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# Evaluation of high-performance liquid chromatography column retentivity using macromolecular probes
## II. Silanophilic interactivity traced by highly polar polymers[☆]

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**Abstract**

A new method of HPLC column retentivity testing utilizes polymeric probes instead of conventional sets of low molar mass substances. The procedure allows at least semiquantitative, separate and independent evaluation of adsorption and partition properties of column packings. In this present work, the method is applied for comparison of the polar interactivities of selected silica gel $C_{18}$ HPLC columns. It is shown that free silanols which remained on the surface of the end-capped silica $C_{18}$ column packings are accessible for interaction with highly polar macromolecules. High molar mass polymeric test probes are adsorbed on the surface silanols and their retention volumes increase. As result, deviations from regular size-exclusion chromatographic (SEC) behavior are observed. The extent of retention volume changes depends on both the nature of polymer probes and on column packing type. Adsorption of macromolecules can be suppressed by addition of a highly polar substance to the mobile phase. The amount of polar additive which is needed to attain regular SEC elution of the polymer probe depends on the column packing type and can be used as a characteristic of silanophilic column interactivity. Courses of dependences of retention volumes on sizes of macromolecules indicate the presence of ''U-turn'' adsorption which allows two and more silanols situated among $C_{18}$ groups to be occupied simultaneously with the same macromolecule.
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*Keywords:* Stationary phases, LC; Macromolecular probes; Column retentivity; Poly(methyl methacrylate); Polystyrene; Polymers

## 1. Introduction

Recently, a new approach for evaluation of HPLC column retention properties was proposed [1]. Instead of commonly used sets of low molar mass test substances (for reviews see Refs. [2–5]), macromolecular probes were applied. Polar interactivities of 15 different columns, largely silica based $C_{18}$ phases, were compared on the basis of elution behavior of medium polar poly(methyl methacrylate)s (PMMAs) and non-polar polystyrenes (PSs) in toluene mobile phase. Due to strong silanophilic interactions PMMA probes were fully adsorbed on the bare silica gels from toluene at 30 °C. Under the same experimental conditions, PS species eluted in the SEC mode without measurable adsorption effects. The tested columns were divided into three groups. PMMA and PS species with matched hydrodynamic sizes eluted with the same retention vol-

[☆]For Part I, see Ref. [1].

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umes ($V_R$) from the well end-capped $C_{18}$ phases (''a'' group of column packings). This indicates the negligible effect of polar interactions between packing and PMMA probes. Slight-to-pronounced increase of $V_R$ for PMMA in comparison with retention volumes for PS was observed for the ''b'' group of columns. Shifts of $V_R$ for PMMA probes were caused by the presence of (active) polar surface groups with either low concentration or with limited adsorption activity. Non-end-capped or poorly end-capped column packings fully trapped PMMA probes not allowing their elution (''c'' group of columns). Evidently, the active groups in the ''b'' and ''c'' types of columns, mainly surface silanols situated among $C_{18}$ groups, were accessible for large PMMA macromolecules. In this way, the presence of adsorptive groups in the reversed-phase HPLC column packings could be easily traced. From the elution characteristics of the PS probes, pore size and pore volume of the HPLC column packings, both average values and distributions, could also be evaluated [1,6,7].

In present study, measurements were extended to more polar polymer probes, namely poly(ethylene oxide)s and poly(2-vinyl pyridine)s in order to visualise small differences among columns of ''a'' and ''b'' group. A column containing surface polar groups in the $C_{18}$ phase was also added. More detailed explanation of this new column evaluation method is presented.

## 2. Principle of method

Two basic retention mechanisms are to be considered in HPLC of small molecules using reversed-phase column packings, viz. enthalpic partition and adsorption. In the case of charged analytes, ion interactions, largely ion exchange, may take place as well. Secondary equilibria such as complexation/micellization of analytes with the eluent components affect the extent of their partition and adsorption.

Conventionally, processes of *adsorption* are those which take place on a *two-dimensional boundary* between two chemically different phases while the term *thermodynamic partition* describes processes which take place within the *volumes* of two chemically different phases.

It is known that all so far commercially available silica gel $C_{18}$ phases contain substantial amounts of non-reacted silanols. The surface concentration of free silanols can be reduced by various end-capping procedures. However, even the most efficient end-capping reactions leave ~50% of initial free silanols unreacted. Silanols, together with other polar groups which may be present on the silica surface, are responsible for polar interactions with analyte molecules which cause their adsorption. The interaction activity of surface silanols is increased by the presence of metal impurities within the silica gel matrix (A type silica gels). Therefore, highly pure silica gels are preferred (B type materials). Strong (polar) eluents which effectively compete with analyte molecules to occupy free silanols, suppress adsorption activity of silica based HPLC column packings. Still, many basic analytes may exhibit excessive adsorption on the packing surface even in strong mobile phases. Adsorption results in the chromatographic peak non-symmetry, tailing.

As known, the characteristics of particular commercial silica gel $C_{18}$ column packings differ remarkably and therefore they are subject to extensive testing and comparisons aimed at the development of improved materials.

The assessment of both partition and adsorption retentivity represents a very important step in HPLC column evaluation. This is usually done by injecting a series of selected low molecular substances containing various polar and non-polar groups and possessing various molecular shapes [2–5]. The general drawback of present column test procedures the impossibility of adjusting just one characteristic of the test substances at a time (e.g. their dipole moments) while keeping other characteristics constant (e.g. size and shape of molecules). Further, it is difficult to largely suppress analyte partition in the course of adsorptivity tests and vice versa, to avoid analyte adsorption while exclusively evaluating partition properties of packing. To mitigate the above problems, and to obtain some additional information on HPLC column retentivities, we proposed the application of macromolecular test probes [1].

Similar to low molar mass substances, enthalpic HPLC retention mechanisms of synthetic uncharged macromolecules include adsorption and partition [8]. Another important HPLC retention mechanism for polymer species is phase separation process. Phase

separation is rarely encountered with low molar mass analytes (also because of their low injected concentration) and will not be considered in the present discussion. Charged macromolecules may exhibit additional retention mechanisms such as ion exchange, ion inclusion and ion exclusion. Charges on polymer chains are created in the course of dissociation (polyelectrolytes) or sorption of low molar mass ions within initially non-charged polymer chains (pseudo-polyelectrolytes). The possible presence of both latter phenomena should be considered when working with highly polar macromolecules, even if the ion interactions are not intentionally included into the column testing protocol.

*Polymer adsorption* is caused by attraction among active moieties situated within column packing and within eluted macromolecules. Sometimes, repulsion between column packing and macromolecules is also observed and may lead to ‘‘negative adsorption’’. Usually, polar active sites are responsible for adsorption of macromolecules. Polar groups can be situated either on the solid surface of HPLC column packing (surface adsorption) or on the end of (non-polar) spacers (interface adsorption). Alternatively, polar groups can be embedded within otherwise non-polar bonded groups. At this stage, we shall discuss the surface adsorptivity of column packings but most conclusions can be applied also to interface adsorption on such bonded phases as -aminopropyl, -propylonitril, -dihydroxypropyl (‘‘glyceryl’’), etc. As regards analytes, polar groups can be situated within an entire polymer sample (a homopolymer with a polar repeating unit), they can be statistically distributed within polymer chains (a statistical copolymer of a polar and a non-polar monomer) or they can create domains within polymer chain (e.g. a block copolymer of a polar and a non-polar monomer). Polar groups can be also situated on one or both ends of a polymer chain. Usually, at least one end group of a macromolecule differs in its chemical nature from the rest of the polymer chain. For the HPLC column evaluation, applications of *homopolymers* possessing *different polarities* is advantageous. Molar mass of the test polymers should be high enough so that the role of end groups can be neglected.

The choice of chromatographic column packings for analyte partition is rather limited. Bonded phases with macromolecules attached to a solid support are rare and so are bonded phases with intrinsically tailored polarities (continuous polar bonded phases) [9]. HPLC is dominated with parafinic bonded phases (mainly $C_{18}$ groups) and their ‘‘apparent polarity’’ is controlled with their (preferential) solvation by molecules of eluent components. As result, we deal with analyte partition between a ‘‘solution’’ of bonded aliphatic groups in eluent (stationary phase) and between mobile phase.

We shall explain and apply the proposed column test procedure using silica gel $C_{18}$ bonded phase as an example.

The extent of interaction of macromolecules with the column packing can be semiquantitatively expressed with the help of the segmental interaction energy parameter, $\epsilon$. Positive $\epsilon$ values denote attraction while negative $\epsilon$ values mean repulsion between segments of dissolved macromolecules and HPLC column packing. $\epsilon \sim 0$ appears in systems where solvent strength is high enough to fully suppress enthalpic interactions between the polymer and packing. This is the case of ‘‘ideal size-exclusion chromatography’’ (SEC) in which retention of macromolecules within HPLC column is controlled exclusively with entropic (exclusion) retention mechanism.

Enthalpic interactions of polymer segments with HPLC column packings are summed so that the resulting interaction of the entire macromolecule rapidly increases with its molar mass. This explains why isocratic enthalpic interaction chromatography is possible only in the area of lower molar masses of macromolecules (oligomers). On the other hand, linear summing of segmental interactions between macromolecules and HPLC column packings is not possible because due to steric reasons all segments of a macromolecule cannot simultaneously interact with the column packing.

The elution behavior of macromolecules is described by dependences of retention volume $V_R$ on either molar mass ($M$) or hydrodynamic size ($V_h$) of polymer species. The plots of log $M$ vs. $V_R$ or log $V_h$ vs. $V_R$ are called ‘‘SEC calibration dependences’’ and ‘‘universal SEC calibration dependences’’, respectively. Hydrodynamic size, or ‘‘hydrodynamic volume’’, of macromolecules is defined as a product [10]:

$$V_h = M[\eta] \quad (1)$$

where $[\eta]$ is limiting viscosity number of polymer in solvent used as eluent.

The relation between $M$ and $[\eta]$ is described by the Kuhn–Mark–Houwink–Sakurada viscosity law:

$$[\eta] = KM^a \quad (2)$$

where $K$ and $a$ are constants for given polymer–solvent system. The $a$ constant characterizes the *thermodynamic quality* of solvent toward macromolecules, as well as *physical structure* of dissolved macromolecules (statistical coils, globules, rods, etc). Most macromolecules form statistical coils in their solutions. In this case, $a$ assumes values from 0.5 (a poor solvent) to ~0.8 (a good solvent). $K$ and $a$ values for electroneutral macromolecules in good solvents only slightly depend on temperature. Values of $a$ below 0.5 indicate the closeness of phase separation (precipitation). Numerous $K$ and $a$ values for various (co)polymer–solvent systems have been reported in the literature and some of them are compiled in the Polymer Handbook [11]. $K$ and $a$ values can be readily determined by SEC, using viscometric detectors.

The plots of log $M$ vs. $V_R$ and log $V_h$ vs. $V_R$ are constructed on the base of experimental data. A series of polymer probes (usually polystyrenes) with known molar masses is one by one or as a mixture injected into an HPLC column and the retention volumes of their peak apexes are determined. Polymer probes should have as narrow molar mass distribution as possible in order to precisely identify the $V_R$ position. Still, one has to keep in mind that all so far available "narrow polymer standards" contain macromolecules of different sizes. The availability of narrow molar mass distribution polymer probes with various chemical natures (and various polarities) is so far rather limited.

Universal calibration dependences of log $M[\eta]$ vs. $V_R$ coincide for different polymers in different eluents for the same column [10] provided enthalpic interactions are absent in the chromatographic system. Enthalpic interactions between macromolecules and column packings strongly affect the courses of universal calibration dependences [8,12]. This means that if polymer probes and eluent are appropriately chosen, enthalpic interactivities of HPLC columns can be evaluated from the mutual shifts of universal calibration dependences.

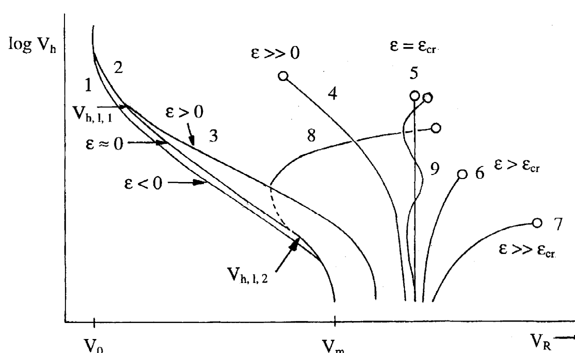


Fig. 1. Schematic representation of the courses of universal calibration dependences log $V_h$ vs. $V_R$. For further explanations see the text.

The situation is schematically depicted in Fig. 1. Curve 1 holds for a system in which repulsion ($\epsilon<0$) (slightly) decreases retention volumes of polymer probes. Curve 2 is the universal calibration dependence for an "ideal SEC system", where $\epsilon \sim 0$. Curve 3 belongs to a system where the segmental interaction energy assumes a positive but low value. Alternatively, interaction sites in the system may not abundant. For example, polar groups on packing surface or on polymer probe which are responsible for adsorption may be present in a low concentration, or active surface of adsorbent is small, or stationary phase available for partition has a small volume. In this case, the calibration curve shift is usually more pronounced for smaller macromolecules. This is explained by increasing packing surface or stationary phase volume which is available for interactions of smaller polymer species because they can also penetrate narrower packing pores.

When $\epsilon$ increases, the shape of calibration dependences is changed (curve 4). It seems that at sufficiently high $\epsilon$ values, polymer species are strongly attracted by the column packing so that they may change their conformation, de-coil, and "reptate" also into narrow pores from which they would be excluded in the regime of low $\epsilon$ [13].

If further increased, segmental interaction energy may reach its critical value, $\epsilon = \epsilon_{cr}$, at which entropic (exclusion) and enthalpic contributions mutually compensate and $\Delta G$ assumes zero value:

$$\Delta G = \Delta H - T\Delta S = 0 \quad (3)$$

In this situation, chromatographic distribution constant $K$ does not depend on polymer molar mass [14,15] (curve 5):

$$K \sim \exp\left(\frac{\Delta G}{RT}\right) \sim 1 \qquad (4)$$

At even higher $\epsilon$ values enthalpic interactions prevail over entropic contribution and the calibration dependences exhibit typical patterns. Retention volumes rise with increasing polymer molar mass (Fig. 1, curves 6 and 7) [8].

When $\epsilon$ reaches a certain limit, polymer probes with higher molar masses may be fully retained within HPLC columns. This is often observed in the systems with dominating adsorption retention mechanism. In the scheme in Fig. 1, the corresponding situation is depicted with open circles.

As mentioned, the overall effect of segmental interactions of macromolecules with HPLC column packings is often not additive. This happens if, for steric reasons, only few segments of a macromolecule may simultaneously interact with the column packing. The relative number of interacting segments may depend on polymer molar mass. As result, unusual shapes of calibration curves may be observed (for example curves 8 and 9 in Fig. 1).

Evaluation of column interactivity with macromolecular probes may allow assessment of the role of molecular size. For example, the $V_{h,1,1}$ value in Fig. 1 would indicate the size of test macromolecules which is just small enough to allow the presence of weak enthalpic interactions with column packing while the $V_{h,1,2}$ value belongs to macromolecules which are large enough to start exerting strong enthalpic interactions.

Macromolecular column test probes may allow, at least to some extend, discrimination of adsorption and partition effects.

If one wants to evaluate polar (adsorption) interactivity of a $C_{18}$ HPLC column, partition effects must be suppressed. This can be attained, if eluent is a good solvent for test polymer probes, much better than the solvated $C_{18}$ phase. In this case, the driving force for partition of macromolecules is small or even absent. At the same time, the eluent is not strong (polar) enough to suppress silanophilic interactions between the polymer probes and the column packing. Comparison of elution behavior of polymer probes differing in their polarity can give information about column adsorptivity. This was demonstrated in our preceding paper [1] and additional results will be presented in this article. In contrast, if partition properties of different $C_{18}$ phases are to be evaluated, adsorption effects should be eliminated. The elution behavior is to be compared for non-adsorbing, non-polar polymer probes using a series of thermodynamically poor, polar mobile phases which promote partition of macromolecules in favor of stationary phase but suppress polymer adsorption. The corresponding results will be shown in the following paper in this series.

## 3. Experimental

The HPLC apparatus and procedures applied have been described in Part I of this series [1]. In brief, the pump was Model 510 (Waters, Milford, MA, USA). It was operated at 1 ml/min. The manual sample injecting valve was Model 7725 (Rheodyne, Cotati, CA, USA) with a sample loop of 50 μl. Polymers in effluent were detected by an evaporative light scattering device DDL-21 (Eurosep, Cergy-Saint-Pontoise, France). Column temperature was kept at 30±0.01 °C using a custom made oven and a water thermostat. The data were processed with the help of Chroma software (Chromtech, Graz, Austria).

A series of different columns was included in this study: ACE from ACT, Cadenza from Imtakt, Extend $C_{18}$ from Agilent, Luna $C_{18}$ and Synergi MAX-RP, both from Phenomenex, Kromasil $C_{18}$ from AKZO Nobel, and TSK gel ODS from Tosoh. All these packings exhibited negligible or low polar interactivities toward poly(methyl methacrylate)s in the previous study [1]. Producers of two of them kindly agreed to publication of the present data namely Tosoh, Tokyo, Japan (TSK gel ODS No. 1 in Table 1 [1]) and AKZO Nobel, Bohus, Sweden (Kromasil $C_{18}$, 100 Å, No. 8 in Table 1 [1]). The latter packing exhibited a small shift of the universal calibration curve for PS and PMMA in toluene (''b'' type) while the courses of above universal calibration curves were practically identical for the former column (''a'' type).

Table 1
$K$ and $a$ values of the viscosity law (Eq. (2)) at 30 °C

| Polymer–solvent system | $K$ (ml/g) | $a$ | Ref. |
|---|---|---|---|
| PS–THF | 0.0128 | 0.712 | [16] |
| PMMA–THF | 0.0128 | 0.69 | [11] |
| PEO–THF | 0.00143 | 0.76 | [17] |
| P2VP–THF[a] | 0.0014 | 0.906 | [18] |
| PS–toluene | 0.0092 | 0.72 | [11] |
| PMMA–toluene | 0.007 | 0.71 | [11] |

[a] $K$ and $a$ values for P2VP in THF are of limited precision only. Solubility of P2VP in THF strongly depends on the presence of a small amount of water [18]. Because of high hydroscopicity of THF, humidity traces may be present in some eluents. High $a$ value for P2VP–THF system indicates a stiff conformation of macromolecules. All other systems exhibit similar $a$ values around 0.7. This is typical for coiled structure of macromolecules and "good" solvent quality.

A column which contained added surface polar groups (Aquasil from Thermo Hypersil-Keystone, Bellefonte, PA, USA) was included in the present study.

Column packing particle sizes were 5 or 10 μm and column sizes ranged from 150×7.8 to 250×4.6 mm.

Analytical grade solvents were used as eluents, viz. toluene from Slavus (Bratislava, Slovak Republic), tetrahydrofuran (THF) from Merck (Darmstadt, Germany) and dimethylformamide (DMF) from Scharlau (Barcelona, Spain). They were vacuum distilled before use. THF was stabilized with 0.02% of butylated $p$-cresol. Mixed eluents were prepared by weighing with a KERN 572-45 (KERN, Ibstadt, Germany) with sensitivity ±0.1 g.

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. Polystyrenes were from Pressure Chemicals, Pittsburgh, PA, USA (molar mass range from 0.666 to 1200 kg/mol), while PMMAs of low stereoregularity were a gift from Dr W. Wunderlich (Röhm, Darmstadt, Germany) ($M$ range from 16 to 613 kg/mol). Poly(ethylene oxide)s were from Toso (Shinnanyo, Japan) ($M$ range from 0.4 to 860 kg/mol), poly(2-vinyl pyridine)s were from Polysciences (Warrington, PA, USA) and PSS (Mainz, Germany) ($M$ range from 3 to 1260 kg/mol).

$K$ and $a$ values in Eq. (2) used for $V_h$ calculations are summarized in Table 1.

## 4. Results and discussion

Universal calibrations obtained for PS and PMMA in toluene, as well for PEO and P2VP in THF for TSK gel ODS, Kromasil $C_{18}$, 100 Å, and Aquasil $C_{18}$, 100 Å are shown in Figs. 2–4, respectively.

For the former two columns, the courses of universal dependences for PS and PMMA probes in toluene are similar. This indicates that the materials are end-capped [1]. The calibration curves for PS and PMMA in toluene for Aquasil, however, exhibit rather different shapes. Polar macromolecules, especially those of PEO and P2VP seem to be fully excluded from the pores of Aquasil which contained additional surface polar groups. In contrast, non-polar PS elutes from Aquasil in toluene in the SEC mode. So far, we have no reasonable explanation for this result. Also, we do not know if the small shifts of "exclusion" (interparticulate) retention volumes in the sequence PMMA>PEO>P2VP can be con-

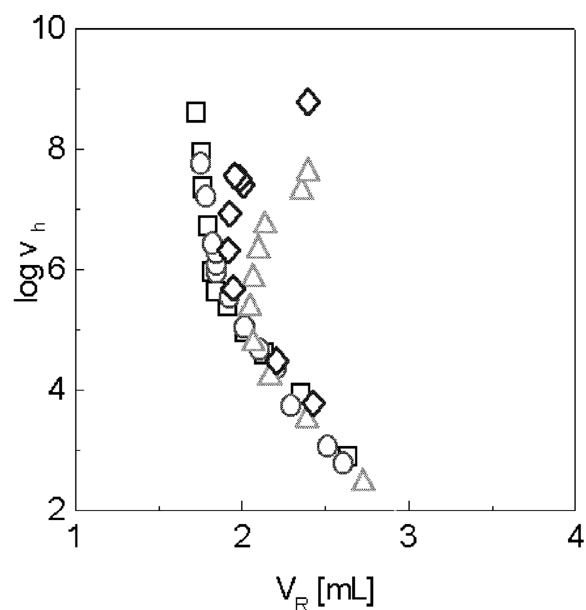


Fig. 2. Universal calibration dependences for PS in toluene (□), PMMA in toluene (○), PEO in THF (△) and P2VP in THF (◇) for TSK gel ODS.

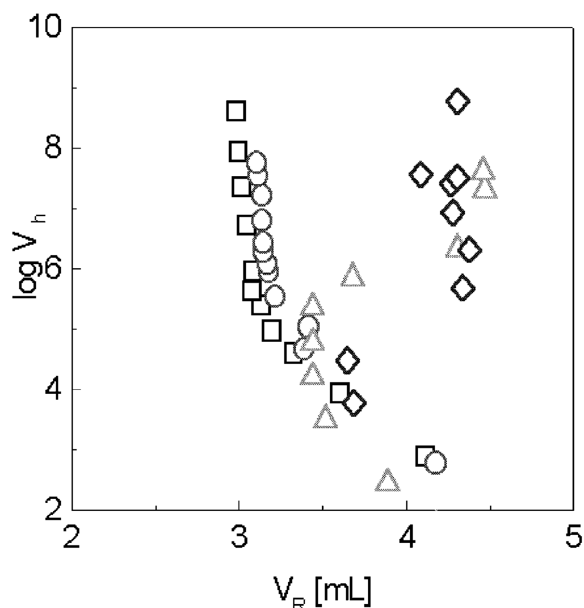


Fig. 3. Universal calibration dependences for PS in toluene (□), PMMA in toluene (○), PEO in THF (△) and P2VP in THF (◇) for Kromasil $C_{18}$, 100 Å.

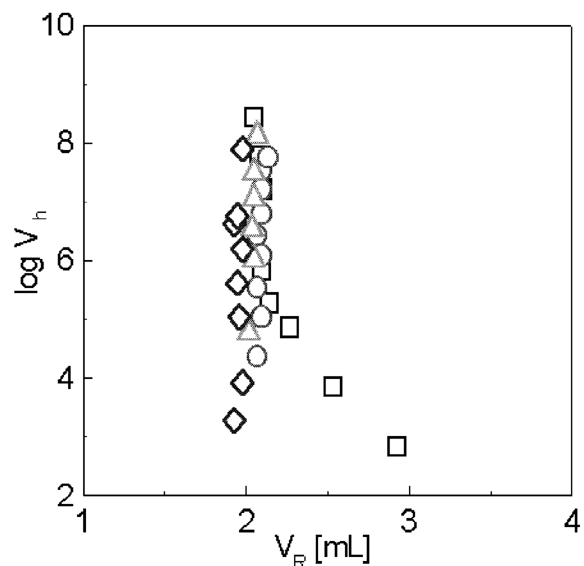


Fig. 4. Universal calibration dependences for PS in toluene (□), PMMA in toluene (○), PEO in THF (△) and P2VP in THF (◇) for Aquasil $C_{18}$, 100 Å.

sidered as a significant manifestation of the repulsion difference. After all, exclusion retention volumes for PS and PMMA coincide in the framework of experimental errors.

THF is a relatively strong eluent concerning silanophilic interactions. It largely though not entirely suppresses adsorption of PMMA on bare silica gels [19,20]. However, this eluent is not able to compete for free silanols with the polar segments of PEO and P2VP. Both latter polymers are fully retained within columns packed with the non-modified silica gels (D. Berek, unpublished results).

We believe that THF is a much better solvent for all polymers used for column tests than a ''solution'' of $C_{18}$ groups in THF. Therefore, partition of macromolecules in favor of solvated $C_{18}$ phase should not augment retention volumes of polymer probes. In contrast, poor solubility of polar macromolecules in the $C_{18}$ stationary phase may cause some decrease of retention volumes. This can be observed for Kromasil columns with PEO probes possessing lower molar masses, below $V_{h,l,2}$ value. Very polar –OH end groups in PEO chains may cause repulsion with the $C_{18}$ phase. The relative role of the end group repulsions decreases with increasing molar mass of the test probe and would be compensated with sample adsorption at the limiting value $V_{h,l,2}$. Above $V_{h,l,2}$ polymer retention volumes start to rise rapidly with increasing molar mass of polymer probes (back-turn courses, Figs. 2 and 3).

This limiting value of $V_h$ for PEO probes is lower for Kromasil than for the TSK column. Similarly unusual shapes of universal calibration dependences can be seen for the P2VP probes in THF, however, the sequence of $V_{h,l,2}$ values is reversed.

It is to be noticed that $K$ and $a$ values were determined for PEO samples in the molar mass range $8.5 \cdot 10^4$–$8.6 \cdot 10^5$. These values were used also for the calculation of $V_h$ values for much lower molar mass values where viscosity law (Eq. (2)) exhibits limited validity [11].

Further speculation about the significance of these results will need highly precise $V_R$ measurements using large volume columns or several small volume columns connected in series. Further appropriate polymer probes should be included as well. It is also important to correlate measurements with polymer probes and with low molar mass test substances, as

well as to consider the possible ion character of P2VP. Macromolecules of PEO can contain further highly polar groups created in the course of their oxidization.

The existence of the back-turn courses of the universal calibration dependences and the $V_{h,l,2}$ values was observed also with other mentioned silica gel $C_{18}$ phases (results not shown) using P2VP and PEO polymer probes and may be considered a general phenomenon. An explanation for this unusual shape of log $V_h$ and log $M$ vs. $V_R$ dependences was proposed in the previous paper [1]. Macromolecules which reptate among $C_{18}$ groups to "find" polar surface groups must be simultaneously attached to several adsorption sites in order for their $V_R$ to start increasing. Due to steric reasons this can be attained only at a certain limiting molar mass ($M_l \rightarrow V_{h,l,2}$) (Fig. 5). The corresponding phenomenon is called "U-turn adsorption".

Next, retention volumes of PEO and P2VP were determined in THF mobile phases to which various amounts of dimethyl formamide were added (Figs. 6–11). DMF very strongly interacts with silanols and efficiently suppresses interactions of many polar polymers including PEO and P2VP with various bare $SiO_2$ based column packings [21] and also with other materials exhibiting polar interactivity. For example, it was found [21] that 15 vol.% of DMF fully suppressed adsorption of PEO containing copolymers on an interactive SEC PS/DVB column packing [22]. Unfortunately, $K$ and $a$ constants in viscosity law are not known in mixtures of THF plus DMF for polymers under study. Therefore, calibration dependences of log $M$ vs. $V_R$ were compared instead of universal calibration dependences of log $M[\eta]$ vs. $V_R$. The comparison of the former calibration dependences is less precise because the effect of thermodynamic quality of eluent on the size of macro-

Fig. 5. Schematic representation of the "U-turn adsorption". For explanation see the text.

Fig. 6. Calibration dependences log $M$ vs. $V_R$ for P2VP in THF (□), THF–DMF (85/15) (○) and THF–DMF (70/30) (△) for Aquasil $C_{18}$, 100 Å.

Fig. 7. Calibration dependences log $M$ vs. $V_R$ for PEO in THF (□), THF–DMF (85/15) (○) and THF–DMF (70/30) (△) for Aquasil $C_{18}$, 100 Å.

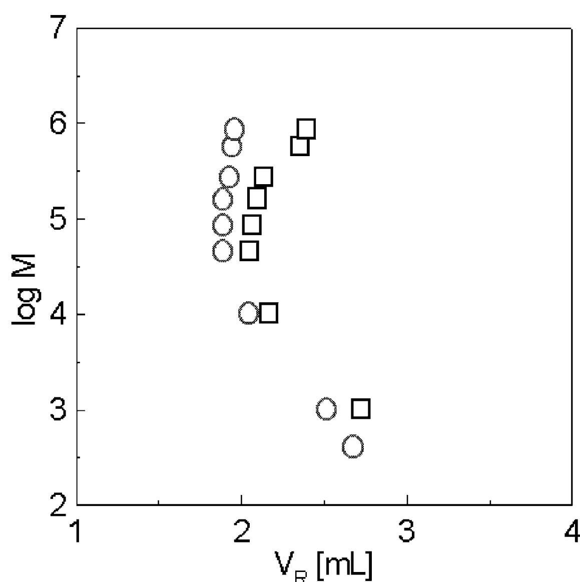


Fig. 8. Calibration dependences log $M$ vs. $V_R$ for PEO in THF (□), and THF–DMF (85/15) (○) for TSK gel ODS.

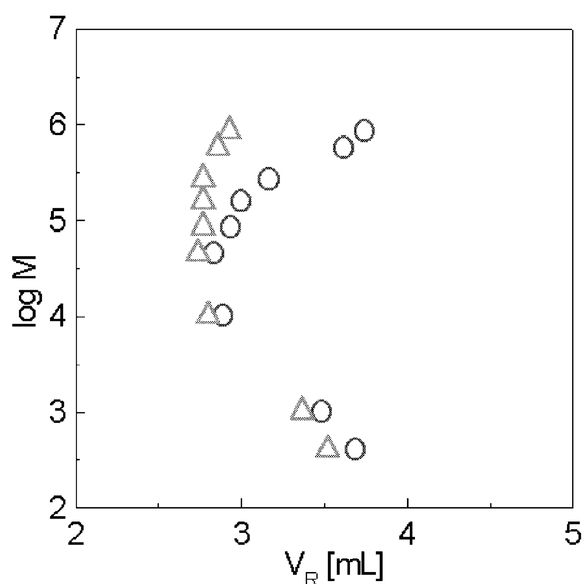


Fig. 10. Calibration dependences log $M$ vs. $V_R$ for PEO in THF–DMF (85/15) (○) and in THF–DMF (70/30) (△) for Kromasil $C_{18}$, 100 Å.

molecules in solution is not considered. Nevertheless, several interesting conclusions can also be drawn in this case.

Penetration of macromolecules of PEO and P2VP into Aquasil with added surface polar groups is allowed with 15% of DMF (Figs. 6 and 7).

The calibration dependences still exhibit a slight back-turn shape for high molar mass probes. Addi-

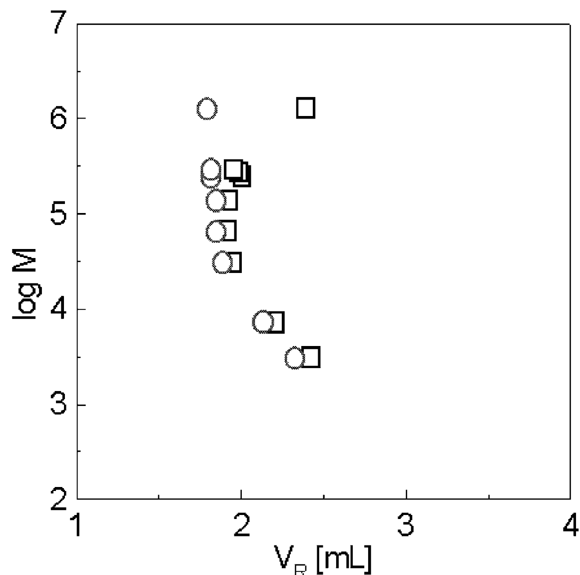


Fig. 9. Calibration dependences log $M$ vs. $V_R$ for P2VP in THF (□), and THF–DMF (85/15) (○) for TSK gel ODS.

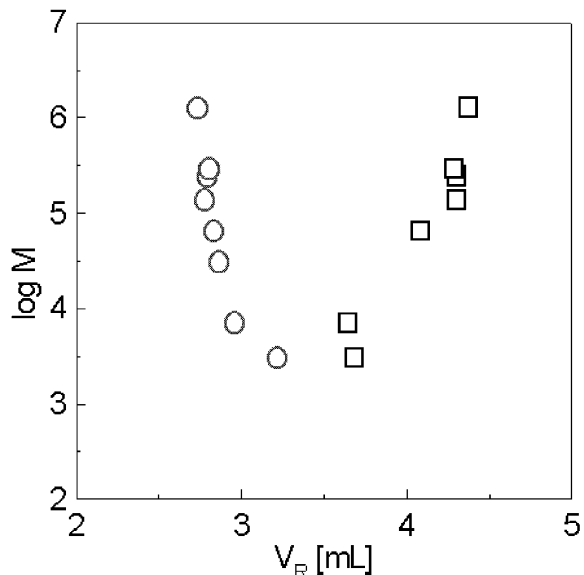


Fig. 11. Calibration dependences log $M$ vs. $V_R$ for P2VP in THF (□) and in THF–DMF 85/15 (○) for Kromasil $C_{18}$, 100 Å.

tion of 30% of DMF lead to a practically regular, non-perturbed SEC behavior. Similar shapes also assume calibration dependences for TSK (Figs. 8 and 9) column.

We believe that the minimum amount of DMF needed for a non-disturbed SEC elution of PEO and P2VP from TSK column lies in the area of 15%. This is a much lower value than in the case of Aquasil. Kromasil seems to exhibit even stronger silanophilic interactivity towards PEO than Aquasil (Figs. 10 and 11).

More than 30% of DMF would be needed to attain a non-disturbed SEC elution of PEO from this column. In this way, higher silanophilic interactivity was confirmed for Kromasil in comparison with TSK. Qualitative comparison of retention volumes for PEO and P2VP at particular molar masses reveals large shifts within each column. As mentioned, however, comparisons of the $V_h$ values will be more conclusive.

Important information on the column retentivity would be obtained also from the polymer probe recoveries. Generally, sample recoveries dropped dramatically with increasing sample molar masses for all systems exhibiting back-turn calibration dependences. Unfortunately, the response of evaporative light scattering detectors in general and that of DDL-21 detector in particular, depends on eluent composition and often also on the polymer molar mass (D. Berek, unpublished results). This complicates quantitative evaluation of sample recovery. It is anticipated that the fractions of polymer probes (the highest molar masses) which remain non-eluted from the HPLC columns may alter retention properties of column packings. Therefore, for a quantitative study, columns should be periodically flushed with a strong solvent to remove macromolecules permanently adsorbed from a weaker eluent. Then, column should be re-equilibrated with initial eluent before further polymer probe injection. This would make the tests less practical.

Solid supports for bonded phases which are largely used for HPLC of small molecules have nominal pore diameter of 10 nm (100 Å) or even only 6 nm. $C_{18}$ groups protruding over support solid surface reduce effective pore size. The SEC exclusion limit of such column packings lies in the area of molar masses from 10 to 40 kg/mol only. Correspondingly, the range of polymer probes which can penetrate the packing pores under weak interaction regime is rather limited. Moreover, these molar masses are situated at the limits of validity of viscosity law for coiled linear polymers (Eq. (2)). This further decreases the feasibility of quantitative comparisons of polymer retention data. Differences in $V_R$ values for "excluded", large macromolecules can be only assessed when interactions between polymer probes and column packing are rather high, that is under strong interaction regime. Evaluation of these strong interactions may be important for numerous practical systems. Precision of the presented method certainly increases also in the area of weak interactions, with increasing packing pore diameter. However, in the case of the wide pore packings (e.g. in the range of 50 nm), comparison of retentivity for polymeric and low molar mass probes may be difficult because of decreased precision of measurements in the low molar mass area. The compromise could represent packings with intermediate pore sizes of 20–30 nm.

## 5. Conclusions

The potential of macromolecular probes for evaluation of HPLC column packings is assessed. Silanophilic interactivities of selected end-capped silica gel $C_{18}$ column packings were compared on the basis of retention volumes of highly polar polyethylene oxide and poly(2-vinyl pyridine) probes possessing different molar masses. Tetrahydrofuran and THF–dimethylformamide mixtures were used as eluents. THF cannot efficiently suppress interactions of highly polar macromolecules with the residual silanols. The addition of appropriate amount of DMF to eluent removes silanophilic interactions so that macromolecular probes elute in the regular, non-perturbed size-exclusion mode.

The results obtained clearly show that large macromolecules can penetrate narrow packing pores and can also reptate along $C_{18}$ groups provided their interactivity with the column packing surface (represented, for example, by the $\epsilon$ value) is high enough. It is supposed that macromolecular probes allow tracing polar, mainly silanophilic interactions in silica $C_{18}$ phases which are responsible for the adsorptive retention of analytes. Polar interactions

can be at least partially discriminated from hydrophobic interactions which lead to the thermodynamic partition of analytes within HPLC columns. In this way, column testing with macromolecular probes may produce valuable additional information on the properties of HPLC stationary phases. Differences among columns from different producers can be easily identified as well.

Unexpected retention behavior of polar polymeric probes was observed in numerous HPLC systems. Lower molar mass species eluted in the non-perturbed SEC mode while larger macromolecules exhibited rising retentivity which rapidly augmented with increasing sample molar mass. This supports the hypothesis of ''U-turn adsorption'' which assumes that macromolecules must be large enough to bend and attach to the packing surface (on free silanols) simultaneously on several sites. Further experiments and calculations will be, however, needed to confirm this hypothesis. The steric hindrances within $C_{18}$ bonded phases and conformational limitations of macromolecular kinks should be considered.

The drawbacks of the proposed method include its relatively low sensitivity. The procedure works in the narrow (SEC) range of retention volumes and columns with larger volumes are needed to improve precision and accuracy of data obtained.

It is expected that application of macromolecular test probes will provide valuable input mainly in the course of development of new and improved HPLC column packings and will help better understanding of the HPLC retention mechanisms.

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## References

[1] D. Berek, J. Chromatogr. A 950 (2002) 75.
[2] H.A. Claessens, M.A. van Straten, C.A. Cramers, M. Jezierska, B. Buszewski, J. Chromatogr. A 826 (1998) 135.
[3] R. Kaliszan, M.A. van Straten, M. Markuszewski, C.A. Cramers, H.A. Claessens, J. Chromatogr. A 855 (1999) 455.
[4] H.A. Claessens, Trends Anal. Chem. 20 (2001) 563.
[5] M.R. Euerby, P. Petersson, LC·GC Eur. 13 (2000) 665.
[6] S.B. Schram, D.H. Freeman, J. Liq. Chromatogr. 3 (1980) 403.
[7] R.N. Nikolov, W. Werner, I. Halasz, J. Chromatogr. Sci. 18 (1980) 207.
[8] D. Berek, Prog. Polym. Sci. 25 (2000) 873.
[9] Discovery HS PEG, Supelco, Bellefonte, PA, 2002
[10] Z. Grubisic, P. Rempp, H. Benoit, J. Polym. Sci. B5 (1967) 753.
[11] M. Kurata, Y. Tsunashima, in: J. Brandrup, E.H. Immergut, E.A. Grulke (Eds.), Polymer Handbook, 4th ed, Wiley, New York, 1999, p. 1, Chapter VII.
[12] D. Berek, D. Bakos, T. Bleha, L. Soltes, Makromol. Chem. 176 (1975) 391.
[13] M. Snauko, D. Berek, presented at the 25th International Symposium on High Performance Liquid Phase Separation and Related Techniques, Maastricht, 17–22 June 2001, book of abstract, 2002, p. 56.
[14] B.G. Belenkii, E.S. Gankina, G.E. Kasalainen, M.Z. Tennikov, in: M. Potschka, P.L. Dubin (Eds.), Strategies in Size-exclusion Chromatography, ACS Symposium Series, Vol. No. 635, American Chemical Society, Washington, DC, 1996, p. 274.
[15] A.A. Gorbunov, A.M. Skvortsov, Adv. Colloid Interface Sci. 62 (1995) 31.
[16] T. Spychaj, D. Lath, D. Berek, Polymer 20 (1979) 437.
[17] D. Lath, Polymer Institute SAS, Bratislava, Slovak Republic, personal communication.
[18] P. Kilz, Polymer Standards Service, Mainz, Germany, personal communication.
[19] O. Chiantore, J. Liq. Chromatogr. 7 (1984) 1.
[20] D. Berek, M. Janco, G.R. Meira, J. Polym. Sci. A: Polym. Chem. 36 (1998) 1363.
[21] S.H. Nguyen, D. Berek, I. Capek, O. Chiantore, J. Polym. Sci. A: Polym. Chem. 38 (2000) 2284.
[22] D. Berek, in: C.S. Wu (Ed.), Column Handbook for SEC, Academic Press, New York, 1999, p. 445, Chapter 15.