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SUPERCritical FLUID CHROMATOGRAPHY OF VARIOUS SAMPLES WITH DIFFERENT POLARITIES

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SUMMARY

The retention behaviour of various samples with different polarities was studied with silica and ODS columns and *n*-hexane as the primary solvent. The effect of adding ethanol as the secondary solvent or modifier was also investigated. The results indicate that a selection of stationary and mobile phases should be made, depending on the polarity and the chemical reactivity of the sample.

INTRODUCTION

Supercritical fluid chromatography (SFC) has been recognized as a powerful technique for separating non-volatile and thermally labile compounds, especially oligomeric species, which are not amenable to gas chromatography (GC)¹⁻⁴. The separation ability of SFC is in some cases superior to that of high-performance liquid chromatography (HPLC).

The advantage of SFC over GC and HPLC arises from the flexibility in optimizing separations⁵. As in HPLC, a choice of stationary and mobile phases greatly influences the separation efficiency in SFC. The solvating power of the supercritical fluid can be easily adjusted simply by changing the pressure, and temperature is also an important operational parameter. Although various stationary and mobile phases have been used for SFC, it is not yet apparent which are the most suitable.

In previous work the surface properties of silica and chemically bonded phases were investigated⁶. The results suggested that a proper selection of stationary and mobile phases is required, depending on the types of compound to be analysed. In this work the retention behaviour of various samples with different polarities was investigated with silica and ODS columns and *n*-hexane as a primary solvent and ethanol as a modifier.

EXPERIMENTAL

The chromatographic system used was basically the same as in previous work⁴. In order to make a change of solvent easy, the solvent delivery system was modified. A gradient elution system reported elsewhere⁷ was installed between the pump and

TABLE I
PHYSICAL DATA FOR COLUMNS

Column No.	Length (cm) (I.D. = 0.2 mm)	Packing	Remarks
1	70	Develosil 100-10	*
2	60	Develosil 100-0	**,**
3	70	Develosil ODS-10	*
4	65	Develosil ODS-10	

* Both ends of the column (each 10 cm) were packed with glass beads.

** No use of ethanol-containing solvent.

the injector. A loop was replaced with a longer stainless tubing (10 m \times 1/16 in. O.D. \times 1 mm I.D.). The volume of the tubing is *ca.* 8 ml. As the flow-rate used was a few μ l/min, sufficient mobile phase was stored in the tubing to operate for one day. Gradient elution can be also carried out with this system, as described elsewhere⁸.

Table I summarizes the physical data for the columns used. All the columns were packed and conditioned in the same manner as in previous work. As the use of ethanol-containing solvents reduced the activity of silica⁴, the other silica column was prepared and used only with *n*-hexane. In order to examine the effect of glass beads on retention, two ODS columns with or without glass beads were prepared. The column temperature was kept at 260°C. Pure *n*-hexane or 10% ethanol in *n*-hexane was used as the mobile phase, unless specified. When necessary, pressure programming was carried out.

The samples used were as follows, in the order of increasing polarity: polystyrene oligomers with an average molecular weight of 2100, OV-17 (polysiloxane, 50% phenyl), dialkylphthalates ($R = C_1-C_{12}$) and Triton X-100 (polyethylene glycol *p*-isooctyl phenyl ether).

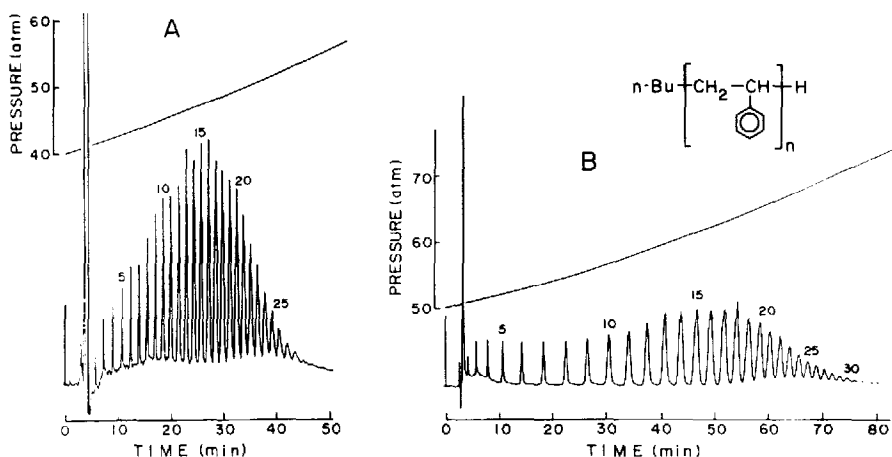


Fig. 1. Separation of polystyrene 2100 on column 1. Mobile phase: (A) *n*-hexane; (B) 10% ethanol in *n*-hexane. Temperature, 260°C.

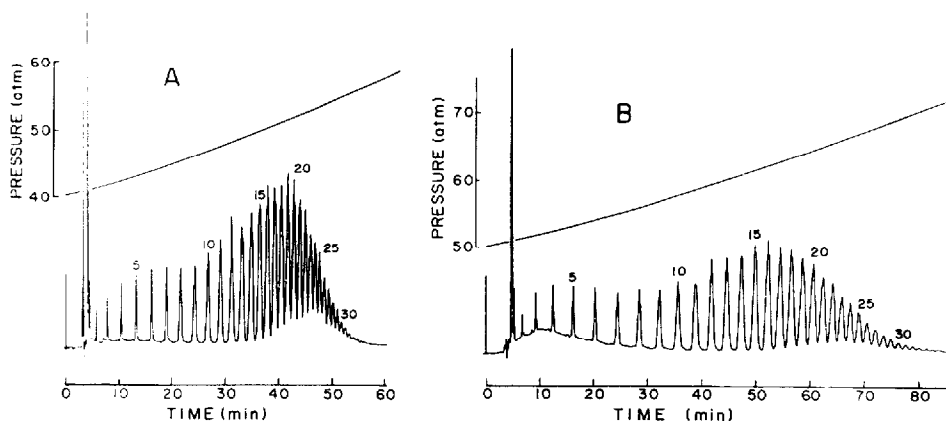


Fig. 2. Separation of polystyrene 2100 on column 3. Mobile phase: (A) *n*-hexane; (B) 10% ethanol in *n*-hexane. Temperature, 260°C.

RESULTS AND DISCUSSION

Fig. 1 shows the pressure-programmed separation of polystyrene oligomers on column 1 with pure *n*-hexane or 10% ethanol in hexane as the mobile phase. As the column was used with 20% ethanol in *n*-hexane before this separation, the silica surface may have been partially deactivated through the formation of ethyl silyl ether as reported⁴. As the addition of ethanol to the mobile phase increased the retention, pressure-programming was started from a higher initial pressure.

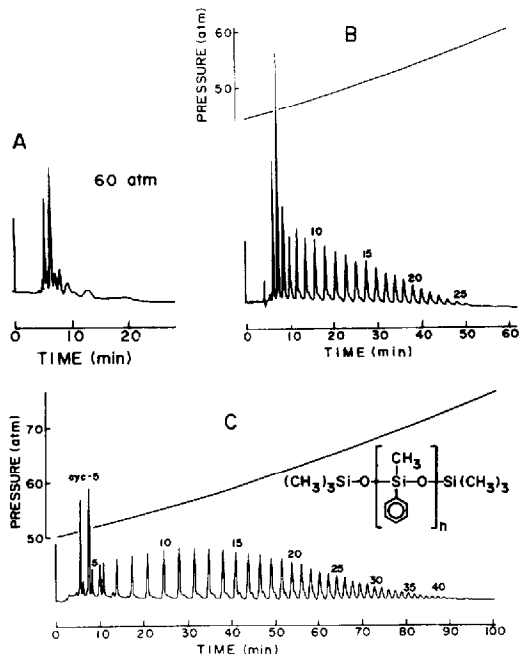


Fig. 3. Separation of OV-17 on column 2 (A) and column 1 (B) and (C). Mobile phase: (A and B) *n*-hexane; (C) 10% ethanol in *n*-hexane. Temperature, 260°C.

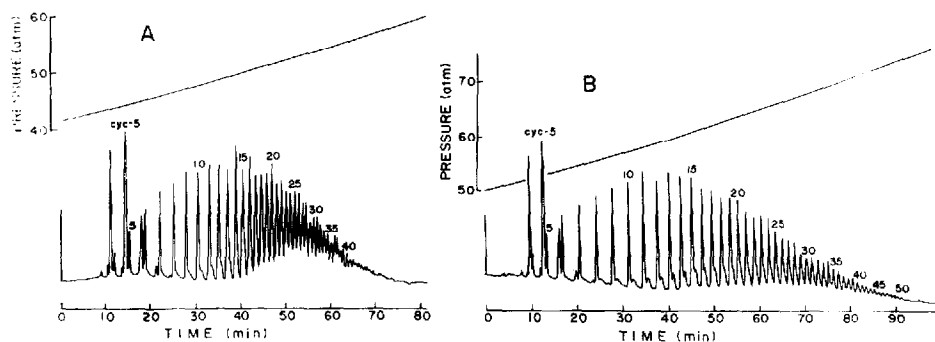


Fig. 4. Separation of OV-17 on column 3. Mobile phase: (A) *n*-hexane; (B) 10% ethanol in *n*-hexane. Temperature, 260°C.

Fig. 2. shows the separation of polystyrene oligomers on column 3, under conditions similar to those in Fig. 1. Although the retention was slightly greater on column 3 than column 1, the two columns showed similar elution behaviour for polystyrene oligomers.

Fig. 3 shows the separation of OV-17, which is more polar than polystyrene. On column 2 with pure *n*-hexane as the mobile phase, most of oligomers are not eluted even at higher pressures. On column 1 under similar conditions, more oligomers are eluted, but the higher ones still seem to be adsorbed on the silica. This indicates that column 2 has a stronger adsorption activity than column 1. Adding ethanol to the mobile phase led to complete elution of OV-17.

All the samples were eluted from column 3 with either pure *n*-hexane or 10% ethanol in *n*-hexane (Fig. 4).

A similar type of polysiloxane has been fractionated into cyclic and linear species by SFC⁹. Here, another series was found, eluted just after the linear species. Furthermore, the cyclic pentamer split into two peaks. This may be due to the higher resolving power of the system, indicating the advantage of using a small-bore column in SFC.

Figs. 5 and 6 show the separation of dialkylphthalates, which are more polar than OV-17. Owing to the higher polarity, they could not be eluted from column 1 with *n*-hexane as the mobile phase. The addition of ethanol to the mobile phase led

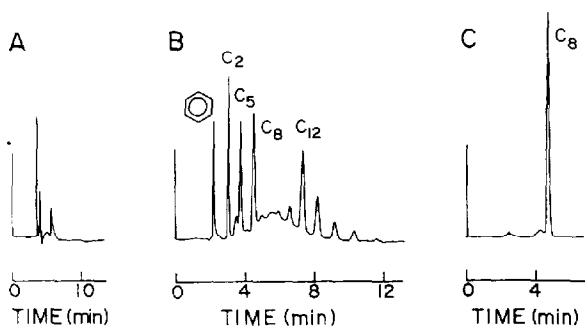


Fig. 5. Separation of dialkylphthalates on column 1. Mobile phase: (A) *n*-hexane; (B and C) 10% ethanol in *n*-hexane. Temperature, 260°C; inlet pressure, 50 atm.

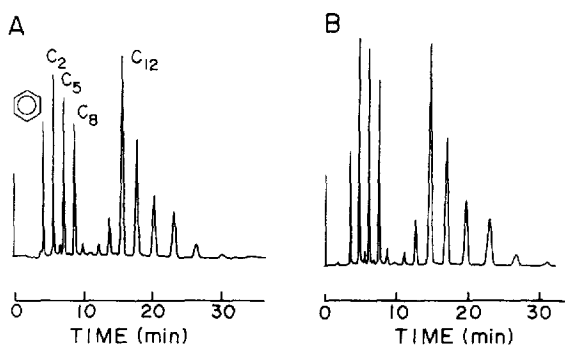


Fig. 6. Separation of dialkylphthalates on column 3. Mobile phase: (A) *n*-hexane; (B) 10% ethanol in *n*-hexane. Temperature, 260°C. Inlet pressure: (A) 40 atm; (B) 50 atm.

to complete elution, but caused decomposition. Fig. 5c shows the separation of dioctylphthalate, where a small broad peak was observed on the leading edge. Diethylphthalate did not show such behaviour. This may be accounted for by an ester-exchange reaction between the sample molecule and ethanol.

Column 3 could resolve all the samples without decomposition, even with 10% ethanol in *n*-hexane as the mobile phase. The addition of ethanol to the mobile phase only increased the retention. The results suggest that the ester-exchange reaction is catalysed by silica, or that ethyl silyl ether formed on the silica surface may play a role.

Fig. 7 shows the separation of Triton X-100, which is the most polar compound in this study. As it was insoluble in *n*-hexane, separation using pure *n*-hexane as the

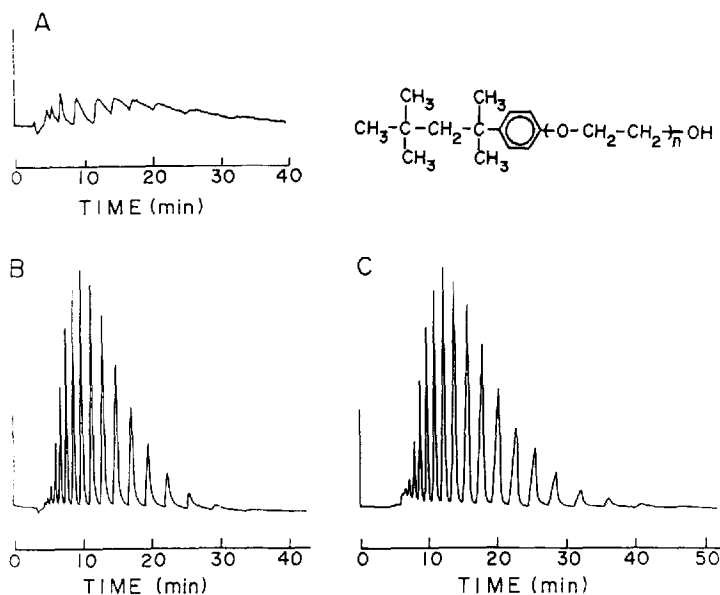


Fig. 7. Separation of Triton X-100 on column 1 (A), column 3 (B) and column 4 (C). Mobile phase: (A) 20% ethanol in *n*-hexane; (B and C) 10% ethanol in *n*-hexane. Temperature, 260°C. Inlet pressure: (A) 55 atm; (B and C) 50 atm.

mobile phase was not possible. Even with 20% ethanol in hexane with column 1, considerable tailing was observed. On column 3, where both ends were filled with glass beads, tailing was greatly reduced, but still present. On column 4, where the entire length was packed with ODS material, no tailing occurred. This indicates that the surface of glass beads is still active for very polar compounds such as Triton X-100.

In conclusion, the stationary and mobile phases should be chosen according to the types of solute to be separated. The chemical reactivity of the sample should be also taken into consideration.

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