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

I. CHARACTERISTICS OF THE TAMPING METHOD FOR PACKING PREPARATIVE COLUMNS*

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SUMMARY

Since no detailed reports on studies of the tamping method for packing preparative columns have been reported in the literature, the subject has now been investigated. Columns of diameter up to 52 mm and length up to 40 cm were used, employing silica gel with particle diameters 22, 33, 65, 124 and 240 μm.

The zone shapes obtained in the columns and in the column effluent were examined using detection of mobile phase fractions collected along the column radius or by investigating the zone shapes in the sections of the packing ejected from the column. On the basis of the above studies the following were established: the efficiency of columns packed by the tamping method depends essentially on the rate of gel feeding; the efficiency of columns packed with the same particles increases with increasing column diameter; and the resultant efficiency in preparative columns is determined primarily by the shape of the substance zones, not by their local width: a particular role is therefore played by uniformity of packings and not by other parameters.

On the basis of the investigations described above the optimum packing conditions for preparative columns packed by the tamping method have been described.

INTRODUCTION

Analytical and preparative chromatographic columns are in principle expected to be of equal efficiency. In either kind of operation, columns are prepared with beds composed of possibly small (spherical) particles, matched for shape and size, and forming compact structures of equal, ideally small, interparticle spaces.

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In preparative operations, more often only those packing characteristics are considered which influence the output of the separation processes, *i.e.* the capacity of the porous layers, primarily as a function of the specific surface of the particles.

The above assumptions allow one to state that the differences between the theoretical plate values of analytical and preparative systems should only be related to the differences in the sample size or, in particular, to the use of amounts of substance exceeding the linear range of isotherms characterizing interactions with the stationary phase. Such systems would, however, be nearly ideal.

In practice, the efficiency of the two kinds of column has been found to differ even if the packing methods and materials are similar. If the differences in the so-called instrumental conditions under which the column efficiency is tested can be eliminated, (*e.g.* injection of substances, inflow and outflow of the mobile phase, etc.), there only will remain the influence of parameters resulting from differences in column size. The first to investigate this subject were Giddings and Robinson[1], Sternberg and Poulson[2] and Knox and Parcher[3] who had found that the ratio of column diameter to particle diameter should be <6. Naturally, these results could not be treated as generalizations of practical importance to be applied to large-diameter columns.

There are no unambiguous data relating to variations in the efficiency of columns with increase in their diameter. Some workers (*e.g.* Sie and Rijnders[4]) have found column efficiency to decrease with increase of diameter. Others, such as Snyder[5] and Stewart *et al.*[6], observed initially no change in efficiency and then a decrease with increasing column diameter. Further experimental data have, however, led to the opinion that column efficiency may even improve with increasing diameter.

It may be of particular importance in this study to investigate the character and extent of the packing area adjacent to column walls. The significance of this area for column efficiency can be inferred from studies performed by Knox and Parcher[3] and De Stefano and Beachell[8,9]. These workers employed the so-called infinite diameter phenomenon to inject solutions of substances in an unconventional manner, *i.e.* so as to make them occupy only the central part of the packing, with exclusion of the wall area. Under such conditions, the above-mentioned authors achieved improved efficiency of large-diameter columns. Unfortunately, the method of infinite diameter cannot be used in practical preparative chromatography, where column output is usually essential.

Reports from the literature usually agree that the area adjacent to the column walls is less compact, and particle distribution in it more irregular, than it is near the centre (axis) of the column. The contribution of that area to separation processes will depend on the ratio of mass of packing in that area to the entire mass of the packing. However, some workers believe that this area extends to the depth of several multiples of particle diameter (*e.g.*, ref. 10: $5\ \bar{d}_p$), while others report much larger values (*e.g.*, ref. 11: $30\ \bar{d}_p$). If the internal column walls are assumed to be of similar smoothness, it seems reasonable to presume that the extent of the area adjacent to walls depends not only on the packing methods employed or on the character of particles, but also on the ratio of wall thickness to column diameter. This ratio determines the character of the column-wall vibrations occurring during tamping. Such vibrations can occur not only during vibrational loading but also in tap-fill methods consisting of tapping a column on a hard surface. Differences in the kind of wall vibrations or in

the particle–wall or particle–particle friction coefficients may alter the structure of the packing in the area adjacent to the walls.

In studying the relationship between the efficiency of chromatographic columns and their diameter, one cannot overlook the effect of particle autosegregation which has been described by a number of workers[12]. The character of interparticle spaces is indirectly dealt with in all equations describing column efficiency in the form of terms for particle size and so-called structural parameters (*e.g.* coefficients $\lambda_3,\lambda_4,\lambda_5$ and $\omega_3,\omega_4,\omega_5$ in Giddings' equations)[13]. However, no information is available on even the approximate relationship between structural parameters and packing methods. The packing methods are described ambiguously and, frequently, selected arbitrarily. This is best illustrated by the fact that Kirkland[14] recommends the use of the so-called modified "tap-fill" method which consists in tapping the column end on a hard surface and simultaneously tapping its sides 80–100 times, without explaining what is meant by "light tapping" or defining from what height the column is to be dropped. According to Kennedy and Knox[15] the column should be dropped from a height of 1 cm. They suggest, without giving a reason, that the packing should be delivered at a rate of 3.3 cm/min, regardless of particle diameter. Halász and Naefe[16] recommend vibrating the column with a frequency of 60 Hz without giving a more precise description of the influence of the frequency and amplitude of vibrations (or mean acceleration).

The studies mentioned above lead to no general conclusions and show especially that simple extrapolations are, in these cases, unacceptable. For this reason comparative studies of the efficiency of preparative columns should be preceded by studies of the relationship between the structure of the packing and the type of packing method.

The purpose of this study was to establish exactly the relationship between the efficiency of chromatographic columns and the kind of packing method employed. The following operations used in column packing were treated as basic: the so-called "dry" methods [(1) tamping along the column axis, (2) vibration along the column axis, (3) mechanical compression of the particle bed along the column axis] and the so-called "wet", *i.e.* filtration, methods [(1) pumping particle suspension into the column, (2) mechanical pressing of particle suspension].

All the experiments were carried out under the assumption that the effect of bed structure on the efficiency of the preparative chromatographic systems should, whenever possible, be investigated under conditions of "non-overloading". Then, the concentration distributions obtained in the zones of the substances approach Gaussian distribution and are relatively unambiguous and easy to interpret quantitatively. This also facilitates preliminary qualitative evaluation by observing peak deformations.

The present paper, which is the first of a series, presents the results of studies dealing with the packing of columns by tamping, *i.e.* by compacting the packing during a free drop and then tapping the column on a hard base.

Loading columns by tamping

Loading columns by tamping has been extensively investigated in gas chromatography (GC)[17–20]. However, owing to the specific requirements of GC resulting from the high values of diffusion coefficients and low viscosity of the gas phase, the

results of these investigations are of little value for the needs of liquid chromatography.

It is noteworthy that reports from the literature describing the tamping method do not only mention tapping of columns on a hard base, but also tapping column walls[14,15] or tapping and vibrating columns[16]. Hence there are difficulties in comparing the described tamping methods. The height from which columns are to be dropped is mentioned in only a few publications. According to Kennedy and Knox[15] it should be of 1 cm, and according to Laird[21] 2–5 mm. Some workers recommend careful vertical positioning of the columns.

A separate problem is feeding of the packing particles during tamping. One must agree with the suggestions of Sie and Van den Hoed[22] that the packing material should be distributed uniformly over the entire internal column surface. One-point feeding, such as were used by Giddings and Fuller[12], causes formation of a particle cone and may contribute to particle autosegregation, as described by these authors. Halász[23] recommends continuous feeding of the packing material. There is still no agreement as to the velocity at which particles are to be delivered. Sie[22] reports 10 cm/min, Laird[21] 1 cm/min and Kennedy and Knox[15] 3.3 cm/min. Huyten[17] has found that the efficiency of columns in gas chromatography is markedly influenced by the rate at which columns are filled. Bayer[19] remarks that a slow rate improves column efficiency in GC. Moreover, Kirkland[14] suggests tapping the columns for another 5 min after packing with gel is completed.

Studies by Halász and Naefe[16] and Kirkland[14] have not revealed any relationship between column length and efficiency during packing with particles of $\bar{d}_p$ 25–90 μm.

The efficiency of columns packed by means of tamping was found to increase with decreasing of particle diameter, although only to a certain extent, which Snyder[5] and Stewart[6] defined as 50–60 μm, Kirkland[14] as 40 μm and Beachell[24] as 30 μm. Laird *et al.*[21] obtained efficient columns packed with particles of $\bar{d}_p$ = 10 μm. They had used non-porous particles of high-density aluminium oxide. It is generally recognized[22,25,26] that particles of less than 20 μm diameter should not be used to fill columns by the tamping method. According to some authors[16,27], the range of particle size ($\Delta\bar{d}_p$) does not affect column efficiency.

In order to properly characterize side effects, silica gel with particles of irregular shape was used in the present investigations. Compact packing of columns is difficult owing to the low density of silica gel and the large friction coefficients between its irregular particles. This results in marked differences between the efficiency of columns packed with Zipax and those packed with Porasil[15], the former being more advantageous. Hence, if the packing parameters reported in the present paper are optimal for silica gel, they may be also expected to be optimal for other packing materials. Columns packed according to these parameters should therefore be expected to be more efficient.

EXPERIMENTAL

Apparatus

An analytical KB-5101 (Kabid, Warsaw, Poland) liquid chromatograph, equipped with a coil system pressurized up to 10.0 MPa, a septum injector, a UV-

detector for wavelength 253.7 nm and stainless-steel columns of 15 cm × 4 mm I.D., was used in the investigations.

A preparative liquid chromatograph, made in our laboratories, equipped with a piston pump yielding 1 l/min at pressures up to 15 MPa, a septum injector and a UV-detector for wavelength 253.7 nm, a five-channel conductance detector with a range from 0.1 S to 0.1 mS and stainless-steel columns of 16.8 and 52 mm I.D. and length 20–40 cm, was also employed in the studies.

Columns were equipped with two equal heads for distributing liquid (see 4–8, Fig. 1); a multi-outlet head (11, 12, 13, 17, 18, 19, Fig. 1) required for some measurements was fastened to the column outlet. One of the electrodes used for detection with a conductometric detector was a porous disc, the other an arbitrary set of tubes of a multi-outlet head.

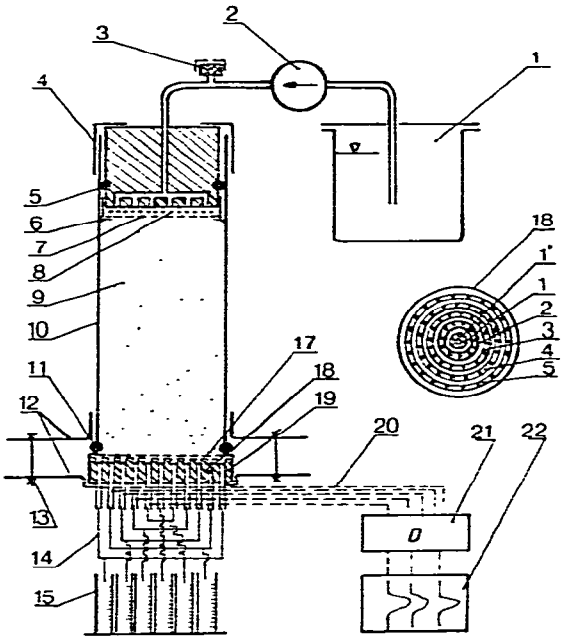
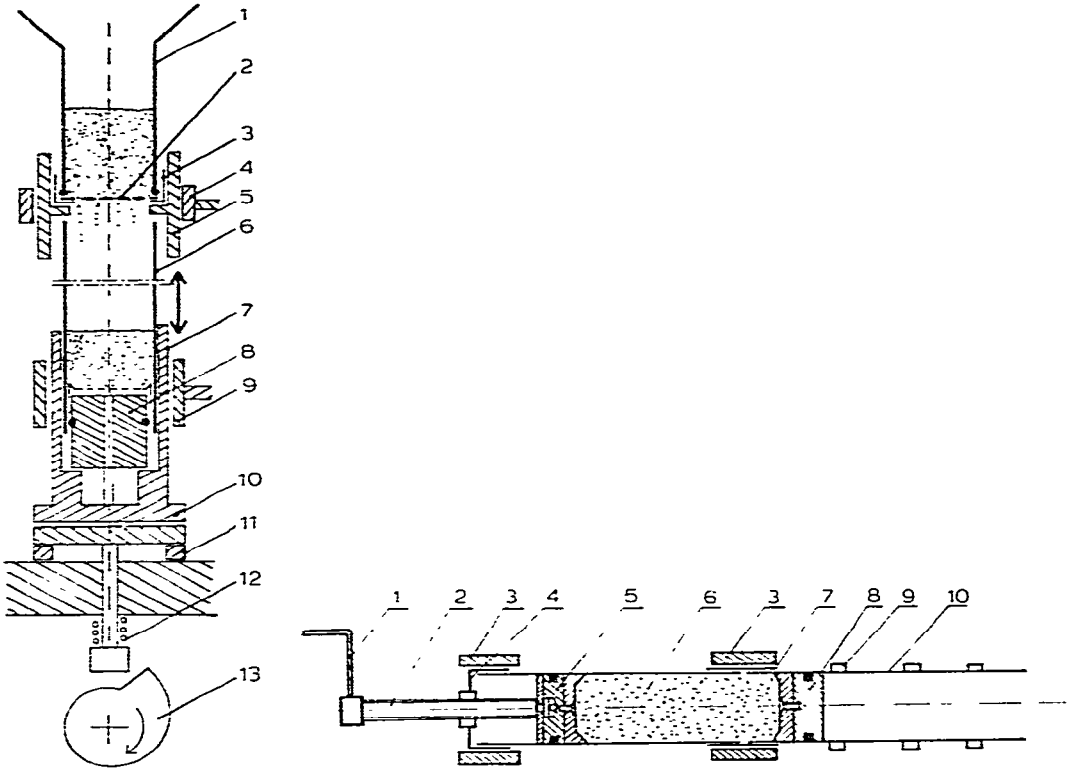


Fig. 1. Diagram of the measuring stand for investigations of the radial distribution of the profile of the velocity and degree of mass dispersion in preparative columns with d_c = 52 mm. 1 = Mobile-phase tank; 2 = pump; 3 = feeding chamber; 4 = nut; 5 = gasket; 6 = nut; 7 = porous sintered metal plate; 8 = steel gauze; 9 = column packing; 10 = 52 mm I.D. tube; 11 = gasket; 12 = flange; 13 = connecting screws; 14 = PTFE tubing; 15 = graduated cylinders; 17 = porous sintered metal plate; 18 = multi-ring stream distributor; 19 = metal tubes 1.5 mm I.D.; 20 = electric leads; 21 = five-channel conductometric detector; 22 = y–t recorder.

A device for packing columns by tamping is shown in Fig. 2. It consists of two adjustable column guides mounted on a stand above a small table serving to raise the columns. The table is lifted by means of a motor-operated cam.

A silica gel container fastened to the columns during loading had a metal gauze or a disc of perforated metal plate at the bottom. The perforated metal disc had at least one opening for each 3 cm^2 of column diameter. The diameter of the opening



10 = sliding table; 11 = hard rubber washer; 12 = spring; 13 = engine-propelled cam.
perforated plate; 3 = nut; 4, 9 = guide rings; 5 = coupling; 6 = column; 7 = nut; 8 = outlet head; 10 = sliding table; 11 = hard rubber washer; 12 = spring; 13 = engine-propelled cam.

Fig. 3. Diagram of the device for ejecting packing layers from columns. 1 = Crank; 2 = feed screw; 3 = vise; 4 = nut; 5 = piston; 6 = packing bed; 7 = coupling; 8 = contra-piston; 9 = elastic clamping ring; 10 = divided cylindric tank.

depended on the velocity of gel feeding required to ensure uniform and uninterrupted filling of the columns.

A device for ejecting the packing layers out of the columns is shown in Fig. 3. The packing was ejected by means of a piston operated by a hand-driven screw into the lengthwise-divided cylindrical container fastened axially to the column.

Materials

Silica gel type H60 (E. Merck, Darmstadt, G.F.R.) was fractionated by sedimentation, first in methanol and then in water. The column of liquid during sedimentation was 26 cm high. The suspension from above the residue was decanted four times. Sedimentation parameters are given in Table I. The distributions of particle size obtained are presented in Fig. 4. After sedimentation, the gel was activated for 2 h at 120°C.

Coloured silica gel was obtained by soaking in 10% cobalt nitrate solution and

TABLE I

CHARACTERISTICS OF SEDIMENTATION CONDITIONS OF SILICA GEL FRACTIONS

Initial material: silica gel type H60 (E. Merck).

$\bar{d}_p$ (μm)	90% w/w d_p (μm) 10% w/w	Sedimentation conditions (min)	Yield (%)
16	11–22	$\tau_s < 95$, CH_3OH $\tau_s > 85$, H_2O	11
22.5	15–27	$85 > \tau_s > 30$, H_2O	22
33	25–46	$30 > \tau_s > 15$, H_2O	19
65	47–85	$\tau_s < 15$, H_2O	39
		losses	9

addition of concentrated ammonia solution. Coloured gel was calcined at 400°C. Colourless gel was also calcined before mixing it with coloured gel.

Silica gel fractions with larger particles were obtained by sieving.

The mobile phase was hexane–dioxan (85:15) [hexane pure (Reachim, U.S.S.R.), dioxan pure (POCh Gliwice, Poland)].

A multi-outlet column was tested using distilled water as mobile phase.

Test substances

The hexane–dioxan mobile phase was tested with the following substances: benzene (analytical-reagent grade, 1.6%) and *m*-nitroaniline (analytical-reagent grade, 0.6%). The coloured substance used for observing zone shape was Sudan I. When water was used as mobile phase, the testing substance was 10% aqueous KNO_3 solution.

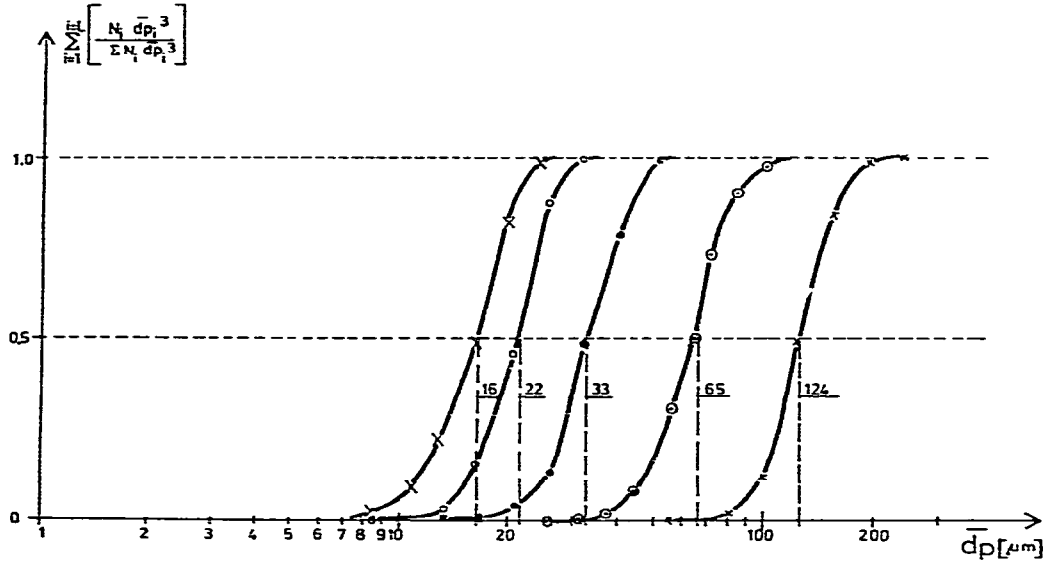


Fig. 4. Granulometric characteristics of silica gel fractions used to study the efficiency of preparative columns.

Procedure

Column packing. A column equipped with a lower head was carefully fastened in a vertical position on two adjustable guide bars. A device for feeding silica gel over the entire internal column surface was fastened in the upper part of the column. After turning on the tamping device, the column was raised by 2 cm* and then dropped. The gel was then poured into the feeder. The loaded column was submitted to tamping for 10 min[14] in order to compact the last portion of injected packing material.

Particle size measurements. Particle size was measured by a microscope, a Coulter counter (Model TAII, Coulter-Electronics Ltd.) or by elutriation (Bakho elutriator). The Krumbeinsch diameter[28] of *ca.* 400 particles was measured with a microscope coupled to a monitor.

Examination of zones in columns. Columns were tested at least four times at different mobile-phase velocities. Special attention was given to study the effect of column heads on the form of the substance zones. We intended to find out whether the heads ensure a uniform inflow of liquid phase into the entire cross-section of the packing in the column. The lack of effect of heads on the form of the substance zones was confirmed by injecting a coloured solution through the heads into the liquid-filled glass tube, and also by examining the form of the zones in the cross section of the column packings obtained directly under the heads with the technique described elsewhere in the present work.

To examine the changes in zone shape, solutions of Sudan I (10%) in dioxan were successively injected onto columns. Subsequently, the column was mounted into a device shown in Fig. 3. The packing was ejected, cut along its axis, whereafter the Sudan I zones were photographed and drawings made. The local theoretical plate height was calculated from some of the photographs or drawings under the assumption that concentration distribution on lines parallel to the column axis is approximately Gaussian (Fig. 5) and the visible zone width equal to 4σ.

The theoretical plate height was calculated from chromatograms, the width of peaks being measured in the middle of their height. Columns of d_c 16.8 mm were packed at least 3–5 times, columns of d_c 52 mm at least twice.

RESULTS AND DISCUSSION

Fig. 6 shows diagrams illustrating changes in theoretical plate height as a function of the rate at which silica gel is poured into the columns (on the rate of increase of silica layer height in columns). As follows from Fig. 6, the curves characterizing the relationship $H = f(u_f)$ for silica gel particles of $\bar{d}_p = 33$ μm show minima for the ranges of *ca.* 0.3–0.5 cm/min for columns of 52 mm I.D. and length 20 cm and *ca.* 0.5–1.2 cm/min for columns of 16.8 mm I.D. and length 20 and 40 cm. For particles of diameter 124 μm these minima are *ca.* 0.8–1.2 cm/min for columns of 16.8 mm I.D. and length 20 cm.

No minimum was observed for a column of 52 mm I.D. and length 20 cm.

The investigations have led to the following general conclusions.

(1) It can be assumed that during tamping packing of columns of *ca.* 50 mm

* The investigations were supported by the Ministry of Science, Higher Education and Technique. The results will be published.

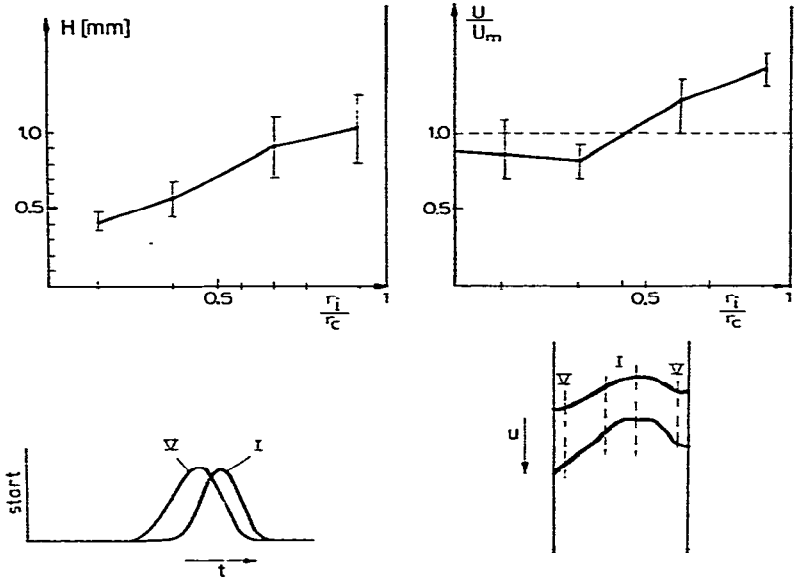


Fig. 5. Plots of efficiency and radial velocity distribution of the liquid phase, obtained in multi-outlet columns: d_c = 52 mm, d_p = .65 μm, u_f = 0.94 cm/min; Sudan I, k' = 0.3.

I.D. with silica gel particles of *ca.* 30–125 μm, the optimum common velocity of gel feeding is *ca.* 0.5–0.8 cm/min.

(2) As particle and column diameters increase, so do the optimum ranges of silica gel packing velocity, so that for particle diameters of 124 μm and column diameters of 52 mm no minimum was observed in the plots of $H = f(u_f)$, *ca.* 0.8–1.2 cm/min for columns of diameter 16.8 mm and length 20 cm.

(3) As the length of columns packed under the conditions described increases, the mean efficiency of columns does not undergo essential changes, but the repeatability of the H values worsens.

Moreover, the investigations described lead to the supposition that the existence of minima found for the $H = f(u_f)$ curves for particles of diameter 33 μm may result from autosegregation of particles according to their size during packing of the columns. Such a conclusion may be partly drawn from an analysis of substance zone shapes presented in Fig. 7 and from the results of investigations using bicoloured particles discussed further in this paper. If these suppositions are correct, then the smaller the differences in particle size of a given fraction (smaller Δd_p values), the greater will be the optimum velocity of column filling. The results of further detailed investigations on this subject will be published later.

Analysis of further data presented in Figs. 7a–d reveals the existence of three various shapes of substance zones resulting from various profiles of liquid-phase flows, *viz.* (1) approximately flat, sometimes somewhat widened near the walls in both directions along the column, (2) with their central part curved in the direction of the mobile-phase flow and (3) with their central part curved in a direction opposite to the mobile-phase flow.

Each zone shape gives a characteristic distribution of substance concentrations

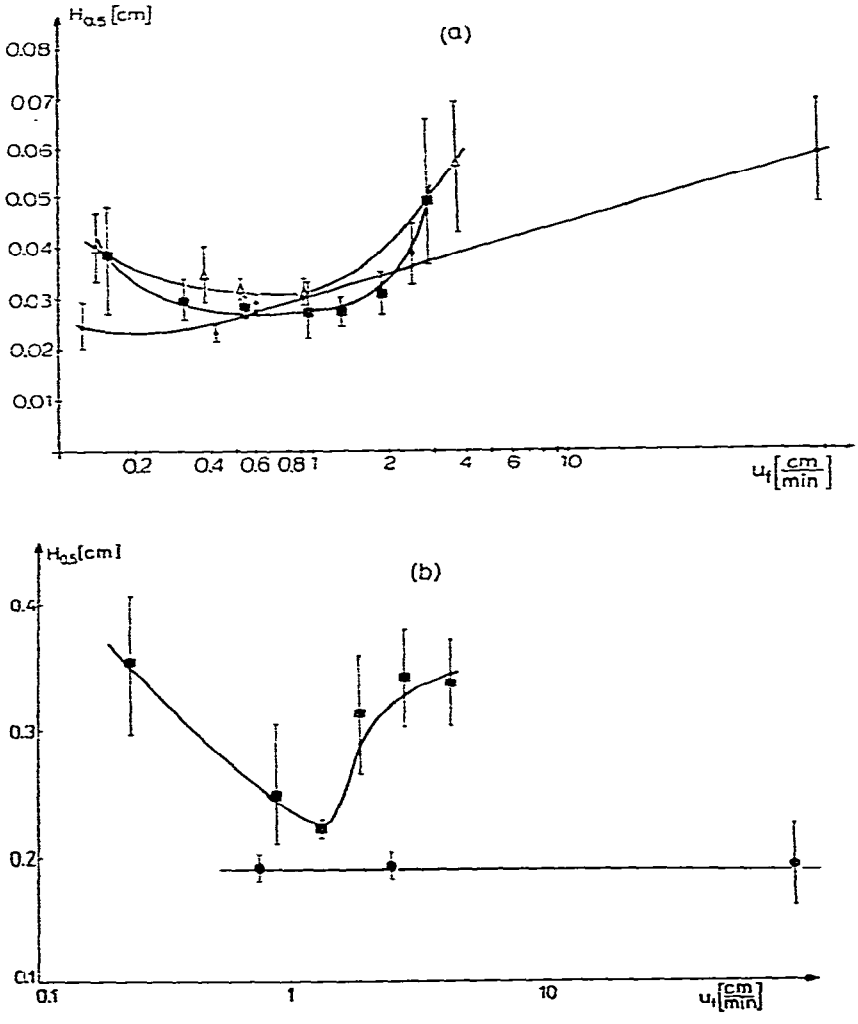


Fig. 6. Dependence of column efficiency on the rate of feeding of silica gel: u_m = 0.5 cm/sec; *m*-nitroaniline, k' = 4. (a) $\bar{d}_p$ = 33 μm; ●, d_c = 52 mm, L = 20 cm; ■, d_c = 17 mm, L = 20 cm; △, d_c = 17 mm, L = 40 cm. (b) $\bar{d}_p$ = 124 μm; ●, d_c = 52 mm, L = 20 cm; ■, d_c = 17 mm, L = 20 cm.

which effect the shape of the chromatographic peaks as shown in Fig. 7a–d. The first kind gives a symmetrical concentration distribution with a Gaussian shape to the curve, possibly with small "tails" at the base line. The second and third kinds show asymmetric peaks on the side opposite to the zone bulge. The degree of peak asymmetry depends on the degree of zone deformation. As can be seen from Fig. 7c and d, the dependences discussed were less marked in columns with d_c = 52 mm. In particular the effect of column-packing conditions on the shape of zones for cases presented in Fig. 7d, *i.e.* for a column with d_c = 52 mm and $\bar{d}_p$ = 124 μm, was little pronounced. In addition, it was observed that zone shapes depend on the rate at which the silica gel is poured. Thus, when gel is fed in at a rate greater than that

established as optimum, there predominate zones curved in the centre towards the mobile-phase flow (*e.g.* Fig. 7a, zones e and f; at a lower rate than that established as optimum, there predominate zones curved in the centre in a direction opposite to the mobile-phase flow (*e.g.* Fig. 7a, zones a and b). Feeding gel into the columns at a rate approximately the same as the optimum rate gave zones nearly flat or slightly curved towards the mobile-phase flow (*e.g.* in Fig. 7a, zones c and d). The column with d_c = 52 mm differed from that with d_c = 16.8 mm by oscillation of the zone border lines (*e.g.* Fig. 7c) superimposed on the shape described above. Despite local changes in the zone shapes resulting from the oscillating shape of the border lines, there are no difficulties in attributing each of these zones to one of the three kinds given above.

The question arises of whether the zones differ significantly not only in their shape, but also in their local widths. As can be seen from Fig. 7, the local zone

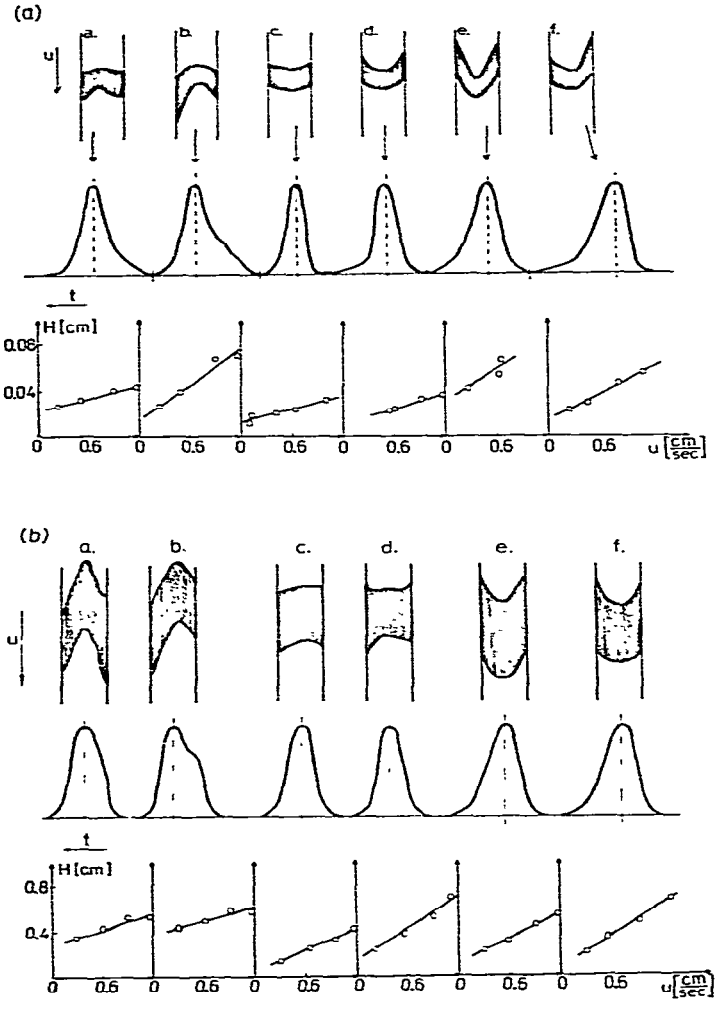


Fig. 7.

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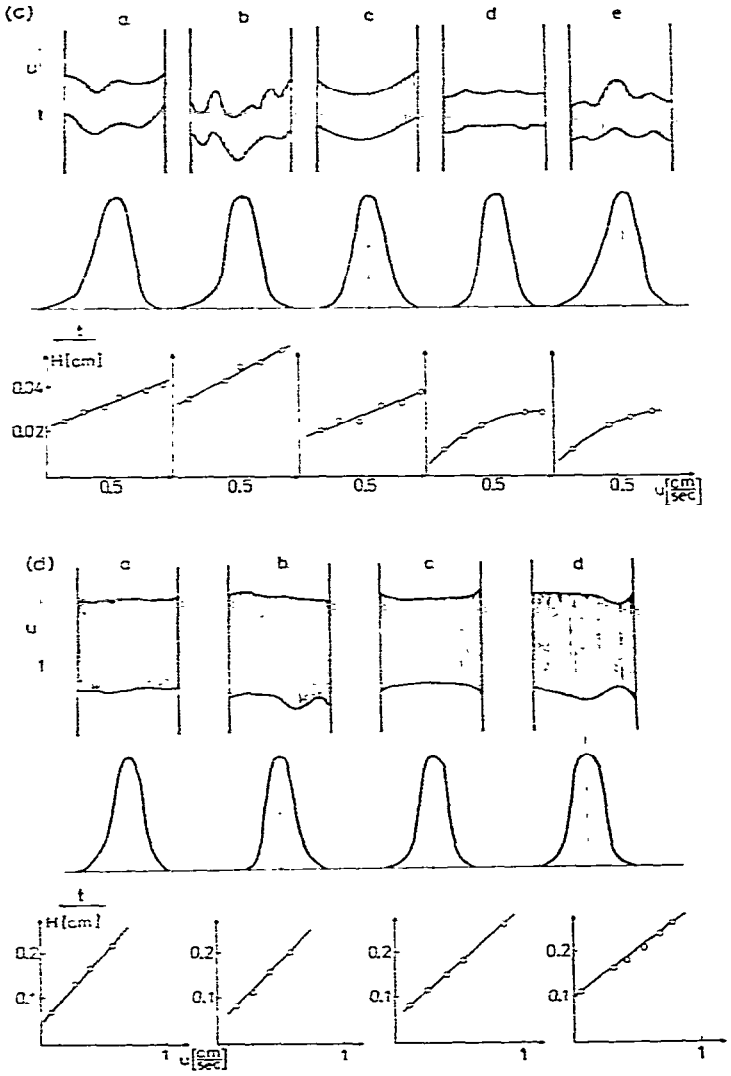

Fig. 7. Dependence of column efficiency on the mobile-phase flow-rate (*m*-nitroaniline, $k' = 4$) and shapes of zones of coloured substance Sudan I ($k' = 0.3$) and corresponding chromatographic peaks as a function of feeding rate of silica gel. (a) $d_c = 16.8$ mm; $u_m = 0.22$ cm/sec; $\bar{d}_p = 33$ μm. u_f (cm/min): a, 0.17; b, 0.42; c, 0.93; d, 0.96; e, 1.9; f, 1.97. (b) $d_c = 16.8$ mm; $u_m = 0.22$ cm/sec; $\bar{d}_p = 124$ μm. u_f (cm/min): a, 0.33; b, 0.8; c, 1.0; d, 1.92; e, 3.1; f, 4.63. (c) $d_c = 52$ mm; $u_m = 0.12$ cm/sec; $\bar{d}_p = 33$ μm. u_f (cm/min): a, 2.3; b, 3.36; c, 0.6; d, 0.42; e, 0.12. (d) $d_c = 52$ mm; $u_m = 0.12$ cm/sec; $\bar{d}_p = 124$ μm; u_f (cm/min): a, 2.16; b, 3.0; c, 0.77; d, 0.74.

widths do not essentially differ in columns packed under optimum or non-optimum conditions. This is illustrated by the data presented in Table II.

The general conclusion drawn from this is that if the final efficiency of preparative columns (calculated from the chromatogram) depends markedly on the packing rate it is primarily due to the effect of both the width and the shape of substance zones

TABLE II

COMPARISON OF THE MEAN LOCAL THEORETICAL PLATE HEIGHT WITH THAT CALCULATED FROM THE KNOX FORMULA FOR $A = 1$ AND $A = 1.7$

d_c (mm)	$\bar{d}_p$ (μm)	$\bar{H}_{lx}$ for not optimal parameter (mm)	$\bar{H}_{lx}$ for optim. parameter (mm)	H from the Knox formula for $A = 1$ (mm)	H from the Knox formula for $A = 1.7$ (mm)	u (cm/sec)
17	33	0.27	0.24	0.19	0.27	0.22
17	130	1.62	1.45	1.88	2.4	0.22
52	33	0.2	0.2	0.13	0.23	0.12
52	130	1.0	1.0	1.23	1.65	0.12

on the efficiency. Unsymmetrical peaks are obtained even when, as follows from the exemplary data presented in Fig. 5, the local concentration distributions are symmetrical and approximately Gaussian. As follows from the studies presented in Fig. 5, detection of the effluent from concentric rings provided information on the retention time of that part of the zone which has a toroidal shape, and on the concentration distribution in that part of the zone. Fig. 5 shows the peaks obtained from a zone situated in the vicinity of the wall and in the centre, along with the velocity profile in the column and the radial distribution of efficiency. It must be noted that even the peak obtained for the zone situated near the wall approximates to a Gaussian curve. The non-uniform flow profile in the columns illustrated in the plots in Fig. 5 may be due to particle autosegregation or to local changes in the packing density. A number of experiments were carried out to find out whether it was due to particle autosegregation. Sampled particles were measured by the microscopic method and with a Coulter counter. The results were within limits of error for either of the methods for particles collected from near the walls or from the centre of columns. Therefore, it was decided to fill the columns with a mixture of two silica gel fractions with $\bar{d}_p = 33$ and 65 μm, each dyed with a different colour. The longitudinal section of the packing of a column of d_c 16.8 cm is shown in Fig. 8 which shows a complicated picture of particle segregation. In the vicinity of the column walls there are aggiomerations of smaller (Fig. 8b) or larger (Fig. 8b) particles, depending on the filling rate during packing. Moreover, autosegregation was either radial (Fig. 8b) or radial and along the column (Fig. 8a), with the particle sizes recurring periodically in the zones. More detailed study of particle autosegregation will be published later.

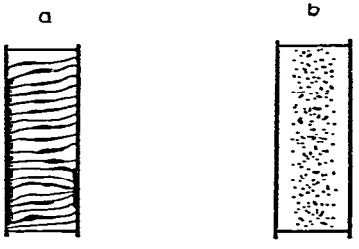

Fig. 8. Characteristics of the distribution of particle size in columns of 16.8 mm I.D. packed by the tamping method with two-coloured silica gel mixture (1:1) $\bar{d}_p = 33$ μm (white) and $\bar{d}_p = 65$ μm (black). a, $u_f = 0.3$ cm/min; b, $u_f = 1$ cm/min.

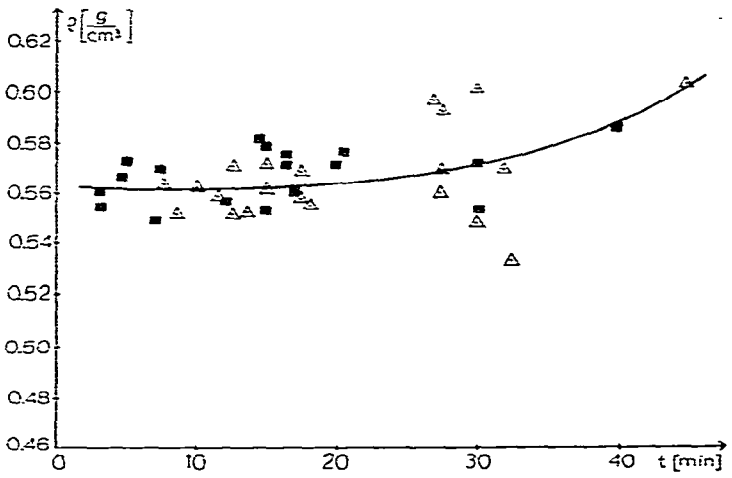

Fig. 9. Effect of time of tamping on the density of packing; d_c = 16.8 mm. ■, $\bar{d}_p$ = 124 μm; △, $\bar{d}_p$ = 33 μm.

As was to be expected, the density of the packing also depends on the filling rate (Fig. 9) and is found to rise slightly with increasing time of column tamping (tamping time has been assumed to be one half of the packing time plus the time of additional tapping). Figs. 10a and b show the relationship between reduced theoretical plate height and reduced mobile-phase rate in the form of $\log h = f(\log \nu)$ for columns packed under optimal conditions. Some data from the literature[15,3] are also shown for comparison. As may be seen from Fig. 10, the course of the function $\log h = f(\log \nu)$ for particles of d_p = *ca.* 30–125 μm can be illustrated by the same curve.

Contrary to expectations, somewhat more efficient columns have been obtained for much larger particles with $\bar{d}_p$ = 240 μm, whereas the theoretical plate heights for particles with $\bar{d}_p$ = 22 μm were decidedly greater than for larger particles. These results confirm the opinion of workers who believe that the limiting value of $\bar{d}_p$ determining the limit of packing by "dry" methods is $\bar{d}_p \geqslant 25$ μm. This is also confirmed by the plot in Fig. 11 where decrease of the reduced theoretical plate height is only insignificant with increase of the silica gel particle size to more than $\bar{d}_p$ = *ca.* 30 μm. For particles with $\bar{d}_p$ = 30 μm there is a rapid increase in theoretical plate height. It must, however, be remembered that the value of $\bar{d}_p$ = 30 μm is only approximately a boundary value because the effects of "dry" packing will depend on other properties of the particles, such as their shape, surface roughness, range of diameter for a given fraction, etc.

Fig. 12 shows the dependence of the theoretical plate height on the diameter of the column for u = 0.5 cm/sec. A marked improvement in efficiency is seen for all three particle sizes (22, 33 and 124 μm) along with increase in column diameter, particularly within the range of small column diameters.

CONCLUSIONS

Since the data reported in the literature characterize tamping methods used for packing preparative columns only fragmentarily and ambiguously, the subject was

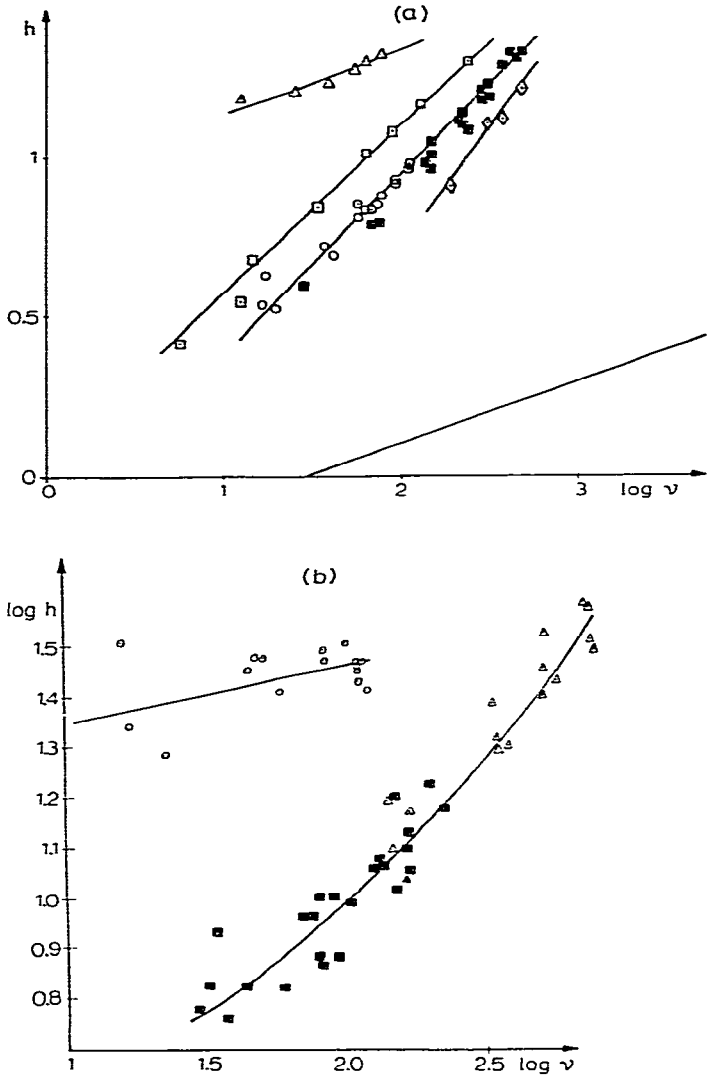

Fig. 10. Plot of log h *vs.* log ν for columns packed under optimum conditions (*m*-nitroaniline, $k' = 4$). (a) $d_c = 52$ mm; $\bar{d}_p$ (μm): ◇, 240; ■, 124; ⊙, 33; △, 22; —, 480 (ref. 3); ⊡, 48 (ref. 15) ($d_c = 2$ mm); (b) $d_c = 16.8$ mm; $\bar{d}_p$ (μm); ■, 33; △, 124; ⊙, 22.

systematically studied in the present work for columns up to diameter 52 mm packed with silica gel particles up to 125 μm in diameter. These investigations have led to the following conclusions.

(1) The efficiency of chromatographic columns packed by the tamping method depends to a great extent (in contrast to opinions reported in the literature[15,22]) on the packing rate (increase in the packing layer). However, a decrease of that dependence is observed along with increase of column and particle diameters.

(2) An increase in column diameter, particularly within the range up to $\bar{d}_p =$

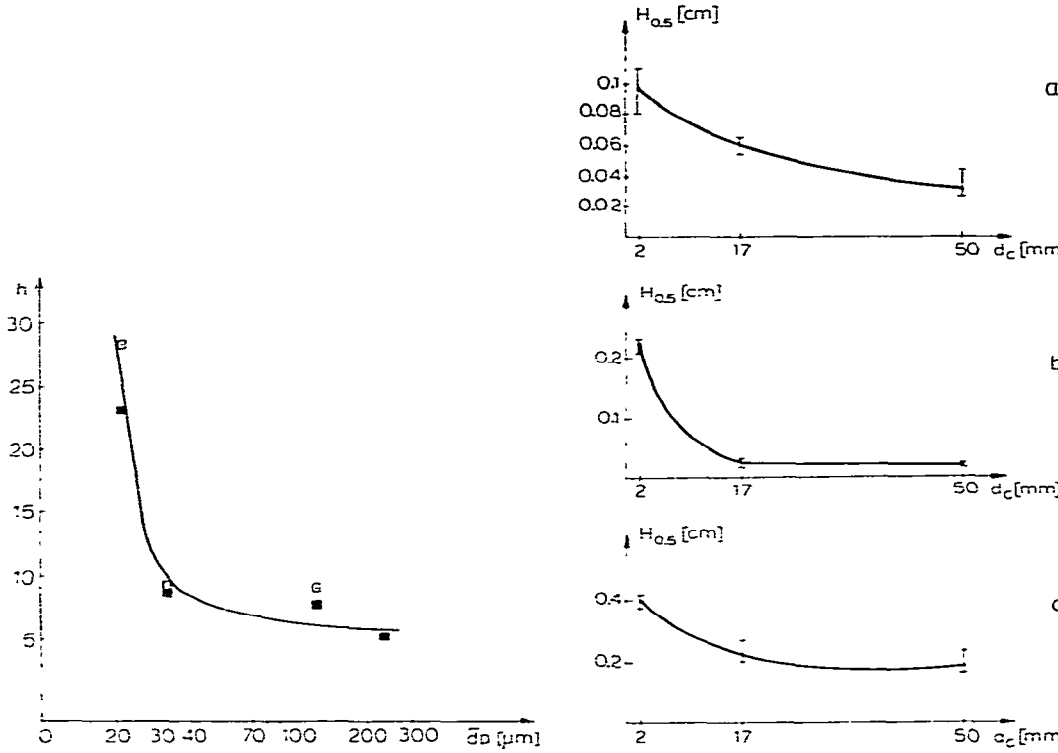

Fig. 11. Dependence of the reduced theoretical plate height on the mean size of particles for columns packed by the tamping method under optimum conditions: $v = 100$; *m*-nitroaniline, $k = 4$. d_c: ■, 52 mm; □, 16.8 mm.

Fig. 12. The effect of column diameter on theoretical plate height for optimum packing parameters (*m*-nitroaniline, $k' = 4$); $u = 0.5$ cm sec. $\bar{d}_p$: A, 22 μm; B, 33 μm; C, 124 μm.

ca. 10 mm (*i.e.* for analytical to micropreparative columns) leads to a decrease in the theoretical plate height. The increase in efficiency observed is more significant, the smaller the size of particles used in the packing.

(3) The resultant efficiency (calculated on the basis of chromatograms) in preparative columns packed with similar particles, depends more on the shape of the substance zones than on their local widths; consequently it depends on the non-homogeneity resulting from the complicated autosegregation of particles and the interparticle space in the layer constituting the packing. The same observations have been made for cases of symmetrical distribution of substance concentrations, approximating to Gaussian distributions, in all regions of the zone.

(4) The shape of substance zones achieved at optimum filling rates approximates to a flat shape, characteristic of the piston profile of liquid-phase rate in the column section. At gel-filling rates lower than optimum, the zone shapes are curved in the centre in a direction opposite to the liquid-phase flow, and at higher velocities the zones are curved in a direction consistent with that of the liquid-phase flow.

(5) The optimum packing conditions for preparative columns are as follows. Silica gel should be poured onto the entire section surface of a vertically positioned

column which is raised and then dropped from a height of 2 cm. It can be assumed that, for columns of *ca.* 50 mm packed by tamping with particles of *ca.* 30–125 μm, the optimum gel filling rates are of *ca.* 0.5–0.8 cm/min, these optimum rates increasing with increase of particle and column diameters. The mean column efficiency is not affected by an increase in the column length.

(6) Particles of diameter *ca.* 30 μm seem particularly advantageous for most uses in preparative chromatography because the columns are easy to pack by the tamping method. (An efficiency of 6000 plates per min has been obtained at a linear velocity of 0.2 cm/sec and at a pressure below 10 MPa/m.)

SYMBOLS

c concentration of test substances (%)
d_c internal column diameter (mm)
$\bar{d}_p$ mean particle size (μm)
h reduced height equivalent to a theoretical plate, $h = \frac{H}{\bar{d}_p}$
H height equivalent to a theoretical plate (cm)
$H_{0.5}$ height equivalent to a theoretical plate measured at 0.5 cm/sec (cm)
H_{loc} mean local height equivalent to a theoretical plate for several columns (cm)
k' partition ratio
L length of column packing (cm)
r_c internal radius of column (mm)
r_i distance of a point from column axis (mm)
t time (sec and min)
u linear velocity of mobile phase (cm/sec)
u_m mean linear velocity of mobile phase (cm/sec)
u_f linear velocity of stationary phase feeding (cm/min)
ϱ density of packing (g/cm^3)
v reduced velocity
σ standard deviation in the distribution of zone concentration (mm)
τ_s sedimentation time (min)

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