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PREPARATION AND APPLICATION OF POROUS PACKING MATERIALS FOR LIQUID–LIQUID CHROMATOGRAPHY

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SUMMARY

A method is described for preparing reasonable quantities of porous supports, in size fractions with a mean particle diameter of from 5 to 30 μ, from an inexpensive starting material. Several columns filled with these materials have been examined in order to determine some of their characteristics which are of importance in liquid–liquid chromatography. The columns have a relatively high permeability and a small plate height. The material gives fast separations at low pressure gradients.

INTRODUCTION

Reduction of the particle size of the porous support used in liquid–liquid chromatography generally gives better and faster separation, especially if mass transfer phenomena make an important contribution towards peak broadening. Porous support materials of small particle size are difficult to prepare in practice. Fractions with a mean particle size of 30 μ or over can be obtained by dry-screening; the preparation of fractions of smaller particles, however, calls for entirely different techniques, *e.g.* sedimentation or elutriation. HUBER[1] used diatomaceous earth particles of about 30 μ diameter. The same author[2] recently described the use of a 5–15 μ diameter porous support obtained by a decantation method. However, this method is suited only for the preparation of small quantities of packing material. A more general application of high-speed, high-efficiency liquid–liquid chromatography can be achieved only if suitable porous support materials of small particle size (*e.g.* 10 μ) can be prepared in sufficient quantities and at low cost.

THEORY

HUBER AND QUAADGRAS[3] derived the following relation between plate height H and interstitial linear velocity v_e of the eluent.

$$H = \frac{A_1 d_p}{1 + A_2[D_m/(v_e d_p)]^{1/2}} + \frac{B}{v_e} + C\left(\frac{k^*}{1 + k^*}\right)^2 d_p^{3/2} \cdot v_e^{1/2} + D\frac{k^*}{(1 + k^*)^2}\frac{d_p^2 v_e}{D'_s} \quad (1)$$

where

$$k^* = \frac{\varepsilon_l + \varepsilon_s K}{\varepsilon_e} \quad (1a)$$

The first and second terms on the right-hand side represent the peak broadening due to dispersion and molecular diffusion in the interstitial mobile phase. The third term makes allowance for the finite rate of mass exchange between the interstitial mobile phase and the stationary fluid retained in the support material, the latter corresponding to the volume fraction $\varepsilon_i + \varepsilon_s$. The last term denotes the contribution of the slow mass transfer in the stationary liquid.

At the eluent velocities normally employed in liquid–liquid chromatography the interstitial mixing terms contribute a value of between 2 and 10 times the particle diameter d_p to the plate height H, provided that the column is properly packed[4–6]. At high eluent velocities, the plate height will always be determined by the mass-transfer in the stationary liquid, *i.e.* by the last term in eqn. 1. A decrease in particle size will result in lower H-values, which in its turn, permits the use of higher fluid velocities. If a separation between the components A and B requires N_r theoretical plates, the retention time t_{RB} of the component to be eluted last (the analysis time) can be calculated from

$$t_{RB} = N_r(1 + k^*_B)\frac{H}{v_e} \tag{2}$$

with

$$N_r = \frac{16\,R^2(\varepsilon_e + \varepsilon_i + \varepsilon_s K_B)}{\varepsilon_s^2(K_B - K_A)} \tag{3}$$

where R represents the required resolution between the peaks of the components A and B. Fast analysis calls for a low value of H/v_e; this can be obtained only at high fluid velocities and small mean particle sizes[7].

The flow volume under conditions of laminar flow through packed beds can be calculated from Darcy's law

$$\Phi = K_o \cdot \frac{Q\Delta p}{\eta L} \tag{4}$$

where Q is the column cross-section, Δp the pressure drop, η the viscosity of the mobile phase and L the column length. For the linear interstitial velocity v_e we then find

$$v_e = \frac{\Phi}{\varepsilon_e Q} = \frac{K_0}{\varepsilon_e} \cdot \frac{\Delta p}{\eta L} \tag{5}$$

The specific permeability K_0 can be calculated from

$$K_0 = \frac{d^2_p}{\Psi} \frac{\varepsilon^3_e}{(1 - \varepsilon_e)^2} \tag{6}$$

where Ψ is a constant, which depends *inter alia* on the particle shape. Substituting (6) in (5) and replacing L by NH we get

$$\frac{\Delta p}{N} = \frac{\Psi \eta v_e H}{d_p^2}\left(\frac{1 - \varepsilon_e}{\varepsilon_e}\right)^2 \tag{7}$$

HORVATH[8] and HUBER[7] indicated that the conditions for high-speed, high-efficiency liquid–liquid chromatography can be derived from eqns. 1, 2 and 7. Δp is taken as high as the operating conditions allow. The number of plates needed to achieve the required resolution follows from eqn. 3. The maximum value of $\Delta p/N$ is thus defined.

At a given value of ε_e, $v_e H/d_p^2$ will then be constant; a decrease of d_p will therefore involve a decrease of $v_e H$. It can be seen from eqn. 1 that in the velocity region where mass exchange contributes substantially to H, reduction of the particle size d_p at constant velocity v_e results in a more than proportional decrease of H: in consequence, the quotient H/v_e and hence the time of analysis will diminish upon a decrease of d_p at constant Δp and ε_e. The interparticle porosity strongly influences $\Delta p/N$. Normally, packed beds prepared from the usual chromatographic support materials have an interparticle porosity of about 0.4. In packed capillaries as are used in gas chromatography stable packed beds with interparticle porosities of between 0.6 and 0.8 can be obtained by reduction of the column to particle diameter ratio. An increase of the interparticle porosity results in higher column permeability and lower pressure drops.

Packed beds in chromatographic columns composed of small irregularly shaped particles usually show higher interparticle porosities and, hence, higher permeabilities than packed beds[9,10] of large, smooth particles. It is quite obvious, however, that for successful application of the former type of packing in liquid chromatography two conditions have to be fulfilled. The packing structure has to be sufficiently regular to avoid channelling, which strongly affects the convective dispersion expressed in the first term of eqn. 1; further, the packed bed must be sufficiently stable at the pressure drops needed for elution at the desired fluid velocities.

PREPARATION OF SUPPORT MATERIALS

Sizing of porous support materials by dry sieving can be achieved down to 30 μ. Below this range use has to be made of other techniques. Batch separation by elutriation of an ion-exchange resin into size fractions with a mean particle diameter of less than 40 μ has been described by HAMILTON[11]. SCOTT[12] developed a continuous elutriation method for preparing narrow size fractions of spherical ion-exchange beads in the size region between 5 and 40 μ. The material to be fractionated is fed continuously as a suspension into an upward flow of fluid. Small particles are entrained, whereas large

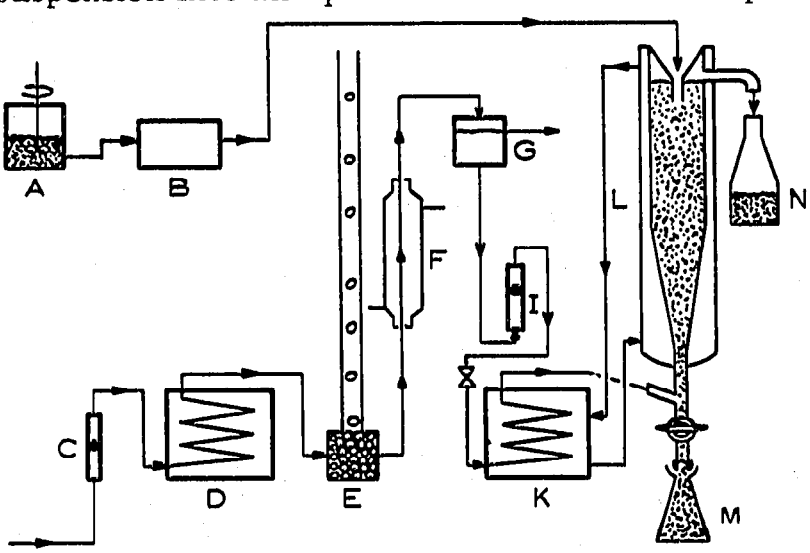

Fig. 1. Apparatus for preparation of porous supports by continuous elutriation. A = feed supply; B = slurry pump; C = rotameter; D = thermostat; E = degasser; F = cooler; G = constant level water supply; I = rotameter; K = thermostat; L = elutriation tube; M = bottom product receiver; N = overhead product receiver.

ones are allowed to settle. A special shape of the elutriation tube eliminates problems due to the laminar flow. We used a slightly modified version of this method for separating porous support materials.

It appeared to be necessary to extend SCOTT's equipment with an effective device for degassing the water to be used in the elutriation and to thermostat the elutriation tube (Fig. 1).

The starting material was Hyflo Super-Cel, a flux-calcinated diatomaceous earth of John's Manville with 80 % of the particles smaller than 20 μ. In a first separation, the flow rate was adjusted so as to permit collection of particles smaller than 15 μ in the overhead receiver. The flow conditions for a specific separation were always calculated from Stokes formula on the assumption of a flat flow profile in the elutriation tube and spherical particle shape. The overhead product was submitted to a subsequent elutriation under such conditions that particles smaller than 10 μ were washed out. The particle size distribution of the product collected in the bottom receiver was determined by means of a Coulter-counter; the distribution curve was nearly symmetrical and showed a mean particle size of 9.2 μ and a standard deviation of about 3 μ (Fig. 2). Other sized fractions have been prepared after suitable variation of the flow rate.

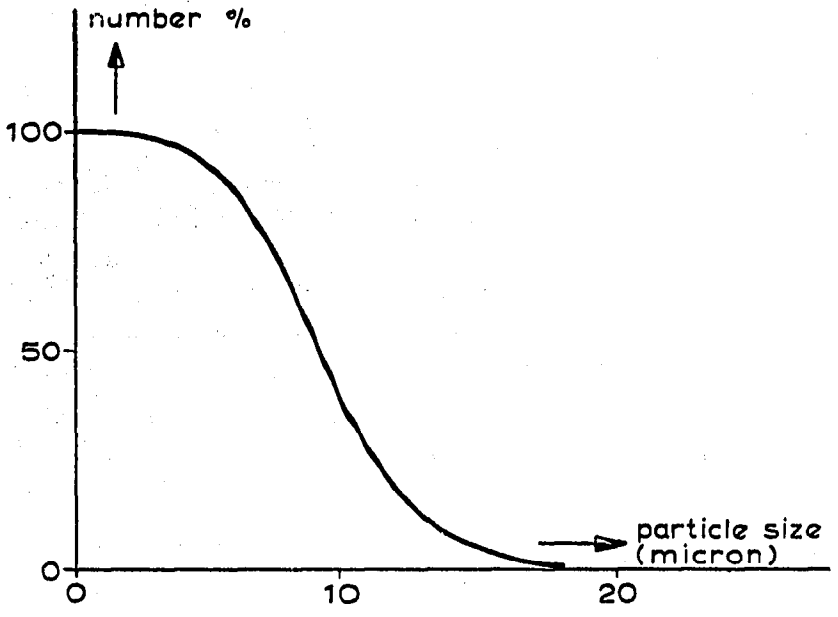

Fig. 2. Particle size distribution curve for a size fraction of porous support material prepared from Hyflo Super-Cel by continuous elutriation.

The method yields reasonable quantities of sized materials with a minimum of the operator's effort. Two runs of 48 h each yielded 200 g of material with a mean size of 9.2 μ.

CHROMATOGRAPHIC EXPERIMENTS

The apparatus for liquid-liquid chromatography was constructed in our laboratory. The eluent was pumped by a Milton Roy Minipump, while pressure pulsations were damped by means of a Bourdon spring combined with a capillary restriction[1].

The eluent was equilibrated with the fixed bed in a precolumn, placed between the damping device and the injection port; this precolumn was filled with a coated packing of the same type but of a much coarser mesh size than that used in the separation column. The separation column was made of thick-walled precision-bore straight glass tubes with an internal diameter of 4 mm and a length of 20 or 40 cm. The columns were thermostated. Low dead-volume end-pieces were used to avoid extra column peak-broadening. The design of the injection port was similar to that described by HALASZ *et al.*[13]. Injection was done with a Hamilton 10-μl syringe; good results were obtained only if during injection the tip of the needle was placed exactly in the upper layer of the packing material. The detector was a Zeiss PMQ-II spectrophotometer equipped with a low dead-volume flow-cell[17]. Connections between column and detector were made of 0.5 mm I.D. teflon tubing.

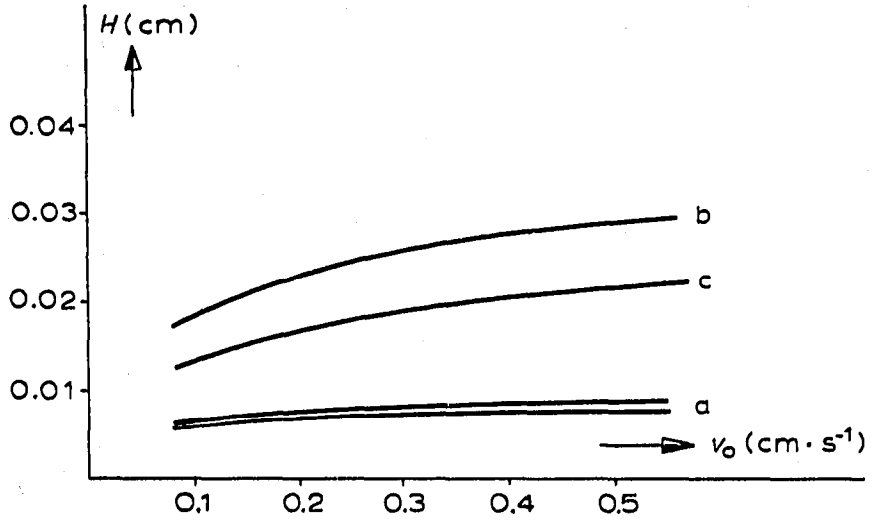

Fig. 3. H–v_0 curves for butylbenzene in a column (20 cm × 4 mm) filled with uncoated support material (d_p = 9.2 μ). (a) Normal curves, quantity of packing material 640–680 mg; (b) poor-quality column, quantity of packing material 600 mg; (c) poor-quality column, quantity of packing material 770 mg.

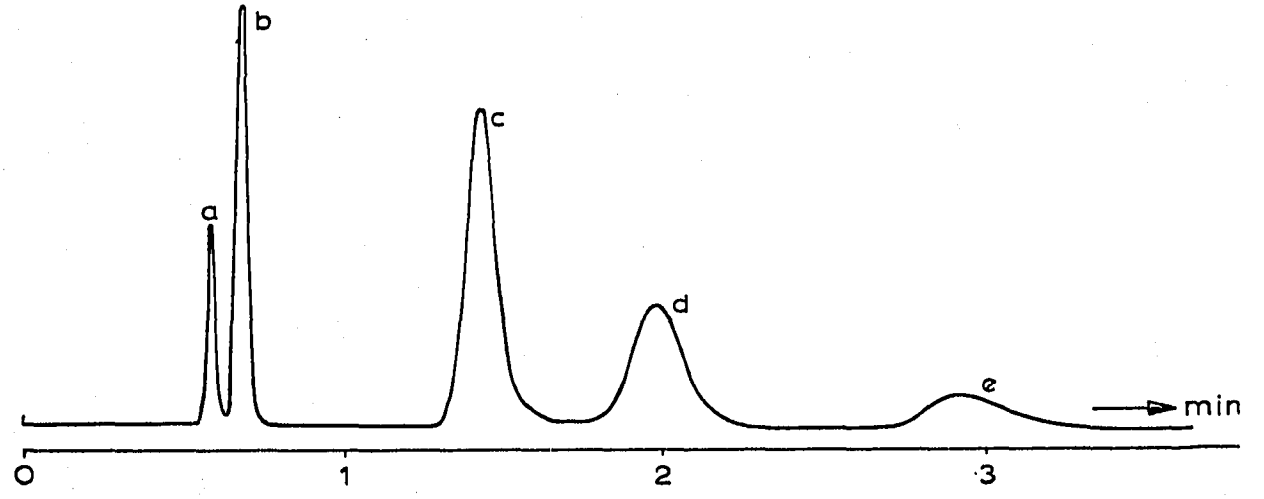

Fig. 4. Separation of phenol and nitrophenol isomers. Column 18 cm × 4 mm, inlet pressure 3.2 atm. (a) Butylbenzene ($k' \sim 0$); (b) *o*-nitrophenol; (c) phenol; (d) *m*-nitrophenol; (e) *p*-nitrophenol. Injection: 1 μl solution in eluent ($\varepsilon_e + \varepsilon_i$ = 0.80, ε_e = 0.07).

A ternary liquid–liquid system was prepared from 2,2,4-trimethylpentane–ethanol–water (85:12.5:2.5, w/w); the less-polar upper layer was used as eluent and the polar lower layer as stationary phase. The use of these ternary systems in liquid–liquid chromatography has been described by HULSMAN[14]. The porous support was coated by addition of small quantities of the stationary liquid to weighed samples of the dry support and subsequent shaking. Columns were filled with small portions of the coated porous support; every portion was compressed with a glass rod fitting closely in the column.

To examine the quality of the packed beds containing the small-size porous support, plate-height curves were determined for butylbenzene on a column filled with uncoated material (Fig. 3). As expected, the packing density strongly influences the peak dispersion caused by convection.

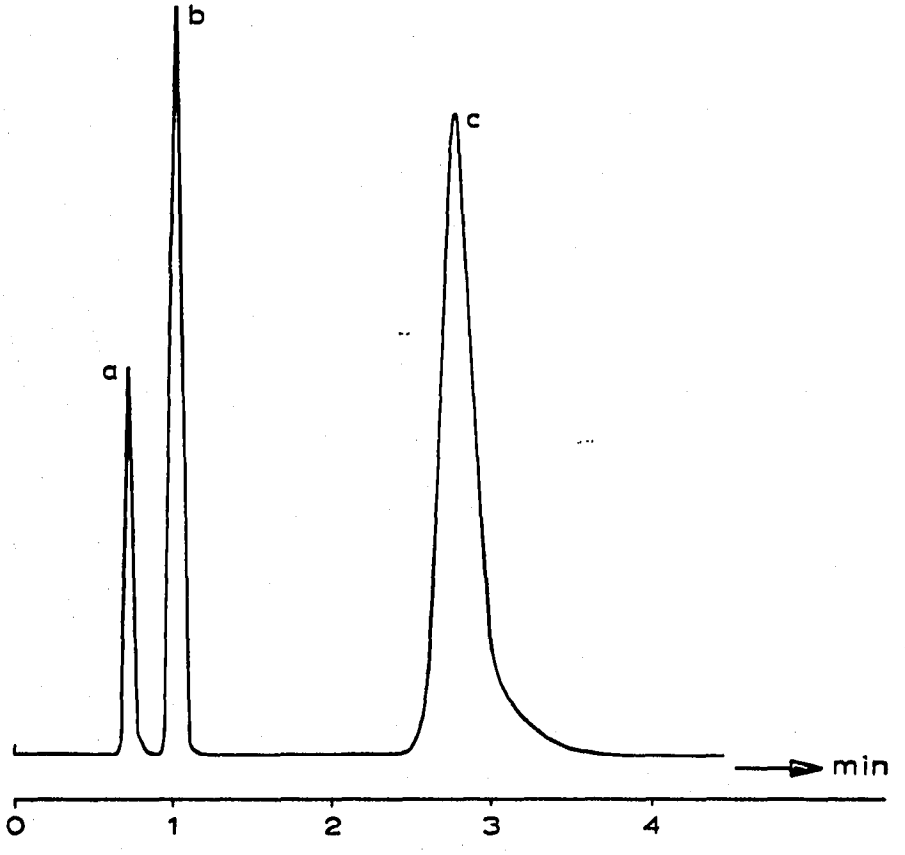

Fig. 5. Example of application of the porous support material in fast liquid chromatography for separation of: (a) octahydrophenazine; (b) cyclohexanone-oxime and (c) caprolactam. For detection of (a): λ = 290 nm. For detection of (b) and (c): λ = 210 nm. Column 18 cm × 4 mm, inlet pressure 3 atm. Injection: 1 μl solution in eluent.

Some examples of separations in columns containing coated supports are given in Figs. 4 and 5; a plate-height curve for such a column is shown in Fig. 6. The linear velocity of the eluent, v_0, was calculated from the retention time of a substance with $k' \sim 0$; the relation between v_0 and the interstitial linear velocity v_e is given by $v_0 = \varepsilon_e v_e/(\varepsilon_e + \varepsilon_t)$.

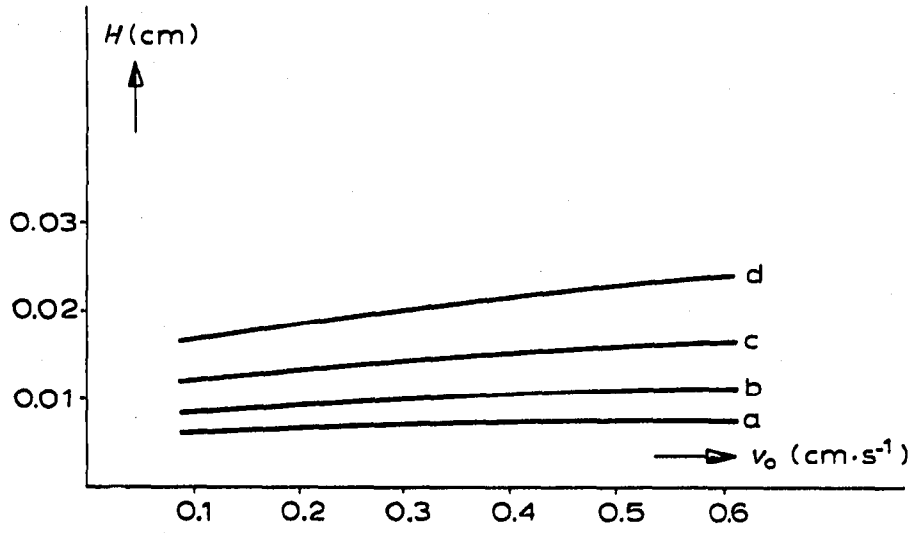

Fig. 6. H–v_0 curve for the column shown in Figs. 4 and 5. (a) Butylbenzene ($k' \sim 0$); (b) *o*-nitrophenol ($k' = 0.16$); (c) phenol ($k' = 1.45$); (d) *m*-nitrophenol ($k' = 2.50$).

DISCUSSION

High permeability is a characteristic feature of the columns packed with the porous support material described in this work (Table I).

TABLE I

PERMEABILITY OF COLUMNS PACKED WITH VARIOUS SUPPORT MATERIALS USED IN LIQUID CHROMATOGRAPHY

Support	*Size* (μ)	$K_0 \times 10^8$ (cm^2)
Chromosorb W	50–63	10
Silica gel	60–90	14
Hyflo Super-Cel	9 ± 3	1.1

The interparticle porosity ε_e of packed beds of Hyflo Super-Cel calculated by means of eqns. 4 and 6 on the assumption that $\Psi \approx 180$ will be approximately 0.6. These packings have been found to retain their stability at pressure gradients up to 1 atm·cm^{-1} (Fig. 7). Higher pressure gradients tend to compress the packing and lower the permeability.

As can be observed by electron-microscopy, the finished packing material consists of irregularly shaped, almost flat particles with a large number of perforations of 0.1–1 μ diameter. This irregular shape of the individual particles results in a more or less irregular structure of the packed bed, which in its turn gives rise to an increase in convective dispersion[9,15].

However, the reduced plate height for inert substances in a column packed with a coated 9.2 μ Hyflo Super-Cel support was found to be slightly greater than that measured by HUBER[1] on a very homogeneous 28–32 μ fraction of diatomaceous earth material (Fig. 8). This indicates a rather uniform packing of the small size support.

The contributions to the plate height from mass exchange and mass transport phenomena, as expressed in the third and the fourth term in eqn. 1 will be low, partly owing to the small particle size, partly to the structure of the packing material and the consequent favorable distribution of the stationary phase.

Compared with other supports, the packing material described in this work gives a very good performance, even if the pressure gradient has to be kept below a

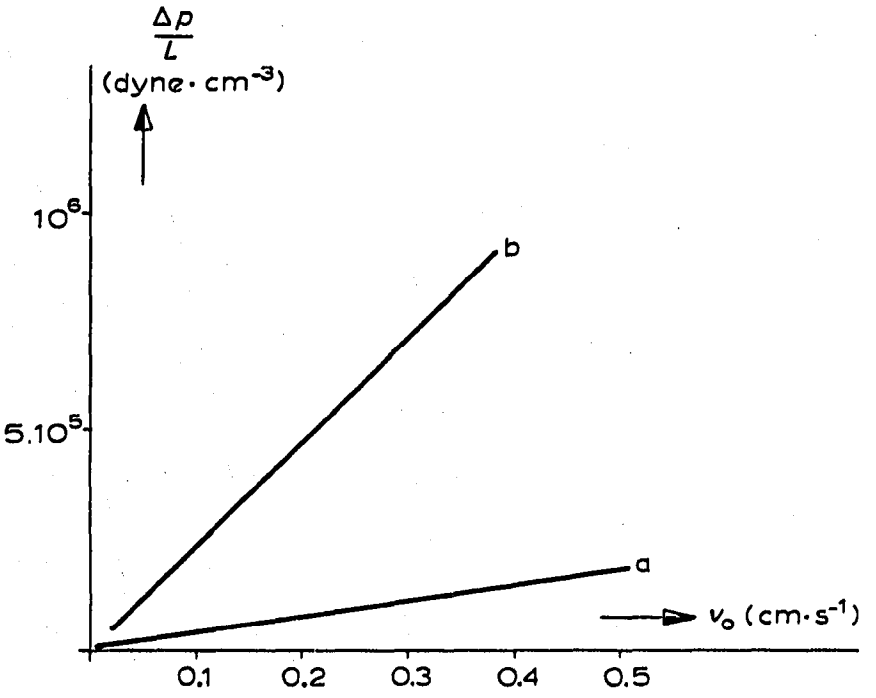

Fig. 7. Relation between liquid flow and pressure gradient for 9.2-μ support material. Mobile and stationary phase prepared from the ternary system 2,2,4-trimethylpentane–ethanol–water (85:12.5:2.5). (a) Mobile phase: a-polar layer; (b) mobile phase: polar layer (reversed phase chromatography on silanized support).

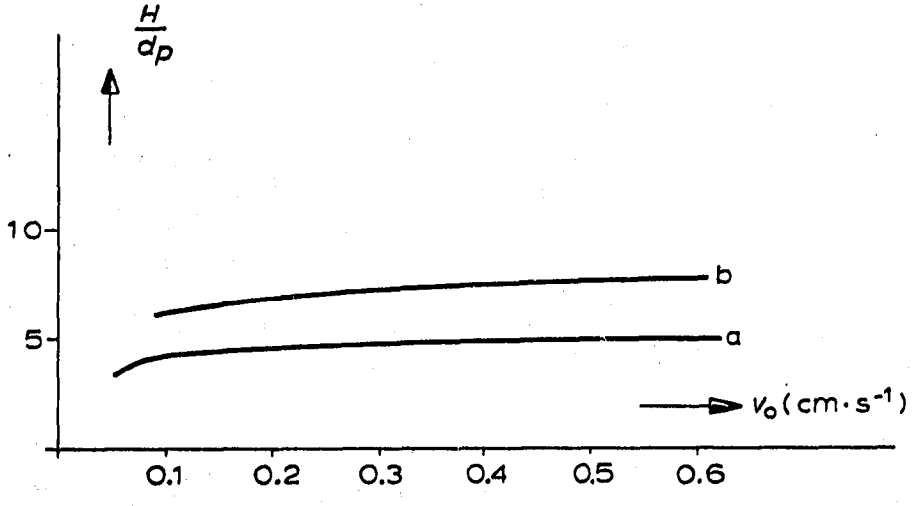

Fig. 8. Comparison of reduced plate heights of inert components in columns filled with a porous support material. (a) Diatomaceous earth[1] 28–32 μ; (b) Hyflo Super-Cel, mean particle size 9.2 μ.

given limit. As shown in Fig. 9, H/v_0–v_0 curves are comparable to those reported for other porous supports[1,2] and for the controlled surface porosity (C.S.P.) packings[16]; the material is very suitable for use in fast analysis work.

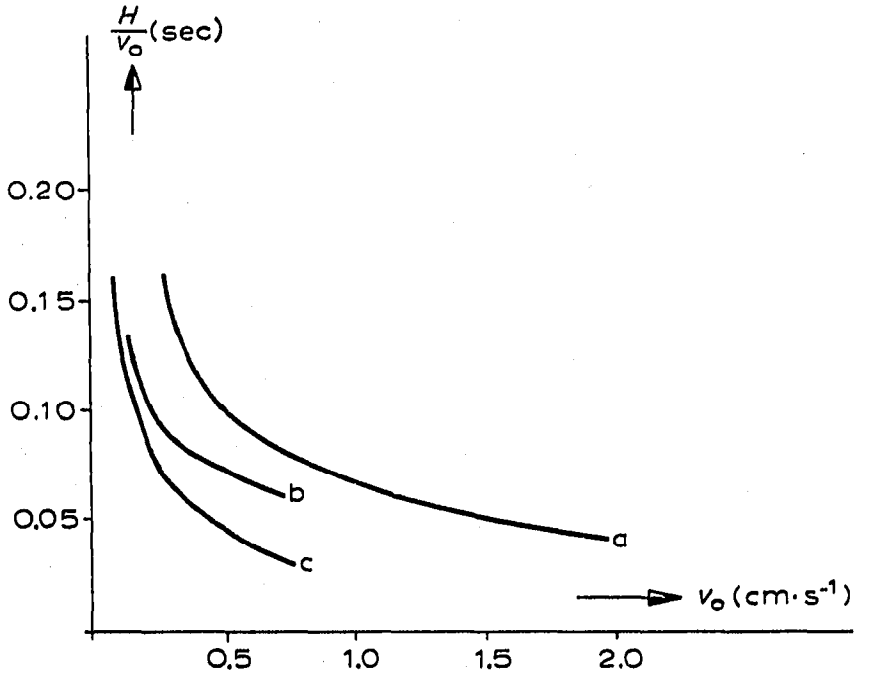

Fig. 9. Comparison of H/v_0–v_0 curves for some packing materials. (a) C.S.P. packing, $k' = 1.4$ (ref. 16); (b) diatomaceous earth 5–15 μ, $k' = 9.4$ (ref. 2); (c) Hyflo Super-Cel, mean particle size 9.2 μ; $k' = 3.4$.

LIST OF SYMBOLS

A_1, A_2	coefficient	—
B	coefficient	$cm^2 \cdot sec^{-1}$
C	coefficient	$sec^{\frac{1}{2}} \cdot cm^{-1}$
d_p	particle size	cm
D	coefficient	—
D_m	binary diffusion coefficient of the component in the mobile phase	$cm^2 \cdot sec^{-1}$
D_s'	apparent diffusion coefficient of the component in the stagnant fluid	$cm^2 \cdot sec^{-1}$
H	plate height	cm
k^*	capacity factor, according to (1a)	—
k'	capacity factor $\varepsilon_s K/(\varepsilon_e + \varepsilon_i)$	—
K	partition coefficient	—
K_0	specific permeability	cm^2
L	column length	cm
N	plate number	—
Q	surface of the cross section of the column	cm^2
t_{RX}	retention time of component X	sec
v_e	linear interstitial velocity	$cm \cdot sec^{-1}$

v_0	linear velocity of a component with $K = 0$	$cm \cdot sec^{-1}$
Δp	pressure drop	$dyne \cdot cm^{-2}$
ε_e	interparticle porosity	—
ε_i	intraparticle porosity, portion filled with mobile phase	—
ε_s	intraparticle porosity, portion filled with stationary phase	—
η	viscosity of the mobile phase	$dyne \cdot sec \cdot cm^{-2}$
Φ	volume-flow	$cm^3 \cdot sec^{-1}$
Ψ	constant	—

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