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Effect of organic modifier concentration on retention in reversed-phase ion-pair liquid chromatography

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ABSTRACT

The concentration of organic modifier in the eluent is one of the most important factors that affect the retention of ionized solutes in reversed-phase ion-pair liquid chromatography (RP-IPC). Linear regression analysis of $\ln k'_{ip}$ *vs.* methanol concentration (C_b) according to the equation $\ln k'_{ip} = \ln k^w_{ip} + c_{ip}C_b$ was carried out; $\ln k^w_{ip}$ and c_{ip} are constants for a given solute with a given column system, where $\ln k^w_{ip}$ is determined mainly by the electrostatic and non-electrostatic free-energy change of retention at $C_b = 0$, and c_{ip} is mainly determined by the interaction behaviour between the ion-pair reagent, the ionized solute and the mobile phase. This equation has been confirmed experimentally. The absolute values of $\ln k^w_{ip}$ and c_{ip} in RP-IPC are much larger than those in RP high-performance liquid chromatography (HPLC), which means that there is a much stronger effect of methanol concentration on retention in RP-IPC than in RP-HPLC. On the other hand, ionized compounds with the same kind and number of charges show almost the same value of electrostatic free-energy change, and $\ln k'_{ip}$ and $\ln k^w_{ip}$ in RP-IPC can be well correlated with $\ln k'_{rp}$ and $\ln k^w_{rp}$ in RP-HPLC.

INTRODUCTION

Reversed-phase ion-pair liquid chromatography (RP-IPC) is widely used in separations of ionized organic compounds and inorganic ions [1–3]. The retention can be regulated by the properties and concentrations of the organic modifier and counter ion and also by a competing ion with the same charge as the analyte. Many models of the so-called "mechanism" of RP-IPC have been published [4–10].

Most of the proposed models, including the ion-pair model [4] and the dynamic ion-exchange model [4,8,9] are stoichiometric, *i.e.*, they construct a reaction scheme and the corresponding equilibrium constants express the interaction between the oppositely charged ion-pair reagent and analyte ions in the system. By combining these constants with the Langmuir isotherm, equations are obtained for the capacity factor as a function of different variables. Recently, the Gouy–Chapman theory in combination with a modified Langmuir isotherm [11,12] and statistical thermodynamic method [13] in combination with the Freundlich isotherm were applied to ion-pair liquid chromatography. These treatments are complicated. Jandera *et al.* [14] used the equation $\ln k'_{ip} = \ln k^w_{ip} - c_{ip}C_b$, where $\ln k^w_{ip}$ and c_{ip} are constants for a given solute with a given column system and C_b is the concentration of organic modifier, to

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correlate ln k' with organic modifier concentration in RP-IPC. In this paper, linear regression analysis of ln k'_{ip} *vs.* methanol concentration (C_b) according to this equation was carried out, and it was observed that the absolute value of ln k^w_{ip} and c_{ip} in RP-IPC are much larger than those in RP high-performance liquid chromatography (HPLC). For compounds with the same kind and number of charges, ln k'_{ip} and ln k^w_{ip} in RP-IPC can be quantitatively correlated with ln k'_{Rp} and ln k^w_{Rp} in RP-HPLC. These results showed that both the hydrophobic and the electrostatic interaction play an important role in the retention of solutes in RP-IPC.

RETENTION EQUATION TO DESCRIBE THE EFFECT OF ORGANIC MODIFIER CONCENTRATION ON CAPACITY FACTORS IN RP-IPC

Jandera *et al.* [14] used the following equation to describe the effect of organic modifier concentration on capacity factor in RP-IPC:

$$\log k' = A - Bc \tag{1}$$

where A and B are constants for a given solute with a given column system and c is the concentration of organic modifier. We used a thermodynamic method in combination with an empirical relationship to derive the retention equation in RP-IPC as follows:

$$\ln k'_{ip} = \ln k^w_{ip} + c_{ip}C_b \tag{2}$$

where ln k^w_{ip} is the extrapolated logarithm of the capacity factor at $C_b = 0$ and c_{ip} is mainly determined by the interaction behaviour between the ion-pair reagent, ionized solute and the mobile phase, C_b is the concentration of organic modifier (expressed as methanol to buffer ratio, v/v), ln k^w_{ip} and c_{ip} are the constants at a given column system and can be expressed as

$$\ln k^w_{ip} = \ln \Phi - (\Delta G^\circ_R + \Delta G^\circ_e)/RT \tag{3}$$

$$c_{ip} = -(\gamma x_2 ZF + x_1)/RT \tag{4}$$

$$\Delta G^\circ_e = (\beta + \gamma \ln N^\circ_s)ZF \tag{5}$$

where R and T are the gas constant and absolute temperature, respectively, ΔG°_R and ΔG°_e are the non-electrostatic and electrostatic free-energy change of retention at $C_b = 0$, x_1 and x_2 are mainly determined by the interaction behaviour between the solute, the ion-pair reagent and the mobile phase and are constants for a given column system, Z and F are the charge number of the solute and Faraday constant, respectively, γ and β are empirical constants and N°_s is amount of the ion-pair reagent adsorbed on the surface at $C_b = 0$ (these were given by Deelder and Van den Berg [15]) and Φ is the phase ratio.

EXPERIMENTAL

Materials

The compounds analysed (listed in Table I) were obtained from the Dyestuff Laboratory, Chemical Engineering Department, Dalian University of Science and

Technology. Standard solutions were prepared in water. Doubly distilled water was used throughout. Tetrabutylammonium iodide (Beijing Chemical Reagent Factory, Beijing, China), methanol, NaH_2PO_4, NaOH and HCl were of analytical-reagent grade. The capacity factors were calculated using the equation $k' = (t_r - t_0)/t_0$, where t_r is the retention time of the solute and t_0 is the dead time of the column, which was measured as retention time of the methanol peak at a detection wavelength 220 nm in RP-HPLC.

Apparatus

HPLC was carried out at room temperature (23 ± 1°C) regulated by a room cooling system and using a stainless-steel column (200 × 4.0 mm I.D.) that contained a Spherisorb C_{18} reversed-phase packing material with 5-μm particle diameter (Phase Separations, Deeside, U.K.), which was packed at the Dalian Chromatographic R & D Centre of China. The mobile phase was delivered by two Waters Model 510 pumps. The ratio of methanol to phosphate buffer in eluents A and B was 0.95:0.05 and 0.60:0.40 (v/v), respectively, and the concentration of the ion-pair reagent tetrabutylammonium iodide, 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 controlled and regulated by a NEC APCIV computer with a Waters System Interface Module (Waters Assoc., Milford, MA, U.S.A.) by changing the ratio of eluent A to eluent B. The eluates were detected by 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 eluent was 1.0 ml/min. The eluent pH was measured with an SA-720 pH meter (Orion Research, Chicago, IL, U.S.A.). All experimental data were processed on an NEC APCIV personal computer.

RESULTS AND DISCUSSION

RP-IPC is often used to separate ionized organic compounds. Figs. 1 and 2 show the separation of some phenylamine- and naphthylaminesulphonic acids by RP-IPC. Table I lists the capacity factors of sixteen of these compounds at different organic modifier concentrations (methanol to buffer ratios). We obtained a linear regression according to eqn. 2 for the experimental data shown in Table I. Ln k'^{w}_{ip} and c_{ip} values and the regression coefficient r are listed in Table II. It can be seen that the regression coefficient for all solutes is larger than 0.99, which strongly supports the relationship shown in eqn. 2.

Table III shows the capacity factors of seven phenylamine- and naphthylamine-sulphonic acids at different methanol concentrations in RP-HPLC, which were taken from a previous paper [16]. It has been generally accepted that the effect of the organic modifier concentration (C_b) on the logarithm of the capacity factor (k') in RP-HPLC can be described by

$$\ln k'_{Rp} = \ln k^{w}_{Rp} + c_{rp}C_b \qquad (6)$$

where k^{w}_{Rp} is the capacity factor measured when pure water (or buffer) is used as the mobile phase and c_{rp} is mainly determined by the molecular interaction between the solute and the mobile phase. Table IV lists ln k^{w}_{Rp}, c_{Rp} calculated from the data in Table

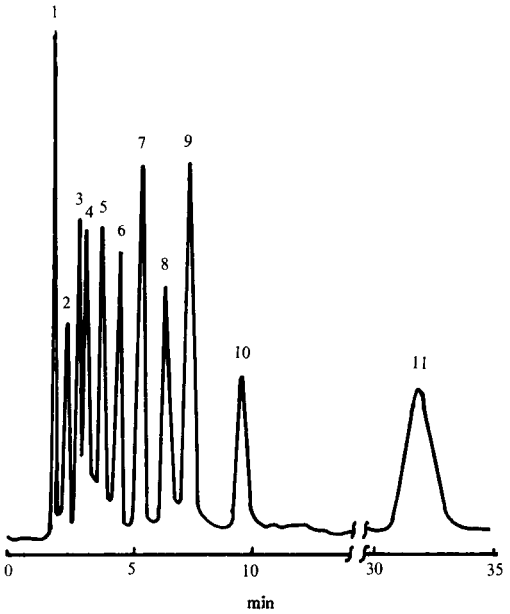

Fig. 1. Chromatogram of a mixture of eleven phenylamine- and naphthylaminesulphonic acids. Mobile phase, methanol–phosphate buffer (0.281:0.719) containing 10 mmol/l NaH_2PO_4, ion-pair reagent 4 mmol/l tetrabutylammonium iodide (pH 7.15). Peaks: 1 = phenylamine-4-sulphonic acid; 2 = phenylamine-3-sulphonic acid; 3 = phenylamine-2,5-disulphonic acid; 4 = phenylamine-2-sulphonic acid; 5 = 4-methylphenylamine-3-sulphonic acid; 6 = 2-aminonaphthalene-3-sulphonic acid; 7 = 2-aminonaphthalene-3,6-disulphonic acid; 8 = 2-aminonaphthalene-6-sulphonic acid; 9 = 4-nitrophenylamine-2-sulphonic acid; 10 = 2-aminonaphthalene-1-sulphonic acid; 11 = naphthylamine-8-sulphonic acid.

III and the values of $\Delta c = c_{ip} - c_{Rp}$ and $\Delta \ln k = \ln k^w_{ip} - \ln k^w_{Rp}$ for seven phenylamine- and naphthylaminesulphonic acids. It can be seen that the absolute values of $\ln k^w_{ip}$ and c_{ip} in RP-IPC are much larger than those in RP-HPLC, which is as expected. The values of $\Delta \ln k$ taken from Jandera *et al.* [14] are listed in Table V. It can also be seen that $\ln k^w_{ip}$ in RP-IPC was much larger than those in RP-HPLC when both C_{18} and C_8 are used as packing materials. Ln k^w_{ip} in RP-IPC tends to increase with increasing charge number of the solutes, which agrees with our results for the thermodynamic treatment. It is necessary to investigate the effect of the charge number of the solute on $\ln k^w_{ip}$ and c_{ip}. Ln k^w_{ip} and c_{ip} in RP-IPC and $\ln k^w_{Rp}$ and c_{rp} in RP-HPLC calculated from the experimental data of Bartha and Vigh [17] are listed in Table VI. It can also be seen that $\ln k^w_{ip}$ and c_{ip} in RP-IPC are larger than those in RP-HPLC.

The larger value of $\ln k^w_{ip}$ means that there is a much larger free-energy change of retention at $C_b = 0$ in RP-IPC than in RP-HPLC. It is known that in addition to the contribution of non-electrostatic free-energy change to $\ln k^w_{ip}$ in RP-IPC, similarly to that in RP-HPLC, $\ln k^w_{ip}$ also has a contribution from the electrostatic free-energy change of retention. The values of Δc in Table IV are almost constant, which is mainly due to the interaction behaviour between the ion-pair reagent and the mobile phase, and should not change substantially for different solutes. Although this phenomenon cannot be seen from the data of Jandera *et al.* [14], it may be implied from the high concentration of salt used in RP-HPLC. The parameter c_{ip} is always negative, which

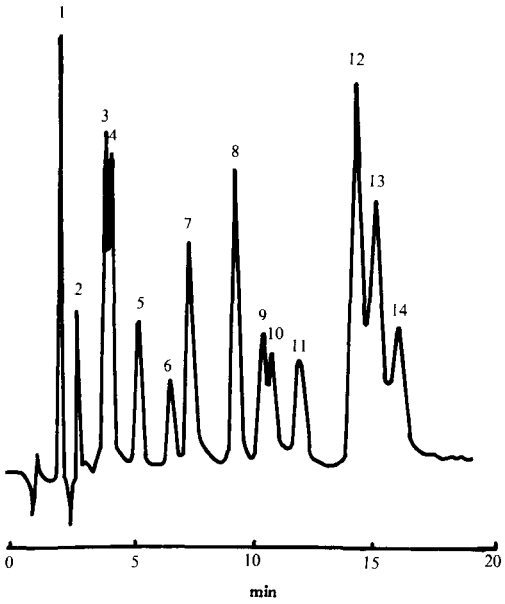

Fig. 2. Chromatogram of a mixture of fourteen phenylamine- and naphthylaminesulphonic acids. Mobile phase, methanol–phosphate buffer (0.239:0.761) containing 10 mmol/l NaH_2PO_4, ion-pair reagent 4 mmol/l tetrabutylammonium iodide (pH 7.15). Peaks: 1 = phenylamine-4-sulphonic acid; 2 = phenylamine-3-sulphonic acid; 3 = phenylamine-2,5-disulphonic acid; 4 = phenylamine-2-sulphonic acid; 5 = 4-methylphenylamine-3-sulphonic acid; 6 = naphthylamine-5-sulphonic acid; 7 = 2-aminonaphthalene-5-sulphonic acid; 8 = 2-aminonaphthalene-3,6-disulphonic acid; 9 = 2-aminonaphthalene-6-sulphonic acid; 10 = 4-nitrophenylamine-2-sulphonic acid; 11 = 4-methylphenylamine-2-sulphonic acid, 12 = 2-aminonaphthalene-4,6,8-trisulphonic acid; 13 = 2-aminonaphthalene-3,6,8-trisulphonic acid; 14 = 2-aminonaphthalene-1-sulphonic acid.

TABLE I

CAPACITY FACTORS OF 16 PHENYLAMINE- AND NAPHTHYLAMINESULPHONIC ACIDS AT DIFFERENT ORGANIC MODIFIER CONCENTRATION IN RP-IPC

For experimental conditions, see Experimental.

Solute	Methanol to buffer ratio (v/v)			
	0.325	0.281	0.239	0.198
Phenylamine-2-sulphonic acid	0.461	1.00	1.60	3.04
Phenylamine-3-sulphonic acid	0.255	0.53	0.84	1.34
Phenylamine-4-sulphonic acid	0.152	0.301	0.489	1.01
4-Methylphenylamine-2-sulphonic acid	1.54	3.27	5.69	9.86
4-Methylphenylamine-3-sulphonic acid	0.70	1.45	2.35	4.09
4-Methoxyphenylamine-2-sulphonic acid	0.751	1.56	2.46	4.97
4-Methoxyphenylamine-3-sulphonic acid	0.189	0.339	0.554	1.15
4-Nitrophenylamine-2-sulphonic acid	1.41	3.23	5.38	10.25
6-Chlorophenylamine-3-sulphonic acid	1.45	3.15	5.29	9.65
4-Chlorophenylamine-3-sulphonic acid	0.498	0.985	1.62	3.07
1,3-Diaminophenyl-4-sulphonic acid	0.142	0.283	0.433	0.948
Naphthylamine-5-sulphonic acid	0.620	1.28	2.22	4.21
2-Aminonaphthalene-1-sulphonic acid	–	4.53	8.04	18.34
Naphthylamine-8-sulphonic acid	8.67	17.41	–	–
2-Aminonaphthalene-5-sulphonic acid	0.779	1.63	2.94	5.75
2-Aminonaphthalene-6-sulphonic acid	1.23	2.80	5.01	9.85

TABLE II

LN k_{ip}^{w} AND c_{ip} REGRESSED BY EXPERIMENTAL DATA SHOWN IN TABLE I

Solute	Ln k_{ip}^{w}	c_{ip}	r
Phenylamine-2-sulphonic acid	3.982	−14.50	0.9967
Phenylamine-3-sulphonic acid	2.887	−12.88	0.9946
Phenylamine-4-sulphonic acid	2.853	−14.57	0.9972
4-Methylphenylamine-2-sulphonic acid	5.190	−14.49	0.9979
4-Methylphenylamine-3-sulphonic acid	4.135	−13.67	0.9973
4-Methoxyphenylamine-2-sulphonic acid	4.441	−14.48	0.9967
4-Methoxyphenylamine-3-sulphonic acid	2.839	−13.95	0.9960
4-Nitrophenylamine-2-sulphonic acid	5.351	−15.25	0.9963
6-Chlorophenylamine-3-sulphonic acid	5.206	−14.72	0.9976
4-Chlorophenylamine-4-sulphonic acid	3.894	−14.08	0.9986
1,3-Diaminophenyl-4-sulphonic acid	2.745	−14.46	0.9939
Naphthylamine-5-sulphonic acid	4.384	−14.89	0.9963
2-Aminonaphthalene-1-sulphonic acid	6.197	−16.83	0.9939
2-Aminonaphthalene-6-sulphonic acid	4.828	−15.57	0.9994
2-Aminonaphthalene-5-sulphonic acid	5.493	−16.14	0.9984
Naphthylamine-8-sulphonic acid	7.309	−15.84	1.0000

TABLE III

CAPACITY FACTORS OF SEVEN PHENYLAMINE- AND NAPHTHYLAMINESULPHONIC ACIDS MEASURED AT DIFFERENT METHANOL TO BUFFER RATIOS WITH 10 mmol/l NaH_2PO_4 (pH 6.8) AS THE MOBILE PHASE

Stainless-steel column (200 × 4.0 mm I.D.) packed with Polygosile-C_{18} with particle diameter 5 μm obtained from Macherey, Nagel & Co. (Düren, Germany).

Solute	Methanol to buffer ratio (v/v)					
	0	0.05	0.1	0.15	0.2	0.25
Phenylamine-3-sulphonic acid	1.03	0.59	0.44	0.33	0.26	0.21
4-Methylphenylamine-3-sulphonic acid	2.97	1.05	0.77	0.57	0.42	0.32
4-Methoxyphenylamine-2-sulphonic acid	6.62	2.93	1.86	1.25	0.93	0.74
4-Nitrophenylamine-2-sulphonic acid	9.51	4.54	2.95	1.97	1.60	1.11
2-Aminonaphthalene-6-sulphonic acid	12.02	4.84	2.72	1.66	1.28	0.86
2-Aminonaphthalene-1-sulphonic acid	15.95	6.32	3.63	2.35	1.74	1.13
Naphthylamine-8-sulphonic acid	30.00	14.03	8.72	6.01	4.71	3.64

TABLE IV

LN k_{Rp}^{w} AND c_{Rp} CALCULATED FROM THE DATA IN TABLE III AND THE VALUES OF ΔLN k AND Δc FOR SEVEN PHENYLAMINE- AND NAPHTHYLAMINESULPHONIC ACIDS

Solute	Ln k_{Rp}^{w}	Δln k	c_{Rp}	Δc
Phenylamine-3-sulphonic acid	−0.295	3.182	−5.184	−7.70
4-Methylphenylamine-3-sulphonic acid	0.388	3.793	−5.966	−7.70
4-Methoxyphenylamine-2-sulphonic acid	1.343	3.098	−6.891	−7.59
4-Nitrophenylamine-2-sulphonic acid	1.802	3.549	−6.934	−8.32
2-Aminonaphthalene-6-sulphonic acid	1.899	2.929	−8.418	−7.72
2-Aminonaphthalene-1-sulphonic acid	2.187	4.010	−8.357	−8.47
Naphthylamine-8-sulphonic acid	2.882	4.427	−6.626	−9.21

TABLE V

VALUES OF ΔLN k FOR AROMATIC SULPHONIC ACIDS ON OCTADECYLSILICA (C_{18}) AND OCTYLSILICA (C_8) COLUMNS

$\Delta\ln k$ was calculated using $\Delta\ln k = 2.30(A_{ip} - A_{Rp})$. All experimental data were taken from Jandera *et al.* [14].

Acid	$\Delta\ln k$	
	C_{18} column	C_8 column
2-Naphthalenesulphonic	2.22	3.12
1,5-Naphthalenedisulphonic	6.71	7.19
1,6-Naphthalenedisulphonic	5.18	5.69
2,6-Naphthalenedisulphonic	5.83	5.66
2,7-Naphthalenedisulphonic	5.19	5.42
1-Naphthylamine-4-sulphonic	2.57	2.54
1-Naphthylamine-5-sulphonic	2.53	2.57
1-Naphthylamine-6-sulphonic	1.80	2.05
1-Naphthylamine-7-sulphonic	2.24	2.64
2-Naphthylamine-6-sulphonic	2.71	–
1-Naphthylamine-8-sulphonic	4.84	5.96
2-Naphthol-1-sulphonic	–	4.43
1-Naphthol-4-sulphonic	3.08	3.38
2-Naphthol-6-sulphonic	2.69	2.70
R-acid	5.80	6.48
G-acid	5.48	6.08
2-Amino-5-naphthol-7-sulphonic	2.34	–
1-Amino-8-naphthol-3,6-disulphonic	2.56	–
2-Amino-5-naphthol-1,7-disulphonic	5.72	–
1-Anthraquinonesulphonic	1.44	–
1,5-Anthraquinonedisulphonic	5.10	6.07
2,6-Anthraquinonedisulphonic	4.110	5.05
1,8-Anthraquinonedisulphonic	3.88	4.66

TABLE VI

LN k^w_{ip} AND c_{ip} IN RP-IPC WITH 2 mmol/l TETRABUTYLAMMONIUM BROMIDE AS MOBILE PHASE AND LN k^w_{Rp} AND c_{Rp} IN RP-HPLC CALCULATED FROM THE EXPERIMENTAL DATA OF BARTHA AND VIGH (17)

The effect of mobile phase pH on k' was neglected in regression analysis. The range of C_b (methanol to buffer ratio) for DCSA is 0–0.6, for PTSA 0–0.5, for $BuSO_3$ 0–0.6, for $HexSO_3$ 0.375–0.6 and for $HepSO_3$ 0.375–0.7 in RP-IPC and RP-HPLC. The ranges of C_b for $PeSO_3$ in RP-IPC and RP-HPLC are 0.1–0.375 and 0.1–0.25, respectively.

Solute[a]	Ln k^w_{Rp}	c_{Rp}	r	Ln k^w_{ip}	c_{ip}	r
DCSA	2.223	−6.901	0.9918	3.152	−8.390	0.9980
PTSA	2.973	−7.373	0.9983	3.342	−7.870	0.9984
$BuSO_3$	1.080	−5.326	0.9897	1.533	−5.866	0.9998
$PeSO_3$	2.42	−6.460	1.0000	3.063	−7.374	0.9993
$HexSO_3$	3.688	−6.888	0.9989	4.205	−7.702	0.9990
$HepSO_3$	5.172	−8.163	0.9990	5.533	−8.653	0.9989

[a] DCSA = *d,l*-10-camphorsulphonic acid; PTSA = *p*-toluenesulphonic acid; $BuSO_3$ = sodium butylsulphonate; $PeSO_3$ = sodium pentanesulphonate; $HexSO_3$ = sodium hexanesulphonate; $HepSO_3$ = sodium heptanesulphonate.

means that the capacity factor of a solute decreases with increasing organic modifier concentration; the larger absolute value of c_{ip} in RP-IPC means that there is a much stronger effect of organic modifier concentration on the retention of a solute in RP-IPC than in RP-HPLC.

It has been observed that the capacity factor of phenylamine- and naphthylaminesulphonic acids increases with increasing salt concentration in the mobile phase in RP-HPLC, which may be caused by the salting-out effect [14] or changes in mobile phase surface tension [18]. In RP-IPC, the mobile phase containing an ion-pair reagent will increase the capacity factors of phenylamine- and naphthylaminesulphonic acids by the salting-out effect. However, in general, the salting-out effect of an ion-pair reagent makes only a minor contribution to the retention owing to the lower concentration of the ion-pair reagent in RP-IPC. We consider that the main contribution of the ion-pair reagent to retention is from the electrostatic interaction between the solute and ion-pair reagent. For solutes with the same kind and number of charges, there should be almost the same electrostatic free-energy change to the retention value and $\ln k_{ip}^{w}$ in RP-IPC, and therefore it is possible to correlate $\ln k'_{ip}$ and $\ln k_{ip}^{w}$ in RP-IPC with $\ln k'_{Rp}$ and $\ln k_{Rp}^{w}$ in RP-HPLC by the following equations:

$$\ln k'_{ip} = a_1 + b_1 \ln k'_{Rp} \tag{7}$$

or

$$\ln k_{ip}^{w} = a_2 + b_2 \ln k_{Rp}^{w} \tag{8}$$

Table VII lists the capacity factors of sixteen phenylamine- and naphthylaminesulphonic acids measured in RP-HPLC with phosphate buffer as the mobile phase. The quantitative correlations between $\ln k'_{ip}$ at different ratios of methanol to buffer in RP-IPC and $\ln k'_{Rp}$ in RP-HPLC for phenylamine- and naphthylaminesulphonic acids are as follows:

$C_b = 0.325$: $\ln k'_{ip} = -1.492 + 0.8357 \ln k'_{Rp}$; $n = 15$, $r = 0.9388$
$C_b = 0.281$: $\ln k'_{ip} = -0.7877 + 0.8471 \ln k'_{Rp}$; $n = 16$, $r = 0.9449$
$C_b = 0.239$: $\ln k'_{ip} = -0.2558 + 0.7886 \ln k'_{Rp}$; $n = 15$, $r = 0.9548$
$C_b = 0.198$: $\ln k'_{ip} = 0.3960 + 0.7924 \ln k'_{Rp}$; $n = 15$, $r = 0.9618$

TABLE VII

CAPACITY FACTORS OF SIXTEEN PHENYLAMINE- AND NAPHTHYLAMINESULPHONIC ACIDS WITH AQUEOUS BUFFER CONTAINING 10 mmol/l NaH_2PO_4 (pH 6.8) AS THE ELUENT

Column as in Table III.

Solute	Capacity factor (k')	Solute	Capacity factor (k')
Phenylamine-2-sulphonic acid	2.31	6-Chlorophenylamine-3-sulphonic acid	8.64
Phenylamine-3-sulphonic acid	1.03	4-Chlorophenylamine-3-sulphonic acid	1.87
Phenylamine-4-sulphonic acid	0.484	1,3-Diaminophenyl-4-sulphonic acid	0.510
4-Methylphenylamine-2-sulphonic acid	8.29	Naphthylamine-5-sulphonic acid	4.37
4-Methylphenylamine-3-sulphonic acid	2.87	Naphthylamine-7-sulphonic acid	18.56
4-Methoxyphenylamine-2-sulphonic acid	6.86	Naphthylamine-8-sulphonic acid	29.85
4-Methoxyphenylamine-3-sulphonic acid	1.43	2-Aminonaphthalene-5-sulphonic acid	10.73
4-Nitrophenylamine-2-sulphonic acid	9.51	2-Aminonaphthalene-6-sulphonic acid	11.22

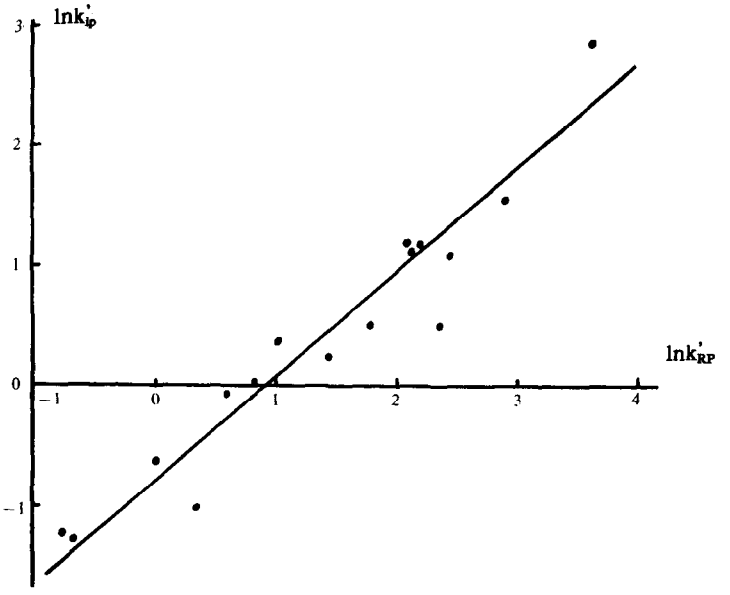

Fig. 3. Linear regression of ln k'_{ip} in RP-IPC with methanol–phosphate buffer (0.281:0.719) containing ion-pair reagent 4 mmol/l tetrabutylammonium iodide as the eluent *vs.* ln k'_{Rp} shown in Table VI in RP-HPLC for sixteen phenylamine- and naphthylaminesulphonic acids. Ln $k'_{ip} = -0.7947 + 0.8576$ ln k'_{Rp}; $r = 0.9412$.

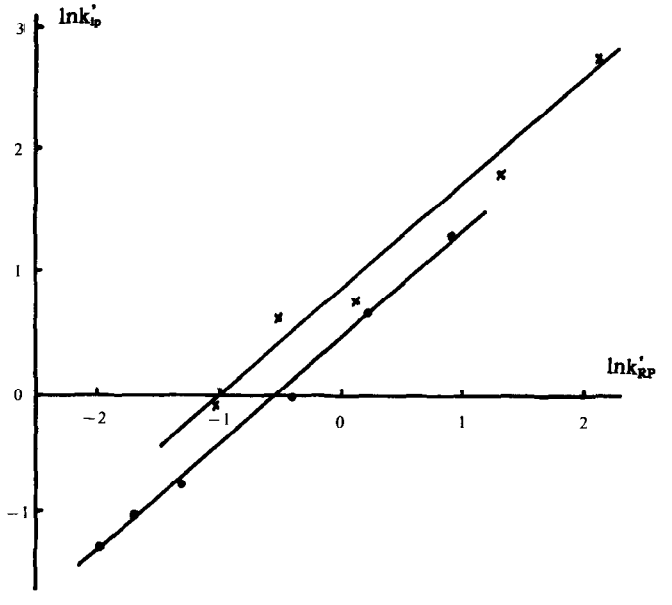

Fig. 4. Linear regression of ln k'_{ip} in RP-IPC *vs.* ln k'_{Rp} in RP-HPLC. (×) For DCSA, PTSA, $BuSO_3$, $HexSO_3$ and $HepSO_3$ with 37.5% methanol in phosphate buffer (pH 2.75) and that containing ion-pair reagent 20 mmol/l tetrabutylammonium bromide as eluent in RP-HPLC and RP-IPC, respectively. Ln $k'_{ip} = 0.777 + 0.868$ ln k'_{Rp}; $r = 0.987$. (●) For DCSA, PTSA, $BuSO_3$, $HexSO_3$, $HepSO_3$ and $OcSO_3$ (sodium octanesulphonate) with 60% methanol in phosphate buffer (pH 3.12) and that containing ion-pair reagent 35 mmol/l tetrabutylammonium bromide as eluent in RP-HPLC and RP-IPC, respectively. Ln $k'_{ip} = 0.433 + 0.891$ ln k'_{Rp}; $r = 0.998$. Experimental data were taken from Bartha and Vigh [17].

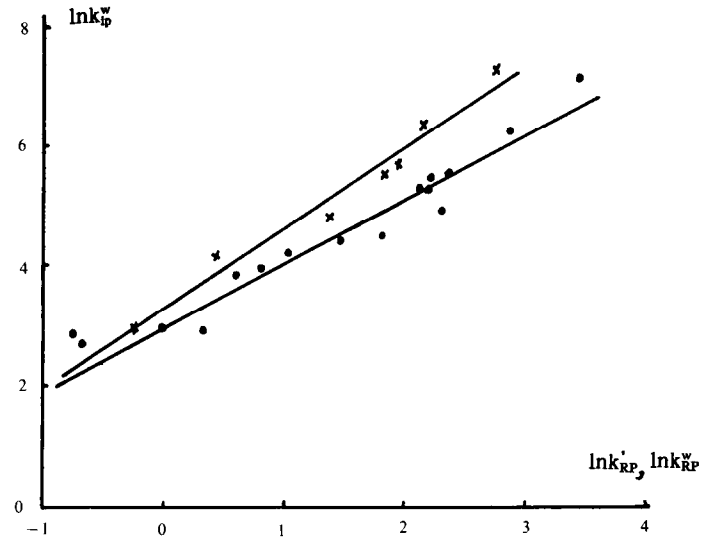

Fig. 5. Linear regression of ln k^w_{ip} in RP-IPC *vs.* ln k'_{Rp} [●; with 10 mmol/l phosphate buffer (pH 6.8) as eluent] and ln k^w_{ip} (×) in RP-HPLC for sixteen and seven phenylamine- and naphthylaminesulphonic acids, respectively. (●) Ln k^w_{ip} = 3.076 + 1.009 ln k'_{Rp}; r = 0.9563. (×) Ln k^w_{ip} = 3.256 + 1.282 ln k^w_{Rp}; r = 0.9710.

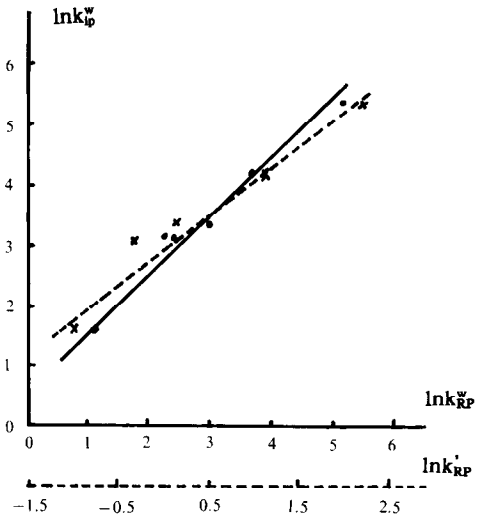

Fig. 6. Linear regression of ln k^w_{ip} in RP-IPC shown in Table VI *vs.* ln k'_{Rp} in RP-HPLC with 37.5% methanol in phosphate buffer as mobile phase for DCSA, PTSA, $BuSO_3$, $HexSO_3$ and $HepSO_3$ (×), ln k^w_{ip} = 0.400 + 2.460 ln k'_{Rp}, r = 0.9631; and ln k^w_{ip} in RP-IPC *vs.* ln k^w_{Rp} in RP-HPLC shown in Table VI (●), ln k^w_{ip} = 0.716 + 0.940 ln k^w_{Rp}, r = 0.9896.

It can be seen from above regression equations that there is a slight change in slope with change in organic modifier concentration, which may be caused by the fact that different column packings were used and the slight difference in pH between the two sets of experiments. The intercepts increase with decreasing methanol concentration, which implies an increase the capacity factors with decreasing methanol concentration. Figs. 3 and 4 illustrate the linear regression results of $\ln k'_{ip}$ at $C_b = 0.281$ in RP-IPC *versus* $\ln k'_{Rp}$ in RP-HPLC for sixteen phenylamine- and naphthylaminesulphonic acids, and the experimental data taken from Bartha and Vigh [17], respectively. Figs. 5 and 6 demonstrate the quantitative correlation between $\ln k^{w}_{ip}$ in RP-IPC and the $\ln k^{w}_{Rp}$ and $\ln k'_{Rp}$ in RP-HPLC calculated from our experimental data and those of Bartha and Vigh [17]. It can be seen that the relationships shown in eqns. 7 and 8 should basically exist. The above results mean that the non-electrostatic free energy change of retention in RP-IPC is paralleled by that in RP-HPLC.

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