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Experimental evaluation of sorbent bed homogeneity in high-performance liquid chromatographic columns

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ABSTRACT

Base-line perturbations, eigenzones, are generated in a high-performance liquid chromatographic (HPLC) column by sudden pressure variations if particular binary eluents are used. The proposed interpretation of the shape of these eigenzones allows the semiquantitative evaluation of the packing density distribution along the column. Common HPLC equipment allows the necessary experimental data to be obtained, and their evaluation is simple and straightforward. However, the correction of eigenzones for spreading is necessary in order to draw more reliable, quantitative conclusions.

INTRODUCTION

The regular arrangement of sorbent particles within a high-performance liquid chromatographic (HPLC) column, *i.e.*, homogeneity of the sorbent bed, is one of the necessary conditions for its high efficiency and stability. The average packing density of most real HPLC columns is not very high. The interstitial porosity ε_0, defined as the ratio of the interstitial volume and the total column volume, varies in the range $0.35 < \varepsilon_0 < 0.45$ [1], and is usually higher than 0.4. However, the theoretical value of ε_0 for arrays of spherical particles of equal size with maximum possible density, *i.e.*, for rhombohedral and face-centred cubic arrangements, is only 0.259 [2]. This means that in common HPLC columns the sorbent particles are either loosely packed along the whole column length or the bed of sorbent contains local heterogeneities such as zones with very low packing density or tears, channels, holes, etc. It is widely accepted, however, that the presence of such packing irregularities adversely influences the column quality in a decisive way. It is therefore advantageous both for developing efficient column packing methods and for routine testing of available columns to possess a procedure for determining column packing density variations along the column.

We have recently proposed the use of the pressure dependence of preferential sorption from mixed mobile phases [3] for this purpose [4,5]. The role of pressure in sorption processes has long been overlooked. There have been only a few studies of the influence of pressure on the sorption equilibria in static [6–9] and dynamic (HPLC) [3–5,10–21] systems. In a series of papers [3–5,16–18,21], we studied the effects of pressure on the extent of preferential sorption. The term "preferential sorption" denotes the phenomenon that the composition of a two-component liquid differs in the bulk phase and in the phase sorbed in the domain of a solid sorbent. It was found that sudden changes in pressure cause variations in the sorption equilibrium in many systems consisting of common LC sorbents and two-component mobile phases. Consequently, rapid desorption–extraction processes take place, resulting in the perturbation of the column effluent composition. These perturbances can be easily visualized by a non-specific detector such as a differential refractometer and have the form of fairly well defined "eigenzones" [16].

The height of the eigenzone, *h*, depends on the particular pressure change, ΔP, and on the difference in the refractive indices of the mobile phase components. Similarly to preferential sorption it-

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self, *h* is also a function of the nature and composition of both the column packing and the mobile phase [3,16,17] and of temperature and the amount of sorbent within the column [5]. On the other hand, *h* seems not to depend on the mobile phase flow-rate [5]. Under appropriate experimental conditions, *h* reaches 200 mm at ΔP = 10 MPa. Single liquids under similar experimental conditions produce very small or undetectable baseline perturbations [4].

The width of the eigenzone, with a few exceptions so far observed [18], corresponds roughly to the total volume of liquid within the column [3,4]. The shape of eigenzones depends on the manner in which the pressure change was generated. For example, the pressure strokes produced by a sudden mobile phase flow reversal (column backflushing) yield Z-shaped eigenzones that have been utilized in the qualitative evaluation of both the homogeneity and the stability of the packing bed [4]. On the other hand, the pressure variations induced by inserting a capillary with a large hydrodynamic resistance behind the column give rise to tetragonally shaped eigenzones [5,18].

The advantage of the latter procedure is that the pressure increases almost simultaneously throughout the whole volume of the column to approximately the same, well defined extent, provided that the sorbent bed geometry remains unaffected in the course of the experiments. The shape of such eigenzones gives directly at least qualitative information concerning the column packing geometry [5].

In this paper, we present further experimental results obtained on the generation of eigenzones by the insertion of a hydrodynamic resistor behind the column. We also describe an attempt to evaluate semi-quantitatively the inhomogeneities within LC column packings from the shapes of eigenzones.

EXPERIMENTAL

We used two experimental assemblies, shown in Fig. 1a and b. Liquids were transported either by an FR-30 reciprocating double-piston pumping system (RP) (Knauer KG, Bad Homburg, Germany) (Fig. 1a) or by an HPP 4001 positive displacement (syringe) pumping system (PP) (Laboratory Instruments, Prague, Czechoslovakia) (Fig. 1b). By switching the six-port, three-way valve V (Workshops of Czechoslovak Academy of Sciences, Prague, Czechoslovakia) the resistor capillary CA, 50 or 100 m × 0.25 mm I.D., was inserted behind the column C. The resulting pressure changes were monitored by a flow-through pressure gauge (Institute for Chemical Process Fundamentals, Czechoslovak Academy of Sciences, Prague, Czechoslovakia), G, and recorded by a recorder, RC, simultaneously with the variations of effluent composition that were measured with an RIDK 101 differential refractometer (Laboratory Instruments), RI. The single-piston displacement pump cannot be used in the assembly in Fig. 1a because, owing to the compression of the eluent in the container of the pumping system, the pressure variations within column are slow and not well defined. On the other hand, the system shown in Fig. 1b allows pressure variations to be produced within the column without a change in pressure at the outlet of the pumping system.

All measurements were made at 22 ± 1°C. Stainless-steel columns (100 × 6 mm I.D. or 100 × 8 mm I.D.) (Laboratory Instruments) were packed either with bare silica gel Silpearl (Glassworks Kava-

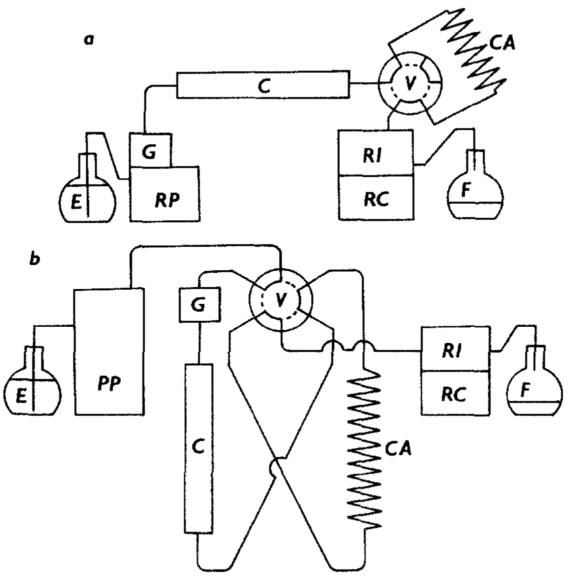

Fig. 1. Schemes of the LC assemblies: E = eluent container; RP = reciprocating pump; PP = piston pump; G = pressure gauge; C = column; V = valve in position a (solid line) and b (dashed line); CA = capillary; RI = detector; RC = recorder; F = waste.

lier, Votice, Czechoslovakia) or with bare silica gel CH (Lachema, Brno, Czechoslovakia). Some samples of Silpearl were fired at different temperatures in order to decrease their specific surface area. The physical characteristics of the sorbents are given in Table I. The sorbents were packed into the column by the upward slurry method from methanol (Silpearl) or dry tapped (silica gel CH). At the end of experiments, some columns were emptied and the amount of dry sorbent within column was determined by weighing.

The eluent was tetrahydrofuran (THF)–water (80:20, v/v). THF (Laborchemie, Apolda, Germany) was distilled from KOH and stabilized by 0.1 g/l of butylated *p*-hydroxytoluene. Water was redistilled after oxidation of organic admixtures with an alkaline solution of $KMnO_4$ for 1 week. The use of pure eluent components is necessary because admixtures could participate in sorption processes and complicate the shapes of eigenzones. The amount of the THF stabilizer used, however, was small enough not to produce measurable eigenzones.

The density of the sorbent matrix was determined using a pycnometer and appropriate mixed liquids. The specific pore volume was calculated from GPC data, the density of the sorbent matrix and the mass sorbent within the column.

RESULTS AND DISCUSSION

Extent of pressure effect

Switching the valve V into the position marked with the full line in Fig. 1 and inserting the capillary CA into the eluent path cause an increase in pressure within the column C. Conversely, removal of the capillary CA (valve V in the position marked with a dashed line in Fig. 1) leads to a sudden drop in pressure. Fig. 2a and b show typical eigenzones produced by such pressure changes. Whereas the eigenzone in Fig. 2a is fairly symmetrical, the eigenzone in Fig. 2b does not show a flat top. Such asymmetric eigenzones indicate poor sorbent bed homogeneity. Note the large time delay in the appearance of eigenzone when the capillary CA was inserted behind the column: this corresponds to the volume of capillary CA, which is much larger than the volume of the connecting capillaries between the column and detector. The apparent time delay between the signal of the pressure gauge and the differential refractometer trace is due to the construction of the two-pen recorder used.

Water molecules are preferentially sorbed on the surface of bare silica gel from THF–water (80:20, v/v) at pressures of 0.1–10 MPa [3]. The amount of preferentially sorbed water, however, decreases with increasing pressure. This means that in an HPLC column flushed with THF–water the pressure increase leads to desorption of water and extraction of THF from the mobile phase. As water has a lower refractive index (RI) than THF, the RI detector response to the corresponding effluent composition perturbation is negative (Fig. 2a and b). The process is fully reversible and a subsequent decrease in pressure to the initial value leads to reestablishment of the initial sorption equilibrium and, consequently, desorption of THF from the column packing and extraction of water from the mobile phase. The eigenzone proper to the latter effluent composition perturbation has a positive sign (Fig. 2). For further evaluation, only columns producing repeatable eigenzones were considered, as a changing eigenzone shape indicates instability of the sorbent bed [4].

Fig. 3 shows the dependence of the areas of the eigenzones on the extent of pressure change ΔP for three different silica gels. The dependences can to a first approximation be represented by straight lines (correlation coefficients 0.997, 0.982, 0.984) and we can consider, at least for small pressure variations, the extent of the column effluent composition change to be directly proportional to ΔP.

In a previous paper [5], we showed that apart from ΔP, the eigenzone size is approximately directly proportional to the amount of sorbent in the column and inversely proportional to the volume of liquid in the column. Sorption processes evidently take place on the sorbent surface and this parameter should be considered in the quantitative evaluation of pressure effects (*cf.*, Fig. 3). In the following series of experiments, the eigenzones were generated in columns containing silica gels with different specific surface areas. These sorbents had different pore sizes and pore volumes. The differences in the physical structures of the sorbent particles together with different average packing densities of particular columns resulted in different volumes of the mobile phase available for compositional

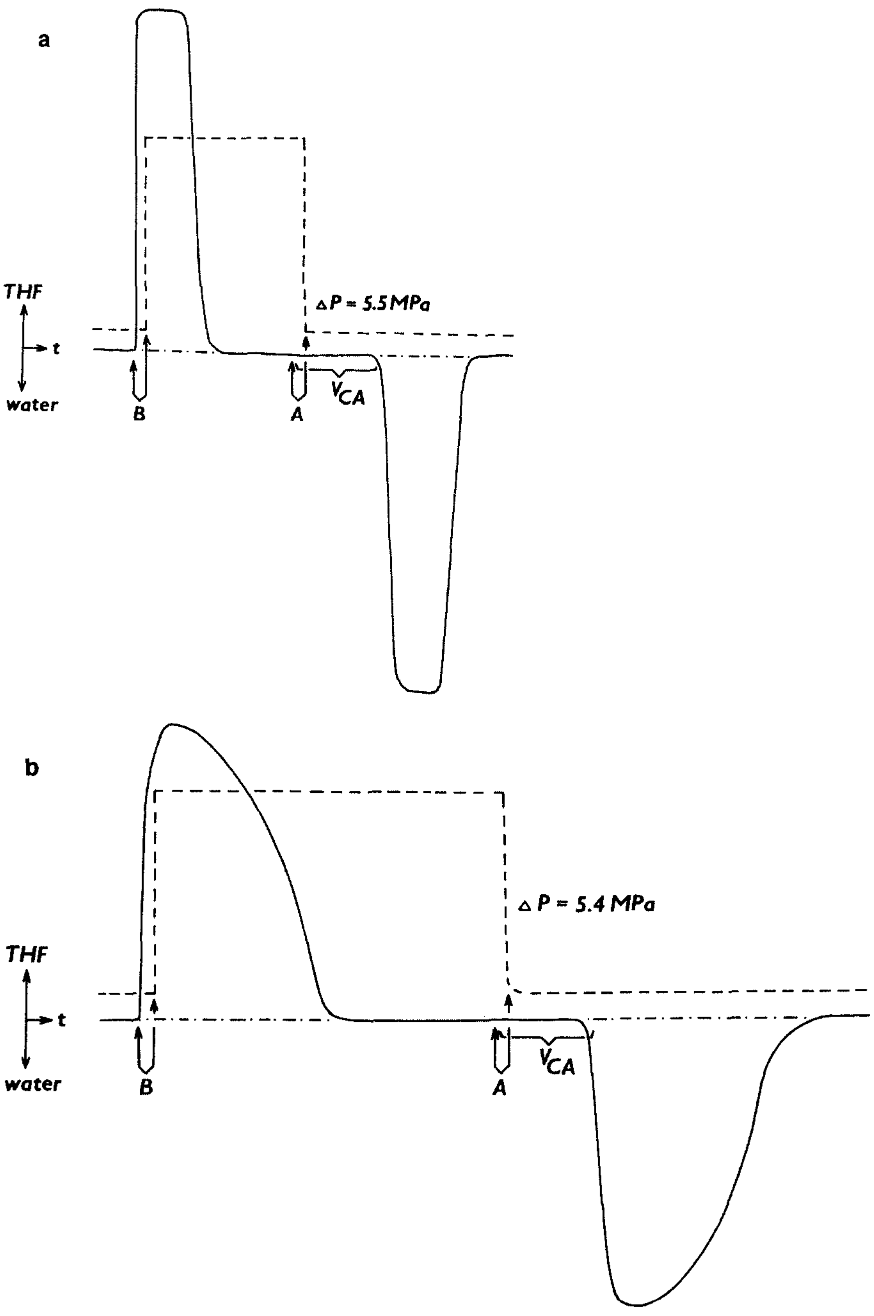

Fig. 2. Examples of eigenzones produced by pressure variations due to inserting (or removing) a capillary with large hydrodynamic resistance in (from) the eluent path. Eluent, THF–water (80:20, v/v); flow-rate, 0.6 ml/min. Column: (a) Silpearl, 13 μm (10 × 0.6 cm I.D.); (b) silica gel CH, 32–70 μm (10 × 0.6 cm I.D.). A, At this time valve V was switched from position b to a (Fig. 1); B, valve V was switched from position a to b; t = elution time; dashed line, response of pressure gauge G; solid line, response of RI detector; V_{CA} = volume of hydrodynamic resistor, capillary CA. The sign of the detector response for the excess of particular eluent components is shown.

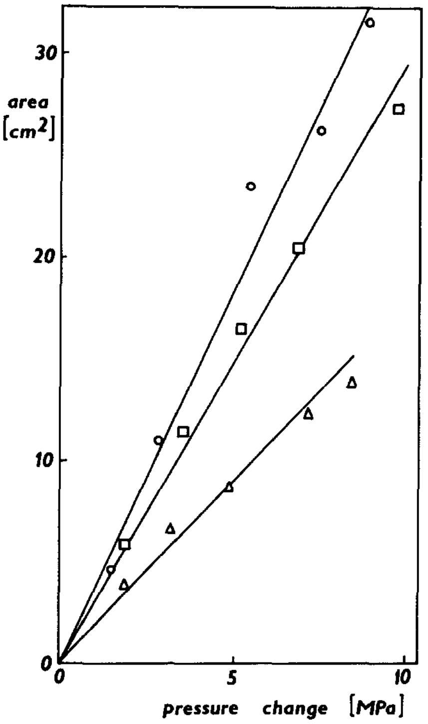

Fig. 3. Dependence of the area of the eigenzone on the pressure change. Eluent, THF–water (80:20, v/v). Column △ = silica gel CH, 70–100 μm; □ = silica gel CH, 100–170 μm; ○ = Silpearl, 13 μm.

changes. This is especially pronounced with sample No. 7, which lost most of its porosity in the course of thermal treatment. The results are collected in Fig. 4 and Table I. Fig. 4 shows a plot of $Y = A(\Delta P)^{-1}V^{-1}$ *versus* $X = SV^{-1}$, where A is the area of the eigenzone, ΔP the pressure variation, V the volume of liquid within the column and S the surface area of the corresponding sorbent. For evaluating the material balance, the detector response was calibrated by means of a set of test mixtures of THF and water of known composition. From the eigenzone area, the amount of water released at ΔP = 1 MPa from 1 m^2 of the sorbent surface was then calculated (Table I). The scatter of the data in Fig. 4 and Table I is pronounced, but one has to bear in mind that the surface concentration of free silanol groups for starting silica gels CH and the thermally treated Silpearl probably differed. On the other hand, the starting Silpearl with a surface area of 615 m^2 g^{-1} at a pore volume of *ca.* 0.5 ml g^{-1} evidently contained a substantial amount of micropores not accessible to solvent molecules. Under these circumstances, and neglecting the possible influence of the pore diameter on the sorption processes and the role of temperature variations in the columns packed with particles of different size, we can, at least for low values of X and Y and to a first approximation, consider the dependence in Fig. 4 to be linear (correlation coefficient 0.949). The roughly estimated mean amount of water desorbed from 1 m^2 of the silica gel surface in the mixed eluent THF–water (80:20, v/v) is about $(7 \pm 2) \cdot 10^{-4}$ mg per ΔP = 1 MPa. Generally, we can say that the height of the eigenzone reflects, under otherwise identical conditions, the surface area of the sorbent that is available to the desorption–extraction process when the pressure is varied. This means that the local changes in the packing surface (*i.e.*, in the packing density) along the column will influence the shape of the eigenzone. To prove this idea one would need columns containing defined inhomogeneities. However, it is difficult to prepare such HPLC columns in practice. Still, it is possible to

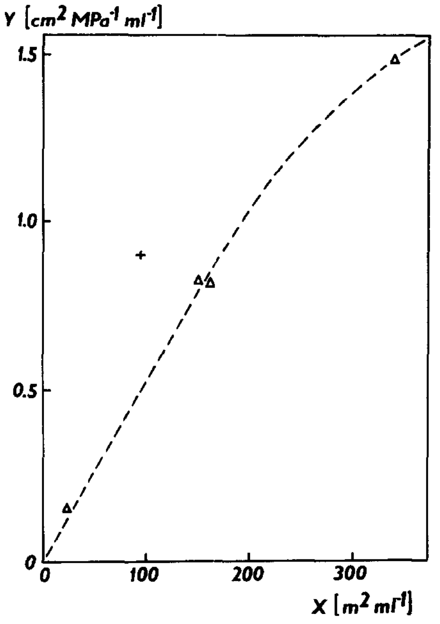

Fig. 4. Relationship between the area of the eigenzone generated by a 1 MPa pressure change per 1 ml of liquid within the column (Y) and the surface area of sorbent per 1 ml of liquid within the column (X). + = Silica gel CH; △ = thermally modified Silpearl.

TABLE I

PHYSICAL PROPERTIES OF THE SORBENTS AND EXTENT OF THE CHANGE OF AMOUNT OF PREFERENTIALLY ADSORBED WATER WITH PRESSURE

Sorbent No.[a]	Particle diameter (μm)	Specific surface area (m^2/g)	Mass of desorbed water/pressure change/mass of sorbent (mg MPa^{-1} g^{-1})	Mass of desorbed water/pressure change/surface of sorbent (mg MPa^{-1} m^{-2})	Mass of sorbent/volume of liquid within column (g/ml)
1	7	612	0.32	$5.24 \cdot 10^{-4}$	0.76
2	13	480	0.34	$7.04 \cdot 10^{-4}$	0.81
3	10	440	0.25	$5.66 \cdot 10^{-4}$	0.77
4	10	210	0.15	$7.00 \cdot 10^{-4}$	0.75
5	10	188	0.14	$7.34 \cdot 10^{-4}$	0.79
6	70–100	178	0.16	$9.32 \cdot 10^{-4}$	0.67
7	10	13	0.001	$0.75 \cdot 10^{-4}$	1.79

[a] Sorbents 1–5 and 7, Silpearl; sorbent 6, silica gel CH.

simulate the column inhomogeneity by a set of short columns containing sorbents with different specific surface areas [5]. An example of eigenzones obtained with trains of various columns is shown in Fig. 5. This illustrates that the eigenzone shape reflects the variations in the packing surface. After changing the column sequence, the shape of eigenzones is correspondingly changed [5].

The interpretation of eigenzone shape

For the sake of simplicity, we shall assume that insertion or deletion of the capillary CA produces the same changes in pressure ΔP at any point in the column. This means that the changes in viscosity and density [22] of the eluent with pressure and the variations in temperature within the column due to friction [23] will be neglected. Further, we assume

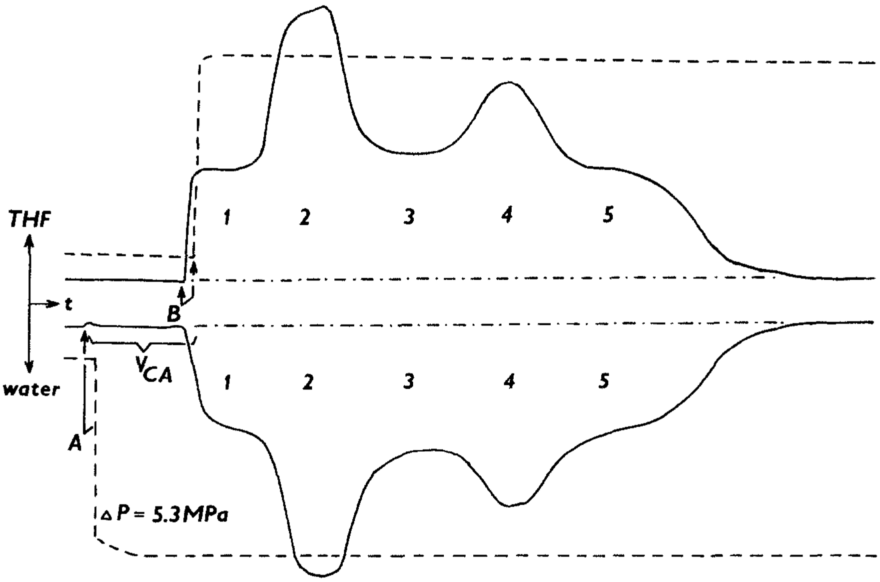

Fig. 5. Eigenzones corresponding to a train of five different columns in series. Eluent, THF–water, 80:20 (v/v); columns, 10 × 0.6 cm I.D. Sorbents: 1 = silica gel CH, 32–70 μm (178 m^2/g); 2 = Silpearl, 13 μm (480 m^2/g); 3 = silica gel CH, 70–100 μm (178 m^2/g); 4 = Silpearl, 7 μm (612 m^2/g); 5 = silica gel CH, 100–170 μm (178 m^2/g).

that the physical and chemical properties of particles of packing along the column, *i.e.*, their pore volume, pore size, specific surface area and concentration of active sites, where the preferential sorption takes place, remain constant. On the basis of the experimental data collected so far [3–5,16,17], we can write for a perfectly homogeneously packed column with one kind of silica gel sorbent:

$$h = \Delta P \rho K \tag{1}$$

where h is the height of eigenzone, ΔP is the pressure change, ρ is the packing density of the column and K is a constant; ρ is expressed as the mass of sorbent per millilitre of liquid situated in both the pores and the interstitial volume of the column. The value of K depends on the nature of both the sorbent and the eluent and on temperature and the detector used. K must be determined by independent experiments for each sorbent–eluent–detector system. For the sake of simplicity, the mass of sorbent is considered in eqn. 1 instead of its specific surface area.

Eqn. 1 allows the distribution of sorbent in the inhomogeneous bed of packing to be estimated by a simple procedure, as follows:

1. Divide the eigenzone into equally small and identical volume elements of eluent, V_i (Fig. 6), so that one can assume that the sorbent bed within each volume element is homogeneous. We chose V_i equal to 0.2 ml to illustrate the calculation procedure.

2. Calculate M_i, *i.e.*, the mass of sorbent corresponding to each volume element of eluent V_i:

$$M_i = V_i \rho_i = V_i \cdot \frac{h_i}{\Delta P K} \tag{2}$$

where V_i is the size of eluent volume element in ml, ρ_i is the mass of sorbent corresponding to 1 ml of eluent, h_i is the mean height of the eigenzone within the volume element V_i, ΔP is the overall pressure change and K the constant in eqn. 1.

3. Calculate the volume of sorbent matrix W_i that corresponds to the mass of sorbent M_i within the eluent volume V_i:

$$W_i = M_i / \rho_s \tag{3}$$

where ρ_s is the density of sorbent matrix (*e.g.*, 2.2 g cm^{-3} for bare silica gel).

4. Calculate the length of column L_i that corresponds to the volume of eluent V_i plus the volume of sorbent matrix W_i:

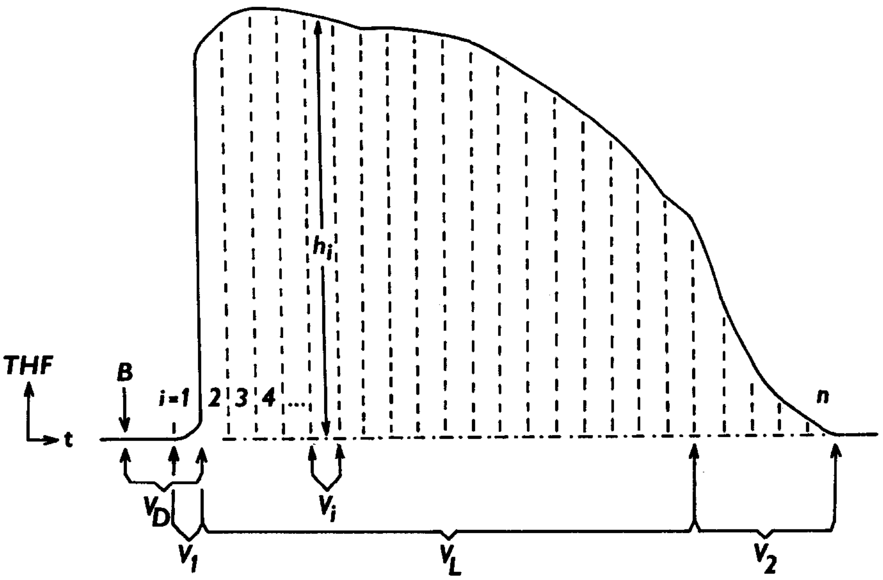

Fig. 6. Example of eigenzone divided into volume elements. V_i = volume element with indices $i = 1 \ldots n$; h_i = height of eigenzone; V_L = volume of effluent equal to the volume of liquid within the column; V_D = volume between the column outlet (column end) and the detector cell; V_1, V_2 = parts of volume of eigenzone due to broadening processes during passage of the eigenzone through the capillaries and the column.

$$L_i = \frac{V_i + W_i}{\pi \cdot \frac{D^2}{4}} \tag{4}$$

where D is column inside diameter.

5. Construct the plot

$$\frac{\rho_i V_i}{\pi r^2 L_i} = \mathrm{f}\left(\sum_{i=1}^{i=n} L_i\right) \tag{5}$$

which represents the differential distribution of sorbent within the column, *i.e.*, the mass of sorbent per unit column volume as a function of the length of column L. The dependence

$$\sum_{i=1}^{i=n} (\rho_i V_i) = \mathrm{f}\left(\sum_{i=1}^{i=n} L_i\right) \tag{6}$$

express integral distribution of sorbent mass within the column. Both kinds of distribution calculated for two different columns are shown in Fig. 7a and b.

Theoretically, the sum of L_i should be equal to

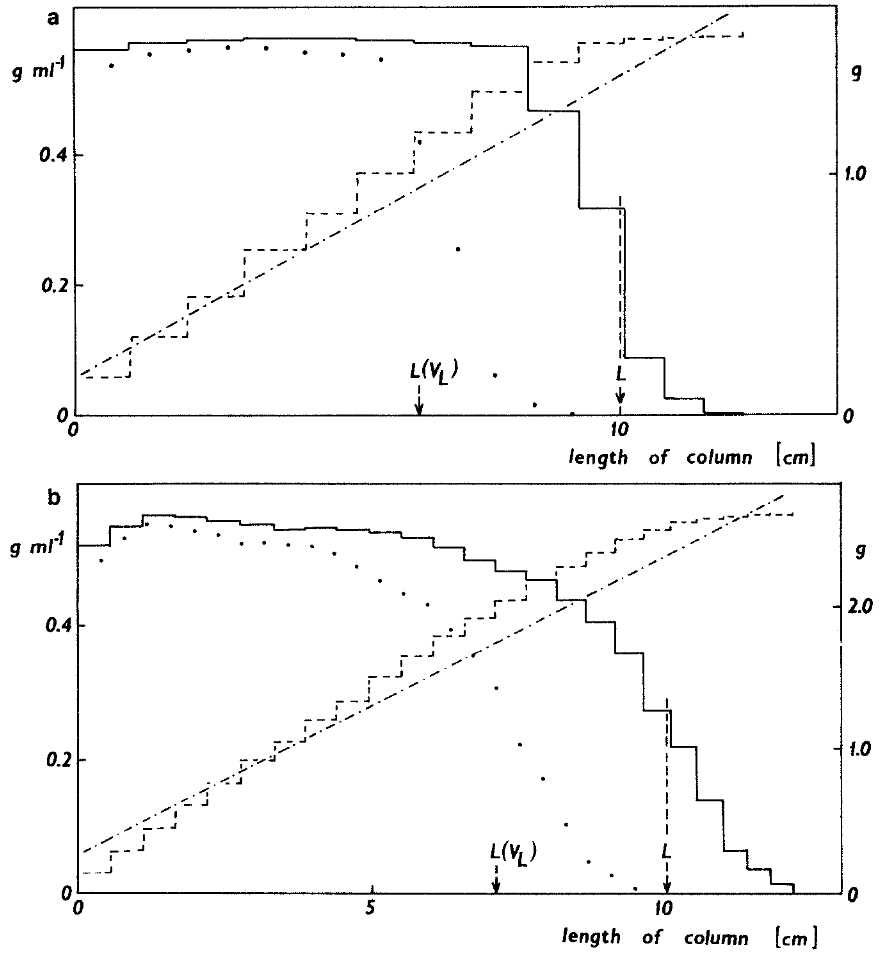

Fig. 7. Example of the calculated sorbent distribution within the column on the base of the whole eigenzone. Left-hand ordinate = grams of sorbent per 1 ml of column volume; right-hand ordinate = grams of sorbent. (a) From the eigenzone in Fig. 2a. Calculated length of column, 12.25 cm; correlation coefficient calculated for integral distribution, 0.9783; calculated average packing density, ρ = 0.601; experimental ρ = 0.797. (b) From the eigenzone in Fig. 2b. Calculated length of column, 12.15 cm; correlation coefficient calculated for integral distribution, 0.9828; calculated average packing density, ρ = 0.550; experimental ρ = 0.709. Dashed line, differential distribution of sorbent; solid line, integral distribution of sorbent; dot-dashed line, calculated by least-squares method; $L(V_L)$ = length corresponding to the volume of liquid within the column; L = actual length of column. Points corresponding to the eigenzone which were used for the calculation are also shown.

the total column length L and the sum of M_i values must correspond to the mass of sorbent within column:

$$L = \sum_{i=1}^{i=n} L_i \quad (7)$$

$$M = \sum_{i=1}^{i=n} M_i \quad (8)$$

In practice, however, $L < \Sigma L_i$, as the volume of eigenzone is larger than the total volume of liquid within the column owing to the spreading processes in the packing, column end-pieces, connecting capillaries, valve and detector. For illustration, both the length of the column $L(V_L)$ corresponding to the volume of liquid within the column V_L and corresponding positions of points of eigenzone, which were used for calculation, are shown in Fig. 7a and b.

The spreading effect increases with increasing path length of the eigenzone within the column. The front of the eigenzone presents its least distorted part as it arises in the very end of the sorbent bed and has the shortest path into the detector, whereas the tail of the eigenzone is its most distorted part. The effect of the zone broadening within the capillary resistor CA is especially evident when comparing the shapes of the front parts of eigenzones in Figs. 2 and 5. The uncorrected results are therefore approximate. The comparison of the actual (L) and calculated (ΣL_i) lengths of the column gives semiquantitative information on band broadening effects within the system studied.

The influence of the spreading effects was tentatively decreased in the following way. Let us assume that the eigenzone is not influenced by the spreading process. In this event the volume of the eigenzone (V_E) equals the volume of liquid within the corresponding column (V_L). It is possible to locate the position of the non-distorted eigenzone on a chromatogram if one knows the volume of the parts of HPLC system between the end of the packing bed and the detector measuring cell (V_D), *i.e.*, the volume of the column end-piece and connecting capillaries and the volume of liquid within the column, V_L (*cf.*, Fig. 6). As a consequence of the broadening process, the height of the eigenzone decreases and its width increases. The parts of the eigenzone produced by the broadening within both the column and the HPLC hardware are designated in Fig. 6 as V_1 and V_2. The influence of the broadening on the constant K calculated from eqn. 1 is eliminated by applying the relationship

$$K = \frac{h}{\Delta P \cdot \frac{M}{V_L}} = \frac{\frac{\sum_{i=1}^{i=n} h_i}{(n-x_1-x_2)}}{\Delta P \cdot \frac{M}{V_L}} \quad (9)$$

where h_i is the average height of the eigenzone in the volume element V_i, n is the number of volume elements V_i in the total volume of the eigenzone V_E, x_1 and x_2 are the number of volume elements V_i in volumes V_1 and V_2 (*cf.*, Fig. 6) and M is the mass of sorbent within the column. For calculation of K from eqn. 9 only that volume of eluent (V_L) is considered which corresponds to the eigenzone at the moment of its generation within the column. In other words, the number of volume elements n is deliberately reduced considering the effect of the zone broadening. On the other hand, the whole area of the eigenzone is used for calculating the constant K because it is assumed that the area of an eigenzone does not depend on its broadening. We used constants K calculated from eqn. 9 in eqn. 1.

Fig. 8a and b show examples of the sorbent distribution curves calculated from that part of the eigenzones which belonged to the volume of liquid within the column. In this instance, the calculated column length approaches the actual length of the column (L) (*cf.*, calculated L in Figs. 7 and 8).

The integral distribution of the sorbent (see Fig. 7a and b) for an ideal, homogeneously packed column would be a straight line. The correlation coefficient of the actual experimental dependence evaluated by the least-squares method can also be a measure of the inhomogeneity of a real column packing.

In our opinion, the effective density of the sorbent matrix, ρ_{eff}, should be used in calculations (eqn. 3) instead of the true density ρ_s; ρ_{eff} is determined in given eluent whereas the ρ_s values are obtained by using liquid media with very small molecules, such as helium. In addition to closed pores, also micropores of the sorbent may be inaccessible to larger molecules of the eluent and ρ_{app} will be lower than ρ_s. Hence the use of ρ_s may lead to errors

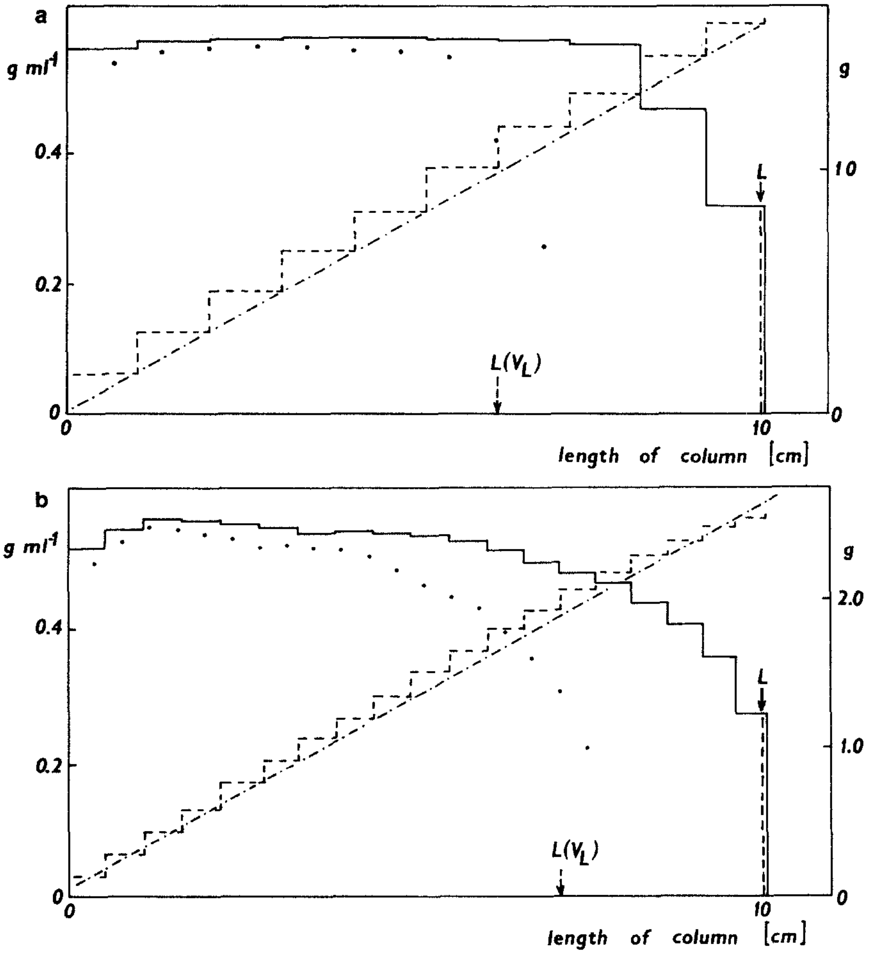

Fig. 8. Distribution of sorbent within the column calculated from that part of the eigenzone which corresponds to the volume of liquid within the column. (a) From the eigenzone in Fig. 2a. Calculated length of column, 10.08 cm; correlation coefficient calculated for integral distribution, 0.9988; calculated average packing density, ρ = 0.7690; experimental ρ = 0.7975. (b) From the eigenzone in Fig. 2b. Calculated length of column, 10.06 cm; correlation coefficient calculated for integral distribution, 0.9983; calculated average packing density, ρ = 0.668; experimental ρ = 0.709. Symbols as in Fig. 7. For explanation, see text.

in calculations of the column bed density distribution.

CONCLUSIONS

The eigenzones, *i.e.*, the baseline perturbations due to changes in composition of mixed column effluents, are generated by sudden changes in pressure within HPLC columns. A simple experimental assembly was designed to generate non-destructive and fully repeatable pressure strokes within columns and to create well defined eigenzones.

The shape of the eigenzones was interpreted on the basis of the experimentally found direct proportionality between the height or the area of the eigenzone (*i.e.*, the amount of extracted or desorbed substance) and both the pressure change and the concentration of sorbent within the column. The concentration of sorbent is defined as the mass of sorbent (*i.e.*, its surface area) per unit volume of liquid in the column. A procedure was proposed that permits the calculation of the mass of sorbent at any point along the column.

To obtain more precise data, however, a correlation of the eigenzone shape with respect to the broadening effects will be necessary, especially if the columns contain larger sorbent particles. On the other hand, the closer the volume of the eigenzone approaches the volume of liquid within the column, the more precise data about the packing distribution may be expected. Of course, high sensitivity of the adsorption equilibrium towards pressure changes and good detectability of the resulting effluent composition changes are also required.

The method proposed enables information to be obtained about the inhomogeneity of HPLC column beds. It may help in defining relationships between the sorbent bed structure and its chromatographic efficiency and allow the optimization of column packing procedures.

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