

Porous Microspheres, Copolymers of Bis[4-(2-hydroxy-3-methacryloyloxypropoxy)phenyl]sulfide, and Divinylbenzene as Stationary Phase for HPLC

Beata Podkościelna, Andrzej Bartnicki, Barbara Gawdzik

Faculty of Chemistry, MCS University, pl. Marii Curie-Skłodowskiej 5, 20-031 Lublin, Poland

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ABSTRACT: Synthesis of highly crosslinked methacrylate copolymers of in form of microspheres is presented. They are prepared from tetrafunctional methacrylate derivative of bis(4-hydroxyphenyl)sulfide, bis[4-(2-hydroxy-3-methacryloyloxypropoxy)phenyl]sulfide (BES-DM), and divinylbenzene (DVB). In chemical structure of these copolymers exhibit hydroxyl and ester groups of hydrophilic nature. Additionally, copolymer contains sulfur atoms coming from BES-DM monomer. Porous structure of the copolymers in the dry and wet state was studied.

Microspheres possessing the largest pore volume and specific surface area were subjected to chromatographic investigations. The results show that polar functional groups existing in the chemical structure of the studied microspheres have influence on reversed-phase HPLC retention mechanism. © 2008 Wiley Periodicals, Inc. *J Appl Polym Sci* 111: 1257–1267, 2009

Key words: bis(4-hydroxyphenyl)sulfide; divinylbenzene; copolymerization; porous microspheres; surfaces; HPLC

INTRODUCTION

Porous polymeric packings in high-performance liquid chromatography (HPLC) have their history. The use of first rigid macroporous copolymers for HPLC has its origins in the work carried out by Sober and Peterson.¹ In 1954, they made the observation that proteins could be adsorbed by diethylaminoethyl-derivatized cellulose and then subsequently eluted by the eluent of an increasing ionic strength.

In 1964, Moore² synthesized macroporous poly(styrene-divinylbenzene) (PS-DVB) materials with a high percentage of divinylbenzene (DVB) playing a role of crosslinker. He carried out suspension polymerization in the presence of a porogen, a compound which is soluble in the monomers but insoluble in the polymer. In consequence, he obtained rigid spherical particles containing large pores in their structure. Technology originally developed by Moore is still commonly used.³

Early experiments with polymer packing materials as high efficiency counterparts to alkyl-bonded silica failed. Unlike silica, most of these early polymers were not enough rigid and compressed under the high eluent linear flow velocities.⁴ The resulting

high pressure rendered them unsuitable for most applications. Other polymers exhibited greater rigidity, but their mass transfer characteristics were poor, and the resulting long analysis times were unacceptable. Simultaneously, these materials were stable with eluents from pH 1–14.

This feature caused growing interest in polymeric packings for use in HPLC. In a short time scientists and producers improved their mechanical properties. To achieve high mechanical stability, modern synthetic technologies produce these polymeric beads with a high degree of crosslinking, up to 90%.⁵ The obtained highly crosslinked PS-DVB microspheres were not only chemically stable, inert, ion free, insoluble in all nonoxidizing solvents but also physically rigid and capable of higher mass transfer than conventional polystyrene packings.

Practical application of PS-DVB columns caused that their important shortcomings were revealed. They were associated with their inherent hydrophobicity and swelling in organic solvents, which is especially troublesome when solvent gradients were used.⁶

Recently searching for more hydrophilic materials and good mechanical resistance have been made. These new materials can be obtained from monomers containing polar functional groups or by chemical modification of the nonpolar ST-DVB polymeric matrix.

This article is one of the series presenting synthesis of highly crosslinked methacrylate copolymers of

Correspondence to: B. Gawdzik (barbarag@hermes.umcs.lublin.pl).

in form of microspheres.⁷⁻⁹ They are prepared from tetrafunctional methacrylate derivative of bis(4-hydroxyphenyl)sulfide, bis[4-(2-hydroxy-3-methacryloyloxypropoxy)phenyl]sulfide (BES-DM) and DVB. Use of two tetrafunctional monomers in their synthesis lead to high crosslinkage degree and as a consequence ensure them good mechanical stability. In chemical structure of these copolymers exhibit hydroxyl and ester groups of hydrophilic nature. Additionally, copolymer contains sulfur atoms coming from BES-DM monomer.

This article presents chromatographic properties of these unique microspheres.

EXPERIMENTAL

Chemicals and eluents

Bis(2-ethylhexyl)-sulfosuccinate sodium salt (DAC,BP), 1-decanol, tetrahydrofuran (THF), and methacrylic acid were from Fluka AG (Buchs Switzerland). Bis(4-hydroxyphenyl)sulfide, α,α' -azoisobisbutyronitrile, and DVB (62.2% of 1,4-DVB, 0.2% of 1,2-DVB, and ethylvinylbenzene) washed with 3% aqueous sodium hydroxide solution before use were obtained from Merck (Darmstadt, Germany). Reagent grade acetone, methanol, chloroform, *p*-xylene, 2-propanol 1,4-dioxane, dichloromethane, toluene, sodium hydroxide, potassium hydroxide, 2-(chloromethyl)oxirane and hydroquinone were from POCh (Gliwice, Poland).

Methanol and acetonitrile were of LiChlorosolv quality from Merck (Darmstadt, Germany). The akryl 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, citric acid, sodium citrate, sodium nitrate, and potassium dihydrogenophosphate were reagent grade from POCh (Gliwice, Poland).

Phosphate buffer solution of pH 7 was prepared from 0.0029 mol disodium hydrogenorthophosphate (0.500 g) and 0.0022 mol potassium dihydrogenophosphate (0.301 g) in 1 L redistilled water. Citrate buffer solution of pH 7 was prepared by dissolving 23 g of citric acid in 1 L of redistilled water, and 29.4 g of sodium citrate in 1 L of redistilled water. Next, 15 mL of citric acid and 985 mL of sodium citrate solutions were mixed in 1 L calibrate flask.

All buffers were diluted five times using Millipore-Q water. The pH of the buffer solutions were verified by a pH meter Model 720A (Orion, Boston, MA). All eluents were filtered through suitable filters in a Sartorius apparatus (Sartorius, Göttingen, Germany).

Preparation of bis[4-(2,3-epoxypropoxy)phenyl]sulfide (BES-EP)

In a 500 cm³ round-bottomed three-necked flask equipped with a mechanical stirrer, a thermometer, and a dropper, 0.1 mol of bis(4-hydroxyphenyl)sulfide (21.8 g) was placed together with 1 mol (92.5 g) of 2-(chloromethyl)oxirane, and 0.75 mol (45.0 g) of 2-propanol and the whole content was heated over a water bath at 70°C. Then, at the same temperature 3 g of NaOH (catalyst) were added during 10 min, another portion of 13.5% solution of NaOH was added (0.65 mol or 25.8 g) and the stirring was continued for 5 min. When the reaction was over, the content of the flask was placed into the dropper, the water layer was separated along with the sodium chloride in it, and the organic layer was distilled at low pressure (1.6 kPa) to separate 2-propanol and the excess of 2-(chloromethyl)oxirane. The remaining 2-(chloromethyl)oxirane and water were distilled at low pressure after 50 g of toluene was added into it. The obtained epoxide compound was filtered off. The yield of the reaction was about 98.5%; its epoxide number was close to the theoretical value.

Preparation of bis[4(2-hydroxy-3-methacryloyloxypropoxy)phenyl]sulfide (BES-DM)

The reaction between the epoxide groups of bis[4-(2,3-epoxypropoxy)phenyl]sulfide and the carboxylic ones of methacrylic acid went on at 90°C. The synthesis was carried out in a 500 cm³ round-bottomed flask equipped with a thermometer, a mechanical stirrer, and a heater. In such a flask 0.5 mol (165 g) of BES-EP, 1 mol (86.09 g) of 2-methacrylic acid, 2 g of triethylbenzylammonia TEBA (a catalyst),⁸ and 0.02 g hydroquinone (polymerization inhibitor) were added. The progress of the reaction was controlled by determination of the acidic number. The acidic number determines the amount of KOH milligrams used to neutralize the free acids contained in 1 g of substance. The reaction was considered to be over when the acidic number was below 3. Its rate is higher when carried out at higher temperatures but a considerable exothermic effect can result in losing control of the reaction and appearing of some products of darker color.

Chemical structure of the new monomer was confirmed by spectroscopic methods.

Copolymerization

Copolymerization with DVB was performed in the aqueous medium. Redistilled water (150 mL) and 1.4 g of bis(2-ethylhexyl)sulfosuccinate sodium salt (surfactant) were stirred for 0.5 h at 80°C in a three-necked flask fitted with a stirrer, a water condenser, and a thermometer. Then, the solution containing

TABLE I
Experimental Parameters of the Syntheses of the
BES-DVB. Initiator = 0.18 g. Ratio of monomers (w/w);
BES : DVB = 10 : 2.6 (g)

Synthesis no.	Diluent (mL)		1-Decanol
	Toluene	Product	
1	16.00	0	Coagulated
2	14.00	2.00	Microspheres
3	12.00	4.00	Microspheres
4	10.00	6.00	Microspheres
5	8.00	8.00	Microspheres
6	4.00	12.00	Microspheres
7	6.00	10.00	Microspheres
8	4.00	12.00	Microspheres
9	2.00	14.00	Microspheres
10	0	16.00	Microspheres

10 g of the BES-DM and 2.6 g of DVB, the constant amount of initiator: 0.18 g of α, α' -azoisobutyronitrile, and the mixture of pore-forming diluents (toluene and 1-decanol) were added while stirring the aqueous medium (Table I). Copolymerization was performed for 18 h at 80°C. The obtained copolymers in the form of porous microspheres were washed with distilled water, filtered off, dried, and extracted in a Soxhlet apparatus with boiling acetone and methanol. Microscopic examination showed that in most cases particles of perfectly spherical shapes with diameters in the range 5–30 μm were obtained.

Uniform particles (5–15 μm) used in further studies were isolated by sedimentation from acetone-methanol (90 : 10, *v/v*).

Characterization

GC-MS was made on a Thermo-Finnigan DSQ spectrometer (Finnigan, USA) connected with a gas chromatograph Trace GC-Ultra equipped with a fused-silica RTX-5 capillary column (20 m \times 0.18 mm I.D., film thickness 0.20 μm). The conditions were as follows: injector PTV-split 1 : 20, program temperature 35–320°C with the rate 20°C min^{-1} ; MS electron ionization at 70 eV, and temperature of ion volume is 220°C.

^1H NMR spectra were recorded on a Bruker 300 MSL instrument (Bruker, Germany) operating at the ^1H resonance frequency of 300 MHz. Chemical shifts were referred to chloroform serving as an internal standard. ^{13}C NMR spectrum of BES-EP and BES-DM in chloroform was made using the same apparatus.

Swellability coefficients, B , were determined by equilibrium swelling in acetone, methanol, acetonitrile, THF, dichloromethane, using the centrifugation method. B is expressed as¹⁰:

$$B(\%) = \frac{V_s - V_d}{V_d} \times 100$$

where V_s is the volume of the copolymer after swelling, whereas V_d is the volume of the dry copolymer.

As the porous BES-DM microspheres will be used as HPLC column packing, their pH stability was studied. To do this, microspheres wetted by methanol were maintained in aqueous solutions of pH 3 and 13. After 5 and 24 h, FTIR spectra and thermogravimetry analyses were made. The results were compared with those for the reference sample.

Chromatographic measurements

The column (100 mm \times 4.1 mm I.D.) was packed according to the procedure described earlier.¹¹ The diameter of the polymeric beads packed in the column was 5–15 μm . Methanol-phosphate buffer pH 7 (70 : 30; *v/v*), methanol-citrate buffer pH 7 (70 : 30; *v/v*), acetonitrile-phosphate buffer pH7 (50 : 50; *v/v*), and acetonitrile-citrate buffer pH 7 (50 : 50; *v/v*) were used as mobile phases. The eluent flow rate was 0.5 mL min^{-1} .

The column void volume was determined by injecting 10 μL of a saturated solution of sodium nitrate.¹²

Separations of alkyl aryl ketones, alkylbenzenes, *N*-alkylanilines, alkyl aryl ethers, alkyl benzoates were determined using 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 copolymer. Test compounds (toluene, nitrobenzene, *p*-cresol, 2-phenylethanol, *N*-methylaniline) were injected separately.

Retention indices for alkylbenzenes, *N*-alkylanilines, alkyl aryl ethers, alkyl benzoates, and test compounds (toluene, nitrobenzene, *p*-cresol, 2-phenylethanol, *N*-methylaniline) were calculated according to the following equation¹³:

$$I_x = 100 \log(t'_{R,x}/t'_{R,z}) / \log(t'_{R,z+1}/t'_{R,z}) + 100z$$

in which $t'_{R,x}$ denotes the reduced retention time of the studied compound,

$t'_{R,z}$ —the reduced retention time of the homologous alkyl aryl ketone used as standard substance with the nearest shorter retention time,

$t'_{R,z+1}$ —the reduced retention time of the next higher homologue eluted after homologue z ,

z —the number of carbon atoms in the alkyl aryl ketone.

In exclusion chromatography (EC) experiment, retention volumes of toluene, alkylphenones, phthalates, and polystyrene standards were determined

using the same chromatograph. THF was the mobile phase at a flow rate 1 mL min^{-1} . Each substance was injected separately as 0.1% (*v/v*) solution in THF. The column was thermostated at 30°C .

Characterization of porous structure

Such parameters as specific surface areas, pore volumes, pore size distributions, and average pore diameters were determined by the method of nitrogen adsorption on the surface of the studied stationary phases in a dry state. The specific surface areas were calculated by the BET method, assuming that the area of a single nitrogen molecule is 16.2 \AA^2 . These determinations were made using an adsorption analyzer ASAP 2405 (Micrometrics Inc., USA). The measurements of the surface properties of the copolymers were preceded by activation of the samples at 200°C for 2 h. The beads were also examined using an atomic force microscope (AFM), AFM Nanoscope III (Digital Instruments, USA) operating in the contact mode. The images presented in this article contain 512×512 data points which were obtained within a few seconds. The typical force applied to obtain these images ranged from 1.0 to 100 nN.

In a swollen state, the beads were characterized by the inverse EC technique introduced by Halasz and Martin.¹⁴

The cumulative pore size distribution was determined from the plot $1 - K_0(\text{EC})$ versus $\lg \Phi$, where $K_0(\text{EC})$ is the distribution constant in EC calculated from the following equation¹⁴:

$$K_0(\text{EC}) = \frac{V_R - V_0}{V_p} = \frac{V_R - V_0}{V_i - V_0}$$

where V_R —the retention volume of the probe

V_0 —the interstitial volume equal to the retention volume of a totally excluded molecule

V_i —the retention volume of a totally included molecule

$V_p = V_i - V_0$ —the pore volume.

RESULTS AND DISCUSSION

Figure 1 presents the synthesis of the new aromatic tetrafunctional methacrylate monomer 4,4'-(2-hydroxy-3-methacryloyloxypropoxy)sulfide (BES-DM) and its copolymerization with DVB. The obtained copolymers were in the form of porous microspheres. For their synthesis, suspension-emulsion polymerization was applied. All syntheses were carried out in the presence of pore-forming diluents such as toluene and 1-decanol. The detailed information about the experimental parameters of all syntheses is given in Table I.

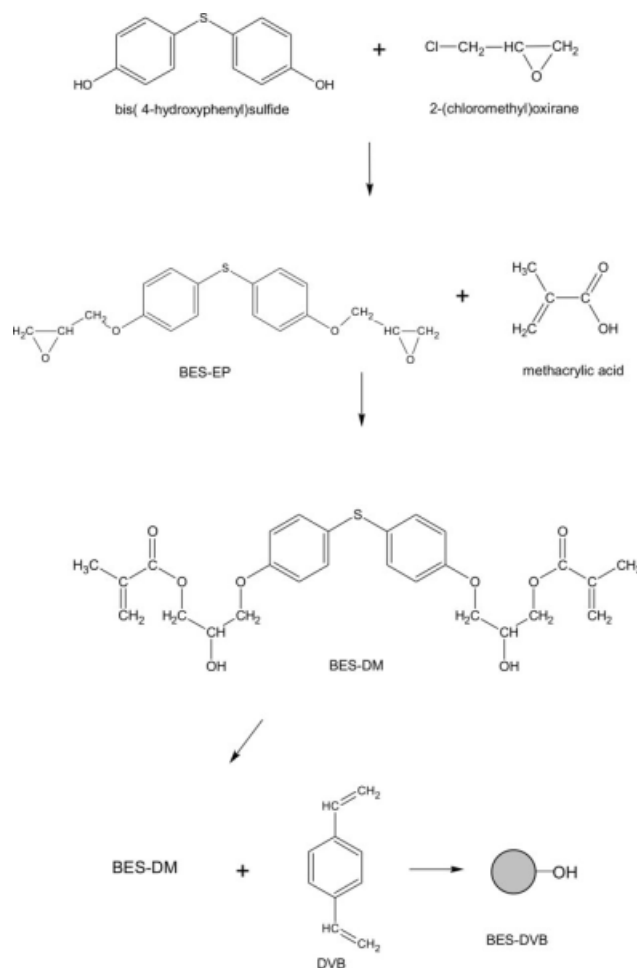


Figure 1 Synthesis and chemical structure of the compound used for copolymerization.

NMR Spectroscopy

The ^1H NMR spectra of both BES-EP and BES-DM were obtained. In the ^1H NMR spectrum of the BES-EP protons in the methylene group ($-\text{CH}_2-\text{O}$) connected with a oxygen give four shape bands at $\delta = 3.891, 3.910, 3.928, 3.947$ ppm and $\delta = 4.178, 4.188, 4.215, 4.225$ ppm, whereas protons from the terminal methylene group ($-\text{CH}_2-$) at $\delta = 2.742\text{--}2.894$ ppm. The bands at $\delta = 6.823\text{--}6.874$ ppm and $7.233\text{--}7.283$ ppm correspond to the phenyl ring.

In the ^1H NMR spectrum of BES-DM in chloroform protons of methylene group ($-\text{CH}_2-$) give signals at $\delta = 4.005\text{--}4.035$ ppm and $\delta = 4.336\text{--}4.360$ ppm; proton in hydroxyl group ($-\text{OH}$) at $\delta = 2.829\text{--}2.845$ ppm; and protons near the double bond ($-\text{CH}=\text{CH}_2$) give signals at $\delta = 5.605$ and $\delta = 6.140$ ppm.

The ^{13}C NMR spectra of these compounds are presented in Figure 2. Both spectra show set of three peaks at 76.7, 77.1, and 77.6 ppm. These peaks come from the solvent, chloroform and can be neglected

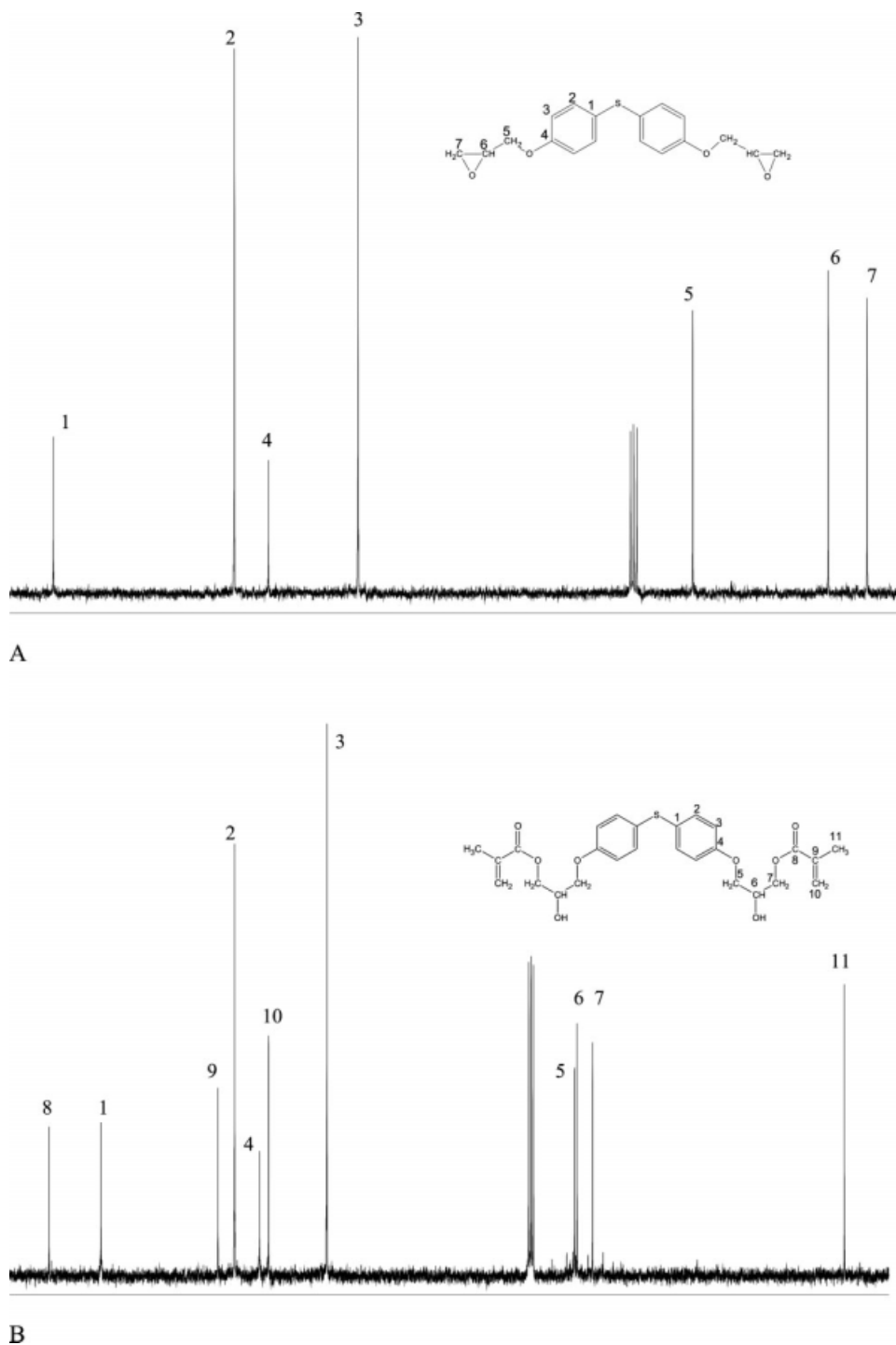


Figure 2 ^{13}C NMR spectra of the BES-EP (A) and BES-DM (B).

in interpreting spectra. In the spectrum of BES-EP, seven peaks are visible. Carbon atom in the terminal methylene group ($-\text{CH}_2$) gives signal at 44.6 ppm, in methylene group ($-\text{O}-\text{CH}_2-$) connected with oxygen at 68.9 ppm, and carbon of epoxide group ($-\text{CH}-$) at 50.1 ppm. The signals at 115.4, 128.0, 132.7, and 157.9 ppm come from four different types of carbon atoms of the aromatic ring.

In the spectrum of BES-DM, eleven peaks are visible. The terminal carbon atom of the double bond absorbs at 126.4 ppm and the more highly substituted one at 135.9 ppm. Carbon of the methyl group absorbs at 18.3 ppm. The signal for the carbonyl group of ester appears at 167.6 ppm, whereas that of methylene group carbon at 65.6 and 69.0 ppm. The most highly deshielded carbon atom (69.3 ppm) is

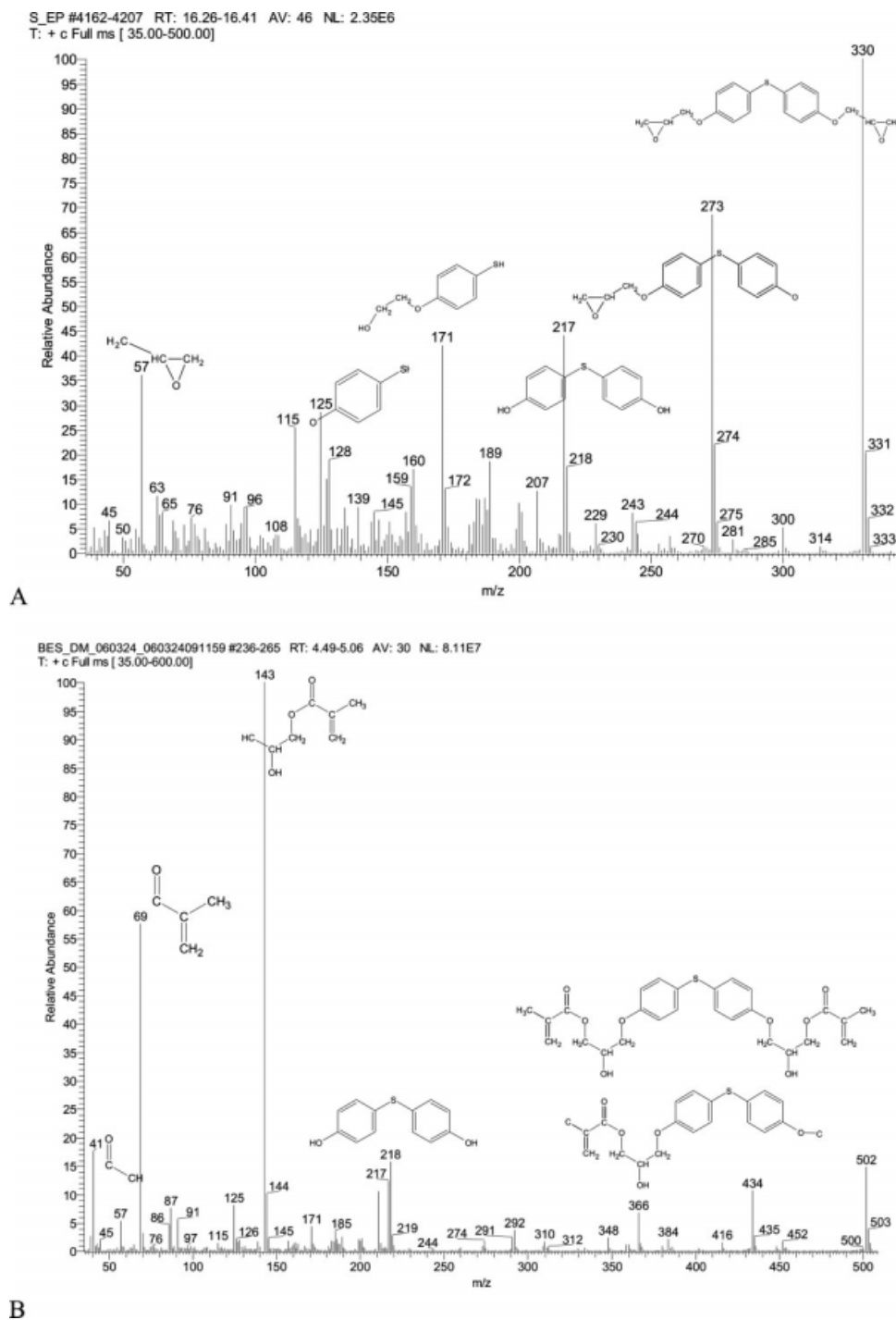


Figure 3 MS spectra of the BES-EP (A) and BES-DM (B).

bonded to the oxygen atom in hydroxyl group ($-\text{OH}$). Four different types of carbon atoms of the aromatic ring give signals at 115.5, 128.1, 132.8, and 157.8 ppm.

GC-MS

Chemical structures of BES-DM and its precursor BES-EP were also confirmed by the GC-MS analysis.

As their spectra were not available in the library, identification was achieved analyzing molecular and fragmentary ions. In the spectra presented in Figure 3, molecular ions corresponding to the calculated molecular weights of bis[4-(2,3-epoxypropoxy)phenyl]sulfide (330) and bis[4-(2-hydroxy-3-methacryloyloxypropoxy)phenyl]sulfide (502) are well visible. Besides, a large number of fragmentary ions are also noticeable. The most important ones are identified, and their meaning is presented in both the spectra.

TABLE II
Characterization of the Porous Structure
of the Obtained Porous Microspheres

Synthesis no.	Specific surface area (m ² /g)	Pore volume (cm ³ /g)	Average pore diameter (Å)
2	136	0.27	108
3	86	0.24	111
4	128	0.44	136
5	163	0.53	130
6	96	0.32	134
7	72	0.27	149
8	81	0.24	118
9	97	0.24	101
10	29	0.09	124

Porous structure characterization

In Table II characterization of the porous structure of the obtained microspheres in the dry state is presented. From these data, one can see that the obtained beads are characterized by rather low porosities. The sample no. 5 possesses the largest pore volume and specific surface area, and it was chosen for more detailed studies. The fragment of the surface of BES-DM microspheres showing its porous structure is presented in Figure 4.

As porous microspheres of BES-DM will be studied as HPLC packing to characterize their porous structure inverse EC is used. By this method, copolymer in a wetted state is studied that resembles real chromatographic conditions. The obtained data are presented in Table III and Figure 5.

Table III lists diameters, Φ , and retention volumes of the compounds used as the pore-probes, whereas Figure 5 presents cumulative and differential pore size distribution curves. To prepare EC pore size distribution an assumption that in the good solvent chains of macromolecules form coils of a diameter corresponding to the polymer molecular weights was made. 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.^{15,16} Constant retention volume corresponding to the interstitial volume for the studied polymer is achieved for polystyrene 750,000 (point no. 20).

From these data and the weights of the copolymer in the chromatographic column (Table IV), pore volume and volume of micropores for the studied copolymer are determined (Table V). According to Nevejans and Verzele,¹⁶ the term microporosity defines the pores with a diameter smaller than 20 Å. In the studies presented here, differences between the retention volumes of toluene having a molecule diameter $\Phi = 9$ Å and the molecule of dinonyl phthalate ($\Phi = 22$ Å) indicate the contribution of micropores to the internal structure of the copolymer.^{17,18}

The existence of micropores is well visible on differential pore size distribution curve. This curve has two maxima. The smaller maximum is associated with the contribution of micropores in the copolymer internal structures. Diameter of the most probable micropores is 17 Å. The larger maximum resembles the contribution of mezopores. Their diameters are in the range of 400 Å.

Changes of its porous structure in comparison to that of dry copolymer are caused by interactions of its net with THF. This good solvent swells the polymeric net. In consequence, some micropores become detectable. In a dry copolymer, micropores are not accessible for the nitrogen molecule, because their diameters are smaller than that of single nitrogen molecule.^{18,19}

Swelling studies

Swelling behavior of BES-DVB microspheres in the range of diluents of different intermolecular interactions was also studied. Their swellability coefficients

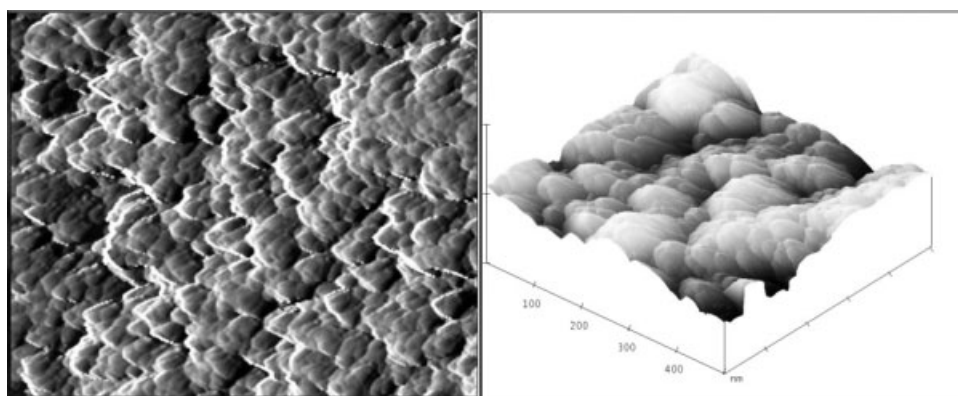


Figure 4 Contact-mode AFM images for the copolymer BES-DVB, magnification: $\times 135,000$.

TABLE III
Diameter (ϕ) and Retention Volume (V_R) of the Probes
on the Porous BES-DVB Copolymer

No.	Probe	Molecular weight, M (g)	ϕ (Å)	Retention volumes V_R (mL)
				BES-DVB
1	Toluene	92.14	9.1	0.948
2	Acetophenone	120.15	10.6	0.940
3	Butyrophenone	148.20	12.0	0.910
4	Dimethyl phthalate	194.19	14.1	0.906
5	Diethyl phthalate	222.24	15.3	0.868
6	Dipropyl phthalate	250.30	16.3	0.834
7	Dibutyl phthalate	278.35	17.4	0.824
8	Dipentyl phthalate	306.41	18.5	0.801
9	Diethyl phthalate	390.57	21.3	0.779
10	Dinonyl phthalate	418.62	22.2	0.765
11	Dilauryl phthalate	502.78	24.7	0.735
12	Polystyrene	580	26.9	0.730
13	Polystyrene	2,450	62.9	0.655
14	Polystyrene	5,100	97.0	0.600
15	Polystyrene	11,600	157.5	0.529
16	Polystyrene	30,300	277.6	0.457
17	Polystyrene	68,000	447.2	0.425
18	Polystyrene	120,000	625.3	0.417
19	Polystyrene	390,000	1253.3	0.416
20	Polystyrene	750,000	1843.4	0.413
21	Polystyrene	1,260,000	2503.6	0.412
22	Polystyrene	2,750,000	3847.3	0.412
23	Polystyrene	3,840,000	4831.7	0.412

in methanol, acetonitrile, acetone, THF, toluene, and hexane are presented in Table VI. The largest values of polymer swellability coefficients, equal or exceeding 30%, are achieved in polar THF and methanol. The smallest volume increase of the studied microspheres is observed in nonpolar hexane and toluene.

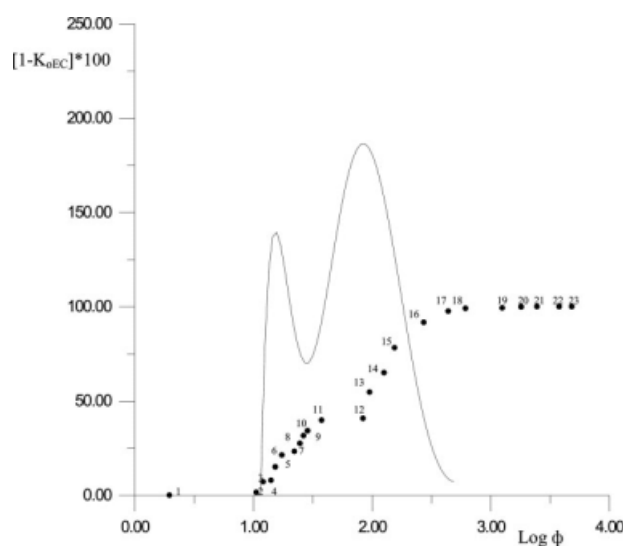


Figure 5 Cumulative and differential pore size distribution curves for the studied polymers. Numbers correspond to the probe-compounds from Table III.

Chromatographic properties

In characterization of chromatographic properties of porous BES-DVB microspheres the method proposed by Smith and Garside^{20,21} was used. In this method retention indices of selected compounds are used for quantitative description of the packing selectivity properties. Their values permit a direct comparison of different columns using the same eluents. Following their works, the retention indices of five sets of homologous compounds and the selectivity test compounds (toluene, nitrobenzene, *p*-cresol, 2-phenylethanol, *N*-methylaniline) were measured. Retention index scale was based on alkyl aryl ketones. Beside, methanol-phosphate buffer pH 7 (70 : 30; v/v) and acetonitrile-phosphate buffer pH 7 (50 : 50; v/v) previously used,^{11,12} methanol-citrate buffer pH 7 (70 : 30; v/v) and acetonitrile-citrate buffer pH 7 (50 : 50; v/v) were applied.

TABLE IV
Properties of the Microspheres Packed in the Column

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)
BES-DVB	0.7821	163	127

TABLE V
Properties of the Studied Copolymer
in the Swollen State

Copolymer		Pore volume (cm ³ /g)	Contribution of micropores (cm ³ /g)	Most probable pore diameter (Å)
BES-DVB	BET	0.530	0	250
	EC	0.628	0.183	20/110

pH stability studies show that the BES-DVB microspheres are stable in solvents of pH 3 and 13. It means that the studied copolymer can be used as

TABLE VI
Copolymer Swelling Studies

Copolymer	Swellability coefficient, <i>B</i> (%)				
	Acetone	Methanol	THF	Toluene	Hexane
BES-DVB	29	32	35	23	20

HPLC stationary phase in both acidic and basic mobile phases.

Exemplary chromatograms are presented in Figures 6 and 7. Figure 6 presents separation of alkyl

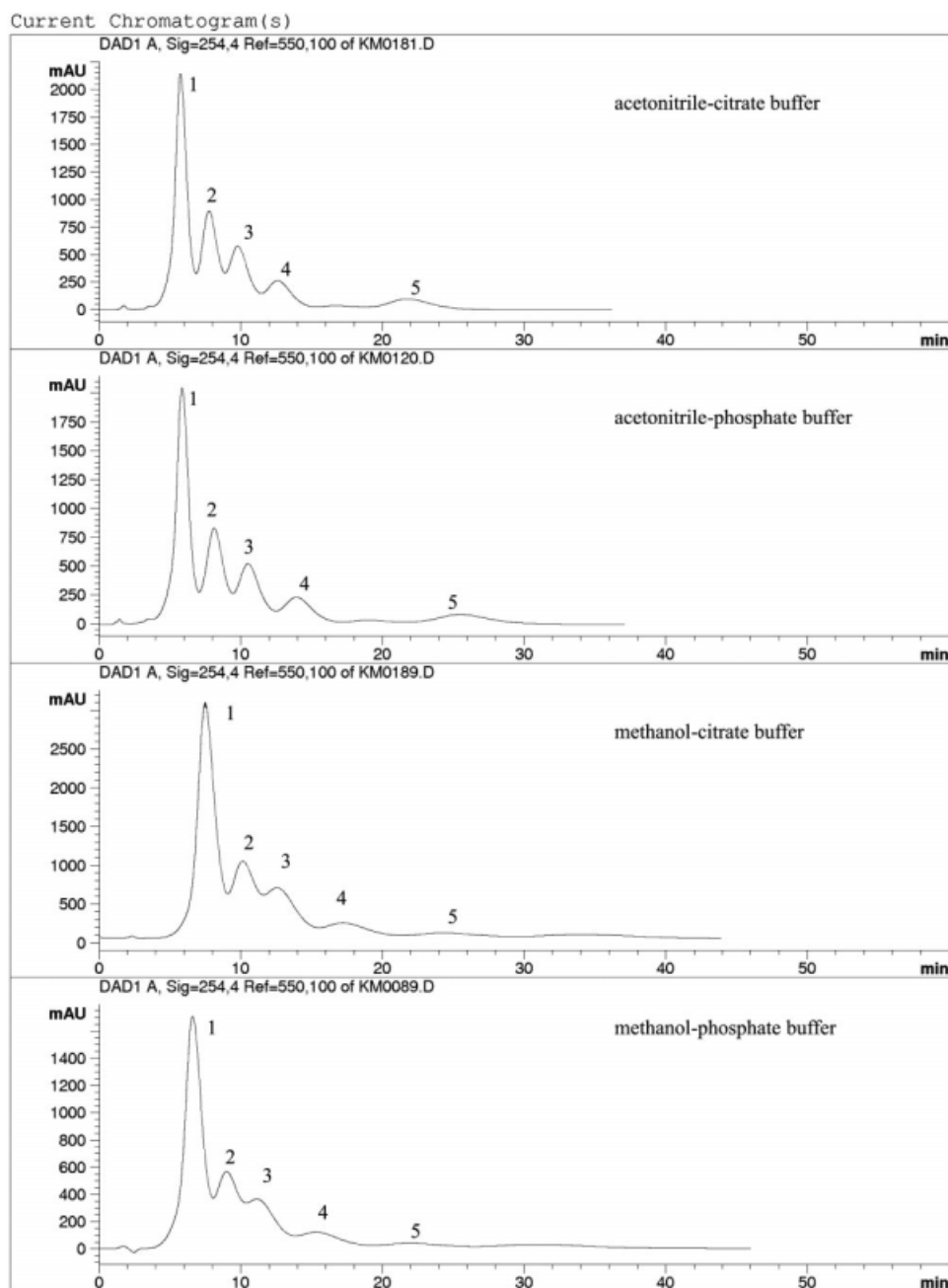


Figure 6 Chromatogram of homologous alkyl and aryl ketones in different mobile phases. Peaks: 1, acetophenone; 2, propiophenone; 3, butyrophenone; 4, valerophenone; and 5, hexanophenone.

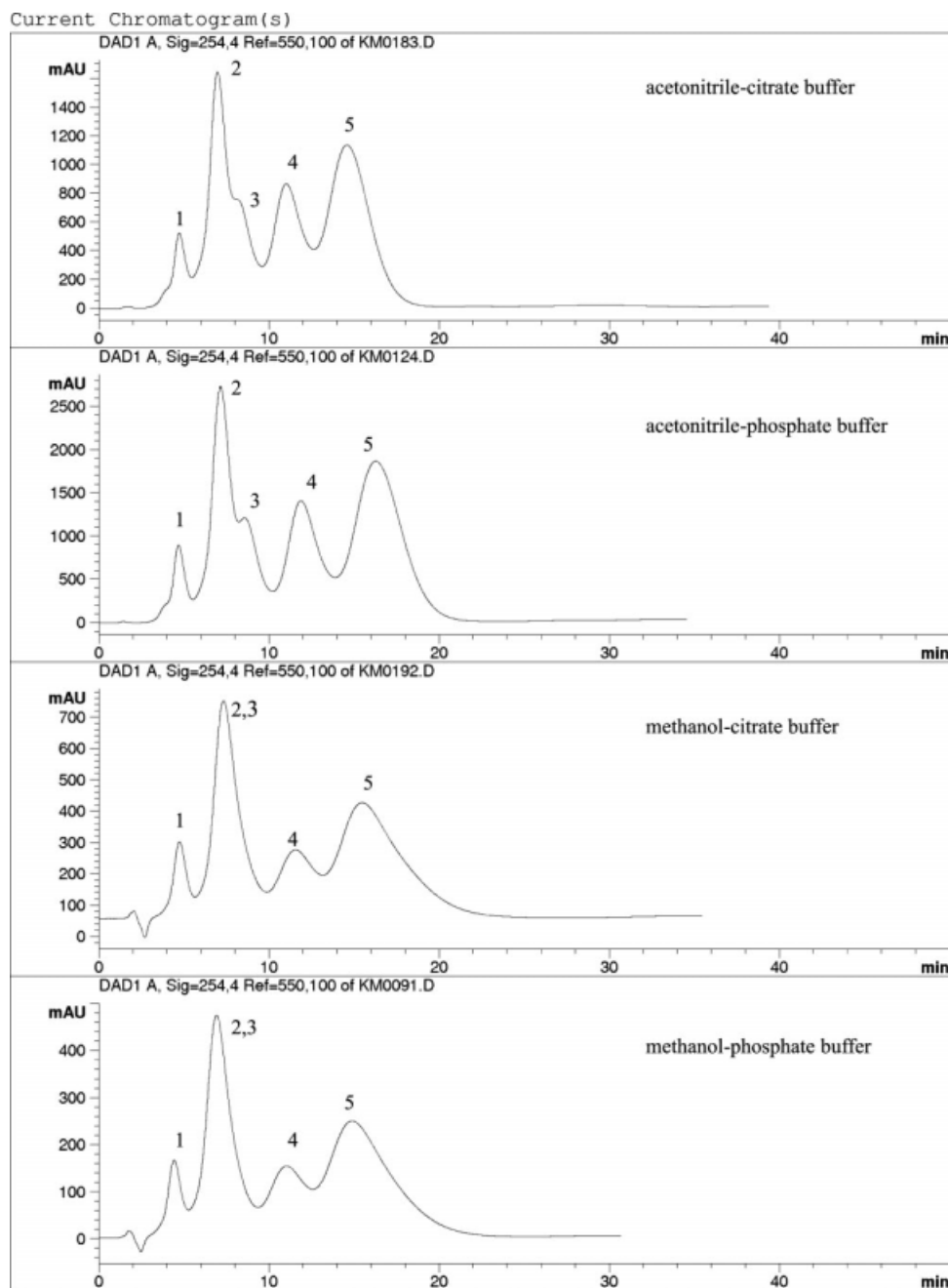


Figure 7 Chromatograms of homologous *N*-alkylanilines in the studied mobile phases. Peaks: 1, aniline; 2, *N*-methylaniline; 3, *N*-ethylaniline; 4, *N*-propylaniline; 5 = *N*-butylaniline.

aryl ketones used as standards in retention index calculations, whereas Figure 7 shows separation of homologous *N*-alkylanilines in all mobile phases. Generally, better separations are obtained in mobile phases containing acetonitrile. In citrate buffer mobile phases insignificantly better resolution of peaks is visible. Independently of rather small theoretical plate numbers peak shapes are symmetrical. This is especially important for *N*-alkylanilines which exhibit tailoring on most HPLC stationary phases.

In Table VII, the retention indices of the selected test compounds obtained on the studied copolymer with use of different mobile phases. These compounds represent solutes enable to specific interactions with different stationary phases. Values of their retention indices permit a direct comparison of different columns in reversed-phase HPLC providing that the same eluents are used. In all studied mobile phases, nitrobenzene is retained most significantly. The retention indices of *p*-cresol, representing a compound of acidic character, have

TABLE VII
Retention Indices of Test Compounds on the Studied Porous Copolymer with Use of Different Eluents

Eluent	Retention index				
	Toluene	Nitro-benzene	<i>p</i> -Cresol	2-Phenyl-ethanol	<i>N</i> -Methyl-aniline
Methanol citrate buffer	908	959	610	939	793
Acetonitrile citrate buffer	887	930	405	602	879
Methanol phosphate buffer	916	981	656	927	822
Acetonitrile phosphate buffer	836	907	415	629	866

the lowest values. *N*-methylaniline, representing compounds of basic nature, is retained a little weaker than nonpolar toluene. In the case of 2-phenylethanol different interactions with stationary phase are observed. In methanol-buffer mobile phases, its interactions are significantly stronger than those in acetonitrile containing eluents. The results for column test compounds indicate that dipolar interactions and proton donation effects play important role in reversed-phase HPLC mechanism on the BES-DVB microspheres.

CONCLUSIONS

Synthesis of the new aromatic tetrafunctional methacrylate monomer bis[4(2-hydroxy-3-methacryloyloxypropoxy)phenyl]sulfide (BES-DM) is described. This monomer was copolymerized with DVB in the presence of pore-forming diluents (toluene + 1-decanol) to obtain highly crosslinked porous microspheres. In chemical structure of these copolymers exhibit hydroxyl and ester groups of hydrophilic nature. Additionally, copolymer contains sulfur atoms coming from BES-DM monomer.

Chromatographic investigations confirm that the chemical structure of the polymeric stationary phase is responsible for some specific interactions with the separated compounds. On the BES-DVB polymeric microspheres, dipolar interactions and proton dona-

tion effects play important role in reversed-phase HPLC retention mechanism.

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