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HIGH-PERFORMANCE LIQUID AND SUPERCRITICAL FLUID CHROMATOGRAPHIC SEPARATION OF VINYL OLIGOMERS BY GRADIENT ELUTION*

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SUMMARY

Gradient elution with various eluent pairs was used to separate some vinyl oligomers on silica as the stationary phase. For the high-performance liquid chromatographic separation of vinylpyridine oligomers changing the primary component (in which the solute is less soluble) of the eluent mixture distinctly influenced the separation; for example, nitrous oxide–methanol yielded a better separation with respect to the degree of oligomerization than pentane–methanol.

In supercritical fluid chromatography the separation efficiency was greatly influenced by the column temperature and/or pressure and by the eluent composition. Combined programming of eluent composition and pressure was applied to elute N-vinylcarbazole oligomers, and combined programming of eluent composition and temperature was used to improve the resolution of homologous series.

INTRODUCTION

The aim of our research is the chromatographic separation of the individual members of a homologous series of oligomers. In such a series, the relative differences between successive members decrease with increasing molecular weight, *i.e.* with increasing degree of polymerization. As an example, this applies to the size of the random coil in solution, which leads to the fundamental limit for the chromatographic resolution of two neighbouring members of a homologous series by size-exclusion chromatography. The pore diameters of the stationary phase and their distribution are fixed parameters which cannot be changed during a separation. However, coupling of columns packed with materials having different pore diameters and repeated recycling of the eluate have yielded some improvement of separations at the expense of increased analysis times. Nevertheless, improvement of the separation of homologues of higher molecular weight was difficult to achieve.

Contrary to this, chromatographic procedures based on adsorption or parti-

* Dedicated to Prof. Dr.-Ing. Dr. h. c. mult. H. Zahn on the occasion of his 70th birthday.

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tion are easier to adjust to a given separation problem. Separations of mixtures containing components which have different solubilities can be achieved by increasing the solvent strength (the "thermodynamic quality") of the eluent during a chromatographic run. In supercritical fluid chromatography (SFC), this can be achieved by raising the mobile phase density through increasing the column pressure or, at least in principle, by decreasing the column temperature. On the other hand, the solvent strength of the eluent can be enhanced by increasing the amount of the better solvent in an eluent mixture. This procedure is applicable to both SFC and high-performance liquid chromatography (HPLC), since the transport of solute molecules through the column is based on interactions between the solute and the mobile phase. To achieve optimum separation of oligomers, the concentration of the better solvent must increase continuously, but the slope of the gradient must decrease due to the decreasing relative differences between successive members of the homologous series. Gradients with a linear increase in the solvent ratio have been shown suitable^{1,2}.

Gradient elution has been applied to the analysis of oligomers over a long period of time. In 1969, Jentoft and Gouw³ reported on the pressure-programmed SFC separation of oligostyrenes. Later, Van der Maeden *et al.*⁴ separated several oligomers by HPLC by using eluent gradients, and this technique has been used for oligomer separations by SFC since 1981⁵. In our work, some examples will be presented for the separation of vinyl oligomers by means of eluent gradients, both in HPLC and SFC. The influence of the temperature, pressure, and the nature of the primary component of the eluent mixture on the separations will be discussed.

EXPERIMENTAL

The HPLC instrument was a Model 1084B from Hewlett-Packard (Waldbronn, F.R.G.). For SFC operation, this instrument was modified as described previously^{1,6}. The eluents (except carbon dioxide and nitrous oxide) were distilled from sodium, filtered through Fluoropore filters (FALP 10 μm ; from Millipore, Eschborn, F.R.G.) and degassed before use. 1,4-Dioxane was stored in dark bottles in order to prevent the formation of peroxides. Nitrous oxide (99.5% purity; from Linde, Höllriegelskreuth, F.R.G.) and carbon dioxide (99.995% purity; from Messer Griesheim, Hürth, F.R.G.) were passed from the pressure cylinders through a filter system before they entered the chromatograph. Eluents containing a low-boiling primary component were treated as described elsewhere^{2,7}. The stainless-steel columns (25 cm \times 4.6 mm I.D.) were packed with LiChrosorb Si 100, 10 μm (for oligovinylpyridine separations) or Si 60, 10 μm (both from Merck, Darmstadt, F.R.G.) using a slurry method¹. For reequilibration, the columns were flushed with high concentrations of the strong component of the eluent mixture (*i.e.* dioxane or methanol) subsequent to each run. The flow-rate was 1 ml/min, measured at the pumps in the liquid state, except for the separation of N-vinylcarbazole oligomers.

Conditions for the oligomerization reactions were as described previously^{1,2,8}. Pertinent data are given in Table I. With anionic oligomerizations, butyl lithium was used as the initiator and tetrahydrofuran as the solvent, while radical oligomerizations were initiated by azobisisobutyronitrile in toluene. Samples of 20–60 μl of oligomer solutions in methanol (vinylpyridine oligomers) or in 1,4-dioxane (all other oligomers) were injected in concentrations of 1–5 g/100 ml. The detection wave-

TABLE I
OLIGOMERIZATION REACTIONS

Monomer	Monomer concentration (mg/ml)	Monomer-initiator ratio	Initiation	Reaction temp. (°C)	Reaction time (min)	Fig.
2-Vinylpyridine	20	10	Anionic	-20	2	1
4-Vinylpyridine	10	1	Radical	90	60	1
1-Vinylnaphthalene	17.5	1	Anionic	-20	3	2
4-Vinylbiphenyl	25	0.75	Anionic	-20	3	3, 6
4-Vinylbiphenyl	25	10	Radical	100	30	3
N-Vinylcarbazole	25	0.5	Anionic	-20	5	4
N-Vinylcarbazole	25	5	Radical	90	60	4

lengths were 254 nm for oligomers of vinylpyridines and 4-vinylbiphenyl, 278 nm for vinylnaphthalene oligomers and 295 nm for N-vinylcarbazole oligomers.

RESULTS AND DISCUSSION

For the chromatography of vinylpyridine oligomers, methanol was chosen as the strong component of the binary eluent mixture. Only methanol and ethanol were found suitable for eluting more than just one or two members of the homologous vinylpyridine series. This is in agreement with the solution behaviour of vinylpyridine oligomers, which are soluble primarily in methanol and ethanol, or in solvents containing one of these alcohols. Two different weak solvents for the eluent mixtures were tested, pentane and nitrous oxide. HPLC chromatograms, obtained with pentane-methanol and nitrous oxide-methanol are shown in Fig. 1.

4-Vinylpyridine oligomers proved to be more difficult to elute from the silica stationary phase than 2-vinylpyridine oligomers. This can be explained by the better accessibility of the pyridine nitrogen atom in the 4-position. Thus, interaction between the solute and the stationary phase is enhanced². It can be seen from the chromatograms that more than one homologous series was formed, because for each degree of oligomerization a group of peaks is observed.

Comparing the chromatograms with pentane as the primary component (Fig. 1a and c) with those obtained with nitrous oxide (Fig. 1b and d), the separation between the groups of peaks that correspond to the same degree of oligomerization (*i.e.* that contain equal numbers of repetitive units) is found to be greater with nitrous oxide. This improvement applies also to the separation within the respective groups of peaks as far as 2-vinylpyridine oligomers are concerned (Fig. 1c and d). However, for the 4-vinylpyridine oligomers this seems to be reversed: with pentane, separations within a given degree of oligomerization seem to be better than with nitrous oxide. These differences in the elution behaviour may be due to competition of the solute molecules and the mobile phase molecules for interaction with the stationary phase².

Separations of vinylpyridine oligomers could also be achieved by SFC. However, silica stationary phases were found to show poor stability when supercritical fluids with high alcohol contents were used⁶. Thus, the separation was inferior to

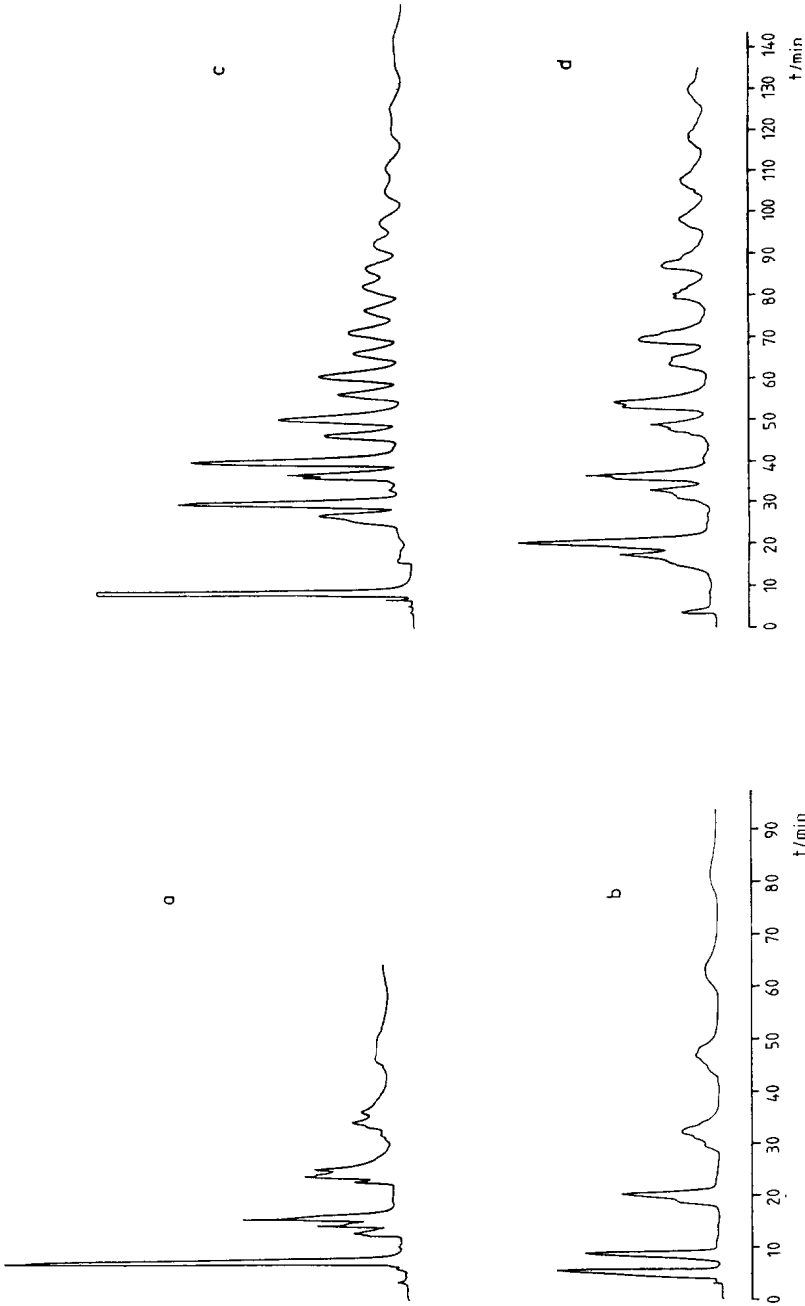


Fig. 1. HPLC separations of 4-vinylpyridine (a, b) and 2-vinylpyridine (c, d) oligomers. Column: 25 cm \times 4.6 mm I.D., packed with Si 100, 10 μ m; eluents: pentane-methanol (a, c) or nitrous oxide-methanol (b, d); gradients of increasing methanol content from 10 to 60% (v/v) in 100 min (a, b); increasing methanol content from 5 to 40% in 160 min after an initial isocratic period of 10 min at 5% methanol (c, d).

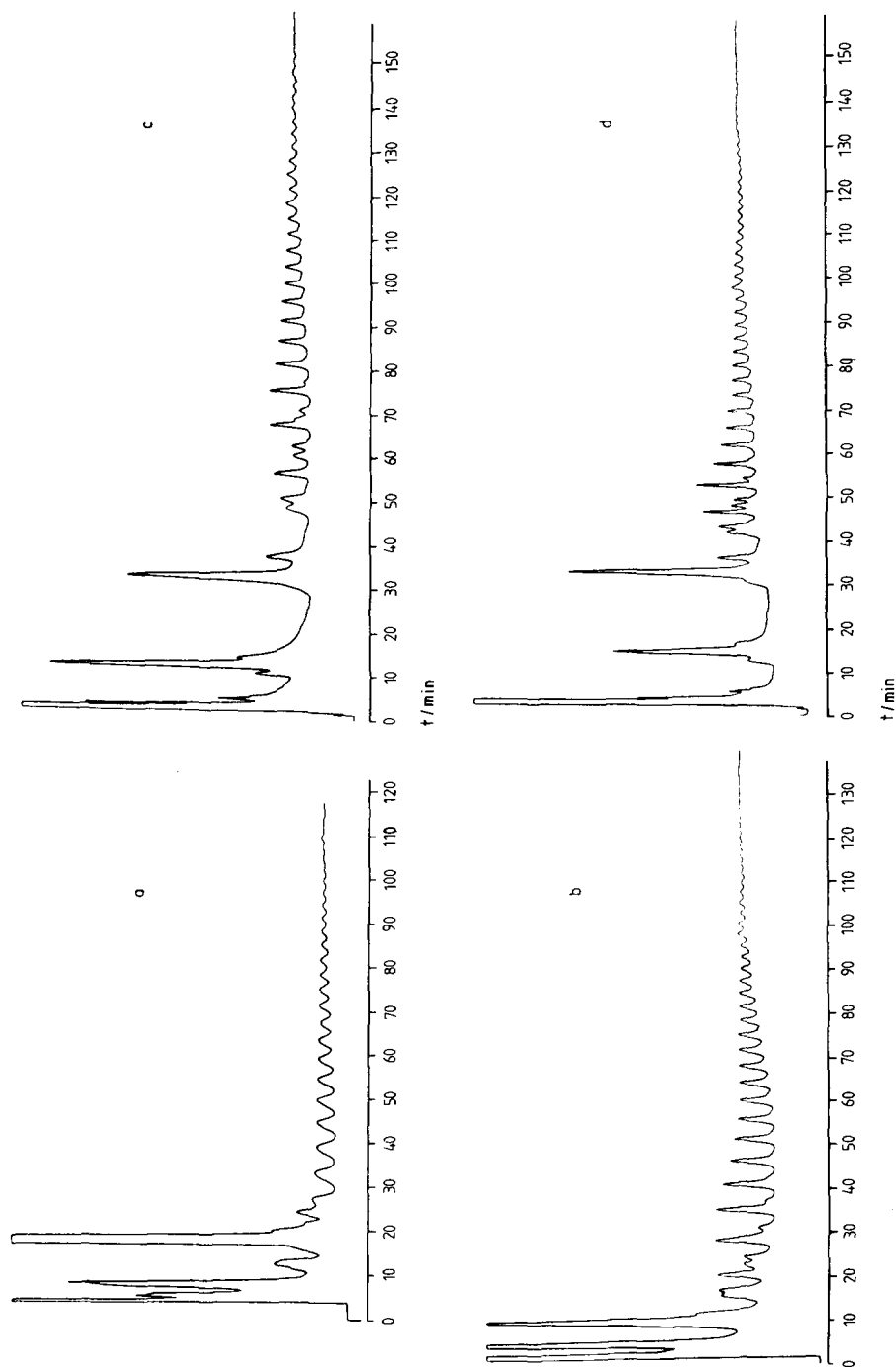


Fig. 2. Separations of 1-vinylnaphthalene oligomers by (a) HPLC and (b-d) SFC. Column, 25 cm \times 4.6 mm I.D., packed with Si 60, 10 μ m; eluents: pentane-1,4-dioxane; column inlet pressures at the start of the chromatograms: (a) 49, (b) 69, (c) 50 and (d) 46 bar; column temperatures: (a) ambient, (b and c) 270 and (d) 250°C; gradient: 5-55% (v/v) dioxane in 180 min.

that obtained by HPLC. Although silica and silica-based stationary phases are known to be dissolved also by polar liquid mobile phases, this occurs to a much lesser extent than in the case of polar supercritical fluids.

When the strong solvent component of the eluent pair is of lower polarity than methanol or ethanol, silica stationary phases are stable in SFC and chromatograms are easily obtained. For vinyl oligomers with aromatic side groups cyclohexane and, especially, 1,4-dioxane have proven to be suitable. Thus, separations by means of eluent gradients have been applied to oligomers of styrene^{5,6,9,10}, vinylnaphthalenes^{1,8}, 4-vinylbiphenyl¹, and N-vinylcarbazole¹.

In Fig. 2, separations of 1-vinylnaphthalene oligomers by HPLC and SFC are compared. It can be seen that the resolution obtained by HPLC is distinctly poorer than that obtained by SFC, even when retention times are comparable (Fig. 2a and b). The resolution, R_s , is related to plate numbers, and thus, to plate heights. From Van Deemter plots it is known that the plate heights are lower at the usual flow-rates with supercritical fluids than they are with liquid eluents^{11,12}. This is due to the considerably higher diffusion coefficients for supercritical fluids compared to those of liquids¹³. Thus, equilibrium between the mobile and the stationary phase is more rapidly established and, at a given mobile phase velocity, resolution is superior in SFC.

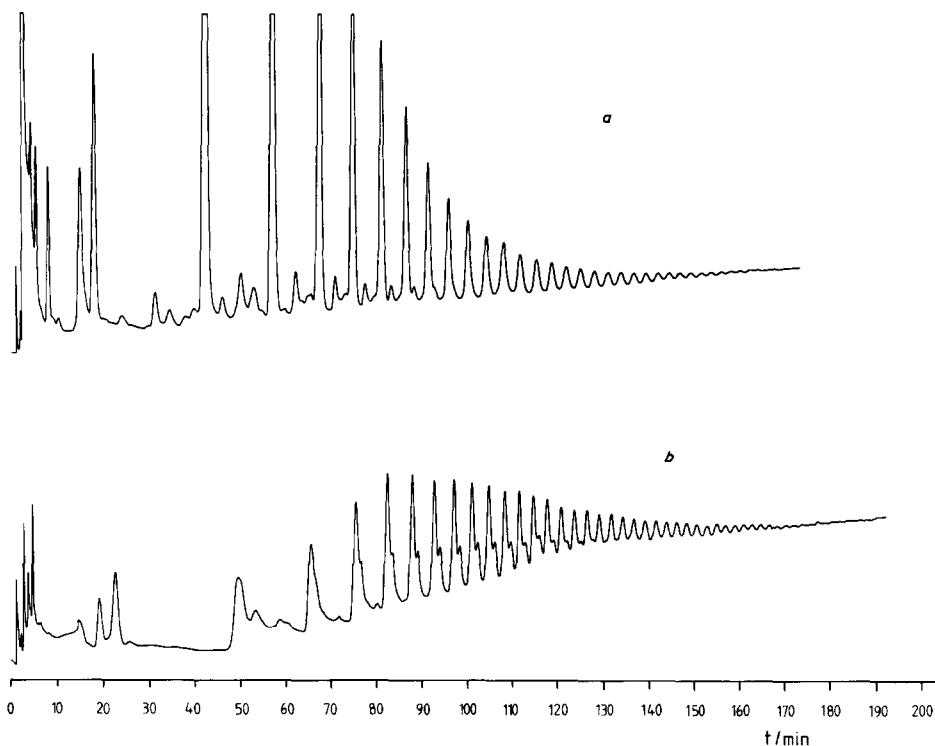


Fig. 3. SFC separations of 4-vinylbiphenyl oligomers, prepared by (a) radical and (b) anionic initiation. Column: 25 cm \times 4.6 mm I.D., packed with Si 60, 10 μ m; eluents: pentane-1,4-dioxane; column inlet pressures at the start of the chromatograms, (a) 51 and (b) 48 bar; column temperature: 270°C; gradient, 5-68% (v/v) dioxane in 300 min.

Whereas higher column pressures have only little effect on liquid eluents, owing to their low compressibility², it has been found that in SFC, the best resolution may be obtained when the pressure is not too far from the critical pressure at the start of an oligomer separation¹⁰, if low-molecular-weight components are to be resolved. When the eluent pair pentane-dioxane is used, the pressure at the column inlet increases during a run if the valve setting that controls the pressure at the column outlet remains unaltered, due to the higher viscosity of dioxane. The pressure increase is sufficient to maintain the mobile phase at supercritical conditions, although the critical pressure of the eluent mixture increases with increasing dioxane content.

The chromatograms shown in Fig. 2 demonstrate the effects of pressure and temperature on a separation in SFC. Reducing the pressure leads to higher retention times and improved resolution (Fig. 2b and c), while a decrease in temperature results in lower retention times (Fig. 2c and d).

The good SFC separations obtained for vinyl arene oligomers could be used

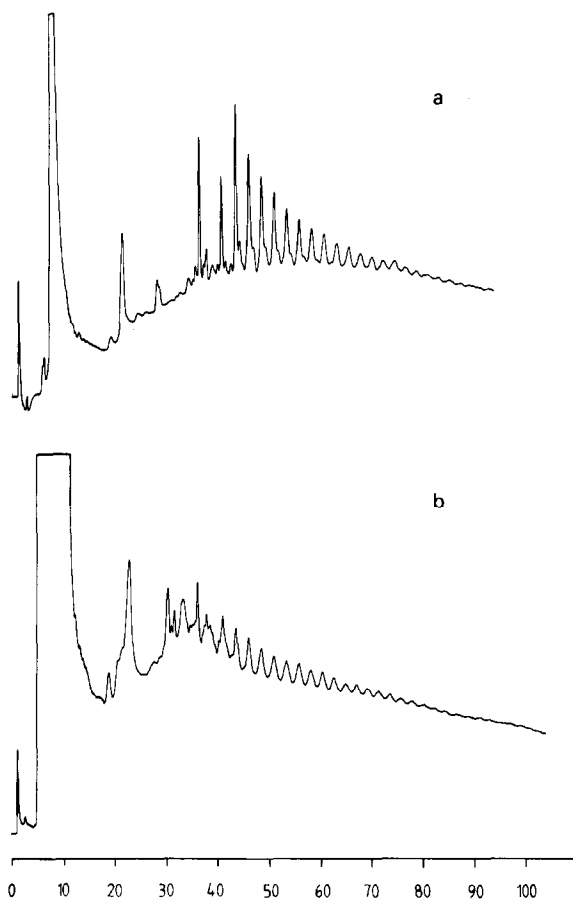


Fig. 4. SFC separation of N-vinylcarbazole oligomers, prepared by (a) radical and (b) anionic initiation. Column: 25 cm \times 4.6 mm I.D., packed with Si 60, 10 μ m; eluents: pentane-1,4-dioxane; column temperature: 270°C; flow-rate kept constant at 0.75 ml/min for 10 min and then increased to 1.65 ml/min at 130 min. For eluent and pressure gradients see Fig. 5.

for comparing oligomerization reactions, initiated radically and anionically. Fig. 3 shows chromatograms obtained with 4-vinylbiphenyl oligomers. These chromatograms show, in analogy to the oligomerizations of vinylnaphthalenes⁸ and vinylpyridines², that more homogeneous products were obtained from the radically initiated oligomerization than from anionically initiated oligomerization under the reaction conditions of Table I.

One of the advantages of SFC is the possibility of combining different gradient techniques, if needed, to a specific separation problem. Due to their poor solubility, N-vinylcarbazole oligomers were found difficult to elute. Neither eluent gradients nor pressure gradients were found to elute and separate these oligomers effectively. As can be seen from Fig. 4, the solution to this problem was to combine an eluent gradient with a pressure gradient¹, the latter being generated by increasing the flow-rate at a given setting of the outlet valve (*i.e.* at a constant flow-resistance). The gradients are shown in Fig. 5.

While the combination of gradients of pressure and eluent composition can be used to speed up the elution, combining either of these two gradient techniques with a temperature gradient can yield an improvement in resolution. This was demonstrated some years ago by Klesper and Hartmann¹⁴, who combined gradients of increasing pressure and increasing temperature. The reason for such improvements in resolution is to be found in the strong dependence of the chromatographic properties of eluents on pressure, temperature, and eluent composition. For instance, at a given pressure, a temperature can be found where resolution shows a maximum^{7,15-18}. This maximum is shifted to higher temperatures with increasing solute molecular weight, increasing pressure, and increasing concentration of a higher boiling component in the eluent mixture. Thus, to maintain conditions of high resolution during a gradient separation, the temperature should be increased simultaneously^{7,18}.

In Fig. 6, separations of vinylbiphenyl oligomers obtained with an eluent gradient at isothermal conditions (6a) are compared to those with combined programming of eluent composition and temperature (6b), the binary mobile phase being carbon dioxide-dioxane. The gradient obtained in the first and the second example

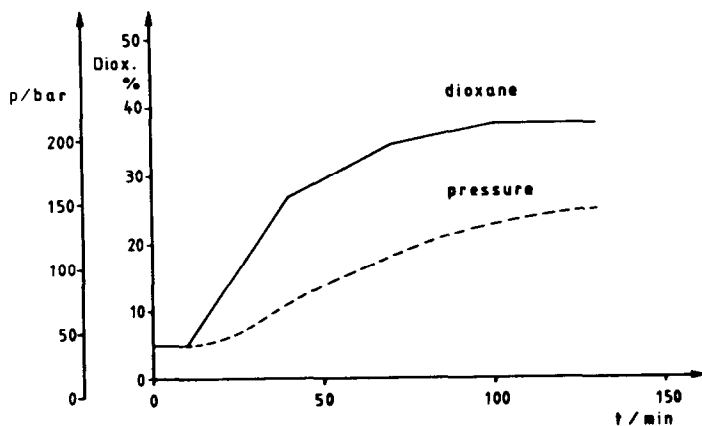


Fig. 5. Eluent and pressure gradients for the chromatograms in Fig. 4. Solid line: dioxane content (% v/v) in the eluent mixture; broken line: pressure at column inlet.

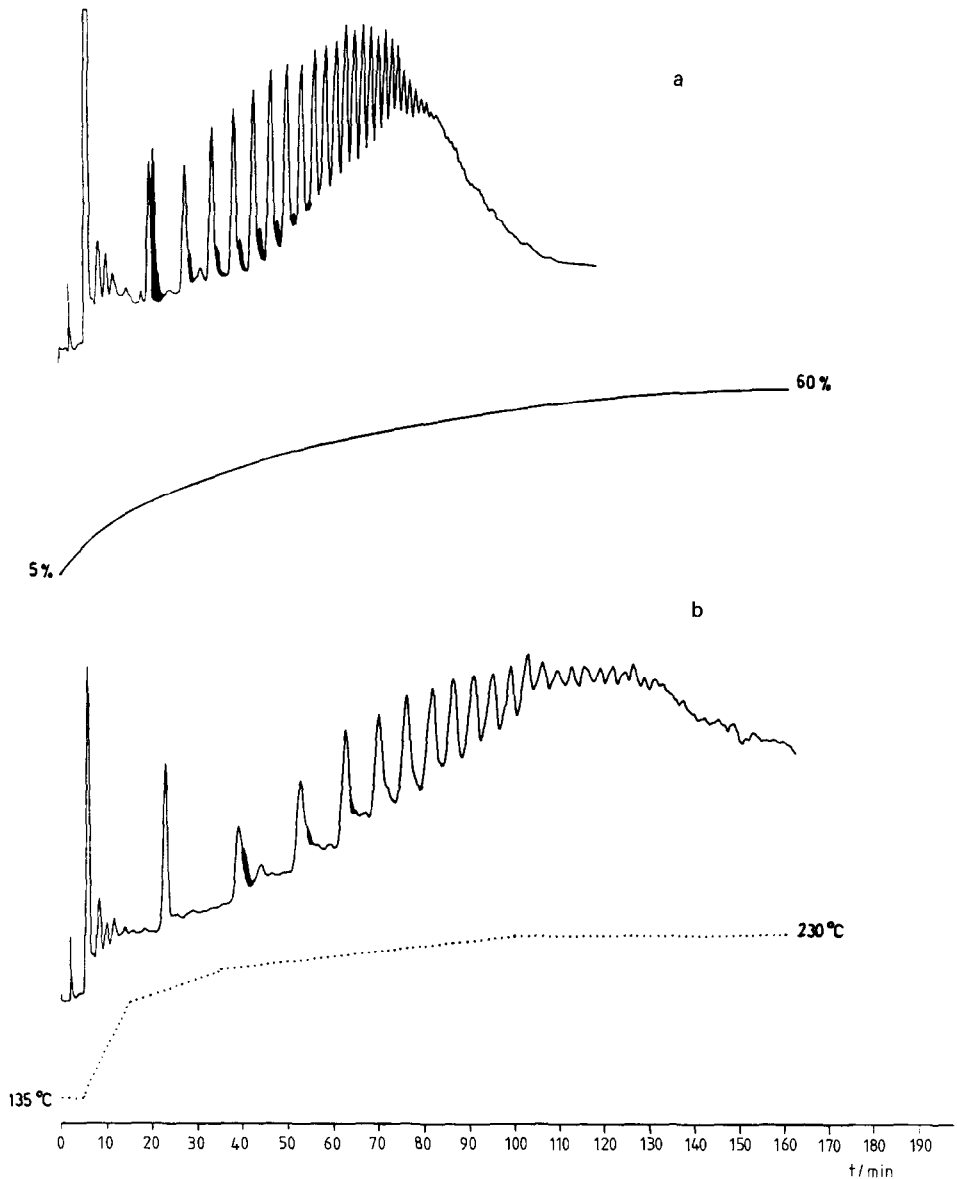


Fig. 6. SFC separations of 4-vinylbiphenyl oligomers (a) with eluent composition gradient at 135°C and (b) with a dual temperature-eluent composition program. Column: 25 cm \times 4.6 mm I.D., packed with Si 60, 10 μ m; eluents: carbon dioxide-1,4-dioxane; column exit pressures at the start of the chromatograms: (a) 217 and (b) 220 bar; gradients of temperature (for chromatogram b) and eluent composition are shown in the figure.

is a double and a triple gradient, respectively, since, as noted already, a pressure increase occurred during the separation because of the higher viscosity of 1,4-dioxane compared to carbon dioxide and the unchanged setting of the flow-restriction valve.

The "inherent" pressure gradients were linear, the pressure increase was about 60 bar during the separations. Comparing the two chromatograms, it is observed that the distance between the groups of peaks was increased with the temperature-program. A similar enhancement of resolution can also be obtained by combining pressure programming and temperature programming^{14,19}. However, the resolution was generally not improved, as is seen when comparing the shaded and unshaded parts of the peak groups in Fig. 6a and b. Since the temperature dependence of resolution differs for chemically different solutes, this may indicate distinct chemical differences between the different homologous series contributing to the peak groups.

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