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GRADIENT ELUTION WITH NORMAL PHASES ON SILICA

A COMPARISON BETWEEN HIGH-PERFORMANCE LIQUID AND SUPERCRITICAL FLUID CHROMATOGRAPHY

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SUMMARY

Gradient elution on silica in supercritical fluid chromatography (SFC) and high-performance liquid chromatography (HPLC) was compared. The instrumental set-up for the measurement of the equilibration volumes in SFC and HPLC is described. It was demonstrated by k' measurements that 300–1000 column volumes in normal-phase HPLC are needed in order to achieve constant chromatographic conditions after a change of eluent. For this reason normal-phase gradient elution is an impracticable and irreproducible HPLC method for routine application. On the other hand, pressure gradient elution with supercritical carbon dioxide on silica showed very small equilibration volumes (*ca.* 2 column volumes). Thermodynamic equilibration in the column is achieved instantly. The modifier change in SFC needs only 10–30 column volumes. Here the equilibration volume depends strongly on the density and diffusivities in the supercritical fluid. Consequently, the use of pressure and modifier gradients in SFC has great advantages over the use of gradient elution in normal-phase HPLC.

INTRODUCTION

In recent years, interest in supercritical fluid chromatography (SFC) has increased continuously as it combines promising kinetic properties[1] with interesting solvent behaviour of the fluids. The solvent power and therefore the retention of substances in SFC depend strongly on the density of the solvent, and pressure programmed SFC[2] is comparable to gradient elution in high-performance liquid chromatography (HPLC).

Supercritical carbon dioxide at low densities possesses solubility parameters[3] similar to those of liquid pentane and at high densities of liquid toluene. Therefore, carbon dioxide is an advantageous eluent in normal-phase chromatography. A major disadvantage of normal phases in HPLC is the sensitive dependence of the selectivity on the amount of water; normal-phase HPLC often seems to be an irreproducible method and gradient elution on pure silica is nearly impossible. The equilibration

volumes needed to obtain stable chromatographic conditions are between 300 and 1000 column volumes. Polar bonded phases are better than pure silica[4] and normal-phase HPLC was gradually replaced by reversed-phase HPLC (RP-HPLC), although many separation problems such as the separation of isomeric compounds are often better solved by normal-phase HPLC than by RP-HPLC. With supercritical carbon dioxide as the eluent one should be able to combine the advantages of both SFC and normal-phase HPLC. The aim of these investigations was to compare gradient elution on silica in SFC and HPLC with respect to the equilibration volumes necessary for constant and reproducible chromatographic conditions.

EXPERIMENTAL

Apparatus

The determination of the pressure gradient equilibration volumes in SFC was carried out on the instrumental set-up shown in Fig. 1. This made possible an immediate change of the pressure conditions in the column without any change in flow-rate. Two independent SFC systems at different pressure levels were coupled by a Rheodyne 7010 switching valve. LC 420 and LC 410 pumps for carbon dioxide were obtained from Kontron (Zürich, Switzerland). The pump heads for the carbon dioxide-delivering pumps were equipped with head exchangers that allowed the carbon dioxide to be cooled to below $-10°C$. Pressure was monitored by LCP 501 pressure transducers (Innovativ-Labor, Wallisellen, Switzerland). The injector was
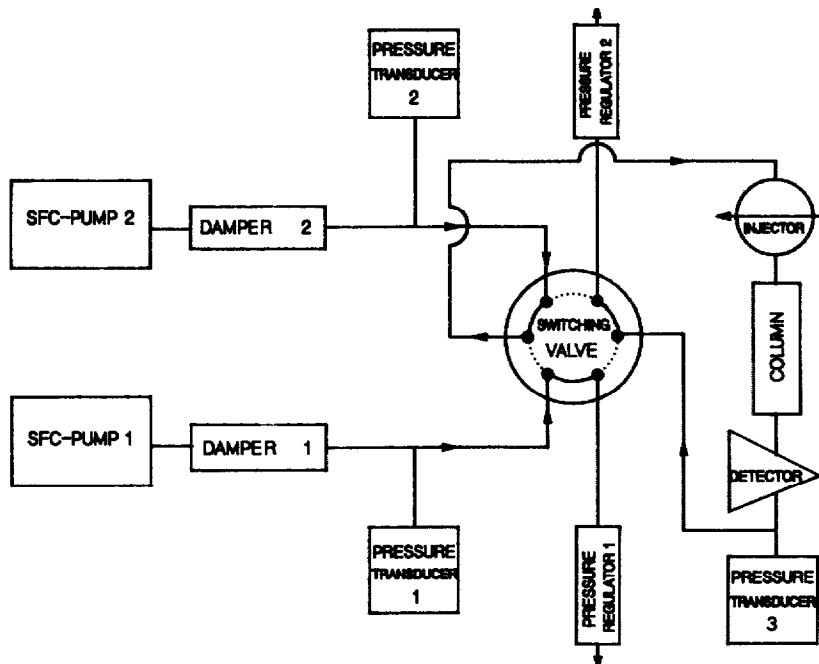

Fig. 1. Instrumental set-up for the measurement of relaxation time and pressure gradient equilibration time in SFC.

a Rheodyne 7010 valve. Uvikon 720 LC UV detector (Kontron) equipped with laboratory-made high-pressure-resistant cell was used. The system back-pressure was maintained and adjusted with 26-1761 pressure regulators (Tescom, Elk River, MA, U.S.A.).

For the measurement of the equilibration volumes in solvent-programmed HPLC the SFC set-up shown in Fig. 1 was used without pressure regulators and pressure transducers. One pulse damper was replaced with an alumina column (200 × 30 mm I.D.) in order to guarantee a constant small amount of water in the n-heptane.

Chemicals

Carbon dioxide (48 grade) was obtained from Carba Gas (Basle, Switzerland), and n-heptane (HPLC grade) from Merck (Darmstadt, F.R.G.). Wet n-heptane, directly from the bottle, contained 80 ppm of water. The dried n-heptane (50 ppm of water) was pumped through a column filled with neutral alumina (Woelm, Eschwege, F.R.G.), pretreated in an oven at 600°C for 4 h. Methanol was obtained from Rathburn (Walkerburn, U.K.). Anthracene, naphthalene, perylene, nitrobenzene and 1,2-diphenylethandione were purchased from Merck. In all experiments silica columns from Brownlee Labs. (Santa Clara, CA, U.S.A.) were used. The columns (100 × 4.6 mm I.D.) were packed with 5-μm particles.

RESULTS AND DISCUSSION

It is of practical importance to know the equilibration times in pressure programmed SFC to prevent irreproducible results being obtained owing to a lack of thermodynamic equilibrium in the column. The reproducibility of retention times could be influenced by the heat generated by the compression of the carbon dioxide during the pressure gradient. The dependence of the retention on the pressure drop was described by Schoenmakers and Verhoeven[5]. It might be that at high pressures an effect similar to capillary condensation causes the existence of a liquid carbon dioxide phase within the pores of the packing material[5]. Water dissolved in the carbon dioxide[6] could be adsorbed on to the silica surface. All these effects would influence the retention and, hence, the reproducibility of the separation. The faster the chromatographic equilibrium is obtained the better is the reproducibility. The disturbance of the equilibrium can be caused by a change of pressure in SFC or of solvent in silica HPLC. The solvent volume that is necessary to give constant capacity factors (k') after changing the chromatographic conditions serves as a criterion of reproducibility. This equilibration volume is expressed as the number of column volumes.

Modifier change in normal-phase HPLC

The influence of water on retention is demonstrated in Fig. 2. More than 1000 column volumes of dried n-heptane (40 ppm of water) are necessary to give stable conditions after changing the eluent from wet (80 ppm of water) to dry n-heptane. The water is strongly adsorbed on the polar silica surface[7] and also it has very low solubility in n-heptane. Both of these factors are responsible for the slow exchange of adsorbed water. A problem arises when solvents are used in gradient elution with different solubilities of water. It is very time consuming to establish the initial chromatographic
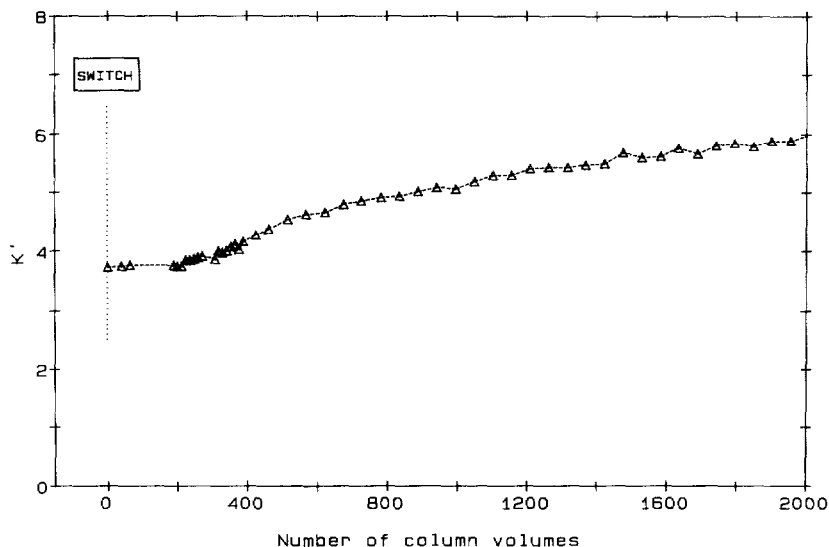
Fig. 2. Relaxation volumes in HPLC on silica. Switch from n-heptane (80 ppm of water) to n-heptane (50 ppm of water); flow-rate, 3.0 ml/min; sample, nitrobenzene.

conditions because of the insufficient solubility of water in the weaker eluent. Hence it is essential to control exactly the content of water in the solvents.

   With methanol as a modifier in n-heptane the new equilibrium seems to be obtained after nearly 300 column volumes (Fig. 3). Methanol is more soluble in n-heptane. However, the retarded recovery of the equilibrium may indicate that the
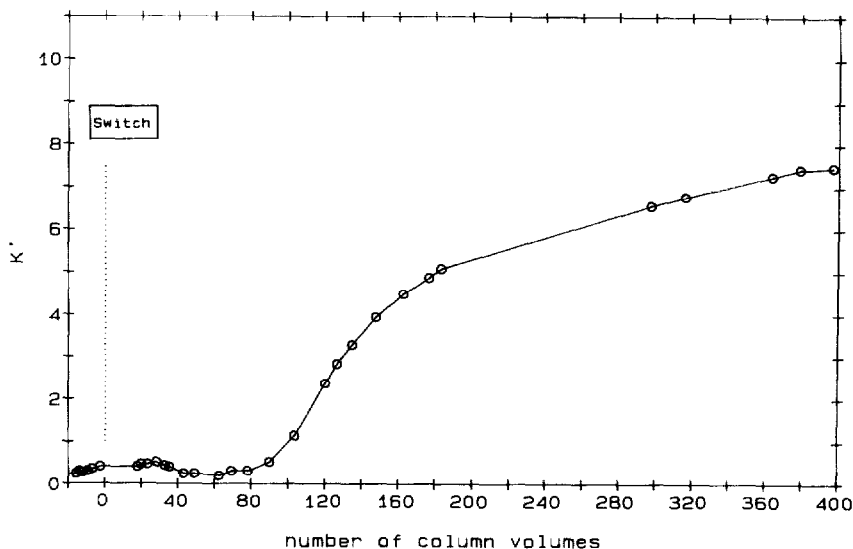

Fig. 3. Relaxation volumes in HPLC on silica. Switch from n-heptane–methanol (98.4:1.6) to n-heptane; flow-rate, 3.5 ml/min; sample, nitrobenzene.

equilibrium between methanol and *n*-heptane is superimposed by an equilibrium between adsorbed water from the methanol and the *n*-heptane.

Pressure change in normal-phase SFC

Fig. 4 shows that in contrast to solvent gradients in silica HPLC, pressure changes in silica SFC result in a very rapid establishment of the equilibrium. For all substances constant chromatographic conditions are obtained within two column volumes after switching the pressure from 150 to 180 bar. Apparently the heat of compression has no significant influence on retention. One gains considerable time in
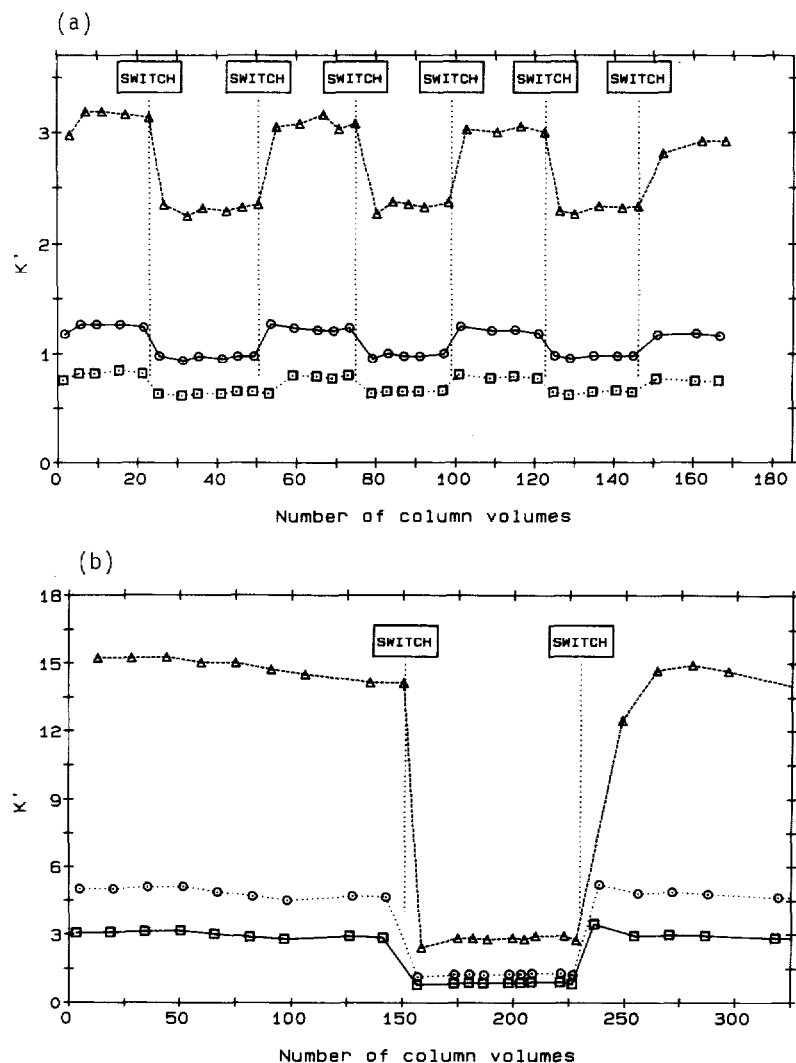

Fig. 4. Relaxation volumes in SFC on silica. (a) Switch from 150 to 180 bar; temperature, 40°C; flow-rate, 3.0 ml/min. (b) Switch from 100 to 200 bar. Conditions as in (a). △ = Perylene; ○ = pyrene; □ = anthracene.
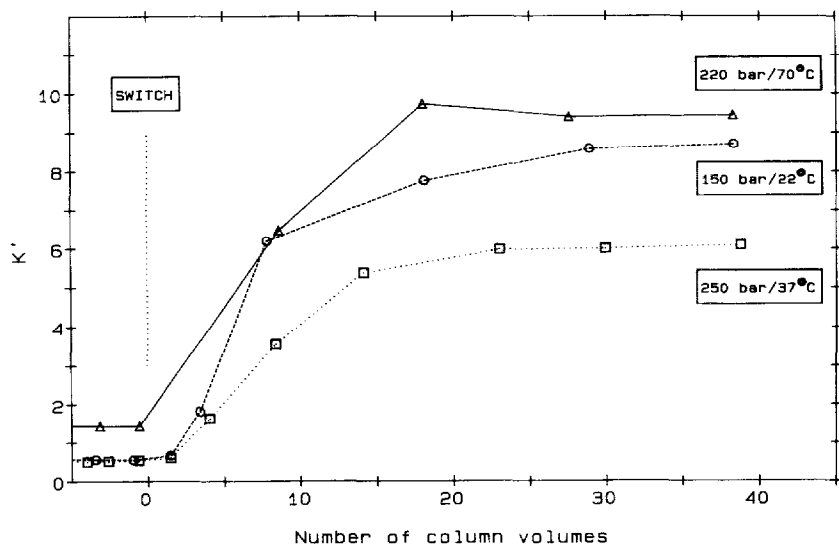
Fig. 5. Relaxation volumes in SFC on silica at different densities and diffusivities. Switch from carbon dioxide–methanol (98:2) to carbon dioxide; flow-rate, 4.0 ml/min; sample, 1,2-diphenylethandione.

SFC not only in gradient chromatography but also when optimizing an isocratic constant pressure method. Within a few seconds new constant chromatographic conditions are obtained.

Modifier change in SFC

Often it is essential to add polar modifiers to the carbon dioxide in order to improve the retention behaviour of the samples. It is important to know mainly during the optimization when the chromatographic equilibrium has been obtained. Fig. 5 and Table I demonstrate that after 10–30 column volumes completely constant chromatographic conditions are obtained. The number of equilibration volumes depends on the density of the fluid. There is a faster exchange of modifier at lower densities and consequently at higher diffusivities. The diffusion coefficients in carbon dioxide increase from 0.9 to 0.7 g/cm^3 and from lower temperature to higher temperature[8]. It

TABLE I

COMPARISON OF THE EQUILIBRATION VOLUMES (NUMBER OF COLUMN VOLUMES) FOR PRESSURE CHANGE AND MODIFIER CHANGE IN SFC AND MODIFIER CHANGE IN NORMAL-PHASE HPLC

Method	No. of column volumes
Pressure change in SFC	< 2
SFC 1 (70°C/220 bar), CO_2 + 2% methanol	10
SFC 2 (37°C/250 bar) → pure CO_2	18
Subcritical (22°C/150 bar)	28
HPLC 1 [n-heptane–methanol (98.4:1.6) to n-heptane]	300
HPLC 2 [n-heptane + 80 ppm water to n-heptane + 50 ppm water]	> 1000

seems possible that because of the 10-fold higher diffusivities of supercritical fluids compared with those of liquid heptane, the rate of desorption of the polar modifier molecules from the pores of the silica into the mobile phase is also much higher.

CONCLUSION

The retention in HPLC on silica is heavily dependent on the water content. Gradient silica HPLC is a time-consuming and often irreproducible chromatographic method because equilibrium is obtained only after about 300–1000 column volumes.

In contrast there are very short equilibrium times in pressure gradient SFC compared with gradients in silica HPLC. This leads to shorter times of analysis in pressure gradient SFC and higher reproducibility in normal-phase SFC. Moreover, modifier changes in SFC are much faster than in normal-phase HPLC. Therefore, the times necessary to develop optimized separations are much shorter in SFC than in HPLC. In addition, there are more possibilities in SFC of optimizing separations because both the density and the organic modifier affect selectivity.

Hence the most important objectives of a chromatographic method, namely reproducibility of retention times, time of analysis and time to develop a separation method, are much better fulfilled in SFC than in HPLC. A combination of SFC with the undisputed advantages of normal phases should be a powerful alternative to normal-phase HPLC.

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