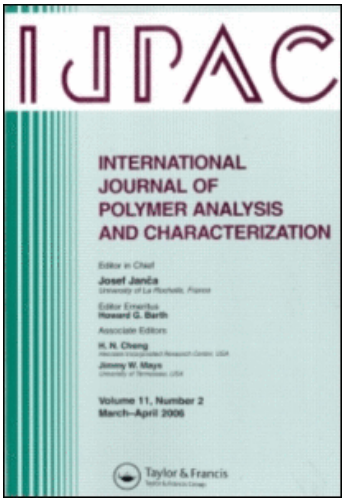

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Liquid Chromatography of Macromolecules Under Limiting Conditions of Solubility (LC LCS): A Mechanistic Study

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Liquid Chromatography of Macromolecules Under Limiting Conditions of Solubility (LC LCS): A Mechanistic Study

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A coupled liquid chromatographic technique combining an enthalpic contribution, precipitation, and the entropic size exclusion effect is reported. Liquid chromatography under limiting conditions of solubility (LC LCS) results in the elution of the polymer solute on the front shoulder of the injection zone. In the experiments carried out herein 70/30–65/35 wt.% THF/n-hexane mixtures were found to provide a retention independent of molar masses for poly(methyl methacrylate)s up to molar mass of approximately 1000 K daltons. This far exceeds the feasible range of liquid chromatography at the critical adsorption point. The effects of the mobile phase, composition and further flow rate,

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injected concentration, and temperature, were investigated. The polymer retention volumes were found to be dependent on the eluent composition and temperature and to a lesser extent also on injected polymer concentration and eluent flow rate. At high molar masses and high injected concentrations, the system can be overloaded, shifting the balance in favor of precipitation, while at low molar masses and flow rates the macromolecules have time to equilibrate and the balance shifts in favor of solubility. In LC LCS the enthalpic effects leading to adsorption and partition may be sufficiently strong to result in large retention of high molar mass compounds on the stationary phase, implying that the choice of the column packing is critical.

Keywords: Adsorption; Critical conditions; Limiting conditions of solubility; Liquid chromatography; Partition; Polymethylmethacrylate; Size exclusion chromatography

INTRODUCTION

Since Belenkii's pioneering experiments, which illustrated that the entropic and enthalpic separation mechanisms could be offset within a liquid chromatographic arrangement[1], a series of systems have been investigated at the critical adsorption point (CAP), both in thin layers and columns[2–4]. This technique, which is generally termed "liquid chromatography under critical conditions" (LC CC), belongs to the family of coupled liquid chromatographic methods at the point of exclusion-adsorption transition (LC PEAT)[5]. These methods permit the separation and characterization of functionalized oligomers[2], block copolymers[3,6–9], statistical polymers[10,11], and polymer blends[3], as well as homopolymers differing in their microstructure[12,13]. Generally, the LC PEAT methods achieve a molar mass independent retention by balancing enthalpic interactions and exclusion by varying the eluent blend, although the critical adsorption point can also be achieved by varying the temperatur[14] and, recently, using single eluents[15].

Several recent reviews summarize the current potential, weaknesses, and experimental protocol of LC coupled techniques[3–5,16,17], and provide extensive surveys of the polymer-eluent-sorbent systems studied to date[3,4]. In brief, these methods involve the selection of a (usually binary) mobile phase in which a given polymer elution is independent of its molecular size, for a particular stationary phase and temperature. Numerous polymers possess multiple molecular heterogeneity, as manifested in superposition of molar mass, chemical composition, stereoregularity, branching, and

functional group distributions, and the coupled techniques offer the possibility of separating such complex polymers according to only one of their distributions. Historical interest in LC CAP has been aimed at the fundamental elucidation of the separation mechanism or increasing the molar mass range[18,19]. Moreover, detector hyphenation, including refractive index-density combinations[20] or MALDI-TOF measurements[21], have been attempted. Mathematical manipulations (deconvolution) and experimental techniques such as orthogonal chromatography[22] have also been applied with limited success.

The preceding LC CAP method involves the injection of a polymer in the mobile phase utilized for separation. An alternative to this technique, referred to as liquid chromatography under limiting conditions of solubility (LC LCS)[4,18,19,23–25] involves the utilization of an injection zone is a good that solvent for the polymer solute while eluent is, de facto, a weak non solvent. Under particular, "limiting" conditions, the separation proceeds through microgradient processes of exclusion, precipitation, and redissolution. Due to their partial exclusion from the packing pores, macromolecules travel faster along the column than the small molecules of eluent. As result, polymer leaves the injection zone, encounters and interacts with the mobile phase, precipitates, and is then redissolved as the injection zone "catches up" to the solute. The net result is that the polymer elutes just in the front part of the solvent peak, as has been well documented using a variety of differential refractive index (DRI), UV, and evaporative light scattering detectors[18,19,23]. The principal advantage of LC LCS is that it can enable a molar mass-independent elution or vertical "calibration curve" for molar masses ranging to high polymers of over one million daltons. This has been a limitation of the LC CAP method, which is generally feasible for molecules up to the order of 10^5 daltons[16]. However, the LC LCS system, due to the harness of the precipitation and enthalpic interactions between column packing and macromolecules (adsorption, enthalpic partition) can exhibit extensive polymer retention on the column. Therefore, the present investigation sought to examine some mechanistic features of LC LCS. Furthermore, since results to date have exclusively utilized silica gel based sorbent, in this article the role of column packing has been investigated. The sensitivity of LC LCS data to variables such as the injection concentration, mobile phase flow rate, and temperature has been assessed, as well.

EXPERIMENTAL

Mobile and Stationary Phases

Spectranalyzed grade tetrahydrofuran (THF) (Fisher, Norcross, Ga., USA) and HPLC grade n-hexane (Fisher) were used as received.

THF/n-hexane mobile phase compositions from 75/25 to 65.1/39.9 wt.% were applied. A Shodex (JM Science, Grand Island, N.Y., USA) linear GPC 806 L column (0.8×30 cm) packed with 10 mm polystyrene-co-divinylbenzene (PS/DVB) particles was utilized for all experiments.

Polymer Standards

Narrow molar mass distribution atactic poly(methyl methacrylate)s (PMMA) with molar masses between 6 and 1,000 $kg \cdot mol^{-1}$ daltons were purchased from American Polymer Standards Corporation (Mentor, Oh., USA) and used as received.

Liquid Chromatograph

An L-6000 (Hitachi Instruments, Tokyo, Japan) pump coupled with a Hitachi L-4000 UV detector operating at a wavelength of 234 nm was utilized in all experiments. A Rheodyne type 7725i valve (Cotati, Calif., USA) with injection loops of 10, 15, and 20 μL was employed. Chromatograms were collected on a PC running Viscotek GPC PRO Version 4.01 software (Houston, Tex., USA). The standard separation involved a 20 μL sample loop, 1.5 mL/min flow rate, a solute concentration of 1.0 mg/mL, and a 2 cm tubing connection length between the valve and column. Sample loops of 10 and 50 μL were also employed in some measurements. These parameters were systematically varied in the mechanistic study as shown in the figures and the text. All experiments were performed at ambient temperature ($22 \pm 1°C$).

RESULTS AND DISCUSSION

In the experiments carried out herein, the eluent components, tetrahydrofuran (THF) and n-hexane (hexane), were chosen since they do not absorb UV light at 234 nm. This permitted the monitoring of PMMA by means of a UV photometer irrespective from the system (solvent) peak. THF is a good solvent for PMMA and polystyrene (PS) and is anticipated to preferentially solvate the PS-based column packing in contact with mixtures of THF/hexane. In this way we attempted to prevent the formation of a "nonsolvent layer" on the column packing surface, which would be created if a nonsolvent eluent component preferentially solvated column packing. Such a nonsolvent layer decreases the effective pore size and supposedly also the polymer recovery, especially in the higher molar mass (M) area. Due to preferential sorption, however, the overall composition of eluent within the pores of gel differs from the mobile phase composition. The liquid within the gel pores, a quasi-stationary phase, is a thermodynamically better solvent for

macromolecules than mobile phase, and we must consider the additional separation mechanism, namely, the enthalpic partition[26].

The solubility of PMMA in THF/hexane mixtures was assessed applying the cloud point measurements at a concentration of polymer equal to 1 mg/mL[23]. The results showed that even lowest PMMA was insoluble in mixed solvents THF/hexane containing more than 54.5 wt.% of hexane. LC LCS measurements were carried out at 34.9 wt.% of n-hexane, that is, in a weak nonsolvent eluent, especially for higher molar mass PMMA.

The influence of eluent composition on the PMMA retention volume was investigated. Specifically, Figure 1 shows the dependence of log M versus V_R for PMMA over a styrene-divinylbenzene polymeric column

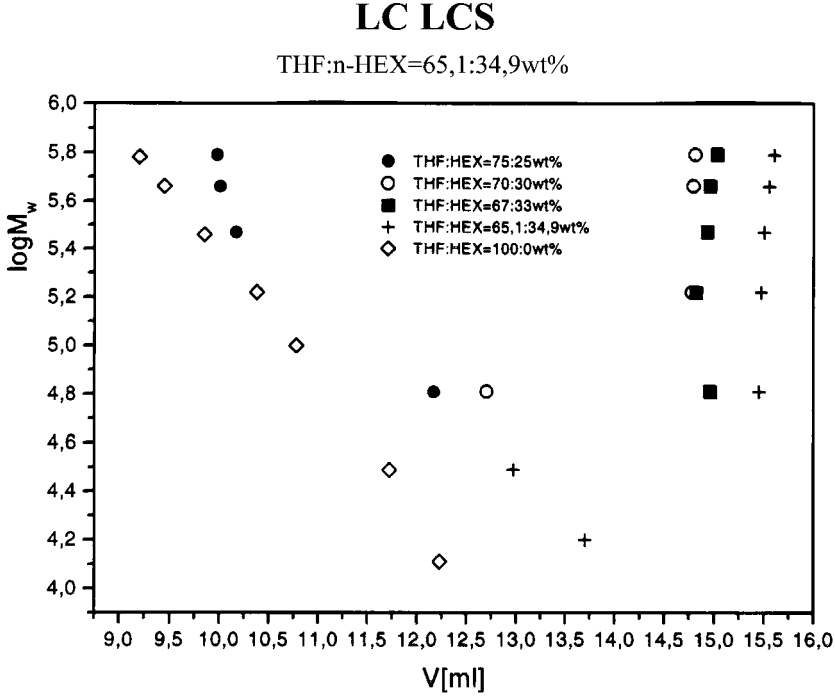

FIGURE 1 Logarithm of the molar mass (log M, g/mol) as a function of the retention volume (V_R, mL) for narrow PMMA standards in a mixed eluent (THF/n-hexane) and for various eluent compositions. Measurements were carried out under standard conditions, at a flow rate of 1.5 mL/min, with a 20 μL injection loop and a 2 cm tubing connecting the injector and column. The injected polymer concentration was 1.0 mg/mL.

packing as a function of the volume fraction of hexane in THF/hexane mixed eluents. The retention volumes grow only slightly up to 30 wt.% of hexane. However, by adding a further 5 wt.% of hexane to eluent, that is, when reaching conditions under which high molar mass poly(methyl methacrylate)s are no longer soluble, the dependence of log M versus V_R changes abruptly. Macromolecules elute in a single retention volume independently of their M. It seems that lowest molar masses of PMMA were soluble in eluent applied. Therefore, the true molar mass independent elution (LC LCS) appears at higher molar masses only. In order to avoid problems with adsorption/partition of PMMA onto/within PS gel, we have limited our further experiments to a relatively high THF content in eluent, 65.1 vol.%, and polymers with the lowest molar masses below 60 K daltons were abandoned. Unlike LC CAP systems where the molar mass retention independence of polymer molar mass is usually localized below molar masses 100 K daltons, a molar mass-independent retention is observed up to one million daltons for this LC LCS. This result supports the basic hypothesis on the LC LCS mechanism that is the role of limited polymer solubility or even local precipitation that is combined with the size exclusion of macromolecules. In our previous work[12], we have identified limiting conditions for PMMA on a bare silica gel column packing at eluent composition 81 wt.% of THF in a mixture with hexane. Evidently, the adsorption was a decisive retention mechanism in this case, and, in fact, we worked under limiting conditions of adsorption[27].

The change of hexane amount in eluent from 33 to 34.9 wt.% brings about a surprisingly large increase of polymer retention volumes, although the principle of LCS behavior does not alter: macromolecules elute essentially at the same retention volume, irrespectively of their M. Concomitant with the rise of V_R, the apparent sample recovery decreased. There are three tentative explanations for the increase of polymer retention volume:

1) The effect of adsorption/partition of macromolecules onto/within the column packing: extent of the attractive enthalpic interactions of PMMA with the column packing is expected to increase with increasing amount of the weak, adsorption-promoting hexane in eluent.
2) A strong shift of the retention volume of the solvent zone is observed due to retention of THF molecules within the column packing. In fact, the affinity of THF to the PS/DVB gel is much higher than that of hexane. Consequently the retention volume of the THF probe is expected to rise with decreasing strength of eluent, that is, with increasing content of hexane. Since macromolecules cannot leave the zone of their THF solvent, the sample V_R will rise with the retention volume of tetrahydrofuran.
3) Macromolecules require higher concentrations of THF in the sample solvent zone to be eluted from the column in a more efficiently

precipitating eluent containing more hexane. Therefore, macromolecules cannot be situated at the very edge of the THF zone in the latter eluent. They find appropriate conditions for elution more in the center of the THF zone, and, consequently, they elute at higher V_R compared to a less efficient eluent that "allows" macromolecules to approach the limit of the THF zone.

Figure 2 illustrates the effect of the injected solution zone broadening, which took place in the capillary connecting injector and column. The volume of the connecting capillary was changed between 10 and 50 μL. There is essentially no dependence of the LC LCS retention volume on the volume of the connecting capillary, over the range investigated. This

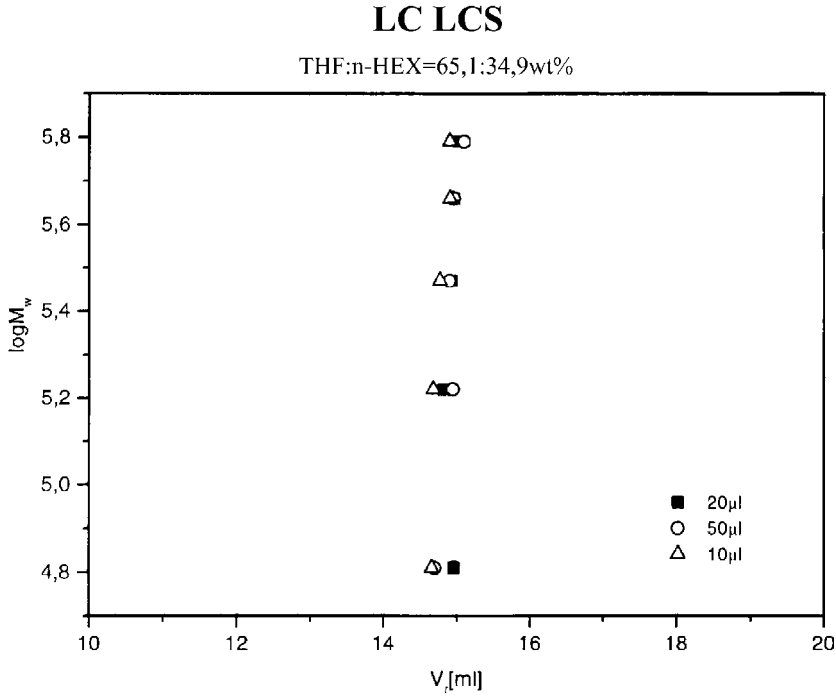

FIGURE 2 Logarithm of the molar mass (log M, g/mol) as a function of the retention volume (V_R, mL) for narrow PMMA standards in a mixed eluent (THF/n-hexane 65.1/34.9 wt.%). Measurements were carried out at a flow rate of 1.5 mL/min with various volume injection loops (10, 20, 50 μL) and a 2 cm tubing connecting the injector and column. The injected polymer concentration was 1.0 mg/mL.

indicates that the broadening of the injected band, in the connecting capillary, is not large.

Figure 3 shows the effect of the mobile phase flow rate on the LC LCS. Higher flow rates shift the LC LCS to higher retention volumes, although the effect is subtle and only marginally exceeds experimental errors. A 50% increase or decrease in flow rate changes the LC LCS only by 5% at low molar masses, with essentially no dependence on the retention volumes in the high molar mass range. As mentioned, the rapid progression of macromolecules is hampered in LC LCS by the (nonsolvent) mobile phase. The polymer sample is forced to move within the "slow" THF zone and is expected to accumulate in its front part. Increased eluent flow rate causes more broadening of the sample solvent zone. This

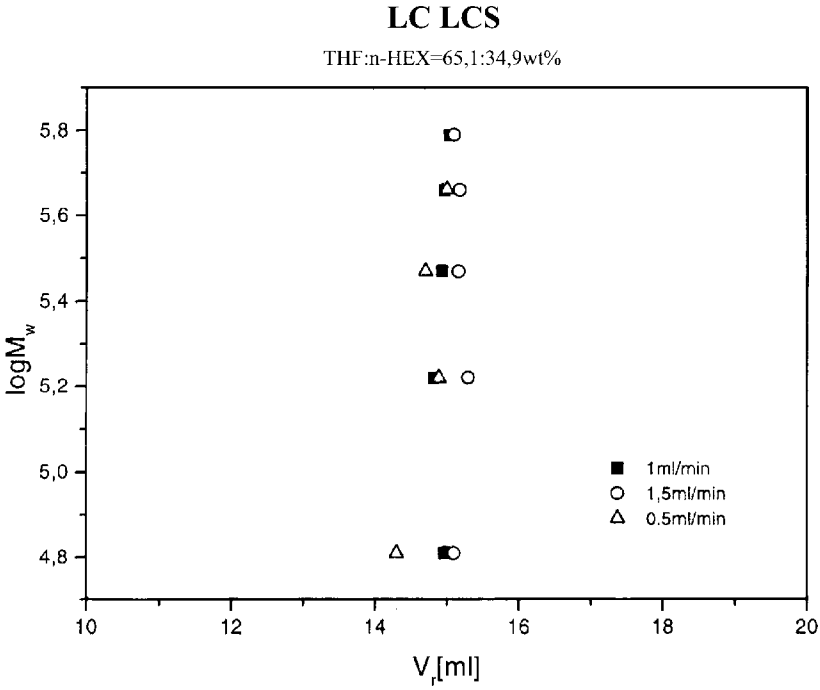

FIGURE 3 Logarithm of the molar mass (log M, g/mol) as a function of the retention volume (V_R, mL) for narrow PMMA standards in a mixed eluent (THF/n-hexane 65.1/34.9 wt.%). Measurements were carried out at varying mobile phase flow rates (0.5, 1.0, 1.5 mL/min), with a 20 μL injection loop and a 2 cm tubing connecting the injector and column. The injected polymer concentration was 1.0 mg/mL.

indicates that macromolecules should have more "space" within THF and can elute earlier, that is, within reduced retention volume. The situation may, however, reverse itself if the eluent is a very effective nonsolvent for polymer species. Macromolecules may require higher THF concentration, near to the center of the THF zone, and, consequently, their retention volumes can slightly increase for broadened injected zones.

Figure 4 shows the influence of the concentration of polymer injected on the elution behavior. As the concentration of polymer within the injection zone increases (1 to 3 mg/mL) there is a very small shift toward higher retention volumes. This may result from a shift from the thermodynamic equilibrium, which can be obtained in LC LCS at modest

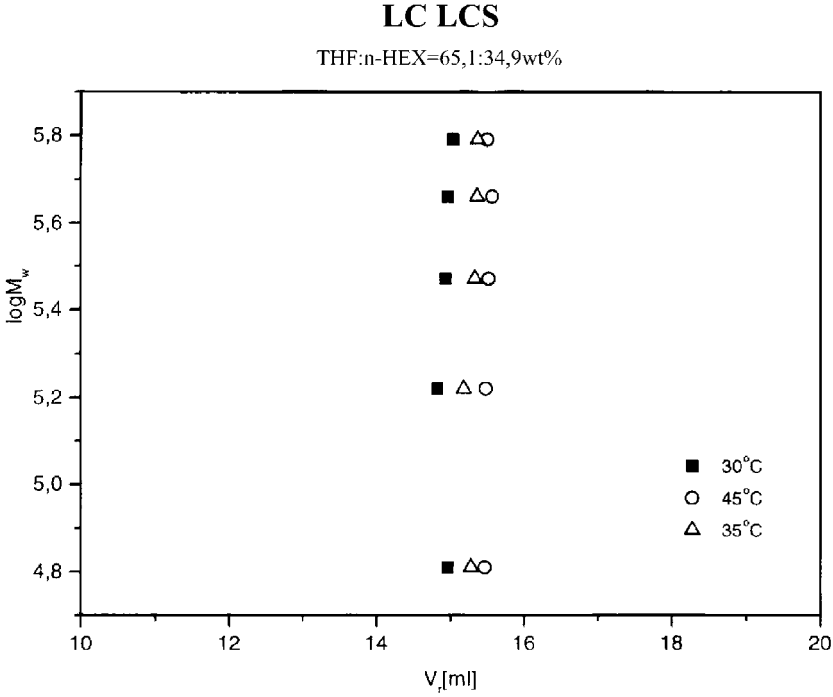

FIGURE 4 Logarithm of the molar mass (log M, g/mol) as a function of the retention volume (V_R, mL) for narrow PMMA standards in a mixed eluent (THF/n-hexane 65.9/34.9 wt.%). Measurements were carried out at a flow rate of 1.5 mL/min, with a 20 μL injection loop and a 2 cm tubing connecting the injector and column. The injected polymer concentration was varied from 1 to 3 mg/mL.

injection concentrations, to a kinetically controlled regime at higher concentrations.

Figure 5 illustrates the effect of temperature on LC LCS. Retention volumes of PMMA are systematically higher as temperature increases. We can speculate that the broadening of injected zone increases with decreasing temperature, and the broader THF zone allows faster progression of macromolecules. Moreover, the resulting polymer retention depends on the extent of its insolubility within mobile phase. The lower the polymer solubility, the slower its progression along the LC LCS column under otherwise identical conditions and the lower are the measured retention volumes. This is clearly demonstrated also in Figure 1. The present explanation would indicate that the system PMMA/THF/hexane exhibits lower critical

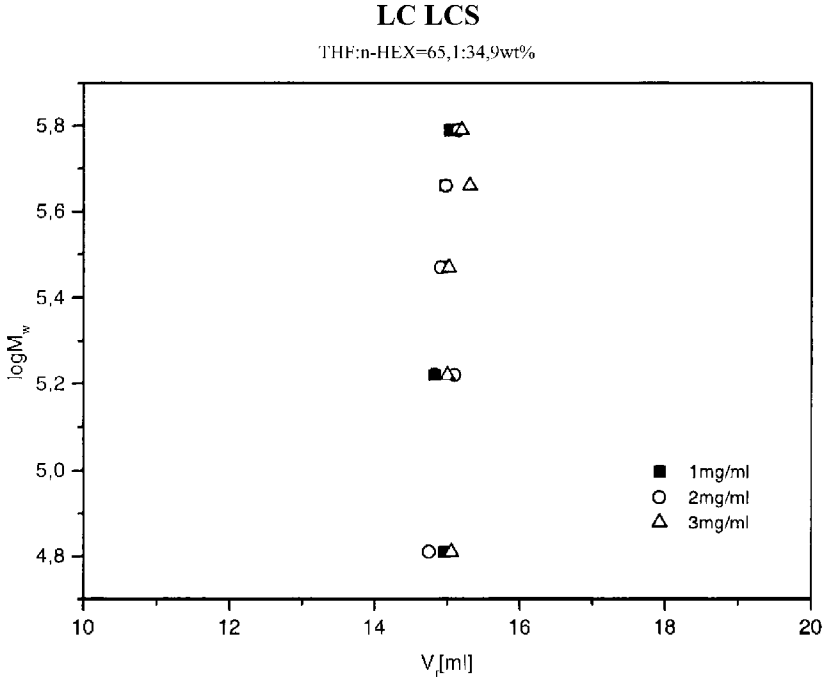

FIGURE 5 Logarithm of the molar mass (log M, g/mol) as a function of the retention volume (V_R, mL) for narrow PMMA standards in a mixed eluent (THF/n-hexane 65.9/34.9 wt.%). Measurements were carried out at a flow rate of 1.5 mL/min, with a 20 μL injection loop and a 2 cm tubing connecting the injector and column.

solution temperature, with solubility of macromolecules improving with decreasing temperatures.

CONCLUSIONS

The mechanism of LC LCS appears to be a microgradient process during which the solute macromolecules leave the injected solution, precipitate in eluent, and redissolve, when again reached by the initial solvent zone. This proceeds many times within the column. Therefore, as would be expected, the LC LCS situation is quite sensitive to temperature and, to a lesser extent, also to the parameters that affect the width of the injected zone, such as the eluent flow rate. However, although the positions of sample retention volumes are shifted due to experimental variables, they remain independent of polymer molar mass under defined conditions. Therefore, LC LCS is experimentally more feasible than the common liquid chromatography under critical conditions (LC CC) because it is less sensitive to minute changes in the mobile phase composition. LC LCS also provides a molar mass-independent retention up to higher molar masses than does LC CC. This implies that the LC LCS method can be easily coupled to other chromatographic techniques. At high molar masses, and at high injected concentrations, the column can be, however, overloaded, shifting the balance of solubility/insolubility.

As adsorption and partition may also play a role in LC LCS, and are difficult to suppress entirely, it is to be expected that the selection of the stationary phase will be important.

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