

Measurement of partition coefficients by reversed-phase ion-pair liquid chromatography

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ABSTRACT

Sixteen phenylamine- and naphthylaminesulphonic acids which are negatively charged and weakly retained or non-retained in reversed-phase high-performance liquid chromatography were used as model compounds to examine the quantitative relationship between the calculated *n*-octanol–water partition coefficient ($\log P$) and the retention value ($\log k'$) (or $\log k_w$) and solute charges in reversed-phase ion-pair liquid chromatography (RP-IPC). It was observed that the correlation of $\log P$ vs. $\log k'$ (or $\log k_w$) for solutes with one negative charge has moderate regression coefficients of *ca.* 0.80, but, the correlation of $\log P$ vs. $\log k'$ ($\log k_w$) and solute charges (n_s) for solutes with different charges has a regression coefficient higher than 0.98. The $\log k'$ (or $\log k_w$) value in RP-IPC always make a positive contribution and the solute charges always makes a negative contribution to $\log P$ values. The proposed relationship between the $\log P$ value and the $\log k'$ value and solute charges makes it possible to predict the *n*-octanol–water $\log P$ values of ionic compounds from the retention values in RP-IPC, and is also useful in elucidating the retention mechanism in RP-IPC.

INTRODUCTION

Partition coefficients, a medicinally relevant physico-chemical property, play an influential role in many biological processes and therefore find numerous applications in quantitative structure–activity relationship (QSAR) studies [1–3] and in the determination of environmental parameters [4–6]. Partition coefficients have been measured in nearly 100 solvent–water systems, mainly by means of the traditional shake-flask method [3]. *n*-Octanol–water is widely accepted as the reference system because of its analogy with biomembranes [3]. However, practical disadvantages and the limitation of $\log P$ values to between -2 and $+4$ have led researchers to investigate other methods for measuring partition coefficients [7,8]. In recent years, reversed-phase high-performance liquid chromatography (RP-

HPLC) has become a popular alternative, capacity factors frequently being used as substitutes for *n*-octanol–water partition coefficients in QSAR studies.

The measurement of partition coefficients by RP-HPLC is based on the principle of the partition of a solute between a polar eluent and a stationary phase of low polarity [9–11]. It should be noted that many ionic organic compounds such as drugs are weakly or not retained in RP-HPLC even with pure buffer or water as the eluent, which makes it difficult to measure partition coefficients from the capacity factors in RP-HPLC. However, reversed-phase ion-pair liquid chromatography (RP-IPC) has become a well established method for the separation of ionic compounds, in which the retention can be regulated by the properties and concentration of organic modifier and ion-pair reagent and also by a competing ion with the same charges as the analyte [12–16]. Recent studies [17–19] on the retention process in RP-IPC showed that both electrostatic and hydrophobic interactions make im-

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portant contributions to retention. The former is directly related to the solute charges and the latter to solute hydrophobicity, which is paralleled with the partition coefficient of a solute. It is necessary to investigate the possibility of calculating partition coefficients from the capacity factors in RP-IPC if the contribution of the solute charge on the retention is corrected. In this work, the quantitative correlation of partition coefficients with capacity factors in RP-IPC and solute charges was studied.

THEORETICAL

Relationship between log P (P = partition coefficient) values and log k' (k' = capacity factor) values and solute charges

To establish a common hydrophobicity scale characterizing the partitioning of solutes in various organic–water systems, the free energies of partition should linearly related [20]:

$$\log P = -(\Delta G_{\text{pm}}^0 + \Delta G_{\text{pe}}^0)/RT \quad (1)$$

where ΔG_{pm}^0 and ΔG_{pe}^0 are the free energy of a solute transition from water to the organic phase, related to molecular and electrostatic interactions, R is the gas constant and T is absolute temperature. It can be expected that there will be a much weaker electrostatic interaction in the partitioning of an ionic compound or a natural compound in *n*-octanol–water system, and we then have

$$\log P = -\Delta G_{\text{pm}}^0/RT \quad (2)$$

Eqn. 2 means that the log P values of natural and ionic compounds are mainly determined by the molecular interaction, and related to ΔG_{r}^0 (free energy of a solute transition from mobile phase to stationary phase in RP-HPLC) or retention in RP-HPLC. In RP-IPC, according to the electrostatic interaction model, the free energy change in the adsorption or partitioning of an ionic solute between the mobile and stationary phase can be divided into two parts, that is, free energy changes related to molecular and electrostatic interactions (ΔG_{im}^0 and ΔG_{ie}^0), hence the capacity factor of an ionic solute can be expressed as [21]

$$\log k' = -(\Delta G_{\text{im}}^0 + \Delta G_{\text{ie}}^0)/RT \pm \log \phi \quad (3)$$

where ϕ is the mobile/stationary phase ratio. For a given RP-IPC column system, ΔG_{im}^0 depends on the

hydrophobicity of the solute and should be paralleled with the ΔG_{pm}^0 of log P value; ΔG_{ie}^0 can be expressed as

$$\Delta G_{\text{ie}}^0 = n_e F \psi_0 / RT \quad (4)$$

where ψ_0 is the electrical potential on the stationary surface and is constant under given conditions, n_e is the apparent charges of the ionic solute and F is the Faraday constant. Therefore, we have

$$\log P = A \log k' + B n_e + C \quad (5)$$

where A , B and C are constants under given conditions. It can be seen that the partition coefficient of an ionic compound in the *n*-octanol–water system is related to the capacity factor and solute charges in RP-IPC.

Calculation of n-octanol–water partition coefficient

According to Hansch and co-workers [22,23], the *n*-octanol–water partition coefficient (log P) can be expressed as

$$\log P = \sum_1^n a_n f_n + \sum_1^m b_m F_m \quad (6)$$

where f_n is the hydrophobic fragmental constant, the lipophilicity contribution of a constituent part of a structure to the total lipophilicity, F_m represents factors affecting the partition coefficient and a_n and b_m are numerical factors indicating the incidence of

TABLE I

THE FRAGMENTAL CONSTANTS (f_n) USED TO CALCULATE THE *n*-OCTANOL–WATER PARTITION COEFFICIENTS OF SULPHONIC ACIDS

The fragmental constants are taken from Lyman [6] and Wang [24].

Functional group	f_n
f_{CH}	0.355
f_{c}	0.255
$f_{\text{SO}_3^-}^{\oplus}$	-4.53
$f_{\text{NH}_2}^{\oplus}$	-1.00
$f_{\text{-O-}}^{\oplus}$	-0.61
$f_{\text{CH}_3}^{\oplus}$	0.89
f_{Cl}^{\oplus}	0.94
f_{H}	0.23
$f_{\text{C}_6\text{H}_5}$	1.90

the given fragmental and other factors indicating the incidence of the given fragmental and other factors in the structure of complex substance. Table I lists the fragmental constants used to calculate the partition coefficient of sixteen phenylamine- and naphthylaminesulphonic acids.

Below we give three examples of the calculation of log *P* values for the sulphonic acids:

1-aminobenzene-2-sulphonic acid:

$$\begin{aligned}\log P &= f_{C_6H_5} + f_{NH_2} + f_{SO_3^-} + F_{p_2} - f_H \\ &= 1.90 - 1.0 - 4.53 + 0.08 (4.53 + 1) - 0.23 \\ &= -3.42\end{aligned}$$

1-amino-4-methoxybenzene-2-sulphonic acid:

$$\begin{aligned}\log P &= f_{C_6H_5} + f_{NH_2} + f_{SO_3^-} - 2f_H + f_{-O-} + f_{CH_3} \\ &\quad + F_b + F_{p_2} = 1.90 - 1.0 - 4.53 - 0.46 \\ &\quad - 0.61 + 0.89 - 0.12 + 0.08(4.53 + 1) = 3.61\end{aligned}$$

2-aminonaphthalene-4,6,8-trisulphonic acid:

$$\begin{aligned}\log P &= 8f_{CH} + 2f_c - 4f_H + 3f_{SO_3^-} + f_{NH_2} \\ &= 8 \times 0.355 + 2 \times 0.255 - 4 \times 0.23 + 3 \times \\ &\quad (-4.53) - 1.0 = -12.22\end{aligned}$$

where F_{p_2} represents the combined interaction between polar substituted group and polar substituted

TABLE II

CALCULATED *n*-OCTANOL-WATER PARTITION COEFFICIENTS (log *P*) OF SIXTEEN SULPHONIC ACIDS AND THEIR NEGATIVE CHARGES (n_c)

Solute	n_c	Log <i>P</i>
1-Aminobenzene-2-sulphonic acid	1	-3.42
1-Aminobenzene-3-sulphonic acid	1	-3.86
1-Aminobenzene-4-sulphonic acid	1	-3.86
1-Amino-4-methylbenzene-2-sulphonic acid	1	-2.76
1-Amino-4-methoxybenzene-2-sulphonic acid	1	-3.61
1-Amino-6-chlorobenzene-3-sulphonic acid	1	-3.15
1,3-Diaminobenzene-4-sulphonic acid	1	-4.65
1-Aminonaphthalene-5-sulphonic acid	1	-2.70
2-Aminonaphthalene-5-sulphonic acid	1	-2.70
2-Aminonaphthalene-6-sulphonic acid	1	-2.70
1-Aminobenzene-2,5-disulphonic acid	2	-8.18
1,3-Diaminobenzene-4,6-disulphonic acid	2	-8.97
2-Aminonaphthalene-4,8-disulphonic acid	2	-7.46
2-Aminonaphthalene-3,6-disulphonic acid	2	-7.02
2-Aminonaphthalene-4,6,8-trisulphonic acid	3	-12.22
2-Aminonaphthalene-3,6,8-trisulphonic acid	3	-11.86

group in orthogonal position, and F_b is a bond factor. Table II lists the calculated log *P* values and negative charges for sixteen sulphonic acids.

EXPERIMENTAL

Materials

The phenylamine- and naphthylaminesulphonic acids analysed were obtained from the Dyestuff Laboratory, Chemical Engineering Department, Dalian University of Sciences and Technology. Standard solutions were prepared in water. Doubly distilled water was used throughout. Tetrabutylammonium iodide (TBAI) (Beijing Chemical Reagent Factory, Beijing, China), methanol, NaH_2PO_4 , NaOH and HCl were of analytical-reagent grade.

Apparatus

The RP-IPC experiments were performed using a stainless-steel column (200 × 4.0 mm I.D.) that contained a Spherisorb-ODS reversed-phase packing material of 5 mm particle diameter (Phase Separations, Deeside, UK). The column was packed at the National Chromatographic Research and Analysis Centre, Dalian, China. The mobile phase was delivered by two Waters Model 510 pumps. The ratios of methanol to phosphate buffer in eluents A and B were 0.95 : 0.05 and 0.6 : 0.4 (v/v), respectively, and the concentration of the ion-pair reagent TBAI, the NaH_2PO_4 concentration and pH in both eluents were 4 mmol/l, 10 mmol/l and 7.15, respectively. The organic modifier concentration in the eluent was regulated by an NEC-APCIV computer with a Waters System Interface Module (Waters Assoc., Milford, MA, USA) by changing the ratio of eluent A to eluent B. The eluates were detected with a Waters Model 490 programmable multi-wavelength detector set at 254 nm. Samples were loaded with a U6K syringe loading sample injector. The flow-rate of the eluent was 1.0 ml/min. The eluent pH was measured with an SA-720 pH meter (Orion Research, Chicago, IL, USA). All experimental data were processed on a COMPAQ-286 personal computer with Lotus software (Micro-soft).

RESULTS AND DISCUSSION

The capacity factors of sixteen sulphonic acids

TABLE III

CAPACITY FACTORS OF SIXTEEN PHENYLAMINE- AND NAPHTHYLAMINESULPHONIC ACIDS AT DIFFERENT METHANOL CONCENTRATIONS IN RP-IPC

For experimental conditions, see text.

Solute	Methanol-to-buffer ratio (v/v)			
	0.325	0.281	0.239	0.198
1-Aminobenzene-2-sulphonic acid	0.46	1.00	1.64	3.04
1-Aminobenzene-3-sulphonic acid	0.26	0.54	0.84	1.34
1-Aminobenzene-4-sulphonic acid	0.15	0.30	0.49	1.01
1-Amino-4-methylbenzene-2-sulphonic acid	1.54	3.27	5.69	9.86
1-Amino-4-methoxybenzene-2-sulphonic acid	0.75	1.56	2.46	4.79
1-Amino-6-chlorobenzene-3-sulphonic acid	1.45	3.15	5.29	9.65
1,3-Diaminobenzene-4-sulphonic acid	0.14	0.28	0.43	0.95
1-Aminonaphthalene-5-sulphonic acid	0.62	1.28	2.22	4.21
2-Aminonaphthalene-5-sulphonic acid	0.78	1.63	2.94	5.25
2-Aminonaphthalene-6-sulphonic acid	1.23	2.80	5.01	9.85
1-Aminobenzene-2,5-disulphonic acid	0.29	0.68	1.11	2.46
1,3-Diaminobenzene-4,6-disulphonic acid	0.32	0.63	1.03	2.34
2-Aminonaphthalene-4,8-disulphonic acid	0.63	1.63	3.34	9.56
2-Aminonaphthalene-3,6-disulphonic acid	0.76	2.06	4.40	10.24
2-Aminonaphthalene-4,6,8-trisulphonic acid	1.09	3.04	6.98	16.79
2-Aminonaphthalene-3,6,8-trisulphonic acid	1.14	3.25	7.40	17.66

experimentally measured by RP-IPC are presented in Table III. The quantitative correlations between the calculated $\log P$ values and $\log k'$ values in RP-IPC for ten sulphonic acids with one negative

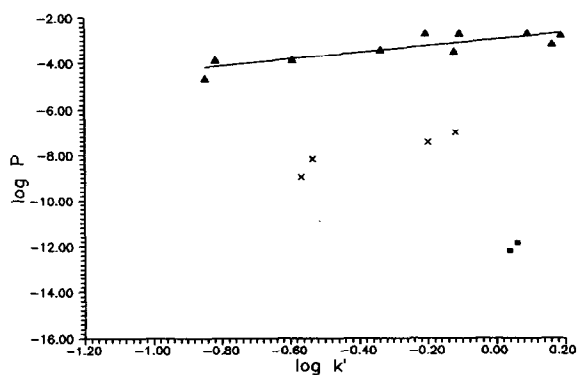


Fig. 1. Quantitative correlation of the calculated n -octanol-water partition coefficients ($\log P$) vs. $\log k'$ and n_e in RP-IPC when the methanol concentration is 0.325; for other experimental conditions, see text. Points \blacktriangle , \times and \blacksquare represent sulphonic acids with one, two and three negative charges, respectively. For ten sulphonic acids with one negative charge: $\log P = -2.899 + 1.541 \log k'$; $r = 0.773$, $n = 10$. For sixteen sulphonic acids with different negative charges: $\log P = -5.830 - 1.333 \log k'$, $r = 0.017$, $n = 16$; $\log P = 1.807 + 1.722 \log k' - 4.606 n_e$, $r = 0.988$, $n = 16$.

charge or sixteen sulphonic acids with different negative charges and the relationship of the calculated $\log P$ values vs. $\log k'$ in RP-IPC and solute negative charges were established, and the results obtained are shown in Figs. 1–4. Very low regression

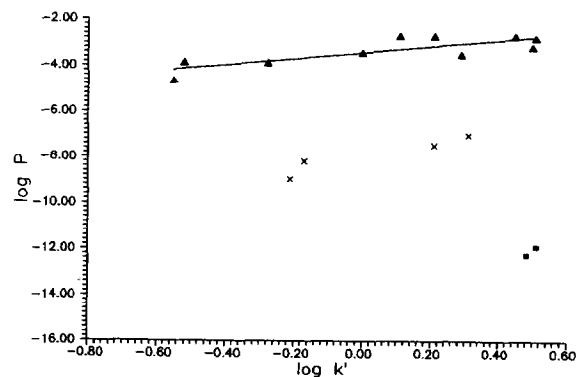


Fig. 2. Quantitative correlation of the calculated $\log P$ vs. $\log k'$ and n_e in RP-IPC when the methanol concentration is 0.281. Experimental conditions and symbols as in Fig. 1. For ten sulphonic acids with one negative charge: $\log P = -3.393 + 1.485 \log k'$, $r = 0.771$, $n = 10$. For sixteen sulphonic acids with different negative charges: $\log P = 5.166 - 2.499 \log k'$, $r = 0.075$, $n = 16$; $\log P = 1.303 + 1.630 \log k' - 4.685 n_e$, $r = 0.989$, $n = 16$.

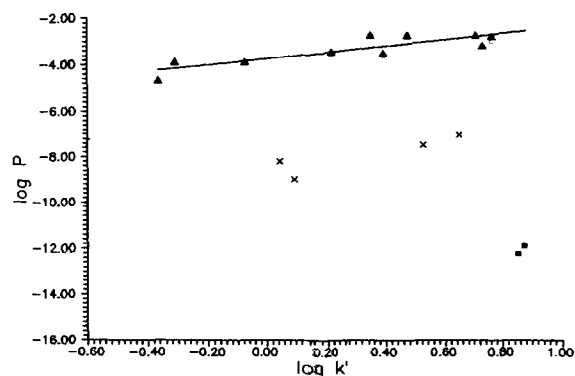


Fig. 3. Quantitative correlation of the calculated $\log P$ vs. $\log k'$ and n_e in RP-IPC when the methanol concentration is 0.239. Experimental conditions and symbols as in Fig. 1. For ten sulphonic acids with one negative charge: $\log P = -3.714 + 1.451 \log k'$, $r = 0.801$, $n = 10$. For sixteen sulphonic acids with different negative charges: $\log P = -4.360 - 2.923 \log k'$, $r = 0.125$, $n = 16$; $\log P = 1.055 + 1.566 \log k' - 4.783n_e$, $r = 0.991$, $n = 16$.

coefficients (<0.20) were obtained for the relationship of $\log P$ vs. $\log k'$ in RP-IPC for sixteen sulphonic acids with different negative charges, but the moderate (0.80) or high regression coefficients (>0.98) were obtained for the relationship of $\log P$ vs. $\log k'$ for ten sulphonic acids with one negative charge or that of $\log P$ vs. $\log k'$ and n_e for sixteen sulphonic acids with different negatives charges.

The retention in RP-IPC is more complex than that in RP-HPLC owing to presence of the ion-pair reagent and may be based on numerous factors [25], but the hydrophobic and electrostatic interactions play the dominant role for rigid compounds. This finding is confirmed by the fact that the correlation is greatly improved by taking into account the solute charges. However, other factors such as steric and configurational factors also play some role, especially for the complex compounds, which may cause the moderate regression coefficients (about 0.80) for ten sulphonic acids with one negative charge. We think that the effects of other factors such as steric and configurational factors on the quantitative correlation may arise in at least two ways: one is through the change in the apparent charges which may affect the electrostatic interaction of the solutes in RP-IPC; the other may result from the fact that there is a difference between the experimental solute hydrophobicity ($\log P$) and the calculated value.

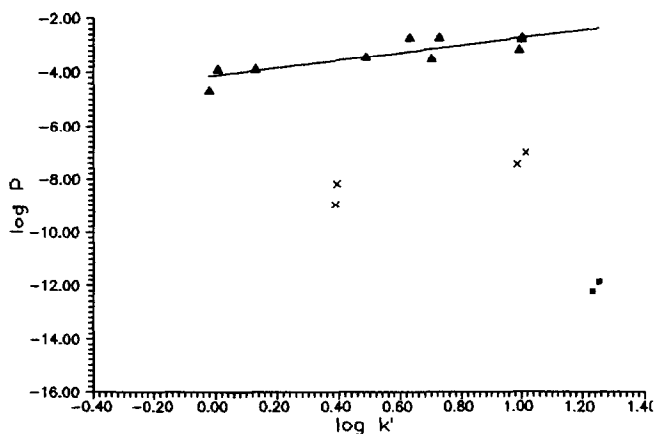


Fig. 4. Quantitative correlation of the calculated $\log P$ vs. $\log k'$ and n_e in RP-IPC when the methanol concentration is 0.198. Experimental conditions and symbols as in Fig. 1. For ten sulphonic acids with one negative charge: $\log P = -4.128 + 1.477 \log k'$, $r = 0.778$, $n = 10$. For sixteen sulphonic acids with different negative charges: $\log P = -2.995 - 3.557 \log k'$, $r = 0.200$, $n = 16$; $\log P = 0.703 + 1.601 \log k' - 4.890n_e$, $r = 0.991$, $n = 16$.

It is necessary to investigate further how these factors affect the quantitative correlation of $\log k'$ vs. $\log P$. In the relationship of $\log P$ vs. $\log k'$ for sulphonic acids with one negative charge and $\log P$ vs. $\log k'$ and n_e for sulphonic acids with different negative charges, the coefficient values of the $\log k'$ and n_e terms (parameters A and B) are always positive and negative, respectively, which means that $\log k'$ and n_e make positive and negative contributions, respectively, to the $\log P$ values. There is no serious effect of methanol concentration on the values of A and B in eqn. 5, but the value of C in eqn. 5 decreases with decrease in methanol concentration, which means that the retention in RP-IPC increases with decreasing methanol concentration.

It has been reported [9,11] that there is a better correlation between extrapolated $\log k_w$ and $\log P$ values than between isocratic $\log k'$ and $\log P$ values in RP-HPLC. It has been proposed that the logarithm of the capacity factors is linearly dependent on the organic modifier concentration in RP-IPC with an adequate range of organic modifier concentrations [17,18,26–28]:

$$\log k' = \log k_w + cC_b \quad (7)$$

where k_w is the extrapolated capacity factor in RP-IPC with pure buffer as the eluent, c is the slope,

TABLE IV

PARAMETERS $\log k_w$ AND c OBTAINED BY REGRESSION ANALYSIS OF THE EXPERIMENTAL DATA SHOWN IN TABLE III

Solute	$\log k_w$	c	r
1-Aminobenzene-2-sulphonic acid	1.73	-6.30	0.997
1-Aminobenzene-3-sulphonic acid	1.26	-5.60	0.995
1-Aminobenzene-4-sulphonic acid	1.24	-6.33	0.997
1-Amino-4-methylbenzene-2-sulphonic acid	2.26	-6.30	0.998
1-Amino-4-methoxybenzene-2-sulphonic acid	1.93	-6.30	0.997
1-Amino-6-chlorobenzene-3-sulphonic acid	2.27	-6.40	0.998
1,3-Diaminobenzene-4-sulphonic acid	1.20	-6.29	0.994
1-Aminonaphthalene-5-sulphonic acid	1.90	-6.47	0.996
2-Aminonaphthalene-5-sulphonic acid	2.10	-6.77	0.999
2-Aminonaphthalene-6-sulphonic acid	2.39	-7.02	0.998
1-Aminobenzene-2,5-disulphonic acid	1.74	-7.28	0.998
1,3-Diaminobenzene-4,6-disulphonic acid	1.81	-7.16	0.998
2-Aminonaphthalene-4,8-disulphonic acid	2.77	-9.16	0.997
2-Aminonaphthalene-3,6-disulphonic acid	2.90	-9.30	0.999
2-Aminonaphthalene-4,6,8-trisulphonic acid	3.07	-9.29	0.999
2-Aminonaphthalene-3,6,8-trisulphonic acid	3.10	-9.30	0.999

mainly determined by the hydrophobic and electrostatic interaction between the solute, ion-pair reagent and the eluent, and C_b is the concentration of the organic modifier in the eluent. It should be noted that there is a quadratic relationship of $\log k'$

vs. C_b in RP-HPLC for a wide range of the organic modifier concentrations [29,30], and this quadratic relationship may also exist in RP-IPC when the range of organic modifier concentration is wide. It has been observed by El Tayar *et al.* [30] that there is no serious difference in the accuracy of prediction of $\log P$ values from $\log k_w$ values of linear and quadratic origin. The $\log k_w$ values derived by linear extrapolation of the isocratic capacity factors k' shown in Table III are given in Table IV. The quantitative correlation of the calculated $\log P$ values vs. $\log k_w$ for ten sulphonic acids with one negative charge and sixteen sulphonic acids with different negative charges and also $\log P$ vs. $\log k_w$ and n_e are shown in Fig. 5. It can be seen that there is no serious difference in the regression coefficients of the quantitative correlation from capacity factors and extrapolated capacity factors. It can be concluded that it is possible to calculate n -octanol-water $\log P$ values from the retention values (both $\log k'$ and $\log k_w$) for rigid compounds in RP-IPC after the electrostatic interaction that is directly related to solute charges has been corrected. In order to improve the quantitative correlation, the effects of other factors such as steric and configurational factors should be taken into account, for which detailed investigations are needed.

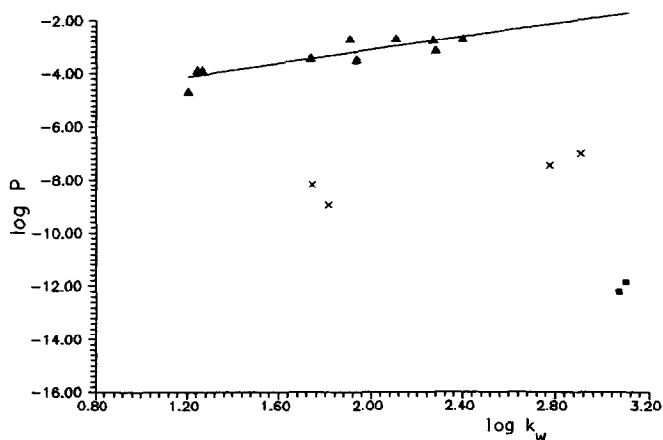


Fig. 5. Quantitative correlation of the calculated $\log P$ vs. $\log k_w$ and n_e in RP-IPC. Experimental conditions and symbols as in Fig. 1. For ten sulphonic acids with one negative charge: $\log P = -5.717 + 1.322 \log k_w$, $r = 0.800$, $n = 10$. For sixteen sulphonic acids with different negative charges: $\log P = 0.563 - 2.902 \log k_w$, $r = 0.301$, $n = 16$; $\log P = -0.4385 + 1.301 \log k_w - 5.198 n_e$, $r = 0.992$, $n = 16$.

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