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TEMPERATURE-PROGRAMMED REVERSED-PHASE LIQUID CHROMATOGRAPHY WITH PACKED FUSED-SILICA COLUMNS

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SUMMARY

Temperature-programmed separations using fused-silica columns with reversed-phase packings are described. The columns were specially designed for temperature programming. This allows their operation at elevated temperatures under high pressure and the use of an UV detector at room temperature without back pressure. Temperature programming has successfully been applied to the separation of *p*-nitrobenzyl esters of fatty acids, which are eluted over a wide range of capacity factors under isothermal conditions. It is also shown that fused-silica columns are superior to glass capillary columns, and 1-m fused-silica columns with 10- and 5-μm packings generate 60,000 and 90,000 theoretical plates, respectively.

INTRODUCTION

In liquid chromatography (LC) there has been an increasing demand for the separation of complex mixtures in biochemical and clinical chemistry and in the analysis of water pollutants and fossil fuels. Such separations require very efficient columns compared with conventional ones (4–8 mm I.D. and 20–50 cm long). Very efficient columns can also resolve closely eluting components of mixtures. If the mixture contains solutes with a wide range of capacity factors a programming technique is essential. Therefore, programmed elution using very efficient columns will be a powerful technique for the separation of highly complex mixtures, although optimization of mobile phase selectivity must still be considered.

Solvent programming (gradient elution) is the most often used of the various programming techniques in LC. Snyder[1] concluded that temperature programming was less effective than gradient elution under given conditions. However, he also suggested that the use of elevated temperatures beyond the boiling point of the mobile phase, and operation of the column at constant pressure, would lead to a substantial increase in efficiency.

When conventional LC columns are employed for temperature programming or operation at elevated temperature, several problems are found. The mobile phase has to be preheated to the column temperature before entering column, a change of column temperature during separation causes a temperature gradient across the

column cross-section and insufficient thermal equilibration often leads to a loss of efficiency and even skewed peaks[2]. This suggests that the use of micro-bore columns may be advantageous for temperature programming. Such columns have been attracting much attention due to their distinct advantages[3–5], the most prominent of which is their ability to achieve very high efficiency. Recently, fused-silica[6,7] and glass capillary columns[8] with inside diameters less than 0.5 mm have proved to be very efficient. Temperature programming using glass capillary columns has also been demonstrated[8], where the column temperature was maintained below the boiling point of the mobile phase.

Here, the use of temperature programming at temperatures beyond the boiling point of the mobile phase is described using fused-silica columns packed with reversed-phase materials. The effect of temperature on the retention of a series of *p*-nitrobenzyl esters of fatty acids was also investigated. A comparison of fused-silica and glass capillary columns is presented in terms of the height equivalent to a theoretical plate (HETP).

EXPERIMENTAL

Chromatographic systems

Two chromatographic systems were used. For the constant-pressure mode, a Twincle pump (Jasco, Tokyo, Japan) operated at constant pressure and a direct sample injection system[4] were employed. For the constant-flow-rate mode, a micro-feeder, Model MF-2 (Azumadenki Kogyo, Tokyo, Japan) equipped with a 500-μl gas-tight syringe and a micro-loop injector (volume 0.08 μl) was used. Detection was performed using a UVIDEC 100-III UV detector (Jasco) with a modified flow-through cell (volume 0.04 μl). Columns were placed into an oven of the gas chromatograph, GC-5A (Shimadzu, Kyoto, Japan), when necessary.

Column preparation

Fused silica with 0.20, 0.25 and 0.30 mm I.D. (Scientific Glass Engineering, Ringwood, Australia) and glass capillary with 0.20, 0.25 and 0.30 mm I.D. drawn using a glass drawing machine, GDM-1B (Shimadzu) were packed with Develosil ODS-10 and 5 (Nomura Kagaku, Seto, Japan). Details of the packing procedure were described elsewhere[8]. Columns used for temperature programming were packed as follows. Usually, a 1-m column was employed. Both ends, 10–15 cm long, were filled with silica gel and the intermediate portion with reversed-phase packing. In temperature programming, the portions packed with silica gel serve as the heating and cooling zones of the mobile phase.

RESULTS AND DISCUSSION

Comparison of glass capillary and fused-silica columns

Glass capillary and fused-silica columns (1 m long) were packed with Develosil ODS-10 or 5 using a slurry method[8]. Their efficiency for the solute *n*-hexylbenzene ($k' = 0.9$) was determined using methanol as a mobile phase. Efficiencies were calculated using the band width at half-height. Curves relating the height equivalent to a theoretical plate (HETP) to the linear velocity were constructed for each column. The

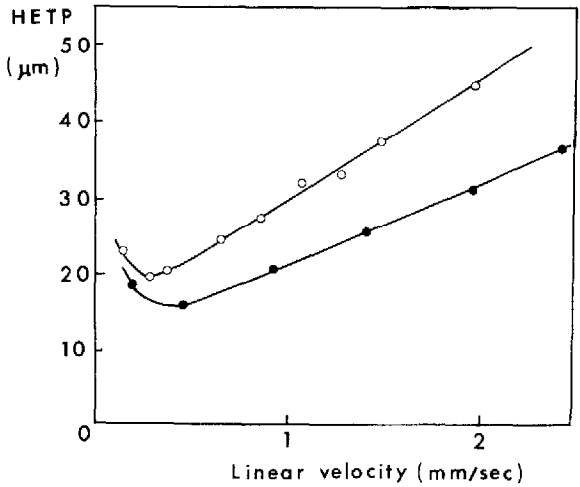

Fig. 1. Van Deemter plots for glass capillary (○) and fused-silica columns (●) (1 m × 0.2 mm I.D.) packed with Develosil ODS-10. Mobile phase: methanol. Solute: *n*-hexylbenzene ($k' = 0.9$).

results are shown in Figs. 1 and 2 for 10- and 5-μm packings, respectively.

The effect of column material on the efficiency is obvious. Fused-silica columns gave lower minimum HETPs for both 10- and 5-μm packings. Minimum HETPs were 20 μm (0.3 mm/sec) and 16 μm (0.4 mm/sec) for glass capillary and fused silica columns, respectively, using 10-μm packings, and 14 μm (0.45 mm/sec) and 11 μm (0.65 mm/sec) for 5-μm packings. The fused-silica columns also gave a smaller slope of the HETP curves, indicating that the resistance to mass transfer was smaller. Using a 1-m fused-silica column, 60,000 and 90,000 theoretical plates can be generated for 10- and 5-μm packings, respectively.

The different results may be due to the differences between the smoothness of the inside surfaces of the glass capillary and fused silica as pointed out before[6], while uniformness of the column diameter throughout the entire column length may also be

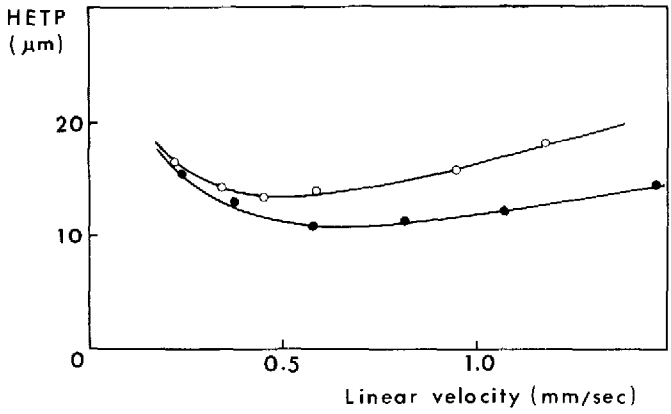

Fig. 2. Van Deemter plots for glass capillary (○) and fused-silica columns (●) (1 m × 0.2 mm) packed with Develosil ODS-5. Conditions as in Fig. 1.

an important factor. Both types of columns can withstand pressures of more than 500 kg/cm^2. High pressure operation of the fused silica column for longer periods caused the mobile phase to penetrate between the fused silica and coating film, and led to a loss of flexibility and mechanical strength. On the other hand, the glass capillary is fragile, so that careful handling is necessary.

Effect of temperature on the capacity factors

The effect of temperature on the capacity factors of a series of *p*-nitrobenzyl esters of fatty acids was examined. A fused-silica column (0.2 mm I.D. × 20 cm) packed with Develosil ODS-5 was used in the constant-flow-rate mode. Acetonitrile–water (70:30) was used as a mobile phase and water as a non-retained solute. Plots of log capacity factor (k') against the number of carbon atoms of the fatty acid moiety are shown in Fig. 3. As the last three solutes of C_{14}, C_{16} and C_{18} were strongly retained under the conditions used, their capacity factors were estimated by extrapolation. Fig. 4 shows a plot of log k' against the reciprocal of column temperature, *i.e.*, a Van 't Hoff plot. From the slope, the enthalpy change of each solute was determined. The values of $-\Delta H$ for each solute were plotted against the number of carbon atoms as shown in Fig. 5. It is seen that $-\Delta H$ increases almost linearly with the number of carbon atoms, indicating that strongly retained solutes are affected to a larger extent by temperature than are weakly retained ones. The values of $-\Delta H$ ranged from 3 to 7 kcal/mole. Therefore, an increase in temperature by 30°C results in a two- to three-fold increase in solute migration rate. At a column temperature of 16°C, the capacity factor of C_{18} exceeds 500. To elute this solute in a reasonable time with sufficient sensitivity, the temperature has to be raised to 200°C, where the capacity factor is 4.4, if the enthalpy change remains constant.

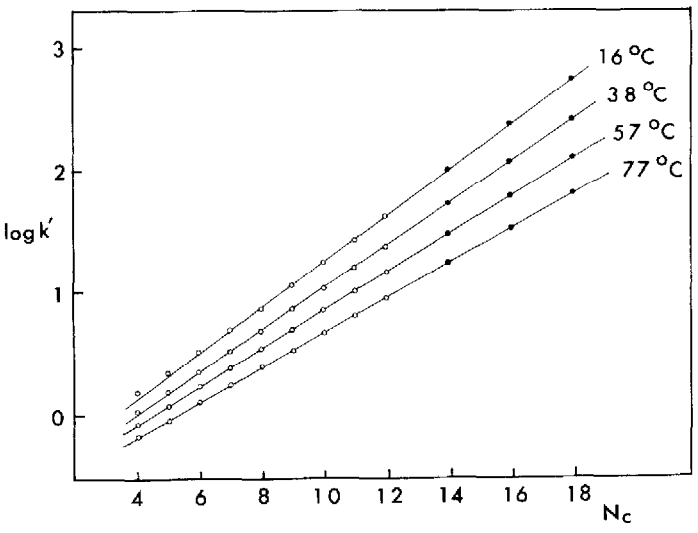

Fig. 3. Plots of log k' *vs.* number of carbon atoms of the fatty acid moiety in *p*-nitrobenzyl esters. Column: fused silica (20 cm × 0.2 mm I.D.) packed with Develosil ODS-5. Mobile phase: acetonitrile–water (70:30). Flow-rate: 2.1 μl/min. Detection wavelength: 254 nm. (○) Measured; (●) calculated.

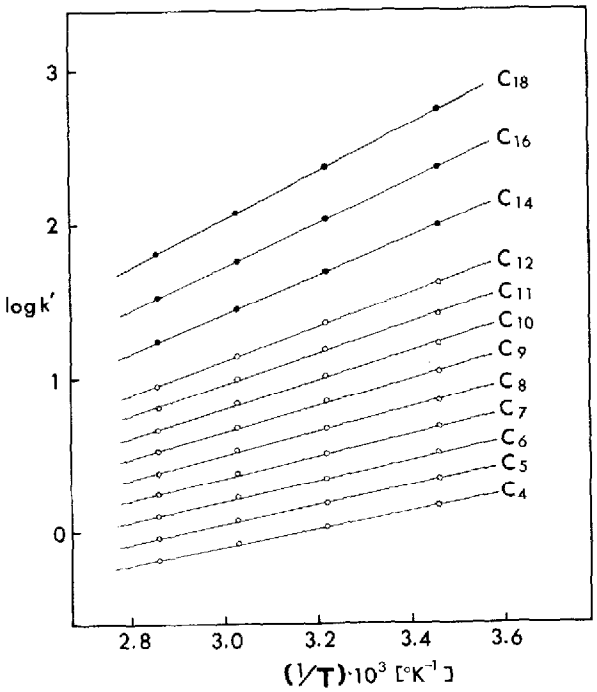

Fig. 4. Van 't Hoff plots for *p*-nitrobenzyl esters of fatty acids. Conditions as in Fig. 3.

Temperature programming

Temperature-programmed separation of a series of *p*-nitrobenzyl esters of fatty acids is shown in Fig. 6. The column temperature was maintained at 30°C for 30 min and then raised to 200°C at a rate of 1°C/min. A 110 × 0.2 mm I.D. fused-silica column was used. Both ends, 15 cm long, were filled with 5-μm silica gel, while the intermediate portion, 80 cm long, was filled with Develosil ODS-5. The column was

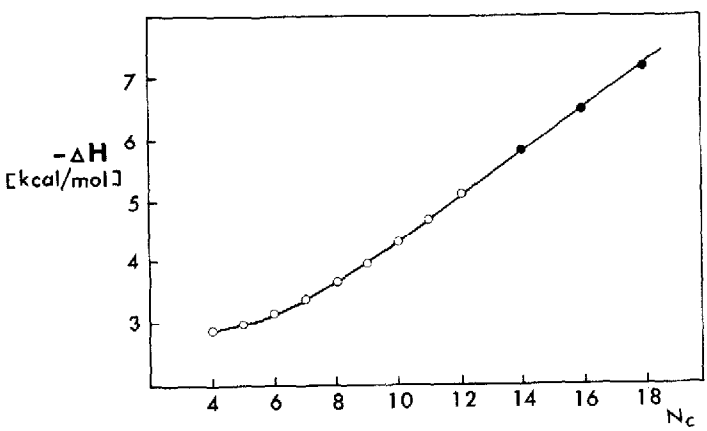

Fig. 5. Plots of $-\Delta H$ *vs.* number of carbon atoms (N_c) of the fatty acid moiety in *p*-nitrobenzyl esters. Conditions as in Fig. 3.

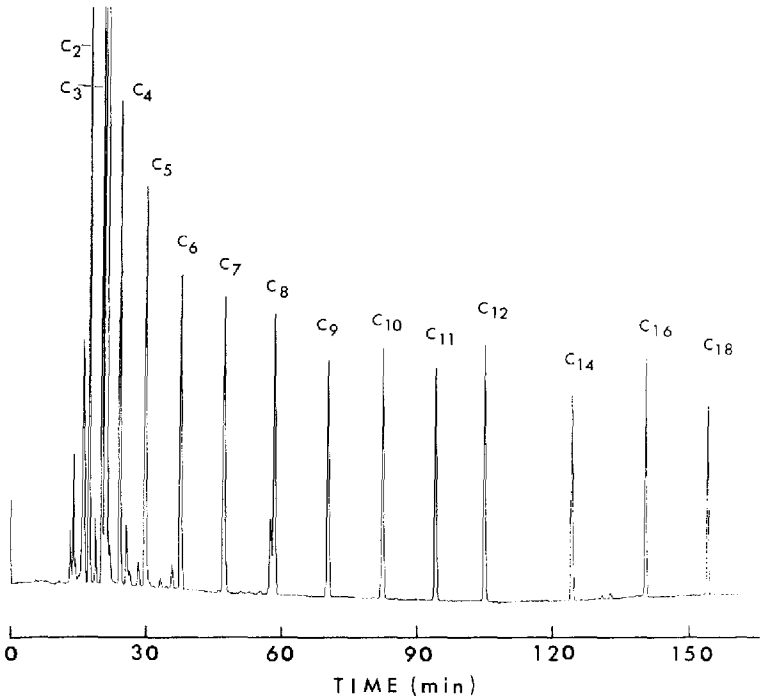

Fig. 6. Temperature-programmed separation of *p*-nitrobenzyl esters of fatty acids. Column: fused-silica column (110 cm × 0.2 mm I.D.) packed with Develosil ODS-5. Mobile phase: acetonitrile–water (70:30). Pressure: 250 kg/cm^2. Detection wavelength: 254 nm. The temperature was maintained at 30°C for 30 min and then raised to 200°C at a rate of 1°C/min.

operated in the constant-pressure mode at 250 kg/cm^2 using acetonitrile–water (70:30) as a mobile phase.

The mobile phase was preheated before entering the column and cooled to the ambient temperature at the column exit. The length of 15 cm was enough to prevent the mobile phase from boiling inside the column. The flow-rate increased from 2.2 to 4.4 μl with increase in temperature from 30 to 150°C, and corresponds to the range 0.88–1.7 ml/min for a conventional column with 4 mm I.D.

Under the same conditions, the separation of fatty acids of coconut oil was carried out, Fig. 7. These chromatograms clearly indicate the separation power of temperature-programmed liquid chromatography using a high efficiency column. Also the solutes which are strongly retained at room temperature can be eluted as narrower peaks with improved sensitivity.

An increase in temperature causes a decrease in viscosity of the mobile phase which results in improvement of efficiency due to a decrease in the mass transfer term. The decrease in mobile phase viscosity also causes an increase in flow-rate as described above, at constant pressure. This results in a combination of temperature programming and flow programming, which substantially increases the efficiency throughout the separation, as pointed out[1]. Moreover, temperature programming is much simpler than gradient elution, because only a one-component mobile phase is required. The baseline can be maintained constant throughout the separation.

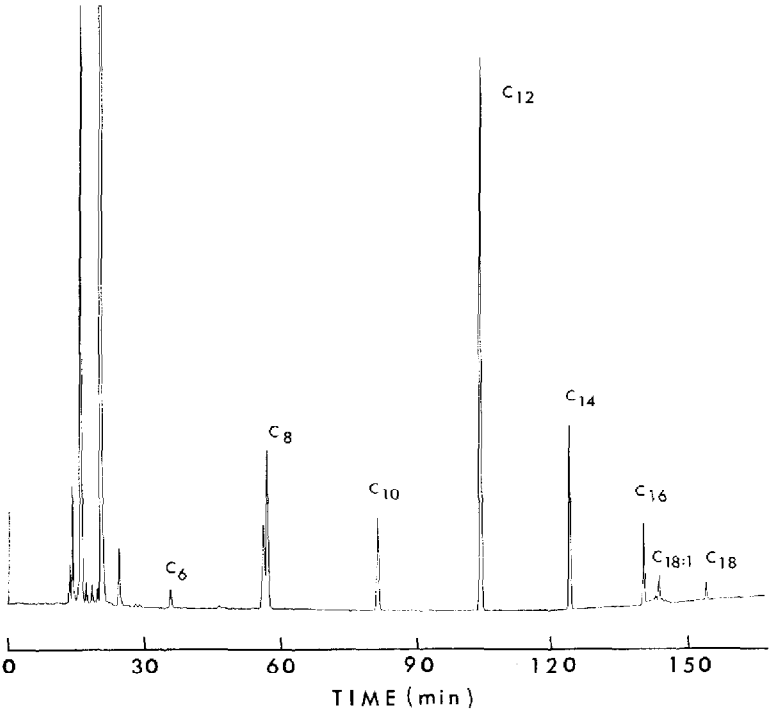

Fig. 7. Temperature-programmed separation of *p*-nitrobenzyl esters of fatty acids of coconut oil. Conditions as in Fig. 6.

Several repeated operations gave reproducible results, although operation over longer periods resulted in a loss of efficiency. This may be due to movement of the packed bed caused by changes in temperature and/or degradation of stationary phase. Silica gel packed into both ends of the column may be more unstable at the elevated temperature relative to ODS packing, because it will dissolve in the mobile phase to a greater extent. Under conditions similar to that in supercritical fluid chromatography, both the reactivity and thermal stability of the mobile phase and sample molecules have also to be considered. Further investigations along these lines are in progress.

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