Enthalpic Partition-Assisted Size Exclusion Chromatography: 1. Principle of Method

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Summary: A novel high performance liquid chromatographic (HPLC) method viz. "enthalpic partition assisted size exclusion chromatography" deliberately combines entropic and enthalpic partition mechanisms. It enables separation of homopolymers according to their molar mass with increased selectivity, as well as discrimination of polymer species differing in their nature/composition. Enthalpic partition of macromolecules takes place between the mobile phase and the stationary "liquid" of a different chemical nature, which is immobilized within pores of an appropriate carrier (a bonded phase). The extent of enthalpic partition depends on the accessibility of bonded phase for macromolecules and on the difference of polymer solubility in the mobile phase and in the solvated bonded phase. The enthalpic partition in favor of column packing arises from better solubility of polymer solutes in the solvated stationary phase compared to the mobile phase. Macromolecules are "pushed" into the solvated stationary phase and their retention volumes (V_R) increase. In the area of high molar masses, the extent of enthalpic partition as rule raises with the increasing size of macromolecules. However, under properly chosen experimental conditions the enthalpic partition may rapidly diminish with the sample molar mass (M), likely due to the solubility changes and/or due to partial exclusion of macromolecules from the pores. As result, the corresponding retention volumes sharply drop within a narrow range of M with the increasing size of macromolecules. This results in the log M vs. V_R dependences, which resemble in their form that for size exclusion chromatography but are much more flat indicating highly selective separations of homopolymers according to their molar masses. In this way, enthalpic partition "assists" entropic partition (size exclusion). Polymer species, which do not undergo enthalpic partition, elute from the HPLC column in the conventional size exclusion mode and can be discriminated from the partitioning species. Enthalpic partition assisted size exclusion chromatography can be utilized in separation and characterization of various homopolymers, and polymer blends.

Keywords: enthalpic partition; liquid chromatography of polymers; size exclusion chromatography

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Introduction

Size exclusion chromatography (SEC) is presently the most popular method for molecular characterization of synthetic polymers. It furnishes important information on molar mass averages and distributions, as well as on long chain branching of many polymeric species. SEC is rather fast, well repeatable and relatively both simple and cheap. Recent developments in the area of SEC involve ultra-fast separations. These are aimed at increased sample throughput, which is needed for example in the combinatorial polymer research and in the on-line polymer production control.

SEC exhibits several shortages challenging to its further investigation and improvements. One of the important problems of SEC is a rather low selectivity of the separation process. This limits practical resolution of SEC and restricts its applicability in both the ultra-fast separations and assessment of minute heterogeneities present in the polymer samples.

Generally, the SEC analyses can be divided into "scouting" and "high resolution" ones. In the former case, low selectivity (universal) "linear" columns are applied which cover broad molar mass area. To increase the SEC resolution, however, highly selective columns are needed, which work only in a narrow molar mass region.

The SEC separation takes place mainly in the pores of the column packing. Due to considerations about mechanical stability of packing matrix, pore volume available for the separation process only exceptionally exceeds 80 % of the volume of packing particles. In practical systems, the interparticle volume assumes about 40% of the packing bed and, consequently, the effective pore volume reaches only about 50% of the total volume of column. Selectivity of SEC separation can be enhanced by extending the column length and by narrowing the packing pore size distribution. Former approach brings about increased both eluent consumption and time of analysis. Moreover, the raised inlet pressure of longer columns decreases the column and pump seals lifetime and thus deteriorates precision and accuracy of measurements. Due to their experimental restrictions, recycling procedures did not find wider application in the analytical SEC of high polymers. Improvements in the SEC selectivity by application of the column packings with narrowed pore size distribution has its theoretical and practical limits, as well. Even the packings with uniform pore sizes would not considerably raise selectivity of SEC separations. [1]

Therefore it is necessary to manipulate retention mechanisms responsible for separation of macromolecules in order to substantially improve resolution of the high performance liquid chromatography of polymers.

Basic Terms

Retention mechanisms operative in the polymer HPLC can be divided into entropic (exclusion) and enthalpic ones. This classification is highly practical though it says only little about molecular backgrounds of processes taking place within the HPLC column. The **exclusion retention mechanism** includes changes in mixing, conformation [2-5] and possibly also orientation [6] entropy of macromolecules during their passage along the HPLC column. These entropy change result from the concentration gradients, as well as from the flow, and diffusion processes within column and lead to partial or full exclusion of macromolecules from the pores or from the outer surface of packing particles. This **entropic partition** of macromolecules is considered basic retention mechanism of conventional SEC. The **enthalpic retention mechanisms** [7] are connected with the attractive or repulsive energetic interactions among column packing, macromolecules, and eluent molecules. These take place in the mobile phase, and on the packing surface or interface, as well as within the (quasi) liquid stationary phase physically immobilized on or chemically bonded to the packing surface. It is useful to consider a set of binary enthalpic interactions in the system,[7] namely between macromolecules and eluent **(thermodynamic quality of eluent),** between macromolecules and packing **(affinity of analytes toward packing),** and between eluent and packing **(eluent strength).** The thermodynamic quality of a solvent determines solubility of macromolecules and in the first approximation it can be characterized by the exponent in the Kuhn-Mark-Houwink-Sakurada viscosity law, a. The effective segmental interaction energy, ε, describes affinity between macromolecules and column packing. The concept of eluent strength introduced by Snyder is based on the considerations about interaction energy between molecules of solvent and solid surfaces. Interaction of eluent molecules with the bonded stationary phase leads to solvation phenomena. In the case of mixed mobile phases the preferential solvation effect should be considered. Above interactions are responsible for the retention mechanisms based on **phase separation, adsorption** and **enthalpic partition** of macromolecules. In the case of charged macromolecules, we encounter also **ion effects,** while **bio-affinity** is to be considered for many biopolymers. Recent developments of polymer HPLC include controlled combinations, **coupling,** of two and more retention mechanisms within the same chromatographic column.

At present, the most often used coupled procedures of polymer HPLC suppress separation of macromolecules according to their molar masses under isocratic and isothermal conditions. The well-known polymer HPLC working under "critical conditions" [8-10] utilizes mutual compensation of entropic (exclusion) and enthalpic contributions to the Gibbs function (ΔG).

In the situation where the resulting $\Delta G = 0$, macromolecules elute from the HPLC column irrespective of their molar mass and the differences in their chemical structure or physical architecture can be assessed [8-12]. The idea of **"critical polymer HPLC"** is very attractive, however, its practical utilization in the area of high molar masses over 100 $kg.mol^{-1}$ is hampered by several recently reviewed experimental problems. [13]

Further coupled procedures of polymer HPLC apply elution of samples in a gradient. One can gradually change either overall eluent composition or temperature. In the former case, polymer species can be separated according to their chemical structure or physical architecture independently of their molar mass. [14-16] In the latter case, highly selective separation of macromolecules according to their molar mass can be achieved [17] and polymer species can also be discriminated according to their nature. [18] Though both eluent and temperature gradient polymer HPLC methods produce interesting results, technical problems, for example those connected with the gradient control and with the quantitative interpretation of results so far complicate their wider application. Therefore, new isocratic methods are still looked for, which could lead to efficient enhancements of polymer separation selectivity according to one of the molecular characteristics of polymers, especially according to their molar masses.

New approach presented in this paper isocratically combines two retention mechanisms. One of them is the conventional entropic partition (size exclusion) of macromolecules, between two liquid phases of the same composition situated in the interstitial volume and in the pore volume. Another retention mechanism is the enthalpic partition of macromolecules between two chemically different phases, namely between the mobile phase (eluent) and the *volume* of stationary (bonded) phase within column packing. The enthalpic partition of polymer species is accompanied by large entropic effects because macromolecules change their conformation as result of energetic interactions. So far, little is known about the latter processes and they will be neglected in this present qualitative discussion.

The enthalpic partition is directly connected with the solubility differences of polymer species in the stationary and in the mobile phases [7]. If macromolecules are (much) better soluble in the mobile phase than in the stationary phase, enthalpic partition mechanism is hardly operative. Macromolecules do not tend entering bonded stationary phase, even though they are small enough to permeate the packing pores. The stationary (bonded) phase, occupies a relatively large fraction of pore volume and therefore the SEC separation selectivity of such systems is reduced. If the sample is similarly soluble in the mobile phase and in the solvated stationary phase, difference is small between retention volumes obtained with the bare and

bonded but otherwise identical column packing because the contribution of enthalpic partition is unimportant. If, however, solubility of macromolecules is (much) higher in the solvated stationary phase than in the mobile phase, macromolecules are "pushed" into and retained by the stationary phase. Consequently, their retention volumes rise. Sample may be even fully retained within stationary phase [19] including that situated on the surface of nonporous particles. [20]

As rule, solubility of macromolecules decreases with their increasing molar mass provided the end-group effect is negligible. The actual contribution of enthalpic partition to variation of HPLC retention volumes with polymer molar masses depends on particular system that is on the different effect of polymer size on the solubility reduction in the mobile phase compared to the stationary phase. If solubility of polymer species drops with their M faster in the mobile phase than it does in the solvated bonded phase, the contribution of enthalpic partition to retention volumes rises with sample molar mass. In this way the enthalpic partition acts antagonistically to the size exclusion retention mechanism. On the contrary, if solubility of polymer species drops with M faster in the stationary phase than in the mobile phase, the contribution of enthalpic partition to the overall V_R diminishes with the sample molar mass. Retention volumes, which were increased by the enthalpic partition in the lower molar mass area would be rapidly reduced with M in the higher molar mass range. In this case, the changes in extent of enthalpic partition may "assist" the size exclusion retention mechanism.

The extent of enthalpic partition most likely decreases with the sample molar mass also if macromolecules become too large to fully enter the pores of packing. Under appropriate experimental conditions, the extent of enthalpic partition may rapidly drop with the increasing size of macromolecules. Here again, the enthalpic partition of macromolecules may support the size exclusion retention mechanism. The overall separation selectivity would strongly increase if the reduction of enthalpic partition appears in the narrow range of molar masses. One speaks about **enthalpic partition assisted size exclusion chromatography (EPA SEC).** The introductory study on EPA SEC is presented in this paper.

Experimental

The HPLC apparatus consisted of the pump Model 510 (Waters, Milford, MA, USA) operated at 1 mL min^{-1}, the manual sample injection valve Model 7725 (Rheodyne, Cotati, CA, USA) provided with the sample loop of 50 μL and the evaporative light scattering detector DDL-21 (Eurosep, Cergy-Saint-Pontoise, France). Polymer samples dissolved in the given eluent at the

concentration of 1 mg mL^{-1} were injected. Relatively large sample volume was applied into columns of small size because of both limited detector sensitivity and necessity to work with rather low polymer concentrations in order to maintain low viscosity of injected solutions. As known, high viscosity of polymer containing samples causes shifts, broadenings and deformations of solute zones. Column temperature was kept in most experiments at 30 ± 0.01 °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 eluent and sample solutions were pre-thermostated. Other temperatures applied are given in Figure 7. The data were processed with help of the software Chroma (Chromtech, Graz, Austria).

The retention behavior of several different well and poorly endcapped silica C-18 phases described in [21,22] was evaluated. The results were similar for different columns and only data obtained with Aquasil C-18, 10 nm pores, 5 μm particle sizes (Thermo Hypersil-Keystone, Bellefonte, PA, USA) and Kromasil C-18, 100Å (10 nm pore size), 5 μm particle size, (Eka Chemicals, Bohus, Sweden) are presented and compared in this paper. Column sizes were 250 x 4.6 mm, and 300 x 7.8 mm, respectively. For comparison, selected results are reported with bare silica gel Kromasil 100 Å, 10 μm, column size 300x7.8 mm. Aquasil column is the commercial product. Kromasil sorbents were slurry packed in this Laboratory. The efficiencies of later two columns, measured with toluene injected into tetrahydrofuran eluent applying refractive index detector, were 29,000 theoretical plates.m^{-1} for bare Kromasil and 16,000 for Kromasil C-18. The column efficiencies for eluents containing viscous dimethylformamide are expected to be lower.

Analytical grade solvents were used as eluents, or eluent components viz. tetrahydrofuran from Merck, Darmstadt, Germany, and dimethylformamide from Scharlau, Barcelona, Spain. They were vacuum distilled before use. Tetrahydrofuran was treated with KOH and Na before distillation and the distilled solvent was stabilized with 0.02% of butylated p-cresol. Mixed eluents were prepared by weighing and the control of eluent composition was better than ± 0.1 wt. %.

Two sets of homopolymers differing in their polarities were applied. They exhibited narrow to medium molar mass distributions. In all cases, the peak retention volumes could be well identified. Polystyrenes (PS) were from Pressure Chemicals Co., Pittsburgh, PA, USA (molar masses ranged from 0.666 to 1,200 kg.mol^{-1}), and poly(methyl methacrylate)s (PMMA) of low stereoregularity were gifts from Dr. W. Wunderlich, Röhm, Darmstadt, Germany [23] and from Dr. J. Herz of Institut Sadron, Strasbourg, France (M ranged from 1.3 to 613 kg.mol^{-1}).

A set of block copolymers PS-b-PMMA was also measured. They were prepared by anionic polymerization. The molar masses of blocks were nearly equal and assumed values about 10, 30, 50 and 90 $kg.mol^{-1}$ (Polymer Standards Services, Mainz, Germany). After each set of experiments the retained macromolecules were removed from columns by an overnight action of the efficient displacer, THF. Columns were re-equilibrated by the fresh eluent before the next series of measurements.

Results and Discussion

To avoid problems with mixed enthalpic retention, the adsorption of macromolecules on the column packing surface must be suppressed. Recently, it was found [21,22] that some medium-, and high-polarity polymers were adsorbed from the low- or medium-polarity eluents on the free silanols, which are abundant on the surface of most commercial silica C-18 bonded phases. For very polar polymers, the extensive adsorption was observed even with the carefully end-capped silica C-18 column packings.[22] However, the adsorption of low polarity macromolecules such as polystyrenes on the end-capped silica C-18 phases is improbable. Moreover, polar dimethyl formamide (DMF) and medium polar tetrahydrofuran (THF), which suppress adsorption of medium polarity macromolecules on silica gel, were applied in our study as eluents. DMF even fully prevented adsorption of high polarity polymers such as poly(2-vinyl pyridine)s and polyethylene oxides on free silanols of bare and bonded silica gels at its concentration of about 30% in a mixed eluent DMF/THF. [22] The low polarity macromolecules of PS are adsorbed on bare silica gel only from non-polar solvents like cyclohexane or carbon tetrachloride. [24,25] Adsorption of PS on bare and bonded silica gels was not observed from medium and low polarity, eluents such as THF and toluene, respectively. We can conclude that the adsorption of PS on the silica gel C-18 column packings from DMF and THF is hardly possible.

The exponents in Kuhn-Mark-Houwink-Sakurada viscosity law, a, for PS in THF at 25-30 °C range from 0.64 to 0.768. [26] This large scatter is explained mainly by the high hygroscopicity of THF, which rapidly absorbs substantial amount of water and it is anticipated that the proper a value for PS in dry THF lies in the area of 0.73. [26] It means that THF is a good solvent for PS, likely much better than the solvated C-18 groups of the bonded phase. Therefore THF should suppress enthalpic partition of polystyrene in favor of the C-18 phase. The dependences of log V_h vs. V_R for PS standards in THF eluent monitored for bare silica gel and for the C-18 phase prepared from the same starting silica gel are compared in Figure 1.

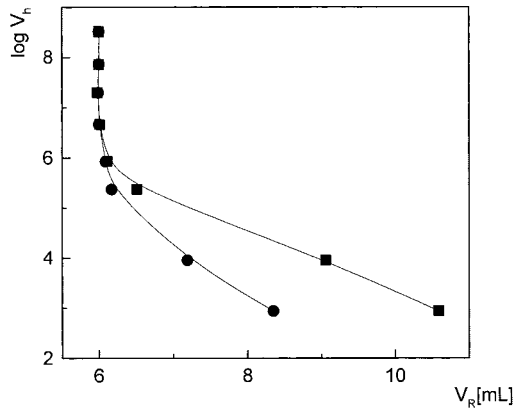

Figure 1. Dependences of log V_h vs. V_R for bare silica gel (■) and for silica gel C-18 phase (●) prepared from the same carrier, Kromasil 100. Polystyrenes were injected in a good solvent, tetrahydrofuran. The curve for silica gel C-18 was normalized considering excluded retention volume (V_0) for bare silica gel. For discussion see the text.

V_h is the hydrodynamic volume of macromolecules [27] expressed: $V_h = M[\eta]$, where M is the most abundant molar mass in the sample and $[\eta]$ is the corresponding limiting viscosity number in eluent. The evaluation of courses of these dependences for the same column but with various polymers and various eluents allows assessment of enthalpic interactivity of HPLC columns [19,21,22] and at least semi-quantitative determination of averages and distributions of packing pore sizes. [28,29] The difference in the shapes of the dependences in Figure 1 is obvious. It reflects unequal pore volume accessible for PS molecules in bare and bonded silica gel. Both the excluded V_h values and the retention volumes of smaller polymer species decreased due to presence of the C-18 groups. It scan be concluded that molecules of PS hardly enter the C-18 bonded phase in THF and both the effective pore size and pore volume become smaller. In a reasonable approximation, enthalpic interactions can be neglected and entropic partition of macromolecules can be considered the only retention mechanism present in this system ("**ideal SEC**"). The course of dependence of log V_h vs. V_R for bare Kromasil also indicates relatively low pore volume of this material (see also [21]), which is therefore generally less suitable for SEC separations. It is supposed that the effective volume of the C-18 phase can be estimated from dependences shown in Figure 1. [19]

DMF is a rather poor solvent for PS. The exponent a at 30 °C is 0.612. [30] DMF strongly promoted enthalpic partition of PS species in favor of C-18 phases [7,19] and molar masses higher than about 1 kg.mol^{-1} were even fully retained within C-18 phases bonded to porous silica. [20]

Basing on the above observations, THF has been chosen as the partition suppressing eluent component and DMF as the partition promoting eluent component for polystyrenes in connection with the silica C-18 phases. It was anticipated that by mixing these two solvents it would be possible to finely control extent of PS partition. The dependences of log M vs. V_R (further only "the Plots") for Aquasil C-18 are depicted in Figure 2.

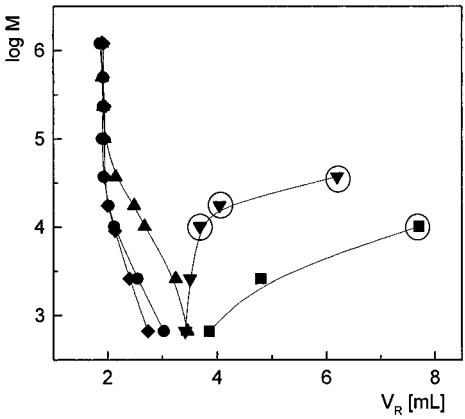

Figure 2. The Plots of log M vs. V_R for polystyrenes eluted from Aquasil C-18 column packing in DMF (■), THF (◆), and in mixed eluents DMF/THF containing 90 (▼), 80 (▲) and 50 (●) wt. % of DMF. The data points for systems exhibiting reduced apparent sample recovery are marked with additional circles. For explanation see the text.

Aquasil contains "embedded" polar groups within the C-18 phase. Polar groups are introduced into some C-18 bonded phases in order to control their polar interactivities and to prevent their collapse in the HPLC eluents containing high concentration of water. The producer did not disclose nature, amount and position of embedded polar groups. The overall retention of polystyrenes in DMF on Aquasil was dominated by enthalpic partition and the retention volumes increased with M. Molar masses over 10 kg mol^{-1} were fully retained within column. Addition of THF to eluent suppressed enthalpic partition and the character of the Plots in Figure 2 changed from the enthalpic partition dominated to the exclusion dominated one for the eluents containing between 10 and 20 wt. % of THF. Addition of 50

wt. % of THF to DMF brought the Plot almost to the coincidence with that for pure THF. Similar courses of the Plots were observed for Kromasil C-18 with different DMF/THF mobile phases compositions (Figure 3), except for the fact that higher molar masses of PS have been eluted from Aquasil in the pure DMF eluent compared to Kromasil C-18. [19] This indicates that presence of the embedded polar groups slightly suppressed enthalpic partition of PS species in favor of the C-18 phase.

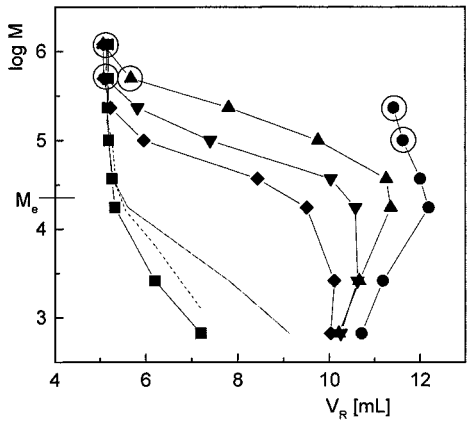

Figure 3. The Plots of log M vs. V_R for PS eluted from Kromasil C-18 with THF (■) and with mixed eluents DMF/THF containing 83 (●); 82 (▲); 81 (▼), and 80 (◆) wt. % of DMF. For comparison also the Plots are shown for PMMA in DMF/THF with 80 wt. % of DMF (- - -) (compare also Figure 5), and for PS in THF on bare Kromasil (—) (normalized to V_0 = 5.17 mL). M_e is the molar mass of PS excluded from the silica C-18 packing in THF that is under SEC conditions. The reduced apparent sample recovery is depicted with additional circles. The sample recovery for eluent containing 80 wt. % of DMF approached 100 %.

Small changes in the eluent composition around 80 wt. % of DMF strongly affected the courses of the Plots in Figure 3. Evidently, the extent of enthalpic partition of polystyrene between mobile phase and solvated bonded C-18 intensively varied in the vicinity of this eluent composition. The courses of the Plots around 80 wt.% of DMF reflected highly selective SEC-like separation of polystyrenes, which extended only over about 1.5 order of magnitude in *M*. This is less than the expected highest theoretical selectivity of separation based solely on size exclusion. The SEC column packings with pores of uniform size should produce the Plots extending over two orders of magnitude in *M*. [1] The excluded molar masses in eluents containing 80 to 82 wt. % of DMF fairly exceeded M_e for the silica C-18 in THF and even that for the bare silica gel. Similarly, retention volumes of polymers with the

lowest *M* were higher than that found for silica C-18 phase, as well as for identical bare silica gel packing. This behavior can be attributed to the effect of the enthalpic partition of polystyrenes in favor of the C-18 phase, which assisted the SEC retention mechanism.

Notice the unexpected back-turn course of the Plots for eluents containing 81 and, especially 82 wt. % of DMF in Figure 3. These Plots are likely composed of two different parts. The enthalpic partition slightly prevailed up to about the SEC excluded molar mass of PS, while above this limit the exclusion process clearly dominated. Similar back-turn courses of the Plots have been observed with several other systems, in which enthalpic partition was combined with exclusion. [7,19] It is evident, that the experimental conditions leading to the back-turn shaped Plots must be avoided in most chromatographic systems applied for analytical purposes.

The high selectivity separation of macromolecules assisted by enthalpic partition (EPA SEC) is documented with a mixture of three polystyrenes in Figure 4.

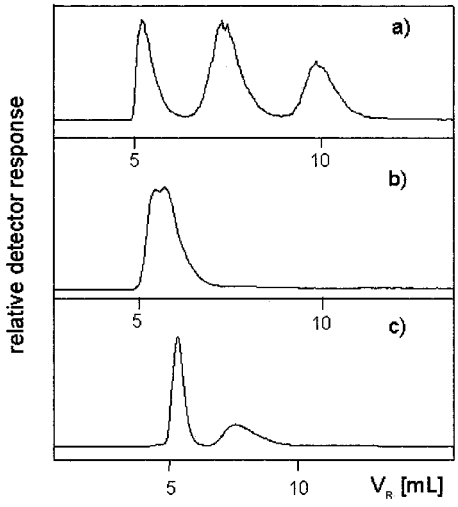

Figure 4. Chromatograms of mixtures of polystyrenes with M 100, 37 and 9 kg.mol^{-1} obtained a) with Kromasil C-18 column 300x7.8 mm and mixed eluent DMF/THF containing 80 wt. % of DMF; b) with the same column as a) and pure THF eluent; c) with bare Kromasil column 300x7.8 mm and pure THF. Interstitial volume for bare silica gel column was slightly higher than that for C-18 column.

The base line separation for polystyrenes with similar molar masses has been easily achieved with one single column in spite of its rather low efficiency (see the Experimental section) and a relatively high viscosity of eluent. For comparison also chromatograms are shown in Figure

4, which were obtained without presence of enthalpic partition. The same mixture of polystyrenes was eluted from bare silica gel and C-18 bonded silica gel using pure THF eluent. Selectivity of separation in the latter cases was much lower and this demonstrates the result of enthalpic partition "assistance".

The Plots of log M vs. V_R obtained for poly(methyl methacrylate)s with the same column and with eluents of similar composition as in Figure 3 are shown Figure 5.

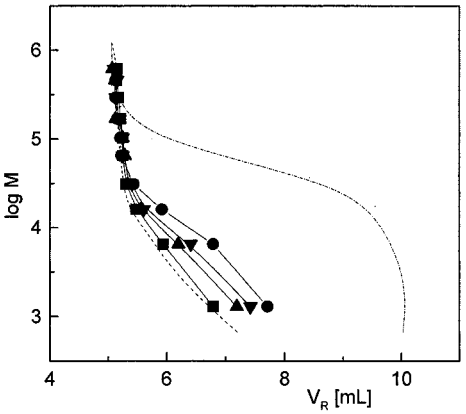

Figure 5. The Plots of log M vs. V_R for PMMA eluted from Kromasil C-18 in THF (■), and in DMF (●), as well as in mixed eluents DMF/THF containing 80 (▲) and 81 (▼) wt. % of DMF. For comparison also the Plots for PS in THF (- - -) and in the mixed eluent containing 80 wt. % of DMF (-.-.-) are depicted.

DMF is only a little better solvent for PMMA (a = 0.625 [31]) than for PS. Still, it seems that the enthalpic partitioning of more polar PMMA in favor of solvated C-18 groups is much less pronounced than in the case of less polar PS in DMF and DMF containing eluents. The enthalpic partition of PMMA appears only in the "SEC non-excluded" molar mass area. This gives a chance for separation of less polar polymers such as PS from the more polar species such as PMMA - even if their molar masses are similar: PMMA samples with M above 30 $kg.mol^{-1}$ will leave the column in the excluded retention volume V_0 while less polar macromolecules of PS will elute much later, under the EPA SEC conditions. An example of such approach is demonstrated in Figure 6.

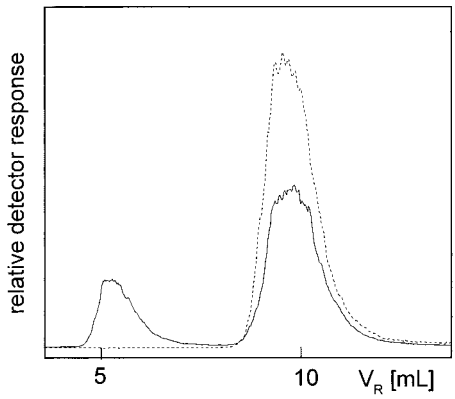

Figure 6. Chromatograms of the mixture of PS (17.5 kg.mol^{-1}) with PMMA (16 kg.mol^{-1}) (—), and of the same single PS (- - -). Column Kromasil C-18, 300x7.8 mm, mixed eluent DMF/THF containing 80 wt. % of DMF.

Polystyrene was separated from poly(methyl methacrylate) possessing almost identical molar mass. The chromatogram of PS remained practically unaffected by presence of PMMA. The PMMA species leaving an EPA SEC column can be subsequently characterized by an appropriate on-line SEC column, which does not exhibit enthalpic partition that is in a two-dimensional polymer HPLC arrangement.

In Figure 7, the effect of temperature on the courses of the Plots is demonstrated for Kromasil C-18 with the eluent containing 80 wt. % of DMF. The flow rate was not corrected for thermal contraction/expansion of eluent. The corrections would not affect tendencies in the courses of the Plots. The Plots changed their shapes from enthalpic partition dominated to size exclusion (entropy) dominated retention with increasing temperature. The system was very sensitive to temperature change in the range from 10 to 25 °C, and especially between 11.5 and 13 °C. The back-turn shaped Plots were observed between 12.7 and 15 °C. The solubility of PS likely improved with temperature faster in eluent than in the solvated C-18 phase and, therefore, the overall extent of enthalpic partition of PS dropped with rising temperature. It is evident that the molar mass range, in which the EPA SEC selective separation takes place, can be easily adjusted by changing the column temperature. A tandem of columns packed with the same material but working at different temperatures can cover a rather broad molar mass area. This is experimentally well feasible, however, more attractive is the opportunity to ad hoc create a tailored EPA SEC system, which would highly selectively discriminate samples just in the desired molar mass area. It is anticipated that the temperature adjustment

will allow to extend the working area of the EPA SEC method to the molar masses well exceeding thousands of kg.mol-1 with relatively narrow pore column packings of less than 100 nm diameter. The work with mechanically instable ultra-wide pore SEC column packings could be avoided in this way. However, one must consider the back-turn courses of some Plots, as well as the possible reduction in the sample recovery (see below). It is not yet clear if the EPA SEC principle will be applicable to oligomers. The method apparently works well only in the domain of molar masses excluded from the given packing under "ideal" SEC conditions. As known, the temperature dependences of retention volumes allow discrimination of enthalpic and enthalpic contributions to polymer retention. The quantitative evaluation, which needs large series of very exact data is under preparation.

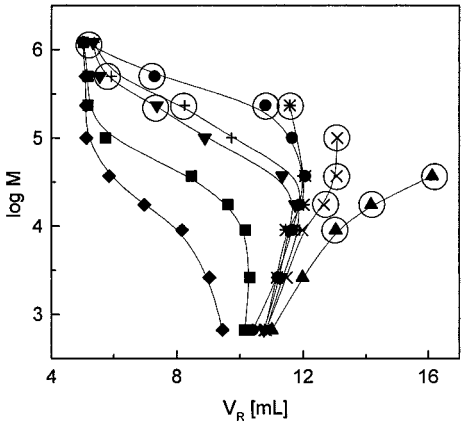

Figure 7. The Plots log M vs. V_R for PS eluted from Kromasil C-18 with mixed eluent DMF/THF containing 80 wt. % of DMF at different temperatures (▲) 10 °C; (×) 11.5 °C; (✳) 12.3 °C; (●) 12.7 °C; (+) 13 °C; (▼) 15 °C; (■) 25 °C, and (◆) 50 °C. The data points for systems exhibiting reduced apparent sample recovery are marked with additional circles.

An important issue for all polymer HPLC techniques, which couple entropic and enthalpic retention mechanism represents sample recovery. This is the case for polymer HPLC under critical [13] and limiting [32] conditions – and also for EPA SEC. In all above methods, macromolecules may exhibit a tendency to stay retained within column irrespectively of the amount of mobile phase pumped through the system ("full retention"). The full retention problem augments with decreasing diameter of the packing pores and with increasing both sample molar mass and enthalpic interaction between macromolecules. Selective full retention of high molar mass fractions affects results of analyses also indirectly because the

sample fractions retained within column alter both the effective pore size and the overall interactivity of packing.

The low sensitivity of evaporative light scattering detector (ELS) used in this study, as well as intrinsic limitations of ELS detection in general - the non linear response toward sample concentration [33,34], and the dependence of response on the eluent nature [34] - prevented quantitative evaluation of sample recovery. The "apparent" sample recovery was considered "decreased" when the peak area monitored by the ELS detector for the EPA SEC system dropped more than 25% when compared to the "ideal" SEC conditions. In this case, data points in Figures are marked with an additional circle (Figures 2, 3 and 7).

The exact molecular backgrounds of processes taking place in the EPA SEC systems are not well understood, yet. One can speculate that the **difference** of solubilities of polymer molecules in the stationary and in the mobile phase rapidly changes in the area of M subject to selective separation. As mentioned, solubility of macromolecules may decrease much faster in the bonded phase than in the mobile phase with rising molar mass. As result, the extent of enthalpic partition would decrease with increasing polymer molar mass. This hypothesis, however, does account neither for the increased excluded molar mass of samples in EPA SEC compared to an identical "ideal" SEC column nor for the back-turn shape of some EPA SEC Plots. Further, there is no apparent reason for abrupt change in the solubility difference to appear just at a relatively high sample molar mass nearly corresponding to that excluded from the packing pores in the "ideal" SEC separation mode.

The alternative explanation of the EPA SEC phenomenon supposes that the enthalpic interactions push also large (the SEC excluded) polymer species into the packing pores. [7] This process intensifies with increasing attractive segmental interaction energy between sample molecules and column packing, ε, which is sensitive to temperature, eluent composition and possibly also to pressure. At very high ε values, and for eluents, which are thermodynamically poor for the polymer under study (low a values in the viscosity law) enthalpic partition in favor of stationary phase may be so large that macromolecules of practically any molar mass stay retained within column and cannot be eluted by any volume of eluent (full retention). It is supposed that both the attractive interactions between packing and macromolecules and the repulsive interactions between eluent and macromolecules force parts of the de-coiled macromolecules to enter even the narrowest pores to create "stems" of the polymer species assuming a "flower like" conformation. [35-37] Under such conditions, macromolecules may be attached to the column packing simultaneously with many adjacent

segments. This attachment may be very strong and difficult to be cancelled by molecules of a displacing liquid [32], also due to slow processes of diffusion.

As the attractive interactions packing – polymer (ε) decrease and the attractive interactions polymer – eluent (a) increase, the retention volumes decrease. At the same time, ever larger macromolecules start eluting (see for example Figure 2). Eventually, we arrive at the above mentioned "critical conditions" where the entropic partition (exclusion) and the enthalpic partition mutually compensate and polymer retention volumes become independent of the sample molar mass. [8-10] The plots of $log\ M$ vs. V_R are vertical. It seems that in the vicinity of critical conditions, the flower like interactions are still feasible. This means that at least a fraction of macromolecules, which are excluded at $\varepsilon \sim 0$ and at high a (the SEC system) may enter some packing pores at $\varepsilon > 0$ and at low a. The dept of such enthalpy forced pore permeation probably rapidly decreases with the sample molar mass. Therefore, the retention volume augmentation due to enthalpic partition sharply drops with the increasing sizes of macromolecules. This process can be responsible for the EPA SEC behavior of macromolecules.

Clearly, the overall retention of macromolecules is dominated by enthalpic partition (V_R's increase with M) when ε exceeds its critical value. Above certain molar mass, however, the effect of enthalpic partition starts decreasing with M, anyway, and so do the retention volumes. As result, the back-turn courses of the Plot appear. In this case, the area of "enthalpic assistance" to exclusion retention mechanism is shifted to the higher sample molar masses.

It is anticipated that the selectivity of the SEC separation of polymers can be augmented also by the adsorption processes. [7,38] The resulting procedure can be termed **adsorption assisted size exclusion chromatography.**

An interesting application of EPA SEC would be the characterization of block copolymers. A chromatographic system can be identified, in which one of the blocks (for example PMMA) remains non-retained (excluded) while another block (for example PS) will be eluted under EPA SEC conditions. In this way, the PS blocks would be characterized irrespective of presence of PMMA blocks. This idea was tested with a series of PS-b-PMMA samples. Contrary to expectations, elution behavior of block copolymers was governed by the PMMA blocks. Samples of block copolymers eluted in the excluded volume of column in the form of rather narrow peaks or at the V_R of PMMA blocks (results not shown). It seems that the presence of PMMA blocks in diblock copolymers PMMA-block-PS prevented enthalpic

partition of PS chains. The interpretation of these results will, however, need further experiments. This last observation may also shed light on the behavior of diblock copolymers in the "critical polymer HPLC", where some deviations from expected behavior were found by Chang et al. [39]

Conclusions

A new member was added to the family of methods of polymer HPLC namely enthalpic partition assisted size exclusion chromatography (EPA SEC). EPA SEC combines enthalpic and entropic partition of macromolecules within the HPLC column to allow a selective separation of polymer species according to their molar mass or chemical nature/composition. Enthalpic partition of macromolecules within appropriate stationary phases is promoted by the low solubility of polymer species in eluent. The extent of enthalpic partition is controlled by the amount of a thermodynamically good solvent added to the mobile phase. The additive suppresses extent of enthalpic partition. Several systems polymer-eluent-column packing, which exhibited excessive enthalpic partition were identified in the studies initially devoted to the tests of enthalpic retentivities of HPLC column packings [19,21,22] and to the evaluation of enthalpic retention mechanisms of macromolecules. [7] These systems included polystyrenes (PS) and poly(n-butyl methacrylate)s (PnBMA) model polymers, dimethylformamide (DMF) and diethylmalonate (DEM) as enthalpic partition promoting solvents, as well as tetrahydrofuran (THF) and toluene as enthalpic partition suppressing liquids – all in combination with a series of different silica C-18 bonded phases. Only few typical results obtained with polystyrenes, DMF/THF mixed eluents and with two different silica C-18 column packings are presented in this paper, however, the behavior of PnBMA polymers and DEM/THF and DEM/toluene mixed eluents was very similar to that reported here. Therefore the generalization of basic conclusions is possible. The tentative explanation of high selectivity separation of homopolymers by EPA SEC assumes

- a presence of enthalpic partition of macromolecules in favor of the (bonded) stationary phase. Enthalpic partition results from better solubility of macromolecules in solvated stationary phase than in the mobile phase

- an increase of polymer retention volumes caused by enthalpic partition compared to that due to the sole entropic partition (exclusion)

- a fast decrease of enthalpic partition of macromolecules with their increasing molar masses under specific experimental conditions. The sharp drop of enthalpic partition appears

above certain value of sample molar mass, M_{ep}, which as rule lies in the vicinity of polymer molar mass fully excluded from the packing pores in the "ideal" size exclusion chromatographic mode (M_e). For a given polymer and column packing the actual value of M_{ep} depends on eluent composition and/or on temperature of experiment.

The mechanism behind EPA SEC may include the molar mass dependent

- pushing the parts of large (SEC excluded) polymer chains by enthalpic interactions into the solvated bonded phase even if the latter is situated in the narrow pores of column packing

- differences between solubilities of macromolecules in the stationary phase and in the mobile phases.

EPA SEC can be used for selective size separations of homopolymers within narrow molar mass areas, as well for discrimination of polymer blend components. Further applications of EPA SEC may include separation of macromolecules according to their composition and architecture. The efficacy and feasibility of EPA SEC must be, however, checked with a series of real systems and with the homogeneous bonded phases of different volumes and polarities. The effects of experimental conditions, for example packing pore size, injected polymer concentration and volume, flow rate and pressure in the system, as well as column temperature must be carefully evaluated together with the role of sample recovery in order to decide about practical applicability of method.

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