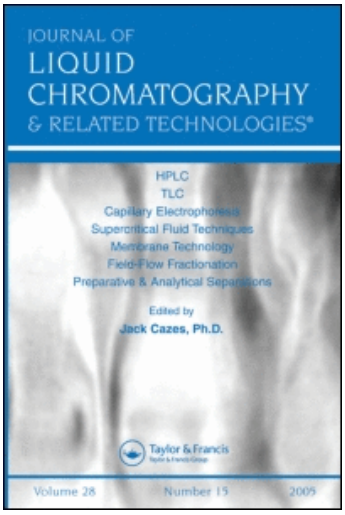

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LIQUID CHROMATOGRAPHY OF MACROMOLECULES UNDER LIMITING CONDITIONS OF ADSORPTION. I. PRINCIPLES OF THE METHOD

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ABSTRACT

A novel separation method for macromolecules, viz. liquid chromatography under limiting conditions of adsorption (LC LCA), is presented. LC LCA is designed for discrimination of complex polymers. It combines exclusion and adsorption mechanisms so that they mutually compensate. This results in the absence of separation of macromolecules according to their size or molar mass. In LC LCA, the eluent is a liquid moderately promoting adsorption of polymer species. The column packing attracts macromolecules and, if the sample were dissolved and injected in eluent, it would be retained within column. Therefore, the polymer is injected in a solvent that effectively suppresses adsorption. Experimental conditions can be identified with regard to the eluent, column packing, temperature as well as sample solvent, and injected sample volume under which macromolecules of various molar masses are eluted together with their initial solvent. In this way, binary polymer blends, with components exhibiting different adsorptive properties can be separated.

The LCA behavior of poly(methyl methacrylate) is presented using bare silica gel or "active" polystyrene/divinyl benzene column packings, tetrahydrofuran as desorption promoting liquid and toluene or chloroform as adsorption promoting liquids.

INTRODUCTION

Complex polymers are composed of species differing not only in their molar mass but also in their chemical nature and/or physical structure. Consequently, both chemical and physical properties of complex polymers are described by more than one mean value and by more than one distribution function. This complicates their full molecular characterization. Selective separation methods must be applied that are able to discriminate macromolecules solely according to one single parameter. As a rule, these selective separation methods combine two or several separation mechanisms. The typical examples for such coupled procedures represent combinations of steric exclusion with solubility[1,2] and exclusion with adsorption.[3-5] Both combinations are aimed at suppressing separation of macromolecules according to their molar masses. As a result, two polymer species with different solubilities in an eluent, or with different adsorption onto the column packing, can be mutually separated even if their molar masses or, more precisely, their molecular sizes in solution, are equal. This is not possible in the conventional size exclusion chromatography.

As is well known, the exclusion of macromolecules from the pores of column packings accelerates their elution and this is the basis of the polymer separation in size exclusion chromatography (SEC). On the other hand, the adsorption of macromolecules retards their progression along the LC column. The adsorption of polymers increases with their molar mass and this offers another opportunity for their separation. The corresponding method is called liquid adsorption chromatography (LAC) (Figure 1).

The exclusion-adsorption transition is also shown in Figure 1. In this case macromolecules possessing identical chemistry, but different molar masses (M), elute in the same retention volume (V_R) that roughly corresponds to the total volume of liquid within the LC column. The experimental conditions that lead to the exclusion – adsorption transition vary with the chemical composition and/or with the physical structure of macromolecules. This implies that when one polymer species elutes at the point of exclusion – adsorption transition and another kind of polymer elutes according to the SEC or LAC mechanism the two kind of chains can be characterized without interference.

To date the most popular approach to the liquid chromatography at the point of exclusion – adsorption transition has been referred to as liquid chromatography at the critical adsorption point (LC CAP).[3-10]

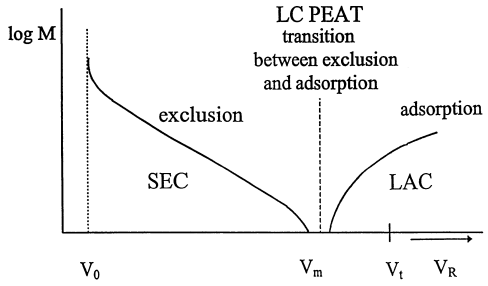

Figure 1. Schematic representation of liquid chromatographic separation mechanisms for macromolecules. For explanation, see the text.

In the LC CAP approach the column packing, eluent, and temperature are chosen so that the macromolecules under study are slightly adsorbed within column. The adsorption of polymer species is just sufficient to compensate for their size exclusion. It is important to stress that the sample is dissolved in the eluent prior its injection.

LC CAP has already found application in the separation of oligomers according to their functionality,[6] as well as in characterization of polymer blends and block copolymers.[7] Unfortunately, LC CAP exhibits several serious shortcomings that complicate its utilization, particularity in the area of high molecular weight polymers.[8] For example, important problems of LC CAP are connected with the high sensitivity of polymer retention in the critical adsorption area toward minute changes in:

- eluent composition,
- column temperature,
- column packing surface chemistry and, possibly, also the
- pressure within the column.

For the preceding reasons alternative exclusion – adsorption combinations have been investigated that could mitigate the drawbacks of the LC CAP. This paper reports one such attempt which is termed liquid chromatography under limiting conditions of adsorption (LC LCA).[9-12] In this case, the eluent is adjusted to more strongly promote adsorption of macromolecules on the column packing than in the LC CAP mode. If a polymer solution were injected in the eluent it would be fully retained. In contrast the polymer sample is dissolved and injected in a single or mixed desorption promoting liquid. This is the important difference between LC LCA and LC CAP. In LC LCA, macromolecules tend to travel faster than their initial solvent along the column due to their partial exclusion from the pores of column packing. However, when the polymer leaves its solvent zone and encounters eluent it is retained by

adsorption until reached by its initial solvent and desorbed to start moving again. An equilibrium situation is eventually established in which macromolecules just travel within the leading portion of their initial solvent zone. The adsorption strength of the eluent and the desorption strength of sample solvent are adjusted so that, for macromolecules of different sizes, their size exclusion and adsorption mutually compensate. Consequently, polymer samples are eluted in the same retention volume independently of their molar mass.

Basic experimental data on the liquid chromatography under limiting conditions of adsorption are presented in this contribution. The potential of the LC LCA method, as well as some of its possible limitations are briefly discussed.

EXPERIMENTAL

The LC LCA experimental assembly is, in principle, identical with an SEC apparatus. Our instrument consisted of a mobile phase container in which eluent was protected against moisture absorption. A Model 510 isocratic pump from Waters (Milford, MA, USA) was employed at the flow rate of 1 $mL.min^{-1}$ along with injection and switching valves from Rheodyne (Cotati, CA, USA). The sample loop had a volume of 50 μL. LC columns (7.8 x 300 mm) were packed either by bare silica gel with pore diameter 10 nm (Labio, Prague, Czech Republic) or by polystyrene divinylbenzene SEC gels ("linear columns") (Polymer Laboratories, Church Stretton, UK, and Waters Corp.).

The detector was either a differential refractometer from Knauer (Berlin, FRG) or an evaporative light scattering device Model DDL-21 from Eurosep (Cergy St. Christophe, France). A column oven was maintained at a constant temperature of 30°C. The data were collected with a PC and processed with the software from Chromtech (Graz, Austria).

Medium-to-broad molar mass distributed poly(methyl methacrylate)s (PMMA)s of low stereoregularity were obtained from Dr. W. Wunderlich (Röhm, Darmstadt, Germany). Their characteristics have been summarized elsewhere.[13]

Both the broad polystyrene (PS) and PMMA were commercial materials. The eluents were toluene, chloroform and tetrahydrofuran (THF) all from Merck (Darmstadt, Germany). Toluene was used as purchased. Chloroform was stabilized with 1% ethanol.

For selected experiments, ethanol was removed and chloroform was stabilized by 50 ppm of amylene. THF was distilled immediately before measurements and used without stabilizer. All mixed eluent compositions are given in weight %.

RESULTS AND DISCUSSION

The effect of sample solvent and eluent composition on polymer retention can be easily visualized by the dependence of retention volumes on the molar mass of injected macromolecules ("calibration curve"). However, the magnitude of the changes in retention volumes indicates the vicinity of the point of exclusion-adsorption transition even without precise knowledge of the molar masses of eluted macromolecules.

As discussed above, for an effective LC LCA system an "active" column packing is needed together with eluent that is an adsorption promoting liquid (ADSORLI). Furthermore, the sample solvent must promote polymer desorption (DESORLI). The easiest way for controlling polymer adsorption is the use of mixed eluents containing appropriate amounts of ADSORLI and DESORLI. The adjustment of temperature usually allows fine tuning of the eluent adsorption strength. If the ADSORLI eluent component does not dissolve the polymer under study it may happen that the mixed eluent with appropriate adsorption strength becomes a nonsolvent for the polymer sample, which is injected in a thermodynamically good DESORLI solvent. In this case, one can arrive at retention mechanism which is termed "limiting conditions of solubility" (LCS).[1,2] To avoid problems with polymer solubility and to exclude the interference of LCA and LCS, thermodynamically good solvents were used as both the ADSORLI and DESORLI eluent components in present study.

The calibration curves for poly(methyl methacrylate)s of low stereoregularity in THF and toluene/THF mixtures with bare silica gel column packing are shown in Figure 2. PMMAs are fully retained by bare silica gel in pure toluene. This implies that toluene is a strong ADSORLI for PMMA with bare silica gel. On the other hand, THF is a DESORLI for PMMA and the calibration curve of this polymer in THF assumes typical SEC patterns. Therefore, pure THF was applied as sample solvent in further experiments. When larger amounts of toluene was added to the THF eluent, the calibration curves began shifting toward higher retention volumes due to adsorption. At 65% of toluene the limiting conditions of adsorption have been reached and the V_R values of PMMA did not depend on the polymer molar mass.

We have also studied the LC CAP mode, in an identical system utilizing bare silica packing and PMMAs probes with toluene/THF mixed eluents.[14] The "near critical adsorption point" was identified for an eluent containing 64% of toluene. Evidently, the difference between CAP and LCA eluent composition is not large and this indirectly demonstrated high sensitivity of the polymer adsorption toward eluent composition and sample solvent changes. High molar mass PMMA did not elute from the silica packed column which was flushed with mixed eluent containing more than 66% of toluene if PMMA was dissolved in eluent (LC CAP approach) instead of pure THF (LC LCA approach).

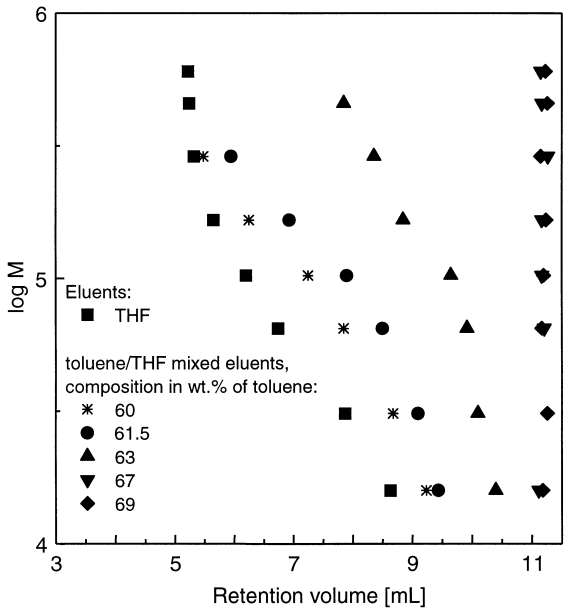

Figure 2. LC LCA calibration curves for PMMAs of low stereoregularity with bare silica gel column packing. Pure THF and THF/toluene mixed eluents, with various amounts of toluene: 60%; 61.5%; 63%. Limiting conditions of adsorption were maintained between 65 and 69% of toluene. Samples were injected in THF.

The LC CAP recovery was reduced even at 64% of toluene when polymer molar mass approached the column packing exclusion limit and the excluded macromolecules were as rule fully retained within the LC CAP column.[14] No decrease in polymer recovery was observed with eluent containing 65% of toluene if samples were injected in THF that is under LC LCA conditions – even for polymer species fully excluded from the packing pores. On the other hand, the polymer recovery was found to decrease also under LC LCA conditions when amounts of toluene in the eluent exceeded 66%.

One can conclude, that polymer recovery was much higher under LCA conditions when compared with the CAP situation. The latter observation was, however, only semiquantitative since the evaporative light scattering detector response depended on eluent composition.[15]

Further increases of the toluene content in the eluent caused only a slight shift of calibration curves to higher retention volumes. Their vertical course remained unchanged. The position of the LCA eluent composition zone evidently depended on the extent of polymer adsorption within the column

packing and on the adsorption strength of eluent and sample solvent. For a given system of polymer – column packing – sample solvent – eluent, both the position and the width of the LCA zone would be influenced by the operational parameters as the volume and concentration of injected polymer solution, and the factors that affect the broadening of injected sample zone.[11,12] The effect of temperature and, possibly, also the pressure drop within column must be considered on the LCA zone position, as well.

In any case, the LC LCA retention volumes remained independent of the polymer molar mass in a rather broad area of eluent compositions (for comparison see also).[16] This is a pronounced advantage of LC LCA over LC CAP where usually a minute difference in the eluent composition in the range of a few tenths of percent may completely change the course of calibration curve toward either SEC or LAC.[8]

Unimodal and narrow PMMA peaks were obtained with the silica gel/THF/toluene systems. More complicated peak patterns were, however, observed in the systems silica gel/THF/$CHCl_3$/PMMA. In this case, chloroform is an ADSORLI for PMMA. While PMMA produced regular, unimodal peaks at low chloroform ADSORLI contents in THE eluent, a pronounced peak splitting appeared at increased chloroform contents. The chromatograms exhibited a large "main" peak and a smaller "ghost" peak. The sizes and positions of both peaks observed by evaporative light scattering detector were fully repeatable for given eluent but they were strongly influenced by eluent composition. The "ghost" peaks always eluted in the LCA – like mode that is their retention volume did not change with molar mass of the polymer injected. Further, the ghost peaks exhibited nearly constant retention volume about 10.2 mL, independent of the eluent composition (chromatograms not shown). The corresponding calibration curves are collected in Figure 3.

Similar peak splitting was also observed with the system PS/DVB column packing/$CHCl_3$/toluene/PMMA. Surprisingly, poly(methyl methacrylate)s do not elute from some PS/DVB columns using toluene as eluent but $CHCl_3$ behaves as a DESORLI in this case. Such behavior can be used for tests of adsorptive properties of SEC packing materials.[17,18] The peak splitting was strongly suppressed and it often completely disappeared if $CHCl_3$ was freshly distilled before use and the solutions of PMMA were prepared immediately before their application.

We do not have any plausible explanation for the appearance of the ghost peak detectable with evaporative light scattering detector. In any case, the observed phenomenon indicates necessity to carefully purify the solvents and especially chloroform used in experiments and to apply fresh polymer solutions.

The LC LCA method can be used to discriminate polymer blends including mixtures of copolymers with their parent homopolymers, provided the constituents exhibit different adsorptive properties. Macromolecules which are

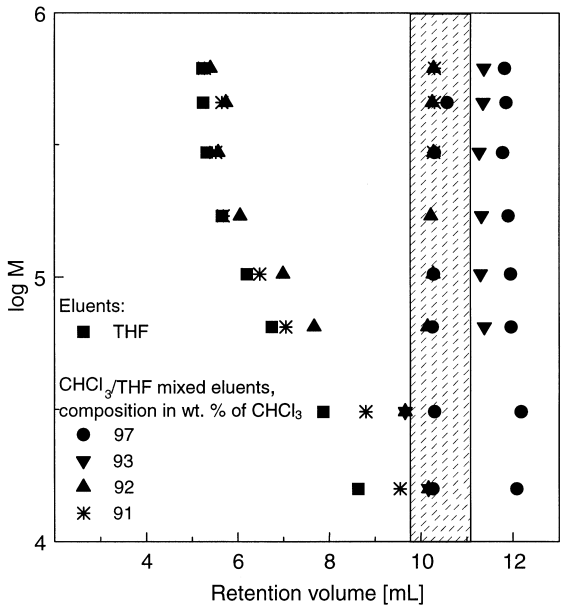

Figure 3. Calibration dependences for PMMAs with bare silica gel column packing. Pure THF and mixed eluents THF/$CHCl_3$ with various amounts of chloroform: 97%; 93%; 92%; 91% were applied. Peak splitting was evident at compositions over 95% of $CHCl_3$. The ghost peak area is shaded. Samples were injected in THF.

more strongly adsorbed within the column packing are eluted in the LCA mode while the less adsorptive macromolecules are eluted in the SEC mode. In this way the interferences in retention volumes of macromolecules with similar sizes in solution but with different chemical structures and architectures can be avoided.

The LCA column can also be coupled with the SEC column(s) (Figure 4). A sample of polymer blends is introduced into LCA column from the injection valve 1. Valves 2 and 3 are simultaneously switched and the elution of retained sample is interrupted when the nonretained macromolecules have left the LC LCA column and entered detector or SEC column via valve 4. Next, the elution of LCA retained macromolecules is completed by operation of valves 2 and 3 and polymer is transported into the SEC column. If necessary, valve 4 also allows introduction of a second DESORLI eluent. If the LC LCA retention volumes of nonadsorbed (unretained) and adsorbed constituents of polymer blend differ sufficiently the interruption of retained sample elution is not necessary.

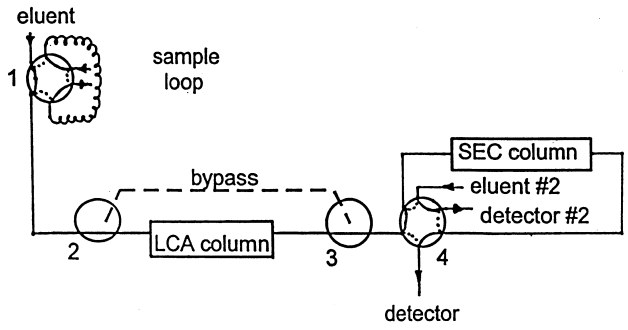

Figure 4. The schematic representation of an LC LCA/SEC coupling: (1) sample injection valve, (2,3) switching valves for bypass, (4) switching valve allowing introduction of eluent #2.

This simplifies assignment of the elution starting point for retained polymer blend constituent and valves 2,3 can be deleted. If a non-retentive SEC column is available, the application of eluent #2 is not necessary and valve 4 can be omitted, as well.

A simple practical utilization of the LC LCA method for discrimination of PS and PMMA of similar molar masses is documented in Figure 5. Polystyrene has been eluted in the SEC mode while PMMA left the column without interference under limiting conditions of adsorption using the interactive PS/DVB column from PL and $CHCl_3$/toluene 23/77% eluent. The molar mass values for polystyrene measured in pure THF and in the LCA eluent in absence or in presence of PMMA were M_w=3.4x10^5, and 3.6x10^5 g.mol^{-1}, respectively. The differences in determined M values lay in the area of expected experimental errors. The molar mass of PMMA leaving the LC LCA column was evaluated by on-line noninteractive SEC column from Waters (cf. Figure 4, without valves 2, 3 and 4). The molar masses M_w and M_n of PMMA determined by SEC independently, were 4.8x10^5 and 2.4x10^5 g.mol^{-1}, respectively, and those measured with the above LC LCA/SEC combination were 4.2x10^5 and 1.8x10^5 g.mol^{-1}, respectively.

The example demonstrates the utility of the LC LCA procedure though the effect of the LC LCA column presence on the PMMA results is hardly negligible. The M values for PMMA are influenced by the additional chromatographic band broadening within the LC LCA column.

When necessary, this effect can be diminished by optimizing the chromatographic system and by introducing the band broadening corrections usual in the conventional SEC.

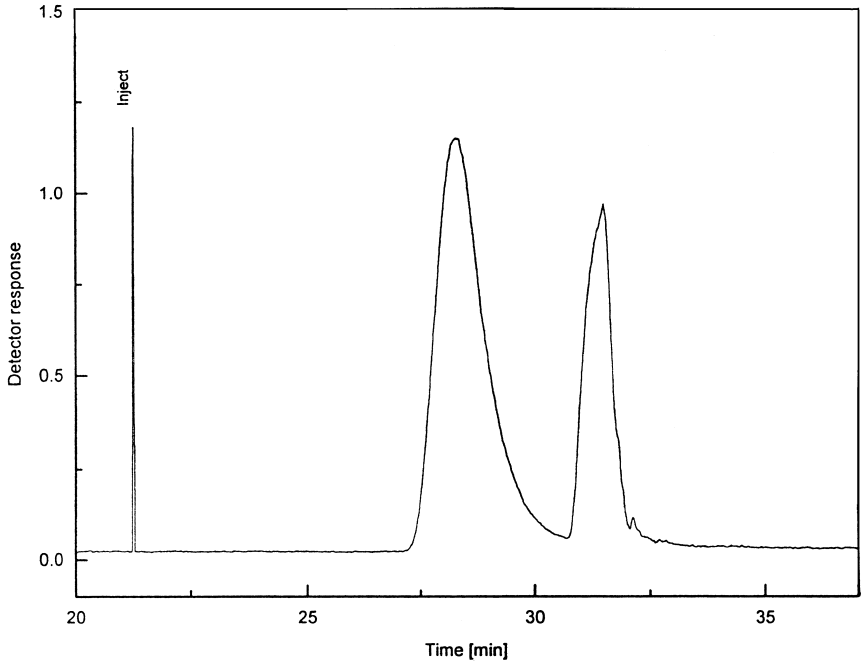

Figure 5. Separation of polystyrene with molar mass 3.4 x 10^5 g.mol^{-1} from poly(methyl methacrylate) 4.8 x 10^5 g.mol^{-1} by means of LC LCA. An interactive PS/DVB column from PL was applied in combination with eluent chloroform/toluene 23/77%. For further explanation see the text.

On the other hand, the observed error in the PMMA molar masses is much smaller than in the case when PS and PMMA co-eluted and their molar masses were determined applying a non-selective detector (differential refractometer) in combination with a selective detector (UV photometer) followed by data reduction.[19]

As mentioned, LC LCA is also operative for macromolecules that are fully excluded from the pores of column packing. This is another advantage of the LC LCA method over LC CAP in which the excluded macromolecules behave irregularly and often are even fully retained within column. We have recently demonstrated that the peak broadening and limited sample recovery may be important drawbacks of some LC CAP systems.[14]

CONCLUSIONS

Liquid chromatography under limiting conditions of adsorption belongs to the coupled LC methods designed for discrimination of complex polymers. The method combines exclusion and adsorption of polymer chains within the LC column and results in the suppression of separation according to their molecular size. In contrast, the nonadsorbed component of a polymer blend is eluted in the SEC mode and can be independently characterized in the usual way. Carefully purified solvents and optimized system of mixed eluent/column packing and temperature must be applied in order to prevent peak splitting which may limit the applicability of method, particularly if both components of polymer blends are to be assessed.

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