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Structure-driven retention model for solvent selection and optimization in reversed-phase thin-layer chromatography

Waruna Kiridena, Colin F. Poole*

Department of Chemistry, Wayne State University, Detroit, MI 48202, USA

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Abstract

A new approach to method development in reversed-phase thin-layer chromatography is proposed based on the use of calculations employing the solvation parameter model. System constants are provided for aqueous binary mobile phase mixtures containing the organic solvents methanol, 2-propanol, 2,2,2-trifluoroethanol, acetone, *N*,*N*-dimethylformamide and acetonitrile on a cyanopropylsiloxane-bonded layer. Good agreement between experimental and predicted R_F values (± 0.03 R_F units) for steroids, phenols and naphthalene derivatives is demonstrated for mobile phase optimization. © 1998 Elsevier Science B.V.

Keywords: Retention models; Optimization; Mobile phase composition; Solvation parameter model; Steroids; Phenols; Naphthalene derivatives

1. Introduction

Modern thin-layer chromatography (TLC) is a highly instrumentalized and partially automated technique using layers with a fine particle size and narrow size distribution to maximize separation performance [1–4]. The slow step in achieving an acceptable separation, however, remains the method development process. Window diagram, simplex optimization, mixture design statistical techniques, and the PRISMA method are the approaches most commonly employed for guided methods development, but these have not succeeded in replacing entirely the time consuming trial-and-error approach [4–13]. Their benefit is the provision of an experimental framework to replace intuition, providing a more reliable possibility of achieving a desired separation, but they retain the vice of being relatively slow. We have sought a different approach to methods development in liquid chromatography employing the solvation parameter model to create retention maps which predict the separation of solutes in previously characterized systems using trivial arithmetic procedures performed by computer for convenience [14–18]. Since the model employed is based on sound thermodynamic principles we anticipate that it will perform better than other structure-driven approaches used for computer-assisted methods development in column liquid chromatography that rely on the estimation of general parameters, such as hydrophobicity, pK_a, etc., and the use of arbitrary rules, to correlate these properties with retention [19].

The solvation parameter model in a form suitable for methods development in reversed-phase liquid chromatography is set out below:

*Corresponding author.

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$$SP = c + mV_X + rR_2 + s\pi_2^H + a\sum\alpha_2^H + b\sum\beta_2^0 \quad (1)$$

where *SP* is the experimentally observed retention property (the R_M value in this case). The solute descriptors are McGowan's characteristic volume V_X (in $cm^3\ mol^{-1}/100$), excess molar refraction R_2 (in $cm^3/10$), π_2^H the ability of the solute to stabilize a neighboring dipole by virtue of its capacity for orientation and induction interactions, and $\Sigma\alpha_2^H$ and $\Sigma\beta_2^0$ and the solute's effective hydrogen-bond acidity and hydrogen-bond basicity, respectively. Solute descriptors are available for more than 2000 compounds with others available through parameter estimates and by computational approaches [20–25]. In other cases the solute descriptors are obtained by calculation (V_X and R_2) or measured experimentally in chromatographic or liquid–liquid distribution systems using standard methods [21,25].

The system constants in Eq. (1) are defined by their complementary interactions with the solute descriptors. The *r* constant determines the difference in capacity of the solvated sorbent layer and mobile phase to interact with solute *n*- or π-electrons; the *s* constant to the difference in capacity of the solvated sorbent layer and mobile phase to take part in dipole–dipole and dipole-induced dipole interactions; the *a* constant is a measure of the difference in hydrogen-bond basicity of the solvated sorbent layer and the mobile phase; the *b* constant is a measure of the difference in hydrogen-bond acidity of the solvated sorbent layer and mobile phase; and the *m* constant is a measure of the relative ease of forming a cavity for the solute in the solvated sorbent layer and mobile phase. For any TLC system, the system constants can be obtained using multiple linear regression analysis of experimental R_M values acquired for a group of varied solutes with known descriptors.

2. Experimental

2.1. Materials

All solvents and water were OmniSolv grade from EM Science (Gibbstown, NJ, USA). The 10×10 cm cyanopropylsiloxane-bonded, silica-based, high-performance TLC plates HPTLC CN F254s (catalog nos. 16464-5) were obtained from EM Separations Technology (Gibbstown, NJ, USA). Prior to use the plates were soaked in methanol for 10 min, dried under a flow of nitrogen, and then stored overnight in a vacuum desiccator [26]. Solutes used for sorbent characterization and confirmation of the retention models were obtained from Aldrich Chemical Co. (Milwaukee, WI, USA) and Sigma Chemical Co. (St. Louis, MO, USA). Triethylammonium phosphate buffer (pH 2.5) was obtained from Regis Chemical Co. (Morton Grove, IL, USA).

2.2. Apparatus and conditions

All separations were carried out in twin-trough developing chambers for 10×10 cm HPTLC plates (Camag, Wilmington, NC, USA) by adding 2.5 ml of solvent to each trough. The mobile phase compositions were prepared on a volume to volume basis using a burette. The natural pH of the water was 5.5. The chamber was allowed to equilibrate for 5 min at ambient temperature (23°C) prior to inserting the plate. The solvent front migration distance was 5 cm. Samples were applied to the layer using a 200 nl Pt-Ir dosimeter held in a glass capillary sleeve using a Nanomat II sample applicator (Camag). The TLC plates were spotted alternately with sample and a solution of potassium iodide used as a marker for the thermodynamic solvent front [27]. Chromatograms were recorded with a Shimadzu CS-910 slit scanning densitometer and U-135 strip chart recorder (Columbia, MA, USA): slit width = 0.3 mm; slit height = 3.0 mm; wavelength = 270 nm; and scan speed = 40 mm/min. The R_F values were determined manually from the chromatograms.

2.3. Calculations

The R_F values were calculated from Eq. (2)

$$R_F = (MD)_S/(MD)_{KI} \quad (2)$$

where $(MD)_S$ is the migration distance from the sample application position to the solute peak maximum recorded on the chromatogram and $(MD)_{KI}$ is the migration distance from the sample application position to the peak maximum of the potassium iodide solvent front marker recorded on the chro-

Table 1
Solute descriptors used in the solvation parameter model

Compound	Solute descriptors				
	V_X	R_2	π_2^H	α_2^H	β_2^0
Solute descriptors used to construct models					
2,6-Dimethylphenol	1.057	0.860	0.79	0.39	0.39
3,5-Dimethylphenol	1.057	0.820	0.84	0.57	0.36
2-Methylphenol	0.916	0.840	0.86	0.52	0.30
3-Methylphenol	0.916	0.822	0.88	0.57	0.34
Phenol	0.775	0.805	0.89	0.60	0.31
3-Cyanophenol	0.929	0.930	1.55	0.77	0.28
2-Chlorophenol	0.898	0.853	0.88	0.32	0.31
4-Chlorophenol	0.897	0.915	1.08	0.67	0.20
3-Bromophenol	0.950	1.080	1.17	0.67	0.20
2-Nitrophenol	0.949	1.015	1.05	0.05	0.37
1,3-Benzenediol	0.834	0.980	1.00	1.10	0.58
4-Chloro-3-methylphenol	1.038	0.920	1.02	0.65	0.23
4-Phenylphenol	1.383	1.560	1.41	0.59	0.45
Eugenol	1.354	0.946	0.99	0.22	0.51
Vanallin	1.131	1.040	1.04	0.33	0.67
1-Naphthol	1.144	1.520	1.05	0.61	0.37
Benzyl alcohol	0.916	0.803	0.87	0.33	0.56
4-Nitrobenzyl alcohol	1.090	1.064	1.39	0.44	0.62
Acetanilide	1.113	0.870	1.40	0.50	0.67
Benzamide	0.972	0.990	1.50	0.49	0.67
2-Nitroaniline	0.991	1.180	1.37	0.30	0.36
4-Nitroaniline	0.991	1.220	1.91	0.42	0.38
Benzenesulfonamide	1.097	1.130	1.55	0.55	0.80
1,2-Dibromobenzene	1.086	1.190	0.96	0	0.05
3-Nitrotoluene	1.031	0.874	1.10	0	0.28
Azobenzene	1.480	0.680	1.20	0	0.44
Piperanal	1.022	0.990	1.60	0	0.52
Caffeine	1.363	1.400	1.55	0	1.34
Coumarin	1.062	1.060	1.79	0	0.46
1-Methylnaphthalene	1.226	1.344	0.90	0	0.20
1-Nitronaphthalene	1.260	1.270	1.50	0	0.30
Acenaphthalene	1.258	1.604	1.04	0	0.20
Biphenyl	1.324	1.360	0.99	0	0.26
Solute descriptors for confirmation of model accuracy					
Estrone	2.156	1.730	3.10	0.56	0.91
Estradiol	2.199	1.800	3.30	0.88	0.95
Estriol	2.258	2.000	3.36	1.40	1.22
Progesterone	2.622	1.450	3.29	0	1.14
Testosterone	2.383	1.540	2.59	0.32	1.19
Hydrocortisone	2.798	2.030	3.49	0.70	1.87
Pentachlorophenol	1.389	1.217	0.88	0.97	0
2,4,6-Trichlorophenol	1.192	1.010	1.01	0.82	0.08
4-Nitrophenol	0.949	1.070	1.72	0.82	0.26
Catechol	0.834	0.970	1.07	0.85	0.52
1-Chloronaphthalene	1.208	1.417	1.05	0	0.13
1-Bromonaphthalene	1.260	1.598	1.13	0	0.13
1-Methylnaphthalene	1.226	1.344	0.90	0	0.20
1-Ethoxynaphthalene	1.426	1.411	1.11	0	0.38

matogram. The R_M value (equivalent to log retention factor in column chromatography) was calculated by Eq. (3)

$$R_M = \log[(1 - R_F)/R_F] \quad (3)$$

The solute descriptors used in the solvation parameter model are summarized in Table 1 [14–17,19–22]. The criteria used for selection of individual solutes at a particular mobile phase composition were as follows: (1) the solute migrated in the mobile phase from the sample application position to a position below the solvent front $0.05 < R_F < 0.95$ with most values $0.1 < R_F < 0.9$; (2) all data sets contained a range of R_M values without clusters; (3) the absence of significant cross-correlation between the solute descriptors in a data set was demonstrated; (4) the selected solutes provided a reasonable range of individual descriptor values for each term in the model (no clustering of values for individual descriptors); and (5) the total number of solutes in a data set was sufficient to exhaustively fit the model in the statistical sense. The system constants were then obtained from the individual data sets by multiple linear regression analysis using the program SPSS/PC+ V5.0 (SPSS, Chicago, IL, USA) on an Epson Apex personal computer (Epson, Torrence, CA, USA). Retention maps were constructed using a spreadsheet program Excel V4.0 (Microsoft, Redmond, WA, USA) or Cricket Graph III (Computer Associates International, Islandia, NY, USA) on a Power Macintosh 7200 personal computer (Apple Computer, Cupertino, CA, USA).

3. Results and discussion

3.1. Solvent selection

The solvent plays an important role in chromatography transcending its primary function as the transport medium. Solute–solvent interactions establish the preference for residence in the mobile phase in competition with solute–stationary phase interactions. For a given stationary phase the chromatographic retention and selectivity is adjusted by varying the mobile phase (solvent) composition and type. Since many solvents have similar properties it

is desirable to identify a reduced number of solvents that singularly (or more usually) when blended, can represent the global properties of all solvents. For reversed-phase liquid chromatography there is the additional requirement that the solvent be completely miscible with water. A list of candidate solvents along with their solvatochromic parameters is given in Table 2 [27–30]. The solvents capacity for dipole-type interactions is given by π_1^*, their hydrogen-bond donor acidity by α_1, and their hydrogen-bond acceptor basicity by β_1. The solvents acetone, acetonitrile, 2-propanol, methanol and 2,2,2-trifluoroethanol provide a convenient range of hydrogen-bond acidity. 2,2,2-Trifluoroethanol has the useful property of being the strongest hydrogen-bond acid of the solvents in Table 2 (stronger than water) with zero hydrogen-bond basicity. Its properties, therefore, should be very different to those of the normal alcohols, which possess both significant hydrogen-bond acid and base properties. Triethylamine ($\pi_1^* = 0.14$, $\alpha_1 = 0$ and $\beta_1 = 0.71$) would be an excellent choice as a hydrogen-bond base solvent, but is not miscible with water, while the water miscible trimethylamine is unsuitable for use in TLC because it degrades the binder fixing the layer to the plate. Acetone has a similar capacity for dipole-type interactions and hydrogen-bond acidity to tetrahydrofuran, and was selected in preference to tetrahydrofuran, since it is a stronger hydrogen-bond base and has better storage properties. *N*,*N*-Dimethylformamide was selected because it is a stronger hydrogen-bond base than acetone and has zero hydrogen-bond acidity. Virtually all of the water miscible solvents in Table 2 have a significant capacity for dipole-type interactions but are simultaneously either significant hydrogen-bond acids or bases, and do not possess high selectivity for this interaction. Acetonitrile was added to the list of selected solvents because of its common use in reversed-phase liquid chromatography. It has a significant capacity for dipole-type interactions accompanied by a modest capacity as a hydrogen-bond acid and base. The above selection procedure resulted in the identification of methanol, 2-propanol, 2,2,2-trifluoroethanol, acetone, *N*,*N*-dimethylformamide, and acetonitrile as our basis set of selective solvents for reversed-phase TLC.

Table 2
Kamlet-Taft solvatochromic parameters for common water miscible solvents with favorable chromatographic properties

Solvent	Solvatochromic parameters		
	π_1^*	α_1	β_1
Water	1.09	1.17	0.18
Methanol	0.60	0.93	0.62
2-Propanol	0.48	0.76	0.95
2,2,2-Trifluoroethanol	0.73	1.51	0
Acetonitrile	0.75	0.19	0.31
Tetrahydrofuran	0.73	0	0.22
Acetone	0.71	0.08	0.48
N,*N*-Dimethylformamide	0.88	0	0.69

3.2. System characterization

The solvation parameter model was fit to the retention data (R_M values) for different groups of varied solutes selected from Table 1 covering the mobile phase composition range from about 10 to 90% (v/v) organic solvent in water at 10% (v/v) increments. The systems constants and statistics for the fit for each solvent are summarized in Table 3. The model fits are acceptable in all cases with the poorest results obtained for those mobile phases containing little water. In this case the selection of solutes from Table 1 was not ideal, resulting in most solutes clustered into a tight, high R_F range. A notable feature of the models is that the *s* system constant is statistically insignificant for all mobile phase compositions independent of solvent identity. Interactions of a dipole-type do not contribute to the retention of organic solutes on the cyanopropylsiloxane-bonded layer. A value of zero for a system constant does not indicate that a particular interaction is absent for the stationary phase, but rather that an equality exists for that interaction in the mobile and stationary phases, and consequently it does not influence the retention process. Similar results for the *s* system constant were observed with a cyanopropylsiloxane-bonded sorbent in reversed-phase column liquid chromatography, and a plausible explanation provided [14].

For the purpose of general interpretation the tabulated results can be plotted in the form of the system constants against the volume fraction of organic solvent in the mobile phase. A representative example is shown for acetonitrile–water mobile

Table 3
System constants for organic solvent–water mobile phases using HPTLC CN F254s plates (*s* constant is 0 in all cases)

Composition % (v/v)	System constants					Statistics*				Solute R_F range
	m	*r*	*a*	*b*	*c*	*ρ*	*S.E.*	*F*	*n*	
Methanol										
0	2.46	0	0	−1.64	−0.86	0.937	0.11	76	23	0.35–0.05
	(0.29)			(0.13)	(0.25)					
10	2.39	0	0	−1.64	−0.93	0.950	0.10	101	24	0.40–0.05
	(0.25)			(0.12)	(0.22)					
20	1.99	0.30	0	−1.63	−0.93	0.972	0.09	126	25	0.45–0.05
	(0.21)	(0.11)		(0.08)	(0.18)					
30	1.72	0.42	0	−1.58	−0.95	0.978	0.07	139	22	0.55–0.10
	(0.19)	(0.10)		(0.08)	(0.16)					
40	1.60	0.40	0	−1.46	−1.06	0.980	0.08	174	25	0.60–0.05
	(0.14)	(0.09)		(0.07)	(0.11)					
50	1.37	0.22	−0.29	−1.30	−0.78	0.990	0.06	218	21	0.70–0.10
	(0.10)	(0.05)	(0.06)	(0.05)	(0.11)					
60	0.99	0.23	−0.35	−1.10	−0.78	0.992	0.04	318	24	0.70–0.20
	(0.08)	(0.04)	(0.04)	(0.04)	(0.08)					
70	0.41	0.39	−0.21	−0.72	−0.87	0.947	0.06	46	25	0.85–0.60
	(0.13)	(0.08)	(0.05)	(0.06)	(0.10)					
80	0	0.57	−0.43	−0.65	−0.86	0.956	0.07	93	29	0.95–0.75
	(0.07)	(0.05)	(0.06)	(0.08)						
90	0	0.52	−0.41	−0.48	−1.18	0.890	0.10	36	31	0.95–0.80
	(0.08)	(0.07)	(0.08)	(0.10)						
2-Propanol										
10	2.31	0.39	0	−2.01	−0.98	0.992	0.04	365	21	0.47–0.04
	(0.12)	(0.09)	(0.06)	(0.13)						
20	2.05	0.35	0	−1.95	−0.80	0.984	0.07	219	25	0.57–0.04
	(0.14)	(0.08)	(0.07)	(0.13)						
30	1.73	0	0	−1.57	−0.59	0.988	0.06	450	25	0.68–0.04
	(0.08)	(0.06)	(0.09)							
40	1.35	0	0	−1.22	−0.71	0.985	0.06	417	28	0.76–0.11
	(0.07)	(0.05)	(0.07)							
50	0.69	0	−0.31	−0.94	−0.40	0.976	0.06	157	27	0.85–0.34
	(0.08)	(0.05)	(0.05)	(0.10)						
60	0.23	0.24	−0.43	−0.84	−0.36	0.977	0.05	91	22	0.88–0.44
	(0.08)	(0.05)	(0.05)	(0.05)	(0.09)					
70	0	0.31	−0.44	−0.69	−0.57	0.955	0.07	52	20	0.91–0.65
		(0.07)	(0.07)	(0.07)	(0.09)					
80	0	0.50	−0.64	−0.32	−1.25	0.957	0.07	66	22	0.96–0.79
		(0.06)	(0.07)	(0.11)	(0.09)					
2,2,2-Trifluoroethanol										
10	2.20	0	−0.45	−1.65	−0.52	0.979	0.06	158	24	0.35–0.05
	(0.15)		(0.06)	(0.07)	(0.14)					
20	1.83	0	−0.53	−1.53	−0.33	0.971	0.07	125	26	0.42–0.06
	(0.13)		(0.07)	(0.08)	(0.13)					
30	1.37	0	−0.38	−1.11	−0.39	0.994	0.04	578	25	0.69–0.06
	(0.07)		(0.04)	(0.03)	(0.09)					
40	1.17	0	−0.31	−1.00	−0.54	0.991	0.04	471	27	0.76–0.13
	(0.06)		(0.03)	(0.03)	(0.08)					

(Cont.)

Table 3. Continued

Composition % (v/v)	System constants					Statistics*				Solute R_F range
	m	*r*	*a*	*b*	*c*	*ρ*	*S.E.*	*F*	*n*	
50	0.72 (0.12)	0.36 (0.07)	−0.20 (0.04)	−0.89 (0.04)	−0.85 (0.10)	0.980	0.05	142	28	0.77–0.24
60	0.36 (0.08)	0.55 (0.05)	0	−1.02 (0.06)	−0.98 (0.09)	0.975	0.06	122	23	0.89–0.47
70	0	0.59 (0.04)	0	−1.16 (0.07)	−0.79 (0.06)	0.981	0.05	242	21	0.91–0.60
80	0	0.39 (0.05)	0	−1.76 (0.07)	−0.57 (0.06)	0.987	0.05	356	21	0.96–0.67
Acetone										
10	1.89 (0.12)	0.45 (0.08)	0	−1.93 (0.06)	−0.72 (0.12)	0.990	0.05	314	23	0.48–0.04
20	1.67 (0.15)	0.50 (0.07)	0	−1.94 (0.07)	−0.64 (0.13)	0.990	0.05	263	20	0.60–0.04
30	1.52 (0.08)	0.41 (0.06)	0	−1.92 (0.06)	−0.57 (0.08)	0.988	0.06	318	26	0.73–0.02
40	1.13 (0.07)	0.12 (0.04)	−0.16 (0.04)	−1.52 (0.04)	−0.20 (0.08)	0.990	0.05	344	31	0.76–0.07
50	0.91 (0.06)	0	−0.36 (0.03)	−1.31 (0.04)	−0.15 (0.07)	0.990	0.04	394	28	0.81–0.16
60	0.68 (0.07)	0	−0.18 (0.04)	−0.97 (0.04)	−0.31 (0.08)	0.984	0.05	234	27	0.85–0.28
70	0.45 (0.03)	0	0	−0.84 (0.02)	−0.39 (0.03)	0.992	0.02	738	26	0.88–0.47
80	0.43 (0.04)	0	0	−0.44 (0.03)	−0.84 (0.04)	0.948	0.03	99	25	0.88–0.71
N,N-Dimethylformamide										
10	2.20 (0.10)	0.28 (0.06)	0	−1.86 (0.06)	−1.00 (0.08)	0.990	0.05	361	24	0.54–0.02
20	1.96 (0.06)	0	0	−1.70 (0.04)	−0.79 (0.07)	0.992	0.05	752	26	0.69–0.03
30	1.63 (0.14)	0	−0.16 (0.07)	−1.56 (0.06)	−0.70 (0.18)	0.990	0.06	311	22	0.80–0.06
40	1.36 (0.06)	0	−0.30 (0.03)	−1.48 (0.04)	−0.65 (0.07)	0.993	0.04	539	25	0.83–0.14
50	1.06 (0.06)	0	−0.34 (0.03)	−1.24 (0.03)	−0.63 (0.07)	0.991	0.04	511	29	0.87–0.27
60	0.85 (0.05)	0	−0.35 (0.03)	−1.13 (0.06)	−0.66 (0.07)	0.990	0.04	306	21	0.91–0.44
70	0.25 (0.08)	0	−0.42 (0.04)	−0.95 (0.04)	−0.36 (0.09)	0.983	0.05	210	26	0.94–0.64
80	0	0	−0.71 (0.04)	−1.00 (0.05)	−0.30 (0.03)	0.978	0.06	262	26	0.97–0.76
Acetonitrile										
1	2.38 (0.19)	0.23 (0.14)	0	−1.87 (0.11)	−0.87 (0.19)	0.978	0.07	116	20	0.37–0.04
5	2.29 (0.10)	0.36 (0.07)	0	−1.93 (0.06)	−0.96 (0.10)	0.994	0.03	425	18	0.39–0.06
10	2.24 (0.22)	0.44 (0.15)	0	−2.02 (0.11)	−0.97 (0.20)	0.975	0.08	119	23	0.43–0.04

Table 3. Continued

Composition % (v/v)	System constants					Statistics*				Solute R_F range
	m	*r*	*a*	*b*	*c*	*ρ*	*S.E.*	*F*	*n*	
20	1.90	0.40	0	−1.83	−0.83	0.973	0.10	121	24	0.57–0.03
	(0.25)	(0.13)		(0.09)	(0.19)					
30	1.66	0	−0.25	−1.53	−0.48	0.985	0.07	296	31	0.66–0.03
	(0.11)		(0.06)	(0.06)	(0.12)					
40	1.14	0	−0.32	−1.16	−0.38	0.990	0.04	396	26	0.70–0.11
	(0.07)		(0.04)	(0.04)	(0.08)					
50	0.89	0	−0.22	−1.07	−0.47	0.993	0.03	446	21	0.83–0.28
	(0.05)		(0.04)	(0.03)	(0.07)					
60	0.46	0	−0.16	−0.62	−0.52	0.956	0.03	75	25	0.84–0.63
	(0.07)		(0.04)	(0.04)	(0.07)					
70	0	0.23	−0.19	−0.53	−0.65	0.962	0.04	99	28	0.91–0.68
		(0.04)	(0.03)	(0.03)	(0.05)					
80	0	0.16	−0.37	−0.49	−0.84	0.944	0.05	74	31	0.95–0.81
		(0.05)	(0.05)	(0.04)	(0.06)					

* ρ = overall correlation coefficient, *S.E.* = standard error in the estimate, $F = F$-statistic, and n = number of solutes. The numbers in parentheses are the standard deviations for the system constants.

phases in Fig. 1. Three general regions can be distinguished. In the region between 0 and about 10% (v/v) organic solvent there are significant changes in the values for some system constants from those in the immediate neighboring region containing a larger volume fraction of organic solvent. This region is dominated by the solvation properties of water and quite probably by changes in the selective absorption of mobile phase components by the stationary phase. Although the changes in the solvation properties can be sharp in this region, the results are quite stable and reproducible. Between about 10 and about 70% (v/v) organic solvent the characteristic solvation properties of water are diminished by the presence of an increasing amount of organic solvent. Water is the most cohesive of the solvents evaluated, and with the exception of 2,2,2-trifluoroethanol, the strongest hydrogen-bond acid. Thus, within this region the primary driving force for retention by the stationary phase is the relative ease of cavity formation (*m* constant). The factor which contributes most to reducing retention in this region is the solute hydrogen-bond basicity. The *b* system constant is negative and becomes more positive with increasing amounts of organic solvent. The *m* and *b* system constants oppose each other in this region with increasing solute size increasing retention and increasing solute hydrogen-bond basicity decreasing retention. Solvent selectivity is dependent on the capacity of the organic solvent to moderate the cohesion and hydrogen-bond basicity of water (discussed subsequently) and small but significant contributions from lone pair electron interactions (*r* system constant) and hydrogen-bond basicity (*a* system constant). For all solvent compositions investigated the *r* system constant is either zero or positive (has no influence or favors retention by the solvated stationary phase) and the *a* system constant zero or negative (has no influence or reduces retention by the solvated stationary phase). The relative importance of these interactions depends on the solute under consideration having the necessary complementary properties for that interaction, but the

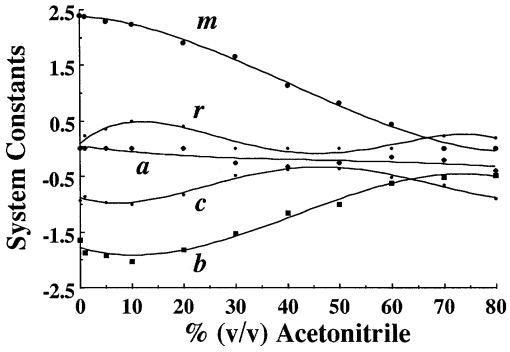

Fig. 1. Plot of the system constants as a function of the acetonitrile–water composition.

dominance of solute size (mV_X) and the solvent hydrogen-bond acidity ($b\Sigma\beta_2^0$) on the general retention mechanism is well illustrated by the data in Table 4 for 1-naphthol and 1-nitronaphthalene in the six solvent systems containing 30% (v/v) organic solvent as the mobile phase. At solvent compositions greater than about 70% (v/v) organic solvent the dominant hand of water is diminished, the *m* system constant is small or zero and the *b* system constant remains negative but approaches zero. This region is dominated by the characteristic properties of the organic solvent. Retention is weak and one of the primary retention mechanisms in this region is the relative capacity of the solvated stationary phase for lone pair electron interactions. At high organic solvent compositions the relative capacity of the mobile and stationary phases for intermolecular interactions have become quite similar and this region is only likely to be used occasionally for separations with water present in the mobile phase.

3.3. Relationship between the m system constant and solvent type

Converting the abscissa from volume fraction to mole fraction provides some insight into the solvent-dependent changes in the *m* and *b* system constants for the middle region, identified above, dominated by reversed-phase type interactions (Fig. 2). Initially there is a region in which both the *m* and *b* system constants change linearly with the mole fraction acetonitrile. The linear range observed for the *b* system constant is usually somewhat shorter than the *m* system constant, and is probably fortuitous, in that it represents a linear portion of a shallow curve. There are two types of behavior for the change in the *m* system constant with choice of organic solvent (Fig. 3). Methanol and acetonitrile show a linear decrease in the *m* system constant for the composition range indicated. The difference in slopes being an indication of solvent selectivity. For acetone, *N*,*N*-dimethylformamide and 2,2,2-trifluoro-

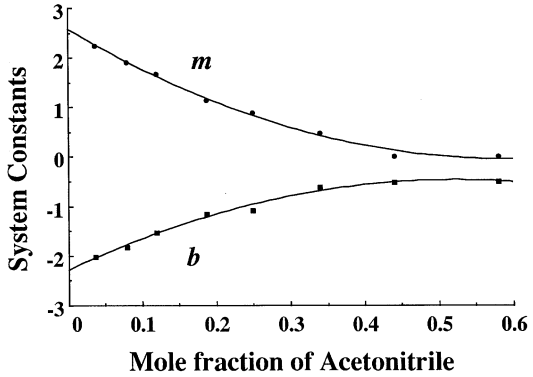

Fig. 2. Plot of the *m* and *b* system constants as a function of the mole fraction of acetonitrile in the mobile phase.

Table 4
Contribution of different intermolecular interactions to retention using 30% (v/v) organic solvent in water as mobile phase on the cyanopropylsiloxane-bonded HPTLC plates (interactions of a dipole-type $s\pi_2^H$ are insignificant for all solvents)

Solvent	Contribution to retention					R_M
	mV_X	rR_2	$a\Sigma\alpha_2^H$	$b\Sigma\beta_2^0$	c	
1-Naphthol						
Methanol	1.968	0.638	0	−0.585	−0.95	1.072
2-Propanol	1.981	0	0	−0.589	−0.59	0.811
2,2,2-Trifluoroethanol	1.567	0	−0.232	−0.411	−0.39	0.535
Acetone	1.739	0.623	0	−0.710	−0.57	1.082
N,*N*-Dimethylformamide	1.865	0	−0.103	−0.577	−0.70	0.607
Acetonitrile	1.899	0	−0.153	−0.566	−0.48	0.614
1-Nitronaphthalene						
Methanol	2.167	0.533	0	−0.474	−0.95	1.277
2-Propanol	2.182	0	0	−0.471	−0.59	1.121
2,2,2-Trifluoroethanol	1.726	0	0	−0.333	−0.39	1.003
Acetone	1.915	0.521	0	−0.576	−0.57	1.284
N,*N*-Dimethylformamide	2.054	0	0	−0.468	−0.70	0.888
Acetonitrile	2.092	0	0	−0.459	−0.48	1.153

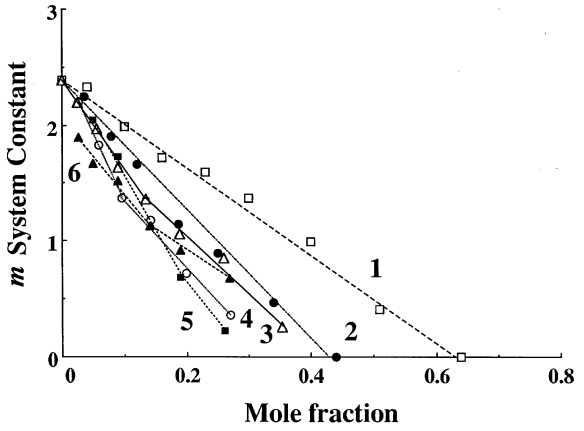

Fig. 3. Plot of the *m* system constant as a function of the mole fraction of organic solvent in the mobile phase. Identification; 1=methanol; 2=acetonitrile; 3=*N*,*N*-dimethylformamide; 4=2,2,2-trifluoroethanol; 5=2-propanol; and 6=acetone.

ethanol there is an initial linear decrease with increasing mole fraction of organic solvent that changes abruptly to a second linear region with a more gentle slope. The situation for 2-propanol is more ambiguous because of the smaller number of data points for which *m* is greater than zero, but it probably falls into the second category. A possible reason for the changes observed is structural reordering of the water hydrogen-bonded network to accommodate the increasing proportion of organic solvent, although because of the complexity of the microstructure of mixed solvents and the influence of selective absorption of solvent by the stationary phase, this explanation remains speculative for the present.

3.4. Method development for steriod separations

Method development is quite straightforward using the solvation parameter model and the system constants determined in Section 3.2. The useful solvent strength range for migration of analytes between $0.1<R_F<0.9$ and the difference in predicted R_F values is obtained by simple calculations using Eq. (1) and data from Table 3. The results can be evaluated in tabulated form or graphically, as desired. For an effective evaluation of the method development approach we have selected a series of test mixtures whose identity is different from those used to construct the models assembled in Table 3. System constants for solvent compositions other than experimental values are obtained by interpolation from plots of the type shown in Fig. 1. In general terms, the change in system constant as a function of mobile phase composition is fitted to a polynomial function, and these relationships are used to estimate the system constants as a continuous function of mobile phase composition.

As a general example we have selected the separation of estrone, estradiol and estriol. The solvation parameter model was used to calculate the R_M values for the six organic solvents used as the basis set. The most difficult to separate pair are estrone and estradiol, and for these steroids a mobile phase of about 50% (v/v) 2-propanol, 50% (v/v) acetone or 60% (v/v) *N*,*N*-dimethylformamide in water provides the best separation with a difference in R_F values ((R_F) of about 0.7. With minimal effort and time the model identifies the experimental domain that could be used for each solvent and, in this case, three solvent systems equally suitable for the separation. These observations are confirmed for the separation of the three estrogens using different compositions of the mobile phase 2-propanol in water (Fig. 4) which demonstrates that both the solvent type and composition were correctly identified for the separation.

For the separation of progesterone, testosterone and hydrocortisone the same methodology was fol-

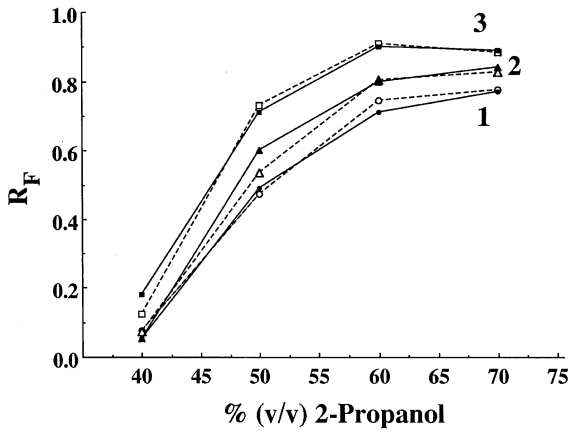

Fig. 4. Plot of the predicted (dotted line) and experimental (full line) R_F values for (1) estrone, (2) estradiol, and (3) estriol as a function of the mobile phase composition for 2-propanol in water.

Table 5
Ranking of solvent systems by ΔR_F values for the separation of progesterone, testosterone and hydrocortisone

Mobile phase values	Predicted ΔR_F values		Experimental ΔR_F values	
60% (v/v) *N*,*N*-Dimethylformamide	0.21	0.26	0.16	0.21
50% (v/v) *N*,*N*-Dimethylformamide	0.17	0.30	0.19	0.23
50% (v/v) 2-Propanol	0.17	0.25	0.16	0.20
50% (v/v) Acetone	0.16	0.25	0.15	0.23
50% (v/v) Acetonitrile	0.15	0.24	0.14	0.21
60% (v/v) Acetone	0.14	0.25	0.15	0.22
60% (v/v) Methanol	0.12	0.18	0.14	0.18

lowed as used for the estrogens. The ΔR_F values for the seven best solvent systems for the separation are ranked in Table 5. Agreement between the predicted and experimental ΔR_F values is very good as indicated. For the repeat separation of steroids in the above chromatographic systems the average error in the determination of R_F values is about 0.02 R_F units. The average difference between the predicted and experimental R_F values is 0.03 ± 0.02 ($n = 24$) R_F units.

3.5. Method development for phenols and substituted naphthalenes

For the separation of the four phenols pentachlorophenol, 2,4,6-trichlorophenol, 4-nitrophenol and catechol a 0.01 *M* triethylammonium phosphate buffer, pH 2.5, was used to minimize dissociation of the acidic phenols. A number of solvent systems provide an adequate separation with a mobile phase containing about 60% (v/v) methanol as the preferred mobile phase, since it provides a somewhat equal spacing between the separated peaks. The good agreement between the predicted and experimental R_F values for the methanol–water system is indicated in Table 6 with the average difference between the predicted and experimental R_F values for the methanol–water mobile phases being 0.03 ± 0.02 ($n = 16$) R_F units.

As well as identifying the optimum separation conditions for possible separations the solvation parameter model provides useful insight into the reasons for failure. Consider the separation of 1-ethoxynaphthalene, 1-methylnaphthalene, 1-chloronaphthalene and 1-bromonaphthalene. Only 2,2,2-trifluoroethanol with a low volume fraction of water and 60% (v/v) 2-propanol in water provide a useful separation of the four compounds. The intermolecu-

Table 6
Ranking of solvent systems by ΔR_F values for the separation of pentachlorophenol, 2,4,6-trichlorophenol, 4-nitrophenol and catechol and the agreement between the experimental and predicted values for the methanol–water system

Mobile phase	Predicted ΔR_F values		
70% (v/v) Methanol	0.16	0.21	0.21
50% (v/v) 2,2,2-Trifluoroethanol	0.16	0.16	0.17
60% (v/v) 2,2,2-Trifluoroethanol	0.16	0.12	0.16
50% (v/v) *N*,*N*-Dimethylformamide	0.13	0.24	0.20
50% (v/v) Acetonitrile	0.12	0.21	0.20

Confirmation of results for the methanol–water system

	Pentachlorophenol		2,4,6-Trichlorophenol		4-Nitrophenol		Catechol	
	Experimental	Predicted	Experimental	Predicted	Experimental	Predicted	Experimental	Predicted
40% (v/v) Methanol	0.01	0.01	0.05	0.05	0.18	0.16	0.49	0.46
50% (v/v) Methanol	0.03	0.02	0.11	0.08	0.26	0.23	0.59	0.55
60% (v/v) Methanol	0.12	0.07	0.26	0.18	0.41	0.39	0.69	0.65
70% (v/v) Methanol	0.28	0.22	0.45	0.38	0.58	0.59	0.82	0.79

lar interactions that contribute to the separation for 80% (v/v) 2,2,2-trifluoroethanol in water, the best solvent systems that contained 2,2,2-trifluoroethanol, and 60% (v/v) 2-propanol in water, the best of the solvent systems that did not contain 2,2,2-trifluoroethanol, are summarized in Table 7. For the 2,2,2-trifluoroethanol system only contributions from lone pair electron interactions and the hydrogen-bond basicity of the solutes contribute to retention. Differences in the capacity for lone pair electron interactions is the main contributor to the separation of 1-chloronaphthalene and 1-bromonaphthalene and differences in solute hydrogen-bond basicity provides the mechanism for the separation of 1-methylnaphthalene and 1-ethoxynaphthalene from each other and from 1-chloronaphthalene and 1-bromonaphthalene. For the 2-propanol system the same interactions are important and supplemented by size differences (*m* was zero for the 2,2,2-trifluoroethanol system). The size differences for these compounds are too small to significantly improve the separation. The more favorable blend of hydrogen-bond acidity and capacity for lone pair electron interactions possessed by the 2,2,2-trifluoroethanol system, provides the better, if less than baseline separation for the mixture of naphthalene compounds. From the solute descriptors for these compounds, Table 1, significant differences in their capacity for dipole-type interactions is indicated suggesting a separation system that combines strong interactions of a dipole-type and solute hydrogen-bond basicity would be more successful. Since all the solvent systems for the cyanopropylsiloxane-bonded layer have a zero *s* system constant a different choice of stationary phase would be the way to proceed. The results discussed here were confirmed by experiment. However, the initial calculations were performed in a spread sheet, in a few minutes, and would have saved a significant amount of laboratory time and materials.

3.6. Further consideration

In the above studies we have used zone spacing to indicate the extent of a separation for convenience. The use of resolution would not be difficult since zone broadening in HPTLC is controlled by molecular diffusion and there is (usually) a linear relationship between peak width and migration distance (R_F value) for solutes of similar molecular mass [2,3,31,32].

The development time is an intrinsic property of the mobile phase composition (viscosity, surface tension) and properties of the layer (permeability, contact angle). The cyanopropylsiloxane-bonded layers can be developed conveniently with water (41 min for a 5 cm development) and for the mixed mobile phases the time required for a 5 cm development are in the range 10–20 min for water–acetonitrile mixtures; 30–36 min for acetone–water mixtures; 30–43 min for methanol–water mixtures; 41–50 min for 2,2,2-trifluoroethanol–water mixtures; 43–50 min for *N*,*N*-dimethylformamide–water mixtures; and 75–80 min for 2-propanol–water mixtures. After development excess solvent is evapo-

Table 7
Intermolecular interactions contributing to the separation of various naphthalene compounds on cyanopropylsiloxane-bonded layers by reversed phase chromatography

Compound	Contribution to retention					Predicted R_F
	mV_X	rR_2	$a\Sigma\alpha_2^H$	$b\Sigma\beta_2^0$	c	
80% (v/v) 2,2,2-Trifluoroethanol in water						
1-Ethoxynaphthalene	0	0.55	0	−0.67	−0.57	0.83
1-Methylnaphthalene	0	0.52	0	−0.35	−0.57	0.71
1-Chloronaphthalene	0	0.55	0	−0.23	−0.57	0.64
1-Bromonaphthalene	0	0.62	0	−0.23	−0.57	0.60
60% (v/v) 2-Propanol in water						
1-Ethoxynaphthalene	0.33	0.34	0	−0.32	−0.36	0.51
1-Methylnaphthalene	0.28	0.32	0	−0.17	−0.36	0.46
1-Chloronaphthalene	0.28	0.34	0	−0.11	−0.36	0.42
1-Bromonaphthalene	0.29	0.38	0	−0.11	−0.36	0.38

rated from the layer by a flow of nitrogen or vacuum evaporation. This process is quite slow when *N*,*N*-dimethylformamide is used, typically requiring about one hour or more to obtain flat and stable densitometric baselines. This is inconvenient, so unless *N*,*N*-dimethylformamide is identified as a uniquely selective solvent for a separation, mobile phases containing one of the other basis solvents are chosen in preference.

There is a good correlation for the *m* and *b* system constants obtained in this study and those reported earlier for a cyanopropylsiloxane-bonded sorbent in column liquid chromatography with methanol–water, 2-propanol–water and acetonitrile–water mobile phases [14]. Fig. 5 shows a plot of log *k* (retention factor in column chromatography) against the R_M value for compounds in common in the two studies with 30% (v/v) 2-propanol in water and 40% (v/v) 2-propanol in water mobile phases. This indicates that TLC data can be used to predict retention in column liquid chromatography and vice versa once the necessary correlation equation is established; and since we have used column liquid chromatographic data to predict breakthrough volumes in solid-phase extraction [14–16], it should be possible to use TLC retention data for the same purpose. Of particular interest in the context of this paper, since the column sorbent contains no binder yet exhibits similar selectivity to the cyanopropylsiloxane-bonded layer, it is reasonable to assume that the presence of the binder in the layer is not influential in controlling selectivity. In addition, since column systems are presumed to be at equilibrium, the good correlation between the column and the layer separation systems is a reasonable indication that equilibrium conditions prevail for the separations obtained by reversed-phase TLC.

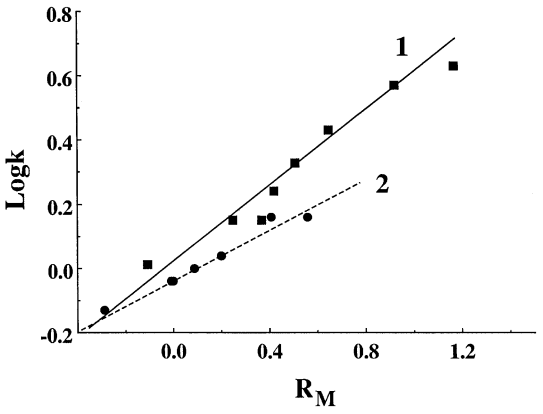

Fig. 5. Plot of log *k* (column chromatography) and R_M value (TLC) for the same compounds using (1) 30% (v/v) 2-propanol in water, and (2) 40% (v/v) 2-propanol in water for cyanopropylsiloxane-bonded phases. The column packing material was a Bakerbond CN packing described in [14].

4. Conclusions

The solvation parameter model provides a convenient method for the prediction of retention in reversed-phase thin layer chromatography from compound characteristic properties. Retention maps can be constructed from plots of the system constants as a function of mobile phase composition provided for six binary solvent mixtures on a cyanopropylsiloxane-bonded layer in this paper. Further studies will extend this approach to include stationary phase selection and solvent optimization using ternary and quaternary mobile phases.

Acknowledgements

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References

[1] C.F. Poole, S.K. Poole, Chromatography Today, Elsevier, Amsterdam, 1991, pp. 649–734.
[2] C.F. Poole, S.K. Poole, J. Chromatogr. A 703 (1995) 573.
[3] C.F. Poole, S.K. Poole, Anal. Chem. 66 (1994) 27A.
[4] J. Sherma, B. Fried, Handbook of Thin-Layer Chromatography, Marcel Dekker, New York, 2nd. ed., 1996.
[5] D. Nurok, Chem. Rev. 89 (1989) 363.
[6] W. Prus, T. Kowalska, J. Planar Chromatogr. 8 (1995) 288.
[7] F.L. Birkinshaw, D.G. Waters, J. Planar Chromatogr. 8 (1995) 319.
[8] C. Cimpoiu, T. Hodisan, H. Nascu, J. Planar Chromatogr. 10 (1997) 195.
[9] J.K. Rozylo, R. Siembida, J. Planar Chromatogr. 10 (1997) 97.
[10] Sz. Nyiredy, Z. Fater, J. Planar Chromatogr. 8 (1995) 341.

[11] Q.S. Wang, B.W. Yan, L. Zhang, Chromatographia 40 (1995) 463.
[12] P. Vuorela, E.-L. Ranko, R. Hiltunen, H. Vuorela, J. Chromatogr. A 670 (1994) 191.
[13] A.-M. Siouffi, J. Chromatogr. 556 (1991) 81.
[14] D.S. Seibert, C.F. Poole, Chromatographia 41 (1995) 51.
[15] D.S. Seibert, C.F. Poole, M.H. Abraham, Analyst 121 (1996) 511.
[16] C.F. Poole, S.K. Poole, D.S. Seibert, C.M. Chapman, J. Chromatogr. B 689 (1997) 245.
[17] W. Kiridena, C.F. Poole, Anal. Comm. 34 (1997) 195.
[18] D. Bolliet, C.F. Poole, Chromatographia 46 (1997) 381.
[19] L.R. Snyder, J.J. Kirkland, J.L. Glajch, Practical HPLC Method Development, Wiley, New York, 2nd ed., 1997, pp. 463–466.
[20] M.H. Abraham, H.S. Chadha, G.S. Whiting, R.C. Mitchell, J. Pharm. Sci. 83 (1994) 1085.
[21] M.H. Abraham, H.S. Chadha, R.C. Mitchell, J. Pharm. Pharmacol. 47 (1995) 8.
[22] M.H. Abraham, Chem. Soc. Rev. 22 (1993) 73.
[23] M.H. Abraham, in: P. Politzer, J.S. Murray (Editors), Quantitative Treatments of Solute/Solvent Interactions, Elsevier, Amsterdam, 1994, pp. 83–134.
[24] A.H. Lowery, C.J. Cramer, J.J. Urban, G.R. Famini, Computers Chem. 19 (1995) 209.
[25] M.H. Abraham, H.S. Chadha, in: V. Pliska, B. Testa, H. van de Waterbeemed (Editors), Lipophilicity in Drug Action and Toxicology, VCH, Weinheim, Germany, 1996, pp. 311–337.
[26] R.J. Maxwell, S.W. Yeisley, J. Unruh, J. Liq. Chromatogr. 13 (1990) 2001.
[27] K. Dross, S. Sonntag, R. Mannhold, J. Chromatogr. 639 (1993) 287.
[28] M.J. Kamlet, J.-L.M. Abboud, M.H. Abraham, R.W. Taft, J. Org. Chem. 48 (1983) 2877.
[29] C. Reichardt, Chem. Rev. 94 (1994) 2319.
[30] A. de Juan, G. Fonrodona, E. Casassas, Trends Anal. Chem. 16 (1997) 52.
[31] C.F. Poole, W.P.N. Fernando, J. Planar Chromatogr. 5 (1992) 323.
[32] C.F. Poole, W.P.N. Fernando, J. Planar Chromatogr. 6 (1993) 357.