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Review

Practitioner's guide to method development in thin-layer chromatography

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Abstract

The authors provide a personal perspective of method development in thin-layer chromatography for the novice and more experienced chromatographer alike. No attempt has been made at a comprehensive survey of the literature. Instead we provide an overview with insights into a smaller number of approaches that the authors have found useful in their own work and indicate the factors responsible for the variation in retention and their control. The main topics covered are the relationship between sorbent chemistry and retention, the selection of primary solvents for mobile phase optimization and mobile phase optimization using the PRISMA and solvation parameter models. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Reviews; Thin-layer chromatography; Method development

Contents

1. Introduction

For as long as samples present themselves in an infinite variety of compositions there will remain a need for method development. In the absence of an

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exact understanding of the kinetic and thermodynamic factors that underlie any chromatographic method the procurement of suitable separations fit for their intended purpose will rely on a blend of human experience and intuition combined with limited information based on established physical principles. The balance between the two complementary inputs reflecting the level of information maturity in a technique. For example, it is possible to make reliable estimates of the influence of column properties on the efficiency of open tubular columns in gas chromatography using computer simulations to compare hypotheses [1]. There is a limited need to perform new experiments for this purpose. On the other hand, to predict retention in liquid chromatography it is usually necessary to gather initial experimental data to estimate retention under other (related) conditions, since our knowledge of the principles of retention does not run to outright calculation of thermodynamic properties from system and solute properties. In chromatography in general, retention is optimized experimentally, and therefore most methods developed for specific separations continue to require an experimental approach.

1.1. Define the problem

If possible we would like to know how many detectable components are present in the sample for this is the only way that it is possible to be certain that a separation is complete. In addition, the zone capacity of thin-layer chromatography (TLC) is limited, Table 1 [2–4], and the number of detectable components and their polarity range determines the choice of development technique. From simple considerations of the theory of statistical overlap in chromatography [5] it is easy to demonstrate that only when the zone capacity of a technique is significantly larger than the number of sample components can a complete separation be obtained. Based on the information in Table 1 it will be difficult or impossible to separate to baseline more than about 8 to 10 components in a single development by thin-layer chromatography. In addition, if the range of component polarities is too wide for separation in a single development then one of the gradient development approaches based on multiple development will be necessary [2,6,7]. Forced flow and automated multiple development provide for the possibility of separating mixtures containing up to about 20 components in a single lane. Equipment for forced flow development is not widely available in the Western Hemisphere [2] and, as a consequence, has become a rarely used option. The greater zone capacity for automated multiple development is a product of the zone refocusing mechanism obtained when solvent gradients are employed. The large zone capacity that is possible for two-dimensional thin-

Table 1
Zone capacity calculated or predicted for different conditions in TLC

Development	Dimensions	Zone capacity
(i) Predictions from theory		
Capillary controlled flow	1	<25
Forced flow	1	<80 (up to 150 depending on pressure limit)
Capillary controlled flow	2	<400
Forced flow	2	Several thousand
(ii) Based on experimental observations		
Capillary controlled flow	1	10–14
Forced flow	1	30–40
Capillary controlled flow (AMD)	1	30–40
Capillary controlled flow	2	≈ 100
(iii) Predictions based on results in (ii)		
Forced flow	2	≈ 1500
Capillary flow (AMD)	2	≈ 1500

layer chromatography might signal that this should be the general approach to separations by thin-layer chromatography. This is not the general case. Large zone capacities are obtained only when the two development techniques are orthogonal and methods for recording two-dimensional chromatograms lag behind those for lane scanning [2,6,8]. In normal laboratory operations thin-layer chromatography should be considered for simple separations using a single development and for more complex mixtures by multiple development. Two-dimensional separations are valuable for qualitative analysis of complex mixtures if a suitable approach for obtaining two orthogonal separation mechanisms can be identified.

The concentration range of relevant components is of interest for two reasons. It indicates whether derivatization techniques will be required for detection of compounds with poor response characteristics and minor components with similar migration properties to major components require greater zone separation for confident quantitation. This is a common problem in purity analysis of a chemical substance, which is essentially a single component containing a small amount of synthetic byproducts and starting materials. The analysis is usually simplified if the minor components are more strongly retained than the major component.

1.2. Sample information

Chemical information is central to the initial selection of chromatographic and detection properties. Many derivatizing reagents, for example, are functional group or compound class selective [9,10]. Reasonable solubility in a volatile solvent is required for sample application by spray-on or contact devices. Solvent selection for sample application is discussed elsewhere, including how to handle difficult samples due to high viscosity or lack of homogeneity [11,12]. The pK_a of easily ionized compounds indicates whether ion-suppression techniques using buffered mobile phases will be successful and the appropriateness of using ion-pair techniques. The limited range of ion-exchange sorbents for thin-layer chromatography sometimes dictates that easily dissociated functional groups and ions are derivatized prior to separation. This is usually the case if silica gel layers are used. Chemically bonded layers are often a better choice for these compounds.

2. Mode selection

A general approach to mode selection is summarized in Fig. 1. Most inorganic oxide and chemically bonded sorbents used in thin-layer chromatography are small pore materials (<10 nm average pore diameter) optimized for the separation of organic compounds with molecular masses below about 700. For the separation of polymers soluble in organic solvents choices are limited. Precipitation chromatography, where separations are based on solubility differences in a solvent gradient generated by mobile phase demixing is generally the best option [13]. Water soluble biopolymers are usually separated on cellulose layers [14], which because of low retention, find few applications to low-molecular-mass organic compounds. Inorganic oxide sorbents are the first choice for the separation of low-molecular-mass organic compounds soluble in typical organic solvents. The inorganic oxides include silica gel, alumina, kieselguhr and Florisil. Of these silica gel is by far the most important. Specific differences in the type and distribution of silanol groups for individual sorbents may result in selectivity differences [15], and it is not always possible to reproduce individual separations on different silica sorbents. The alumina surface is more complex than silica gel containing hydroxyl groups, aluminium cations, and oxide anions. Its apparent pH and hydration level significantly influence separation properties. Greater uncertainty in sorbent properties and poorer reproducibility of separations makes alumina less useful than silica gel in our opinion. It is used only when silica gel fails or produces irregular zone profiles, and in addition, chemically bonded sorbents lack the required selectivity for the separation. We virtually never use kieselguhr or Florisil.

Compounds of low polarity are difficult to separate on silica gel because of weak retention (mobile phase selection is limited because most solvents are too strong for these separations). In addition, very polar compounds are difficult to separate on silica gel because of strong retention (mobile phase selection is limited because most solvents are too weak

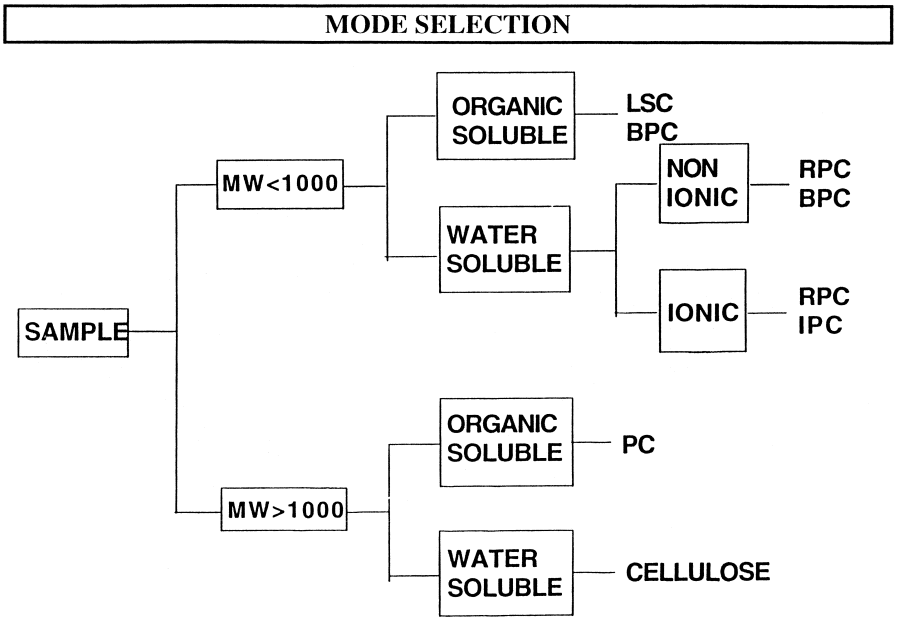

Fig. 1. Separation mode selection guide for thin-layer chromatography. LSC = Liquid–solid chromatography on an inorganic oxide sorbent; BPC = liquid–solid chromatography on a chemically bonded sorbent; RPC = reversed-phase chromatography with a water-containing mobile phase and chemically bonded stationary phase: IPC = ion-pair chromatography with reversed-phase separation conditions: and PC = precipitation chromatography.

for these separations). Ionic compounds and easily ionized compounds are usually separated by reversed-phase chromatography using buffered mobile phases (weak acids and bases) or ion-pair reagents (strong acids and bases). There are only a limited number of stationary phases available for ion-exchange chromatography, and with the exception of the aminopropylsiloxane-bonded silica gel layers, which function as a weak anion exchanger at low mobile phase pH, ion exchange is not a widely used separation mechanism in thin-layer chromatography.

Chemically bonded phases provide access to a range of complementary separation mechanisms to silica gel, Table 2. Alkylsiloxane-bonded silica gel layers with a high level of surface modification are incompatible with mobile phases containing a significant amount of water. They are used mainly for normal-phase separations of compounds of low polarity. Alkylsiloxane-bonded layers with a lower degree of surface bonding, a slightly larger particle size, and with a modified binder are used for reversed-phase chromatography. These layers are referred to as water wettable. The mobile phase velocity for aqueous solutions depends on a complex combination of sorbent, binder and mobile phase properties as indicated by the results in Fig. 2 [16]. Development times can be long as indicated. The polar chemically bonded phases are compatible with water in all proportions and are suitable for use in both normal-phase and reversed-phase chromatography [17]. In addition, mobile phase velocities vary less with changing solvent composition.

2.1. Layer pretreatments

Prior to chromatography it is common practice to prepare the layers for use by any or all of the following steps; washing, activation, conditioning and equilibration. Newly consigned precoated layers are invariably contaminated, or quickly become so, because of residual contaminants from the manufacturing process, contact with packaging materials, and adsorption of materials from the atmosphere. This can result in irregular and drifting densitometric baselines, ghost peaks in the chromatogram, and reduced sample detectability in postchromatographic

Table 2
Retention properties of silica based chemically bonded layers

Type of modification	Functional group	Application
Alkylsiloxane	Si-CH_3 Si-C_2H_5 Si-C_8H_{17} Si-$C_{18}H_{37}$	· For reversed-phase separations generally but not exclusively · Separation of water soluble polar organic compounds (RPC) · Weak acids and bases after ion suppression (RPC) · Strong acids and bases by ion-pair mechanism (RPC) · Homologous and oligomous compounds (RPC) · Hydrocarbon-like and polycyclic aromatic compounds (RPC & NPC)
Phenylsiloxane	Si-C_6H_5	· We have found no useful applications for this layer that cannot be performed on alkylsiloxane layers
Cyanopropylsiloxane	Si-$(CH_2)_3CN$	· Useful for both RPC and NPC · In NPC it exhibits properties similar to a low capacity silica gel. · In RPC it exhibits properties similar to a short-chain alkylsiloxane-bonded layers (it has no selectivity for dipole-type interactions)
Aminopropylsiloxane	Si-$(CH_2)_3NH_2$	· Used mainly in NPC & IEC. Limited retention in RPC · Selectively retains compounds by hydrogen-bond interactions in NPC. Separations unlike those obtained on silica gel. · Functions as a weak anion exchanger in acidic mobile phases (IEC)
Spacer bonded propane diol	Si-$(CH_2)_3OCH_2CH(OH)CH_2OH$	· Used in NPC and RPC but more useful for NPC because of low retention in RPC. · Reasonable retention of polar compounds by hydrogen bond and dipole-type interactions in NPC. More hydrogen-bond acidic and less hydrogen-bond basic then aminopropylsiloxane-bonded layers in NPC. More retentive than aminopropylsiloxane-bonded layers in RPC. · Similar retention to short chain alkylsiloxane-bonded layers but different selectivity for hydrogen-bonding compounds

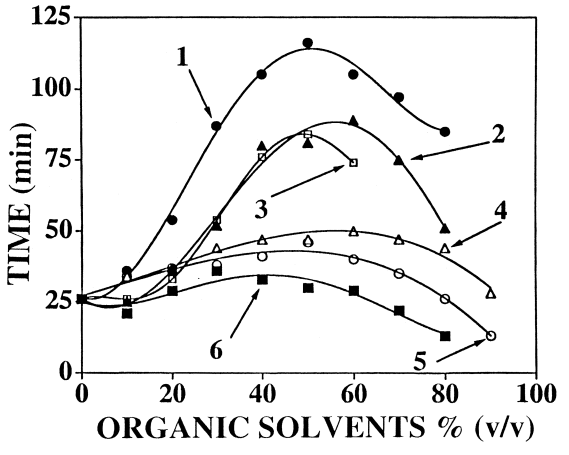

Fig. 2. Plot of the time required for the solvent to migrate 5 cm as a function of the mobile phase composition for (1) 2-propanol, (2) *N*,*N*-dimethylformamide, (3) 2,2,2-trifluoroethanol, (4) methanol, (5) acetone and (6) acetone in water on a Merck HPTLC RP-18 WF254s layer. (From Ref. [16]; ©Research Institute for Medicinal Plants).

derivatization reactions. These problems are easily remedied by prewashing the layers before use. Ascending development followed by immersion in the same polar solvent, e.g., 2-propanol or methanol, provides acceptable performance even for trace analysis [18].

For inorganic oxide sorbents the absolute R_F value and the reproducibility of R_F values depends on the layer activity. The latter is controlled by the adsorption of reagents, most notably water, through the gas phase [19]. There is no consensus, however, on how best to achieve this with some laboratories using elaborate controlled temperature deactivation and others seemingly doing nothing at all. In a modern air conditioned laboratory inorganic oxide layers in our experience achieve a consistent level of activity that should provide for adequate reproducibility for most separations. Equilibration of heat activated layers by exposure to the atmosphere during manipulation will likely undermine any benefit gained from

thermal activation. If deactivation is to be performed this should be done after the sample has been applied to the layer by exposure to a defined gas phase in an enclosed container. Atmospheres of different constant relative humidity can be obtained by using solutions of concentrated sulfuric acid or saturated solutions of various salts [19]. Acid or base deactivation can be carried out in a similar manner by exposure to, for example, ammonia or hydrochloric acid fumes. When such harsh conditions are required to obtain acceptable zone migration and shapes on inorganic oxide sorbents often a better alternative is to try chemically bonded layers for the separation. If successful it generally results in a simpler methodology.

3. Mobile phase selection

Methods of classifying solvents into groups with similar properties have to take account of two features of the role of solvents in the separation process. Empirically it is recognized that solvents differ in strength and selectivity. Strength is a single parameter ranking of the solvent's ability to cause migration in chromatography. It is a composite property of the stationary phase and the solvent and cannot be considered as a fundamental property of the solvent alone. For example, water is a strong solvent when the stationary phase is silica gel and a weak solvent when the stationary phase is a chemically bonded silica gel layer.

Solvent selectivity is seen as the factor that distinguishes individual solvents that have suitable solvent strength for a separation. In reality, separations result from the competition between the mobile and stationary phases for solutes based on the differences of all intermolecular interactions with the solute in both phases. We can attempt to organize solvents on scales of relative selectivity but when applied to separations the outcome must consider the properties of the stationary phase. As useful as selectivity scales are for initial solvent selection there is no more than a qualitative link between solvent selectivity and chromatographic separations. Methods that attempt to model chromatographic separations need to consider simultaneously mobile and stationary phase properties.

Four methods have been used to rank solvents according to their selectivity. The Hidebrand solubility parameter approach has been little used in thin-layer chromatography and will not be considered here [1]. Snyder's solvent selectivity triangle approach is the most widely used but in some respects the least satisfactory. Solvents are classified based on their interactions with three prototypical solutes determined by their gas–liquid distribution constants corrected for differences in solvent molecular mass and dispersion interactions (assumed identical to the interactions of a hypothetical *n*-alkane with the same molar volume) [20]. The sum of the three polar distribution constants provides a measure of solvent strength (P') and the ratio of individual polar distribution constants to their sum a measure of selectivity (x_e, x_d and x_n). Representing each solvent by the three solvent selectivity coordinates and plotting the results on the surface of a triangle ($x_e+x_d+x_n=1$) resulted in the classification of solvents into eight groups. Solvents in the same group are expected to show similar separation properties and are only appropriate for fine tuning separations. Solvents from different groups have different selectivity characteristics and are likely to provide different migration orders. The important contribution made by the selectivity triangle solvent classification was the idea that method development should involve the selection of a typical solvent (or a few solvents only) from individual groups and that all groups should be represented in the method development process to ensure that the full range of available solvent selectivity is explored. A suitable selection of solvents for method development in thin-layer chromatography based on the solvent selectivity triangle is summarized in Table 3 [20].

The most significant limitation of the solvent selectivity triangle approach is the association of an individual intermolecular interaction with the properties of a single solute. Thus, ethanol is used to determine solvent hydrogen-bond basicity (x_e), dioxane hydrogen-bond acidity (x_d) and nitromethane dipole-type interactions (x_n). Ethanol is a stronger hydrogen-bond base than it is an acid and considerably dipolar, while all three solutes are significant hydrogen-bond bases and dipolar. If it is assumed that the solubility of ethanol is predominantly due to its hydrogen-bond acidity then an

Table 3
Solvent strength and selectivity parameters based on Snyder's selectivity triangle (S_T is an empirical solvent strength parameter used in reversed-phase chromatography)

Solvent	Selectivity group	Solvent strength		Solvent selectivity		
		(P')	(S_T)	x_e	x_d	x_n
n-Butyl ether	I	2.1		0.44	0.18	0.38
Diisopropyl ether		2.4		0.48	0.14	0.38
Methyl *tert*.-butyl ether		2.7				
Diethyl ether		2.8		0.53	0.13	0.34
n-Butanol	II	3.9		0.59	0.19	0.25
2-Propanol		3.9		0.55	0.19	0.27
1-Propanol		4.0		0.54	0.19	0.27
Ethanol		4.3	3.6	0.52	0.19	0.29
Methanol		5.1	3.0	0.48	0.22	0.31
Tetrahydrofuran	III	4.0	4.4	0.38	0.20	0.42
Pyridine		5.3		0.41	0.22	0.36
Methoxyethanol		5.5		0.38	0.24	0.38
Dimethylformamide		6.4		0.39	0.21	0.40
Acetic acid	IV	6.0		0.39	0.31	0.30
Formamide		9.6		0.38	0.33	0.30
Dichloromethane	V	4.3		0.27	0.33	0.40
1,1-Dichloroethane		3.5		0.30	0.21	0.49
Ethyl acetate	VI	4.4		0.34	0.23	0.43
Methyl ethyl ketone		4.7		0.35	0.22	0.43
Dioxane		4.8	3.5	0.36	0.24	0.40
Acetone		5.1	3.4	0.35	0.23	0.42
Acetonitrile		5.8	3.1	0.31	0.27	0.42
Toluene	VII	2.4		0.25	0.28	0.47
Benzene		2.7		0.23	0.32	0.45
Nitrobenzene		4.4		0.26	0.30	0.44
Chloroform	VIII	4.3		0.31	0.35	0.34
Dodecafluoroheptanol		8.8		0.33	0.40	0.27
Water		10.2	0	0.37	0.37	0.25

inflated value for the hydrogen-bond basicity of the solvent is obtained and even solvents with limited hydrogen-bond basicity could be classified as moderately strong hydrogen-bond bases due to their capacity for dipole-type interactions. Since it is impossible to find a test solute that is a strong hydrogen-bond acid or base and is not dipolar, it is also impossible to characterize intermolecular interactions based on the solubility properties of single solutes.

The above problems are circumvented in the solvatochromic scale of solvent selectivity based on the studies of Kamlet–Taft and co-workers on the influence of solvent effects on the shift in spectroscopic absorption bands. The solvatochromic parameters are average values for a number of select solutes and somewhat independent of solute identity. Some representative values for the solvatochromic parameters of common solvents used in thin-layer chromatography are summarized in Table 4, where π_1^* is a measure of solvent dipolarity and polarizability, and α_1 and β_1 solvent hydrogen-bond basicity and acidity, respectively. Further values are compiled in Refs. [20,21] together with comments on their use to characterize solvent properties. By

Table 4
Solvatochromic solvent selectivity parameters (italicized solvents are only weakly attached to a group. A ''?'' indicates that the value is unknown or uncertain)

Solvent	Solvatochromic parameters		
	π_1^*	α_1	β_1
n-Butyl ether	0.27	0	0.46
Diisopropyl ether	0.27	0	0.49
Methyl *tert*.-butyl ether			
Diethyl ether	0.27	0	0.47
(*Triethylamine*	*0.14*	*0*	*0.71*)
Pyridine	0.87	0	0.64
Dimethylformamide	0.88	0	0.69
Dimethyl sulfoxide	1.00	0	0.76
(*Nitrobenzene*	*1.01*	*0*	*0.39*)
Dichloromethane	0.82	0.30	0
(*Chloroform*	*0.58*	*0.44*	*0*)
Ethyl acetate	0.55	0	0.45
Methyl ethyl ketone	0.67	0.06	0.48
Dioxane	0.55	0	0.37
Acetone	0.71	0.08	0.48
Tetrahydrofuran	0.58	0	0.55
(*Acetonitrile*	*0.75*	*0.19*	*0.31*)
Toluene	0.54	0	0.11
Benzene	0.59	0	0.10
(*1,1-Dichloroethane*	*0.81*	*0*	*0*)
n-Butanol	0.47	0.79	0.88
2-Propanol	0.48	0.76	0.95
1-Propanol	0.52	0.78	?
Ethanol	0.54	0.83	0.77
(*Methanol*	*0.60*	*0.93*	*0.62*)
Acetic acid	0.64	1.12	?
Formamide	0.97	0.71	?
Water	1.09	1.17	0.18
(*2,2,2-Trifluoroethanol*	*0.73*	*1.51*	*0*)

hierarchical cluster analysis the solvents can be categorized into groups with similar selectivity for polar interactions, Fig. 3. Group membership is similar to Snyder's solvent selectivity groups with a few exceptions. The ethers are weakly dipolar and hydrogen bond basic solvents with no hydrogen-bond acidity. This group is loosely connected to triethylamine, which is a stronger hydrogen-bond base. The second group of solvents headed by pyridine are stronger dipolar/polarizable and hydrogen-bond base solvents than the ethers with no hydrogen-bond acidity. Nitrobenzene is only loosely connected with this group and is a much weaker hydrogen-bond base. Dichloromethane and chloroform are grouped together but with obvious differences. They possess intermediate dipolarity/polarizability and hydrogen-bond acidity but are not hydrogen-bond bases. The large group of solvents headed by ethyl acetate possess intermediate dipolarity/polarizability and hydrogen-bond basicity with no or weak hydrogen-bond acidity. Their properties are similar to the group containing pyridine but less intense. Acetonitrile is loosely connected to this group but is really behaving independently. Toluene and benzene have intermediate dipolarity/polarizability and weak hydrogen-bond basicity and no hydrogen-bond acidity. 1,1-Dichloroethane is loosely connected with this group but is significantly more dipolar/polarizable. The alcohols are strong hydrogen-bond acids and bases with intermediate dipolarity/polarizability. Methanol is detached from the main group because of its greater capacity for intramolecular hydrogen bonding. Water and 2,2,2-trifluoroethanol are only loosely grouped together and differ from the other solvents in being strong hydrogen-bond acids with minimal hydrogen-bond basicity. Individually the two solvents differ in all solvatochromic parameters, so one solvent does not substitute for the other, but from among the solvents clustered 2,2,2-trifluoroethanol is the closest relative to water, albeit that they are readily distinguishable in terms of solvent properties.

The main problem with solvent classification based on the solvatochromic parameters is that it considers only the polar interactions of the solvents and not their cohesion. The transfer of a solute from one solvent to another occurs with (approximate) cancellation of dispersion interactions, but the energy required for cavity formation in the two solvents is not necessarily self-canceling, and when one of these solvents is water, cancellation of the cavity term is unlikely. Solvent selection should also consider cohesion as well as the capacity for polar interactions. For this purpose we need to consider a model that contains a contribution from cavity formation in addition to polar interactions. The solvation parameter model of Abraham, widely used in gas chromatography [22], is suitable for use in solvent classification of experimental gas–liquid distribution con-

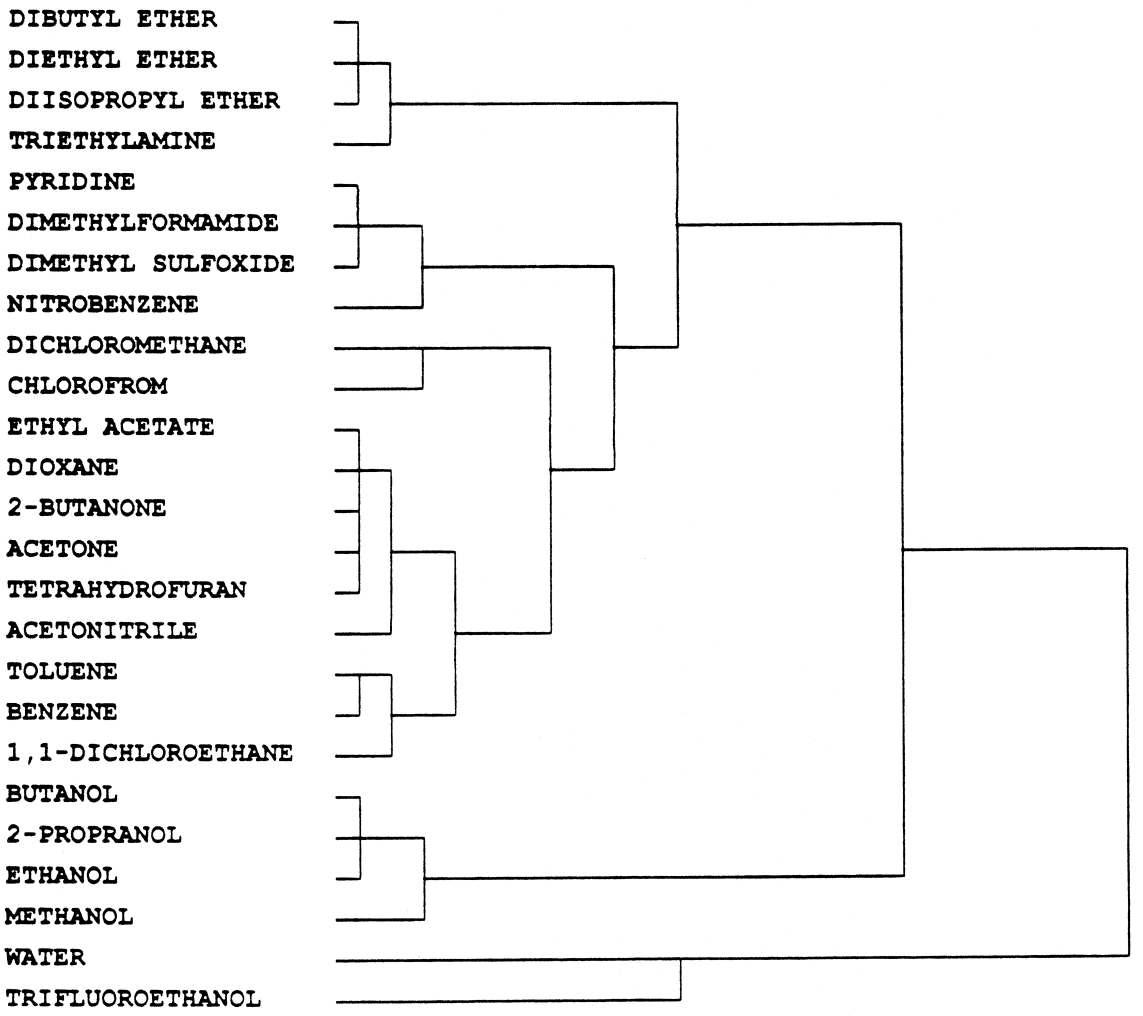

Fig. 3. Average linkage (within-group) cluster dendrogram for solvents with the solvatochromic parameters as variables.

stants [23–25]. Abraham's model is set out below in a form suitable for analyzing gas–liquid distribution systems:

$$\log K_L = c + l \log L^{16} + rR_2 + s\pi_2^H + a\sum\alpha_2^H + b\sum\beta_2^H \quad (1)$$

where $\log K_L$ is the gas–liquid distribution constant and the solute descriptors are $\log L^{16}$ the distribution constant for the solute between a gas and *n*-hexadecane at 298 K, R_2 excess molar refraction (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 $\Sigma\beta_2^H$ the solute's effective hydrogen-bond acidity and hydrogen-bond basicity, respectively. The system constants in Eq. (1) are defined by their complementary interactions with the solute descriptors. The *r* constant refers to the capacity of the solvent for interaction with solute n- or π-electrons and the *s* constant to the solvent's capacity for dipole-type interactions. The *a* constant characterizes the solvent's hydrogen-bond basicity (because a basic solvent will interact with an acidic solute) and the *b* constant the solvent's hydrogen-bond acidity. The *l* constant together with contributions contained in the

model constant (*c* term) accounts for contributions from cavity formation and dispersion interactions. It can be assumed that the gas phase behaves ideally so all contributions measured by the system constants represent interactions in the solvent. The system constants are determined by multiple linear regression analysis of experimental gas–liquid distribution constants for a varied group of solutes, sufficient in number and variety to establish the statistical and chemical validity of the model.

At present there are a limited number of useful compilations of gas–liquid distribution constants for varied solutes in different volatile solvents for classification of all solvents of interest to thin-layer chromatography. The available data are summarized in Table 5 resulting in the cluster dendrogran in Fig. 4. The group headed by benzene has low cohesion, weak hydrogen-bond properties and is moderately dipolar/polarizable. Chloroform and diiodomethane are singular solvents. They have low cohesion and intermediate dipolarity/polarizability but are significantly more hydrogen-bond acidic than the aromatic hydrocarbons. The hydrocarbons and carbon tetrachloride are low cohesive solvents with little capacity for polar interactions. 3-Ethylphenol and 2,2,2-trifluoroethanol are loosely grouped together and really behave independently. These solvents are strong hydrogen-bond acids, moderately cohesive and dipolar/polarizable. *N*-Formylmorpholine and dimethyl sulfoxide are dipolar/polarizable, significantly cohesive, and strong hydrogen-bond bases with no hydrogen-bond acidity. The alcohols have a general blend of all polar interactions and are moderately cohesive. Acetonitrile and water are singular solvents. Water is the most cohesive of the solvents and the strongest hydrogen-bond acid. It is

Table 5
System constants for distribution between the gas phase and solvent (Eq. (1))

Solvent	System constants					
	r	*s*	*a*	*b*	*l*	*c*
Benzene	−0.31	1.05	0.47	0.17	1.02	0.11
Toluene	−0.22	0.94	0.47	0.10	1.01	0.12
Chlorobenzene	−0.55	1.25	0.36	0	1.04	0.05
1,2-Dichloroethane	−0.15	1.44	0.65	0.74	0.94	0.01
Chloroform	−0.60	1.26	0.28	1.37	0.98	0.17
Diiodomethane	0.32	1.34	0.83	1.19	0.87	−0.74
Hexane	−0.17	0	0	0	0.98	0.29
Cyclohexane	0	−0.18	0	0	1.02	0.22
Hexadecane	0	0	0	0	1.00	0
Carbon tetrachloride	−0.20	0.35	0.07	0.27	1.04	0.23
3-Ethylphenol	−0.20	0.87	1.80	3.42	0.90	−1.08
2,2,2-Trifluoroethanol	−0.61	1.46	1.90	4.46	0.63	−0.13
N-Formylmorpholine	0	2.57	4.32	0	0.73	−0.53
Dimethyl sulfoxide	−0.20	2.89	5.46	0	0.73	−0.59
Methanol	−0.22	1.17	3.70	1.43	0.77	0
Ethanol	−0.21	0.79	3.63	1.31	0.85	0.01
Tris(2-ethylhexyl) phosphate	−0.26	0.91	3.47	0	0.96	−0.07
Octan-1-ol	−0.12	0.44	3.69	0.59	0.93	0.07
Acetonitrile	−0.22	2.19	2.38	0.41	0.73	0
Water	0.82	2.74	3.90	4.80	−2.13	−1.27

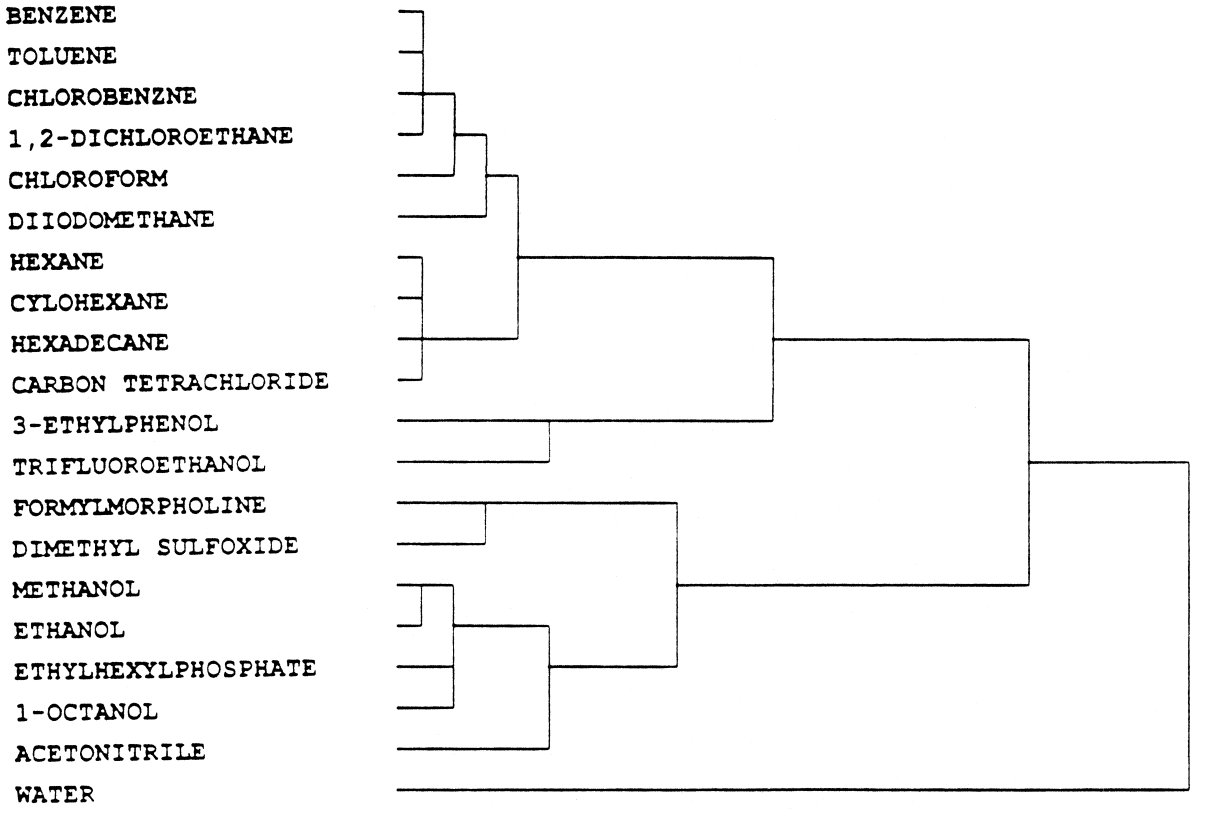

Fig. 4. Average linkage (within-group) cluster dendrogram for solvents with Abraham's system constants as variables.

also a strong hydrogen-bond base and has significant dipolar/polarizability. Its properties more clearly set it apart from the other solvents than any other entry in Table 5. Acetonitrile is moderately cohesive, strongly dipolar/polarizable and quite hydrogen-bond basic with weak hydrogen-bond acidity.

3.1. Selection of primary solvents for mobile phase optimization

Based on the above considerations and practical experience we suggest the following primary solvents for initial use in mobile phase optimization: hexane, toluene, methyl *tert*.-butyl ether, dichloromethane, chloroform, ethyl acetate, acetone, pyridine, triethylamine, acetonitrile, methanol, 2-propanol, 2,2,2-trifluoroethanol, acetic acid and water. For normal-phase chromatography hexane is the strength adjusting solvent for weak and moderately polar compounds. For polar compounds the strongest solvent that fails to result in migration of the sample components is used. This also allows solvents that are immiscible with hexane to be introduced into the mobile phase optimization process.

For reversed-phase chromatography water is always the strength adjusting solvent and the solvents useful for mobile phase optimization are restricted to those solvents that are miscible with water. Suitable solvents are methanol, 2-propanol, 2,2,2-trifluroethanol, acetone, pyridine (or *N*,*N*-dimethylformamide) and acetonitrile. Solvents which are strong hydrogen-bond bases with weak dipole-type properties and miscible with water are uncommon. Triethylamine is the best of these solvents but its low water solubility restricts its practical use to ternary solvent mixtures. Acetone, acetonitrile, 2-propanol, methanol and 2,2,2-trifluoroethanol provide a convenient range of hydrogen-bond acidity. Acetonitrile (or dioxane), acetone (or tetrahydrofuran) and pyridine (or *N*,*N*-dimethylformamide) provide a

reasonable range of hydrogen-bond basicity. Only acetonitrile is a significant hydrogen-bond acid but all are dipolar. *N*,*N*-Dimethylformamide and pyridine are stronger hydrogen-bond bases than the other solvents but otherwise similar to each other in their capacity for polar interactions. *N*,*N*-Dimethylformamide has low volatility and is not easily evaporated from the layer prior to detection while pyridine has an offensive odor and is a stronger protonic base in aqueous solution. These secondary properties tend to dictate which one from the pair of solvents is preferred for a particular separation. The six selected primary water-miscible organic solvents are expected to provide a reasonable range of selectivity when combined with water, but as a group they span only a fraction of the solvent selectivity space available.

4. Mobile phase optimization

Given the similarity in the retention mechanism it is hardly surprising that the principal methods of mobile phase optimization in thin-layer chromatography are similar to those advanced for high-performance liquid chromatography [26–28]. Since detection occurs in the presence of the stationary phase and absence of the mobile phase, a wider range of UV absorbing solvents are commonly used in thin-layer chromatography than is the case for high-performance liquid chromatography. The most significant difference between column and thin-layer methods is that in thin-layer chromatography equilibrium may not be obtained throughout the separation. Using multi-component mobile phases in thin-layer chromatography can result in the formation of a solvent gradient in the direction of development due to demixing. Demixing is characterized by the selective stationary phase sorption of each component in turn from the mobile phase resulting in an advancing solvent front with a different composition to the bulk mobile phase entering the layer [29]. If demixing is complete, then zones with sharp boundaries are formed, separating the chromatogram into sections of different solvent composition and, therefore, selectivity. Demixing effects are less apparent when saturated developing chambers are used. The presence of a vapor phase in thin-layer chromatography further complicates matters with both migration distances and migration order influenced by the saturation level of the developing chamber [30]. These considerations hinder mobile phase optimization strategies based on the composition of the solvents added to the developing chamber.

The selection of a mobile phase to separate simple mixtures may not be a particularly difficult problem and can be arrived at quite quickly by guided trial and error methods [26–28]. A solvent of the correct strength for a single development separation will migrate the sample into the R_F range 0.2–0.8, or thereabouts, and if of the correct selectivity, will distribute the sample components evenly throughout this range. If the sample contains a wide range of sample sizes then the correct mobile phase will ensure adequate separation of the major and minor components rather than an even distribution through the R_F range. Solvent systems based on the selected primary solvents can be screened in parallel using either several development chambers or a device like the Vario-KS chamber. The latter allows the simultaneous evaluation of a number of solvents by allowing each of these to migrate along parallel channels scored on a single layer [29]. Alternatively, sample spots (up to 16 on a standard high-performance thin-layer plate) can be applied at suitable positions on a single layer and automatically developed in sequence with 45 μl of solvent or solvent mixtures using an automated sample applicator [28,31]. The individual circular chromatograms enable rapid identification of solvents with suitable strength and selectivity for the separation. However, whenever the number of components in a mixture exceeds all but a small fraction of the zone capacity for the separation system (see Table 1), a more systematic approach for mobile phase optimization is required. We prefer different approaches to mobile phase optimization using the PRISMA model for silica gel and the solvation parameter model for reversed-phase chromatography. The reasons for this selection will become apparent in the following discussion.

4.1. PRISMA model

The PRISMA model was developed by Nyiredy and co-workers for optimization of multiple-component mobile phases in thin-layer and high-performance liquid chromatography [32,33]. Mobile

phase optimization commences with the identification of a minimum of three solvents found to have the highest selectivity for the separation. These are the solvents, which after adjusting to an operational solvent strength as required, provide the greatest number of separated zones for the sample. These solvents are selected in a rapid, guided, trial and error procedure employing separations in parallel.

Between three and five solvents can be selected for construction of the PRISMA model for solvent optimization. Modifiers such as acids, ion-pair reagents, etc., can be added to improve the separation and reduce tailing. Modifiers are generally used in low and constant concentration so that their influence on solvent strength can be neglected. The actual PRISMA model, Fig. 5, is a three-dimensional geometrical design which correlates the solvent strength with the selectivity of the mobile phase [32]. The model consists of three parts: the base or platform representing the modifier; the regular part of the prism with congruent base and top surfaces; and the irregular truncated top prism (frustum). The lengths of the edges of the prism (S_A, S_B, S_C) correspond to the solvent strengths of the neat solvents (A, B and C). Since the selected solvents usually have different solvent strengths, the edges of the prism are generally of unequal length and the top plane of the prism will not be parallel and congruous with its base. Cutting the prism parallel to its base at the height of the lowest edge (determined by the solvent strength of the weakest solvent, solvent C in Fig. 5), gives a regular prism, where the top and any planes representing weaker solvents diluted with a strength adjusting solvent are parallel equilateral triangles. The upper frustum of the model is used for mobile phase optimization of polar compounds in normal-phase chromatography, while the regular part is used for the separation of nonpolar and moderately polar substances.

For polar compounds optimization is always started on the top irregular triangle of the model, either within the triangle, when three solvents are selected, or along one side, for binary mobile phases. Any solvent composition on the face of the triangle can be represented by a three-coordinate selectivity point (P_S); each coordinate corresponding to the volume fraction of the solvent at that position on the triangle, Fig. 5. Optimization is commenced by selecting solvent combinations corresponding to the center point $P_S = 333$ and three other points close to the apexes of the triangle $P_S = 811$, 181 and 118. If the separation obtained is insufficient other selectivity points are tested around the solvent combination that gave the best separation. On changing the selectivity points on the top triangle the solvent strength changes as well, especially when the solvent strengths of the solvents used to construct the prism are considerably different. The strength of the sol-

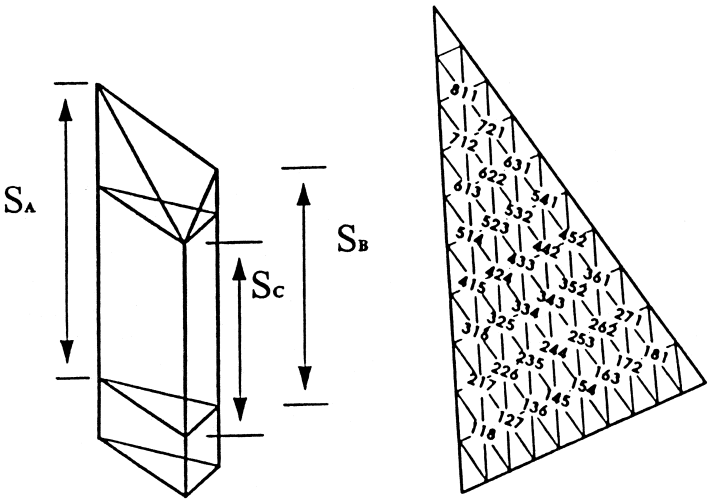

Fig. 5. The PRISMA mobile phase optimization model showing the construction of the prism and the selection of the selectivity points.

vent should be adjusted with the strength adjusting solvent to maintain the separation in the optimum R_F range. It may also be advisable to change the selectivity points by small increments if regular step sizes cause large changes in resolution. To aid optimization experimental data can be fitted to a three-dimensional retardation surface with *x*- and *y*-coordinates as selectivity points and the *z*-coordinate as the R_F value [34].

The regular center portion of the prism is used to optimize the mobile phase composition for the separation of nonpolar and moderately polar compounds. The initial solvent composition corresponds to the center of the triangular top face of the regular prism ($P_S = 333$); this composition is then diluted to bring all sample components into the R_F range 0.2–0.8. At this solvent strength three more chromatograms are run corresponding to the selectivity points close to the apexes of the triangle. These initial runs are then used to choose selectivity points for further chromatograms until the best solvent composition is located. For saturated developing chambers there is a linear correlation between R_F and the solvent strength at a constant P_S value [$\ln R_F = d(S_T) + e$, where S_T is the solvent strength and *d* and *e* are regression constants]. At a constant solvent strength there is a quadratic relationship between R_F and the selectivity points describing the retardation surface [$R_F = a(P_S)^2 + b(P_S) + c$, where *a*, *b* and *c* are regression constants]. These relationships can form the basis of a computer-aided optimization strategy according to a fixed experimental design requiring 18 experimental measurements [33] or a general approach with decisions based on the interpretation of retardation surfaces [35].

The optimization of the solvent strength by varying the selectivity points has to be carried out until the required separation is obtained. If no adequate separation is obtained then a new stationary phase or additional solvents must be selected and the PRISMA model utilized again to optimize the new system.

4.2. Solvation parameter model

The solvation parameter model has been used as the basis of a structure-driven retention model for method development in reversed-phase thin-layer chromatography [16,36–38]. The model should be applicable to normal-phase separations on chemically bonded layers as well, but has not been applied to silica gel layers. For column chromatography it was shown that solute size differences and site-specific surface interactions on silica gel adsorbents are not adequately accounted for by the model and it is likely that these effects would result in poor predictions of retention in thin-layer chromatography as well [39]. Solvent demixing and non-equilibrium affects are further difficulties that plague all bulk solvent composition models for mobile phase optimization on inorganic oxide layers explaining the preference for entirely empirical models like the PRISMA model.

The solvation parameter model is set out below in the form suitable for method development in reversed-phase thin-layer chromatography:

$$R_M = c + mV_X + rR_2 + s\pi_2^H + a\sum\alpha_2^H + b\sum\beta_2^0 \quad (2)$$

The symbols retain the same meaning as indicated for Eq. (1) except that $\log L^{16}$ is replaced by the solute's characteristic volume V_X. The associated system constant (*m*) is a measure of the difference in free energy of cavity formation in the two phases combined with the component from dispersion interactions that is not self-canceling for transfer between the two phases. The new solute descriptor accounts for the fact that two condensed phases are involved in the model whereas for Eq. (1) transfer was from an ideal gas phase and a solute descriptor capable of modeling both cavity formation and dispersion interactions simultaneously was required. The correct free energy related dependent variable is the R_M value, but this is easily transposed into the more familiar R_F value since $R_M = \log (1 - R_F)/R_F$.

Method development for binary mobile phases using the solvation parameter model is based on the use of system maps. A system map is a continuous plot of the system constants obtained from experimental data fit to the solvation parameter model against mobile phase composition. The system map is a permanent record of system properties used in all calculations. Generally speaking the system constants can be fit to low-order polynomial functions of mobile phase composition (%, v/v, organic solvent) to provide continuous functions for the interpolation

Table 6
System constants for pyridine–water mixtures on chemically bonded layers

Pyridine (%, v/v)	System constants					
	m	*r*	*s*	*a*	*b*	*c*
(i) Merck HPTLC RP-18 WF254s						
0	2.83 (±0.12)	0	0	−0.54 (±0.05)	−1.07 (±0.06)	−1.15 (±0.12)
5	1.97 (±0.14)	−0.23 (±0.06)	0	0	−1.50 (±0.08)	−0.08 (±0.12)
10	1.58 (±0.09)	0	−0.23 (±0.05)	0	−1.27 (±0.07)	−0.07 (±0.10)
15	1.41 (±0.08)	0	−0.21 (±0.05)	0	−1.23 (±0.07)	−0.04 (±0.10)
20	1.11 (±0.10)	0.17 (±0.05)	−0.29 (±0.06)	0.15 (±0.06)	−1.19 (±0.07)	0.04 (±0.11)
30	0.85 (±0.10)	0.23 (±0.04)	−0.27 (±0.05)	0.14 (±0.06)	−0.95 (±0.06)	−0.25 (±0.10)
40	0.72 (±0.06)	0.14 (±0.03)	−0.12 (±0.03)	0.30 (±0.04)	−0.83 (±0.04)	−0.54 (±0.07)
50	0.52 (±0.03)	0.12 (±0.01)	−0.07 (±0.02)	0.23 (±0.03)	−0.73 (±0.03)	−0.61 (±0.02)
(ii) Merck HPTLC CN F254s						
0	2.46 (±0.29)	0	0	0	−1.64 (±0.13)	−0.86 (±0.25)
5	2.06 (±0.17)	0.59 (±0.17)	0	0	−2.17 (±0.12)	−0.75 (±0.21)
10	1.86 (±0.15)	0.65 (±0.14)	0	0.30 (±0.10)	−2.04 (±0.10)	−0.87 (±0.16)
15	1.94 (±0.19)	0.43 (±0.15)	0	0.41 (±0.11)	−1.86 (±0.12)	−1.04 (±0.21)
20	1.75 (±0.11)	0.20 (±0.09)	0	0.48 (±0.08)	−1.52 (±0.09)	−1.00 (±0.13)
25	1.59 (±0.10)	0	0	0.42 (±0.07)	−1.31 (±0.08)	−0.88 (±0.11)
30	1.32 (±0.05)	0	0	0.17 (±0.04)	−1.32 (±0.04)	−0.66 (±0.06)
40	0.93 (±0.06)	0	0	0	−1.21 (±0.05)	−0.53 (±0.06)
50	0.52 (±0.07)	0	0	0	−0.64 (±0.07)	−0.55 (±0.08)

of system constants values at any composition and for the calculation of retention maps. System maps for the primary solvents identified earlier on octadecylsiloxane-bonded [16] and cyanopropylsiloxane-bonded silica gel layers [38] are given elsewhere. New results for the construction of system maps for pyridine–water mixtures on octadecylsiloxane-bonded and cyanopropylsiloxane-bonded silica gel layers are given in Table 6 and for acetone–water and methanol–water mixtures on a spacer bonded propanediol silica gel layer in Table 7. An example of a system map for pyridine–water mixtures on a

Table 7
System constants for methanol–water and acetone–water mixtures on a spacer bonded propanediol layer (Merck HPTLC DIOL F254s)

Organic solvent (%, v/v)	System constants					
	m	*r*	*s*	*a*	*b*	*c*
(i) Methanol–water						
0	0.66 (±0.12)	1.15 (±0.09)	−0.18 (±0.05)	−0.40 (±0.07)	−1.48 (±0.06)	−0.59 (±0.12)
10	1.14 (±0.12)	0.66 (±0.08)	0	0	−1.29 (±0.06)	−1.15 (±0.11)
20	0.95 (±0.11)	0.63 (±0.07)	0	0	−1.19 (±0.06)	−1.10 (±0.09)
30	0.63 (±0.12)	0.57 (±0.08)	0	0	−1.11 (±0.06)	−0.88 (±0.09)
40	0.37 (±0.11)	0.56 (±0.07)	0	−0.15 (±0.05)	−1.03 (±0.06)	−0.70 (±0.10)
50	0.21 (±0.09)	0.37 (±0.06)	0	−0.14 (±0.04)	−0.73 (±0.04)	−0.65 (±0.09)
(ii) Acetone–water						
10	0.35 (±0.10)	0.84 (±0.06)	−0.21 (±0.04)	−0.29 (±0.05)	−1.18 (±0.05)	−0.27 (±0.10)
20	0.28 (±0.10)	0.55 (±0.06)	−0.15 (±0.03)	−0.22 (±0.04)	−1.10 (±0.04)	−0.28 (±0.09)
30	0.16 (±0.13)	0.45 (±0.08)	0	−0.21 (±0.06)	−0.98 (±0.05)	−0.35 (±0.10)
40	0	0.44 (±0.04)	0	−0.14 (±0.04)	−0.84 (±0.04)	−0.48 (±0.06)
50	0	0.43 (±0.04)	0	−0.22 (±0.04)	−0.75 (±0.04)	−0.71 (±0.05)
60	0	0.20 (±0.06)	0	−0.50 (±0.05)	−0.45 (±0.05)	−0.80 (±0.07)

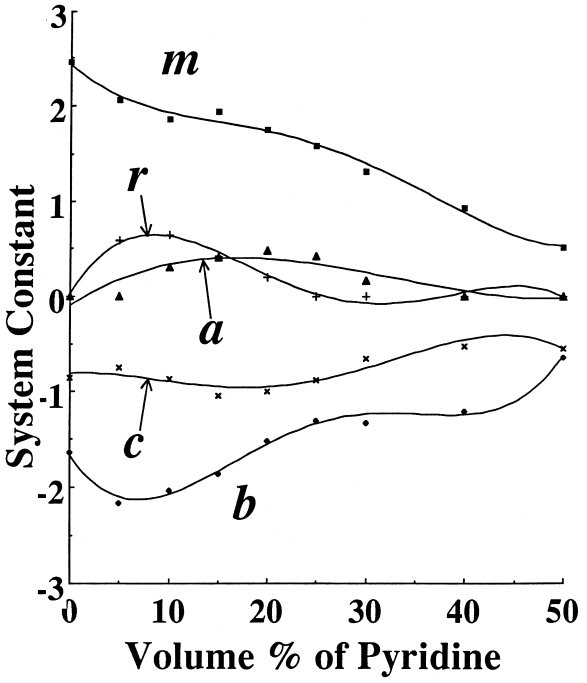

Fig. 6. A system map for pyridine–water mobile phases on a cyanopropylsiloxane-bonded silica gel layer (Merck HPTLC CN F254s).

cyanopropylsiloxane-bonded silica gel layer is shown in Fig. 6. In addition to methods development the system maps provide insight into the retention mechanism. In reversed-phase chromatography the driving force for retention is the difference in cohesion between the mobile and stationary phases, sometimes aided by favorable lone-pair electron interactions with the solvated stationary phase (*m* and *r* system constants are positive). Polar interactions, particularly solute hydrogen-bond basicity, either reduce retention or are not significant (*s*, *a*, *b* system constants are negative or zero). This is a reflection of the dominant properties of water, its high cohesive energy and hydrogen-bond acidity, and the failure of the solvated stationary phase to compete with the aqueous mobile phase in these interactions.

For method development retention maps are created from the system maps for all solutes to be separated. This requires that the solute descriptors are either known or can be conveniently estimated for the compounds of interest. Solute descriptors are available for about 4000 compounds and methods for their experimental determination [22] or estimation from structure by computational approaches [40,41] are well advanced. For any solutes with known descriptors their R_F value as a function of composition are calculated by summing the product terms for the experimental system constants and solute descriptors over the selected composition range using Eq. (2). This is conveniently done using a spreadsheet for the calculations and graphics for evaluation. All mobile phase and stationary phase combinations for which system maps are available can be compared in the search for the optimum system in this way. A typical retention map for the separation of a mixture of steroids on a cyanopropylsiloxane-bonded silica gel layer with mixtures of pyridine–water as mobile phase is shown in Fig. 7. Those solvent compositions resulting in acceptable zone separation are easily identified by visual inspection. From Fig. 7, as an example, mobile phase compositions containing less than 25% (v/v) pyridine are too weak to provide significant migration and those containing more than 50% (v/v) pyridine are too strong to provide sufficient retention. A mobile phase composition of about 40% (v/v) pyridine provides near optimum separation conditions for this mixture. Computer simulation of retention maps allows those systems (defined as a

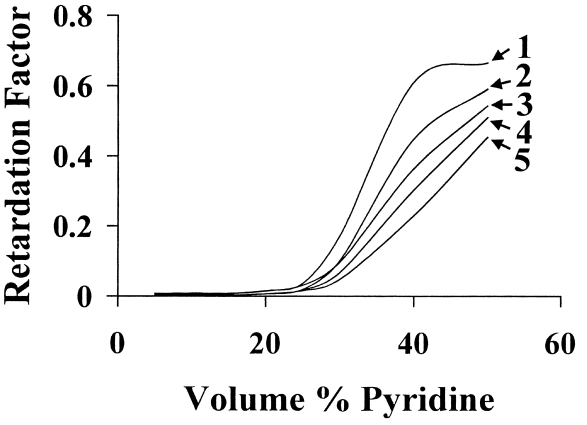

Fig. 7. Retention map for the separation of a five-component steroid mixture on a cyanopropylsiloxane-bonded silica gel layer calculated from the system map in Fig. 6. Steroids: 1=hydrocortisone, 2=estriol, 3=testosterone, 4=estradiol and 5=progesterone.

combination of stationary and mobile phase) likely to provide an acceptable separation to be identified before experimental work commences. This approach to modeling, therefore, is able to direct experimental procedures and not just simply aid in their interpretation. The agreement between model predicted and experimental R_F values is generally good. Differences are typically less than 0.05 R_F units. Retention surfaces for the optimization of ternary solvent systems have only been demonstrated for column chromatography so far [42,43], but should be equally applicable to thin-layer chromatography and are currently being researched.

4.3. Comments on computer simulation models

The solvation parameter model can be considered as a suitable model for computer-aided method development in thin-layer chromatography. Computer-aided strategies for mobile phase optimization using window diagrams, overlapping resolution maps, simplex methods, and iterative procedures have found occasional use in thin-layer chromatography, but not to the extent that they are used in column chromatography [26,27,44]. In these procedures some form of statistical design is used to select a group of solvents for evaluation, or alternatively, the results obtained from the arbitrary selection of a series of solvents are compared to indicate the best separation obtained. In the simplex method an evolving experimental design is used to predict new mobile phase compositions from initial solvent compositions guided by a set of rules that (hopefully) direct the movement of the simplex to a solvent composition providing an acceptable separation. To rank chromatograms for computer interpretation a single-value numerical index of separation quality is required [44]. A large number of mathematical functions have been used for this purpose but none have been found ideal. This is a severe limitation of computer-aided approaches at this time. The selection of the separation quality index can lead to the prediction of different optimum mobile phase compositions for a separation, which is disconcerting. Resolution based functions favored in column chromatography are not useful in thin-layer chromatography because zone widths are difficult to predict in absolute terms and depend on migration distance. More commonly separation quality is defined by an index based on the zone center separation, sometimes combined with a function to characterize the zone distribution throughout the separation.

Computer simulations of separations, at least for binary mobile phases containing a single strong solvent are usually based on one of the functions $R_M = a \log (X_S) + b$ or $R_F = a(X_S)^2 + b(X_S) + c$ where a, b and c are regression constants and X_S is the mole fraction of strong solvent [45]. A certain amount of experimental data is required to statistically define the regression constants after which additional R_F values can be estimated by interpolation. This approach can only estimate results for compounds included in the initial model and new experimental data are required if additional compounds are included. Results for computer simulation of two-dimensional separations based on sequential application of the above equations for one-dimensional separations varied from poor to very good without obvious reasons for the variation [46].

4.4. Affect of sample polarity range

Samples containing components with some reasonable variation in polarity or hydrophobicity are potentially separable by conventional development techniques provided that the number of components is significantly smaller than the zone capacity of the layer. Samples that are difficult to separate in this way are those at either extreme of the property scale, either all components have a narrow range of properties or the components have an excessively wide range of properties. Isocratic multiple development with incremental changes in the solvent entry position is the preferred approach for the separation of samples with a narrow polarity range that tend to migrate as a compact group (e.g., isomers, diastereomers and analogs with minor structural variations) [2,47,48]. The most selective solvent for the separation can be identified from the screening stage of the PRISMA model. Increased resolution of sample zones results from the increase in the zone migration distance achieved, while the normal zone broadening mechanism leading to increased zone widths is effectively counteracted. Maintaining a mobile phase velocity range that is close to the most favorable

value for the separation increases the separation efficiency.

For mixtures containing components spanning a wide retention range, some form of gradient development is required to obtain a separation of all components in either a single chromatogram or in separate chromatograms for successive developments. Continuous solvent composition gradients, as commonly employed in column liquid chromatography, are rarely used in thin-layer chromatography. These require special equipment and experimental conditions that are less convenient than step gradients. In addition, step gradients can be easily constructed to mimic a continuous linear gradient with the added advantage that the zone refocusing effect can be employed to minimize zone broadening.

Incremental multiple development using gradients of increasing solvent strength is used to fractionate complex mixtures by separating just a few components at each step [49]. Compounds of interest are usually quantified by scanning those intermediate steps at which the compounds are adequately separated. In this way the zone capacity can be much greater than predicted for a complete separation recorded as a single chromatogram. On the other hand, this approach can be tedious when many components are of interest, and it is difficult to automate. The alternative approach is the use of incremental multiple development with solvent gradients of decreasing solvent strength. In this case the sample is developed for the shortest distance in the strongest mobile phase with each subsequent, longer development using mobile phases of decreasing solvent strength. This strategy is most useful when the final separation is to be recorded as a single chromatogram; it is, on the other hand, limited in peak capacity because all components must be fitted between the position of the sample origin and the final solvent front. The decreasing solvent strength gradient approach is the operating basis of the automated multiple development chamber [2,6,7]. The two approaches for exploiting solvent strength gradients are complementary, but in practice, the ease of automation and repeatability favor automated multiple development.

Optimized gradients for automated multiple development are usually arrived at by pragmatic means rather than reliance on theory [6,7,44,50]. Two general approaches have been adopted for guided trial and error procedures. The first is based on the use of a universal gradient which commences with methanol, ends with hexane, and uses dichloromethane or methyl *tert*.-butyl ether as the intermediate or base solvent (typically employing 25 steps). By scaling and superimposing the chromatogram of the separation above the theoretical gradient profile, those regions of the chromatogram affecting the separation are easily identified. The gradient shape can then be modified to enhance resolution in those regions of the chromatogram that are poorly separated or to make better use of the zone capacity by minimizing regions devoid of sample zones. For relatively simple mixtures this approach is often satisfactory. The universal gradient is not a linear solvent strength gradient and the abrupt change in solvent strength occurring during the gradient can cause grouping of sample components resulting in poor resolution [51]. Resolution can be inadequate also because of zone distortion, particularly tailing. Adjusting the layer conditioning step or adding a tailing inhibitor in low concentration (e.g., formic acid, water, ammonia, etc.) to those mobile phase compositions that influence the migration of the distorted zones is at times useful. However, in those cases where the resolution remains inadequate after making the above adjustments it is necessary to identify a different gradient composition for the separation. At this point the solvent screening portion of the PRISMA model can be employed to identify more selective solvents for incorporation into the gradient as a replacement for the initial, terminal, or base solvent. Alternatively, if the composition of the sample is known and standards are available, isocratic plots of R_M against the composition of binary solvent mixtures can be used to infer suitable gradient separation conditions [52].

Not all samples are suitable for separation by automated multiple development [7,49,53]. Compounds with significant vapor pressure may be lost during the repeated solvent evaporation steps performed under vacuum. Artifact peaks from chemically unstable compounds may be mistaken for sample components. Compounds that are easily oxidized, photolyzed, or hydrolyzed in contact with the layer to more polar products yield two separated zones in

each development, and after repeated development, the accumulative effect can result in a complex chromatogram from a single component. Solvents of low volatility and/or high polarity should be avoided as mobile phase components since they are only slowly removed from the layer by suction. This can extend the total separation time considerably. Solvent impurities can be a source of ghost peaks (identifiable by scanning between sample lanes).

The large number of variables and our limited knowledge of their interactions combined with the long separation times resulting from the sequential steps of the unitary development process invariably mean that method development for automated multiple development is a lengthy process. For mixtures with more than about 15–20 components of interest it is often faster to develop separate methods in which the least polar and most polar components of the mixture are separated separately using different programs. Once a method is developed the results are surprisingly repeatable over time and the sample throughput is high because several samples can be separated simultaneously.

5. Conclusions

It is easy to get the general impression from the literature that mobile phase optimization is akin to making a spaghetti sauce. The more ingredients in there the better. If solvents are combined without direction by simply mixing or adding the results from less than perfect separations a recipe might be arrived at that provides an acceptable separation but uses a complex mobile phase. Such mobile phases rarely provide rugged separations. Systematic optimization returns control to the analyst and generally results in simpler mobile phase compositions, achieved according to a logical framework and more reliable separations.

It is also clear from the contemporary literature that silica gel is the dominant stationary phase for thin-layer chromatography. This sometimes blinds the user to the benefits of using chemically bonded phases for normal-phase separations. Apart from differences in selectivity it is often possible to simplify mobile phase compositions and to avoid the use of corrosive reagents for layer pretreatment to control zone shape. This almost always provides a more rugged separation.

References

[1] C.F. Poole, S.K. Poole, Chromatography Today, Elsevier, Amsterdam, 1991.
[2] C.F. Poole, J. Chromatogr. A 856 (1999) 399.
[3] G. Guiochon, A.M. Siouffi, J. Chromatogr. 245 (1982) 1.
[4] G. Guiochon, M.F. Gonnord, A.M. Siouffi, M. Zakaria, J. Chromatogr. 250 (1982) 1.
[5] J.M. Davis, J. Chromatogr. A 831 (1999) 37.
[6] C.F. Poole, S.K. Poole, J. Chromatogr. 703 (1995) 573.
[7] C.F. Poole, M.T. Belay, J. Planar Chromatogr. 4 (1991) 345.
[8] M. Petrovic, M. Kostelan-Macan, S. Balic, J. Planar Chromatogr. 11 (1998) 353.
[9] H. Jork, W. Funk, W. Fischer, H. Wimmer, Thin-Layer Chromatography Reagents and Detection Methods, Vol. 1, VCH, Weinheim, 1990.
[10] H. Jork, W. Funk, W. Fischer, H. Wimmer, Thin-Layer Chromatography Reagents and Detection Methods, Vol. 2, VCH, Weinheim, 1992.
[11] C.F. Poole, S.K. Poole, T.A. Dean, N.M. Chirco, J. Planar Chromatogr. 2 (1989) 180.
[12] C.F. Poole, S.K. Poole, J. Chromatogr. 492 (1989) 539.
[13] E.S. Gankina, I.I. Efinova, J.J. Kever, B.G. Belenkii, Talanta 34 (1987) 167.
[14] H.E. Hauck, H. Halpaap, Chromatographia 13 (1980) 538.
[15] D.L. Grumprecht, J. Chromatogr. 595 (1992) 368.
[16] W. Kiridena, C.F. Poole, J. Planar Chromatogr. 12 (1999) 13.
[17] H.E. Hauck, M. Mack, S. Reuke, H. Herbert, J. Planar Chromatogr. 2 (1989) 268.
[18] R.J. Maxwell, A.R. Lightfield, J. Planar Chromatogr. 12 (1999) 109.
[19] E. Hahn-Deinstrop, J. Planar Chromatogr. 6 (1993) 313.
[20] V.J. Barwick, Trends Anal. Chem. 16 (1997) 293.
[21] A. de Juan, G. Fonrodona, E. Casassas, Trends Anal. Chem. 16 (1997) 52.
[22] M.H. Abraham, C.F. Poole, S.K. Poole, J. Chromatogr. A 842 (1999) 79.
[23] M.H. Abraham, G.S. Whiting, W.J. Shuely, R.M. Doherty, Can. J. Chem. 76 (1998) 703.
[24] M.H. Abraham, G.S. Whiting, P.W. Carr, H. Ouyang, J. Chem. Soc., Perkin Trans. 2 (1998) 1385.
[25] M.H. Abraham, J.A. Platts, A. Hersey, A.J. Leo, R.W. Taft, J. Pharm. Sci. 88 (1999) 670.
[26] D. Nurok, Chem. Rev. 89 (1989) 363.
[27] A.-M. Siouffi, J. Chromatogr. 556 (1991) 81.
[28] E. Reich, T. George, J. Planar Chromatogr. 10 (1997) 273.
[29] F. Geiss, Fundamentals of Thin-Layer Chromatography, Hüthig, Heidelberg, 1987.
[30] Sz. Nyiredy, Z. Fater, J. Planar Chromatogr. 7 (1994) 329.
[31] F.L. Birkenshaw, D.G. Waters, J. Planar Chromatogr. 8 (1995) 319.

[32] Sz. Nyiredy, K. Dallenbach-Toelke, O. Sticher, J. Planar Chromatogr. 1 (1988) 336.
[33] Sz. Nyiredy, Z. Fater, J. Planar Chromatogr. 8 (1995) 341.
[34] A. Pelander, K. Sivonen, I. Ojanpera, H. Vuorela, J. Planar Chromatogr. 10 (1997) 434.
[35] P. Vuorela, E.-L. Rahko, R. Hiltunen, H. Vuorela, J. Chromatogr. A 670 (1994) 191.
[36] M.H. Abraham, C.F. Poole, S.K. Poole, J. Chromatogr. A 749 (1996) 201.
[37] W. Kiridena, C.F. Poole, Anal. Commun. 34 (1997) 195.
[38] W. Kiridena, C.F. Poole, J. Chromatogr. A 802 (1998) 335.
[39] W. Kiridena, C.F. Poole, Analyst 123 (1998) 1265.
[40] J.A. Platts, D. Butina, M.H. Abraham, A. Hersey, J. Chem. Inf. Comput. Sci. 39 (1999) 835.
[41] J.A. Platts, M.H. Abraham, D. Butina, A. Hersey, J. Chem. Inf. Comput. Sci. 39 (1999) 835.
[42] W. Kiridena, C.F. Poole, Chromatographia 48 (1998) 607.
[43] D. Bolliet, C.F. Poole, Anal. Commun. 35 (1998) 253.
[44] B. Fried, J. Sherma (Eds.), Handbook of Thin-Layer Chromatography, Marcel Dekker, New York, 1996.
[45] M.C. Frost, T. Lahr, R.M. Kleyle, D. Nurok, J. Chromatogr. A 788 (1997) 207.
[46] D. Nurok, R.M. Kleyle, C.L. McCain, D.S. Risely, K.J. Ruterbories, Anal. Chem. 69 (1997) 1398.
[47] S.K. Poole, C.F. Poole, J. Planar Chromatogr. 5 (1992) 221.
[48] T.H. Dzido, M.A. Hawryl, W. Golkiewicz, E. Soczewinski, J. Planar Chromatogr. 8 (1995) 306.
[49] C.F. Poole, S.K. Poole, W.P.N. Fernando, T.A. Dean, H.D. Ahmed, J.A. Berndt, J. Planar Chromatogr. 2 (1989) 336.
[50] J. Summanen, R. Hiltunen, H. Vuorela, J. Planar Chromatogr. 11 (1998) 16.
[51] P.V. Colthup, J.A. Bell, D.L. Gadsdon, J. Planar Chromatogr. 6 (1993) 386.
[52] G. Lodi, A. Betti, V. Brandolini, E. Menziani, B. Tosi, J. Planar Chromatogr. 7 (1994) 29.
[53] C.F. Poole, S.K. Poole, M.T. Belay, J. Planar Chromatogr. 6 (1993) 438.