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Journal of Chromatography A, 670 (1994) 173–179

JOURNAL OF CHROMATOGRAPHY A

Experimental study of band broadening and solute interferences in preparative supercritical fluid chromatography

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(First received July 21st, 1993; revised manuscript received February 3rd, 1994)

Abstract

The effect of the amount injected on the elution profile of a single solute was used to investigate the shape of the distribution isotherm in overloaded supercritical fluid chromatography. Subsequently, the role of competition between solutes when the column is overloaded with a binary mixture was studied. The band broadening pattern is explained by the difference between the solubilities of the solutes in the supercritical fluid mobile phase.

1. Introduction

The main interest in preparative supercritical fluid chromatography (PSFC) is that the separation of the collected substance from the mobile phase is theoretically easy as a decrease in solvating power of the supercritical fluid mobile phase can be obtained simply by pressure reduction. Although the application of PSFC on a small scale was suggested as early as 1962 [1] and the feasibility of large-scale PSFC was demonstrated in 1982 [2], the technique is not yet widely accepted and used. Its development has been limited by some technological difficulties, more particularly the design of efficient devices for sample injection and solute collection (for a complete bibliography, see the reviews by Berger and Perrut [3] and Kirschner and Taylor [4]). Today, the technological problems seem to have been solved and the establishment of PSFC as an optimized purification tool requires a better understanding of the band broadening mechanism under column overload conditions and in the case of multi-component samples. Such fundamental studies were the origin of the recent and spectacular developments in preparative liquid chromatography (PLC).

This paper presents some results of an experimental study of the competitive adsorption phenomena when the column is overloaded with a binary mixture. The objective was to exhibit the solute elution profiles when the column is heavily overloaded and the eluted bands considerably overlap. For this purpose, the chosen test solutes have large spectral differences so that a judicious adjustment of the detection wavelength in each experiment allowed only one of the two injected solutes to be detected. It was also necessary to eliminate any source of extraneous band broadening and, in order to avoid peak distortion resulting from partial flooding of the column by the liquid solvent of the sample [5], we used the sample solvent evaporation injection technique investigated previously [6,7]:

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0021-9673/94/$07.00
SSDI 0021-9673(94)00118-S

the sample solution is first loaded in a precolumn, then the sample solvent is removed by using a flow of warm helium in a similar manner to gas chromatography and finally the supercritical fluid mobile phase is introduced into the precolumn to sweep the sample on to the separation column.

2. Experimental

2.1. Supercritical fluid chromatograph

Fig. 1 shows the system used for the experiments. The liquid CO_2 pump (4) was a Shimadzu (Kyoto, Japan) LC-6A equipped with a cooling jacket kept at 5°C by means of a Julabo F30-HC circulation bath (Julabo Labortechnik, Seelback, Germany). The modifier pump (5) was a Waters Model 6000A (Waters–Millipore, Milford, MA, USA) modified for delivering micro flow-rates. The mixing between liquid CO_2 and the organic modifier is achieved by placing downstream of the tee-piece a 150 × 4.6 mm I.D. column (6) packed with 100-μm glass beads. The column inlet pressure is monitored with a Chromatem 944 pressure gauge (7) (Touzart et Matignon, Vitry sur Seine, France). The injection device is composed of two Rheodyne (Cotati, CA, USA) sampling valves, Model 7010 (8) and Model 7125 (9), and a 50 × 4.6 mm I.D. loading precolumn (10). The injection device, the 250 × 4 mm I.D. separation column (11) and the back-pressure regulator (14) (Model 26-1724-24; Tescom, Elk River, MN, USA) are immersed in a thermostated water-bath (15) (Polystat 86602; Bioblock Scientific, Illkirch, France). The Shimadzu SPD-6A UV spectrophotometer (12) equipped with a high-pressure cell of volume 3 μl and optical path 6 mm is connected to an integrator (C-R5A; Shimadzu). For collecting a small amount of component, the Tescom back-pressure regulator is by-passed and the solute is trapped in a collection solvent (17) by using a 34 mm × 50 μm I.D. fused-silica restrictor (16). The collection flask (18) is topped by a condenser (19) in order to avoid any loss of solute resulting from formation of an

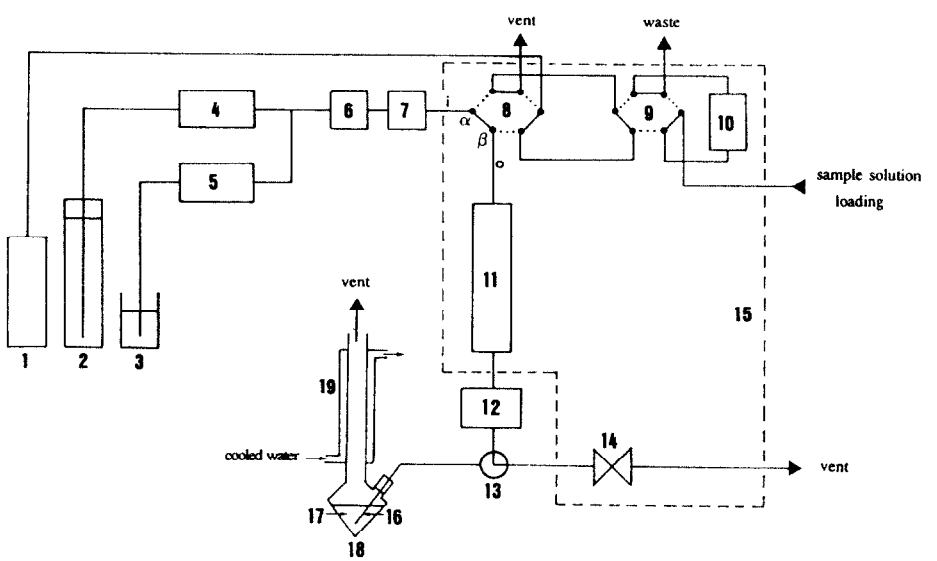

Fig. 1. Schematic diagram of the supercritical fluid chromatograph. 1 = Helium cylinder; 2 = liquid CO_2 cylinder; 3 = modifier solvent tank; 4 = CO_2 pump; 5 = modifier pump; 6 = mixer; 7 = column inlet pressure gauge; 8 and 9 = six-way valves (—— load position; – – – inject position); 10 = precolumn; 11 = column; 12 = UV detector; 13 = three-way valve; 14 = back-pressure regulator; 15 = thermostated bath; 16 = linear fused-silica restrictor; 17 = trapping solvent; 18 = cone-shaped flask; 19 = condenser; i and o = tubings connected to positions α and β of sampling valve 8.

aerosol by decompression of the supercritical fluid inside the trapping solvent.

2.2. Procedures

For evaporating the sample solvent prior to injection, first both valves 8 and 9 are in the load position (see Fig. 1) and the sample solution is introduced directly into the precolumn by means of a syringe (Model 702 or 725; Hamilton, Bonaduz, Switzerland) while the supercritical fluid mobile phase is flowing through the separation column. Second, valve 9 is rotated to the inject position and a regulated flow of helium is delivered through the precolumn to remove the sample solvent. Third, valve 8 is switched to the inject position, the supercritical fluid mobile phase flows through the precolumn in the reverse direction and the solventless sample is swept on to the column with minimum band broadening.

For determination of the purity of the collected solute, the contents of the collection flask were quantitatively analysed with a Shimadzu LC-6A liquid chromatograph using the external standardization method.

2.3. Chemicals and experimental conditions

Liquid CO_2 (99.99% pure) and HP-grade helium were supplied by Carboxyque Française (Venissieux, France). All solvents (methanol, heptane and ethyl acetate) were of HPLC grade (Carlo Erba, Milan, Italy). Acetic acid, orthophosphoric acid and all solutes (benzyl alcohol, methylparaben and vanillin) were of RP grade (Prolabo, Paris, France). The loading precolumn and the separation column were laboratory-packed with Kromasil C_{18}, 5 μm (Eka Nobel, Surte, Sweden).

For supercritical fluid chromatographic experiments, the elution conditions were as follows: the temperatures of the helium flow, precolumn, column and back-pressure regulator were 60°C; the organic modifier was a 0.01 mol l^{-1} methanolic solution of orthophosphoric acid at a flow-rate of 75 μl min^{-1}; the liquid CO_2 flow-rate was 1.7 ml min^{-1}, which corresponded to a mobile phase composition of CO_2–methanolic modifier (95.8:4.2); the opening of the back-pressure regulator and the length of the linear restrictor were adjusted so that the column inlet pressure was maintained at 112 bar; the detection wavelength was chosen for each experiment; and samples were dissolved in methanol.

For liquid chromatographic analysis of the collected fractions, the elution conditions were as follows: the stationary phase was LiChroprep Si 60, 5–20 μm (Merck, Darmstadt, Germany), laboratory-packed in a 150 × 4.6 mm I.D. column; the mobile phase was heptane–ethyl acetate–acetic acid (85:15:1), which was also used as a trapping solvent in supercritical fluid experiments; the elution flow-rate was 1 ml min^{-1}; and the detection wavelength was 254 nm.

3. Results and discussion

3.1. Elution profile of a single solute

Because there is no interference between the migrating solutes, the behaviour of a single solute yields little information on the separation in preparative chromatography. However, in order to gain an insight into the mechanisms that govern the distribution of the solute between the mobile and stationary phases, it is instructive to investigate the variation of the concentration profile of a single solute under overload conditions.

Fig. 2 shows how an increase in the amount injected influences both the dispersion of the peak front and the sharpening of the peak rear. First, it was checked that these fronting peaks were not the result of the combined effects of partial saturation of the stationary phase during the introduction of the sample solution into the precolumn and reversal of the flow through the precolumn during the transfer of the solventless sample from the precolumn to the column. If so, tailing peaks should be obtained with no reversal of the flow direction. Consequently, the same experiments were carried out after connection of tubings (i) and (o) to positions β and α, respectively, of the sampling valve (8) (Fig. 1); with

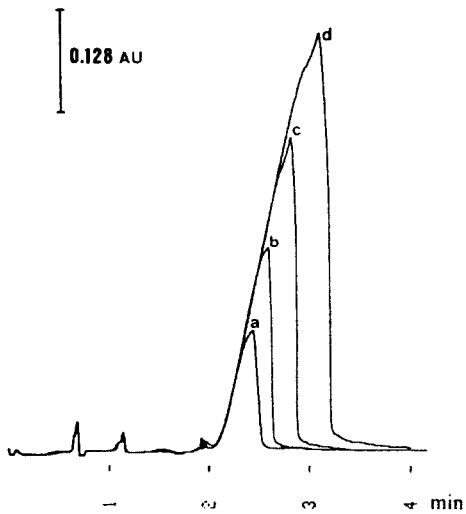

Fig. 2. Effect of amount injected on the peak shape of a single solute. Solute, vanillin; volume injected, 20 μl; amount injected, (a) 1.6, (b) 3.2, (c) 6.4 and (d) 12.8 μmol; detection wavelength, 338 nm.

this configuration, the flow through the precolumn was no longer reversed and the sample was forced to migrate along the whole length of the precolumn before being transferred to the column. Fig. 3 shows that the peaks obtained under these conditions are slightly delayed and distorted, but with a band broadening pattern that is identical with that observed during the previous experiments: as the injected amount is increased, the elution time of the peak front

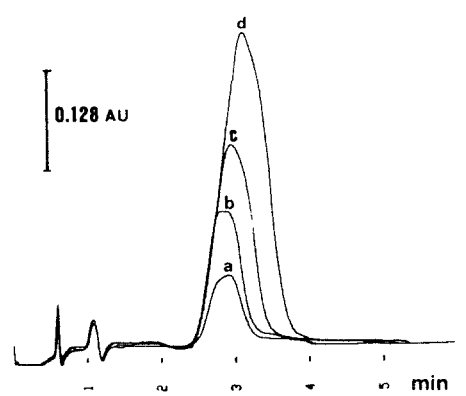

Fig. 3. Same as Fig. 2, except tubings (i) and (o) were connected to positions β and α, respectively, of the sampling valve (8) (see text).

remains constant while the peak rear elutes progressively later. This result confirms that the overload effects observed in Fig. 2 do not depend on some band broadening phenomenon associated with the injection process on the precolumn.

However, two other types of phenomena can explain the change in peak profile with increasing amount of solute. The fronting peak can result from either a concave distribution isotherm due to limited solubility of the solute in the supercritical fluid mobile phase or competition between the solute and the organic modifier of the supercritical fluid mobile phase for the stationary phase surface. Indeed, fronting peaks have been observed in PLC with binary eluents containing a strongly sorbed additive [8,9]. In the latter instance, the peak front should move to earlier retention times when the amount of solute is increased [8], which is not observed in Figs. 2 and 3. Hence the solute follows a true concave distribution isotherm and the overload effects observed in Figs. 2 and 3 only arise from the low solvating power of the supercritical fluid mobile phase.

3.2. *Elution profile of the second-eluted component of a binary mixture*

The chromatograms in Fig. 4a correspond to the separation of benzyl alcohol and vanillin when increasing amounts of benzyl alcohol are co-injected with a constant amount of vanillin. Comparison with the peak obtained when the same amount of vanillin is injected alone (Fig. 4b) indicates clearly that the vanillin peak shifts to higher retention times as the benzyl alcohol load is increased. This phenomenon, known as the restriction effect [10] or the retainment effect [11], is related to limited solubility of the solutes in the supercritical fluid mobile phase: when the load of the more soluble solute 1 is increased, the minor and less soluble solute 2 is forced out of the supercritical fluid mobile phase and, consequently, is more retained than it would be if it was injected as a pure component.

Identical with the displacement effect occurring with convex isotherms in PLC [12], this

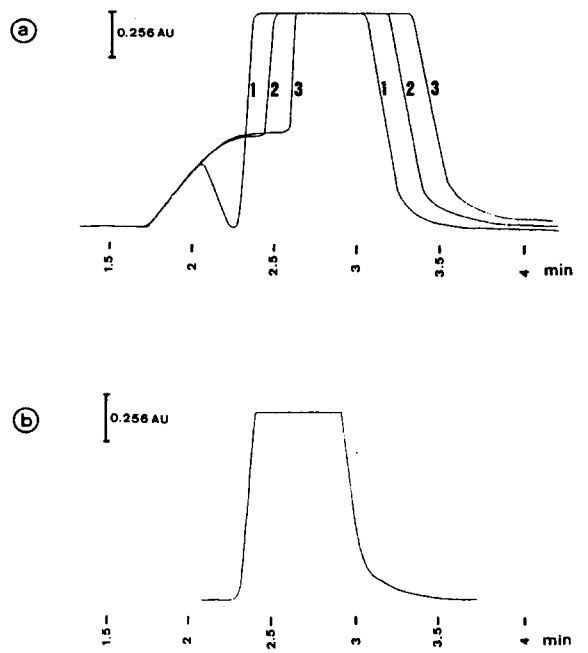

Fig. 4. Effect of amount of the first-eluted component (benzyl alcohol) injected on the elution profile of the second-eluted component (vanillin). Detection wavelength, 243 nm; volume injected, 20 μl; amount of vanillin injected, 10 μmol. (a) Chromatograms of binary mixture with increasing injected amounts of benzyl alcohol: 1 = 10; 2 = 30; 3 = 50 μmol. (b) For comparison, peak corresponding to 10 μmol of vanillin injected alone.

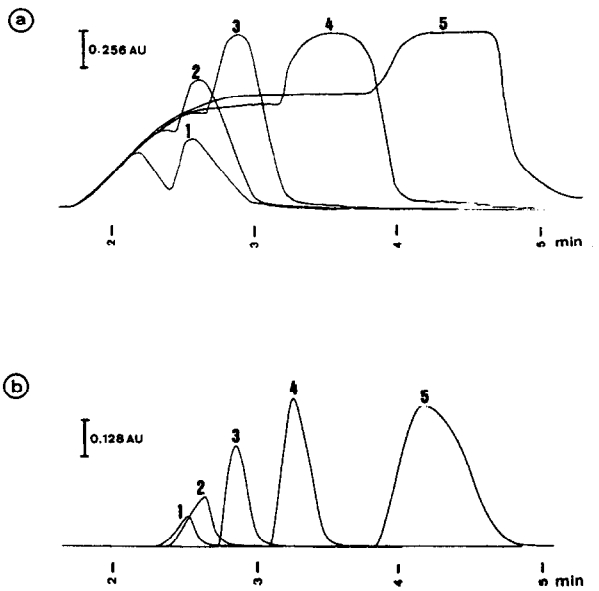

Fig. 5. Effect of sample size on separation of a 25:1 benzyl alcohol–vanillin mixture. (a) Chromatograms monitored at 243 nm; (b) elution profiles of vanillin monitored at 330 nm where benzyl alcohol has no UV absorption. Volume injected and sample concentration: 1 = 20 μl, 0.65 mol l^{-1}; 2 = 20 μl, 1.3 mol l^{-1}; 3 = 20 μl, 2.6 mol l^{-1}; 4 = 40 μl, 2.6 mol l^{-1}; 5 = 80 μl, 2.6 mol l^{-1}.

restriction effect occurring with concave isotherms in PSFC is beneficial and contributes to facilitate the separation. Fig 5a and b illustrate the effect of increasing sample load on chromatograms and elution profiles of the second-eluted component, respectively, for a 25:1 benzyl alcohol–vanillin mixture. The less soluble solute 2 (vanillin) seems to form a zone at the rear of the elution profile of solute 1 (benzyl alcohol) that is clearly driven back as the sample size is increased and the peak rear of solute 1 moves to longer retention times. However, it is difficult to visualize the relative overlapping of the two elution profiles because, on the one hand, the benzyl alcohol elution profile cannot be monitored selectively, and on the other, whatever the detection wavelength chosen, the UV absorptivity of vanillin is so large that it is impossible to have a close representation of the 25:1 sample composition. Therefore, in order to qualify the separation obtained in the different experiments in Fig. 5, solute 2 was totally recovered (detection at 330 nm where solute 1 does not absorb, as in Fig. 5b, allowed the collection of solute 2 as soon as it began to elute) and its purity was determined by quantitative liquid chromatographic analysis of the collected fraction (Table 1). Although the sample size is increased, the purity of solute 2 remains approximately constant, which means that the restriction effect prevents band overlapping even if band broadening is occurring.

3.3. *Elution profile of the first-eluted component of a binary mixture*

The separation of vanillin (solute 1) and methylparaben (solute 2) is used as a model system for the study of the interaction between solutes on the first-eluted component of a binary mixture. Fig. 6 shows the chromatograms and

Table 1
Effect of sample size on purity of vanillin totally recovered from the 25:1 benzyl alcohol–vanillin mixture

Volume injected (μl)	Sample concentration (mol l^{-1})	Sample load (μmol)	Corresponding chromatogram in Fig. 5	Purity of vanillin (%)
20	0.65	13	1	94.9
20	1.3	26	2	94.0
20	2.6	52	3	94.9
40	2.6	104	4	95.3
80	2.6	208	5	95.4

For experimental conditions, see Fig. 5.

the vanillin elution profiles observed for 0.4 μmol of vanillin injected with various larger loads of methylparaben. The vanillin peak shape undergoes broadening and the separation deteriorates as the proportion of methylparaben is increased. This phenomenon, termed the pull-back effect [11], mirrors the tag-along effect encountered in PLC for convex isotherms [12], but its origin is different. The pull-back effect is related to the limited solvating power of the supercritical fluid mobile phase: although the less retained component, solute 1, is more soluble in the mobile phase than is solute 2, the high concentration of solute 2 results in a shift of the distribution equilibrium of solute 1 away from the mobile phase, leading to a higher retention for solute 1 and increased overlapping between solutes 1 and 2.

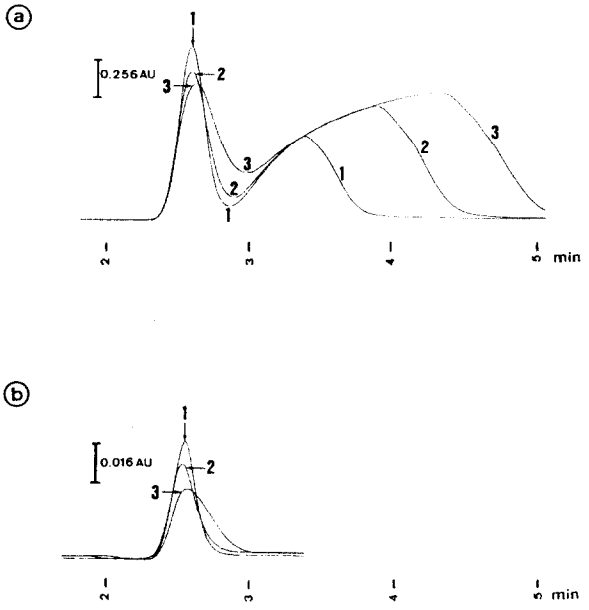

Fig. 6. Effect of amount of the second-eluted component (methylparaben) injected on elution profile of the first-eluted component (vanillin). Volume injected, 20 μl; amount of vanillin injected, 0.4 μmol; amount of methylparaben injected, 1 = 2; 2 = 6 and 3 = 10 μmol. (a) Chromatograms monitored at 285 nm; (b) elution profiles of vanillin monitored at 330 nm (wavelength at which methylparaben has no UV absorption).

Fig. 7a illustrates the overload effect on the elution profile of vanillin for the 1:25 vanillin–methylparaben mixture. Comparison with peaks obtained on injection of the equivalent amounts of vanillin alone (Fig. 7b) indicates clearly that, at high column loading, the pull-back effect can become very detrimental for the separation and recovery of pure solute 2.

4. Conclusions

In supercritical fluid chromatography with the sample solvent evaporation injection technique, the fronting behaviour of peaks observed for a single solute under overload conditions results from the concave isotherm, explained by the limited solubility of solute in the supercritical fluid mobile phase. Investigation of solute interferences when peaks overlap has revealed two effects, the restriction effect and the pull-back effect, which are both related to the limited solvating power of the supercritical fluid mobile phase. Hence band broadening in PSFC is gov-

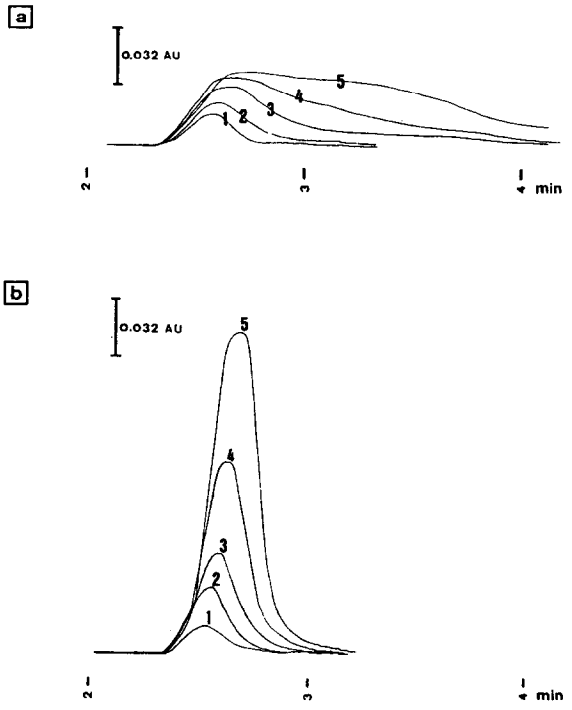

Fig. 7. Effect of sample size on behaviour of the first-eluted component of the 1:25 vanillin–methylparaben mixture. (a) Elution profiles of vanillin monitored at 330 nm (wavelength at which methylparaben has no UV absorption); (b) for comparison, peaks corresponding to the same amounts of vanillin injected alone. Volume injected and sample concentration: 1 = 20 μl, 0.13 mol l^{-1}; 2 = 20 μl, 0.26 mol l^{-1}; 3 = 20 μl, 0.52 mol l^{-1}; 4 = 40 μl, 0.52 mol l^{-1}; 5 = 80 μl, 0.52 mol l^{-1}.

erned by saturation of the mobile phase and a knowledge of the phase diagram for supercritical eluent–solute 1–solute 2 ternary mixture would make it easier to understand the phenomena and optimization of preparative separations.

PLC references in the literature clearly indicate that, owing to the beneficial displacement effect and the detrimental tag-along effect, a separation is easier when the minor solute is less retained than the major solute. In PSFC, the opposite situation seems to apply: owing to the beneficial restriction (or retainment) effect and the detrimental pull-back effect, a separation is easier when the minor solute is more retained than the major solute.

5. References

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