ELSEVIER

Journal of Chromatography A, 686 (1994) 165–177

JOURNAL OF CHROMATOGRAPHY A

Effect of bed compression on high-performance liquid chromatography columns with gigaporous polymeric packings☆

Ruth Freitag[1], Douglas Frey[2], Csaba Horváth*

Department of Chemical Engineering, Yale University, New Haven, CT 06520-8286, USA

First received 29 March 1994; revised manuscript received 25 July 1994

Abstract

The behavior of chromatographic columns packed with gigaporous, highly cross-linked styrenic particles was investigated for use in protein separation by reversed-phase chromatography at high flow velocities. Stainless-steel columns which were 3.5 or 7.5 cm long and had an inner diameter of 0.46 mm were slurry packed with 8 or 20 mm diameter spherical particles of 4000 Å mean pore size by using methanol as the packing fluid. It was found that the conditions employed during the packing process have a dramatic effect on the properties of such columns and that this can be attributed in part to the deformability of the particles. An increase in the packing pressure to approximately 6000 p.s.i. (41 MPa) resulted in a higher mass-transfer efficiency for the column with a concomitant decrease in permeability. This is ascribed to a decrease in the interstitial porosity with increasing packing pressure since the experimentally measured plate heights for these columns were found to agree quantitatively with theoretical predictions that relate changes in the interstitial porosity to intraparticle mass transfer. However, the theoretically derived relationship between porosity, permeability, and efficiency does not hold for columns packed at pressures higher than 6000 p.s.i., in which case the total column porosity was found to be high while the permeability and column efficiency were low. This behavior is explained by the formation of a low-porosity layer of highly compressed particles at the downstream end of the column during high pressure packing so that the assumption of axially uniform column properties used in the theoretical approach leads to very large errors.

1. Introduction

There is a growing need for rapid chromatographic separation of biopolymers such as proteins, nucleic acids and complex carbohydrates. In conventional packed columns, the intrinsically low diffusivity of large molecules has limited the speed of separation due to the low rate of intraparticle mass transfer [1]. In order to circumvent this obstacle, pellicular stationary phases consisting of a fluid-impervious spherical support with a thin retentive layer have been introduced [2,3]. Because of the relatively low surface area available for adsorption with pellicular stationary phases, however, the use of such packings is restricted primarily to high-speed separations in analytical chromatography.

Recently, bidisperse gigaporous particles with

* Corresponding author.

☆ Presented in part at the *14th International Symposium on Column Liquid Chromatography, Boston, 1990.*

[1] Present address: Institut für Technische Chemie der Universität, Hannover, Germany.

[2] Present address: Department of Chemical and Biochemical Engineering, University of Maryland Baltimore County, Baltimore, MD 21228, USA.

0021-9673/94/$07.00
SSDI 0021-9673(94)00697-0

pore diameters on the order of 1000 Å have been introduced for protein chromatography [4,5]. It has been suggested that under appropriate conditions, intraparticle mass transfer is augmented by convective transport within the gigapores [6], so that even though mass transfer to the adsorptive surface in the smaller pores still occurs solely by molecular diffusion, the speed of protein separation is considerably enhanced [7,8]. The theoretical aspects of convective mass transfer within gigaporous stationary phases have been investigated [9–14] with particular regard to the enhancement of column efficiency at high flow velocities where intraparticle convection is expected to yield higher column efficiencies than those obtained with columns packed with conventional porous particles.

Highly cross-linked, polymeric stationary phases having large pores are emerging as an alternative to siliceous and polysaccharide-based stationary phases in liquid chromatography. They are stable in aqueous salt solutions over a wide pH range, which is a considerable advantage in protein chromatography where columns are frequently cleaned with strongly basic solutions. In contact with organic and hydro–organic solvents, however, polymeric stationary phases are less stable mechanically than silica-based sorbents. Solvation of the polymer leads to some swelling and thus softening of the support so that columns packed with porous polymeric particles cannot withstand the entire pressure range attainable with current HPLC equipment [15,16]. In particular, at sufficiently high pressures, bed compression occurs with a concomitant decrease in column permeability. This compression process consists of particles moving, becoming deformed, breaking, or being forced into the frit at the column outlet [17].

At moderate packing pressures, several investigators have observed bed compression, i.e., a reduction in the interstitial porosity, which has favorably affected column efficiency. For example, Hjertén and co-workers [18–22] found that a reduction in the interstitial void volume in columns packed with highly cross-linked agarose beads had a beneficial effect on column efficiency. The results were interpreted using an equation introduced by Janson and Hedman [23] which relates the resolution to the ratio of the intraparticle and interstitial void volumes. Others have found that the efficiency of columns packed with styrenic particles increases in contact with mobile phases which cause particle swelling and have attributed this effect to a reduction in the amount of accessible micropores [24,25]. Meyer and Hartwick [26] investigated the relationship between column efficiency and packing pressure for narrow-bore columns packed with siliceous stationary phase particles and found evidence for the existence of an optimum column packing pressure. However, the authors made no attempt to explain this observation and presented no data concerning the interstitial porosities of the columns obtained at different packing pressures.

To date, we have not found data in the literature on the effect of bed compression in columns packed with polymeric gigaporous particles. This is rather surprising in view of the need to understand the relationship between the conditions for column packing and the magnitude of bed compression, as well as the effect of bed compression on column efficiency, in order to fully exploit the potential of gigaporous adsorbents in macromolecular chromatography.

2. Theory

2.1. Column efficiency

An efficient HPLC column is characterized by a small theoretical plate height, H. In order to facilitate the comparison of different packing materials, dimensionless values for the plate height and flow velocity are often used. The reduced plate height, h, is given by H/d_p, and the reduced velocity, ν, also termed the Peclet number, is defined as ud_p/D_m where d_p is the particle diameter, u is the interstitial fluid velocity and D_m is the diffusion coefficient of the eluite in the mobile phase. In the absence of kinetic resistances at the chromatographic surface, the total reduced plate height is the sum of three major independent contributions as follows [27–31]

$$h = h_{disp} + h_{ext} + h_{int} \quad (1)$$

In Eq. 1, h_{disp} expresses the combined effect of axial dispersion, longitudinal diffusion, flow maldistribution and extra column band-broadening and, in its simplest form, can be written as

$$h_{disp} = A + \frac{B}{\nu} \quad (2)$$

where A and B are constants.

For an unretained eluite, the plate height increment representing external mass-transfer resistances, h_{ext}, can be expressed using standard mass-transfer correlations as [14]

$$h_{ext} = \frac{(1-\alpha)\epsilon^2\alpha^{5/3}}{3.27[\alpha + (1-\alpha)\epsilon]^2} \cdot \nu^{2/3} \quad (3)$$

where ϵ and α are the intraparticular and interstitial porosities, respectively.

For an unretained eluite, the plate height increment arising from internal mass-transfer resistances in conventional porous particles is given as [29–31]

$$h_{int} = \frac{\theta\alpha(1-\alpha)\epsilon}{30[\alpha + (1-\alpha)\epsilon]^2} \cdot \nu \quad (4)$$

where θ is the diffusional tortuosity in the pores of the particles.

Recently, Eq. 4 has been extended to account for convective mass transfer inside a gipaporous particle by defining an apparent diffusivity, D_{app}, which accounts for the effects of both diffusion and convection. Eq. 4 can therefore be modified to yield the following result [6,13,14,32]:

$$h_{int} = \frac{\theta'\alpha(1-\alpha)\epsilon' D'_e}{30[\alpha + (1-\alpha)\epsilon']^2 D_{app}} \cdot \nu \quad (5)$$

where D'_e is the effective diffusivity within the gigapores. Note that the symbols and nomenclature used in this study are identical to those used in Frey et al. [14]. Accordingly, a single prime in Eq. 5 denotes properties associated with the gigapores, e.g., ϵ' denotes the volume fraction of gigapores in the particle. On the other hand, in the equations below, a double prime denotes properties associated with the domains between the gigapores. These domains, which contain the micropores, will be termed here the "subsidiary particles".

As shown by Frey et al. [14], for small mass-transfer rates, a linear driving force approximation applies not only to intraparticle mass transfer by diffusion, but also to intraparticle mass transfer by convection. If these two approximations are compared, the following relation results for the apparent diffusion coefficient

$$\frac{D_{app}}{D'_e} = \left(1 + \frac{2\nu'}{45}\right) \cdot f \quad (6)$$

where $\nu' = u' d'_p / D'_e$ and where the parameter f, which accounts for the diffusional resistance in the subsidiary particles, can be estimated as described in Ref. [33]. Under the restriction that linear driving forces apply for intraparticle mass transfer by both diffusion and convection, Eq. 6 is exact in the limit $\nu' \rightarrow \infty$ and also accurately represent mass-transfer behavior for finite ν' [14].

If it is assumed that the Carman–Kozeny equation (see Eq. 12) applies to both the flow inside the particle and in the region between particles, then the convective velocity in the gigapores, u', can be related to the interstitial flow velocity, u, as [14]

$$\sqrt{\frac{u'}{u}} = \frac{d''_p}{d'_p} \cdot \frac{\epsilon'(1-\alpha)}{\alpha(1-\epsilon')} \quad (7)$$

where d'_p is the particle diameter, and d''_p is the diameter of the subsidiary particles, which is generally taken to be twice the diameter of the gigapore.

2.2. Packing of the column as a filtration process

Slurry packing of chromatographic columns is closely related to the process of filtration —a classical solid–liquid separation method that involves phenomena related to interfacial science, transport in porous media, rheology, fine particle technology and filtration theory [34–36]. The following treatment of the column packing process at constant pressure is based on widely used theories of filtration and assumes one-di-

mensional geometry where only axial gradients in interstitial porosity exist.

The first step in the column packing procedure is the preparation of a stable suspension of the stationary phase in a suitable liquid. The solid content of such a slurry typically ranges from 5 to 20% (w/w). After the empty column tube is connected to the packing apparatus, a reservoir is filled with the packing liquid. The frit at the bottom of the tube serves as a "filter" which is permeable to the liquid, but impermeable to the stationary phase particles. The column tubing and the reservoir are filled with the slurry while the packing liquid is pumped through the column at constant pressure. As the packing process proceeds, the particles form a growing filter cake on the frit as the particles are deposited at the top of the packing and the liquid is forced through the interstices of the bed.

The drag force generated by the liquid flow on each particle is transmitted to the adjacent particles downstream. For simplicity, it is assumed here that the particles are in point contact, the liquid completely fills the voids between the particles and only axial pressure gradients exist. In this case, at any particular point in the bed, the total drag force on all the particles upstream from that point is exactly balanced by a compressive force at the point transmitted through the particles. Furthermore, the net force resulting from the pressure difference between any two column cross-sections equals the total drag force on the particles located between those cross-sections. This implies that

$$F(x) = [P - P_l(x)]A_c \tag{8}$$

where $F(x)$ is the drag force on all the particles upstream from a point a distance x from the inlet, $P_l(x)$ is the liquid pressure at x, P is the applied packing pressure and A_c is the column cross-sectional area.

If the compressive force on the particles at a particular point is divided by the column cross-sectional area to yield an apparent pressure, P_s, it follows that

$$P_s(x) + P_l(x) = P \tag{9}$$

According to Eq. 9, the sum of the pressures on the solid particles and on the packing liquid is at every point in the column equal to the momentary gauge pressure, P, of the packing apparatus. In going towards the column outlet, P_l decreases while P_s increases.

Due to the pressure gradients developed during the packing process, the interstitial porosity and the linear flow velocity of the mobile phase will vary along the column axis. A logarithmic change in the interstitial porosity with bed height is often found in filter cakes formed at low pressures. However, due to the high pressures used in the packing of HPLC columns, the change in porosity is most likely represented by an exponential relationship of the form [37]

$$\alpha = \alpha_0 e^{-cP_s} = \alpha_0 e^{-c(P-P_l)} \tag{10}$$

where α_0 is the interstitial porosity in the absence of a drag pressure, and c is a constant. Differentiation of Eq. 10, assuming P is constant, yields

$$\frac{d\alpha}{dP_l} = -\alpha_0 c\, e^{-c(P-P_l)} \tag{11}$$

For spherical particles, the change in P_l with column length (L) is given by the Carman–Kozeny equation [38] as follows

$$\frac{dP_l}{dL} = \eta u_s \cdot \frac{180(1-\alpha)^2}{\alpha^3 (d_p')^2} \tag{12}$$

where η is the viscosity of the packing fluid and u_s is the superficial flow velocity of the fluid.

In some cases, no simple relation is known for the dependence of the local interstitial porosity and the local pressure. For example, with highly compressible latex particles, α changes little over the major part of the bed, but decreases rapidly adjacent to the filter [37]. Although an axially non-uniform bed structure of this type has a high average interstitial porosity, it exhibits a high flow resistance due to the formation of a very dense layer over the bottom frit. The preceding equations are unlikely to apply under these conditions. However, provided that these types of severe axial non-uniformities do not exist,

Eqs. 11 and 12 can be combined and integrated along the column length to yield an expression for the porosity as a function of distance from the column inlet.

3. Experimental

3.1. Instrumentation

The liquid chromatograph consisted of a Model 100A precision metering pump (Beckman, San Ramon, CA, USA), a Model LC-85 B variable-wavelength detector (Perkin-Elmer, Norwalk, CT, USA) with a 1.4-μl flow cell, a Model 7125 sampling valve (Rheodyne, Cotati, CA, USA) with a 5-μl loop, and a Model B 41 stripchart recorder (Kipp & Zonen, Delft, Netherlands). The extracolumn dead volume was kept to a minimum by using a 0.12 mm I.D. capillary tube to connect the column to the injector and the flow cell of the detector.

3.2. Columns and stationary phases

The columns were prepared by slurry packing 30, 50 and 75 mm long No. 316 stainless-steel tubes of 4.6 mm I.D. and 1/4 in. O.D. (1 in. = 2.54 cm). Highly cross-linked, gigaporous, spherical particles of 8 and 20 μm diameter (PLRP-S), made by co-polymerization of styrene and divinylbenzene, were kindly donated by Polymer Labs. (Shropshire, UK). The average pore size of the particles used in this study was 4000 Å according to the manufacturer.

3.3. Chemicals

Urea was obtained from Mallinckrodt (St. Louis, MO, USA), nitromethane from Fisher (Fair Lawn, NJ, USA). HPLC-quality acetonitrile, methanol and trifluoroacetic acid (TFA) were purchased from J.T. Baker (Phillipsburg, NJ, USA). Carbonic anhydrase from bovine erythrocytes and the tripeptide Phe–Gly–Gly were supplied by Sigma (St. Louis, MO, USA). Water was purified by a Barnsted Nanopure unit (Boston, MA, USA). All eluents were filtrated and degassed by sonification before use.

3.4. Column packing procedure

The particles were dispersed by sonification in 5 ml methanol for the packing of 3- and 5-cm columns and in 10 ml methanol for that of the 7.5-cm columns. The solid content of the slurry was 10% (w/v). The column tubing with a 0.5-μm frit at the bottom end was connected to the reservoir of the packing apparatus. The latter was assembled from a No. DSHF-302 air-driven fluid pump (Haskel, Burbank, CA, USA), a Craftsman 4 HP-20 air compressor, and a laboratory-made 50-ml packing reservoir from stainless steel No. 316 and rated to 25 000 p.s.i. (1 p.s.i. = $6.8 \cdot 10^3$ Pa). The column tubing and the bottom part of the reservoir were filled with slurry, while the rest of the reservoir was filled with methanol. Methanol was pumped into the device from a fluid supply reservoir at a fixed pressure in the range from 1000 to 9000 p.s.i. The applied pressure was regulated within $\pm$ 50 p.s.i. In some cases the column was conditioned at the end of the packing procedure by pumping water through the bed for an additional 20 min at the packing pressure. At the end of the packing procedure, the column inlet and outlet pressures were allowed to equalize, and subsequently the column was disconnected from the apparatus. Finally, a stainless-steel frit was placed at the inlet end of the column.

3.5. Chromatography

Experiments were performed by isocratic elution at room temperature with acetonitrile containing 30% (v/v) water and 0.1% (v/v) TFA as the mobile phase at room temperature. Under these conditions the eluites did not interact with the chromatographic surface and therefore traversed the column without retention. Samples were prepared by dissolving the eluite in the mobile phase to obtain concentrations between 1 and 3 mg/ml. The detection wavelength was 215 nm. The column inlet pressure during the chromatographic experiments was kept below the

packing pressure. Both the linear flow velocity and the volumetric flow-rate were monitored throughout the experiments. For the evaluation of plate heights, urea, nitromethane, L-phenylalanyl-L-glycyl-L-glycine and carbonic anhydrase were employed as non-retained eluites.

3.6. Measurement of permeability, porosity and packing density

The chromatographic flow velocity, u_c, was determined from the residence time, t_0, of an unretained eluite [32]. Under the conditions employed in the study, urea and nitromethane were found to be suitable probes for this purpose. The packing density, i.e., the mass of stationary phase per unit column volume, was determined by carefully removing the packing material from the column and weighing it after drying. The average total porosity of the columns, ϵ_T, was calculated from the relationship

$$\epsilon_T = \frac{t_0 F}{V_0} \tag{13}$$

where F is the volumetric flow-rate and V_0 is the empty column volume. The specific column permeability, B^0, was evaluated as

$$B^0 = \frac{u_c \eta L}{\Delta P} \tag{14}$$

where ΔP is the pressure drop across the column. Plate heights were determined from the peak widths measured at the peak half height. The diffusion coefficients for these calculations were either taken from the literature [39] or calculated using the Wilke–Chang equation [40].

4. Results and discussion

4.1. Properties of columns prepared at different packing pressures

According to Eq. 12, the flow-rate should increase linearly with the applied column inlet pressure as long as the permeability of the bed, i.e., the structure of the packing, remains constant. Fig. 1 illustrates the volumetric flow-rate as a function of the column inlet pressure for 5-cm long columns packed with 20-μm particles using a methanol slurry at various packing pressures. It is seen that the relationship between the flow-rate and inlet pressure is non-linear, indicating that the column permeability decreased significantly at high inlet pressures even though the operating inlet pressure never exceeded the packing pressure. In fact, the relationship between the flow-rate and inlet pressure is non-linear even at pressures well below the typical upper limit specified by manufacturers of columns containing polymeric particles. The change in permeability was, however, reversible, i.e., the column permeability at low flow-rates was the same, before and after exposure to high inlet pressures.

The behavior described above is likely due to the swelling of the polymeric particles in contact with the methanol used as the suspending and

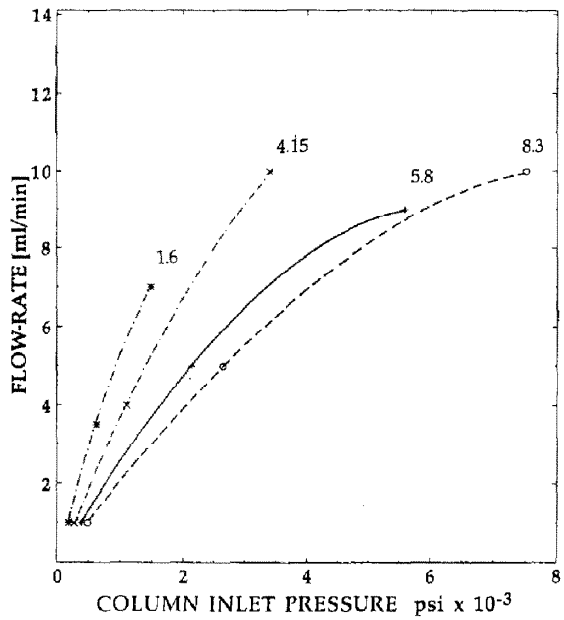

Fig. 1. The volumetric flow-rate as a function of the inlet pressure for columns packed at different pressures. The mobile phase was acetonitrile containing 30% (v/v) water and 0.1% trifluoroacetic acid. The 50 × 4.6 mm columns were slurry packed with 20-μm gigaporous, styrenic particles at the packing pressures indicated. Methanol served as both slurry and packing fluid.

packing fluid [41]. The substitution of water for methanol would have circumvented this swelling problem; however, this substitution was not possible because the particles were poorly wetted by neat water, which precluded the preparation of a suitable slurry. A compromise method was attempted, however, in which the column was packed according to the protocol given above, and afterwards rinsed for 20 min with water at the packing pressure so that most of the methanol was removed and some shrinking of the particles occurred. In subsequent contact with the hydro–organic mobile phase, however, the particles took up the organic modifier and consequently the relationship between the inlet pressure and the flow-rate of aqueous acetonitrile for the columns conditioned with water was essentially the same as that depicted in Fig. 1.

The permeability and total porosity of the columns packed at different pressures with and without water conditioning were determined from the residence time, t_0, of urea at a flow-rate of 1 ml/min as described in the Experimental section. Typical data obtained with 5-cm long columns packed with 20-μm particles and without water conditioning are illustrated in Fig. 2. The dependence of the density of the column packing on the packing pressure is also depicted in Fig. 2. The results show that while the column permeability decreases monotonically with the packing pressure, the total column porosity and the packing density go through a minimum and maximum, respectively. According to additional experiments (not shown here), the observed trends in the changing column properties with increasing packing pressure were independent of the column length or packing procedure.

Comparison of the results obtained with columns packed with or without water conditioning shows that water-conditioned columns have generally higher packing densities, and consequently lower total column porosities, than columns that were not subjected to water conditioning. In contradistinction, the permeability of water-conditioned columns tends to be higher than that of columns packed from methanol only. These trends are likely due to the swelling of the polymer particles in methanol. Presumably, the

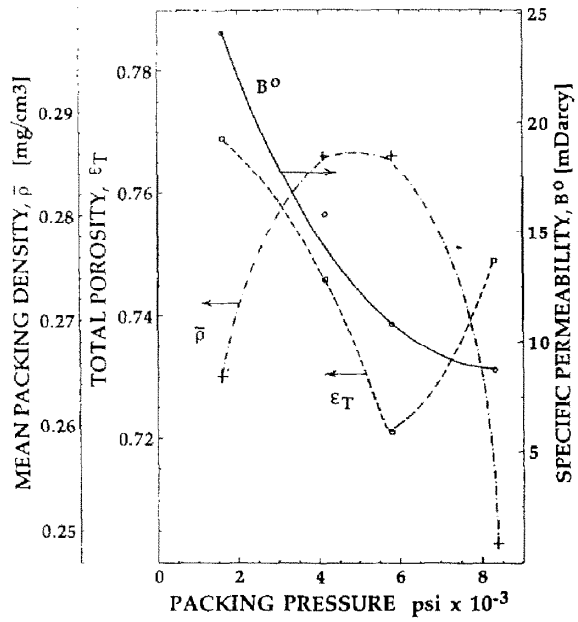

Fig. 2. The packing density, ρ, total column porosity, ϵ_T, and specific permeability, B^0, as a function of the packing pressure for 50 × 4.6 mm columns packed with 20-μm, gigaporous, styrenic particles. Methanol was used as both the slurry and packing fluid. Urea was the unretained tracer and acetonitrile containing 30% (v/v) water and 0.1% TFA the mobile phase.

resulting deformation of these swollen particles accounts for the comparatively high flow resistance of unconditioned columns.

It is seen in Fig. 2, that up to a packing pressure of 6000 p.s.i. the dependence of column porosity and permeability on the packing pressure reflects the behavior predicted by Eqs. 12 and 14 in that both the porosity and permeability decrease with increasing packing pressure. However, for columns packed at pressures higher that 6500 p.s.i., the permeability of the column decreases, albeit at a lower rate, with increasing packing pressure, whereas the porosity of the column increases. This finding suggests that packing pressures higher than 6500 p.s.i. yield columns having a bed structure different from that of columns packed at lower pressures. This difference is not due to particle breakage during the packing procedure since an examination of the particles by scanning electron microscope gave no evidence of particle fragmentation.

Instead, the situation appears to be similar to the filtration behavior of certain compressible latex particles described earlier where the interstitial porosity changes little over the larger part of the bed, but decreases dramatically in the proximity of the filter proper [42]. As a result, the filter cake manifested not only a high average interstitial porosity, but also a high flow resistance. By analogy, the relatively low permeability of the column in our case also originates from a densely packed layer of particles at the top of the frit. Otherwise, most of the column contains a loose and highly porous packing, which would account for the observed behavior of the columns packed at the highest pressures.

For the column packings considered here, the total porosity is the only type of porosity directly measurable by chromatographic methods since we did not have access to an inert tracer with sufficiently large molecular dimensions to ensure its complete exclusion from all gigapores. However, the interstitial porosity can be estimated for a given column by measuring the pressure drop and flow-rate, and substituting those values into Eq. 12. The results are given in Table 1 for packing pressures lower than 6000 p.s.i., where axial gradients in porosity appear to be relatively small, and Eq. 12 can be assumed to apply with reasonable accuracy.

Table 1
Interstitial porosities of columns packed with gigaporous 20-μm styrenic particles at different pressures using methanol as the slurry and packing fluid

Column length (cm)	Packing pressure (p.s.i.)	Interstitial porosity	
		A	B
3	1000	0.18	–
3	2000	–	0.17
3	2500	0.16	–
3	3500	0.14	–
3	5000	0.13	0.14
5	1600	0.19	–
5	2000	–	0.19
5	4150	0.17	–
5	5800	0.15	0.17
7.5	2000	–	0.22
7.5	2450	0.20	–
7.5	5000	–	0.18
7.5	5800	0.17	–

A = Standard packing procedure; B = column conditioned with water after packing procedure.

In most cases, the apparent interstitial column porosities calculated by the above procedure are lower than 0.2, which is significantly less than the value of 0.35–0.40 normally observed for random packings of rigid spherical particles. It is known that the Carman–Kozeny equation does not apply accurately to a compressible bed, since it tends to underestimate the interstitial porosity in such cases [34]. The total porosities of the water-conditioned columns are lower than those of the corresponding unconditioned columns, whereas according to our calculations the reverse is true for the apparent interstitial column porosities. This would appear to indicate that the particles in the water-conditioned packing are less deformed than in the unconditioned bed.

As mentioned previously, the measurement of the average column porosity does not give information on the variation of the local interstitial porosity, α, within the packed bed. In order to illustrate the type of porosity profiles expected in the bed, Eq. 12 was employed assuming a value of $\alpha_0 = 0.2$ at the top of the column, and also assuming an exponential decrease in α in terms of P_s over the length of the column according to Eq. 11. The change in local interstitial porosity with distance in a 5-cm column packed with 20-μm particles was calculated by using a value for the constant c that gave interstitial porosity values closest to the pertinent experimental data. The results are presented in Fig. 3 to illustrate the effect of axial non-uniformity on the increase of the local porosity with the packing pressure for a given stationary phase.

4.2. Column efficiency

In order to investigate the influence of the packing pressure on column efficiency, plate heights were measured at different flow velocities with carbonic anhydrase as the unretained tracer. Our main interest was in examining the plate height dependence on the flow velocity at high flow-rates where intraparticular mass trans-

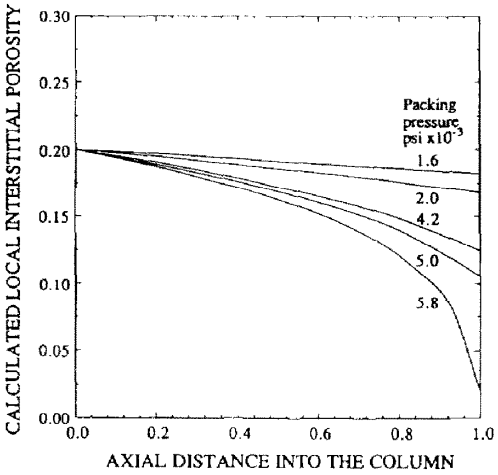

Fig. 3. Calculated local interstitial porosity as a function of axial distance in 50 × 4.6 mm columns packed with 20-μm, gigaporous, styrenic particles at different pressures as indicated. The axial profile of interstitial porosity was calculated using Eqs. 11 and 12 with $\alpha_0 = 0.2$.

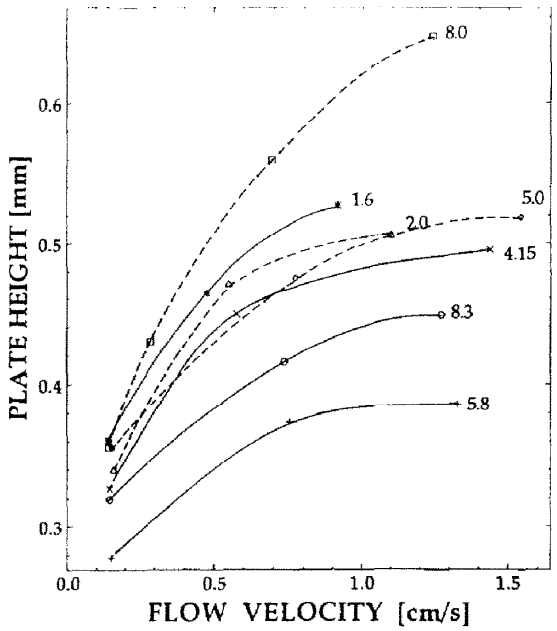

Fig. 4. The plate height as a function of the flow velocity measured with 50 × 4.6 mm columns packed at different pressures. Carbonic anhydrase was used as an unretained tracer, and acetonitrile containing 30% (v/v) water and 0.1% trifluoroacetic acid as the mobile phase. Columns were packed with 20-μm, gigaporous, styrenic particles from a methanol slurry at the pressures indicated in p.s.i. × 10^{-3}. Methanol served as the sole packing fluid for the cases represented by solid lines. A subsequent, water-conditioning step was used in the cases represented by the broken lines.

fer dominates. The results obtained by using 5-cm long columns packed with 20-μm particles in methanol at different packing pressures are depicted in Fig. 4 with the data points connected by solid lines. For several columns, the otherwise identical packing procedure was followed by the water-conditioning step. The plate heights obtained by these columns are also illustrated in Fig. 4 with the data points connected by broken lines. Similar behavior was observed with 7.5-cm columns packed in the same manner (data not shown). Inspection of the results presented in Fig. 4 suggests that, in general, the column efficiency increases with packing pressure up to about 6000 p.s.i., while a further increase in packing pressure results in lower efficiency. Furthermore, water-conditioned columns exhibit higher plate heights, i.e., lower efficiency in the flow-rate range studied, than those without this conditioning. This trend agrees with the calculations of the interstitial porosities from the Carman–Kozeny equation discussed earlier and again indicates that the water-conditioned packing is less deformed than the unconditioned packing.

Plate heights for 3-cm long columns packed with 8- or 20-μm particles were also measured with carbonic anhydrase under conditions of no retention in order to investigate the effect of particle size. The packing pressures were 3500 and 5000 p.s.i. for the 8-μm particles and 2000 and 5000 p.s.i. for the 20-μm particles, and all columns were conditioned with water at the end of the packing procedure. Fig. 5 illustrates the relation between the reduced plate height and the reduced velocity thus obtained. It can be seen that the column efficiency increases with the packing pressure for columns containing either particle size, and that columns packed with 20-μm particles are more efficient in the terms of the reduced plate height in the Peclet number range considered than those packed with 8-μm particles. This is likely due to the fact that the larger particles are less rigid than the smaller particles and therefore deform to a greater

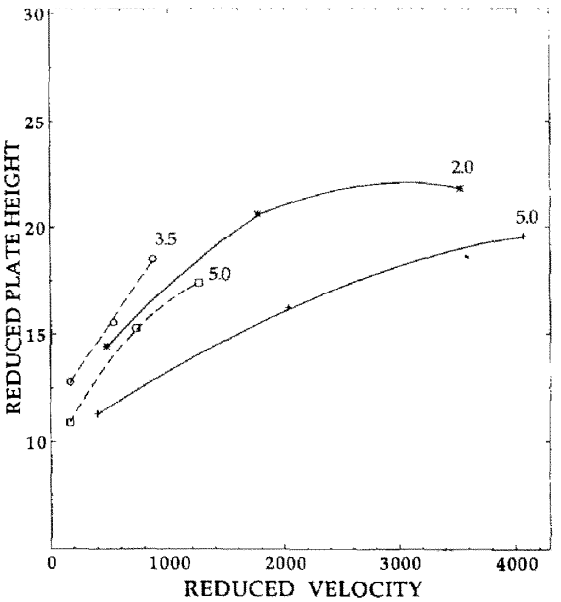

Fig. 5. The reduced plate height as a function of the reduced flow velocity measured with columns packed at different pressures. Columns were 30 × 4.6 mm and packed with either 8- (broken line) or 20-μm (solid line), gigaporous styrenic particles, from a methanol slurry and with methanol as the packing fluid at the pressures indicated in p.s.i. $\times 10^{-3}$. Mobile phase and inert tracer as in Fig. 4.

degree at a given packing pressure. Note that the permeability of columns packed with 20-μm particles is higher than that of those packed with 8-μm particles such that the former type of column can be operated at a higher flow-rate than the latter at a given column inlet pressure.

4.3. Comparison of experimental data and theoretical calculations

According to Eqs. 3 and 5, the interstitial porosity of the column influences both h_{ext} and h_{int} in Eq. 1. In particular, provided that all other parameters are held constant, a decrease in interstitial porosity will reduce the magnitudes of both of these plate height contributions. More importantly, however, in the case of gigaporous particles there is an additional effect; namely, a decrease in the interstitial porosity may result in an increase in the amount of intraparticle fluid flow, and thereby in an enhancement of mass transfer inside the particles.

In order to assess the importance of this effect, as well as to see whether an expression for the plate height employing an average interstitial porosity for the bed can describe the overall column efficiency, Eqs. 5–7 were employed together with the apparent interstitial porosities from Table 1 to calculate the reduced plate height as a function of the flow-rate. In the application of Eqs. 5–7, the volume fraction of the particles occupied by the gigapores, ϵ', was assumed to be 0.4, and for the pore size the value of 4000 Å reported by the particle supplier was used. As discussed above, interstitial porosities could not be calculated from Eq. 12 for columns packed at pressures greater than 6500 p.s.i. since those columns appeared to contain a dense layer of low permeability adjacent to the bottom frit. Thus, plate height calculations are not shown for those columns.

Figs. 6 and 7 illustrate both the experimentally measured and theoretically calculated reduced plate heights as a function of the reduced velocity for 5-cm long columns packed with 20-μm particles with and without subsequent water conditioning. The calculations were performed as described above. In both figures, the reduced plate height calculated for unretained carbonic anhydrase are shown by solid lines, whereas the experimentally determined plate heights are represented by data points. As seen, the calculated values agree well with the experimental results for columns packed at pressures below 6000 p.s.i. This suggests that essentially all the improvement in the column efficiency with increasing packing pressure up to the optimal value is accounted for quantitatively by the effect of changes in interstitial porosity on the magnitude of interparticle convection. Furthermore, the degree to which the theoretical and experimental results agree in Figs. 6 and 7 lends further support to the assumptions underlying Eqs. 5–7, i.e., that intraparticle flow exists under the experimental conditions employed, that the fluid velocity in the particle can be described by an equation analogous to Eq. 12, and that the resulting effect on mass transfer can be described by Eq. 6. The treatment outlined here should also be applicable to other chromatographic

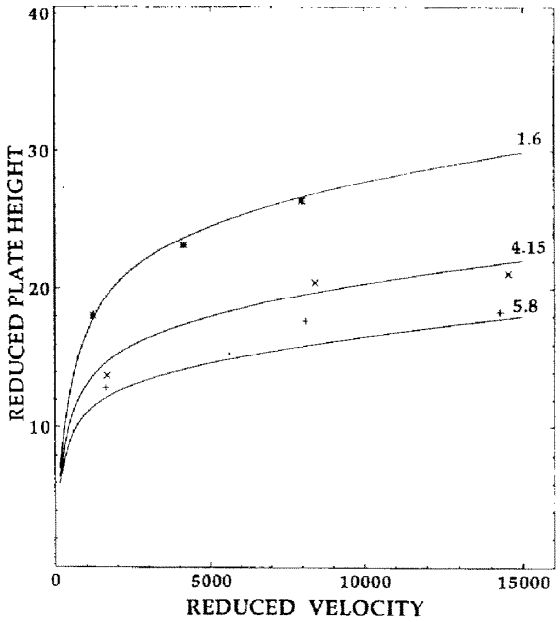

Fig. 6. The reduced plate height as a function of the reduced velocity for 50 × 4.6 mm columns packed at different pressures as indicated in p.s.i. × 10^{-3},with methanol serving as both the slurry and the packing fluid. The mobile phase was acetonitrile containing 30% (v/v) water and 0.1% trifluoroacetic acid. Carbonic anhydrase was used as the inert tracer. The interstitial column porosities were evaluated from experimentally measured permeability data and used together with the parameters $\epsilon' = 0.4$ and $d_p'' = 8000$ Å to calculate the solid lines using Eqs. 5-7. Experimental data are shown by the data points.

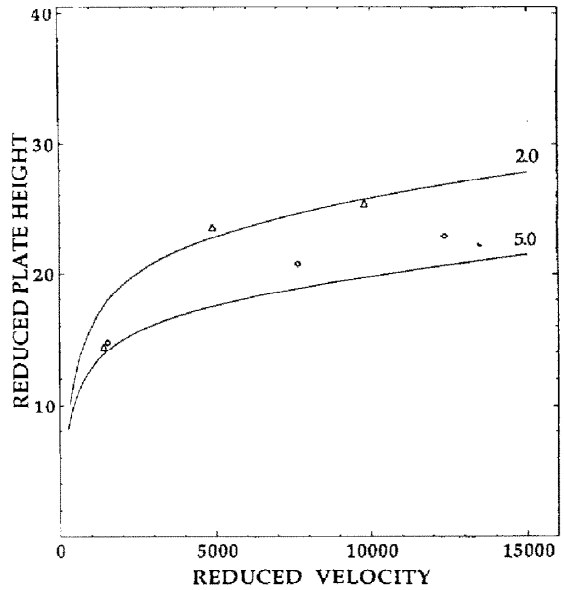

Fig. 7. The reduced plate height as a function of the reduced velocity for 50 × 4.6 mm columns packed at different packing pressures as indicated in p.s.i. × 10^{-3}, using methanol for the slurry and as the packing fluid. The packing process was followed by a water-conditioning step. The calculation of the lines and the measurement of the data points shown were carried out as in Fig. 6.

systems involving columns packed with deformable particles, such as those described by Hjertén and co-workers [18–22] who investigated the use of agarose particles in compressed beds.

5. Conclusions

In the practice of modern liquid chromatography, a wide variety of column packing materials are employed, including stationary phases based on organic polymer, silica or polysaccharide supports. These materials vary considerably in their physical properties so that the study described here does not represent all of the considerations that influence the effect of the packing process on column efficiency. For a given type of packing material, the solvent used in the packing process should be chosen so that it wets the solid surface and has a density close to that of the solid in order to form a stable suspension. As shown in this study, however, another solvent property should also be considered; namely, the ability of the solvent to swell and therefore soften the support particles. In most cases, this type of softening is not desired since it complicates the packing processes. In contrast to this general rule, our results indicate that it may be possible to exploit this type of softening to produce a column which has a high mass-transfer efficiency at the expense of a greater pressure drop.

Although the various phenomena which underlie the effect of the packing conditions on the column efficiency can be difficult to quantify for a given system, we have shown that a relatively simple theoretical approach can be used to explain the effect of the packing pressure on

column efficiency for the particular case of gigaporous styrenic particles where intraparticle convection may facilitate the rate of mass transfer. In particular, we have shown that at moderate packing pressures an increase in the packing pressure leads to a reduction in the interstitial column porosity, which in turn leads to an increase in the magnitude of intraparticle fluid flow relative to the flow-rate. This enhancement of intraparticle convection then brings about a decrease in the plate height. However, as the packing pressure is increased above a certain optimal value, the column porosity becomes highly non-uniform due to the formation of a very dense layer of particles with a low porosity on the bottom frit of the column during the initial stages of the packing process, whereas the remainder of the column has a relatively high porosity. Such a column has a low overall permeability as well as a high average porosity so that its fluid mechanical and mass-transfer behavior cannot be described by a single average interstitial porosity.

Acknowledgements

R.F. is grateful for the support by the Deutsche Forschungsgemeinschaft. The authors are indebted to F.P. Warren of Polymer Laboratories Ltd. for providing the bulk materials of the stationary phases and to A.S. Pooley of Yale University for providing the scanning electron microscopy data. This work was supported by grants No. GM 20993 from the National Institutes of Health US Public Health Service and No. BCS-9014119 from the National Science Foundation, and from the National Foundation of Cancer Research.

References

[1] Cs. Horváth and H.-J. Lin, *J. Chromatogr.*, 149 (1978) 43.
[2] Cs. Horváth and S.R. Lipsky, *J. Chromatogr. Sci.*, 7 (1969) 109.
[3] K. Kalghatgi and Cs. Horváth, *J. Chromatogr.*, 398 (1987) 335.
[4] J.V. Dawkins, L.L. Lloyd and F.P. Warner, *J. Chromatogr.*, 352 (1986) 157.
[5] L.L. Lloyd and F.P. Warner, *J. Chromatogr.*, 512 (1990) 365.
[6] N.B. Afeyan, N.F. Gordon, I. Mazsaroff, L. Varady, S.P. Fulton, Y.B. Yang and F.E. Regnier, *J. Chromatogr.*, 519 (1990) 1.
[7] N. Afeyan, S.P. Fulton, N.F. Gordon, I. Mazsaroff, L. Varady and F.E. Regnier, *Bio/Technology*, 8 (1990) 203.
[8] S.P. Fulton, N. Afeyan, N.F. Gordon and F.E. Regnier, *J. Chromatogr.*, 547 (1991) 452.
[9] A.E. Rodrigues, Z.P. Lu and J.M. Loureiro, *Chem. Eng. Sci.*, 46 (1991) 2765.
[10] A.E. Rodrigues, J.M. Loureiro and R.M.Q. Ferreira, *Chem. Eng. Commun.*, 107 (1991) 21.
[11] G. Carta, M. Gregory, D. Kirwan and H. Massaldi, *Sep. Technol.*, 2 (1992) 62.
[12] A.I. Liapis and M.A. McCoy, *J. Chromatogr.*, 599 (1992) 87.
[13] A.E. Rodrigues, *LC·GC*, 9 (1993) 273.
[14] D. Frey, E. Schweinheim and Cs. Horváth, *Biotech. Prog.*, 9 (1993) 273.
[15] K.A. Tweeten and T.N. Tweeten, *J. Chromatogr.*, 395 (1986) 111.
[16] N. Tanaka, K. Hashizume and M. Araki, *J. Chromatogr.*, 400 (1987) 38.
[17] F.B. Hutton, Jr., *Chem. Eng. Progr.*, 53 (1957) 328.
[18] S. Hjertén, Z.-Q. Liu and D. Yang, *J. Chromatogr.*, 296 (1984) 115.
[19] S. Hjertén, B.-L. Wu and J.-L. Liao, *J. Chromatogr.*, 396 (1987) 101.
[20] S. Hjertén and J.-L. Liao, *J. Chromatogr.*, 457 (1988) 165.
[21] S. Hjertén, J.-P. Li and J.-L. Liao, *J. Chromatogr.*, 475 (1989) 177.
[22] S. Hjertén and D. Yang, *J. Chromatogr.*, 316 (1984) 301.
[23] J.-Ch. Janson and P. Hedman, in A. Fichter (Editor), *Advances in Biochemical Engineering*, Vol. 25, Springer, Berlin, 1982, pp. 43–99.
[24] M. Stuurman, J. Köhler, O. Jansson and A. Litzen, *Chromatographia*, 23 (1986) 341.
[25] D.C. Shelly and Th.J. Edkins, *J. Chromatogr.*, 411 (1987) 185.
[26] R.F. Meyer and R.A. Hartwick, *Anal. Chem.*, 56 (1984) 2211.
[27] J.C. Giddings, *J. Chromatogr.*, 5 (1961) 61.
[28] E. Grushka, L.R. Snyder and J.H. Knox, *J. Chromatogr. Sci.*, 13 (1975) 25.
[29] Cs. Horváth and H.-J. Lin, *J. Chromatogr.*, 126 (1976) 401.
[30] E. Kucera, *J. Chromatogr.*, 19 (1965) 237.
[31] P. Schneider and J.M. Smith, *AIChE J.*, 14 (1968) 763.
[32] A. Rodrigues, J. Lopes, Z.P. Lu, J. Loureiro and M. Dias, *J. Chromatogr.*, 590 (1992) 93.

[33] H.W. Haynes and P.N. Sarma, *AIChE J.*, 19 (1973) 1043.
[34] F.M. Tiller, *Filtr. Sep.*, 4 (1975) 386.
[35] D.C. Shelly, V.L. Antonucci, Th.J. Edkins and T.J. Dalton, *J. Chromatogr.*, 458 (1989) 267.
[36] D.C. Shelly and Th.J. Edkins, *J. Chromatogr.*, 411 (1987) 185.
[37] R.S. Spencer, G.D. Gilmore, R.M. Wiley, *J. Appl. Phys.*, 21 (1950) 527.
[38] P.C. Carman, *Trans. Inst. Chem. Eng.*, 16 (1938) 168.
[39] H.A. Sober (Editor), *CRC Handbook of Biochemistry*, CRC Press, Cleveland, OH, 2nd ed., 1970, p. C-10.
[40] C.R. Wilke and P. Chang, *AIChE J.*, 1 (1955) 264.
[41] Y.-F. Maa and Cs. Horváth, *J. Chromatogr.*, 445 (1988) 71.
[42] F.M. Tiller and T.C. Green, *AIChE J.*, 19 (1973) 1266.