Liquid Chromatography of Synthetic Polymers under Critical Conditions. The Case of Single Eluents and the Role of Θ Conditions

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ABSTRACT: As a rule, critical conditions in liquid chromatography of synthetic macromolecules are adjusted by both mixed mobile phase composition and temperature. The application of single component eluents is attempted in the present study. The enthalpic interactions in the column are controlled exclusively by temperature. Poly(methyl methacrylate)s (PMMA), poly(*n*-butyl methacrylate)s (PnBMA), and polystyrenes (PS) in various solvents are investigated with bare silica gel and silica C18 bonded phases. Using a series of single eluents based on various esters, PMMA probes, and bare silica gel, it is shown that minute variations in eluent nature strongly affect polymer retention, and temperature represents a too weak parameter to adjust critical conditions in thermodynamically good solvents. The idea is proposed and tested that the sensitivity of polymer retention toward temperature variations increases in the vicinity of Θ conditions, where the structure of macromolecules in solution strongly depends on temperature. The critical conditions have been identified in some single eluents which are thermodynamicaly poor solvents for polymers investigated, namely, acetonitrile for PMMA at 66 °C and dimethylformamide for PnBMA at 154 °C and for PS at 95 °C—all with C18 bonded silica gel. A "critical-like" elution was also observed in cyclohexane for PS at 9 °C with silica gel C18 packing, which is well below the Θ temperature. It is demonstrated that poly(methyl methacrylate)s can be separated according to stereoregularity in the area of critical conditions using acetonitrile eluent and silica C18 column packing. The excessive polymer peak broadening and decreased recovery which were observed in some mixed mobile phases are diminished in the single component critical eluents studied, but they are not fully eliminated.

Introduction

High-performance liquid chromatography (HPLC) represents an important group of methods for the molecular characterization of macromolecules including synthetic complex polymer systems. The latter exhibit more than one single distribution of their basic molecular characteristics, namely molar mass, chemical structure/composition, and architecture. Consequently, the best known HPLC of polymers, namely the conventional size exclusion chromatography (SEC), which is based solely on an entropic retention mechanism, can only exeptionally produce unbiased data on molecular characteristics of complex polymers. New HPLC procedures are being developed to solve this problem. The general approach includes methods that allow separation of complex polymers, either exclusively or at least primarily according to one only molecular characteristic. The suppressed or even eliminated characteristic of analyzed samples is usually their molar mass. The suppression of molar mass effect on sample retention volume is achieved by an appropriate coupling of entropic and enthalpic mechanisms within the same HPLC column.[1] Fractions leaving the corresponding HPLC column, and exhibiting narrow distribution of either composition or architecture, are forwarded into another HPLC system, usually into an SEC column, to be separated according to their molar mass. In this way we arrive at the two-dimensional HPLC of macromolecules.[2−4]

An interesting isocratic approach to the coupling of HPLC separation mechanisms represents high-performance liquid chromatography under critical conditions (LCCC). LCCC was initiated in 1970s by a group of researchers in Petersburg.[5−8] It is based on the controlled coupling of entropic and enthalpic retention mechanisms. As is known, the retention volumes of macromolecules (V_R) decrease with polymer molar mass (M) in entropy-driven HPLC (SEC) while V_R increase with M in enthalpy-dominated interactive HPLC methods. Under critical conditions the entropic and enthalpic effects mutually compensate, and macromolecules with different molar masses elute at the same retention volume which is approximately equal to the total volume of liquid in column. This situation is sometimes denoted as "chromatographic invisibility" of the polymer to molar mass.[8] Two enthalpic retention mechanisms are utilized in "critical" HPLC, namely adsorption and partition. Consequently, we speak about LC at the critical adsorption point and LC at the critical partition point. It is necessary to point out that adsorption of macromolecules within column packing may be accompanied by their partition, and vice versa; partition of macromolecules is often associated with important adsorption effects.

Critical conditions for polymer species of particular chemical structure and architecture reflect the balance between entropic and enthalpic interactions of macromolecules with the column packing. The latter interactions are strongly influenced by the nature of mobile phase and partially also by temperature. Furthermore, at least in one experimental system pressure also has an effect on the critical adsorption point.[9] Molecules of mobile phase compete with polymer segments for active

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sites on the column packing surface available for adsorption of macromolecules. Alternatively, the mobile phase may push macromolecules into the (quasi) stationary phase situated either on the packing surface or within the packing pore volume so that the polymer species undergo thermodynamic partitioning. Unfortunately, the choice of appropriate HPLC column packings is rather limited. Microparticulate materials of various pore sizes/surfaces and also monolithic and nonporous column packings are applied. The enthalpic retention based HPLC is dominated by silica gels which may possess different surface silanol concentrations. Silica gel can be used either bare or grafted with various organic groups. Numerous such bonded phases were synthesized, but only a few of them are commercially available. The by far most popular bonded phase represents a dense array of aliphatic C18 groups. On the contrary, the most important SEC column packings are based on organic polymers**,** especially on the meso-, macro-, and gigaporous heavily cross-linked poly(styrene-*co*-divinylbenzene) resins (PS/DVB).

The mobile phases so far applied for the "critical" HPLC are exclusively two- or multicomponent systems containing constituents that either promote or suppress enthalpic interactions. Changing the eluent composition, one can adjust critical conditions and reach the necessary entropy/enthalpy compensation. The fine-tuning of enthalpic interactions between the column packing, macromolecules, and the mobile phase is usually achieved by temperature adjustments—also in the course of one single series of experiments. This is necessary in order to compensate the changes in the packing interactivity due to possible sorbent chemical and physical alteration. The latter may be for example induced by surface oxidization or by irreversible adsorption of impurities either from eluent or from injected samples. Alternatively, the minute changes are corrected in the mobile phase composition which may be caused for example by preferential evaporation or humidity absorption.

The application of mixed mobile phases in high-performance liquid chromatography is generally accompanied by numerous problems which are rather pronounced in the case of macromolecular analytes. Preferential adsorption of mobile phase component(s) on the column packing surface and the following displacement effects,[10] as well as preferential solvation of dissolved macromolecules,[11] may be responsible for appearance of the "ghost" peaks on chromatograms monitored by nonspecific detectors. These peaks belong to the zone(s) of mobile phase with altered composition and are called system peaks. In general, system peaks complicate detection of polymers eluted in mixed mobile phases. Moreover, the zones of mobile phase with changed composition may also affect local retentivity of HPLC columns. The latter effect is anticipated also in LCCC. Here, the samples of homopolymers travel together with their system peaks, and a sort of dynamic equilibrium is reached during sample elution. Characterization of block and graft copolymers by LCCC, however, involves the hybrid retention of macromolecules. One part of the copolymer chain is retained under critical conditions. It would leave the column at constant V_R, together with the system peak. At the same time, the chemically different part of a block or graft copolymer is eluted under SEC conditions and can be conventionally characterized irrespective of presence of "critically retained" or "chromatographically invisible" chains.[3,12−14] This means, however, that the critically retained chains leave their initial solvent zone and travel together with the SEC eluting chains in the fresh eluent. Consequently, the actual retention behavior of the "chromatographically invisible" chains within copolymer may be different from that expected because the critical conditions may be perturbed.[15] The separation of sample macromolecules from the zone of their initial solvent with changed composition can be also coresponsible for unexpected peak broadening and even splitting which was reported in some LCCC experiments with block copolymers.[13,14] On the other hand, LCCC peak broadening was observed also with homopolymers.[16,17] Besides nonspecific detectors such as differential refractometers, also light scattering HPLC detectors can hardly be used with mixed mobile phases due to preferential solvation effects. Evidently, for a deeper understanding of LCCC principles and for the development of method applications it is of interest to utilize single mobile phases.

Experimental Section

The HPLC assembly consisted of a pumping system model 501 (Waters, Milford, MA), a custom-made column oven thermostated with a liquid medium from a thermostat RM6 (Lauda, Koningshofen, Germany) or a Knauer column air oven (Knauer, Berlin, Germany), an injection valve 7725i (Rheodyne, Cotati, CA) provided with the sample loop of 50 μL, and an evaporative light scattering detector DDL-21 (Eurosep, Clergy-St-Christophe, France). Data were processed using software Chroma (Chromtech, Graz, Austria).

The following columns were used: a 250 × 6 mm column was packed with the bare silica gel of 10 μm particle size and 50 nm pore diameter. This material was prepared in Polymer Institute from the narrow pore silica gel Separon SGX (Tessek, Prague, Czech Republic); a 250 × 6 mm column contained silica gel bonded with C18 phase, 5 μm, 30 nm, Nucleosil C18 (Macherey-Nagel, Düren, Germany); a 250 × 4.6 mm column was filled with silica gel bonded and end-capped C18 phase, 5 μm, 10 nm, type Extend (Agilent, Waldbronn, Germany).

Narrow molar mass distribution polystyrenes (PS) were prepared by Dr. G. Hild in Institute Sadron CNRS, Strasbourg, France. Poly(methyl methacrylate)s (PMMA) of low stereoregularity[18] were a gift from Dr. W. Wunderlich from Rohm Co., Darmstadt, Germany. Poly(*n*-butyl methacrylate)s (PnBMA) were bought from Polymer Standards Services (Mainz, Germany). The medium broad molar mass distribution, highly stereoregular samples of PMMA were kindly provided by Prof. K. Hatada and Prof. T. Kitayama from Osaka University, Japan. They had following characteristics: The highly isotactic PMMAs (iPMMA) with mm higher than 97% had mass average molar masses M_w 12.3 × 10^3−157.1 × 10^3 g/mol and highly syndiotactic PMMA (sPMMA) with rr higher than 88% possessed M_w from 12.7 × 10^3 to 153.3 × 10^3 g/mol.[18]

The following mobile phases were used: ethyl acetate and isoamyl acetate (Lachema, Brno, Czech Republic), isopropyl acetate, methyl isobutyrate, and acetonitrile (ACN) (Fluka, Buchs, Switzerland), *n*-butyl acetate, *n*-amyl acetate, and *n*-butyl propionate (Laborchemie, Apolda, Germany), dimethylformamide (DMF) (Reachim, Moscow, Russia), methyl butyrate and ethyl butyrate (Merck-Schuchard, Munich, Germany), isobutyl methyl ketone and cyclohexane (Merck, Darmstadt, Germany). They were either distilled or used as purchased.

Injected polymer concentration was 0.5−1 mg/mL, and the elution rate was in range from 0.4 to 1 mL/min depending on eluent viscosity.

Results and Discussion

In the literature, the authors have not identified any data showing a molar mass independent retention with a single component eluent. In preliminary studies on

Table 1. Chromatographic Behavior of Poly(methyl methacrylate) on a Bare Silica Gel Column (SGX 500) Applying Different Ester-Based Single Mobile Phases and Temperatures

no.	chemical structure of mobile phase	mobile phase	elution mode	column temp [°C]	Θ temp [°C] [ref 27]
1	$CH_3-CH_2-O-CO-CH_3$	ethyl acetate	exclusion	+10; +50	
2	$(CH_3)_2CH-O-CO-CH_3$	isopropyl acetate	exclusion	+10	
3	$CH_3-O-CO-CH(CH_3)_2$	methyl isobutyrate	adsorption	+71	
4	$CH_3-CH_2-CH_2-CH_2-O-CO-CH_3$	*n*-butyl acetate	exclusion	+3; +25; +50	−20
5	$CH_3-CH_2-CH_2-CH_2-CH_2-O-CO-CH_3$	*n*-amyl acetate	adsorpion	−2; +30; +130	+41
6	$CH_3-CH_2-CH_2-CH_2-O-CO-CH_2-CH_3$	*n*-butyl propionate	adsorption	+37; +71	
7	$(CH_3)_2CH-CH_2-CH_2-O-CO-CH_3$	isoamyl acetate[a]	adsorption	+36; +78	+57.5
			exclusion	+36	
8	$(CH_3)_2CH-CH_2-O-CO-CH_3$	isobutyl acetate	exclusion	+36	
9	$CH_3-O-CO-CH_2-CH_2-CH_3$	methyl *n*-butyrate	exclusion	+71	
10	$CH_3-CH_2-O-CO-CH_2-CH_2-CH_3$	ethyl butyrate	adsorpion	−9; +25; +115	

[a] See the text.

the SEC method, several authors observed strong dependence of retention volumes for a given polymer/eluent system when changing column packing surface properties. For example, Klein and Treichel[19] revealed that poly(ethylene glycol)s (PEO) were eluted in 1,4-dioxane from a nonmodified silica gel in the adsorption mode and from a silanized silica gel in the SEC mode. Similarly, Audebert[20] showed that the extent of PEO adsorption on silica gel was reduced when PEO chains were grafted on the column packing surface. Often, polymer samples which were initially fully retained within bare silica gel packed column started eluting in the SEC mode when the column packing surface was appropriately modified (for a review see e.g. ref 21). Evidently, the critical elution behavior of polymer samples would be observed if the activities of column packings were properly adjusted.

Recently, Chang with co-workers[22] proposed the temperature gradient interaction chromatography (TGIC) method for selective separation of macromolecules. TGIC utilizes a temperature gradient to control retention of macromolecules in the vicinity of critical conditions. In addition to mixed mobile phases, single component eluents were also used in TGIC.[22,23]

The aforementioned observations indicate that the critical conditions in single component mobile phases do exist. In fact, elution very near to the critical behavior was observed over 20 years ago in the Laboratory of Liquid Chromatography, PI SAS, using silica gel Porasil (Rhone Poulenc, Pechiney, Saint Gobain, France) for the PMMA/ethyl acetate system at 30 °C.[24] These results were, however, not published because the SEC elution of the same PMMA samples in ethyl acetate was observed in another set of experiments,[25] probably due to both different nature and amount of impurities in eluent.

We began our present studies utilizing a series of various esters and the PMMA samples of low stereoregularity with the bare silica gel column. Adsorption was the governing retention mechanism. The results are summarized in Table 1. We did not identify any single eluent/temperature system corresponding to critical conditions. Moreover, we have observed different behavior—either full adsorption or SEC elution—in the system PMMA/isoamyl acetate at 35 °C depending on the solvent batch and on the way of its purification. The following conclusions can be drawn from this set of experiments:

(a) The extent of polymer adsorption strongly depends on the silica gel column packing activity. Evidently, the Porasil silica gel used in the previous study was more active than our present wide pore silica gel. The latter material was heated to temperatures over 500 °C in the last stage of the pore widening process. This firing procedure certainly reduced the silanol concentration on the sorbent surface.

(b) Polymer adsorption sensitively responds to small variations in the mobile phase nature. For example, one methylene group difference between *n*-butyl acetate (eluent 4 in Table 1) and *n*-amyl acetate (5) or between methyl *n*-butyrate (9) and ethyl *n*-butyrate (10) as well as between *n*-butyl acetate (4) and *n*-butyl propionate (6) causes a transition from the SEC elution mode to the full retention for PMMA samples. Similarly, the minute difference in structure between *n*-amyl acetate (4) and isoamyl acetate (7) or between methyl isobutyrate (3) and methyl *n*-butyrate (9), or even the change of isopropyl group position in esters 2 and 3, lead to a transition from the SEC elution mode to the full retention, or vice versa.

(c) Esters which otherwise allow changing eluent polarity in relative small increments seem not to be appropriate eluents for liquid chromatography in general and for critical HPLC with silica gels in particular. In addition to humidity, they may contain minute amounts of peroxides[26] and also acids created by hydrolysis. These polar admixtures may substantially affect retention of polar macromolecules.

(d) Temperature is a rather weak parameter to control the adsorption of macromolecules on bare silica gel surface if only polymer−column packing interactions are considered. This fact substantially complicates adjusting critical conditions with single eluents as far as the tailored column packings with finely tuned interactivities are not available.

In the second stage, we tried to include the effect of temperature on structure of macromolecules in solution. Therefore, we concentrated our effort on the Θ systems where molecular properties of polymers in solution strongly depend on temperature. It is known that, in the vicinity of Θ conditions (solvent nature, temperature), polymer coils rapidly change their structure.[27] When approaching Θ conditions, macromolecules shrink, and eventually, their coils reach the unperturbed dimensions. This is evident for example from the reduction of limiting viscosity numbers of polymer solutions. The exponent a in the viscosity law rapidly decreases to reach value 0.5 at the Θ point. Further deterioration of the solvent thermodynamic quality, for example due to the temperature decrease below the Θ point, may lead to a collapse of polymer coils. Macromolecules may also start associating to eventually form microphases (droplets of a concentrated "gel" phase). The changes in coil structure induced by temperature variations may cause

Table 2. Chromatographic Behavior of Selected Polymers on Different Column Packings at Different Temperatures

no.	system description	eluent	elution mode	column temp [°C]	Θ temp [°C] [27]
11	PMMA on Nucleosil C18	acetonitrile	critical	+66	+(28−44)
12		2-propanol	phase separation	+15; +35; +70	
13	PMMA on Agilent Extend C18	*n*-amyl acetate	exclusion	+36; +80	+41
14	PnBMA on Nucleosil C18	dimethylformamide	critical	+154	+23.6
15		2-propanol	exclusion	+15; +35; +70	+(20.9−23.7)
16	PS on Nucleosil C18	dimethylformamide	critical	+95	
17		cyclohexane	critical or precipitation	+9	+(35−35)
18	PS on SGX 500	cyclohexane	adsorption	+36; +70	+(34−35)
19	PS on Agilent Extend C18	cyclohexane	critical	+9	+(34−35)

extensive alteration of polymer interactions with the column packing and consequently large changes in adsorption, especially in partition of macromolecules. In fact, many known "critical eluents" are very poor solvents for polymer samples (for review see e.g. ref 28), and it is suspected that some of them are not far from Θ conditions. For example, Baran, Laughier, and Cramail[29] showed that the critical adsorption point in system PS/THF/*n*-hexane was reached at the eluent composition where the exponent *a* approached 0.5. The above system was shown to be rather sensitive to temperature variations, and it is well possible that the dependence critical adsorption point/eluent composition/temperature corresponded with the dependence solvent composition/Θ temperature.

For further experiments we have chosen the systems where mobile phases were poor solvents or even nonsolvents for polymers. We included also two single mobile phases applied by Chang in TGIC.[22] The tabulated Θ points of several these systems were situated in the experimentally feasible region. The following systems were studied (Table 2): PMMA/acetonitrile (11), PMMA/2-propanol (12), PMMA/*n*-amyl acetate (13), PnBMA/DMF (14), PnBMA/2-propanol (15), PS/DMF (16), and PS/cyclohexane (17−19) using both the nonmodified silica gel and silica gels bonded with C18 groups. Systems 5 and 7, from Table 1, can be considered in this series as well. Θ temperatures for the above systems were taken from literature.[27] In some systems the literature data on Θ temperature scatter remarkably, and we quote only the minimum and maximum values. Numerous systems can be also found in the literature[27] which possess two entirely different Θ values. Such systems may exhibit the closed solubility loops, and the phase separation for a given polymer molar mass and concentration takes place at two temperatures (lower and upper critical solution temperature). Nearly critical conditions were found for systems 11, 14, 16, and 19 (see Table 2 and Figures 1−5). The behavior of systems 12 and 17 was not unambiguous. Specifically, system 12 exhibited distinct microphase separation (turbidity). The viscosity of cyclohexane at 9 °C is rather high, and the solvent itself is not far from its freezing point. The critical conditions for system 11, silica C18/PMMA/acetonitrile, have been reached at temperature about 66 °C while those for system 14, silica C18/PnBMA/DMF, are situated around temperature 154 °C. This is far above the tabulated Θ points. The latter system, however, may belong to those exhibiting two Θ points. The difference between Θ temperature and critical temperature may be fortuitous. The eluents used may incidentally exhibit critical temperatures irrespective of the Θ point vicinity. In fact, our hypothesis does not exclude the existence of critical temperature in a thermodynamically good single mobile phase, especially if the decisive retention mechanism is adsorption. Moreover, as we show later, a mixed

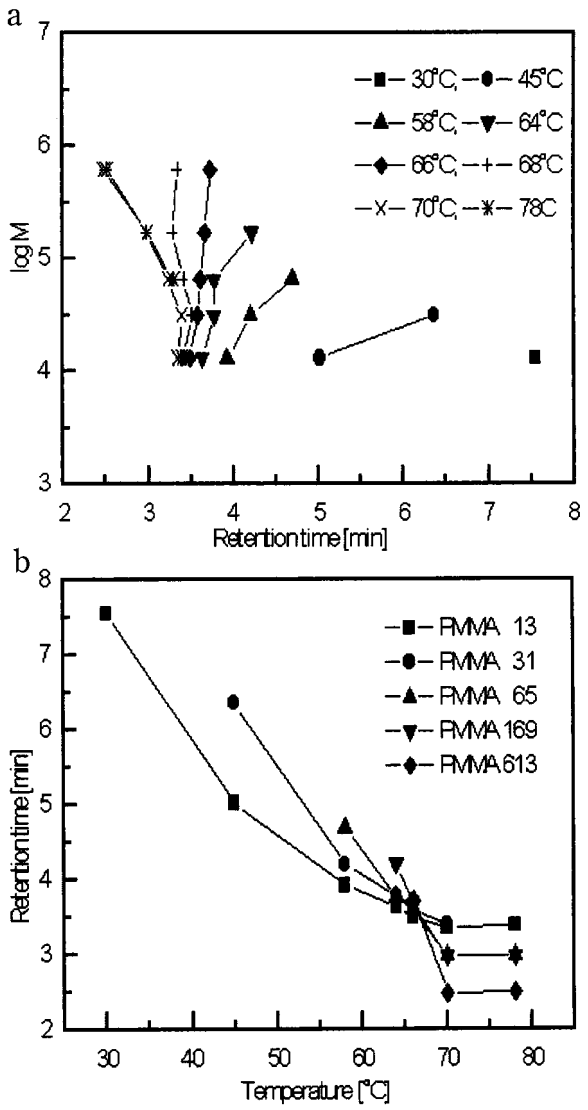

Figure 1. (a) "Calibration dependences" plots of log (molar mass, *M*) vs retention time for system 11 (PMMA/ACN/Nucleosil C18) at various temperatures. (b) Dependence of retention time on the column temperature for system 11 at various polymer molar masses *M* in kg/mol.

retention mechanism that is partition plus adsorption must be anticipated with many silica gel C18 phases. Some LC measurements indicate that change of conformation of alkyl chains bonded on column packing with temperature could influence retention of analyzed solutes.[30] Another tentative explanation of the observed disagreement between Θ temperature and critical temperature may consider minute amounts of impurities present in eluents. Evidently, further experiments are needed with the high-purity mobile phases.

If the sensitivity of polymer retention toward temperature increases in the vicinity of the Θ point, one

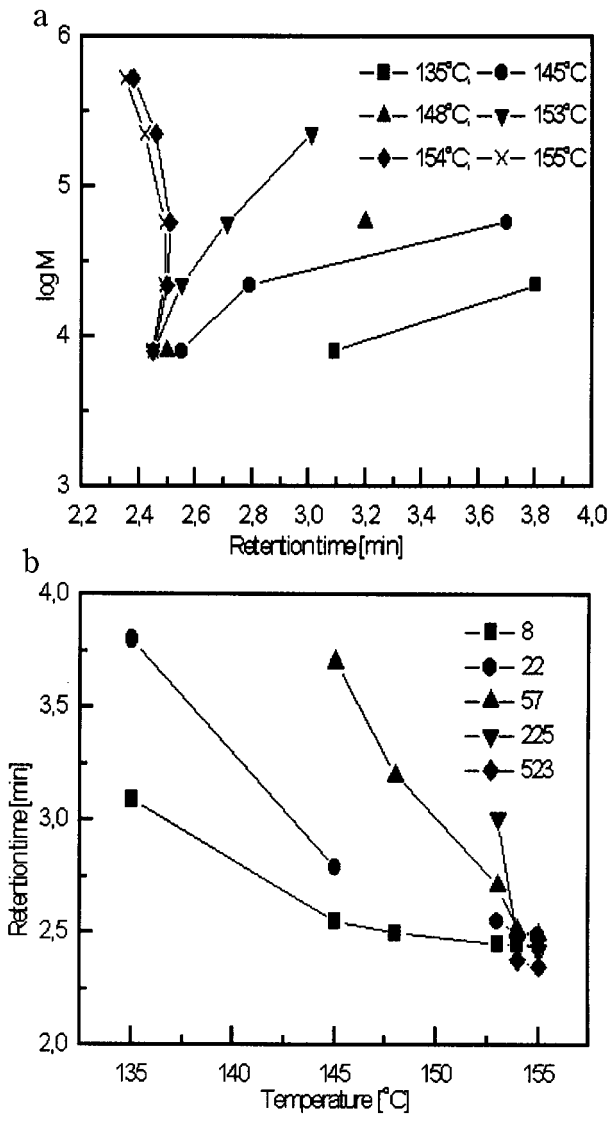

Figure 2. (a) Plots of log *M* vs retention time for system 14 (PnBMA/DMF/Nucleosil C18) at various temperatures. (b) Dependence of the retention time on the column temperature for system 14 at various polymer molar masses in kg/mol.

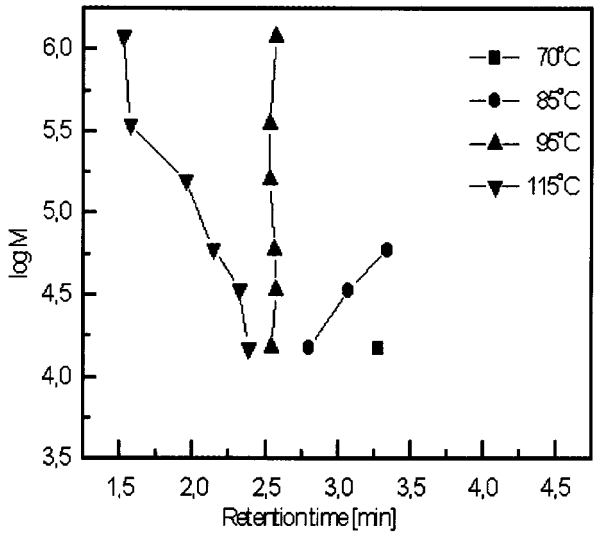

Figure 3. Plots of log *M* vs retention time for system 16 (PS/DMF/Nucleosil C18) at various temperatures.

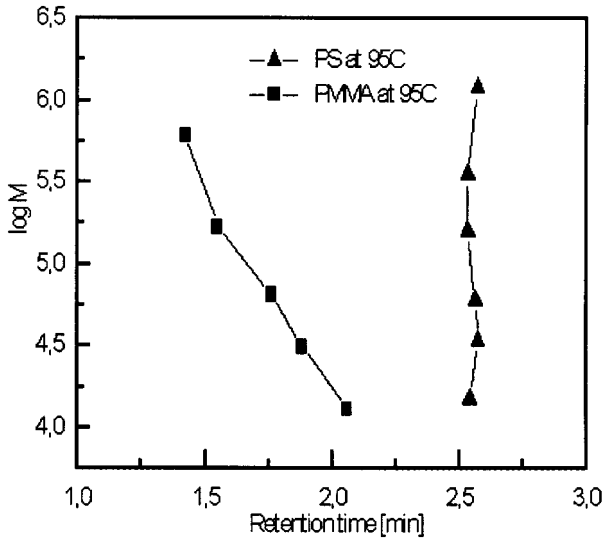

Figure 4. Comparison of plots of log *M* vs retention time the system 16 (PS/DMF/Nucleosil C18) and the system PMMA/DMF/Nucleosil C18 at 95 °C. The leading retention mechanism for polystyrene is enthalpic. The two polymers system can be easily separated under these conditions, irrespective of their molar masses. This system can also be applied for investigation of block and graft copolymers prepared of styrene and methyl methacrylate.

can expect two critical temperatures for the polymer/eluent systems exhibiting two Θ points using appropriate HPLC column packing. This conclusion may apply also for binary mobile phases.

Inspection of the thus far successfully applied TGIC polymer/eluent systems reveals that most of them seem not to be far from Θ conditions. Few of them were single Θ solvents, and others were mixtures of solvents and precipitants for polymer samples. So far only one mixture of two solvents for a polymer sample turned an efficient TGIC eluent, viz. tetrahydrofuran plus isooctane for poly(isoprene) with bare silica gel.[31] The critical conditions were not found in Θ eluents for systems 13, 15, and 18 as well as for systems 5 and 7 from Table 1. It is evident that any transition in the structure of macromolecules produced by temperature variation cannot bring the system to critical conditions if the interaction between macromolecules and column packing is either too strong or too weak.

As was mentioned, the set of PMMA samples used in previous experiments exhibited low stereoregularity. Tentatively, we have injected also some highly stereoregular samples of PMMA. The results are displayed in Figure 6. In agreement with our previous experiments[18,32,33] and with results of Sato et al.,[34] the stereoregularity of macromolecules affects their retention in the regime of weak interactions. Isotactic PMMA is eluted in the adsorption mode while syndiotactic PMMA in the SEC mode. This finding again confirms the possibility for simultaneous determination of molar mass distribution and tacticity distribution for stereoregular polymers applying two-dimensional liquid chromatography.[32,33] It also indicates the potential of TGIC in separation of stereoregular species, including for example the two-dimensional combination of SEC with TGIC.

As mentioned, an important component of any "critical" HPLC system is the column packing. Our recent experiments showed large differences in the polar interactivities of densely bonded silica C18 materials from various producers.[35] The courses of universal SEC

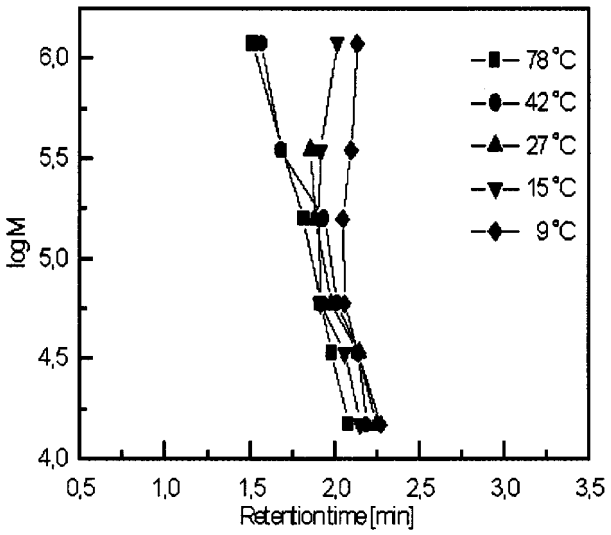

Figure 5. Plots of log *M* vs retention time for system 17 (PS/cyclohexane/Nucleosil C18) at different temperatures. The higher PS molar masses underwent a phase separation, though polymer still eluted under "critical conditions".

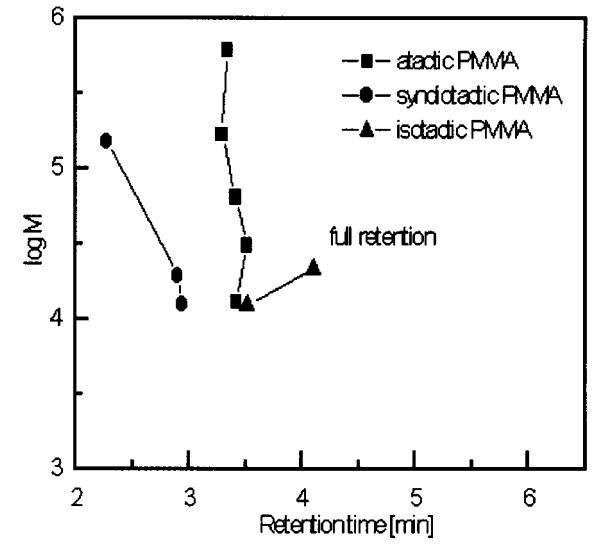

Figure 6. Plots of log *M* vs retention time for system 11 (PMMA/acetonitrile/Nucleosil C18) at 68 °C. A comparison of samples with different stereoregularities is shown.

calibration dependences of log(hydrodynamic volume) vs retention volume[36] were compared for a nonpolar polymer (PS) and a polymer of intermediate polarity (PMMA) using an adsorption promoting eluent (adsorli) toluene. The columns tested can be tentatively divided into three groups:

(a) The universal calibration dependences for PMMA and PS in toluene coincided. The columns did not exhibit polar interactivity toward PMMA in toluene. The column packings were, rather effectively, end-capped.

(b) The universal calibration dependences for PS and PMMA in toluene were mutually shifted. This slight polar retentivity of the PMMA probes indicates either presence of adsorption sites with low activity or a very low concentration of highly active adsorption sites on the bonded silica gel surface.

(c) PMMA was not eluted from the columns in toluene. This shows that silica gel surface bonded with C18 groups bears relatively high amount of very active silanol groups which are accessible for macromolecules of PMMA. The interaction between macromolecules and these groups causes their full retention.

Nucleosil C18 column used in present study belonged to the (b) group. Therefore, we compared its retention behavior with that of the well end-capped narrow-pore Silica C18 Extend column which was situated in the (a) group. We have found a regular SEC elution of PMMA samples in acetonitrile from the Extend column at 66 °C, which is at the critical temperature for the Nucleosil C18 column. This again demonstrates the important role of column packing in HPLC of macromolecules under critical conditions and probably also in TGIC. The possible hydrolysis of C18 groups during the column use on one hand and irreversible adsorption of both polar macromolecules and impurities from eluent on the other hand may successively alter retention characteristics of HPLC columns. As a result, the repeatability and precision of the LCCC results obtained would decrease. Therefore, it must be stressed again that the availability of defined and stable column packings as well as of sorbents with tailored activities may become one of the limiting factors of LCCC and possibly also of TGIC.

The application window of the single eluents for LCCC presented in this paper is rather narrow. For example, acetonitrile, which is a stable solvent, readily available in the HPLC purity and well transparent in the ultraviolet range, barely dissolves polymers less polar than PMMA. On the contrary, more polar polymers which are well soluble in ACN are expected to be strongly retained within many silica-based HPLC packings except for perfectly end-capped bonded phases. The critical temperatures identified in systems PnBMA/DMF (14) and or PS/DMFA (16) are too high for general applications also from the point of view of limited long-term thermal stability of most bonded phases. Moreover, DMF is vulnerable to hydrolysis. The partial solution of these problems would require the application of highly stable, well protected, clean, nonpolar (reversed) HPLC phases which could allow identification of single solvents with lower critical temperatures.

As mentioned in the Introduction, excessive band broadening was reported in LCCC of homo- and copolymers with numerous mixed mobile phases. The broadening was particularity pronounced when working at critical adsorption point with narrow pore column packings and its extent increased with increasing polymer molar mass.[17] Simultaneously, sample recovery was also reduced and full retention was observed for polymer with excluded molar masses. This was surprising because the column packing surface available for adsorption became strongly reduced for large macromolecules, and eventually, only a very small outer surface of packing particles was available for excluded macromolecules unless large macromolecules decoil and penetrate into the narrow pores. This would mean that important kinetic effects should be considered in many "critical systems". Certainly, decreased solubility of high molar mass polymers could also be responsible for reduced recovery in some experimental systems. A drop in sample recovery was, however, observed also with the mobile phase which was a good solvent for polymer sample.[17,37] Both a decrease in sample recovery and peak broadening with rising molar mass was less pronounced in LCCC with single eluents than with mixed mobile phases, though it was not negligible. Figure 7 illustrates the largest peak broadening observed in this work. The effects are noticeable mainly

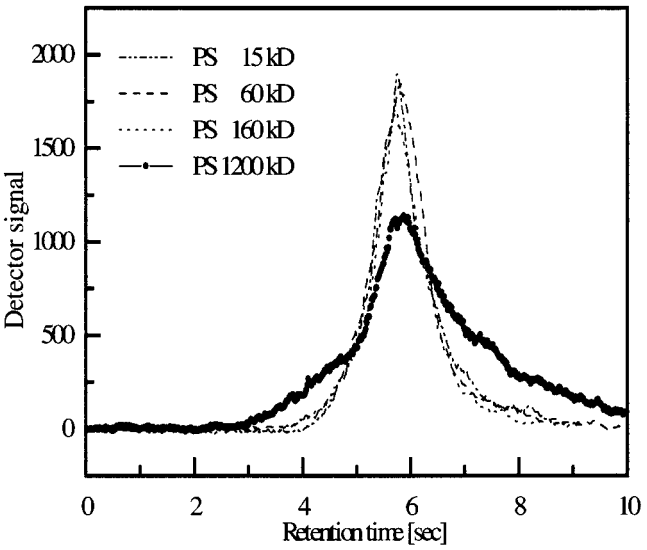

Figure 7. Comparison of chromatograms for system 16 (PS/DMF/Nucleosil) at different polymer molar masses (in kg/mol) and similar polydispersities. The constant injected concentration was nearly 1 mg/mL. The most wide peak belongs to PS 1200 kDa.

for highest molar mass of polystyrene, 1.200 kmol/g. The exclusion limit of the Nucleosil C18 column was fairly below 10^6 g/mol and polystyrene was well soluble in DMF at the temperature of experiment. It is to be stressed again that the width of polymer molar mass distribution should not play any role under critical conditions or in their vicinity. Peak broadening may be, however, somewhat augmented by increased viscosity of the samples containing high molar mass polymers. Still, it seems that the preferential solvation of polymer sample in mixed mobile phases discussed previously may be not the only reason for additional peak broadening in LCCC.

Conclusions

Critical conditions in liquid chromatography of macromolecules with single component eluents are difficult, though feasible, to achieve. An attractive approach namely to adjust the eluent strength by using a series of solvents with slightly changing polarities, such as various esters, in combination with temperature changes is shown to be inefficient. The reason for this situation is the limited effect of temperature on the adsorption induced polymer retention in the case of thermodynamically good solvent and bare silica gel column packing. The temperature adjustments should be more effective in the partition based retention, especially if supported by a fine-tuning of column packing chemical structure/interactivity. This later possibility is, however, so far rather limited as is the choice of commonly available HPLC column packing chemistries.

It is proposed that critical conditions could be easier controlled by adjustments of temperature in the systems where thermodynamic quality of eluent for polymer changes extensively. This happens in poor solvents in the vicinity of Θ temperature where macromolecules rapidly change their structure in solution. This approach may be especially successful when applying thermodynamic partition of macromolecules as leading retention mechanism. Using poor and Θ solvents, critical conditions were identified for four systems polymer/solvent/silica C18 or bare silica gel column packings. The critical temperatures, however, did not exactly coincide with Θ temperatures.

As demonstrated with poly(methyl methacrylate)s of high stereoregularity, LCCC with single eluents can be applied in simultaneous determination of molar mass and tacticity distribution. Both the LCCC peak broadening and loss of sample recovery are reduced, though not fully eliminated, with single eluents. Further experimental material with high-purity mobile phases is needed for quantification of these findings. The authors believe that the data presented in this paper may form a basis for further development of LCCC and TGIC methods.

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