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Review

Optimization methods in chromatography and capillary electrophoresis

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Abstract

Many methods have been developed in order to optimize the parameters of interest in either chromatography or capillary electrophoresis. In chemometric approaches experimental measurements are performed in such a way that all factors vary together. An objective function is utilized in which the analyst introduces the desired criteria (selectivity, resolution, time of analysis). Simplex methods and overlapping resolution maps are declining. Factorial designs and central composite designs are more and more popular in electrodriven capillary separations since the number of parameters to master is much larger than in either GC or LC. The use of artificial neural networks is increasing. The advantage of chemometrics tools is that no explicit models are required, conversely the number of experiments to perform may be high and boundaries of the domain are not straightforward to draw and the approach does more than is required. When models are available optimization is easier to perform by regression methods. Computer assisted methods in RPLC are readily available and work well but are still in infancy in CE. Linear solvation energy relationships seem a very valuable tool but estimates of coefficients still require many experiments.

Keywords: Reviews; Optimization; Chemometrics; Mathematical modelling

Contents

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1. Introduction

Optimization in chromatography and related techniques (electrochromatography and electrophoresis) may involve several concepts. We can distinguish kinetic optimization which is an approach to faster analysis from actual optimization strategies whose aim is to obtain the largest number of good quality information while carrying out a limited number of experiments.

Kinetic optimization often uses reduced (or dimensionless) parameters. One of the most prominent examples is the Knox's equation [1]. A thorough insight on LC kinetic optimization can be found in a chapter from Tijssen in a recent book [2]. An extended discussion which involves electrophoresis and electrochromatography has been published by Poppe [3]. Two comprehensive reviews on speed optimization in gas chromatography (GC) have been published recently [4,5].

The large number of chromatographic parameters and the relationships between them rules out the possibility of empirical optimization by trial and error or intentional variation of one or two parameters. Some of these intentional variations or univariate approach in which one parameter is varying whilst others are kept constant may be successful [6] and are often published under the title 'optimization' but fails to take interactions between two or more parameters into account. Our purpose is to consider multivariate optimization. The problem is difficult to tackle as is evident from two drawings (Fig. 1), one in GC [7] the other in LC [8].

A variety of systematic methods has been developed to optimize the parameters of interest.

In grid search methods a large number of experiments (up to 50 or 100!) are carried out and the best is chosen. In sequential methods a few experiments are performed at given levels of parameters and a search routine is carried out to locate the optimum conditions. In simultaneous methods many experiments are performed at selected levels of parameters and a model is built to estimate the quality of separation. Multivariate methods involve simultaneous alteration of a number of parameters according to a predefined regime.

Methods based on sound theoretical basis would be able to predict the behavior of solutes under fixed separation conditions. A great deal of work is in progress on that topic.

Chemometrics provides a tool to find the optimum of a certain response (or responses) or an adequate compromise if requirements are in conflict (complete separation versus analysis time for example).

At the early beginning in the 70s and 80s chemometrics was understood as data processing in chemistry. Nowadays chemometrics is the science of processing the chemical information.

According to the pioneering work of Berridge [9] three classifications of mixtures are to be considered in chemical analysis:

(i) a mixture containing a known number of constituents n

(ii) a mixture in which the total number of constituents n is unknown but only some solutes must be considered.

(iii) a mixture of totally unknown nature.

The following points list the steps by which optimization may proceed:

(1) Define the problem. Facing a sample the analyst must select which type of separation will be convenient: Gas chromatography (GC)?; liquid chromatography (LC)?; capillary electrophoresis (CE)? For that purpose he must know some basic features of the sample (solubility, UV or IR spectrum, elemental analysis, ionizable groups . . .). A strategic approach to HPLC method selection has been described by Massart et al. [10] which makes a search of the hydrophobic, basic, acidic properties of the sample components.

(2) Screen for the appropriate parameters. Some are of primary importance, some are not and simple basic knowledge of chromatography will suffice. For example, there is no need to include flow-rate in chromatography since it can be easily deduced from the equation for the height equivalent to a theoretical plate (HETP).

(3) Select the domain of variation of the parameters (for example water content in RPLC or pH range in CE), it defines the parameter space within which the variable can range.

(4) Select a criterion to evaluate the quality of the separation. This is not simple and depends on the type of mixture to separate. Dealing with chiral separations the criterion is obviously the resolution R_s of the enantiomers. Emphasis can be on analysis

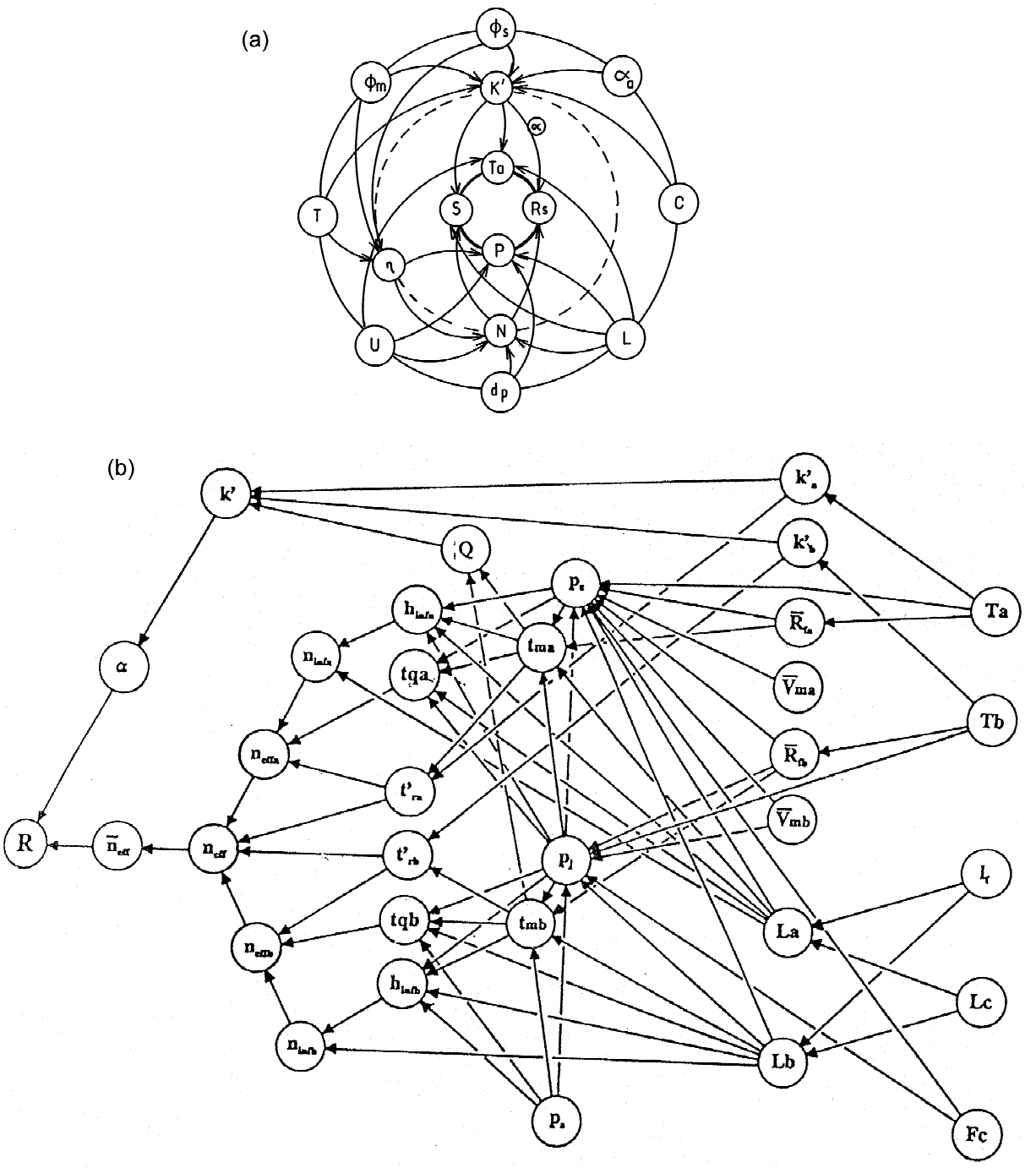

Fig. 1. (a) Interactions between a certain number of parameters, intermediate parameters and criterions of appreciation of chromatographic quality in liquid chromatography. φ_B, φ_M: Volumic fraction of the most strongly eluting solvent and modifying solvent respectively, T: Temperature, u: Flow-rate, d_p: Particle diameter, L: Column length, α_a: Adsorbent activation state, C: Carbon content, k': Retention factor, η: Viscosity, N: Column efficiency, P: Pressure drop, S: Sensitivity (peak height), T_a: Analysis speed, R_s: Resolution, P: Necessary pressure, S: Selectivity; from Ref. [8]. (b) Influence of parameters on the response criterion in GC with mass flow regulation; from Ref. [7].

time as in GC. In complex samples some compounds must be precisely quantitated, others not.

(5) Build a model or algorithm which relates the migration of solutes to the parameters.

(6) Estimate the model and particularly the weight of the parameters. Complications can arise from the mutual interactions of the parameters. Strategies fall into two categories as follows:

(i) Methods based on retention models. In this category, the retention of all solutes in the sample is modeled. A mathematical equation is used to estimate the optimum conditions.

(ii) Methods not based on retention models require a number of experiments. In this case a response function is build that fits the phenomenon within a domain. The response function may or may not fit e.g. retention, if any change in the domain is performed.

Sequential procedures rely on the power of the search algorithm and the effectiveness of the quality criterion that is being used. A response function, e.g. the sum of all resolutions is optimized with a search method such as the simplex method or iterative procedures. Retention mapping methods are also called simultaneous methods or regression methods since the retention is described by a model which is estimated by multiple (non linear) regression. They have been extensively used in isocratic optimization in RPLC where a minimum number of experiments has to be performed at a number of mobile phase compositions, these are the design points. Data gathered from those measurements (in most cases the retention factor k or ln k) are used for modeling the response surface of every solute in the sample. The response function can be predicted at every mobile phase composition in the design space. This in turn allows prediction of a chromatogram and the best chromatogram is selected by an optimization procedure. Knowledge on the separation mechanism is not required.

The goal is to obtain satisfactory separation conditions in the shortest time with only a few experiments. It is common belief that chemometrics methods require much more experiments than modeling. The purpose of this paper is to review the proposed methods in recent literature. There are a great many papers which continue to appear on optimization in LC. We shall only give an overview of the most relevant aspects in this technique, the reader is referred to original papers or a book [11]. Conversely papers on GC are not numerous, it is striking that no paper on the topic appeared in the special issues of the Journal of Chromatography devoted to contemporary capillary GC [12]. Papers on optimization in electrodriven separations are more and more numerous. We shall focus on methods rather than on techniques.

2. Method development using a chemometric approach

2.1. The objective functions

Since in either chromatography or capillary electrophoresis elution of solutes is monitored as peaks on the recording chart, the quality of the separation must be assessed. As was pointed out by Schoenmakers [13], many different terms are used: Response functions, objective functions or optimization functions. Elementary criteria are distinguished from global optimization criteria. When two consecutive peaks are gaussian shaped, the resolution (minimum resolution):

$$R_{si,i+1} = (2(t_{ri+1} - t_{ri})/(w_{i+1} + w_i)) \quad (1)$$

is often selected as criterion. In many cases (see Tables 1–3) the analyst is only interested in resolution R_s or selectivity α. These simple criteria are mainly used with chiral separations or when response maps are drawn. With non symmetrical peaks the peak valley ratio P is sometimes utilized. Selectivity α or minimal relative retention (k_{i+1}/k_i) is a good criterion when efficiency of the column is not known.

These are elementary criteria. When many peaks are concerned a global criteria must be used. Another criterion that may added is the analysis time. Criteria are introduced in objective functions that defines a chromatogram (or electropherogram) and includes (for example) resolution of peak pairs, retention time of the last eluted solute and spacing of peaks.

Since these objective functions were named chro-

Table 1
Literature reports on chemometrics and LC[a]

Solutes	Separation method	Optimization method	Variables	Number of experiments	Response	Ref.
Peptides	RP	FFD (hydrid strategy)	3 pH (trifluoroaetic acid, [TFA]), organic modifier	25 (3 gradients for each mobile phase + one run optimal)	R_s	[49]
Pharmaceuticals (5 solutes)	RP with ion pairing	fFD	7 ACN %, MeOH % THF %, pH, octanesulfonic acid, dimethyloctylamine, temperature	25	*k*, *N*, *As*	[63]
Lipid classes	NP	fFD 2^{3+1}	7 Eluent without additives, acetic acid, tetrahydrofuran, BuOH isooctane, ammonium acetate, temperature	9	CRS	[48]
Fat soluble vitamins (NPLC)	RP[b]	Mixture design	15	15 (10+5 replicates)	R_s and $t_{analysis}$	[41]
Inorganic anions	IC	CCD	3 $HClO_4$, NaOH, Na_2CO_3, coded variables	20	*k* polynomial model	[64]
Nitrate, phosphate	IC	fFD $2^{6-2}+3$	6 pH, ionic strength, in two eluents	19	R_s, *k*	[50]

[a] Hydrid strategy: Optimization of mobile phase system and gradient slope. Retention modelling is combined with direct response optimization, the optimum mobile phase is the one that allows the highest gradient slope.
[b] Multicriteria decision making plot, Pareto optimality.

matographic response function or optimization function there is no marked difference in objective function and response function.

In the early development of optimization procedures, the analyst neglected analysis time.

The result of an optimization process depends on the optimization criterion selected. A striking example has been given by Cela [14] in reversed-phase liquid chromatography (RPLC) (Fig. 2) where it is evidenced that responses can be very different according to the selected response function.

The chromatographic response function (CRF) was introduced by Berridge [15]. It was criticized since the poorly resolved peaks do not influence much the function value and the quality of a chromatogram is determined by well resolved peaks. It is of course valuable to focus on the resolution of unresolved peaks:

$$\mathrm{CRF} = \sum_{i=1}^{\mathrm{L}} R_i + L^{W_1} - w_2|T_\mathrm{A} - T_\mathrm{L}| - w_3(T_1 - T_0) \quad (2)$$

where R_i is the resolution between the *i*th and the

Table 2
Literature reports on chemometrics and CE and MEKC

Solutes	Optimization method	Variables	Number of experiments	Response	Ref.
Testosterone esters	Plackett Burman	7	8	R_s	[55]
Dinitrophenyl amino acids	ORM	3 pH, [SDS], [Tetrabutylammonium]	11	R_s	[42]
Enkephalin related peptides	D optimal design	7 [SDS], pH, temperature, CH_3CN %, voltage, ionic strength, plug	16	t_{eof} t_{mig} N, R_s	[72]
Enkephalin related peptides	CCD 2^k+5+8 design	4 [SDS], ACN %, temperature, ionic strength	25+8	R_s, k migration time window[a]	[74]
Peptides (enkephalin related)	CCD (new model)	6 [Surfactant], organic modifier %, ionic strength, temperature, (injected plug length), (sample solution conc.)	29 then 27	t_{cof} t_{mig}, N, R_s[b]	[71]
Ranitidine and related products	Plackett Burman	6 MeOH %, [αCD], voltage, temperature, pH, $[Na_2HPO_4]$[c]	20 then after $2n\alpha$ CCD 13	CEF	[75]
Sulphonamides β lactanes	Box–Behnken design	3 pH, voltage, [SDS]	15	response plots number of peaks versus pH, voltage, SDS 26 peaks	[59]
Ibuprofen codeine phosphate degradation products	Fractional factorial design 2^{6-2}	6 [SDS], pH, ACN %, temperature, [boric acid], voltage	16	t_{mig} R_s	[56]
Quinidones	ORM	3 Sodium heptanesulfonate ACN %, [sodium cholate]	7	R_s	[43]
Antibacterial and S oxidat. products	CCD[d]	3 Voltage, ACN %, [Tris]	15	CR[e] Optim Criterion COF	[24]
Pesticides	Plackett Burman	6 pH, type of CD, ACN %, [tetraborate], [SDS]	16	Response function based on information theory	[30]

Table 2. Continued

Solutes	Optimization method	Variables	Number of experiments	Response	Ref.
Bis benzyl Quinoline Alkaloids	ORM	3 [Sodium cholate], ACN %, pH	7	R_s	[44]
Methamphetamine	CCD	4	26	R_s	[70]
Amphetamine (ecstasy)	FFD[f]	[Buffer], pH, voltage, temperature			
Catecholamines	fFD	5 Mobile phases with different amounts of alcohols	25	k'[g]	[58]
Sulphonamides	ORM	2 pH [CD]	9	R_s	[95]
Main peak and impurities	CCD	8	32 then 16		[61]
Benzodiazepines	Box–Behnken design then Simplex	3 Organic modifier % in both buffer and simple injection time	13	H (HETP)	[36]
Fungal metabolites	FFD	9 $[PO_4^{3-}]$, $[B_4O_7]^{2-}$, pH, [SDS], ionic strength, [SDC], power, MeOH %, CH_3CN %	35	Number of peaks	[60]
Tropane alkaloids	Doehlert design	3 pH, [SDS], ACN %	15	R_s t_{mig} Power	[78]

[a] PCA of the responses determination of critical micelle concentration.
[b] PLS regression revealed that domain was too large conflict between R_s and t_{mig} while solving for N_{opt}.
[c] Voltage and pH are the most significant factors.
[d] Separation with CEC.
[e] See text.
[f] It is a second degree design.
[g] $1/k = A\mu + B\phi + C\mu\phi + D$.

$(i+1)$th peaks, L is the number of peak appearing in the chromatogram, T_A, T_L, T_1 and T_0 are the maximum acceptable time, retention time of final peak, retention time of first peak and the minimum retention time of first peak respectively, w_1, w_2 and w_3 are weighting parameters selected by the analyst.

The chromatographic resolution statistic (CRS) was proposed by Schlabach and Excoffier [16]:

$$\mathrm{CRS} = \left\{ \sum_{i=1}^{n-1} \left[\frac{(R_{i,i+1} - R_{opt})^2}{(R_{i,i+1} - R_{min})^2 R_{i,i+1}} \right] + \sum_{i=1}^{n-1} \frac{R_{i,i+1}^2}{(n-1)R_{av}^2} \right\} \cdot \frac{t_f}{n} \quad (3)$$

where $R_{i,i+1}$ is the resolution between consecutive solute pairs, R_{av} is the average resolution of all

Table 3
Literature reports on chemometrics and chiral separations

Solutes	Separation method	Optimization method	Variables	Number of experiments	Response	Ref.
Dopamine agonist	LC[a]	RSM	3 Hexane %, temperature, flow-rate	37	R_s	[86]
Leucovorin	LC	Simplex	3 pH, ionic strength, propanol %	14	$P_{\text{criterion}}$ valley to peak	[87]
Omeprazole Hydroxyomeprazole	LC	Fractional factorial design with centre points	4 ACN %, temperature, ionic strength, pH	28 then 24	k_R, k_S α, N, As (As = asymmetry)	[88]
Roxifiban	LC	fFD	5 then 3 Phosphate conc., isopropanol %, temperature	8 then 11	$V_2 \times V_3 / t$	[89]
Mosapride and deriv.	LC	Full factorial design	3 pH, temperature, MeOH %	15	k_R, k_S α	[90]
N-arylthiazolin-2 thione	LC	Full factorial design	4 Spatial steric requirement, lipophilicity, dipole moment, basicity	16	Link	[91]
Oxybutinin Chloride	LC	3 different multivariate techniques	4 pH, temperature, MeOH %, buffer	20	CRF	[21]
Metoprolol analogues	LC[b]	FFD with centre point	3 Isopropanol %, water content, acetic acid	11	k, α	[52]
Dihydro pyrano imidazo pyridines	LC	FFD with centre point	3 ACN %, pH, temperature	11	k, α	[53]
Amino Tetralin analogues	LC	fFD	3 pH, % organic modifier, H bonding	6	α k	[96]
Clenbuterol	CE	Plackett Burmann	5 pH, [CD], ionic strength, temperature, MeOH %	15	R_s, N, t t = time of analysis	[92]

Table 3. Continued

Solutes	Separation method	Optimization method	Variables	Number of experiments	Response	Ref.
Ibuprofen	CE	Contour plot	2 [CD], temperature	–	α	[93]
amlodipine	CE	CCD	3 pH, temperature, [additive]	15	$P_{\text{criterion}}$ R_s CRF	[65]
amlodipine	CE	CCD	Other additive	15	$P_{\text{criterion}}$ R_s CRF	[66]
Ticonazole	CE	CCD	3 pH, temperature, [additive]	15	R_s	[67]
Doxazosin	CE	CCD	3 pH, temperature, [additive]	15	R_s	[68]
Epinephrine enantiomers	CE	Fractional factorial design 2^{5-1}	5 Voltage, BGE Conc., pH, temperature, [CD]	20	R_s	[58]
Amphetamine	CE	FFD with centre point	5 pH, temperature, voltage, buffer conc., chiral selector conc.		R_s time generated power	[69]

[a] Quadratic polynomial model, Smilde's multi criteria decision making.
[b] Preliminary screening of column temperature, 2-propanol %, acetic acid % and amine %.

solute pairs, R_{opt} is the desired (optimum) resolution, t_f is the migration time of the last solute and n is the number of solutes in the sample. It does require the knowledge of n. The optimal chromatographic conditions are found at the minimum of the CRS function. The function is usually split in three parts. The first term approaches zero when resolution is optimal. $R_{i,j+1} = R_{\text{opt}}$. When the resolution is zero, this term is undefined. The second term is a variation of the relative resolution product; it approaches 1 when the resolution of each solute pair is equal to the average resolution which is the case when all peaks are equally spaced; the third term is intended to minimize analysis time.

The above CRF and CRS were criticized by Morris et al. [17] who proposed the chromatographic exponential function (CEF) for GC separation of phenols.

R_{opt} and R_i are selected optimum resolution and the resolution for the ith peak pair

$$\text{CEF} = \left(\left\{ \sum_{i=1}^{n-1} (1 - e^{a(R_{\text{opt}} - R_i)})^2 \right\} + 1 \right) \left[1 + \frac{t_f}{t_{\max}} \right] \quad (4)$$

respectively, $t_{\max}$ and t_f are the maximum acceptable time and the elution time of final peak respectively, a is the slope adjustment factor and n is the number of expected peaks.

According to the authors the CEF has the advantage of not having undefined points and also has less emphasis on elution time over resolution.

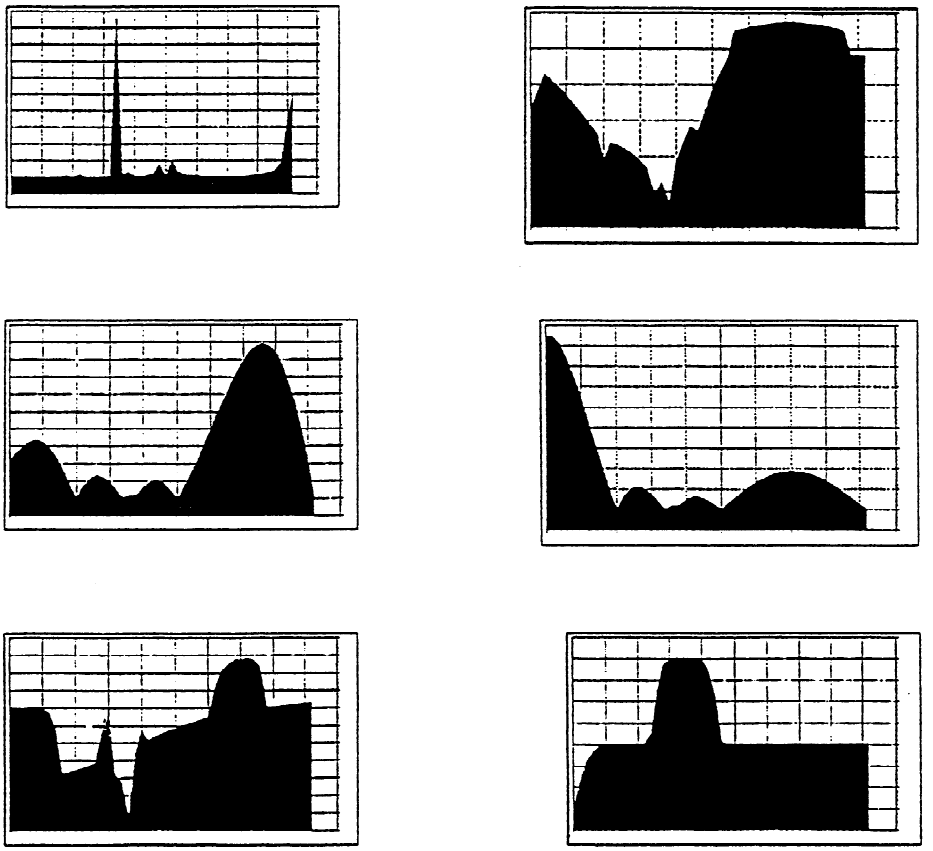

Fig. 2. Retention map for a simulated mixture of five compounds in RPLC and the response surfaces corresponding to isocratic separations using different objective functions. From Ref. [14].

With either CEF or CRS there is no need to identify peaks and peak crossovers do not affect the values of these functions.

Different CRF are proposed under same acronym. Morgan and Deming [18] make use of the *P* criterion

$$\mathrm{CRF} = \sum_{j=1}^{k} \ln(Pi) \tag{5}$$

which was used by Wenclawiak and Hees [19]. Dose [20] proposed the following CRF

$$\mathrm{CRF} = \frac{t_{\mathrm{R},n}}{t_{\mathrm{R,cri}}} + \sum_{i \neq j} \mathrm{e}^{-R_{\mathrm{s},ij}/R_{\mathrm{s,crit}}} \tag{6}$$

where $t_{\mathrm{R},n}$ is the retention time of the last eluting peak, $t_{\mathrm{R,crit}}$ is a user-selected time–cost weighting factor, $R_{\mathrm{s,crit}}$ is a user-selected resolution target value, $R_{\mathrm{s},ij}$ is the resolution between two gaussian peaks *i* and *j*.

A new CRF, developed for enantiomer separations, was proposed by Bylund et al. [21]:

$$\mathrm{CRF} = \sqrt[a+b]{Q^a T^b}; \quad (0 < \mathrm{CRF} < 1) \tag{7}$$

where *T* is a time factor, *Q* is a factor which includes the acceptable resolution and describes the quality of the separation, *a* and *b* are weight constants. For example if *T* and *Q* are of equal importance, *a* and *b* are set to unity.

T is a function of the retention factor, the curve $T = f$(capacity factor k) exhibits a maximum. *Q* is a sigmoidal function of R_{s} the inflection point is at $R_{\mathrm{s}} = 1$ and the plateau at $R_{\mathrm{s}} = 1.5$.

Heinisch et al. [22] proposed a criterion called

CRIT, defined for a given pair of solutes $[i,j]$ in a given binary mobile phase (A) by:

$$\mathrm{CRIT}_A(i,j) = \left[\frac{(t_j/t_i)_{\mathrm{predicted}}}{(t_j/t_i)_{\mathrm{required}}} \right] - 1 \quad (8)$$

A positive value means that the separation of the pair (i,j) is possible using mobile phase (A). When all possible pairs are considered, the criterion value that is retained is the worst CRIT (i,j) corresponding to the less resolved pair. The response function used to select the composition space is the geometric mean of two desirability functions $F1$ and $F2$ varying from 0 to 1.

$F1 = 0$ for $\mathrm{CRIT} \leq 0$,

$F1 = \mathrm{CRIT}/\mathrm{CRIT}_{\max}$ for $\mathrm{CRIT} > 0$

$F2$ takes into account the analysis time.

Peyrin and Guillaume [23] proposed that the quality of the separation of all solutes would be assessed by means of a new response function ξ defined as:

$$\xi = \mathrm{Min}(R_s) \text{ if } \mathrm{Min}(R_s) \leq R_1$$
$$\xi = R_1 + 1/t_a \text{ if not} \quad (9)$$

$\mathrm{Min}(R_s)$ is the resolution for the worst separated pair of peaks, R_1 is the minimum value of the resolution accepted, t_a is the analysis time. The function is maximal when both efficient separation conditions and minimal time are obtained.

Miyawa et al. [24] utilized in capillary electrochromatography (CEC) a criterion:

$$\mathrm{Cr} = 10(\alpha_{\mathrm{av}}/t_{\mathrm{m}})f \quad (10)$$

where α_{av} is the average selectivity, t_{m} is the migration time of the slowest eluting solute, f is a factor taking in account the number of separated peaks. They compared it with the well known COF (chromatographic optimization function) from Glajch et al. [25]:

$$\mathrm{COF} = \sum_{i=1}^{n} A_i \ln(R_i/R_{id}) + B(t_{\mathrm{m}} - t_{\mathrm{n}}) \quad (11)$$

where R_i is the resolution for the ith pair, R_{id} the desired resolution for the ith pair set at unity, t_{m} the desired maximum analysis time, t_{n} the time of the last eluted peak, A_i and B are weighting factors. They concluded that both Cr or COF are more appropriate indices of separation quality than average selectivity. A response function can be derived from the information theory [26,27]. If a mixture of n components is separated and the resulting chromatogram (or electropherogram) is composed of k_1 singlets, k_2 doublets and k_p p-multiplets with $\Sigma\,[pk_p] = n$ the contribution of the p-multiplets to the quantity of information is given by:

$$I_p = (pk_p/n)\log_2(n/p) \quad (12)$$

where pk_p/n is the appearance frequency and $\log_2(n/p)$ is the quantity of specific information obtained by the identification of a component in a p-multiplet.

The total information from the chromatogram is the sum of the I_ps of each peak group:

$$Ic = \sum_p (k_p p/n)\log_2(n/p) \quad (13)$$

The value of Ic varies between 0, all the peaks together ($p = n$, $k_p = 1$), and $\log_2(n)$, all the peaks separated, ($p = 1$, $k_p = n$).

This function was formerly utilized in HPLC [28], in HPTLC [29] and is proposed in micellar electrokinetic capillary electrophoresis (MEKC) [30].

Studying the ruggedness of optimization criteria in RPLC, De Aguiar et al. [31] proposed the CR criterion defined as:

$$\mathrm{CR} = y/\Delta y/\Delta x \quad (14)$$

where y is the response to be optimized (α or R_s min) and $\Delta y/\Delta x$ represents the variation Δy around this response value as a function of a variation Δx in the mobile phase composition.

Four derived criteria were proposed and it was demonstrated that optimal conditions selected through these criteria are Pareto optimal or agreed well with Derringer's desirability function. An experiment is Pareto optimal if there is no other experiment in the selected space which gives a better result for one criterion, without giving a worse result on at least one other.

The overall desirability is:

$$D = (d_1 d_2 \ldots d_n)^{1/n} \quad (15)$$

where $d_1 = 0$ for $y_i < y_{\mathrm{u}}$ and $d_i = 1$ for $y_i > y_{\mathrm{d}}$ where y_{u} is the undesirable value of the response y and y_{d} is the desirable value.

It appears that there is no definitive response function. When all compounds in a sample are

known it seems that responses based on simple resolution are sufficient. When dealing with samples with unknown compounds, the CRS function seems convenient.

2.2. Simplex

At the very beginning of optimization the simplex algorithm, which is useful for any type and number of parameters, was the method of choice.

The Simplex method has serious limitations mainly derived from its limiting searching capability. The procedure is a hill climbing method in which the direction and increment of advance is dependent only on the experimental responses. The simplex method will find an optimum but it might be a local optimum and no reversal order is detected. A possible route to avoid that a local optimum may be considered as a global optimum is to repeat the procedure with a different starting position. By consequence, super modified Simplex, weighted centroid methods have been advocated. Another drawback of the Simplex method is the large number of experiments needed to reach the optimum. A new sequential procedure called optimization procedure by search point (OPSP) in HPLC [32] required 11 experiments in the study of the separation of five pesticides. When dealing with CE, Castagnola et al. [33] used a weighted simplex to speed up the procedure, nevertheless 10 to 15 steps were necessary and incomplete separation of 20 amino acids was obtained.

Snijders et al. [34,35] developed an off line optimization of temperature programmed GC separations. The procedure is based on extracting thermodynamic values (enthalpy and entropy terms) from published Kovats retention indices. A numerical approach models the solute chromatographic process into very small segments of equal time. From the column characteristics and the solute data a Simplex is created (parameters, boundaries, fixed values). The calculated retention times and peak widths are used to calculate the CRF for each vertex. The optimal temperature program is thus determined. The requirement is that the analyst must know the identities of all solutes.

In CE, Peyrin and Guillaume [36] used a simplex to monitor the use of acetonitrile as organic modifier in the separation of ten benzodiazepines. The response (plate height value H) was calculated for m sets of starting conditions where m was given by the number of parameters to be optimized (organic modifier in both buffer and sample solution and time of injection) plus 1. The point corresponding to the lowest value of H was then reflected in relation to the surface defined by the three other points to give another set of starting conditions and the process is sequentially repeated.

2.3. Overlapping resolution maps

The ‘overlapping resolution maps’ scheme (ORM) was proposed by Glajch et al. [25] in RPLC. It is a Scheffe mixture design. The aim is to predict the best mobile phase composition consisting of mixtures of water with various proportions of three common organic modifiers, methanol, acetonitrile and tetrahydrofuran.

Resolution between two peaks is the aim of the analyst. The calculated resolutions are fitted by a second order polynomial equation (canonical equation)

$$R_s = \alpha_1 x_1 + \alpha_2 x_2 + \alpha_3 x_3 + \alpha_{12} x_1 x_2 + \alpha_{13} x_1 x_3 + \alpha_{23} x_2 x_3 \quad (16)$$

or a cubic model. Seven to ten experiments are performed. Experiments at the vertices use mobile phase of similar elution strength. From preliminary runs with a binary mixture the analyst subsequently uses the Schoenmakers [37] transfer rules to determine the volume fractions for other isoeluotropic mixtures. Overlapping the calculated resolutions for every pair in the parameter space or using a Venn diagram permits the location of the optimum mobile phase.

From the published optimization procedures making use of ORM, it appeared that in most cases optimum was located close to a vertex. It means that one solvent is not playing any role or at least a very minor one. ORM was plagued by the number of experiments to carry out. The method is now rarely advocated in HPLC. Some papers appeared from the Singapore group [38–40]. In ORM procedure the chromatographer is looking for the separation quality and does not care too much on analysis time. It was considered by Nsengiyumva et al. [41] in their

proposed optimization of the fat soluble vitamin separation. They carried out 15 experiments (ten runs with five replicates) and used a cubic model to build a contour plot of minimum effective resolution and maximum retention time as a function of mobile phase composition. An interesting feature is the right angle triangle graph that is simple for visual inspection. A Pareto optimal plot helps the analyst to make his decision.

The ORM procedure has been extended to electrophoresis separations and particularly MEKC. For the separation of 16 dinitrophenyl derivatized amino acids, Yik and Li [42] performed 11 experiments: Parameters are pH, sodium dodecyl sulfate (SDS) concentration and buffer concentration (sodium tetraborate). An optimization of electrophoretic separation of quinolone antibacterials has been published [43] where two additives (sodium cholate and sodium heptane sulfonate) with volume percentage of acetonitrile were selected as parameters. In a subsequent paper [44] the same authors used same methodology to optimize the electrophoretic separation of bisbenzylisoquinoline alkaloids with pH, one single additive and acetonitrile. To perform preliminary screening of important parameters affecting resolution, Wu et al. [45] used an orthogonal array design prior to ORM. In this mode they could select pH, sodium phosphate concentration and methanol percentage as parameters and 11 experiments were conducted to draw the ORM.

Advantage of ORM as compared to Simplex is the location of a global optimum but actual migration of solutes is not followed. In HPLC use of ORM is declining. Reliable peak tracking is required, the selection of parameter space is not obvious. Since one can directly optimize through a gradient run (see further) there is no need for ORM. In CE, the number of parameters is too important. Moreover dependence of the migration behavior on the experimental parameters is highly non linear.

2.4. Factorial designs

The Plackett–Burman designs are useful for screening the effects of a large number of potential parameters, they are saturated designs based on balanced incomplete blocks. Plackett Burman designs have a built-in ability to detect large main effects. One of the major disadvantages of these schemes is the assumption that all interaction effects are negligible.

When some influent parameters are reported, the simplest experimental design is a full factorial design (FFD) in which each factor can assume two levels (in coded units $+1$ or -1). A two level full factorial design is a design where all combinations of parameter levels are made. If, for example, six parameters of interest are identified (see example in Ref. [55]) the number of experiments needed is 64. This type of design would allow the determination of all main effects as well as all interaction effects. A two parameter interaction effect occurs when the main effect of one parameter is different at both levels of the second one. A three parameter effect occurs when the two parameter interaction effects are different at both levels of the third one etc. Since it is time consuming to perform as many experiments, a fraction of the full factorial design is utilized. Fractional factorial design (fFD) is a fraction of a full factorial design and, whilst a reduction in the number of experiments leads to some loss of information, this procedure maintains the statistical ability to identity the influence of each parameter and to check possible interactions between parameters. The linear model taking account interaction effects between factors obtained from a two level design may lead to erroneous conclusions about factor effects in the case where curvatures (second order effects) are neglected. In that case, a three level factorial design can be used.

FFD or fFd have been utilized in all major types of separation GC, LC, CE, SFC [94]. The main feature of factorial design is that one does not need to know the retention mechanisms in GC, LC or CE. The response is most often the retention factor. The number of parameters is increasing with the technique according to the complexity of the mobile phase. Since in GC gas phase only acts as a carrier, a three parameter FFD (rate of programming, linear velocity of the carrier gas and sample concentration) is sufficient to optimize the GC separation of some phospholipids [46]. A GC optimization of the separation of pesticides was published [47] where helium pressure drop (and by consequence flow-rate) is introduced as a variable. To target the search area in normal/phase liquid chromatography (NPLC), Ar-

noldsson and Kaufman [48] performed a screening of the physical properties of mixtures of solvents through non linear mapping. A FFD with nine experiments was further used to check the modifiers. In LC separation of peptides Lundell and Markides [49] used a FFD with three parameters (pH, trifluoroacetic acid concentration and organic modifier percentage) to determine the optimum mobile phase that is the one that allows the highest gradient slope. Combination with the predictive capability of gradient theory allows to model retention from three gradients. In ion chromatography a fFD was utilized to select the appropriate eluent in the determination of nitrate and phosphate in sea waters [50]. With chiral LC on chiral stationary phase a FFD with four parameters (pH, ionic strength, organic modifier percentage and temperature) was performed to determine low levels of impurities ($\leq$0.1%) and reversal of retention order could be detected [51,52]. Nystrom and Karlsson [53] could detect some unusual effects of mobile phase (pH and column temperature) on enantiomeric separation. A three level factorial design was applied for the optimization of separation in gradient elution in RPLC [54]. The retention times of all mixture components are measured at three levels of both the gradient time and initial solvent composition, resulting in nine values for each component. The empirical model is written as

$$t_r = a_0 + a_1.t_g + a_2.(\%B) + a_{11}.t_g^2 + a_{22}.(\%B)^2 + a_{12}.t_g.(\%B) \quad (17)$$

t_g is gradient time and %B is initial solvent composition, a_0, a_1, a_2, a_{11}, a_{22}, a_{12} are coefficients. The number of parameters is ever increasing: voltage, background electrolyte (BGE), pH with CE, surfactant concentration and even chiral additive concentration with MEKC (micellar electrokinetic chromatography). This may lead to seven parameters. Vindevogel and Sandra [55] were first to use a Plackett Burman design in optimization of the CE separation of testosterone esters. Since only eight runs were carried out and seven parameters were considered the method was not fully successful. All parameters can be significant [56], in this case it not possible to reduce the number of parameters and a full factorial design is necessary. Torres Lapasio et al. [57] described the retention of adrenaline as a function of the micelle concentration and the alcohol concentration. The response is a slightly asymmetric hyperbolic shape, the minimum of the function is located at the lower surfactant and propanol concentrations.

Fanali et al. [58] first carried out a fractional factorial design for the study of five parameters then this design has been augmented with additional experiments to conduct to a response surface modeling. Responses (resolution, migration times of enantiomers) were transformed into an appropriate desirability scale to balance between desired resolution and minimization of analysis time.

In sulfonamide separations, a Box–Behnken design helped to select the proper conditions to obtain the desired number of peaks [59]. The use of a fFD may lead to a great number of experiments since 39 experiments with nine parameters were necessary to obtain the complete resolution of some fungal metabolites [60]. Altria and Filbey [61] used FFD to search for the effects of parameters on the robustness of the response.

FFD and fFD are applicable under different experimental circumstances they are not transferable. A change in separation conditions changes the whole procedure. From Tables 1–3, it is clear that FFD is of great help in selecting proper conditions in MEKC but much less than CCD.

2.5. Response surface methodology

A central composite design (CCD) consists of a 2^d factorial runs (or fractional factorial of resolution V), $2d$ axial or star runs and n_C center runs.

$$\eta = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 \quad (18)$$

In the above equation, η is the response model selected for $d=3$, X_i are the coded parameters selected for the CCD.

Six replicates at the center point of the design and a pair of experiments along each coordinate axis permit to calculate the experimental error of the process and to determine response surfaces and the corresponding contour plots.

For design with few variables the experiments to

choose are obvious but for larger series computer aided selections are mandatory.

Data from a CCD can be evaluated and plotted as a response surface by combining a statistical procedure that fits a quadratic response surface model to the data. Response surfaces can provide a graphical representation of the data over the range of the key parameters under study.

Guillaume and Guinchard [62] used a desirability function along with a CCD and Simplex optimization to investigate the effects of gas flow-rate and column head pressure on the separation of eight *p*-hydroxybenzoic esters by GC.

CCD is useful to monitor the effects of additives in LC and in chiral LC particularly.

Loading plots describe how the responses are affected by the descriptors. Some effects are evidenced but some are hidden. For example in the paper from Andersson et al. [63] ion pairing effect was not discovered in LC separation of some pharmaceutical substances.

A CCD was used to investigate a ternary eluent (perchloric acid, sodium hydroxide sodium carbonate) in anion chromatography [64].

Fell and co-workers in a series of papers [65–68] demonstrated the usefulness of CCD for enantiomeric separations in both LC and CE and they pointed out that insight of the chiral recognition mechanism may be revealed.

A complete and quite general physicochemical model in CE is still missing. CCD is used for systematic optimization and it offers an efficient route for rapid optimization of resolution with multiple interacting parameters. Varesio et al. compared FFD and CCD procedures in analysis of amphetamine [69] and amphetamine derivatives found in ecstasy [70]. FFD permitted determination of the significant parameters and CCD enabled modeling of the separation. The problem is even more complex in MEKC. Thorsteindottir et al. published extensively on optimization of MEKC with fFD [71–74]. They checked band broadening, resolution R_s, retention factor k and migration time window. They pointed out that resolution in MEKC is highly complex and describes a non linear function of the experimental parameters. They also investigated the sample solution concentration and the injected plug length. Principal component analysis (PCA) of the responses could reveal some features for example the highly non linear influence of acetonitrile modifier. They also could determine the critical micelle concentration (CMC).

From the published papers (see Tables 1–3) one can see that the number of experimental parameters that are considered varies from three to seven and includes pH, temperature, background electrolyte or buffer concentration, ionic strength, organic modifier percentage, voltage and surfactant concentration.

A CCD was applied in CEC [24]. Three parameters were examined: applied potential, buffer concentration and volume fraction of acetonitrile modifier. From the results a high buffer concentration can be utilized. The correlation coefficients generated from the multivariate regression of the CCD data were then used as measures of suitability for the optimization criterion.

In some cases [75], a Plackett Burman is used at the beginning of the study and from the data a CCD is constructed.

Doehlert design was introduced into the optimization of HPLC methods by Hu and Massart [76]. A Doehlert design with two parameters forms an hexagon where each point represents an experiment. A feature of the Doehlert design is that the number of levels for each experimental parameter is not the same. Applications of Doehlert design are not numerous. Bourguignon et al. [77] optimized both pH and organic modifier content of the mobile phase in RPLC separation of chlorophenols. Mateus et al. [78] used a Doehlert design to search for the region where pH, SDS concentration and organic modifier percentage are simultaneously optimized for the tropane alkaloids separation with MEKC.

2.6. Neural networks

An Artificial Neural Network (ANN) is a data processing system consisting of a large number of simple, highly interconnected processing elements in an architecture inspired by the structure of the brain. The network is made out of neurons organized in layers. A neuron receives inputs. After summation, these input values t_j are modified by the transfer function which is often a sigmoid function. It produces an output value T_j of the neuron. Between the input and the output layers is located at least one

hidden layer. A description of the working process of ANNs and their application in selection of optimal design can be found in [79]. No prior knowledge is required about the relations among the parameters.

Metting and Coenengracht [80] compared the responses (ln k) for different sets of compounds including ionizable solutes where polynomial models are failing. They obtained promising response surfaces. Marengo et al. [81] used ANNs to investigate the effects of five parameters in ion interaction chromatography.

Comparison of the prediction power of ANNs and mathematical modeling has been studied by Sacchero et al. [82] and more extensively by Havel et al. [83–85] in ion chromatography. Sacchero et al. pointed out that a similar prediction power was obtained with both models when number of data was sufficiently large (more than 17). In a series of papers devoted to separations of ions and metal complexes, Havel et al. demonstrated that retention times predicted with ANNs are better than those predicted by mathematical models. They pointed out that ANN modeling does not provide any numerical values for physical parameters. Tables 1–3 (with references up to [96]) summarize the published optimization procedures with chemometrics tools. A special emphasis is placed on chiral separations.

3. Methods based on models

3.1. Computer-assisted optimization

As we could see in the above sections statistical approaches are time consuming. If the analyst gets information from a limited number of experiments, he can model the behavior of the solutes of interest through a computer program with help of empirical equations or models. The recent introduction of electronic pneumatic control in commercial GC together with fast temperature programming and enhanced performances of capillary columns permit carrying out a lot of trial and error runs in a short time. It may explain why optimization through computer in GC is not as popular as in LC. The off line optimization from Snijders [34,35] simulate and optimize both single and multiramp temperature programmed GC separations. It is efficient but Kovats indices should be known. There is no optimization scheme when a set of both identified and non identified solutes should be separated.

Most of computer assisted procedures have been developed for HPLC separations and mainly for RPLC. Some are commercially available, others are not.

In RPLC the variation of the retention factor with solvent strength has usually been described by:

$$\ln k = \ln k_w - S\varphi \tag{19}$$

where k is the retention factor, k_w is the hypothetical value of the solute retention factor for an aqueous mobile phase, S the slope of the relationship and φ the volume fraction of the organic modifier. In fact this model fails to describe the solute retention for wide variations of the organic modifier content of the mobile phase. Using solubility parameters, Schoenmakers et al. [97] derived a quadratic equation as:

$$\ln k = a + b\varphi + c\varphi^2 \quad \text{or}$$

$$\ln k = A\varphi^2 - S\varphi + \ln k_w \tag{20}$$

3.1.1. Optimization without gradient runs in LC

A computer-assisted system relies on hydrophobicity: The Eluex

The Eluex expert system has been introduced in 1993 [98]. It is able to predict a starting mobile phase composition without performing experiments. The analyst must know the chemical structures of the compounds to be separated to assess their polarity. The system calculates 1-octanol–water partition coefficients P and recognizes the ionizable groups. The program is based on the assumption that ln k is linearly related to log P. The system relies on three equations:

$$\text{if } \ln k = 0 \quad \text{then} \quad \log P = a\varphi_0 + b \tag{21a}$$

φ_0 is the organic modifier percentage (ACN = acetonitrile; MeOH = methanol) which yields ln k = 0:

$$\varphi_{0\,\mathrm{ACN}} = 16 \log P + 26 \tag{21b}$$

$$\varphi_{0\,\mathrm{MeOH}} = 13 \log P + 42 \tag{21c}$$

To calculate log P values, the Rekker's fragmental constants [99] are used.

In the case of ionizable solutes [100], pK_a values

must be known. To cope with the mixed aqueous–organic medium, a correction on the pK_a values is performed:

$$pK_a^* = pK_a + a\varphi \quad (22)$$

φ is the volume fraction of organic solvent in binary mobile phase.

pK_a values are predicted using either the Hammett equation (aromatic acids and bases) or Taft equation (aliphatic acids and bases).

Input data are preferred organic solvent, minimum resolution, pH range, plate number. The Eluex system is mainly suitable for isocratic separations but calculations may drive to a gradient.

When $\ln k = 0$, ϕ_0 is the volume percentage of organic phase concentration in the binary mobile phase by which the retention time is twice the dead time. If the linear relationship holds true then:

$$(\phi_0) = -\ln k_w / S \quad (23)$$

By consequence if $\ln k_w$ is determined through another method and if (ϕ_0) is experimentally obtained, it would be possible to calculate retention.

(ϕ_0) [101] was proposed as a new hydrophobicity index with the claimed advantage that it is more applicable to interlaboratory and column to column comparison than in $\ln k_w$. It shows a significant correlation to the traditional octanol–water partition coefficient.

Using linear free energy relationship solvation equation with a set of 62 solutes, Du et al. [102] observed that log P is totally insensitive to solute hydrogen bond acidity whereas (ϕ_0) (and $\ln k_w$) are all influenced by hydrogen bond acidity. Authors are aware of the parabolic profiles of plots of $\ln k$ versus ϕ and they consider the linear portion of this function keeping $\ln k$ values between 1.2 and -0.5.

We believe that we cannot gather in the same set solutes that exhibit such differences in behavior. It is experimentally demonstrated that deviations from linearity occur especially at the extreme values of ϕ. Sandi and Szepesy [103] published a large amount of data of retention (as In k) versus ϕ for a set of 31 solutes on six different columns with methanol and acetonitrile as modifier. Fig. 3 displays the behavior of caffeine. In Du's data some solutes such as theophylline and pyridine are far from fitting a linear plot; the more polar the solute the less it fits a linear regression equation. The linear model is a tangent to the quadratic relationship. For the same solute, the linear model will lead to different values of S and $\ln k_w$. From the slopes and $\ln k_w$ obtained through linear regression some confusing predictions are possible, for example peak cross over occurs between phenol and aniline at 46% acetonitrile–water instead of 40%.

The (ϕ_0) values are varying from column to column. With data from [102] for example, for toluene ϕ_0 values lie between 44.5 and 53.0 and phenol values lie between 66.0 and 72.0. Data from these authors demonstrate that using linear relationship for a large set of solute is overfitting the regression. As was pointed out by Krass et al. [104] determination of $\ln k_w$ by linear gradient elution should be accompanied by standards exhibiting similar molecular interactions as compared to the sample.

Al-Haj et al. [105] performed regressions on some test solutes to derive a predictive equation for $\ln k_w$. They used three approaches: One with the linear solvation energy relationship (LSER), one with structural descriptors from molecular modeling (the total dipole moment DIP, the maximum electron excess on a most charged atom, the water accessible molecular surface area), one with linear correlation between $\ln k_w$ and log P. They conclude that three series of reference analytes should be selected for interlaboratory comparison of stationary and mobile phases. Each series should be designed specifically for a given quantitative structure retention relationship.

The Prisma optimization [106] model developed by Nyiredy is mainly used in planar chromatography but it is currently in progress to be used in HPLC. The name comes from the geometrical interpretation in the form of a prism. It is a three dimensional geometrical design which correlates the solvent strength (St) with the selectivity of the mobile phase. The Prisma optimization scheme consists of three parts:

(i) Selection of the basic parameters of the chromatographic procedure (columns and pure solvent). The strategy of solvent selection is based on the solvent classification by Snyder [107]. Ten solvents from different solvent groups are tested in the preassays. Adjusting the strength of weak solvents is

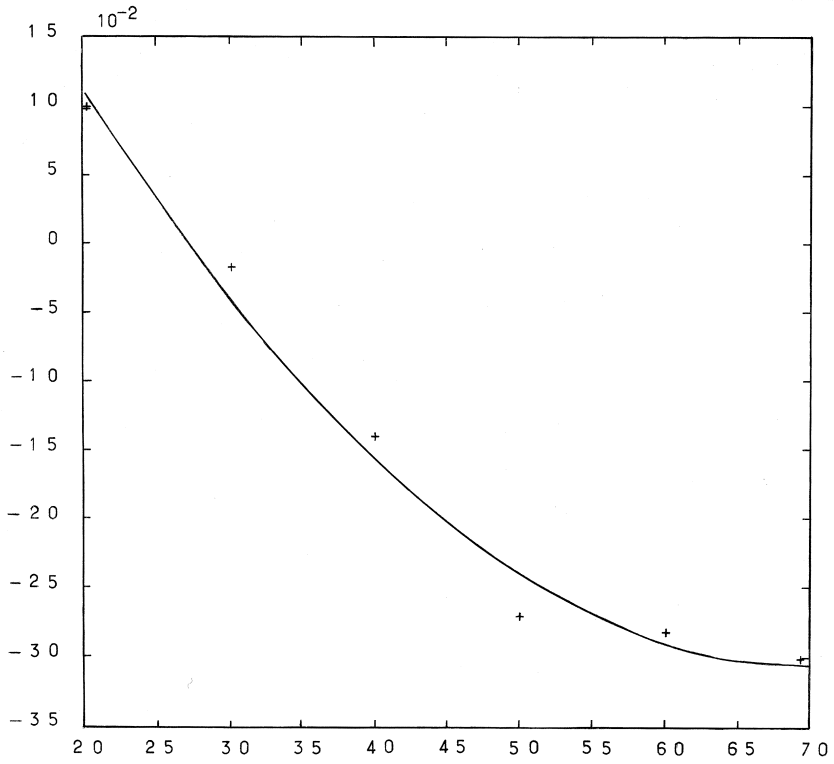

Fig. 3. Plot of ln k versus volume fraction of acetonitrile. Solute: Caffeine. Data from Ref. [103].

performed by addition of polar modifiers such as water, diethylamine or acetic acid. Conversely adjusting the strength of strong solvents is performed by dilution with hexane. The analyst performs TLC experiments and adjusts the solvent strength so that the R_F of the target compounds should be between 0.2 and 0.6. There is no need to know the number and the structure of the analytes. Selection of the best three solvents or binary mixtures is performed to obtain a selectivity triangle.

(ii) The analyst constructs a prism (Fig. 4) which has three parts: an irregular top part used for optimization in normal-phase chromatography of polar solutes, a regular middle part and a lower part which represents the modifiers. The lengths of each edge of the prism represent the strengths of the pure solvents A, B and C. Another triangle parallel to the original is drawn at a height equal to the strength of the weakest of the three solvents.

(iii) When the best possible mobile phase composition has been achieved, the appropriate development mode is selected. These procedures are rather long, for that reason use of gradients is attractive since a lot of information can be gathered in a short time.

3.1.2. Optimization through gradients in LC

In RPLC the Drylab computer simulation approach which appeared ten years ago [108] is probably the most widespread approach to optimization of operation parameters. Here the retention data of two initial gradient runs are used to adjust subsequently the steepness and the range of the gradient and if necessary other working parameters. The trial gradient runs should be made under conditions in which the gradient times differ by a factor of 3–4 but with the same gradient range for both runs. A similar procedure was published by Heinisch et al. [109] where linear and non-linear variations of the binary mobile phase composition versus time were considered.

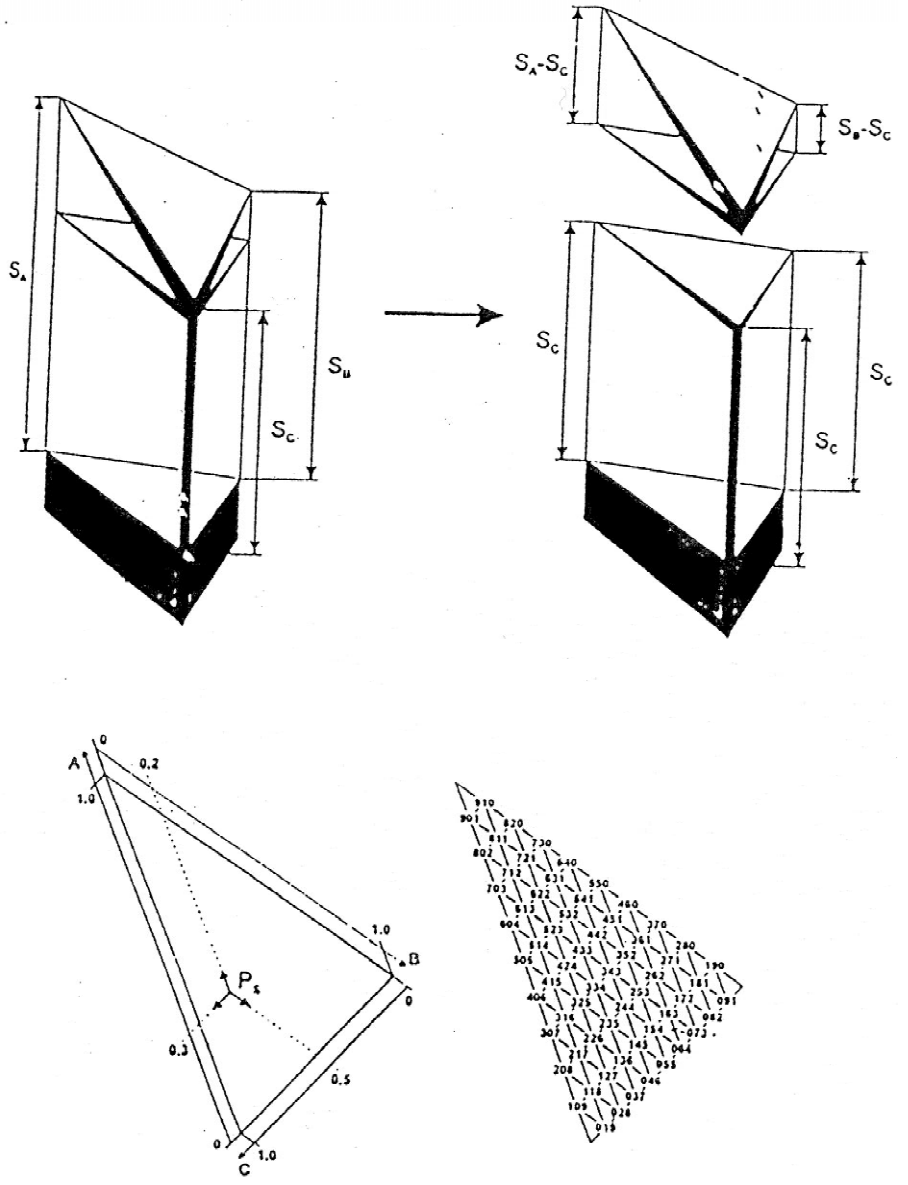

Fig. 4. Geometrical interpretation of the Prisma model.

The retention time in gradient elution can be expressed as:

$$t_R = (t_0/b)\log(2.3k_0b + 1) + t_0 + t_D \quad (24)$$

$$b = V_m \Delta\varphi S/(t_g F) \quad (25)$$

in which k_0 is the retention factor at the initial eluent composition of the gradient, t_D is the dwell time of the system, t_0 is the column dead time, b is the gradient steepness parameter where $\Delta\varphi$ is the change in organic modifier in the eluent program, V_m is the column dead volume, F is the flow-rate, t_g is the

gradient time, S has been defined (Eq. (19)), it is a constant for each solute.

Temperature effects were observed on LC separation of some peptides and the effect was complementary to gradient steepness [110,111]. In a series of papers [112–115], Zhu et al. extended the area of optimization with temperature modulation. A linear relationship:

$$t_R = a + bT \tag{26}$$

relates gradient retention time t_R to column temperature T (other conditions kept constant). A design with two gradients and two temperatures is advocated. The retention times of only four experimental runs are entered into the program. The other required parameters are the column data (length, particle size and plate number), t_M the dead time and V_d the dwell volume. Polar neutral compounds exhibit large changes in a when temperature or gradient steepness was varied.

It must be kept in mind that selectivity adjustment through temperature is much less sensitive than through solvent change. The software calculates the resolution map for the compounds under study and allows the convenient optimization of T and the gradient time tg with the use of a contour map. The two gradients based (no consideration of temperature) method permit calculation of S and $\ln k_w$ and use of Eq. (19). In a slightly different approach, Chaminade et al. [116] used an iterative method: The estimated S and $\ln k_w$ from the first gradient are utilized to compute the second gradient. The retention model can be extended to a more accurate quadratic form. They also pointed out that an improvement in accuracy of retention time prediction is observed when the two gradients varied by a limited difference in solvent composition. From Schoenmakers [13] a correlation links together the parameters S and $\ln k_w$.

$$S = p + q \ln k_w \tag{27}$$

These two constants are depending on the organic modifier, the stationary phase and the chemical structure of the solute. The correlation is only valid for compounds from homologous series. If a linear relationship links $\ln k_w$ with $\log P$, then:

$$S = a + b \log P \tag{28}$$

This relationship was exploited by Verbruggen et al. [117] for fractionation of aromatic micropollutants. The authors were aware of the fact that acetonitrile solvated stationary phases are less like octanol than the methanol solvated ones since the dispersive attraction by acetonitrile is stronger than the one by methanol.

Yoshida and Okada [118] demonstrated that prediction of peptides retention times in NPLC can be performed with a single run when two equations hold true:

$$\ln k(\varphi) = \ln k_{(0)} - S \ln \varphi \tag{29}$$

and

$$S = p \ln k_{(0)} + q \tag{30}$$

where $k_{(0)}$ is the retention factor for $\varphi = 0.01$, φ is the volume fraction of the more polar solvent in the binary mobile phase. As in RPLC this relationship is only valid when structurally similar compounds are considered.

When dealing with ionizable solutes, Drylab limp was developed for the determination of pK_a values from three chromatographic runs by varying the pH [119].

An interesting comparison was performed by Larew et al. [120] between predictions made by Drylab I/mp and a factorial design for the separation of some benzodiazepine derived compounds. ln this difficult separation where buffer, pH and surfactant percentage are added parameters, the empirical equations derived from the statistical analysis performed better in the prediction of the chromatographic behavior. In this case Drylab did not take into account the ion pairing formation. In their procedure, values of $\ln k_{[0]}$ were calculated numerically from an equation they derived by inputting a single gradient experimental data. S is further calculated. They pointed out that isocratic retention times prediction is much less accurate (14.4% average error) and the method cannot predict R_s values.

Outinen et al. [121] compared Eluex, Prisma and Drylab for the separation of biogenic amines. They concluded that Eluex is a good method for a rapid screening which gives some idea of the HPLC conditions, Drylab is a universal method for optimizing solvent strength in both isocratic and gradient

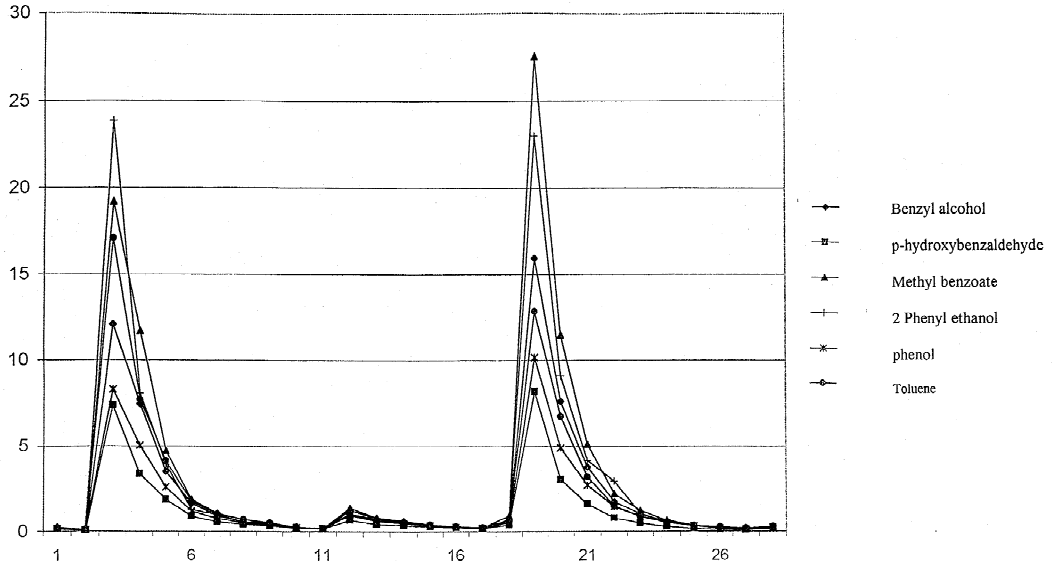

Fig. 7. Behavior of six solutes in a ternary mixture of solvents [water, methanol and acetonitrile] from Nemrod software [168]. Data from Schoenmakers [167]. Responses versus number of experiments, each number represents a ternary mixture.

scriptors versus the tabulated solute parameters Solute descriptors are available for more than 2000 compounds, some of empirical origin, others from computational chemistry supported by molecular modeling. The calibration of the LSER model at one mobile phase composition cannot be transferred to a second mobile phase composition because at different mobile phase composition a different set of coefficients are needed to fit the LSER model [162].

Poole et al. [163–166] published a huge amount of data in LC and TLC and used statistical mixture design to draw surface responses for v (m in Poole's papers), a, b, r, and ln k_0 (constant c in Poole's papers). A plot of the system constants as a function of solvent composition provides a system map to calculate retention maps for mobile phase optimization. The system constants in binary phases are not truly linear but exhibit smooth changes.

With binary mobile phases, the v (or m) constant is decreasing when organic modifier volume increases, v (or m) is always positive. Plots of the v (or m) constant versus methanol percentage are more linear than those versus acetonitrile percentage. There are three regions with acetonitrile, an approximate straight line between 30 and 70% and curved regions beyond. Values of a and c constants are not too far from zero.

From these results, if the constants in LSER equation are varying with the volume percentage of organic modifier (φ), we may take into account the variation and write:

$$\ln k = \ln k_0 + (v_0 + v_1\varphi)V_x + (s_0 + s_1\varphi)\pi_2^H + (r_0 + r_1\varphi)R_2 \quad (42)$$

Considering only four solutes (toluene, ethylbenzene, anisole and hydroquinone) and data from Sandi and Szepesy [103] and solute descriptors from [101], we performed a linear regression to obtain values of ln k_w and S in Eq. (19). We neglected the coefficients that are close to zero in Poole's data but calculation should theoretically be performed with all coefficients.

It yields a straight line (Fig. 5) with the correlation coefficient $R^2 = 0.840$.

$$\ln k_w = \ln k_0 + (v_0 V_x) + (s_0 \pi_2^H) + (r_0 R_2) \quad (43)$$

$$S = [(v_1 V_x) + (s_1 \pi_2^H) + r_1 R_2]\varphi \quad (44)$$

Calculated S of toluene yields $S = -0.292$. From

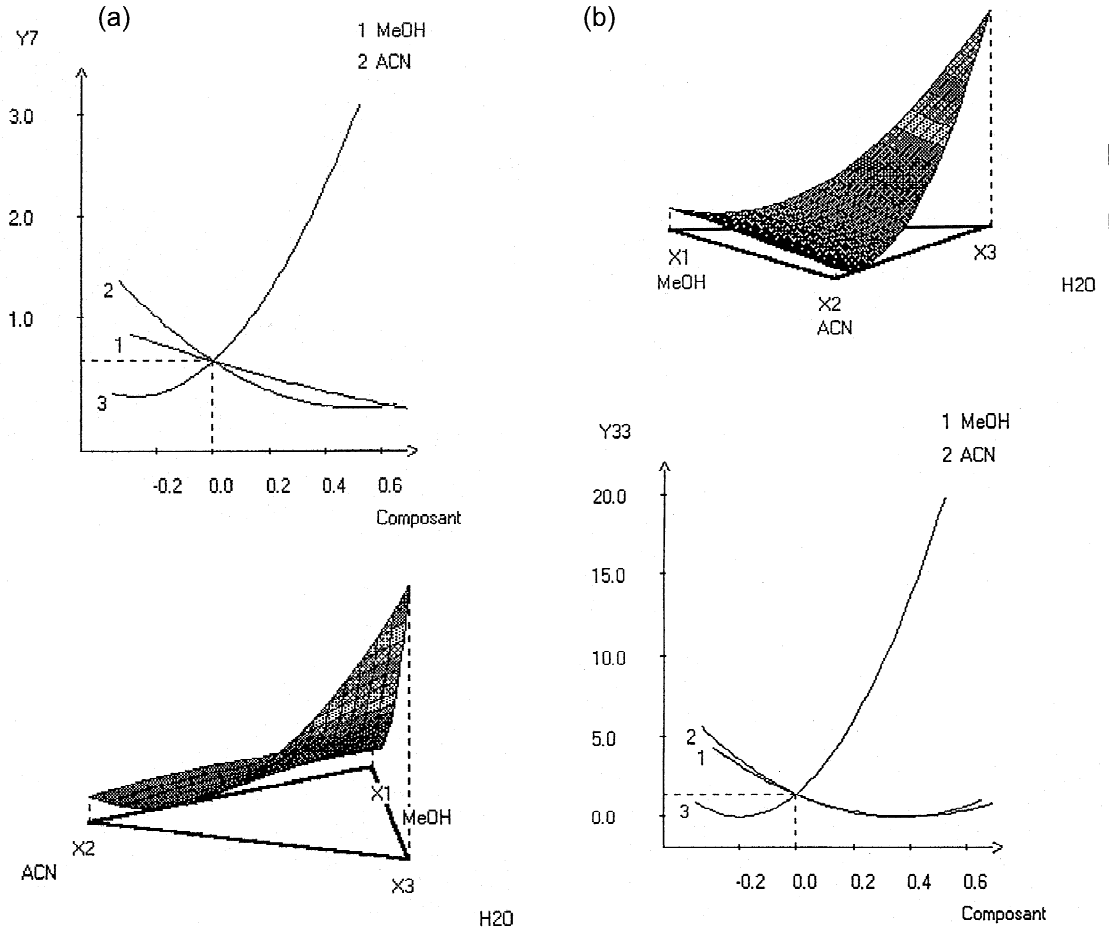

Fig. 8. Response surfaces and relative influence of water, methanol and acetonitrile on retention of some solutes [benzyl alcohol (a), methyl benzoate (b), *p*-hydroxybenzaldehyde (c)] in ternary mixtures. Data from Ref. [167].

data from [103], $S=-0.298$ and from data from [101], $S=-0.20$.

If we introduce caffeine which behaves non linearily (Fig. 3) together with the above four solutes in the regression we use now a quadratic relationship:

$$\ln k = \ln k_0 + (v_0 + v_1\varphi + v_2\varphi^2)V_x + (s_0 + s_1\varphi + s_2\varphi^2)\pi_2^H + (r_0 + r_1 + r_2\varphi^2)R_2 \quad (45)$$

The fit is good ($R^2=0.89$) (Fig. 6).

Papers with ternary mixtures in RPLC are rather scarce.

From the data published by Schoenmakers in 1981 [167], Kiridena and Poole drew system surfaces for a ternary mixture of solvents namely acetonitrile–methanol–water. As usual in statistical mixture design they described the surfaces by the general model equation:

$$y = A\varphi_{Me} + B\varphi_{ACN} + C\varphi_w + D\varphi_{Me}\varphi_{ACN} + E\varphi_{Me}\varphi_w + F\varphi_{ACN}\phi_w \quad (46)$$

where y is the response for the constants in LSER equation, subscripts Me, ACN and w denote methanol, acetonitrile and water respectively.

Response surfaces for v (or m) and b are smooth

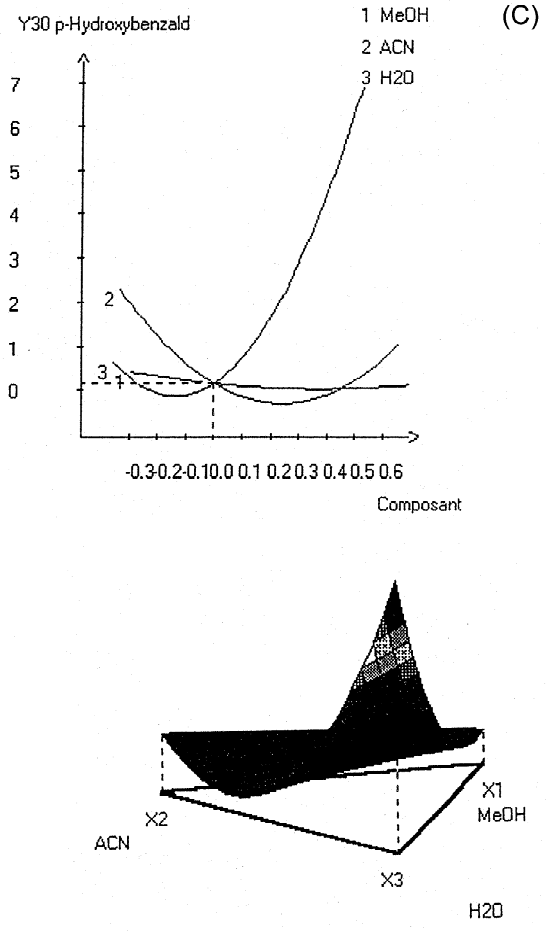

Fig. 8. (*continued*).

and they can be approximated by a straight line in the range 30–70% of water. Calculation of ln k can be performed and Poole obtained a good fit for ln k (experimental) versus ln k (calculated) with a three component solvent mixture. Calculation with the published equation by Schoenmakers yields some differences.

$$\ln k = A_1\varphi_1^2 + A_2\varphi_2^2 + B_1\varphi_1 + B_2\varphi_2 + C + D\varphi_1\varphi_2 \tag{47}$$

It seems surprising that ln k only depends on the two volume fractions of organic modifier φ_1 and φ_2. In fact

$$\varphi_1 + \varphi_2 + \varphi_3 = 1 \quad \text{and} \quad \varphi_3 = 1 - \varphi_1 - \varphi_2 \tag{48}$$

The solubility parameter for water is included in constants of the equation.

As it is, the equation does not account for the influence of the different solvents. This can be emphasized with use of another statistical treatment (Fig. 6). We selected six solutes among the 49 solutes that were considered by Schoenmakers (see Appendix 2 in Ref. [167]): Benzyl alcohol, *p*-hydroxybenzaldehyde, methyl benzoate, 2-phenylethanol, phenol and toluene. These solutes represent a wide variation in polarity and data are given for almost any solvent mixture. We checked the influence of the different solvent composition on the response (ln k) by means of the Nemrod software [168]. Responses are plotted versus the 28 solvent compositions reported by the authors. Solutes qualitatively behave in a similar way but responses are quantitatively different which means that a single model from chemometrics is able to predict the general trend of solutes but is not able to accurately predict retention for all solutes. Chemometrics does not derive the mathematical model which would be able to predict the behavior differences between the solutes. An increase in retention with increasing proportion of water cannot be modeled in this way. The influence of water is overwhelming. It is interesting to note that in some experiments a low percentage of acetonitrile is used with a large proportion of water.

In Fig. 7, we can see the behavior differences between methanol and acetonitrile with different solutes. This is well known for a long time [169] and was emphasized in the separation of benzodiazepines [170].

Data from Schoenmakers are not recent and for example, some results with ACN–water (50:50, v/v) and MeOH–ACN–water (25:25:50, v/v) are not fully consistent. Nevertheless we can see the behavior differences between benzyl alcohol, *p*-hydroxybenzaldehyde and methyl benzoate (Fig. 8). With methyl benzoate there is similar behavior with either acetonitrile or methanol. Differences are increasing with the polarity of solutes. It means that we need a theoretical model to account for solute behavior in ternary mixtures to determine whether it is worth to perform a ternary gradient to optimize the separation or not.

4. Conclusion

Optimization in separation sciences is still an important demand from analysts who look for a desired resolution or a desired selectivity with a limited number of experiments in minimum time. The number of parameters to master is increasing from GC to chiral MEKC.

Several approaches are possible. Chemometrics provide valuable tools for studying the influence of parameters and for determining which are those of primary importance. In this mode, factorial designs and central composite designs are very often utilized in CE and MEKC. Doehlert designs yield good insight of retention but are seldom proposed. The trend is towards the use of artificial neural networks but they are still in infancy when applied in separations. It must be kept in mind that the chemometrics derived model represents the phenomenon within the selected parameter space and it can be completely unfit beyond the boundaries. In many cases these models are not predictive.

Computer-assisted procedures are reliable and well established methods in LC.

In RPLC the gradient approach saves much time. Practical approaches rely on linear relationships between solute retention as ln k and mobile phase composition for example volume fraction of the organic modifier or eluent concentration in IC. However the linear approach is often too simple.

Use of ternary gradients is rare probably because the majority of problems can be solved through binary mixtures. A fine selectivity tuning may be obtained through ternary mixtures but such an optimization relies on the different behavior of solutes in the solvents and at present time it is difficult to predict such a behavior.

Other practical approaches rely on the knowledge of the identities of solutes and the retrieval of some physicochemical properties such as log P. The variable log P can be correlated with ln kw but it is a correlation with some scatter as in any correlation. A remarkable exception is the Prisma model where nothing is required. The only drawback is the number of preliminary experiments which is rather large. ln every practical approach peak tracking is needed.

If one wants a reliable predictive model, he must rely on a model built on sound theoretical basis. In this mode the parametric modulation and the LSER approach seem the best performing.

A great deal of work has been performed on LSER and still in progress. As was pointed out by Bolliet and Poole [163], the combination of chemometrics methods with hard modelling will certainly bring some improvements in the prediction of retention.

In any case it is easier to optimize the separation of non electrolyte solutes than ionic ones. In this case equations are very complex and computer assisted methods are only emerging.

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