

Contactless conductivity detection of synthetic polymers in non-aqueous size-exclusion electrokinetic chromatography

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Abstract

A capacitively coupled contactless conductivity detection (CCD) system has been applied for the detection of neutral synthetic polymers in capillary size-exclusion electrokinetic chromatography (SEEC). Polystyrene standards, that were used as a model compounds, were separated on a capillary column packed with porous 10 μm silica particles with an electrokinetically driven mobile phase, and detected by CCD and UV detection simultaneously. 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 polystyrenes on the sensitivity was small. Therefore, the signals obtained could not 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.

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1. Introduction

While in the last decade the use of miniaturized liquid chromatography (LC) systems in (bio)chemical and pharmaceutical analysis 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 [6,7]. In polymer analysis the smaller sample volume is an issue only in exceptional cases [8]. 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) polymers in micro-scale size-exclusion systems are still limited. In most applications micro LC systems are combined with an UV detector, which limits their use to specific polymers. A refractive index detector has been developed for small-scale SEC [9], but it is not (yet) commercially available. Another option is to use a miniaturized evaporative light scattering detector, as has been done in capillary reversed-phase chromatography [10].

Capacitively coupled contactless conductivity detection (CCD) was introduced by Zemmann et al. [11] in capillary electrophoresis in 1998. In the same year Fracassi da Silva and do Lago [12] presented a similar detection system. Modifications of these designs have been proposed to provide a high sensitivity for the detection of various ionic species [13]. Limits of detection reported were at a low-ppb level for small ions. Recently, a miniaturized contactless conductivity detection cell with similar sensitivity as the conventional cells was developed (Fig. 1) [14].

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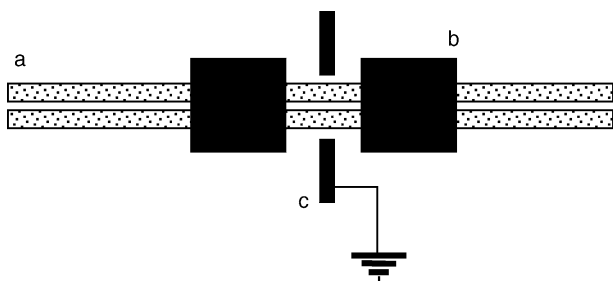


Fig. 1. Scheme of the CCD device: (a) capillary; (b) ring electrodes; (c) grounded copper foil.

A CCD setup is 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 electrodes) an ac voltage generated by an oscillator is applied on the inlet electrode giving a current through the background solution, which is picked up by the second electrode and amplified, rectified and recorded [15]. When an analyte zone with a conductivity different from that of the background electrolyte passes the detection gap, a change in the ac signal over the amplification unit is 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 CCD [16]. The principle of the conductivity response was unclear. According to the authors, it might be based on the effect of the dielectric constant 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 CCD for the detection of synthetic polymers.

In this paper, preliminary experiments on the application of CCD for the detection of neutral synthetic polymers are described. Size-based separations of polystyrene standards were performed by size-exclusion electrochromatography (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.

2. Experimental

2.1. Chemicals and materials

Narrow polystyrene standards with molar masses (MMs) between 2100 and 675 000 were purchased from different manufacturers (Polymer Labs., 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 particle diameter of 10 μm and nominal pore diameters of 300 Å.

2.2. CCD setup

The design of the sensor cell and the electronic components were similar as in the detector setup developed by Mayrhofer et al. [15]. Two cylindrical electrodes glued to Perspex holders that fitted the capillary column 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. In most experiments a detection gap with a width of approximately 1 mm was used. The input signal applied was a square wave with a frequency of 50 kHz and an amplitude of 8 V.

The aluminium CCD housing was placed in a modified capillary cartridge to accommodate both the cell and the electrical connections as described elsewhere [17]. Data acquisition and processing was carried out with Maxima software of Waters Chromatography.

2.3. SEEC system

SEEC experiments were performed on an Agilent CE system (Waldbron, Germany) equipped with a diode-array detection (DAD) system. The Chemstation software was used for the control of the instrument and for data acquisition of the UV detection, which was carried out at a wavelength of 260 nm. Fused-silica capillaries were packed using a slurry packing method as has been described previously [18]. Columns were 250 mm long. Close behind the outlet frit of the column the CCD cell was placed. A UV detection window was made 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. 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. Injec-

tions were performed electrokinetically typically at 15 kV for 5 s. Separations were performed at a voltage of 15 kV. During the separations a 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 algorithm was used. The algorithm included baseline construction, translation of the time axis in an MM axis using calibration plot data, and the calculation of the centralized moments of the peaks.

3. Results and discussion

3.1. CCD of polystyrenes

With the system used it is possible to compare the CCD signal for a polystyrene standard with its UV response. Fig. 2 shows the SEEC separation, with on-line detection by CCD and UV, of a polystyrene standard with an average molar mass of 30 000 at a concentration of 20 g l⁻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 main peak detected by CCD is clearly related to the elution of the polystyrene standard. Close to t_0 the CCD signal shows a series of positive and negative deviations that are not well defined; they may have been caused by the 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 a specific analyte 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 18 700 standard, at

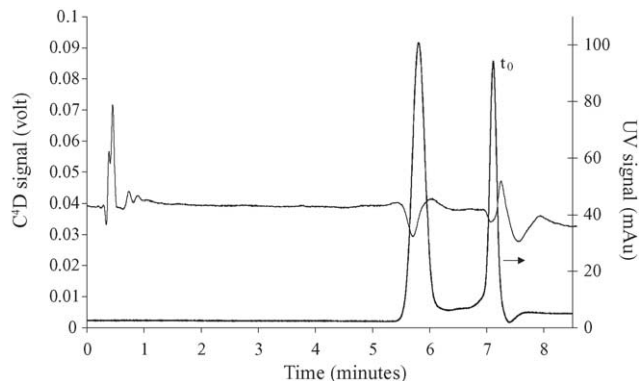


Fig. 2. SEEC chromatogram of polystyrene 30 000 simultaneously detected by CCD and UV absorption. Conditions: column: Nucleosil 300-10 (25 cm × 100 μm i.d.), eluent: 10⁻⁴ M LiCl in DMF; injection 15 kV, 5 s; voltage 15 kV.

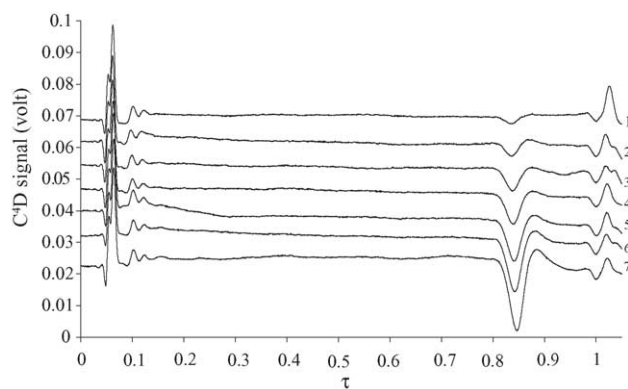


Fig. 3. Separations of polystyrene 18 700 at sample concentrations of (1) 5 g l⁻¹, (2) 10 g l⁻¹, (3) 15 g l⁻¹, (4) 20 g l⁻¹, (5) 30 g l⁻¹, (6) 40 g l⁻¹ and (7) 50 g l⁻¹. Conditions as in Fig. 2.

concentrations of 5–50 g l⁻¹, are shown in Fig. 3. For clarity, in this figure the time-axes of the chromatograms were standardized by translation into the retention factor τ , which is defined as the retention volume divided by that of the unretained solute. The concentration-calibration curves with peak heights and peak areas were linear with values for $R^2 > 0.99$ for both the CCD and the UV detector.

Experiments on the stability and repeatability of the detection signal were performed. Samples of polystyrene 18 700 at concentrations of 20 and 50 g l⁻¹ were injected seven times each. The relative standard deviation (R.S.D.) of τ , the peak heights and peak areas obtained with CCD in this repeatability study were compared with the results obtained with UV detection (Table 1). The variation in τ observed with conductivity detection was slightly higher than that with UV detection. The reason for this might be that the determination of t_0 in the CCD signal was less straightforward than in the UV signal. With both CCD and UV detection spreading in the values for the peak heights and peak areas of polystyrene were <5%.

3.2. Mass-calibration curves

For the construction of mass-calibration curves, polystyrene standards with average molar masses between 2100 and 675 000 were injected. All sample solutions contained a polymer concentration of 20 g l⁻¹. With low-MM polystyrenes symmetrical peaks were obtained, while for the standards with the highest MM values broad and sometimes

Table 1
Repeatability (as R.S.D., %, $n = 7$) of the analysis of polystyrene 18 700 at different sample concentrations

Concentration (g l ⁻¹)	τ		Peak height		Peak area	
	CCD	UV	CCD	UV	CCD	UV
20	0.17	0.03	2.7	1.5	3.7	1.6
50	0.09	0.04	2.4	3.0	2.7	2.4

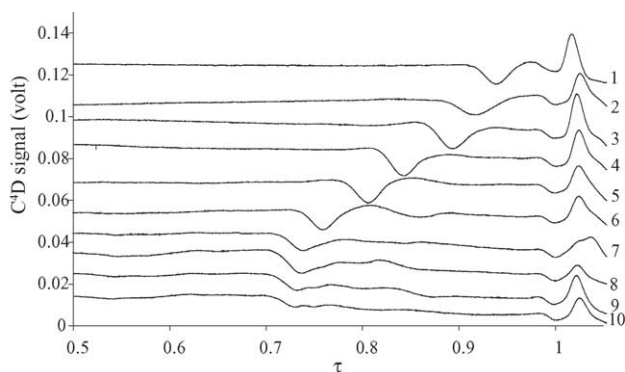


Fig. 4. Separations of polystyrene standards with molar masses of (1) 2100 (2) 3680 (3) 4000 (4) 7000 (5) 18 700 (6) 30 000 (7) 76 700 (8) 212 400 (9) 325 000 (10) 400 000 and (11) 675 000. Conditions as in Fig. 2.

double peaks were observed (see Fig. 4). It should be noted that even the highest-MM standards used were below the exclusion limit of the column.

Mass-calibration curves were constructed using the relative retention τ from both the CCD and the UV signals. Fig. 5 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 CCD signal. Both curves could be used to determine the polydispersity of the polystyrene standards. For the calculation of the molar mass distribution a laboratory-made algorithm developed in Excel was used. With the CCD system a polydispersity value of 1.06 was found for the 30 000 standard, while with UV detection a slightly higher value was found (1.08).

3.3. The origin of the CCD signal

The conductance of an electrolyte solution is related to the charge and concentration of the ionic species in solution and their electrophoretic mobility. A possible explanation for the observed sensitivity of CCD for neutral polymers could be that the higher viscosity of the solution in the polymer

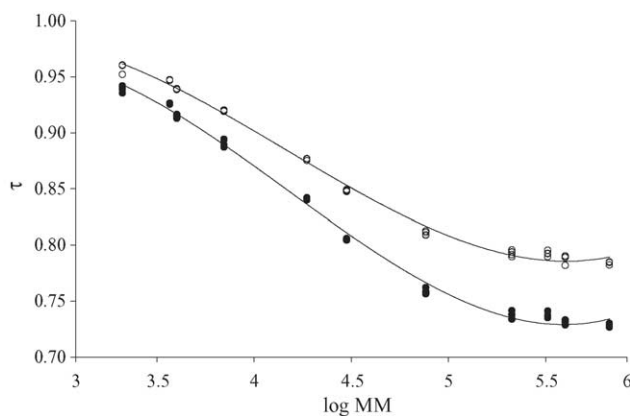


Fig. 5. Mass-calibrations curves for polystyrene obtained with CCD (●) and UV detection (○).

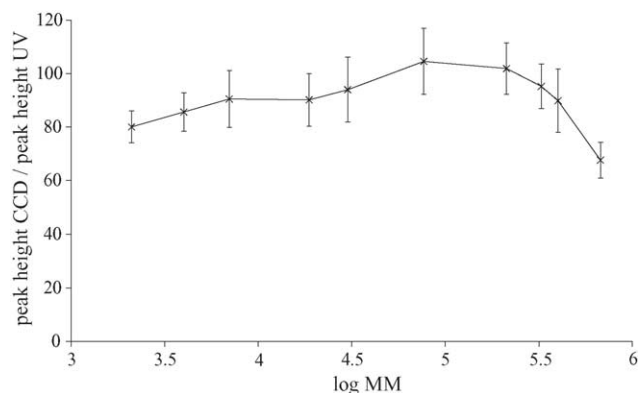


Fig. 6. Dependency of the sensitivity of CCD on the average molar mass of the polystyrene standards.

containing zone affects the mobility of the background electrolyte ions, and therewith the response of the detector. Since the viscosity of a polymer solution is related not only to the concentration of the polymer but also to its size, it was expected that the CCD response depends 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 (MM, 2100–675 000). 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 CCD were divided by the UV peak heights. The relative peak heights are plotted against the molar mass of the polymers in Fig. 6. 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 weeks. For the low-MM polystyrenes the magnitude of the conductivity signal increased with increasing molar mass. However, the dependency of the conductivity signal on the polymeric size was lower than might be expected from the relation 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 CCD signal with the molar mass of the polystyrenes was found. Apparently, the hypothesis that the CCD signal is related to changes in the (bulk) viscosity of the solution is not correct.

3.4. Conclusions and suggestions for further research

The experimental work performed so far has shown clearly that CCD can be used to monitor the elution of neutral synthetic polymers from a micro separation system. Repeatable signals were obtained, and a mass-calibration curve could be constructed. However, fairly high sample concentrations (in the order of grams per litre) were required to obtain useful results. Signal-to-noise ratios obtained with the CCD were at least one order of magnitude lower than those obtained with UV detection. Therefore, the main prospect of CCD 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.

A further optimization 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 CCD response is that the polymeric compounds affect the electrical double layer at the surface of the (silica) capillary wall, as has been suggested in another study with neutral analytes [16]. To study this possibility, capillaries of different materials could be compared, and the effect of the mobile phase composition on the sensitivity could be studied. Moreover, experiments with different types of polymers could shed light on this unresolved question on the mechanism of the CCD sensitivity for neutral compounds.

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