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COMPARISON OF CHROMATOGRAPHIC BEHAVIOUR OF SILICA AND CHEMICALLY BONDED PHASES IN SUPERCRITICAL FLUID CHROMATOGRAPHY

YUKIO HIRATA

School of Materials Science, Toyohashi University of Technology, Toyohashi 440 (Japan)

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SUMMARY

The chromatographic behaviour of silica and chemically bonded phases was investigated under supercritical conditions using *n*-hexane-ethanol as the mobile phase. The results showed that the silica surface was modified with ethanol through the formation of ethyl silyl ether. At low concentrations of ethanol (less than 1%) adsorption was a predominant mechanism on the silica column, whereas at the higher concentrations (greater than 1%) partition similar to that for chemically bonded phases was predominant.

INTRODUCTION

Various stationary and mobile phases have been used for supercritical fluid chromatography (SFC)¹⁻³. Adsorbents such as silica, alumina, and chemically bonded phases have been generally used. The conventionally coated phases used in gas chromatography are unsuitable for SFC, because the liquid film is easily stripped off by the supercritical mobile phase. Therefore, cross-linked stationary phases have been preferred⁴. Carbon dioxide and *n*-pentane have been most widely used as the mobile phase. Although many solvents can be used for SFC, as indicated by Asche⁵, information about their properties under supercritical conditions is at present limited.

Owing to the non-polar nature of carbon dioxide and *n*-pentane, they are of limited usefulness as a chromatographic solvent. The use of more polar solvents has helped to improve selectivity^{6,7}. Alternatively, mixtures containing 2-propanol or methanol have been frequently used. Several workers have investigated the effect of the modifier on retention⁸⁻¹¹, but the results with silica as stationary phase are sometimes contradictory. A reprecipitation-redissolution mechanism has been suggested⁸. The surface properties of silica have been contrasted with those of chemically bonded phases⁹. However, the associated retention mechanism is not at present well understood.

In this work the chromatographic behaviour of silica and various carbonaceous bonded phases was investigated. Surface properties of silica with *n*-hexane-ethanol as the mobile phase, were also studied.

EXPERIMENTAL

The chromatograph system, which is described in detail elsewhere¹¹, consisted of a Du Pont LC-840 and Jasco Twincle liquid chromatograph pumps, a Jasco micro valve injector, a Shimadzu GC-5A gas chromatograph oven and a Jasco UVIDEC 100-III UV spectrophotometer. A fused-silica column (12 cm \times 200 μ m I.D.) packed with Develosil ODS-7 and connected to the outlet of the detector, was used as a restrictor throughout the study. A UV flow-cell with an effective volume of *ca.* 60 nl was prepared from 200- μ m I.D. fused silica.

Mobile phase solvents were *n*-hexane and ethanol. Polystyrene oligomers with an average molecular weight of 580 were used as a sample, dissolved in *n*-hexane. The following packing materials were used: Develosil 100-10, Develosil ODS-10 and Develosil C₈-10 (Nomura Kagaku, Japan); Finesil C₁₈-10, Finesil C₂ and Finesil C₁ (Jasco, Japan). The particle diameter of all the packing materials was 10 μ m.

Column preparation, conditioning and operation

The column was prepared from 0.2-mm I.D. fused silica with a slurry method using methanol as a slurry solvent. Both ends of the column (each 10 cm) were packed with glass beads and the intermediate portion (40–60 cm) with the packing material. This enables uniform heating of the column bed. The column was completely washed with dichloromethane and then *n*-hexane to remove methanol, because heating silica

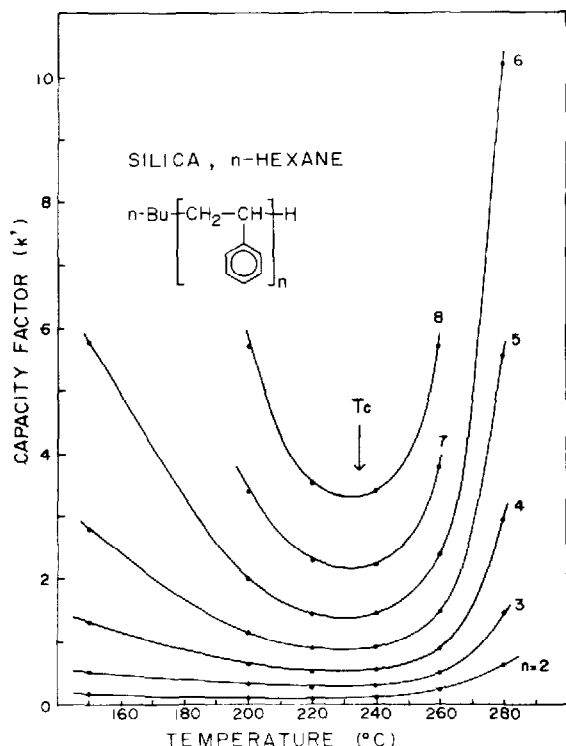


Fig. 1. Effect of temperature on retention on Develosil 100-10 column. Inlet pressure, 50 atm.

with methanol caused a change of surface properties as indicated later. The column was then heated to 200°C and kept for several hours under a flow of *n*-hexane to activate silica and cooled to room temperature.

All the data were obtained in the order of increasing temperature from room temperature to 280°C, and also of increasing concentration of ethanol from 0 to 20%. The inlet pressure was kept at 50 atm. Measurements of the flow resistance of the restrictor indicated that the mobile phase was in the liquid state below the critical temperature and in the supercritical fluid state above the critical temperature. For later case, the outlet pressure was around 40 atm. Capacity factors were calculated using benzene as a non-retained solute.

RESULTS AND DISCUSSION

Retention behaviour using n-hexane as the mobile phase

Figs. 1 and 2 show the effect of temperature on the retention of polystyrene oligomers on the Develosil 100-10 and Develosil ODS-10 columns, respectively. Pure *n*-hexane with a critical temperature (T_c) of 234.2°C was used as the mobile phase. Fig. 1 indicates that minimum k' values are obtained around T_c . Below T_c the separation mode is ordinary liquid-solid chromatography, where the retention decreases with increasing temperature. Above T_c it should be called "fluid-solid" chromatography¹². In this range the retention increases with increasing temperature, because

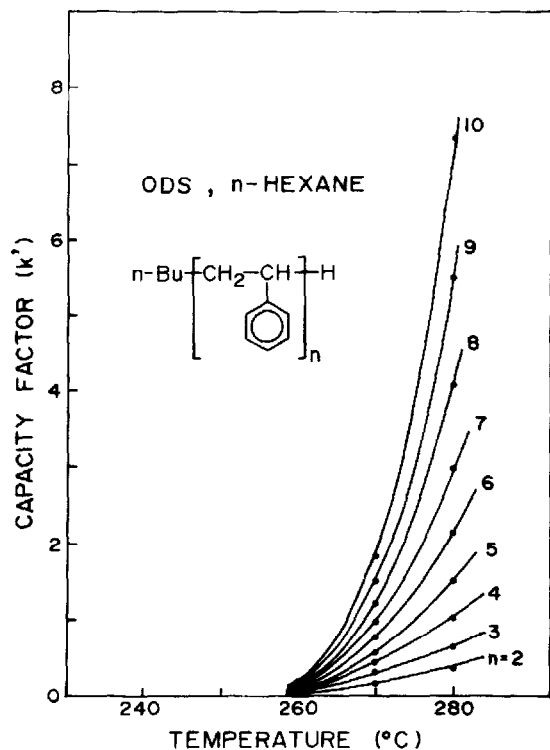


Fig. 2. Effect of temperature on retention on Develosil ODS-10 column. Inlet pressure, 50 atm.

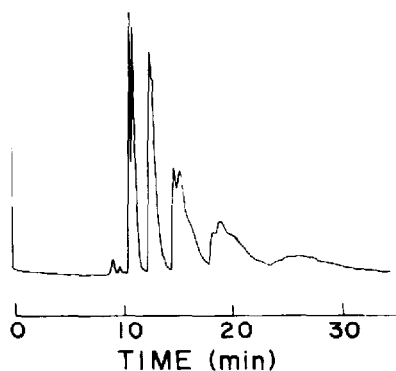


Fig. 3. Separation of polystyrene 580 on Finesil C_{18-10} column. Mobile phase, *n*-hexane; temperature, 50°C.

the density of the mobile phase decreases with increasing temperature. On the silica column adsorption will be a predominant mechanism both below and above T_c .

Polystyrene oligomers were not retained on the Develosil ODS column below T_c but increasingly retained with increasing temperature above T_c . The Develosil C_8 column also showed the same behaviour. These results indicate that these packings are sufficiently inert for polystyrene oligomers below T_c . Therefore, "partition" or adsorption on the ODS surface may be a predominant mechanism above T_c . The other ODS column (Finesil C_{18-10} , no end-capping) retained polystyrenes even below T_c , as shown in Fig. 3, and retained them more strongly than the Develosil ODS column above T_c . Here, both adsorption and partition may be included under the

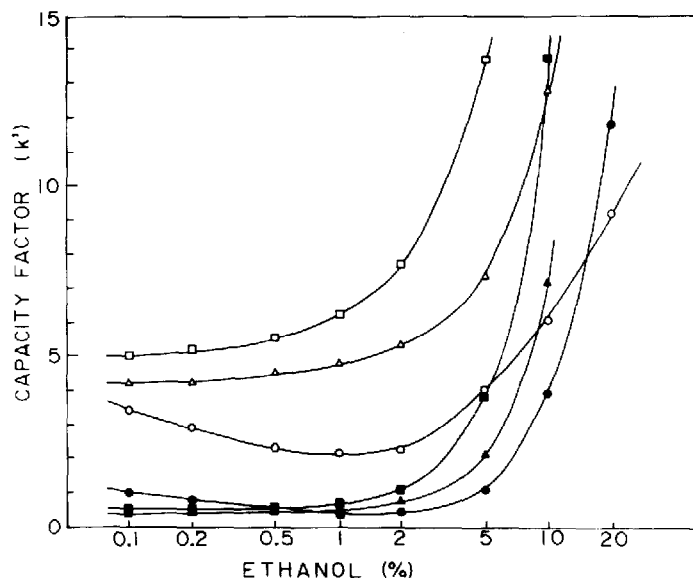


Fig. 4. Dependence of k' value on ethanol concentration for Develosil 100-10 (circles), Develosil ODS-10 (triangles) and Finesil C_2 (squares) columns. Solute, polystyrene ($n = 6$); inlet pressure, 50 atm. Closed symbols, 260°C; open symbols, 280°C.

supercritical conditions. Two other bonded phases, Finesil C₁ and C₂, were both slightly more active than Develosil ODS.

As the adsorption activity is related to the residual silanol groups and complete deactivation is difficult, more polar solutes may be retained even on the Develosil ODS column.

Effect of modifier on retention

In adsorption chromatography, a small amount of polar solvent is often added to a non-polar primary solvent as a moderator. The concentration is typically less than 1%. Since the polar moderator is preferentially adsorbed, the retention decreases with increasing concentration. Similar effects are also observed in SFC (Fig. 4). On the silica column, the retention was greatly reduced by adding 0.1% ethanol to *n*-hexane and gradually by further additions up to 1%. However, additions beyond 1% increased the retention with increasing concentration.

In contrast, both on the Develosil ODS and the Finesil C₂ columns, retention was increased with increasing concentration over the entire range.

With bonded phases, partition may be a predominant mechanism as discussed above. As ethanol is a poor solvent for polystyrene, addition of ethanol to *n*-hexane will decrease the solubility of polystyrene in the mobile phase and then increase retention. For the silica column, adsorption activity may be neglected at higher concentrations. Partition into ethanol adsorbed on the silica surface is not unlikely. However, ethanol can react with silanol on the silica surface to yield ethyl silyl ether.

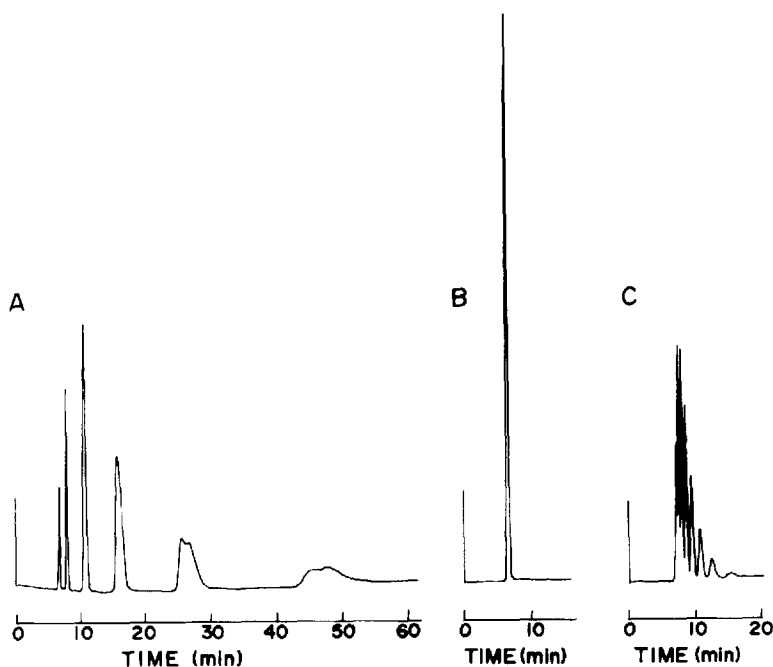


Fig. 5. Activity change of Develosil 100-10 column under adsorption LC mode. Mobile phase, *n*-hexane; temperature, 150°C; sample, polystyrene 580. (A) Before using ethanol; (B) after using 20% ethanol in *n*-hexane; (C) after washing with water-acetonitrile (50:50).

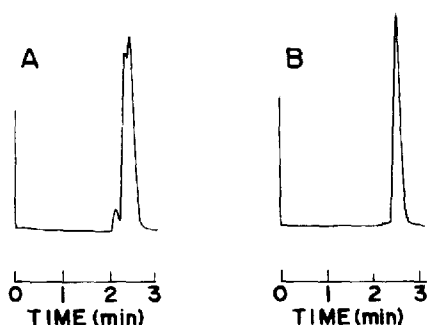


Fig. 6. Separation of polystyrene 580 on Develosil 100-10 after SFC with 20% ethanol in *n*-hexane. Mobile phase, acetonitrile; temperature, ambient. (A) First injection after washing with dichloromethane and acetonitrile; (B) second injection.

Such a bonded phase will behave in a similar manner to the C_2 phase. These considerations are supported by the following results.

When 20% ethanol in hexane was used and the column was washed with pure *n*-hexane, the activity of the silica column was almost completely lost (Fig. 5). Further washing with *n*-hexane at 200°C for 15 h resulted in little change. The column was then washed with dichloromethane and acetonitrile and submitted to reversed-phase separation of polystyrenes. An initial injection just after washing resulted in partial separation, but a second injection did not lead to the separation (Fig. 6). This observation may be due to the decomposition of ethyl silyl ether formed on the silica surface. In order to effect decomposition, the column was washed with water-acetonitrile (50:50) and then used for SFC with *n*-hexane as the mobile phase after conditioning as described in Experimental section. Fig. 5 indicates that the activity is partially recovered by this treatment, although the surface area may be different.

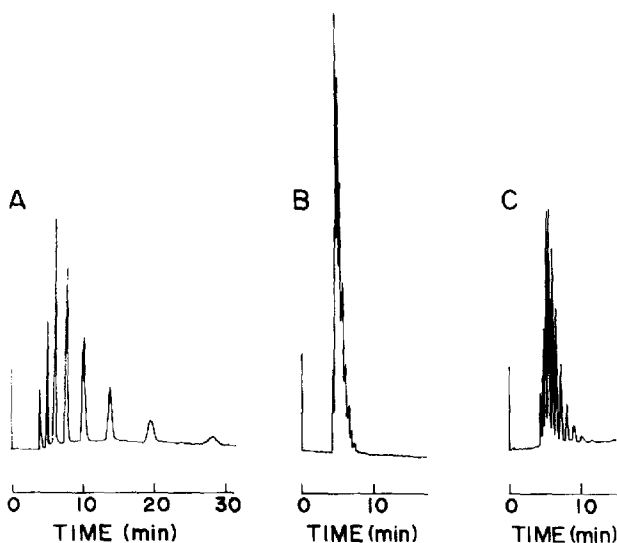


Fig. 7. Activity change of Develosil 100-10 column under supercritical conditions. Mobile phase, *n*-hexane; temperature, 260°C; inlet pressure, 50 atm; sample, polystyrene 580; (A), (B) and (C) same as in Fig. 5.

Similar behaviour was also observed under supercritical conditions (Fig. 7).

These results indicated that addition of ethanol to *n*-hexane changes the surface properties of silica under supercritical conditions. This behaviour will play an important role in separation, depending on the types of compound.

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