The Selectivity and Polarity of Carbon Layer Open Tubular Capillary Columns Modified with a Polar Liquid Phase

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Summary

The polarity of carbon layer open tubular (CLOT) columns coated with a layer of non-porous graphitized carbon black (Carbopack B) modified with an appropriate amount of polar polyglycol liquid phase has been evaluated and compared with that of standard polar (Supelcowax-10) and non-polar (SPB-1) bonded phase open tubular columns. The efficiency and selectivity were measured at various temperatures and the polarity of the columns was evaluated by use of *McReynolds'* constants and the difference in apparent carbon number, ΔC of linear alkanes and alcohols. The polarity of the CLOT column was found to depend on temperature, and changing the analytical conditions therefore enabled the separation of compounds of different polarity whose reciprocal position and resolution were affected by temperature. The application of calculation methods which enable programmed temperature retention times to be predicted from isothermal data was also found to be possible when the polarity of the CLOT column changes with temperature.

1 Introduction

Graphitized carbon black has previously been suggested as a matrix for gas solid chromatography (GSC) [1–4] and as a support for gas liquid chromatography (GLC) [5] and gas liquid solid chromatography (GLSC). The influence of the degree of graphitization on the adsorption process has been investigated [6,7] and the role of the polarity of the liquid phase, the thickness and uniformity of the coating, and the choice of carbon blacks of different origin have been tested [8–11]. Owing to the advantage in resolution and analysis speed offered by capillary columns, glass capillary columns coated with graphite or carbon black have been prepared by various techniques and their performances evaluated [12–20].

Recently, capillary columns with internal walls coated with a thin layer of graphitized carbon black, Carbopack B, and partially deactivated and modified with an appropriate amount of liquid phase have become commercially available. These columns, designated Carbon Layer Open Tubular or CLOT columns, and were shown to separate compounds by a mixed mechanism of adsorption and partitioning. *Sidisky* and *Robillard* [21] evaluated the performance of these columns by measuring: the reproducibility of carbon layer thickness; the efficiency as a function of the carrier gas velocity; and the polarity by measuring changes in the values of the capacity factor, k, of *n*-dodecane as a function of temperature.

The behavior of CLOT Carbopack B columns modified with a polyglycol/terephthalic acid layer was investigated in previous research [22]. In this work the polarity of the same CLOT columns has been further evaluated by using two polarity indicators: the sum of the *McReynolds'* constants of the first five polarity probes (benzene, *n*-butanol, 2-pentanone, nitropropane, and pyridine) [23] and the difference in apparent carbon number of linear alkanes and alcohols, ΔC [24,25]. The results are compared with those obtained on stand-

ard bonded phase open tubular columns coated with polar polyglycol (Supelcowax-10) and non-polar polydimethylsiloxane (SPB-1) liquid phases.

2 Materials and Methods

The experiments were performed with 30 m × 0.32 mm i.d. fused silica Carbopack B carbon layer open tubular (CLOT), Supelcowax-10, and SPB-1 bonded phase columns (Supelco, Bellefonte, PA, USA). The two bonded phase columns had film thicknesses of 0.25 μm. In the figures the Supelcowax-10 column will be indicated as WAX-10.

The columns were installed in a Varian model 3600 gas chromatograph (Varian Associates, Palo Alto, CA, USA) equipped with a split/splitless injector and a flame ionization detector. Helium was used as carrier gas; its linear velocity under the different analytical conditions was monitored by using the built-in electronic flow-meter and by measuring the dead time on the chromatograms. Isothermal conditions were set with an accuracy of ± 0.3 °. The retention times were measured with an accuracy of ± 0.005 min by using a Varian DS-650 Data System, and verified by using the interactive graphical program of this integrator to check that the retention values automatically calculated corresponded to the maximum of each peak and to measure the asymmetry and the tailing factors of the peaks.

The efficiency and the selectivity of the columns were measured at various temperatures by calculating the number of theoretical plates; the resolution of selected pairs of compounds was calculated with the formula

$$R = 2d/(W_1 + W_2) \qquad (1)$$

where d is the difference between the retention times and W_1 and W_2 are the base widths of two adjacent peaks. The tailing factor TF [26] was calculated by comparing the width of the first half of each peak, a, with that of the second half, b, both measured at 10 % of peak height. The formula

$$TF = 100 \, a/b \qquad (2)$$

gives values ranging from 100 for perfectly symmetrical peaks to zero for infinitely tailing peaks. Its use was preferred to the classical empirical skew ratio

$$n = A/B$$

where A and B are measured at baseline level between the perpendicular drawn from the peak apex and the intercepts between baseline and the tangents at the inflection points of the leading (first eluted) and trailing (last eluted) edges of the peak. The a and b

values measured at 10 % of the peak height are not exactly proportional to the slopes of the trailing and leading edges, as are A and B, but as no simple relationship exists between the skew ratio, n, and the more statistically meaningful skewness, S, i.e. the ratio of the third central moment to the cube of the standard deviation [27], the TF gives an indication sufficiently approximate to the average skewness of the peak, its calculation being independent of statistical considerations or on the drawing of tangents to the peak edges, which is subject to large errors if applied to narrow or very tailing peaks. As the TF value is, furthermore, often used to measure the inertness of the support in packed columns before and after silanization [26] or the interaction with the silica walls in capillary columns, its use is a convenient means of establishing the effect of the mixed gas–solid and gas–liquid interaction of the CLOT columns.

Table 1
The compounds used as standards, their boiling points, b.p., and the average slopes of the lines shown in Figures 1–3.

	Compound	b.p. [°C]	Average slope on:		
			CLOT	SPB-1	Supelco-wax-10
0	n-C_9	150.8	–	2562	–
1	n-C_{10}	174.1	3919	3249	–
2	n-C_{11}	195.9	4390	3885	–
3	Chlorobenzene	132	3820	2148	2386
4	n-C_{12}	216.3	4867	4373	–
5	Bromobenzene	156	4637	2634	3148
6	n-C_{13}	235.4	5314	5059	2973
7	1,3-Dichlorobenzene	173	4618	3120	3552
8	1,4-Dichlorobenzene	174	4716	3120	3659
9	1,2-Dichlorobenzene	180.5	4811	3220	3860
10	n-C_{14}	253.7	5797	–	3463
11	1,3,5-Trichlorobenzene	208	5039	3646	4213
12	n-C_{15}	270.6	6303	–	4064
13	1,2,4-Trichlorobenzene	213.5	5434	3979	4615
14	Nitrobenzene	210.8	5526	3263	4993
15	n-C_{16}	287	6784	–	4714
16	1,2,3-Trichlorobenzene	218–9	5655	4058	4940
17	Naphthalene	218	5559	3913	4993
18	1-Chloro-3-nitrobenzene	235–6	6161	4058	5715
19	2-Chloroaniline	208.8	6416	3564	6095
20	1-Chloro-4-nitrobenzene	242	6299	4128	5872
21	1-Chloro-2-nitrobenzene	246	6401	4256	6101
22	n-C_{18}	316.1	7767	–	5841
23	4-Chloroaniline	232	7204	3979	7046
24	3-Chloroaniline	229.9	7246	3979	7092
25	n-C_{20}	343	8758	–	6764
26	n-C_{22}	368.6	–	–	7798
27	Benzene	80.1			
28	2-Pentanone	154–5			
29	1,4-Dioxane	101			
30	1-Butanol	117.2			
31	Pyridine	115.5			
32	1-Nitropropane	130.5			

The polarity was evaluated by two methods: measurement of McReynolds' constants [23] and the difference between the apparent carbon number of linear alkanes and alcohols, ΔC [24,25]. **Table 1** lists the boiling points of the standard compounds used; benzene, n-butanol, 2-pentanone, 1-nitropropane, and pyridine were used in order to measure the McReynolds' constants and to obtain the average polarity; n-alkanes (C_{11}–C_{22}) and n-alcohols (C_4–C_{13}) were used to measure the retention indices and to calculate the ΔC values. Naphthalene and other aromatic compounds with various substituent groups (halogen, nitro, or amino) were also used as reference standards.

The prediction of the programmed temperature retention times starting from three isothermal values has previously been performed by different methods [28–31] all of which give accurate results when there is a linear relationship between the logarithm of the adjusted retention times or of the capacity factor k (i.e. the ratio between the adjusted retention time and the dead time) and the reciprocal of absolute column temperature. In this instance, owing to the different slopes of the plots for compounds of different polarity, linear interpolation using only three isothermal data points obtained at constant temperature intervals over the whole range tested (60–190 °C) did not enable correct prediction of the programmed temperature retention times. The trapezoid method previously described [31–35] was therefore applied; several values measured in isothermal analyses performed within the range of the desired programmed run were used for integration.

3 Results and Discussion

Figure 1 shows the most peculiar characteristic of the CLOT column modified with a polar liquid phase, i.e. the change in the order of elution of compounds of different polarity when the column temperature changes. Owing to the different slopes, many of the plots for the polar compounds intersect with those for linear alkanes. The change of retention index values with temperature is greater for the CLOT column than for the columns coated with the other
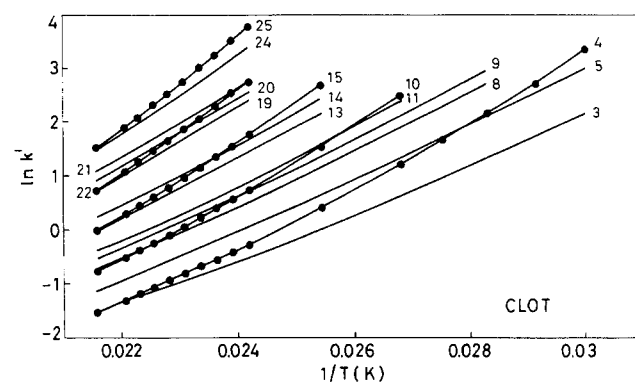

Figure 1
The logarithm of the capacity factor, k, on the CLOT column as a function of the reciprocal of absolute temperature; numbers refer to the compounds listed in Table 1; n-alkanes are also indicated with black dots.

liquid phases, as is shown by the behavior of the same compounds on the Supelcowax-10 and SPB-1 columns (**Figures 2** and **3**). The plots representing the behavior of the CLOT column are more linear than those of the wall coated capillary columns. In order to compare the changes in the slopes of compounds of different polarity, the values of ln k in the central portion of the range tested were interpolated in order to obtain the average slope. Table 1 shows these values as a function of the reciprocal of the absolute temperature averaged within the range 90–160 °C. On Supelcowax-10 the values measured for n-alkanes and other compounds are similar; on SPB-1, however, they differ markedly owing to the greater interaction of the alkanes with the non-polar phase. The CLOT column shows intermediate behavior.

Changes of relative retention and inversion of order of elution are observed for many of the compounds. Complete separation of closely eluting compounds also depends on both column efficiency and peak symmetry. The resolving power of the column for compounds with similar polarities and close retention values is shown
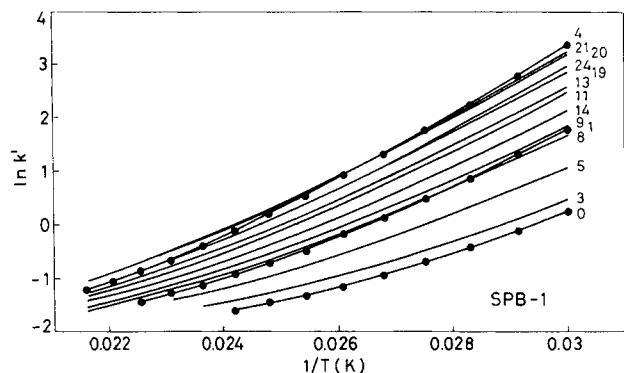
Figure 2
The logarithm of the capacity factor, k, on the non-polar SPB-1 column as a function of the reciprocal of absolute temperature; numbers refer to the compounds listed in Table 1; n-alkanes are also indicated with black dots.
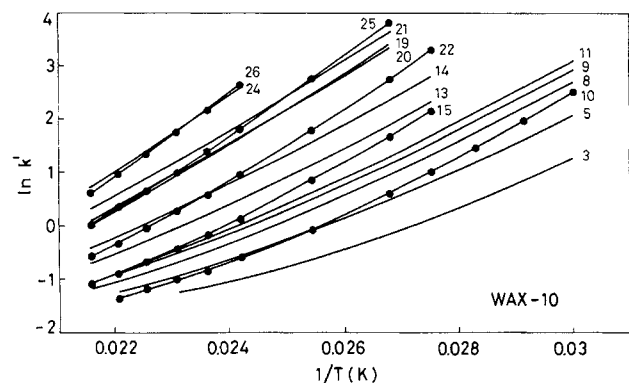

Figure 3
The logarithm of the capacity factor, k, on the polar Supelcowax-10 column as a function of the reciprocal of absolute temperature; numbers refer to the compounds listed in Table 1; n-alkanes are also indicated with black dots.
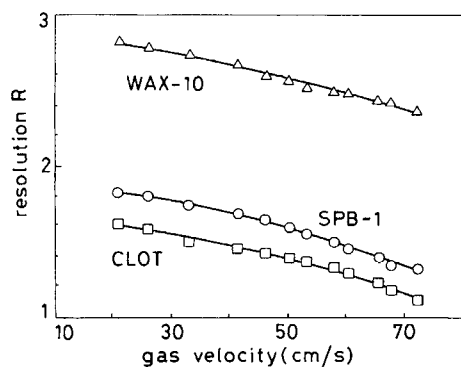

Figure 4
Resolution, R, of naphthalene and 1,2,3-trichlorobenzene as a function of the linear velocity of the carrier gas; column temperature 160 °C.

by the resolution R between the adjacent peaks of naphthalene and 1,2,3-trichlorobenzene (**Figure 4**); the value slowly decreases as the flow rate is increased. The efficiency of the columns, expressed as a plot of $HETP$ as a function of the linear velocity of the carrier gas, is shown in **Figure 5**. The optimum flow rate for the CLOT column is lower than for standard bonded liquid phase columns, and the portion of the curve to the right of the minimum seems to be straighter and of lower slope than those of SPB-1 and Supelcowax-10. This behavior has previously been found to be characteristic of graphitized carbon black columns in which the gas–solid
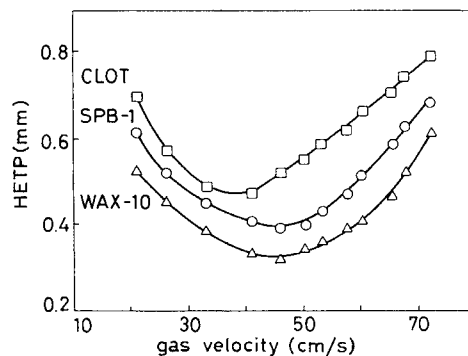

Figure 5
Height equivalent to a theoretical plate ($HETP$) for naphthalene as a function of the linear velocity of the carrier gas; column temperature, 160 °C.

interaction is the prevailing separation mechanism. As the amount of liquid phase in CLOT columns is increased, the gas–solid interactions decrease and behavior similar to that of GLC is enhanced [13,15–17]. The same change from gas–solid to gas–liquid–solid and gas–liquid mechanisms is also observed at high temperature, when the behavior of CLOT column becomes similar to that of Supelcowax-10.

The $HETP_{opt}$ value for the CLOT column (ca 0.47 mm) was higher than that for the Supelcowax-10 column (ca 0.32 mm), despite the latter's being coated with a similar liquid phase. This can be attributed to the greater value of the A term of the Van Deemter equation (eddy diffusion or multiple path contribution) owing to the presence of the carbon layer which substantially increases the roughness and porosity of the inner surface of the column in comparison with those of the uncoated silica capillary. The smaller abscissa of the minimum $HETP$ value, i.e. of the crossing point between the hyperbolic molecular diffusion term (which depends solely on the diffusivity of the solute in the gas phase, i.e. on the density of the carrier gas, because the tortuosity factor can be neglected in open tubular columns) and the linear resistance to mass transfer term, C, is indicative of the greater contribution of the resistance to mass transfer to the $HETP$ value of the CLOT column.

The equation which describes the dependence of the C term on the linear gas velocity includes a term containing the square of the liquid phase film thickness; the diffusivity of the solute in the liquid phase is also important. The distribution of the polyglycol within the carbon layer, rather than as a uniform thin film on the column wall will therefore appreciably influence mass transfer phenomena.

The simultaneous action of the gas–solid and gas–liquid mechanisms also influences the peak shape: axial diffusion, multiple path effect, and non-equilibrium owing to resistance to mass transfer between phases, are responsible for terms in the van Deemter expression which tend to spread a peak virtually symmetrically ($TF \approx 100$), provided that the column contains a large number of plates and the solute is at infinite dilution. For the capillary GLC columns used, the degree of symmetry is reduced by the finite column length and the amount injected; it remains high, however, for non-polar hydrocarbons on both SPB-1 and Supelcowax-10 (**Figure 6**).

When slow desorption occurs from sites of high adsorption energy, as in gas–solid interactions, these slow kinetic processes in addition to the normal rapid equilibration between mobile and stationary phases can produce gross peak spreading and tailing as described by the two-site model of column behavior [36–38], where the solute is distributed between the gas phase and two types of stationary phase 'site'. Normal and predominant sites are the location of the
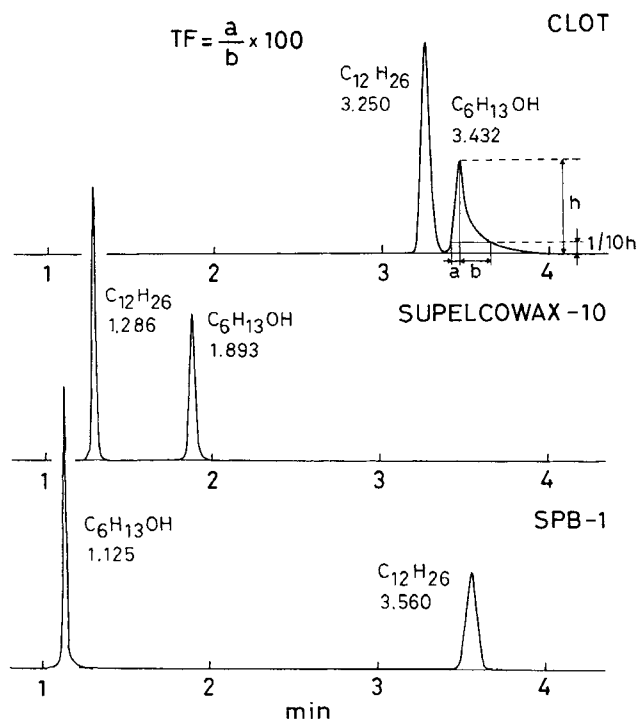
Figure 6
Typical peak shapes for *n*-dodecane and 1-hexanol at 100 °C on the three columns tested; the procedure followed for the determination of the tailing factor, *TF*, is shown.

usual fast exchange of solute between liquid and gas phases in GLC columns and in the liquid modifier of the CLOT columns. When the solute molecules are captured by the second type of site they are held, on average, for a longer time and on desorption, therefore, form a peak with a tail (Figure 6). **Table 2** shows the *TF* values at 60 °C and their variation with temperature, expressed as $\Delta TF/\Delta T$, of an *n*-alkane and a 1-alcohol on the three columns.

Table 2
Tailing Factor at 60 °C, *TF*60, of dodecane and hexanol on CLOT and GLC columns, and average increments of *TF*60 with temperature in the range 60–190 °C.

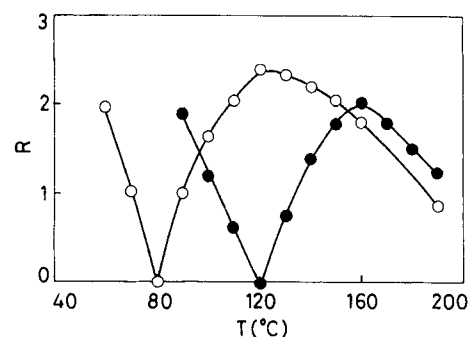|	Dodecane		Hexanol	
	*TF*60	$\Delta TF/\Delta T$	*TF*60	$\Delta TF/\Delta T$
CLOT	86.4	0.364	20.0	–0.103
Supelcowax-10	93.3	0.190	73.5	0.210
SPB-1	87.5	0.014	88.9	0.056

Figure 7
Resolution, *R*, on CLOT column, as a function of the temperature of the column, for pairs of compounds of similar retention time; white circles, *n*-hexanol and *n*-dodecane; black dots, naphthalene and 1,2,3-trichlorobenzene.

The change of polarity of the CLOT column with temperature causes a shifting of peaks of compounds with different polarities; this is shown in **Figure 7** by the resolution between *n*-hexanol and *n*-dodecane and between naphthalene and 1,2,3-trichlorobenzene. The first pair of compounds has the same retention at 80 °C, the second at 120 °C; at these temperatures the resolution is obviously equal to zero. At temperatures lower than the interaction point of the *Arrhenius* plots, (Figure 1) the resolution increases, and the elution order is, respectively, hexanol then dodecane and naphthalene then trichlorobenzene. At higher temperatures, the elution order is reversed. The maximum resolution is obtained at 120 °C for hexanol and dodecane and at 160 °C for naphthalene and trichlorobenzene. This diagram resembles those obtained with the 'window method' of *Laub* and *Purnell* [39] by plotting the absolute value of the ratio between the retention times of every pair of compounds in the sample. In this instance, however, the information obtained from the plot of *R* values is more detailed, because the effective separation of the two compounds is given, whereas classical window diagrams show the difference in retention only and further data are therefore required to establish if a given pair of compounds can be resolved.

For an evaluation of the general behavior of the CLOT column, the comparison with classical non-polar and polar liquid phases should be made by using standard polarity indexes. The change in polarity was evaluated previously in the range 70–154 °C [21] by using the slope of the logarithm of *k* values as a function of the reciprocal of absolute temperature for *n*-dodecane. The same measurements were performed over a wider range of temperature (60–190 °C) at 10° intervals. As pointed out above, the resulting plots show appreciable curvature in the temperature range tested, but the mean slope values increase from Supelcowax-10 to SPB-1 to CLOT column, in accordance with the results published previously.

Table 3
Retention index at 60 °C, *I*60, of five *McReynolds'* probes on CLOT and WCOT columns, and average increments of *I*60 with temperature in the range 60–110 °C.

McReynolds' probe	CLOT		Supelcowax-10		SPB-1	
	*I*60	$\Delta I/\Delta T$	*I*60	$\Delta I/\Delta T$	*I*60	$\Delta I/\Delta T$
Benzene	835.0	0.789	955.5	0.455	642.7	0.109
2-Pentanone	871.5	0.570	990.2	0.340	648.6	0.502
1-Butanol	1000.0	0.292	1147.5	0.542	687.9	0.242
Pyridine	1030.5	1.018	1187.0	0.716	706.2	0.201
1-Nitropropane	1048.8	0.864	1216.4	0.542	724o5	0.275

Other parameters were tested in order to measure the change in polarity. The use of the McReynolds' constants [23] for benzene, n-butanol, 2-pentanone, nitropropane, and pyridine is restricted to a relatively narrow range of temperatures, because above 110 °C the retention times of the peaks are too small to be measured with sufficient precision. In the temperature range 60–110 °C the behavior of I values is linear for all the columns tested. **Table 3** lists the I values measured on the three columns at 60 °C and the temperature coefficients of the retention index, $\Delta I/\Delta T$. The order of elution of the probes is the same for all three columns; the values of I do not depend appreciably on temperature, and the CLOT column shows behavior intermediate between that of SPB-1 and Supelcowax-10. Following McReynolds' method the average polarity can be expressed as the sum of the differences between the I values of these probes on the polar columns and the SPB-1 column used as the non-polar reference. This is not completely correct because the standard ΔI values for the McReynolds' constants are referred to squalane, and the polydimethylsiloxane liquid phase (SPB-1) leads to I values appreciably greater than those measured on squalane. The differences between SPB-1 and the Supelcowax-10 or CLOT column relative to squalane are, therefore, smaller than the theoretical McReynolds' constants but, as the effect of temperature is evaluated here on a relative basis, this difference can be neglected. For the CLOT column the average polarity obtained from the sum of the differences in the range 60–110 °C increases from 1375 to 1512. The values of the summation for Supelcowax-10, which change from 2088 to 2149, show that the average polarity of the polyglycol liquid phase column is about 45 % greater than that of the modified CLOT, but remains fairly constant at different temperatures.

Evaluation of the change in polarity over a greater temperature range is possible by using the difference in apparent carbon number of linear alkanes and alcohols, ΔC [25]; this also enables measure-

ment of the polarity of SPB-1, without the need for any reference liquid phase. For the CLOT column the ΔC value increases linearly from 6.00 to 6.45 in the range 60–190 °C, following the relationship

$$\Delta C = 0.0034\ T_c + 5.790 \tag{3}$$

where T_c [°C] is the column temperature; in the same temperature interval the linear equations for SPB-1 and Supelcowax-10 are, respectively:

$$\Delta C = 0.000084\ T_c + 2.688 \tag{4}$$

and

$$\Delta C = 0.000050\ T_c + 7.512 \tag{5}$$

The $\Delta C/\Delta T$ values of the GLC columns, 8.4×10^{-6} and $5.0 \times 10^{-6}\ K^{-1}$, respectively, are negligible compared with that of the CLOT column $(3.4 \times 10^{-3}\ K^{-1})$. For practical purposes the polarities of the two GLC columns can be regarded as constant over a wide range of temperatures, with ΔC values of 2.70 ± 0.009 for SPB-1 and 7.52 ± 0.011 for Supelcowax-10.

The polarity of the CLOT column probably increases with temperature because the gas–liquid interaction with the polar polyglycol layer becomes more important relative to gas–solid adsorption on the carbon black substrate which, being an exothermic process, prevails at low temperature. The observed change of the order of elution of substances of different polarity enables optimization of the resolution by adjustment of the column temperature. The interpolation of the retention values as a function of the reciprocal of absolute temperature on plots similar to those shown in Figures 1, 2 and 3; the application of the 'window method' [39] to retention values or to capacity factors k; and the use of diagrams of resolution values (e.g. Figure 7) enable prediction of the conditions which give the best resolution in isothermal analysis.

Table 4
Retention times obtained from three different temperature programmed runs.

Compound	$t_R^{e\ d)}$	Program 1 [a)] $t_R^{c\ e)}$	$E\ [\%]$ [f)]	t_R^e	Program 2 [b)] t_R^c	$E\ [\%]$	t_R^e	Program 3 [c)] t_R^c	$E\ [\%]$
n-C$_{10}$	2.526	2.533	0.277	3.844	3.854	0.260	1.516	1.525	0.594
n-C$_{11}$	3.929	3.913	0.407	5.707	5.674	0.578	2.177	2.186	0.413
Chlorobenzene	3.646	3.681	0.961	5.153	5.178	0.485	2.230	2.256	1.166
n-C$_{12}$	5.855	5.835	0.343	7.613	7.552	0.801	3.310	3.319	0.272
Bromobenzene	5.766	5.829	1.094	7.385	7.424	0.528	3.575	3.611	1.007
n-C$_{13}$	8.122	8.085	0.457	9.432	9.332	1.060	5.022	5.029	0.139
1,3-Dichlorobenzene	7.333	7.389	0.765	8.770	8.787	0.193	4.714	4.764	1.061
1,4-Dichlorobenzene	7.914	7.975	0.772	9.246	9.247	0.010	5.172	5.229	1.102
1,2-Dichlorobenzene	8.841	8.897	0.633	9.963	9.963	0	5.984	6.041	0.953
n-C$_{14}$	10.535	10.411	1.177	11.147	10.988	1.426	7.255	7.241	0.193
1,3,5-Trichlorobenzene	9.961	9.983	0.222	10.802	10.761	0.379	6.981	7.046	0.931
n-C$_{15}$	12.951	12.757	1.499	12.769	12.632	1.072	9.814	9.739	0.764
1,2,4-Trichlorobenzene	12.735	12.671	0.504	12.769	12.672	0.759	9.814	9.761	0.540
Nitrobenzene	13.978	13.933	0.323	13.635	13.556	0.579	11.106	11.119	0.117
n-C$_{16}$	15.320	15.117	1.325	14.331	14.153	1.242	12.530	12.391	1.109
1,2,3-Trichlorobenzene	14.594	14.577	0.116	14.064	13.977	0.618	11.782	11.814	0.272
Naphthalene	14.594	14.573	0.145	14.064	14.001	0.447	11.782	11.819	0.314
1-Chloro-3-nitrobenzene	17.919	17.677	1.352	16.251	16.069	1.119	15.566	15.356	1.349
2-Chloroaniline	18.035	17.871	0.909	16.251	16.131	0.738	15.715	15.584	0.834
1-Chloro-4-nitrobenzene	18.766	18.609	0.838	16.811	16.656	0.922	16.579	16.441	0.832
1-Chloro-2-nitrobenzene	19.740	19.595	0.734	17.463	17.306	0.899	17.705	17.601	0.587
n-C$_{18}$	19.780	19.615	0.834	17.274	17.056	1.262	17.924	17.749	0.976
4-Chloroaniline	22.670	22.590	0.354	19.301	19.156	0.751	21.256	21.236	0.094
3-Chloroaniline	22.827	22.710	0.514	19.384	19.244	0.722	21.432	21.399	0.154
n-C$_{20}$	24.068	23.890	0.741	20.052	19.769	1.411	23.099	22.976	0.532

[a)] Programmed from 70 °C at 5 °min^{-1}. [b)] 60 °C for 2 min then programmed at 8 °min^{-1}. [c)] 100 °C for 2 min the programmed at 4 °min^{-1}. [d)] Experimental retention time. [e)] Calculated retention time. [f)] $E\% = 100(t_R^c - t_R^c)/t_R^e$.

The change of polarity with increasing temperature which enables the separation of closely eluting peaks simply by changing the analytical conditions, may be a source of problems in qualitative analysis, as the order of elution of the peaks changes during temperature programming, depending on the choice of the initial temperature, the length of the initial isothermal period, and the programming rate. In order to avoid the use of authentic samples to identify all the peaks whenever the analytical conditions are changed, the prediction of the programmed temperature retention times by means of suitable computer programs is necessary. The application to CLOT chromatography of the methods used previously to predict temperature programmed retention times on standard bonded phase capillary columns [28–31], starting from three isothermal values when the dependence of the logarithm of the adjusted retention times or the ln k values on the reciprocal of absolute column temperature are linear, does not give correct values in this instance, owing to the different slopes obtained for compounds of different polarity. Integration performed using only three isothermal data values obtained at constant temperature intervals over the whole range tested (60–190 °C) must, therefore, be modified.

The trapezoid method [31–35] was therefore applied; several isothermal values measured within the range of the desired programmed run were used for integration. In this instance values at 10° intervals were measured in order to follow the behavior of the column as a function of the temperature, and were therefore used for calculations. The method gives satisfactory results notwithstanding the different slopes observed for compounds of different polarity (Figure 1 and Table 1). **Table 4** shows the results of the prediction of retention times for three different temperature programs, with and without an initial isothermal period. The absolute values of the percent error are also reported and show that the method is sufficiently accurate to enable the correct prediction of elution order and retention times.

4 Conclusion

The polarity of the CLOT column was found to depend on temperature and suitable choice of the isothermal analysis conditions therefore enables the separation of compounds of different polarity because their reciprocal positions and resolution values vary with temperature. During temperature programming the column behaves differently depending on the initial temperature, the length of the initial isothermal period, and the rate of temperature increase. The prediction of programmed temperature retention times by use of computer programs and isothermal data is possible using the procedure proposed and confirms the validity of the calculation method. The application of the suggested prediction program enables selection of the best temperature program for the separation of critical pairs of compounds.

Acknowledgments

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