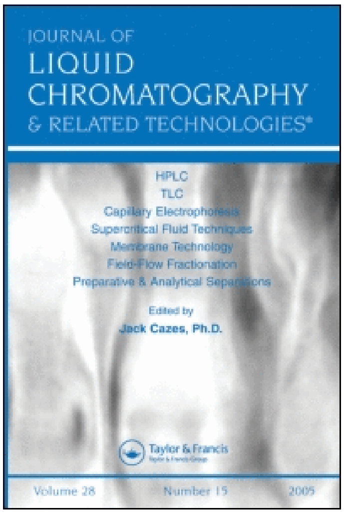

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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T. Macko[a]; D. Berek[a]
[a] Polymer Institute of the Slovak Academy of Sciences Dübravská cesta, Bratislava, Slovakia

To cite this Article Macko, T. and Berek, D.(1998) 'Experimental Determination of Longitudinal Pressure Profile Within HPLC Column', Journal of Liquid Chromatography & Related Technologies, 21: 15, 2265 — 2279
To link to this Article: DOI: 10.1080/10826079808000537
URL: http://dx.doi.org/10.1080/10826079808000537

J. LIQ. CHROM. & REL. TECHNOL., 21(15), 2265–2279 (1998)

EXPERIMENTAL DETERMINATION OF LONGITUDINAL PRESSURE PROFILE WITHIN HPLC COLUMN

T. Macko, D. Berek*

Polymer Institute of the Slovak Academy of Sciences
Dúbravská cesta 9
84236 Bratislava, Slovakia

ABSTRACT

A new method for evaluation of pressure gradient along an HPLC column is presented. The method utilizes the dependence on pressure of the sorption equilibrium between sorbent surface and a two-component liquid. Sudden changes of pressure generates a change of sorption equilibrium of mixed eluent within the column packing and an eigenzone, i.e. the zone of effluent with changed composition, is observed on the detector record. The interpretation of the shape of eigenzone allows both evaluating distribution of sorbents along the column and calculating pressure profile within the column.

INTRODUCTION

It is known that pressure and its changes influence both numerous properties of materials and courses of many physico-chemical processes.[1] Elevated pressure is an attribute of most high performance liquid chromatography (HPLC) separations and the role of pressure in liquid chromatography was discussed in many articles.[2-22]

Various effects of pressure on HPLC results were studied, including dependence of analyte retention on pressure.[2-7,21,22] The retention may depend on pressure either directly (due to pressure dependence of adsorption equilibrium) or indirectly, as a result of column heating by friction.[23-25] Information on the pressure profile within an HPLC column is also important from the point of view of column diagnostics, e. g. in the course of column packing procedures optimization. The nonlinearities in the pressure profile along the column indicate inhomogeneities within the sorbent bed.

We present a procedure for experimental assessment of pressure profile within HPLC columns. The procedure is based on the fact that the sorption equilibrium of mixed liquids on the solid surface depends on pressure.[12,26,27] Sudden pressure changes were shown to produce rapid and repeatable desorption of a defined amount of one liquid component. This means that the composition of both the adsorbed liquid layer and the interstitial liquid changes with pressure variations.[12] Consequently, well defined and repeatable base line perturbations i.e. changes of effluent composition called eigenzones[13] are formed as the manifestation of the controlled pressure variations in the HPLC systems. The eigenzones can be detected by eluent-specific or by non-specific detectors such as a differential refractometer. The appropriate two component eluent for a given column packing is to be used. The sign, size, and shape of eigenzones depend on the nature and composition of both eluent and sorbent; further, on the extent and course of pressure change, as well as on temperature and on the amount of sorbent and liquid within column.[12-17,19,20] Size and shape of the eigenzone obtained at defined conditions enable one to determine the mass distribution of sorbent particles within column,[20] and as we shall show in this paper, it also allows evaluation of the pressure profile within the HPLC column.

EXPERIMENTAL

Instrumentation

The experimental arrangement used is shown in Fig. 1a. The pumping system (RP) was model FR-30 (Knauer Co., Berlin, Germany), the pressure gauge (G) was custom made in the Institute of Chemical Processes Fundamentals, Academy of Sciences of Czech Republic, Prague). High pressure valves (V1, V2), differential refractometer RIDK (RI), line recorder TZ 4200 (RC), stainless column (C) 25 x 0.6cm I.D. or 10 x 0.6cm I.D., and capillary 100m x 0.25mm I.D. were products of Laboratory Instruments Co., Prague, Czech Republic. Valve V_1 was used to inject some test substances in the course of the characterization of particular columns. For eigenzone

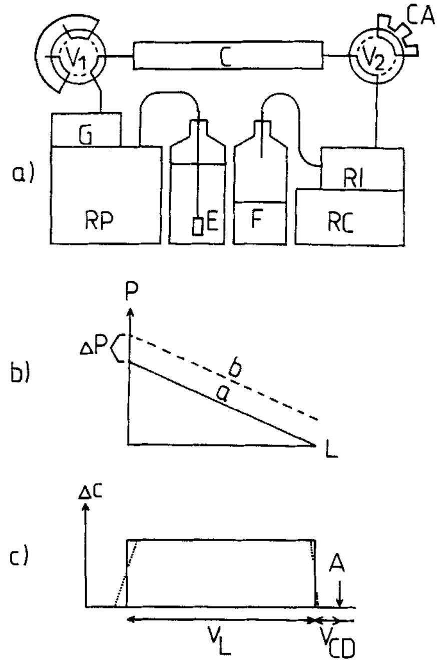

Figure 1. a) Scheme of the LC assembly: E = eluent container; RP = pump; G = pressure gauge; V_1 = injection valve; V_2 = switching valve in position a (solid line) and b (dashed line), C = column; CA = capillary; RI = detector; RC = recorder, F = waste. b) Schematic representation of pressure change within column after switching the valve V_2 from position a to b or vice versa: Line a corresponds to the valve position represented by the solid line in Fig. 1a; P = pressure; ΔP = pressure change; L = column length. c) The eigenzone proper with changed eluent composition due to pressure variation within the column (solid line), the eigenzone shape after leaving the column (dotted line) and corresponding volumes: V_{CD} = volume between the column and the detector measuring cell; V_L = volume of mobile phase within the column. Δc = change of mobile phase composition; A = in this moment the valve V_2 was switched to position represented by full line in Fig. 1a, i.e. the capillary CA was deleted from the flow path.

generation exclusively switching valve V_2 was operated and no extra solute was injected. The insertion or deletion of the appropriate hydrodynamic resistor CA situated behind the column caused an increase or decrease of pressure which was equal within any site of the column (Fig. 1b). In order to achieve sudden pressure change in the HPLC a pumping system with small volumes of piston chambers and without a pressure damper was used. The eigenzones (see Fig. 1c) produced by inserting a capillary CA into the flow path (valve V_2 switched to the position represented by the broken line in Fig. 1a) were additionally broadened due to mixing within the capillary CA. Therefore, we evaluated only the eigenzones produced by decreasing the pressure, i.e. the eigenzones generated by deleting capillary CA from the flow path (valve V_2 switched to the position represented by the full line in Fig. 1a). In this case the eigenzone was broadened within the column proper and within capillaries between column and detector cell only. The detector signal was digitalized by means of an A/D module (Waters, Milford, MA).

Materials and Method

We have used various bare and bonded silica gels as column packings. They differed in particle size and shape as well as in porosity and surface area. However, we quantitatively evaluated only the data obtained with two bare silica gels, namely with Silpearl (from Kavalier Glass Works, Votice, Czech Republic) with mean particle diameter, d_p, 13 mm, surface area 440 m^2/g and specific pore volume Vp, 0.43 mL/g and with experimental spherical silica gel SG-7 (prepared in our Institute) and having d_p = 12 mm, surface area 420 m^2/g and pore volume 2 mL/g.

Azeotropic mixture of benzene and methanol containing 39.6 weight % of methanol[28] was used as eluent. Both solvents were of analytical grade and were purchased from Lachema, Brno, Czech Republic. The boiling point of the azeotrope at atmospheric pressure was 58.3°C and the eluent could be readily regenerated by distillation. The advantage of the present mixed eluent was the high sensitivity of its sorption equilibrium to the pressure variations[15,16] as well as large differences of the components refractive indices that enhanced the sensitivity of refractometric detection.

The density of the sorbent matrix was determined by pycnometry using benzene plus a methanol mixture with a known density. The mass of sorbent within the column was determined by its weighing after the column had been emptied and the sorbent was dried at 100°C for six hours. Alternatively, the mass of sorbent within column was determined from the density and volume of the sorbent matrix present in the column. The latter value was estimated from retention volume of an excess of benzene, which corresponded to the total volume of the liquid within the column.

The experimental value of interstitial volume of the column was obtained from the retention volume of high molar mass (1.2×10^6 dalton) polystyrene which was excluded from the pores of silica gel flushed with tetrahydrofuran. The above data was corrected for volume of capillaries connecting column with the detector cell (V_{CD} in Fig. 1c). The latter value was determined by injecting benzene into the system containing no column.

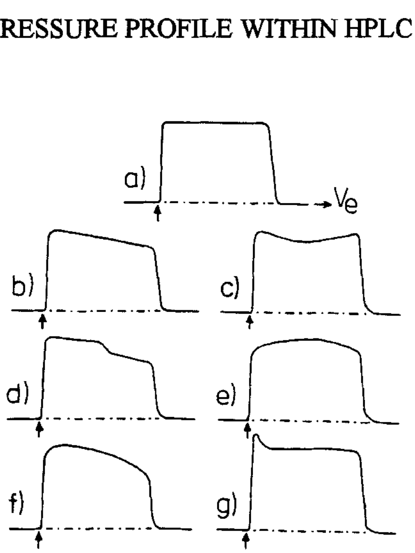

Figure 2. Schematic survey of various shapes of eigenzones produced by pressure variations within columns packed by silica gel sorbents: y-axis corresponds to detector response, x-axis corresponds to elution volume V_e. The arrow indicates a moment, in which pressure decreased due to deleting capillary CA from the flow path.

The pressure drop ΔP within the column packing bed was calculated as the difference between pressure drop of the LC system equipped with the packed column and pressure drop of an identical LC system equipped with an empty column. In this way, contributions from capillaries, valve, frits, and detector cell to the value of total pressure drop measured with the pressure gauge (Fig. 1) were eliminated.

RESULTS AND DISCUSSION

Generation and Shape of Eigenzones

Figure 2 shows a survey of typical shapes of eigenzones observed with various columns used in the course of our studies. The height of eigenzones generated under otherwise identical conditions is directly proportional to the concentration of sorbent within a given volume part of column, i.e. to the mass of sorbent per volume of liquid.[15,20] Consequently, the perfectly homogeneously packed column would give a quite regular zones (Fig. 2a) that are only slightly distorted due to the above mentioned diffusion and mixing processes within both column packing and connecting capillaries. Each pronounced eigenzone distortion indicates inhomogeneity of column packing (Fig. 2b-g). Pretty regular zones could be obtained exclusively with silica gel sorbents with small

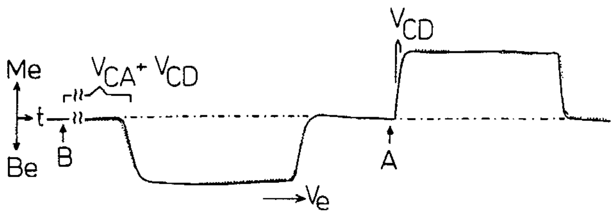

Figure 3. Examples of experimental eigenzones produced by pressure variations within a uniformly packed and stable column packing. Column: Silica gel Silpearl, 13 mm (10x0.6 cm I.D.). Flow rate: 0.5 ml/min. Pressure change: 6 MPa. Eluent: Benzene (Be)/methanol (Me), azeotropic mixture. Symbols: A = in this moment capillary CA was deleted from the flow path; B = capillary CA was inserted into the flow path; t = elution time; V_e = elution volume; V_{CA} = volume of hydrodynamic resistor, capillary CA; V_{CD} = volume of connecting capillaries between column and detector. The sign of the detector response indicating the excess of particular eluent components is shown in figure. Solid line: Response of refractometer due to pressure change within the column flushed in one direction. Dashed line: Detector response due to pressure change, when the column was flushed in reversed direction.

particles of narrow size distributions. Silica gels with large particles having wide size distributions yielded, as a rule, irregular eigenzones. Recently, we have shown that the shape of eigenzones reflects the regularity of a particular packing bed.[20] This may also be proven by reversing the flow direction within the column. Under otherwise constant experimental conditions, but with the column turned up, we generated the eigenzones that presented almost perfect mirror images of eigenzones obtained in reversed flow direction (Fig. 3). On the other hand, the packings with inhomogeneous geometry gave eigenzones with the shapes depending on the flow direction. Moreover, if the bed of packing was instable, its structure was affected when flow direction was changed. In this case, the shape of eigenzones not only depended on flow direction but also changed steadily when the experiment was repeated (Fig. 4).

Eigenzones are caused by the changes in sorption equilibrium between adsorbent and binary liquid due to pressure variations. Evidently, above sorption equilibrium is influenced also by temperature and by presence of a third component within eluent. Typical unwanted admixture to the binary LC eluents is water which is extensively adsorbed on many surfaces including silica gel used in the present study. The deteriorating effect of water on the eigenzone hysteresis is illustrated in Fig. 5 for the system of silica gel and methanol plus benzene eluent. In this case, the eigenzones produced by pressure rise and by pressure drop did not represent the mirror images. The eigenzone produced by the pressure decrease was much larger, than the eigenzone caused by the

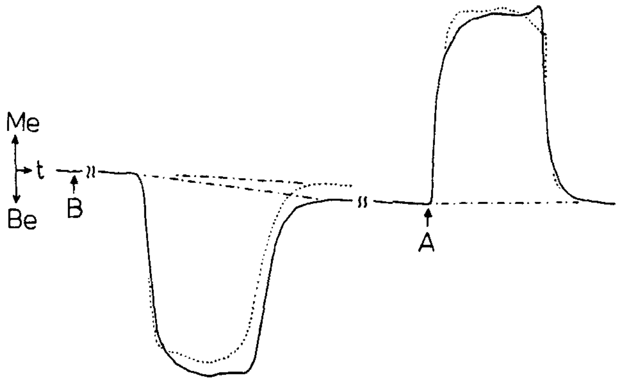

Figure 4. Examples of experimental eigenzones produced by pressure variations within an inhomogeneous and instable column packing. Column: Silica gel SG-7, 12 mm (25x0.6 cm I.D.) Flow rate: 0.83 ml/min. Pressure change: 14.2 MPa. Eluent: Benzene (Be)/methanol (Me), azeotropic mixture. Symbols as in Fig. 3.

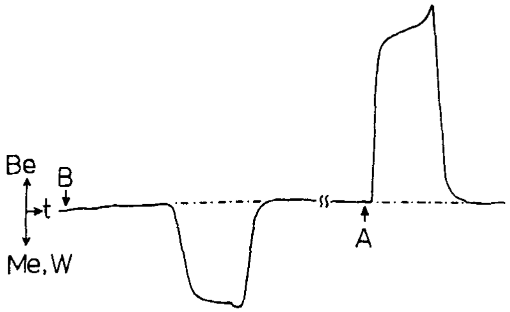

Figure 5. Influence of moisture in mixed eluent on the shape of eigenzones. Column: Silica gel Silpearl, 13 mm (10x0.6cm I.D.). Flow rate: 0.9 ml/min. Pressure change: 6.4 MPa. Eluent: Benzene/methanol/water = 60/40/0.5 vol. Symbols as in Fig. 3.

pressure increase. It is evident that dry liquids or, generally, pure two-component mixtures must be used for preparation of mixed eluents applied in experiments in order to avoid irrepeatable results. The size and shape of eigenzones may be influenced also by the traces of eluent with other compositions that were retained by the sorbent in the course of previous measurements.

Calculation of Interstitial Porosity and Pressure Profile of Column Packing

The overall pressure drop along the HPLC column can be calculated by using Carman-Kozeny equation[29-31] describing the flow of liquid through a layer of particles:

$$\Delta P = \frac{150.v.\eta.L.(1-\varepsilon_0)^2.\Psi^2}{D^2.\varepsilon_0^3} \qquad (1)$$

where ΔP is the overall pressure drop along the column, v is the linear flow rate of eluent (i.e. the volumetric flow rate divided by the column cross-section), h is the eluent viscosity, ε_o is the interparticular porosity of the column with a length of L, D is the mean diameter of the sorbent particles having shape factor Ψ^2. $\Psi^2 = 1$ for porous spherical particles and $\Psi^2 = 1.7$ for porous irregular particles.[30] According to the literature, the values of ΔP calculated from Eq. 1 may differ from experimental values by about ±10%. Still, the Carman-Kozeny equation so far best describes above complicated physical system.

Equation 1 was derived for particles of uniform sizes but it can be applied, in good approximation, for packings with very narrow particle size distributions that are usual in today's HPLC analytical column technology. Carman-Kozeny equation was, however, also applied quite successfully to the beds of particles having rather broad particle size distribution, e.g. to some preparative LC columns. In this case one has only to assume that the particles are arranged randomly and not ordered according to their size.

For the sake of simplicity, we will neglect both the dependence of eluent viscosity on pressure and the flow rate variations due to eluent compressibility. Also eluent heating due to friction[23-25] and consequent changes of flow rate and viscosity due to temperature changes have not been considered. Finally, the diffusion and mixing processes that take place within the column and connecting capillaries have been neglected, as well.

The latter processes cause the eigenzone broadening and skewing[20] that is often quite pronounced, especially on the tail of eigenzone. This is quite conceivable: The tail of the eigenzone was formed near the column entrance and had to travel along the whole column length. For this reason, we considered only that part of the eigenzone volume which was equal to the volume of the liquid within the column V_L. The position of this "reduced" eigenzone was identified on the chromatogram in the following way: To the point that corresponded to the moment of pressure change, the volumes V_{CD} and V_L were added (cf. Fig. 1c).

Further, we assumed the validity of Eq. 1 for each segment of column having volume V_i and containing sorbent with the mass m_{Si} and liquid with volume V_{Li}. The ratio of interstitial volume to column volume is called interstitial porosity ε_o. One can write for each column segment:

$$\varepsilon_{oi} = \frac{V_{Li} - (m_{Si} \cdot V_p)}{\Pi . r^2 . L_i} \tag{2}$$

where V_p is the specific pore volume [mL g^{-1}]. The mass of sorbent m_{Si} within the volume of column segment can be calculated from the material balance of the adsorption-desorption process.

A part of methanol molecules initially adsorbed on the sorbent surface A [m^2] were transferred into mobile phase volume V_L [mL] after pressure decreased within the column. Consequently, both the surface concentration of methanol Γ [mole.m^{-2}] and the total methanol concentration in the mobile phase c [mole.mL^{-1}] has been changed and it follows:

$$\Delta\Gamma . A = \Delta c . V_L \tag{3}$$

Total surface of sorbent within the column is proportional to the mass of sorbent, m_S [g], and to its specific surface A_{SPEC} [m^{2}g^{-1}]. Introducing pressure variations ΔP into Eq. 3 we obtain:

$$\Delta c = \frac{\Delta\Gamma}{\Delta P} . \frac{A_{SPEC} \cdot m_S}{V_L} . \Delta P = K_S . \frac{m_S}{V_L} . \Delta P \tag{4}$$

where K_S is a constant for the particular sorbent.

The overall eluent composition change is proportional to the size of eigenzone, i.e. to either its area or its mean height, h:

$$\Delta c = h . K_{RI} \tag{5}$$

where K_{RI} is the coefficient of proportionality. If we incorporate the constants for the detector, K_{RI} and for the sorbent, K_S from the Eq. 4 and 5 into a new constant K, we obtain the relation:

$$h = K . \frac{m_s}{V_L} . \Delta P \tag{6}$$

Equation 6 well describes the obtained experimental data:[15-20] The overall change of eluent composition Δc, i.e. the area or mean height of eigenzone is directly proportional to the mass of sorbent m_S, to its specific surface A_{SPEC} and to pressure change ΔP. On the contrary, Δc is indirectly proportional to the volume of liquid within the column, V_L. The constant K can easily be determined for each system sorbent plus mixed liquid by a set of calibration measurements at given temperature and detector sensitivity. The constant K gives mean height of the eigenzone corresponding to unit sorbent concentration within the column at the unit pressure change, e.g. 1 gram of sorbent per 1 mL of liquid at $\Delta P = 1$ MPa.

Let us apply this equation to a small column segment V_i with the length L_i and diameter r containing a volume of liquid V_{Li} and the sorbent with matrix volume V_{Si} and matrix mass m_{Si}. The change of the mixed liquid composition in each segment of column is proportional to the mean height of that part of eigenzone h_i which corresponds to that segment.

After rearrangement of Eq. 6 we obtain the equation for calculating the mass of sorbent within a particular column segment, m_{Si}:

$$m_{Si} = \frac{h_i \cdot V_{Li}}{K.\Delta P_i} \tag{7}$$

For the sake of simplicity, it is an advantage to divide the eigenzone into volume segments V_{Li} with equal volumes, e.g. 0.1 mL and then to calculate the mass of sorbent m_{Si} within particular segments. Similarly, one can calculate the volume of sorbent matrix within one segment of column $V_{Si} = m_{Si}/\rho_S$ where ρ_S is the density of sorbent matrix. The amount of sorbent and liquid calculated in this way corresponds to the column segment with the length L_i:

$$L_i = \frac{V_{Si} + V_{Li}}{\Pi . r^2} = \frac{\frac{m_{Si}}{\rho_s} + V_{Li}}{\Pi . r^2} \tag{8}$$

From the calculated values of L_i (Eq.8) and ε_{oi} (Eq. 2) one can construct a curve showing the distribution of interstitial volume along the column (Fig. 6). Using calculated ε_{oi} values, ΔP_i can be calculated from Eq. 1. The graphical representation of the dependence of ΔP_i on L_i represents pressure profile within the column packing (Fig. 6). Typical experimental and calculated values of pressure drop ΔP, interstitial porosity ε_o, length of the column L and mass of sorbent m_S are compared in Table 1.

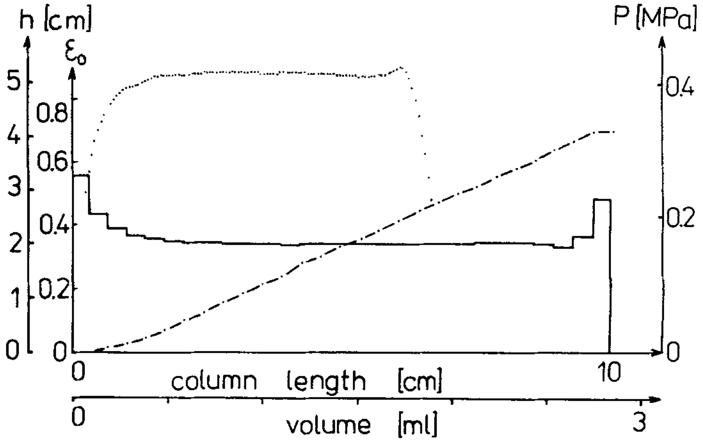

Figure 6. Example of both the calculated pressure profile (dashed line) and the corresponding distribution of interparticle porosity (solid line) within the column. Left-hand ordinate = interparticle porosity, right-hand ordinate = pressure drop. The eigenzone (dotted line) is also shown in the figure what was used for calculation. Column: Silica gel Silpearl, 13 mm (10x0.6 cm I.D.). Flow rate: 1.0 ml/min. Pressure change: 10 MPa. Eluent: Benzene/methanol, azeotropic mixture.

Table 1

Experimental Data and Calculated Values from the Eigenzone in Figure 6*

Parameter	Experimental Value	Calculated Value
ΔP	0.4 Mpa	$\Sigma\Delta P_I = 0.33$ MPa
ε_o	0.335	$\Sigma\varepsilon_{oi} = 0.358$
L	10.0 cm	$\Sigma l_I = 10.0$ cm
m_s	2.07 g	$\Sigma m_{si} = 2.00$ g

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* For the explanation see the text.

The calculated and experimental data, including the value of overall pressure drop, are in good agreement, if we consider that several effects were neglected, as mentioned above. Similar agreement between the experimental and the calculated values have been also obtained for other columns that produced repeateable and fairly regular eigenzones.

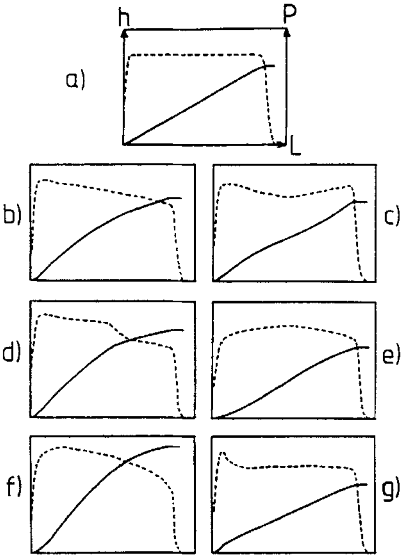

Figure 7. Pressure profiles calculated from the eigenzones shown in Fig. 2. For the calculations procedure see the text.

We have also tried to calculate pressure profiles for columns with more irregular or unusual eigenzones (Fig. 2). Some experimental parameters were not available for the experimental eigenzones since the columns were repacked in the meantime. To overcome this problem and to compare final results, the zones in Fig. 2 were normalized, so that each eigenzone had the same area. It corresponded to the situation where the amount of the sorbent within the column was constant but its distribution (i.e. the shape of eigenzone) within the column varried.

The same parameters as for the above described column, except the shape of the eigenzone, were then introduced into the described calculations. The calculated pressure profiles corresponding to the eigenzones in Fig. 2 are shown in Fig. 7.

As illustrated in Fig. 7, inhomogeneities of the column packing cause measurable deviations of pressure profile from linearity. Thus, the described procedure may be also used for detection of pressure "defects" caused with locally changed packing density. We suppose that the sensitivity of the procedure can be increased using a more sensitive detector or a more suitable pair of solvents.

CONCLUSIONS

The sorption equilibrium depends on pressure in the system containing a sorbent and an appropriate mixture of liquids. This phenomenon may be studied by any HPLC instrument provided with an additional valve and with a narrow capillary situated behind the column. Such an arrangement allows changing pressure within the column packing in a controlled and repeatable way. Pressure variations produced in the column cause defined changes in the effluent composition and eigenzones appear. The shapes of eigenzones give information on both the distribution of interstitial porosity and the pressure gradient along the column provided sorbent particles possess readily narrow size distribution.

Similarly, as in other methods based on sorption phenomena (e.g. the measurement of surface area of porous materials), the choice of sorbate - in our case, the choice of a suitable pair of liquids - is critically important for the applicability of the procedure described.

The evaluation of the eigenzones provides a valuable insight into HPLC columns, and presents an analogy with the x-ray examination of the bodies non-transparent to the light so that it is possible to see "invisible". In our case the average longitudinal distribution of particles within the column is observable. On the other hand, the radial sorbent distributions are not detectable in this way. By analogy with the x-ray examination, too large or too numerous pressure changes may destroy the column packing.

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Received June 5, 1997
Accepted June 20, 1997
Manuscript 4502