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CONSTRUCTION OF COLUMNS FOR LIQUID CHROMATOGRAPHY WITH VERY LARGE PLATE NUMBERS

THEORY AND PRACTICE

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SUMMARY

A new method of characterizing the performance of liquid chromatographic columns of different types, with respect to the compromise between separation speed, pressure drop and number of theoretical plates, by plotting H/v versus $\Delta P/N$ (pressure drop per plate) is proposed. The practical value of this representation in choosing the optimal column type and constructing columns with large plate numbers is demonstrated. The possibility of creating large plate numbers on narrow-bore columns (I.D. 2.8 mm) by the coupling of shorter columns was investigated. The separating power of a column with 50,000 theoretical plates is illustrated by some difficult separations of test and natural mixtures.

INTRODUCTION

Any attempt to generate a large number of theoretical plates in a liquid chromatograph within a reasonable time immediately raises the problem of the compromise between the pressure drop and the quality and speed of the separation, and we therefore need a measure for characterizing columns in this respect. Many proposals for the characterization of a column by means of a single quantity have been made, e.g., the performance index¹, the performance factor², the peak capacity³ and the product of retention time and pressure for a specified plate number⁴. In comparing columns of different types, e.g., with widely varying particle sizes or superficially and totally porous materials, these proposed quantities are not useful for the estimation of the compromise between pressure and separation speed, because these proposals either ignore the pressure limitation or depend on crude simplifying expressions for the plate height.

An important parameter of the separation process, the linear velocity of the eluent, cannot be changed at will because the available input pressure is not infinite. In order to find the optimal situation such that, with the available pressure drop, the separation is possible in the shortest time with the particle size as an adjustable parameter, several methods are in use, e.g.:

(i) The dependence of plate height on flow-rate and particle size is simplified to fractional powers such as $H = d_p^n$ or $H = v^m$. These methods, which were applied by Snyder² and Majors and MacDonald⁵, are suitable only within a relatively narrow range of parameter values, as shown by Huber⁶.

(ii) One can measure the plate height at a specified linear velocity of the eluent, e.g., 1 cm/sec. This again gives a reasonable comparison between different columns only within narrow ranges of parameters where the stated velocity is meaningful.

(iii) It is assumed that the plate height curve, when plotted in the dimensionless parameter $v = vd_p/D_{im}$ and $h = H/d_p$, is independent of the particle size. When this is correct, the compromise between pressure drop and separation speed can be found very elegantly, as shown by Knox⁷ and Knox and Saleem⁸. The optimal operation is then at the minimum of the reduced plate height curve, with a particle size such that the available pressure drop is necessary. However, the solution predicts optimal particle sizes for liquid chromatography that are between 0.5 and 5 μm , which unfortunately lie in the range where packing difficulties occur. In this range, the reduced plate height is not constant, thereby invalidating the mathematical treatment of the problem by Knox and Saleem⁸.

Summarizing the above discussion, we can state that: (a) characterization of the performance of columns with a single quantity is of limited use in practice, and (b) the compromise between speed of separation and pressure drop cannot be found easily. These conclusions are especially valid when one tries to vary the column packing material and particle size over a wide range.

THEORETICAL

We propose here a procedure by which columns can be compared from the operational viewpoint of the analytical chemist and purely experimentally. The operational viewpoint can be expressed as follows. A certain mixture has to be separated and, of different phase systems investigated one or a few are chosen that have the best selectivity. The necessary number of theoretical plates, N_{req} , can then be calculated with the well known equation

$$N_{\text{req}} = (r_{jt} - 1)^{-2} \cdot R^2 \left(\frac{k' + 1}{k'} \right)^2 \quad (1)$$

where r_{jt} = selectivity factor; R = specified resolution; and k' = capacity ratio.

The question for the analyst is now how to achieve this plate number in the shortest time with equipment that is capable of a certain input pressure. To solve this problem, we first have to distinguish two cases: (i) the separation problem is on the trace level with a limited amount of sample available; or (ii) the separation problem is on the analytical level with an unlimited amount of sample available.

In the case (i), on the one hand the analyst desires a very low plate height in order to minimize the sample dilution in the column, e.g., he wants to keep a low detection limit, while on the other hand he needs a certain number of plates in order to resolve his peaks. In case (ii) he can consider the column as a black box and he is not concerned with the inside of the column, e.g., particle size, plate height, length and velocity of the eluent; if it will give the required plate number in a short time but with a plate height of, say, 1 cm, he will be satisfied.

The retention time of the components will be proportional to H/v , according to the equation⁹

$$t_{Rj} = (r_{Hj} - 1)^{-2} \cdot R^2 \frac{(k' + 1)^3}{(k')^2} \cdot \frac{H}{v} \quad (2)$$

Now, H/v is dependent on v , generally decreasing with increasing v . Thus, with a certain type of column (by a column type we mean columns with a certain packing material and packing technique but without a specified length), one desires to work at the highest value of v that is in accord with the maximum pressure available (P_{\max}) and the number of plates required (N_{req}). In the ideal case, a pressure drop of exactly P_{\max} will exist for a column with a length such that the plate number is exactly N_{req} .

For the characterization of column performance, it is therefore logical to plot H/v as a function of $\Delta P/N = p$, the pressure drop per theoretical plate. This allows the analyst to find the point where the whole pressure capacity of his instrument is used by calculating p as the quotient of the known parameters P_{\max} and N . Moreover, different particle sizes, pellicular or totally porous particles can be characterized in one graph. The compromise between pressure drop and speed of separation and the performance of columns can be made very clear in this way.

In order to gain some insight into the experimental curves, which will be presented later, it is useful to discuss the curves obtained when certain models of the permeability and plate height behaviour are adopted. For the dependence of theoretical plate height on parameters such as velocity, particle size and capacity ratio, complicated mathematical expressions are in use. The more complete and exact the expressions are, the more mathematical difficulties result in optimization procedures. For the discussion of the theoretical predictions in the plots of H/v versus p , a simplified model is more useful:

$$H = \alpha \cdot \frac{D_{im}}{v} + \beta d_p + \gamma \cdot \frac{d_p^2 v}{D_{ip}} \quad (3)$$

where α , β and γ are constants depending on the bed geometry and capacity ratio, D_{im} is the diffusion coefficient in the mobile phase, D_{ip} the diffusion coefficient in the stationary bed and d_p the mean particle size.

When we combine the equations with the expression for the pressure drop along the column:

$$\Delta P = \frac{\delta \eta L v}{d_p^2} \quad (4)$$

where δ is a constant of the order of 800, we can obtain a representation of the plot of $\Delta P/N$ as abscissa and H/v as an ordinate:

$$\frac{\Delta P}{N} = \delta \eta \left(\alpha \cdot \frac{D_{im}}{d_p^2} + \beta \cdot \frac{v}{d_p} + \gamma \cdot \frac{v^2}{D_{ip}} \right) \quad (5)$$

$$\frac{H}{v} = \alpha \cdot \frac{D_{im}}{v^2} + \beta \cdot \frac{d_p}{v} + \gamma \cdot \frac{d_p^2}{D_{ip}} \quad (6)$$

We can distinguish two extreme situations. At extremely low velocity, the pressure drop per plate becomes a constant, $\delta\eta\alpha D_{im}/d_p^2$, and the separation speed decreases with the square of the velocity. With a certain particle size and packing structure, a smaller pressure drop per plate can never be obtained. This corresponds to the critical pressure necessary to carry out a separation, as discussed by Giddings^{10,11} and Knox¹². This critical pressure was calculated to be^{10,11}:

$$\frac{\Delta P_{crit}}{N} = \frac{4\phi\gamma\eta D_{im}}{d_p^2}$$

which turns out to agree exactly with our result for the lowest pressure drop per plate, $\delta\eta\alpha D_{im}/d_p^2$.

At extremely high velocity, we have:

$$\frac{\Delta P}{N} \approx \delta\eta\gamma \cdot \frac{v^2}{D_{ip}} \text{ and } \frac{H}{v} \approx \gamma \cdot \frac{d_p^2}{D_{ip}}$$

which shows the high price that must be paid in pressure, proportional to v^2 , to attain high-speed separations.

As can be seen from Fig. 1, each of the extreme cases shows straight-line asymptotic behaviour. The total curve will lie higher and more to the right than the two linear extrapolations because both the first and the last term contribute and because of the contribution of the convection and mobile phase exchange terms. The effects of decreasing the particle size while assuming the same packing geometry are now two-fold:

(a) The right-hand side asymptote, $H/v \rightarrow (\gamma d_p^2/D_{ip})$, is displaced downwards. As

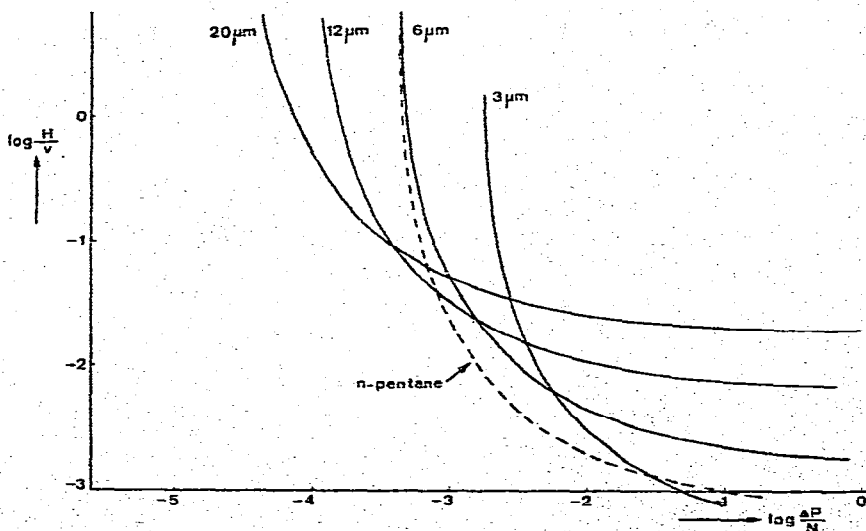


Fig. 1. Theoretically calculated H/v versus $\Delta P/N$ plots. H calculated according to $H = 1.5D/v + 2d_p + 0.1 d_p^2/v$. $\Delta P/N$ calculated according to $\Delta P/N = 800 Hvd_p^{-2}\eta$. Eluent: 2,2,4-trimethylpentane.

long as enough pressure is available and the packing structure remains the same, we can speed up the separation.

(b) The vertical asymptote, $p \rightarrow (\delta\eta\alpha D_{im}/d_p^2)$, is displaced to the right. The critical pressure is raised and with a limited pressure we can no longer obtain very high plate numbers.

Curves for different particle sizes with an assumed constancy of α , β and γ (h constant) will have an intersection point. In practice, this means that in order to obtain very small separation times small particles are to be preferred; on the other hand, very high plate numbers can be obtained only with larger particles with associated low p values.

Finally, Fig. 1, drawn for $d_p = 30, 10$ and $3 \mu\text{m}$, shows that for the fastest realization of a certain plate number with a fixed pressure drop a certain optimal particles size exists. This is the result that Knox and Saleem⁸ obtained previously. Martin *et al.*^{13,14} discussed the same point, together with the practical ramifications.

The dashed line in Fig. 1 shows the effect of a decrease in the viscosity of the mobile phase on the H/v versus p plot. The viscosity change has no influence on the position of the vertical asymptote, $\delta\eta\alpha D_{im}/d_p^2$, because the product ηD_{im} is a constant, according to the Wilke-Chang equation. On the other hand, the horizontal asymptote, $\gamma d_p^2/D_{im}$, shifts downwards (except in the unusual case where the pores of the particles are completely filled with stationary liquid, when no shift will occur).

In conclusion, we can say that our theoretical explanations contain nothing new; all of the results are well documented in the literature and they are given here only to illustrate the significance of the H/v versus p plots. The importance of the representation of this plot is the fact that column performances can be compared with it without any assumptions other than: (a) proportionality of time variance with column length, (b) proportionality of pressure drop with column length and (c) small influence of the capacity ratio k' . Therefore, the representation, although interpretable in theoretical terms, is based purely on experimental results.

EXPERIMENTAL

Apparatus

The liquid chromatographic experiments were carried out on a home-made liquid chromatograph constructed from a reciprocating pump (Orlita, Type AE-10-4.4), a Bourdon-type manometer, a high-pressure injection valve (Valco, HPSV CV-6-UHPA) and a UV spectrophotometer (Zeiss, PM2 DLC). In all of the experiments, stainless-steel columns of I.D. 2.8 mm and length 25 cm were used.

In order to minimize band broadening, special home-made PTFE-filled Swagelok reducing unions with a frit were used to connect the injection port and detector with 0.25-mm I.D. stainless-steel capillary tubing to the column. To couple columns, 1/16-in. zero dead volume Swagelok connectors were used. Most experiments were carried out at a wavelength of 260 nm. In order to prevent contamination of the column, a stainless-steel pre-column (50 \times 1 cm) was installed in front of the injection port.

Materials

The materials used were 2,2,4-trimethylpentane, *n*-pentane, acetone, diethyl

ether, tetrabromoethane and chloroform (Merck, Darmstadt, G.F.R.). Silica gel (Merck, SI-60) was ground and classified in different particle size ranges by means of an air classifier (Alpine MZR, Augsburg, G.F.R.). The fractions were sedimented in acetone-water (1:1) in order to remove fines.

Procedures

The different columns were filled by a balanced slurry technique. About 10% (w/w) of silica gel was slurried in a mixture of tetrabromoethane and chloroform of sp.gr. 2.10, and 10 ml of this slurry were placed into a metal stock tube (30×1 cm) to which the column was connected. The slurry was pumped into the column as rapidly as possible with a high-pressure pump (Burdosa, Type V410) at a maximum pressure of 1000 atm with 2,2,4-trimethylpentane. After filling, the columns were eluted successively with acetone, diethyl ether and 2,2,4-trimethylpentane (about 100 ml each). Finally, the columns were eluted with 2,2,4-trimethylpentane plus 0.5% of *n*-butanol as a modifier until the capacity ratios of the test samples were constant.

To calculate the number of theoretical plates of the columns, four methods for measuring the standard deviation (σ_t) were used: (1) half the peak width at 0.607 of the peak height; (2) one quarter of the peak width at 0.135 of the peak height; (3) one quarter of the peak width at the base by drawing the tangents; and (4) according to the normalized central second moment. The retention times were measured to the peak maximum with a stop-watch. The particle sizes were determined visually from photographs made with a scanning electron microscope (S.E.M.). Special attention was paid to minimizing the external peak broadening caused by the detector, injector and connection tubes. The variance caused by these components was found to be $36 \mu^2$. No correction for this external effect was applied.

RESULTS AND DISCUSSION

To show the usefulness of H/v versus p plots for characterizing the performance of columns, some plots were constructed from data available in the literature (Fig. 2). Some of the data were incomplete and had to be extrapolated. Despite this, Fig. 2 shows clearly the value of such H/v versus p plots. The graph for porous layer beads (curve a) from the data of Done *et al.*¹⁵ is noteworthy. It shows that porous layer beads act as an excellent column material and should be preferred for the attainment of very large plate numbers with a reasonable pressure drop, compared with the completely porous supports, because of the favourable pressure drop per plate. However, porous-layer beads have some disadvantages which cannot be seen directly from the H/v versus p plots: (i) the sample capacity of porous layer beads is two orders of magnitude smaller than that of completely porous materials, so that such columns are easily overloaded, resulting in asymmetrical peaks; and (ii) the theoretical plate height of this type of column is high because of the large particle size, which results in a greater dilution of the sample on its passage through the column and may lead to detection problems in trace analysis.

The completely porous supports have a large sample capacity and the particle size can be very small, which is favourable for extremely small plate heights. This type of support must be preferred in most instances, despite the larger pressure drop needed to obtain reasonable separation speeds. In order to demonstrate more precisely

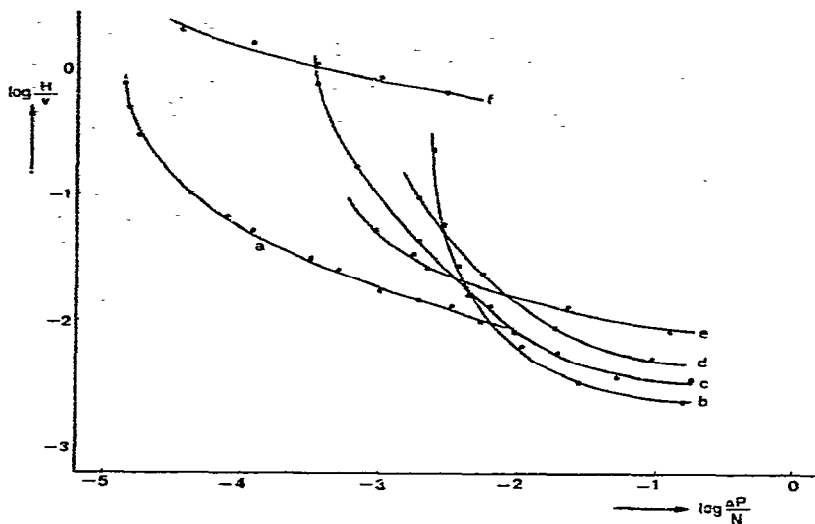


Fig. 2. Some H/v versus $\Delta P/N$ plots constructed from data from the literature.

Curve	d_p (μm)	Eluent	Support	Coating	Ref.
a	29	Hexane	Porous layer bead	2% β, β' -Oxydipropionitrile	15
b	4.2	<i>n</i> -Pentane	Spherical silica	—	16
c	5–6	Hexane	Spherical silica	30% β, β' -Oxydipropionitrile	17
d	4.2	Heptane	Spherical silica	—	16
e	5–6	Hexane	Spherical silica	—	18
f	63–80	Isooctane	Diatomaceous earth	8% Fractonitril III	19

the relationship between separation speed and pressure drop, a number of columns were filled with silica gel of different particle sizes by a balanced density method and H/v versus p plots were constructed, as shown in Fig. 3. The curves agree very well

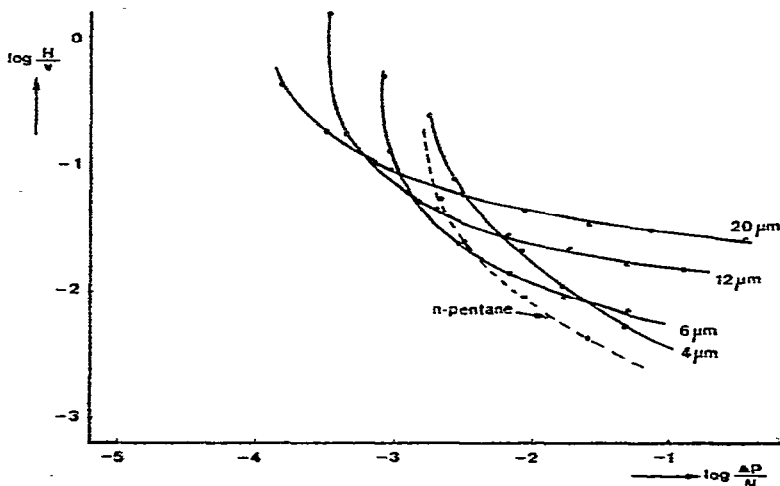


Fig. 3. Experimentally measured H/v versus $\Delta P/N$ plots. Support: irregular silica. Eluent: 2,2,4-trimethylpentane + 0.5% butanol. Solute: *m*-dinitrobenzene ($k' = 2.8$).

TABLE I
EFFECT OF METHOD OF CALCULATION ON THE THEORETICAL PLATE NUMBER MEASURED

No. of columns coupled	Compound	Procedure						2nd moment		
		0.607 of peak height		0.135 of peak height		Tangents		Measured	Expected	
		Measured	Expected	Measured	Expected	Measured	Expected			
1	Toluene ($k' = 0$)	10,000	—	9500	—	9600	—	6000	—	
2		20,500	20,100	19,400	17,900	19,400	19,200	13,600	11,500	
3		29,700	28,700	27,200	25,600	27,800	27,400	21,700	16,800	
4		40,800	37,800	38,800	34,300	39,000	36,300	—	—	—
5		51,100	46,800	47,500	42,600	48,800	44,700	—	—	—
1	<i>p</i> -Nitrotoluene ($k' = 0.5$)	11,100	—	10,400	—	10,500	—	7400	—	
2		21,100	23,500	18,700	19,800	19,700	21,900	15,900	14,200	
3		28,900	33,600	25,900	28,100	27,600	31,300	22,500	20,500	
4		40,000	43,600	35,000	37,600	35,100	40,900	—	—	
5		47,100	54,200	42,400	46,700	44,100	50,900	—	—	

with the theoretical predictions, as discussed before. It can be seen that the fastest separations can be achieved on columns filled with very small particles, as was pointed out earlier by some workers, but the price one has to pay for a reasonable number of theoretical plates is very high. This is especially the case for the 4- μm particles, because the reduction in plate height is no longer proportional to the particle size, at least with our packing method. In our opinion, the very small particles (<5 μm) are interesting only for trace analyses on very short columns in those instances where the number of theoretical plates need only be small because of large selectivity factors.

Influence of the viscosity of the mobile phase on H/v versus p plots

According to eqn. 4, the pressure drop is proportional to the viscosity of the eluent. An increase in the viscosity has no effect on the asymptote $\delta\eta\alpha D_{im}d_p^{-2}$ (ηD_{im} is constant). According to eqn. 3, the theoretical plate height also depends on the diffusion coefficients, D_{ip} and D_{im} . D_{ip} is an overall coefficient, built up from D_{im} (stagnant mobile phase) and D_{is} (stationary phase). An increase in the viscosity of the mobile phase will increase the theoretical plate height. As a result the plots are shifted upwards in this case. The change in the H/v versus p plots due to a change in the viscosity of the eluent can be seen from Fig. 3, where the dashed line shows the shift if the eluent is changed from 2,2,4-trimethylpentane ($\eta = 0.50$) to *n*-pentane ($\eta = 0.23$) for 4- μm particles (see also curves b and d in Fig. 2). If different columns are compared by our proposed method, one has to take account of large differences in the viscosity of the mobile phase.

Effect of the procedure on the number of theoretical plates measured

Recent results obtained with very fine particles in short columns by various workers suggest reasonable elution times for plate numbers in the range 50,000–100,000. There is some doubt, however, whether these plate numbers can be extrapolated to larger total column lengths. In particular, the fact that the peak shapes on these short columns are frequently asymmetrical raises this doubt. In order to elucidate this point, we investigated the effect of the procedure for the measurement of the plate number on the results obtained.

The linearity of the distribution isotherm was first checked by varying the sample size over two orders of magnitude. No effect on the capacity ratio was found. Nevertheless, all peaks showed a small degree of tailing. This effect could be due to the injection profile and to the so-called wall effect, as discussed by Knox and Parcher²⁰. Four different methods of determining the peak standard deviations were applied, *viz.*, measurements of the peak width at 0.607 and 0.135 of the maximal peak height, the tangent method and the normalized central second moment. With the last procedure, reproducible results could be obtained only with small column lengths, because problems with noise and drift rejection arose with larger column lengths. The results of the measurements, on a number of separate and coupled columns for an unretained and a retained component, are presented in Table I.

Two trends can be seen in Table I:

(i) For the retained compound (*p*-nitrotoluene), the situation is the simplest, because the extra-column effects play a minor role. It can be seen that on extrapolation from a 25-cm column length to five times this length larger plate numbers are predicted than are actually measured. This is the case for both measurements via the peak width

at a certain peak height and for the tangent method. On the basis of our experiments, it is possible to conclude only that the second moment gives a better fit up to a combination of three columns. It is reasonable to suppose that with asymmetrical peaks, with a tail in the 1-5% maximal peak height region, the first three methods overestimate the separation power of long columns; such tails do not affect the measurement on one column, but when a number of columns are coupled the repeated convolution of elution curves will bring the effect of the tails into the higher parts of the elution curve, *e.g.*, the peak width at a certain peak height increases.

(ii) With the unretained component (toluene), this effect is overshadowed by the extra-column effects. As a result, the single-column (25 cm) experiments give systematically low results. When a number of columns are coupled, the effect decreases. As a result, the actual plate numbers measured are greater than the extrapolated values.

Construction of columns with large plate numbers

Although in practice a large number of separations can be carried out effectively on columns with 5000-10,000 plates, in some instances more plates are very useful, if not really necessary, such as:

(1) in those instances where, despite the fact that the phase system is optimized as well as possible, for some components of the sample the selectivity factors are still very small;

(2) for those separation problems where the capacity factors of the solutes are very small and cannot be increased;

(3) for the separation of very complex mixtures; optimization of the phase system in such difficult separations is almost impossible because any improvement in the selectivity factor of two solutes in the mixture usually worsen the selectivity factor of another pair.

In order to demonstrate that it is possible to create columns with really large plate numbers (50,000) at a certain separation speed ($H/v = 7 \cdot 10^{-3}$ sec) and a fixed pressure drop (350 atm), a number of 25-cm columns were coupled. According to Fig. 3, 6- μ m silica gel particles were selected as being the best choice for this problem. The final column length required in order to fulfil the stated demands was 125 cm. This result proves the possibility of creating really large plate numbers on narrow-bore coupled columns and shows the value of H/v versus $\Delta P/N$ plots for selecting the best column material. Fig. 4 shows some separations of test and natural mixtures on this 50,000-plate column and confirms the large separation power that one can expect.

CONCLUSIONS

The main conclusions can be summarized as follows:

(1) Comparison of columns with respect to separation speed and pressure drop by constructing H/v versus p plots is very valuable for a large range of parameters, because it is based exclusively on experimental results.

(2) One has to be careful when extrapolating plate numbers based on measurements on short columns.

(3) Large plate numbers can be created by coupling short narrow-bore columns.

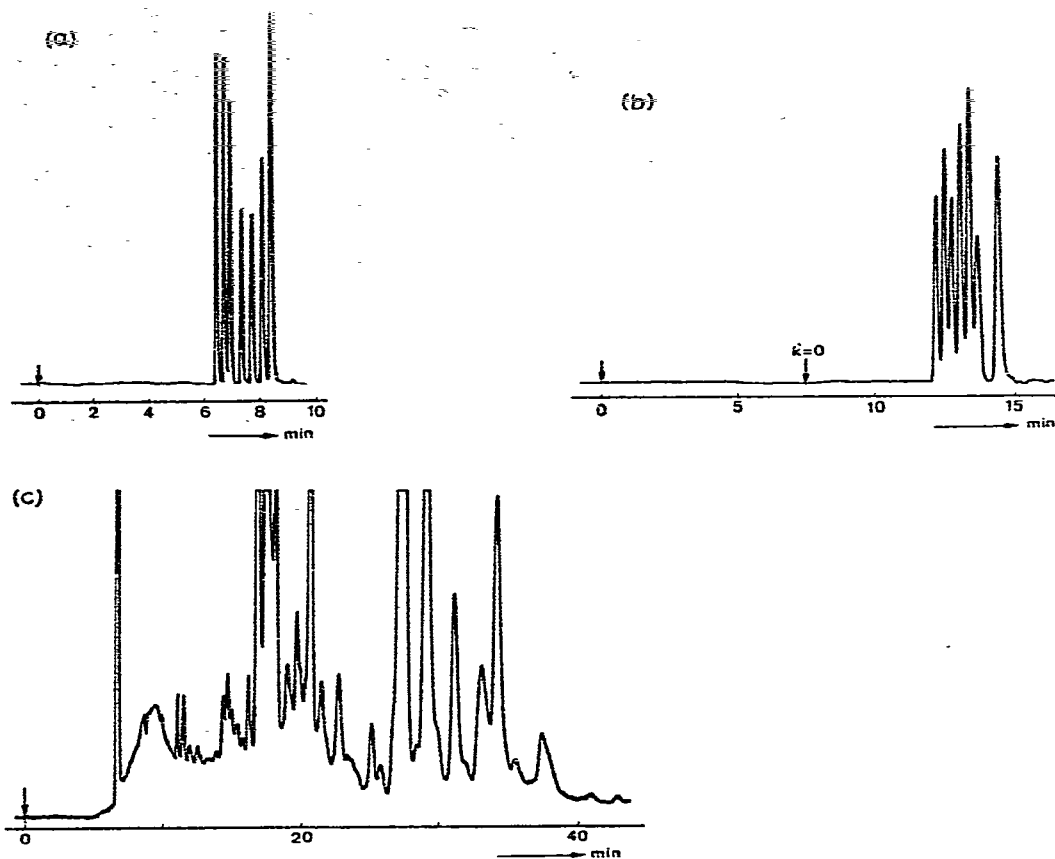


Fig. 4. Separations on a 50,000-plate column. Support: irregular silica ($d_p = 6 \mu\text{m}$). Column length, 125 cm; I.D., 2.8 mm. Eluent: *n*-pentane + 0-0.2% butanol. (a) Test mixture of aromatic hydrocarbons: dodecylbenzene, butylbenzene, toluene, naphthalene, anthracene, fluoranthene, chrysene ($k' = 0.31$). (b) Test mixture of alkylbenzenes: C_{12} , C_{10} , C_8 , C_6 , C_4 , C_1 , benzene. (c) Extract of the ash from a refuse incinerator.

(4) Standardization or at least a description of theoretical plate height measurement methods is recommended.

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