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OPTIMIZATION AND EVALUATION OF PACKED CAPILLARY COLUMNS FOR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

Packed capillary columns were systematically optimized for adsorption high-performance liquid chromatography, and their performance was evaluated using conventional plate height *vs.* linear velocity curves and the Knox separation impedance. Column performance was improved by decreasing the inner diameter, nominal particle size, and mean pore size of the silica adsorbent. Irregularly shaped silica particles gave consistently higher efficiencies than spherical materials. The performance of packed capillary columns, as measured by the Knox separation impedance, is currently equal to or exceeds that of conventional or microbore packed columns.

INTRODUCTION

The development of miniaturized systems in high-performance liquid chromatography (HPLC) has generated a great deal of interest during recent years. The HPLC microcolumns, with reduced inner diameters and increased column lengths, have considerably higher chromatographic efficiency than their conventional counterparts. Yet another desirable attribute of these microcolumns is their characteristically low flow-rates, which result in significantly reduced consumption of both sample and mobile phase. The economic and environmental impacts of the reduced solvent consumption are immediately obvious, but additional advantages are emerging with the increased development and use of micro-HPLC[1]. First, "exotic" mobile phases, which are economically prohibitive in conventional HPLC, may be utilized to improve either the separation or detection of solutes. In addition, new detection and ancillary techniques become available which could not be previously developed with conventional columns; a directly interfaced HPLC–mass spectrometer[2] and selective flame-based detectors[3,4] are among representative examples.

The HPLC columns that are currently under development in different laboratories, *i.e.*, open tubular, packed capillary, and packed microbore column types, require extensive evaluation for various analytical purposes. The theoretical potential and analytical merit of different microcolumn types have been under continual discussion[5–8], but, clearly, much remains to be done in this potentially important area. Meaningful column investigations have frequently been hindered by inadequate instrumentation.

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While open tubular columns have the greatest potential performance, there are numerous technological problems associated with their use[8]. We currently view the packed capillary column[9] as a practical intermediate between conventional columns and the highly efficient open tubular columns. Packed capillaries have slightly larger inner diameters than open tubular columns; hence, injection and detection volumes (on the order of 100 nl) are not as critical. In addition, the greater overall surface area of packed capillaries allows the injection of larger samples without column overloading, and the sensitivity requirements of the detection systems are thereby reduced. Most importantly, the high efficiencies and low flow-rates (typically 1 μl/min) characteristic of open tubular columns are maintained with packed capillaries.

Packed capillary columns for HPLC have not been adequately characterized prior to this communication. Hence, we report here our investigation of various structural parameters which influence the efficiency of such columns. Column performance was evaluated using both conventional Van Deemter plots and the Knox separation impedance[10]. Comparisons were made with some previously reported results for both conventional and open tubular columns.

The concept of a "performance index" was first discussed by Golay[11], when comparing the efficiencies of open tubular and packed columns in gas chromatography. Such a performance index allows the comparison of different column types under dissimilar chromatographic conditions, and concurrently permits the correlation of experimental results with theoretical column performance. The Knox separation impedance[10] appears to provide a suitable equivalent for HPLC investigations. It is defined as:

$$E = \frac{t_r \, \Delta P}{N^2 \eta \, (1 + k)} = \frac{H^2}{K^0} = h^2 \varphi' \tag{1}$$

The Knox separation impedance (E) evaluates column performance not only on the basis of total plate number (N), but also considers the time of analysis (t_r) and pressure (ΔP) required to achieve the separation. In addition, a correction factor is included for the apparent effects of solvent viscosity (η) and capacity factor (k). It can be easily shown that E is equal to the square of the plate height (H) divided by the chromatographic permeability (K^0), or, alternatively, the square of the reduced plate height ($h = H/d_p$, where d_p = particle diameter) multiplied by a column resistance factor ($\varphi' = d_p^2/K^0$). Optimum column performance is thus achieved with minimum plate height and maximum permeability.

Theoretical minimum values for E can be estimated from the minimum reduced plate height (h_{min}) and typical column permeability[7]. For conventional and microbore packed columns. h_{min} is 2, and the column resistance factor is typically between 500 and 1000; thus, the minimum E value is predicted to be approximately 2000. According to Knox[7], packed capillary columns should have the same h_{min} value, but much higher permeability ($\varphi' \approx 150$); hence, the E value could be as low as 600. Open tubular columns exhibit a lower h_{min} value ($h_{min} = 0.8$), as there is no contribution from multiple flow paths. In addition, the column resistance parameter is significantly reduced ($\varphi' = 32$), and the E value may be as low as 20 under optimal conditions. Thus, open tubular columns should have a 100-fold advantage in performance over conventional or microbore packed columns. This advantage may be

manifested in several ways, at the discretion of the analyst. For example, if time of analysis and inlet pressure are relatively unimportant, then extremely high plate numbers may be achieved. Conversely, if moderate chromatographic efficiency is acceptable, then very fast separations are possible.

EXPERIMENTAL

In this investigation, it was imperative to carefully control all variables either known or suspected to have an influence upon the chromatographic band-broadening processes. Adsorption chromatography was utilized to eliminate the variability in surface coverage of the solid support which may occur with bonded or mechanically-coated stationary phases. Moreover, the silica packing materials were all of the same origin in order to minimize intercolumn variation in chemical composition, particle structure and porosity. LiChrosorb and LiChrospher siliceous materials, manufactured by E. Merck Reagents (Darmstadt, G.F.R.), were utilized exclusively in this investigation.

Packed capillary columns were prepared by first dry-packing standard-bore Pyrex glass tubing with the silica adsorbent and, subsequently, extruding the capillary with a glass-drawing machine (Model GDM-1B; Shimadzu Seisakusho, Kyoto, Japan). The column inner diameter, particle size, particle shape, and pore size were systematically varied, while the column length (26.4 m) and coiling radius (14 cm) remained constant.

The model sample used to evaluate column performance contained toluene, nitrobenzene, acetophenone, indole and 1-chloro-2,4-dinitrobenzene, and was eluted with 0.3% methanol in hexane as the mobile phase. All reagent-grade solutes were obtained from Aldrich (Milwaukee, WI, U.S.A.), and were further purified by vacuum-line distillation or recrystallization. Spectral-grade solvents were purchased from Fisher Scientific (Fair Lawn, NJ, U.S.A.). Perfluorohexane, obtained from PCR Research Chemicals (Gainesville, FL, U.S.A.), was utilized to measure the elution time of a non-retained solute.

The chromatographic system utilized in this investigation was similar to that described previously by Tsuda and Novotný[9]. A syringe pump (Model 8500; Varian Instrument Division, Palo Alto, CA, U.S.A.), operated under the conditions of constant pressure, provided uniform flow through the packed capillary column with minimal pulsation. Direct on-column sample introduction was achieved with a novel low-volume injection system[12], which delivered typically 100-nl samples for the presented data. The solutes were detected using a variable-wavelength UV-absorbance detector (Uvidec 100-II; Japan Spectroscopic Co., Tokyo, Japan), with a modified 0.15-μl cell.

For each column, the test chromatogram (Fig. 1) was recorded in triplicate at several linear velocities between 0.3 and 2.0 cm/sec. The data from these chromatograms were utilized to generate plate height (H) *versus* linear velocity (u) curves, which were compared with the Taylor equation for an open tubular column of the same inner diameter. The Taylor equation[13] expresses the relationship between plate height and velocity for a non-retained peak in an open tube:

$$H = \frac{r^2}{24D_m} u \tag{2}$$

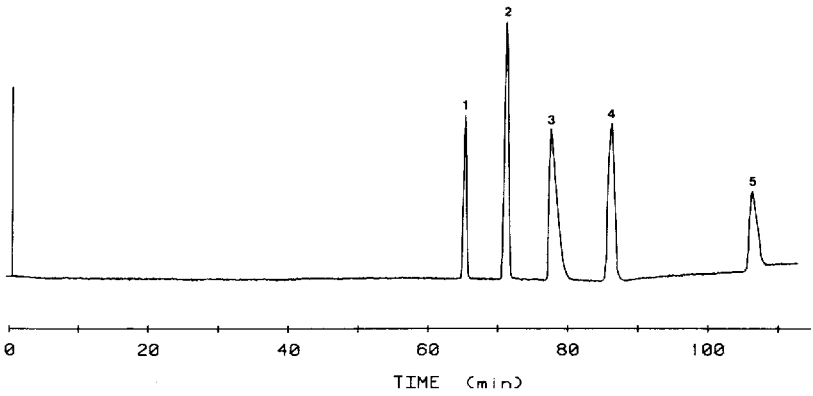

Fig. 1. Test chromatogram for the evaluation of packed capillary column performance. Column: 30-μm LiChrosorb Si-100, 26.4 m × 100 μm I.D. Mobile phase: 0.3 % methanol in hexane at 0.72 cm/sec. Solutes: 1 = toluene (k = 0.01); 2 = nitrobenzene (k = 0.10); 3 = acetophenone (k = 0.20); 4 = indole (k = 0.33); 5 = 1-chloro-2,4-dinitrobenzene (k = 0.64). Detector: Jasco Uvidec 100-II (254 nm).

where r is the column radius, and D_m is the diffusion coefficient of the solute in the mobile phase. The binary solute–solvent diffusion coefficients were estimated from the Wilke–Chang equation[14] to be $3.7 \cdot 10^{-5}$ cm^2/sec for toluene, $3.8 \cdot 10^{-5}$ cm^2/sec for nitrobenzene, $3.6 \cdot 10^{-5}$ cm^2/sec for acetophenone, $4.0 \cdot 10^{-5}$ cm^2/sec for indole, and $3.5 \cdot 10^{-5}$ cm^2/sec for 1-chloro-2,4-dinitrobenzene in hexane at 20°C. The chromatographic data were also utilized to determine the relationship between H and k for each column. These curves were graphically compared with the mobile phase resistance-to-mass-transfer term calculated from the Golay equation[11]:

$$H_m = \frac{1 + 6k + 11k^2}{24\,(1 + k)^2} \cdot \frac{r^2}{D_m} \cdot u \tag{3}$$

This comparison does not account for the contributions to the total plate height from the multipath and longitudinal diffusion terms, which are thought to be independent of the capacity ratio, nor does it consider the effect of resistance to mass transfer in the stationary phase. Ohmacht and Halász[15] have shown for various silica packing materials that stationary phase mass-transfer processes are negligible in comparison with those in the mobile phase. Nevertheless, only the general shape of the theoretical curve should be used for comparison, and not the absolute magnitude.

To further characterize the packed capillary columns, K^0 was calculated:

$$K^0 = \frac{L^2\,\eta}{t_m\,\Delta P} \tag{4}$$

where L is the column length, η is the mobile phase viscosity, t_m is the elution time of a non-retained solute, and ΔP is the difference between column inlet and outlet pressures. The E value was then calculated using eqn. 1, and the packed capillary columns were subsequently compared with the theoretical performance of conventional packed and open tubular columns.

The interparticle porosity was estimated from the modified Kozeny–Carman equation[16,17]:

$$K^0 = \frac{d_p^2}{180\ \Psi^2} \frac{\varepsilon_u^3}{(1 - \varepsilon_u)^2\ (\varepsilon_u + \varepsilon_i)} \tag{5}$$

where ε_u and ε_i are the inter- and intra-particle porosity, and Ψ^2 is a structural constant, equal to approximately 1.7 for porous, non-spherical packings. The intra-particle porosity was estimated by Ohmacht and Halász[18] to be 0.37–0.38 for Li-Chrosorb Si-100 and Si-60 materials.

RESULTS AND DISCUSSION

For conventional packed columns, it is well-established that particle size is the characteristic column dimension to be used in theoretical calculations. It is equally evident that column diameter is the dimension to be considered for open tubular columns. However, the situation for packed capillary columns is not so straightforward, since the column diameter is typically only two to five times larger than the particle size. This ambiguity is exemplified by our initial use of reduced parameters (h, $\nu = ud_p/D_M$, φ') to describe column performance. If the particle size is chosen as the characteristic column dimension (as is normally done for conventional packed columns), reduced plate heights are unreasonably large and the column resistance parameter is frequently less than that of an open tube ($\varphi' = 32$). If the inner diameter is chosen as the characteristic column dimension (as is consistent with the evaluation of open tubular columns), reduced plate heights less than the theoretical minimum ($h = 2$) are obtained. We have attempted to circumvent these anomalous conclusions by the use of conventional parameters (H, u, K^0) and the Knox separation impedance to describe column performance. Unfortunately, the ambiguity in choosing the appropriate dimension (r or d_p) will also extend to the plate-height equation as well. The H values obtained experimentally are less than theoretically predicted by the Van Deemter equation using column diameter, which overestimates the magnitude of the multipath term, but greater than predicted by particle size, which underestimates the contribution from resistance to mass transfer in the mobile phase. Hence, the efficiency of packed capillary columns cannot be predicted theoretically with the conventional treatment for packed or open tubular columns.

In the following results, the packed capillaries have been compared with an open tubular column of the same inner diameter. Some contribution to the total plate height will result from multiple paths around the loosely packed particles; thus, the plate height will always be greater than the theoretical model. The presence of large stagnant regions of mobile phase between particles may increase the resistance-to-mass-transfer term relative to the open tubular model; however, enhanced radial mass transport could simultaneously reduce the effects of slow diffusion in the mobile phase.

Effect of inner diameter on column performance

The Golay equation clearly indicates that a reduction in inner diameter will decrease the plate height for an open tubular column. A conventional packed column

may show an increase in plate height due to imperfect packing technology, but normally should not be affected by a decrease in column diameter.

Packed capillary columns, having inner diameters of 100, 70 and 60 μm, were prepared with LiChrosorb Si-100 of 30 μm nominal particle size. As shown in Fig. 2, a statistically significant reduction in plate height was observed with decreasing column diameter. Similar results were obtained for columns packed with 20- and 10-μm silica particles.

The decrease in plate height is not as large as predicted for an open tubular column, and this may result from several factors. First, the effect of tortuosity or multiple paths may become proportionately more significant as inner diameter is decreased. In addition, it is possible that particles do not pack as efficiently in small diameter columns. However, ε_u, evaluated using the Kozeny–Carman equation (eqn. 5) varied from 0.72 for the 100 μm I.D. column to 0.71 for the 60 μm I.D. column. This indicates that the fractional volume occupied by the mobile phase and adsorbent (phase ratio) remained essentially constant. Hence, the smaller-bore columns seem to be packed with the same efficiency as those of larger inner diameter.

K^0, calculated using eqn. 4 decreased slightly with the reduction of inner diameter, as shown in Table I. Despite this decrease in permeability, the overall column performance (as measured by E) improved significantly with smaller-diameter columns. The values of E in Table I are not minimum values, but are quoted at a convenient mobile-phase velocity, since no minimum in the H *vs.* u curve was observed in the range of velocities investigated. It should be noted that all columns examined in this study performed better than the minimum values predicted by Knox[7] for conventional or microbore packed columns ($E_{min} = 2000$).

There are two limitations to the further improvement of column performance by reducing the column diameter. First, it becomes technologically difficult to prepare columns with inner diameters less than twice the particle size. Secondly, as k increases, the reduction in plate height with inner diameter rapidly becomes less significant; at $k = 1$, all columns were essentially identical in performance (Fig. 3). This dependence on k suggests that the column diameter may be primarily reflected in the resistance-to-mass-transfer term of the Van Deemter equation. However, contrary to accepted theory, Ohmacht and Halász[15] have reported the experimental dependence of the multipath term on k for various silicious materials. Thus, no conclusive arguments can be made concerning the origin of the effect of inner diameter on column efficiency from the available data, yet this effect is beyond dispute[19].

TABLE I

EFFECT OF INNER DIAMETER ON MICROCOLUMN PERFORMANCE

Columns are described in Fig. 2. Mobile phase velocity 0.35 cm/sec. I.D. = Inner diameter; N = total plate number; H = plate height; K^0 = specific column permeability; ε_u = interparticle porosity; E = Knox separation impedance.

I.D. *(μm)*	*N*	*H* *(mm)*	K^0 *(mm²)*	ε_u	*E*
100	$1.65 \cdot 10^5$	0.160	$1.3 \cdot 10^{-5}$	0.72	1970
70	$1.89 \cdot 10^5$	0.140	$1.2 \cdot 10^{-5}$	0.71	1630
60	$2.20 \cdot 10^5$	0.120	$1.1 \cdot 10^{-5}$	0.71	1310

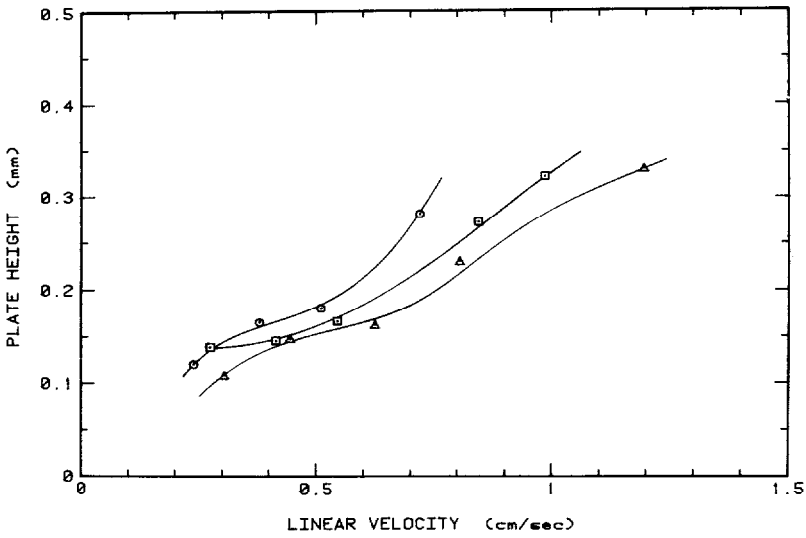

Fig. 2. Plate height *versus* linear velocity curve as a function of column inner diameter. Columns: 30-μm LiChrosorb Si-100, 26.4 m, (○) 100 μm I.D.; (□) 70 μm I.D.; (△) 60 μm I.D. Mobile phase: 0.3% methanol in hexane. Solute: toluene (k = 0.01).

Effect of particle size on column performance

Packed capillary columns, of 75 μm I.D., were prepared with LiChrosorb Si-100 adsorbent of 30 and 10 μm nominal particle size. As shown in Fig. 4, plate height decreased with particle size, as predicted by the Van Deemter equation. However, the improvement in efficiency was not as great as predicted theoretically for packed columns. This may be the result of dead volume in the injection or detection systems that becomes more significant for the smaller particles.

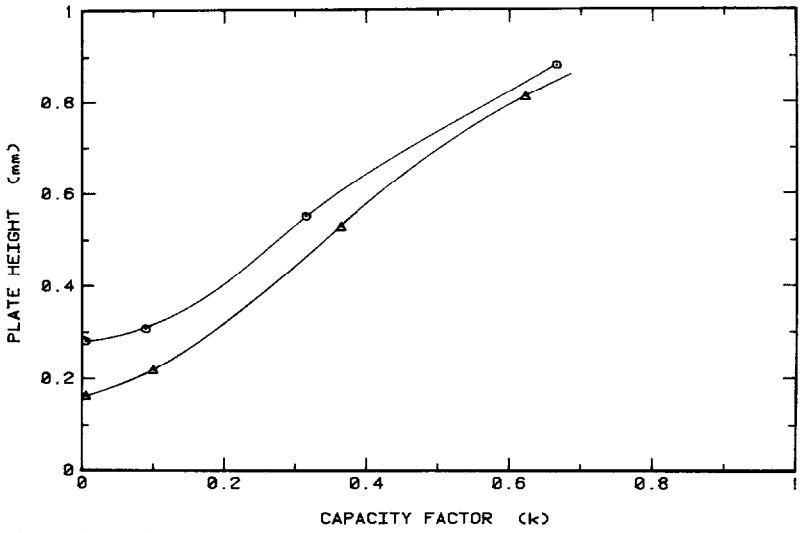

Fig. 3. Plate height *versus* capacity factor curve as a function of column inner diameter. Columns: 30-μm LiChrosorb Si-100, 26.4 m, (○) 100 μm I.D.; (△) 60 μm I.D. Mobile phase: 0.3% methanol in hexane at 0.6–0.7 cm/sec.

TABLE II

EFFECT OF PARTICLE SIZE ON MICROCOLUMN PERFORMANCE

Columns are described in Fig. 4. Mobile phase velocity 0.28 cm/sec. Symbols are defined in Table I.

d_p (μm)	N	H (mm)	K^0 (mm^2)	ε_u	E
30	$1.96 \cdot 10^5$	0.135	$1.2 \cdot 10^{-5}$	0.71	1520
10	$3.10 \cdot 10^5$	0.085	$7.7 \cdot 10^{-6}$	0.85	940

It is also probable that less efficient packing of the smaller particles will influence the plate height, as amply demonstrated for conventional packed columns. The interparticle porosity for columns packed with 30-μm particles was 0.71, while those packed with 10-μm particles had porosity of 0.85. To some extent, the amount of packing contained in the column can be increased by using standard-bore glass tubing of slightly larger inner diameter, followed by extrusion at a larger drawing ratio than currently employed. While it may be possible to pack the smaller particles more efficiently, it will be at the expense of column permeability. The K^0 value of the columns packed with 10-μm particles was considerably lower than those with 30-μm particles, as shown in Table II. By reducing the interparticle porosity from 0.85 to 0.71, the permeability of the column packed with 10-μm particles, calculated from eqn. 5, will be reduced from $7.7 \cdot 10^{-6}$ to $1.3 \cdot 10^{-6}$ mm^2. At this permeability, a plate height of 0.035 mm would be required to maintain the present level of column performance ($E = 940$, at 0.28 cm/sec). This plate height is less than that of an open tube of the same inner diameter (75 μm), and is not likely to be attained with packed capillary columns. It is most probably not desirable to seek an increase in packing

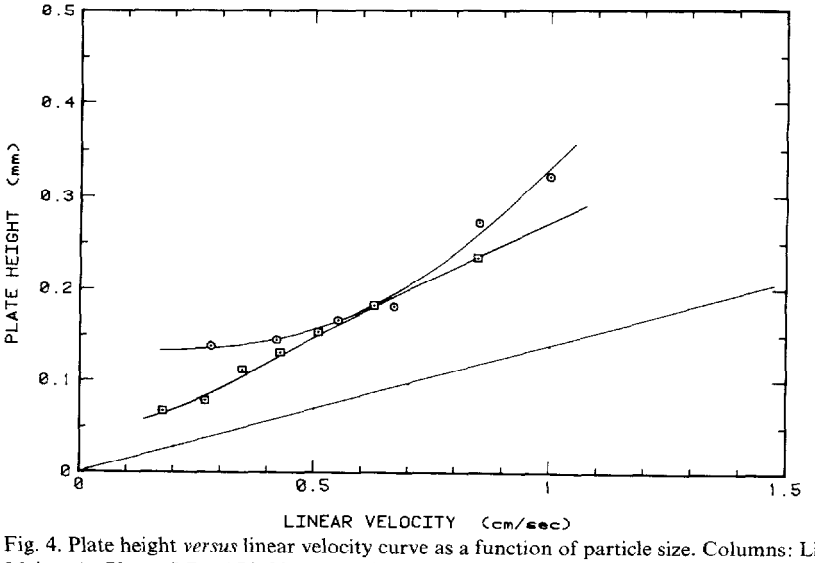

Fig. 4. Plate height *versus* linear velocity curve as a function of particle size. Columns: LiChrosorb Si-100, 26.4 m × 70 μm I.D., (○) 30 μm d_p, (□) 10 μm d_p. Mobile phase: 0.3% methanol in hexane. Solute: toluene ($k = 0.01$). Solid line represents the Taylor equation (eqn. 2) for a 70 μm I.D. open tubular capillary.

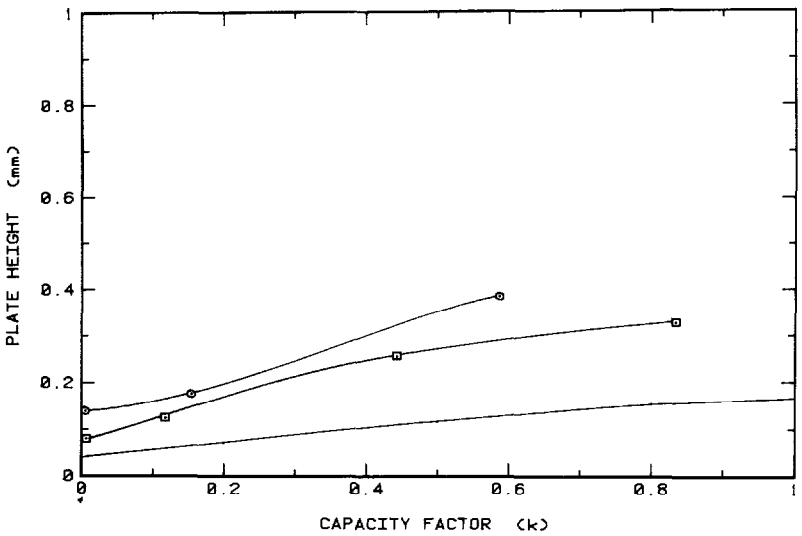

Fig. 5. Plate height *versus* capacity factor curve as a function of particle size. Columns: LiChrosorb Si-100, 26.4 m × 70 μm I.D., (○) 30 μm d_p; (□) 10 μm d_p. Mobile phase: 0.3% methanol in hexane at 0.3 cm/sec. Solute: toluene (k = 0.01). Solid line represents the mobile phase resistance-to-mass-transfer term for a 70 μm I.D. open tube, using eqn. 3.

efficiency, because, ultimately, the performance would be no better than that of a conventional packed column. The advantages of column "openness" have been widely acknowledged for both gas and liquid chromatography[5,11].

The improvement in performance for columns packed with smaller particles was maintained as capacity factor increased, as shown in Fig. 5. The distance between H *vs.* k curves for 30- and 10-μm particle sizes remained relatively constant within the range investigated ($0 < k < 1$). This indicates that particle size is most likely reflected in the multipath term, and not in the resistance-to-mass-transfer term.

Effect of particle shape on column performance

Several years ago, Unger *et al.*[20] compared the performance of spherical and irregularly shaped silica packings (1–10 μm particle size) in conventional HPLC columns. The columns exhibited nearly identical reduced plate height *vs.* reduced velocity curves; however, those packed with spherical particles had significantly higher permeability than those packed with angular silica. Thus, the E value was lower for the spherical materials, indicating improved overall column performance.

Packed capillary columns, with inner diameters of 100, 75 and 60 μm, were prepared with LiChrosorb and LiChrospher Si-100 (30 μm) silica adsorbents. The columns prepared with irregularly shaped particles had consistently lower plate heights than those prepared with spherical materials, as shown in Fig. 6. With large-bore columns, the Van Deemter curves were significantly lower for the angular materials. However, as the inner diameter was reduced, the distinction became less significant; for 60 μm I.D. columns, angular materials performed only slightly better than spherical particles. Although the mean pore size is the same for both materials (100 Å), the spherical particles have slightly less surface area than those of irregular

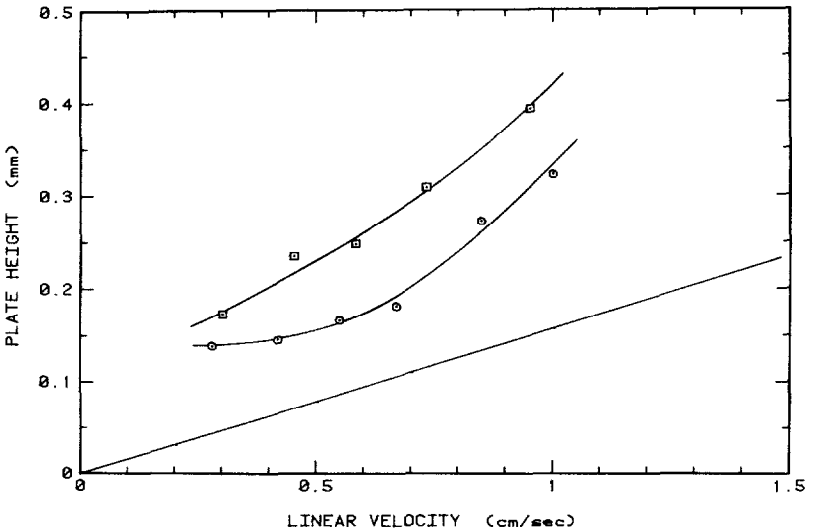

Fig. 6. Plate height *versus* linear velocity curve as a function of particle shape. Columns: 30-μm Si-100, 26.4 m × 75 μm I.D., (○) LiChrosorb (irregular), (□) LiChrospher (spherical). Mobile phase; 0.3% methanol in hexane. Solute: toluene (k = 0.01). Solid line represents the Taylor equation (eqn. 2) for a 75 μm I.D. open tubular capillary.

shape (250 and 420 m^2/g, respectively[21]). The higher plate height for spherical materials was not due to column overload, as a reduction in sample size did not influence the column efficiency. In addition, the difference in efficiency between spherical and irregular particles was maintained as capacity factor increased, as demonstrated in Fig. 7.

The specific column permeability was considerably lower for the column packed with spherical particles (Table III). At the same time, the interparticle porosity from the Kozeny–Carman equation was larger. This suggests that flow obstruction may occur more easily with the spherical materials. These particles may not become firmly imbedded in the column wall during the process of extrusion, since they lack the necessary surface roughness. When high pressure is applied to the column, the loosely packed spherical particles may be dislodged and rearranged to form obstructions. The column permeability decreased markedly with increasing pressure for the columns packed with spherical particles, but not for those containing irregularly shaped particles.

TABLE III

EFFECT OF PARTICLE SHAPE ON MICROCOLUMN PERFORMANCE

Columns are described in Fig. 6. Mobile phase velocity 0.30 cm/sec. Symbols are defined in Table I.

Shape	*N*	*H* (*mm*)	K^0 (mm^2)	ε_u	*E*
Irregular	$1.96 \cdot 10^5$	0.135	$1.2 \cdot 10^{-5}$	0.71	1520
Spherical	$1.51 \cdot 10^5$	0.175	$8.6 \cdot 10^{-6}$	0.76	3560

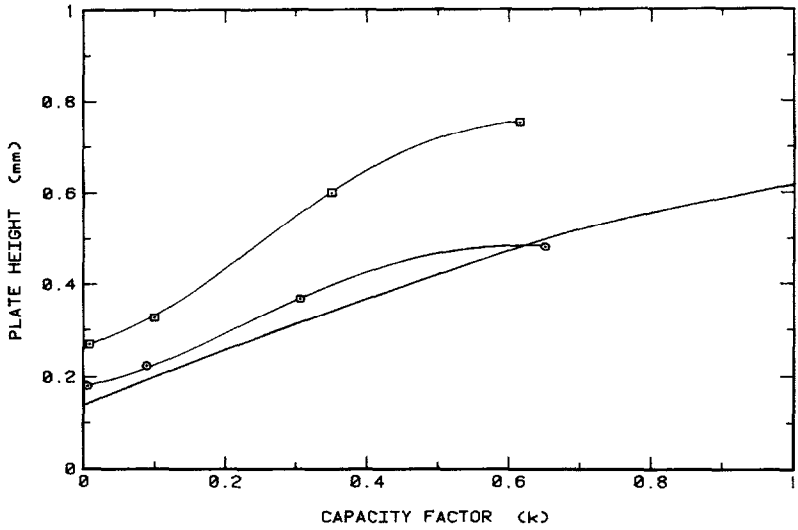

Fig. 7. Plate height *versus* capacity factor curve as a function of particle shape. Columns: 30-μm Si-100, 26.4 m × 75 μm I.D., (○) LiChrosorb (irregular), (□) LiChrospher (spherical). Mobile phase: 0.3% methanol in hexane at 0.5 cm/sec. Solute: toluene (k = 0.01). Solid line represents the mobile phase resistance-to-mass-transfer term for a 75 μm I.D. open tube, using eqn. 3.

Effect of pore size on column performance

LiChrosorb Si-60 and Si-100 were utilized to prepare packed capillary columns of 30 μm nominal particle size and 75 μm inner diameter. LiChrosorb Si-60 has a specific surface area of 550 m^2/g and mean pore size of 60 Å, whereas LiChrosorb Si-100 has 420 m^2/g of specific surface area and 100 Å mean pore size[21].

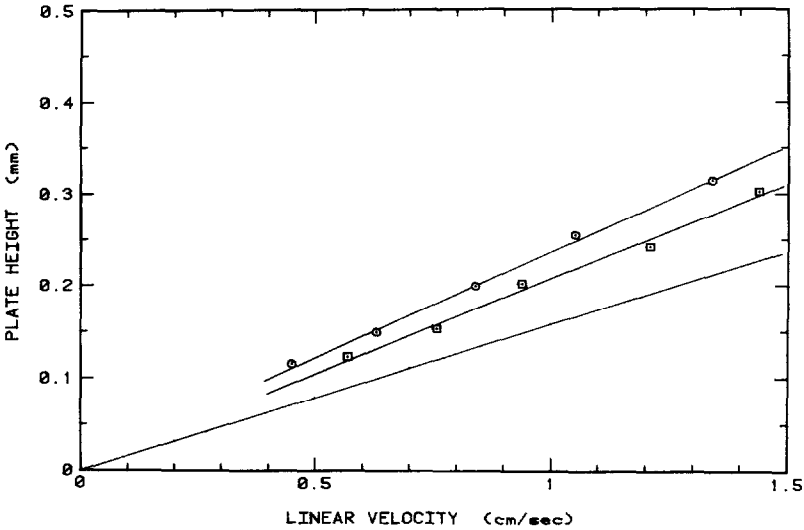

Fig. 8. Plate height *versus* linear velocity curve as a function of particle pore size. Columns: 30-μm LiChrosorb, 26.4 m × 75 μm I.D., (○) Si-100 (100 Å mean pore diameter), (□) Si-60 (60 Å mean pore diameter). Mobile phase: 0.3% methanol in hexane. Solute: toluene (k = 0.01–0.03). Solid line represents the Taylor equation (eqn. 2) for a 75 μm I.D. open tubular capillary.

The effect of pore size or surface area on efficiency is shown in Fig. 8; a significant reduction in plate height was achieved with the Si-60 packing material. It should be noted that model solutes were more strongly retained by LiChrosorb Si-60 adsorbent than by the Si-100 packing material utilized in previous studies. Hence, the difference in column efficiency shown in Fig. 8 would be even greater for solutes of the same capacity factor. More importantly, this advantage in plate height was maintained as capacity factor increased.

The improved column efficiency may be attributed to the surface structure of the silica adsorbent. Ogan and Scott[22] have determined that intraparticle characteristics, specifically pore size, may contribute to the mass-transfer term of the Van Deemter equation. This contribution is considered to be a result of intraparticle tortuosity or stagnant-layer mass transfer. For large-pore silica adsorbents, the intraparticle contribution dominates the total resistance-to-mass-transfer term. This effect becomes less significant as pore size is reduced, and is not a function of k.

The permeability of the column packed with LiChrosorb Si-60 was slightly lower than that packed with Si-100 (Table IV). This is primarily a result of the reduced interparticle porosity of the column packed with Si-60. Under these experimental conditions, the performance of Si-60 and Si-100 adsorbents was similar. However, superior column performance is expected of the Si-60 packing material, provided that capacity factor and packing density remain constant.

TABLE IV

EFFECT OF MEAN PORE SIZE ON MICROCOLUMN PERFORMANCE

Columns as described in Fig. 8. Mobile phase velocity 0.5 cm/sec. Symbols are defined in Table I.

Pore (Å)	N	H (mm)	K^0 (mm^2)	ε_u	E
100	$2.11 \cdot 10^5$	0.125	$1.7 \cdot 10^{-5}$	0.75	920
60	$2.34 \cdot 10^5$	0.113	$1.3 \cdot 10^{-5}$	0.72	980

CONCLUSIONS

The performance of packed capillary columns in HPLC, as measured by the Knox separation impedance, is currently equal to or exceeds that of conventional or microbore packed columns. Packed capillaries cannot, however, compete with open tubular columns on the basis of performance, since plate height will always be higher and permeability always lower than that of an open tube of the same I.D. Nonetheless, they possess other practical advantages which may make them more desirable. Packed capillaries have greater sample capacity than do open tubular columns, in terms of both mass and volume of injection. Consequently, the sensitivity and dead volume requirements of the detection system are less prohibitive. Yet, they still maintain the advantages of very low flow-rates, on the order of 1 μl/min, which are necessary for the special detection methods[3,4]. Thus, packed capillary columns could be a practical intermediate between conventional packed columns and the highly promising[5], but frequently elusive, open tubular microcolumns.

The performance of packed capillary columns containing silica adsorbent may be improved by decreasing the column inner diameter, particle size and mean pore

size. Irregularly shaped silica particles yield lower plate heights than spherical materials. The best column evaluated in this study, prepared with 10-μm LiChrosorb Si-60 (70 μm I.D.), achieved a total plate number of 390,000 at 0.18 cm/sec linear velocity, and an E value of 580. Further improvement of column performance based on these principles appears feasible. Greater control of column technology and packing uniformity will be necessary to achieve consistently high performance and analytical reproducibility.

It should be noted that these conclusions only rigorously apply in the case of adsorption chromatography on silica gel. The thermodynamics of different retention mechanisms preclude the immediate extrapolation of these results to partition chromatography.

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