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Retention of Ionizable Compounds on HPLC. 4. Mobile-Phase pH Measurement in Methanol/Water

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The different procedures used in HPLC to measure the pH of a mobile phase are evaluated in terms of the rigorous IUPAC definition of pH. The three procedures evaluated are as follows: measurement of the pH of the aqueous HPLC buffer before mixing it with the organic modifier, measurement of the pH of the HPLC buffer after mixing it with the organic modifier using a pH electrode system calibrated with aqueous buffers, and measurement of the pH of the HPLC buffer after mixing it with the organic modifier but calibrating the electrode system with reference buffers prepared in the same mixed solvent used as mobile phase. Following IUPAC definitions and recommendations, the three pH values can be related with the pH scales: $^{w}_{w}$pH, $^{s}_{w}$pH, and $^{s}_{s}$pH, respectively. The relationships between these three pH scales are also presented. The retention of several compounds with acid/base behavior in a C-18 and a polymeric column with buffered methanol/water as mobile phase is related to the mobile phase pH value measured in the three pH scales. It is demonstrated that the $^{s}_{w}$pH and $^{s}_{s}$pH scales give better relationships than the $^{w}_{w}$pH scale (pH measured in the aqueous buffer before mixing it with the organic modifier), commonly used on HPLC. The $^{s}_{w}$pH scale is specially recommended because of its simplicity of measurement: the pH is measured after mixing the aqueous buffer with the organic modifier, but the pH calibration is performed with the common aqueous reference buffers.

The optimization of HPLC separations of many analytes requires an accurate control of mobile-phase pH.[1−9] Although the IUPAC has endorsed rules and procedures for the measurement of pH in organic solvents and binary aqueous organic solvent mixtures,[10−12] such as those used as mobile phases in HPLC, these are seldom applied in practical liquid chromatography.

There are several procedures used to measure the pH of a mobile phase. The most common procedure consists of measuring the pH of the aqueous buffer before mixing it with the organic modifier. A more rigorous procedure, recommended by the IUPAC, is to measure the pH of the mobile phase after mixing the aqueous buffer and the organic modifier. In this instance, the electrode system used to measure pH can be calibrated either with aqueous buffers or with buffers prepared in the same solvent composition used as the mobile phase. The latter requires knowledge of the pH value of reference buffers prepared in different aqueous/organic solvent mixtures. The IUPAC reports pH values of buffers at several methanol/water compositions,[10,12] and pH values for some buffers in acetonitrile/water mixtures can be found in the literature.[13,14] Recently, we evaluated the accuracy of the available pH buffers and p*K* values of acids in acetonitrile/water.[9]

This paper describes the basis of the different procedures for mobile-phase pH measurement and critically compares them. The HPLC retention of acids and bases is related to the pH of several methanol/water mobile phases measured according to the three procedures.

pH DEFINITION, NOTATION, AND TERMINOLOGY

The concept of pH was first introduced as

$$\mathrm{pH} = -\log c_{\mathrm{H}} \tag{1}$$

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

$$\mathrm{pH} = -\log a_{\mathrm{H}} \tag{2}$$

where a_{H} is the hydrogen ion activity.[10]

Activity and pH are dimesionless quantities, but activity must be referred to a concentration scale and so is pH. The most used concentration scales, accepted by the IUPAC for pH definition, are molarity (c) and molality (m, in mol kg^{-1}). This leads to two definitions of pH, either in the molarity scale (pH$_c$)

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$$pH_c = -\log(c_H\gamma_{c,H}/c^0) \quad (3)$$

or in the molality scale (pH_m)

$$pH_m = -\log(m_H\gamma_{m,H}/m^0) \quad (4)$$

where c^0 and m^0 are arbitrary constants, representing the standard-state condition, numerically equivalent to either 1 mol dm^{-3} or 1 mol kg^{-1}, respectively, and $\gamma_{c,H}$ and $\gamma_{m,H}$ are the single ion activity coefficients of hydrogen ion on the two scales, respectively.[10] For dilute solutions, molarity and molality are directly related through the density (ρ) of the solution, and therefore, the pH on one scale can be easily converted to pH on the other scale through

$$pH_c = pH_m + \log \rho/\rho^0 \quad (5)$$

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

The density of water is close to 1 kg dm^{-3} in the usual range of working temperatures, and therefore, pH_c and pH_m are practically identical. However, the density of some aqueous/organic solvent mixtures can be quite different from 1 kg dm^{-3}, and the transfer term log ρ can achieve several tenths of pH units. Table 1 reports the value of the log ρ term for several methanol/water compositions. Equations to compute molar volume (V_M) for any methanol/water mixture are given in the literature.[7] Density can be easily calculated from the molar volume and solvent composition ($\rho = (32.04x_{MeOH} + 18.01x_{H_2O})/V_M$, where x_{MeOH} and x_{H_2O} are the mole fractions of methanol and water mixed).

The concentration scale used in the particular pH definition must be carefully stated and controlled, even for the pH buffers used in the electrode system calibration. The IUPAC prefers the molality scale because molality does not change with the temperature of the solution. However, in HPLC practice, molarity is almost always used because of its simplicity for preparation of solutions, and we shall use this scale in this work.

The IUPAC remarks that the above definitions of pH are only notional because they involve a single ion activity (a_H), which is immeasurable.[10] To obtain the pH value, an extrathermodynamic assumption is necessary.[11,12] This is usually the Debye–Hückel equation, which allows estimation of the activity coefficient of hydrogen ion

$$\log \gamma_H = -AI^{1/2}/(1+a_0BI^{1/2}) \quad (6)$$

In eq 6, I is the ionic strength of the solution, A and B are solvent and temperature-dependent parameters, and a_0 is the ion size parameter, which is assigned a value fixed by the Bates–Guggenheim convention extended to the general solvent s.[11,12,15]

$$({}^sa_0{}^sB) = 1.5[{}^w\epsilon^s\rho/({}^s\epsilon^w\rho)]^{1/2} \quad (7)$$

where ${}^sB$ is the classical Debye–Hückel constant of eq 6 for the solvent s (e.g., mobile phase), ${}^w\epsilon$ and ${}^s\epsilon$ are the relative permittivities of pure water (superscript w) and of the solvent s (superscript s), and ${}^w\rho$ and ${}^s\rho$ are the corresponding densities. If s is water itself, eq 7 reduces to $a_0B = 1.5$, which is the form of the Bates–Guggenheim convention that was introduced originally for pH standardization in pure water.[15] Table 1 reports values of A and a_0B terms for several methanol/water compositions. Equations to estimate the terms for any methanol/water composition are also given in the literature.[7]

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Table 1. Macroscopic Properties of Methanol/Water Mixtures

% MeOH (v/v)	x_{MeOH}	ρ (g/mL)	log ρ	A^a	a_0B^a	δ	pK_{ap}
0	0.000	0.9948	−0.002	0.53	1.50	0.00	14.00
10	0.047	0.9826	−0.008	0.56	1.53	0.01	14.08
20	0.100	0.9693	−0.014	0.59	1.57	0.02	14.08
30	0.160	0.9548	−0.020	0.64	1.61	0.04	14.07
40	0.229	0.9388	−0.027	0.70	1.66	0.08	14.09
50	0.308	0.9209	−0.036	0.77	1.72	0.13	14.14
60	0.400	0.9008	−0.045	0.87	1.79	0.19	14.23
70	0.509	0.8780	−0.057	1.01	1.88	0.22	14.39
80	0.640	0.8518	−0.070	1.20	1.99	0.10	14.63
90	0.800	0.8216	−0.085	1.48	2.13	−0.28	15.04
100	1.000	0.7870	−0.104	1.87	2.31	−2.24	16.77

[a] Calculated by use of the following:[7] $A = 0.53 + 1.34x_{MeOH} - 0.37(2.73 - \sqrt{7.45-10.92x_{MeOH}(1-x_{MeOH})}$; $a_0B = 1.50 + 0.81x_{MeOH} - 0.06(2.73 - \sqrt{7.45-10.92x_{MeOH}(1-x_{MeOH})}$.

Since pH is defined in terms of activity, it depends not only on the concentration scale chosen but also on the standard state of activity. In water, the standard state for a_H is infinite dilution of hydrogen ion in water (i.e., pure water), for which $\gamma_H \rightarrow 1$. In the solvent s, two different standard states can be chosen. One is infinite dilution of the ion in the same solvent s, and the other is infinite dilution of the ion in water. This leads to two different pH scales, one relative to each particular solvent, and the other relative to water, which is also called "absolute pH scale".

To distinguish between the two pH scales, the IUPAC recommends the notation used by Robinson and Stokes for their discussion of the effect of the medium on transferring a binary electrolyte from water (w) to a nonaqueous or mixed solvent (s), already partially used in eq 7. Thus, lower-case left-hand superscripts indicate the solvent (w or s) in which measurements are being made; lower-case left-hand subscripts indicate the solvent in which the ionic activity coefficient γ is referred to unity at infinite dilution (w or s).[10] Also, the notation ${}^s_w\gamma^0_H$ is recommended for the primary medium effect (related to the standard Gibbs energy change) for the transfer of the H^+ ion from water (w) to the solvent s. Thus, the ${}^s_spH$ value measured in solvent s relating to the pH scale specific to the solvent might be expressed as ${}^s_wpH$ on an "intersolvental" or "absolute" scale with ultimate reference to the solvent water[10] and be meaninfully compared with the latter by

$${}^s_wpH = {}^s_spH - \log({}^s_w\gamma^0_H) \quad (8)$$

where ${}^s_w\gamma^0_H \rightarrow 1$ as s $\rightarrow$ w.

In practice, the standard state at which the pH measured is related depends on the calibration of the electrode system. It is universally agreed that the definition of pH difference is an operational one.[10] The pH of a test solution (pH_X) is determined

by comparison of the electromotive forces E_X and E_S of two appropriate potentiometric cells. The two cells must be equal except for that one contains the test solution X and the other a standard reference solution S of known pH (pH_S). The pH_X is determined from

$$pH_X = pH_S - (E_X - E_S)/k \qquad (9)$$

where $k = (RT/F)$ ln 10 and R is the gas constant, T the thermodynamic temperature, and F the Faraday constant, ignoring a term $\Delta E_J = E_{JX} - E_{JS}$, which is called the residual liquid junction potential.[10] Formerly, the IUPAC recommended the potentiometric cells to be composed of a hydrogen and a reference electrode, together with a bridge solution of concentrated potassium chloride ($c \geq 3.5$ mol kg^{-1} for aqueous solutions). The most recent edition of the *Compendium of Analytical Nomenclature*,[10] however, allows the hydrogen electrode to be replaced by another hydrogen ion-responsive electrode, such as a glass electrode.

Therefore, if a potentiometric cell used to measure the pH of an aqueous organic solvent mixture s (e.g., an HPLC mobile phase) is standardized with pH buffers prepared in the same solvent mixture s, the standard state for a_H is the solvent mixture s and the ${}^{s}_{s}pH$ quantity will be obtained. But, if the electrode system is calibrated with aqueous buffers, the standard state is water and the ${}^{s}_{w}pH$ quantity will be obtained when the pH of the test solution in solvent s is measured.

RELATIONSHIPS BETWEEN THE DIFFERENT pH QUANTITIES

From the pH definitions above, it is clear that the three procedures mentioned in the introduction to measure the pH of HPLC mobile phases lead to three different pH quantities. If one calibrates the electrode system with aqueous buffers and measures the pH of the HPLC aqueous buffer before mixing it with the organic modifier, ${}^{w}_{w}pH$ is obtained. However, the pH of the solution changes after dilution of the aqueous buffer with the organic modifier. If the electrode system is calibrated with aqueous buffers, but the pH is measured after mixing the HPLC aqueous buffers with the organic modifier, ${}^{s}_{w}pH$ is directly obtained. Finally, if the electrode system is calibrated with buffers prepared in the same solvent composition used as mobile phase, and the pH is measured in this same mobile-phase composition (i.e., after mixing aqueous buffer and organic modifier), the quantity obtained is ${}^{s}_{s}pH$.

${}^{s}_{w}pH$ can be converted to ${}^{s}_{s}pH$ by means of eq 8 provided that the medium effect (the log $({}^{s}_{w}\gamma^{0}_{H})$ term) is known. In fact, the difference between the measured ${}^{s}_{w}pH$ and ${}^{s}_{s}pH$ values includes the difference of the liquid junction potentials (or $\overline{E_j}$, expressed in pH units and assumed to be constant), together with the primary medium effect $-\log({}^{s}_{w}\gamma^{0}_{H})$. The addition of the two terms leads to the useful quantity δ,[16] since

$$\delta = \overline{E_j} - \log({}^{s}_{w}\gamma^{0}_{H}) = {}^{s}_{w}pH - {}^{s}_{s}pH \qquad (10)$$

Figure 1 shows the variation of this term with the methanol/water composition. The original values were measured in the molality scale,[17−19] but we have converted them to the molarity scale by means of eq 5. It can be observed that the term is small (~0.2 or less) for methanol contents up to 80% (v/v), but it changes abruptly for higher methanol contents to reach a −2.24 value for pure methanol.

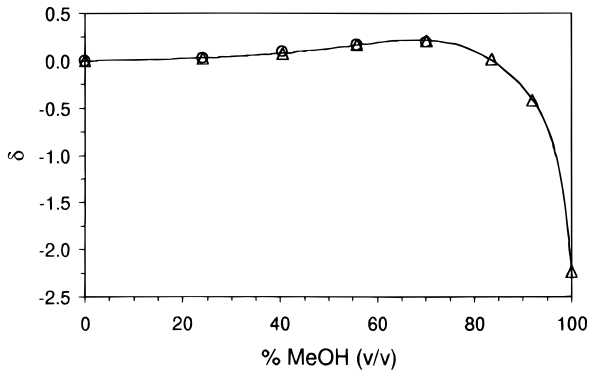

Figure 1. Variation of the δ quantity in molar scale with solvent composition in methanol/water mixtures: (△) data from ref17; (○) data from ref18.

The difference between ${}^{s}_{w}pH$ and ${}^{s}_{s}pH$ is a constant value for each mobile-phase composition. However, the difference between ${}^{w}_{w}pH$ and ${}^{s}_{s}pH$ (or ${}^{s}_{w}pH$) depends not only on the mobile-phase composition but also on the particular buffering solution employed. Some examples will illustrate this:

If an aqueous buffering solution is prepared from a strong (completely dissociated) acid, and this solution is mixed with an organic modifier, the pH increases from ${}^{w}_{w}pH$ to ${}^{s}_{s}pH$ because of the dilution. There is also a pH variation caused by the variation on the activity coefficients of hydrogen ion, but this is meaningless as compared with the pH variation by dilution.

If the buffering solution is prepared from a strong base, the dilution decreases pH, but the increase in the pK_{ap} value (see Table 1) partially balances it.

A very common case on HPLC is use of buffers prepared from an acid at concentration c_a and its conjugated base at concentration c_b (e.g., acetic/acetate, NH_4^+/NH_3, etc.). The hydrogen ion activity is approximately

$$a_H = K_a c_a / c_b \qquad (11)$$

and the pH variation

$$\Delta pH = {}^{s}_{s}pH - {}^{w}_{w}pH = \Delta pK_a \qquad (12)$$

where ΔpK_a is the difference between the pK_a values of the acid in the mobile-phase solvent and in water (${}^{s}_{s}pK_a - {}^{w}_{w}pK_a$).

The pH difference does not include the dilution term because it affects c_a and c_b to the same degree.

The above examples show that the pH difference between the true pH of the mobile phase and the pH of the aqueous buffer is

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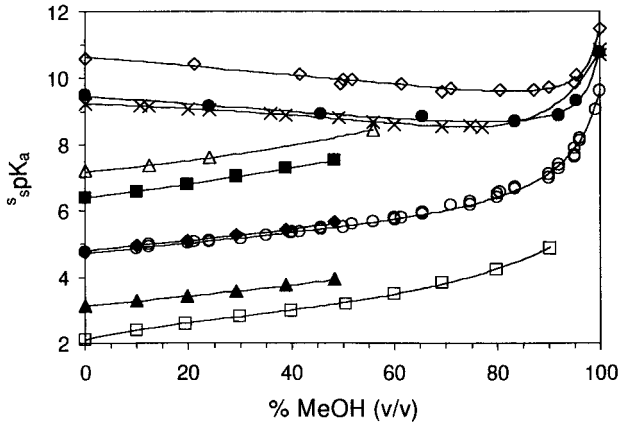

Figure 2. Variation of the $^{s}_{s}pK_a$ of acids in methanol/water mixtures with solvent composition. Neutral and anionic acids: (□) pK_{a1} of phosphoric acid; (▲) pK_{a1} of citric acid; (○) acetic acid; (◆) pK_{a2} of citric acid; (■) pK_{a3} of citric acid; (△) pK_{a2} of phosphoric acid. Protonated bases: (×) ammonia; (●) ethanolamine; (◇) butylamine.

very dependent on the buffer solution. Even if buffers of the same type are used (e.g., acid/conjugated base), different acids and bases with different pK_a values will be needed to cover an extensive pH range. Figure 2 presents the variation of pK_a values of several acids and bases in methanol/water mixtures.[22] The pK_a of neutral (phosphoric, acetic, citric) and anionic (dihydrogen citrate, hydrogen citrate, hydrogen phosphate) acids increases with the methanol contents, but the pK_a of cationic acids (protonated amines) decreases with the methanol contents up to ~80% methanol. Therefore, in the range 0−80% of methanol, ΔpH is positive for buffers prepared from neutral and anionic acids and its conjugated base, but negative for buffers prepared from neutral bases and its conjugated acid (cationic acid).

HPLC RETENTION AND PH

The retention factor k of a compound that undergoes an acid/base equilibrium of the type

$$HA^z \leftrightarrow H^+ + A^{z-1} \qquad K_a = a_H a_A / a_{HA} \qquad (13)$$

can be given as an average of the retention factors of the two species (k_{HA} and k_A) according to the mole fraction of each species at the mobile-phase pH

$$k = ([HA]k_{HA} + [A]k_A)/([HA] + [A]) = (k_{HA} + k_A 10^{pH-pK_a'})/(1 + 10^{pH-pK_a'}) \quad (14)$$

where pK_a' is the acidity pK in terms of the concentration of the two species, instead of activities, i.e.

$$pK_a' = -\log K_a' = -\log a_H[A]/[HA] = -\log K_a \gamma_{HA}/\gamma_A \qquad (15)$$

The variation of k with the mobile-phase pH produces a sigmoidal plot with an inflection point at pH = pK_a'. Depending on the pH scale used, different pK_a' values may be obtaine. If k is plotted and fitted to $^{s}_{s}$pH, the value obtained is $^{s}_{s}pK$, the acid/base constant relative to the mobile-phase standard state. But if $^{s}_{w}$pH is used, the value obtained should be $^{s}_{w}pK_a'$, the constant referred to water as standard state. Both pK_a' values should differ in the constant δ term. However, if the pH scale used is $^{w}_{w}$pH, i.e., the pH is measured in the aqueous buffer before mixing it with the organic modifier, the pK_a' obtained cannot be usually related to any thermodynamic pK. Only, if ΔpH would remain constant for all the used buffers with pH close to pK_a' and for the analyte acid chromatographed, would the pK_a' value obtained agree with the pK_a' of the acid in water ($^{w}_{w}pK_a'$).

Equation 14 can be also written in terms of retention time from

$$k = t'_R/t_M = (t_R - t_M)/t_M \qquad (16)$$

where t_R, t'_R, and t_M are retention time, adjusted retention time and holdup time, respectively.

From eqs 14 and 16

$$t'_R = (t'_{R(HA)} + t'_{R(A)} 10^{pH-pK_a'})/(1 + 10^{pH-pK_a'}) \qquad (17)$$

$$t_R = (t_{R(HA)} + t_{R(A)} 10^{pH-pK_a'})/(1 + 10^{pH-pK_a'}) \qquad (18)$$

In fact, these equations may provide better fits than eq 14. It must be pointed out, that k is not a true thermodynamic constant. It is related to the partition constant K through the phase ratio V_M/V_S, where V_M is the mobile-phase volume (proportional to t_M) and V_S the stationary-phase volume. When k is calculated from t'_R and t_M, usually slightly different t_M values are obtained for each buffer, which indicates different phase ratios. In this instance, it seems more appropriate to relate retention to pH through eqs 17 or 18, rather than through eq 14.

Another factor to take into account, is that the ionic strength of each buffer is different and therefore each buffer would require a different pK_a' parameter. However, activity coefficient correction is small (e.g., $\log \gamma_H = -0.07$ for $I = 0.01$ M and 50% methanol) and the difference in $\log \gamma$ values between different buffers is insignificant. A more rigorous model that considers the activity coefficient of each buffer and also a different holdup time for each species can be found in the literature.[8] The model has been setted only for neutral acids in C-18 columns and at present it cannot be generalized to other columns or solutes (such as neutral bases).

EXPERIMENTAL SECTION

Apparatus. Potentiometric and pH measurements were taken with a Ross combination electrode Orion 8102 in a Crison micropH 2002 potentiometer with a precision of ±0.1 mV (±0.002 pH unit). The retention data were measured on a 25 cm × 4.0 mm i.d. Merck LiChrospher 100 RP-18 column (5 μm) or on a 15 cm × 4.6 mm i.d. Interaction Chromatography PRX-1 POLY RP (styrene divinylbenzene, 10 μm) column with a flow of 1 mL min^{-1} in an ISCO model 2350 dual-pump system with a 20-μL loop valve. The detectors used were a variable-wavelength V[4] absorbance detector (ISCO) set at 254 nm for the test acids and base, 210 nm for the

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Table 2. Determination of the δ Term for 50% Methanol (v/v)

buffer solution	${}^{s}_{s}\mathrm{pH}_{theo}$	${}^{s}E$ (mV)	${}^{s}E_0 + {}^{s}E_{jX}$	${}^{s}_{s}\mathrm{pH}_{exp}$	${}^{s}_{w}\mathrm{pH}$	δ
HCl 0.1 M	1.16	322.2	390.8	1.10	1.25	0.15
HCl 0.01 M	2.07	265.6	387.8	2.06	2.20	0.14
HCl 0.001 M	3.02	208.8	387.6	3.02	3.15	0.13
0.05 M H_3PO_4/0.05 M $H_2PO_4^-$	3.11	205.4	389.4	3.08	3.21	0.13
0.05 M H_3Cit/0.05 M H_2Cit^-	3.78	166.6	390.1	3.73	3.87	0.14
0.05 M HAc/0.05 M Ac^-	5.40	64.4	384.1	5.46	5.59	0.13
0.05 M H_2Cit^-/0.05 M $HCit^{2-}$	5.09	85.2	386.2	5.11	5.23	0.12
0.05 M $HCit^{2-}$/0.05 M Cit^{3-}	6.37	12.5	389.1	6.34	6.46	0.12
0.05 M $H_2PO_4^-$/0.05 M HPO_4^{2-}	7.66	−67.5	385.7	7.69	7.80	0.11
0.05 M NH_4^+/0.05 M NH_3	8.89	−142.0	383.7	8.95	9.07	0.12
0.05 M $BuNH_3^+$/0.05 M $BuNH_2$	10.07	−209.5	386.5	10.09	10.20	0.11
KOH 0.001 M	11.12	−248.7	409.0	10.75	10.88	0.13
KOH 0.01 M	12.07	−327.4	386.8	12.08	12.17	0.09
KOH 0.1 M	12.98	−384.7	383.2	13.05	13.13	0.08

C-18 holdup time marker potassium bromide (0.01%), and 200 nm for pure methanol, which was used as marker in the polymeric column. All data were taken in triplicate at 25 °C with the potentiometric cell and columns thermostated with water jackets.

Chemicals. Methanol for chromatography from Merck and water purified by the Milli-Q plus system from Millipore were used. Other chemicals were reagent grade or better and obtained from Fluka, Aldrich, Merck, or Carlo Erba.

Procedure. To determine the δ quantity for 50% methanol as mobile phase the ${}^{s}_{s}\mathrm{pH}$ values of some buffers in 50% methanol were calculated from the ${}^{s}_{s}\mathrm{p}K$ values of the buffer components. The algorithm used for pH calculation is similar to that described by de Levie[23] and is explained elsewhere.[9] The ${}^{s}_{s}\mathrm{p}K$ values of the acids in 50% methanol were interpolated from the ${}^{s}_{s}\mathrm{p}K$ of the acids at different methanol/water compositions (plotted in Figure 2), obtained from the literature.[22] To account for errors in the literature ${}^{s}_{s}\mathrm{p}K$ values, the potencial of each buffer (${}^{s}E_X$) was measured with the potentiometric system and related to ${}^{s}_{s}\mathrm{p}K_X$ through the Nernst equation:[10]

$$ {}^{s}E_X = ({}^{s}E_0 + {}^{s}E_{jX}) - k\left({}^{s}_{s}\mathrm{pH}_X\right) \qquad (19) $$

where ${}^{s}E_0$ is the standard potential (constant at each temperature), ${}^{s}E_{jX}$ is the liquid junction potential (assumed to be negligible or at least constant for all buffers), and $k = (RT/F)\ln 10$ (see eq 9) is the Nernst constant ($k = 0.059\,157$ at 25 °C).

The $({}^{s}E_0 + {}^{s}E_{jX})$ constant was calculated for each buffer from the measured ${}^{s}E_X$ potential and the theoretically calculated ${}^{s}_{s}\mathrm{pH}_X$ value. The overall $({}^{s}E_0 + {}^{s}E_{jX})$ constant of the electrode system in 50% methanol was calculated by averaging the individual constants of different buffers, and from this averaged constant a new ${}^{s}_{s}\mathrm{pH}$ value was calculated for each buffer from the measured potential. These experimentally calculated ${}^{s}_{s}\mathrm{pH}$ values provided better results than the initial ${}^{s}_{s}\mathrm{pH}$ values calculated from literature ${}^{s}_{s}\mathrm{p}K$ values. Later the ${}^{s}_{w}\mathrm{pH}$ value of each buffer was measured with the electrode system set to pH measurements, after calibration with the usual aqueous standard reference buffers of potassium hydrogen phthalate (${}^{w}_{w}\mathrm{pH} = 4.00$) and potassium dihydrogen phosphate/disodium hydrogen phosphate (${}^{w}_{w}\mathrm{pH} = 7.02$). The δ quantity for each buffer was obtained by subtraction of the experimental ${}^{s}_{s}\mathrm{pH}$ from the measured ${}^{s}_{w}\mathrm{pH}$ (eq 10).

RESULTS AND DISCUSSION

Determination of δ for 50% Methanol. Table 2 reports the results obtained in the determination of δ with the buffers studied. These buffers include solutions of strong acids (HCl) and bases (KOH) and solutions prepared from mixtures of weak acids (phosphoric, citric, acetic, dihydrogen citrate, hydrogen citrate, dihydrogen phosphate, ammonia, and butylammonia) and their conjugated base, and they encompass a wide pH range. Table 2 gives the ${}^{s}_{s}\mathrm{pH}$ theoretically calculated from the literature ${}^{s}_{s}\mathrm{p}K$ values (${}^{s}_{s}\mathrm{pH}_{theo}$), the potential measured for each buffer (${}^{s}E$), the calculated ${}^{s}E_0 + {}^{s}E_{jX}$ term, the experimental ${}^{s}_{s}\mathrm{pH}$ value (${}^{s}_{s}\mathrm{pH}_{exp}$), the measured ${}^{s}_{w}\mathrm{pH}$ value, and the δ quantity obtained by subtraction of the two last terms. KOH buffers gave inconsistent results, possibly because of CO_2 absorption, and they were excluded from the average parameters. The mean ${}^{s}E_0 + {}^{s}E_{jX}$ value was 387 ± 2 mV, and the mean δ value was 0.12 ± 0.01. The δ value agrees very well with the values reported in the literature (see Table 1) obtained from hydrogen electrode measurements. This agreement and the wide agreement between the different ${}^{s}E_0 + {}^{s}E_{jX}$ values calculated suggest that the liquid junction potential is negligible as compared with the medium effect $-\log({}^{s}_{w}\gamma^0_H)$. If this is also confirmed for other electrode systems and mobile-phase compositions, then the ${}^{s}_{w}\mathrm{pH}$ values obtained from different electrode systems would be directly comparable without need of δ determination (which would be constant and known for each mobile phase).

Relationships between HPLC Retention and Mobile-Phase pH Quantities. Knowledge of the δ quantities allows the calculation of the ${}^{s}_{s}\mathrm{pH}$ value of any buffer from the measured ${}^{s}_{w}\mathrm{pH}$ value, without need of calibration of the electrode system with buffers of known ${}^{s}_{s}\mathrm{pH}$ for each mobile-phase composition. Therefore, the pH values of a particular mobile phase in the three pH scales can be determined in the three ways explained with calibration of the electrode system with the usual aqueous buffers: The ${}^{w}_{w}\mathrm{pH}$ value is obtained by measuring the pH of the aqueous buffer before mixing it with the organic modifier; the ${}^{s}_{w}\mathrm{pH}$ value is determined by measuring the pH of the buffer after mixing it with the organic modifier; and the ${}^{s}_{s}\mathrm{pH}$ is obtained by subtracting δ from the ${}^{s}_{w}\mathrm{pH}$ value (eq 10). Alternatively, ${}^{s}_{s}\mathrm{pH}$ can be directly determined if the electrode system is calibrated with reference buffers of known ${}^{s}_{s}\mathrm{pH}$ value, but the process is

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Table 3. pH Quantities for Some HPLC Buffers in Water ($^{w}_{w}$pH) and Diluted to 50% Methanol ($^{s}_{w}$pH and $^{s}_{s}$pH)

buffer	description	$^{w}_{w}$pH	$^{s}_{w}$pH	$^{s}_{s}$pH	ΔpH
A	0.01 M H_3PO_4	2.20	2.72	2.60	0.40
B	0.0065 M H_3Cit/0.0035 M H_2Cit^-	2.97	3.76	3.64	0.67
C	0.0086 M HAc/0.0014 M Ac^-	3.99	4.81	4.69	0.70
D	0.0035 M HAc/0.0065 M Ac^-	4.99	5.93	5.81	0.82
E	0.0056 M $HCit^{2-}$/0.0044 M Cit^{3-}	6.03	7.29	7.17	1.14
F	0.0051 M $H_2PO_4^-$/0.0049 M HPO_4^{2-}	7.05	8.29	8.17	1.12
G	0.0095 M NH_4^+/0.0005 M NH_3	8.01	7.53	7.41	−0.60
H	0.0065 M NH_4^+/0.0035 M NH_3	8.97	8.58	8.46	−0.51
I	0.0056 M H_3BO_3/0.0044 M $H_2BO_3^-$	9.02	9.28	9.16	0.14
J	0.008M $BuNH_3^+$/0.002 M $BuNH_2$	9.88	9.16	9.04	−0.84
K	0.001M H_3BO_3/0.09 M $H_2BO_3^-$	10.00	10.20	10.08	0.08
L	0.0019M $BuNH_3^+$/0.0081 M $BuNH_2$	11.02	10.46	10.34	−0.68

more complex and only feasible for some mobile phases. The retention of an ionizable compound for a particular HPLC system can be related to any of the three pH quantities through eqs 14, 17, or 18.

Table 3 presents the different pH quantities for some HPLC buffers we have prepared and used to relate retention of acids and bases with pH. The table also includes the ΔpH value, which is the difference between $^{s}_{s}$pH and $^{w}_{w}$pH. It may be observed that, conversely to δ, this term is not constant. For buffers prepared from neutral acids and their conjugated anionic base (solutions B−D, I, and K in Table 3) it is ∼0.7, except for $H_3BO_3/H_2BO_3^-$, which is only ∼0.1. For buffers prepared from anionic acids and their conjugated base (solutions E and F) it is larger, ∼1.1. For buffers prepared from cationic acids and their conjugated (neutral) base (solutions G, H, J, and L) it is negative, between approximately −0.5 and −0.8. These ΔpH variations can be well explained by the theory proposed by Izmailov[24,25] for dissociation of an acid in a solvent. The most simplified form of the equation is

$$^{s}_{s}pK_a = {}^{0}pK_A - {}^{0}pK_{SH^+} - B(z-1)/\epsilon_s \qquad (20)$$

where $^{0}pK_A$ and $^{0}pK_{SH^+}$ are the intrinsic dissociation pK values of the acid and protonated solvent (constant for any medium), B is a constant that depends on the temperature and average radius of the ions, ϵ_s is the dielectric constant of the medium, and z is the charge of the acid. From eqs 12 and 20

$$\Delta pH = {}^{0}pK_{H_3O^+} - {}^{0}pK_{SH^+} - B(z-1)(\epsilon_s^{-1} - \epsilon_w^{-1}) \qquad (21)$$

For solutions G, H, J, and L of Table 3, $z = +1$ and ΔpH should be approximately equal to the difference in the intrinsic pK value of protonated water and protonated 50% methanol (conjugated acids of water and 50% methanol mixed solvent). According to the results, this difference seems to be negative. For solutions B−D, I, and K, $z = 0$ and since $\epsilon_s^{-1} \gg \epsilon_w^{-1}$ an additional positive term is added to the negative $^{0}pK_{H_3O^+} - {}^{0}pK_{SH^+}$ term giving a positive ΔpH term. For solutions E and F, $z < 0$ and the positive term is larger. Equation 20 is only qualitative. The complete model[24,25] includes more terms, such as specific solvation effects, that contribute to the ΔpH variation.

The pH variation for buffering solutions prepared from strong acids and diluted from water to 50% methanol should be −log (0.5) = 0.3 pH unit. Although not completely dissociated, H_3PO_4 (buffer A) is quite a strong acid, and the pH variation is close to 0.3.

The retention of several test solutes was related to the different pH values of the buffers of Table 3 through eq 17. Results obtained by using t_R and eq 18, instead of t'_R and eq 17, were similar, but results obtained using k and eq 14 were slightly worse. The retention data obtained are presented in Table 4. Initially, retention of benzoic acid, 2,4-nitrophenol, and 2,6-dinitrophenol was measured on a C-18 column and KBr was used as holdup time marker. However, the particular column used was not stable at basic pH values and did not give reproducible and symmetric peaks for bases. Then, a polymeric column was used to measure retention of 3-nitrophenol and aniline. Potassium bromide gave large retention times in the polymeric column, and it was replaced by methanol as holdup time marker. This gave consistent t_M values, similar to those obtained for KBr in the C-18 column (see Table 4).

Table 5 presents the results obtained using the $^{s}_{w}$pH and $^{s}_{s}$pH values of the buffers. The results obtained are identical, except for the pK_a values ($^{s}_{w}$pK and $^{s}_{s}$pK, respectively) which differ in the δ amount. The fits are very good in all instances, and the $^{s}_{s}$pK values obtained are in very good agreement with the literature pK values of the acids in 50% methanol.[22] 3-Nitrophenol and aniline give negative t'_R values for their ionized forms, but in fact, the standard deviation is larger than the absolute value of the parameter and therefore t'_R is not significantly different from zero.

Table 6 reports the results obtained using the $^{w}_{w}$pH values of the buffers, i.e., the pH measured before mixing the aqueous buffer with the organic modifier. It can be observed that the statistics of the fits obtained for benzoic acid, 2,4-dinitrophenol, and 2,6-dinitrophenol in the C-18 column are only slightly worse than those of Table 5. The variation of retention time is between 3 and 6 $^{s}_{w}$pH values, or between 2.5 and 5.5 $^{w}_{w}$pH values, approximately. This pH range is covered by the buffer solutions B−D of Table 3. For these three buffers, the ΔpH value is rather constant, since the three have been prepared from neutral acids. It can be also observed that the difference between the fitting

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Table 4. Retention Times of Some Test Solutes in C-18 or Polymeric Columns and the Buffers of Table 3 as Mobile Phase (50% methanol)

	retention time (min)						
buffer	KBr (t_M)[a]	benzoic acid[a]	2,4-dinitrophenol[a]	2,6-dinitrophenol[a]	methanol (t_M)[b]	3-nitrophenol[b]	aniline[b]
A	2.22	7.74	9.41	7.08	2.27	34.06	2.35
B	2.11	7.62	8.15	5.69	2.05	35.51	4.94
C	1.91	6.71	5.22	3.66	2.23	34.40	11.34
D	2.14	3.51	2.96	2.76	2.33	35.53	14.26
E	2.24	2.68	3.42	3.32	2.31	34.88	14.00
F	2.29	2.49	3.13	3.09	2.33	31.62	14.77
G					2.33	34.89	14.61
H					2.12	29.50	14.63
I					2.17	14.72	14.64
J					2.23	19.41	14.35
K					2.22	4.75	14.13
L					2.17	4.13	13.71

[a] In C-18 column. [b] In polymeric column.

Table 5. Retention Parameters for Test Solutes Obtained Using Eq 25 and the ${}^{s}_{w}$pH and ${}^{s}_{s}$pH Values of Buffers of Table 3

	$t'_{R\,(HA)}$	$t'_{R(A)}$	${}^{s}_{w}pK$	${}^{s}_{s}pK$	sd	F	${}^{s}_{s}pK_{lit.}$
benzoic acid[a]	5.64 ± 0.13	0.23 ± 0.13	5.36 ± 0.08	5.24 ± 0.08	0.19	470	5.23
2,4-dinitrophenol[a]	7.14 ± 0.26	0.88 ± 0.18	4.50 ± 0.10	4.38 ± 0.10	0.29	228	4.41
2,6-dinitrophenol[a]	4.93 ± 0.26	0.84 ± 0.14	4.09 ± 0.14	3.97 ± 0.14	0.24	124	4.14
3-nitrophenol[b]	32.87 ± 0.24	−0.26 ± 0.56	9.21 ± 0.03	9.09 ± 0.03	0.61	2145	8.94
anilinium ion[b]	−0.04 ± 0.36	12.12 ± 0.12	4.32 ± 0.07	4.20 ± 0.07	0.36	733	4.23

[a] In C-18 column. [b] In polymeric column.

Table 6. Retention Parameters for Test Solutes Obtained Using Eq 25 and the ${}^{w}_{w}$pH Values of Buffers of Table 3

	$t'_{R\,(HA)}$	$t'_{R(A)}$	${}^{w}_{w}pK$	sd	F	${}^{s}_{s}pK - {}^{w}_{w}pK$	${}^{w}_{w}pK_{lit.}$
benzoic acid[a]	5.67 ± 0.17	0.16 ± 0.18	4.57 ± 0.10	0.24	277	0.66	4.19
2,4-dinitrophenol[a]	7.18 ± 0.29	0.86 ± 0.19	3.75 ± 0.11	0.32	195	0.66	4.10
2,6-dinitrophenol[a]	5.00 ± 0.29	0.84 ± 0.14	3.33 ± 0.15	0.25	115	0.81	3.73
3-nitrophenol[b]	32.28 ± 1.93	3.27 ± 4.08	9.22 ± 0.27	4.95	28	−0.28	8.43
anilinium ion[b]	12.20 ± 0.12	−0.47 ± 0.40	3.46 ± 0.07	0.35	765	0.77	4.60

[a] In C-18 column. [b] In polymeric column.

parameter ${}^{w}_{w}pK$ and the literature ${}^{s}_{s}$pH is close to the ΔpH value for buffers B−D.

For the polymeric column, the results obtained for aniline with ${}^{w}_{w}$pH (Table 6) are also similar to those obtained with ${}^{s}_{w}$pH and ${}^{s}_{s}$pH (Table 5), because the variation of retention time of aniline (Figures 3 and 4) encompasses a pH range similar to the variation for the acids studied in the C-18 column. This range is covered by buffer solutions B and C, for which ΔpH is constant (Table 3). The difference between ${}^{w}_{w}pK$ and literature ${}^{s}_{s}pK$ values is also close to the ΔpH term for these buffers. However, the fits obtained for 3-nitrophenol with ${}^{w}_{w}$pH are much worse than those obtained with ${}^{s}_{w}$pH and ${}^{s}_{s}$pH. Figures 3 and 4 show that the variation of retention time for this phenol is produced in the pH range covered by buffers G−L. Buffers I and K have positive ΔpH values, but buffers G, H, J, and L have negative ΔpH values. The ΔpH term is not constant in this pH range, and this produces the bad fit observed in Figure 4. The results obtained for 3-nitrophenol clearly demonstrate the need to use the rigorous ${}^{s}_{w}$pH and ${}^{s}_{s}$pH quantities to obtain good relationships when buffers prepared from different types of acids are used. In addition, Table 6 shows that there is not direct relationship between the ${}^{w}_{w}pK$ value obtained from HPLC measurements and the true ${}^{w}_{w}pK$ value of the acid in pure water. HPLC-obtained ${}^{w}_{w}pK$ values of benzoic acid and 3-nitrophenol are higher than the pK value of the acids in water, but the HPLC ${}^{w}_{w}pK$ values of 2,4-dinitrophenol, 2,6-dinitrophenol,

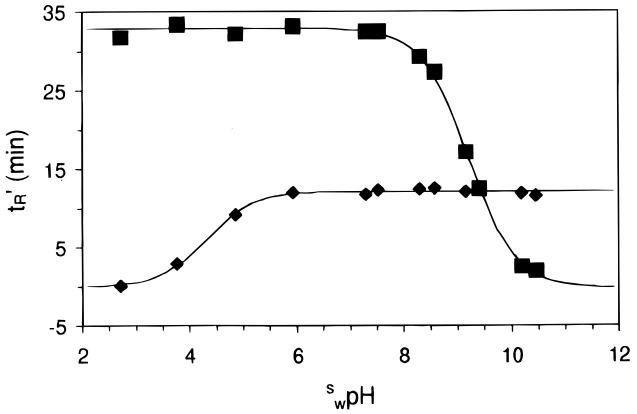

Figure 3. Variation of the adjusted retention time of (■) 3-nitrophenol and (◆) aniline in a polymeric column with the 50% methanol mobile-phase pH measured after mixing the aqueous buffer with the organic modifier with electrode calibration with aqueous buffers (${}^{s}_{w}$pH scale).

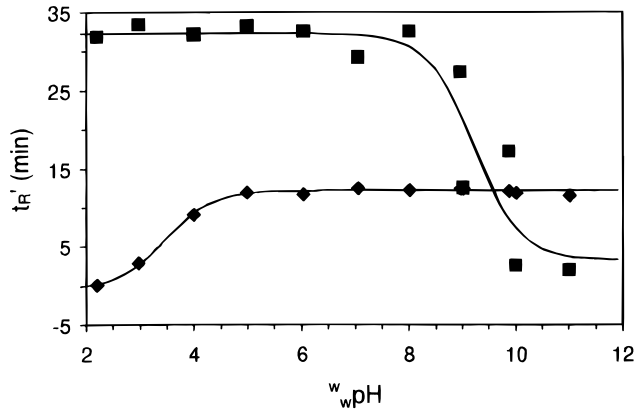

Figure 4. Variation of the adjusted retention time of (■) 3-nitrophenol and (◆) aniline in a polymeric column with the 50% methanol mobile-phase pH measured before mixing the aqueous buffer with the organic modifier ($^{w}_{w}$pH scale).

and anilinium are lower. Therefore, it does not have much sense to use the pH of the aqueous buffer before mixing it with the organic modifier and the p*K* values of the test acid in water to optimize HPLC separation methods through eqs 14, 17, or 18.

CONCLUSIONS

pH is a relative quantity that depends on the concentration scale used and on the reference state (solvent) in which the ionic activity coefficient is referred to unity at infinite dilution. These two factors must be clearly reported in pH measurements of HPLC mobile phases.

The common practice of measuring the pH of the aqueous HPLC buffer before mixing it with the organic modifier ($^{w}_{w}$pH scale) gives consistent relationships of the type of eqs 14, 17, or 18 only when HPLC buffers of the same type are used in the pH range close to the pK_a value of the chromatographied solute. Even in this instance it is difficult to relate the p*K* value obtained in the fit with the true thermodynamic p*K* values of the solute in water or in the solvent used as mobile phase.

It is recommended to measure the pH of the HPLC buffer after mixing the aqueous buffer with the organic modifier. The electrode system used can be calibrated with aqueous buffers and the pH measured will be $^{s}_{w}$pH. The $^{s}_{w}$pH scale, related to the $^{s}_{s}$pH scale through the δ constant, provides fits as good as those obtained with the $^{w}_{w}$pH scale when buffers of the same type are used and much better fits for buffers of diverse type. Moreover, the $^{s}_{w}$p*K* values obtained in the fits can be directly related to the thermodynamic $^{s}_{s}$p*K* value of the chromatographied solute in the mobile phase by means of the δ constant. The use of the $^{s}_{w}$pH scale is specially recommended in optimization of the HPLC separation of mixtures of acids and bases covering a wide range of p*K* values.

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