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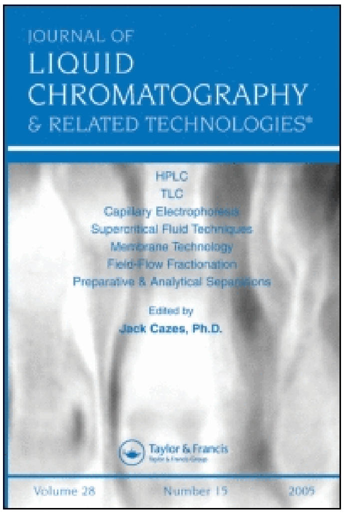

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Centrifugal Partition Chromatography. vi. Temperature Effects

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CENTRIFUGAL PARTITION CHROMATOGRAPHY. VI. TEMPERATURE EFFECTS

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ABSTRACT

Temperature changes induce numerous physico-chemical changes in liquids. Vapor pressure, density, refractive index, viscosity and surface tension are such temperature dependent parameters. The mutual solubilities of the stationary and mobile phase liquids are also temperature dependent. The ternary diagram of the water-methanol-hexane system is presented for two temperatures, 20° and 40°C. It is shown that a flow behavior change, as determined by an efficiency study, is temperature dependent because the viscosities of the liquids are modified by the temperature. A solute's partition coefficient is also dependent upon temperature. Using Van't Hoff plots, it is possible to determine the enthalpy of transfer of the solute from the mobile phase to the stationary phase. In the last part of this study, additional problems resulting from temperature changes in CPC are discussed.

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INTRODUCTION

Centrifugal partition chromatography (CPC) is a variation of countercurrent chromatography (CCC). As described in previous papers of this series (1,2), two immiscible liquids are used. One is the stationary phase, the other is the mobile phase. In each paper of this series specific results were presented and discussed (1-5); however, all results were obtained at 25°C.

It is well known that temperature changes induce numerous physicochemical solution parameter changes. Vapor pressure, density, refractive index, viscosity, surface tension are typical temperature dependent parameters. In CPC, when two immiscible liquids are put in contact with each other, any temperature change has a further influence on the mutual solubility and the interfacial tension of one liquid versus the other. When a solute is injected into the system, its partition coefficient and kinetics of transfer between the two liquids are also temperature dependent.

The aim of the present paper is to study the temperature effect in CPC. The general influence of temperature on some liquid systems is presented. The ability to link temperature-induced changes in CPC chromatograms to fundamental thermodynamic parameters is demonstrated. The variation of efficiency with temperature is related to the kinetics of solute transfer between liquid phases. The last part of this study presents some additional side effects that can be caused by temperature changes in CPC.

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EXPERIMENTAL SECTION

The experiments were performed using a Sanki centrifugal partition chromatograph, Model CPC-NMF, from Sanki Laboratories Inc., Sharon Hill, PA. This apparatus consists of up to 12 cartridges placed in the rotor of a centrifuge. Each cartridge, model 250 W, contains 400 channels of 50 uL each, as described in a previous paper (1). A LC pump (Shimadzu, model LC-6A) and a UV-vis detector (Shimadzu, model SPD-6A) with a preparative cell were connected to the CPC system to circulate the liquids and to detect the solutes, respectively. The rotor of the CPC apparatus was enclosed in a constant temperature box adjustable from 15 to 35°C. The two liquids of both systems, octanol-water and methanol (water 1% v/v)-hexane, were equilibrated in an ultrasonic bath at the working temperature. Octanol was supplied by Aldrich Chemical Company (Milwaukee, WI) and used as received. HPLC-grade water, methanol and hexane were from Fisher Scientific (Pittsburgh, PA). All solutes were of highest available purity and were obtained from Aldrich.

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RESULTS AND DISCUSSION

1-Physico-chemical Parameters

Table I presents the physico-chemical parameters: molecular weight, boiling point, density, viscosity and surface tension of the pure liquids used in this work. By definition, the molecular weight and boiling point of a liquid are not temperature dependent. The surface tension is somewhat temperature dependent. For example, the surface tension of methanol is 24.5 dynes/cm at 0°C, 22.6 and 20.2 dynes/cm at 20 and 50°C, respectively (6). The surface tension of water is 73.5; 72.0 and 70.4 dynes/cm at 15, 25 and 35°C, respectively. For pure organic liquids, the surface tension decreases by about 1 dyne/cm with every 10°C temperature increase (6).

The density and, particularly, viscosity are significantly modified by temperature changes. Both parameters decrease when the temperature increases. Between 15 and 35°C, the density of water decreases by 0.26 mg/cm^3 (= 0.26 °/oo) with every 1°C temperature increase. The density of

Table I - Physical-Chemical Parameters of Liquids Used.

Compounds	m.w. g/mol	b.p. °C	ρ g/cm^3	μ cP	σ dynes/cm
Methanol	32	65	0.791	0.55	22.6
Hexane	86	69	0.660	0.29	18.4
Octanol	130	194.5	0.827	5.0	27.5
Water	18	100	0.999	0.89	73.0

The viscosity values (μ) were measured at 20°C, the density (ρ) and surface tension (σ) values at 25°C, and the boiling points at 1 atmosphere.

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methanol decreases by 0.85 mg/cm^3 (= 0.11%) with every 1°C temperature increase (6).

Figure 1 shows the relationship between viscosity and temperature for three liquids. As will be shown, these variations are particularly important as far as CPC, or any counter current chromatography technique, is concerned. For example, the viscosity of octanol is 50% less when the temperature is increases by 10°C.

2- *Mutual Solubility*

When two liquids, 1 and 2, are immiscible, it means that their mutual solubility is very low. However, a finite amount of liquid 1 always dissolves in Liquid 2 and vice versa. This mutual solubility is very sensitive to temperature, increasing when the temperature increases. Many of the so-called immiscible liquid-liquid systems exhibit an upper critical solution

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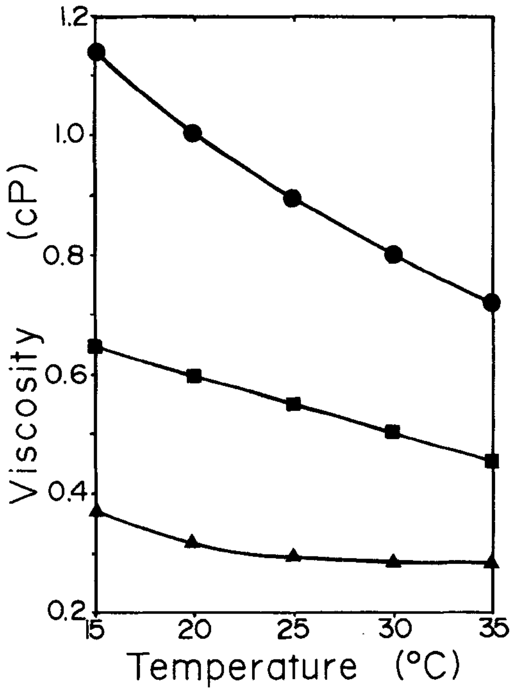

Figure 1: Viscosity changes versus temperature. ● water; ■ methanol; ▲ hexane. The octanol viscosity is listed in Table III.

temperature (CST) phenomenon. For any temperature above the CST, a binary liquid system becomes a monophase (7).

The CST point of the binary system methanol-hexane is 35°C. Indeed, the mutual solubility of hexane in methanol is very temperature dependent. For example, at 20°C, 100 mL of methanol can dissolve 90.8 mL of hexane to form saturated solution of methanol-hexane (composition 52.4-47.6 v/v) while at 30°C, 100 mL of methanol can dissolve about 500 mL of hexane; the corresponding saturated solution composition is methanol-hexane 17.3-82.7 v/v. As shown in a recent work (3), the addition of water

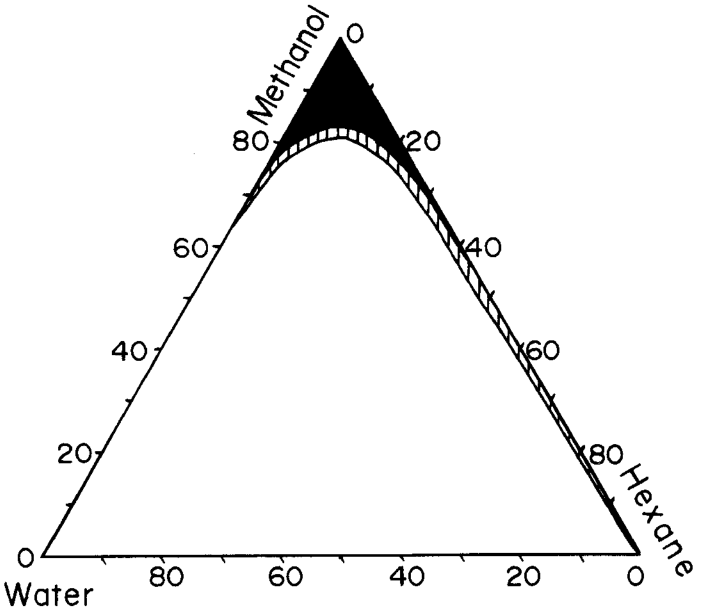

Figure 2: Mass diagram of the ternary system water-methanol-hexane. Black area: monophase domain at 20°C. Black + hatched area: monophasic domain at 40°C.
The solubility of the hexane in methanol is very much modified by temperature (Critical solubility temperature = 35°C, see text).

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dramatically decreases the hexane solubility in methanol. Figure 2 shows the solubility diagram of the ternary system water-methanol-hexane at 20°C and 40°C in mass percentage. With only 2% v/v of water added to the methanol (2.5% w/w), at 20°C, the saturated solution composition is (i) in volume: methanol (+ water 2% v/v)-hexane 74-26% v/v, or (ii) in mass: methanol (+ water 2.5% w/w)- hexane 77.5-22.5% w/w. At 30°C, the respective compositions are 73.1-26.9% v/v or 76-24% w/w.

The CST point of the binary system octanol-water is 182°C (7). This high CST value means that the variation of the mutual solubility of these two

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liquids between 15 and 35°C is relatively low (in the percentage range). Also, it should be noted that there are binary systems that have a lower CST. There even are a few that have both upper and lower CSTs.

3- Efficiency

The dependence of the efficiency on the temperature was studied with the binary system octanol-water because of the large viscosity changes of octanol in the temperature range 15-35°C, and because the CST point of the methanol-hexane system was too low (35°C, see previous paragraph). Figure 3 is a 3-dimensional plot of the results obtained with octanol as the mobile phase and water as the stationary phase. Figure 3A corresponds to the unretained solute pentachlorophenol (2,5). Figure 3B corresponds to the solute hydroquinone. In both cases, the efficiency increases with the temperature, as shown by Table II for the 3 mL/min flow rate. The pentachlorophenol efficiency first decreases as the flow rate increases and, above a minimum plate value, the efficiency increases with the flow rate. This phenomenon was noted and described in Part II of this series (2). To make a comparison with the classical van Deemter plot, the ratio 2400/N, that corresponds to the number of channels needed to obtain one theoretical plate, was plotted versus the flow rate. The ratio 2400/N in CPC corresponds to the HETP in liquid chromatography. Figure 4 shows the temperature dependence of the CPC "van Deemter" plots corresponding to Figure 3.

The maxima of the CPC "van Deemter" plots (or the minima of efficiency) are temperature dependent for the solute pentachlorophenol. Table III shows the coordinates of the maxima of the van Deemter plots and the corresponding Reynold's number, Re. The Re value can be calculated using:

$$\mathrm{Re} = (4\,F\,\rho)/(\pi\,d\,\mu) \qquad (1)$$

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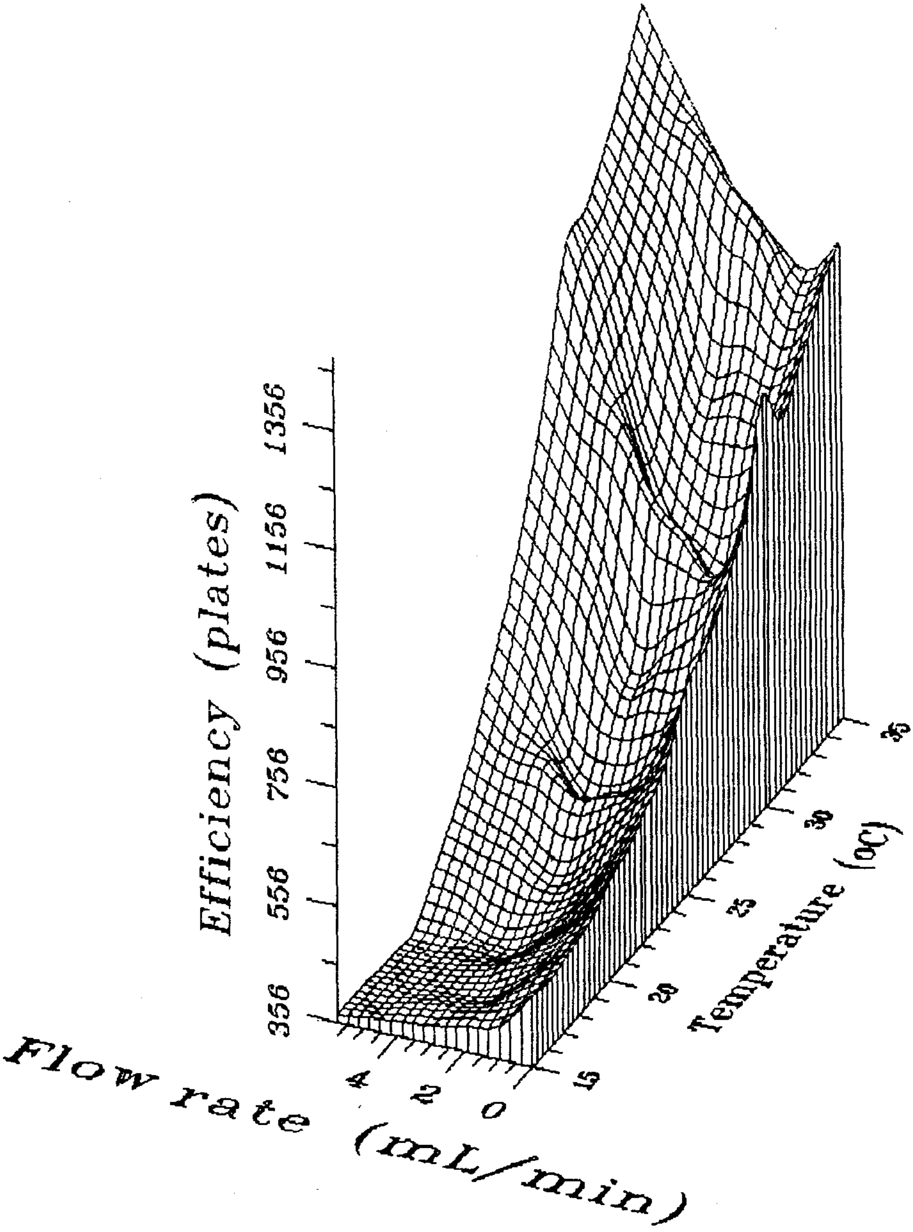

Figure 3: 3d-plot of the efficiency (vertical axis) versus the flow rate and the temperature (horizontal axises).
A - solute pentachlorophenol; B - solute hydroquinone.

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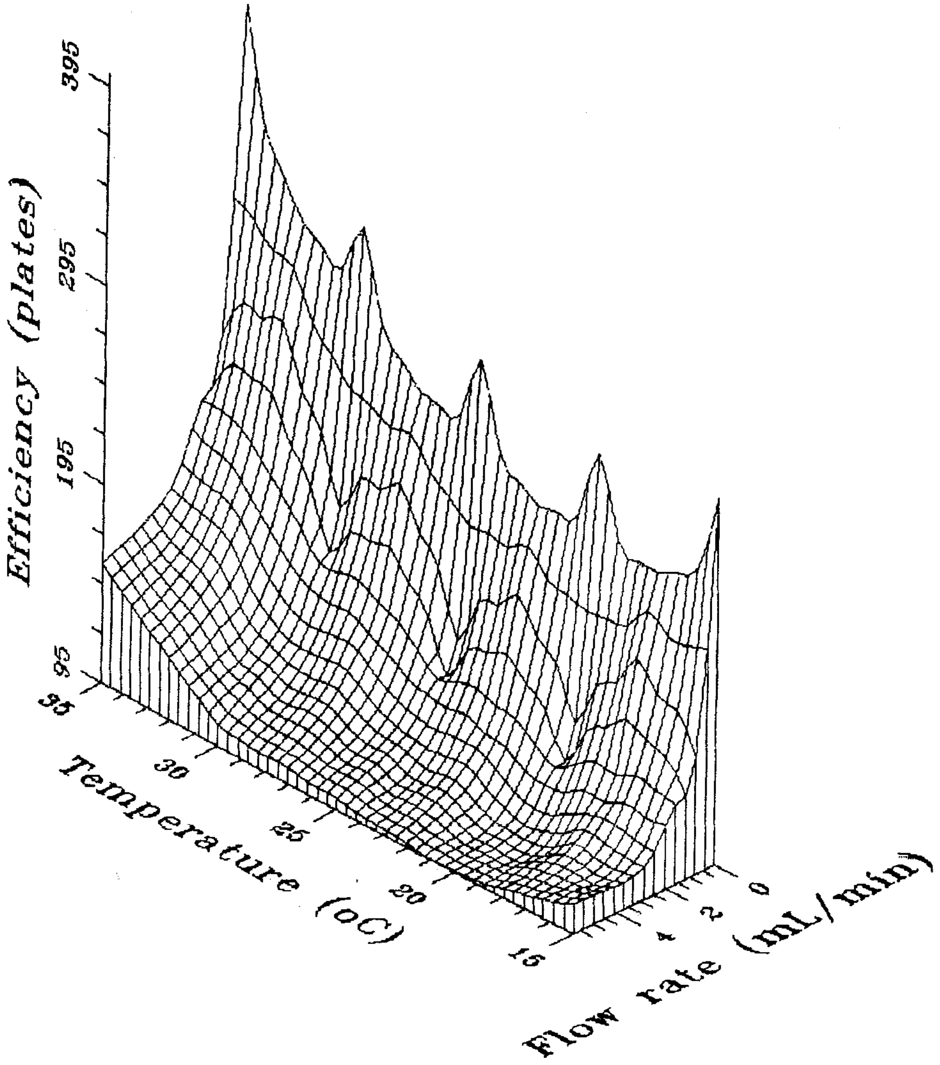

FIGURE 3B

Table II - Evolution of Efficiency and Retention Volume with Temperature[a]

Temperature ^{o}C	Pentachlorophenol v_r mL	Pentachlorophenol N plates	Hydroquinone v_r mL	Hydroquinone N plates	K^b
15	59.4	390	77.0	90	0.269
20	64.2	350	81.6	100	0.286
25	68.1	450	85.8	110	0.311
30	60.6	790	82.2	125	0.336
35	64.8	1190	86.4	180	0.359

[a] Flow rate 3 mL/min, mobile phase-octanol, Stationary phase -water.

[b] Because water was the stationary phase, K is the water-octanol partition coefficient. The classical octanol-water partition coefficient is the reverse ($K_{oct} = 1/K$).

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in which F is the flow rate (in mL/s), ρ is the liquid density (g/cm^3), d is the equivalent linear pipe diameter (cm) and μ is the liquid viscosity (Poise or g/(cm x 2)). The channel equivalent pipe diameter was estimated to be 3.4 mm (1,2).

Table III shows that the Re number that corresponds to the efficiency minima (or to the van Deemter maxima) is always about the same value regardless of the temperature. This indicates that some flow state change occurs around a Re number of 2.9 that is sufficient to reverse the efficiency versus flow rate trend (Figure 4). For a homogeneous solution in a linear pipe, the Re value of 2.9 is far too low to correspond to a flow change from

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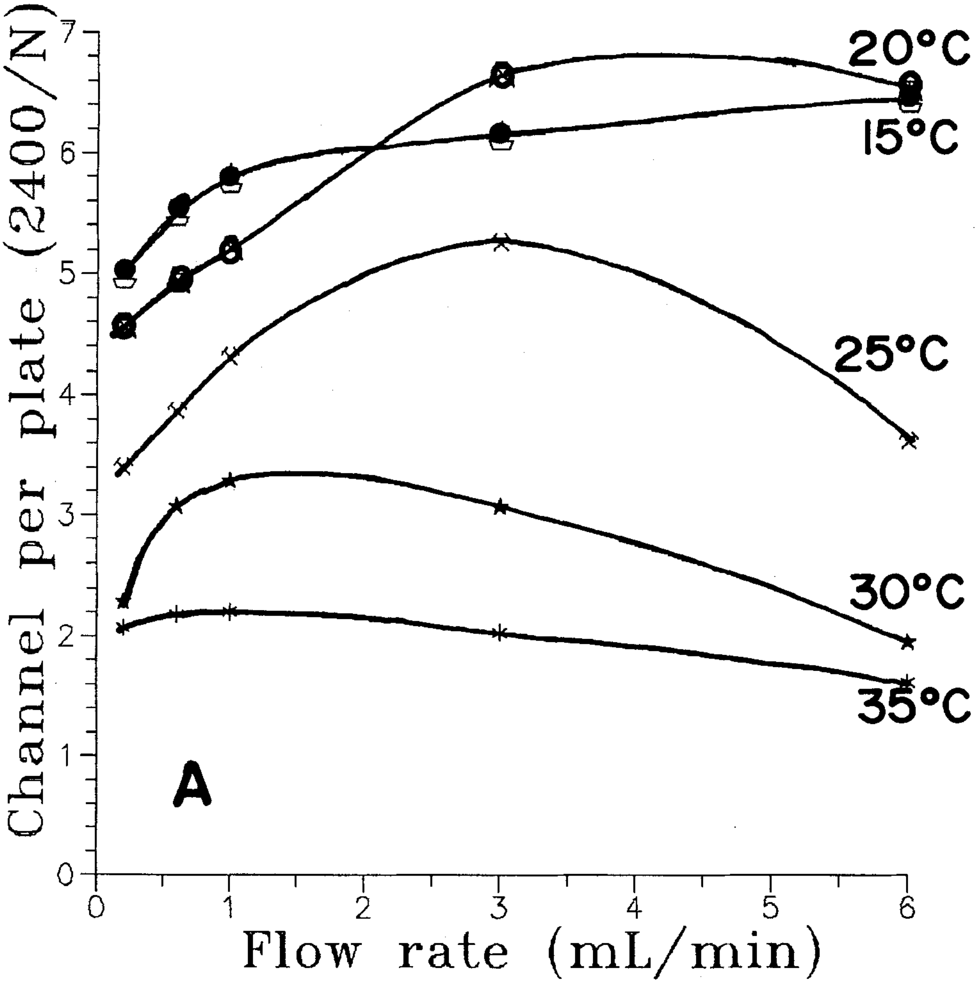

Figure 4: The CPC "van Deemter" plot at five different temperatures.
A - solute: pentachlorophenol; B - solute: hydroquinone.

(continued)

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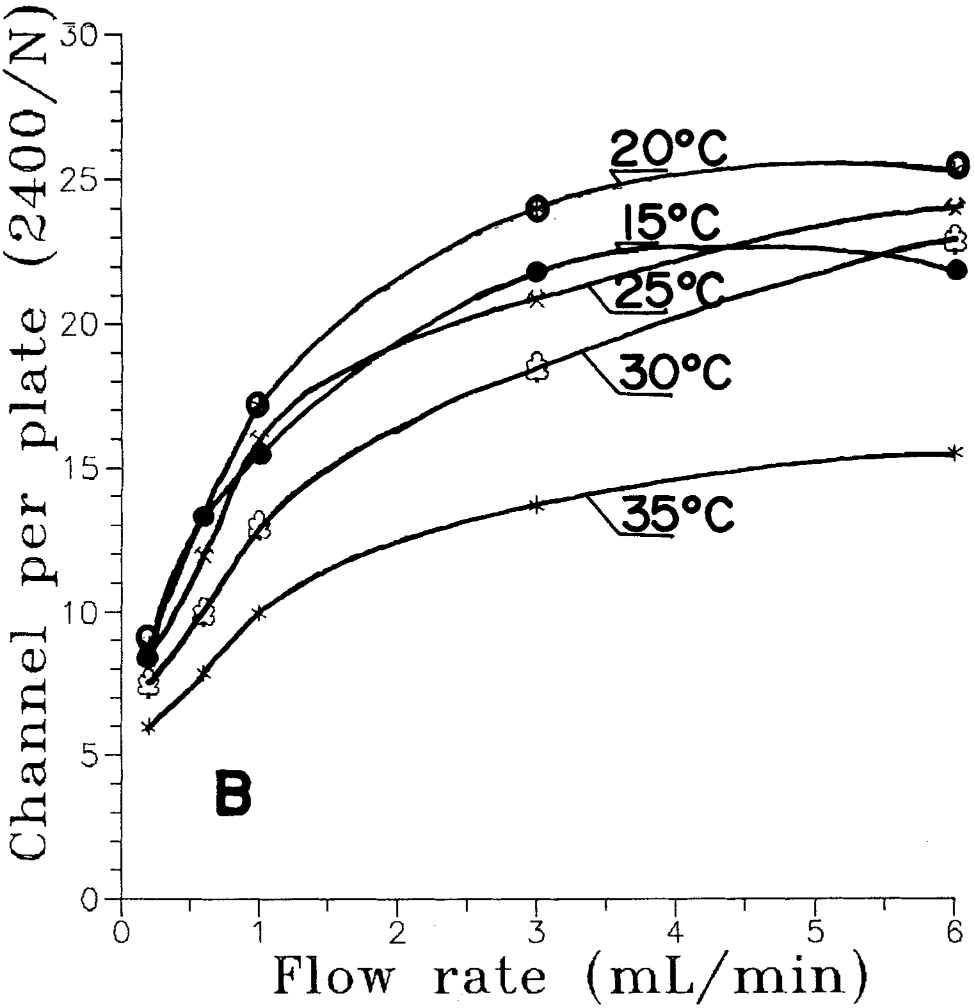

FIGURE 4B

Table III - Coordinates of the Maxima of the van Deemter Plots

Temperature o C	Flow Rate mL/min	N plates	2400/N ch/plt	Re	μ octanol cP
15	> 6	-	-	> 2.9	10.6
20	4.0	340	7.1	2.9	7.2
25	2.8	450	5.3	2.9	5.0
30	2.0	710	3.4	2.6	3.9
35	1.4	1090	2.2	2.7	2.7

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laminar to turbulent, which occurs for a Re value of 2000. However, given the shape of the CPC channels and the biphasic nature of the liquids in them, it is possible that some kind of an emulsified layer forms at the interface of the two liquids for a Re number as low as 2.9. As shown in Table III, the flow rate corresponding to the efficiency minimum decreases as the temperature increases. This is due to the decrease in the octanol viscosity (Figure 1).

The efficiency, calculated for the hydroquinone peak, is much lower than the one for the pentachlorophenol peak (Figure 3 and Table II). The exchange of hydroquinone between the two phases is slow, producing a low efficiency. An increase of temperature produces two effects: (i) an increase in efficiency and (ii) an increase in retention volume (Table II). The temperature increase enhances the hydroquinone exchange between phases, mainly because the decrease of the octanol viscosity induces an increase in the hydroquinone diffusion coefficient. The increase in retention volume is due to the increase of the partition coefficient (Table II). Plotting the ln K values versus the inverse of the corresponding absolute temperature (Van't Hoff plot) produced a straight line (regression coefficient 0.998) with a slope of -1500 K. With the Van't Hoff equation:

$$d(\ln K) / dT = \Delta H_0 / (RT^2) \qquad (2)$$

we can estimate the variation of enthalpy, ΔH_0, corresponding to the transfer of hydroquinone from octanol to water, to be -12.5 kJ/mol or -3 kcal/mol. It should be noted that in this approach it is assumed that the composition of the two phases does not change significantly over the temperature range used.

The partition coefficient, K, of pentachlorophenol is very low (about 10^{-5}). As described in a previous paper (2), this compound was used as a dead volume tracer. It was not possible to study its partition coefficient variation with temperature. The pentachlorophenol retention variations, observed on Table II, were not produced by temperature changes but by stationary phase volume changes (1).

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4-Other Temperature Effects

There are two additional temperature effects that affect detection in CPC. The first of these effects was noticed when doing experiments at 35°C with the octanol-water solvent system. It was found that a CPC chromatogram could not be recorded under these conditions because of extreme UV detector noise. Also, it was noticed that the solution emanating from the detector port was turbid. We suspected that the noise was produced by rapid cooling of the water-saturated, octanol mobile phase exiting the heated rotor chamber. Upon exiting the warm rotor chamber (35°C) the saturated octanol cooled (to 21°C) became supersaturated with water and

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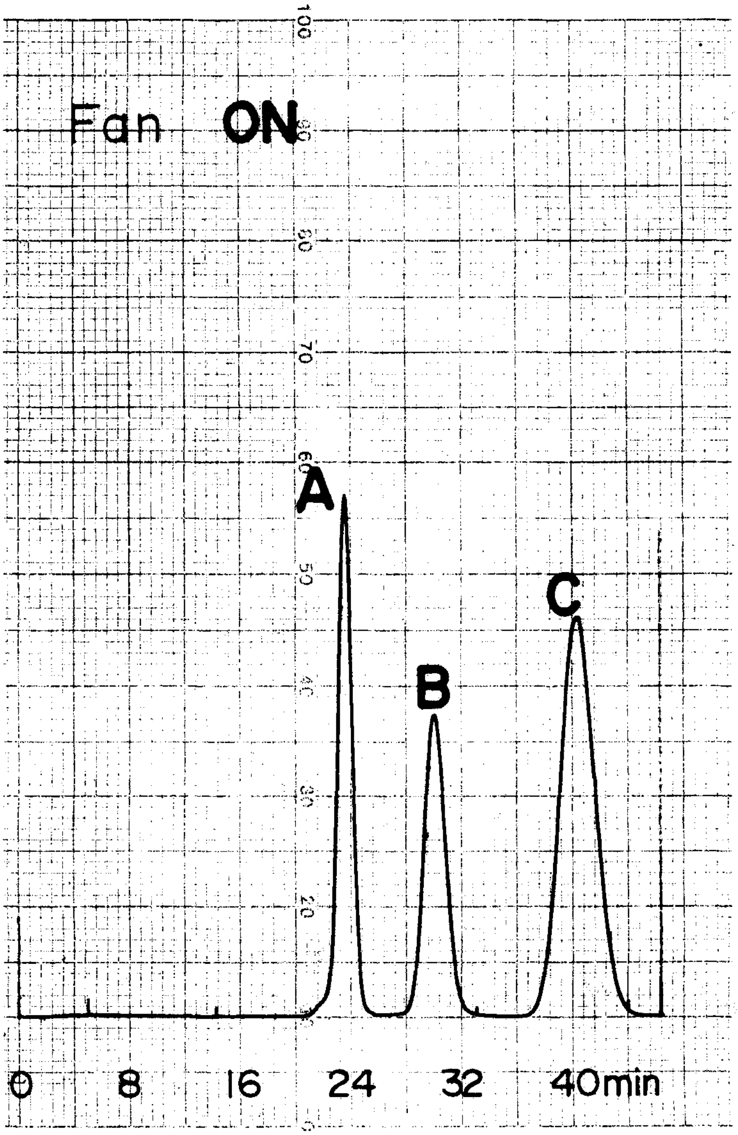

Figure 5: Actual CPC chromatogram of: A - 1naphthol (20 μg); B - DDD (50 μg); C - DDT (100μg). Stationary phase: hexane; mobile phase: methanol (+ water 1% v/v); flow rate: 3 mL/min; descending mode; spin rate: 1300 rpm; pressure: 26.5 kg/cm^2 (380 psi); absorbance: 0.08; wavelength: 230 nm; temperature 25 °C. A fan was used to cool the bottom rotary seal.

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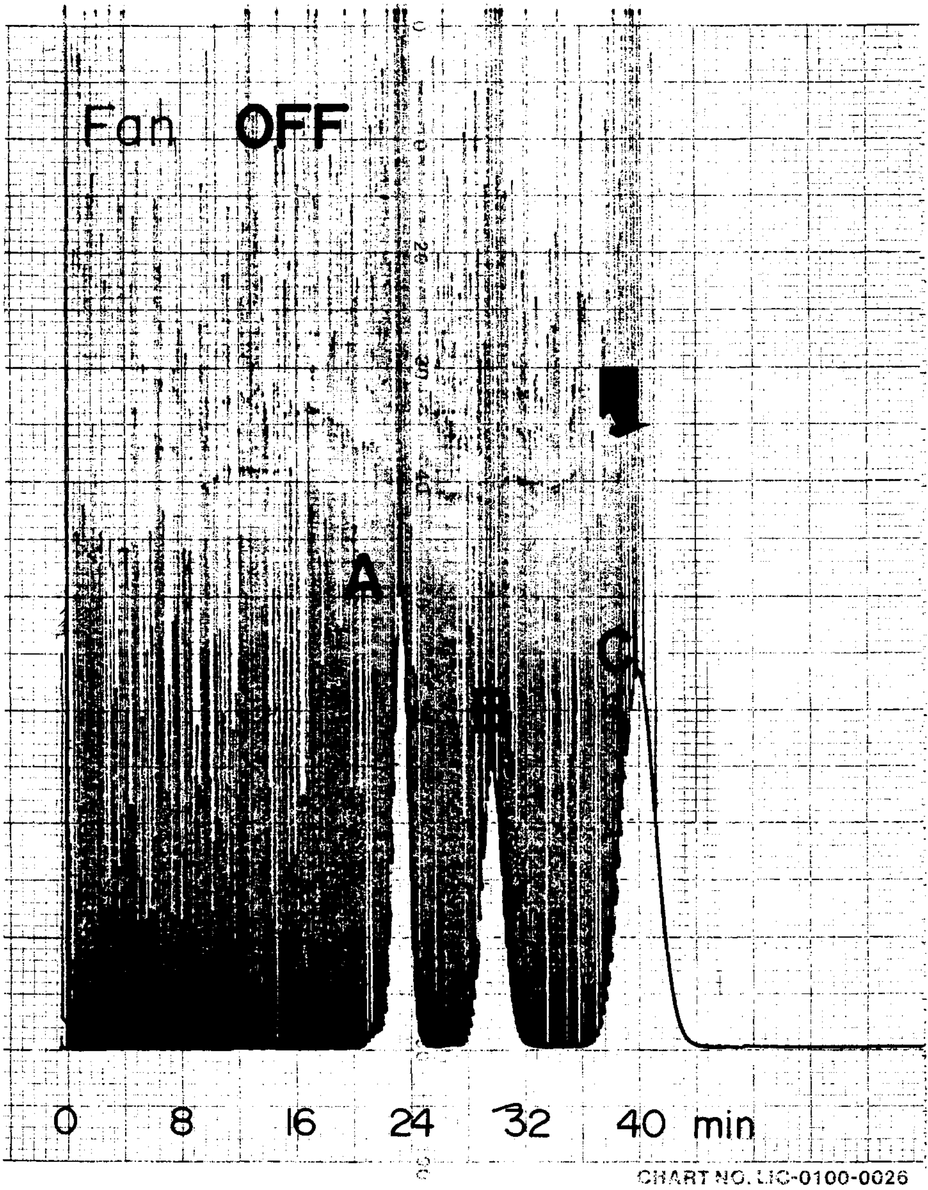

Figure 6: Actual CPC chromatogram obtained with exactly the same conditions as in Figure 5, but the fan was turned off. It was turned on again at Time=38 min (see arrow).

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subsequently produced aqueous microdroplets. The microdroplets scattered the UV light in the detector producing the noise. This problem was completely eliminated by heating the tubing between the rotor chamber and the detector to ≥35°C with a hair drier.

A second effect was observed with the methanol-hexane solvent system. With this system it was impossible to obtain CPC chromatograms at temperatures ≥30°C because of detector noise. In this case it was small gas bubbles that disturbed UV detection. At 25°C and 20°C it was possible to obtain CPC chromatograms, but some bubbles were still present. Also, it was noted that the "bubble problem" decreased as the flow rate increased. It was found that the bubbles were produced from the residual heat generated by the rotary seal. The heat is the natural result of mechanical friction between the rotating and stationary parts of the seal (8). The boiling points of methanol and hexane are 65°C and 69°C, respectively. Because each liquid is saturated with the other, the actual boiling point of each phase is even lower. The heat generated by the end rotary seal was sufficient to induce local vaporization that produced the numerous observed bubbles. Figure 5 shows a CPC chromatogram obtained with a fan located close to the tubing exiting the end rotary seal. This fan cooled the seal avoiding any local vaporization, thereby producing a smooth chromatogram. Figure 6 shows the CPC chromatogram obtained under the same conditions as Figure 5, but with the fan off. The arrow indicates the point of which the fan was turned on again. After a relaxation time needed to cool the rotary seal, the bubbles again disappeared.

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