Review

Carboxylic acids: prediction of retention data from chromatographic and electrophoretic behaviours

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Abstract

A review of the main results reached in the prediction of retention data of carboxylic acids, inferred by their chromatographic and electrophoretic behaviour, is presented. Attention has been focused on the main separation methods used in carboxylic acids analysis, that is ion-exclusion, anion-exchange, reversed-phase (RP) liquid chromatography and capillary electrophoresis. Papers proposing mechanistic models as well as chemometric and multilayer feed-forward neural network analysis of ion chromatography (IC) and RP chromatographic retention data were reviewed. Principal component analysis, PCA, sequential simplex method and simultaneous modelling of response surfaces through simple nonlinear models (not related to equilibria involved in retention) have been considered. Computer simulations for the prediction of retention data have also been discussed. A quick overlook on the prediction of capacity factors of analytes by less common determination methods such as thin-layer, gas chromatography and supercritical fluid chromatography has also been done. © 1998 Elsevier Science B.V. All rights reserved.

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1. Introduction

Carboxylic acids are hydrophilic weak electrolytes belonging to a class of compounds of biological and application importance. In the pharmaceutical industry, they are used as antioxidants, acidifiers and drug adsorption modifiers. Carboxylic acids are also extensively used for manufacturing technical products. Carboxylates can also be found as natural compounds or additives as preservers in food and beverages.

The most widely used methods for the determination of carboxylic acids include liquid chromatographic techniques based on the anion-exchange (IE), ion-exclusion (IEC) and reversed-phase (RP) mechanisms. Among separative analysis, capillary electrophoresis has also become a powerful technique owing to its high separation ability.

In every separation technique, it is desirable to have the option of manipulating a variety of parameters affecting retention in order to control the quality of a separation.

This review will focus on the main results reached in the prediction of retention data of carboxylic acids, focusing on the main separation methods characteristic for carboxylic acids analysis, previously cited.

Ion-exclusion chromatography has been extensively used by several researchers for carboxylic acid determination and applicative purposes. Literature dealing with the separation of weak acids, and particularly with prediction of retention data of carboxylic acids, agrees in stating that in addition to the charge on the solute, several other factors (e.g.: size and hydrophobicity) play a part in the whole retention process. The main contributions to this topic will be discussed.

Ion-exchange of organic ions with a hydrophilic polar group and a hydrophobic part or chain involves a mechanism that is not easy to understand or to handle in order to optimize selectivity. Papers by several researchers are discussed.

Wide emphasis has been devoted to the prediction results obtained in capillary electrophoresis. In fact this field proved to be very active in optimizing experimental conditions and in relating experimental parameters to electrophoretic resolution.

A quick overview of the prediction of capacity factors of analytes by less common determinations has also been done.

2. Prediction of carboxylic acids retention data

2.1. Ion-exclusion chromatography

Ion-exclusion chromatography (IEC) [1,2] is a versatile approach in which retention of a single compound depends on the ratio of concentration of its ionized and neutral forms. The charge of dissociated ion-exchange resin functional groups has the same sign of the charge of the analyzed ionic compound.

When the water flows through the column, water molecules hydrate the ionic functional groups of the resin, and constitute the stationary phase together with water contained in the pores of the resin [3].

The hydrated resin network behaves like a semipermeable membrane between stationary and mobile phase through which species are freely exchanged. Retention according to a pure ion-exclusion mechanism is based on the following considerations. Neutral, uncharged molecules can enter the resin network and are eluted in a volume corresponding to the sum of inner (volume of stationary phase) and dead volumes of the column. Similarly, charged compounds are electrostatically repulsed and excluded by the resin phase according to the Donnan membrane equilibrium principle and elute at the dead volume of the column.

Focusing our attention on separations by negatively charged functional groups, acids of intermediate strength, such as carboxylic acids, can be partially ionized depending upon the pH of the medium and they can be separated on a cation-exchange resin in the H^+ form using an acidic eluent. Since its first introduction by Wheaton and Bauman [4], ion-exclusion chromatography has been extensively used for separation of hydrophilic, weakly ionized compounds (e.g.: hydrogencarbonates, phosphates [5], and condensed phosphate [5–7], aromatic and aliphatic carboxylic acids [5,8–11], alcohols and carbohydrates [12] from strong electrolytes and for applicative purposes.

In most applications, polystyrene–divinylbenzene (PS–DVB) gels with low crosslinking (2–8%) or

very soft gels have been employed. These materials have a loose matrix and hence interactions between solute molecules and the resin matrix through Van der Waals forces are very weak, so that retention of weak acids on these columns is mainly controlled by the Donnan exclusion effect [13]. So far, only a few applications have been reported on the determination of carboxylic acids on highly crosslinked PS–DVB (even up to 50%) [13–16] and unmodified silica-gel-based columns [11].

Current literature agrees that additional hydrophobic interactions and adsorptions, besides pure ion-exclusion effect, are involved in retention of carboxylic acids when polymeric resins are used. This point is of great importance in predicting retention data of analytes and maybe much more work should be devoted to characterising the retention behaviour of acids with hydrophobic properties on stationary phases at different degrees of crosslinking and hence at different reversed-phase characters.

The first relationship between the distribution coefficient (K_d) of different acidic compounds with their pK_a values has been qualitatively illustrated by Tanaka et al. [17], who used a hydrogen-form cation-exchange resin (degree of crosslinking 8%) and water as the eluent, proving that retention volumes of weak acids increased proportionally to the increase of pK_a, even if deviations from linearity occurred for ethanoic and propionic acids. This deviation was ascribed to adsorption effects.

In the field of understanding the mechanism involved during separation by IEC and in the prediction of retention data, the work of Glód and Kemula is relevant. In fact, as far as in 1986, Glód and Kemula [18] attempted a more quantitative description of the ion-exclusion mechanism of weak acids. Due to its importance in elucidating the retention mechanism in ion-exclusion chromatography and being the improvement in a further model, we will show hereafter the basic statements on which the model is based on.

Dissociation of a weak acid HR (Eq. (1)) and the existence of a dissociated and undissociated form in both phases are first considered:

$$HR \underset{}{\overset{K_a}{\rightleftharpoons}} H^+ + R^- \tag{1}$$

Under thermodynamic equilibrium, the chemical potential of the species at both sides of a membrane (Donnan membrane equilibrium accepted) are equal and under dilute acidic elutions can be expressed through the concentrations:

$$[H^+]_m[R^-]_m[HR]_m = [H^+]_s[R^-]_s[HR]_s \tag{2}$$

Dissociation of HR in both phases can be expressed by the following equation:

$$K_a = \frac{[H^+]_m[R^-]_m}{[HR]_m} = \frac{[H^+]_s[R^-]_s}{[HR]_s} \tag{3}$$

Considering the electroneutrality conditions and the concentration of dissociated functional groups (SO_3^- in this instance) it can be written:

$$[H^+]_m = [R^-]_m \tag{4}$$

$$C_{SO_3^-} + [R^-]_s = [H^+]_s \tag{5}$$

The definition of the distribution coefficient K_d is:

$$K_d = \frac{[R^-]_s + [HR]_s}{[R^-]_m + [HR]_m} \tag{6}$$

The distribution constant can be expressed as a function of Eqs. (3) and (4) and as the concentration of the sample at peak maximum, c defined as:

$$c = [R^-]_m + [HR]_m + [R^-]_s + [HR]_s \tag{7}$$

The distribution constant can be rewritten as:

$$K_d = \frac{c - [R^-]_m - \dfrac{[R^-]_m^2}{K_a}}{[R^-]_m + \dfrac{[R^-]_m^2}{K_a}} \tag{8}$$

and under the reasonable assumption $c \ll c_{SO_3^-}$, the following final form can be derivatised:

$$K_d = \frac{1 + \dfrac{2c}{K_a} - \sqrt{1 + 8\dfrac{c}{K_a}}}{\dfrac{2c}{K_a} - 2} \tag{9}$$

Starting from Eq. (9), using the experimental data (c/K_a of 24 carboxylic acids) of Ref. [18], we have calculated K_d values and deviations from experimental K_d. Results are shown in Table 1. A fair agreement of experimental and calculated values is

Table 1
Comparison of experimentally obtained K_d (K_d meas) with those calculated (K_d calc) by Eq. (9), Ref. [18]

Analytes	c/K_a	K_d calc	K_d meas	Error[a] %
Oxalic	0.00127	0.00127	0.04	96.8
Maleic	0.00599	0.00589	0.05	88.2
o-Nitrobenzoic	0.012	0.011	0.00	–
Chloroacetic	0.042	0.041	0.08	48.9
Phthalic	0.055	0.048	0.07	31.4
Salicylic	0.057	0.049	0.13	62.2
Fumaric	0.067	0.056	0.07	19.6
Tartaric	0.070	0.058	0.06	2.69
Citric	0.089	0.070	0.07	0.19
p-Nitrobenzoic	0.150	0.108	0.15	28.1
m-Nitrobenzoic	0.181	0.124	0.11	12.4
Terephthalic	0.216	0.140	0.07	100.1
Formic	0.306	0.176	0.15	17.6
Lactic	0.421	0.214	0.12	78.4
Benzoic	0.654	0.272	0.33	17.5
Ascorbic	0.809	0.302	0.20	51.2
Succinic	0.830	0.306	0.19	61.1
Metoxyphenylacetic	0.849	0.309	0.32	3.34
p-Acetylsalicylic	1.950	0.435	0.17	155.6
Acetic	2.280	0.458	0.35	31.0
Isobutyric	2.330	0.462	0.45	2.61
Valeric	2.250	0.456	0.52	12.2
Propionic	2.670	0.482	0.43	12.2
Caproic	2.170	0.451	0.65	30.6

Concentration of acids: 1 mM; column: 150×4-mm I.D. LiChrosorb KAT; mobile phase: water.
[a] Calculated from $100 \cdot abs(K_d \text{ calc} - K_d \text{ meas})/K_d \text{ meas}$.

obtained, showing that the retention process cannot be described only by ion-exclusion, even if this proves to be the primary mechanism governing retention.

The parameter most difficult to evaluate in Eq. (9) is c for sure. The paper does not give any hint how to obtain it, or the value of the number of theoretical plates N of the column used. The worth of Glód and Kemula work lies on their pioneering attempt to give a quantitative description of the ion-exclusion mechanism, and therefore is unquestionable.

Throughout the years, the model has been improved by the same authors [19] who clearly state the difficulty of the evaluation of c (that can be evaluated only approximately or has to be determined experimentally). Most of their work aims at the description of the retention behaviour of acidic compounds at different K_a, injected sample concentration and functional group concentrations by

two different approaches: the first one is the *global approach* and is based on thermodynamic chromatographic relationships. It represents the improvement of their previous work. In these conditions, the distribution coefficient can only be evaluated numerically from a nonlinear set of equations as an implicit function of the parameters characterizing the system. This approach is unjustified for a nonlinear partition isotherm. The second approach, the *local approach*, is a computer simulation in which chromatographic equilibria are applied locally to small fragments of the column corresponding roughly to theoretical plates.

They found that the addition of an acidic buffer eliminates the dependence of the retention volume on the sample concentration and that the computer simulation is more accurate and physically correct than the global approach.

Though these are improvements in weak acids modelling, results clearly show that some considerations on the whole retention behaviour are missing and that additional interactions between analytes and mainly stationary phase should be taken into account.

As previously mentioned, these interactions originate in a secondary retention mechanism, especially for neutral, large molecules, like aromatic and long-chain aliphatic compounds. In particular, the especially large interaction of aromatic compounds has been attributed to their π-electron interactions with the network when a polystyrene–divinylbenzene resin is used. Referring to interactions causing an increase in solute retention in respect to a pure ion-exclusion mechanism, the term *adsorption* is generally accepted in the current literature.

Among the attempts to include the adsorption interactions in the development of retention models, the works of Zhao and Liu [20], Glód and Stafiej [22], represent two good quantitative approaches.

Zhao and Liu's paper [20] is mainly focused on retention of dicarboxylic acids and for this reason it represents a good generalization of retention behaviour of analytes with multiple charge. Assuming $[HR^-]_s$ and $[R^{2-}]_s = 0$ (reasonable simplification if the functional group concentration is in excess with respect to the concentration of the solute), and that the neutral form of the solute is partitioned between the stationary and the adsorption phases, the follow-

ing equation for K_d expressing two contributions (adsorption, H and ion-exclusion, I) can be derived:

$$K_d = \frac{K_H + 1}{1 + \dfrac{K_{a1}}{C_E} + \dfrac{K_{a1}K_{a2}}{C_E^2}}$$

$$= \frac{K_H}{1 + \dfrac{K_{a1}}{C_E} + \dfrac{K_{a1}K_{a2}}{C_E^2}} + \frac{1}{1 + \dfrac{K_{a1}}{C_E} + \dfrac{K_{a1}K_{a2}}{C_{E^2}}}$$

$$= K_d(H) + K_d(I) \qquad (10)$$

C_E = concentration of the eluent.

Eq. (10) has been used to predict the retention volumes of C_4–C_9 dicarboxylic acids (see Fig. 1) on a sulfonated styrene–divinylbenzene copolymer (8% degree of cross-linking) cation-exchange column eluted by 0.2 mM HCl. The very good agreement between experimental and calculated retention volumes support the validity of the model derived and the great contribution of adsorption interactions that reaches 87% for the longer chain dicarboxylic acid studied. The paper also presents K_d, $K_d(H)$ and $K_d(I)$

values for a direct comparison. In this model, the same authors have further included a mass balance equation according to Glod et al. [19] obtaining good prediction of retention data [21] with average relative error of 3.1%.

In Glód and Stafiej's paper [22], their previously developed model, discussed in this review, was generalized to include adsorption of the solute in the mass (m) conservation equation on a theoretical plate distributed in the mobile (v_m), stationary (v_s) and adsorption (v_A) phase volumes, according to the relation:

$$m = ([R^-]_m + [HR]_m)v_m + ([R^-]_s + [HR]_s)v_s$$
$$+ [HR]_A v_A \qquad (11)$$

Under the assumptions of Zhao and Liu's work [20] and explicitly the Nernstian law or, equivalently, the linear Henry isotherm for the partitioning of the neutral form of the solute between stationary and the adsorption phases ([HR]$_A$ = K_H[HR]$_s$), they obtained that solute retention depends on the term $K_H V_A$ (V_A = volume of the adsorbed layer on one theoretical
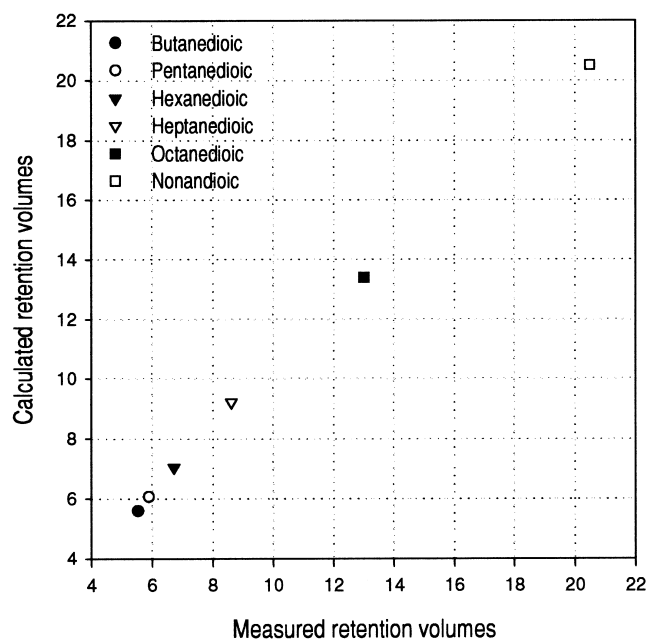

Fig. 1. Experimental vs retention volumes of C_4–C_9 dicarboxylic acids calculated by Eq. (10). Column: 500×3-mm I.D. packed with sulfonated styrene–divinylbenzene copolymer (8% degree of crosslinking) macroporous cation exchange resin. Eluent: 0.2 mM HCl; flow-rate: 0.87 ml/min; concentration of sample solutions: 100 ppm. Data from Ref. [20] with permission.

plate). Some examples of the dependence of retention volumes on the adsorption term are provided and adsorption constants for acetic, propionic, butyric, valeric, caproic, gallic, *o*-nitrobenzoic, acetylsalicylic, salicylic, *m*-nitrobenzoic are calculated from literature experimental data. The data indicate an increase in adsorption with increasing chain length and that aromatic compounds show strong adsorption and their retention is mainly governed by the adsorption mechanism. Moreover, it is shown how strong adsorptions induce increased asymmetry of the peak shape and how asymmetry can be theoretically predicted.

Since adsorption seems to play an important role in ion-exclusion chromatography of hydrophobic compounds like long-chain and aromatic carboxylic acids, some authors have investigated how to govern it [3]. Hydrophobic adsorption can be decreased by addition of an organic modifier [8,10–12,23] in the mobile phase and increased by addition of an ion interaction reagent (tetrabutylammonium bromide, TBABr). It has been shown [3] that retention of isomeric nitrobenzoic acids increases in the order *o*-<*p*-<*m*-nitrobenzoic when analytes are eluted with 0.5 m*M* TBABr instead of pure water in a Bio-Rad Aminex polystyrene–divinylbenzene copolymer (8% crosslinking). The increased retention is certainly due to increased hydrophobic interactions via ion-pairs which are probably more sterically favourite for the *meta* isomer.

Although some works dealing with eluent modifiers in ion-exclusion chromatography of carboxylic acids are present, no attempt has yet been made on modelling (empirically or mechanistically) retention data in the presence of organic solvent. This probably derives from the difficulty in the interpretation of organic solvents effects on such a separation. The organic modifier added in the mobile phase is supposed to be adsorbed on the polymeric resin, but clearly other effects are present. In our opinion, in fact, other parameters, such as the degree of ionization of all ionizable species of the system in the presence of an organic solvent, and swelling and shrinking of the resins should be, in some way, taken into account.

In order to account for the change of pK_a values with solvent content, at least of the analytes (in our case carboxylic acids) from which retention is strongly dependent according to what is reviewed until now, we propose to include the following simplified relationship developed by Bosch et al. [24,25] to the previous ones described:

$$pK_{(solvent)} = pK_{(H_2O)} + \frac{ax_2^0}{1 + bx_2^0} \qquad (12)$$

where *a* and *b* are characteristics of the acidic solute and x_2^0 is the mole fraction of the organic solvent. In Table 2, we report the changes in pK_a values for some carboxylic acids.

In order to control hydrophobic interactions between higher aliphatic, aromatic carboxylic acids and PS–DVB resins, Ohta et al. [11] have shown how the K_d of analytes with hydrophobic characteristics can be reduced using an unmodified silica-gel column (Develosil 30-5, functionality-SiOH) when compared with conventional PS–DVB columns (TSKgel SCX, functionality-SO$_3$H), as shown in Table 3. The silanol group on the surface of silica, in fact, acts as a weakly acidic cation-exchanger at pH>2 and is expected to behave as an ion-exclusion support for elutions by neutral or moderately acidic eluents. Hydrophobic interactions can also be controlled and reduced using a silica-based cation-exchanger functionalized with phenylsulfonic and propylsulfonic groups [16], even if secondary interactions such as adsorption phenomena on the silica gel still occur.

A different approach to optimization of chromatographic methods is the chemiometric one. Calull et al. [26] compared a super-modified simplex method and a modelling response surface method for the

Table 2
Effect of organic solvent on pK_a of some carboxylic acids according to Ref. [24]

Acid		p$K_{(H_2O)}$	p$K_{(50\%CH_3OH)}$	ΔpK
Citric	pK_1	3.13	3.98	0.85
	pK_2	4.78	5.70	0.92
	pK_3	6.39	7.59	1.20
Succinic	pK_1	4.20	5.00	0.80
	pK_2	5.60	6.71	1.11
Trichloroacetic	pK_1	0.65	1.61	0.96
Formic	pK_1	3.73	4.35	0.62
Acetic	pK_1	4.77	5.54	0.77
Benzoic	pK_1	4.19	5.23	1.04

Table 3
Distribution coefficients of some carboxylic acids on two ion-exclusion chromatography columns according to Ref. [11]

Analytes	Develosil 30-5 K_d	TSKgel SCX K_d
Formic	0.47	0.42
Acetic	0.95	0.81
Propionic	1.26	1.22
Butyric	1.66	2.03
Valeric	2.31	3.10
Caproic	3.01	9.67
Heptanoic	3.73	21.8
Caprylic	4.54	v.l.
Nonanoic	5.72	v.l.
Capric	7.87	v.l.
Lactic	0.59	0.42
Oxalic	0.55	0.03
Malonic	0.25	0.15
Tartaric	0.23	0.14
Malic	0.32	0.23
Succinic	0.55	0.48
Citric	0.25	0.11
Benzoic	0.48	4.74
Phthalic	0.05	0.14
Trimellitic	0.01	0.06
Pyromellitic	0.01	0.01

Mobile phase: water. For column characteristics see Section 2.1.
v.l. = very large value.

optimization of the major carboxylic acids found in wine (tartaric, lactic, malic, acetic, citric and succinic acids) by ion-exclusion chromatography. Considering pH (sulphuric acid), the flow-rate of the mobile phase and column temperature as variables, the two approaches lead to similarly good results. Details on chemometric approaches and on the chromatographic response functions will be given in the following sections.

Finally, a qualitative in-depth study on regularities in the retention of aliphatic carboxylic acids has been performed by Medved' et al. [27] who pointed out the significance of some factors affecting the retention of organic acids by IEC, like acidity, length of the hydrocarbon chain, the effect of substituents and the presence of double bonds in the acid molecule.

2.2. Anion-exchange chromatography

The equilibria which govern separations through pure ion-exchange separation are well understood

and therefore retention models have been developed in order to provide a quantitative description of factors governing retention mainly of inorganic simple ions.

Considering the equilibrium:

$$y R_x - E + x A^{y-} \overset{K_{A/E}}{\rightleftharpoons} x R_y - A + y E^{x-} \tag{13}$$
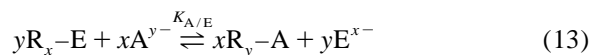
where A^{y-} is the analyte ion, E^{x-} is the eluent ion, R is the resin matrix, $K_{A/E}$ is the ion-exchange equilibrium constant, it is possible to obtain the expression:

$$\log k'_a = const - \frac{y}{x} \log[E^{x-}] \tag{14}$$

Eq. (14) expresses the capacity factor of a monoanionic species using monoanionic eluents and has been systematically applied for regressions of retention data of a wide variety of ions. More complex approaches can be followed in order to account for separations in the presence of multiple eluent competing anions, namely the *dominant equilibrium* approach, the *competing ion 'effective charge'* approach, and *dual eluent* species approach. These have been reviewed and discussed for general applications by Haddad and Jackson [2].

We will focus our attention on later developments on retention modelling in ion-exchange when applied to the prediction of retention data of carboxylic acids.
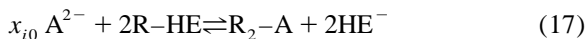
A model relating the capacity factors of analytes to stationary and mobile phase variables has been presented by Mongay et al. [28], and reviewed by Janoš [29]. The model has been developed taking into account the presence of a polyprotic eluent and monoanionic and dianionic sample ions. This approach considers that each species of eluent ion can displace each form of analyte ions, leading to a general equation that, at least in theory, can account for k' variations as a function of pH in any ion-exchange process of weak acids. The equation has been tested to monoanionic analytes, in a simplified linearized form:

$$\ln k' = \ln P - j \sum_{i=1}^{n} \left(\frac{x_i}{i} \right) \ln C \tag{15}$$

and for dianionic analytes in the following form:

$$k' = \frac{P_1}{C \sum\limits_{1=1}^{n} \left(\frac{2x_{i0}}{i}\right)} + \frac{P_2}{C \sum\limits_{1=1}^{n} \left(\frac{x_{i1}}{i}\right)} \qquad (16)$$

where P, P_1, P_2 are constants including selectivity coefficient, sample and eluent protonation constants, pH, dead volume, resin dry mass and capacity, j is the analyte charge, i, the eluent species charge, C, the total eluent concentration, x_i, the contribution of eluent species to displacement of analyte ions. For dianionic samples these contributions are expressed by x_{i0} and x_{i1} according to the equilibria:

$$x_{i0} \, A^{2-} + 2R\text{–}HE \rightleftharpoons R_2\text{–}A + 2HE^- \qquad (17)$$

$$x_{i1} HA^- + 1/2R_2\text{–}E \rightleftharpoons R\text{–}HA + 1/2E^{2-} \qquad (18)$$

Application of Eqs. (15) and (16) at different eluent concentrations allows the determination of the contribution x_i of each exchange reaction and a global selectivity coefficient defined for divalent anions as:

$$E_0 = \frac{[R_2A] \prod [H_{n-i}E^{i-}]^{\frac{2x_{i0}}{i}}}{[A^{2-}] \prod [R_iH_{n-i}E]^{\frac{2x_{io}}{i}}} \qquad (19)$$

$$E_1 = \frac{[RHA] \prod [H_{n-i}E^{i-}]^{\frac{x_{i1}}{i}}}{[HA^-] \prod [R_iH_{n-i}E]^{\frac{x_{i1}}{i}}} \qquad (20)$$

In Fig. 2, we have plotted the contributions calculated according to Eqs. (15) and (16) for succinic, malic, tartaric, oxalic, acetic and lactic acids. For monovalent anions, x_i has been considered as x_{i1}. It is possible to see that for dianionic species – except for oxalic acid – the elution power of phthalate ion for both ionized species of analytes is greater than the hydrogenphthalate ion, as expected. Oxalic acid seems to behave as the monoanionic analyte for which the contribution of hydrogenphthalate ion to elution is greater than phthalate ion ($x_{11} > x_{21}$). The agreement between experimental and calculated k' as a function of pH has only been shown qualitatively and seems indeed fair. A comparison with contributions of Cl^-, F^-, NO_2^-, Br^-, NO_3^- obtained at the same experimental conditions (for inorganic ions: $x_{11} < x_{21}$) and with the best fitting between experimental and calculated k', lead us to suppose that other additional mechanisms (e.g.: nonpolar interactions with the stationary phase especially at low pH values) could be involved in the retention of all carboxylic acids.

A less investigated parameter in anion-exchange of carboxylic acids is temperature, although a lot of work has been presented in reversed-phase liquid chromatography. The van't Hoff plots ($\ln k'$ vs $1/T$) permit the standard enthalpy for the transfer of the solute ion from mobile to stationary phase to be
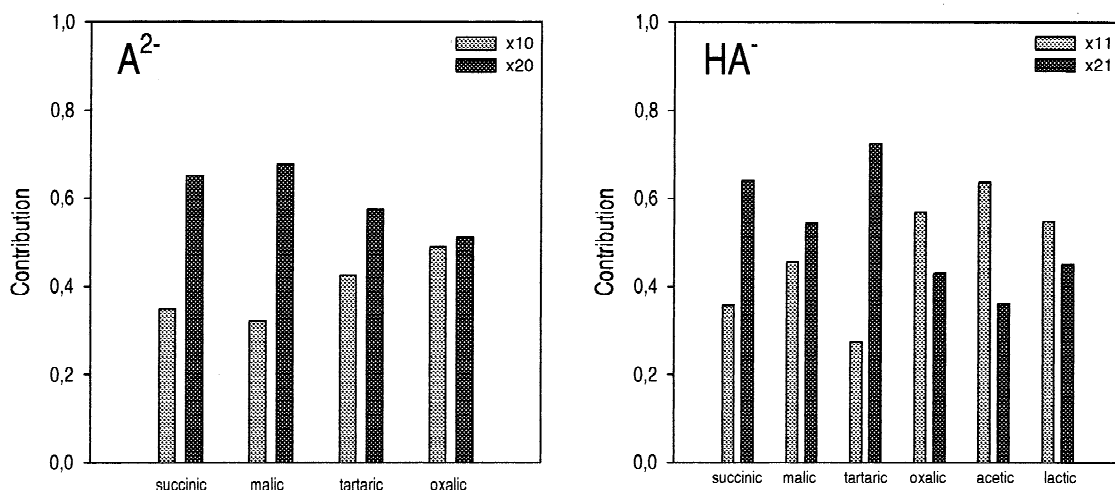

Fig. 2. Contribution coefficients of dianionic and monoanionic species calculated by Eqs. (16) and (15) respectively, according to Ref. [28]. Column: 100×4.6-mm I.D. packed with quaternary ammonium polymetacrylate (particle size 12.5 μm, 0.42 g dry resin, $Q = 0.050$ mequiv/g). Eluent: phthalic acid.

obtained, if linearity occurs, and give valuable information on the univocity of the mechanism involved in retention. Lee and Hoffman [30] studied the effect of temperature on separation of phenyl-acetic, 3-phenylpropanoic, 4-phenylbutyric and 5-phenylvaleric acids by PRP-X100 (spherical poly-(styrene–divinylbenzene) trimethylammonium anion-exchange column). Measurements performed in the temperature range 273–328 K, showed a slope change of the k' vs $1/[NO_3^-]$, meaning that a change in the ion-exchange equilibrium constant occurs. This is also confirmed by the lack of linearity obtained in the van't Hoff plots, with shapes almost identical for each concentration of nitrate ion as the eluent tested. The fact that shape is a function of the type of eluent ion, and not of its concentration denotes changes in the ion-exchange equilibrium constant. Eluent and analyte ions compete for the cationic sites on the stationary phase. The effect of temperature on the free energy for the transfer from the mobile to the stationary phase can be different for the two ions. Although all aspects of the effect of temperature have not been rationalized, it seems unlikely that a stationary phase transition, usually observed in reversed-phase liquid chromatography, is the cause of irregular shapes of carboxylate anions.

As additional interactions occur in the anion-exchange separations of carboxylic acids (formic, acetic, propionic, lactic, pyruvic, oxalic, malonic, succinic, tartaric, fumaric, maleic acids) a combination of ion-exchange and ion-exclusion mechanism on highly cross-linked latex-based anion-exchange stationary phase (IonPac AS4A-SC) has been recently proposed by Révész et al. [31]. In fact, when they applied Eq. (14) to the retention data obtained by NaOH eluents (1–75 mM), they found that the calculated slopes were lower than predicted on the basis of the stoichiometric pure ion-exchange model and that a second order polynomial function fitted the experimental data well. Considering the configuration of the resin used (a surface-sulfonated region on a highly crosslinked core, covered by an aminated latex layer), they suggest the presence of two sites of interactions, namely the anion-exchange in latex-bonded layer and electrostatic repulsion (ion-exclusion) in an under-layer which excludes anions through the Donnan potential. To account for this additional effect, the k' values calculated according

to the pure ion-exchange model (k'_{iex}) should be corrected (subtracted) for the contribution of an ion-exclusion term (k'_{excl}) in order to obtain the observed k' (k'_{obs}):
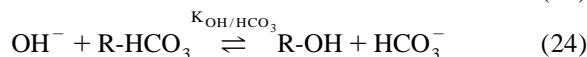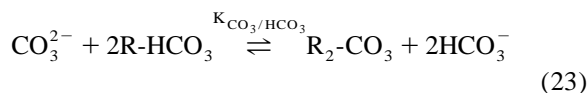
$$k'_{obs} = k'_{iex} - k'_{excl} \tag{21}$$

It was observed that k'_{obs} and k'_{iex} are equal, that is, carboxylic acids are retained by the pure ion-exchange mechanism, only at high NaOH concentrations. A residual cation-exchange capacity (about 10 μeq/col) in columns made by electrostatically bonding an anion-exchange latex to a cation-exchange surface has also been proven by our group [32]. Besides this additional repulsion to retention, in our opinion some other interactions (e.g., hydrogen bond with the alkanol functionality of the resin) should not be neglected as well.

One of the main applications of compounds containing carboxylic groups in ion-exchange chromatography is the use of complexing agents for the elution of metal ions. Extensive work on the use of EDTA as a ligand and on the elucidation of equilibria involved in complexation and elution has been done by Hajós et al. [33–36]. Other applications include lactate, oxalate [37] and PDCA [38].

A retention model [33,39] accounting for the presence of multiple species eluent, such as carbonate buffer, has been recently proposed and applied for the prediction of retention data of carboxylic acids [40]. The model uses the definition of the distribution coefficient D_A^{y-} related to the equilibrium [Eq. (13)]:

$$D_{A^{y-}} = \frac{(A^{y-})}{[A^{y-}]} = K_{A/E}^{1/x} \left(\frac{(E^{x-})}{[E^{x-}]} \right)^{y/x} \tag{22}$$

In the presence of multiple species eluent, such as the carbonate buffer system studied, E^{x-} is the result of the following inter-eluent ion-exchange equilibria:

$$CO_3^{2-} + 2R\text{-}HCO_3 \overset{K_{CO_3/HCO_3}}{\rightleftharpoons} R_2\text{-}CO_3 + 2HCO_3^- \tag{23}$$

$$OH^- + R\text{-}HCO_3 \overset{K_{OH/HCO_3}}{\rightleftharpoons} R\text{-}OH + HCO_3^- \tag{24}$$

Introducing the capacity of the column, defined as:

$$Q = 2(CO_3^{2-}) + (HCO_3^-) + (OH^-) \qquad (25)$$

with a few mathematical rearrangements, the final form of the model for retention behaviour of monovalent (Eq. (26)) and divalent (Eq. (27)) anions is obtained.

$$D_{A^-} = K_{A^-/HCO_3} \frac{-p + \sqrt{p^2 + q}}{4K_{CO_3/HCO_3}[CO_3^{2-}]} \qquad (26)$$

$$D_{A^{2-}} = K_{A^{2-}/HCO_3} \left(\frac{-p + \sqrt{p^2 + q}}{4K_{CO_3/HCO_3}[CO_3^{2-}]} \right)^2 \qquad (27)$$

where p and q are defined as:

$$p = \lfloor HCO_3^- \rfloor + K_{OH/HCO_3} \lfloor OH^- \rfloor \qquad (28)$$

$$q = 8 \cdot K_{CO_3/HCO_3} \lfloor CO_3^{2-} \rfloor Q \qquad (29)$$

For analytes that undergo partial protonation, the molar fractions of the different solute species must also be considered. The application of a nonlinear regression on experimental data allows the values of ion-specific ($K_{A/E}$) and inter-eluent (K_{OH/HCO_3}, K_{CO_3/HCO_3} constants to be obtained which in turn permit prediction of retention volumes. We have applied the developed equation to retention of monovalent and divalent analytes on an IonPac AS4A-SC column ($[HCO_3^-] + [CO_3^{2-}] = 2.5$–$7.5$ mM, pH 9.8–10.7) and the results are shown in Fig. 3. Although more physically consistent modelling based on the Gouy–Chapman theory for electrical double layer has been proposed [41], the ion-exchange computational difficulties and the neglect of ion-correlation effects for multiply charged ions in the Poisson–Boltzmann equation make the stoichiometric models more easy to use. The values of the ion-specific constants provide a relative comparison among retention of analytes, in fact, although the absolute values should be considered with care, they carry information on the retention mechanism involved. In fact, if ion-exchange is the only (or at least the main) interaction involved, $K_{A/E}$ should be related to the selectivity of the ion-exchanger and hence to the retention order. That is what we observed using the IonPac AS4A-SC column. In order to clarify the retention mechanism involved, we studied the performance of different ion-exchangers extending the
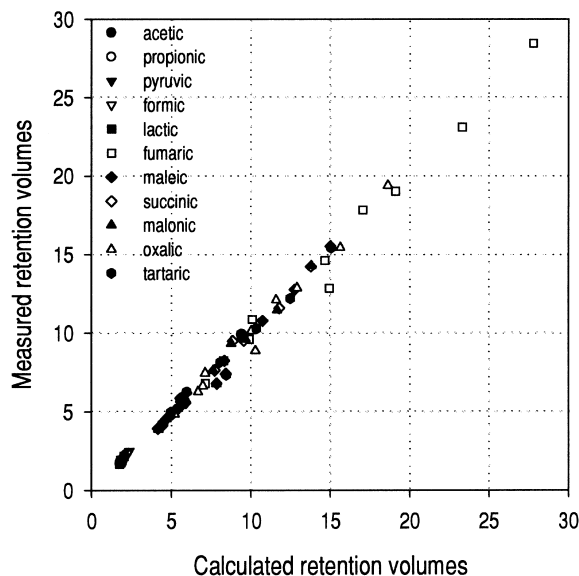

Fig. 3. Comparison between measured retention volumes and calculated according to Eqs. (26) and (27). Column: IonPac AS4A-SC 250×4-mm I.D. Eluent: carbonate buffer. Data taken from Ref. [40].

study to carboxylic acids of different hydrophilicity (quinic, *trans*-muconic and *trans*-β-hydromuconic acids were added as analytes) [42,43], proving different selectivities according to the ion-exchanger properties and analyte characteristics, with ion-specific constants related to the retention order when additional interactions, besides ion-exchange, with stationary phase where reduced to a minimum. It is evident that the retention models developed up to now need some improvement, mainly in the treatment of hydrophobic and other specific analyte–stationary phase interactions, steric effects (e.g.: partial volume exclusion of bulky biomolecules) and, in some cases, heterogeneity of the resin matrix. A description of multicomponent ion-exchange equilibria for weak electrolytes using a general thermodynamic framework based on the identification of the surface excess properties in the resin phase and on an assumed distribution function of functional groups has been presented by van der Wielen et al. [44]. The experimental results obtained for acetic, acetylmethionine, 6-aminopenicillanic and phenylacetic acids through batch and column experiments on a relatively low capacity (217 mol/m^3) strong-

base acrylate based anion-exchange resin (Macro-Prep Q resin from Bio-Rad) were correlated with the model developed using a nonlinear parameter estimation technique. The average deviation of the prediction of ion-exchange equilibria for acetic acid is 14%. An advantage of the model proposed is that prediction can be performed in a wide range of analyte concentration whose superior limit strongly exceeded resin capacity (in this application from 0 to about 0.5 kmol/m^3). A comparison with the uptake predicted by conventional models, showing the convergence of the two approaches only in the dilute region of analyte content in the resin, is available.

A different approach to describe retention behaviour is represented by chemometric analysis of retention data via factor analysis. Principal component analysis (PCA) and correspondence factor analysis (CFA) have been used to evaluate the complexity of the retention mechanism and search for optimization in an ion chromatographic analysis [45]. The ion chromatographic behaviour of formic, acetic, propionic, butyric and pentanoic acids was studied using a carbonate buffer mobile phase and a resin copolymer of 2-hydroxyethyl methacrylate and ethylene dimethacrylate coated with a pellicular surface ion-exchanger of formula: -$\{CH_2CH[C_6H_4CH_2N^+(CH_3)_3]\}_m$. The application of chemometric methods can reveal hidden factors important from a mechanistic and optimization point of view. PCA analysis proved that only a major retention mechanism is responsible for the observations, as only one factor is necessary to calculate retention times. A more in-depth analysis by CFA shows that although no special regularities can been seen on a factorial plane, the length of the alkyl chain governs selectivity in this ion-exchange system. CFA maps allow estimation of selectivity which turns out to be a function of both eluent concentration and of the carbonate/bicarbonate ratio. This kind of approach can show only a qualitative mechanism of retention of carboxylic acids and it has not been used to predict retention data.

Modified simplex methods, with the use of proper chromatographic response functions (CRF) have been used to identify the optimum working conditions for the optimization of separations. This approach allowed the selection of phthalic acid as the eluent giving the wider chromatographic separation

among acetic, lactic, succinic, malic, citric, tartaric acids in a Shimpack IC-AI column (low capacity polymeric phase with quaternary ammonium polymethacrylate) [46].

2.3. Reversed-phase chromatography

Retention of organic compounds on an alkylchain-bonded silica gel is related to the hydrophobicity ($\log P$) of the solutes expressed as the partition coefficient in an octanol–water system. In reversed-phase modes, it has been verified that $\log P$ is in linear relation with $\log k'$, while this correlation is absent in ion-exchange and ion-pair systems, and therefore chromatographic data have often been used to calculate hydrophobicity. Conversely, it has been shown how to use hydrophobicity parameters to predict retention data and to optimize reversed-phase mode liquid chromatography. Hanai and Huber [47] used the $\log P$ values of indoleacetic, benzoic, phenylacetic, DL-mandelic and hippuric acids to predict their retention on polystyrene gel and octadecyl packings by using the following relationship:

$$k' = \frac{k'_M + k'_I \dfrac{K_a}{[H^+]}}{1 + \dfrac{K_a}{[H^+]}} \tag{30}$$

where k'_M is the retention of acid under its molecular form and related to hydrophobicity of the solute and k'_I is the retention of its ionic form measured at high pH values. Besides the difficulty to correctly evaluate k'_I in a reversed-phase system, the main drawback of this approach is the measurement of pH in the presence of organic solvents whose use is required in RP systems. This disadvantage has been overcome by Bosch et al. [24] who proposed a general equation to calculate the pH of a buffered solution in the presence of organic solvents and further applied it to the quantitative description of weak acids retention [25] also considering the decrease of the activity coefficients as the dielectric constant of the medium decreases, that is with the increase of the organic modifier percentage. The equation proposed to describe retention of weak acids considering the contribution of their ionized

(A$^-$) and molecular (HA) form according to Ref. [25] is:

$$t_R = \frac{(t'_{R(HA)} + t_{0(HA)})y_{A^-}10^{pK_a-pH} + (t'_{R(A^-)} + t_{0(A^-)})}{y_{A^-}10^{pK_a-pH} + 1}$$

(31)

where $t'_R = t_R - t_0 =$ adjusted retention time, and $y =$ activity coefficient.

Eq. (31), virtually applicable to the prediction of retention of all ionizable species in any buffer system, has been tested with benzoic, 2-nitrobenzoic, 3-nitrobenzoic, 4-nitrobenzoic acids in methanol mixtures (40, 60, 80% CH_3OH) on a 5-μm Li-Chrospher 100 RP-18 column. Introducing the measured retention times, pH, activity coefficients and $t_{0(A^-)}$ (it has been proven that column holdup time changes with buffer composition) in the equation developed, pK_a and adjusted retention times of neutral and anionic base can be calculated. Such an approach leads to an optimization in terms of separation factor of two adjacent peaks but not of their resolution.

Owing to additional interactions such as adsorption phenomena between reversed-phase supports and polar solutes their retention is usually higher than predicted by the liquid–liquid distribution models and sometimes peaks also show considerable tailing. It has been shown that the liquid–liquid distribution model of some carboxylic acids (benzoic, 4-hydroxybenzoic acids) is obeyed when supports without highly acidic, isolated residual, silanol groups (μ-Bondapak type like) are used or if the liquid stationary phase has a hydrophobic group and a polar function as well [48]. The modelling surface response method has also been used to achieve optimization of carboxylic acids separation. After the choice of the variable space at which the response surface has to be modelled, this kind of approach allows the use of general mathematical equations (e.g.: third-order linear model with interactions relating variables and response criterion) without the knowledge of the exact relationship between retention behaviour and mobile phase parameters (variables). Nevertheless, the success of an optimization process depends on the model selected, on its validity to fit experimental data and on the experimental response chosen. As a selected applica-

tion [49], Eq. (32) is the chromatographic response function (CRF), used as the optimization response, to obtain an optimum separation of malic, tartaric, acetic, lactic, succinic, citric, as phenacyl bromide derivatives, in wine samples by a 5-μm Spherisorb ODS-2 column with the minimum analysis time accepted.

$$CRF = a \sum R_{i,j} + b(t_m - t_1)$$

(32)

$R_{i,j}$ is the resolution of each pair of peaks i and j, t_m is the maximum acceptable retention time of the last peak, t_1 the retention time of the last peak and a and b are weighting factors. $\sum R_{i,j}$ and $t_m - t_i$ can be then expressed as linear combinations (the model) of the selected mobile phase variables (see above). The use of computer programs allows the coefficients of the selected model and the isoresponse surfaces to be obtained, that show the variation of the summands of CRF as a function of the variables of the mobile phase chosen, thus allowing the chose of the optimum values of the terms of the CRF criterion. In the application selected, taking into account that derivatized carboxylic acids separation is achieved by organic modifier gradient, starting mobile phase composition (methanol %) and change in the solvent strength (methanol % min^{-1}) have been chosen as mobile phase variables.

Chemometrically driven retention prediction systems in RP chromatography have also been developed by constructing solvent parameters or by correlating a compound's molecular structure and properties to its retention behaviour, developing compound structure–retention relationships and solvent composition–retention relationships. This approach, coupled with a multilayer feed-forward neural-networks analysis, has been used in the prediction of chiral chromatographic separations of several racemic carboxylate derivatives by the construction of quantitative structure–enantioselective retention relationships (QSERRs) [50], as shown in Fig. 4. This kind of relationships takes an extrathermodynamic approach to identification and isolation of the most important structural characteristics responsible for the observed retention. Development of statistically significant equations allows for the possibility of extracting physically meaningful information relating to the main solute–stationary phase
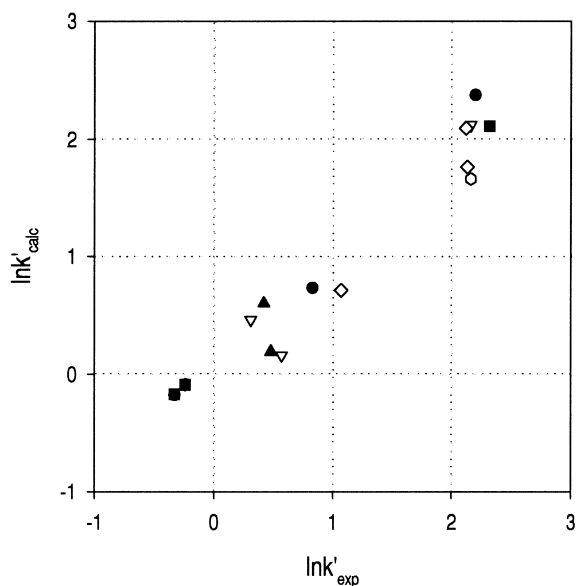
Fig. 4. Comparison between experimental and network-predicted retention factors for 2-(4-chlorophenoxy)propionic, 2-phenyl-4-methylpentanoic, α-hydroxyphenylacetic acids on Chiralpak AD, amylose (S)-phenylethyl carbamate (Chiralpak AS) and amylose (R)-phenylethyl carbamate (Chiralpak AR). Eluent: hexane-2-propanol (95:5, v/v) plus 1% trifluoroacetic acid, temperature 27℃. Data taken from Ref. [50] with permission.

interactions. Neural networks have proven to be capable of higher *predictive* power than multivariate regression, although the *descriptive* power of the networks is limited and must be compensated by regression equations, through which it is possible to identify factors (e.g.: charge transfer, electrostatic, lipophilic, dipole interactions) describing retention and, in this instance, enantioselectivity. The general equation used by Booth et al. [50] in multivariate regression analysis was:

$$\ln k' = f_1(\text{LUMO}) + f_2(\text{MEP}) + f_3(\text{MLP})$$
$$+ f_4(\text{DIP}) + f_5 \tag{33}$$

where f_i = regression coefficients, LUMO = energy of the lowest unoccupied molecular orbital, MEP = average molecular electrostatic potential, MLP = average molecular lipophilic potential, DIP = total dipole moment. Correlation coefficient values were obtained ranging from 0.874 to 0.972 for the 23 solutes and the three columns studied: Chiralpak AD, amylose (S)-phenylethyl carbamate (Chiralpak AS)

and amylose (R)-phenylethyl carbamate (Chiralpak AR).

An alternative to the errors associated with imperfect models and parameters has been seen in the entire elimination of the model and in the reliance on relationships defined by the data themselves. PCA analysis, for its ability to produce abstract factors that are intrinsic to the data set, and target transformation factor analysis (TTFA) have proven to be successful in the description of retention properties of compounds independent of any column or mobile phase and conversely in the description of the retention properties, separation systems can be described independently of any particular compound [51]. Based on the development of a universal retention index system, prediction of retention of different compounds (dimethylphthalate as a carboxylate compound) was achieved with satisfactory accuracy. Other applications of multivariate analysis demonstrated that uncharged modifiers (methanol, acetonitrile, tetrahydrofuran) and pH effect retention times, while column temperature and pH are important for the column efficiency [52]. The use of a partial least squares model allowed the prediction of retention times of a solute with a carboxylic functionality (amoxicillin) within a −4% error.

Among computer-aided methods for prediction of retention data, computer simulations based on proper equations have been developed. The DryLab I/mp software has been developed [53] and used for predicting high-performance liquid chromatographic separations of acidic [53–55] and basic solutes as a function of changes in mobile phase pH, based on Eq. (34):

$$k' = k^0(1 - F^{\pm}) + k^+ F^{\pm} \tag{34}$$

where k_0 and k^+ refer to k' values for the non-ionized (^{0}) and ionic ($^{\pm}$) forms and $F^{\pm}$ is the fraction of solute molecules that are ionized according to the following expressions:

$$F^+ = 1/\{1 + K_a/[\text{H}^+]\} \quad \text{for a basic solute} \tag{35}$$

$$F^- = 1/\{1 + [\text{H}^+]/K_a\} \quad \text{for an acidic solute} \tag{36}$$

This strategy has proven to be a powerful tool for prediction of retention of monoprotic benzoic acids (errors in t_R ranging from 1 to 7%, average accuracy

in α prediction $\pm 1\%$), but lead to greater errors for polyprotic acids, due to the fact that their retention could not be described exactly by Eqs. (34)–(36).

As an improvement, the same authors considered the simultaneous effect of pH and solvent strength [54] according to Eq. (37):

$$\log k' = A\varphi^2 + B\varphi + C \qquad (37)$$

where A, B, C are constants for a given solute and φ is the volume fraction of organic solvent in mobile phase. Prediction of band width, resolution and selectivity is presented. Taking into account that most of the literature deals only with the effect of one variable at a time, the combination of two mobile phase variables has to be noted, because it is well known that retention is the result of several interactions among solute, mobile and stationary phase, making optimization procedures not always straightforward and obvious.

2.3.1. Ion interaction chromatography (IIC)

Ion interaction chromatography has been widely used for the separation of polar, charged compounds. The general operative mode includes a proper pairing ion added to the mobile phase which is considered to give a dynamic equilibrium between the eluent and a reversed-phase stationary phase. Retention is due to electrostatic and hydrophobic interactions between the pairing ion and an analyte of opposite charge in a stationary phase that behaves like an ion-exchanger.

Retention and selectivity in IIC are influenced by several experimental parameters such as concentration of ion interaction reagent, ionic strength, concentration of organic modifier. Due to its high efficiency and to the wide range of eluent variables which can be used to manipulate the retention of solutes, IIC is often applied to the resolution of difficult mixtures of solutes. If the number of mobile phase variables turns out to be advantageous for achieving separation, on the contrary it becomes a drawback in the modelling of the retention process, due to the difficulty of quantitatively attributing the effect of each parameter to k'. Since the first developments of the technique, great efforts have been devoted in characterizing retention mechanism. The main basic mechanisms proposed have been examined in detail by Haddad and Jackson [2].

One of the first approaches to ion interaction chromatography has been attempted by Iskandarani and Pietrzyk [56] who derived the following equation accounting for the major equilibria that influence the retention of organic analytes:

$$\frac{1}{k'_x} = \frac{1}{A[Q^+]} + B[C^-] + F[X^-] \qquad (38)$$

with A, B and F constants including the phase ratio, the sorption capacity of the column, the equilibrium constants of the retention of the pairing ion, and of the ion pair formed by the pairing ion with the analyte. Although experimentally tested for p-hydroxybenzoic and o-phthalic acids eluted by an acetonitrile–water mixture containing tetrapentylammonium chloride, NaOH and NaCl on a PS–DVB column (10 μm PRP-1), Eq. (38) has been fitted by varying one variable at a time and no quantitative regression results have been presented. Despite this, this theory constitutes a strong background for further stoichiometric model developing in IIC.

Retention of p-hydroxybenzoic, o-toluic, sorbic and p-toluic acids in the presence of tetrabutyl- and tetrapropyl ammonium on a 5-μm C_{18} irregular shaped column has been studied by Del Rey and Vera-Avila [57]. Experimental results are discussed in view of previously proposed mechanisms and a two-member expression [Eq. (39)] relating k' to adsorbed pairing ion concentration is proposed.

$$k'_X = k_{0,X} - Z + \left\{ \Phi K_{ip} + \frac{\Phi K_{ie}}{[C^-]_m} \right\} [Q^+]_{st} \qquad (39)$$

$k_{0,X}$ is the capacity factor in the absence of the pairing ion (Q^+), Z is a constant term depending on the nature of solute (X^-) and pairing ion, Φ is the phase relation, C^- is a counter-ion. The main statements in developing the retention model are the attraction of the solute ion by the ionic surface to get into a diffuse ionic cloud displacing a counter-ion, governed by the constant K_{ie}, and the presence of hydrophobic effects experienced by the solute in the mobile phase and by the adsorbed pairing ions that can promote the formation of ion pairs on the surface of the packing, governed by the constant K_{ip}. The retention model, tested at constant Q^+ concentration when varying C^- concentration gave satisfactory results. In fact, when tetrabutylammonium is used as

pairing ion, the percentage difference between the experimental slope of the linear plot k' vs $[Q^+]_{st}$ (obtained varying $[Q^+]_{st}$) and that calculated by Eq. (39) (obtained by varying $[C^-]_m$) is 6.2%.

A very different approach in IIC is followed by Bartha and Ståhlberg [58] who developed an electrostatic theory which accounts for electrostatic interactions resulting from long-range forces. A simplified electrostatic model has been used to predict the slope of the linear plots k' vs ionic strength (I) of dissociated carboxylic acids separated on LiChrosorb RP-18 column using a mobile phase containing a buffer phosphate and hexylamine as pairing ion. A generally good agreement, shown only qualitatively, has been obtained for 3,5-dihydroxybenzoic, 4-hydroxymandelic, 2,4-dihydroxybenzoic and mandelic acids using Eq. (40), derived in case surface concentrations are relatively low and no specific adsorption of the electrolytic counter ions occurs.

$$\ln k'_X = K + \frac{1}{2}\left(\frac{z_Q z_X}{z_Q^2 + 1}\right) \cdot \ln I - \left(\frac{z_Q z_X}{z_Q^2 + 1}\right) \cdot \ln [Q] \tag{40}$$

The term K is a constant depending on the respective charges and hydrophobicity of the solute and the pairing ion and on the organic modifier concentration, while z_Q and z_X are the charge of pairing ion and solute, respectively. The electrostatic theory formulated in the general form:

$$\left(\frac{k'_X}{k'_{0X}}\right)^{-\frac{z_Q}{x_Z}} = f([Q]) \tag{41}$$

k'_{0X}, the capacity factor of solute ion when the electrostatic potential and the pairing ion concentration are equal to zero, can be used to evaluate the charge of the eluting species or in turn to confirm the applicability of the dynamic ion-exchange to experimental data. This approach has been followed by Sacchero et al. [59] for the elution of singly charged chelates of Bi (III), Co(III) and Fe(III) with EDTA and for the doubly charged complexes of Ni(II), Cu(II) and Pb(II) with the same ligand. A modified form of Eq. (41):

$$\frac{k'_{X^-}}{k'_{0X^-}} = \left(\frac{k'_{X^{2-}}}{k'_{0X^{2-}}}\right)^{1/2} \tag{42}$$

has been applied to retention data of chelates obtained on a LiChrospher 100 RP-18 column at a constant tetrabutylammonium concentration ($z_Q = 1$, $z_X^- = -1$ and $z_{X2-} = -2$) gave a satisfactory correlation value ($r = 0.990$).

A permanent coating IIC method for carboxylic acids has been presented by Jun et al. [60]. The difference from conventional IIC mechanism is that the stationary phase is initially equilibrated by the pairing ion which now is not added to the mobile phase. Since the stationary phase in this case is converted into an ion-exchanger, the eluents used in the separation are identical to those employed with conventional fixed-site ion-exchange materials and so virtually do the theoretical relationships. The work of Jun et al. [60] showed that in the separation of propionic and butyric acids on a permanently coated polymeric column (PRP-1 column) with hexadecyltrimethylammonium, the low capacity ion-exchange column obtained (0.30 mmol per column) facilitates a dual retention mechanism of ion-exchange and surface adsorption. The latter interaction has been proven throughout the application of Eq. (40) in the simplified form:

$$\frac{1}{k'} = C_1 + \frac{C_2}{\sqrt{I}} \tag{43}$$

where C_1 and C_2 are constants. This relationship, derived for the adsorption of organic ions onto a nonionic adsorbent according to the Stern–Gouy–Chapman theory of double layer adsorption, has been applied to retention data of propionic and butyric acids together with chloride ion. The good regression coefficient obtained for the carboxylic acids ($r^2_{propionic} = 0.981$ and $r^2_{butyric} = 0.951$) and the total lack of fit for chloride ion indicates that surface adsorption was involved in the retention of propionate and butyrate on the coated column. A schematic representation of the retention process of the carboxylic acids to stationary from mobile phase as a function of mobile phase pH is presented. Although the work proposes the contribution of the two mechanisms only from a qualitative point of view and applies to a very limited number of organic analytes, it represents a good discussion of retention of organic acids and of extra-column factors influencing retention of the surface adsorption of analyte ions.

The predictive power of a stoichiometric model [Eq. (44)] for anionic (including as carboxylic compounds propionic and oxalic acids), neutral and cationic species, retained by an IIC mechanism, has been shown by Sarzanini et al. [61].

$$k'_{X^{2-}} = \frac{(ads)}{1 + [X^{2-}] \cdot (ads) + (exch)} \cdot b \cdot e^{c\varphi} \qquad (44)$$

with *ads* being the term including the interaction in the stationary phase between analyte-pairing ion and between analyte-opposite charge ion, and *exch* the term accounting for dynamic ion-exchange of analyte ion with other counter-ions of the same charge. b and c are constants and φ is the fraction of the organic modifier in the mobile phase.

The main contribution of this retention model is the possibility to predict retention data of analytes when the main mobile phase parameters (pairing-ion, ionic strength and organic modifier concentrations) are simultaneously changed. The good predictive ability is confirmed by the errors between calculated and experimental k' (%error$_{propionic}$ = 12%).

IIC has been also used for correlation between $\log k'$ and $\log P$ and biological activity of *N*-phenylamides substituted in the positions 2,3,4 by one carboxylic functionality [62]. These compounds owing to their biological activity can be used in studies of quantitative structure–activity relationships (QSAR). The relatively high correlation coefficients obtained for retention data of eight *N*-phenylamides (ODS column, pairing ion: di(2-ethylhexyl)orthophosphoric acid, pK_a = 1.3) show a good analogy between $\log k' - \log P$ and biological activity, in this example expressed by the inhibition of synthetase of prostaglandins and the strength of binding to albumin according to Collander's equation [63]:

$$-\log C = b \cdot \log k' + a \qquad (45)$$

where C is the molecular concentration of the compound producing an equivalent biological effect. The positive slopes obtained indicate the importance of the hydrophobicity of compounds in the quantitative structure–activity relationships.

2.4. Capillary electrophoresis

In recent years, capillary electrophoresis has emerged as an increasingly powerful separation tool that complements HPLC and IC for its high separation efficiency, selectivity and short analysis time. Many applications concerning organic acids analysis have been reported in the literature [64].

The simplest method of peak identification in capillary zone electrophoresis is the comparison of the migration times of the sample peaks with those of standard species analyzed under the same conditions. Any change in the operational conditions from run to run, either as selected by the operator or due to the aging of the capillary, brings about principal changes in the migration times and prevents their direct comparison. As an example, in the separation of DNA fragments, even the difference of several seconds is important to assign the true base pair number to a peak [65]. Vespalec et al. [66] proposed a procedure for the calculation of migration times, compensating for the changes in temperature, electroosmosis, capillary length and diameter, voltage applied and running buffer. The actual effective mobility of analyte X (μ_X), can be determined by Eq. (46) using the detection times of analyte and of two standards, A and B (t_A, t_B, t_X) of known mobilities (μ_B, μ_A), from the same run, providing that the electroosmotic velocity (ν_{eo}) and the term $l\chi S/I$ remains constant in one run (l = detection length, χ = specific conductivity of the background electrolyte, S = capillary cross-section, I = electric current).

$$\bar{\mu}_X = \mu_A + (\mu_B - \mu_A) \cdot \frac{t_B}{t_X} \cdot \frac{t_A - t_X}{t_A - t_B} \qquad (46)$$

The actual corrected mobilities of sulfosalicylic, phthalic, D,L-dinitrophenylglutamic, D-dibenzoyltartaric, salicylic, anthranilic, picric, cinnamic, β-coumarineacetic, indolyllactic, D,L-benzoylmethionine, D,L-benzoylphenylalanine, D,L-tryptophan cyclohexylamine acids, calculated in a wide range of experimental conditions (different background electrolytes, temperature and voltage applied), proved to be constant at every experimental condition evaluated and in good agreement with those tabulated.

Optimization of separation conditions of polyvalent weak acids (3,5-dimethoxybenzoic, 1,2-1,3-1,4-benzenedicarboxylic, and 1,2,3-1,2,4-1,3,5-benzenetricarboxylic acids) throughout resolution of each

pair of peaks (R_{ji}) has been reached by Friedl and Kenndler [67,68]. The introduction of resolution in CZE according to Giddings definition, without electroosmosis, and longitudinal diffusion being the only process causing peak dispersion, it was possible to derive the following expression:

$$R_{ji} = (r_{ij} - 1) \cdot \frac{1}{\dfrac{1}{z_i^{1/2}} + \dfrac{r_{ij}}{z_j^{1/2}}} \cdot \left(\frac{e_0 U}{8KT} \right)^{1/2} \qquad (47)$$

where r_{ij} = selectivity (ratio of the effective mobilities) = $\mu_i^{\text{eff}} / \mu_j^{\text{eff}}$, z_i is the effective charge number approximated as the summation of the degree of dissociation of the individual ionic subspecies, e_0 is the electric charge, U is the voltage, K is the Boltzmann constant and T is the absolute temperature. From Eq. (47) it follows that voltage and pH are the two only parameters existing in order to adjust resolution in CZE. Depending on the mutual sequence of actual mobility and pK_a value, the curve of resolution can fall to a resolution of zero at a certain pH, this effect being independent on the efficiency. Excellent agreement between experimental and calculated resolution was found, allowing one to forecast the resolution for any pair of sample components as a function of pH. Limiting factors, not specifically related to the theoretical approach, like reliability of effective mobility, pK_a values (pK_a values are used for calculation of mobilities and charge number), and the occurrence of other sources of peak dispersion besides longitudinal diffusion (e.g.: wall adsorption of small ions, contained for instance as impurities in the buffer can generate active sites on the capillary wall, whose effects are not reproducible) reduce the possibility to predict selectivity and efficiency with sufficient accuracy.

For weak electrolytes like carboxylic acids, pH is a crucial parameter, especially for ampholytes whose direction towards electrodes can be reversed according to buffer pH. The artifactual peak splitting (each peak corresponding to the neutral, cationic and anionic states, this phenomenon being enhanced at pH close to pI) of small amphoteric compounds (histidine and tryptophan) caused by overloading phenomena, when the concentration of sample is comparable with that of background electrolyte, has been modelled by Ermakov et al. [69]. In general

good qualitative and quantitative agreement between experimental and simulated electropherograms has been found. The most significant discrepancies were found in the prediction of the height of the neutral peak, probably due to the approximations adopted in deriving the model (e.g.: the dissociation of each group is independent of the others) and again the approximation data on the pK values. Though the working conditions in CZE generally use small amounts of sample, the model can be applied when preparative-scale electrophoresis and preconcentration procedures are performed.

The possibility to identify unknown compounds in a sample by separative analytical methods is very attractive. Wronski [70] showed a procedure to calculate absolute mobilities and pK_a values, based on isothachophoretic measurements. By means of a relationship between mobilities, determined in three different buffers, and the pK_a of 46 acidic compounds having a pK_a ranging from 2.20 (2,3-dibromopropionic acid) and 6.18 (cacodylic acid) empirical relationships were derived. Different equations have to be used for monovalent acids according to the value of the mobilities in the buffers (a range of validity is shown) and for divalent acids as well. Even if the agreement between calculated pK_2 and the literature values is rather good (see Table 4), from the previous discussion it comes out that in view of separation optimizing, minute differences of pK values can be decisive. Moreover, it seems important to state the number of initial compounds necessary to evince the relationship between μ_B / μ_A and pK.

Separation of a mixture of carboxylic acids has proved to be difficult in borate buffer, where these analytes exhibit similar ionic mobilities, and their detection by direct UV is dependent on the carrier electrolyte. Ion mobilities can be manipulated by organic solvent affecting solvation [71,72], by pH affecting the dissociation [73,74], and by complexing reagents affecting the charge [75,76]. A model for the migration of carboxylic acids in the presence of tetradecyltrimethylammonium (establishing coelectroosmotic separation conditions), barium and cyclodextrin as complexing agents has been reported by Buchberger and Winna [76]. The presence of barium ions improved separations of carboxylic acids owing to the complexation by two vicinal carboxylate

Table 4

Comparison between pK_2 of some carboxylic acids taken from the literature and calculated on the basis of mobilities determined in buffers A (β-alanine pH_A 3.25), B (ε-aminocaproic acid pH_B 4.50), C (histidine pH_C 6.25), according to equations [70]

$$pK_2 = \frac{pH_C - pH_B}{\mu_c - \mu_b}(0.75 \cdot \mu_{02} - \mu_B) + pH_B$$

$$pK_2 = \frac{pH_B - pH_A}{\mu_B - \mu_A}(0.75 \cdot \mu_{02} - \mu_A) + pH_A$$

(when $0.75 \cdot \mu_{02} < \mu_B$)

Analytes	pK_2 lit.	pK_2 calc.
Oxalic	4.27	4.00
Malonic	5.70	5.43
Succinic	5.64	5.80
Glutaric	5.27	5.67
Adipic	5.28	5.74
Mailc	5.05	4.98
Fumaric	4.38	4.29
Ketoglutaric	5.01	5.13
Tartaric	4.37	4.19
Phthalic	5.41	5.54
Terephthalic	4.46	4.48
3-Ethylglutaric	5.30	5.68
Ethylmalonic	5.83	5.93
Glutaconic	5.08	5.39
Itaconic	5.45	5.64

groups, while cyclodextrin can be used in CZE for fine-tuning of separation, taking advantage from the known benefits introduced by cyclodextrin in ion-exclusion chromatography of carboxylic acids. Considering the effective electrophoretic mobility μ_{eff} of a carboxylate anion in the presence of barium ions and cyclodextrin as the sum of the mobility of the free carboxylate anion (μ_A), the mobility of the barium-carboxylate complex (μ_{BA}) and the mobility of the cyclodextrin-carboxylate complex (μ_{CA}), each multiplied by the respective molar fraction α, it can be finally obtained:

$$\mu_{eff} = \frac{\mu_A + \mu_{CA}K_{CA}C_C}{1 + K_{BA}C_B + K_{CA}C_C} \tag{48}$$

where K_{BA} and K_{CA}, are the complex formation constants for the barium-carboxylate and cyclodextrine-carboxylate complexes, respectively, C_B and C_C are the concentrations of barium ions and cyclodextrin, respectively. Values for μ_A, μ_{BA}, μ_{CA}, K_{CA}

and K_{BA} can be obtained with five experiments. Eq. (48) enables a quick and efficient optimization of the carrier electrolyte composition and shown to be appropriate (in the separation of 18 di- and tri-charged carboxylic acids) for cyclodextrin concentrations higher than 1 mM, while below this value, ion pairs between carboxylate and tetradecyltrimethylammonium ions are present whose formation has not be taken into account by the model.

Method development and optimization procedures in capillary electrophoresis for small ions (formate, propionate and oxalate have been included as applications) have been discussed by Jimidar et al. [77] who reviewed the selection of initial conditions and optimization of selectivity focusing on the variables involved (system, sample and buffer electrolyte related).

A charged-based transient-state model for isotachophoresic separations of a multi-component system has been described by Dolník et al. [78] and applied to the separation of acetic, butyric and lactic acids. The model enables the calculation of the column hold-up required for the full separation of a multi-component sample and the analysis time. The inputs in the computer program are the amount of separated components, their ionic mobilities and dissociation constants.

Most of the procedures considered here have in common the fact that they provide guidelines to achieve adequate selectivity with a minimum number of experiments. Preselection of parameters and the parameter space to be optimized, a model or algorithm to describe the migration behaviour of the solutes and a criterion to evaluate the resulting electropherograms are indispensable for this purpose. A network of artificial neurons, usually called ANN, is a data processing system of a large number of simple, highly interconnected processing elements in an architecture inspired by structure of the brain. The characteristics that make ANN systems different from traditional computing and artificial intelligence are (i) learning by example, (ii) distributed associative memory, (iii) fault tolerance and (iv) pattern recognition. The application of ANN in optimization of CZE analysis has been examined by Havel et al. [79] who developed a method based on the combination of experimental design (central composite design) and ANN methods. Starting from two simple

cases (system 1: two weak acids with equal mo-
bilities for the anions and zero mobilities for the
nondissociated molecules, system 2: two weak acids
with different mobilities of all the species) the
authors showed that ANN can predict the best
separation conditions without any necessity to use
whichever *hard* model. The combination of the
experimental design and artificial neural network
applied to the prediction of the optimal separation
conditions of mixture of metal ions by CZE is indeed
quite general and can be used for the optimization of
any other separation processes.

Separation selectivity in capillary electrophoresis
is determined by the mobility of a solute and thus
can be changed by varying medium properties.
Nonaqueous media have been primarily used to
enhance and modify selectivity. Other practical
advantages are that the lower mobility of ions in
some organic solvents with high viscosity allows the
use of high concentration electrolyte solutions, the
use of capillaries with large diameters and finally the
application of large sample volume [80]. An interest-
ing work on CE separation of some Brønsted acids
(including carboxylic acids) in acetonitrile has been
performed by Okada [81]. Undissociated analytes
when interacting with an inorganic anion (X^-) added
to a running solution form heteroconjugates anions
migrating towards the anode. The extent of
heteroconjugation (K_{HAX}=formation constant) is
dependent on the charge density in the oxygen atoms
of the carboxyl group. The following equation has
been proposed to quantitatively interpret the electro-
phoretic behaviour of the heteroconjugated anion:

$$\frac{1}{\dfrac{1}{t_{app}} - \dfrac{1}{t_f}} = \left(\frac{1}{K_{HAX}C} + 1\right) \cdot \frac{LF}{E\lambda_{hetero}} \qquad (49)$$

where t_{app} and t_f are the apparent and the flow time
of migration, C is the concentration of the anion in a
running solution, L is the effective length of the
capillary, F is the Faraday constant, E is the strength
of the electric field, and λ_{hetero} is the molar ionic
conductivity of the heteroconjugated anion (that has
to be corrected for ionic strength by the Onsager's
equation). The fitting of parameters of Eq. (49)
allows one to obtain the values of K_{HAX} that were
found to be related to the experimental electro-

phoretic migration order of the solutes studied.
Linear relationships for *o*-, *m*- and *p*-substituted
carboxylic acids between log K_{HACl} and pK_a have
been found. Although this approach permits predic-
tion of separation in nonaqueous media from the
acidity of the acids (use of aqueous pK_a values in
nonaqueous solution is reasonable since for homolo-
gous series of compounds a linear relationship
between aqueous and nonaqueous pK_a values exists)
and the nature of the anion used as a running
electrolyte, the method has not be tested towards this
direction.

A one-dimensional dynamic computer model,
based on electroneutrality and conservation of mass
and charge, has been used by Thormann et al. [82]
for treating of biprotic ampholytes, weak and strong
monovalent acids (salicylic acid) and bases, and
proteins. Based on the dissociation of the silanol
surface groups of the capillary wall, the model is
able to predict the temporal behaviour of electroos-
mosis and the electroosmotic pumping activity of
each fluid element along the capillary column at
specified time points after power application. Good
qualitative agreement between simulation and ex-
perimental data were obtained at pH 6.0 in two
setups featuring open-tubular fused-silica capillaries
of 50–75 μm ID.

Even if not exactly related to the prediction of
retention data of carboxylic acids, but to their
chemical structure, Ishihama et al. [83] introduced a
migration index (MI) concept as a novel scale for
measuring the hydrophobicity of anionic solutes by
microemulsion electrokinetic chromatography, a
branch of CE, and capillary zone electrophoresis
measurements. Though for carboxylic acids, the
relationship between MI and log P deviated from
that of other compounds, the MI values correlated
more quantitatively, by a quadratic relationship, with
the bioactivity ($r^2_{bioactivity/MI}$=0.854), such as human
skin permeability, than log P ($r^2_{bioactivity/\log P}$=0.687)
in quantitative structure–activity relationships
studies.

Capillary electrophoresis with proper chiral resolv-
ing agents, like charged cyclodextrins (CCDs), has
been used to resolve enantiomeric separations of
racemic mixtures, including also analytes with car-
boxylic functionalities. Analytical expressions have
been derived by Williams and Vigh [84] in order to

calculate effective charge, effective mobility, separation selectivity and peak resolution values for various enantiomeric analyte classes, in the presence of CCD. Omitting the equilibria between CCD and the background electrolyte constituents (reasonable assumption if their concentrations are much higher than those of analytes, and considering the protonation and complexation reactions, a charged resolving agent migration model (the CHARM model) has been derived. For weak electrolyte enantiomers, the following expression for separation selectivity can be written:

$$\alpha =$$

$$\frac{\mu_R^0 + \mu_{RCD}^0 K_{RCD}[CD] + \mu_{HRCD}^0 \dfrac{[H_3O^+]}{K_a} K_{HRCD}[CD]}{\mu_S^0 \mu_{SCD}^0 K_{SCD}[CD] + \mu_{HSCD}^0 \dfrac{[H_3O^+]}{K_a} K_{HSCD}[CD]} \cdot$$

$$\frac{1 + K_{SCD}[CD] + \dfrac{[H_3O^+]}{K_a}(1 + K_{HSCD}[CD])}{1 + K_{RCD}[CD] + \dfrac{[H_3O^+]}{K_a}(1 + K_{HRCD}[CD])} \qquad (50)$$

where RCD and SCD, HRCD and HSCD indicate the complexes between cyclodextrin, CD, and the deprotonated and protonated forms of enantiomers R and S.

When charged and neutral cyclodextrins are used three typical, fundamentally different, separation types (*desionoselective*, *ionoselective* and *duoselective*) can be distinguished for weak electrolytes. In a *desionoselective* separation, only the undissociated forms of enantiomers complex selectively ($K_{RCD} = K_{SCD}$, $K_{HRCD} \neq K_{HSCD}$, $\mu^\circ_R = \mu^\circ_S$, $\mu^\circ_{RCD} = \mu^\circ_{SCD}$, $\mu^\circ_{HRCD} \neq \mu^\circ_{HSCD}$). In these conditions, the resolution surface can show that rugged separations can be obtained at low pH values as long as a sufficient high concentration of CD is present. In an *ionoselective* separation only the dissociated forms of the enantiomers complex selectively ($K_{RCD} \neq K_{SCD}$, $K_{HRCD} = K_{HSCD}$, $\mu^\circ_R = \mu^\circ_S$, $\mu^\circ_{RCD} \neq \mu^\circ_{SCD}$, $\mu^\circ_{HRCD} = \mu^\circ_{HSCD}$) and it can be shown that very stable separations can be obtained at high pH and high CD concentrations. In a *duoselective* separation, both the dissociated and undissociated forms of the enantiomers complex selectively ($K_{RCD} \neq K_{SCD}$, $K_{HRCD} \neq K_{HSCD}$, $\mu^\circ_R = \mu^\circ_S$, $\mu^\circ_{RCD} \neq \mu^\circ_{SCD}$, $\mu^\circ_{HRCD} \neq$

μ°_{HSCD}) and Eq. (50) cannot be simplified. The corresponding selectivity surface, at low pH, shows the features of a desionoselective separation, while at high pH, those of an ionoselective separation, with resolution being flat at high CD concentrations. By taking advantage of the predictions of the CHARM model, the utility of a large number of charged cyclodextrins can be evaluated in a short period of time for any chiral analyte, as done by Vincent et al. [85] with the synthesis of the sodium salt of hepta-6-sulfato-β-cyclodextrin for the chiral separation numerous analytes including those with carboxylic functionalities.

2.5. Other methods

For the sake of completeness, in this paragraph, less conventional analytical procedures used for the prediction of retention properties of carboxylic acids will be briefly reviewed.

Normal-phase thin-layer chromatography (TLC) measurements have been used by Levin et al. [86] for predicting retention of methyl- and phenylsalycilate in liquid chromatography with multicomponent mobile phases, based on experiments with corresponding binary ones, using the multicomponent, mobile-phase, active-component concept. Thin-layer chromatography with a chiral stationary phase has been used by Pyka [87] to separate carboxylic hydroxy acids and amino acids into their L- and D-enantiomers and to derive a new optical topological index that can be used to predict R_M values and to distinguish between isomers of D and L configuration. In Fig. 5, we plot the experimental R_M values and those predicted for hydroxy acids (A) and amino acids (B) by simple regression analysis equations containing topological indexes. In order to justify the poor agreement between experimental and calculated R_M values for hydroxy acids the author states that these analytes were not separated according to the optimum chromatographic conditions.

Polar and hydrophilic compounds, like carboxylic acids, can be analyzed by gas chromatography (GC) as such or derivatized, in case their peaks result skewed and asymmetrical. A method for the prediction of retention index (I) from chemical structure using the number of atoms in the molecule, the increment for atom addition (A) and the group
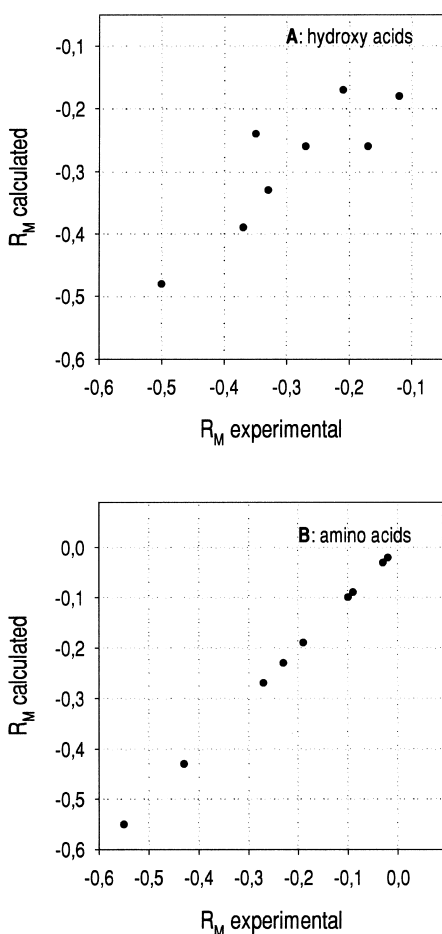
Fig. 5. Experimental and calculated R_M values for A: carboxylic hydroxy acids optical isomers (lactic, mandelic, hydroxyvaleric and hydroxycaproic acids) and B: amino acids (methionine, valine, leucine, serine and isoleucine). Experimental: Chir HPTLC plates. Eluent A: acetonitrile–water (3:2, v/v). Eluent B: methanol–water–acetonitrile (1:1:4, v/v). Data taken from Ref. [87] with permission.

retention factors (GRFs) of the functional groups substituents on polar as well as nonpolar columns to within 3% error has been shown by Peng et al. [88]. Accurate A and GRFs values seem to be essential to the prediction. The magnitude of the GRF is dependent not only upon the polarity and polarizability of the substituent and functional group but also on the stationary phase. Among *n*-alkanoids acids, differences between observed and predicted *I* values for acetic, *n*-propionic, *n*-butanoic, *n*-pentanoic, 2-methylpentanoic, *n*-hexanoic, *n*-heptanoic, *n*-oc-

tanoic, *n*-nonanoic, *n*-decanoic, lauric, myristic, cyclobutane carboxylic, cyclopentane propionic and cyclopentane carboxylic acids are shown. The same authors showed that silylated derivatives of acids behave chromatographically as hydrocarbons, and that their retention indexes can be readily predicted from their base values [89]. In this work it is pointed out that the difference between the retention index of analytes on polar and nonpolar columns is minimal for the silylated derivatives in comparison to that observed for the same underivatized solutes. Silylation, in fact, minimizes the intra-molecular interaction between functional groups and facilitates the prediction of retention indexes, due to the chromatographic characteristics of analytes more similar now to aliphatic hydrocarbons. Application of these methods is useful for routine separation, analysis and tentative identification of unknown components in mixtures.

Supercritical fluid chromatography (SFC) occupies the middle ground between GC and LC. Solute volatility (depending on column temperature) and the strength of the mobile phase (depending on its state of compression, or density) play important roles in determining retention. According to the operating temperature ranges, GC-type and LC-type [90] behaviours have been identified, and owing to the difficulties in modeling GC- and LC-type behaviour, no comprehensive predictive model has been yet proposed. Mitra et al. [91], emphasizing the complexity of rigorous thermodynamic equations, presented a simple empirical approach for prediction of the k' as a function of mobile phase reduced density (density/critical density) and reduced temperature (temperature/critical temperature in degrees Kelvin), according to the following equation:

$$\ln k' = a + b\rho_R + cT_R \qquad (51)$$

The term $b\rho_R$ accounts for the solvating effect of the mobile phase, and cT_R for the volatility effect, while the magnitude of constants b and c (depending on analyte and on experimental conditions) determines the relative dependence of the capacity factor on temperature and density. For nonanoic acid, using a capillary column and CO_2 as the mobile phase, the following set of values has been obtained: $a = 12.2 \pm 0.93$, $b = -3.84 \pm 0.40$, $c = -8.38 \pm 0.61$, $r^2 = 0.96$.

Acknowledgements

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