

Note

Effect of solute concentration on retention in supercritical fluid chromatography

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Solute retention in supercritical fluid chromatography (SFC) has been demonstrated to be a complex function of pressure, temperature and density¹⁻⁴. The effect of solute concentration on retention as a function of pressure and/or temperature in SFC remains uncertain. Two major types of (ad)sorption isotherms in gas and liquid chromatography⁵⁻⁷ have been described, Langmuir and anti-Langmuir isotherms; both are caused by solute overloading of the stationary phase, but are manifested as different effects on the peak shape and the retention time of the concentration maximum.

The quantitative study of adsorption isotherms in both liquid and gas chromatography has been described^{5,7}. A qualitative description, combining a mathematical analysis of peak shape in non-linear liquid chromatography and the effect of the adsorption isotherm on peak shape has recently been published by Jaulmes *et al.*⁸. In the technique outlined by Jaulmes *et al.*, the change in peak shape and the variation in retention time for the peak maxima with concentration at various temperatures was studied and related to thermodynamic behavior (*e.g.*, concave or convex isotherms) for reversed-phase liquid chromatography.

In SFC, both pressure and temperature can impact (ad)sorption isotherms and affect solute retention times based upon peak maxima. In this article, we report investigations of solute isotherms in capillary SFC, based on the method of Jaulmes *et al.*⁸, at different pressures for two temperatures with two probe molecules.

EXPERIMENTAL

A Varian 8500 high-pressure syringe pump under computer control operating in a pressure-regulated mode, delivered a precise, pulse-free flow of fluid to the capillary column in a temperature-regulated oven. A Valco valve (C14W) with a 0.2- μ l sample loop was used for solute introduction. The capillary column (10 m \times 50 μ m) was coated and cross-linked with an SE-54 type methyl phenylpolysiloxane stationary phase (film thickness 0.2 μ m). SFC-grade carbon dioxide (Scott Speciality Gases) was used as the mobile phase. Solute probes for the isotherm studies were 2-nitroanisole and 4-ethylnitrobenzene (Aldrich) and were used without any further purification. The (sample) solutes were detected using a flame ionization detector, and the

output was recorded using a HP 3390A reporting integrator. The integrator was used to record peak shape and retention times of the concentration maxima of the peak. Further details on the experimental apparatus and technique have been given by Smith and co-workers^{9,10}.

RESULTS AND DISCUSSION

As shown in the work of Jaulmes *et al.*⁸ and others¹¹, once the non-linear region of the sorption isotherm is attained, the retention time of the concentration maxima of a peak shifts with increasing solute concentration. This shift in retention time is dependent on the isotherm shape (convex or concave). An anti-Langmuir (concave) sorption isotherm manifests itself in fronting solute peaks, with the retention time increasing for the concentration maxima of the peak. A Langmuir (convex sorption) isotherm gives rise to tailing peaks where retention time decreases for the concentration maxima of the peak.

In SFC where solute retention is dependent on pressure and temperature, one could envision the solute isotherm showing a pressure dependence. This pressure dependence could manifest itself in one of two ways. The peaks could begin to front or to tail with increasing solute concentration, with the retention times for the peak maxima increasing or decreasing respectively.

The solute concentration for 2-nitroanisole and 4-ethylnitrobenzene is proportional to the injected quantity if the (ad)sorption isotherm is linear. Fig. 1 shows a plot of $\log[\text{peak height}]$ as a function of $\log[\text{injected solute concentration}]$ for the two probe molecules at pressures where their sorption isotherms were linear. If the flame ionization detector was responding linearly to solute concentration, one would expect a straight line with slope 1 for the solute under these conditions. Indeed, the detector response was linear for an initial injected solute concentration range of 1 M to $1 \cdot 10^{-2}\text{ M}$ and 1 M to $5 \cdot 10^{-3}\text{ M}$ with slopes of 1.09 and 0.998 for 2-nitroanisole and 4-ethylnitrobenzene, respectively. The sample was split in an approximately 1:4 ratio before introduction to the column. The approximate solute concentration

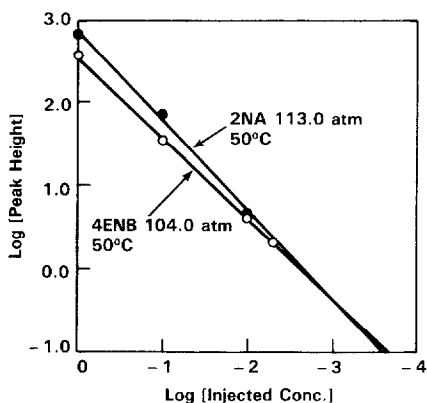


Fig. 1. Plot of $\log[\text{peak height}]$ versus $\log[\text{injected solute concentration}]$ (M) for 2-nitroanisole (2NA) at 113.0 atm, 50°C and 4-ethylnitrobenzene (4ENB) at 104.0 atm, 50°C.

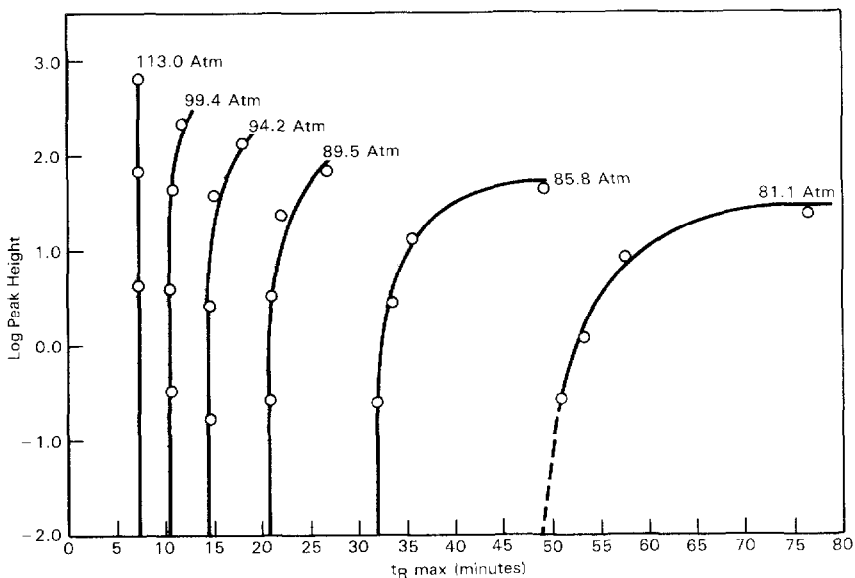


Fig. 2. Plot of $\log[\text{peak height}]$ versus retention time for the concentration maxima of the solute peak (t_R^{max}) for 2-nitroanisole at 50°C and various pressures.

range seen by the flame ionization detector was calculated to be $3.7 \cdot 10^{-2} M$ to $3.7 \cdot 10^{-4} M$ and $3.3 \cdot 10^{-2} M$ to $1.58 \cdot 10^{-4} M$ for 2-nitroanisole and 4-ethylnitrobenzene, respectively. These values are based on the following equation

$$[C_{\text{Detector}}] = 2.354 [C_{\text{injected}}] (\text{Split ratio}) \left\{ \frac{V_{\text{injector}} (N/L)^{1/2}}{V_{\text{column}} (1 + k')} \right\}$$

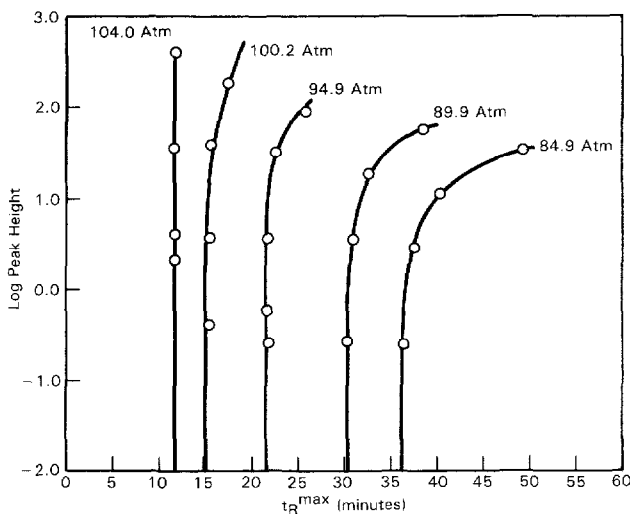


Fig. 3. Plot of $\log[\text{peak height}]$ versus retention time for the concentration maxima of the solute peak (t_R^{max}) for 4-ethylnitrobenzene at 50°C and various pressures.

and a measured efficiency of *ca.* 160 plates/m for a capacity factor (k') of 0.18 for 2-nitroanisole at 113.0 atm and a k' value of 0.34 for 4-ethylnitrobenzene at 104.0 atm, both at 50°C. This corresponded to a sample size range of *ca.* 8.0 μg –*ca.* 30 ng of solute being injected onto the column.

Figs. 2 and 3 show the effect of concentration on the retention time of the peak maxima for the solute peaks at various pressures for supercritical carbon dioxide at 50°C. One can see from these figures that the retention time of the peak maxima increases with concentration for both of the solutes studied. Therefore, the isotherm shape for these solutes under SFC conditions can be inferred to be anti-Langmuir (concave sorption isotherms). Under these experimental conditions at 50°C the stationary phase can become overloaded with solute molecules possibly due to the saturation of the solute in the fluid mobile phase. The solute sorption mechanism may be controlled by multi-layer adsorption of solute molecules. Solubility of the solutes in carbon dioxide at these conditions remains to be measured to determine the nature of the sorption process. It is interesting to note that as the pressure increases, the linear region of the sorption isotherm extends to higher concentrations. This is not unexpected since the solvent strength of the fluid is proportional to density¹². Therefore, the solubility of the solute in the fluid phase increases with pressure (density) and is the likely origin of this effect.

Figs. 4 and 5 show the isotherm behavior of 2-nitroanisole and 4-ethylnitrobenzene at 80°C as a function of pressure and solute concentration. Comparing Figs. 4 and 5 to Figs. 2 and 3, one immediately notes the higher pressures needed at 80°C to assure linear isotherm behavior. This is caused by the density difference between the two temperatures at similar pressures. For 2-nitroanisole data equal densities are achieved at 89.5 atm, 50°C and 117.1 atm, 80°C and also at 85.8 atm, 50°C and 109.4 atm, 80°C. The corresponding densities are *ca.* 0.29 g/ml and *ca.* 0.26 g/ml respec-

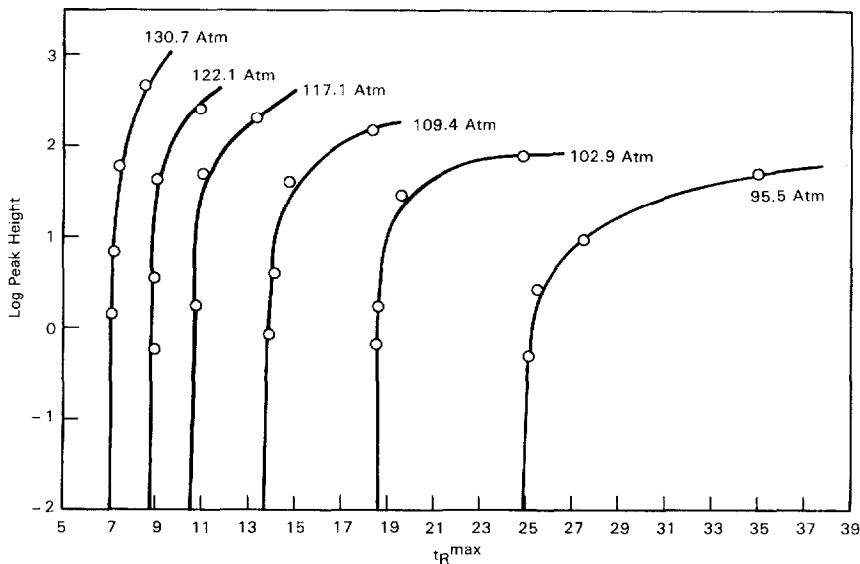


Fig. 4. Plot of $\log[\text{peak height}]$ versus retention time for the concentration maxima of the solute peak (t_R^{max}) for 2-nitroanisole at 80°C and various pressures.

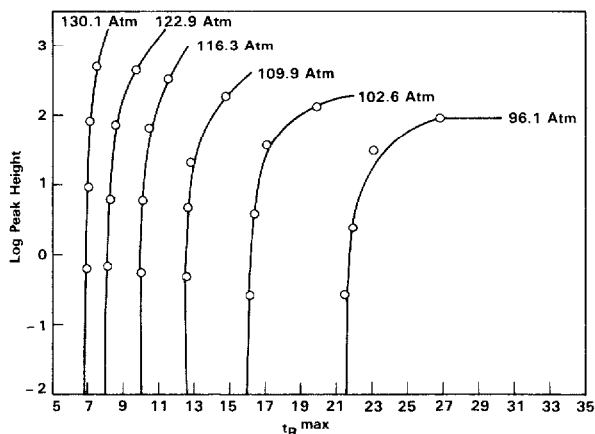


Fig. 5. Plot of $\log[\text{peak height}]$ versus retention time for the concentration maxima of the solute peak (t_R^{max}) for 4-ethylnitrobenzene at 80°C and various pressures.

tively. For 4-ethylnitrobenzene data equal densities are achieved at 94.9 atm, 50°C and 130.1 atm, 80°C, and also at 84.9 atm, 50°C and 109.9 atm, 80°C. The corresponding densities are *ca.* 0.34 g/ml and *ca.* 0.26 g/ml respectively. At constant density for SFC the onset of the non-linear region of the adsorption isotherm seems to occur at a higher concentration as one proceeds to higher temperatures (compare Figs. 3 and 5). This effect is likely caused by the combined effect of pressure and temperature on solute solubility, and therefore, on the sorption isotherm for SFC.

At low pressures (densities) in SFC, the stationary phase can become saturated with solute molecules such that solute-solute interactions begin to dominate in the retention process leading to the increased retention time for the concentration maxima of the solute peak. This phenomenon is caused by the limited solubility of the solute in the fluid mobile phase at low densities. Thus, one must be aware of the fact that when pressure programming in SFC, one is cutting across a range of sorption isotherms and for a particular concentration, and the isotherms could pass from a non-linear to a linear regime. Therefore, during a pressure program one would want a rapid pressure program rate through the non-linear isotherm regions coupled to a slower ramp rate as one proceeds through the linear region of the sorption isotherms as a function of pressure. This can be advantageous in terms of chromatographic efficiency and choosing an optimum pressure ramp, where one can maintain an optimum ramp rate to keep all sample components in their linear sorption region.

CONCLUSIONS

In SFC, the sorption isotherms of the solute have been shown to have a pressure dependence, where at lower pressures the non-linear region of the isotherm is reached at a lower solute concentration than for higher pressures (densities). This is due to the lower solubility of the solute in a fluid at lower densities. The influence of isotherm non-linearity on the retention time of the concentration maxima for a solute peak was shown to be a sensitive probe of the isotherm shape, be it Langmuir (con-

vex) or anti-Langmuir (concave). Pressure programming in SFC has the effect of passing through a region of non-linear to linear isotherm behavior as a function of increasing pressure for a fixed solute concentration, which can be used to advantage. Thus, one can determine the optimum pressure conditions (linear isotherm region) to obtain maximum resolution during pressure programmed or isobaric separations for SFC.

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