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Short communication

Alternative sample-introduction technique to avoid breakthrough in gradient-elution liquid chromatography of polymers

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ABSTRACT

Gradient-elution liquid chromatography (GELC) is a powerful tool for the characterization of synthetic polymers. However, gradient-elution chromatograms often suffer from breakthrough phenomena. Breakthrough can be averted by using a sample solvent as weak as the mobile phase. However, this approach is only applicable to polymers for which a sufficiently strong solvent exists which is at the same time a weak eluent. Finding such a solvent for a given polymer can be laborious or may even be impossible. Besides, when working with comprehensive two-dimensional LC the effluent of the first dimension is the injection solvent of the second dimension. In this case, it is not possible to avoid breakthrough by adjusting the eluent strength of the second-dimension injection solvent. Therefore, another strategy to avert breakthrough has to be implemented. In this work, we successfully avoided breakthrough in GELC by mixing the mobile phase not before, but after the autosampler. This was demonstrated measuring a blend of poly(methyl methacrylate) standards with different molecular-weights as model mixture with comprehensive two-dimensional GELC × size-exclusion chromatography. The strategy is thought to be applicable to all substances with a sufficiently strong dependence of retention on mobile-phase composition. This typically applies to large molecules (synthetic and natural polymers) and allows efficient refocusing. Unretained and barely retained substances are not refocused and therefore suffer in the proposed setup from peak broadening.

1. Introduction

Polymers are omnipresent in our every-day-life. They are easy to manipulate, durable, light-weight, and have an excellent cost-performance ratio, which makes them the material of choice for numerous applications. Polymers, especially copolymers, are highly complex samples. Quality control as well as research and development makes their thorough characterization necessary. Size-exclusion chromatography (SEC) separates according to the hydrodynamic volume and thereby provides insight into the molecular-weight-distribution (MWD). SEC is already well-established and high-temperature systems enable the handling of hardly soluble polymers.

Philipsen [1] and Chang [2] have reviewed the many contributions in which the potential of gradient-elution liquid chromatography (GELC) for the characterization of polymers was demonstrated. In GELC all or a part of the sample is immobilized on the top of the column at the conditions that prevail during injection. Immobilized substances are subsequently eluted from the column by changing the composition of the mobile phase. However, accurate separation is often hampered by breakthrough [1,3,4]. Breakthrough is observed when part of the polymer sample travels with the injection band through the column without interacting with the stationary phase [4]. The sample solvent and the polymer concentration, the injection volume, the (initial) composition of the mobile phase, and the column temperature all affect whether or not breakthrough occurs.

To avoid breakthrough, Jiang et al. [4] suggest to choose a sample solvent as weak as possible and to minimize the injected volume. However, to adjust the eluent strength of the sample solvent is only possible if – for the polymer in question – a sufficiently strong solvent exists, which at the same time is a weak eluent. This will not always be the case. Besides, there are situations where the eluent strength of the sample solvent cannot be manipulated freely. This is the case in comprehensive two-dimensional LC where the effluent of the first dimension serves as the second-dimension sample solvent. Thus, an alternative strategy to avoid breakthrough is necessary.

In the present work such an alternative approach to avoid breakthrough was evaluated. The components of the mobile phase were mixed after (rather than before) the autosampler. The success of

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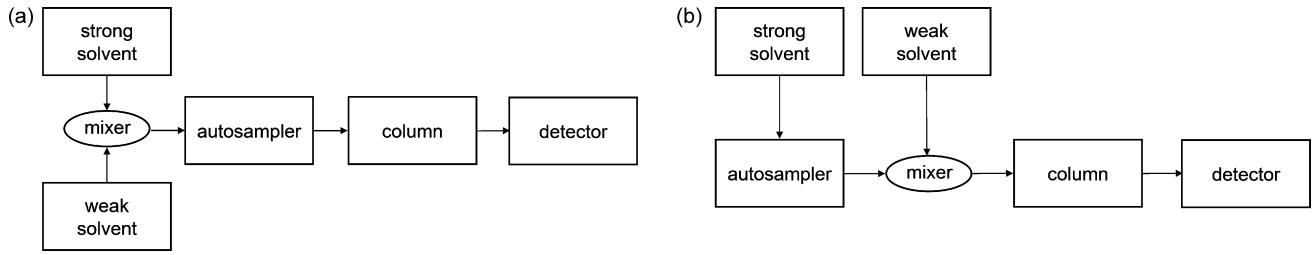

Fig. 1. Schematic drawing of the HPLC system, where the mobile phases are mixed (a) in front of the autosampler (conventional setup) and (b) after the autosampler.

this alternative arrangement was verified with GELC × SEC experiments.

2. Experimental

2.1. Samples and chemicals

Three poly(methyl methacrylate) standards (M_W 14.9 kDa, 49.6 kDa and 141.5 kDa, respectively) were generously donated by PSS (Mainz, Germany).

Chloroform (HPLC grade, containing 1% of ethanol for stabilization) and methanol (HPLC grade, absolute) were both purchased from Biosolve (Valkenswaard, The Netherlands).

2.2. Instrumentation

The setup for the LC experiments consisted of an autosampler (SIL-9A), a column oven (CTO-10A VP), a controller (CBM-20A), and three LC pumps (LC-10AD VP), all from Shimadzu (Kyoto, Japan), as well as a two-position ten-port switching valve (Valco VICI International, Schenkon, Switzerland). Two 100-μL sample loops were connected to the switching valve according to van der Horst et al. [5] (see Figure Sup-1 in the supplementary material). Substances were detected with a charged-aerosol detector (esa CAD plus; ESA, Chelmsford, MA, USA).

In a conventional LC setup, the mobile phase is mixed in front of the autosampler (see Fig. 1a). Alternatively, only the pump delivering the strong solvent (which was also utilized as sample solvent) was connected to the autosampler (see Fig. 1b). The weak solvent was added after the autosampler (i.e. in front of the column). A mixer with a volume of 150 μL was used for mixing the mobile phase.

Two-dimensional chromatograms were constructed with an in-house written Matlab program (version R2007b, Mathworks, Natick, MA, USA).

2.3. Chromatographic conditions

A blend of the three poly(methyl methacrylate) standards with a concentration of about 0.5 mg/mL of each were prepared in chloroform. GELC × SEC experiments were performed at 30 °C. The injection volume was 50 μL. Gradient-elution LC was carried out on a column (150 mm × 4.6 mm ID) packed with non-porous 40-μm glass beads, the flow was 50 μL/min. The composition of the mobile phase at the beginning of each run was 10% chloroform and 90% methanol by volume. After 30 min, the gradient program was started: the concentration of chloroform was increased from 10% up to 100% at a rate of 1%/min. The final conditions were held for 10 min; subsequently the column was equilibrated with 10% chloroform and 90% methanol at a flow of 0.5 mL/min for 2 min. The switching valve was actuated with an interval of 1 min. The second-dimension SEC runs were carried out on a PLgel 5-μm Mix-C column (100 mm × 4.6 mm ID, Polymer Labs/Varian, Church Stretton, Shropshire, UK) with pure chloroform as the mobile phase at 1 mL/min.

3. Results and discussion

3.1. Avoiding breakthrough by an alternative mixing of the mobile phase

If the weak and the strong solvent are mixed before the autosampler (as it is the case in Fig. 1a), analytes arrive at the column within a solvent band consisting of sample solvent. If the sample solvent is stronger than the mobile phase, the retention factor of the analytes within this solvent plug may be significantly smaller than the retention factor of the analytes in the mobile phase. Under these circumstances, breakthrough is possible [4].

In the alternative setup the weak component of the mobile phase is added directly after the autosampler (i.e. in front of the column; see Fig. 1b) with a mixer. In this setup the analytes are exclusively transported by the strong solvent to the mixer. At the beginning of the run, the concentration of strong solvent in the eluent and, thus, also the flow rate of the strong solvent before the mixer are low. Therefore, the initial conditions of the gradients have to be held long (or the flow rate has to be increased [6]) to assure that all the injected material reaches the column during the initial conditions of the gradient. The big advantage of the new setup is that no injection plug with a solvent stronger than the mobile phase exists. Breakthrough should therefore not be an issue. Only those substances, which are not retained on the column at the beginning of the gradient, will elute at t_0. Sufficiently retained analytes should be refocused at the top of the column and be eluted during the gradient.

3.2. Evaluation of the alternative sample-introduction technique

It is characteristic for breakthrough that the breakthrough peak is representative of the entire injected sample [4]. If this is not the case, the peak eluting around t_0 does not derive from a genuine breakthrough phenomenon, but from a fraction that is not retained at the initial gradient conditions.

For the mixture of the three poly(methyl methacrylate) standards this means that – if breakthrough occurs – the unretained peak contains all three different molecular-weight poly(methyl methacrylate)s. If only lower-molecular weight components elute unretained, breakthrough is not occurring. The molecular weight of every eluting fraction can conveniently be investigated by hyphenating GELC with SEC. Therefore, GELC × SEC experiments were carried out to elucidate whether breakthrough occurred. The scheme of the employed GELC × SEC system can be seen in Figure Sup-1 in the supplementary material.

3.3. Interpretation of GELC × SEC experiments

The glass-bead-packed column used in GELC showed neither any adsorptive interactions with the poly(methyl methacrylate) nor any size-exclusion effects. Any separation, therefore, resulted from on-line precipitation and subsequent dissolution and was exclusively controlled by the composition of the mobile phase.

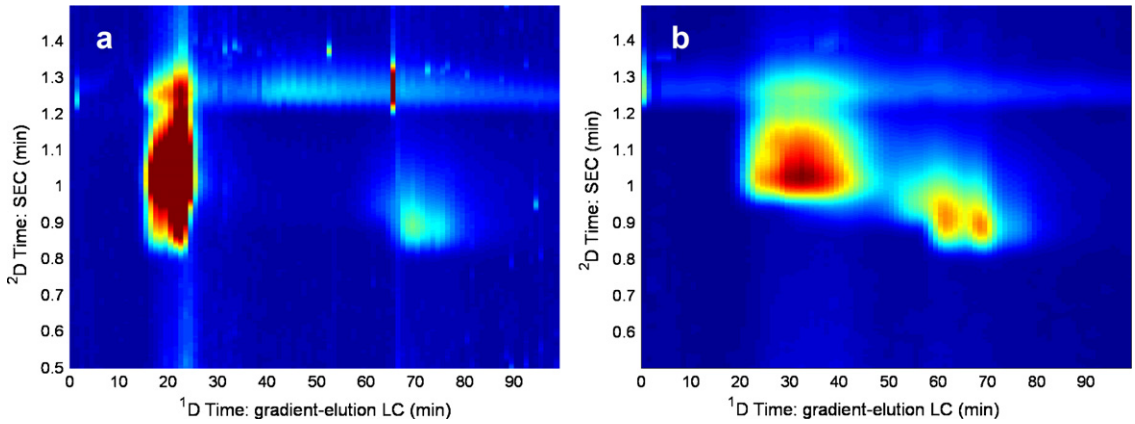

Fig. 2. Effect of mixer location in GELC proven by comprehensive two-dimensional GELC × SEC. If the mixer is placed before the autosampler (a), breakthrough occurs. If the strong and the weak eluent are mixed after the autosampler (b), breakthrough can be avoided and only low-molecular weight compounds elute around the dead time.

In a GELC × SEC experiment using a conventional setup for GELC (see Fig. 2a) substances eluting around the dead time (t_0) included low-molecular weight as well as high-molecular weight poly(methyl methacrylate) as they eluted at high as well as at low SEC retention volumes. This observation provided evidence of breakthrough [4].

In GELC × SEC, where the mobile phase was mixed after the autosampler, only low-molecular weight poly(methyl methacrylate) (eluting at high SEC retention volume) eluted unretained (see Fig. 2b) in GELC. This peak did not arise from a breakthrough phenomenon, but rather from the relatively small effects of mobile-phase composition on retention for low-molecular weight poly(methyl methacrylate)s. High-molecular weight poly(methyl methacrylate) was successfully refocused on the top of the column and eluted later in the gradient. When we increased the initial conditions to more than 90% methanol, even low-molecular weight poly(methyl methacrylate) could be effectively refocused on the top of the column and the intensity of the peak observed around t_0 diminished.

In the conventional setup the unretained peak appeared around 20 min (see Fig. 2a); in the new setup the unretained peak appeared around 30 min and was considerably broader (see Fig. 2b). These differences in retention time and peak width in Fig. 2a and b were caused by different flow rates in different parts of the system. The flow through the autosampler in the new arrangement (see Fig. 1b) was only 5 μL/min (i.e. 10% of the total flow of 50 μL/min). In the conventional arrangement (see Fig. 1a) the entire flow (also 50 μL/min) passed through the injection loop. Therefore, the injection plug arrived in the new arrangement later at the column and spread across a longer time. Accordingly, the unretained peak in chromatogram 2b eluted later and was broader than the unretained peak in 2a. Additional peak broadening might have been caused by the packing quality of the glass-bead column. However, this aspect was considered to be less relevant for this work which focused on the development of a basic strategy for avoiding breakthrough. As the glass-bead column was used as the first dimension in Fig. 2a and b, it contributed to peak broadening in both cases to the same extent.

3.4. Potential and limitations of the alternative sample-introduction technique

The setup described in Fig. 1b is a straightforward approach to avoid breakthrough. Its main advantage is that it is applicable to all polymeric samples, regardless of whether or not they are soluble in the weak eluent. Moreover, the present approach is especially promising for comprehensive LC × GELC. Here, an off-line sample-dilution approach cannot be applied as the composition of the injection solvent of the second-dimension is determined by the effluent composition of the first dimension.

If a sufficiently strong solvent exists which is at the same time a weak eluent the proposed sample-introduction approach can be compared with an on-line dilution of the sample solvent and is just as efficient as preparing the sample in a weaker sample solvent. Samples of polymers which are insoluble in mixtures containing high percentages of weak solvent can neither be prepared in a weaker sample solvent nor diluted off-line with a weaker eluent. The polymer would not dissolve respectively precipitate before being injected. With some HPLC systems, this dilution step can also be carried out in the injection loop. For polymers that are insoluble in the initial mobile-phase, this strategy may lead to system blockages.

The present arrangement requires a mixer after the autosampler. This introduces compared to the conventional arrangement additional dead volume resulting in significant broadening of unretained peaks (compare Fig. 2b). Therefore, this strategy is limited to analytes which can be efficiently refocused on the column (either by adsorptive interaction or by on-line precipitation). This implies that the slope of the retention vs. composition curves has to be sufficiently steep. However, for polymers above a certain molecular weight this is usually the case [7].

Another limitation of this approach is that it can be only realized with high-pressure gradient systems, as mixing is accomplished in the high-pressure part of the instrument.

4. Conclusions

Breakthrough phenomena often hamper the accurate characterization of polymeric samples. In this work a straightforward approach is presented to avoid breakthrough.

When the weak and the strong component of the eluent were mixed after – instead of before – the autosampler, no breakthrough was observed for a blend of three poly(methyl methacrylate) standards. If the mobile phase was mixed in front of the autosampler (as is usual practice in LC), massive breakthrough was observed under the same conditions.

This approach is applicable to samples that are either soluble or insoluble in the initial mobile phase, provided that they have sufficiently steep retention vs. composition curves. Peaks of unretained or barely retained substances were significantly broadened. For polymers soluble in the initial mobile-phase composition, the presented approach is comparable with an on-line dilution of the sample. For insoluble analytes off-line dilution would not be

possible and the presented approach is thus an efficient way to avert breakthrough. Furthermore, it is suitable for comprehensive LC × GELC, where off-line manipulation of the transferred factions is not possible.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2010.07.073.

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