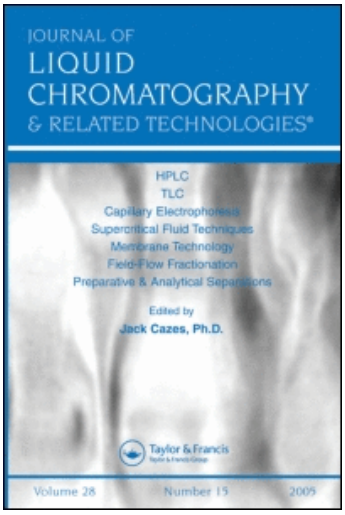

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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Online publication date: 31 May 2001

To cite this Article Macko, Tibor and Berek, Dušan(2001) 'PRESSURE EFFECTS IN HPLC: INFLUENCE OF PRESSURE AND PRESSURE CHANGES ON PEAK SHAPE, BASE LINE, AND RETENTION VOLUME IN HPLC SEPARATIONS', Journal of Liquid Chromatography & Related Technologies, 24: 9, 1275 — 1293
To link to this Article: DOI: 10.1081/JLC-100103447
URL: http://dx.doi.org/10.1081/JLC-100103447

J. LIQ. CHROM. & REL. TECHNOL., 24(9), 1275–1293 (2001)

PRESSURE EFFECTS IN HPLC: INFLUENCE OF PRESSURE AND PRESSURE CHANGES ON PEAK SHAPE, BASE LINE, AND RETENTION VOLUME IN HPLC SEPARATIONS

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ABSTRACT

High sensitivity of modern detectors enables monitoring and utilisation of very small chromatographic effects caused by pressure. Till now, these effects have been often overlooked. Taking the influence of pressure on the HPLC system into account, numerous unexplained chromatographic phenomenona may be elucidated. Moreover, some pressure effects may be exploited for HPLC column diagnostics, and pressure may even be employed to control retention behaviour of substances. Last but not least, ultrahigh pressure enables transporting eluent through long microparticulate column packings to reach extremely high efficiency of HPLC separations.

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INTRODUCTION

A substantial part of liquid chromatography (LC) separations bears the designation - High Pressure Liquid Chromatography. It was the pumping of liquids under high pressure that enabled the exploitation of the high separation efficiency of very small sorbent particles, thereby, allowing High Performance LC separations. Consequently, pressure is an important experimental parameter, which is connected with the successful development of liquid chromatography.

Usually, the chromatographers wish to work at constant pressure. In chromatographic practice, however, pressure variations occur. If the pump works in constant flow mode, the pressure within a column and/or a precolumn can *gradually* change as a result of:

i) partial blockage of frits within the column fittings and/or of part of the column packing bed with the nondissolved fraction of injected samples or with fragments of sorbent;
ii) partial blocking of connecting capillaries or on-line filters;
iii) variations of temperature which cause changes in viscosity and density of the mobile phase and, consequently, in the hydrodynamic resistance of the column.

Pressure may *abruptly* change

a) eluent flow, i.e., due to sample injection, recycling, backflushing, column switching, etc.,
b) when the flow rate is suddenly altered,
c) as result of viscous sample injection,
d) due to temporary pump failure (for example, due to air bubbles in eluent).

The frequency of such pressure changes depends substantially on the type of LC equipment used. Many modern LC pumps equipped with continuous pressure monitoring and an automatic performance adjustment, autosamplers causing only very short flow interruptions, effective filters, as well as effective degassers and column thermostats successfully eliminate, or at least decrease, these undesirable pressure variations. Nevertheless, deliberately or accidentally generated changes of pressure, as we will show, may be manifested on chromatograms in a characteristic way.

Numerous new procedures are being continuously introduced into research and production chemical units. They are, for example, reactions within over pressurized chemical reactors, purifications of substances within adsorption towers, as well as filtration and industrial chromatography. In many cases, specific pressure effects may be present.

RESULTS AND DISCUSSION

Action of Pressure in HPLC

Generally, pressure affects the density and viscosity of liquids[1] and, consequently also the diffusion of solutes and flow velocity of mobile phase.[2,3] Mechanical action of pressure may cause changes in both the size and shape of some column packing particles, as well as in their pore structure. Friction connected with movement of liquid along the column packing also causes an increase in temperature of both the mobile phase and column.[4-7]

Naturally, pressure is not uniform within the chromatographic column where a certain pressure gradient does exist. This means that the physical parameters mentioned above possess different values in different parts of the column. Gradients of basic parameters within a homogeneously packed column are schematically shown in Figure 1. The actual values of density and viscosity at a given pressure depend on the eluent nature and on temperature within the column, which in turn, is affected by the heat dissipation conditions. Moreover,

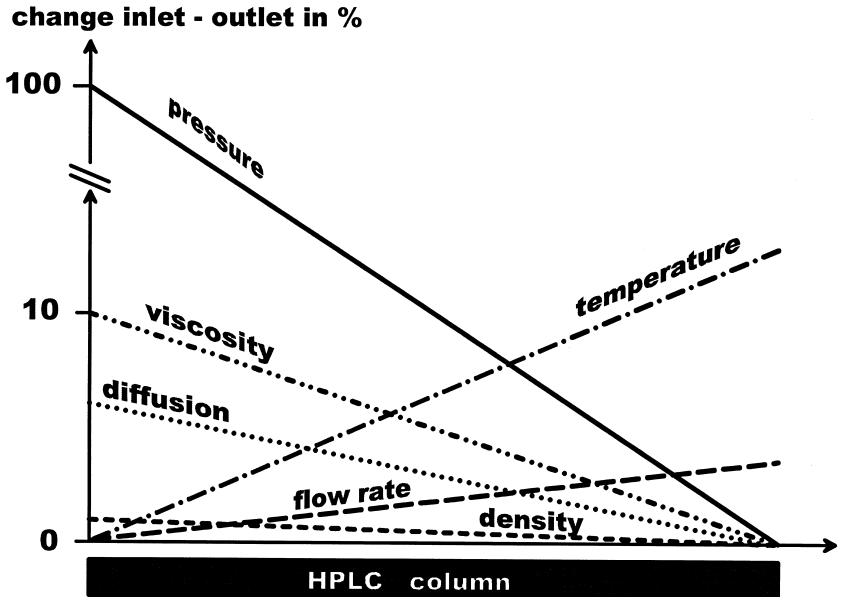

Figure 1. Schematic representation of longitudinal profile of pressure, flow velocity, viscosity, density, temperature, and diffusion within a homogeneously packed non-thermostated HPLC column (Constructed according to data from refs. 2, 3, and 5).

some parameters mutually affect each other, for example, increase of temperature reduces the viscosity and density of eluent in contrast to increase in viscosity and density due to direct pressure action.

The dependence of compressibility, viscosity, flow velocity, and diffusion on pressure and its impact on retention volumes and retention times has been theoretically evaluated by Martin et al.[2,3] It was shown, that variation of the above parameters with pressure under the usual experimental conditions, that is at pressure as high as 10 to 15 MPa, causes only relatively small, and often practically negligible, variations in retention volumes (a few percent) of test solutes. The change of temperature within the column, resulting from the heat generated by friction[4-6] has usually also only a very small influence on retention behavior[7] of small molecules.

Taking all this into account, one should not expect considerable variations in retention of low molar mass substances with change of pressure, unless interactions in the system mobile phase - sorbent - sample are affected.[1] Certainly, the situation in size exclusion chromatography of macromolecules may be different. Variations in retention volumes as small as 5% due to pressure changes in the range of 10 MPa may bring about important errors in molar mass values determined.

It was confirmed experimentally, that pressure influences the behaviour of many substances in solutions, for example, the ionization equilibrium,[1] size of solvated macromolecules,[8,9] aggregation of macromolecules,[10] adsorption of both liquids[11,12] and solutes[13] on adsorbent surfaces.

As we will show in the next sections, the action of pressure on the interaction of components within the chromatographic system manifests itself in a characteristic manner.

Influence of Pressure on the Chromatographic Base Line

Several reports[14-16] can be found in the literature concerning reproducible temporary perturbations of the base line after a sudden change of flow velocity, i.e. pressure, within the HPLC. Here, the eluents consisted of more than one component.

In our laboratory, we have observed reproducible perturbations of the base lines caused by sudden pressure changes – if two component eluents and porous column packing were used. The perturbations were monitored by a refractometric detector at constant overall eluent flow rate. We revealed that the pressure changes in the range of 0.1 and 10 MPa caused well detectable alterations in effluent composition. The shape of the effluent zones or eigenzones[17-22] with altered composition depended on the method employed for the pressure change generation. For example, inserting a long capillary with a large hydrodynamic

resistance behind a LC column gave rise to a nearly rectangular zone (Figure 2); a sudden change of flow direction caused a Z-shaped zone (Figure 3); a sudden increase in flow velocity lead to a triangular zone (Figure 4); a sudden flow interruption resulted in the appearance of new peaks (Figure 5), and the alternate recycling with mixed eluent generated bipolar eigenzones (Figure 6). It is necessary to stress, that in the experiments no sample was injected.

The total volume of eigenzones corresponded with the volume of liquid within the particular column – though it was systematically increased due to broadening effects. The position of the eigenzone is shifted on a chromatogram when inserting a long capillary (with volume V_{CA}, Figure 2) . When the packed column was replaced with a long capillary no eigenzones appeared. Similarly, columns containing non-porous solid particles did not give measurable eigenzones,[20] due to the strong decrease of the surface of system available for adsorption.

The origin of eigenzones and their shape can be explained by the dependence of preferential adsorption of mixed eluent components on pressure. Pressure changes influence the adsorption equilibrium of eluent components on the sorbent surface – possibly due to changes in intermolecular interactions within mixed eluents. The amount of preferentially adsorbed or desorbed molecules of liquids on/from the sorbent surface is a function of the extent of pressure

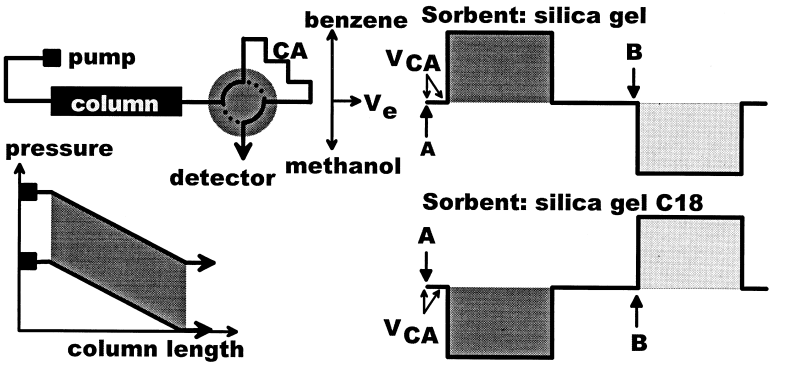

Figure 2. The shape of zones (schematically) generated by insertion of long capillary behind the column. Benzene/methanol azeotropic mixture (39.6 % wt. of methanol) was used as eluent. The sign of the detector response is shown in the figure. Pressure change: 5 MPa. Flow rate: 1ml/min. Symbols: V = valve in position a (—) and b (- - -); A = valve V was switched from position b to a; B = valve V was switched from position a to b; CA = hydrodynamic resistance, long capillary; V_{CA} = volume of capillary CA; V_e = elution volume. Eigenzone generated by an increase of pressure = dark grey area, by a decrease of pressure = light grey area.

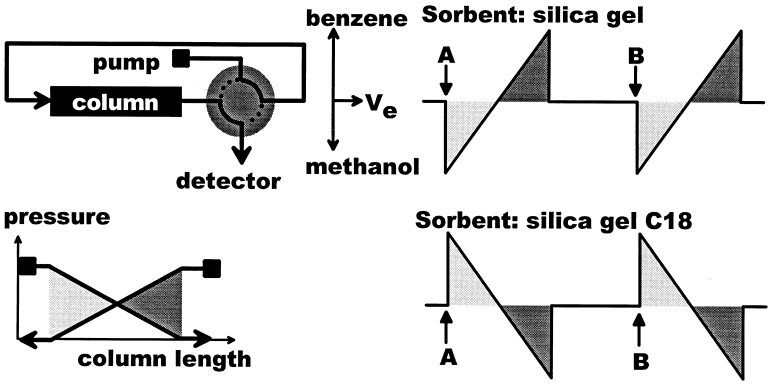

Figure 3. The shape of zones (schematically) generated by column backflushing. Symbols as in Fig. 2.

change and also the chemical nature of all constituents. Therefore, the shape of eigenzone reflects the changes of the pressure profile within the column packing (Figures 2-6).

It is well known, that after a mobile phase flow velocity adjustment it is sometimes necessary to wait for the base line stabilization. One of the possible reasons for this phenomenon is the dependence of the adsorption equilibrium on pressure. This effect is typical for the commonly used HPLC separation systems, for example water/methanol and tetrahydrofuran/water mixed mobile phases with bare or chemically modified silica gel sorbents.[17,18,20] Similar effects were also observed with other types of sorbents, e.g. with organic polymers (acrylonitrile-ethylene glycoldimethacrylate, styrene-ethyleneglycoldimethacrylate, hydroxy-

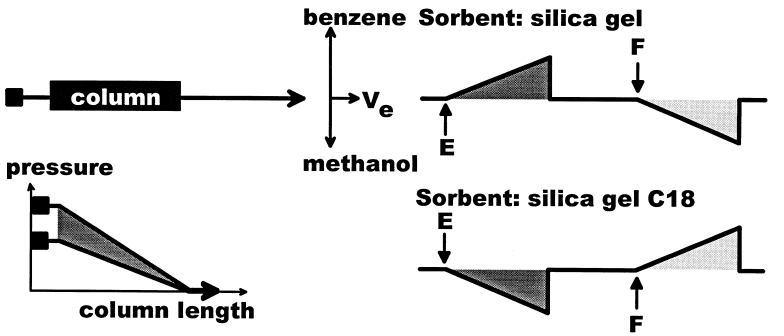

Figure 4. The shape of zones (schematically) generated by sudden change in flow rate. E = increase in flow velocity; F = decrease in flow velocity. Symbols as in Fig. 2.

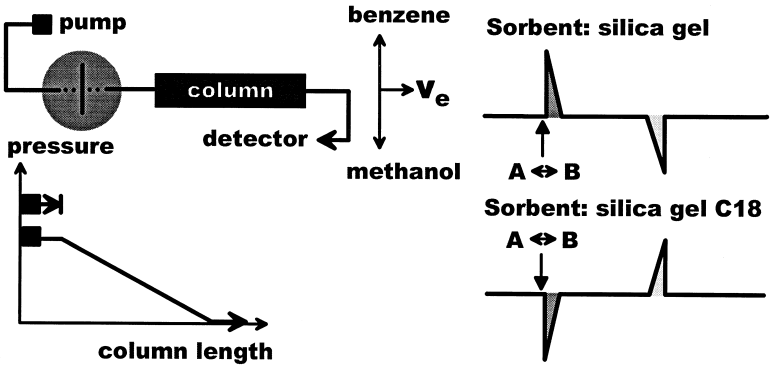

Figure 5. The shape of zones (schematically) generated by short interruption of flow applying a valve situated before column. Symbols as in Fig. 2. For detail explanation see ref. 25.

ethyl methacrylate), as well as carbon based packing materials.[23] In this way, the drift of the base line may be explained as a result of pressure changes either during a longer period of time or suddenly, for example, after the injection of a viscous sample.

HPLC is used also for the dynamic measurement of adsorption isotherms. For example, Eltekov et al.[24] determined the adsorption isotherm for the system,

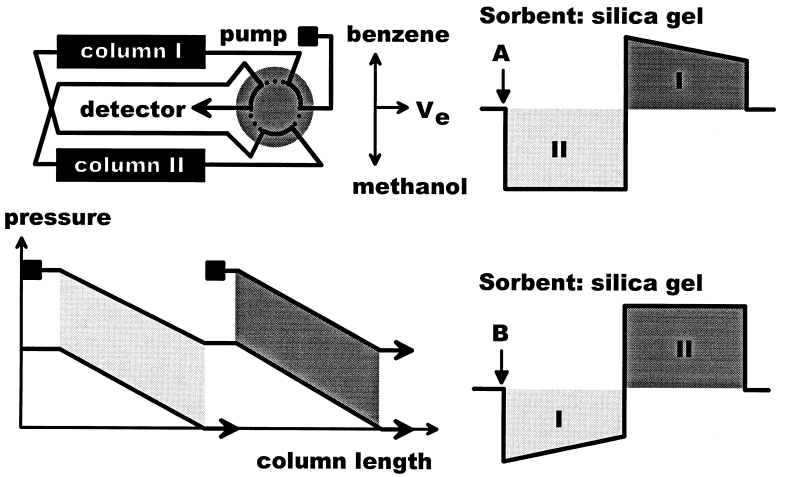

Figure 6. The shape of zones (schematically) generated by alternate recycling. Symbols as in Fig. 2.

silica gel - benzene - heptane by the frontal method. They observed that the amount of adsorbed benzene decreased with the increasing eluent flow velocity. As a pressure gradient exists within each HPLC column, the question arises as to which value of pressure corresponds to a measured adsorption isotherm. In the best case, it should be the average value of pressure within the column.

As we have shown, the appearance of eigenzones is of practical interest since pressure changes can be encountered upon recycling, backflushing, column switching, etc. Therefore, it is useful to understand and possibly to control the associated detector signal perturbations.

Let us discuss the case of flow interruption more in detail. We observed the appearance of two new peaks on the chromatogram[25] when flow was interrupted (Figure 5) in various HPLC systems. One of two ghost peaks is narrow and appears almost immediately after the reestablishment of eluent flow and the second is a wide peak in the area of the column dead volume.[25] The second peak elutes in the part of the chromatogram, where, as a rule, components of the eluent elute, i.e. near retention volume of "solvent peak," "system peak," "ghost peak." These peaks are well detectable with non-specific detectors but may remain unnoticed when photometers are applied. The peak with low retention volume can be found in many published chromatograms (cf. for example ref. 26-28) and its cause is usually not discussed. Perturbation of the adsorption equilibrium by sudden pressure changes may again be an adequate explanation of the appearance of this extra peak. The manual injection of a sample into an HPLC column is usually connected with the flow interruption.[29,30]

The pressure variation due to flow interruption can be eliminated or decreased either by using an injection valve with a bypass or an electrically on pneumatically actuated injection valve. This is one of the reasons why autosamplers should be preferred to manual injection.

Naturally, the gradual or sudden change of pressure may also desorb some impurities from the column and cause additional baseline instability or appearance of irreproducible peaks.

Influence of Pressure on Retention Volume and Peak Shape

Numerous papers discuss influence of pressure on diffusion rate, viscosity, and temperature within the LC column and, consequently, on sample retention volumes. Following basic experimental studies of Rogers and co-workers,[31-36] theoretical analysis[2,3] and additional experimental measurements,[37] an interest in the pressure role in LC has been periodically revived.[38-49]

Dependence of retention volume on pressure within the column was observed for almost all LC modes (Table 1). Unfortunately, rather limited research has been carried out in this field and the action of very high pressures

Table 1. LC Systems Where Pressure Dependence of Retention Was Described

LC Modus (Ref.)	Sorbent	Eluent	Solute	P [MPa]	Observed Effect	Possible Cause According to Authors
Normal phase adsorption LC (31,33)	Silica gel	Water	Methyl orange, ethyl orange	241	$-\Delta k$=13% at ΔP=34 MPa; $+\Delta k$=50% at ΔP=151 MPa; First $-\Delta k$ then $+\Delta k$ at ΔP for both solutes; Change of peak shape	Change of water structure with pressure, thus change of hydratation of solute
SEC (32)	Porous glass	Deionized water	Bovine plasma albumin	343	Change of peak shape	Denaturation of protein with pressure
SEC (34)	Porous glass	Water/NaCl, 0.1M,	Sodium dodecyl sulphate	369	$+\Delta k$=50% at ΔP=282 MPa; Reversible change of peak shape	Change of equilibrium monomer-micelle, reversible dissociation of micelles with pressure
Ion-exchange LC (35)	Anion-exchange resin	Deionized water	Lead nitrate, potassium nitrite, chloride and bromide	353	$-\Delta k$=58% at ΔP=353 MPa; $+\Delta k$ =16% at ΔP=353 MPa; $-\Delta k$ for nitrate anion; $+\Delta k$ for remaining anions;	Change of dissociation of complexes solute-water with pressure; compressibility leads to larger volumetric flow
Reverse ion-pairing LC (36)	Silica gel C_{18}	Methanol /water, buffer	Methyl orange, methyl red	345	$+\Delta V_R$=320% at ΔP=241 MPa; above 345 MPa total adsorption of the solute	Dependence of ion-pairing and structure of solvents on pressure,
LC at the point of exclusion-adsorption transition (37)	Porous glass	Chloroform/ tetrachloro-methane	Polystyrene standards	20	$-\Delta V_R$=30% at ΔP=19 MPa; $+\Delta V_R$=13 at ΔP=19 MPa; $-\Delta V_R$ or $+\Delta V_R$ in dependence on molar mass of the polymer	Change of adsorption with pressure

(continued)

Table 1. Continued

LC Modus (Ref.)	Sorbent	Eluent	Solute	P [MPa]	Observed Effect	Possible Cause According to Authors
Ion-exchange LC (38)	Silica gel C_{18}	Methanol/ water, buffer	Benzoic acid, p-nitrophenol, 2,6-dimethylpyridine, N-methylaniline	20	$-\Delta k$=12% at ΔP=20 MPa; $-\Delta k$ for all solutes	Change of dissociation of both the solute and the buffer species with pressure
Reverse phase LC (40,42)	Silica gel C_{18}	Methanol	Derivatized fatty acids	33	$+\Delta k$=24% at ΔP=23 MPa $+\Delta k$ for all solutes	Change of interaction energy with density
Chiral LC (41)	ß-cyclo-dextrin-bonded to silica	Methanol/ water, buffer	Positional isomers of nitrophenol	34	$-\Delta k$=35% at ΔP=28 MPa; $-\Delta k$ for all solutes	Dissociation of the cyclodextrin-solute complex with pressure
Reverse phase LC (44)	Silica gel C_{18}	ACN/water/ trifluoro-acetic acid	Positional isomers of hydroxybenzene, 4-methylcatechol, 4-ethylresorcinol, 2,3-dihydroxy-naphthalene	410	$+\Delta k$=50% at ΔP=375 MPa; $+\Delta k$ for all solutes	Change of retention with pressure

Chiral LC (45)	ß-cyclodextrin-bonded to silica	ACN or methanol/ water/acetic acid/ triethylamine	Hexobarbital, methobarbital, ibuprofen, chlorthalidone, benzoin, propranolol hydrochloride, metoprolol, warfarin	30	−Δk=20% at ΔP=30 MPa; +Δk=12% at ΔP=30 MPa; −Δk for first four solutes; +Δk for last four solutes; Plate heights increase with pressure up to 240%; Pressure-induced hysteresis effect in retention	Dissociation of the cyclodextrin-solute complex with pressure
Chiral LC (46)	ß-cyclodextrin-bonded to silica	Methanol/ water/ acetic acid/ triethylamine	Hexobarbital, mephobarbital	24	+Δk=11% at ΔP=25 MPa +Δk for all solutes	Equilibrium complexation shifts with pressure
Reverse phase LC (46)	ß-cyclodextrin-bonded to silica	Methanol/ water;	Positional isomers of nitrophenol, naphthol;	32	−Δk=14% at ΔP=27 MPa; +Δk=3% at ΔP=27 MPa; −Δk for nitrophenols; +Δk for naphthol	Pressure-induced shifts in solute equilibria
Reverse phase LC (49)	Silica gel C_{18}	Methanol/ water or methanol/ water/ ß-cyclodextrin	Positional isomers of nitrophenol	28	+Δk=12% at ΔP=22 MPa; +Δk for nitrophenols	Pressure-induced shifts in complexation, partitioning and solute ionization

(continued)

Table 1. Continued.

LC Modus (Ref.)	Sorbent	Eluent	Solute	P [MPa]	Observed Effect	Possible Cause According to Authors
Reverse phase LC (48)	Silica gel C_8	Acetonitrile/ water or ethanol/water	Insulin	28	$+\Delta k$=33% at ΔP=13 MPa	Adsorption
Reverse phase LC (47)	Silica gel C_{18}	ACN/water/ trifluoro-acetic acid	Positional isomers of hydroxybenzene, 4-methylcatechol, resorcinol, peptides	500	$+\Delta k$=53% at ΔP=500 MPa $+\Delta k$ for all solutes	Change of retention with pressure

Symbols: P = the largest value of pressure studied; Δk = the largest observed change of capacity factor ($-\Delta$ = decrease, $+\Delta$ = increase; 100% = value at smaller pressure); ΔP = increase of pressure; ΔV_R = the largest found change of retention volume corresponding to ΔP. (For an easier comparison: 1 MPa ≈ 145 psi ≈ 10 atm ≈ 10 kg/cm^2 ≈ 10 bar)

over 300 MPa was investigated only briefly. Recently, Jorgenson's group[44,47] reported experiments using mobile phase pressure as high as 410 - 500 MPa.

The retention of the injected solutes either increased or decreased with rising pressure[31,35,36,38-41,44,47] (Table 1). In most cases, capacity ratio increased linearly with pressure, but some authors[36,39,40] found linearity between logarithm of capacity ratio and pressure. The logarithm of capacity ratio also increased linearly with the number of methylene groups within the solute molecule.[39,40] A minimum retention volume for two model solutes was identified at a specific pressure.[31,33] Other groups[32-34,45] have even observed a change of peak shape if pressure was varied.

So far the largest change of retention time with pressure (3.2 times) has been found by Prukop and Rogers[36] (Table 1).

Taking into account the relatively small pressure change (19 MPa), pronounced changes of polymer elution volume with pressure (20-30%) were revealed by Nefedov and Zhmakina.[37] They found that in the system porous glass - chloroform / tetrachloromethane (4.5/95.5 v/v), the retention volumes of polystyrene standards depended on pressure (Figure 7).

It is known that both the size of macromolecules in solution and their diffusion rate diminish with increasing pressure. Consequently, the size exclusion retention volumes of polystyrene should increase with increasing pressure. This was observed in a lesser extent for polystyrenes with polymerization degree up to 100 (Figure 7). However, for high molar masses an opposite effect was found. For example, retention volumes of high molar mass polystyrenes decreased with pressure (Figure 7). It is known that polystyrenes adsorb on the silica surface from tetrachloromethane while adsorption is negligible from chloroform.

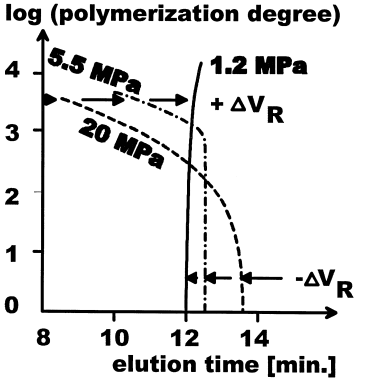

Figure 7. Dependence of elution behaviour of polystyrenes on pressure**.** Eluent: chloroform / tetrachloromethane, 4.5/95.5, v/v. Column packing: Porous glass. Figure constructed according to data.[37]

Therefore, we may expect that an increase of concentration of chloroform on the surface of sorbent due to pressure change would decrease retention of polystyrene and vice versa.[50] The sign of eigenzone generated within the system bare silica – tetrachloromethane – chloroform, confirmed that an increase in pressure caused excess of tetrachloromethane within the effluent.[50] This means that the amount of chloroform on the sorbent surface increased with rising pressure and, therefore, the retention volumes of polystyrenes decreased.

The change in preferential sorption of eluent components on the column packing surface with pressure, and the effect of pressure on both diffusion and chain dimension, may induce comparable effect on retention volumes but their signs may be opposite. Which effect prevails on resulting retention volume, depends on the molar mass of polymer under study.

Nefedov and Zhmakina[37] employed a composition of the chloroform / tetrachloromethane mixed eluent in which exclusion and adsorption of macromolecules mutually compensated. In this exclusion-adsorption transition or critical adsorption point for polystyrene, the elution of PS did not depend on molar mass of PS. It is known (for review see ref. 51) that at the critical adsorption point the retention volumes are very sensitive to the structure of the macromolecules, temperature, and as shown above, also to pressure. In most liquid chromatographic separations, mixed eluents are used (see also Table 1). Therefore, the pressure-dependent preferential sorption of eluent components on the sorbent surface[17-19] may often contribute to the retention behaviour of analysed substances.

Pressure in LC systems is changed deliberately in the course of the van Deemter or Knox-Bristow plot construction. It is known that these plots have a characteristic shape, but many deviations were observed.[52] The role of pressure cannot be excluded here, as well.

Several authors[44,47] worked with pressures as high as 140 MPa to transport mobile phase through a 50 cm long capillary packed with sorbent particles of 1.5 μm diameter. They generated as much as 200 000 theoretical plates/m for slightly retained compounds and over 200 000 plates for more retained compounds (k' about 2). For the gradient separation of peptides, a peak capacity of 300 at 30 min. analysis was demonstrated.[47]

These results are so attractive that the ultrahigh-pressure LC assemblies may be commercialized and intensively exploited in the future. Naturally, in such ultra-high-pressure range, all above mentioned pressure effects will have higher practical significance and may be readily observable.

Exploring Changes Caused by Pressure Variations

Marshall et al.[53] used the pressure jump from several thousand psi (several tens of MPa) to ambient pressure for direct *measurements of desorption rates* of

solutes in a liquid chromatographic system. The differences in desorption kinetics were found to correlate with the differences in stationary phase efficiency measured under standard reverse phase conditions, i.e. the shorter sorption-desorption equilibrium times, the higher chromatographic efficiency.

McGuffin at al.[40,42] directly measured retention of solutes in several locations of a high-pressure region of a glass capillary column. If solution retention is a significant function of pressure, a retention gradient, which arises along the LC column as a consequence of the pressure gradient, can be examined in this way. The LC conditions were carefully chosen so that the net effect of pressure was isolated from other factors influencing solute retention. Single eluents were used. The experimental data collected under different pressures for a homologous series of solutes were applied for the *evaluation of a thermodynamic model* describing retention in LC. The comparison of theoretical relations with the experimental results revealed that not only mobile phase, but also solute and stationary phase, must be considered compressible.

Ringo and Evans[43,54] demonstrated that pressure-controlled LC represents a valuable tool for sensitive evaluation of pressure-dependent perturbations in chiral complexation. The measurements of the capacity factors were used for *estimation of changes in partial molar volume due to solute complexation.*

Diagnostics of LC Column Packings

Pressure dependence of sorption equilibrium may be used for diagnostics of LC column packings. As mentioned, the appearance of eigenzones is a result of preferential adsorption and desorption perturbation due to pressure variation.[17] Therefore, the size (area or height) of the eigenzone is proportional not only to the extent of pressure change, but also to the amount (concentration) of the sorbent within the particular locus of the column.[20-22] This fact is pronouncedly manifested when the same pressure change is generated in all parts of the column, e.g., using an assembly presented in Figure 2, a homogeneously packed column would produce a rectangular zone with a tail due to broadening effects (Figure 8a). The non-homogeneity of column packing is reflected in the eigenzone shape deformation (Figure 8). At first glance, the shape of the zone indicates in which part of the column the concentration of the sorbent is higher, i.e., at the both ends of the column (Figure 8b), near the column entrance (Figure 8c), in its central part (Figure 8d), or if there is a gradient in packing density along the column (Figure 8e).

The interpretation of the eigenzone shapes and the quantitative evaluation of sorbent distribution within the column is based on the following experimental dependence.[20]

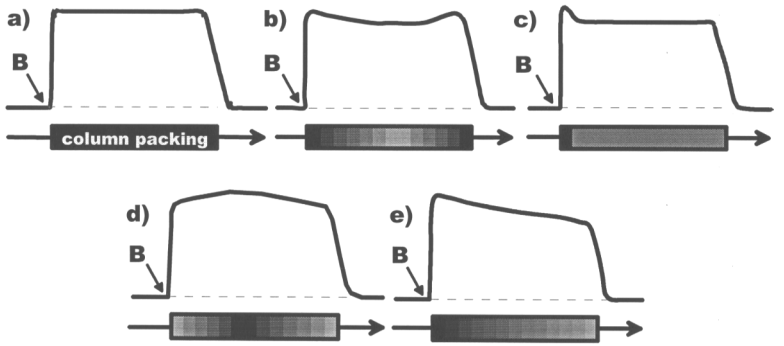

Figure 8. Dependence of zone shape on column packing density. Smaller packing density of sorbent in column = light gray area, larger packing density = dark gray area.

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

where h is the average height of a very small section of the eigenzone, m_s is mass of the corresponding sorbent, V_L is volume of the liquid corresponding to eigenzone section, and ΔP is pressure difference which generates the eigenzone. In other words, the eigenzone area is directly proportional to both pressure change and sorbent amount within the column.

The value of the constant K for the particular sorbent, eluent temperature, and detector sensitivity can be determined by means of an independent experiment with the exact known amounts of both sorbent and liquid within the column.[21] The quantitative interpretation of the eigenzone size and shape enables an evaluation of the distribution of the sorbent along the column[21] and distribution of interparticle volume.[22] The application of Carman-Kozeny equation describing the flow of liquid through a layer of particles, allows the calculation of the pressure profile along the HPLC column.[22] The agreement of the data calculated from the shape of eigenzones with the actual column parameters, depends on the eigenzone broadening and deformation. For precise column evaluation, adequate zone broadening corrections should be introduced.

For the application of the described column diagnostics, the choice of the eluent components is very important. It must be optimised for each column packing type and detector applied. Naturally, too large or too many pressure shocks may destroy the tested column packing. On the other hand, by analogy with x-ray examination, when applied under moderate conditions, this chromatographic diagnostic method enables "to see invisible," that is, it reflects longitudinal distribution of the sorbent within the column.

CONCLUSION

High pressure in the HPLC columns brings about some unexpected effects, which should be taken into account during interpretation of separation results. Pressure variations may explain the surprising changes of retention volumes or peak shapes, as well as the appearance of new peaks or base line perturbations. At the same time, pressure may also allow control of the retention of substances in some systems.

Pressure-controlled liquid chromatography may become a tool for evaluation of solute properties upon complexation, partitioning, and adsorption. Evaluation of small compositional changes within the effluent caused by pressure changes can be exploited in testing of stability and homogeneity of HPLC columns packings.

ACKNOWLEDGMENTS

This work was partially supported with the VEGA Grant Agency (Project No. 2-7037-20).

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Received August 26, 2000 Manuscript 5401
Accepted September 27, 2000