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PROGRAMMING OF PRESSURE AND MOBILE PHASE COMPOSITION AT CONSTANT FLOW-RATE USING A SELF-ADJUSTING VALVE IN SUPERCRITICAL-FLUID CHROMATOGRAPHY

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SUMMARY

Programming of pressure and mobile phase composition were compared for the separation of styrene oligomers on silica. At a constant flow-rate, *i.e.*, constant pump feed rate, composition gradients were found to be superior to pressure gradients, the latter being produced by means of a self-adjusting valve. For optimization the two types of gradient may be combined. Additionally, it is shown that optimized resolution can also be obtained by superimposing a negative pressure gradient on a composition gradient.

INTRODUCTION

Recently, supercritical-fluid chromatography (SFC) has become a rapidly expanding technique, owing especially to the steadily growing interest of applications chemists looking for a chromatographic technique complementary to gas (GC) and liquid chromatography (LC). As has been demonstrated¹⁻⁴, a need for such a technique actually exists, and a growing number of application "niches" for SFC are being found with regard to the solution of problems that are very difficult or even impossible to solve by either GC or LC.

A unique feature of SFC is the greater variety of gradient techniques, which have a large effect on the separation: temperature programming with increasing temperature, as in GC, or with decreasing temperature; composition programming, as in LC; flow programming, as in GC or LC; and pressure/density programming (unique to SFC). These different gradient techniques can be used either as single gradients or as coupled multiple gradients (for a detailed discussion, see recent reviews⁵⁻⁸). So far, however, coupled gradients have often been used involuntarily, that is, a gradient of one variable was produced by changing a second variable. As an example, pressure programming is most frequently effected by increasing the feed rate of the pump against a non-adjustable flow resistance (restrictor), regardless of the effect of the changing linear flow-rate in the column on the separation.

In this paper we present results obtained using pressure and/or composition

gradients. The feed rate of the pumps was kept constant, using a self-adjusting valve at the outlet for changing the pressure. The self-adjustment was based on a pressure feedback loop. The optimization of the separation of a styrene oligomer sample under these conditions is demonstrated.

EXPERIMENTAL

The chromatographic instrumentation used was similar to that described previously⁹. The central part of the SFC instrument was an HP 1084B chromatograph (Hewlett-Packard, Waldbronn, F.R.G.), which provides a constant volume flow-rate of liquid eluent, irrespective of the system pressure or of the relative flow pumped by either of the two pumps, *i.e.*, irrespective of the mobile phase composition. The mobile phase compositions given with the chromatograms refer to the volume feed ratio for the two liquid mobile phase components. The carbon dioxide eluent (99.995%; Messer Griesheim, Köln, F.R.G.) was fed to one pump from a cylinder added to the original HP 1084B, prepressurized by helium, and turned upside down. The 1,4-dioxane eluent was delivered from the storage bottles provided with the chromatograph. Column heating was as described previously⁹, and the column (25 cm × 4.6 mm I.D.) was packed with LiChrosorb Si 100 (10 μm) using a slurry packing procedure¹⁰; the column was conditioned with 1,4-dioxane in order to produce a uniform silica surface with improved resolution characteristics¹¹. The detector and pressure regulators, which were different to the design previously described⁹, are described in more detail below.

The UV detector used in these experiments was a Model LC 75 (Perkin-Elmer, Düsseldorf, F.R.G.) equipped with a pressure-resistant detector cell. However, when working with low-boiling mobile phases, leaks frequently occurred, and retightening of the cell assembly often resulted in destruction of the cell window. Therefore, the

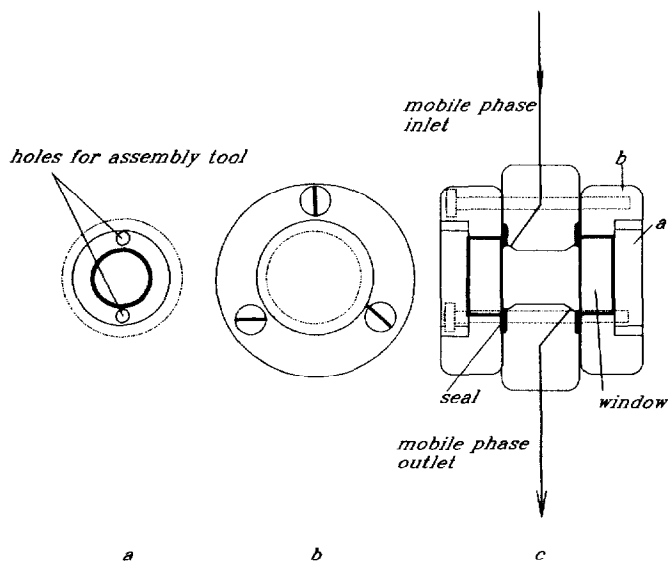


Fig. 1. Design of the UV detector cell. (a) Screw; (b) cover (front view); (c) cell assembly.

cell assembly was redesigned in order to prevent breakage of the windows. This assembly is shown schematically in Fig. 1. The window is pressed against the seal by means of the central screw, and thus the pressure applied is distributed nearly equally. This permits very effective tightening of the cell assembly and therefore the application of high working pressures (*ca.* 350 bar) for long periods of time without leaks.

The pressure regulation was performed downstream of the detector, using an electronically regulated valve of the back-pressure regulating type (HITEC P732; Bronkhorst, Ruurlo, The Netherlands, distributed by Mättig Mess- und Regeltechnik, Unna, F.R.G.). The valve assembly contains a pressure sensor, a feedback loop and the valve proper, which consists of a plunger oscillating in a solenoid. The strength of the magnetic field is controlled by the feedback loop, which compares the actual value from the pressure sensor with an external preset value. This external value can be obtained from a personal computer, in our case from an IBM AT (IBM, Stuttgart, F.R.G.) via a D/A interface. Hence the pressure can not only be held constant but can also be programmed via a simple BASIC program. With the applied flow-rate of 1 ml min^{-1} (liquid), the regulating times are short, which allows the use of steeply ramped pressure programmes. Problems will arise, however, from the high dead volume of the regulating valve (a few millilitres) when distinctly lower flow-rates are used, as is commonly done with microbore or even open-tubular capillary columns.

Pressure programming by means of the PC is demonstrated by the screen hardcopy shown in Fig. 2. Trace A (shifted to lower pressure by 10 pixels for the sake of better inspection) shows a calculated pressure programme from 230 to 300 bar within 75 min, corresponding to a linear pressure ramp of $0.93 \text{ bar min}^{-1}$, and in trace B the

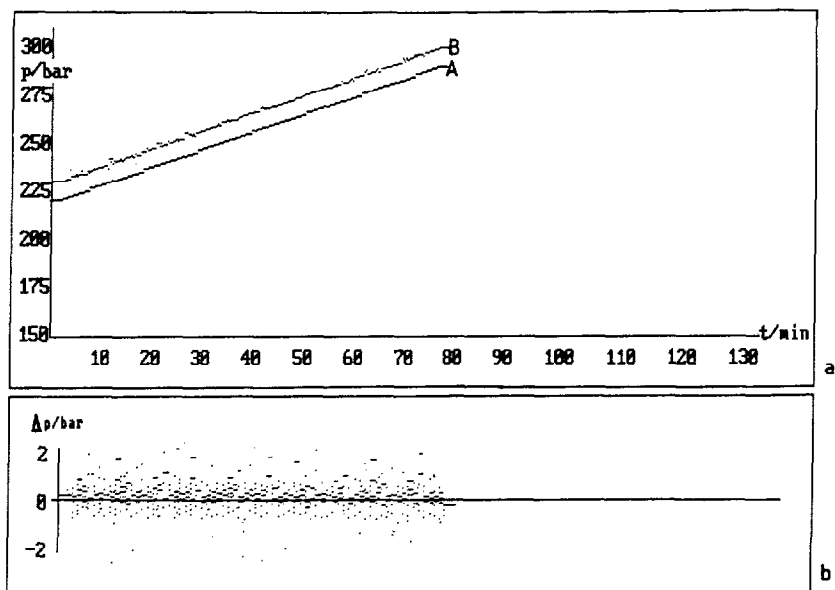


Fig. 2. Hardcopy of pressure-control screen. (a) Pressure programme (A) and actual gradient (B). For better inspection, trace A is arbitrarily shifted to lower values. (b) Actual pressure difference between programme and resulting gradient (the short lines are series of points).

actual data as measured by the pressure sensor of the valve assembly are given. In Fig. 2b actual pressure differences between traces A and B in Fig. 2a are shown, measured at a frequency of 1 s^{-1} . The pressure variations are seen to be in the range of ± 2 bar.

As the analyte we chose an oligostyrene sample (polystyrene PS 800 from Pressure Chemical, obtained from Pfannenschmidt, Hamburg, F.R.G.). The sample concentration was 65 mg ml^{-1} , the solvent being 1,4-dioxane. The sample was injected by means of a Rheodyne 7125 valve equipped with a $20\text{-}\mu\text{l}$ sample loop. The injection time was manually controlled at 3 s, which can prevent the occurrence of double peaks¹². For all chromatograms shown here the temperature was kept at 150°C ; this proved to be reasonably near the optimum of resolution in preliminary experiments based on data from fundamental studies with mobile phases composed of carbon dioxide and 1,4-dioxane¹³. The flow-rate was 1 ml min^{-1} (liquid), as measured by the flow controllers provided with the HP 1084B.

RESULTS

The reasons for the choice of sample, mobile phase and stationary phase were as follows. First, styrene oligomers were selected as the analyte because of their ready availability as highly standardized samples. Further, the separation of oligomers has become a domain of SFC owing to its advantages over GC and LC. Second, a mobile phase composed of carbon dioxide and 1,4-dioxane was chosen as this combination has proved suitable for the separation of styrene oligomers¹⁴. Finally, experimental data¹³ are available for this eluent combination and its chromatographic behaviour with respect to pressure, temperature and 1,4-dioxane content with silica as the stationary phase.

Separations using composition gradients

Applying a composition gradient of 5 to 40% (v/v) 1,4-dioxane with a linear increase of the volume ratio¹⁰ within 80 min, chromatograms were obtained at pressures of 230, 265 and 300 bar (Fig. 3). The pressure was kept above 220 bar because at lower pressures mobile phase demixing must be considered when the 1,4-dioxane content exceeds 30%¹³. The chromatograms show decreasing analysis times with increasing pressure, and baseline separations were obtained except at the lowest pressure. The effect of increasing pressure on retention differences appears more pronounced for the lower members of the homologous series and thus more equal spacing results over the entire chromatogram; at the end of the chromatogram, the unresolved peaks at lowest pressure (Fig. 3a) become baseline resolved at 265 bar (Fig. 3b). A further increase in pressure to 300 bar then reduces the analysis time required for separating fifteen oligomers by an additional 10 min (Fig. 3c), maintaining the good resolution obtained at 265 bar. With increasing pressure, the 1,4-dioxane content needed for eluting an individual oligomer is reduced, and this effect seems to improve the quality for this specific separation.

Separations using pressure gradients

Pressure-programmed separations with a linear gradient from 230 to 300 bar within 75 min were performed at constant dioxane contents of 5, 15, 20, 25 and 30% (v/v). The resulting chromatograms are shown in Fig. 4. At the lowest 1,4-dioxane

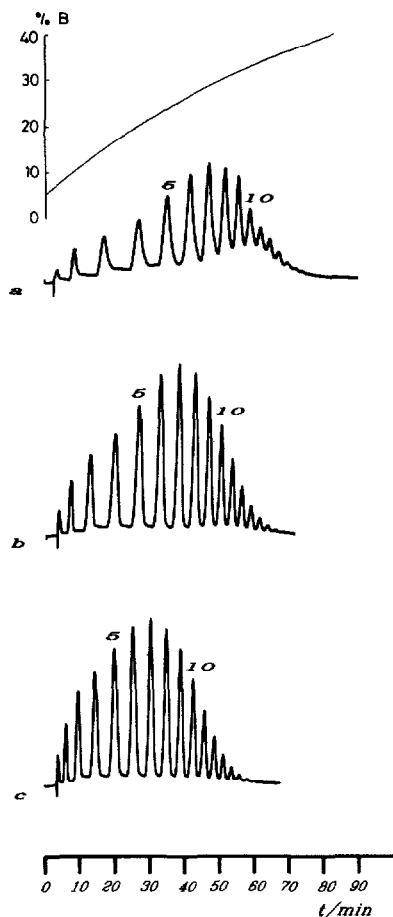


Fig. 3. Composition-gradient separation of styrene oligomers; 5–40% (v/v) 1,4-dioxane in carbon dioxide within 80 min at a constant pressure of (a) 230, (b) 265 and (c) 300 bar. Temperature, 150 °C; flow-rate, 1 ml min⁻¹. For further details, see Experimental.

content the pressure programme elutes only six oligomers within 2 h. Increasing the 1,4-dioxane content reduces the retention times substantially. However, 1,4-dioxane contents exceeding 25% are not suitable for a pressure gradient separation in this instance as is evident from the inferior resolution in Fig. 4e.

The quality of the pressure-gradient separations is poorer than those of the composition-gradient separations in Fig. 3; in fact, in the present instance, pressure gradients are not very effective. This is evident from Fig. 5, showing a comparison between an isocratic pressure-programmed separation (Fig. 5a) and an isocratic-isobaric separation (Fig. 5b). Although the effect of the pressure gradient is clearly visible (*e.g.*, by comparison of retention times for the decamer, $n=10$), this effect is not very large. Extending the pressure gradient to higher pressures would yield a further reduction in retention, but as the gain in density for a given pressure interval

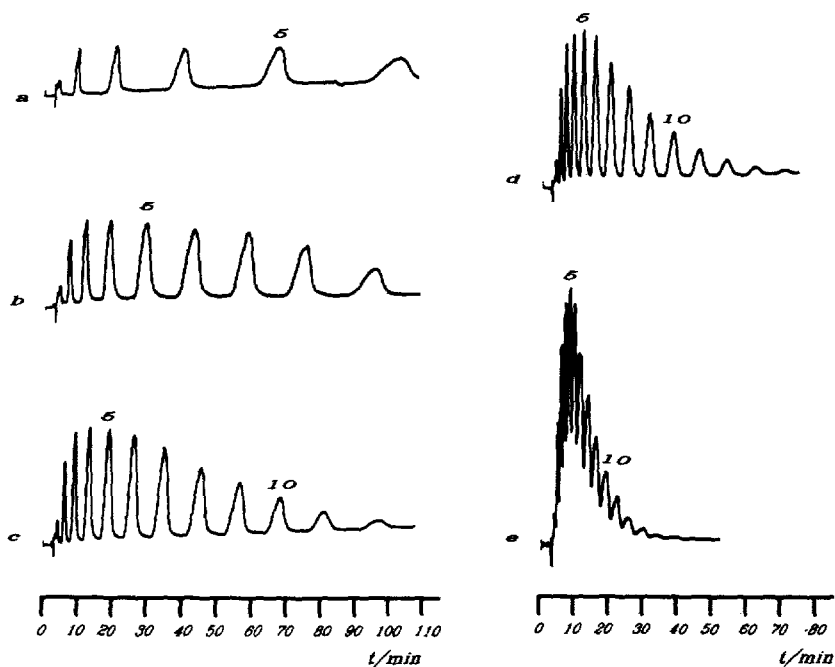


Fig. 4. Pressure-gradient separation of styrene oligomers; 230–300 bar linear increase within 75 min with a constant 1,4-dioxane content of (a) 5, (b) 15, (c) 20, (d) 25 and (e) 30% (v/v) in carbon dioxide. Other conditions as in Fig. 3.

becomes smaller at increasing pressure levels, it can be predicted that for separating higher oligomers pressure gradients will be less effective with this chromatographic system of solute, mobile phase and stationary phase, at a constant flow-rate.

Frequently, non-linear pressure gradients have been applied for oligomer separations. With the pressure-control device used in this work, the use of non-linear gradients is also possible. Such a separation is shown in Fig. 6. With this “asymptotically shaped” pressure gradient, a pressure of 350 bar is obtained after 45 min. It is

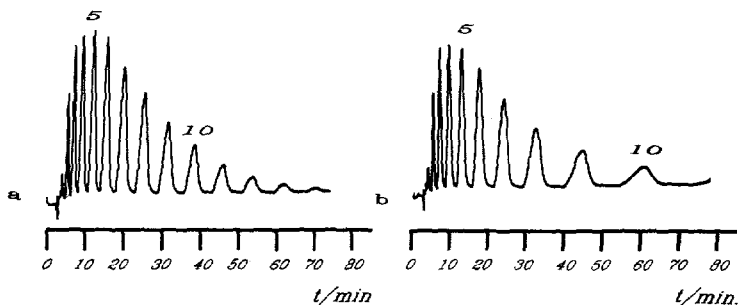


Fig. 5. Comparison of (a) isocratic pressure-programmed separation (*cf.*, Fig. 4d) with (b) isobaric-isocratic separation of styrene oligomers. Mobile phase, carbon dioxide–dioxane (75:25, v/v); pressure, 230 bar (b), 230–300 bar (a). Other conditions as in Fig. 3.

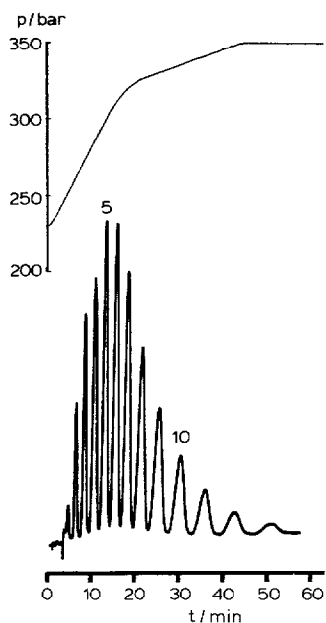


Fig. 6. Separation of styrene oligomers with an "asymptotic" pressure gradient from 230 to 350 bar. Mobile phase, carbon dioxide-1,4-dioxane (80:20, v/v). Other conditions as in Fig. 3.

seen that the more steeply ramped gradient combined with a higher final pressure yields a considerable decrease in retention (*cf.*, Fig. 4c). Nevertheless, this pressure effect appears insufficient for eluting species of higher molecular weight within a reasonable analysis time.

Multiple gradient separations

Superimposing pressure and composition gradients results in a combined effect of reduction of retention times, as demonstrated several years ago^{9,10}. Fig. 7 shows a separation obtained by the simultaneous application of the composition gradient used for the separations shown in Fig. 3 and the pressure gradient in Fig. 4. The combined effects are obvious, but the peak spacing is unequal with large inter-peak distances in the first part and unresolved peaks in the second part of the chromatogram.

Better results are obtained when at least one of the two gradients is modified. Fig. 8 shows an example. Here the composition gradient is modified and increases, after a 5-min isocratic period, from 20% to 40% within 50 min, keeping the pressure gradient unchanged as in Figs. 4 and 7. The chromatogram shows an almost baseline separation for sixteen oligomers within 45 min.

Alternatively, negative pressure gradients (*i.e.*, gradients of decreasing pressure) can be used for refinement of a composition gradient. This appeared possible as the reduction in retention times with increasing pressure was more pronounced for the lower than for the higher oligomers (*cf.*, Fig. 3). Accordingly, a decrease in pressure during the separation resulted in only a slight increase in analysis time, as shown in

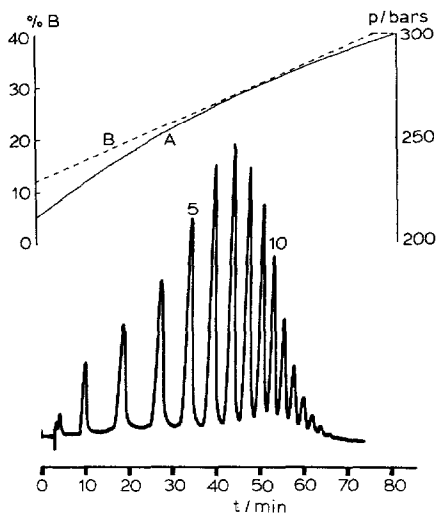


Fig. 7. Separation of styrene oligomers with combined composition (full line, A) and pressure (broken line, B) gradients as used for separations in Figs. 3 and 4. Other conditions as in Fig. 3.

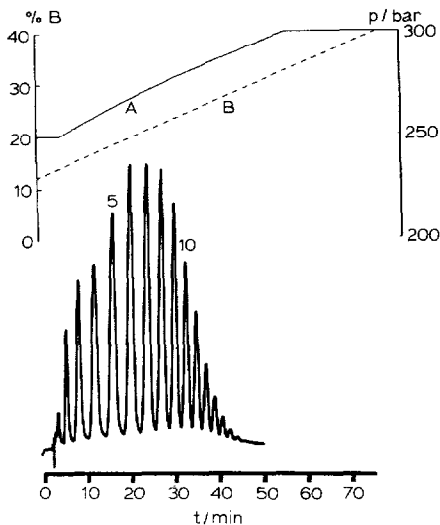


Fig. 8. Separation of styrene oligomers with pressure gradient (broken line, A) as in Fig. 4 and modified composition gradient (full line, B): 5 min isocratic with carbon dioxide-1,4-dioxane (80:20), then from 20 to 40% (v/v) 1,4 dioxane within 50 min. Other conditions as in Fig. 3.

Fig. 9 for a composition gradient as in Figs. 3 and 7 but with a negative pressure gradient from 290 to 230 bar within 75 min. Compared with Fig. 3c, which was obtained at a constant pressure of 300 bar, the peak widths are identical, but the spacing between the peaks is increased in Fig. 9. In other words, the chromatogram in Fig. 9 shows a higher resolution than that in Fig. 3c. This may be of interest for the separation of more complex samples than the styrene oligomer sample separated here.

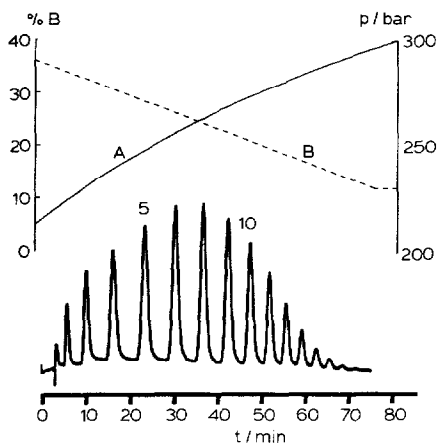


Fig. 9. Separation of styrene oligomers with composition gradient (full line, A) as in Fig. 3 and negative (linear) pressure gradient (broken line, B) from 290 to 230 bar within 75 min. Other conditions as in Fig. 3.

DISCUSSION

The results demonstrate that programming of pressure or density is less suitable for the separation of styrene oligomers on a polar silica stationary phase and with a mobile phase of carbon dioxide plus a constant amount of modifier if the (liquid) volume flow-rate (or the mass flow-rate) is kept constant throughout the run. Composition gradients and combinations of composition and pressure gradients were found to yield distinctly better results.

One reason for this partial failure of single pressure gradients may be the decrease in linear velocity in the separation column, as indicated by the corresponding change in dead time, if the pressure is increased at a constant mass flow-rate or at a constant volume flow-rate (which is virtually identical with a constant mass flow-rate with moderate pressure changes and pumping of the mobile phase in the liquid state). Hence an improvement in the effectiveness of pressure gradients may be possible if the decrease in the linear velocity in the column is counteracted or even balanced by a continuous change in the mobile phase feed rate, *i.e.*, by a flow gradient. However, previous results with combined pressure-flow gradients¹⁵ do not seem promising. With composition gradients, changes in linear velocity in the column at a constant pump feed rate are only small and can be neglected to a first approximation.

By means of the pressure-control system used in this study, pressure control was very easy to achieve and pressure changes could be carried out instantaneously. It also provides high flexibility for pressure and density programming. These statements are valid as long as flow-rates are applied that are typical for normal-bore packed columns, *i.e.*, around 1 ml min^{-1} . With substantially lower flow-rates the large dead volume of the valve assembly may cause serious problems. For such low flow-rates a valve assembly as described by Saito and co-workers^{16,17} might be more suitable. This valve, being similar in principle to that used in this study, is stated to have a very low dead volume of only a few microlitres. Nevertheless, the use of both valves is restricted to post-detector regulation, such as with UV (or IR or fluorescence) detection. For use upstream of the detector, as is necessary, *e.g.*, with flame ionization detection, the dead volumes of both valves are too high; also, a substantial reduction in dead volumes might be inevitable for pre-column use.

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REFERENCES

- 1 B. A. Charpentier and M. R. Sevenants (Editors), *Supercritical Fluid Extraction and Chromatography. Techniques and Applications (ACS Symposium Series, No. 366)*, American Chemical Society, Washington, DC, 1988.
- 2 R. M. Smith (Editor), *Supercritical Fluid Chromatography*, Royal Society of Chemistry, London, 1988.
- 3 C. M. White (Editor), *Modern Supercritical Fluid Chromatography*, Hüthig, Heidelberg, Basle, New York, 1988.
- 4 M. Yoshioka, S. Parvez, T. Miyazaki and H. Parvez (Editors), *Supercritical Fluid Chromatography and Micro-HPLC (Progress in HPLC, Vol. 4)*, VSP, Utrecht, 1988.

- 5 E. Klesper and F. P. Schmitz, in C. M. White (Editor), *Modern Supercritical Fluid Chromatography*, Hüthig, Heidelberg, Basle, New York, 1988, p.1.
- 6 F. P. Schmitz and E. Klesper, *J. Chromatogr.*, 388 (1987) 3.
- 7 E. Klesper and F. P. Schmitz, *J. Chromatogr.*, 402 (1987) 1.
- 8 E. Klesper and F. P. Schmitz, *J. Supercrit. Fluids*, 1 (1988) 45.
- 9 F. P. Schmitz, H. Hilgers and E. Klesper, *J. Chromatogr.*, 267 (1983) 267.
- 10 F. P. Schmitz, H. Hilgers, B. Lorenschat and E. Klesper, *J. Chromatogr.*, 346 (1985) 69.
- 11 F. P. Schmitz, D. Leyendecker and D. Leyendecker, *J. Chromatogr.*, 389 (1987) 245.
- 12 D. Thiebaut, M. Caude and R. Rosset, *Analisis*, 15 (1987) 528.
- 13 D. Leyendecker, D. Leyendecker, F. P. Schmitz, B. Lorenschat and E. Klesper, *J. Chromatogr.*, 398 (1987) 105.
- 14 F. P. Schmitz, D. Leyendecker, D. Leyendecker and B. Gemmel, *J. Chromatogr.*, 395 (1987) 111.
- 15 F. P. Schmitz, *Chromatographie mit überkritischen verdichteten Gasen zur Trennung von Oligomeren und Polymeren*, Profil, Munich, 1988.
- 16 M. Saito, T. Hondo and Y. Yamauchi, in R. M. Smith (Editor), *Supercritical Fluid Chromatography*, Royal Society of Chemistry, London, 1988, p. 203.
- 17 M. Saito, Y. Yamauchi, H. Kashiwazaki and M. Sugawara, *Chromatographia*, 25 (1988) 801.