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Hypercrosslinked polystyrene as a novel type of high-performance liquid chromatography column packing material Mechanisms of retention

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Abstract

An experimental material, Chromalite 5HGN (Purolite, UK), that represents hypercrosslinked polystyrene as a new type of neutral stationary phase for HPLC was examined. The material contains no functional groups, but is compatible with any kind of nonpolar and highly polar mobile phase, and even with water. It is chemically resistant and thermally stable. When using aqueous organic mobile phases, Chromalite 5HGN works similar to standard C_{18} reversed-phase packings, but is characterized by much greater hydrophobicity and, sometimes, unusual selectivity. When using nonpolar mobile phases, i.e. under ''quasi normal-phase'' conditions, the retention is mostly governed by the interactions between π-electronic systems of the adsorbent and adsorbate. Adding highly polar, even hydrophilic solvents into the mobile phase, leads to a shift of retention times toward the ''reversed-phase'' kind of chromatography, which gives an additional possibility in fine tuning the column selectivity.

Keywords: Stationary phases, LC; Retention mechanism; Principal component analysis; Polystyrene stationary phases

1. Introduction

The creation of new stationary phases has always been one of the primary lines of research in chromatographic science, in particular in HPLC. Over 500 HPLC packings have been described in the literature. Nevertheless, as the result of years of development, only a limited number of types of stationary phases remained on the market. Most of the conventional HPLC separations today are performed using C_4, C_8, C_{18} bonded silicas in RP-HPLC and cyano-propyl, amino-propyl-bonded silicas or bare silica in NP-HPLC. The drawbacks of silica-based materials are well known. Most serious among them is reduced hydrolytic stability in aqueous and aqueous–organic media, which practically eliminates the possibility of regenerating a contaminated column by an intensive acidic or alkaline wash. Porous silica-based HPLC column packings have also a reduced lifetime under high pressure drops combined with flow pulsation, which results from both fracturing and re-structurization of the rigid silica matrix in highly stressed positions [1]. Polymeric packings, in particular conventional styrene–divinylbenzene copolymers, though chemically resistant and more resilient mechanically, are on the contrary, too compressible. Besides, they

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show a tendency to swell in one type of solvent and collapse in the others, which dramatically reduces the palette of allowable mobile phases [2]. Only highly crosslinked macroporous polystyrene packings in combination with appropriate eluents are used in HPLC separations [3–8].

An ''ideal'' HPLC polymeric packing should be mechanically robust, inert, pH stable, compatible with both polar and non-polar organic solvents and even water. These conditions are best met by the new generation of polymeric adsorbent materials, hypercrosslinked polystyrene [2]. Principally differing from conventional styrene–divinylbenzene copolymers, hypercrosslinked polystyrene is obtained by an extensive post-crosslinking of long polystyrene chains in the presence of excess of good solvent, preferably by introducing methylene ($-CH_2-$) bridges between phenyl groups [9]. Rigid, expanded, open-work-type hypercrosslinked networks display extremely high apparent inner surface area (up to 1000–1500 m^2/g) and almost identical solvent uptake in both polar and non-polar media, which explains good compatibility of the material with all mobile phases, from hexane to methanol and water. The structure of hypercrosslinked polystyrene is a three-dimensional network; the main structural element of this network is a spatially non-planar cycle formed by crosslinking bridges and very short chain segments confined between the branching points. The whole interior of the hypercrosslinked polystyrene bead is accessible to analytes, as if the rather homogeneous network is composed of small ''pores'' of about 2.0 to 4.0 nm in diameter. (So-called ''biporous'' materials contain large transport pores, in addition to the small pores).

Hypercrosslinked polystyrene materials have already found wide application for large scale adsorption technologies (Macronet Hypersol, Purolite) and for solid-phase extraction (Purosep, Purolite; Isolute-ENV+, IST, UK; LiChrolut EN, Merck, Darmstadt, Germany). However, there are only a few examples illustrating the applicability of this material in the capacity of stationary phases in HPLC [2,10–13].

Here, we present initial chromatographic information obtained on a HPLC column packed with experimental Chromalite 5HGN (Purolite). This is a monosized 5 μm beaded ''Macronet'' material of the hypercrosslinked (H) polystyrene family, of the gel-type (G), non-activated (N).

Due to the compatibility with any type of mobile phase, the Macronet Gel packing may be used for either reversed-phase or normal-phase separations of both polar and nonpolar compounds. In the case that the mobile phase is composed of water, acetonitrile, THF and other polar organic components, the polystyrene packing in general works similar to a standard silica-based reversed-phase packing, displaying, however, an extremely high hydrophobicity. If the mobile phase is based on nonpolar solvents such as hexane, Macronet Gel is found to predominantly work like normal-phase packings, since many organic compounds are strongly retained and well separated. We have to call this chromatographic mode ''quasi normal-phase HPLC'', pointing out the absence of any polar groups on the polystyrene stationary phase.

Preliminary experiments showed that in quasi normal-phase mode the hypercrosslinked polystyrene retains only substances with π-systems of electrons: aromatic rings, carbonyl groups and so on. In order to explain the phenomenon we assumed that the main driving force of retention on the polystyrene packing in nonpolar media is the formation of labile π-complexes between aromatic fragments of the stationary phase and π-systems of adsorbates (see also Ref. [11]). The major aim of this study was to prove this suggestion and, in addition, to estimate the possible contribution of π–π interactions to the retention under reversed-phase conditions.

2. Factor analysis

In the general case, retention of an analyte is the sum of different types of interaction with the stationary phase. In an attempt to discriminate these types and estimate the contribution of the ''pure'' π–π interactions ''adsorbent–adsorbate'', principal factor analysis (PFA) was applied to processing experimental retention data. There are only a few examples of application of this mathematical method in chemistry, and particularly in chromatography [14–16]. For this reason, we have to touch upon its basic idea.

The main assumption of PFA is that the matrix of observed quantitative characteristics of a given sys-

tem can be represented as a combination of a smaller amount of hypothetical unobserved, linearly-independent characteristics, called factors. Thus, if there are any strong correlations between observed characteristics of the system, PFA will reduce the amount of initial experimental data, without any loss in self-descriptiveness. In this way, all experimental data will be described by the variety of extracted factors, which can be considered as ''objective'' tendencies of a system. However, in order to get some meaningful information from the experimental data matrix, one must interpret the factors calculated. No doubt, this step of PFA is the most difficult and important for the researcher.

In our experiments, two matrixes of retention data, those in the RP and NP chromatography modes, for a series of analytes as a function of the mobile phase composition were obtained. On a given stationary phase, the mobile phase composition presents the most important variable that influences the column selectivity. Selectivity depends on the retention mechanisms, which are characterized by the factors to be evaluated in the PFA method.

In order to obtain reliable information on the retention mechanisms, separation selectivity must be varied in a broad range. For this reason tetrahydrofuran (THF), dichloromethane and chloroform were tested as components of the eluent in the reversed-phase mode of chromatography. The first compound has a cyclic structure, the last two incorporate chlorine atoms which are known to enter specific interactions with aromatic π-electron systems.

To consider the mathematical details of the method, let $\mathbf{Y} = (y_{ij})$ be the matrix of initial data $m \times n$ (m objects$\times n$ parameters). First, one is to convert the initial matrix into a ''standard'' matrix $\mathbf{Z} = (z_{ij})$ in accordance with the rule $z_{ij} = (y_{ij} - y_i^{av})/s_i$, where y_i^{av} are average values, s_i are standard deviations, and z_{ij} are standard scores. The aim of factor analysis is to represent every element of that matrix $\mathbf{Z}$ as a linear combination of r hypothetical linearly independent variables called factors: $z_{ij} = a_{i1}p_{1j} + a_{i2}p_{2j} + \cdots + a_{ir}p_{rj}$; then, $\mathbf{Z} = \mathbf{AP}$. Here, $\mathbf{A} = (a_{il})$ is the matrix $m \times r$ to be calculated. It is called the factor pattern, and its coefficients are called factor loadings. $\mathbf{P} = (p_{lj})$ is the matrix $r \times n$, its coefficients are called factor scores. Secondly, from the standard matrix $\mathbf{Z}$ one calculates a correlation matrix $\mathbf{R}$. It is a quadratic symmetric matrix $n \times n$ formed by correlation coefficients of n parameters. The problem of extraction of factors from $\mathbf{Z}$ leads to the problem of calculation of eigenvalues of the corresponding correlation matrix $\mathbf{R}$: $\mathbf{R}\alpha_l = \lambda_l\alpha_l$, where λ_l are eigenvalues of $\mathbf{R}$, α_l are its eigenvectors. Factors are proportional to eigenvectors: $a_{il} = \alpha_{il}(\lambda_l/\alpha_{1l}^2 + \alpha_{2l}^2 + \cdots + \alpha_{ml}^2)^{1/2}$.

3. Experimental

A 250×4.6 mm I.D. HPLC column was packed with experimental monosized spherical 5 μm Macronet Gel neutral hypercrosslinked polystyrene beads, Chromalite 5HGN (Purolite).

The instrument used was composed of a HPLC pump (Bischoff) with a Rheodyne injector loop and a UV detector (Knauer).

The first series of experiments was carried out on the Macronet Gel column under quasi normal-phase conditions. Three mobile phases, pentane–CH_2Cl_2–2-propanol of different compositions were tested—(60:20:20), (70:20:10) and (85:5:20), at a flow-rate of 1.0 ml/min. The experimental test mixture was composed of 13 adsorbates: four aromatic hydrocarbons (benzene, toluene, naphthalene and anthracene), eight monosubstituted benzenes (acetophenone, benzaldehyde, phenol, anisol, aniline, acetanilide, bromobenzene, nitrobenzene), and acetone. All these compounds are well retained on the Macronet Gel packing under the normal-phase (as well as under reversed-phase) chromatographic conditions. All analytes can be easily detected at 254 nm. The chromatograms obtained are shown in Fig. 1. The retention results are summarized in Table 1. Initial perturbation of the baseline on the chromatogram (''minor disturbance method'' [17]) was used for calculating the k' values. Similar chromatograms result on replacing $CHCl_3$ for CH_2Cl_2 in the mobile phase.

The second experimental series was carried out on Macronet Gel and silica-bonded Zorbax SB-C_{18} packings under reversed-phase conditions. The test mixture was composed of eight adsorbates: benzene, toluene, naphthalene, acetophenone, phenol, acetanilide, bromobenzene, nitrobenzene. When using Macronet Gel as stationary phase, three mobile

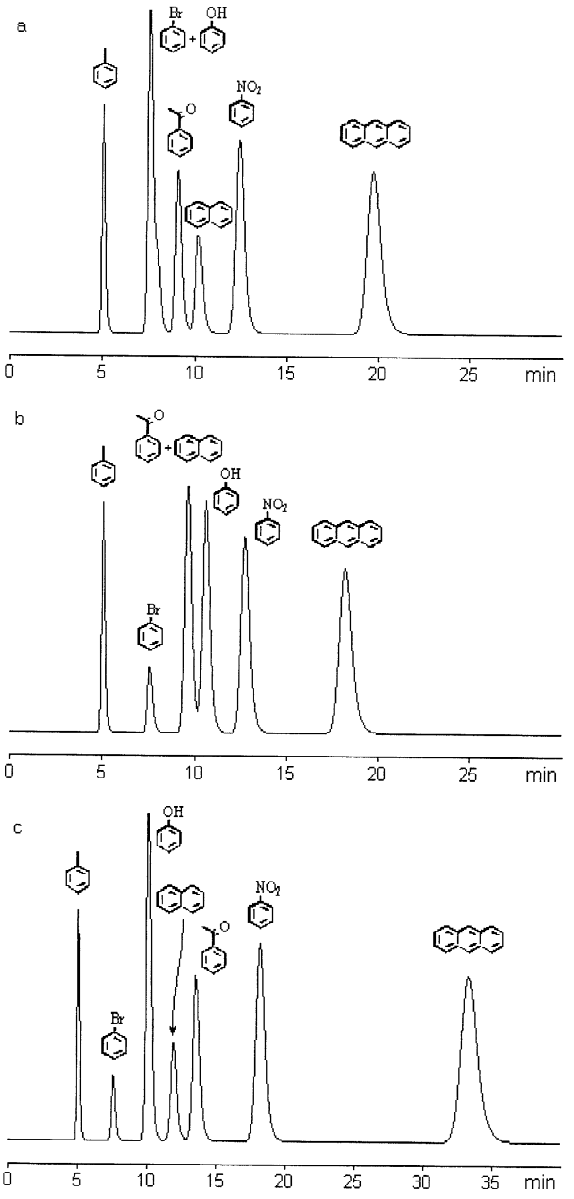

Fig. 1. HPLC separation of a mixture of five substituted benzenes, naphthalene and anthracene under quasi-normal-phase conditions. Column, Macronet Gel, 5 μm (250 mm×4.6 mm I.D.). Mobile phases, pentane–CH_2Cl_2–2-propanol (a) 60:20:20, (b) 70:20:10, (c) 75:5:20. Flow rate 1 ml/min.

phases were tried: acetonitrile–THF–water (70:10:20), acetonitrile–CH_2Cl_2–water (80:10:10) and acetonitrile–2-propanol–water (70:20:10). In the experiment with Zorbax SB-C_{18}, two mobile phases were tried: acetonitrile–water of 70:30 and 60:40, respectively. The results are collected in Table 2.

The retention data of Table 1 (matrix table 13×3, 13 analytes characterized under three different conditions) was processed by the method of principal factor analysis. Two factors F11 and F12 with contributions 95.76% and 3.76%, respectively, were extracted from these data describing retention on Macronet Gel under the quasi normal-phase conditions. Obviously, two linearly independent tendencies, one major and another substantially less important, govern the analyte–sorbent interactions under the quasi normal-phase conditions. Only one factor F2 with contribution 99.85% was extracted from the data of Table 2 (matrix table 8×3) describing retention on Macronet Gel under the reversed-phase conditions. Similarly, one single factor F3 with contribution 99.96% was derived from the data (matrix table 8×2, Table 2) describing the experiment carried out on Zorbax SB-C_{18} bonded silica. Let us assume this factor F3 (Zorbax SB-C_{18}, RP-HPLC) to be the criterion of ''hydrophobic'' interactions in a chromatographic system with an aqueous–organic mobile phase.

4. Results and discussion

The initial assumption of this work is that the major driving force for the retention of analytes examined under the quasi normal-phase conditions (stationary phase:hypercrosslinked polystyrene, hexane-based mobile phases) is π–π interactions ''adsorbent–adsorbate'', i.e. the formation of labile π–π complexes [18]. Their stability at first approximation must be proportional to the difference between the π-densities of aromatic fragments of the adsorbent and the π-system of the corresponding adsorbate.

Let us consider π-complexes of ''polystyrene-monosubstituted benzene'' type as the most simple structures. Retention of the substituted benzene should be generally enhanced by the π-electron-donating or π-electron-accepting ability of the adsorbate, relative to the constant π-electron density of the polystyrene sorbent. Least difference in the π-electron densities can be expected for benzene and toluene. Indeed, these analytes are found to be least retained on Macronet Gel under the quasi normal-

Table 1
Data matrix for quasi-normal phase conditions; Macronet Gel

Compound	ln k'			F11	F12
	Pentane–CH_2Cl_2–2-propanol 60:20:20	Pentane–CH_2Cl_2–2-propanol 70:20:10	Pentane–CH_2Cl_2–2-propanol 85:5:10		
Naphthalene	0.699	0.636	1.424	0.198	−0.783
Benzaldehyde	0.676	0.783	1.647	0.372	−0.215
Anthracene	1.598	1.516	2.633	1.845	−1.403
Aniline	0.907	1.207	1.86	0.855	0.327
Acetanilide	0.671	1.494	1.904	0.88	2.242
Anisole	0.296	0.288	1.006	−0.455	−0.362
Benzene	−0.169	−0.209	0.262	−1.387	−0.17
Phenol	0.228	0.799	1.214	−0.12	1.649
Nitrobenzene	0.956	1.03	1.957	0.837	−0.492
Acetophenone	0.531	0.665	1.592	0.187	−0.051
Toluene	−0.223	−0.439	0.271	−1.545	−0.76
Bromobenzene	0.344	0.296	0.885	−0.478	−0.508
Acetone	−0.223	−0.057	0.563	−1.193	−0.526

phase conditions (Fig. 1, Table 1). Both electron-donating and electron-accepting substituents in the aromatic ring of the analyte must change the π-density of the latter and facilitate retention of the analyte in the column. In our opinion, the Hammet–Taft σ_p^o constant, which is responsible for the resonance of the reacting center of the aromatic ring with its substituent in the *para*-position, can be used as the quantitative estimation of the π-donating (π-accepting) ability of the substituent. The Hammet–Taft σ_p^o constants thus should reflect not only the intramolecular effects, but also the ability of the analyte to enter donor–acceptor molecular interactions with polystyrene. We used therefore the correlations with σ_p^o, in order to recognize, among the principal factors obtained above, those reflecting the π–π interactions ''polystyrene-monosubstituted benzene''.

Predictably, the dominant factor F11 (Macronet Gel, quasi normal-phase HPLC) was found to correlate strongly with Hammet–Taft constant σ_p^o, revealing itself as the factor responsible for π–π interactions in the chromatographic system. In fact, there are two particular correlations between F11 and σ_p^o, one for π-donating and one for π-accepting substituents (Fig. 2), correlation coefficients R_{xy} being 0.986 and 0.994, respectively. Excellent correlations obtained for F11 and σ_p^o thus prove that F11, the ''π'' factor, with a contribution of 95.76% dominates the retention of substituted benzene on hypercross-

Table 2
Data matrix for reversed phase conditions

Compound	Macronet Gel				Zorbax SB-C_{18}
	ln k'			F2	F3
	Acetonitrile–THF–water 70:10:20	Acetonitrile–CH_2Cl_2–water 80:10:10	Acetonitrile–2-propanol–water 70:20:10		
Naphthalene	2.667	1.587	1.865	1.48	1.229
Acetanilide	0.245	−0.266	−0.057	−0.876	−1.621
Benzene	1.419	0.588	0.758	0.176	0.324
Phenol	0.36	−0.325	−0.045	−0.859	−1.318
Nitrobenzene	1.625	0.688	0.977	0.375	−0.158
Acetophenone	1.207	0.435	0.659	0.002	−0.476
Toluene	1.725	0.803	0.985	0.461	0.863
Bromobenzene	2.175	1.204	1.435	0.968	1.000

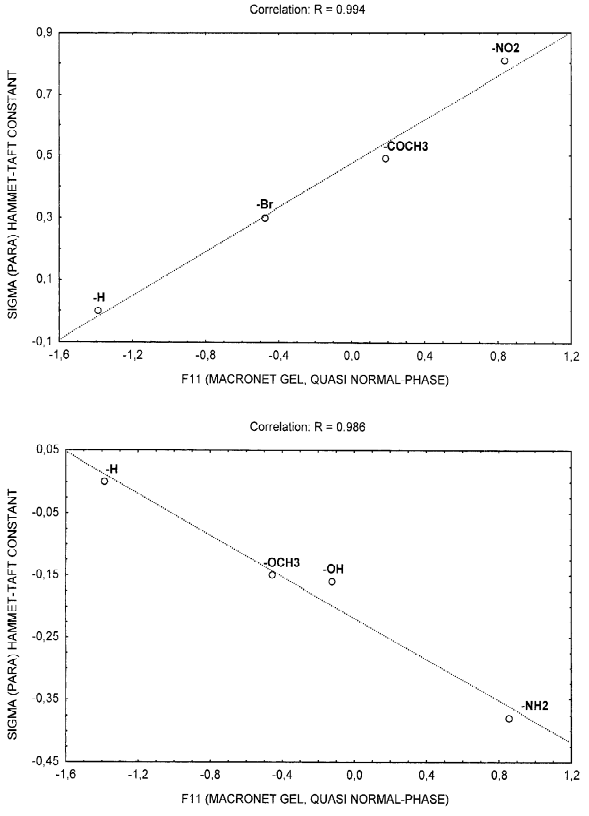

Fig. 2. Correlations between F11 factor (Macronet Gel, quasi-NP) and σ_p^o Hammet–Taft constant for electron-accepting and electron-donating substitutes.

linked polystyrene packings in quasi normal-phase chromatography.

A correlation coefficient of 0.948 was obtained between factors F2 (Macronet Gel, reversed-phase HPLC) and F3 (Zorbax SB-C_{18} in RP HPLC), see Fig. 3. So F2 can be named the ''reversed-phase'' factor for polystyrene packing. In fact, the mobile phase being composed of polar organic solvents such as acetonitrile, THF, 2-propanol and even water (reversed-phase conditions), Macronet Gel works similar to silica-based RP packing, and the contribution of the factor F2 is 99.85%. Obviously, in the above examined polar media, the principal factor analysis of the experimental data does not reveal any noticeable contribution from $\pi-\pi$ interactions to the analyte retention, though the unusual selectivity of Macronet Gel under the RP conditions still remains to be explained.

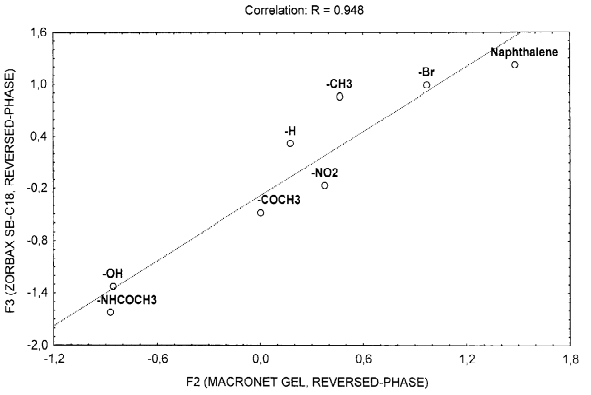

Fig. 3. Correlation between F2 factor (Macronet Gel, RP) and F3 factor (Zorbax SB-C_{18}, RP).

Reversed-phase HPLC separations of a test mixture of 10 compounds on Macronet Gel and Zorbax SB-C_{18} are shown in Fig. 4. In spite of basic similarity, one can also notice that the polystyrene packing sometimes reveals unusual selectivity, especially for anisole and nitrobenzene.

The minor factor F12 (Macronet Gel, quasi-NP-HPLC) with the contribution of 3.76% satisfactorily correlates (R_{xy} being equal 0.930) with F2 (Macronet Gel, RP-HPLC), see Fig. 5. This factor thus reveals itself as the ''reversed-phase'' factor contributing under the normal-phase chromatographic conditions, in addition to the major ''π'' factor F11. In spite of the relatively small contribution, about 4% of ln k' values, F12 makes it possible to regulate the selectivity in quasi normal-phase chromatography by changing the mobile phase composition. The addition and increase in content of a highly polar additive, such as 2-propanol, in the basically nonpolar mobile phase, leads to the shift of retention times in the ''reversed-phase'' kind of way.

This possibility, revealed due to the principal factor analysis, can be clearly demonstrated by the example of separation of aromatic compounds (Fig. 6): benzene, toluene, *o*-xylene, naphthalene, acenaphthene, 2,7-dimethylnaphthalene, phenanthrene, anthracene. When using ''pure'' reversed-phase conditions, i.e. in the water-containing polar mobile phase, all peaks are well resolved, with the alkyl substituted compounds retained longer than unsubstituted compounds (Fig. 6a). On the other hand, in the hexane–$CHCl_3$ mobile phases, peaks of alkyl substi-

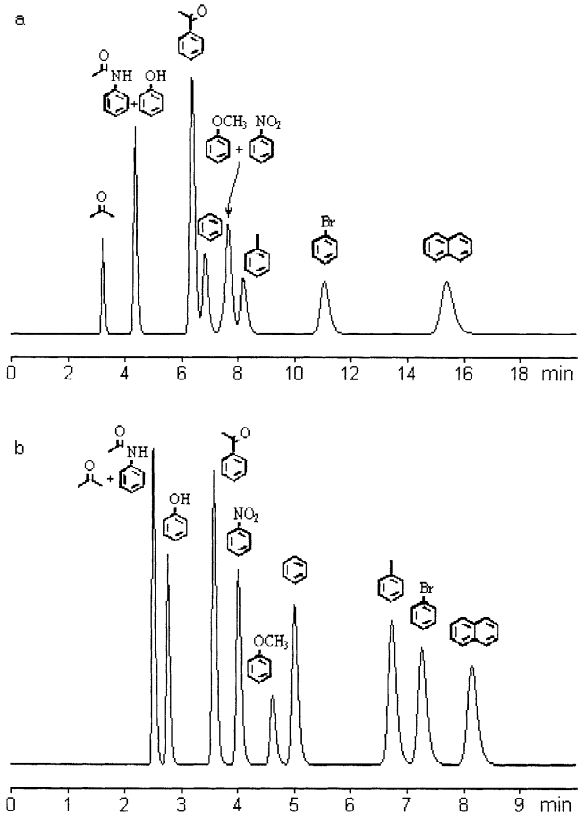

Fig. 4. HPLC separation of a mixture of eight substituted benzenes, acetone and naphthalene under reversed-phase conditions. (a) Column, Macronet Gel, 5 μm (250 mm×4.6 mm I.D.); mobile phase, acetonitrile–THF–water (80:10:10). (b) Column, Zorbax SB-C_{18}, 5 μm (250 mm×4.6 mm I.D.); mobile phase, acetonitrile–water (60:40). Flow rate 1 ml/min.

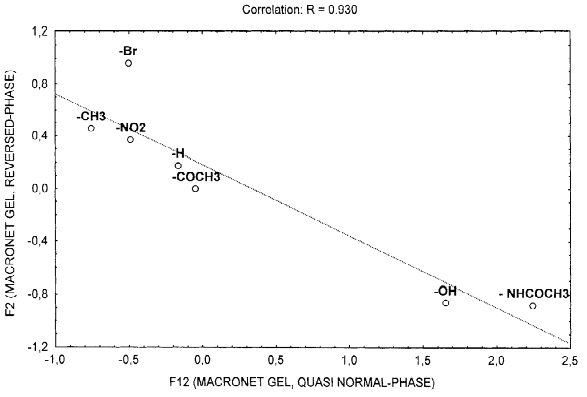

Fig. 5. Correlation between F12 factor (Macronet Gel, quasi-NP) and F2 factor (Macronet Gel, RP).

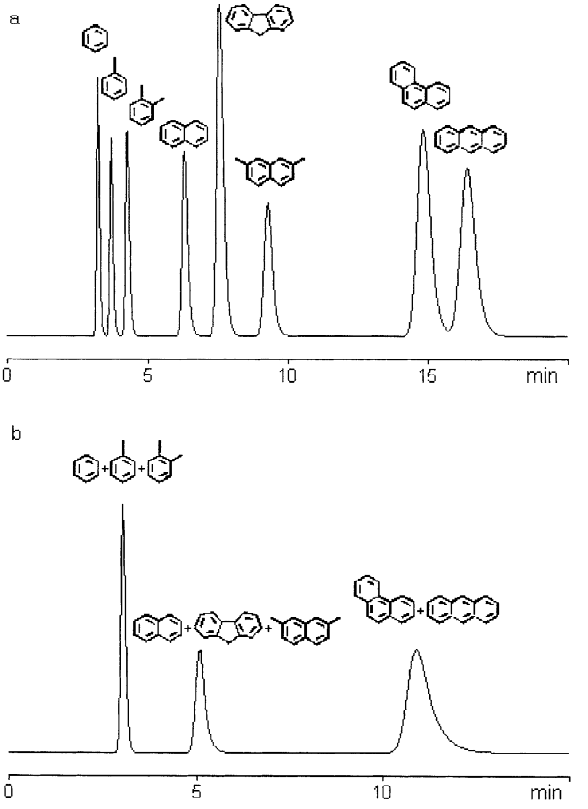

Fig. 6. HPLC separation of a mixture of aromatic compounds. Column, Macronet Gel, 5 μm (250 mm×4.6 mm I.D.). Mobile phases, (a) acetonitrile–CH_2Cl_2–water (85:10:5), flow-rate 1.5 ml/min, (b) hexane–$CHCl_3$–2-propanol (30:20:50), flow-rate 1 ml/min.

tuted compounds tend to precede peaks of unsubstituted ones. By simply adding 2-propanol under these quasi-NP conditions, all peaks of alkyl substituted benzene are forced to overlap. As a result, only compounds having a different number of aromatic rings will separate. In this way, one can see the opportunity to update the normal-phase conditions, in order to suppress undesired resolutions and obtain a group chromatogram of this mixture, consisting of only three peaks which correspond to mono-, bi- and tri-cyclic aromatics, respectively (Fig. 6b). Because of the effect of combining all peaks of aromatic compounds having identical number of aromatic rings, the method provides higher sensitivity in the group analysis of trace amounts of gasolines and oils.

Fig. 7 illustrates the application of hypercrosslinked polystyrene packing for the separation of 10 phenols in a ''standard'' reversed-phase HPLC mode, with either isocratic or gradient elution. It is worth mentioning, that in general, the application of polystyrene packing in reversed-phase HPLC should prove especially rewarding in combination with the on-line pre-concentration of trace analytes on solid-phase extraction (SPE) cartridges packed with the same hypercrosslinked polystyrene-type material (for instance, ''Purosep'', Isolute-ENV+, LiChrolut EN, the most powerful SPE materials). Effective on-column pre-concentrations should also be possible.

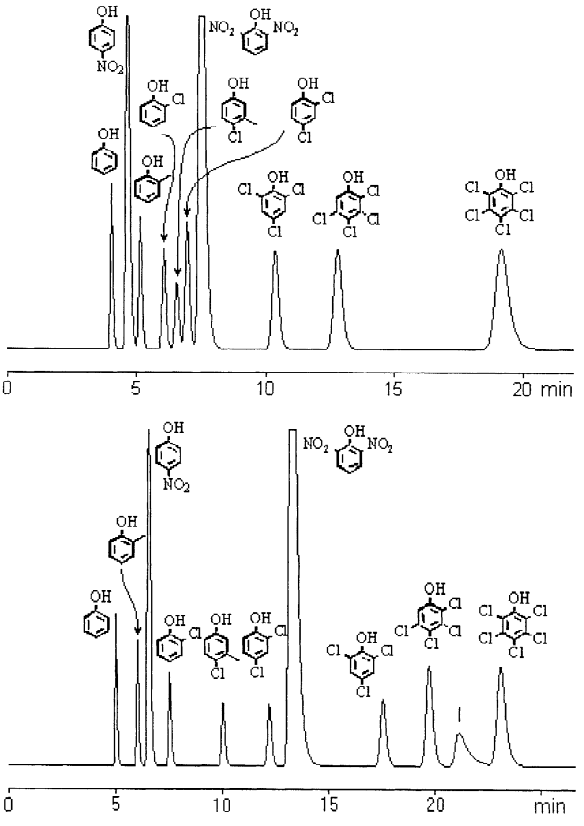

Fig. 7. HPLC separation of 10 phenols using isocratic and gradient elution under reversed-phase conditions. Column, Macronet Gel, 5 μm (250 mm×4.6 mm I.D.). (a) Isocratic elution. Mobile phase, acetonitrile–THF–water–acetic acid (80:10:10:1.5), flow-rate 1 ml/min. (b) Gradient elution. Solvent A, acetonitrile–THF–water–acetic acid (45:15:40:2); solvent B, acetonitrile–THF–acetic acid (85:15:0:2); 0–20% B in 15 min, 20–100% B in 10 min; flow-rate 1.5 ml/min. Detection, UV 254 nm.

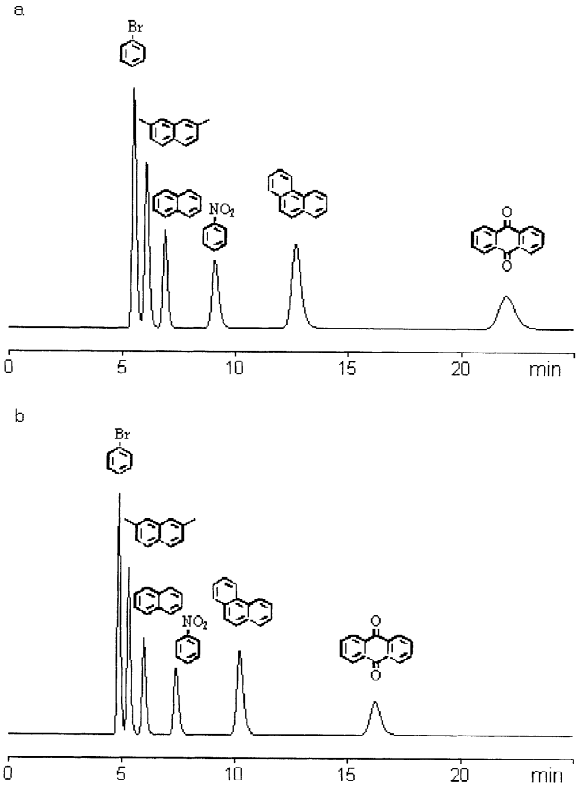

Fig. 8. Influence of temperature on the separation of a test mixture. Column, Macronet Gel, 5 μm (250 mm×4.6 mm I.D.). Mobile phase, hexane–CH_2Cl_2–2-propanol (70:20:10). Flow rate, 1 ml/min. Temperature, (a) ambient, (b) 60 °C.

Another perspective area of application of the hypercrosslinked polystyrene packing is the modern high-temperature HPLC. Enhanced temperatures, through facilitating the mass transfer, substantially increase the column efficiency, and reduce analysis time. Conventional silica-based materials, because of their insufficient chemical resistance, are least suitable for that purpose, while neutral hypercrosslinked polystyrene easily withstands temperatures of 200–220 °C. Fig. 8 illustrates evident benefits of increasing the column temperature from ambient to 60 °C on chromatography of a test mixture under quasi-NP

conditions. Besides shorter run times, significant progress in efficiency (an increase in plate numbers of up to 60%) is achieved.

5. Conclusions

The hypercrosslinked polystyrene packing Macronet Gel presents a new type of stationary phase for HPLC. It is compatible with both hydrophobic solvents such as hexane, chloroform, and hydrophilic and highly polar, such as acetonitrile, methanol, and even water.

In the first case, when using nonpolar solvents as mobile phases, the retention is mostly due to π-interactions between π-systems of the adsorbent and adsorbate. This chromatographic mode was named ''quasi normal-phase HPLC''. Adding highly polar, even hydrophilic solvents into the mobile phase may change selectivity of separation. The increase in content of the polar additive such as 2-propanol in the nonpolar mobile phase, leads to a shift of retention times toward the ''reversed-phase'' kind of chromatography.

When using hydrophilic solvents as mobile phases, Macronet Gel works similar to standard C_{18} reversed-phase packing. However, the new polystyrene packing is characterized by much greater hydrophobicity and unusual selectivity.

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