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Retention characteristics of an immobilized artificial membrane column in reversed-phase liquid chromatography

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Abstract

Retention for a varied group of compounds on an immobilized artificial membrane column (IAM PC DD2) with a methanol–water mobile phase is shown to fit a second-order model for the retention factor ($\log k$) as a function of the volume fraction of organic solvent. The numerical value of the intercept obtained by linear extrapolation to zero organic solvent ($\log k_w$) is shown to depend on the range of mobile phase composition used for the extrapolation. Each series of intercepts so obtained represents a different hypothetical distribution system as identified by the system constants of the solvation parameter model. Although a linear model is a poor fit for isocratic retention data, the linear solvent strength gradient model provides a reasonable estimate of isocratic retention factor values that are (slightly) larger than experimental values, but provide the same chemical information for the system. These preliminary results suggest that gradient elution may prove to be a rapid and useful method for creating system maps for column characterization and method development. In this work a system map is provided for methanol–water compositions from 0 to 60% (v/v) methanol and additional system constants for acetonitrile–water compositions containing 20 and 30% (v/v) acetonitrile. It is shown that the main factors contributing to retention on the IAM PC DD2 column are favorable cavity formation and dispersion interactions, electron lone pair interactions and the hydrogen-bond basicity of the sorbent. The latter feature more than any other distinguishes the IAM column from conventional chemically bonded phases. Interactions of a dipole-type (weakly) and inability to compete with the mobile phase as a hydrogen-bond acid reduce retention. A comparison of system constant ratios is used to demonstrate that the retention properties of the IAM column are not easily duplicated by conventional chemically bonded phases. The retention characteristics of the IAM column, however, are strongly correlated with the retention properties of pseudostationary phases used for micellar electrokinetic chromatography, which provide a suitable alternative to IAM columns for physical property estimations. By the same comparative method it is shown that retention on the IAM column possesses some similarity to biomembrane absorption processes, allowing suitable correlation models to be developed for the estimation of certain biopartitioning properties.

Keywords: Retention characteristics; Immobilized artificial membrane column; Stationary phases, LC

1. Introduction

Immobilized artificial membranes (IAMs) are solid-phase membrane mimetics prepared by covalently bonding a monolayer of a phospholipid, typically phosphatidylcholine, to a porous silica

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substrate suitable for liquid chromatography. They are used mainly for the isolation of cell membrane proteins and as a chromatographic model for the estimation of biomembrane transport properties of primary interest for drug development in the pharmaceutical industry. Thus, for example, the retention factor on IAM columns was shown to correlate with analyte partition coefficients in fluid liposome systems [1,2]; to predict drug permeability through caco-2 cells [1,3]; to predict skin permeability to organic compounds [4]; to predict drug transport across the blood–brain barrier [5,6]; and to predict intestinal absorption of organic compounds [1]. These and other quantitative structure–activity relationship studies are reviewed elsewhere [7–10]. It is the possibility of using a convenient and rapid process like liquid chromatography to screen compound libraries as an indication of membrane absorption properties that sustains general interest in IAM sorbents.

For general orientation, Fig. 1 provides simplified structures of the IAM column packing materials commonly used in drug discovery. In this report an IAM PC DD2 material, consisting of diacyl double chain ester phosphatidylcholine ligands surface-bonded to an aminopropylsiloxane-bonded silica substrate and endcapped by mixed propionic and decanoic alkylamide groups, is used in all retention studies. Several earlier studies report results for a IAM.PC.DD column packing consisting of a single acyl chain phosphatidylcholine ligand without a glycerol backbone and endcapped as described above. This material has been withdrawn from production in favor of IAM PC DD2. Materials containing diacyl double chain ester phosphatidylcholine ligands similar to IAM PC DD2 without endcapping (IAM PC) or endcapped with methyl glycolate (IAM PC MG) complete the range of commercially available IAM columns. Although chemically bonded phases dominate research using IAM columns, good results have been reported for sorbents prepared by dynamically coating phospholipids on conventional alkylsiloxane-bonded stationary phases [11,12].

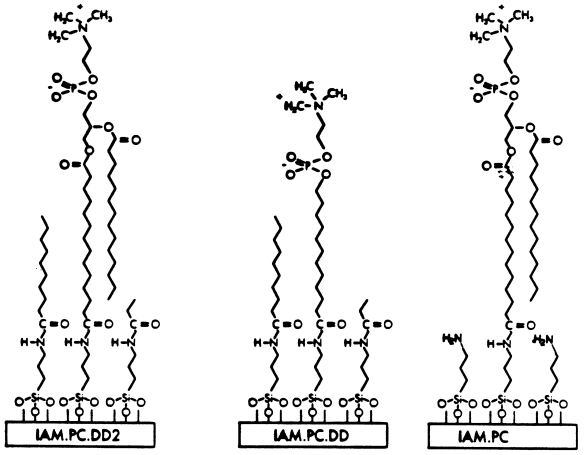

Fig. 1. Structures of IAM phases.

The general approach used for column characterization used in these studies is based on Abraham's solvation parameter model, indicated below in the form suitable for reversed-phase liquid chromatography [13,14]:

$$\log k = c + vV + eE + sS + aA + bB \quad (1)$$

where k is the solute retention factor. The model equation is made up of product terms representing solute properties (descriptors) and sorbent and mobile phase properties (system constants). The solute descriptors are McGowan's characteristic volume V, the excess molar refraction E, the solute's dipolarity/polarizability S, and the solute's effective hydrogen-bond acidity and hydrogen-bond basicity, A and B, respectively. Solute descriptors are available for about 4000 compounds [15,16], with others available through parameter estimates and computational approaches [13,17].

The system constants in Eq. (1) are defined by their complementary interactions with the solute descriptors. Each system constant characterizes the difference in capacity of the solvated sorbent and mobile phase for a specific interaction. The e constant is determined by the contribution from electron lone pair interactions, the s constant by dipole–dipole and dipole–induced dipole interactions and the a and b system constants by hydrogen-bond basicity and acidity, respectively. The v constant is a measure of the dispersion interactions that fail to cancel when the solute is transferred from one phase to the other, together with contributions from the difference in the ease of cavity formation in each phase. System constants have been determined for a

large number of stationary phases in reversed-phase liquid chromatography and their relationship to stationary and mobile phase properties are reviewed elsewhere [16,18,19].

Abraham et al. [20] determined the system constants for an IAM PC MG column with a mobile phase containing 10% (v/v) acetonitrile in a pH 7 buffer. The solvated IAM PC MG sorbent was shown to be less hydrogen-bond acidic than the mobile phase, but more polarizable, more hydrogen-bond basic and less cohesive than the mobile phase. The retention properties of the sorbent were shown to be rather different to typical octadecylsiloxane-bonded silica sorbents with the same mobile phase and were not well correlated with either $\log P$ (octanol–water distribution constant) or $\log P_{alk}$ (alkane–water distribution constant). Al-Haj et al. [21] determined system constants for a IAM PC DD column using the intercept ($\log k_{we}$) as the dependent variable. The latter was obtained by linear extrapolation of isocratic retention factors ($\log k$) for the composition range 80:20 to 20:80% (v/v) acetonitrile in a pH 7 phosphate buffer. Retention was shown to be dominated by the differences in hydrogen-bond acidity and cohesion of the solvated sorbent and the mobile phase. The dipolarity/polarizability and hydrogen-bond basicity of the solvated sorbent and the mobile phase were deemed to be equivalent. These results are quite different to what might be expected based on the results for the similar IAM PC MG column. The model is statistically weak and possibly adversely affected by the use of an extrapolation method to determine $\log k_{we}$ and inclusion of partially ionized solutes in the data set. Valko et al. [22] determined system constants for an IAM PC DD2 column with a mobile phase containing 20% (v/v) acetonitrile in a pH 7.4 ammonium acetate buffer and for $\log k_{we}$ obtained by linear extrapolation for unspecified compositions in the same acetonitrile–buffer system. The results demonstrate that the statistical fit for the extrapolated model is not as good as for the isocratic acetonitrile–buffer system, but the general trends in the system constants for both models are consistent. In particular, both models indicate that the solvated sorbent is a stronger hydrogen-bond base than the mobile phase. The same group has proposed a method of stationary phase characterization based on gradient elution and the chromatographic hydrophobicity index (CHI) [23,24] which was applied to IAM columns [22,25,26]. This approach requires calibration of the column under gradient elution conditions with a series of compounds with known isocratic CHI values. The linear model is then used to convert gradient retention times for a varied group of compounds into CHI values, which are then entered as the dependent variable in the solvation parameter model. In this approach the gradient retention time is correlated with the isocratic solvent strength determined as the volume fraction of organic solvent required to achieve an equal distribution of the compound between the mobile and stationary phase, corresponding to $\log k = 0$. This approach seems to lack a simple sense of the equilibrium phase properties associated with the system constants, confusing any constructive interpretation since it expresses retention as a difference in mobile phase composition. This composition, however, must be linearly related to the change in retention for an undefined isocratic mobile phase composition and is a useful method for determining solute descriptors [25,26]. The theory of linear solvent strength gradients can be used to relate gradient retention times to solute properties in isocratic binary mobile phase compositions and, subsequently, the calculation of system constants at several isocratic binary mobile phase compositions [27–30]. This approach is attractive, since gradient elution methods provide faster and more convenient separation conditions for compounds with a wide range of retention properties and allow estimation of isocratic retention factors for different mobile phase compositions from two gradient separations.

The solvation parameter model has been used to characterize a large number of biopartitioning processes of interest in drug development and toxicity assessment [18,31]. These include the octanol–water distribution constant [32,33], blood–brain permeation and distribution constants [34–36], skin–water permeation and distribution constants [36–38], cell permeation [39], intestinal absorption [40] and non-specific toxicity in bacteria [41], fish [42] and tadpole [43] models. Experiments in biological systems can be difficult for technical and ethical reasons, as well as costly to perform. For these reasons, models able to predict the outcome of

biological tests are welcome as a means of providing useful biological property information in a timely and economic manner. Chromatographic models are well suited to this purpose. A chromatographic model is able to emulate a biological process when the system constant ratios of the solvation parameter model are (nearly) identical for the compared systems [18,31]. The dependent variables for the two systems can then be correlated through a first-order linear equation. It should be possible, therefore, to consider the usefulness of the IAM columns to predict biological properties by comparison of their system constant ratios to those of the previously characterized biological processes. A comparison of system constant ratios with those of conventional liquid chromatographic sorbents should provide an indication of whether IAM sorbents provide unique sorption properties or simply duplicate the properties of readily available materials.

2. Experimental

Methanol, acetonitrile and water were OmniSolv grade from EM Science (Gibbstown, NJ, USA). Other common chemicals were reagent grade or better and obtained from several sources. The 15 cm×4.6 mm I.D. IAM PC DD2 column, 12 μm average particle size and 30 nm average pore diameter, was obtained from Regis Technologies (Morton Grove, IL, USA).

A Varian Vista 5500 liquid chromatograph (Walnut Creek, CA, USA) with a 10 μl rotary injection valve and variable-wavelength UV detector was used for liquid chromatography. Chromatograms were recorded using a Hewlett-Packard 3390A computing integrator (Wilmington, DE, USA). The column hold-up time was determined using an aqueous solution of sodium nitrate (26 mg/ml) as an unretained solute. The gradient dwell volume for this instrument was 3.30 ml.

To estimate isocratic retention factors by gradient elution, two linear gradients from 0 to 100% (v/v) acetonitrile in 13 min, gradient 1, and 45 min, gradient 2, were used. Sample injection was delayed after the start of the gradient to correspond to the time required for the mobile phase to sweep out the dwell volume. The method used to calculate the isocratic retention factors is described in detail elsewhere [28–30]. Briefly, the procedure is as follows. The gradient steepness parameter, b^* (written here with an asterisk to distinguish it from the b system constant of the solvation parameter model), is calculated for each compound from the second gradient by:

$$b_2^* = [\log k_1 - \log k_2]/[(t_{g2}/t_M) - \beta(t_{g1}/t_M)] \quad (2)$$

where t_g is the gradient retention time, t_M the column hold-up time, k the retention factor $[(t_g - t_M)/t_M]$, $\beta = t_{G2}/t_{G1}$ and t_G is the gradient run time. Once b_2^* is calculated then b_1^* is obtained from the relationship $b_1^* = \beta b_2^*$. Then for both gradients an estimate of $\log k_{we}$ for each compound is obtained from:

$$\log k_{we} = \log k + b^*(t_g/t_M) \quad (3)$$

and the average of the estimate for each gradient taken as the final value of $\log k_{we}$, where k_{we} is the retention factor of each compound with water as the mobile phase, equivalent to the isocratic retention factor obtained by linear extrapolation of $\log k$ against the volume fraction of acetonitrile to zero acetonitrile composition. The S-value is then calculated for each compound in both gradients by:

$$S = (b^* t_G)/(t_M \Delta\phi) \quad (4)$$

and the average of the two estimates ($b^* = b_1^*$ and b_2^*) taken as the final value of S, where $\Delta\phi$ is the change in the volume fraction of strong solvent over the course of the gradient. Isocratic retention factor values for any specified mobile phase composition are then determined from:

$$\log k = \log k_{we} - S\phi \quad (5)$$

where ϕ is the volume fraction of acetonitrile.

Multiple linear regression analysis and statistical tests were performed on a Gateway G6-333 personal computer (North Sioux City, SD, USA) using the program SPSS/PC+ v. 10 (SPSS, Chicago, IL, USA). The solute descriptors used in the solvation parameter model were taken from an in-house database and are summarized in Table 1 together with the isocratic experimental retention factors determined for methanol–water and acetonitrile–water

Table 1
Solute descriptors and retention factors (log k) for solutes used to characterize the IAM PC DD2 column

Solute	Descriptor					Methanol (%, v/v)								Acetonitrile	
	V	E	S	A	B	0	10	20	30	40	50	60	70	20	30
Acetanilide	1.113	0.870	1.40	0.50	0.67	0.631	0.373	0.207	−0.042	−0.246	−0.541	−0.842	−1.377	−0.095	−0.375
Acetophenone	1.014	0.820	1.01	0	0.48	0.819	0.588	0.415	0.150	−0.100	−0.375	−0.717	−1.252	0.139	−0.159
2-Aminophenol	0.875	1.110	1.10	0.60	0.66	0.275	0.100	−0.050	−0.192	−0.380	−0.531	−0.695		−0.218	−0.423
Anisole	0.916	0.710	0.75	0	0.29	0.933	0.764	0.650	0.430	0.250	−0.030	−0.335	−0.690	0.435	0.157
Benzaldehyde	0.873	0.820	1.00	0	0.39	0.629	0.430	0.297	0.088	−0.122	−0.405	−0.706	−1.155	0.073	−0.188
Benzamide	0.973	0.990	1.50	0.49	0.67	0.245	0.023	−0.133	−0.383	−0.580	−0.879			−0.377	−0.688
Benzene	0.716	0.610	0.52	0	0.14	0.704	0.603	0.526	0.389	0.213	−0.036	−0.335	−0.672	0.415	0.164
Benzonitrile	0.871	0.740	1.11	0	0.33	0.695	0.509	0.347	0.167	−0.089	−0.360	−0.695		0.186	−0.107
Benzyl alcohol	0.916	0.803	0.87	0.33	0.56	0.292	0.146	0.036	−0.138	−0.306	−0.578	−0.889	−1.377	−0.161	−0.393
Biphenyl	1.324	1.360	0.99	0	0.26	2.541	2.328	2.097	1.814	1.377	0.924	0.449	0.023	1.647	1.093
1-Bromo-naphthalene	1.260	1.598	1.13	0	0.13	2.904	2.695	2.459	1.999	1.660	1.160	0.662	0.211	1.906	1.304
3-Bromophenol	0.950	1.060	1.15	0.70	0.16	1.707	1.533	1.358	1.092	0.794	0.441	0.073	−0.306	0.938	0.555
4-Chlorophenol	0.898	0.920	1.08	0.67	0.20	1.483	1.291	1.062	0.915	0.643	0.321	0.064	−0.389	0.784	0.428
Cinnamyl alcohol	1.155	1.090	1.04	0.38	0.60	1.022	0.825	0.682	0.437	0.168	−0.149	−0.510	−0.947	0.355	0.029
Coumarin	1.062	1.060	1.79	0	0.46	0.994	0.727	0.489	0.263	−0.041	−0.315	−0.646	−1.060	0.173	−0.151
Cyclohexanone	0.861	0.403	0.86	0	0.56	0.077	−0.098	−0.255	−0.417	−0.640	−0.863			−0.348	−0.559
Diethyl phthalate	1.711	0.729	1.40	0	0.88	1.634	1.267	1.014	0.651	0.272	−0.131	−0.556	−1.065	0.640	0.220
2,6-Dimethylphenol	1.057	0.860	0.79	0.39	0.39	1.252	1.072	0.900	0.663	0.411	0.101	−0.213	−0.572	0.604	0.243
4-Hydroxybenzyl alcohol	0.975	0.998	1.15	0.88	0.85	0.124	−0.082	−0.261	−0.417	−0.666	−0.889			−0.538	−0.750
2-Methylphenol	0.916	0.840	0.86	0.52	0.30	1.019	0.866	0.714	0.488	0.280	−0.009	−0.319	−0.666	0.432	0.150
4-Methylphenol	0.916	0.820	0.87	0.57	0.31	1.015	0.861	0.709	0.498	0.251	−0.043	−0.357	−0.690	0.419	0.088
Naphthalene	1.085	1.340	0.92	0	0.20	2.032	1.795	1.658	1.313	1.043	0.649	0.238	−0.168	1.282	0.803
1-Naphthol	1.144	1.520	1.05	0.61	0.37	2.080	1.850	1.665	1.315	0.981	0.650	0.198	−0.169	1.118	0.700
2-Naphthol	1.144	1.520	1.08	0.61	0.40	1.991	1.753	1.551	1.221	0.887	0.486	0.100	−0.272	1.028	0.633
2-Nitroaniline	0.990	1.180	1.37	0.30	0.36	1.117	0.896	0.799	0.570	0.332	0.045	−0.259	−0.550	0.433	0.107
4-Nitroaniline	0.990	1.220	1.91	0.42	0.38	1.011	0.831	0.713	0.472	0.224	−0.035	−0.334	−0.644	0.343	0.018
Nitrobenzene	0.891	0.871	1.11	0	0.28	0.913	0.748	0.646	0.447	0.222	−0.030	−0.321	−0.701	0.410	0.137
4-Nitrobenzyl alcohol	1.090	1.064	1.39	0.44	0.62	0.717	0.520	0.366	0.223	−0.052	−0.291	−0.587	−0.943	0.101	−0.203
1-Nitrobutane	0.846	0.227	0.95	0	0.29	0.422	0.280	0.184	0.029	−0.156	−0.397	−0.697		0.092	−0.142
4-Nitrotoluene	1.032	0.870	1.11	0	0.28	1.351	1.118	0.994	0.750	0.492	0.179	−0.167	−0.521	0.701	0.365
Phenol	0.775	0.810	0.89	0.60	0.30	0.615	0.465	0.366	0.174	−0.006	−0.259	−0.544	−0.907	0.121	−0.103
2-Phenylethanol	1.057	0.811	0.91	0.30	0.65	0.533	0.367	0.220	0.046	−0.174	−0.429	−0.747	−1.194	0.037	−0.259
4-Phenylphenol	1.383	1.560	1.41	0.59	0.45	2.563	2.295	2.048	1.642	1.238	0.798	0.353	−0.077	0.899	1.432
Toluene	0.857	0.601	0.52	0	0.14	1.153	1.015	0.908	0.707	0.522	0.227	−0.108	−0.484	0.757	0.436

mobile phase compositions. Additional solute descriptors used in the gradient model to extend the retention range of the compounds studied are summarized in Table 2 together with their experimental isocratic retention factors for 20% (v/v) acetonitrile–water. Gradient retention times for the acetonitrile–water gradients and parameters estimated from the linear solvent strength gradient model are summarized in Table 3.

3. Results and discussion

3.1. Selection of the dependent variable

For separations employing binary mixtures of a single organic solvent in water the change in solute retention factors with composition in reversed-phase liquid chromatography can be adequately described by Eq. (6) [18,44]. For a restricted composition

Table 2
Additional solute descriptors used in the gradient retention model and experimental isocratic retention factors in 20% (v/v) acetonitrile–water

Solute	Descriptor					$\log k_{20\%}$
	V	*E*	*S*	*A*	*B*	
Butyrophenone	1.296	0.800	0.95	0	0.51	0.908
Dibutyl phthalate	2.274	0.700	1.40	0	0.86	2.013
α-Estradiol	2.199	1.800	3.30	0.88	0.95	1.623
Fluorene	1.357	1.588	1.06	0	0.25	1.628
Hexanophenone	1.578	0.720	0.95	0	0.50	1.603
Octanophenone	1.859	0.720	0.95	0	0.29	2.151
Phenanthrene	1.454	2.055	1.29	0	0.26	2.093
Progesterone	2.622	1.450	3.29	0	1.14	1.689
Propiophenone	1.155	0.800	0.95	0	0.51	0.578
Valerophenone	1.437	0.800	0.95	0	0.50	1.265

range a simple linear equation will often suffice, Eq. (5):

$$\log k = a_0 + a_1\phi + a_2\phi^2 \quad (6)$$

where ϕ is the volume fraction of organic solvent. For the purpose of retention modeling, any of the free energy related parameters ($\log k$, $\log k_w$, $\log k_{we}$, *S*-value) could be used as the dependent variable in the solvation parameter model. The meaning of $\log k$ is unambiguous for any defined mobile phase composition. To describe retention as a function of mobile phase composition, system constants are required for a number of different compositions that are fit to an algebraic model expressing the change in system constants as a continuous function of the volume fraction of the strong solvent, called a system map [16,18]. Selection of $\log k_{we}$ and the *S*-value as dependent variables is attractive because Eq. (5) indicates that system properties could be specified at any composition from two independent models. Some authors have asserted that $\log k_{we}$ is the preferred parameter for column characterization using the solvation parameter model [21,45–47]. The main concern with the use of either $\log k_{we}$ or the *S*-value as a dependent variable in the solvation parameter model is that both terms lack a clear thermodynamic definition [18] and a rigorous and self-consistent method for their determination. Also, models developed using $\log k_{we}$ as a dependent variable are generally not as good in terms of descriptive statistics as similar models for a single mobile phase composition [16,18,28,45–48]. Caldwell et al. [49] demonstrated a significant difference between experimental $\log k_w$ values and those obtained by extrapolation for mobile phases containing 10–40% (v/v) acetonitrile on an IAM PC DD column. For a limited number of compounds, Demare et al. [50] reported a linear correlation between $\log k$ and the volume fraction of acetonitrile for IAM PC DD and IAM PC DD2 columns. However, extrapolated values of $\log k_{we}$ showed improved agreement with experimental values for water as a mobile phase when the pH and the ionic strength of the acetonitrile-containing mobile phases were adjusted to the same value as the purely aqueous mobile phase. In most studies it is either implied or stated explicitly that the intercept for Eq. (5) is equal to the isocratic retention factor for water as the mobile phase. This is not an essential interpretation of Eq. (5), and even if $\log k_{we}$ was no more than an abstract term, it could still be used for model development, so long as it is related in one way or another to the free energy of the system. But to be useful for column comparison purposes, $\log k_{we}$ still requires a suitable definition in terms of system properties.

3.2. Extrapolated retention factors for water as the mobile phase

The retention factor values summarized in Table 1 were generated to allow an assessment of the influence of mobile phase composition on general

Table 3
Gradient elution retention times and parameters predicted from the linear solvent strength gradient model for acetonitrile–water mobile phases

Solute	Gradient retention time (min)		Calculated parameters				
	t_{g1}	t_{g2}	b_1^*	b_2^*	$\log k_{we}$	S	$\log k_{20\%}$ [a]
Acetophenone	3.46	5.05	0.171	0.049	0.626	1.640	0.298
Anisole	4.15	6.42	0.157	0.045	0.794	1.506	0.493
Benzene	4.03	5.43	0.101	0.029	0.592	0.965	0.399
Biphenyl	6.95	18.29	0.397	0.115	2.651	3.807	1.889
1-Bromonaphthalene	7.66	20.41	0.375	0.108	2.785	3.599	2.065
3-Bromophenol	5.90	12.88	0.261	0.075	1.653	2.503	1.152
Butyrophenone	5.13	11.11	0.295	0.085	1.561	2.828	0.995
4-Chlorophenol	5.51	11.40	0.240	0.069	1.458	2.300	0.998
Cinnamyl alcohol	4.01	6.85	0.216	0.063	0.931	2.076	0.515
Coumarin	3.60	5.58	0.200	0.058	0.740	1.914	0.357
Dibutyl phthalate	7.02	18.78	0.419	0.121	2.793	4.023	1.989
Diethyl phthalate	4.87	9.96	0.269	0.078	1.381	2.583	0.864
2,6-Dimethylphenol	4.91	9.06	0.209	0.060	1.165	2.002	0.764
α-Estradiol	7.01	18.00	0.361	0.104	2.485	3.459	1.793
Fluorene	7.29	18.15	0.312	0.090	2.313	2.996	1.714
Hexanophenone	6.73	17.07	0.358	0.103	2.371	3.430	1.685
2-Methylphenol	4.42	7.10	0.162	0.047	0.879	1.550	0.569
4-Methylphenol	4.35	7.02	0.171	0.049	0.884	1.643	0.556
Naphthalene	6.54	15.76	0.305	0.088	2.051	2.921	1.467
1-Naphthol	6.36	14.82	0.290	0.084	1.921	2.787	1.364
2-Naphthol	5.92	14.27	0.345	0.100	2.035	3.313	1.372
Nitrobenzene	4.11	6.51	0.170	0.049	0.823	1.634	0.496
4-Nitrotoluene	5.16	9.89	0.216	0.062	1.261	2.072	0.847
Octanophenone	7.66	21.15	0.440	0.127	3.153	4.220	2.309
Phenanthrene	7.83	21.12	0.385	0.111	2.902	3.690	2.164
4-Phenylphenol	6.58	16.98	0.390	0.113	2.482	3.746	1.733
Progesterone	6.71	17.44	0.391	0.113	2.534	3.752	1.783
Propiophenone	4.45	7.78	0.199	0.057	1.012	1.909	0.630
Toulene	5.16	9.64	0.197	0.057	1.198	1.889	0.820
Valerophenone	6.08	14.37	0.314	0.091	1.949	3.010	1.347

[a] Estimated isocaratic value of $\log k$ for a mobile phase containing 20% (v/v) acetonitrile–water.

retention for the IAM PC DD2 column and the suitability of extrapolated $\log k_{we}$ values as a dependent variable in the solvation parameter model. The solutes used for column evaluation were selected to represent a wide range of properties and to be predominantly in a neutral form under the conditions of the experiment. There are two reasons for the latter qualification. The solvation parameter model is set up to model the retention of neutral compounds and ionizable compounds in their neutral form. Without extension it does not model correctly the retention characteristics of ionic compounds in reversed-phase liquid chromatography [51,52]. The IAM PC DD2 stationary phase contains zwitterionic groups that may influence the retention of ionized compounds by specific electrostatic interactions unrelated to general retention of neutral compounds.

Some representative plots of retention as a function of the mobile phase composition are shown in Fig. 2. These plots show various degrees of curvature with a general flattening of the curves as $\phi \rightarrow 0$. For all compounds there is a good fit to the second-order model, Eq. (6), with an average coefficient of determination of 0.997 ± 0.007 (Table 4). The first-order model, Eq. (5), provides only a modest fit with an average coefficient of determination of 0.978 ± 0.013.

There are no established guidelines for the selec-

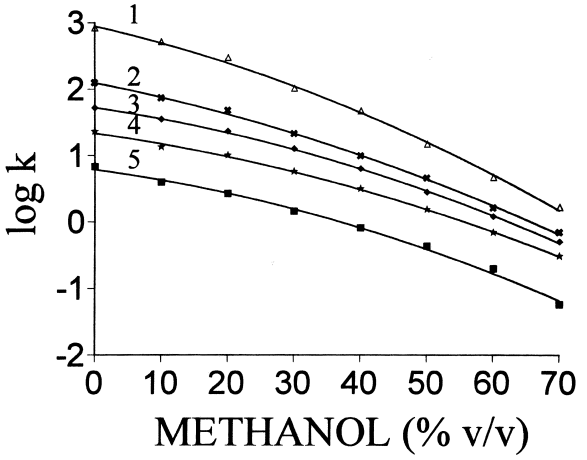

Fig. 2. Plot of the isocratic retention factor (log k) against the volume fraction of methanol in the mobile phase. Identification: 1 = 1-bromonaphthalene; 2 = 1-naphthol; 3 = 3-bromophenol; 4 = 4-nitrotoluene; and 5 = acetophenone.

tion of the mobile composition range to use for extrapolation. Typically, three and sometimes four mobile phase compositions in which the compounds of interest exhibit convenient retention are generally selected. In the same vein we arbitrarily selected three different composition ranges for comparison that span a reasonable segment of the total composition range for which acceptable experimental values could be obtained for all solutes. The slope and intercept for a linear extrapolation of experimental log k values for the composition ranges 10–30% (v/v), 20–50% (v/v) and 30–50% (v/v) are summarized in Table 5. As a general trend the extrapolated $\log k_{we}$ values and the slope increase in value at higher initial values of the strong solvent chosen for the extrapolation. Since the linear extrapolation represents a smoothing of the natural curvature in the individual plots, this would be expected. A linear extrapolation of the individual composition ranges is acceptable with an average coefficient of determination of 0.979±0.010 for 10–30% (v/v), 0.994±0.006 for 20–50% (v/v) and 0.994±0.006 for 30–50% (v/v) methanol–water mobile phases. For consistency in using the extrapolation method with the IAM PC DD2 column it is important that the same composition range is used for all solutes in each data set to avoid additional uncertainty from dispersion of $\log k_{we}$ values associated with different extrapolation ranges for the same compound.

3.3. Comparison of extrapolated and experimental retention factors for water as a mobile phase

Taking water as a reference mobile phase for comparison purposes the experimental $\log k_w$ values (Table 1) can be compared with the extrapolated $\log k_{we}$ values (Table 5) for the different mobile phase composition ranges used for extrapolation. A comparison is made in the form of linear regression models for 10–30% (v/v):

$$\log k_{we} = 1.041(\pm 0.008) \log k_w + 0.036(\pm 0.011)$$
$$r^2 = 0.998 \quad SE = 0.034 \quad F = 16464 \quad n = 34 \qquad (7)$$

for 20–50% (v/v):

$$\log k_{we} = 1.102(\pm 0.012) \log k_w + 0.073(\pm 0.015)$$
$$r^2 = 0.997 \quad SE = 0.049 \quad F = 8848 \quad n = 34 \qquad (8)$$

and for 30–50% (v/v) methanol–water:

$$\log k_{we} = 1.093(\pm 0.018) \log k_w + 0.149(\pm 0.024)$$
$$r^2 = 0.991 \quad SE = 0.075 \quad F = 3651 \quad n = 34 \qquad (9)$$

For all models, r^2 is the coefficient of determination, SE the standard error in the estimate, F the Fischer statistic and n the number of solutes. Compared with water as a mobile phase the extrapolated values for $\log k_{we}$ increase in magnitude for extrapolations initiated from higher volume fractions of methanol. The correlation equations, Eqs. (7) to (9), indicate that this increase results from a change in two factors. A fixed effect factor leading to a larger intercept and a change in a chemical factor reflected in the higher slopes. It is likely that changes in the intercept values largely result from changes in the phase ratio and changes in the slope from differences in solute interactions with the solvated stationary phase.

The chemical factors should be identifiable from changes in the system constants of the solvation parameter model (Table 6). With water as a mobile phase the main factors responsible for retention are

Table 4
Retention models for the fit of the retention factor (log k) on the IAM PC DD2 column with methanol–water mobile phases to Eqs. (5) and (6)

Solute	Non-linear model (Eq. (6))				Linear model (Eq. (5))		
	a_0	a_1	a_2	r^2	$\log k_{we}$	$-S$	r^2
Acetanilide	0.576	−1.334	−1.934	0.993	0.711	2.688	0.973
Acetophenone	0.779	−1.344	−2.210	0.996	0.926	2.815	0.974
2-Aminophenol	0.269	−1.568	−0.064	0.999	0.272	1.607	0.999
Anisole	0.919	−0.967	−1.863	0.998	1.041	2.271	0.973
Benzaldehyde	0.591	−0.995	−2.062	0.997	0.736	2.439	0.969
Benzamide	0.157	−0.283	−4.170	0.989	0.264	2.194	0.993
Benzene	0.682	−0.265	−2.374	0.999	0.942	1.927	0.942
Benzonitrile	0.676	−1.236	−1.717	0.999	0.762	2.266	0.982
Benzyl alcohol	0.247	−0.375	−2.674	0.995	0.434	2.246	0.942
Biphenyl	2.540	−1.855	−2.582	0.998	2.721	3.662	0.978
1-Bromo-naphthalene	2.938	−2.296	−2.375	0.998	3.104	3.959	0.984
3-Bromophenol	1.699	−1.452	−2.053	0.999	1.843	2.889	0.979
4-Chlorophenol	1.473	−1.257	−1.965	0.997	1.611	2.632	0.976
Cinnamyl alcohol	1.001	−1.188	−2.252	0.999	1.159	2.764	0.974
Coumarin	0.966	−2.014	−1.198	0.999	1.050	2.852	0.992
Cyclohexanone	0.067	−1.404	−0.900	0.999	0.097	1.854	0.994
Diethyl phthalate	1.569	−2.154	−2.213	0.990	1.697	3.778	0.994
2,6-Dimethylphenol	1.230	−1.406	−1.667	0.999	1.347	2.573	0.982
4-Hydroxybenzyl alcohol	0.112	−1.666	−0.652	0.998	0.133	1.992	0.995
2-Methylphenol	0.966	−1.127	−1.773	0.999	1.120	2.368	0.977
4-Methylphenol	0.966	−1.165	−1.790	0.999	1.122	2.418	0.977
Naphthalene	2.023	−1.644	−2.155	0.999	2.173	3.153	0.980
1-Naphthol	2.062	−1.941	−1.826	0.997	2.190	3.219	0.985
2-Naphthol	1.977	−2.073	−1.684	0.998	2.095	3.251	0.987
2-Nitroaniline	1.098	−1.359	−1.450	0.998	1.200	2.370	0.983
4-Nitroaniline	1.004	−1.341	−1.462	0.999	1.106	2.365	0.984
Nitrobenzene	0.891	−0.886	−1.948	0.999	1.027	2.250	0.970
4-Nitrobenzyl alcohol	0.689	−1.173	−1.624	0.998	0.803	2.310	0.979
1-Nitrobutane	0.400	−0.679	−1.875	0.998	0.493	1.804	0.966
4-Nitrotoluene	1.327	−1.410	−1.767	0.999	1.451	2.647	0.981
Phenol	0.591	−0.745	−1.955	0.999	0.728	2.114	0.966
2-Phenylethanol	0.498	−0.802	−2.223	0.997	0.654	2.360	0.963
4-Phenylphenol	2.585	−2.647	−1.719	0.999	2.705	3.850	0.991
Toluene	1.132	−0.648	−2.357	0.999	1.297	2.230	0.959

more favorable cavity formation and dispersion interactions, electron loan pair interactions and greater hydrogen-bond basicity of the solvated stationary phase with respect to water. An unusual feature is the positive sign of the system constant, indicating that IAM PC DD2 is more hydrogen-bond basic than water, while for conventional chemically bonded phases the opposite is generally true [16,18,19]. Factors that reduce retention are the hydrogen-bond basicity and dipolarity/polarizability of the solute, since water is a stronger hydrogen-bond acid and more dipolar/polarizable than the solvated IAM PC DD2 stationary phase. Compared with $\log k_w$ the extrapolated values of $\log k_{we}$ indicate a solvated stationary phase that is less cohesive, hydrogen-bond acidic and dipolar/polarizable. These changes increase for values of $\log k_{we}$ extrapolated from higher initial volume fractions of methanol in the mobile

Table 5
Linear retention models for the fit of Eq. (5) to different methanol–water composition ranges for the IAM PC DD2 column

Solute	Methanol–water composition								
	10–30% (v/v)			20–50% (v/v)			30–50% (v/v)		
	$\log k_{we}$	$-S$	r^2	$\log k_{we}$	$-S$	r^2	$\log k_{we}$	$-S$	r^2
Acetanilide	0.594	2.075	0.987	0.701	2.448	0.995	0.723	2.495	0.987
Acetophenone	0.822	2.190	0.986	0.940	2.620	1.000	0.942	2.625	0.999
2-Aminophenol	0.245	1.460	1.000	0.283	1.631	0.997	0.310	1.695	0.996
Anisole	0.949	1.670	0.968	1.102	2.220	0.992	1.137	2.300	0.984
Benzaldehyde	0.614	1.710	0.984	0.775	2.316	0.994	0.840	2.465	0.993
Benzamide	0.241	2.030	0.982	0.359	2.435	0.994	0.378	2.480	0.986
Benzene	0.720	1.070	0.974	0.925	1.862	0.982	1.039	2.125	0.990
Benzonitrile	0.683	1.710	0.999	0.848	2.377	0.992	0.960	2.635	1.000
Benzyl alcohol	0.299	1.420	0.983	0.457	2.010	0.985	0.539	2.200	0.981
Biphenyl	2.594	2.570	0.997	2.938	3.956	0.990	3.138	4.450	0.998
1-Bromo-naphthalene	3.080	3.480	0.967	3.302	4.236	0.995	3.263	4.150	0.986
3-Bromophenol	1.769	2.205	0.986	1.988	3.049	0.996	2.078	3.255	0.998
4-Chlorophenol	1.513	2.060	0.973	1.609	2.750	0.975	1.814	2.970	0.998
Cinnamyl alcohol	1.036	1.940	0.977	1.251	2.762	0.997	1.324	2.930	0.998
Coumarin	0.957	2.320	1.000	1.050	2.716	0.997	1.125	2.890	0.999
Cyclohexanone	0.062	1.595	1.000	0.173	2.047	0.995	0.252	2.230	1.000
Diethyl phthalate	1.593	3.080	0.989	1.786	3.814	0.999	1.828	3.910	1.000
2,6-Dimethylphenol	1.287	2.045	0.992	1.446	2.649	0.996	1.516	2.810	0.996
4-Hydroxybenzyl alcohol	0.082	1.675	0.998	0.188	2.133	0.992	0.287	2.360	0.999
2-Methylphenol	1.067	1.890	0.987	1.200	2.377	0.995	1.247	2.485	0.991
4-Methylphenol	1.052	1.815	0.991	1.230	2.503	0.995	1.317	2.705	0.997
Naphthalene	2.070	2.410	0.942	2.320	3.297	0.995	2.330	3.320	0.989
1-Naphthol	2.054	2.335	0.923	2.355	3.379	1.000	2.312	3.325	1.000
2-Naphthol	2.040	2.660	0.981	2.271	3.529	0.998	2.335	3.675	0.997
2-Nitroaniline	1.081	1.630	0.948	1.312	2.500	0.997	1.366	2.625	0.997
4-Nitroaniline	1.031	1.795	0.962	1.216	2.492	1.000	1.234	2.535	1.000
Nitrobenzene	0.915	1.505	0.967	1.110	2.255	0.997	1.167	2.385	0.999
4-Nitrobenzyl alcohol	0.667	1.485	1.000	0.848	2.246	0.985	0.988	2.570	0.998
1-Nitrobutane	0.415	1.255	0.982	0.590	1.928	0.990	0.677	2.130	0.994
4-Nitrotoluene	1.322	1.840	0.966	1.550	2.703	0.997	1.616	2.855	0.997
Phenol	0.626	1.455	0.967	0.788	2.055	0.994	0.836	2.165	0.991
2-Phenylethanol	0.532	1.605	0.998	0.674	2.167	0.993	0.764	2.375	0.998
4-Phenylethanol	2.648	3.265	0.981	2.885	4.154	1.000	2.914	4.220	0.999
Toulene	1.185	1.540	0.970	1.371	2.228	0.988	1.445	2.400	0.983

phase. Changes in the *e* and *a* system constants are small and not readily interpreted. The observed changes in the system constants are compatible with the sorption of methanol by the stationary phase. In that case, $\log k_{we}$ can be defined as characteristic of a hypothetical distribution system in which the mobile phase is water and the stationary phase is the solvated sorbent with a composition typical of the (average) equilibrium value for the mobile phase composition range used for the extrapolation [18,48,53]. The system is thermodynamically defined but considered hypothetical because the composition of the two phases cannot coexist in a real equilibrium. On the other hand, the volume of methanol associated with the stationary phase depends on the mobile phase composition range taken for the ex-

Table 6
System constants for $\log k_w$ and $\log k_{we}$ as the dependent variable in the solvation parameter model

System	System constants						Statistics[a]			
	v	*e*	*s*	*a*	*b*	*c*	ρ	SE	*F*	*n*
$\log k_w$	2.92	0.66	−0.24	0.23	−2.50	−1.28	0.995	0.080	525	34
	(0.11)	(0.07)	(0.06)	(0.06)	(0.10)	(0.08)				
$\log k_{we}$ 10–30% (v/v) methanol	3.01	0.69	−0.28	0.26	−2.65	−1.29	0.994	0.089	466	34
	(0.12)	(0.08)	(0.06)	(0.07)	(0.11)	(0.09)				
$\log k_{we}$ 20–50% (v/v) methanol	3.17	0.75	−0.32	0.24	−2.80	−1.23	0.996	0.077	701	34
	(0.10)	(0.07)	(0.06)	(0.06)	(0.10)	(0.08)				
$\log k_{we}$ 30–50% (v/v) methanol	3.21	0.67	−0.33	0.29	−2.87	−1.12	0.994	0.095	451	34
	(0.13)	(0.08)	(0.07)	(0.08)	(0.12)	(0.10)				
Literature values										
IAM PC DD2 with $\log k_{we}$ obtained by linear extrapolation from different acetonitrile–acetatae buffer (pH 7.4) compositions [22]										
	2.53	0.28	−0.08	0.30	−2.65	−0.37	0.964	0.27	101	44
	(0.17)	(0.13)	(0.12)	(0.13)	(0.15)	(0.28)				
IAM PC DD with $\log k_{we}$ obtained by linear extrapolation in acetonitrile–phosphate buffer (pH 7) for 20 to 80% (v/v) acetonitrile [21]										
	2.82	0.47	−0.18	−0.32	−2.63	−0.67	0.958	0.23	71	54
	(0.29)	(0.20)	(0.29)	(0.21)	(0.31)	(0.35)				

[a] ρ, overall multiple correlation coefficient; SE, standard error in the estimate; *F*, Fischer's statistic; *n*, number of solutes; and values in parentheses are the standard deviations in the system constants.

trapolation. It is quite likely that the solvent composition adsorbed by the stationary phase depends on its structure so that even if $\log k_{we}$ was defined with respect to a fixed mobile phase composition range it could not be considered a reliable property for the comparison of different stationary phases. This makes $\log k_{we}$ a poor choice for comparing column properties. While there is no theoretical objection to $\log k_w$ as a system property it is not easy to determine for all stationary phases due to excessive retention or poor peak shapes. For these reasons we prefer to use system maps for characterizing stationary phase properties in reversed-phase liquid chromatography [16,18]. There is only modest agreement with literature models for $\log k_{we}$ for the same or slightly different stationary phases using acetonitrile–aqueous buffer mobile phases for the extrapolation (Table 6). Based on the above discussion, this is not unexpected, but the descriptive statistics for these models suggest they are not as reliable as those presented in this work and a detailed comparison does not seem warranted.

3.4. Solvation parameter models (system maps) for isocratic mobile phases

The system constants for different methanol–water and acetonitrile–water mobile phase compositions are summarized in Table 7. A system map for methanol–water compositions is shown in Fig. 3. The cohesion of the mobile phase and its hydrogen-bond acidity with respect to the properties of the solvated stationary phase are reduced significantly by addition of methanol. The contribution from electron lone pair interactions changes little at first and more noticeably at higher methanol compositions. It is notable that the hydrogen-bond acidity and dipolarity/polarizability of the system is hardly influenced by the composition of the mobile phase, at least for the composition range studied. Solutes that differ in

Table 7
System constants for different methanol–water and acetonitrile–water mobile phase compositions with log k as the dependent variable

Organic solvent (%, v/v)	System constant						Statistics			
	v	e	s	a	b	c	ρ	SE	F	n
Methanol										
10	2.73	0.66	−0.28	0.23	−2.52	−1.24	0.995	0.073	592	34
	(0.10)	(0.06)	(0.05)	(0.06)	(0.09)	(0.07)				
20	2.57	0.65	−0.31	0.22	−2.50	−1.20	0.997	0.056	965	34
	(0.08)	(0.05)	(0.04)	(0.04)	(0.07)	(0.06)				
30	2.25	0.60	−0.29	0.23	−2.38	−1.14	0.995	0.066	615	34
	(0.09)	(0.05)	(0.05)	(0.05)	(0.08)	(0.07)				
40	2.00	0.53	−0.31	0.22	−2.28	−1.08	0.996	0.053	765	34
	(0.07)	(0.05)	(0.04)	(0.04)	(0.07)	(0.05)				
50	1.66	0.49	−0.28	0.20	−2.07	−1.12	0.993	0.067	372	34
	(0.09)	(0.06)	(0.05)	(0.05)	(0.08)	(0.07)				
60	1.32	0.45	−0.21	0.24	−1.86	−1.22	0.984	0.081	148	31
	(0.11)	(0.07)	(0.06)	(0.07)	(0.11)	(0.08)				
Acetonitrile										
20	2.17	0.47	−0.34	0.10	−2.26	−0.95	0.995	0.059	617	34
	(0.18)	(0.05)	(0.04)	(0.05)	(0.07)	(0.06)				
30	1.78	0.37	−0.32	0.11	−2.00	−0.91	0.994	0.056	497	34
	(0.08)	(0.05)	(0.04)	(0.04)	(0.07)	(0.06)				
Literature values										
IAM PC DD2 with 20% (v/v) acetonitrile in a pH 7.4 ammonium acetate buffer [22]										
	1.89	0.23	−0.20	0.22	−2.03	−0.83	0.971	0.17	125	44
	(0.11)	(0.08)	(0.12)	(0.09)	(0.17)	(0.23)				
IAM PC MG with 10% (v/v) acetonitrile in a pH 7.0 buffer [20]										
	1.87	0.81	−0.42	0.69	−2.00	−1.04	0.993	0.12	287	27

size and hydrogen-bond basicity will show the greatest change in retention with increasing methanol content of the mobile phase.

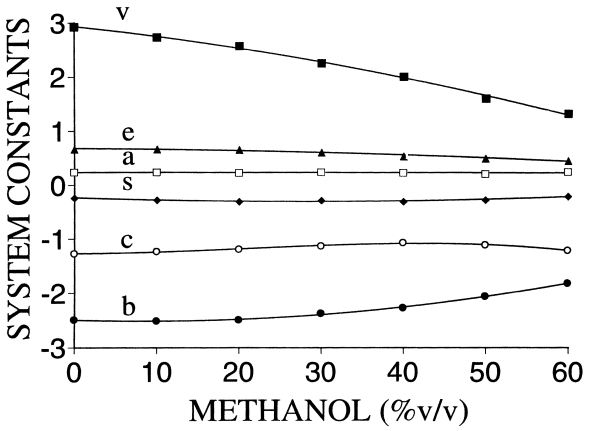

Fig. 3. System map for methanol–water mobile phase compositions.

System constants for 20 and 30% (v/v) acetonitrile–water mobile phases are also summarized in Table 7. These results can be compared with those for 20 and 30% (v/v) methanol–water mobile phases. Differences in the system constants result from changes in both the mobile and stationary phases. For the acetonitrile–water system the contribution of cavity formation and dispersion interactions, electron lone pair interactions and interactions as a hydrogen-bond base are less favorable, while hydrogen-bond acid interactions are more favorable for retention than for the methanol–water system. The contributions of dipole-type interactions to retention are about the same in both systems.

Qualitatively, there is reasonable agreement between the results presented in Table 7 and the system constants reported by Valko et al. [22] for an IAM PC DD2 column with a mobile phase containing 20% (v/v) acetonitrile in an ammonium acetate pH 7.4 buffer, also shown in Table 7. Differences in the

system constants are probably explained by differences in the ionic strength of the mobile phase and the inclusion of some partially ionized solutes in the model proposed in Ref. [22]. In terms of the descriptive statistics the model proposed by Valko et al. [22] is less reliable than the model we present. For the IAM PC MG column a direct comparison with our results is not possible because of the different mobile phase compositions used for the measurements. There is a general indication, however, that the IAM PC MG and IAM PC DD2 columns have a similar character but are not identical in their retention properties.

3.5. Solvation parameter models from gradient elution separations

Gradient elution is attractive for column characterization as it offers a method to reduce significantly the time needed to collect the experimental retention data required for the solvation parameter model [22–28]. The linear solvent strength model of gradient elution is based on the co-linear relationship between the change in solvent strength during the gradient separation and the approximate effect of the strong solvent on retention in isocratic reversed-phase liquid chromatography. The theory was briefly explained in the Experimental section. Here we evaluate whether the general theory of linear solvent strength gradients provides a realistic estimate of $\log k$ and $\log k_{we}$ values for a varied group of compounds using retention times determined in two different gradients. The data set used to evaluate column characteristics under isocratic separation conditions was not wholly appropriate for gradient elution measurements due to the weak retention of a number of solutes. Solutes with weak retention were removed from the data set and others added (Table 2) to better occupy the gradient retention factor range available. The retention data and the parameters for the linear solvent strength model estimated from the data are summarized in Table 3. Regression analysis of the estimated isocratic $\log k_{we}$ values from the gradient model against the experimental $\log k_w$ values indicated good agreement:

$$\log k_{we} = 1.054(\pm 0.031) \log k - 0.173(\pm 0.052)$$
$$r^2 = 0.984 \quad SE = 0.090 \quad F = 1099 \quad n = 21 \qquad (10)$$

The slope is close to unity, indicating that the chemical sense of the two variables is the same. The intercept is significantly larger than zero, but this is not unusual for extrapolated values for $\log k_{we}$ and agrees with the results indicated for the comparison of isocratic $\log k_{we}$ values with experimental $\log k_w$ values presented earlier. The linear solvent strength gradient model was also used to estimate isocratic retention factor values ($\log k_{est}$) for a 20% (v/v) acetonitrile–water mobile phase, which can be compared with the experimental isocratic retention factor values for the same mobile phase composition, resulting in the following regression model:

$$\log k_{est} = 0.997(\pm 0.026) \log k + 0.145(\pm 0.031)$$
$$r^2 = 0.981 \quad SE = 0.086 \quad F = 1457 \quad n = 30 \qquad (11)$$

The slope is essentially unity but the model contains a significant intercept. The plot is shown in Fig. 4 and indicates good general agreement but with obvious scatter about the best fit line through the data. The estimated isocratic retention factor values were taken as the dependent variable in the solvation parameter model resulting in:

$$\log k_{est} = 2.32V + 0.44E - 0.39S + 0.31A - 2.33B - 0.89$$
$$(0.11) \quad (0.08) \quad (0.07) \quad (0.08) \quad (0.21) \quad (0.08)$$
$$\rho = 0.986 \quad SE = 0.114 \quad F = 166 \quad n = 30 \qquad (12)$$

For comparison purposes a similar model was con-

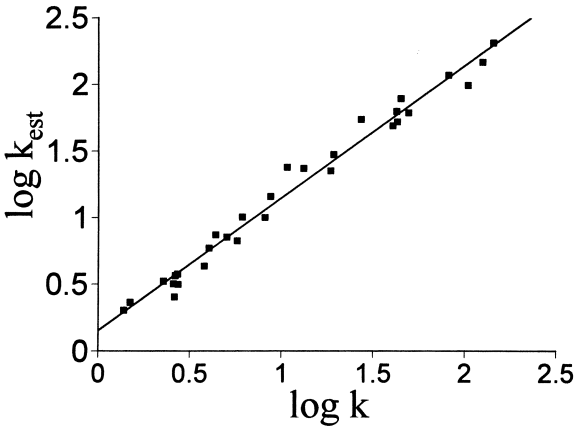

Fig. 4. Plot of the estimated retention factors ($\log k_{est}$) by gradient elution against the experimental isocratic retention factors ($\log k$) for 20% (v/v) acetonitrile–water as the mobile phase.

structed for the same compounds determined by isocratic measurements:

$$\log k = 2.38V + 0.33E - 0.35S + 0.18A - 2.47B - 0.96$$
$$(0.08)\quad (0.06)\quad (0.05)\quad (0.06)\quad (0.15)\quad (0.06)$$
$$\rho = 0.992\quad \mathrm{SE} = 0.083\quad F = 312\quad n = 30 \qquad (13)$$

Model (13) is statistically more reliable than model (12) but the differences are not large. We can see this more clearly if we use both models to estimate the experimental retention factor values and then form a regression model (Fig. 5 and Eq. (14)):

$$\log k_{\mathrm{est(SPM)}} = 1.012(\pm 0.020) \log k_{\mathrm{(SPM)}} + 0.137(\pm 0.024)$$
$$r^2 = 0.989\quad \mathrm{SE} = 0.065\quad F = 2498\quad n = 30 \qquad (14)$$

The subscript SPM is used to indicate that the retention factors have been estimated from the solvation parameter models indicated as Eqs. (12) and (13). Both models essentially predict the same results as the experimental data with the intercept reflecting a systematic difference between the isocratic and gradient predicted retention factors.

3.6. Surrogate chromatographic models for biopartitioning

The main use of IAM columns is to estimate biopartitioning properties of general interest for assessing the properties of candidate compounds at an early stage of the drug development process. A relationship between two distribution systems, the biological and chromatographic system, for example, is usually established through a correlation model for a limited number of model compounds. Such models are generally limited by the structural diversity represented by the model compounds. The solvation parameter model provides a more comprehensive approach. In this case, two distribution systems are expected to correlate when the ratios of their system constants are (nearly) identical [16,18,31]. Division by the v system constant is commonly used to normalize the data. The system constant ratios for the IAM PC DD2 column for different isocratic mobile phase compositions together with selected chromatographic and biopartitioning systems for comparison are summarized in Table 8.

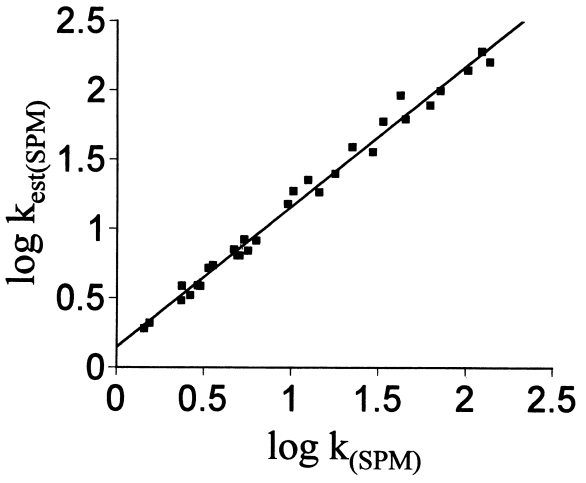

Fig. 5. Plot of the estimated retention factors ($\log k_{\mathrm{eff}}$) calculated by the solvation parameter model (Eq. (12)) for gradient elution against the retention factors calculated by the solvation parameter model (Eq. (13)) for 20% (v/v) acetonitrile–water.

Probably the most widely used solute descriptor in quantitative structure–activity relationships (QSAR) is the octanol–water distribution constant ($\log P$). Confusion exists as to whether retention on IAM columns mimics or represents a different distribution property to $\log P$ [1–3,7,8,12,20]. From the system constant ratios in Table 8, wet octanol is generally less dipolar/polarizable and has a lower capacity for electron lone pair interactions than the solvated IAM PC DD2 sorbent. The hydrogen-bonding terms are reasonably well matched. Thus it is quite likely that reasonable correlation models for compounds of limited descriptor diversity can be found. The best models are expected for compounds belonging to a homologous series or of weak-to-moderate dipolarity/polarizability. Certainly, better chromatographic models for $\log P$ than the IAM PC DD2 column are available [18,33,54]. The dimyristoylphosphatidylcholine–water (DMPC–water) distribution system was suggested as a better model than $\log P$ for membrane absorption [55]. Compared to the IAM PC DD2–water system, wet DMPC is slightly more dipolar/polarizable and significantly more hydrogen-bond acidic and less hydrogen-bond basic. The two systems are not close in their distribution properties in spite of superficial similarities in chemical structure. These differences are probably explained by structural differences imposed on the immobilized phosphatidyl groups compared to the liquid state, the

Table 8
System constant ratios for the IAM PC DD2 column and selected chromatographic and biopartitioning systems for comparison

System	System constant ratios				Ref.
	e/v	s/v	a/v	b/v	
IAM PC DD2					
Water	0.226	−0.082	0.079	−0.856	
10% (v/v) Methanol	0.242	−0.103	0.084	−0.923	
20% (v/v) Methanol	0.253	−0.121	0.086	−0.973	
30% (v/v) Methanol	0.267	−0.129	0.102	−1.058	
40% (v/v) Methanol	0.265	−0.155	0.110	−1.140	
50% (v/v) Methanol	0.295	−0.169	0.120	−1.247	
60% (v/v) Methanol	0.341	−0.159	0.182	−1.407	
20% (v/v) Acetonitrile	0.217	−0.156	0.046	−1.041	
30% (v/v) Acetonitrile	0.208	−0.180	0.062	−1.124	
Water–organic solvent liquid–liquid distribution					
Octanol	0.146	−0.273	0.008	−0.901	[32]
Dimyristoylphosphatidylcholine	0.207	−0.155	0.249	−1.198	[42]
Reversed-phase liquid chromatography					
Poly(styrene)-coated zirconia					
30% (v/v) Acetonitrile	0.20	−0.12	−0.12	−1.15	[16]
Poly(butadiene)-coated zirconia					
30% (v/v) Acetonitrile	0.07	−0.20	−0.13	−1.16	[16]
Zorbax SB 300 CN					
30% (v/v) Acetonitrile	0.18	−0.23	−0.21	−1.12	[16]
Dipalmitoylphosphatidylcholine-coated silica					
20% (v/v) Acetonitrile	0.255	−0.224	−0.003	−1.153	[20]
30% (v/v) Acetonitrile	0.189	−0.307	−0.029	−0.995	[20]
Micellar electrokinetic chromatography					
Sodium *N*-dodeconyl-*N*-methyltaurine	0.18	−0.12	0.14	−0.82	[56]
Sodium taurodeoxycholine	0.26	−0.17	0	−0.83	[56]
Sodium lauryl sulfoacetate	0.16	−0.13	0.04	−0.82	[16]
Sodium *N*-dodeconyl-methyltaurine–Brij 35 (5:2) with 10% (v/v) acetonitrile	0.246	−0.157	0.104	−1.007	[57]
Sodium *N*-dodeconyl-methyltaurine–Brij 35 (5:2) with 20% (v/v) methanol	0.208	−0.125	0.110	−0.956	[57]
Biopartitioning					
Intestinal absorption	0.28	0.39	−2.05	−1.99	[40]
Blood–brain distribution	0.354	0	−0.148	−1.086	[34]
Skin–water distribution	0	−0.198	0.177	−0.898	[38]
Non-specific toxicity					
Fathead minnow	0.071	0	0.118	−1.077	[42]
Guppy	0.180	0	0.108	−0.946	[42]
Tadpole	0.243	−0.219	0	−0.746	[43]
Vibrio fischeri (Microtox test)	0.900	0	0	−0.480	[41]
Tetrahymena pyriformis (Tetratox test)	0.222	0	0	−0.872	[41]

presence of amide groups in the bonded phase and differences in the concentration of water attracted into the interphase region for the IAM PC DD2 sorbent compared with the equilibrium solubility of water in DMPC.

Although there is no comprehensive database of system constant ratios for chemically bonded phases operated under reversed-phase conditions, some large compilations are available [16,18,19]. A comparison with the system constant ratios for the IAM PC DD2 column indicates that it would be difficult to duplicate the retention properties of the IAM PC DD2 column with a conventional chemically bonded phase. The most significant difference is that the a system constant is generally ≤ 0 for chemically bonded phases and is ≥ 0 for the IAM PC DD2 systems. System constant ratios are available for the widest range of stationary phase types with mobile phase compositions of 50% (v/v) methanol–water and 30% (v/v) acetonitrile–water [16]. For conventional chemically bonded phases and 50% (v/v) methanol–water as the mobile phase: the e/v ratio is generally smaller or of opposite sign (except for porous graphitic carbon and cyanopropylsiloxane-bonded and propanediolsiloxane-bonded silica sorbents); the s/v ratio is similar to the alkylsiloxane-bonded silica sorbents but smaller than for porous graphitic carbon, cyanopropylsiloxane-bonded and propanediolsiloxane-bonded silica sorbents; the a/v ratio is ≤ 0; and the b/v ratio is significantly larger and negative compared to the IAM PC DD2 systems. For 30% (v/v) acetonitrile there is reasonable agreement for a number of phases for the e/v and s/v ratios but generally poor agreement for the a/v and b/v ratios. Except for hydrogen-bond acidic solutes there is reasonable agreement for the other system constant ratios for poly(styrene)-coated and poly(butadiene)-coated zirconia and a cyanopropylsiloxane-bonded silica sorbent (Zorbax SB 300 CN) (Table 8). There is also fairly good agreement for the system constant ratios for a dipalmitoylphosphatidylcholine-coated silica column and the IAM PC DD2 column with 20 or 30% (v/v) acetonitrile–water as the mobile phase (Table 8). Similar conclusions were reached by Hanna et al. [12] for a comparison of retention factors on IAM PC MG and IAM PC DD columns with phospholipid-coated octanylsiloxane-bonded silica columns.

The retention properties of the IAM PC DD2 column are strongly correlated with typical surfactant pseudostationary phases used in micellar electrokinetic chromatography (MEKC) [16,18,56–58]. Only a few examples are entered into Table 8, but in general it would be a simple task to provide adequate MEKC models for the chromatographic systems indicated for the IAM PC DD2 column. MEKC, therefore, provides a suitable alternative to reversed-phase liquid chromatography on a IAM PC DD2 column for the estimation of biopartitioning data for those laboratories that prefer this approach.

A number of biopartitioning processes have been modeled using the solvation parameter model and their system constant ratios are summarized in Table 8 [33–43]. For intestinal absorption there are significant differences in the system constant ratios, suggesting that retention on IAM PC DD2 columns is not a good model for this system. Modest correlation models should be possible for blood–brain and skin–water distributions. For blood–brain distribution there are significant differences (Δ) in the s/v ($\Delta = 0.13$) and a/v ($\Delta = 0.25$) system constant ratios for the IAM PC DD2 column with 30% (v/v) methanol–water as the mobile phase, but for moderately dipolar and weak hydrogen-bond acid solutes, reasonable agreement could be expected. For skin–water distribution there is a significant difference in the e/v ratio ($\Delta = 0.24$) but smaller differences for the other system constant ratios ($\Delta \leq 0.09$) for the IAM PC DD2 column with 10% (v/v) methanol–water as the mobile phase. Since we do not have retention factor values for the IAM PC DD2 column for the same compounds used to determine the skin–water distribution constant ($\log K_M$), a correlation model was generated by using the IAM PC DD2 solvation parameter model to estimate the retention factors ($\log k_{est(SPM)}$) for the compounds with experimental skin–water distribution constants. This provided the model:

$$\log K_M = 0.51(\pm 0.03) \log k_{est(SPM)} + 0.49(\pm 0.03)$$

$$r^2 = 0.893 \quad SE = 0.123 \quad F = 310 \quad n = 39 \qquad (14)$$

which demonstrates reasonable predictive properties for a biological process as indicated by the standard

error of the estimate. There is little in common between the system constant ratios for non-specific toxicity measured by the Microtox test or the Tetratox test (Table 8) and the IAM PC DD2 column is an unlikely model system for these tests. There is modest agreement with the system constant ratios for non-specific aquatic toxicity to fish. For the guppy the largest difference in the system constant ratios for the 10% (v/v) methanol–water mobile phase is for s/v ($\Delta = 0.1$) with $\Delta \leq 0.06$ for the other system constant ratios. The agreement in system constant ratios is not quite as good for the fathead minnow and the 30% (v/v) methanol–water mobile phase, but it should still be possible to develop a reasonable correlation model.

4. Conclusions

The above discussion confirms two important features of IAM column packings. Retention data are not easily duplicated on more commonly used chemically bonded phases, providing a rationale for their existence. Secondly, there is an element of similarity in the retention properties of the IAM column and membrane absorption properties, suggesting that these columns should be suitable for estimating some biopartitioning properties. It is also indicated that reversed-phase retention on the IAM column has more in common with retention in MEKC than reversed-phase liquid chromatography on conventional stationary phases. Retention on the IAM PC DD2 column as a function of mobile phase composition is better represented by a second-order model with the volume fraction of organic solvent as the independent variable. Linear extrapolation methods result in intercept values ($\log k_{we}$) that are dependent on the mobile phase composition range used for the extrapolation and represent a hypothetical distribution system that is not identical to experimental systems with water as the mobile phase. The linear solvent strength model of gradient elution is quite successful at estimating isocratic retention factors that provide similar information to experimental isocratic retention factors in the solvation parameter model. With further work this may provide a rapid approach to the construction of system maps for column characterization and method development.

References

[1] S. Ong, H.L. Liu, C. Pidgeon, J. Chromatogr. A 728 (1996) 113.
[2] S.W. Ong, H.L. Liu, X.X. Qiu, G. Bhat, C. Pidgeon, Anal. Chem. 67 (1995) 755.
[3] C. Pidgeon, S.W. Ong, H.L. Liu, X.X. Qiu, M. Pidgeon, A.H. Dantzig, J. Munroe, W.J. Hornbeck, J.S. Kasher, L. Gluntz, T. Sszczerba, J. Med. Chem. 38 (1995) 590.
[4] A. Nasal, M. Sznitowska, A. Bucinski, R. Kaliszan, J. Chromatogr. A 692 (1995) 83.
[5] A. Reichel, D.J. Begley, Pharm. Res. 15 (1998) 1270.
[6] T. Salminen, A. Pulli, J. Taskinen, J. Pharm. Biomed. Anal. 15 (1997) 469;
W.J. Hornback, J.S. Kasher, L. Glunz, T. Szczerba, J. Med. Chem. 38 (1995) 590.
[7] C.Y. Yang, S.J. Cai, H. Liu, C. Pidgeon, Adv. Drug Deliv. Rev. 23 (1996) 229.
[8] B.H. Stewart, O.H. Chan, J. Pharm. Sci. 87 (1998) 1471.
[9] R. Kaliszan, Trends Anal. Chem. 18 (1999) 400.
[10] T.-H. Lee, M.-I. Aguilar, Adv. Chromatogr. 41 (2001) 175.
[11] K. Miyake, F. Kitaura, N. Mizuno, H. Terada, J. Chromatogr. 389 (1987) 47.
[12] M. Hanna, V. de Biasi, B. Bond, P. Camilleri, A.J. Hutt, Chromatographia 52 (2000) 710.
[13] M.H. Abraham, Chem. Soc. Rev. 22 (1993) 73.
[14] M.H. Abraham, J.A. Platts, J. Org. Chem. 66 (2001) 3484.
[15] M.H. Abraham, C.F. Poole, S.K. Poole, J. Chromatogr. A 842 (1999) 79.
[16] C.F. Poole, S.K. Poole, J. Chromatogr. A (in press).
[17] J.A. Platts, D. Butina, M.H. Abraham, A. Hersey, J. Chem. Inf. Comput. Sci. 39 (1999) 835.
[18] C.F. Poole, S.K. Poole, A.D. Gunatilleka, Adv. Chromatogr. 40 (2000) 159.
[19] M.H. Abraham, M. Roses, C.F. Poole, S.K. Poole, J. Phys. Org. Chem. 10 (1997) 358.
[20] M.H. Abraham, H.S. Chadha, R.A.E. Leitao, R.C. Mitchell, W.J. Lambert, R. Kaliszan, A. Nasal, P. Haber, J. Chromatogr. A 766 (1997) 35.
[21] M.A. Al-Haj, R. Kaliszan, A. Nasal, Anal. Chem. 71 (1999) 2976.
[22] K. Valko, C.M. Du, C.D. Bevan, D.P. Reynolds, M.H. Abraham, J. Pharm. Sci. 89 (2000) 1085.
[23] C.M. Du, K. Valko, C. Bevan, D. Reynolds, M.H. Abraham, Anal. Chem. 70 (1998) 4228.
[24] K. Valko, C. Bevan, D. Reynolds, Anal. Chem. 69 (1997) 2022.
[25] K. Valko, M. Plass, C. Bevan, D. Reynolds, M.H. Abraham, J. Chromatogr. A 797 (1998) 41.
[26] M. Plass, K. Valko, M.H. Abraham, J. Chromatogr. A 803 (1998) 51.

[27] J. Li, B. Cai, J. Chromatogr. A 905 (2001) 35.
[28] A. Wang, L.C. Tan, P.W. Carr, J. Chromatogr. A 848 (1999) 21.
[29] L.R. Snyder, J.W. Dolan, Adv. Chromatogr. 38 (1998) 115.
[30] J.D. Krass, B. Jastorff, H.G. Genieser, Anal. Chem. 69 (1997) 2575.
[31] M.H. Abraham, J.M.R. Gola, R. Kumarsingh, J.E. Cometto-Muniz, W.S. Cain, J. Chromatogr. B 745 (2000) 103.
[32] M.H. Abraham, H.S. Chadha, in: V. Pliska, B. Testa, H. van de Waterbeemd (Eds.), Lipophilicity in Drug Action and Toxicology, VCH, Weinheim, 1996, p. 311.
[33] J.A. Platts, M.H. Abraham, A. Hersey, D. Buttina, J. Chem. Inf. Comput. Sci. 40 (2000) 71.
[34] M.H. Abraham, K. Takavs-Novak, R.C. Mitchell, J. Pharm. Sci. 86 (1997) 310.
[35] J.A. Gratton, M.H. Abraham, M.W. Bradbury, H.S. Chadha, J. Pharm. Pharmacol. 49 (1997) 1211.
[36] M.H. Abraham, H.S. Chadha, F. Martins, R.C. Mitchell, M.W. Bradbury, J.A. Gratton, Pestic. Sci. 55 (1999) 78.
[37] M.H. Abraham, H.S. Chadha, R.C. Mitchell, J. Pharm. Pharmacol. 47 (1995) 8.
[38] M.H. Abraham, F. Martina, R.C. Mitchell, J. Pharm. Pharmacol. 49 (1997) 858.
[39] J.A. Platts, M.H. Abraham, A. Hersey, D. Butina, Pharm. Res. 17 (2000) 1013.
[40] Y.H. Zhao, J. Le, M.H. Abraham, A. Hersey, P.J. Eddershaw, C.N. Luscombe, D. Boutina, G. Beck, B. Sherborne, I. Cooper, J.A. Platts, J. Pharm. Sci. 90 (2001) 749.
[41] A.D. Gunatilleka, C.F. Poole, Analyst 125 (2000) 127.
[42] A.D. Gunatilleka, C.F. Poole, Anal. Commun. 36 (1999) 235.
[43] M.H. Abraham, C. Rafols, J. Chem. Soc., Perkin Trans. 2 (1995) 1843.
[44] T. Baczek, M. Markuszewski, R. Kaliszan, M.A. van Straten, H.A. Claessens, J. High Resolut. Chromatogr. 23 (2000) 667.
[45] R. Kaliszan, M.A. van Straten, M. Markuszewski, C.A. Cramers, H.A. Claessens, J. Chromatogr. A 855 (1999) 455.
[46] B. Buszewski, R.M. Gadzata-Kopciuch, M. Markuszewski, R. Kaliszan, Anal. Chem. 69 (1997) 3277.
[47] M.A. Al-Haj, R. Kaliszan, B. Buszewski, J. Chromatogr. Sci. 39 (2001) 29.
[48] D. Bolliet, C.F. Poole, Chromatographia 46 (1997) 381.
[49] G.W. Caldwell, J.A. Masucci, M. Evangelisto, R. White, J. Chromatogr. A 800 (1998) 161.
[50] S. Demare, D. Roy, J.Y. Legendre, J. Liq. Chromatogr. Relat. Technol. 22 (1999) 2675.
[51] D. Bolliet, C.F. Poole, M. Roses, Anal. Chim. Acta 368 (1998) 129.
[52] M. Roses, D. Bolliet, C.F. Poole, J. Chromatogr. A 829 (1998) 29.
[53] M.-M. Hsieh, J.G. Dorsey, J. Chromatogr. 631 (1993) 31.
[54] S.K. Poole, D. Durham, C. Kibbey, J. Chromatogr. B 745 (2000) 117.
[55] W.H.J. Vaes, E.U. Ramos, H.J.M. Verhaar, J.L.M. Hermens, Environ. Toxicol. Chem. 17 (1998) 1380.
[56] S.K. Poole, C.F. Poole, Analyst 122 (1997) 267.
[57] S.K. Poole, C.F. Poole, Anal. Commun. 34 (1997) 57.
[58] C.F. Poole, S.K. Poole, M.H. Abraham, J. Chromatogr. A 798 (1998) 207.