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Reversed-phase high-performance liquid chromatography on porous copolymers of different chemical structure

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Abstract

The influence of chemical structure of porous polymers on the chromatographic properties of high-performance liquid chromatography columns was studied. Columns were packed with four different porous copolymers: di(methacryloyloxymethyl)naphthalene–divinylbenzene containing ester functional groups, 4,4′-bis(maleimido)diphenylmethane–divinylbenzene with imide groups, di(4,4′-dimethacrylphenyl)sulfone–divinylbenzene which contains sulphonyl groups, and styrene–divinylbenzene with any functional groups. Using the alkyl aryl ketone scale, the retention indices of five homologous series (alkylbenzenes, alkyl aryl ketones, *N*-alkylanilines, alkyl aryl ethers, alkylbenzoates) and column test compounds (toluene, nitrobenzene, *p*-cresol, 2-phenylethanol, *N*-methylaniline) were calculated. Their values were used for comparison of the selectivities of the studied polymeric packings. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Retention index scale; Porous copolymers; Polar functional groups; Selectivity; Stationary phases, LC; Alkyl aryl ketones

1. Introduction

Macroreticular porous polymer sorbents are widely used in solid-phase extraction for the isolation of organic compounds from air and water and as stationary phases in gas, liquid and supercritical fluid chromatography. In liquid chromatography they are used in ion-exchange and size-exclusion chromatography, but less frequently in normal and reversed-phase high-performance liquid chromatography (HPLC), where silica-based packings dominate [1–8].

The commercially available porous polymers are macroporous copolymers of styrene cross-linked with divinylbenzene. These modern column packings are characterized by good mechanical stability as a result of the high percentage of cross-linking [9–12]. Additionally, they have a reasonably homogeneous chemical structure, deprived of strong binding sites.

Besides advantages such as resistance to pH changes and unlimited lifetime the polymeric packings have some shortcomings. The main drawback of the polystyrene phases is their sensitivity to solvent changes associated with the presence of micropores in their internal structure [9,13–15]. The microporosity of the polystyrene phases is not constant, but can change with the nature of the eluent and sample. Even rigid, highly cross-linked polystyrene packings have an undesirable microporosity which appears in contact with strong eluents such as tetrahydrofuran or acetonitrile. In consequence, these packings can shrink or swell with a change of solvent. This

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problem becomes less important when eluents with a high water content are used because, under these conditions, the microporous structure is closed or unavailable. Despite this, polymeric reversed-phase HPLC packing materials seem to be excellent alternatives to the alkyl-bonded silicas.

Among the techniques used to characterize polymeric stationary phases, the retention of standards based on the alkyl aryl ketone scale, introduced by Smith [16–18], is the most promising. In order to determine the selectivity of the HPLC column, Smith selected a set of column test compounds (toluene, nitrobenzene, *p*-cresol, 2-phenylethanol and *N*-methylaniline) reflecting the effects of specific interactions with different stationary phases. Column test compounds are chosen as the aromatic equivalents of the standards used by Rohrschneider [19] and McReynolds [20] in gas chromatography and represent solutes of different polarity. Retention indices of these compounds permit a direct comparison of different columns in reversed-phase HPLC.

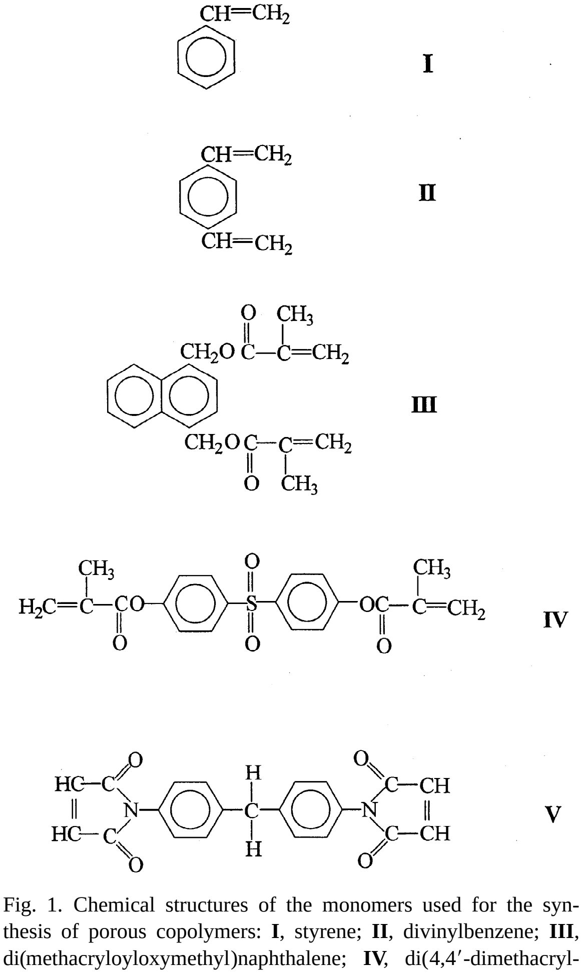

Fig. 1. Chemical structures of the monomers used for the synthesis of porous copolymers: **I**, styrene; **II**, divinylbenzene; **III**, di(methacryloyloxymethyl)naphthalene; **IV**, di(4,4′-dimethacrylphenyl)sulfone; **V**, 4,4′-bis(maleimido)diphenylmethane.

In this paper the influence of the chemical nature of the polymeric stationary phase on their selectivities is studied. Besides non-polar styrene–divinylbenzene (ST–DVB) porous copolymer, three others synthesized by us were used: di(methacryloyloxymethyl) naphthalene – divinylbenzene (DMN–DVB) which contains ester functional groups, 4,4′-bis(maleimido)diphenylmethane–divinylbenzene (BM–DVB) containing the imide functional group and di(4,4′-dimethacrylphenyl)sulfone–divinylbenzene (DMS–DVB) with sulphonyl groups. The chemical structures of the monomers used are shown in Fig. 1.

2. Experimental

2.1. Chemicals and eluents

Methanol and acetonitrile were of LiChrosolv quality from Merck (Darmstadt, Germany). The alkyl aryl ketone retention index standards, homologous alkylbenzenes, *N*-alkylanilines, alkyl aryl ethers, alkyl benzoates and test compounds were laboratory reagent grade from a range of sources. Disodium hydrogenorthophosphate and potassium dihydrogenphosphate were reagent grade from POCh (Gliwice, Poland). Buffer solution (pH 7) was prepared from 0.0029 *M* disodium hydrogenorthophosphate (0.500 g), 0.0022 *M* potassium dihydrogenphosphate (0.301 g) and 1 L bidistilled water. The pH of the buffer solution was verified by a pH meter Model 720A (Orion, Boston, MA, USA). All eluents were filtered through suitable filters in a Sartorius apparatus (Sartorius, Göttingen, Germany).

2.2. HPLC measurements

Separations were carried out using a Hewlett-Packard HP-1050 liquid chromatograph equipped with a UV diode-array detector, a Rheodyne Model 7125 injection valve with a sample loop of 20 μL and a 100 mm×4 mm I.D. column packed with porous copolymers. Columns were packed according to the procedure described elsewhere [10].

As previously, methanol–phosphoric buffer pH 7 (70:30; v/v) and acetonitrile–phosphoric buffer pH 7 (50:50; v/v) were used as mobile phases [12]. The eluent flow-rate was 0.5 mL $\min^{-1}$. The sieve fraction of the polymeric particles in the columns was always 5–15 μm. The column void volume was determined by injecting 10 μL of a saturated aqueous solution of sodium nitrate. All substances studied were injected as ca. 0.1% solutions in the proper mobile phase. Data acquisition and integration were controlled by a Hewlett-Packard ChemStation.

2.3. Preparation of porous copolymers

All porous copolymers were obtained by combined suspension-emulsion polymerization from an equivalent mole fraction of monomers [21]. For copolymerization with divinylbenzene the following monomers were used: styrene (Merck, Germany),

Table 1
Retention factors (k) and specific retention factors ($k_s = k/S_c$) for alkyl aryl ketones, alkylbenzenes, and *N*-alkylanilines with methanol–buffer (70:30, v/v) eluent

Compound	ST–DVB		DMN–DVB		BM–DVB		DMS–DVB	
	k	$k_s \cdot 10^3$	k	$k_s \cdot 10^3$	k	$k_s \cdot 10^3$	k	$k_s \cdot 10^3$
Alkyl aryl ketones								
Acetophenone	0.39	2.78	0.30	3.73	0.21	7.37	0.31	2.35
Propiophenone	1.22	8.70	1.15	14.28	0.71	24.91	1.00	7.57
Butyrophenone	2.93	20.90	2.23	27.70	1.14	40.00	2.00	15.15
Valerophenone	6.09	43.44	4.31	53.54	2.00	70.17	4.06	30.76
Hexanophenone	11.26	80.31	8.46	105.09	3.36	117.89	7.75	58.71
Heptanophenone	20.81	148.43	16.62	206.45	5.50	192.98	13.38	101.36
Relative interactions between heptanophenone and porous copolymer (%)		100		139		130		68
Alkylbenzenes								
Benzene	0.48	3.42	0.54	6.70	0.08	2.80	0.13	0.98
Toluene	1.71	12.20	1.00	12.42	0.21	7.36	0.75	5.68
Ethylbenzene	3.61	25.75	2.15	26.70	0.50	17.54	1.44	10.90
n-Propylbenzene	6.74	48.07	3.92	48.70	0.86	30.17	2.44	18.48
n-Butylbenzene	13.52	96.43	7.54	93.66	1.50	52.63	4.50	34.09
Relative interactions between *n*-butylbenzene and porous copolymer (%)		100		97		55		35
N-Alkylanilines								
Aniline	0.48	3.42	0.30	3.73	0.32	11.22	0.14	1.06
N-Methylaniline	0.93	6.63	0.89	11.05	0.40	14.03	0.51	3.86
N-Ethylaniline	2.06	14.69	1.15	14.29	0.57	20.00	0.81	6.14
N-Propylaniline	4.51	32.17	2.31	28.70	1.21	42.47	2.00	15.51
N-Butylaniline	7.02	50.07	5.54	66.82	2.00	70.18	3.88	29.40
Relative interactions between *N*-butylaniline and porous copolymer (%)		100		133		140		59
Correlation coefficient for alkyl aryl ketones								
Acetophenone–heptanophenone	0.94954		0.99021		0.98381		0.99192	
Butyrophenone–heptanophenone	0.99903		0.99998		0.99960		0.99939	

di(methacryloyloxymethyl)naphthalene [22], 4,4′-bis(maleimido)diphenylmethane [23], and di(4,4′-dimethacrylphenyl)sulfone [24]. They were polymerized in the following way: mixtures of the monomers containing 0.5 mol fractions of each monomer with α,α′-azobisisobutyronitrile were added to an aqueous solution of Aerosol OT-75 and polymerized at 80°C for 20 h. After polymerization the product was washed with hot water and extracted with acetone, methanol and toluene in a Soxhlet apparatus. A uniform fraction of 5–15 μm copolymer beads was isolated by sedimentation from 90:10 (v/v) methanol–acetone. The specific surface areas were measured on an adsorption analyser ASAP 2010N (Micrometrics Inc., USA) using a standard nitrogen adsorption method.

3. Results and discussion

To compare the chromatographic properties of porous copolymers containing different functional

Table 2
Retention factors (k) and specific retention factors ($k_s = k/S_c$) for alkyl aryl ketones, alkylbenzenes, and N-alkylanilines with acetonitrile–buffer (50:50, v/v) eluent

Compound	ST–DVB		DMN–DVB		BM–DVB		DMS–DVB	
	k	$k_s \cdot 10^3$	k	$k_s \cdot 10^3$	k	$k_s \cdot 10^3$	k	$k_s \cdot 10^3$
Alkyl aryl ketones								
Acetophenone	0.08	0.57	0.08	0.99	0.17	5.96	0.08	0.61
Propiophenone	0.18	1.28	0.45	5.59	0.35	12.28	0.20	1.51
Butyrophenone	0.34	2.43	1.04	12.92	0.57	20.00	0.42	3.18
Valerophenone	1.34	9.56	2.16	26.83	0.96	33.68	0.92	6.97
Hexanophenone	4.58	32.67	4.08	50.68	1.57	55.09	2.00	15.15
Heptanophenone	10.86	77.46	8.25	102.48	2.41	97.18	3.93	29.77
Relative interactions between heptanophenone and porous copolymers (%)		100		132		125		38
Alkylbenzenes								
Benzene	0.24	1.71	0.54	6.70	0.23	0.81	0.11	0.83
Toluene	0.75	5.34	0.79	9.81	0.50	17.54	0.28	2.12
Ethylbenzene	2.37	16.90	1.42	17.64	0.84	29.47	0.71	5.38
n-Propylbenzene	5.37	38.30	2.37	29.44	1.53	53.68	1.30	9.85
n-Butylbenzene	11.34	80.88	4.28	53.16	2.04	71.58	2.29	17.34
Relative interactions between *n*-butylbenzene and porous copolymers (%)		100		66		88		22
N-Alkylanilines								
Aniline	0.09	0.64	0.38	3.60	0.30	10.52	0.04	0.03
N-Methylaniline	0.12	0.85	0.49	6.08	0.40	14.03	0.07	0.53
N-Ethylaniline	0.20	1.43	0.70	8.70	0.70	24.50	0.25	1.89
N-Propylaniline	1.03	7.35	2.00	24.84	1.35	47.36	0.79	5.98
N-Butylaniline	3.53	25.17	3.67	45.59	2.12	74.39	1.68	12.73
Relative interactions between *N*-butylaniline and porous copolymers (%)		100		181		295		50
Correlation coefficient for alkyl aryl ketones								
Acetophenone–heptanophenone	0.98879		0.98995		0.99856		0.98566	
Acetophenone–butyrophenone	0.99962		0.99965		0.99952		0.99940	

groups the method proposed by Smith et al. [16–18,25] was used. The method allows comparison of different HPLC columns as it is based on the retention index scale. In order to use the alkyl aryl ketones index scale the relation between the logarithm of the retention factor and the carbon number was studied. The logarithms of the retention factors versus carbon number were plotted for alkyl aryl ketones using different eluents and the linearity of the plots was determined by regression analysis of the relationship:

$$\log k = an_{\mathrm{C}} + c$$

(n_{C} is the number of carbon atoms, c a constant and a the slope) for each homologous series (Tables 1 and 2).

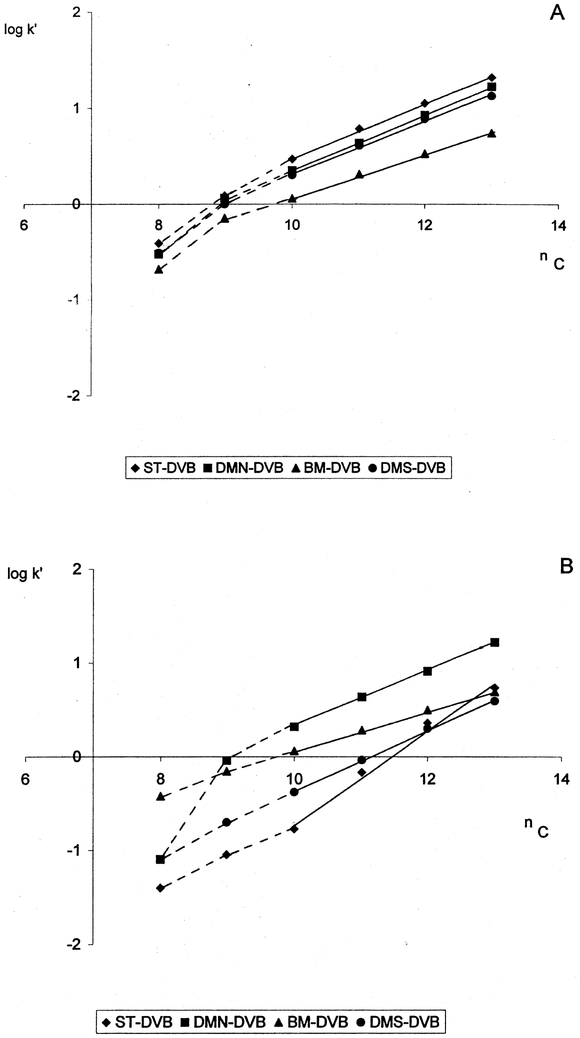

Fig. 2. Variation of $\log k'$ with the carbon number (n_{C}) of alkyl aryl ketones on the studied porous copolymers with different mobile phases: (A) methanol–buffer; (B) acetonitrile–buffer.

As expected from previous work [10], the first members of the alkyl aryl ketone homologous series show some deviations from linearity on all the studied copolymers (Fig. 2). Thus, the retention indices were calculated using the linear relationship for alkyl aryl ketones from butyrophenone to heptanophenone. Fortunately, on all the studied columns, retention increase of acetophenone confirming the effect of molecule inclusion was not observed. Tables 1 and 2 present the values of the retention factors (k) for alkyl aryl ketones, alkylbenzenes, and N-alkylanilines. To make a direct comparison of the columns, the values of the retention factors with reference to the surface area of the copolymer in the column (k_{s}) were calculated. Taking into account the values of the specific surface areas and the weights of the copolymers in the columns (Table 3), the increase of the k values indicates that the strength of the sample–sorbent interactions also increases. The strongest interactions are observed for non-polar alkylbenzenes on the ST–DVB copolymer. These interactions have typical hydrophobic character [26]. Lets us assume that the value of k_{s} for n-butylbenzene on ST–DVB is equal to 100%; thus the values obtained on other copolymers reflect a decrease of hydrophobic interactions. This effect is especially visible on DMS–DVB despite the fact that the values of S_{c} of this copolymer and non-polar ST–DVB are very similar (Table 3). For polar N-alkylanilines (calculations for N-butylaniline), stronger interactions are observed on copolymers containing ester (DMN–DVB) and imide (BM–DVB) groups. The weak interactions with DMS–DVB are associated with the chemical character of the DMS monomer. Probably, the sulfur atom weakens the hydrophobicity of the aromatic rings. In MeOH–buffer the contribution of hydrophobic interactions between n-alkylbenzenes and DMN–DVB copolymer are comparable to those with ST–DVB. In this case, the naphthalene ring of strong hydrophobic character plays an important role. These results are in accordance with the earlier studies of Kimata et al. [27] and Tchapla et al. [28] concerned

Table 3
Properties of the copolymers studied

Copolymer	Weight of copolymer in the column (g)	Specific surface area, S (m^2/g)	Surface area of the copolymer in the column, S_c (m^2)
ST–DVB	0.469	299.0	140.2
DMN–DVB	0.763	105.5	80.5
BM–DVB	0.814	35.0	28.5
DMS–DVB	0.635	208.0	132.0

with the contribution of the polar group in the stationary phase to retention selectivity in reversed-phase HPLC.

Tables 4 and 5 show the retention indices for the five homologous series and test compounds. In both mobile phases, the retention indices of alkylbenzenes have the highest values on the ST–DVB porous copolymer. It is not very visible for benzene but this

Table 4
Retention indices measured on ST–DVB, DMN–DVB, BM–DVB, and DMS–DVB copolymers with methanol–buffer (70:30, v/v) eluent

Compound	Retention index			
	ST–DVB	DMN–DVB	BM–DVB	DMS–DVB
Alkyl aryl ketones				
Acetophenone	768	759	777	701
Propiophenone	860	902	907	885
Butyrophenone	995	1000	997	994
Valerophenone	1107	1099	1103	1105
Hexanophenone	1202	1200	1202	1206
Heptanophenone	1297	1300	1297	1294
Alkylbenzenes				
Benzene	717	788	677	557
Toluene	911	880	747	839
Ethylbenzene	1027	996	839	942
n-Propylbenzene	1123	1085	941	1025
n-Butylbenzene	1230	1183	1049	1122
N-Alkylanilines				
Aniline	658	707	688	663
N-Methylaniline	716	849	864	852
N-Ethylaniline	818	902	1008	994
N-Propylaniline	940	1005	1103	1098
N-Butylaniline	1061	1137	1200	–
Alkyl aryl ethers				
Methyl phenyl ether	725	758	619	720
Ethyl phenyl ether	883	953	809	903
n-Propyl phenyl ether	1004	1056	970	1012
n-Butyl phenyl ether	1106	1163	1074	1122
Alkylbenzoates				
Methyl benzoate	802	826	809	811
Ethyl benzoate	920	902	997	903
n-Propyl benzoate	1033	1020	1096	1012
n-Butyl benzoate	1132	1126	1172	1117
n-Pentyl benzoate	1209	1220	1268	1211
Void volume for sodium nitrate (mL)	0.92	0.48	0.47	0.53

Table 5
Retention indices measured on ST–DVB, DMN–DVB, BM–DVB, and DMS–DVB columns with acetonitrile–buffer (50:50, v/v) eluent

Compound	Retention index			
	ST–DVB	DMN–DVB	BM–DVB	DMS–DVB
Alkyl aryl ketones				
Acetophenone	779	713	770	768
Propiophenone	874	920	892	755
Butyrophenone	991	998	997	997
Valerophenone	1107	1104	1102	1102
Hexanophenone	1213	1197	1204	1205
Heptanophenone	1290	1300	1297	1296
Alkylbenzenes				
Benzene	932	902	870	898
Toluene	1020	956	967	942
Ethylbenzene	1120	1043	1075	1066
n-Propylbenzene	1202	1118	1179	1146
n-Butylbenzene	1277	1204	1257	1223
N-Alkylanilines				
Aniline	715	650	802	666
N-Methylaniline	864	902	936	876
N-Ethylaniline	946	992	1066	957
N-Propylaniline	1092	1093	1172	1064
N-Butylaniline	1186	1182	1265	1162
Alkyl aryl ethers				
Methyl phenyl ether	843	831	807	810
Ethyl phenyl ether	1035	932	983	973
n-Propyl phenyl ether	1134	1130	1110	1102
n-Butyl phenyl ether	1203	1212	1224	1184
Alkylbenzoates				
Methyl benzoate	850	902	770	861
Ethyl benzoate	911	950	913	849
n-Propyl benzoate	1092	1019	1085	1036
n-Butyl benzoate	1174	1130	1194	1145
n-Pentyl benzoate	1232	1218	1272	1210
Void volume for sodium nitrate (mL)	0.82	0.47	0.43	0.47

compound did not behave as the zeroth member of this homologous series [17,18]. In MeOH–buffer, *N*-alkylanilines have the largest retention indices on the BM–DVB and DMS–DVB porous copolymers. Ethers have an increased retention on the DMN–DVB copolymer containing ester groups, whereas esters (alkylbenzoates) on the BM–DVB copolymer. In the ACN–buffer mobile phase, the retention indices of polar *N*-alkylanilines are the largest on the BM–DVB columns. In this phase, weak interactions with the DMS–DVB copolymer containing sulphonyl functional groups are observed. Ethers and esters have the largest retention on the copolymer with imide groups.

Fig. 3 shows the separation of alkyl aryl ketones on all the columns studied. In MeOH–buffer the most effective separation is obtained on the DMN–DVB copolymer. Theoretical plate numbers are rather small independent of the mobile phase used (Table 6).

Table 7 shows the retention indices of the test compounds obtained on the studied porous copolymers. As stressed previously [10] for all test compounds extrapolation can deform the real values of the retention indices. Despite this, a comparison of different columns is possible, assuming that each mobile phase is studied separately to remove the effects of sample–solvent interactions [16,17]. In both mobile phases, toluene is retained most significantly compared with other compounds on the ST–DVB column. Nitrobenzene has the smallest retention on this column, while it has the largest on BM–DVB containing imide groups. The retention indices of *p*-cresol, representing a compound of acidic character, have rather low values. In MeOH–buffer, different interactions of this compound and the studied porous copolymers are observed, while in ACN–buffer these differences disappear. A similar phenomenon is observed for 2-phenylethanol. *N*-Methylaniline has the smallest retention index on the ST–DVB column in both mobile phases. This is especially visible in MeOH–buffer. In ACN–buffer it clearly has the highest value on the BM–DVB porous copolymer.

The results presented here suggest that the chemical structure of the polymeric stationary phase is responsible for some specific interactions with the separated compounds. Discussion of the nature of these interactions is complicated because retention indices of the compounds are based on standards which possess polar functional groups. It will be easier to define some of the interactions when alkylbenzenes are used as retention index standards.

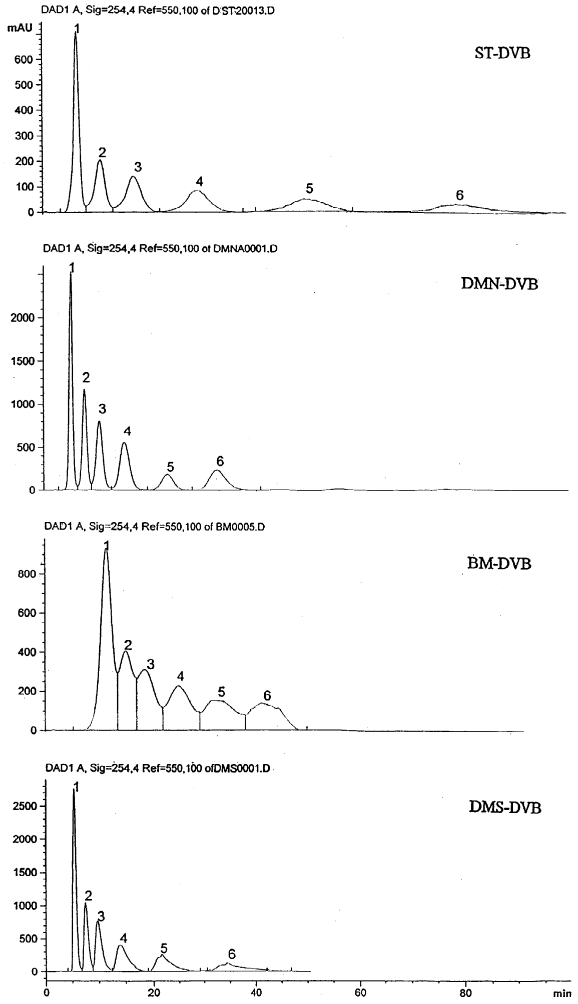

Fig. 3. Chromatograms of the homologous alkyl aryl ketones. Mobile phase: methanol–buffer (70:30, v/v).

Acknowledgements

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Table 6
Theoretical plate number of the columns (*N*)

Copolymer	*N*			
	ST–DVB	DMN–DVB	BM–DVB	DMS–DVB
Methanol–buffer				
Toluene	1020	2300	550	1200
Nitrobenzene	1500	3000	620	1400
N-Methylaniline	950	2200	560	840
Acetonitrile–buffer				
Toluene	750	1000	440	850
Nitrobenzene	880	1000	500	800
N-Methylaniline	450	950	340	700

Table 7
Retention indices of test compounds on the studied porous copolymers

Copolymer	Retention index				
	Toluene	Nitrobenzene	*p*-Cresol	2-Phenylethanol	*N*-Methylaniline
Methanol–buffer					
ST–DVB	911	875	540	448	716
DMN–DVB	880	966	705	626	849
BM–DVB	747	1048	712	552	864
DMS–DVB	839	1032	588	649	852
Acetonitrile–buffer					
ST–DVB	1020	921	637	546	864
DMN–DVB	956	949	692	561	902
BM–DVB	967	1118	662	582	936
DMS–DVB	952	942	651	538	876

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