Composition changes of the mixed mobile phase within LC column due to pressure variations

A possibility of estimating column packing homogeneity

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Dedicated to Academician V Kellö, in honour of his 70th birthday

Sudden changes of pressure within liquid chromatographic (LC) column cause perturbations in the composition of mixed effluent — eigenzones. The extent of this effect depends on the nature of column packing, on the nature and composition of mixed mobile phase, as well as on the relative pressure variation and on the sorbent surface available per volume unit of column, *i.e.* on the packing density. On the other hand, the size of eigenzones is independent of the eluent flow rate. It is possible to use deliberately generated pressure changes within columns for estimating the homogeneity of their packing beds.

Внезапные изменения давления в хроматографической колонке вызывают изменения в составе двухкомпонентного элюента — возникновение зон. Величина этого явления зависит от природы наполнителя в колонке, от природы и состава смешанной подвижной фазы, от величины изменения давления и от поверхности сорбента в единице объема колонки, то значит от плотности ее упаковки. Величина зон независит от скорости потока элюента. Целесообразные изменения давления возможно использовать для оценки гомогенности упаковки колонки.

In most multicomponent eluents, the LC column packing is surrounded by an excess of molecules of one type, *i.e.* one of the eluent components is preferentially sorbed on the sorbent surface. For given column packing and eluent, the extent of preferential sorption is a function of both the relative concentration of the mobile phase components and the column temperature.

Recently, we have found that under conditions common in HPLC, the extent of preferential sorption in the packing can also be altered by sudden pressure change in the column [1, 2]. For example, from binary mixtures water—tetrahydrofuran (THF), methanol (Me)—benzene (Be), water—acetonitrile (ACN), water—methanol, the former eluent component is preferentially sorbed on the surface of the unmodified, bare silica gel. After sudden pressure increase in the column, the effluent zone with perturbed composition, “eigenzone” [2] leaves the column. The eigenzone contains the increased concentration of water (in the eluent THF—water ($\varphi_r = 80:20$) and in ACN—water ($\varphi_r = 70:30$)) or benzene (in Be—Me ($\varphi_r = 61:39$)) or methanol (in Me—water ($\varphi_r = 90$ 10)), in comparison with the composition of eluent entering the column. The eigenzone can easily be detected by a nonspecific detector, *e.g.* by a differential refractometer.

The volume of eigenzone is about equal to the volume of the liquid in the column. The process is fully reversible, *i.e.* after reduction of pressure in the column to the original value, the original sorption equilibrium is established and the mirror-image eigenzone is produced.

In our previous experiments [1—4], pressure changes were generated within columns in different ways, *i.e.* by alteration of the elution order of two columns connected in a series [1], by changing either eluent flow direction [2, 4], or flow rate [3], or, finally, by inserting the capillary with large hydrodynamic resistance behind the column [3].

These experiments showed that the size and shape of eigenzones was a function of not only numerous parameters governing the static sorption equilibrium, *e.g.* the nature of sorbent, nature and composition of eluent and temperature but it depended also on the extent and course of the pressure variation within the column, *i.e.* on the method used for generation of pressure changes.

At the same time, we have observed that the shape of eigenzones often considerably differed for "identical" systems, *i.e.* for the columns packed with the same sorbent and using the same mixed eluent under otherwise equal experimental conditions as temperature, extent of pressure change, and applied LC assembly. This may be explained by the difference in the overall and local column packing density within particular columns. Thus, the shape of eigenzone may be characteristic of the LC column packing geometry. Consequently, in the foregoing work [4] we proposed to test both homogeneity and stability of a column packing considering the shape of eigenzones.

The aim of present study was to give further experimental support to the above proposal. The dependence of eigenzone size is investigated on the extent of pressure changes, on eluent flow rate and on packing density. The absolute changes in effluent composition are evaluated and some secondary effects are discussed.

Experimental

The solvents used were purified by distillation. Tetrahydrofuran (Laborchemie, Apolda, GDR) was stabilized by butylated *p*-cresol. The mixture tetrahydrofuran and water ($\varphi_r = 80:20$) was prepared by direct mixing of appropriate volumes of liquids, the azeotropic mixture of benzene and methanol ($\varphi_r = 61:39$) [5] was prepared by distillation.

Apparatus

Fig. 1 shows the scheme of the apparatus used. The eluent is pumped (Pumping system F-30, Knauer, Bad Homburg, GFR) through a capillary pressure gauge (Institute for Chemical Processes Fundamentals of the Czechoslovak Academy of Sciences, Prague, Czechoslovakia) into a metal column (250 mm × 6 mm or 100 mm × 8 mm, Laboratorní přístroje, Prague).

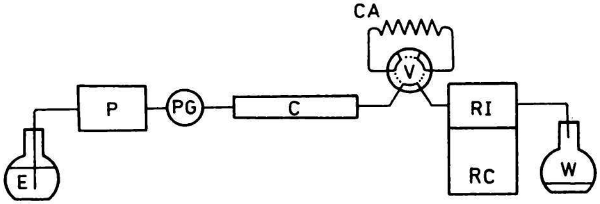

Fig. 1. Scheme of the liquid chromatographic assembly.
E — eluent container; P — pump; CA — capillary; PG — pressure gauge; V — valve in position A (———) and in position B (   ); C — column; RI — detector; RC — recorder; W — waste.

Switching the six-port three-way LC valve V (Spectra Physics, Santa Clara, CA, U.S.A.) allows to insert a metal capillary CA with I.D. = 0.25 mm into the circuit. The capillaries 9, 28, 37, 50, 78, 100, 128, and 168 m long were used. Concentration changes in the effluent were monitored with a differential refractometer (RIDK 101, Laboratorní přístroje, Prague). The responses of both the detector and the pressure gauge were recorded by a TZ 4200 recorder (Laboratorní přístroje, Prague).

Column packings

Bare silica gel designated "CH" with particles 70—200 μm and octadecyl bonded silica gel Silipor C_{18}, 125—150 μm were purchased from Lachema, Brno. Bare silica gel Silpearl with particles 8—12 μm was from Kavalier, Votice and bare silica gel SI VSK, 8—10 μm from Laboratorní přístroje, Prague. Nonporous glass beads larger than 100 μm were from BDH Chemicals, Great Britain.

Relatively large particles of sorbent were chosen in some experiments in order:

(i) to reach negligible pressure gradient within the column, *i.e.* all particles are at identical pressure within the column,

(ii) to minimize amount of heat which is generated by flow of liquid through the sorbent bed [6, 7], because the changes of temperature may influence sorption equilibrium within the column.

Amount of sorbent within particular columns was determined by weighing. The total volume of liquid within column was calculated from the known total volume of the column and experimentally determined density of sorbent matrix.

Determination of the coefficient of preferential sorption

The extent of preferential sorption from a two-component liquid under atmospheric pressure was measured by static experiments. To a small, known amount of the sorbent a known volume of the mixed solvent was added. After sorption equilibrium has been established, the composition of the supernatant was determined by the differential refractometer (Brice Phoenix Model BP-2000-V, Virtis, Cardiner, NY, U.S.A.).

The corresponding coefficient of preferential sorption (λ) expressed in cm^3 of the solvent, which is sorbed in excess on the surface of one gram of sorbent, was calculated from the material balance.

Results and discussion

After switching the valve V in the assembly shown in Fig. 1 from position A to B, the pressure in the column rises as a result of insertion of the high hydrodynamic resistance — capillary CA into circuit. After rotating the core of the valve V back from B to A position, the column pressure suddenly decreases to the initial value. In the system containing a sorbent plus a two-component eluent the increase of pressure causes desorption of one eluent component from the sorbent surface into mobile phase while the pressure decrease leads to an extraction of that eluent component from the eluent.

The examples of composition changes, eigenzones, which were generated within the columns containing silica gel particles Silpearl 8—12 µm or silica gel "CH" 70—200 µm and mixed eluent benzene—methanol or THF—water are shown in Figs. 2*a*—*c*.

1. Relations between the height of eigenzones and extent of the pressure change or eluent flow rate

The column selected for these experiments contained bare silica gel particles. Eigenzones were generated by inserting capillaries of various lengths at various eluent flow rates. Since eigenzones had fairly rectangular shape (Figs. 2*a*, *b*), it was easy to assign an average height (h) to each eigenzone. The h values were then plotted against the pressure changes (Δp) measured at the column inlet at almost constant flow rate ($1\ cm^3\ min^{-1}$) (Fig. 3) or against the flow rates at almost constant value of the pressure change in the column ($\Delta p = 15.6$ MPa) (Fig. 4).

As seen from Fig. 3, the dependence of the height of eigenzone on the pressure variation is fairly linear and passes through the origin. This means that the height of an eigenzone produced at any point of the column can be directly correlated with the concrete value of the pressure change at that point.

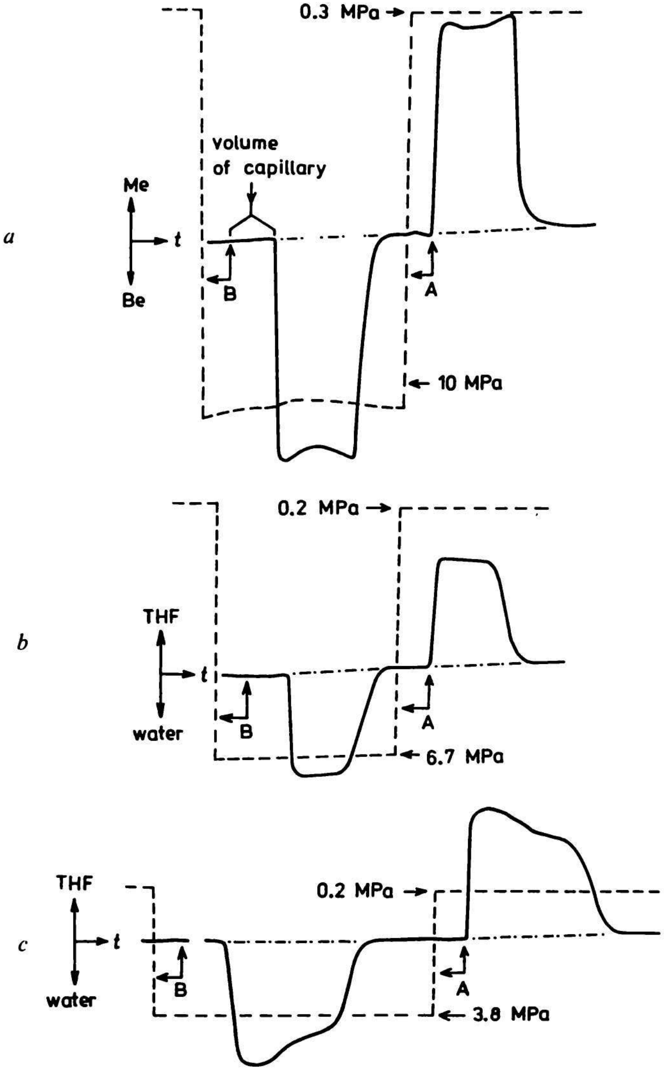

Fig. 2. Examples of eigenzones.
Signal of refractometer: ————; signal of pressure gauge: – – – ; A — valve V was switched from position B to A; B — valve V was switched from position A to B; *t* — elution time.
The sign of the detector response for the excess eluent components is shown in the figure.
a) Sorbent: Silica gel Silpearl, particle size 8—12 µm. Eluent: Benzene—methanol ($\varphi_r = 61:39$). Length of the capillary: 78.5 m.
b) Sorbent: Silica gel "CH", particle size 70—200 µm. Eluent: THF—water ($\varphi_r = 80:20$). Length of the capillary: 78.5 m.
c) Sorbent: Silica gel "CH", particle size 70—200 µm. Eluent: THF—water ($\varphi_r = 80:20$). Length of the capillary: 50 m.

On the other hand, the height of the eigenzone does not depend on the flow rate (Fig. 4). A nonzero hypothetic eigenzone corresponds to extrapolated zero flow rate. Extrapolated "static" conditions of the experiments reflect the situation, when no friction heat [6, 7] is formed in the column and the pressure effect on sorption equilibrium is not accompanied with changes in temperature.

The data in Figs. 3 and 4 show rather high reversibility of the process since the absolute heights of eigenzones practically do not depend on the sign of pressure change.

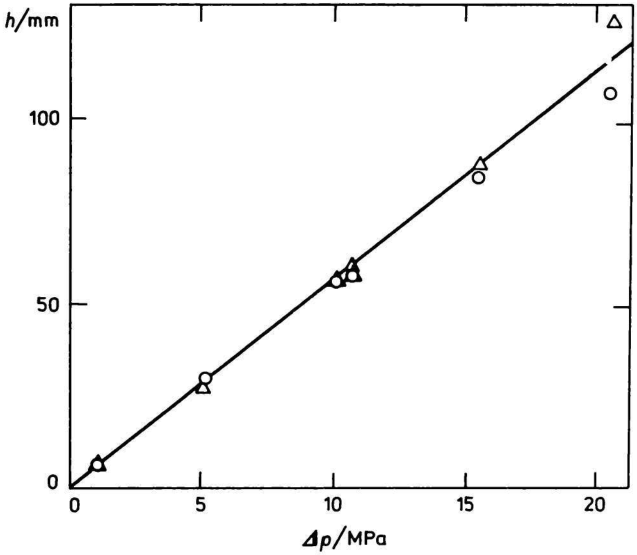

Fig. 3. Relationship between the height of eigenzones generated at constant flow rate ($1\ \mathrm{cm^3\ min^{-1}}$) and pressure change.

△ Pressure increase; ○ pressure decrease.

Sorbent: Silica gel Silpearl, particle size 8—12 μm. Eluent: Benzene—methanol ($\varphi_r = 61:39$).

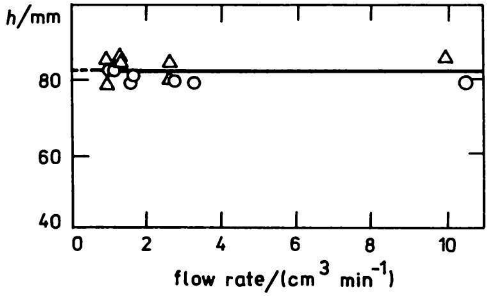

Fig. 4. Dependence of the height of eigenzones generated at constant pressure change (15.6 MPa) on the flow rate. Symbols as in Fig. 3.

Sorbent: Silica gel Silpearl, particle size 8—12 μm. Eluent: Benzene—methanol ($\varphi_r = 61:39$).

2. Relation between the amount of sorbent in the unit column volume and the height of eigenzone

The relation was examined by model experiments. One column was packed with nonporous glass beads and some columns with mixture of silica gel particles and nonporous glass in the known volume ratios. If pressure was changed in the column packed exclusively with nonporous glass, no eigenzones were observed, because glass particles had very small surface for adsorption. On the contrary, eigenzones were generated in the columns containing besides nonporous glass also silica gel.

The heights of eigenzones obtained at pressure changes $\Delta p = 5$ MPa, 10 MPa, and 15 MPa were correlated with the amount of silica gel present in the columns. In the case when the amount of silica gel was expressed as the volume present in 1 cm^3 of the bed of packing, nonlinear dependence was obtained (Fig. 5*a*). If, however, the heights of eigenzones were plotted against the mass of silica gel per volume of eluent within the column, almost linear dependence was obtained (Fig. 5*b*). Evidently, the glass beads not only "dilute" silica gel but decrease also markedly the volume of mobile phase to which the sorbent releases or from which it extracts eluent components after the pressure change.

From the above experiment it results that the height of eigenzone depends on both the amount of sorbent or more precisely the surface area of sorbent and the volume of liquid present within the corresponding volume unit of the column.

For column packed with one type of sorbent these two quantities are connected: The larger amount of sorbent, the smaller volume of liquid present in the volume unit of the column.

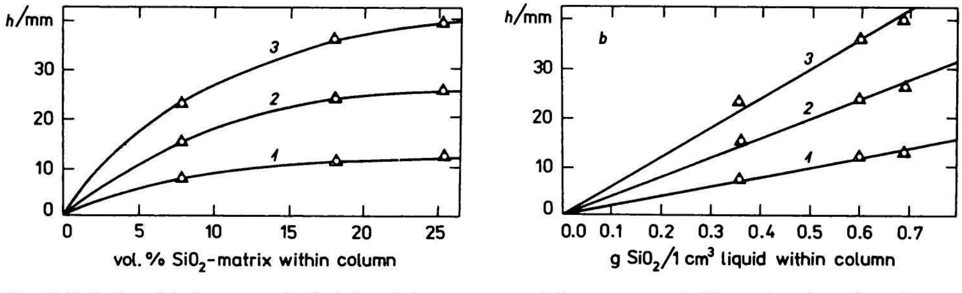

Fig. 5. Relationship between the height of eigenzones and the amount of silica gel sorbent in column. Amount of sorbent is expressed as: *a*) volume of silica gel per unit column volume. *1.* $\Delta p = 5$ MPa; *2.* $\Delta p = 10$ MPa; *3.* $\Delta p = 15$ MPa; *b*) mass of silica gel per unit eluent volume in the column. *1.* $\Delta p = 5$ MPa; *2.* $\Delta p = 10$ MPa; *3.* $\Delta p = 15$ MPa. Packing of columns: Silica gel "CH", particle size 70—200 μm and nonporous glass, particle size ≈ 100 μm, in various ratios. Eluent: Benzene—methanol ($\varphi_r = 61:39$). For explanation see the text.

In typical HPLC columns, the packing density may vary as a result of both micro- and macroheterogeneities like cavities, channels, and cracks in the packing bed. Presence of these irregularities within sorbent bed is difficult to control independently. However, properties of a long heterogeneously packed column can be roughly modelled by a series of short columns packed with different materials. Fig. 6 shows eigenzones belonging to three columns connected in series. The influence of column sequence on the shape of eigenzones is evident.

On the basis of described experiments, the shape of eigenzones can be explained as follows: By inserting capillary CA into the eluent flow path, pressure increases at any point of the column by a constant value. If the column is packed homogeneously the eigenzone will be symmetrical, *i.e.* approximately rectangularly shaped (Figs. 2*a*, *b*) and its shape will be distorted only by usual diffusion and mixing processes. The situation, however, differs in nonhomogeneous beds of packing. In the denser parts of packing bed which contains more

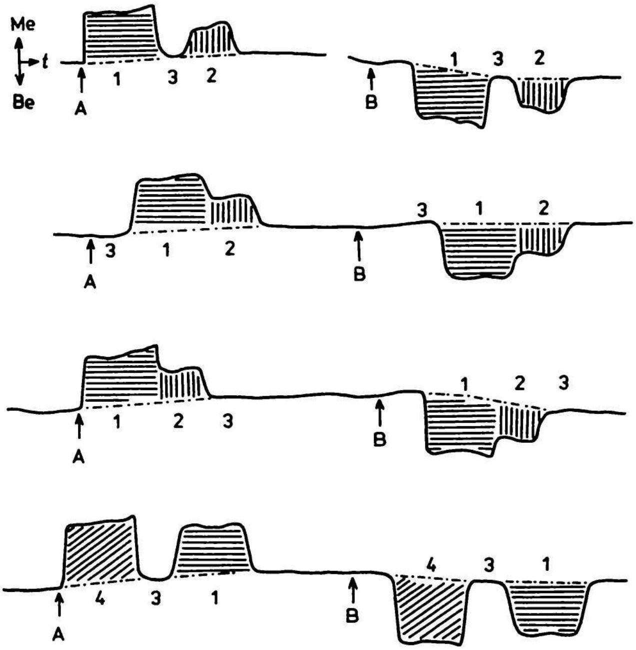

Fig. 6. Eigenzones corresponding to three different columns in series. Columns 250 mm × 6 mm: No. *1* — silica gel SI VSK, particle size 8—10 µm; No. *2* — silica gel "CH", particle size 70—200 µm and nonporous glass, particle size ≈ 100 µm in the ratio 4.6 g/7.3 g; No. *3* — nonporous glass, particle size ≈ 100 µm; No. *4* — silica gel SI VSK, particle size 8—10 µm.
Eluent: Benzene—methanol ($\varphi_r = 61 : 39$). Length of the capillary: 100 m. Pressure change: 8 MPa. Symbols as in Fig. 2.

sorbent per column volume unit, more solvent is desorbed from sorbent due to pressure variation. Consequently, the corresponding part of eigenzone is higher (Fig. 2*c*). Thus the shape of eigenzone reflects differences in sorbent bed density along the column.

The present procedure, however, allows to reveal only large heterogeneities in the bed of packing — unless more pressure sensitive mixed eluents and more effective detectors are applied.

3. Extent of effluent composition changes

In view of a more complete evaluation of the nature of eigenzones it is necessary to determine also absolute changes of the composition of the mobile phase (Δc) due to pressure variations Δp in the bed of a sorbent.

Table 1 contains the results obtained by the calculation based on the heights of eigenzones, calibration of the detector response by injecting liquid mixtures with the known composition into detector and on the material balance of the columns.

The values of coefficient of preferential sorption λ measured at atmospheric pressure are also given in Table 1.

The material balance reveals that although the eigenzones are often very distinct and easily observable, the coefficient of preferential sorption λ changes a few percents only (Table 1) due to pressure changes as large as 10 MPa. That is probably why no pressure effect on elution volumes in HPLC has been so far observed.

We have generated similarly large eigenzones as those presented in this paper also with other types of sorbents, *e.g.* with various surface modified silica gels, organic copolymers (acrylonitrile—ethylene glycol dimethacrylate, styrene—ethylene glycol dimethacrylate), and carbon based LC packing materials.

Data about pressure dependence on preferential sorption are rather scarce in literature. Recently, *Ozawa et al.* [8] theoretically and experimentally described the pressure dependence of coefficients of preferential sorption on carbon sorbent in various binary liquids at static conditions up to 490 MPa. A comparison of their results with our data in Table 1 reveals similar extent of influence of pressure on sorption equilibria, *i.e.* the change of parameter λ presents only few percents when pressure change $\Delta p = 10$ MPa is applied.

4. Secondary effects

As it was shown, the height and the shape of the eigenzones depends on amount of sorbent within the column and on the value of pressure changes. However, in some extent also other — secondary — effects may influence the shape of the eigenzones. They are:

Table 1

Changes of coefficient of preferential sorption λ induced by 10 MPa increased pressure

Sorbent	Eluent φ_r	Zone height at detector sensitivity 16× mm	λ/mm^3 g^{-1} at 0.1 MPa	Δc/mm^3 g^{-1}	$\Delta\lambda$/mm^3 g^{-1}	$\Delta\lambda$/%
Silica gel "CH"	THF—water 80 : 20	82	47.0 water	1.9 water	−3.0 water	−6.5 water
Silica gel Silipor C_{18}		8	40.7 THF	0.7 THF	−0.7 THF	−1.7 THF
Silica gel "CH"	Be—Me 61 : 39	50	174.2 Me	4.7 Be	+3.7 Me	+2.1 Me
Silica gel Silipor C_{18}		52	25.2 Be	1.7 Me	+1.6 Be	+6.5 Be

Notes: λ — Coefficient of preferential sorption (volume of preferentially adsorbed eluent component per one gram of sorbent). Be — Benzene, Me — methanol, THF — tetrahydrofuran. Δc — Amount of THF (or water ...) desorbed from column packing into one gram of eluent within the column. $\Delta\lambda$ — Change of coefficient λ (100 % = λ at 0.1 MPa).

(i) Broadening and skewing of eigenzones during their passage through the column packing.

(ii) Establishment of secondary sorption equilibria. Each part of the eigenzone in the column is in equilibrium with that part of packing, in which it was generated. During its passage through the column, however, the eigenzone interacts also with the parts of packing, with which it is not in sorption equilibrium. The establishment of new equilibria might affect the resulting shape of the detected eigenzone. The latter effect, however, is expected to be small.

(iii) Changes in the packing structure. In a series of preliminary experiments with glass-wall columns we observed that repeated pressure strokes necessary for the generation of eigenzones led sometimes to the formation of craks or cavities in the column. In that case repeatability of size and shape of the eigenzones was deteriorated. The probability of the bed destruction is proportional to the extent and number of pressure changes and depends also on the method of pressure stroke generation. On the other hand, fairly large eigenzones may be produced — without destruction of packing structure — by rather small pressure variations (2—3 MPa), if both the detection and the nature and composition of the mobile phase are properly chosen.

Conclusion

The influence of some experimental variables on the formation of perturbations in effluent composition, eigenzones, generated by pressure changes in LC columns, was examined.

Measurements with mixed eluents tetrahydrofuran—water and benzene—methanol and with columns packed with bare and C_{18}-bonded silica gel revealed that the height of an eigenzone is a function of the chemical nature and the effective surface of sorbent present in the volume unit of column as well as of the extent of the pressure change. On the other hand, the height of eigenzones does not depend on the flow rate of the eluent.

The absolute changes in the extent of preferential sorption on the sorbent surface due to pressure changes in the system studied are not large. Variation in the coefficient of preferential sorption λ by $\pm 4\,\%$ at pressure change $\Delta p = 10$ MPa indicates the desorption or additional adsorption of only a few molecules per one hundred preferentially adsorbed molecules. That is probably why the effect of pressure on retention is hardly to be found in common LC systems. On the other hand, some unexpected chromatographic results may be explained considering the pressure dependence of preferential sorption on column packing [3].

Our experiments confirmed potentiality of utilizing the effect described for estimation of the homogeneity of the sorbent bed in the column.

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References

1. Berek, D., Chalányová, M., and Macko, T., *J. Chromatogr. 286,* 185 (1984).
2. Chalányová, M., Macko, T., Kandráč, J., and Berek, D., *Chromatographia 18,* 667 (1984).
3. Macko, T., Chalányová, M., and Berek, D., *J. Liq. Chromatogr. 9,* 1123 (1986).
4. Macko, T. and Berek, D., *J. Chromatogr. Sci. 25,* 17 (1987).
5. Horsley, L. H., *Anal. Chem. 19,* 508 (1947).
6. Poppe, H., Kraak, J. C., Huber, J. F. K., and Van der Berg, J. H. M., *Chromatographia 14,* 515 (1981).
7. Katz, E., Ogan, K., and Scott, R. P. W., *J. Chromatogr. 260,* 277 (1983).
8. Ozawa, S., Kawahara, K., Yamabe, M., Unno, H., and Ogino, Y., *J. Chem. Soc., Faraday Trans. 1 80,* 452 (1980).

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