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Determination of the porosities of monolithic columns by inverse size-exclusion chromatography

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Abstract

Retention data of polystyrene samples of narrow molecular size distribution and known average molecular mass were measured on several monolithic columns (Chromolith Performance, Merck) and one conventional packed column (Luna C_{18}, Phenomenex) by size-exclusion chromatography. These data were used to determine the external, the internal, and the total porosities of these columns. These data provided also information on the pore-size distribution of the adsorbent medium. The external and the total porosities of these columns are much higher than those of conventional packed columns. The results illustrate the profound changes brought by monolithic columns to the balance of the hydrodynamic and the mass transfer kinetic properties of chromatographic columns. Classical methods of comparison between column performance must be re-evaluated.

Keywords: Porosity; Monolithic columns; Inverse size-exclusion chromatography; Silica C_{18}; Polystyrene standards

1. Introduction

The recent invention and development of monolithic columns[3] is a major technological change in column technology, indeed the first original breakthrough to have occurred in this area since Tswett invented chromatography, a century ago [1]. This new process of manufacturing columns holds great promises of further improvements of the performance of analytical and, possibly, preparative separations. There are now two very different methods of preparing monolithic columns, by synthesis of an appropriate polymeric skeleton [2–5] or by preparation of a suitable silica monolith [6–9] that can be later, if desired, derivatized by following one of the classical approaches for the preparation of silica bonded phases. While the former approach was the first to be tackled successfully, the latter one seems at present to give more practical results and was the first to achieve broad commercial diffusion.

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[3]These columns are sometimes called rod columns, silica rod columns, or continuous porous silica columns. There are many different preparation recipes, most proprietary, patented, and, for silica columns at least, difficult to reproduce.

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Monolithic columns are made of one single piece of an adsorbent material (porous silica or polymer) that fills the entire length and width of the column. This piece of adsorbing material contains two interconnected networks of pores, the macropores and the mesopores. The macropores, also called the throughpores, have dimensions in the 1.5–2-μm range. Their network provides flow paths through and along the column and ensures access of the sample molecules to the whole network of mesopores. The density of the macropore network causes monolithic columns to have a high external porosity. This high porosity combined with the relatively large value of the average size of these pores gives to monolithic columns a high permeability [9–13]. There is a consistent agreement in the literature suggesting that the macropore network accounts for approximately 80% of the total porosity. The mesopore network represents 10–15% of the total porosity. Their average size is generally between 10 and 20 nm. The specific surface area of the monolith is essentially accounted for by that of the mesopore network [6,14]. A small percentage of the total porosity corresponds to micropores [10,15].

The combination of the properties of these two pore networks gives monolithic columns unusual hydraulic, thermodynamic and kinetic characteristics. Because the specific surface area of the mesopores is similar to that of conventional porous particles, the retention factors observed are of the same order of magnitude. However, the large porosity contribution of the macropores gives to these columns a higher permeability and a higher efficiency than those of the conventional columns made of packed particles. Accordingly, monolithic columns of length and efficiency comparable to those of conventional columns may be operated at higher velocities to carry out faster analyses. Alternately, far longer, hence more efficient columns can be operated by connecting several monolithic columns than would be possible using the conventional particle technology. These long column series enable the relatively easy achievement of extremely high column efficiencies [3,15,16].

The investigation of the properties of monolithic columns is complicated by the characteristics of the macropore network. This network does not have a scale that is determined by the size of the packing particles. The classical correlations between the bed properties (external porosity, permeability, column efficiency) and the particle size are no longer valid. The external porosity of the column or porosity of the macropore network is no longer close to 0.40 as it is in particulate beds. The important properties of a column must be measured separately, which raises new problems.

Monolithic columns could be studied with the same theoretical and experimental methods than conventional chromatographic columns. In principle, their porosity could be determined by measuring the amount of nitrogen sorbed on the monolith at the temperature of its atmospheric boiling point as a function of the partial pressure of nitrogen [17]. Unfortunately, the monolith has dimensions that are not compatible with those of conventional nitrogen-sorption instruments. In spite of the high permeability of the monolithic column, there will be a significant pressure difference between the column inlet and outlet, hence the isotherm data will not be measured at constant pressure, causing serious difficulties in the interpretation of the data. Mercury porosimetry also could give the size distribution of the mesopore and macropore networks [17]. However, the column tubing cannot withstand the required pressure and the monolith is not compatible with the sample holder of available instruments. More modern methods, such as small-angle X-ray scattering, neutron scattering, nuclear magnetic resonance, coulometric measurements, and MRI, are well suited for investigations of the properties of particulate matter. They are difficult to apply to the study of the properties of large monoliths. Admittedly, the monolith can be removed from the column, broken into pieces of adequate size, and investigated using any one of these methods. This approach is often used by those who produce monoliths. It can give accurate results provided the powder of the monolith is sufficiently coarse. The external porosity of this powder is the same as that of conventional particles, so a third mode is introduced to the material porosity distribution. This mode must be well separated from the other two that we want to investigate. Yet, a major drawback subsists; the analyst, unfortunately, cannot use the destroyed column anymore.

Among the conventional methods of determination

of the column porosities, only inverse size-exclusion chromatography (ISEC) [17,18] can be used conveniently with monolithic columns. We used this method to measure the porosities of several monolithic columns and report here on the results obtained.

2. Theory

Size-exclusion chromatography (SEC) is one of the modes of application of liquid chromatography. It differs from all the other chromatographic modes because, in SEC, the separation does not depend on chemical interactions, but it depends on a physical sieving process (i.e., on the molecular volume of the analytes) [19–23]. It is based on the influence of the ratio of the average size of a pore and of the size of the solute molecule on the entropy of this solute in the mobile phase. When this ratio is large, the solution behaves similarly in the pore and in the bulk. When the ratio becomes small, the entropy of the solute is lower in the pore than in the bulk and the solute tends to be excluded from the pores, i.e., its equilibrium constant becomes lower than 1. SEC is used to separate mixtures of macromolecules, e.g., of polymers, after their molecular size. Although this size is correlated to the molecular mass for compounds of a given structure, the primary factor controlling retention is actually the ''hydrodynamic volume'' of the molecules. The compounds with larger molecules are excluded from a larger fraction of the pores than those with smaller molecules. Therefore, they are eluted earlier. Correlations have been derived between the retention data in SEC and the molecular size.

Conversely, inverse size-exclusion chromatography uses these correlations (see later) to derive information on the structure of the pores of the packing material from the retention data of a series of known probe compounds, e.g., samples of polymers of narrow molecular mass distribution and known average molecular mass [18]. This simple principle was used to determine the external, total and internal porosities of columns by injecting the members of a long series of standard polymers (e.g., standard polystyrene samples) of narrow molecular mass distributions [17].

The use of ISEC requires that pure size-exclusion data are acquired. There should be no adsorption of the probe compounds on the surface of the adsorbent studied. SEC should be carried out with a mobile phase that is at least as strongly adsorbed as the probe samples used. Thus, it is possible, in principle to use either biochemical polymers in an aqueous solution or polystyrene gels and THF or dichloromethane as the mobile phase. Furthermore, the equilibrium between the mobile phase held within the pores of the adsorbent and the stream of mobile phase percolating through the column should be established rapidly (i.e., the column efficiency under the experimental conditions selected must be high). Finally, the injected sample should be injected rapidly to avoid peak broadening and its size should be small so that the column is not overloaded; the elution peak should have a nearly Gaussian profile, with a low asymmetry factor; the matrix of the adsorbent should be rigid and should not shrink or swell when the eluent is changed [18]; and the temperature and the mobile phase flow-rate must remain constant during the whole experiment.

The total porosity (ε_T) of the column, its external porosity (ε_e), and the internal porosity of the adsorbent (ε_i) can be determined from the ISEC data. These three porosities are defined as follows:

$$\begin{aligned} \epsilon_T &= \frac{V_T}{V_g} \\ \epsilon_e &= \frac{V_e}{V_g} \\ \epsilon_i &= \epsilon_T - \epsilon_e \end{aligned} \tag{1}$$

where V_T is the retention volume of an unretained tracer, usually assumed to be that of the smallest injected molecule (in this work, we used toluene ($M=92$ g/mol) or benzene ($M=78$ g/mol) for this purpose), V_e is the retention volume of the excluded molecular mass (see below), and V_g is the geometrical volume (or empty separation volume) of the column. Since the column can be considered as a long cylindrical tube of radius r, its geometrical volume is given by:

$$V_g = \pi r^2 L \tag{2}$$

where L is the column length. The retention volume

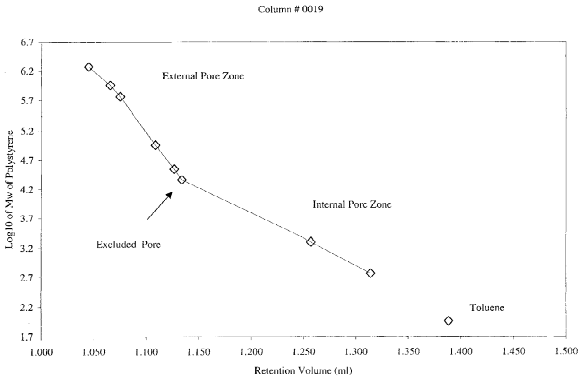

Fig. 1. ISEC plot or plot of the logarithm of the molecular masses of the polystyrene standards versus their retention volume for the monolithic Chromolith Performance column UM 0019.

of the excluded molecular mass is the volume corresponding to the intersection point of the interpolated straight lines corresponding to the internal and the external pore zones in the calibration curve (see Fig. 1).

ISEC allows also the determination of the pore-size distribution. Let us define as the volume V_n, the fractional volume of the pores that have a size equal to or larger than ϕ_n, n being the rank of the polymer standard used (in order of increasing or decreasing molecular mass). Similarly, the fractional volume of the pores that have a size equal to or larger than ϕ_{n+1} is $V_{n+1}(\phi_{n+1} > \phi_n)$. This means that the fractional volume of the pores that have a size larger than ϕ_n and smaller than ϕ_{n+1} is given by:

$$\Delta V_{n+1,n} = V_{R,n+1} - V_{R,n} \quad (3)$$

where $\Delta V_{n+1,n}$ can be obtained easily from the ISEC data [18]. In order to relate the molecular mass M_w of a polystyrene sample and the size of the pores from which it is just excluded, we used the following correlation:

$$M_w = 2.25\phi_n^{1.7} \quad (4)$$

where the pore size diameter, ϕ, is in Å [17,18,23].

3. Experimental

3.1. Equipment

The measurements were carried using an HP 1100 Liquid Chromatograph (Hewlett-Packard, Palo Alto, CA, USA), equipped with a manual sample injection system, a solvent delivery system, a mobile phase degasser, a temperature-controlled column compartment, and a variable wavelength UV detector. This equipment operates under the control of a ChemStation computer software and has a computerized data acquisition system. The main feature of this instrument is the high stability and the accuracy of the solvent delivery system that allows the on-line preparation of mobile phases of the requested composition and its delivery at a constant flow-rate.

3.2. Columns

Six monolithic columns (serial # UM 0019-0024, 10×0.46 cm) were made available to us (Merck, Darmstadt, Germany). Each column is filled with a porous silica monolith, wrapped inside a PEEK tube using a proprietary process that avoids leaks between the tube wall and the monolith. The silica surface in this column was bonded to a monomeric C_{18} layer obtained by reaction with monofunctional octadecylsilanes, using a proprietary in situ surface-modification process.

For comparison purposes, we carried out the same measurements on a conventional 25×0.46 cm column packed in the laboratory with 10-μm particles of Luna Prep Silica C_{18} (Phenomenex, Torrance, CA) [24].

3.3. Chemicals

Polystyrene standards with molecular masses ranging from 2000 to 1 860 000 were purchased from Supelco (Bellefonte, PA, USA). Polystyrene standards with molecular masses ranging from 550 to 2000 were purchased from Scientific Polymers Products (Ontario, NY, USA). Tetrahydrofuran was used as the mobile phase in analytical measurements, acetonitrile as the washing solvent, and toluene as a small molecule tracer. These compounds were all HPLC grade and purchased from Fisher Scientific (Suwanee, GA, USA).

3.4. Procedures

Samples of 25 μl of each individual polystyrene standard dissolved in the mobile phase were manually injected into each column, at a flow-rate of

1.0 ml/min. Each analysis was repeated three times successively and each result or data point reported later in this work is the average of the three retention times measured. A 25-μl sample of toluene was also injected, to determine the total accessible porosity of the column [17,18]. All the chromatograms were recorded at the wavelength of 254 nm at which the response for polystyrene was satisfactory. All the peaks were symmetrical. The retention time or volume corresponding to each injection was determined from the peak maximum [18]. All retention volumes were corrected for the extra-column volume of the equipment (0.67 ml).

For each column, the logarithm of the molecular mass of each polystyrene standard was plotted versus the retention volume of its peak. The graphs obtained, referred to later as the ISEC plots, consist of two nearly straight lines, showing that there is a bimodal pore size distribution. These two lines correspond to the macropore or external pore zone (the steeper line) and to the mesopore or internal pore zone (the less steep line). The intersection point of the two straight lines gives the excluding pore diameter or diameter of the largest pores of the mesopore network. The polystyrene molecules that have a diameter smaller than the excluding pore diameter are separated depending on the fraction of the mesopore network to which these molecules have access. Those that have a larger diameter are similarly separated in the macropore network. The excluding pore diameter is the (artificial) boundary between these two pore networks. The fractional volume of the pores in the macropore network is the external porosity, that of the mesopores, the internal porosity.

Chromatographers report the internal porosity (ε_i^{Chrom}) as the fraction of the column volume that is occupied by the pores contained inside the particles. Chemical engineers report this porosity (ε_i^{ChemE}) as the fraction of the particle volume that is occupied by the pores contained inside the particles. Obviously, they are related by $\varepsilon_i^{Chrom} = (1 - \varepsilon_e)\ \varepsilon_i^{ChemE}$. We followed here the chromatography definition.

4. Results and discussion

4.1. Column porosities

The ISEC plots obtained for all the monolithic columns studied are nearly identical in shape and quantitatively very close to the one shown in Fig. 1. Each plot includes two series of data points, grouped around two separate straight lines. These two lines correspond to the two different types of pores, the external and the internal pores. The two pore types are probed and scaled by polystyrene standards of different molecular masses, as explained earlier. The steeper line, with five data points, corresponds to the macropores. The other line has three data points, toluene being excluded because it can penetrate all the pores. It corresponds to the internal pores. The external porosity of the column corresponds to the excluded molecular mass (see data in Table 1) and is derived from the coordinates of the intersection point of the two straight lines.

The coefficients R^2 of the linear regressions of the different sets of data obtained with all the columns were equal to 0.985 or larger. The total porosity of the column is derived from the retention volume of toluene. The internal porosity is derived from the difference between total and external porosities. A similar result was obtained for the particulate column (Fig. 2). The essential difference is only in the numerical values of the porosities.

The values obtained for the total (ε_T), the external (ε_e), and the internal (ε_i) porosities of all the columns are reported in Table 2. The values of the total and external porosities of the monolithic columns, and particularly that of the external porosity, are large, much larger than the typical value obtained for the conventional packed column that is most similar to those used traditionally in HPLC [12,25,26]. In conventional columns, the external porosity is typically 0.40, with values ranging between 0.37 and 0.42 [25], and rarely exceeding 0.45, a value that is achieved only for some special

Table 1
Pore diameter (ϕ) corresponding to the excluded molecular mass

	ϕ (Å)	RSD %	M_w (g/mol)	RSD %
# 19	223±6	2.6	21 700±200	0.90
# 20	279±15	5.5	33 000±150	0.50
# 21	204±5	2.3	18 100±200	1.10
# 22	217±6	2.9	19 900±160	0.85
# 23	208±6	2.9	19 400±120	0.65
# 24	255±10	4.0	29 800±190	0.65
Luna	220±7	3.2	21 800±1000	4.7

Mobile phase, THF; flow-rate, 1.0 ml/min on all columns.

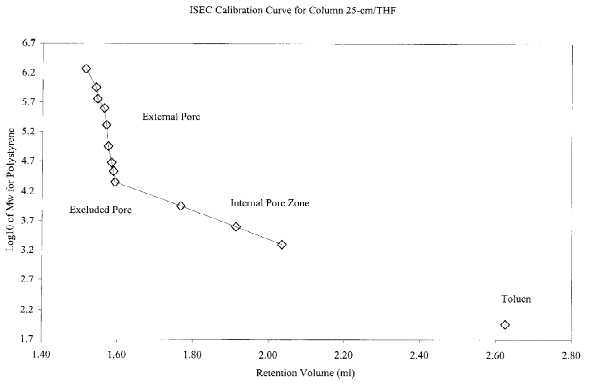

Fig. 2. ISEC plot or plot of the logarithm of the molecular masses of the polystyrene standards versus their retention volume for the classical Phenomenex column.

purposes [27], and more often with narrow bore columns than with standard ones. The result obtained with the single packed column studied here, at $\varepsilon_e = 0.38$ (Table 2) is in agreement. By contrast, the porosity of the macropore network, which in monolithic columns plays the same role as the external or interparticle pore network does in packed columns, is typically around 0.70 or even, but exceptionally, larger [3,8–14,28]. These high values are consistent with those measured using a different method on the same columns [13,28]. They will be explained in the next section.

4.2. Pore-size distributions of the monolithic column

There are different methods available to report the pore-size distributions of the various columns studied [29]. In this work, we express these distributions as histograms of the pore-size diameters versus the volume fraction [18]. ISEC assumes an empirical relationship, Eq. (3), between the diameter of the largest pores into which a molecule can penetrate and the diameter of this molecule [17,18]. This result is consistent with our understanding of the mechanism of SEC [17]. By applying Eq. (3) to the retention volumes of the polystyrene standards studied, we can determine the volume of the pores having a range of diameters into which the molecules of polystyrene standards can fit. The retention volume of toluene is considered as equal to the total pore volume, V_T. Because of its small molecular volume, this compound can penetrate into almost all the pores. Accordingly, the volume fraction will be reported as $\Delta V_{n+1,n}/V_T$ (%).

The larger the number of polystyrene standards that are used in a porosity study, the narrower and the more accurate the definition of the pore distribution obtained will be. It is particularly important to use a wide range of molecular masses to achieve a reasonably accurate determination of the volume fractions of the micropores ($\phi < 15$ Å), the mesopores ($15 < \phi < 500$ Å), and the macropores ($\phi > 500$ Å) of the column.

Unfortunately, the range of molecular mass of the standards available is limited and their molecular mass range is relatively wide. This restricts the amount of information that can be derived using the ISEC method. For the lack of suitable standards, we were unable to obtain sufficient information in the transition range between the micropore and the mesopore domains, i.e., in the size range between 0.9 and 2.6 nm.

Fig. 3 shows the pore size distribution (PSD) for one of the monolithic columns, UM # 0019-0024. The results obtained with the other ones are very similar, as expected [12], and differ essentially by small variations in the proportions of pores having a

Table 2
Total (ε_T), external (ε_e), and internal (ε_i) porosities of the monolithic columns and their respective relative standard deviations

	ε_T	RSD %	ε_e	RSD %	ε_i	RSD %
# 19	0.830±0.01	1.2	0.685±0.011	1.5	0.145±0.001	0.70
# 20	0.855±0.01	1.2	0.678±0.011	1.5	0.176±0.001	0.60
# 21	0.826±0.01	1.2	0.688±0.010	1.4	0.138±0.001	0.70
# 22	0.857±0.02	2.3	0.713±0.020	2.8	0.143±0.001	0.70
# 23	0.850±0.01	1.2	0.706±0.020	2.8	0.142±0.001	0.70
# 24	0.868±0.02	2.3	0.688±0.011	1.4	0.180±0.002	1.10
Luna	0.617±0.01	2.7	0.386±0.023	6.0	0.238±0.012	5.0

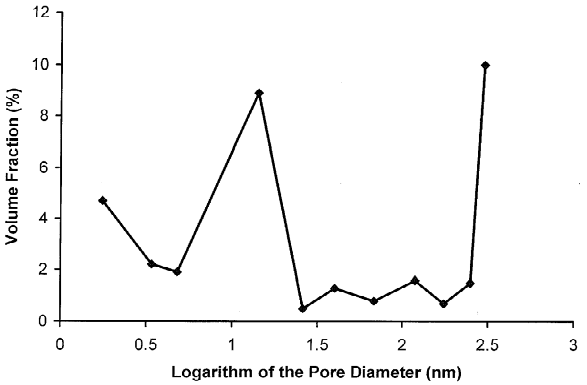

Fig. 3. Average pore size distribution for the monolithic Chromolith Performance column UM 0019. Fractional volume of pores above 300 nm, 76%.

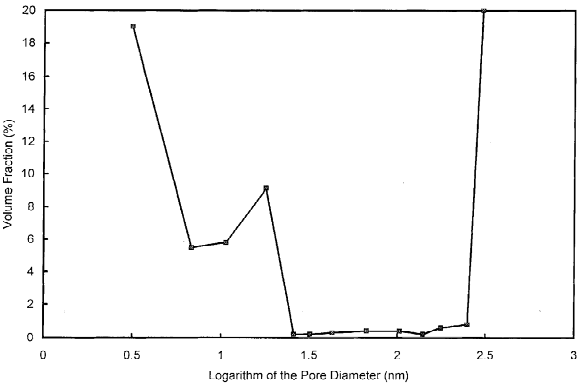

Fig. 4. Average pore size distribution for the classical Phenomenex column. Fractional volume of pores above 300 nm, 58%.

certain size range. These differences are in part actual, in part the results of the errors of measurements. Table 3 reports the incremental pore size distributions of the six monolithic columns studied. A large volume fraction (75–80%) of all the monolithic columns is composed of large macropores having a diameter of 0.3 μm or larger, a result that is in agreement with their large external porosity. In addition, there is a small fraction of pores in the range between 50 and 300 nm (ca. 3%) that still qualify as macropores. The volume fraction of the micropores is probably less than a few percent but a more precise figure cannot be obtained at this stage. The volume fraction of the mesopores is of the order of 10–12%. The volume fraction of the micropores and mesopores is in agreement with the value of the internal porosity of the monoliths.

Fig. 4 shows the PSD for the conventional column. The striking difference between the PSDs of the monolithic and the conventional columns is in the external porosity. It is nearly twice larger in the former than in the latter columns. The network of silica walls in the column bed has been reduced considerably. The specific surface area is 300 m^2/g [7]. A 10×0.46-cm column with a total porosity of 85% contains 0.25 ml of silica or approximately 0.65 g, for a total surface area of 195 m^2. Yet, the advantages of the monolithic columns arise from their very high porosity.

4.3. Figure of merit of monolithic columns

The essential difference between monolithic and particulate columns is in the nature and structure of the macropore network. The internal pore or meso-

Table 3
Incremental pore distribution of the monolithic columns

M_w (g/mol)	ϕ (Å)	Range (Å)	# 19	# 20	# 21	# 22	# 23	# 24	Luna
92	9	9–26	4.68	3.08	4.88	4.78	4.75	7.98	27.2
590	26	26–42	2.21	2.03	2.04	2.18	2.12	1.94	
1300	42	42–54	1.92	2.03	1.97	1.90	1.98	1.87	
2000	54	54–228	8.92	2.10	8.75	8.36	8.50	8.33	8.2
23 000	228	228–292	0.54	2.29	0.51	0.56	0.50	0.83	4.2
35 000	292	292–509	1.31	4.52	1.24	1.19	1.42	1.32	0.5
90 000	509	509–862	0.83	0.46	0.80	0.98	0.85	0.90	0.2
220 000	862	862–1517	1.60	1.51	1.53	1.26	1.35	1.39	1.0
575 000	1517	1517–1974	0.69	0.59	0.80	0.84	0.64	0.62	0.2
900 000	1974	1974–3025	1.49	0.46	1.68	1.55	1.84	0.76	1.0
1 860 000	3025	3025–	75.78	80.94	75.80	76.39	76.06	74.05	57.7

pore network appears to be quite similar in both type of columns because the synthesis of the porous silica and the chemical nature of the adsorbent obtained are very similar. In conventional columns, the macropores are the channels between particles. These channels are very tortuous and have a high degree of constriction: they expand and narrow at the scale of a particle diameter. This characteristic explains the high hydraulic resistance of particulate beds [30–32]. Macropores in monoliths seem to have a far smaller degree of constriction; hence, for similar average dimensions would present a lower hydraulic resistance. To compare the two types of columns and their performance, essentially the permeability and efficiency of a bed of a given length, we must resort to a simple model. Let us assume that the network of macropores can be considered as a bundle of parallel tubular channels. In packed columns, these channels are interconnected, at a length scale that is of the order of a particle diameter.

In monolithic columns, there is also a strong degree of channel anastomosis. As a first approximation, we will neglect the influence of this property on the characteristics of the bundle of channels.

The flow-rate through a packed bed is given by:

$$F_v = uS = \frac{k_0 d_p^2 \Delta P}{\eta L} \pi R_c^2 \epsilon_e \quad (5)$$

where u is the mobile phase velocity, S the cross-section area available to the mobile phase in the column ($S = \pi R_c^2 \varepsilon_e$), k_0 the permeability coefficient, d_p the diameter of the particles in the packed column of radius R_c, ΔP the inlet pressure, and L the length common to the column and to the equivalent bundle of capillaries, η the mobile phase viscosity, and ε_e the external column porosity. The flow-rate through the equivalent bundle of n capillaries of radius r_c is:

$$F_v = uS = n\pi \frac{r_c^4 \Delta P}{8\eta L} \quad (6)$$

Hence, equaling these flow-rates, we have a relationship between the particle size, on the one hand, the diameter of the capillary and their number on the other hand:

$$r_c^4 = \frac{8 k_0 d_p^2 R_c^2 \epsilon_e}{n} \quad (7)$$

A second relationship results from the condition that the volume of the two bundles be equal. Hence,

$$\pi R_c^2 L \epsilon_e = n \pi r_c^2 L \quad (8)$$

Proper combination of these two equations gives:

$$r_c = \sqrt{8 k_0} d_p \quad (9)$$

In practice, the permeability coefficient is approximately $k_0 = 0.001$. Thus, the diameter of the capillary in the bundle is approximately $2r_c = 0.18 d_p$ and the number of capillaries in the bundle is $n = (R_c^2 \varepsilon_e)/(8 k_0 d_p^2)$.

As an approximation, we can assume that the macropores in a monolith are about as tortuous as those in a packed column but much less constricted. So, we assume that their diameter is nearly constant. The number of capillaries in the bundle is proportionately larger since the column external porosity is larger and this will account for part of the difference in flow-rate that is obtained through a monolithic and through a particulate column when their inlet pressures are the same. The comparison between the performance of the two types of columns is summarized in Eq. (9). Because the macropores of the monolith are little constricted, the hydraulic resistance of the monolithic column is much less than that of the conventional column. A column packed with 11-μm spherical particles would have about the same hydraulic resistance than a monolith having macropores with an average diameter of 2 μm. The results of this simple model agree well with our observations. On the other hand, because the external porosity of the monolith is twice as large as that of the conventional column, the density of capillaries in the bundle is much higher, hence the apparent thickness of the porons or blocks of silicagel between adjacent channels is markedly smaller. This explains the low values of the column HETP that are observed [6–16].

5. Conclusion

A fast, easy, direct, and inexpensive method to determine some important properties of chromatographic columns, inverse size-exclusion chromatography, has been successfully applied to monolithic

columns. The results obtained with six monolithic columns confirm that these columns are highly reproducible [12] and have values for the total and the external porosities that are much larger than those observed for conventional particulate columns. The external porosity is almost twice as large for the monoliths as for the beds of particles. The internal porosity of a monolithic column appears smaller than that of a conventional column (0.15 for the Chromolith Performance from Merck versus 0.24 for the Luna C_{18} column). This is essentially explained by the fact that these values are given using the chromatographer's convention ($\varepsilon_T = \varepsilon_e + \varepsilon_i$). If we use the chemical engineer definition (see end of Section 3), we obtain values that are much closer, 0.40 and 0.48, respectively. This confirms our assumption that the poron blocks are made of porous silica with similar properties in both column types.

The results in this work confirm our earlier conclusions [12], that the monolithic columns appear to be the ultimate perfusive columns with an exceptional potential to produce columns offering a markedly improved compromise between two opposite sets of requirements. First, in order to achieve a high column efficiency, we need silica porons that have a small dimension, so mass transfer inside and out of these porons be fast because it is in the porons that retention, hence separation, takes place. On the other hand, we need columns that have a low hydraulic resistance because flow-rates under high pressure are costly.

As illustrated by the history of the progress of HPLC in the last third of a century, packed columns offer little room for compromise. Monolithic columns do not seem to offer more room for compromise but there are several areas in which some progress is possible. First, a further increase in the external porosity is possible, although we may not be now far from the optimum. Second, as in packed columns, the distribution of the density of macropores should be as homogeneous as possible across the monolith. It definitively must be so in the radial direction. Otherwise, the band is warped during its migration along the column and, in practice, this has the same results as back-mixing.

Hard data on radial homogeneity of monoliths are missing. However, a comparison between the available data on the average size of the porons and the values reported for the column HETP suggests that monolithic columns should be markedly more efficient that they are.

Finally, preparative chromatography dearly needs monoliths. We hope that it will be possible in the near future to solve the difficulties encountered in the synthesis of large monoliths.

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