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Evaluation of liquid chromatography column retentivity using macromolecular probes IV. Poly(ethylene glycol) bonded phase

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Abstract

Interaction properties of the novel HPLC silica gel–poly(ethylene glycol) (PEG) bonded phase were evaluated applying polymeric test substances, viz. polystyrenes, poly(methyl methacrylate)s, poly(ethylene oxide)s and poly(2-vinyl pyridine)s, and eluents of different polarities. Silanols on the silica gel surface are well shielded by the PEG phase, and silanophilic adsorption of macromolecules is suppressed in comparison with most silica C_{18} bonded phases. The adsorption of solutes on the –OH groups of the PEG phase seems to be low as well. The partition of macromolecules in favor of the PEG phase is inferior to that observed in case of the silica C_{18} phases. The volume of the PEG bonded phase is small and it is supposed that the PEG chains assume flat conformation on the silica gel surface.

Keywords: Retentivity; Macromolecular probes; Poly(ethylene glycol)

1. Introduction

It has been shown [1–3] that macromolecular probes can provide useful information on the properties of high-performance liquid chromatography (HPLC) columns. Several series of well-defined narrow molar mass distribution polymers are successively injected into the HPLC column under evaluation and the corresponding retention volumes are monitored. The dependence of $\log M$ versus V_R or $\log V_h$ versus V_R are constructed in different eluents and compared for polymers of different polarities, where M is the most abundant molar mass present in the polymer probe, and V_R is its peak retention volume. V_h is the hydrodynamic volume of polymer probe, defined as a product of M and $[\eta]$, where $[\eta]$ is the limiting viscosity number of macromolecules with molar mass M in the eluent [4]. The relation between M and $[\eta]$ for linear, coiled macromolecules is described by the well-known Kuhn–Mark–Houwink–Sakurada viscosity law

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

where K and a are constants for a given polymer–solvent–temperature combination. K and a values for many systems can be found in literature and several of them are compiled in *Polymer Handbook* [5]. Exponent a characterizes the thermodynamic quality of solvent toward macromolecules and assumes values from 0.5 for theta solvent, where macromolecules are unperturbed, to about 0.65 for low quality (poor) solvents, and over 0.65 for good and very good solvents, in which the macromolecular coils are well-expanded. In addition, the exponent a depends also on the stiffness (compact spheres, flexible coils, rigid rods) and on the architecture (linear, branched) of polymer species. However, for most linear flexible-coiled macromolecules, the a values depend only on the thermodynamic quality of the solvent. This allows direct comparison of $\log V_h$ versus V_R dependence for different (linear) polymer–solvent systems in the same column provided the pore geometry remains unaffected by the eluent nature. In a given column and in absence of enthalpic interactions between polymer and column packings, the dependence of $\log V_h$ versus V_R

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coincide for different polymers and different mobile phases. Their mutual shifts and to the same extent also the shifts of the plots of $\log M$ versus V_R indicate presence of enthalpic interactions between macromolecular probes and column packing. In this way, enthalpic retentivity of the HPLC columns can be qualitatively evaluated and the effect of analyte size can be assessed. As a side product, both effective pore size and volume, as well as their distributions, can be estimated using the so-called inverse size exclusion chromatography [6,7].

The described procedure has been applied in the study of the interactivity of various silica C_{18} phases [1–3]. It is believed that the adsorption on the column packing solid surface, which is caused, for example, by the silanophilic interactions, can be assessed practically irrespectively of the enthalpic partition between the eluent and the C_{18} phase. A series of polar polymer probes of different molar masses is eluted in less polar but still thermodynamically good eluents. In this case, enthalpic partition of sample molecules in favor of the bonded stationary phase is suppressed because the solvated C_{18} groups represent a poorer "solvent" for polymer probes compared with the eluent. Still, the C_{18} phase seems to be well permeable for macromolecules. Large polymer species appear to penetrate along the C_{18} groups to "find" free silanols for adsorption. The extent of end-capping of silica C_{18} phases can be evaluated applying moderately polar polymer probes as poly(methyl methacrylate)s (PMMAs) in eluents of low polarity (e.g. toluene). The well-end-capped C_{18} phases do not retain PMMA from toluene [1]; however, more polar polymer probes such as poly(2-vinyl pyridine)s (P2VPs) and poly(ethylene oxide)s (PEOs) may still exhibit a well-measurable adsorption even from a more polar, stronger eluent—tetrahydrofuran (THF) [2]. A pronounced retention of P2VPs and PEOs appears only above molar masses of a few or tens of kilograms per moles. A hypothesis was proposed that macromolecules must reach a particular "limiting" molar mass in order to be able to bend around the C_{18} groups so that they are attached simultaneously to several silanols ("U-turn adsorption") [2].

Macromolecular probes may also allow studies of enthalpic partition between the mobile and the solvated C_{18} phases, while suppressing the effect of silanophilic adsorption [3]. In this case, polymer probes are as non-polar as possible and eluents are as polar as possible—and poor for the test polymers. Poor solvents push macromolecules into solvated C_{18} phases and this results in large enthalpic partition effects. The appropriate systems represent for example polystyrene (PS) and poly(*n*-butyl methacrylate) probes in combination with polar eluents such as diethylmalonate (DEM) or dimethylformamide (DMF) [3]. In these eluents, macromolecules are strongly retained within column packing by enthalpic partition in favor of stationary phase and possibly also by interfacial adsorption of macromolecules between the C_{18} phase and the eluent. A small amount of a good solvent for polymer probes such as toluene or THF added to the eluent suppresses enthalpic partition so that macromolecules start eluting; however, their retention volumes still increase with molar mass (enthalpic-partition-dominated behavior). The effect of enthalpic partition decreases with increasing amount of a good solvent added to the eluent and, eventually, the critical eluent composition is reached [8–10] at which entropic and enthalpic retention mechanisms mutually compensate and V_R does not depend on polymer molar mass. Further increase of good solvent concentration in eluent leads to the size-exclusion-dominated situation, where polymer retention volumes decrease with increasing M. Differences in the critical eluent composition as well as effects of both eluent composition and temperature on the shapes of $\log M$ versus V_R plots indicate different partition properties of certain C_{18} phases [3].

Recently, a novel silica bonded phase was commercialized by Supelco Inc, which contains bonded poly(ethylene glycol) chains [11]. It was of interest to compare retention behavior of this material with the silica C_{18} phases applying the described procedures with macromolecular test probes.

2. Experimental

The HPLC apparatus consisted of a pump, Model 510 (Waters, Milford, MA, USA), operated at 1 ml/min, a manual sample injection valve, Model 7725 (Rheodyne, Cotati, CA, USA), provided with the sample loop of 50 μl, and an evaporative light scattering detector DDL-21 (Eurosep, Cergy-Saint-Pontoise, France). Relatively large sample volumes were applied owing to both limited detector sensitivity and the necessity to work with lower polymer concentrations to maintain as low a viscosity of injected solutions as possible. High viscosity of polymer containing samples causes shifts, broadening, and deformations of solute zones. Column temperature was kept at $30 \pm 0.01\,^{\circ}C$ using a custom-made air oven with duplex walls connected to a water thermostat. The data were processed with the help of Chroma software (Chromtech, Graz, Austria).

As mentioned, a large partition in favor of the bonded C_{18} phase was observed in the PS/DMF/THF system [3] and it was of interest to extend the study to the PEG phase also. The constants K and a in the viscosity law (Eq. (1)) for ternary solutions of PS in DMF/THF mixtures were not found in literature. Therefore, we determined them in a series of independent measurements. A size exclusion chromatography (SEC) system equipped with a viscosimetric detector was used for assessing limiting viscosity numbers $[\eta]$ at various eluent compositions. The SEC system was a GPCV2000 from Waters that uses two on-line detectors: a differential viscometer (DV) and a differential refractometer (DRI) as concentration detector. The detailed description of this SEC–DV system and data evaluation in terms of $[\eta]$ calculation has been given elsewhere [12]. The experimental conditions were as follows: two mixed Styragel columns 300 mm × 7.8 mm (HR5E and HR4E) from Waters, flow

Table 1
Summary of the K and a coefficients of the Kuhn–Mark–Houwink–Sakurada equation for polystyrenes in THF/DMF mixed solvents at 35 °C

DMF/THF (v/v)	Composition (w/w)	K ($\times 10^2$ ml/g)	a
0/100	0/100	1.551	0.700
10/90	10.6/89.4	1.531	0.697
20/80	21.1/78.9	1.527	0.695
30/70	31.5/68.5	1.517	0.692
50/50	51.7/48.3	1.594	0.681
70/30	71.4/28.6	1.710	0.667
80/20	81.1/18.9	1.933	0.653
100/0[a]	100/0	3.180	0.603

[a] Data from [5], 30 °C.

rate of 0.8 ml/min, temperature 35 °C, and injection volume 200 μl. The molar mass dependence of [η] was determined using 10 narrow PS standards, with M ranging from 10.9 to 3140 kg/mol from Polymer Standard Services, Mainz, Germany. Mixed eluents were used, in which the DMF/THF composition (v/v) changed progressively from 80/20, 70/30, 50/50, 30/70, 20/80, and 10/90 to pure THF. The corresponding K and a values are listed in Table 1. In order to prevent damage of the poly(styrene-*co*-divinylbenzene) columns, measurements in pure DMF were not performed. The data for pure DMF were taken from literature [5]. For experimental reasons, temperature of viscosity measurements was higher (35 °C) than that applied in HPLC (30 °C). In the first approximation, the V_h values we calculated for 35 °C were applied for the construction of dependence of $\log V_h$ versus V_R, with V_R measured at 30 °C. The K and a constants used for the calculation of actual V_h values were interpolated from the data in Table 1.

The 250 mm × 10 mm Discovery HS PEG column was provided by Supelco, Bellefonte, PA, USA [11] (further "HS PEG column"). Silica gel used for bonding of poly(ethylene glycol) (PEG) had a pore diameter of 12 nm. The molar mass and amount of PEG in the bonded phase were not disclosed. The behavior of the HS PEG column was compared with silica C_{18} material Kromasil C-18, 100A (mean pore size 10 nm), 5 μm particles, EKA Chemicals (Akzo Nobel) Bohus, Sweden. The column sized 300 mm × 7.8 mm was slurry packed in Polymer Institute, Bratislava, Slovakia.

Analytical grade solvents were used as eluents, or eluent components viz. THF from Merck, Darmstadt, Germany; toluene from Central Chem, Bratislava, Slovakia; acetonitrile (ACN) from Merck, Darmstadt, Germany; and DMF from Scharlau, Barcelona, Spain. They were vacuum-distilled before use. THF solvent was treated with KOH before distillation and the distilled solvent was stabilized with 0.02% of butylated *p*-cresol. Mixed eluents for HPLC were prepared by weighing, with precision better than 0.1%.

In the column retentivity studies, four 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. The V_R values are averages from two independent injections. Polystyrenes were from Pressure Chemicals Co., Pittsburgh, PA, USA (molar mass ranged from 0.666 to 1200 kg/mol); poly(methyl methacrylate)s of low stereoregularity were gifts from Dr. W. Wunderlich, Röhm, Darmstadt, Germany, and Dr. J. Herz from Institut Sadron, Strasbourg, France (M ranged from 1.3 to 613 kg/mol) [13]; poly(ethylene oxide)s were from Tosoh Co., Shinannyo, Japan (M ranged from 0.4 to 860 kg/mol); and poly(2-vinyl pyridine)s (P2VP) were from Polymer Standards Services, Mainz, Germany (M ranged from 3 to 1.26 kg/mol). All injected polymers were dissolved in the given eluent at a concentration of 1 mg/ml. After each set of experiments the retained macromolecules were removed from columns by an overnight static action of an efficient displacer for the given polymer, THF or DMF. Columns were re-equilibrated by the fresh eluent before further measurements.

3. Results and discussion

The plots of $\log M$ versus V_R (only "Plots" hereafter) and $\log V_h$ versus V_R for PS eluted from the Discovery HS PEG column in various eluents are shown in Figs. 1 and 2, respectively.

Comparison of Figs. 1 and 2 illustrates the effect of eluent quality on the Plot. The dependences of $\log V_h$ versus V_R nearly coincide. Fig. 2 shows that the enthalpic interactions of PS with the PEG column packing are only a little dependent on the eluent polarity. This is remarkably different from Kromasil C-18 (Fig. 3), where a pronounced enthalpic partition of the PS species was observed in favor of stationary phase from the DMF-containing eluents (see also [3,14,18]).

The result can be explained by low the "solubility" of PS species in the PEG bonded phase and/or by smaller effective volume of the PEG bonded phase compared to

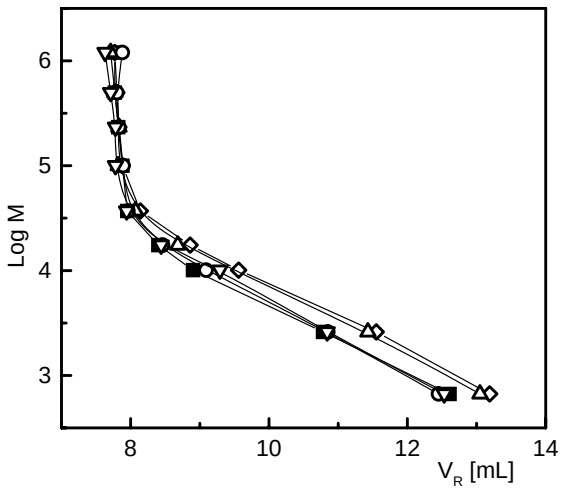

Fig. 1. The plots of $\log M$ vs. V_R for Discovery HS PEG column and PS probes in DMF (◇), toluene (○), THF (■), and DMF/THF mixed eluents containing 50 wt.% (▽) and 83 wt.% (△) of DMF.

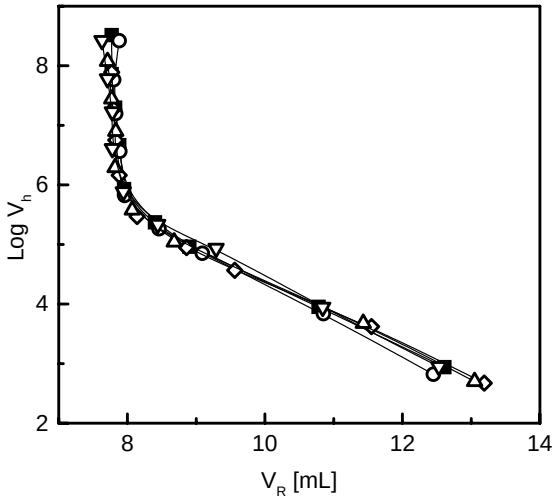

Fig. 2. The dependence of log V_h vs. V_R for Discovery HS PEG column and PS probes. Eluents and symbols as in Fig. 1.

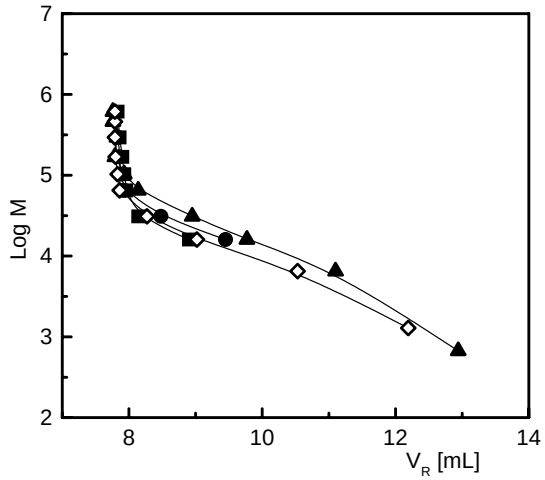

Fig. 4. The plots of log M vs. V_R for Discovery HS PEG columns and PMMA probes in DMF (◇), THF (■), toluene (●), and acetonitrile (▲).

the C_{18} phase. Excluded molar mass for the nonpartitioning species lies in the range of 50 kg/mol for the HS PEG column (Fig. 1), while it is only about 10 kg/mol for Kromasil C-18 [14]. Since the pore diameter of starting silica gel was in both cases similar (10 nm for Kromasil and 12 nm for HS PEG), it can be concluded that the effective volume of C_{18} phase is indeed much larger than that of the PEG phase. Moreover, retention volumes for the lowest polymer species are higher for the HS PEG column compared with the C_{18} bonded phase, even if the difference in the column sizes is considered (Figs. 1 and 3). This can be, however, caused also by the larger pore volume of starting silica gel used in preparation of the PEG phase because Kromasil is known for its low pore volume. It is concluded that the bonded PEG chains possess flat conformation, being spread on the silica gel surface rather than forming a "brush-like" structure.

The Plots for poly(methyl methacrylate)s, poly(ethylene oxide)s and poly(2-vinyl pyridine)s in various eluents for the HS PEG column are shown in Figs. 4–6, respectively. For PMMA (Fig. 4), the shapes of the Plots well resemble that for PS in Fig. 1.

This is an important difference compared to both bare silica gel and not well-end-capped silica C_{18} phases, where PMMA were fully retained in columns using toluene eluent [1]. There is some shift of V_R in acetonitrile, which can be explained with the reduced coil dimensions of the PMMA probes because ACN is a poor solvent for PMMA, with a theta temperature in the range of 45 °C [5]. In fact, the mutual shifts of dependence of log V_h versus V_R for PMMA in single eluents hardly exceed experimental errors in the area of higher M (Fig. 7).

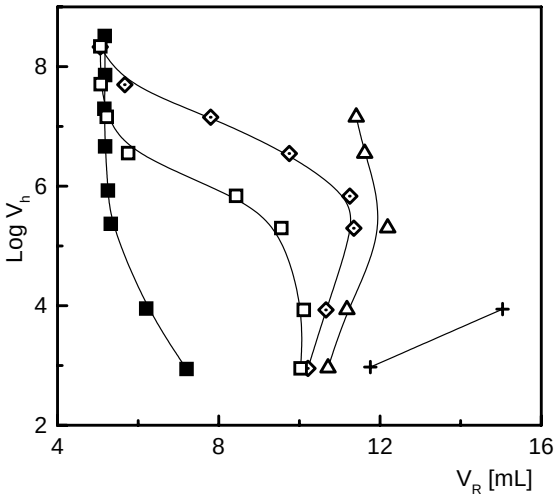

Fig. 3. The dependence of log V_h vs. V_R for Kromasil C-18 column and PS probes in THF (■) and in mixed eluents DMF/THF containing 80 (□), 82 (◇), 83 (△), and 90 (+) wt.% of DMF.

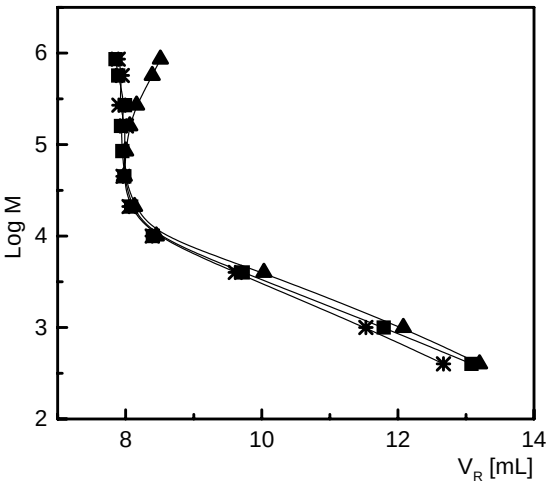

Fig. 5. The plots of log M vs. V_R for Discovery HS PEG columns and PEO probes in THF (■), acetonitrile (▲), and a mixed eluent (*) DMF/THF containing 70 wt.% of DMF.

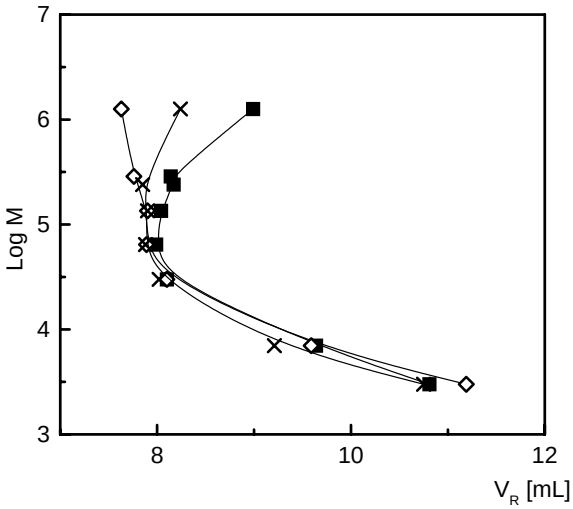

Fig. 6. The plots of log M vs. V_R for Discovery HS PEG column and P2VP probes in DMF (◇), THF (■), and mixed eluent (×) DMF/THF containing 30 wt.% of DMF.

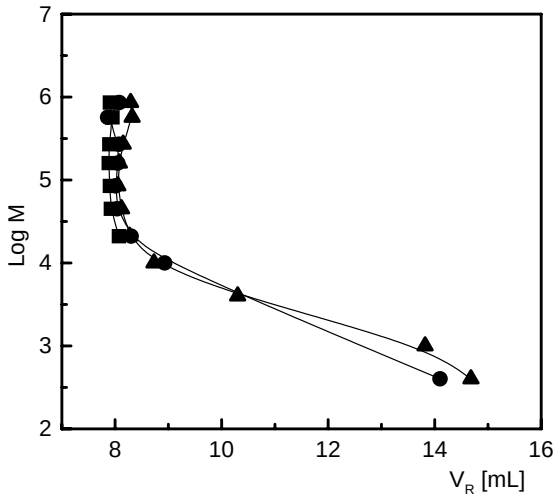

Fig. 8. The plots of log M vs. V_R for Discovery HS PEG column and PEO probes in THF (■) as well as in mixed eluents THF/toluene containing 10 (▲) and 20 (●) wt.% of THF.

Unfortunately, the viscosity law is often invalid below M values of about 10 kg/mol [5]. Therefore our calculated V_h values for polymers with lower M may be subject to large errors.

In conclusion, free silanols seem to be rather well shielded in the HS PEG phase. This would support the idea about a flat deposition of the PEG groups on the silica gel surface. If the PEG groups assumed a "brush-like" structure, they could hardly shield silanols on the silica gel surface unless the material is very carefully end-capped.

The Plot in Fig. 5 indicates a weak U-turn adsorption of PEO in ACN. Contrary to the silica C_{18} bonded phases [2], no U-turn adsorption is observed for PEO in both the THF and THF/DMF mixed eluents. The apparent sample recovery of PEO in toluene, which is a poor solvent promoting adsorption of this polymer, has been very low. Addition of a small amount of THF to toluene allows elution of PEO; however, retention volumes increase in the area of oligomers compared to more polar eluents (Fig. 8).

This may be caused by the end-group interactions of injected PEO in combination with increased (bonded) surface available for macromolecules of reduced size. The increase in retention volumes for lower members of the polymer homologous series augments selectivity of oligomer separation [15,16].

The Plot for P2VP in THF (Fig. 6) exhibits a pronounced U-turn shape. The phenomenon is less distinct in Discovery HS PEG than in Kromasil C-18 [2]. The increase of retention with M appears above the excluded molar mass of polymers. This is explained by the "flower-like interactions" of polymer coils [14]. If the attractive enthalpic interactions between macromolecules and column packing are large enough, the conformational entropy of polymer coils can be surmounted. Macromolecules "de-coil" and reptate into the pores, from which they would be otherwise fully excluded. The de-coiled macromolecules assume a "flower-like conformation" [17]. The "stem" of such a "flower" is situated in the pore and the "crown" is formed by the rest of the coil, which stays excluded from the pores.

For comparison, the Plots for PS and PMMA in mixed eluent DMF/THF 83/17 (w/w) are displayed in Fig. 9 for the HS PEG and C-18 phases.

As demonstrated in Fig. 3, the enthalpic partition of PS in favor of the C_{18} phase is very large in the mixed eluent DMF/THF containing 87 wt.% of DMF, while the enthalpic partition of PS in the same eluent on the HS PEG phase is almost negligible. The retention of PMMA on the HS PEG column is similar to that of PS.

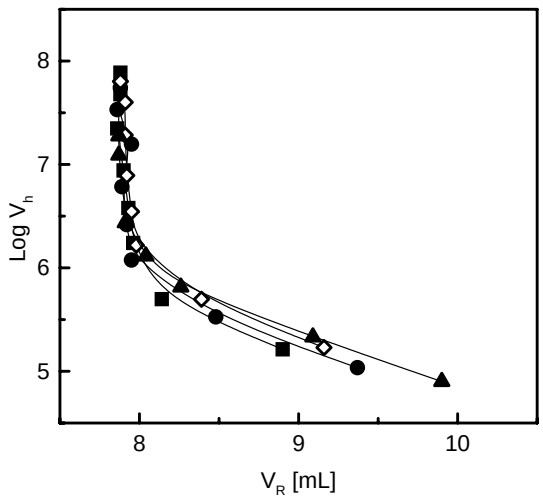

Fig. 7. The dependence of log V_h vs. V_R for Discovery HS PEG column and PMMA probes in DMF (◇), THF (■), toluene (●), and acetonitrile (▲). K and a values from literature [5] were used in the calculation of V_h in DMF, THF, and toluene at 30 °C and in acetonitrile at 45 °C.

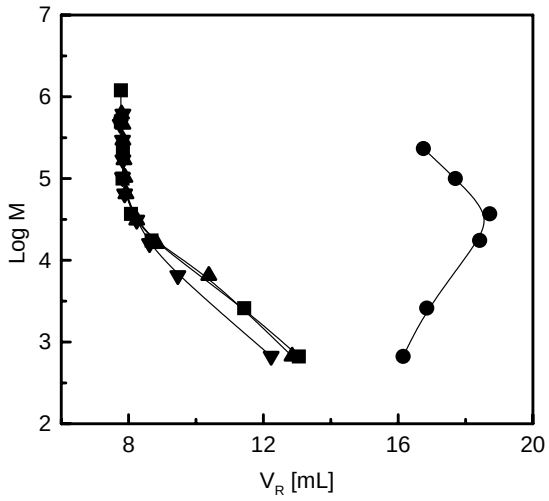

Fig. 9. The plots of $\log M$ vs. V_R for Discovery HS PEG and Kromasil C-18 for PS and PMMA in mixed eluent DMF/THF containing 87 wt.% of DMF; PMMA on Kromasil C-18 (▼) and on HS PEG (▲); PS on Kromasil C-18 (●) and on HS PEG (■). The plots for Kromasil C-18 are normalized to the interstitial volume of the HS PEG column (7.8 ml).

4. Conclusions

The retentive properties of Discovery HS PEG column toward macromolecular probes applied in this study differ substantially from those of bare silica gel and silica gel C_{18} phases. Retention of the moderately polar polymer PMMA, as well as polar PEO and P2VP, caused by their adsorption on free silanols is much lower compared with bare silica gel and it is also inferior to the end-capped C_{18} phases. The adsorption activity of –OH groups, which may be present in the PEG phase itself, is weaker than expected. The extent of enthalpic partition is low for nonpolar PS and moderately polar PMMA probes with respect to the PEO bonded phase from the polar mobile phases containing DMF. The volume of bonded PEG phase seems to be small. These observations indicate high density of the PEG chains attached to the surface of the silica gel. The PEG species probably assume a flat conformation, exhibiting long trains and only a few loops and free ends protruding over silica gel surface. This sets limitations for the use of HS PEG phase in enthalpic-partition-assisted size exclusion chromatography separation of macromolecules [18]. It also explains a rather efficient shielding of surface silanols by the PEG phase, as well as low interactivity of hydroxyl end-groups of bonded PEG chains. The latter groups are probably deactivated by a mutual hydrogen bonding within the densely packed PEG phase.

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