Liquid Chromatography of Macromolecules under Limiting Conditions of Desorption. 1. Principles of the Method

D. Berek

Polymer Institute, Slovak Academy of Sciences, 842 36 Bratislava, Slovakia

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ABSTRACT: The liquid chromatography of macromolecules under limiting conditions of desorption (LC LCD) is based on a combination of exclusion and adsorption separation mechanisms. Eluent promotes desorption of macromolecules that are dissolved in an adsorption-promoting liquid and injected into a liquid chromatographic (LC) column packed with porous adsorptive packing. The zone of sample solvent forms an "adsorption promoting barrier". Under properly chosen, "limiting", conditions the transport of adsorbing polymer species along the column is slowed since they cannot pass the above adsorption-promoting barrier. As result, macromolecules of various sizes and molar masses leave LC column in the form of a rather narrow band immediately behind the zone of their initial solvent. At the same time, other kinds of polymer chains which exhibit lower affinity toward column packing and less adsorption surmount the zone of their original solvent. They are eluted in the size exclusion chromatographic mode. In this way macromolecules with different chemical structures can be discriminated. The LC LCD idea has been tested with a model system comprising poly(methyl methacrylate) probes, silica gel column packing, toluene (adsorbing liquid), and tetrahydrofuran (desorbing liquid). Some applications of this novel LC procedure have been proposed. They include separation of two-component and multicomponent blends, various kinds of copolymers, and oligomers.

Introduction

Complex polymers that are built of more than one kind of monomer (blends, copolymers) usually exhibit multiple distributions in their molar mass, chemical structure, and architecture. Various nonuniformities in chemical composition are encountered also in the case of functionalized polymers. As known, distribution in the architecture of macromolecules often appears even in homopolymers, referring, e.g., to their stereoregularity, long- and short-chain branching, etc.

Molecular characterization of complex polymers usually includes their separation by liquid chromatography (LC) or mass spectrometry. The most commonly used LC method, size exclusion chromatography (SEC), separates macromolecules according to their size in solution. Since this parameter depends on all above-mentioned characteristics of macromolecules, SEC can only exceptionally afford simultaneous and unambiguous information on the molar mass distribution (MMD), chemical composition distribution, and molecular architecture distribution for all constituents of complex polymers. Therefore two or even several LC separation mechanisms are being combined with the aim to enhance the selectivity of separation according to one single parameter. At the same time, separation according to other parameters should be suppressed.

A typical example of such combined or "coupled" separation mechanisms represents liquid chromatography at the point of exclusion−adsorption transition (LC PEAT). In this case, the exclusion and adsorption of macromolecules is combined in such a way that they mutually compensate. This compensation results in the loss of separation selectivity according to the polymer molar mass.[1] Generally, polymer is eluted from the LC column packing with given pore sizes either in the SEC, in the liquid adsorption chromatographic (LAC), or in the transition (PEAT) mode. The actual elution mode is governed by the extent of adsorption between macromolecules and column packing which can be controlled mainly with eluent adsorption strength, further with temperature, and possibly also with pressure. The eluent adsorption strength depends on molecular interactions of solvent molecules with column packing surface. It predetermines whether polymer segments are adsorbed onto or displaced from the LC column packing.

So far two approaches to the LC PEAT were proposed. The first one is based on the pioneering experimental works of Belenkii, Gankina, and Tennikov[1−5] and on the theoretical considerations of Skvortsov and Gorbunov.[5−10] This procedure is called liquid chromatography at the critical adsorption point (LC CAP) and utilizes a eluent/sample solvent system that slightly promotes adsorption of macromolecules, just to compensate for their exclusion. Following theoretical predictions,[6−8] LC CAP was successfully applied to discrimination and characterization of oligomers differing in the nature of their functional groups and possessing functionality distribution,[11−13] as well as to separation and characterization of binary polymer blends and block copolymers.[13] It was shown that graft copolymers with short side chains can also be characterized with the LC CAP.[14] In all these cases, one kind of polymer chain is eluted at the critical adsorption point while other chains elute under SEC or LAC conditions.

Unfortunately, LC CAP exhibits numerous drawbacks that complicate its application to high polymers.[15,16] These drawbacks involve enormous sensitivity of critical adsorption point toward experimental conditions[16] including even the effect of column packing pore sizes.[17] A pronounced peak broadening and even peak splitting was observed[15] in some LC CAP systems, especially for macromolecules excluded from the pores of column packing.[17]

Therefore, other approaches to the LC PEAT were looked for. The recently proposed alternative to the LC CAP is called liquid chromatography under limiting

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conditions of adsorption (LC LCA).[18−21] In this case, eluent rather strongly promotes adsorption of macromolecules. These are, however, dissolved and injected in the solvent that promotes polymer desorption (a DESORLI). Due to their partial exclusion from the packing pores, macromolecules tend to move faster along the LC column than the zone of their original solvent. However, when polymer leaves the DESORLI zone, it is retained and again displaced only after it has been reached with the DESORLI zone. The conditions can be found under which all macromolecules will be eluted just within their initial solvent zone irrespective of their molar mass. These are limiting conditions of adsorption (LCA). Evidently, LCA depends mainly on the affinity of macromolecules toward column packing and on both the adsorbing strength of eluent and desorbing strength of sample solvent. The operational parameters such as temperature and width of sample solvent zone also influence the LCA position.[21] Both system nature and operational parameters must be carefully optimized to attain controlled and repeatable LCA elution.

In this paper, we propose the third mode of the liquid chromatography at the point of exclusion−adsorption transition. We shall call it liquid chromatography under limiting conditions of desorption (LC LCD). In this case, the LC eluent promotes desorption of polymer analyzed; it is a DESORLI. Macromolecules are dissolved and introduced into the LC column in an adsorption-promoting liquid (an ADSORLI). This means that polymer is temporarily retained near the column inlet after its injection. Macromolecules are desorbed from the column packing when their initial solvent moves away in the course of the experiment. At this moment polymer starts eluting. Due to their partial exclusion, macromolecules would move faster than the zone of their initial solvent, however, they cannot pass this local eluent gradient, that is the "adsorption-promoting barrier". Under specific, "limiting" conditions all macromolecules will elute as a narrow band just at the tail of their original ADSORLI solvent peak—independent of their molar masses.

For the sake of clarity the three modes of LC PEAT, namely LC CAP, LC LCA, and LC LCD, are schematically depicted in Figure 1. Note that the peak retention volumes exhibit opposite tendency when compared with the peak position.

In this work we present the first experimental proof of the LC LCD approach and discuss its potential.

Experimental Section

The common LC/SEC apparatus was used. It consisted of a pumping system Model 501 (Waters, Milford, MA), a six-port, three-way injection valve (Rheodyne, Cotati, CA) equipped with a 50 μL sample loop. In all experiments, the entire loop volume was injected. Columns 4 × 250 mm from Knauer (Berlin, Germany) were packed with 10 μm silica gels Biospher SI-120 (Labio, Prague, Czech Republic) and Separon SGX-1000 (Tessek, Prague, Czech Republic) with pores of 12 and 100 nm, respectively. Further specifications of the columns used are given elsewhere.[17] The columns were kept at 30 ± 0.1 °C except for a few experiments indicated in Figure 4. A column oven (Chroma, Graz, Austria) was connected with a water thermostat, Model RM6 (Lauda, Königshofen, Germany). Detectors were differential refractometer, Model RIDK 101 (Laboratory Instruments, Prague, Czech Republic) and evaporative light scattering detector (ELSD) Model DDL-21 (Eurosep, Cergy-St. Christophe, France) in series. The data were collected and processed with SEC/HPLC software from Chroma.

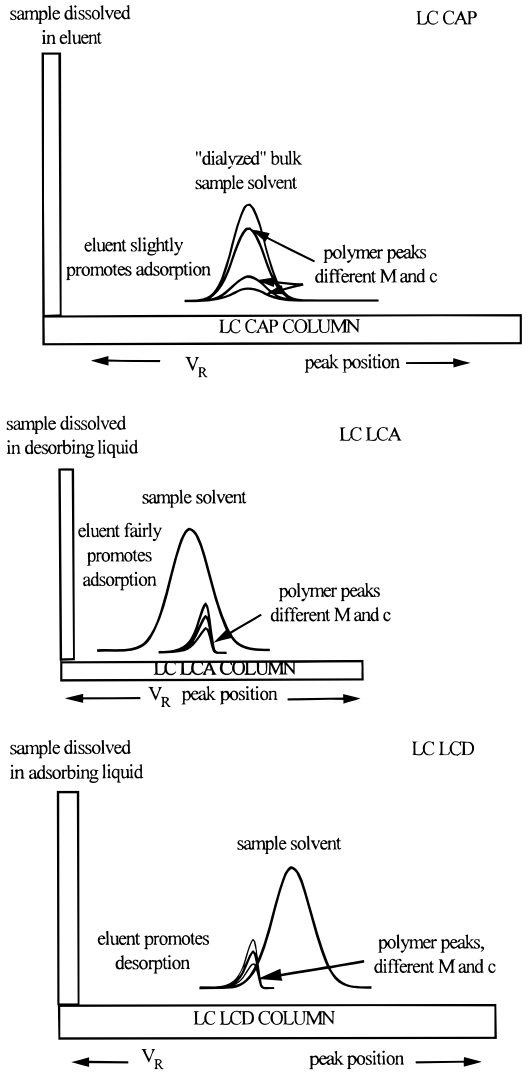

Figure 1. Schematic representation of LC CAP, LC LCA, and LC LCD modes of elution. For detailed explanation, see the text.

Poly (methyl methacrylate)s (PMMA)s of low tacticity and of medium broad molar mass distributions[22] were used as model polymers. Solvents were tetrahydrofuran (THF) (a strong DESORLI) and toluene (a strong ADSORLI) for PMMA/silica gel system. PMMAs injected in toluene into toluene eluent were fully retained within columns. Both THF and toluene are thermodynamically good solvents for PMMA. They were purchased from Merck, (Darmstadt, Germany) and distilled immediately before use. THF was stabilized with 0.1 wt % of butylated *p*-cresol after distillation. All eluent compositions are given in weight percent. A constant flow rate of 1 mL·min^{-1} was maintained. All eluent compositions are given in weight percent.

Results and Discussion

The dependences of log M vs retention volumes (the "calibration curves") for PMMAs are shown in Figures 2−4 for different elution modes. Figure 2 compares the SEC calibration curve (sample solvent and eluent was THF DESORLI) with the calibration curves obtained when sample solvent was toluene ADSORLI and the adsorbing ability of eluent or more precisely the inherent adsorption of eluent on the silica gel surface has been gradually enhanced by addition of toluene. In the framework of experimental errors, the calibration curves were linear up to about 20% of toluene. A surprisingly

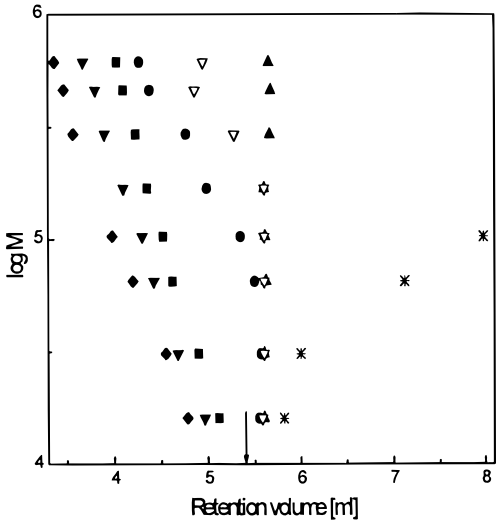

Figure 2. Calibration dependences for PMMAs eluted in THF (▼) and in THF/toluene mixed eluents (wt %): 80/20 (■); 60/40 (●); 55/45 (▽); 50/50 (▲, LC LCD mode); and 35/65 (∗, LAC mode). Sample solvent was toluene. For comparison also the calibration dependence for PMMA dissolved in THF and eluted in THF is shown (◆). An asterisk indicates retention volume of toluene excess injected into eluent THF/toluene 60/40 wt %.

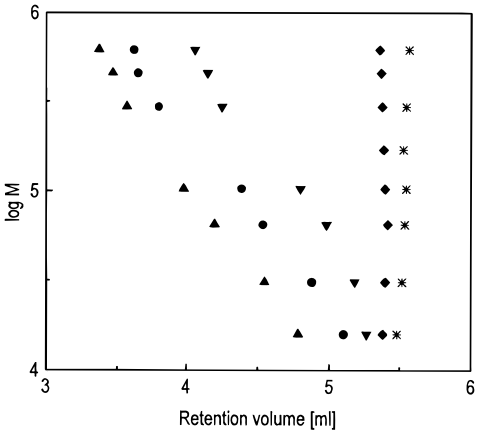

Figure 3. Calibration dependences for PMMAs eluted in THF (▲) and in mixed eluents THF/toluene: 50/50 (●); 40/60 (▼); 35/65 (◆, LC LCA mode); 5/95 (∗, LC LCA mode). Samples solvent was THF.

large shift of retention volumes was observed when toluene was used as sample solvent instead of THF, applying pure THF as eluent. The calibration curves converged in the area of high retention volumes. This situation differs from that often observed in the slightly adsorbing SEC systems where sample solvent equals eluent and the SEC elution order is still maintained. For example, calibration curves diverged in the range of high retention volumes for PS and PMMA probes using THF sample solvent/eluent and bare silica gel column packing.[17,23] The latter behavior is explained with increasing pore surface being accessible for smaller macromolecules. This effect prevails over rising polymer adsorption with its molar mass. The tentative explanation of present results is as follows: if an adsorption-promoting liquid is used as sample solvent, macromolecules are temporarily retained at the column inlet. Polymer is again desorbed when the concentration of DESORLI in eluent reaches a "desorption threshold". The composition of low molecular displacer at the desorption treshold strongly depends on the molar

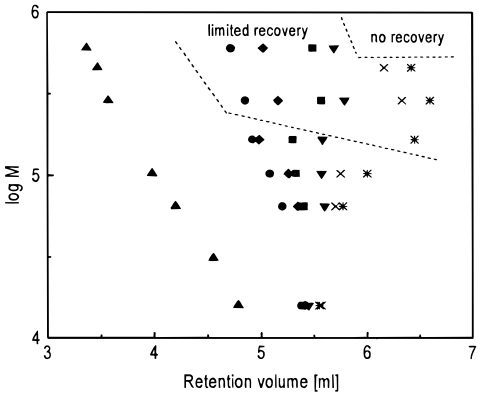

Figure 4. Calibration dependences for PMMAs eluted in mixed eluents THF/toluene (wt %): 39/61 (●); 38/62 at 25 °C (◆); 38/62 at 30 °C (■); 38/62 at 35 °C (▼, nearly LC CAP); 37/63 (×); 36.5/63.5 (∗). Eluent was used as sample solvent. For comparison, also calibration dependence for samples dissolved and eluted in pure THF is shown (▲). The area of limited sample recovery (less than about 70% of initial sample was eluted) and the area of total sample retention are indicated.

mass of polymer: larger macromolecules need a stronger displacer than small macromolecules to desorb and start moving.[24] Therefore the retention volumes of polymers with higher molar masses are more shifted toward higher values.

The results in Figure 2 apparently differ from our previous finding[25] that the retention volumes of polystyrenes did not depend on presence of various admixtures in the sample solvent—this was also true if these admixtures were either poor solvents for polymer such as dimethylformamide or strong nonsolvents for polymer samples such as methanol or water, using THF as eluent and porous glass column packings. The abovementioned admixtures in injected sample solutions promoted polymer desorption due to their polarity. We can conclude that the thermodynamic quality of sample solvent plays a less important role than its adsorbing strength in the case of adsorptive macromolecules and active column packing.

Up to 20% content of ADSORLI toluene, eluent was still able to rather effectively suppress adsorption effects. The toluene "barrier" was rapidly and effectively diluted with eluent so that macromolecules could surmount it and were eluted essencially in the SEC mode. The situation, however, rapidly changed when the amount of toluene in eluent further increased. Retention of both macromolecules and injected pulse of toluene rised substantially (Figure 2). For example, the retention volume of toluene increased from 5.07 mL in pure THF up to 5.42 mL in eluent containing 60% of toluene and approached the total volume of the column which was 6.18 mL. The accessibility of packing pore walls started to play a decisive role and the retention volumes of larger macromolecules increased less than that of smaller macromolecules which eventually stayed behind the toluene "barrier". Eventually, we arrived at the eluent composition (50% of toluene) where macromolecules of all sizes were unable to pass the "barrier" of the ADSORLI sample solvent. In other words, all polymer species eluted essencially within the same retention volumes and just behind the "adsorption-promoting barrier" of their original solvent, irrespective of their molar mass. These were the limiting conditions of desorption. Evidently, the actual eluent composition

needed to reach LCD would depend on the adsorbing properties of both column packing and polymer and further on the desorbing properties of eluent, as well as on the experimental conditions such as volume of injected ADSORLI sample solvent, column length, and mixing/diffusion rate of ADSORLI sample solvent and eluent. In present system, the area of LCD eluent composition was rather broad and assumed to be at least 10 wt %: eluent containing 60 wt % toluene still exhibited LCD properties. Further increase of the ADSORLI amount in the eluent promoted adsorption of macromolecules to such extent that at 65 wt % toluene, PMMAs eluted in the LAC mode (Figure 2).

For comparison, selected results on the LC LCA elution are shown in Figure 3 for the same polymers, column packing, and THF/toluene eluents but with THF DESORLI sample solvent. Up to 60% of toluene in eluent the PMMA elution was predominantly controlled by SEC mechanism though adsorption effects on V_R were evident. Between 65 and 95 wt % content of toluene ADSORLI in eluent, which is within an enormously broad eluent composition area, the system attained limiting conditions of adsorption and macromolecules eluted within the zone of their initial DESORLI solvent irrespective of their molar masses.

The results obtained in the LC CAP elution mode are shown in Figure 4. Again, the same column and PMMA probes were used in THF/toluene mixed eluents as in previous experiments. In this case, however, sample solvent and eluent were identical. Present experiments confirmed our observations made with similar polymer probes and eluents studying the effect of column packing pore sizes.[17] The vertical PEAT calibration plot could hardly be attained, the system was sensitive toward small variations in sample solvent/eluent composition and temperature and the sample recoveries dropped substantially with increasing molar mass of PMMA. The overall LC CAP retention was controlled with the small pore size column and as soon as macromolecules became excluded from the column packing pores the whole system began to behave irregularly.[17]

The areas of SEC, LC LCD, LC CAP, and LC LCA are schematically depicted in Figure 5 showing their dependence on eluent composition.

The repeatability of retention volumes changed in the series SEC ~ LC LCD ~ LC LCA > LC CAP ≫ LAC.

Let us compare retention volumes and peak shapes for particular elution modes. PMMA 103K with a M_w/M_n value of 1.27 has been chosen as a typical example, and corresponding chromatograms are collected in Figure 6. The behavior of other PMMAs has been similar, except for the 35/65 THF/toluene eluent composition, in which higher molar masses were fully retained within column, and on the contrary, the peaks of PMMAs with smaller molar masses have been much less broadened. Due to partial exclusion of macromolecules, sample retention volume was lowest in the SEC mode (sample dissolved and eluted in THF). It followed the LC CAP mode (sample dissolved and eluted in THF/toluene 38/62 "critical eluent"). In this case, macromolecules were accompanied with their original solvent. In the LC LCA mode (sample dissolved in DESORLI THF and injected into ADSORLI THF/toluene 10/90 eluent), the peak retention volume slightly increased but polymer still eluted within its THF original solvent. In the case of LC LCD (sample injected in the ADSORLI toluene into a DESORLI THF/toluene 40/60 eluent)

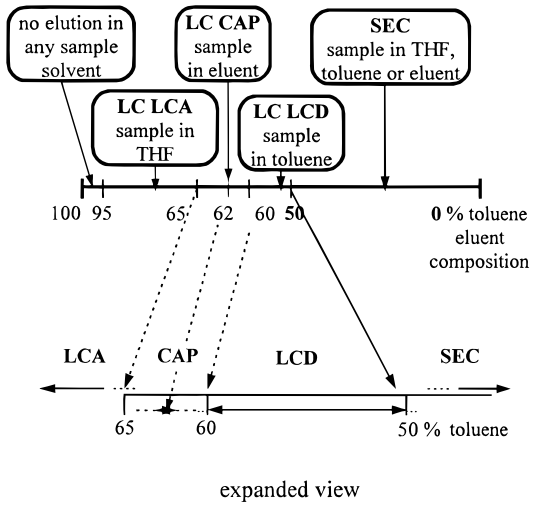

Figure 5. Schematic representation of SEC, LC LCD, LC CAP, and LC LCA elution modes for selected sample solvents and eluent compositions for bare silica gel packed columns THF/toluene eluents and PMMA probes. Note that the actual elution mode depends also on experimental conditions such as temperature, volume of injected solution, column length and efficiency, etc.

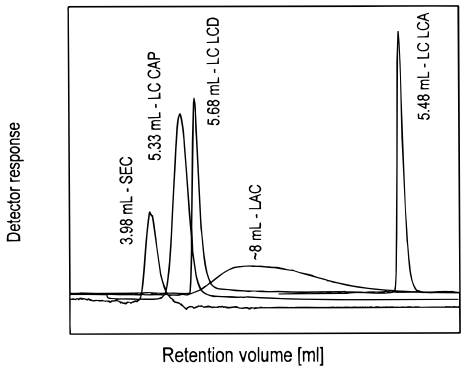

Figure 6. Chromatograms of PMMA 103 K eluted in different LC modes. For the sake of clarity, the start of the LC LCA chromatogram was shifted. For detailed discussion see the text.

macromolecules were accumulated at the tail of their original solvent peak (toluene) so that their retention volume further increased. Finally, the PMMA retention volume rose in a pronounced manner in the LAC mode (sample dissolved in a strong ADSORLI toluene and eluted in a weakly adsorbing eluent THF/toluene 35/65).

The differences in the peak shapes/widths were also rather large for particular elution modes. In the first approximation, we can assume that the peak widths were only slightly affected by different viscosities of eluent components THF and toluene. Moreover, temperature and eluent flow rate were practically constant and the entire LC system hardware was identical. Therefore, the peak broadening caused by extracolumn (injector, connecting capillaries, detector) and intracolumn (diffusion, mixing) nonseparation effects were at least comparable and we can conclude that observed variances in the peak shapes were mainly due to intrinsic features of particular chromatographic separation processes. This is obvious in the case of SEC peak which is broadened due to size separation of polymer. On the other hand, there was no size separation in the LC CAP system and the observed excessive peak broadening remained so far unexplained. As men-

tioned, the LC CAP peak broadening has been experimentally observed already before.[15,17] The peak broadening in the LAC mode is enormous, probably also due to very strong effect of molar mass on the polymer retention volume (see also Figure 2). The latter phenomenon complicates practical utilization of the isocratic LAC in the molar region over 10K g·mol^{-1}. As expected, the LC LCA and LC LCD peaks were rather narrow due to the "peak compression processes". The front parts of both peaks were very steep because of the "impermeability" of both eluent in the case of LC LCA and sample solvent zone in the case of LC LCD. The LC LCA peaks were, however, less tailed than those produced by LC LCD. This can be understood when considering processes taking place in LC column. Macromolecules are confined in the zone of their initial solvent in LC LCA and their adsorption is rather limited. Polymer accumulates within the front part of the DESORLI sample solvent zone but the nonexcluded macromolecules are slightly retained by the SEC (entropic) processes. Therefore the "peak compression" or "peak sharpening" process is expected to reach its maximum when all macromolecules are fully excluded from the pores of packing.

In LC LCD, a certain adsorption-promoting activity of eluent is necessary. Otherwise, sample solvent, that is the ADSORLI barrier would have to be very broad to preserve its impermeability for macromolecules after its diluting with eluent. Therefore, in addition to the exclusion phenomena, adsorption of macromolecules may cause some additional peak broadening. This shortcoming can be at least partially suppressed by optimization of LC LCD experimental conditions.

Conclusions

Elution behavior of macromolecules under limiting conditions of desorption (LCD) has been proven for a model system PMMA−silica gel−toluene−tetrahydrofuran. This result can be readily generalized to conclude that the idea of liquid chromatography of macromolecules under limiting conditions of adsorption (LC LCD) is operative.

LC LCD was compared with other two modes of liquid chromatography at the point of exclusion−adsorption transition (LC PEAT), namely with liquid chromatography at the critical adsorption point (LC CAP) and with liquid chromatography under limiting conditions of adsorption (LC LCA). It has been shown that LC LCD similarly as LC LCA is much more robust and therefore experimentally more feasible than LC CAP. The limiting conditions of desorption are expected to depend on the column packing and eluent nature and also on the operational parameters of experiment. All these variables must be independently adjusted and carefully optimized to attain molar mass independent polymer elution.

The polymer peaks in the LC LCD and LC LCA modes are much narrower than in the LC CAP mode and we can speak about peak compression processes in the former two procedures. In contrast with LC CAP, both LC LCD and LC LCA methods are expected to be applicable also to the macromolecules that are excluded from the pores of column packing. The peak compression is assumed to be even more effective for the excluded polymer species. Separation of macromolecules with different chemical compositions could be possible using a narrow pore LC LCD packing utilizing only the difference between exclusion of eluent molecules and polymer molecules irrespective of the size or molar mass of latter species.

It is expected that the LC LCD method can be applied not only to the separation of binary polymer blends but also to the discrimination of multicomponent polymer systems including various types of copolymers and oligomers: One can generate a series of barriers applying adsorption promoting liquids with adjusted adsorption strengths and "permeability" so that macromolecules of different chemical compositions and thus of different adsorptive properties will be discriminated more or less irrespectively of their molar mass.

The common advantage of all three LC PEAT procedures is the easiness of their further coupling with other liquid chromatographic methods, e.g. with SEC, and LAC and also with the gradient elution based LC modes.

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