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COMPARATIVE SUPERCRITICAL-FLUID CHROMATOGRAPHIC PROPERTIES OF CARBON DIOXIDE AND SULPHUR HEXAFLUORIDE-AMMONIA MIXTURES WITH PACKED COLUMNS

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SUMMARY

The separation of polar compounds by supercritical-fluid chromatography is difficult with carbon dioxide as mobile phase. In this work, we tested mixtures of ammonia with sulphur hexafluoride as mobile phase and we have compared the solvating power of these mixtures with carbon dioxide for five solutes. The results show that the solvating power of sulphur hexafluoride–ammonia is weak in comparison with carbon dioxide, except for aniline. Ammonia plays only a role as silanol masking agent. Furthermore, ammonia is harmful for the pumping system.

INTRODUCTION

The development of supercritical-fluid chromatography (SFC) was mainly accomplished with carbon dioxide (CO_2) as the mobile phase¹. This fluid has numerous advantages: low cost, mild critical parameters and non-toxic nature. However, CO_2 behaves as a non-polar mobile phase (solubility parameters varying between hexane and dichloromethane depending on the pressure and the temperature). Therefore, very few results for polar compounds and particularly basic compounds² have been reported with pure CO_2 . For these compounds and with packed columns, polar modifiers are commonly added to CO_2 ^{3–6}. However, the advantages of SFC over liquid chromatography (higher diffusion coefficients, lower viscosities and higher efficiencies per unit time) can be partly lost in this way when the concentration of the polar modifier becomes significant. Further, when modified phases are used it results in a poor or no sensitivity with a flame ionization detector.

It is now recognized that a more polar mobile phase (compatible with a flame ionization detector) is necessary. Sulphur hexafluoride (SF_6) has not been used extensively as a mobile phase for SFC^{7,8} owing to its weak solvating power⁹. On the contrary, ammonia (NH_3) is known as a polar supercritical fluid, but very few results

are available in SFC^{10,11} owing to its critical parameters ($T_c = 132.4^\circ\text{C}$; $P_c = 111.3$ bar) and because it requires a stationary phase and a device specially designed to work with such an harmful medium¹². Therefore, a mixture of NH_3 with an inert fluid, such as SF_6 , might be a more convenient way to obtain a polar supercritical fluid. This mixture would be less corrosive, have more easily obtainable critical parameters (SF_6 , $T_c = 45.5^\circ\text{C}$; $P_c = 37.1$ bar) and would be compatible with a flame ionization detector. In this work, we tested three mixtures of NH_3 with SF_6 for the elution of some model compounds and have compared the results with those obtained with CO_2 .

EXPERIMENTAL

Apparatus

The design has been described elsewhere¹³. CO_2 and the SF_6 - NH_3 mixture are contained in a cylinder with an eductor tube and supply the pump of a Varian 5500 chromatograph (Varian, Palo Alto, CA, U.S.A.). Cooling of the pump head with cold ethanol (5°C) is necessary to improve the pump efficiency. Temperature control of the fluid and the chromatographic column was achieved by using a constant-temperature water-bath (Polystat 22; Bioblock Science, Illkirch, France). The injector was a Rheodyne 7125 six-way switching valve with a loop of $10\ \mu\text{l}$, its rotor being made of Tefzel to be compatible with basic media. A Varian UV 2550 spectrophotometer was used with a detection cell modified in order to withstand pressures up to 350 bar. The pressure in the system was monitored by a back-pressure regulator (Model 26-3220-24004; Tescom, Minneapolis, MN, U.S.A.).

The stainless-steel columns ($15\text{ cm} \times 0.46\text{ cm I.D.}$) were packed with Nucleosil-100 bare silica ($5\ \mu\text{m}$) (Macherey, Nagel & Co., Düren, F.R.G.) and with MCH-5 reversed-phase RP-18 silica ($5\ \mu\text{m}$) (Varian).

Chemicals and reagents

CO_2 (N45 grade) and three SF_6 - NH_3 mixtures, containing 1, 2 and 5% (w/w) of ammonia, were obtained from Air Liquide (Paris, France). The ammonia content of the three mixtures was determined by bubbling the SF_6 - NH_3 through citrated hydrochloric acid solutions and titrating the excess of acid with sodium hydroxide solution. The ammonia contents of the three mixtures were found to be 1.1 ± 0.1 , 2.3 ± 0.3 and $5.2 \pm 0.5\%$ (w/w) ($n = 3$), respectively.

Benzonitrile, nitrobenzene, methyl benzoate, phenol and aniline were obtained from Merck (Darmstadt, F.R.G.) and were used without purification. Solutions of solutes in hexane were injected directly into the loop.

RESULTS AND DISCUSSION

In order to compare the properties of the two supercritical fluids, solutes were chosen on the basis of their adsorption energies on bare silica¹⁴ and of the presence of typical basic or acidic functions such as NH_2 or OH , which can interact strongly with the silica surface or with NH_3 itself.

Separations were first done on bare silica with CO_2 as the supercritical mobile phase. Then, with SF_6 - NH_3 mixtures, bare silica and octadecyl-bonded silica were

used. With both fluids, the influence of pressure and temperature on the retention times of solutes was studied.

Results with CO₂

On bare silica, all compounds were eluted with CO₂ as the supercritical mobile phase, as shown in Fig. 1. The order of elution follows the adsorption energies¹⁴, as in normal-phase liquid chromatography.

Aniline is the most retained solute, because of its strong adsorption energy and the presence of an amino function, which can strongly interact with silanol groups of the silica surface in an apolar medium^{15,16}.

For all compounds the capacity factors decrease with increasing pressure at constant temperature. Likewise, at constant pressure, the capacity factors of the solutes increase with increasing temperature, except for aniline, as shown in Table I. Indeed, the density of the mobile phase plays the major role in the retention mechanism, as mentioned previously^{1,17}. However, for aniline, the slight decrease in capacity factor with increasing temperature can be explained mainly by its high volatility¹⁷.

Results with SF₆-NH₃ [1, 2 and 5% (w/w) NH₃]

On bare silica. On this stationary phase the order of elution is modified in comparison with the results obtained with CO₂, methyl benzoate being eluted first (Fig. 1). Phenol is not eluted even with a 5% NH₃ content. The strong retention of phenol can be attributed to the substitution of silanol groups by NH₃ which modifies the acidic character of the support surface. This modification of the stationary phase is clearly confirmed by the results obtained with aniline, where the peak tailing is

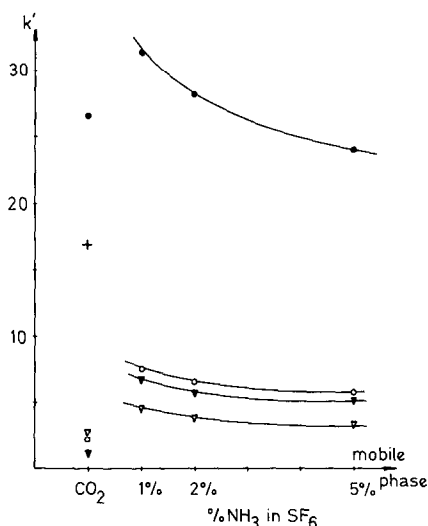


Fig. 1. Capacity factors of test solutes as a function of the supercritical mobile phase. Stationary phase, bare silica, 5- μ m Nucleosil-100; temperature, 50°C; pressure, 190 bar. Mobile phases: CO₂ (P_r = 2.61, T_r = 1.06); SF₆-NH₃ containing 1, 2 and 5% (w/w) of NH₃ (P_r = 4.20, T_r = 0.99; P_r = 3.69, T_r = 0.97; P_r = 3.01, T_r = 0.93, respectively); flow-rate, 4 ml min⁻¹. Detection: UV at 254 nm. Solute: ●, aniline; ○, benzonitrile; ▼, nitrobenzene; ▽, methyl benzoate; +, phenol.

TABLE I

CAPACITY FACTORS (k') OF TEST SOLUTES ON BARE SILICA WITH CO₂ AS MOBILE PHASE AS A FUNCTION OF PRESSURE AT CONSTANT TEMPERATURE (313 K) AND AS A FUNCTION OF TEMPERATURE AT CONSTANT PRESSURE (190 bar)

Pressure (bar)	Temperature (K)	Aniline	Methyl benzoate	Benzonitrile	Nitrobenzene
165	313	35.1	2.7	2.4	1.3
215		29.8	2.3	1.9	1.1
265		28.6	2.3	1.9	1.1
288		25.2	1.9	1.6	1.0
190	323	26.5	2.6	2.2	1.1
	333	26.2	2.7	2.7	1.5
	343	26.3	3.1	3.0	1.7
	353	22.6	3.5	3.4	2.1

minimized in comparison with the results obtained with CO₂¹⁶, the interaction of amino functions with silanols being masked. Moreover, the influence of NH₃ concentration is more important for aniline (Fig. 1) than for other solutes without basic or acidic functions.

The solvating power of SF₆-NH₃ mixtures is governed by two factors, the composition and the density. The calculated critical parameters of the mixtures are reported in Table II¹⁷. From these values, it is observed that when the percentage of NH₃ increases, the critical density of the mobile phase decreases. In addition, as NH₃ is far less dense than SF₆ at any given pressure and temperature, the higher the percentage of NH₃ in the mixture, the lower is the final density. As shown in Fig. 1, as the NH₃ concentration increases, the capacity factors decrease. Hence, the change in mobile phase composition has a more important effect on solute retention than density, and finally the mobile phase polarity is enhanced by addition of NH₃. Nevertheless, as shown in Fig. 1, it is not necessary to increase the NH₃ concentration in SF₆ above 5% as the capacity factor plotted against percentage of NH₃ becomes constant. Indeed, at this percentage the density fall balances the effect of NH₃.

Hence, under our thermodynamic test conditions, the solvating power of SF₆-NH₃, at any percentage of NH₃, appears to be lower than that of CO₂ for the tested solutes. Only aniline has similar capacity factors in both fluids (Fig. 1). SF₆, having a weak solvating power in comparison with CO₂⁸ [solubility parameters: SF₆ = 5.5

TABLE II

CRITICAL PARAMETERS OF SF₆-NH₃ MIXTURES AND OF CO₂

Parameter	CO ₂	SF ₆ -NH ₃		
		1.1% NH ₃	2.3% NH ₃	5.2% NH ₃
ρ_c (g cm ⁻³)	0.464	0.694	0.653	0.577
P_c (bar)	72.9	43.6	49.6	60.8
T_c (K)	304.3	326.2	333.2	346.4

(cal cm⁻³)^{1/2}; CO₂ = 7.5 (cal cm⁻³)^{1/2}], a small addition of NH₃ to SF₆ appears to be insufficient to give a more polar mobile phase than CO₂.

Further, the weak solvating power of SF₆-NH₃ results in the volatility of the solutes having a more pronounced influence on capacity factors in SF₆-NH₃ than in CO₂, as shown in Fig. 2 for aniline. Therefore, if the temperature increases, the capacity factors decrease dramatically, as already mentioned elsewhere⁸.

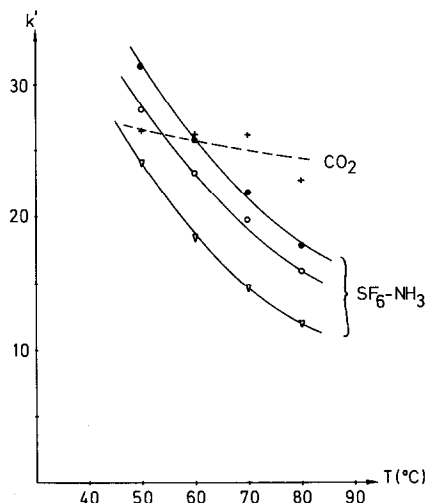


Fig. 2. Influence of temperature at constant pressure ($P = 190$ bar) on aniline capacity factors as a function of the mobile phase. Other conditions as in Fig. 1. NH₃ concentration: ●, 1%; ○, 2%; ▽, 5% (w/w); +, CO₂.

On reversed-phase silica. On octadecyl-bonded silica and under the same operating conditions with SF₆-NH₃ as the mobile phase, all solutes are less retained than on bare silica. These results can be explained by the small number of silanol groups available on this stationary phase. Moreover, the elution order for nitrobenzene, benzonitrile and methyl benzoate follows the inverse polarity order as in reversed-phase liquid chromatography; it is exactly the reverse of that obtained on bare silica with supercritical CO₂ as the mobile phase (Fig. 3).

Consequently, two mechanisms can exist on this surface, the most important being a silanophilic interaction which can be decreased by ammonia impregnation as on bare silica (Fig. 3), and the other based on non-polar-non-polar partition.

Practical considerations of the use of SF₆-NH₃

No degradation of the silicas has been observed and the SF₆-NH₃ mixtures were homogeneous as the capacity factors for test solutes remained constant.

The low solvent power of SF₆-NH₃ causes problems during the injection process, some solutes diluted in hexane being precipitated in the injection valve. This phenomenon has been observed with some morphinic alkaloids, benzaldehyde and some other moderately polar compounds. These observations indicate that ammonia plays the role of a silanol masking agent rather than of a polar modifier.

The most important drawback to SF₆-NH₃ mixtures is their corrosive nature

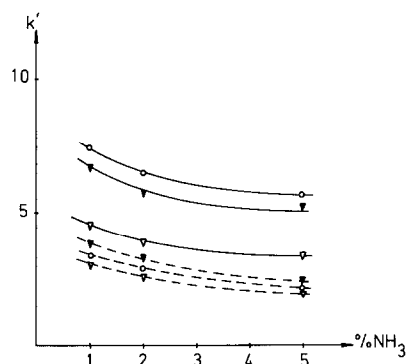


Fig. 3. Influence of percentage of NH_3 on solute capacity factors on two stationary phases: solid lines, bare silica; and broken lines, reversed-phase silica. Conditions as in Fig. 1. Solutes: \circ , benzonitrile; \blacktriangledown , nitrobenzene; ∇ , methyl benzoate.

toward the pumping system material, even when using a low percentage of NH_3 . In 1 month, we had to change the inlet-valve seal and the spring-loaded seal twice and the piston itself once.

CONCLUSION

The solvating power of $\text{SF}_6\text{-NH}_3$ (even containing 5% of NH_3) is weak in comparison with CO_2 , the capacity factors for all the tested solutes being higher in $\text{SF}_6\text{-NH}_3$ than in CO_2 . Further, this weak solvating power induces the precipitation of some solutes in the injection valve. The role of ammonia under these conditions is mainly limited to that of a silanol masking agent and therefore it is unnecessary to increase its concentration in SF_6 above 5% (w/w).

Finally, only basic molecules, such as aniline, show a better behaviour with $\text{SF}_6\text{-NH}_3$ than with CO_2 , the silanophilic interactions with the amino groups being minimized by ammonia impregnation.

REFERENCES

- 1 R. M. Smith (Editor), *Supercritical Fluid Chromatography*, Royal Society of Chemistry, London, 1988.
- 2 S. M. Fields and K. Grolimund, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 11 (1988) 727.
- 3 J. R. Wheeler and M. E. McNally, *J. Chromatogr.*, 410 (1987) 343.
- 4 P. Carraud, D. Thiebaut, M. Caude, R. Rosset, M. Lafosse and M. Dreux, *J. Chromatogr. Sci.*, 25 (1987) 395.
- 5 J. L. Janicot, M. Caude and R. Rosset, *J. Chromatogr.*, 437 (1988) 351.
- 6 J. B. Crowther and J. D. Henion, *Anal. Chem.*, 57 (1985) 2711.
- 7 J. C. Giddings, M. N. Myers, L. McLaren and R. A. Keller, *Science (Washington, D.C.)*, 162 (1968) 67.
- 8 J. W. Hellgeth, M. G. Fessehaie and L. T. Taylor, *Chromatographia*, 25 (1988) 172.
- 9 R. D. Smith, B. W. Wright and C. R. Yonker, *Anal. Chem.*, 60 (1988) 1323 A.
- 10 K. Grolimund, W. P. Jackson, M. Joppich, W. Nussbaum, K. Anton and H. M. Widmer, in D. Ishii, K. Jinno and P. Sandra (Editors), *Proceedings of the Seventh International Symposium on Capillary Chromatography*, Gifu, Nagoya, May 11–14, 1986, The University of Nagoya Press, Nagoya, 1986, p. 625.
- 11 J. C. Kuei, K. E. Markides and M. L. Lee, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 10 (1987) 257.
- 12 P. J. Schoenmakers and F. C. C. J. G. Verhoeven, *Trends Anal. Chem.*, 6 (1987) 10.

- 13 P. Mourier, P. Sassiati, M. Caude and R. Rosset, *Analisis*, 12 (1984) 229.
- 14 L. R. Snyder, *Principles of Adsorption Chromatography*, Marcel Dekker, New York, 1968.
- 15 K. E. Bij, Cs. Horváth, W. R. Melander and A. Nahum, *J. Chromatogr.*, 203 (1981) 65.
- 16 J. H. Phillips and R. J. Robey, *J. Chromatogr.*, 465 (1989) 177.
- 17 P. A. Peaden, *J. Liq. Chromatogr.*, 5 (1982) 179.