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Separation of parent homopolymers from diblock copolymers by liquid chromatography under limiting conditions of desorption 3. Role of column packing

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ABSTRACT

The novel separation method, liquid chromatography under limiting conditions of desorption, LC LCD enables rapid one-step discrimination of both parent homopolymers from diblock copolymers. The low-molecular admixtures/impurities can be base-line separated, as well. The general rules for selection of the LC LCD columns are reviewed. Bare silica gel column packings are discussed in detail. Selected examples of separation are presented. They demonstrate that the principle of LC LCD separation is not affected by the particle size and initial purity of bare silica gel column packing nor by its effective pore diameter and volume. However, appropriate choice of the packing pore size facilitates base-line separation of particular sample constituents. Important may be the column history; columns saturated with previously adsorbed polymers may lose their performance. Up to a certain limit, success of the LC LCD separation does not depend on the column efficiency and reasonable results can be obtained even with the columns packed with rather big particles. This indicates possibility of the large-scale preparative applications and feasibility of the high-speed LC LCD separations.

1. Introduction

Most researchers who work in polymer synthesis characterize their products by size exclusion chromatography, SEC. The attractive features of SEC are speed, wide availability and experimental simplicity. Low separation selectivity of SEC, however does not enable discrimination of multicomponent polymer systems with constituents of similar molecular sizes. Limited sample capacity and insufficient sensitivity of detectors prevents identification of sample constituents of low relative concentration. Moreover, the results of SEC depend simultaneously on all molecular characteristics of macromolecules, not only on their molar mass. Only few researchers are willing to invest time in scouting for suitable separation procedure among the experimentally rather complicated *critical* or *gradient* methods of liquid chromatography to attain optimum separation and comprehensive characterization of their products. Consequently, presence of parent homopolymers in block copolymers often remains non-disclosed. Simple, efficient and robust alternative separation procedures are needed. One of the candidates is liquid chromatography under limiting conditions of adsorption, LC LCD [1–7]. LC LCD employs the difference between elution rates of small molecules of mobile phase and separated macromolecules within the liquid chromatographic column packed with porous particles. Small molecules permeate most pores of the column packing and therefore their elution rate is low. On the contrary, macromolecules are partially or fully pore-excluded and therefore they tend to elute rapidly. Certain low-molecular substances foster adsorption of particular kind of macromolecules within the liquid chromatographic column. If introduced into column in front of sample, the adsorption promoting substance may create a slowly moving *barrier* that selectively decelerates transport of the adsorbing macromolecules. The latter accumulate on the barrier edge and elute behind the barrier irrespectively of their size in solution. At the same time, the fast transport of the non-adsorbed polymer species remains non-hindered. In this way, the distinct constituents of complex polymer systems can be mutually separated independently of their molar mass. To facilitate applications of LC LCD, the basic rules are presented in this series of papers for selection and optimization of appropriate LC LCD systems. Potential column packings for LC LCD are specified in present study, and their actual role is demonstrated on the model commercial lipophilic diblock copolymers. The commonly available silica-based column packings are applied though also some home-made silica gels are considered. It is likely that the conclusions presented can be generalized about numerous other complex polymer systems, and about other LC LCD methods.

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2. Columns for LC LCD of block copolymers – general considerations

2.1. Basic properties of column packings

The LC LCD column packings must be mechanically and also chemically stable to withstand impact of elevated pressure, as well as of mobile phase components and sample constituents. Soft, swollen gels may alter geometry of their beds when either pressure or polarity of environment is suddenly altered. For this reason the homogeneously crosslinked organic gels are practically inapplicable in LC LCD. The densely (heterogeneously) crosslinked organic porous particles are much more mechanically stable and the extent of their swelling is low. However, topology of their surface is not well defined as many free polymer chains protrude over pore walls. A layer of quasi-liquid phase can be formed on the surface of pores [8,9]. As a result, besides *adsorption retention mechanism* also the *enthalpic partition* (absorption) of sample molecules may play important role [10]. Therefore inorganic column packings are preferred in LC LCD, while the so far dominating material is bare porous silica. Alternative inorganic porous materials such as titania, zirconia, hafnia, and alumina that have already been applied in HPLC of low-molecular substances are still waiting for their evaluation in LC LCD.

The important issue of the LC LCD column packings is their *interactivity* with separated macromolecules. The packing is to be active enough to fully retain by adsorption at least one kind of polymer chains present in the block copolymer under study. Bare silica gel is known for its intensive attractive interactions with numerous different macromolecules of medium-to-high polarity.

Further significant parameter of the LC LCD column packings is their *pore size*. The actual geometry of pores in silica gel is unknown. Therefore it is more useful to speak about the (mean) *pore size* than about the (mean) *pore diameter*. On the other hand, the term *pore size* also includes *volume* of pores, which is an important parameter in liquid chromatography under limiting conditions of desorption. The terms *effective* (mean) *pore diameter* and *pore volume* will be used in this work.

It is intuitively assumed that the narrow-pore column packings would be advantageous for the LC LCD separations. In this case the non-retained macromolecules would be mostly excluded from the packing pores to elute in the interstitial volume of the column. Since the retained macromolecules elute behind the barrier that is approximately in the total volume of liquid within the column, the difference between the actual retention volumes would be large for the non-adsorbed (SEC eluted) and for the adsorbed (LC LCD eluted) sample constituents. This would bring about increased separation selectivity. However, the details of retention processes in LC LCD are not yet fully understood. There may be a substantial difference in the extent of interactions of macromolecules with the column packings in dependence on their effective pore diameters. For example, the actual retention of the medium-polarity macromolecules may be insufficient for the pore-excluded species because only the outer packing surface and pore orifices are available for their *weak adsorption* within the column. This may negatively affect the blocking power of the barrier. In contrast, the strongly interacting macromolecules may partially "snake-into" the narrow pores to assume *the flower-like conformation* [11–13] and to stay fully retained within the column. The equilibration of pore walls with the adsorption promoting liquid, an *adsorli* is the necessary condition for such "reptation" process, which is likely augmented by the osmotic effects [14]. Flower-like interactions may substantially decrease sample recovery. Therefore wider pores could be advantageous for liquid chromatography methods that apply adsorption retention mechanism, including LC LCD. However, actual effect of pore size on the extent of adsorption of macromolecules is not clear. On the base of simulations, Wang et al. [15] have shown that the wide-pore silica gels should be more interactive than the narrow-pore column packings. Still, it is difficult to quantitatively prove this conclusion because the silica gels with distinct pore sizes but with identical surface properties are not available. In any case, only a part of the coiled polymer chain comes into contact with the inner walls of a pore in the course of the LC LCD process – even if the pore is large enough to accommodate entire macromolecule.

The pores with very large effective diameters bring about specific limitation. The retention volume of the non-retained, SEC eluted macromolecules depends on the extent of their pore exclusion. Obviously, the non-retained sample constituents with lower molar masses leave the wide-pore LC LCD columns in the retention volumes larger than the interstitial volume of column. Therefore they could co-elute with the macromolecules transported behind the barrier, near the total volume of liquid within column. The application of the packings with increased *pore volume* could largely mitigate the latter problem because they would provide increased space on the LC LCD chromatograms for accommodation of the late-eluted peaks of the non-adsorbed macromolecules, as well as of the low-molecular additives/impurities. In this way, pore volume of the packings may substantially improve overall selectivity of the LC LCD columns. Therefore, the role of the effective pore diameter should be considered in conjunction with the packing pore volume. It is anticipated that efficacious LC LCD separations can be obtained with packings that exhibit very large pore volume even if their pores are rather wide. It could be even possible to directly characterize the non-adsorbed, LC LCD discriminated homopolymers, which were eluted in the SEC mode. In that case, the molar mass and molar mass distribution of the non-retained, base-line separated polymer could be directly calculated from the corresponding peak of the LC LCD chromatogram with help of the conventional SEC software. Column packings with large pore volumes are especially welcome in the LC LCD separations of multicomponent polymer systems that necessitate application of multiple barriers.

As to effective diameters, the materials with pores in the range of 6–30 nm may represent appropriate compromise for LC LCD separation of high molar mass polymers. The column packings with 3–5 nm pores may be applicable for oligomers that inevitably much less suffer from the limited sample recovery than polymers with molar masses over 10 kg mol^{-1}. Unfortunately, most commercially available totally porous and mechanically strong column packings with the above pore diameters exhibit rather small pore volume in the range of 1 mL g^{-1} and less. Moreover, the pore volume of porous materials as a rule rapidly diminishes with the effective pore diameter below 6 nm.

As known, the *size* of packing *particles* and their *pore structure* extensively affect the extent of broadening of chromatographic zones that is the efficiency of HPLC columns. If it is accepted that the LC LCD retention processes take place mostly in the neighborhood of pore orifices and on the outer surface of particles, the main reason for peak broadening, namely the slow mass transfer within pores would be obviated. Further, as follows from the principle of LC LCD, the method produces well-focused peaks because during polymer elution, the fast transported rear part of the injected zone is catching up its decelerated front part [2–4]. Therefore, it is expected that larger packing particles can be used in LC LCD and the known problems connected with application of very small HPLC packing particles could be avoided or at least mitigated - such as high backpressure and danger of column blocking by the solid microparticles possibly present in samples, as well as extensive production of heat due to friction [16]. It is likely that the 10 μm particles may present a good choice for many practical LC LCD separations.

2.2. Silica gel column packings

As suggested, bare silica gels are column packings of choice for LC LCD. Numerous different HPLC silica gels are commonly available. They show varied topology and concentration of active silanols, as well as distinct degree of purity. Consequently both their surface properties and adsorption ability are unequal. Particular HPLC silica gels also exhibit various exterior (particle size, shape, and roughness) and also interior (pore size and geometry) structure.

It is supposed that a quite large fraction of the inner surface of the LC LCD column packing may remain in equilibrium with the adsorption suppressing mobile phase, with the *desorli* because the contact of packing with the narrow zone of the barrier liquid is rather brief [13]. The latter idea is supported with the preliminary experimental finding that the LC LCD sample recovery on silica gel based column packings only moderately depends on the packing pore size and on the molar mass of the separated polymer [4,13]. This is an important difference between LC LCD and other coupled methods of polymer liquid chromatography such as for example liquid chromatography under critical conditions, and various procedures of gradient polymer HPLC. The latter procedures often suffer from an incomplete sample recovery, which tends to worsen as the polymer molar mass increases and the packing pore size decreases [17–20].

As mentioned, enthalpic interactivity is an important requirement of the LC LCD columns. Bare silica gels are expected to satisfy most demands of the LC LCD separations of lipophilic polymers of medium-to-high polarity, as far as they are soluble in organic solvents. Presumably the adsorption of medium- and high-polarity polymer segments on the silanol groups of silica gel is largely responsible for the sample retention. Silica gels with polar bonded groups such as for example nitril-, amino-, cyano-, or dihydroxypropyl- may be suitable for application of the interphase adsorption retention mechanism [21]. In that case, however the overall polymer retention may be also affected by the non-polar spacers, usually *n*-propyl- groups. Adsorption of the non-polar polymers on bare silica gel or on silica bonded with polar groups may be rather weak. Nonpolar solvents are to be applied as adsorlis. Alternatively, alkyl bonded silica gels can be utilized for the selective retention of non-polar sample constituents. However, the retention mechanism of macromolecules on silica gel bonded with longer alkyl groups is as a rule switched from the *interphase adsorption* to the *enthalpic partition* (*absorption*) [22] and the corresponding barrier method is called *liquid chromatography under limiting conditions of unpartition*, LC LCU [23]. Chemical nature and the resulting enthalpic interactivity of the column packings play less important role in the barrier procedures that employ *phase separation* (*precipitation*) *retention mechanism* [24–26]. Still, sizeable enthalpic interactions between the separated macromolecules and the column packing are to be suppressed also in the latter case to prevent the mixed retention mechanism [26].

Preferential sorption of a mixed mobile phase component on the packing surface may bring about formation of the layer of liquid with a changed composition. Enthalpic partition of macromolecules may take place between the mobile phase and such *quasi-stationary* phase [27] and to affect sample retention. However, the resulting changes in LC LCD retention volumes are anticipated to be small. The preferential displacement processes may appear of particular eluent component from the surface layer when in contact with sample molecules [28,29]. They can give rise to *system peaks*, which are however negligible when compared with the mobile phase composition perturbations caused by the barriers themselves.

In summary, the mesoporous bare silica gel column packings with as large pore volume as possible are expected to fulfill most needs of the LC LCD separations of complex polymer systems that contain at least one constituent of medium-to-high polarity. The well defined, largely uniform adsorption retention mechanism is to be maintained. The effects of undesired mixed enthalpic retention mechanisms that may include enthalpic partition and/or phase separation (insolubility) should be considered when unexpected results occur.

2.3. Column size

The typical feature of LC LCD is its large sample capacity in terms of both sample concentration and volume [2]. The peak focusing that takes place on the barrier edge during polymer elution ameliorates detectability of solutes and it is expected to be beneficial in two-dimensional polymer liquid chromatography [30–32]. Another advantage of the optimized LC LCD is generally high separation selectivity, which permits application of relatively short columns. In order to save solvents and time, very small columns can be utilized in the course of the LC LCD method development that is during identification of appropriate column packing, eluent and barrier composition, as well as temperature of experiment. The column size should be increased if multiple barriers are to be applied and if presence of low molecular or oligomeric impurities in the sample is expected. In both cases, space for several peaks is to be provided on the LC LCD chromatograms [5]. Still, fast LC LCD separations can be achieved also with larger columns with help of increased elution rate [7]. High elution rate may, however cause excessive heat evolution due to flow-induced friction [16] even with rather large packing particles. In order to allow the unhindered heat transfer, the column diameter should be small. The extent of heat dissipation can be easily assessed from temperature of the column effluent.

The overall column size also must match up with the barrier volume(s). When adjusting appropriate volume of barrier(s), the dilution process should be taken into account. A barrier with too small volume may become permeable for separated macromolecules due to its dilution during its travel along the column. The safest LC LCD procedures apply barrier volumes large enough so that the composition of barrier at the column outlet attains the initial composition [33]. However, the application of multiple barriers cannot be realized if their volumes are too large. Evidently a compromise must be sought. The detailed study of barrier volume effect on the LC LCD separation will be presented in the future study.

The so far applied LC LCD column sizes varied between (250 × 4) and (300 × 7.5) mm. It is anticipated that the analytical LC LCD separations with the smaller columns and the preparative LC LCD applications with the correspondingly larger columns will be well feasible, as well.

3. Experimental

The detailed description of apparatus used, as well as the arrangement of experiments is given in Parts I and II of this series [6,7], respectively. Therefore, only basic information will be given in present paper. It is important to note that most experiments were done with evaporative light scattering detector from PL – Agilent, Model 1000. The alternative detector was a differential refractometer.

With one exception, columns were slurry packed with different bare silica gels at pressure of 40 MPa. The actual column dimensions are given in the Figure captions. The basic characteristics of the silica gel column packings applied in present study are given in Table 1. The size of the column packing particles was 10 μm, the exceptions were Develosil and SG-10 material with particles of 5 μm and Kromasil 100 with particles of 16 and 25 μm. Columns were thermostated to 30 ± 0.1 °C.

Table 1
Properties of silica gels applied in present study.

Column	Designation supplier	Effective pore diameter[a] [nm]	Pore volume[b]	Purity[c]	Excluded molar mass [kg mol^{-1}][f]	N_T[g]
1	Develosil	3–4	0.3	B	5	26,000
2	Silpearl	5	0.5	A[d]	7	14,800
3	Kromasil 60	6	0.9	B	40	37,400
4	Kromasil 100	10	0.9	B	80	27,900
5	Kromasil 300	30	0.9	B	500	40,000
6	Biospher 100	10	1.1	A	100	32,100
7	Kromasil 100[h]	10	0.9	A	40	19,900
8	Kromasil 100[i]	10	0.9	A	40	10,300
9	Separon SGX	10	1.2	A	20	40,000
10	SG-7	10	1.0	B[e]	100	27,200
11	SG-10	30	1.5	B[e]	400	35,400

[a] Nominal values provided by producers.
[b] Estimated from the bulk density.
[c] Standard HPLC designation: a silica gels containing metal impurities. As a rule, they were prepared from water glass.
[d] Silpearl was prepared from silica sol in presence of Ca ions [34]. B silica gels of high purity were engineered from the sol of silicic acid, or from tetraethylethylsilane. Alternatively, the metal traces were removed from silica gel with help of a proprietary procedure.
[e] [35].
[f] Values of excluded molar mass were estimated from the log M *vs.* V_R dependences for narrow molar mass distribution polystyrenes in tetrahydrofuran, THF.
[g] Theoretical plate numbers of the columns were determined by toluene probes in neat THF eluent with help of refractive index detector and re-calculated for 1 m column length. Nominal particle size was 10 μm with exception of Develosil (5 μm).
[h] Theoretical plate numbers of the columns were determined by toluene probes in neat THF eluent with help of refractive index detector and re-calculated for 1 m column length. Nominal particle size was 10 μm with exception of Develosil (5 μm and Kromasil 60 with 16 μm).
[i] Theoretical plate numbers of the columns were determined by toluene probes in neat THF eluent with help of refractive index detector and re-calculated for 1 m column length. Nominal particle size was 10 μm with exception of Develosil (5 μm and Kromasil 60 with 25 μm).

A tandem of three six-port two-way injection valves was applied. In dependence on the column size, the *sample injection valve* was provided with the loops of 20 or 50 μL. It was followed by *the barrier valve*, which was used for independent injection of the barrier(s) into the system before the sample. The barrier valve was equipped with the loops either 200 or 1000 μL. The third, *displacement valve* was situated between the pumping system and the sample injector. It served for the qualitative assessment of the sample recovery by the injections of 1500 μL of *N*,*N*-dimethylformamide displacer. The latter solvent is very strong considering bare silica gel and it quantitatively releases many soluble polar polymers fully retained on its surface from the weak solvents [39]. The estimated sample recovery in most present systems was higher than 80%. The exceptions were polar polymers injected into Silpearl column packing and non-polar polymers eluted in eluents that contained cyclohexane. The injected polymer concentration was about 1 mg mL^{-1}, and the flow rate was 1 mL min^{-1}. The actual barrier compositions are listed in Figure captions. The entire volumes of barrier, sample, and displacer loops were injected.

PS-*b*-PMMA copolymers were from Polymer Laboratories – Agilent. Their total molar masses given by the producer were 39 kg mol^{-1} (sample Nr. 2) according to the designation applied in [6], and 140 kg mol^{-1} (sample Nr. 4), respectively. The content of PS chains in samples Nr. 2, and Nr. 4 was 55.0, and 76.6 wt.%, respectively. The weight average molar masses of the PS precursors in above block copolymers determined by means of SEC by the producer were 21.45, and 107 kg mol^{-1}, respectively. The content of homopolymers in samples 2, and 4 as estimated from the introductory LC LCD measurements was 2.1, and 6 wt.% for PS, and 0.4, and 0.7 wt.%, for PMMA respectively [6]. Poly(isoprene)s, PI and block copolymer polystyrene-block-poly(isoprene), PS-*b*-PI were prepared in the Department of Chemistry, Pohang University of Science and Technology, South Korea.

Narrow molar mass distribution polystyrenes were from Pressure Chemicals, USA. Their molar masses lay between 0.88 and 2000 kg mol^{-1}. Medium-broad molar mass distribution, low tacticity poly(methyl methacrylate)s were from Merck, Germany. Their molar masses ranged from 16 to 613 kg mol^{-1} [40]. Narrow molar mass distribution PMMA with molar masses 1.3 and 8.0 kg mol^{-1} were from Institut Sadron, Strasbourg, France. Narrow molar mass distribution homopolymers of PI were the precursors of the block copolymers. The homopolymers were used for identification of appropriate eluent and barrier compositions according to procedures described in papers [6,7], as well as for the spiking of copolymer samples. The molar masses of PS and PMMA were chosen similar to those of the corresponding blocks in the block copolymers as given by the suppliers. The model admixture/impurity was tricresylphosphate, TCP from Slavus, Slovakia. In the eluents used, TCP did not adsorb on the silica gel and because of its low volatility it could be well detected with the ELS detector provided medium temperature of evaporator was applied.

Tetrahydrofuran, THF, toluene, cyclohexane, and dichloromethane, DCM, were applied as eluent and barrier components while DMF served as an efficacious displacer of macromolecules trapped within LC LCD columns. Because of insolubility of PI chains in DMF, THF was applied as the displacer for PS, PI and diblock copolymer PS-*b*-PI. Analytical grade THF, and toluene were from CentralChem, Slovakia, and DMF of HPLC grade was from Scharlau, Spain. THF and toluene were distilled immediately before use. THF was stabilized with 0.2 g L^{-1} of 2,6-di-tert-butyl-4-methyl phenol.

In most LC LCD experiments temperature of the ELSD evaporator was set at 80 °C ("Method 1") except for measurements when DMF displacer was used. In the latter cases, temperature of evaporator was 180 °C ("Method 6"). Otherwise, the detector responded to the DMF zone. At this temperature, TCP could not be detected.

Liquids that dissolved polymers under study were applied as eluent and barrier components. In connection with bare silica gel column packings, neat toluene is a desorli for polystyrene, and poly(isoprene). THF is a desorli for all polymers chains under study. Toluene is a desorli for PS but it is an adsorli for PMMA chains while cyclohexane is appropriate adsorli for PS. The eluents were desorli/adsorli mixtures and their compositions were adjusted so that they acted as desorlis for all sample constituents; they promoted the SEC or SEC-like elution of homo- and block- copolymers under study. Barriers for the adsorbing homopolymers and for the corresponding block copolymers were desorli/adsorli mixtures with increased concentration of adsorli. Two barriers with distinct deceleration ability were used [6,7]. 100% toluene or cyclohexane adsorli was used as barrier #1 for the block copolymers. Barriers #2 were less powerful; they had to let-through block copolymers while decelerating the corresponding homopolymers. In most cases, the composition of barriers for PS-*b*-PMMA copolymers was the same as applied previously [7], while the appropriate barrier compositions for PS-*b*-PI copolymers were identified with a series of preliminary experiments with barriers of different compositions [39]. The actual mobile phase and barrier compositions are specified in Figure captions.

Different time delays between injections were applied [7]. The actual injection times of barriers and sample are quoted in Figure captions.

The role of eluent and barrier nature and composition in LC LCD will be in detail discussed in the following papers of this series.

3.1. Important notes

Mobile phase and barrier compositions are given in the weight parts. Desorli component is always quoted first. Samples were dissolved and injected in eluent. The chromatograms were recorded from the time of sample (not barrier) injection. However, the overall timing of experiments started in the moment of the first barrier introduction. The time delays between introduction of sample and barrier or between two barriers are given in minutes and seconds, for example "0–2–5:30" denotes the situation when barrier #2 was injected 2 min after barrier #1 and sample was injected 3 min and 30 s after barrier #2. This means that sample was injected 5 min and 30 s after barrier #1 and in that moment the V_R measurement started. Time delay designation 0–0:36–0 means that sample was introduced immediately after barrier #2, 36 s after barrier #1.

4. Results and discussion

4.1. Effect of particle size and column efficiency

Three different particle sizes of Kromasil 100 were compared, namely 10, 17, and 25 μm (Table 1). The slurry-packed Kromasil 100 with particles 25 μm exhibited very low efficiency expressed as a number of theoretical plates N_T, about 900 per meter. The resulting LC LCD peaks were broad and skewed (results not shown). Therefore, the latter material was dry-packed with the "tap-method" The plate number increased but it was still substantially lower than N_T of the columns packed with 10 and 16 μm particles (Table 1). The LC LCD results for block copolymers sample Nr. 4 obtained with 10, 16, and 25 μm particles are depicted in Fig. 1. The peaks for particular particle size are mutually shifted and roughly follow the differences in retention volumes of excluded PS homopolymers, which were 6; 5.4, and 5.5 mL for the particles 10, 16, and 25 μm, respectively. The peaks obtained with large column packing particles are surprisingly narrow. Irrespective of particle size, the peaks of block copolymers were often split. This phenomenon could be well observed when samples were injected at low concentration so that their peaks could be displayed in full. In that case, however, the peaks of homopolymers become non-detectable. The LC LCD peak splitting was observed also with some homopolymers [5]. Its reason is so far unknown. The answer to this question may bring the detailed study of the barrier shape and also results of the two-dimensional LC LCD × SEC experiments, which are under preparation. It is, however evident that the LC LCD separation principle has been maintained irrespectively of the particle size and that good separation could be achieved with columns of rather low efficiency packed with large particles.

The LC LCD experiments with PMMA homopolymers lead to the same conclusion when narrow pore silica gel columns of different efficiencies were compared [4]. However, it is expected that there may be minimum column efficiency, below which the reasonable LC LCD separation is no more possible. According to our experience, this minimum column efficiency may be situated in the range of 6–8000 theoretical plates per meter, as determined with a low molar mass substance. Below this limit, the barrier edge may be poorly defined. The results demonstrate suitability of the LC LCD method for preparative applications and indirectly also for the high-speed separations [7], in which the column efficiency inevitably diminishes.

4.2. Effect of pore size - effective pore diameter and pore volume

Intuitively chosen bare silica gels Silpearl and Kromasil 60 with the effective pore diameters 5 and 6 nm, and pore volumes 0.5 and 0.9 mL g^{-1}, respectively were used in our previous LC LCD separations of parent homopolymers from diblock copolymers [6,7]. In present study, the range of both the effective pore diameter and the pore volume was substantially extended (Table 1).

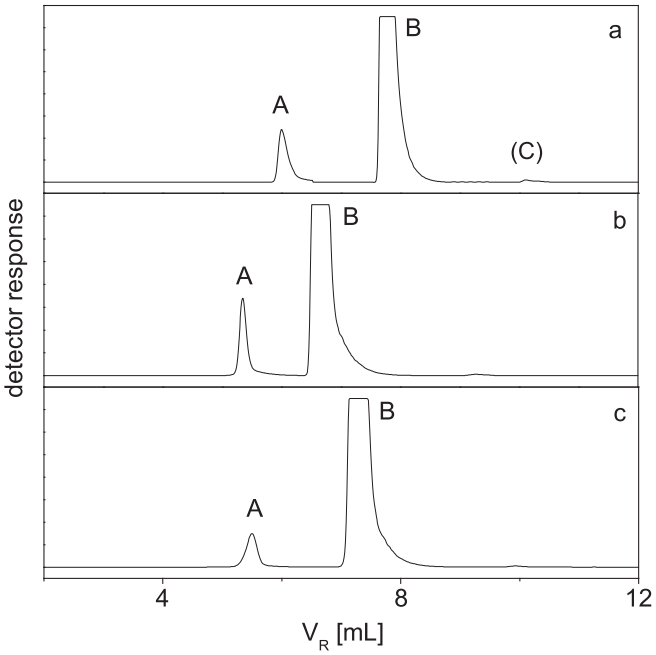

Fig. 1. LC LCD chromatograms of sample Nr. 4 obtained with Kromasil 100 columns (300 × 7.5) mm packed with different particle sizes. $v_i = 50$ μL; $c_i \sim 1$ mg mL^{-1}; eluent THF/toluene: 70/30; barrier #1: neat toluene; barrier #2: THF/toluene 35/65; injection delays: 0–3–5 min. A – PS; B – block copolymer; (C) – PMMA. Particle sizes: a – 10 μm; b – 16 μm; c – 25 μm.

The effective pore volumes of commercial silica gels are provided by producers. The actual pore volume can be estimated from the bulk density of materials. The pore volume, V_p of all Kromasil packings is about 0.9 mL g^{-1}, while it is about 1 mL g^{-1} for Biospher SI-100, Separon SGX and SG-7, and even over 1.5 mL g^{-1} for the SG-10 material, which was prepared with a proprietary procedure [38]. Pore volume of Develosil given by the producer is 0.5 mL g^{-1} but the value calculated from the bulk density indicates pore volume only about 0.3 mL g^{-1}.

Valuable information on the pore sizes of HPLC column packings can be extracted from the dependences log M *vs.* V_R. These are obtained with help of the polymer probes with known molar masses and narrow molar mass distributions. A thermodynamically good desorli solvent for polymer probes is applied as mobile phase. The log M *vs.* V_R dependences enable to estimate both the distribution of effective diameters and the volume of packing pores, and also to assess the bed density (interstitial volume) of particular columns. The log M *vs.* V_R plots for the silica gels under study are depicted in Figs. 2 and 3. Polystyrene standards with the neat THF mobile phase were used in the measurements, mostly with the refractive index detector. Benzene was used as a probe to assess the very small pores. In spite of similar particle sizes, nearly identical column packing procedure, and equal overall column size except for Biospher 100 and SG-7, the interstitial volumes of individual columns fairly deviate from each other. The particular pore volumes are well different, too and, as a result, the total volumes of liquid within columns are rather unequal. Therefore, the LC LCD retention volumes obtained with specific packings under otherwise identical experimental conditions can be hardly compared *ex directo*.

The excluded molar mass of silica gel SG-10 and also the shape of its log M *vs.* V_R dependence resemble Kromasil 300 but the pore volume of SG-10 material is uniquely large. As explained in the part "General considerations", this property may be valuable for

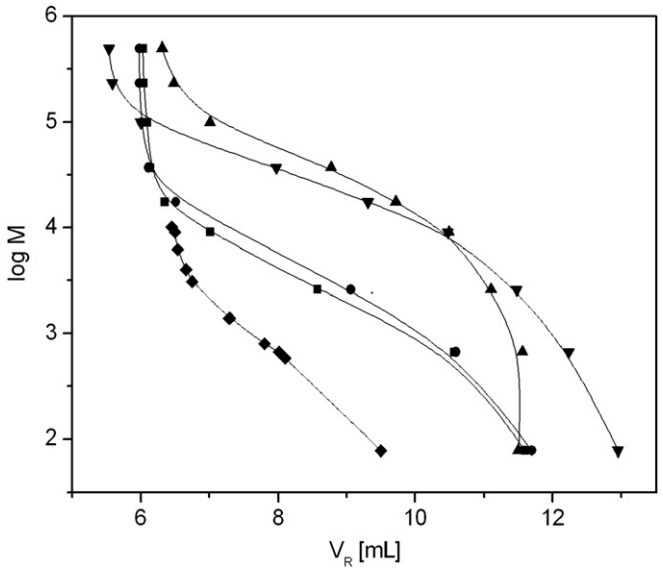

Fig. 2. Plots of log *M vs.* V_R as determined with PS standards and benzene in THF. $v_i = 50$ μL; $c_i \sim 1$ mg mL^{-1}. Column size (300 × 7.5) mm. Develosil (◆); Kromasil 60 (■); Kromasil 100 (●); Kromasil 300 (▲); SG – 10 (▼).

numerous LC LCD applications. The effective pore diameter of Develosil is small and the excluded molar mass of PS is situated well below 10 kg mol^{-1}. This would be favorable for the LC LCD separations of macromolecules with low molar masses. However, the small pore volume of Develosil makes it inapplicable for simultaneous separation of both parent homopolymers from diblock copolymers because the tandem of two distinct barriers can be hardly applied. Therefore, Develosil was eliminated from the following LC LCD experiments with diblock copolymers. The excluded molar masses of Kromasil 60, 100 and 300 lie in the range of 40, 80 and 500 kg mol^{-1}, respectively. Except for the slightly different excluded molar mass of PS, the overall courses of the log *M vs.* V_R dependences for Kromasil 60 and 100 are rather similar. It is likely that Kromasil 60 contains larger fraction of very small pores, which are no more permeable for the PS probes. Small pores substantially contribute to the surface area of porous materials and thus they decrease the calculated effective mean pore diameter. Pore volume of all three Kromasil packings is similar (Fig. 3).

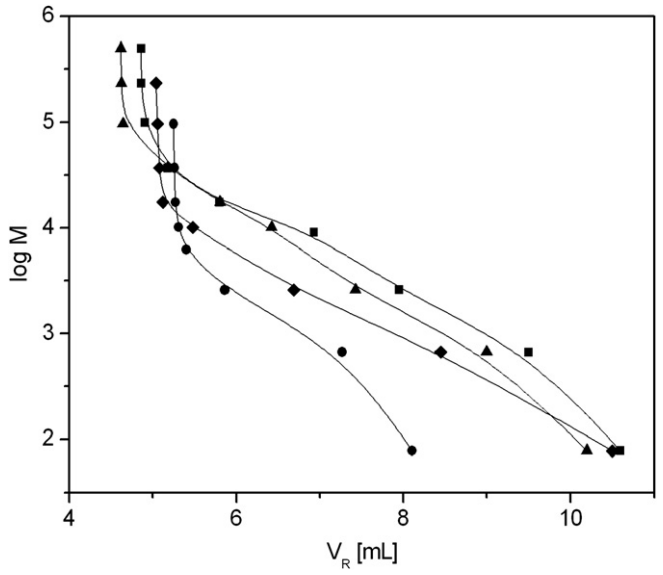

Fig. 3. Plots of log *M vs.* V_R measured with PS standards and benzene in THF. $v_i = 50$ μL; $c_i = 1$ mg mL^{-1}. Silpearl (●) – column size (300 × 7.5) mm; Separon SGX (◆) – column size (300 × 7.5) mm. Biospher SI-100 (▲) – column size (250 × 8) mm; SG-7 (■) – column size (250 × 8) mm.

The courses of the log *M vs.* V_R dependences for Biospher SI-100 and for SG-7 indicate that the effective pore diameters of these materials are situated between that of Kromasil 100 and Kromasil 300. Their excluded molar masses lie in the region of 100 kg mol^{-1} and also their pore size distribution is similar. The main distinction is purity of both materials. The excluded molar mass and the pore size distribution of Separon SGX seems to be conform with that of Kromasil 60 and again the later two silica gels differ primarily in their purity.

The results shown in Figs. 2 and 3 demonstrate that the introductory SEC measurements can help judge suitability of the particular column packing for LC LCD from the point of view of its both effective pore diameter and volume.

Kromasil 60, 100 and 300 packings together with SG-10 material were chosen for assessment of role of effective pore diameter and pore volume. It is likely that all three Kromasil silica gels were made under similar conditions, except for the licensed pore size enlargement process [41] applied in the production of commercial Kromasil 300, the material with 30 nm pores. Therefore, it was supposed that the surface properties, which – in absence of metals – are dictated by both concentration and topology of the free silanols situated on the silica gel surface, would be similar for all three Kromasil column packings.

The LC LCD chromatograms for sample Nr. 4 obtained with two barriers and appropriate injection time delays [7] on the Kromasil column packings of three different pore sizes are depicted in Figs. 4–6, and chromatograms for sample Nr. 2 on Kromasil 60 are shown in Fig. 7.

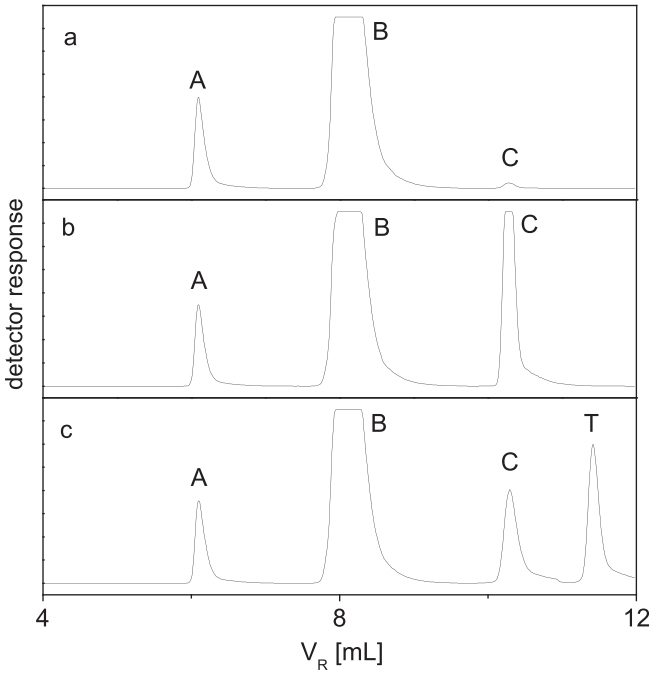

Fig. 4. Effect of pore size. LC LCD chromatograms of sample Nr. 4. Column (300 × 7.5) mm packed with Kromasil 60. Eluent: THF/toluene 70/30; $v_i = 50$ μL; $c_i \sim 2$ mg mL^{-1}. Barrier #1 neat toluene; barrier #2 THF/toluene 30/70 – both with volume of 1000 μL; injection time delays: 0–3–5 min. A – PS homopolymer; B – block copolymer; C – initial and spiked PMMA homopolymer; T – spiked tricresylphosphate. a – initial sample; b – sample spiked with PMMA 16 kg mol^{-1}; c – sample spiked with PMMA 294 kg mol^{-1} and with TCP.

The optimum composition of barrier #2 was separately checked for each particular packing, and if necessary it was correspondingly adjusted. Kromasil 300 with larger effective pore diameter needed a little more desorli in the barrier to let-through the block copolymer while the PMMA homopolymer remained decelerated. This is in agreement with the conclusion of Wang et al. [15] on increased adsorptive activity of silica gel with larger effective pore diameter. It is evident that the barrier effect should be verified for the actual pore size of the column packing. The peaks of spiked polystyrenes with lower, non excluded molar masses were shifted to higher retention volumes due to the permeation effect. As to the general shape of chromatograms of sample Nr. 4 with higher molar mass, the results obtained for Kromasil silica gels with different pore sizes are similar. This reflects relatively small effect of silica gel pore size on the principle of the LC LCD separation proper.

With help of the injection time delay adjustment, the retention volume of block copolymer can be shifted to higher retention volume [7] but a relatively large space on chromatogram would be sacrificed, into which the peak(s) of PMMA homopolymer and low-molecular impurities could be placed.

The peaks of polymers eluted behind barriers are slightly tailed. As a result, the peaks of copolymers may easily interfere with the peaks of PMMA. This is obvious when one considers the mechanism of LC LCD. The adsorbed macromolecules accumulate on the barrier edge and the front of peak is focused. At the same time, the rear part of the polymer zone elutes in the SEC-like mode and therefore it may be broadened.

Polystyrenes with molar masses than 37 kg mol^{-1} added to sample Nr. 4 could not be well discriminated from the block copolymer if simultaneously also PMMA homopolymer and low molar mass marker have to be base-line separated (results not shown). On the other hand, polystyrenes with higher molar masses are well separated even on silica gel with 30 nm effective mean

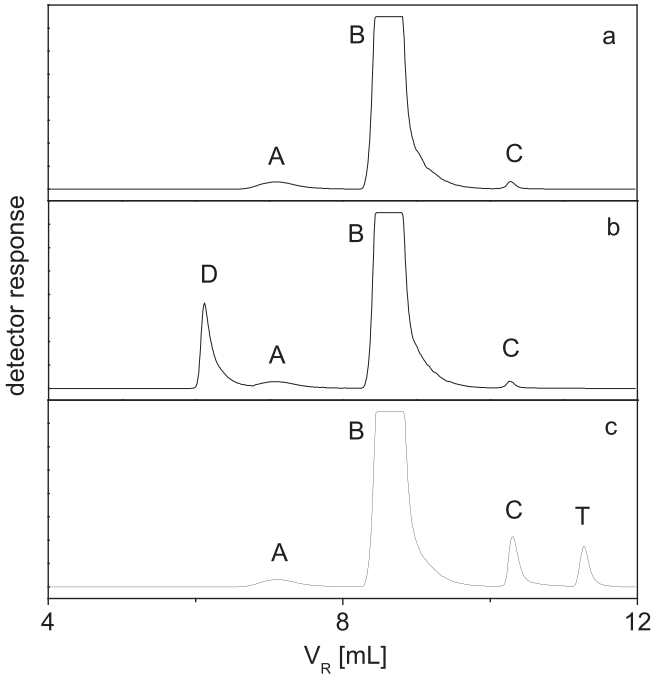

Fig. 6. Effect of pore size. LC LCD chromatograms of sample Nr. 4. Column (300 × 7.5) mm packed with Kromasil 300. Other experimental conditions as in Fig. 4, except for composition of barrier #2: THF/toluene 35/65. A – PS homopolymer; B – block copolymer; C – initial and spiked PMMA homopolymer; D – spiked PS; T – spiked tricresylphosphate. a – initial sample; b – sample spiked with PS 2000 kg mol^{-1}; c – sample spiked with PMMA 613 kg mol^{-1} and with TCP. Notice the broadening of PS peaks A due to the SEC effect. The peak molar mass of PS is 106 kg mol^{-1}, which corresponds with the value given by the producer [6].

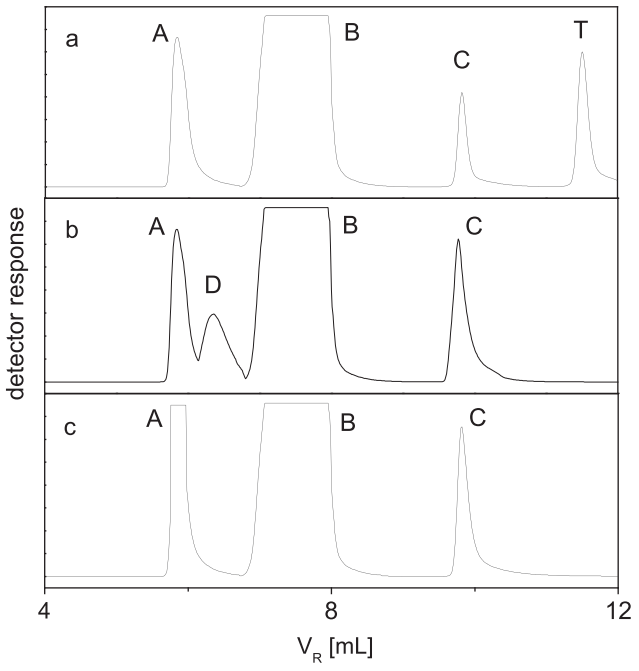

Fig. 5. Effect of pore size. LC LCD chromatograms of sample Nr. 4. Column (300 × 7.5) mm packed with Kromasil 100. Other experimental conditions as in Fig. 4. A – original, and D spiked PS homopolymer; B – block copolymer; C – initial and spiked PMMA homopolymer. a – initial sample; b – sample spiked with PS 17.5 mol^{-1}, and with PMMA 16 kg mol^{-1}; c – sample spiked with PS 498 kg mol^{-1}, and with PMMA 294 kg mol^{-1}.

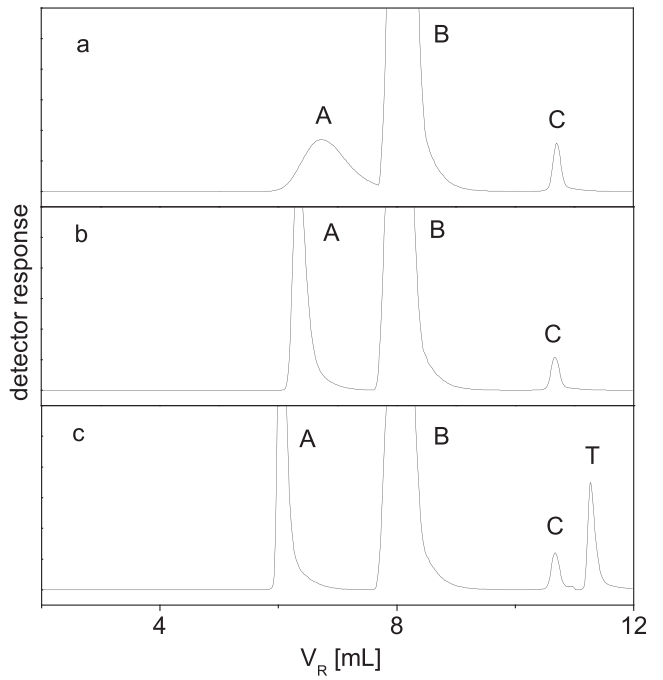

Fig. 7. Effect of pore size. LC LCD chromatograms of sample Nr. 2. Column (300 × 7.5) mm packed with Kromasil 60. Other experimental conditions as in Fig. 4. A – initial and spiked PS homopolymer; B – block copolymer; C – initial PMMA homopolymer; T – spiked tricresylphosphate. a – sample spiked with PS 10 kg mol^{-1}; b – sample spiked with PS 17.5 kg mol^{-1}; c – sample spiked with PS 233 kg mol^{-1} and with TCP.

pore diameter. There is enough space on the chromatograms to also accommodate peaks of block copolymer, of adsorbed homopolymer, and of low-molecular impurities. The molar mass averages and distributions of non-adsorbed homopolymers eluted in the regular SEC mode can be evaluated in conventional way. Contrary to PS, the retention volumes of PMMA homopolymers do not depend on their molar mass because they are eluted behind barrier #2 irrespective of the effective pore diameter of the column packing. Base line separation of TCP marker is feasible even on the wide-pore column packing.

Polystyrene of rather low molar mass 10 kg mol^{-1} added to sample Nr. 2 was well separated on Kromasil 60 (Fig. 7). The retention volumes and shapes of peaks of spiked polystyrenes demonstrate the advantage of the narrow pore packings though the PS homopolymer with molar mass below 4 kg mol^{-1} has already started to interfere with the peak of the block copolymer even with Kromasil 60 column packing.

The effect of pore volume is demonstrated with the SG-10 material. The LC LCD chromatograms of initial and spiked sample Nr. 2 are shown in Fig. 8. Applying appropriate injection time delay, as well as increased both sample concentration and volume, the non-adsorbed PS in the molar mass range between about 5 and 200 kg mol^{-1} can be easily base-line separated from the block copolymer and, as stated above, their molar mass values can be directly determined with help of conventional SEC software. In this way, the comprehensive molecular characterization of some diblock copolymers by two-dimensional liquid chromatography can be simplified because the "second-dimension" that is the SEC separation need not to be applied for the non-retained homopolymer.

In summary, the principle of LC LCD separation little depends on the pore size of the column packing, both effective mean pore diameter and pore volume. The optimum effective pore diameter of packing depends on the molar mass of the non-adsorbed homopolymer. For many practical separations it lies in the range of 6–10 nm. Wide pore LC LCD columns may permit direct characterization of the non-adsorbed homopolymer with higher molar mass. Large volume of packing pores would facilitate application of multiple barriers, for example in separation of triblock copolymers and multicomponent polymer blends.

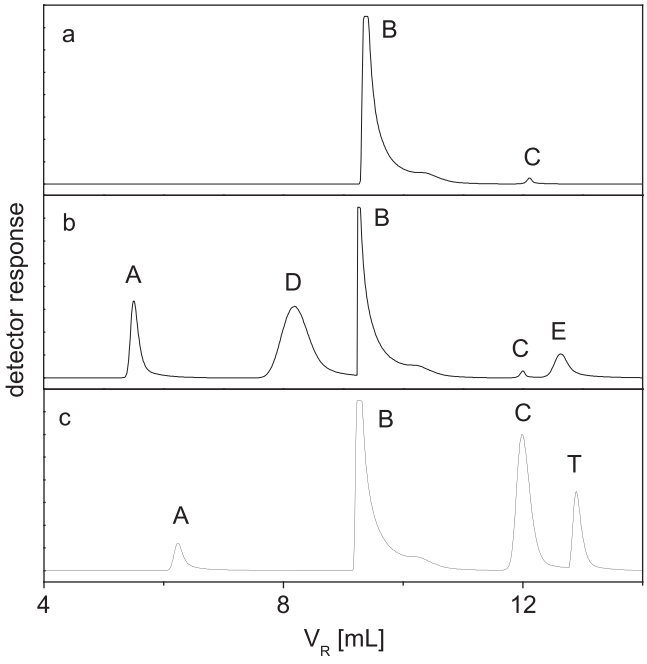

Fig. 8. Effect of pore size. LC LCD chromatograms of sample Nr. 2. Column (300 × 7.5) mm packed with SG-10 material. Eluent: THF/toluene 70/30; $v_i = 20$ μL; ci = 1 mg mL^{-1}. Barrier #1: neat toluene; barrier #2: THF/toluene 28/72 – both with volume of 1000 μL; injection time delays: 0–3–5 min. A, D, and E – spiked PS; B – block copolymer; C – initial and spiked PMMA, T – spiked tricresylphosphate. a – initial sample; b – sample spiked with PS 1200; 10; and 0.7 kg mol^{-1}; c – sample spiked with PS 97 kg mol^{-1}, with PMMA 16 kg mol^{-1}, and with tricresylphosphate. Notice small amounts of polymer injected and that low molar mass PS eluted behind both barriers [7].

4.3. Effect of the column packing purity

It is well known that silica gel HPLC column packings of diverse origin and distinct purity exhibit pronounced differences in their interactivity with separated analytes. Typically, silica gels that contain metal impurities, the "A-type" materials show rather poor properties in separation of basic low-molecular substances. The aim of present study was to test possible influence of silica gel purity on the results of LC LCD separations. A series of bare silica gels of varied degree of cleanliness was applied.

Silpearl, applied in the previous work [6], was a narrow pore material of low purity, which was prepared in presence of Ca ions [34]. The surface and adsorption properties of Silpearl are expected to well differ from other A-type HPLC silica-based column packings applied in this study, Biospher and Separon SGX. The latter two A-type materials were prepared from water glass [36,37] necessarily contain substantial concentration of various metal impurities, especially sodium and potassium. It is known that once the silica hydrogel is transformed into the aerogel, metals embedded in the gel matrix cannot be any more fully removed with the known methods, for example by washing with acids at elevated temperature. The B-type silica gels are high purity materials prepared either from tetraethylsilicate or from pure silica sols (Kromasil materials) or applying a special method, in which the purification of matrix was performed in the hydrogel state (SG-7, SG-10) [38]. As revealed by Buszewski [42] the corresponding SG-7 material exhibited extremely high purity. SG-10 was purified in the same way as SG-7.

Typical LC LCD chromatograms obtained for sample Nr.4 on silica gels of A-type with effective pore diameters of about 6 and 10 nm are shown in Fig. 9. Compared with B-type materials of similar effective pore diameter and pore volume (Figs. 4 and 5) the overall shape of chromatograms is alike. The chromatograms obtained with the low purity Silpearl [6], and the high purity SG-7 has been conformable, as well (results not shown). The eluent and barrier compositions were always the same. It can be concluded that the silica gel purity does not affect principle of the LC LCD separation. It is however anticipated that difference in activity of silica gel may play a role if macromolecules with similar adsorptivity are to be mutually separated, for example polymers of distinct stereoregularity [43] and macromolecules of low polarity.

It is likely that more important than small quantity of metal atoms present in the silica gel matrix is the "column history" that is the amount and nature of polymers, which have been trapped within column in the course of previous separations. Though the sample recovery was generally high in present experiments, after dozens of the LC LCD separations, which were not followed with consequent column cleansing with help of the DMF displacer, the peaks successively changed their both position and shape, and often they were significantly split (Fig. 10). The repeatability of measurements was completely lost. The column history may be an important issue for all coupled methods of polymer HPLC.

4.4. Effect of column size

Columns of different sizes were applied in present study. As evident from the comparison of results in previous papers [6,7] and

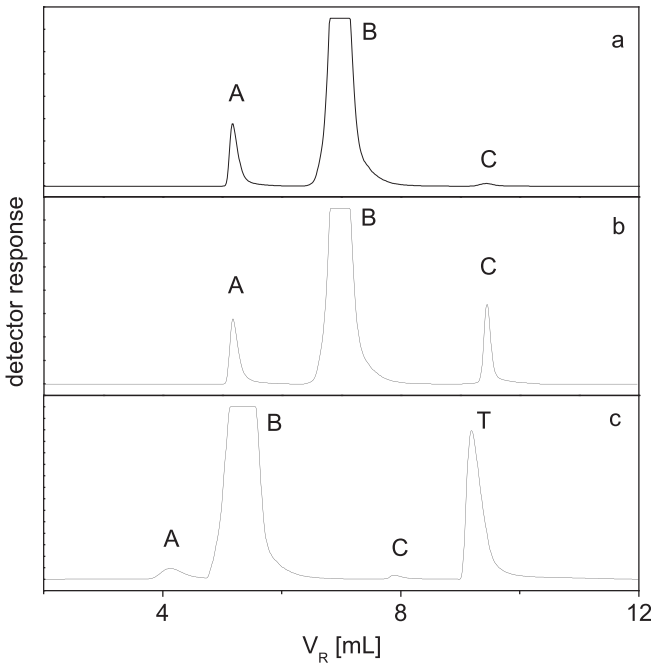

Fig. 9. Effect of silica gel purity. LC LCD chromatograms of sample Nr. 4. Eluent: THF/toluene 70/30. Other experimental conditions as in Fig. 4, except for injection time delays and column dimensions. A – PS; B – block copolymer; C – initial and spiked PMMA. a – and b – column packed with Separon SGX (300 × 7.5) mm., injection time delays: 0–3–5 min; c – column packed with Biospher 100 (250 × 8) mm, injection time delays: 0–3.5–6 min a – initial sample; b – sample spiked with PMMA 394 kg mol^{-1}; c – sample spiked with TCP.

in this work, no effect was observed of the column size on the LC LCD separation proper. The short columns enable fast separations and consumption of eluent is decreased. On the other hand, the short columns such as those packed with Silpearl in previous paper [6] do not allow introduce time delay between sample and barrier injection [7], which is needed for discrimination of low molecular admixtures/impurities. Sample is injected immediately after the barrier #2 and flows through the barrier loop. Therefore its peak may be extensively broadened. This does not represent a serious problem for polymers eluted behind barriers because the sample zones are re-focused during their passage along the LC LCD column. However, the SEC separated peaks may be distorted. In the short columns, there is no space for accommodation of broad, SEC separated peaks of non-adsorbed homopolymers. It is necessary to evaluate the gain in the separation time and in the eluent consumption, which can be achieved with the short columns and to compare it with the advantages of larger columns. The miniaturization of method by applying the micro-columns is well possible, as well. As it is obvious from the present results, also (very) large preparative LC LCD columns can be easily operated.

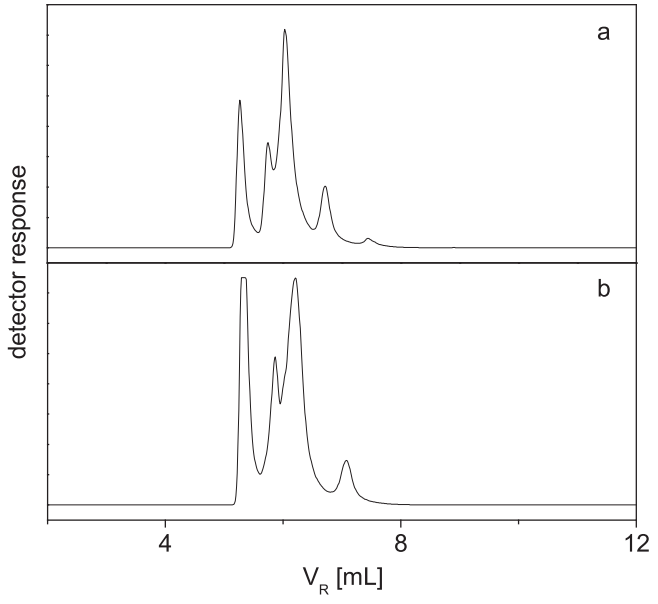

Fig. 10. Effect of column purity. LC LCD chromatograms obtained with column packed with Silpearl (300 × 7.5) mm nearly saturated with retained PMMA and block copolymers. Eluent: THF/toluene 50/50, barrier #1: neat toluene, barrier #2: THF/toluene 30/70. Injection delays: 0–2–4 min a – sample Nr. 2; b – sample Nr. 4.

4.5. LC LCD separation of block copolymers that contain non-polar chains

As shown above, bare silica gel column packings perform well in the LC LCD separation of medium polarity. Binary polymer blends can be separated even if the adsorptivity difference between particular constituents is very small [43]. LC LCD separation of high-polarity polymers such as poly(ethylene oxides) on bare silica gels is also non-problematic provided an appropriate desorli can be found [39]. It was of interest to essay application of bare silica gel for the LC LCD separation of block copolymers, which contain a combination of low-polarity and non-polar chains. Evidently, in order to solve this task, the system must be identified, in which the low-polarity chains are well adsorbed while the non-polar chains remain non-adsorbed. The extent of adsorption of low-polarity polymer chains on bare silica gel under static conditions is rather limited [44]. Situation in the dynamic LC LCD system may be even more complex. Nonpolar, chromatographically very weak solvents are to be used to achieve the adsorli effect for the low-polarity polymers in connection with bare silica gel. As to polystyrene, the suitable adsorli is for example carbon tetrachloride or cyclohexane [45]. CCl_4 is a suspect carcinogen and cyclohexane is a poor solvent for PS with theta temperature about 34 °C. The latter solvent was used as an adsorli for PS in the exploratory attempt to separate parent homopolymers from the diblock copolymer polystyrene-block-poly(isoprene), PS-*b*-PI. Toluene, THF, and dichloromethane were applied as desorlis with distinct desorbing efficacy for both homopolymers. Kromasil 60 was the column packing. Temperature of experiments was 35 °C. Eluent compositions were THF/cyclohexane 25/75, DCM/cyclohexane 30/70, and toluene/cyclohexane 80/20. In these mobile phases, both PI and PS homopolymers and also the block copolymer with the composition of about 50/50 eluted in the SEC mode. Most promising was the system THF/cyclohexane. The barrier composition THF/cyclohexane 20/80 decelerated fast elution of PS but it allowed the break-through of PI so that the mutual separation of both homopolymers was feasible. However, the system was too sensitive toward barrier composition and the repeatability of separation of block copolymer was poor. The peaks of block copolymer obtained with barriers of different composition were broad and distorted. It is likely that the difference in adsorptivity of PS and PI on bare silica gel was not sufficient to allow easy and efficient separation of the parent homopolymers from the block copolymer. Moreover, the sample recovery as estimated by injections of THF zones was surprisingly bad, lower than 50%. Evidently, the applicability of LC LCD with bare silica gel may be rather restricted if the block copolymer contains low-polarity and non-polar chains. It is anticipated that the enthalpic partition retention mechanism with silica alkyl bonded phases as the column packings and with thermodynamically poor solvents for the less polar poly(isoprene) chains would give better results.

5. Conclusions

The detailed discussion is presented of the general requirements, which are to be met by the columns suitable for liquid chromatography under limiting conditions of desorption, LC LCD intended for separation of parent homopolymers from diblock copolymers. The emphasis was laid on the bare silica gel based column packings with different particle and pore sizes, as well as with distinct purity. Typical results of the LC LCD separations of model diblock copolymers with blocks of unequal polarity and adsorptivity were used for comparison of different column packings. It can be concluded that – at least for bare silica gels under investigation and for PS-*b*-PMMA diblock copolymers in THF/toluene eluent and barrier system – the above packing characteristics do not affect the LC LCD separation principle. The column packing properties may however play certain role from the viewpoint of the optimum eluent and barrier composition. For example, pore volume of the column packing plays important role; the larger pore volume the more selective the LC LCD separation. The packings with larger both effective pore diameter and pore volume can be applied for the direct SEC characterization of the non-adsorbed homopolymers that were separated from the block copolymers. A specific issue is the chromatographic history of packing, which reflects its previous applications. Columns saturated with adsorbed polymers may completely lose their separation ability. Bare silica gel column packings are well suitable for the LC LCD separation of parent homopolymers from the diblock copolymers that contain blocks of different adsorptivity and the results confirm large robustness and experimental feasibility of the method. On the other hand, application of bare silica gel to separate parent homopolymers from diblock copolymers that contain non-polar or low-polarity blocks is difficult. Numerous experiments with block copolymers spiked with their parent homopolymers provide further proof of the LC LCD separation principle. Depending on the size of macromolecules to be separated, silica gels with effective pore diameters between 6 and 30 nm seem to be the materials of choice for LC LCD of many high polymers.

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References

[1] Berek D. Macromolecules 1998;31:8517–21.
[2] Berek D. Progr Polym Sci 2000;25:873–908.
[3] Šnauko M, Berek D. J Chromatogr A 2005;1094:42–8.
[4] Šnauko M, Berek D. Macromol Chem Phys 2005;206:938–44.
[5] Berek D. Eur Polym J 2009;45:1798–810.
[6] Berek D. Macromol Chem Phys 2008;209:695–706.
[7] Berek D. Macromol Chem Phys 2008;209:2213–22.
[8] Heitz W, Kern W. Angew Makromol Chem 1967;1:150–73.
[9] Heitz WZ. Anal Chem 1975;277:323–33.
[10] Dawkins JV. Pure Appl Chem 1979;51:1473–81.
[11] Belenkii BG, Valchikhina MD, Vakhtina IA, Gankina ES, Tarakanov OG. J Chromatogr 1976;129:115–24.
[12] Skvortsov AM, Klushin LT, Gorbunov AA. Macromolecules 1997;30:1818–27.
[13] Šnauko M, Berek D. Chromatographia 2003;57:S55–9.
[14] Teraoka I, Lee D. High osmotic pressure chromatography. In: Wu C-S, editor. Handbook of size exclusion chromatography and related techniques. Chromatographic sciences series, vol. 91. New York: Marcel Dekker, Inc.; 2004. p. 657–75.
[15] Orelli S, Jiang W, Wang Y. Macromolecules 2004;37:10073–8.
[16] Poppe H, Kraak JC, Huber JFK, Van den Berg JHM. Chromatographia 1981;14:515–23.
[17] Berek D, Jančo M, Meira GR. J Polym Sci Part A Polym Chem 1998;36:1363–71.
[18] Berek D, Russ A. Chem Pap 2006;60:249–52.
[19] Beaudoin E, Favier A, Galindo C, Lapp A, Petit C, Gigmes D, et al. Eur Polym J 2008;44:514–22.
[20] Favier A, Petit C, Beaudoin E, Bertin D. E-Polymers 2009;009:1.
[21] Berek D. Chromatographia 2003;57:S45–54.
[22] Berek D. Macromol Chem Phys 2005;206:1915–27.
[23] Berek D, Capek I, Mendichi R, Labátová S. Macromol Chem Phys 2006;207:2074–83.
[24] Berek D. Chem Pap 2006;60:71–3.
[25] Berek D. Macromol Chem Phys 2006;207:893–902.
[26] Bartkowiak A, Hunkeler D, Berek D, Spychaj T. J Appl Polym Sci 1998;69:2549–57.
[27] Bakoš D, Bleha T, Ozimá A, Berek D. J Appl Polym Sci 1979;23:2233–44.
[28] Solms DJ, Smuts TW, Pretorius V. J Chromatogr Sci 1971;9:600–3.
[29] Šlais K, Krejčí MJ. Chromatographia 1974;91:161–6.
[30] Pasch H, Trathnigg B. HPLC of polymers. Berlin: Springer; 1998.
[31] Berek D. Macromol Symp 2001;174:413–34.
[32] Berek D. Two-dimensional liquid chromatography of synthetic polymers. Anal Bioanal Chem 2010;396:421–41.
[33] Šnauko M, Berek D. J Sep Sci 2005;28:2094–103.
[34] Anon. Silpearl silica gel for HPLC. Votice, Czech Republic: Sklárny Kavalier; 1990.
[35] Novák I, Berek D. US Pat. 4,382,070.
[36] Anon. Biospher silica gels for high performance liquid chromatography. Prague, Czech Republic: Labio; 1991.
[37] Anon. Separon column packings for HPLC. Prague, Czech Republic: Tessek; 1987.
[38] Berek D, Novák I. US Pat. 4,255,286.
[39] Berek D. unpublished results.
[40] Berek D, Jančo M, Kitayama T. Hatada K Polym Bull 1994;32:629–35.
[41] Berek D. Process for pore extension of ultra-pure HPLC silica gels. Patents pending.
[42] Buszewski B. Chromatographia 1992;34:573–80.
[43] Berek D, Kitayama T, Hatada K, Hirano T, Ihara H, Capek I, et al. Liquid chromatography under limiting conditions of desorption IV. Separation of macromolecules according to their stereoregularity. Polym J 2009;41:1144–51.
[44] Lipatov YuS, Sergeeva LM. Adsorption of polymers. , New York: Wiley; 1974.
[45] Russ A, Berek D. J Sep Sci 2007;30:1852–9.