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POSSIBILITIES OF DETERMINATION AND PREDICTION OF SOLUTE CAPACITY FACTORS IN REVERSED-PHASE SYSTEMS WITH PURE WATER AS THE MOBILE PHASE

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SUMMARY

A sorption and an elution experimental method were developed and tested for the determination of solute capacity factors in pure water as the mobile phase in reversed-phase systems. The experimental values determined by the sorption method are generally higher than those determined by the elution method, but the two types of values are strongly correlated. These correlations may be used for corrections of the experimental capacity factors to suit either chromatographic or sorption conditions.

The log k' dependencies on methanol concentration are adequately described by quadratic equations for various pesticides tested over a range of mobile phase compositions from 2 to 90% methanol in water, but the extrapolation to zero methanol concentration yields systematically lower extrapolated capacity factors, k'_{H_2O}. The extrapolated values depend strongly on the range of the experimental k' and on the range of methanol concentrations used for the determination of the experimental data points, and neither linear nor quadratic extrapolation yield correct k'_{H_2O} values. The accuracy of predicted extrapolated k'_{H_2O} improves as the concentration range of methanol approaches zero, but the time of determination and experimental difficulties increase simultaneously. The accuracy of the predicted k'_{H_2O} values from the low methanol concentration range can be improved using correlations with the k'_{H_2O} values determined experimentally. The correlation equations are determined for a set of (at least three) standard compounds and apply only for a given sorbent.

Correlations of log k'_{H_2O} with solute lipophilic indices, n_{ce}, yield better predictions than similar correlations with logarithms of solute solubilities, with comparable errors in the predicted k'_{H_2O} values as for the values extrapolated from the data measured in the range of medium methanol concentrations.

INTRODUCTION

The values of the capacity factors of sample solutes in pure water, k'_{H_2O}, in reversed-phase systems are useful both for correlations with the parameters characterizing solute lipophilicities[1–9] and for the evaluation of various materials as sorbents

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for solid-phase extraction enrichment techniques treating dilute aqueous samples[9–12].

Quantitative characterization of the lipophilicity is important in quantitative structure–activity relationship (QSAR) investigations. Solute lipophilicities are most often characterized by the partition coefficients P in *n*-octanol–water partitioning systems, which are determined conventionally using laborious and time-consuming shaking flask methods. More recent and more rapid determination of P is based on the Collander-type correlation equations[13] between log P and the logarithms of sample capacity factors, $k' = V_R/V_0 - 1$, in reversed-phase high-performance liquid chromatographic (HPLC) systems[14] (V_R is the elution volume of a sample solute and V_0 is the column void or dead volume, *i.e.*, the elution volume of a non-retained solute). Some workers have found that log k'_{H_2O} determined by the extrapolation of experimental log k' *versus* φ plots to pure water yield better correlations with log P than the log k' values measured at a single concentration of methanol, φ, in volume units, in the mobile phase[1–4,8,9]. From the known values of k'_{H_2O}, breakthrough volumes and sorbent capacity may be calculated for the trace enrichment of organic compounds from aqueous samples on non-polar sorbents[11].

The direct experimental determination of k'_{H_2O} is unfortunately fraught with serious difficulties, as these values are of the order of magnitude of 10^2–10^5 or even greater, depending on the size and polarity of the solute. Predictive calculations of k'_{H_2O} based on the correlations with some physical constants or with the retention data in mixed aqueous–organic mobile phases in reversed-phase systems would speed up and facilitate the determination of k'_{H_2O}.

Theories based both on solubility parameters[15,16] and on interaction indices[17] predict quadratic dependences of solute log k' on φ in reversed-phase systems:

$$\log k' = a - m\varphi + d\varphi^2 \tag{1}$$

where a, m and d are constants depending on the solute and the chromatographic system. Eqn. 1 may often be simplified by neglect of the quadratic term over a limited range of mobile phase compositions[15,18,19]:

$$\log k' = a - m\varphi \tag{2}$$

Even the quadratic eqn. 1 does not give an accurate description of the solute retention in mobile phases with concentrations of the organic solvent close to zero, which is attributed to preferential sorption of the organic solvent on the non-polar surface of the column packing material[20], and it was suggested that an additional term with $\sqrt{\varphi}$ be added to the right-hand side of eqn. 1 to compensate for this effect[21].

In practice, curved log k' *versus* φ plots are observed if the full range of mobile phase compositions is investigated[6,21–23] and even minima of retention occur with some strongly polar[22] or partially ionized[23] solutes at certain φ, owing to a mixed retention mechanism in methanol-rich mobile phases. Consequently, the values of k'_{H_2O} determined by extrapolation may be subject to gross errors and would depend not only on the function fitted to the experimental data, but also on the range of concentrations, φ, over which these data are measured.

In addition to the extrapolation of the experimental data measured in mixed aqueous–organic mobile phases, several other methods for the prediction of k'_{H_2O} have

been reported. Werkhoven-Goewie *et al.*[11] tried to calculate k'_{H_2O} from a single value of k' measured at a given concentration φ of the organic solvent in the mobile phase, using the correlation between the parameters a and m in eqn. 2[24,25]:

$$m = p + qa = p + q \log k'_{H_2O} \tag{3}$$

After combination of the eqns. 2 and 3 they obtained the following equation for the predictive calculation of k'_{H_2O} from a single value of k' measured at a given mobile phase composition, φ:

$$\log k'_{H_2O} = \frac{\log k' + p\varphi}{1 - q\varphi} \tag{4}$$

This method can be applied only with a series of structurally closely related compounds, as it has been found that p and q are really constant only in a given homologous[26] or oligomeric[27] series.

Wells and co-workers[5,6] employed the retention model based on the solvophobic theory[28] for calculations of k'_{H_2O} from an equation correlating log k' with the surface tension and the dielectric constant of the mobile phase and a microscopic cavity factor depending on the solute, after extrapolation of the correlation plots to $\varphi = 0$. These studies were limited to structurally related homologous N-alkylbenzamides[5] and 5,5-disubstituted barbiturates[6] in acetonitrile–water mobile phases and the failure of this approach for methanol–water mobile phases has been reported[6].

Thurman *et al.*[29] found a significant correlation ($r_k = 0.9$) between log k'_{H_2O} and $-\log S$ (S = solubility in water) for twenty aromatic, aliphatic and alicyclic compounds on a polymeric organic sorbent, Amberlite XAD-8, and suggested the use of such correlations for the prediction of k'_{H_2O}.

The determination of the solute capacity factors in pure water is still a difficult problem, which we found useful to investigate in more detail in connection with research aimed at the development of new HPLC methods for the trace determination of pesticides and related compounds in connection with sample enrichment by solid-phase extraction. Because of the weak elution strength of pure water in reversed-phase systems, columns as short as possible should be used to reduce the time necessary for the experimental determination of k'_{H_2O}[21]. We have developed and compared an elution and a sorption method for this purpose. As these methods are more time consuming than a conventional HPLC determination of k' in a mixed aqueous–organic mobile phase and because a special instrumental set-up is required, we investigated the possibility of extrapolating k'_{H_2O} from the experimental data in mixed aqueous–organic mobile phases in various composition ranges. For this purpose, "common" HPLC conditions, *i.e.*, the range from 30 to 80% methanol, would be especially useful, as they do not impose excessive elution times. Further, we investigated the possibility of improving the accuracy of prediction using various correlations between the experimentally determined k'_{H_2O} and either the extrapolated parameters a in eqns. 1 and 2 or some physical constants, such as solubilities and lipophilic indices.

EXPERIMENTAL

Instrumentation

(A) The equipment used for the determination of the capacity factors of sample solutes in mobile phases containing 15–90% methanol in water consisted of a Model 6000A pump, a U6K variable-volume injector, a Model 440 UV detector operated at 254 nm (all from Waters–Millipore, Milford, MA, U.S.A.), a TZ 4221 line recorder and a CI 100 computing integrator (both from Laboratory Instruments Works, Prague, Czechoslovakia) and a stainless-steel column (300 × 3.6 mm I.D.) packed in the laboratory with Silasorb C_{18} octadecylsilica gel (7.5 μm) (Lachema, Brno, Czechoslovakia) using a high-pressure slurry packing technique.

(B) For the determination of the capacity factors of sample solutes in mobile phases containing 0–20% methanol in water, the equipment in (A) was modified as follows. (1) A micro flow-through cell with a 1.9-μl inner volume was used in the M 440 detector instead of the standard flow-through cell with a 15.5-μl inner volume. (2) An LCI-30 sampling valve (Laboratory Instrument Works) with an inner sample loop of volume 0.783 μl was used instead of the U6K injector. The exact volume of the sample loop was determined using comparison of the peak areas of a standard solution injected via the LCI-30 valve with the peak areas of the same compounds obtained when several different known volumes of the standard solution were injected via the U6K injector. (3) A stainless-steel microcolumn (30 × 1 mm I.D. packed in the laboratory with Silasorb C_{18} (7.5 μm) was used instead of the conventional analytical column. (4) The M 6000 pump was controlled by an M 660 solvent programmer via a micro-flow module (Waters), which made it possible to reduce the conventional operating range of the pump (0.1–9.9 ml/min) to 10% or 1% of these values.

The reproducibility of the flow-rate was *ca.* 1% relative at the flow-rates used (50–200 μl/min); the exact value of the flow-rate was determined using a microburette connected to the outlet of the detector cell and a stop-watch.

(C) The equipment for the determination of k'_{H_2O} by the sorption method was as in (A), with the following modifications. (1) A two-channel M 440 UV detector with a conventional flow-through cell in one channel and with a micro flow-through cell in the other was used. Both channels were operated at 254 nm. The channel with the conventional cell was used to monitor the sorption process and the channel with the micro cell served to detect the compounds eluted from the analytical column. (2) The analytical column was stainless steel (297 × 2.17 mm I.D., packed in the laboratory with Silasorb SPH C_{18} (7.5 μm) (Lachema). (3) An LCI-30 sampling valve used instead of the U6K injector was adapted by inserting the same microcolumn as in (B) as the sorption column instead of the external sample loop, using the shortest connecting capillaries possible (30 mm × 0.12 mm I.D.), shown in Fig. 1A (filling position) and Fig. 1B (sorption position). (4) An auxiliary MHPP 20 micro pump (Laboratory Instruments Works) was connected to the LCI-30 sampling valve via the inlet capillary (N in Fig. 1). The pump employed a special high-pressure glass syringe of 7.0-ml inner volume for solvent delivery and was used to feed the solutions of the solutes to be sorbed onto the sorption microcolumn.

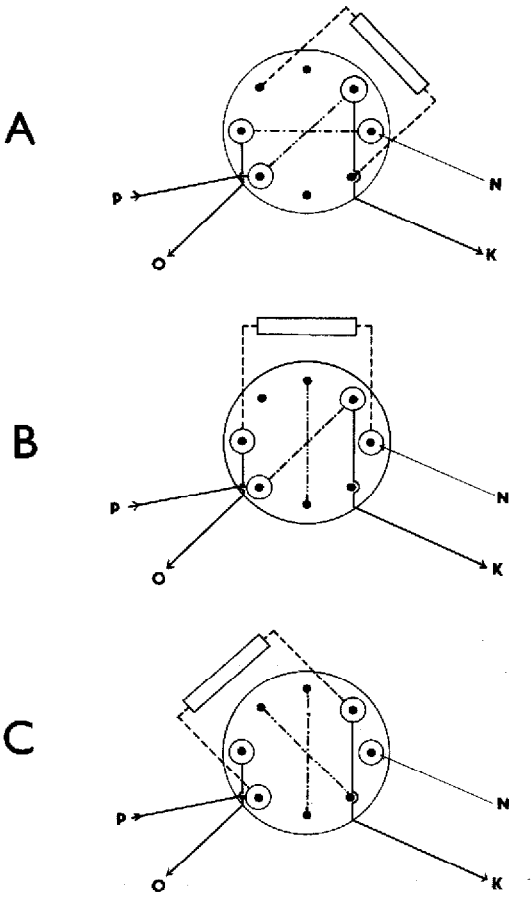

Fig. 1. The LCI-30 sampling valve with a sorption microcolumn instead of the external sampling loop. (A) Filling the internal sampling loop (0.783 μl, dash-dotted line) with the sample. (B) Injection of the sample from the internal loop on to the column or enrichment of the sample on the sorption microcolumn (dashed lines, capillary inlets of the microcolumn). (C) Desorption of the sample solute from the sorption microcolumn to the analytical column and chromatographic separation. P = Inlet from the high-pressure pump; K = outlet to the analytical column; N = filling port of the internal sampling loop for manual injection, or inlet from the auxiliary micro pump; O = outlet to the second channel of the UV detector, equipped with a conventional flow-through cell.

Chemicals

The mobile phases were either pure water or mixtures prepared by mixing water with methanol (spectroscopic grade; Lachema) in the required volume ratios. Water was deionized, doubly distilled in glass with addition of potassium permanganate and purified on a column (100 × 20 mm I.D.) packed with octadecylsilica gel Silipore 300 C_{18} (125–160 μm) (Lachema). Water and methanol were filtered using a 0.45-μm filter (Millipore) before mixing and the mobile phases were degassed by ultrasonication before use.

Table I lists the triazine, phenylurea and carbamate pesticides and related

TABLE I

LIST OF PESTICIDES AND RELATED SAMPLE COMPOUNDS USED

The values of the solute solubilities, S, are taken from ref. 30 and the values of the solute lipophilic indices, n_{ce}, from ref. 31.

No.	*Solute*	*IUPAC name*	*S (mol/l)*	n_{ce}
1	Methomyl	S-Methyl-N-(methylcarbomoyloxy)thioacetimidate	$3.58 \cdot 10^{-1}$	−2.07
2	Hydroxy-metoxuron	3-(3-Chloro-4-hydroxyphenyl)-1,1-dimethylurea	–	−0.92
3	Metoxuron	3-(3-Chloro-4-methoxyphenyl)-1,1-dimethylurea	$2.96 \cdot 10^{-3}$	0.0
4	Monuron	3-(4-Chlorphenyl)-1,1-dimethylurea	$1.16 \cdot 10^{-3}$	–
5	Carbofuran	2,3-Dihydro-2,2-dimethylbenzofuran-7-yl methylcarbamate	$3.16 \cdot 10^{-3}$	0.55
6	Simazine	6-Chloro-N,N′-diethyl-1,3,5-triazinediyl-2,4-diamine	$2.47 \cdot 10^{-5}$	0.30
7	Fluometuron	1,1-Dimethyl-3-(α,α,α-trifluoro-*m*-tolyl)urea	$4.52 \cdot 10^{-4}$	1.24
8	Atrazine	6-Chloro-N-ethyl-N′-isopropyl-1,3,5-triazinediyl-2,4-diamine	$1.39 \cdot 10^{-4}$	1.18
9	Desmetryne	N-Isopropyl-N′-methyl-6-methylthio-1,3,5-triazine	$2.72 \cdot 10^{-3}$	1.26

compounds used as the sample solutes. Stock solutions of the sample solutes were prepared in methanol as the solvent and were diluted 50-fold with the mobile phase to give working sample solutions for the experiments with methanol-containing mobile phases; working sample solutions for the experimental determination of k'_{H_2O} were prepared in pure water as the solvent.

Determination of the capacity factors using the elution method

Capacity factors in mobile phases containing 15% or more of methanol in water were measured using the equipment with the conventional analytical column (*Instrumentation*, A) at a mobile phase flow-rate of 1 ml/min. The elution volumes, V_R, were evaluated from the distance of the peak maximum from the injection point on the chromatogram and the known chart speed of the recorder and from the data of the integrator and the flow-rate of the mobile phase measured with a burette and a stop-watch. The k' values were calculated from the well known equation

$$k' = V_R/V_0 - 1 \tag{5}$$

The column dead volume, V_0, was determined in each mobile phase using ^2H_2O as the dead volume marker and an R-401 differential refractometer (Waters) as the detector. The V_0 values were in the range 2.18–2.25 ml, *i.e.*, the porosities ε_T were in the range 0.71–0.74 in mobile phases containing 15–90% methanol.

The equipment described under *Instrumentation* (B) with the short microcolumn was used for the determination of k'_{H_2O} and k' values in mobile phases containing 20% or less methanol in water. The flow-rates were set to 0.05–0.2 ml/min, depending on the retention of a solute. The chromatograms were evaluated as in the experiments with the conventional analytical column, but the column dead volume, V_0, was determined using a saturated solution of potassium bromide as the unretained marker and detection with an M 440 UV detector equipped with a micro flow-through cell, as the

volume of the unexchangeable cell in the R-401 refractometer was too large for work with the microcolumn. The volume V_M determined in this way was corrected for the extra-column contribution, V_d, which was determined from the elution volume of potassium bromide in the instrument with the LCI-30 sampling valve connected directly to the inlet capillary of the micro flow-through detector cell (V_d = 0.0103 ml): $V_0 = V_M - V_d$. The experimental values of V_0 were in the range 0.0133–0.0154 ml for mobile phases containing 0–20% methanol. The capacity factors were calculated as

$$k' = \frac{V_R - V_M}{V_0} = \frac{V_R - V_M}{V_M - V_d} \tag{6}$$

The total porosity of the microcolumn was *ca.* 10% relative lower than that of the analytical column (ε_T = 0.59–0.68). The reason for these discrepancies may be as follows: (a) different packing densities in the two columns; (b) dependence of ε_T on the mobile phase composition; (c) differences between the potassium bromide and ^2H_2O methods of determination of V_0[32]; (d) errors connected with the experimental determination of very small volumes (both the total inner volume and the dead volume of the microcolumn used were only a few microlitres, *viz.*, 23 and 13–15 μl, respectively).

As no systematic shift between the capacity factors measured on the two columns in the same mobile phases was observed and the k' values on the two columns were similar, these discrepancies are not likely to influence the results seriously.

Determination of k'_{H_2O} using the sorption method

This method is based on the equation for the definition of the capacity factor:

$$k' = \frac{m_s}{m_m} = \frac{m_s}{V_0 c_M} \tag{7}$$

where m_s is the mass of the solute in the stationary phase and m_m that in the mobile phase under equilibrium conditions.

A solution of a sample solute is pumped through the column and when the equilibrium distribution of the solute along the whole column has been achieved, m_m can be calculated as $m_m = V_0 c_M$, where c_M is the concentration of the solute in the solution pumped through the column and m_s can be determined by an appropriate analytical method after desorption by a strong solvent. For the determination of m_s we developed a method similar to that used by May *et al.*[33] for the determination of sorption isotherms. A short microcolumn (1 mm I.D.) used for the sorption of sample solutes was connected on-line via a switching valve to an analytical column (2 mm I.D.) for the determination of the desorbed solute by HPLC. This combination of columns was used in order to suppress the effect of band broadening in the sorption column on the results. Use of the same microcolumn as in the elution method for the determination of k'_{H_2O} should provide a better comparability of the results given by the two methods.

The equipment used is described under *Instrumentation* (C) and the procedure is shown schematically in Fig. 1. The capillary inlet P of the LCI-30 valve was connected to the M 6000 A pump and the inlet N to the auxiliary MHPP 20 micro pump; the

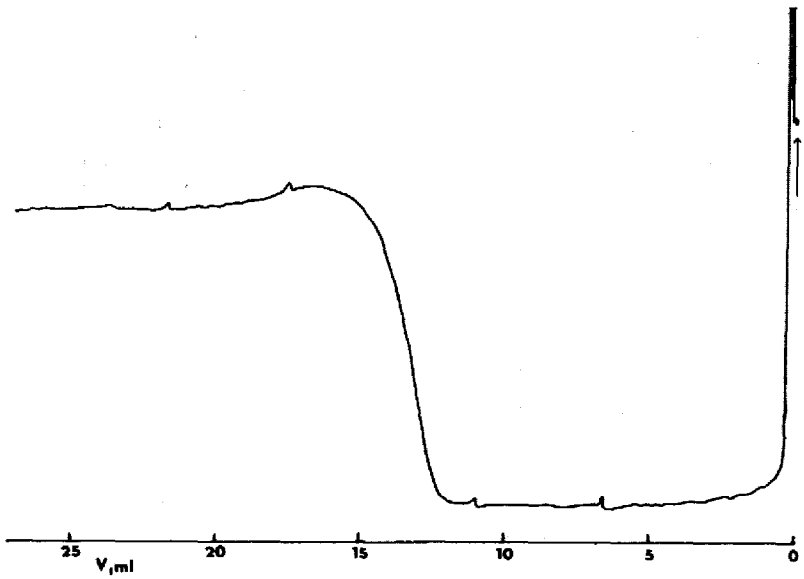

Fig. 2. Example of a sorption curve monitored in the second channel of the UV detector. Sample solution, $1.965 \cdot 10^{-7}$ mol/l monuron in water; sorption column, Silasorb C_{18}, 7.5. μm (30 × 1 mm I.D.); flow-rate of sample solution, 0.2 ml/min, from the auxiliary micro pump; detector range, 0.01 a.u.f.s. V = Volume of the solution passed through the microcolumn from the start of the experiment (marked with an arrow).

outlet capillary O was connected to the channel of the M 440 UV detector equipped with the conventional flow-through cell, while the outlet capillary K was connected via the analytical column to the other channel with the micro flow-through cell installed.

The LCI-30 valve was first switched to position B in Fig. 1 and the aqueous solution of sample solute was pumped by the micro pump through the microcolumn to the detector. The signal from the detector was registered in order to monitor the sorption curve. In the meantime, the analytical column was washed with the mobile phase from the pump M 6000 A. The end of the sorption was indicated by stabilization of the detector signal at the plateau of the sorption curve (Fig. 2). Then the valve was switched to position C and the solute was desorbed and eluted onto the analytical column and to the micro flow-through cell of the UV detector. Because of very low concentrations of the solute in the solution used for the sorption, the amount of the solute in the liquid in the column is very small in comparison with the amount of the sorbed solute, m_s, and can be neglected. The peak area of the desorbed solute was integrated using the CI-100 integrator and the amount m_s of the solute sorbed on the microcolumn was determined from the calibration graph measured under the same conditions using conventional injections of known sample amounts. After each experiment, the sorption microcolumn was washed with 1–2 ml of methanol. As was found in preliminary experiments, the sorption capacity decreased with time when the column was in continuous use without washing. The washing step using 100% methanol proved sufficient for obtaining reproducible results and maintaining the column stability. Each determination was repeated three times with different volumes of the sorbed solution, to ensure that the sorption equilibrium has been really attained.

In a set of preliminary experiments with various concentrations of the solutes in solutions to be sorbed, we found that the experimental capacity factors are independent of the concentration of sample solutes below 10^{-5} mol/l. Hence 10^{-7}–10^{-6} mol/l solution of sample solutes were used for the determination of k' by the sorption method. Mobile phases used for the desorption and HPLC determination of sorbed solutes contained 30–50 vol.% of methanol to ensure approximately equal and short elution times of the various solutes tested, but also a sufficient retention to allow the separation of sample solutes from possible impurities.

Evaluation of the results

Linear or quadratic regression curves were fitted to the experimental data sets using a programmable TI-58 calculator (Texas Instruments, Houston, TX, U.S.A.).

RESULTS AND DISCUSSION

Direct experimental determination of k'_{H_2O} values

Table II gives the experimental capacity factors of the tested pesticides and related compounds over the full composition range of mixed mobile phases (from 0 to 90% methanol) measured on a conventional analytical column (I) and on a microcolumn (II) packed with Silasorb C_{18}. The data measured on the conventional column (V) are in sufficient agreement with the data measured on the microcolumn (M), which can be considered as evidence of the reliability of the experimental methods of determination using the two columns (Fig. 3).

Table II also compares the experimental capacity factors in pure water determined using (1) the elution method with the microcolumn $k'_{H_2O}(E)$ and (2) the sorption method, $k'_{H_2O}(S)$ (method III). The experimental $k'_{H_2O}(S)$ values are approximately 30% relative higher than the $k'_{H_2O}(E)$ values. The two types of the experimental capacity factors are strongly correlated for the compounds studied by the equation

$$\log k'_{H_2O}(S) = 0.0059 + 1.0405 \log k'_{H_2O}(E) \quad (8)$$

with a correlation coefficient $r_k = 0.9993$ (Fig. 4). The slope of the correlation eqn. 8 is close to unity, which means that an approximately linear proportionality exists between $k'_{H_2O}(S)$ and $k'_{H_2O}(E)$. This suggests that the reason for the differences between the two types of experimental values is independent of the solute structure.

To eliminate possible instrumental sources of errors in the experimental k'_{H_2O} values, we used the same microcolumn in both the elution and the sorption methods. In our opinion, the most likely explanation of the differences between the experimental $k'_{H_2O}(S)$ and $k'_{H_2O}(E)$ values may be connected with different environments surrounding the sorbent in the two experimental methods. In contrast to the elution method, where the mass of a solute that comes into contact with the sorbent is very small, the sorption method makes use of the full sorption capacity of the sorbent, so that multilayer sorption on the previously sorbed sample molecules is much more probable.

Anyway, $k'_{H_2O}(E)$ values determined using the elution method underestimate the real sorption capacity of octadecylsilica for the compounds studied. This means that the

TABLE II

EXPERIMENTAL AND PREDICTED CAPACITY FACTORS, k', OF PESTICIDES ON SILASORB C_{18} (7.5 μm) IN MOBILE PHASES CONTAINING VARIOUS CONCENTRATIONS (φ, VOL.% $\cdot 10^{-2}$) OF METHANOL IN WATER AND IN PURE WATER AS THE MOBILE PHASE (k'_{H_2O})

Methods: (I) direct determination by elution method, k'_{H_2O}(E), conventional column, 300 × 3.6 mm I.D.; (II) direct determination by elution method, k'_{H_2O}(E), microcolumn, 30 × 1 mm I.D.; (III) direct determination by sorption method, k'_{H_2O}(S), see Experimental; (IV) k'_{H_2O}(S) predicted for the sorption method from the experimental k'_{H_2O}(E) values determined by the elution method (II) using the correlation equation $\log k'_{H_2O}(S) = 0.0308 \pm 1.0287 \log k'_{H_2O}(E)$ (correlation coefficient $r_k = 0.99966$), based on three standards only; (V)–(X) k'_{H_2O}(E) predicted by extrapolation from the intercepts a of eqns. 1 (V–VIII) and 2 (IX and X) [$k'_{H_2O}(E) = 10^a$], using volume concentrations, φ (V, VII, IX and X), and molar fractions, x (VI and VIII), of methanol for fitting the data in the range from 2 to 90% methanol (V and VI) and from 30 to 80% methanol and $\log k'$ from −0.5 to 1.3, *i.e.*, in the "common conditions" range, (VI, VII and IX) and finally from 10 to 20% methanol (X); the regression equations fitted to the experimental data for the individual compounds tested are listed in Table III; (XI)–(XIX) k'_{H_2O}(E) predicted using the following correlation equations based on three standards: (XI) $a_V = -0.1953 + 1.0169 \log k'_{H_2O}(E)$; $r_k = 0.99984$; (XII) $a_{VI} = -0.2381 + 0.9889 \log k'_{H_2O}(E)$; $r_k = 0.99993$; (XIII) $a_{VII} = 0.5472 + 0.5810 \log k'_{H_2O}(E)$; $r_k = 0.85945$; (XIV) $a_{VIII} = 0.4620 + 0.5414 \log k'_{H_2O}(E)$; $r_k = 0.91503$; (XV) $a_{IX} = -0.0898 + 0.7764 \log k'_{H_2O}(E)$; $r_k = 0.98218$; (XVI) $a_X = -0.4610 + 1.0522 \log k'_{H_2O}(E)$; $r_k = 0.99908$; (XVII) $\log k'(0.4) = -1.6219 + 0.8218 \log k'_{H_2O}(E)$; $r_k = 0.98837$; (XVIII) $n_{ce} = -5.3095 + 1.7220 \log k'_{H_2O}(E)$; $r_k = 0.99638$; (XIX) $\log S = 0.6679 - 0.19311 \log k'_{H_2O}(E)$; $r_k = 0.89250$; where a_V–a_X are the extrapolated parameters a of the eqns. 1 and 2 found by regression analysis of the experimental data using methods V–X as characterized above; $k'(0.4)$ is the value of k' in 40% methanol as the mobile phase; n_{ce} is the solute lipophilic index; and S is the solute solubility in water (Table I); e is the mean error of prediction expressed as the mean value (absolute) of deviations of the individual predicted capacity factors in water from the experimental k'_{H_2O}(E) determined by the elution method (% relative). The numbers of the compounds 1–9 are as in Table I. The values of k'_{H_2O} for the standards used as the basis for the correlation equations are marked by asterisks.

Method	*e*	φ	k'								
			1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
I		0.90	–	–	0.07	0.14	0.10	0.19	0.13	0.26	0.32
I		0.80	0.04	0.03	0.18	0.32	0.26	0.39	0.36	0.59	0.72
I		0.70	0.08	0.34	0.36	0.65	0.61	0.79	0.88	1.29	1.64
I		0.60	0.18	0.23	0.80	1.41	1.37	1.63	2.23	2.90	3.88
I		0.50	0.32	0.53	1.85	3.20	3.55	3.63	6.18	7.36	8.48
I		0.40	0.69	1.21	4.76	7.93	9.36	8.88	16.04	18.81	24.18
I		0.30	1.53	2.91	12.72	19.07	27.48	23.25	45.65	55.05	70.78
I		0.20	3.81	7.95	37.19	45.18	82.44	58.45	110.5	161.6	–
II		0.20	4.48	8.60	37.24	45.67	73.8	60.38	102.6	156.7	206.4
I		0.15	6.27	13.53	69.08	75.08	143.6	105.5	185.1	–	–
II		0.15	6.82	14.61	62.90	72.83	134.6	101.6	171.1	261.9	398.9
II		0.10	12.22	26.34	144.2	126.1	274.4	210.3	318.1	566.5	825.6
II		0.05	24.37	63.08	364.9	271.2	742.2	477.8	708.4	1443	2056
II		0.02	38.43	96.04	750.6	471.1	1249	833.8	1122	2468	3412
II	–		71.28	171.0	1502	792.8	1999	1394	1714	3589	5621
III	–		83.9	–	1929	1103	2882	1954	2370	5286	7447
IV	5		86*	213	1989	1031*	2669	1842	2278	4873	7730*
V	28		50	128	879	541	1479	993	1294	2851	4256
VI	46		40	108	589	413	1064	711	1035	2046	2999
VII	60		28	40	383	407	1104	625	1191	1656	329
VIII	79		22	59	188	196	398	191	495	522	223
IX	79		19	36	170	211	355	189	716	565	540
X	51		32	80	531	347	1000	706	966	1959	3251
XI	6		73*	184	1222	758*	2038	1378	1787	3886	5762*
XII	8		72*	198	1101	770*	2004	1333	1949	3882	5714*
XIII	463		36*	66	3192	3553*	19763	7425	22524	39699	2458*
XIV	198		43*	260	2232	2409*	8891	2290	13318	14686	3044*
XV	55		57*	130	975	1285*	2511	1114	6203	4570	4307*
XVI	8		75*	176	1066	711*	1946	1399	1883	3687	5967*
XVII	30		60*	119	628	1169*	1434	1341	2754	3343	4538*
XVIII	67		76*	354	1211*	–	2527	1809	6358	5868	6531*
XIX	84		40*	–	2775	2835*	2769	2874	2859	2870	2783*

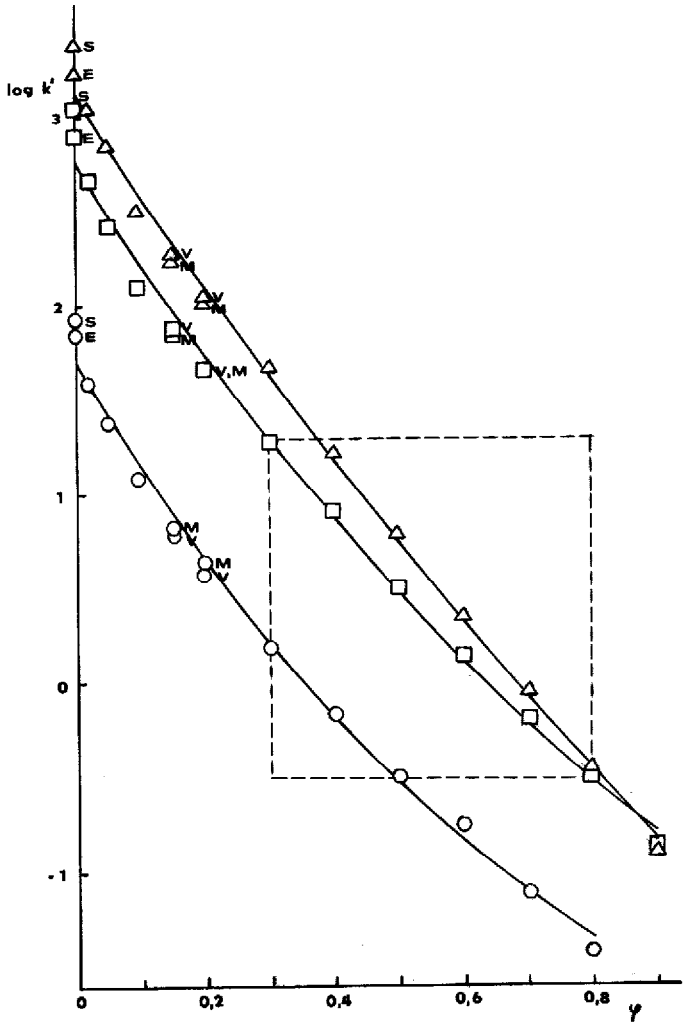

Fig. 3. Plots of log k' of pesticides *versus* the concentration of methanol in the mobile phase, φ (vol.% · 10^{-2}) over the full mobile phase composition range. Data measured: (1) using a conventional column, 300 × 3.6 mm I.D. (V); (2) using a microcolumn, 30 × 1 mm I.D. (M); both packed with Silasorb C_{18}, (7.5 μm). The experimental values of log k'_{H_2O} were measured using the elution (E) and sorption (S) methods, both on the microcolumn. The curves were fitted to the experimental data points using quadratic regression (eqn. 1). Dashed lines limit the range of the "common conditions" in HPLC. Solutes: (△) fluometuron; (□) monuron; (○) methomyl.

experimentally determined k'_{H_2O}(E) values are only suitable for the characterization of the capacity factors in water for the purposes of analytical chromatography working with very dilute sample solutions, whereas the k'_{H_2O}(S) values are only suitable for the characterization of the sorption capacity of a sorbent for sample enrichment purposes or, possibly, its behaviour in preparative chromatography. A correlation equation such as eqn. 8 can be used for the prediction of one type of k'_{H_2O} values from the other

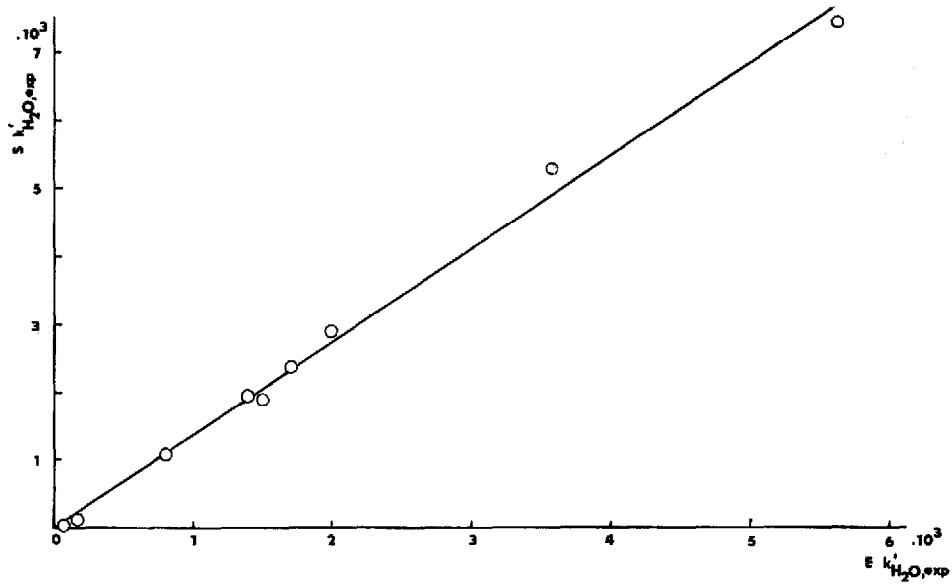

Fig. 4. Correlations between the experimental capacity factors of the nine solutes tested measured in pure water as the mobile phase by the sorption (S $k'_{H_2O,exp}$) and elution (E $k'_{H_2O,exp}$) methods.

type, if necessary. This possibility is illustrated by method IV in Table II, where the correlation equation based on the experimental data for only three compounds used as standards, namely methomyl, monuron and desmetryne, were used to predict the "sorption" capacity factors, k'_{H_2O}(S), of the remaining six pesticides from their "elution" capacity factors, k'_{H_2O}(E). The mean difference of *ca.* 30% relative between the two types of values was thus reduced to 5%.

Extrapolation of k' data in mixed aqueous–methanolic mobile phases to zero methanol concentration

The direct determination of k'_{H_2O} is time consuming because of the long elution times in the elution method and the long times necessary to achieve equilibrium between the sorbent and the solution of the compound being sorbed. Both methods require an instrumental modification of the standard equipment for HPLC. Hence, it would be more convenient to determine k'_{H_2O} from some data acquired rapidly using conventional HPLC instrumentation. This is only possible if mixed organic–aqueous mobile phases are employed.

The most straightforward approach is to use a linear extrapolation to zero methanol concentration of the plots fitted to two or more experimental data points measured at different concentrations of methanol or another organic solvent in the mobile phase. As discussed in the Introduction, these plots unfortunately are curved at low methanol concentrations. Consequently, the extrapolated k'_{H_2O} values may be subject to significant errors and their values can be expected to depend strongly both on the type of curve fitted to the experimental data points and on the range of mobile phase compositions used for the measurement of the experimental data. We considered it worthwhile to investigate these aspects in more detail.

TABLE III

PARAMETERS OF THE LOG k' *VERSUS* CONCENTRATION OF METHANOL DEPENDENCES DESCRIBED BY FUNCTIONS FITTED TO THE EXPERIMENTAL DATA POINTS USING LINEAR AND QUADRATIC REGRESSION ANALYSIS

Type of regression function: V, VII, $\log k' = a - m\varphi + d\varphi^2$ for the range of volume fractions of methanol, φ, in vol.% $\cdot 10^{-2}$, from 0.02 to 0.9 (V) and from 0.3 to 0.8, $\log k'$ in the range from -0.5 to 1.3 (VII); VI, VIII, $\log k' = a - mx + dx^2$ for the range of molar fractions of methanol, x, from 0.009 to 0.8 (*i.e.*, from 2 to 90%) (VI) and from 0.16 to 0.64 (*i.e.*, from 30 to 80% methanol, $\log k'$ in the range from -0.5 to 1.3 (VIII); IX, X, $\log k' = a - m\varphi$, for the range of φ from 0.3 to 0.8, $\log k'$ in the range from -0.5 to 1.3 (IX) and φ from 0.1 to 0.2 (X). The numbers of functions agree with the numbers of methods for prediction of k'_{H_2O} by extrapolation in Table II. r_k = correlation coefficient; n = number of the experimental data points. Compounds as in Tables I and II.

Function	*n*	*Parameter*	*Compound*								
			1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
V	11–13	*a*	1.698	2.108	2.944	2.733	3.170	2.997	3.112	3.455	3.629
		m	5.574	6.194	6.811	5.305	6.333	6.107	5.172	6.364	6.600
		d	2.218	2.551	2.664	1.544	2.004	2.285	0.872	2.174	2.339
		r_k	0.9978	0.9979	0.9969	0.9981	0.9987	0.9984	0.9990	0.9989	0.9989
VI	11–13	*a*	1.600	2.032	2.770	2.616	3.027	2.852	3.015	3.311	3.477
		m	9.174	10.917	10.255	8.488	10.013	9.383	8.811	9.975	10.267
		d	7.245	9.912	6.990	5.377	6.484	6.398	5.076	6.629	6.887
		r_k	0.9932	0.9946	0.9894	0.9939	0.9940	0.9928	0.9966	0.9942	0.9939
VII	4–6	*a*	1.454	1.605	2.583	2.610	3.043	2.796	3.076	3.219	2.517
		m	4.699	3.895	5.385	4.725	5.850	5.222	4.923	5.404	2.911
		d	1.625	0.275	1.529	1.046	1.700	1.529	0.650	1.371	−0.525
		r_k	1.0000	0.9999	1.0000	0.9999	0.9999	0.9999	0.9999	0.9999	0.9999
VIII	4–6	*a*	1.348	1.769	2.275	2.293	2.600	2.281	2.695	2.718	2.348
		m	8.547	9.725	8.285	7.049	8.400	6.763	7.398	7.297	5.259
		d	8.393	10.160	5.834	4.223	5.636	4.027	3.908	4.227	2.133
		r_k	0.9999	0.9999	0.9999	0.9999	1.0000	0.9996	1.0000	0.9999	0.9993
IX	4–6	*a*	1.276	1.552	2.231	2.324	2.550	2.276	2.855	2.752	2.732
		m	3.561	3.647	3.856	3.574	3.980	3.388	4.143	3.758	3.593
		r_k	0.9991	0.9999	0.9989	0.9990	0.9992	0.9985	0.9997	0.9990	0.9998
X	3	*a*	1.511	1.902	2.725	2.540	3.000	2.849	2.985	3.292	3.512
		m	4.360	4.860	5.880	4.430	5.700	5.410	4.910	5.580	6.010
		r_k	0.9957	0.9996	0.9917	0.9987	0.9988	0.9953	0.9985	0.9934	0.9996

Table III lists the regression equations fitted to the experimental data in various manners in order to find the best possibility for the determination of k'_{H_2O} by extrapolation of the experimental k' dependences on methanol concentration in the mobile phase. The extrapolated k'_{H_2O} values are given in Table II as the results of methods V–X. The correlation coefficients in Table III show that the best fit to the experimental data was achieved using the quadratic function eqn. 1 in the "common" range of methanol concentrations most frequently used for practical reversed-phase chromatographic separations, *i.e.*, 30–80%, where the experimental data can be measured fairly rapidly using "conventional" analytical columns. A slightly less good fit to the experimental data was observed for the linear function eqn. 2 in the

"common" range of methanol concentrations and for the quadratic function eqn. 1 in the wide range of methanol concentrations, from 2 to 90%. The fit did not depend on the concentration units employed, either volume concentrations, φ, or molar fractions, x.

All the values of k'_{H_2O} extrapolated from the data measured in aqueous–organic mobile phases were subject to significant systematic negative errors, which depended both on the type of the regression function and the range of the experimental data as is illustrated in Table II. The mean error of prediction was significantly higher for concentrations given in molar fractions than for those in volume concentrations. The average relative error of the extrapolated k'_{H_2O} was the least, *i.e.*, *ca.* 30% relative, for the quadratic function eqn. 1 fitted to the wide range of methanol concentration from 2 to 90%, followed by the linear function eqn. 2 fitted to the data in the range 10–20% methanol and by the quadratic function eqn. 1 fitted to the "common" range of methanol concentrations from 30 to 80%. The worst k'_{H_2O} predicted by extrapolation, which was subject to a mean error of approximately 80% relative, resulted when the linear function eqn. 2 was fitted to the experimental data in the "common" range of methanol concentrations, which method, however, has been most frequently used in practice for the prediction of k'_{H_2O}. These results indicate that the k'_{H_2O} values extrapolated in the usual manner may be highly unreliable. The relative error of the extrapolated capacity factors can be reduced if the experimental data measured in the range of low methanol concentrations are used for prediction by extrapolation or if the quadratic function is fitted to the data over a wide range of methanol concentrations.

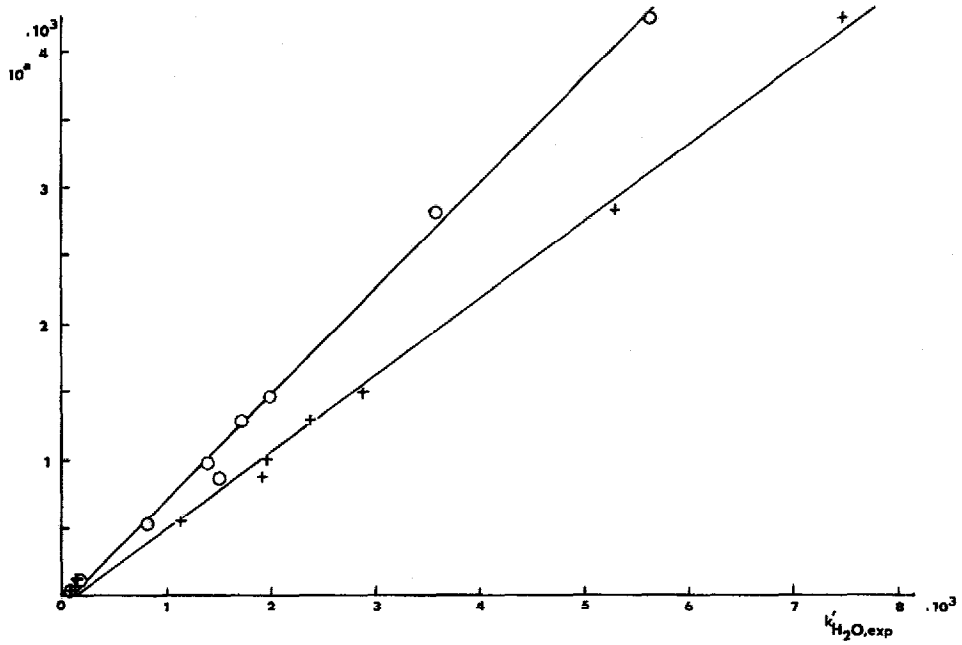

Fig. 5. Correlations between the experimental k'_{H_2O} values determined by the elution (○) and the sorption (+) methods and the parameter a of the quadratic function eqn. 1 fitted to the experimental data over the full range of mobile phase compositions (2–90% methanol).

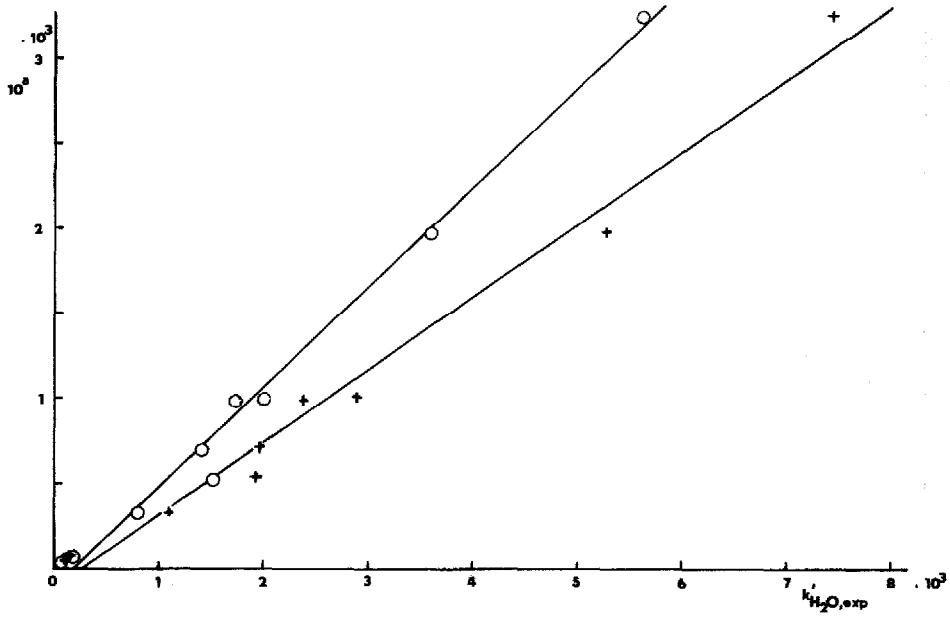

Fig. 6. Correlations between the experimental k'_{H_2O} values and the parameter a of the linear function eqn. 2 fitted to the experimental data in the range of methanol concentrations from 10 to 20%, determined on the microcolumn. Symbols as in Fig. 5.

Unfortunately, the time necessary for the determination of capacity factors increases rapidly as the concentration of the organic solvent in the mobile phase is decreased.

Using correlations for prediction of k'_{H_2O}

We attempted to improve the accuracy of predictions using the extrapolated data on the basis of correlations of the extrapolated parameters a of the quadratic (eqn. 1) and linear (eqn. 2) functions with the experimental log k'_{H_2O} values of three standard compounds. Various correlations investigated for this purpose are given in Table II as methods XI–XVI. The standards used for the construction of the correlation equations are marked by asterisks. Generally, the extrapolated k'_{H_2O} values that were subject to least errors showed most significant further improvement from the correlation approach. Thus the predicted k'_{H_2O} from the correlation equations showed the least mean error when the quadratic function eqn. 1 was used for the extrapolation of the data acquired over a wide range of methanol concentrations in the mobile phase from 2 to 90% (methods XI and XII) (Fig. 5). Similar results were obtained for correlations using the parameters a of the linear extrapolation function eqn. 2 fitted to the experimental data in mobile phases with low methanol concentrations from 10 to 20% (method XVI) (Fig. 6), but only a marginal improvement resulted for linear extrapolation of the data measured in the "common" range from 30 to 80% methanol (method XV). On the other hand, the accuracy of the prediction is seriously impaired for the correlations of log k'_{H_2O} with the parameter a of the quadratic function eqn. 1 fitted to the experimental data in the "common" range (methods XIII and XIV) (Fig. 7).

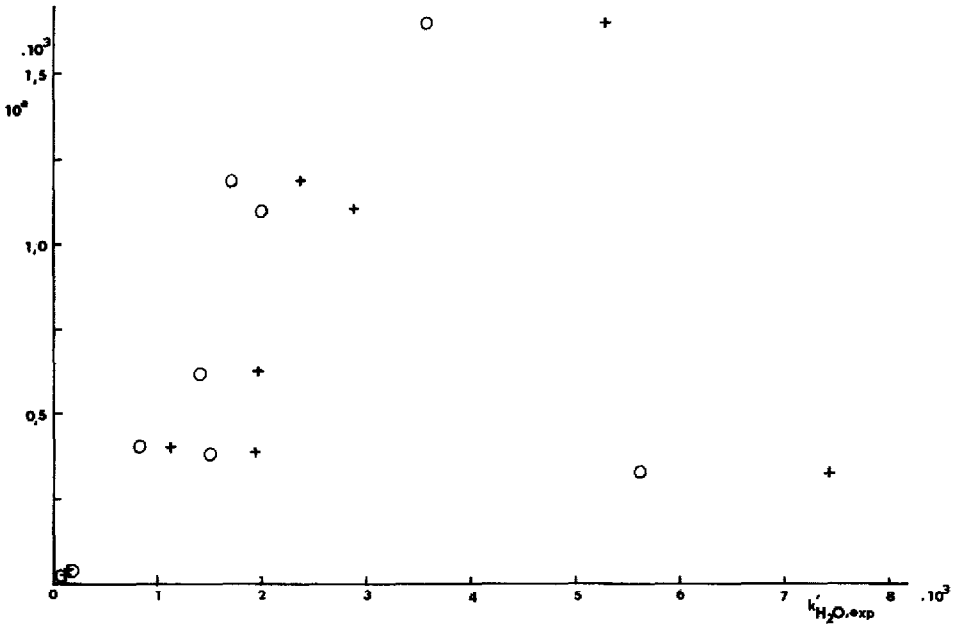

Fig. 7. Correlations between the experimental k'_{H_2O} values and the parameter a of the quadratic function eqn. 1 fitted to the experimental data in the range of $\log k'$ from −0.5 to 1.3 and from 30 to 80% methanol as the mobile phase. Symbols as in Fig. 5.

Better correlations with a smaller mean error in the predicted k'_{H_2O} than for the parameters a extrapolated from the data in the "common" concentration range were obtained for the capacity factors measured at a single methanol concentration close to the lower limit of the "common" concentration range, *e.g.*, at 40% methanol (method XVII).

The prediction of k'_{H_2O} based on the correlation of $\log k'_{H_2O}$ of the standards with the lipophilic indices, n_{ce}, (method XVIII) (Fig. 8) is subject to a slightly smaller error than the prediction based on a similar correlation with the logarithms of solubilities in water, S, (method XIX) and the average error of prediction is comparable to the error of k'_{H_2O} values predicted from direct extrapolation from the data in mixed aqueous–organic mobile phases measured in the "common" range from 30 to 80% methanol.

CONCLUSIONS

The capacity factors of the sample solutes in pure water, k'_{H_2O}, can be determined experimentally on short microcolumns using either a direct elution method or a sorption method, where the total amount of the solute sorbed is determined by on-line HPLC on a conventional column. Similar equipment can be used in the two methods. The k'_{H_2O} values determined by the sorption method are higher than those found by the elution method, but the two types of k'_{H_2O} are strongly intercorrelated. The capacity factors determined by the sorption method are likely to characterize more accurately the sorption behaviour, *i.e.*, the capacity of a sorbent and breakthrough

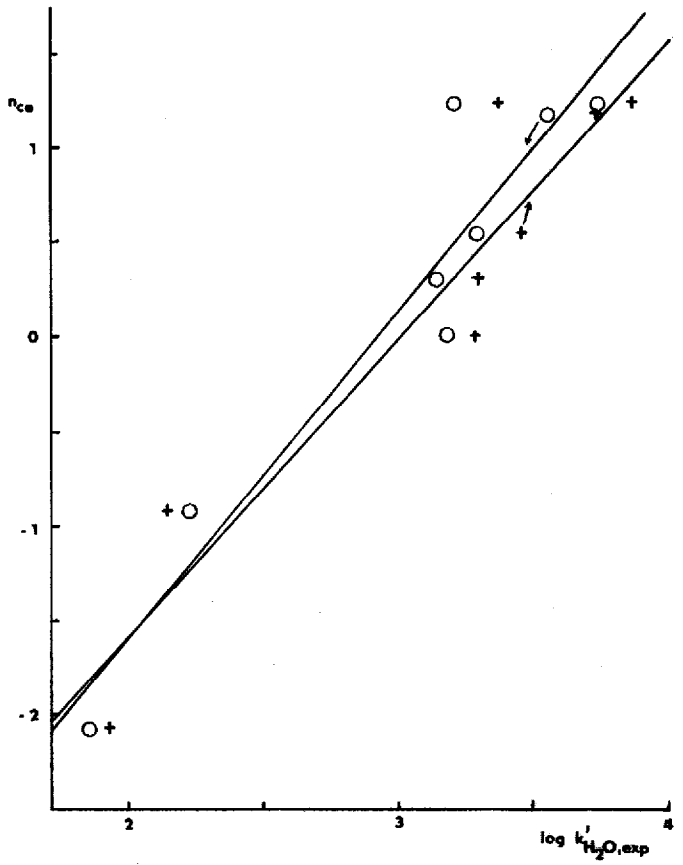

Fig. 8. Correlations between the experimental k'_{H_2O} values and the lipophilic indices, n_{ce}[31]. Symbols as in Fig. 5.

volumes, of the sorbents to be used for the enrichment of aqueous samples by solid-phase extraction techniques and can be predicted from the experimental k'_{H_2O} values determined by the elution method using the correlation equation applying for a given sorbent. The experimental k'_{H_2O} values determined by the latter method are suited for correlations with the structural parameters of solutes in QSAR studies or for the investigation of the retention mechanism in reversed-phase systems.

Although the retention over the full composition range of methanol–water mobile phases from 2 to 90% methanol is adequately described by the quadratic function of log k' in dependence on the methanol concentration (eqn. 1), the values of k'_{H_2O} determined from this regression function by extrapolation differ significantly from the experimental k'_{H_2O} values determined using both the sorption and the elution methods, but are strongly correlated with them. This correlation can be utilized for predictions of one type of k'_{H_2O} values from the other type.

The commonly used extrapolation of the linear plots of log k' *versus* methanol concentration fitted to the experimental data in a limited range of mobile phase

compositions can yield only rough estimates of k'_{H_2O} with an accuracy of an order of magnitude, unless only the range of low methanol concentrations is used or the quadratic function of log k' *versus* methanol concentration is plotted for the experimental data over the whole range of mobile phase compositions. Because the k' values are fairly high in the mobile phases with low contents of organic solvents, very short conventional columns or microcolumns are necessary to accomplish the data acquisition in a reasonably short time, like those used for the direct elution determination of k'_{H_2O}.

Based on the correlation equations between the experimental logarithms of k'_{H_2O} and the parameters a of the functions log k' *versus* methanol concentration fitted to the experimental data in aqueous–organic mobile phases for a few standard compounds, k'_{H_2O} of other solutes can be predicted from their corresponding values of a. However, this prediction approach yields more accurate k'_{H_2O} values than the prediction by extrapolation only for the parameters a obtained from the data at low methanol concentrations in the mobile phases and there is no sense in using this predictive method in connection with the "common" range of methanol concentrations for the acquisition of the experimental data. On the other hand, it is possible to use the capacity factors determined at a single mobile phase composition with a low methanol concentration for this correlation predictive method with a smaller error than when the parameter a extrapolated from the "common" concentration range is used for this purpose.

The lipophilic indices, n_{ce}, may be used for the approximate estimation of k'_{H_2O} from the correlation equations with log k'_{H_2O} with a mean error of prediction comparable to that for the direct extrapolation from the "common" concentration range.

The test compounds used in this work were selected so as to represent "practically useful" compounds (pesticides) and simultaneously to cover relatively broad combinations of basic structures and functional groups. Because of time-consuming methods of determination, this selection is necessarily limited. Therefore, the correlation predictive approach should be used carefully, as another group of sample solutes would probably require a different set of standards to those used here. Also, the correlation approach should be verified for other than octadecylsilica types of sorbents. It is almost certain that each sorbent would require its own correlation equation even for the same set of standards.

It is hoped that the results of this work may be useful in giving an idea of various possibilities for the experimental determination and prediction of capacity factors in pure water as the mobile phase.

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