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ROLE OF COLUMN TEMPERATURE IN OPEN-TUBULAR MICROCAPILLARY LIQUID CHROMATOGRAPHY

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SUMMARY

The influence of column temperature on chromatographic performance was examined for reversed-phase, normal-phase and cation-exchange columns in open-tubular microcapillary liquid chromatography. Linear relationships between the logarithm of capacity factor and the reciprocal of column temperature were generally observed. Operation at higher column temperature had the tendency to give higher column efficiency owing to the decrease in the viscosity of the mobile phase.

INTRODUCTION

Column temperature is one of the most significant parameters in gas chromatography (GC) and gradient elution with temperature is a popular and powerful technique in GC. In contrast, it has often been neglected in liquid chromatography (LC), which has sometimes caused poor reproducibility of retention of solutes or incorrect results. Some workers have examined the hydrodynamic and thermodynamic roles of column temperature in LC[1–15]. In reversed-phase chromatography, it has been reported that operation at elevated temperature leads to a decrease in the viscosity of the mobile phase and consequently an increase in column efficiency[3–5,15]. However, a few workers concluded that operation at subambient or low temperature improves the selectivity[8]. However, the logarithm of the capacity factor was proportional to the reciprocal of column temperature in the reversed-phase mode.

In normal-phase LC, the effect of temperature on solute retention and selectivity has been discussed[1,2,12–14]. The direction of the dependence of solute retention on column temperature was reversed with variation of temperature.

Temperature-gradient separations have been tried in LC, and these reduced the analysis time comparable to conventional solvent gradient separations[2,7,15].

The larger difference in diffusion speed in the liquid and gaseous states is the main reason why open-tubular capillary LC has not generally been so successful as glass capillary GC[16]. The employment of a narrow-bore capillary column is necessary in order to solve this problem, as can be appreciated from the following equation:

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

where H is 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 and the stationary phase, respectively.

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

$$H = \frac{r_c^2\,u}{24D_m} \equiv H_m \qquad (2)$$

Eqn. 1 indicates that a system with larger D_m and D_s can also generate a higher efficiency as the first term in the equation is negligible under the usual conditions. The third term in eqn. 1, based on the resistance of mass transfer in the mobile phase, is dominant in open-tubular capillary LC[17,18]. Hence the viscosity of the mobile phase should be given particular attention in open-tubular capillary LC.

This paper describes the influence of column temperature on chromatographic characteristics for reversed-phase, normal-phase and cation-exchange columns in open-tubular microcapillary LC.

EXPERIMENTAL

All reagents were obtained from Wako (Osaka, Japan), unless stated otherwise.

A liquid chromatograph was assembled from a pumping system, a capillary column, a column oven and a detection system. The pumping system included a Micro Feeder (Azumadenki Kogyo, Tokyo, Japan) and a 100-μl gas-tight syringe (Hamilton, Reno, NV, U.S.A.). The column oven was home-made and consisted of asbestos boards equipped with a heater and a micro fan. The temperature was adjusted by altering the applied voltage with a sliding rheostat, and could be kept within $\pm 1°C$ during each chromatographic run. A Uvidec-100 UV spectrophotometer (Japan Spectroscopic Co., Tokyo, Japan) was employed as the detector. A flow cell was arranged for capillary LC, as described previously[19]. A schematic diagram of the apparatus is shown in Fig. 1.
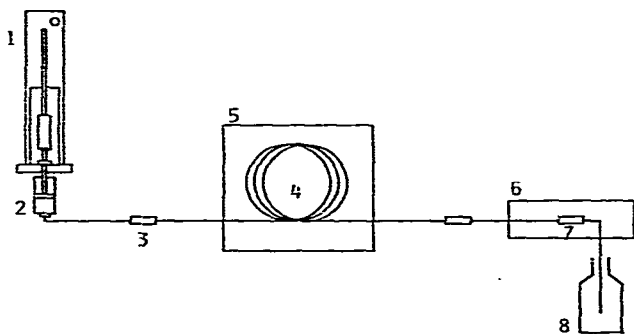

Fig. 1. Schematic diagram of the apparatus. 1 = Micro Feeder; 2 = gas-tight syringe; 3 = inlet; 4 = capillary column; 5 = column oven; 6 = UVIDEC 100 UV spectrophotometer; 7 = micro flow cell; 8 = waste reservoir.

In this work, capillary columns containing octadecylsilane (ODS), γ-amino-propylsilane (NH$_2$), silica gel (Si) and 2-sulphoethylsilane (CEX) were prepared as described earlier[18,20,21]. The preparation procedures for these columns are presented in Fig. 2. First, soda-lime glass capillaries were treated with 1 N sodium hydroxide solution for 2 days at 45–50°C. This treatment produced quasi-silica gel on the surface of the glass capillaries, the properties of which were dependent on the treatment temperature and time[20]. Subsequent to washing and drying, the surface of the glass capillaries was reacted with octadecyltrichlorosilane[18], γ-aminopropyltriethoxysilane and 2-mercaptoethyltriethoxysilane[21] (Tokyo Chemical Industry Co., Tokyo, Japan). For the CEX column, mercato groups were oxidized to sulpho groups by treatment with potassium permanganate solution.
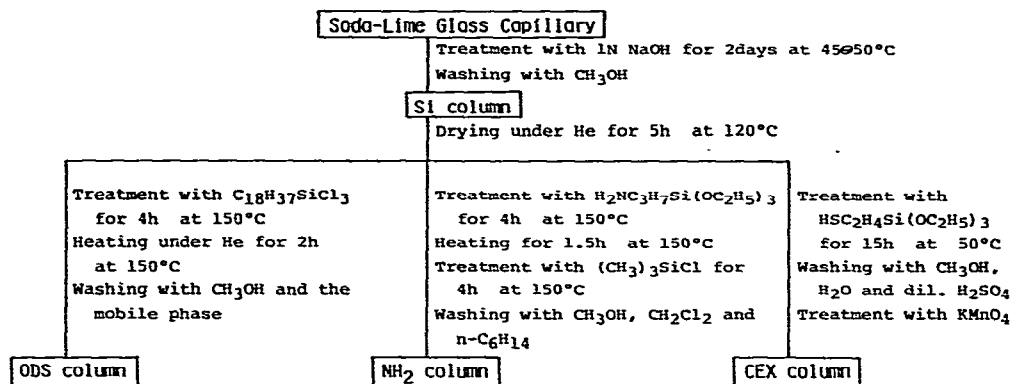
| Soda-Lime Glass Capillary |
| Treatment with 1N NaOH for 2days at 45⊖50°C |
| Washing with CH$_3$OH |
| Si column |
| Drying under He for 5h at 120°C |

Treatment with C$_{18}$H$_{37}$SiCl$_3$ for 4h at 150°C	Treatment with H$_2$NC$_3$H$_7$Si(OC$_2$H$_5$)$_3$ for 4h at 150°C	Treatment with HSC$_2$H$_4$Si(OC$_2$H$_5$)$_3$ for 15h at 50°C
Heating under He for 2h at 150°C	Heating for 1.5h at 150°C	Washing with CH$_3$OH,
Washing with CH$_3$OH and the mobile phase	Treatment with (CH$_3$)$_3$SiCl for 4h at 150°C	H$_2$O and dil. H$_2$SO$_4$
	Washing with CH$_3$OH, CH$_2$Cl$_2$ and n-C$_6$H$_{14}$	Treatment with KMnO$_4$
ODS column	NH$_2$ column	CEX column

Fig. 2. Preparation procedures for open-tubular capillary columns. ODS = octadecyltrichlorosilane; NH$_2$ = γ-aminopropylsilane; CEX = 2-sulphoethylsilane; Si = silica gel.

Each column was set in the column oven and the effect of column temperature was studied. The ODS column was examined in both reversed- and normal-phase systems; the Si and NH$_2$ columns were examined in the normal-phase system and the CEX column in a cation-exchange system.

RESULTS AND DISCUSSION

Reversed-phase system
    The diffusion coefficient of a solute in the mobile phase (D_m) is highly dependent on the temperature and composition of the mobile phase. The effect of column temperature and mobile phase composition on H for a non-retained solute is illustrated in Fig. 3. H_m is inversely proportional to D_m, as represented by eqn. 2. In other words, H_m corresponds to the viscosity of the mobile phase. Therefore, Fig. 3 indicates that a methanol solution containing *ca.* 60% of water has the maximal viscosity, whereas the viscosity of acetonitrile solution varies monotonously with mobile phase composition. In both instances, the higher the column temperature, the smaller is H, which suggests that a higher efficiency will be obtained at elevated temperature for retained solutes. However, it should be remembered that the retention of solutes generally decreases with increasing column temperature and therefore a water-rich solution should be employed at elevated temperature.
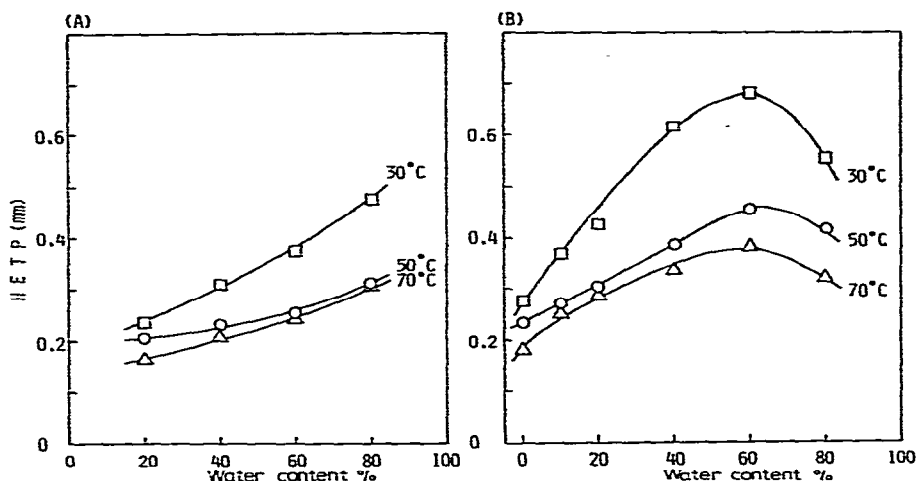
Fig. 3. Effect of column temperature and mobile phase composition on H. Column: 7.6 m × 72 μm I.D. Mobile phase: (A) acetonitrile–water; (B) methanol–water. Flow-rate: 1.7 μl/min. Sample: phenol in the mobile phase.

The dependence of k' on temperature is given by

$$\ln k' = \frac{-\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} + \ln\left(\frac{V_s}{V_m}\right) \qquad (3)$$

where ΔH° and ΔS° are the enthalpy and entropy, respectively, of transfer of a solute from the mobile phase to the stationary phase, R is the gas constant, T is absolute column temperature, V_s is the total volume of the stationary phase and V_m is the interstitial volume in the column. Eqn. 3 indicates that the logarithm of capacity factor is proportional to the reciprocal of absolute column temperature and ΔH° can be calculated from the slope of ln k' versus $1/T$ plots.

The order of retention of some polynuclear aromatic hydrocarbons reverses with change in column temperature. Fig. 4 demonstrates an example of the change in elution sequence with temperature change. 1,3,5-Triphenylbenzene elutes before 3,4-benzopyrene at low temperature (40°C), whereas the elution sequence is reversed at higher temperature (71°C); this is ascribed to the difference in the enthalpies of the two solutes. The flow-rate depends slightly on column temperature owing to the expansion of the eluent. As only the column was heated, the flow-rate at higher temperature was higher than original value.

Figs. 5 and 6 show the relationship between ln k' and $1/T$ using acetonitrile–water as the mobile phase. The slope of ln k' versus $1/T$ plots is peculiar to the structure of the solute. Compact solutes such as chrysene, perylene and 3,4-benzopyrene give smaller slopes than non-compact solutes such as p-terphenyl, 9-phenylanthracene and 1,3,5-triphenylbenzene. Similar results have been reported for packed column LC[7,15].

Fig. 7 shows the linear relationship between ln k' and $1/T$ using methanol–water as the mobile phase. Nearly the same tendency was obtained as in the acetonitrile–water system (Fig. 6), except for p-terphenyl. p-Terphenyl is obviously not
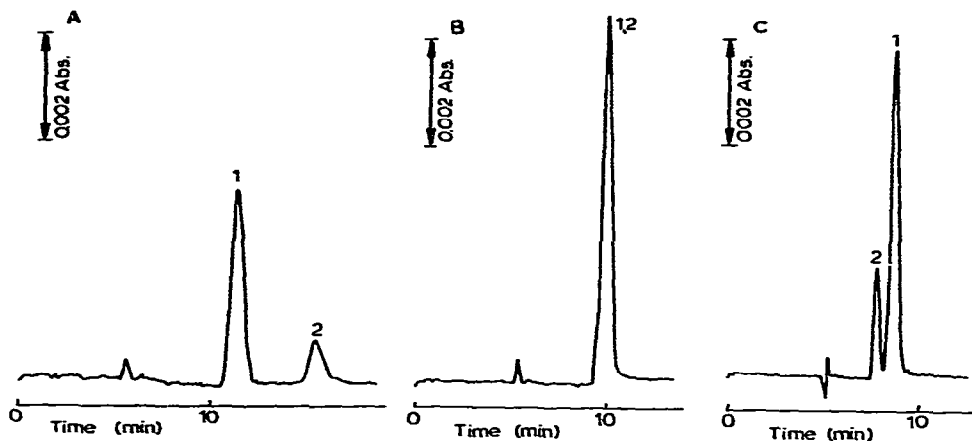
Fig. 4. Change in elution sequence with change in column temperature. Column: ODS, 5.3 m × 46 μm I.D. Mobile phase: acetonitrile–water (1:1). Flow-rate: 1.7 μl/min. Column temperature: (A) 40°C; (B) 50°C; (C) 71°C. Sample: 1 = 1,3,5-triphenylbenzene; 2 = 3,4-benzopyrene. Wavelength of detection (UV): 254 nm.
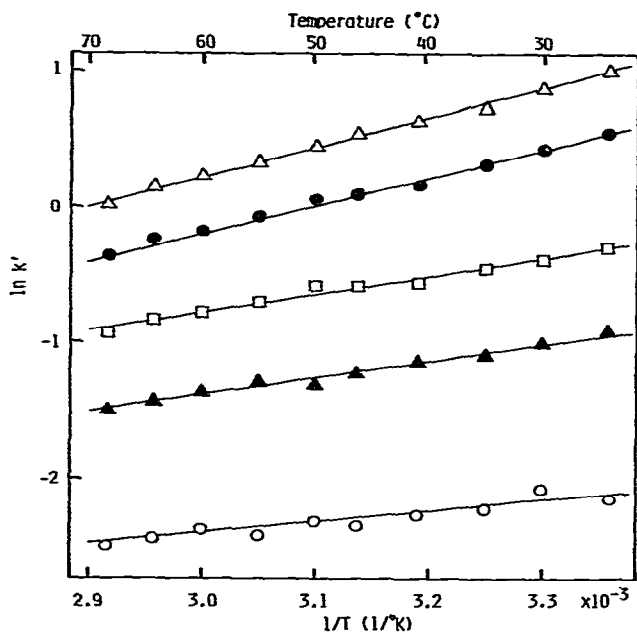

Fig. 5. Relationship between ln k' and $1/T$. Column: ODS, 5.3 m × 46 μm. Mobile phase: acetonitrile–water (35:65). Sample: O = benzene; ▲ = naphthalene; □ = biphenyl; ● = anthracene; △ = pyrene.

compact, but has a larger slope than other non-compact solutes. Snyder[9] considered that the common factor for "irregularity" seems to be the relative departure from a flat, straight molecule on the one hand, versus a bulky three-dimensional or spherical shape on the other. Hence polyaryls become less flat and become more compact or spherical with increasing intermolecular crowding, which may be dependent on the solvent system.
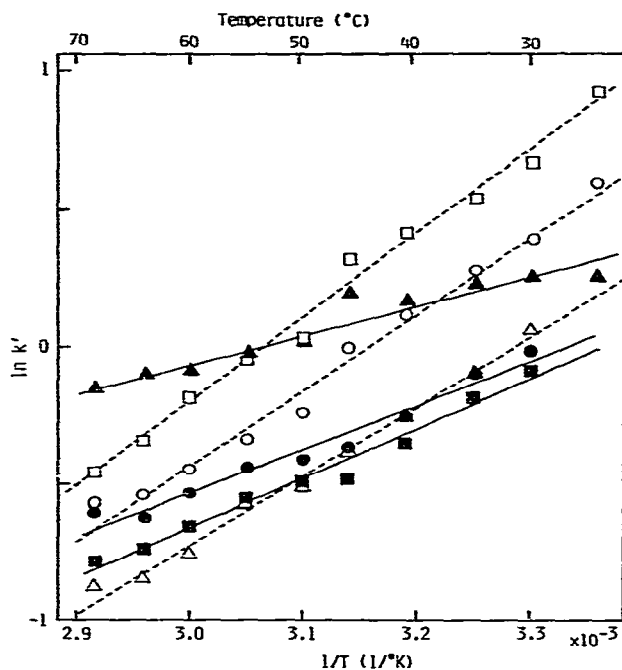
Fig. 6. Relationship between ln k' and $1/T$. Column: as in Fig. 5. Mobile phase: acetonitrile–water (1:1). Sample; ■ = p-terphenyl; △ = chrysene; ● = 9-phenylanthracene; O = perylene; ▲ = 1,3,5-triphenyl-benzene; □ = 3,4-benzopyrene.
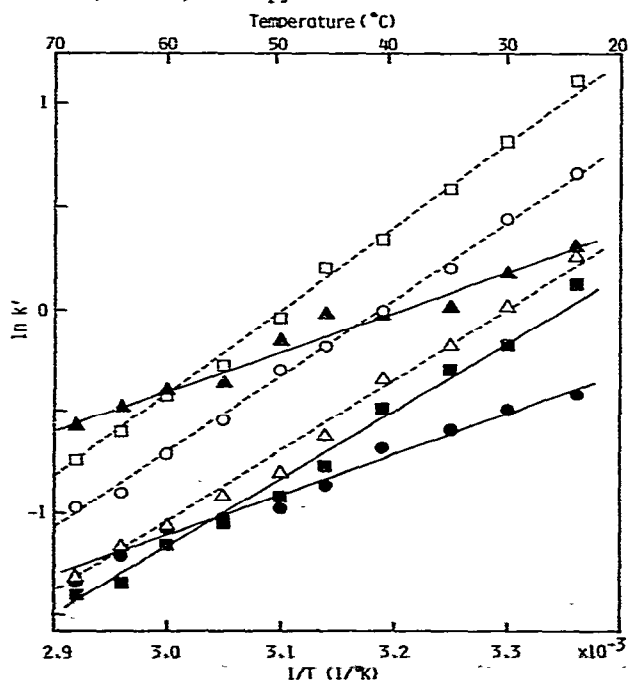

Fig. 7. Relationship between ln k' and $1/T$. Operating conditions as in Fig. 6, except the mobile phase (methanol–water, 7:3).

$\Delta H°$ values for polynuclear aromatic hydrocarbons are given in Table I. With the acetonitrile–water system, the $\Delta H°$ values obtained were nearly the same as those in the literature[7]. $\Delta H°$ is dependent on the kind of organic solvent and the water content. As described above, as the retentions of polynuclear aromatic hydrocarbons are individually dependent on column temperature, the $\Delta H°$ values become qualitative and temperature-programmed separation will be favoured in capillary LC as in GC.

TABLE I

$\Delta H°$ VALUES FOR POLYNUCLEAR AROMATIC HYDROCARBONS

Column: ODS, 5.3 m × 46′μm I.D.

Solute	Mobile phase	$-\Delta H°$ (kJ/mol)
Benzene	Acetonitrile–water (35:65)	7.0
Naphthalene		10.3
Biphenyl		11.3
Anthracene		16.0
Pyrene		17.6
p-Terphenyl	Acetonitrile–water (1:1)	15.2
Chrysene		20.7
9-Phenylanthracene		13.0
Perylene		23.2
1,3,5-Triphenylbenzene		8.5
3,4-Benzopyrene		25.7
p-Terphenyl	Methanol–water (7:3)	29.1
Chrysene		29.7
9-Phenylanthracene		17.1
Perylene		31.9
1,3,5-Triphenylbenzene		16.5
3,4-Benzopyrene		34.4

Selectivity is highly dependent on column temperature. Fig. 8 shows the effect of column temperature on the resolution (R_s) of solutes, which is defined by

$$R_s = \frac{2(t_2 - t_1)}{w_1 + w_2} \tag{4}$$

where t_1 and t_2 are retention times of solutes 1 and 2 ($t_2 > t_1$) and w_1 and w_2 are the peak widths (time units) of the two solutes. A pair of solutes can be resolved when $R_s \geqslant 1$ and they overlap when $R_s = 0$.

The effect of column temperature on efficiency is shown in Fig. 9. The theoretical plate number (N) increases with increasing column temperature. N doubles with a temperature increase of 30°C. On the other hand, the effective theoretical plate number (N_{eff}) varies only slightly with temperature because the retention of solutes decreases with increasing column temperature. Operation at temperatures higher than ambient gives higher efficiencies.

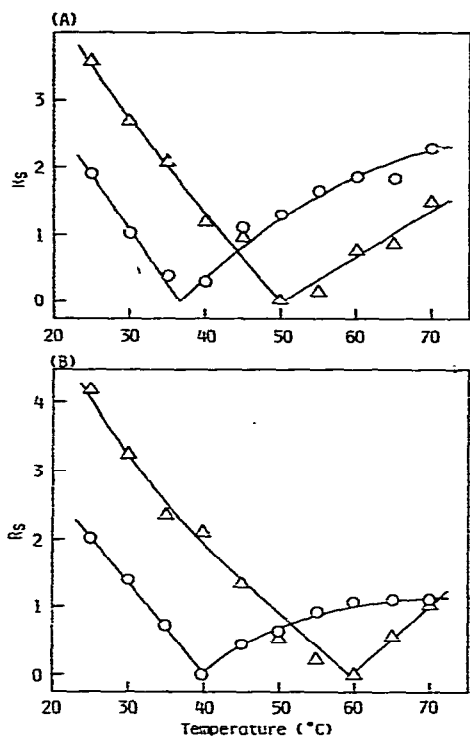A typical separation of polynuclear aromatic hydrocarbons on an ODS capil-

Fig. 8. Effect of column temperature on resolution of solutes. Resolution: O = perylene–1,3,5-triphenylbenzene; △ = 1,3,5-triphenylbenzene–3,4-benzopyrene. Flow-rate: 1.7 μl/min. Mobile phase: (A) acetonitrile–water (1:1); (B) methanol–water (7:3).
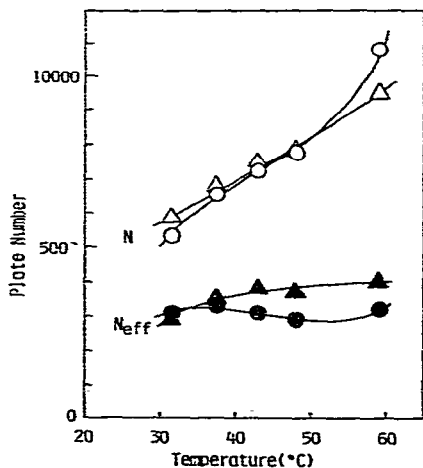

Fig. 9. Effect of column temperature on efficiency. Column: ODS, 7.2 m × 32 μm I.D. Mobile phase: acetonitrile–water (45:55). Flow-rate: 0.42 μl/min. Sample: O, ● = 3,4-benzopyrene; △, ▲ = 1,3,5-triphenylbenzene.

lary column is shown in Fig. 10. *p*-Terphenyl and chrysene overlapped at 43°C but fourteen compounds could be resolved at 13 or 32°C. A theoretical plate number of 19,000 was attained for 1,3,5-triphenylbenzene (Fig. 10).
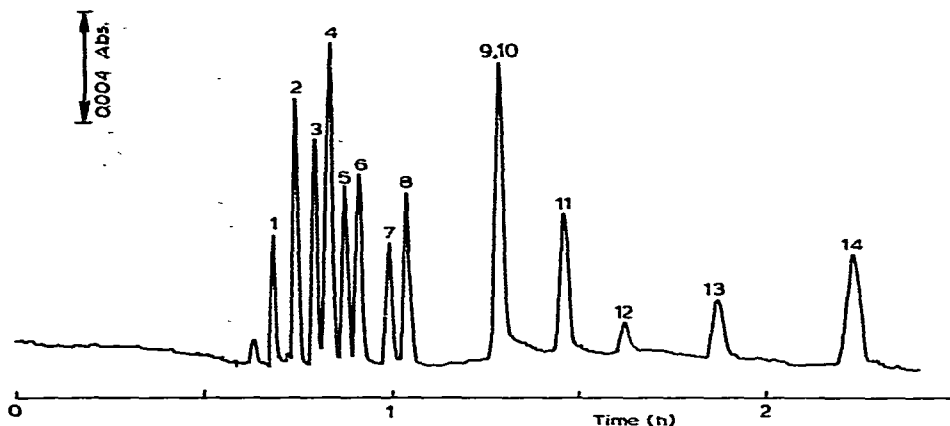

Fig. 10. Separation of polynuclear aromatic hydrocarbons on ODS capillary column. Column: ODS, 7.2 m × 32 μm I.D. Mobile phase: acetonitrile–water (45:55). Flow-rate: 0.14 μl/min. Sample: 1 = benzene; 2 = naphthalene; 3 = biphenyl; 4 = fluorene; 5 = phenanthrene; 6 = anthracene; 7 = fluoranthene; 8 = pyrene; 9 = *p*-terphenyl; 10 = chrysene; 11 = 9-phenylanthracene; 12 = perylene; 13 = 3,4-benzopyrene; 14 = 1,3,5-triphenylbenzene. Column temperature: 43°C. Wavelength of detection (UV): 254 nm.

Normal-phase system

Si, NH_2 and ODS columns were prepared and the effect of column temperature on the retention of aromatic amines was examined in the normal-phase system. Although ODS columns are usually employed in the reversed-phase mode, residual silanol groups in ODS capillary columns worked as adsorbents and permitted separation also in the normal-phase mode.

Figs. 11–13 show relationships between ln k' and $1/T$ for three types of columns. The mobile phase was *n*-hexane containing 0.5% (v/v) of acetonitrile as the moderator. Linear relationships were observed with the NH_2 and ODS columns, but for the Si column the relationship deviated from linearity. These results are not consistent with results in the literature[12,14]. In normal-phase chromatography using packed columns, the relationship between ln k' and $1/T$ is complex and far from linear.

Likewise in the reversed-phase system, $\Delta H°$ can be calculated from the data in Figs. 12 and 13. The results are given in Table II, which also gives $\Delta H°$ values using other solvents as the moderator for the NH_2 column. The $\Delta H°$ values are dependent on both the moderator and the stationary phase. In particular the $\Delta H°$ values for N-phenyl-α- and N-phenyl-β-naphthylamine obtained on the ODS column and those obtained using *n*-hexylamine as the moderator are characteristic.

The difference in the selectivity with the three types of columns was considered. Separation factors, defined as k_1'/k_2', are given in Table III. The difference in selectivity may be ascribed to the modification of the stationary phase.

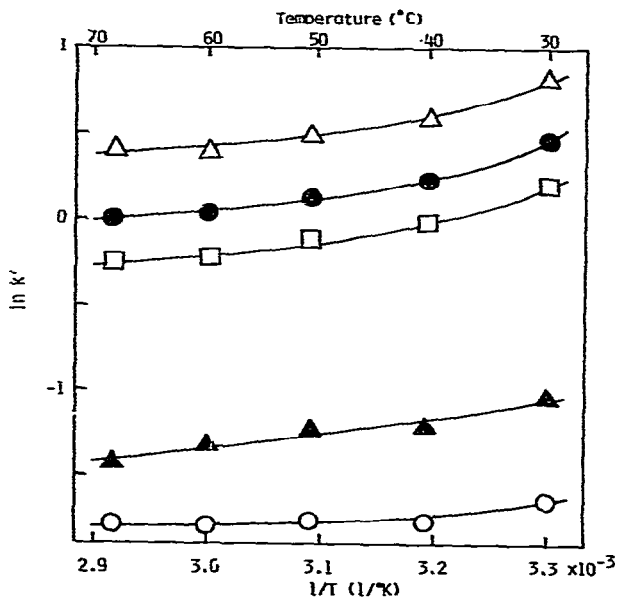The separation of aromatic amines on the ODS capillary column is shown in Fig. 14.

Fig. 11. Relationship between ln k' and $1/T$ for Si column. Column: Si, 5.2 m × 46 μm I.D. Mobile phase: n-hexane containing 0.5% of acetonitrile. Sample: O = N-phenyl-α-naphthylamine; ▲ = N-phenyl-β-naphthylamine; □ = aniline; ● = α-naphthylamine; △ = β-naphthylamine.
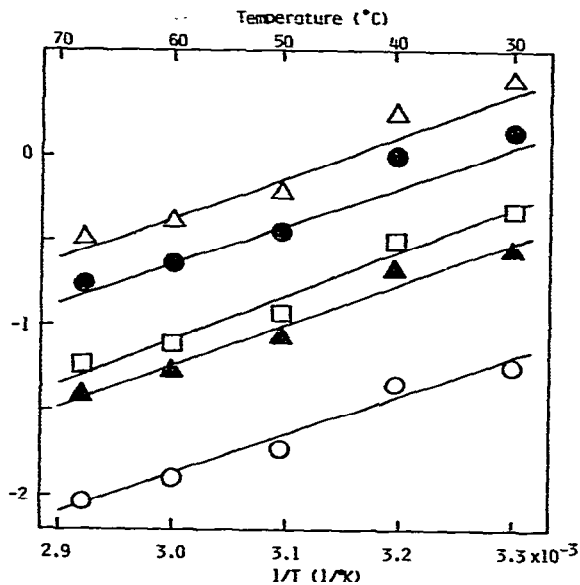
Fig. 12. Relationship between ln k' and $1/T$ for NH$_2$ column. Column: NH$_2$, 5.2 m × 55 μm. Other conditions as in Fig. 11.

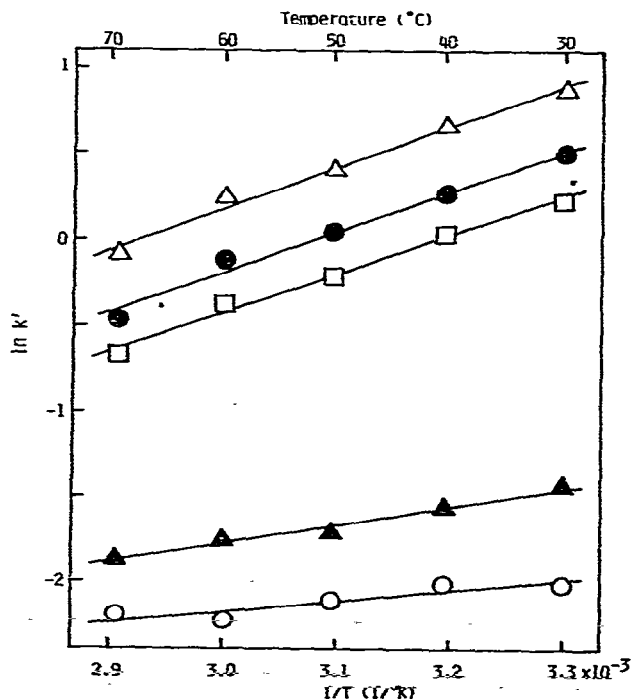

Fig. 13. Relationship between ln k' and $1/T$ for ODS column. Column: ODS, 5.3 m × 46 μm I.D. Other conditions as in Fig. 11.

TABLE II

$\Delta H°$ VALUES FOR AROMATIC AMINES

Columns: NH_2, 5.2 m × 55 μm I.D., and ODS, 5.3 m × 46 μm I.D. Mobile phase: n-hexane containing the moderator indicated.

Column	Moderator	Solute*	$-\Delta H°$ kJ/mol
NH_2	CH_3CN (0.5%)	NPαNA	18.8
		NPβNA	20.1
		A	21.1
		αNA	21.0
		βNA	21.1
NH_2	CH_3CN (0.2%) + CH_2Cl_2 (5%)	NPαNA	12.8
		NPβNA	15.4
		A	19.5
		αNA	18.7
		βNA	19.0
NH_2	n-$C_6H_{13}NH_2$ (0.2%)	NPαNA	5.2
		NPβNA	6.4
		A	—
		αNA	6.5
		βNA	6.1
ODS	CH_3CN (0.5%)	NPαNA	5.1
		NPβNA	9.2
		A	19.0
		αNA	19.1
		βNA	20.1

* NPαNA = N-phenyl-α-naphthylamine; NPβNA = N-phenyl-β-naphthylamine; A = aniline; αNA = α-naphthylamine; βNA = β-naphthylamine.

TABLE III

SEPARATION FACTORS OBTAINED IN THE NORMAL-PHASE SYSTEM

Mobile phase: n-hexane containing 0.5% of acetonitrile.

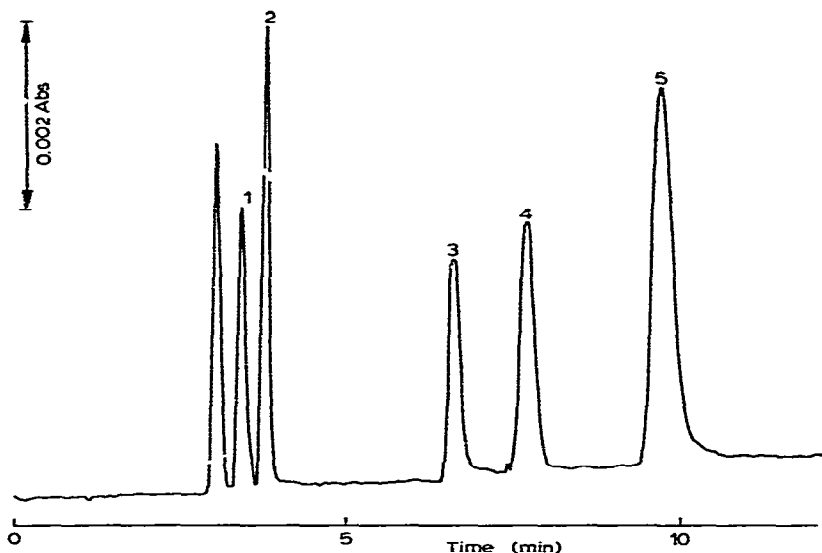| Column	Temperature (°C)	Separation factor*		
		k' (A)/k' (NPβNA)	k' (αNA)/k' (NPβNA)	k' (βNA)/k' (NPβNA)
Si	30	3.5	4.5	6.3
NH_2	30	1.2	2.0	2.6
ODS	30	5.2	6.9	10.0
Si	50	3.0	3.9	5.3
NH_2	50	1.1	1.8	2.4
ODS	50	4.6	5.9	8.6

* Abbreviations in parentheses as in Table II.

Fig. 14. Separation of aromatic amines on ODS column. Column: ODS, 5.3 m × 46 μm I.D. Mobile phase: n-hexane containing 0.5 % of acetonitrile. Sample: 1 = 2.2 ng of N-phenyl-α-naphthylamine; 2 = 2.6 ng of N-phenyl-β-naphthylamine; 3 = 3.1 ng of aniline; 4 = 1.7 ng of α-naphthylamine; 5 = 1.5 ng of β-naphthylamine. Temperature: 35°C. Wavelength of detection (UV): 235 nm.

Cation-exchange

Diffusion coefficients of solutes in the mobile phases employed in ion-exchange chromatography are so small that it is preferable to operate at higher temperature. The effect of temperature on column efficiency was examined in this work and previously[21], and a higher efficiency was attained at *ca.* 60°C for retained solutes.
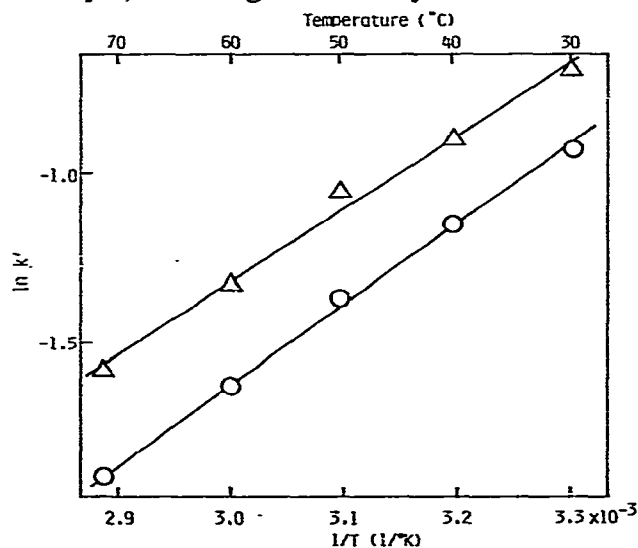

Fig. 15. Relationship between ln k' and $1/T$ for CEX column. Column: CEX, 5.4 m × 47 μm I.D. Mobile phase: $1 \cdot 10^{-3}$ M ammonium formate adjusted to pH 3.5 with formic acid. Sample: O = adenosine; △ = cytidine.

A linear relationship between ln k' and $1/T$ is also observed in cation-exchange capillary LC. The results are shown in Fig. 15. The $\Delta H°$ values for adenosine and cytidine were calculated to be -19.9 and -18.2 kJ/mol, respectively.

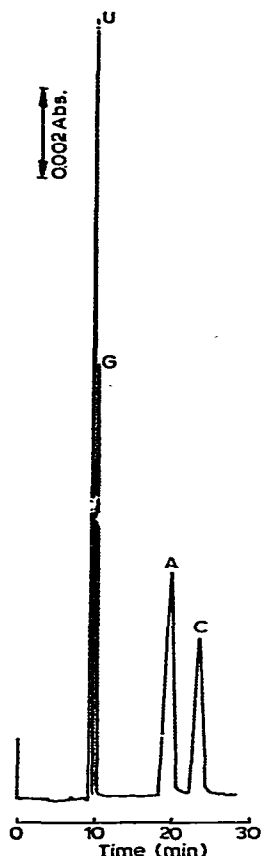The separation of four nucleosides on the CEX column is shown in Fig. 16.

Fig. 16. Separation of nucleosides on CEX column. Column: CEX, 6.0 m × 39 μm I.D. Mobile phase: 2 × 10^{-3} M ammonium formate adjusted to pH 3.4 with formic acid. Sample: U = uridine; G = guanosine; A = adenosine; C = cytidine. Temperature: 43°C. Wavelength of detection (UV): 260 nm.

CONCLUSION

Temperature determines the retention of solutes, selectivity and column efficiency in open-tubular capillary LC. Linear relationships between ln k' and $1/T$ are generally obtained in reversed-phase, normal-phase and cation-exchange capillary LC. Operation at higher temperature leads to a decrease in the viscosity of the eluent, which results in an increase in column efficiency and a decrease in inlet pressure.

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