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CHROMATOGRAPHY WITH SUB- AND SUPERCRITICAL ELUENTS

INFLUENCE OF THE SEPARATION CONDITIONS ON SELECTIVITY, PLATE NUMBER AND RESOLUTION

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(Received December 4th, 1985)

SUMMARY

The contributions of the selectivity, α , and plate number, n , to the dependence of chromatographic resolution on the pressure and temperature have been studied. It is demonstrated that these contributions depend on the separation conditions and on the substrates, with changes in α being in some cases responsible for changes in resolution rather than changes in n . Increasing pressure may cause the selectivity to increase, while plate numbers are lowered. For the separation of oligomers, a pressure may be found where a maximum in the overall resolution occurs.

INTRODUCTION

Studies on the chromatographic behaviour of various mobile phases in different physical states (liquid, gaseous and supercritical) with respect to the parameters temperature and pressure have produced interesting results¹⁻⁷. In some of these studies^{2,3,5,7}, particular attention was paid to the effects of pressure and temperature on the chromatographic resolution, R , as a measure of the separation quality. Since R is influenced both by the elution times of the peaks relative to each other, *i.e.*, the selectivity, α , and by the peak widths, the latter information being contained in the plate number, n , knowledge of changes in the values of α and n are of additional interest. In this communication, some results are presented concerning the relationship between α , n and R and its dependence on the temperature and pressure.

THEORETICAL

For determination of resolution, a modified resolution equation was used:

$$R_{ij}^* = P_{ij} + \frac{d_{ij}}{w_i' + w_j'} \sqrt{\ln 4} \quad (1)$$

with $d_{ij} > 0$

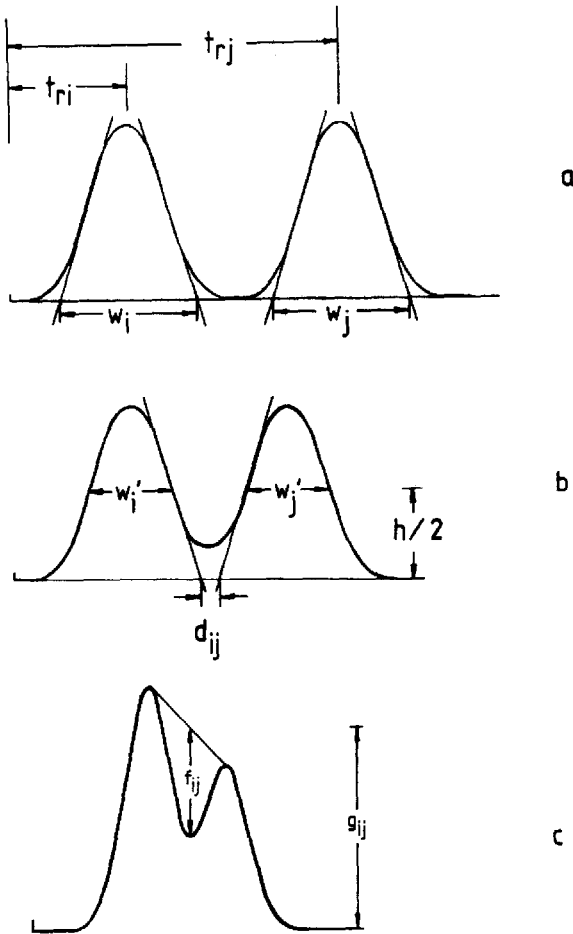


Fig. 1. Different types of peak pairs: (a) fully resolved; (b) incompletely resolved; (c) severely overlapped.

Here, P_{ij} is the ratio of the depth of a peak valley to the average peak height⁸ (*cf.*, Fig. 1c)

$$P_{ij} = f_{ij}/g_{ij} \quad (2)$$

d_{ij} is the portion of the baseline intercepted between the tangents to two peaks and w' is the peak width at half height (*cf.*, Fig. 1b). For $d_{ij} = 0$ and $d_{ij} < 0$, R^* was calculated according to:

$$R_{ij}^* = P_{ij} \quad (3)$$

Eqn. 1, which resembles an equation proposed by Bhattacharjee⁹, was derived from the well known resolution equation (*cf.*, Fig. 1a)

$$R_{ij} = \frac{2(t_{rj} - t_{ri})}{w_i + w_j} \quad (4)$$

with eqn. 5 (*cf.*, Fig. 2a)

$$t_{rj} - t_{ri} = d_{ij} + \frac{w_i + w_j}{2} \quad (5)$$

yielding:

$$R_{ij} = 1 + \frac{2 d_{ij}}{w_i + w_j} \quad (6)$$

Replacing peak widths, w , by peak widths at half height, w' , leads to:

$$R_{ij} = 1 + \frac{d_{ij}}{w'_i + w'_j} \sqrt{\ln 4} \quad (7)$$

For the calculation of resolution, peaks are generally treated as having trapezoidal shapes (*cf.*, Fig. 2b) for which both R_{ij} and P_{ij} are equal to unity when $d_{ij} = 0$. The value of P_{ij} remains equal to unity when d_{ij} assumes positive values. Thus, eqn. 7 may be written in the form of eqn. 1.

Calculating R^* by means of eqn. 1 for peak pairs where $d_{ij} > 0$ and by eqn. 3 when $d_{ij} \leq 0$ has the advantage that in plots of resolution *vs.* a selected chromatographic parameter no abrupt change is produced, since there is a smooth transition from eqn. 1 to eqn. 3. Compared to eqn. 4, the resolution values calculated using eqn. 1 are more sensitive to peak asymmetries with closely neighbouring peaks. Thus, from a practical point of view, the R^* values are more "realistic". The difficult construction of tangents to overlapped peaks is avoided when using the procedure described above.

For peaks which are resolved so poorly that their widths cannot be determined, the plate numbers can be estimated from resolution values using the relation

$$n_j = 16 \left(\frac{t_{rj}}{t_{rj} - t_{ri}} \right)^2 R_{ij}^2 \quad (8)$$

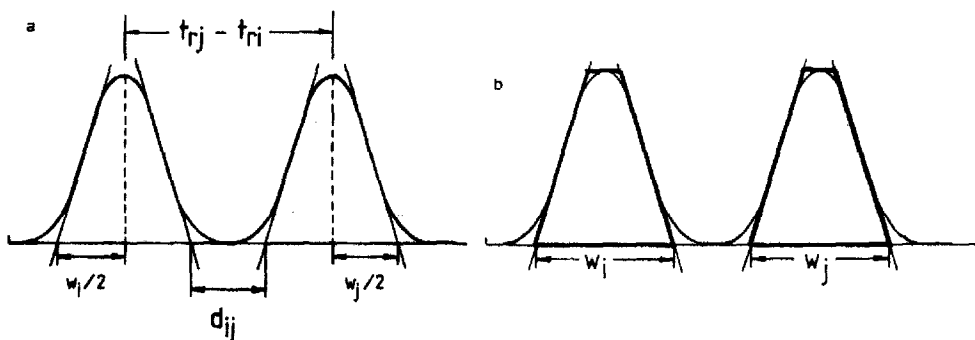


Fig. 2. (a) Relationship between $(\Delta t_r)_{ij}$ and d_{ij} . (b) Trapezoidal peak shapes.

which can be obtained from eqn. 9

$$R_{ij} = \frac{1}{4} \cdot \frac{\alpha - 1}{\alpha} \cdot \frac{k_j}{1 + k_j} \sqrt{n_j} \quad (9)$$

which is valid for closely neighbouring peaks.

Here, α is the selectivity

$$\alpha = \frac{t_{rj} - t_0}{t_{ri} - t_0} \quad (10)$$

k_j is the capacity factor of the j th peak

$$k_{rj} = \frac{t_{rj} - t_0}{t_0} \quad (11)$$

and t_0 is the dead time.

EXPERIMENTAL

The instrument used for supercritical-fluid chromatography (SFC) has been described previously¹⁰. It consisted of a high-performance liquid chromatograph, Type 1084 B, from Hewlett-Packard, which had been modified for SFC.

Stainless-steel columns (25 cm \times 4.6 mm I.D.) were packed with LiChrosorb Si 60, 10 μ m (Merck, Darmstadt, F.R.G.) using a slurry method¹¹. UV detection (254 nm) was carried out at ambient temperature in the liquid state. The flow-rate was 1 ml/min at the pumps at ambient temperature. The eluents *n*-pentane and 1,4-dioxane were purified before use by drying, distillation and degassing. The chromatographic substrates were PS 800, an oligostyrene sample with a number-average molecular weight of 810 g/mol (Pressure Chemical, Pittsburgh, PA, U.S.A.), and a mixture of the aromatic compounds naphthalene, anthracene, pyrene and chrysene (*cf.*, ref. 2); the sample solvent was *n*-hexane.

The chromatographic data used for the calculation of resolution values shown in Fig. 3 are taken from studies reported previously^{3,7}.

RESULTS AND DISCUSSION

Fig. 3 shows isobaric plots of resolution *vs.* temperature for a sample mixture consisting of naphthalene, anthracene, pyrene and chrysene, chromatographed with *n*-pentane as the eluent. Curves 1 and 3 show the temperature dependence of the average resolution, $\overline{R^*}$, calculated according to

$$\overline{R^*} = \frac{\sum R_{ij}^*}{n} \quad (12)$$

where n = total number of adjacent peak pairs. Curves 2 and 4 are plots of the

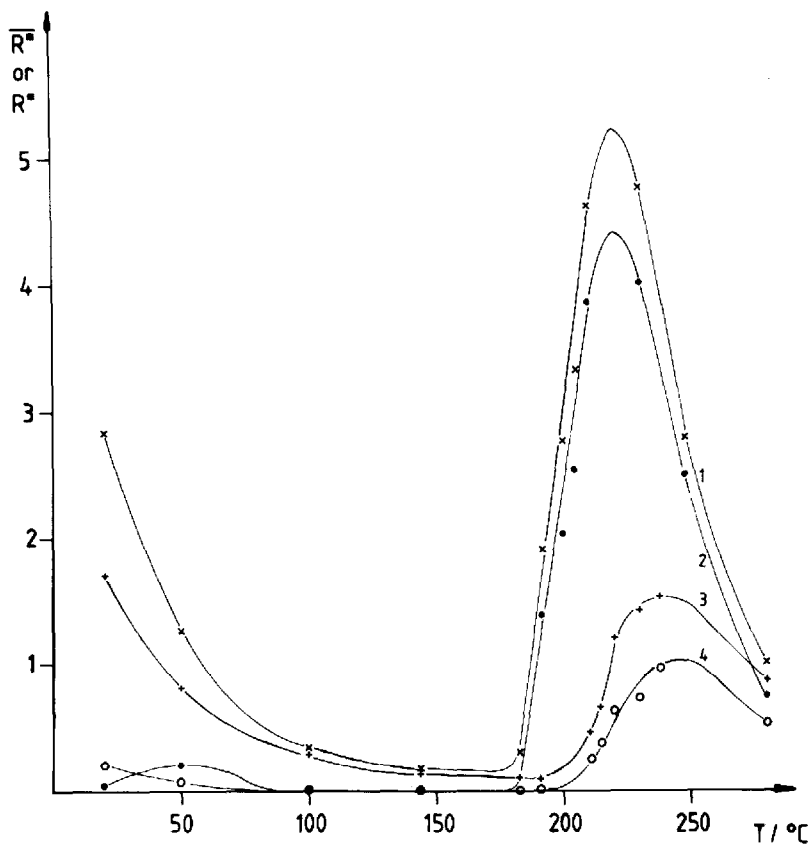


Fig. 3. Plots of mean resolution, \bar{R}^* , vs. temperature for a mixture of naphthalene, anthracene, pyrene and chrysene in *n*-pentane as the eluent at 20 bar (curve 1) and 36 bar (curve 3), and of resolution, R_{ij}^* , vs. temperature for anthracene/pyrene at 20 bar (curve 2) and 36 bar (curve 4).

resolution, R_{ij}^* , between anthracene and pyrene. Values of R_{ij}^* were calculated by the combination of eqns. 1 and 3 as described above.

As is seen from Fig. 3, the overall resolution, \bar{R}^* , for the three peak pairs naphthalene/anthracene, anthracene/pyrene and pyrene/chrysene decreases when the temperature is raised, as long as the mobile phase is in the liquid state (curves 1 and 3). Beyond the boiling or the critical temperature, \bar{R}^* increases again and exhibits a maximum. Further increase in temperature leads to decreasing resolution. The lower the pressure the more pronounced is this behaviour, (*cf.*, refs. 5, 7). In contrast, the separation of anthracene and pyrene is rather poor in the liquid phase. Good separation can be achieved only when the mobile phase is in the sub- or supercritical gaseous state.

This behaviour can be ascribed partly to changes in the plate numbers, which were estimated by means of eqn. 8. In the experiments at 20 bar (curves 1 and 2), plate numbers obtained at 230°C, *i.e.*, near the maximum of the resolution curve.

TABLE I

RELATIVE VALUES FOR PARAMETERS AT THE RESOLUTION MAXIMA (*cf.*, FIG. 3)

Referred to corresponding values in the liquid phase at ambient temperature. N = Naphthalene; A = anthracene; P = pyrene; C = chrysene.

Conditions	Peak pair	n (rel.)	$\frac{\alpha - 1}{\alpha}$ (rel.)	$\frac{k_j}{1 + k_j}$ (rel.)
20 bar.	N/A	1.6	1.9	3.4
230°C	A/P	2.3	5.2	5.7
	P/C	2.2	1.2	6.8
36 bar.	N/A	0.6	1.5	1.0
	A/P	0.8	4.5	1.4
	P/C	0.7	0.8	1.1

exceed those obtained in the liquid phase by a factor of about two (*cf.*, Table I). This is accompanied by increasing values of both the α and k terms of the resolution equation 9, with the increase in α for anthracene/pyrene being particularly high. Contrary to this, at 36 bar, the plate numbers at 20 and 240°C are comparable, even for the pair anthracene/pyrene, for which the resolution is considerably higher at 240°C. Here, the increase in resolution is due to changes in the selectivity of the separation, as is seen from the increase in the selectivity term. Similar results are obtained for the lower alkanes butane and propane.

It has been shown that, for SFC separations of oligomers, in addition to pres-

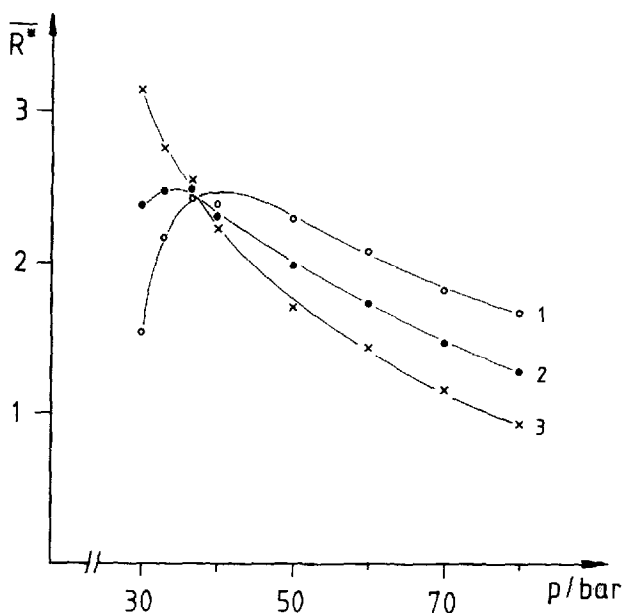


Fig. 4. Resolution of oligostyrenes as a function of column pressure. Curves: 1, overall resolution, \bar{R}^* , of peak 10-18; 2, overall resolution, \bar{R}^* , for peaks 1-18; 3, overall resolution, \bar{R}^* , for peaks 1-9. $T = 210^\circ\text{C}$. Pressure, p , at the column exit at the start of the chromatographic experiments.

TABLE II
GRADIENT PROGRAM FOR THE POLYSTYRENE SEPARATIONS

	Time (min)								
	0	10	20	30	40	50	60	70	80
Dioxane (%, v/v)	5.0	7.1	9.1	11.0	12.9	14.6	16.2	17.9	19.4

sure programming which is most frequently used, gradients in eluent composition can be applied¹⁰. Fig. 4 shows the separations of oligostyrenes with *n*-pentane-1,4-dioxane as the eluent; the dioxane content in the eluent mixture is given in Table II. While the gradient and the temperature were kept the same, at the start of the chromatograms the pressure at the column exit was varied between 30 and 80 bar. It is seen from Fig. 4 that the average resolution for the first nine peaks decreases steadily with increasing pressure. Nevertheless, for both the average resolution of the last nine peaks and the overall resolution, maxima occur at pressures of *ca.* 35–40 bar.

These resolution effects are produced by changes in the selectivity, α , and plate number, *n*, the capacity factor term (*cf.*, eqn. 9) being only of minor importance. For the higher oligomers, *i.e.*, from a degree of oligomerization of *ca.* 6, the plate numbers were found to decrease with increasing pressure, while increasing α values were observed. Thus, with increasing pressure, the quality of the oligostyrene separations was governed more and more by selectivity. From these results it is obvious that, for an oligomer separation, a pressure may be found where the separation is optimal. A comparison of curves 1 and 2 in Fig. 5 shows that such optimum pressures are shifted to higher values with increasing molecular weight of the sample.

It should be mentioned that, with the oligostyrene separation shown here, the critical temperature of the eluent mixture may exceed the applied temperature of 210°C owing to the increase in dioxane content (*cf.*, Table III). Thus, a transition of the mobile phase from a dense (supercritical) gas to a liquid occurs during the experiment, especially where the pressure is low and, therefore, higher dioxane contents are necessary to elute the whole sample (*cf.*, Table IV). Yet, a transition from a state with a lower density to one where the density is higher does not seem to have a detrimental effect on the separation¹¹. In contrast, pressures below the critical pres-

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

Calculated according to the methods described by Chueh and Prausnitz¹².

	Dioxane (%, v/v)				
	0	5	10	15	20
T_c (°C)	196.5	203.9	211.2	218.3	225.2
P_c (bar)	33.7	37.4	40.8	43.9	46.7

TABLE IV
DATA FOR THE OLIGOSTYRENE SEPARATIONS

P_e (bar)*	t_{max} ** (min)	Dioxane content at t_{max} (%, v/v)	P_c at t_{max} (bar)
30	78.1	18.4	41
33	68.0	16.7	43
37	56.2	14.8	46
40	52.9	14.2	49
50	35.4	11.1	57
60	31.7	10.5	66
70	25.5	9.2	75
80	22.9	8.7	85

* Column exit pressure at the start of the chromatogram.

** Retention time of the 18th peak.

sure might have a greater effect. Since, however, the column pressure is set at the start of the experiment by use of a valve at the column outlet and since the increasing dioxane content leads to a higher viscosity of the mobile phase, the pressure increases to some extent during the experiment.

This pressure increase due to the higher dioxane contents is almost parallel to the dioxane content in the eluent mixture¹³; the pressures resulting at the column exit at the elution time of the last peak, t_{max} , are summarized in Table IV. It is seen from Tables III and IV that if at the start of the chromatogram the pressure, p_e , is above the critical pressure of the eluent mixture, it will remain above critical during the separation irrespective of the increasing dioxane content. Thus, the increase in pressure prevents the transition to the subcritical gaseous state which would have occurred in some instances.

For Fig. 4, the pressure at the column exit, set at the start of the separation, was chosen as the parameter for two reasons. First, this pressure is of interest from a practical point of view since it can easily be set and controlled. Secondly, for the quality of a separation, this pressure is most important in SFC because elution is slowest at the column exit due to the lower eluent density compared to that in the preceding parts of the column; the pressure drop along the column was in the range of 10–15 bar.

ACKNOWLEDGEMENTS

Thanks are expressed to Professor E. Klesper for kindly supporting this work, to Mr. B. Lorenschat for experimental aid and to the Arbeitsgemeinschaft Industrieller Forschungsvereinigungen (AIF) for financial support.

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