Russian Chemical Reviews **77** (4) 373 – 379 (2008) © 2008 Russian Academy of Sciences and Turpion Ltd

DOI 10.1070/RC2008v077n04ABEH003754

Monolithic stationary phases in liquid and gas chromatography

A Yu Kanatyeva, A A Kurganov, E N Viktorova, A A Korolev

Contents

I.	Introduction	373
II.	Brief historical survey	373
III.	The use of monolithic stationary phases in liquid chromatography and electrochromatography	374
IV.	The use of monolithic stationary phases in ion chromatography	376
V.	The use of monolithic stationary phases in gas chromatography	377
VI.	Conclusion	378

Abstract. The results of studies devoted to the use of stationary phases based on monolithic materials in chromatography are summarised. The attention is focused on the advanced methods for preparation of monolithic columns and the use of these columns in various chromatographic separations ranging from analytical to preparative. The bibliography includes 97 references.

I. Introduction

Since the first chromatographic separation performed by M Tsvet more than a century ago, columns filled with various types of granulated sorbents have been used in chromatography. An increase in the efficiency of columns and improvement of selectivity of sorbents have always been the most important goals in this field. The traditional method for increasing the performance of columns packed with granulated sorbents is associated with the use of sorbents of progressively more narrow grain size composition and finer and finer particles. However, the use of fine sorbents results in a significant increase in the pressure that should be applied to ensure the optimal flow of the mobile phase. High pressure poses technical problems in practical work, that is why a search for alternative ways of increasing the performance of chromatographic analysis is topical.

Approximately at the same time, when the foundations of modern high-performance liquid chromatography (HPLC) were laid, studies of another type of sorbents have been started; these sorbents represent a single polymeric macroporous block that has been termed as a monolith. Studies of these materials that are not traditional for chromatography have been going on more or less successfully for recent decades,[1,2] but only recently their production has been started. Monolithic columns are successfully used for fast and efficient separation of complicated mixtures of biological molecules,[3–7] inorganic and organic ions,[8–13] polyaromatic hydrocarbons (PAHs),[14–16] as well as for analyses related to the monitoring of the environment.[17–21]

A Yu Kanatyeva, A A Kurganov, E N Viktorova, A A Korolev
A V Topchiev Institute of Petrochemical Synthesis, Russian Academy of Sciences, Leninsky prosp 29, 119991 Moscow, Russian Federation.
Fax (7-495) 633 85 20, tel. (7-495) 955 41 84,
e-mail: kanatieva@ips.ac.ru (A Yu Kanatyeva), victorova@ips.ac.ru (E N Viktorova), akorolev@ips.ac.ru (A A Korolev),
tel. (7-495) 955 42 91, e-mail: kurganov@ips.ac.ru (A A Kurganov)

Received 5 October 2007
Uspekhi Khimii **77** (4) 393 – 400 (2008); translated by M G Esernitskaya

II. Brief historical survey

The history of monoliths in chromatography started five decades ago with the works of R L M Synge[22,23] awarded with the Nobel prise in chemistry in 1952 for the contribution into the separation science. In the 1950s he was the first to describe[22,23] the structures that are now termed as monoliths. However, they appeared to be mechanically fragile and crashed under hydrostatic pressure. Later, Kubin *et al.*[24] proved this fact in the studies of the monolith prepared on the basis of 2-hydroxyethyl methacrylate hydrogel. The flow rate through the columns was insufficient for their practical use, and the performance appeared to be extremely low [552 theoretical plates (TP) per m for the column prepared by radical-induced polymerisation of 22% aqueous solution of 2-hydroxyethyl methacrylate containing 0.2% ethylene glycol dimethacrylate in a glass tube with an inner diameter of 25 mm]. In the early 1970s, monolithic columns attracted attention again, but that time for the use in gas chromatography (GC).[25–27] Monolithic columns based on polyurethane foams were obtained by polymerisation *in situ* (*i.e.* directly in the tube). The columns were successfully used for the GC-separation of various hydrocarbon mixtures (for example, alkanes C_6-C_9).[25] It was shown that a satisfactory separation can also be achieved in liquid chromatography.[26,27] However, at that time, this line was not further developed due to low stability of the phases obtained, which did not form chemical bonds with column walls and, therefore, easily lost the contact with the column. For the next 15 years, there have been virtually no publications devoted to monolithic columns.

Renaissance in studies of monolithic stationary phases has been observed since the mid-1980s when a new approach to the synthesis of porous monolithic media was developed on the basis of the production process of ultrafiltration membranes. In 1989, novel monolithic stationary phases were suggested as a result of joint research of the Institute of Macromolecular Compounds of the Academy of Sciences of the USSR (Leningrad) and the Institute of Macromolecular Chemistry of the Academy of Sciences of Czechoslovakia (Prague).[28–31] These phases were tested[32] in the separation of macromolecular biological objects: peptides, proteins and nucleic acids. The authors applied their rich experience in separation of biological samples on polymeric membranes[33,34] to the separation on monolithic columns.[35,36] The number of monomers used for the synthesis of monolithic columns has been substantially increased during the next several years.[37,38] Convenient methods for the synthesis of monoliths were suggested,[39] which allowed the preparation of monoliths directly in glass or quartz tubes used later as chromatographic columns. Application of these methods ruled out the complicated task of placing the monolith into the chromatographic column. Due to slow thermally initiated polymerisation performed in a vertical tube, the initially formed cross-linked polymer could shrink, but the liquid polymerisation mixture remained around and above the polymeric core. This mechanism successfully compensated potential radial shrinkage of the polymer, which otherwise would result in the formation of cavities between the monolith and the inner surface of the tube, which, in turn, would adversely affect chromatographic properties of the monolithic sorbent obtained.[40–42]

Although monolithic columns based on organic polymers showed good results in separation and purification of macromolecular sorbates including proteins,[4,43–45] nucleic acids[6] and synthetic polymers,[36–38] their efficiency in separation of low-molecular analytes remained insufficient. Taking into account the extreme popularity of silica-based sorbents in HPLC, in the mid-1990s silica gel monolithic columns were suggested.[46] The method for their preparation was based on the sol–gel process developed earlier.[47,48] A distinguishing feature of the new method was that in the course of polymerisation, coral-like structures with a bimodal pore distribution were formed: micropores ensured a significant area of the adsorption surface, whereas macropores favoured easy flow of the mobile phase through the monolith structure.[49]

Recently the pore structure and size distribution in silica-based monoliths have been comprehensively studied.[50–52] Monoliths were synthesised by polycondensation of tetramethoxysiloxane in the presence of poly(ethylene glycol), urea and other reagents.[17,53–56] In the course of the reaction, spontaneous phase separation occurred and the structures arose with silica-rich zones alternating with aqueous zones. Subsequent hydrothermal treatment of the product yielded micropores in the skeleton of the forming inorganic monolith. After elimination of the aqueous phase, the final product was obtained, which consisted of the silica skeleton with through-channels with a diameter of ~ 1 μm, the walls of which were covered by pores with a diameter of 10–20 nm. Porous silica-based monoliths were modified by the treatment with dimethyloctadecyl(*N*,*N*-diethylamino)silane;[47] the efficiency of the modified phases obtained was estimated under conditions of reversed-phase high-performance liquid chromatography (RP-HPLC).

These modified monoliths allowed efficient separation of both low-molecular [the height equivalent to a theoretical plate (HETP) was 10–15 μm for aromatic hydrocarbons] and macromolecular sorbates (HETP 20–30 μm for insulin). Notably high performance of standard silica-based monolithic columns is retained at high flow rate of the mobile phase, especially in the case of macromolecular sorbates. The Van Deemter plots for standard monolithic columns have smaller slope as compared to those for the columns filled with granulated sorbents; in addition, the minimum HETP value for monolithic columns is achieved in the range of higher flow rate of the mobile phase. As was mentioned above, the most important condition for high-performance separations is the tight cohesion of the monolith with column walls.[47] If there are cavities between the monolith and the column walls, the mobile phase will go through these cavities bypassing the monolith layer. This is the reason why the column material should have the same thermal expansion coefficient as the monolith or the monolith should be bound to the column walls by chemical bonds. In the former case, the silica gel monolith is placed into polymeric cartridges [for example, from thermocontractable poly(ester ether ketone) (PEEK)]. In the latter case, glass or quartz tubes and capillaries with the premodified surface are used. Both methods ensure tight cohesion of the monolith with the column walls, however, each method has limitations. The use of PEEK limits the number of eluents, which can be applied for separations, because dichloromethane, for instance, causes swelling. Chemical graft to the column walls by silylation is unstable in alkaline media and is destroyed at pH > 10.

III. The use of monolithic stationary phases in liquid chromatography and electrochromatography

1. Separation of proteins and peptides

Investigations into proteomics of a cell is a topical task in biochemistry because formation and destruction of proteins are interrelated dynamic processes allowing the cells to adapt to changing environmental conditions such as temperature, pH, the presence of biologically active substances. In view of this, studies of proteins and their methabolic ways are the most important problems.[57] Taking into consideration the complexity of most biological objects, different methods for separation of proteins have been developed, the most popular being gel electrophoresis, capillary electrophoresis (CE) and liquid chromatography (LC). Gel electrophoresis is a classical method and is widely used in biochemical practice.[58,59] Two-dimesional (2D) gel electrophoresis is considered today as the most efficient method for separation of multicomponent mixtures present in the cell, though it has a number of drawbacks.[60–62] For example, the method has a limited dynamic range, which complicates the analysis of very small samples or samples containing proteins with very high molecular mass.

Recently, a new analytical method for investigating protein composition of the cell has been suggested based on the combination of liquid chromatography and time-of-flight mass spectrometry (MALDI-TOF),[63–65] monolithic columns based on poly(styrene–divinylbenzene) were used, which belong to the last generation of columns for protein determination. The major advantages of these columns are high mechanical strength and selectivity in separation of peptides and proteins.[66–71] A chromatographic method for separation of bovine serum albumin, egg albumin and lysozyme on a monolithic capillary column with a length of 5 cm and an inner diameter of 200 μm in combination with tryptic hydrolysis of separated proteins is reported.[63] The protein fraction after chromatographic separation was collected in the ionisation

chamber of a mass spectrometer, where the tryptic hydrolysis was performed. Thus, all peptides of the tryptic hydrolysate could unambiguously be ascribed to a given protein, which, in principle, allows a comparative analysis of all isoforms of proteins. This method appeared to be faster as compared with electrophoresis in polyacrylamide gel in the presence of sodium dodecyl sulfate (1D SDS–PAGE) or with two-dimensional electrophoresis in polyacrylamide gel with isoelectric focusing in the presence of sodium dodecyl sulfate (1D IF SDS–PAGE). The method can be improved in view of separation of phosphorylated forms by the use of different ion-pair reagents,[69] two-dimensional LC on ion-exchange columns or affinity chromatography for the primary separation under conditions of LC.[70, 72]

Monolithic capillary columns based on a copolymer of methylstyrene (MS) and 1,2-bis(*p*-vinylphenyl)ethane (BVPE) were suggested for separation of biopolymers.[4] Monolithic capillary columns showed extremely high mechanical stability and the minimum swelling in organic solvents. The possibility of using the poly(MS/BVPE) monolith as a stationary phase in separation of proteins and peptides by RP-HPLC and in separation of nucleic acids under conditions of ion-pair RP-HPLC has been studied. Permeability and chromatographic performance of monolithic capillary columns appeared to depend strongly on the monomer fraction in the initial polymerisation mixture. Columns with various permeability were prepared and the possibility of fast separation of biopolymers was demonstrated. A protein test mixture containing ribonuclease A, cytochrome *c*, α-lactalbumin, β-lactalbumin B and egg albumin has been completely separated in 35 s, and a homologous series of phosphorylated oligothymidylic acids in less than 2 min.

Another method for very fast separations of proteins under different conditions [for example, under conditions of ion-exchange hydrophilic interaction liquid chromatography (HILIC) or affinity chromatography] consists in the use of short monolithic columns in which convective mass transfer occurs.[73] These are known under the trademark CIM-monoliths (Convective Interaction Media). CIM-discs and CIM-tubes have high sorption capacity for large molecules and allow the operation with high rates of the mobile phase.[73, 74] The use of protein A or protein G as ligands allows the operation with large amounts of complicated biological mixtures containing, for instance, antibodies.[75] The application of short monolithic columns for isolation of membrane proteins has been described.[76] Several types of antibodies have been isolated from plasma proteins of liver membranes by virtue of their binding to protein A or protein G supported on CIM-discs.[77] The immunoaffinity columns obtained in this way were used, in turn, for target isolation of the antigen. It was shown [76] that similar approach can be used for high-selective enrichment of proteins of low content from large volumes of complicated biological mixtures such as extracts of membrane proteins of rat liver. Solubilised membrane proteins were first separated using a system containing one anion-exchange (with diethylaminoethyl groups, DEAE) and one cation-exchange monolithic CIM-discs.[78] The separation of monoclonal antibodies (mAb) was carried out on a monolithic CIM-disc with G-protein. However, the antigen was eluted together with the antibody and should be separated by an additional process with the use of 1D SDS–PAGE. An example of the chromatogram (obtained under conditions of ion-exchange chromatography) of the protein fraction with the approximate molecular mass of ~110 kDa isolated from the tryptic hydrolysate SDS–PAGE is presented in Fig. 1.[76]

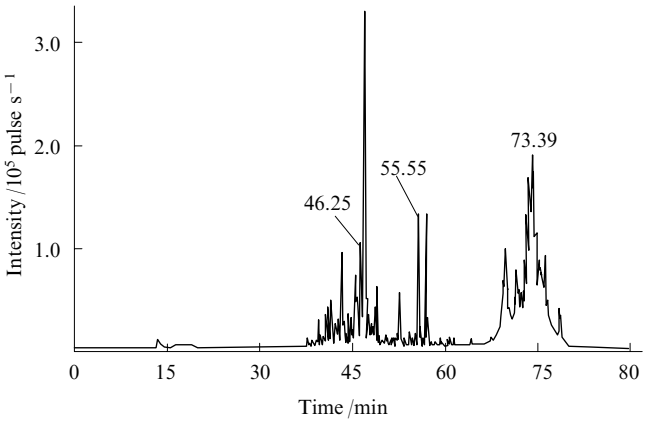


Figure 1. Identification of proteins by LC–MSC/MS.[76]
The chromatogram of a tryptic hydrolysate of a protein fraction with the average molecular mass of about 110 kDa obtained by SDS–PAGE.

Recently the dynamic adsorption of large protein-carrying particles on monolitic CIM-discs containing DEAE groups and quaternary ammonium groups has been reported.[79] This study was aimed at investigating the peculiarities of dynamic adsorption of nanoparticles. Virus-like synthetic particles carrying different proteins on the outer surface were specially prepared by two different methods. The first method was based on chemical cross-linking of proteins, whereas the second method was based on the modification of the surface of spherical polystyrene microparticles by proteins. Two chromatographic methods were used for experimental studies of dynamic adsorption, *viz*. ion exchange chromatography and affinity liquid chromatography on supershort monolithic columns (CIM-discs with the DEAE groups or with the quaternary ammonium groups). The values of the maximum dynamic adsorption capacity and their dependence on the flow rate were determined. The permeability of the monolithic porous medium appeared to be sufficient for efficient separation of large particles.

2. The use of monolithic columns for separation of polyaromatic hydrocarbons

Polyaromatic hydrocarbons are widespead xenobiotics formed upon burning of fossil fuels and always appear as a mixture of components. Due to low solubility in water, PAHs are usually present in aqueous samples at insignificant concentrations, but sometimes they are found in large amounts in hydrophobic media such as vegetable oils and animal fats. Some PAHs, for example, benz[*a*]pyrene, are strong carcinogens and decompose very slowly under the aerobic conditions. According to hygienic and environmental requirements, the concentration of PAHs in air, water and foodstuffs should be carefully controlled. The most commonly used methods for separation and analysis of PAHs are LC and GC.[80]

The method for preparation of a silica gel monolithic column with grafted n-octyl (C_8) groups is suggested [16] for reversed-phase capillary electrochromatography.[81] The monolithic silica gel stationary phase was obtained in two steps: by acid-base hydrolysis and by co-condensation of tetraethoxysilane (TEOS) and n-octyltriethoxysilane (C_8-TEOS). An increase in the TEOS : C_8-TEOS ratio resulted in smaller pores and increased pore volume. The optimal TEOS : C_8-TEOS ratio was 90 : 50 (by volume); the average pore diameter

in the monolith achieved ~1.0 μm with the overall pore volume of 3.25 $cm^3\ g^{-1}$. Aromatic compounds including alkylbenzenes, PAHs and phenols were successfully separatedon the modified high-performance (180 000 TP m^{-1}) C_8-monolithic column.

Monolithic capillary columns based on organic polymers are also widely used for separation of PAHs. In capillary electrochromatography (CEC), it was suggested[82] to use porous polymeric monoliths on the basis of butyl acrylate obtained in a quartz capillary. In order to estimate the performance of the columns, the Van Deemter plots were constructed for chrysene at different operating temperatures. The minima in the plots were shifted to the higher flow rate range with an increase in temperature, whereas the HETP value in the minima slightly increased. The performance of the columns was reproduced for two different columns of different diameter used in different chromatographic systems but prepared under the same conditions. Homogeneity of the monolith along the column length was studied using the standard mixture of polycyclic aromatic hydrocarbons (EPA 610). The values of PAH retention persisted indicating that the columns were homogeneous along the length. For substances with the PAH retention factor from 2 to 12, the performance observed was 65 000 – 85 000 TP m^{-1}.

Later monolithic capillary columns based on butyl acrylate – dodecyl acrylate were suggested[14] CEC. As was expected, the introduction of a small amount of a hydrophobic monomer yielded the columns on which the neutral analytes were retained more strongly. However, further increase in the percentage of the hydrophobic monomer in the polymerisation mixture decreased the retention values. Unusual behaviour of hydrophobic columns is probably associated with the non-uniform structure of the monolith containing the more hydrophobic monomer. Nevertheless, the Van Deemter curves plotted for the columns studied showed the minimum HETP value in the range from 8.7 to 9.1 μm (115 000 – 110 000 TP m^{-1}) and revealed no contradictions.

Determination of PAHs in real samples usually requires preconcentration. Modern applications of monoliths for preconcentration and solid-phase extraction are reviewed.[20] In addition to the traditional laboratory method, which is not widely applied to monolithic materials, the combination of preconcentration with high-performance liquid chromatography, electrochromatography, electrophoresis and on-line enzymatic hydrolysis is considered. Recently, on-line concentration of trace amounts of carcinogenic PAHs on a microcolumn under LC conditions, when large volumes of specimens are injected has been suggested.[15] The possibility of using this system for preconcentration of 14 PAHs under conditions of microcolumn chromatography was evaluated. The authors compared micro precolumns filled with spherical silica C_{30} with silica monolithic columns with the C_{18} groups and microcolumns filled with C_{30}-coated quartz fibres. No significant differences between the columns modified with C_{30} and C_{18} groups used for PAH separation were found, however, for further tests, columns with C_{30} were selected because of more reproducible results obtained. Of three sorbents used, better preconcentration was achieved with the columns filled with granulated silica C_{30} and with monolithic capillary columns with C_{18}-silica. From the viewpoint of concentration and separation of PAHs, the use of monolithic precolumn with an inner diameter of 0.1 mm gave better results as compared to that with an inner diameter of 0.2 mm. In addition, the injection of up to 0.5 ml of the sample is possible, *i.e.* the ~2500-fold enrichment was achieved without deterioration in separation. The system developed was later used for determination of trace amounts of PAHs in the soil the samples of which were occasionally collected near highway.

IV. The use of monolithic stationary phases in ion chromatography

Determination of soluble ionic compounds in natural and industrial water is performed routinely in various analytical laboratories and is an important task in both the monitoring of the environment and the control of industrial processes. At present, simple inorganic ions are mainly determined by ion chromatography (IC), capillary zone electrophoresis (CZE) and by potentiometric methods with ion-selective electrodes. Ion chromatography is inferior to CZE in the analysis time, but far exceeds it in selectivity and especially in reproducibility of the results.[83] It is possible to decrease the IC analysis time using short chromatographic columns. In this case, however, in order to achieve acceptable resolution, fine sorbents should be used, which results in an increase in the pressure required for the optimal work of the column. Jiang *et al.* attempted[84] to develop a low pressure systems based on short columns with a length of 3 – 6 cm for efficient ion separation; difficulties are described that can be overcome by the use of monolithic columns.

Ion-exchange monolithic columns can be obtained on the basis of modified silica or organic polymers. Ion-exchange properties of the monolith can be achieved by the treatment of a finished monolithic matrix containing reactive groups by different reagents or by adsorption of ionogenic compounds on the surface of the monolith.[85] The use of monolithic sorbents provided the development of new approaches to ion separation, namely, low- and medium-pressure chromatography, superfast IC, gradient IC, capillary IC and, finally, multicolumn multidimensional IC.[8] Recently the separation of simple anions on ultrashort (1 cm) monolithic columns with the eluent supply by a microperistaltic pump has been demonstrated.[9] Although the sensitivity of this method is not high yet and should be optimised, the authors managed to separate simple anions (fluoride, chloride, nitrate, sulfate) in aqueous samples using a low pressure gradient. The chromatograms obtained on monolithic anion-exchange columns with the size 1 × 0.4 cm are shown in Fig. 2.[9] The same authors reported[10] the modification of silica monolithic columns (Onyx C_{18}, 150 mm × 0.1 mm) by the amphoteric surfactant (*N*-dodecyl-*N*,*N*-dimethylammonio)undecanoate and estimated the ability of the column to separate inorganic ions using a contactless conductivity detector. Chromatographic performance of the column under the optimal conditions was 56 200 TP m^{-1} (7025 TP per column at the detection point). Direct connection of the capillary column to a microinjector and on-column detection excluded peak broadening associated with extracolumn effects. This permitted the researchers to plot more accurate Van Deemter curves for the monolithic capillary columns obtained and to determine the value of the coefficient *C* in the Van Deemter equation, which appeared to be quite low, thus indicating the possibility of utilisation of the columns at elevated flow rates of the eluent without significant losses in efficiency of the column.

Monoliths based on organic polymers are characterised by higher chemical stability as compared to that of silica-based monoliths; they allow the work with aqueous eluents. Recently experiments have been conducted[86, 87] aimed at creating capillary columns for ion chromatography based on organic copolymers of poly(ethylene glycol dimethacrylate/glycidyl

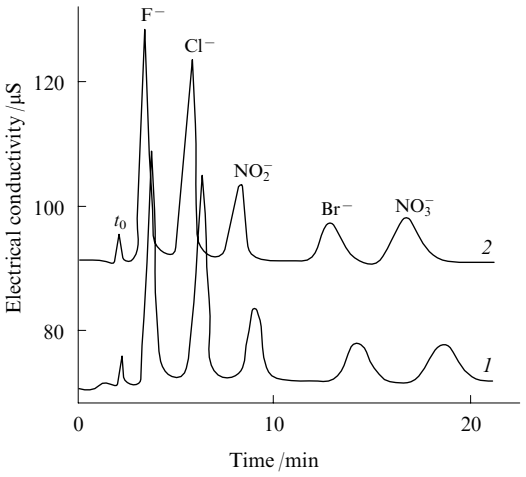


Figure 2. Separation of a standard anion mixture on a monolithic anion-exchange column with a size of 1.0×0.4 cm.[9]
Eluent is 0.002 M aqueous solution of *p*-hydroxybenzoic acid, pH 6.5, eluent flow rate 0.33 ml min^{-1}, conductivity detector: (*1*) room temperature, (*2*) eluent is heated to 45 °C. Concentration of F^-, Cl^-, NO_2^-, Br^- and NO_3^- ions in the standard 50 mg $litre^{-1}$.

methacrylate) [poly(EDMA/GMA] and poly(divinylbenzene/chloromethylstyrene). Monolithic matrices were obtained by polymerisation *in situ* in a quartz capillary with an inner diameter of 100 μm. Before polymerisation, the inner surface of the capillary was silanized with 3-(trimethoxysilyl)propyl methacrylate in order to ensure its covalent binding to the monolith.[86,87] In addition, the effect of the polymerisation conditions (temperature, duration, *etc.*) on the efficiency of monolithic columns was studied. The matrices that demonstrated the maximum efficiency were then modified in order to introduce quarternary nitrogen atoms in the structure of the monolith.[88] The separation of a model anion mixture on a monolithic capillary column obtained is presented in Fig. 3. The anion mixture is separated to the base line in about 2.5 h,

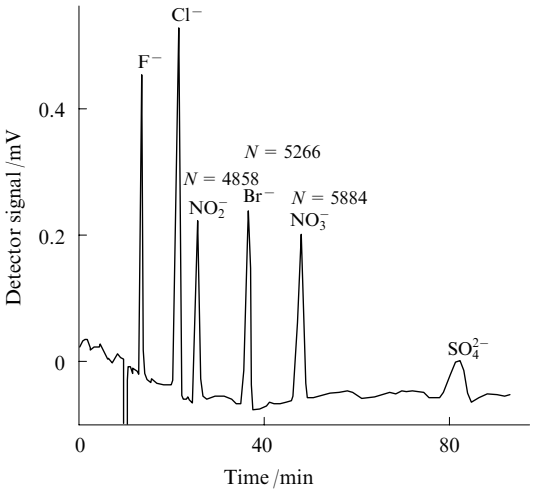


Figure 3. Separation of an anion mixture on a monolithic capillary column based on poly(EDMA/GMA).[88]
Inner diameter of the column 100 μm, the column surface is modified by amine; eluent is 0.005 M aqueous solution of potassium phthalate, pH 4.25, flow rate 0.1 ml min^{-1}, pressure 700 kPa, *N* is the number of TP per column.

which is significantly longer than in the above-mentioned on silica columns. The authors note that the value of the *C* coefficient in the Van Deemter equation for polymeric monolithic columns is an order of magnitude higher than that for silica-based columns, which prevents rapid and efficient separation on these columns. At the same time, selectivity of ion separation on polymeric columns appears to be substantially higher than on silica columns thus decreasing the analysis time to 15–20 min with complete separation of all ions of the standard mixture.[88]

V. The use of monolithic stationary phases in gas chromatography

First studies devoted to the use of monolithic columns in gas chromatography date back to the late 1960s–early 1970s.[25–27] However, the efficiency of the columns obtained was low, they were unstable, and soon were completely expelled by open capillary columns. Recently, monolithic columns for gas-adsorption chromatography based on poly(divinylbenzene) (PDVB) have been reported.[39,89] The columns were prepared by the new method of polymerisation and are termed as 'molded' monolithic columns.[89] According to the data from thermogravimetric analysis, the monolith in the column did not undergo degradation until 380 °C. High thermal stability of the monolith allows routine operation with these columns at ~300 °C without deterioration in their characteristics. Synthesis and properties of monolithic columns for gas chromatography have been comprehensively considered.[90–97] Permeability,[90,91] porosity,[90,91] load characteristics[96] and performance[92,93] have been studied for monolithic capillary columns based on silica and organic polymers. Monolithic capillary columns based on silica are characterised by high porosity (to 95%). Despite the fact that the monolith itself occupies a smaller part of the overall volume of the column, it resists significantly to the carrier gas flow. As a result, the permeability of the monolithic capillary columns studied was three orders of magnitude lower than that of the parent open capillaries. At the same time, due to high porosity values, the permeability of monolithic columns appeared to be one to two orders of magnitude higher than that of the columns filled with granulated sorbents with the grain size equal to the size of monolith domains. The estimation of porosity and permeability of the columns by the gas chromatographic method permitted researchers to estimate the differences in the structure of the sorbent obtained.[95]

Load characteristics of monolithic columns have been also considered. As is known, the load characteristic of the column depends on the amount of the stationary phase in the column and its porosity.[96] The specific load of the monolithic capillary columns (*i.e.*, the load per unit column length) exceeded that of open capillary columns with the same inner diameter 7–10-fold. This property of monolithic columns allowed the use of relatively short columns (0.5 m and less) with standard injection systems without risk of overloading the column. In addition, it was shown that the specific efficiency of monolithic capillary columns is significantly higher than those of open capillary columns. The HETP values obtained on monolithic capillary columns strongly depended on the nature of gas-carrier gas.[94] Thus the substitution of helium for carbon dioxide resulted in a 10-fold decrease in the HETP value. The HETP values obtained under the optimal chromatographic conditions for monolithic columns with the optimised monolith structure were equal to 10–15 μm, which is comparable with those for the same columns under HPLC conditions. Low

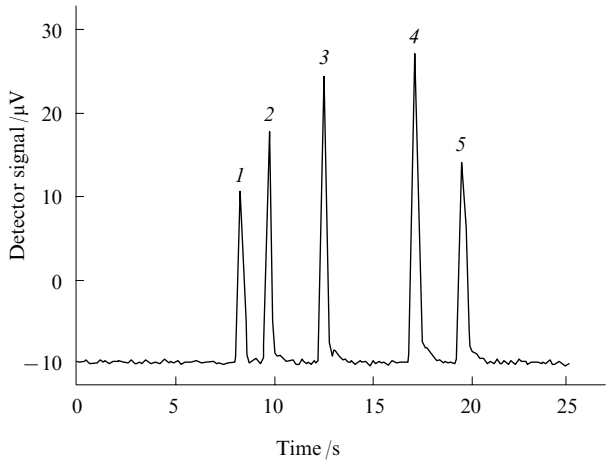


Figure 4. Separation of a mixture of light hydrocarbons on a monolithic capillary column (47 cm × 50 μm) based on PDVB.
(*1*) Methane, (*2*) ethane, (*3*) propane, (*4*) isobutane, (*5*) n-butane. Carrier gas CO_2, pressure 9 MPa, 80 °C.

HETP values and high load capacities of monolithic columns allow the use of short columns in gas chromatography and ensure highly efficient and fast separations. The separation of a test mixture of light hydrocarbons on monolithic columns with a length of 47 cm and the inner diameter of 50 μm using the traditional detecting system is presented in Fig. 4. All the components of the mixture were separated to the base line over a period of up to 20 s with efficiency of $\sim$900 TP s^{-1} (for n-butane).

VI. Conclusion

Monolithic stationary phases represent a new type of sorbents for liquid and gas chromatography, capillary electrophoresis, different systems for sample preparation, solid-phase extraction, *etc*. They possess large porosity and better permeability as compared with traditional columns filled with granulated sorbents with the same graining. Numerous monomers that can be used for the synthesis of monoliths as well as a diversity of reagents for the modification of the surface of the monoliths obtained allow the creation of monolithic columns with different properties required for solution of particular problems. Due to high specific efficiency, monolithic columns can be short; moreover, monolithic columns often have a flat Van Deemter plot, which provides possibility to operate at high flow rates of the mobile phase, which opens the way to fast and efficient separations.

Together with undoubted advantages mentioned above, monolithic sorbents have drawbacks. The major drawback is that the reproducible synthesis of the monolith is very difficult. This problem can be overcome by strict control of the synthesis parameters (temperature and polymerisation time, the composition of the polymerisation mixture). The possibility of conducting the reproducible synthesis would allow serial production of different monolithic columns.

Recently, different columns with monolithic sorbents have been put on the market. These include columns for HPLC, IC, columns based on silica and organopolymer matrices, columns of the standard analytical and capillary formats, as well as monolithic CIM-discs used mainly in the analysis of biological objects. Fast development of new methods for preparation of chromatographic sorbents allow the users to solve a variety of problems without recourse to the instrumentation for operation with high and superhigh pressure.

References

1. F Švec, C G Huber *Anal. Chem.* **78** 2100 (2006)
2. B G Belenkii *Bioorg. Khim.* **32** 360 (2006) [a]
3. P Jandera, J Urban, D Moravcová *J. Chromatogr., A* **1109** 60 (2006)
4. L Trojer, S H Lubbad, C P Bisjak, G K Bonn *J. Chromatogr., A* **1117** 56 (2006)
5. L Rieux, D Lubda, H A G Niederländer, E Verpoorte, R Bischoff *J. Chromatogr., A* **1120** 165 (2006)
6. P Holdšvendová, J Suchánková, M Bunček, V Bačkovská, P Coufal *J. Biochem. Biophys. Methods* **70** 23 (2007)
7. R Skudas, B A Grimes, E Machtejevas, V Kudirkaite, O Kornysova, T P Hennessy, D Lubda, K K Unger *J. Chromatogr., A* **1144** 72 (2007)
8. B Paull, P Nesterenko *TrAC — Trends Anal. Chem.* **24** 295 (2005)
9. D Victory, P Nesterenko, B Paull *Analyst (Cambridge, U.K.)* **129** 700 (2004)
10. C Ó Ríordáin, E Gillespie, D Connolly, P N Nesterenko, B Paull *J. Chromatogr., A* **1142** 185 (2007)
11. E Sugrue, P N Nesterenko, B Paull *J. Chromatogr., A* **1075** 167 (2005)
12. L Barron, P N Nesterenko, D Diamond, M O'Toole, K T Lau, B Paull *Anal. Chim. Acta* **577** 32 (2006)
13. T Ikegami, K Horie, J Jaafar, K Hosoya, N Tanaka *J. Biochem. Biophys. Methods* **70** 31 (2007)
14. B L Waguespack, S A Hodges, M E Bush, L J Sondergeld, M M Bushey *J. Chromatogr., A* **1078** 171 (2005)
15. L W Lim, Y Okouchi, T Takeuchi *Talanta* **72** 1600 (2008)
16. L-J Yan, Q-H Zhang, Y-Q Feng, W-B Zhang, T Li, L-H Zhang, Y-K Zhang *J. Chromatogr., A* **1121** 92 (2006)
17. N Tanaka, H Kobayashi, N Ishizuka, H Minakuchi, K Nakanishi, K Hosoya, T Ikegami *J. Chromatogr., A* **965** 35 (2002)
18. T Ikegami, N Tanaka *Curr. Opin. Chem. Biol.* **8** 527 (2004)
19. Z Jiang, N W Smith, P D Ferguson, M R Taylor *J. Biochem. Biophys. Methods* **70** 39 (2007)
20. F Švec *J. Chromatogr., B* **841** 52 (2006)
21. A Maruška, O Kornyšova *J. Chromatogr., A* **1112** 319 (2006)
22. D L Mould, R L M Synge *Analyst (Cambridge, U.K.)* **77** 964 (1952)
23. D L Mould, R L M Synge *Biochem. J.* **58** 571 (1954)
24. M Kubin, P Spacek, R Chromecek *Collect. Czech. Chem. Commun.* **32** 3881 (1967)
25. W D Ross, R T Jefferson *J. Chromatogr. Sci.* **8** 386 (1970)
26. F D Hileman, R E Sievers, G C Hess, W D Ross *Anal. Chem.* **45** 1126 (1973)
27. H Schnecko, O Bieber *Chromatographia* **4** 109 (1971)
28. T B Tennikova, B G Belenkii, F Švec *J. Liq. Chromatogr.* **13** 63 (1990)
29. US P. 4889632A (1989)
30. US P. 4952349 (1990)
31. US P. 4923610 (1990)
32. D Josić, J Reusch, K Löstner, O Baum, W Reutter *J. Chromatogr., A* **590** 59 (1992)
33. T B Tennikova, M Bleha, F Švec, T V Almazova, B G Belenkii *J. Chromatogr., A* **555** 97 (1991)
34. T B Tennikova, F Švec *J. Chromatogr., A* **646** 279 (1993)
35. K Hosoya, Y Kishii, K Kimata, T Araki, N Tanaka, F Švec, J M J Fréchet *J. Chromatogr., A* **690** 21 (1995)
36. M Petro, F Švec, J M J Fréchet *J. Chromatogr., A* **752** 59 (1996)
37. Q C Wang, F Švec, J M J Fréchet *J. Chromatogr., A* **669** 230 (1994)
38. S Xie, F Švec, J M J Fréchet *J. Chromatogr., A* **775** 65 (1997)
39. S Xie, R W Allington, J M J Fréchet, F Švec *Adv. Biochem. Eng. Biotechnol.* **76** 86 (2002)
40. F Švec, J M J Fréchet *Anal. Chem.* **64** 820 (1992)

41. F Švec, J M J Fréchet *Ind. Eng. Chem. Res.* **38** 34 (1999)
42. S Hjertén, J-L Liao, R Zhan *J. Chromatogr., A* **473** 273 (1989)
43. S El Deeb, L Preu, H Wätzig *J. Pharm. Biomed. Anal.* **44** 85 (2007)
44. J M Armenta, B Gu, C D Thulin, M L Lee *J. Chromatogr., A* **1148** 115 (2007)
45. J P Hutchinson, E F Hilder, M Macka, N Avdalovic, P R Haddad *J. Chromatogr., A* **1109** 10 (2006)
46. H Minakuchi, K Nakanishi, N Soga, N Ishizuka, N Tanaka *Anal. Chem.* **68** 3498 (1996)
47. H Minakuchi, K Nakanishi, N Soga, N Ishizuka, N Tanaka *J. Chromatogr., A* **762** 135 (1997)
48. N Ishizuka, H Minakuchi, K Nakanishi, K Hirao, N Tanaka *Colloids Surf., A* **187–188** 273 (2001)
49. N Ishizuka, H Minakuchi, K Nakanishi, N Soga, N Tanaka *J. Chromatogr., A* **797** 133 (1998)
50. W Gao, G Yang, J Yang, H Liu *Turk. J. Chem.* **28** 379 (2004)
51. R Takahashi, K Nakanishi, N Soga *J. Sol-Gel Sci. Technol.* **17** 7 (2000)
52. M Al-Bokari, D Cherrak, G Guiochon *J. Chromatogr., A* **975** 275 (2002)
53. C Xie, J Hu, H Xiao, X Su, J Dong, R Tian, Z He, H Zou *Electrophoresis* **26** 790 (2005)
54. C Xie, J Hu, H Xiao, X Su, J Dong, R Tian, Z He, H Zou *J. Sep. Sci.* **28** 751 (2005)
55. C Xie, M Ye, X Jiang, W Jin, H Zou *Mol. Cell. Proteom.* **5** 454 (2006)
56. K Nakanishi, H Shikata, N Ishizuka, N Koheiya, N Soga *J. High Resolut. Chromatogr.* **23** 106 (2000)
57. M Lubeck, U Schweiger-Hufnagel, C Baessmann, R Rabus, M Shen *LC–GC Eur.* (Suppl. 5) 12 (2003)
58. F Vanrobaeys, R van Coster, G Dhondt, B Devreese, J van Beeumen *J. Proteome Res.* **4** 2283 (2005)
59. W-H Jin, J Dai, S-J Li, Q-C Xia, H-F Zou, R Zeng *J. Proteome Res.* **4** 613 (2005)
60. H Liu, D Lin, J R Yates III *BioTechniques* **32** 898 (2002)
61. C C Wu, J R Yates III *Nat. Biotechnol.* **21** 262 (2003)
62. X Li, Y Gong, Y Wang, S Wu, Y Cai, P He, Z Lu, W Ying, Y Zhang, L Jiao, H He, Z Zhang, F He, X Zhao, X Qian *Proteomics* **5** 3423 (2005)
63. C Hunzinger, A Schrattenholz, S Poznanović, G P Schwall, W Stegmann *J. Chromatogr., A* **1123** 170 (2006)
64. E Nagele, M Vollmer *Rapid Commun. Mass Spectrom.* **18** 3008 (2004)
65. E Mirgorodskaya, C Braeuer, P Fucini, H Lehrach, J Gobom *Proteomics* **5** 399 (2005)
66. H S Chen, T Rejtar, V Andreev, E Moskovets, B L Karger *Anal. Chem.* **77** 2323 (2005)
67. C Legido-Quigley, N Marlin, N W Smith *J. Chromatogr., A* **1030** 195 (2004)
68. H Toll, R Wintringer, U Schweiger-Hufnagel, C G Huber *J. Sep. Sci.* **28** 1666 (2005)
69. A Tholey, H Toll, C G Huber *Anal. Chem.* **77** 4618 (2005)
70. B M Mayr, O Kohlbacher, K Reinert, M Sturm, C Gröpl, E Lange, C Klein, C G Huber *J. Proteome Res.* **5** 414 (2006)
71. A C A Veloso, N Teixeira, I M P L Ferreira *J. Chromatogr., A* **967** 209 (2002)
72. T S Nühse, A Stensballe, O N Jensen, S C Peck *Mol. Cell. Proteom.* **2** 1234 (2003)
73. T B Tennikova, J Reusch *J. Chromatogr., A* **1065** 13 (2005)
74. D Forcic, K Branovic-Cakanic, J Ivancic, R Jug, M Barut, A Strancar *J. Chromatogr., A* **1065** 115 (2005)
75. T V Gupalova, O V Lojkina, V G Pálágnuk, A A Totolian, T B Tennikova *J. Chromatogr., A* **949** 185 (2002)
76. M Rucevic, J G Clifton, F Huang, X Li, H Callanan, D C Hixson, D Josic *J. Chromatogr., A* **1123** 199 (2006)
77. M M Comegys, S-H Lin, D Rand, D Britt, D Flanagan, H Callanan, K Brilliant, D C Hixson *J. Biol. Chem.* **279** 35063 (2004)
78. D Josic, M K Brown, F Huang, H Callanan, M Rućević, A Nicoletti, J Clifton, D C Hixson *Electrophoresis* **26** 2809 (2005)
79. I V Kalashnikova, N D Ivanova, T G Evseeva, A Yu Menshikova, E G Vlakh, T B Tennikova *J. Chromatogr., A* **1144** 40 (2007)
80. H-P Nirmaier, E Fischer, A Meyer, G Henze *J. Chromatogr., A* **730** 169 (1996)
81. *Capillary Electrochromatography* (Eds Z Deyl, F Švec) (Amsterdam: Elsevier, 2001)
82. L J Sondergeld, M E Bush, A Bellinger, M M Bushey *J. Chromatogr., A* **1004** 155 (2003)
83. M I H Helaleh, T Korenaga *J. Chromatogr., B* **744** 433 (2000)
84. X Jiang, X Zhang, M Liu *J. Chromatogr., A* **857** 175 (1999)
85. J Weiss, D Jensen *Anal. Bioanal. Chem.* **375** 81 (2003)
86. E N Viktorova, A Yu Kanatyeva, A A Korolev, A A Kurganov *Zh. Fiz. Khim.* **81** 507 (2007) [b]
87. A Yu Kanatyeva, A A Korolev, E N Viktorova, A A Kurganov *Zh. Fiz. Khim.* **81** 568 (2007) [b]
88. A Yu Kanatyeva, A A Korolev, E N Viktorova, A A Kurganov, in *Vserossiiskii Simpozium 'Khromatografiya v Khimicheskom Analize i Fiziko-khimicheskikh Issledovaniyakh', Moskva, Klyaz'ma, 2007* (All-Union Symposium 'Chromatography in Chemical Analysis and Physicochemical Investigations', Moscow, Klyazma, 2007)
89. D Sykora, E C Peters, F Švec, J M J Fréchet *Macromol. Mater. Eng.* **275** 42 (2000)
90. A A Korolev, T P Popova, V E Shiryaeva, A A Kurganov *Zh. Fiz. Khim.* **79** 543 (2005) [b]
91. A A Korolev, T P Popova, V E Shiryaeva, A A Kurganov *Zh. Fiz. Khim.* **80** 132 (2006) [b]
92. A A Korolev, V E Shiryaeva, T P Popova, A A Kurganov *Zh. Fiz. Khim.* **80** 709 (2006) [b]
93. A A Korolev, V E Shiryaeva, T P Popova, A A Kurganov *Zh. Fiz. Khim.* **80** 896 (2006) [b]
94. A A Korolev, V E Shiryaeva, T P Popova, A A Kurganov *Zh. Fiz. Khim.* **80** 1290 (2006) [b]
95. A A Korolev, V E Shiryaeva, T P Popova, A V Kozin, A A Kurganov *Vysokomol. Soedin., Ser. A* **48** 1373 (2006) [c]
96. A A Korolev, T P Popova, V E Shiryaeva, A V Kozin, A A Kurganov *Zh. Fiz. Khim.* **81** 552 (2007) [b]
97. A V Kozin, A A Korolev, V E Shiryaeva, T P Popova, A A Kurganov *Zh. Fiz. Khim.* **81** 512 (2007) [b]

[a] — *Russ. J. Bioorg. Chem. (Engl. Transl.)*
[b] — *Russ. J. Phys. Chem. (Engl. Transl.)*
[c] — *Polym. Sci. (Engl. Transl.)*