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SEPARATION OF OLIGOMERS WITH UV-ABSORBING SIDE GROUPS BY SUPERCRITICAL FLUID CHROMATOGRAPHY USING ELUENT GRADIENTS

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SUMMARY

The chromatographic separation of oligomers prepared from vinyl arene compounds was achieved using eluent gradients with the eluent pair *n*-pentane–1,4-dioxane, which had previously been applied to oligostyrene separations. Separations were found to occur not only with respect to degree of oligomerization, but also between sub-series of oligomeric species. Transition from the supercritical to the liquid state, which may occur during an eluent gradient run if the selected column temperature is relatively little above T_c at the beginning of the gradient, does not seem to reduce the resolution considerably. The combination of two gradient techniques, a pressure gradient and a gradient of eluent composition, was applied for the separation of the difficult to elute N-vinylcarbazole oligomers. Supercritical fluid chromatograms showed considerably superior separation compared with high-performance liquid chromatograms obtained at ambient temperature using the same eluent gradient.

INTRODUCTION

The use of adsorption chromatographic techniques for the separation of oligomers has been increasing for some years. In addition to chromatography with liquid eluents, [high-performance liquid chromatography (HPLC)], chromatography with supercritical mobile phases [supercritical fluid chromatography (SFC)] has been shown to be applicable. The viscosity of supercritical fluids is lower than that of liquids and diffusion coefficients in supercritical gaseous phases are considerably higher than those in liquids^{1–3}. This favours equilibrium processes in the distribution of substrates between mobile and stationary phases and thus chromatographic separations can be improved and accelerated.

For the separation of substrate mixtures with greatly differing solubilities, gradient techniques have to be applied that enhance the solvating power of the eluent during the chromatographic run and thus lead to reasonable analysis times. With homologous systems such as oligomer mixtures, solubility decreases with increasing molecular weight. In SFC, the solvating power of an eluent can be enhanced by lowering the temperature, thereby increasing the density, increasing the pressure or changing the chemical composition of the mobile phase.

While changes in temperature are of lesser importance for gradient separations⁴, changes in pressure or density and in mobile phase composition are of considerable importance in SFC. Oligomer separations by means of pressure gradients have frequently been reported⁵⁻¹⁰; for improving the resolution still further, simultaneous pressure and temperature gradients have been applied¹¹.

It has also been shown that gradients of eluent composition, which are common in HPLC, are also applicable to SFC^{4,12,13}. With eluent gradients, using an increasing content of a component with higher viscosity there may simultaneously occur a pressure increase, depending on the instrumentation used, *i.e.*, with an unchanged setting of the pressure-reducing valve at the outlet of the apparatus. Hence the effect of the applied gradient is enhanced¹³. For the separation of oligostyrenes, the eluent system *n*-pentane-1,4-dioxane has been found suitable^{12,13}, the stationary phase being normal-phase silica. In this paper it is shown that this eluent system can also be used for the separation of related oligomers.

EXPERIMENTAL

The SFC apparatus consists of a modified HPLC instrument¹³ (1084B, Hewlett-Packard); the modifications consist mainly of an additional oven, which is capable of operating at higher temperatures, valves for setting the pressure levels at the outlet of the separation column and of the detector, and an additional loop injector.

1,4-Dioxane and *n*-pentane were distilled from sodium and degassed. Dioxane was stored in dark bottles in order to prevent the formation of peroxides.

The stainless-steel separation columns (25 cm × 4.6 mm I.D.) were packed with LiChrosorb Si 60, 10 μm (Merck, Darmstadt, F.R.G.) using a slurry method. The slurry consisted of a suspension of the stationary phase (2.5 g) in a mixture of

TABLE I
DATA FOR THE CHROMATOGRAMS SHOWN IN FIGS. 2-6

Chromatogram	Substrate*	λ (nm)**	T (°C)	p_i (bar)***	Dioxane programme [§]	Type
Fig. 2a	OVBP	254	250	49	5; 60; 220	SFC
Fig. 2b	OVBP	254	270	45	5; 60; 220	SFC
Fig. 3a	O1VN	278	250	46	5; 55; 180	SFC
Fig. 3b	O1VN	278	270	50	5; 55; 180	SFC
Fig. 4a	O2VN	278	240	52	5; 40; 160	SFC
Fig. 4b	O2VN	278	270	51	5; 50; 160	SFC
Fig. 5a	O1VN	278	Ambient	49	5; 55; 180	HPLC
Fig. 5b	OVBP	254	Ambient	50	5; 60; 220	HPLC
Fig. 6a	ONVC	295	270	40	See Fig. 7a	SFC
Fig. 6b	ONVC	295	270	41	See Fig. 7b	SFC

* Abbreviations: OVBP = oligo(4-vinylbiphenyl); O1VN = oligo(1-vinylnaphthalene); O2VN = oligo(2-vinylnaphthalene); ONVC = oligo(N-vinylcarbazole).

** Detection wavelength.

*** Column inlet pressure at the start of the chromatogram.

§ Initial dioxane content (% v/v); final dioxane content (% v/v); programme time (min). For programme type, see text and Fig. 1.

TABLE II
OLIGOMERIZATION REACTIONS

Monomer	Solvent	Monomer concentration (g/ml)	Monomer/initiator ratio	Initiation	Reaction temperature (°C)	Reaction time (min)
1	Tetrahydrofuran	0.025	1:1.33	Anionic	-20	3
2	Tetrahydrofuran	0.0175	1:1	Anionic	-20	3
3	Tetrahydrofuran	0.050	1:2	Anionic	-20	3
4	Toluene	0.025	5:1	Radical	90	60

toluene (20 ml) and cyclohexanol (30 ml), which was packed by pumping heptane on to the slurry in a reservoir at the top of the column.

After each chromatographic run, the column had to be flushed with high concentrations of dioxane to elute high-molecular-weight compounds present in the oligomer mixtures. Owing to their broad molecular weight distribution, this is especially necessary for oligomer samples prepared by radical initiation.

The flow-rate was 1 ml/min for the chromatograms shown in Figs. 2-5, measured at the pumps in the liquid state at ambient temperature. Further chromatographic conditions are summarized in Table I.

Anionic oligomerizations (Table II) took place in tetrahydrofuran at -20°C , using *n*-butyllithium as initiator. Reaction times were 3 min, the reactions being stopped by addition of methyl iodide. Radical oligomerization was performed in toluene, using azobisisobutyronitrile as initiator (Table II).

RESULTS AND DISCUSSION

Members of homologous series such as oligomers become less soluble with increasing molecular weight, requiring gradient techniques for chromatographic separations. However, with increasing molecular weight, the solubility differences decrease on going from a degree of oligomerization n to $n + 1$. Consequently, the slope of the gradient should decrease during a chromatographic run in order to obtain equally spaced peaks. It was found experimentally that on linear variation of the ratio Q_B the resulting gradients were suitable for oligomer separation:

$$Q_B = \frac{P_B}{1 - P_B}$$

where P_B is the volume fraction of eluent B, measured at the pumps in the liquid state. The resulting gradients of %B vs. time have the general shape shown in Fig. 1.

The oligomers obtained from the monomers 4-vinylbiphenyl (I), 1-vinylnaphthalene (II), 2-vinylnaphthalene (III) and N-vinylcarbazole (IV) were soluble in 1,4-dioxane, analogous to oligostyrenes. It was expected that for the chromatographic separation of these oligomers the eluent pair *n*-pentane-1,4-dioxane, which had previously been found suitable for oligostyrenes, could be applied.

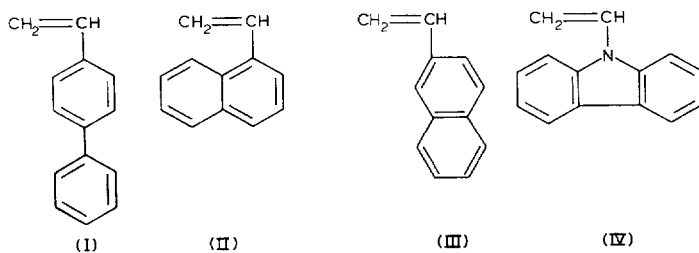


Fig. 2a shows the separation of oligomers prepared by anionic oligomerization of 4-vinylbiphenyl (OVBP). About 45 peaks or peak groups can be observed in this chromatogram; using higher detector sensitivities up to 55 oligomers could be detected. The chromatographic conditions were chosen such that the early eluting peaks of the oligomer series were eluted with large spacing in order to separate the broad peaks (indicated by arrows), which do not seem to belong to the oligomeric series. At least two different sub-series can be observed, which differ in their elution behaviour. In the course of the separation, a smaller side peak separates from the main peak, having a degree of oligomerization n , and finally merges into the peak corresponding to a degree of oligomerization $n + 1$. As the structural assignments of the different oligomeric sub-series are not known at present, it is possible that the differences between these sub-series are due to variations in the repeating units of the oligomer chains or to different end groups.

In Figs. 3 and 4, separations of oligomers from anionic oligomerization of 1-vinylnaphthalene (O1VN) (Fig. 3a) and 2-vinylnaphthalene (O2VN) (Fig. 4a) are shown. As with the separation of vinylbiphenyl oligomers, separation into sub-series was achieved. Particularly for the 2VN oligomers, the "wandering" of a peak from one peak group to the next can be observed during the separation. Up to three oligomeric sub-series were separated; however, the irregular peak shapes that can be observed for the lower oligomers suggest that further sub-series or by-products may

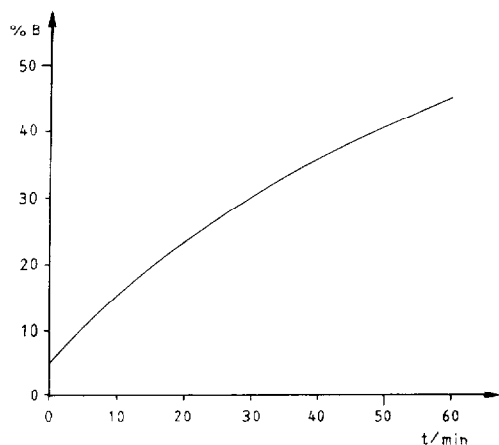


Fig. 1. General shape of gradients in eluent composition applied for the separations shown in Figs. 2–5. Programme data for this example: initial dioxane content, 5%; final dioxane content, 45%; programme time, 60 min.

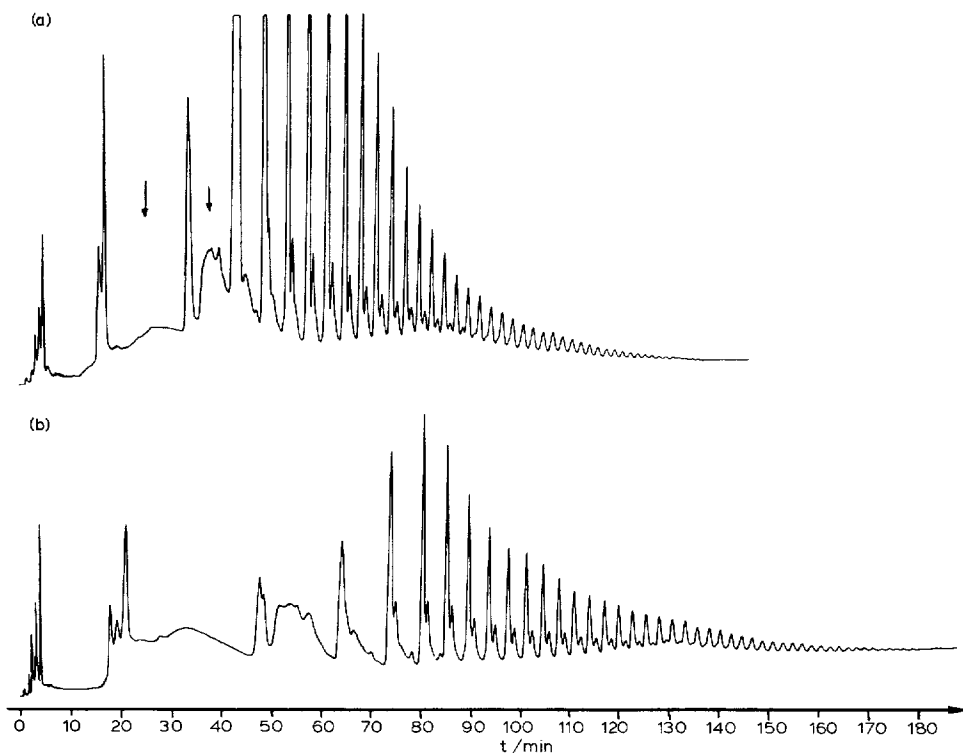


Fig. 2. SFC separations of 4-vinylbiphenyl oligomers. Column temperatures: (a) 250°C; (b) 270°C. See Table I and Experimental.

be present in the samples. For higher degrees of oligomerization, n , the different homologous sub-series merge into one peak per n .

Increasing the dioxane content leads to increasing critical values for temperature and pressure for the resulting eluent mixtures (Table III). Thus, during the separations shown in Figs. 2a, 3a and 4a, a transition from the supercritical to the liquid state occurs during the chromatographic run owing to the increasing dioxane content. To determine whether such a phase transition has a detrimental effect on the separation, the same chromatograms were run at 270°C, but with slightly different pressures. At this temperature, the mobile phase remains in the supercritical state until a dioxane content of about 55% is reached. Figs. 2b and 3b show that the separations were similar to those obtained at 250°C. However, the analysis times were longer and the separations, especially those into sub-series, were enhanced. The latter effect is not surprising, because resolution has been reported to show maxima at slightly supercritical temperatures¹⁵⁻¹⁸.

Elution can be accelerated to yield analysis times comparable to those obtained at lower temperatures in two ways. First, the column pressure at the start of the chromatogram can be increased, which leads to higher eluent densities, thereby compensating for the effect of the decreasing density at increasing temperature. Increasing the column pressure by about 20 bar leads to analysis times being even shorter than those at 250°C, but a loss in information is observed only for the early eluting peaks.

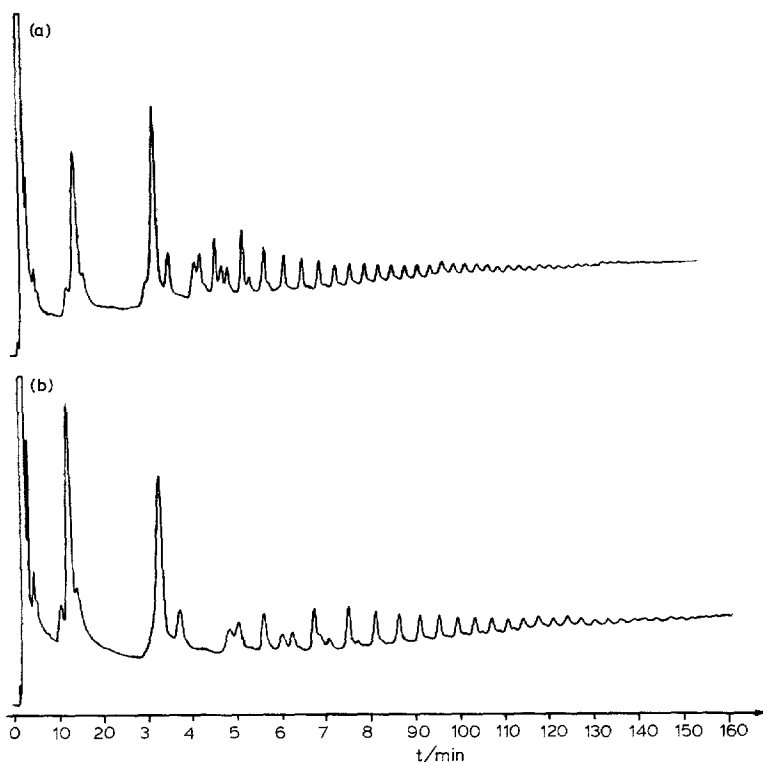


Fig. 3. SFC separations of 1-vinylnaphthalene oligomers. Column temperatures: (a) 250°C; (b) 270°C. See Table I and Experimental.

The second means of reducing analysis times at higher temperatures is to increase the slope of the gradient. Fig. 4b shows a separation of the O2VN sample at 270°C, where the slope of the gradient has been increased compared with the chromatogram at 240°C (Fig. 4a). Again, the analysis times become comparable, but the chromatograms show some differences. First, at the higher temperature the resolution of the sub-series is lower. This may be attributed to temperature-dependent changes in selectivity¹⁹. Second, more oligomer species are eluted under the conditions applied for the chromatogram in Fig. 4b. It seems that for the elution of the higher oligomers the chemical composition of the mobile phase is more effective than the density.

TABLE III

CRITICAL DATA FOR MIXTURES OF *n*-PENTANE AND 1,4-DIOXANE

The data were calculated according to procedures described by Chueh and Prausnitz (*cf.*, ref. 14).

Parameter	Value											
B (%)*	0	5	10	20	30	40	50	60	70	80	90	100
T_c (°C)	196.5	203.9	211.2	225.2	238.4	250.9	262.8	274.1	284.8	295.0	304.7	313.9
p_c (bar)	33.7	37.4	40.8	46.7	51.4	54.9	57.1	58.2	58.1	56.9	54.9	52.1

* Content of 1,4-dioxane in *n*-pentane (% v/v).

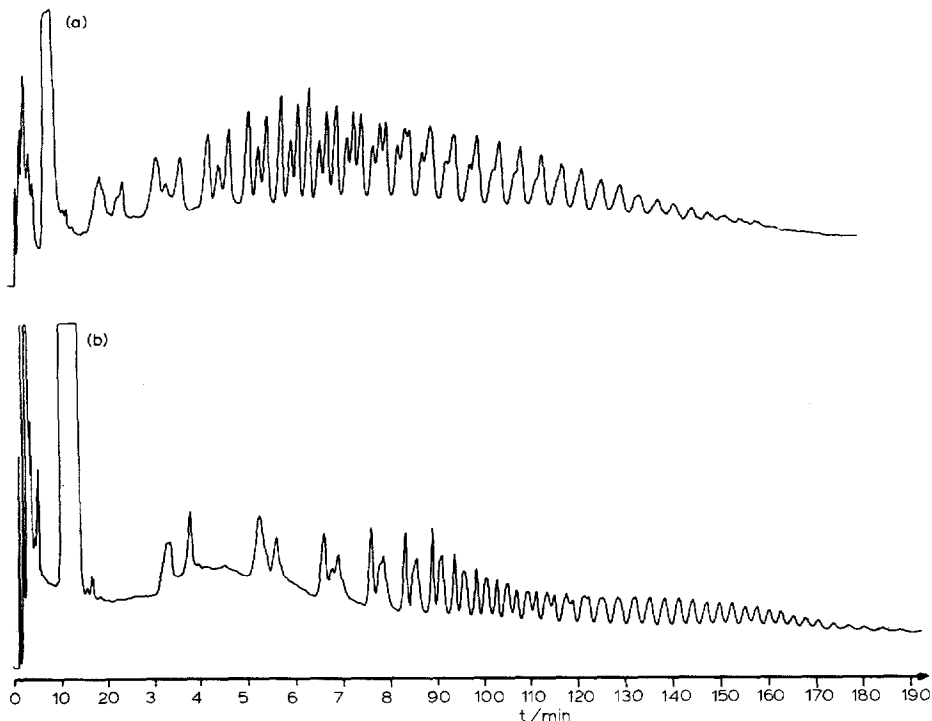


Fig. 4. SFC separations of 2-vinylnaphthalene oligomers. Column temperatures: (a) 240°C; (b) 270°C. Gradients: (a) 5–40% dioxane within 160 min; (b) 5–50% dioxane within 160 min. See Table I and Experimental.

The eluent system *n*-pentane–1,4-dioxane is not only suitable for the separation of vinylarene oligomers under supercritical conditions but also, in principle, at ambient temperature, *i.e.*, under HPLC conditions. However, as can be seen from Fig. 5, the resolution obtained is smaller than that in the SFC chromatograms. Fig. 5a shows an HPLC separation of the O1VN sample where the gradient and the column pressure were similar to those applied for the chromatograms shown in Fig. 3. The oligomer peaks are broad but no separation into the sub-series can be observed. Comparison of the OVBP separations in Figs. 5b (HPLC) and 2 (SFC) demonstrates that the separation under HPLC conditions is considerably poorer than that under SFC conditions. Decreasing the pressure for the HPLC separation does not yield a substantial enhancement of resolution.

For the separation of *N*-vinylcarbazole oligomers (ONVC), the application of a gradient of eluent composition was not sufficient for obtaining good separations. It was found that an eluent gradient had to be combined with a pressure gradient; this pressure gradient was generated by increasing the flow-rate at a given setting of the outlet valve. The applied flow-rates ranged from 0.75 to 1.9 ml/min. With such flow-rates good to at least usable resolutions can be obtained, as has already been found with *n*-pentane as the eluent¹⁸. Fig. 6a shows a separation obtained by application of a pressure gradient with a slightly decreasing slope (*cf.*, Fig. 7a). The pro-

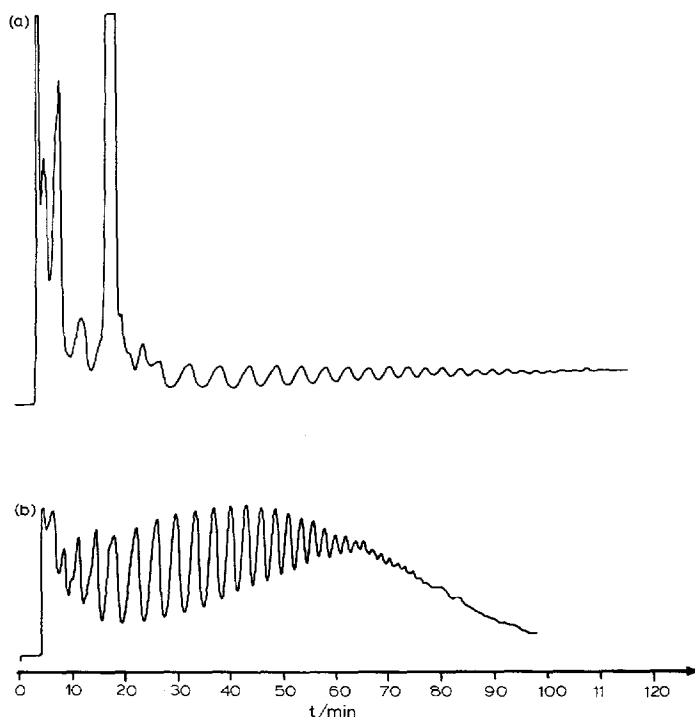


Fig. 5. HPLC separation of (a) 1-vinylnaphthalene oligomers and (b) 4-vinylbiphenyl oligomers. See Table I and Experimental.

grammes were started 10 min after the start of the chromatogram in order to separate low-molecular-weight compounds, which appear at the beginning of the chromatogram, from the homologous oligomer series. About 25 oligomers can be recognized in the chromatogram, which also shows a separation into some sub-series for the lower members of the oligomer series. At the beginning of the chromatogram the mobile phase is under slightly supercritical or even at subcritical conditions with respect to pressure; elution of the homologous series takes place with the transition to supercritical pressures. This elution behaviour is even more pronounced in the chromatogram shown in Fig. 6b, where a sharp rise in pressure (*cf.*, Fig. 7b) causes immediate start of elution of the NVC oligomers.

In conclusion, it can be stated that the combination of an alkane with 1,4-dioxane as the eluent system is not only applicable to SFC separations of oligostyrenes but also to separations of chemically related oligomers. Although these eluent pairs can also be used in the liquid state, *i.e.*, under HPLC conditions, the resolution of the HPLC separations was found to be distinctly inferior to that obtained under supercritical conditions using the same eluent gradient. In contrast, a transition from the supercritical to the liquid state, while still near T_c , which may occur owing to an increasing content of the higher boiling component in the eluent, does not seem to reduce the resolution greatly.

Changes in the chromatographic conditions (temperature, pressure, shape and slope of the gradient) may affect the selectivity α of a separation and thus lead to

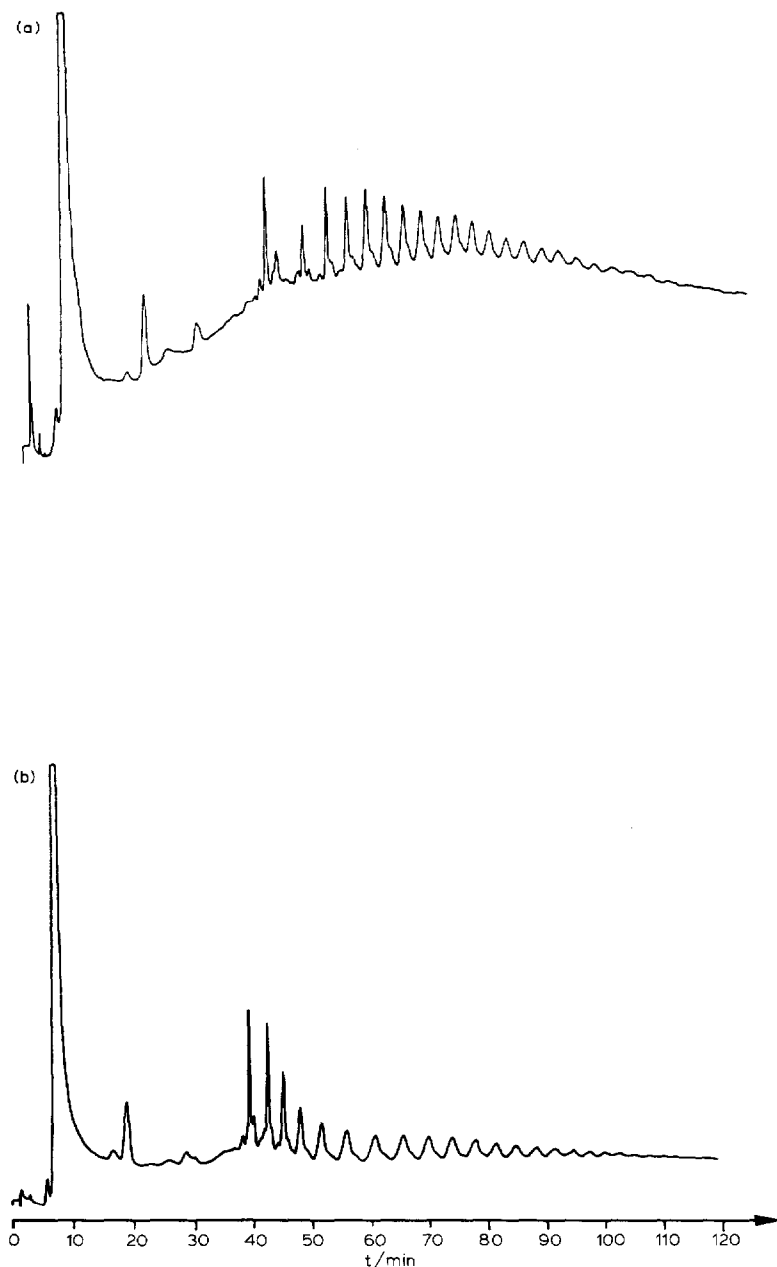


Fig. 6. SFC separations of N-vinylcarbazole oligomers. (a) Column temperature, 270°C. Flow-rate kept constant at 0.75 ml/min for 10 min and then increased to 1.65 ml/min at 130 min. For eluent and pressure gradients, see Fig. 7a; for further chromatographic conditions, see Table I and Experimental. (b) Column temperature, 270°C. Flow-rate, increased from 0.75 ml/min at the start of the chromatogram to 1.9 ml/min at 120 min. For eluent and pressure gradients see Fig. 7b; for further chromatographic conditions, see Table I and Experimental.

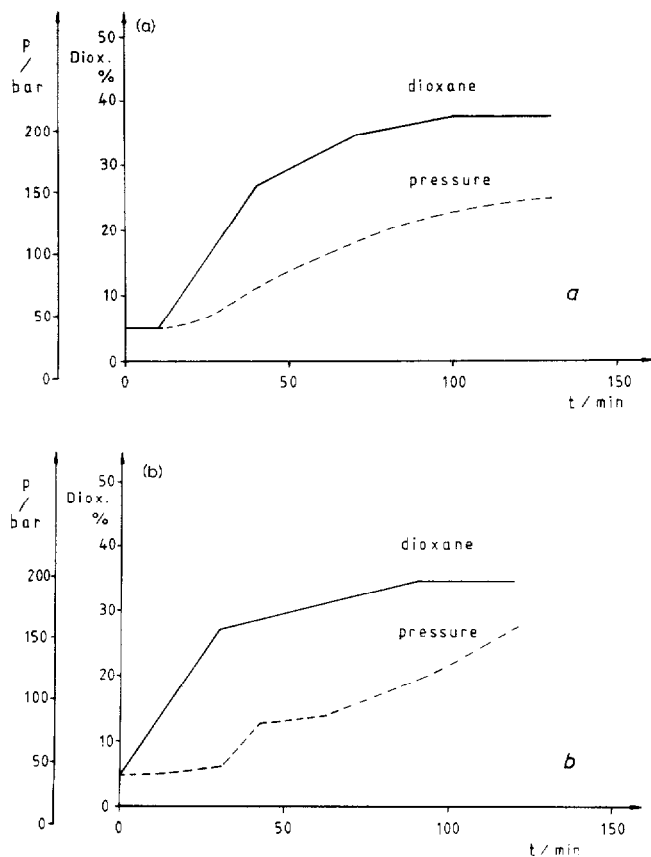


Fig. 7. Eluent and pressure gradients for the chromatograms in Figs. 6a (a) and 6b (b). Solid line, dioxane content (% v/v) in the eluent mixture; broken line, pressure at column inlet.

changes in the order of elution of sub-series within the homologous oligomer series. This opens up the possibility of optimizing separations, either with respect to the separation of such sub-series or with respect to a separation according to the degree of oligomerization.

Finally, for substrates that are difficult to elute, combined programmes of eluent composition and pressure may be applied in order to effect a particular separation.

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