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Study of physico-chemical properties of some packing materials. I. Measurements of the external porosity of packed columns by inverse size-exclusion chromatography

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Abstract

Previous work has shown that the beds of packing materials in conventional packed columns consolidate to a marked extent during an initial period. A detailed investigation of this phenomenon requires the development of a procedure for the accurate and precise determination of the external porosity of columns. This data can be obtained by inverse size-exclusion chromatography. We used a series of polystyrene standards with molecular masses ranging from 456 to 20 600 000 and injected them into four series of five chromatographic columns packed with Kromasil, Vydac, YMC, and Zorbax, respectively (all 10-μm particles of C_{18} bonded silica). The internal and external pore volumes (hence, porosities) of the columns can easily be determined with accuracy from the plots of the logarithm of the molecular mass of the probes versus their retention volume. This procedure offers a simple, fast, and convenient method for the determination of the external porosity of columns. This porosity correlates with retention data and equilibrium isotherms.

Keywords: Stationary phases, LC; Porosity; Pore-size distribution; Inverse size-exclusion chromatography

1. Introduction

The practical importance of the pores located inside solid materials has long been recognized [1–19]. Porosity plays an active role in the performance of packed beds [1], construction materials [2,6], chemical reactors [3,8], membranes [4,12], gas chromatographic columns [7], filtration systems [10], or in the choice of machining direction [18], etc. Various studies have been carried out on porous materials, both theoretically [20–24] and experimentally [11,25–29]. Much effort has been devoted to the control of the pore-size distribution of solid substrates [30–34]. It is only recently that the importance of pore-size distribution on the performance of stationary phases for gas or liquid chromatography has been recognized as being profound. One good example is the development of 'perfusion' chromatography [35–38] and the attention given to the related issue of pore connectivity [39]. The detailed investigation of the influence of the pore-size distribution on column performance requires a convenient, accurate and rapid method for its determination.

Various methods have been used or proposed to determine the pore volume or the pore-size dis-

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tribution [40–56]. The most common and traditional methods of porosity determination are nitrogen BET [40], mercury porosimetry [41], the K method [45], and the flow method [46]. These methods have a limited accuracy [44,57] or may damage the sample [58]. In recent years, new methods such as neutron scattering [48,49], capillary condensation hysteresis [50], nuclear magnetic resonance [52], coulometric measurement [53], X-ray [54], and MRI [56] have been proposed. However, these methods are still at the development stage.

Among the numerous methods used for the porosity determination which are listed above, none uses a liquid chromatographic technique. This is a serious inconvenience for the determination of the internal or external porosity of chromatographic columns. An additional instrument is required if any of the above methods is to be used. The result may not be accurate because the pore-size distribution measured from bulk packing material may be quite different from that of a packed liquid chromatographic column [59]. This is especially true for the external pore-size distribution.

In 1975 Halász and co-workers [60–65] introduced the idea of using inverse size-exclusion chromatography to determine the porosity inside a liquid chromatographic column. In size-exclusion chromatography (SEC) [66–68], sample mixtures that are dissolved in the mobile phase/solvent can be separated according to their different molecular sizes. Large molecules can only enter large pores; therefore they have a shorter elution time than small molecules, which are able of entering small pores. Classical SEC uses the known pore structure of a selected adsorbent to determine the molecular mass distribution of a polymer mixture. Conversely, if the molecular mass of a series of polymer standards is known, we can determine the pore-size distribution of an unknown stationary phase from the distribution of their retention times. For our purpose, the advantage of this method is that it operates simply on the very column investigated. The same instrument is used as for conventional HPLC studies. Compared with the traditional porosimetry methods, this procedure is less time-consuming and gives more accurate and dependable results.

Knox and co-workers [69,70] showed that it is possible to convert the calibration graphs to pore-size distributions following a complex procedure. For our purpose, however, the semi-empirical method of Halász and Martin [61] was chosen. As it constitutes a mere linear transformation of the calibration graph, the resulting distributions are too broad and the mean pore size determined may not agree well with the one determined by other methods. However, the procedure is simple, practical, and convenient to compare columns which differ by their degree of consolidation.

In their study, Halász and Martin [61] applied inverse SEC to the determination of the internal porosity of the column packing. In our current work, however, we are more interested in the column external porosity. In recent studies [71], we have shown that the deviation between the equilibrium isotherms measured on columns packed with the same ODS phase can be corrected by the ratio of the respective column external porosities. This means that we can derive the equilibrium isotherm for a preparative-scale liquid chromatographic column by normalizing the equilibrium isotherm obtained on an analytical column packed with the same stationary phase by the ratios of their external porosities. The significance of this work is important for applications in preparative chromatography.

2. Theory

The principle of our method of determination of the external porosity of a column is an extension of the procedure described by Halász and Martin [61] for the determination of the internal porosity of packing materials. Conventional size-exclusion chromatography (SEC) consists in separating the components of a polymer sample after their molecular mass on a column packed with an adsorbent whose volume pore-size distribution is known to encompass the molecular volumes of the sample components [67,68]. Provided that standard samples of known molecular masses derived from a known monomer are available for calibration, experiments show that there is a correlation between the average molecular mass (or the degree of polymerization) of these polymers, M_{r_n}, and the average diameter of the pores from which they are excluded, ϕ_n, assuming that all

the polymeric chains remain in the same conformation of a random coil [67]. For polystyrene dissolved in methylene chloride, we have [61]:

$$M_{r_n} = 2.25\phi_n^{1.7} \quad (1)$$

where ϕ_n is given in angstrom (Å). This correlation is based on the fact that the access of molecular coils into pores is limited by steric hindrance caused by the wall [72]. The retention volume of macromolecules of increasing size decreases as the proportion of the column porosity which corresponds to the volume of the layer against the pore wall having a thickness equal to the molecular radius. For each packing material, there are two thresholds, a low and a high one. Molecules larger than the high threshold have no access to any significant fraction of the internal porosity; they are totally excluded from it. Molecules smaller than the low threshold have access to the entire pore volume; they are not excluded. Molecules of intermediate size have access to part of the pores, as indicated by Eq. 1. The validity of this correlation relies on the assumption that the polymer samples used, although having different molecular masses have the same structure and that the same solvent is used.

Conversely, in inverse SEC solutions of known polymeric samples are injected into a column packed with an unknown adsorbent. The retention behavior of these solutes can be correlated with the pore-size distribution of the packing material. For polystyrene samples eluted by methylene chloride through a bed of chemically bonded C_{18} silica, the correlation is obtained by solving Eq. 1 for ϕ_n. It becomes [61]:

$$\phi_n \simeq 0.621(M_{r_n})^{0.588} \quad (2)$$

Thus, inverse SEC will provide the pore-size distribution of the sample of adsorbent studied. If we assume that all the pores that have a size equal to or larger than ϕ_n have a volume V_n and that all the pores that have a size equal to or larger than ϕ_{n+1} have a volume V_{n+1} ($\phi_{n+1} > \phi_n$), the volume of the pores that have a size larger than ϕ_n and smaller than ϕ_{n+1} is given by:

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

$\Delta V_{n+1,n}$ can be derived from the inverse SEC data. We have to note, however, that axial dispersion and mass transfer resistance are omnipresent in chromatography and blur the data derived from ISEC. Furthermore, the derivation of a pore-size distribution (psd) from the retention volume behavior of a series of polymeric samples of different molecular weights is a convolution problem which do not have a unique solution. The use of a large number of polymeric samples would improve significantly the probability of achieving a reasonably close approximation of the psd, as indicated by Eq. 3.

The total volume of a liquid chromatographic column, V_k, can be written as the sum of three contributions:

$$V_k = V_e + V_i + V_U \quad (4)$$

where V_e is the inter-particle, interstitial, or external pore volume; V_i is the internal pore volume; and V_U is the unaccessible volume. This last volume can be split into three different contributions: V_s, the stationary-phase solid volume; V_c, the closed pore volume; and V_a, the volume of the layer of C_{18} bonded chains. These three volumes, which are being discussed separately [73], can be lumped together into the unaccessible volume, V_U, for all practical purposes of this study except for the calculation of the apparent density of the packing.

In order to use inverse SEC to determine the porosity of chromatographic columns, the following conditions must be met [61]. (1) There is no adsorption, whether irreversible or reversible, of the polymeric samples used on the stationary phase studied. (2) The standard polymer molecules remain discrete and do not agglomerate in the solvent used as mobile phase. (3) There is instantaneous, quasi-reversible equilibrium between the two phases during the whole experiment. (4) The elution peak profile is close to Gaussian, with an asymmetry factor smaller than 2. (5) The stationary phase studied is rigid. (6) The mobile phase flow-rate is constant (±1%).

To satisfy the first two requirements, good solvents of polystyrene, such as methylene chloride or tetrahydrofuran, should be used. Polystyrene samples do not aggregate in the dilute methylene chloride solutions used in this work [67]. To satisfy the third requirement, the columns studied should have a minimum efficiency. Although the value of this threshold is arguable, the columns used in the

present work having an efficiency in excess of 2500 theoretical plates [71] do meet this condition. The peaks obtained in this work were close to symmetrical. Finally, the silica particles used are rigid and the equipment used in this work delivers flow-rates stable within better than 0.1% [74].

3. Experimental

3.1. Equipment

All measurements were carried out with a Hewlett-Packard (Palo Alto, CA, USA) HP1090M liquid chromatograph equipped with a diode-array UV detector, an automatic sampling system, and a computerized data acquisition system. One of the feature of this apparatus, which is critically important in the present study, is the high stability and accuracy of the solvent delivery system [74]. Accordingly, the reproducibility of the measurements of the retention volumes was better than 0.1% and their accuracy is between 0.1 and 0.2%.

3.2. Columns

The empty stainless-steel columns (10×0.46 cm I.D.) were purchased from Alltech (Deerfield, IL, USA). Samples of four 10-μm spherical C_{18} ODS packing materials were obtained, Kromasil from Eka-Nobel (Stratford, CT, USA), Vydac from The Separation Group (Hesperia, CA, USA), YMC from YMC (Wilmington, NC, USA), and Zorbax from BTR (Wilmington, DE, USA). Each material was packed into five columns by conventional slurry packing [75], under the pressure recommended by the packing manufacturer. All the columns containing the same phase were packed on the same day, starting with column 1 and ending with column 5. The lot numbers of each material and the column packing conditions are listed in Table 1.

3.3. Chemicals

Polystyrene standards with molecular masses ranging from 2000 to 1 860 000 were obtained from Supelco (Bellefonte, PA, USA). Polystyrene standards with molecular masses of 456, 1050, 3 840 000, 5 840 000, and 20 600 000 were obtained from Tosoh (Tokyo, Japan). Polystyrene standards with molecular masses of 8 420 000 and 10 200 000 were obtained from American Polymer Standards (Mentor, OH, USA). Benzene (M_r 78.11) was purchased from EM Science (Gibbstown, NJ, USA).

Methylene chloride (J.T. Baker, Phillipsburg, NJ, USA) was used both as the mobile phase and the sample solvent. Unless specified otherwise, all measurements were carried out with UV detection at 254 nm, at a mobile phase flow-rate of 1.000 ml/min.

Table 1
Column packing conditions[a]

Brand Lot. No.	Kromasil DT0080	Vydac 920714-28-1	YMC EC16717	Zorbax B32110
Pore size (Å)	100	90	120	150
Slurry solvent	30% CH_2Cl_2 and 70% isopropanol	60% $CHCl_3$ and 40% acetone	CH_2Cl_2	30% CH_2Cl_2 and 70% isopropanol
Pushing solvent	Same as above	33% isopanol and 67% methanol	Methanol	Same as above
Pressure increase	Direct	Step	Direct	Direct
Maximum pressure (MPa)	69	48	28	55
Ca. packing time (min)	5	20	10	5
Ca. settle time (min)	5	30	5	5

[a] Particle size, 10 μm; column dimensions, 10×0.46 cm I.D.

3.4. Procedures

A total of 19 polystyrene standards with molecular masses ranging from 456 to 20 060 000 were injected into each column. Benzene was used for calibration and for the determination of the total accessible porosity of the column. Each injection was repeated at least three times. The results listed are the average of these three retention volumes. Plots of the logarithm of the probe molecular mass versus its retention volume showed that for each packing material there is a bimodal pore-size distribution (see next section and Figs. 1–5). These two distributions correspond to the internal porosity (between ca. 10 and 150 Å) and the external porosity (above a few hundreds Å), respectively. In all cases, the borderline between the two porosities is around $M_{r_n} = 23\,000$, corresponding to an average pore size, ϕ_n, of 228 Å (Eq. 2).

The total pore volume was taken as the retention volume of benzene. It was practically equal to that of uracil under reversed-phase chromatography conditions (see later). The external pore volume was derived from the intermediate point between the two pore-size distributions (see later). The porosities were calculated using the total column volume, V_k, itself derived from the geometrical dimensions of the column tubing:

$$V_k = \pi r^2 l \tag{5}$$

where r is the column radius (here, r=0.23 cm) and l the column length (l=10.0 cm).

Before packing, the mass, W_1, of each empty column was obtained on an analytical balance. After the end of the whole series of experiments, each column was dried under a stream of ultra-pure nitrogen (inlet pressure, ca. 30 p.s.i.=ca. 207 kPa), at a temperature of ca. 50°C, for at least 8 h, after what its mass was measured as W_2. The mass of solid material inside the column is $W_s = W_1 - W_2$. This mass and the relative mass concentrations of silica and alkyl bonded chains are used to calculate the volume of support and of stationary phase inside the column, V_s and V_a [73].

The volume fraction of the external pores, or external porosity ϵ_e, corresponding to a retention volume V_e, hence to the exclusion pore size, ϕ_e, and the total pore volume fraction or total porosity, ϵ_T, are defined by:

$$\epsilon_e = \frac{V_e}{V_k} \quad \epsilon_T = \frac{V_T}{V_k} \tag{6}$$

Two values are used for V_T, the retention volume of benzene in dichloromethane and the retention volume of uracil in methanol. The values of the column total porosity corresponding to these retention volumes are respectively $\epsilon_{T,b}$ and $\epsilon_{T,u}$. They are very close to each other.

The internal porosity, ϵ_i, is better defined by referring it to the actual volume of the particles, excluding the external porosity. In this way, the influence of variations of the external porosity due to a different extent of consolidation of the packing [59] is eliminated. It comes:

$$\epsilon_i = \frac{V_i}{V_k(1 - \epsilon_e)} \tag{7}$$

Total, external, and internal porosities are related by:

$$\epsilon_T = \epsilon_i(1 - \epsilon_e) + \epsilon_e \tag{8}$$

This balance can be used to derive ϵ_i from independent determinations of ϵ_T and ϵ_e, as done in this work. It will be used in a forthcoming paper to ascertain the consistency of the results obtained when these three porosities are obtained from different, independent measurements [79].

4. Results and discussion

The results of the determinations of the values of the different porosities of concern here are reported in Tables 2–5. They include the total, internal, and external porosities. The total porosity is derived from the retention volume of either benzene in methylene chloride or uracil in methanol. Both methods give results which are in close agreement, albeit slightly different, as discussed separately [73].

4.1. Column internal and external porosities

Plots of the logarithm of the molecular mass of the polystyrene probes versus their elution volume are

given for the four stationary phases studied, Kromasil (Fig. 1), Vydac (Fig. 2), YMC (Fig. 3), and Zorbax (Fig. 4). The numbers on each figure indicates the corresponding column. The degree of column-to-column reproducibility of the experimental data is generally excellent. Only the data for the first columns packed with Kromasil (Fig. 1) and, to a lesser degree, Vydac deviate significantly from the data obtained with the other four columns.

In each case, the plot obtained consists of two curves, each one approximately linear. These two curves correspond to the retention contributions of the two different types of pores in the column packing, the pores located inside the packing particles (internal pores) and the pores which are between the packing particles (external pores), respectively. If we disregard the five points corresponding to benzene, the polymer with M_r 23 000, and the three largest polystyrene samples (see rational below), we observe that the remaining fifteen points are located on two different straight lines whose slopes and intercepts are easily obtained by proper linear regressions. The coordinates of the intercepts (the exclusion pore size, ϕ_e, and the elution volume, V_e) of the two lines are listed in Table 2 (Kromasil), Table 3 (Vydac), Table 4 (YMC), and Table 5 (Zorbax). They are in rows 1 and 2, respectively.

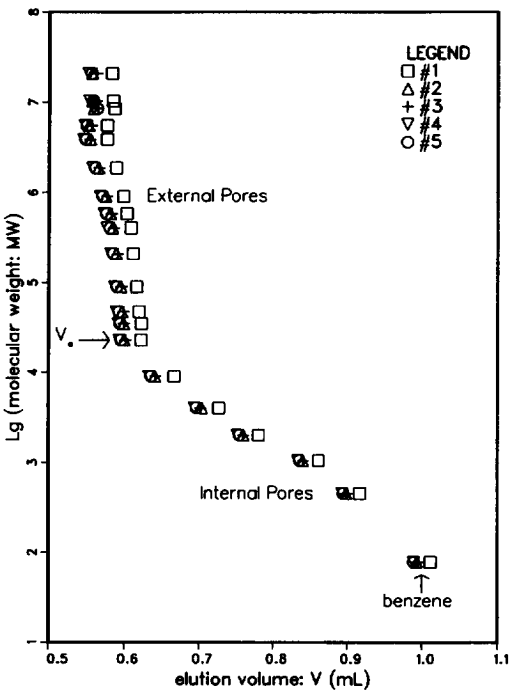

Fig. 1. Plot of the logarithm of molecular masses of the polystyrene standards injected versus their elution volumes at 1.000 ml/min flow-rate of methylene chloride. Kromasil columns. (□) column 1; (△) column 2; (+) column 3; (▽) column 4; (○) column 5.

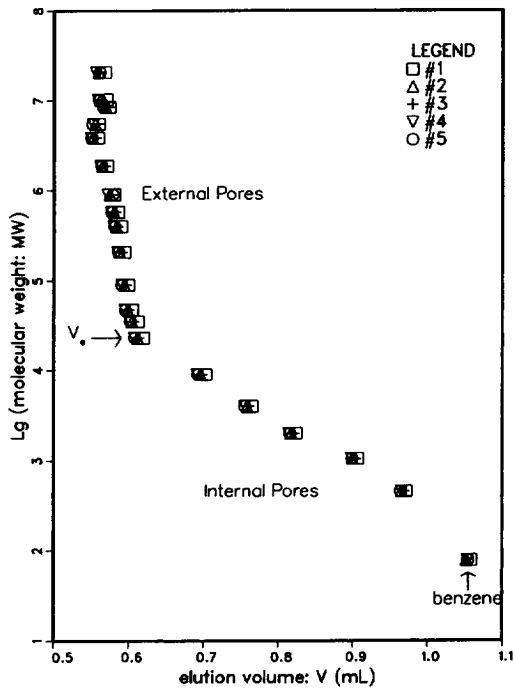

Fig. 2. Plot of the logarithm of molecular masses of the polystyrene standards injected versus their elution volumes at 1.000 ml/min flow-rate of methylene chloride. Vydac columns. Symbols, see Fig. 1.

We assume that all the pores inside the column having a size equal to or larger than ϕ_e belong to the inter-particle or external porosity, while the pores smaller than ϕ_e belong to the intra-particle or internal porosity. The corresponding elution volume, V_e, is the external pore volume of the column, from which the external porosity is derived (Eq. 6).

The reasons to eliminate benzene and the compound at M_{r_n} = 23 000 from the linear regression are as follows. Benzene is a small molecule which is outside the range of selectivity of the packing

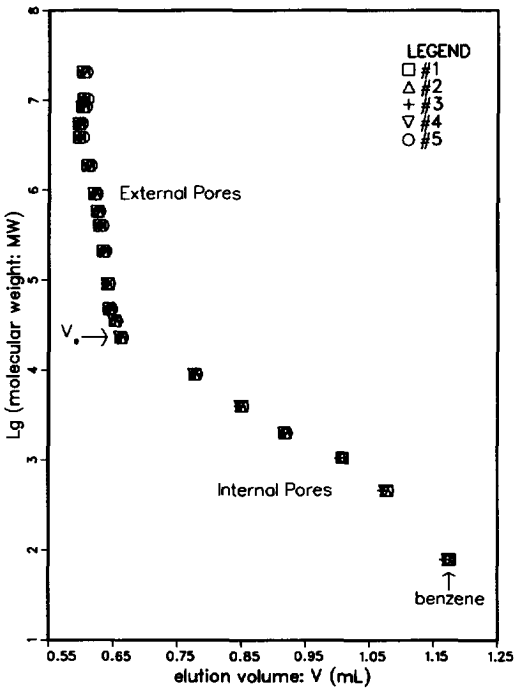

Fig. 3. Plot of the logarithm of molecular masses of the polystyrene standards injected versus their elution volumes at 1.000 ml/min flow-rate of methylene chloride. YMC columns. Symbols, see Fig. 1.

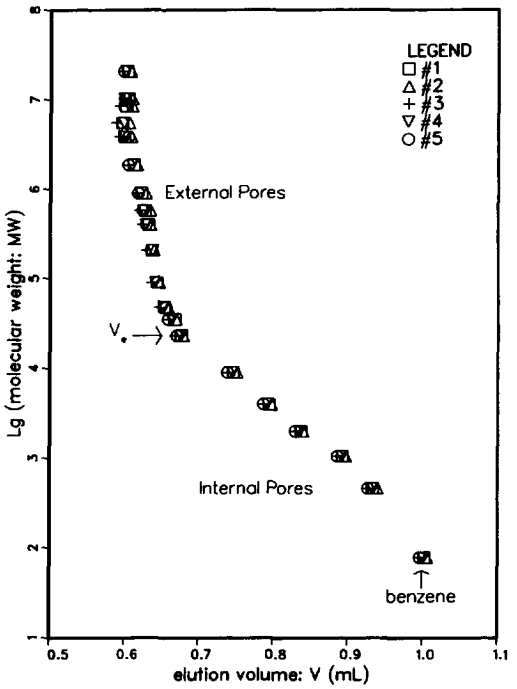

Fig. 4. Plot of the logarithm of molecular masses of the polystyrene standards injected versus their elution volumes at 1.000 ml/min flow-rate of methylene chloride. Zorbax columns. Symbols, see Fig. 1.

materials used (see Figs. 1–4). Its usefulness is in giving the column total porosity. The probe at M_{r_n} = 23 000 (ϕ_n=228 Å, Eq. 2) is intermediate. It seems to be already slightly excluded from the external porosity, without being completely excluded from the internal porosity. So, it does not belong to either correlation. This is related to the ambiguity found in defining these two porosities and their boundary. Some large pores, e.g., cavities located at the surface of the particles, cannot be simply assigned to one or the other type of pores. The three heaviest probes have retention volumes which tend to be slightly larger than those of the lighter polystyrene standards and than those predicted by extrapolation of the second straight line. These volumes are a function of the flow velocity and sample size (see later), although data acquired at very low sample size and flow velocities seem to be consistent with the other data pertaining to the external porosity. This abnormal behavior is observed for all four phases and is especially obvious in Figs. 1 and 2. It can be explained by the influence of the mobile-phase flow velocity on the retention of large random coil polymers as classically observed in size-exclusion chromatography [67,68] or in hydrodynamic chromatography [76,77].

To verify this assumption, we randomly chose the Zorbax column 2 (Fig. 5). We had previously injected all the polystyrene standards into this column at a flow-rate of 1.0 ml/min. The results were used for the determination of the column porosity (see Fig. 4 and Table 5). The experiment was repeated at a flow-rate of 5.0 ml/min. Afterward, the column was dried for weighing (see Section 3) and stored after closing both ends by plastic stoppers. A week later the column was retrieved and the injection of the whole series of polystyrene standards was repeated, at flow-rates of 0.05, 0.2, and 1.0 (for reproducibility check) ml/min. The results are shown in Fig. 5.

Table 2
Kromasil porosities

	Columns				
	1	2	3	4	5
Intersection ϕ_e (Å)	153	149	147	147	147
Coordinates V_e (ml)	0.637	0.615	0.617	0.608	0.611
$\epsilon_{T,b}$	0.608	0.597	0.598	0.595	0.595
ϵ_i	0.365	0.360	0.361	0.361	0.359
ϵ_e	0.383	0.370	0.371	0.366	0.368
$\epsilon_{T,u}$	0.588	0.580	0.584	0.581	0.582

ϕ_e (Å)=pore diameter in angstrom units; V_e (ml)=external pore volumes; ϵ_i=internal porosity; ϵ_e=external porosity; $\epsilon_{T,b}$=total porosity (retention volume of benzene in dichloromethane); $\epsilon_{T,u}$=total porosity (retention volume of uracil in methanol).

Table 3
Vydac porosities

	Columns				
	1	2	3	4	5
Intersection ϕ_e (Å)	213	212	213	210	210
Coordinates V_e (ml)	0.619	0.612	0.613	0.608	0.609
$\epsilon_{T,b}$	0.637	0.633	0.635	0.634	0.635
ϵ_i	0.422	0.419	0.421	0.423	0.423
ϵ_e	0.372	0.368	0.369	0.366	0.366
$\epsilon_{T,u}$	0.634	0.632	0.638	0.634	0.641

ϕ_e, V_e, ϵ_i, ϵ_e, $\epsilon_{T,b}$, $\epsilon_{T,u}$: as in Table 2.

Table 4
YMC porosities

	Columns				
	1	2	3	4	5
Intersection ϕ_e (Å)	253	252	256	252	253
Coordinates V_e (ml)	0.655	0.655	0.654	0.654	0.658
$\epsilon_{T,b}$	0.707	0.706	0.703	0.705	0.706
ϵ_i	0.516	0.515	0.509	0.514	0.513
ϵ_e	0.394	0.394	0.393	0.393	0.396
$\epsilon_{T,u}$	0.698	0.701	0.697	0.697	0.700

ϕ_e, V_e, ϵ_i, ϵ_e, $\epsilon_{T,b}$, $\epsilon_{T,u}$: as in Table 2.

Table 5
Zorbax porosities

	Columns				
	1	2	3	4	5
Intersection ϕ_e (Å)	272	275	268	272	257
Coordinates V_e (ml)	0.664	0.666	0.657	0.661	0.661
$\epsilon_{T,b}$	0.603	0.606	0.599	0.603	0.600
ϵ_i	0.338	0.342	0.337	0.340	0.335
ϵ_e	0.399	0.401	0.395	0.398	0.398
$\epsilon_{T,u}$	0.593	0.595	0.590	0.594	0.590

ϕ_e, V_e, ϵ_i, ϵ_e, $\epsilon_{T,b}$, $\epsilon_{T,u}$: as in Table 2.

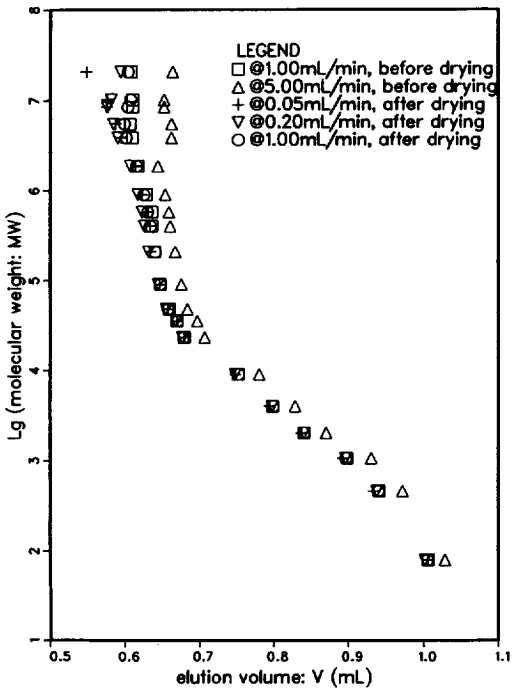

Fig. 5. Plot of the logarithm of molecular masses of the polystyrene standards injected versus their elution volumes at 0.050, 0.200, 1.000, and 5.000 ml/min flow-rates. Zorbax column 2.

There are no differences between the two series of experimental results obtained at a flow-rate of 1.0 ml/min, demonstrating the excellent consistency of the solvent delivery system and the stability of the structure of the column packing. However, the elution volumes of all the polystyrene standards at 5.0 ml/min appear to be about 0.025 ml larger than at lower flow-rates, up to M_r 1 860 000. For higher M_rs, these volumes increase significantly, indicating spurious effects possibly related to the influence of differential viscous drag on the conformation of the polymer backbone or, more probably, to viscous shear degradation of these polymers. On the other hand, a reduction of the flow-rate has no effect on the retention volumes of the polystyrene standards, except at high molecular masses. At flow-rates of 0.05, 0.2 and 1.0 ml/min, the elution volumes of the standards up to M_r 1 860 000 are independent of the velocity. However, the retention volumes of the three heaviest standards increase with increasing flow-rate from 0.05 to 0.2 and 1.0 ml/min, demonstrating a larger exclusion degree and confirming the assumption of shear degradation at high flow-rates. This is the justification for eliminating the corresponding data from the regression. In future studies, measurements made with polymers having more than M_r 1 000 000 should be made at 0.1 ml/min or at a lower flow-rate.

The total accessible pore volume inside the column, V_T, hence, the total column porosity, is derived from the elution volume of benzene. Having derived successively the total and the external porosity, we can solve Eq. 8 for the internal porosity. The results are listed in Tables 2–5, as values of the internal and external porosities (Eqs. 6 and 7).

The values of the total porosity derived from the retention volume of uracil [71], $\epsilon_{T,u}$, are also listed in Tables 2–5. Uracil is commonly used as the non-retained compound in reversed-phase liquid chromatography. The values of the total porosities derived from the retention volumes of benzene and uracil on all columns are nearly equal, the former being on average 2.7%, 0.1%, 1%, and 1.6% larger than the latter for Kromasil, Vydac, YMC, and Zorbax, respectively. These differences are nearly constant for the five columns, with very small standard deviations. Thus, they may probably be explained by a difference in the differential amounts of methanol and methylene chloride adsorbed by the chemically bonded layers of octadecyl groups on the different phases and may relate to the structure of the layer of bonded alkyl chains. In order to distinguish between these two porosities, we call them *benzene and uracil total porosities*, respectively [71].

4.2. The pore-size distributions of a chromatographic column

Based upon Eq. 3, the pore distribution inside the column can be expressed as the percentage of the total pore volume V_T accounted for by $\Delta V_{n+1,n}$, i.e., the volume fraction occupied by the pores larger than ϕ_n and smaller than ϕ_{n+1} (i.e., $\Delta V_{n+1,n}/V_T$, %). The results are listed in Table 6, Table 7, Table 8, and Table 9. The first column gives the boundary of the fraction considered. The blank line indicates the separation between the internal and the external pore-size distributions. For columns packed with the same ODS phase, there are some small but signifi-

Table 6
Incremental pore distribution on Kromasil columns (% of total pore volume)

$\Delta\phi$ (Å)	Columns				
	1	2	3	4	5
8 –22.7	9.33	9.50	9.53	9.67	9.57
22.7–37.1	5.47	5.87	5.87	6.03	5.93
37.1–54.2	8.01	8.12	8.19	8.32	8.25
54.2–81.5	5.37	5.67	5.80	5.86	5.90
81.5–131	5.93	6.31	6.04	6.17	6.13
131 –228	4.35	4.13	4.06	4.01	4.04
228 –292	0.00	0.07	0.20	0.10	0.10
292 –349	0.33	0.24	0.03	0.24	0.17
349 –508	0.33	0.13	0.27	0.24	0.27
508 –831	0.43	0.64	0.54	0.54	0.51
831 –1222	0.33	0.47	0.50	0.51	0.40
1222 –1513	0.56	0.30	0.30	0.37	0.51
1513 –1969	0.40	0.64	0.60	0.51	0.51
1969 –3017	0.96	0.97	1.04	1.04	1.01
3017 –4620	1.22	1.07	1.27	1.21	1.28
4620 –	56.99	55.86	55.75	55.17	55.42

Table 8
Incremental pore distribution on YMC columns (% of total pore volume)

$\Delta\phi$ (Å)	Columns				
	1	2	3	4	5
8 –22.7	8.23	8.21	8.30	8.39	8.07
22.7–37.1	5.99	5.80	5.68	5.60	5.94
37.1–54.2	7.77	7.65	7.62	7.90	7.59
54.2–81.5	5.59	5.94	5.85	5.83	5.82
81.5–131	6.21	6.14	6.08	6.11	6.08
131 –228	9.96	9.84	9.96	9.95	9.91
228 –292	0.77	0.82	0.74	0.77	0.77
292 –349	0.77	0.68	0.71	0.63	0.68
349 –508	0.06	0.31	0.37	0.26	0.28
508 –831	0.71	0.45	0.43	0.54	0.45
831 –1222	0.45	0.43	0.43	0.34	0.37
1222 –1513	0.26	0.28	0.29	0.43	0.43
1513 –1969	0.00	0.00	0.00	0.00	0.00
1969 –3017	0.91	0.77	0.86	0.68	0.80
3017 –4620	1.13	1.22	1.03	1.17	1.02
4620 –	51.21	51.45	51.67	51.41	51.79

cant differences in the pore-size distributions. As shown in a previous publication [71], these differences are at the origin of the systematic column-to-column fluctuations observed for various parameters and the values of the external porosity derived from our measurements can be used as a basis for correction.

The average pore-size distributions of the four phases are shown in Fig. 6 (Kromasil), Fig. 7 (Vydac), Fig. 8 (YMC), and Fig. 9 (Zorbax). These

Table 7
Incremental pore distribution on Vydac columns (% of total pore volume)

$\Delta\phi$ (Å)	Columns				
	1	2	3	4	5
8 –22.7	8.15	8.24	8.15	8.35	8.53
22.7–37.1	6.17	6.24	6.13	6.39	6.19
37.1–54.2	7.78	7.86	7.87	7.94	7.9
54.2–81.5	5.57	5.61	5.69	5.60	5.75
81.5–131	5.86	5.96	6.01	6.07	5.94
131 –228	7.87	7.95	7.93	7.94	8.00
228 –292	0.63	0.70	0.66	0.60	0.54
292 –349	0.72	0.57	0.66	0.63	0.66
349 –508	0.50	0.41	0.32	0.38	0.44
508 –831	0.50	0.63	0.54	0.57	0.41
831 –1222	0.44	0.38	0.57	0.47	0.51
1222 –1513	0.35	0.32	0.22	0.32	0.38
1513 –1969	0.50	0.54	0.54	0.47	0.00
1969 –3017	0.94	0.95	0.82	0.89	1.39
3017 –4620	1.13	1.24	1.17	1.20	1.26
4620 –	52.87	52.42	52.72	52.17	52.09

Table 9
Incremental pore distribution on Zorbax columns (% of total pore volume)

$\Delta\phi$ (Å)	Columns				
	1	2	3	4	5
8 –22.7	6.78	6.49	6.80	6.69	6.89
22.7–37.1	4.02	4.21	4.12	4.09	4.08
37.1–54.2	5.59	5.73	5.72	5.69	5.72
54.2–81.5	4.29	4.21	4.32	4.39	4.32
81.5–131	4.82	4.61	4.79	4.66	4.85
131 –228	7.02	7.22	7.16	6.99	6.86
228 –292	0.86	0.96	0.77	1.20	1.14
292 –349	1.36	1.06	1.34	1.10	0.33
349 –508	1.00	1.16	1.00	1.13	1.14
508 –831	0.70	0.73	0.70	0.33	0.84
831 –1222	0.43	0.43	0.54	0.90	0.67
1222 –1513	0.43	0.03	0.40	0.33	0.43
1513 –1969	0.00	0.00	0.00	0.00	0.00
1969 –3017	0.96	1.16	1.14	1.10	1.30
3017 –4620	1.30	0.76	1.07	1.13	0.00
4620 –	60.43	61.23	60.13	60.25	61.43

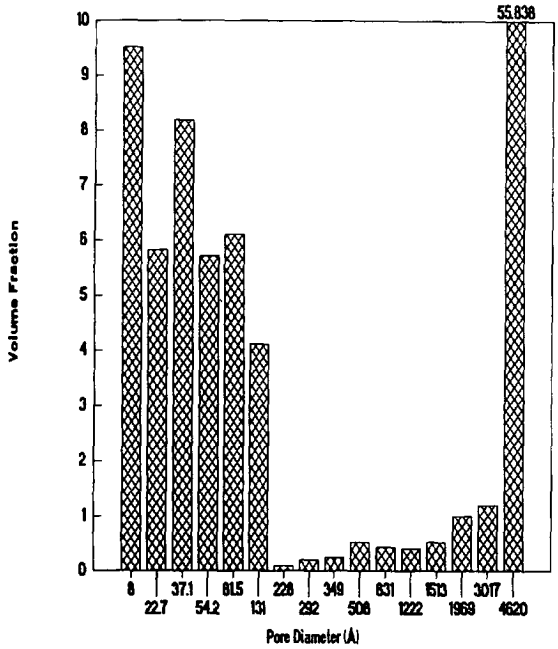

Fig. 6. Average pore-size distribution of Kromasil columns.

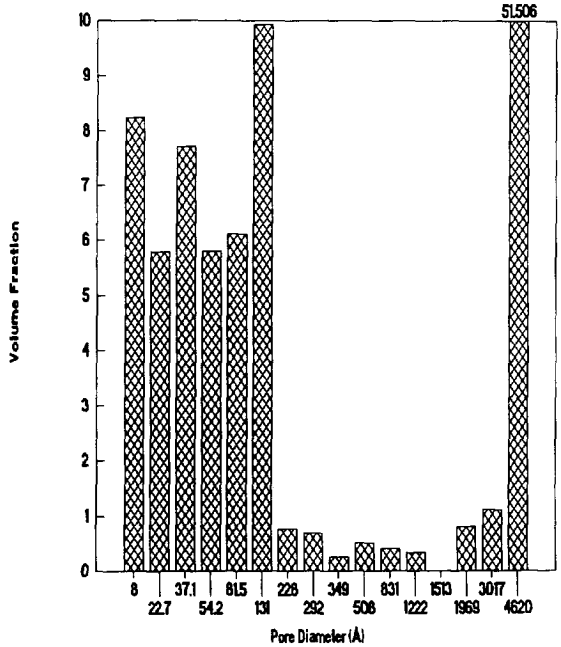

Fig. 8. Average pore-size distribution of YMC columns.

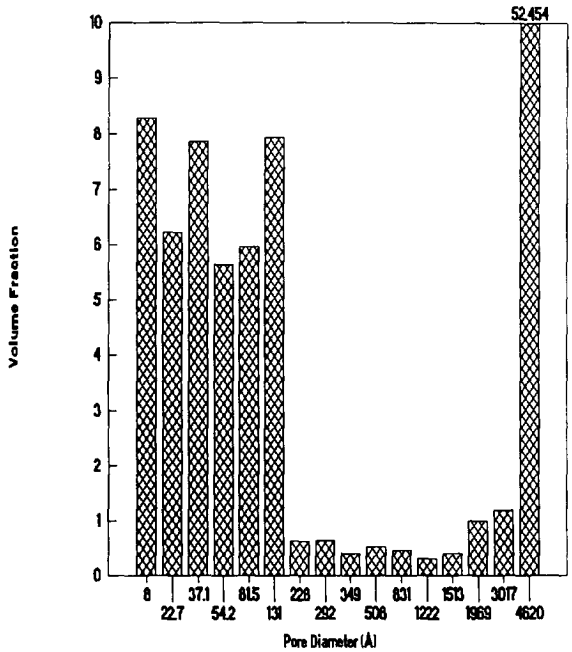

Fig. 7. Average pore-size distribution of Vydac columns.

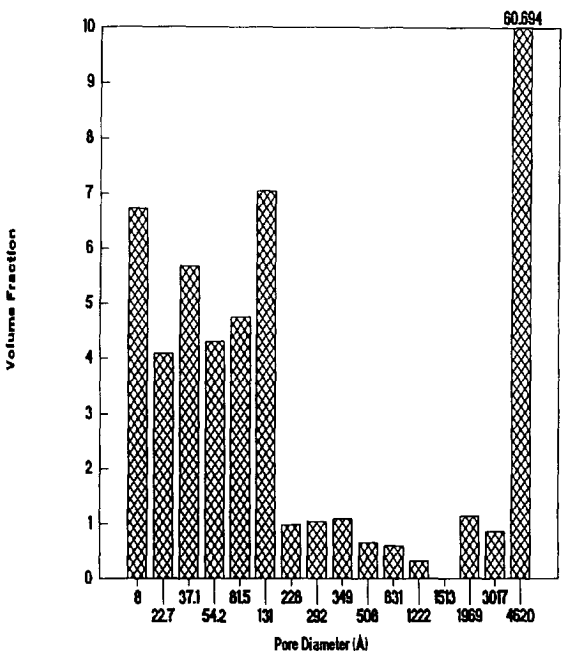

Fig. 9. Average pore-size distribution of Zorbax columns.

distributions are in good agreement with the pore distributions measured by the BET method, using nitrogen, which were performed by the manufacturers of the stationary phases studied here [78]. From the data in Tables 6–9, we see that the external porosity includes the fraction at 228 Å for Kromasil and Vydac but does not include it for YMC and Zorbax. However, the contribution of this fraction to the total porosity is small, so this is a minor difference.

In all cases, the contribution to the total porosity of the pores having an average size equal to 228 Å or larger and equal to 3000 Å or lower is small, between 5.5% (Zorbax) and 3.5% (Kromasil). Data in Tables 6–9 show that the pores larger than 0.4 μm account for more than 91% of the external porosity for these spherical materials having all a nominal particle size of 10 μm. The fact that these materials have different particle size distributions and different actual shapes [73] may contribute to explain the differences in the external pore-size distributions in the low range. For reasons explained above in the discussion of Fig. 5, it is doubtful that meaningful distributions of pores larger than 0.4 μm can be obtained by inverse SEC. Other methods, such as mercury porosimetry [41] should be used. A comparison between the results obtained for the external psd using these different methods will be published shortly [79].

Little is known on the relationships between the external porosity and the packing conditions [59], between the pore-size distribution of the packing (i.e., of the external pores), the particle shape, the average particle size, and the size distribution of the particles, or between any of these parameters and the column performance. Thus, our results open a new approach for the investigation of column properties and performance.

5. Conclusion

An accurate estimate of the values of the internal, external, and total porosities of a column, as well as of the external pore-size distribution of that column can be derived from the retention volumes of a series of polystyrene standards with a wide range of known molecular masses. This method is faster and more accurate than the traditional methods used for the determination of the porosity on a solid-phase. In a chromatographic laboratory, it does not require any special equipment.

The systematic measurements made demonstrate that there exist significant differences between the pore-size distributions of individual columns in a series, even when they are packed successively with the same packing material. These differences may explain, at least in part, the lack of reproducibility of the column performance which has been previously observed and reported for these columns [71]. Thermodynamic properties should depend only on the amount of surface area of stationary phase per unit volume of solvent. The external porosity measured as described in this work was used to correct satisfactorily for the deviation observed between the equilibrium isotherms measured for these columns [71].

In further work, the ISEC results obtained in this report will be compared to those obtained by other methods of investigation [79].

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