Adsorption and Enthalpic Partition Retention Mechanisms in Liquid Chromatography of Noncharged Synthetic Polymers. Two Critical Ranges[†]

Dušan Berek*

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

Received March 12, 2004; Revised Manuscript Received May 4, 2004

ABSTRACT: Two independent liquid chromatographic critical ranges were identified in systems composed of the non-end-capped silica gel C-18 column packing, poly(methyl methacrylate)s (PMMAs), and mixed eluents containing acetonitrile (ACN) and toluene at 35 °C. ACN suppressed surface adsorption of PMMA on free silanols and promoted enthalpic partition of polymer species in favor of a solvated C-18 bonded phase. On the contrary, toluene promoted adsorption of PMMA on silanols and suppressed its enthalpic partition in favor of the C-18 phase. Changing the eluent composition, it was possible to separately control both the above enthalpic retention mechanisms. Consequently, a critical adsorption range was reached at one eluent composition with a negligible or only small effect of enthalpic partition, and vice versa, compensation of enthalpic partition and exclusion retention mechanisms was attained in eluent-suppressing adsorption. In this way, the existence was indirectly proved of surface adsorption and enthalpic partition as two similar but independent enthalpic retention mechanisms operative in high-performance liquid chromatography of polymers. In many cases adsorption and enthalpic partition act concurrently in a synergetic or an antagonistic way, and this may strongly affect the selectivity of polymer separation.

Introduction

As known, distribution of solute molecules between a solution and a (solid) *surface* or a (liquid−liquid) *interface* is commonly termed *adsorption* while distribution of solute molecules between two *volumes* of liquids or quasi-liquids is designated *partition* or absorption. Adsorption and partition represent important retention mechanisms in high-performance liquid chromatography of polymers (polymer HPLC). Adsorption of macromolecules is a typical enthalpic process, which is usually accompanied by large entropic effects. Partition of polymer species can be governed either by entropic or by enthalpic phenomena. Changes of entropy are responsible for partition of macromolecules between a liquid confined within pores of a solid substance and a free liquid of the same nature. One speaks about *entropic partition*, which is the basic retention mechanism of size exclusion chromatography (SEC). Partition, which takes place between two liquids of unlike chemical nature, is a rather overlooked retention mechanism in polymer HPLC. It is composed of an entropic contribution, similar to but not identical with SEC, and of an enthalpic contribution, called *enthalpic partition*.

In the first part of this series[1] it was suggested that (i) adsorption of macromolecules on the surface of solid column packing particles or on the interface between the mobile phase and the chemically bonded phase and (ii) enthalpic partition of polymer species between the mobile phase and the C-18 alkane bonded phase are similar but independent enthalpic retention mechanisms operative in polymer HPLC. The difference between adsorption and enthalpic partition was demonstrated on the dissimilar courses of the dependences of log M vs V_R for different polymer−eluent systems in conjunction with silica gel C-18 bonded phases.[1−4] Here M is the molar mass most abundant in the polymer sample, exhibiting narrow molar mass distribution, and V_R is the corresponding retention volume.

The *eluent strength*, which is the affinity between eluent molecules and the (polar) column packing surface, controlled adsorption of macromolecules on the surface free silanols of bare and bonded silica gels. Strong eluents such as dimethylformamide, which efficiently competed with polymer segments for active sites on the column packing surface, suppressed adsorption of medium and highly polar macromolecules such as poly(2-vinylpyridine)s and poly(ethylene oxide)s. Weaker solvents, for example, toluene, less extensively interacted with silanols and allowed adsorption of medium-polarity polymer species such as poly(methyl methacrylate)s on the packing surface.[1−3] Very weak cyclohexane or carbon tetrachloride is known to promote adsorption of low-polarity polystyrene on bare silica gel.[5,6] The affinity of eluent molecules toward the solid surface of the column packing can be estimated from the *solvent strength parameter*, ϵ^0, or from the *solvent polarity index*, P, both proposed by Snyder.[7,8]

The *thermodynamic quality* of the eluent for the polymer, which is the affinity between macromolecules and eluent molecules, seemed to be less important for adsorption control. On the contrary, the thermodynamic quality of the eluent for macromolecules, its *solvation power*, strongly affected enthalpic partition of polymer species between the mobile phase and the solvated C-18 bonded stationary phase. Poor solvents such as dimethylformamide and diethyl malonate pushed macromolecules of low polarity such as polystyrene or poly(*n*-butyl methacrylate) into the solvated C-18 alkane bonded stationary phase and promoted enthalpic partition of polymer species in favor of column packing.[1,4] The effect of enthalpic partition of macromolecules was likely augmented by their adsorption on the interface between the eluent and C-18 phase. At the same time,

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* To whom correspondence should be addressed. E-mail: dusan.berek@savba.sk.

[†] The first part of this series is ref 1.

10.1021/ma0400564 CCC: $27.50

eluents, which were good solvents for polymers, prevented their enthalpic partition in favor of the C-18 bonded phase. In strong and good eluents, macromolecules eluted from the C-18 bonded phase in the "ideal" size exclusion mode, practically without influence of enthalpic interactions.

The solvation power for macromolecules can be approximated by the exponent, *a*, of the Kuhn−Mark−Houwink−Sakurada viscosity law

$$[\eta] = KM^a \qquad (1)$$

which is valid for coiled, noncomplexing, uncharged linear macromolecules of sufficiently high molar mass, above about 10 kg·mol^{-1}.[9] $[\eta]$ is limiting viscosity number of the polymer sample with molar mass M, while K and a are constants for a given polymer−solvent system at a given temperature. Macromolecules, which are more soluble in the solvated bonded phase than in the eluent, are partitioned in favor of column packing.

Unfortunately, all above parameters exhibit large source-to-source variability. Their theoretical backgrounds are often questioned. Therefore, discussions about enthalpic retention mechanisms in polymer HPLC are only semiquantitative. Generally, it can be said that the larger surface and interface adsorption or enthalpic partition of macromolecules onto/in favor of column packing, the larger are their retention volumes compared with those of the noninteracting species of the same size. As a rule, the extents of both adsorption and enthalpic partition rise with increasing molar mass of the macromolecules.

Column packings represent an important problem in polymer HPLC. So far the only available column packings covered with a defined homogeneous "quasi-liquid" that is with solvated bonded groups of uniform composition are silica gels bearing alkyl groups. Such phases are suitable for enthalpic partition of macromolecules. Most other commercial bonded phases are composed of a polar group, for example, nitrile, amine, etc., and of an *n*-propyl spacer. It is anticipated that the interfacial adsorption on the polar groups together with the surface adsorption on free silanols would dominate the sample retention on the latter phases. A recently introduced poly(ethylene glycol) (PEG) bonded phase does not exhibit properties appropriate for enthalpic partition of macromolecules, either. The PEG groups seem to lie flat on the silica surface instead of exhibiting a brushlike topology.[10] The effective volume of the PEG phase is likely rather small compared with that of the C-18 phase, which assumes in tetrahydrofuran about 50% of the pore volume.[4]

Commercial C-18 bonded phases, even the end-capped ones, contain a high concentration of free silanols on the silica gel surface. These silanols are surprisingly well accessible for large polymer species.[1−3] It is supposed that polar macromolecules in the weak and medium strong eluents may decoil and reptate along the C-18 groups to be adsorbed on the active silanols. This process is well pronounced above a certain "limiting" polymer molar mass because macromolecules must be large enough to bend above and among the bonded C-18 groups to attach simultaneously on several silanols[1−3,11] ("U-turn adsorption").

The suggested dissimilarity between surface adsorption and enthalpic partition retention mechanisms of macromolecules in polymer HPLC needs further experimental evidence. Important proof would provide liquid chromatographic systems, in which the above two enthalpic retention mechanisms could be independently controlled by a simple adjustment of experimental conditions, for example, by changing the composition of the eluent, which contains the same solvents. In such systems, the elution behavior of homogeneous polymers would be governed once by the surface adsorption and then by the enthalpic partition augmented by interfacial adsorption, and this may result in two *critical points* or at least in two *critical ranges*.

The concept of liquid chromatography under critical conditions was created by the Belenkii group.[12,13] Its theory[14,15] suggests that, at the critical point, entropic (exclusion) and enthalpic (interaction) retention mechanisms mutually exactly compensate and the resulting Gibbs function equals zero. At the critical point, macromolecules elute from the column irrespectively of their molar mass. If one accepts the existence of two simultaneous and independent enthalpic retention mechanisms in the same chromatographic system, two critical points may exist. One of them is a manifestation of the compensation between exclusion and surface adsorption, and another one is due to the compensation between exclusion and enthalpic partition. Correspondingly, we can speak about the *critical adsorption point* and about the *critical enthalpic partition point*.

The results of the experimental study in this direction are described in the present paper.

Experimental Part

The HPLC apparatus consisted of a pump, model 510 (Waters, Milford, MA), operated at 1 mL·min^{-1}, of a manual sample injection valve, model 7725 (Rheodyne, Cotati, CA), provided with a 50 μL sample loop, and of an evaporative light scattering detector, DDL-21 (Eurosep, Cergy-Saint-Pontoise, France). Polymer samples were dissolved and injected in the given eluent at a concentration of 1 mg·mL^{-1}. The column temperature was kept at 35 ± 0.1 °C using a custom-made column oven with a duplex wall connected to a water thermostat. The pump was operated at ambient temperature, but both the eluent and sample solutions were prethermostated. The actual flow rate was controlled by a buret. The data were processed with the help of the software Chroma (Chromtech, Graz, Austria).

The retention behavior of two different silica C-18 phases was evaluated, namely, a poorly-end-capped silica C-18 phase, Separon SGX C-18 (18.20% carbon), Tessek, Prague, Czech Republic, and a custom-synthesized non-end-capped Kromasil C-18 (starting material from EKA Chemicals, Bohus, Sweden) with a low degree of coverage (10.66% carbon) (University of Torun, Poland).[16] The column sizes were 250 × 3.2 and 150 × 5 mm, respectively. For comparison, retention of selected polymer species was evaluated using the bare Kromasil 100 Å (column 300 × 7.8 mm) and the end-capped Kromasil C-18 (column 300 × 7.5 mm) as well as bare Separon SGX (column size 250 × 4 mm) in various eluents. All Kromasil sorbents had identical starting silica gel matrixes, and the same applied for both Separon SGX materials. Most Kromasil sorbents were slurry packed in this laboratory, the non-end-capped Kromasil C-18 was packed in Torun, and the Separon SGX and SGX C-18 columns were commercial products.

Analytical grade solvents were used as eluents, or eluent components, viz., acetonitrile from Merck, Darmstadt, Germany, tetrahydrofuran from Polskie Odczynniki Chemiczne S.A., Gliwice, Poland, and toluene from Centralchem, Bratislava. Solvents were vacuum distilled before use. Mixed eluents were prepared by weighing, while the control of eluent composition was better than ±0.1 wt %. Anionic poly(methyl methacrylate)s were gifts from Dr. W. Wunderlich, Röhm, Darmstadt, Germany, and from Dr. J. Herz of the Institut Sadron, Strasbourg, France (M ranged from 1.3 to 613 kg·mol^{-1}). The polymers exhibited a low degree of stereoregularity and narrow to medium molar mass distributions.[17] Anionic polystyrene standards were purchased from Pressure Co., Pittsburgh, PA. Their molar masses ranged from 0.666 to 498 kg·mol^{-1}. For all polymers used, the peak retention volumes could be well identified. After each set of experiments the retained macromolecules were removed from the columns by an overnight action of the efficient displacer tetrahydrofuran. The columns were reequilibrated with 20 volumes of the fresh eluent before the next series of measurements.

Identification of the Chromatographic System

On the basis of the results presented in previous papers[1-4] and on a series of preliminary experiments, the following components of the chromatographic system were chosen for detailed tests: (i) Non-end-capped and poorly-end-capped silica gel C-18 column packings, in which both enthalpic partition and adsorption could be achieved. (ii) Medium polar polymer probes, poly(methyl methacrylate)s (PMMAs), which were expected to be susceptible both to adsorption on free silanols and to enthalpic partition on the solvated C-18 groups. (iii) Acetonitrile (ACN), a polar, strong solvent, which should suppress adsorption of PMMA on silanols and act as a *desorli*. At the same time, ACN is a very poor Θ solvent for PMMA with $a = 0.5$ (eq 1) between 30 and 45 °C[9] so that it should promote enthalpic partition of macromolecules in favor of the C-18 solvated bonded phase. The difference in experimental Θ temperatures for PMMA in ACN published by various authors is remarkable. (iv) Toluene, a weak, low-polarity solvent, which promoted adsorption of PMMA on silanols[2] and acted as an *adsorli*. Toluene is a thermodynamically good solvent for PMMA (the a value lies between 0.680 and 0.720[9,18]) so that it should suppress enthalpic partition of PMMA in favor of the bonded solvated C-18 phase.

The hydrodynamic volume of macromolecules in solution, V_h, is the size parameter governing the HPLC retention of many polymer species, which is controlled solely by entropic partition (exclusion). V_h is defined by the simple relation

$$V_h = M[\eta] \tag{2}$$

and it forms the basis of the famous Benoit universal calibration concept, which is widely used in size exclusion chromatography.[19] Macromolecules possessing the same hydrodynamic volume elute from the same column in the same retention volume provided enthalpic retention mechanisms are not operative. This means that the dependences of log V_h vs V_R coincide. On the contrary, mutual shifts of the above dependences signal the presence of adsorption or enthalpic partition of polymer species and allow the enthalpic retentivity of the HPLC columns to be qualitatively evaluated.[2-4]

The dependences of log V_h vs V_R for PMMAs in tetrahydrofuran (THF) and ACN for bare silica gel Kromasil are plotted in Figure 1. The K and a values necessary for calculation of limiting viscosity numbers were taken from the literature[9,18] (Table 1).

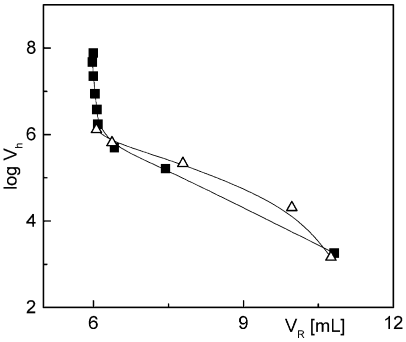

Figure 1. Dependences of log V_h vs V_R for a column packed with bare Kromasil, 100 Å, 10 μm, 300 × 7.8 mm. PMMA samples in THF (■) and ACN (△) eluents.

Table 1. Selected K and a Values from the Viscosity Law (Eq 1) for Polymers Used in This Study

polymer	solvent	temp (°C)	K (10^2 mL·g^{-1})	a	ref
PMMA	toluene	25	1.910	0.680	19
PMMA	toluene	30	0.700	0.710	9
PMMA[a]	toluene	39	0.724	0.720	9
PMMA	THF	25	0.750	0.720	9
PMMA[a]	THF	35	1.220	0.690	9
PMMA[a]	ACN	30	0.393	0.500	19
PMMA	ACN	45	0.480	0.500	9
PS	toluene	30	1.570	0.695	19
PS[a]	toluene	35	1.260	0.710	9
PS[a]	THF	25	1.100	0.725	9
PS	THF	25	1.400	0.700	9
PS[a]	THF	35	1.258	0.715	19
PS[a]	DMF	35	3.180	0.603	9
PS[b]	ACN				

[a] Values used for calculation of V_h. [b] Nonsolvent in the available temperature range.

Compared to that obtained with THF, the curve obtained with ACN eluent is slightly shifted to higher retention volumes. This would indicate that the medium strong ACN ($\epsilon^0 = 0.5$)[7] less efficiently prevents adsorption of PMMA on silica gel than the somewhat weaker THF (($\epsilon^0 = 0.35$).[7] This apparent discrepancy may be caused by large differences in the published K and a values for PMMA/ACN solutions and also by limited validity of the viscosity law (eq 1) below molar mass 10 kg·mol^{-1}. In any case ACN can be considered a (medium efficient) desorli for PMMA with silica gel.

Poly(methyl methacrylate)s injected into bare silica gel in toluene were fully retained within the column, irrespective of their molar mass (see also refs 2 and 3). Therefore, toluene is considered an efficient adsorli for PMMA on the nonbonded silica gels. PMMA was eluted in toluene from several commercial C-18 bonded silica gels; however, the polymer species were slightly retained (increased retention volumes) or even fully retained (“infinitive” retention volumes) within some common C-18 materials.[2,3] It was concluded that these column packings were non-end-capped or poorly-end-capped. For example, higher PMMAs were fully retained within both Separon SGX C-18 and Zorbax ODS columns. The latter non-end-capped material contained a diisopropyl octadecyl bonded phase. Evidently, isopropyl groups did not effectively shield free silanols. Still, the adsorption activity was reduced for PMMA on the majority of silica gels bonded with the C-18 groups when

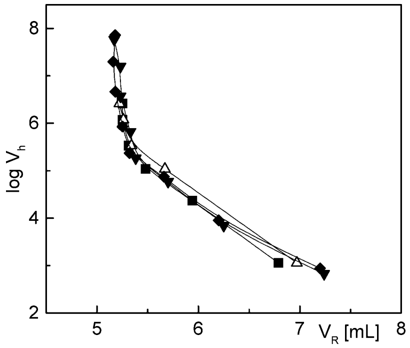

Figure 2. Dependences of log V_h vs V_R for a column packed with Kromasil C-18, 100 Å, 300 × 7.5 mm. PS samples in toluene (▼) and THF (◆). PMMA samples in toluene (△) and THF (■).

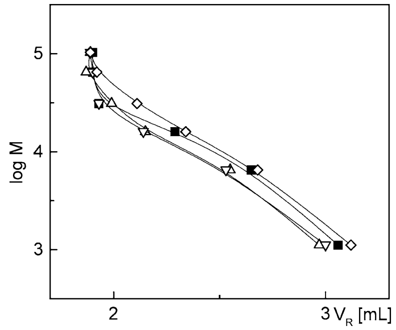

Figure 3. log M vs V_R for a column packed with bare silica gel Separon SGX, 10 μm, 250 × 4 mm. PMMA samples in ACN/toluene mixed eluents at compositions 20/80 (no elution), 50/50 (◇), 70/30 (▽), 80/20 (△), and 90/10 (■).

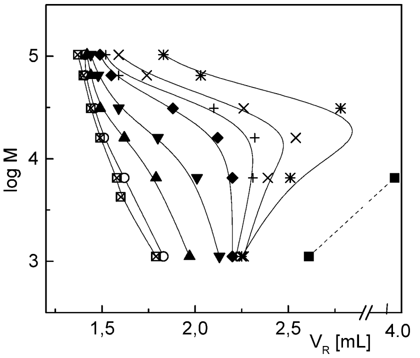

Figure 4. log M vs V_R for a column packed with Separon SGX C-18, 5 μm, 250 × 4 mm. PMMA samples in ACN (■) and ACN/toluene mixed eluents at compositions 20/80 (boxed times sign), 30/70 (○), 70/30 (▲), 80/20 (▼), 85/15 (◆), 87/13 (+), 88/12 (×), and 90/10 (∗).

compared with bare silica gels. This applied also for the non-end-capped silica gel C-18 packings. The most active silanols may preferentially react with chloro- or ethoxysilanes during silanization reaction, while the less active silanols remain unreacted. This is why a non-end-capped bonded silica gel was also included in our present study together with the poorly-end-capped Separon SGX C-18.

It is widely accepted that polystyrene is not adsorbed on bare silica gel from THF. The dependences of log V_h vs V_R for PS probes in THF and toluene ($\epsilon = 0.22$)[7] obtained with bare silica gel coincided well (results not shown). It can be concluded that polystyrene does not adsorb on free silanols from toluene. Therefore, also adsorption of PS from toluene on the free silanols present in the silica C-18 column packing is highly improbable.

The dependences of log V_h vs V_R for the PS and PMMA probes in toluene and THF are depicted in Figure 2 for the rather well end-capped reversed phase Kromasil C-18. Both adsorption and enthalpic partition of PMMA on silanols and within the C-18 phase, respectively, would increase polymer retention volumes. The extent of adsorption of PMMA on bare silica gel from THF is low.[20] Therefore, the similar courses of log V_h vs V_R curves for PMMA and PS in THF and toluene in Figure 2 can be interpreted—at least in the first approximation—as a proof of low or negligible enthalpic partition of PMMA in favor of the C-18 phase from both eluents. Therefore, toluene seems to be a good choice for an eluent component, which would fairly suppress enthalpic partition of PMMA in favor of the solvated C-18 phase.

PMMA was extensively retained within the C-18 column packings in ACN, and only lowest molar mass probes were eluted from the C-18 phases tested (Figures 4 and 6 below). Because ACN is a desorli for PMMA from bare silica gel (Figure 1), this result indicates large enthalpic partition of PMMA in favor of the solvated C-18 phase. Evidently, even medium-polarity macromolecules can be strongly retained within the C-18/ACN phase. In the case of PMMA, the C-18/ACN "mixed" phase may exhibit a certain cosolvency effect and become a better "solvent" for polymer species than a very poor single solvent, acetonitrile.

Results and Discussion

K and *a* values are unknown for PMMA in the mixed eluents ACN/toluene. Therefore, the dependences of log M vs V_R are further considered instead of those of log V_h vs V_R. The former dependences do not allow quantitative evaluations, which, however, were not aimed at in this stage of research. On the other hand, the dependences of log M vs V_R (further only "the plots") illustrate well the retention *tendencies*, which are sufficient for our present discussion.

The plots for bare silica gel Separon SGX, PMMA probes, and the mixed eluents ACN/toluene are depicted in Figure 3. The mutual shifts of the plots were caused by simultaneous changes of the eluent in both strength and quality. The desorption ability of ACN for PMMA was again clearly manifested. While PMMA was fully retained in the eluent containing 20 wt % ACN, 70 wt % ACN seemed to entirely suppress adsorption. Simultaneously, eluent quality deteriorated with increasing amount of ACN in the eluent. As a result, the sizes of the macromolecules decreased and their retention volumes slightly increased.

The plots for the system Separon SGX C-18/PMMA/ACN/toluene are shown in Figure 4. Let us start our discussion from the pure ACN eluent. As mentioned, enthalpic partition of PMMA in favor of the C-18 phase was strong and prevented elution of molar masses higher than 6 kg·mol^{-1}. An increased amount of a good solvent for PMMA in eluent, toluene, suppressed enthalpic partition. At about 15 wt % toluene the critical partition range was reached for macromolecules with molar mass up to about 10 kg·mol^{-1}, which were not large enough to be fully excluded from the packing pores under the "ideal" SEC conditions. Here the enthalpic and entropic partition nearly compensated. Above the SEC exclusion limit, the plot suddenly changed its course. It assumed an SEC-like shape, and retention

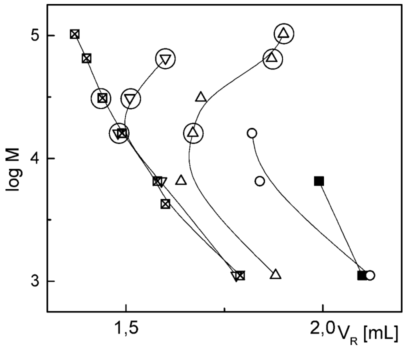

Figure 5. log M vs V_R for Separon SGX C-18 (see Figure 5). PMMA samples in toluene (■) and ACN/toluene mixed eluents at compositions 1/99 (○), 2.5/97.5 (△), 10/ 90 (▽), and 20/80 (boxed times sign). Data points for peaks with areas reduced more than 25% due to limited sample recovery are provided with additional circles.

volumes rapidly decreased with increasing polymer molar masses. This phenomenon was observed in several systems comprising interactive polymers, active column packings, and eluents promoting enthalpic interactions. It could be utilized for high-selectivity SEC-like separations.[21] The resulting methods were designated *"enthalpic partition* (or *adsorption) assisted SEC"*. The surface adsorption of PMMA on free silanols likely did not yet play an important role in the plots depicted in Figure 4 because addition of toluene to the eluent caused a decrease and not an increase of the retention volumes. Still, the surface adsorption of polymer species could not be completely ruled out despite the presence of ACN in the eluent, especially for high molar masses of PMMA. The lowest retention volumes were observed at the eluent composition 20/80 w/w ACN/toluene. At an even higher content of toluene, adsorption on the free silanols likely started to be operative and retention volumes increased, especially for higher molar masses of PMMA (Figure 5). The plots showed the typical back-turn shape, which was observed for silica C-18 phases with poly(2-vinylpyridine) and poly(ethylene oxide) probes in THF.[3]

Sample recovery decreased for higher molar mass samples, and the peak areas decreased for interacting systems. The data points in Figure 5 (and similarly in Figures 6 and 7) for systems where the peak areas were reduced by more than 25% are denoted with an additional circle. Retention volumes were highly sensitive to ACN content, and already 1% ACN in the eluent affected the plot rather pronouncedly. The conventional critical range was not reached because adsorption of PMMA from pure toluene was insufficient for smaller macromolecules.

The retention of PMMA was similar with the non-end-capped column in the area of high ACN concentration in the eluent (Figure 6). Surprisingly, the extent of enthalpic partition of PMMA in the ACN-rich eluents did not extensively depend on the degree of coverage of silica gel by the C-18 groups. The critical partition range for lower molar mass samples was attained in the eluent containing 15 wt % toluene, and the enthalpic partition assisted SEC behavior was confirmed. The "regular" SEC plot was observed with eluent containing 80 wt % toluene.

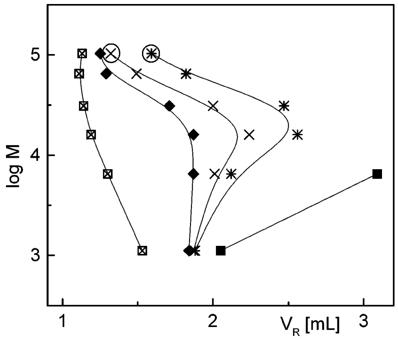

Figure 6. log M vs V_R for a column packed with low-density coverage non-end-capped C-18 silica gel, 150 × 5 mm. PMMA samples in ACN (■) and mixed eluents ACN/toluene at compositions 20/80 (boxed times sign), 85/15 (◆), 88/12 (×), and 90/10 (∗). The data points for peaks with areas reduced more than 25% due to limited sample recovery are provided with additional circles.

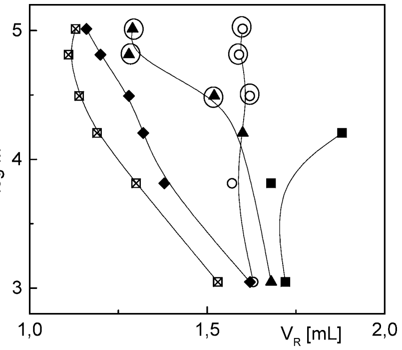

Figure 7. log M vs V_R for a column as in Figure 6. PMMA samples in toluene (no elution) and mixed eluents ACN/toluene at compositions 4/96 (no elution), 9/91 (■), 9.7/90.3 (○), 10/90 (▲), 11/89 (◆), and 20/80 (boxed times sign). Data points for peaks with areas reduced more than 25% due to limited sample recovery are provided with additional circles.

The adsorption of PMMA on the non-end-capped silica gel was much more pronounced than on the poorly-end-capped C-18 material (Figure 7). There was no elution of even the lowest molar mass PMMA probes in pure toluene and in the eluent containing 4 wt % ACN. The critical adsorption range was achieved at 9.7 wt % ACN in the surprisingly wide molar mass area; however, the sizes of the polymer peaks were strongly reduced for high molar mass samples.

In this way, two independent critical ranges were identified on the non-end-capped silica gel C-18 column packing. This result confirms the previous conclusion[1,3] that enthalpic partition of macromolecules in favor of the C-18 phase on the one hand and their silanophilic surface adsorption on the other hand can be independently controlled in appropriate polymer HPLC systems. The inherent difference and mutual independence of these two enthalpic retention mechanisms immediately evoke some practical conclusions.

(a) Both enthalpic retention mechanisms, namely, silanophilic surface adsorption and enthalpic partition, can be simultaneously operative in many real polymer HPLC systems. All so far commercially available silica gel bonded phases contain large amounts of unreacted silanols, which may exhibit measurable adsorption of macromolecules. Adsorption-active sites are present also on the surface of many column packings synthesized from rather nonpolar organic monomers such as polystyrene/divinylbenzene gels.[22,23] On the other hand, enthalpic partition can play a certain role also on bare surfaces, such as nonbonded silica gels in mixed eluents.[24] Due to preferential solvation of the packing surface with one of the eluent components, the composition of liquid differs in the free mobile phase and in the vicinity of the packing surface. Macromolecules can be retained due to their enthalpic partition in favor of such a quasi-stationary phase. Still, the volume of the quasi-stationary phase produced by preferential solvation is very small, and the resulting changes in retention volumes may be hardly measurable.

The noncontrolled simultaneous presence of two concurrent retention mechanisms, which exhibit unlike effects of polymer molar mass, could be responsible for the unexpected behavior of some systems in the area of critical conditions: the dependences of log M vs V_R sometimes lose their vertical courses.

(b) A common critical point or at least a critical range can exist for two polymer chains of different chemical nature or physical architecture—one due to surface adsorption/exclusion compensation and another one as a result of enthalpic partition/exclusion compensation. These “binary critical conditions” could be applied in the molecular characterization of block copolymers composed of three chemically different blocks or in the separation of ternary blends of polymers including blends of species differing in their stereoregularity. Identification of a binary critical range or even a binary critical point may, however, represent a formidable task.

(c) Enthalpic partition and surface adsorption retention mechanisms can be independently controlled by temperature.

(d) The existence of two different enthalpic retention mechanisms is important for selection of appropriate chromatographic systems needed to solve particular separation problems. Surface adsorption and enthalpic partition acting concurrently may either deteriorate or improve the selectivity of separation in polymer HPLC. Let us consider a chromatographic system which is aimed at separation of a copolymer according to its chemical composition. Let the copolymer contain polar (P) and nonpolar (N) segments or chains. The chromatographic system should discriminate macromolecules according to the P/N concentration. The column packing and eluent combination should be chosen, which promotes retention of one component and supports elution of another one. If both kinds of segments or chains are simultaneously retained, for example, P by surface adsorption and N by enthalpic partition, the overall separation selectivity may be deteriorated or even completely lost. The concurrent action of surface adsorption and enthalpic partition explains numerous so far not well understood experimental observations in polymer HPLC. For example, Glöckner and van den Berg[25] separated statistical copolymers of styrene and methyl methacrylate [P(S-*stat*-MMA)] according to their composition using the silica gel C-8 bonded phase (Nucleosil C-8). Evidently, this column packing was based on the A-type silica gel, and its free silanols were highly active. There is no information available about the end-capping of the column packing used, but it is very probable that the concentration of free silanols accessible for macromolecules was rather high. The authors applied two kinds of mixed eluents in the eluent gradient mode, namely, THF with isooctane (ISO) and THF with methanol (MET). Both ISO and MET are nonsolvents for styrene and MMA segments. ISO is a weak liquid ($\epsilon^0 = 0.01$),[7] a typical adsorli, while methanol is a strong liquid ($\epsilon^0 = 0.73$)[7] acting as an efficient desorli for many polar species. As can be seen from Table 1, THF is a relatively good solvent for both MMA and styrene segments. Glöckner and van den Berg started from eluents containing a high concentration of a nonsolvent, MET or ISO, and gradually increased amounts of solvent (THF). It is anticipated that the leading retention mechanism was enthalpic partition of styrene segments in the case of THF/MET eluent while surface adsorption of the MMA segments should be low. Due to their polarity, enthalpic partition of MMA segments in favor of the C-8 phase should be lower than that of styrene segments. Moreover, the mixed solvent THF/MET would be generally better for the MMA segments than for the styrene segments. Consequently, enthalpic partition of styrene segments should be more intensive. In fact, copolymers with increasing concentration of styrene segments eluted at higher retention volumes. This means that a higher concentration of the good solvent THF was needed to allow elution of copolymers high in styrene. The resulting separation selectivity was high. On the contrary, both enthalpic retention mechanisms were likely operative in the low-polarity THF/ISO eluent, namely, the surface silanophilic adsorption for the MMA segments and the enthalpic partition for the styrene segments. In this case, the overall selectivity of separation deteriorated because only the *difference* of retention between MMA and styrene segments was responsible for the copolymer discrimination.

Similar results were obtained by Teramachi et al.[26] with P(S-*stat*-MMA) copolymers. The eluent THF/cyclohexane did not produce satisfactory fractionation according to composition using the silica C-18 phase, while the same eluent gave good results with the $-NH_2$ and $-CN$ bonded phases.

The results of Sato et al.[27] can also be explained considering a cooperative action of surface adsorption and enthalpic partition on the retention of some complex polymers. Sato separated various copolymers of styrene using gradient elution in mixtures of dichloromethane (DCM) with either *n*-hexane or ACN. As for P(S-*stat*-MMA) copolymers, DCM is a solvent for both kinds of segments while *n*-hexane and ACN are the nonsolvents for styrene segments. *n*-Hexane is a nonsolvent and ACN is a poor solvent for MMA segments. Evidently, *n*-hexane is a more efficient nonsolvent for MMA segments, and ACN preferentially precipitates styrene segments. Column packings were polar, acrylonitrile/ethylene dimethacrylate (PAN), and nonpolar, styrene/divinylbenzene (PS), heterogeneously cross-linked materials. It is supposed that short free chains protrude over the pore walls of gels based on the heterogeneously cross-linked organic polymers and form a “quasi-liquid phase” bonded to both the inner and the outer surfaces of the column packing.[23] Such a quasi-liquid phase is suitable for the enthalpic partition of macromolecules. As mentioned, many PS gels including commercial SEC column packings exhibited pronounced polar interactivity.[22,23] The latter may be caused by polar components of polymerization systems, especially by protective colloids, built into the gel matrix. In Sato’s systems, copolymers with the highest content of styrene units eluted first in the DCM/*n*-hexane eluent while the elution order was reversed in the DCM/ACN eluent. Similar to Glöckner and van den Berg, also Sato et al. evaluated correlations between the compositions of the solvent (eluent) mixtures at the cloud point (solubility threshold) and at the peak retention volume for P(S-*stat*-MMA) copolymers of different compositions. These correlations were very close for the PS column in the DCM/*n*-hexane eluent and for the PAN column in the DCM/ACN eluent. This may suggest that almost the cloud point concentration of the nonsolvent in the eluent was needed in both latter cases to attain sufficient retention due to enthalpic partition—or that retention was due to a phase separation based mechanism. In any case, the enthalpic partition did not efficiently retain copolymers in the above systems. *n*-Hexane promoted adsorption and ACN suppressed adsorption of polar segments on the polar sites of the column packing. The overall separation selectivity was low in both cases. The decisive retention mechanism was probably (a weak) adsorption of MMA segments in the PS/DCM/*n*-hexane system and (a weak) enthalpic partition of the MMA segments in the PAN/DCM/ACN system. On the contrary, cloud point curves did not coincide well with the chromatographic retention volumes for the PS column with DCM/ACN eluent and for the PAN column with DCM/*n*-hexane eluent. The retention appeared at much lower nonsolvent concentration. This indicates that the enthalpic partition could be much more efficient than adsorption in the latter cases, and this was the reason why selectivity of separation was high. Adsorption likely supported the retention of MMA segments on PAN gel in DCM/*n*-hexane eluent.

Very recently, Trathnigg et al.[28] observed two critical points for lower poly(ethylene glycol)s with the silica gel C-6 and C-18 bonded phases and with the mixed eluents containing water and acetone. They explained the existence of two critical points considering interactions with the surface silanols of either ethoxamer groups (at a low concentration of acetone) or −OH groups (in eluents high in acetone). The finding of Trathnigg rather well supports the hypothesis about simultaneous existence of two independent retention mechanisms, namely, surface adsorption of terminal −OH groups of oligomers on the free silanols in eluents containing an excess of acetone and enthalpic partition of central ethoxamer units in favor of an aliphatic bonded phase in the eluents high in water. Acetone is a medium-strength solvent regarding polar surfaces, with the ϵ^0 parameter equaling 0.43.[7] Surprisingly, water may be a rather poor solvent for the low molar mass poly(ethylene oxide)s up to 1 kg·mol^{-1} with an *a* value of 0.5[9] and high strength ($\epsilon^0 > 0.73$[7]), though the *a* value for such a low molar mass must be considered with caution. Trathnigg et al. did not identify two critical points in mixed eluents composed of water and methanol. In fact, methanol is stronger than acetone so that it more efficiently suppressed adsorption of −OH groups, and the broad critical range observed by Trathnigg et al. may be due to enthalpic partition of oligomer species.

In the oligomer area, the relative importance of the end groups is expected to increase with decreasing molar mass. This should be reflected in the increasing adsorptivity and, especially, in the decreasing solubility of oligomeric species in the less polar eluents when the molar mass drops. Therefore, poly(ethylene glycol)s with molar masses below a few kg·mol^{-1} cannot be considered homogeneous species. The situation is different with poly(methyl methacrylate)s. Macromolecules of PMMA contain one nonsymmetric end group, which is usually a nonpolar rest of the anionic polymerization initiator. It was the *tert*-butyl group in the case of our samples.[17] The nonpolar end groups likely influence enthalpic partition of samples, but this effect should be very low for molar masses above a few kg·mol^{-1}. Therefore, high molar mass samples of PMMA can be in a good approximation considered chemically homogeneous.

Interphase adsorption between the mobile phase and the C-18 phase can augment the effect of enthalpic partition. Kazakevich et al. suggested[29] that the C-18 phase would be collapsed even in organic solvents. In that case, interphase adsorption would replace enthalpic partition. However, the volume of the C-18 phase estimated from the elution behavior of macromolecules in tetrahydrofuran roughly corresponds with the brush-like topology of alkyl groups.[4] Moreover, it is difficult to explain how polar macromolecules permeate thru a rather dense array of the collapsed C-18 groups to be adsorbed on the free surface silanols of silica gel.[1−3] The volume of the poly(ethylene glycol) phase bonded to silica gel is much smaller than the volume of the C-18 phase.[10] The increase of polymer retention on the PEG phase in poor solvents compared to good ones is hardly measurable. This applies also to the PMMA/ACN system and suggests a less important role of interfacial adsorption. To answer this question, however, further experiments are needed which will include bonded groups of different lengths and column packings of different pore sizes.

Conclusions

Further indirect proof is furnished of the difference between surface adsorption and enthalpic partition based retention mechanisms in high-performance liquid chromatography of macromolecules (polymer HPLC). PMMAs, which were eluted from the non-end-capped silica gel C-18 column packings, exhibited two different critical ranges in the mixed eluents composed of acetonitrile (ACN) and toluene at different compositions. ACN was a very poor solvent for PMMA, promoting enthalpic partition of macromolecules in favor of the solvated C-18 phase. At the same time, ACN was a strong, polar solvent considering silica gel surface and active silanol groups. Therefore, ACN suppressed adsorption of PMMA on the silica gel column packings. Toluene was a thermodynamically good solvent which suppressed enthalpic partition of PMMA in favor of the solvated C-18 phase. Low-polarity molecules of toluene did not intensively interact with free silanols present on the silica gel surface. Consequently, toluene promoted adsorption of PMMA on the silica-based column packing. One critical range for PMMA on the non-end-capped and poorly-end-capped silica C-18 packings appeared at an increased concentration of ACN in the eluent, where the surface adsorption of macromolecules was negligible. These were conditions under which the enthalpic partition and size exclusion of macromolecules nearly compensated. Another critical range for PMMA was observed for the non-end-capped low-coverage C-18 column packing at the same temperature, applying a high concentration of toluene in the eluent, at which enthalpic partition was small and the leading retention mechanism was silanophilic adsorption. This was the critical range at which exclusion of macromolecules was nearly compensated by their adsorption on the surface silanols.

Surface adsorption and enthalpic partition based retention mechanisms can act concurrently in the course of polymer HPLC, either enhancing or suppressing selectivity of separation. Therefore, it is useful to separately consider possible effects of both these retention mechanisms when identifying experimental conditions for separation of a complex polymer according to their chemical structure or chemical architecture.

The existence of two similar but still different retention mechanisms in polymer HPLC does the following: (i) Helps to explain unexpected unevenness in some log M vs V_R dependences in the vicinity of critical conditions. As a result, the true *critical point* often cannot be identified, and the system hardly exhibits even a *critical range*. (ii) Suggests possible existence of one common critical range for two different kinds of polymer chains in the same column and eluent and at the same temperature. This would allow liquid chromatographic characterization of some complex ternary polymer systems applying the critical polymer HPLC. (iii) Elucidates so far not well understood behavior of some eluent gradient HPLC systems such as enhanced or decreased selectivity of copolymer separation. (iv) Simplifies selection of column packings and eluents for solving particular separation problems in polymer HPLC.

Acknowledgment. This work was supported by the Slovak Grant Agency Vega, Project 2-301 123. I thank Drs. W. Wunderlich and J. Herz for samples of poly(methyl methacrylate)s, Prof. B. Buszewski for the non-end-capped silica C-18 phase, Eka Chemicals (Akzo Nobel) for the Kromasil materials, and Mrs. J. Tarbajovska for her excellent technical assistance.

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MA0400564