

CHROM. 14,688

HIGH-BACK-PRESSURE LIQUID CHROMATOGRAPHY

III. OPEN-TUBULAR MICROCAPILLARY LIQUID CHROMATOGRAPHY USING LIQUEFIED ALKANES AS THE MOBILE PHASE

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(Received December 30th, 1981)

SUMMARY

Open-tubular microcapillary liquid chromatography using lower alkanes as the mobile phase was examined. The diffusivity of the solutes increased with decreasing carbon number of the solvents, leading to higher column efficiency. Some typical separations of standard solutes are demonstrated.

INTRODUCTION

Lower alkanes such as propane and butane can be easily liquefied at relatively low pressure (5–10 atm). If the pressure of the entire chromatograph is kept high enough to exceed the vapour pressure of the now liquid solvents, even such low-boiling solvents can be employed as the mobile phase in liquid chromatography (LC)¹. In previous work¹, micro-high-performance liquid chromatography (MHPLC) using C₃–C₆ alkanes was investigated and useful results were obtained owing to their low viscosity, which encouraged us to use low-boiling alkanes as the mobile phase in LC. In addition, the resolution increased with decreasing carbon number of the alkanes for the separation of polynuclear aromatic hydrocarbons on a silica gel column.

The larger difference in diffusion speed in the liquid and gaseous states is the main reason why open-tubular capillary LC has not been so successful as capillary gas chromatography². Thus the employment of liquefied alkanes of low viscosity as the mobile phase will be advantageous in capillary LC.

The basic equation expressing column performance in open-tubular capillary LC is

$$H = \frac{2D_m}{u} + \frac{2k'd^2u}{3(1+k')^2D_s} + \frac{(11k'^2 + 6k' + 1)r_c^2u}{24(1+k')^2D_m} \quad (1)$$

where H is the height equivalent to a theoretical plate, u is the linear velocity of the mobile phase, k' is the capacity factor, d is the thickness (or depth) of the stationary

phase, r_c is the radius of an open tube and D_m and D_s are the diffusion coefficients of a solute in the mobile phase and the stationary phase, respectively.

For non-retained solutes ($k' = 0$), eqn. 1 is simplified under normal conditions to

$$H = \frac{2D_m}{u} + \frac{r_c^2 u}{24D_m} \approx \frac{r_c^2 u}{24D_m}$$

From plate theory, H is represented by the following equation:

$$H = \frac{L}{N} = \frac{L}{16} \left(\frac{V_w}{V_t} \right)^2 \quad (3)$$

where L is column length, N is theoretical plate number, V_w is the peak width in volume units and V_t is the retention volume.

Combining eqns. 2 and 3 and rewriting, we obtain

$$V_w^2 = \frac{2V_t^2 r_c^2}{3L} \cdot \frac{u}{D_m} \quad (4)$$

The first term in eqn. 1 is negligible under the usual conditions. Hence the selection of a system with large D_m and D_s as well as the use of a narrow-bore capillary column is necessary in order to attain high efficiency. As the third term in eqn. 1, based on the resistance to mass transfer in the mobile phase, is dominant in open-tubular capillary LC^{3,4}, particular attention should be paid to the viscosity of the mobile phase and the column bore. It is relatively easy to deal with the former problem, whereas the latter suffers from technical problems associated with the preparation of narrow-bore columns.

The viscosity of the mobile phase generally decreases with increasing column temperature. The influence of column temperature on column efficiency and the retention of solutes was examined in open-tubular capillary LC⁵. It was found that operation at higher temperature had a tendency to give a higher column efficiency owing to the decrease in the viscosity of the mobile phase.

This paper examines the use of lower alkanes as the mobile phase in open-tubular capillary LC in order to attain higher column efficiencies using low-viscosity mobile phases.

EXPERIMENTAL

All reagents were supplied by Wako (Osaka, Japan) or Tokyo Chemical Industry (Tokyo, Japan).

A liquid chromatograph was assembled from a Micro Feeder (Azumadenki Kogyo, Tokyo, Japan) equipped with a gas-tight syringe, Model MS-GAN 025 (250 μ l) or 050 (500 μ l) (Terumo Co., Tokyo, Japan), as a pump, a three-way valve [Japan Spectroscopic Co. (JASCO), Tokyo, Japan], a guard column, a saturation column, a micro valve injector (JASCO), a gas cylinder and a back-pressure pump. A diagram of the apparatus is shown in Fig. 1.

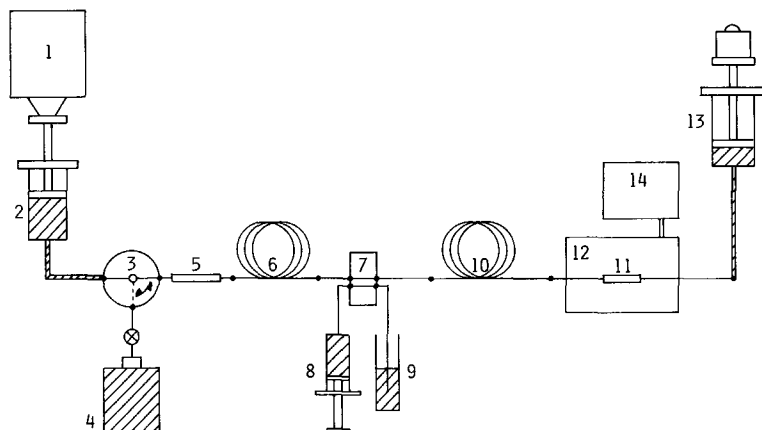


Fig. 1. Diagram of the apparatus. 1 = Micro feeder; 2 = gas-tight syringe; 3 = three-way valve; 4 = gas cylinder; 5 = guard column; 6 = saturation column; 7 = micro valve injector ($0.02 \mu\text{l}$); 8 = sample; 9 = waste reservoir; 10 = separation column; 11 = micro flow cell; 12 = UV detector; 13 = back-pressure pump; 14 = recorder.

A low-boiling solvent was collected by the method described previously¹. The guard column ($3 \text{ cm} \times 0.5 \text{ mm I.D.}$) was made of PTFE tubing and was packed with Develosil spherical porous silica (Nomura Chemical, Seto-shi, Japan), which removed impurities from the solvent. β, β' -Oxydipropionitrile (BOP) columns were generally employed in this work. The preparation method was the same as described previously³. When a physically coated column is employed as the separation column, the mobile phase should be saturated with the stationary phase. An open-tubular capillary saturation column, *ca.* $5 \text{ m} \times 60 \mu\text{m}$, physically coated with BOP worked well for saturating the mobile phase with BOP *in situ*. Samples could be loaded with good reproducibility using the micro valve injector, in spite of the small volume ($0.02 \mu\text{l}$).

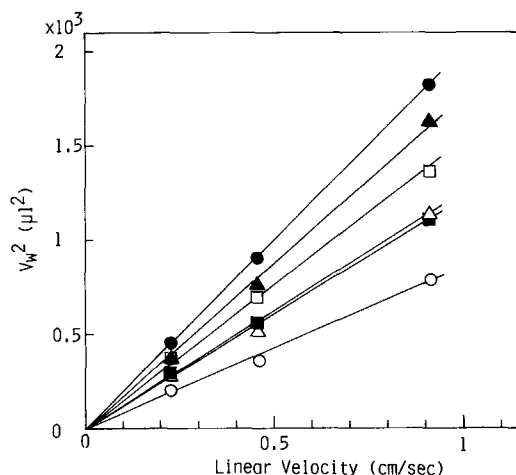


Fig. 2. Dependence of the square of the band broadening on linear velocity of the mobile phase. Column: $3.8 \text{ m} \times 0.35 \text{ mm I.D.}$ Mobile phases: ● = *n*-hexane; ▲ = neopentane; □ = *n*-pentane; ■ = *n*-butane; △ = isobutane; ○ = propane. Sample: benzene. Temperature: 18°C .

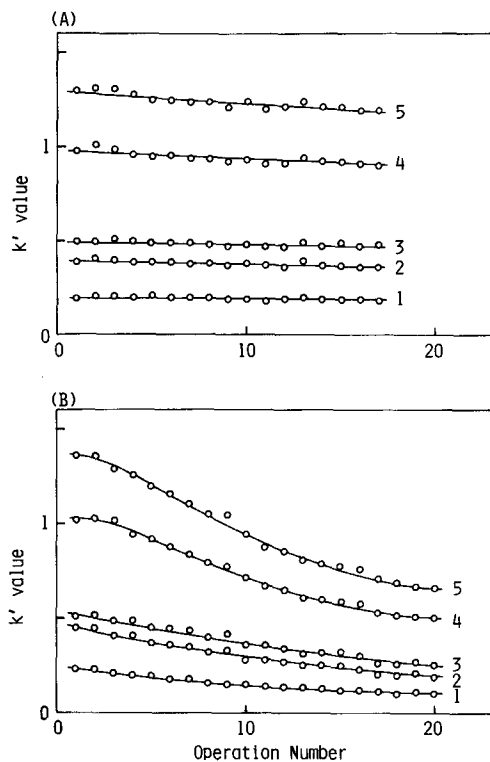


Fig. 3. Durability of column performance: (A) with saturation column; (B) without saturation column. Separation column: BOP, 7.4 m \times 44 μ m I.D. Saturation column: BOP, 5 m \times 60 μ m I.D. Mobile phase: *n*-butane. Flow-rate: 2.1 μ l/min. Samples: 1 = N-phenyl- α -naphthylamine; 2 = N-phenyl- β -naphthylamine; 3 = aniline; 4 = α -naphthylamine; 5 = β -naphthylamine.

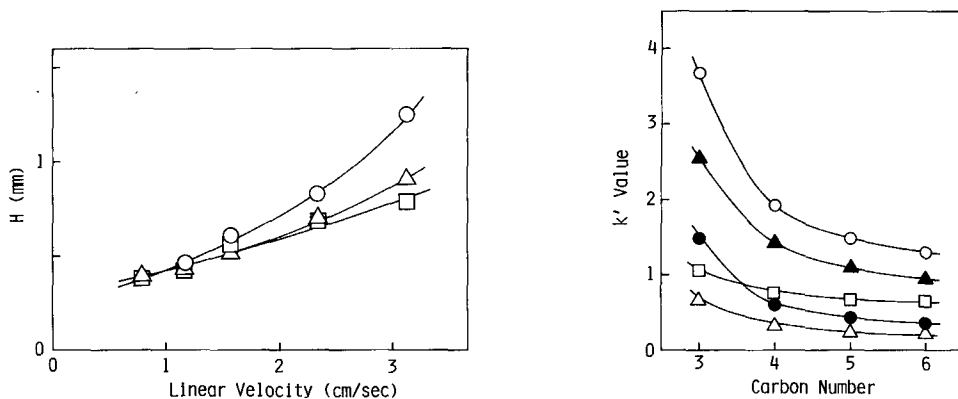


Fig. 4. Dependence of column efficiency on linear velocity and type of *n*-alkane. Column: BOP, 5.5 m \times 44 μ m I.D. Mobile phases: \circ = *n*-hexane; \triangle = *n*-pentane; \square = *n*-butane. Sample: β -naphthylamine. k' values: 0.64 (*n*-hexane); 0.79 (*n*-pentane); 1.05 (*n*-butane).

Fig. 5. Relationship between retention of aromatic amines and carbon number of *n*-alkanes. Column as in Table I. Samples: \triangle = N-phenyl- α -naphthylamine; \bullet = N-phenyl- β -naphthylamine; \square = aniline; \blacktriangle = α -naphthylamine; \circ = β -naphthylamine. Temperature: 25°C.

The pressure in the detector and the column was kept sufficiently high to exceed the vapour pressure of the solvent by applying pressure with the back-pressure pump, consisting of a gas-tight syringe and a weight, as described previously^{1,6}.

RESULTS AND DISCUSSION

As described in the preceding section, the diffusion coefficient of solutes in the mobile phase plays an important role in open-tubular capillary LC. In other words, the viscosity of the mobile phase directly affects the column efficiency. Eqn. 4 indicates that the square of the peak width of a non-retained solute is proportional to the linear velocity of the mobile phase and inversely proportional to the diffusion coefficient of a solute in the mobile phase. Hence, if the peak width of a solute flowing through a capillary tubing is measured, D_m can be calculated.

Fig. 2 illustrates relationship between V_w^2 and u for various low-boiling alkanes using benzene as the test solute. The slope of these plots decreases with decreasing carbon number of the alkanes, *i.e.*, the lower the carbon number, the smaller is the viscosity. Diffusion coefficients of benzene in various solvents can be calculated from the data in Fig. 2, *viz.*, $4.4 \cdot 10^{-5}$ cm²/sec in *n*-hexane, $4.8 \cdot 10^{-5}$ cm²/sec in neopentane, $5.5 \cdot 10^{-5}$ cm²/sec in *n*-pentane, $6.9 \cdot 10^{-5}$ cm²/sec in *n*-butane, $7.0 \cdot 10^{-5}$ cm²/sec in isobutane and $1.0 \cdot 10^{-4}$ cm²/sec in propane. These results encouraged us to employ lower alkanes as the mobile phase in open-tubular capillary LC.

Physically coated columns were generally employed as separation columns, necessitating saturation of the mobile phase with the stationary phase. Solvents that are liquid under ambient conditions can be easily pre-saturated with the stationary phase in the batch method³, whereas liquefied solvents should be saturated with the stationary phase in the closed system, as described in the experimental section. Fig. 3

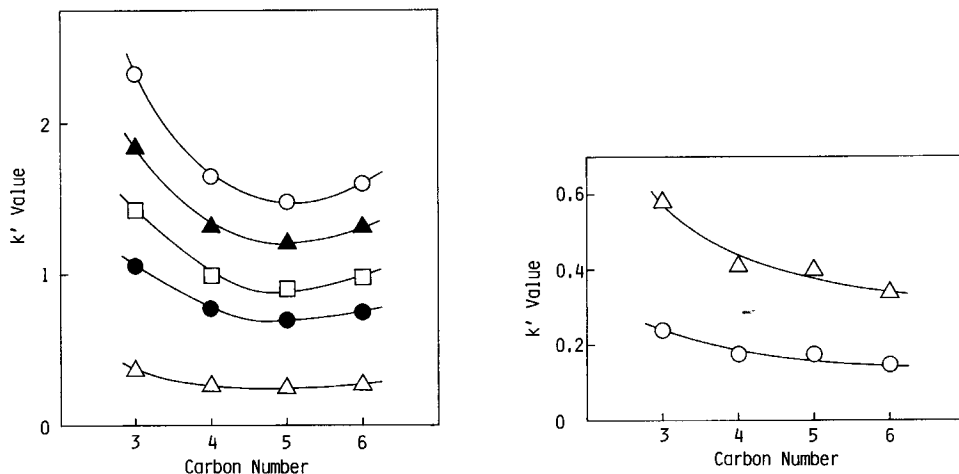


Fig. 6. Relationship between retention of xyleneol isomers and carbon number of *n*-alkanes. Column: BOP, 5.1 m \times 42 μ m I.D. Samples: Δ = 2,6-xyleneol; \bullet = 2,5-xyleneol; \square = 2,3-xyleneol; \blacktriangle = 3,5-xyleneol; \circ = 3,4-xyleneol. Temperature: 27°C.

Fig. 7. Relationship between retention of N-alkylanilines and carbon number of *n*-alkanes. Column: BOP, 5.4 m \times 44 μ m I.D. Samples: \circ = N-ethylaniline; Δ = N-methylaniline. Temperature: 30°C.

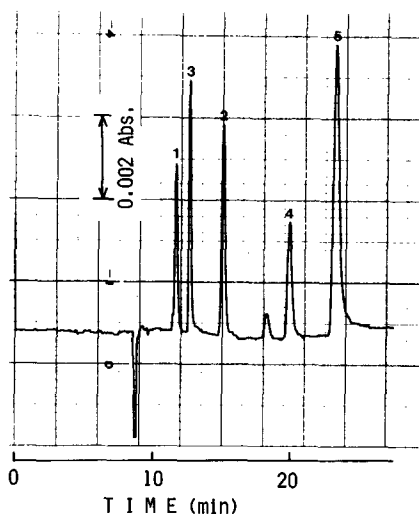
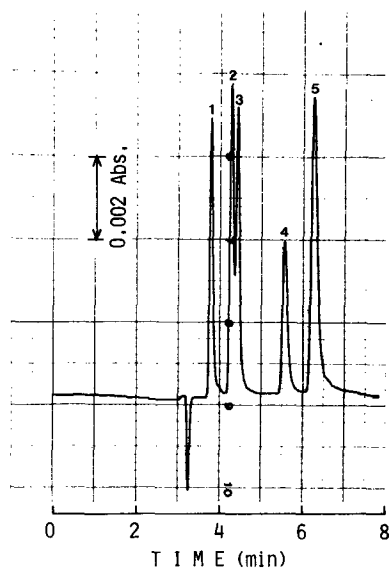


Fig. 8. Separation of aromatic amines. Column: BOP, 5.5 m \times 44 μ m I.D. Mobile phase: *n*-butane. Flow-rate: 2.8 μ l/min. Samples: 1 = N-phenyl- α -naphthylamine; 2 = N-phenyl- β -naphthylamine; 3 = aniline; 4 = α -naphthylamine; 5 = β -naphthylamine. Wavelength of UV detection: 235 nm.

Fig. 9. Separation of aromatic amines. Column: BOP, 7.4 m \times 44 μ m I.D. Mobile phase: propane. Flow-rate: 1.4 μ l/min. Samples as in Fig. 8. Wavelength of UV detection: 235 nm.

illustrates the variation of the retention of solutes in successive operations during a few days. The k' value of each solute decreases slightly with the saturation column, whereas the decrease in k' is significant without the saturation column. Chemically bonded open-tubular columns will solve the above problems.

The results obtained for non-retained solutes suggest that a higher column efficiency can be attained for retained solutes with lower alkanes as the mobile phase. Fig. 4 shows H versus u relationships obtained by using *n*-alkanes with 4–6 carbon atoms as the mobile phase. As the carbon number decreases a lower dependence of H on u is observed, in spite of the larger retention. Eqn. 1 indicates that H increases with

TABLE I

RETENTION OF AROMATIC AMINES ON A BOP COLUMN

Column: BOP, 5.1 m \times 42 μ m. Temperature: 25°C. NP α NA = N-phenyl- α -naphthylamine; NP β NA = N-phenyl- β -naphthylamine; A = aniline; α NA = α -naphthylamine; β NA = β -naphthylamine.

Mobile phase	Sample				
	NP α NA	NP β NA	A	α NA	β NA
<i>n</i> -Hexane	0.18	0.34	0.65	0.94	1.30
<i>n</i> -Pentane	0.24	0.42	0.66	1.10	1.48
Neopentane	0.50	0.99	0.94	2.08	2.86
<i>n</i> -Butane	0.32	0.61	0.75	1.42	1.92
Isobutane	0.50	0.99	0.93	2.05	2.78
Propane	0.70	1.49	1.06	2.55	3.69

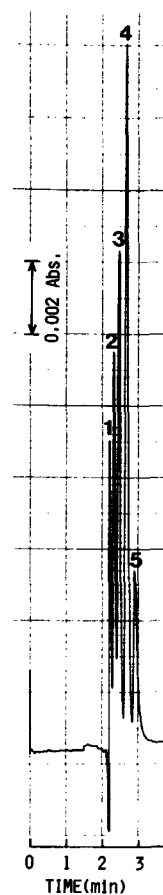
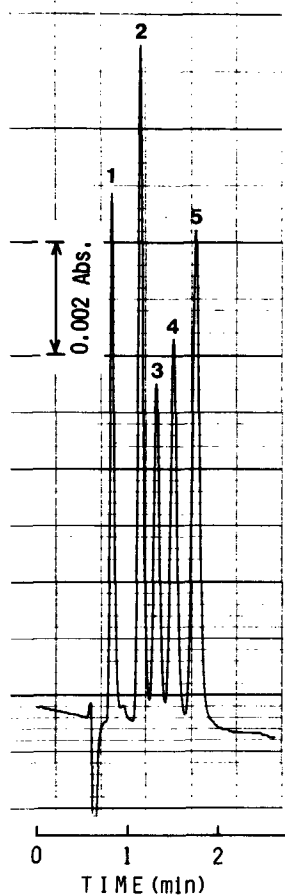


Fig. 10. Rapid separation of xylene isomers. Column: BOP, 3.4 m \times 33 μ m I.D. Mobile phase: propane. Flow-rate: 5.6 μ l/min. Samples: 1 = 2,6-xylene; 2 = 2,5-xylene; 3 = 2,3-xylene; 4 = 3,5-xylene; 5 = 3,4-xylene. Wavelength of UV detection: 280 nm.

Fig. 11. Separation of aromatic hydrocarbons on a silica gel column. Column: 5.4 m \times 44 μ m I.D., prepared by treatment with 1 *N* sodium hydroxide solution for 2 days at 50°C. Mobile phase: isobutane. Flow-rate: 4.2 μ l/min. Samples: 1 = benzene; 2 = naphthalene; 3 = biphenyl; 4 = anthracene; 5 = pyrene. Wavelength of UV detection: 254 nm.

increasing k' of a solute when the third term is dominant. Hence the above results strongly recommend the use of lower alkanes as the mobile phase and indicate their advantages in open-tubular capillary LC.

The retention behaviours of typical aromatic compounds were examined using BOP columns. The retention of the solutes was dependent on the concentration of BOP in the coating solution, the coating speed of BOP or conditions of pre-treatment with sodium hydroxide solution. The retention of solutes increased with increasing BOP concentration, coating speed of BOP and pre-treatment temperature. The coating solution and pre-treatment temperature adopted in this work were 15% (w/w) of BOP in dichloromethane containing 0.15% (w/w) of sodium tetraphenylborate and

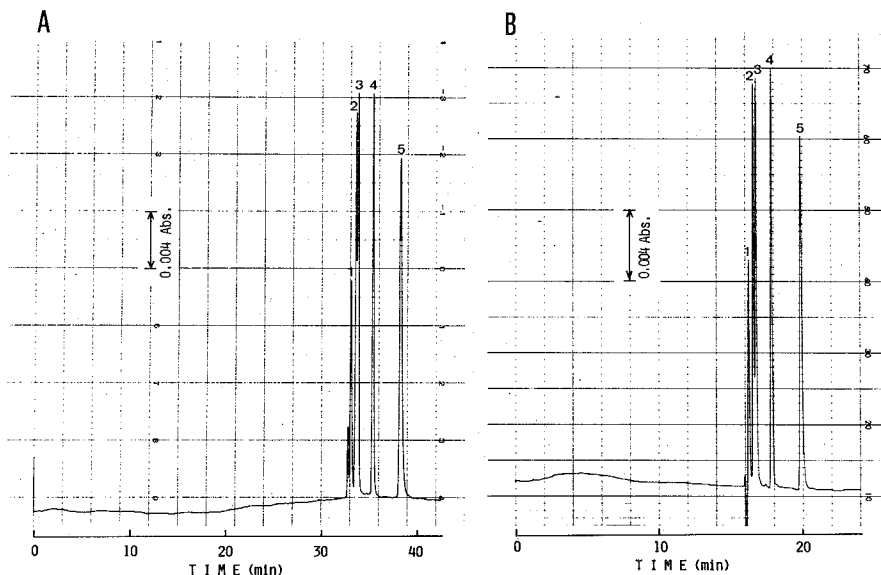


Fig. 12. Separations of N-alkylanilines. Column: BOP, 19.2 m \times 37 μ m I.D. Mobile phases: (A) *n*-butane; (B) propane. Flow-rates: (A) 0.69 μ l/min; (B) 1.4 μ l/min. Samples: 1 = N,N-dichylaniline; 2 = N,N-dimethylaniline; 3 = N-*n*-butylaniline; 4 = N-ethylaniline; 5 = N-methylaniline. Wavelength of UV detection: 235 nm.

4% (w/w) methanol, and 20–30°C, respectively, unless indicated otherwise.

Table I shows the retention of aromatic amines on a BOP column. The retention of each solute generally increased with decreasing carbon number but the order of elution of N-phenyl- β -naphthylamine and aniline changed, depending on the carbon number or structure of the solvent. In addition, a branched-chain solvent gave a larger k' value than the corresponding straight-chain solvent, these results being similar to those obtained in MHPLC employing packed columns¹.

The relationship between the k' values of aromatic amines and the carbon number of *n*-alkanes is shown in Fig. 5.

Figs. 6 and 7 show the relationships between k' and carbon number of *n*-alkanes for xylene isomers and N-alkylanilines, respectively. In the former instance *n*-pentane gives the minimum k' values, whereas the k' values decrease monotonously with increasing carbon number in the latter.

Figs. 8 and 9 show typical separations of aromatic amines on a BOP column using *n*-butane and propane, respectively, as the mobile phase. Symmetrical chromatographic peaks are observed.

A low viscosity of the mobile phase facilitates rapid separations. Fig. 10 demonstrates the rapid separation of xylene isomers on a BOP column. The linear velocity of propane was about 11 cm/sec.

Fig. 11 shows the separation of aromatic hydrocarbons on a silica gel column, prepared by treatment with 1 *N* sodium hydroxide solution for 2 days at 50°C. The retention of solutes generally increased with decreasing carbon number also for silica gel columns.

Liquefied alkanes with low viscosity favour the operation of longer columns.

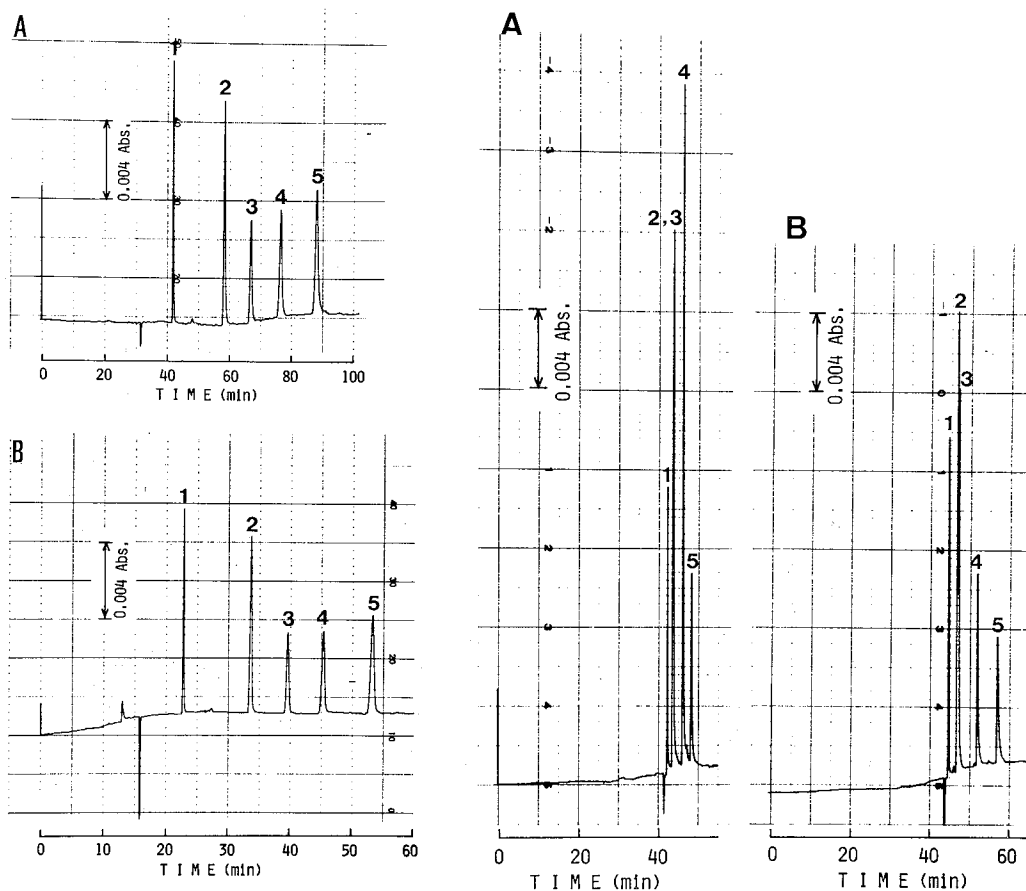


Fig. 13. Separations of xylene isomers. Operating conditions, except samples and wavelength of detection, as in Fig. 12. Samples: 1 = 2,6-xylene; 2 = 2,5-xylene; 3 = 2,3-xylene; 4 = 3,5-xylene; 5 = 3,4-xylene. Wavelength of UV detection: 280 nm.

Fig. 14. Separations of aromatic hydrocarbons. Column: BOP, 30.6 m \times 34 μ m I.D. Mobile phases: (A) *n*-butane; (B) propane. Flow-rate: 0.69 μ l/min. Samples as in Fig. 11. Wavelength of UV detection: 254 nm. Efficiency: (A) (solute, k' , N): benzene, 0.02, 300,000; anthracene, 0.12, 190,000; pyrene, 0.17, 140,000. (B) (solute, k' , N): benzene, 0.02, 340,000; anthracene, 0.19, 120,000; pyrene, 0.31, 94,000.

Figs. 12 and 13 show separations of *N*-alkylanilines and xylene isomers, respectively, on a BOP column (19.2 m \times 37 μ m). Fig. 14 shows separations of aromatic hydrocarbons on a BOP column (30.6 m \times 34 μ m) in which 100,000–300,000 theoretical plates are attained within 1 h.

CONCLUSION

The use of liquefied alkanes as the mobile phase in open-tubular capillary LC led to higher column efficiency; 100,000–300,000 theoretical plates were easily attained on BOP columns. The development of chemically bonded capillary columns with high efficiency and the use of various kinds of low-viscosity solvents as the mobile phase will advance this field.

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