

CHROMSYMP. 210

DEPENDENCE OF PREFERENTIAL SOLVATION OF LIQUID CHROMATOGRAPHIC SORBENTS IN MIXED ELUENTS ON PRESSURE

D. BEREK*

Polymer Institute, Centre of Chemical Research, Slovak Academy of Sciences, 84236 Bratislava (Czechoslovakia)

M. CHALÁNYOVÁ

Chemical Institute, Comenius University, 84215 Bratislava (Czechoslovakia)

and

T. MACKO

Polymer Institute, Centre of Chemical Research, Slovak Academy of Sciences, 84236 Bratislava (Czechoslovakia)

SUMMARY

Pronounced baseline perturbations were observed in alternating recycling liquid chromatographic experiments when mixed eluents and a refractometric detector were applied. These perturbations were caused by local variations in the effluent composition most probably due to changes in the extent of preferential solvation of the column packings with pressure. Experimental support of this hypothesis is presented and the possible practical aspects of this phenomenon are briefly discussed.

INTRODUCTION

It is well known that the composition of a liquid chromatographic (LC) mixed eluent usually differs considerably in the vicinity of the column packing material and in the mobile phase outside the sorbent particles¹⁻⁴. This phenomenon is a result of the differences in affinity of the eluent components toward the column packing and is alternatively called selective or preferential solvation, sorption, extraction, etc. Similarly, differences in the interactions of the eluent components with the solute molecules cause preferential solvation of the sample injected into a LC system.

Generally, the extent of preferential solvation in any multicomponent system is determined by the chemical nature of all components as well as by the temperature, pressure and, in a dynamic system, possibly also by kinetic effects.

Evidently, the preferential solvation of both the column packing and the sample molecules substantially influences the corresponding retention characteristics/and in some cases also the shape of the chromatographic peaks⁴. Other consequences of preferential solvation of the column packing are the selective displacement effects that bring about local changes in the effluent composition and the appearance of so-called vacancy peaks on chromatograms⁴⁻¹¹. However, similar local changes in the

effluent composition in comparison with the initial mobile phase composition are caused also by evaporation of one eluent component from the injected solution¹² and by preferential solvation of the sample molecules^{11,13,14}.

The selective displacement effects can be distinguished from both preferential evaporation and preferential solvation by means of solute recycling^{13,14}: the second passage of the sample through the column is accompanied only by selective displacement.

In the course of our alternate recycling experiments devoted to the study of selective displacement we have observed pronounced baseline perturbances, resulting in "ghost zones" that appeared on the chromatograms when the recycling valve was switched —no matter whether the sample was or was not injected into the column system. This phenomenon can be explained by considering the influence of the mobile phase pressure changes on the extent of preferential solvation of the column packing.

EXPERIMENTAL

Apparatus

A diagram of the chromatographic assembly used for the alternate recycling¹⁵ is shown in Fig. 1. The single piston reciprocating membrane pump (Model VCM 150; Workshop of the Czechoslovak Academy of Sciences, Prague, Czechoslovakia) was equipped with a pulse damper as described in ref. 16. Both the injection and the recycling valves were low-dead-volume six-port three-way valves designed at the Institute for Chemical Processes Fundamentals of the Czechoslovak Academy of Sciences, Prague. In some experiments, a commercial six-port injection valve (Valco Universal HPLC Sample Valve; Valco, Houston, TX, U.S.A.) was applied for the sample recycling.

The detector was a deflection type differential refractometer (RI detector) (Model 7050/25; Knauer, Berlin) and its response was recorded by means of a potentiometric recorder (Model 7127; Hewlett-Packard, Palo Alto, CA, U.S.A.).

The elution volumes were measured by means of a drop counter (Model 1002; Laboratory Instruments Works, Prague, Czechoslovakia). The particular hydraulic parts of the LC assembly were connected with the capillaries of 0.25 mm I.D.

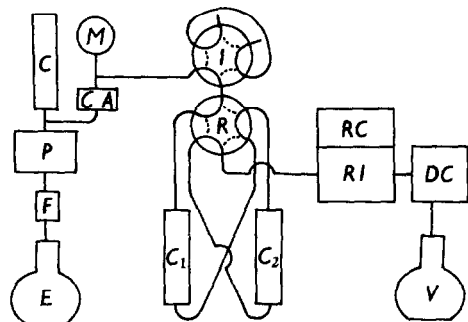


Fig. 1. Diagram of the LC assembly: E = eluent container; F = filter; P = pumping system; C (column) and CA (capillary) = pulse damper; M = pressure gauge; I = injection valve; R = recycling valve in position a (---) and in position b (—); C₁ and C₂ = columns; RI = detector; DC = drop counter; RC = recorder; V = vessel for effluent collection.



Fig. 2. Example of ghost zones. Columns: Separon Six CN. Eluent: THF-water (80:20, v/v). Elution rate: 0.3 ml/min. Pressure: 6 MPa. Sensitivity of detector: $16 \times$. Counts: 15 drops of effluent, *i.e.*, about 0.4 ml. A, Recycling valve R switched from position b to a. B, valve R switched from position a to b; C₁, zone from column C₁; C₂, zone from column C₂.

Fig. 3. Example of ghost zones. Columns: Separon Six. Experimental conditions as in Fig. 2.

Compact Glass Columns for HPLC, 150×3.2 mm I.D. (Laboratory Instruments Works), were packed either with unmodified $5\text{-}\mu\text{m}$ spherical silica gel Separon Six or with chemically bonded silica-based phases Separon Six CN, Six NH₂ and Six C₁₈ (Laboratory Instruments Works) or LiChrosorb Diol (E. Merck, Darmstadt, F.R.G.). In each set of recycling experiments, columns packed with the same sorbent type were applied. The columns were thermostatted in a water-bath at $25 \pm 0.1^\circ\text{C}$; however, the temperature of the other parts of the liquid chromatographic assembly was not controlled.

The concentrations of the supernatant in the static experiments were measured by a differential refractometer Brice Phoenix Model BP-2000-V (Virtis, Cardiner, NY, U.S.A.) at a wavelength of 436 nm.

Reagents

Redistilled water and tetrahydrofuran (THF) (VEB Laborchemie, Apolda, G.D.R.) purified as described¹⁷ and stabilized by 0.01% 2,6-di-*tert.*-butyl-4-methylphenol were mixed in the ratios 20:80 and 35:65 (v/v), respectively.

RESULTS AND DISCUSSION

As mentioned, "ghost zones" were generated on chromatograms by switching the recycling valve (R in Fig. 1) either from the position a to the position b or *vice versa*. Typical examples of such ghost zones are shown in Figs. 2-6. The shape of the ghost zones depended on both the sorbent nature and the eluent composition as well as on the elution rate. In all cases, the ghost zones appeared almost immediately after switching the recycling valve and they were split into two parts with responses of different signs on the differential refractometer (*cf.*, Figs. 2-6). The width of each part of the ghost zone corresponded to the elution volume of the peaks generated by the injection of a small excess of either THF or water into the column in the given mixed eluent in the course of independent non-recycling measurements.

On the other hand, both the size and the shape of the ghost zones was independent of the position of the injection valve. The injection of pure eluent or any sample did not cause any pronounced perturbations of the baseline unless the recycling valve was operated simultaneously.

Series of preliminary experiments confirmed that the chromatographic

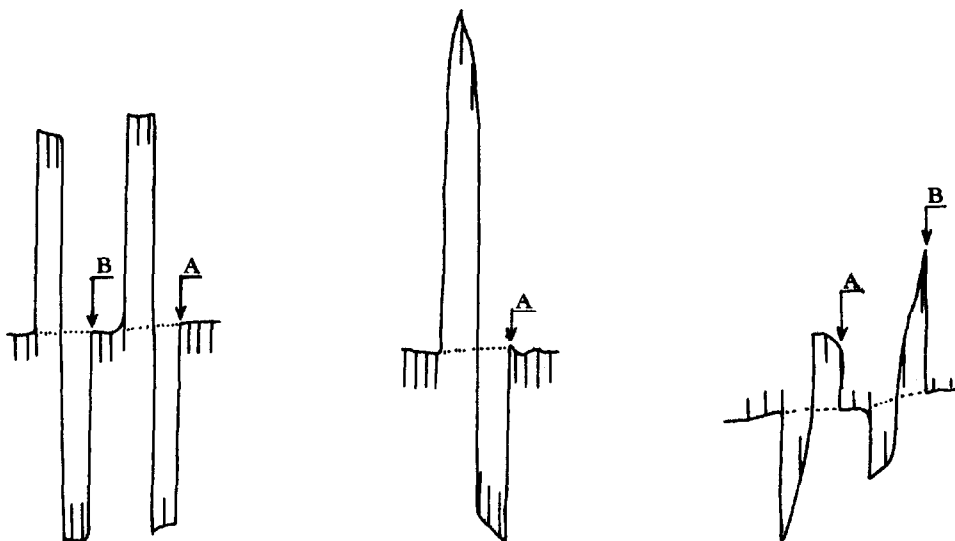


Fig. 4. Example of ghost zones. Columns: LiChrosorb Diol. Experimental conditions as in Fig. 2.

Fig. 5. Example of ghost zones. Columns: Separon Six NH_2 . Experimental conditions as in Fig. 2.

Fig. 6. Example of ghost zones. Columns: Separon Six C_{18} . Eluent: THF-water (65:35, v/v). Other experimental conditions as in Fig. 2.

assembly was "tight", *i.e.*, the ghost zones were not produced by leaking or by evaporation of the eluent eventually by sucking air or moisture into the LC system. Almost identical results were obtained with both types of the recycling valves so that the ghost zones were not caused by bleeding of the seals in valves.

The pressure dependence of the effluent refractive index could not be responsible for the appearance of the ghost zones since the pressure on the detector inlet was not affected by the position of the recycling valve (*cf.*, Fig. 1). Also the flow direction remained constant when the recycling valve was switched.

Finally, we conclude that the ghost zones were produced by local changes in the effluent composition caused by the variations in the extent of preferential solvation of the column packing with pressure due to switching the recycling valve (*cf.*, Fig. 7).

For comparison, the extent of preferential solvation of the particular sorbents was measured by static experiments: 30–150 mg of the carefully dried sorbent were weighed into a glass vial, 3 ml of the mixed solvent were added and after 4 h of occasional gentle mixing, the composition of the supernatant was determined refractometrically. The extent of preferential solvation was expressed by λ parameters: λ reflects the excess of one solvent component in the domain of sorbent in comparison with the composition of the starting solvent and is expressed in ml per gram of dry sorbent. The results are given in Table I where positive λ values mean that preferential solvation of the sorbent occurs by THF and negative λ values by water.

Evidently, the λ values obtained under static conditions should be considered as a first approximation of the real situation in the column since the preferential solvation equilibrium may be different under static and dynamic conditions and is

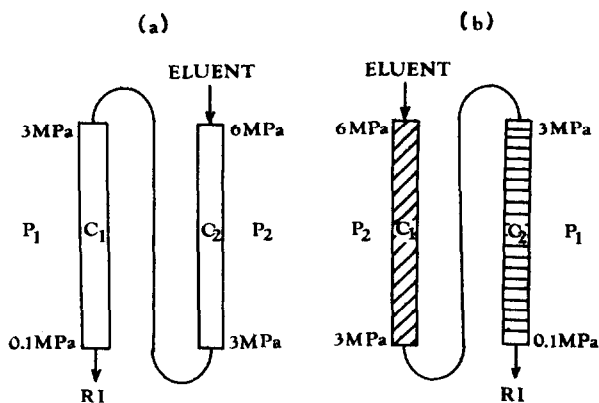


Fig. 7. Representation of the pressure variations in the course of recycling experiments. The corresponding positions of the recycling valve are denoted in (a) and (b).

expected to be dependent on the solvent pressure. Moreover, the sorbents used for the static experiments were fresh and in contact with the eluent for the first time, while the surface of the sorbents packed in the columns could be saturated with molecules of the substances used for stabilization of THF as well as by various impurities present in the percolating eluent. Finally, the sorbents used for the static and dynamic experiments were from different batches and their surface properties could differ substantially¹⁸.

The typical pressure relationships in the column system are represented in Fig. 7a. For simplicity, let us neglect the pressure drop within the particular column, the recycling valve and the connecting tubings. Let the mean pressure in the columns C_1 and C_2 be P_1 and P_2 , respectively. We further assume that the column packing is preferentially solvated by THF at any eluent pressure, however, the extent of such solvation is pressure dependent. Let the coefficients of preferential solvation at the pressures P_1 and P_2 be λ_1 and λ_2 , respectively, λ_1 being larger than λ_2 . When switching the recycling valve, the pressures in the columns are suddenly changed (Fig. 7b).

TABLE I

HEIGHTS OF GHOST ZONES AND λ PARAMETER OF PREFERENTIAL SOLVATION OF SORBENTS

Eluent: THF-water (80:20, v/v), except where indicated otherwise.

Sorbent	Heights of zones (cm)	λ (ml/g)
Separon Six C_{18}	≈ 0	+0.094
Separon Six	0.6-1.0	+0.023
Separon Six CN	0.5-0.7	-0.081
Separon Six NH_2	3.7-6.5	-0.188
LiChrosorb Diol	3.8-4.3	-
Separon Six C_{18}	1.4-2.7*	+0.100*

* Eluent: THF-water (65:35, v/v).

In comparison with the initial situation (Fig. 7a), the pressure in column C_1 is now P_2 and this column must release some THF and extract water molecules from the eluent in order to reach the preferential solvation equilibrium characterized by λ_2 . Similarly, the column C_2 which is now under pressure P_1 must extract some THF from the eluent and release some water so that the new equilibrium preferential solvation corresponding to λ_1 is established. The zone of the mobile phase containing the excess of THF released from column C_1 as well as the zone containing an excess of water due to the THF extraction by column C_2 are now successively eluted from the column system into the detector and produce the baseline perturbation.

It seems, however, that both columns C_1 and C_2 are not completely identical so that the amount of THF released by column C_1 is not equal to the amount of THF extracted by column C_2 from the eluent. Moreover, the zones of eluent with perturbed compositions will interact with the column packing during their elution so that complicated composition patterns are formed (Figs. 2-6). Consequently, both parts of a ghost zone are not of equal size and show extensive deformations in shape.

The non-identity of the columns may be brought about by the different densities of the packings and by the resulting differences in both the pressure drop within the columns and the active surface of the sorbents available for preferential solvation.

When switching the valve R back to the starting position (a), all processes are reversed and the initial solvation equilibrium is re-established. The resulting ghost zone is now an exact mirror image of that obtained when the valve R was switched into position b. This means that the amount of THF released by column C_1 in the step $a \rightarrow b$ equals the amount of THF extracted by the same column in the step $b \rightarrow a$.

The heights of the ghost zones can be roughly correlated with the λ values given in Table I: The higher the value of λ the higher are the ghost zones. Separon Six C_{18} with a small positive λ value produced only very small ghost zones. On the other hand, pronounced ghost zones were produced by the same sorbent with the eluent THF-water (65:35, v/v) where the preferential solvation under static conditions slightly increased (*cf.*, Table I).

Our further experiments supported the hypothesis of a dependence of the sorbent preferential solvation on the eluent pressure:

(1) If the column C_2 was substituted by a short capillary with a small pressure drop, switching of the valve R did not result in any ghost zones. Evidently, in this case, switching the valve R caused only a negligible pressure variation within column C_1 .

(2) The height of the ghost zones decreased with decreasing linear velocity of the mobile phase, *i.e.*, with decreasing pressure variations.

CONCLUSIONS

The described experimental results lead to the hypothesis that the preferential solvation of LC sorbents in mixed eluents depends to a detectable extent on pressure. This may be somewhat surprising since the internal pressure of common liquids represents hundreds of MPa while the pressure variations produced in the LC assembly by switching the recycling valve are about two orders of magnitude lower. On the other hand, so far, very little is known about the pressure relationships between

liquids and the surfaces of bodies immersed in them.

Pressure changes due to switching the recycling valve may act also indirectly, causing a change in other parameters, *e.g.*, temperature. A more detailed explanation of these results would need further studies which are now in progress in this laboratory.

Finally, some practical aspects of the observed phenomenon are as follows:

(i) The course of the adsorption isotherms measured under static conditions at atmospheric pressure should differ systematically from that determined in the chromatographic column at elevated pressure.

(ii) The extent of preferential solvation of a sorbent and, consequently, also its retention characteristics will—under otherwise identical experimental conditions—depend on the mobile phase flow-rate, on the geometry of both the sorbent particles themselves and the column packing as a whole, as well as on other parameters that determine the pressure drop within the column, *e.g.*, the flow resistance of the column outlet end piece. These effects will be very small and hardly detectable in the common LC systems and under usual eluent pressures, but may be observable when working under high pressures.

(iii) Any change of pressure in an LC system containing a mixed eluent will produce a zone with changed mobile phase composition. For example, the change in effluent composition within the ghost zone for LiChrosorb Diol columns upon varying the pressure by about 6 MPa reached about 0.4% (v/v).

Within this zone, the preferential solvation of the sorbent will be further modified and, as a consequence, some sort of microgradients with rather complicated patterns will be formed. All these processes are usually not detectable by means of a specific detector, however, they may complicate the interpretation of results when a non-specific detector such as a differential refractometer is applied. Moreover, the sample components that penetrate the column together with such zones or within accompanying microgradients may exhibit measurably different retention characteristics.

ACKNOWLEDGEMENTS

We are indebted to Dr. T. Bleha and Dr. I. Novák for their interest in this work and for stimulating discussions, and to Dr. J. Čoupek and Dr. S. Vozka for the Compact Glass Columns.

REFERENCES

- 1 L. R. Snyder, *Principles of Adsorption Chromatography*, Marcel Dekker, New York, 1968.
- 2 P. J. Schoenmakers, H. A. H. Billiet and L. de Galan, *J. Chromatogr.*, 282 (1983) 107.
- 3 T. Bleha and D. Berek, *Chromatographia*, 14 (1981) 163.
- 4 D. Berek, in preparation.
- 5 D. J. Solms, T. W. Smuts and V. Pretorius, *J. Chromatogr. Sci.*, 9 (1971) 600.
- 6 K. Šlais and M. Krejčí, *J. Chromatogr.*, 91 (1974) 161.
- 7 R. M. McCormick and B. L. Karger, *J. Chromatogr.*, 199 (1980) 259.
- 8 R. M. McCormick and B. L. Karger, *Anal. Chem.*, 52 (1980) 2249.
- 9 R. P. W. Scott and P. Kucera, *J. Chromatogr.*, 142 (1977) 213.
- 10 R. P. W. Scott and P. Kucera, *J. Chromatogr.*, 149 (1978) 93.
- 11 W. R. Melander, J. F. Erard and Cs. Horváth, *J. Chromatogr.*, 282 (1983) 211.

- 12 A. Mat'ášeje, T. Bleha and D. Berek, in preparation.
- 13 D. Berek, T. Bleha and Z. Pevná, *J. Chromatogr. Sci.*, 14 (1976) 560.
- 14 D. Berek, T. Bleha and Z. Pevná, *J. Polym. Sci., Polym. Lett. Ed.*, 12 (1976) 323.
- 15 J. A. Biesenberger, M. Tan and I. Duvdevani, *J. Polym. Sci., Part B*, 9 (1971) 353.
- 16 D. Berek, *J. Chromatogr.*, 132 (1977) 128.
- 17 T. Sychaj, D. Lath and D. Berek, *Polymer*, 20 (1979) 437.
- 18 I. Halász, *Anal. Chem.*, 52 (1980) 1393A.