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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC STUDIES ON N-ALKYLPHTHALIMIDES

II. A METHOD FOR CALCULATING CAPACITY FACTORS FOR MEMBERS OF A HOMOLOGOUS SERIES AT DIFFERENT MOBILE PHASE COMPOSITIONS*

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SUMMARY

The high-performance liquid chromatographic behaviour of non-branched N-alkylphthalimides on LiChrosorb RP-8 was studied as a function of the alkyl carbon number and the methanol–water ratio. The linear dependences of log k' on alkyl carbon number and log k' on log $c_{methanol}$ have been used as a basis for deriving a general formula for calculating the capacity factor for any member of the homologous series at any methanol–water mobile phase composition. A good agreement was observed between the measured and the recalculated data.

INTRODUCTION

In our previous paper[1] we described the high-performance liquid chromatographic (HPLC) behaviour of N-alkylphthalimides on various columns. The data led us to the conclusion that the behaviour of the compounds is given by shielding of the polar –CO–N–CO– group of phthalimide by a non-polar or slightly polar substituent, depending on the structure and length of the alkyl carbon chain. In the homologous series of non-branched N-alkylphthalimides (and other derivatives) a linear dependence of log k' on carbon number was observed. This linearity was used to derive a formula for calculating the capacity factor of any member of the homologous series under the same chromatographic conditions. The agreement of the measured and the calculated data was excellent.

Furthermore, the data suggested the possibility of elucidation of the dependence of log k' on the mobile phase composition. In the range of methanol–water ratios from 50:50 to 80:20 (v/v) on LiChrosorb RP-18 the dependence of log k' on the volume per cent of methanol was nearly linear. Similar results were observed recently by Grushka *et al.*[2], whereas others advocate a quadratic relationship[3,4].

* Part I See ref. 1.

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However, the mobile phase concentration interval and/or the number of members of homologous series were too small to resolve this question definitely.

To decide whether the dependence of log k' on the mobile phase composition is linear, quadratic or otherwise, we measured the retention data of all available non-branched N-alkylphthalimides in the widest range of methanol–water ratios possible on a LiChrosorb RP-8 column.

EXPERIMENTAL

N-Alkylphthalimides were prepared according to Mitsunobu[5,6] as described earlier[1]. Double-distilled water and reagent grade methanol (Lachema, Brno, Czechoslovakia) were used in the mobile phase.

The measurements were performed on a Spectra-Physics SP 8700 liquid chromatograph equipped with a LC 871 UV-VIS detector. The wavelength selected for all measurements was 290 nm (absorbance maximum of phthalimide). The retention time measurements were performed using a Minigrator (Spectra-Physics).

A stainless-steel column packed with LiChrosorb RP-8 (Chrompack, The Netherlands) (10 μm, 250 × 4.6 mm I.D.) was used at room temperature without temperature control with a methanol–water mobile phase (ratios from 40:60 to 100:0, v/v) at a flow-rate of 2.0 ml/min.

To achieve equilibrium on the column each change in the mobile phase composition was followed by a few hours of passing the new mobile phase through the column until the retention times stabilized. The t_0 was measured as a retention time of methanol determined at 200 nm after injecting onto a column with 100% water mobile phase. The value found was 81 sec.

RESULTS AND DISCUSSION

The capacity factors (k') of non-branched N-alkylphthalimides C_1–C_{12}, C_{14}, C_{16} and C_{18} on LiChrosorb RP-8 with a methanol–water mobile phase at ratios ranging from 40:60 to 100:0 (v/v) are summarized in Table I. As expected, the k' values increase with increasing amount of water in the mobile phase as well as with increasing alkyl carbon number. A plot of log k' *vs.* carbon number at all mobile phase compositions studied is linear, with the exception of the lowest members of the homologous series (Fig. 1).

It has been shown[1] that the capacity factor of a homologue containing $n + j$ carbon atoms in a non-branched alkyl chain can be calculated according to the equation

$$k'_{n+j} = k'_n \cdot K^j = k'_n \cdot \left(\frac{k'_{n+i}}{k'_n}\right)^{j/i} \quad (1)$$

where k'_n and k'_{n+i} are the measured capacity factors of homologues containing n and $n + i$ alkyl carbon atoms, respectively, and K is a constant characterizing the column, mobile phase composition, temperature and type of homologous series.

The values of the constant K for non-branched N-alkylphthalimides on Li-

TABLE I

CAPACITY FACTORS OF NON-BRANCHED N-ALKYLPHTHALIMIDES

Column, LiChrosorb RP-8; mobile phase, methanol–water; flow-rate, 2.0 ml/min.

Alkyl group	*Methanol in mobile phase (%)*											
	40	*50*	*55*	*60*	*65*	*70*	*75*	*80*	*85*	*90*	*95*	*100*
H	1.80	0.93	0.63	0.57	0.46	0.36	0.27	0.26	0.23	0.20	0.19	0.16
Methyl	3.81	1.80	1.21	0.98	0.77	0.58	0.43	0.37	0.32			
Ethyl	8.36	3.46	2.19	1.60	1.20	0.86	0.62	0.51	0.41			
Propyl	17.35	6.26	3.70	2.58	1.80	1.23	0.83	0.64	0.48			
Butyl		12.16	6.94	4.46	2.91	1.84	1.20	0.86	0.63			
Pentyl			12.62	7.62	4.63	2.74	1.69	1.16	0.78	0.56	0.40	
Hexyl				13.00	7.47	4.14	2.42	1.54	0.99	0.65	0.44	0.32
Heptyl					12.19	6.31	3.49	2.09	1.26	0.80	0.51	0.35
Octyl					19.47	9.54	5.01	2.80	1.60	0.96	0.58	0.38
Nonyl						14.47	7.17	3.74	2.04	1.17	0.65	0.42
Decyl						21.96	10.26	5.02	2.58	1.41	0.75	0.46
Undecyl						33.47	14.73	6.78	3.28	1.70	0.86	0.49
Dodecyl							21.21	8.98	4.20	2.05	0.99	0.54
Tetradecyl									6.85	3.01	1.31	0.65
Hexadecyl									11.27	4.44	1.72	0.78
Octadecyl										6.53	2.25	0.96

Chrosorb RP-8 with various mobile phase compositions calculated according to the formula $K = k'_{n+1}/k'_n$ or $K = (k'_{n+2}/k'_n)^{1/2}$ are given in Table II. The K values at the chosen mobile phase composition vary very little, with the exception of the first four non-linear members of the homologous series, *i.e.*, phthalimide, N-methyl-, N-ethyl- and N-propylphthalimides. This non-linearity can be explained by ineffective shielding of a polar –CO–N–CO– group by a short alkyl chain (*cf.* ref. 1). It is noteworthy that at most mobile phase compositions the K values decrease in the sequence k'_{Me}/k'_H, k'_{Et}/k'_{Me}, k'_{Pr}/k'_{Et}. At k'_{Bu}/k'_{Pr} the K value increases and holds constant for higher homologues. This suggests that the electronic effect of the nitrogen atom becomes negligible only in C_3 and longer alkyl chains. It also accounts for the ineffective shielding of the polar group of phthalimide in the case of N-methyl- and N-ethylphthalimides.

Fig. 1 indicates that the constant K depends on the mobile phase composition. A plot of log K (mean values) *vs.* per cent methanol in the mobile phase is shown in Fig. 2. A linear dependence of log K (log K = log k'_{n+1} − log k'_n, *cf.* eqn. 1) on the percentage of methanol thus also suggests a linear dependence of log k' on log (per cent methanol) in mobile phase. The plot of log k' values from Table I *vs.* log (per cent methanol) in Fig. 3 confirms this suggestion.

The logarithmic dependence of log k' on per cent methanol in the mobile phase accounts for the previous observation of the authors who advocate a linear[2] or quadratic[3,4] relationship. In a narrow concentration interval the logarithmic dependence could seem to be nearly linear or quadratic, depending on the concentration interval and the carbon number of the homologues under study.

The dependence of log k' on log (per cent methanol) has been used as a basis

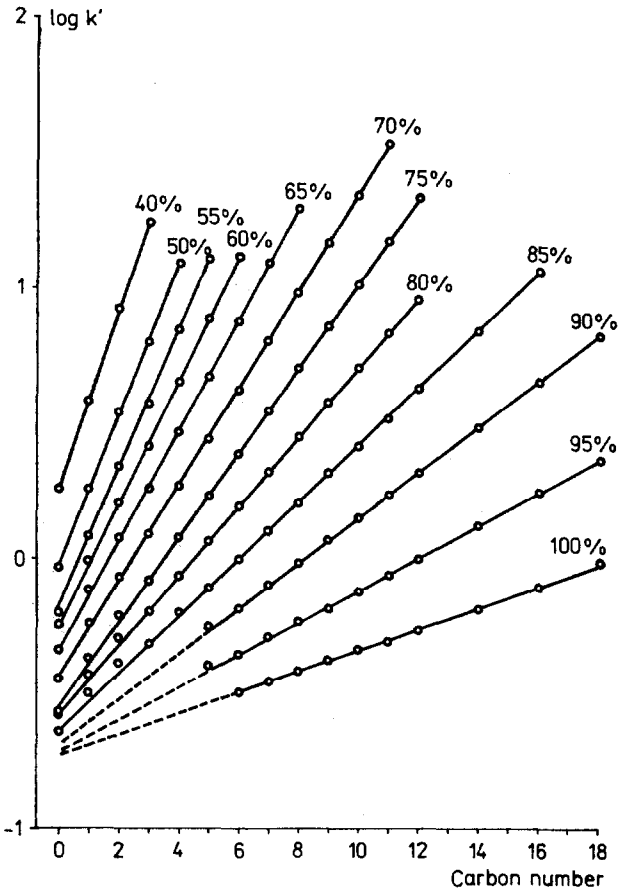

Fig. 1. Dependence of log k' on the number of carbon atoms in the alkyl group of N-alkylphthalimides with a non-branched carbon chain at various concentrations of methanol in the methanol–water mobile phase. Column, LiChrosorb RP-8.

for extending the formula for calculating the capacity factors in a homologous series (eqn. 1) to involve the methanol concentration.

It follows from Fig. 3 that the slope, B_{n_1}, for a homologue containing n_1 alkyl carbon atoms, is given by eqn. 2:

$$\log k'_{n_1c_1} - \log k'_{n_1c_2} = B_{n_1} (\log c_2 - \log c_1) \text{ and hence}$$

$$B_{n_1} = \frac{\log (k'_{n_1c_1}/k'_{n_1c_2})}{\log (c_2/c_1)} \tag{2}$$

B_{n_1} depends on the carbon number, temperature and type of column and homologous series, c_1 and c_2 are per cent proportions of methanol in mobile phase, $k'_{n_1c_1}$ and $k'_{n_1c_2}$ are capacity factors of a homologue containing n_1 carbon atoms at methanol concentrations c_1 and c_2, respectively.

TABLE II

CALCULATED VALUES OF K [k'_{n+1}/k'_n OR $(k'_{n+2}/k'_n)^{1/2}$] FOR NON-BRANCHED N-ALKYLPHTHALIMIDES AT VARIOUS METHANOL PROPORTIONS IN THE MOBILE PHASE

n	*Methanol in mobile phase (%)*											
	40	*50*	*55*	*60*	*65*	*70*	*75*	*80*	*85*	*90*	*95*	*100*
0	2.12	1.94	1.92	1.72	1.67	1.61	1.59	1.57	1.42			
1	2.19	1.92	1.81	1.63	1.56	1.48	1.44	1.38	1.28			
2	2.08	1.81	1.69	1.61	1.50	1.43	1.34	1.25	1.17			
3		1.94	1.88	1.73	1.62	1.49	1.45	1.34	1.31			
4			1.82	1.71	1.59	1.49	1.41	1.35	1.24			
5				1.71	1.61	1.51	1.43	1.33	1.27	1.16	1.10	
6					1.63	1.52	1.44	1.36	1.27	1.23	1.16	1.09
7					1.60	1.51	1.44	1.34	1.27	1.20	1.14	1.09
8						1.52	1.43	1.34	1.28	1.22	1.12	1.11
9						1.52	1.43	1.34	1.26	1.21	1.15	1.10
10						1.52	1.44	1.35	1.27	1.21	1.15	1.07
11							1.44	1.32	1.28	1.21	1.15	1.10
12									1.28*	1.21*	1.15*	1.10*
14									1.28*	1.21*	1.15*	1.10*
16										1.21*	1.14*	1.11*
$\bar{x}$	2.13	1.90	1.82	1.69	1.60	1.51	1.44	1.34	1.28	1.21	1.14	1.10
s	0.06	0.06	0.09	0.05	0.05	0.04	0.06	0.04	0.05	0.02	0.02	0.01

* From $K = (k'_{n+2}/k'_n)^{1/2}$.

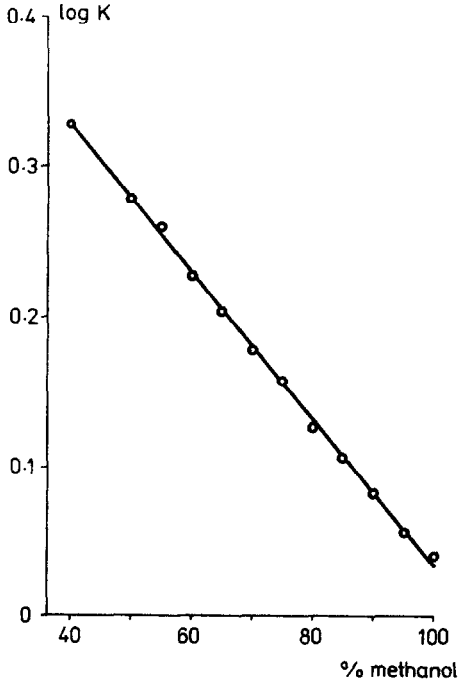

Fig. 2. Dependence of log K (mean values) on methanol concentration in the mobile phase.

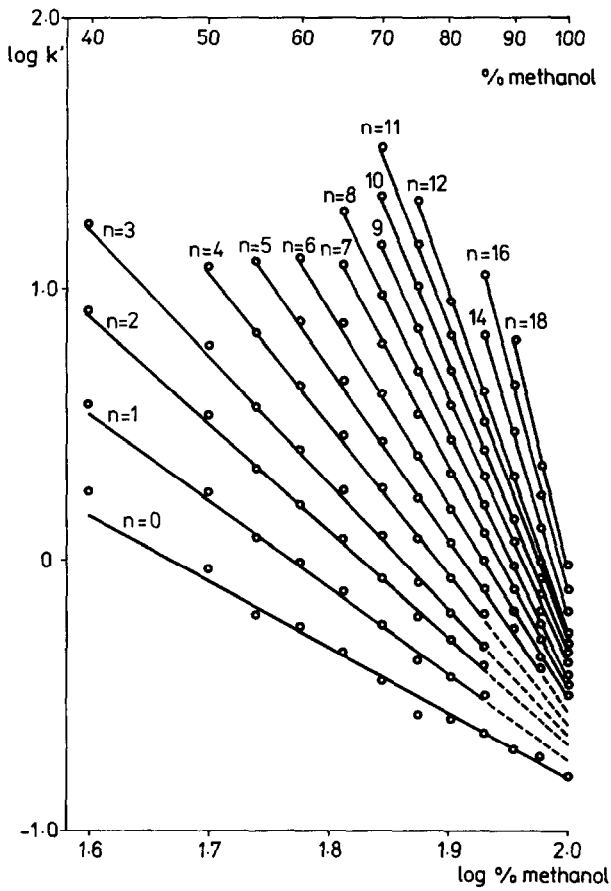

Fig. 3. Dependence of log k' on log (per cent methanol) for non-branched N-alkylphthalimides with alkyl chains containing n carbon atoms.

Fig. 4 shows the plot of B_n *vs.* n (carbon number). It is evident that the slope B_n is a linear function of carbon number and thus

$$B_{n_2} - B_{n_1} = \beta(n_2 - n_1) \tag{3}$$

and using eqn. 2

$$\beta = \frac{B_{n_2} - B_{n_1}}{n_2 - n_1} = \frac{\log (k'_{n_2c_1}/k'_{n_2c_2}) - \log (k'_{n_1c_1}/k'_{n_1c_2})}{(n_2 - n_1) \log (c_2/c_1)} \tag{4}$$

The capacity factor $k'_{n_3c_3}$ of a homologue containing n_3 carbon atoms at methanol concentration c_3 can be expressed according to eqn. 2 as

$$\log k'_{n_3c_3} = B_{n_3} \log (c_1/c_3) + \log k'_{n_3c_1} \tag{5}$$

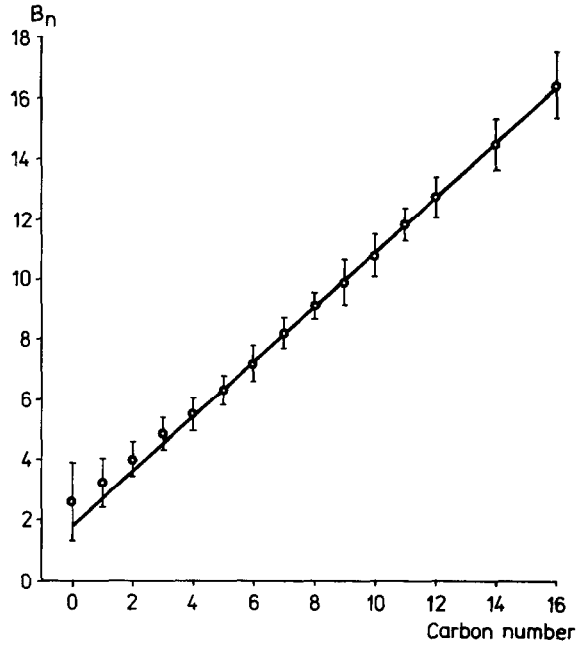

Fig. 4. Dependence of factor B_n (eqn. 2) on alkyl carbon number. Bars indicate standard deviations.

Using eqn. 4

$$B_{n_3} = \beta(n_3 - n_1) + B_{n_1} =$$

$$= \frac{B_{n_2} - B_{n_1}}{(n_2 - n_1)}(n_3 - n_1) + B_{n_1} =$$

$$= \frac{(n_2 - n_3)\log(k'_{n_1c_1}/k'_{n_1c_2}) + (n_3 - n_1)\log(k'_{n_2c_1}/k'_{n_2c_2})}{(n_2 - n_1)\log(c_2/c_1)} \quad (6)$$

According to eqn. 1, $k'_{n_3c_1}$ is equal to

$$k'_{n_3c_1} = k'_{n_1c_1} K_{c_1}^{(n_3 - n_1)}, \text{ hence} \quad (7)$$

$$\log k'_{n_3c_1} = \log k'_{n_1c_1} + (n_3 - n_1)\log K_{c_1} =$$

$$= \log k'_{n_1c_1} + \frac{(n_3 - n_1)}{(n_2 - n_1)}\log\frac{k'_{n_2c_1}}{k'_{n_1c_1}} \quad (8)$$

Inserting eqns. 6 and 8 (expressions for B_{n_3} and $\log k'_{n_3c_1}$, respectively) into eqn.

TABLE III

CALCULATED k' VALUES OF NON-BRANCHED N-ALKYLPHTHALIMIDES

Alkyl group	*Methanol in mobile phase (%)*																		
	10	*15*	*20*	*25*	*30*	*35*	*40*	*45*	*50*	*55*	*60*	*65*	*70*	*75*	*80*	*85*	*90*	*95*	*100*
H	16.19	7.24	4.09	2.63	1.83	1.35	1.03	0.82	0.66	0.55	0.46	0.39	0.34	0.30	0.26	0.23	0.21	0.19	0.17
Methyl			19.01	10.00	5.91	3.79	2.58	1.84	1.36	1.03	0.80	0.64	0.52	0.42	0.35	0.29	0.25	0.21	0.18
Ethyl					19.14	10.70	6.46	4.14	2.78	1.94	1.40	1.03	0.78	0.60	0.47	0.38	0.30	0.25	0.20
Propyl						30.16	16.17	9.33	5.71	3.66	2.44	1.68	1.19	0.86	0.64	0.48	0.37	0.29	0.22
Butyl							40.46	21.02	11.70	6.88	4.24	2.72	1.80	1.23	0.86	0.61	0.45	0.33	0.25
Pentyl								47.33	23.97	12.96	7.39	4.41	2.73	1.75	1.15	0.78	0.54	0.38	0.27
Hexyl										24.39	12.89	7.14	4.14	2.50	1.55	0.99	0.65	0.44	0.30
Heptyl											22.41	11.58	6.29	3.56	2.09	1.27	0.79	0.51	0.33
Octyl												18.78	9.54*	5.08	2.82	1.62	0.96*	0.59	0.37
Nonyl												30.44	14.47	7.24	3.79	2.06	1.16	0.68	0.40
Decyl													21.96*	10.33	5.11	2.63	1.41*	0.78	0.45
Undecyl													33.32	14.74	6.87	3.36	1.71	0.90	0.49
Dodecyl														21.03	9.26	4.28	2.07	1.04	0.54
Tridecyl														30.00	12.46	5.46	2.51	1.20	0.60
Tetradecyl															16.78	6.97	3.04	1.39	0.66
Pentadecyl															22.60	8.89	3.69	1.60	0.73
Hexadecyl																11.33	4.47	1.85	0.80
Heptadecyl																14.46	5.41	2.14	0.89
Octadecyl																18.44	6.56	2.47	0.98
Nonadecyl																	7.95	2.85	1.08
Eicosyl																	9.64	3.29	1.19

* Values used as reference data.

5 we obtain:

$$\log k'_{n_3c_3} = \frac{(n_2 - n_3)\log(k'_{n_1c_1}/k'_{n_1c_2}) + (n_3 - n_1)\log(k'_{n_2c_1}/k'_{n_2c_2})}{(n_2 - n_1)} \times$$

$$\frac{\log(c_1/c_3)}{\log(c_2/c_1)} + \log k'_{n_1c_1} + \frac{(n_3 - n_1)}{(n_2 - n_1)} \log \frac{k'_{n_2c_1}}{k'_{n_1c_1}} \qquad (9)$$

Eqn. 9 is a general formula for calculating the capacity factor $k'_{n_3c_3}$ of any homologue containing n_3 carbon atoms at any concentration c_3 of methanol in the mobile phase. The only data necessary for the calculation are four measured capacity factors of two homologues containing n_1 and n_2 carbon atoms at two different mobile phase compositions c_1 and c_2, respectively, on the same column and the same temperature. In practice, it requires two injections of a mixture of the two homologues at two mobile phase compositions.

The right-hand side of eqn. 9 consists of three terms; the first one expresses the concentration dependence of the capacity factor and the other two are logarithmic expressions of eqn. 1 giving the capacity factor dependence on the carbon number. If $c_3 = c_1$, eqn. 9 simplifies to eqn. 1.

On the other hand, eqn. 9 can be used to calculate the mobile phase composition for a homologue containing n_3 carbon atoms and eluting at a chosen retention time given by capacity factor $k'_{n_3c_3}$ (eqn. 10).

$$\log c_3 = \log c_1 - \frac{(n_2 - n_1)\log(k'_{n_3c_3}/k'_{n_1c_1}) - (n_3 - n_1)\log(k'_{n_2c_1}/k'_{n_1c_1})}{(n_2 - n_3)\log(k'_{n_3c_3}/k'_{n_1c_2}) + (n_3 - n_1)\log(k'_{n_2c_1}/k'_{n_2c_2})} \times$$

$$\log \frac{c_2}{c_1} \qquad (10)$$

Because of the non-linearity of the lowest homologues it is more suitable to use values of the capacity factors of higher homologues for the calculation ($n \geq 3$).

Table III summarizes the capacity factor values recalculated according to eqn. 9 based on values $n_1 = 8$, $n_2 = 10$, $c_1 = 70\%$ methanol, $c_2 = 90\%$ methanol. Hence from Table I $k'_{n_1c_1} = 9.54$, $k'_{n_1c_2} = 0.96$, $k'_{n_2c_1} = 21.96$ and $k'_{n_2c_2} = 1.41$, *e.g.* the reference compounds were N-octyl- and N-decylphthalimides.

The measured data agree well with those calculated according to eqn. 9 (*cf.* Table I and III), especially for higher members of the homologous series and at high percentages of methanol.

It is necessary to emphasize that all the data were obtained without temperature control. Along with the problems of achieving equilibrium on the column, this might cause the slight deviations observed. The difficulties with determining accurate values of the dead retention time t_0 (discussed elsewhere[2]) can also affect the capacity factor values, especially those of homologues eluting at short retention times.

Eqn. 9 has been based on the assumption of a linear dependence of $\log k'$ on the percentage methanol in the mobile phase. It is probable that a more exact expression could be written by using polarity or another related thermodynamic quantity

instead of concentration. Even so, the agreement of the calculated and measured capacity factors listed in Tables I and III is fairly good.

The importance of eqn. 9 consists in the possibility of estimating the approximate retention behaviour of any member of a homologous series at any mobile phase composition. The acquisition of the data necessary is a simple matter of two injections.

The validity of this equation for other homologous series is under further investigation.

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