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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

initial packing saturation left the column in the course of experiments with the unretained PS, during which several hundreds of milliliters of eluent were transported through the column. The pronounced base line perturbations were, however, not observed in the course of PS elution. The amount of PEO 50 kg mol^{-1} adsorbed in column 1 from toluene and calculated considering volume and concentration of injected solutions was 0.64 g, which is comparable with PMMA of similar molar mass, 65 kg mol^{-1}.

3. Results and discussion

Column efficiencies N_T measured with hexane decreased remarkably for columns saturated with adsorbed polymers. For example N_T value for column 1 and bare silica gel in toluene was about 15,000 theoretical plates, while for the same column saturated with PEO from THF it dropped to less than 9000 plates. Peaks of non-retained polymers were also broadened in the saturated columns compared to the original ones. Moreover, split peaks were observed for hexane for column 1 saturated with PMMA 65 kg mol^{-1} (Fig. 1) and with PEO 50 kg mol^{-1} from toluene. Similarly, high molar mass ("SEC excluded") PS probes produced split peaks when eluted from column 1 saturated with PMMA 65 kg mol^{-1} from toluene (see Fig. 2). However, only PS 233 and 498 kg mol^{-1} produced split peaks using the same column saturated with PEO from toluene. It seems as if a large negative peak of unknown origin interfered with sample peaks. Such situation often appears when system peaks interfere with sample peaks. System peaks are caused by a local excess of one component of mixed eluent. They are detected with the non-specific detector such as refractive index measuring devices but they are not visible by evaporative light scattering detectors. Moreover, retention volumes

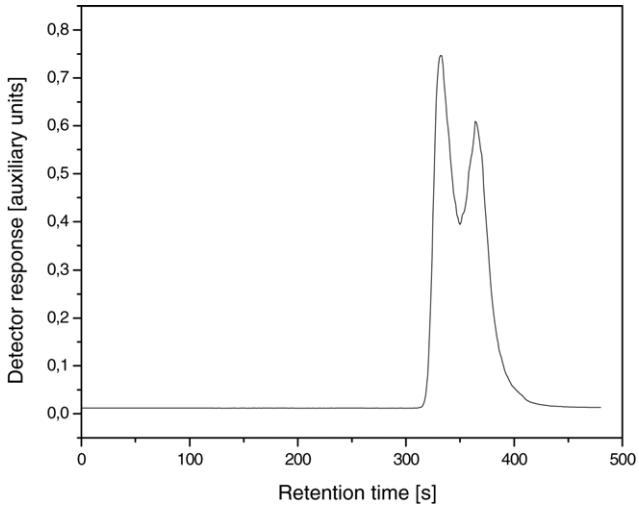

Fig. 2. Elution patterns of PS 233 kg mol^{-1} in toluene obtained with the column 1 saturated with PMMA 65 kg mol^{-1}. ELSD detector, sample concentration: 1 mg mL^{-1}.

of system peaks are higher than those of polymers fully excluded from the packing pores. Surprisingly, columns saturated with PMMA 16 and 613 kg mol^{-1}, as well as with the medium broad industrial PMMA from toluene and with PEO from THF produced unimodal and symmetrical sample peaks. Reasons for the behavior of column 1 saturated with PMMA 65 kg mol^{-1}are so far not unknown.

Universal calibration curves for polystyrenes in toluene determined with bare silica gel and with silica saturated with PMMA are displayed in Fig. 3. Both apparent retention volumes of the split peaks are plotted for high molar mass polystyrenes above molar mass 100 kg mol^{-1}. The actual peak retention volumes may be situated between these two values.

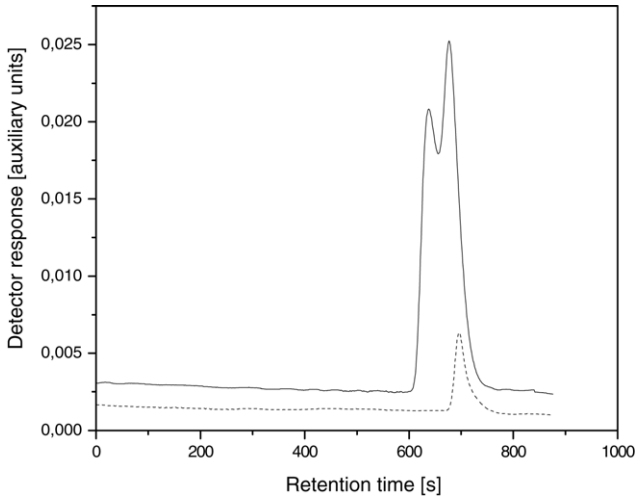

Fig. 1. Elution patterns of hexane in toluene eluent obtained with the column 1 saturated with PMMA 65 kg mol^{-1} (solid line) and with the bare silica gel (dash line). RI detector, sample concentration: 10 μL mL^{-1}. For the sake of clarity, the peak for bare silica gel was electronically reduced.

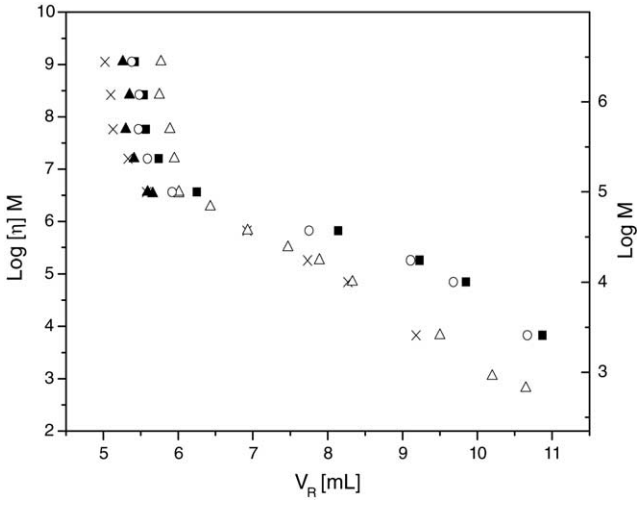

Fig. 3. Universal calibration curves for PS in toluene, column 1. (■) original silica, (○) silica saturated with PMMA 613 kg mol^{-1}, (×) silica saturated with PMMA 16 kg mol^{-1} and (▲ and △) silica saturated with PMMA 65 kg mol^{-1}.

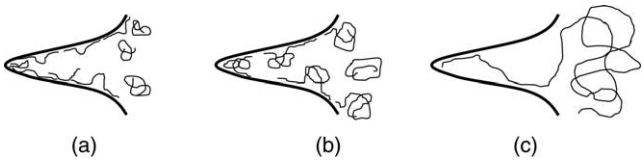

Fig. 4. Schematic representation of SEC column packing pores with adsorbed macromolecules. The smallest (a), medium (b) and the largest (c) molar mass PMMA species adsorbed on the surface of silica gel.

It is evident that macromolecules of PMMA fully retained within silica gel by adsorption strongly alter packing pore volume and this brings about large shifts in retention volumes of unretained polystyrenes. For example, for the column 1 with retained PMMA 65 kg mol^{-1} the decrease of V_R for PS 10.1 kg mol^{-1} represents almost 15%. This corresponds to the M_p value 26.6 kg mol^{-1} considering the calibration curve of original silica gel.

The extent of V_R shifts for polystyrenes depends on the molar mass of adsorbed PMMA. It is more pronounced for PMMA with medium molar masses and it is relatively small for high molar mass PMMA. Actually, PMMA 613 kg mol^{-1} would be fully excluded from the column packing under ideal SEC conditions that is in absence of enthalpic interactions between macromolecules and column packing (compare the right-hand axis of Fig. 3 where log M values are plotted). However, in presence of strong attractive interactions between pore walls and polymer species, very large, "SEC excluded" macromolecules de-coil and reptate into the pores like a snake [10]. The tentative explanation of this result is schematically presented in Fig. 4. Small sized (16 kg mol^{-1}) macromolecules of PMMA freely penetrate most of the pores and (partially) de-coil to adsorb on the silica surface. Medium sized macromolecules of adsorbed PMMA still occupy a rather large part of the pore volume. The largest "SEC excluded" macromolecules much less affect effective packing pore volume.

As rule, peaks of polymers eluted from columns saturated with adsorbed macromolecules are broadened. Typical examples are shown in Fig. 5, where chromatograms are displayed for PS with molar mass 17.5 kg mol^{-1} eluted from bare silica gel, as well as from silica gel saturated with PMMA 65 and 613 kg mol^{-1} (column 1). The peak broadening observed with the columns saturated with PMMA is remarkable. The molar mass of PS 17.5 kg mol^{-1} lies in the area of highest selectivity of separation for this column and large portion of its peak is situated in the nearly linear part of calibration dependence (compare Fig. 3). On the other hand, shifts of retention volume for PS 17.5 kg mol^{-1} is not that large as with polystyrenes possessing lower molar masses. The number and weight molar mass averages $\overline{M}_n$ and $\overline{M}_w$ for PS 17.5 kg mol^{-1} calculated from chromatograms before and after saturation and using calibration curve for virgin, as well as for PMMA saturated silica gel are collected in Table 1 (column 1). Considering calibration curve for original silica gel, apparent $\overline{M}_n$ and $\overline{M}_w$ values increased about 78% compared

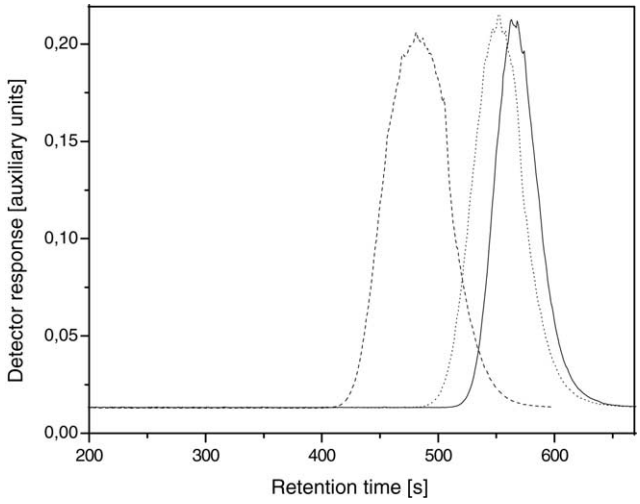

Fig. 5. Chromatograms for PS with molar mass 17.5 kg mol^{-1} eluted in toluene from bare silica gel (solid line), from silica gel saturated with PMMA 65 kg mol^{-1} (dash line) and from silica gel saturated with PMMA 613 kg mol^{-1} (dotted line) (column 1).

to the actual ones for sample eluted from the column saturated with PMMA 65 kg mol^{-1}. A rise of $\overline{M}_w/\overline{M}_n$ value calculated from the real calibration curve of the column saturated with PMMA 65 kg mol^{-1} is to be noticed, as well.

Results with column 1 saturated with PEO from toluene or THF confirmed previous observations. For example, calibration dependences for PS and PMMA obtained with column 1 saturated with PEO from toluene are displayed in Fig. 6.

PEO adsorbed on silica blocked most free silanols on the surface and allowed elution of PMMA in toluene in the form of symmetrical peaks (results not shown). Molar mass values for PS 17.5 kg mol^{-1} obtained with column 1 saturated with PEO are collected in Table 2.

In order to mimic real situation column 2 was saturated with the medium broad industrial PMMA in the next series

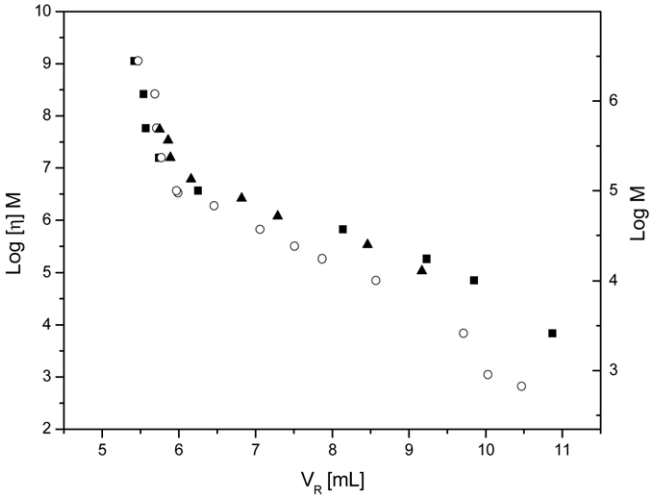

Fig. 6. Universal calibration curve for PS and PMMA in toluene, original and PEO saturated column 1. (■) original silica (PS), (◯) saturated silica (PS) and (▲) saturated silica (PMMA).

Table 1
Retention volumes and molar mass values for polystyrene 17.5 kg mol^{-1} eluted from both virgin and PMMA saturated column 1

Sample eluted from	V_R (mL)	Calibration curve considered	$\overline{M}_n$ (kg mol^{-1})	$\overline{M}_w$ (kg mol^{-1})	$\overline{M}_w/\overline{M}_n$
Original column	9.18	For original column	17.6	18.3	1.04
Column saturated with PMMA 16 kg mol^{-1}	7.71	For original column	33.0	33.9	1.03
Column saturated with PMMA 16 kg mol^{-1}	7.71	For column saturated with PMMA 16 kg mol^{-1}	17.5	18.7	1.07
Column saturated with PMMA 65 kg mol^{-1}	7.86	For original column	31.4	32.3	1.03
Column saturated with PMMA 65 kg mol^{-1}	7.86	For column saturated with PMMA 65 kg mol^{-1}	14.6	17.4	1.19
Column saturated with PMMA 613 kg mol^{-1}	9.02	For original column	20.1	21.0	1.04
Column saturated with PMMA 613 kg mol^{-1}	9.02	For column saturated with PMMA 613 kg mol^{-1}	17.2	18.2	1.05

Table 2
Retention volumes and molar mass values for polystyrene 17.5 kg mol^{-1} eluted from toluene from both virgin and PEO saturated column 1

Sample eluted from	V_R (mL)	Calibration curve considered	$\overline{M}_n$ (kg mol^{-1})	$\overline{M}_w$ (kg mol^{-1})	$\overline{M}_w/\overline{M}_n$
Original column	9.18	For original column	17.6	18.3	1.04
Column saturated with PEO 50 kg mol^{-1}	7.84	For original column	30.8	31.6	1.03

Table 3
Retention volumes and molar mass values for polystyrene 17.5 kg mol^{-1} eluted from both virgin and medium broad industrial PMMA saturated column 2

Sample eluted from	V_R (mL)	Calibration curve considered	$\overline{M}_n$ (kg mol^{-1})	$\overline{M}_w$ (kg mol^{-1})	$\overline{M}_w/\overline{M}_n$
Original column	9.37	For original column	16.9	18.8	1.11
Column saturated with medium broad industrial PMMA	8.43	For original column	52.8	53.8	1.02
Column saturated with medium broad industrial PMMA	8.43	For column saturated with medium broad industrial PMMA	17.3	18.8	1.08

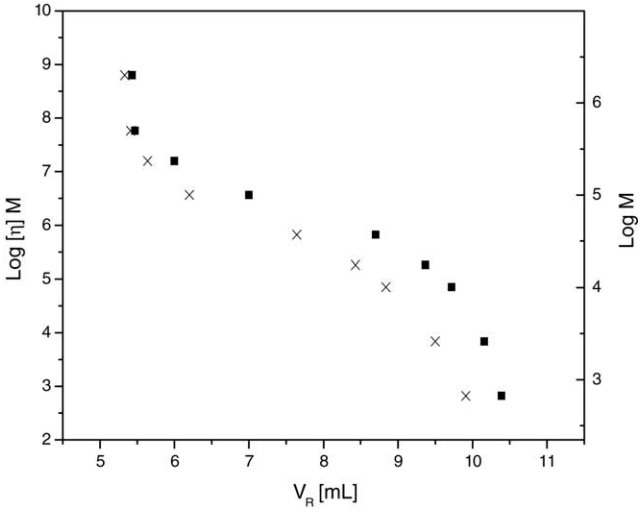

Fig. 7. Universal calibration curves for PS in toluene. Column 2 saturated with medium broad industrial PMMA (×), original silica (■).

of experiments. Universal calibration curves before and after column saturation are compared in Fig. 7 and in Table 3. The results are similar to those obtained with the medium polydispersity PMMA, which were presented in Fig. 3 and in Table 1.

It is accepted that the fully excluded molar mass or hydrodynamic volume of non-retained macromolecules reflects the largest pores present in the SEC column packing. Universal calibration curves shown in Figs. 3, 6 and 7 do not allow

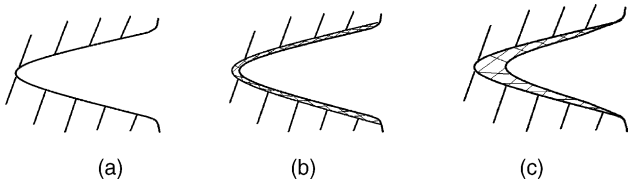

Fig. 8. Schematic representation of pores in a SEC column packing. (a) original silica gel, (b) silica gel coated with an equally thick "layer" of adsorbed macromolecules, (c) silica gel coated with an asymmetrical layer of adsorbed macromolecules, which are accumulated mainly in the narrow parts of pores.

quantitative evaluation of changes in the effective diameter of the largest pores due to the saturation with polymers. Still, it seems that the size of the largest pores was only slightly affected by the adsorbed macromolecules of PMMA or PEO. This means that the shift of retention volumes for the unretained macromolecules is preferentially caused not only by relatively but also by absolutely larger decrease in volume of narrow parts of pores. Such situation is schematically represented in Fig. 8c. This phenomenon deserves further study.

4. Conclusions

Pronounced changes were observed in the courses of calibration dependences for the unretained polystyrene probes measured before and after saturation of silica gel columns with adsorbed poly(methyl methacrylate) or polyethylenox-

ide. This indicates alteration of the packing pore volume. The extent of the shifts of PS retention volumes depended on the molar mass of polymer adsorbed. The effective pore volume decreased also if adsorbed polymer possessed molar mass, which was large enough to be fully excluded from the pores in the "ideal" SEC mode that is if eluent efficiently prevented adsorption of macromolecules. However, in this case, the pore volume reduction was small. The adsorbed macromolecules also reduced selectivity of the SEC columns.

The observed phenomena represent a part of the "SEC column history". Small portions of injected samples can be successively adsorbed and fully retained within the SEC packing in the course of routine molar mass measurements. Reduced sample retention volumes of polymer species eluted from the column packing, which contains retained macromolecules may lead to erroneously high values of the calculated apparent molar masses of the non-adsorbed analyzed polymers if the calibration dependences monitored with the "virgin" columns are applied. Due to additional band broadening, errors in determined molar mass values can appear also if the actual calibration curve is considered that is the $\log [\eta]M$ versus V_R dependences monitored with columns saturated with adsorbed polymer species. This means that application of light scattering SEC detectors would mitigate but often not completely remove the above problems, especially in terms of $\overline{M}_n$ values determined for the wide molar mass distribution polymers. Therefore, SEC columns should be not only often re-calibrated but also the retained macromolecules must be periodically removed.

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References

[1] D. Berek, R. Bruessau, D. Lilge, I. Mingozzi, S. Podzimek, E. Robert, Reproducibility of molar mass values of commercial polymers determined with size-exclusion chromatography, in: Proceedings of the International Symposium on High Performance Liquid Phase Separations and Related Techniques, Maastricht, P-2110, 2001, p. 307.
[2] H. Benoit, Z. Grubisic, P. Rempp, D. Decker, J. Zilliox, J. Chim. Phys. 63 (1966) 1507.
[3] M. Kurata, Y. Tsunashima, in: J. Brandrup, E.H. Immergut (Eds.), Polymer Handbook, fourth ed., J. Wiley and Sons, New York, 1999, p. VII/1.
[4] M. Jančo, D. Berek, T. Prudskova, Polymer 36 (1995) 3295.
[5] D. Berek, M. Jančo, G.R. Meira, J. Polym. Sci., A. Polym. Chem. 36 (1998) 1363.
[6] S.H. Nguyen, D. Berek, Chromatographia 48 (1998) 65.
[7] O. Chiantore, J. Liq. Chromatogr. 7 (1984) 1.
[8] S.H. Nguyen, D. Berek, Colloid Polym. Sci. 277 (1999) 318.
[9] S.H. Nguyen, D. Berek, Colloid Polym. Sci. 277 (1999) 1179.
[10] M. Šnauko, D. Berek, Chromatographia 57 (2003) 5.