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RELATIONSHIP BETWEEN LOG P_{OW} SHAKE-FLASK VALUES AND CAPACITY FACTORS DERIVED FROM REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY FOR *n*-ALKYLBENZENES AND SOME OECD REFERENCE SUBSTANCES

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SUMMARY

The capacity factors (k') of eight *n*-alkylbenzenes and eleven reference substances used in the OECD Chemicals Testing Programme were determined using reversed-phase high-performance liquid chromatography (RP-18) at different compositions of the mobile phase (methanol–water). All substances except urea show a good linear relationship ($r \geqslant 0.995$) between the ln k' values and the volume fraction (φ_{CH_3OH}) of the organic modifier in the range 0.6–0.95.

Correlation of *n*-octanol–water partition coefficients (shake-flask values) given in the literature and from the OECD/EEC laboratory comparison tests with the experimental log k' values results in a good linear relationship for the *n*-alkylbenzenes ($r = 0.998$) and a slightly poorer relationship for the OECD reference substances ($r = 0.967$–0.986). If the log P_{OW} values are related to extrapolated log k^0 values with pure water as the eluent, the regression lines of both the homologues and the reference substances are nearly identical and can reciprocally be used to predict their log P_{OW} values with reasonable accuracy.

INTRODUCTION

As numerous experiments have shown, there is a close relationship between the tendency of certain chemicals to bioconcentrate or bioaccumulate and their partition behaviour between a lipophilic phase and water[1–6]. The partition coefficient as a measure of lipophilicity is, moreover, closely related to the relative biological activity of pharmaceuticals and therefore plays an important part in the quantitative structure–activity relationship (QSAR)[7–9]. Also, for determining the adsorption constant (K_{OC}) of chemicals and for carrying out exposure analysis, the partition coefficient is one of the basic parameters[10,11].

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It has been established in a large number of experiments that the partitioning system *n*-octanol–water in particular is very suitable for characterizing the interactions between chemical substances and biological systems[7,9].

For these reasons, the Physical Chemistry Expert Group of the OECD, working within the Chemicals Testing Programme of the OECD[12] for the harmonization of testing methods for chemicals, drew up a test guideline[13] for the determination of the partition coefficient (*n*-octanol–water) and subjected it to an international laboratory comparison test[14].

P_{OW} is one of the chemical properties laid down by the OECD as part of the MPD (*M*inimum *P*re-marketing set of *D*ata) and is also one of the physico-chemical parameters of the base set, set forth in Annex VII of EEC Directive 79/831/EEC (ref. 15). As a result, the P_{OW} has to be measured in all EEC Member Countries before a chemical is marketed for the first time.

As new experiments[4] and the OECD/EEC laboratory comparison tests (*cf.*, Table I) have shown, the results of the shake-flask method may have a large variation range. Incorrect results occur particularly for very lipophilic substances with log $P_{OW} \geqslant 4$[4,16–19]. Moreover, the method may be very sensitive to impurities, is time consuming, involves numerous experimental difficulties and is applicable to a limited extent only to compounds that are volatile, dissociate and/or associate. For a given chemical, varying log P_{OW} values are found in the literature. A few examples are given in Table II.

To eliminate the disadvantages of the shake-flask method, chromatographic techniques, especially thin-layer chromatography and reversed-phase high-performance liquid chromatography (HPLC), have been used to quantitate hydrophobic properties and to correlate log P_{OW} values with retention data.

Basically, the chromatographic determination of unknown partition coefficients for the system *n*-octanol–water is performed as follows:

TABLE I

RESULTS OF THE OECD/EEC LABORATORY COMPARISON TESTS[14] FOR THE SHAKE-FLASK METHOD

The test was performed by using a former version of the now finalized test guideline.

Substance	*Log P_{OW}**	*Overall mean value*	*Standard deviation of the mean value*
Aniline	0.87, 0.91, 0.92, 0.92, 0.96, 1.02, 1.06, 1.25, 1.45	1.08	±0.19
Dibutylphthalate**	3.90, 4.12, 4.17, 4.22, 4.45, 4.45	4.08	±0.42
1,2-Dichlorobenzene**	3.19, 3.19, 3.31, 3.53, 3.60, 3.84, 4.15, 4.36	3.61	±0.42
Di(2-ethylhexyl)phthalate	4.66, 4.94, 4.95, 5.18, 5.44	5.03	±0.29
Urea	−1.70, −1.59, −1.53, −1.51, −1.50	−1.57	±0.08
Hexachlorobenzene	5.02, 5.20, 5.41, 5.51, 5.55, 5.66, 5.92	5.47	±0.29
4-Nitrophenol	1.49, 1.66, 1.91, 1.99, 2.02, 2.05, 2.05	1.88	±0.22
Pentachlorophenol	2.68, 2.86, 3.31, 3.67, 3.94	3.29	±0.53
Trichloroethylene	2.71, 2.79, 3.49, 3.57	3.14	±0.45

* Mean values reported by the participants.

** EEC.

TABLE II

VARIATION RANGE OF LOG P_{OW} LITERATURE VALUES[20]

Substance	*Log P_{OW} range reported*	*Log P_{OW} calculated according to Rekker*[18]	*Remarks*
Trichloroethylene	2.24–3.19	2.28	Volatile
Pentachlorophenol	3.81–5.86	5.19	Ionic
Hexachlorobenzene	4.13–6.18	6.44	Highly lipophilic
p,p'-DDT	3.98–6.19	7.35	Highly lipophilic

(1) Measurement of the retention times, t_R, or R_F values for a number of reference substances (preferably homologues or structurally related compounds) with well-known P_{OW} values.

(2) Calculation of log k' or R_M values for the reference compounds by using the equation

$$\log k' = \log \left(\frac{t_R - t_0}{t_0} \right) \tag{1}$$

or

$$R_M = \log \left(\frac{1 - R_F}{R_F} \right) \tag{2}$$

(3) Determination of the relationship between log k' and R_M or log P_{OW} by regression analysis (e.g., $\log P_{OW} = a + b \log k'$).

(4) Measurement of the t_R or the R_F value of the test substance and calculation of log k' (R_M).

(5) Calculation of log P_{OW} of the test substance using the regression equation obtained under (3).

The chromatographic separation systems used by different workers can basically be divided into two groups:

(a) Octanol separation systems[21–24]. Here, thin-layer plates or HPLC packing material are covered with octanol; the eluent consists of octanol-saturated water or buffers.

(b) Reversed-phase separation systems[2,16,17,21,25–29]. Use of chemically modified carrier material, *e.g.*, octadecylsilica, as the stationary phase and binary solvent mixtures consisting of water and an organic modifier as eluents.

As can be seen from Table III, octanol separation systems truly imitate the properties of octanol–water partitioning by the shake-flask method. Thus, correlations of log P_{OW} and log k' are perfectly linear, and the slope of the regression lines is nearly 1.0. On the other hand, octanol systems have the disadvantage of a limited measuring range from $\log P_{OW} = -1$ to 3, and particularly substances of high lipophilicity, which tend to bioaccumulate, cannot be measured by using this method.

TABLE III

LITERATURE REGRESSION DATA FOR THE RELATIONSHIP LOG P_{OW} = a + b LOG k'

Class of substance	*a*	*b*	*r*	*n*	*Log P_{OW} range*	*Reference*	*Separation system**
Miscellaneous**	1.15	1.086	0.982	21	1.1–2.7	26	ODS; 1% TEA in W
Miscellaneous**	1.28	1.081	0.927	21	1.1–2.7	26	ODS; 15 ACN–85 W
Phenols**	1.92	1.907	0.961	9	1.5–2.6	30	ODS; acetone–W
Anilines**	1.44	2.240	0.968	12	0.9–2.7	30	ODS; acetone–W
Sulphonamides	−3.03	0.98	0.937	11	−1.2–1.1	21	ODS; buffer, pH 4.0
Barbiturates	−0.64	8.72	0.956	5	0.7–2.2	21	ODS; 10 ACN–buffer, pH 5.0
Chlorobenzenes Chlorotoluenes Chloroanilines	2.12	2.50	0.989	20	0.9–5.0	17	ODS; 70 CH_3OH–30 W; 35°C
Pesticides	1.88	2.32	0.976	20	1–6	4	Reversed phase
Pyridazinones	−0.38	0.901	0.992	8	1.1–2.7	16	ODS; CH_3OH–W; 100 W
Miscellaneous***	−0.42	1.160	0.967	27	0.9–4.2	31	ODS; CH_3OH–W; 100 W
Miscellaneous***	−2.13	2.503	0.972	29	0.9–4.2	31	ODS; ACN–W; 100 W
Miscellaneous***	−1.38	0.835	0.850	29	0.9–4.2	31	ODS; THF–W; 100 W
Miscellaneous	1.80	4.23	0.976	15	1.2–6.2	28	ODS; 75 ACN–25 W
1,4-Benzodiazepines	−0.13	0.894	0.968	9	–	32	ODS; CH_3OH–W; 100 W
Phenols	−0.31	0.848	0.979	29	0.6–5.0	25	ODS; CH_3OH–buffer 100 W
Miscellaneous	2.37	2.70	0.987	37	−0.4–6.3	27	ODS; 75 CH_3OH–25 W
Miscellaneous	3.33	3.02	0.963	15	2.1–6.3	27	ODS; 85 CH_3OH–15 W
Miscellaneous	0.62	0.994	0.999	7	0.6–2.1	22	S-oct; B-oct
Miscellaneous	1.51	1.036	0.996	10	0.6–2.4	23	S-oct; B-oct, pH 2.0
Miscellaneous	0.80	1.025	0.987	33	−0.2–3.4	24	ODS-oct; B-oct. pH 7.0

* ACN = acetonitrile; ODS = octadecylsilica; B-oct = *n*-octanol-saturated buffers; S-oct = *n*-octanol-loaded silica; TEA = triethylamine; THF = tetrahydrofuran; W = water; 100 W = extrapolated from binary solvent mixtures (or buffers) to 100% water.

** Regression analysis was performed by us on the basis of the published k' and log P_{OW} values.

*** The published log k' values were correlated with literature log P_{OW} values.

With reversed-phase separation systems, very lipophilic compounds with log P_{OW} up to about 8 can be measured, and in most instances good linear relationships between log P_{OW} and log k' are obtained (*cf.*, Table III). For some substances, problems may arise through selective effects of the stationary phase. This is probably due to free silanol groups present on the surface of the octadecylsilica[33,34].

In order to improve determinations of unknown log P_{OW} values, it is necessary to standardize chromatographic measurements, if possible, so that different classes of compounds show the same slope of the regression line between log P_{OW} and log k'. For this purpose, Biagi *et al.*[35], in thin-layer chromatography, at first extrapolated R_M values to an eluent composition of 100% water. The same method was shown to be applicable to reversed-phase HPLC by Braumann and Grimme[16] for some pyridazinones and by Butte *et al.*[25] for a number of phenols.

The aim of this study was to investigate the possibilities of reciprocally predicting log P_{OW} values from two different reference systems at different compositions of the mobile phase methanol–water and in this way to compare the results of the OECD laboratory comparison test for the shake-flask method with those of the HPLC method.

EXPERIMENTAL

Equipment

The HPLC system consisted of a Series 3 liquid chromatograph and a Model LC-75 variable-wavelength UV–visible detector with an autocontrol unit (all from Perkin-Elmer). A 5-μm C-18-SIL-X-5 column (25 cm × 4.0 mm I.D.) (Perkin-Elmer) was used without further treatment. Retention times were measured with an Autolab System 1 calculator (Spectra-Physics). The integrator and column pressure signal were simultaneously recorded with a Philips PM 8221 two-line recorder.

Chemicals

n-Alkylbenzenes (98 + % pure) were obtained from Polyscience Corp. All other compounds tested, generally 99 + % pure, were obtained from Ferak, Fluka, E. Merck and Riedel de Haen. Chromatographic-grade methanol (ChromAR, Mallinckrodt) and water, doubly distilled in quartz, were used to prepare the mobile phases.

Conditions

The eluting solvents were vacuum-degassed separately. Isocratic elution with different methanol–water mixtures (95–60% methanol, in 5% steps) was maintained with the gradient programme of the liquid chromatograph at a flow-rate of 1 ml/min. The column temperature was kept at 23 ± 0.3°C by means of a thermostatically controlled water-bath. The detection wavelengths used were 202 nm for urea and trichloroethylene and 254 nm for all other compounds. Solutions of the substances in methanol were prepared in concentrations that permitted their UV detection with injection of up to 20 μl. The stability of the separation system was checked by repeated injections of *n*-alkylbenzene standard solutions during the measuring period of 4 months. The coefficients of variation of the retention times thus found were about 1% for benzene and 2% for decylbenzene ($n = 17$) at identical compositions of the eluent.

Log k' values obtained for each solvent mixture and each reference substance were, in most instances, calculated from the mean value of 3–5 retention time measurements with coefficients of variation of less than 1%.

Column dead-time

The determination of an "accurate" dead time is of particular importance for the calculation of capacity factors, especially if the column dead time depends on the eluent composition. In this study, dead time was therefore determined in three different ways*: by injection of a substance that is expected to be unretarded (urea); by dead time iteration of the retention times of homologous *n*-alkylbenzenes; and by injection of 10 μl of pure methanol.

For urea no statistically significant relationship was determined between dead time and water content of the eluent. However, both the iteration and the methanol method showed, with increasing water content, an increase in dead time between 0 and 40%. The iterated dead time was found to be dependent not only on the water

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* A comparison of the results will be published in a separate paper.

content but also on the homologues used for the iteration, whereas only for methanol was a linear increase in dead time with increasing water content measured (about 3 sec per 10% increase in water content). Therefore, we used the retention time of pure methanol as the dead time (t_0) in our subsequent calculations.

CALCULATIONS

The capacity factors (k') were calculated according to eqn. 1.

All linear regression analyses (we used the method of least-squares fitting according to Sachs[36]), as well as dead time iterations and data storage and handling were performed on a Tektronix 4051 graphic computer system with BASIC programs of our own.

RESULTS AND DISCUSSION

ln k' and φ_{CH_3OH}

The regression analyses performed in this study were based on the assumption that a linear relationship exists between ln k' and the methanol concentration in the eluent, at least in the measuring range from 60 to 95% (v/v) methanol.

As was shown by several investigators, including Snyder *et al.*[37], this is true for many substances. Deviations may occur if substances interact with the free silanol groups of the stationary phase or if they are not retarded.

The relationship for methanol given by Snyder *et al.* is

$$\ln k' = \ln k^\circ - S\,\varphi_{\mathrm{CH_3OH}} \tag{3}$$

where k° represents the k' value for a compound if pure water is used as eluent ($\varphi_{\mathrm{CH_3OH}} = 0$). Normally, this value is the intercept of the ordinate, obtained by extrapolation. The quantity S depends both on the solvent strength and on the specific interactions between solute, the stationary phase and the mobile phase[31]. $\varphi_{\mathrm{CH_3OH}}$ is the volume fraction of the methanol in the mobile phase.

Table IV shows that the above-stated relationship produced very good linear regression lines ($r \geqslant 0.995$) for all substances. It should be noted that, for substances with "ionic behaviour", such as aniline and 4-nitrophenol, the determined degree of linearity is lower than that for the other substances investigated.

In addition to the substances listed in Table IV, we also investigated urea. For urea, which was not retarded on the separation column, a linear relationship between ln k' and $\varphi_{\mathrm{CH_3OH}}$ could not be proved statistically. Therefore, the following mean value was calculated on the basis of the ln k' values and used in the following correlations:

$$\ln k'_{\mathrm{urea}} = -4.38 \pm 1.23 \qquad (n = 29) \tag{4}$$

Fig. 1a illustrates that the homologous n-alkylbenzenes were eluted in identical sequence, irrespective of the water content of the mobile phase, and that a CH_2 group contributed to the ln k° values by *ca.* 1.4. In contrast, the regression lines of the OECD reference substances intersect several times (*cf.*, Fig. 1b).

TABLE IV

RELATIONSHIP BETWEEN ln k' AND THE VOLUME FRACTION OF METHANOL, φ_{CH_3OH} ln k' = ln k° − $S\,\varphi_{CH_3OH}$

Values in parentheses give the standard deviations for the regression coefficients.

Substance	*ln k°*	*S*	*r**	$s_{y \cdot x}$**	*n*
Aniline	3.22 (0.07)	5.44 (0.10)	−0.9954	0.063	32
Biphenyl	9.85 (0.06)	10.71 (0.08)	−0.9994	0.035	23
Dibutylphthalate	11.45 (0.05)	12.60 (0.06)	−0.9997	0.026	23
1,2-Dichlorobenzene	8.32 (0.03)	9.27 (0.04)	−0.9997	0.025	30
p,p'-DDT	15.28 (0.11)	15.98 (0.13)	−0.9996	0.028	16
Di(2-ethylhexyl)phthalate	20.62 (0.19)	20.71 (0.21)	−0.9996	0.034	8
Fluoranthene	11.23 (0.08)	11.43 (0.10)	−0.9995	0.027	15
Hexachlorobenzene	13.76 (0.06)	13.33 (0.06)	−0.9999	0.012	13
4-Nitrophenol	4.54 (0.09)	6.78 (0.11)	−0.9960	0.072	31
Trichloroethylene	6.62 (0.03)	7.81 (0.03)	−0.9997	0.021	30
Benzene	5.50 (0.03)	6.79 (0.03)	−0.9992	0.029	73
Toluene	6.97 (0.03)	8.10 (0.03)	−0.9992	0.039	99
Ethylbenzene	8.14 (0.03)	9.13 (0.03)	−0.9995	0.030	73
n-Propylbenzene	9.61 (0.03)	10.47 (0.04)	−0.9996	0.033	72
n-Butylbenzene	10.96 (0.03)	11.65 (0.03)	−0.9997	0.023	88
n-Hexylbenzene	13.70 (0.04)	14.05 (0.04)	−0.9996	0.029	73
n-Octylbenzene	16.46 (0.05)	16.45 (0.06)	−0.9996	0.028	67
n-Decylbenzene	19.26 (0.10)	18.89 (0.10)	−0.9993	0.029	48

* Correlation coefficient.

** Standard error of estimate[36].

To simplify the calculation of the log P_{HPLC} values presented below, we determined the entire equation for the *n*-alkylbenzenes to be as follows:

$$\ln k' = f(n_C) - \varphi_{CH_3OH} f'(n_C) \tag{5}$$

where

$$f(n_C) = \ln k^\circ_{(\text{benzene})} + n_C \ln k^\circ_{(-CH_2-)} \tag{6}$$

and

$$f'(n_C) = S_{(\text{benzene})} + n_C S_{(-CH_2-)} \tag{7}$$

This was done by linear regression, using the ln k° and S values listed in Table IV. The results of the regressions are given in Table V.

Thus, the entire formula for the homologous series of *n*-alkylbenzenes, under the above-stated separation conditions, is:

$$\ln k' = 5.493 + 1.372 n_C - \varphi_{CH_3OH}(6.812 + 1.206 n_C) \tag{8}$$

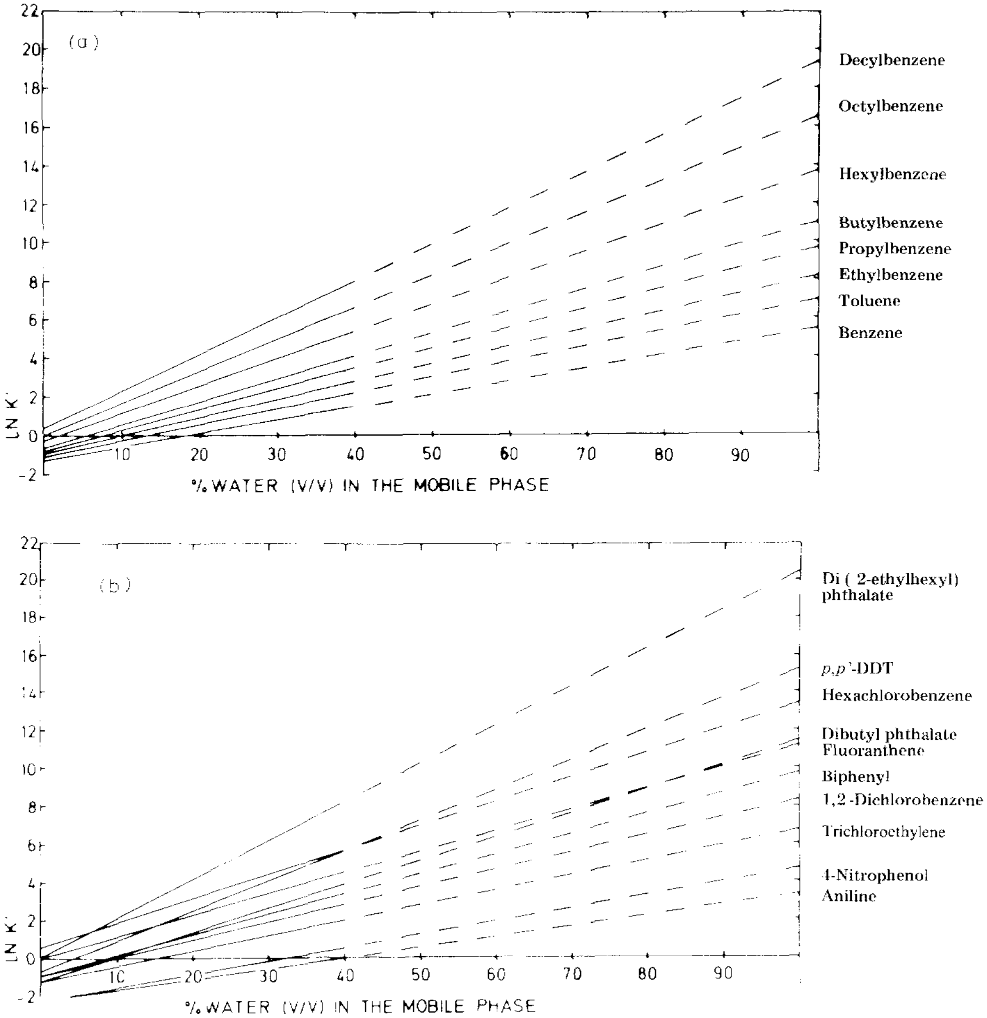

Fig. 1. Relationship between ln k' and the water concentration in the mobile phase. (a) *n*-Alkylbenzenes; (b) OECD reference substances.

TABLE V

RESULTS OF LINEAR REGRESSION ANALYSIS

Function	a	s_a*	b	s_b*	r	$s_{y \cdot x}$
f	5.493	±0.036	1.3722	±0.0067	0.9999	±0.0622
f'	6.812	±0.032	1.2065	±0.0059	0.9999	±0.0545

* s_a and s_b are the standard deviations for the regression coefficients a and b, respectively; $s_{y \cdot x}$ is the standard error of the estimate.

TABLE VI

CORRELATION OF LOG k' AND LOG P_{OW} FOR n-ALKYLBENZENES AT DIFFERENT COMPOSITIONS OF THE MOBILE PHASE

Log $P_{OW} = a + b \log k'$. Log k' values calculated from the entire equation for n-alkylbenzenes. Log P_{OW} literature data according to Hansch and Leo[20]. For all volume fractions: $r = 0.998$; $s_{y \cdot x} = \pm 0.05$.

φ_{CH_3OH}	a	b
0*	0.05	0.873
0.10*	0.13	0.957
0.20*	0.23	1.059
0.30*	0.36	1.185
0.40*	0.51	1.346
0.50*	0.72	1.557
0.60	1.00	1.847
0.70	1.42	2.269
0.80	2.08	2.942
0.90	3.29	4.181
1.00	6.27	7.226

* Extrapolated.

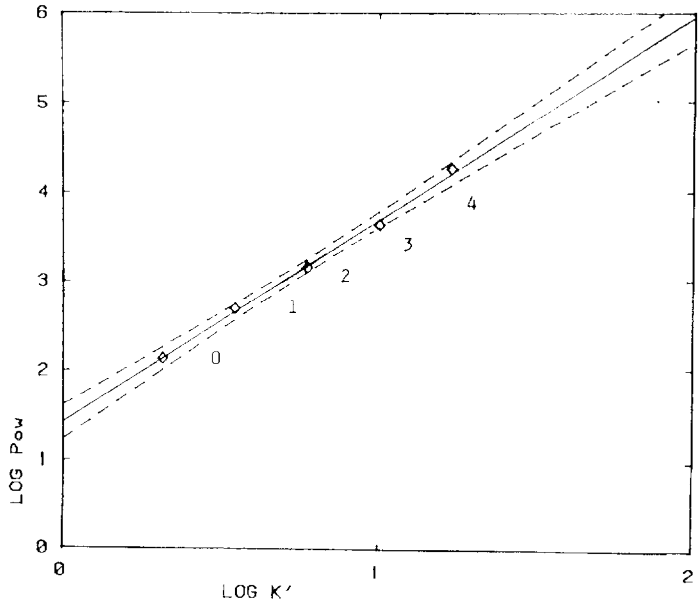

Fig. 2. Correlation of literature log P_{OW} data[20] with experimental log k' values for n-alkylbenzenes and a methanol–water mobile phase (70:30, v/v). Dashed lines mark the confidence interval calculated for 95% significance. Log P_{OW} = 1.42 + 2.27 log k'. $r = 0.998$; $n = 5$; $s_{y \cdot x} = \pm 0.052$. 0 = Benzene; 1 = toluene; 2 = ethylbenzene; 3 = propylbenzene; 4 = butylbenzene.

Log P_{OW} versus log k'

To perform the liquid chromatographic determination of P_{OW}, the HPLC capacity factors of the investigated substances were correlated with the corresponding octanol–water partition coefficients. For this, we used the P_{OW} values given in the literature[20] and also those from the OECD/EEC laboratory comparison test.

n-Alkylbenzenes. The basis for the correlation were the log k' values calculated from the entire equation given above, as well as the log P_{OW} values given by Leo and Hansch and Leo[20] (*cf.*, Table VIII).

As can be seen from Table VI, good linear relationships between log P_{OW} and log k' were obtained at all eluent compositions, with a correlation coefficient of $r = 0.998$ and a standard error of the estimate of $s_{y \cdot x} = \pm 0.05$.

As an example of the correlations, Fig. 2 represents the regression line determined for a methanol–water eluent of composition 70:30 (v/v).

OECD reference substances. The log k' values of the reference substances required for correlation were calculated by using the relationships between ln k' and φ_{CH_3OH} listed in Table IV. The corresponding log P_{OW} values were the mean values obtained in the OECD/EEC ring test by means of the shake-flask method (*cf.*, Table I).

The values for di(2-ethylhexyl)phthalate were not included in the correlations, as the log P_{OW} values measured with the shake-flask method were *ca.* 3 log *P* units lower than the value calculated from the regression lines for the seven other reference substances. Also, the value estimated according to Rekker[18] was *ca.* 4 log *P* units higher than the values determined in the laboratory intercomparison test.

Table VII shows the parameters of the straight-line equation calculated for the seven reference substances at different eluent compositions. We obtained correlation coefficients of $r \geq 0.97$ and standard errors of the estimate of $s_{y \cdot x}$ from ± 0.36 to ± 0.54 log *P* units.

TABLE VII

CORRELATION OF LOG k' AND LOG P_{OW} FOR SEVEN OECD/EEC REFERENCE SUBSTANCES AT VARYING COMPOSITIONS OF THE MOBILE PHASE

Log $P_{OW} = a + b$ log k'. Log k' values calculated according to Table VI. Log P_{OW} results from the laboratories which participated in the ring test (*cf.*, Table I).

φ_{CH_3OH}	a	b	r	$s_{y \cdot x}$
0*	0.08	0.900	0.983	0.39
0.1*	0.21	0.976	0.984	0.38
0.2*	0.37	1.064	0.984	0.38
0.3*	0.56	1.167	0.985	0.37
0.4*	0.79	1.294	0.985	0.37
0.5*	1.08	1.453	0.986	0.36
0.6	1.44	1.651	0.986	0.36
0.7	1.93	1.913	0.986	0.36
0.8	2.60	2.270	0.984	0.38
0.9	3.57	2.777	0.980	0.43
1.0	5.06	3.534	0.967	0.54

* Extrapolated.

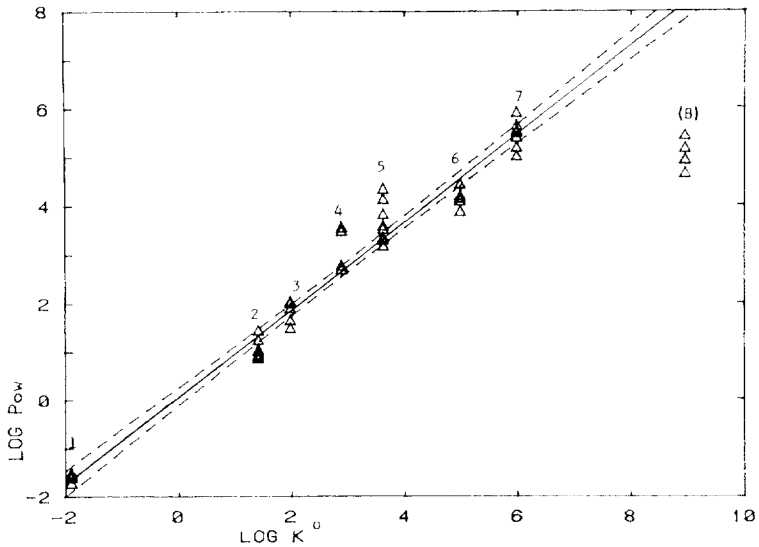

Fig. 3. Correlation of log P_{OW} mean values from OECD/EEC laboratory comparison tests with log k° values obtained by HPLC. Dashed lines mark the confidence interval calculated for 95% significance. Log $P_{OW} = 0.08 + 0.900 \log k^\circ$. $r = 0.983$; $n = 46$; $s_{y \cdot x} = \pm 0.389$. 1 = Urea; 2 = aniline; 3 = 4-nitrophenol; 4 = trichloroethylene; 5 = 1,2-dichlorobenzene; 6 = dibutylphthalate; 7 = hexachlorobenzene; 8 = di(2-ethylhexyl)phthalate.

Fig. 3 exemplifies the correlations by showing the regression line for a mobile phase of 100% water (extrapolated).

If one considers the wide range of values produced by the shake-flask method in the ring test, this would explain the considerably higher standard error of (log P_{HPLC}) estimates when compared with the *n*-alkylbenzenes.

Calculation of log P_{HPLC} values

For each of the two reference systems, we calculated the partition coefficients of all substances by using the correlations, determined as described above, for log P_{OW} and log k' (cf., Tables VI and VII) as well as the log k' values calculated for each eluent and the respective substance according to Table IV.

Figs. 4 and 5a and b show the shapes of the curves of these log P_{HPLC} values as a function of the water content of the mobile phase. These figures illustrate that, for most substances, an approximately linear relationship between log P_{HPLC} and water content was obtained only at a water content of at least 30% (v/v). The most significant relative changes in log P_{HPLC} values were found within the range 0–30% (v/v) water in the eluent, with partly degressive and partly progressive slopes.

On comparing our data with OECD and literature values, the relative hydrophobicity of the substances for both reference systems appeared to be best reflected at a high water content of the mobile phase. This result is plausible, considering that the hydrophobic behaviour of substances in reversed-phase separation systems can surely be best shown between water and the apolar alkyl chains of the octadecylsilica. Accordingly, the regression lines between log P_{OW} and log k°, which we determined

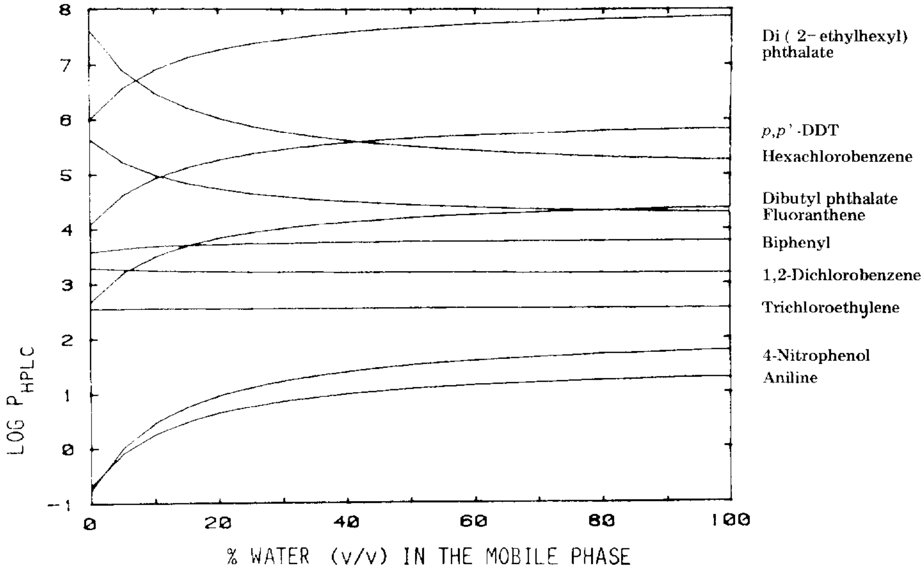

Fig. 4. Variation of log P_{HPLC} values with the water content in the mobile phase. Reference system: *n*-alkylbenzenes.

(extrapolated) for pure water as the mobile phase, were nearly identical in both reference systems:

n-alkylbenzenes:

$$\log P_{OW} = 0.05\ (0.10) + 0.87\ (0.01) \log k^{\circ}$$
$$r = 0.998;\ n = 5;\ s_{y \cdot x} = \pm\ 0.05 \qquad (9)$$

OECD/EEC reference substances:

$$\log P_{OW} = 0.08\ (0.09) + 0.90\ (0.01) \log k^{\circ}$$
$$r = 0.983;\ n = 46;\ s_{y \cdot x} = \pm\ 0.39 \qquad (10)$$

Table VIII compares the log P_{OW} values determined experimentally with those calculated from fragment constants according to Rekker[18] and some literature values. The log P_{HPLC} values listed in the table were extrapolated to a mobile phase of 100% water. It can be seen that the log P_{HPLC} values of the reference substances, which were determined from the reference system of *n*-alkylbenzenes, deviate by a maximum of 0.6 log *P* units from the mean values obtained by the shake-flask method in the OECD/EEC ring test. This does not take into account the DEHP (Δlog P_{HPLC} = 3) for the above-mentioned reasons. However, these relatively large deviations can by no means be attributed to the HPLC method. Rather, the literature values show that they are caused by the wide range of variation obtained in the laboratory comparison test. This is also shown by the log P_{HPLC} values for the *n*-alkylbenzenes, which were determined in the same fashion from the OECD ring test reference system, and which deviate from literature values by a maximum of 0.2 log *P* units.

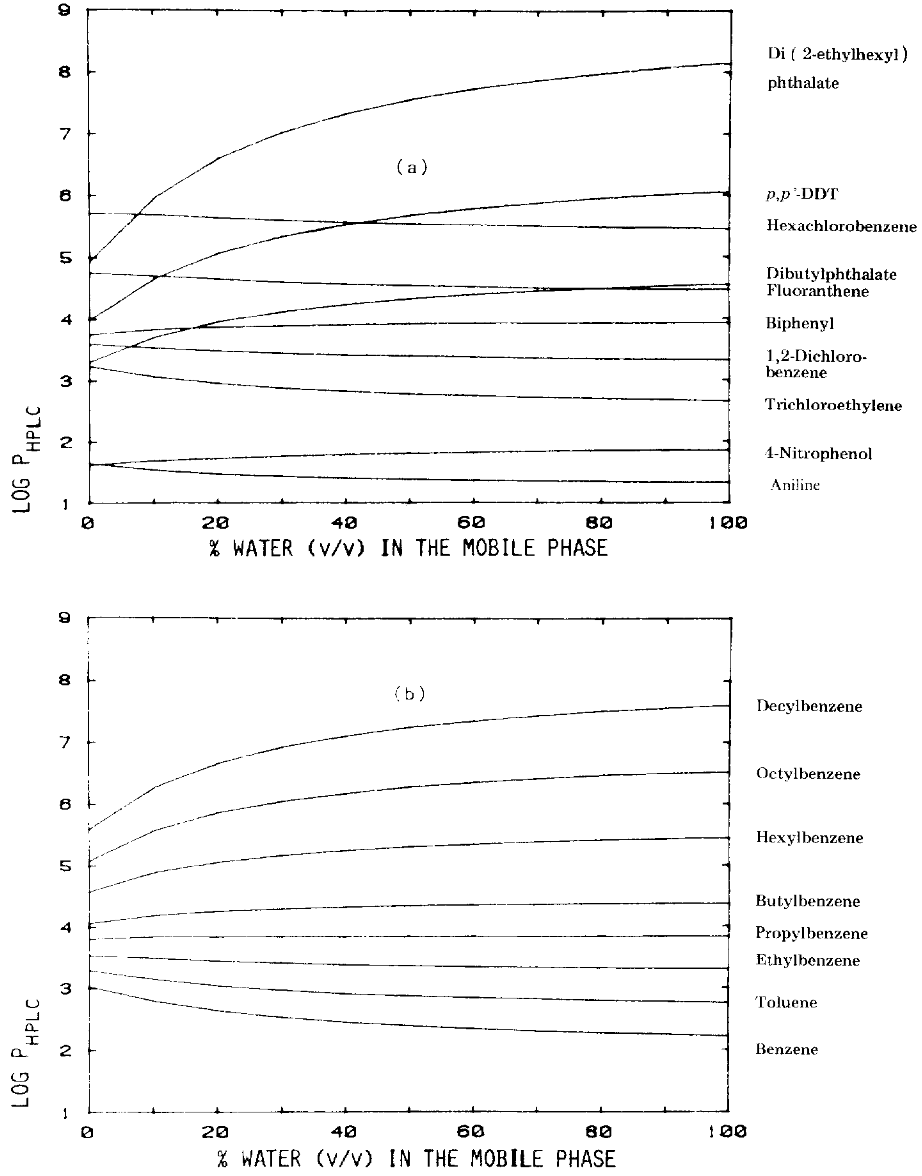

Fig. 5. Variation of log P_{HPLC} values with the water content of the mobile phase. Reference system: substances tested in the OECD/EEC laboratory comparison tests. (a) OECD reference substances; (b) *n*-alkylbenzenes.

In a final evaluation of the results obtained in this study by HPLC, it must be taken into consideration that most of the substances investigated in the laboratory comparison test represent "problem substances" of the shake-flask method. In this sense, aniline and 4-nitrophenol are ionic, trichloroethylene is highly volatile and DEHP, hexachlorobenzene and dibutylphthalate are highly hydrophobic.

If one considers the wide range of variation of the log P_{OW} values used for correlation in the laboratory comparison test, there is surprisingly good agreement between log P_{HPLC} values determined in both reference systems and the other values listed in Table VIII. For most of the substances investigated in the OECD/EEC laboratory comparison test the log P_{HPLC} values lie within the range of variation of the shake-flask method. It is true that this fact, in view of the wide range of variation in the laboratory comparison test, does not say much about the quality of the HPLC method. However, for substances the log P_{HPLC} values of which do not lie within the variation range of the laboratory comparison study, such as dibutylphthalate, DEHP and trichloroethylene, the values determined chromatographically seem to be considerably more reliable than those obtained by the shake-flask method. This is evident in the literature and experimental data in Table VIII.

Comparison of the methods

The compilation of the advantages and disadvantages of the different methods for the determination of the log P_{OW} values presented in Table IX shows that, in comparison with the shake-flask method, the chromatographic methods offer several advantages. Thus, measurements can be taken quickly, with relative ease, and are highly reproducible. There is no need for a time-consuming quantitative determi-

TABLE VIII

COMPILATION OF LOG P_{OW} VALUES DETERMINED BY DIFFERENT METHODS

Substance	*HPLC* (reference substances)*	*HPLC* (alkyl-benzenes)*	*OECD/EEC** (shake-flask method)*	*Rekker*** (calculated)*	*Literature§*	*Miscellaneous*
Aniline	1.34	1.27	1.08	1.03	0.90	0.98[29]
Biphenyl	3.93	3.78		4.04	4.06	3.76[29]
Dibutylphthalate	4.56	4.39	4.08	5.43		5.15[29]
1,2-Dichlorobenzene	3.34	3.20	3.61	3.53	3.38	
p,p'-DDT	6.06	5.84		7.35	(4.98)	6.19[29]
Di(2-ethylhexyl)phthalate	8.15	7.86	5.03	9.7		
Fluoranthene	4.47	4.31		4.9		
Urea	−1.63	−1.61	−1.57	−1.35	(−1.09)	
Hexachlorobenzene	5.46	5.26	5.47	6.51	(4.13)	6.18[29]
4-Nitrophhenol	1.86	1.77	1.88	(1.27)	1.95	1.96§§
Trichloroethylene	2.67	2.56	3.14	2.28	2.25	
Benzene	2.23	2.13		2.13	2.13	
Toluene	2.77	2.65		2.60	2.69	
Ethylbenzene	3.30	3.17		3.13	3.15	
Propylbenzene	3.84	3.69		3.66	3.63	
Butylbenzene	4.38	4.21		4.19	4.26	
Hexylbenzene	5.45	5.25		5.25		
Octylbenzene	6.52	6.29		6.31		
Decylbenzene	7.60	7.33		7.37		

* Values for a mobile phase of 100% water (extrapolated).

** Mean values of the OECD/EEC laboratory comparison test (*cf.* Table I).

*** Calculated from fragment constants[18].

§ According to Hansch and Leo[20].

§§ The value was determined in this study.

TABLE IX

COMPARISON OF METHODS FOR THE DETERMINATION OF LOG P_{OW}

Method	*Advantages*	*Problems/disadvantages*
OECD shake-flask method in the system *n*-octanol–water	Good reflection of the characteristics of biological lipid phases. Highly sensitive for changes in hydrophobicity. Values available for *ca.* 5000 substances. Many studies available on structure–activity relationship and bioaccumulation. Standardized test method available. Measuring range of *ca.* −2 to +6.	Not applicable to surface-active and organometallic substances. Applicable to a limited extent to: Ionic substances. Association/dissociation. Volatile substances. Problems: Purity of the substance. Formation of emulsions. Phase separation. Highly hydrophobic substances. Disadvantages: Very time consuming owing to the need to carry out a quantitative determination of the test substance in both phases.
Reversed-phase HPLC	Fast method. Suitable for substances containing impurities and substance mixtures. Requires no quantitative determination. Applicable to volatile substances (gases). Well suited to hydrophobic substances. Highly reproducible. Measuring range of *ca.* 1–8.	Not applicable to organometallic substances. Applicable to a limited extent to surface-active and ionic substances. Problem: selective separation mechanisms are possible for some substances. Disadvantage: requires a reference system.
Reversed-phase TLC	Same as for HPLC, but requires considerably less lab. equipment, is less reproducible and less sensitive.	Same as for HPLC, but not for volatile substances.

nation of the test substance in both phases of the partition system. Volatile substances, including gases, mixtures and substances containing impurities, can be investigated by HPLC without much difficulty. Even for highly hydrophobic substances, where log P_{OW} determination is particularly time consuming and difficult by the shake-flask method, P_{OW} values can be determined without extra effort by means of chromatographic methods.

A laboratory comparison test undertaken by NORDFORSK[38] (Nordic Cooperative Organization for Applied Research) in 1980 also confirmed that the chromatographic methods HPLC and HPTLC are on a par with the shake-flask method. In this ring test, dimethylphthalate and methoxychlor were investigated by using both the shake-flask and the chromatographic methods. The chromatographic separation system consisted of octadecylsilica as the stationary phase and a mixture of acetonitrile and water as the mobile phase. The following substances were used as reference substances, with log P_{OW} values: phenoxyethanol, 1.16; benzophenone, 3.18; biphenyl, 4.09; 4,4′-dichlorobiphenyl, 5.49; and *p,p*′-DDT, 6.19.

The results of this ring test have shown that the chromatographic methods require considerably less effort and render more precise results than the direct OECD method. For dimethylphthalate, which is only slightly hydrophobic (log $P_{OW} \approx 2$), all three methods produced comparable results. For the hydrophobic methoxychlor (log $P_{OW} \approx 4$), the results of the shake-flask method vary by a factor of 12. In contrast, the chromatographic results show a relative standard deviation of 17–26% and concur, in both methods, with the literature value.

CONCLUSIONS

The results of this study, together with the literature results presented in Table III for comparison, confirm that the partition coefficient (*n*-octanol–water) can be determined by reversed-phase HPLC with an accuracy equivalent to that of the shake-flask method. This can be done for homologues and structurally related compounds by means of a single correlation by using HPLC capacity factors at a composition of the mobile phase with a high water content ($\geqslant$ 30%, v/v). It should be noted, however, that the correlation determined for a given substance class cannot be extrapolated automatically to other substances and separation systems, as selective interactions may occur between the solute and the separation system.

A possibility for avoiding such interactions seems to be the measurement of the k' values at different compositions of the mobile phase and extrapolating a ln k° value to pure water in the manner described above. It is likely that for a multitude of different substances this methodology produces k° values that are based mainly on hydrophobic interactions. This assumption is supported by the nearly identical regression lines obtained in this study of the *n*-alkylbenzenes and the OECD reference substances, which make it possible to predict reciprocally their partition coefficients with reasonable accuracy.

Owing to its simplicity and speed, the good reproducibility of the results and its broader applicability, the liquid chromatographic determination method investigated is a good alternative or complement to the conventional shake-flask method. We believe that it may well be used for the determination, as required under the EEC-wide chemicals legislation, of the order of magnitude of the partition coefficient of a chemical newly placed on the market in order to obtain an estimate of its bioaccumulation behaviour. The method is particularly suitable for highly hydrophobic substances (log $P_{OW} \geqslant 4$).

The statement, "It cannot be too strongly emphasized that, on all the available evidence, the only true model for octanol is octanol itself"[22], is still regarded by some authors to hold true. However, there is some evidence that, when a chemical's bioaccumulation is estimated on the basis of HPLC parameters, the "detour" via the system octanol–water could be avoided in future. This would become possible if, by using reference substances, one could succeed in defining the order of magnitude of the capacity factor above which a substance will presumably bioaccumulate.

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