COMPARISON OF THE EFFICIENCY OF LIQUID CHROMATOGRAPHY SYSTEMS WITH MIXED ADSORBENTS WITH THAT OF SYSTEMS WITH CONNECTED ADSORBENT LAYERS

JERZY S. KOWALCZYK and BARBARA ŚLEDZIŃSKA

Department of Chemical Engineering and Measuring Techniques, Technical University of Gdańsk, 80-952 Gdańsk (Poland)

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SUMMARY

Processes that determine band broadening in systems with mixed beds and those with connected bed layers were investigated. The correctness of theoretical considerations was confirmed experimentally by plotting the dependences of the theoretical plate height or the apparent theoretical plate height on the weight percentage of alumina for systems that contain alumina plus silica gel. The final conclusion reached was that it is only in systems with mixed beds that additional phenomena occur which make it impossible to predict the efficiency of the systems on the basis of simple additivity of the values that characterize pure adsorbents. The dependences of efficiency on the composition of stationary phases in the latter systems will therefore always show positive deviations from linear plots. The plots of the above functions in systems with connected bed layers may show either negative or positive deviations. The parameters that influence the character of the deviations of these functions were determined.

INTRODUCTION

The chromatographic separation of polar and non-polar compounds or of polar compounds that contain functional groups with a weakly acidic or basic character may sometimes be very difficult to achieve in a single operation. In such instances the gradient technique is generally used, and chromatographic systems with multi-component stationary phases can also be useful. These can be different phases in a series of connected columns or in a single column that contains two adsorbent layers. Mixtures of particles of several types of phases (mixed beds) can also be used.

Connected columns, columns with connected layers and columns containing mixed beds have so far not been used in liquid chromatography. The use of mixed beds in thin-layer chromatography has been reported only in a few instances[1-7] where, however, no attempts were made to draw general conclusions or to establish rules for the application of multi-component stationary phases. This has been attempted only in investigations with gas chromatography[8,9].

The purpose of the liquid chromatographic study described here was to establish the characteristics of two-adsorbent systems in columns containing mixed beds or connected bed layers and to compare their efficiencies. Both of these systems were heterogeneous, differing mainly in the type of border surface on which the properties of the system undergo step changes. With mixed beds, these step changes occur only on the particle surfaces of the various adsorbents, whereas in connected bed layers they occur in the space between the layers that form various sets of packing material. The efficiency of each of these systems must first be characterized.

EFFICIENCIES OF SYSTEMS WITH SINGLE-COMPONENT ADSORBENTS AND THOSE WITH MULTI-COMPONENT ADSORBENTS IN LIQUID CHROMATOGRAPHY

The efficienciencies of systems with single-component phases and those with mixed phases are functions of similar parameters. For both of these systems, the well known Giddings[10] equation for the height of a theoretical plate applies:

$$H = (C_k + C_{m_2})u + \cfrac{1}{\cfrac{1}{A} + \cfrac{1}{\sum_i C_{m_i} u}} \tag{1}$$

where

$A = 2 \sum_i \lambda_i d_p$ = eddy diffusion;

$C_k = \dfrac{2(1-R)^2}{k_a}$ = kinetics of the sorption–desorption processes;

$C_{m_i} = \dfrac{\omega_i d_p^2}{D_m}$ = diffusion in the mobile phase, C_{m_2} referring to diffusion in the part of the mobile phase deposited in pores of adsorbent particles;

u = linear flow-rate of the mobile phase;
R = retention coefficient;
λ_i, ω_i = structural parameters;
D_m = diffusion coefficient;
k_a = adsorption constant;
d_p = particle diameter.

In considering the various terms in eqn. 1, one has to bear in mind that the systems investigated are not strictly homogeneous but are real systems which, even with single-component adsorbents, contain sets of particles that differ not only in size, shape and structure but also in the packing density at different positions in the column. Mean values that characterize the various sets of particles cannot be used because efficiency is not an effect that results from simple addition of component values.

The heterogeneity of stationary phases influences the efficiency of systems with single-component adsorbents and of those with mixed adsorbents. In the latter, however, additional differences in the properties of the adsorbents may appear that do not exist in single-component systems, and some of these properties are considered below.

According to Giddings[10] estimate of the parameters λ_i and ω_2 (see eqn. 1), the efficiency of single-component systems at typical flow-rates of the liquids is influenced by eddy diffusion. Eddy diffusion can be of even greater importance to the efficiency of systems with mixed phases, as a result of additional differences in the properties of the column packing. For example, different particle wettabilities in the two types of adsorbent may increase the velocity differences in the liquid, thus increasing the influence of inter-particle, trans-particle and trans-channel effects on the value of term A in eqn. 1. An increased influence of the trans-column effect can also result from different specific gravities of the particles of the two adsorbents or from different surface friction coefficients.

The above findings also concern diffusion phenomena in mobile phases (component C_{m_t} in eqn. 1) because of the analogy between the coefficients λ in the expression for eddy diffusion and the coefficients ω.

A comparative estimation of the influence of sorption–desorption kinetics (term C_k in eqn. 1) on either of the systems must be preceded by estimation of the range of variations in the values of R and k_q. The influence of these parameters in systems with single-component stationary phases increases with increase in the differences in adsorption energy for the different surface sites. In such instances, Giddings[10] established the equation

$$C_k = \frac{2(1 - R)^2}{\alpha \left(\dfrac{z}{n}\right)\left(\dfrac{A}{V_m}\right)} \cdot \lambda \tag{2}$$

where

α = sticking coefficient;

λ = heterogeneity coefficient;

z = number of collisions of compound molecules with unit surface area of the stationary phase;

n = number of molecules in unit volume of the mobile phase;

V_m = volume fraction of the mobile phase in the layer.

Sorption–desorption kinetics in systems with mixed phases can be assumed to be influenced by two factors:

(a) a factor originating from mass exchange kinetics on the surface of each type of particle the values of which result from additivity of component characteristics; and

(b) a factor resulting from macroheterogeneity of the system according to the differences in adsorption energy on each surface of the mixed stationary phase; by analogy with λ in eqn. 2, this factor can be called the macroheterogeneity coefficient, λ_m.

Thus, the influence of sorption–desorption kinetics on the efficiency of systems with mixed two-component stationary phases can be expressed by

$$C_k = \left[X_1 \cdot \frac{2(1 - R_1)^2}{\alpha_1 \left(\dfrac{z}{n}\right)\left(\dfrac{A_1}{V_{m_1}}\right)} \lambda_1 + X_2 \cdot \frac{2(1 - R_2)^2}{\alpha_2 \left(\dfrac{z}{n}\right)\left(\dfrac{A_2}{V_{m_2}}\right)} \lambda_2 \right] \lambda_m \tag{3}$$

where X_1 and X_2 are the weight fractions of components 1 and 2, and the other symbols are as in eqn. 2, with subscripts indicating the adsorbent.

To summarize, it must be stated that liquid chromatographic systems with mixed adsorbents are less efficient than would result from simple additivity of the corresponding values for single-component packings, because the fact that the particles of various adsorbents are mixed has an additional influence on the values of all terms in eqn. 1.

EFFICIENCIES OF CHROMATOGRAPHIC SYSTEMS CONTAINING CONNECTED LAYERS OF DIFFERENT ADSORBENTS

If the band-broadening processes in the component layers are assumed to be independent, then the apparent theoretical plate height in the systems discussed here is determined by the equation[10,11]

$$\hat{H} = \frac{\Sigma L_i \Sigma \tau_i^2}{(\Sigma t_i)^2} \tag{4}$$

where

L_i = length of the layer (or column) with a stationary phase i;
τ_i = standard deviation of zone profile obtained for the compound leaving layer i;
t_i = retention time of the compound leaving layer i.

A problem to be considered is the efficiency of the systems discussed, a measure of which is the apparent plate height depending on the relative length of the two layers and their efficiencies. Introduction into eqn. 4 of known dependences, adequately transformed, gives the following equation for systems with two connected bed layers:

$$\hat{H} = \frac{H_1 \cdot \dfrac{L_1}{L} + H_2 \cdot \dfrac{L_2}{L} \cdot M^2}{\left(\dfrac{L_1}{L} + \dfrac{L_2}{L} \cdot M\right)^2} \tag{5}$$

where

H_1, H_2 = theoretical plate heights in layers 1 and 2, respectively;
M = ratio of the retention parameters for the compound zones in layers 1 and 2, which equals R_1/R_2 if the migration rates are assumed to be $u_1 = u_2$.

Substitution in eqn. 5 of the expression for H_1 and $H_2 = f(u)$ from eqn. 1 gives an equation that can be written in a simplified form as

$$\hat{H} = (\hat{C}_k + \hat{C}_{m_2}) u + \frac{1}{\dfrac{1}{\hat{A}} + \dfrac{1}{\Sigma \hat{C}_{m_i} u}} \tag{6}$$

where $\hat{A}$, $\hat{C}_k$, $\hat{C}_{m_2}$, $\hat{C}_m$ are expressions for the apparent eddy diffusion, mass transfer resistances and diffusion in the mobile phase, respectively, all of which depend on L_1/L, L_2/L and M, as does $\hat{H}$ in eqn. 5.

It can be seen from eqn. 6 that the plot of $\hat{H} = f(u)$ will agree with that given by Giddings (eqn. 1). It must be borne in mind, however, that the various components of eqn. 6 no longer have the meaning given to them by the theory of random-walk processes for systems with a single-component stationary phase.

In the systems discussed here, the apparent plate height will be a linear function of the relative layer (column) length only for $M = 1$. In situations that are of interest in practice, *i.e.*, when $M > 1$, the plot for the above function will deviate from linearity, the magnitude and direction of the deviation depending on the relative values of M, H_1, H_2 and L_1/L in eqn. 5.

Negative deviations of the function $\hat{H} = f(L_1/L)$ will occur if the following condition is satisfied:

$$\frac{\frac{L_1}{L} \cdot H_1 + \frac{L_2}{L} \cdot M^2 H_2}{\left(\frac{L_1}{L} + \frac{L_2}{L} \cdot M\right)^2} < \frac{L_1}{L} \cdot H_1 + \frac{L_2}{L} \cdot H_2 \tag{7}$$

It can be stated that after adequate transformation of eqn. 7, negative deviations from the linearity of the function $\hat{H} = f(L_1/L)$ will occur when

$$\frac{H_1}{H_2} > \frac{\frac{L_2}{L}\left[\left(\frac{L_1}{L} + \frac{L_2}{L} \cdot M\right)^2 - M^2\right]}{\frac{L_1}{L}\left[1 - \left(\frac{L_1}{L} + \frac{L_2}{L} \cdot M\right)^2\right]} \tag{8a}$$

Similarly, positive deviations will occur when

$$\frac{H_1}{H_2} < \frac{\frac{L_2}{L}\left[\left(\frac{L_1}{L} + \frac{L_2}{L} \cdot M\right)^2 - M^2\right]}{\frac{L_1}{L}\left[1 - \left(\frac{L_1}{L} + \frac{L_2}{L} \cdot M\right)^2\right]} \tag{8b}$$

In order to establish how the dependence $\hat{H} = f(L_1/L)$ is influenced by the H_1/H_2 values, the values of the right-hand side of inequalities 8a and 8b were plotted (Fig. 1) as a function of L_1/L for parameter values usually encountered in practice. This function is valid for $L_1/L = 0.05$–0.95 (for more extreme values it reaches the limits 0 and ∞, which do not enter into consideration).

It can be seen from Fig. 1 that inequality 8b is always satisfied for $H_1/H_2 < 1$. In such instances, the efficiency of the chromatographic systems considered here is lower for the whole range of L_1/L values discussed and for all assumed values that might result from the linear plot for the dependence $\hat{H} = f(L_1/L)$.

The curve $\hat{H} = f(L_1/L)$ for $H_1/H_2 > 1$, however, can be characterized, depending on the values of this ratio and of M, by a positive deviation from linearity, or initially by a negative deviation and then by a positive deviation within the range of L_1/L values. The latter two types of deviation are, of course, characterized by the presence of a point of inflection at a certain value of L_1/L. For example, as can be seen from Fig. 1 for $M = 5$, which is a value likely to occur in practice, positive
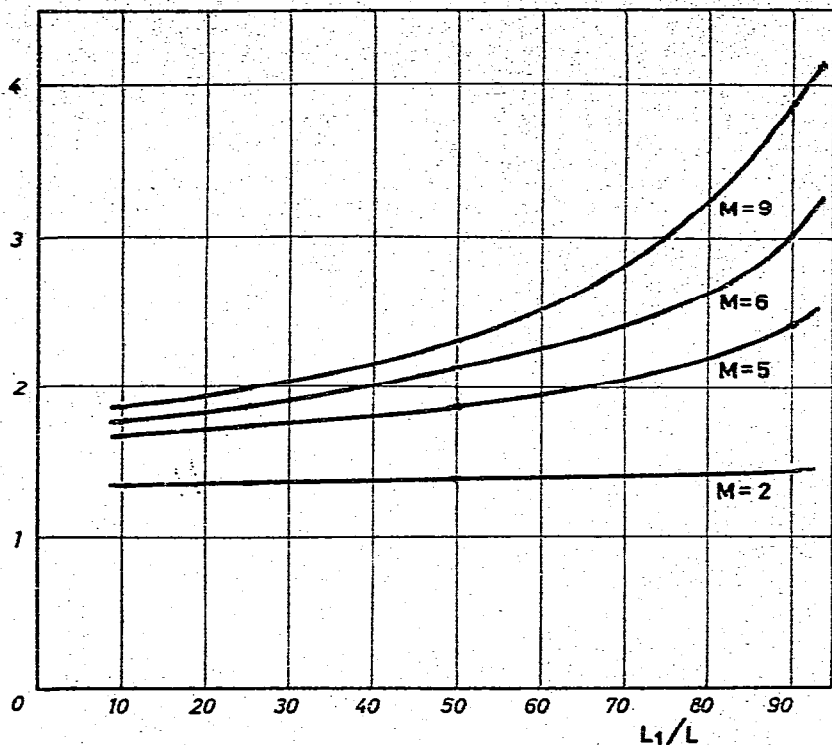
Fig. 1. Right-hand side of inequalities 8a and 8b as functions of L_1/L for various values of $M = R_1/R_2$ (for more detailed explanation, see text).

deviations occur within the whole of the L_1/L range for $H_1/H_2 > 1.67$. If, however, $H_1/H_2 = 2$, then for $L_1/L < 0.6$ the above function will show negative deviations from linearity, while for $L_1/L > 0.6$ they will be positive. On the other hand, for $H_1/H_2 > 2.5$, the deviations will be negative over the whole of the L_1/L range examined.

EXPERIMENTAL

Apparatus and materials

Measurements were made with a DuPont Model 830 liquid chromatograph, using a 253.7-nm UV detector and 0.2 × 50 cm steel columns. The mixed phases and the connected layers contained silica gel H nach Stahl (Type 60) (Merck, Darmstadt, G.F.R.) and alumina (neutral; Woelm, Eschwege, G.F.R.) in various weight proportions.

Fig. 2 shows the curves characterizing the particle size distribution in these adsorbents. The results were obtained on a Quantimet 720 apparatus (Imanco).

The following test compounds were utilized: 8-methylquinoline, α-naphthoquinoline, o-nitroaniline, p-nitroaniline, o-nitrophenol, p-methyl-o-hydroxyazobenzene and azobenzene.
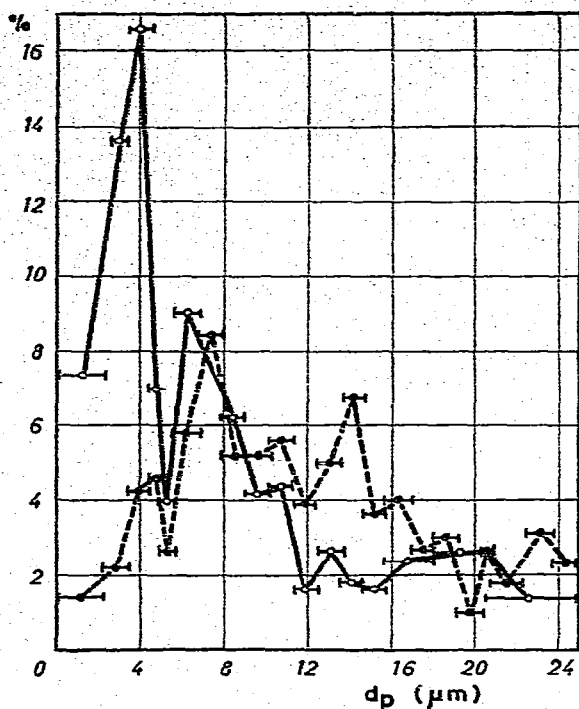
Fig. 2. Curves for particle size distribution (d_p) for the adsorbents alumina (solid line) and silica gel (broken line).

Methods

Mixed stationary phases were prepared as follows. An aqueous suspension of a mixture of the two adsorbents in appropriate weight ratios was placed on a glass plate and activated for 2 h at 125°, then cooled in a desiccator. The layer obtained was removed and utilized as the column packing.

Connected layers were prepared by placing a suspension of each of the adsorbents on a glass plate and activating them under similar conditions. The adsorbents were removed and both were used as the column packing. In the column two layers in suitable weight ratios were formed, each consisting of a different adsorbent; the upper layer was always silica gel.

The columns with mixed stationary phases and those with connected phases were gradually filled with small portions of packing material. Each time ca. 50 mg of adsorbent had been inserted into the column, its contents were subjected to an argon pressure of 35 atm in order to compact the packing materials.

Before taking measurements, a solvent was passed through the columns so as to balance the phase properties until the retention volume of the test compound was constant.

Mobile phase

Ethylene chloride was distilled, then dried by passing it through a column packed with 0.2–0.5-μm silica gel (Merck). The compounds investigated were injected

as 0.01 M solutions. The volume of a sample was 1–3 μl, depending on the intensity of light absorption.

For columns with mixed phases, each value of the theoretical plate number plotted was the mean of at least three measurements taken from three independently packed columns. Considering that it is hardly possible to pack exactly the assumed weight proportion into columns with connected bed layers each time, the measurements referring to these systems were plotted as average values of at least three results from each column.
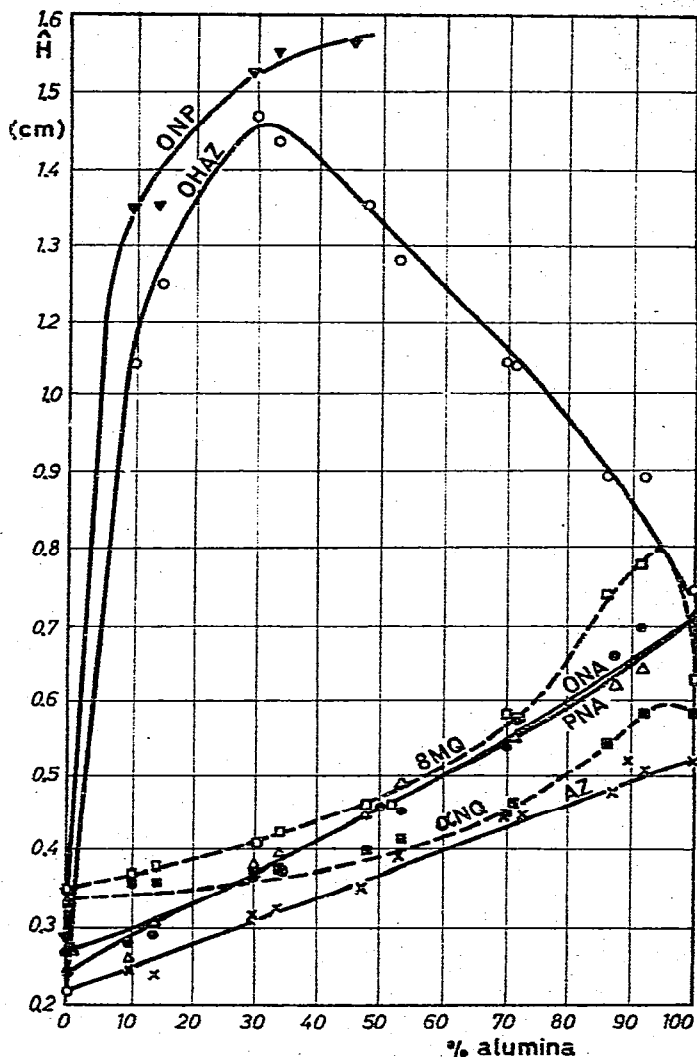
Fig. 3. Dependence of apparent theoretical plate height ($\hat{H}$) on the percentage by weight of alumina for connected bed layers containing alumina + silica gel. Mobile phase, ethylene chloride; flow-rate, 0.23 cm/sec. Curves were calculated on the basis of eqn. 5; points were obtained experimentally (only the curve for ONP was plotted merely on the basis of experimental data). ONA = o-nitroaniline; PNA = p-nitroaniline; α-NQ = α-naphthoquinoline; 8MQ = 8-methylquinoline; OHAZ = p-methyl-o-hydroxyazobenzene; ONP = o-nitrophenol; AZ = azobenzene.

TABLE I
VALUES OF RETENTION COEFFICIENT RATIO (M) AND HETP FOR BOTH AD-SORBENT LAYERS FOR OBTAINING DEPENDENCES PLOTTED IN FIG 3

Compound	$M = R_1/R_2$	H_1	H_2	H_1/H_2
p-Methyl-o-hydroxyazobenzene	6.4	0.22	0.75	0.29
p-Nitroaniline	1.4	0.27	0.71	0.38
o-Nitroaniline	1.3	0.24	0.71	0.34
8-Methylquinoline	5.4	0.67	0.36	1.86
α-Naphthoquinoline	2.7	0.63	0.33	1.91
Azobenzene	1.0	0.22	0.52	—

RESULTS AND DISCUSSION

Fig. 3 shows the curves defining the changes that occur in the apparent theoretical plate height depending on the proportions of alumina and silica gel in the columns that contain connected bed layers. The curves were calculated from eqn. 5, taking into account the experimental data obtained for 100% of alumina or 100% silica gel. The points obtained experimentally are also shown in Fig. 3. The curve for o-nitrophenol (ONP) was obtained only experimentally because retention data for 100% alumina could not be obtained.

It can be seen from Fig. 3 that there is good agreement between the experimental and calculated data. This confirms the fact that the band-broadening processes in the two adsorbent layers are independent of each other, as predicted.

Table I shows the M and H_1/H_2 values obtained for the compounds included in Fig. 3.

Subsequently attempts were made to predict the course of the function $\hat{H} = f(\%Al_2O_3)$ for connected bed layers, taking into account the data in Table I and information obtained from the dependences plotted in Fig. 1. The results obtained were compared with those in Fig. 3. Good agreement was found between the courses of the dependences $\hat{H} = f(\%Al_2O_3)$ that were predicted on the basis of Fig. 1 and plotted in Fig. 3. It could therefore be predicted that, depending on the values of M and H_1/H_2, the curves in Fig. 3 would show positive deviations from linearity over the whole range of measurements for p-methyl-o-hydroxyazobenzene, negative deviations for α-naphthoquinoline, and that there would be points of inflection for 8-methylquinoline. On the other hand it could be predicted for compounds with $M \approx 1$ (for azobenzene and o- and p-nitroaniline) that the dependences $\hat{H} = f(\%Al_2O_3)$ would be virtually rectilinear.

Fig. 4 shows the dependences $H = f(\%Al_2O_3)$ in systems containing mixed beds, i.e., alumina + silica gel. The influence of retention data on the theoretical plate heights was observed much less in systems with mixed beds than in those with connected bed layers. According to the above prediction, the plots in Fig. 4 are similar for all compounds, i.e., they all show positive deviations from linearity, and only the magnitude of the deviations depends on the type of compound involved. It should be noted that the curves $H = f(\%Al_2O_3)$ for systems with mixed beds showed deviations from linearity also for compounds whose R values were nearly identical in both adsorbents. This effect was also in agreement with the prediction
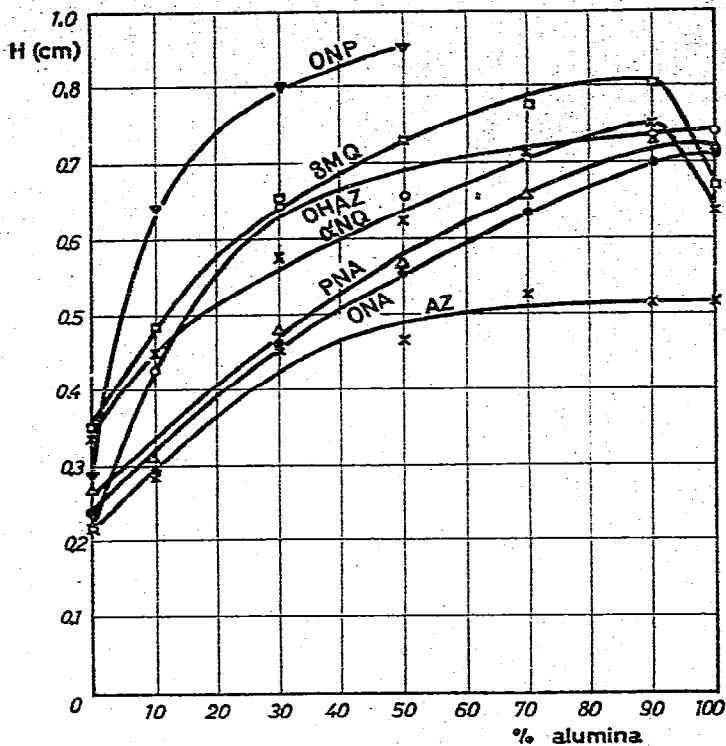
Fig. 4. Experimental dependence of the theoretical plate height (*H*) on the percentage by weight of alumina for mixed bed systems containing alumina + silica gel. Mobile phase, ethylene chloride; flow-rate, 0.23 cm/sec. Abbreviations as in Fig. 3.

that, in contrast to systems with connected bed layers (see Fig. 3), the dependences for mixed beds are never linear.

In order to characterize the efficiency of systems with mixed beds better, the dependences $H = f(u)$ were plotted, taking into consideration the various components of the equation for the theoretical plate height. As the dependences $H = f(u)$ within the range of values investigated were not linear, they were interpreted on the basis of the equation
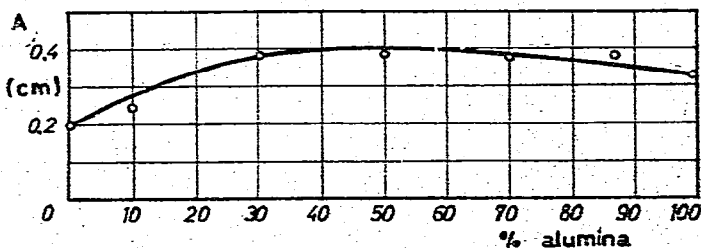
$$H = A + (C_k + C_{m2})u \qquad (9)$$

Fig. 5. Dependence of eddy diffusion (*A*) on the percentage by weight of alumina for mixed bed systems containing alumina + silica gel. Mobile phase, ethylene chloride.
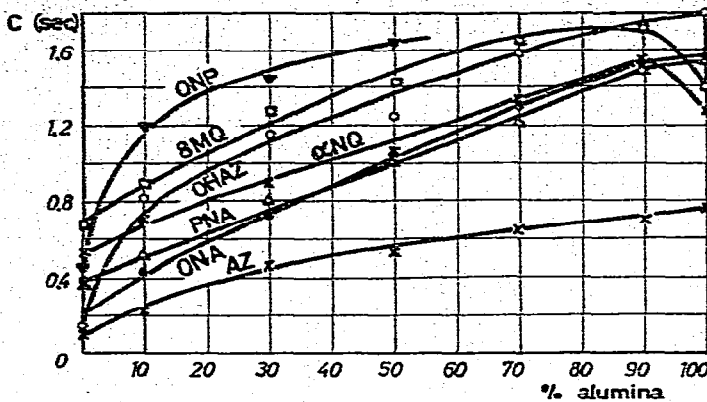
Fig. 6. Dependence of mass transfer resistance (C) on the percentage by weight of alumina for mixed bed systems containing alumina + silica gel. Mobile phase, ethylene chloride. Abbreviations as in Fig. 3.

Fig. 5 shows the curves that characterize eddy diffusion for various proportions of alumina and silica gel in columns packed with mixed beds. The curves indicate that additional parameters affect the systems discussed and change the flow conditions of the liquid phases.

Changes in the parameters that characterize mass transport resistance (C_k + C_{m2}), together with changes in the composition of the stationary phases, are shown in Fig. 6. The curves were found to be significantly dependent on the type of compounds involved, which indicates a significant participation of the component and illustrates the influence of sorption–desorption kinetics (C_k). The values of C_k were found by using the coefficient of mass transport resistance determined for a virtually non-adsorbing compound, namely azobenzene (curve AZ in Fig. 6). The mass transport resistances for azobenzene could be assumed to depend only on the diffusion component in the part of the mobile phase deposited in inaccessible pores of the packing. The values of C_{m_2} obtained in this way can be regarded as constant for all compounds investigated in the systems described here. The values of C_k calculated on the above basis as a function of alumina content in mixed beds are shown in Fig. 7.
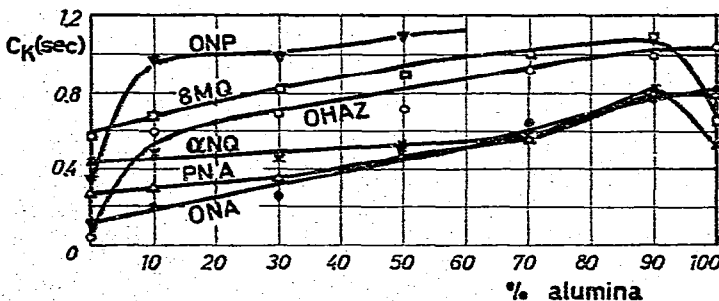

Fig. 7. Dependence of the adsorption–desorption kinetics term (C_k) on the percentage by weight of alumina for mixed bed systems containing alumina + silica gel. Mobile phase, ethylene chloride. Abbreviations as in Fig. 3.
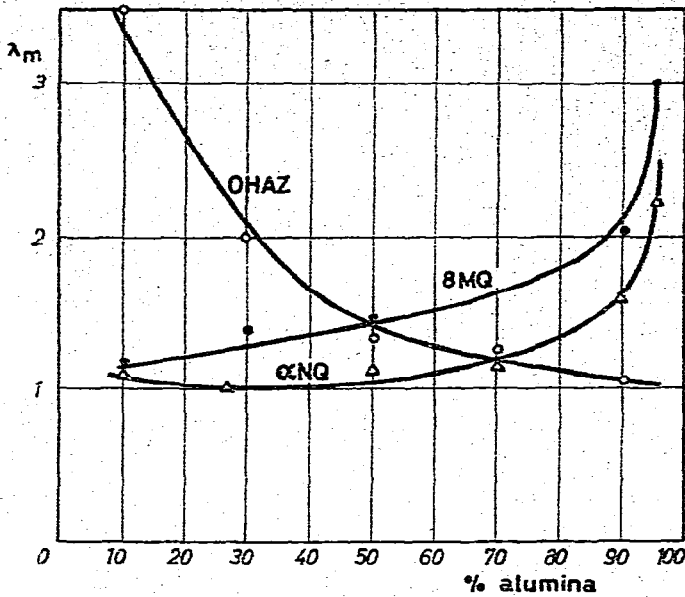
Fig. 8. Dependence of the macroheterogeneity factor (defined by eqn. 3) on the percentage by weight of alumina for mixed bed systems containing alumina + silica gel. Mobile phase, ethylene chloride. Abbreviations as in Fig. 3.
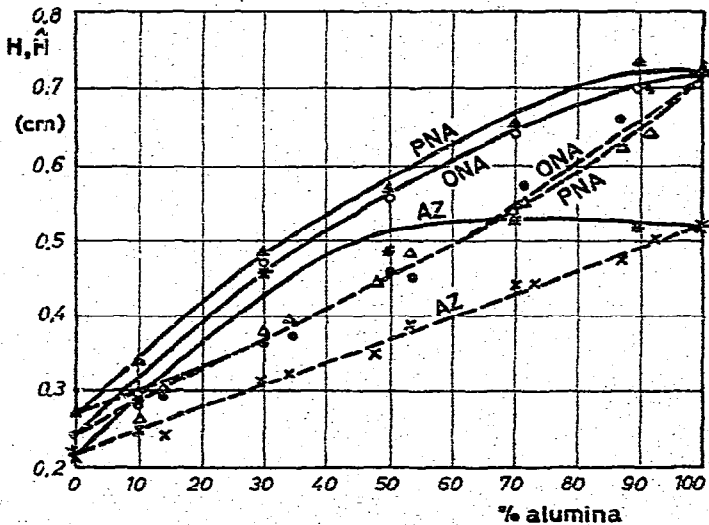

Fig. 9. Comparison of the efficiencies of connected bed layers and mixed beds in systems containing silica gel + alumina, plotted as functions $\hat{H}$ or $H = f(\%Al_2O_3)$ for $M = 1$ and $H_1 \neq H_2$. Mobile phase, ethylene chloride; flow-rate, 0.23 cm/sec. Abbreviations as in Fig. 3. Solid lines, mixed bed systems; broken lines, connected bed layer systems.

The curves shown in Fig. 8 refer to mixed beds and illustrate the changes in the so-called macroheterogeneity coefficient, λ_m (eqn. 3) as a function of the composition of the stationary phase. It can be seen from the shape of these curves that the phenomenon called here macroheterogeneity, whose influence on the mass transport resistance generated by sorption–desorption kinetics is represented by the coefficient λ_m, results from the fact that the column packing contains particles that differ considerably in their adsorption capability. The macroheterogeneity is greatest when a layer contains only small portions of the stronger adsorbent.
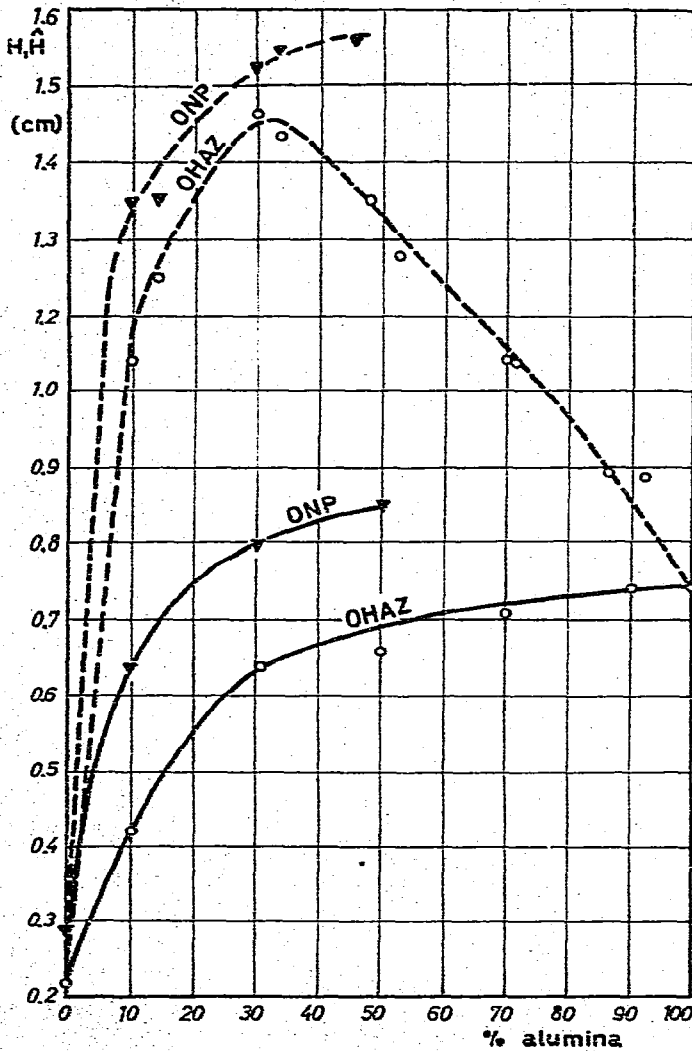

Fig. 10. Comparison of the efficiencies of connected bed layers and mixed beds in systems containing alumina + silica gel, plotted as functions $\hat{H}$ or $H = f(\%Al_2O_3)$ for $M > 1$, $H_1/H_2 < 1$. Mobile phase, ethylene chloride; flow-rate, 0.23 cm/sec. Abbreviations as in Fig. 3. Solid lines, mixed bed systems; broken lines, connected bed layer systems.

Fig. 11. Comparison of the efficiencies of connected bed layers and mixed beds in systems containing silica gel + alumina, plotted as functions $\hat{H}$ or $H = f(\%Al_2O_3)$ for $M > 1$, $H_1/H_2 > 1$. Mobile phase, ethylene chloride; flow-rate, 0.23 cm/sec. Abbreviations as in Fig. 3. Solid lines, mixed bed systems; broken lines, connected bed layer systems.
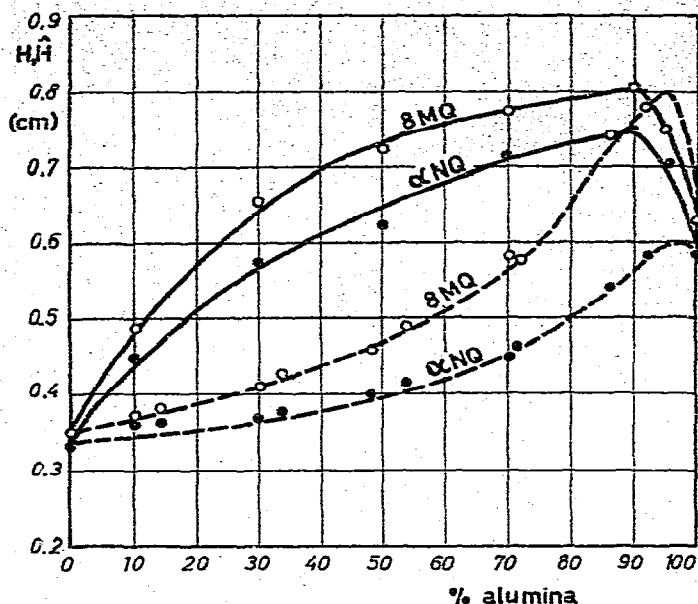
As the efficiencies of the two systems compared here, *i.e.*, those with mixed beds and those with connected bed layers, have been shown to be influenced differently by the retention parameters of the compounds being tested, the corresponding curves for the three basic cases are presented in Figs. 9, 10 and 11. These cases are:

(a) $M \approx 1$; $H_1 \neq H_2$ (Fig. 9);
(b) $M > 1$; $H_1/H_2 < 1$ (Fig. 10);
(c) $M > 1$; $H_1/H_2 > 1$ (Fig. 11).

It can be seen that the cases (a) presented in Fig. 9 show higher efficiencies for columns with connected phases; cases (b) presented in Fig. 10 show considerably higher efficiencies for columns with mixed phases; and cases (c) presented in Fig. 11 show higher efficiencies for columns with connected bed layers.

CONCLUSIONS

It can be concluded that the efficiencies of chromatographic systems with mixed phases and of those with connected stationary phases must be established in different ways, for two reasons:
(a) the influences of features that characterize the components of the stationary phase are summed differently in each of the two systems; additional phenomena that

appear in mixed beds make it impossible to predict their properties on the basis of simple addition of values that characterize the individual components of the stationary phase;

(b) retention data have substantially different significances in the two systems: in the expression that defines the efficiency of mixed beds (eqn. 1), only the term C_k depends on the parameter R, while all components of the expression that defines the efficiency of connected bed layers depend additionally on the value of $M = R_1/R_2$.

For this reason, the plots for those efficiency changes which depend on the proportion of each of the adsorbents in mixed beds will always have positive deviations. The plots for connected phases will show deviations only for $M > 1$, and their magnitude and direction will depend on the M, H_1 and H_2 and on the range of L_1/L, as predicted in discussing the data presented in Fig. 1. Further investigations aimed at predicting quantitatively the apparent theoretical plate height on the basis of the dependences in Fig. 1 are being carried out.

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