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Review

# Hypercross-linked polystyrene and its potentials for liquid chromatography: a mini-review

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## Abstract

Hypercross-linked polymeric adsorbing materials are obtained under conditions that (i) their polymeric network is formed in the presence of large amounts of a thermodynamically good solvent (porogen) and (ii) the network is rigid. Hypercross-linked polystyrene is a transparent microporous low-density material with an apparent inner surface area of over 1000 m<sup>2</sup>/g and an unprecedented adsorption capacity. To enhance the mass transfer, the adsorbent beads may be provided with large transport pores, in addition to the inherent micropores; these beads are opaque. Hypercross-linked polystyrene sorbents are widely used for large scale adsorption of organic compounds from aqueous and gaseous media and for solid-phase extraction of trace components. Novel perspective application areas of the materials are high-performance liquid chromatography column packings and blood purification. Present mini-review summarises basic principles of obtaining hypercross-linked materials, their structural peculiarities and distinguishing properties, as well as major application areas. Important new unpublished data are also included.

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## 1. Introduction

Modern liquid chromatography involves a whole series of really large, but rather independent areas such as water treatment, hydrometallurgy, ion-exchange, sample preparation, high-performance liquid

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chromatography (HPLC), ion chromatography, affinity chromatography, hemoperfusion, etc. Some of these areas exploit entirely frontal analysis, others are based either on elution chromatography or on displacement chromatography. Even the terminology used by specialists of the different areas may differ substantially. However, column packing materials used in several different techniques are often identical, at least with respect to their chemical nature. Such as ion exchanging materials, activated carbons and neutral polystyrene-type resins.

## 2. Three generations of polystyrene-type adsorbing materials

First styrene–divinylbenzene copolymers were synthesised in 1934 by Staudinger and Heuer [1], but they were converted into useful chromatographic column packing materials, namely, ion exchanging resins [2], one decade later. At the beginning, the beaded styrene copolymers were of the gel-type. This first generation of copolymers was synthesised by conducting the crosslinking copolymerization of styrene with relatively small amounts of divinylbenzene (below 8%) in the absence of any solvent. Gel-type copolymers are able to swell in thermodynamically good non-polar media; such as, toluene or ethylene dichloride, only. Ion exchangers with highly polar sulfonic or tertiary ammonium groups introduced into phenyl rings of polystyrene, swell, on the contrary, in aqueous media or strongly polar alcohols. In nature swelling is an essential condition of the gel-type material in order to function in the adsorption process or chromatographic systems, as the outer surface of the beads is negligibly small and the interior of the beads becomes accessible to solute molecules in swollen materials, only. From this point of view, the first type of copolymers are also called materials with hidden porosity. In fact, when in dry state, the copolymer beads are transparent and impermeable even for gases.

Permanent porosity is characteristic of macroporous styrene copolymers, i.e. materials of second generation. They emerged in 1952 as a result of the efforts of several independent research groups, starting with the first USA patent by McBurney [3], as

described in the history related review by Abrams and Millar [4]. Permanent porosity results from micro-phase separation of the initially homogeneous mixture consisting of styrene, enhanced the quantity of DVB (above 8–15%) and also a porogen that is miscible with monomers, but precipitates growing chains of the styrene copolymer. Micro volumes occupied by the separated porogen after evaporation, leave voids in the copolymer structure that represent interconnected macropores. Macroporous materials are opaque and display a total pore volume of about 1 ml/g and an inner surface area of about 20–300 m<sup>2</sup>/g (larger values of surface area imply the presence of small pores, in addition to macropores). The pores can be filled with any liquid media, both good from the thermodynamic viewpoint and non-solvents for polystyrene. These materials can serve as excellent hydrophobic adsorbents for organic molecules dispersed in aqueous media, which is well known for Amberlite XAD-4, one of the best commercially available adsorbing materials of the second generation styrene copolymers. An important benefit of these materials is that they do not change the net volume of the column packing in adsorption–regeneration cycles. The same is true for sulfonated or aminated cation or anion-exchangers, respectively, based on the macroporous copolymers which can function in any liquid media and do not noticeably change their volume. Last, it should be noted that the pore diameter of the “macroporous” styrene–DVB copolymers lies in the range above 200 Å, though it is more common to refer to “macro” pores as larger than 500 Å in diameter.

A substantially different network structure results from crosslinking polymerisation in the presence of a thermodynamically good solvent taken as the porogen. Here, no micro-phase separation occurs because of the full compatibility of the porogen with the polymer. An extended microporous network builds up under these conditions in the form of a homogeneous gel swollen with the porogen. Removing the porogen results in a low-density transparent microporous polymer of an extremely high inner surface area of over 1000 m<sup>2</sup>/g and pores of less than 20 Å in diameter. However, the microporosity of the dry material can be preserved only under the condition that the network rigidity is high enough to prevent the collapse of the extended network con-

struction under the action of strong intermolecular short-distance attraction forces between the polymer segments. It should be noted that in the macroporous material the walls of the pores are relatively thick and, consisting of a moderately crosslinked polymeric phase, they easily withstand any contraction strains (the between-wall attractions across the macropores are weak). Contrary to this, in the microporous network under discussion, the tendency to contraction is much stronger, whereas the construction units of the network are represented by individual polymeric chains and cross-bridges. These units are much more flexible than the bulk polymeric phase in the walls of the macroporous copolymer, so that a very high extent of crosslinking, over 40%, and high conformational rigidity of the cross bridges are needed, in order to provide the microporous network with sufficient rigidity. Even then, the whole network shrinks on removing the porogen and swells back to the initial volume on contacting the dry material with any liquid. As shown by Davankov and Tsyurupa [5] who first introduced [6] the hypercross-linked polymers, the specific property of such a network is that it swells with any liquid media, not just with thermodynamically good solvents. It thus remains permeable in any state and, once swollen, it is rather insensitive to changing eluents. The volume changes of the hypercross-linked material can be further reduced by making its porous structure heterogeneous, i.e. by introducing large macropores in addition to the intrinsic microporosity of the polymer. Hypercrosslinked polystyrene-type materials thus represent a fundamentally new class of polymeric networks and form the base for the third generation of polystyrene-type adsorbing materials. They are distinguished by an extremely high apparent inner surface area, unprecedented adsorption capacities, combined with an easy regeneration and reuse.

### 3. Hypercross-linked polystyrene in large-scale liquid adsorption chromatography

A whole series of hypercross-linked polystyrene-based adsorbing materials is manufactured by Puro-lite International (Wales, UK) since 1993 under the trade name “Macronet Hypersol”. The list includes

neutral hydrophobic adsorbents such as MN-200, as well as a series of chemically functionalized materials such as sulfonated resin MN-500. Some of the resins contain large transport macropores for facilitating kinetically limited processes and treating large volumes at high flow-rates. The other group like MN-100, are without macroporosity and efficiently discriminate large molecules from small molecules. Adsorption properties of hypercross-linked materials were summarised in a review [7]. Adsorbents are widely used in de-colorization of sweeteners syrups, especially starch hydrolysates [8], thus replacing long-established activated carbon technology. They are also highly perspective in waste water treatment, removing dyes from effluents of textile dyeing plants, de-bittering of orange juice by selective removal of limonin and polyphenols, for isolation of valuable components from microbiological fermentation liqueurs, etc. Fig. 1 presents a view of an operational chloroform removal plant with three columns, 10 m<sup>3</sup> each. With high adsorption capacity, up to 100 g CHCl<sub>3</sub> per litre resin, and steam regeneration at temperatures as low as 100 °C, there were significant savings in chloroform use and waste management costs.

Of rapidly increasing importance could be fine polishing of potable water. As Street et al. [9] showed, huge amounts of water, up to 150 000–300 000 bed volumes, can be purified completely from pesticides such as atrazine, simazine, chlorotoluron, isoproturon and diuron present in the parts per billion (ppb) range. More importantly, the authors demonstrated efficient, complete regeneration of the resins with alcohol, which is in marked contrast to activated carbons where pyrolysis seems to be the only effective regeneration method.

Unexpectedly, neutral hypercross-linked polystyrene having no functional groups was found to display a high affinity to heavy metal ions. Thus, mercury(II) ions slowly adsorb from acetate or nitric acid solutions in amounts of up to 400 mg Hg per 1 g of the polymer [10]. Large amounts of Ag<sup>+1</sup>, Pb<sup>+2</sup> and Bi<sup>+3</sup> ions can also be adsorbed. We suppose that sorption results from the formation of complexes between the metal cation and  $\pi$ -electron systems of the liquid-exposed phenyl rings of polystyrene. Moreover, it has been recently found that simple inorganic salts and acids separate in a frontal analy-



Fig. 1. The Guzina plant chloroform removal unit.

sis manner. Fig. 2 shows that sodium chloride migrates faster through the column as compared to hydrochloric acid, so that a significant amount of

neutral sodium chloride solution can be obtained from the initial mixture of the salt and acid. On further eluting the column with pure water, sodium

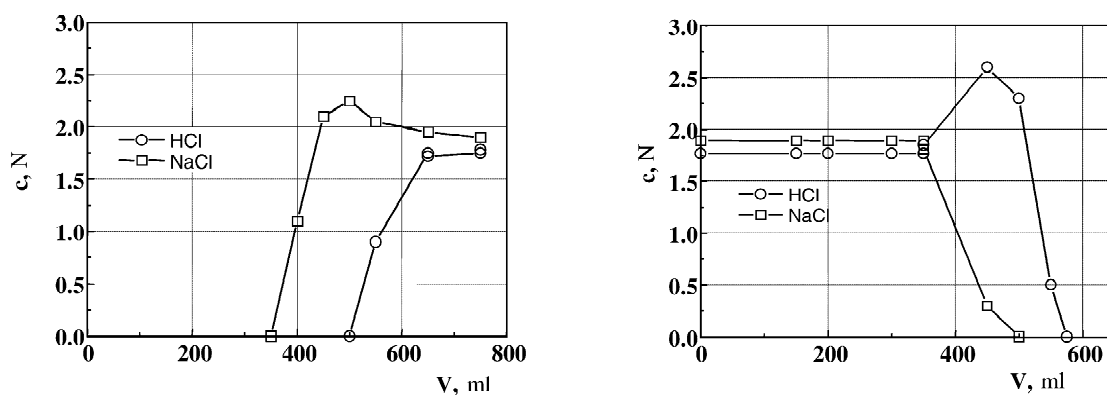


Fig. 2. Discrimination between sodium chloride and hydrochloric acid on a neutral sorbent MN-270 (dp circa 500  $\mu\text{m}$ ) column. A mixture of 1.8 N HCl and 1.9 N NaCl was applied onto a 500-ml column (left panel) and then eluted with pure water (right panel).

free hydrochloric acid can be obtained at the tail of eluting bands. It seems that protons enter into some kind of interaction with the electron donating aromatic systems of the hypercross-linked polystyrene, and that protons move slower than the sodium ions. An additional noticeable increase in concentrations of first sodium chloride and then hydrogen chloride was found to occur. This finding opens up new perspectives to removing salts from acids in a novel reagent free process [11]. Conventional ion-exchange does not offer such possibilities.

#### 4. Biomedical applications of hypercross-linked sorbents

Formation of hypercross-linked networks has finally become a well understood and safely controlled process, which provides several possibilities for fine tuning the porous structure of the final material. Many variables can be operated, such as the amount, functionality, molecular length and conformational rigidity of cross-links, and also the amount and thermodynamic quality of the porogens. As already mentioned, precipitating porogens lead to macroporous materials, whereas thermodynamically good solvents produce microporosity. This regularity leads to a suggestion that  $\Theta$ -solvents (the border between good solvents and precipitating media) when used as porogens during the formation of the hypercross-linked network, may result in the formation of an intermediate-size pores, between 20 and 200 Å in diameter. Such “mesoporous” polymers could prove efficient in the adsorption of middle-size molecules. One such acute problem is known to exist in the dialysis treatment of patients with malfunctioning kidneys. Dialysis procedures easily remove excess water and small toxic molecules, like urea, from patient’s blood, whereas large toxic molecules as  $\beta_2$ -microglobulin ( $\beta_2$ M) cannot filtrate through the membrane. The toxic protein has a molecular mass of 11 800 Da and a molecular diameter of ca. 33.5 Å. Increasing the mean diameter of membrane pores to a sufficient size would unavoidably result in an unacceptable loss of albumin that is less than twice as big (60 Å in diameter, 65 kDa). Only selective adsorption could, it is hoped, efficiently remove the

toxin that builds up in the organs of dialysis patients and deposits in their joints and vessels, thus causing high morbidity and mortality of renal failure patients. Indeed, applying mixtures of good solvents and precipitating media as porogens during the polymerisation of a DVB-rich monomer mixture, allowed us to obtain a mesoporous adsorbent [12,13]. Fig. 3 shows that the polymer efficiently removes  $\beta_2$ -microglobulin from patient’s blood plasma, whereas the concentration of albumin and other large proteins remains largely unaffected. Both smaller molecular size and higher hydrophobicity may contribute to the tendency of  $\beta_2$ M to adsorb on the mesoporous material with such relatively high apparent selectivity. We call this selectivity “apparent”, since in fact the total amount of adsorbed albumin is larger than that of adsorbed  $\beta_2$ M, due to a difference in their concentrations by several orders of magnitude. Current clinical trials [14] show excellent hemocompatibility of the surface-modified polymer and a very positive reaction from patients to the new treatment. Remarkably, no reduction of platelet and white blood cell counts occurred during the hemoperfusion session with patients and only an insignificant one in canines (Fig. 4) whose blood is more sensitive to foreign surfaces than human blood.

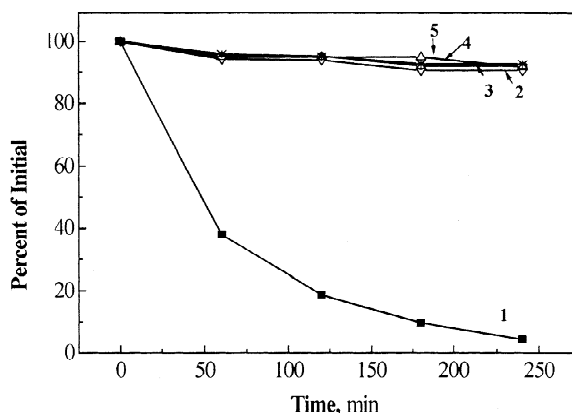


Fig. 3. Apparent selectivity of adsorption of  $\beta_2$ -microglobulin in a dynamic experiment with  $\beta_2$ M-spiked plasma. Plasma is continuously pumped through a column with hypercrosslinked “Betisorb100” polymer. Polymer–plasma (1:20, v/v). Initial concentrations:  $\beta_2$ M, 63.5 mg/l (1); albumin, 31.5 g/l (2); prealbumin, 300 mg/l (3); transferrin, 2170 mg/l (4); and total protein, 62 g/l (5).

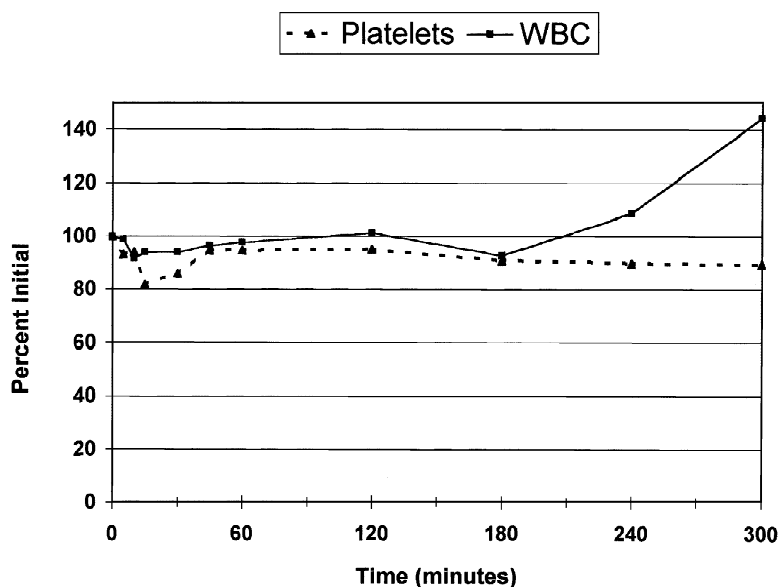


Fig. 4. Platelet and white blood cells counts during a hemoperfusion session on an experimental dog. Column, 100 ml. Blood flow-rate, 200 ml/min.

## 5. Solid phase extraction processes

Extremely high adsorption capacity and the ability to be easily regenerated makes hypercross-linked polystyrene the material of choice for concentrating trace organic compounds by solid-phase extraction (SPE) technique. Indeed, a series of novel SPE materials appeared on the market, namely, Purosep (Purolite, Wales, UK), Isolut-ENV+ (International Sorbent Technology, Cambridge, UK), LiChrolut EN (Merck, Germany). It has been repeatably shown that they effectively adsorb the whole range of organic contaminants. They proved to be especially suitable for medium to highly polar substances. Unmodified phenol readily adsorbs on Purosep-200 and can be quantitated in a simple on-line system [15]. Even acidic pesticides like 2,4,5-trichlorophenoxy acetic acid, dicamba [16], aromatic sulfonates [17] as well as chlorophenols were extracted quantitatively, sometimes without acidification of the sample. Many other examples of successful application of the new generation of SPE materials can be found in the literature. More recently, we also showed that concentration of volatile organic contaminants from indoor and outdoor air with the new material [18] displays marked advantages over well

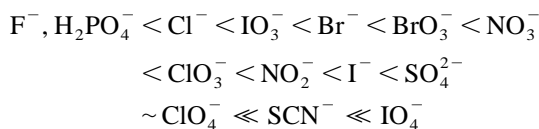
established Tenax materials, especially with respect to most polar and highly volatile components, like pentane and dichloromethane. Both thermal desorption and solvent extraction of analytes can then be applied to hypercrosslinked polystyrene SPE materials.

## 6. Hypercross-linked polystyrene as HPLC column packing

One potentially perspective, but barely examined thus far area of application of hypercross-linked polystyrene is HPLC column packing materials. Due to an extremely high degree of crosslinking and high mechanical strength, the microbeads should easily withstand high pressure drops in the column. Indeed, this has been shown to be the case [19] with a 150×3 mm column packed with disintegrated to 15 μm particles of the MN-200 neutral sorbent that has been exposed to a 12 MPa pressure drop showing no noticeable deviations from a linear pressure/flow-rate dependence. Spherical microbeaded sorbents, more recently prepared in monodisperse form as experimental batches, Chromalite HMN (Purolite Int., UK), are even more pressure resistant. Another

crucial advantage of hypercross-linked materials that distinguishes them from other polymers and relates them to mineral packings, is that the volume of the packing does not depend on the nature of the eluent. This follows from the inherent ability of the material to swell to almost equal extent with any kind of liquid media. (The column packing, however, collapses when the solvent is allowed to evaporate). Due to high chemical resistance of polystyrene, aqueous eluents of any pH can be also safely applied, which is known to be impossible with silica packings.

All the above properties of hypercross-linked polystyrene make the packing rather versatile, useful for several different kinds of chromatography. First of all for reversed-phase, distribution, size-exclusion chromatography modes. The mechanism of retention on the packing remains to be examined in detail. There are no measurable amounts of polar and charged groups on the polymer surface [19]. Nevertheless, the material was unexpectedly found to effectively separate complex mixtures of cations and/or anions [20] in a quasi ion-chromatography mode. This finding correlates with the above described separation of acids and salts and the earlier found [21] ability of the polymer to separate Pb–Bi and Zn–Cd–Pb ion mixtures due to complex interactions of exposed aromatic systems with heavy metal ions. Still, when eluted with 0.2 mM perchloric acid, the sequence of elution of anions



differs from that in ion-exchange chromatography and shows a strong contribution from dispersion interactions with ions prone to polarisation.

The strong hydrophobic character of the hypercross-linked polystyrene packing and its expressed tendency to enter  $\pi$ – $\pi$  interactions with the solutes have been revealed by analytical scale chromatography experiments with several classes of compounds, such as substituted phenols, phenylene diamines, aliphatic and aromatic acids, dialkyl phthalates, including aqueous organic and aqueous eluents with pH values varying from strongly acidic to 11.5 [22]. The highest efficiency observed thus far for a

25 cm column was 7.000–9.000 theoretical plates for solutes of the size of toluene and acetophenone. This corresponds to a reduced plate height of about 3, which seems to be an acceptable efficiency for microporous 10  $\mu\text{m}$  particles at an applied flow-rate of 1 ml/min. Fig. 5 gives an idea that the efficiency drops to about 5.000 plates for a more bulky anthracene. Still, without any special optimisation, this level of efficiency permits the observation of 14 peaks for a standard mixture (PAH 610-M) of 16 polyaromatic hydrocarbons (Fig. 6A). Chromatography was carried out in a  $\text{CH}_3\text{CN}$ – $\text{CH}_2\text{Cl}_2$  gradient, pointing to some kind of adsorption interaction with solutes, rather than exclusion phenomena, since larger solutes elute last. Actually, these conditions are absolutely inappropriate for chromatography on conventional macroporous polystyrene–divinylbenzene sorbents, analogous to PLRP-S materials (Fig. 6B). Note that in the last case the bead size was 5, not 10  $\mu\text{m}$ . This comparison displays once more a basic difference of the network structure and the properties of the new and well-established polymeric sorbents of the same chemical nature.

An outstanding and surprising property of the new packing material is that the efficiency of the column was found to drop only insignificantly with the flow-rate of the eluent increasing to very high values

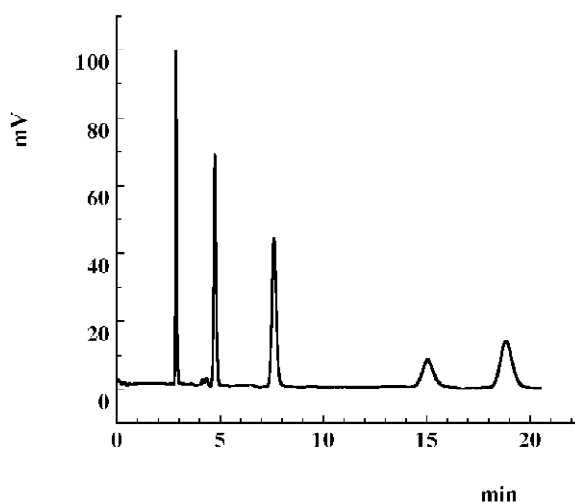
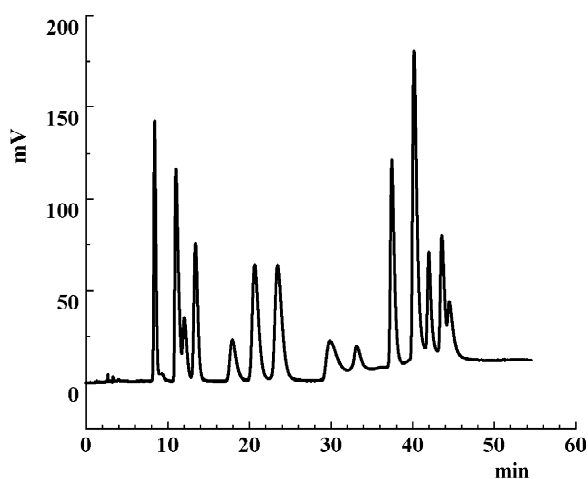
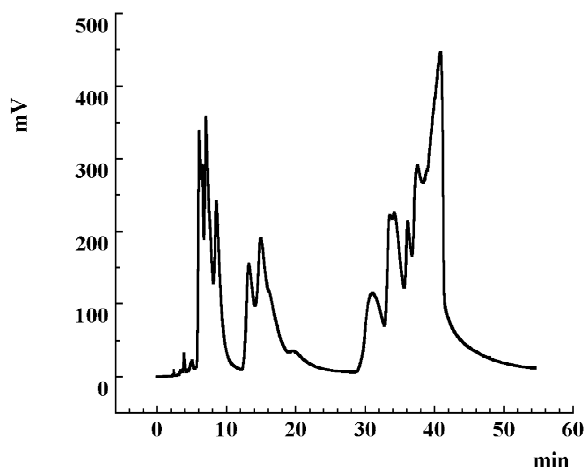


Fig. 5. Test chromatogram of a 250×4 mm column packed with 10  $\mu\text{m}$  monosized beads in acetonitrile at a flow-rate of 1 ml/min. Peak sequence, acetone, toluene, naphthalene, unknown, anthracene.



(A)



(B)

Fig. 6. Test chromatogram of a standard mixture of 16 poly-aromatic hydrocarbons (EPA method 610 mixture) on two  $250 \times 4$  mm columns at a flow-rate of 1 ml/min. Eluent,  $\text{CH}_3\text{CN}$  for 25 min, then linear gradient to  $\text{CH}_2\text{Cl}_2$  for additional 10 min. (A) Hypercross-linked  $10 \mu\text{m}$  beads. (B) Macroporous  $5 \mu\text{m}$  beads.

[19]. This property stands in contrast to the behaviour of conventional macroporous polystyrene sorbents and RP silica packings that were tested under identical conditions. Having a microporous open-work structure with an additional system of large transport pores, beads of the new material are possibly permeable to the eluent and allow some kind of permeation chromatography.

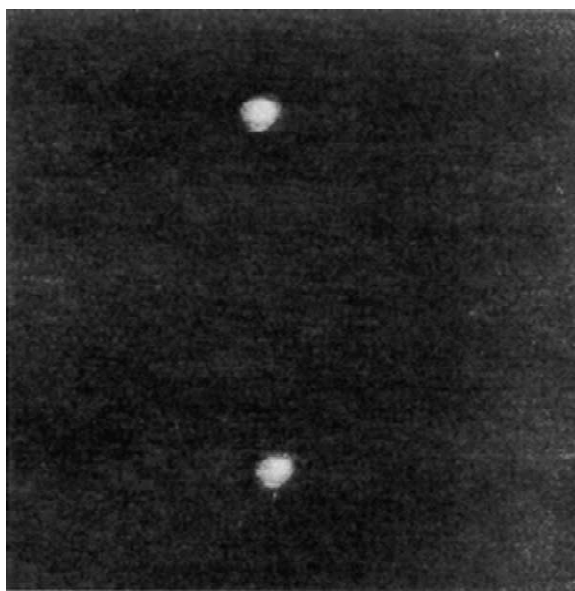


Fig. 7. Nanosponge, intramolecularly hypercross-linked polystyrene. Diameter 17 nm, molecular mass 360–370 kDa.

Finally, it could be mentioned that scaling down the size of beads of expanded hypercross-linked polystyrene networks can be continued to nanosponges (Fig. 7), new macromolecular soluble species [23] that, probably, could compete in the future with hydrophobic micelles so often used in capillary electrophoretic separations.

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#### References

- [1] H. Staudinger, W. Heuer, *Chem. Ber.* 67 (1934) 1164.
- [2] G.F. D'Alelio, *Pat. USA* 2366007 (1945); G.F. D'Alelio, *Chem. Abstr.* 39 (19) (1945) 4418.
- [3] C.H. McBurney, *Pat. USA*, 2591573 (1952).
- [4] I.M. Abrams, J.R. Millar, *React. Funct. Polymers* 35 (1997) 7.



- [5] V.A. Davankov, M.P. Tsyurupa, *React. Polymers* 13 (1990) 27.
- [6] V.A. Davankov, S.V. Rogozhin, M.P. Tsyurupa, Pat. USSR 299165 (1969), Pat. USA 3729457; V.A. Davankov, S.V. Rogozhin, M.P. Tsyurupa, *Chem. Abstr.* 75 (1971) 6841B.
- [7] M.P. Tsyurupa, L.A. Maslova, A.I. Andreeva, T.A. Mrachkovskaya, V.A. Davankov, *React. Polymers* 25 (1995) 69.
- [8] J.A. Dale, N.V. Nikitin, R. Moore, D. Opperman, G. Crooks, D. Naden, E. Belsten, P. Jenkins, in: J.A. Greig (Ed.), *Ion Exchange at the Millennium, Proceedings of IEX 2000*, Imperial College Press, London, 2000, p. 261.
- [9] M. Streat, L.A. Sweetland, D.J. Horner, *Trans. IChemE* 76 (1998) 115.
- [10] M.P. Tsyurupa, O.G. Tarabaeva, A.V. Pastukhov, V.A. Davankov, *Int. J. Polymer Mater.*, in press.
- [11] N.B. Ferapontov, M.P. Tsyurupa, V.A. Davankov, N.L. Strusovskaya, VIII All-Russia Symposium on Liquid Chromatography and Capillary Electrophoresis, Moscow, October 2001, p. 20.
- [12] V. Davankov, M. Tsyurupa, L. Pavlova, Pat. USA 6,156,851, (2000).
- [13] V. Davankov, L. Pavlova, M. Tsyurupa, J. Brady, M. Balsamo, E. Yousha, *J. Chromatogr. B* 739 (2000) 73.
- [14] C. Ronco, A. Brendolan, J.F. Winchester, E. Golds, J. Clemmer, H.D. Polaschegg, T.E. Muller, G. LaGreca, N.W. Levin, *Blood Purif.* 19 (2001) 260.
- [15] M.P. Tsyurupa, M.M. Ilyin, A.I. Andreeva, V.A. Davankov, *Fresenius J. Anal. Chem.* 352 (1995) 672.
- [16] R. Curini, A. Gentili, S. Marchese, A. Marino, D. Perret, *J. Chromatogr. A* 874 (2000) 187.
- [17] R. Loos, M. Alonso, D. Barcelo, *J. Chromatogr. A* 890 (2000) 225.
- [18] M.P. Baya, P.A. Panayotis, V.A. Davankov, *J. AOAC Int.* 83/3 (2000) 579.
- [19] N.A. Penner, P.N. Nesterenko, M.M. Ilyin, M.P. Tsyurupa, V.A. Davankov, *Chromatographia* 50 (1999) 611.
- [20] N.A. Penner, P.N. Nesterenko, *J. Chromatogr. A* 884 (2000) 41.
- [21] R.M.C. Sutton, S.J. Hill, P. Jones, *J. Chromatogr. A* 789 (1997) 389.
- [22] N.A. Penner, PhD Thesis, Moscow State Lomonosov-University, Moscow, 2000.
- [23] V.A. Davankov, M.M. Ilyin, G.I. Timofeeva, M.P. Tsyurupa, I.V. Yaminsky, *J. Polymer. Sci., Part A: Polymer Chem.* 37 (1999) 1451.