Pressure effects in high performance liquid chromatography

Dušan Berek and Tibor Macko

Polymer Institute CCR, Slovak Academy of Sciences,
842 36 Bratislava, Czechoslovakia

Abstract - The effect of pressure on physico-chemical
parameters of HPLC systems below critical conditions is
briefly summarized. The pressure dependence of preferential
sorption from the two component eluents on the column
packing is discussed in detail from the viewpoint of base
line perturbations accompanying sudden pressure changes
within the HPLC columns. It is shown that the above
phenomenon may be responsible for various artificial LC
results. On the other hand, it can be utilized e.g. in the
HPLC column diagnostics.

GENERAL EFFECTS OF PRESSURE IN HPLC

Most HPLC columns are packed with very small sorbent particles.
Consequently, high pressure has to be applied to reach the eluent flow rate
required. Elevated pressure not only puts high demands on the HPLC apparatus
but the extensive pressure changes (ΔP) during experiments inherently
influence several parameters of the HPLC systems. As a result, the retention
characteristics and the peak widths of analytes can be affected and base
lines can be perturbed.

Generally, pressure influences
a/ density and viscosity of the mobile phase,
b/ diffusion rate of the analyte molecules,
c/ size and shape of the packing particles,
d/ eluent flow rate and temperature of the system,
e/ interactions among eluent components, column packing and analytes.

Apart from the changes of pressure in the whole system one should also
consider the pressure drop along the column.

An excellent analysis of several parameters mentioned above was published
by Martin, Blu and Guiochon (ref. 1) already in the first stage of the high
pressure liquid chromatography development and, more recently, by some
other authors (refs. 2-7). The conclusions can be summarized as follows:

The volume of eluent decreases with increasing pressure i.e. the density of
the mobile phase rises by about $10^{-4}/0.1$ MPa (ref. 1). As pressure drops
along the column, the eluent expands and, consequently, the elution rate is
higher at the column end than at the column inlet. On the other hand, with
the exception of water below ca 20 °C, viscosity of eluent increases with
pressure by about $10^{-3}/0.1$ MPa and diffusion coefficient falls by about
$10^{-3}/0.1$ MPa (ref. 2). According to the conclusions of Martin et al (ref.1)
who also considered the changes in liquid compressibility with pressure,
the effect of increasing viscosity with pressure dominates and, for example,
with by n-heptane as eluent, it may change the retention time of the non
retained substances by almost 25 % at $\Delta P = 50$ MPa. The change of viscosity
with pressure affects, however, only the retention time but not the
retention volume while the change of eluent density with pressure affects
mainly the retention volume.

The rising pressure causes an increase in the column diameter and
a decrease in both the particle and the pore size of sorbent. Consequently,
the column porosity increases. The compressed particles of packing may
block the eluent flow and the partly collapsed pores have the decreased
surface available to the molecules of analyte. Both effects limit the
working pressure for some types of sorbents.

Friction of the mobile phase within bed of sorbent produces rather large amount of heat (refs. 3-7). For example, Poppe et al (ref. 5) observed the 4.5 °C rise of temperature at 20 MPa pressure drop within column packed with 5 μm particles. The actual increase of temperature within column depends not only on the eluent nature and the flow rate but also on the particle size, packing density as well as on the column material and the heat dissipation conditions.

The changes in pressure influence also various interactions between solutes and eluents, for example ionization equilibria of electrolytes (ref. 8), sizes of solvated macromolecules in solution (refs. 9-11) and also interactions between solute and sorbent, e.g. solute adsorption from diluted solution (refs. 12-15). As a result, retention volumes of the corresponding systems in ion chromatography and gel permeation chromatography are sometimes pressure dependent (refs. 16-21).

Few experimental data have so far been published concerning the pressure effects on the sorption equilibrium in the systems containing a two component liquid and a sorbent. Ogino and coworkers (ref. 22) observed changes in preferential sorption on a carbon sorbent from nine mixtures of different liquids when pressure was raised to 400 MPa under static conditions. In the series of chromatographic experiments we have found that the extent of preferential sorption from two-component liquids is often pressure dependent. In the following, we collect the most important experimental results so far obtained, generalize the effects observed, show their negative influence on the base line stability and propose their utilization in column diagnostics.

EFFECT OF PRESSURE ON SORPTION EQUILIBRIUM OF BINARY ELUENT IN HPLC COLUMN

In the systems containing a sorbent and a two component liquid, one liquid component is usually preferentially sorbed on the solid surface. In the HPLC column, an equilibrium is established between the interstitial mobile phase and the stagnant liquid layer within the column packing in contact with the mixed eluent. We have found that this equilibrium can be perturbed when pressure is changed (ref. 23) so that one of the eluent components is released from the sorbent and the other is extracted by the sorbent. As a result, the variation in composition of the column effluent accompanies the pressure change. This effect can be easily observed by a non specific detector (e.g. differential refractometer) as a base line perturbation, which we call "eigenzone" (ref. 24) in order to stress that this phenomenon is related exclusively to the property of the mixed mobile phase. The height or size and sign of eigenzones is given by the extent of extraction - - release process induced by the pressure change as well as by the applied detection principle and detector sensitivity. After the liquid with the changed composition has left the column, the base line is reestablished. When pressure is changed back to its initial value, the original sorption equilibrium is reached by a new extraction and releasing process. The size and shape of the eigenzone now produced is the same but the sign of the detector response is reversed so that we obtain the mirror image eigenzone.

Figure 1 shows the typical examples of eigenzones produced by various methods of pressure variations. The process is perfectly repeatable unless mixed eluent contains impurities, temperature of the system is constant and the column packing does not change its geometry in the course of pressure changes.

Practically no eigenzones are generated when mixed eluent is replaced by pure single liquid, when column is either packed with nonporous material or replaced with a capillary. The volume of eigenzones - with few exceptions only - equals to the volume of liquid within column (ref. 25).

There are several possible explanations for the observed phenomenon. Generally, the partition coefficient must depend on pressure since

$$\frac{\partial(\Delta G)}{\partial P} = \Delta V ,$$

(1)

where ΔG and ΔV are the variations of the free energy and the partial molar volume, resp., associated with the transfer processes between mobile

Scheme of the LC assembly   Sorbent: Silica gel   Silica gel modified
                                                           with C_{18}-alkyl groups

a/ Insertion of long capillary
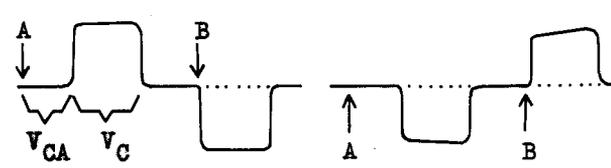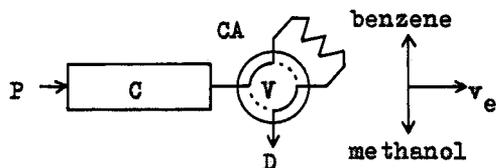
b/ Backflushing
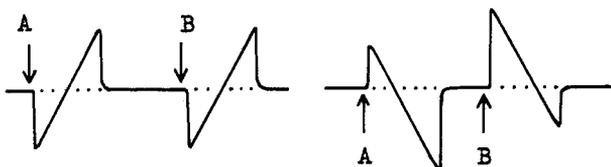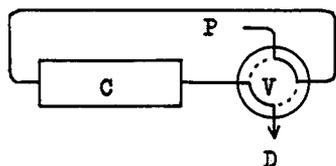
c/ Change of flow velocity
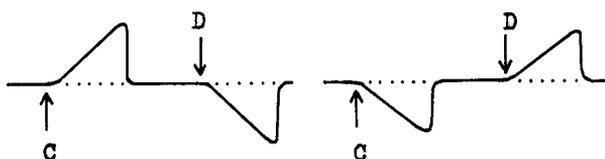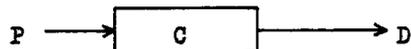
d/ Short interruption of flow - after column
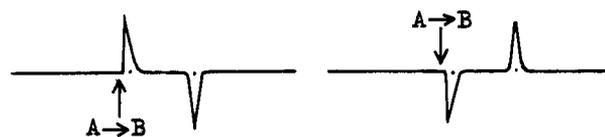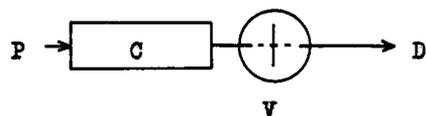
e/ Short interruption of flow - before column
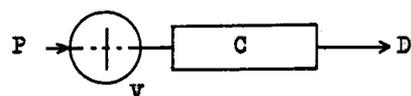
f/ Alternate recycling   Sorbent: Silica gel in both columns.
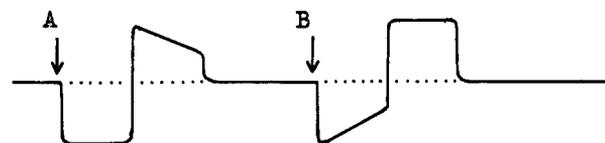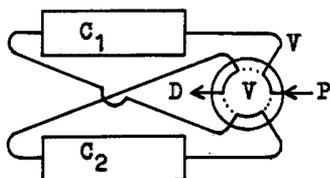
Fig. 1 Different possibilities of pressure changes generation
        and the shape of the corresponding eigenzones.

        Symbols: P=pump, C=column, V=valve in position a (———)
        and b (- - -), CA=long capillary, D=detector,
        A=valve V was switched from position b to a,
        B=valve V was switched from position a to b,
        C=sudden increase of flow velocity,
        D=sudden decrease of flow velocity,
        V_{CA}=volume of capillary CA, V_C=volume of liquid within
        column C, v_e=elution volume.
        The sign of the detector response corresponds to the
        experiments.

and stationary phase. Ogino et al (ref. 22) expressed the pressure
dependence of the surface excess, Γ_A, of the component A of the mixed
liquid A + B starting from the Schay theory of adsorption. Their analysis
showed that $\partial \Gamma_A / \partial P$ may assume negative, positive or zero values in
dependence on the value $\triangle_{AB}$ defined:

$$\triangle_{AB} = \partial \sigma_A^o / \partial P - \partial \sigma_B^o / \partial P , \qquad (2)$$

where σ_A^o and σ_B^o are the interfacial tensions between the sorbent
surface and pure components A and B, resp. If $\triangle_{AB}$ takes a large positive
value, $\partial \Gamma_A / \partial P$ is negative, if $\triangle_{AB}$ takes a large negative values,
$\partial \Gamma_A / \partial P$ is positive, and, the preferential sorption is not pressure
sensitive if $\triangle_{AB}$ is small. Still, the effects observed by us are larger
than expected from theory and other reasons have to be considered – such as
for example the selective compression of one component of mixed liquid into
the micropores of sorbent or the effects of the temperature changes during
pressure variations. Our experiments, however, seem to contradict the
hypothesis of the selective compression since in some systems larger
molecules are increasingly sorbed when pressure rises. However, to answer
this question definitively, experiments are needed with non associating
liquids. Also, the temperature variations alone cannot be responsible for
the observed effects since pronounced eigenzones were produced with large
packing particles and already at relatively small pressure variations of
few of MPa i.e. in the systems where the temperature effects are expected
negligible. An attractive hypothesis involves the influence of pressure on
the intermolecular interactions in mixed liquids. Actually, the large
sensitivity of both boiling point and composition of benzene/methanol
azeotrope (ref. 26) somewhat supports this idea since the above mixture
also produces extremely large pressure induced eigenzones. The definitive
answer to this question deserves further studies.

The size, shape and sign of eigenzones depend on the

        a/ chemical nature and physical structure of sorbent,
        b/ chemical nature and composition of eluent,
        c/ temperature,
        d/ extent of pressure change,
        e/ course of pressure change,
        f/ amount of both sorbent and liquid within column.

Let us discuss briefly the particular points:

a/ We have produced eigenzones with various kinds of sorbents differing in
their chemical composition, pore size and pore volume. They were bare and
chemically bonded HPLC silica gels (refs. 23-25, 27-32), macroporous carbon
sorbents and packings based on copolymers of polyhydroxyethylmethacrylate
crosslinked with glycol dimethacrylate (ref. 27). The height of eigenzones
generated with silica gels decreased with the decreasing specific surface
area of sorbent and nonporous glass microspheres did not produce any
eigenzones (ref. 27). The differences in polarity of sorbent surface usually
manifest themselves in the change of the sign of zone (see Fig. 1).

b/ Both the detectability of effluent composition changes and the pressure
effect itself depend on the chemical nature and composition of the eluent.
Sometimes, even the sign of the extraction – release process changes with
varying eluent composition. So far example, in a column packed with
3-glycerylpropyl bonded silica gel in the mixed eluent THF/water 80/20
vol/vol, the increased pressure caused desorption of water while THF was
desorbed in the same mixture with composition 30/70 vol/vol (ref. 30). It
is necessary to note again that pressure may induce either a further
increase or a decrease of the concentration of a component which is in
excess on the sorbent surface. In other words, the extent of preferential
sorption may either increase or decrease with rising pressure (refs. 29,
31).

c/ Temperature of the column as the parameter of sorption equilibrium
fairly influences the size of eigenzones (ref. 30).

d/ The height of eigenzones increases linearly with pressure change (refs.
23, 27). The excessive pressure strokes may, however, lead to the
destruction of the sorbent bed. This is accompanied with the changes of

eigenzone shape during repeated experiments. This fact may be utilized for a destructive testing of column stability (ref. 28).

e/ The characteristic eigenzone shapes obtained by different methods of pressure change generation can be seen in Fig. 1. Generally, the shapes of eigenzones reflect the difference between pressure profiles within the column before and after switching the valve (cf. Fig. 1a,b,f) and/or changing the eluent flow velocity (Fig. 1c,d,e). From the point of view of interpretation simplicity, the most suitable way for eigenzone generation presents the insertion/deletion of the capillary with high hydrodynamic resistance into the eluent flow path (Fig. 1a). In this case the induced pressure change is the same along the column. More detailed discussion of the interpretation of eigenzone shape can be found elsewhere (refs. 23, 27, 28).

Two subsequent pressure changes of the opposite sign produce two eigenzones that may interfere and the resulting base line perturbation has the shape of two or more distinct system peaks. For example, the short blocking of the eluent flow (Fig. 1d,e) produces two system peaks with different sign of detector response. The former system peak appears immediately after the flow has been reestablished, the retention volume of the latter system peak corresponding to the column dead volume (ref. 32).

f/ The height of eigenzone is approximately directly proportional to the amount of sorbent and indirectly proportional to the amount of liquid within column (ref. 27). The columns containing the same sorbent packed in different ways give eigenzones of different shape. We have found that valuable conclusions concerning the packing density variations within column can be drawn from the eigenzone shapes using the assembly according to Fig. 1a (ref. 27).

The effluent composition changes caused by pressure variations are usually fairly small and represent only few microliters of one mobile phase component in one milliliter of eluent when ΔP equals 10 MPa. This corresponds to a few percent change in the extent of preferential sorption (ref. 27). Still this effect may produce eigenzones 200-300 mm high under appropriate conditions. On the other hand, the changes of composition of the stagnant liquid on the sorbent surface so far generated are too small to produce pronounced changes of retention volumes in common liquid chromatographic systems. Anyway, the described phenomenon can be responsible for some unexpected results in both retentive and exclusion liquid chromatography with mixed mobile phases (ref. 25). The unwanted pressure changes within the column can be produced due to the partial blocking of the column outlet by the sample components or by the microparticles of column packing, due to sample injection (ref. 32) and column switching or recycling (ref. 19). The pressure dependence of preferential sorption should be therefore considered when erroneous or strange HPLC results are to be explained.

REFERENCES

1. M. Martin, G. Blu and G. Guiochon, *J. Chromatogr. Sci.*, 11, 641-654 (1973).
2. M. Martin and G. Guiochon, *Anal. Chem.*, 55, 2302-2309 (1983).
3. I. Halasz, R. Endele and J. Asshauer, *J. Chromatogr.*, 112, 37-60 (1975).
4. H. Poppe and J.C. Kraak, *J. Chromatogr.*, 282, 399-412 (1983).
5. H. Poppe, J.C. Kraak, J.F.K. Huber and J.H.M. van den Berg, *Chromatographia*, 14, 515-523 (1981).
6. M. Martin, C. Eon and G. Guiochon, *J. Chromatogr.*, 110, 213-232 (1975).
7. E. Katz, K. Ogan and R.P.W. Scott, *J. Chromatogr.*, 260, 277-295 (1983).
8. S.D. Hamann, *J. Solution Chem.*, 11, 63-67 (1982).
9. D. Gaeckle and D. Patterson, *Macromolecules*, 5, 136-141 (1972).
10. G.H. Hammel, G.V. Schulz and M.D. Lechner, *Eur. Polym. J.*, 15, 209-222 (1979).
11. G.V. Schulz and M. Lechner, *J. Polym. Sci. A2*, 8, 1855-1860 (1970).
12. D.B. Marshall, J.W. Burns and D.E. Connolly, *J. Chromatogr.*, 360, 13-24 (1986).
13. V.L. Bogatyryov, *J. Chromatogr.*, 364, 125-133 (1986).
14. V.A. Avramenko, V.Yu. Glushenko, V.L. Zhubacevskaya, V.M. Teplyuk and B.P. Shipacev, *Zh. Fiz. Chim.*, 60, 931-935 (1986).

15. M.E. Zawadski, A.W. Adamson, M. Fetterolf and H.W. Offen, Langmuir, 2, 541-543 (1986).
16. B.A. Bidlingmeyer, R.P. Hooker, C.H. Lochmüller and L.B. Rogers, Separ. Sci., 4, 439-446 (1969).
17. B.A. Bidlingmeyer and L.B. Rogers, Sep. Sci., 7, 131-158 (1972).
18. G. Prukop and L.B. Rogers, Sep. Sci., 13, 59-78 (1978).
19. Sj. van der Wal, Chromatographia, 22, 81-87 (1986).
20. N. Tanaka, T. Yoshimura and M. Araki, J. Chromatogr., 406, 247-256 (1987).
21. P.P. Nefedov and T.P. Zhmakina, Vysokomol. soed., A. 23, 276-282 (1981).
22. S. Ozawa, K. Kawahara, M. Yamabe, H. Unno and Y. Ogino, J. Chem. Soc., Faraday Trans., 80, 1059-1068 (1984).
23. D. Berek, M. Chalányová and T. Macko, J. Chromatogr., 286, 185-192 (1984).
24. M. Chalányová, T. Macko, J. Kandráč and D. Berek, Chromatographia, 18, 668-672 (1984).
25. T. Macko, M. Chalányová and D. Berek, J. Liq. Chromatogr., 9, 1123-1140 (1986).
26. L.H. Horsley, Anal. Chem., 19, 508-600 (1947).
27. T. Macko, A. Potmáková and D. Berek, Submitted to Chem. Papers.
28. T. Macko and D. Berek, J. Chromatogr. Sci., 25, 17-21 (1987).
29. T. Macko, D. Berek and M. Chalányová, Physical optics of dynamic phenomena and processes in macromolecular systems, ed. B. Sedláček, W. de Gruyter, Berlin, 521-524 (1985).
30. T. Macko, Ph. D. Thesis, Bratislava, 108-109 (1987).
31. T. Macko, M. Chalányová and D. Berek, In: Modified polymers, Polymer Institute, Bratislava, 667-670 (1984).
32. T. Macko, L. Šoltés and D. Berek, in preparation.