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Evaluation of high-performance liquid chromatography column retentivity using macromolecular probes I

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Abstract

The application of macromolecular probes is proposed for evaluation of HPLC column retentivity. The idea is tested with a set of different commercial silica C_{18} reversed-phases. For comparison, porous glass C_{18} and polystyrene/divinylbenzene column packings are also included. Polar, mainly silanophilic interactions are evaluated. The retention volumes of a series of narrow molar mass distribution polystyrenes (PS) and poly(methyl methacrylate)s (PMMA) in toluene eluent are compared. Toluene is a weak mobile phase concerning silica gel surface and it promotes adsorption of PMMA on silanols, while PS is not adsorbed from toluene. Simultaneously, toluene is a thermodynamically good solvent for both polymers so that extensive partition in favour of stationary phase is not probable. Differences in retention behaviour of PS and PMMA indicate presence of abundant free silanols on the surface of some reversed-phases. These silanols are accessible even for large macromolecules of PMMA. Pore diameter and pore volume of the column packing can be semiquantitatively evaluated from the elution data of PS in toluene in the course of retentivity tests.

Keywords: Stationary phases, LC; Macromolecular probes; Column retentivity; Polystyrene; Poly(methyl methacrylate)

1. Introduction

The retention properties of high-performance liquid chromatographic (HPLC) columns belong to their most important characteristics. To test and compare the HPLC column retentivities, a series of low molar mass probes bearing various functional groups is eluted and the differences in retention volumes V_R are evaluated. The nature of functional groups, overall polarity, basicity/acidity, etc., of the test substances is considered as well as the size and shape of their molecules (for reviews see e.g., [1–4]). Valuable information on the HPLC column retentivities are obtained in this way. The conclusions drawn from different sets of test substances may, however, differ. This is, for example, the case when evaluating silanophilic interactions of various silica gel C_{18} phases [1]. Many presently available HPLC column packings are heterogeneous as to their surface/bonded phase properties [5]. The accessibility of the heterogeneities may be selective and this could be one of the reasons for lacking unambiguity of the present column test results. It is anticipated that valuable complementary data on the column retentive properties can be obtained applying series of chemical homologs as test probes, for example, paraffins or esters. The latter substances possess the same polar functional group, however, their effective

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molecular sizes differ. Alternatively, the sets of probes could be applied containing repeated functional groups viz. oligomers and polymers. The problems with oligomeric testing probes are caused by their end groups which usually differ in chemical nature from the macromolecular chain. The relative role of end groups is reduced when the molar mass of test substances increases. Eventually, the end group effect may become negligible in the high polymer area. A general problem with the synthetic oligomer and polymer test substances presents the non-uniformity in the size of their molecules. One has to consider the mass and number ''averages'' of molar masses ($\overline{M}_w$ and $\overline{M}_n$, respectively) that is the mean values of molar masses (MMM) and also the molar mass ''distributions'' (MMD) of polymers. The width of molar mass distribution of polymers can be represented by the ratio $\overline{M}_w/\overline{M}_n$. Presently, several chemically different ''narrow polymer standards'' are readily available. These possess different MMM values and narrow MMD with $\overline{M}_w/\overline{M}_n$ values between 1.01 and 1.2. Polymers with broader but symmetrical MMD can be still used for testing column retentivity. In a reasonable approximation, the peak retention volumes, V_R, of such polymers are supposed to correspond to the molar mass of macromolecules most abundant in the test sample.

In this contribution we discuss introductory results of the new approach to HPLC column evaluation using the high molar mass polymer probes.

2. Experimental

The HPLC assembly consisted of an eluent container protected from the moisture absorption, a pumping system Model 510 (Waters, Milford, MA, USA), a manual sample injecting valve Model 7725i (Rheodyne, Cotati, CA, USA), a custom-made column oven (Chromtech, Graz, Austria) connected with a water thermostat Model RM6 (Lauda, Koningshofen, Germany) and an evaporative light scattering detector DDL-21 (Eurosep, Cergy-Saint-Pontoise, France). The data were collected and processed using software Chroma (Chromtech). The flow-rate was set to 1 ml/min. The pumping resettability was controlled by a burette. Injected volume was 50 μl, and sample concentration was 1 mg ml^{-1}. Temperature of column oven was kept at 30±0.01 °C. The eluent was preheated using a capillary loop of over 2 ml volume.

Fifteen different columns from the following producers were tested (alphabetically): Advanced Chromatography Technologies (ACT), (Aberdeen, UK); Agilent (Waldbronn, Germany); Akzo Nobel (Bohus, Sweden); Imtakt (Kyoto, Japan); Macherey-Nagel (Duren, Germany); Lenchrom (St Petersburg, Russia); Phenomenex (Torrance, CA, USA); Polymer Laboratories (Church Stretton, UK); Tessek (Prague, Czech Republic); Tosoh (Tokyo, Japan).

Most columns were filled with C_{18} silica gels both end-capped and non-end-capped. Their nominal pore sizes were in the range of 10 nm except for two columns with the pore diameter of 30 nm and 50 nm, respectively. For comparison, we evaluated also one column packed with C_{18} porous glass and one column packed with polystyrene/divinylbenzene (PS/DVB) mesoporous HPLC resin. The columns are denoted with numbers (Table 1) which, however, do not correspond with the sequence of producers given above. Our intention was to test the idea of column evaluation with polymers rather than produce the assessment of particular columns. Only one column of each kind was evaluated and most of them were gifts, anyway. The author is, however, ready to furnish further information on the columns tested. Table 1 gives some column characteristics available.

Analytical grade toluene (Slavus, Bratislava, Slovakia) was used as mobile phase. It was distilled immediately before use.

Two sets of model polymers with different mean molar masses were applied. They were prepared by anionic polymerization. Narrow molar mass distribution polystyrenes (PS) were bought from Pressure Chemicals, Pittsburgh, PA, USA. Their MMM ranged from 0.666 to 1,260 kg/mol and their polydispersity values ($\overline{M}_w/\overline{M}_n$) laid between 1.06 and 1.12. Poly(methyl methacrylate)s (PMMA) of low stereoregularity and of medium broad molar mass distribution [6] were a gift from Dr W. Wunderlich (Rohm, Darmstadt, Germany). Their MMM were between 16 and 613 kg/mol and polydispersites $\overline{M}_w/\overline{M}_n$ ranged between 1.14 and 1.85.

3. Results and discussion

In this stage of the HPLC column testing by

Table 1
Some characteristics of HPLC column tested

Column no.	Remarks	Column size (mm)	Particle size (μm)	End-capping	PMMA in toluene	Column history
1		250×4	5	Yes	Shift of V_R	New
2	(c)	2×(150×4)	8	Yes	Similar to PS	New
3	(d)	250×4	5	Yes	Shift of V_R	Used
4	(e)	250×4	7	Yes	Shift of V_R	Used
5		250×4.6	5	Yes	Similar to PS	New
6		250×4.6	5	No	Full retention	New
7		250×4.6	5	Yes	Similar to PS	New
8		150×7.8	5	Yes	Shift of V_R	New
9	(f)	75×4.6	5	Yes	Shift of V_R	New
10		250×4	10	Yes	Full retention	New
11	(a)	250×4	10	No	Full retention	New
12		2×(150×3.2)	5	?	Full retention	New
13		250×4.6	5	Yes	Similar to PS	New
14		250×4.6	4	Yes	Similar to PS	New
15	(b)	150×4.6	8	–	Shift of V_R	New

Remarks: (a) Polymeric phase; (b) PS/DVB packing; (c) porous glass; (d) pore diameter 30 nm; (e) pore diameter 100 nm; (f) end-capping with a polymer.

polymer probes the ''SEC universal calibration dependences'' were evaluated; that is the plots of logarithm of polymer hydrodynamic volumes V_h versus retention volume V_R [7]. In the absence of enthalpic interactions between macromolecules and column packing the universal calibration curves coincide for various kinds of polymers and eluents. One speaks about ideal size exclusion chromatographic behaviour. On the contrary, mutual shifts of universal calibration dependences indicate a presence of enthalpic interactions in the chromatographic system [8].

The mean value of V_h is defined as a product of the most abundant molar mass (M) in the polymer sample and the corresponding limiting viscosity number [η]. The values of [η] can be either directly determined in solvent which is used as eluent at the temperature of the experiment or calculated from the Kuhn–Mark–Houwink–Sakurada viscosity law:

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

where K and a are constants for given polymer/solvent system and temperature. Numerous K and a values can be found in literature and many of them are collected in the ''Polymer Handbook'' [9]. We applied values $K = 9.2\times10^{-3}$ ml/g and $a = 0.72$ for polystyrene and $K = 7\times10^{-3}$ ml/g and $a = 0.71$ for poly(methyl methacrylate). Evidently, Eq. (1) allows us to interpolate and to some extent also to extrapolate the [η] values. However, the validity of Eq. (1) for polymer molar masses below about 10 kg/mol is limited. This applies also for some PS samples used in this study. The PS probe with MMM 10.1 kg/mol is denoted with an arrow in our Figures.

Retention volumes of PS and PMMA samples in toluene were compared. Toluene is a weak solvent considering bare silica gel. It promotes adsorption of many medium polarity polymers including PMMA [10] on the silica gel surface. One can say that toluene is an effective ''adsorli'' for PMMA. As a result high PMMA polymers are fully retained from toluene within the bare silica particles [10] irrespective of their porosity. We have found that even large macromolecules which are excluded from the narrow pores of column packings in the purely entropic separation mode (size exclusion chromatography) can ''reptate'' into the narrow packing pores provided eluent is weak enough, and strong attractive interactions do exist between column packing and macromolecules [11]. The reptation process is accompanied with large conformational changes of polymer chains (''de-coiling'' of macromolecules). In contrast to PMMA, polystyrene is a rather nonpolar polymer, which is not retained by adsorption on the silica gel surface from toluene.

It is believed that toluene does not promote

partition of PS and PMMA in favour of C_{18} phase because aliphatic alkanes are non-solvents for both polymers. Further, toluene is a similarly ''thermodynamically'' good solvent for both PS and PMMA. Therefore, we anticipate that the differences between retention of PS and PMMA in toluene on the silica C_{18} phases are associated mainly with the polar interactions of PMMA with the free silanols present on the silica gel surface. In other words, the polar (silanophilic) interactivity of the C_{18} column packings can be evaluated comparing retention volumes of PMMA and PS in toluene. Evidently, other appropriate pairs of polymers can be applied in connection with eluents of low to intermediate polarities.

It may be possible to estimate also the non-polar interactivity of the C_{18} bonded phases using polymer probes. In this case, the mobile phases should be applied, which strongly interact with free silanols and thus prevent adsorption of macromolecules on the silica gel surface. If such mobile phases are thermodynamically poor solvents for polymer probes they will promote partition of macromolecules in favour of solvated bonded phase. The extent of partition will depend on the polymer probe nature, on the thermodynamic quality of eluent for polymer probes and on the bonded phase properties.

The choice of appropriate single solvents for testing the HPLC column interactivity is, unfortunately, rather limited. Both the eluent strength for packing investigated and the thermodynamic quality for polymer probes can be adjusted by mixing two or several appropriate liquids. The corresponding *K* and *a* parameters must be, however, determined for each mobile phase either in the conventional way or applying an on-line HPLC viscometric detector.

Based on the results obtained with toluene eluent the columns tested in present work can be divided into the three groups:

(a) The universal calibrations of PS and PMMA in toluene coincide (an example is in Fig. 1). This behaviour is typical for the well end-capped materials in which the majority of accessible silanols is efficiently blocked.

(b) The universal calibrations for PS and PMMA are mutually shifted as demonstrated in Figs. 2 and 3. This behaviour indicates presence of adsorption sites on the packing surface which are accessible for

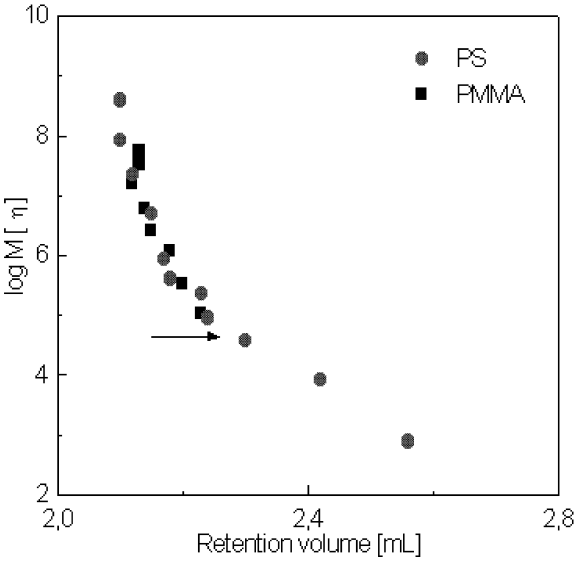

Fig. 1. Column no. 5. Universal calibration plots for PS and PMMA in toluene. The MMM 10.1 kg/mol of PS is denoted with arrow.

macromolecules. The effect of these adsorption sites on polymer retention is however relatively small, because accessible silanols are either not too active or not abundant. The latter possibility is more

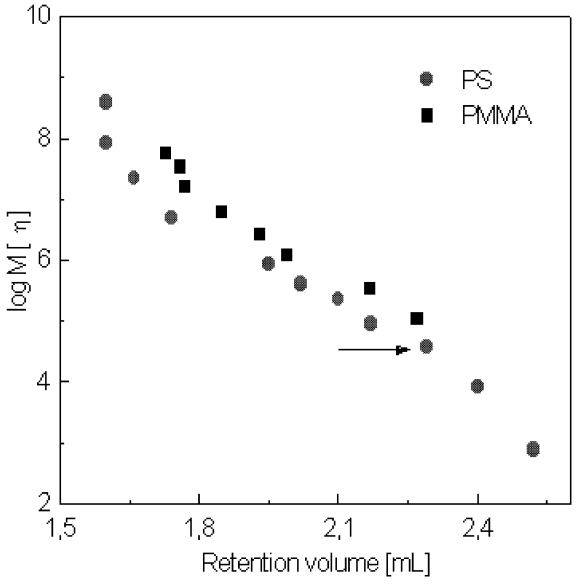

Fig. 2. Column no. 3. Universal calibration plots for PS and PMMA. The MMM 10.1 kg/mol of PS is denoted by an arrow.

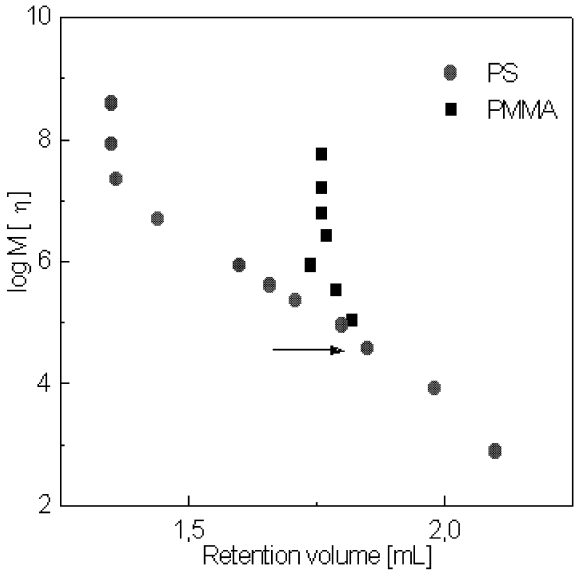

Fig. 3. Column no. 15. Universal calibration plots for PS and PMMA. Arrow denotes MMM 10.1 kg/mol of PS. PMMA elution resembles the ''critical'' behaviour [14], which is otherwise relatively rare with single component mobile phases [15]. Extensive peak broadening was observed for higher molar mass PMMA probes.

feasible for silica gel-based column packings because all kinds of silanols strongly interact with PMMA in toluene eluent [11]. On the contrary, both explanations may apply to the PS/DVB column packing. Surprisingly, the surface of this latter packing seems to accommodate a non-negligible amount of polar groups. This was observed for a series of size exclusion chromatographic packings from different producers [12,13]. The polar groups on the packing surface interact with PMMA in mobile phases of low polarity and bring about its unexpected retention. The polar groups can be introduced into the non-polar PS/DVB materials by auxiliary compounds used during the polymer microbeads synthesis. One source of polar sites may be the ''protective colloids''. These are selected water soluble polymers, added to the polymerization mixtures in order to improve sphericity of packing particles. The protective colloids, as well as further additives to the polymerization systems such as detergents, initiators, transfer agents, etc. may be incorporated into the column packing matrix.

(c) PMMA probes are fully retained within column packing from the toluene mobile phase. In other words, polymer probes are not eluted from columns and their retention volumes are ''infinite''. The retained macromolecules can be quantitatively released by an appropriate desorption promoting liquid (a desorli), for example, by tetrahydrofuran in the case of PMMA/silica gel system [10,11]. The full retention behaviour is typical for the non-end-capped or poorly end-capped column packings in which the free silanol concentration exceeds a certain limiting value.

An inspection of SEC calibration dependences for an unretained polymer allows to estimate also effective mean pore size and pore size distribution of the packing, as well as to evaluate the pore volume and interparticle volume of the HPLC column [16–18]. In other words, the semiquantitative values of several physical parameters of the column packing matrices can be obtained as a side-product of the retentivity tests using polymer probes.

The PS universal calibration curves are compared for five different column packings in Fig. 4. The retention volumes of some columns were normalized considering a common value of interparticle volume to compare columns of different sizes. The pore geometry of the columns packings no. 13 and no. 14

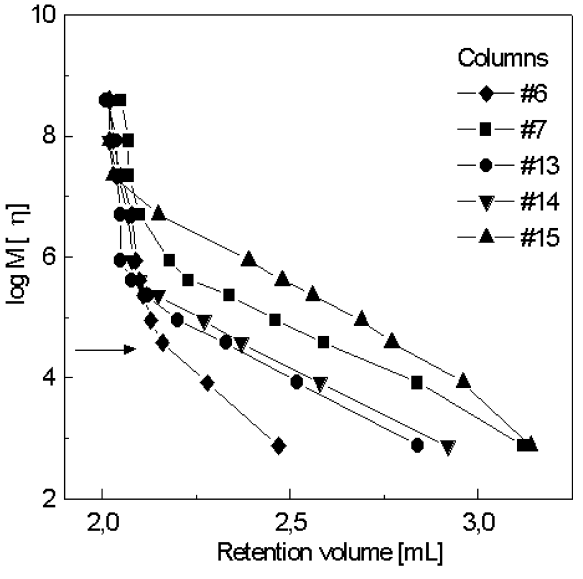

Fig. 4. Universal calibration plots for PS in toluene for selected columns.

is practically identical. Most probably the same starting silica gel was used for the synthesis of these reversed-phases. The pore diameter and pore size distribution of the packings no. 13 and no. 14 are similar to the packing no. 7; the latter possesses, however, larger pore volume. Both the pore size and pore volume of the column no. 6 is much lower than with other columns. This is typical for globular silica gels prepared by agglutination of silica sols. Pore size of the column no. 15 (PS/DVB) is the largest of the set investigated. Pore volumes of column packings no. 7 and no. 15 are comparable.

4. Conclusions

The results presented demonstrate that the differences both in retentivity and physical properties of column packings can be easily traced using polymeric test probes. It is also shown that the free surface silanols present in most silica gel C_{18} phases are accessible for large macromolecules. This indicates that macromolecules are able to de-coil and reptate among C_{18} groups to reach the silica gel surface. Alternatively, structure of particular C_{18} bonded phases may be irregular exhibiting ''patches''. Macromolecules can hardly be retained in full by attachment of just one single segment on the packing surface. Therefore ''U-turn'' adsorption of macromolecules simultaneously on two and more packing adsorption sites should be considered together with patched structure to explain full retention of test polymer probes within the HPLC silica gel C_{18} column packings.

We believe that further experimental material will reveal full potential of the HPLC column testing with polymers, especially when results will be correlated for low molecular and macromolecular test species.

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References

[1] H.A. Claessens, M.A. van Straten, C.A. Cramers, M. Jezierska, B. Buszewski, J. Chromatogr. A 826 (1998) 135.
[2] R. Kaliszan, M.A. van Straten, M. Markuszewski, C.A. Cramers, H.A. Claessens, J. Chromatogr. A 855 (1999) 455.
[3] H.A. Claessens, Trends Anal. Chem., (in press).
[4] M.R. Euerby, P. Petersson, LC–GC Europe 13 (2000) 665.
[5] B. Buszewski, M. Jezierska, M. Welniak, D. Berek, J. High Resolut. Chromatogr. 21 (1998) 267.
[6] D. Berek, M. Jančo, T. Kitayama, K. Hatada, Polym. Bull. 32 (1994) 629.
[7] Z. Grubisic, P. Rempp, H. Benoit, J. Polym. Sci. B5 (1967) 753.
[8] D. Berek, D. Bakoš, T. Bleha, L. Šoltes, Makromol. Chem. 176 (1975) 391.
[9] M. Kurata, Y. Tsunashima, Chapter VII in: J. Brandrup, E.H. Immergut, E.A. Grulke (Eds.), Polymer Handbook, 4th Edition, Wiley, New York, 1999, p. 1.
[10] S.H. Nguyen, D. Berek, Colloids Surface, A: Phys.-Chem. Eng. Aspects 162 (2000) 75.
[11] M. Šnauko, D. Berek, in: Proceedings of HPLC 2001, Maastricht (CD version).
[12] D. Berek, in: C.-s. Wu (Ed.), Column Handbook for Size Exclusion Chromatography, Academic Press, New York, 1999, p. 445.
[13] D. Berek, J. Tarbajovská, in: Proceedings of the Waters International Symposium on Gel Permeation Chromatography, Las Vegas, USA, Oct. 2000.
[14] B.G. Belenkii, E.S. Gankina, G.E. Kasalainen, M.Z. Tennikov, in: M. Potschka, P.L. Dubin (Eds.), ACS Symp. Ser., Vol. 635, 1996, p. 274.
[15] T. Macko, D. Hunkeler, D. Berek, Macromolecules, (in press).
[16] S.B. Schram, D.H. Freeman, J. Liq. Chromatogr. 3 (1980) 403.
[17] R.N. Nikolov, W. Werner, I. Halász, J. Chromatogr. Sci. 18 (1980) 207.
[18] K. Jeřábek, A. Revillon, E. Puccilli, Chromatographia 36 (1993) 259.