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On the Effect of Organic Solvent Composition on the pH of Buffered HPLC Mobile Phases and the pK_a of Analytes—A Review

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On the Effect of Organic Solvent Composition on the pH of Buffered HPLC Mobile Phases and the pK_a of Analytes—A Review

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Abstract: A review about the analyte pK_a and buffer pH variations in RP-HPLC mobile phases with the changes in the organic modifier content (acetonitrile or methanol) is presented. A model to accurately predict the pH of particular mobile phases for several commonly used buffers (acetic, citric and phosphoric acid and ammonia systems) in acetonitrile-water and methanol-water mixtures is described. Linear relationships are also presented for several families of acid-base compounds (aromatic and aliphatic carboxylic acids, phenols, amines and pyridines) to estimate pK_a values of analytes in methanol-water and acetonitrile-water from their corresponding aqueous pK_a. From both, the estimated pH of the mobile phase and the estimated pK_a of acid-base analytes, it is possible to predict their degree of ionization and, therefore, the analyte chromatographic retention.

Keywords: Mobile phase composition, methanol–water mixtures, acetonitrile–water mixtures, pH, pK_a, buffers, chromatographic retention, ionization degree

INTRODUCTION

The use of buffered mobile phases in liquid chromatography is very common for separation of analytes with acid-base properties. For monoprotic acids

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there is a well known relationship between the retention factor (k), the pK_a of the analyte at the working ionic strength and the pH of the mobile phase (1):

$$k = \frac{k_{HA} + k_A 10^{pH-pK_a}}{1 + 10^{pH-pK_a}} \quad (1)$$

where k_{HA} and k_A are the retention factors obtained when the analyte is completely in its acidic or basic form, respectively. Eq. (1) defines a sigmoidal plot for the retention as a function of the pH of the mobile phase, with a pronounced jump around the analyte pK_a. Therefore, slight variations in the pH of the mobile phase at pH near the analyte pK_a result in significant changes in retention and, thus, two similar analytes with small differences in their pK_a values can be successfully separated by a proper control of mobile phase pH. Expressions equivalent to Eq. (1) can be obtained if retention is measured in retention time (t_R) or adjusted retention time ($t'_R = t_R - t_M$) if the holdup time (t_M) is independent of the buffer (2–4). If the analyte has more than one acid-base equilibria more complex expressions should be considered (1, 4, 5).

When an organic modifier is added to an aqueous buffer to prepare the mobile phase there is a change in the pK_a of the buffering acid and in the autoprotolysis constant of the solvent, which is responsible of the pH range of the pH scale. Consequently there is a variation in the pH of the hydroorganic mixture in relation to the aqueous pH of the buffer. Moreover, the pK_a of the analyte also changes. These variations affect the ionization degree of acid-base analytes and, therefore, they may produce important changes in chromatographic retention and selectivity. The sign and extent of the pH variation when adding an organic solvent to an aqueous buffer depend not only on the organic fraction of the mixture, the aqueous pH and buffer concentration, but also on the nature of the buffering system (3, 6–12). The example given in Figure 1 illustrates these statements.

The order of elution of the ionizable analytes is clearly different, even though in both cases we have mobile phases containing a 60% of methanol (v/v) prepared from aqueous buffers of the same pH (8.00) and concentration (0.01 mol $\cdot$ L^{-1}). In this instance, the difference lies in the nature of the buffer: in one case it is ammonium/ammonia and in the other it is dihydrogenphosphate/hydrogenphosphate. Obviously the acid-base constant of the analytes in the particular mobile phase plays an important role, but in contrast to the mobile phase pH, it only depends on the organic solvent fraction in the mobile phase. The effect of both the pH and the pK_a on ionization degree and therefore on retention times in HPLC has been already extensively reported (3, 6–26). In this review we present the models developed in our research group to estimate the pH values of the most commonly used buffering systems in RP-HPLC at any fraction of organic solvent in a particular acetonitrile- and methanol-water medium up to 60% and 80% (v/v), respectively. The model we proposed to estimate the pK_a of a compound in

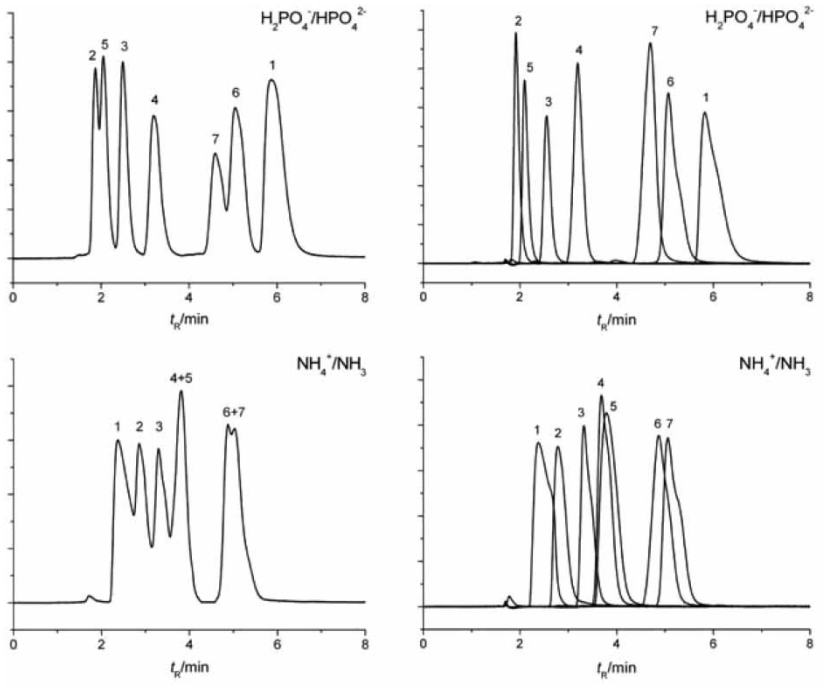

Figure 1. Chromatograms of individual acid-base compounds and their corresponding eluted mixture in a 60% (v/v) methanol mobile phase prepared from $H_2PO_4^-$-HPO_4^{2-} and NH_4^+-NH_3 aqueous buffers of concentration $0.01\ \text{mol} \cdot \text{L}^{-1}$ and pH = 8.00. Compounds: (1) *N*,*N*-dimethylbenzylamine; (2, 4)-nitrophenol; (3) 3-nitrophenol; (4) 2-chlorophenol; (5) 2-nitrophenol; (6) 2,4,6-trimethylpyridine; (7) 3-bromophenol. From ref. (44), with permission, © 2007 Elsevier.

a particular methanol-water or acetonitrile-water from its corresponding aqueous pK_a is also presented.

pH DEFINITION IN ORGANIC SOLVENT-WATER MIXTURES

Looking for a friendly way to write small hydrogen ion concentrations, the pH definition was first introduced by Sørensen (27) in 1909 in terms of the negative decimal logarithm of the hydrogen ion concentration. Some years later Sørensen found that the electrodes used to measure the pH responded to hydrogen ion activity (a_H) instead of concentration, so pH was redefined as (28):

$$\text{pH} = -\log a_H \tag{2}$$

Although activity and pH are dimensionless quantities, activity must be referred to a particular concentration scale. In fact, activity can be related to concentration through an activity coefficient (γ). This means that the same solution may have different pH values depending on the scale in which hydrogen ion concentration is measured. In analytical chemistry practice, including chromatography, the pH definition in the molarity scale (moles of hydrogen ion per liter of solvent, $\text{mol} \cdot \text{L}^{-1}$) (29, 30) is commonly used because of its simplicity for preparation of solutions. The pH definition of Eq. (2) is only notional because it involves single ion activity, which is immeasurable (29–35). Therefore an operational definition of pH was established. The pH of a solution is obtained by comparison of the electromotive force of a sample solution in an appropriate potentiometric cell in relation to the electromotive force of standard reference solutions of known pH in the same cell (29–41).

In analytical practice pH is commonly measured using a glass electrode combined with a reference electrode (very often silver-silver chloride). Usually the reference electrode contains a highly concentrated KCl solution. In this solution the cation and the anion are equitransferent (i.e., they diffuse at nearly the same rate), and thus the liquid junction potential (i.e., a potential difference formed at the boundary between two different compositions) between the reference electrode and the sample or standard calibration solutions is minimized. The temperature of calibration standards and sample solutions should be at least roughly controlled, because of the dependence of the glass electrode potential with the temperature.

Three different procedures are used to measure the pH of hydroorganic mobile phases in HPLC (3, 6–12). A typical one consists on calibrating the electrode systems with commercial aqueous standard buffers, and then measuring the pH of the aqueous buffer before mixing it with the organic modifier. This way the pH value is obtained in the ${}^{\text{w}}_{\text{w}}\text{pH}$ scale (19). In our opinion this is not the best option because the pH of the solution changes after dilution of the aqueous buffer with the organic modifier. If the electrode system is calibrated with standard buffers prepared in the same solvent composition used as mobile phase and the pH is measured in this particular mobile phase composition, the ${}^{\text{s}}_{\text{s}}\text{pH}$ value is obtained. Working in the ${}^{\text{s}}_{\text{s}}\text{pH}$ scale requires a careful preparation and maintenance of the standard buffers and electrodes, and often these standards are not commercially available. Finally, when pH is measured in the hydroorganic mixture, but the electrode system is calibrated with aqueous buffers, the ${}^{\text{s}}_{\text{w}}\text{pH}$ values are obtained.

Notice that here the IUPAC nomenclature (15) has been used: the left hand superscript indicates the medium where the quantity is measured (*w* for water and *s* for hydroorganic mixture), and the subscript indicates the standard state medium (i.e., the solvent where activity coefficients are taken as equal to unity at infinite dilution), which means in practice, the solvent (*w* or *s*) in which electrode systems are calibrated. It has been widely reported that better

results are obtained when the pH in the mobile phase is considered instead of the aqueous pH of the buffer (6–8, 12–14, 17, 23–26). ${}^{s}_{s}$pH can be easily converted to ${}^{s}_{w}$pH by means of δ parameter (9, 36, 37):

$$ {}^{s}_{w}\mathrm{pH} = {}^{s}_{s}\mathrm{pH} + \delta \tag{3} $$

The δ term is a constant value for each mobile phase composition. It includes the primary medium effect and the difference between the liquid junction potential of the electrode system in the hydroorganic mobile phase and in water. The primary medium effect (related to the standard Gibbs energy change for the transfer of the H^+ ion from water to the non-aqueous or hydroorganic solvent at infinite dilution) depends only on the mobile phase solvent composition, but the liquid junction potential depends also on the particular electrode system, pH standards, and sample composition. Therefore, general interlaboratory conversion between both pH scales is only possible if the different electrode systems are designed to have a negligible residual liquid junction potential. In practice, this requirement is fulfilled using a combination electrode containing a reference electrode with a concentrated KCl solution in water as a salt bridge. These δ values for methanol-water mixtures were studied by various authors (7, 9, 42, 43) and they can be estimated from the solvent composition through the following empirical Equation (9):

$$ \delta = \frac{0.09\phi_{\mathrm{MeOH}} - 0.11\phi^2_{\mathrm{MeOH}}}{1 - 3.15\phi_{\mathrm{MeOH}} + 3.51\phi^2_{\mathrm{MeOH}} - 1.35\phi^3_{\mathrm{MeOH}}} \tag{4} $$

where ϕ_{MeOH} is the volume fraction of methanol in the hydroorganic mixture. δ values for acetonitrile-water mixtures up to 60% (v/v) of organic modifier can be also estimated from the solvent composition through the Equation (4, 8):

$$ \delta = \frac{-0.446\phi^2_{\mathrm{MeCN}}}{1 - 1.316\phi_{\mathrm{MeCN}} + 0.433\phi^2_{\mathrm{MeCN}}} \tag{5} $$

The relationship between ${}^{s}_{s}$pH and ${}^{w}_{s}$pH depends on the organic solvent fraction in the mixture, whereas the difference between ${}^{w}_{w}$pH and ${}^{s}_{s}$pH (or ${}^{s}_{w}$pH) depends not only on the mobile phase composition but also on the particular buffering solution employed. δ values are also useful to convert ${}^{s}_{w}\mathrm{p}K_a$values to ${}^{s}_{s}\mathrm{p}K_a$, and ${}^{s}_{w}\mathrm{p}K_{ap}$ to ${}^{s}_{s}\mathrm{p}K_{ap}$, where $\mathrm{p}K_a$ refers to the analyte acid-base constant and $\mathrm{p}K_{ap}$ to the autoprotolysis constant of the solvent (organic solvent-water mixture).

Then to obtain precise information about the pH of a particular mobile phase it is convenient to measure pH directly in the hydroorganic mixture, rather than in the aqueous buffer. When the measurement of pH in the mobile phase is not easy, e.g. in the case of highly automated HPLC experiments where independent reservoirs of buffer and organic solvent are pumped into and mixed within the apparatus, it may be very useful to estimate the pH variation for a particular buffer when the organic modifier is added.

pH VARIATION OF THE BUFFER WITH THE ADDITION OF ACETONITRILE OR METHANOL

It has been shown that when acetonitrile is added to an aqueous buffer, the pH variation can be considered linearly related to the volume fraction of the organic modifier (ϕ_{MeCN}) (18):

$$ {}^{s}_{w}\mathrm{pH} - {}^{w}_{w}\mathrm{pH} = m_{pH}\phi_{MeCN} \tag{6} $$

where m_{pH} is the proportionality coefficient for the pH change. A similar equation has been proposed to relate the pH variation with the volume fraction of methanol (ϕ_{MeOH}) (44):

$$ {}^{s}_{w}\mathrm{pH} - {}^{w}_{w}\mathrm{pH} = m_{pH}\phi_{MeOH}^{d_{pH}} \tag{7} $$

The difference between Eqs. (6) and (7) is the d_{pH} parameter. This empirical parameter is assumed to be equal to 1 for cationic buffering acids ($BH^+ \rightleftarrows B + H^+$), and 5/4 for neutral ($HA \rightleftarrows H^+ + A^-$) and anionic buffering acids ($HA^{-z} \rightleftarrows H^+ + A^{-z-1}$). m_{pH} is a proportionality coefficient which depends on the particular buffering system used, and on the aqueous pH value and concentration of the buffer before adding the organic modifier. The variation of m_{pH} with the initial aqueous ${}^{w}_{w}$pH of the buffer for acetonitrile and methanol-water mixtures can be described by means of Eq. (8) (18, 44):

$$ m_{pH} = \frac{a_0 + \sum_{i=1}^{n} a_i 10^{s_i({}^{w}_{w}\mathrm{pH} - b_i)} + a_{n+1} 10^{s_i((n+1){}^{w}_{w}\mathrm{pH} - b_{n+1})}}{1 + \sum_{i=1}^{n} 10^{s_i({}^{w}_{w}\mathrm{pH} - b_i)} + 10^{s_i((n+1){}^{w}_{w}\mathrm{pH} - b_{n+1})}} \tag{8} $$

where the a_0 term in the numerator and the 1 value in the denominator predominate over the other terms at low pH values, when the solution is buffered by strong acids.

The $(n + 1)$ term predominates at very basic pH values (buffers with strong bases). The intermediate terms prevail in the pH zones close to the acid-base conjugate equilibria of the buffered system, represented by their n pK_a values. a_i values are associated to the pK_a variation of the buffer when adding the organic modifier and b_i values are related to the pK_a values of the corresponding acid-base pairs of the system. s_i are fitting parameters that account for the sharpness of the transitions (22) between the different pH zones buffered by the different acid-conjugate base pairs of the system. A linear tendency is observed in the graphical representations of the parameters s_i, a_i and b_i value against the logarithm of the aqueous concentration of the buffer (log c_T), before adding the organic modifier. These linear equations for ammonium and acetic, citric and phosphoric acid systems in acetonitrile and methanol-water mixtures are shown in Tables 1 and 2.

Tables 3 and 4 show calculated ${}^{w}_{s}$pH values in acetonitrile and methanol-aqueous buffer mixtures for the most commonly used buffering systems in

Table 1. Linear variation of the s_i, a_i and b_i parameters in acetonitrile-water mixtures for some buffering systems depending on the aqueous buffer concentration, c_T ($0.001 < c_T < 0.1$ mol · L^{-1})

Parameter	Acetic acid system	Ammonia system
s_i	$0.20 \log c_T + 3.56$	$0.20 \log c_T + 3.71$
a_0	0.00	0.00
a_1	2.28	−0.60
a_2	1.81	1.81
b_1	$-0.52 \log c_T + 2.33$	$-0.45 \log c_T + 4.84$
b_2	$-0.07 \log c_T + 11.53$	$0.06 \log c_T + 16.52$
	Phosphoric acid system	Citric acid system
s_i	$-0.04 \log c_T + 1.99$	$0.29 \log c_T + 2.59$
a_0	0.00	0.00
a_1	$0.53 \log c_T + 2.40$	$0.14 \log c_T + 1.63$
a_2	$-0.06 \log c_T + 1.63$	$-0.06 \log c_T + 1.56$
a_3	1.81	$-0.16 \log c_T + 1.67$
a_4	—	1.81
b_1	$-0.69 \log c_T + 0.93$	$-0.58 \log c_T + 1.47$
b_2	$-0.97 \log c_T + 5.16$	$-0.79 \log c_T + 4.94$
b_3	$-0.61 \log c_T + 15.34$	$-1.12 \log c_T + 9.53$
b_4	—	$-0.75 \log c_T + 19.25$

RP-HPLC, in the pH range of good buffer capacity. The m_{pH} values have been calculated by means of Eq. (8), and the ${}^{w}_{s}$pH values through Eqs. (6) and (7) for acetonitrile and methanol, respectively.

BUFFER CAPACITY

Buffer capacity (β) is a quantitative measurement of the buffer ability to keep pH constant. It can be calculated by means of the differential Equation (36, 37):

$$\beta = \frac{dc_b}{d(pH)} = -\frac{dc_a}{d(pH)} \tag{9}$$

where c_b and c_a are the concentrations of the buffering base and acid, respectively. Buffer capacity is, in rough terms, the strong base or strong acid amount (expressed in equivalents) required to produce one pH unit change in the buffer solution. For a weak acid-weak base buffer, maximum buffer capacity of a protolyte occurs when the acid species concentration is equal to the concentration of its conjugate base. It means that the apex of buffer capacity is achieved when the pH of the solution is equal to the pK'_a (the pK_a value at the working ionic strength) of the buffering species.

Table 2. Linear variation of the s_i, a_i and b_i parameters in methanol-water mixtures for some buffering systems depending on the aqueous buffer concentration, c_T ($0.001 < c_T < 0.1$ mol · L^{-1})

Parameter	Acetic acid system	Ammonia system
s_1	$0.22 \log c_T + 3.07$	$0.05 \log c_T + 1.45$
s_2	$0.13 \log c_T + 2.19$	$0.16 \log c_T + 2.18$
a_0	1.03	0.91
a_1	$-0.03 \log c_T + 2.18$	$0.01 \log c_T - 0.67$
a_2	0.00	0.00
b_1	$-0.51 \log c_T + 2.35$	$-0.45 \log c_T + 4.79$
b_2	$-0.50 \log c_T + 8.86$	$0.53 \log c_T + 18.68$
	Phosphoric acid system	Citric acid system
s_1	$0.73 \log c_T + 3.38$	$0.03 \log c_T + 1.05$
s_2	$0.02 \log c_T + 2.11$	$0.03 \log c_T + 1.05$
s_3	$0.02 \log c_T + 1.73$	$0.03 \log c_T + 1.05$
s_4	—	$0.03 \log c_T + 1.05$
a_0	1.03	1.03
a_1	$0.57 \log c_T + 3.55$	$0.18 \log c_T + 2.52$
a_2	$-0.00 \log c_T + 2.91$	$-0.10 \log c_T + 2.30$
a_3	0.00	$-0.15 \log c_T + 2.57$
a_4	—	0.00
b_1	$-0.64 \log c_T + 0.97$	$-0.57 \log c_T + 1.51$
b_2	$-1.89 \log c_T + 3.32$	$-0.73 \log c_T + 5.05$
b_3	$-2.12 \log c_T + 9.64$	$-1.02 \log c_T + 9.73$
b_4	—	$-0.76 \log c_T + 19.13$

The addition of the organic solvent produces a shift of the maximum of buffer capacity towards higher ${}^{s}_{w}$pH values for neutral or anionic acid buffers (acetic, citric and phosphoric buffering systems), but towards lower ${}^{w}_{s}$pH values for the cationic acid buffer (ammonia system). These trends have been already explained in terms of electrostatic interactions that contribute to the pK_a values of the buffering species (45, 46). The acid-base constants reported in the literature are normally thermodynamic pK_a values, which are given for zero ionic strength. Table 5 shows calculated aqueous pH values of equimolar mixtures of acid/conjugate base for several buffers at different concentrations and, consequently, ionic strength. Each pH value is related to the maximum buffer capacity achievable in aqueous solutions. It is especially significant the pH variation in case of dihydrogenphosphate/hydrogenphosphate and hydrogencitrate/citrate due to the increase of the ionic strength with the concentration because of the high charge of the buffering species. For the rest of the buffers, no dramatical changes are observed. Figure 2 shows the buffer capacity of commonly used buffering systems at several methanol-water compositions, and Figure 3 reproduces the buffer

Table 3. pH variation of acetonitrile-aqueous buffer mixtures

Buffering system	Aqueous concentration	${}^{w}_{w}pH$	m_{pH}	${}^{s}_{w}pH$ at MeCN volume fraction of 0.1	0.2	0.3	0.4	0.5	0.6
${}^{s}_{w}pH = {}^{w}_{w}pH + m_{pH}\,\phi_{MeCN}$									
Acetic acid	0.01 mol · L^{-1}	3.50	1.64	3.66	3.83	3.99	4.16	4.32	4.48
		4.00	2.26	4.23	4.45	4.68	4.90	5.13	5.36
		4.50	2.28	4.73	4.96	5.18	5.41	5.64	5.87
		5.00	2.28	5.23	5.46	5.68	5.91	6.14	6.37
		5.50	2.28	5.73	5.96	6.18	6.41	6.64	6.87
		6.00	2.28	6.23	6.46	6.68	6.91	7.14	7.37
	0.05 mol · L^{-1}	3.50	2.23	3.72	3.95	4.17	4.39	4.62	4.84
		4.00	2.28	4.23	4.46	4.68	4.91	5.14	5.37
		4.50	2.28	4.73	4.96	5.18	5.41	5.64	5.87
		5.00	2.28	5.23	5.46	5.68	5.91	6.14	6.37
		5.50	2.28	5.73	5.96	6.18	6.41	6.64	6.87
		6.00	2.28	6.23	6.46	6.68	6.91	7.14	7.37
Citric acid	0.01 mol · L^{-1}	2.50	0.48	2.55	2.60	2.64	2.69	2.74	2.79
		3.00	1.15	3.12	3.23	3.35	3.46	3.58	3.69
		3.50	1.38	3.64	3.78	3.91	4.05	4.19	4.33
		4.00	1.56	4.16	4.31	4.47	4.62	4.78	4.94
		4.50	1.67	4.67	4.83	5.00	5.17	5.34	5.50
		5.00	1.75	5.18	5.35	5.53	5.70	5.88	6.05
		5.50	1.91	5.69	5.88	6.07	6.26	6.46	6.65
		6.00	1.98	6.20	6.40	6.59	6.79	6.99	7.19
		6.50	1.99	6.70	6.90	7.10	7.30	7.50	7.69
		7.00	1.99	7.20	7.40	7.60	7.80	8.00	8.19
		7.50	1.99	7.70	7.90	8.10	8.30	8.50	8.69
	0.05 mol · L^{-1}	2.50	1.16	2.62	2.73	2.85	2.96	3.08	3.20
		3.00	1.43	3.14	3.29	3.43	3.57	3.72	3.86
		3.50	1.49	3.65	3.80	3.95	4.10	4.25	4.39
		4.00	1.60	4.16	4.32	4.48	4.64	4.80	4.96
		4.50	1.65	4.67	4.83	5.00	5.16	5.33	5.49
		5.00	1.75	5.18	5.35	5.53	5.70	5.88	6.05
		5.50	1.86	5.69	5.87	6.06	6.24	6.43	6.62
		6.00	1.88	6.19	6.38	6.56	6.75	6.94	7.13
		6.50	1.88	6.69	6.88	7.06	7.25	7.44	7.63
		7.00	1.88	7.19	7.38	7.56	7.75	7.94	8.13
		7.50	1.88	7.69	7.88	8.06	8.25	8.44	8.63
Phosphoric acid	0.01 mol · L^{-1}	2.21	0.51	2.26	2.31	2.36	2.41	2.47	2.52
		3.00	1.29	3.13	3.26	3.39	3.52	3.65	3.77
		3.50	1.34	3.63	3.77	3.90	4.04	4.17	4.30
		6.50	1.75	6.68	6.85	7.03	7.20	7.38	7.55

(*continued*)

Table 3. Continued

Buffering system	Aqueous concentration	${}^{w}_{w}$pH	m_{pH}	${}^{s}_{w}$pH at MeCN volume fraction of 0.1	0.2	0.3	0.4	0.5	0.6
		7.00	1.75	7.18	7.35	7.53	7.70	7.88	8.05
		7.50	1.75	7.68	7.85	8.03	8.20	8.38	8.55
		8.00	1.75	8.18	8.35	8.53	8.70	8.88	9.05
		8.50	1.75	8.68	8.85	9.03	9.20	9.38	9.55
	$0.05\ mol \cdot L^{-1}$	2.21	1.47	2.36	2.50	2.65	2.80	2.95	3.09
		3.00	1.70	3.17	3.34	3.51	3.68	3.85	4.02
		3.50	1.71	3.67	3.84	4.01	4.18	4.36	4.53
		6.50	1.71	6.67	6.84	7.01	7.18	7.36	7.53
		7.00	1.71	7.17	7.34	7.51	7.68	7.86	8.03
		7.50	1.71	7.67	7.84	8.01	8.18	8.36	8.53
		8.00	1.71	8.17	8.34	8.51	8.68	8.86	9.03
		8.50	1.71	8.67	8.84	9.01	9.18	9.36	9.53
Ammonia	$0.01\ mol \cdot L^{-1}$	8.00	−0.60	7.94	7.88	7.82	7.76	7.70	7.64
		8.50	−0.60	8.44	8.38	8.32	8.26	8.20	8.14
		9.00	−0.60	8.94	8.88	8.82	8.76	8.70	8.64
		9.50	−0.60	9.44	9.38	9.32	9.26	9.20	9.14
		10.00	−0.60	9.94	9.88	9.82	9.76	9.70	9.64
	$0.05\ mol \cdot L^{-1}$	8.00	−0.60	7.94	7.88	7.82	7.76	7.70	7.64
		8.50	−0.60	8.44	8.38	8.32	8.26	8.20	8.14
		9.00	−0.60	8.94	8.88	8.82	8.76	8.70	8.64
		9.50	−0.60	9.44	9.38	9.32	9.26	9.20	9.14
		10.00	−0.60	9.94	9.88	9.82	9.76	9.70	9.64

capacity variation for acetonitrile as organic modifier. In both types of mixtures, the buffer capacity presents a similar profile. The buffer capacity decreases when the organic solvent is added to the aqueous buffer, due to the decrease of the buffer concentration on increasing the volume of the solution. The addition of the organic solvent produces a shift of the maximum of buffer capacity towards higher ${}^{w}_{s}$pH values for neutral or anionic acid buffers (HAc/Ac, H_3Cit/H_2Cit-, $H_2Cit^{-}/HCit^{2-}$, $HCit^{2-}/Cit^{3-}$, $H_3PO_4/H_2PO_4^{-}$, $H_2PO_4^{-}/HPO_4^{2-}$...), and towards lower ${}^{w}_{s}$pH values for cationic acid buffers (NH_4^{+}/NH_3...).

Quantitative values of β are different in both figures, because of the different initial aqueous concentration of the buffers. As a well known rule, the higher the concentration of the buffer, the higher the buffer capacity. It is noteworthy a broad poorly buffered zone between the first and the second pK_a of the phosphoric system, around pH 5. It is also remarkable a wide range of excellent buffer capacity of the citric acid system from pH 3 to pH 7 (18, 22, 44). In this buffering system, the different extent in the variation of the three pK_a values when increasing the organic solvent fraction in the mixture is also remarkable.

Table 4. pH variation of methanol-aqueous buffer mixtures

Buffering system	Aqueous concentration	${}^{w}_{w}pH$	m_{pH}	${}^{s}_{w}pH$ at MeOH volume fraction of 0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8
${}^{s}_{w}pH = {}^{w}_{w}pH + m_{pH}\,\phi\,{}^{d_{pH}}_{MeOH}$											
Acetic acid	0.01 mol · L^{-1}	3.50	1.85	3.60	3.75	3.91	4.09	4.28	4.48	4.69	4.90
		4.00	2.22	4.12	4.30	4.49	4.71	4.93	5.17	5.42	5.68
		4.50	2.25	4.63	4.80	5.00	5.22	5.45	5.69	5.94	6.20
		5.00	2.25	5.13	5.30	5.50	5.72	5.95	6.19	6.44	6.70
		5.50	2.25	5.63	5.80	6.00	6.22	6.45	6.69	6.94	7.20
		6.00	2.25	6.13	6.30	6.50	6.71	6.94	7.19	7.44	7.70
	0.05 mol · L^{-1}	3.50	2.17	3.62	3.79	3.98	4.19	4.41	4.65	4.89	5.14
		4.00	2.22	4.13	4.30	4.49	4.71	4.94	5.17	5.42	5.68
		4.50	2.23	4.63	4.80	4.99	5.21	5.44	5.68	5.93	6.18
		5.00	2.23	5.13	5.30	5.49	5.71	5.94	6.18	6.43	6.68
		5.50	2.23	5.63	5.80	5.99	6.21	6.44	6.68	6.93	7.18
		6.00	2.23	6.13	6.30	6.49	6.71	6.94	7.18	7.42	7.68
Citric acid	0.01 mol · L^{-1}	2.50	1.52	2.59	2.70	2.84	2.98	3.14	3.30	3.48	3.65
		3.00	1.88	3.11	3.25	3.42	3.60	3.79	3.99	4.20	4.42
		3.50	2.16	3.62	3.79	3.98	4.19	4.41	4.64	4.88	5.13
		4.00	2.35	4.13	4.31	4.52	4.75	4.99	5.24	5.50	5.78
		4.50	2.49	4.64	4.83	5.05	5.29	5.55	5.81	6.09	6.38
		5.00	2.61	5.15	5.35	5.58	5.83	6.10	6.38	6.67	6.98
		5.50	2.73	5.65	5.87	6.11	6.37	6.65	6.94	7.25	7.57
		6.00	2.81	6.16	6.38	6.62	6.89	7.18	7.49	7.80	8.13
		6.50	2.84	6.66	6.88	7.13	7.40	7.70	8.00	8.32	8.65
		7.00	2.83	7.16	7.38	7.63	7.90	8.19	8.50	8.81	9.14
		7.50	2.76	7.66	7.87	8.11	8.38	8.66	8.96	9.27	9.59
	0.05 mol · L^{-1}	2.50	1.86	2.60	2.75	2.91	3.09	3.28	3.48	3.69	3.91
		3.00	2.15	3.12	3.29	3.48	3.68	3.90	4.13	4.37	4.62
		3.50	2.30	3.63	3.81	4.01	4.23	4.47	4.71	4.97	5.24
		4.00	2.39	4.13	4.32	4.53	4.76	5.01	5.26	5.53	5.81
		4.50	2.48	4.64	4.83	5.05	5.29	5.54	5.81	6.09	6.37
		5.00	2.58	5.15	5.35	5.57	5.82	6.08	6.36	6.65	6.95
		5.50	2.68	5.65	5.86	6.09	6.35	6.63	6.91	7.21	7.53
		6.00	2.73	6.15	6.37	6.61	6.87	7.15	7.44	7.75	8.07
		6.50	2.75	6.65	6.87	7.11	7.37	7.66	7.95	8.26	8.58
		7.00	2.74	7.15	7.37	7.61	7.87	8.15	8.45	8.76	9.07
		7.50	2.70	7.65	7.86	8.10	8.36	8.63	8.92	9.23	9.54
Phosphoric acid	0.01 mol · L^{-1}	2.11	1.51	2.19	2.31	2.44	2.59	2.74	2.91	3.08	3.25
		3.00	2.36	3.13	3.32	3.52	3.75	3.99	4.25	4.51	4.79
		3.50	2.40	3.64	3.82	4.03	4.27	4.51	4.77	5.04	5.32
		6.50	2.99	6.67	6.90	7.16	7.45	7.76	8.08	8.41	8.76
		7.00	2.98	7.17	7.40	7.66	7.95	8.25	8.57	8.90	9.25

(*continued*)

Table 4. Continued

Buffering system	Aqueous concentration	${}^{w}_{w}$pH	m_{pH}	${}^{s}_{w}$pH at MeOH volume fraction of 0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8
		7.50	2.94	7.67	7.89	8.15	8.44	8.74	9.05	9.38	9.73
		8.00	2.85	8.16	8.38	8.63	8.91	9.20	9.51	9.83	10.16
		8.50	2.62	8.65	8.85	9.08	9.33	9.60	9.88	10.18	10.48
	0.05 mol · L^{-1}	2.21	2.54	2.25	2.45	2.67	2.92	3.18	3.45	3.73	4.03
		3.00	2.81	3.16	3.38	3.62	3.89	4.18	4.48	4.80	5.12
		3.50	2.81	3.66	3.88	4.12	4.39	4.68	4.99	5.30	5.63
		6.50	2.96	6.67	6.90	7.16	7.44	7.74	8.06	8.40	8.74
		7.00	2.95	7.17	7.40	7.66	7.94	8.24	8.56	8.89	9.24
		7.50	2.94	7.67	7.89	8.15	8.43	8.73	9.05	9.38	9.72
		8.00	2.88	8.16	8.39	8.64	8.92	9.21	9.52	9.84	10.18
		8.50	2.73	8.65	8.87	9.11	9.37	9.65	9.94	10.25	10.57
Ammonia	0.01 mol · L^{-1}	8.00	−0.69	7.93	7.86	7.79	7.73	7.66	7.59	7.52	7.45
		8.50	−0.69	8.43	8.36	8.29	8.22	8.16	8.09	8.02	7.95
		9.00	−0.69	8.93	8.86	8.79	8.72	8.66	8.59	8.52	8.45
		9.50	−0.69	9.43	9.36	9.29	9.23	9.16	9.09	9.02	8.95
		10.00	−0.66	9.93	9.87	9.80	9.73	9.67	9.60	9.53	9.47
	0.05 mol · L^{-1}	8.00	−0.68	7.93	7.86	7.80	7.73	7.66	7.59	7.52	7.45
		8.50	−0.68	8.43	8.36	8.30	8.23	8.16	8.09	8.02	7.95
		9.00	−0.68	8.93	8.86	8.80	8.73	8.66	8.59	8.52	8.45
		9.50	−0.68	9.43	9.36	9.30	9.23	9.16	9.09	9.02	8.95
		10.00	−0.68	9.93	9.86	9.80	9.73	9.66	9.59	9.52	9.46

$d_{pH} = 5/4$ for acetic, citric and phosphoric acid systems.
$d_{pH} = 1$ for ammonia system.

Table 5. pH values at different buffer concentrations corresponding to maximum buffer capacity in aqueous solutions, calculated from de ${}^{w}_{w}pK_a$ (54) of the buffering species

Buffer	${}^{w}_{w}pK_a$	Equimolar concentration (mol · L^{-1}) 0.001	0.01	0.05	0.1
Acetic acid/acetate	4.76	4.74	4.72	4.69	4.67
Ammonium/ammonia	9.25	9.26	9.28	9.32	9.34
Phosphoric acid/dihydrogenphosphate	2.16	2.15	2.13	2.09	2.07
Dihydrogenphosphate/hydrogenphosphate	7.21	7.14	7.01	6.85	6.76
Citric acid/dihydrogencitrate	3.13	3.12	3.10	3.06	3.04
Dihydrogencitrate/hydrogencitrate	4.76	4.69	4.56	4.40	4.31
Hydrogencitrate/citrate	6.40	6.21	5.91	5.59	5.44

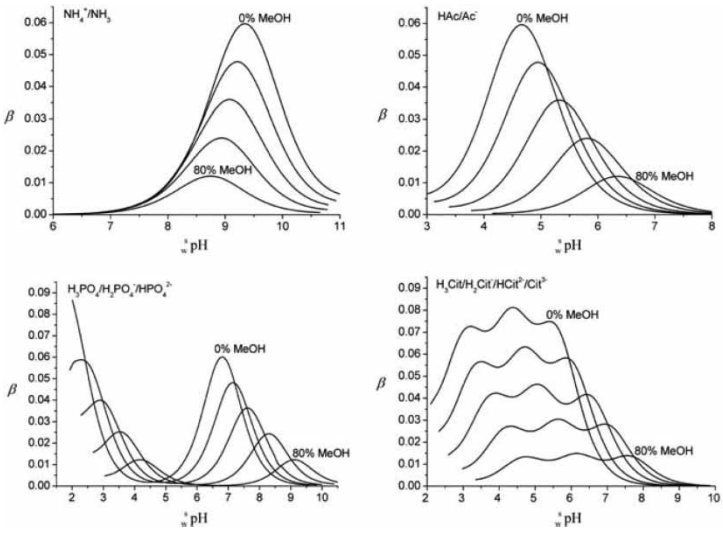

Figure 2. Buffer capacity variation of the ammonia, acetic acid, phosphoric acid and citric acid systems for 0, 20, 40, 60 and 80% (v/v) methanol-water compositions and an initial aqueous buffer concentration of 0.1 mol · L^{-1}. From ref. (44), with permission, © 2007 Elsevier.

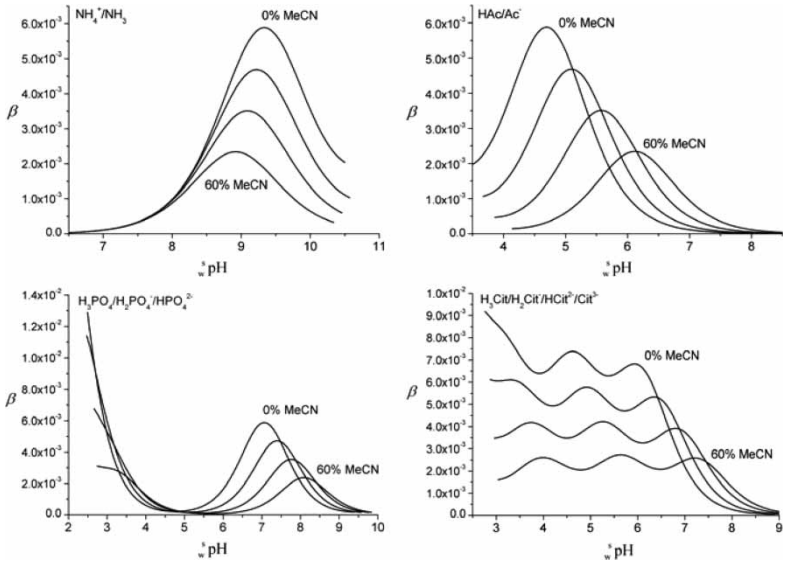

Figure 3. Buffer capacity variation of the ammonia, acetic acid, phosphoric acid and citric acid systems for 0, 20, 40 and 60% (v/v) acetonitrile-water compositions and an initial aqueous buffer concentration of 0.01 mol · L^{-1}. From ref. (22), with permission, © 2004 Elsevier.

For example, in pure water the difference between the first and the third pK_a value is about 3.3 units, whereas for methanol and acetonitrile at 60% this difference increases up to 3.7 pK_a units.

pK_a VARIATION OF THE ANALYTES WITH THE ADDITION OF ACETONITRILE OR METHANOL

For the most common families of analytes, linear relations have been established for pK_a values in the hydroorganic mobile phases in relation to their aqueous pK_a. Rived et al. (46–48) and Espinosa et al. (22, 49) developed equations to estimate ${}^{s}_{s}\mathrm{p}K_a$ from ${}^{w}_{w}\mathrm{p}K_a$values of pyridines, amines, carboxylic aromatic acids, carboxylic aliphatic acids and phenols in methanol-water and acetonitrile-water, respectively. They proposed the same general equations:

$$ {}^{s}_{s}\mathrm{p}K_a = a_s\, {}^{w}_{w}\mathrm{pK}_a + b_s \tag{10} $$

with

$$ a_s = \frac{1 + a_{s1}\,\phi_{Org} + a_{s2}\,\phi^2_{Org}}{1 + a_{s3}\,\phi_{Org} + a_{s4}\,\phi^2_{Org}} \tag{11} $$

$$ b_s = \frac{b_{s1}\,\phi_{Org} + b_{s2}\,\phi^2_{Org}}{1 + b_{s3}\,\phi_{Org} + b_{s4}\,\phi^2_{Org}} \tag{12} $$

where ϕ_{Org} is the volume fraction of organic solvent (acetonitrile or methanol) in the hydroorganic mixture, and a_{s1}, a_{s2}, a_{s3}, a_{s4}, b_{s1}, b_{s2}, b_{s3} and b_{s4} are fitting constants for all acids of the same family at any organic solvent-water composition. These a_{si} and b_{si} values are shown for methanol in Table 6 and for acetonitrile in Table 7. The analyte pK_a in the hydroorganic mobile phase can be expressed in the ${}^{s}_{w}\mathrm{p}K_a$ scale, instead of the ${}^{s}_{s}\mathrm{p}K_a$, through the already known δ parameter (Eqs. (4) or (5)). Therefore Eq. (10) is converted to the following expression:

$$ {}^{s}_{w}\mathrm{p}K_a = a_s\, {}^{w}_{w}\mathrm{pK}_a + b_s + \delta \tag{13} $$

Tables 8 and 9 show several examples of calculated ${}^{s}_{w}\mathrm{p}K_a$ values for families of compounds when increasing the acetonitrile or the methanol fraction in the hydroorganic mixture. ${}^{s}_{w}\mathrm{p}K_a$ of neutral acids or anionic acids (aliphatic and aromatic carboxylic acids and phenols) increase when acetonitrile or methanol is added, whereas the ${}^{s}_{w}\mathrm{p}K_a$ of cationic acids (amines and pyridines) decreases, mainly due to electrostatic interactions that contribute to the pK_a value (36, 45).

Table 6. Parameters for the prediction of the slope a_s Eq. (11) and the intercept b_s Eq. (12) of the linear correlation between ${}^{s}_{s}pK_a$ values in methanol-water and ${}^{w}_{w}pK_a$ in water Eq. (10) (46)

Family of compounds	a_{s1}	a_{s2}	a_{s3}	a_{s3}	b_{s1}	b_{s2}	b_{s3}	b_{s4}
Phenols	−0.656	−0.030	−0.844	0.133	−0.454	0.866	−0.017	−0.865
Aliphatic carboxylic acids	−1.406	0.680	−1.551	0.827	1.034	−0.898	−1.250	0.277
Aromatic carboxylic acids								
With *ortho*-substituents	−1.189	0.190	−1.424	0.425	0.449	−0.429	−1.674	0.677
Without *ortho*-substituents	−1.101	0.103	−1.516	0.518	−0.178	0.187	−1.699	0.702
Amines	−0.476	0.209	−0.400	0.158	−0.458	0.477	−1.674	0.690
Pyridines	2.617	0.000	2.809	0.000	−1.733	1.763	−1.214	0.272

Valid equations up to 100% (v/v) of methanol.

ESTIMATION OF THE DEGREE OF IONIZATION AND VARIATION ON CHROMATOGRAPHIC RETENTION OF ANALYTES

The retention of acid-base analytes in RP-HPLC mainly depends on their hydrophobicity and ionization degree (1–3, 6–7, 9, 14, 50–53). Whereas the hydrophobicity of a substance is a property inherent to the own nature of the analyte, the degree of ionization depends on both, analyte dissociation

Table 7. Parameters for the prediction of the slope a_s Eq. (11) and the intercept b_s Eq. (12) of the linear correlation between ${}^{s}_{s}pK_a$ values in acetonitrile-water and ${}^{w}_{w}pK_a$ in water Eq. (10) (22, 49)

Family of compounds	a_{s1}	a_{s2}	a_{s3}	a_{s4}	b_{s1}	b_{s2}	b_{s3}	b_{s4}
Aliphatic carboxylic acids	9.97	−8.59	8.83	−8.72	−0.68	9.94	8.45	−8.59
Aromatic carboxylic acids	52.04	−10.93	49.33	−32.69	−5.32	8.99	22.56	−23.21
Phenols	10.05	−10.04	7.97	−8.37	−5.33	9.95	0.19	−0.70
Amines	−0.73	−0.27	−0.87	−0.12	−1.82	2.25	−1.75	0.90
Pyridines	−1.67	0.67	−1.66	0.67	−1.78	1.89	−0.58	−0.40

Valid equations up to 60% (v/v) of acetonitrile (100% for pyridines).

Table 8. pK_a variation of analytes in acetonitrile-water mixtures

Family of analytes	${}^{w}_{w}pK_a$	${}^{s}_{w}pK_a$ at MeCN volume fraction of 0.1	0.2	0.3	0.4	0.5	0.6
Aliphatic carboxylic acids	2.00	2.14	2.28	2.43	2.61	2.82	3.09
	2.50	2.67	2.83	3.00	3.19	3.41	3.70
	3.00	3.21	3.38	3.56	3.76	4.01	4.32
	3.50	3.74	3.93	4.12	4.34	4.60	4.94
	4.00	4.27	4.47	4.68	4.92	5.19	5.55
	4.50	4.80	5.02	5.24	5.49	5.79	6.17
	5.00	5.33	5.57	5.81	6.07	6.38	6.78
Aromatic carboxylic acids	2.00	2.02	2.12	2.23	2.35	2.47	2.57
	2.50	2.57	2.69	2.84	3.00	3.16	3.32
	3.00	3.11	3.27	3.45	3.64	3.85	4.08
	3.50	3.65	3.84	4.05	4.29	4.55	4.83
	4.00	4.20	4.41	4.66	4.94	5.24	5.58
	4.50	4.74	4.99	5.27	5.58	5.94	6.33
	5.00	5.28	5.56	5.88	6.23	6.63	7.08
Phenols	7.00	7.35	7.40	7.49	7.70	8.07	8.64
	7.50	7.90	7.97	8.08	8.30	8.67	9.26
	8.00	8.46	8.55	8.67	8.89	9.28	9.88
	8.50	9.02	9.13	9.26	9.49	9.89	10.49
	9.00	9.57	9.71	9.85	10.09	10.50	11.11
	9.50	10.13	10.28	10.44	10.69	11.10	11.73
	10.00	10.68	10.86	11.03	11.29	11.71	12.34
	10.50	11.24	11.44	11.62	11.89	12.32	12.96
	11.00	11.79	12.02	12.21	12.49	12.93	13.58
Amines	7.00	6.90	6.76	6.59	6.39	6.18	6.02
	7.50	7.41	7.28	7.11	6.92	6.72	6.55
	8.00	7.91	7.79	7.63	7.44	7.25	7.08
	8.50	8.42	8.30	8.15	7.97	7.78	7.62
	9.00	8.93	8.82	8.67	8.49	8.31	8.15
	9.50	9.43	9.33	9.19	9.02	8.84	8.69
	10.00	9.94	9.84	9.71	9.55	9.37	9.22
	10.50	10.45	10.36	10.23	10.07	9.90	9.76
	11.00	10.95	10.87	10.75	10.60	10.43	10.29
Pyridines	4.00	3.82	3.64	3.46	3.25	3.01	2.70
	4.50	4.32	4.14	3.95	3.75	3.50	3.19
	5.00	4.82	4.64	4.45	4.24	3.99	3.68
	5.50	5.32	5.14	4.95	4.74	4.49	4.16
	6.00	5.82	5.64	5.45	5.23	4.98	4.65
	6.50	6.32	6.13	5.94	5.73	5.47	5.14
	7.00	6.82	6.63	6.44	6.22	5.96	5.63
	7.50	7.32	7.13	6.94	6.72	6.46	6.12
	8.00	7.82	7.63	7.43	7.21	6.95	6.60

Table 9. $p{K_a}$ variation of analytes in methanol-water mixtures

		${}^{s}_{w}p{K_a}$ at MeOH volume fraction of							
Family of analytes	${}^{w}_{w}p{K_a}$	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8
Aliphatic carboxylic acids	2.00	2.15	2.32	2.50	2.72	2.96	3.21	3.45	3.62
	2.50	2.66	2.83	3.03	3.25	3.50	3.76	3.99	4.16
	3.00	3.16	3.35	3.55	3.78	4.04	4.30	4.54	4.70
	3.50	3.67	3.86	4.08	4.32	4.58	4.85	5.09	5.24
	4.00	4.18	4.38	4.60	4.85	5.12	5.40	5.63	5.77
	4.50	4.69	4.90	5.13	5.38	5.66	5.94	6.18	6.31
	5.00	5.19	5.41	5.65	5.92	6.20	6.49	6.73	6.85
Aromatic carboxylic acids									
With *ortho*-substituents	2.00	2.11	2.23	2.38	2.56	2.78	3.03	3.29	3.51
	2.50	2.62	2.76	2.93	3.12	3.36	3.63	3.91	4.15
	3.00	3.13	3.29	3.47	3.68	3.93	4.22	4.53	4.79
	3.50	3.65	3.81	4.01	4.23	4.51	4.82	5.14	5.44
	4.00	4.16	4.34	4.55	4.79	5.08	5.41	5.76	6.08
	4.50	4.67	4.86	5.09	5.35	5.65	6.01	6.38	6.72
	5.00	5.18	5.39	5.63	5.90	6.23	6.60	6.99	7.36
Without *ortho*-substituents	2.00	2.08	2.17	2.28	2.41	2.56	2.73	2.87	2.91
	2.50	2.60	2.72	2.85	3.01	3.20	3.41	3.59	3.69
	3.00	3.12	3.26	3.43	3.62	3.84	4.09	4.32	4.47
	3.50	3.65	3.81	4.00	4.22	4.48	4.77	5.05	5.25
	4.00	4.17	4.36	4.57	4.83	5.12	5.45	5.77	6.03
	4.50	4.69	4.90	5.15	5.43	5.76	6.13	6.50	6.81
	5.00	5.21	5.45	5.72	6.04	6.40	6.81	7.22	7.59
Phenols	7.00	7.10	7.23	7.37	7.54	7.73	7.93	8.13	8.27
	7.50	7.61	7.75	7.90	8.07	8.27	8.48	8.68	8.83
	8.00	8.12	8.27	8.43	8.61	8.81	9.03	9.24	9.39
	8.50	8.63	8.78	8.96	9.15	9.36	9.58	9.79	9.94
	9.00	9.14	9.30	9.48	9.68	9.90	10.13	10.35	10.50
	9.50	9.65	9.82	10.01	10.22	10.44	10.68	10.90	11.05
	10.00	10.16	10.34	10.54	10.75	10.99	11.23	11.45	11.61
	10.50	10.67	10.86	11.07	11.29	11.53	11.78	12.01	12.17
	11.00	11.18	11.38	11.59	11.83	12.08	12.33	12.56	12.72
Amines	7.00	6.91	6.82	6.74	6.66	6.59	6.52	6.41	6.20
	7.50	7.41	7.32	7.23	7.15	7.08	7.00	6.89	6.68
	8.00	7.90	7.81	7.72	7.64	7.56	7.49	7.37	7.16
	8.50	8.40	8.30	8.21	8.12	8.05	7.97	7.85	7.64
	9.00	8.90	8.79	8.70	8.61	8.53	8.45	8.33	8.12
	9.50	9.39	9.29	9.19	9.10	9.02	8.94	8.82	8.61
	10.00	9.89	9.78	9.68	9.59	9.50	9.42	9.30	9.09
	10.50	10.38	10.27	10.17	10.07	9.99	9.90	9.78	9.57
	11.00	10.88	10.77	10.66	10.56	10.47	10.39	10.26	10.05
Pyridines	4.00	3.77	3.57	3.38	3.20	3.05	2.91	2.76	2.58

(*continued*)

Table 9. Continued

Family of analytes	${}^{w}_{w}pK_a$	${}^{s}_{w}pK_a$ at MeOH volume fraction of							
		0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8
	4.50	4.27	4.06	3.86	3.69	3.53	3.39	3.24	3.06
	5.00	4.76	4.54	4.35	4.17	4.01	3.86	3.72	3.54
	5.50	5.25	5.03	4.83	4.65	4.49	4.34	4.19	4.01
	6.00	5.74	5.52	5.32	5.13	4.97	4.82	4.67	4.49
	6.50	6.24	6.01	5.80	5.61	5.45	5.30	5.15	4.97
	7.00	6.73	6.50	6.29	6.10	5.93	5.78	5.63	5.44
	7.50	7.22	6.98	6.77	6.58	6.41	6.26	6.10	5.92
	8.00	7.71	7.47	7.25	7.06	6.89	6.74	6.58	6.39

constant and mobile phase pH. As a general rule for analytes of similar hydrophobicity, the higher the degree of ionization, the lower the retention.

For a compound that has a unique acid-base equilibrium ($HA^z - A^{z-1}$), ruled by an acidity constant (K_a), its ionization degree (α), i.e., the mole fraction of the ionized species, can be calculated by:

$$\alpha_A = \frac{[A^{z-1}]}{[HA^z] + [A^{z-1}]} = \frac{1}{1 + 10^{pK_a - pH}} \tag{14}$$

or

$$\alpha_{HA} = \frac{[HA^z]}{[HA^z] + [A^{z-1}]} = \frac{1}{1 + 10^{pH - pK_a}} \tag{15}$$

where α_A is the ionization degree of a neutral acid ($z = 0$) and α_{HA}corresponds to the ionization degree of a neutral base ($z = 1$). Strictly, pH and pK_a should be ${}^{s}_{s}pH$ and ${}^{s}_{s}pK_a$. However, we can use ${}^{w}_{s}pH$ and ${}^{s}_{w}pK_a$ values because ${}^{s}_{s}pH - {}^{s}_{s}pK_a = {}^{w}_{s}pH - {}^{s}_{w}pK_a$ since ${}^{w}_{s}pH - {}^{s}_{s}pH = {}^{s}_{w}pK_a - {}^{s}_{s}pK_a = \delta$.

Inserting the estimated values of both the analyte pK_a and the mobile phase pH in Eqs. (14) or (15) we are able to predict the ionization degree of an analyte in a particular mobile phase. Now we are capable of explaining the retention changes observed in the chromatograms of Figure 1, in which two different buffering systems of initial aqueous concentration of 0.01 mol $\cdot$ L^{-1} and ${}^{w}_{w}pH$ 8.00 prepared from dihydrogenphosphate/hydrogenphosphate and ammonium/ammonia were considered. The pK_a values of the chromatographied acid-base analytes were relatively close to 8 (with their corresponding ${}^{w}_{w}pK_a$values in brackets (11, 54)): 4-nitrophenol (7.15), 2-nitrophenol (7.23), 2,4,6-trimethylpyridine (7.43), 3-nitrophenol (8.36), 2-chlorophenol (8.56), *N,N*-dimethylbenzylamine (8.91), and 3-bromophenol (9.03). The hydrophobicities of these compounds were quite similar. Figure 4 shows the calculated ionization degrees Eqs. (14) or (15) for the analytes from their estimated pK_a Eq. (13) and mobile phase pH Eqs. (6) or (7) at several

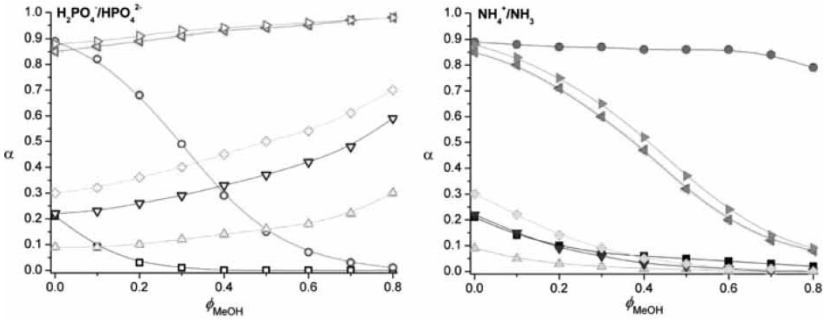

Figure 4. Variation of the ionization degree of acid-base compounds with the addition of methanol to $H_2PO_4^-$-HPO_4^{2-} and NH_4^+-NH_3 aqueous buffers of ${}^{w}_{w}$pH 8.00 and concentration 0.01 mol · L^{-1}. Legend: (■, □) 2,4,6-trimethylpyridine, (●, ○) *N*,*N*-dimethylbenzylamine, (▲, △) 3-bromophenol, (▼, ▽) 2-chlorophenol, (◀, ◁) 2-nitrophenol, (◆, ◇) 3-nitrophenol, and (►, ▷) 4-nitrophenol. From ref. (44), with permission, © 2007 Elsevier.

fractions of methanol. At 60% (v/v) of methanol the ${}^{w}_{s}$pH of the dhydrogenphosphate/hydrogenphosphate and ammonium/ammonia mobile phases were 9.51 and 7.59, respectively, and the ${}^{s}_{w}$pK_a of the analytes in both mobile phases were, 8.10, 8.19, 6.19, 9.43, 9.65, 8.37, and 10.17, respectively. In case of pyridines and amines the ionization degree is high when the pH of the mobile phase is lower than the analyte pK_a ($BH^+ \rightleftarrows B + H^+$), and in the rest of the cases (aromatic and aliphatic carboxylic acids and phenols) the ionization is high when the pH is higher than the pK_a ($HA \rightleftarrows H^+ + A^-$).

ESTIMATION OF CHROMATOGRAPHIC RETENTION OF IONIZABLE ANALYTES

The pH and pK_a models exposed above can be used to achieve quantitative information about the retention of weak acid-base analytes Eq. (1). It is possible to predict the retention from both, the estimated buffer pH and solute pK_a, and from the retentions of the pure acidic and basic forms of the analyte. These retention times can be measured in mobile phases with a pH at least two or three units lower and higher than the pK_a of the analyte. In a recent paper (26), several drugs with known aqueous pK_a were studied to test this retention time estimation model in acetonitrile-aqueous buffer mobile phases: diclofenac, ibuprofen and naproxen (nonsteroidal anti-inflammatory drugs), codeine (narcotic analgesic), trazodone, imipramine, nortriptyline and maprotiline (antidepressants). Figure 5 shows the differences between the experimental and the estimated retention times at several measured aqueous pH. Generally, there is a very good correspondence between the

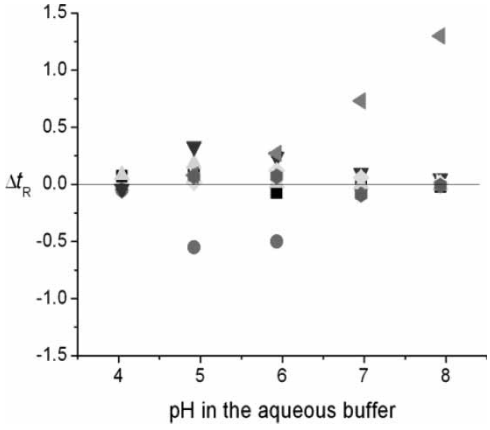

Figure 5. Differences between the experimental and the estimated retention times at several measured ${}^{w}_{w}$pH ($\Delta t_R = t_R^{est} - t_R$). Estimated retention times were calculated through Eq. 1, where ${}^{s}_{w}pK_a$ were estimated from the literature ${}^{w}_{w}pK_a$ values, and ${}^{s}_{w}$pH were estimated from measured aqueous ${}^{w}_{w}$pH. Buffer aqueous concentration was, in all cases, 0.01 mol $\cdot$ L^{-1}. Legend: (▼) trazodone, (■) diclofenac, (◆) codeine, (▲) naproxen, (●) ibuprofen, (◄) imipramine, (★) maprotiline, (►) nortriptyline. From ref. (26), with permission, © 2006 Elsevier.

estimated and the experimental retention times. Except for ibuprofen and imipramine, the average of the absolute error for all the analytes and studied pH values is less than 5%.

These differences in retention times for imipramine and ibuprofen can be attributed to a mismatch between the chromatographically obtained ${}^{s}_{w}pK_a$ values and the estimated ones. We must take into account that when the difference in retention times of the neutral and fully ionized species is large, this pK_a mismatch has a significant effect on retention estimation. When no experimental aqueous pK_a value is available in the literature for a particular analyte, it is possible to resort to computational programs, e.g., SPARC (55) and ACD/Labs (56). The former is freely accessed through Internet, and the latter is embedded in the SciFinder Scholar 2006 data base research tool.

Sometimes it is not possible to measure both of the pure acidic and basic forms of the analyte, either because the required pH value is not recommended for the column (e.g., high pH values in silica based columns) or because the k value is too high and the solute can not be detected in a reasonable analysis time. In these cases it is recommended to resort to models able to infer the chromatographic behaviour of the analytes upon changes in the experimental factors. Once the models are built with data obtained from sets of experiments, molecular modelling or other

approaches, they can be applied to predict the performance of new conditions (57).

CONCLUSIONS

When adding acetonitrile or methanol to an aqueous buffer to prepare a mobile phase, the pH of the hydroorganic mixture depends on the nature of the buffering species, the organic solvent content, and the aqueous pH and concentration of the buffer. Models have been developed to allow and accurate prediction of this pH change for several commonly used buffers in RP-HPLC (acetic, citric and phosphoric acid and ammonia systems) in acetonitrile-water and methanol-water mobile phases. Both models cover initial aqueous concentrations between 0.001 and 0.1 $mol \cdot L^{-1}$, and organic solvent contents up to 60% in volume for acetonitrile and 80% for methanol.

The buffer capacity decreases when the organic solvent is added, due to the dilution effect of the mixture, and their maximum values shift together with the pK_a variation of the buffer species.

Linear relationships have been also modelled between the pK_a values of acid-base analytes in methanol-water and acetonitrile-water and their corresponding pK_a values in water. The pK_a variation depends on the nature of family of compounds, the aqueous pK_a and the organic solvent content in the mixture. These linear relations have been established for the most common families of acid-base analytes: aromatic and aliphatic carboxylic acids, phenols, amines and pyridines. In acetonitrile-water these relations are applicable up to 60% in volume of organic modifier (100% for pyridines).

From both the analyte pK_a and the mobile phase pH, the analyte ionization degree, which plays an important role in the chromatographic retention of acid-base compounds, can be easily calculated. Moreover, with the measured retention times of neutral and fully ionized species this approach is able to estimate the retention times of weak acids and bases at any hydroorganic pH.

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