CHROM. 22 262

Retention studies in supercritical fluid chromatography

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SUMMARY

The effect of fluid density on solute retention using a mobile phase containing 3% (v/v) propan-2-ol in pentane has been investigated. A linear relationship was established between solute retention times and mobile (fluid) phase density. A new method for the determination of the critical temperature of mobile phase mixtures is described and the chromatographic integrity of gas, supercritical fluid and high-performance liquid chromatography is demonstrated.

INTRODUCTION

Supercritical fluid chromatography (SFC) is a rapidly developing analytical technique for the analysis of non-volatile and thermally labile compounds. SFC was first described by Klesper[1] and further studies by Giddings and Myers[2–4] and by Sie and co-workers[5–9] using carbon dioxide, isopropanol and n-pentane as mobile phases have been published. Recently, interest in SFC has increased due to its potential ability to provide faster analysis and higher resolving power when compared to high-performance liquid chromatography (HPLC).

Many of the advantages of gas and liquid chromatography are exploited in SFC. These include: densities ranging between a gaseous and liquid mobile phase, higher solute diffusivities and lower mobile phase viscosities. The solvation power of a supercritical fluid is controllable, because it is principally a function of density (although mobile phase modifiers may also be used to enhance separation). This allows pressure-programming techniques to be used in SFC[10], similar to temperature programming in gas chromatography.

This paper reports the effect of fluid density (attained through temperature and pressure programming) on solute retention and efficiency for a packed-column SFC system using 3% (v/v) propan-2-ol in pentane as the mobile phase. The effect of fluid density using normal paraffins (hexane, heptane, octane, nonane and decane) as the mobile phase is also described. The effect of mobile phase modifiers, n-butylamine, and propan-2-ol on solute retention is discussed.

EXPERIMENTAL

Apparatus

The supercritical fluid **chromatograph** used has been described previously' [1].

Reagents

HPLC-grade **propan-2-ol,** pentane, hexane, heptane and octane were obtained from Fisons (Loughborough, U.K.). Nonane and **decane** were obtained from BDH (Poole, U.K.) together with the other chemicals used in this work.

Table I gives a list of solvents and their critical parameters.

TABLE I

CRITICAL PARAMETERS OF MOBILE PHASES

T_c = Critical temperature; P_c = critical pressure; ρ_c = critical density; ρ = density.

Solvent	T_c *(°C)*	P_c *(atm)*	ρ_c *(g cm^{-3})*	ρ *at 200°C and 60 kg cm^{-2} inlet pressure (g cm^{-3})*
Pentane	196.5	33.3	0.237	0.459
Hexane	234.2	29.3	0.234	0.525
Heptane	267.0	27.0	0.232	0.587
Octane	295.6	24.54	0.232	0.613
Nonane	321.4	22.8	–	0.636
Decane	344.3	20.72	–	0.653
Propan-2-ol	235.1	47.0	0.273	
Carbon dioxide	31.3	72.9	0.468	
Methanol	240.0	78.5	0.272	
Propan-2-ol–pentane (3:97)	197.94	33.8	0.238	
Methanol-carbon dioxide **(3:97)**	37.4	73.03	0.466	

RESULTS AND DISCUSSION

Effect of varying the column temperature of constant inlet pressure

The retention behaviour of aniline and pyridine on a 250 x 4.6 mm I.D. column packed with 7-μm silica particles with a mobile phase composition of 3% (v/v) **propan-2-ol** in pentane was investigated. The temperature of the column was varied between 160 and 240°C at constant inlet and outlet pressure to observe the behaviour of the test solutes with the fluid below and above its critical temperature. The results obtained are shown in Fig. 1.

The retention times of the solutes decreased approximately linearly with the increase in temperature at a constant inlet pressure of 60 kg cm^{-2}. This observation confirms results obtained by Schoenmakers[12]. The decrease in solute retention observed was principally a result of the increase in the linear mobile phase velocity with temperature through the chromatographic column. Although a slight change in solute partition coefficient does occur with the increase in column temperature at constant inlet and outlet pressure, the effect of this slight change in partition **coefficient** on
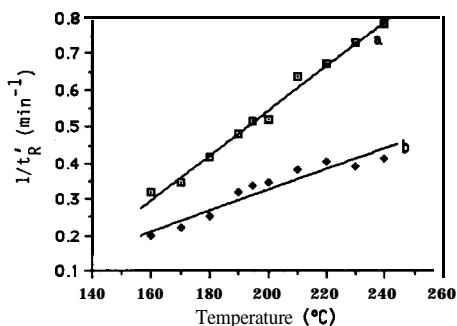
Fig. 1. Effect of column temperature on the reciprocal retention time. Lines: a = aniline (correlation coefficient 1.00); b = pyridine (correlation coefficient 0.96).

solute retention is secondary to the effect of the change in the linear mobile phase velocity through the chromatographic column.

Variation of the column inlet pressure at constant temperature and outlet pressure

The effect of varying the column inlet pressure at constant temperature and outlet pressure on the retention of the test solutes aniline and pyridine was investigated on a 250 x 4.6 mm I.D. column packed with 7-μm silica particles. The mobile phase was propan-2-ol–pentane (3:97).

Fig. 2 illustrates the relationship between the solute retention and the fluid density which is a function of the inlet and outlet pressure of the system. The experiments were performed at three different temperatures: 180,190 and 200°C. The inlet pressure was varied between 50 and 90 kg cm^{-2} and the outlet restriction was maintained constant concomitant with the inlet pressure. The pressure drop over the chromatographic column for the above experiments was too small (from 1 to *ca.* 1.5 kg cm-") to be measured accurately and, hence, was considered negligible.
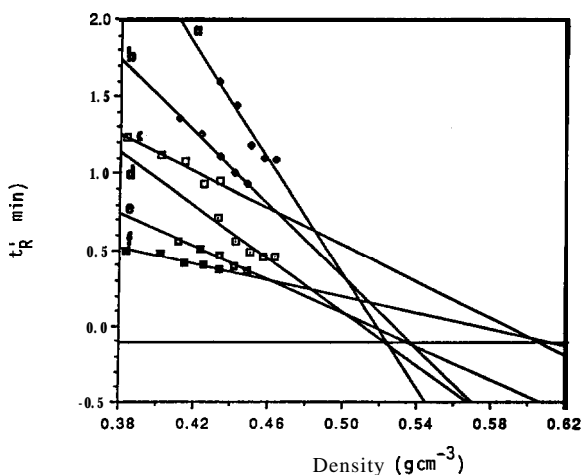

Fig. 2. Plots of adjusted retention time versus fluid density at 180, 190 and 200°C. Lines: a = aniline, 200°C; b = aniline, 190°C; c = aniline, 180°C; d = pyridine, 200°C; e = pyridine, 190°C; f = pyridine, 180°C.

A linear decrease in the adjusted solute retention time $(t_R' = t_R - t_0,$ where t_R is the retention time and t_0 the dead time) was observed as a result of the increase in density of the mobile phase[11]. From this plot, it is clear that there are differences in the retention behaviour of the test solutes above and below the critical temperature (197.8°C) of the mobile (fluid) phase (see Table I).

From the extrapolation of the relationships in Fig. 2, lines a + d, b + e and c + f intersect at a nearly constant negative adjusted retention time. This may be due to inaccuracy in measuring the dead times of the operational systems and could provide a measure of the dead times of the column in the various operational systems and hence a measure of the dead volume of the column and the volumetric flow-rates. Further work is needed to substantiate this.

The rate of decrease in solute retention with density in the supercritical state (lines c + f) is considerably less than that observed below the critical temperature of the mobile phase (lines a + d and b + e).

In order to explain the general trend of variation in retention time with density observed in these results, the effect of fluid density on solute retention was further studied. The density of the mobile phase was changed using the higher alkanes as the solvents keeping all other operational parameters constant. If the solute retention was observed to decrease linearly with increase in solvent density, it would then be clear that the density of the mobile phase was a principal controlling factor of retention in SFC. This would not take into consideration the effect of changing the polarity of the mobile phase which, under the conditions, would be substantially constant.

Variation in the fluid density by change of solvent

SFC was performed with a series of n-alkanes as the mobile (fluid) phase. The first fluid, pentane, was pumped at a constant flow-rate of 1 $cm^3 min^{-1}$ and the temperature of the system was maintained at 200°C, *i.e.* above the critical temperature of pentane. The mobile phase was changed to the higher alkanes (up to *n*-decane) successively while all the other experimental conditions were maintained constant. Under these conditions, the higher n-alkanes were below their critical temperatures (see Table I). The test solute used was chrysene on a column packed with 40-μm silica particles (250 x 4.6 mm I.D.). With the same chromatographic column the retention characteristics of this solute were also determined at 200°C using helium as the fluid phase in a gas chromatographic system using a thermal conductivity detector.

Fig. 3 shows that a general decrease in solute retention was obtained with increasing fluid density. These observations confirm that there is a linear relationship between mobile phase density and the solute retention and show that a continuity exists between gas, supercritical, and liquid chromatography and that this is a direct function of the density of the mobile phase.

From the above result it is clear that the use of the higher alkanes which possess higher fluid densities would enable the separation and elution of high-molecular-weight non-polar solutes. This work clearly establishes that there is no real need to operate in the supercritical state with SFC and that the control of the fluid density (achieved through controlling temperature and pressure) is the key factor to attain fast and efficient separations.
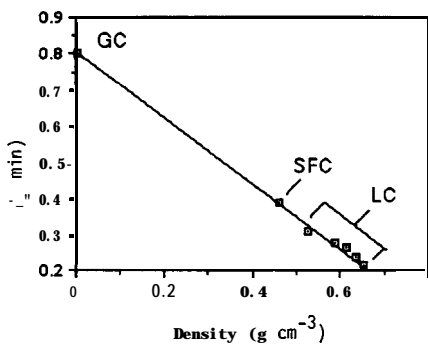
Fig. 3. Plot of adjusted retention time *versus* density of alkanes. Correlation coefficient 0.99.

Effect of column temperature on the dead time in SFC

The effect of the column temperature on the chromatographic dead time was studied using a 250 x 4.6 mm I.D. column packed with 7-μm silica particles.

The SFC system was maintained at a constant inlet pressure of 60 kg cm^{-2}, *i.e.* above the calculated critical pressure for this mobile phase composition (pentane with 3% propan-2-ol). The dead time was obtained using a fixed sample size of potassium nitrate as the unretained solute.

Fig. 4a shows that the dead volume decreases rapidly with temperature until the temperature reaches 196°C, when the rate of decrease in dead volume was significantly reduced up to the maximum temperature employed (240°C). Between 160 and 190°C the mobile phase exists as a liquid phase while above this temperature it exists in the fluid state. This difference in the retention time of the unretained solute is due to the increase in fluid velocity as the critical temperature of the mobile phase is approached. In the supercritical state, the fluid velocity only increases at a rate concomitant with the "expansion" of the fluid with increasing temperature. The critical temperature of the mobile phase mixture was estimated by drawing a vertical line to the x-axis, at the point of interception of the two lines (first line of points obtained below the critical temperature and the second line of points above the critical temperature) (Fig. 4a).

To confirm the above finding, the experiment was repeated for a different mobile phase mixture (3% methanol in carbon dioxide). The temperature of the supercritical fluid chromatograph was varied between 15 and 70°C and a similar behaviour was observed for the rate of change of chromatographic dead time with temperature (Fig. 4b).

This confirms that this approach is indeed useful in obtaining a practical estimate of the critical temperature of a given solvent mixture.

Effect of modifier concentration on solute retention

Organic modifiers may be used to reduce the retention of highly polar solutes in a similar manner as in LC. The effect of including two organic modifiers, n-butylamine and isopropanol, in the mobile phase on the retention of aniline using a 250 x 4.6 mm I.D. column packed with 7-μm silica particles was studied. During the experiments the inlet pressure was maintained at 60 kg cm^{-2} and the chromatographic column was maintained at a temperature of 200°C. Fig. 5a shows the effect of the modifier

concentration on solute retention with pentane (above its critical temperature, $T_c =$ 196.5°C) and Fig. 5b with hexane (below its $T_c = 234.2°C$) as mobile (fluid) phases.

From these plots a decrease in the retention of aniline is observed with an increase in the modifier concentration (n-butylamine and isopropanol) in the mobile fluid. It is most unlikely that at this operating temperature (200°C) the modifiers are irreversibly adsorbed onto the surface of the silica, but the observed effect is related to a competition for the adsorption sites on the silica surface between the modifier and the solute molecules[13]. Since the modifier is present in excess with respect to the solute, this will preferentially, but transiently occupy the adsorption sites rendering them unavailable for simultaneous interaction with the solute molecules. This explains why the retention time of the solute molecule decreases with increase in modifier concentration.
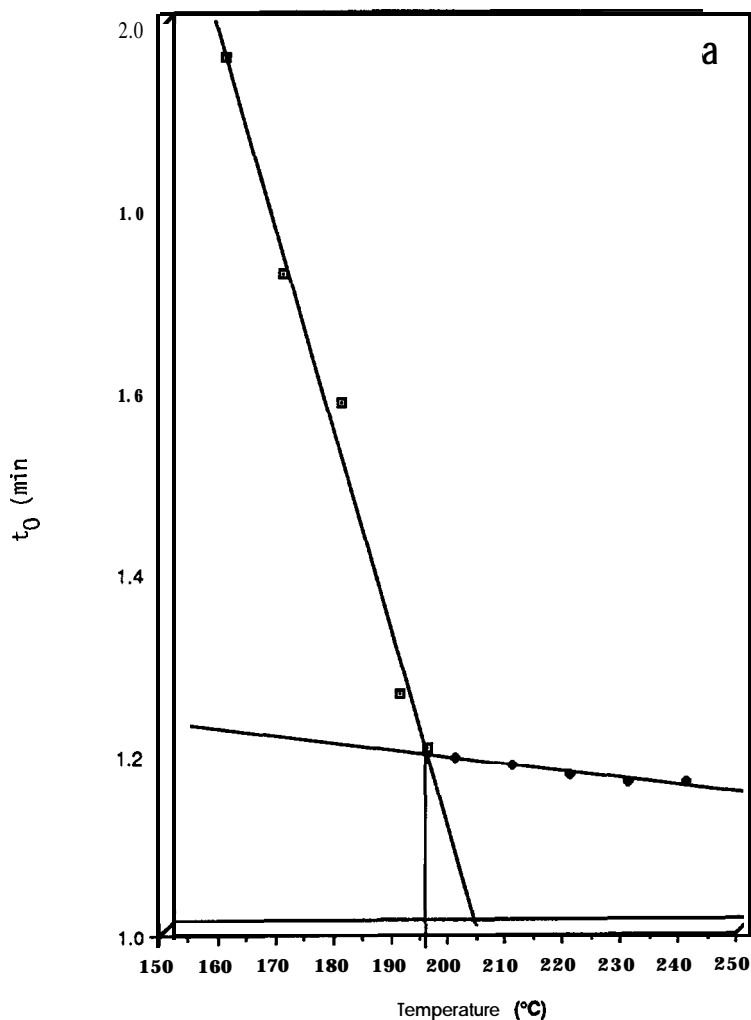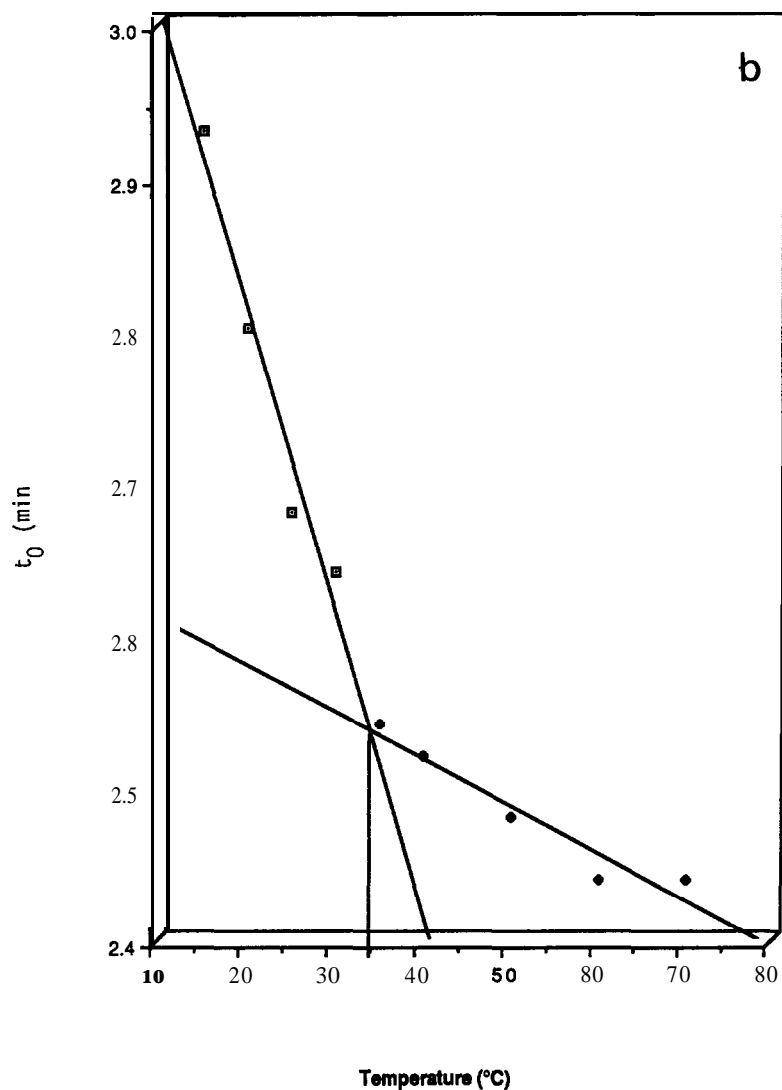

Fig. 4.

Fig. 4. Effect of column temperature on chromatographic dead time. (a) Mobile phase, propan-2-ol–pentane (3:97); □ = below critical temperature; ● = above critical temperature. Lines intersect at 196°C. Critical temperature 197.9°C (calculated). (b) Mobile phase, methanolcarbon dioxide (3:97); □ = below critical temperature; ● = above critical temperature. Lines intersect at 35°C. Critical temperature 37.4°C (calculated).

This study was carried out on a silica column. However, other workers have studied modifier effects on cyano, amine and diol stationary phases with different solutes[14–16]. The general trend of their results correlates well with those reported above.

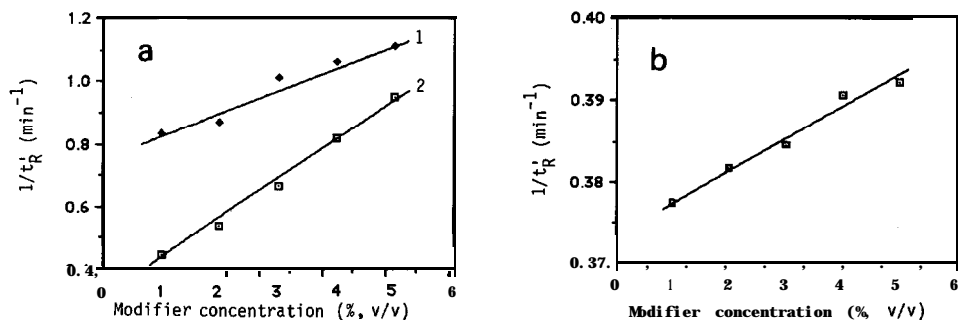Fig. 5. Effect of **propan-2-ol** and n-butylamine concentration on solute retention. (a) Modifier, **propan-2-ol**; lines: 1 = pentane (correlation **coefficient** 0.99); 2 = hexane (correlation coefficient 0.98). (b) Modifier, n-butylamine; pentane (correlation **coefficient** 0.98).

CONCLUSION

A linear relationship has been established between solute retention and mobile (fluid) phase density. From the experiment (Fig. 3), it is clear that the mobile (fluid) phase density and solubility parameter are the factors that control solute retention in SFC and that separations need not be performed above the critical temperature of the mobile phase. Also observed from Fig. 3, is continuity in the three separation techniques, gas chromatography, SFC and HPLC.

Studies on the effect of the mobile (fluid) phase temperature on the dead volume resulted in an interesting observation that varying the mobile phase temperature (at a constant inlet pressure) could be a means of determining the critical temperature of the mobile (fluid) phase mixture.

ACKNOWLEDGEMENTS

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