Macromolecules
ARTICLE

Macromolecules **2010**, *43*, 9627–9634 **9627**
DOI: 10.1021/ma101853g

Comprehensive Molecular Characterization of Complex Polymer Systems by Sequenced Two-Dimensional Liquid Chromatography. Principle of Operation

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Received August 12, 2010; Revised Manuscript Received October 1, 2010

ABSTRACT: It is shown that numerous complex polymer systems consisting of macromolecules, which exhibit not only distinct sizes but also chemical composition, can be comprehensively characterized by a novel simple and efficient method of sequenced two-dimensional liquid chromatography. In the first step, macromolecules are separated according to their chemical composition, irrespectively of their molar mass, with help of the unconventional method of liquid chromatography under limiting conditions of desorption. Thus-obtained fractions are in their entireties successively online forwarded into the size exclusion column for separation according to their molar mass. The effectuation of procedure is elucidated.

Introduction

Size exclusion chromatography (SEC)[1,2] is at present the most important method for molecular characterization of synthetic polymers in terms of their molar mass averages and distributions. The well-performed SEC measurements give reliable, quantitative information on the above molecular characteristics of linear homopolymers. SEC also provides valuable estimates of molar masses for complex polymers exhibiting more than one distribution in their molecular characteristics, such as branched polymers, copolymers, and functional oligomers. However, the SEC data obtained for the complex polymers can be considered orientational only. This is because SEC separates macromolecules according to their size in solution, which simultaneously depends on all molecular characteristics of polymers, besides molar mass also on chemical structure (composition) and on physical architecture (topology). To assess the latter two molecular characteristics, coupled methods of liquid chromatography are to be used. These combine two or several different retention mechanisms within the same liquid chromatographic column.[3−5] Appropriate coupling of entropy-controlled (exclusion) and enthalpy-driven (interaction) retention mechanisms enables suppression of the molar mass effect so that the resulting separation proceeds mainly or exclusively according to other molecular characteristic. Alternatively, different separation and characterization principles are hyphenated, for example liquid chromatography with mass spectrometry or NMR.[6,7] To provide comprehensive molecular characterization of complex polymer systems, which are mixtures of macromolecules of distinct nature and exhibit discrete distributions in their molecular characteristics, all constituents must be discriminated and then independently characterized. However, constituents of complex polymer systems can be mutually separated with help of SEC only if they possess well different molar masses and if their relative concentrations in system are similar. Limited both selectivity and sample capacity of SEC do not enable to assess minor macromolecular constituents ($< 1\%$ and often even $< 10\%$) in complex polymer systems even if their size in solution well differs from that of polymer matrix.[1,2]

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In order to solve this task, procedures of two-dimensional liquid chromatography (2D-LC) are to be applied, which utilize two different chromatographic systems.[8,9] In 2D-LC, fractions obtained in the first-dimension (1-d) column are further discriminated in the self-existent second-dimension (2-d) column, which applies distinct separation mechanism(s). With advantage, the first-dimension separation is performed irrespectively of sample molar mass by means of suitable coupled retention mechanism. Fractions from the 1-d column that possess similar chemical composition or physical architecture are successively separated according to their molar mass/molecular size in the 2-d SEC column. Effluent from the 1-d column can be transferred into the 2-d column either off-line or online.[9−13] In the off-line approach, the fractions from the 1-d column are collected and successively reinjected into the 2-d column. The preseparated macromolecules within particular fractions are mixed, and the entire procedure is laborious and slow. Various approaches were elaborated for the online transfer of fractions from the 1-d column into the 2-d column. Often, the fractions from the 1-d column are cut into narrow parts that are one-by-one gradually transported into the 2-d SEC column for independent characterization. This is the method of choice if the first-dimension separation produces broad peaks, such as liquid chromatography under critical conditions of enthalpic interactions (LC CC).[3,4,14−18] The exacting requirement of the latter online 2D-LC approach is that the elution rate in the 1-d column must be slow and the separation process in the 2-d SEC column has to be so fast that entire effluent leaving the first-dimension column can be continuously processed in the 2-d column, without flow interruption.[19,20] These requisites may bring about experimental problems. These may be solved with help of the stop-and-go elution method,[12,21−23] in which the flow in the first-dimension column is periodically interrupted to allow time for the second-dimension separation. Alternatively, the fractions from the 1-d column can be stored in a set of the capillaries or in the full-retention-elution columns,[24−26] where they wait for their transfer into the second dimension column. The full-retention-elution approach could even enable the reconcentration of fractions diluted in the course of the first-dimension separation.[26,27] Eventually, only a selected part

Published on Web 11/04/2010

of fractions from the first-dimension column is processed in the second-dimension column (the "heart-cut method"[23]). A favorable situation occurs if the 1-d column produces narrow, baseline-separated fractions of macromolecules of distinct composition irrespectively of their molar mass. In the event of very high selectivity of the first-dimension separation, entire segments of effluent from the 1-d column can be directly forwarded into the 2-d column. Even if fractions contain similar molar masses, they would not overlap. In the sequenced two-dimensional liquid chromatography, S2D-LC the baseline discriminated polymer peaks from the 1-d column are successively transported into the online 2-d column. The stop-and-go approach can be easily applied or the first-dimension separation is repeated several times, once for each distinct sample constituent. Always one particular fraction from the 1-d column is forwarded into the 2-d column, and the nonused fractions go to waste. The concentrations or volumes of sample introduced into the 1-d column in the course of particular injections are so adjusted that the polymer detection of the 2-d column effluent is feasible. The group of methods which well fulfill the above conditions for the first-dimension separation is liquid chromatography under limiting conditions of enthalpic interactions (LC LC).[4,28−39] LC LC utilizes the significantly different elution rates of small, pore-permeating solvent molecules and large, partially or fully pore-excluded macromolecules. Appropriately chosen slowly eluting low-molecular-mass substances promote interactions of certain macromolecules in the chromatographic system. If eluted in front of polymer sample, the interaction promoting small molecules can decelerate fast elution of interacting macromolecules. The most suitable so far tested LC LC approach employs adsorption retention mechanism. It is denoted liquid chromatography under limiting conditions of desorption (LC LCD).[4,28−39] In the well-flexible LC LCD arrangement, mobile phase promotes SEC elution of all sample constituents. A narrow zone of adsorption promoting liquid, an adsorli, is injected prior to sample. Adsorli hinders fast elution of adsorptive polymer species and acts as a slowly progressing barrier. At the same time the nonadsorbed macromolecules freely elute in the exclusion mode. As a result, macromolecules with uneven polarities are separated in dependence on the extent of their adsorption on the active porous column packing, for example, on bare silica gel.[4,34] Important features of LC LCD include its simplicity and robustness, which result in experimental feasibility of method. A further attribute of LC LCD is high selectivity of separation according to chemical structure or physical architecture of macromolecules, while the molar mass effect is small or negligible. The method exhibits remarkably high sample recovery and produces narrow, focused peaks of macromolecules eluted behind the barrier. High sample capacity of LC LCD in terms of sample volume and/or concentration enables to create fractions large enough to be detected in the 2-d column effluent even if the initial sample contains less than 1% or even less than 0.1% of polymer to be characterized.

Principle and basic experimental features of two-dimensional combination of liquid chromatography under limiting conditions of desorption with SEC, LC LCD × SEC are discussed in this study. Selected complex polymer systems have been applied as the separation examples. Besides polymer blends, particular attention has been paid to the block copolymers that served as practically important model systems because they often contain one or both parent homopolymers.

Experimental Section

Instruments. The common HPLC instrumentation was applied in present work. Two pumping systems were P 102 from Watrex, Czech Republic, and two evaporative light scattering detectors (ELSD) were model 1000 and model 380 LC, both from PL-Agilent

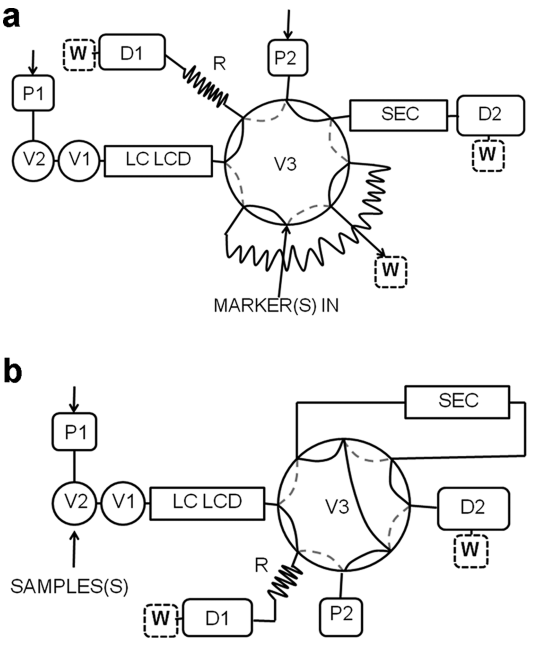

Figure 1. Schematic representation of S2D-LC system LC LCD × SEC. Injection valves for LC LCD column are: V1, barrier; V2, sample. Switching/injection valve for SEC column is V3. P1 and P2are pumping systems for LC LCD and for SEC, respectively. D1 and D2 are detectors for LC LCD and SEC, respectively. R is hydrodynamic (capillary) resistor, and W stands for waste. In position (---) of valve V3, effluent from the LC LCD column is transported directly into detector D1, and SEC column is flushed with eluent delivered by pump P2. In position (—), effluent from LC LCD column is transported into SEC column. Valve V3 in (a)is provided with additional loop for marker or independent sample introduction in the arrangement.

UK−USA. Temperature of evaporators in both detectors was set at 80 °C (method 1 for model 1000). At this temperature, detectors did not respond to tetrahydrofuran and toluene solvents. The gas flow rates were 1.5 mL min^{-1} for model 1000 and 1 mL min^{-1} for model 380. The sensitivity of the ELSD model 380 LC was about 20 times higher compared to model 1000. The values of retention volumes determined under identical experimental conditions with different detectors in the course of introductory measurements were equal. The LC LCD columns were thermostated to 30 °C with help of an air oven provided with water jacket. A tandem of two-way, six-port manual injection valves from Rheodyne was applied for barrier (V1) and sample (V2) injection into the LC LCD column (Figure 1). Sample injection valve V2 was equipped with an electric trigger to start the LC LCD data collection. Data collection in SEC column was started with a hand-operated electric trigger. Volumes of barrier and sample loops were 1000 and 100 μL, respectively. The 1-d LC LCD column (250 × 8 mm) was home packed with bare silica gel Kromasil 60 from Eka Chemicals, Sweden with effective pore diameter 6 nm and 10 um particle size. Entire effluent or its sequences that is the selected sample fractions from the LC LCD column were forwarded into the 2-d SEC columns by means of an eight-port two-way switching valve from Valco Vici, V3. A set of two SEC linear columns (300 × 7.5) mm was from American Polymer Standards. They were chosen because of their low polar interactivity.[40] Poly(methyl methacrylate) (PMMA) eluted not only in THF but also in toluene from the latter SEC columns in the exclusion mode, without adsorption effect. Chromatographic data were collected and processed with help of software WinGPC from PSS, Germany, in terms of retention volumes/concentrations from the LC LCD column and also in terms of polymer molar masses from the SEC columns.

A scheme of the LC LCD × SEC instrument, which was used in the method testing, is depicted in Figure 1a. Besides common injection valves V1 and V2 applied for barrier and sample injections,

respectively, the system was provided with an extra valve V3, which enabled introduction of entire effluent or its selected sequence from the LC LCD column into the SEC columns. Valve V3 was equipped by an additional loop with volume of 50 μL. This arrangement allowed independent injection of the control samples and the appropriate markers into the SEC columns. Alternatively, fractions from the LC LCD column could be (practically) simultaneously introduced into SEC columns together with the markers with help of V3. A scheme of instrument applied for the actual sequenced two-dimensional LC LCD × SEC separations is shown in Figure 1b. Two ELS detectors were applied in the LC LCD × SEC instruments. Detector D1 was used for exact determination of retention volumes of fractions leaving LC LCD column and also for assessment of concentration of particular sample constituents. On the basis of such introductory measurements, times for injections of particular sequences from the LC LCD were adjusted, exact retention volumes of LC LCD peak apexes were determined, and appropriate injected sample concentrations were chosen. Detector D2 was applied for monitoring the SEC effluent. Note the hydrodynamic resistor R in both S2D-LC systems located in front of detector D1. The flow resistance of resistor capillary of internal diameter of 0.1 mm was similar to that of the set of SEC columns. The role of resistor was to prevent sharp pressure fluctuations due to sudden introduction/elimination of the SEC column into/from the flow circuit. Volume of the resistor capillary was considered in determination of retention volumes of polymers leaving the LC LCD column. Actual flow rate produced by pumps was regularly checked with a buret-like volumeter.

Materials. Narrow molar mass distribution polystyrenes (PS) with molar masses from 0.88 to 612 kg mol^{-1} were from Pressure Chem. Co. and ARRO Laboratories. The ultrahigh molar mass PS with molar mass of 30 × 10 kg mol^{-1} was from Polysciences (M_w/M_n = 1.3). It was used as marker together with tri-*p*-tolyl phosphate (TTP) from Acros Organics, Belgium. The retention volumes of markers were chosen not to interfere with retention volumes of polymers under study. Molecules of TTP were small enough to permeate most pores of LC LCD column while they were still detectable with ELSD at temperature of evaporator of 80 °C. Homopolymers of poly(methyl methacrylate) (PMMA) with molar masses from 16 to 613 kg mol^{-1} were from Rohm, Germany. They exhibited medium broad molar mass distribution and low stereoregularity.[35] Narrow molar mass distribution samples of PMMA with molar masses 1.8 and 5.7 kg mol^{-1} were from Institut Sadron Strasbourg, France. Polystyrene-*block*-poly(methyl methacrylate) copolymer (PS-*b*-PMMA) was from PL-Agilent UK−USA. It is designated "sample Nr.3" in order to correspond with the designation used in paper.[36] According to producer, the copolymer contained 71.4 wt % of PS, and the weight-average molar mass M_w of PS blocks was 75.7 g mol^{-1}. Its M_{peak} was 106 kg mol^{-1}, and apparent M_w/M_n value was 1.13.

Tetrahydrofuran (THF) and toluene of analytical grade from POCH, Poland, and AFT, Slovakia, respectively, were applied as eluent and barrier components. Both solvents were distilled immediately before use. THF was stabilized by 0.2 g L^{-1} of 2,6-di-*tert*-butyl-4-methylphenol. The action of THF, toluene, and mixed solvents THF/toluene in LC LCD of PS, PMMA, and PS-*b*-PMMA copolymers was studied in previous works.[36,37,39] It was shown that THF prevents adsorption of both PS and PMMA on silica gel; is acts as a desorli for these polymers. Toluene is a desorli for PS, but it promotes adsorption of PMMA on bare silica gel, it is an adsorli for the latter polymer. Eluent was a mixture of THF and toluene 70/30. It is a desorli for both polymers and allows their SEC elution from bare silica gel. Compositions of all mixed solvents are given in weight parts.

For the sake of simplicity, identical mobile phase composition was used in both separation dimensions. This was enabled by large difference between the high adsorption activity of bare silica gel in the LC LCD column and the low adsorption activity of styrene/divinylbenzene packing applied in the SEC columns. Naturally, the experimental arrangements in Figure 1 allow application of different mobile phases in the LC LCD and SEC columns. Pecularities of such eluent switching will be discussed in the future parts of this series.

Procedures. The eluent flow rate was 1 mL min^{-1}, and polymer concentrations injected into LC LCD column c_i varied between 0.03 and 30 mg mL^{-1}. The actual concentration depended on the amount of constituent analyzed and also on the detector applied. The actual values of c_i are given in the figures. Polymer samples have been always dissolved and injected in eluent. For separation of blends of PS and PMMA, one barrier of neat toluene has been applied. It has allowed unhindered SEC elution of PS but decelerated fast elution of PMMA. The barrier has been injected in front of polymer sample, which followed after certain time delay. The time delay between barrier and sample allowed returning the barrier valve V1 into its initial position so that sample solution did not flow through the barrier loop.[37] Moreover, the time delay enabled to appropriately adjust retention volume of polymer eluted behind barrier. The larger the time delay, the lower sample retention volume because the longer time the analyzed polymer has rapidly eluted in the SEC mode, unaffected by barrier. Eventually, time delay between barrier and sample injection created space on chromatogram to accommodate peak of low-molecular marker and to prevent interference between sample and possible low-molecular-mass impurities.[37,38] In the case of block copolymers, two barriers were introduced into LC LCD column. The zone of neat toluene has been injected as first (the first barrier, B#1). It hindered fast elution of not only PMMA but also of block copolymer PS-*b*-PMMA. After appropriate time delay the zone with composition THF/toluene 30/70 (the second barrier, B#2) has been introduced into the column. It decelerated elution of PMMA homopolymer, but it allowed SEC elution of PS and block copolymers.[36,37,39] Sample has been applied after certain time delay after B#2. Starting with B#1 introduction, the overall time of the LC LCD separation has been manually monitored, but the polymer retention time has been automatically measured from the instant of sample injection. Time delay(s) for the barrier(s) and sample injection are given in minutes. For a single barrier the code "0−4.5" means that sample was injected 4.5 min after barrier. In the case of two barriers, the sign "0−3−5" indicates that B#2 has been applied 3 min after B#1 and sample was injected 2 min after B#2.

Results and Discussion

Figure 2 shows elution behavior of PS (a) and PMMA (b) from Kromasil 60 in mixed eluent THF/toluene 70/30 in absence of barrier. Polymers with different molar masses were separately injected into the column. The molar mass exclusion limit of Kromasil 60 lies in the range of 60 kg mol^{-1}. Consequently, the peaks of high-molar mass, excluded polymers are narrow. The peaks of polymers with lower molar masses become broadened because of their size separation. This is the well-known, regular SEC behavior.

Figure 2c demonstrates the action of barrier of THF/toluene 30/70 on elution of PMMA homopolymers. In spite of content of 30 wt % of THF in barrier, it still fully decelerates elution of PMMAs—in principle irrespectively of their molar masses. The PMMA peaks exhibit narrow focused form and the reconcentration effect of barrier is evident—though some tailing takes place, especially for the PMMA sample with the lowest molar mass 1.8 kg mol^{-1} (the peak which is situated on the right side of set of peaks). This is likely the result of exclusion processes in the rear part of the polymer zone that elutes behind barrier. Macromolecules with lowest molar masses could not fully catch the barrier edge. For illustration, the peaks of ultrahigh-molar-mass PS (Figure 2c) and TTP markers are shown as well. The actual retention volume of TTP is shifted in comparison with PMMA,

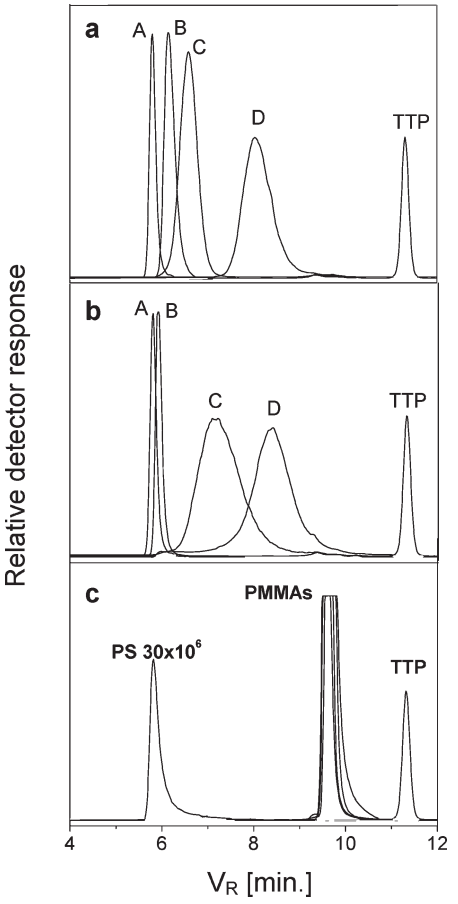

Figure 2. Chromatograms obtained with the LC LCD column. Injected polymer concentration c_i ranged from 0.15 to 3 mg mL^{-1}. PS (a) and PMMA (b) eluted without barrier (SEC mode). Molar masses of polymers in kg mol^{-1}: PS: A, 612; B, 100; C17.5; D, 4; PMMA: A, 613; B, 103; C, 16; D, 1.8. (c) Chromatograms of PMMA as in (b) with the decelerating effect of barrier of THF/toluene 30/70 on PMMA homopolymer at time delay 0−3. Markers in (c) are PS with molar mass of 30×10^3 kg mol^{-1} and tri-*p*-tolyl phosphate.

which was eluted behind barrier. This is due to time delay introduced between barrier and sample injection.[37] Retention volumes of markers have remained unaffected with the zone of THF/toluene 30/70—similar as PS with lower molar masses did not respond on the zones of neat toluene injected in front of them (results not shown).

As evident from Figure 2a,b (peaks D), PS 17.5 kg mol^{-1} and PMMA 16 kg mol^{-1} would not be mutually separated by LC LCD column working in the SEC mode. The same applies for the couple of PS 100 kg mol^{-1} with PMMA 103 kg mol^{-1} as well as PS 612 kg mol^{-1} with PMMA 613 kg mol^{-1}. Selectivity of the SEC columns would be insufficient for size separation of mixtures of these polymers. Each couple was injected into LC LCD column and baseline separated by a barrier THF/toluene 30/70. The results are depicted in Figure 3a,b. Next the LC LCD effluent containing separated polymers was online transported into SEC columns. The resulting chromatograms are displayed in Figure 3c,d. Evidently, the discrimination of PS and PMMA of similar molar masses in the LC LCD column of present size and under present experimental conditions has been ample enough to allow direct transfer of the LC LCD column effluent into the SEC column. This is the most straightforward, sequenced two-dimensional arrangement LC LCD × SEC. Molar mass values for all four polymers calculated from SEC chromatograms thus obtained well agreed with the data determined for the polymers separately. Quantitative processing of SEC results in terms of

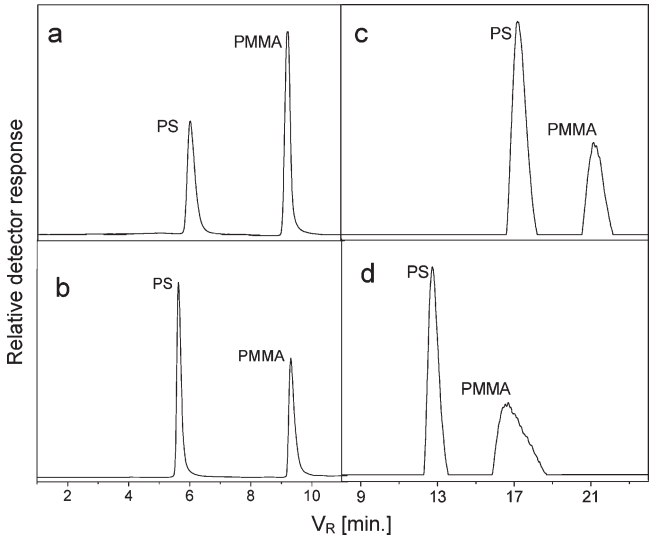

Figure 3. Chromatograms of PS plus PMMA mixtures separated by LC LCD column by means of a barrier THF/toluene 30/70 with a time delay 0−3. Molar masses (in kg mol^{-1}) of PS were 17.5 and PMMA 16 (a) or PS 612 and PMMA 613 (b). The same blends after separation in LC LCD column were directly online transported into SEC columns, chromatograms (c) and (d), respectively. Injected polymer concentration c_i was 0.03 mg mL^{-1}.

polymer molar masses is in more detail discussed in the following part of this work.

Figures 4 and 5 demonstrate potential of separation and molecular characterization of minor constituents from binary polymer blend by means of LC LCD × SEC. Figure 4 depicts the case of blend of PS and PMMA with similar molar masses (17.5 and 16 kg mol^{-1}, respectively) that contains 1% of PMMA. Elution of polymer mixture from the LC LCD column plus two SEC columns without barrier (Figure 4a) did not show any indication of separation. LC LCD with barrier of THF/toluene 30/70 enabled baseline separation of both polymers (Figure 4b).[38] The peak of PS matrix was discarded, and the peak of PMMA was transported into the online SEC columns (Figure 4c). The polystyrene equivalent molar mass averages were 1.4 and 1.3 kg mol^{-1} for M_w and M_n, respectively. The inverse situation is demonstrated in Figure 5. A blend of the same PS and PMMA contained 1% of PS. Again, there was no chance to observe presence of minor constituent on the chromatogram obtained with help of tandem of three columns: one LC LCD plus two SEC columns without barrier (Figure 5a). Minor PS was easily separated from the PMMA matrix by the barrier of THF/toluene 30/70 (Figure 5b) and its molar mass consecutively determined by means of SEC columns without interference from PMMA, which was discarded (Figure 5c). M_w and M_n values calculated from the corresponding SEC chromatogram were 1.7 and 1.68 kg mol^{-1}, which is in agreement with data given by producer. These results confirm ability of barrier to selectively and quantitatively retain small amount of adsorptive polymer from a large excess of the non-adsorptive polymer matrix and vice versa to allow free elution of minor nonadsorptive blend constituent from an excess of adsorptive polymer retained by barrier.[38] The LC LCD separated minor constituents can be easily characterized with help of the online SEC instrument. It is clear that the direct introduction of minor constituent separated by LC LCD together with the major blend constituent into the SEC column is hardly possible. The sample must be injected into the LC LCD column two times at different concentrations c_i to obtain appropriate amounts of particular polymers suitable for the online SEC separation.

Many block copolymers, even those prepared by sequential anionic polymerization, contain important amounts of parent

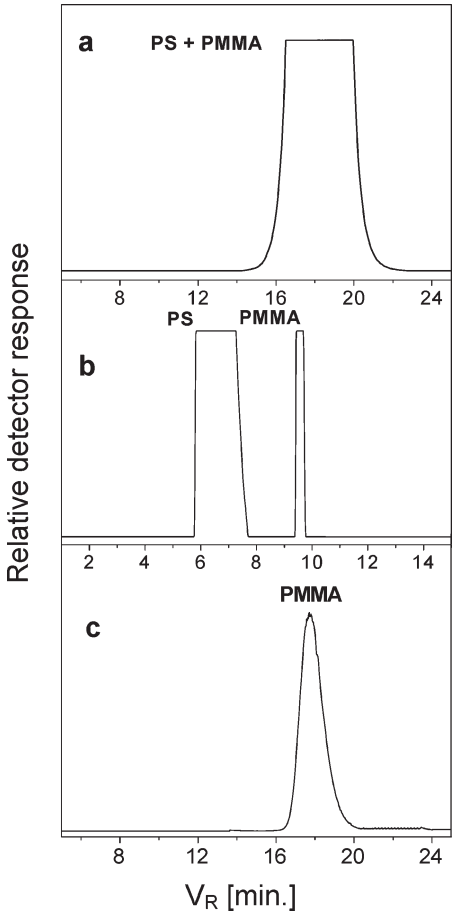

Figure 4. LC LCD separation and SEC characterization of minor (1%) PMMA constituent from its mixture with PS matrix by means of LC LCD × SEC. Molar masses of polymers were almost identical: PS = 17.5 and PMMA = 16 kg mol^{-1}. Total c_i injected into LC LCD column was 0.03 mg mL^{-1}. (a) SEC chromatogram from a tandem of LC LCD (without barrier) plus SEC columns. (b) LC LCD chromatogram of blend; barrier was THF/toluene 30/70. Time delay was 0−3. (c) SEC chromatogram of minor constituent PMMA obtained after its separation from PS matrix in the LC LCD column. Separated PS matrix was discarded. A detailed explanation is in the text.

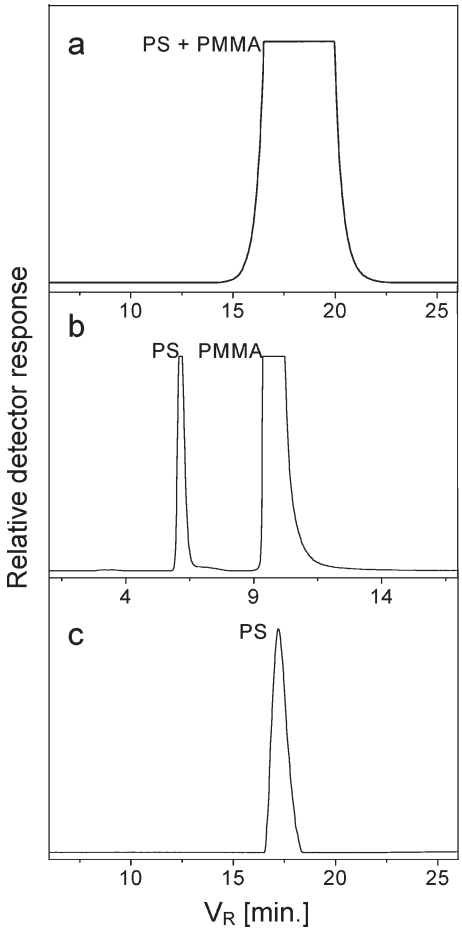

Figure 5. LC LCD separation and SEC characterization of minor (1%) PS constituent from its mixture with PMMA matrix by means of LC LCD × SEC. Polymer molar masses and other experimental conditions as well as designation of chromatograms as in Figure 4a; however, the peak of PS minor constituent is shown in (c). PMMA matrix separated in LC LCD column was discarded. A detailed explanation is in the text.

homopolymers. Knowledge of amount and molecular characteristics of parent homopolymers would enable optimization of polymerization conditions. Fast and selective separation of parent homopolymers from the block copolymers so far represented a challenge. The problem has been considered even more exacting if the concentration of parent homopolymers lay well below 1%. Recently,[34,36,37,39] it was shown that the LC LCD method is able to efficiently discriminate both parent homopolymers from diblock copolymers in one single step. Very low concentrations of parent homopolymers could be monitored and quantified as well. It was inviting to determine molar masses of parent homopolymers separated with help of LC LCD by means of online SEC. The corresponding S2D-LC approach is demonstrated in Figures 6 and 7.

The SEC chromatogram of original block copolymer sample Nr.3 is shown in Figure 6a. Block copolymer has been eluted together with parent homopolymers, and no conclusions about their presence could be done. This is a typical situation encountered in numerous works that describe synthesis and properties of block copolymers. Changes of injected concentration did not affect extent of information, which could be extracted from the SEC chromatograms. The same sample has been subject of LC LCD separation (Figure 6b). Two barriers were applied, namely B#1 of neat toluene and B#2 of mixture THF/toluene 30/70. As explained in the Experimental Section, B#1 decelerated elution of block copolymer, while B#2 blocked fast elution of the PMMA homopolymer but let through block copolymer. Elution of PS was not affected by the preceding barriers. The presence of both parent homopolymers, PS and PMMA, has been evidenced, and three baseline separated peaks appeared on the LC LCD chromatogram. In the previous papers[34,36,37] the peaks of homopolymers present in block copolymers PS-*b*-PMMA were identified by spiking experiments. The peaks of PS were selectively monitored also by a UV detector.[39] In order to avoid peak interference in the SEC chromatogram, the peaks of both homopolymers were discarded and only the peak of block copolymer was transported into SEC columns. The resulting chromatogram is depicted in Figure 6c. In the next step, sample Nr.3 was subject to the LC LCD separation with only one barrier of neat toluene. Two peaks were created: one for PS homopolymer and the other one contained the block copolymer and the PMMA homopolymer (Figure 7a). The latter peak was discarded, and the peak of PS was online forwarded into SEC column. Again, a well-defined chromatogram was created (Figure 7b). A similar procedure was utilized for SEC characterization of PMMA homopolymer. In this case a single barrier of THF/toluene 30/70 was applied in the LC LCD column. PS and the block copolymer eluted together without deceleration, but fast elution of PMMA homopolymer was blocked by barrier (Figure 7c). Eventually, the peak of PMMA was forwarded into the SEC columns (Figure 7d). Molar mass values for both homopolymers as well as for block copolymer

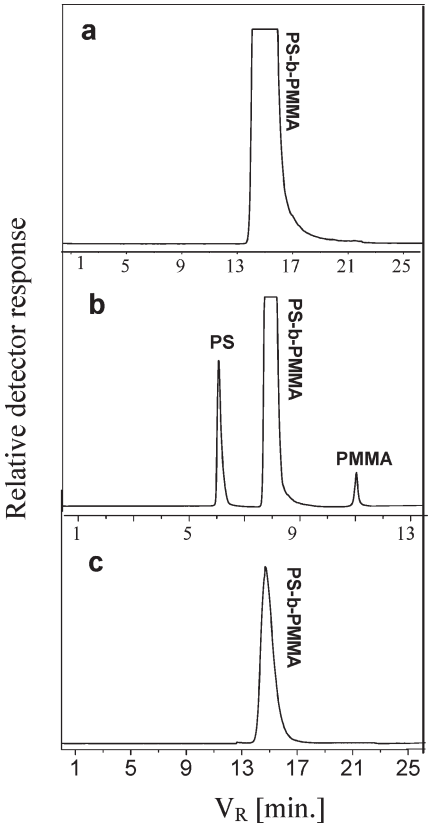

Figure 6. Demonstration of potential of S2D-LC; the combination of LC LCD with SEC for separation and molecular characterization of diblock copolymers that contain parent homopolymers. c_i was always 0.015 mg mL^{-1}. (a) SEC chromatogram of block copolymer sample Nr.3. (b) LC LCD chromatogram of sample Nr.3. Barrier B#1 was neat toluene, and B#2 was the mixture THF/toluene 30/70. Time delays were 0−3.5−5 min. (c) SEC chromatogram of sole block copolymer without parent homopolymers transferred from the LC LCD column into the SEC columns. Further explanation is in the text.

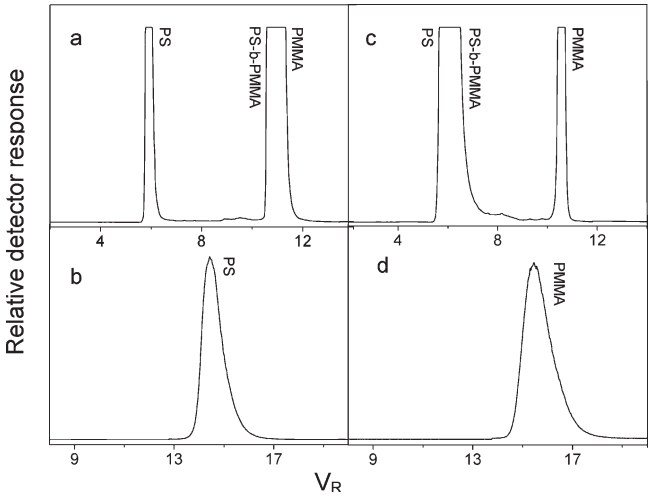

Figure 7. Demonstration of potential of LC LCD × SEC for separation and molecular characterization of parent homopolymers in diblock copolymer Nr.3 at c_i of 0.03 mg mL^{-1}. (a) LC LCD chromatogram, separation of PS from PMMA and block copolymer. One barrier of neat toluene was applied with time delay 0−2 min. (b) SEC chromatogram of PS homopolymer transported from the LC LCD column into the SEC column. (c) LC LCD chromatogram, separation of PMMA from PS and block copolymer. One barrier of THF/toluene 30/70 was applied with time delay 0−2 min. (d) SEC chromatogram of PMMA homopolymer transported from the LC LCD column into the SEC column. Further explanation is in the text.

Table 1. SEC Data for Block Copolymer Sample Nr.3 Obtained with S2D-LC and Combination of LC LCD with SEC

	PMMA	PS-*b*-PMMA	PS
V_r [min]	17.26	14.96	15
M_w [kg/mol]	1.84×10^4	7.89×10^4	6.32×10^4
M_n [kg/mol]	1.69×10^4	6.53×10^4	5.73×10^4
M_w/M_n	1.08	1.19	1.13
M_p	1.95×10^4	8.84×10^4	6.27×10^4

were calculated from the calibration of SEC columns with polystyrene standards obtained with THF/toluene eluent 70/30. Molar mass averages of PS correspond well with the data given by producer (Table 1). Evidently, these values were determined with PS precursor. The polystyrene equivalent molar mass values for the block copolymer and for the PMMA homopolymer are also collected in Table 1. The data for PMMA indicate that homopolymer could be present already in monomer used in block copolymer synthesis. It can be concluded that sequential two-dimensional liquid chromatography based on combination of LC LCD × SEC enabled comprehensive molecular characterization of parent homopolymers present in block copolymer and estimation of molar mass averages of block copolymer unaffected with parent homopolymers.

Exact retention volumes are needed for calculation of polymer molar masses from the SEC chromatograms. Therefore, the process of introduction of each sequence of the LC LCD effluent into the SEC column must be precisely monitored. The introductory measurements with instrumental arrangement in Figure 1a have shown that in the framework of experimental errors the molar masses of narrow and broad polystyrenes calculated from the SEC chromatograms well agreed for samples injected directly into SEC column from the loop in valve V3 or via the LC LCD column, when applying the corresponding calibration that is either only for SEC columns or for all three columns, LC LCD plus SEC. In these cases, both the elution and the data processing start in SEC columns were precisely known. The situation became more complicated when a sequence of column effluent from the LC LCD column was transported into SEC columns and the exact start of data processing in the latter columns had to be located.

The variation of retention volumes in dependence on start of elution and data processing in the SEC column is demonstrated in Figure 8. Figure 8a shows a model LC LCD chromatogram of a polymer eluted in the exclusion mode. Times in minutes *X*, *Y*, and *Z* design the instant, in which the V3 was switched to forward effluent from the LC LCD column into the SEC columns. Simultaneously, the data collection from the SEC columns has started by means of a hand driven electric trigger. Valve V3 has been switched back always at 7.2 min. The real SEC chromatograms for PS 100 kg mol^{-1} are shown in Figure 8b together with peaks of markers, which were injected in the moment of V3 switching (compare Figure 1a). The peaks *X* and *Y* are identical in size and shape, while the peak *Z* is smaller, because in the latter case only one-half of polymer was forwarded from the LC LCD column into the SEC column. As expected, the instant of V3 switching was not important from the point of view of the SEC both peak size and shape—provided the entire sample had been transferred into the SEC column. The only important issue for correct molar mass calculation has been the exact start of SEC data processing. The shift of SEC peaks in dependence on the instant of data collection start is evidenced in Figure 8c. The peak designated *YZ* was injected in the instant *Y* (Figure 8a), but the data collection has started in the instant *Z*. A series of experiments with selected PS and PMMA samples were performed, in which the sequences with different volumes were forwarded from the LC LCD column into the SEC columns. Both the instant of V3 switching and start of data collection were changed. As expected,

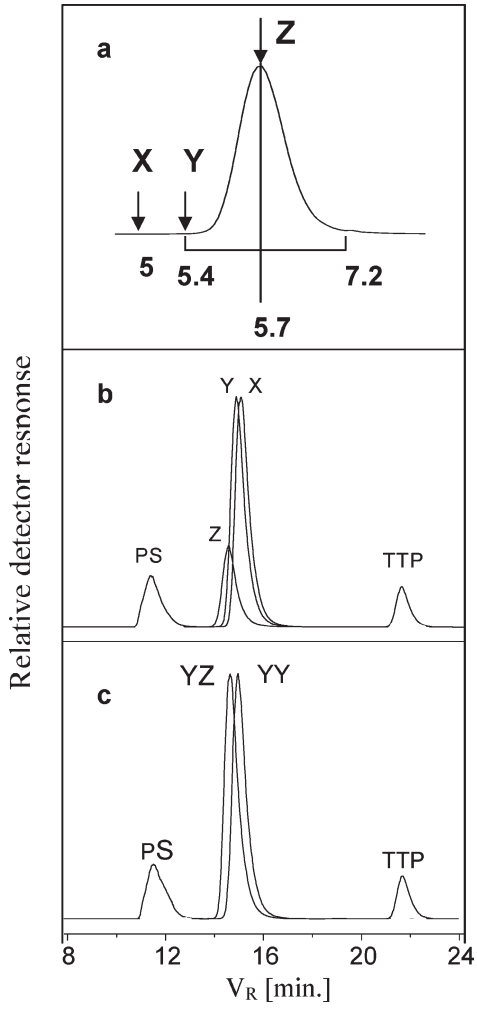

Figure 8. Relation between SEC peak position and size, on the one hand, and parameters of transfer of effluent from the LC LCD column into the SEC column, on the other hand. Illustration of effect of instant and duration of transfer of effluent from the LC LCD column into the SEC column. Model chromatogram of PS eluted from LC LCD column is considered (a) *X*, *Y*, and *Z* are instants of V3 switching. (b) Real SEC chromatograms of PS 100 kg mol^{-1} injected in instances *X*, *Y*, and *Z* from (a) are designated *X*, *Y*, and *Z*, respectively. PS and TTP markers were introduced from V3. (c) Role of software start is visualized. Sample transfer started at instant *Y*. Data collection started at instant *Z* (*YZ*) or *Y* (*YY*). A detailed explanation is in the text.

the LC LCD peak apex was confirmed as appropriate start of SEC data collection. SEC molar masses obtained in this way exhibited very good agreement with the values obtained by conventional SEC analyses.

In Figure 9, SEC chromatograms of PMMA with molar masses 16 and 103 kg mol^{-1} are compared for different experimental arrangements. Solid line depicts the situation when PMMA has been directly injected into SEC column, dashed line is valid for the LC LCD plus SEC column combination, and the dotted line shows the data from the LC LCD × SEC system with PMMA eluted behind the barrier of THF/toluene 30/70. The dashed chromatogram of PMMA 16 kg mol^{-1} has been broadened due to the SEC contribution of the LC LCD column, while PMMA 103 kg mol^{-1} was excluded from the LC LCD column packing. The molar mass data calculated from chromatograms are given in Table 2. The mutual agreement is well reasonable, and this result demonstrates applicability of sequential two-dimensional liquid chromatography LC LCD × SEC for quantitative molar mass determination of those constituents of complex polymer systems that can be discriminated by the LC LCD method.

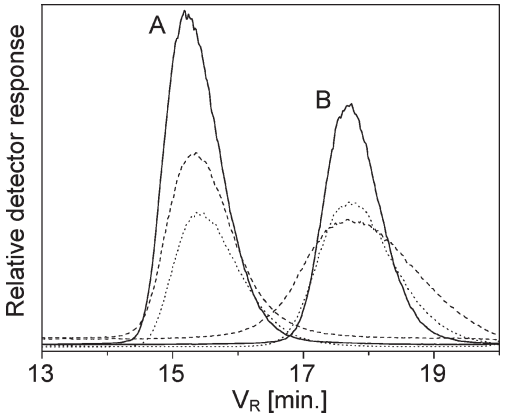

Figure 9. Comparison of SEC chromatograms for PMMA with molar masses 103 kg mol^{-1} (A) and 16 kg mol^{-1} (B), which were obtained in different experimental arrangements: (—) samples were directly injected into SEC columns from V3; (- - -) samples eluted via the LC LCD column were transferred into the SEC columns without barrier (···) samples eluted from LC LCD column behind barrier THF/toluene 30/70 were transferred into SEC columns. A detailed explanation is in the text.

Table 2. SEC Data for PMMA 16 and 103 kg mol^{-1} Determined with Direct Injection of Sample into Sole SEC Column or into a Tandem of LC LCD Column without Barrier plus SEC Columns (LC LCD + SEC) or into the Tandem of LC LCD Column with Barrier of THF/Toluene 30/70 plus SEC column (LC LCD × SEC)

PMMA (16 kg mol^{-1})	SEC	LC LCD + SEC	LC LCD × SEC
V_r [min]	17.7	17.7	17.7
M_w [kg/mol]	1.4×10^4	1.4×10^4	1.4×10^4
M_n [kg/mol]	1.3×10^4	1.1×10^4	1.1×10^4
M_w/M_n	1.1	1.3	1.2
PMMA (103 kg mol^{-1})	SEC	LC LCD + SEC	LC LCD × SEC
V_r [min]	15.18	15.23	15.29
M_w [kg/mol]	9.2×10^4	9.2×10^4	8.9×10^4
M_n [kg/mol]	8.2×10^4	7.7×10^4	7.4×10^4
M_w/M_n	1.1	1.3	1.2

Conclusions

Sequenced two-dimensional liquid chromatography of polymers (S2D-LC) is a procedure in which whole amounts of particular sample constituents separated in the first-dimension column are online forwarded into the second-dimension column. A necessary condition for a successful S2D-LC is the high selectivity and sample capacity of separation in the first-dimension column. Comprehensive separation and molecular characterization of model complex polymer systems that contain macromolecules of different compositions have been attained by a S2D-LC that combines liquid chromatography under limiting conditions of desorption (LC LCD) and size exclusion chromatography (SEC). High separation selectivity for polymers of distinct chemical structure and large sample capacity as well as robustness and experimental feasibility of LC LCD together with universality and experimental simplicity of SEC allow fast and efficacious discrimination, identification, and molecular characterization of numerous complex polymer systems including those containing minor macromolecular constituents (well below 1%). The limitation of LC LCD includes necessity of appropriate difference in adsorptivity of sample constituents and their good solubility in solvents of distinct polarities. The so far open question is the applicability of LC LCD to complex polymers that exhibit continuous distribution in their chemical structure or physical architecture such as statistical copolymers and stereoregular polymers. The work in this direction is in progress in this laboratory. Important application of the above S2D-LC procedure is separation and molecular characterization of parent homopolymers present in

block copolymers. Moreover, molar mass of block copolymers estimated with help of S2D-LC is not affected by presence of homopolymers. The authors are ready to assist the method introduction to potential users, especially concerning identification of appropriate LC LCD conditions.

Acknowledgment. Authors acknowledge financial support from the Slovak Grant Agencies VEGA (project 2/0171/09) and APVV (project 0592-07). Thanks also belong to Dr. W. Wunderlich of Rohm, Darmstadt, Germany, and to Dr. J. Herz of Institut Sadron, Strasbourg, France, for PMMA samples as well as EKA Chemicals, Gothenburg, Sweden, for the gift of Kromasil column packing.

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