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Retention of ionizable compounds on high-performance liquid chromatography

III. Variation of pK values of acids and pH values of buffers in acetonitrile–water mobile phases

Elisabeth Bosch*, Sonia Espinosa, Martí Rosés*

Departament de Química Analítica, Universitat de Barcelona, Diagonal, 647, E-08028 Barcelona, Spain

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Abstract

A critical compilation of the literature pK data for acetonitrile–water is presented and equations and parameters that allow estimation of the pK of these compounds for any acetonitrile–water mixture up to 60% of acetonitrile by volume are proposed. The data and equations have been used to calculate the pH of different buffers, which have been prepared and used to calibrate a potentiometric system for several acetonitrile–water mixtures. The measured potential vs. pH plots follow the Nernst equation for all solvent mixtures after elimination of some outliers. This procedure allows us to identify the most reliable pK data and from them to propose buffered solutions of accurate pH. These solutions have been used for every-day calibration of the pH-meter in solute retention–mobile phase pH relationships. A model developed in earlier works has been used to relate the retention of benzoic acid in a C_{18} column with the measured pH of the mobile phase (60% acetonitrile). The results obtained demonstrate that the best relationships are obtained when the retention is related with the true pH of the buffer in the mobile phase, instead of the pH value of the buffer in water as it is usually done. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Mobile phase composition; pH values; Dissociation constants; Ionizable solutes; Benzoic acid

1. Introduction

The optimization of HPLC separations requires an accurate control of mobile phase parameters such as solvent composition and pH [1]. These two parameters are not independent because the pH value for an specific acid–base buffer varies with the solvent composition. Methanol–water and acetonitrile–water are the most used solvents in the preparation of mobile phases for reversed-phase liquid chromatog-

raphy. In a previous study, we presented equations that allow calculation of the pH value of some of the most used HPLC buffers for any methanol–water composition [2]. We also derived rigorous equations that relate the retention of a weak acid with the pH value of the buffer and solvent composition [3]. The use of the pH value of the buffer in the particular methanol–water mixture that constitutes the mobile phase gives better relationships with the solute retention than the traditional method that uses the pH value of the buffer in water.

There are many pK data of acids and bases in

*Corresponding authors.

methanol–water mixtures that can be used to prepare buffers of known pH value for HPLC separations or every-day pH-electrode calibrations [2,4]. However, the available p*K* data for acetonitrile–water mixtures is very limited [5–11]. In this article, we present a critical compilation of these data and propose equations to calculate the p*K* of the acid, and from this the pH of any buffer prepared from this acid, for any acetonitrile–water mixture up to 60% of acetonitrile by volume. The confidence in the p*K* and pH values for the acids and buffers is checked by potentiometric measurements of buffered solutions and this allows us to identify the most accurate pH reference buffers for calibration of potentiometric systems in acetonitrile–water. The better performance of pH measurements in the acetonitrile–water mobile phases in comparison with pH measurements in water is demonstrated for the retention of benzoic acid in a C₁₈ column.

2. Theory and analysis of literature data

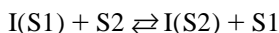
2.1. p*K* of acids in acetonitrile–water

There have been very few studies about the dissociation p*K* values of acids in acetonitrile–water mixtures. As far as we know, only seven references from different authors report p*K* data for neutral or anionic acids, and none for neutral bases [5–11]. The data have been obtained by different analytical techniques, such as potentiometry, conductometry and spectrophotometry and for diverse acetonitrile–water compositions up to a maximum mole fraction of acetonitrile of 0.637 [5]. Apparently there are no literature data for mixtures with higher contents in acetonitrile, except for pure acetonitrile, probably because of the solubility and homoconjugation problems of ionic solutes in acetonitrile-rich mixtures.

Since the p*K* data from different authors have been obtained for different acetonitrile–water mixtures, and the composition of these mixtures seldom agree with the desired mobile phase composition, it is necessary to fit the p*K* data to some equation that relates the p*K* with the solvent composition. The fitted equation will allow estimation of the p*K* value of the acid for any acetonitrile–water composition.

There are several models available for this purpose

that consider the solute–solvent and solvent–solvent interactions in binary solvent mixtures [2,12–15]. The simplest model is based on the solvent exchange process



where S1 and S2 are the two solvents that constitute the solvent mixture and I(S1) and I(S2) refer to the solute (I) solvated by these solvents. The constant of this process is the preferential solvation parameter $f_{2/1}$ that measures the tendency of the solute to be solvated by solvent S2 (acetonitrile) in reference to solvent S1 (water).

$$f_{2/1} = \frac{x_2^s/x_1^s}{x_2/x_1} \quad (1)$$

In Eq. (1), x_1^s and x_2^s are the mole fractions of solvents 1 and 2 solvating the solute [i.e. I(S1) and I(S2), respectively] and x_1 and x_2 the mole fractions of solvent S1 and S2 mixed.

The ΔG^0 of dissociation of the acid in the mixed solvent can be considered as an average of the ΔG^0 in pure solvents S1 and S2, according to the mole fractions of these solvents that solvate the acid (x_1^s and x_2^s). Since the acid–base p*K* is directly related with the ΔG^0 , we can write:

$$pK = x_1^s pK_{(S1)} + x_2^s pK_{(S2)} \quad (2)$$

where $pK_{(S1)}$ and $pK_{(S2)}$ are the acidity p*K* values of the acid in solvents S1 (water) and S2 (acetonitrile). Taken into account that the sum of the two mole fractions equals 1 and replacing Eq. (1) in Eq. (2), the following equation, which relates the p*K* value of the acid with the solvent composition (x_2), is obtained:

$$pK = \frac{(1 - x_2)pK_{(S1)} + x_2 f_{2/1} pK_{(S2)}}{1 - x_2 + x_2 f_{2/1}} \quad (3)$$

In fact, Eq. (3) is a simplified form of a more general equation [14,15] and its applicability is limited to some special cases. However, one of these cases is when the solute data (p*K*) cover a limited range of solvent compositions, such as for the p*K* data in acetonitrile–water mixtures. Because the lack

of pK data in acetonitrile-rich mixtures prevents application of more complex equations, Eq. (3) has been used to fit the pK data of acids in acetonitrile–water mixtures to solvent composition (x_2).

Some of the acids had been studied by two or even three different authors and the different sets of pK data do not always agree. We may observe three examples in Fig. 1. Whereas the pK data for acetic acid determined by Moreau [5] and Barbosa et al. [8] agree very well, the data presented by Azab et al. [6] show a different trend. The pK data obtained by Barbosa et al. [8] for hydrogenphthalate is also in very good agreement with the data from Rondinini and Nese [10]. However, the pK data for benzoic acid obtained by Azab et al. [6] and Niazi and Ali [7] do not agree at all. Because of these discrepancies the pK data obtained by each author were analyzed separately from the pK data for the same acid from other authors and the results for the different series are presented in Table 1.

In order to determine the reliability of the pK data for the different series, buffers of equimolar mixtures of the acids in Table 1 and their corresponding conjugate bases (sodium or potassium salt) were prepared in acetonitrile–water mixtures with 0, 10, 20, 30, 40, 50, and 60% of acetonitrile (v/v). The potential of these buffered solutions was measured and related to their calculated pH.

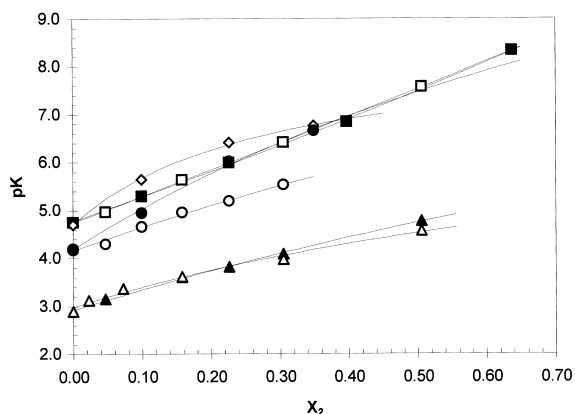


Fig. 1. Variation of pK values of acids with solvent composition in acetonitrile–water mixtures. Acetic acid: (□) Ref. [8], (●) Ref. [5], (◇) Ref. [6]. Benzoic acid: (○) Ref. [7], (●) Ref. [6]. Phthalic acid: (△) Ref. [8], (▲) Ref. [10].

2.2. pH calculation in acetonitrile–water mixtures

Eq. (3) and the parameters of Table 1 allow calculation of the pK value of the acids at any acetonitrile–water composition. The pH value of a buffer prepared from a particular acid in an acetonitrile–water mixture can be easily calculated from the pK value and the buffer composition.

The range of solvent composition studied covers up to a 60% in volume of acetonitrile (0.339 in mole fraction) and in this water-rich media, homoconjugation and ion pair formation can be neglected. Therefore, the acid–base equilibria is similar to that in water and the same procedures can be used to calculate the pH of a solution. The particular procedure used is similar to that described by De Levie [16] for acid–base titrations of arbitrary mixtures. The activity coefficients, however, must be considered for an accurate pH calculation because of the lower dielectric constant in acetonitrile–water than in pure water. Molar activity coefficients (γ) are calculated from the ionic strength (I) of the solution by using the Debye–Hückel equation:

$$\log \gamma = - \frac{Az^2\sqrt{I}}{1 + Ba_0\sqrt{I}} \quad (4)$$

where z is the charge of the ion and the Bates–Guggenheim convention assigns a constant value of 4.56 Å to the a_0 parameter [17,18]. A and B are the Debye–Hückel parameters, which can be calculated from the absolute temperature ($T=298.2$ K) and the dielectric constant of the medium through the equations

$$A = \frac{1.8246 \cdot 10^6}{(\epsilon T)^{3/2}} \quad (5)$$

$$B = \frac{50.29}{(\epsilon T)^{1/2}} \quad (6)$$

Analysis of the literature data [7,19,20] on dielectric constants of acetonitrile–water mixtures at 25°C shows that there is a good linear relationship between the reverse of the dielectric constant and the solvent composition in mole fraction of acetonitrile (x_2):

$$\epsilon^{-1} = 0.01305 + 0.01547 x_2 \quad r^2 = 0.9941$$

S.D. = 0.00039 (7)

Table 1
Coefficient values of Eq. (3) for the acids studied and reliability of the buffered solutions prepared from them

Acid	p <i>K</i> _(s1)	p <i>K</i> _(s2)	<i>f</i> _{2/1}	S.D.	<i>n</i>	<i>x</i> ₂ Range	Ref.	Sol.	S.D. ^a (mV)	Reliability
2,3-Dichloropropionic	2.29	7.34	1.23	0.00	4	0.099–0.637	[5]	B	6	Good
2,3-Dibromopropionic	2.19	7.26	1.49	0.07	5	0.000–0.637	[5]	C	4	Good
2-Nitrobenzoic	2.19	5.08	4.16	0.04	7	0.000–0.397	[7]	D	18	Bad
Chloroacetic	2.80	5.12	2.61	0.06	8	0.000–0.506	[11]	E	18	Bad
2-Chloropropionic	2.92	8.23	1.16	0.07	5	0.000–0.637	[5]	F	4	Good
Citric (p <i>K</i> ₁)	3.16	6.30	1.36	0.04	6	0.000–0.506	[8]	G	2	Good
3-Nitrobenzoic	3.47	7.39	0.93	0.09	6	0.000–0.305	[7]	H	32	Very bad
4-Nitrobenzoic	3.41	7.54	0.89	0.08	6	0.000–0.305	[7]	I	33	Very bad
3-Bromopropionic	4.03	9.34	1.19	0.09	5	0.000–0.637	[5]	J	14	Fair
Tartaric (p <i>K</i> ₁)	3.06	7.25	0.98	0.03	6	0.000–0.506	[8]	–	–	Unknown
Tartaric (p <i>K</i> ₂)	4.38	9.10	0.80	0.03	6	0.000–0.506	[8]	K	11	Fair
Benzoic	4.18	9.29	1.80	0.11	4	0.000–0.349	[6]	L	9	Fair
	4.15	7.64	1.49	0.07	6	0.000–0.305	[7]		31	Very bad
Citric (p <i>K</i> ₂)	4.79	9.21	0.99	0.04	6	0.000–0.506	[8]	M	4	Good
Cinnamic	4.57	24.50	0.22	0.25	4	0.000–0.349	[6]	N	11	Fair
Acetic	4.77	10.73	0.84	0.05	5	0.000–0.637	[5]	O	3	Good
	4.70	7.64	4.50	0.05	4	0.000–0.349	[6]		18	Bad
	4.74	10.48	0.95	0.02	6	0.000–0.506	[8]		1	Good
Propionic	4.90	10.84	0.95	0.06	5	0.000–0.637	[5]	P	5	Good
Valeric	4.82	9.48	1.85	0.07	4	0.000–0.349	[6]	Q	2	Good
Isobutyric	4.87	10.04	1.98	0.09	4	0.000–0.349	[6]	R	16	Bad
Phthalic (p <i>K</i> ₁)	2.92	6.02	1.42	0.03	6	0.000–0.506	[8]	–	–	Unknown
	2.98	5.32	1.95	0.09	6	0.000–0.506	[10]		–	Unknown
Phthalic (p <i>K</i> ₂)	5.39	11.17	1.67	0.08	6	0.000–0.506	[8]	S	4	Good
Citric (p <i>K</i> ₃)	6.42	10.61	1.34	0.04	6	0.000–0.506	[8]	T	6	Good
Phosphoric (p <i>K</i> ₂)	7.23	11.19	1.19	0.06	6	0.000–0.506	[9]	U	4	Good
Boric	9.20	12.83	2.72	0.06	6	0.000–0.506	[8]	V	6	Good

^a Calculated from Eq. (9).

which can be used to calculate the dielectric constant for any solvent mixture.

The calculation of the pH value of particular buffers allows calibration of potentiometric systems. The relationship between measured potential and pH for a set of buffered solutions should follow the Nernst equation:

$$E = E^0 - g \text{pH} \quad (8)$$

where E^0 includes the standard and junction (assumed to be constant) potentials and g is the Nernst constant (59.16 mV at 25°C).

3. Experimental

3.1. Apparatus

Potentiometric measurements were taken with a

Ross Combination Electrode Orion 8102 in a Crison micropH 2002 potentiometer with a precision of ± 0.1 mV. The retention data were measured on a 25 cm \times 4.0 mm I.D. Merck LiChrospher 100 RP-18 column (5 μ m) with a flow of 1 ml min⁻¹ in an ISCO (Lincoln, NE, USA) Model 2350 dual-pump system with a 10- μ l loop valve and a variable-wavelength V⁴ absorbance detector (ISCO) set at 254 nm for benzoic acid and 210 nm for the hold-up time marker potassium bromide (0.01%). All data was taken by triplicate at 25°C with the potentiometric cell and column thermostated with a water jacket.

3.2. Chemicals

Acetonitrile was HPLC ultra gradient grade from Baker and water purified by the Milli-Q plus system from Millipore. Other chemicals were reagent grade

or better and obtained from Fluka, Merck or Carlo Erba.

3.3. Procedure

For the potentiometric measurements in acetonitrile–water mixtures, the buffers were prepared at 0, 10, 20, 30, 40, 50, and 60% of acetonitrile by volume. Hydrochloric acid and potassium hydroxide buffers were prepared by dilution of concentrated aqueous solutions with acetonitrile and water. All other buffers were prepared at equal concentrations of acid and base by solving the appropriate amounts of acid and conjugated base in the acetonitrile–water mixture. If the conjugated base of the acid was not commercially available, a concentrated solution of the acid was prepared in water (or in a water–acetonitrile mixture if it is not soluble in water), half-neutralized with potassium hydroxide, and diluted with the appropriate volume of acetonitrile (or water). The composition of the different buffers prepared is given in Table 2. In general, the concentration of the acid and the base has been 0.05 *M*, but because of limited solubility the concentration is lower for some buffers in some solvent mixtures.

4. Results and discussion

4.1. pH of buffers in acetonitrile–water

The potential of the studied buffered solutions of Table 2 has been measured and related to the calculated pH through Eq. (8). The pH value of the solution has been computed from the buffer composition and the p*K* value of the acid. The p*K* of the acid at each solvent composition has been estimated from Eq. (3) and the parameters of Table 1. Since the p*K* of some acids was determined by more than one author, several different pH values were obtained in some instances for the same buffered solution. For example, the acetic–acetate solution (solution O in Table 2) led to only one measured potential value for each solvent composition, but to three different pH values calculated from the p*K* data of Refs. [5,6,8]. Since a priori the most correct pH value is not known, the different pH values for the

same solution were considered as different points in the correlation of *E* with pH.

The results obtained in these correlations are presented in Table 3 for each solvent composition. From these correlations, the most outstanding outliers (± 2.5 S.D. in at least one solvent composition) were identified and removed from the correlations. The outliers removed have been all data obtained from Refs. [7,11] and the data for acetic and isobutyric acids from Ref. [6]. The improved correlations are also presented in Table 3 with a minor number of points (*n*). One graphical example of the correlations obtained is presented in Fig. 2 for 60% of acetonitrile, including the outliers.

Table 3 shows that the effect of the outliers increases with the percentage of acetonitrile in the mixture. For pure water, removal of the outliers practically does not have any effect on the correlation. However, for 60% of acetonitrile the S.D. decreases to a half part after removal of the outliers. It may be also observed that very good correlations are obtained, with slopes close to the theoretical value of 59.16 mV, when the outliers are removed.

For a better identification of the outliers we present in Fig. 3 the variation of the differences between the measured potentials and those calculated from Eq. (8) and the parameters of Table 3, after removing the most important outliers. Fig. 3 shows that the scatter of the differences increase with the percentage of acetonitrile because of the decrease of the precision of measurements (both p*K* determination and potential readings) when the organic solvent contents increase. Even so, it may be easily noticed that the largest differences correspond to the data obtained from Refs. [7,11] and to some data from Ref. [6] (acetic and isobutyric acids). This can be also observed in Table 1 where we have included the overall S.D.s for each buffer and author over the full range of solvent compositions, obtained from:

$$\text{S.D.} = \sqrt{\frac{\sum (E_{\text{exp}} - E_{\text{cal}})^2}{n - 1}} \quad (9)$$

where E_{exp} and E_{cal} are the experimentally measured and calculated from Eq. (8) potentials, respectively, and *n* the number of solvent mixtures measured for each buffer.

Table 1 indicates that the most reliable p*K* data

Table 2

Mixtures of acids and their conjugate bases (potassium or sodium salts) studied as buffered solutions in acetonitrile–water mixtures

Solution	Acid	C_a (M)	C_b (M)	% MeCN (v/v)
A1	Hydrochloric acid	0.1	–	0–60
A2	Hydrochloric acid	0.05	–	0–60
A3	Hydrochloric acid	0.01	–	0–60
A4	Hydrochloric acid	0.005	–	0–60
A5	Hydrochloric acid	0.001	–	0–60
B	2,3-Dichloropropionic acid	0.05	0.05	0–60
C	2,3-Dibromopropionic acid	0.05	0.05	0–60
D	2-Nitrobenzoic acid	0.05	0.05	10–60
E	Chloroacetic acid	0.05	0.05	0–60
F	2-Chloropropionic acid	0.05	0.05	0–60
G	Citric acid	0.05	0.05	0–60
H	3-Nitrobenzoic acid	0.05	0.05	10–60
I	4-Nitrobenzoic acid	0.0025	0.0025	10
		0.005	0.005	20–30
		0.01	0.01	40–60
J	3-Bromopropionic acid	0.05	0.05	0–60
K	Hydrogen tartrate	0.014	0.014	0–10
		0.01	0.01	20–30
		0.008	0.008	40
		0.007	0.007	50
		0.003	0.003	60
L	Benzoic acid	0.05	0.05	10–60
M	Dihydrogen citrate	0.05	0.05	0–50
		0.025	0.025	60
N	Cinnamic acid	0.005	0.005	10
		0.01	0.01	20
		0.02	0.02	30–60
O	Acetic acid	0.05	0.05	0–60
P	Propionic acid	0.05	0.05	0–60
Q	Valeric acid	0.05	0.05	0–60
R	Isobutyric acid	0.05	0.05	0–60
S	Hydrogen phthalate	0.05	0.05	0–60
T	Hydrogen citrate	0.05	0.05	0–50
		0.0125	0.0125	60
U	Dihydrogen phosphate	0.05	0.05	0–50
		0.025	0.025	60
V	Boric acid	0.05	0.05	0–50
		0.025	0.025	60
W5	Water ^a	–	0.001	0–60
W4	Water ^a	–	0.005	0–60
W3	Water ^a	–	0.01	0–60
W2	Water ^a	–	0.05	0–60
W1	Water ^a	–	0.1	0–60

^a Potassium hydroxide as conjugate base.

for preparation of buffers of known pH in acetonitrile–water are those obtained by Moreau [5] and by Barbosa and coworkers [8,9]. The less accurate pK data is that obtained by Niazi and Ali [7,11]. We

believe the reason for the poor accuracy of the data from these authors is that pK values were simultaneously computed with limiting molar conductances (Λ_0) of the acids from conductometric mea-

Table 3

Fits of the Nernst equation (Eq. (8)) for the buffered solutions of Table 2 in different acetonitrile–water mixtures for the whole data and after removal of some outliers (see Section 4.1)

% MeCN	With outliers					Without outliers				
	E^0 (mV)	g	S.D.	r^2	n	E^0 (mV)	g	S.D.	r^2	n
0	357.2	58.9	1.9	0.9999	28	357.6	58.9	1.7	0.9999	26
10	363.4	58.9	4.4	0.9995	34	364.7	59.0	3.5	0.9997	28
20	361.0	58.7	7.5	0.9986	34	363.6	58.9	4.4	0.9996	28
30	360.5	58.5	10.1	0.9976	34	364.1	58.9	6.7	0.9991	28
40	362.4	58.2	12.3	0.9965	34	368.3	58.7	7.8	0.9988	28
50	363.6	57.7	13.5	0.9959	34	371.2	58.3	8.1	0.9988	28
60	369.9	57.4	16.7	0.9939	34	381.8	58.4	8.4	0.9987	28

surements. A_0 is calculated by extrapolation of conductance measurements to zero concentration. The weaker the acid, the lower the conductance and the accuracy in the A_0 . Since pK is calculated simultaneously with A_0 it has low accuracy too. This would explain why the accuracy of that pK data decreases with the contents in acetonitrile, since the dissociation of the acid decreases too. We have encountered the same problem with pK data of the same authors for tetrahydrofuran–water mixtures [21], where we suggested A_0 to be calculated independently of the pK of a weak acid from the A_0

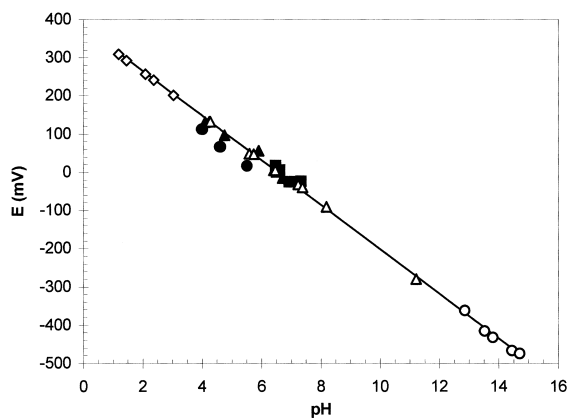


Fig. 2. Plot of measured potential against pH value in 60% acetonitrile for the buffered solutions presented in Table 1. The pH value of the buffered solutions has been calculated from the pK data of references: (▲) [5], (■) [6], (●) [7] and [11], (△) [8] and [9], (◇) HCl solutions (A1–A5) and (○) KOH solutions (W1–W5).

value of a salt of the acid and the A_0 values of a strong acid and its corresponding salt.

From the results presented in Table 1 and Fig. 3, we have deduced the reliability of the parameters of Table 1 for pK estimation of acids and preparation of buffers of known pH. This is indicated in the last column of Table 1. The pK data used to prepare buffers which show an overall S.D. about 6 mV (0.1 pH units) or lower have been rated as good. If the S.D. is between 6 and 12 mV (0.2 pH units), the data is rated as fair. When the S.D. is between 12 and 30 mV (0.5 pH units), the data is considered to be bad and if the S.D. exceeds 30 mV, very bad. The S.D. values for the solutions prepared with strong acids (solutions A1–A5) and strong bases (solutions W1–

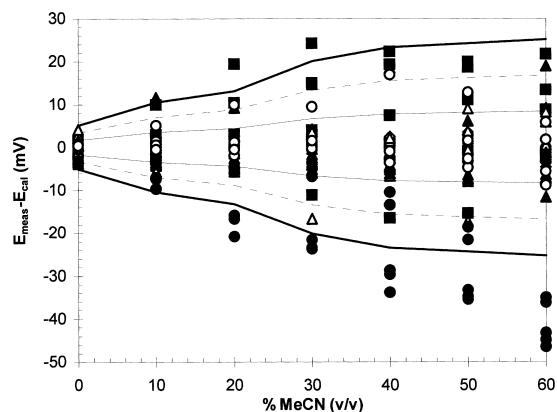


Fig. 3. Differences between measured and calculated (Table 3, Eq. (8)) potentials for the buffered solutions of Table 1. Symbols as in Fig. 2. — $\pm 1S.D.$, - - - $\pm 2S.D.$, ··· $\pm 3S.D.$

W5) are not included in Table 1, but they are lower than 5 mV except for the most diluted KOH solution (W5) which is 11 mV. Therefore, reliable buffers can be also prepared from strong acids (HCl) and bases (KOH).

4.2. Relationships between HPLC retention of acids and mobile phase pH

Identification of the reliable pH buffers in acetonitrile–water allows standardization of potentiometric systems and exact measurement of pH. In particular, we have used the citric–dihydrogencitrate, acetic–acetate and dihydrogenphosphate–hydrogenphosphate (solutions G, O and U, respectively, in Table 2) buffers to standardize a potentiometric system in 60% acetonitrile. After standardization, we have measured the pH values of several HPLC buffers, previously used in methanol–water [3], in this mobile phase and related the measured pH to the retention of benzoic acid in a C₁₈ column using those buffers.

The pH values of the buffers in pure water (0% acetonitrile) and 60% acetonitrile and the retention times of benzoic acid and the hold-up time marker potassium bromide (0.01%) are presented in Table 4.

Most authors (e.g. [22–27]) relate the retention of the ionizable compound (benzoic acid) to the pH value of the buffer in water, instead of relating it to the pH value in the mobile phase used (60% acetonitrile in this instance). Table 4 shows that the

difference between pH in water and in 60% acetonitrile is buffer dependent, e.g., buffers III and IV have almost the same pH value in water, but differ in about half pH unit in 60% acetonitrile. Also, the pH order of buffers VIII, IX and X is reversed in 60% acetonitrile in reference to the order in water. This is caused because the variation of the pK values of the acids that compose the different buffers with the acetonitrile contents is different for each acid. The behaviour is typical for organic solvent–water mixtures and has already been reported for methanol–water mobile phases [2,3].

The general model most widely used for the influence of ionization on the retention of weak acids [3,22–28], considers the observed retention factor k (or retention time t_R , or adjusted retention time t'_R) as an average of the corresponding values of the neutral (k_{HA} , $t_{R(HA)}$, or $t'_{R(HA)}$) and ionic (k_{A^-} , $t_{R(A^-)}$, or $t'_{R(A^-)}$) forms of the solute according to the fractions of these forms in the mixture

$$k = (1 - \alpha)k_{HA} + \alpha k_{A^-} \quad (10a)$$

$$t_R = (1 - \alpha)t_{R(HA)} + \alpha t_{R(A^-)} \quad (10b)$$

$$t'_R = (1 - \alpha)t'_{R(HA)} + \alpha t'_{R(A^-)} \quad (10c)$$

where α is the degree of dissociation of the acid, which depends on the pH of the mobile phase (usually taken as pH in water) and the pK_a of the acid (also in water).

Table 4
pH values of buffers in water (0%) and in 60% acetonitrile and retention times of benzoic acid (t_R) and potassium bromide ($t_{0(A^-)}$) in a C₁₈ column and 60% acetonitrile as a mobile phase

Buffer		pH		t_R (min)	
		0%	60%	t_R	$t_{0(A^-)}$
I	0.01 M H ₃ PO ₄	2.26	3.20	3.34	1.83
II	0.005 M H ₃ Cit–0.005 M KH ₂ Cit	3.10	4.50	3.35	1.92
III	0.001 M H ₃ PO ₄ –0.009 M KH ₂ PO ₄	3.34	4.72	3.35	2.00
IV	0.01 M HAc	3.39	5.25	3.34	1.72
V	0.009 M HAc–0.001 M NaAc	3.86	5.87	3.11	1.75
VI	0.005 M KH ₂ Cit–0.005 M KNaHCit	4.60	6.22	2.90	2.04
VII	0.005 M HAc–0.005 M NaAc	4.74	6.63	2.44	1.86
VIII	0.009 M KH ₂ PO ₄ –0.001 M Na ₂ HPO ₄	6.09	7.68	2.11	1.95
IX	0.005 M KNaHCit–0.005 M Na ₃ Cit	6.02	7.71	2.12	2.09
X	0.001 M HAc–0.009 M NaAc	5.68	7.75	2.03	1.91
XI	0.005 M KH ₂ PO ₄ –0.005 M Na ₂ HPO ₄	7.01	8.51	1.95	1.94

Ac = Acetate, Cit = Citrate.

$$\alpha = \frac{[A^-]}{[HA] + [A^-]} = \frac{K_a}{[H^+] + K_a}$$

$$= \frac{1}{10^{(pK_a - pH)} + 1} \quad (11)$$

We have proposed a more rigorous model [3] that modifies the equations in three points:

1. It uses the pH and pK_a values in the organic solvent used as mobile phase, instead of the values in water.
2. Takes into account the activity coefficients (y) in the definition of the degree of dissociation

$$\alpha = \frac{[A^-]}{[HA] + [A^-]} = \frac{K_a}{[H^+]y^2 + K_a}$$

$$= \frac{1}{y10^{(pK_a - pH)} + 1} \quad (12)$$

3. It considers different hold-up times for the neutral ($t_{0(HA)}$) and ionic ($t_{0(A^-)}$) forms of the acid. Because of ion-exclusion effects, the volume of stationary phase available to neutral and ionic compounds is different, and for ionic compounds it changes with the ionic buffer composition.

$$t_{R(A^-)} = t_{R(A^-)}^F - t_{0(A^-)} \quad (13)$$

$$t'_{R(HA)} = t_{R(HA)} - t_{0(HA)} \quad (14)$$

where $t'_{R(A^-)}$ and $t'_{R(HA)}$ are the adjusted retention times of the ionized and neutral forms of the acid, respectively, and $t_{R(A^-)}$ and $t_{R(HA)}$ the retention times of the same species. $t_{0(A^-)}$ is measured with 0.01% potassium bromide in each buffer (see Table 4) and $t_{0(HA)}$ is measured with potassium bromide in an unbuffered 60% acetonitrile mobile phase (1.67 min in this work) [3].

Fig. 4 compares the results obtained for benzoic acid (data in Table 4) using the traditional model (pH in water, no activity coefficients and the same hold-up time for all buffers and species) with the results obtained using the model we propose (pH in 60% acetonitrile, activity coefficients and different hold-up times). It may be observed that the fit is better when the pH in 60% acetonitrile is used. The numerical results obtained for benzoic acid in the HPLC system studied when the traditional model is used are $t'_{R(HA)} = 1.71$ min, $t'_{R(A^-)} = 0.33$ min, $pK_a =$

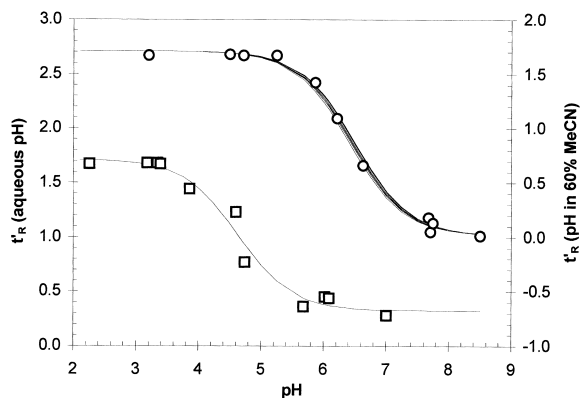


Fig. 4. Variation of the adjusted retention time of benzoic acid with the pH of the mobile phase in 60% acetonitrile: (□) pH of the aqueous buffer before mixing it with acetonitrile (left hand ordinate), (○) true pH of the buffer in the 60% acetonitrile mobile phase (right hand ordinate).

4.63 and S.D.=0.11 min. And for the proposed rigorous model, $t'_{R(HA)} = 1.71$ min, $t'_{R(A^-)} = 0.02$ min, $pK_a = 6.51$ and S.D.=0.05 min. These results confirm that the fit of the rigorous model is better and also show the effect of the different modifications on the traditional model. Both models give the same adjusted retention time for the neutral species because they use the same hold-up time for this species (1.67 min), but they give different adjusted retention times for the ionic species. The traditional model gives an appreciable retention of the benzoate ion (0.33 min) because it uses the same hold-up time as for the neutral acid (1.67 min). However, the rigorous model predicts that the ionic species is practically not retained (0.02 min) because it uses an ionic solute (KBr) as hold-up marker in the same studied buffers. The pK_a values obtained with both models are also different. An estimation of the pK_a values of benzoic acid by means of the most reliable data in Table 1 (data from Ref. [6]) gives 4.18 and 6.63 for water and 60% acetonitrile, respectively. The traditional model gives a value of 4.63, that does not agree with the value in 60% acetonitrile, nor with the value in water. In fact, the application of the traditional model only leads to an estimate of inflection point on the k vs. pH curve. The location of this point is influenced by two effects: change of pK of buffer constituents and change of pK value of

the analyte, both as a consequence of the presence of organic solvent in the mobile phase [27].

The rigorous model gives a value of 6.51, quite close to the value estimated for the mobile phase used (60% acetonitrile). The effect of taking into account the activity coefficients is very small and it may be observed in Fig. 4, which shows a set of close lines, one for each buffer, when they are considered.

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