Novel Methods for the Characterization of Water-Soluble Polymers

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ABSTRACT: The liquid chromatography of four water-soluble polymers [pullulan, polyacrylamide, poly(ethylene glycol), and poly(ethylene oxide)] have been investigated and used to categorize separation processes which couple enthalpic and entropic mechanisms. Experiments were carried out with a binary mobile phase which combined a thermodynamically good solvent (water or aqueous $0.02M$ Na_2SO_4) and nonsolvent (methanol). The polymer solute was injected in a good solvent. By varying the solvent–nonsolvent ratio in the eluent, conditions could be obtained where the free energies of exclusion and solvation were balanced. This has been given the nomenclature "liquid chromatography under limiting conditions of solubility" (LC-LCS) since the polymer elutes just in front of the system peak at the "limit" of its solubility. Conditions can also be identified where exclusion is balanced with adsorption ("liquid chromatography under limiting conditions of adsorption," or LC-LCA). To our knowledge, these are the first experimental reports of LC-LCS for any polymer and the first LC-LCA observation on water-soluble macromolecules. All measurements were carried out over a poly(hydroxymethylacrylate) sorbent. Cloud point curves were found to generally distinguish the regions where LC-LCA or LC-LCS dominate. The data illustrate the need to consider the polymer when analyzing LC-LCA. Conversely, polymer adsorption may play an important role in LC-LCS. © 1998 John Wiley & Sons, Inc. J Appl Polym Sci 69: 2549–2557, 1998

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INTRODUCTION

Liquid chromatography at the point of the elution-adsorption transition (LC-PEAT) results in a retention volume that is independent of the molecular weight of macromolecules.[1] It is one of the family of methods where entropic separation mechanisms, such as exclusion, are balanced with enthalpic processes (adsorption, solubility). LC-PEAT conditions can be obtained using a binary eluent for the macromolecule where one component of the eluent is normally a thermodynamically good solvent and the second is a poor solvent, nonsolvent, or desorption promoting liquid. Since the binary eluent composition where molecular-weight-independent retention is observed can be

a nonsolvent for the polymer probe, the solute is injected in a thermodynamically good solvent. Therefore, as the solvent zone passes through the column, the polymer excludes and encounters a mobile phase under which conditions its coil size is diminished. The polymer then adsorbs, reversibly, onto the sorbent, only to redissolve as the injection zone "catches up" to the excluded polymers. This microgradient process of exclusion, adsorption, and redisollution occurs several times throughout the column, resulting in the polymer eluting on the leading front of the injection zone.[2] Since the polymer elutes on the limit of its adsorption, such conditions have been termed "liquid chromatography under limiting conditions of adsorption" (LC-LCA) and are the topic of this investigation. In contrast, if polymer solubility also plays a role in the enthalpic mechanism, and molecular-weight-independent retention is observed, the polymer excludes from the injection zone and is retained due to a change in chain solubility, which reduces the coil size and decreases the elution rate. Under such conditions, the macromolecule elutes on the limit of its solubility, again at the leading edge of the injection zone. Such conditions are termed "liquid chromatography under limiting conditions of solubility" (LC-LCS).[3]

A recent article[4] details the specifics of LC-PEAT. Another review[1] distinguishes LC-LCA from other competing techniques, including liquid chromatography at the critical adsorption point (LC-CAP). This has been called "critical conditions" in historical reference to Belenkii's pioneering work in a thin layer arrangement.[5,6] LC-CAP involves the combination of two solvents for a polymer probe, with the macromolecule dissolved and injected in the mobile phase, as is common in high-pressure liquid chromatography (HPLC). The LC-CAP point is, however, very sensitive to minute changes in eluent composition and temperature (hence, the terminology "critical"), whereas molecular-weight-independent retention occurs over a broader range of eluent compositions in LC-PEAT methods, such as LC-LCA[7] and LC-LCS.[8] LCCAP and LC-PEAT[4] conditions have been documented in several polymer–binary eluent–stationary phase systems. The latter have been shown to be influenced by the temperature. Thus far, water-soluble polymers have not been characterized by LC-LCA in aqueous eluents, although poly(ethylene glycol) (PEG) has been investigated in a binary organic solvent system.[9] This article will present the first experimental evidence of LC-LCS for any polymer and the first observations on water-soluble polymers characterized in
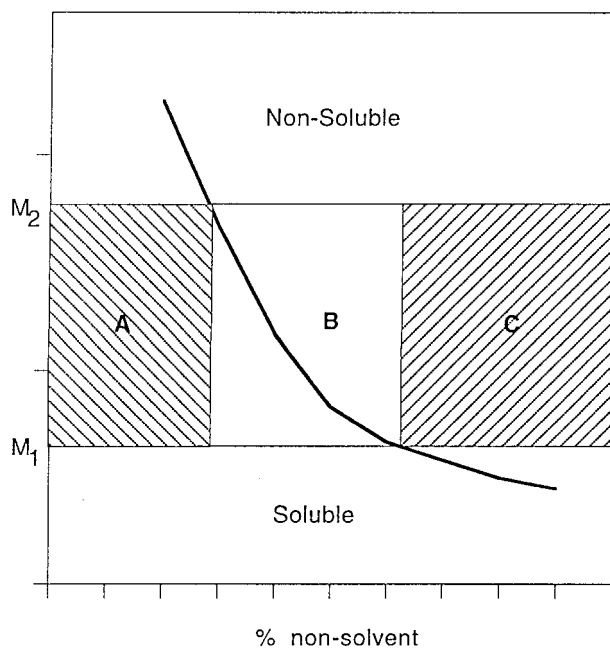

Figure 1 A schematic plot of the solubility of polymer standards in a mixed eluent (solvent plus nonsolvent) system, as this relates to interactive liquid chromatography experiments. In domain (A), adsorption is the operative enthalpic mechanism, which is balanced with exclusion (LC-LCA), while in domain (C), the polymer–solvent solubility dominates the enthalpy. Domain (B) is a hybrid where the entropic exclusion forces are balanced by both adsorption and solubility. Note that M_1 and M_2 represent the range where the retention volume is independent of the polymer molecular weight.

aqueous media. (Other papers have studied organically soluble[4] and stereoregular[10] polymers through mechanisms that are now clearly recognized as LC-LCA, though they were, at the time, believed to be and identified as LC-LCS.)

Using Cloud Point Curves to Distinguish Separations Mechanisms

Recently, Bartkowiak et al. proposed that the solubility of the polymers in a binary eluent (cloud point curve) can be used as a means to identify whether a molecular-weight-independent calibration curve operates in the LC-LCA or LC-LCS modes.[7] Provided the eluent is a weak nonsolvent for the solute, at each eluent composition, there is a maximum molecular weight above which the polymer precipitates (Fig. 1). If this limit is higher than the largest polymer molecular weight to be characterized (M_2), or the molecular weight

Table I   Properties of the Water-Soluble Polymers Used for HPLC Measurements

Polymer	Manufacturer	Range of MW	Polydispersity
Polyacrylamide	American Polymer Standards (Mentor, OH)	7950–725,000	1.8–3.0
Pullulan	Showa–Denko (Japan)	5800–1,600,000	< 1.2
Poly(ethylene glycol)	For Waters Corp. by Polymer Standards Service (Mainz, Germany)	106–23,000	< 1.2
Poly(ethylene oxide)	For Waters Corp. by Polymer Standards Service (Mainz, Germany)	25,300–825,000	< 1.2

corresponding to the exclusion limit of the column, then one operates in the soluble S domain, where the only operative enthalpic mechanism is adsorption (that is, LC-LCA). This is depicted by zone (A) in Figure 1. However if the calibration curve crosses the solubility threshold, as is shown in zone (B) of Figure 1, then both solubility changes, and adsorption occur within the column. Therefore, the process is a hybrid of the LC-LCA and LC-LCS mechanisms. In zone (C) of Figure 1, all polymers within the $M_1–M_2$ range precipitate. This is the postulated for requirement limiting conditions of solubility,[8] and one can say that polymer solubility is the primary enthalpic mechanism occurring in zone (C). Theoretically, one may also have a "pure" LC-LCS system in which the adsorption of a the polymer within the column is negligible. In this work, water-soluble polymers were utilized as models in order to elucidate the mechanisms of LC-LCA and LC-LCS.

EXPERIMENTAL

HPLC

Chromatographic measurements were carried out on a poly(hydroxymethacrylate) gel packed in a 300-mm stainless column with 8-mm internal diameter. The Shodex OHpak SB-804 HQ column was obtained from Showa-Denko (Tokyo, Japan). The HPLC system consisted of Waters 510 HPLC pump (Waters, Milford, MA), a Waters R401 Differential Refractometer, and a Rheodyne 7725I injector (Cotati, CA). The flow rate was 0.5 mL/min and 20 μL of a 0.05 wt % aqueous polymer solutions was injected. Polymer samples were injected in a pure solvent. All measurements were carried out at ambient temperature (23 ± 1°C). Type I deionized water with a resistivity $\geq$ 16.7 mΩ-cm (Continental Water, San Antonio, TX) was filtered through a 0.2-mm nylon membrane

filter. HPLC-grade methanol was purchased from Fisher Scientific (Norcross, GA).

Four types of water-soluble polymers were utilized in this investigation, the properties of which are given in Table I. For polyacrylamide (PAM), a 0.05M aqueous sodium sulfate solution was used as a solvent in place of water for the chromatographic measurements.

Cloud Point Measurements

The solubility of pullulan and PAM were determined by measuring the required volume of methanol to precipitate the polymer (0.05 wt %) from a 5-mL aqueous solution. The solubility of PEG and poly(ethylene oxide) (PEO) standards in methanol was determined in the same manner. All measurements were carried out at a temperature of 23 ± 1°C. (The authors are aware of the temperature sensitivity of chromatographic measurements and cloud point curves. In regard to the latter, cloud point measurements have been performed exclusively in order to distinguish LC-LCA and LC-LCS conditions and not to provide quantitative measures of solubility, for which strict temperature control would be required.) The onset of turbidity was observed visually and was used as the metric for precipitation. Samples were agitated with magnetic stirring bars.

RESULTS

Polyacrylamide Characterization

The LC calibration curves for PAM in mixtures of methanol and 0.05M aqueous sodium sulfate is shown in Figure 2. For the mobile phase composition of 58/42 vol % methanol–0.05M aqueous sodium sulfate, the retention volume for PAM standards (range 5000–197,000) is nearly constant. Figure 3 shows that the cloud point curve for the

PAM standards. While Elias has found that the critical level of nonsolvent required to precipitate a polymer has a semilogalithmic dependence on the polymer concentration,[11-13] such a trend was not observed herein. Indeed, our cloud point curves were independent of polymer concentration between 0.1 and 0.5 mg/mL. In Figure 3, the cloud point curve operates over a 14% range in eluent composition (47 to 61% of methanol). Clearly, the calibration curve at 58% methanol crosses the solubility threshold. Therefore, according to Figure 1, this system primarily operates in zone B, where a hybrid mechanism of exclusion–adsorption (LC-LCA) and exclusion–solubility–adsorption (LC-LCS) occur. The highest molecular weight point on the calibration curve, at 58 vol % methanol, has a vertical distance of 180,000 daltons from solubility boundary. This corresponds to a horizontal distance of 9 vol %. The relevance of this phenomenon will be described following the presentation of the pullulan results.

Pullulan Characterization: Effect of Dilution and Zone-Broadening

At a composition of 70/30 vol % methanol–water, the retention volume for pullulan standards is
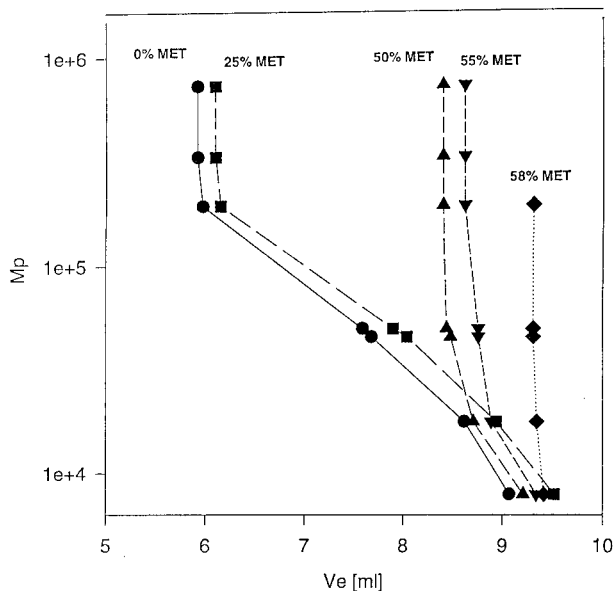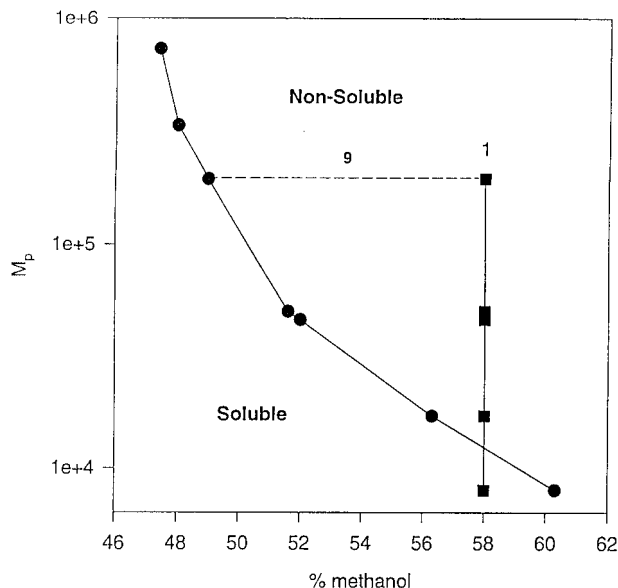

Figure 3 A plot of the solubility of PAM standards in a mixed eluent ($0.05 M$ aqueous Na_2SO_4 plus methanol) system. PAM of various molecular weights are soluble to the left of cloud point curve (S zone) and insoluble to the right of solid line (NS zone). Line (1) represents the retention-independent M_W condition (LC-LCA and LC-LCS) for PAM observed on a poly(hydroxymethacrylate) gel (data in Fig. 2).

Figure 2 A plot of the molecular weight (M_W; g/mol) as a function of retention volume (mL). The calibration curves for polyacrylamide standards in a mixed eluent ($0.05 M$ aqueous Na_2SO_4 plus methanol) are shown at various binary eluent compositions (% methanol in methanol–$0.05 M$ aqueous Na_2SO_4). A retention-independent elution is observed at 58% methanol–42% $0.05 M$ Na_2SO_4.

constant over the range 5000–25,000 daltons (Fig. 4). Figure 5 shows the cloud point curve of pullulan in water–methanol solutions as a function of molecular weight [solid line (1)]. The solid line, labeled (2), represents a 70/30 solution where retention volume of pullulan standards is independent of molecular weight. This line cross the cloud point curve, with 1 point (pullulan molecular weight 5450) below and 2 points (12,200 and 25,300 molecular weight) above the solubility threshold. This indicates that, as in case of PAM, a hybrid LC-LCA and LC-LCS mechanism exclusion–adsorption–solubility is observed for pullulan. For all these experiments, pullulan was injected in a good solvent (deionized water).

The highest point on the calibration curve for pullulan, in 60/40 vol % methanol–water mixture, has a horizontal distance 9 vol % from the solubility boundary. This is the same distance observed for the highest eluted molecular weight PAM at 58/42 vol % of methanol–water (Fig. 3). The fact that one can observe a polymer eluting in the insoluble domain (above the cloud point curve) leads us to postulate that a dilution, or zone broadening, mechanism was occurring in LC-LCS. That is, the polymer remains soluble
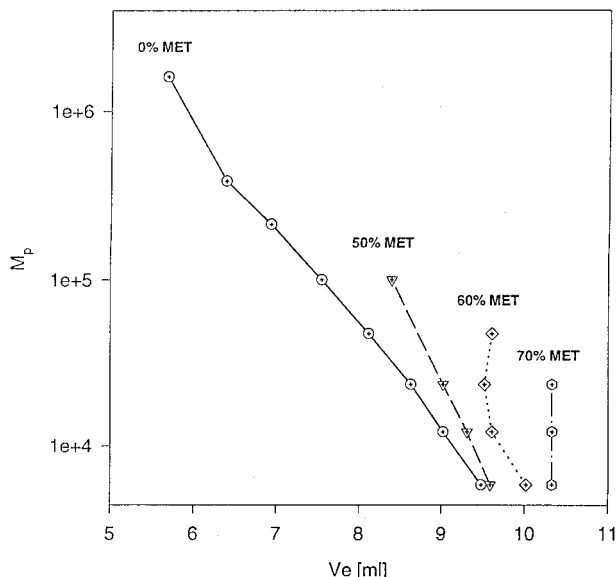
Figure 4 A plot of the molecular weight (M_W; g/mol) as a function of retention volume (mL). The calibration curves for narrow pullulan standards in a mixed eluent (water plus methanol) are shown at binary eluent various compositions (% methanol in methanol−water). A retention-independent elution is observed at 70% methanol−30% water, though clearly, the higher molecular weight samples were highly interacting, and the recovery of such injected samples was limited.

when eluted, and the solubility threshold is exceeded due to the mixing of the injection zone with the eluent. Furthermore, it seems reasonable that the zone broadening would be constant for given binary mobile phase−sorbent−injection solvent combination, as is observed for PAM and pullulan. An elementary calculation will be used to quantify this phenomena. The following data are required.

1. A 0.02-mL injection loop is used containing 100% solvent (water).
2. The theoretical volume of the eluent (mL), which "ideally" mixes with the injection solvent, is assumed to be 0.1 mL. That is, the peak broadens by 0.1 mL.
3. The percentage of solvent (water) in the binary eluent is given by the variable X.
4. The percentage of solvent in the eluent after mixing (0.02 mL of the injection solvent + 0.1 mL of eluent) is given by the variable X'.

Based on the preceding assumptions, the following mass balance can be easily calculated:

$$(0.02 + 0.1)\cdot X'\% = 0.1\cdot X\% + 0.02\cdot 100\% \quad (1)$$

In this equation, the left side represents the mass of solvent (water) in the eluent after mixing with the injection zone. The right side of eq. (1) is the total quantity of solvent in the portion of the eluent that mixes with the injection zone ($0.1\cdot X$) and the volume of solvent in the injection zone ($0.02\cdot 100\%$). If one solves eq. (1) for a binary eluent mixture containing 40% water as a solvent ($X = 40\%$), then X' can be easily calculated to be 50%. This calculation indicates that an eluent that is 40/60 vol % water−methanol will dilute, due to mixing from the injection zone, to 50/50 vol % water−methanol. That is, dilution makes the mobile phase less of a nonsolvent for the polymer probe, by 10 vol % ($X' - X$), in quite good agreement with the experimental data (9%). It is important to note that the 9% shift is not expected to be universal and is a value particular to the system (binary eluent, polymer, sorbent) at hand.

The pullulan cloud point curve in Figure 5 operates over a 40% range of the eluent composition. Therefore, some of the high-molecular-weight pullulan standards appear in the insoluble zone (NS) for the eluent compositions of 50, 60, and 70 vol % of methanol. Figure 5 also plots the highest-molecular-weight points for 3 pullulan calibration
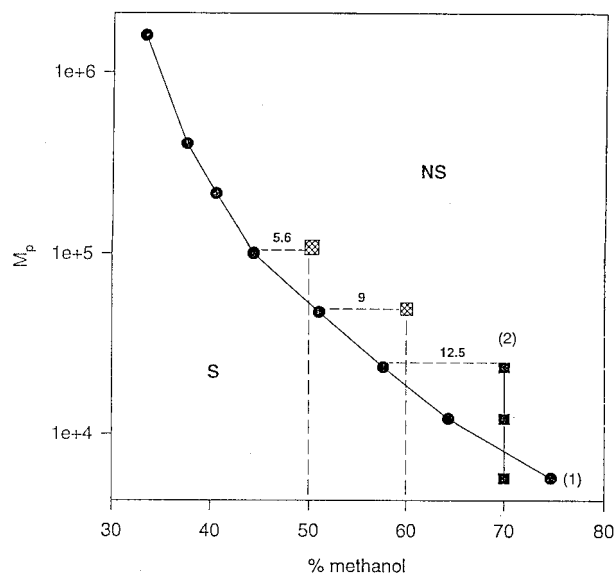

Figure 5 A plot of solubility of pullulan standards in a mixed eluent (water plus methanol) system [line (1)]. Pullulan of various molecular weights are soluble to the left of could point curve (S zone) and insoluble to the right of solid line (NS zone). Line (2) represents the retention-independent elution at the hybrid condition (LC-LCA and LC-LCS) for pullulan on a polyhydroxymethacrylate gel (data in Fig. 4).

curves measured at 50/50, 60/40, and 70/30 vol % methanol−water. For molecular weights above these points, the polymer precipitated in the eluent within the column. The horizontal distance from the highest molecular weight on the calibration curve to the solubility threshold (line 1) decreases from 12.3 for 70/30 methanol−water eluent to 5.6% for a 50/50 binary mixture of methanol−water. Specifically, the horizontal distance data imply that the injection of an aqueous pullulan solution into the column causes a decrease in the methanol concentration in the mobile phase due to the dilution effect and that the severity of the dilution is dependent on the binary eluent composition.

The horizontal distance between the maximum point on the pullulan calibration curve and the cloud point curve has been observed to increase as a function of the nonsolvent (methanol) content in the eluent, as is shown in Figure 5. Indeed, the distance increases from 5.6 to 9% and ultimately 12.5% for 50, 60, and 70% methanol in the eluent. This is due to the fact that additions of the same volume of solvent (water) into the column with higher concentration of methanol will cause respectively larger decreases of methanol concentration simply by a mass balance effect. This is demonstrated by a second calculation, which is based on 2 cases with differing concentrations of solvent in the eluent ($X_A = 30\%$, $X_B = 50\%$). The mass balance of the eq. (1) can be expressed as

$$(0.02 + Y) \cdot X'\% = 0.1 \cdot X\% + 0.02 \cdot 100\% \quad (2)$$

From eq. (2) one may easily calculate the value of X' for each value of X:

$$X'_A = 41.6\% \quad \text{and} \quad X'_B = 58.3\%$$

That is, eluent A will concentrate from 30 to 41.6% due to peak broadening. In contrast, the more solvent-rich eluent B will only concentrate from 50 to 58.3%. Therefore, the higher concentration of nonsolvent in eluent, the larger the decrease in concentration. This calculation provides a reasonable explanation for why the horizontal distance, in Figure 5, decreases with reduced methanol levels. It appears that the dilution phenomena causes a change in the concentration of the solution and a shift in the "effective solubility" threshold, as is shown in Figure 6. This effective solubility threshold thereby sets the limit for the maxi-
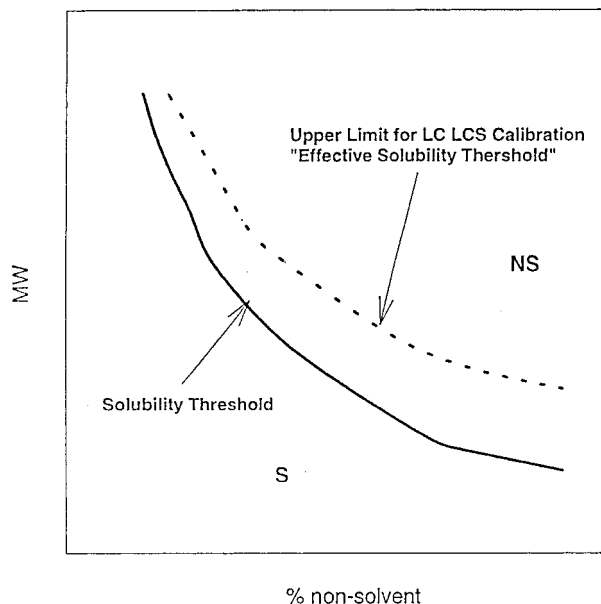

Figure 6 A schematic of the effect of dilution on the solubility threshold (effective cloud point curve) for pullulan in methanol−water. Mixing of the injection zone with the eluent increases the concentration of solvent in the region surrounding the polymer improving solubility. Hence, LC-LCAs can be carried out at conditions in the insoluble portion of the cloud point curve.

mum molecular weight observed in the calibration curves of Figure 3 (PAM) and Figure 4 (pullulan).

Effect of the Slope of the Calibration Curve on Limiting Conditions

By comparing the result obtained for PAM and pullulan, one can postulate that the upper limit of molecular weight in LC-LCA, or the lower limit in LC-LCS, depends on the local slope of cloud point curve. This can be explained as follows: for a similar range of molecular weight standards, the solubility curve for pullulan (Fig. 5) operates over a 40% of methanol concentration range (slope* = −0.042), while for polyacrylamide (Fig. 3), this range is limited to 14% methanol (slope* = −0.116). As a result, at 60 vol % methanol, the calibration curve for pullulan extends up to 25,000 daltons, while at 58% methanol, the calibration curve of polyacrylamide is operative until 193,000 daltons. We have previously postulated that the dilution of the methanol−water is a function of the sample solvent volume (water) and is independent of the chemistry polymer probe. Therefore, the effect of a 10% dilution on the maximum molecular weight for a calibration curve de-

pends on the slope of the solubility threshold. As the local slope of the cloud point curve increases, the vertical distance above the solubility threshold a polymer can still dissolve, due to dilution, rises. This is shown schematically in Figure 7. Clearly distance $M'_B - M_B$ for polymer B exceeds that of distance $M'_A - M_A$ for polymer A, which is why solubility plays a stronger role in the separation of acrylamide $(M'_B - M_B)$ than in the case of pullulan $(M'_A - M_A)$, as is shown schematically in Figure 7 and quantitatively in Figures 3 and 5, respectively.

Poly(ethylene Oxide) and Poly(ethylene Glycol)

PEO and PEG samples are soluble in both methanol and water; therefore, for the water–methanol system, it is only possible to observe LC-LCAs. In our experiments, the PEO and PEG were injected into the column in an aqueous solution (good solvent), such as that employed in a typical LC-LCS experiment due to the difficulty in dissolving of
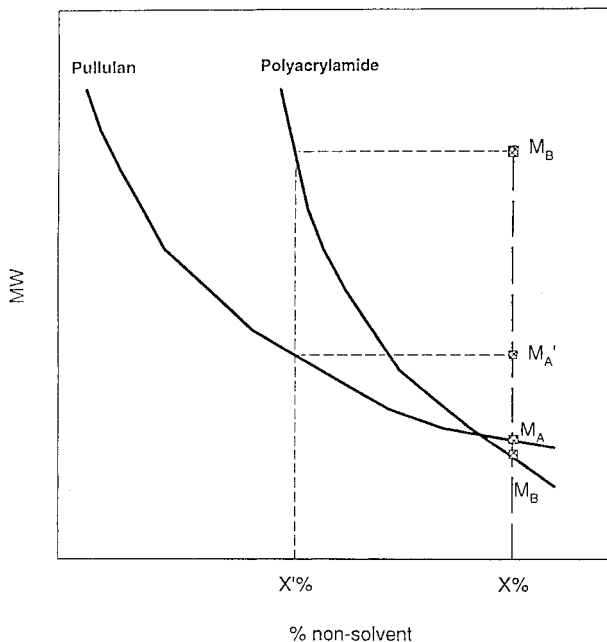
Figure 8 A plot of the molecular weight (M_W; g/mol) as a function of retention volume (mL). The calibration curves for narrow PEO standards in a mixed eluent (% methanol in methanol–water system) are shown at various compositions. A retention-independent elution is observed at 97% methanol–3% of water.

Figure 7 A schematic plot of the solubility of 2 polymers in a mixed eluent (solvent plus nonsolvent) system. Methanol is weak nonsolvent for pullulan (polymer A) and strong nonsolvent for polyacrylamide (polymer B). $X'–X$ represents the dilution zone, which is characteristic for eluent with $X\%$ composition. The difference $(M'_A - M_A)$ is related to molecular weight range where the critical conditions of solubility (LC-LCS) are observed for pullulan (A) while $(M'_B - M_B)$ represents the LC-LCS range for polyacrylamide (B).
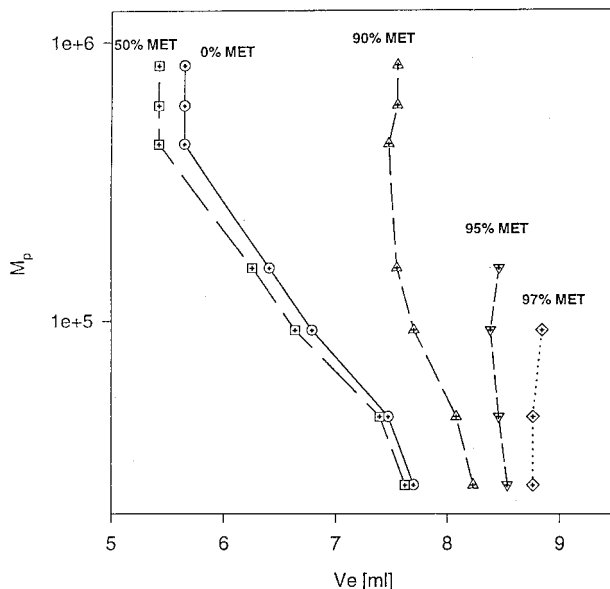
PEOs in methanol without increasing the temperature. It was found that at an eluent composition of 97/3 vol % methanol–water, the retention volume was independent of polymer molecular weight in range from 5000 to 12,000 daltons (Fig. 8). For PEG in 100% methanol with a molecular weight above 5000 daltons, a retention volume independent of MM was observed. However, below 5000 daltons, the column still separated PEG according to the size of the coil (Fig. 9). One could, therefore, refer to these as near limiting conditions. The results for PEO and PEG indicate that there is adsorption on poly(xydroxymethacrylate) gel in the water–methanol system. For these polymers, true LC-LCA conditions remain to be identified. Future experiments could use methanol–water as a sample solvent while employing a stronger nonsolvent to achieve the molecular-weight-independent retention.

The results of HPLC measurements for the four water-soluble polymers in the water–methanol system are summarized in Table II.

DISCUSSION

From the results obtained for pullulan and PAM, some general conclusions can be drawn. Figure
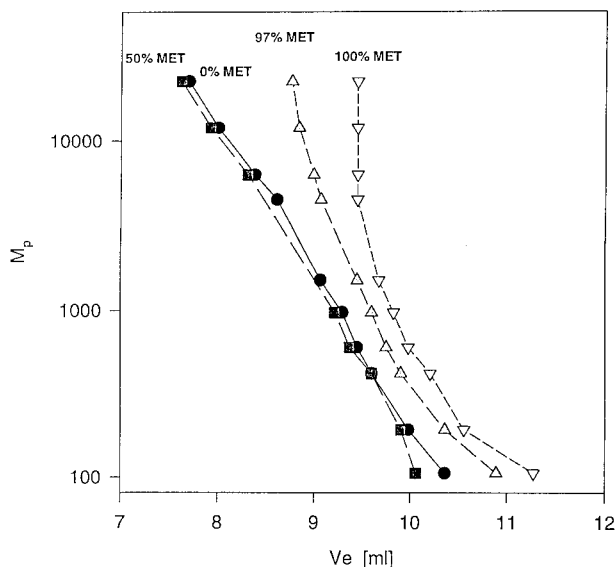
Figure 9 A plot of the molecular weight (M_W; g/mol) as a function of retention volume (mL). The calibration curves for narrow PEG standards in a mixed eluent (% methanol in methanol–water) are shown at various compositions. A retention-independent elution is observed for molecular weight above 8000 daltons at 100% methanol. However, for molecular weights below 5000 daltons, LC-LCA were not observed.

10 presents two zones above the solubility curve. The first zone (I) is a region where the polymers in range (M_1-M_2) are insoluble in an eluent with $X_A\%$ of nonsolvent. The fact that the polymer is injected in good solvent results in a concentration driving force and a dilution effect. The sample zone mixes with eluent zone and the final compo-
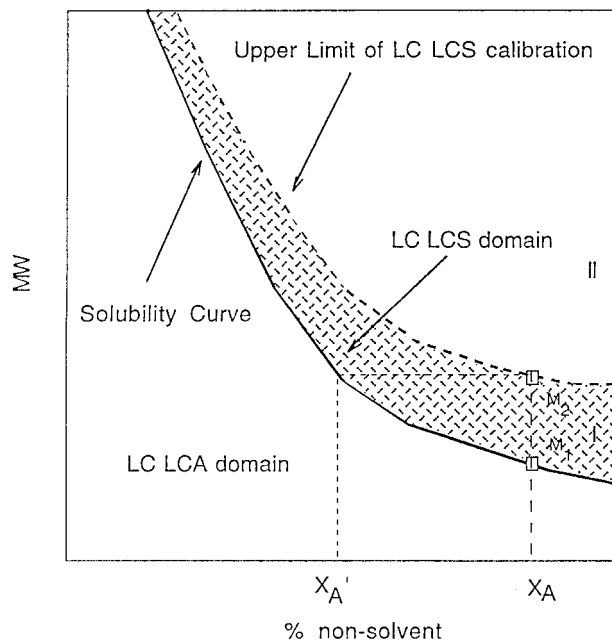

Figure 10 A theoretical plot of the solubility of polymer standards in a mixed eluent (solvent–nonsolvent). In zones I and II, polymers are insoluble; whereas to the left of the solubility curve, the polymers are soluble. X'_A-X_A represents the dilution zone that is characteristic for eluent with $X_A\%$ composition. Limiting conditions of solubility (LC-LCS) are only observed in the zone I.

sition of the injection zone has a lower concentration of nonsolvent ($X'_A\%$) than the mobile phase. Such a mixture is still a solvent for polymers in range (M_1-M_2). However, above M_2 (zone II), all polymers injected into this system are insoluble.

Table II   Systems of Polymer Plus Solvent Plus Nonsolvent Where LC–LCA and LC–LCS Have Been Observed

Polymer	Eluent	Conditions[a] (vol %/vol %)	Molecular Weight Range[b] (Daltons)	Mechanism
Pullulan	Methanol–water	70/30	5800–23,700	Hybrid mechanism (LC–LCA + LC–LCS)
Polyacrylamide	Methanol–0.05M aq. Na$_2$SO$_4$	58/42	7950–173,000	Hybrid mechanism (LC–LCA + LC–LCS)
Poly(ethylene oxide)	Methanol–water	97/3	25,300–92,000	(LC–LCA)
Poly(ethylene glycol)	Methanol–water	100/0	4450–23,000	(LC–LCA)

[a] Conditions for retention-independent calibration curve.
[b] Molecular weight range over which retention-independent calibration curves were observed.

In other words, the dilution causes in outward shift in the effective solubility threshold or cloud point curve. It has been also observed that the horizontal distance between solubility curve and the curve which separates zone I and II (that is, $X_A - X'_A$), increases with increasing concentrations of nonsolvent (methanol) in the eluent. The results for pullulan and polyacrylamide indicate that the shift $(X_A - X'_A)$ for different polymers with the same sorbent and mobile phase composition is equivalent. While one anticipates a shift for each solvent–nonsolvent system, the magnitude will be dependent on the polymer, eluent, and sorbents employed.

Figure 7 represents a theoretical example of two polymers (A and B) that have different solubility curves (different slopes). Both curves have the limiting conditions at the same eluent composition ($X\%$). If the shift for the same composition is constant (for example, $X'\%$ composition), we can determine the maximum molecular weight for a retention-independent exclusion is M_A and M_B, respectively. These points are on the border between zone I and zone II (Fig. 10) and show the highest molecular weight at which the polymers are still eluting from the column. Since the negative slope of cloud point curve for polymer B is greater than for A (Fig. 7), the range where polymer B operates under limiting conditions is larger ($M_B > M_A$). This has implications in methods development and suggests that, if one wishes to have a molar-mass-independent retention over several orders of magnitude, a solvent–nonsolvent system should be selected providing a relatively sharp (large slope) cloud point curve.

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