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Monodisperse Microbeads of Hypercrosslinked Polystyrene for Liquid and Supercritical Fluid Chromatography

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Abstract—Monodisperse styrene–divinylbenzene (1 wt %) copolymer microbeads are obtained via the elaborate method of high-productivity precipitation polymerization. The crosslinking of this copolymer with chloromethyl methyl ether in the presence of Friedel–Crafts catalyst yields porous hypercrosslinked polymers with degrees of crosslinking that range from 200 to 500%. Microbead sorbents are shown to be suited for selective stationary phases for high-performance liquid chromatography and supercritical fluid chromatography.

Keywords: hypercrosslinked polystyrene, precipitation polymerization, liquid chromatography, supercritical fluid chromatography.

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INTRODUCTION

Hypercrosslinked polystyrene is well known as a neutral hydrophobic absorbent that perfectly sorbs polar and nonpolar organic substances from aqueous solutions and gas media. These include all priority phenols, numerous pesticides, chlorinated aliphatic and aromatic hydrocarbons, polynuclear aromatic hydrocarbons, different drug substances, aromatic sulfonic acids, amines, and many other compounds. Hypercrosslinked polystyrene sorbents are therefore used successfully both in a number of industrial processes and in the quantitative analysis of toxic organic environmental pollutants [1].

The wide range of retained compounds predetermined the interest in using hypercrosslinked polystyrene as the stationary phase for high-performance liquid chromatography (HPLC). The first systematic studies along these lines were performed by Davankov and Sychoy [2, 3], who showed that the neutral hydrophobic hypercrosslinked sorbent Chromalite 5HGN can separate mixtures of substances in both the reversed-phase and quasinormal-phase modes of HPLC. Pilot batches of this sorbent's microbeads with diameters of around 5 μm were obtained by Purolite Int. through the additional crosslinking of the swollen macroporous styrene copolymer divinylbenzene and vinylbenzyl chloride in the presence of Friedel–Crafts catalyst. The same tercopolymer, along with copolymer also containing oxystyrene fragments, were used by Svec et al. [4–6] to prepare the solid-cast hyper-

crosslinked polystyrene phase for a capillary column. It is highly effective in separating uracil and alkylbenzenes in the reversed-phase mode.

In all cases, the degree of sorbent crosslinking was no more than 200%. We recently showed that increasing the degree of linking between the phenyl rings of the initial polystyrene chains to 300, 400, and 500% (when each phenyl ring is linked with adjacent rings through 3, 4, and 5 methylene groups, respectively) does not deprive the hypercrosslinked polymer of capability to absorb organic compounds, but it also gives it the capability of retaining even inorganic acids, salts, and bases [7]. It is therefore of great interest to study the chromatographic properties of highly crosslinked sorbents in more detail. However, synthesizing microspheric beads with a very narrow (monodisperse) size distribution remains a key problem in using polymer sorbents as the stationary phase for HPLC.

At present, there are two fundamentally different approaches to synthesizing micron-size monodisperse spherical particles. Ugelstad et al. [8] created a multi-stage process for the preparation of such particles using a monodisperse (e.g., linear polystyrene) latex as a nucleation center for future beads. A stable dispersion of such latex in the aqueous phase containing a large amount of a mixture of different stabilizers is mixed with a stable emulsion of a mixture of monomers (styrene and divinylbenzene), porogenes, and free-radical polymerization initiators in an aqueous

medium of the same composition. The mixture is slowly stirred until, due to diffusion through the aqueous phase, the starting suspension and emulsion forms a single uniform system in which the disperse phase is a solution of linear polystyrene in the mixture of added comonomers and porogene. Subsequent polymerization of the monomers at high temperatures produces monodisperse crosslinked microspheric particles. Finally, the removal of stabilizers from the surface and the extraction of linear polystyrene yield macroporous copolymer particles. Although this method allows us to obtain desirable microspheric sorbent beads with a rather narrow size distribution, it is very time consuming and labor intensive with low productivity and reproducibility.

Dispersion polymerization [9] is simpler single-step method for synthesizing monodisperse microspheres. A monomer, a radical polymerization initiator, and a stabilizer of the future suspension of polymer particles are dissolved in an organic solvent (e.g., aliphatic hydrocarbon). As polymerization proceeds, the resulting polymer chains precipitate as spherical particles from the dispersion medium, which serves as their precipitating agent. Correct selection of the precipitating medium, concentrations of components, and process conditions allow us to obtain size-uniform polymer microspheres. At present, the convention is to distinguish between dispersion polymerization and precipitation polymerization. Dispersion polymerization is understood to mean the synthesis of linear polymers in the presence of suspension stabilizers and, as when preparing crosslinked polymer microbead without using a stabilizer, it is referred to as precipitation polymerization [10, 11]. This division is somewhat arbitrary and does not consider the mechanism of particle formation (which is undoubtedly identical in both cases), but the term "precipitation polymerization" is now commonly accepted in the literature.

In this work, we present the results from a high-throughput procedure for precipitation polymerization that we developed for preparing monodisperse microbeads of styrene-divinylbenzene (DVB) copolymer. The copolymer beads were then additionally crosslinked with 1, 1.5, 2, and 2.5 moles of chloromethyl methyl ether (CMME) up to degrees of crosslinking of 200, 300, 400, and 500%, respectively. We discuss some physicochemical aspects of the synthesis of microspherical styrene-DVB copolymers, their properties, and the properties of crosslinked sorbents based on them. The chromatographic properties of an HPLC column filled with crosslinked (300%) polystyrene microbead sorbents are tested using the example of separating the model mixture in the isocratic and gradient modes, and under the conditions of supercritical fluid chromatography.

EXPERIMENTAL

Reagents and Materials

1,2-Dichloroethane was dried by refluxing over phosphoric anhydride for two hours and distilled over a fresh portion of drying agent. A 65% solution of divinylbenzene (Merck) in styrene was purified from inhibitors by washing with 10% KOH and water until a neutral reaction. The organic solution was then passed through alumina.

HPLC-grade acetonitrile, reagent-grade methanol (LabScan), and food-grade carbon dioxide (Linde-Gas Rus) were used in our chromatographic experiments.

Synthesis of Chloromethyl Methyl Ether

A four-necked flask equipped with a stirrer, dropping funnel, thermometer, and reflux condenser was loaded with methanol (32 g, 1 mol) and pre-ground paraform powder (30 g, 1 mol). The mixture was stirred thoroughly and NaCl (30 g) was added. The reaction mass was cooled to -5°C and concentrated hydrochloric acid (120 mL) was added dropwise for 1 h at this temperature. Concentrated sulfuric acid (120 mL) was also added slowly at -5°C . Stirring and cooling continued for an additional 5 h until the dissolution of paraform was complete. When the reaction finished, the upper layer was separated and distilled to collect the fraction boiling at $59\text{--}61^{\circ}\text{C}$. The yield of ether was 60%. In conducting the procedure, we must also remember that CMME (CAS 107-30-2) is a class 3 hazard compound and irritates the respiratory tract [11].

Synthesis of Styrene-Divinylbenzene (1%) Copolymer Microbeads

PES-5 polydiethylsiloxane liquid (63 mL) and *n*-octane (63 mL) were mixed in a flask. A mixture of styrene (25 mL), DVB (0.5 mL), and azobisisobutyronitrile (AIBN) (0.46 g) were added to this solution. The reaction mixture was stirred until a clear solution was obtained; it was then heated at 70°C for 4 h. At the end of this period, the mixture was left at room temperature for 20 h. The resulting beads were filtered off, washed on a filter with hexane and ethanol, and (if necessary) dried in air.

The synthesis of hypercrosslinked polymers was described in detail in [12].

Polymer swelling by weight (K_{wt}) was determined as follows: Polymer swollen in toluene (or water) was separated from the excess of solvent via filtration and the beads were dried on a Petri dish until they started to roll on a glassy surface and were quickly transferred to three preweighed weighing cups. The polymer was weighed in the swollen state and dried at 100°C until a constant weight was achieved. The swelling was calculated as the amount of solvent absorbed per one gram

of polymer. The reproducibility of measurements was $\pm 3\%$.

The swellings by volume (K_{vol}) in toluene and water were measured in a graduated glass test tube. The tube was loaded with a weighed portion of the dry polymer and centrifuged for 5 min at 4000 rpm; the volume of the dry bead layer then was measured. The polymer was submerged under toluene, stirred, and, after several minutes, centrifuged under the same conditions. The upper layer of the sorbent was stirred carefully and the tube was again centrifuged for 10 min at the same rate. The last operation was performed twice to obtain a flat horizontal upper layer of the sorbent. The volume of swollen polymer layer was then measured. The swelling of polymer by volume was calculated as the ratio between the volumes of swollen and dry polymers. The reproducibility of measurements was $\pm 5\%$. Bulk volume V of the dry polymer layer was calculated from its weight and volume.

The apparent internal specific surface area (S_{app}) of the hypercrosslinked polymers was measured via thermal argon desorption and calculated according to the BET theory.

Micrograph of the sorbent beads was obtained using a MIN-8 optical microscope (Russia). To obtain a reliable pattern, the toluene-swollen beads were placed onto a slide plate as a monolayer and dried.

Packing of Chromatographic Columns

The precolumn of a packing bed connected to a steel analytical column with dimensions of 75×4.0 mm was filled with a suspension of the polymer in an acetone–water (1 : 2, v/v) mixture. A mixture of the same composition was fed into the system by slowly raising the pressure up to 70–75 bar.

Reversed-Phase HPLC

For primary evaluation of the suitability of monodisperse microspherical hypercrosslinked polystyrenes with high degrees of crosslinking for use as HPLC sorbents, a test mixture (acetone, benzene, naphthalene, and anthracene) was separated under the isocratic conditions of reversed-phase chromatography. The polar mobile phase (acetonitrile or an acetonitrile–water (70/30) mixture) was fed into the column at 20°C and a flow rate of 0.5 mL/min. The working pressure at this flow rate was no more than 26 bar. The chromatograms were obtained on a LC Agilent 1000 chromatograph with UV detection of sorbates at 254.8 nm.

Supercritical Fluid Chromatography (SFC)

The columns were tested using an Acquity UPC² analytical chromatograph (Waters Corporation). The chromatograph consisted of a pumping unit capable of

feeding a mixture of liquid carbon dioxide and liquid cosolvent at flow rates of up to 4 mL/min, a temperature-controlled autosampler, a column thermostat, diode matrix detector, and a pressure controller that maintained the pressure downstream of the column in the detector. Solutions (~ 1 mg/mL) of toluene and *p*-nitrophenol in isopropanol or 2,6-dinitrophenol and phenanthrene in hexane were used as our model sorbates. Carbon dioxide was used as the main component of the mobile phase, and methanol was used as the polar cosolvent. Elution was performed in the isocratic mode and the co-solvent content was preliminarily optimized in order to minimize the time of analysis while maintaining the separation. The volume ratio of methanol in the mobile phase was 25% in the experiments with the elution of phenol and its nitro derivatives, and 30% in the experiments to plot the Van Deemter curves for phenanthrene. The column was equilibrated at 40°C using a flow of the mobile phase and a sample with a volume of 1 μ L was injected. The pressure in the system was maintained by the output controller at a level of 105 bar (the minimum value for this instrument). Detection was performed at 254 nm. In the experiments on the elution of phenols, the volumetric flow rate was equal to 2.5 mL/min; in the experiments to plot the HETP against the flow rate, it varied from 0.25 to 4 mL/min at intervals of 0.25 mL/min.

RESULTS AND DISCUSSION

In its final version, the synthesis of monodisperse styrene–DVB copolymer microbeads by precipitation polymerization was very simple; however, we must correctly choose a medium for synthesis that allows us to obtain spherical microparticles of a desired size. After testing many solvents and their mixtures, we decided in favor of a PES-5 polydiethylsiloxane liquid–*n*-octane mixture [12–14]. Fairly close densities of the mixture components (PES-5 and octane, styrene, DVB, and their copolymer, equal to 1.0, 0.703, 0.906, 0.92, and 1.04 g/cm³, respectively) in combination with high-viscosity silicone (200–500 cm²/s) favor the slow precipitation of growing polymer particles and thus prevent their aggregation. In precipitation (as in dispersion) polymerization, the dissolving power of the precipitation agent (i.e., the precipitation agent's thermodynamic affinity for the polymer) is of utmost importance. The higher its dissolving power, the coarser the beads that form. The optimum condition is apparently an affinity of the dispersion medium to the growing polymer that is close to a θ -solvent (at the temperature of synthesis) [9]. The dissolving power of the precipitation agent can be increased by adding a solvent that is thermodynamically advantageous for the growing polymer. The advantageous solvent in our system was the monomer (initial and unreacted). Consequently, an increase in the styrene concentration in the dispersion medium must favor an

Physicochemical properties of microspherical hypercross-linked polymers

Polymer	S_{sp} , m ² /g	Residual chlorine, %	V , cm ³ /g	K_{wt} , mL/g		K_{vol} , toluene
				water	toluene	
MS-200	1500	1.68	3.20	2.85	3.85	1.35
MS-300	1140	1.51	2.59	2.16	2.62	1.33
MS-400	1180	4.08	2.50	1.85	2.30	1.30
MS-500	680	7.61	2.36	1.50	1.75	1.28

increase in both the polymerization rate and the microbead size. Unfortunately, the current state of the precipitation (dispersion) polymerization theory allows no a priori determination of the synthetic conditions that guarantee the production of microbeads of identical desired size. We must select these conditions experimentally.

The copolymerization of styrene with 1 mol % of DVB in silicone oil, taken in the amount of 5 volumes of oil per 1 volume of the monomeric mixture (monomer concentration, 17 vol %), results in the formation of small particles with diameters of 1 μm and smaller, with virtually complete aggregation of the particles. Diluting the PES-5 with an equal volume of *n*-octane obviously increases the dissolving power of the precipitation medium for synthesis and favors the production of larger monodisperse microspheres. After 4 h of heating the mixture at 70°C, followed by holding at room temperature for 20 h, the size of the beads removed from the reaction medium was around 4 μm . It fell to 3.0–3.2 μm after thorough removal of the residual reaction medium by washing the beads on a filter with hexane and ethanol; this suggests the inclusion of monomeric styrene and/or components of the dispersion medium in significant amounts. As Fig. 1 shows, the obtained and dried copolymer beads were quite monodispersed. Their size, determined by measuring the diameters of 100 beads, was 3.2 μm .

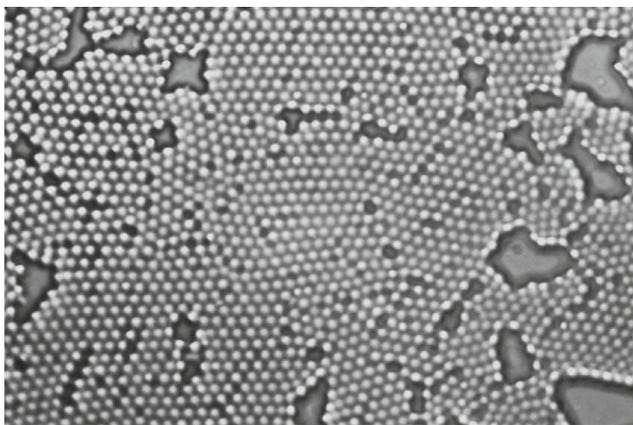


Fig. 1. Micrograph of styrene–1% DVB copolymer beads.

It is interesting that the yield of polymer under all conditions of synthesis was about 50%. Performing polymerization in a medium of inert gas, raising the temperature of synthesis up to 85°C after 2 h of heating at 70°C, prolonging the time of heating at 70°C to 7 h, holding at room temperature for 90 h, doubling the concentration of initiator, and replacing AIBN with benzoyl peroxide neither increased the bead size nor improved the yield of copolymer. Changing the volumetric ratio of the dispersion medium and monomers from 5 : 1 to 4 : 1 or 3 : 1 also had no appreciable effect on the yield of copolymer. Only after 4 h of polymerization at 70°C, holding the reaction mixture for 20 h at room temperature, and reheating it at 70°C for 3 h did the yield of polymer rise to 70%. However, the yield grew not because of the growth of particles that had formed, but due to the formation of fine new beads, resulting in the complete aggregation of all microspheres. The reason for the termination of precipitation polymerization even at a 50% conversion of styrene requires more detailed study; however, we believe that the reason should be sought in the poor distribution of styrene between the dispersion medium (PES-5–*n*-octane mixture) and the growing polystyrene particles. Nevertheless, a 50% yield based on styrene should be considered as more than sufficient, considering the monodispersity of the produced microbeads.

The formed monodisperse microbeads of styrene–DVB copolymer were easily filtered on a glass filter from a rather viscous silicone liquid, though their micron size would have allowed them to penetrate through the filter's pores. It is noteworthy that polydispersed mixtures of particles with the same size blocked the filter and ended filtration. It is not recommended that beads washed of traces of PES-5 be dried, since they often agglomerate into large conglomerates. In addition, the beads restore their individuality after swelling in a solvent thermodynamically advantageous for polystyrene. It is also interesting that suspensions of microbeads in ethanol form a stable thixotropic structure after ultrasound treatment.

Monodisperse microspheres (MS) of styrene–1% DVB copolymer were additionally crosslinked with chloromethyl methyl ether (CCME) up to nominal degrees of crosslinking of 200 (MS-200), 300 (MS-300), 400 (MS-400), and 500% (MS-500). The characteristics of the resulting hypercrosslinked polymers are given in the table.

Judging from the results of the gas chromatographic analysis of the reaction medium, chloromethyl methyl ether is almost completely consumed in the reaction with polystyrene chains; as the degree of network crosslinking increases, however, the possibility of it acting as a bifunctional crosslinker decrease: MS-400 and especially MS-500 contain a large amount of unreacted chloromethyl groups; the residual chlorine content in MS-500 can be as high as 7.6%.

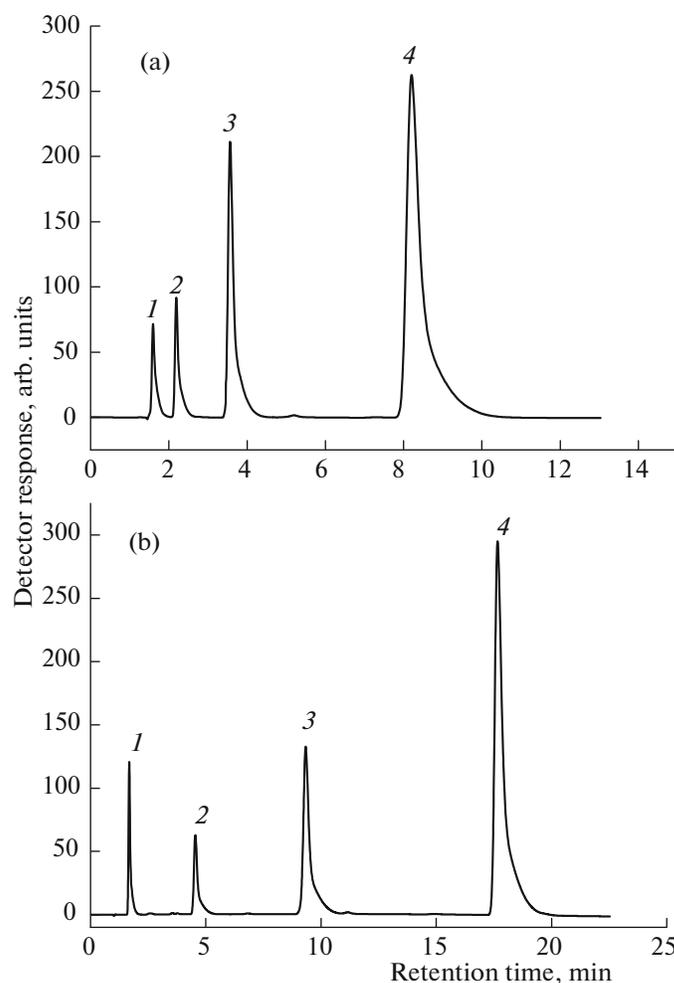


Fig. 2. Chromatograms of the test mixture: (1) acetone, (2) benzene, (3) naphthalene, and (4) anthracene. Column, 75×4 mm; MC-500 sorbent. Detection at 254.8 nm; flow rate of the mobile phase, 0.5 mL/min; temperature, 20°C. (a) Isocratic elution with pure acetonitrile; pressure, 15 bar. (b) Gradient elution from 70% (in water) to 100% of acetonitrile in 15 min; working pressure, 15 bar.

Consequently, the actual degrees of crosslinking for MS-400 and MS-500 are less than the nominal values.

All microspherical polymers are highly porous products with apparent specific internal surface areas of 1500 to 680 m²/g. Upon raising the degree of crosslinking from 200% to the maximum possible value, the amount of bead-absorbed toluene falls steadily but remains high (about 2–4 mL/g) in all cases. It is noteworthy that all polymers increase their volumes only to a small but identical degree (by about 1.3 times) after absorbing the maximum amount of toluene. This great difference between the weight and volume swellings of microspheres could only be due to large volume of pores whose filling is not associated with an increase in polymer volume. We might assume that water absorption would correspond approximately to the volume of such pores; however, hypercrosslinked polystyrene is prone to a certain amount of swelling in water. Indeed, all polymers absorb large amounts of

water, though less than those of toluene. We succeeded in measuring the volume swelling of MS-500 in water by treating it with acetone and replacing acetone with water while separating the beads by centrifugation. MS-500 was found to increase its volume in water (due to swelling of the hypercrosslinked polymer phase) by only 1.13 times. This means that upon swelling in water the bead diameter (and possibly the pore size) grew only by 4%, so the pore volume of MS-500 can be taken equal to the weight swelling of polymer in water, 1.5 cm³/g. A much greater pore volume was characteristic of the MS-200 sample.

Due to the emergence of a large free volume (no less than 1.5 cm³/g) in the styrene–divinylbenzene copolymer network as a result of its hypercrosslinking in the dichloroethane-swollen state, and to an additional nearly 1.3-fold increase in the volume of hypercrosslinked beads as a result of swelling in the water–acetone mixture, the working size of beads packed into

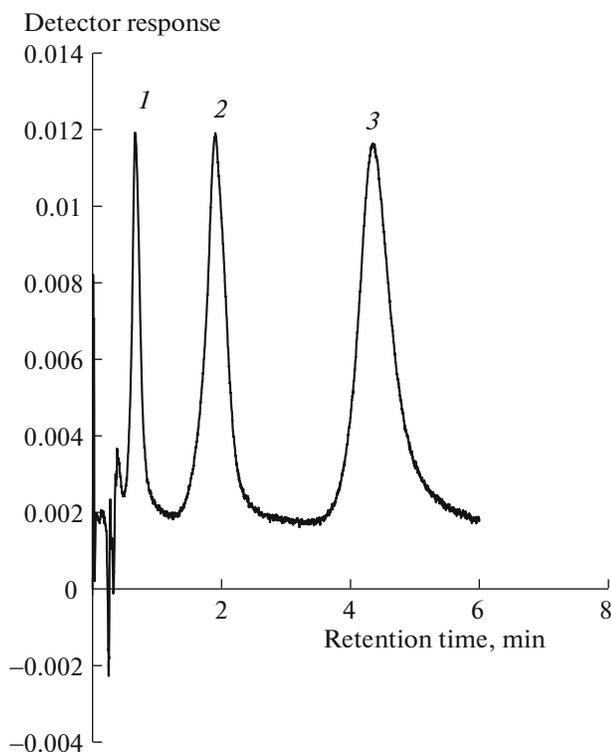


Fig. 3. Chromatogram of the test mixture: (1) toluene, (2) *p*-nitrophenol, (3) 2,6-dinitrophenol. Column, 75 × 4 mm; sorbent, MC-300. Detection at 241 nm; eluent, CO₂-MeOH (75–25%); pressure, 105 bar; flow rate of the mobile phase, 2.5 mL/min; temperature, 40°C.

the analytical chromatographic column grew from the initial 3.2 to 4.0 nm. This size corresponds to the optimum value for standard HPLC.

The data of Fig. 2a indicate extremely high retention of aromatic hydrocarbons by the sorbent even in pure acetonitrile (in the mobile phase), where hydrophobic sorbent–sorbate interactions are virtually suppressed. The retention times of the analytes grew rapidly as the number of condensed rings in the molecule rose (up to 9 min for anthracene). This was undoubtedly due to strong π – π -interactions between the aromatic electronic systems. The intensity of these interactions clearly surpassed the exclusion effects expected for anthracene-size molecules in a microporous sorbent matrix. Even in pure acetonitrile (Fig. 2b), the separation time of the mixture was only reduced to 20 min.

Although low flow resistance and sharp chromatographic zone fronts undoubtedly indicate adequate packing of the microparticles in the column, the peaks are obviously asymmetric. The tailing of peaks testify to the energy heterogeneity of the adsorption centers of MS-500 sorbent and the presence of sites with a dramatically increased affinity for the sorbate molecules. It seems this effect is an inevitable consequence of the internal structure of a hypercrosslinked microporous sorbent.

Instead of the uniform solid homogeneous surface typical of macroporous reversed-phase silica gel sorbents, hypercrosslinked polystyrene with an increased degree of crosslinking in its openwork network produces a sorbate molecule with a considerable number of nanosized pockets in which the molecule interacts simultaneously with several aromatic electronic systems and is therefore retained for an abnormally long time. If we ignore the tailing of the peaks and estimate the peak width at half height, the number of theoretical plates of the column varies from 3000 to 4000 for different sorbents; this corresponds to $(4\text{--}6) \times 10^4$ tp/m, which for these sorbates is only 50% lower than the performance of today's best surface-modified microspherical silica gel sorbents.

Elution in the gradient mode (Fig. 2b) considerably eliminates the peak tailing effect.

The high chemical and hydrolytic stability of polystyrene, the high affinity for sorbates capable of electronic interactions, and the high selectivity and adequate efficiency of their separation suggest that hypercrosslinked polystyrene microbeads could be used in the HPLC analysis of specific groups of low-molecular weight compounds.

It is believed that chromatography in a supercritical carbon dioxide fluid could reduce peak asymmetry and increase column performance, since the rate of sorbate diffusion in the fluid would be much higher than in the liquid mobile phase used in HPLC. The same columns were therefore tested in the mode of supercritical fluid chromatography (SFC) using low-molecular-weight aromatic compounds. Figure 3 shows a chromatogram from the separation of monocyclic aromatic compounds on MS-300 sorbent with a 300% degree of crosslinking.

It is clear that in the mode of supercritical fluid chromatography, the MS-300 polystyrene sorbent also exhibits extremely high selectivity with respect to aromatic compounds. In this context, the selectivity of the sorbent with respect to nitrophenol/dinitrophenol pairs is instructive. Despite the lack of peak tailing, however, the tested column with hypercrosslinked polystyrene had abnormally low performance. The number of theoretical plates in all our experiments was no more than 800, and was often 400–600. This number is approximately one-tenth of those shown by these columns in liquid chromatography.

To study this phenomenon in more detail, we plotted the Van Deemter curves (plots of heights equivalent to those of theoretical plates vs. the volume flow rate) in SFC for two columns based on hypercrosslinked polymers with different degrees of crosslinking and compared them to similar data for conventional reversed-phase silica gel sorbents (Fig. 4).

As can be seen from a comparison of these dependences, the tested polymer sorbents do show very low performance in SFC compared to both conventional sorbents and their performance in HPLC. The ran-

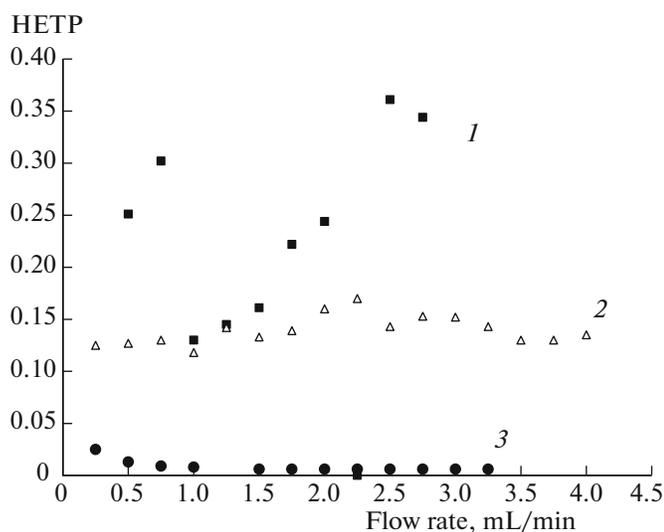


Fig. 4. Van Deemter curves for two hypercrosslinked polystyrene sorbents, (1) MS-300 and (2) MS-500, and for (3) octadecyl silica gel sorbent HSS C18 (Waters Corp.) in the SFC mode. The test compound was phenanthrene. Columns, 75 × 4 mm; bead size, 4 μm (1, 2); column, 100 × 3 mm; bead size, 1.8 μm (3). The mobile phase was CO₂–MeOH (70–30% by volume) for polymer sorbents and pure CO₂ for HSS C₁₈. For the remaining conditions, see Fig. 3.

dom scatter of experimental data reveals no Van Deemter dependences, which must have smooth shapes (Fig. 4). We believe this indicates destruction of the polymer microbead packing in the column under the conditions of SFC. We may assume that hypercrosslinked polystyrene beads in a medium of supercritical fluid are in a swollen plasticized state that considerably reduces their mechanical strength. The sorption layer inside the column can thus be displaced under the action of hydraulic impact when the mobile phase is first fed in at a pressure of 65–70 bar (the pressure in a CO₂ cylinder). Inspection of the analytical column packed with the copolymer with a 300% degree of crosslinking revealed considerable compaction of the sorbent layer after a free space with a height of 6 mm appeared in the upper part of the column. The destruction of the sorption layer of course affected the column's performance, though the high selectivity of the sorbent remained unchanged. Unfortunately, the design of the SFC instruments available to us did not allow a gradual increase from atmospheric pressure to working pressure.

The literature describes many examples of using polymeric stationary phases in SFC as surface layers fixed on rigid porous mineral or polymer carriers [15–17]. We found only one work in which a polymer material without a supporting base was used as the sorbent in SFC [18]. In this work, a molecularly imprinted polymer that had proven useful in HPLC was tested in SFC for the separation of enantiomeric 1,2-amino

alcohols. The authors described observations very close to those obtained in our work. After several days of operating in SFC, the columns lost their selectivity with respect to the imprinted target compounds up to the complete confluence of peaks for two enantiomers. Nevertheless, some residual selectivity was restored when this sorbent was returned to HPLC, while the retention times of the analytes fell notably.

CONCLUSIONS

We developed a highly effective version of precipitation polymerization to obtain monodisperse microspherical styrene–divinylbenzene copolymers on whose basis hypercrosslinked polystyrenes with extremely high degrees of crosslinking were prepared. The polymeric microbeads were shown to be suitable for use as stationary phases in high-performance liquid chromatography. Considering the chemical stability, microporous structure, and electronic properties of hypercrosslinked polystyrenes, promising fields of application for our sorbents are the chromatography of small polar molecules and the analysis of complex systems in which small molecules of analytes are present in a sample alongside high-molecular-weight components. Such components must be excluded from the sorbent phase. One such problem of interest is the direct analysis of toxins and drug substances and their metabolites in blood plasma or other physiological liquids.

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