Brief Communications

Porous structure of the monolithic sorbent and separation properties of monolithic capillary columns in gas and liquid chromatography

*A. A. Korolev, V. E. Shiryaeva, T. P. Popova, E. N. Viktorova, and A. A. Kurganov**

A. V. Topchiev Institute of Petrochemical Synthesis, Russian Academy of Sciences,
29 Leninsky prosp., 119991 Moscow, Russian Federation.
E-mail: kurganov@ips.ac.ru

Macroporous polymer based on polydivinylbenzene was used for the preparation of monolithic capillary columns with the diameter from 0.01 to 0.53 mm for separations by gas and liquid chromatography. The separation properties of the columns were studied by analysis of model systems of aromatic (in liquid chromatography) and light (in gas chromatography) hydrocarbons. The permeability was determined and the *C* parameter of the Van-Deemter equation was found for each column. The permeability of the majority of columns determined by gas chromatography is independent of the column diameter. The permeability of the same columns in liquid chromatography is also almost constant for the columns 0.53—0.1 mm in diameter; however, the permeability decreases sharply on going to columns of smaller diameter. In gas chromatography the value of the *C* parameter reflecting the effect of the mass transfer of the sorbate between the mobile and stationary phases on the smearing of a chromatographic peak in the column approximately the same for all columns. In liquid chromatography the value of the *C* coefficient in the Van-Deemter equation for the same capillary columns changes with a change in the column diameter and reaches a minimum for the columns 0.1 mm in diameter. The differences observed for the characteristics of the columns in gas and liquid chromatography are due to different structures of the macroporous monolith formed in columns of different diameter and to the effect of solvation of the monolith by the mobile phase under the conditions of liquid chromatography.

Key words: monolithic columns, gas chromatography, liquid chromatography, capillary columns.

The dependence of the efficiency of an open capillary column on the flow rate of the carrier gas is described by Golay´s equation.[1,2] It follows from this equation that the minimum height of a theoretical plate for an open capillary column, which can be achieved under optimal conditions, is approximately equal to its diameter.[1,2] Thus, a decrease in the diameter of the capillary column from 0.53 to 0.05 mm should enhance the column efficiency by

Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 8, pp. 1616—1619, August, 2010.
1066-5285/10/5908-1660

an order of magnitude. However, the use of capillary columns of this diameter creates a series of problems related to their very low loading capacity, because the internal surface of such a capillary is very small. To avoid overloading of the column, it is necessary to use samples with the weight not more than several fractions of nanogram, which is unfavorable for the detection of the sample by standard detectors. Therefore, to date columns of small diameters were not widely used in chromatography. In modern gas chromatography open capillary columns with the diameter ≥ 0.1 mm are predominantly used. The diameter of columns usually used in liquid chromatography is still larger (1—2 mm and more).

The loading capacity of the column can be increased replacing an open capillary column with a column packed with the sorbent. According to the theory of chromatography, the efficiency of packed columns is determined by the diameter of sorbent particles rather than the diameter of the column.[2,3] At the same time, several publications[4] present experimental data favoring the fact that the column diameter exerts a certain effect on its properties even in the case of packed columns. There are two main reasons explaining a deviation of experimental results from the theoretical model. This first reason is the so-called near-wall effect. It is related to the fact that the sorbent layer near the walls of the column has always a looser structure than that in the main bulk of the packed layer. The influence of this factor is lower for columns of large diameter but can be significant for capillary columns. The second factor is due to a proper technique used to pack columns with the sorbent. This parameter can hardly be evaluated theoretically and is related, to a greater extent, to experimental skill of the researcher. The general regularity, for example, in liquid chromatography is that the best packing is usually attainable for columns of analytical size standard for HPLC (4—8 mm in diameter), whereas considerable effort is needed to achieve high quality of the columns on going to large diameters and/or capillary columns.

This problem is eliminated to some extent for monolithic columns, because the quality of packing in them is determined, in fact, by the monolith structure, which, in turn, is specified by synthesis conditions. It is more simply to control the synthesis parameters than conditions used to pack columns with the sorbent, which makes it possible to expect better reproducibility of the properties of the obtained columns. In addition, there are reports[5] that during the synthesis of the monolith a denser monolith layer than in the major bulk is formed on the capillary walls. This makes it possible to expect that the near-wall effect of monolithic columns is absent or strongly weakened.

At the same time, an analysis of literature data shows that we cannot deny the influence of the diameter of monolithic columns on the properties, at least in liquid chromatography. For the separation of low-molecular-weight sorbates the capillary monolithic columns (0.53 and 0.2 mm in diameter) are more efficient[6] than analogous 1- and 3-mm diameter columns. This effect is nearly unobservable for the gradient separation of high-molecular-weight objects, such as proteins and nucleic acids. The weak dependence of the column efficiency on the flow rate of the mobile phase observed[7,8] for analytical monolithic columns in HPLC makes it possible to use these columns at high flow rates without efficiency loss and it is not retained for capillary monolithic columns. To achieve a proper efficiency with monolithic capillary columns, one has to use in HPLC very low linear flow rates.[9,10] The need to use low linear rates of the mobile phase to achieve high efficiency indicates that the mass exchange in monolithic capillary columns is constrained, but the reason for this remains unclear.

One of the factors responsible for different properties of standard and capillary monolithic columns can be the difference in the structure of monoliths. Evidently, the temperature conditions of polymerization should depend on the diameter of the capillary in which the monolith is synthesized. It has previously been shown[11] that the structure of the monolith obtained is very sensitive even to slightest temperature changes during the synthesis.

The purpose of this study is to synthesize monolithic sorbents in capillary columns of different diameter and to disclose the effect of the structure of the formed monolithic sorbent on the separation properties of the chromatographic columns obtained.

Experimental

All monolithic columns are based on quartz capillaries having different internal diameters and the same (350 μm) external diameter. Prior to the synthesis of the monolith, the internal surface of the capillary was rehydroxylated with 1% hydrofluoric acid. Then the capillary was washed with deionized water and acetone, dried in a helium flow, and silanized. 3-(Trimethoxysilyl)propyl methacrylate was used for silanization, which was able to provide further covalent binding of the monolith with the capillary surface. The capillary was filled with a 30% (wt.) solution of 3-(trimethoxysilyl)propyl methacrylate in acetone, and the solution was kept inside the capillary for 24 h. Then the capillary was again washed with acetone and dried in a helium flow. The silanized capillary was used further for the preparation of monolithic columns.

A polymerization mixture was prepared by the dissolution of the initiator in a mixture of the monomer and pore-forming agent. Divinylbenzene (DVB) was used as the monomer. The pore-forming agent was a mixture of dodecan-1-ol and toluene (27 : 4, vol.). The initiator of the radical reaction was 2,2′-azodiisobutyronitrile (AIBN), whose content was 1 wt.% of the monomer amount. To carry out polymerization the quartz silanized capillary was filled with a polymer solution *in vacuo*, the capillary ends were sealed, and the column was placed in a

water thermostat. The polymerization temperature was 75 °C, and the polymerization duration was 1 h. After the end of polymerization, the column was washed with methanol to remove residues of the polymerization mixture and polymer particles, which are not chemically bound to the main structure of the monolith.

Capillary monolithic columns were tested under the conditions of liquid chromatography on an isocratic liquid chromatograph (Shimadzu, Japan) consisting of an LC-10AD high-pressure pump and an SPD-10A UV detector with a variable wavelength equipped with a capillary cell. A weighed sample was introduced with a dosing valve (Reodyne, USA) equipped with a 2-μL loop. Since the pump can push the mobile phase with a rate not lower than 10 μL min^{-1}, a flow divider, which split the sample in a ratio of ~1 : 50, was mounted in front of the column to decrease the mobile phase consumption and the volume of the injected sample. Data were collected from the detector using the ChromStar, version 4 software (SCPA GmbH, Germany).

Gas chromatographic experiments were carried out on an LKhM-8D chromatograph modified for the work under elevated pressure. A weighed sample was introduced with a dosing valve (Valco, USA) equipped with a 2-μL loop. In all experiments, the column temperature was 80 °C and a flame-ionization detector from the LKhM-8D package was used for detection.

All solvents used had purity grade not lower than analytical grade and they were used without additional purification. A solution of acetonitrile in water (3 : 1) served as a mobile phase.

Uracil was used as the non-retained sorbate in the determination of the dead time and dead volume of the column, and benzene and nitrobenzene served as test components. All reagents were analytical grade.

Divinylbenzene used for the preparation of the monolith was available from Merck (Germany) and contained 85% of a mixture of divinylbenzene isomers and 15% of its precursors (ethylstyrene, diethylstyrene).

Results and Discussion

All capillary columns were prepared for separations in the same manner for both GC and LC (they were washed, dried, *etc.*). The permeabilities of the monolithic columns in GC and LC were calculated by the Poiseuille—Darcy equations for compressible and incompressible mobile phases[2] (Table 1). In GC for the most part of columns the permeability value was well reproduced and lied around $(2.2\pm0.2)\cdot10^{-9}$ cm^2, indicating that the monolith structures are of the same type. At the same time, noticeably higher permeability values were obtained for three columns (0.53, 0.01, and 0.25 mm) in GC. The highest permeability was observed for the column that had the smallest diameter studied in this work (0.01 mm). This is consistent with the results,[13] indicating that for the monolithic columns prepared under identical conditions the column structure changes from the monolithic structure to the structure of columns of the PLOT type if the column diameter decreases from 0.05 to 0.01 mm and then to 0.005 mm.

Table 1. Geometric characteristics and permeability of the monolithic capillary columns studied in the work (standard deviations are given in parentheses)

Column diameter	Column length	$B^a\cdot10^9$/cm^2		$C^b\cdot10^3$/s	
		GC	LC	GC[c]	LC[d]
mm					
0.530	477	2.9(2)	1.7(5)	1.9(2)	171(10)
0.320	467	2.3(1)	1.6(4)	0.8(1)	29(5)
0.250	477	3.3(2)	1.7(5)	0.8(1)	17(4)
0.100	475	2.1(1)	1.4(4)	0.7(1)	13(2)
0.05	475	2.2(1)	1.2(3)	0.9(1)	16(4)
0.025	469	2.2(1)	0.8(3)	0.7(1)	18(4)
0.010	472	5.3(2)	0.3(3)	1.2(2)	—

[a] Permeability.
[b] Parameter of the Van-Deemter equation.
[c] *n*-Butane is the sorbate, nitrogen is the carrier gas, and the temperature is 80 °C.
[d] Nitrobenzene is the sorbate, acetonitrile—water (3 : 1) mixture is eluent, ~20 °C.

In LC the permeability of the same columns changed slightly with the diameter changing from 0.53 to 0.1 mm, but it decreased monotonically with the further decrease in the diameter from 0.1 to 0.01 mm (Fig. 1). The permeability of the columns determined in LC is always lower than that of the same columns in GC. Moreover, the column, which is most permeable in GC, was least permeable in LC (see Table 1). A specific feature of the formation of monolithic columns should be emphasized: their structure changes from monolithic to the structure of the PLOT type with a decrease in the capillary diameter. This process should increase the column permeability. At the same time, for open capillary columns and columns of the PLOT type the permeability decreases proportionally to the squared decrease of the capillary diameter.[2] Probably, the decrease in the capillary diameter and the change in the monolith structure with a change in the capillary diameter exert differently directed effects on the permeability of the column under the con-

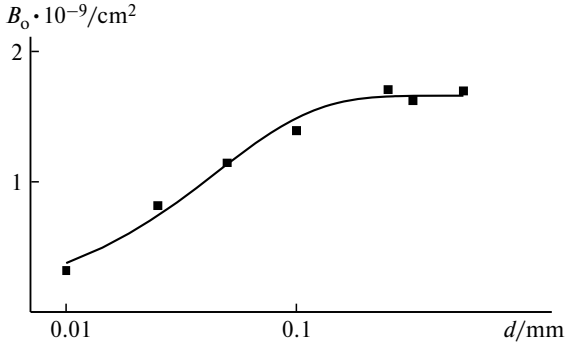

Fig. 1. Permeability of the monolithic capillary columns (B_o) *vs* column diameter (d) in LC; the abscissa is logarithmic.

ditions of GC and LC, which can explain the discrepancy observed.

The separation properties of chromatographic columns are usually characterized by the Van-Deemter dependence, which relates the height equivalent to a theoretical plate (HETP) to the linear flow rate of the mobile phase ($\bar{u}$). The Van-Deemter curves measured in GC and in LC for the columns of different diameter prepared in our group are shown in Figs 2 and 3, respectively. As can be seen for the presented plots, in GC, when *n*-butane is used as the sorbate, the least value of HETP of ~0.05 mm was observed for the column 0.32 mm in diameter, whereas for other columns in GC (except for the narrowest 0.01-mm diameter columns) the minimum HETP was in rather narrow range from 0.05 to 0.09 mm. In LC we were able to determine the minimum HETP value and the optimum value of the flow rate only for the column 0.25 mm in diameter, whereas for other columns we failed to observe a minimum in the Van-Deemter curve even at the minimally possible flow rate (see Fig. 3). For the same flow rate in all columns, a decrease in the column

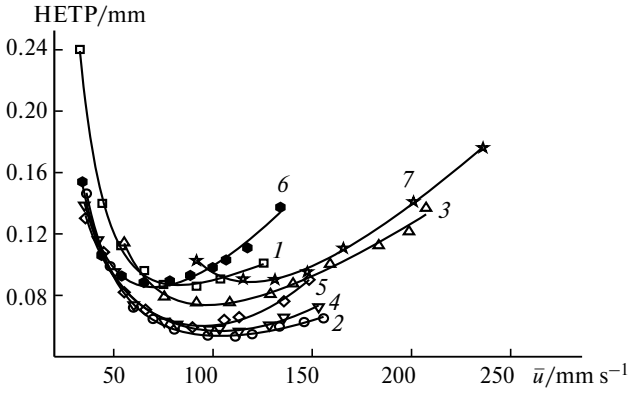

Fig. 2. The plots of the HETP *vs* average linear rate ($\bar{u}$) of the carrier gas for the monolithic capillary columns with the diameter 0.53 (*1*), 0.32 (*2*), 0.25 (*3*), 0.10 (*4*), 0.05 (*5*), 0.025 (*6*), and 0.010 mm (*7*) in GC. *n*-Butane is the sorbate, and nitrogen is the carrier gas.

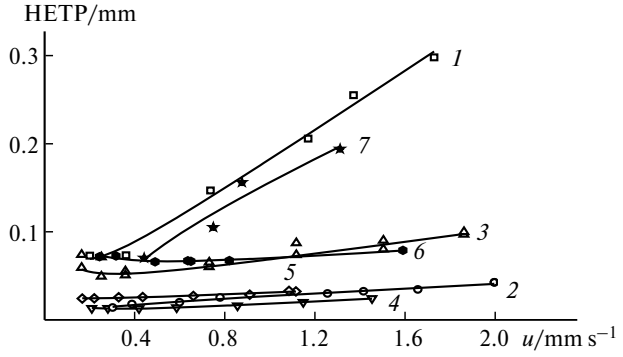

Fig. 3. The plots of the HETP *vs* linear rate of the mobile phase for the monolithic capillary columns of different diameter in LC. Nitrobenzene is the sorbate, and acetonitrile—water (3 : 1) mixture as eluent. For designations of the curves, see Fig. 2.

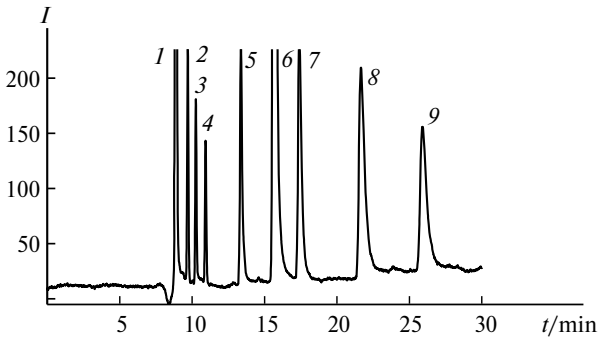

Fig. 4. Chromatogram of separation of a standard mixture of oxygen-containing compounds and aromatic hydrocarbons on the monolithic capillary column (diameter 100 μm, length 470 mm). Separation temperature 20 °C, UV detector (250 nm); sorbates: uracil (*1*), acetone (*2*), ethyl methyl ketone (*3*), ethyl acetate (*4*), dinitrobenzene (*5*), nitrobenzene (*6*), benzene (*7*), toluene (*8*), and ethylbenzene (*9*).

diameter from 0.53 to 0.1 mm decreases the HETP from 0.073 to 0.013 mm. The further decrease in the diameter of the columns induces an increase in HETP in LC (see Fig. 3). Since the efficiency of the columns remained unchanged upon a decrease in the amount of nitrobenzene injected in the column from 0.2 to 0.0015 μg, the absence of overloading and high loading capacity of the column are evident. It is most likely that the change in the minimum HETP reflects the difference in the structures of the monoliths obtained in capillaries of different diameters. In this case, the structure of the monolith should affect the mass transfer rate between the mobile and stationary phases, which is characterized by the *C* coefficient in the Van-Deemter equation.[2]

In GC, except for the largest (0.53 mm) and smallest (0.01 mm) capillaries, the *C* parameter for all columns was near $(0.8\pm0.1)\cdot10^{-3}$ s for *n*-butane. These values approximately an order of magnitude exceed the *C* parameter values for the packed columns[4] and indicate the high mass transfer rate in the monolithic sorbent. In LC the *C* coefficient is by one—two orders of magnitude higher than that in GC.

The higher mass transfer rate for the monolithic columns is due to specific features of the structure of the stationary phase. The monolith structure is formed by very fine (< 1 μm) primary particles, and transport pores inside the monolith that are in the size range of several micrometers.[5] This structure of the stationary phase favors the fast transfer of the sorbate from the mobile to the stationary phase and back.

Unlike open capillary columns, monolithic capillary columns are considerably less sensitive to a change in the column diameter, and in this parameter they are closer to packed columns. Monolithic columns with the optimum structure of the monolithic sorbent make it possible to carry out the fast and efficient separation of mixtures of compounds to be analyzed (Fig. 4).

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Received November 19, 2009;
in revised form June 15, 2010