

Effects of molecular structure on parameters a , b and c in the fundamental retention equation

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ABSTRACT

An important aspect of chromatography is the prediction of peak positions. For high-performance liquid chromatography (HPLC), a fundamental retention equation $\ln k' = a + b \ln C_b + cC_b$ has been derived by statistical thermodynamics. The effects of molecular structure on the parameters a , b and c in this equation were investigated theoretically and proved experimentally. The parameter b in reversed-phase (RP) HPLC approaches a low constant value, because there is very weak displacement caused by adsorption. However, in normal-phase (NP) HPLC, the displacement by adsorption is strong, and plays an important role in retention. The parameter b in NP-HPLC changes slightly when different batches of packing of the same size and with the same mobile phase are used, and the absolute value of this parameter increases slightly and approaches a constant value with increase in carbon number for homologous compounds. The parameter c in RP-HPLC is mainly determined by the difference between interactions in solute–strong solvent and solute–weak solvent systems and approaches a constant value even when column systems with the different packings and the same mobile phase are used. The parameter c in RP-HPLC can also be quantitatively correlated with structural parameters of the solute such as Van der Waals volume (V_w), dipole moment (μ_A) and hydrogen bond energy (X_{AH}). For non-polar or homologous compounds, a simplified linear relationship between the parameter c and V_w can be established. The parameter c in NP-HPLC is affected not only by factors valid in RP-HPLC, but also by the displacement caused by adsorption of the solute on the surface. The parameter a in both RP- and NP-HPLC follows similar rules to parameter c . A linear relationship between k' and amount of C_{18} in RP-HPLC was also found. A good linear relationship between the parameters a and c occurs only when two of the three structural parameters V_w , μ_A and X_{AH} for solutes are equal or close.

INTRODUCTION

One of the most important aspects in developing software for expert systems in chromatography is the prediction of peak positions^{1,2}, which means predicting the capacity factor of a given compound with changes in the operating conditions. For high-performance liquid chromatography (HPLC) the capacity factor and selectivity are changed substantially when the mobile phase composition is changed. Therefore, to solve this problem, we need first to derive an equation that describes the variation of the capacity factor of a given compound when the mobile phase composition is changed over a wide range of concentration, and then to find a means of predicting the values of the parameters in this equation from the molecular structure of the solute.

The capacity factor is determined by the partition coefficient, which is a macro-property of a system. In order to predict macro-properties from the molecular structure, the correct approach is to use statistical thermodynamics (STM)^{1,3,4}. There are, of course, other ways of solving this problem, *e.g.*, using classical thermodynamics to obtain the retention equation⁵⁻⁸ and directly correlating ΔH and ΔS with molecular structure. However, the theoretical correlation between the parameters in the retention equation and molecular structure can only be predicted by STM and statistical quantum chemistry methods⁹.

A fundamental retention equation in HPLC has been derived by STM^{1,4}. The validity of this equation with changing mobile phase composition over a broad range has been demonstrated in both reversed-phase (RP) HPLC¹⁰⁻¹² and normal-phase (NP) HPLC¹³⁻¹⁵. In this work, a further investigation of effects of molecular structure on the parameters in the fundamental retention equation was carried out. The validity of the theoretical predictions was confirmed experimentally.

FUNDAMENTAL RETENTION EQUATION

The fundamental retention equation valid for high-performance liquid chromatography has been derived as follows^{1,4}:

$$\ln k' = a + b \ln C_b + cC_b \quad (1)$$

where

$$a = \ln \frac{N_S}{V_S} - \ln \beta + \frac{\partial N_{B_1}}{\partial N_A} \ln \left[\left(\frac{h^2}{2\pi m_{B_1} kT} \right)^{3/2} / k_1 V_1 \right] + \frac{3}{2} \ln \frac{h^2}{2\pi m_A kT} + \frac{1}{kT} \left\{ (K_{AB_0} - K_{AB_0}^a) X_{AB_0} - X_A^a + \frac{\partial N_{B_1}}{\partial N_A} \left[(K_{B_1B_0} - K_{B_1B_0}^a) X_{B_1B_0} - X_{B_1}^a \right] \right\} \quad (2)$$

$$b = \frac{\partial N_{B_1}}{\partial N_A} \left(1 - \frac{1}{n_1} \right) \quad (3)$$

$$c = \frac{1}{kT} \left\{ (K_{AB_1} - K_{AB_1}^a) X_{AB_1} - (K_{AB_0} - K_{AB_0}^a) X_{AB_0} + \right. \\ \left. + \frac{\partial N_{B_1}}{\partial N_A} \left[(K_{B_1B_1} - K_{B_1B_1}^a) X_{B_1B_1} - (K_{B_1B_0} - K_{B_1B_0}^a) X_{B_1B_0} \right] \right\} \quad (4)$$

where A and B_i ($i = 0, 1$) are solute and solvent i (weak and strong solvent), h and k are the Planck and Boltzmann constants, k_1 and n_1 are constants in the Freundlich adsorption isotherm, m_A and m_{B_i} are the molecular weight of solute A and solvent B_i , T is absolute temperature, V_{B_1} is the molar volume of solvent B_1 , the X_{AB_1} , X_{AB_0} , X_A^a , $X_{B_1B_1}$, $X_{B_1B_0}$ and $X_{B_i}^a$ are values for interactions of solute–strong solvent, solute–weak solvent, solute–stationary phase, strong solvent–strong solvent, strong solvent–weak solvent and strong solvent–stationary phase, respectively, where the hydrogen bond energy is proportional to the concentration of solvent as assumed if comparing the independence of concentration of solvent in the original papers^{1,4} and β is the phase ratio. It can be seen from eqns. 2–4 that the parameter a is mainly determined by the difference between solute–stationary phase and solute–weak solvent interactions as well as solvent–stationary phase interactions, the parameter b is determined by displacement caused by adsorption on the adsorbent surface and the parameter c is mainly determined by the difference between solute–weak solvent and solute–strong solvent interactions as well as solvent–solvent interactions. The validity of eqn. 1 has been proved by describing the variation of the capacity factor by changing the mobile phase composition over a broad range of concentration in both RP-HPLC and NP-HPLC^{10–15}. The advantage of this model is that the effects of solute–stationary phase and solute–mobile phase interactions as well as displacement caused by adsorption are simultaneously considered. In a given chromatographic system, the solvent–solvent and solvent–stationary phase interactions are constant, and the solute–stationary phase and solute–solvent B_i interactions can be described by the following equations:

$$X_A^a = -\frac{1}{r^6} \left[\left(\frac{3\alpha_a I_A I_a}{2(I_A + I_a)} + \mu_a^2 \right) \alpha_A + \left(\frac{2\mu_a^2}{3kT} + \alpha_a \right) \mu_A^2 \right] + Z_a X_{AH} \quad (5)$$

$$X_{AB_i} = -\frac{1}{r_{AB_i}^6} \left[\left(\frac{3\alpha_{B_i} I_A I_{B_i}}{2(I_A + I_{B_i})} + \mu_{B_i}^2 \right) \alpha_A + \left(\frac{2\mu_{B_i}^2}{3kT} + \alpha_{B_i} \right) \mu_A^2 \right] + Z_{AB_i} X_{AH} \quad (6)$$

where I_A , α_A , μ_A , I_a , α_a , μ_a and I_{B_i} , α_{B_i} , μ_{B_i} are the approximate ionization energy, polarizability and dipole moment of the solute, stationary phase and solvent i , respectively, X_{AH} the contribution of the hydrogen bond energy of the solute, Z_a and Z_{AB_i} are parameters related to the behaviour of the stationary phase and solvent i and r and r_{AB_i} are the interaction distance of solute–stationary phase and solute–solvent i , respectively, and to a first approximation are assumed to be constant for different compounds.

FACTORS AFFECTING THE PARAMETER b

It can be seen from eqn. 3 that the parameter b is determined by n_1 and $\partial N_{B_1}/\partial N_A$, where n_1 is a parameter of the Freundlich adsorption isotherm ($N_{B_1}^a = k_1 C b^{1/n_1}$) on a stationary surface for a strong solvent and relates to the behaviours of the adsorbent surface and a strong solvent, and $\partial N_{B_1}/\partial N_A$ indicates how many strong solvent molecules are displaced by the adsorption of one solute molecule. For a given system, the strength of the solute-adsorbent interaction and the solute molecular size are the factors that determine $\partial N_{B_1}/\partial N_A$. The stronger the solute-adsorbent interaction and the larger the molecular size of the solute, the more strong solvent molecules can be displaced by adsorption of one molecule of the solute on the adsorbent surface. In RP-HPLC a polar solvent molecule is less easily adsorbed on a C_{18} surface than a polar solvent molecule on silica in the NP-HPLC, so that $\partial N_{B_1}/\partial N_A$ approaches a small constant value. Table I lists the b values observed in RP-HPLC, which agree with the theoretical values.

The value of C_b in the most RP-HPLC separations is higher than 0.1 volume fraction of the strong solvent, but in most NP-HPLC separations C_b is less than 0.1 or even 0.001 volume fraction of the strong solvent. The absolute value of $\ln C_b$ in RP-HPLC is much smaller than that in NP-HPLC in most separations, so the contribution of term $b \ln C_b$ in eqn. 1 to the retention value in RP-HPLC can be neglected in the case of small concentrations of the strong solvent. However, in NP-HPLC the solute-adsorbent interaction, which is mainly due to interactions between polar groups of the solute and silanol groups, is strong and the absolute value of $\ln C_b$ is large, so the contribution of $b \ln C_b$ to the retention value in NP-HPLC is significant. For a homologous series, the parameter b is mainly determined by the interaction between polar groups and silanol groups on the surface, and the absolute value of b should increase slightly with increasing number of methylene groups in the solute molecule and approach a constant value at a certain number of carbon atoms. The validity of this conclusion is supported by the experimental data given in Table II for the homologous series of benzoates of n -alkanols.

TABLE I

PARAMETER b OBSERVED IN RP-HPLC WITH DIFFERENT C_{18} PACKINGS

Solute	YQG ^a	ES ^b	Nucleosil	LiChrosorb	B4 ^c	B8 ^c
Benzene	0	-0.001	-0.003	-0.001	-0.003	-0.001
Naphthalene	-0.01	-0.003	-0.007	-0.001	-0.003	-0.003
Biphenyl	-0.01	-0.005	-0.007	-0.002	-0.004	-0.001
Fluorene	-0.005	-0.007	-0.003	-0.003	-0.002	0
Phenanthrene	-0.003	-0.005	-0.005	-	-	-
Anthracene	-0.003	-0.007	-0.005	-	-	-
Chrysene	-0.004	-0.003	-0.002	-	-	-
Toluene	-0.005	-0.002	-0.005	-0.006	-0.003	0
Ethylbenzene	-0.004	-0.003	-0.005	-0.01	-	-

^a YQG- C_{18} is made by Tijing Chemical Reagent Factory (China).

^b ES- C_{18} is made by ES Industry Inc.

^c B4 and B8 are homemade silica-based C_{18} packing materials.

TABLE II

PARAMETER b OBSERVED IN THE ADSORPTION CHROMATOGRAPHY OF BENZOATES OF HOMOLOGOUS n -ALKANOLS IN THE SYSTEM OF YWG-SILICA GEL ($5 \mu\text{m}$) PACKING AND HEXANE-ETHYL ACETATE MOBILE PHASE (ETHYL ACETATE FROM 0.1 TO 3.0%, v/v)

Data recalculated from ref. 16.

n_c	b	n_c	b
1	-0.64	5	-0.75
2	-0.69	6	-0.74
3	-0.72	7	-0.77
4	-0.74	8	-0.77

TABLE III

PARAMETER b FOR EIGHT POLAR COMPOUNDS OBSERVED IN A COLUMN SYSTEM WITH DIFFERENT BATCHES OF YWG-SILICA GEL ($5 \mu\text{m}$) PACKING AND HEXANE-ETHYL ETHER MOBILE PHASE

Compound	Parameter ^a		
	b'	b''	b'''
2-Methylnitrobenzene	-0.41	-0.42	-0.50
Nitrobenzene	-0.37	-0.37	-0.40
Ethoxybenzene	-0.54	-0.50	-
4-Chloronitrobenzene	-0.49	-0.50	-0.51
2-Chloronitrobenzene	-0.27	-0.26	-0.32
n -Butyl benzoate	-0.78	-0.82	-
n -Pentyl benzoate	-0.78	-0.81	-
Ethyl benzoate	-0.74	-0.79	-

^a b' recalculated from ref. 17; mobile phase composition: ethyl ether from 2.0 to 70% (v/v); b'' recalculated from ref. 14; mobile phase composition: ethyl ether from 0.1 to 60% (v/v); b''' recalculated from ref. 15; mobile phase composition: ethyl ether from 0.2 to 60% (v/v).

The parameter b changes very little when different batches of packing material with the same grain size and the same mobile phase are used, because the behaviour of the adsorbing surface is very similar. Table III gives the values of the parameter b observed in adsorption chromatography, which are in good agreement with the theoretical values.

FACTORS AFFECTING PARAMETER c

The parameter c in RP-HPLC is mainly determined by the difference between solute-strong solvent and solute-weak solvent interactions, and is almost independent of the behaviour of the solute adsorbed on the surface. It is a constant when different packings and the same mobile phase are used. Table IV gives the values of the parameter c observed with different C_{18} packings for various solutes.

On the other hand, the polarizability of a solute is proportional to its Van der Waals volume:

$$\alpha_A = e + fV_w \quad (7)$$

TABLE IV

PARAMETER c OBSERVED WITH DIFFERENT C_{18} PACKINGS WITH WATER-METHANOL MOBILE PHASE (METHANOL FROM 60 TO 95%, v/v) FOR VARIOUS COMPOUNDS

<i>Solute</i>	<i>YQG</i>	<i>ES</i>	<i>Nucleosil</i>	<i>LiChrosorb B4</i>	<i>B8</i>
Benzene	-2.69	-2.62	-2.71	-2.80	-2.75
Naphthalene	-3.50	-3.49	-3.62	-3.59	-3.67
Biphenyl	-4.14	-4.10	-4.26	-3.83	-4.27
Phenanthrene	-4.54	-4.53	-4.30	-4.35	-4.46
Anthracene	-4.44	-4.50	-4.41	-	-
Chrysene	-5.09	-5.04	-5.18	-	-
1,4-Diphenylbenzene	-5.59	-5.55	-5.57	-	-
Anisole	-2.70	-2.63	-2.74	-2.82	-2.71
Benzyl alcohol	-2.05	-1.93	-2.00	-2.12	-
Acetophenone	-2.30	-2.18	-2.30	-	-
<i>p</i> -Nitrotoluene	-3.00	-2.97	-3.05	-	-
<i>n</i> -Butyl benzoate	-4.05	-4.00	-4.10	-	-

On substitution of eqns. 5 and 6 together with eqn. 7 into eqn. 4, the parameter c can be expressed as:

$$c = k_2 + k_3 V_w + k_4 \mu_A^2 + k_5 X_{AH} \quad (8)$$

where

$$k_2 = \frac{e}{kT} \left[(K_{AB_0} - K_{AB_0}^a) \frac{1}{r_{AB}^6} \left(\frac{3\alpha_{B_0} I_A I_{B_0}}{2(I_A + I_{B_0})} + \alpha_{B_0} \right) - (K_{AB_1} - K_{AB_1}^a) \frac{1}{r_{AB_1}^6} \left(\frac{3\alpha_{B_1} I_A I_{B_1}}{2(I_A + I_{B_1})} + \alpha_{B_1} \right) \right] \quad (9)$$

$$k_3 = \frac{f}{kT} \left[(K_{AB_0} - K_{AB_0}^a) \frac{1}{r_{AB_0}^6} \left(\frac{3\alpha_{B_0} I_A I_{B_0}}{2(I_A + I_{B_0})} + \alpha_{B_0} \right) - (K_{AB_1} - K_{AB_1}^a) \frac{1}{r_{AB_1}^6} \left(\frac{3\alpha_{B_0} I_A I_{B_0}}{2(I_A + I_{B_0})} + \alpha_{B_0} \right) \right] \quad (10)$$

$$k_4 = \frac{1}{kT} \left[(K_{AB_0} - K_{AB_0}^a) \frac{1}{r_{AB_0}^6} \left(\frac{\mu_{B_0}^2}{3kT} + \alpha_{B_0} \right) - (K_{AB_1} - K_{AB_1}^a) \frac{1}{r_{AB_1}^6} \left(\frac{\mu_{B_1}^2}{3kT} + \alpha_{B_1} \right) \right] \quad (11)$$

$$k_5 = \frac{1}{kT} [(K_{AB_1} - K_{AB_1}^a) Z_{AB_1} - (K_{AB_0} - K_{AB_0}^a) Z_{AB_0}] \quad (12)$$

It can be seen from eqn. 8 that the parameter c is determined by the Van der Waals volume, dipole moment and hydrogen bond energy of the solute in a given mobile phase system. V_w can be calculated by Bondi's method¹⁸, dipole moment data are available for some solutes, but data on hydrogen bond energies are scarce¹⁹. In fact, the hydrogen bond energy between molecules is also affected by the surrounding functional groups. For example, if the parent compound to which a hydroxy group is attached is a hydrocarbon, then the hydrogen bond energy between molecules can be 0.5–1.0 kcal/mol higher than that between water molecules²⁰. On the other hand, the possibility of hydrogen donors and/or acceptors for the solute and solvent in chromatography must be considered simultaneously¹⁹.

Tables V and VI show the values of V_w , μ_A and X_{AH} for some solutes and a comparison of calculated and experimental values of parameter c . The results in Tables V and VI show the good correlation between the parameter c and structural parameters of the solute.

If a solute is non-polar, then $\mu_A = 0$ and $X_{AH} = 0$ and there should be a linear relationship between the parameter c and Van der Waals volume of the solute (V_w).

TABLE V

VALUES OF V_w , μ_A AND X_{AH} FOR VARIOUS SOLUTES AND COMPARISON OF CALCULATED AND EXPERIMENTAL c VALUES WITH METHANOL–WATER MOBILE PHASE (METHANOL FROM 40 TO 70%, v/v)

Data are recalculated from ref. 21.

Solute	V_w	μ_A	X_{AH}	c' (exp.) ^a	c' (calc.) ^{a,b}	Δ
Benzene	43.36	0	0	-2.95	-2.67	0.28
Chlorobenzene	57.48	1.58	0	-3.75	-3.45	0.39
Toluene	59.51	0.43	0	-3.56	-3.56	0
Bromobenzene	60.96	1.53	0	-3.93	-3.64	0.29
Iodobenzene	65.48	1.28	0	-4.13	-3.89	0.24
Ethylbenzene	69.74	0.35	0	-4.06	-4.13	-0.07
<i>o</i> -Xylene	70.66	0	0	-3.87	-4.18	-0.31
Aniline	56.38	1.56	15	-2.38	-2.28	0.10
Phenol	53.88	1.55	12	-2.66	-2.36	0.30
Methoxybenzene	63.21	1.25	6	-3.16	-3.32	-0.16
Benzaldehyde	60.06	2.80	10	-2.62	-2.63	-0.01
Benzyl alcohol	64.11	1.67	16	-2.79	-2.84	-0.05
Nitrobenzene	62.64	3.93	8	-3.03	-3.12	-0.09
Benzonitrile	60.54	3.93	8	-3.05	-3.00	0.05
Benzyl cyanide	70.77	3.50	12	-3.27	-3.27	0
1-Chloro-4-nitrobenzene	72.12	2.60	9	-3.45	-3.58	-0.13
1-Bromo-4-nitrobenzene	75.23	1.94	9	-3.63	-3.76	-0.13
4-Nitrophenol	68.14	4.76	16	-2.83	-2.82	0.01
1,4-Dimethoxybenzene	77.06	1.70	16	-3.27	-3.34	-0.07
2,4-Dinitrobenzene	88.07	4.33	20	-3.33	-3.62	-0.29
4-Methoxybenzaldehyde	74.41	3.26	16	-3.17	-3.18	-0.01
4-Hydroxybenzaldehyde	65.58	4.22	20	-2.69	-2.39	0.30

^a $c' = c/\ln 10$.

^b $c' = -0.2887 + 0.07414 X_{AH} - 0.05502 V_w - 0.001535 \mu_A^2$; S.D. = 0.2021, average error = 0.1535, $N = 22$.

TABLE VI

VALUES OF V_w , μ_A AND X_{AH} FOR PHENOLS AND CARBOXYLIC ACIDS AND COMPARISON OF CALCULATED AND EXPERIMENTAL c VALUES WITH ACETONITRILE-WATER MOBILE PHASE (ACETONITRILE FROM 20 TO 40%, v/v)

Data are recalculated from ref. 22.

Solute	V_w	μ_A	X_{AH}	c' (exp.) ^a	c' (calc.) ^{a,b}	Δ
2-Methylphenol	65.03	1.41	6.5	-3.11	-3.11	0
3-Methylphenol	65.03	1.54	6.5	-3.17	-3.12	-0.05
4-Methylphenol	65.03	1.54	6.5	-3.17	-3.12	-0.03
2,3-Dimethylphenol	76.18	1.25	7.0	-3.68	-3.76	-0.08
2,4-Dimethylphenol	76.18	1.39	7.0	-3.76	-3.77	-0.01
2,5-Dimethylphenol	76.18	1.44	7.0	-3.73	-3.78	-0.05
3,4-Dimethylphenol	76.18	1.53	7.0	-3.73	-3.79	-0.06
3,5-Dimethylphenol	76.18	1.76	7.0	-3.83	-3.82	0.01
Benzoic acid	63.38	1.72	7.5	-3.34	-3.31	0.03
3-Methylbenzoic acid	76.51	2.75	7.5	-4.09	-4.15	-0.06
2-Methylbenzoic acid	76.51	1.70	7.5	-3.82	-3.95	-0.13
2-Chlorobenzoic acid	74.84	2.49	7.5	-3.67	-4.01	-0.34
2-Bromobenzoic acid	77.96	1.91	7.5	-3.77	-4.06	-0.29
3-Bromobenzoic acid	77.96	2.17	7.5	-4.51	-4.19	0.32
4-Bromobenzoic acid	77.96	2.10	7.5	-4.59	-4.10	0.41
<i>trans</i> -Cinnamic acid	82.32	2.26	7.5	-4.33	-4.33	0

^a $c' = c/\ln 10$.

^b $c' = 1.7794 - 0.2490 X_{AH} - 0.04895 V_w - 0.04216 \mu_A^2$; S.D. = 0.2026, average error = 0.1294, $N = 16$.

Fig. 1 shows the relationship between c and V_w observed in an RP-HPLC experiment.

If solutes belong to a homologous series, the same dipole moments and hydrogen bond energies are assumed and Van der Waals volume can be described as:

$$V_w = \sum_i n_i \Delta V_{w(i)} + n_c \Delta V_{w(\text{CH}_2)} \quad (13)$$

where n_i and $\Delta V_{w(i)}$ are the numbers of group i and the Van der Waals volume contributed by group i and n_c and $\Delta V_{w(\text{CH}_2)}$ are the numbers of methylene groups and the Van der Waals volume contributed by a methylene group. Substitution of eqn. 13 together with μ_A and X_{AH} in eqn. 8 gives the parameter c for homologous compounds:

$$c = k_6 + k_7 n_c \quad (14)$$

where

$$k_6 = k_2 + k_4 \mu_A^2 + k_5 X_{AH} + k_3 \left[\sum_i n_i \Delta V_{w(i)} \right] \quad (15)$$

$$k_7 = k_3 [\Delta V_{w(\text{CH}_2)}] \quad (16)$$

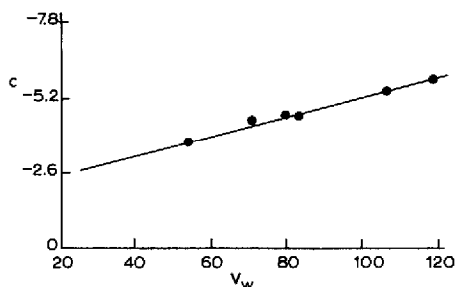


Fig. 1. Linear relationship between c and V_w for some polynuclear aromatic hydrocarbons observed in an RP-HPLC column system with Nucleosil-ODS packing and water-methanol mobile phase (methanol from 60 to 95%, v/v).

Eqns. 14–16 mean that there is a linear relationship between c and carbon number for homologous compounds, as shown by the data in Table VII.

In NP-HPLC, $\partial N_{B_1}/\partial N_A$ is large and the effect of displacement of a solute by adsorption on the parameter c cannot be neglected. For homologous compounds in adsorption chromatography, the relationship between carbon number and the parameter c shown in eqn. 14 must be corrected for the contribution of adsorption of the solute:

$$c = k_6 + k_7 n_c + mb \quad (17)$$

where

$$m = \frac{1}{kT \left(1 - \frac{1}{n_1}\right)} [(K_{B_1 B_1} - K_{B_1 B_1}^a) X_{B_1 B_1} - (K_{B_1 B_0} - K_{B_1 B_0}^a) X_{B_1 B_0}]$$

TABLE VII

LINEAR RELATIONSHIPS BETWEEN c AND CARBON NUMBER FOR HOMOLOGOUS SERIES

Data recalculated from ref. 23. Homologues: A, 3,5-dinitrobenzoates of n -alkanols; B, p -bromophenacyl esters of lower saturated carboxylic acids; C, 1,2-naphthoylbenzimidazole 6-sulphonamides of n -alkylamines. Binary mobile phases: M_1 = methanol-water (methanol from 70 to 90%, v/v); M_2 = dioxane-water (dioxane from 50 to 75%, v/v); M_3 = acetonitrile-water (acetonitrile from 60 to 80%, v/v).

Mobile phase	Homologous series								
	A			B			C		
	k_6	k_7	r^a	k_6	k_7	r^a	k_6	k_7	r^a
M_1	-6.50	-1.01	0.9984	-6.49	-0.944	0.9955	-6.59	-0.999	0.9984
M_2	-6.89	-1.30	0.9963	-6.13	-1.29	0.9991	-8.36	-1.06	0.9938
M_3	-6.62	-0.327	0.9924	-5.79	-0.398	0.9868	-2.97	-0.529	0.9780

^a Regression coefficient.

TABLE VIII

COMPARISON OF EXPERIMENTAL AND CALCULATED c VALUES FOR HOMOLOGOUS BENZOATES OF n -ALKANOLS

Data from ref. 16.

Parameter	n_c			
	1	2	3	4
c (exp.)	-0.39	-0.45	-0.58	-0.83
c (calc.)	-0.39	-0.43	-0.60	-0.83

and k_6 and k_7 are as in eqns. 15 and 16. Table VIII gives the results for the comparison of experimental measured parameters c with those calculated by eqn. 17 for a homologous series of benzoates of n -alkanols. The results in Table VIII describe well the variation of the parameter c for homologous derivatives separated by NP-HPLC.

FACTORS AFFECTING THE PARAMETER a

The parameter a is influenced by the behaviours of the stationary phase, solvent and solute. In a given reversed-phase system, the parameter a can also be correlated with structural parameters of the solute and can be expressed as:

$$a = k'_2 + k'_3 V_w + k'_4 \mu_A^2 + k'_5 X_{AH} \quad (18)$$

where

$$k'_2 = \ln \left(\frac{N_s}{V_s} \right) - \ln \beta + \frac{3}{2} \ln \left(\frac{h^2}{2\pi m_A kT} \right) + \frac{e}{kT} \left\{ (K_{AB}^a - K_{AB_0}) \frac{1}{r_{AB_0}^6} \left[\frac{3\alpha_{B_0} I_A I_{B_0}}{2(I_A + I_{B_0})} + \alpha_{B_0} \right] - \frac{1}{r^6} \left[\frac{3\alpha_a I_A I_a}{2(I_A + I_a)} + \alpha_a \right] \right\} \quad (19)$$

$$k'_3 = \frac{f}{kT} \left\{ \frac{1}{r^6} \left[\frac{3\alpha_a I_A I_a}{2(I_A + I_a)} + \alpha_a \right] - (K_{AB_0} - K_{AB_0}^a) \frac{1}{r_{AB_0}^6} \left[\frac{3\alpha_{B_0} I_A I_{B_0}}{2(I_A + I_{B_0})} + \alpha_{B_0} \right] \right\} \quad (20)$$

$$k'_4 = \frac{1}{kT} \left[\left(\frac{2\mu_A^2}{3kT} + \alpha_a \right) - (K_{AB_0} - K_{AB_0}^a) \left(\frac{2\mu_{B_0}^2}{3kT} + \alpha_{B_0} \right) \right] \quad (21)$$

$$k'_5 = \frac{1}{kT} [Z_a - (K_{AB_0} - K_{AB_0}^a) Z_{AB_0}] \quad (22)$$

It is possible to correlate the parameter a quantitatively with the structural parameters V_w , μ_A and X_{AH} of a solute as discussed above. Table IX illustrates the quantitative correlation between parameter a and V_w , μ_A and X_{AH} observed in RP-HPLC with methanol-water for 22 compounds and acetonitrile-water for 16 compounds, and demonstrates the validity of the theoretical deduction.

TABLE IX

COMPARISON OF EXPERIMENTAL AND CALCULATED a VALUESValues of V_w , μ_A and X_{AH} for solutes and mobile phase compositions as in Tables V and VI.

Methanol-water				Acetonitrile-water			
Solute	a' (exp.) ^a	a' (calc.) ^{a,b}	Δ	Solute	a' (exp.) ^a	a' (calc.) ^{a,c}	Δ
Benzene	2.45	2.53	0.08	2-Methylphenol	1.67	1.68	0.01
Chlorobenzene	3.22	2.97	-0.25	3-Methylphenol	1.65	1.67	0.02
Toluene	3.12	3.12	0	4-Methylphenol	1.65	1.67	0.02
Bromobenzene	3.42	3.16	-0.26	2,3-Dimethylphenol	2.10	2.09	-0.01
Iodobenzene	3.68	3.42	-0.26	2,4-Dimethylphenol	2.14	2.09	-0.05
Ethylbenzene	3.68	3.67	-0.01	2,5-Dimethylphenol	2.14	2.09	-0.05
<i>o</i> -Xylene	3.60	3.72	0.12	3,4-Dimethylphenol	2.12	2.07	-0.05
Aniline	1.36	1.43	0.07	3,5-Dimethylphenol	2.04	2.08	0.04
Phenol	1.56	1.59	0.03	Benzoic acid	1.47	1.42	-0.05
Methoxybenzene	2.55	2.70	0.15	3-Methylbenzoic acid	2.00	1.95	-0.05
Benzaldehyde	1.92	2.04	0.12	2-Methylbenzoic acid	1.87	2.01	0.14
Benzyl alcohol	1.57	1.74	0.17	2-Chlorobenzoic acid	1.69	1.89	0.20
Nitrobenzene	2.06	2.23	0.17	2-Bromobenzoic acid	1.80	2.06	0.26
Benzonitrile	2.06	2.12	0.06	3-Bromobenzoic acid	2.33	2.05	-0.28
Benzyl cyanide	2.15	2.67	0.52	4-Bromobenzoic acid	2.38	2.05	-0.33
1-Chloro-4-nitrobenzene	2.82	2.80	-0.02	<i>trans</i> -Cinnamic acid	2.08	2.24	0.16
1-Bromo-4-nitrobenzene	3.00	3.01	0.01				
4-Nitrophenol	1.77	1.61	-0.16				
1,4-Dimethoxybenzene	2.55	2.44	-0.11				
2,4-Dinitrobenzene	2.62	2.36	-0.26				
4-Methoxybenzaldehyde	2.23	2.32	0.09				
4-Hydroxybenzaldehyde	1.36	1.16	-0.20				

^a $a' = a/\ln 10$.^b $a' = -0.07132 + 0.05372 V_w - 0.01747 \mu_A^2 - 0.09885 X_{AH}$; S.D. = 0.1812, average error = 0.1400, $N = 22$.^c $a' = -0.1094 + 0.04441 V_w - 0.01305 \mu_A^2 + 0.07414 X_{AH}$; S.D. = 0.1821, average error = 0.1389, $N = 16$.

If the solutes are non-polar, then $\mu_A = 0$ and $X_{AH} = 0$ and eqn. 18 can be simplified to:

$$a = k'_2 + k'_3 V_w \quad (23)$$

Eqn. 23 means that there should be a linear relationship between the parameter a and V_w for non-polar compounds. The validity is confirmed by the results shown in Fig. 2.

If the solutes belong to a homologous series, there should be a linear relationship between the parameter a and the carbon numbers of the homologous substances:

$$a = k'_6 + k'_7 n_c \quad (24)$$

where

$$k'_6 = k'_2 + k'_3 \left[\sum_i n_i \Delta V_{w(i)} \right] + k'_4 \mu_A^2 + k'_5 X_{AH} \quad (25)$$

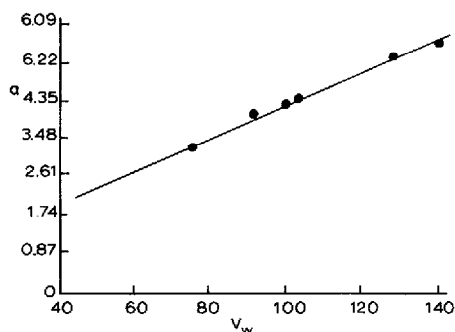


Fig. 2. Linear relationship between a and V_w for some non-polar compounds observed with the same system as in Fig. 1.

$$k'_7 = k'_3 [\Delta V_{w(\text{CH}_2)}] \quad (26)$$

Table X gives the results of the linear regression of parameter a and carbon numbers of the homologous series, showing consistency with theory.

In adsorption chromatography, the effect of displacement by adsorption on the parameter a must be considered for a homologous series. Then the parameter a can be expressed as:

$$a = k'_6 + k'_7 n_c + m'b \quad (27)$$

where

$$m' = \frac{1}{kT \left(1 - \frac{1}{n_1}\right)} [(K_{B_1, B_0} - K_{B_1, B_0}^a) X_{B_1, B_0} - X_{B_1}^a]$$

and k'_6 and k'_7 are as in eqns. 25 and 26. Eqn. 27 derived theoretically describes well the dependence of the parameter a on the carbon numbers n_c and the parameter b (Table XI).

TABLE X

LINEAR RELATIONSHIP BETWEEN a AND CARBON NUMBERS FOR HOMOLOGOUS SERIES

Homologues A-C and mobile phases M_1 - M_3 as in Table VII. Data recalculated from ref. 23.

Mobile phase	Homologous series								
	A			B			C		
	k'_6	k'_7	r^a	k'_6	k'_7	r^a	k'_6	k'_7	r^a
M_1	4.04	1.18	0.9991	4.00	1.10	0.9970	4.89	1.10	0.9994
M_2	3.93	1.26	0.9990	3.27	1.23	0.9998	3.80	1.05	0.9971
M_3	3.51	0.592	0.9991	3.09	0.628	0.9967	1.59	0.677	0.9883

^a Regression coefficient.

TABLE XI

COMPARISON OF EXPERIMENTAL AND CALCULATED (EQN. 27) a VALUES FOR HOMOLOGOUS BENZOATES OF n -ALKANOLS $k'_6 = -2.22$, $k'_7 = -0.161$, $m' = -0.105$. Data from ref. 16.

n_c	a (exp.)	a (calc.)	Δ
1	-2.14	-2.31	-0.17
2	-2.50	-2.47	0.03
3	-2.76	-2.63	0.13
4	-2.95	-2.94	0.01
5	-3.03	-2.95	0.08
6	-3.13	-3.11	0.02
7	-3.31	-3.26	0.04
8	-3.28	-3.42	-0.14

Another problem is to establish how the amount of bonded hydrocarbon chain (N_s) covering the stationary phase affects the retention value in RP-HPLC. From eqns. 1-4, it can be seen that the effect of N_s on the retention is given by the change in the parameter a when the other operating conditions remain constant:

$$a = \ln N_s + C \quad (28)$$

where

$$C = -\ln V_s - \ln \beta + \frac{\partial N_{B_1}}{\partial N_A} \ln \left[\left(\frac{h^2}{2\pi m_B kT} \right)^{3/2} / k_1 V_1 \right] + \frac{3}{2} \ln \left(\frac{h^2}{2\pi m_A kT} \right) + \frac{1}{kT} \left\{ (K_{AB_0} - K_{AB_0}^a) X_{AB_0} - X_A^a + \frac{\partial N_{B_1}}{\partial N_A} [(K_{B_1 B_0} - K_{B_1 B_0}^a) X_{B_1 B_0} - X_{B_1}^a] \right\} \quad (29)$$

By substituting eqn. 28 together with eqns. 2-4 into eqn. 1 and rearrangement, we obtain the following expression:

$$k' = S N_s \quad (30)$$

where

$$S = \exp (b \ln C_b + c C_b + C) \quad (31)$$

Eqn. 31 means that there is a linear relationship between k' and N_s which passes through the origin (Figs. 3 and 4).

RELATIONSHIP BETWEEN PARAMETERS a AND c

It has been reported that a linear relationship between the parameters a and c was observed for some compounds^{7,23}. From the results discussed in the previous two

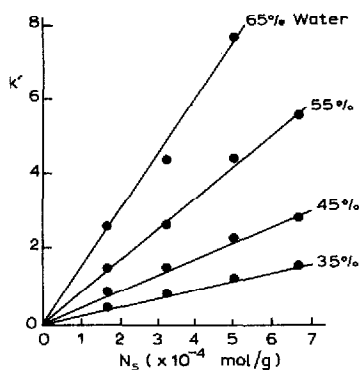


Fig. 3. Linear relationship between k' and N_s for nitrobenzene at different compositions of methanol–water mobile phase. Data replotted from ref. 24.

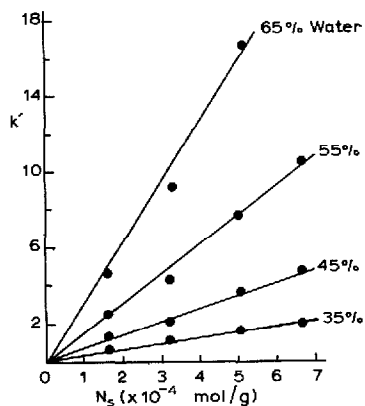


Fig. 4. Linear relationship between k' and N_s for methyl benzoate at different compositions of methanol–water mobile phase. Data replotted from ref. 24.

sections, it can be concluded that for a given RP-HPLC system it is possible to obtain a good linear relationship between parameters a and c if two of the three structural parameters V_w , μ_A and X_{AH} are equal or close. For non-polar compounds and homologous series of compounds this relationship can be expressed by the following equations:

$$\text{non-polar compounds: } a = k_8 + k_9c \quad (32)$$

$$\text{homologous series: } a = k'_8 + k'_9c \quad (33)$$

where

$$k_8 = k'_2 - k'_3k_2/k_3; \quad k_9 = k'_3/k_3 \quad (34)$$

$$k'_8 = k'_6 - k'_7k_6/k_7; \quad k'_9 = k'_7/k_7 \quad (35)$$

It can be seen from eqns. 34 and 35 that the slope of the linear relationship between a and c is mainly determined by the ratio of the difference between the solute–stationary phase and solute–weak solvent dispersive interactions to the difference between the solute–strong solvent and solute–weak solvent dispersive interactions, the intercept being related to the various physico-chemical behaviours of the stationary phase, solute and solvents. Figs. 5 and 6 show the linear relationship between a and c for non-polar compounds and homologous series, respectively. Table XII gives the results of a comparison of k_8 and k'_9 calculated by eqn. 35 and those obtained by regression from a and c directly for homologous series. These results for non-polar and homologous compounds confirm the theoretical data.

It must be pointed out that k'_9 for homologues in a dioxane–water binary mobile phase is less than -1 , which means that

$$-k'_7 \geq k_7 \quad (36)$$

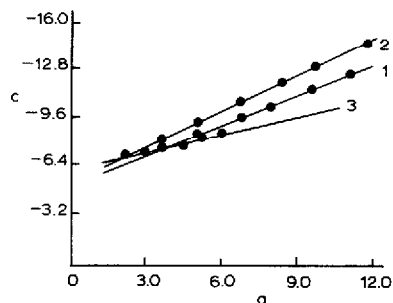
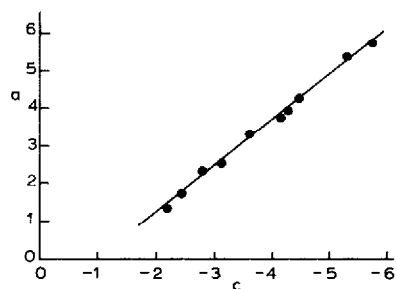


Fig. 5. Linear relationship between a and c for ten non-polar compounds observed with the same column system as in Fig. 1.

Fig. 6. Linear relationship between a and c for homologous 3,5-dinitrobenzoates of n -alkanols in different binary mobile phases: 1 = methanol-water; 2 = acetonitrile-water; 3 = dioxane-water (see Table VII). Data replotted from ref. 22.

After substitution of eqns. 16 and 27 into eqn. 36 and rearrangement, we obtain:

$$\frac{1}{r^6} \left(\frac{3\alpha_a I_A I_a}{2(I_A + I_a)} + \mu_a^2 \right) \leq \frac{1}{r_{AB_1}^6} (K_{AB_1} - K_{AB_1}^a) \left(\frac{3\alpha_{B_1} I_A I_{B_1}}{2(I_A + I_{B_1})} + \mu_{B_1}^2 \right) \quad (37)$$

Eqn. 37 means that the dispersive interaction between the solute and the stationary

TABLE XII

COMPARISON OF EXPERIMENTAL AND CALCULATED k'_8 AND k'_9 VALUES (EQN. 32) FOR HOMOLOGOUS SERIES

Data recalculated from ref. 23. Homologues A-C and mobile phases M_1 - M_3 as in Table IV.

Mobile phase	Homologous series								
	A			B			C		
	k'_9 (exp.)	k'_9 (calc.)	Δ (%) ^a	k'_9 (exp.)	k'_9 (calc.)	Δ (%) ^a	k'_9 (exp.)	k'_9 (calc.)	Δ (%) ^a
M_1	-0.859	-0.856	0.07	-0.859	-0.855	0.47	-0.908	-0.911	-0.27
M_2	-1.037	-1.035	0.24	-1.050	-1.049	0.11	-1.011	-1.009	0.24
M_3	-0.558	-0.554	0.71	-0.643	-0.634	1.34	-0.802	-0.780	2.78
	k'_8 (exp.)	k'_8 (calc.)	Δ (%)	k'_8 (exp.)	k'_8 (calc.)	Δ (%)	k'_8 (exp.)	k'_8 (calc.)	Δ (%)
M_1	-3.039	-3.046	-0.20	-3.048	-3.031	-0.07	-2.144	-2.135	0.40
M_2	-2.807	-2.828	-0.75	-2.702	-2.710	-0.31	-4.50	-4.526	-0.58
M_3	-4.651	-4.675	-0.42	-3.827	-3.779	1.27	-1.692	-1.737	-2.68

^a Relative error: Δ (%) = $\frac{k'_i(\text{exp.}) - k'_i(\text{calc.})}{k'_i(\text{exp.})} \cdot 100$.

phase is weaker than that between the solute and a strong solvent. If a methanol–water or acetonitrile–water binary mobile phase is used, then $k'_q \geq -1$, which means that:

$$\frac{1}{r^6} \left[\frac{3\alpha_a I_A I_a}{2(I_A + I_a)} + \mu_a^2 \right] \geq \frac{1}{r_{AB_1}^6} (K_{AB_1} - K_{AB_1}^a) \left[\frac{3\alpha_{B_1} I_A I_{B_1}}{2(I_A + I_{B_1})} + \mu_{B_1}^2 \right] \quad (38)$$

Eqn. 38 shows that the dispersive interaction between the solute and a strong solvent is weaker than that between the solute and the stationary phase. These results confirm that the dispersive interaction of dioxane is stronger than that of methanol and acetonitrile and the stationary phase affects the retention and selectivity in both NP- and RP-HPLC.

CONCLUSION

The parameter b in RP-HPLC approaches to a small constant value because the displacement by adsorption is very weak. In NP-HPLC, this displacement is strong and the parameter b changes slightly when different batches of packing of the same size and the same mobile phase are used; the absolute value of the parameter b increases slightly and approaches a constant value with increasing carbon numbers for homologous series. The parameter c in RP-HPLC is mainly determined by the difference between solute–strong solvent and solute–weak solvent interactions and approaches a constant value when different packings and the same mobile phase are used. The parameter c in RP-HPLC can be correlated with structural parameters of the solute such as Van der Waals volume (V_w), dipole moment (μ_A) and hydrogen bond energy (X_{AH}). For compounds such as non-polar or homologous series, a simplified linear relationship between the parameters c and V_w can be established. The parameter c in NP-HPLC is also influenced by displacement of the solute and the relationships discussed for parameter c in RP-HPLC must be modified to take account of this effect. The parameter a in both RP- and NP-HPLC follows similar relationships to the parameter c . A linear relationship between k' and coverage of C_{18} in RP-HPLC has been derived theoretically and confirmed experimentally. There is a good linear relationship between the parameters a and c only when two of the three structural parameters V_w , μ_A and X_{AH} of solutes are equal or close and the effect of displacement of solute by adsorption on the parameters a and c can be neglected.

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