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Evaluation of liquid chromatography column retentivity using macromolecular probes III. Partition properties of C_{18} phases traced by polymers

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Abstract

Application of polymeric probes was proposed for evaluation of partition properties of the high performance liquid chromatographic stationary phases. The approach was tested with selected silica gel C-18 column packings. Polystyrene (PS) and poly(*n*-butyl methacrylate) (PnBMA) narrow molar mass standards of low polarity were applied to avoid adsorption of macromolecules on silanols and other polar groups present within column packings. Polar eluent components further reduced contingency of silanophilic interactions. The major eluent component was dimethylformamide (DMF), a thermodynamically poor solvent for polymer probes, which strongly promoted enthalpic partition of macromolecules in favor of the C_{18} bonded phase. Methyl ethyl ketone (MEK) and diethyl malonate (DEM) were also tested as the partition promoting eluent components. With polystyrenes, MEK was rather inefficient as a partition promoter while DEM was similarly active as DMF. A thermodynamically good solvent for polymer probes, viz. tetrahydrofuran (THF) was added to eluent to reduce and control the extent of partition. The differences in elution behavior of column tested indicate their unlike partition properties.

Keywords: Column retentivity; Macromolecular probes; Stationary phases, LC; Entropic partition; Enthalpic partition; Polystyrene; Polymers; Poly(*n*-butyl methacrylate)

1. Introduction

New procedure for evaluation of HPLC column retentivity utilizes macromolecular probes [1,2] instead of commonly applied sets of low molar mass substances. A homologous series of appropriate, well characterized linear homopolymer "standards" with known molar mass averages and narrow molar mass distributions is injected into column tested and the dependences of $\log M$ versus V_R or $\log M[\eta]$ versus V_R, are constructed, where $[\eta]$ is limiting viscosity number in eluent of the polymer probe with the most abundant molar mass M and V_R the corresponding peak retention volume. The product of M and $[\eta]$ is called hydrodynamic volume of macromolecules, V_h [3]. M and $[\eta]$ are mutually related with the well known Kuhn–Mark–Houwink–Sakurada viscosity law:

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

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where K and a are constants for a given polymer–solvent pair at a given temperature. They are tabulated for numerous systems [4].

The exponent a characterizes the extent of interactions between macromolecules and eluent, i.e. the *thermodynamic quality* of a solvent toward given polymer at a given temperature. It assumes values about 0.65–0.8 for linear, coiled macromolecules if attractive interactions between polymer segments and solvent molecules are favorable. This means that polymer segments prefer contacts with solvent molecules and polymer coils are expanded. Corresponding solvent is termed thermodynamically *good* for the polymer. The solvents with $0.5 < a < 0.65$ values for a polymer are denoted thermodynamically *poor* while $a = 0.5$ holds for the *theta* solvents, in which polymer segment–solvent and segment–segment interactions are equal. In the poor and theta solvents, polymer coils shrink compared to the situation in the good solvents. Values of $a < 0.5$ indicate a metastable situation, i.e. a vicinity of the coil collapse, or aggregation and, eventually, the onset of phase separation.

The courses of dependences: $\log V_h$ versus V_R and $\log M$ versus V_R reflect both entropic and enthalpic processes taking place in the HPLC columns. In absence of attractive or repulsive enthalpic interactions between polymer segments and column packing, the dependences of $\log V_h$ versus V_R are governed exclusively with entropic (exclusion) processes within the HPLC column. The courses of $\log V_h$ versus V_R curves depend only on the overall column geometry, on the mean size and size distribution of the packing pores, on the pore volume, and further on the average packing bed density. They coincide for the same column for different polymers and different eluents provided the pore and packing bed geometry does not change with the eluent nature. Therefore, the $\log V_h$ versus V_R curves are termed “universal calibration dependences” in size exclusion chromatography (SEC).

On the contrary, the courses of the plots of $\log M$ versus V_R depend also on the polymer nature and on the thermodynamic quality of eluent for given polymer probes. Nevertheless, also these plots can furnish valuable information about the HPLC columns. They are used if the constants in the viscosity law (Eq. (1)) are unknown. In absence of enthalpic interactions, both kinds of the above dependences are rather insensitive toward temperature and eluent flow rate variations.

The mutual shifts of the dependences of $\log V_h$ versus V_R and in many cases also of the plots of $\log M$ versus V_R for different polymer-eluent systems indicate presence of enthalpic interactions in the HPLC column. We believe that it could be possible to at least semi-quantitatively evaluate the above shifts, to correlate them with the data obtained from measurements with low molar mass test probes, and to compare them for various HPLC columns to assess differences in their retentivities.

In contact with two mutually immiscible, phase separated solvents, solute molecules as rule prefer the phase exhibiting larger attractive interactions with the dissolved species, i.e. the *thermodynamically better solvent*. One can say that solute molecules undergo *enthalpic partition* in favor of a better solvent. Unlike small molecules, partition equilibrium of macromolecules can be strongly shifted toward one solvent so that one phase would hardly contain any polymer species. Similar situation is to be anticipated in the HPLC column, for example, the one packed with the silica C_{18} phase. Macromolecules eluted in the mobile phase, which is their good solvent likely show little tendency to get partitioned in favor of the solvated C_{18} phase if the latter is their poor “solvent”. This idea was applied in preceding papers [1,2] where eluents were as good solvents as possible for the polar polymeric test probes in order to suppress enthalpic partition effects. At the same time, mobile phases were weak enough to allow interactions between polymer segments and free silanols on the silica surface, situated among C_{18} groups. Surprisingly, silanophilic interactions were strong enough to pull polar macromolecules through the C_{18} phase so that they were adsorbed on the silica surface. This means that at least parts of adsorbed macromolecules were in contact with solvated C_{18} groups and underwent a sort of the “forced” enthalpic partition. Both processes of adsorption and “forced” enthalpic partition have been inevitably accompanied with large entropic effects because macromolecules changed their conformation. Nevertheless, it is assumed that in the first approximation, the overall retention volumes of polymer probes reflect mainly the net adsorption of macromolecules depending on the amount, accessibility and activity of the free silanols, which remained unoccupied with the bonded C_{18} groups and with the end-capping agent. This gives an opportunity to test and compare

the silanophilic interactivities of various silica C_{18} column packings [1,2]. The addition of a polar, strong solvent to eluent suppresses adsorption of macromolecules. An additive, which strongly interacts with free silanols, should be again a rather good solvent for polymer probes not to promote their enthalpic partition. The amount of a strong additive which is needed to fully prevent adsorption of macromolecules can be one of the measures of silanol groups both activity and concentration/accessibility.

An interesting phenomenon was observed when highly polar macromolecules such as polyethylene oxides (PEO) and poly(2-vinyl pyridine)s (P2VP) were eluted from the silica C_{18} phases in a medium strength eluent, tetrahydrofuran (THF). Only a relatively unimportant enthalpic retention was observed for lower molar mass probes so that they eluted in the SEC mode. However, the retention volumes grew rapidly with *M* for higher molar mass probes (an "anti-SEC behavior"). It was hypothesized that after reaching a certain limiting molar mass, the macromolecules were able to bent around the C_{18} groups to be simultaneously attached to several distant silanols ("U-turn adsorption") [1,2]. Here again, the changes of polymer conformation, which accompanied adsorption represented important entropic contribution to the thermodynamics of the overall process.

In this present study, the possibility for an independent assessment of the enthalpic partition processes within the silica C_{18} phases is evaluated. It should be noted that partition of macromolecules takes place between eluent portions possessing identical composition but situated within the pores and in the interstitial volume also without presence of enthalpic interactions. This is the main retention mechanism operative in the size exclusion chromatographic columns. Such *entropic partition* results from the confinement of macromolecules within pores [5] and it is accompanied with large conformational changes of macromolecules. In this work, the additional partition processes, are of interest. These are promoted by enthalpic interactions between macromolecules, eluent and the solvated C_{18} phase. Certainly, these *enthalpic partition processes* are again composed of both enthalpic and entropic contributions. The discrimination of these contributions will be attempted in the following papers. In this stage, possible differences between entropic effects in the particular C_{18} phases are neglected and it is supposed that the enthalpic effects dominate under applied experimental conditions. When further speaking about partition of macromolecules, only the enthalpic processes will be considered.

In order to suppress their adsorption on silanols and to promote their partition in favor of the C_{18} phase, the macromolecular probes should be as non-polar as possible and the mobile phases as polar as possible. Consequently, the eluents are *thermodynamically poor* solvents for macromolecules, which are therefore "pushed" from the mobile phase into the C_{18} phase. As a result, polymer species undergo enthalpic partition. Their retention volumes increase and even their full retention within C_{18} phase can be observed. It is well known that solubility of macromolecules in poor solvents rapidly decreases with their increasing molar mass. In a HPLC system, this may refer to both solvated C_{18} phase and eluent. Therefore, both the extent of enthalpic partition and the measured retention volumes, should largely depend on polymer molar mass. Addition of a good solvent for the polymer probe into the eluent would reduce the extent of enthalpic partition.

The resulting partition processes of macromolecules may reflect specific properties of bonded phases also from the point of view of low molar mass analytes. For example, the extent of partition under otherwise identical conditions should depend on the volume of C_{18} phase available for both small and large molecules. In this paper, first results are presented on study of enthalpic partition of macromolecules within the silica C_{18} phases based on the above idea.

2. 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). The air pressure in DDL-21 detector was set at 1 kPa and temperature varied from 60 to 90 °C depending on the boiling point of the mobile phase components. Large sample volumes were applied due to both limited detector sensitivity and necessity to

work with relatively low polymer concentrations to keep low viscosities of injected solutions. High viscosity of polymer containing samples causes shifts, broadening and deformations of solute zones. Most injections were repeated twice and the averages of retention volumes were considered. Column temperature was kept in most experiments at $30 \pm 0.01\,^{\circ}C$ using a custom made air-oven connected to a water thermostat. Other temperatures applied are given in Figs. 7 and 8. The data were processed with help of the software Chroma (Chromtech, Graz, Austria).

Several different well endcapped silica C_{18} phases [1] were studied, however, here we present and compare only the data obtained with Kromasil C-18, 100 Å (10 nm pore size), 5 μm particle size, two different batches, the carbon content 20.2 and 20.3% (Akzo Nobel, Bohus, Sweden) and TSK gel ODS, 10 nm pores, the carbon content 18.8%, 10 μm particle size (TOSO, Shinnanyo, Japan) because these two companies agreed with publishing the data. Column sizes were 150 mm × 7.8 mm or 250 mm × 4.6 mm. For comparison, selected results are reported for bare silica gel Kromasil 100 Å, 10 μm, column size 300 mm × 7.5 mm.

Analytical grade solvents were used as eluents, or eluent components, viz. tetrahydrofuran from Merck, Darmstadt, Germany, methyl ethyl ketone (MEK) and toluene from Slavus, Bratislava, Slovakia, diethyl malonate (DEM) from Acros Organics, Geel, Belgium, and dimethylformamide (DMF) from Scharlau, Barcelona, Spain. They were vacuum distilled before use. Tetrahydrofuran was treated with KOH before distillation and the distilled solvent was stabilized with 0.02% of butylated *p*-cresol. Mixed eluents were prepared by weighing at sensitivity of balances 0.1 g. The eluents were protected from moisture.

Three sets of polymers differing in their polarities were applied. They exhibited narrow to medium molar mass distributions. In all cases, the peak retention volumes could be unambiguously identified. Polystyrenes (PS) were from Pressure Chemicals Co., Pittsburgh, PA, USA (molar mass ranged from 0.666 to 1200 kg mol^{-1}), poly(methyl methacrylate)s (PMMA) of low stereoregularity were a gift from Dr. W. Wunderlich, Röhm, Darmstadt, Germany (*M* ranged from 16 to 613 kg mol^{-1}) [6] and poly(*n*-butyl methacrylate)s (PnBMA) were purchased from Polymer Standards Services, Mainz, Germany (*M* ranged from 8.4 to 723 kg mol^{-1}). All injected polymers were dissolved in the given eluent at the concentration of 1 mg ml^{-1}. After each set of experiments the retained macromolecules were removed from columns by an overnight action of an efficient displacer, tetrahydrofuran. Columns were re-equilibrated by the fresh eluent before the next measurement.

3. Results and discussion

The first step was identification of appropriate eluents for the available non-polar probes of polystyrenes and poly(*n*-butyl methacrylate)s. For comparison, retention volumes of poly(methyl methacrylate)s were also determined in selected systems. Methyl ethyl ketone, dimethylformamide and diethyl malonate were tested as the potential partition promoting solvents for PS and PnBMA. Both MEK and DMF are polar liquids, which extensively interact with the free silanols. They are “strong” toward silica gel and rather efficiently suppress adsorption of medium polar poly(methyl methacrylate)s on bare silica gel and porous glass [7]. DMF fully prevents also adsorption of highly polar polymers, polyethylene oxides and poly(2-vinyl pyridine)s on silica based column packings. Already 20–30% addition of DMF to THF precludes adsorption of PEO and P2VP on silanols [7]. It is known that polystyrenes are adsorbed on bare silica gel from carbon tetrachloride and cyclohexane [8,9]. On the other hand, THF, chloroform and even toluene prevent adsorption of PS on bare silica gel. Therefore, the adsorption of low polarity polymers on the silica C_{18} packings should not be enhanced by adding THF or toluene into more polar eluents. DEM is a solvent not commonly used in HPLC and its strength toward silica gel is not known. Therefore, the retention of PS and PMMA was checked on the bare silica gel from this solvent. The dependences of $\log V_h$ versus V_R and $\log M$ versus V_R for PS in DEM and in THF are shown in Fig. 1.

The $[\eta]$ values for the systems PS-THF and PS-DEM were calculated applying Eq. (1) with the literature data for *K* and *a*, see later. The courses of both kinds of dependences are very similar. One can conclude that DEM rather efficiently suppresses adsorption of PS on bare silica gel. It is well probable that the silanophilic interactions of PS in DEM can be

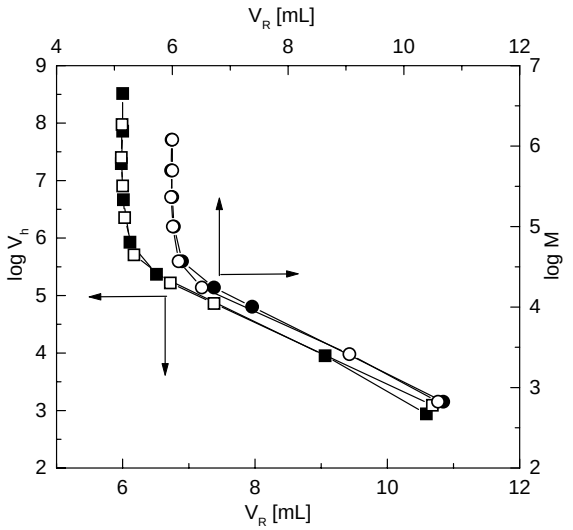

Fig. 1. Dependences of $\log V_h$ vs. V_R and $\log M$ vs. V_R for polystyrenes in diethyl malonate (○) and (●); as well as in tetrahydrofuran (□) and (■); respectively. Bare Kromasil 100 Å, 10 μm, column size 300 mm × 7.5 mm. The similarity of the plots of $\log V_h$ vs. V_R and $\log M$ vs. V_R is incidental. The coincidence of the dependences of $\log V_h$ vs. V_R for THF and DEM indicates that adsorption of PS in DEM can be neglected.

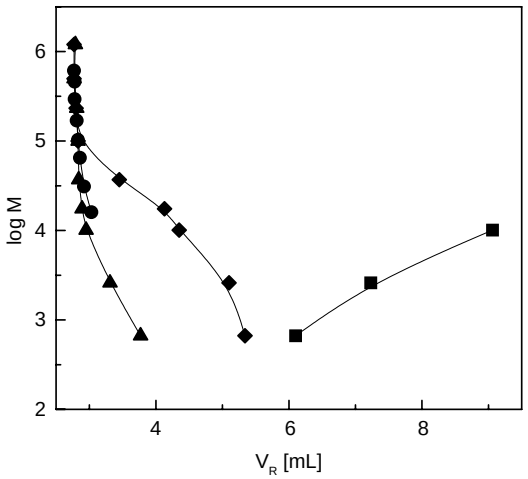

Fig. 2. The plots of $\log M$ vs. V_R for PS in DEM (■); PMMA in DEM (●); PS in THF (▲) and PS in mixed eluent DEM/THF (90 wt.% of DEM) (◆). Column 150 mm × 7.8 mm packed with Kromasil C-18, 100 Å, 5 μm.

neglected also in the case of silica gel C_{18} phases. On the other hand, PMMA was fully retained within silica gel from DEM but eluted likely without enthalpic interactions from the Kromasil C-18 column in DEM because the plots of dependences of $\log M$ versus V_R for PMMA in DEM and PS in THF well coincided (Fig. 2).

MEK and DMF are poor solvents for both polymers. a values (Eq. (1)) for PS in MEK at 30 °C and in DMF at 35 °C are 0.620 and 0.603, respectively [4]. DEM is a theta solvent for PS at 34.2 °C ($a = 0.5$) [4]. DMF is a theta solvent for PnBMA at 23.6 °C [10]. It was expected that MEK, DMF and DEM may support partition of low polarity polymers PS and PnBMA in favor of the C_{18} phase.

Tetrahydrofuran is a thermodynamically good solvent for polystyrene: a ranges from 0.64 to 0.768 at 25–30 °C [4,11]. Large scatter of experimental K and a values is attributed to presence of water in THF, which is highly hygroscopic [11]. The azeotropic mixture of THF and water contains about 5 wt.% of water and its boiling point differs less than 3 °C from the boiling point of dry THF (65.9 °C) at atmospheric pressure. The a values for PS determined in dry THF lie around 0.73 [11]. It is supposed that THF is a good solvent also for PnBMA. Toluene is a good solvent for PnBMA ($a = 0.77$ [12]) and also for PS ($a > 0.7$ at 25–35 °C [4]) and most probably also for PnBMA. It was expected that both THF and toluene may prevent or at least reduce partition of PS and PnBMA in favor of the C_{18} phase.

The dependences of $\log M$ versus V_R (further only "the Plots") for PS standards in DEM, and in DEM/THF mixed eluent 90/10 wt. for Kromasil C-18 are also presented in Fig. 2. The comparisons of their courses for PS in DEM on bare silica and silica C_{18} in Figs. 1 and 2, as well as of those for PS in pure THF and in the DEM containing eluents in Fig. 2 evidence extensive partition of PS in favor of the C_{18} phase promoted by DEM. Unfortunately, diethyl malonate (sometimes non-appropriately called ethylmalonate) possesses physical properties which are not advantageous for liquid chromatography. It has rather high viscosity and intermediate refractive index ($n_D = 1.414$ at 20 °C). High boiling point makes its purification difficult and relatively high price further decreases its attractivity. Non-polar polymers such as poly(dimethyl siloxane)s and highly polar polymers such as polyvinyl chlorides, or poly(2-vinyl pyridine)s are practically insoluble in DEM.

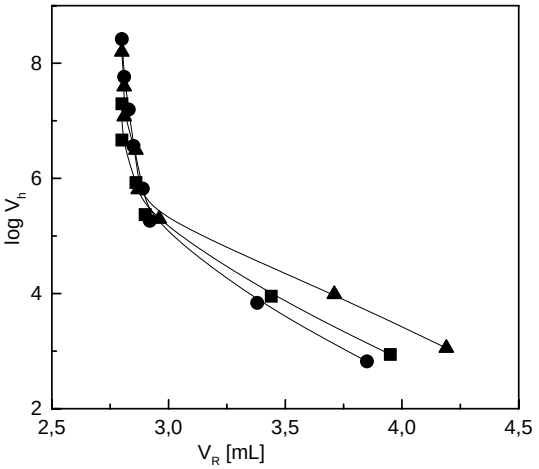

Fig. 3. The dependences of $\log V_h$ vs. V_R for PS in toluene (●); tetrahydrofuran (■) and in methyl ethyl ketone (▲). Column 250 mm × 4.6 mm packed with TSK gel ODS, 100 Å, 10 μm.

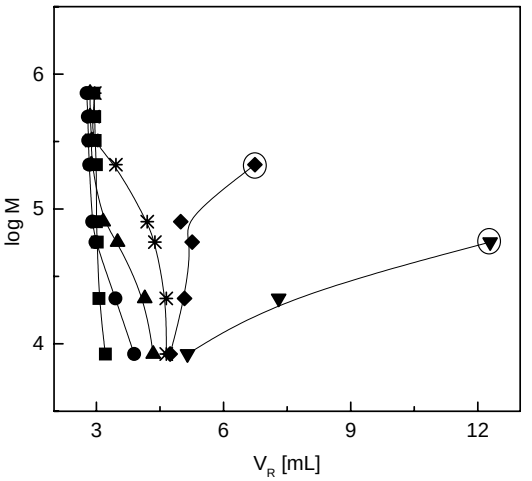

Fig. 4. The plots of $\log M$ vs. V_R for poly(*n*-butyl methacrylate)s in pure THF and in mixtures of THF with dimethylformamide (in wt.% of DMF): 0 (■); 50 (●); 55 (▲); 57 (✳); 58 (◆); 60 (▼). Column 150 mm × 7.8 mm packed with Kromasil C-18, 100 Å, 5 μm. The reduced sample recovery is marked with an additional circle.

The dependences of $\log V_h$ versus V_R for PS standards in the partition suppressing toluene ($a = 0.72$) [4] and in MEK for Kromasil C-18 are shown in Fig. 3.

The curves diverge in the area of lower molar masses of polymer probes. A small effect of enthalpic partition for PS in THF cannot be ruled out. The shift between curves for toluene and THF, however, only slightly exceeds experimental errors. The shift for MEK is somewhat more pronounced. The enthalpic contribution to the retention of PS in MEK seems to increase with the decreasing molar masses of polymer species. This is typical for weak interactions between macromolecules and the column packing [1,2,13] and can be explained by increasing accessibility of the C_{18} phase situated in the relatively narrow pores of silica for smaller polymer species. In any way, MEK does not extensively promote enthalpic partition of PS in favor of the C_{18} phase and therefore it is not suitable for the present retentivity studies.

The next solvent tested was dimethylformamide. DMF is relatively often used as eluent in size exclusion chromatography. Its advantage is a relatively high solubility of inorganic salts like LiCl, LiBr or KSCN. Therefore, the DMF/salt eluents are utilized in separation of highly polar and highly hydrogen bonded polymers, as well as for macromolecules, which carry dissociating groups. On the other hand, DMF is rather toxic, and highly hygroscopic, it possesses a high UV cutoff, and exhibits unfavorably high refractive index (1.425), boiling point and viscosity. From the point of view of the control of enthalpic interactions within column, the important disadvantages of DMF are possible changes of moisture content, and its low stability: DMF easily decomposes into both formic and oxalic acids, as well as into diamines [14].

The plots for PnBMA standards in DMF and in mixtures DMF/THF are shown for Kromasil C-18 in Fig. 4.

Similar results were obtained also with TSK gel ODS column (results not shown). The extent of enthalpic partition of PnBMA polymer in favor of the C_{18} phase is very large. Lower molar masses of PnBMA start eluting only at 40 wt.% of THF in eluent while higher molar masses are fully retained or their apparent sample recovery is rather low. The estimated reduction in polymer recovery is manifested by the decreased peak sizes. With the increasing content of THF in eluent, the enthalpic partition dominated retention of polymer changes to the exclusion dominated one and the apparent sample recovery improves remarkably. The plots in mixed eluents tend to coincide with that for pure THF when amount of THF in eluent increases. In other words, at a certain limiting

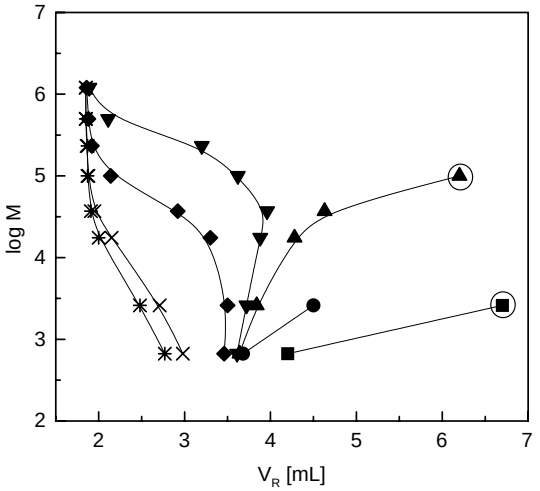

Fig. 5. The plots of log M vs. V_R for PS in DMF (■); THF (✳) and in mixed eluents DMF/THF (in wt.% of DMF): 90 (●); 83 (▲); 82 (▼); 80 (◆); and 50 (×). Column 250 mm × 4.6 mm packed with TSK gel ODS, 100 Å, 10 μm. The reduced sample recovery is marked with an additional circle.

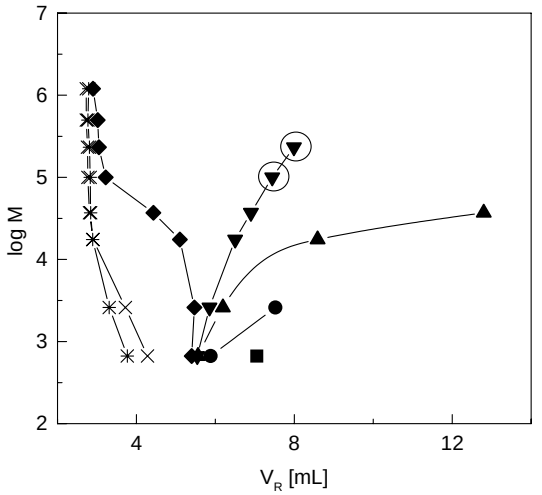

Fig. 6. The plots of log M vs. V_R for PS in DMF (■); THF (✳) and in mixed eluents DMF/THF (in wt.% of DMF): 90 (●); 85 (▲); 82 (▼); 80 (◆); and 50 (×). Column 150 mm × 7.8 mm packed with Kromasil C-18, 100 Å, 5 μm.

content of THF in DMF, the enthalpic partition of macromolecules may be practically suppressed.

The PS "standards" are better characterized, narrower as to theit molar mass distribution and easier accessible than the PnBMA ones. Therefore, the study was extended to narrow polystyrenes (Figs. 5 and 6).

Enthalpic partition of polystyrenes in favor of the C_{18} phase is very large in dimethylformamide and it is suppressed upon addition of tetrahydrofuran. The difference in the courses of the plots is evident for Kromasil and TSK gel ODS. Polystyrene with higher molar masses elute in DMF—though only with a decreased apparent sample recovery—from TSK gel ODS compared with Kromasil C-18. Further, transition from the enthalpic partition dominated retention to the exclusion dominated one (the "critical point") [15,16] appears at a higher content of THF in eluent in the case of Kromasil. This signalizes larger extent of polystyrene partition in favor of C_{18} phase for Kromasil C-18 compared to TSK gel ODS.

Another parameter characterizing the extent of partition could be the composition of the mixed eluent at which the plot coincides with that for pure THF. In this respect, however, the Kromasil C-18 and TSK gel ODS seem to exhibit only a relatively small difference (Figs. 5 and 6).

The effect of temperature on the courses of the plots for the eluent containing 83 wt.% of DMF is represented in Figs. 7 and 8. No correction for temperature expansion of eluent was made. It is evident that the

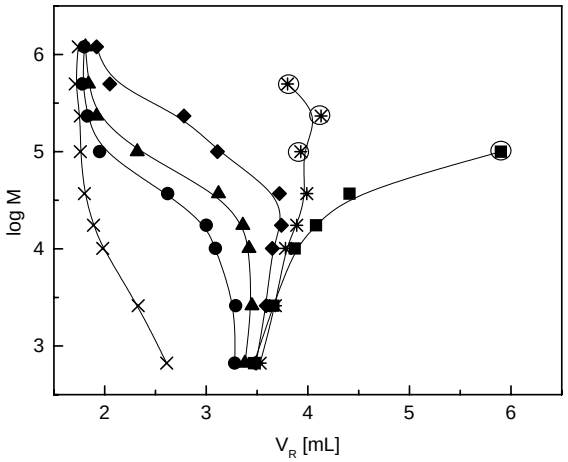

Fig. 7. The plots of log M vs. V_R for polystyrenes in mixed eluents DMF/THF containing 87 wt.% of DMF. Temperature dependence: 50 °C (●); 40 °C (▲); 35 °C (◆); 31 °C (✳); and 30 °C (■). For comparison, also the plot for pure THF at 30 °C (×) is depicted. Column 250 mm × 4.6 mm packed with TSK gel ODS, 100 Å, 10 μm. The reduced sample recovery is marked with an additional circle.

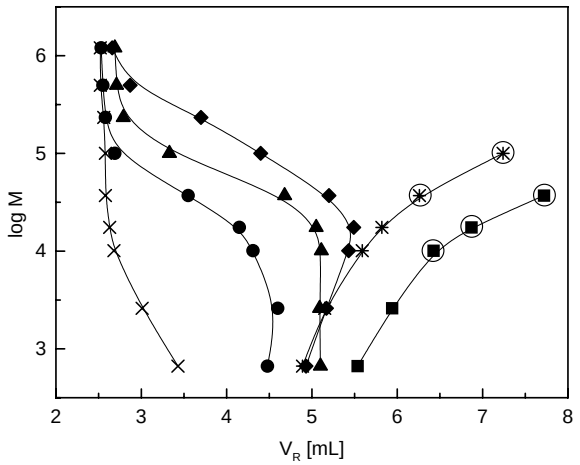

Fig. 8. Effect of temperature on the courses of the plots of log M vs. V_R for polystyrenes in mixed eluents DMF/THF containing 87 wt.% of DMF and in pure THF. The symbols and temperature are the same as in Fig. 7. Column 150 mm × 7.8 mm packed with Kromasil C-18, 100 Å, 5 μm.

courses of the plots are very sensitive toward temperature variation.

The extent of partition of macromolecules in favor of the C_{18} phase rises with decreasing temperature, i.e. with the anticipated decrease of PS solubility in the mixed eluent. It is anticipated that the drop of solubility of PS in the solvated stationary phase with lowering temperature is less pronounced. The transition from the enthalpy dominated retention to the entropy dominated one ("critical temperature") appears at a slightly higher temperature for Kromasil C-18 than for TSK gel ODS. Again, Kromasil C-18 column packing exhibits stronger tendency to the enthalpic partition of PS macromolecules than TSK gel ODS. For a more detailed analysis of temperature effects on enthalpic partition of macromolecular probes, larger series of highly precise data are needed.

The effect of temperature on the course of the plots has also been compared for two different batches of Kromasil C-18 (Fig. 9).

The overall similarity of shapes of the plots indicates that the enthalpic partition properties of both batches may be alike. Their differences could be explained by the non-identical both pore volumes of starting silica gels and packing bed densities.

The above results coincide with a little higher carbon content of the Kromasil C-18 compared with TSK gel ODS. The detailed analysis should, however, consider also the surface area of the starting silica gels.

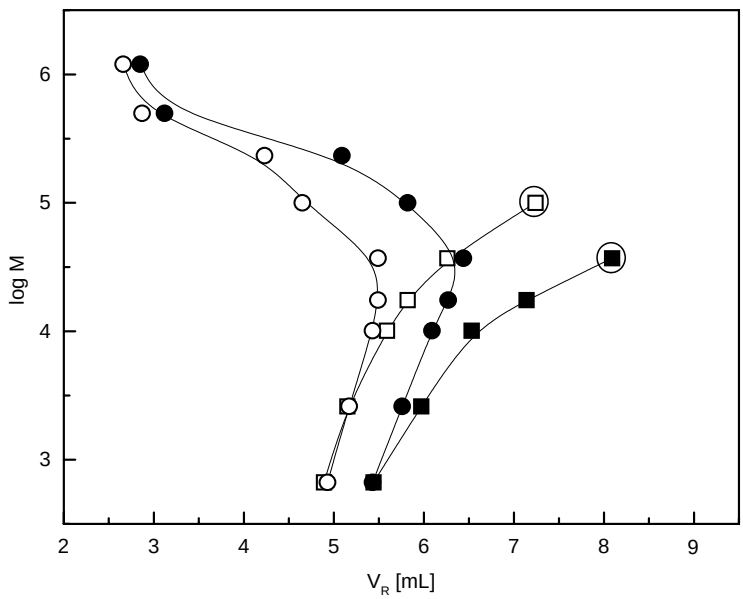

Fig. 9. Comparison of the plots log M vs. V_R for two different batches of Kromasil C-18, 100 Å, 5 μm (the batch DT 0268—empty symbols and the batch DT 0336—full symbols). Equal column size 150 mm × 7.8 mm was used. Eluent contained 83 wt.% of DMF. Temperatures, 31 °C (□) and (■) and 35 °C (○) and (●). The reduced sample recovery is marked with an additional circle.

In any case, the results indicate feasibility of studying the enthalpic partition of macromolecules in favor of C_{18} phases in the arrangement proposed and also possibility to compare various C_{18} phases.

It is observed (Figs. 2 and 5–9) that large changes of V_R are caused by small variations in polymer molar masses in certain areas of eluent composition and temperature for both PnBMA and PS probes, i.e. in the systems exhibiting extensive enthalpic partition. This indicates highly selective separation process. The highest possible selectivity of size exclusion chromatography separation not affected by enthalpic interactions assumes two orders of magnitude in sample molar mass for the packing pores of (almost) uniform size, such as controlled porosity glass [7]. In some of our present systems, the separation range covers only one order of magnitude of polymer molar masses and extends well over sizes of polymer species, which would be fully excluded from the packing pores if only entropic (exclusion) retention mechanism are operative ("ideal SEC"). High separation selectivity for macromolecules in the area of dominating exclusion retention mechanism assisted with enthalpic effects can be used for analytical purposes [17]. This important observation will be evaluated and discussed in another series of papers.

A comparison of the dependences of $\log V_h$ versus V_R and $\log M$ versus V_R monitored for starting bare and bonded silica gels can be used for estimation of the bonded phase volume. Entire pore volume of bare silica gel matrix is available for exclusion processes of macromolecules. At the same time, above dependences are shifted to lower V_h, or M as well to lower V_R for the silica C_{18} packings because of impermeability of the bonded phase for the macromolecules, which are not subject to enthalpic partition (compare the corresponding dependences in Figs. 1 and 2). In this way the effective volume of the C_{18} phase could be estimated. The non-partitioning and non-adsorbing (on silanols) polymer probes with different molar masses and narrow molar mass distributions, must be identified and their retention volumes determined. Alternatively, mobile phases efficiently suppressing both partition and adsorption of polymer probes must be used for construction of $\log V_h$ versus V_R or $\log M$ versus V_R dependences. As far as the same kind of polymer probes and eluent is applied, the application of $\log M$ versus V_R dependences would be sufficient. The dependence of $\log V_h$ versus V_R may allow also assessing the changes in effective volume of the C_{18} phase depending on the eluent and temperature applied. Application of V_h would also permit evaluation of accessibility of the C_{18} phase situated within the packing pores for analytes of different sizes. If the starting bare silica gel is not available for comparison, the C_{18} phase can be removed, for example, by pyrolysis applying mild conditions at which the structure of silica gel is not altered. The outlined procedure will be evaluated in the following contributions of this series.

4. Conclusions

Enthalpic partition properties of silica C_{18} column packings for high performance liquid chromatography can be at least semi-quantitatively assessed independently of the packing adsorptivity by means of macromolecular probes of low polarity applying as polar eluents as possible. The latter are thermodynamically poor solvents for non-polar polymer probes, which are therefore "pushed" into the solvated C_{18} phase. Narrow molar mass distribution polystyrenes and poly(*n*-butyl methacrylate)s are appropriate macromolecular probes and dimethylformamide and diethyl malonate efficiently promote their enthalpic partition in favor of the C_{18} phase. Addition of a thermodynamically good solvent for polymer species at a given temperature, to mobile phase, for example, tetrahydrofuran at 30 °C, diminishes extent of partition and thus allows its fine control. Partition can be controlled also by temperature of experiment. A detectable difference has been observed between partition of polystyrenes from dimethylformamide/THF mixtures for Kromasil C-18 and TSK gel ODS column packing.

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