

CHROM. 17,080

## REVERSED-PHASE LIQUID CHROMATOGRAPHY OF HOMOLOGOUS SERIES

### A GENERAL METHOD FOR PREDICTION OF RETENTION

PAVEL JANDERA

*University of Chemical Technology, Leninovo nám. 565, Pardubice (Czechoslovakia)*

(Received July 13th, 1984)

---

#### SUMMARY

The model of interaction indices is used as the basis for the derivation of equations describing the retention of homologous series in reversed-phase liquid chromatography. Linear relationships between the retention and the mobile phase composition can be used to a first approximation in binary mobile phases containing 40–50% or more of methanol, acetonitrile or 1,4-dioxane in water. For these mobile phases, a general equation was derived that makes possible predictions of capacity factors of various homologues in binary mobile phases with different proportions of organic solvent to water, based on a single experimental capacity factor of one homologue. The meaning of the constants of this equation and their dependence on various factors are discussed.

---

#### INTRODUCTION

The retention mechanism and the possibility of predicting the retention in reversed-phase liquid chromatography have attracted considerable attention, but the subject remains controversial. Various retention mechanisms have been suggested and tested—the models based on molecular connectivity<sup>1–5</sup>, on Hildebrand solubility parameters<sup>6–9</sup>, the molecular statistical theory of Martire and Boehm<sup>10</sup> and the solvophobic theory of Horváth *et al.*<sup>11,12</sup> based on the solvophobic theory of Sinanoglu<sup>13</sup>.

It has been generally accepted that mobile phase interactions are of principal importance in the control of retention in reversed-phase systems, whereas the non-polar stationary phase can be regarded, to first approximation, as a passive solute acceptor. This has led to the derivation of a retention model based on interaction indices, in which the stationary phase interactions are neglected and the interaction indices of appropriate test compounds are used for the calibration of reversed-phase systems<sup>14–16</sup>. However, stationary phase interactions also contribute to retention and influence capacity factors to a certain extent<sup>17–19</sup>. A scale of retention indices, similar to Kováts indices, has been suggested for the calibration of reversed-phase systems<sup>20</sup>,

based either on the alkyl aryl ketones<sup>21,22</sup> or on alkan-2-ones<sup>23</sup> as calibration compounds, but this seems to be of rather limited application.

Homologous series are particularly useful for the investigation of retention mechanisms in reversed-phase systems and potentially attractive for the calibration of retention in such systems, because they allow differentiation between the non-specific contribution to retention, caused by a regular increase in the length of the aliphatic chain, and the specific contribution, caused by the interactions of the molecular residue of the homologous series with the mobile and stationary phase<sup>2,24,25</sup>. A regular linear increase of the log of the capacity factors with increasing number of carbon atoms in a homologous series has been reported for a number of homologous series and reversed-phase systems<sup>2,17,18,20-34</sup>. This is to be expected from the Martin rule of additivities of the molecular increments to retention, and has also been observed with series where the number of structural units other than methylene groups increases regularly, such as ethylene oxide units in phenyloligoethylene glycols<sup>35</sup>, or in oligostyrene series<sup>36</sup>.

Any general model for the prediction of retention should consider the influence of the mobile phase composition. Both from the theory of solubility parameters<sup>6-9,27</sup> and interaction indices<sup>14</sup>, quadratic equations can be derived for description of the dependence of the logarithms of capacity factors on the volume fraction of the organic solvent in reversed-phase chromatography using binary aqueous-organic mobile phases. Simplified linear forms of this dependence are often adequate over a limited range of concentrations of organic solvents in the mobile phase<sup>8,9</sup>, as has been confirmed from a number of experimental data<sup>1,8,9,11,27,33,34,37,38</sup>. However, this dependence is non-linear over the full composition range of the mobile phase, and even quadratic equations cannot fully describe the retention in mobile phases containing organic solvents in low concentrations (less than 5-10%)<sup>39,40</sup>.

Most studies of the behaviour of homologous series reported so far have either investigated either a single homologous series on a given column in mobile phases of one or a few compositions only, or dealt with rather simple homologous series containing a single small functional group as the molecular residue (*e.g.* OH or Cl). On the basis of the results with the homologous series of N-alkylphthalimides in methanol-water mobile phases, Dufek<sup>33</sup> suggested a method for calculating the capacity factors of any member of the homologues series at any methanol concentration in the mobile phase, based on the experimental values of four capacity factors,  $k'$ , of two homologues in mobile phases of two different compositions. Unfortunately, his method is based on the assumption of a linear dependence of  $\log k'$  on the logarithm of the concentration of methanol in the mobile phase, in contrast with generally published and theoretically derived semilogarithmic relationships; thus general validity of this approach is questionable.

The purpose of the present work is to compare the behaviour of several homologous series in mobile phases containing various organic solvents and on different alkylsilica bonded phases, to get a better understanding of general rules of retention behaviour and thus to suggest a simple general equation for the calculation of the retention of individual homologues in mobile phases of different compositions. Homologous series containing a UV-absorbing or fluorescent chromophore in the molecule, which are often prepared by pre-column derivatization of aliphatic compounds so as to improve detection, are used in these investigations. These homolo-

gous series are usually chromatographed in organic solvent-rich mobile phases, containing more than 40–50% of the organic solvent.

#### THEORETICAL

The retention model for reversed-phase liquid chromatography based on interaction indices<sup>14</sup> can be readily applied to the separation of the members of a homologous series. According to this model, the capacity factor  $k'$  may be calculated from the Gibbs' free energy  $\Delta G$  that is proportional to the molar volume of the sample solute  $V_x$  and to the difference of the products of the interaction indices (the interaction index of the mobile phase is  $I_M$  and that of the sample solute is  $I_x$ ).

$$\log k' = \log \varphi - \frac{\Delta G}{2.3 RT} = \log \varphi + \frac{V_x}{2.3 RT} (c_M^2 I_M^2 - c_M c_x I_M I_x) \quad (1)$$

where  $\varphi$  is the phase ratio  $V_S/V_M$  (the ratio of the volume of the stationary,  $V_S$  and the mobile,  $V_M$ , phases in the column),  $R$  is the gas constant,  $T$  is the temperature (in kelvins) and  $c_M$  and  $c_x$  are proportionality constants relating to the mobile phase and to the solute, respectively. In a mobile phase composed of water (interaction index  $I_{H_2O}$ ) and one or more organic solvents in concentrations  $x_i$  (interaction indices  $I_{org,i}$ ), the interaction index of the mobile phase  $I_M$  is given as:

$$I_M = \left(1 - \sum_i x_i\right) I_{H_2O} + \sum_i x_i I_{org,i} \quad (2)$$

In a homologous series, both the molar volumes  $V_x$  and the interaction indices  $I_x$  increase with increasing number of carbon atoms in the aliphatic saturated straight chain,  $n_c$ :

$$V_x = V_{0x} + \Delta V_x n_c \quad (3)$$

$$I_x = I_{0x} + \Delta I_x n_c \quad (4)$$

The combination of the eqns. 1, 3 and 4 yields the following expression for the capacity factors of the members of a homologous series:

$$\begin{aligned} \log k' &= \log \varphi + \frac{V_{0x} c_M}{2.3 RT} (c_M I_M^2 - c_x I_M I_{0x}) + \frac{\Delta V_x c_M}{2.3 RT} (c_M I_M^2 - c_x I_M I_{0x}) n_c \\ &\quad - \frac{V_{0x} c_M c_x}{2.3 RT} I_M \Delta I_x n_c + \frac{\Delta V_x c_M c_x}{2.3 RT} I_M \Delta I_x n_c^2 = \\ &= \log \beta + (\log \alpha) n_c + (\log \gamma) n_c^2 \end{aligned} \quad (5)$$

This is a quadratic equation with respect to  $n_c$ .

Because of the low values of the product of the increments  $\Delta V_x \Delta I_x$ ,  $\log \gamma$  is generally low enough so that the quadratic term may be neglected in most practical

systems and the relationships between  $\log k'$  and  $n_c$  are linear:

$$\log k' = \log \beta + (\log \alpha)n_c \quad (5a)$$

As we have reported elsewhere<sup>18</sup>, the relationships between the product  $V_x I_x$  and  $n_c$  are generally linear, which means that the product  $\Delta I_x \Delta V_x$  can be neglected, because:

$$V_x I_x = V_{0x} I_{0x} + (I_{0x} \Delta V_x + V_{0x} \Delta I_x) n_c + \Delta I_x \Delta V_x n_c^2 \quad (5b)$$

However, it is possible that some deviations of the experimental  $\log k' = f(n_c)$  plots from the linear relationships are caused by the contribution of the product  $\Delta I_x \Delta V_x$  and of the term  $\log \gamma$ .

If we consider a binary mobile phase composed of water and one organic solvent with the interaction index  $I_{org}$ , eqn. 5 can be rewritten in the following form (neglecting the term  $\log \gamma$ ):

$$\log k' = \beta_0 - \beta_1 x + \beta_2 x^2 + (\alpha_0 - \alpha_1 x + \alpha_2 x^2) n_c \quad (6)$$

where

$$\beta_0 = \log \varphi + \frac{V_{0x} c_M I_{H_2O}}{2.3 RT} \cdot (c_M I_{H_2O} - c_x I_{0x}) \quad (6a)$$

$$\beta_1 = \frac{V_{0x} c_M}{2.3 RT} (2c_M I_{H_2O} - c_x I_{0x}) (I_{H_2O} - I_{org}) \quad (6b)$$

$$\beta_2 = \frac{V_{0x} c_M^2}{2.3 RT} (I_{H_2O} - I_{org})^2 \quad (6c)$$

$$\alpha_0 = \frac{c_M I_{H_2O}}{2.3 RT} [(c_M I_{H_2O} - c_x I_{0x}) \Delta V_x - c_x \Delta I_x V_{0x}] \quad (6d)$$

$$\alpha_1 = \frac{c_M (I_{H_2O} - I_{org})}{2.3 RT} [(2c_M I_{H_2O} - c_x I_{0x}) \Delta V_x - c_x \Delta I_x V_{0x}] \quad (6e)$$

$$\alpha_2 = \frac{c_M^2 \Delta V_x}{2.3 RT} (I_{H_2O} - I_{org})^2 \quad (6f)$$

The quadratic form of the eqn. 6 with respect to  $x$  is in agreement with the conclusions of both the interaction indices<sup>14</sup> and solubility parameters<sup>6-9</sup> theories that predict the following form of the retention-mobile phase composition equation:

$$\log k' = a - mx + dx^2 \quad (7)$$

From a comparison of the eqns. 5-7 it follows that:

$$\log \beta = \beta_0 - \beta_1 x + \beta_2 x^2; \log \alpha = \alpha_0 - \alpha_1 x + \alpha_2 x^2 \quad (8a,b)$$

$$a = a_0 + a_1 n_c; m = m_0 + m_1 n_c; d = d_0 + d_1 n_c \quad (9a-c)$$

and

$$a_0 = \beta_0; a_1 = \alpha_0; m_0 = \beta_1; m_1 = \alpha_1; d_0 = \beta_2; d_1 = \alpha_2 \quad (10a-f)$$

It has been shown that in many reversed-phase systems the quadratic term can be neglected over the limited composition range of the mobile phase useful for practical separations. Here,  $d_0 = \beta_2 \approx 0$  and  $d_1 = \alpha_2 \approx 0$ , and eqn. 6 simplifies to:

$$\begin{aligned} \log k' &\cong a - mx = \beta_0 - \beta_1 x + (\alpha_0 - \alpha_1 x)n_c = \\ &= a_0 + a_1 n_c - (m_0 + m_1 n_c)x \end{aligned} \quad (11)$$

After elimination of  $n_c$  from the eqns. 9a and b, we obtain:

$$m = m_0 - m_1 \cdot \frac{a_0}{a_1} + \frac{m_1}{a_1} \cdot a = \beta_1 - \beta_0 \cdot \frac{\alpha_1}{\alpha_0} + \frac{\alpha_1}{\alpha_0} \cdot a = q + p \cdot a \quad (12)$$

After combination with eqns. 6a, b, d and e, the equations for  $p$  and  $q$  are derived as follows:

$$p = \left(1 - \frac{I_{\text{org}}}{I_{\text{H}_2\text{O}}}\right) \left[ 1 + \frac{1}{1 - \frac{c_x(I_{0x}\Delta V_x + \Delta I_x V_{0x})}{c_M I_{\text{H}_2\text{O}} \Delta V_x}} \right] \quad (13)$$

$$\begin{aligned} q &= \frac{c_M V_{0x}}{2.3 RT} [(2c_M I_{\text{H}_2\text{O}} - c_x I_{0x})(I_{\text{H}_2\text{O}} - I_{\text{org}}) \\ &\quad - p I_{\text{H}_2\text{O}}(c_M I_{\text{H}_2\text{O}} - c_x I_{0x})] - p \log \varphi \end{aligned} \quad (14)$$

It can be seen that as far as the increments  $\Delta V_x$  and  $\Delta(I_x V_x) = I_{0x}\Delta V_x + \Delta I_x V_{0x}$  are approximately constant for various homologous series, the value of  $p$  should also be constant for various homologous series in mobile phases containing water and a given organic solvent and should not be much influenced by the alkyl-silica column used. On the other hand,  $q$  is expected to vary to a certain extent with the type of the homologous series and to depend on the column.

If we introduce eqn. 12 into eqn. 11, we obtain the following relationships for  $\log k'$  (assuming a linear dependence of  $\log k'$  on  $x$ ):

$$\log k' = (a_0 + a_1 n_c)(1 - px) - qx \quad (15)$$

or:

$$\log k' = (m_0 + m_1 n_c) \left( \frac{1}{p} - x \right) - \frac{q}{p} \quad (16)$$

It has been observed that plots of  $\log k'$  vs.  $n_c$  measured in binary mobile phases containing various proportions of organic solvent and water show a common intersection point. We have reported previously that this effect can be predicted using the model of interaction indices. The common intersection point originates in linear plots of  $\log k'$  vs.  $x$ . In the terminology of the present work, the following equations can be derived for the coordinates  $n_c^*$  and  $\log k^*$  of the intersection point:

$$\log k^* = \beta_0 - \frac{\alpha_0 \beta_1}{\alpha_1} = - \frac{q}{p} \quad (17)$$

and

$$n_c^* = \left( \frac{c_x \Delta I_x}{2c_M I_{H_2O} - c_x I_{Ox}} - \frac{\Delta V_x}{V_{Ox}} \right)^{-1} \quad (18)$$

Hence,  $n_c^*$  should not depend significantly on the mobile phase used, but rather on the type of the homologous series, whereas  $\log k^*$  can be expected to vary more significantly with the type of the organic solvent used in the mobile phase than with the type of the homologous series.

## EXPERIMENTAL

The equipment used included a Model 6000A pump, an U6K injector and a Model 440 UV detector operated at 254 nm; a differential refractometer R-401 was used for measuring column dead volumes (all from Waters Assoc., Milford, MA, U.S.A.). Stainless steel columns were all packed in the laboratory using a high-pressure slurry-packing technique with octadecylsilica and octylsilica materials: (1) 300 × 4.2 mm I.D. freshly packed with Silasorb C<sub>18</sub>, 10 μm (Lachema, Brno, Czechoslovakia); (2) 300 × 4.6 mm I.D. packed with the same material but after two years of periodical use; (3) 300 × 4.2 mm I.D. packed with LiChrosorb RP C<sub>18</sub>/Si 100, 10 μm (Merck, Darmstadt, F.R.G.); (4) 300 × 3.8 mm I.D. packed with LiChrosorb RP C<sub>18</sub>/Si 60, 10 μm (Merck); (5) 150 × 4.2 mm I.D. packed with Hypersil C<sub>18</sub>, 5 μm (Shandon, Sewickley, U.S.A.); (6) 300 × 3.8 mm I.D. packed with Silasorb C<sub>8</sub>, 7.5 μm (Lachema). Three other commercial columns were used: (7) 300 × 3.9 mm I.D., stainless steel, packed with μBondapak C<sub>18</sub>, (Waters); (8) 250 × 4.0 mm I.D., stainless steel, packed with LiChrosorb RP C<sub>8</sub>, 7 μm (Merck); (9) a glass cartridge column, 150 × 3.9 mm I.D., packed with Separon Six C<sub>18</sub>, 5 μm (Laboratory Instruments, Prague, Czechoslovakia). The column dead volumes,  $V_M$ , were determined as the retention volumes of <sup>2</sup>H<sub>2</sub>O measured with the aid of a differential refractometer.

The mobile phases were prepared by mixing water (double distilled in glass

with addition of potassium permanganate) with the organic solvents in the required volume ratios. Methanol, acetonitrile, 1,4-dioxane and tetrahydrofuran were all spectroscopic or pro-analysis grade (Lachema).

The homologous series of (1) 3,5-dinitrobenzoates of saturated *n*-alcohols ( $C_1$ – $C_6$ ), (2) *p*-bromophenacyl esters of saturated lower carboxylic acids ( $C_2$ – $C_8$ ), (3) 1,2-naphthoylenebenzimidazole-6-sulphonamides of aliphatic *n*-amines ( $C_1$ – $C_8$ ) were synthesised in the laboratory and purified by recrystallization; (4) homologous *n*-alkylbenzenes ( $C_1$ – $C_{12}$ ) were purchased or obtained as a gift from Dr. H. Colin, École Polytechnique, Palaiseau, France.

The capacity factors were calculated as  $k' = V_R/V_M - 1$  from the arithmetic means of two or three experimental retention volumes  $V_R$  in various mobile phases ( $V_M$  = column dead volume).

## RESULTS AND DISCUSSION

### *Comparison of retention and selectivity for various columns*

In order to compare the retention and selectivity of various columns for the separation of homologous series, the retentions of homologous 3,5-dinitrobenzoates of saturated *n*-alcohols and of homologous *p*-bromophenacyl esters of lower aliphatic carboxylic acids were measured in 70% (v/v) methanol in water. The plots of  $\log k'$  vs.  $n_c$  were fairly linear (correlation coefficients 0.9995 – 0.9999) and therefore the quadratic term ( $\log \gamma$ ) in eqn. 5 could be neglected. The values of  $\log \alpha$  and  $\log \beta$  in eqn. 5 were determined from the linear regression of the experimental  $\log k' = f(n_c)$  plots and are given in Table I. The term  $\alpha$  represents the retention ratio between the two neighbouring members of a homologous series. The values of  $\log \alpha$  are slightly lower for the *p*-bromophenacyl esters than for the homologous series of 3,5-dinitrobenzoates on all the columns tested. The term  $\beta$ , which is a measure of specific selectivity in a homologous series, has the meaning of  $k'$  of the residue of the molecule ( $n_c = 0$ ) and is also lower for *p*-bromophenacyl esters than for 3,5-dinitrobenzoates. The values of  $\log \alpha$  for a given homologous series are within  $\pm 3\%$  on various materials (LiChrosorb, Hypersil, Silasorb, Separon) of equal lengths of the bonded alkyl chains, with the exception of  $\mu$ Bondapak  $C_{18}$ , and are lower on octylsilica than on octadecylsilica columns. Values of  $\log \alpha$  for the 2-year old Silasorb  $C_{18}$  column were ca. 5% lower than those for the freshly packed column. The  $\mu$ Bondapak  $C_{18}$  column yielded  $\log \alpha$  values between those of the  $C_{18}$  and  $C_8$  columns. As expected, the relative differences between the  $\log \beta$  values determined on various columns are larger than those between the  $\log \alpha$  values.

These results suggest that, to the first approximation, an approximately equal non-specific selectivity in a given homologous series can be expected on various columns of the same type (same bonded alkyls).

### *The dependence of retention on $n_c$ and on the composition of the mobile phase: the validity of eqn. 11 in organic solvent-rich mobile phases*

In a variety of mobile phases tested, containing methanol–water, acetonitrile–water, 1,4-dioxane–water and tetrahydrofuran–water in different proportions, fairly linear plots of  $\log k'$  vs.  $n_c$  were found experimentally (correlation coefficients usually better than 0.9995). Fig. 1 illustrates a few examples of such plots

TABLE I

THE INFLUENCE OF THE COLUMN PACKING MATERIAL ON SELECTIVITY AND RETENTION IN REVERSED-PHASE CHROMATOGRAPHY OF HOMOLOGOUS 3,5-DINITROBENZOATES OF ALIPHATIC *n*-ALCOHOLS (I) AND OF HOMOLOGOUS *p*-BROMOPHENACYL ESTERS OF LOWER ALIPHATIC CARBOXYLIC ACIDS (II)

Mobile phase: 70% (v/v) methanol in water.

Column	Homologous series			
	I		II	
	$\log \beta$	$\log \alpha$	$\log \beta$	$\log \alpha$
Silasorb C <sub>18</sub> , 10 $\mu$ m, 300 $\times$ 4.2 mm I.D. freshly packed	-0.230 $\pm 0.006$	0.229 $\pm 0.002$	-0.234 $\pm 0.014$	0.216 $\pm 0.003$
Silasorb C <sub>18</sub> , 10 $\mu$ m, 300 $\times$ 4.6 mm I.D. after two years of use	-0.227 $\pm 0.011$	0.207 $\pm 0.003$	-0.257 $\pm 0.014$	0.196 $\pm 0.003$
LiChrosorb RP C <sub>18</sub> /Si 100, 10 $\mu$ m, 300 $\times$ 4.2 mm I.D. freshly packed	-0.171 $\pm 0.009$	0.215 $\pm 0.002$	-0.210 $\pm 0.014$	0.208 $\pm 0.003$
LiChrosorb RP C <sub>18</sub> /Si 60, 10 $\mu$ m, 300 $\times$ 3.8 mm I.D. freshly packed	-0.200 $\pm 0.008$	0.218 $\pm 0.002$	-0.217 $\pm 0.014$	0.209 $\pm 0.003$
Hypersil C <sub>18</sub> , 5 $\mu$ m, 150 $\times$ 4.2 mm I.D. freshly packed	-0.250 $\pm 0.006$	0.224 $\pm 0.002$	-0.283 $\pm 0.014$	0.214 $\pm 0.003$
Separon Six C <sub>18</sub> , 5 $\mu$ m, 150 $\times$ 3.9 mm I.D. commercial glass column	-0.161 $\pm 0.010$	0.208 $\pm 0.002$	-0.167 $\pm 0.014$	0.200 $\pm 0.004$
$\mu$ Bondapak C <sub>18</sub> , 300 $\times$ 3.9 mm I.D. commercial stainless-steel column	-0.412 $\pm 0.006$	0.195 $\pm 0.002$	-0.429 $\pm 0.017$	0.182 $\pm 0.004$
Silasorb C <sub>8</sub> , 7.5 $\mu$ m, 300 $\times$ 3.8 mm I.D. freshly packed	-0.435 $\pm 0.005$	0.170 $\pm 0.001$	-0.395 $\pm 0.012$	0.162 $\pm 0.003$
LiChrosorb RP C <sub>8</sub> , 7 $\mu$ m, 250 $\times$ 4.0 mm I.D. commercial stainless-steel column	-0.419 $\pm 0.008$	0.177 $\pm 0.002$	-0.384 $\pm 0.014$	0.167 $\pm 0.003$

measured at different compositions of 1,4-dioxane-water on the column packed with Silasorb C<sub>18</sub>. Analogous plots were found for other homologous series, columns and mobile phases tested, in agreement with a number of linear  $\log k'$  vs.  $n_c$  plots reported earlier. Table II lists the constants  $\log \beta$  and  $\log \alpha$  of eqn. 5a, determined using linear regression analysis of the experimental  $\log k'$  vs.  $n_c$  plots for two homologous series and various binary mobile phases; similar results were obtained for other combinations of homologous series, columns and mobile phases.



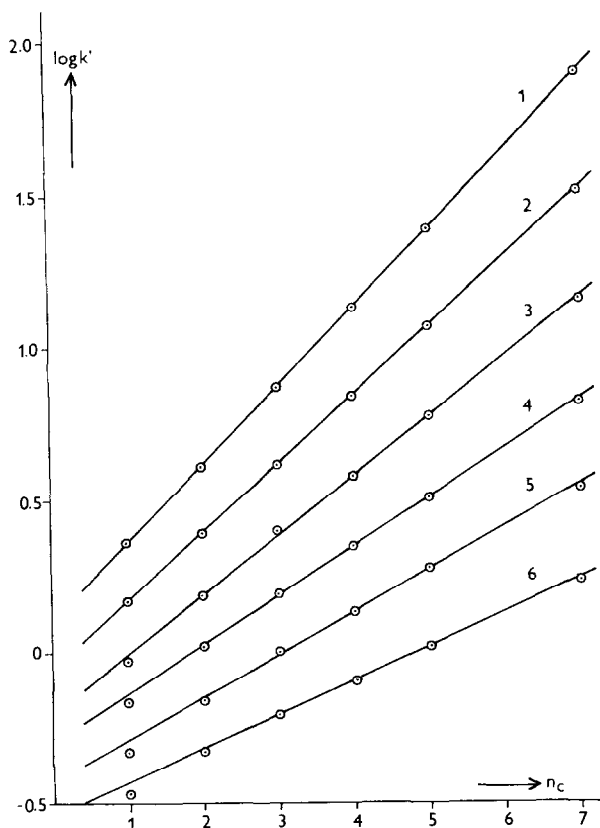


Fig. 1. Plots of logarithms of capacity factors  $k'$  of *p*-bromophenacyl esters of lower aliphatic carboxylic acids in dependence on the number of carbon atoms in the aliphatic chains of the esters,  $n_c$ , on Silasorb  $C_{18}$  ( $10\ \mu\text{m}$ ) in 1,4-dioxane–water mobile phases containing 50% (1), 55% (2), 60% (3), 65% (4), 70% (5) and 75% (6) 1,4-dioxane (v/v).

The experimental  $\log k'$  vs.  $x$  plots are approximately linear for various homologous series tested in the mobile phases composed of methanol and water, whereas slight deviations from linearity occur in 1,4-dioxane–water and in acetonitrile–water, and the plots are distinctly curved in tetrahydrofuran–water mobile phases (*e.g.* Figs. 2 and 3). The constants  $a$  and  $m$  of eqn. 11 determined by linear regression analysis of the experimental  $\log k'$  vs.  $x$  plots for two homologous series on Silasorb  $C_{18}$  column in binary mobile phases containing four different organic solvents are listed in Table III. Analogous results were obtained for other homologous series and columns tested.

According to eqns. 9a and b, the constants  $a$  and  $m$  of the  $\log k'$  vs.  $x$  relationships should increase in a linear manner with increasing  $n_c$  in a given homologous series. This was confirmed experimentally for all the mobile phases and homologous series tested (correlation coefficients were better than 0.999 for  $a$  vs.  $n_c$  relationships, and 0.991–0.999 for  $m$  vs.  $n_c$  relationships). The constants  $a_0$ ,  $a_1$  and  $m_0$ ,  $m_1$  in eqns. 9a and b determined by linear regression analysis of  $a$  vs.  $n_c$  and  $m$  vs.  $n_c$  plots are

TABLE II

VALUES OF CONSTANTS  $\log \beta$  AND  $\log \alpha$  OF EQN. 5a

Determined by linear regression of experimental  $\log k'$  vs.  $n_c$  plots and constants  $\beta_0$ ,  $\beta_1$  and  $\alpha_0$ ,  $\alpha_1$  of  $\log \beta$  vs.  $x$  and  $\log \alpha$  vs.  $x$  plots for (A) 1,2-naphthoylenebenzimidazole-6-sulphonamides of aliphatic  $n$ -amines and (B) 3,5-dinitrobenzoates of aliphatic  $n$ -alcohols on a Silasorb C<sub>18</sub> column, 10  $\mu$ m, 300  $\times$  4.2 mm I.D., in various binary mobile phases;  $x$  = %vol. of the organic solvent in the mobile phase  $\times 10^{-2}$ ;  $R$  = correlation coefficient.

Mobile phase composition	$\log \beta$	$\log \alpha$	$R$	$\beta_0$	$\beta_1$	$R$	$\alpha_0$	$\alpha_1$	$R$
<i>Homologous series A</i>									
Methanol-water									
75:25	-0.0195	0.1523	0.9999						
80:20	-0.1627	0.1291	0.9997	2.170	2.918	0.99998	0.467	0.420	0.9993
90:10	-0.4566	0.0888	0.9994						
Dioxane-water									
50:50	-0.1426	0.2271	0.9995						
55:45	-0.3597	0.1995	0.9999						
60:40	-0.5279	0.1687	0.9999	1.426	3.213	0.9955	0.463	0.481	0.9953
65:35	-0.6711	0.1460	0.99999						
70:30	-0.8415	0.1303	0.9999						
75:25	-0.9494	0.1048	0.9997						
Acetonitrile-water									
70:30	-0.2767	0.1378	0.9995						
75:25	-0.3322	0.1239	0.9986	0.974	1.770	0.9776	0.247	0.159	0.9190
80:20	-0.4537	0.1219	0.9993						
<i>Homologous series B</i>									
Methanol-water									
70:30	-0.2297	0.2080	0.99998						
75:25	-0.3799	0.1835	0.99999						
80:20	-0.4789	0.1535	0.9995	1.713	2.773	0.9972	0.517	0.446	0.9922
90:10	-0.7912	0.1192	0.9998						
Dioxane-water									
50:50	0.2150	0.2658	0.9999						
55:45	0.0644	0.2307	0.99998						
60:40	-0.0923	0.1987	0.9999	1.700	2.980	0.9997	0.551	0.580	0.9980
65:35	-0.2492	0.1717	0.9997						
70:30	-0.3819	0.1472	0.9993						
75:25	-0.5288	0.1183	0.9992						
Acetonitrile-water									
60:40	-0.1960	0.1736	0.99995						
65:35	-0.3464	0.1606	0.99996	1.544	2.901	0.9998	0.253	0.137	0.9719
70:30	-0.4806	0.1552	0.99997						
80:20	-0.7786	0.1447	0.9998						
Tetrahydrofuran-water									
45:55	0.3600	0.1879	0.99999						
50:50	0.1545	0.1562	0.9999						
55:45	-0.0335	0.1284	0.9995	1.845	3.357	0.9958	0.338	0.372	0.9866
60:40	-0.1760	0.1180	0.9998						
65:35	-0.3140	0.0984	0.9997						

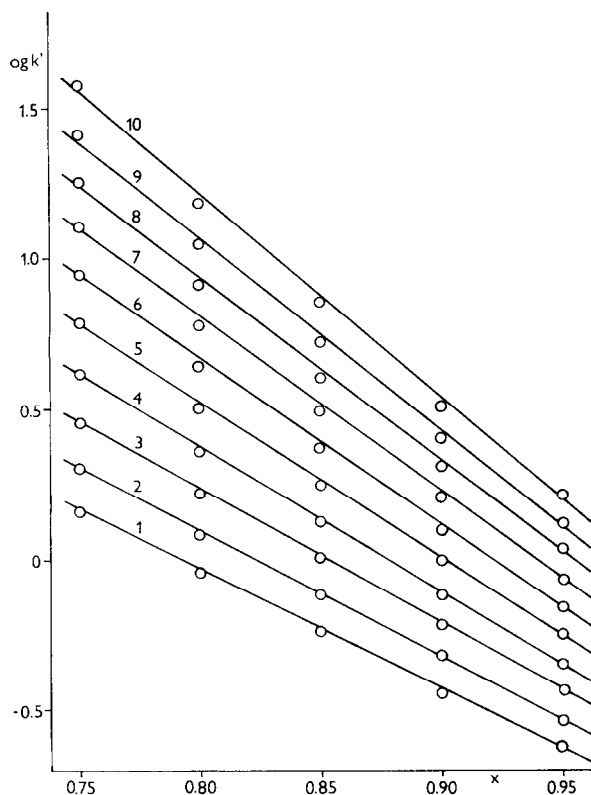


Fig. 2. Plots of logarithms of capacity factors  $k'$  of 1,2-naphthoylebenzimidazole-6-sulphonamides of saturated aliphatic  $n$ -amines in dependence on the concentration ( $x$ , % v/v  $10^{-2}$ ) of methanol in water as the mobile phase on Silasorb  $C_{18}$  ( $10 \mu\text{m}$ ). The numbers of the plots are the numbers of carbon atoms in the aliphatic chains of the amides.

given in Table III for two homologous series and various mobile phases, and in Table IV for other combinations of homologous series, columns and mobile phase. Examples of  $a$  vs.  $n_c$  and  $m$  vs.  $n_c$  plots are shown in Figs. 4 and 5.

According to eqn. 11, the constants  $\log \beta$  and  $\log \alpha$  of the  $\log k'$  vs.  $n_c$  relationships should decrease in a linear manner with increasing  $x$  for the homologous series and mobile phases, where linear  $\log k'$  vs.  $x$  relationships apply. The correlation coefficients in Table II indicate that the plots  $\log \alpha$  vs.  $x$  and  $\log \beta$  vs.  $x$  are almost linear for methanol-water and 1,4-dioxane-water mobile phases, whereas the correlation is somewhat poorer for acetonitrile-water and tetrahydrofuran-water. Experimental constants  $\alpha_0$ ,  $\alpha_1$ ,  $\beta_0$  and  $\beta_1$  for the homologous series, mobile phases and columns tested are listed in Table II and in Table IV, and plots of  $\log \alpha$  vs.  $x$  and  $\log \beta$  vs.  $x$  are shown in Figs. 6 and 7.

To check further the validity of the eqn. 11 we compared the constants  $a_0$ ,  $a_1$ ,  $m_0$  and  $m_1$  obtained from linear regression analysis of the experimental  $a$  vs.  $n_c$  and  $m$  vs.  $n_c$  plots with the constants  $\alpha_0$ ,  $\alpha_1$ ,  $\beta_0$  and  $\beta_1$  determined using linear regression analysis of the experimental  $\log \alpha$  vs.  $x$  and  $\log \beta$  vs.  $x$  plots. If eqn. 11 is to be valid,

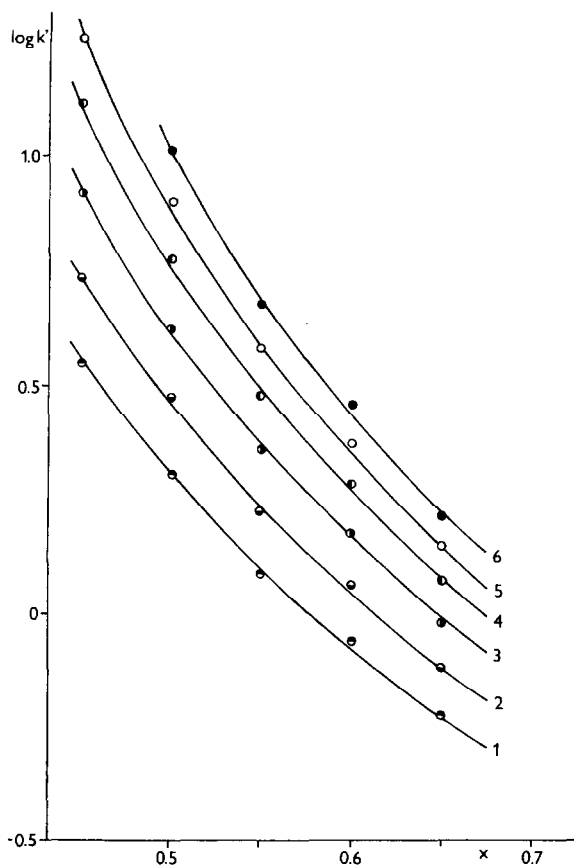


Fig. 3. Plots of logarithms of capacity factors  $k'$  of 3,5-dinitrobenzoates of carboxylic acids in dependence on the concentration ( $x$ , %v/v  $10^{-2}$ ) of tetrahydrofuran in water as the mobile phase on Silasorb  $C_{18}$  (10  $\mu$ m). The numbers of the plots are the numbers of carbon atoms in the aliphatic chains of the carboxylic acids.

then the equalities 10a–d must apply ( $a_0 = \beta_0$ ,  $a_1 = \alpha_0$ ,  $m_0 = \beta_1$ ,  $m_1 = \alpha_1$ ). The constants  $a_0$ ,  $a_1$ ,  $m_0$ ,  $m_1$ ,  $\alpha_0$ ,  $\alpha_1$ ,  $\beta_0$  and  $\beta_1$  obtained in this way for four different homologous series on two octadecylsilica columns (LiChrosorb RP  $C_{18}$ /Si 100 and Silasorb  $C_{18}$ ) in various binary mobile phases containing methanol–water, acetonitrile–water, 1,4-dioxane–water, and tetrahydrofuran–water in different proportions (50 and more per cent of the organic solvent) are compared in Table IV.

In the mobile phases methanol–water and 1,4-dioxane–water, the agreement between the experimental constants  $a_0/\beta_0$ ,  $a_1/\alpha_0$ ,  $m_0/\beta_1$  and  $m_1/\alpha_1$  is generally good (better than  $\pm 3\%$  rel., with the exception of  $a_1/\alpha_0$  and  $m_1/\alpha_1$  for *p*-bromophenacyl esters). In mobile phases composed of acetonitrile and water, good agreement was found only for 3,4-dinitrobenzoates and 1,2-naphthoylene-benzimidazole-6-sulphonamides; differences of 10% and more were found for the two other homologous series and even greater differences arose in tetrahydrofuran–water mobile phases. An attempt to improve the agreement by using non-linear (quadratic) regression analysis

TABLE III

VALUES OF CONSTANTS  $a$  AND  $m$  OF EQN. 11

Determined by linear regression of experimental  $\log k'$  vs.  $x$  plots and constants  $a_0$ ,  $a_1$  and  $m_0$ ,  $m_1$  of  $a$  vs.  $n_c$  plots and  $m$  vs.  $n_c$  plots for (A) 1,2-naphthoylenebenzimidazole-6-sulphonamides of aliphatic  $n$ -amines and (B) 3,5-dinitrobenzoates of aliphatic  $n$ -alcohols on a Silasorb C<sub>18</sub> column, 10  $\mu$ m, 300  $\times$  4.2 mm I.D., in various binary mobile phases;  $n_c$  = number of carbon atoms in the aliphatic chain of a member of a homologous series;  $x$  = %vol. of the organic solvent in the mobile phase  $\times 10^{-2}$ ;  $R$  = correlation coefficient.

Mobile phase	$n_c$	$a$	$m$	$R$	$a_0$	$a_1$	$R$	$m_0$	$m_1$	$R$
<i>Homologous series A</i>										
Methanol-water	1	2.571	3.256	0.9998						
	4	4.071	4.645	0.9998						
	6	5.082	5.572	0.99999	2.170	0.467	0.9989	2.918	0.420	0.9980
	7	5.404	5.822	0.9999						
	8	5.928	6.312	0.99997						
	10	6.770	7.037	0.9998						
Dioxane-water	1	1.933	3.754	0.9950						
	4	3.171	4.937	0.9980						
	6	4.204	6.093	0.9962	1.420	0.463	0.9990	3.185	0.482	0.9971
	7	4.688	6.605	0.9964						
	8	5.137	7.058	0.9959						
Acetonitrile-water	4	1.976	2.409	0.9981						
	6	2.427	2.693	0.99999						
	7	2.741	2.951	0.9987	0.974	0.247	0.9980	1.770	0.159	0.9913
	8	2.902	2.985	0.9961						
	10	3.463	3.370	0.9980						
<i>Homologous series B</i>										
Methanol-water	1	2.249	3.235	0.9991						
	2	2.754	3.673	0.9993						
	3	3.251	4.103	0.9998	1.713	0.517	0.9997	2.773	0.446	0.9994
	4	3.749	4.518	0.9999						
	5	4.283	4.979	0.9996						
	6	4.852	5.488	0.9996						
Dioxane-water	1	2.289	3.629	0.9995						
	2	2.775	4.096	0.9995						
	3	3.330	4.675	0.9993	1.701	0.551	0.9997	2.980	0.580	0.9991
	4	3.904	5.285	0.9992						
	5	4.463	5.888	0.9992						
	6	5.021	6.494	0.9991						
Acetonitrile-water	1	1.820	3.075	0.9997						
	2	2.043	3.162	0.9997						
	3	2.293	3.297	0.9992	1.537	0.251	0.9992	2.882	0.137	0.9942
	4	2.523	3.402	0.9990						
	5	2.803	3.584	0.9989						
	6	3.115	3.798	0.9977						
Tetrahydrofuran-water	1	2.228	3.808	0.9945						
	2	2.599	4.224	0.9942						
	3	2.974	4.656	0.9939	1.876	0.364	0.9989	3.388	0.424	0.9986
	4	3.371	5.140	0.9924						
	5	3.660	5.468	0.9927						

TABLE IV

COMPARISON OF THE CONSTANTS  $a_0$ ,  $a_1$ ,  $m_0$ ,  $m_1$  AND  $\beta_0$ ,  $\beta_1$ ,  $\alpha_0$ ,  $\alpha_1$  FOR VARIOUS HOMOLOGOUS SERIES, MOBILE PHASES AND OCTADECYLSILICA COLUMNS

Homologous series: (a) 3,5-dinitrobenzoates of aliphatic *n*-alcohols; (b) *p*-bromophenacyl esters of lower aliphatic carboxylic acids; (c) 1,2-naphthoylenebenzimidazole-6-sulphonamides of aliphatic *n*-amines; (d) *n*-alkylbenzenes. Columns: I, LiChrosorb RP C<sub>18</sub>/Si 100, 10  $\mu$ m, 300  $\times$  4.2 mm I.D.; II, Silasorb C<sub>18</sub>, 10  $\mu$ m, 300  $\times$  4.2 mm I.D. Mobile phases: A, methanol-water; B, dioxane-water; C, acetonitrile-water; D, tetrahydrofuran-water.

Mobile phase and column	Homologous series	$a_0$	$\beta_0$	$a_1$	$\alpha_0$	$m_0$	$\beta_1$	$m_1$	$\alpha_1$
A, I	b	1.721	1.715	0.519	0.484	2.896	2.889	0.448	0.403
	c	2.588	2.632	0.432	0.420	3.410	3.519	0.365	0.351
A, II	a	1.713	1.713	0.517	0.517	2.773	2.773	0.446	0.446
	b	1.735	1.674	0.478	0.502	2.818	2.746	0.410	0.439
	c	2.170	2.170	0.467	0.467	2.918	2.918	0.420	0.420
	d	1.915	1.917	0.536	0.536	2.628	2.637	0.466	0.465
Average values for A (column II):						$m_0 = 2.768$	$m_1 = 0.442$		
B, II	a	1.701	1.700	0.551	0.551	2.980	2.980	0.580	0.580
	b	1.402	1.402	0.535	0.534	2.637	2.637	0.563	0.563
	c	1.420	1.426	0.463	0.463	3.185	3.213	0.482	0.481
Average values for B (column II):						$m_0 = 2.943$	$m_1 = 0.541$		
C, I	b	1.318	1.487	0.303	0.266	2.502	2.737	0.204	0.152
	c	1.066	1.062	0.263	0.263	2.018	2.013	0.164	0.164
C, II	a	1.537	1.544	0.251	0.253	2.882	2.901	0.137	0.137
	b	1.417	1.312	0.258	0.286	2.606	2.466	0.155	0.191
	c	0.974	0.974	0.247	0.247	1.770	1.770	0.159	0.159
	d	1.393	1.344	0.277	0.297	2.196	2.135	0.165	0.189
Average values for C (column II):						$m_0 = 2.318$	$m_1 = 0.169$		
D, I	c	1.783	1.798	0.213	0.288	4.248	4.323	0.187	0.338
D, II	a	1.876	1.845	0.364	0.338	3.388	3.357	0.424	0.371

of the  $\log k'$  vs.  $x$  plots was unsuccessful because of the large error in the determination of constants by non-linear regression of only five experimental data points for each plot.

It can be concluded that eqn. 11 can be used to describe the retention in homologous series in the methanol-water and 1,4-dioxane-water mobile phases, whereas certain deviations of the calculated data are to be expected with some homologous series in acetonitrile-water mobile phases. Eqn. 11 is clearly not well suited for tetrahydrofuran-water mobile phases.

It should be noted that these conclusions relate to mobile phases containing 50% or more of the organic solvent, and that more significant curvatures of  $\log \alpha$  vs.  $x$  and  $\log \beta$  vs.  $x$  plots should be expected in more water-rich mobile phases<sup>25</sup>.

From a comparison of the constants in Table IV for different homologous series one can see that, in methanol-water and 1,4-dioxane-water and, to a certain extent, in acetonitrile-water, the constants  $m_1 = \alpha_1$ ,  $m_0 = \beta_1$  and  $a_1 = \alpha_0$  do not depend significantly on the character of the homologous series (or of the column). It is the constant  $\beta_0$  that is the main contributor to the specific selectivity for a given

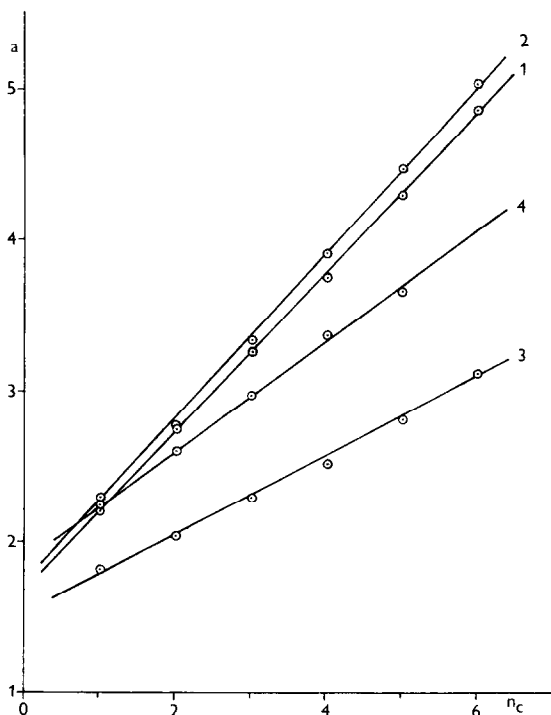


Fig. 4. Plots of the constants  $a$  of eqn. 11 vs. the number of carbon atoms,  $n_c$ , in the chains of 3,5-dinitrobenzoates of aliphatic carboxylic acids. Column: Silasorb  $C_{18}$  (10  $\mu$ m); mobile phases: 1, methanol-water; 2, 1,4-dioxane-water; 3, acetonitrile-water; 4, tetrahydrofuran-water.

homologous series. This constant has the meaning of  $\log k'$  of the molecular residue in a homologous series extrapolated to pure water. (The differences in  $\beta_0$  values for various organic solvents in the mobile phase with a given homologous series are caused by the extrapolation of a quasi-linear part of non-linear  $\log \beta$  vs.  $x$  plots of different curvatures to  $x = 0$ .)

#### Verification of the validity of eqn. 15 for prediction of retention in homologous series

From eqn. 12, it follows that the constants  $m$  and  $a$  for various members of a given homologous series should be correlated. The correlation between  $m$  and  $a$  has been reported earlier by Schoenmakers *et al.*<sup>41</sup>, who found good correlation in aqueous methanol ( $R = 0.98$ ), poorer correlation in aqueous tetrahydrofuran ( $R = 0.76$ ) and practically no correlation in aqueous acetonitrile for various organic compounds with different functional groups. Table V shows the results of the linear regression analysis of the experimental  $m$  vs.  $a$  plots for four different homologous series on two octadecylsilica columns in binary mobile phases containing various proportions of methanol, acetonitrile, 1,4-dioxane and tetrahydrofuran as the organic solvent in water (50–90%). The values of correlation coefficients  $R = 0.995$ – $0.9999$  confirm the linear dependence of  $m$  and  $a$  in all the systems studied (Fig. 8).

The reason is that the linear equation (12) between  $m$  and  $a$  applies only for

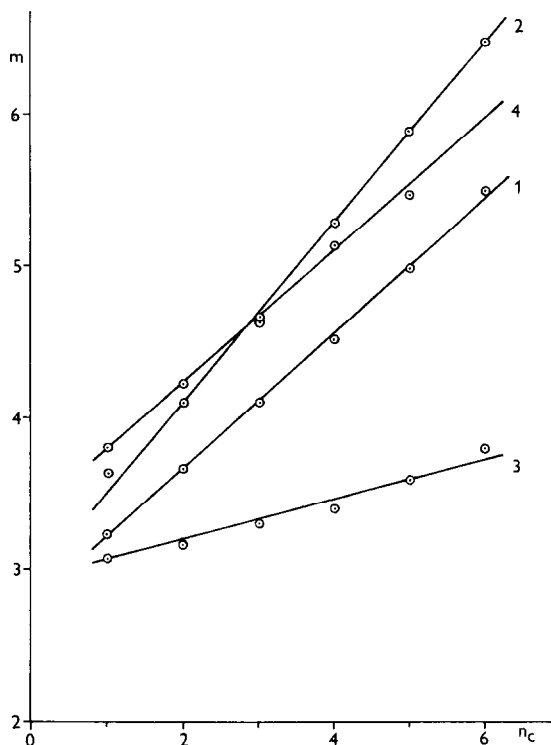


Fig. 5. Plots of the constants  $m$  of eqn. 11 vs. the number of carbon atoms,  $n_c$ , in the chains of 3,5-dinitrobenzoates of aliphatic carboxylic acids. Column and mobile phases as in Fig. 4.

such series of compounds as possess one common functional group and differ structurally only in the hydrocarbonaceous part of the molecule, such as the members of a certain homologous series. Here, eqns. 13 and 14 control the constants  $p$  and  $q$  of eqn. 12, and the values  $I_{0x}$ ,  $\Delta I_x$ ,  $V_{0x}$ ,  $\Delta V_x$ ,  $c_x$  are constant for the individual homologues. This is not true for compounds that contain different functional groups, which applies above all for the parameter  $q$ . From eqn. 13 it follows that the value in square brackets is independent of the solute [the value  $\Delta(I_x V_x) = I_{0x} \Delta V_x + \Delta I_x V_{0x}$  is small in comparison with  $I_{H_2O} \Delta V_x$ ] and close to 2:

$$p \approx 2 \left( 1 - \frac{I_{org}}{I_{H_2O}} \right) \quad (19)$$

Consequently, the slopes  $p$  of the  $m$  vs.  $a$  plots should be almost constant for binary mobile phases containing a given organic solvent, which is in approximate agreement with experimental results (Table V and Fig. 8). The constants  $q$  differ much more significantly one from another for different homologous series and, if we consider each compound as a potential member of a homologous series, we obtain points scattered around the "average"  $m$  vs.  $a$  plot for compounds with various functional groups.



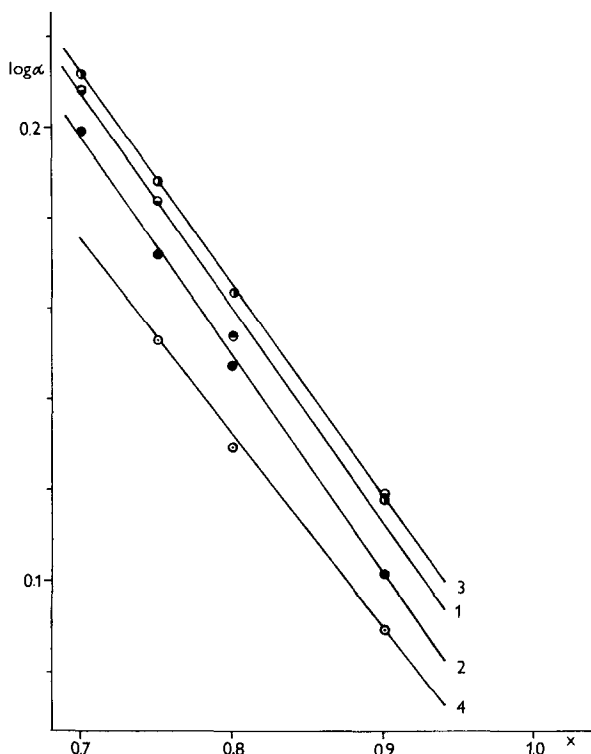


Fig. 6. Plots of the constants  $\log \alpha$  of eqn. 5a vs. the concentration  $x$  (%v/v  $10^{-2}$ ) of methanol in the mobile phase for the following homologous series: 1, 3,5-dinitrobenzoates of aliphatic  $n$ -alcohols; 2,  $p$ -bromophenacyl esters of aliphatic carboxylic acids; 3, 1,2-naphthoylenebenzimidazole-6-sulphonamides of aliphatic  $n$ -amines; 4,  $n$ -alkylbenzenes. Column: Silasorb  $C_{18}$  ( $10 \mu\text{m}$ ).

Because the parameter  $q$  is influenced by the interaction index of the organic solvent,  $I_{\text{org}}$  (eqn. 14), the amount of scattering depends on the character of the organic solvent in the mobile phase.

The parameter  $p$  is non-specific with respect to different homologous series and is related to the interaction index of the organic solvent in the mobile phase,  $I_{\text{org}}$ , which is a measure of polarity of the organic solvent. It is interesting to compare average experimental values of  $I_{\text{org}}/I_{\text{H}_2\text{O}}$  from eqn. 19 with the ratios of polarities,  $P'_{\text{org}}/P'_{\text{H}_2\text{O}}$  calculated from the tabulated values of  $P'^{42}$ :

	methanol- water	acetonitrile- water	dioxane- water	tetra- hydro- furan- water
$I_{\text{org}}/I_{\text{H}_2\text{O}}$	0.57	0.69	0.5	0.5
$P'_{\text{org}}/P'_{\text{H}_2\text{O}}$	0.50	0.56	0.47	0.39

The experimental verification of the eqns. 15 and 16 is demonstrated in Table VI, where the experimental values of capacity factors,  $k'$ , are compared with the

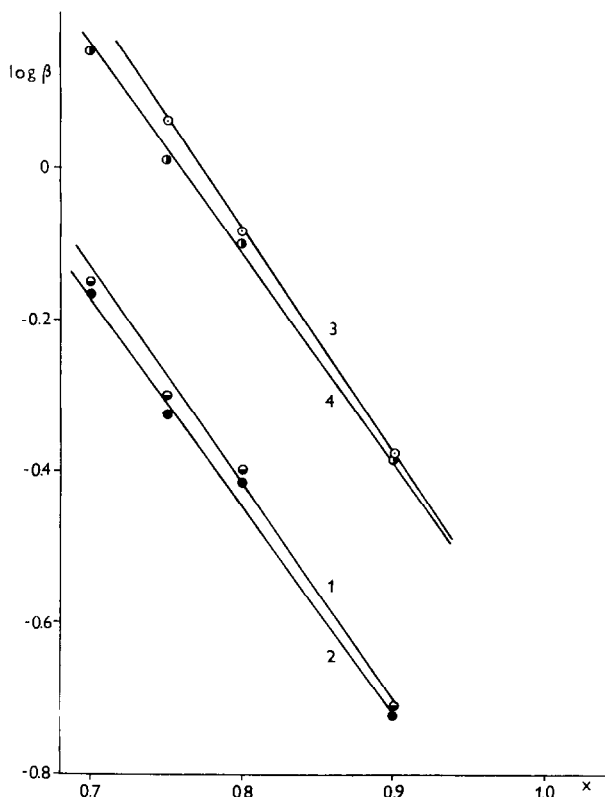


Fig. 7. Plots of the constants  $\log \beta$  of eqn. 5a vs. the concentration  $x$  (%v/v  $10^{-2}$ ) of methanol in the mobile phase. Column and homologous series as in Fig. 6.

values calculated from these equations using constants  $a_0$ ,  $a_1$ ,  $m_0$ ,  $m_1$ ,  $p$  and  $q$  from Tables IV and V. The agreement between the experimental and calculated values is very good for methanol-water, is within *ca.* 10% rel. for 1,4-dioxane-water and is within *ca.* 5% rel. for acetonitrile-water mobile phases.

Eqn. 16 offers an interesting possibility for calculations of retention of various homologues in mobile phases with different proportions of the organic solvent and water, based on a single experimental value of the capacity factor of any arbitrarily chosen homologue measured in one mobile phase. Because the constants  $m_0$ ,  $m_1$ , and  $p$  are almost independent of the character of a homologous series, the average values of these constants may be used for various homologous series. Using these average constants (Tables IV and V) and an experimental capacity factor ( $n_c$  and  $x$  are known), the value of  $q_i$  can be calculated for a given homologous series from eqn. 16. Then eqn. 16 can be used to calculate capacity factors for various homologues and mobile phase compositions. The values of  $k'$  calculated in this way are also compared with the experimental capacity factors in Table VI. The agreement between the experimental and calculated values is in most instances better than 10–15% rel.

Because there are no very significant differences between the constants  $m_0$ ,  $m_1$  and  $p$  measured for two different octadecylsilica columns (Tables IV and V), it seems

TABLE V

CONSTANTS  $p$  AND  $q$  OBTAINED USING LINEAR REGRESSION ANALYSIS OF THE EXPERIMENTAL  $m$  vs.  $a$  PLOTS (EQN. 12) FOR VARIOUS HOMOLOGOUS SERIES, MOBILE PHASES AND OCTADECYLSILICA COLUMNS

Mobile phases, columns and homologous series as in Table IV.

Mobile phase and column	Homologous series	$p$	$q$	$R^*$	Average $\bar{p}$
A, I	b	0.920	1.403	0.9998	
	c	0.813	1.378	0.99995	
A, II	a	0.862	1.296	0.99996	0.873
	b	0.859	1.324	0.9996	
	c	0.901	0.960	0.9999	
	d	0.871	0.960	0.99995	
B, II	a	1.053	1.189	0.9998	1.043
	b	1.054	1.158	0.9998	
	c	1.022	1.765	0.9993	
C, I	b	0.674	1.613	0.9996	
	c	0.624	1.352	0.9987	
C, II	a	0.558	2.028	0.9954	0.611
	b	0.641	1.645	0.9969	
	c	0.646	1.138	0.9973	
	d	0.599	1.357	0.9976	
D, I	c	0.990	2.298	0.9991	
D, II	a	1.047	1.213	0.99993	

\* Correlation coefficient.

probable that these constants as determined for one octadecylsilica column can be used for calculations of retention on other octadecylsilica columns from various manufacturers, and that the differences between the retention on different columns influence mainly the constant  $q_i$  that should be determined for each column from the experimental capacity factor of one homologue in a single mobile phase (eqn. 16). This assumption was verified experimentally for six different octadecylsilica columns in aqueous methanolic mobile phases for 3,5-dinitrobenzoates of saturated aliphatic  $n$ -alcohols (Table VII). Here, the constants  $m_0$ ,  $m_1$  and  $p$  determined for the Silasorb C<sub>18</sub> column were used for calculations of  $k'$  on all the columns studied, and the values  $q_i$  were calculated for each column from the experimental  $k'$  of  $n$ -butyl-3,5-dinitrobenzoate using eqn. 16 —values marked with asterisks. The differences between the calculated and experimental  $k'$  of other homologues on other columns were somewhat higher than those measured on Silasorb C<sub>18</sub> column, but they did not exceed 15% rel. This agreement suggests that, to the first approximation, the capacity factors  $k'$  may be estimated using  $m_0$ ,  $m_1$  and  $p$  determined for one column without the need for the time-consuming re-determinations for each octadecylsilica column.

TABLE VI

## EXPERIMENTAL AND CALCULATED CAPACITY FACTORS

Calculated values from  $a_0$ ,  $a_1$ ,  $m_0$ ,  $m_1$ ,  $p$  and  $q$  listed in Tables IV and V using eqn. 15 (I) and 16 (II) and from the average values of  $m_0$ ,  $m_1$ , and  $p$  and  $q_i$  calculated from the experimental  $k'$  of one homologue measured in one mobile phase (the values marked with asterisks) using eqn. 16 (III);  $x = \% \text{vol.} \times 10^{-2}$  of the organic solvent in the mobile phase;  $n_c =$  number of carbon atoms in the aliphatic chain of the homologues. Homologous series: A, 3,5-dinitrobenzoates of saturated aliphatic  $n$ -alcohols; B,  $p$ -bromophenacyl esters of lower saturated carboxylic acids; C, 1,2-naphthoylebenzimidazole-6-sulphonamides of saturated aliphatic  $n$ -amines. Column: Silasorb C<sub>18</sub>, 10  $\mu\text{m}$ , 300  $\times$  4.2 mm I.D.

$n_c$	Methanol-water					Dioxane-water					Acetonitrile-water				
	$x$	$k'_{\text{exp}}$	I	II	III	$x$	$k'_{\text{exp}}$	I	II	III	$x$	$k'_{\text{exp}}$	I	II	III
Homologous series A: $q_i = 1.243$															
1	0.7	0.96	0.95	0.95	1.01	0.5	3.05	2.96	2.96	3.47	$q_i = 1.594$	0.6	0.95	0.94	0.93
	0.75	0.64	0.66	0.66	0.70	0.6	1.27	1.31	1.30	1.55	0.65	0.65	0.66	0.68	0.70
	0.8	0.48	0.45	0.45	0.48	0.7	0.57	0.58	0.57	0.70	0.7	0.47	0.47	0.48	0.53
2	0.9	0.21	0.22	0.22	0.23	0.75	0.38	0.38	0.38	0.47	0.8	0.23	0.23	0.24	0.30
	0.7	1.54	1.52	1.52	1.60	0.5	5.48	5.40	5.40	6.14	0.6	1.42	1.38	1.41	1.39
	0.75	0.97	1.00	1.00	1.05	0.6	2.01	2.08	2.08	2.43	0.65	0.95	0.96	0.98	1.02
3	0.8	0.66	0.65	0.66	0.69	0.7	0.82	0.80	0.80	0.96	0.7	0.68	0.67	0.68	0.76
	0.9	0.28	0.28	0.28	0.30	0.75	0.51	0.50	0.50	0.61	0.8	0.33	0.32	0.33	0.41
	0.7	2.42	2.44	2.44	2.51	0.5	10.26	9.85	9.85	10.87	0.6	2.12	2.03	2.06	2.09
4	0.75	1.46	1.52	1.52	1.57	0.6	3.19	3.32	3.32	3.80	0.65	1.36	1.38	1.41	1.51
	0.8	0.94	0.95	0.95	0.98	0.7	1.17	1.12	1.12	1.33	0.7	0.97	0.95	0.96	1.09
	0.9	0.36	0.37	0.37	0.38	0.75	0.69	0.65	0.65	0.78	0.8	0.46	0.44	0.44	0.57
5	0.7	3.95*	3.91	3.92	3.95*	0.5	19.26*	17.97	17.95	19.26*	0.6	3.13*	2.97	3.00	3.13*
	0.75	2.25	2.32	2.32	2.34	0.6	5.14	5.30	5.30	5.94	0.65	1.97	2.00	2.02	2.21
	0.8	1.34	1.37	1.37	1.39	0.7	1.66	1.56	1.56	1.83	0.7	1.38	1.35	1.36	1.57
6	0.9	0.49	0.48	0.48	0.49	0.75	0.90	0.85	0.85	1.02	0.8	0.64	0.61	0.61	0.79
	0.7	6.45	6.27	6.29	6.22	0.5	35.25	32.76	32.73	34.09	0.6	4.66	4.37	4.37	4.68
	0.75	3.45	3.53	3.53	3.51	0.6	8.01	8.46	8.45	9.29	0.65	2.85	2.89	2.89	3.25
7	0.8	1.95	1.98	1.99	1.99	0.7	2.26	2.18	2.18	2.53	0.7	1.96	1.91	1.91	2.26
	0.9	0.64	0.63	0.63	0.63	0.75	1.16	1.11	1.11	1.32	0.8	0.87	0.84	0.84	1.09
	0.7	10.58	10.06	10.08	9.79	0.5	63.92	59.45	59.66	60.38	0.6	7.08	6.42	6.37	7.00
8	0.75	5.30	5.37	5.38	5.24	0.6	12.40	13.50	13.48	14.52	0.65	4.16	4.18	4.18	4.77
	0.8	2.82	2.87	2.88	2.81	0.7	3.08	3.05	3.05	3.49	0.7	2.82	2.72	2.70	3.25
	0.9	0.84	0.82	0.82	0.81	0.75	1.48	1.45	1.45	1.71	0.8	1.22	1.15	1.15	1.51

*Homologous series B:  $q_i = 1.285$*

1	0.7	0.93	0.90	0.91	0.91	0.5	2.30	2.17	2.17	2.43	0.6	1.00	1.11	1.21	0.99
	0.75	0.60	0.62	0.62	0.63	0.6	0.94	1.04	1.04	1.09	0.65	0.71	0.81	0.88	0.74
	0.8	0.45	0.43	0.43	0.43	0.7	0.47	0.50	0.50	0.49	0.7	0.54	0.59	0.64	0.56
	0.9	0.20	0.20	0.20	0.21	0.75	0.35	0.34	0.34	0.33	0.8	0.30	0.32	0.34	0.31
2	0.7	1.38	1.40	1.40	1.43	0.5	4.11	3.89	3.89	4.31	0.6	1.53	1.59	1.71	1.48
	0.75	0.87	0.92	0.92	0.94	0.6	1.54	1.64	1.64	1.71	0.65	1.07	1.14	1.22	1.09
	0.8	0.64	0.61	0.61	0.62	0.7	0.69	0.69	0.69	0.68	0.7	0.79	0.82	0.87	0.80
	0.9	0.26	0.26	0.26	0.27	0.75	0.47	0.45	0.45	0.43	0.8	0.40	0.42	0.45	0.43
3	0.7	2.16	2.17	2.18	2.25	0.5	7.48	6.97	6.96	7.64	0.6	2.27	2.30	2.41	2.21
	0.75	1.27	1.36	1.36	1.40	0.6	2.47	2.57	2.57	2.67	0.65	1.53	1.62	1.69	1.60
	0.8	0.88	0.86	0.86	0.88	0.7	0.99	0.95	0.95	0.93	0.7	1.12	1.14	1.19	1.15
	0.9	0.34	0.34	0.34	0.34	0.75	0.62	0.58	0.58	0.55	0.8	0.54	0.56	0.59	0.60
4	0.7	3.54*	3.37	3.37	3.54*	0.5	13.52*	12.48	12.46	13.52*	0.6	3.33*	3.31	3.40	3.33*
	0.75	1.86	2.02	2.02	2.10	0.6	3.79	4.05	4.04	4.17	0.65	2.16	2.29	2.34	2.34
	0.8	1.22	1.21	1.21	1.24	0.7	1.35	1.31	1.31	1.29	0.7	1.56	1.58	1.62	1.66
	0.9	0.43	0.43	0.43	0.44	0.75	0.80	0.75	0.75	0.72	0.8	0.74	0.75	0.77	0.83
5	0.7	5.71	5.22	5.23	5.77	0.5	24.50	22.36	22.28	23.95	0.6	4.93	4.77	4.78	4.95
	0.75	2.78	2.98	2.98	3.14	0.6	5.91	6.37	6.35	6.52	0.65	3.10	3.24	3.24	3.44
	0.8	1.70	1.70	1.70	1.77	0.7	1.87	1.81	1.81	1.78	0.7	2.20	2.19	2.20	2.39
	0.9	0.55	0.56	0.56	0.56	0.75	1.03	0.97	0.97	0.93	0.8	1.00	1.01	1.01	1.15
7	0.75	6.48	6.52	6.52	7.02	0.5	79.03	71.70	71.34	75.10	0.65	6.57	6.47	6.20	7.41
	0.8	3.46	3.39	3.39	3.57	0.6	14.11	15.75	15.69	15.95	0.7	4.48	4.22	4.06	4.95
	0.9	0.93	0.91	0.91	0.93	0.7	3.44	3.46	3.45	3.39	0.8	1.87	1.80	1.73	2.21
						0.75	1.69	1.62	1.62	1.56					

*Homologous series C:  $q_i = 1.058$*

1	0.75	1.36	1.36	1.37	1.14	0.5	1.26	1.09	1.07	0.97	0.7	0.80	0.75	0.75	0.65
	0.8	0.91	0.93	0.93	0.79	0.6	0.43	0.47	0.46	0.44	0.75	0.68	0.60	0.60	0.49
	0.9	0.44	0.43	0.43	0.38	0.7	0.18	0.20	0.20	0.20	0.8	0.58	0.48	0.48	0.36
4	0.75	3.82*	3.88	3.88	3.82*	0.5	5.40*	5.22	5.24	5.40*	0.7	1.93*	1.90	1.90	1.93*
	0.8	2.30	2.29	2.29	2.27	0.6	1.32	1.61	1.61	1.67	0.75	1.50	1.44	1.44	1.37
	0.9	0.77	0.79	0.79	0.80	0.7	0.48	0.50	0.50	0.51	0.8	1.11	1.09	1.09	0.97
6	0.75	7.80	7.80	7.79	8.54	0.5	16.64	14.80	15.16	16.92	0.7	3.48	3.54	3.53	4.00
	0.8	4.21	4.17	4.17	4.58	0.6	3.02	3.67	3.74	4.07	0.75	2.56	2.59	2.58	2.73
	0.9	1.17	1.19	1.19	1.31	0.7	0.91	0.91	0.92	0.98	0.8	1.87	1.89	1.89	1.86
7	0.75	11.28	11.06	11.03	12.78	0.5	28.40	24.93	25.78	29.98	0.7	4.78	4.83	4.82	5.76
	0.8	5.32	5.63	5.62	6.51	0.6	4.50	5.54	5.69	6.36	0.75	3.31	3.47	3.46	3.85
	0.9	1.49	1.46	1.46	1.69	0.7	1.17	1.23	1.26	1.35	0.8	2.43	2.49	2.48	2.57
8	0.75	15.73	15.67	15.63	19.11	0.5	48.24	41.99	43.85	53.09	0.7	6.61	6.60	6.60	8.29
	0.8	7.49	7.60	7.58	9.25	0.6	7.92	8.37	8.67	9.95	0.75	4.45	4.65	4.63	5.44
	0.9	1.77	1.79	1.79	2.16	0.7	1.60	1.67	1.71	1.87	0.8	3.32	3.27	3.26	3.56

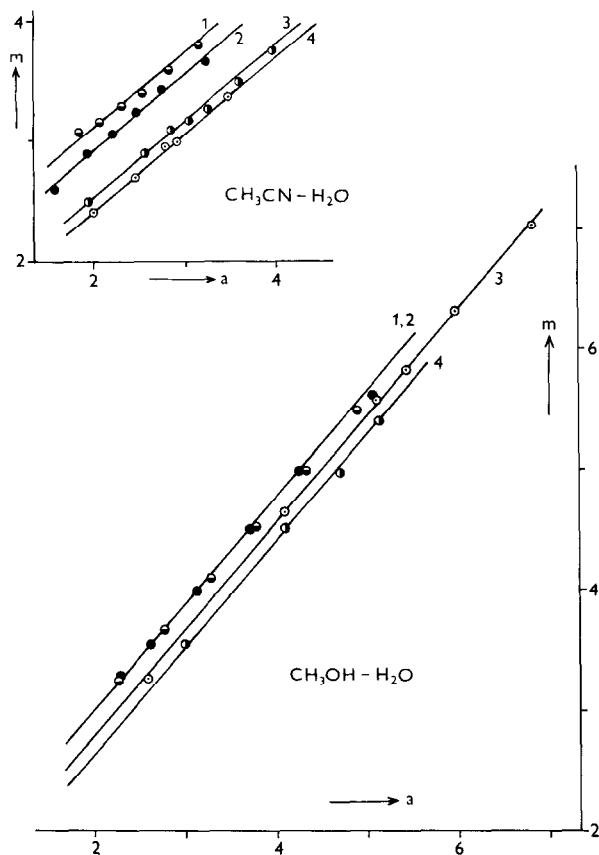


Fig. 8. Relationships between the constants  $m$  and  $a$  of the eqn. 11 for aqueous methanol and aqueous acetonitrile as the mobile phases. Column and homologous series as in Fig. 6.

## CONCLUSIONS

(1) From the retention model of interaction indices for reversed-phase liquid chromatography, a quadratic equation can be derived for the relationship between  $\log k'$  and the number of carbon atoms in aliphatic chains of the individual homologues,  $n_c$ . The quadratic term of this equation is usually very small and generally can be neglected, but it could possibly explain some deviations from the linearity of the above relationships observed experimentally in certain systems.

(2) The plots of  $\log k'$  of the individual homologues *versus* the concentration,  $x$ , of the organic solvent in the mobile phase, are generally non-linear, and so are the plots of  $\log \alpha$  and  $\log \beta$  (constants of the  $\log k'$  vs.  $n_c$  eqn. 5a) vs.  $x$ . However, in mobile phases containing 40-50% or more of the organic solvent, these plots are almost linear in aqueous methanolic mobile phases and are slightly curved in 1,4-dioxane- and acetonitrile-containing mobile phases, where they may be approximated as straight lines. The linear approximation is not valid for aqueous tetrahydrofuran as the mobile phase.

TABLE VII

## EXAMPLES OF CALCULATED CAPACITY FACTORS ON DIFFERENT COLUMNS

The  $k'_{calc}$  values are calculated from the average values of  $m_0$ ,  $m_1$  and  $p$  determined for the Silasorb  $C_{18}$  column and methanol-water mobile phases (Tables IV and V) and  $q_i$  determined from the experimental  $k'$  value for the  $n$ -butyl homologue ( $n_c = 4$ ) on each column (the values marked with asterisks) using eqn. 16. Homologous series, 3,5-dinitrobenzoates of saturated aliphatic  $n$ -alcohols;  $k'_{exp}$ , experimental capacity factors determined for each column; mobile phase, methanol-water (70:30).

$n_c$	Silasorb $C_{18}$ $q_i = 1.243$		Separon Six $C_{18}$ $q_i = 1.187$		Hypersil $C_{18}$ $q_i = 1.202$		$\mu$ Bondapak $C_{18}$ $q_i = 1.448$		LiChrosorb Si 100/ $C_{18}$ $q_i = 1.170$		LiChrosorb Si 60/ $C_{18}$ $q_i = 1.181$	
	$k'_{exp}$	$k'_{calc}$	$k'_{exp}$	$k'_{calc}$	$k'_{exp}$	$k'_{calc}$	$k'_{exp}$	$k'_{calc}$	$k'_{exp}$	$k'_{calc}$	$k'_{exp}$	$k'_{calc}$
1	0.96	1.01	1.14	1.18	0.96	1.13	0.62	0.59	1.13	1.23	1.07	1.19
2	1.54	1.60	1.80	1.85	1.57	1.78	0.95	0.93	1.81	1.93	1.72	1.88
3	2.42	2.51	2.81	2.91	2.60	2.80	1.46	1.46	2.91	3.04	2.79	2.96
4	3.95*	3.95*	4.59*	4.59*	4.40*	4.40*	2.30*	2.30*	4.79*	4.79*	4.65*	4.65*
5	6.45	6.22	7.53	7.21	7.45	6.93	3.65	3.62	8.02	7.54	7.82	7.32
6	10.58	9.79	12.46	11.34	12.67	10.90	5.80	5.70	13.42	11.87	13.13	11.53

(3) In the region where linear  $\log k'$  vs.  $x$  relationships can be used, the constants  $a$  and  $m$  of these equations are strongly correlated for the members of a given homologous series, and  $m$  is a linear function of  $a$ . The slope  $p$  of this relationship (eqn. 12) depends in practice only on the organic solvent in the mobile phase and is not significantly influenced by the character of the homologous series or by the column used; however, these do influence the constant  $q$  of eqn. 12. Like the constant  $p$ , the constants  $m_0$  and  $m_1$  of linear  $m$  vs.  $n_c$  relationships depend in practice only on the character of the organic solvent in the mobile phase.

(4) The non-specific selectivity,  $\log \alpha$ , does not depend significantly on the character of the column used, given the same character (chain length) of the alkyl-bonded phase. The constants  $\alpha_0$ ,  $\alpha_1$  of the  $\log \alpha$  vs.  $x$  plots depend in practice only on the character of the organic solvent used and not on the type of the homologous series or on the column.

(5) Based on these conclusions, a simple equation (16) can be derived that describes the linear dependence of  $\log k'$  on  $n_c$  and  $x$  for various homologous series. This equation can be used for mobile phases containing more than 40–50% of methanol, 1,4-dioxane or acetonitrile. The three constants of this equation ( $m_0$ ,  $m_1$  and  $p$ ) are related to the non-specific selectivity and only the constant  $q$ , which relates to the specific selectivity, depends on the character of the homologous series and the column used. Eqn. 16 can be used for prediction of retention in homologous series in organic solvent-rich mobile phases from a single experimental capacity factor of a member of the homologous series in a mobile phase of certain composition. These predictions are feasible for various columns of a certain type, such as octadecylsilica, using the constants  $m_0$ ,  $m_1$  and  $p$  determined with a single alkyl-bonded column.

## REFERENCES

- 1 B. L. Karger, J. R. Gant, A. Hartkopf and P. H. Weiner, *J. Chromatogr.*, 128 (1976) 65.

- 2 H. Colin and G. Guiochon, *J. Chromatogr. Sci.*, 18 (1980) 54.
- 3 M. J. M. Wells, C. R. Clark and R. M. Patterson, *J. Chromatogr.*, 235 (1982) 61.
- 4 R. E. Koopmans and R. F. Rekker, *J. Chromatogr.*, 285 (1984) 267.
- 5 M. J. M. Wells and C. R. Clark, *J. Chromatogr.*, 244 (1982) 231.
- 6 R. Tijssen, H. A. H. Billiet and P. J. Schoenmakers, *J. Chromatogr.*, 122 (1976) 185.
- 7 P. J. Schoenmakers, H. A. H. Billiet, R. Tijssen and L. de Galan, *J. Chromatogr.*, 149 (1978) 519.
- 8 P. Jandera and J. Churáček, *J. Chromatogr.*, 91 (1974) 207.
- 9 P. Jandera and J. Churáček, *Advan. Chromatogr.*, 19 (1981) 125.
- 10 D. E. Martire and R. E. Boehm, *J. Liquid Chromatogr.*, 3 (1980) 753.
- 11 Cs. Horváth, W. Melander and I. Molnár, *J. Chromatogr.*, 125 (1976) 129.
- 12 Cs. Horváth, W. Melander and I. Molnár, *Anal. Chem.*, 49 (1977) 142.
- 13 O. Sinanoglu, in B. Pullman (Editor), *Molecular Associations in Biology*, Academic Press, New York, 1968, p. 427.
- 14 P. Jandera, H. Colin and G. Guiochon, *Anal. Chem.*, 54 (1982) 435.
- 15 H. Colin, G. Guiochon and P. Jandera, *Anal. Chem.*, 55 (1983) 442.
- 16 H. Colin, G. Guiochon and P. Jandera, *Chromatographia*, 17 (1983) 83.
- 17 H. Colin, A. Krstulović, G. Guiochon and Z. Yun, *J. Chromatogr.*, 255 (1983) 295.
- 18 H. Colin, A. Krstulović, M. F. Gonnord, G. Guiochon, Z. Yun and P. Jandera, *Chromatographia*, 17 (1983) 9.
- 19 W. Melander, J. Stoveken and Cs. Horváth, *J. Chromatogr.*, 199 (1980) 35.
- 20 J. K. Baker, *Anal. Chem.*, 51 (1979) 1693.
- 21 R. M. Smith, *J. Chromatogr.*, 236 (1982) 313.
- 22 R. M. Smith, *Anal. Chem.*, 56 (1984) 256.
- 23 J. K. Baker and C.-Y. Ma, *J. Chromatogr.*, 169 (1979) 107.
- 24 H. Colin, G. Guiochon and J. C. Diez-Masa, *Anal. Chem.*, 53 (1981) 146.
- 25 H. Colin, G. Guiochon, Z. Yun, J. C. Diez-Masa and P. Jandera, *J. Chromatogr. Sci.*, 21 (1983) 179.
- 26 K. Karch, I. Sebastian and I. Halász, *J. Chromatogr.*, 122 (1976) 3.
- 27 P. Jandera, J. Churáček and L. Svoboda, *J. Chromatogr.*, 174 (1979) 35.
- 28 A. Nakae and K. Kunihiro, *J. Chromatogr.*, 152 (1978) 137.
- 29 A. Nakae and K. Kunihiro, *J. Chromatogr.*, 156 (1978) 167.
- 30 A. Nakae, K. Kunihiro and G. Muto, *J. Chromatogr.*, 134 (1977) 459.
- 31 B. A. Bidlingmeyer, S. N. Deming, W. P. Price, B. Sachock, Jr. and M. Petrusek, *J. Chromatogr.*, 186 (1979) 419.
- 32 P. Dufek and E. Smolková, *J. Chromatogr.*, 257 (1983) 247.
- 33 P. Dufek, *J. Chromatogr.*, 281 (1983) 49.
- 34 P. Jandera, H. Pechová, D. Tocksteinová, J. Churáček and J. Královský, *Chromatographia*, 16 (1982) 275.
- 35 W. R. Melander, A. Nahum and Cs. Horváth, *J. Chromatogr.*, 185 (1979) 129.
- 36 P. Jandera, in preparation.
- 37 L. R. Snyder, W. J. Dolan and J. R. Gant, *J. Chromatogr.*, 165 (1979) 3.
- 38 J. W. Dolan, J. R. Gant and L. R. Snyder, *J. Chromatogr.*, 165 (1979) 31.
- 39 P. J. Schoenmakers, H. A. H. Billiet and L. de Galan, *J. Chromatogr.*, 282 (1983) 107.
- 40 M. J. Wells and C. R. Clark, *J. Chromatogr.*, 235 (1982) 31.
- 41 P. J. Schoenmakers, H. A. H. Billiet and L. de Galan, *J. Chromatogr.*, 185 (1979) 179.
- 42 L. R. Snyder and J. J. Kirkland, *Introduction to Modern Liquid Chromatography*, Wiley, New York, 1979, 2nd ed., p. 248.