

CHROM. 14,132

## HIGH-BACK-PRESSURE LIQUID CHROMATOGRAPHY

### I. DEVELOPMENT OF MICRO-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY USING LIQUEFIED ALKANES AS THE MOBILE PHASE

TOYOHIDE TAKEUCHI\*, YOUICHI WATANABE, KIYONARI MATSUOKA and DAIDO ISHII  
*Department of Applied Chemistry, Faculty of Engineering, Nagoya University, Chikusa-ku, Nagoya-shi 464 (Japan)*

(Received June 22nd, 1981)

---

#### SUMMARY

Alkanes with 3-6 carbon atoms are employed as the mobile phase in liquid chromatography. Compounds with low boiling points are pressurized and liquefied, and the pressure of the system is kept sufficiently high to exceed the vapour pressure of the now liquid mobile phase. Polynuclear aromatic hydrocarbons and phthalates are well separated by employing pressurized liquefied fluids. Propane gave the highest efficiency of the examined solvents.

---

#### INTRODUCTION

In supercritical fluid chromatography (SFC), the mobile phase is a fluid compressed somewhat above its critical temperature which therefore possesses the properties of both a gas and a liquid<sup>1-7</sup>. Such a fluid has a solvent strength that is sufficiently high to induce migration of even very-high-molecular-weight substances on chromatographic columns. Styrene oligomers<sup>2-5</sup>, polynuclear aromatic hydrocarbons<sup>1,2</sup> and phthalates<sup>5</sup> which cannot be subjected to conventional gas chromatography (GC) are satisfactorily resolved by a pressure and temperature program in SFC. Usually, carbon dioxide, *n*-pentane, isopropanol, methanol and diethyl ether have been employed as mobile phases.

On the other hand, a substance which is gaseous at ambient conditions can be liquefied if it is pressurized above its vapour pressure at ambient temperature. If the whole chromatograph (including pumping, injection, column and detection parts) is kept at a pressure higher than the vapour pressure of a substance, even a low-boiling substance can be used as the mobile phase for liquid chromatography (LC).

Lower alkanes such as butane or propane can be liquefied relatively easily. Boiling points of some hydrocarbons with 3-6 carbon atoms are listed in Table I.

Table II shows boiling points of propane at various pressures<sup>8</sup>. Values in Table II indicate that propane can be used as the mobile phase for LC at ambient temperature if the entire chromatograph is kept at about 10 atm.

TABLE I  
BOILING POINTS OF HYDROCARBONS (1 atm)

<i>Hydrocarbon</i>	<i>Boiling point</i> (°C)
<i>n</i> -Hexane	69
<i>n</i> -Pentane	36
Neopentane	9.5
<i>n</i> -Butane	-0.5
Isobutane	-12
Propane	-42

TABLE II  
BOILING POINTS OF PROPANE AT VARIOUS PRESSURES

<i>Pressure</i> (atm)	<i>Boiling point</i> (°C)
1	-42.1
2	-25.6
5	+1.4
10	+26.9
20	+58.1

Liquefied alkanes are expected to have lower viscosity, which leads to rapid separation and higher column efficiency. The authors examined the use of pressurized liquefied alkanes as the mobile phase in LC.

#### EXPERIMENTAL

In employing a low-boiling solvent as the mobile phase in LC or when operating at elevated temperature, a liquid chromatograph should be a closed or a semi-closed system in order to detect solutes stably<sup>9</sup>. One should be exceedingly careful to prevent the vaporization of the mobile phase in the detector.

The apparatus employed in this work is illustrated in Fig. 1. It was assembled from a micro feeder (Azumadenki Kogyo, Tokyo, Japan) or Familic 100N (JASCO; Japan Spectroscopic, Tokyo, Japan) as a pump, a three-port valve (JASCO), a cylinder containing a low-boiling solvent (Tokyo Chemical Industry, Tokyo, Japan), a guard column, a pressure gauge (JASCO), micro valve injector (0.1  $\mu$ l, JASCO), a separation column, UV spectrophotometer UVIDEC-100III (JASCO), a back-pressure pump and a recorder. The pressure in the detector is kept sufficiently high so as to exceed the vapor pressure of the solvent by applying pressure with back-pressure pump. The back-pressure can be varied easily by altering a weight on the pump.

Both guard and separation columns were made of PTFE tubing packed with commercially available materials. The packing technique was the same as in a previous work<sup>10</sup>. Spherical porous silica Develosil (Nomura Chemical, Seto-shi, Japan)

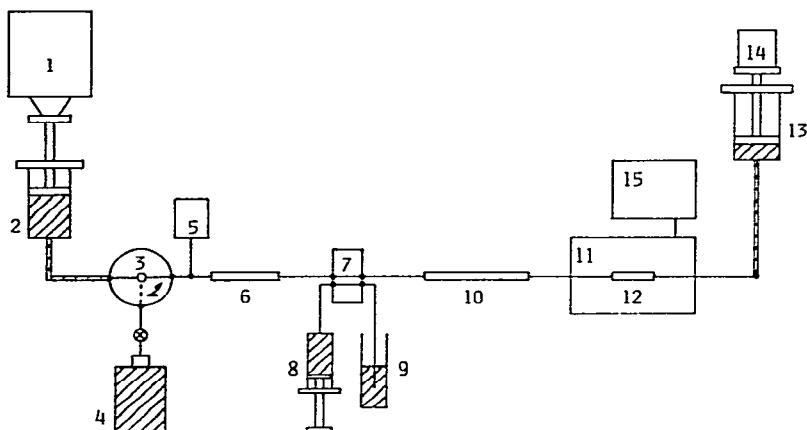


Fig. 1. Schematic diagram of the apparatus. 1 = Micro feeder; 2 = gas-tight syringe; 3 = three-port valve; 4 = gas cylinder; 5 = pressure gauge; 6 = protection column; 7 = micro valve injector; 8 = sample; 9 = waste reservoir; 10 = separation column; 11 = UV spectrophotometer UVIDEC 100-III; 12 = micro flow cell; 13 = back-pressure pump; 14 = weight; 15 = recorder.

was packed in the guard column, which removed impurities from the solvent. The separation column was packed with Develosil ( $5\ \mu\text{m}$ ) or a porous polymer HP-01 ( $10\ \mu\text{m}$ , JASCO).

Low-boiling solvent was collected in a gas-tight syringe as follows. A gas-tight syringe was connected to the gas cylinder through a three-port valve and filled with carrier gas. Then, the gas-tight syringe was cooled by cold water in order to liquefy the carrier gas. After an appropriate volume of the partially liquefied solvent was collected, the three-port valve was changed and the solvent was completely liquefied by applying pressure manually.

## RESULTS AND DISCUSSION

The dynamic viscosity of liquefied solvents is expected to be smaller than that of solvents commonly used in LC. In other words, diffusion coefficients of solutes in liquefied solvents are larger, which leads to higher column efficiency.

Diffusion coefficients of solutes in liquefied solvents were determined from Ouano's equation<sup>11</sup> by measuring the band spreading of solutes flowing through capillary tubing. The results are listed in Table III. The diffusion coefficients in *n*-alkane increase with decreasing carbon number of the solvent.

Rough values of diffusion coefficients in the mobile phase employed in reversed-phase LC, SFC and GC are also shown in Table III. Diffusivity in propane is 20 times that in methanol-water and less than that in a supercritical fluid or a gas by a factor of 10 and 1000, respectively.

Fig. 2 shows the dependence of retention of solutes on the carbon number of *n*-alkane for a silica column. The capacity factors ( $k'$ ) of polynuclear aromatic hydrocarbons had a tendency to increase with decreasing carbon number. Thus, resolution of solutes was high when employing propane as the mobile phase.

Column performances obtained with various solvents are compared in Table

TABLE III

DIFFUSION COEFFICIENTS IN ALKANES AND IN MOBILE PHASES FOR REVERSED-PHASE LC, SFC AND GC

Solute, benzene; temperature of alkanes, 18°C.

Fluid	Diffusion coefficient ( $\text{cm}^2/\text{sec}$ )
<i>n</i> -Hexane	$4.4 \cdot 10^{-5}$
<i>n</i> -Pentane	$5.5 \cdot 10^{-5}$
Neopentane	$4.8 \cdot 10^{-5}$
<i>n</i> -Butane	$6.9 \cdot 10^{-5}$
Isobutane	$7.0 \cdot 10^{-5}$
Propane	$1.0 \cdot 10^{-4}$
Methanol-water (1:1)	<i>ca.</i> $5 \cdot 10^{-6}$
Supercritical fluid	<i>ca.</i> $10^{-3}$
Gas	<i>ca.</i> $10^{-1}$

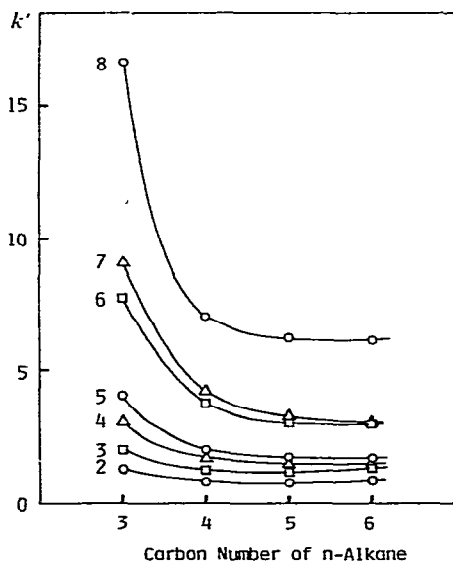


Fig. 2. Dependence of retention of solutes on the carbon number of the *n*-alkane. Conditions: column, 12.5 cm  $\times$  0.5 mm I.D., packed with Develosil ( $5 \mu\text{m}$ ); mobile phase, *n*-alkane. Sample: 2 = naphthalene; 3 = biphenyl; 4 = anthracene; 5 = pyrene; 6 = chrysene; 7 = 3,4-benzopyrene; 8 = 1,3,5-triphenylbenzene.

IV. Retention of solutes was dependent upon the structure of the solvent as well as the carbon number. A branched-chain solvent gave larger retention than a straight-chain solvent with the same carbon number. Height equivalent to a theoretical plate (HETP) also varied with solvents. Variation of HETP was dependent upon the diffusion coefficient. The solvent with a larger diffusion coefficient had a tendency to give a smaller HETP value, as shown in Tables III and IV.

TABLE IV  
COMPARISON OF COLUMN PERFORMANCE

Conditions: column, 12.5 cm  $\times$  0.5 mm I.D., packed with Develosil (5  $\mu$ m); sample, 1,3,5-triphenylbenzene; flow-rate, 10  $\mu$ l/min.

Solvent	$k'$	HETP (mm)
<i>n</i> -Hexane	6.2	0.048
<i>n</i> -Pentane	6.1	0.043
Neopentane	9.1	0.060
<i>n</i> -Butane	7.0	0.038
Isobutane	12	0.040
Propane	17	0.033

Typical separations of polynuclear aromatic hydrocarbons on a silica column are shown in Figs. 3–5. With propane the resolution of the solutes was exceedingly high. Peak shape was symmetric even for a strongly retained solute. Pressure drop over the column was 12–14 atm for the operations shown in Figs. 3–5.

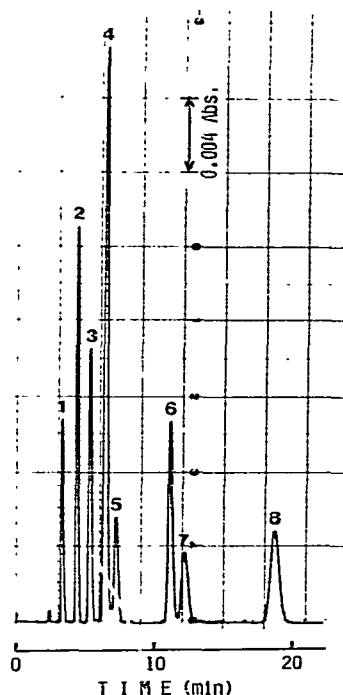
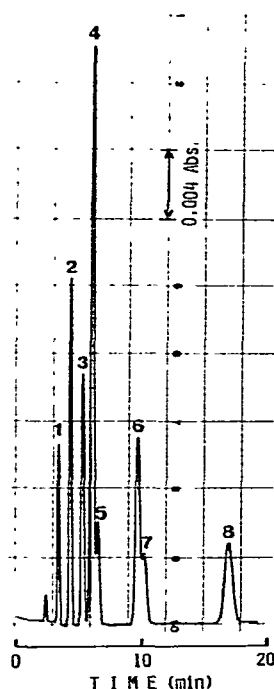


Fig. 3. Separation of polynuclear aromatic hydrocarbons. Conditions: column, the same as in Fig. 2; mobile phase, *n*-pentane; flow-rate, 10  $\mu$ l/min; sample volume, 0.1  $\mu$ l; and, detection wavelength (UV), 254 nm. Peaks: 1 = 0.97% of benzene; 2 = 0.10% of naphthalene; 3 = 0.020% of biphenyl; 4 = 0.006% of anthracene; 5 = 0.021% of pyrene; 6 = 0.012% of chrysene; 7 = 0.0081% of 3,4-benzopyrene; 8 = 0.010% of 1,3,5-triphenylbenzene.

Fig. 4. Separation of polynuclear aromatic hydrocarbons. Peaks and conditions as in Fig. 3, except the mobile phase was *n*-butane.

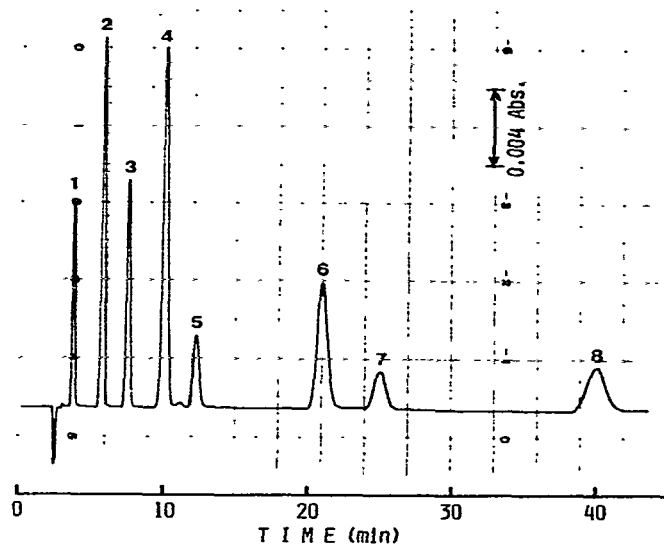


Fig. 5. Separation of polynuclear aromatic hydrocarbons. Peaks and conditions as in Fig. 3, except the mobile phase was propane.

Fig. 6 illustrates the dependence of retention of phthalates on the chain length of the *n*-alkane used as the mobile phase for a polystyrene column. The  $k'$  values of phthalates increased with decreasing carbon number, similarly to the tendency for the silica column. A branched-chain solvent gave a larger  $k'$  value than a corresponding straight-chain solvent, *e.g.*,  $k'$  values of dimethylphthalate were 6.7 and 11.5 for

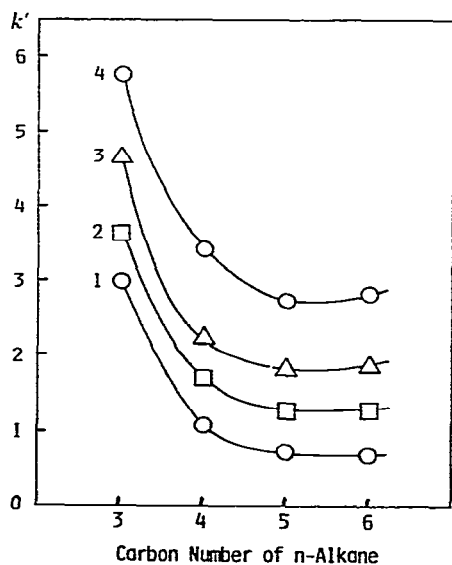


Fig. 6. Dependence of retention of phthalates on the carbon number of *n*-alkane. Conditions: column, 16.2 cm  $\times$  0.5 mm I.D., packed with HP-01; mobile phase, *n*-alkane. Sample: 1 = dioctyl-; 2 = dibutyl-; 3 = diethyl-; and, 4 = dimethylphthalate (in isooctane).

isobutane and neopentane, respectively. These values are larger than those in Fig. 6. In the case of a polystyrene column, tailing of chromatographic peaks was observed.

Comparison of column performance is shown in Table V. The polystyrene column showed nearly the same tendency as the silica column. HETP was dependent upon the diffusion coefficient of the mobile phase.

TABLE V  
COMPARISON OF COLUMN PERFORMANCE

Column, 16.2 cm × 0.5 mm I.D., packed with HP-01. Flow-rate, 8.3 μl/min.

Mobile phase	Diethylphthalate (DOP)		Dimethylphthalate (DMP)	
	$k'$	HETP (mm)	$k'$	HETP (mm)
<i>n</i> -Hexane	0.70	0.24	2.8	0.28
<i>n</i> -Pentane	0.74	0.20	2.7	0.27
Neopentane	3.9	0.41	11.5	0.49
<i>n</i> -Butane	1.1	0.18	3.4	0.20
Isobutane	2.3	0.19	6.7	0.43
Propane	3.0	0.14	5.8	0.19

## CONCLUSION

Pressurized liquefied fluids could be employed as mobile phases in LC. They had larger diffusivity, which led to higher column efficiency. Experiments with low-boiling solvents could be performed without any danger since the flow-rate of the mobile phase was very low. The use of volatile solvents in LC will favor the combination of a liquid chromatograph and an infrared spectrophotometer or a mass spectrometer. Modification of the mobile phase and the use of other kinds of low-boiling solvents are currently under investigation.

## REFERENCES

- 1 M. Novotný, W. Bertsch and A. Zlatkis, *J. Chromatogr.*, 61 (1971) 17.
- 2 T. H. Gouw and R. E. Jentoft, *J. Chromatogr.*, 68 (1972) 303.
- 3 E. Klesper and W. Hartman, *J. Polym. Sci., Polym. Lett. Ed.*, 15 (1977) 9.
- 4 E. Klesper and W. Hartmann, *Eur. Polym. J.*, 14 (1978) 77.
- 5 E. Klesper, *Angew. Chem., Int. Ed. Engl.*, 17 (1978) 738.
- 6 W. Asche, *Chromatographia*, 11 (1978) 411.
- 7 J. A. Graham and L. B. Rogers, *J. Chromatogr. Sci.*, 18 (1980) 75.
- 8 M. Windholz and D. S. Leahy, in P. G. Stecher (Editor), *The Merck Index*, Merck, Rahway, NJ, 8th ed., 1968, p. 871.
- 9 T. Takeuchi, Y. Watanabe and D. Ishii, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, (1981) 4 (1981) 300.
- 10 D. Ishii, K. Asai, K. Hibi, T. Jonokuchi and M. Nagaya, *J. Chromatogr.*, 144 (1977) 157.
- 11 A. C. Ouano, *Ind. Eng. Chem., Fundam.*, 11 (1972) 268.