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# HIGH-PRECISION APPARATUS FOR PHYSICO-CHEMICAL MEASUREMENTS BY CAPILLARY SUPERCRITICAL FLUID CHROMATOGRAPHY

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## SUMMARY

A capillary chromatograph suitable for physico-chemical measurements by near- or supercritical fluid chromatography is described. The apparatus makes it possible to control the column temperature and the column inlet pressure to within ±0.01 K and ±0.2 bar, respectively. The system's precision has been evaluated for the measurements of certain diffusion and thermodynamic characteristics. The factors affecting precision are further discussed.

## INTRODUCTION

Chromatographic techniques have often been utilized to acquire various thermodynamic and/or kinetic characteristics of a chromatographic system[1,2]. The major advantages of such techniques over more conventional aproaches are the possibilities to work with very small amounts and/or at very low concentrations of the compounds studied. In the case of the thermodynamic (*i.e.*, phase-equilibrium) studies by chromatography, there is a fundamental theoretical difficulty caused by the intrinsically dynamic (*i.e.*, non-equilibrium) nature of the chromatographic process. However, there is ample evidence[1,2] that the results of thermodynamic studies by chromatography generally agree with the results of conventional static methods to within the limits of experimental error.

With the recently growing importance of supercritical fluid and dense-gas media in both technological and fundamentally scientific directions, there is a need for the acquisition of reliable data on various solute transport and thermodynamic parameters. Since the first report[3] on supercritical fluid chromatography (SFC) in 1962, a number of physico-chemical studies with near- and supercritical mobile phas-

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es have been reported[4]. Diffusivities of various solutes in compressed mobile phases[5–10] and the effects of temperature and pressure on solute retention[8,9,11–30] were investigated. The systems described generally involved a modified commercial gas chromatograph together with some type of a pump serving to deliver the compressed phase. While the described operational controls of such instruments may be satisfactory for most analytical applications of SFC, more precise controls are desirable to acquire meaningful physicochemical parameters.

In this communication, we describe a high-precision capillary chromatograph that is suitable for both diffusion and thermodynamic studies with mobile phases at elevated pressures. The advantages of a capillary system are stressed, as are the precise temperature and pressure controls, and the sample introduction through an extraction vessel. The most critical factors affecting the system's reproducibility have been investigated, as shown with the model diffusion and thermodynamic measurements. More recently, the value of this system has been demonstrated on measuring the diffusion characteristics of isomeric polycyclic hydrocarbons[32].

## DESCRIPTION OF THE APPARATUS

A schematic diagram of the apparatus is shown in Fig. 1. An injection valve (type C14WHC, Valco Instruments, Houston, TX, U.S.A.), splitter T-connection, and a fused-silica capillary column are immersed in the Therminol 55 heat transfer medium (Monsanto, St. Louis, MO, U.S.A.) in a circulating bath (Type N3-B, Haake Mess-Technik, Karlsruhe, F.R.G.). The injection valve is operated from outside the oil bath by a helical-drive air actuator (Type A 90 6, Valco Instruments). The mobile phase is fed to the injection valve from a syringe pump (Type 8500, Varian Assoc., Palo Alto, CA, U.S.A.) through a 2-$\mu$m filter union (Tye ZUFR1, Valco Instru-

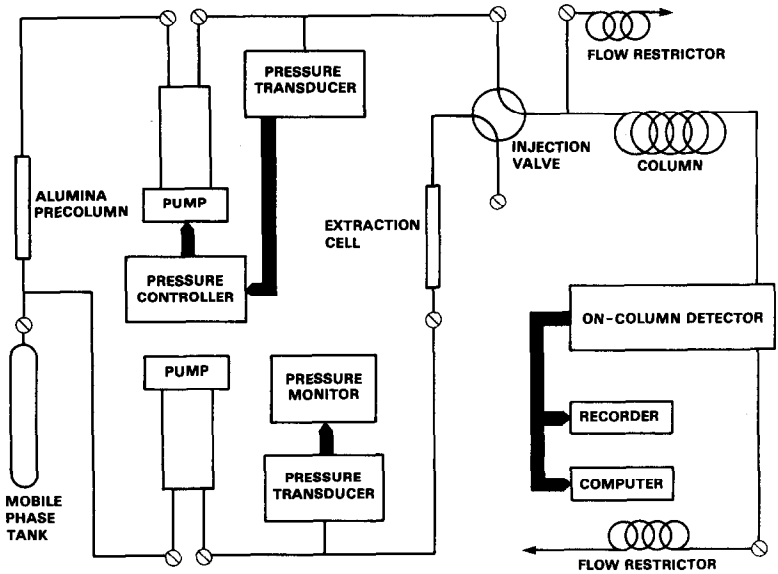


Fig. 1. Schematic diagram of the apparatus.

ments). The pump communicates with a pressure-controlling computer (TRS-80 Color Computer, Radio Shack, Ft. Worth, TX, U.S.A.) through a home-made interface. The column-inlet pressure is read by a capacitance-type sensor (Model 204, Setra Systems, Acton, MA, U.S.A.). The flow-rates of the mobile phase through the column and through the splitter vent are controlled pneumatically by 50 $\mu$m I.D. glass capillary restrictors of proper lengths. The restrictors are connected to the system through miniature on–off valves (Type 15MW-A200, Scientific Systems, State College, PA, U.S.A.). The current instrument set-up includes an on-column fluorometric detector (modified type FS 950 Fluoromat, Kratos Analytical Instruments, Ramsey, NJ, U.S.A.). The detection-cell housing is kept at the column temperature by circulating the column-bath oil through it. The 20–25 mm long on-column detection cell is formed by burning-off the polyimide coating of the fused-silica capillary column. The signal from the detector is fed to a strip-chart recorder (Type Fisher Recordall Series 5000, Houston Instruments, Austin, TX, U.S.A.), and, optionally, to a computer (type IBM PC, IBM Instruments, Danbury, CT, U.S.A.) for subsequent data processing. It is possible to inject either liquid solutions at barometric pressure or fluid solutions at elevated pressures. In Fig. 1, the latter alternative is shown. The fluid mixture to be injected is prepared and stored in an extraction cell. The internal volume of the cell is 28 ml, and, if necessary, it can be reduced by cylindrical inserts of proper sizes. Both the body of the cell and the inserts were machined from 316 stainless steel. The temperatures of the extraction cell, of the connection between the cell and the injection valve, and of the connection between the oil bath and the detection cell are controlled independently by electronic control units (6100 Series temperature controllers, Omega Engineering, Stamford, CT, U.S.A.). The solvent used to prepare the solution to be injected is delivered to the extraction cell from a syringe pump (Type 4100, Varian Assoc.). The pressure in the cell is monitored by a pressure transducer (Type P 2501, Schaevitz Engineering, Pennsauken, NJ, U.S.A.).

## APPLICATIONS OF THE APPARATUS

### *Diffusion measurements*

In the first series of experiments, the apparatus was applied to the measurement of limiting diffusion coefficients of three- and four-ring polycyclic aromatic hydrocarbons in near- or supercritical propane by the Taylor dispersion technique. The results of this study and the discussion of the major sources of error are given elsewhere[32]. In accordance with the detailed analysis given by Alizadeh *et al.*[33], much effort was spent to minimize deviation of the operation of the diffusion apparatus from an ideal description of it.

Here, we describe briefly the results of the preliminary tests of the performance of the apparatus when used for the diffusion measurements.

Fig. 2 shows the plot of the variance of the diffusion peak of phenanthrene in supercritical propane (111.2°C, 1500 p.s.i.) as a function of the logarithm of the concentration of the solution injected. The solvent employed was a mixture of xylenes, while phenanthrene concentration ranged from 0.015 to 2 mg/ml; the volume of the injection loop in the Valco valve was 100 nl; and the splitting ratio was 4:1. The diffusion tube used for these experiments was an 18.8-m long empty fused-silica column of 0.2 mm I.D. Fig. 2 suggests that concentrations close to the upper limit of

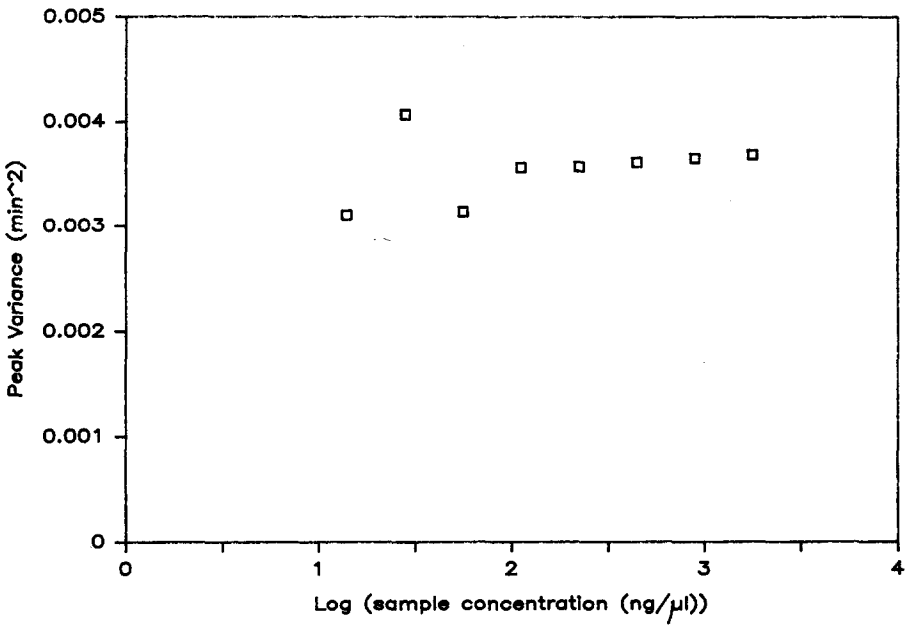


Fig. 2. Variance of the diffusion peak of phenanthrene *vs.* the logarithm of the concentration of the solution injected.

the range investigated are preferable. On decreasing the concentration of the injected solution, the concomitant decrease of the signal-to-noise ratio can contribute significantly to uncertainty in the resulting peak variance.

The volume of the injection loop in the Valco valve is probably more important than the concentration of the injection solution, as it is the volume injected (together with the splitting ratio), rather than the concentration, that actually controls the initial pulse-width. Injection loops with volumes of 100, 200, and 500 nl were tested in experiments similar to that shown in Fig. 2. The interdiffusion coefficients obtained from the measurements with 100 and 200 nl loops were nearly identical, while those from the 500 ml loop were significantly lower. This indicates that, with the largest loop, the width of the injected concentration pulse contributes significantly to the variance of the resulting peak. Consequently, the 100 nl loop was used throughout the remaining measurements.

The effect of the time constant of the fluorometric detector on the variance of the resulting peak is shown in Fig. 3. The conditions of the experiment were the same as in Fig. 2. Within the range investigated, the peak width appears invariant with respect to the detector time constant. The detector was difficult to operate at time constants less than 0.5 s. Therefore, the time constant of 0.5 s was used throughout the diffusion measurements.

The results of the preliminary tests shown in Figs. 2 and 3 were obtained with the injection of liquid solutions. The interdiffusion coefficients obtained from such experiments would be those of an aromatic hydrocarbon in an undefined mixture of propane and the solvent employed, rather than those in pure propane. In the recently

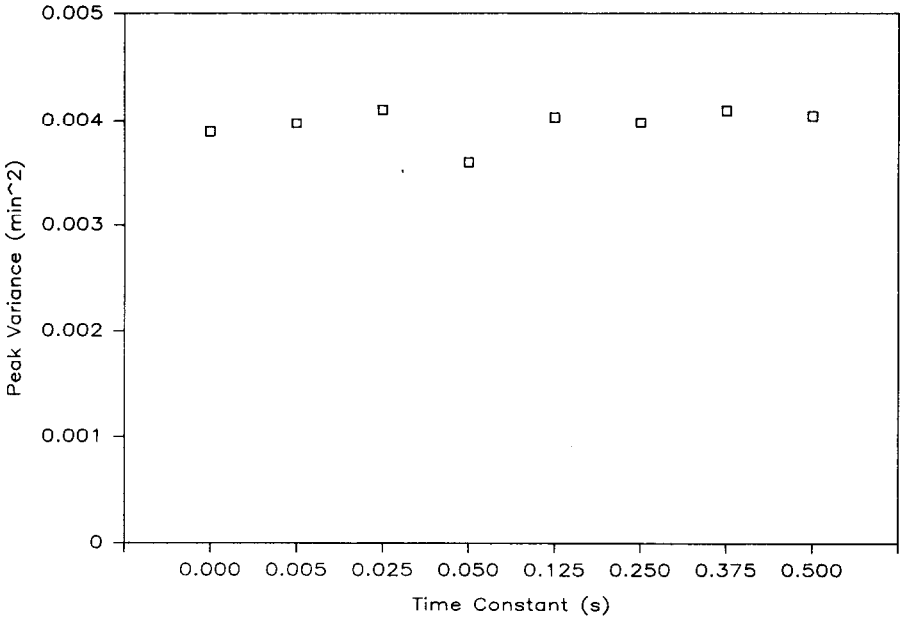


Fig. 3. Variance of the diffusion peak of phenanthrene *vs.* the time constant of the detector.

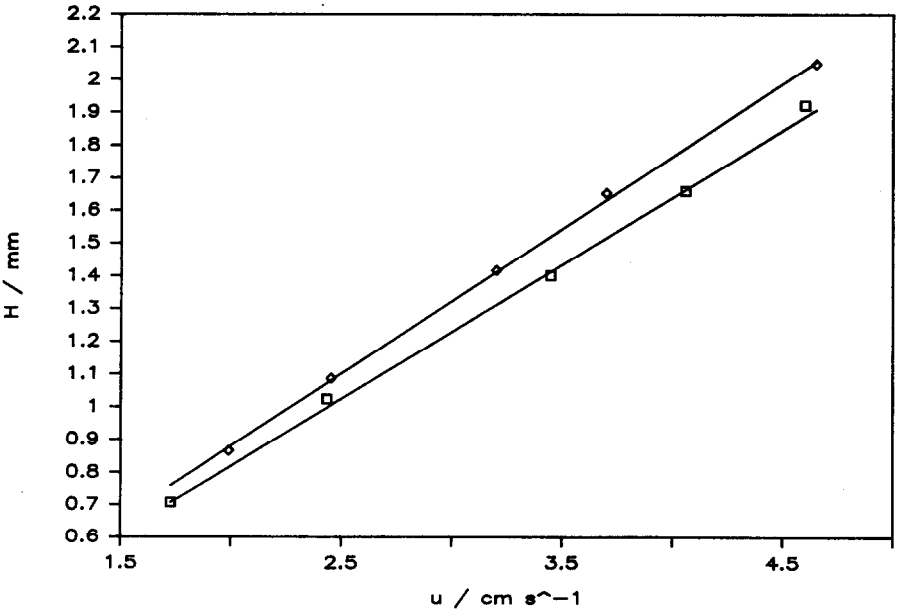


Fig. 4. Anthracene peak variance per unit column length ($H$) *vs.* the mean linear velocity of propane (density 0.3771 g $cm^{-3}$) in the diffusion column. (◇) 84.6°C, (□) 111.2°C.

reported diffusion measurements[32], a fluid mixture of propane and an aromatic hydrocarbon was injected. The mixture was prepared in the extraction cell (see Fig. 1) and kept at the temperature and pressure which were close to those in the diffusion tube itself (for most measurements, 111.2°C and 1500 p.s.i.). The amount of aromatic hydrocarbon deposited into the cell was intended to produce a concentration close to 2 mg/ml, but the actual concentration of the aromatic hydrocarbon was determined by its solubility in propane under given conditions. To our knowledge, no solubility data are available for aromatic hydrocarbons in supercritical propane, but an estimate based on recently published solubilities in supercritical ethane[34] suggests that, with the possible exception of phenanthrene, the actual concentration of an aromatic hydrocarbon in the mixture injected was lower than 2 mg/ml.

The day-to-day reproducibility of the interdiffusion coefficients was generally within the experimental error, as illustrated, for instance, by three consecutive values of limiting interdiffusion coefficient of anthracene in propane at 111.2°C and 1500 p.s.i.: $(1.73 \pm 0.02) \cdot 10^{-4}$, $(1.74 \pm 0.03) \cdot 10^{-4}$, and $(1.74 \pm 0.01) \cdot 10^{-4}$ cm$^2$ s$^{-1}$, obtained on different days.

The effect of temperature on interdiffusion coefficients at a constant density of propane was also studied. Fig. 4 shows a plot of the peak variance per unit column length ($H$) against the mean linear velocity of propane in the diffusion column for anthracene at two different temperatures. Each of the data points shown in Fig. 4 represents the mean of 4–5 injections.

*Chromatographic retention measurements*

For the investigation of the effects of column temperature and pressure on solute retention in supercritical fluid chromatography, capillary columns are generally preferable to packed columns. Besides the fundamental advantage of their larger permeability (see Introduction), capillary columns also require much lower mobile phase volumetric flow-rates as compared to packed columns. This results in several practical advantages, such as compatibility with flame-based detectors (if necessary), increased safety when working with flammable or toxic mobile phases, and decreased cost when working with expensive media.

The apparatus was applied to the study of the temperature and pressure (or density) effects on the retention of phenanthrene in an SE-30 silicone elastomer with carbon dioxide as the mobile phase. Two capillary columns were used: column A: 633 cm × 0.32 mm I.D., 2600 nm film of SE-30; and column B: 1049 cm × 0.20 mm I.D., 200 nm film of SE-30.

The SE-30 films were cross-linked with azo-*tert.*-butane[35]. The cross-linking procedure was repeated three times to ensure nonextractability of the stationary phase. With column A, the retention of phenanthrene was measured at 90°C and 100°C, while temperatures of 35, 40, 50, and 70°C were employed with column B. The solution of phenanthrene in xylenes (10 mg/ml) was injected through a Valco injection valve with a 100-nl injection loop. With the fluorometric detector, it proved to be impossible to find a compound that could serve as a marker of the dead retention time. Therefore, an indirect procedure was adopted. The volumetric flow-rate of the carbon dioxide mobile phase was measured with a soap-bubble flow-meter at the outlet of the column flow restrictor at the ambient temperature and pressure. This value, $\dot{V}_{amb}$, was converted to mass flow-rate, $\dot{m}$, using the equation

$$\dot{m} = P_{amb}\dot{V}_{amb}M/Z_{amb}RT_{amb} \quad (1)$$

where $P_{amb}$ is the ambient pressure, $T_{amb}$ is the ambient temperature, $Z_{amb}$ is the compressibility factor of carbon dioxide at the ambient temperature and pressure, $M$ is the molecular weight of carbon dioxide, and $R$ is the molar gas constant. The mass flow-rate of carbon dioxide throughout the system is invariant with respect to temperature and pressure, so that it is possible to calculate the volumetric flow-rate of carbon dioxide at the column temperature and pressure, $\dot{V}_{col}$, from the equation

$$\dot{V}_{col} = \dot{m}/\rho_{col} \quad (2)$$

where $\rho_{col}$ is the density of carbon dioxide at the column temperature and pressure. The quantities $Z_{amb}$ and $\rho_{col}$ have been calculated from the Lee-Kesler correlation[36] using the critical constants and acentric factors given by Reid *et al.*[37]. Provided that the column free cross-section and column length are known, the value of $\dot{V}_{col}$ can easily be converted to either the mean linear flow velocity in the column or the dead retention time. The procedure described suffers from the fact that it neglects the swelling of the stationary phase as the mobile phase dissolves in the stationary film at elevated pressures. The resulting error in the calculated dead retention time is negligible with column B (thin film), but it might be significant with column A. The mean linear flow velocities employed in the measurements ranged from 2 to 15 cm $s^{-1}$.

Figs. 5 and 6 show the plot of the logarithm of phenanthrene capacity ratio against the column inlet pressure for columns A and B. It is apparent from the plots

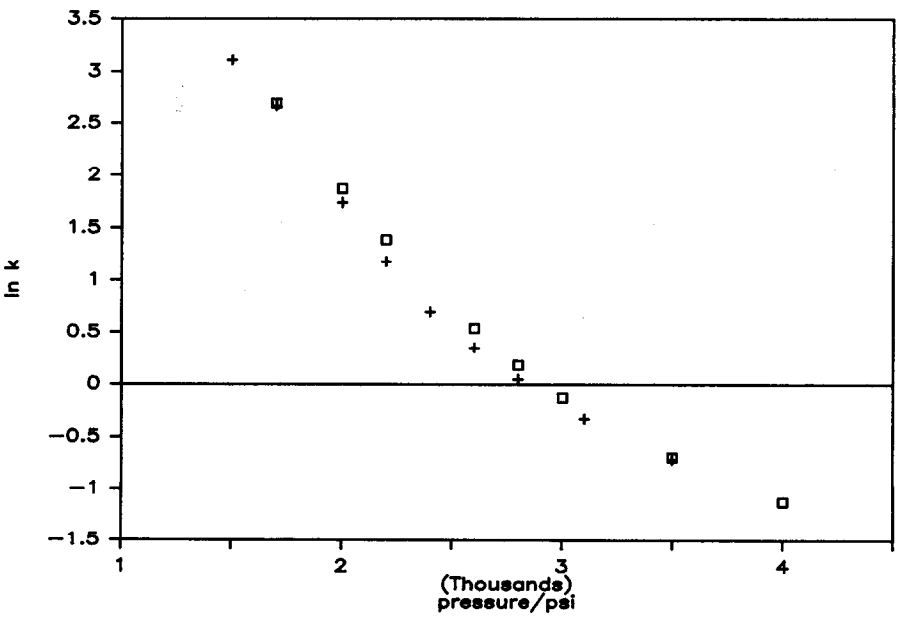


Fig. 5. Logarithm of phenanthrene capacity ratio *vs.* the column inlet pressure. Column A: 633 cm × 0.32 mm I.D., 2600 nm film of SE-30. (+) 90°C, (□) 100°C.

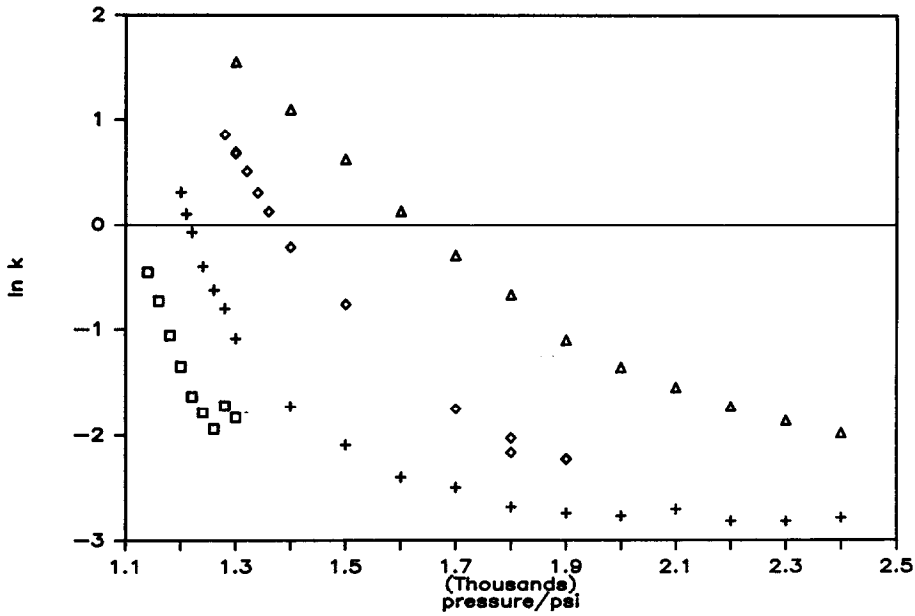


Fig. 6. Logarithm of phenanthrene capacity ratio *vs.* the column inlet pressure. Column B 1049 cm × 0.20 mm I.D., 200 nm film of SE-30. (□) 35°C, (+) 40°C, (◇) 50°C, (△) 70°C.

that the curvature of the isotherms decreases when increasing the temperature. This behavior can readily be interpreted considering the shape of the isotherms in the pressure-density diagram of carbon dioxide.

To date, retention in supercritical fluid chromatography (SFC) has generally been expressed in terms of the capacity ratio,

$$k = (t_R - t_0)/t_0 \tag{3}$$

where $t_R$ is the retention time of the compound in question and $t_0$ is the dead retention time. The capacity ratio depends on both the thermodynamic partition coefficient and the column geometry (the phase ratio). For the purposes of interlaboratory comparison of SFC retention data, as well as for the thermodynamic interpretation of the SFC retention, absolute retention data (*i.e.*, partition coefficients) are more desirable. In the course of the preparation of a capillary column, both the void volume of the column, $V_0$, and the mass of the stationary phase in the column, $m_s$, can be controlled to a fair accuracy. If these two quantities are known, it is possible to express the retention in terms of the partition coefficient given by

$$K = q_s/c_m = k(V_0/m_s) \tag{4}$$

where $q_s$ is the concentration (moles/mass) of the solute in the stationary phase and $c_m$ is the concentration (moles/volume) of the solute in the mobile phase. The partition coefficient defined by eqn. 4 has the dimension of volume/mass. Fig. 7 shows

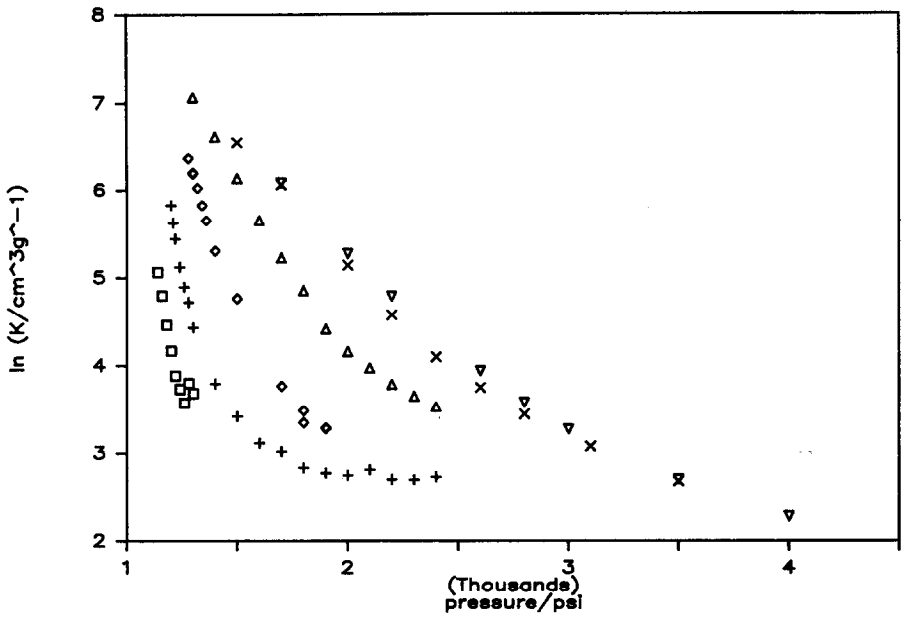


Fig. 7. Logarithm of phenanthrene partition coefficient *vs.* the column inlet pressure. (□) 35°C, (+) 40°C, (◇) 50°C, (△) 70°C, (×) 90°C, (▽) 100°C.

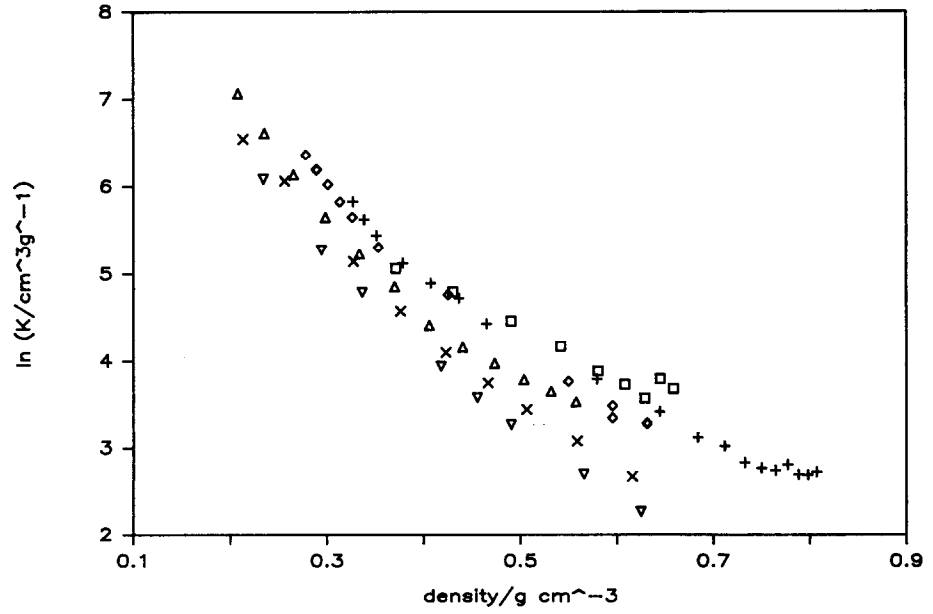


Fig. 8. Logarithm of phenanthrene partition coefficient *vs.* the density of carbon dioxide in the column. (□) 35°C, (+) 40°C, (◇) 50°C, (△) 70°C, (×) 90°C, (▽) 100°C.

a plot of the logarithm of the partition coefficient for phenanthrene against the column inlet pressure. The plot indicates a fair degree of consistency between the data obtained with the two columns. In Fig. 8, the plot is shown of the logarithm of the partition coefficient for phenanthrene against the density of carbon dioxide in the column.

The preliminary results shown in Figs. 5–8 illustrate the applicability of the present apparatus to retention studies in SFC. The application of this apparatus to the study of the specific intermolecular solute–stationary phase interactions in SFC systems is currently underway in this laboratory.

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