

CHROM. 6778

EXPERIMENTAL RESULTS AND PHYSICO-CHEMICAL ASPECTS OF SUPERCRITICAL FLUID CHROMATOGRAPHY WITH CARBON DIOXIDE AS THE MOBILE PHASE

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SUMMARY

In fluid chromatography, compressed fluids at temperatures in the critical range are used as mobile phases, particularly for the separation of substances with low migration rates and/or low thermal stabilities. Some results of isobaric and pressure-programmed separations with supercritical CO₂ as the mobile phase are presented. These results are discussed with respect to the phase behaviour and the transport properties of fluid CO₂ mixtures at elevated pressures and compared with the results of other workers. The experimental HETP values agree fairly well with data calculated from a modified Van Deemter equation with dimensionless numbers.

INTRODUCTION

Supercritical fluid chromatography (SFC) is a relatively new chromatographic technique in which compressed fluids at slightly supercritical temperatures are used as mobile phases. Review papers by Myers and Giddings¹ and Gouw and Jentoft² have given a variety of theoretical, practical and instrumental details of this technique and it has been shown that its use makes possible the improved separation of several substances whose gas or liquid chromatographic separation is difficult or even impossible. These substances are mainly higher boiling or thermolabile compounds, *e.g.*, macromolecules, polymers, biochemicals and natural substances.

In order to give a better understanding of the complex processes involved in SFC, some basic physico-chemical aspects are considered in this paper. Our own results and those of other workers are discussed with respect to the phase behaviour and the transport properties of fluid CO₂ mixtures at high pressures.

PHASE-THEORETICAL ASPECTS OF SOLUBILITY IN THE SUPERCRITICAL REGION

Pressure-induced solubility effects of dense gases in the supercritical region with respect to their application in SFC have been discussed by Giddings and co-workers³⁻⁵, who explained the solvent power of a supercritical mobile phase by means

of Hildebrand's solubility parameter. A detailed discussion⁶, however, has shown that the treatment of solubility phenomena in the critical region for very different substances with the aid of the solubility parameter should be classified as an approximation.

In order to discuss these phenomena in greater detail, the different types of phase behaviour of binary mixtures will first be reviewed. Component I corresponds to the supercritical mobile phase and component II to the less volatile sample. As we have used only CO₂ as the mobile phase, we consider only binary CO₂ systems, but in general this treatment is relevant for all types of mobile phases and substances.

The variety of phase behaviour of fluid binary systems can be classified by the different forms of the critical $p(T)$ locus curves*. For the following discussion, it is sufficient to know that the critical locus curve is the boundary between regions of limited and complete miscibility.

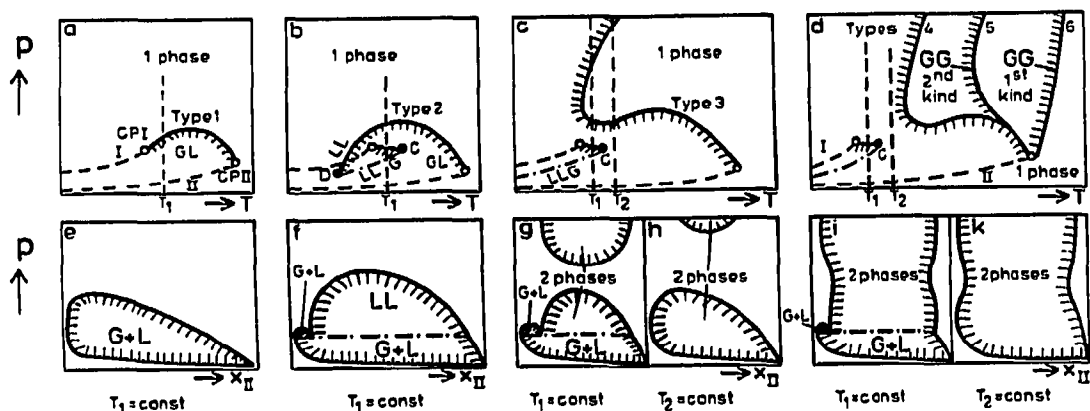


Fig. 1. Phase behaviour of binary CO₂ systems: $p(T)$ projections (a-d) and isothermal $p(x)$ diagrams (e-k) (schematic; x = mole fraction; see text).

In Fig. 1 (a-d), the critical $p(T)$ locus curves for some typical binary CO₂ systems are represented schematically. Characteristic $p(x)$ sections (x = mole fraction) for constant separation temperatures (T_1 , T_2) slightly above the critical temperature of pure CO₂ are also given in Fig. 1 (e-k). The two-phase region is shaded in each case.

Type 1 (Fig. 1a) is the most familiar form of critical phase behaviour in a binary system. The critical locus curve connects the critical points CP of the two pure components. Here the curve runs through a pressure maximum and these maxima commonly lie below 300 bar; extreme values of temperature or a monotonous shape are also possible. This type is characteristic of binary CO₂ systems with non-polar components of low and medium molecular weight and with some polar low-molecular substances showing weak association in the liquid state, e.g., for mixtures of CO₂ with n -alkanes (from methane up to n -undecane⁷⁻¹³), cyclohexane, toluene, some un-

* At a critical point, two phases in equilibrium become identical. The line that connects the critical points of all binary mixtures of a system in the p - T - x space is the critical curve (x = mole fraction). The phase equilibria and critical phenomena can be advantageously discussed with the aid of $p(T)$ projections of these critical curves (the so-called critical $p(T)$ locus curves).

saturated straight-chain hydrocarbons, dimethyl ether, methanol, acetic acid, acetaldehyde, acetonitrile, *n*-propanol, *n*-amyl alcohol, methyl chloride and chlorobenzene^{7,14,15}. Additionally, at lower temperatures, regions of liquid-liquid immiscibility can exist (*e.g.*, for CO₂ mixtures with *n*-alkanes from *n*-octane to *n*-undecane¹¹).

Type 2 (Fig. 1b) has been found for mixtures of CO₂ with nitrobenzene, halogenated nitrobenzenes, *o*-nitrophenol, thymol and urethane^{7,16}. Here the critical locus curve is interrupted; its two branches end at the critical end-points D and C on a three-phase line LLG where two liquid phases and one gaseous phase are in equilibrium. For mixtures of aromatic amines, a more complicated phase behaviour has been found, because of the appearance of solid phases and the formation of solid compounds^{14,16}.

For type 3 (Fig. 1c) and for types 4-6 (Fig. 1d), the right-hand branch of the critical locus curve shows different shapes according to the decreasing mutual miscibility, *e.g.*, for CO₂-*n*-hexadecane (type 3), CO₂-water (type 5) and He-CO₂ (type 6)⁸. Type 4 corresponds to a transition type. Types 5 and 6 are related to the so-called gas-gas equilibria of the first kind (type 6) and of the second kind (type 5). Further details are given in the literature^{7,8}.

PRESSURE-INDUCED SOLUBILITY EFFECTS AND FLUID CHROMATOGRAPHIC PERFORMANCE

According to one of the main principles of column chromatography, the sample should enter the column as a narrow "plug" achieved in normal gas and liquid chromatography by well known techniques. In SFC, however, this achievement is linked with the important condition that the amount of the sample injected must be dissolved completely in the amount of the mobile phase present in the injection chamber under operational conditions.

It follows from the $p(x)$ sections for constant separation temperature (T_1 , T_2) in Fig. 1 (e-k) that CO₂ systems of type 1 (Fig. 1e) can become completely miscible by an isothermal compression only. Type 2 systems (Fig. 1f) also conform with this pressure-induced complete mutual miscibility*. Type 3 systems also become totally miscible with increasing pressure if the separation temperature lies above the temperature minimum of the right-hand branch of the critical $p(T)$ locus curve (Fig. 1g and 1h).

For all of these systems, a rapid increase in mutual solubility and consequently in the migration rate can be deduced if the pressure approaches and exceeds the values corresponding to the pressure maxima in the isothermal $p(x)$ sections. This rapid increase has been confirmed in SFC experiments for a series of substances¹⁷⁻¹⁹. Giddings³ has characterized this effect by the term "threshold pressure". As these pressures commonly lie in the neighbourhood of the critical pressure of pure CO₂ for many compounds, a loss of selectivity is unfortunately inevitable. Therefore this effect shows the necessity for a sensitively controlled pressure programme for the separation of very similar substances^{6,17,18}; on the other hand, a pressure programme is

* Except that the right-hand branch of the critical $p(T)$ locus curve ends at a crystallization surface in the p - T - x space above the separation temperature.

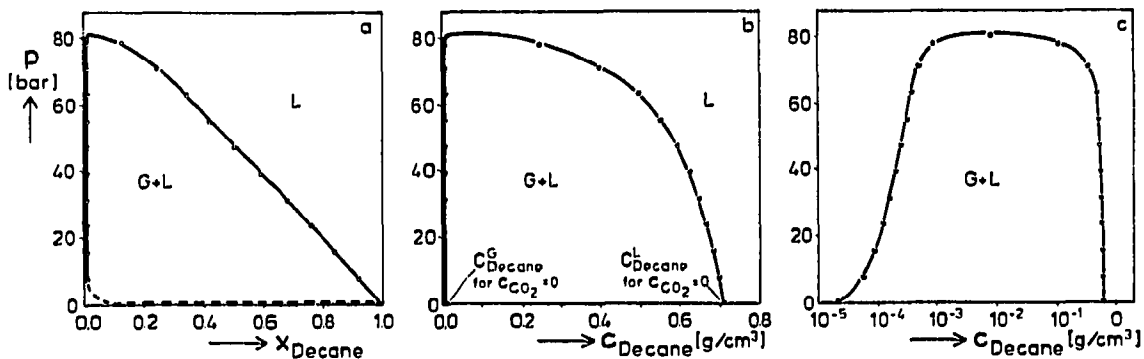


Fig. 2. Pressure-composition diagrams for the system CO_2 -*n*-decane at 37.8° according to results of Reamer and Sage²⁰ (x = mole fraction; c = concentration in mole/cm³). (a) Pressure versus mole fraction; (b) pressure versus concentration; (c) as (b), on a logarithmic scale.

of advantage for the separation of substances having a wide range of molecular weights, boiling points, polarities, etc.*^{2,6}.

The solubility of the samples in CO_2 , corresponding to the left-hand side of the $p(x)$ sections in Fig. 1 (e-k) can be extremely low. This is demonstrated in Fig. 2a, where the isothermal $p(x)$ section for the system CO_2 -*n*-decane is plotted at 37.8° according to the experiments of Reamer and Sage²⁰. Figs. 2b and 2c show the same diagram with the concentration plotted in grams per cubic centimetre. For substances with lower solubilities, it can lie even in the parts per million range. When normal amounts of these samples (0.1–5 μl) are introduced into an injection chamber of normal internal volume (5–20 mm³), peak broadening is always found because the amount injected cannot be dissolved completely in the amount of mobile phase present. In extreme cases, only a very diffuse peak can be detected. Types 4–6 of the CO_2 systems show such a strongly reduced mutual miscibility. Furthermore, it follows from their $p(x)$ sections (Fig. 1i and 1k) that the miscibility gap cannot be closed by an increase in pressure only.

A typical example showing the decrease in solubility in a sequence of similar compounds is given in Fig. 3. The chromatogram gives evidence of sufficient miscibility for the *n*-alkanes from *n*-C₅ to *n*-C₂₀, whereas *n*-C₂₂ shows a diffuse peak that is certainly caused by the solidification of this substance in the injection chamber. Other higher *n*-alkanes in this sequence could not be detected, and even an increase in the pressure up to 500 bar did not improve the results. An analogy was found by Jentoft and Gouw²¹ in separating the *n*-alkylbromides**. In such cases, an increase in solubility can often be obtained by raising the separation temperatures¹⁷ or by changing the operational conditions to those of liquid chromatography²².

In the ambient temperature region (which is of particular interest for biochemical applications), additionally ethane, ethylene, some Freons^{23,24}, sulphur hexa-

* In contrast to liquid chromatography, however, the pressure drop in an SFC column must be small otherwise de-mixing effects can occur and produce unsatisfactory results. Therefore, the regulation of the flow-rate of the mobile phase by varying the pressure drop seems to be an unreliable method in SFC.

** A typical example of a chromatogram for a substance of very low solubility was given by Giddings and co-workers^{1,3}, showing once again that the application of pressure alone cannot guarantee sufficient solubility.

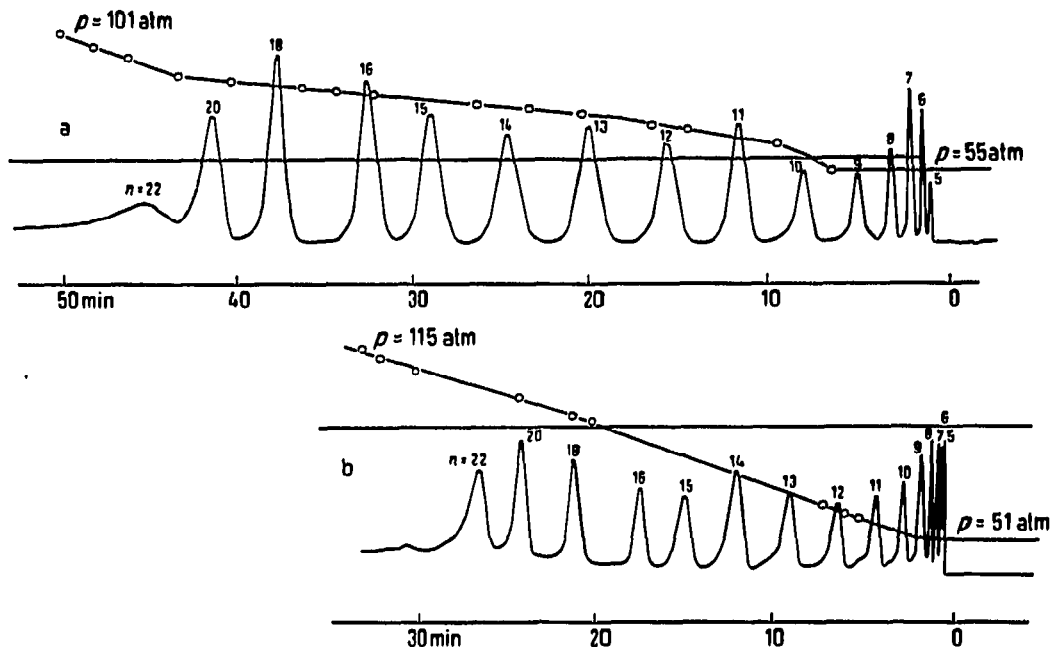


Fig. 3. Chromatograms of *n*-alkanes¹⁷. Column: steel, 3 m × 1.7 mm I.D., Carbowax 400/Porasil C. Mobile phase: CO₂. (a) 40°, 1.00 l/min (N.T.P.); (b) 55°, 2.00 l/min (N.T.P.).

fluoride and nitrous oxide would be suitable as mobile phases^{1,22}. For temperatures near 200°, several mobile phases exist; some workers^{22,25-28} have shown that here SFC is a useful tool for the separation of polycyclic hydrocarbons, polymers, etc. Furthermore, Novotný *et al.*¹⁰, Doran²² and Jentoft and Gouw²⁸ have pointed out that in this temperature range the addition of a small amount of a polar liquid to a non-polar supercritical mobile phase causes a significant reduction in the retention times; similar moderator techniques also seem to be possible for CO₂ in the ambient temperature region.

THERMODYNAMIC ASPECTS OF SFC

In partition chromatography, pressure-induced solubility phenomena can be discussed by means of the isothermal pressure dependence of the so-called capacity ratio, k_i , of a sample i :

$$k_i = \frac{c_i''}{c_i'} \cdot \frac{V''}{V'} = K_i \frac{V''}{V'} \quad (1)$$

where

$$K_i = c_i'' / c_i' \quad (2)$$

is the partition coefficient and V'' , V' and c_i'' , c_i' are the volumes and the concen-

trations of the stationary (double prime) and mobile (single prime) phases, respectively. Normally k_i can be determined experimentally by means of the relationship

$$k_i = \frac{t_i' - t^0}{t^0} \quad (3)$$

where t_i' and t^0 are the retention times of the sample and an inert substance, respectively.

From the isothermal pressure dependence of the partition equilibrium of the sample i at infinite dilution, it follows from eqn. 1 for $V' \gg V''$ and $\kappa' \gg \kappa''$ that

$$\left(\frac{\partial \ln k_i}{\partial p} \right)_T = \frac{V_i^{\infty'} - V_i^{\infty''}}{RT} - \kappa' \quad (4)$$

where $V_i^{\infty'}$ and $V_i^{\infty''}$ are the partial molar volumes of the sample i at infinite dilution (symbol ∞) in the mobile (single prime) and stationary (double prime) phase, respectively, and κ' and κ'' are the compressibilities of these phases. Numerical values of κ and V_i can be calculated from very accurate pVT data, no sufficiently accurate values being hitherto obtainable from statistical theories; some interesting new aspects have been published elsewhere^{29,30}.

Eqn. 4 includes gas and liquid chromatography as limiting cases. For liquid chromatography, the mobile phase is a liquid and eqn. 4 can be written in the form

$$\left(\frac{\partial \ln k_i}{\partial p} \right)_T \approx \frac{(V_i^{E\infty})' - (V_i^{E\infty})''}{RT} \quad (4a)$$

where $(V_i^{E\infty})'$ and $(V_i^{E\infty})''$ are the partial molar excess volumes of the sample i at infinite dilution and $\kappa' \approx 0$. For gas chromatography the mobile phase is a gas and eqn. 4 can be transformed into

$$\left(\frac{\partial \ln k_i}{\partial p} \right)_T \approx \frac{2B_{1i} - V_i''}{RT} \quad (4b)$$

where the validity of the equation of state

$$V' = \frac{RT}{p} + B'_m$$

is assumed for the mobile phase (where $B'_m = x_1^2 B'_{11} + 2 x_1 x'_i B'_{1i} + x_i'^2 B'_{ii}$, B'_{11} and B'_{ii} are the second virial coefficients of the pure mobile phase 1 and the sample i , respectively, and B'_{1i} is the so-called mixed second virial coefficient); eqn. 4b has already been derived by Sie *et al.*³¹ A detailed discussion of eqn. 4 will be published elsewhere.

Fig. 4 shows some k values that have been determined from our experimental results. At low pressure, the $\log k$ versus p curve is approximately a straight line, agreeing with the results of other workers^{26,31}. With increasing pressure, however, it shows a negative curvature for *n*-decane, *trans*-5-decene and 1-decene (see also

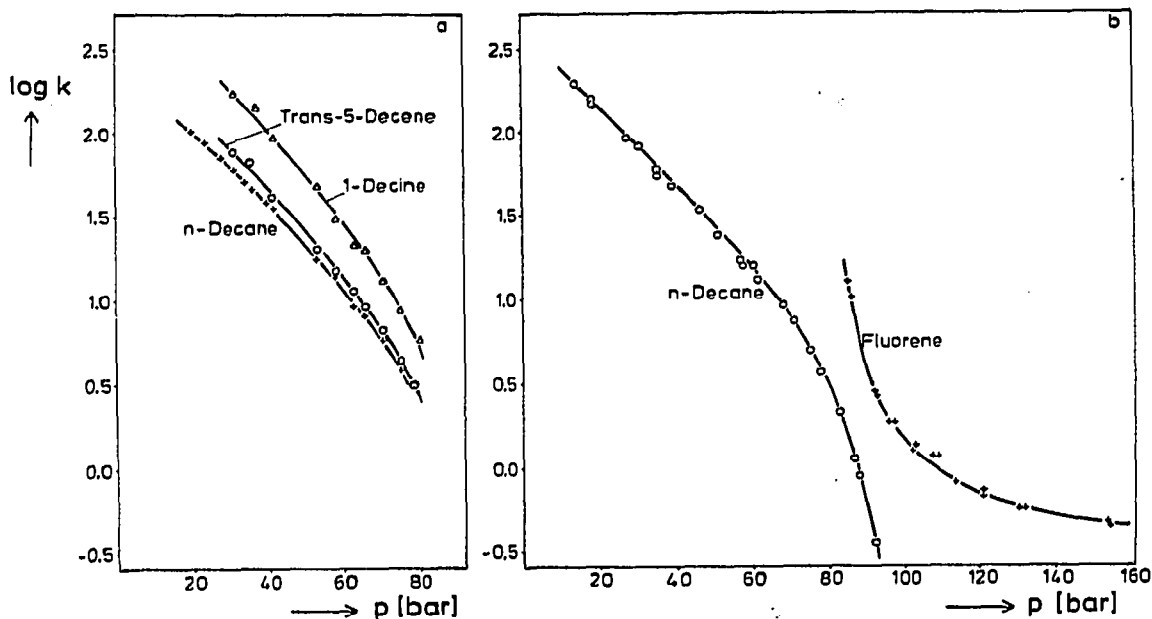


Fig. 4. Pressure dependence of the capacity ratio, k , of some hydrocarbons. Column: steel, 2 m \times 1.4 mm I.D. Mobile phase: CO_2 , 40°, 0.5 l/min (N.T.P.). (a) Stationary phase: OPN/Porasil C, 80–100 mesh. (b) Stationary phase: Carbowax 400/Porasil C, 100–120 mesh.

Ref. 31) and a positive curvature for fluorene. The former can be explained by the rapid increase in mutual solubility with supercritical CO_2 when the pressure is increased to a region of complete miscibility (e.g., above 80 bar for the CO_2 -*n*-decane system; see Fig. 2a), whereas the latter can be attributed to a phase characteristic of the type shown in Fig. 1i or 1k for the CO_2 -fluorene system.

MASS TRANSFER ASPECTS IN SFC

Based on a detailed discussion of elementary processes in a packed column for partition chromatography, Wicke³² has expressed the Van Deemter equation in a more generalized form. It is to be presumed that the assumptions for this equation are also applicable to SFC. Additionally, the following assumptions were introduced⁶:

(1) An estimation of thickness of the boundary layer of the fluid phase according to Prandtl's theory is possible.

(2) For chemically bonded stationary phases, the mass transfer resistance in the stationary liquid film is negligible in comparison with the resistance in the pores of the support and the boundary layer.

(3) The physical properties of the mobile phase (e.g., its density, ρ' ; viscosity, η' ; and diffusion coefficient, D') can be represented by the corresponding values of the pure mobile phase.

With these assumptions, the Van Deemter equation can be written in the fol-

lowing form:

$$\text{HETP} = B \cdot Re_p \cdot d_p + \frac{1.3 d_p}{Pe_p^*} + \frac{1.5 k}{(1+k)^2} \cdot g^* \cdot Pe_p^* \quad (5)$$

where

B is a coefficient of the packing geometry of the column (order of magnitude normally 0.1);

Re_p is the Reynolds number, related to the average particle diameter d_p of the support; $Re_p = (\bar{u} \cdot d_p \cdot \rho') / \eta'$, where \bar{u} = linear velocity of the mobile phase in the column;

Pe_p^* is the Peclet number of mass transfer, also related to the average particle diameter; $Pe_p^* = (\bar{u} \cdot d_p) / D'$;

k is the capacity ratio (see eqn. 1);

g^* is defined as $\frac{\text{volume of stationary phase}}{\text{active area of mass exchange}}$.

Eqn. 5 shows the three typical terms of the classic Van Deemter equation; the first two are related to the convective and the molecular dispersion effects, and the last to the retarded set-up of the partition equilibrium.

The estimation of the convective or eddy diffusion term is taken from a model described by Schlünder³³ and the results are compared with values published by Wicke³².

As a first approximation the pressure-induced parameters in eqn. 5 are only Re_p , Pe_p^* and k ; the first two can easily be calculated, while the last has to be determined experimentally (see Fig. 4).

The viscosity, η' , and the product $D' \cdot \rho'$ of the diffusion coefficient and the density of fluid CO_2 at pressures up to *ca.* 100 bar are not very different from the corresponding values in the diluted gaseous state. The low viscosity and the relatively high diffusivity are the great advantages of mass transfer in SFC in comparison with high-pressure liquid chromatography.

An estimation of the orders of magnitude of Re_p and Pe_p^* for typical SFC experiments reveals that the second term on the right-hand side can be neglected at all pressures of interest for SFC (see also refs. 19 and 34). Therefore, eqn. 5 can be rewritten in the following form:

$$\text{HETP} = \left(\frac{B \cdot d_p}{Sc} + \frac{1.5 k}{(1+k)^2} g^* \right) Pe_p^* \quad (6)$$

where $Sc = Pe/R_p^*$ is the Schmidt number of mass transfer.

Fig. 4 indicates that high k values are related to low pressures and *vice versa*. Therefore, at low pressures, normally the first term in parentheses in eqn. 6 prevails; HETP values in the range 2–6 mm result from our experiments and calculations. At higher pressures (70–100 bar), the k values of substances that show sufficient migration under operational conditions decrease to the order of 1 or less and the influence of the second term increases. At still higher pressures (which are of minor

importance for SFC, according to our experience), the HETP is mainly determined by the rapid increase in Pe_p^* , whereas the influence of both of the terms in parentheses in eqn. 6 becomes further reduced.

Generally, the HETP increases if Pe_p^* rises with increasing pressure. According to the parameters that determine Pe_p^* , it follows that the pressure-induced drop in the diffusion coefficient can be almost completely compensated for by an appropriate decrease in the linear velocity of the mobile phase in the column in the pressure range up to ca. 100 bar. The flow-rate of the mobile phase (reduced to N.T.P. conditions) behind the exit of the column was therefore controlled to constant values for all pressures^{6,19}. Hence our HETP results are not directly comparable with the data of Novotný *et al.*¹⁹ and Sie and Rijnders³⁴.

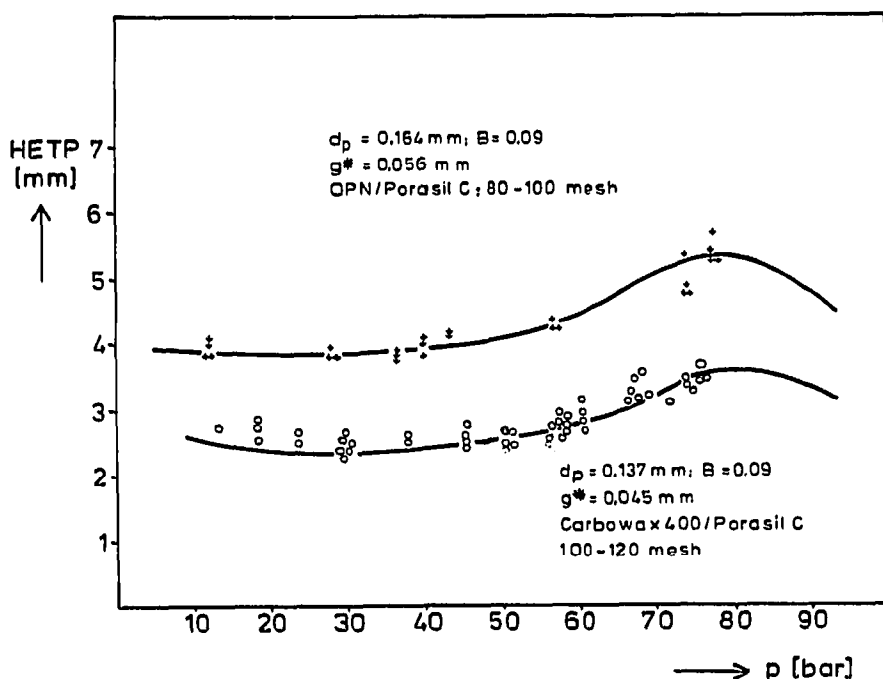


Fig. 5. Pressure dependence of HETP for two different columns. Test substance, *n*-decane; full lines = calculated values according to eqn. 6. Column: steel, 2 m \times 1.4 mm I.D. Mobile phase: CO₂, 40°, 0.5 l/min (N.T.P.).

Fig. 5 shows the experimental results and the curves calculated from eqn. 6 for *n*-decane as a representative sample. Two different stationary phases were used, demonstrating the influence of different values of d_p , g^* and k . For the calculations, the k values from Fig. 4 were used; since B and g^* cannot be obtained directly, the values were adjusted to the experiment. The calculated maxima of the HETP *versus* p curves in Fig. 5 correspond to the extreme value of the function $k/(1+k)^2$ for $k = 1$.

For $k < 1$, the accurate experimental determination of k and HETP becomes difficult, but this region is not of interest for practical separations. Our results show that no great increase in HETP values is caused by increasing the pressure to *ca.* 100 bar.

CONCLUSIONS

Considering the phase-theoretical and mass transfer aspects discussed above, it follows that SFC can be a useful tool for the separation of various substances. However, for separations with CO₂ as the mobile phase in the ambient temperature region, its application is limited and the variation of pressure alone does not always produce satisfactory results. Therefore investigations with other fluid phases or with CO₂ with the addition of a moderator have to be carried out. The non-existence of an on-line detector operating under elevated pressures did not prove to be a disadvantage in our experiments; we used a modified flame-ionization detector where the expansion takes place only a few millimetres below the nozzle and no disturbance effects were found^{6,17}.

ACKNOWLEDGEMENTS

The authors thank Mr. U. Van Wasen for his assistance with the experimental investigations. Financial support from the Deutsche Forschungsgemeinschaft and the Fonds der Chemie is gratefully acknowledged.

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