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Liquid chromatography of polymers under limiting conditions of adsorption IV. Sample recovery

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Abstract

The high performance liquid chromatography of polymers under limiting conditions of adsorption (LC LCA) separates macromolecules, either according to their chemical structure or physical architecture, while molar mass effect is suppressed. A polymer sample is injected into an adsorption-active column flushed with an adsorption promoting eluent. The sample solvent is a strong solvent which prevents sample adsorption. As a result, macromolecules of sample elute within the zone of their original solvent to be discriminated from other, non-adsorbing polymer species, which elute in the exclusion mode. LC LCA sample recovery has been studied in detail for poly (methyl methacrylate)s using a bare silica gel column and an eluent comprised toluene (adsorli) and tetrahydrofuran (desorli). Sample solvent was tetrahydrofuran. It was found that a large part of injected sample may be fully retained within the LC LCA columns. The amount of retained polymer increases with decreasing packing pore size and with higher sample molar masses and, likely, also with the column diameter. The extent of full retention of sample does not depend of sample volume. An additional portion of the injected desorli sample solvent (a tandem injection) does not fully eliminate full retention of the sample fraction and the reduced recovery associated with it. The injected sample is retained along the entire LC LCA column. The reduced sample recovery restricts applicability of many LC LCA systems to oligomers and to discrimination of the non-adsorbing minor macromolecular components of complex polymer mixtures from the adsorbing major component(s). The full retention of sample molecules within columns may also complicate the application of other liquid chromatographic methods, which combine entropic and enthalpic retention mechanisms for separation of macromolecules.

Keywords: Chromatography of polymers; Barrier methods; Limiting conditions of adsorption; Sample recovery

1. Introduction

The liquid chromatography of polymers under limiting conditions of adsorption (LC LCA) belongs to the "barrier" methods of high performance liquid chromatography of polymers (polymer HPLC) [1–8]. It utilizes intrinsic difference between mobilities of small molecules in the HPLC eluents and the sample macromolecules. The former species permeate the packing pores and their transport along the column is slow. On the contrary, macromolecules travel along the column much faster because they are partially or fully excluded from the packing pores. If the eluent promotes adsorption of macromolecules on an active column packing, it is 'an adsorli', and sample is injected in a strong solvent, which prevents its adsorption, in 'a desorli', the system may exhibit the LC LCA behavior. Fast moving macromolecules accumulate on the leading edge of the slowly moving desorli zone without breaking into the bulk volume of adsorli eluent. As result,

Abbreviations: ELSD, evaporative light scattering detector; HPLC, high performance liquid chromatography; *M*, molar mass; PMMA, poly (methyl methacrylate); PS, polystyrene; LC LCA, liquid chromatography of polymers under limiting conditions of adsorption; RI, refractive index; SEC, size exclusion chromatography; THF, tetrahydrofuran; UV, ultraviolet; v_i, injected volume; $v_{i,max}$, maximum injected volume; $v_{i,min}$, minimum injected volume; $v_{i,s}$, safe injected volume; V_0, interstitial volume of column; V_M, volume of mobile phase within column; V_R, retention volume

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polymer species elute from the column irrespectively of their molar mass in the form of a narrow, focused peak. If the sample contains both adsorbing and non-adsorbing species, the latter leave the zone of their original solvent and elute in conventional size exclusion chromatography (SEC) mode at lower retention volume. In this way, polymer species possessing different adsorption properties can be discriminated, and independently characterized, for example using an on-line SEC column.

The role of experimental conditions in LC LCA was studied in several different systems [3–6]. When an appropriately chosen column packing, eluent and sample solvent for a given polymer were combined, the following behavior was observed:

(a) Retention volumes of macromolecules were independent of their size in a very broad molar mass range.
(b) Narrow, focused peaks with similar retention volumes were generated for different injected sample volumes and sample concentrations. LC LCA columns packed with common silica gel sorbents tolerated large sample sizes. Up to about 40% of the total column volume and at least up to 100 mg ml^{-1} concentration could be injected into the column of 250 mm × 4 mm size. This is important for tracing and characterization of minor components (even below 1%) in polymer blends applying two-dimensional liquid chromatography.
(c) Narrow-bore, long and efficient columns gave the best results.
(d) The effect of temperature was insignificant for poly (methyl methacrylate)s (PMMA) eluted from bare silica gel in the zone of tetrahydrofuran applying tetrahydrofuran/toluene 35/65 (w/w) eluent.

However, reduced sample recovery was observed in some LC LCA systems using a narrow pore column packing [7]. It was, therefore, of interest to study in detail the problem of sample recovery in LC LCA.

2. Experimental

2.1. Chromatograph

The chromatograph consisted of following components. A Model 510 isocratic pump (Waters, Milford, MA, USA) was employed at the flow rate of 1 ml min^{-1} in most experiments. For extremely low flow rates the isocratic pump Model 64 was used (Knauer, Berlin, Germany) equipped with a micro pump head. The actual flow rate was checked by a burette. An autosampler MIDAS (Spark Holland, Emmen, The Netherlands) was applied for sample injection. Columns were kept at constant temperature in an air oven (Knauer, Berlin, Germany) or in a custom made oven with a duplex wall connected to a water thermostat. The temperature of most experiments was 30 °C. The experimental arrangement of the chromatograph is shown in Fig. 1. For selected recovery

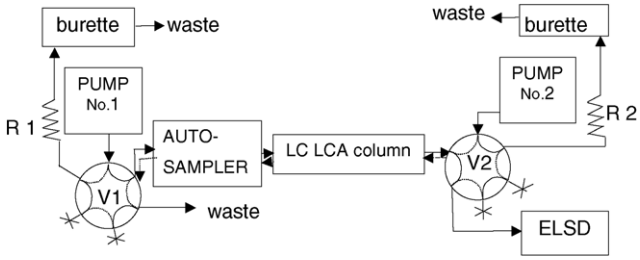

Fig. 1. Experimental arrangement of the LC LCA chromatograph employed. R1 and R2 are hydrodynamic resistors (capillaries), V1 and V2 are the switching valves, ELSD is an evaporative light scattering detector. For details see the text.

assessments, the backflush experiments were applied. Using valves V1 and V2 (Institute of Chemical Technique Fundamentals, Academy of Sciences of Czech Republic, Prague, Czech Republic), after a standard LC LCA experiment had been completed, the column was slowly filled with certain volume of tetrahydrofuran (THF) desorli from the pump no. 2. After a pre-selected time of desorption, the valves V_1 and V_2 were operated again and elution in the original direction was restarted. Evaporative light scattering detectors (ELSD) (Models PL 960 and PL 1000 from Polymer Laboratories, Shropshire, Church Stretton, UK) were used for detection of polymer probes. PMMA eluted within the sample solvent zone and therefore the conventional refractive index (RI) detector was inapplicable. Due to the problems with the quantitative processing of the ELSD response [9,10], the experiments were evaluated only semi-quantitatively. An ultraviolet (UV) detector (Laboratory Instruments Works, Prague, Czech Republic) functioning at 254 nm wavelength was applied for detection of polystyrenes (PS) in a selected series of experiments. Peaks of *n*-hexane used for determination of the column efficiencies and the volume of the mobile phase within columns were monitored by an RI detector Model 198 (Knauer, Berlin, Germany). Experimental data were processed with Baseline (Waters, Milford, MA, USA) or Chroma (Chroma, Graz, Austria) PC softwares.

2.2. Stationary and mobile phases

Bare silica gels were chosen for this study to avoid extensive enthalpic partition and interphase adsorption of macromolecules in favor of bonded stationary phases [11]. Thus, adsorption of macromolecules onto surface silanols of silica gel was anticipated the main enthalpic retention mechanism coupled with the exclusion retention mechanism. Narrow pore (6 nm) spheroidal silica gels Silpearl (Glasswork, Votice, Czech Republic) with particle diameters 7 and 10 μm, were applied in most experiments. Macroporous 10 μm spherical silica gels with pore diameters 10, 30 and 60 nm (Biospher from Labio, Prague, Czech Republic), as well as 100 nm (Separon SGX 1000 from Tessek, Prague, Czech Republic) were utilized for evaluation of the

Table 1
Specifications of the columns used

Column number	Column packing (particle diameter, μm)	Column dimensions (mm)	Pore diameter (nm)	V_M (ml)
1	Silpearl (10)	300 × 7	6	9.04
2	Silpearl (7)	250 × 4	6	2.92
3	Biospher Si 100 (10)	250 × 4.5	10	2.66
4	Biospher Si 300 (10)	250 × 4.5	30	2.70
5	Biospher Si 600 (10)	250 × 4.5	60	2.81
6	Separon SGX 1000 (10)	250 × 4.5	100	2.74

pore diameter influence on the polymer sample recovery. Sorbents were packed into stainless steel columns of various dimensions (see Table 1) in this Laboratory.

Analytical grade tetrahydrofuran (Slavus, Bratislava, Slovakia), was chosen as a medium effectivity desorli for PMMA in combination with bare silica gel. In fact, a weak adsorption of PMMA in THF was observed with the non-modified silica gels [12,13]. The retention volumes of PMMA probes in THF slightly increased in comparison with the non-adsorbed PS, and this effect was more pronounced for lower molar masses. Analytical grade toluene (Slavus, Bratislava, Slovakia), which prevented elution of PMMA from the bare silica gel was chosen as an efficient adsorli [13]. It should be noted, that both THF and toluene are thermodynamically almost equally good solvents for PMMA [14], and the deterioration of the mixed solvents quality due to a co-nonsolvency effect was improbable. Both solvents were dried and distilled before use. THF was stabilized immediately after distillation with 0.02 wt.% of butylated *p*-crezol. According to our previous results [5,6] the optimum LC LCA for PMMA eluent contained 35 wt.% THF. It was used in all present experiments with PMMA probes. Analytical grade, distilled cyclohexane (Spolchim, Bratislava, Slovakia) was used as adsorption promoting liquid for polystyrenes. Cyclohexane is a poor (theta) solvent for PS at 34.8 °C [14], however the addition of 10% THF well improved solubility of PS polymer samples, even those with high molar masses in mixed eluent at ambient temperature. Mixed eluents were prepared by weighing of components with a precision better than 0.1%.

2.3. Polymers, injected solutions

Poly (methyl methacrylate)s of medium polydispersity and low stereoregularity with the most abundant molar masses (*M*) ranging from 0.67 to 16.0 kg mol^{-1} were employed. They were gifts of Dr. W. Wunderlich (Röhm, Darmstadt, Germany) and Dr. J. Herz (Institut Sadron, CNRS, Strasbourg, France). Narrow molar mass distribution polystyrenes 10.1, 37.0, 233.0 and 498.0 kg mol^{-1} from Pressure Co, (Pittsburgh, PA, USA) were applied in the LC LCA systems monitored by a UV detector. All samples were dissolved and injected in pure desorli THF. The injection volumes varied from 20 to 2000 μl, however, the standard injection volume was 50 μl. Concentration of solutions was generally 1 mg ml^{-1} except for experiments, in which constant polymer amounts were injected in different sample volumes.

3. Results and discussion

The sample volume, v_i, applicable in LC LCA must be situated between two values, namely between the minimum and the maximum allowed injected sample volumes. The minimum allowed injected sample volume $v_{i,min}$ for a given column depends on the strengths of both eluent and desorli sample solvents. Sample solvent is diluted during sample elution due to band broadening processes. Still, concentration of desorli in the sample zone must not decrease below certain value, which is just able to prevent sample adsorption [6]. Therefore, $v_{i,min}$ is affected not only by the mutual interactivity of column packing with the sample molecules, but also by size and by the efficiency of the column used. The maximum allowed injected sample volume, $v_{i,max}$ depends on the interstitial volume and pore volume within column [6]. It is defined as $v_{i,max} \sim V_M - V_0$, where V_M is total volume of liquid within column and V_0 is interstitial volume. $v_{i,max}$ used in this study was 3 and 1 ml for the columns nos. 1 and 2, respectively. If sample volume exceeds $v_{i,max}$, the rear part of the injected sample zone is unable to catch the front of desorli zone. As a result, LC LCA peaks are broadened and deformed [6]. To demonstrate the effect of injected sample volume on the sample recovery, some typical elution profiles are displayed in Fig. 2a and b.

Below $v_i \sim 35$ μl, sample peak rapidly decreased. No elution of PMMA was observed for 20 μl injected volume. Too small zone of THF sample solvent did not permit sample elution. The same amount of PMMA injected in volumes of THF above 50 μl produced peaks of comparable but not equal sizes. It is evident that a part of sample was still

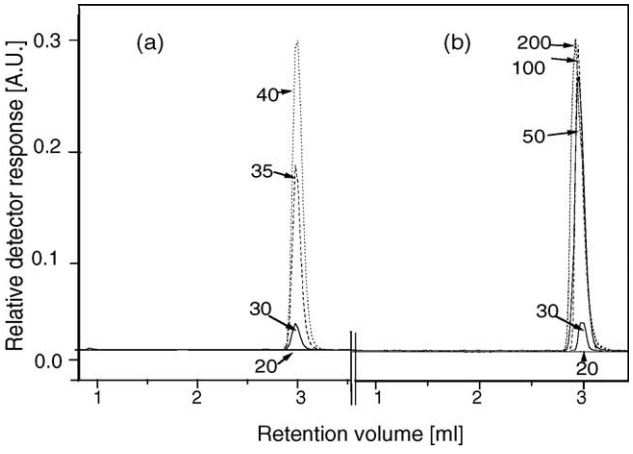

Fig. 2. Elution profiles of PMMA 294 kg mol^{-1} eluted in the LC LCA mode from the column no. 2. (a) Different injected sample volumes of the same concentration, 1 mg ml^{-1}. Numbers designate the sample volumes. (b) Different injected sample volumes, sample weight was constant, 10 μg. Numbers designate the sample volumes.

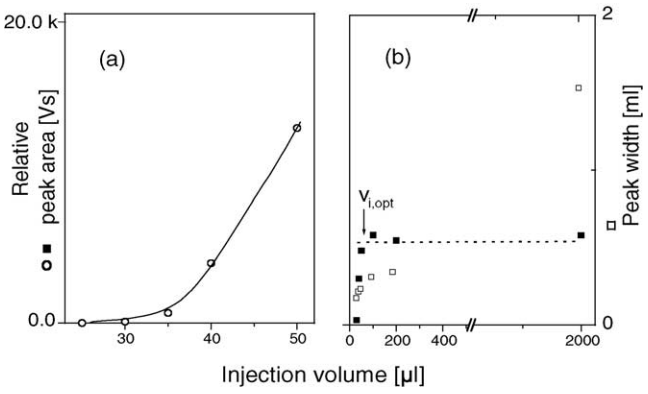

Fig. 3. Characteristics of peaks for PMMA 294 kg mol^{-1} in column no. 2. (a) Dependences of peak areas on the injected volume for constant sample concentration. (b) Dependences of peak parameters on the injected sample volume for constant injected weight of the polymer. Full squares represent polymer peak areas, while open squares designate polymer peak widths.

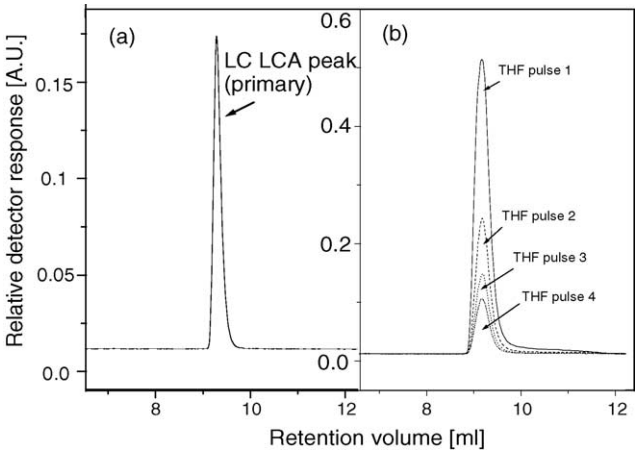

Fig. 4. (a) Chromatogram of PMMA 169 kg mol^{-1} eluted under LC LCA conditions from the column no. 1. (b) Elution profiles of same polymer sample desorbed from the same column by the subsequent 2 ml THF pulses. Pulses of THF were introduced immediately after the LC LCA experiment has been completed.

retained within column. Dependences of peak area versus injected volume are shown in Fig. 3a and b. Fig. 3a shows peak areas for experiments with constant concentration of polymer sample, but different injected volumes. In Fig. 3b, the amount of polymer sample was almost constant for different injected volumes.

In both cases, one can identify nearly linear parts. A deviation from linearity results from low desorption efficiency of a too small desorli zone. Clearly, application of sample volumes only slightly larger than $v_{i,min}$ can lead to large errors in LC LCA. One can define also the apparent "safe" sample volume, $v_{i,s}$. It lies above 40 μl in this case. Evidently, $v_{i,s}$ must be determined for each system studied by independent introducing experiments. Since LC LCA peaks are focused and their width only a little depends on v_i, it is advisable to inject a little larger sample volume than the $v_{i,s}$. Application of injected volume above $v_{i,s}$, however, does not necessarily secure full sample recovery. Therefore, in the next series of experiments, the actual PMMA recovery was checked. After the regular LC LCA experiment had been completed (Fig. 4a), large, 2 ml pulses of pure THF were repeatedly injected into the LC LCA column and eluted employing the original eluent. Surprisingly large residual, slightly broadened peaks were observed (Fig. 4b).

Their retention volumes corresponded to V_R of the original LC LCA peak. The area of peak no. 1 (produced by the first additional THF pulse) was about four times larger than that for the original peak. The areas of residual peaks produced by successive THF pulses gradually decreased but even fourth THF pulse gave rise to a quite well defined peak (Fig. 4b). Comparing the area of the original LC LCA peak with the total area of the residual peaks created by four THF pulses, it can be estimated that more than about 90% of PMMA remained within column no. 1 in the course of the original LC LCA elution. This is very low sample recovery, indeed. On the contrary, the same experiment carried out on column no. 2 produced higher sample recovery. Surprisingly, only about 30% of PMMA was retained within the column during the LC LCA elution in this case (result not shown). This indicates a possible dependence of sample recovery on the LC LCA column diameter.

Similar results as shown in Fig. 4a and b were obtained for different injected sample volumes. Even maximum injected sample volume $v_{i,max}$ did not prevent reduction of sample recovery. The reduction of LC LCA recovery can be explained by a slow equilibration of the column packing with the desorli solvent [6]. A rather high concentration of adsorli eluent seems to remain within the narrow pores of packing during passage of desorli zone containing sample. Macromolecules are "pulled" into the pores filled by adsorli eluent and stay adsorbed during passage of desorli zone. This may be responsible for the recovery reduction.

In order to monitor the role of THF zone extension, a tandem injection was performed. Immediately following a 50 μl sample, a 1.5 ml tandem zone of pure THF was injected. Column no. 1 was used, for which the total volume of THF injected (that is the sample plus the tandem THF) was still below $v_{i,max}$. LC LCA chromatograms obtained with a 1.5 ml tandem zone of THF and chromatogram of PMMA desorbed by the following new, additional 2 ml pulse of THF are shown in Fig. 5.

Comparing chromatograms obtained without and with the tandem THF zone (Figs. 4a,b and 5), it is evident that the tandem zone of THF increased sample recovery but still a large part of PMMA remained in the column. The establishment of solvation equilibrium on the column packing surface should be faster as the pore diameter raises. Therefore, the effect of pore diameter on the sample recovery was investigated. The molar mass independent LC LCA retention was observed for all packings under study (results not shown). This means that in agreement with [5] the eluent composition THF/toluene

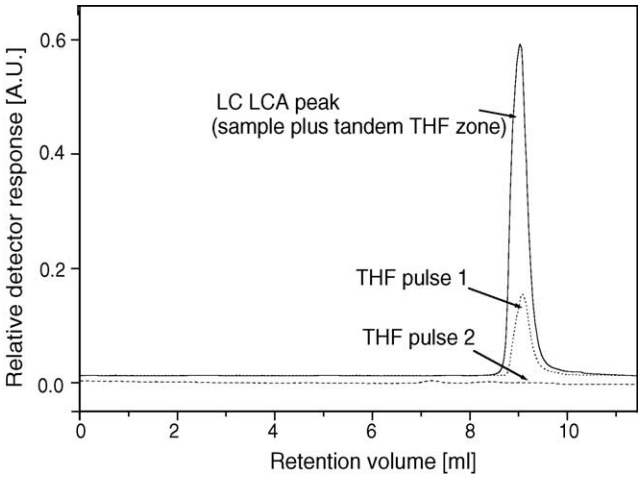

Fig. 5. Elution profiles of PMMA 169 kg mol^{-1} eluted under LC LCA conditions from column no. 1. The solid line represents the LC LCA (primary) polymer peak produced by a tandem injection of 1.5 ml THF zone immediately after polymer sample. Dotted lines indicate polymer desorbed from the column by the additional 2 ml pulse of THF injected after LC LCA experiment had been finished.

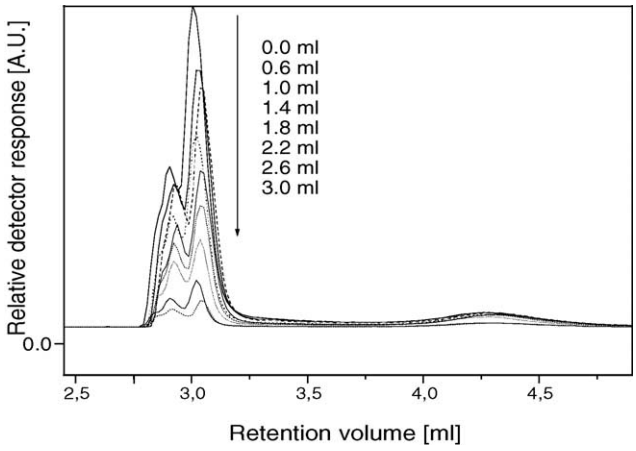

Fig. 7. Elution profiles of PMMA 294 kg mol^{-1} retained within column no. 2 in the course of an LC LCA experiment and then released applying an additional pulse of THF. Prior to the injection of a THF pulse, the column was filled by various volumes of THF in the backward direction and then flushed by the eluent.

35/65 (w/w) worked for different packing pore sizes. Selected chromatograms are shown in Fig. 6a and b.

It is evident that the amount of retained polymer was much larger in the narrow pore column no. 3 (10 nm) (Fig. 6b) than in the wide pore column no. 6 (100 nm) (Fig. 6a). However, the amount of PMMA retained in the 100 nm packing is still remarkable. The results obtained with columns nos. 4 and 5 (30 and 60 nm pores, respectively) confirm that the amount of retained polymer dropped with increasing pore size (results not shown). The peaks of high molar mass PMMA desorbed from the narrow pore column packing are extremely broadened and bimodal. A comparison of results for PMMA with

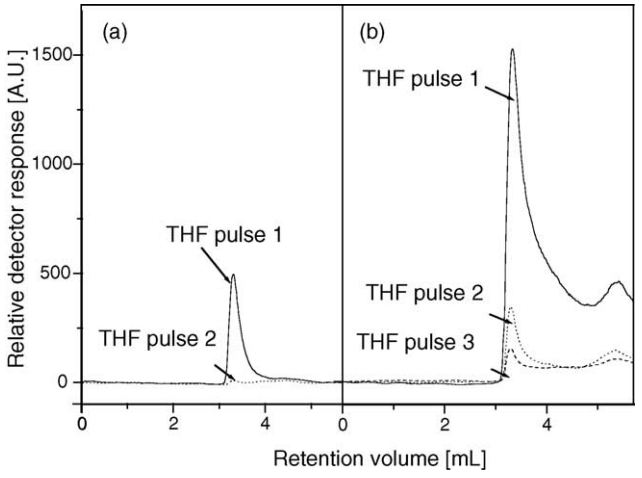

Fig. 6. Elution profiles of PMMA 613 kg mol^{-1} retained in the LC LCA columns and eluted by an additional 2 ml pulse of THF. Primary LC LCA peaks are not shown. (a) Wide pore (100 nm) column (no. 6). (b) Narrow pore (6 nm) column (no. 3).

various molar masses in Figs. 2–6 shows that the full retention of macromolecules in the course of the LC LCA experiments raises with increasing molar masses. Macromolecules, which would be fully excluded from the narrow pores of Silpearl (column no. 1, 6 nm) in the non-interactive size exclusion chromatography (SEC) mode, were extensively retained by adsorption in the course of the LC LCA experiments. This again confirms the hypothesis that large macromolecules decoil and reptate into the packing pores if the attractive interactions are strong enough [7,15,16].

The question immediately arises, in which part of the LC LCA column the macromolecules are retained. Three possibilities can be considered. The full retention takes place:

(i) near the column inlet. This is improbable because in this case entire sample would be successively retained in a large enough (e.g. no. 1) column;
(ii) near the column outlet, when the desorli zone becomes diluted;
(iii) within the entire volume of column.

To answer this question, the backflush experiments were performed (see Section 2). After the normal LC LCA experiment had been completed, the column was successively filled by different volumes of THF via its outlet. Pumping of THF desorli was stopped for about 1 min and the elution was restarted with original eluent in the original direction into detector (Fig. 1). Afterwards a pulse of THF has been introduced into the column, which resulted in desorption of polymer that was not desorbed in backflush experiment. Polymer amounts desorbed by these pulses are depicted in Fig. 7.

PMMA was found in all portions of column and one can conclude that polymer was adsorbed within entire volume of the sorbent. Still, the fact that the largest amount of PMMA was eluted at the end of experiment indicates that probably largest portion of sample is retained near the column inlet.

The desorption process is evidently rather complex and it results in multimodal peaks.

A series of quasi static experiments was performed to evaluate polymer desorption rate. Immediately after the LC LCA experiment had been concluded, the column was completely filled via its inlet with the volume of THF, which corresponded to V_M of the column used. Then, the flow was stopped for a time ranging from a few minutes to several hours. Next, the column was shortly flushed by eluent. Eventually, a new pulse of THF (2 ml) was introduced from autosampler into the column to release the still retained polymer. Our results showed that static desorption of polymer from narrow pores by pure THF was a very slow process since approximately 20 h time was needed for a quantitative release of PMMA from the column packed with 6 nm silica gel. It is also interesting to note that a relative short (2 ml) dynamic pulse of THF displaced PMMA, which was not desorbed in the course of long (several hours) static action of the same desorli.

It is evident that reduced sample recovery may bring about important problems in many liquid chromatography systems working under limiting conditions of adsorption. An exception represent the LC LCA experiments aimed at simple discrimination of two polymers whereas the non retained, that is the SEC eluted polymer is subject to further analysis and the LC LCA eluted species are discarded. A typical example is analysis and characterization of minor macromolecular admixtures [6,17], where only the minor component (a less polar polymer) is characterized either directly or applying an on-line SEC column. Another option is analysis of oligomers, in which the full retention of sample and resulting decreased sample recovery is less probable.

In attempt to quantitatively assume the sample recovery reduction, PS/bare silica gel/cyclohexane-THF system was investigated. THF (15 wt.%) in cyclohexane formed an efficient adsorli for PS on bare silica gel and this mixture was used as an LC LCA eluent. PS was dissolved and injected in the THF desorli. Column no. 1 was applied. UV detection was used because of transparency of eluent components. It is well known that linearity of the UV detector response is generally better compared to ELSD and evaluation of results was expected to be more accurate. Unfortunately, the changing refractive index of system during the desorli zone passage lead to appearance of split peaks (results not shown) and prevented quantitative evaluation of results. Applying additional pulses of THF after completion of LC LCA experiments we have, however, revealed that sample recovery was well below 100% also in this system.

The reduced sample recovery was also observed in liquid chromatography under limiting conditions using silica gel C 18 phase with polystyrene [17] and eluents composed of dimethylformamide and THF. In this case, former eluent component is a poor solvent for PS and "pushes" macromolecules into the C 18 bonded phase, while THF is a good solvent for PS and promotes its elution. Eluents were composed of a poor solvent (*N*,*N*-dimethyl formamide) which promoted sample retention and a good solvent (THF), which promoted elution of polystyrenes [11].

4. Conclusions

Reduced sample recovery was observed in liquid chromatography under limiting conditions of adsorption (LC LCA). A major part of polymer was fully retained within the entire volume of the LC LCA column packing. This phenomenon can badly affect results of analyses if the LC LCA eluting component of a polymer mixture should be further analyzed. On the other hand, LC LCA is suitable for analysis of minor macromolecular component(s) in multicomponent polymer systems provided the latter are eluted in the SEC modus and the LC LCA mechanism is used only for their discrimination from the major component(s). Macromolecules retained within the LC LCA column may affect its retentivity. Therefore, LC LCA columns should be periodically flushed with an efficient desorli, for a sufficiently long time. In any way, sample recovery should be evaluated in practical LC LCA systems before their application.

The full retention of macromolecules within column and resulting reduced sample recovery may represent a formidable problem also in many gradient polymer HPLC procedures. Surprisingly, the impact of this phenomenon was so far fully overlooked. For example, we have observed the "ghost" chromatograms of PMMA eluted from bare silica gel column packing in the eluent gradient from toluene to THF, when gradient was repeated without any sample injection [18]. The size of sample peaks was small but their retention volumes corresponded with those of original experiment. Similarly, Chang [19] observed ghost chromatograms also in some blank temperature gradient interaction chromatography experiments. The question of reduced sample recovery in the polymer HPLC employing enthalpic retention mechanism certainly deserves a detailed study.

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References

[1] D. Berek, Proceedings of the 5th Latin American Polymer Symposium, Mar del Plata, 1996, p. 37.
[2] A. Bartkowiak, D. Hunkeler, D. Berek, T. Spychaj, J. Appl. Polym. Sci. 69 (1998) 2549.
[3] A. Bartkowiak, R. Murgasova, M. Janco, D. Berek, T. Spychaj, D. Hunkeler, Int. J. Polym. Anal. Charact. 5 (1999) 137.
[4] D. Berek, D. Hunkeler, J. Liq. Chrom. Rel. Technol. 22 (1999) 2867.

[5] D. Berek, Macromolecules 31 (1998) 8517.
[6] M. Šnauko, D. Berek, J. Appl. Polym. Sci., submitted for publication.
[7] M. Šnauko, D. Berek, Chromatographia Suppl. 57 (2003) S-55.
[8] T. Macko, D. Hunkeler, Adv. Polym. Sci. 163 (2003) 61.
[9] B. Trathnigg, M. Kollroser, J. Chromatogr. A 768 (1997) 223.
[10] B. Trathnigg, M. Kollroser, D. Berek, S.H. Nguyen, D. Hunkeler, in: T. Provder (Ed.), Chromatography of Polymers: Hyphenated and Multidimensional Techniques (ACS Symp. Ser., No. 731), American Chemical Society, Washington, DC, 1999, p. 95.
[11] D. Berek, Chromatographia Suppl. 57 (2003) S-45.
[12] O. Chiantore, J. Liq. Chromatogr. 7 (1984) 1.
[13] D. Berek, M. Jančo, G. Meira, J. Polym. Sci., Part A: Chem. 36 (1998) 1363.
[14] M. Kurata, Y. Tsunashima, in: J. Brandrup, E.H. Immergut, E.A. Grulke (Eds.), Polymer Handbook, fourth edition, Wiley, New York, 1999 (Chapter VII).
[15] B.G. Belenkii, M.D. Valchikina, I.A. Vakhtina, E.S. Gankina, O.G. Tarakanov, J. Chromatogr. 129 (1976) 115.
[16] T. Macko, J.F. Denayer, H. Pasch, L. Pan, J. Li, A. Raphael, Chromatographia 59 (2004) 461.
[17] M. Šnauko, D. Berek, unpublished results.
[18] D. Berek, unpublished results.
[19] T. Chang, POSTECH University, Pohang, South Korea, personal communication.