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Reversed-phase high-performance liquid chromatography in the investigation of the hydrophobicity of selected ketones

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SUMMARY

Reversed-phase high-performance liquid chromatography was used to compare the hydrophobicities of ketones, the capacity factor, which was determined by extrapolation to 100% water in the mobile phase, being applied as the hydrophobic parameter. Different methods (linear, quadratic and exponential) of performing the extrapolation were compared. The chromatographic data obtained were compared with the solubilities in water, the topological indexes, the partition coefficients calculated on the basis of Rekker fragmentary constants and the electron density, which was calculated by quanto-mechanical methods

INTRODUCTION

In studies of the hydrophobicity of organic compounds[1,2] the logarithm of the capacity factor (log k'_w) extrapolated to 100% water as the eluent is widely used. In a previous study[3], log k'_w values were utilized for the determination of the hydrophobicities of isomeric methylquinolines. Attention was paid to the deviations from the linear relationship between log k' and the mobile phase composition.

According to Snyder *et al.*[4], in most instances there is a determined field of the mobile phase composition in which the following equation is valid:

$$\log k' = a\varphi + b \tag{1}$$

where φ is the volumetric fraction of the organic modifier in the mobile phase.

The utilization of the solubility parameter concept by Schoenmakers *et al.*[5,6] resulted in the quadratic equations

$$\log k' = a\varphi^2 + b\varphi + c \tag{2}$$
$$\log k' = a\varphi^2 + b\varphi + c + e\sqrt{\varphi} \tag{3}$$

Taking into account the double mechanism of retention[7], the following relationship has been introduced:

$$k' = a \exp (b\varphi) + (c + d\varphi)^{-1} \tag{4}$$

Many other relationships between the capacity factor and the composition of the mobile phase have been established[8,9].

Deviations from linearity are mainly caused by the double mechanism of retention[7,10] or by intermolecular interactions[11,12] and in most instances they decrease with increase in the mobile phase polarity.

In this work, an attempt was made to determine the range of mobile phase compositions in which linear extrapolations could be carried out with the required accuracy.

EXPERIMENTAL

The investigated ketones were of analytical-reagent grade from E. Merck (Darmstadt, F.R.G.), Fluka (Buchs, Switzerland) and Koch-Light Labs. (Colnbrook, U.K.). Mobile phases were prepared from analytical-reagent grade methanol and redistilled water in different volume proportions. The chemically bonded stationary phases were used, one with an irregular particle shape and a carbon content of 17.5% (Separon SIX C_{18}) and the other with spherical particles and a carbon content of 18.0% (Separon SGX C_{18}), both from Laboratorní přístroje (Prague, Czechoslovakia). The columns were 150 mm × 3.3 mm I.D. and the particle diameter was *ca.* 5 μm. A liquid chromatograph produced by Laboratorní přístroje with a UV detector set at 254 nm was used. The column void volume was determined by the injection of sodium nitrite dissolved in methanol. The mobile phase flow-rate was 0.7 ml/min. The results obtained represent the means of five measurements.

In order to determine the solubility, saturated solutions of the ketones in water were prepared. After shaking the ketones with water in test-tubes for 5 min, the test-tubes were left for 24 h at 20°C for equilibration. A series of standard ketone solutions in water was prepared and the calibration graph at maximum absorption was constructed. Next, a specified volume of the saturated aqueous solution was taken and its ketone content was determined by spectrophotometry.

RESULTS AND DISCUSSION

The capacity factors were determined for methanol–water volume fractions from 1.0:0 to 0.4:0.6. The investigations were carried out with a series of fifteen ketones, including five isomeric ketone pairs on two stationary phases. The relationship between log k' and mobile phase composition for the five selected ketones is presented in Fig. 1.

The lines in Fig. 1 intersect, so that a change of selectivity is observed while changing the mobile phase composition. The data in Table I for log k'_w extrapolated from the various mobile phase composition ranges depend on the range considered and on the nature of the stationary phase used. It was our aim to extrapolate reliably the log k' values for solutes chromatographed in pure water.

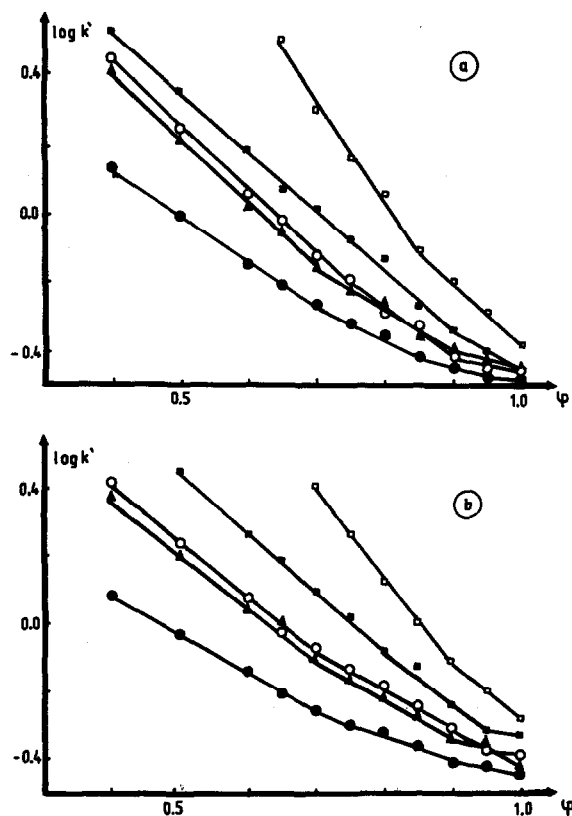Trials were made to solve this problem with two models, as follows.

Fig. 1. Log k' vs. volumetric fraction of methanol in water, on (a) Separon SIX C_{18} and (b) Separon SGX C_{18}. ● = Ethyl methyl ketone; ○ = diethyl ketone; ▲ = isopropyl methyl ketone; ■ = isobutyl methyl ketone; □ = phenyl isobutyl ketone.

TABLE I

VALUES OF LOG k'_w OBTAINED FROM THE STRAIGHT-LINE EXTRAPOLATION IN THE VARIOUS RANGES OF THE MOBILE PHASE COMPOSITION FOR THE SELECTED PAIR OF ISOMERS

Range of the mobile phase composition (volume fraction of methanol)	Log k'_w			
	Separon SIX C_{18}		Separon SGX C_{18}	
	Diethyl ketone	Isopropyl methyl ketone	Diethyl ketone	Isopropyl methyl ketone
1.00–0.80	0.413	0.499	0.645	0.589
0.85–0.65	0.977	0.882	0.737	0.785
0.70–0.40	1.040	0.992	1.060	0.998

Model I

According to the assumption made, the value of the capacity factor is influenced, apart from by incidental factors, also by the systematic factor caused by interactions as described above. The result is a deviation of the experimental points from simple regression. These deviations are minor for the polar mobile phase but they rise following an increase in the methanol content. Consequently, the points corresponding to the mobile phase compositions with high organic modifier contents lie beyond those confidence curves which are calculated for the chosen field of probability. The rejection of those points results in abandoning the systematic error and shifting towards the mobile phase with a greater polarity.

The regression line

$$\log k' = \alpha\varphi + \beta \tag{5}$$

was determined and then the confidence interval for $\log k'$ according to published equations[13]. The value of the Student's variable t_y was taken from tables for the confidence coefficient $1 - \gamma = 95\%$ and for $n - 2$ degrees of freedom. Calculations were made according to the algorithm in Fig. 2. The highest value of the volumetric fraction of methanol in the mobile phase φ_i, at which the $\log k'_i$ lies inside the error channel, and the value of $\log k'_w = \beta$ were determined. β is the value of the intersection of the regression line in the interval $\langle \varphi_1, \varphi_i \rangle$; in our case $\varphi_1 = 0.4$. The values of φ_i and the extrapolated value of β are given in Table II.

A trial to correlate the φ_i value with the partition coefficients calculated on the basis of Rekker fragmentary constants[14], $\log P_{Rek.}$, was made. For the results achieved using the Separon SIX C_{18} column, the following relationship was obtained:
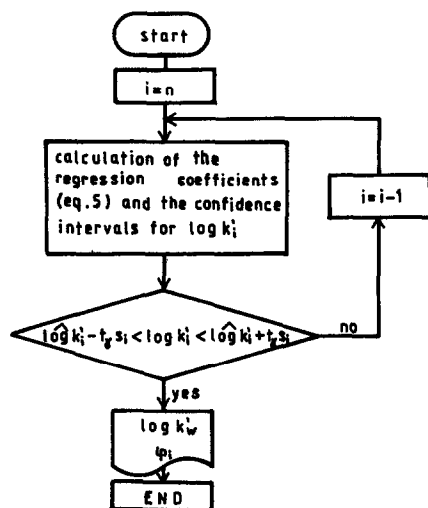

Fig. 2. Algorithm for estimation of the mobile phase composition φ_i for which the corresponding $\log k'_i$ value appears within the confidence interval; $\varphi_n = 1.0$; step $= 0.05$; $\log k'$ is the value determined from the regression eqn. 5.

TABLE II

CALCULATED CORRELATION PARAMETERS FOR EQN. 5 FROM MODEL I AND FOR EQNS. 1, 2 AND 11 FROM MODEL II ON TWO STATIONARY PHASES

Substance	Stationary phase[a]	Model I		Model II		
		φ_i	Eqn. 5: β	Eqn. 1: b	Eqn. 2: c	Eqn. 11: $a' + c'$
Diethyl ketone	1	0.80	1.04	0.92	1.32	1.26
	2	0.75	1.04	0.88	1.32	1.24
Isopropyl methyl ketone	1	0.85	0.96	0.87	1.24	1.18
	2	0.90	0.92	0.86	1.23	1.16
Ethyl propyl ketone	1	0.90	1.31	1.24	1.54	1.49
	2	0.95	1.27	1.23	1.52	1.48
Isobutyl methyl ketone	1	0.95	1.22	1.18	1.43	1.38
	2	0.95	1.21	1.23	1.55	1.43
Dipropyl ketone	1	0.90	1.98	1.90	2.48	2.36
	2	0.95	1.96	1.91	2.67	2.52
Methyl pentyl ketone	1	0.85	2.18	2.01	2.80	2.65
	2	0.95	2.02	1.96	2.68	2.58
Phenyl ethyl ketone	1	0.90	1.90	1.80	3.33	2.99
	2	0.90	1.91	1.81	2.50	2.37
Benzyl methyl ketone	1	0.85	1.66	1.37	2.83	2.54
	2	0.85	1.62	1.55	2.20	2.10
Phenyl isobutyl ketone	1	0.90	2.38	2.17	3.89	3.52
	2	0.95	2.36	2.26	4.29	3.84
Benzyl ethyl ketone	1	0.90	1.99	1.80	3.44	3.08
	2	0.95	1.92	1.72	3.03	2.97
Ethyl methyl ketone	1	0.75	0.53	0.42	1.12	0.96
	2	0.70	0.54	0.39	0.82	0.71
Cyclohexanone	1	0.80	0.83	0.51	1.61	1.48
	2	0.85	0.81	0.47	1.13	1.01
Diisobutyl ketone	1	1.00	2.36	2.36	3.71	3.37
	2	1.00	2.50	2.50	3.23	3.04
Cyclopropyl methyl ketone	1	0.80	0.74	0.54	1.35	1.16
	2	0.85	0.59	0.49	1.00	0.87
Phenyl cyclobutyl ketone	1	1.00	2.43	2.43	3.79	3.40
	2	0.95	2.41	2.35	2.94	2.80
Average correlation coefficient (r)		–	0.991	0.934	0.988	0.997

[a] 1 = Separon SIX C_{18}; 2 = Separon SGX C_{18}.

$$\varphi_i = 0.070 \log P_{Rek.} + 0.77 \qquad (6)$$
$$n = 15, \qquad r = 0.810$$

and for the Separon SGX C_{18} column:

$$\varphi_i = 0.072 \log P_{Rek.} + 0.79 \tag{7}$$
$$n = 15, \qquad r = 0.788$$

These equations allow the prediction of the composition of the mobile phase for which the dependence of $\log k'$ vs. φ is rectilinear. This refers to any substance. Moreover, minor difference in the composition of the stationary phases do not have much importance as regards the results achieved. There are similar coefficients in eqns. 6 and 7.

The procedure described above can be regarded as a model for the approximate determination of the lower limit of the mobile phase composition from which linear extrapolation can be carried out.

Model II

The relationship $\log k' = f(\varphi)$ is parabolic, with a minimum for high concentrations of methanol in the mobile phase. The relationship is well depicted in this field by eqn. 2; with an increase in the polarity of the mobile phase, the linear eqn. 1 becomes valid. If the coefficients in eqn. 2 are changed and the equation is presented in the form

$$\log k' = \frac{a'}{2} \varphi^2 + (a' + b')\varphi + (a' + c') \tag{8}$$

then

$$\log k' = a'\left(1 + \varphi + \frac{\varphi^2}{2}\right) + b'\varphi + c' \tag{9}$$

in which the expression

$$1 + \varphi + \frac{\varphi^2}{2} \tag{10}$$

is an approximate development of the $\exp(\varphi)$ function into a Taylor series. As φ takes its values from the $\langle 0,1 \rangle$ interval, this approximation can be applied successfully, and then eqn. 9 takes the form

$$\log k' = a' \exp(\varphi) + b'\varphi + c' \tag{11}$$

Eqn. 11 is a good approximation of a straight line for low values (it has its oblique asymptote at $\varphi \to -\infty$); at higher values it is a good approximation of a parabola, especially for $\varphi \approx 1$.

In Table II the $\log k'_w$ values have been presented, with the following terms: the coefficient b from eqn. 1, the coefficient c from eqn. 2 and the sum of the coefficients $a' + c'$ from eqn. 11. In this instance the $\log k'_w$ values are higher than those calculated on the basis of eqn. 1 and lower than those calculated from eqn. 2. It can be seen from the comparisons of the correlation coefficients that the best estimation of the $\log k'$ vs. φ relationship was obtained by applying eqn. 11. However, the $\log k'_w$ hydrophobic

TABLE III

DIFFERENT PHYSICO-CHEMICAL PARAMETERS USEFUL IN A COMPARISON OF HYDROPHOBICITY

Substance	Log $P_{Rek.}$	Log c_M	Wiener's index, W	Connectivity index, χ	Balaban's index, IB	Net charge on carbonyl carbon atom, q_c	Net charge on oxygen atom, q_o	Difference of net charges on the carbonyl atoms, $q_c - q_o$
Diethyl ketone	0.768	-0.194	27.8	2.53	3.04	0.261	-0.280	0.0658
Isopropyl methyl ketone	0.652	-0.110	25.8	2.35	3.32	0.250	-0.263	0.0731
Ethyl propyl ketone	1.290	-0.703	46.1	3.03	3.04	0.268	-0.284	0.0650
Isobutyl methyl ketone	1.180	-0.640	44.1	2.82	3.16	0.247	-0.264	0.0761
Dipropyl ketone	1.880	-1.428	70.5	3.53	3.09	0.250	-0.269	0.0646
Methyl pentyl ketone	1.880	-1.558	74.5	3.47	2.85	0.244	-0.264	0.0647
Phenyl ethyl ketone	2.130	-1.438	94.8	4.58	2.86	0.265	-0.269	0.0612
Benzyl methyl ketone	1.430	-1.335	99.8	4.48	2.67	0.237	-0.258	0.0713
Phenyl isobutyl ketone	2.540	-1.946	127.3	4.97	2.86	0.258	-0.271	0.0668
Benzyl ethyl ketone	1.960	-1.728	140.3	5.04	2.58	0.254	-0.263	0.0699

parameters obtained by extrapolation according to eqn. 2 and likewise eqn. 11 result in excessive and divergent values compared with the two stationary phases being investigated. The best conformity of the results was obtained when applying a linear extrapolation in the range of mobile phase compositions determined according to model I. This means that in order to obtain better hydrophobic data in terms of quantity, the linear extrapolation must be carried out in an interval of log k' vs. φ relationship such that deviations of the points can only be the result of incidental errors.

In Table III, log $P_{Rek.}$ and values of the Wiener topological index[15], Balaban's index[16], the connectivity index χ[17] and the solubilities in water, log c_M (c_M is the molar concentration of the saturated ketone in water), are given. The q_C, q_O and $q_{CO} = q_C - q_O$ values are also given, where q_C is the net charge on the carbon atom of the carbonyl group, q_O is the net charge on the oxygen atom and q_{CO} is the difference of net charges on the carbonyl C and O atoms.

The net charges were calculated following the CNDO/2 method. The parametric characteristic was drawn up on data taken from the literature[18]. Of the net charges on various atoms, the value of q_{CO} showed the greatest conformity with the hydrophobicity sequence for isomer pairs.

The following equations express the relationships between log k'_w determined according to model I on the Separon SIX C_{18} phase and the data in Table III:
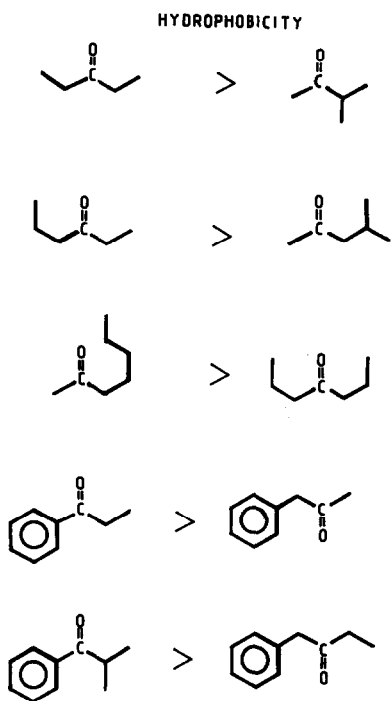

Fig. 3. Schematic comparison of hydrophobicities of the investigated isomers.

$$\log k'_w = 1.18 \log P_{Rek.} - 0.38; \qquad r = 0.959 \qquad (12a)$$
$$\log k'_w = -1.13 \log c_M + 0.84; \qquad r = 0.966 \qquad (12b)$$
$$\log k'_w = 0.015 \, W + 0.50; \qquad r = 0.804 \qquad (12c)$$
$$\log k'_w = 1.62 \, \chi + 1.0; \qquad r = 0.787 \qquad (12d)$$
$$\log k'_w = -0.28 \, IB + 3.41; \qquad r = 0.616 \qquad (12e)$$
$$\log k'_w = -49 \, q_{CO} + 5.03; \qquad r = 0.454 \qquad (12f)$$

The comparisons that were carried out showed that the chromatographic data produced gave good correlations in terms of quantity with such hydrophobic parameters as solubility in water and the partition coefficient between n-octanol and water. Topological indices and quanto-mechanical data do not result in good correlations with $\log k'_w$ for a whole group of compounds. They can, however, be used successfully for qualitative comparisons of hydrophobicity within the field of isomer pairs.

From the comparisons of the results obtained, it can be concluded (Fig. 3) that the hydrophobicity of organic compounds is smaller for isomers with branched hydrocarbon chains and greater for those isomers which have only one, but a considerably longer, hydrocarbon chain, e.g., see methyl pentyl ketone and dipropyl ketone. The hydrophobicity is also smaller for those molecules in which diffusion of the electron charge due to inductive or resonance effects occurs.

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