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Studies on high-performance size-exclusion chromatography of synthetic polymers

I. Volume of silica gel column packing pores reduced by retained macromolecules

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Abstract

Macromolecules, which stay adsorbed within the active size-exclusion chromatography (SEC) column packings may strongly reduce effective volume of the separation pores. This brings about a decrease of retention volumes of the non-retained polymer samples and results in the increased apparent molar mass values. The phenomenon has been demonstrated with a series of poly(methyl methacrylate)s (PMMA) and a polyethylenoxide (PEO) fully retained by adsorption within macroporous silica gel SEC column from toluene or tetrahydrofuran, respectively. The non-retained probes were polystyrenes (PS) in toluene and both PS and PMMA in THF eluents. The errors in the peak molar mass values determined for the non-retained polymer species using a column saturated with adsorbed macromolecules and considering calibration curves monitored for the original "bare" column packing assumed up to several hundreds of percent. Errors may appear also in the weight and number averages of molar masses calculated from calibration dependences obtained with columns saturated with adsorbed macromolecules. Moreover, the SEC peaks of species eluted from the polymer saturated columns were broadened and in some cases even split. These results demonstrate a necessity not only to periodically re-calibrate the SEC columns but also to remove macromolecules adsorbed within packing in the course of analyses.

Keywords: Size-exclusion chromatography; Reduction of effective packing pore volume; Adsorbed macromolecules

1. Introduction

Size-exclusion chromatography (SEC) is the most commonly used method for molecular characterization of synthetic polymers. It is fast, experimentally feasible, relatively cheap and well precise in terms of intra-laboratory repeatability. The long term practice, however, shows that SEC of high polymers offen suffers from limited inter-laboratory reproducibility and low data accuracy. This was confirmed by recent round robin testing, which was organized under auspices of International Union of Pure and Applied Chemistry and included four commercial polymers and one oligomer [1]. Possible reasons of such situation will be analyzed in the following series of papers. An aspect of the SEC "column history" will be discussed in this first part, namely the effect of changing column packing pore volume due to presence of retained macromolecules. A macroporous silica gel was chosen as a rather adsorption-active column packing. Poly(methyl methacrylate)s (PMMA) of different molar masses were fully retained by adsorption within silica gel from toluene. On the contrary, polystyrenes (PS) were eluted in toluene eluent under "ideal" SEC conditions both from the original and the PMMA saturated columns. Their retention behavior on bare and PMMA saturated column, as well as calculated molar mass values were compared. Similarly, a polyethylenoxide (PEO) was adsorbed on silica gel from toluene and tetrahydrofuran, while PMMA was not adsorbed from THF within bare or PEO saturated silica gel column. Molar mass values for PS determined in toluene on bare sil-

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ica gel and on the PEO saturated silica gel were also confronted.

2. Experimental

2.1. Instrument and materials

Common HPLC instrument was used, with a pumping system Model 510 (Waters, Milford, MA, USA) operated at the flow rate of 1 mL min^{-1}. The injected volume and sample concentration were 50 μL and 1 mg mL^{-1}, respectively. An autosampler MIDAS (Spark Holland, Emmen, The Netherlands), an evaporative light scattering detector (ELSD) Model 1000 (Polymer Laboratory, Church Stretton, Shropshire, UK) and a refractive index detector (RI) Model 7515A (ERC Inc., Nishi Aoki, Kawaguchi City, Saitama, Japan) were employed. Columns were thermostated to 30 °C in a hot-air column oven (Knauer, Berlin, Germany).

Two silica gel packed columns 300 mm × 7.8 mm were used. The first one (designated as column 1) contained silica gel with 10 μm particles and with the pore size of 30 nm (Tessek, Prague, Czech Republic). The second one (column 2) was packed with 10 μm silica gel also from Tessek. The original 10 nm pores of this material were extended in this laboratory to about 30 nm using a proprietary process, which was recently licenced to Eka Chemicals (Gothenburg, Sweden) to form a basis for their Kromasil 300 silica. The smallest pores of latter material were partially removed so that it exhibited increased selectivity of separation in a narrower range of molar masses (compare Figs. 3 and 7).

The analytical grade toluene was from Slavus, Bratislava, Slovakia, tetrahydrofuran (THF) was from Merck, Darmstadt, Germany, and dimethylformamide (DMF) was from Scharlau, Barcelona, Spain. Toluene and THF were distilled before use. THF was stabilized with 0.02 wt.% of butylated *p*-cresol.

Polystyrene standards with weight average molar masses ($\overline{M}_w$) from 0.67 to 2000 kg mol^{-1} were from Pressure Chemicals Co., Pittsburgh, PA, USA. Their $\overline{M}_w/\overline{M}_n$ values determined by producer ranged between 1.06 and 1.2. Medium polydispersity poly(methyl methacrylate)s were a gift from Dr. W. Wunderlich, Röhm, Darmstadt, Germany. Their $\overline{M}_w$ determined with conventional SEC ranged from 16 to 613 kg mol^{-1} and $\overline{M}_w/\overline{M}_n$ values from 1.14 to 1.85. A medium broad industrial sample of PMMA with weight average molar mass 108 kg mol^{-1} and $\overline{M}_w/\overline{M}_n$ 1.5 was from Mitsubishi Rayon Co., Tokio, Japan. Poly(ethylene oxide) with $\overline{M}_w$ 50 kg mol^{-1} was a commercial product from Novácke chemické závody, Nováky, Slovakia.

The chromatographic data were processed with help of Baseline (Waters, Milford, MA, USA) and Clarity (DataApex, Prague, Czech Republic) softwares. The universal calibration curves log $M[\eta]$ versus V_R [2] were constructed for both the original and the saturated columns, where M is the most abundant (“peak”) molar mass and $[\eta]$ is the limiting viscosity number for unretained polymer in eluent used. $[\eta]$ values were calculated applying Kuhn–Mark–Houwink–Sakurada viscosity law $[\eta] = KM^a$ with K and a constants at 30 °C for PS/toluene 0.0092 mL g^{-1}, 0.72 [3] and for PMMA/toluene 0.007 mL g^{-1}, 0.71 [3], respectively.

Column efficiencies were assessed by injecting solution of hexane in eluent (10 μL mL^{-1}). In this case, peaks were monitored by a refractive index detector.

2.2. Column saturation

For the column saturations, medium polydispersity PMMA with the weight average molar masses 16, 65 and 613 kg mol^{-1}, as well as medium broad industrial sample of PMMA from Mitsubishi and PEO were used.

It is known that toluene, which is a rather non polar “weak” solvent promotes adsorption and full retention of PMMA on the silica gel surface at 30 °C [4,5]. It is an efficient adsorli for this polymer considering silica gel. Similarly, THF is an adsorli for PEO on silica gel surface at 30 °C [6] though this solvent supresses adsorption of PMMA on silica that means it is a desorli for the latter polymer [5,7]. Dimethylformamide fully desorbs PS, PMMA and PEO from the silica gel surface [8]. Zones of hexane used for determination of column efficiencies did not displace any adsorbed polymer.

Columns were saturated by polymers applying repeated injections of various volumes of diluted solutions ranging from 50 μL to 5 mL with concentration of 2.5, 5 or 10 mg mL^{-1} at the flow rate of 1 mL min^{-1}. As rule, entire amount of polymer was adsorbed and fully retained within column at the beginning of the saturation process. Later, however, each new portion of injected polymer displaced a part of initially adsorbed polymer, which left the column in the form of ill shaped large zones with non-defined and changing retention volumes. This was especially evident for PMMA 65 and 613 kg mol^{-1} adsorbed from toluene. Such “stepwise mechanism” of the retention–elution process is so far not well understood. It may be connected with the exchange processes when larger macromolecules displace the smaller ones from the packing surface [9]. When the saturation of both the inner and the outer surface of column packing was completed, PMMA started to elute in form of rather symmetrical peaks with constant retention volumes. At this stage, the saturation process was concluded and the experiments with unretained polymers were performed. When the latter measurements had been finished, the adsorbed PMMA and PEO were stripped from the packing with THF or DMF, respectively. The amounts of adsorbed polymers in the moment when first portions started to leave the column 1 were calculated from known volumes and concentrations of injected solutions. These were 1.35, 0.73, and 0.22 g for PMMA 16, 65 and 613 kg mol^{-1}, respectively. The amount of PMMA 16 kg mol^{-1} determined by stripping with THF and drying in vacuo at 70 °C was, however, much lower, namely 0.92 g. This indicates that certain amount of polymer used for the