

Retention of ionizable compounds in high-performance liquid chromatography

14. Acid–base pK values in acetonitrile–water mobile phases

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Abstract

Linear relationships between ${}^s pK_a$ values in acetonitrile–water mixtures and ${}^w pK_a$ values in pure water have been established for five families of compounds: aliphatic carboxylic acids, aromatic carboxylic acids, phenols, amines, and pyridines. The parameters (slope and intercept) of the linear correlations have been related with acetonitrile–water composition. The proposed equations allow accurate estimation of the pK_a values of any member of the studied families at any acetonitrile–water composition up to 60% of acetonitrile in volume (100% for pyridines). Conversely, the same equations can be used to estimate aqueous pK_a values from chromatographic pK_a values obtained from any acetonitrile–water mobile phase between the composition range studied. Estimation of pK_a values have been tested with chromatographic literature data. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

In previous works, the importance of proper pH measurement in liquid chromatography mobile phases has been highlighted [1–8]. Measurement of the pH in the mobile phase after mixing aqueous buffer and organic modifier has been recommended [3–8]. This procedure provides fits of analyte retention to mobile phase pH much better than those obtained when the pH is measured in the aqueous buffer before mixing it with the organic modifier. In

addition, the pK_a value obtained from the fits to pH values measured after mixing is the thermodynamic pK_a value of the analyte in the mixed solvent used as mobile phase. This is a significant advantage over pK_a parameters obtained from pH measurements before mixing, which are only fitting parameters without any physical meaning [9].

With proper measurement of mobile phase pH, known thermodynamic pK_a values can be used to predict ionization of the analytes in the mobile phase and thus, retention in the chromatographic system. These pK_a values refer always to the solvent system used as mobile phase, not to the pK_a values in water. This is an important restriction to practical applicability of the method because the number of

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known pK_a values in water–organic mobile phases is very limited. However, in previous studies [10,11] we established linear relationships between pK_a values in methanol–water mixtures and pK_a values in water for several families of compounds. These relationships provide a good estimation of the pK_a value of the compound in any methanol–water mixture from the pK_a value of the compound in water, which can be used to predict the ionization of the compound in a chromatographic system that uses pH buffered methanol–water mobile phases.

In this paper, we extend these relationships to acetonitrile–water mobile phases. Methanol–water and acetonitrile–water solvent mixtures are by far the most used mobile phases in liquid chromatography. The relationships between pK_a values established for these two solvent systems can be extremely useful for prediction of retention and optimization of chromatographic separations of acid–base compounds. Additionally, they can be used for determination of the aqueous pK_a value from the fitting pK_a value obtained by measurement of retention at several mobile phase pH values. This method has been recently used to estimate the aqueous pK_a values of water insoluble arilpropionic acids with antiinflammatory properties from the chromatographic pK_a values in methanol–water mobile phases [12].

2. Theory

The approach followed is based in the linear relationship proposed by Chantooni and Kolthoff [13] to measure the “resolution of acid strength” of a family of compounds in a solvent (s) in reference to water (w). In this approach, the pK_a values of a series of related compounds (commonly compounds that share the same charge and functional acidic group) in the solvent s (${}^s pK_a$) are plotted against the pK_a values of the same compounds in water (${}^w pK_a$). A straight line (Eq. (1)) is usually obtained with a slope value (a_s) that measures the “resolution of acid strength” for the compounds in solvent s as regards to water (slope unity), i.e. the ability of the solvent to differentiate between the acidities of the compound’s set

$${}^s pK_a = a_s {}^w pK_a + b_s \quad (1)$$

The intercept of the correlation (b_s) is related to the differences in basicities, dielectric constants, and specific solvation interactions of the solute (e.g. hydrogen bonding) between solvent s and water. The first two differences depend only on the solvents considered (s and w), but the specific solvation differences depend also on the family of compounds studied. The slope of the correlation (a_s) is related to differences between specific solvation interactions, which depend 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. Detailed explanations about theoretical derivation of Eq. (1) have been given in previous studies [10,11,14].

We shall use here the same notation recommended by the IUPAC [15] for pK and pH definition we have used in previous works. A lower-case left-hand superscript in pH or pK term indicates the solvent (w or s) in which measurements are being made; a lower-case left-hand subscript indicates the solvent in which the ionic activity coefficient γ is referred to unity at infinite dilution (w or s). Notice that the possibility of choosing two different standard state solvents for the ionic activity coefficients leads to two different scales for pH measurement in non-aqueous or mixed solvents [16]. The ${}^s pH$ scale refers to pH measured in solvent s with pH standardization in the same solvent s, and the ${}^s_w pH$ scale refers to pH measurement in solvent s with pH standardization with aqueous reference buffers. ${}^w pH$ scale refers to pH measurement in water.

Linear relationships for resolution of acid strength have been well established for the pK values of families of compounds in different solvents in reference to the pK values in water [10,11,14,17–23]. In two previous studies, we applied them to the available ${}^s pK_a$ data in methanol–water mobile phases [10,11]. The ${}^s pK_a$ values of 121 acid–base compounds belonging to six different chemical families in several methanol–water compositions were fitted to Eq. (1) 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 (v_{MeOH}), the equations take the forms:

$$a_s = \frac{1 + a_1 v_{\text{MeOH}} + a_2 v_{\text{MeOH}}^2}{1 + a_3 v_{\text{MeOH}} + a_4 v_{\text{MeOH}}^2} \quad (2)$$

$$b_s = \frac{b_1 v_{\text{MeOH}} + b_2 v_{\text{MeOH}}^2}{1 + b_3 v_{\text{MeOH}} + b_4 v_{\text{MeOH}}^2} \quad (3)$$

where a_1 , a_2 , a_3 , a_4 , b_1 , b_2 , b_3 , and b_4 were fitting parameters constant for all acids of the same family at all methanol–water mixtures.

This approach shall be applied here to the available data for acetonitrile–water mixtures in order to establish equations for the different families of compounds, which should allow an accurate estimation of the compound $\text{p}K_a$ value at a given acetonitrile–water mobile phase from the $\text{p}K_a$ value of the compound in water.

3. Results and discussion

3.1. $\text{p}K_a$ values in pure acetonitrile

The resolution of acid strength for the families of compounds studied was first investigated in pure acetonitrile. The available literature ${}^s\text{p}K_a$ values in pure acetonitrile [24–26] were collected (Table 1) and plotted against its ${}^w\text{p}K_a$ values in water [27,28]. Fig. 1 depicts the plots obtained. It is evident that the compounds can be divided in five families that follow five different straight lines, with only four outliers of the 101 pairs of $\text{p}K_a$ data points plotted. These five families are aromatic carboxylic acids, aliphatic carboxylic acids, phenols, pyridines, and amines. The parameters of the straight lines obtained are given in Table 2.

All families present a resolution of acid strength in acetonitrile larger than in water ($a_s > 1$). This behaviour is usual in solvents with a poor hydrogen bond donor acidity, such as acetonitrile. In a good hydrogen bond donor solvent, e.g. water, the negative charge of the anion (carboxylic acids and phenol families) or the lone electron pair of the neutral base (amines and pyridines) are stabilized in the oxygen or nitrogen atom of the acid–base group by hydrogen bonding. However, in a poor hydrogen bond donor solvent, the negative charge or lone electron pair can be easily delocalized along the structure of the

molecule. In this instance the effect of the substituents in the molecule is more important and produces a larger variation of the acid–base strength ($\text{p}K_a$ values) [10,11,13,14].

The families with a larger resolution of acid strength are aliphatic carboxylic acids and phenols, whereas aromatic carboxylic acids and the protonated bases, amines and pyridines present a lower resolution of acid strength. In contrast with pure methanol [14] and other alcohols [17,19], *ortho* effect (variation of the resolution of acid strength caused by substituents in *ortho* position) was not observed in acetonitrile.

3.2. Acetonitrile–water mobile phases

The same compound families investigated in pure acetonitrile were studied in acetonitrile–water mixtures. The available ${}^s\text{p}K_a$ data in acetonitrile–water mixtures were compiled for round percentages of acetonitrile in volume and they are given in Table 3. Most data were taken from a previous compilation [29]. The equations given in that study for each compound were used to calculate the ${}^s\text{p}K_a$ value of each compound at each round percentage of acetonitrile. The early compilation for neutral acids was complemented with the ${}^s\text{p}K_a$ data of some amines [31] and substituted pyridines [32]. The ${}^s\text{p}K_a$ data for these substituted pyridines were determined in the molality scale at a constant ionic strength of 0.01 mol kg⁻¹. These ${}^s\text{p}K_a$ data were converted to thermodynamic ${}^s\text{p}K_a$ values (zero ionic strength) in the molarity scale used through all this work by using the densities and Debye–Hückel parameters for acetonitrile–water mixtures given elsewhere [4]. Finally, the linear equations proposed in a previous study [6] and the cubic equations given by Sarmini and Kendler [30] to relate the ${}^w\text{p}K_a$ values of some compounds to acetonitrile composition were used to calculate the ${}^w\text{p}K_a$ of these compounds at the round acetonitrile percentages. ${}^s\text{p}K_a$ values of the compounds were calculated from the ${}^w\text{p}K_a$ values and the δ parameter of the corresponding acetonitrile–water mixture (determined in a previous work [4]) by means of the following relationship:

$${}^s\text{p}K_a = {}^w\text{p}K_a - \delta \quad (4)$$

The a_s and b_s parameters of the correlations

Table 1
Acid–base pK_a values of compounds in pure water and pure acetonitrile

Acid	H ₂ O w_pK_a	MeCN s_pK_a	Ref.	Acid	H ₂ O w_pK_a	MeCN s_pK_a	Ref.
Aliphatic carboxylic acids				Amines			
Trifluoroacetic acid	0.23	12.7	[24]	2-Nitroaniline	2.17	4.9	[24]
Trichloroacetic acid	0.63	10.6	[24]	3-Nitroaniline	3.49	7.6	[24]
Oxalic acid (pK_1)	1.27	14.5	[24]	Aniline	4.61	10.6	[25]
Dichloroacetic acid	1.33	13.2	[24]	<i>p</i> -Toluidine	5.08	11.3	[24]
Dichloroacetic acid	1.33	15.8 ^b	[25]	Benzylamine	9.33	16.8	[24]
Cyanoacetic acid	2.46	18.0	[24]	Trimethylamine	9.81	17.6	[24]
Malonic acid (pK_1)	2.85	15.3	[24]	1,5-Pentanediamine (pK_1)	10.25	19.1	[24]
Chloroacetic acid	2.87	18.8	[25]	1,3-Propanediamine (pK_1)	10.30	19.7	[24]
Fumaric acid (pK_1)	3.02	18.6	[24]	Isobutylamine	10.43	17.9	[24]
Tartaric acid (pK_1)	3.04	14.9	[24]	Butylamine	10.61	18.3	[25]
Glycolic acid (pK_1)	3.83	19.3	[24]	Methylamine	10.62	18.4	[24]
Succinic acid (pK_1)	4.16	17.6	[24]	Propylamine	10.69	18.2	[24]
Glutaric acid (pK_1)	4.34	19.2	[24]	Ethylamine	10.70	18.4	[25]
Adipic acid (pK_1)	4.43	20.3	[24]	Dimethylamine	10.73	18.7	[24]
Nonanedioic acid (pK_1)	4.53	20.9	[24]	Triethylamine	10.78	18.7	[24]
Acetic acid	4.76	22.3	[25]	Triethylamine	10.78	18.5	[25]
Butyric acid	4.82	22.7	[24]	1,4-Butanediamine (pK_1)	10.80	20.1	[24]
Aromatic carboxylic acids				Tributylamine	10.90	18.1	[25]
2,6-Dinitrobenzoic acid	1.14	15.8	[24]	Diethylamine	10.98	18.8	[25]
2,6-Dihydroxybenzoic acid	1.22	12.6 ^b	[24]	Dibutylamine	11.30	18.3	[25]
2,4-Dinitrobenzoic acid	1.42	16.1	[24]	Pyridines			
2-Nitrobenzoic acid	2.22	18.2	[24]	2-Chloropyridine	0.49	6.8	[26]
2,4-Dichlorobenzoic acid	2.72	18.4	[24]	2-Bromopyridine	0.71	7.0	[26]
3,5-Dinitrobenzoic acid	2.82	17.2	[24]	2-Hydroxypyridine	1.25	8.3	[26]
3,5-Dinitrobenzoic acid	2.82	16.9	[25]	3-Cyanopyridine	1.38	8.0	[26]
2-Chlorobenzoic acid	2.94	19.0	[24]	4-Cyanopyridine	1.90	8.5	[26]
<i>o</i> -Phthalic acid (pK_1)	2.95	14.3 ^b	[24]	2-Acetylpiperidine	2.76	9.6	[26]
2-Hydroxybenzoic acid	2.98	16.9 ^b	[24]	3-Bromopyridine	2.84	9.5	[26]
4-Nitrobenzoic acid	3.43	18.7	[25]	3-Chloropyridine	2.84	10.0	[26]
2,4,6-Trimethylbenzoic acid	3.44	20.5	[24]	3-Acetylpiperidine	3.55	10.8	[26]
3-Nitrobenzoic acid	3.49	19.2	[24]	3-Hydroxypyridine	4.75	12.6	[26]
3,4-Dichlorobenzoic acid	3.60	19.0	[25]	Pyridine	5.17	12.6	[26]
<i>m</i> -Phthalic acid (pK_1)	3.62	19.3	[24]	Pyridine	5.17	12.3	[25]
3-Bromobenzoic acid	3.82	19.5	[24]	3-Methylpyridine	5.58	13.7	[26]
3-Bromobenzoic acid	3.82	20.3	[25]	4-Ethylpyridine	5.87	13.6	[25]
4-Bromobenzoic acid	3.97	20.3	[24]	2-Methylpyridine	5.91	13.9	[26]
Benzoic acid	4.21	20.1	[24]	4-Methylpyridine	5.93	14.5	[26]
Benzoic acid	4.21	20.7	[25]	3-Aminopyridine	6.03	14.4	[26]
3,4-Dimethylbenzoic acid	4.41	21.2	[25]	3,5-Dimethylpyridine	6.15	13.9	[26]
4-Hydroxybenzoic acid	4.58	20.8	[24]	3,4-Dimethylpyridine	6.47	14.7	[26]
Phenols				2,3-Dimethylpyridine	6.57	14.8	[26]
2,4,6-Trinitrophenol	0.65	11.0	[25]	2-Aminopyridine	6.66	14.7	[26]
4-Chloro-2,6-dinitrophenol	2.97	15.0	[24]	2,6-Dimethylpyridine	6.68	14.4	[26]
2,6-Dinitrophenol	3.71	16.0	[24]	2,4-Dimethylpyridine	6.70	15.0	[26]
2,4-Dinitrophenol	4.02	16.0	[24]	4-Aminopyridine	9.06	18.4	[26]
3,4-Dinitrophenol	5.42	17.9	[24]				
4-Nitrophenol	7.16	20.7	[24]				

Table 1. Continued

Acid	H ₂ O ^w pK _a ^a	MeCN ^s pK _a	Ref.	Acid	H ₂ O ^w pK _a ^a	MeCN ^s pK _a	Ref.
2-Nitrophenol	7.24	22.0	[24]				
4-Cyanophenol	7.95	22.7	[24]				
3-Nitrophenol	8.39	23.8	[24]				
2-Bromophenol	8.42	23.9	[24]				
3,4-Dichlorophenol	8.63	24.0	[24]				
3-Chlorophenol	9.08	25.0	[24]				
4-Bromophenol	9.37	25.5	[24]				
4-Chlorophenol	9.42	25.4	[24]				
Phenol	9.98	26.6	[24]				
3-Methylphenol	10.00	26.5	[24]				
4-Methylphenol	10.25	27.5	[24]				
2-Methylphenol	10.28	27.5	[24]				

^a From Refs. [27,28].

^b Excluded from the correlations.

between ^spK_a values in acetonitrile–water and ^wpK_a values in water (Eq. (1)) are given in Table 2 for the families and solvent compositions studied. The variation of these parameters for the families studied with the composition of acetonitrile–water mixtures, compared with the variation of the same parameters in methanol–water mixtures, is depicted in Figs. 2 and 3. As compared with methanol–water, acetonitrile–water mixtures exhibit larger *a_s* values (except for aromatic carboxylic acids), that demonstrate a larger resolution of acid strength. This is already

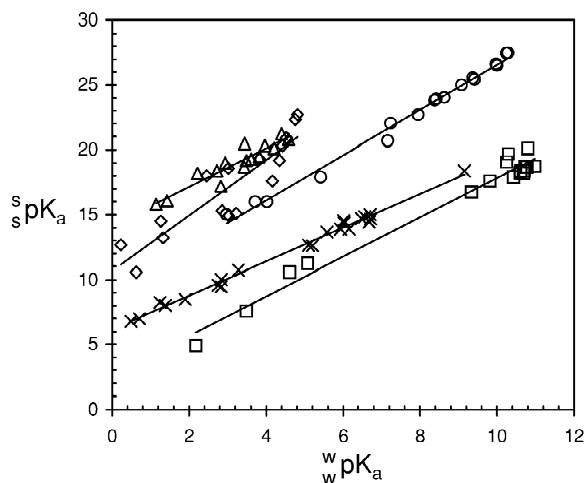


Fig. 1. Plot of the ^spK_a values in pure acetonitrile vs. the ^wpK_a values in pure water. Symbols: (◇) aliphatic carboxylic acids, (△) aromatic carboxylic acids, (○) phenols, (□) amines, (×) pyridines.

expected because the resolution of acid strength in pure acetonitrile is much larger than in pure methanol.

The *a_s* and *b_s* values have been fitted to solvent composition in terms of the volume fraction of acetonitrile according to Eqs. (2) and (3). The parameters obtained for the fits are given in Tables 4 and 5.

The parameters of Tables 4 and 5 allow calculation of the slope and intercept of the correlation between ^spK_a values in acetonitrile–water mixtures and ^wpK_a values in water for the families of compounds studied. The ^spK_a value of any member of these families, including compounds not studied in the original set, in any acetonitrile–water composition can be precisely estimated from the pK_a value of the compound in water. Conversely, the pK_a value of the compound in water can be calculated from the ^spK_a value of the compound at any acetonitrile–water composition. The studied ^spK_a data cover the range 0–60% of acetonitrile in volume (plus pure acetonitrile), except for the pyridines studied by Pawlak [32] which comprise all the acetonitrile–water composition range. Thus, accurate estimation of pK_a values is restricted to these acetonitrile–water composition ranges.

3.3. Estimation of pK_a values and degrees of protonation of pyridines in acetonitrile–water mixtures

Estimation of pK_a values and degrees of ionization

Table 2
Parameters of linear Eq. (1) for each family of compounds studied and acetonitrile–water composition

Acetonitrile (v/v)	0%	10%	20%	30%	40%	50%	60%	100%
Aliphatic carboxylic acids								
a_s	1.000	1.076	1.095	1.118	1.146	1.183	1.235	2.135
b_s	0.000	-0.089	0.091	0.289	0.507	0.744	0.997	10.784
SD	-	0.18	0.21	0.24	0.29	0.33	0.39	1.62
r	-	0.987	0.984	0.979	0.972	0.965	0.956	0.893
Aromatic carboxylic acids								
a_s	1.000	0.953	0.933	0.930	0.998	1.046	1.193	1.511
b_s	0.000	0.374	0.717	1.008	1.130	1.313	1.273	14.095
SD	-	0.13	0.19	0.24	0.28	0.30	0.33	0.60
r	-	0.981	0.960	0.939	0.931	0.926	0.929	0.933
Phenols								
a_s	1.000	-	1.163	1.186	1.215	1.213	1.216	1.706
b_s	0.000	-	-0.804	-0.672	-0.508	-0.120	0.378	9.455
SD	-	-	0.15	0.13	0.17	0.16	0.21	0.42
r	-	-	0.998	0.998	0.998	0.998	0.996	0.996
Amines								
a_s	1.000	1.011	1.029	1.044	1.050	1.073	1.080	1.479
b_s	0.000	-0.144	-0.418	-0.661	-0.760	-1.011	-1.007	2.842
SD	-	0.14	0.12	0.16	0.19	0.23	0.28	0.81
r	-	0.999	0.999	0.999	0.998	0.997	0.996	0.983
Pyridines								
	0%		42.20%	66.07%	81.41%	92.11%	94.30%	100%
a_s	1.000		1.008	1.000	0.999	0.970	1.042	1.314
b_s	0.000		-0.570	-0.848	-0.779	-0.166	-0.071	6.136
SD	-		0.15	0.14	0.10	0.16	0.14	0.29
r	-		0.997	0.997	0.998	0.996	0.997	0.996

in acetonitrile–water mixtures with the method proposed is illustrated with the set of 16 pyridines studied by McCalley [33,34] by liquid chromatography. The pyridines were studied in isoelutropic mixtures of methanol (55%), acetonitrile (40%) and tetrahydrofuran (25%) in combination with a phosphate buffer at aqueous pH 7.0. Table 6 presents the aqueous pK_a values of the pyridines as reported by McCalley [33,34]. We have also calculated the degree of ionization of these pyridines (α_{HB}) at the aqueous pH 7.0 of the phosphate buffer through the equation:

$$\alpha = \frac{HB^+}{B + HB^+} = \frac{1}{1 + 10^{pH - pK_a}} \quad (5)$$

According to the aqueous pK_a and pH of the buffer, pyridines should be partially protonated, especially the most basic dimethylpyridines. However, McCalley found that pyridines behaved chro-

matographically as completely unprotonated in 55% methanol, 40% acetonitrile and 25% tetrahydrofuran mobile phases [34]. Based on potentiometric and spectrophotometric measurements he deduced that the pK_a value of pyridines decreased and the pH value of the buffer increased with the addition of the organic modifier to the aqueous buffer, resulting in a much lower degree of protonation of the pyridines.

From Eqs. (1)–(3) and the parameters of Tables 4 and 5 and the similar parameters given in the literature [11] for methanol–water one can calculate the ${}^s pK_a$ values of the pyridines in 40% acetonitrile and 55% methanol mobile phases. The equations give $a_s = 0.959$ and $b_s = -1.013$ for 55% methanol and $a_s = 0.996$ and $b_s = -0.583$ for 40% acetonitrile, which led to the ${}^s pK_a$ values given in Table 6. pK_a values in 40% acetonitrile and 55% methanol are about 0.6 and 1.3 pK units lower than in water, respectively. The variation of the pH of the aqueous buffer by addition of methanol or acetonitrile can be

Table 3
 $^s pK_a$ values of the compounds studied in acetonitrile–water mixtures

Acetonitrile (v/v)	0%	10%	20%	30%	40%	50%	60%	Ref.
Aliphatic carboxylic acids								
2,3-Dibromopropionic acid	2.17	2.46	2.76	3.10	3.48	3.90	4.39	[29]
Chloroacetic acid	2.87	3.01	3.22	3.44	3.67	3.89	4.13	[29]
2,3-Dichloropropionic acid	2.89	2.52	2.77	3.06	3.40	3.79	4.25	[29]
2-Chloropropionic acid	2.90	3.14	3.40	3.69	4.03	4.43	4.90	[29]
Tartaric acid (pK_1)	3.03	3.21	3.38	3.59	3.83	4.11	4.46	[29]
Citric acid (pK_1)	3.13	3.31	3.49	3.68	3.90	4.16	4.45	[29]
3-Bromopropionic acid	4.02	4.26	4.52	4.82	5.16	5.57	6.04	[29]
Cinnamic acid	4.44	4.74	4.94	5.19	5.52	5.97	6.59	[29]
Acetic acid	4.76	4.95	5.17	5.42	5.73	6.10	6.57	[29]
Valeric acid	4.80	5.13	5.46	5.82	6.20	6.63	7.09	[29]
Isobutyric acid	4.85	5.23	5.62	6.03	6.48	6.96	7.48	[29]
Propionic acid	4.88	5.11	5.35	5.63	5.96	6.36	6.85	[29]
Aromatic carboxylic acids								
2-Nitrobenzoic acid	2.19	2.58	2.95	3.29	3.60	3.89	4.16	[29]
<i>o</i> -Phthalic acid (pK_1)	2.89	3.08	3.26	3.45	3.68	3.93	4.23	[29]
4-Nitrobenzoic acid	3.45	3.50	3.82	4.10	4.45	4.85	5.39	[29]
3-Nitrobenzoic acid	3.49	3.54	3.75	4.01	4.39	4.77	5.31	[29]
1-Naphthoic acid	3.69	4.07	4.44	4.79	5.24	5.67	6.26	[6]
3-Chlorobenzoic acid	3.79	3.88	4.06	4.29	4.64	4.99	5.49	[30]
3-Hydroxybenzoic acid	4.00	4.23	4.50	4.79	5.19	5.57	6.10	[30]
3-Methylbenzoic acid	4.21	4.41	4.68	4.98	5.39	5.78	6.34	[30]
Benzoic acid	4.21	4.49	4.77	5.05	5.44	5.75	6.25	[6]
Phenols								
Resorcinol	9.81	–	10.51	10.81	11.13	11.48	11.92	[6]
Phenol	9.98	–	10.80	11.13	11.69	11.89	12.38	[6]
2,4-Dichlorophenol	7.85	–	8.18	8.56	9.02	9.50	10.14	[6]
2,4-Dinitrophenol	4.07	–	4.07	4.25	4.51	4.81	5.25	[6]
β -Naphthol	9.57	–	10.27	10.72	11.32	11.59	12.08	[6]
2-Nitrophenol	7.24	–	7.40	7.71	8.06	8.57	9.20	[6]
3-Bromophenol	8.87	–	9.63	10.02	10.46	10.80	11.25	[6]
4-Chlorophenol	9.42	–	10.11	10.47	10.90	11.21	11.66	[6]
3-Methylphenol	10.00	–	11.06	11.35	11.73	12.11	12.65	[6]
3-Aminophenol (phenol)	9.99	–	10.87	11.20	11.57	12.14	12.81	[6]
Amines								
2,6-Dimethylaniline	3.95	3.79	3.60	3.43	3.36	3.21	3.24	[6]
4-Chloroaniline	4.00	3.68	3.58	3.40	3.25	3.27	3.39	[6]
Aniline	4.61	4.56	4.38	4.20	4.10	3.99	4.03	[6]
<i>p</i> -Toluidine	5.08	5.07	4.86	4.73	4.72	4.53	4.54	[6]
<i>N</i> -Ethylaniline	5.12	5.28	4.98	4.77	4.71	4.41	4.33	[6]
<i>N,N</i> -Dimethylbenzylamine	8.91	8.73	8.54	8.35	8.29	8.11	8.14	[6]
Ammonia	9.29	9.27	9.21	9.17	9.19	9.21	9.34	[31]
Ethanolamine	9.48	9.47	9.45	9.41	9.38	9.44	9.60	[31]
Triethylamine	10.66	10.63	10.54	10.41	10.30	10.33	10.33	[31]
Pyridines								
	0%		42.20%	66.07%	81.41%	92.11%	94.30%	
4-Aminopyridine	9.06		8.61	8.30	8.31	8.63	9.39	[32]
2-Aminopyridine	6.66		5.91	5.56	5.74	6.21	6.78	[32]
4-Methylpyridine	5.93		5.47	5.13	5.17	5.59	6.10	[32]
2-Methylpyridine	5.91		5.38	5.03	5.10	5.55	6.08	[32]
3-Methylpyridine	5.58		5.07	4.72	4.81	5.20	5.72	[32]
Pyridine	5.17		4.78	4.39	4.44	4.86	5.32	[32]
3-Hydroxypyridine	4.75		4.39	4.10	4.13	4.77	5.17	[32]
3-Acetylpyridine	3.55		2.87	2.62	2.68	3.10	3.49	[32]

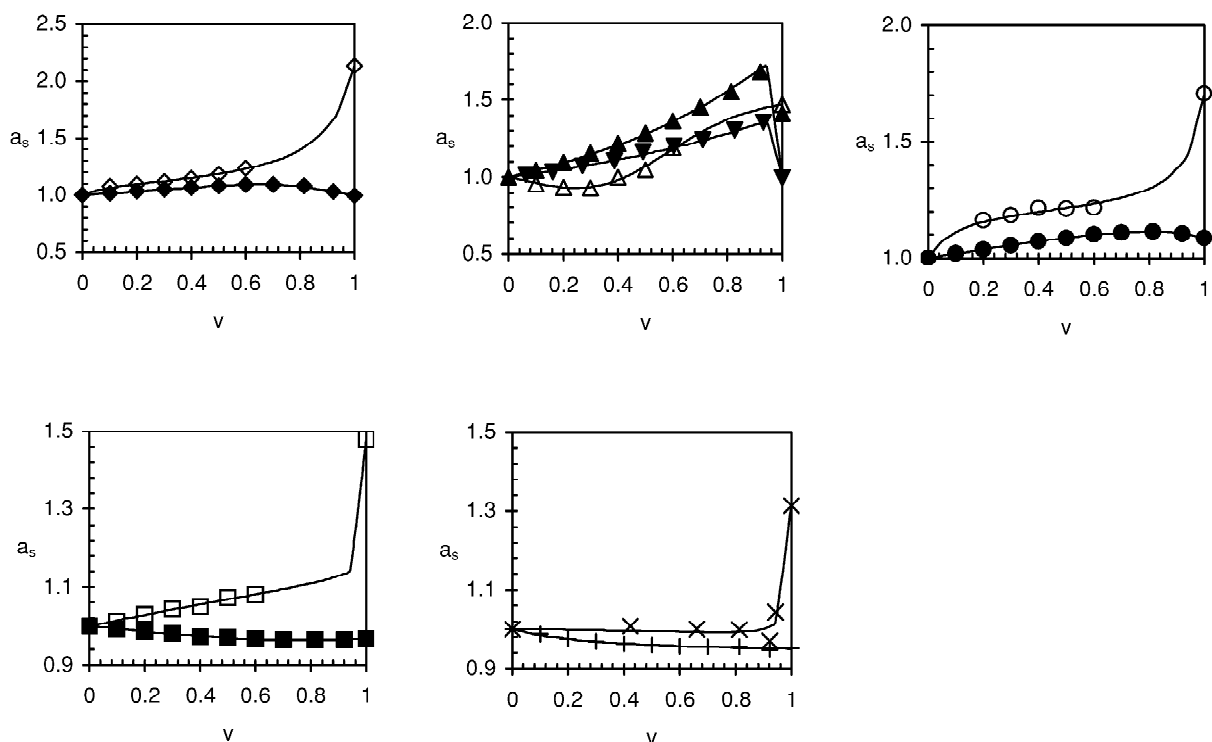


Fig. 2. Variation of the slope of the linear correlations between ${}^s\text{p}K_a$ values in acetonitrile–water or methanol–water and the ${}^w\text{p}K_a$ in pure water with solvent composition for the studied series of acids. Acetonitrile–water, symbols as in Fig. 1. Methanol–water: (♦) aliphatic carboxylic acids, (▲) aromatic carboxylic acids without *ortho* substituents, (▼) *ortho*-substituted aromatic carboxylic acids, (●) phenols, (■) amines, (+) pyridines.

also calculated from $\text{p}K$ variation. The pH of a dihydrogen phosphate/hydrogen phosphate buffer can be calculated in a good approximation by the Henderson equation, which follows:

$$\text{pH} = \text{p}K_a + \log \left(\frac{[\text{HPO}_4^{2-}]}{[\text{H}_2\text{PO}_4^-]} \right) \quad (6)$$

Since the $[\text{HPO}_4^{2-}]/[\text{H}_2\text{PO}_4^-]$ ratio remains constant with the addition of organic modifier, the pH variation is equal to the $\text{p}K_a$ variation of dihydrogen phosphate acid. The variation of the $\text{p}K_a$ value of dihydrogen phosphate in methanol–water and acetonitrile–water has been studied in previous studies [2,29]. From the equations developed in these works, we can calculate that the addition of methanol up to 55% in volume or acetonitrile up to 40% increases the $\text{p}K_a$ value of dihydrogen phosphate in 1.22 $\text{p}K_a$ units or 0.85 $\text{p}K_a$ units, respectively. Thus, the ${}^s\text{pH}$ values of the phosphate aqueous buffer of pH 7.0

should be 8.22 and 7.85 in 55% methanol and 40% acetonitrile, respectively. The methanol calculation agrees very well with the potentiometric measurements of McCally [33] who determined an ${}^s\text{p}K_a$ value of 8.25 for the same buffer in 55% methanol. The acetonitrile buffer was not measured.

From the estimated ${}^s\text{p}K_a$ values of pyridines and ${}^s\text{pH}$ values of the buffer, the degree of ionization of the pyridines in the 55% methanol and 40% acetonitrile mobile phase has been calculated. The results are given in Table 6 and they demonstrate that the pyridines are practically unprotonated (less than 2% protonated) in those mobile phases.

3.4. Estimation of aqueous $\text{p}K_a$ values from liquid chromatography retention data

The proposed equations that relate $\text{p}K_a$ values in acetonitrile–water mixtures with $\text{p}K_a$ values in water

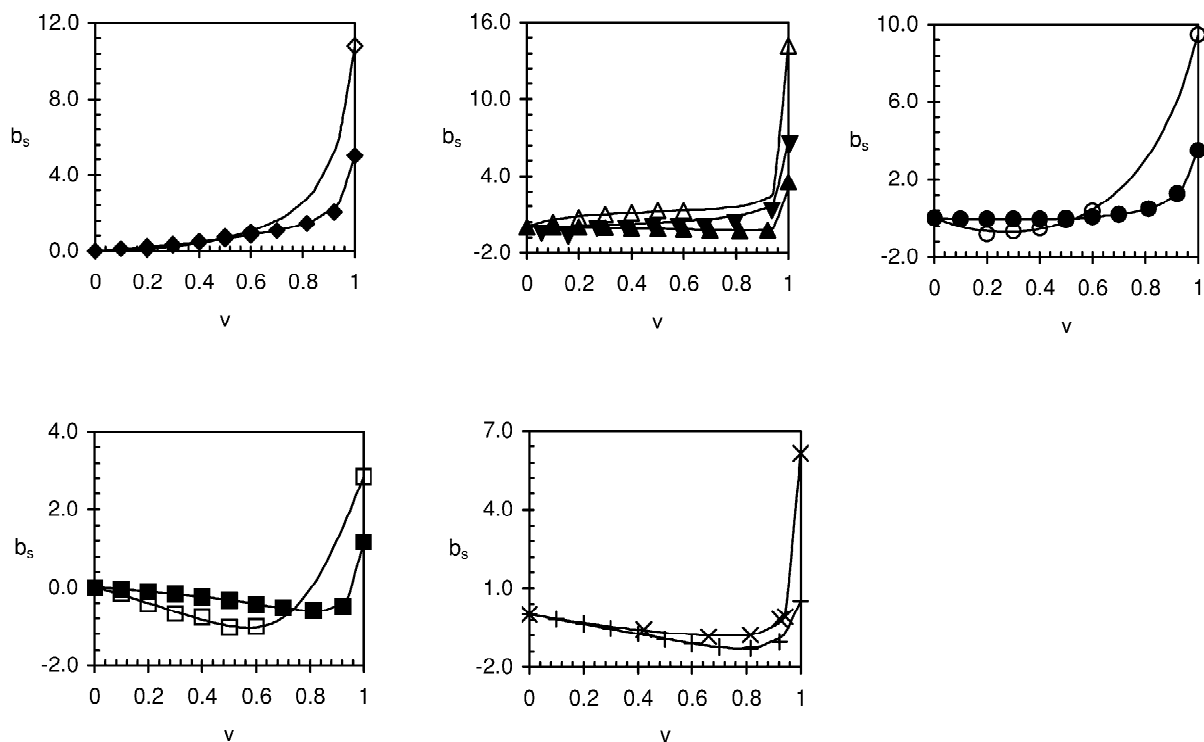


Fig. 3. Variation of the intercept of the linear correlations between ${}^s\text{p}K_a$ values in acetonitrile–water or methanol–water and the ${}^w\text{p}K_a$ in pure water with solvent composition for the studied series of acids. Symbols as in Fig. 2.

Table 4

Parameters for prediction of the slope (a_s) of the linear correlations between ${}^s\text{p}K_a$ values in acetonitrile–water and the ${}^w\text{p}K_a$ values in pure water (Eqs. (2) and (3))

	a_1	a_2	a_3	a_4	SD	F
Aliphatic carboxylic acids	9.97	−8.59	8.83	−8.72	0.01	5464
Aromatic carboxylic acids	−2.42	3.14	−1.98	2.12	0.02	362
Phenols	10.05	−10.04	7.97	−8.37	0.02	386
Amines	−0.73	−0.27	−0.87	−0.12	0.00	3476
Pyridines	−1.67	0.67	−1.66	0.67	0.03	38

Table 5

Parameters for prediction of the intercept (b_s) of the linear correlations between ${}^s\text{p}K_a$ values in acetonitrile–water and the ${}^w\text{p}K_a$ values in pure water (Eqs. (2) and (3))

	b_1	b_2	b_3	b_4	SD	F
Aliphatic carboxylic acids	−0.68	9.94	8.45	−8.59	0.08	5152
Aromatic carboxylic acids	9.97	−9.12	5.96	−6.90	0.14	2607
Phenols	−5.33	9.95	0.19	−0.70	0.11	2406
Amines	−1.82	2.25	−1.75	0.90	0.05	1559
Pyridines	−1.78	1.89	−0.58	−0.40	0.10	1293

Table 6

pK_a values of protonated pyridines in water and in isoelutropic methanol–water (55:45 v/v) and acetonitrile–water (40:60 v/v) mixtures and degree of ionization (α_{HB}) of pyridines in the three solvents prepared from an aqueous phosphate buffer of ${}^w pK_a = 7.00$

Compound	H_2O ${}^w pK_a = 7.00$		MeOH– H_2O (55:45 v/v) ${}^s pK_a = 8.22$		MeCN– H_2O (40:60 v/v) ${}^s pK_a = 7.85$	
	${}^w pK_a$	α_{HB}	${}^s pK_a$	α_{HB}	${}^s pK_a$	α_{HB}
Pyridine	5.17	0.01	3.94	5.3E–05	4.57	5.2E–04
2-Methylpyridine	5.96	0.08	4.70	3.0E–04	5.35	3.2E–03
3-Methylpyridine	5.68	0.05	4.43	1.6E–04	5.07	1.7E–03
4-Methylpyridine	6.00	0.09	4.74	3.3E–04	5.39	3.5E–03
2-Ethylpyridine	5.89	0.07	4.63	2.6E–04	5.28	2.7E–03
3-Ethylpyridine	5.80	0.06	4.55	2.1E–04	5.19	2.2E–03
4-Ethylpyridine	5.87	0.07	4.61	2.5E–04	5.26	2.6E–03
2,3-Dimethylpyridine	6.57	0.27	5.28	1.2E–03	5.96	1.3E–02
2,4-Dimethylpyridine	6.74	0.35	5.45	1.7E–03	6.13	1.9E–02
2,6-Dimethylpyridine	6.71	0.34	5.42	1.6E–03	6.10	1.7E–02
3,4-Dimethylpyridine	6.47	0.23	5.19	9.3E–04	5.86	1.0E–02
3,5-Dimethylpyridine	6.09	0.11	4.82	4.0E–04	5.48	4.3E–03
2-Propylpyridine	6.30	0.17	5.03	6.4E–04	5.69	6.9E–03
4-Isopropylpyridine	6.02	0.09	4.76	3.4E–04	5.41	3.6E–03
4-tert.-Butylpyridine	5.99	0.09	4.73	3.2E–04	5.38	3.4E–03

for families of compounds can be used to estimate the aqueous pK_a values of members of the studied families from the chromatographically determined pK_a values in particular acetonitrile–water mobile phases. Tables 4 and 5 show that the standard deviations of the fits of the experimental a_s and b_s parameters for the different families to mobile phase composition are 0.03 or less for a_s and between 0.05 and 0.14 for b_s . According to Eq. (1), these standard deviations should produce errors about 0.2 pK units or less in pK_a estimation, which is the precision

expected for pK_a measurement in non-aqueous and mixed solvents.

The accuracy of the method for pK_a estimation in water from pK_a values determined in chromatographic mobile phases has been tested with the chromatographic pK_a data obtained in a previous work for several compounds at different acetonitrile–water mobile phases [4]. The compounds belong to four different chemical families (aromatic carboxylic acids, pyridines, phenols, and amines). The results obtained are presented in Table 7. It can be observed

Table 7

Estimation of aqueous ${}^w pK_a$ values from chromatographic ${}^s pK_a$ values in different mobile phases by Eqs. (1)–(3)

Compound	Solvent	a_s	b_s	${}^s pK_a$	${}^w pK_a$ (lit)	${}^w pK_a$ (calc.)	$\Delta {}^w pK_a$
Benzoic acid	20% MeCN	0.932	0.717	4.70	4.21	4.27	–0.06
	40% MeCN	0.979	1.130	5.48	4.21	4.44	–0.23
	60% MeCN	1.183	1.273	6.43	4.21	4.36	–0.15
Pyridine	20% MeCN	0.998	–0.323	4.95	5.17	5.28	–0.11
	40% MeCN	0.996	–0.583	4.75	5.17	5.35	–0.18
	60% MeCN	0.993	–0.765	4.49	5.17	5.29	–0.12
3-Nitrophenol	40% MeCN	1.199	–0.560	9.55	8.43	8.43	0.00
Triethylamine	40% MeCN	1.055	–0.822	9.86	10.66	10.12	0.54
Benzoic acid	50% MeOH	1.279	–0.130	5.24	4.21	4.20	0.01
2,4-Dinitrophenol	50% MeOH	1.087	–0.014	4.38	4.02	4.04	–0.02
2,6-Dinitrophenol	50% MeOH	1.087	–0.014	3.97	3.71	3.66	0.05
3-Nitrophenol	50% MeOH	1.087	–0.014	9.09	8.39	8.37	0.02
Aniline	50% MeOH	0.970	–0.327	4.20	4.61	4.67	–0.06

that the predictions of aqueous pK_a values for benzoic acid, pyridine and 3-nitrophenol agree with the literature aqueous pK_a values in 0.2 pK units or less. However, the pK_a values for triethylamine differ in 0.5 pK units. This discrepancy is attributed to the value of the original chromatographic pK_a data in 40% acetonitrile (9.86) which differ considerably from the potentiometric pK_a data for the same solvent composition (10.30, see Table 3).

Estimation of aqueous pK_a values from acetonitrile–water mobile phases can be compared with estimation from methanol–water mobile phases. Table 7 reports also estimations of pK_a values from 50% methanol mobile phase [3] for five compounds (one aromatic carboxylic acid, three phenols, and one amine). The agreement between aqueous literature and estimated pK_a values is better than for acetonitrile–water (less than 0.1 pK units of difference for 50% methanol). This is not surprising because the pK data analyzed in methanol–water mixtures was much more extensive and the values of the fitting parameters are expected to be more robust.

4. Conclusions

It has been demonstrated that linear relationships between ${}^s pK_a$ values in acetonitrile–water mixtures and ${}^w pK_a$ values in water (Eq. (1)) hold for at least five different families of compounds: aliphatic carboxylic acids, aromatic carboxylic acids, phenols, amines and pyridines. The slopes and intercepts of these correlations can be related to acetonitrile–water composition by means of Eqs. (2) and (3). Combination of Eqs. (1)–(3) leads to simple relationships between the aqueous ${}^w pK_a$ value of any member of the studied families (even of those members not included in the original compound sets) and the ${}^s pK_a$ value of this member at any acetonitrile–water composition up to 60% of acetonitrile in volume.

The established relationships have two main chromatographic applications. On one hand, they can be used to estimate the ${}^s pK_a$ value of a compound in a particular acetonitrile–water mobile phase from its aqueous pK_a value. From the estimated ${}^s pK_a$ value and the pH of the mobile phase, the degree of ionization of the acid–base compound in this particular mobile phase can be easily estimated. An

accurate estimation of the compound ionization can be very useful in optimization of conditions of separation of complex mixtures of acid–base compounds (mobile phase pH and composition). The simplicity of the equations proposed allows an easy implementation in optimization algorithms and computer programs. On the other hand, the equations proposed can be used for estimation of aqueous pK_a values from chromatographically determined pK_a values in a particular acetonitrile–water mobile phase.

However, it must be emphasized that the relationships have been established between the aqueous ${}^w pK_a$ scale and the solvent dependent ${}^s pK_a$ scale. This means that the application of the estimation methods to practical liquid chromatography problems requires a proper measurement of the pH of the mobile phase. This pH must be measured in the particular acetonitrile–water mixture used as mobile phase. The pH-electrode system can be calibrated with ${}^s pK_a$ standards prepared in the same acetonitrile–water mixture used as mobile phase or with the usual aqueous standards and converted to ${}^s pK_a$ through δ values [4].

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References

- [1] E. Bosch, P. Bou, H. Allemann, M. Rosés, *Anal. Chem.* 68 (1996) 3651.
- [2] M. Rosés, I. Canals, H. Allemann, K. Siigur, E. Bosch, *Anal. Chem.* 68 (1996) 4094.
- [3] I. Canals, J.A. Portal, E. Bosch, M. Rosés, *Anal. Chem.* 72 (2000) 1802.
- [4] S. Espinosa, E. Bosch, M. Rosés, *Anal. Chem.* 72 (2000) 5193.
- [5] I. Canals, F.Z. Oumada, M. Rosés, E. Bosch, *J. Chromatogr. A* 911 (2001) 191.
- [6] S. Espinosa, E. Bosch, M. Rosés, *J. Chromatogr. A* 947 (2002) 47.
- [7] I. Canals, J. Portal, M. Rosés, E. Bosch, *Chromatographia* 55 (2002) 565.

- [8] S. Espinosa, E. Bosch, M. Rosés, J. Chromatogr. A 945 (2002) 83.
- [9] R.M. Lopes Marques, P.J. Schoenmakers, J. Chromatogr. 592 (1992) 157.
- [10] M. Rosés, F. Rived, E. Bosch, J. Chromatogr. A 867 (2000) 45.
- [11] F. Rived, I. Canals, E. Bosch, M. Rosés, Anal. Chim. Acta 439 (2001) 315.
- [12] F.Z. Oumada, C. Ràfols, M. Rosés, E. Bosch, J. Pharm. Sci. 91 (2002) 991.
- [13] M.K. Chantooni, I.M. Kolthoff, Anal. Chem. 51 (1979) 133.
- [14] F. Rived, M. Rosés, E. Bosch, Anal. Chim. Acta 374 (1998) 309.
- [15] IUPAC Compendium of Analytical Nomenclature, 3rd ed., Definitive Rules 1997, Blackwell, Oxford, 1998.
- [16] R.G. Bates, in: 2nd ed, Determination of pH. Theory and Practice, Wiley, New York, 1973.
- [17] E. Bosch, M. Rosés, Talanta 36 (1989) 627.
- [18] J. Barbosa, V. Sanz-Nebot, Talanta 36 (1989) 837.
- [19] E. Bosch, C. Ràfols, M. Rosés, Talanta 36 (1989) 1227.
- [20] C. Ràfols, M. Rosés, E. Bosch, Anal. Chim. Acta 338 (1997) 127.
- [21] A. Fini, P. De Maria, A. Guarnerini, L. Varoli, J. Pharm. Sci. 76 (1987) 48.
- [22] A.G. González, M.A. Herrador, Anal. Chim. Acta 356 (1997) 253.
- [23] D. Barrón, S. Butí, J. Barbosa, Anal. Chim. Acta 403 (2000) 349.
- [24] K. Izutsu, IUPAC: Acid–base Dissociation Constants in Dipolar Aprotic Solvents, Blackwell, Oxford, 1990.
- [25] K. Sarmini, E. Kenndler, J. Biochem. Biophys. Methods 38 (1999) 123.
- [26] D. Augustin-Nowacka, L. Chmurzynski, Anal. Chim. Acta 381 (1999) 215.
- [27] G. Kortüm, W. Vogel, K. Andrussov, Dissociation Constants of Organic Acids in Aqueous Solution, Butterworths, London, 1961.
- [28] D.D. Perrin, Dissociation Constants of Organic Bases in Aqueous Solution, Butterworths, London, 1965, Supplement, 1972.
- [29] E. Bosch, S. Espinosa, M. Rosés, J. Chromatogr. A 824 (1998) 137.
- [30] K. Sarmini, E. Kenndler, J. Chromatogr. A 833 (1999) 245.
- [31] S. Espinosa, E. Bosch, M. Rosés, Anal. Chim. Acta 454 (2002) 157.
- [32] Z. Pawlak, J. Chem. Thermodyn. 19 (1987) 443.
- [33] D.V. McCalley, J. Chromatogr. A 664 (1994) 139.
- [34] D.V. McCalley, J. Chromatogr. A 708 (1995) 185.