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INVESTIGATION OF THE RELATIONSHIP BETWEEN PACKING METHODS AND EFFICIENCY OF PREPARATIVE COLUMNS

II*. CHARACTERISTICS OF THE SLURRY METHOD OF PACKING CHROMATOGRAPHIC COLUMNS**

M. KAMIŃSKI*, J. KLAWITER and J. S. KOWALCZYK

Institute of Inorganic Chemistry and Technology, Technical University of Gdańsk, ul. Majakowskiego 11-12, 80-952 Gdańsk (Poland)

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SUMMARY

The usefulness of the slurry method of packing preparative columns in liquid chromatography, a subject only fragmentarily treated in the literature, has been studied. The procedure selected as optimal was the use of a suspension of particles in a liquid of low viscosity and density which was forced into columns at a constant flow-rate in an upward vertical direction.

Columns of diameter 2, 8, 17 or 52 mm and of length 10–50 cm, filled with irregularly shaped silica gel particles of 16, 22, 33 and 124 μm mean particle diameter (\bar{d}_p) have been investigated. Column efficiency was measured on the basis of chromatograms obtained during flow of the liquid through the detector cell. Mass dispersion inside the columns was examined using the multichannel measurement of testing concentration and on the basis of zone shapes fixed in a section of column packing. The influence of the suspension flow-rate on column efficiency was examined. There was found to be an optimum flow-rate for preparative columns, and the efficiency of analytical columns was found to improve with increasing suspension flow-rate.

These studies have also shown that the plots of $H = f(d_c)$, when $u = \text{constant}$ for columns packed by the slurry method, depend on the particle size, and that there is a minimum for the smallest particles, whereas the dependence for largest-diameter particles is nearly linear indicating improvement of column efficiency with increase in column diameter. Our studies, as well as reports from the literature, indicate that, in all preparative columns, there occurs additional zone broadening which is characteristic of the slurry method and is observed in the form of peak tailing. It was found that this phenomenon results from the different structures of the packing layers near the walls.

* For Part I, see ref. 1.

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INTRODUCTION

The present paper is the second of a series of publications dealing with studies on the dependence of the efficiency of chromatographic columns, mainly preparative, on packing conditions. The first concerned "dry-packing"¹ of columns and also contained methodical information on the experiments described in the present paper.

The "slurry method" has often been described by the synonyms "filtration method" or "wet packing method".

There is quite a large literature dealing with studies on the selection of optimum conditions for these packing methods²⁻²⁵ which have mainly been used to fill analytical and micropreparative columns ($d_c = 12$ mm). Fairly detailed reviews of papers dealing with variants of slurry-packing methods for analytical columns have been given by Elgass *et al.*¹⁶, Martin and Guiochon²⁶ and Majors²⁷.

These and other studies^{28,29} have permitted the procedures to be classified according to the manner used to stabilize the packing particles, *viz.*, (1) adjusting the density of the liquid to that of the packing material (the so-called balance-density method)^{7,11,13,14,17}; (2) adjusting the viscosity and density of the liquid to obtain stability of the suspension (viscosity method)^{10,15,17}; (3) mechanical stirring of the suspension in the reservoir and pumping it with simultaneous dilution^{3,19,20}; and (4) minimizing the time during which the suspension remains in the reservoir to prevent its sedimentation and fractionation, despite the low density and viscosity of the liquid^{4,6,8,9,16}.

According to the literature the slurry method should be used to pack columns with particles of less than 20–30 μm ^{16,26,28}, as then the liquid prevents particle agglomeration due to electrostatic charges present on their surface. (These charges are supposed not to play an essential role during packing with larger-size particles with a different mass-to-surface ratio²⁶.)

To break agglomerations, all authors recommend ultrasonic vibrations or at least vigorous shaking.

The results of earlier studies on the effect of properties of liquids forming suspensions of packing particles on column efficiency have so far been ambiguous, if not contradictory. Thus, for example, some authors^{15,9,2} recommended the use of non-polar liquids for silica gel, while others^{3,6,8,16} thought polar liquids best.

There is also no agreement as to the optimum concentration of the suspensions. According to some authors, there exist optimum concentrations^{3,4}, whereas others only state that too high a concentration should be avoided^{2,6}. Some workers recommend a constant flow-rate of the suspension^{16,17}, while according to others, columns should be packed at a constant pressure^{2,6,7,13}. It is generally believed that the flow-rate of the liquid should significantly exceed both the velocity of sedimentation of the largest particles⁸ and the flow-rate of the liquid during separation^{8,16}.

According to most literature reports, this latter method is suitable for packing analytical columns of no more than 25–30 cm in length, but a few cases are reported^{6,8} where efficient columns of length 1 m have been obtained. Opinions also vary regarding pressures used for pumping suspensions into columns. Thus, some authors think that there exist optimum pressures beyond which column efficiency is not improved^{6,8,13,14,23} and may even decrease^{2,4}. Others^{7,15,16,22} believe that column efficiency increases with pressure. Some reports have recently stressed that smoothness

of the internal column walls¹⁷ is of essential importance. Neither are there any doubts that columns should be carefully positioned vertically during packing^{18,20}. Only a few reports are known on detailed investigations of the effect during packing on column efficiency of liquid flow-rate, a problem extremely important for large-bore columns, particularly where determination of the lower limit of useful flow-rates is concerned.

The geometry of the suspension reservoir is also an unresolved problem, although according to Elgass *et al.*¹⁶ it is of no importance for analytical columns.

In conclusion it can be said that a number of authors have obtained similar column efficiencies ($H = 2-3 \bar{d}_p$ for u_{opt}), even though their opinions on slurry packing of analytical columns do not agree in all details. Hence there is a general conclusion that there probably exists an allowable range of parameters determining packing conditions, and still another conclusion, that only unambiguous findings may lead to further improvements in column efficiency and, particularly, to a general optimization of packing procedures. A review of the literature has shown that only few reports^{21,24,25} have been concerned with the dependence of column efficiency on the diameter of columns packed by the slurry method. Column diameters should be expected to affect the hydrodynamic conditions during the flow of the suspension in these columns (flow profile, reverse currents, etc.).

The importance of efficient packing procedures is evident from a number of reports where column packings had been found satisfactory despite frequent peak tailing^{21,25}.

The authors of the present study aimed to give a more detailed characterisation of the dependences that are essential for optimizing column packing by the "wet method". To draw more substantial conclusions, it was decided to investigate column efficiency in relation to the structure of packings and the shape of substance zones in the packing bed, with particular consideration being given to the dependence of efficiency on the conditions under which preparative columns of various diameters are packed with variously sized particles of silica gel. The balanced-density and viscosity methods for suspension stabilization were excluded because in the light of recent research^{6,16,23} they have been found to have no particular merit when compared with those employing liquids of low density and viscosity. These methods do not present any technical problems associated with excessively high packing pressures, and allow the toxicity of liquids to be limited and the cost of packings to be lowered whenever liquids of high viscosity necessitate the use of organic bromo derivatives with high density. These remarks particularly concern large-diameter preparative columns.

EXPERIMENTAL

Materials

Column packings. Silica gel with irregular medium-pore particles of mean diameter (Krumbein diameter²⁹) 6 nm was used $\bar{d}_p = 16, 22, 33, 65, 124 \mu\text{m}$. A more precise granulometric characteristic of silica gel fraction has been presented in an earlier report¹ in which also the methods of obtaining these packings were described.

Liquids. The following liquids were employed: *n*-hexane (Reachim, U.S.S.R.), dioxan, methanol and isopropanol (POCH, Gliwice, Poland) and distilled water.

Test substances. The following test substances were employed: benzene and *m*-nitroaniline solution in hexane-dioxan (85:15) (16 μl of benzene and 6 mg of *m*-

nitroaniline in 1 cm³); aqueous KNO₃ solution (0.1 g/cm³); solution of Sudan dye I in hexane (0.05 g/cm³); saturated aqueous KMnO₄.

Apparatus

Chromatographic columns of stainless steel were made in our laboratory. Internal column diameters: 2, 8, 17, 52 mm. Column lengths: analytical and semi-preparative ($d_c = 2$ and 8 mm), $l_c = 10, 25, 50$ cm; preparative ($d_c = 17$ and 52 mm), column length was limited by the height of the packing bed within the ranges of 12–25 or 35–45 cm.

Heads for distributing and collecting liquids in preparative columns were movable along the columns in such a manner as to adhere very closely to the packing surface. As mentioned earlier¹, these heads always ensured a flat profile of the material injected. This was carefully checked by injecting a coloured solution through the head placed in a liquid-filled glass tube, and also on the basis of photographs showing zone shapes obtained directly under the head and investigated in the cross-section of the column packing. More detailed data on preparative columns were presented earlier¹.

The chromatographic columns used in this study had an internal diameter of 23.6 mm, and a length of 25 cm, and were packed by the producer (Du Pont) with Zorbax SIL ($\bar{d}_p = 7 \mu\text{m}$) particles, using the suspension method.

Column-packing devices. Fig. 1 shows the main characteristics of a device for filtration packing of chromatographic columns. The device had been made in two variants, one adapted to columns with diameters of 2 and 8 mm, fitted with a three-head membrane pump, output 0–170 cm³/min, $P_{\text{max}} = 40$ MPa, or with a constant-pressure pump within the 0–35 MPa range, and another pump adapted to columns with a diameter of 16.8 mm, fitted with a piston pump, output 0–1 dm³/min, $P_{\text{max}} = 20$ MPa (ZDZ, Toruń, Poland). Columns with a diameter of 52 mm were packed using only device *a* shown in Fig. 1, which was fitted with a displacement pump, output 0–4 dm³/min at a maximum counterpressure of 12 MPa (ZDZ).

Column-testing apparatus. Columns with diameters 2 and 8 mm were tested with a KB-5101 chromatograph (KABID, Warsaw, Poland) consisting of a pneumatic device with a pumping pressure of up to 10 MPa, a membranous injector, a UV-254 detector with a sensitivity of 0.01 a.u.f.s., a measuring cell with a volume of 16 μl , and a y - t recorder (Rodelkis, Hungary).

Preparative columns were tested in a self-produced set fitted with a four-head piston pump, output 0–1 dm³/min, $P_{\text{max}} = 20$ MPa (ZDZ), a membrane injector, a UV-254 detector, sensitivity 0.01 a.u.f.s. and flow-cell volume 32 μl , and a y - t recorder (Radelkis).

Some columns with a diameter of 52 mm were tested on a test stand fitted with a piston pump, an injector and a five-channel conductometric detector enabling five-channel recording of mass dispersion on the column outlet. The characteristic features of the method used in the study, a diagram of the measuring stand and data on the structure of columns prepared for that purpose were described earlier¹. The diagram for two variants of mechanical devices for packing ejection (for columns with diameters of 17 and 52 mm) was also presented.

Apparatus for granulometry. A microscope with a type MP3 projecting screen (PZO, Warsaw, Poland) and a type TAI Coulter counter (Coulter Electronic) were employed.

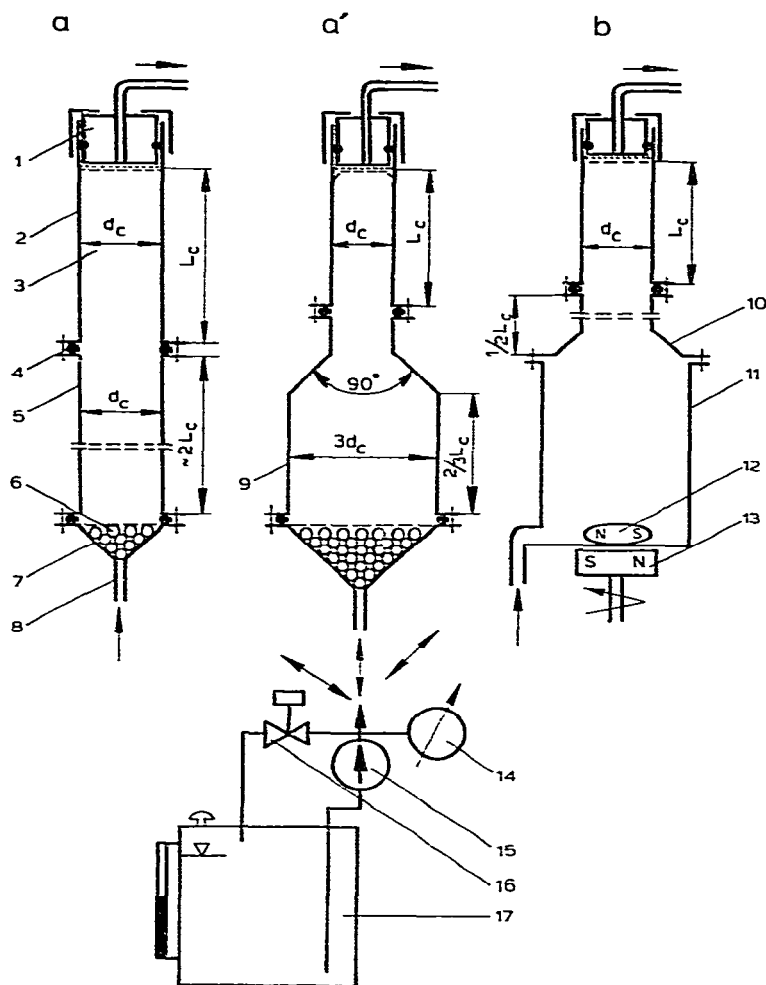


Fig. 1. Diagrams of devices used for packing columns by the suspension method. 1 = Column outlet head; 2 = column; 3 = silica gel suspension; 4 = washer; 5 = tank, diameter equal to column diameter; 6 = wire gauze, mesh diameter, $d = 0.04$ mm; 7 = cone head with beads, $d = 3.5$ mm; 8 = inlet tubing connected to pump; 9 = tank of $3d_c$ diameter; 10 = column-tank connecting element; 11 = tank of 100-cm^3 capacity for columns having $d_c = 2$ and 8 mm, and of 3 dm^3 for columns having $d_c = 17$ mm; 12 = magnetic stirrer; 13 = mixer; 14 = manometer; 15 = pump (one of the pumps described in text, depending on column diameter); 16 = by-pass valve; 17 = pure liquid tank.

Procedures

Packing of columns. Columns were cleaned and degreased and both heads were taken off. The columns were then mounted on the packing stand according to one of the variants shown in Fig. 1. Into the reservoir so formed (diagram *a*, *a'* or element 11 in diagram *b*) was introduced a suspension of silica gel previously degassed and dispersed in an ultrasonic bath [usually in methanol-dioxan (1:1)]. Head 1 was then rapidly mounted on top of the column, the pump was started and readings on the manometer (14) were observed (valve 16 closed). The liquid was pumped until the

pressure remained constant but not less than 15 column volumes. After disconnection of the column the upper head was mounted and pressed down to the surface of the column packing.

Testing of columns. Columns were tested by recording chromatograms of benzene ($k' = 0$) and *m*-nitroaniline ($k' = 4$) which had been separated in the hexane-dioxan (85:15) mobile phase at five selected flow-rates (0–1 cm/sec).

The sample was injected with a syringe during flow of the liquid into the column. The size of the sample depended on the column diameter, *i.e.* 1 μ l for columns of diameter 2 and 8 mm, 4 μ l for columns of diameter 17 mm and 15 μ l for columns of diameter 52 mm. As was established earlier, doubling the sample size did not affect the efficiency of the columns obtained. Ejection of packing material out of columns in order to test zone shapes was preceded each time by two or three injections of Sudan I dye (1 μ l for $d_c = 17$ mm or 9 μ l for $d_c = 52$ mm each time) performed at short time intervals, at mobile phase flow-rates of 0.1–0.2 cm/sec. Injections were made in such a manner as to have coloured-substance zones evenly distributed along the column length. The packing bed was ejected out of the column by means of a piston, the packing was cut along its axis in the plane of the section and the dimensioned zone image in the column was plotted and photographed.

Radial mass distribution in the zones was also measured using a multichannel conductometric detector and injecting 0.1–0.5 cm³ of aqueous potassium nitrate into columns by means of a syringe. Aqueous potassium permanganate was injected simultaneously (similarly as for the Sudan I solution) in order to fix the image of substance distribution in the packing bed.

Other methods. Another method employed in the present study was injection of substances directly into the packing bed of columns in their axis or along their walls.

In order to determine the effect of particle size of the silica gel on the sedimentation rate the initial sedimentation rates of the dilute suspensions were investigated, assuming that in this way the sedimentation rate of particles of size d_p 10% could be observed from the distribution curve.

Experiments with columns packed with mixtures of fractions with $\bar{d}_p = 33$ and 65 μ m were also performed, one of the fractions ($\bar{d}_p = 65$ μ m) having been dyed blue with cobalt compounds.

Data evaluation

The height equivalent to a theoretical plate (HETP) was calculated on the basis of chromatograms from the known dependence concerning peak widths at half height. The parameter so obtained was treated as a measure of column efficiency regardless of the tailing of peaks at their bases. Asymmetry coefficients for *m*-nitroaniline peaks were also calculated on the basis of the peak width at half height and at 0.1 of its height³⁰.

The plots of $H = f(u)$ and $As_{0.5} = f(u)$, were made for each column. The values of the parameter Og were also calculated for selected columns and plots $Og = f(u)_{k'=4}$ were prepared according to the equation

$$Og = \frac{S_2}{S_1 + S_2} \cdot 100\%$$

where S_2 = surface of the "tailing" part of the peak and S_1 = surface of the "peak core".

RESULTS AND DISCUSSION

The data presented in Tables I and II have led to the conclusion that analytical and preparative columns are more efficient when packed by pumping the suspension vertically upwards than when pumping it vertically downwards. This opinion has been put forward previously⁶, but not in relation to preparative columns. Results not presented here have shown that the relative differences in efficiency between columns packed vertically upwards and those packed downwards are smaller for low mobile phase flow-rates, *e.g.* for $u = 0.1$ cm/sec. The results presented in Tables I and II indicate that pumping the suspension under constant pressure drop ($\Delta p = \text{const.}$) is more advantageous than pumping it at a constant flow-rate, particularly in columns difficult to pack (*e.g.* small diameter columns with $d_c = 2$ mm). Large diameter columns ($d_c = 17$ mm), whether obtained under conditions of $\Delta P = \text{const.}$ or $w = \text{const.}$, showed no statistically significant differences in efficiency. Therefore, in investigating preparative columns, we assumed a constant suspension flow-rate during vertical upstream packing.

TABLE I

MEAN HETP VALUES (mm) FOR *m*-NITROANILINE ($k' = 4$; $u = 0.5$ cm/sec) IN PREPARATIVE COLUMNS PACKED BY SCHEME *a* IN FIG. 1 DURING FLOW OF SILICA GEL SUSPENSION IN METHANOL-DIOXAN (1:1) UP OR DOWN THE COLUMN

$d_c = 17$ mm; $l_c = 35$ cm.

\bar{d}_p	$u_f = 1.5$ cm/sec		$u_f = 2.5$ cm/sec		$\Delta P_j = 20.0$	$MPa = \text{const.}$
	↑	↓	↑	↓	↑	↓
22	0.31	0.31	0.22	0.27	0.23	0.26
33	0.44	0.45	0.42	0.53	0.44	0.5
65	1.75	2.0	0.95	1.05	—	—
124	2.5	2.7	2.25	3.2	—	—

TABLE II

MEAN HETP VALUES FOR *m*-NITROANILINE ($k' = 4$; $u = 0.5$ cm/sec) DURING SUSPENSION FLOW UP OR DOWN ANALYTICAL COLUMNS

$d_c = 2$ mm. $l_c = 10$ cm (Fig. 1b)

d_p (μm)	u_f (cm/sec)	HETP			
		$u_f = \text{const.}$		$P_f = \text{const.} = 35.0$ MPa	
		↑	↓	↑	↓
16	3	0.8	4.0	0.5	0.6
22	12	0.85	1.2	0.7	0.8
65	24	1.6	2.0	2.1	2.0

The results presented in Table III conclude that the kind of liquid used to form a suspension does not significantly affect the efficiency of columns with diameters of $d_c > 15$ mm packed with silica gel particles of $d_p > 20 \mu\text{m}$. Greater scatter of HETP values was only observed when methanol was used as dispersing liquid. For that reason methanol has not been used, even though it offers some advantages, such as low viscosity, high polarity, relatively low toxicity and inexpensiveness. The liquid eventually adopted (on the basis of results presented in Table III and literature data) to form the silica gel suspension was methanol-dioxan (1:1).

TABLE III

COMPARISON OF HETP (mm) VALUES OBTAINED FOR *m*-NITROANILINE ($u = 0.5$ m.sec; $k' = 4$) WITH THE USE OF FOUR LIQUIDS AS MEDIA FOR DISPERSING SUSPENSION

Conditions: suspension flow upwards, $d_c = 17$ mm; $l_c = 25$ cm; $u_f = 1.5$ – 2.5 cm/sec.

\bar{d}_p (μm)	<i>Isopropanol</i> ($\eta = 2.4$ cP, $\epsilon_d = 19.5$)	<i>Methanol</i> ($\eta = 0.6$ cP, $\epsilon_d = 33.5$)	<i>Methanol-dioxan</i> (1:1) ($\eta = 0.94$ cP, $\epsilon_d = 20$)	<i>Hexane-dioxan</i> (85:15) ($\eta = 0.5$ cP, ($\epsilon_d = 2$)
22	0.23 ± 0.05	0.3 ± 0.09	0.22 ± 0.04	0.25 ± 0.07
33	0.33 ± 0.03	0.4 ± 0.1	0.33 ± 0.03	0.33 ± 0.04

Surprisingly, the results obtained for hexane-dioxan (85:15) were no worse than those obtained for methanol-dioxan (1:1) despite agglomeration of particles with $\bar{d}_p = 22$ or $33 \mu\text{m}$ in hexane-dioxan during sedimentation, which then becomes more rapid than should be expected considering the viscosity and density of the liquid and the concentration of the suspension. It is obvious that these recent observations must be verified for preparative columns with smaller-diameter particles, e.g. $7 \mu\text{m}$. This seems the more important, as it would be of great advantage to use the main component of the mobile phase as the suspension-forming liquid, to circumvent waste of liquid and conditioning of columns after packing. However, considering the main subject of the present study and the difficulty of obtaining sufficient amounts of packings having $d_p = 7 \mu\text{m}$, this question is now not going to be investigated further.

As can be seen from the results summarized in Table IV for columns with diameters of 17 cm, column efficiency is affected neither by the manner the suspension is introduced nor by the geometry of the suspension reservoir. Conclusions concern-

TABLE IV

COMPARISON OF HETP (mm) MEAN VALUES OBTAINED WITH THREE VARIANTS OF THE SUSPENSION PACKING METHOD

Dispersing liquid methanol-dioxan (1:1) and other data as in Table III.

\bar{d}_p (μm)	For diagram a in Fig. 1	For diagram a' in Fig. 1	For diagram b in Fig. 1
22	0.22 ± 0.04	0.22 ± 0.03	0.21 ± 0.035
33	0.33 ± 0.03	0.35 ± 0.03	0.34 ± 0.03

ing the geometry of the reservoir are consistent with the observations of Elgass *et al.*¹⁶ who fed the suspension into analytical columns from a reservoir with a diameter equal to that of the column or from a reservoir with a much larger diameter. The use of a suspension reservoir with a diameter larger than that of the column, as proposed for analytical columns^{2,6,7,9}, would be particularly disadvantageous for large-diameter preparative columns, as the mean linear velocity of the liquid decreases in the reservoir in proportion to $(d/d_c)^2$.

Another conclusion drawn from the data given in Table IV was that, since there is no difference in efficiency between columns packed according to diagrams *a* and *b* in Fig. 1, the latter procedure has been excluded from further studies. It is inconvenient because stabilizing the suspension by stirring it in the reservoir is not a suitable method for packing preparative columns. An appropriate reservoir is technically difficult to make and requires large amounts of liquid which cause crushing of the silica gel particles by the stirrer. Preliminary studies have shown that changing the concentration of the silica gel suspension in methanol-dioxan (1:1) within the range of 1–15% (v/v) does not produce a statistically significant effect on the efficiency of preparative columns.

Further experiments dealt with the effect of the suspension flow-rate on column efficiency. The results of these studies, *i.e.* the mean efficiencies obtained for $u_f = 0.5$ cm/sec, are presented in Fig. 2. Experiments were performed under conditions of $w = \text{const.}$, with methanol-dioxan (1:1) as dispargating medium. Fig. 3 shows a plot of the sedimentation rate of silica gel particles in methanol-dioxan (1:1) as a function of particle size. It gives an idea of the minimum suspension flow-rates which can be used for a particular particle size.

It can be seen from Fig. 2 that mean column efficiency is variously affected by the suspension flow-rate during packing. It is markedly decreased by a decreased flow-rate in columns packed with particles of $d_p = 22 \mu\text{m}$, in columns with $d_c = 17$ mm at flow-rates below 1.5 cm/sec and in columns with 52-mm diameter at flow-rates as low as 0.8 cm/sec. A similar decrease in efficiency was also observed during packing of columns with 33- μm particles, *viz.* for 17-mm diameter columns at suspension flow-rates less than 1 cm/sec and for 52-mm diameter columns at flow-rates below 0.5 cm/sec. The efficiency remained unchanged for columns packed with particles of $d_p = 124 \mu\text{m}$, with suspension flow-rates higher than the sedimentation rate of the largest particles of this packing fraction.

As can be seen from Fig. 2, preparative columns of length 35–45 cm have efficiencies similar to those of shorter ones of length 15–25 cm. It should be pointed out that columns with an efficiency best suited for a given packing have been obtained within the whole range of suspension flow-rates (u_1) investigated. However, at flow-rates below the lower limit (*i.e.* below the technical optimum), the mean HETP values were found to increase and the reproducibility of results to become worse. It can therefore be assumed that there exists a technically optimum suspension flow-rate for packing columns by the suspension technique. At the same time it must be said that increased suspension flow-rates markedly improve column efficiency when packings consist of small particles ($\bar{d}_p = 22 \mu\text{m}$).

The plots presented in Figs. 4–6 show the mean efficiency values and ranges obtained for columns with various diameters packed with silica gel particles of various size under conditions previously established as optimum.

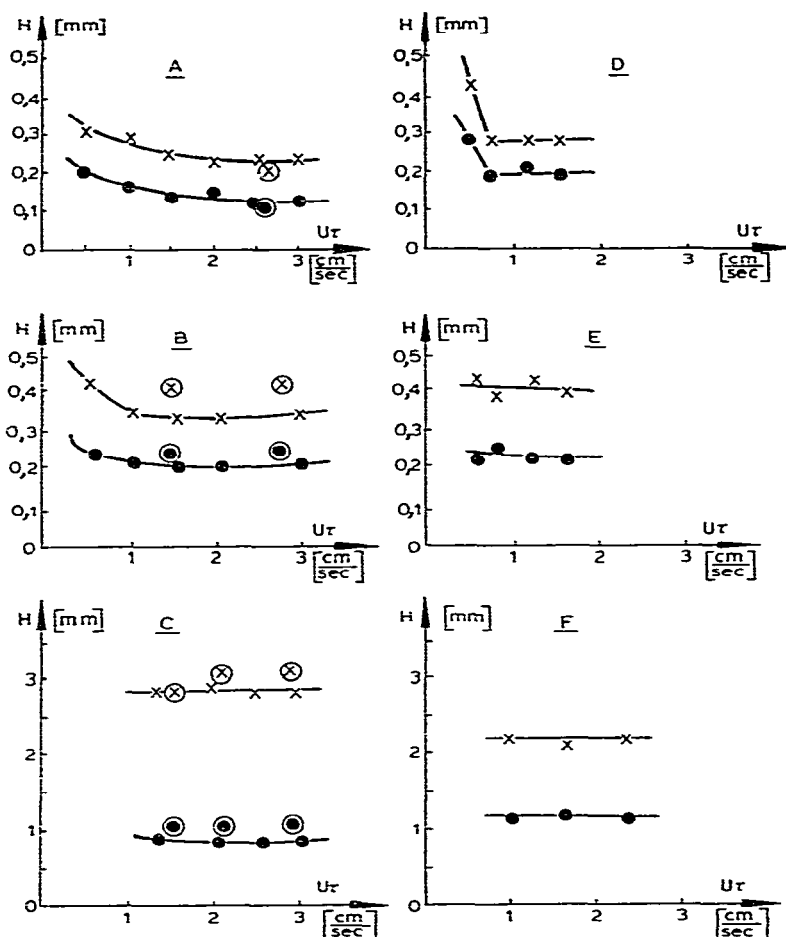


Fig. 2. Effect of particle suspension flow-rate on the average value of HETP during packing of columns with silica gel. Column diameters: $d_c = 17$ mm (A-C), 52 mm (D-F). Particle diameters: $\bar{d}_p = 22$ μ m (A and D), 33 μ m (B and E), 124 μ m (C and F). Slurry medium: methanol-dioxan (1:1). Mobile phase: hexane-dioxan (85:15), $u = 0.5$ cm/sec. Symbols: \bullet , \odot = benzene ($k' = 0$); \times , \otimes = *m*-nitroaniline ($k' = 4$); \bullet , \times , $l_c = 15$ -25 cm; \odot , \otimes , $l_c = 35$ -45 cm.

The plot $\log H_{0.5} = f(\log d_p)$ in Fig. 4 concerning preparative columns with $d_c = 17$ and 52 mm is linear within the entire \bar{d}_p range. The n index values calculated for the semi-empirical Snyder equation^{31,32} $H = D \bar{d}_p^n u^k$ are within the range 1.30-1.45. However the relationship for analytical columns with $d_c = 2$ mm deviates from linearity and clearly indicates that these columns are less efficient than are preparative columns despite the higher suspension rates in the former.

As can be seen from Fig. 5 showing plots of $H = f(d_c)$ for substances with $k' = 4$, column efficiency is usually improved with increasing diameter owing to superposition of two effects: (i) increase of column diameter diminishes the unfavourable influence of the column walls and (ii) the curvatures of the column walls become less pronounced owing to the increase in diameter, thus diminishing the relative significance of effects occurring in the vicinity of the column walls.

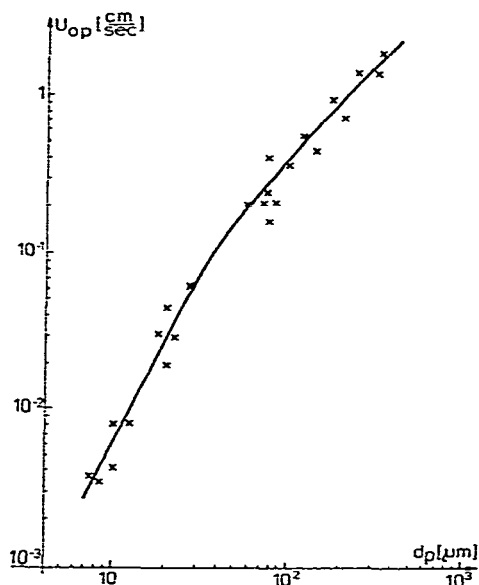


Fig. 3. Dependence of silica gel sedimentation rate on particle size. Liquid phase: methanol-dioxan (1:1) $\rho = 0.91 \text{ g/cm}^3$, $\eta = 0.94 \text{ cP}$; mean value of intraparticle porosity of packing (ϵ_i) = 0.6 ± 0.05 .

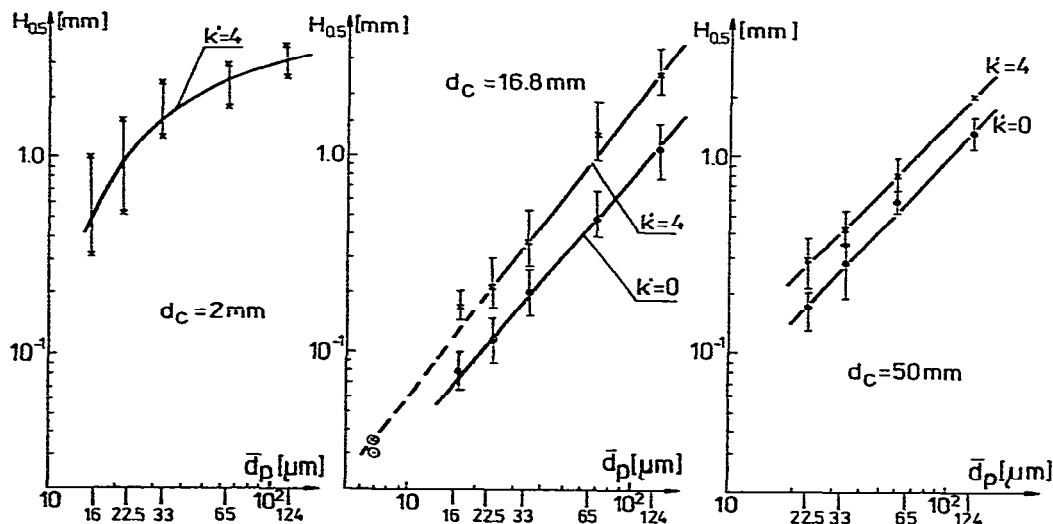


Fig. 4. Dependence of HETP on diameter of silica gel particles for columns packed by the suspension method; $u_r = 1.5\text{--}2.5 \text{ cm/sec}$; $l_c = 15\text{--}45 \text{ cm}$; other conditions as shown in Fig. 2. Determinations: \bullet = benzene ($k' = 0$); \times = *m*-nitroaniline ($k' = 4$); \circ , \otimes , $d_c = 23.6 \text{ mm}$, Sorbax SIL, $\bar{d}_p = 7 \mu\text{m}$; $l_c = 25 \text{ cm}$. Suspension tank as in Fig. 1a.

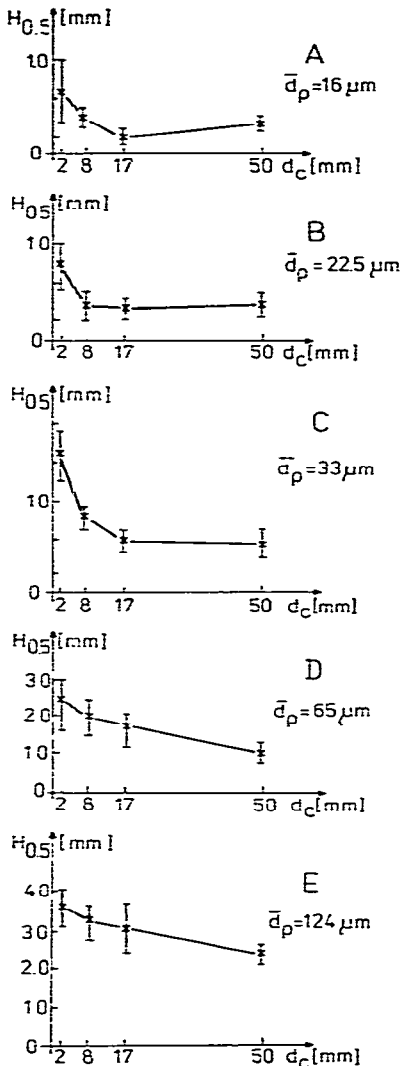


Fig. 5. Dependence of HETP on diameters of columns packed with silica gel by the suspension method. Test substance: *m*-nitroaniline. Other conditions as in Fig. 4.

Efficiency is somewhat decreased in columns with diameters of $d_c = 52$ mm with respect to those with diameters of $d_c = 17$ mm for packings of $\bar{d}_p = 16$ and $22 \mu\text{m}$, probably owing to the fact that it is difficult to maintain uniform hydrodynamic conditions within the entire cross-section of these columns.

Fig. 6 shows the mean values and ranges of variance for the parameters of eddy diffusion (A^z) and mass-transfer resistance (C^z) obtained according to the method of Halász and Naefe³³. These relationships were plotted as a function of \bar{d}_p for columns with $d_c = 17$ and 52 mm. The values of the parameter A^z for the non-adsorbed substance ($k' = 0$) differ considerably from the values for the markedly adsorbed substance ($k' = 4$). Such differences do not occur when columns are dry-packed³³.

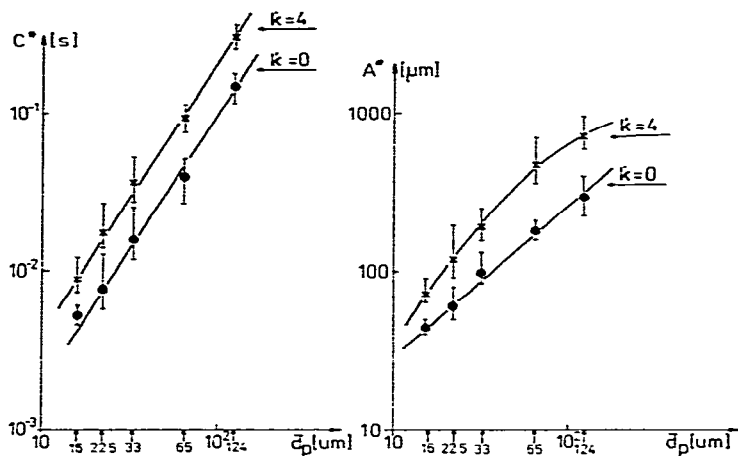


Fig. 6. Values of eddy diffusion (A^*) and mass-transfer resistance (C^*) as a function of silica gel particle size, obtained for benzene ($k' = 0$) and *m*-nitroaniline ($k' = 4$) in columns with $d_c = 17$ and 52 mm I.D. and $l_c = 15$ – 25 cm packed by the slurry method.

This effect may be related to the tailing of chromatographic peaks previously discussed. The values of mass-transfer resistance C^* for preparative columns differ from those reported in the literature for analytical columns, packed with similar particles.

The exponent for the hypothetical dependence $C^* = k \bar{d}_p^m u^k$ was calculated to be $m = 1.75$, i.e. to have a somewhat lower value relative to the value of 1.88 obtained by Snyder^{31,32}.

Fig. 7 shows examples of the relationship $H = f(u)$ obtained for columns with $d_c = 17$ mm packed with particles used in the present work.

The relationship for the reduced parameters $h = f(v)$ in Fig. 8 plotted for 17-mm columns allows one to draw the conclusion that the efficiency of columns tested with the utilization of the total packing surface differs from the efficiencies that might have been attained if a column had been operated under conditions of the so-called "infinite diameter". As can be seen from these plots, the values for HETP vary quite significantly for substances with $k' = 0$ and $k' = 4$. For packings with $\bar{d}_p = 22$ and $33 \mu\text{m}$ nearly identical plots of the relationship $h = f(v)$ were obtained. Only for columns packed with particles of $\bar{d}_p = 124 \mu\text{m}$ were the results similar to those obtained under conditions of the "infinite diameter".

Allowable variations in packing conditions are greater for preparative columns ($d_c = 17$ and 52 mm) than for smaller-diameter columns with $d_c = 6$ and 2 mm. Efficient preparative columns with particles larger than $20 \mu\text{m}$ have been obtained quite often at suspension flow-rates of more than 1.0 cm/sec. The optimum flow-rates for smaller particle packings are probably higher. Because of technical considerations, this condition will be difficult to meet for columns with diameter of 100 mm or more, as the simultaneous pressure drop in them would exceed the level of 0.1 MPa/cm, as can be calculated from Fig. 7. It seems therefore that, although the suspension-packing method may be used for columns with diameters of up to *ca* 50 mm, it will be technically inconvenient for larger-diameter columns.

The correctness of this conclusion has also been confirmed by the observation

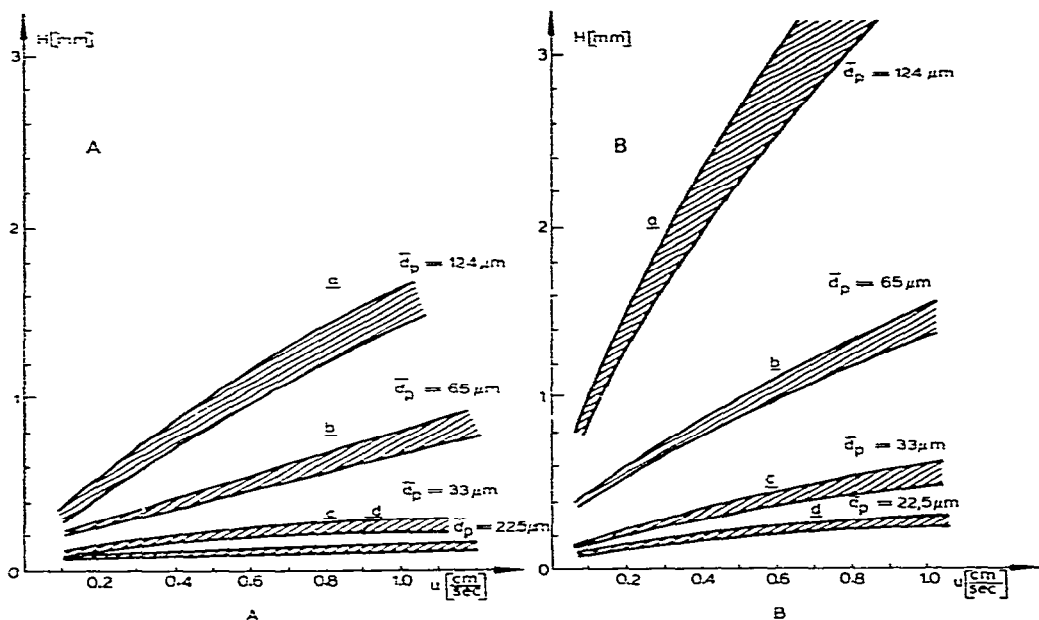


Fig. 7. Ranges of $H = f(u)$ values obtained for packing columns by the suspension method under optimum conditions; $d_c = 17$ mm; $l_c = 15$ – 45 cm. Mobile phase: hexane-dioxan (85:15). A, benzene; B, *m*-nitroaniline.

$$h = \frac{H \cdot \bar{c}_p}{100 \cdot c_p}$$

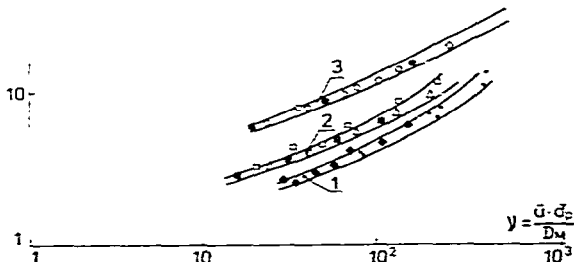


Fig. 8. Relationship $h = f(y)$ for columns with $d_c = 17$ mm and $l_c = 15$ – 45 cm packed by the suspension method. $D_{\text{benzene}} = 2.85 \cdot 10^{-5}$ cm²/sec. $D_{m\text{-NA}} = 2.3 \cdot 10^{-5}$ cm²/sec calculated according to ref. 36. Designations: ■, ●, $\bar{d}_p = 22$ μm; △, ○, $\bar{d}_p = 33$ μm; ·, ◇, $\bar{d}_p = 124$ μm; ◆, □, for columns tested under conditions of "infinite diameter" ($\bar{d}_p = 22$ and 33 μm), for benzene ($k' = 0$) and *m*-nitroaniline ($k' = 4$), respectively.

that the packing bed in columns with diameters of 52 mm has sometimes shown degradation after testing of the column had begun. This phenomenon is due to the fact that the density of the packing material is lower and its porosity greater in columns packed by the slurry method (Table V) than it is in columns packed by the tamping method¹. When the stabilizing effect of the column walls decreases with increase of column diameter, there may occur an avalanche degradation of the layer structure during flow of the liquid in the column.

TABLE V

COMPARISON OF MEAN VALUES CHARACTERIZING DENSITIES AND POROSITIES OF PACKINGS IN PREPARATIVE COLUMNS, $d_c = 17$ AND 52 mm. PACKED BY THE SUSPENSION METHOD UNDER OPTIMUM CONDITIONS (SEE TEXT)

$$\bar{\rho}_w = 0.5 \pm 0.02; \bar{\epsilon}_T = 0.79 \pm 0.03$$

\bar{d}_p (μm)	$d_c = 17$ mm		$d_c = 52$ mm	
	ρ_w (g/cm^3)	ϵ_T	ρ_w (g/cm^3)	ϵ_T
16	0.515	0.79	0.49	0.8
22	0.51	0.78	0.5	0.79
33	0.49	0.78	0.48	0.8
65	0.49	0.80	0.48	0.78
124	0.49	0.79	0.47	0.81

The studies discussed here did not reveal any statistically significant relationship of the permeability of columns, the density of the packing material or the overall porosity of the layer on the kind of packing conditions. The mean values obtained for the porous layer parameters are presented in Table V and Fig. 9. The lower permeability of preparative compared to analytical columns reported by Beck and Halász²⁴ has not been confirmed in the present study.

The discussion has so far been based on data obtained from calculating peak width at half height, the tailing effect at the peak bases in preparative columns being

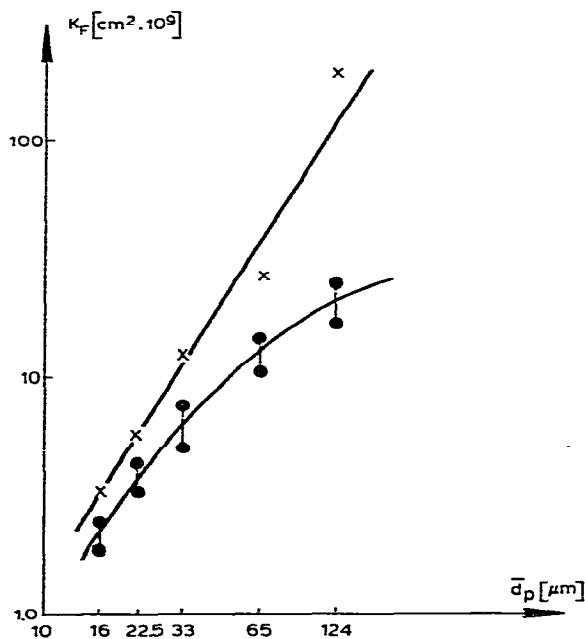


Fig. 9. The effect of silica gel particle diameters on permeabilities for the bed in columns (x) or for packed columns (●) of diameter 17 and 52 mm, packed by the suspension method.

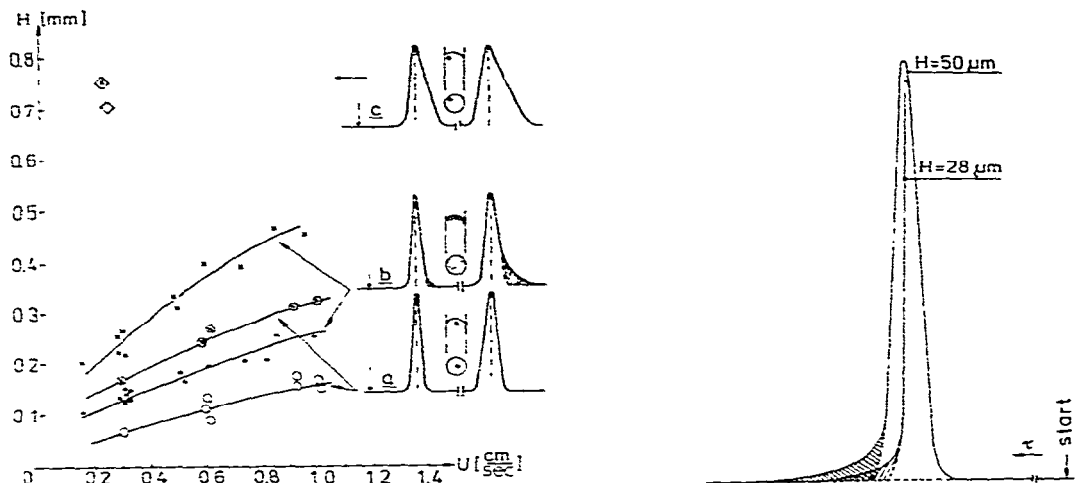


Fig. 10. Comparison of results of the various testing modes for columns with $d_c = 17$ mm, $l_c = 20$ cm, $\bar{d}_p = 33$ μ m, packed under optimum conditions. Condition as shown in Fig. 2. (a) "infinite diameter" mode. (b) injection of substance as a narrow zone on the entire surface of the packing or through the membrane injector; (c) injection of substance near the column wall.

Fig. 11. Examples of shapes of chromatographic peaks obtained for *m*-nitroaniline ($k' = 4$) in two preparative columns with $l_c = 25$ cm, $d_c = 23.6$ mm, manufactured by Du Pont de Nemours, packed by the filtration method by the manufacturer with silica gel Sorbax SH ($\bar{d}_p = 7$ μ m).

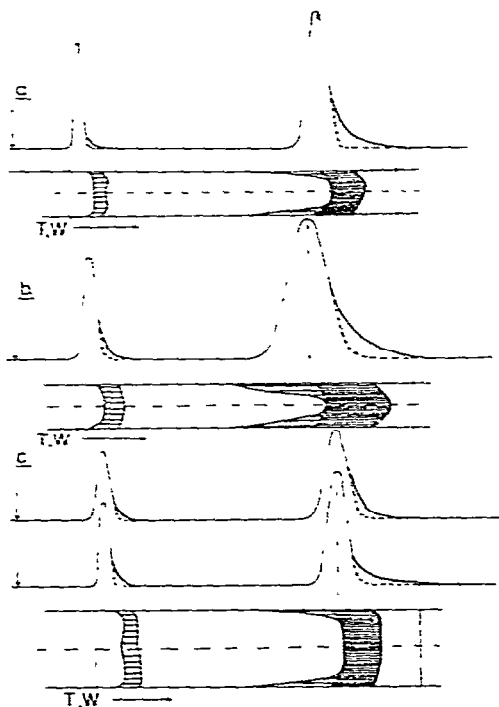


Fig. 12. Examples of typical chromatograms for benzene ($k' = 0$) and *m*-nitroaniline ($k' = 4$) and typical zone shapes for Sudan I for columns packed by the suspension method under optimum conditions. Designations: a, $d_c = 17$ mm, $\bar{d}_p = 22$ μ m; b, $d_c = 17$ mm, $\bar{d}_p = 124$ μ m; c, $d_c = 52$ mm, $\bar{d}_p = 33$ μ m.

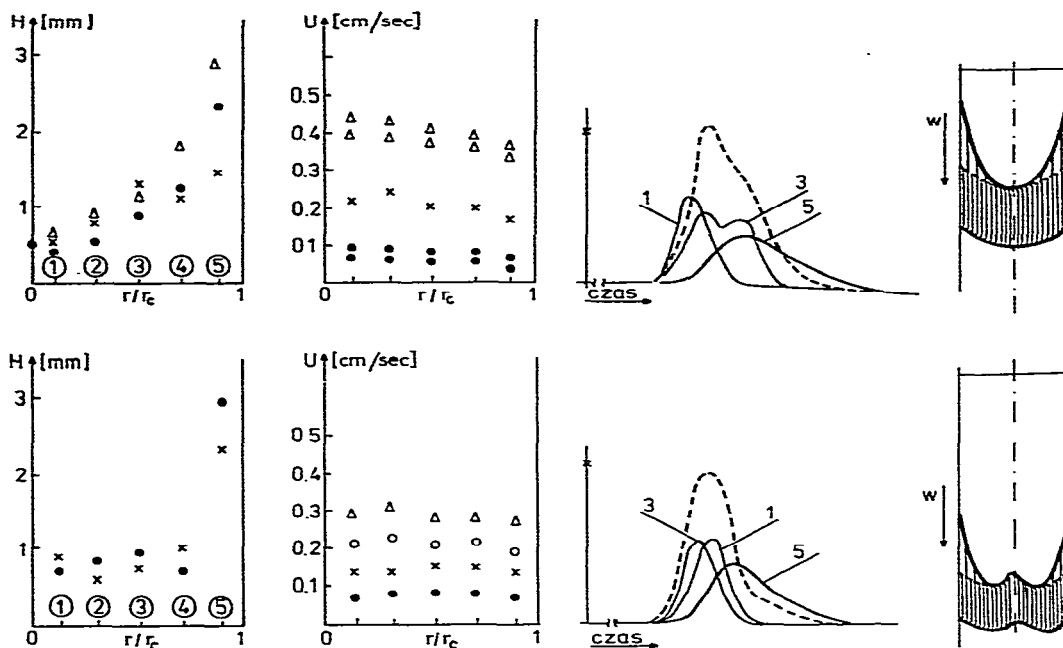


Fig. 13. Flow profiles and mass dispersion along radii in column with $d_c = 52$ mm. (a): $u_f = 1.2$ cm/sec; $\bar{d}_p = 65$ μ m. (b): Test substance, KNO_3 ; mobile phase, distilled water. $u_f = 1.6$ cm/sec; $\bar{d}_p = 124$ μ m.

ignored, though it was always observed in the present study. Consideration of the conditions under which our earlier experiments were performed¹ and close analysis of the data from Fig. 10 make it evident that broadening in the vicinity of walls had not been due to instrumental effects, such as injection, column structure, etc. Furthermore, some reports²¹ and our own experiments with commercially made columns (Fig. 11) have confirmed that these disturbances are quite common in preparative columns. Figs. 10, 12 and 13 show that the reason for this effect is to be sought in the

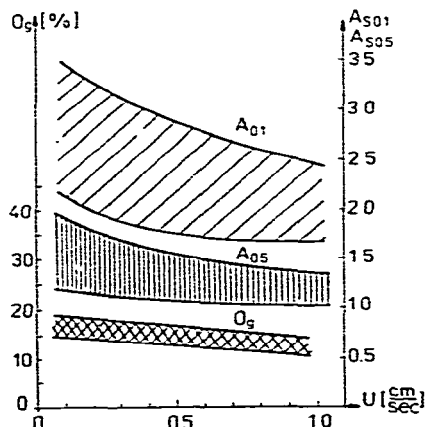


Fig. 14. General characteristics of degree of asymmetry and chromatographic peak tailing in columns with $d_c = 17$ mm packed by the filtration method under optimum conditions for *m*-nitroaniline as test substance ($k' = 4$).

region adjacent to the column walls. According to some reports in the literature, column efficiency is decreased by the disadvantageous effect of the near-wall region²¹; this influence has been attributed by Knox *et al.*³⁴ to the loosening of the bed layer. This view has not been confirmed by our experiments, as that part of the substance which is near the walls is seen in Figs. 10, 12 and 13 to migrate more slowly, whereas, if the bed were loosened, it would migrate more rapidly owing to decreased flow resistance. Furthermore, it is evident from the results of studies with the use of a multichannel detector (Fig. 13) that the concentration distribution in the near-wall regions had been asymmetric, in contrast to the symmetric distribution in the other regions of the column packing. A comprehensive explanation of these observations will be published as soon as investigations are completed.

A quantitative characterisation of the observations is given in Fig. 14, showing the ranges of parameter values which determine peak asymmetries for $h/2$ and $h/10$ ($As_{0.1}$ and $As_{0.5}$) and for the parameter Og calculated for columns of diameter 17 mm and length 15–45 cm filled with gel fractions. Evaluation of the data for each column indicated that the values of the above parameters were unrelated to packing conditions, particle size and column length. Only asymmetry coefficients were found to decrease during testing of a column with an increased rate of liquid flow.

CONCLUSIONS

Investigation of the utility of the slurry method for filling preparative columns has led to the following conclusions.

(1) The relationship $\log H = f(\log d_p)$ for $u = 0.5$ cm/sec is linear with slope $m = 1.30$ – 1.45 . On this basis, the slurry method has been found applicable for filling preparative columns ($d_c > 12$ mm) with particles of sizes ranging from at least \bar{d}_p 16 to 124 μm .

(2) The applicability of the slurry method may, however, be limited by the fact that substance zones often have irregular shapes which are observed as tailing at the peak bases. These irregularities are due to the different bed structure near to the column walls. The dependences discussed here deal with measurements of peak width at half height.

(3) On the basis of $H = f(d_c)$ plots, column efficiency has been found to improve more with increase of column diameter, the larger the particles that were used. It was, however, difficult to obtain efficient columns of more than 20-mm diameter for very fine particles with $d_p < 20$ μm ;

(4) It was found that the flow of the particle slurry through preparative columns must be kept at an appropriate minimum velocity, hence the conclusion that there is no need for attempting to reach maximum flow-rates in these columns as recommended by some workers for loading analytical columns ($d_c < 12$ mm) (correctly also in our view).

(5) The efficiency of preparative columns was found to be unrelated to the density ($\rho_w = 0.48$ – 0.51 g/cm³) and total porosity ($\epsilon_T = 0.78$ – 0.82) of the packing.

(6) The following optimum conditions for filling preparative columns with silica gel by the slurry method have been established: maximum 15% (w/v) suspension of packing particles in methanol–dioxan (1:1, v/v); upward vertical direction of suspension flow; feeding device according to diagram *a* in Fig. 1; minimum time of

residence of suspension in reservoir without flow of liquid; linear rates of slurry flow in columns with $d_c = 52$ mm: $u_f \geq 0.75$ cm/sec, $u > u_{op}(d_{p(max)})$; in columns with $d_c = 17$ mm: $u_f > 1.5$ cm/sec for $\bar{d}_p = 22$ μ m and $u_f > 1.0$ cm/sec for $\bar{d}_p = 33$ μ m.

SYMBOLS

$As, As_{0.1}, As_{0.5}$	peak asymmetry factors calculated for 0.1 or 0.5 peak heights, respectively ³⁰
D_m	diffusion coefficient calculated from Wilke–Chang correlation ³⁶
d_c	column diameter
d_p	diameter of packing particles
\bar{d}_p	mean particle diameter
$d_p^{10\%}$	particle diameter corresponding to 10% in the plot of granulometric distribution
d	other diameters
h	reduced theoretical plate height $\left(h = \frac{H}{d_p}\right)$
H	packing height equivalent to a theoretical plate (HETP)
$H_{0.5}$	height equivalent to a theoretical plate determined for $u = 0.5$ cm/sec
K_F	column permeability ²⁴
l_c	column length
Og	degree of peak "tailing" $\left(Og = \frac{S_2}{S_1 + S_2} \cdot 100\%\right)$
ΔP	pressure drop
ΔP_f	pressure drop during packing
r	radius
r_c	column radius
S_1, S_2	corresponding surfaces: "cores" and "tailing" part of peak
u, \bar{u}	mean linear liquid flow-rate during column testing
u_f	suspension flow-rate during packing of columns calculated for unpacked columns
u_{op}	sedimentation rate of particles in the liquid
w	liquid flow-rate
v	reduced mobile-phase flow-rate
ϵ_d	dielectric constants of liquid
ϵ_T	total porosity of layer
ϵ_i	intraparticle porosity of the gel
η	viscosity of liquid
t	time
ρ	density of liquid
ρ_w	density of packing in column

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