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## Use of chromatographic models for computerized optimization of coupling-point pressure in dual-column gas chromatography

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### ABSTRACT

A novel computer-assisted procedure is presented for optimization of the coupling-point pressure ( $p_m$ ) of a column for the gas chromatographic separation of multi-component samples on two capillary columns of fixed dimensions and stationary phase polarities operated at constant temperature with constant inlet ( $p_i$ ) and outlet ( $p_o$ ) pressures. The retention of all solutes in the system is monitored by the chromatographic model

$$k_{s,i} = k_{A,i} + x_B (k_{B,i} - k_{A,i})$$

where  $k$  is the capacity factor obtained on the polar column (A), the non-polar column (B) and on the columns coupled in series (S) and  $x_B$  is the weight factor determined from column characteristics and carrier gas pressures ( $p_i$ ,  $p_m$  and  $p_o$ ). The optimization procedure is monitored by a dependence of the optimization criterion ( $C_p$ ) against the column coupling-point pressure ( $p_m$ ):

$$C_p = f(p_m)$$

for both column series AB and BA. The optimization criterion is based on a number of peaks resolved equal to or better than the required resolution factor (primary part) in the shortest analysis time (secondary part).

Comparison of the novel optimization procedure with those based on window diagrams shows that the new model is superior as it gives unambiguously the maximum number of peaks that can be resolved under the given conditions equal to or better than the required resolution in the shortest analysis time.

## INTRODUCTION

It is well known that optimization of selectivity is the key to high-resolution gas chromatography<sup>1-4</sup>. For directly coupled columns, a study of selectivity tuning by variation of column length was performed by Purnell and co-workers<sup>5-7</sup>, with particular emphasis on a correction for compressibility effects.

Selectivity optimization or tuning can be performed by coupling two columns of fixed dimensions and different polarities in series<sup>1-4,8-15</sup>. The overall polarity of serially coupled columns can be adjusted as required by tuning the carrier gas flow-rates through the individual columns<sup>2-4,8,9,14,15</sup>, which was first proposed by Deans and Scott<sup>8</sup>, by adjusting the temperatures (isothermal, programmed temperature or a combination) of both columns<sup>1-4,10,11,13,14</sup> or by simultaneous tuning of carrier gas flow-rates and temperatures<sup>2,14</sup>. If the selectivity of two coupled capillary columns is tuned, all solutes pass through both columns and no intermediate trapping is used. The two coupled columns act as a single column, the selectivity of which is a composite of the selectivities of the individual columns. Sandra *et al.*<sup>2</sup> have described the selectivity tuning of two directly coupled capillary columns. They stated that a OV-1-PEG 20M tandem is less polar than a PEG 20M-OV-1 tandem. The retention is higher owing to gas compressibility effects in the polar column when this column is placed first and thus a higher polarity is obtained<sup>2</sup>. Equal polarities were obtained for PEG 20M-OV-1 and OV-1-PEG 20M columns in series when the corresponding carrier gas pressure drops were kept constant on changing the column order<sup>2</sup>. Thus, if the gas flow-rates of each column can be adjusted independently, gas compressibility effects are avoided.

For the separation of a multi-component sample, Hinshaw and Etre<sup>3,4</sup> proposed optimizing the overall selectivity of two columns coupled in series and placed in one oven by tuning the carrier gas flow-rates in the individual columns and/or the oven isothermal temperatures. An optimization procedure based on a chromatographic model and on window diagrams according to Laub and Purnell<sup>12</sup> was used.

Computer-assisted procedures for the adjustment of optimum selectivity by tuning the intermediate pressure<sup>14,15</sup> or temperatures<sup>13,14</sup> for serially coupled columns placed in a separately heated dual oven system have been described<sup>13-15</sup>. The optimization procedures were based on mathematical models in which the dependences of the retention indices of all solutes on the optimized parameters were described by polynomial equations. New threshold criteria for the evaluation of the gas chromatographic (GC) separation of multi-component samples were advanced<sup>15</sup>.

The aim of this paper is to introduce a computer-assisted procedure for the optimization of selectivity by tuning the coupling-point pressure ( $p_m$ ) of two capillary columns of different polarities (A and B) coupled in series (either AB or BA) and operated at constant temperature. The optimization procedure is based on a chromatographic model which is evaluated by a threshold criterion. The validity of the optimization procedure has been verified by the separation of a mixture of 33 hydrocarbons and the new procedure is compared with the computer-assisted procedure based on window diagrams.

## THEORY

It has been shown by Smuts *et al.*<sup>16</sup>, Hinshaw and Ettre<sup>3</sup> and Purnell and Williams<sup>5,7</sup> that the capacity factor of a solute, separated in a system (S) consisting of two columns coupled in series ( $k_{i,S}$ ), can be calculated from the capacity factors of this solute in the individual columns ( $k_{i,A}$  and  $k_{i,B}$ ):

$$k_{i,S} = x_A k_{i,A} + x_B k_{i,B} \quad (1)$$

where  $x_A$  and  $x_B$  are weight factors calculated from the following equations<sup>3,14</sup>:

$$x_A = \frac{t_{M,A}}{t_{M,A} + t_{M,B}} = \frac{L_A \bar{u}_B}{L_A \bar{u}_B + L_B \bar{u}_B} \quad (2)$$

and

$$x_B = \frac{t_{M,B}}{t_{M,A} + t_{M,B}} = \frac{L_B \bar{u}_A}{L_A \bar{u}_B + L_B \bar{u}_B} \quad (3)$$

hence

$$x_A + x_B = 1 \quad (4)$$

From eqns. 1–3 it follows that solute retention in columns in tandem ( $k_{i,S}$ ) can be tuned by changing both physical and thermodynamic parameters.

From eqns. 1–3, it is obvious that the capacity factor found for a solute in columns in tandem does not depend on the column order. However, if two capillary columns of fixed lengths ( $L_A$ ,  $L_B$ ) and inner diameters ( $r_A$ ,  $r_B$ ) are coupled in series and operated with constant inlet ( $p_i$ ) and outlet pressures ( $p_o$ ), owing to the carrier gas compressibility different carrier gas flow-rates are established in both individual columns for column orders AB and BA.

If two columns of fixed dimensions are coupled in series, the  $k_{i,S}$  value can be tuned by changing parameters that influence the carrier gas flow-rates and capacity factors in the individual columns. The carrier gas flow-rates in both individual columns can be tuned by changing the nature of the carrier gas and the inlet ( $p_i$ ), column coupling-point ( $p_m$ ) and outlet ( $p_o$ ) pressures and temperatures ( $T_A$ ,  $T_B$ ). The capacity factor of a solute in the individual columns of a fixed column series can be influenced only by temperature.

Kaiser *et al.*<sup>9</sup> and Hinshaw and Ettre<sup>3,4</sup> have shown that the overall selectivity of two capillary columns coupled in series can be effectively tuned by changing the column coupling point pressure ( $p_m$ ), keeping all other variables constant.

For an average carrier gas flow-rate ( $\bar{u}$ ) in a single capillary column operated under isothermal conditions, Guiochon<sup>17</sup> derived the following equation:

$$\bar{u} = \frac{3}{4} \cdot \frac{K_o}{\eta L} \cdot \frac{(p_i^2 - p_o^2)^2}{p_i^3 - p_o^3} \quad (5)$$

where  $K_0$  is the column permeability,  $\eta$  the dynamic viscosity of the carrier gas,  $L$  the column length and  $p_i$  the inlet and  $p_o$  the outlet pressures. The permeability of a capillary column ( $K_0$ ) can be calculated from the column radius ( $r$ ) by<sup>17</sup>

$$K_0 = r^2/8 \quad (6)$$

Guiochon<sup>17</sup> and Tóth and Garay<sup>11</sup> have shown that the average carrier gas flow-rates, calculated from eqns. 5 and 6, differ considerably from experimentally obtained values. As the overall selectivity of columns coupled in series strongly depends on the carrier gas flow-rates in the individual columns, these values should be known precisely. It was shown that solute retention in two columns in tandem can be predicted accurately if the permeability of the individual columns is determined experimentally instead of by calculation<sup>11,17</sup>.

From eqn. 5, it follows that the average carrier gas flow-rate increases with increase in the driving force ( $P$ ) and decrease in the resistance ( $R$ ):

$$\bar{u} = \frac{P}{R} \quad (7)$$

where  $P = (p_i^2 - p_o^2)/(p_i^3 - p_o^3)$  and  $R = 4\eta L/3K_0$ . Substitution of  $\bar{u} = L/t_M$  into eqn. 7 gives

$$R = Pt_M/L \quad (8)$$

which can be substituted into eqns. 2 and 3 for calculation of  $x_A$ :

$$x_A = \frac{R_A L_A}{R_A L_A + (P_A/P_B)R_B L_B} \quad (9)$$

and  $x_B$ :

$$x_B = \frac{R_B L_B}{R_A L_A + (P_A/P_B)R_B L_B} \quad (10)$$

$P_A/P_B$  can be written as

$$\frac{P_A}{P_B} = \frac{(p_i^2 - p_m^2)^2(p_m^3 - p_o^3)}{(p_i^3 - p_m^3)(p_m^2 - p_o^2)^2} \quad (11)$$

Eqn. 8 allows the resistance ( $R_A$  and  $R_B$ ) to be determined from experimental data ( $P$ ,  $t_M$ ,  $L$ ). The values of  $x_A$  and  $x_B$  can be predicted for two columns in tandem at any  $P_A/P_B$  ratio.

## EXPERIMENTAL

### Gas chromatographic instrumentation

The gas chromatographic system consisted of two independently controlled

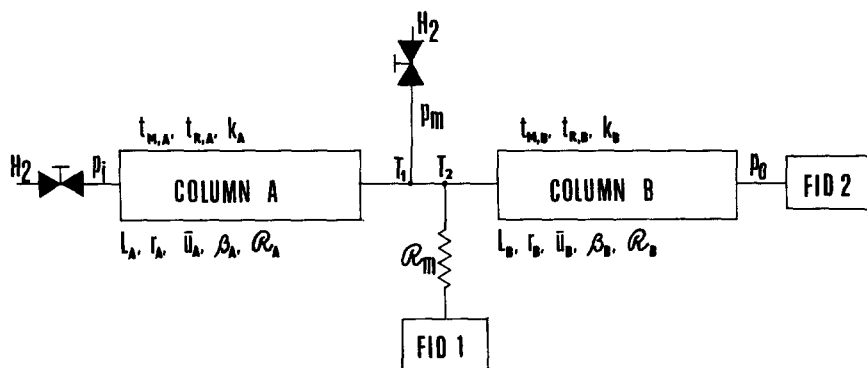


Fig. 1. Experimental set-up for a two column system with mid-point pressure tuning.

ovens (Fractovap 2350 and Fractovap 4180; Carlo Erba, Milan, Italy) interfaced with a separately heated stainless-steel tube as described by Laub and Purnell<sup>12</sup>. The basic scheme of the column series polar column A (Nukol)–non-polar column B (PONA) in the order AB is shown in Fig. 1. In this work both columns were placed in the Fractovap 4180 GC oven as the oven temperature control is better than that with the Fractovap 2350 GC oven.

A sample of hydrocarbons was injected into the first column by using an all-glass inlet stream splitter port mounted in the Fractovap 2350 instrument. Two flame ionization detectors were used to monitor the solutes eluted from the first (FID 1 with the Fractovap 4180) and second (FID 2 with the Fractovap 2350) columns. The list of sample constituents with their capacity factors on individual columns is given in Table I. The inlet ( $p_i$ ) and column coupling-point ( $p_m$ ) pressures were controlled by a pressure controller with the Fractovap 2350 and Fractovap 4180 instruments, respectively.

The signals from FID 1 and FID 2 were recorded by an HP 3392 integrator (Hewlett-Packard, Palo Alto, CA, U.S.A.) and by a Chromatopac CR-3A computing integrator (Shimadzu, Kyoto, Japan), respectively. Two T press-fit connectors,  $T_1$  and  $T_2$  (MEGA, Milan, Italy), were used for coupling the columns with the pressure controller  $p_m$  and FID 1.

**Capillary columns.** Column A was a 60 m  $\times$  0.25 mm I.D. fused-silica capillary column coated with a 0.25- $\mu$ m film of Nukol stationary phase (Supelco, Bellefonte, PA, U.S.A.). Column B was a 50 m  $\times$  0.20 mm I.D. fused-silica capillary column coated with a 0.5- $\mu$ m film of immobilized polydimethylsiloxane (PONA) (Hewlett-Packard).

### Operating conditions

Hydrogen was used as the carrier gas with constant inlet ( $p_i = 320$  kPa) and outlet ( $p_o \approx 101$  kPa) pressures for the column orders AB and BA. The carrier gas flow-rates through the individual columns were changed by varying the intermediate pressure (for AB series from  $p_m = 250$  to 290 kPa and for BA series from  $p_m = 210$  to 250 kPa) in steps of 10 kPa. Both columns were operated isothermally with an arbitrarily chosen temperature  $T_A = T_B = 70^\circ\text{C}$ .

A sample of hydrocarbons (Table I) was diluted with *n*-pentane to 0.03% (v/v) of each compound. A 1- $\mu$ l volume was injected with a 10- $\mu$ l Hamilton syringe.

TABLE I

CAPACITY FACTORS FOUND FOR SAMPLE COMPONENTS AT 70°C ON POLAR ( $k_A$ ) AND NON-POLAR ( $k_B$ ) FUSED-SILICA CAPILLARY COLUMNS BY CORRELATING  $k_S$  AND  $x_B$  ACCORDING TO EQN. 17

Peak No.	Compound	$k_A$	$k_B$
1	2,3,4-Trimethylpentane	0.151	1.816
2	2,2,5-Trimethylhexane	0.138	2.330
3	<i>n</i> -Octane	0.195	2.593
4	2,3,5-Trimethylhexane	0.198	2.986
5	2,4-Dimethylheptane	0.198	3.140
6	4,4-Dimethylheptane	0.223	3.216
7	3,5-Dimethylheptane	0.233	3.497
8	3,3-Dimethylheptane	0.244	3.545
9	2,3-Dimethylheptane	0.279	4.062
10	$\alpha$ -3,4-Dimethylheptane <sup>a</sup>	0.298	4.148
11	$\beta$ -3,4-Dimethylheptane <sup>a</sup>	0.298	4.148
12	3,4-Diethylheptane	0.406	4.752
13	Isopropylbenzene	2.689	6.152
14	<i>n</i> -Nonane	0.369	5.611
15	4,4-Dimethyloctane	0.405	6.629
16	<i>n</i> -Propylbenzene	3.369	7.633
17	2,6-Dimethyloctane	0.423	7.342
18	3,3-Dimethyloctane	0.455	7.417
19	3,4-Diethylhexane	0.501	7.649
20	2-Methyl-3-ethylhexane	0.480	7.761
21	1,3,5-Trimethylbenzene	4.250	8.545
22	1,2,4-Trimethylbenzene	5.395	10.216
23	<i>tert.</i> -Butylcyclohexane	1.058	10.319
24	1,2,3-Trimethylbenzene	7.608	12.372
25	<i>n</i> -Decane	0.709	12.066
26	<i>sec.</i> -Butylcyclohexane	1.414	13.847
27	1,3-Diethylbenzene	5.963	15.368
28	<i>n</i> -Butylbenzene	6.307	16.036
29	<i>n</i> -Butylcyclohexane	1.365	14.679
30	1,4-Diethylbenzene	6.389	16.169
31	1,2-Diethylbenzene	7.226	16.613
32	1,2-Dimethyl-4-ethylbenzene	8.641	18.988
33	<i>n</i> -Undecane	1.377	25.644

<sup>a</sup>  $\alpha$  and  $\beta$  are diastereoisomers.

### Computation

Software for the optimization procedure and also for the window method was written in Turbo Pascal language for a Toshiba T1100 Plus IBM PC/XT-compatible computer.

### RESULTS AND DISCUSSION

The resistances of the individual columns ( $R_A$  and  $R_B$ ) were determined at 70°C by substituting the experimental data in eqn. 8. The results obtained were  $R_A = 8.7 \text{ kPa min m}^{-1}$  ( $\sigma = 0.3 \text{ kPa min m}^{-1}$ ) for column A and  $R_B = 7.8 \text{ kPa min m}^{-1}$  ( $\sigma = 0.6 \text{ kPa min m}^{-1}$ ) for column B.

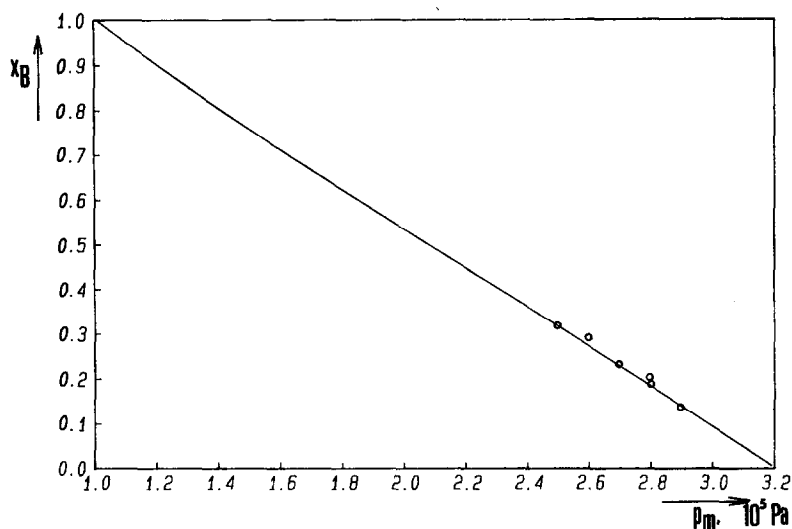


Fig. 2. Dependence of  $x_B$  on  $p_m$ , constructed for the column series AB using data calculated from eqn. 13. The circles correspond to data found for corresponding  $p_m$  values from eqn. 8.

The individual columns were then coupled in AB or BA series and operated at 70°C with constant hydrogen inlet ( $p_i = 320$  kPa) and outlet ( $p_o \approx 101$  kPa) pressures, changing the column coupling-point pressure for series AB from  $p_m = 250$  to 290 kPa and for series BA from  $p_m = 210$  to 250 kPa in steps of 10 kPa.

The dependence of  $x_B$  on  $p_m$ , constructed from the corresponding data calculated with eqn. 8 for the column series AB, is shown in Fig. 2. The circles in Fig. 2 represent values of  $x_B$  found for  $p_m$  from eqn. 3 using gas hold-up times ( $t_{M,A}$  and  $t_{M,B}$ ) measured in the corresponding series using the signals from FID 1 and FID 2. The deviations between the predicted and experimental  $x_B$  values probably arise from the poor accuracy and reproducibility of pressure tuning on the available pressure controllers. Similar results were obtained for the column series BA.

From the results obtained it follows that the changes in  $p_m$  in the chosen experimental ranges lead to the changes in  $x_B$  from 0.15 to 0.35 for column series AB and from 0.40 to 0.60 for column series BA. Deans and Scott<sup>8</sup>, Smuts *et al.*<sup>16</sup>, Kaiser *et al.*<sup>9</sup> and Hinshaw and Ettre<sup>3,4</sup> have shown that, if necessary,  $x_B$  can be varied in a broader range both for AB and BA column series if another experimental arrangement as shown in Fig. 1 is used. It should be noted, however, that the carrier gas flow-rates ( $\bar{u}_A, \bar{u}_B$ ) are usually too far from the optimum values if  $x_B$  is varied in too broad a range.

A separation of sample components can be achieved by tuning the selectivity of a column series if the sample is not too complex. In that case an optimum  $x_B$  can be found for both AB and BA column series. If the sample is very complex, however, both optimum selectivity and maximum separation power are needed. In this event a column order should be chosen that gives the highest separation power at the optimum selectivity.

A combination of eqns. 1 and 4 leads to

$$k_{i,S} = k_{i,A} + x_B(k_{i,B} - k_{i,A}) \quad (12)$$

which shows that the dependence of the overall solute capacity factor in columns in series ( $k_S$ ) theoretically can vary from the value  $k_A$  (for  $x_B = 0$ ) to the value  $k_B$  (for  $x_B = 1$ ) both for column series AB and BA. From eqn. 12, it follows that for the elucidation of the dependence of  $k_S$  on  $x_B$ , capacity factors of the considered solute on the individual columns ( $k_A, k_B$ ) can be applied. If these values are not known, they can be found by linear regression analysis of data ( $k_S$  and  $x_B$ ) found for the solute on column series AB and BA. Fig. 3 shows the dependence of  $k_S$  on  $x_B$  obtained for 2-methyl-3-ethylhexane (peak No. 20). A very good correlation between  $k_S$  and  $x_B$  was obtained for both column series AB and BA, allowing Fig. 4 to be constructed, where the dependences of  $k_S$  on  $x_B$  for all sample components are shown. The plots shown in Fig. 4 were used as a basis for the computer-assisted optimization procedure with the aim of finding an  $x_B$  value (a perpendicular on the  $x_B$  axis) at which the maximum number of peaks will be resolved equally or better than required within the shortest analysis time.

The resolution factors of all peak pairs ( $R_{ji}$ ) for each  $x_B$  value in the range  $x_B = 0$  to  $x_B = 1$  in steps of 0.001 were calculated by the following equation:

$$R_{ji}(x_B) = \frac{t_{Rj,S}(x_B) - t_{Ri,S}(x_B)}{2[\sigma_{j,S}(x_B) + \sigma_{i,S}(x_B)]} \quad (13)$$

where  $t_{RS}(x_B)$  is a retention time and  $\sigma_S(x_B)$  the standard deviation of a peak obtained by separating a solute in the column series with  $p_m$  which corresponds to  $x_B$ .

The retention times [ $t_{RS}(x_B)$ ] needed in eqn. 13 were calculated from the system

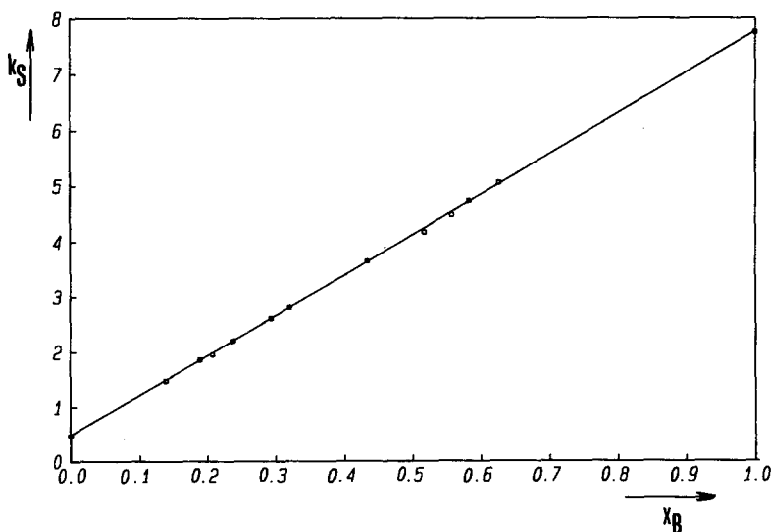


Fig. 3. Dependence of  $k_S$  on  $x_B$  for 2-methyl-3-ethylhexane (peak No. 20).



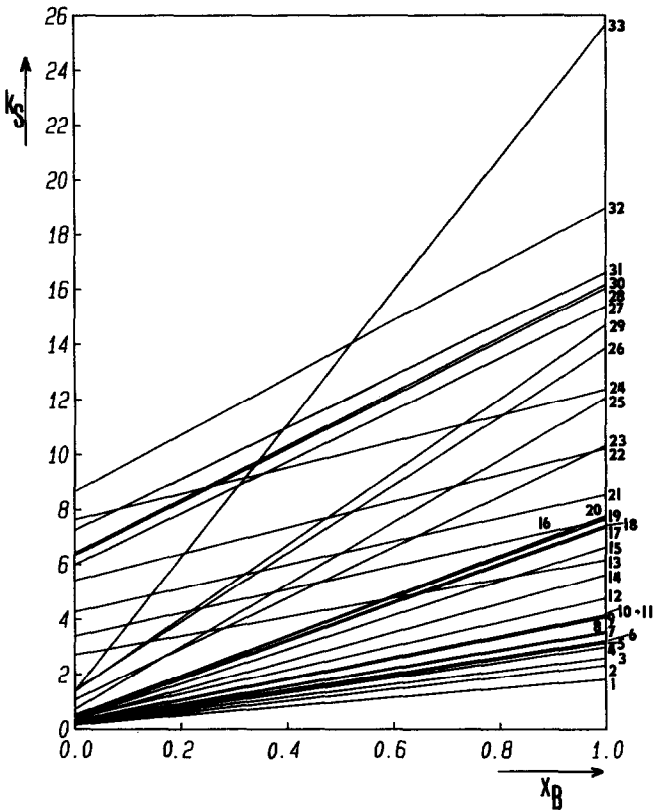


Fig. 4. Dependence of  $k_s$  on  $c_B$  for all sample constituents. For identification see Table I.

capacity factors  $[k_s(x_B)]$  and gas hold-up times  $[t_{MS}(x_B)]$  from the equation

$$t_{Ri,S}(x_B) = t_{M,S}(x_B)[1 + k_{i,S}(x_B)] \quad (14)$$

$t_{M,S}(x_B)$  was calculated as the sum of  $t_{M,A}(x_B)$  and  $t_{M,B}(x_B)$  using eqn. 8.

Correlating the standard deviations ( $\sigma_s$ ) of all peaks obtained from all experiments in a system with the experimental variables ( $p_m, x_B, t_{RS}$ ), we found that the standard deviation of any peak in a chromatogram can be predicted from the following linear equation:

$$\sigma_s = a + bt_{RS} \quad (15)$$

where  $a$  and  $b$  are coefficients which, in the experimental  $p_m$  ranges given above, did not depend on either  $x_B$  or the column order.

From the resolution factors of all peak pairs calculated from eqn. 13 at each  $x_B$  value, an optimization criterion  $C_p$  was determined from an equation already described<sup>14,15</sup>:

$$C_p = \sum^n m_i + (t_{\max} - t_{R,n})/t_{\max} \quad (16)$$

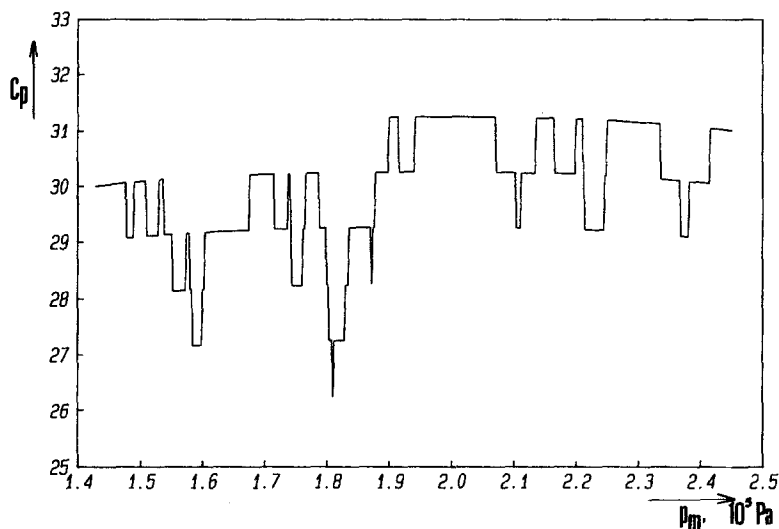


Fig. 5. Dependence of the optimization criterion ( $C_p$ ) on column coupling point pressure ( $p_m$ ) obtained for column series BA.

where  $n$  is the number of solutes in a sample,  $m_i = 1$  if  $R_{ji} > R_{ji,req}$  for  $j = i \pm 1$ , otherwise  $m_i = 0$ ,  $t_{R,n}$  is the retention time of the last-eluting peak and  $t_{max}$  is the maximum acceptable analysis time, which is arbitrarily chosen so that  $t_{max} > t_{R,n}$ . In this work  $R_{ji,req} = 1.00$  and  $t_{max} = 120$  min were chosen for both column series. The retention time of the last peak was read from the retention times calculated by eqn. 14 for all  $x_B$  values.

As in this work  $p_m$  was an independently variable parameter, for practical reasons  $p_m$  was used instead of  $x_B$  in the final computer-assisted optimization procedures. Moreover, as follows from eqns. 13 and 14 and from Fig. 2, it is very easy to recalculate  $x_B$  values to  $p_m$  values.

The dependence of the optimization criterion ( $C_p$ ) on the column coupling-point pressure is shown in Fig. 5 for column series BA. The dependence of  $C_p$  on  $p_m$  for column series AB looks like a mirror image of Fig. 5, owing to the similar column resistances and efficiencies. From these results it follows that 31 peaks can be resolved equal to or better than  $R_{ji,req}$  at different  $p_m$  values with both AB and BA column orders. The second term on the right-hand side of eqn. 16 helps to localize the global optimum among the local optima, in order to choose a  $p_m$  value such that 31 peaks are sufficiently resolved within the shortest time. A maximum  $C_p$  thus corresponds to an optimum  $p_m$ . Different  $C_p$  maxima are obtained for column orders AB and BA: the maximum  $C_p$  is localized in the  $p_m$  range  $2.20 \cdot 10^5$ – $2.35 \cdot 10^5$  Pa for AB and of  $1.95 \cdot 10^5$ – $2.05 \cdot 10^5$  Pa for BA. Both optima are, however, flat and broad enough to allow the  $p_m$  value to be reset in optimum pressure ranges as the set-up reproducibility of the pressure regulator built in the Fractovap 4180 instrument is  $\pm 2.5 \cdot 10^3$  Pa.

A window diagram<sup>12</sup> could be used as an alternative optimization procedure to localize the optimum  $p_m$  value. The basis of this is the construction of the dependences

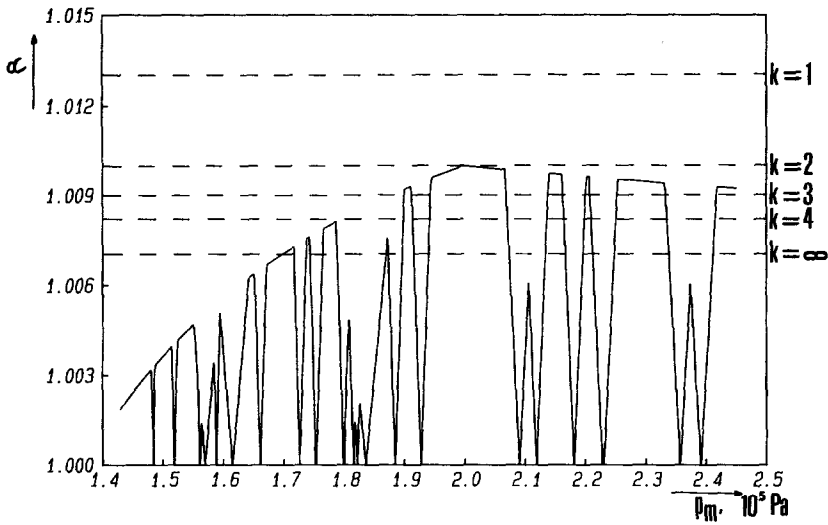


Fig. 6. Dependence of relative retentions ( $\alpha$ ) on column coupling-point pressure ( $p_m$ ) obtained for column series BA (window diagram for BA column series).

of the relative retentions ( $\alpha_{ji}$ ) of all peak pairs against the column coupling-point pressure ( $p_m$ ). Relative retention can be calculated, e.g., from capacity factors ( $k$ ):

$$\alpha_{ji} = k_j/k_i \tag{17}$$

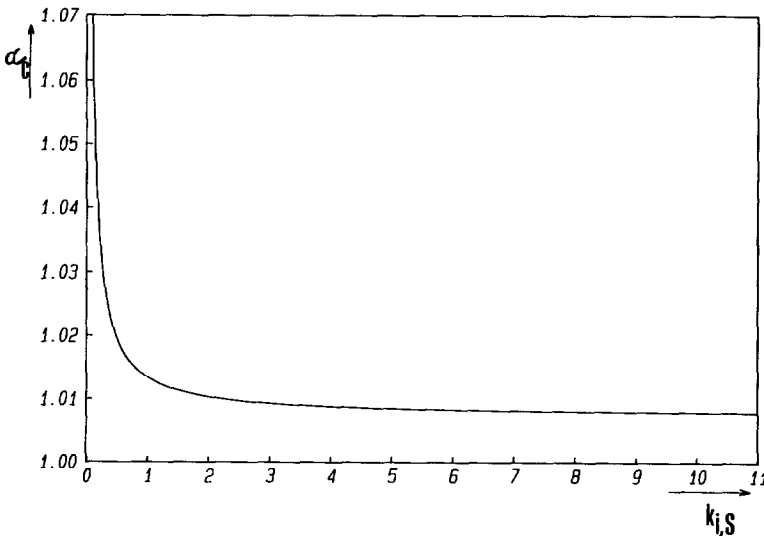


Fig. 7. Relationship between critical relative retention ( $\alpha_c$ ) corresponding to  $R_{j,req} = 1.00$  and capacity factor of the first peak in a considered pair ( $k_{s,i}$ ) for both column series AB and BA.

Hence the window diagram could be drawn from the data taken from Fig. 4 as the dependence of  $\alpha_{ji}$  against  $x_B$ . As  $p_m$  was the optimized parameter, window diagrams were constructed for the dependence of  $\alpha_{ji}$  versus  $p_m$ . The window diagram for column series BA is given in Fig. 6. According to Laub and Purnell<sup>12</sup>, the optimum of  $p_m$  corresponds to the tallest window. At this optimum value of  $p_m$ , the best possible separation of the least separated pairs of compounds is expected<sup>12</sup>. The same optimum intervals of  $p_m$  values were found from window diagrams as were found above with the proposed method both for column order AB ( $2.20 \cdot 10^5$ – $2.35 \cdot 10^5$  Pa) and for column order BA ( $1.95 \cdot 10^5$ – $2.05 \cdot 10^5$  Pa). As the maximum value of  $\alpha$  corresponding to  $p_{m,opt}$  in Fig. 6 is relatively small ( $\alpha < 1.01$ ), from the window diagram it is not possible to deduce if peaks  $j$  and  $i$  with such a relative retention are sufficiently resolved or not, because window diagrams do not contain any information about the separation power. The value of relative retention ( $\alpha_c$ ) which corresponds to the required resolution ( $R_{ji,req}$ ) is not constant and depends on the number of theoretical plates ( $n_i$ ) and the capacity factor of the first peak ( $k_i$ ) according to

$$R_{ji,req} = \frac{\sqrt{n_i}}{4} \cdot \frac{k_i}{k_i + 1} \cdot (\alpha_c - 1) \quad (18)$$

The relationship between the critical relative retention ( $\alpha_c$ ) corresponding to  $R_{ji,req} = 1.00$  and the capacity factor of the first peak in a considered pair ( $k_{i,s}$ ) is the same for both column orders AB and BA and is given in Fig. 7. From Fig. 7 it is evident that for

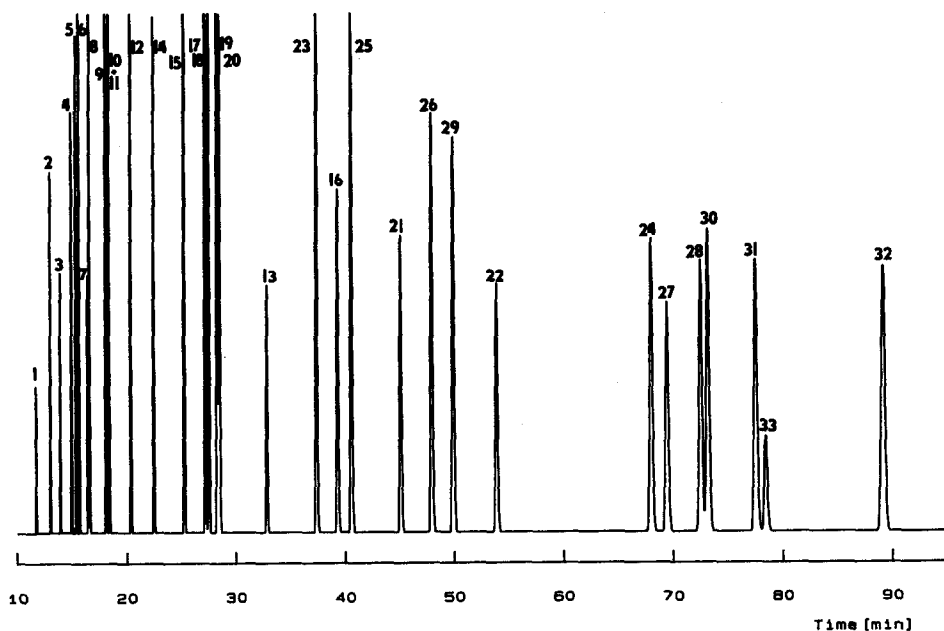


Fig. 8. Computer-simulated separation of the hydrocarbon sample for column series BA at  $p_{m,opt} = 2.0 \cdot 10^5$  kPa. For peak identification see Table I.

the peak pairs eluted at very low capacity factors a relatively high  $\alpha_C$  corresponds to  $R_{j_i, req} = 1.00$  and as we do not know from window diagrams at which capacity factors peak pairs are eluting, we also do not know if peaks are sufficiently resolved or not.

From the dashed lines drawn in Fig. 6 for different capacity factors ( $k$ ) which correspond to  $\alpha_C$ , it can be concluded that the peak pair separated in the window ( $p_m = 1.95 \cdot 10^5 - 2.05 \cdot 10^5$  Pa) will be resolved with  $R_{j_i, req} = 1.00$  only if  $k_i > 2$ . The use of a proper optimization criterion can help to distinguish if the peaks in the windows are sufficiently resolved or not<sup>18</sup>.

Fig. 8 shows the chromatogram of the sample separation simulated by computer for column series BA at  $p_{m, opt} = 2.0 \cdot 10^5$  Pa. All compounds in the sample, except the diastereoisomers, are resolved with  $R_{j_i}$  better than 1.00 within 90 min. For the separation of diastereoisomers lower temperatures are needed<sup>15</sup>.

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