GRADIENT ELUTION IN SUPERCRITICAL FLUID CHROMATOGRAPHY

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SUMMARY

The applicability to supercritical fluid chromatography (SFC) of elution by gradients in eluent composition is demonstrated, using alkanes and diethyl ether as the primary eluent and alcohols, cyclohexane and dioxane as the secondary component. For the separation of styrene oligomers, the combination of an alkane with dioxane turned out to be the most efficient. The SFC apparatus used was a modified high-performance liquid chromatographic instrument.

INTRODUCTION

Solutions to chromatographic problems with respect to optimization of resolution and analysis time often require the application of gradients. Among the substrates requiring gradient techniques are oligomer and polymer systems. The need to work with non-linear gradients arises from the fact that within a homologous series the differences in physical properties of the molecules, *e.g.,* solubility, become smaller with increasing molecular weight.

In supercritical fluid chromatography (SFC), gradients in pressure, temperature and eluent composition can be used. Whereas the usefulness of pressure gradients has already been established for a longer period of time (*cf.* recent reviews[1–3]), temperature gradients have been shown to be of less importance[4,5]. Gradients in eluent composition have been applied to SFC only lately but have already shown encouraging results[6,7]. In this paper, we report some results on the application of eluent gradients to oligomers of styrene and 2-vinylpyridine using diethyl ether and alkanes as the primary eluents.

EXPERIMENTAL

The SFC apparatus used for the investigation was a modified high-performance liquid chromatographic (HPLC) instrument (Model 1084B, Hewlett-Packard). It closely resembles the SFC apparatus described previously[5] and is shown schematically in Fig. 1.

Eluents that boil below 50–70°C are pre-pressurized in steel storage tanks (1) by applying a helium pressure. Alternatively, pre-pressurization can be accomplished
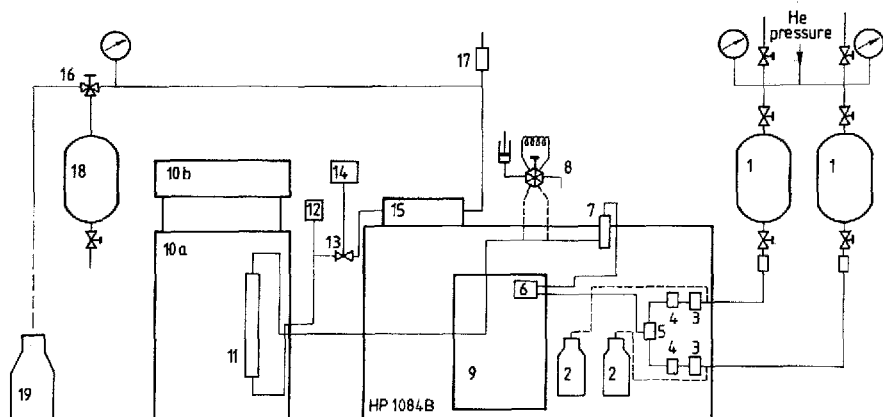
Fig. 1. Schematic diagram of SFC apparatus.

by increasing the temperature of the storage tank above ambient, using an external heating tape. If one of the components of the mobile phase possesses a sufficiently high boiling point, it can be fed without pre-pressurization from the glass bottles (2) provided with the HPLC instrument. The components are metered separately in the liquid state by the pumps of the Model 1084B instrument (3) and consecutively pass the damping system combined with a device for measuring the feed rate (4), the mixing chamber (5), a filter (6) and the variable-volume injection system (7). For work with low-boiling eluents, e.g., propane and butane, as a primary component, a multiport-valve (8) is used for injection instead of the variable-volume injector. The stream then enters the oven of the Model 1084B instrument (9).

For SFC operation, the stream is usually led directly out of the unheated oven to a larger external oven (10a) with forced air circulation (W. C. Heraeus, Hanau, F.R.G.; UT-5042 EK). This external oven can be heated to higher temperatures (300°C) and also accommodates larger columns. In addition, the oven can be temperature programmed by a programmer (10b) (W. C. Heraeus, Kelvitron TPG 2) equipped with a Pt-100 temperature sensor. The stream of mobile phase which is in the supercritical state within the oven passes through the separation column (11) and is allowed to liquefy after having left the oven. The pressure at the column end is monitored by an electric pressure transducer (12) (Siemens, Messumformer Teleperm D, M 56 441), while the pressure upstream of the column is measured by the pressure transducer of the Model 1084B instrument itself. Control of the pressure at the column end is provided by a needle metering valve (13). For creating pressure gradients, the stem of this valve can either be connected to a motor which is controlled by a pressure feedback loop or more simply with the potentiometer axis of a 'cut-a-curve' electromechanical time programmer (14). The eluate is then returned to the Model 1084B instrument, specifically to its UV detector. An external metering valve (16) allows the pressure in the detector to be raised to a level that prevents the formation of gas in the mobile phase. To keep the pressure constant and to protect the detector, an adjustable spring-actuated safety valve is provided (17). The eluent is finally collected in a pressure-resistant metal container as a liquid under its own vapour pressure or, in the case of higher boiling eluents, in a glass bottle (19).

The mobile phases, except for butane and propane, were distilled from sodium and degassed. Diethyl ether and 1,4-dioxane were treated with iron (II) sulphate in addition in order to remove peroxides. Butane (99.5%) and propane (99.5%) (Linde, Höllriegelskreuth, F.R.G.) were used without pre-treatment. Pentane and diethyl ether were fed to the chromatograph with pre-pressures of 0.2–1 bar, and propane and butane of 12–15 bar.

The stainless-steel columns (25 cm × 4.6 mm I.D.) were packed with Li-Chrosorb Si 100 (Merck, Darmstadt, F.R.G.) by means of a slurry method. The oligostyrene sample used in this investigation was PS 800 (number-average molecular weight, $\bar{M}_n$ = 810 g/mol; ratio of weight-average molecular weight to number-average molecular weight, $\bar{M}_w/\bar{M}_n$ ⩽ 1.12; from Pressure Chemical, Pittsburgh, PA, U.S.A.; the data are as given by the manufacturer). The oligo(2-vinylpyridine) sample was prepared by azobisisobutyronitrile-initiated radical oligomerization as described in detail elsewhere[8].

The baseline shifts in the chromatograms are due to different UV absorptions of the different eluent components; this effect is less pronounced in Figs. 4–6 according to higher detector attenuation.

Chromatograms were either run with the column end pressure kept constant, *i.e.* variable valve setting, or else keeping the valve setting constant, thereby incurring a change in column end pressure during the run.

RESULTS

The primary components diethyl ether and linear alkanes are known to be solvents for low-molecular-weight oligostyrenes. In particular, pentane has been shown to be a suitable eluent for the separation of oligostyrenes by SFC. The choice of the secondary eluents cyclohexane and dioxane was based on the increased solution power of these solvents for oligo- and polystyrenes compared with linear alkanes and ethers. Thus an increasing content of cyclohexane or dioxane in the eluent mixture is expected to reduce the retention times, especially for the higher members of the homologous oligostyrene series. Alcohols, on the other hand, are poorer solvents than diethyl ether and linear alkanes for oligostyrenes, and therefore a decrease in alcohol content should show a comparable effect. For oligo(2-vinylpyridine), however, the solubility behaviour in alkanes and alcohols is different to that with oligostyrenes: the solubility in alkanes is negligibly small and methanol is an excellent solvent. Therefore, chromatography of oligo(2-vinylpyridines) has to be carried out with an increasing methanol content. The results obtained for the two oligomer systems are described below for each component pair.

Diethyl ether–ethanol

As mentioned, a negative ethanol gradient had to be applied for oligostyrenes in order to increase the elution power of the eluent. A typical chromatogram is shown in Fig. 2a. This eluent pair turned out to be difficult. Although steep eluent composition gradients were applied, it was difficult to compensate for the broadening of the peaks with elution times longer than 10 min. Also, the pressure decrease due to a decreasing ethanol content with a given valve setting (valve 13 in Fig. 1) necessitated pressure regulating. Regulating the pressure, however, was difficult, as erratic pressure changes occurred. These pressure changes probably arise from particles of
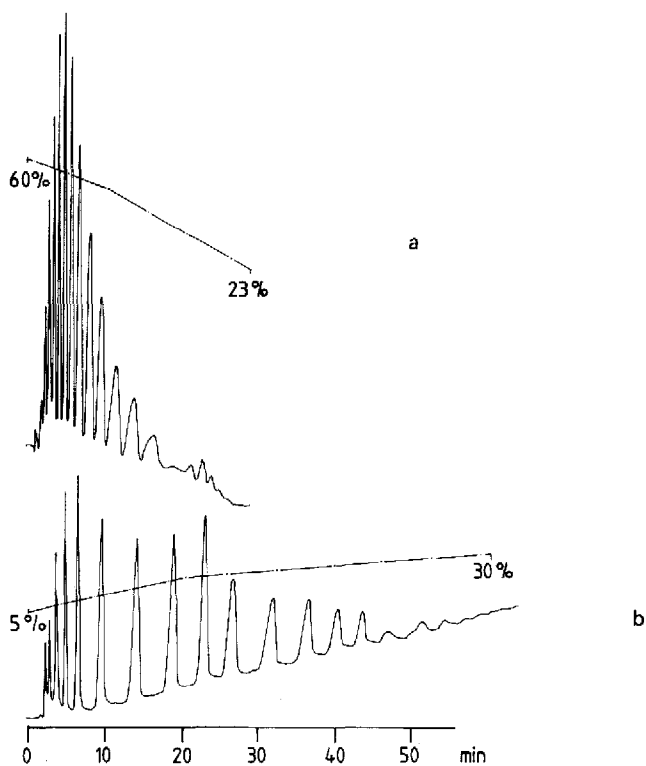
Fig. 2. Chromatograms of PS 800 with diethyl ether as the primary component. (a) Diethyl ether–ethanol; initial column end pressure 70 bar, increased after 20 min to 90 bar to shorten the analysis time. (b) Diethyl ether–dioxane; initial column end pressure 56 bar. ———, Detector signal; – · – · – · , content of ethanol and dioxane in the eluent mixture. Stationary phase: LiChrosorb Si 100, 10 μm. Detection: UV, 254 nm. Sample solvent: dioxane. Flow-rate: 1 ml/min. Oven temperature: 250°C.

silica gel that arise from a significant dissolution process involving the stationary phase in ethanol-containing supercritical mobile phase.

Diethyl ether–dioxane

This eluent pair was much more suitable for SFC experiments with oligostyrenes than the preceding one. The eluent composition gradients could be less steep and the pressure increased with increasing dioxane content at a given setting of the valve at the column end. Thus the effect of the eluent gradient was not counteracted as was the case with diethyl ether–ethanol. Also, no erratic pressure changes were observed. Fig. 2b shows a separation of the PS 800 sample. The applicability of this eluent pair was not restricted to low-molecular-weight oligostyrenes, and oligomers up to a degree of oligomerization of about 40 could be resolved fairly well.

Pentane–cyclohexane

The significant difference in the solubilities of oligostyrenes for the two components of this eluent pair led to gradients similar to those with diethyl ether–dioxane, as is demonstrated in Fig. 3. In order to eliminate any doubts about whether the
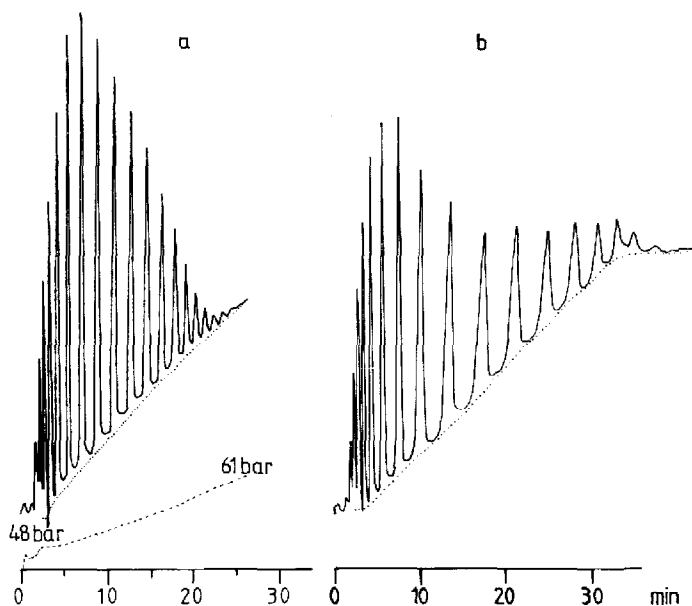
Fig. 3. Chromatograms of PS 800 with pentane–cyclohexane. Linear gradient: 5 to 30% cyclohexane within 30 min. (a) Run with pressure increase at a fixed valve setting; (b) run at constant pressure of 50 bar. ————, Detector signal; ......, baseline; --------, column end pressure. Stationary phase: LiChrosorb Si 100, 7 μm. Detection: UV, 262 nm. Sample solvent: hexane. Flow-rate: 1 ml/min. Oven temperature: 250°C.

chromatographic resolution obtained is due to the eluent gradient or to the pressure increase during the gradient, both causing better solubility of the sample, runs were carried out with the column end pressure maintained constant (Fig. 3b). It can be seen that the pressure increase is indeed responsible for part of the effect. The main influence, however, is due to the increasing amount of cyclohexane, whereby equidistant peaks may be obtained without a pressure increase by changing the gradient programme.

Alkanes–dioxane

This combination proved to be most suitable for oligostyrenes, as shown by the examples in Figs. 4–6. A comparison between the four alkanes is made in Figs. 4 and 5. It was found that the pressure and/or the dioxane content needed for baseline resolution within 80 min or less for PS 800 decreases on going from propane to hexane. The column temperature of 240°C was the same for all the chromatograms in Figs. 4 and 5, except for Fig. 4b, where the temperature had to be increased to 275°C on account of the relatively high critical temperature of hexane. The critical data of the eluent components are given in Table I.

Another interesting feature can be derived from Figs. 4 and 5: it appears to be easier to achieve good resolution for low-molecular-weight oligomers with low-boiling alkanes as primary eluents. On the other hand, elution of oligomers of high molecular weights with the higher alkanes requires lower working pressures. At the end of the chromatogram in Fig. 4a, the column end pressure reached about 140 bar,
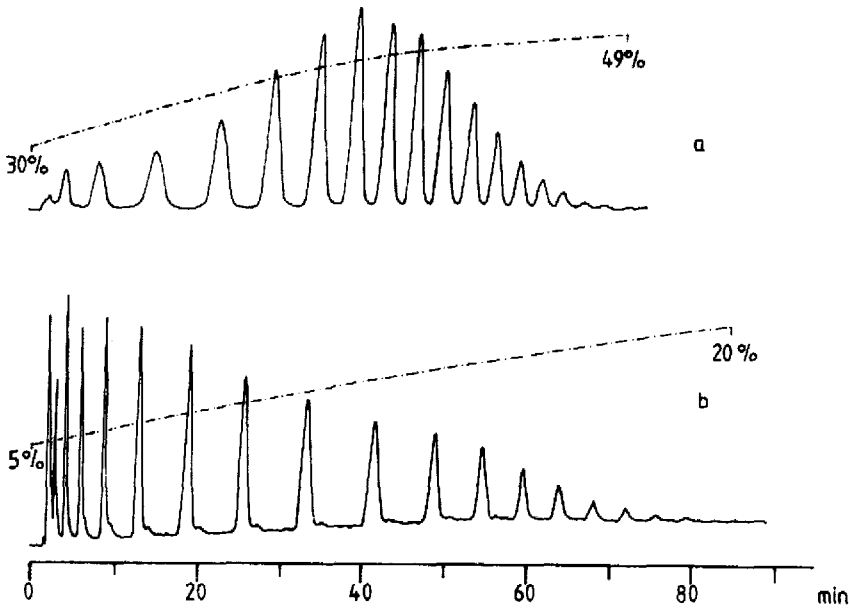
Fig. 4. Chromatograms of PS 800 with (a) propane–dioxane and (b) hexane–dioxane. ———, Detector signal; – · – – – · , content of dioxane in the eluent mixtures. Stationary phase: LiChrosorb Si 100, 10 μm. Detection: UV, 258 nm. Sample solvent: hexane. Flow-rate: 1 ml/min. Oven temperature: (a) 240°C; (b) 275°C. Initial column end pressure: (a) 80 bar; (b) 41 bar.
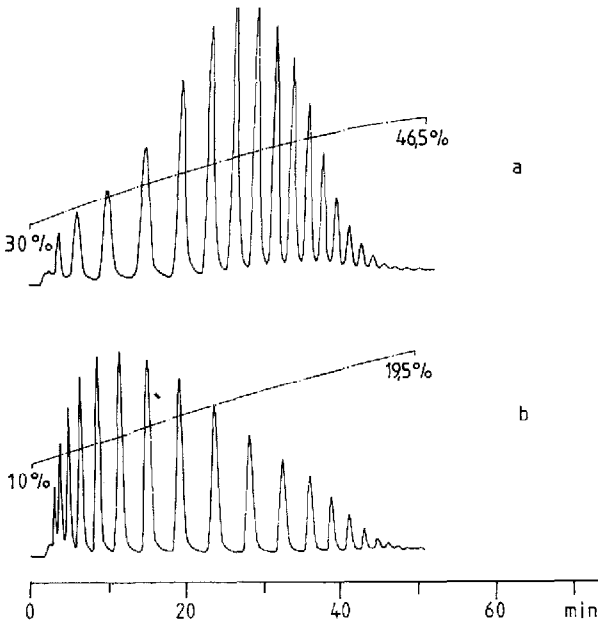

Fig. 5. Chromatograms of PS 800 with (a) butane–dioxane and (b) pentane–dioxane. ———: Detector signal; – · – – – · , content of dioxane in the eluent mixtures. Stationary phase: LiChrosorb Si 100, 10 μm. Detection: UV, 258 nm. Sample solvent: hexane. Flow-rate: 1 ml/min. Oven temperatures: 240°C. Initial column end pressure: (a) 69 bar; (b) 50 bar.

TABLE I

CRITICAL DATA OF THE COMPONENTS OF THE ELUENTS UNDER INVESTIGATION (COMPILED FROM LITERATURE DATA[10,11])

Compound	T_c $(^{\circ}C)$	P_c (bar)	ρ_c $(g\ cm^{-3})$
Propane	96.8	42.6	0.225
Butane	152	38.0	0.228
Pentane	196.6	33.7	0.232
Hexane	234.7	30.3	0.234
Diethyl ether	192.6	36.1	0.267
Cyclohexane	279.9	40.3	0.270
1,4-Dioxane	314.8	52.1	0.360
Methanol	240	79.5	0.275
Ethanol	243.1	63.9	0.280

whereas the value for the chromatogram in Fig. 4b was only about 60 bar. However, column temperatures for the lower alkanes can be decreased significantly while still staying above T_c, which in turn leads to decreased pressures for the same dissolution power of the mobile phase for a given alkane.

Fig. 6 demonstrates the influence of the dioxane programme on the separation of PS 800. Using otherwise identical conditions as for Fig. 5b, the higher initial dioxane content and the steeper gradient shorten the analysis time from 50 to 20 min with a still reasonable resolution.

Pentane–methanol

For separating oligo(2-vinylpyridines) a positive methanol gradient has to be applied. A chromatogram of a radically oligomerized sample is shown in Fig. 7. The sample is well separated with respect to the degree of oligomerization, n. The assignment of groups of peaks for an individual degree of oligomerization was carried out by means of oligomers having a defined degree of oligomerization and defined tacticity[8]. Within a group of peaks of given n, further differentiation, probably tactic isomers, can be observed, as Fig. 7 also shows.
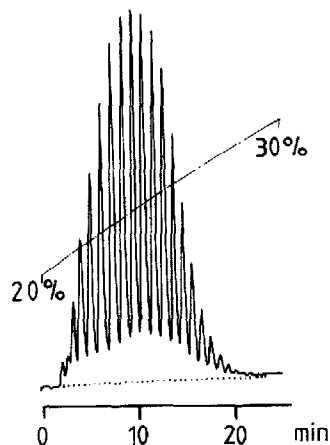

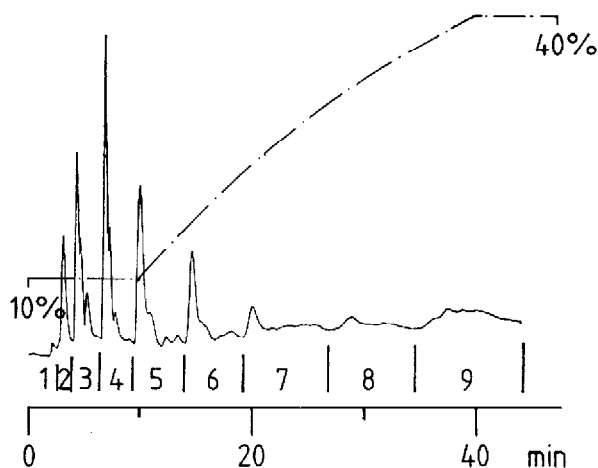Fig. 6. Chromatogram of PS 800 with pentane–dioxane. ....., Baseline. Other conditions as in Fig. 5.

Fig. 7. Chromatogram of an oligo(2-vinylpyridine) sample with pentane–methanol. ———, Detector signal; – · – · – · , content of methanol in the eluent mixture. Stationary phase: LiChrosorb Si 100, 10 μm. Detection: UV, 262 nm. Sample solvent: methanol. Flow-rate: 1 ml/min. Oven temperature: 250°C. Initial column end pressure: 66 bar. The numbers below the detector signal trace correspond to the degree of oligomerization, n.

It should be pointed out, however, that the difficulties that arose with supercritical ethanol are even more pronounced with methanol. After two or three chromatographic runs, the supercritical phase has dissolved enough silica gel to make the loss in stationary phase visibly obvious. Moreover, pressure irregularities caused by particles in the stream passing the valve at the column exit become troublesome and dissolution of the stationary phase can be expected to result in a decreased resolution.

DISCUSSION

The results show that alkanes and ethers are well suited as eluents for SFC. Alcohols are less suitable because of their ability to dissolve the silica gel stationary phase, especially when used at higher concentrations. This may be observed with methanol and also to a lesser extent with ethanol. At lower alcohol concentrations (5–10%, v/v) the dissolution problems are not as severe and may even be disregarded. For chromatographing specifically oligostyrene, eluents composed of alkanes and dioxane yield particularly good resolutions.

In this study, the applicability of SFC has been extended to a greater number of mixed eluents for the technique of gradient elution[6]. Thus, two main gradient techniques are applicable to SFC: pressure gradients, where the mobile phase density is the parameter altered to change the analysis time and resolution, and eluent gradients, where the eluent composition is altered. If the column end pressure is not kept constant during a run, combined effects of the two gradient techniques are obtained. Additionally, it could be shown that the instrumental expenditure when working with SFC is not as large as might be believed. The development of SFC instruments does not need to be a development of completely novel instrumentation. Commercial HPLC instruments additionally equipped with an oven capable of work-

ing at temperatures up to 300°C and with a pressure-regulating device would satisfy many of the requirements for SFC, as has also been demonstrated recently by Gere *et al.*[9].

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