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Review

Influence of mobile phase acid–base equilibria on the chromatographic behaviour of protolytic compounds

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Abstract

A review about the influence of mobile phase acid–base equilibria on the liquid chromatography retention of protolytic analytes with acid–base properties is presented. The general equations that relate retention to mobile phase pH are derived and the different procedures to measure the pH of the mobile phase are explained. These procedures lead to different pH scales and the relationships between these scales are presented. IUPAC rules for nomenclature of the different pH are also presented. Proposed literature buffers for pH standardization in chromatographic mobile phases are reviewed too. Since relationships between analyte retention and mobile phase pH depends also on the pK_a value of the analyte, the solute pK_a data in water–organic solvent mixtures more commonly used as chromatographic mobile phase are also reviewed. The solvent properties that produce variation of the pK_a values with solvent composition are discussed. Chromatographic examples of the results obtained with the different procedures for pH measurement are presented too. Application to the determination of aqueous pK_a values from chromatographic retention data is also critically discussed.

Keywords: Reviews; Acid–base equilibria; Mobile phase composition; pH; Protolytic compounds

Contents

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1. Introduction

Development and optimization of analytical separation methods by liquid chromatography require the manipulation of retention and selectivity through variation of suitable parameters. In liquid chromatography, manipulation is usually performed through modification of the composition of the mobile phase. This can be achieved by change of the type and percentage of the organic modifier, and for solutes with acid–base properties, by variation of the pH of the mobile phase.

In order to develop reliable optimization strategies for separation of acid–base compounds, the theoretical relationships between analyte retention and mobile phase pH needs to be well known. The correct design of experimental procedures for the establishment of these optimization strategies requires the knowledge of the meaning of pH quantities and the proper measurement of these quantities in the mobile phase. Although the IUPAC has endorsed rules and procedures for the measurement of pH in aqueous organic solvent mixtures, the lack of appropriate reference pH data in some mixed solvents used as liquid chromatography mobile phases and some common misunderstandings on pH definition have limited the application of these procedures in practical liquid chromatography.

This review addresses these subjects and some others related with the analyte acid–base properties that affect chromatographic retention. It also shows the theoretical and practical differences between the different procedures used to measure the pH of the mobile phase and how the method of pH measurement influences the interpretation of the results obtained. Determination of aqueous pK_a values from the observed retention data and the measured mobile phase pH is also critically discussed.

2. Chromatographic retention as a function of mobile phase pH and solute pK_a

The rigorous thermodynamic constant that rules distribution of a component between the mobile and stationary phases, is the distribution constant that relates the activities of this component in these two phases. Since it would be extremely difficult to estimate the activity of ionic species in the stationary phase, IUPAC rules for chromatography define the distribution constant in terms of the analytical concentration of the component in the stationary (c_S) and mobile (c_M) phases. Because the component may be present in more than one form (e.g. associated and dissociated forms), the analytical concentration refers to the total amount present without regard to the existence of various forms [1,2]. This definition is equivalent to the definition of the distribution coefficient, or more correctly named distribution ratio, for liquid–liquid distribution, but not to the definition of distribution constant or partition ratio for liquid–liquid distribution [1].

In liquid–liquid chromatography, the concentrations are expressed per unit volume of the phase, and the distribution constant, K_c is defined by the following expression:

$$K_c = \frac{c_S}{c_M} = \frac{W_{i(S)}/V_S}{W_{i(M)}/V_M} \quad (1)$$

where $W_{i(S)}$ and $W_{i(M)}$ are the amounts of component i in the stationary and mobile phases, while V_S and V_M are the volumes of the stationary and mobile phases, respectively. For an analyte with an acid–base equilibrium in the mobile phase solvent (S) of the type:

$$HA^z + S \Leftrightarrow HS + A^{z-1} \quad \text{Scheme 1}$$

the thermodynamic acidity constant K_a relates the activities and concentrations of the different species in the mobile phase according to:

$$K_a = \frac{a_H a_A}{a_{HA}} = \frac{a_H [A]\, \gamma_A}{[HA]\, \gamma_{HA}} \quad (2)$$

where a indicates the activity and γ the mean ionic activity coefficient of the subscript species, and [HA] and [A] the concentrations of the acidic and basic forms of the component in the mobile phase. Ion charges and the subscript M for the mobile phase are

omitted for simplicity.

In this instance, the distribution constant can be expressed as:

$$K_c = \frac{[HA]_S + [A]_S}{[HA] + [A]} \quad (3)$$

From Eq. (2):

$$\frac{[A]}{[HA]} = \frac{K_a \gamma_{HA}}{a_H \gamma_A} = \frac{\gamma_{HA}}{\gamma_A} 10^{pH - pK_a} \quad (4)$$

Defining two individual distribution constants, one for each compound form:

$$K_{c(HA)} = \frac{[HA]_S}{[HA]} \quad (5)$$

$$K_{c(A)} = \frac{[A]_S}{[A]} \quad (6)$$

and replacing Eqs. (4)–(6) in Eq. (3), the following expressions that relate the distribution constant with the acidity of the mobile phase are obtained:

$$K_c = \frac{K_{c(HA)} \gamma_{HA}^{-1} + K_{c(A)} \gamma_A^{-1} K_a a_H^{-1}}{\gamma_{HA}^{-1} + \gamma_A^{-1} K_a a_H^{-1}} \quad (7)$$

$$K_c = \frac{K_{c(HA)} \gamma_{HA}^{-1} + K_{c(A)} \gamma_A^{-1} 10^{pH - pK_a}}{\gamma_{HA}^{-1} + \gamma_A^{-1} 10^{pH - pK_a}} \quad (8)$$

In the derivation of Eqs. (7) and (8) it has been assumed that the unique equilibria present in the medium that affect retention are the analyte acid–base equilibrium in the mobile phase (K_a) and the partition equilibria of the two acid–base species between mobile and stationary phase ($K_{c(HA)}$ and $K_{c(A)}$). If there are other side equilibria, they may also contribute to the final expression. In particular many silica based columns have residual silanols in the surface that ionize at basic pH values. The ionization of silanols leads to size exclusion effects for anions and ion-exchange processes between the analyte cations and the other cations of the mobile phase (e.g. buffer components) retained by electrostatic interactions with the ionized silanols. Since silanol ionization increases with the increase of the mobile phase pH, size exclusion and ion-exchange increase too. Size exclusion decreases retention of anions, whereas ion-exchange increases retention of cations. Ion-exchange is also affected by the concentration of the cations of the buffer. These effects are comparable to a variation of the distributions constants of the analyte individual ionic species ($K_{c(HA)}$ or $K_{c(A)}$) with the mobile phase conditions, in particular pH. In general, variation of the contribution to retention of size exclusion and ion-exchange with mobile phase pH is small as compared with variation of partition and averaged individual distribution constants for the different analyte ions can be taken.

If there are n consecutive acid–base equilibria defined by n acidity constants $K_{a1}, K_{a2}, \ldots, K_{an}$, the following general expressions equivalent to those given by Hardcastle and Jano [3] can be as well derived:

$$K_c = \frac{\sum_{r=0}^{n} K_{c(H_{n-r}A)} \gamma_{H_{n-r}A}^{-1} \left(\prod_{i=0}^{r} K_{ai} \right) a_H^{-r}}{\sum_{r=0}^{n} \gamma_{H_{n-r}A}^{-1} \left(\prod_{i=0}^{r} K_{ai} \right) a_H^{-r}} \quad (9)$$

$$K_c = \frac{\sum_{r=0}^{n} K_{c(H_{n-r}A)} \gamma_{H_{n-r}A}^{-1} 10^{r pH - \sum_{i=0}^{r} pK_{ai}}}{\sum_{r=0}^{n} \gamma_{H_{n-r}A}^{-1} 10^{r pH - \sum_{i=0}^{r} pK_{ai}}} \quad (10)$$

where $\prod_{i=0}^{r} K_{ai} = K_{a0} \times K_{a1} \times \cdots \times K_{ar}$ and $\Sigma_{i=0}^{r} pK_{ai} = pK_{a0} + pK_{a1} + \cdots + pK_{ar}$. In these expressions it must be taken into account that $K_{a0} = 1$ and therefore $pK_{a0} = 0$.

In fact, Eqs. (9) and (10) can be written in more elegant and simple forms if overall protonation constants (β), similar to those used in complex formation equilibria, are used instead of acid–base dissociation constants (K_a). The derivation of these equations is given in the appendix. However, these kind of equations and constants are not common in liquid chromatography studies.

Eqs. (7)–(10) are the general expressions that should be used to relate retention of an acid–base compound with the pH of the mobile phase. However, they are usually simplified by neglecting the contribution of the activity coefficients. This is equivalent to use a concentration acidity constant, K_a', instead of the thermodynamic acidity constant K_a, in the derivation of Eqs. (7)–(10). Both constants are related through the activity coefficients

according to:

$$K'_a = \frac{a_H[A]}{[HA]} = K_a \frac{\gamma_{HA}}{\gamma_A} \quad (11)$$

which leads to the common general expressions:

$$K_c = \frac{\sum_{r=0}^{n} K_{c(H_{n-r}A)} \left(\prod_{i=0}^{r} K'_{ai} \right) a_H^{-r}}{\sum_{r=0}^{n} \left(\prod_{i=0}^{r} K'_{ai} \right) a_H^{-r}} \quad (12)$$

$$K_c = \frac{\sum_{r=0}^{n} K_{c(H_{n-r}A)} 10^{rpH - \sum_{i=0}^{r} pK'_{ai}}}{\sum_{r=0}^{n} 10^{rpH - \sum_{i=0}^{r} pK'_{ai}}} \quad (13)$$

Determination of HPLC distribution constants requires measurement of the mobile and stationary phase volumes, which is not so simple. Therefore, retention parameters, such as retention factor (k), adjusted retention time (t'_R), and retention time (t_R) are used in practice. These retention parameters can be related to distribution constant through the stationary (V_S) and mobile (V_M) phase volumes, the mobile phase flow (F_C) and the column hold-up time (t_M):

$$K_c = \frac{kV_M}{V_S} = \frac{kF_C t_M}{V_S} = \frac{t'_R F_C}{V_S} = \frac{(t_R - t_M) F_C}{V_S} \quad (14)$$

If V_S, F_C and t_M remain constant, expressions analogous to (7)–(10) and (12)–(13) can be derived for the different retention parameters. Among these, the most used is the dimensionless retention factor, which requires measurement of the retention time of the component and hold-up time of the column:

$$k = \frac{t_R - t_M}{t_M} \quad (15)$$

The general expression that relates retention to mobile phase pH in terms of this parameter is:

$$k = \frac{\sum_{r=0}^{n} k_{H_{n-r}A} 10^{rpH - \sum_{i=0}^{r} pK'_{ai}}}{\sum_{r=0}^{n} 10^{rpH - \sum_{i=0}^{r} pK'_{ai}}} \quad (16)$$

From the pioneering works of Horváth and Melander [4,5] and Van de Venne et al. [6], the retention factor has been widely used to relate retention of acid–base solutes with the pH of the mobile phase using equations similar to Eq. (16). Systematization of pH effects on retention through equations of the same type was more recently studied by Schoenmakers and co-workers [7–9] and Lewis et al. [10]. Some recent reviews about retention and separation of acids [11,12], and determination of dissociation constants [13] or hydrophobicity of acidic xenobiotics [14] by reversed-phase liquid chromatography present equations like (16) as the most used in retention–pH relationships.

However, it must be remarked that rigorous relationships between retention and mobile phase pH are established for the distribution constant, and they can be extended to the retention factor through Eq. (14) only if the phase ratio V_M/V_S remains constant in all measurements. This is especially troublesome because V_M is estimated through the hold-up time t_M and there are several methods to do so that lead to different values [15–20].

One of the most simple and widely used methods to estimate hold-up time is to measure the elution time of inorganic salts. It has been argued that the behaviour of the ions produced by an unretained salt would match the interactions of the ionic forms of the acid–base compound with the column much better than the behaviour of a neutral hold-up time marker, which often gives elution time larger than the retention time of the ionized form of the acid–base compound [21]. However, in silica based columns the t_M value measured by an inorganic salt often varies with the pH and composition of the buffered mobile phase [21], because of the different exclusion and ionic exchange effects with the ionized silica and buffer ions retained [22]. Use of different measured t_M values to calculate k at each mobile phase pH through Eq. (15) implies the assumption that the phase ratio changes with pH and buffer composition. In this instance, there is not a proportionality between K_c and k, and therefore Eq. (16) is only approximate.

A modification of Eq. (8) for the retention of a neutral acid HA that uses two different hold-up times for each pH buffer has been proposed [21]. For the ionized form of the acid (A^-) the hold-up time ($t_{M(A-)}$) measured in the particular buffer with KBr is used, because the behaviour of Br^- would match

the interactions of A^- with the stationary phase (mostly size exclusion effects). But, for the neutral form of the acid (HA), the hold-up time of KBr measured in an unbuffered mobile phase should be used, since for this form ion-exchange and size exclusion effects do not apply. The equation was developed in terms of adjusted retention time, rather than retention factor, because the relationship between K_c and t'_R does not imply the mobile phase volume (Eq. (14)), and it can be written as:

$$t_R = \frac{(t'_{R(HA)} + t_{M(HA)}) + (t'_{R(A)} + t_{M(A)})\,\gamma_A^{-1}10^{(pH-pK_a)}}{1 + \gamma_A^{-1}10^{(pH-pK_a)}} \quad (17)$$

where the fitting parameters are the adjusted retention times of the neutral and anionic forms of the compound ($t'_{R(HA)}$ and $t'_{R(A)}$) and the pK_a value of the neutral acid. The ionic activity coefficient of the uncharged form of the compound (γ_{HA}) was taken as the unity.

Fig. 1 gives an example of the quality of the curve fit obtained with this approach. The two fitting lines shown for each acid correspond to the maximum effect for the activity coefficients γ_A (i.e. the fitting lines obtained with the buffers of largest and lowest ionic strength). The effect of the variation of the activity coefficients is very small and likely close to or lower than the experimental error in retention measurements. Therefore, the simplification of neglecting its variation and using the concentration constants K'_a, such as in Eqs. (12), (13) and (16), seems usually justified.

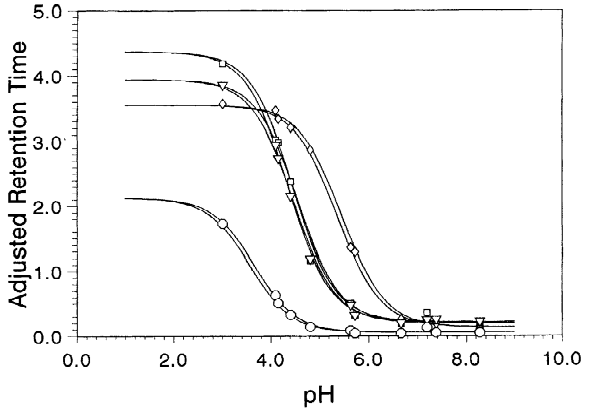

Fig. 1. Adjusted retention times of benzoic acids in 60% of methanol: (◇) benzoic acid, (○) 2-nitrobenzoic acid, (△) 3-nitrobenzoic acid, and (□) 4-nitrobenzoic acid. Lines are computed by means of Eq. (17). Only the two limiting lines are presented for each acid. The lines of the other buffers lay between these two. Reprinted with permission from Analytical Chemistry [21]. Copyright 1996 American Chemical Society.

Eq. (17) was developed for the retention of neutral acids in silica based columns and extension to others columns and compounds, such as neutral bases, has not been done. In particular, it has not yet been proposed an appropriate hold-up time marker for the acidic forms (BH^+) of the neutral bases because they interact strongly with the ionized silanols of silica based columns and in most instances are retained by ion-exchange. An excellent review of these interactions has been already published [23]. Given the difficulties in extension of Eq. (17) to other systems and the small effect of activity coefficient variation, the most appropriate general equations to relate retention with mobile phase pH are likely Eqs. (12) and (13). The retention parameter used can be retention time, adjusted retention time or retention factor (Eq. (16) in this instance). Excellent results have been obtained by direct use of retention time since for this parameter estimation of the column hold-up time is not required [24–26].

The main factor that influence the reliability of equations that relate retention to mobile phase pH, is the measurement of this pH. There are several ways to measure the pH of water–organic solvent mixtures, such as chromatographic mobile phases, that lead to different pH scales. The most used procedure in reversed-phase liquid chromatography consists of measuring the pH of the aqueous buffer before mixing it with the organic modifier. However, when this organic modifier is added, the pH changes, and so does the pK'_a value of the chromatographed compound. If the pK'_a of the compound and the pH of all buffers involved change in same the value, the term $r\mathrm{pH} - \Sigma_{i=0}^{r}\,\mathrm{p}K'_{ai}$ of Eq. (13) remains constant for each buffer and Eq. (12) and (13) hold in this instance. However, this is not usually the case, and especially the constant variation is not fulfilled for basic compounds, whose pK'_a value decreases with the addition of organic modifier, whereas the pK'_a values of neutral acids (from which the mobile phase buffers are mainly prepared in the pH range 2–7)

increase [24–27]. To clarify this point, it seems convenient to revise the IUPAC pH definition and the relationships between the different pH scales.

3. pH scales in water–organic solvent mixtures used as mobile phases

3.1. Notional definition of pH, notation and terminology

The concept of pH was first introduced as:

$$\mathrm{pH} = -\log c_{\mathrm{H}} \tag{18}$$

where c_{H} is the hydrogen ion concentration (in molarity, mol dm^{-3}), but it was later modified to:

$$\mathrm{pH} = -\log a_{\mathrm{H}} \tag{19}$$

where a_{H} is the hydrogen ion activity [1].

Activity and pH are dimensionless quantities, but activity must be referred to a concentration scale and so is pH. The most used concentration scales, accepted by the IUPAC for pH definition, are molarity (c, in mol dm^{-3}) and molality (m, in mol kg^{-1}). This leads to two definitions of pH, either in the molarity scale ($\mathrm{pH_c}$) or in the molality scale ($\mathrm{pH_m}$). Since it is not correct to write, in isolation, the logarithm of a quantity other than a dimensionless number, the full forms of the equations for pH definition are:

$$\mathrm{pH_c} = -\log\left(c_{\mathrm{H}}\gamma_{\mathrm{c,H}}/c^0\right) \tag{20}$$

$$\mathrm{pH_m} = -\log\left(m_{\mathrm{H}}\gamma_{\mathrm{m,H}}/m^0\right) \tag{21}$$

where c^0 and m^0 are arbitrary constants, representing the standard state condition, numerically equivalent to either 1 mol dm^{-3} or 1 mol kg^{-1}, respectively, and $\gamma_{\mathrm{c,H}}$ and $\gamma_{\mathrm{m,H}}$ are the single-ion activity coefficients of the hydrogen ion in the two scales, respectively [1]. For dilute solutions, molarity and molality are directly related through the density (ρ) of the solution, and therefore pH in one scale can be easily converted to pH in the other scale through:

$$\mathrm{pH_c} = \mathrm{pH_m} + \log \rho/\rho^0 \tag{22}$$

with $\rho^0 = 1$ kg dm^{-3}.

The density of water is close to 1 kg dm^{-3}, and therefore $\mathrm{pH_c}$ and $\mathrm{pH_m}$ are practically identical (the pH difference is about 0.001 at 298.15 K rising to 0.02 at 398.15 K). However, the density of some aqueous organic solvent mixtures can be quite different from 1 kg dm^{-3}, and the transfer term $\log \rho$ can achieve several tenths of pH units. Tables 1–3 report the value of the $\log \rho$ term for several compositions of the most used HPLC mobile phases, as well as some other relevant macroscopic properties [24–40]. Equations to estimate these properties from the solvent composition are also given in the references. Different models have been proposed to relate macroscopic properties to solvent composition [27–29], although for practical purposes fits to polynomial equations provide excellent results. The solvent composition of binary solvent mixtures may be given in mole (x), weight (w) or volume (ϕ) fraction. All these quantities can be easily interre-

Table 1
Macroscopic properties of relevant interest for pH measurement in methanol–water mixtures at 25 °C [24,26–34]

% MeOH (v/v)	x_{MeOH}	ρ (kg dm^{-3})	$\log \rho/\rho^0$	A	$a_o B$	$\delta \approx -\log({}^{\mathrm{s}}_{\mathrm{w}}\gamma^0_{\mathrm{H}})$	$\mathrm{p}K_{\mathrm{ap}}$
0	0.000	0.995	−0.002	0.53	1.50	0.00	14.00
10	0.047	0.983	−0.008	0.56	1.53	0.01	14.08
20	0.100	0.969	−0.014	0.59	1.57	0.03	14.08
30	0.160	0.955	−0.020	0.64	1.61	0.05	14.07
40	0.229	0.939	−0.027	0.70	1.66	0.09	14.09
50	0.308	0.921	−0.036	0.77	1.72	0.13	14.14
60	0.400	0.901	−0.045	0.87	1.79	0.18	14.23
70	0.509	0.878	−0.057	1.01	1.88	0.18	14.39
80	0.640	0.852	−0.070	1.20	1.99	0.05	14.63
90	0.800	0.822	−0.085	1.48	2.13	−0.34	15.04
100	1.000	0.787	−0.104	1.87	2.31	−2.00	16.77

Table 2
Macroscopic properties of relevant interest for pH measurement in acetonitrile–water mixtures at 25 °C [25,32,35]

% MeCN (v/v)	x_{MeCN}	ρ (kg dm^{-3})	$\log \rho/\rho^0$	ϵ	A	a_oB	$\delta \approx -\log({}^{s}_{w}\gamma^0_H)$	${}^{s}_{s}pK_{ap}$
0	0.000	0.995	−0.001	76.6	0.528	1.52	0.00	14.00
10	0.040	0.983	−0.007	73.2	0.566	1.55	−0.01	14.24
20	0.079	0.968	−0.014	70.1	0.604	1.59	−0.02	14.47
30	0.130	0.950	−0.022	66.4	0.655	1.63	−0.06	14.74
40	0.186	0.931	−0.031	62.8	0.712	1.68	−0.13	15.08
50	0.260	0.908	−0.042	58.6	0.791	1.74	−0.26	15.48
60	0.339	0.885	−0.053	54.7	0.877	1.80	−0.44	15.90
70	0.440	0.859	−0.066	50.4	0.992	1.87	–	16.42
80	0.578	0.829	−0.081	45.5	1.156	1.97	–	17.14
90	0.755	0.802	−0.096	40.4	1.378	2.09	–	17.13
100	1.000	0.782	−0.107	35.1	1.707	2.24	–	34.40

lated by means of the following equations where the subscripts 1 and 2 indicate each one of the two components of the mixture:

$$x_i = \frac{w_i/M_i}{w_1/M_1 + w_2/M_2} \quad (23)$$

$$x_i = \frac{\phi_i/\nu_{M_i}}{\phi_1/\nu_{M_1} + \phi_2/\nu_{M_2}} \quad (24)$$

M and ν_M are the molecular mass and molar volume, respectively, of the pure components of the mixture, with $\nu_M = M/\rho$. For water, methanol, acetonitrile and tetrahydrofuran M values are 18.02, 32.04, 41.05, and 72.11 g mol^{-1}, respectively, and ν_M is 18.1, 40.7, 52.9, and 81.6 cm^3 mol^{-1} at 25 °C, respectively.

The concentration scale used in the particular pH definition must be carefully stated and controlled, even for the pH buffers used in the electrode system calibration. The IUPAC prefers molality scale because molality does not change with the temperature of the solution. However, in HPLC practice molarity is almost always used because of its simplicity for preparation of solutions. Unless otherwise stated, pH measured in the molarity scale will be assumed here. In this instance, the subscript c will be omitted for simplicity.

The IUPAC remarks that the above definitions of pH are only notional because they involve a single ion activity (a_H) which is immeasurable [1]. In order to obtain the pH value an extrathermodynamic assumption is necessary [41,42]. This is usually the Debye–Hückel equation, which allows estimation of the activity coefficient of the hydrogen ion:

Table 3
Macroscopic properties of relevant interest for pH measurement in tetrahydrofuran–water mixtures at 25 °C [36–40]

% THF (v/v)	x_{THF}	ρ (kg dm^{-3})	$\log \rho/\rho^0$	ϵ	A	a_oB	${}^{s}_{s}pK_{ap}$
0	0.000	0.991	−0.004	78.5	0.51	1.50	14.00
10	0.024	0.993	−0.003	72.4	0.58	1.56	14.12
20	0.053	0.989	−0.005	65.9	0.66	1.64	14.31
30	0.087	0.983	−0.007	58.6	0.79	1.74	14.52
40	0.129	0.974	−0.011	50.5	0.99	1.87	14.79
50	0.182	0.964	−0.016	42.3	1.29	2.04	15.13
60	0.250	0.950	−0.022	34.2	1.77	2.27	15.56
70	0.342	0.937	−0.028	26.4	2.61	2.59	16.14
80	0.471	0.921	−0.036	19.4	4.14	3.01	–
90	0.667	0.903	−0.045	13.2	7.41	3.66	–
100	1.000	0.882	−0.055	7.4	17.64	4.89	34.7

$$\log \gamma_H = -AI^{1/2}/(1 + a_0 BI^{1/2}) \quad (25)$$

In Eq. (25) I is the ionic strength of the solution, A and B are solvent and temperature dependent parameters and a_0 is the ion size parameter of the solvated ion, which is assigned a value fixed by the Bates–Guggenheim convention extended to the general solvent s [41–43]:

$$({}^{s}a_0\,{}^{s}B) = 1.5[{}^{w}\epsilon\,{}^{s}\rho/({}^{s}\epsilon\,{}^{w}\rho)]^{1/2} \quad (26)$$

where ${}^{s}B$ is the classical Debye–Hückel constant of Eq. (25) for the solvent s (e.g. mobile phase), ${}^{w}\epsilon$ and ${}^{s}\epsilon$ are the relative permittivities of pure water (superscript ^{w}) and of the solvent s (superscript ^{s}), and ${}^{w}\rho$ and ${}^{s}\rho$ are the corresponding densities. If s is water itself, Eq. (26) reduces to $a_0 B = 1.5$, which is the form of the Bates–Guggenheim convention introduced originally for pH standardization in pure water [43]. Tables 1–3 report values of A and $a_0 B$ terms for several methanol–water, acetonitrile–water and tetrahydrofuran–water compositions.Since pH is defined in terms of activity, it does not only depends on the concentration scale chosen, but also on the standard state of activity. In water, the standard state for a_H is infinite dilution of hydrogen ion in water (i.e. pure water), for which $\gamma_H \to 1$. In the solvent s (e.g. an HPLC mobile phase), two different standard states can be chosen. One is infinite dilution of the ion in the same solvent s, and the other is infinite dilution of the ion in water. This leads to two different pH scales, one relative to each particular solvent, and the other relative to water, which is also called ''absolute pH scale''.

In order to distinguish between the two pH scales, the IUPAC recommends the notation used by Robinson and Stokes [1,44] for their discussion of the effect of the medium on transferring a binary electrolyte from water (w) to a nonaqueous or mixed solvent (s). Thus, lower-case left-hand superscripts indicate the solvent (w or s) in which measurements are being made; lower-case left-hand subscripts indicate the solvent in which the ionic activity coefficient γ is referred to unity at infinite dilution (w or s) [1]. Also, the notation ${}^{s}_{w}\gamma^{0}_{H}$ is recommended for the primary medium effect (related to the standard Gibbs energy change) for the transfer of the H^+ ion from water (w) to the solvent s. The value of this parameter determines the shift of the ${}^{s}_{w}$pH scale in reference to the ${}^{s}_{s}$pH, since the two pH scales are related by means of the following equation:

$${}^{s}_{w}\mathrm{pH} = {}^{s}_{s}\mathrm{pH} - \log({}^{s}_{w}\gamma^{0}_{H}) \quad (27)$$

where ${}^{s}_{w}\gamma^{0}_{H} \to 1$ as s→w.

Thus, the ${}^{s}_{s}$pH value measured in solvent s relating to the pH scale specific to the solvent might be expressed as ${}^{s}_{w}$pH on an ''intersolvental'' or ''absolute'' scale with ultimate reference to the solvent water [1].

The relative ${}^{s}_{s}$pH scale has been traditionally indicated by pH*****, whereas the absolute ${}^{s}_{w}$pH scale has been sometimes indicated by pA or $\mathrm{pH}^{\mathrm{app}}$ [31,33,34,45–47]. However, the new notation adopted by the IUPAC is much clearer and it is recommended for any pH measurement involving non-aqueous or mixed solvents, such as those used in liquid chromatography. The same notation used for pH definition should be applied to any related quantity, such as acid–base constants (pK_a). For example, the acid–base constant of Eq. (2) in a solvent s may be given as ${}^{s}_{s}pK_a$ if the hydrogen activity is measured in the ${}^{s}_{s}$pH scale or as ${}^{s}_{w}pK_a$ if it is measured in the ${}^{s}_{w}$pH scale. It turns out that both constants are related by an equation similar to Eq. (27):

$${}^{s}_{w}pK_a = {}^{s}_{s}pK_a - \log({}^{s}_{w}\gamma^{0}_{H}) \quad (28)$$

Notice that in the definition of ${}^{s}_{w}pK_a$ the standard state for hydrogen ion is infinite dilution in water (w), but the standard state for the other species implied in the equilibrium (HA and A) is infinite dilution in the non-aqueous or mixed solvent s.

3.2. Operational definition of pH

It is universally agreed that the definition of a pH difference is an operational one [1]. The pH of a test solution ($\mathrm{pH_X}$) is determined by comparison of the electromotive forces E_X and E_S of two appropriate potentiometric cells. The two cells must be equal except for that one contains the test solution X and the other a standard reference solution S of known pH ($\mathrm{pH_S}$). The $\mathrm{pH_X}$ is determined from:

$$\mathrm{pH_X} = \mathrm{pH_S} - (E_X - E_S)/g \quad (29)$$

where $g = (RT/F)\ln 10$ and R is the gas constant, T

the thermodynamic temperature, and F the Faraday constant, ignoring a term $\Delta E_J = E_{JX} - E_{JS}$, which is called the residual liquid junction potential [1]. The recommended symbol for the term $(RT/F)\ln 10$ is k, but we shall use g to avoid any confusion with retention factors. The standard chosen should have a pH_S value as close as possible to the pH_X value of the sample in order to minimize the residual junction potential. In practice, very often the pH is measured by calibration of the electrode with two standards that also contribute to minimize the error of the residual junction potential [1].

Formerly, the IUPAC recommended the potentiometric cells to be composed of a hydrogen and a reference electrodes, together with a bridge solution of concentrated potassium chloride ($c \geq 3.5$ mol kg^{-1} for aqueous solutions). The most recent edition of the ''Compendium of Analytical Nomenclature'' [1], however, allows the hydrogen electrode to be replaced by another hydrogen-ion-responsive electrode, such as a glass electrode, which is more practical.

The operational pH definition for aqueous solutions can be extended to any other solvent s by use of the appropriate pH scale. The procedure implies the measurement of the electromotive forces (^sE_X and ^sE_S) of two potentiometric cells, one containing the test solution X in solvent s and the other a standard reference solution S prepared in the same solvent s and of known ^s_spH ($^s_spH_S$). The $^s_spH_X$ is determined from:

$$^s_spH_X = {}^s_spH_S - (^sE_X - {}^sE_S)/g \quad (30)$$

ignoring, again, the residual liquid junction potential:

$$\Delta E_J = {}^sE_{JX} - {}^sE_{JS} \quad (31)$$

This procedure requires the assignment of reference ^s_spH values to standard solutions prepared in a solvent of exactly the same composition as the solvent where the pH will be measured. There are only a few reference ^s_spH values reported for aqueous–organic solvent mixtures. Regarding HPLC mobile phases, the IUPAC [1,42,48] reports only data of 0.05 M potassium hydrogen phthalate buffer for some methanol–water and acetonitrile–water at various compositions, a few other buffers in 50% w/w methanol, and oxalate and succinate buffers for several compositions of methanol–water mixtures. Notice, that all these data are given in the molality pH scale, and thus a correction according to Eq. (22) is needed if they are used as standards to measure the pH in the molarity scale, which is the most usual concentration scale in HPLC studies.

Some more ^s_spH reference data in the molarity scale have been determined by Barbosa and coworkers [49–52] for acetonitrile–water and tetrahydrofuran–water [37] which can be directly used for ^s_spH standardization in these mobile phases. The ^s_spH reference values for the buffers were fitted to solvent composition in weight, volume and mole fraction through a polynomial equation. The fits obtained for volume fraction (v) are given in Tables 4 and 5 and they can be directly used to calculate the ^s_spH value of the buffer at any acetonitrile or tetrahydrofuran percentage between the range studied.

The ^s_wpH scale may also be used for pH measurement in non-aqueous or mixed solvents. In this instance, the ^s_wpH of the test sample ($^s_wpH_X$) is determined by measuring the electromotive forces of the test sample in the solvent s (^sE_X) and the standard reference solution in water (^wE_S) of know

Table 4
Relationships between ^s_spH values of reference buffers in acetonitrile–water mixtures and the volume percentage of acetonitrile (ϕ) in the solvent mixture up to 70% of acetonitrile in weight ($\phi = 0.75$) [49,52] (Correlation coefficient (R) is also given)

Buffer	Relationship	R
Saturated potassium hydrogen tartrate (25 °C)	$^s_spH = 3.546 + 2.49\times10^{-2}\phi - 2.60\times10^{-4}\phi^2 + 4.01\times10^{-6}\phi^3$	0.999
0.05 mol kg^{-1} potassium dihydrogen citrate	$^s_spH = 3.775 + 1.74\times10^{-2}\phi - 2.72\times10^{-7}\phi^2 + 1.15\times10^{-6}\phi^3$	0.999
0.05 mol kg^{-1} potassium hydrogen phthalate	$^s_spH = 3.998 + 2.75\times10^{-2}\phi - 5.02\times10^{-5}\phi^2 + 1.38\times10^{-6}\phi^3$	0.999
0.1 mol l^{-1} acetic acid + 0.1 mol l^{-1} sodium acetate	$^s_spH = 4.643 + 1.88\times10^{-2}\phi + 1.26\times10^{-4}\phi^2 - 7.23\times10^{-7}\phi^3$	0.999
0.025 mol kg^{-1} Na_2HPO_4 + 0.025 mol kg^{-1} KH_2PO_4	$^s_spH = 6.885 + 3.16\times10^{-2}\phi - 5.84\times10^{-4}\phi^2 + 6.55\times10^{-6}\phi^3$	0.995
0.03043 mol kg^{-1} Na_2HPO_4 + 0.008695 mol kg^{-1} KH_2PO_4	$^s_spH = 7.417 + 2.23\times10^{-2}\phi - 8.29\times10^{-5}\phi^2 + 1.23\times10^{-6}\phi^3$	0.999
0.01 mol kg^{-1} $Na_2B_4O_7\cdot10H_2O$	$^s_spH = 9.178 + 3.35\times10^{-2}\phi - 1.51\times10^{-5}\phi^2 + 2.59\times10^{-7}\phi^3$	0.999
0.025 mol kg^{-1} $NaHCO_3$ + 0.025 mol kg^{-1} Na_2CO_3	$^s_spH = 10.014 + 0.145\phi - 4.43\times10^{-3}\phi^2 + 4.44\times10^{-5}\phi^3$	0.999

Table 5
Relationships between ${}^{s}_{s}$pH values of reference buffers in tetrahydrofuran–water mixtures and the volume percentage of tetrahydrofuran (ϕ) in the solvent mixture up to 70% of tetrahydrofuran in weight ($\phi = 0.72$) [37] (Correlation coefficient (R) is also given)

Buffer	Relationship	R
Saturated potassium hydrogen tartrate (25 °C)	${}^{s}_{s}\mathrm{pH} = 3.575 + 9.99\times10^{-3}\phi + 2.12\times10^{-4}\phi^2$	0.999
0.05 mol kg^{-1} potassium dihydrogen citrate	${}^{s}_{s}\mathrm{pH} = 3.772 + 1.26\times10^{-2}\phi + 1.09\times10^{-4}\phi^2$	0.999
0.05 mol kg^{-1} potassium hydrogen phthalate	${}^{s}_{s}\mathrm{pH} = 3.983 + 2.83\times10^{-2}\phi + 1.55\times10^{-5}\phi^2$	0.999
0.1 mol l^{-1} acetic acid + 0.1 mol l^{-1} sodium acetate	${}^{s}_{s}\mathrm{pH} = 4.655 + 1.18\times10^{-2}\phi + 2.10\times10^{-4}\phi^2$	0.998
0.025 mol kg^{-1} Na_2HPO_4 + 0.025 mol kg^{-1} KH_2PO_4	${}^{s}_{s}\mathrm{pH} = 6.858 + 1.70\times10^{-2}\phi - 8.15\times10^{-5}\phi^2$	0.995
0.03043 mol kg^{-1} Na_2HPO_4 + 0.008695 mol kg^{-1} KH_2PO_4	${}^{s}_{s}\mathrm{pH} = 7.412 + 1.19\times10^{-2}\phi + 2.29\times10^{-5}\phi^2$	0.999
0.01 mol kg^{-1} $Na_2B_4O_7\cdot10H_2O$	${}^{s}_{s}\mathrm{pH} = 9.188 + 2.46\times10^{-2}\phi + 8.76\times10^{-5}\phi^2$	0.999
0.025 mol kg^{-1} $NaHCO_3$ + 0.025 mol kg^{-1} Na_2CO_3	${}^{s}_{s}\mathrm{pH} = 9.982 + 6.77\times10^{-2}\phi - 5.47\times10^{-4}\phi^2$	0.990

pH (${}^{w}_{w}\mathrm{pH_S}$), i.e. an aqueous buffer. The pH will be given by:

$$ {}^{s}_{w}\mathrm{pH_X} = {}^{w}_{w}\mathrm{pH_S} - ({}^{s}E_X - {}^{w}E_S)/g \quad (32) $$

ignoring, the residual liquid junction potential:

$$ \Delta E_J = {}^{s}E_{JX} - {}^{w}E_{JS} \quad (33) $$

This procedure is still simpler than that for the ${}^{s}_{s}$pH scale, since it does not require preparation of a pH reference buffer at each working solvent composition. The same buffers used for pH standardization in water (${}^{w}_{w}$pH) can be used [1,53]. Notice that to avoid any confusion and in consonance with the IUPAC notation, the pH scale in water should be written as ${}^{w}_{w}$pH. This is not usually required when working only in water, where it is allowed and even advisable to use simply pH.

3.3. Relationships between the different pH scales and the normal range of pH

pH values measured in the ${}^{s}_{w}$pH scale can be converted to the ${}^{s}_{s}$pH scale through the value of the primary medium effect ($-\log({}^{s}_{w}\gamma^{0}_{H})$) by means of Eq. (27). In fact, the difference between the two operational ${}^{s}_{w}$pH and ${}^{s}_{s}$pH scales includes also the differences between the residual liquid junction potentials. The difference has been called δ term [1,31], which is defined as:

$$ \delta = \overline{E_j} - \log({}^{s}_{w}\gamma^{0}_{H}) = {}^{s}_{w}\mathrm{pH} - {}^{s}_{s}\mathrm{pH} \quad (34) $$

with

$$ \overline{E_j} = ({}^{s}E_{JX} - {}^{w}E_{JS})/g \quad (35) $$

The δ values have been determined for methanol–water [26,31,33,34] in the whole range of solvent composition and for acetonitrile–water mixtures up to 60% of acetonitrile in volume [25] and they are presented in Tables 1 and 2 for some mixtures. The agreement between the different δ values for methanol–water obtained by different authors with different electrode systems suggests that the residual liquid junction potential term $\overline{E_j}$ is meaningless as compared with the medium effect and $\delta = -\log({}^{s}_{w}\gamma^{0}_{H})$. In this instance Eq. (34) is equivalent to (27) and the notional and operational pH scales agree. However, a well designed salt bridge containing a solution of an equitransferent binary salt at much higher concentration than the sample and standard solutions must be used to assure the residual liquid junction potential is insignificant [1]. For example, a 3 *M* KCl salt bridge in water can experience junction potentials on the order of 1 mV (about 0.02 pH units), which would be usually partially balanced by a similar junction potential in the pH measurement in the mobile phase. Therefore, the error introduced by the residual liquid–junction potentials can be estimated to be about 0.01 pH units or less, which is indeed a low error for practical liquid chromatography measurements [25]. Thus, conversion between ${}^{s}_{w}$pH and ${}^{s}_{s}$pH values can be easily done with the δ values of Tables 1 and 2, which should be valid for any well designed electrode system. For methanol–water and acetonitrile–water mobile phases, δ values can be estimated through the equations [24,25]:

$$ \delta = (0.09\phi_{MeOH} - 0.11\phi^2_{MeOH})/(1 - 3.15\phi_{MeOH} + 3.51\phi^2_{MeOH} - 1.35\phi^3_{MeOH}) \quad (36) $$

for methanol–water, and:

$$\delta = -3.81x_{MeCN}^2 \quad (37)$$

for acetonitrile–water up to 60% of acetonitrile in volume. By replacing Eq. (24) into Eq. (37), Eq. (38) is obtained that gives the δ value for any acetonitrile–water mixture directly from the volume fraction of acetonitrile in the mixture:

$$\delta = -0.11\phi_{MeCN}^2/(1-1.316\phi_{MeCN}+0.433\phi_{MeCN}^2) \quad (38)$$

In these equations x and ϕ have the meanings of mole and volume fraction, respectively, of the subscript species (MeOH for methanol and MeCN for acetonitrile). Unfortunately, δ values for tetrahydrofuran–water mixtures have not yet been determined.

The difference between ${}^{s}_{w}$pH and ${}^{s}_{s}$pH is a constant value for each mobile phase composition. However, the difference between ${}^{w}_{w}$pH and ${}^{s}_{s}$pH (or ${}^{s}_{w}$pH) depends not only of the mobile phase composition, but also of the particular buffering solution measured [24]. One example will illustrate this problem. A 0.010 *M* solution of HCl in water has an ionic activity coefficient (calculated from Eq. (25) and the values of Table 2) of $\gamma_H = 0.900$. By using Eq. (20) the ${}^{w}_{w}$pH of this solution is calculated to be ${}^{w}_{w}\text{pH} = -\log(0.010\times 0.900) = 2.046$. If a solution of the same concentration of HCl is prepared in 50% acetonitrile, the activity coefficient is 0.856 and the ${}^{s}_{s}$pH value is 2.068. The pH variation is 0.022, due only to the variation of the activity coefficient. However, if the solution is 0.010 *M* in KOH, the pH value depends also on the autoprotolysis constant (K_{ap}) of the medium, since $a_H = K_{ap}/a_S$. The lyate (OH^-) activity of the aqueous solution is $a_S = 0.010\times 0.900 = 0.00900$, $a_H = 10^{-14.00}/0.009 = 1.11\times 10^{-12}$ and ${}^{w}_{w}\text{pH} = 11.954$. In 50% acetonitrile, $a_S = 0.010\times 0.856 = 0.00856$, $a_H = 10^{-15.48}/0.00856 = 3.87\times 10^{-14}$, and ${}^{s}_{s}\text{pH} = 13.412$. For this basic solution the pH variation is 1.458, quite more considerable than for the solution of HCl.

The example above shows that general conversion between pH scales in different solvents (e.g. water and one water/organic solvent mobile phase) is not possible, but it also shows the importance of the autoprotolysis constant of the solvent in pH determination.

The value of the autoprotolysis constant determines the ''normal range of pH'' in each solvent and it is needed to achieve complete and effective pH standardization in non-aqueous and mixed solvents [31,41,42]. It is well known that in water the normal range of pH goes from 0 to 14, which is the pK_{ap} of water. In a water–organic solvent mixture used as mobile phase, the normal range of pH can be measured in the ${}^{s}_{s}$pH or ${}^{s}_{w}$pH scales. In the ${}^{s}_{s}$pH scale, it ranges from 0 to the ${}^{s}_{s}pK_{ap}$ value of the solvent mixture. Since the ${}^{s}_{w}$pH scale is shifted the δ value from the ${}^{s}_{s}$pH scale, the normal range of pH goes from δ to ${}^{s}_{w}pK_{ap}$, which is equivalent to ${}^{s}_{s}pK_{ap}+\delta$ [25].

Tables 1–3 report the ${}^{s}_{s}pK_{ap}$ values of methanol–water, acetonitrile–water and tetrahydrofuran–water mobile phases. Literature reports equations to calculate the ${}^{s}_{s}pK_{ap}$ values for other compositions not given in the tables [32,54,55]. These equations are based on the thermodynamics of the chemical autoprotolysis equilibria present in a binary aqueous solvent. In a mixture of water with an organic solvent up to four proton transfer equilibria can contribute to autoprotolysis: autoionization of water, proton transfer from water to the organic solvent, proton transfer from the organic solvent to water and autoionization of the organic solvent. Only three of these equilibria are independent and their combination gives the autoprotolysis constant of the solvent mixture [32].

The limits of the normal pH range are only indicative of the common pH values achieved for most analytical applications. A pH value of 0 in water would correspond to a strong acidic solution with a hydrogen ion activity of unity, whereas a pH of 14 would correspond to a strong basic solution with a hydroxide ion activity of unity. Liquid chromatography columns do not usually allow working at these extreme pH values. Thus, the useful pH ranges in liquid chromatography are smaller. Two studies have been published about the extension of the useful pH ranges for chromatography columns in water to methanol–water [27] and tetrahydrofuran–water [37]. Figs. 2 and 3 include the variation of these pH limits measured in the ${}^{s}_{s}$pH scale in methanol–water and tetrahydrofuran–water mobile phases for a common silica based column which can be used in the ${}^{w}_{w}$pH range between 1 and 8. Although not published, it is evident that for acetonitrile–water

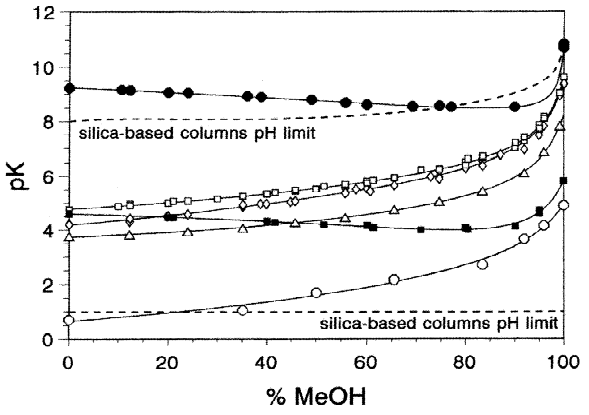

Fig. 2. Variation of pK_a values of acids with the methanol–water composition: (○) trichloroacetic acid, (△) formic acid, (◇) benzoic acid, (□) acetic acid, (■) anilinium, and (●) ammonium. Reprinted with permission from Analytical Chemistry [27]. Copyright 1996 American Chemical Society.

mobile phase the useful pH limits can be estimated in the same way. Thus, for 50% acetonitrile, the useful pH range for the same silica based column would range from 1 to 9.5 because the ${}^{s}_{s}pK_{ap}$ value for 50% acetonitrile increases about 1.5 pH units in reference to the pK_{ap} value of pure water. In the ${}^{s}_{w}$pH scale, the useful pH range should be corrected by the δ value and for the same column in 50% acetonitrile, the range would go from 0.7 to 9.2 because $\delta \approx -0.3$.

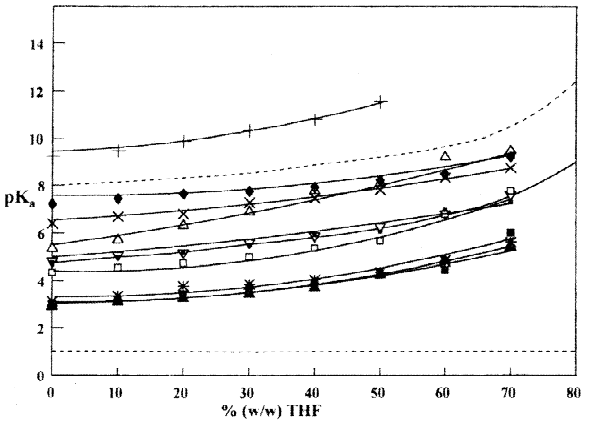

Fig. 3. Variation of pK_a values of acid components of standard buffer solutions with tetrahydrofuran–water composition. Symbols: (■) pK_1 tartaric acid; (□) pK_2 tartaric acid; (*) pK_1 citric acid; (■) pK_2 citric acid; (×) pK_3 citric acid; (♦) pK_2 phosphoric acid; (+) pK_a boric acid; (▲) pK_1 phthalic acid; (△) pK_2 phthalic acid; (▽) pK_a acetic acid. Reprinted from Analytica Chimica Acta [37]. Copyright 1999, with permission from Elsevier Science.

4. Solute pK_a values in water and in water–organic solvent mobile phases

4.1. Solute pK_a values in water

Part 2 of this review has shown that chromatographic retention of acid–base compounds depends on both the pH of the mobile phase and the pK_a value of the solute. Although the rigorous application of Eqs. (13), (16) or (17) requires pH and pK_a values in the particular mobile phase used (${}^{s}_{s}$pH or ${}^{s}_{w}$pH scales), the knowledge of the pK_a value of the analyte in water may give valuable information about the behaviour of the analyte. If the analyte pK_a value is much higher or lower than the pH of the mobile phase, a small change in the pH of this mobile phase should not affect retention. However, if the pK_a is close to the pH, a small change in the pH may produce large changes in retention. In fact, many workers measure the pH of the aqueous buffer before mixing it with the organic modifier (${}^{w}_{w}$pH scale) and use the aqueous pK_a values to calculate ionization of the analyte in this buffer, and from this to get a broad estimation of analyte retention.

Acid–base constants in water have been determined for thousands of compounds and there are many compilations of pK_a values. One of the most known, is the series of books published by the IUPAC in the 1960s to report aqueous literature pK_a values of organic acids [56], inorganic acids and bases [57], and organic bases [58]. A supplement for the organic bases was also published in 1972 [59]. Another important compilation was done in Tartu University (Estonia) in the 1970s directed by Palm. The compilation tried to include all published pK_a data not only in water but also in other solvents and solvent mixtures, as well as many other equilibrium and kinetic data. Two initial volumes were published with pK_a data of acids [60] and bases [61], that were later extended with two supplements [62,63].

When the pK_a value is not directly available, it can be often estimated from the chemical structure of the compound. Nowadays, there are different available computer programs with graphical interfaces to draw the structure and algorithms to calculate the pK_a value from it. Many of them incorporate also a more or less extended literature pK_a database.

Most algorithms are based on the Hammett–Taft equations:

$$pK_a = pK_a^0 - \rho \sum \sigma \quad (39)$$

where pK_a refers to the acidity constant of an organic compounds, pK_a^0 is the pK_a of the unsubstituted parent compound, ρ is the reaction constant for a particular acid–base center and σ is a constant assigned to a specific substitutent. The pK_a is obtained by addition (Σ) of the constants of all substituents in the molecule. The Hammett and Taft parameters (pK_a^0, ρ, and σ) have been compiled for many parent compounds and substituents [64–66].

Pallas system from Compudrug [67] is a commercial classical computer package that uses the Hammett–Taft equations. For a given structure, it estimates not only pK_a values, but also other physicochemical and biological parameters such as octanol–water partition coefficients or toxicities. ACD labs [68] has also developed commercial computer packages to calculate pK_a and many other parameters. Internet versions are also available that provide an immediate pK_a estimation after submission of the structure. Payment is per structure o for a period of time. The popular Scifinder literature searcher program from Chemical Abstracts Service includes also and Internet connection to ACD labs for estimation of aqueous pK_a and other properties of some drug-like substances [69].

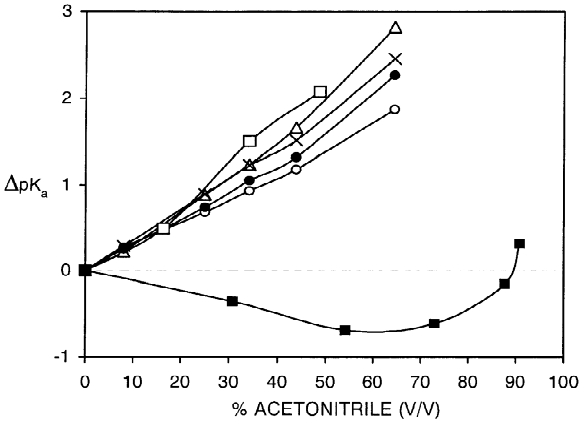

Fig. 4. Variation of the pK_a of acids ($\Delta pK_a = {}^{s}_{s}pK_a - {}^{w}_{w}pK_a$) in acetonitrile–water mixtures with solvent composition: (○) pK_{a1} of citric acid, (●) pK_{a2} of citric acid, (×) pK_{a3} of citric acid, (△) acetic acid, (□) benzoic acid, (■) pyridinium. Reprinted with permission from Analytical Chemistry [25]. Copyright 2000 American Chemical Society.

A different approach has been followed by the SPARC (Sparc Performs Automatic Reasoning in Chemistry) program, developed by collaboration of the University of Athens (Georgia) and the Environmental Protection Agency of the United States [70]. SPARC uses algorithms based on fundamental chemical structure theory that combines principles of Quantitative Structure Activity Relationships (QSAR), Linear Free-Energy Relationships (LFER) and perturbation theory from quantum chemistry [71]. A free internet site is available [72] that allows estimation of the pK_a value and many other parameters of environmental interest of any drawn structure at any temperature.

4.2. Variation of solute pK_a values with the mobile phase composition

The addition of an organic solvent to a solution of an acid–base compound in water changes the acidity pK_a value of the compound. Figs. 2–4 show plots of the ${}^{s}_{s}pK_a$ variation of some representative acids and bases with the composition of chromatographic mobile phases [25,27,73]. The variation of pK_a values is in principle different for each compound. In mixed solvents, preferential solvation of the compound by the components of the solvent mixture affects the pK_a variation. Several rather complex equations have been proposed to account for the preferential solvation in binary solvent mixtures and to relate the ${}^{s}_{s}pK_a$ values to solvent composition, usually in mole fraction of one of the components [27,74–76]. For practical purposes the variation of pK_a values can be fitted to solvent composition in mole, volume or weight fraction by polynomial equations [49,77]. For a limited range of methanol–water and acetonitrile–water compositions the variation of pK_a values with volume fraction of organic modifier may fit well even a straight line, although the fits for ${}^{s}_{w}pK_a$ seem slightly better than for ${}^{s}_{s}pK_a$ [78–81].

Although the variation of pK_a values with solvent composition of mobile phases is different for each compound, there are common trends that can be observed in Figs. 2–4. The ${}^{s}_{s}pK_a$ values of neutral (e.g. acetic acid) and anionic acids (e.g. dihydrogen citrate) always increase with the increase in the percentage of organic solvent in the mixture. For

polyprotic acids, the variation of the ${}^{s}_{s}pK_a$ values increase when the negative charge of the acid increases, i.e. in Fig. 4, the variation of the pK_{a3} of citric acid is larger than that of the pK_{a2}, and the variation of pK_{a2} larger than that of pK_{a1}. The ${}^{s}_{s}pK_a$ values of cationic acids (protonated neutral bases such as ammonia or pyridine) decrease with the addition of organic solvent and then they increase up to a ${}^{s}_{s}pK_a$ value for the pure organic solvent higher than for water. The minimum of ${}^{s}_{s}pK_a$ is reached for about a 90% of methanol or a 60% of acetonitrile in volume. The pK_a values for cationic acids in tetrahydrofuran–water mixtures decrease with the addition of tetrahydrofuran in the range of solvent compositions studied (0–50% of tetrahydrofuran in volume) [36,82,83]. They are expected to increase in pure tetrahydrofuran since the ${}^{s}_{s}pK_a$ values of neutral acids are much higher in tetrahydrofuran than in water [84–86].

These trends can be explained on the basis of the theory established by Izmailov [46,74,75,87,88]. The theory is based on the estimation of the medium effects for the different species that participate in the acid–base equilibria of an acid of charge z in a solvent s (Scheme 1): the acid form (HA^z), the basic form (A^{z-1}) and the solvated hydrogen ion (HS^+). The full equation is too complex to be useful, but some simplifications can be usually taken that are described in detail elsewhere [46,75]. The vacuum, where there is no specific solvation effects, is taken as reference medium. The equation obtained relates the acidity constant of the acid (pK_{HA}) in the solvent to the acidity constant of the acid in the vacuum (pK_{HA}), the acidity constant of the protonated solvent in the vacuum (pK_{HS^+}), the dielectric constant of the solvent (${}^{s}\epsilon$), and the specific energies of solvation of the different species that participate in the equilibria ($\Sigma^{s}_{s}G^{0}_{SOLV}$). The equation can be written as follows:

$$ {}^{s}_{s}pK_a = {}^{vac}_{vac}pK_{HA} - {}^{vac}_{vac}pK_{HS+} - \frac{e^2(z-1)}{2.303r({}^{s}\epsilon)\,kT} - \frac{\sum {}^{s}_{s}G^{0}_{SOLV}}{2.303RT} \quad (40) $$

where e, k, R, and T have the usual meanings of electron charge, Boltzmann and gas constants, and absolute temperature. r is the average radius of all ionic species involved in the equilibrium.

A neutral acid has $z=0$ and therefore the electrostatic term $-\frac{e^2(z-1)}{2.303r({}^{s}\epsilon)\,kT}$ is positive and contributes to increase the ${}^{s}_{s}pK_a$ value when the dielectric constant of the medium decreases by addition of an organic solvent of dielectric constant lower than that of water, such as methanol, acetonitrile or tetrahydrofuran. If the acid is anionic $z<0$ and the electrostatic term is larger. However, the conjugated acid of a neutral base has z = 1 and the electrostatic term has no contribution to the acidity ${}^{s}_{s}pK_a$ value of this acid. Only the variation of the basicity of the solvent (${}^{vac}_{vac}pK_{HS^+}$) and of the specific solvation effects $\left(\frac{\sum {}^{s}_{s}G^{0}_{SOLV}}{2.303RT}\right)$ determine the variation of the ${}^{s}_{s}pK_a$ value of the base when the solvent composition is changed. In methanol–water, acetonitrile–water and in other aqueous–organic solvent mixtures these effects decrease the ${}^{s}_{s}pK_a$ values of bases.

It is interesting to apply Eq. (40) to the variation of the ${}^{s}_{s}pK_a$ value of an acid between water (w) and a particular solvent or mobile phase composition (s) [75]. The equation obtained is:

$$ {}^{s}_{s}pK_{HA} - {}^{w}_{w}pK_{HA} = {}^{vac}_{vac}pK_{H_3O^+} - {}^{vac}_{vac}pK_{HS^+} - \frac{e^2(z-1)}{2.303rkT}\left(\frac{1}{{}^{s}\epsilon} - \frac{1}{{}^{w}\epsilon}\right) - \frac{\left(\sum {}^{s}_{s}G^{0}_{SOLV} - \sum {}^{w}_{w}G^{0}_{SOLV}\right)}{2.303RT} \quad (41) $$

This equation shows that the ${}^{s}_{s}pK_a$ variation depends on some solvent properties (acidities of the protonated water and solvent and dielectric constants of water and solvent s) and on some solute properties (charge and radius of the acid and specific solvation effects). If the differences in the specific solvation effects between water and solvent s could be neglected, acids of the same charge and size would have the same variation of ${}^{s}_{s}pK_a$ when transferred from water to a particular mobile phase composition.

Anyway, for acids belonging to the same family, the differences in the specific solvation effects in solvent s and in water can be linearly related to the acidity of the acid measured by the pK_{HA} value. In this instance, it has been demonstrated that Eq. (41) predicts linear relationships between the pK values of the acids of the same family in a particular mobile phase and in water [74]:

Table 6
Parameters for prediction of the slopes and intercepts of the linear correlations between the pK_{HA} values in methanol–water and the pK_{HA} values in pure water (Eqs. (43) and (44)) [75]

	a_1	a_2	a_3	a_4	SD	F	b_1	b_2	b_3	b_4	SD	F
Aliphatic carboxylic acids	−1.406	0.680	−1.551	0.827	0.005	190	1.034	−0.898	−1.250	0.277	0.003	9.3E+05
Aromatic carboxylic acids:												
with ortho substituents	−1.189	0.190	−1.424	0.425	0.031	80	0.449	−0.429	−1.674	0.677	0.020	2.8E+04
without ortho substituents	−1.101	0.103	−1.516	0.518	0.009	4600	−0.178	0.187	−1.699	0.702	0.029	4.7E+03
Phenols	−0.656	−0.030	−0.844	0.133	0.001	4100	−0.454	0.866	−0.017	−0.865	0.003	5.9E+05
Amines	−0.476	0.209	−0.400	0.158	0.0002	8500	−0.458	0.477	−1.674	0.690	0.002	1.5E+05
Pyridines	2.617	0.000	2.809	0.000	0.002	920	−1.733	1.763	−1.214	0.272	0.001	7.4E+05

$$ {}^{s}_{s}pK_{HA} = a_{s}\,{}^{w}pK_{HA} + b_{s} \quad (42) $$

The intercept of the correlation (b_s) is related to the differences between solvent s and water of the three terms of Eq. (41): differences in the acidities in the vacuum of protonated solvent and water, differences in the dielectric constants of solvent s and water, and differences in the specific solvation in solvent s and water. The first two differences depend only on the solvents considered, but the specific solvation differences depend also on the compound (or family of compounds) studied. The slope of the correlation (a_s) depends only on the specific solvation term, which depends on the solvent and family of compounds studied. The larger the specific solvation of the compound in solvent s, in reference to water, the larger the slope value.

According to Chantooni and Kolthoff [89], the slope value (a_s) measures the ''resolution of acid strength'' of the family of compounds in the solvent in reference to water. The approach has been well established for the pK_a values of families of compounds in different solvents in reference to the pK_a values in water [74,75,85,86,89–97] and it was applied to all available ${}^{s}_{s}pK_a$ data in methanol–water mobile phases [74,75]. The ${}^{s}_{s}pK_a$ values of 121 acid–base compounds belonging to six different chemical families in several methanol–water compositions were fitted to Eq. (42) and the a_s and b_s parameters of the equation were obtained for each compound family and solvent composition. The a_s and b_s sets of values obtained for each family were related to solvent composition through polynomials. For mobile phase compositions measured in volume fraction of methanol (ϕ_{MeOH}), the equations take the forms:

$$ a_{s} = \frac{1 + a_1\phi_{MeOH} + a_2\phi^2_{MeOH}}{1 + a_3\phi_{MeOH} + a_4\phi^2_{MeOH}} \quad (43) $$

$$ b_{s} = \frac{b_1\phi_{MeOH} + b_2\phi^2_{MeOH}}{1 + b_3\phi_{MeOH} + b_4\phi^2_{MeOH}} \quad (44) $$

where a_1, a_2, a_3, a_4, b_1, b_2, b_3, and b_4 are fitting parameters constant for all acids of the same family at all methanol–water mixtures. Their values are given in Table 6 for the different families studied. Therefore, the pK_{HA} value of any member of these families, including compounds not studied in the original set, can be estimated at any methanol–water composition with a reasonable accuracy simply knowing the pK_{HA} value of the compound in water. Conversely, the pK_{HA} value of the compound in water can be calculated from the pK_{HA} value of the compound at any methanol–water composition. The prediction of pK_{HA} values in methanol–water from pK_{HA} in water was successfully tested from the data of compounds not included in the original sets studied because of their small number of ${}^{s}_{s}pK_a$ data [75]. It was also tested by comparison of the predicted ${}^{s}_{s}pK_a$ values in 50% methanol for a series of phenols [74] with those obtained from the chromatographic retention of the phenols in a polymeric column with the same mobile phase composition at several ${}^{s}_{s}pH$ values [98]. The plot obtained is given in Fig. 5. The agreement between calculated and chromatographically measured pK_a values was between 0.4 pK units, except for two outlayers with doubtful pK_a values.

The recent compilation of almost all published pK_a data in methanol–water mixtures [74,75] can be very useful for chromatographic optimization. The degree

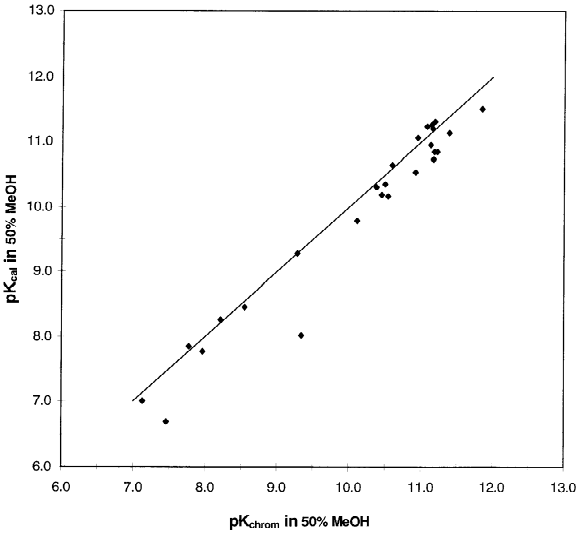

Fig. 5. Plot of pK_a calculated from Eqs. (42)–(44) and the pK_a value in water versus pK_a determined from HPLC retention of phenols in a polymeric column with 50% methanol as mobile phase. Reprinted from Journal of Chromatography A [74]. Copyright 2000, with permission from Elsevier Science.

Table 7
Parameters for prediction of the slopes and intercepts of the linear correlations between the pK_{HA} values in acetonitrile–water and the pK_{HA} values in pure water (Eqs. (43) and (44)) [102]

	a_1	a_2	a_3	a_4	SD	F	b_1	b_2	b_3	b_4	SD	F
Aliphatic carboxylic acids	9.97	−8.59	8.83	−8.72	0.01	5464	−0.68	9.94	8.45	−8.59	0.08	5152
Aromatic carboxylic acids	−2.42	3.14	−1.98	2.12	0.02	362	9.97	−9.12	5.96	−6.90	0.14	2607
Phenols	10.05	−10.04	7.97	−8.37	0.02	386	−5.33	9.95	0.19	−0.70	0.11	2406
Amines	−0.73	−0.27	−0.87	−0.12	0.00	3476	−1.82	2.25	−1.75	0.90	0.05	1559
Pyridines	−1.67	0.67	−1.66	0.67	0.03	38	−1.78	1.89	−0.58	−0.40	0.10	1293

of ionization of the acid–base compound in a particular methanol–water mobile phase can be easily calculated from the known ${}^{s}_{s}pK_a$ value and the measured ${}^{s}_{s}pH$ value of the mobile phase and this would be very helpful for the estimation of the retention of the compound. The establishment of equations to calculate ${}^{s}_{s}pK_a$ values in methanol–water from known or estimated aqueous pK_a (Eqs. (43) and (44) and Table 6) would be also very useful for a general prediction of retention. These equations could be easily implemented in optimization programs such as DryLab or ChromSword [10,99,100].

The number of ${}^{s}_{s}pK_a$ data in acetonitrile–water is much smaller than that in methanol–water [76–79,81,101], but it has been enough to establish ${}^{s}_{s}pK_a$ estimation equations for some families of compounds in a recent work [102]. The parameters of Eqs. (43) and (44) for acetonitrile–water mixtures are given in Table 7.

The published pK_a data in tetrahydrofuran–water is even scarcer than in acetonitrile–water. Only the ${}^{s}_{s}pK_a$ values of a few neutral and anionic acids [36,37,73,103] and of a group of substituted pyridines [82,83] are available for a range of solvent compositions up to 50–80% of tetrahydrofuran in volume. There are also some ${}^{s}_{s}pK_a$ data for neutral acids in pure tetrahydrofuran [84–86], which do not seem to be of chromatographic interest.

5. Chromatographic applications

5.1. Practical measurement of pH in liquid chromatography mobile phases

It is commonly accepted that Horváth and Melander [4,5] did the first systematic studies about the effect of the pH of the mobile phase on the retention of ionizable compounds. They derived equations equivalent to (16) for different types of solutes (monoprotic acids and bases and diprotic acids and zwitterions [4]). They clearly indicated that both the acidity constant and the hydrogen ion concentration must be measured in the mobile phase used [5], although they only studied aqueous mobile phases without organic modifiers for which pH measurement was not a problem [4].

Van de Venne et al. [6] extended the retention–pH studies to methanol–water mobile phases. They recommended the measurement of pH in the mobile phase after calibration with standard buffer solutions of the same solvent composition as the mobile phase if they were available (${}^{s}_{s}pH$ scale). However, as the preparation of standard buffer solutions for different aqueous–organic mixtures is time consuming, they suggested to calibrate the electrode system with the aqueous standard buffer solutions (${}^{s}_{w}pH$ scale) and convert the ${}^{s}_{w}pH$ values (named pH^{app} in the study) to ${}^{s}_{s}pH$ (named pH^{*}) by means of the δ values (Eq. (34)). The ${}^{s}_{s}pH$ of the standard buffer solutions and δ values determined by de Ligny et al. for methanol–water solutions were used in the Van Venne et al. study [104–107].

The lack of pH values of standard buffer solutions and δ values for mobile phases other than methanol–water hindered the practical use of the ${}^{s}_{s}pH$ and ${}^{s}_{w}pH$ scales. Thus, the common practice of measuring the pH of the mobile phase in the aqueous buffer before mixing it with the organic modifier was extended among many workers. Some misunderstandings about pH scales and pH measurement in non-aqueous and mixed solvents contributed to the extension of this practice [108]. It was also argued that the glass electrode could be damaged or give poor reproducibility when used in aqueous–organic mobile phases. In fact, it is well known that the water

content of the glass membrane has a marked effect on the pH function of the electrode [31] and thus it is not recommended to use the glass electrode in dehydrating organic solvents. However, chromatographic mobile phases have usually high water contents and the glass electrode has been successfully used in them, even in pure methanol [31].

Nowadays, there is still no general consensus on how to measure and report the pH value of the mobile phase. The pH is most often measured before mixing the buffer with the organic modifier ($^{w}_{w}$pH scale), sometimes after mixing buffer and organic modifier as an operational pH with electrodes calibrated in water ($^{s}_{w}$pH scale) or as a ''true'' or thermodynamic pH with electrodes calibrated in the same mobile phase solvent ($^{s}_{s}$pH scale) [8,47]. We shall review both the advantages and shortcomings of these three procedures.

Measurement of the pH in the aqueous buffer before mixing it with the organic modifier is the most extended practice. It has the advantage that reduces the number of pH measurements, because pH only has to be measured once for each different buffer [8]. The pH is always the same for all mobile phases prepared from the same aqueous buffer, regardless of the amount and type of organic modifier added. This is a practical advantage, especially for automated systems where it is technically difficult to measure the pH of the eluent after mixing.

The major shortcomings are that the pK_a values obtained in the fits of retention to pH through equations of the type of Eq. (16) do not have a physical meaning [8,9] and very bad fits may be obtained when mobile phase buffers of different type are used [24–26,79]. These two shortcomings come out from the different variation of the pH values of the buffers and the pK_a value of the analyte with addition of the organic modifier.

Sýkora et al. [47] studied the effect of mobile phase pH measured in the aqueous buffer ($^{w}_{w}$pH) in the retention of neutral bases. They observed apparent shifts of the retention vs. pH plots toward pH values more acidic than the $^{w}_{w}$pK_a value of the base. They demonstrated that the shifts were a combination of the two individual shifts caused by the change in the dissociation of the buffer (which produces a mobile phase pH change) and by the change in the pK_a of the basic analyte. These shifts for normalized

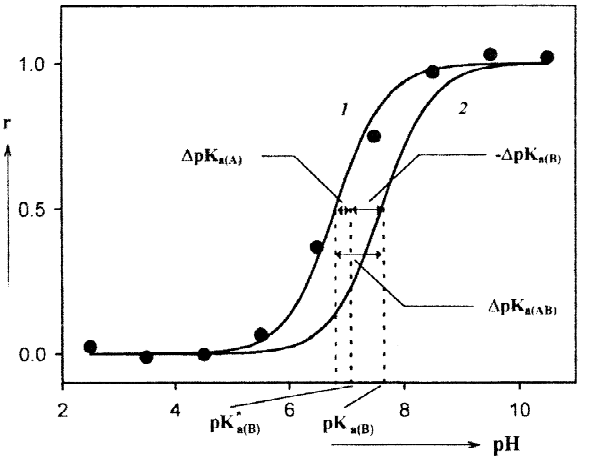

Fig. 6. Influence of methanol on the shift of normalized retention (r) versus pH dependence. Stationary phase: HEMA-BIO 1000 C_{18}. Mobile phase: methanol–25 mM sodium phosphate buffer (20:80, v/v). Analyte: (β) 2,4,6-collidine. Curve 1: Fitted to the experimental data. Curve 2: Theoretical dependence according to the $^{w}_{w}$pH value of the aqueous buffer and the aqueous $^{w}_{w}$pK_a value of the analyte. $\Delta pK_{a(A)}$ change in the pK_a value of the phosphate buffer ($^{s}_{s}pK_{a(A)} - {}^{w}_{w}pK_{a(A)}$); $-\Delta pK_{a(B)}$ change in the pK_a value of the basic analyte ($^{s}_{s}pK_{a(B)} - {}^{w}_{w}pK_{a(B)}$); $\Delta pK_{a(AB)}$ overall apparent pK change (−pH shift); $pK^{*}_{a(B)} \equiv {}^{s}_{s}pK_{a(B)}$; $pK_{a(B)} \equiv {}^{w}_{w}pK_{a(B)}$. Reprinted from Journal of Chromatography A [47]. Copyright 1997, with permission from Elsevier Science.

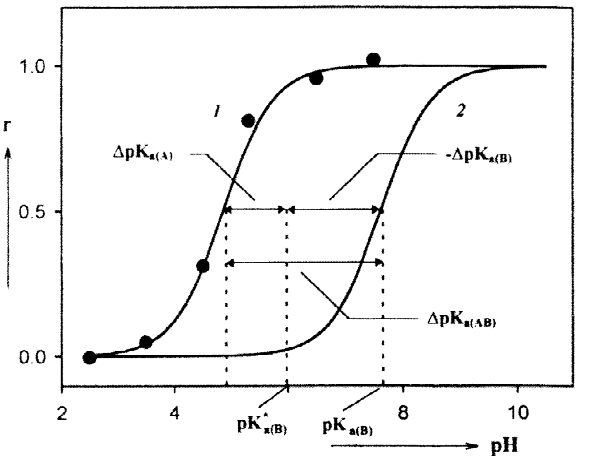

Fig. 7. Influence of methanol on the shift of normalized retention (r) versus pH dependence. Stationary phase: Symmetry C_{18}. Mobile phase: methanol–25 mM sodium phosphate buffer (60:40, v/v). Analyte: (●) 2,4,6-collidine. Curves 1 and 2 and other symbols as in Fig. 6. Reprinted from Journal of Chromatography A [47]. Copyright 1997, with permission from Elsevier Science.

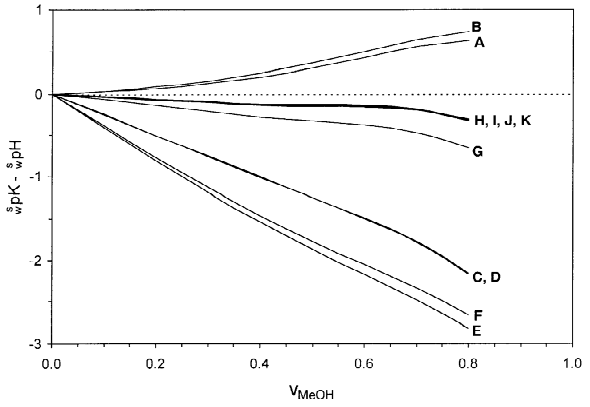

Fig. 8. Differences between the variation of the ${}^{s}_{w}pK_a$ value of the compounds and the ${}^{s}_{w}pH$ value of a buffer with the same pH in pure water than the aqueous pK_a of the compound. Test compounds: (A) benzoic acid, (B) *tert*.-butylbenzoic acid, (C) aniline, (D) 4-*tert*.-butylaniline, (E) 4-*tert*.-butylpyridine, (F) papaverine, (G) lidocaine, (H) ephedrine, (I) 4-*tert*.-butylbenzylamine, (J) 4-*tert*.-butylbenzethylamine and (K) phentermine. Reprinted with permission from Analytical Chemistry [109]. Copyright 2001 American Chemical Society. $v_{MeOH} \equiv \phi_{MeOH}$.

retention (r) are depicted in Figs. 6 and 7 for two different mobile phase compositions. It can be observed that the shift increases with the percentage of organic modifier because of the increase of both individual shifts. These individual shifts are different for each analyte and buffer.

Fig. 8 shows the effect of the addition of methanol on the differences between the ${}^{s}_{w}pK_a$ values of different analytes and the ${}^{s}_{w}pH$ value of a buffer prepared in water at a ${}^{w}_{w}pH$ value equal to the ${}^{w}_{w}pK_a$ value of the analyte. The buffer was prepared from ammonium acetate. The effect of the variation of the differences for four representative solutes in the normalized retention vs. aqueous buffer ${}^{w}_{w}pH$ plots for several mobile phase composition is presented in Fig. 9.

Benzoic acid has a pK_a value close to that of acetic acid, but its pK_a variation with the addition of methanol is slightly larger than the variation of the pK_a of acetic acid (see Fig. 2 too). Thus, the ${}^{s}_{w}pH$ value of the acetic–acetate buffer increases slightly

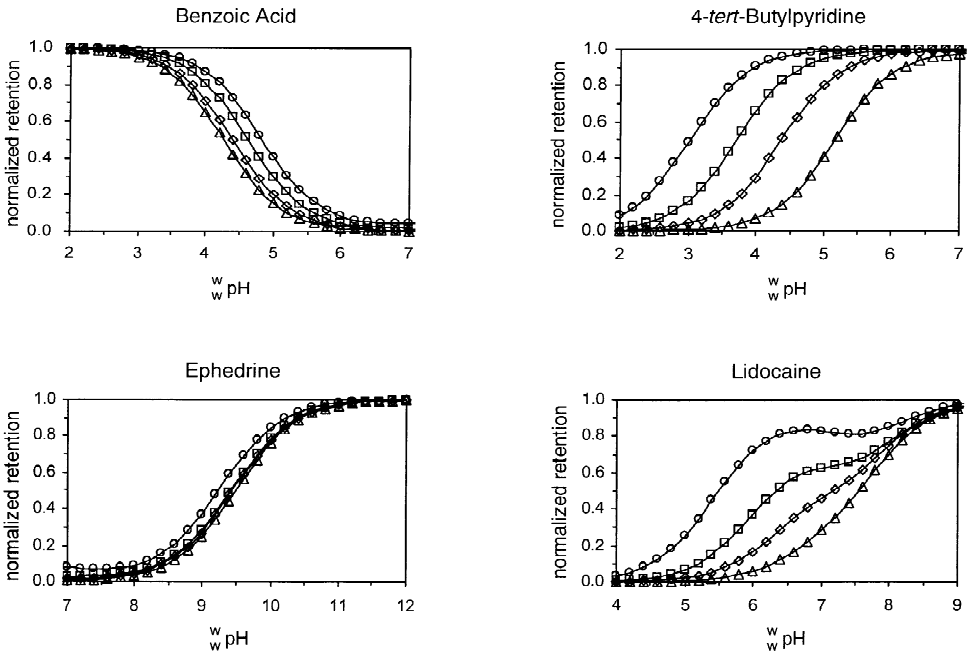

Fig. 9. Calculated retention plots for selected compounds in several isocratic methanol–water mobile phases. Methanol concentrations: (△) 20%, (◇) 40%, (□) 60%, and (○) 80%. Reprinted with permission from Analytical Chemistry [109]. Copyright 2001 American Chemical Society.

less than the ${}^{s}_{w}pK_a$ value of benzoic acid and the ${}^{s}_{w}pK_a - {}^{s}_{w}pH$ term increases slightly with the addition of methanol (Fig. 8). This produces a slight shift of the retention vs. pH plot towards higher ${}^{w}_{w}pH$ values when methanol percentage in the mobile phase increases. 4-*tert*.-Butylpyridine has also a pK_a value close to that of acetic acid, but whereas the ${}^{s}_{s}pK_a$ value of acetic acid and the ${}^{s}_{s}pH$ values of its buffers increase with the addition of methanol, the ${}^{s}_{s}pK_a$ values of neutral bases, such as 4-*tert*.-butylpyridine, decrease. Thus, a large decrease of the ${}^{s}_{w}pK_a - {}^{s}_{w}pH$ difference with addition of methanol is observed in Fig. 8, which is reflected as a large shift towards lower ${}^{w}_{w}pH$ values in the retention vs. pH plots. The ${}^{s}_{s}pK_a$ value of ephedrine (neutral base) decreases with the addition of methanol too, but this compound has a pK_a value close to that of ammonia and therefore the solution is buffered by ammonium/ammonia. Since the ${}^{s}_{s}pK_a$ of ammonia decreases with addition of methanol in a similar way than that of ephedrine, almost no variation of the ${}^{s}_{w}pK_a - {}^{s}_{w}pH$ difference in Fig. 6 and almost no shift of the plot in Fig. 9 are observed with the increase of methanol percentage in the mobile phase. The behaviour of lidocaine (neutral base) is more complex. Its pK_a value is between that of acetic and ammonium acids and it may be buffered by one or other pair. For small additions of methanol, it is buffered by ammonium/ammonia and small variations of the ${}^{s}_{w}pK_a - {}^{s}_{w}pH$ differences and shifts of the plot are observed (such as ephedrine). For larger additions of methanol, the decrease of the ${}^{s}_{s}pK_a$ value of lidocaine and the increase of the pH of acetic–acetate buffers determines that this acid–base pair buffers lidocaine solutions. Then, a larger decrease of the ${}^{s}_{w}pK_a - {}^{s}_{w}pH$ difference and a larger shift toward lower pH values are observed (such as 4-*tert*.-butylamine). A more detailed explanation is given in the original publication [109].

Since most chromatographic buffers are prepared from neutral or anionic acids (e.g. acetic, citric or phosphoric acids), the pK_a variation of these acids and the pH variation of the buffers prepared from them may match approximately the pK_a variation of acid analytes with addition of organic modifier, but not the one of bases. Literature reports good agreements between the aqueous pK_a values of acids [10,99,110] estimated by HPLC by measurement of pH before addition of organic modifier and the accepted thermodynamic values, but the agreement worsen for bases [10,99].

McCalley [111–113] has studied the protonation of bases in methanol–water, acetonitrile–water and tetrahydrofuran–water with phosphate buffers and concluded that half-protonation is produced at aqueous pH much lower than the aqueous ${}^{w}_{w}pK_a$ value of the base. Fig. 10 presents plots of absorbance at selected wavelength vs. pH of the buffer (methanol–phosphate buffer 55:45, v/v) for pyridine and 2,4-dimethylpyridine. The ${}^{w}_{w}pK_a$ values of these two bases are 5.17 and 6.74, respectively. However, it can be seen that even 2,4-dimethylpyridine appears to be unprotonated in methanol–phosphate buffer of ${}^{w}_{w}pH$ 7, measured before mixing (plots a and c). When the pH is measured after mixing phosphate buffer and methanol and converted to ${}^{s}_{s}pH$ (pH* in the plots) by means of the corresponding δ value (plots b and d), the buffer of ${}^{w}_{w}pH$ 7 becomes ${}^{s}_{s}pH$ 8.25. At this ${}^{s}_{s}pH$ value, the figure shows that both bases are fully unprotonated as expected from their ${}^{w}_{w}pK_a$ value. Moreover, the plots show that the pK_a values of the bases decrease to ${}^{s}_{s}pK_a$ values of 4.3 and 5.8 for pyridine and 2,4-dimethylpyridine, respectively, in methanol–phosphate (55:45, v/v) mobile phase [112]. Since protonation of the base causes tailing and peak asymmetry in many C_{18} columns because of the interactions with the ionized surface silanols, it is possible to obtain good efficiencies for bases at mobile phase ${}^{w}_{w}pH$ values much lower than expected from the aqueous ${}^{w}_{w}pK_a$ values of the bases.

Kele and Guiochon [114] reported good batch-to-batch reproducibilities for the basic compounds amitriptyline and propanolol on a C_{18} column and a methanol–water (65:35, v/v) mobile phase with a phosphate buffer of pH 7 measured before addition of methanol. At this pH value the silanol groups of the column are dissociated, whereas the aqueous ${}^{w}_{w}pK_a$values of the amines (9.4 and 9.5) suggest that they should be still completely protonated. Thus, strong ionic interactions are expected between the silica surface of the column and the amines, that should produce pronounced differences between the column batches. However, the differences observed were not as strong as expected. This was attributed to both the increase of the aqueous pH of the buffer and the decrease of the pK_a value of the amines,

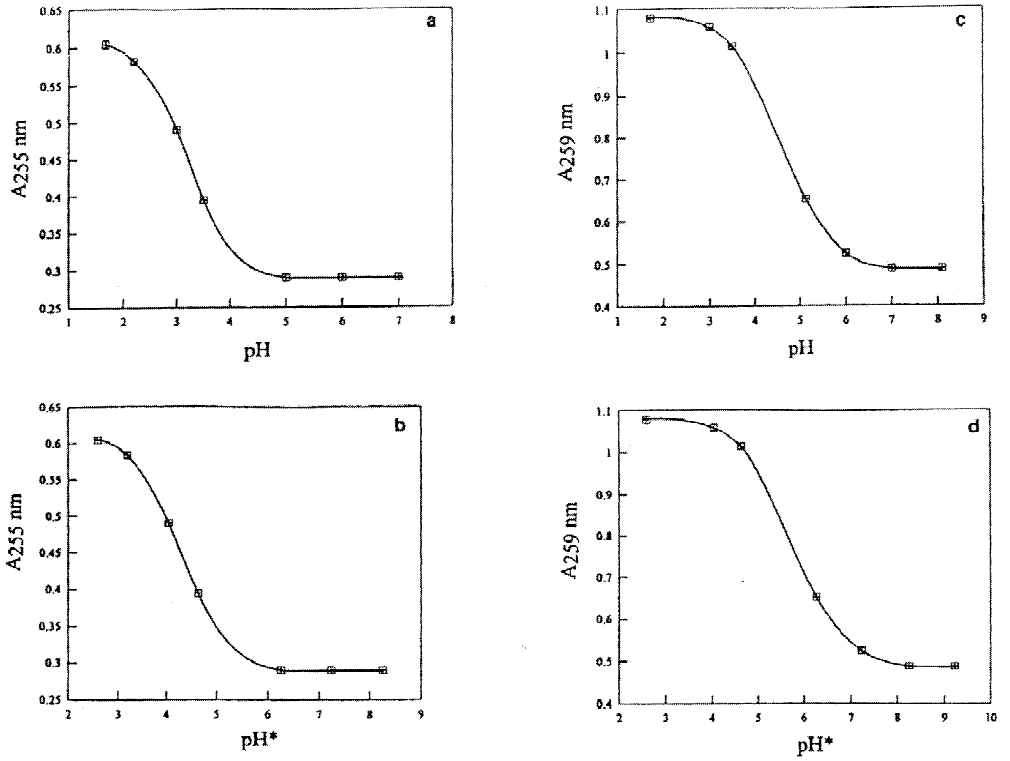

Fig. 10. Plot of UV absorbance against pH. (a) Pyridine in methanol–phosphate buffer (55:45, v/v); pH measured *before* organic solvent addition ($^{w}_{w}$pH). (b) Pyridine (same concentration) in methanol–phosphate buffer (55:45, v/v); pH***** measured *after* organic solvent addition ($^{s}_{s}$pH). (c) 2,4-Dimethylpyridine in methanol–phosphate buffer (55:45, v/v); pH measured *before* organic solvent addition ($^{w}_{w}$pH). (d) 2,4-Dimethylpyridine (same concentration) in methanol–phosphate buffer (55:45, v/v); pH***** measured *after* organic solvent addition ($^{s}_{s}$pH). Reprinted from Journal of Chromatography A [112], Copyright 1994, with permission from Elsevier Science.

caused by the addition of the organic modifier, that combine to get only half-protonation of the amines, and therefore a decrease on the amine–silica interactions.

The same argument was used by Neue et al. [115] for the same amines and mobile phase on a C_8 column to explain the large shifts obtained (relative to acenaphthene) for small variations on the mobile phase conditions (buffer concentration, pH, methanol percentage, and temperature). At aqueous mobile phase pH of 7.0 and for aqueous pK_a values of the bases close to 9, the bases should be completely protonated. In this instance, a small variation on the mobile phase conditions should not influence ionization of the bases and therefore the relative retention should not change. However, the ''apparent'' pK_a values of the bases (caused by combination of the buffer pH and base pK_a variations) were found to be around 6.5–7, i.e. around the aqueous pH value of the buffer. Therefore, in fact the bases were more or less half-protonated and small variations of the conditions cause appreciable variation of ionization and retention.

The variation of the pH value of the buffer is different for each type of buffer. As explained above, the pH values of buffers prepared from acetic acid, and in general from neutral or anionic acids (phosphoric, citric, etc.) increase with the addition of methanol, whereas the pH values of buffers prepared from neutral bases (e.g. ammonia) decrease. This is reflected in the fitting pK_a value obtained for the retention vs. aqueous pH plots, which corresponds to the pH of the inflection point of the plot. However, if buffers of different type are used in the range of variation of retention with pH, the different pH variation of buffers when organic modifier is added

Fig. 11. Variation of the retention time of 3-nitrophenol and triethylamine in the polymeric column with the 40% acetonitrile mobile phase pH measured before mixing the aqueous buffer with the organic modifier ($^{w}_{w}$pH scale): (○) 3-nitrophenol in neutral and anionic acid buffers, (●) 3-nitrophenol in ammonia and butylamine buffers, (□) triethylamine in neutral and anionic acid buffers, (■) triethylamine in butylamine buffers. Neutral acid buffers: phosphoric acid, citric acid + dihydrogen citrate, acetic acid + acetate, and boric acid + borate. Anionic acid buffers: dihydrogen citrate + hydrogen citrate, hydrogen citrate + citrate, dihydrogen phosphate + hydrogen phosphate, hydrogen phosphate + phosphate, and phosphate. Reprinted with permission from Analytical Chemistry [25]. Copyright 2000 American Chemical Society.

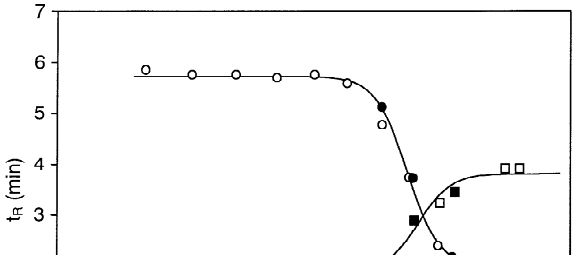

Fig. 12. Variation of the retention time of 3-nitrophenol and triethylamine in the polymeric column with the 40% acetonitrile mobile phase pH measured after mixing the aqueous buffer with the organic modifier ($^{s}_{s}$pH scale). Symbols as in Fig. 11. Reprinted with permission from Analytical Chemistry [25]. Copyright 2000 American Chemical Society.

may result in bad fits to Eq. (13). This can be observed by comparison of Figs. 11 and 12 for 3-nitrophenol and triethylamine. Three different buffers (from NH_4^+/NH_3, $H_2PO_4^-/HPO_4^{2-}$, and $H_3BO_3/H_2BO_3^-$) were prepared at the same aqueous $^{w}_{w}$pH value of 9.00. However, the retention of 3-nitrophenol in these three buffers was very different: t_R was 5.08 min with NH_4^+/NH_3, 3.74 min with $H_2PO_4^-/HPO_4^{2-}$, and 2.38 min with $H_3BO_3/H_2BO_3^-$. It is impossible that the line fitted through Eq. (13) in terms of retention time for the aqueous $^{w}_{w}$pH (measured before mixing) crosses these three points (Fig. 11). However, the $^{s}_{s}$pH values of NH_4^+/NH_3, $H_2PO_4^-/HPO_4^{2-}$, and $H_3BO_3/H_2BO_3^-$ buffers measured after mixing were 8.86, 9.60, and 10.39, respectively, and the fitting line crosses very nicely these three points (Fig. 12). The same effect was observed for triethylamine which was more retained with a borate buffer of $^{w}_{w}$pH 9.00 (but $^{s}_{s}$pH 10.30) than with a butylamine buffer of $^{w}_{w}$pH 10.00 (but $^{s}_{s}$pH 9.60).

Figs. 11 and 12 show that the fits of retention to pH through equations of the type of (13) or (16) can be much better when the pH is measured after mixing aqueous buffer and organic modifier in the $^{s}_{s}$pH or $^{s}_{w}$pH scales. In addition, the fitting pK_a values have a physical meaning, since they agree with the thermodynamic pK_a values of the analytes in the mobile phase solvent [21,24–26,98]. The main shortcoming of direct measurement of the $^{s}_{s}$pH value of the mobile phase is that it requires calibration of the pH electrode system with standard buffers prepared in exactly the same solvent composition that the mobile phase has and with a reference $^{s}_{s}$pH value known for this solvent composition. This handicap limits the practical applicability of this procedure. Even so, several authors have used it to develop HPLC analytical methods for determination of pyrazolidines of pharmaceutical interest [116], prediction of the retention of quinolones and peptides [117–120], or modelling of the retention of diuretics [121]. The pH electrode system was calibrated by the standards studied by De Ligny et al. [33] and Mussini and co-workers [42,122] for methanol–water or Barbosa and co-workers [50–52] for acetonitrile–water.

In our opinion, the procedure of measuring the pH of the mobile phase after mixing the aqueous buffer

of (16). In many circumstances the knowledge of the approximate aqueous pH of the buffer will suffice. In some others, such as interlaboratory testing of procedures, the buffer pH should be accurately measured. This can be done in the aqueous phase before mixing, provided that all laboratories use the same buffer and mobile phase.

5.2. Determination of solute pK_a values by liquid chromatography

The determination of solute pK_a values by liquid chromatography has been discussed in the review of Hardcastle and Jano [13]. They presented the theoretical basis and experimental methodologies needed to determine the pK_a values of compounds of pharmaceutical and biological interest. They remarked that liquid chromatography is more useful than potentiometry or spectrophotometry when only small amounts of the analyte are available. We would add that as separation technique, it might also be very useful for mixtures of analytes that would give overlapping spectra or titration curves [124].

The theoretical basis of the determination of pK_a values is Eq. (13), usually in terms of retention factor (Eq. (16)) or retention time. The procedure consists in measuring the retention time or retention factor of the analyte in an appropriate column and mobile phases for several pH values of this mobile phase. The pK_a value or values of the analyte, as well as the retention times or factors of the different acid–base species, are obtained by fitting the retention–pH data to Eq. (16), usually by non-linear regression. Nowadays, there are many commercial computer programs to perform these fits, including the Solver add-ins in Microsoft Excel worksheets. The accuracy and precision of the pK_a values obtained depend evidently on the reliability of the mobile phase pH measurement, and some concerns regarding this measurement and the results obtained will be discussed in this review.

The most common procedure consists on measuring the pH of the aqueous buffer before mixing it with the organic modifier. It was thought in principle that this procedure would provide the aqueous pK_a value of the analyte. However, it was soon evident that important deviations between the fitting pK_a parameter and the aqueous pK_a values were obtained, especially for bases [10,99]. We have already commented that these deviations originate in the different variation of the pH of the buffer and the pK_a of the analyte with the addition of organic modifier. Only if the same type of buffer were used for all pH points and the variation of the pH of this buffer would exactly match the pK_a variation of the analyte, an accurate aqueous pK_a value would be obtained [25]. The larger the difference between pK_a and pH variation, the larger the difference between the fitting pK_a and the true aqueous pK_a. Since the difference between pK_a and pH increases with the percentage of organic modifier in the mobile phase (see Fig. 8), the bias of the fitting pK_a value in reference to the true pK_a value increases too [25,26]. Sometimes empirical corrections, some of them dependent on the percentage of organic modifier, are applied to obtain the aqueous pK_a value [12,126]. We must emphasize that these corrections will depend on both the analyte and the buffer used if the pH is measured before mixing aqueous buffer and organic modifier.

Measurement of the pH after mixing aqueous buffer and organic modifier, either with aqueous calibration ($^{s}_{w}$pH) or with calibration with standards in the same mobile phase composition ($^{s}_{s}$pH) provides pK_a values independent of the buffer used. However, the values change with the composition of the mobile phase [21,24–26,79–81,120]. In fact, the fitting pK_a values obtained are the true thermodynamic pK_a values of the analyte in the solvent composition used as mobile phase. The disadvantage is the method does not provide the aqueous pK_a value of the analyte. To obtain the aqueous pK_a value extrapolation from the $^{s}_{w}$pK_a or $^{s}_{s}$pK_a values obtained at several mobile phase compositions or semiempirical corrections are required [125,126].

Estimation of aqueous pK_a values of water insoluble drugs from pK_a values in mixed organic solvents is a common practice in pharmaceutical applications, because the aqueous pK_a value of a pharmaceutical drug is a relevant parameter that determines its bioavailability. Aqueous pK_a values are commonly obtained from potentiometric titrations in water–organic solvent mixtures. Traditionally, methanol–water mixtures are used [127–130], although recently other mixed solvents (including acetonitrile–water and tetrahydrofuran–water) have been successfully tested [130]. The same extrapolation procedures

commonly used for the potentiometric pK_a values can be applied to the chromatographic pK_a values to obtain the aqueous pK_a value. This procedure has been used for evaluation of the pK_a values of different drugs in acetonitrile–water mixtures by liquid chromatography and comparison between them and the pK_a data obtained from potentiometry, spectrophotometry and capillary electrophoresis [119,131].

Sometimes, the aqueous pK_a value ($^{w}_{w}pK_a$) is obtained by extrapolation of the plot of $^{s}_{s}pK_a$ values in various organic–solvent mixtures vs. the weight percent of organic solvent. However, these plots often show hockey stick or bow shaped curves [129]. More commonly Yasuda–Shedlovsky plots are used because they give linear relationships that lead to accurate $^{w}_{w}pK_a$ values. The Yasuda–Shedlovsky relationship can be easily derived from Eq. (40) assuming that the specific energy of solvation (ΣG^{0}_{SOLV}) remains constant for all solvent mixtures. With this assumption Eq. (40) becomes:

$$^{s}_{s}pK_a = \frac{a}{^{s}\epsilon} + b \quad (45)$$

The plot of $^{s}_{s}pK_a$ vs. the inverse of the dielectric constant of the solvent should give a straight line. Yasuda reasoned that the usual definition of $^{s}_{s}pK_a$ in the molarity concentration scale according to Eq. (2) does not include the activity of water as solvent [132], which is constant in pure water, but changes with solvent composition in water–organic solvent mixtures. Inclusion of water activity in the molarity scale in Eq. (45) gives:

$$^{s}_{s}pK_a + \log[H_2O] = \frac{a}{^{s}\epsilon} + b \quad (46)$$

Plots of this corrected $^{s}_{s}pK_a$ value against the inverse of the dielectric constant of the solvent gave very good straight lines for methanol–water and dioxane–water mixtures for values of $1/^{s}\epsilon$ lower than 0.02 (water rich portions of the mixtures with dielectric constants larger than 50). Yasuda justified ignoring the activity of methanol or dioxane because water is a much better hydrogen acceptor than methanol or dioxane [130,132].

The arguments of Yasuda were wrong, because the activities of the two solvents (water and organic solvent) are implicitly included in the $^{s}_{s}pK_a$ definition. Independently of the concentration scale used, the activity of the solvents must be always given in the molar fraction scale, and in this scale the sum of all solvent activities is always 1. However, just 1 month after publication of Yasuda's paper, Shedlovsky presented a more rigorous theoretical derivation of Eq. (46) [133]. The approach is commonly known as Yasuda–Shedlovsky plots and it has been used to obtain accurate estimations of the aqueous $^{w}_{w}pK_a$ values of water insoluble drugs [127–130]. The $^{w}_{w}pK_a$ value is obtained by extrapolation of the plot to the inverse of 78.3 (the dielectric constant of water) taking into account that for this point $\log[H_2O] = \log 55.5$ (the molar concentration of pure water) [129]. A validation study for 25 compounds in methanol–water mixtures in the interval 15–65 wt% of methanol demonstrated that the pK_a data extrapolated to zero methanol content from the total interval examined or from the water-rich region agree with the measured aqueous pK_a value in 0.05–0.07 pK units. The extrapolation from methanol-rich regions gave errors in aqueous pK_a not larger than ± 0.2 for weak acids and ± 0.1 for weak bases [129].

Another possibility to estimate the $^{w}_{w}pK_a$ value of a compound consists of measuring the $^{s}_{s}pK_a$ value of the compound and of a series of related compounds in a unique chromatographic mobile phase and use Eq. (42) to calculate the $^{w}_{w}pK_a$ value from the $^{s}_{s}pK_a$ value. The $^{w}_{w}pK_a$ values of the related compounds must be known or measured to calculate the a_s and b_s values of the correlation. If these parameters are already known, calibration with the related compounds is not needed. One recent publication uses the parameters of Table 6 and Eqs. (42)–(44) to estimate the aqueous pK_a values of water insoluble arylpropionic acids with antiinflammatory properties from the chromatographic $^{s}_{w}pK_a$ values in methanol–water mobile phases [134].

6. Conclusions

The pH of the mobile phase has a strong influence in the chromatographic retention of compounds with acid–base properties. The relationship between analyte retention and mobile phase pH is described by a sigmoidal function of the pK_a value of the analyte and the limiting retentions of the different acid–base

forms of the analyte, i.e. by Eq. (10). The general relationship has to be derived in terms of a distribution constant to measure retention and must take into account the activity coefficients of the different analyte species in the different pH buffers. If all pH buffers have similar ionic strengths, the effect of the ionic activity coefficients can be included in the pK_a parameters (pK'_a) and a more practical equation without activity coefficients can be used, i.e. Eq. (13). For practical purposes it is convenient to replace the distribution constant by the retention factor or retention time, provided that the same proportional or linear relationship between these parameters is applied to all data points.

The proper measurement of mobile phase pH is a crucial point to get good fits of the data to the model and fitting pK_a or pK'_a parameters with a physical meaning. Three different procedures, which lead to three different pH scales, are used in liquid chromatography to measure the pH of the mobile phase. Measurement of pH in the aqueous buffer before mixing it with the organic modifier ($^{w}_{w}$pH scale) does not give good retention–pH relationships unless all pH buffers are prepared from the same acid–base pair or with different acid–base pairs that show a similar variation of pH with the addition of organic modifier. Even in this instance, the fitting pK_a parameter will not agree with the true aqueous pK_a value of the analyte unless the pK_a variation of the analyte with addition of organic modifier matches that of the buffers.

Measurement of the pH after mixing aqueous buffer and organic modifier should provide good fits of retention to pH and meaningful fitting pK_a values regardless of the pH buffers used. This procedure should provide a better interpretation of the results obtained and even quantitative predictions of retention from known pK_a data.

Therefore it is recommended, in agreement with IUPAC rules and suggestions for pH measurement in non-aqueous and mixed solvents, to measure the pH of the mobile phase after mixing the aqueous buffer and the organic modifier when the chromatographic retention of ionizable compounds needs to be rigorously related to the pH of the mobile phase. The electrode system used can be calibrated with reference standards prepared in the same solvent mixture used as mobile phase ($^{s}_{s}$pH scale). Reference standards for the $^{s}_{s}$pH scale have been proposed for methanol–water, acetonitrile–water (up to 75% of acetonitrile) and tetrahydrofuran–water (up to 72% of tetrahydrofuran) mobile phases. It is also equally rigorous and more easy to calibrate the pH electrode system with the common aqueous reference standards and measure the pH after mixing the aqueous buffer and organic modifier ($^{s}_{w}$pH scale). $^{s}_{w}$pH and $^{s}_{s}$pH scales are related through the δ term, which is a constant for each mobile phase composition, and thus the two pH scales can be easily interconverted. Relationships between $^{s}_{w}$pH or $^{s}_{s}$pH and $^{w}_{w}$pH are buffer dependent and thus, it is not possible to create a general interconversion between pH values measured before mixing aqueous buffer and organic modifier and pH value measured after mixing. If the pH must be measured before mixing, such as in gradient elution, the results obtained in different runs will be comparable only if the same type of buffer has been used in all measurements.

7. Nomenclature

K_c	Distribution constant
a_i	Activity of the subscripted species (i)
γ_i	Activity coefficient of the subscripted species (i)
K_a	Thermodynamic acidity constant
K'_a	Concentration acidity constant
ρ	Density
pH_c	pH in molarity scale
pH_m	pH in molality scale
x	Mole fraction
w	Weight fraction
ϕ	Volume fraction
M	Molecular weight
ν_M	Molar volume
A	First parameter of Debye–Hückel equation for ionic activity coefficients
B	Second parameter of Debye–Hückel equation for ionic activity coefficients
a_0	Ion size parameter for Debye–Hückel equation
I	Ionic strength of the solution
ϵ	Relative permittivity
$-\log(^{s}_{w}\gamma^{0}_{H})$	Primary medium effect for the transfer of the H^+ ion from water (w) to the solvent s
$^{w}_{w}$pH	pH value in water

${}^{s}_{w}\mathrm{pH}$	pH value in solvent s in reference to water (w) as standard state solvent. In practice, pH measured in a mobile phase with an electrode system calibrated with aqueous standards
${}^{s}_{s}\mathrm{pH}$	pH value in solvent s in reference to the same solvent as standard state solvent. In practice, pH measured in a mobile phase with an electrode system calibrated with standards prepared in the same mobile phase
${}^{w}_{w}\mathrm{p}K$	Aqueous pK value
${}^{s}_{w}\mathrm{p}K$	pK value in solvent s in the ${}^{s}_{w}\mathrm{pH}$ scale
${}^{s}_{s}\mathrm{p}K$	pK value in solvent s in the ${}^{s}_{s}\mathrm{pH}$ scale
E	Electromotive force of a potentiometric cell
ΔE_{J}	Residual liquid junction potential
δ	Term for interconversion between ${}^{s}_{s}\mathrm{pH}$ and ${}^{s}_{w}\mathrm{pH}$ scales
$\overline{E_j}$	Residual liquid junction potential in pH units in pH measurement in a mobile phase with electrode calibration in water
K_{ap}	Autoprotolysis constant of a solvent
a_{s}	Slope of the correlation of the ${}^{s}_{s}\mathrm{p}K_{\mathrm{a}}$ values of a family of compounds in a given mobile phase with the ${}^{w}_{w}\mathrm{p}K_{\mathrm{a}}$ values of the compounds in water
b_{s}	Intercept of the correlation of the ${}^{s}_{s}\mathrm{p}K_{\mathrm{a}}$ values of a family of compounds in a given mobile phase with the ${}^{w}_{w}\mathrm{p}K_{\mathrm{a}}$ values of the compounds in water
β	Overall protonation constant
β'	Concentration overall protonation constant

In general, a left hand superscript in a symbol refers to the solvent where the property is measured and a left hand subscript to the solvent taken as standard state for the ionic activity coefficients at infinite dilution.

Appendix

Simpler forms of Eqs. (9), (10), (12), (13) or (16) can be derived by using overall protonation constants (β_i) instead of consecutive acid–base dissociation constants (K_{ai}). The β_i constants correspond to the different protonation equilibria of the most basic form of the analyte (A^{-z}) to give the different acid base species (up to H_nA^{-z+n}):

$$A^{-z} + iH^{+} \Leftrightarrow H_iA^{-z+i}$$

$$\beta_i = \frac{a_{H_iA} a_H^i}{a_A} = \frac{[H_iA]\,\gamma_i a_H^i}{[A]\,\gamma_0}$$

where the subscript i indicates the number of acidic hydrogen ions of the species. $-z$ is the charge of the most basic species (negative or zero). The overall protonation constants (β_i) are related to the consecutive acid–base dissociation constants (K_{ai}) according to the following equations for $i>0$:

$$\beta_i = \prod_{r=0}^{i-1} K_{a_{n-r}}^{-1}$$

$$\log \beta_i = \sum_{r=0}^{i-1} \mathrm{p}K_{a_{n-r}}$$

The overall retention factor (k) can be written as:

$$k = \frac{\sum_{i=0}^{n} [H_iA]_S\, V_S}{\sum_{i=0}^{n} [H_iA]\, V_M}$$

and considering the individual retention factors of the different species:

$$k_i = \frac{[H_iA]_S\, V_S}{[H_iA]\, V_M}$$

the following equations are derived:

$$k = \frac{\sum_{i=0}^{n} k_i \beta_i \gamma_i^{-1} a_H^i}{\sum_{i=0}^{n} \beta_i \gamma_i^{-1} a_H^i}$$

$$k = \frac{\sum_{i=0}^{n} k_i \gamma_i^{-1} 10^{\log \beta_i - i\mathrm{pH}}}{\sum_{i=0}^{n} \gamma_i^{-1} 10^{\log \beta_i - i\mathrm{pH}}}$$

If the ionic strength of the buffers remains more or less constant, the activity coefficient terms γ_i can be

included in the conditional protonation constants (β_i'), and the equations can be written as follows:

$$k = \frac{\sum_{i=0}^{n} k_i \beta_i' a_H^i}{\sum_{i=0}^{n} \beta_i' a_H^i}$$

$$k = \frac{\sum_{i=0}^{n} k_i 10^{\log \beta_i' - i\mathrm{pH}}}{\sum_{i=0}^{n} 10^{\log \beta_i' - i\mathrm{pH}}}$$

Notice that the equations include a β_0 constant (or β_0') to account for the A^{-z} species, i.e. $\beta_0 = a_A a_H^0 / a_A = 1$.

Provided that V_M remains constant, the same type of equations are obtained for distribution constant, retention time or adjusted retention time simply replacing k by K_C, t_R, or t_R', respectively.

References

[1] IUPAC, Compendium of Analytical Nomenclature. Definitive Rules 1997, 3rd ed, Blackwell, Oxford, 1998. http://www.iupac.org/publications/analytical_compendium
[2] L.S. Ettre, Pure Appl. Chem. 65 (1993) 819.
[3] J.E. Hardcastle, I. Jano, J. Chromatogr. B 717 (1998) 39.
[4] C. Horváth, W. Melander, I. Molnár, Anal. Chem. 49 (1977) 142.
[5] C. Horvath, W. Melander, J. Chromatogr. Sci. 15 (1977) 393.
[6] J.L.M. Van de Venne, L.H.M. Hendrikx, R.S. Deelder, J. Chromatogr. 167 (1978) 1.
[7] P.J. Schoenmakers, S. van Molle, C.M.G. Hayes, L.G.M. Uunk, Anal. Chim. Acta 250 (1991) 1.
[8] R.M. Lopes Marques, P.J. Schoenmakers, J. Chromatogr. 592 (1992) 157.
[9] P.J. Schoenmakers, R. Tijssen, J. Chromatogr. A 656 (1993) 577.
[10] J.A. Lewis, J.W. Dolan, L.R. Snyder, I. Molnar, J. Chromatogr. 592 (1992) 197.
[11] M.V. Brunzzoniti, E. Mentasti, C. Sarzanini, J. Chromatogr. B 717 (1998) 3.
[12] M. Waksmundzka-Hajnos, J. Chromatogr. B 717 (1998) 93.
[13] J.E. Hardcastle, I. Jano, J. Chromatogr. B 717 (1998) 39.
[14] R. Kaliszan, J. Chromatogr. B 717 (1998) 125.
[15] G.E. Berendsen, P.J. Schoenmakers, L. de Galan, J. Liq. Chromatogr. 3 (1980) 1669.
[16] R.M. McCormick, B.L. Karger, Anal. Chem. 52 (1980) 2249.
[17] K. Jinno, N. Ozaki, T. Sato, Chromatographia 17 (1983) 341.
[18] L.G. Daignault, D.C. Jackman, D.P. Rillema, Chromatographia 27 (1989) 156.
[19] K.S. Yun, C. Zhu, J.F. Parcher, Anal. Chem. 67 (1995) 613.
[20] H. Engelhardt, H. Müller, B. Dreyer, Chromatographia 19 (1984) 240.
[21] M. Rosés, I. Canals, H. Allemann, K. Siigur, E. Bosch, Anal. Chem. 68 (1996) 4094.
[22] M. Rosés, F.Z. Oumada, E. Bosch, J. Chromatogr. A 53 (2000) 667.
[23] J. Nawrocki, J. Chromatogr. A 779 (1997) 29.
[24] I. Canals, J.A. Portal, E. Bosch, M. Rosés, Anal. Chem. 72 (2000) 1802.
[25] S. Espinosa, E. Bosch, M. Rosés, Anal. Chem. 72 (2000) 5193.
[26] I. Canals, F.Z. Oumada, M. Rosés, E. Bosch, J. Chromatogr. A 911 (2001) 191.
[27] E. Bosch, P. Bou, H. Allemann, M. Rosés, Anal. Chem. 68 (1996) 3651.
[28] E.D. Katz, K. Ogan, R.P.W. Scott, J. Chromatogr. 352 (1986) 67.
[29] E.D. Katz, C.H. Lochmüller, R.P.D. Scott, Anal. Chem. 61 (1989) 349.
[30] A. Arroyo, Revue Roumaine de Chimie 35 (1990) 523.
[31] R.G. Bates, Determination of pH. Theory and Practice, 2nd ed, Wiley, New York, 1973.
[32] M. Rosés, C. Ràfols, E. Bosch, Anal. Chem. 65 (1993) 2294.
[33] C.L. De Ligny, M. Rehbach, Rec. Trav. Chim. 79 (1960) 727.
[34] R.G. Bates, M. Paabo, R.A. Robinson, J. Phys. Chem. 67 (1963) 1833.
[35] M.S.K. Niazi, J. Ali, Bull. Chem. Soc. Jpn. 63 (1990) 3619.
[36] U. Muinasmaa, C. Ràfols, E. Bosch, M. Rosés, Anal. Chim. Acta 340 (1997) 133.
[37] J. Barbosa, D. Barrón, S. Butí, Anal. Chim. Acta 389 (1999) 31.
[38] S. Taniewska-Osinska, B. Nowicka, A. Kacperska, A. Bald, Phys. Chem. Liq. 25 (1993) 113.
[39] F.E. Critchfield, J.A. Gibson Jr., J.L. Hall, J. Am. Chem. Soc. 75 (1953) 6044.
[40] M. Rosés, Anal. Chim. Acta 285 (1994) 391.
[41] T. Mussini, A.K. Covington, P. Longhi, S. Rondinini, Pure Appl. Chem. 57 (1985) 865.
[42] S. Rondinini, P. Longhi, P.R. Mussini, T. Mussini, Pure Appl. Chem. 59 (1987) 1693.
[43] R.G. Bates, E.A. Guggenheim, Pure Appl. Chem. 1 (1960) 163.
[44] R.A. Robinson, R.A. Stokes, Electrolyte Solutions, 2nd revised ed, Butterworths, London, 1965.
[45] IUPAC, Compendium of Analytical Nomenclature. Definitive Rules 1987, 2nd ed, Blackwell, Oxford, 1987.
[46] L. Sücha, S. Kotrlý, Solution Equilibria in Analytical Chemistry, Van Nostrand Reinhold, London, 1972.
[47] D. Sýkora, E. Tesařová, M. Popl, J. Chromatogr. A 758 (1997) 37.
[48] P.R. Mussini, T. Mussini, S. Rondinini, Pure Appl. Chem. 69 (1997) 1007.
[49] J. Barbosa, V. Sanz-Nebot, Anal. Chim. Acta 283 (1993) 320.

[50] J. Barbosa, V. Sanz-Nebot, Mikrochim. Acta 116 (1994) 131.
[51] J. Barbosa, S. Butí, V. Sanz-Nebot, Talanta 41 (1994) 825.
[52] J. Barbosa, V. Sanz-Nebot, Fresenius J. Anal. Chem. 353 (1995) 148.
[53] A.K. Covington, R.G. Bates, R.A. Durst, Pure Appl. Chem. 57 (1985) 531.
[54] G. Fonrodona, C. Ràfols, E. Bosch, M. Rosés, Anal. Chim. Acta 335 (1996) 291.
[55] E. Bosch, G. Fonrodona, C. Ràfols, M. Rosés, Anal. Chim. Acta 349 (1997) 367.
[56] G. Kortüm, W. Vogel, K. Andrussow, Dissociation Constants of Organic Acids in Aqueous Solution, Butterworths, London, 1961.
[57] D.D. Perrin, Dissociation Constants of Inorganic Acids and Bases in Aqueous Solution, IUPAC, Butterworths, London, 1969.
[58] D.D. Perrin, Dissociation Constants of Inorganic Acids and Bases in Aqueous Solutions: IUPAC, Butterworths, London, 1969.
[59] D.D. Perrin, Dissociation Constants of Inorganic Acids and Bases in Aqueous Solutions: Supplement. IUPAC, Butterworths, London, 1972.
[60] V.A. Palm (Ed.), Tables of Rate and Equilibrium Constants of Heterolytic Organic Reactions, Vol. I (1), Viniti, Moscow, 1975.
[61] V.A. Palm (Ed.), Tables of Rate and Equilibrium Constants of Heterolytic Organic Reactions, Vol. II (1), Viniti, Moscow, 1976.
[62] V.A. Palm (Ed.), Tables of Rate and Equilibrium Constants of Heterolytic Organic Reactions, Supplementary vol. I (1–2), Tartu, Estonia, 1984.
[63] V.A. Palm (Ed.), Tables of Rate and Equilibrium Constants of Heterolytic Organic Reactions, Supplementary vol. I (3), Tartu, Estonia, 1985.
[64] C. Hansch, A. Leo, Exploring QSAR, Fundamentals and applications in Chemistry and Biology, ACS Professional Reference Book, American Chemical Society, Washington, DC, 1995.
[65] D.D. Perrin, B. Dempsey, E.P. Serjeant, pK_a Prediction for Organic Acids and Bases, Chapman and Hall, London, 1981.
[66] J. Shorter, Substituent effect parameters and models applied in organic chemistry, in: R.I. Zalewski, T.M. Krygowski, J. Shorter (Eds.), Similarity Models in Organic Chemistry, Biochemistry and Related Fields, Elsevier, Amsterdam, 1991, Chapter 2.
[67] Compudrug Ltd., http://www.compudrug.com
[68] Advanced Chemistry Development Inc., http://www.acdlabs.com
[69] SciFinder, Chemical Abstract Service, http://www.cas.org/SCIFINDER
[70] S.H. Hilal, S.W. Karickhoff, L.A. Carreira, Quant. Struct.-Activ. Relat. 14 (1995) 348.
[71] S. Borman, Chem. Eng. News 2 (Feb.) (1990) 20.
[72] SPARC, University of Georgia, Athens, GA, http://nt2lac.chem.uga.edu/sparc
[73] D. Barrón, S. Butí, M. Ruiz, J. Barbosa, PCCP 1 (1999) 295.
[74] M. Rosés, F. Rived, E. Bosch, J. Chromatogr. A 867 (2000) 45.
[75] F. Rived, I. Canals, E. Bosch, M. Rosés, Anal. Chim. Acta 439 (2001) 315.
[76] E. Bosch, S. Espinosa, M. Rosés, J. Chromatogr. A 824 (1998) 137.
[77] K. Sarmini, E. Kenndler, J. Chromatogr. A 833 (1999) 245.
[78] S. Espinosa, E. Bosch, M. Rosés, Anal. Chim. Acta 439 (2001) 315.
[79] S. Espinosa, E. Bosch, M. Rosés, J. Chromatogr. A 947 (2002) 47.
[80] I. Canals, J. Portal, M. Rosés, E. Bosch, Chromatographia 55 (2002) 565.
[81] S. Espinosa, E. Bosch, M. Rosés, J. Chromatogr. A 945 (2002) 83.
[82] R. Reynaud, C. R. Acad. Sci. Paris C 263 (1966) 905.
[83] R. Reynaud, Bull. Chem. Soc. Fr. 1 (1967) 4597.
[84] J. Barbosa, D. Barrón, E. Bosch, M. Rosés, Anal. Chim. Acta 264 (1992) 229.
[85] J. Barbosa, D. Barrón, E. Bosch, M. Rosés, Anal. Chim. Acta 265 (1992) 157.
[86] D. Barrón, J. Barbosa, Anal. Chim. Acta 403 (2000) 339.
[87] N.A. Izmailov, V.N. Izmailova, Zh. Fiz. Khim. 29 (1955) 1050.
[88] N.A. Izmailov, Electrokhimiya Rastvorov (Electrochemistry of Solutions), State University, Kharkov, 1959.
[89] M.K. Chantooni, I.M. Kolthoff, Anal. Chem. 51 (1979) 133.
[90] E. Bosch, M. Rosés, Talanta 36 (1989) 627.
[91] J. Barbosa, V. Sanz-Nebot, Talanta 36 (1989) 837.
[92] E. Bosch, C. Ràfols, M. Rosés, Talanta 36 (1989) 1227.
[93] C. Ràfols, M. Rosés, E. Bosch, Anal. Chim. Acta 338 (1997) 127.
[94] A. Fini, P. De Maria, A. Guarnerini, L. Varoli, J. Pharm. Sci. 76 (1987) 48.
[95] A.G. González, M.A. Herrador, Anal. Chim. Acta 356 (1997) 253.
[96] F. Rived, M. Rosés, E. Bosch, Anal. Chim. Acta 374 (1998) 309.
[97] D. Barrón, S. Butí, J. Barbosa, Anal. Chim. Acta 403 (2000) 349.
[98] M. Rosés, D. Bolliet, C.F. Poole, J. Chromatogr. A 829 (1998) 29.
[99] J.A. Lewis, D.C. Lommen, W.D. Raddatz, J.W. Dolan, L.R. Snyder, I. Molnar, J. Chromatogr. 592 (1992) 183.
[100] T. Baczek, R. Kaliszan, H.A. Claessens, M.A. van Straten, LC–GC Europe 14 (2001) 304.
[101] Z. Pawlak, J. Chem. Thermodyn. 19 (1987) 443.
[102] S. Espinosa, E. Bosch, M. Rosés, J. Chromatogr. A 964 (2002) 55.
[103] M.S.K. Niazi, A. Hassan, M.Z.I. Khan, S.S. Shah, J. Ali, J. Chem. Eng. Data 37 (1992) 470.
[104] C.L. de Ligny, P.F.M. Luykx, Rec. Trav. Chim. 77 (1958) 154.
[105] C.L. de Ligny, P.F.M. Luykx, M. Rehbach, A.A. Wienecke, Rec. Trav. Chim. 79 (1960) 699.
[106] C.L. de Ligny, P.F.M. Luykx, M. Rehbach, A.A. Wienecke, Rec. Trav. Chim. 79 (1960) 713.
[107] W.J. Gelsema, C.L. de Ligny, A.G. Remijse, H.A. Blijleven, Rec. Trav. Chim. 85 (1966) 647.

[108] B.A. Bidlingmeyer, J. Chromatogr. Sci. 31 (1993) 347.
[109] I. Canals, K. Valkó, E. Bosch, A. Hill, M. Rosés, Anal. Chem. 73 (2001) 4937.
[110] P. Chaminade, A. Baillet, D. Ferrier, B. Bourguignon, D.L. Massart, Anal. Chim. Acta 280 (1993) 93.
[111] D.V. McCalley, LC-GC Europe 12 (1999) 638.
[112] D.V. McCalley, J. Chromatogr. A 664 (1994) 139.
[113] D.V. McCalley, J. Chromatogr. A 708 (1995) 185.
[114] M. Kele, G. Guiochon, J. Chromatogr. A 855 (1999) 423.
[115] U.D. Neue, E. Serowik, P. Iraneta, B.A. Alden, T.H. Walter, J. Chromatogr. A 849 (1999) 87.
[116] C. Herrenknecht, D. Ivanovic, E. Guernet-Nivaud, M. Guernet, J. Pharm. Biomed. Anal. 8 (1990) 1071.
[117] J. Barbosa, R. Bergés, V. Sanz-Nebot, J. Chromatogr. A 719 (1996) 27.
[118] J. Barbosa, V. Sanz-Nebot, I. Toro, J. Chromatogr. A 725 (1996) 249.
[119] V. Sanz-Nebot, I. Toro, J. Barbosa, J. Chromatogr. A 933 (2001) 45.
[120] J. Barbosa, R. Bergés, V. Sanz-Nebot, J. Chromatogr. A 823 (1998) 411.
[121] R. Bergés, V. Sanz-Nebot, J. Barbosa, J. Chromatogr. A 869 (2000) 27.
[122] P. Longhi, T. Mussini, S. Rondinini, Anal. Chem. 58 (1986) 2290.
[123] S. Espinosa, E. Bosch, M. Rosés, Anal. Chem. 74 (2002) 3809.
[124] K. Valkó, Measurement of physical properties for drug design in industry, in: K. Valkó (Ed.), Handbook of Analytical Separations, Vol. 1, Elsevier, Amsterdam, 2000, Chapter 12.
[125] T. Hanai, K.C. Tran, J. Hubert, J. Chromatogr. 239 (1982) 385.
[126] T. Hanai, K. Koizumi, T. Kinoshita, R. Arora, F. Ahmed, J. Chromatogr. A 762 (1997) 55.
[127] L.Z. Benet, J.E. Goyan, J. Pharm. Sci. 56 (1967) 665.
[128] A. Avdeef, J.E.A. Comer, S.J. Thomson, Anal. Chem. 65 (1993) 42.
[129] K. Takács-Novács, K.J. Box, A. Avdeef, Int. J. Pharm. 151 (1997) 235.
[130] A. Avdeef, K.J. Box, J.E.A. Comer, M. Gilges, M. Hadley, C. Hibbert, W. Patterson, K.Y. Tam, J. Pharm. Biomed. Anal. 20 (1999) 631.
[131] J. Barbosa, D. Barrón, E. Jiménez-Lozano, V. Sanz-Nebot, Anal. Chim. Acta 437 (2001) 309.
[132] M. Yasuda, Bull. Chem. Soc. Jpn. 32 (1959) 429.
[133] T. Shedlovsky, in: B. Pesce (Ed.), Electrolytes, Pergamon Press, New York, 1962.
[134] F.Z. Oumada, C. Ràfols, M. Rosés, E. Bosch, J. Pharm. Sci. 91 (2002) 991.