Characterization of the Porous Structure of Polymeric Packings for HPLC

CHROMATOGRAPHIA

2001, *54*, 595–599

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Key Words

Column liquid chromatography
Inverse exclusion chromatography
Polymeric packings
Porous structure

Summary

Using inverse exclusion chromatography, the porous structure of four polymeric stationary phases has been investigated. Three of the copolymers were characterized by a high degree of crosslinking because they were synthesized from two tetrafunctional monomers. The fourth copolymer was obtained from styrene (bifunctional monomer) and divinylbenzene (tetrafunctional monomer) and has a significantly smaller degree of crosslinking.

To determine the pore size distribution of the copolymers, toluene, alkylphenones, phthalates, and polystyrene standards were used as probes. Additionally, changes of the volume of the copolymers when changing from water to tetrahydrofuran were determined. The results show that more stable porous structures were obtained from tetrafunctional monomers. All polymeric stationary phases have micropores in their internal structure but their contributions are different.

Introduction

The increasing interest in porous polymer beads in recent years is due to their diversified applications as starting materials for the synthesis of ion-exchange resins and column packings for exclusion chromatography (EC), and high performance liquid chromatography (HPLC). The first polymers were suitable only for EC. In the mid-1980s copolymers of enhanced rigidity were also applied in reversed-phase HPLC. The most popular polymers for HPLC are macroporous copolymers of styrene crosslinked with divinylbenzene (ST-DVB). These materials are characterized by good mechanical stability caused by the high degree of cross-linking [1–9].

Porous copolymers used as stationary phases in HPLC should maintain constant volume and geometry during changes of mobile phase composition because the porous structure of the stationary phase has a great influence on its chromatographic properties.

Unfortunately, porous polymers besides advantages such as resistance to pH changes and unlimited life have some shortcomings. The main drawback of the polystyrene phases is their sensitivity to solvent changes connected with the presence of micropores in their internal structure [10, 11]. The microporosity of the polystyrene phases is not constant, but can change with the nature of the eluent and sample.

For separations in eluents with a high water content the microporosity is less important, because under these conditions the microporous structure is closed or unaccessible. In this case only meso- and macropores are open for diffusion by the solute [11].

The results from inverse EC measurements indicate that even highly cross-linked polystyrene phases have an open structure caused by the spatial structure of the polymeric matrix. The existence of pores smaller than 20 Å in the internal structure of the polystyrenes is due to the size of the monomeric divinylbenzene units [11]. These pores allow the solutes to penetrate into the whole bulk mass of the polymer. This phenomenon is especially observed when good solvents such as tetrahydrofuran or acetonitrile are used as mobile phases.

One of the approaches to achieve rigid polymeric networks is associated with the use of copolymers obtained from two tetrafunctional monomers [12]. These copolymers are characterized by a higher degree of cross-linking than the ST-DVB.

The results for copolymers of di(methacryloyloxymethyl)naphthalene – divinylbenzene (DMN-DVB) indicate that the contribution of micropores depends on the method of synthesis. Our previous results show that the common methods used for preparation of porous materials do not

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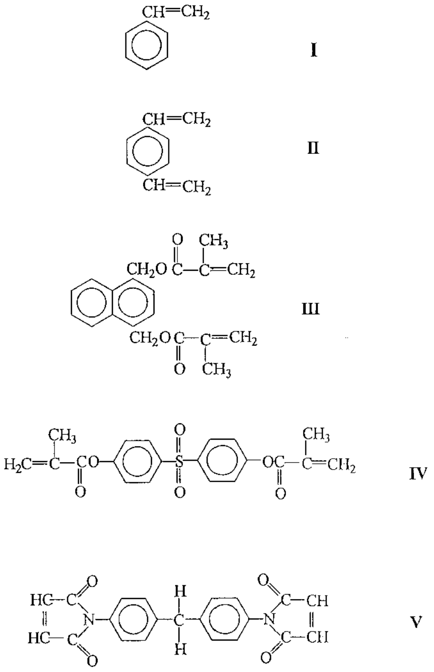

Figure 1. Chemical structures of the monomers used for the synthesis of porous copolymers: I = styrene; II = divinylbenzene; III = di(methacryloyloxymethyl)naphthalene; IV = di(4,4′-dimethacrylphenyl)sulfone; V = 4,4′-bis(maleimido)diphenylmethane.

prevent the formation of some micropores in the copolymer internal structure. In consequence, completely cross-linked porous copolymers (DMN-DVB) swell less than ST-DVB but still have some micropores [12].

In this paper, the porous structure of four polymeric packings has been studied. Besides DMN-DVB these are: di(4,4′-dimethacrylphenyl)sulfone – divinylbenzene (DMS-DVB), 4,4′-bis(maleimido)diphenylmethane – divinylbenzene (BM-DVB), and styrene – divinylbenzene (ST-DVB) [13]. All of them were synthesized from equivalent mole fractions of monomers. Only the copolymer ST-DVB is obtained from bi- and tetrafunctional monomers; thus its crosslinking degree is significantly smaller. To characterize the porous structure of these copolymers inverse EC and nitrogen adsorption/desorption methods were applied.

Experimental

Chemicals and Eluents

Tetrahydrofuran was HPLC grade from Merck (Darmstadt, Germany). Acetone and methanol (reagent grade) came from POCh (Gliwice, Poland). Toluene, alkylphenones, and phthalates were laboratory reagent grade, obtained from a number of sources. Polystyrene standards were from Toyo Soda (Tokyo, Japan), and Merck (Darmstadt, Germany).

Preparation of Porous Copolymer Beads

All the studied copolymers were synthesized by combined suspension-emulsion polymerization in the presence of pore-forming diluents. Mixtures of equivalent mole fractions of monomers were first dissolved in diluents to form an organic phase, in which the initiator, α,α′-azobisisobutyronitrile, was added. Homogenous organic phase was then added in a 1:1.8 volume ratio to the aqueous phase containing Aerosol OT-75, while stirring. Polymerization lasted 20 h at 80 °C. The obtained copolymers in the shape of beads were washed with hot water and then toluene, acetone, and methanol in a Soxhlet apparatus. Uniform particles (5–15 µm) were isolated by sedimentation from methanol-acetone (90:10; *v*/*v*). The columns (100 mm × 4.1 mm I. D.) were packed according to the procedure described earlier [14].

Chemical structures of the monomers used are presented in Figure 1.

Chromatographic Measurements

Retention volumes of toluene, alkylphenones, phthalates, and polystyrene standards were determined with a Hewlett-Packard HP-1050 liquid chromatograph equipped with a diode array UV detector, a Rheodyne 7125 injection valve with 20 µL sample loop and columns packed with the copolymers. Each substance was injected separately as a 0.1% *v*/*v* solution in tetrahydrofuran (THF). The columns were thermostated at 30 °C.

In the HPLC and EC measurements THF was the mobile phase at 0.5 mL min^{-1}. To determine the swelling propensity factors, twicedistilled water, at the same flow rate, was used as the mobile phase. The given retention volumes always represent the mean of three determinations.

Characterization of Porous Structure

Characterization of porous structures was made using inverse EC and nitrogen adsorption-desorption measurements. The copolymers, in a dry state, were characterized by means of an adsorption analyser ASAP 2405 (Micrometrics Inc, USA). Determinations were based on the measurements of adsorption and desorption of nitrogen on the surface of the studied copolymer while cooling it with liquid nitrogen. The specific surface areas were calculated by the BET method, assuming that the area of a single nitrogen molecule is 16.2 Å^2.

In a swollen state the copolymers were characterized by an inverse EC technique introduced by Halász and Martin [15]. The main assumption in this method is that, in the good solvent, chains of macromolecules form coils of diameter corresponding to the polymer molecular weight. The diameter of the probe molecules (Φ, in Å) was calculated from the equation [15]:

$$\Phi = 0.62\, M_w^{0.59}$$

where M_w is the gram-molecular weight of the probe.

The diameter of the probe molecule is associated with a pore diameter (Φ) which corresponds to the smallest pore allowing unhindered access for the probe of a given molecular weight. Toluene, alkylphenones, phthalates, and polystyrenes were used as the pore-size probes [11, 12].

The cumulative pore size distribution (PSD) was determined from the plot 1 – K_o(EC) versus lg Φ, where K_o(EC) is the distribution constant in exclusion chromatography calculated from the equation [15, 16]:

$$K_o(\mathrm{EC}) = \frac{V_R - V_o}{V_p} = \frac{V_R - V_o}{V_i - V_o}$$

where V_R – the retention volume of the probe
V_o – the interstitial volume equal to the retention volume of a totally excluded molecule
V_i – the retention volume of a totally included molecule
V_p = $V_i - V_o$ – the pore volume

As previously, V_i is equal to the retention volume of toluene [12].

The differential pore size distribution W(Φ), was obtained by computer differentiation of the polynomial fitted cumulative pore size distribution curves (PSD) versus the logarithm of the probe pore diameter, lgΦ. Six- to ten-order polynomials were used for fitting the experimental data.

Swelling propensities of the copolymers (SP factors) were calculated according to Nevejans and Verzele's method [17]:

$$\mathrm{SP} = \frac{p(\mathrm{THF}) - p(\mathrm{H_2O})}{p(\mathrm{H_2O})}$$

where $p = P/\eta$ is the pressure relative to the mobile phase viscosity, η, and P is the column inlet pressure when THF and water were used as mobile phases.

Table I. Characterization of the porous structure of the copolymers in the dry state.

Copolymer	Specific surface area (m^2g^{-1})	Pore volume (cm^3g^{-1})	Average pore diameter (Å)	Weight of the copolymer in the column (g)
ST-DVB	299.0	0.54	90	0.469
DMN-DVB	105.5	0.26	85	0.763
DMS-DVB	208.0	0.25	50	0.635
BM-DVB	35.0	0.12	155	0.814

Table II. Properties of the studied copolymers in the swollen state.

Copolymer	Pore volume (cm^3g^{-1})	Contribution of micropores (cm^3g^{-1})	Swelling propensity (SP factor)
ST-DVB	1.25	0.076	1.06
DMN-DVB	0.81	0.022	0.90
DMS-DVB	0.85	0.250	0.93
BM-DVB	0.45	0.010	0.67

Results and Discusion

Porous structure characterization of the studied copolymers obtained from nitrogen adsorption/desorption measurements is listed in Table I. These results refer to polymeric samples in a dry state. Under these conditions, the micropores existing in the copolymer structure are not accessible to the nitrogen molecules, because their diameters are smaller than the diameter of the nitrogen molecule [18]. In the EC column, polymeric packing is wetted by a good solvent like THF, which strongly interacts with the aromatic rings, causing loosening of the polymeric spatial net. In consequence, the copolymer swells and its porous structure changes completely. In a swollen state the porous structure of the copolymer is similar to that under liquid chromatography conditions. From this point of view, inverse EC is (Table II) more informative in the characterization of HPLC packings [19, 20].

In Table III the retention volumes of the compounds used as the pore-probes and their diameters are presented. From these data and the weights of the copolymer in the columns (Table I) pore volume and volume of micropores for the studied copolymers are determined (Table II). According to Nevejans and Verzele [11] the term microporosity defines the pores with a diameter smaller than 20 Å. In the studies presented here, differences between the retention volumes of molecules having diameters smaller than 20 Å and of the molecule of dinonyl phthalate (Φ = 22 Å) indicate the contribution of micropores to the internal structure of the copolymer.

The contribution of micropores is the highest for the copolymer DMS-DVB and the swelling propensity for this copolymer is the smallest. Among the studied copolymers the highest value of SP factor is for the copolymer ST-DVB. This means that the volume and pore structure of the ST-DVB copolymer changes significantly when water is displaced by THF. The obtained value is typical for polystyrene adsorbents [21]. The behaviour of DMS-DVB and ST-DVB copolymers is un-

Table III. Diameter (ϕ) and retention volume (V_R) of the probes on the porous copolymers.

No.	Probe	Molecular weight (g)	ϕ(Å)	Retention volumes (mL)			
				ST-DVB	DMN-DVB	DMS-DVB	BM-DVB
1	Toluene	92.14	9.1	1.014	0.927	1.067	0.985
2	Acetophenone	120.15	10.6	1.018	0.929	1.066	0.984
3	Butyrophenone	148.20	12.1	1.006	0.920	1.065	0.983
4	Dimethyl phthalate	194.19	14.1	0.994	0.916	1.064	0.984
5	Diethyl phthalate	222.24	15.3	0.985	0.914	0.978	0.984
6	Dibutyl phthalate	278.35	17.4	0.979	0.912	0.926	0.985
7	Dinonyl phthlate	418.62	22.2	0.978	0.910	0.908	0.977
8	Didodecyl phthalate	502.78	24.7	0.965	0.836	0.871	0.850
9	Polystyrene	580	26.5	0.964	0.835	0.856	0.848
10	Polystyrene	666	28.7	0.956	0.792	0.828	0.845
11	Polystyrene	1,050	37.6	0.953	0.690	0.684	0.825
12	Polystyrene	4,000	84.1	0.906	0.570	0.670	0.775
13	Polystyrene	5,100	95.5	0.863	0.558	0.594	0.771
14	Polystyrene	8,000	126.5	0.866	0.537	0.558	0.731
15	Polystyrene	11,600	155.0	0.843	0.530	0.540	0.719
16	Polystyrene	30,300	273.2	0.750	0.494	0.532	0.673
17	Polystyrene	68,000	440.1	0.691	0.466	0.529	0.634
18	Polystyrene	120,000	615.3	0.574	0.442	0.529	0.628
19	Polystyrene	390,000	1,253.3	0.470	0.335	0.529	0.623
20	Polystyrene	750,000	1,814.2	0.430	0.310	0.530	0.620
21	Polystyrene	1,260,000	2,463.8	0.427	0.310	0.529	0.620
22	Polystyrene	2,610,000	3,786.3	0.427	0.310	0.530	0.620
23	Polystyrene	3,840,000	4,831.7	0.427	0.310	0.529	0.620

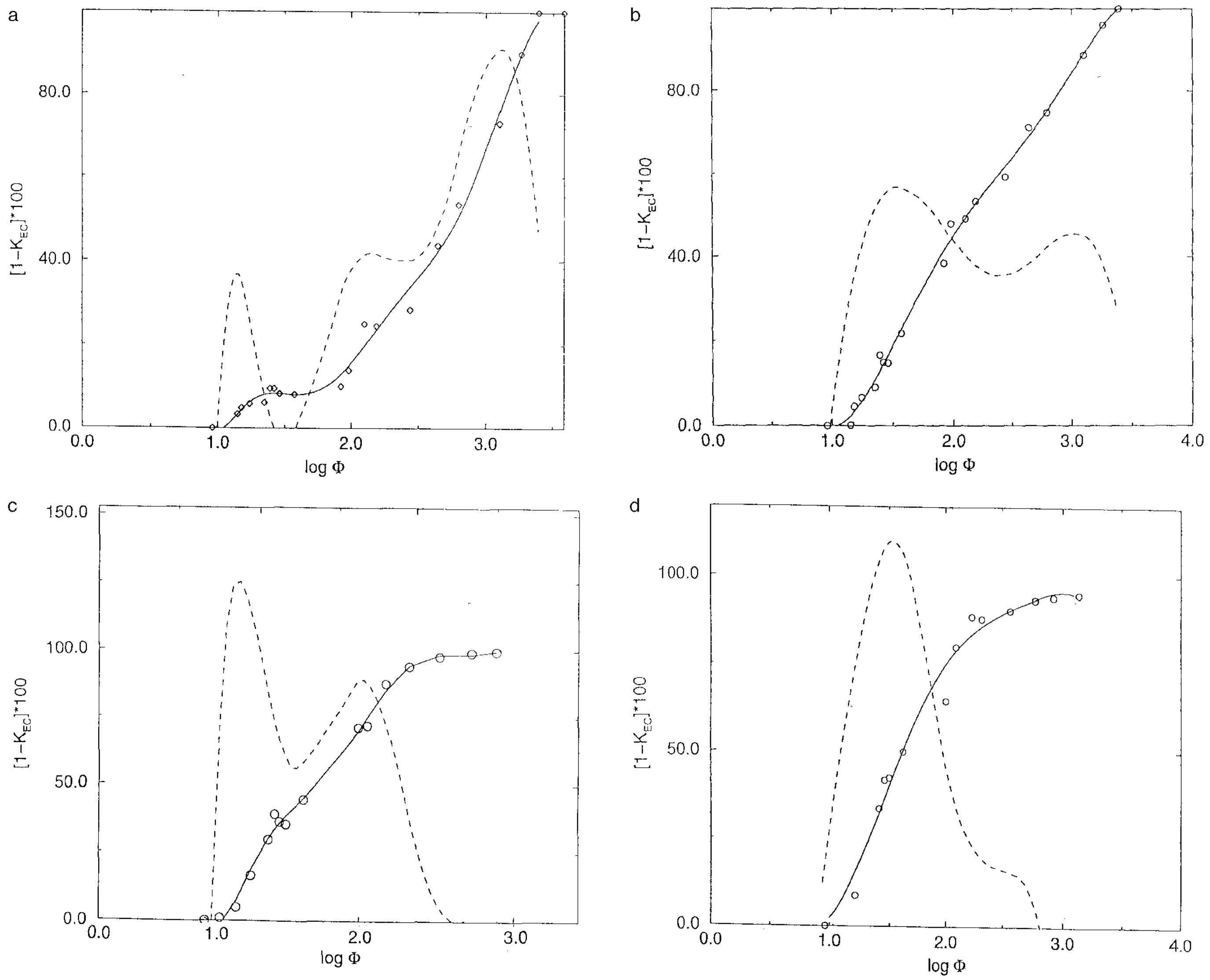

Figure 2. Cumulative and differential pore size distribution curves for the studied copolymers: **a)** ST-DVB; **b)** DMN-DVB; **c)** DMS-DVB; **d)** BM-DVB.

doubtedly associated with different degree of crosslinking. For copolymers obtained from two tetrafunctional monomers swelling propensities are rather low [22–24].

The differences in the copolymer internal structures are presented in Figures 2a–d. The Figures show cumulative and differential pore size distributions. The differential curves (dashed lines) indicate that, except for the BM-DVB, all the studied copolymers have two-dispersive structures. The first maximum is for micropores and the second for mezo- or macropores. Pore size distributions for the copolymers DMN-DVB and DMS-DVB are very similar but contribution of micropores in the internal structure of the DMS-DVB copolymer is significantly higher. Also the second maximum on the differential curves corresponding to the diameters of the most probable macropores is located in a different place. For the copolymer DMN-DVB the most probable macropores have a diameter of 1,000 Å, whereas for DMS-DVB porous copolymer the pores have a diameter of c.a. 100 Å. Porous structure distribution for the copolymer ST-DVB shows that diameters of the pores change over a wide range from c.a. 20 Å to more than 1,000 Å. Differential pore size distribution for the copolymer BM-DVB has a regular Gaussian shape with the most probable pore diameters of 30 Å. The poor chromatographic behaviour of this copolymer suggests that the pores are too small [13].

The results presented here confirm that independently of the degree of cross-linking, the porous structure of polymeric sorbents is quite different in dry and wet states. All porous polymers have micropores but their contribution is different.

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Received: May 11, 2001
Accepted: Jul 18, 2001