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POLYMER-BASED PACKING MATERIALS WITH ALKYL BACKBONES FOR REVERSED-PHASE LIQUID CHROMATOGRAPHY

PERFORMANCE AND RETENTION SELECTIVITY

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SUMMARY

Polymer-based packing materials for reversed-phase liquid chromatography, including poly(styrene–divinylbenzene), poly(alkyl methacrylate) and esterified poly(vinyl alcohol), were examined with respect to their performance and retention characteristics for a variety of hydrocarbons in aqueous–organic mobile phases. Materials with alkyl backbones showed performance comparable with silica C₁₈ under optimized conditions. The performance is dependent on the molecular shape, rigidity and aromatic character of the solutes as well as on the organic solvents in the mobile phase. Better performance was generally seen with alkyl compounds, compared with aromatic compounds. In a mobile phase containing tetrahydrofuran, in which most packing materials showed better performance than in methanol–water, packing materials with aromatic functionality showed lower efficiencies only for planar polynuclear aromatic hydrocarbons. All the polymer-based packing materials showed preferential retention of aromatic solutes, especially for those with rigid, planar structure rather than flexible, bulky compounds, when these packing materials are compared with a silica C₁₈ phase. Polystyrene and poly(vinyl alcohol)-based packing materials showed a greater preference than did the other alkyl-type polymer gels, although this preference was reduced in tetrahydrofuran–water. The retention selectivity and the differences in column performance of the polymer-based phase were explained by the biporous structure of the polymer gels and the solvation of the polymer chains with organic solvents.

INTRODUCTION

An increasing demand for packing materials more stable than silica C₁₈ in reversed-phase liquid chromatography (RPLC) has prompted the recent development of polymer-coated silica packings^{1–3} and organic polymer-based packing materials, such as poly(styrene–divinylbenzene) (PS)^{4–6}, poly(alkyl methacrylate) (PAM)⁷,

esterified poly(vinyl alcohol) (PVA)^{8,9}, alkylated poly(acrylamide)¹⁰ and poly(hydroxyalkyl acrylate or methacrylate) (PHA), which can be substituted for silica C₁₈ when appropriate.

Among the modern polymer-based phases, the most popular PS gels have been used in RPLC for a decade, mainly for the separation of basic compounds and biopolymers¹¹. These separations often need severe separation conditions, which may dissolve silica gels. Although PS packing materials sometimes showed lower efficiencies for aromatic solutes than did silica-based packing materials, the performance was adequate for many separations of small molecules and excellent in the separations of polar compounds and polypeptides¹²⁻¹⁹.

The performance and retention characteristics of PS have been studied with various mobile phase compositions, temperatures, organic solvents and pore sizes of packing materials. The results were explained in terms of the aromatic character and the solvation of the polymer structures as well as the microporosity in the gel structure²⁰⁻³².

We recently showed that alkylated PHA and PVA gels provided preferential retention of rigid, planar compounds rather than bulky flexible solutes compared with silica C₁₈³³. It is agreed that the biporous structures, microporous primary structures composing macroporous particles, are responsible for the potentially poor performance and preferential retention of rigid solutes shown by polymer gels^{22,25,33,34}.

Recently some new polymer-based packing materials for RPLC have become available. They include octadecanoylated PVA gel, commercially available as Asahipak ODP-50, PAM gel, available as Shodex DE-613 (described as short alkyl methacrylate by the manufacturer), and alkylated PHA gel, available as TSK C₁₈-4PW. It was thought desirable to assess the performance and retention characteristics of these packing materials, and compare them with those of PS gel and silica C₁₈. Accordingly we examined four polymer-based packing materials, one PS gel and three polymer gels with alkyl backbones, as well as silica C₁₈, with respect to their performance and retention characteristics for a wide range of hydrocarbons with various sizes, shapes, rigidities and planarities. Each polymer-based packing material showed different retention characteristics and performance which are explained by the chemical as well as the micropore structures of these polymer gels.

EXPERIMENTAL

The high-performance liquid chromatography (HPLC) system consisted of an 880 PU pump, 875 UV detector, 830 refractive index (RI) detector (JASCO, Tokyo, Japan) and a 7000A data processor (System Instruments, Tokyo, Japan). The column packing materials, PLRP-S 300 (Polymer Laboratories, Church Stretton, U.K.) and TSK Octadecyl-4PW (abbreviated as C₁₈-4PW; Tosoh, Tokyo, Japan), and the packed columns (both 15 cm × 6 mm I.D.) of Shodex RS pak DE-613 (Showa Denko, Tokyo, Japan) and Asahipak ODP-50 (Asahi, Kawasaki, Japan) were gifts from the suppliers. Silica-based packing materials were prepared as previously described³³. Silica C₁₈, C₈, PLRP-S and TSK C₁₈-4PW were packed into stainless-steel columns (10 cm × 4.6 mm I.D.).

Samples used for the examination of the effect of molecular shape on the reten-

tion and column performance include pentane (1), hexane (2), heptane (3), octane (4), decane (5), cyclohexane (6), adamantane (7), *trans*-decalin (8), naphthalene (9), anthracene (10), pyrene (11), benz[*a*]pyrene (12), diphenylmethane (13), 1,2-diphenylethane (14), *o*-terphenyl (15), triphenylene (16), triptycene (17), triphenylmethane (18), fluorene (19), benzene (20), toluene (21), ethylbenzene (22), propylbenzene (23), butylbenzene (24) and amylbenzene (25). (Structures were shown in a previous report³³.) Polystyrene standard materials were obtained from Pressure Chemicals (Pittsburg, PA, U.S.A.). Chromatographic measurement and size-exclusion chromatographic (SEC) analysis of the pore size distribution were performed as reported previously^{33,35}. Nitrogen adsorption measurement was carried out at the Shiseido Toxicological and Analytical Research Center (Yokohama, Japan).

RESULTS AND DISCUSSION

Table I lists the results of nitrogen adsorption measurement with polymer-based packing materials as well as with some silica gel particles with 30–50 nm pores. The surface areas of the polymer gels were generally larger than those of silica particles of similar pore sizes, although some of the silica particles were found to be mixtures of particles of different pore sizes³⁵. The pore size distribution can be measured by SEC^{36–38} as well as by the nitrogen adsorption method for these highly cross-linked organic polymer gels. All the polymer gels showed the presence of micropores less than 3 nm diameter in both methods. Fair agreement between the results of the two methods including a micropore region was seen for all the packing materials, as shown in Fig. 1.

In SEC, all the polymer-based packing materials showed selective permeation, or a second plateau, in molecular weight–elution volume curves, at a molecular weight range less than 500 in tetrahydrofuran (THF)³⁵. The mean pore size in the meso- and macropore regions decreases in the order TSK C₁₈-4PW, PLRP-S 300, Asahipak ODP-50 and Shodex DE-613. The fraction of the pore volume occupied by

TABLE I
POLYMER AND SILICA-BASED PACKING MATERIALS^a

<i>Packing material</i>	<i>Surface area (m²/g)</i>	<i>Pore size (nm)</i>	<i>Pore volume (ml/g)</i>	<i>Particle size (μm)</i>
Shodex DE-613	(354)		(0.41)	6
TSK C ₁₈ -4PW	(48)	(50)	(0.60)	7
Asahipak ODP	(421)	(25)	(1.06)	5
PLRP-S 300	(380)	30 (60)	(1.26)	8
LiChrospher 500 ^b	50	50	0.8 (0.90)	10
Nucleosil 300 ^b	100 (109)	30	0.8 (0.81)	5
Hypersil 300 ^b	60	30	0.6	5
Spherisorb 300 ^b	190	30	1.5	5
Vydac TP ^b	80	30	0.6	10

^a Specifications given by the suppliers (results experimentally obtained by nitrogen adsorption are given in parentheses).

^b The results for the silica particles are reported in ref. 35.

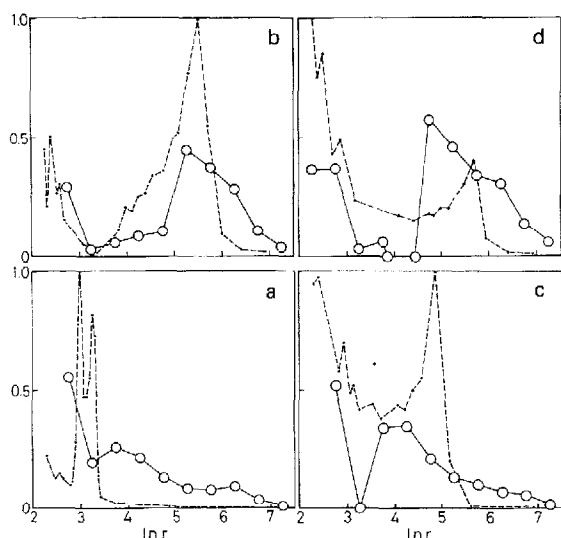


Fig. 1. Pore size measurement by nitrogen adsorption (---) and SEC (—). (a) Shodex DE-613; (b) TSK C₁₈-4PW; (c) Asahipak ODP-50; (d) PLRP-S 300. The vertical axis corresponds to the fraction of pore volume, normalized in the case of inverse SEC, and the abscissa corresponds to the logarithm of the pore radius (Å).

micropores increases in the order PLRP-S 300, TSK C₁₈-4PW, ODP-50 and DE-613, as shown in Fig. 1.

The nitrogen adsorption measurement stresses the presence of micropores on ODP-50 and PLRP-S particles. The size of the meso- or macropores determined by nitrogen adsorption increases in the order Shodex DE-613, Asahipak ODP-50, TSK C₁₈-4PW and PLRP-S 300, in fair agreement with the results of SEC. The difference between the results of the two pore-size determination methods in the micropore region is presumably due to the presence of THF in SEC. The results imply that some micropores of ODP-50 and PLRP-S 300 allowed nitrogen to enter, but not larger molecules in SEC. THF causes swelling of these highly cross-linked gels and at the same time fills the micropores²². The about 10–15% increases in flow resistance observed with polymer-packed columns compared with silica-packed columns with a change in mobile phase from 80% methanol to 70% acetonitrile or 40% THF, shown in Table II, indicate that these polymers gels are swollen to some extent in these solvents.

As shown in Table II, polymer-based packing materials with C₁₈ derivatization possess less hydrophobic properties than does silica C₁₈, presumably due to the presence of hydrophilic functional groups. The hydrophobic selectivity estimated by comparing $\alpha(\text{CH}_2)$ values³⁹, or the retention increase caused by one methylene group, is greater in 80% methanol than in 40% THF with silica C₁₈ and PLRP-S, while the opposite result was obtained with DE-613, C₁₈-4PW and ODP-50 having hydrophilic functional groups as well as silica C₈ and C₁. Incorporation of THF molecules into these less hydrophobic stationary phases resulted in an increase in hydrophobic property. The relatively hydrophilic polymer gels, however, showed considerable retention

TABLE II
 PROPERTIES OF POLYMER PACKING MATERIALS RELATED TO THE MOBILE PHASE

	Silica			Shodex	TSK	Asahipak	PLRP-S
	C ₁	C ₈	C ₁₈	DE-613	C ₁₈ -4PW	ODP-50	300
<i>Hydrophobicity [retention increment for one methylene group, $\alpha(\text{CH}_2)$]^a</i>							
80% Methanol	0.098 (0.54)	0.142 (0.78)	0.183 (1.0)	0.094 (0.51)	0.114 (0.62)	0.133 (0.73)	0.146 (0.80)
70% Acetonitrile	0.083 (0.49)	0.127 (0.75)	0.170 (1.0)	0.068 (0.40)	0.126 (0.74)	0.133 (0.78)	0.144 (0.85)
40% THF	0.144 (0.88)	0.159 (0.97)	0.164 (1.0)	0.103 (0.63)	0.150 (0.91)	0.139 (0.85)	0.125 (0.76)
<i>Retention of phenyl group, b^a</i>							
80% Methanol	-0.570 (-0.54)	-0.182 (-0.15)	-0.029 (0)	0.206 (0.24)	-0.330 (-0.30)	0.148 (0.18)	0.435 (0.46)
70% Acetonitrile	-0.137 (-0.32)	0.082 (-0.11)	0.187 (0)	0.291 (0.10)	-0.396 (-0.58)	0.105 (-0.08)	0.270 (0.08)
40% THF	0.499 (-0.24)	0.634 (-0.10)	0.736 (0)	0.850 (0.11)	0.363 (-0.37)	0.647 (-0.09)	0.579 (-0.16)
<i>Flow resistance (column back pressure at 1 ml/min)^b</i>							
80% Methanol	92 (1.0)	86 (1.0)	94 (1.0)	46 (1.0)	85 (1.0)	92 (1.0)	54 (1.0)
70% Acetonitrile	50 (0.54)	47 (0.55)	52 (0.55)	28 (0.61)	47 (0.55)	59 (0.64)	34 (0.63)
40% THF	137 (1.49)	129 (1.50)	140 (1.49)	76 (1.65)	142 (1.67)	153 (1.66)	88 (1.63)

^a Calculated from the linear relationship between the log k' values and the carbon number of alkylbenzenes, ethylbenzene to amylbenzene: $\log k' = \alpha(\text{CH}_2)C_n + b$. The slope, $\alpha(\text{CH}_2)$, indicates the hydrophobic property of the stationary phase, and the intercept, b , the retention of an imaginary phenyl group. Relative values are indicated in parentheses taking the values on silica C₁₈ as the standard.

^b Column back pressure (kg/cm²) at a flow-rate of 1 ml/min calculated by plotting pressure reading against flow-rate. Relative back pressure, taking the pressure in 80% methanol as a standard for each packing material, in parentheses.

of rigid planar hydrocarbons, as shown by the retention of the phenyl group in Table II. The results indicate the wide variation of the polymer gels and the participation of solvent molecules incorporated in polymer gels in solute retention.

Table III lists the k' values in methanol-water of hydrocarbons with structural features. The greatest selectivity based on molecular shape was seen in methanol-water as the mobile phase which provides the least solvation of the polymer chains. Polymer-based packing materials showed much less retention of alkanes than did silica C₁₈. Decane has no functional groups, and is expected to undergo minimum specific interactions with the stationary phase except hydrophobic interaction. All the polymer phases except DE-613 showed a clear preference for rigid, planar polynuclear aromatic hydrocarbons (PAHs) compared with the bulky aromatic compounds with rotational freedom of phenyl groups, which in turn were retained more favourably than saturated hydrocarbons, when compared with silica C₁₈. The retention of PAHs on polymer gels is very strong, taking into account the low hydrophobic prop-

TABLE III
RETENTION CHARACTERISTICS OF POLYMER-BASED PACKING MATERIALS IN 80% METHANOL

Packing material	$k'(\alpha)^a$				
	Decane	Adamantane	Pyrene	Triphenylmethane	Triptycene
Silica C ₁₈	31.7	11.3 (0.36)	7.29 (0.23)	7.24 (0.23)	3.01 (0.10)
Shodex DE-613	3.48	4.58 (1.32)	15.0 (4.31)	16.7 (4.80)	12.5 (3.58)
TSK C ₁₈ -4PW	4.68	2.54 (0.54)	5.16 (1.10)	2.46 (0.53)	2.05 (0.44)
Asahipak ODP-50	14.8	7.23 (0.49)	7.90 (1.21)	11.3 (0.76)	6.97 (0.47)
PLRP-S 300 ^b	10.5	7.18 (0.68)	33.3 (3.17)	19.4 (1.84)	5.67 (0.54)

^a The α values (given in parentheses) were calculated by dividing the k' values of each solute by the k' value of decane.

^b In 90% methanol.

TABLE IV
SOLUBILITY PARAMETERS OF SOLVENTS AND POLYMERS⁴⁵

	<i>Poly(methyl methacrylate)</i>	<i>PS-DVB^a</i>	<i>Poly(vinyl alcohol)</i>	<i>Methanol</i>	<i>Acetonitrile</i>	<i>THF</i>	<i>Water</i>
Solubility parameter	9.0–9.5	9.1	12.6	14.5	11.9	9.1	23.4

^a Copolymer of styrene and divinylbenzene.

erties of the polymer-based packing materials. Cycloalkanes were also favoured by the polymer-based phases compared with linear alkanes having more flexibility. This tendency can be generalized as the preference by polymer gels toward solutes with rigid, compact structure and aromatic character³³.

In the case of alkyl-bonded silica-based phases, the preference for planar solutes was related to the alkyl chain length⁴⁰ and the surface density of the bonded moieties⁴¹ as well as the functionality of the silylating reagent⁴². Aromatic groups bonded to the silica gel surface also showed preference for aromatic compounds⁴³. Such preference on the polymer-based stationary phases, however, was not affected by the alkyl chain length of the stationary phase or the size of the macropores of the polymer packing materials³³. DE-613 and C₁₈-4PW, or the polymer gels with alkyl backbones, showed preferential retention of aromatic compounds in spite of their saturated structures. The preference toward rigid compounds over flexible ones was also seen with saturated compounds having no functional group such as adamantane. Therefore the shape selectivity associated with polymer gels can be attributed, at least partially, to the structural matching between the solute and the polymer chain structure, or the micropores^{22,33}.

In order to show the difference in selectivity of the polymer-based packing materials more clearly, the $\log k'$ values on the polymer-based packing materials are plotted against those on silica C₁₈, and also against those on Shodex DE-613 in Fig. 2. A group of compounds with similar structural features behaved similarly. These

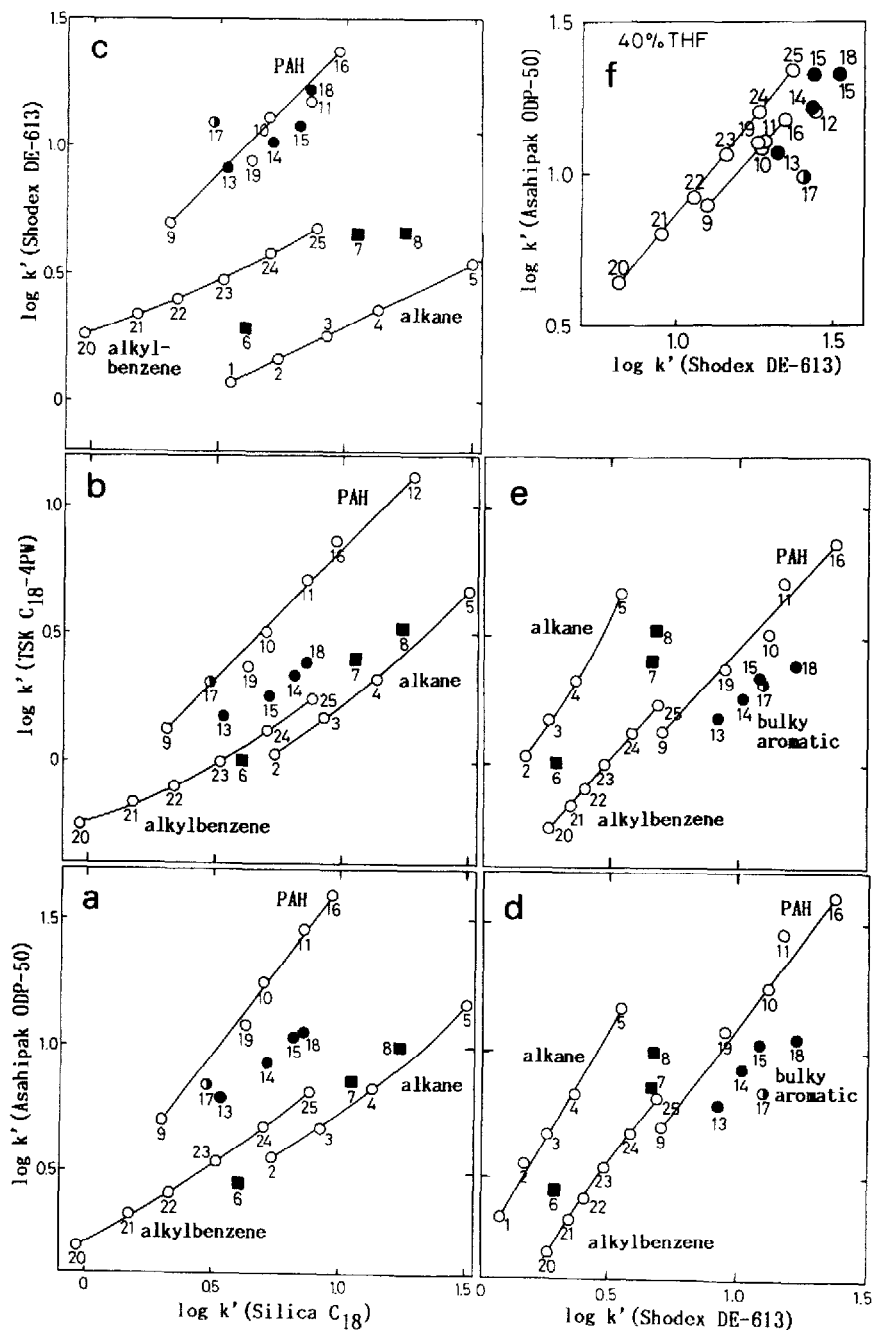


Fig. 2. Comparison of retention selectivity. Plots of $\log k'$ values on Asahipak ODP-50, TSK C₁₈-4PW and Shodex DE-613 against $\log k'$ values on silica C₁₈. (a), (b) and (c), respectively, and plots of $\log k'$ values on Asahipak ODP-50 and TSK C₁₈-4PW against $\log k'$ values on Shodex DE-613, (d) and (e), respectively in 80% methanol. Plots of $\log k'$ values on ODP-50 against $\log k'$ values on DE-613 in 40% THF (f). All measurements were carried out at 30°C. Curves are drawn to indicate the location of a group of compounds of similar structural features. Solid circles indicate aromatic compounds with phenyl groups having rotational freedom, or bulky compounds, and solid squares, cycloalkanes. See Experimental for the identification of the solutes.

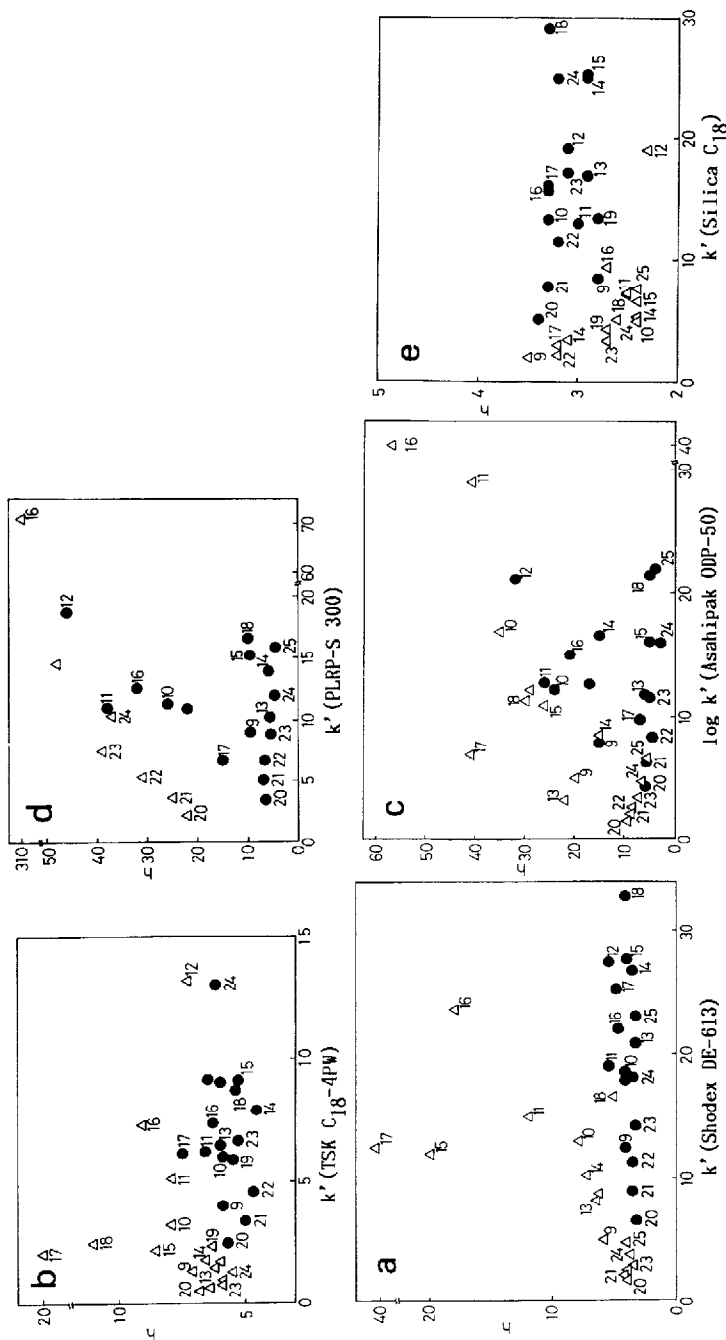


Fig. 3. Plots of reduced plate heights, h_r , against retention, k' , of hydrocarbons on Shodex DE-613 (a), TSK C_{18} -4PW (b), Asahipak ODP-50 (c), PLRP-S 300 (d) and silica C_{18} (e), in 80% methanol (\bullet) and in 40% THF (Δ). Values of h at a flow-rate of 1 ml/min for each column. See Experimental for the identification of the solutes.

plots can afford more reliable information about the selectivity of packing materials than simple comparisons of k' values of individual compounds. The polymer-based phases showed considerably different solute selectivities from silica C_{18} and from each other, which must be related to the chemical and the three-dimensional structure of these polymer gels. For example, DE-613 showed preference for aromatic compounds having more than one phenyl group compared to alkylbenzenes and for cycloalkanes compared to linear alkanes, while ODP-50 showed clear preference for planar PAHs compared to bulky aromatic compounds (13–15, 18), when these packing materials are compared with silica C_{18} . The selectivity of polymer-based packings is very similar to that of a 2-(1-pyrenyl)ethyl-bonded silica phase which possesses very rigid pyrene rings on the silica surface⁴³. The polymer gels with alkyl backbones are supposed to provide such selectivity based on the rigid polymer network. The C_{18} type polymer packings, ODP-50 and C_{18} -4PW, showed greater retention of alkyl compounds than did DE-613 having short alkyl groups, as shown in Fig. 2d and e.

The preference for planar solutes over bulky solutes increases in the order silica C_{18} , Shodex DE-613, TSK C_{18} -4PW, Asahipak ODP-50 and PLRP-S 300. The PAM gel, Shodex DE-613, actually showed favourable retention of bulky aromatic compounds such as triptycene (17) compared to PLRP-S 300, C_{18} -4PW and ODP-50. The results indicate a looser micropore structure of the polymer-based packing materials from alkyl monomers, especially with DE-613, than of PVA or PS gel. The results also indicate that the nitrogen adsorption measurements better explain the structural selectivity in 80% methanol based on the presence of micropores. Although the bulky solutes were preferentially retained by DE-613 compared to ODP-50, the structural selectivity was reduced in 40% THF as shown in Fig. 2f. The effect of the polymer structure is not so obvious in THF in both SEC and in RPLC.

A change in mobile phase resulted in a greater change in the selectivity on the polymer-based packing materials than on the silica C_{18} . As seen in Table II, the retention of a phenyl group on PLRP-S or ODP-50 in 40% THF relative to silica C_{18} was much less than in 80% methanol. Similarly, PLRP-S and ODP-50 showed a large decrease in retention of PAHs, 10–12 and 16, upon changing the mobile phase from 80% methanol to 40% THF, as shown in Fig. 3. This change was accompanied by an increase in retention of other aromatic compounds with bulkiness or flexibility such as 15 and 17. In the case of DE-613, the same change in the mobile phase from 80% methanol to 40% THF resulted in a similar extent of retention of PAHs, and a much greater increase for other aromatic compounds.

The greater preference for planar PAHs shown by ODP-50 and PLRP-S 300 than by other packing materials in methanol–water, and the loss of such a preference in THF–water, suggest that the retention of PAHs on PLRP-S and ODP-50 in methanol–water is dominated by the contribution of the polymer network structure. In agreement with this, nitrogen adsorption measurement indicates the presence of narrower pores on ODP-50 and PLRP-S 300 than on the other materials. The mobile phase effects are understandable in terms of the combination of the facts that microscopic swelling occurs in polymer gels in THF due to better solvation which would favour the retention of bulky compounds, and that THF selectively binds to micropores which otherwise would preferentially bind rigid, planar compounds^{22,25}. A structural matching between the polymer chain structure and the solutes, in other words a steric compatibility, seems to exist, especially with PLRP-S, which may lead

to slower diffusion, or lower column performance for aromatic compounds in methanol–water.

When the column performance was examined, the silica C₁₈ phase showed a good performance for all the solutes tested in the three mobile phases, 80% methanol, 80% acetonitrile and 40% THF. In 80% methanol, C₁₈-4PW, DE-613 and ODP-50 showed similar plots. While consistent reduced plate height, *h*, values were obtained for alkanes and alkylbenzenes, increases in *h* were seen with increasing *k'* values for PAHs, and more so for bulky aromatic compounds. The efficiency of PLRP-S 300 is considerably lower than those of the other materials in this mobile phase, but still showed a similar tendency. The results are understandable in terms of the restricted diffusion of bulky solutes in the polymer network structure.

In 40% THF, however, evidently different features in column performance were seen between the two groups of polymer-based packing materials. DE-613 and C₁₈-4PW, alkyl ester type gels, showed consistently good performance for all the hydrocarbons tested as did silica C₁₈, although the *h* values were slightly larger than those on silica C₁₈ at comparable flow-rates. Conversely, ODP-50 and PLRP-S 300 showed poorer performance, that is an increase in *h* values with increasing *k'* values, only for planar PAHs in THF–water and in acetonitrile water. In the same mobile phases, these packing materials showed better performance for more bulky compounds, such as *o*-terphenyl (15), triptycene (17) and triphenylmethane (18) than in methanol water.

Note that ODP-50 and PLRP-S showed better performance for bulky molecules than for PAHs in THF–water, while this was not the case with DE-613 and C₁₈-4PW. The difference between the two groups of packing materials was also seen in the mobile phase effect on the retention selectivity and in the pore-size distribution determined by nitrogen adsorption, as mentioned above.

The differences in the structural effect of the solutes on column performance can be explained based on the differences in the chemical structure of the polymer chains. DE-613 and C₁₈-4PW are alkyl-type gels, containing no aromatic functionality, while PLRP-S contains aromatic groups and ODP-50 contains a cyclic cross-linking reagent, an isocyanuric derivative which assumes a planar structure⁴⁴. These functionalities can provide interactions with aromatic compounds via π -electrons, especially when the solutes are planar. The fact that the retention of bulky compounds on ODP-50 and PLRP-S is relatively weaker than that of PAHs compared with DE-613 and C₁₈-4PW in similar mobile phases indicates that the latter possess looser network structures than does ODP-50, and are well solvated with THF to give consistent *h* values.

On the contrary, ODP-50 possesses a smaller, more rigid micropore structure which restrict bulky hydrocarbons from entering into the micropores to give higher preference for PAHs in methanol–water, as is the case with PS gel²². With better solvation in THF–water and in acetonitrile–water, only PAHs showed slightly lower performance due to the interaction with the polymer backbones of ODP-50 and PLRP-S containing aromatic groups. With alkyl monomers and alkyl cross-linking reagents, C₁₈-4PW and DE-613 did not show such an effect.

As shown in Fig. 4, higher *h* values were obtained with polymer packings in 80% methanol than with silica C₁₈, although the efficiencies of the alkyl-type gels are much better than that of PS-based gel in this mobile phase. The differences are much

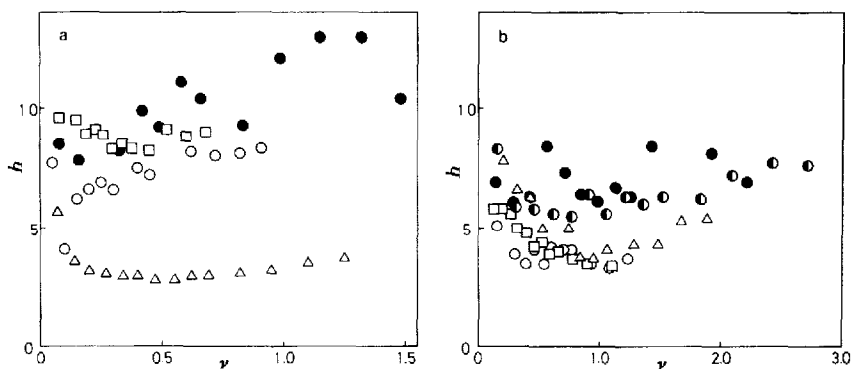


Fig. 4. Dependence of reduced plate heights, h , on the reduced velocity of the mobile phase, v , in 80% methanol (a) and in 40% THF (b). Solute: propylbenzene. Packing materials: silica C₁₈ (Δ); Shodex DE-613 (\circ); TSK C₁₈-4PW (\bullet); Asahipak ODP-50 (\square); PLRP-S 300 (\bullet).

smaller, especially at low flow-rates, in 40% THF. The results agree well with those obtained by others on PS-based packing materials^{22,25,32}. The performance of the alkyl-type gels was comparable with that of silica C₁₈, as shown in Fig. 5. This suggests that these packing materials can have wide applicability in RPLC, although the scope of application is yet to be explored.

It is commonly accepted that polymer gels show lower performance with increasing solute retention. This is shown to be true for a series of compounds with common structural features such as rigidity and planarity which match the chemical and dimensional structure of the polymer micropores. Such an effect strongly depends on the structures of the organic solvent and of the solute.

The PVA-based gel ODP-50 showed excellent performance for alkyl compounds in all the solvent systems, as reported earlier^{8,9}. Although h values for polyaromatic compounds are larger than those for alkyl compounds on this stationary phase, the performance was significantly improved in THF-water and more so in

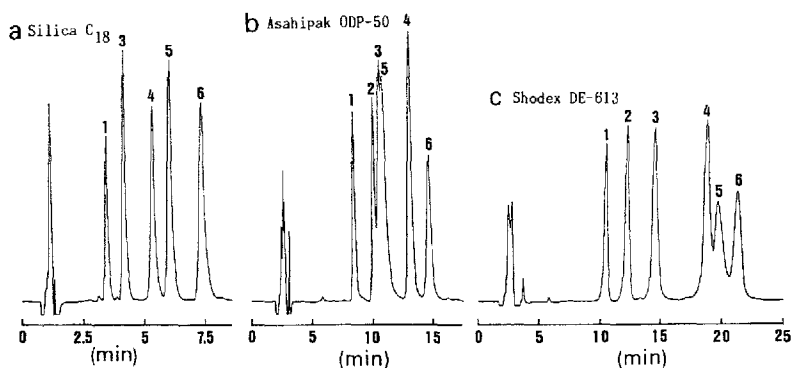


Fig. 5. Performance of polymer-based packing materials for hydrocarbons. Mobile phase: 50% THF. Flow-rate: 1 ml/min. Temperature: 30°C. (a) Silica C₁₈; (b) ODP-50; (c) DE-613. Solutes: benzene (1); toluene (2); naphthalene (3); diphenylmethane (4); triptycene (5); *o*-terphenyl (6).

acetonitrile–water, which gave the best results for this packing material. Solvation of the polymer chains reflected in the matching of solubility parameters seems to be important in determining the performance in each system. The solubility parameter of PVA is similar to that of acetonitrile, as seen in Table IV⁴⁵. The two C₁₈-type polymer packings, ODP-50 and C₁₈-4PW, showed relatively similar retention selectivity, although ODP-50 showed slightly greater retention of aromatic compounds than did C₁₈-4PW. The differences in retention selectivity and structure dependency of the column efficiency are most likely caused by the cross-linking reagents used in these polymer gels. These packing materials showed selectivities similar to that of silica C₁₈ in the presence of acetonitrile or THF.

Although PLRP-S 300, a PS gel, showed relatively poor performance for aromatic hydrocarbons, especially for PAHs in 80% methanol, the performance was much better in 40% THF. In spite of the lower performance than for the other alkyl-type polymer-based phases for low-molecular-weight aromatic compounds, PLRP-S gels have been widely used for the separations of basic compounds and polypeptides, where stability of stationary phases is required¹¹. This material is reported to show excellent performance for high-molecular-weight compounds such as polypeptides which cannot enter into the micropores^{18,19}.

The present results suggest that the abundance of micropores which barely permit the slow diffusion of solutes causes band broadening. The results of the pore-size determination provide evidence to support this interpretation. The actual pore volume in the micropore region existing in a column is the largest with DE-613 in a solvating medium, followed by ODP-50, C₁₈-4PW and PLRP-S 300 in this order. Yet DE-613, C₁₈-4PW and ODP-50 showed much better performance than did PLRP-S 300. The latter, with a very small pore volume in this region, showed a very large effect of microporosity on the retention and performance for PAHs due to the presence of styrene and divinylbenzene units as well as the polymer gel structure allowing the solute to be trapped²². Column performance is related more to the chemical as well as the three-dimensional structures of the micropores than to the total volume of such micropores in the polymer gels.

CONCLUSION

Determination of the pore size by nitrogen adsorption and SEC provides useful information about the performance and retention selectivity of polymer-based packing materials for RPLC. Polymer-based packing materials, including PS, PAM and esterified PVA, showed selective retention of solutes with structural rigidity compared with silica C₁₈, the preference order being PAHs, flexible aromatic compounds, cycloalkanes and linear alkanes. The column efficiencies provided by polymer gels with alkyl backbones are much better than those of PS-packed columns, and comparable with those provided by silica-based phases under optimized conditions. The change in mobile phase from methanol–water to acetonitrile–water or THF–water resulted in higher column efficiencies and loss of structural selectivity accompanied by slight swelling of gels. The results can be explained by the contribution of chemical as well as the three-dimensional structures of the polymer micropores. As more stable packing materials are desired for RPLC⁴⁶, the use of polymer-based packing materials will increase with understanding of their chromatographic properties.

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