchased from Polymicro Technologies (Phoenix, AZ, USA). The unmodified silica particles Nucleosil 300-10 used as packing material were obtained from Machery-Nagel. The particles had a nominal diameter of 10 μm and nominal pore diameters of 300 Å.
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C^4D set up

Figure 6.1 shows a picture of the C^4D sensor cell used in this study. The design of the sensor cell and the electronic components were similar to the detector set-up described by Mayrhofer et al. [14]. Two cylindrical electrodes glued to Perspex holders fitted the capillary column and were connected with the oscillator or amplifier and rectifier. A copper-foil with a hole slightly wider than the outside diameter of the capillary was placed vertically between the electrode holders and connected to ground, to prevent capacitive leakage between the electrodes. Even though the distance between the electrodes could be varied, in most experiments a detection gap with a width of approximately 1 mm was used. The oscillator produced a sine or square wave in the frequency range of 2.5 – 200 kHz, with an amplitude variable up to 20 V peak-to-peak. In most cases, the input signal applied was a square wave with a frequency of 50 kHz and amplitude of 8 V. The aluminium C^4D housing was placed in a modified capillary cartridge to accommodate both the cell and the electrical connections as described elsewhere [16]. Data acquisition and processing was carried out with Maxima software of Waters Chromatography (Milford, MA, USA).

Figure 6.1 C^4D cell including (1) two electrodes with a detection gap of 1 mm, (2) grounded copper foil, (3) capillary column, (4) aluminium housing, (5) connector oscillator, (6) connector amplifier and rectifier.
SEEC experiments were performed on an Agilent CE system (Waldbornn, Germany) equipped with a diode-array detector. The Agilent Chemstation software was used for control of the instrument and for data acquisition of the UV-absorbance detection, which was carried out at a wavelength of 260 nm. The eluent consisted of DMF containing $10^{-4}$ M LiCl. Before each series of experiments the column was flushed with eluent for 10 min at an inlet pressure of 10 bar, followed by electrokinetic flushing at 15 kV for 15 min. Injections were performed electrokinetically typically at 15 kV for 5 s. Separations were performed at a voltage of 15 kV. During the separations, a high pressure (10 bar) was applied on both ends of the capillary column in order to prevent gas-bubble formation. Separations were carried out at a temperature of 25°C.

For the interpretation of the chromatograms a home-written Excel program was used. The program included baseline construction, translation of the time axis in a molar mass axis using calibration plot data, and the calculation of the centralized moments of the peaks.

**Column preparation**

Fused-silica capillaries were packed using a slurry packing system as described previously [17]. At one end of a capillary a temporary frit was prepared by tapping it into a pile of dry silica particles and sintering the particles in place with the gas flame of a lighter. A slurry of Nucleosil 300-10 silica particles with a concentration of 50 g l$^{-1}$ was prepared in methanol. At a pressure of 600 bar the particles were driven into the capillary using methanol as the displacement liquid. The high pressure was maintained for about 15 min, after which the pressure was reduced and the excess of slurry solution in the reservoir was removed. The column was then flushed with water at 400 bar for at least 1 h. For preparing the permanent frits the pressure was reduced to 100 bar. In- and outlet frits were sintered at a distance of 250 mm from each other using a hot metal strip device. The excess of silica in the capillary beyond the outlet frit was removed by flushing the column with water at a low pressure. The C$^{4}$D cell was placed just after the outlet frit of the column. A UV detection window was prepared after the conductivity cell at a distance of 52 mm from the outlet frit. After installation in the CEC system, the column was flushed with the eluent by an external high pressure of 10 bar for 1 h. Electrokinetic conditioning was carried out by a ramped voltage gradient up to 20 kV.
Results and discussion

C\textsuperscript{4}D of polystyrenes

To investigate the suitability of the C\textsuperscript{4}D as an indirect viscosity detection system for (neutral) polymers it was required to combine the detection unit with a separation system. SEEC is an electrokinetic separation method in which the electro-osmotic flow (EOF) drives the mobile phase through a capillary column packed with porous material. It has been shown that SEEC can separate polystyrenes according to size [17]. The system used consisted of a capillary column of 100 \mu m I.D. packed with 10 \mu m bare silica particles and an eluent consisting of 10^{-4} M LiCl in DMF. In the original published method detection of polystyrene was carried out by measuring the UV absorbance at 260 nm. The UV signals for polystyrene standards were independent of the degree of polymerisation of the polymers. The C\textsuperscript{4}D set-up could be easily combined with the original SEEC system, which made it possible to compare the C\textsuperscript{4}D signal of polystyrene with its UV response. Figure 6.2 shows the SEEC separation, with on-line monitoring by C\textsuperscript{4}D and UV detection, of a polystyrene standard with an average molar mass of 30,000 Da at a concentration of 20 g l\textsuperscript{-1}. The times-axis of the UV signal was adapted in the figure to correct for the difference in the positions of the conductivity and UV detectors along the capillary.

It can be seen that the peak detected by C\textsuperscript{4}D was clearly related to the elution of the polystyrene standard. The C\textsuperscript{4}D signal close to \( t_0 \) can have been caused by the unretained marker (toluene) or by the matrix of the sample solution. An instability of the conductivity signal was observed at the start of all runs. This instability could not be related to the presence of specific analytes or to a specific experimental parameter, including the type of injection, the applied voltage or the pressure on the system.

Both conductivity and UV peak heights were found to be linear with the sample concentration. Chromatograms obtained with samples of the polystyrene standard of 18,700 Da, at concentrations of 5 - 50 g l\textsuperscript{-1}, are shown in Figure 6.3. For clarity, in this figure the time axes of the chromatograms were standardized by translation into the retention factor \( \tau \), which is defined as the retention volume of the polymeric compound divided by that of the unretained solute. The concentration-calibration curves for peak heights and peak areas were linear with values for \( R^2 > 0.99 \) for both the C\textsuperscript{4}D and the UV detector.
Figure 6.2 SEEC chromatograms of polystyrene 30,000 Da simultaneous detected by C^4D and UV absorption. Conditions: column: Nucleosil 300-10 (250 mm x 100 μm I.D.), eluent: 10^{-4} M LiCl in DMF, injection 15kV x 5s, voltage 15kV.

Figure 6.3 Separations of polystyrene 18,700 at sample concentrations of (1) 5 g l^{-1}, (2) 10 g l^{-1}, (3) 15 g l^{-1}, (4) 20 g l^{-1}, (5) 30 g l^{-1}, (6) 40 g l^{-1}, (7) 50 g l^{-1}. 
Experiments on the stability and repeatability of the detection signal were performed. Samples of polystyrene 18,700 Da at concentrations of 20 and 50 g l\(^{-1}\) were injected 7 times each. Figure 6.4 shows the chromatograms obtained with the polystyrene samples at 50 g l\(^{-1}\). As can be seen in this figure, the conductivity response was stable.

The coefficient of variation (%CV) of the relative retention $\tau$, peak heights and peak areas obtained with C\(^4\)D in this repeatability study were compared with the results obtained with UV detection (Table 6.1). The variations in $\tau$ obtained with conductivity detection were slightly higher than that with UV detection. The reason for this may be that the determination of $t_0$ in the C\(^4\)D signal was less straightforward than in the UV signal. With both C\(^4\)D and UV detection spreading in the values for the peak heights and peak areas of polystyrene were < 5%.

<table>
<thead>
<tr>
<th>Concentration (g l(^{-1}))</th>
<th>$\tau$</th>
<th>peak height</th>
<th>peak area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C(^4)D</td>
<td>UV</td>
<td>C(^4)D</td>
</tr>
<tr>
<td>20</td>
<td>0.17</td>
<td>0.03</td>
<td>2.7</td>
</tr>
<tr>
<td>50</td>
<td>0.09</td>
<td>0.04</td>
<td>2.4</td>
</tr>
</tbody>
</table>

Figure 6.4 Chromatograms of a repeatability study (n=7) obtained with polystyrene 18,700 at a concentration of 50 g l\(^{-1}\).
Molar-mass calibration curves

For the construction of a molar-mass calibration curve, polystyrene standards with average molar masses between 2,100 and 675,000 Da were injected. All sample solutions had a polymer concentration of 20 g l\(^{-1}\). With low-MM polystyrenes (< 212,000 Da) symmetrical peaks were obtained, while for the polystyrenes that eluted close to the exclusion limit, peak splitting was observed (see Figure 6.5).

Molar-mass calibration curves were constructed using the relative retention \(\tau\) from both the C\(^4\)D and the UV signals. Figure 6.6 shows that the shapes of the curves are similar. The differences between the two curves may again be explained as the result of an inaccurate determination of \(t_0\) in the C\(^4\)D signal. Both curves could be used to determine the polydispersity of the polystyrene standards. For the calculations of the polydisperisty a homemade program developed in Excel was used. As an illustration, Figure 6.7 shows the molar-mass distribution as calculated from the C\(^4\)D and UV chromatograms of polystyrene 30,000. With the C\(^4\)D system a polydispersity value of 1.06 was found for the polymer standard, while with UV detection a slightly higher value was determined (1.08).
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Figure 6.6  Molar-mass calibrations curves for polystyrene obtained with C\textsuperscript{13}D (•) and UV-detection (○) after separation with SEEC.

Figure 6.7  Polydispersity of polystyrene 30,000 determined by C\textsuperscript{13}D and UV detection after separation by SEEC as shown in Figure 6.2.
Origin of the C^4D signal

The conductance of an electrolyte solution (at a constant temperature) is related to the charge and concentration of the ionic analytes in solution and to their electrophoretic mobility [18]. A possible explanation for the observed sensitivity of C^4D for neutral polymers could be that the higher viscosity of the solution in the polymer-containing zone affects the mobility of the background ions, and therewith the response on the detector. Since the viscosity of a polymer solution is related not only to the concentration, but also to its size, it was expected that the C^4D response would depend on the molar mass of polystyrene. To test this hypothesis, the possible effect of the viscosity on the conductivity response was studied with separations of polystyrene standards with average molar masses in a wide range between 2,100 and 675,000 Da (as shown in Figure 6.5). In order to correct for variations of the injection volume, the sample concentration and peak dilution, the peak heights for the polystyrene standards as obtained with C^4D were divided by the UV peak heights. The relative peak heights were plotted against the molar mass of the polymers in Figure 6.8. The error bars in the figure indicate the variation of the results obtained in different series of experiments performed over a time period of several days. For the low-MM polystyrenes the magnitude of the conductivity signal increased with increasing molar mass of polystyrene. However, the dependency of the conductivity signal on the polymeric size was lower than might be expected from the relation

![Figure 6.8 Plot of the C^4D peak heights corrected with the UV peak heights versus the logarithm of the molar mass of the polymers.](image-url)
between the intrinsic viscosity and the molar mass observed for polystyrene in DMF (\([\eta] = 0.0318 \times \text{MM}^{0.603} [19]\)). For the higher-molar-mass polystyrene standards even a decrease of the conductivity signal with the size of the polymeric molecules was found. Apparently, the hypothesis that the C\(^4\)D signal is related to changes in (bulk) viscosity of the solution is not correct.

**Conclusions, remarks and suggestions for further research**

The experimental work performed so far has shown clearly that C\(^4\)D can be used to monitor the elution of neutral synthetic polymers from a micro-separation system. Repeatable signals were obtained, and a molar-mass calibration curve could be constructed. However, fairly high sample concentrations (in the order of grams per litre) were required to obtain useful results. Therefore, the main prospect of C\(^4\)D in polymer analysis (for the characterization of neutral synthetic polymers) will be for separation systems in the capillary or chip format, when other detection possibilities are not available. In these systems the separations are usually performed with electrokinetic driven mobile phases, although the pressure driven mode could be used either. To demonstrate that the C\(^4\)D set-up is also useful in this mode a separation of polystyrene 7,000 was carried out using an inlet pressure at 10 bars (Figure 6.9). The polystyrene standard could clearly be detected, although the baseline was rather unstable.

![Figure 6.9](image-url) **Figure 6.9** Pressure-driven µSEC with C\(^4\)D of polystyrene 7,000 Da.
A further optimisation of the detector performance, e.g., by changing the input signal, was hampered by a lack of understanding of the mechanism of the detector response. Clearly, the change of the solution viscosity in polymer containing zones was not the main cause of the appearance of peaks. An alternative hypothesis for the C^4D response is that the polymeric compounds affect the electrical double layer at the surface of the fused-silica capillary wall, as has been suggested in another study with neutral analytes [15]. To study this possibility, capillaries of different materials such as poly(etheretherketone) (PEEK) could be compared. Moreover, experiments with different types of polymers could shed light on this unresolved question on the mechanism of C^4D sensitivity for neutral compounds.

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References