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Retention of ionizable compounds on HPLC. 6. pH measurements with the glass electrode in methanol–water mixtures

Immaculada Canals, Fadoua Z. Oumada, Martí Rosés, Elisabeth Bosch*

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

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Abstract

The relationship, δ values, between the two rigorous pH scales, ${}^s\text{pH}$ (pH measured in a methanol–water mixture and referred to the same mixture as standard state) and ${}^w\text{pH}$ (pH measured in a methanol–water mixture but referred to water as standard state), in several methanol–water mixtures was determined ($\delta = {}^s\text{pH} - {}^w\text{pH}$). δ values were measured using a combined glass electrode and a wide set of buffer solutions. The results are consistent with those obtained with the hydrogen electrode. This confirms the aptness of the glass electrode to achieve rigorous pH measurements in methanol–water mixtures. An equation that relates δ and composition of methanol–water mixtures, and allows δ computation at any composition by interpolation, is proposed. Therefore, ${}^s\text{pH}$ can be achieved from the experimental ${}^w\text{pH}$ value and δ at any mobile phase composition. ${}^s\text{pH}$ (or ${}^w\text{pH}$) values are related to the chromatographic retention of ionizable compounds through their thermodynamic acid–base constants in the methanol–water mixture used as mobile phase. These relationships were tested for the retention variation of several acids and bases with the pH of the mobile phase. Therefore, the optimization of the mobile phase acidity for any analyte can be easily reached avoiding the disturbances observed when ${}^w\text{pH}$ is used. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: pH measurements; Glass electrode; Mobile phase composition; Ionizable compounds

1. Introduction

An accurate measurement and control of mobile phase pH is required, in many instances, for efficient separations of ionizable compounds by HPLC [1–10]. A revision and critical evaluation of the common procedures used to measure the pH of chromatographic mobile phases in terms of the IUPAC definitions and rules was recently published [11,12]. Literature shows numerous pH and p*K* data con-

cerning methanol–water mixtures [13,14], which are very common mobile phases [1,2], and most of the available data reported were properly analysed [8,9,15–18].

In this paper, the medium effect, δ term, calculated as the difference between the two rigorous pH values (${}^w\text{pH}$ and ${}^s\text{pH}$) for several organic contents methanol–aqueous buffer mixtures is determined. Moreover, pH measurement procedures that should be used in methanol–water mobile phases to obtain accurate chromatographic retention–pH relationships are used. These rigorous procedures involve the measurement of the mobile phase pH after mixing the aqueous buffer and the organic modifier. In the

*Corresponding author. Tel.: +34-93-4021-284; fax: +34-93-4021-233.

E-mail address: bosch@apolo.qui.ub.es (E. Bosch).

first one an electrode system calibrated with aqueous buffers (which leads to the ${}^s\text{pH}$ scale) is used. In the second procedure the potentiometric system is calibrated with buffers prepared in the same methanol–water solvent composition used as mobile phase (which leads to the ${}^s\text{pH}$ scale). Some chromatographical examples that show the improvement achieved with the use of any of the two rigorous scales (${}^s\text{pH}$ or ${}^s\text{pH}$) instead of the widely used ${}^w\text{pH}$ scale (pH measured in the aqueous buffer before mixing it with the organic modifier with the electrode system calibrated with standard aqueous buffers) are presented.

2. pH scales in methanol–water mixtures

The quantity pH is defined in terms of the hydrogen ion activity (a_{H}) [19]:

$$\text{pH} = -\log a_{\text{H}} \quad (1)$$

which is related to the hydrogen ion concentration through the activity coefficient of hydrogen ion (γ_{H}). Therefore, although pH and activity are dimensionless, their value depends on the concentration scale and standard state chosen for activity. This leads to different pH scales, pH_c and pH_m , (pH scales referred to molarity and molality, respectively) which are related by means of

$$\text{pH}_c = \text{pH}_m + \log(\rho/\rho^0) \quad (2)$$

ρ is the solvent density that can be calculated from

the molar volume, V_M , and solvent composition, and ρ^0 is 1 kg dm^{-3} . Parameters to compute V_M and ρ at any intermediate solvent composition are given in Refs. [8] and [11], respectively. Table 1 shows the value of these quantities for several methanol–water mixtures. All the pH measurements reported in this paper were taken in the molarity scale that is the most used in analytical work. For simplicity, the right-hand subscript c in the pH notation is deleted.

To report pH measurements in non-aqueous and mixed solvents, the standard state for the activity, namely the medium in which the ionic activity coefficients, γ , are referred to unity at infinite dilution, must be specified. For pH measurements in water the solvent for standard state is water itself (w), but for measurements in another solvent, methanol–water mixtures in this instance, the most common solvents for the standard state can be the same solvent mixture (s), or water (w). This leads to two different pH scales, for which the notation recently recommended by the IUPAC [11,19] is followed, despite other nomenclatures previously used [20,21]. Thus, lower-case left-hand superscripts will indicate the solvent (w or s) in which measurements are being made and 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). The two pH scales in methanol–water are represented as ${}^s\text{pH}$ if the standard state solvent is water or ${}^s\text{pH}$ if the standard state solvent is the same methanol–water mixture where pH measurements are being made. When the pH measurements are taken in water, the pH scale is written as ${}^w\text{pH}$.

Table 1
Macroscopic properties of methanol–water mixtures [8]

% MeOH (v/v)	x_{MeOH}	V_M^a	ρ (g/ml)	$\log \rho$	A	$a_o B$	$\text{p}K_{\text{ap}}$
0	0.000	18.11	0.9948	−0.002	0.53	1.50	14.00
10	0.047	19.01	0.9826	−0.008	0.56	1.53	14.08
20	0.100	20.03	0.9693	−0.014	0.60	1.57	14.08
30	0.160	21.22	0.9548	−0.020	0.64	1.62	14.07
40	0.229	22.61	0.9388	−0.027	0.70	1.67	14.09
50	0.308	24.25	0.9209	−0.036	0.78	1.73	14.14
60	0.400	26.23	0.9008	−0.045	0.88	1.80	14.23
70	0.509	28.65	0.8780	−0.057	1.02	1.88	14.39
80	0.640	31.69	0.8518	−0.070	1.21	1.99	14.63
90	0.800	35.58	0.8216	−0.085	1.48	2.13	15.04
100	1.000	40.71	0.7870	−0.104	1.87	2.31	16.77

^a Molar volume.

As explained before [11,19], the two methanol–water pH scales differ in the primary medium effect, which is related to the standard Gibbs energy change for the transfer of the H^+ ion from water (w) to the solvent (s), ${}^s_w\gamma_H^0$, according to

$${}^s_w\text{pH} = {}^s\text{pH} - \log({}^s_w\gamma_H^0) \quad (3)$$

Since the pH scale in each solvent is determined by the autoprotolysis constant $\text{p}K_{\text{ap}}$ of the medium [15], the ${}^s\text{pH}$ scale ranges from 0 to ${}^s\text{p}K_{\text{ap}}$ and the ${}^s_w\text{pH}$ scale between $-\log({}^s_w\gamma_H^0)$ and ${}^s\text{p}K_{\text{ap}} - \log({}^s_w\gamma_H^0)$. Values of ${}^s\text{p}K_{\text{ap}}$ for methanol–water mixtures can be calculated by the equations given in literature [15,16] and some of them are included in Table 1.

The IUPAC remarks that the above definitions of pH are only notional because they involve a single ion activity (a_H) which is immeasurable [19,22,23] but it can be estimated from the hydrogen ion concentration and activity coefficient (γ_H). The latter is usually computed by the Debye–Hückel equation:

$$\log \gamma_H = -AI^{1/2}/(1 + a_0BI^{1/2}) \quad (4)$$

where 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 to a value fixed by the Bates–Guggenheim convention extended to the general solvent s [22,23]. Table 1 reports values of A and a_0B terms at 25°C for several methanol–water compositions that allow estimation of the hydrogen ion activity coefficient. Since this coefficient cannot be measured, the strict IUPAC pH definition is operational, namely, in terms of the operation or method used to determine pH. This method consists in measuring the e.m.f. of an appropriate potentiometric cell (E_X) and comparing it with the e.m.f. (E_S) of the same potentiometric cell except for the sample solution is replaced by a standard solution of assigned pH (pH_S). Then,

$$\text{pH}_X = \text{pH}_S - (E_X - E_S)/g \quad (5)$$

where pH_X is the pH of the sample solution and g is the Nernst constant [19]. If the potentiometric system is calibrated with standards prepared in water, with assigned pH in water, the operational pH obtained is in the ${}^s_w\text{pH}$ scale. But, if the standards are prepared in the methanol–water mixture and have assigned pH

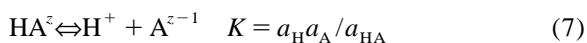
values in these mixtures, the obtained value is in the ${}^s\text{pH}$ scale. The operational difference between both scales is the δ term:

$$\delta = \overline{E}_j - \log({}^s_w\gamma_H^0) = {}^s_w\text{pH} - {}^s\text{pH} \quad (6)$$

which includes the primary medium effect, $-\log({}^s_w\gamma_H^0)$, and the difference of the liquid-junction potentials, \overline{E}_j , expressed in pH units and assumed to be constant. Eq. (6) only agrees with Eq. (3) if \overline{E}_j is negligible as compared with $-\log({}^s_w\gamma_H^0)$. It must be remarked here that the primary medium effect, $-\log({}^s_w\gamma_H^0)$, depends only on the solvent s at which pH is measured, but that the liquid-junction potential depends also on the particular electrode system, pH standards, and sample used. Therefore, general inter-laboratory conversion between both pH scales is only possible if the different electrode systems are designed to have negligible liquid-junction potentials, namely, if $\delta = -\log({}^s_w\gamma_H^0)$. Usually this can be achieved with a salt bridge containing a solution of an equitransferent binary salt (e.g., KCl) at a much higher concentration than the sample and standard solutions [19]. The most recent IUPAC publication [19] encourages the use of the glass electrode instead of the formerly recommended hydrogen electrode. So, δ values measured with the glass electrode in methanol–water mixtures must be compared with the published values earlier determined with the hydrogen electrode. This will allow the evaluation of the \overline{E}_j contribution to the δ quantity and, therefore, the aptness of δ values to calculate ${}^s\text{pH}$ from the experimental ${}^s_w\text{pH}$ value when the measurements in methanol–water are taken using a glass electrode.

3. Chromatographic retention and mobile phase pH scales

The retention factor k of a compound with an acid–base equilibrium of the type



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

$$k = \frac{([\text{HA}]k_{\text{HA}} + [\text{A}]k_{\text{A}})/([\text{HA}] + [\text{A}])}{(k_{\text{HA}} + k_{\text{A}}10^{\text{pH}-\text{pK}'})/(1 + 10^{\text{pH}-\text{pK}'})} \quad (8)$$

where pK' is the acidity pK in terms of the concentration of the two species, instead of activities. Since retention factor is related to the adjusted retention time (t'_{R}) and to the retention time (t_{R}) of the solute through the column hold-up time (t_{M}) by means of

$$k = t'_{\text{R}}/t_{\text{M}} = (t_{\text{R}} - t_{\text{M}})/t_{\text{M}} \quad (9)$$

the following expressions can be easily derived provided the hold-up time does not change with pH:

$$t'_{\text{R}} = (t'_{\text{R}(\text{HA})} + t'_{\text{R}(\text{A})}10^{\text{pH}-\text{pK}'})/(1 + 10^{\text{pH}-\text{pK}'}) \quad (10)$$

$$t_{\text{R}} = (t_{\text{R}(\text{HA})} + t_{\text{R}(\text{A})}10^{\text{pH}-\text{pK}'})/(1 + 10^{\text{pH}-\text{pK}'}) \quad (11)$$

Eqs. (8), (10), and (11) predict a sigmoidal relationship between the retention of the solute and the pH of the mobile phase which can be measured in different pH scales [11,20]. If the $^{\text{s}}\text{pH}$ scale is used, the inflection point of the sigmoidal plot must agree with the pK' value of the solute in the particular methanol–water mixture and referred also to this mixture as standard state ($^{\text{s}}\text{pK}'$). If $^{\text{w}}\text{pH}$ is used, the pH of inflection must agree with the pK' value of the solute in the methanol–water mixture but referred to water as standard state ($^{\text{w}}\text{pK}'$). The relationship between both quantities is

$$^{\text{s}}\text{pK}' - ^{\text{w}}\text{pK}' = \delta \quad (12)$$

However, it has been demonstrated that the difference between $^{\text{w}}\text{pH}$ and $^{\text{s}}\text{pH}$ or $^{\text{s}}\text{pH}$ is buffer dependent [11]. Therefore, Eqs. (8), (10), and (11) do not hold for the $^{\text{w}}\text{pH}$ scale. Only approximate relationships, without any thermodynamic meaning for the inflection point, can be obtained if buffers of the same type are used in the pH range of variation of retention.

4. Experimental

4.1. Apparatus

pH measurements were taken with a Ross Combi-

nation Electrode Orion 8102 in a Crison micropH 2002 potentiometer with a precision of ± 0.1 mV (± 0.002 pH units). Retention data were measured on a 25-cm \times 4.0-mm I.D. Merck LiChrospher 100 RP-18 column (5 μm) with a flow of 1 ml min^{-1} or on a 15-cm \times 4.6-mm I.D. Polymer Labs PLRP-S 100 \AA column (15–20 μm) with a flow of 3 ml min^{-1} in an ISCO Model 2350 dual-pump system with a 20- μl loop valve. A variable-wavelength V⁴ absorbance detector (ISCO) set at 254 or 282 nm was used. All data were taken in triplicate at 25°C with the potentiometric cell and columns thermostated with water jackets.

4.2. Chemicals

Methanol for chromatography from Merck and water purified by the Milli-Q plus system from Millipore were used. Benzoic acid (Merck, p.a., >99.95%); 2,4-dinitrophenol (Doesder); 2,4,6-trimethylpyridine (Merck, p.a., >99%); *N,N*-dimethylbenzylamine (Merck, p.s., >98%) were used too. Buffers were prepared from acetic acid (Merck, p.a., 100%); sodium acetate anhydrous (Merck, p.a., >99%); phosphoric acid (Merck, p.a., 85%); sodium acetate anhydrous (Merck, p.a., >99%); phosphoric acid (Merck, p.a., 85%); potassium dihydrogen phosphate (Merck, p.a., >99.5%); sodium hydrogen phosphate anhydrous (Merck, p.a., >99%); citric acid (Fluka, p.a., $\geq 99.5\%$); potassium dihydrogen citrate (Fluka, MicroSelect, >99%); trisodium citrate dihydrate (Merck, p.a., >99%); butylamine (Aldrich, 99.5%); sodium tetraborate decahydrate (Aldrich, ACS); hydrochloric acid (Merck, p.a., 25%); ammonia solution (Merck, p.a., 25%) and ammonium chloride (Merck, p.a., >99.8%), ammonium acetate (Carlo Erba, RPE, 98%) and potassium hydroxide (Panreac, p.a., 85%).

4.3. Procedure

The experimental procedure already described for methanol–water mixtures at 50% in volume [11] was used. $^{\text{s}}\text{pH}$ values were calculated from the $^{\text{s}}\text{pK}$ of buffer components at 20, 40, 60 and 80% in volume of methanol. These $^{\text{s}}\text{pK}$ values were interpolated from $^{\text{s}}\text{pK}$ of the buffering acids at different methanol–water compositions obtained from literature

[14]. In order to account for errors in the literature ${}^s\text{p}K$ values, the potential of each buffer (sE_X) was measured and related to its ${}^s\text{pH}_X$ through the Nernst equation [19]:

$${}^sE_X = ({}^sE_0 + {}^sE_{jX}) - k({}^s\text{pH}_X) \quad (13)$$

where sE_0 is the standard potential, ${}^sE_{jX}$ the liquid junction potential (assumed to be negligible or at least constant for all buffers), and k is the Nernst constant (0.059157 at 25°C). The $({}^sE_0 + {}^sE_{jX})$ quantity was calculated for each buffer from the measured sE_X potential and the theoretically calculated ${}^s\text{pH}_X$ value. The overall $({}^sE_0 + {}^sE_{jX})$ constant of the electrode system in each methanol percentage was calculated averaging the individual constants of different buffers, and from this averaged constant a new ${}^s\text{pH}$ value was calculated for each buffer from the measured potential. These new buffer ${}^s\text{pH}$ values provided better results than the initial ones.

In addition, the ${}^w\text{pH}$ value of each buffer was measured after calibration of the electrode system with the usual aqueous standard reference buffers of potassium hydrogenphthalate (${}^w\text{pH}=4.00$) and potassium dihydrogen phosphate/disodium hydrogen phosphate (${}^w\text{pH}=7.02$). The δ quantity for each buffer was obtained by subtraction of the experimental ${}^s\text{pH}$ from the measured ${}^w\text{pH}$ according to Eq. (6).

5. Results and discussion

5.1. Buffer solutions

The buffer solutions selected to determine δ values are given in Tables 2–5 and they encompass a wide pH interval, from about 3 to 10.5, which is the best response range of glass electrode. The first eight buffer solutions are the ones recommended by the IUPAC in aqueous solution [19] and they have been complemented with several appropriate buffers selected previously [11]. Nevertheless, buffers from biphthalate were omitted because it does not offer reliable ${}^s\text{p}K$ values in methanol–water mixtures. Table 6 shows the ${}^w\text{p}K$ values of the buffering acids as well as their ${}^s\text{p}K$ at several methanol percentages, which were interpolated from the experimental ones at several methanol contents mixtures. The interpolation was made by means of equations developed elsewhere [18] that relate ${}^s\text{p}K$ to the mole fraction of methanol in the binary solvent. However, although phosphoric and citric acids are very common chemicals in the preparation of buffered mobile phases, their values at 60 and 80% in volume of methanol have been determined potentiometrically here because of the lack of literature values above 50% of methanol. The obtained results at 60 and 80% of methanol were, respectively, 8.55 and 9.53 for ${}^s\text{p}K_2$

Table 2
Determination of the δ term for 20% methanol (v/v)

Buffer solution	${}^s\text{pH}_{\text{theo}}$	sE (mV)	${}^sE_0 + {}^sE_{jX}$	${}^s\text{pH}_{\text{exp}}$	${}^s\text{pH}$	δ
0.1 m H_2Cit^-	4.01	170.8	407.8	4.00	4.00	0.00
0.1 M $\text{HAc}/0.1$ M Ac^-	4.89	119.0	408.5	4.87	4.88	0.01
0.1 M $\text{HAc}/0.01$ M Ac^-	3.97	174.9	409.7	3.93	3.93	0.00
0.025 m $\text{H}_2\text{PO}_4^-/0.025$ m HPO_4^{2-}	7.16	-20.1	403.5	7.22	7.26	0.04
0.0087 m $\text{H}_2\text{PO}_4^-/0.0304$ m HPO_4^{2-}	7.70	-52.5	403.3	7.77	7.82	0.05
0.01 m $\text{H}_2\text{PO}_4^-/0.04$ m HPO_4^{2-}	7.73	-54.1	403.2	7.80	7.85	0.05
0.05 m $\text{B}_4\text{O}_7^{2-}$	9.23	-139.1	407.1	9.23	9.30	0.07
0.01 m $\text{B}_4\text{O}_7^{2-}$	9.22	-137.8	407.4	9.21	9.28	0.07
0.05 M $\text{H}_3\text{PO}_4/0.05$ M H_2PO_4^-	2.56	262.1	413.5	2.45	2.43	-0.02
0.05 M $\text{H}_3\text{Cit}/0.05$ M H_2Cit^-	3.28	215.8	410.0	3.24	3.23	-0.01
0.05 M $\text{HAc}/0.05$ M Ac^-	4.91	118.3	408.8	4.88	4.90	0.01
0.05 M $\text{H}_2\text{Cit}^-/0.05$ M HCit^{2-}	4.62	133.7	407.0	4.62	4.63	0.01
0.05 M $\text{HCit}^{2-}/0.05$ M Cit^{3-}	5.87	59.6	406.8	5.88	5.90	0.02
0.05 M $\text{H}_2\text{PO}_4^-/0.05$ M HPO_4^{2-}	7.04	-12.8	403.7	7.10	7.14	0.04
0.05 M $\text{NH}_4^+/0.05$ M NH_3	9.16	-134.0	407.8	9.15	9.22	0.07
0.05 M $\text{BuNH}_3^+/0.05$ M BuNH_2	10.46	-211.6	407.5	10.46	10.54	0.08

Table 3
Determination of the δ term for 40% methanol (v/v)

Buffer solution	${}^s\text{pH}_{\text{theo}}$	sE (mV)	${}^sE_0 + {}^sE_{jX}$	${}^s\text{pH}_{\text{exp}}$	${}^s_w\text{pH}$	δ
0.1 m H_2Cit^-	4.34	134.0	390.7	4.33	4.41	0.08
0.1 M HAc/0.1 M Ac^-	5.22	82.4	391.2	5.20	5.29	0.09
0.1 M HAc/0.01 M Ac^-	4.30	137.7	391.9	4.27	4.34	0.07
0.025 m H_2PO_4^- /0.025 m HPO_4^{2-}	7.59	-62.0	387.3	7.65	7.77	0.12
0.0087 m H_2PO_4^- /0.0304 m HPO_4^{2-}	8.14	-93.4	388.1	8.18	8.31	0.13
0.01 m H_2PO_4^- /0.04 m HPO_4^{2-}	8.16	-95.8	387.1	8.22	8.35	0.13
0.05 m $\text{B}_4\text{O}_7^{2-}$	9.24	-161.7	384.8	9.33	9.49	0.15
0.01 m $\text{B}_4\text{O}_7^{2-}$	9.29	-160.5	389.3	9.31	9.46	0.15
0.05 M H_3PO_4 /0.05 M H_2PO_4^-	2.91	223.2	395.5	2.82	2.85	0.03
0.05 M H_3Cit /0.05 M H_2Cit^-	3.60	180.7	393.7	3.54	3.58	0.04
0.05 M HAc/0.05 M Ac^-	5.23	82.0	391.2	5.21	5.29	0.07
0.05 M H_2Cit^- /0.05 M HCit^{2-}	4.91	99.7	390.3	4.91	4.99	0.07
0.05 M HCit^{2-} /0.05 M Cit^{3-}	6.23	26.6	395.2	6.15	6.24	0.09
0.05 M H_2PO_4^- /0.05 M HPO_4^{2-}	7.42	-50.2	388.8	7.45	7.57	0.13
0.05 M NH_4^+ /0.05 M NH_3	8.99	-141.5	390.6	8.99	9.13	0.14
0.05 M BuNH_3^+ /0.05 M BuNH_2	10.20	-214.4	389.2	10.22	10.38	0.15

of phosphoric acid and 4.10 and 4.72 for ${}^s\text{p}K_1$, 5.93 and 6.59 for ${}^s\text{p}K_2$ and 7.74 and 8.56 for ${}^s\text{p}K_3$ of citric acid.

5.2. δ Parameter

Tables 2–5 show the parameters obtained from different buffers. All solvent mixtures gave con-

sistent results. The quantity (${}^sE_0 + {}^sE_{jX}$) shows standard deviations of 5 mV or less but the scattering of the results is higher for higher methanol contents since the measurements are slightly less reproducible at high contents of organic component. Obtained δ quantities are also consistent for each mixture showing standard deviations of 0.04 pH units or less. The mean values of these quantities and their standard

Table 4
Determination of the δ term for 60% methanol (v/v)

Buffer solution	${}^s\text{pH}_{\text{theo}}$	sE (mV)	${}^sE_0 + {}^sE_{jX}$	${}^s\text{pH}_{\text{exp}}$	${}^s_w\text{pH}$	δ
0.1 m H_2Cit^-	4.74	106.5	387.2	4.74	4.87	0.13
0.1 M HAc/0.1 M Ac^-	5.61	53.1	385.0	5.64	5.79	0.15
0.1 M HAc/0.01 M Ac^-	4.69	107.8	385.4	4.72	4.85	0.14
0.025 m H_2PO_4^- /0.025 m HPO_4^{2-}	8.16	-96.0	386.9	8.16	8.37	0.21
0.0087 m H_2PO_4^- /0.0304 m HPO_4^{2-}	8.71	-129.7	385.3	8.73	8.94	0.21
0.01 m H_2PO_4^- /0.04 m HPO_4^{2-}	8.73	-130.8	385.4	8.75	8.96	0.21
0.05 m $\text{B}_4\text{O}_7^{2-}$	9.38	-172.1	382.8	9.45	9.66	0.21
0.01 m $\text{B}_4\text{O}_7^{2-}$	9.44	-171.5	387.0	9.44	9.65	0.21
0.05 M H_3PO_4 /0.05 M H_2PO_4^-	3.37	201.4	400.7	3.13	3.21	0.08
0.05 M H_3Cit /0.05 M H_2Cit^-	3.93	155.1	387.5	3.92	4.02	0.10
0.05 M HAc/0.05 M Ac^-	5.60	53.6	384.9	5.63	5.78	0.15
0.05 M H_2Cit^- /0.05 M HCit^{2-}	5.27	75.5	387.2	5.26	5.40	0.14
0.05 M HCit^{2-} /0.05 M Cit^{3-}	6.46	5.7	387.6	6.44	6.61	0.17
0.05 M H_2PO_4^- /0.05 M HPO_4^{2-}	7.90	-77.8	389.3	7.85	8.04	0.19
0.05 M NH_4^+ /0.05 M NH_3	8.75	-137.8	379.9	8.87	9.05	0.18
0.05 M BuNH_3^+ /0.05 M BuNH_2	9.96	-202.0	387.2	9.95	10.15	0.19

Table 5
Determination of the δ term for 80% methanol (v/v)

Buffer solution	${}^s\text{pH}_{\text{theo}}$	sE (mV)	${}^sE_0 + {}^sE_{\text{JX}}$	${}^s\text{pH}_{\text{exp}}$	${}^s\text{pH}_w$	δ
0.1 m H_2Cit^-	5.38	74.2	392.2	5.39	5.43	0.04
0.1 M $\text{HAc}/0.1 \text{ M Ac}^-$	6.28	19.2	390.5	6.32	6.38	0.06
0.1 M $\text{HAc}/0.01 \text{ M Ac}^-$	5.36	73.6	390.8	5.40	5.44	0.04
0.025 m $\text{H}_2\text{PO}_4^-/0.025 \text{ m HPO}_4^{2-}$	9.13	-146.8	393.4	9.13	9.23	0.11
0.0087 m $\text{H}_2\text{PO}_4^-/0.0304 \text{ m HPO}_4^{2-}$	9.67	-178.5	393.5	9.66	9.78	0.12
0.01 m $\text{H}_2\text{PO}_4^-/0.04 \text{ m HPO}_4^{2-}$	9.69	-178.9	394.1	9.67	9.77	0.10
0.05 m $\text{B}_4\text{O}_7^{2-}$	9.70	-171.7	401.9	9.55	9.66	0.12
0.01 m $\text{B}_4\text{O}_7^{2-}$	9.76	-171.1	406.2	9.54	9.66	0.13
0.05 M $\text{H}_3\text{PO}_4/0.05 \text{ M H}_2\text{PO}_4^-$	4.19	141.4	389.2	4.25	4.27	0.02
0.05 M $\text{H}_3\text{Cit}/0.05 \text{ M H}_2\text{Cit}^-$	4.63	120.8	394.5	4.60	4.63	0.03
0.05 M $\text{HAc}/0.05 \text{ M Ac}^-$	6.22	22.3	390.5	6.27	6.33	0.06
0.05 M $\text{H}_2\text{Cit}^-/0.05 \text{ M HCit}^{2-}$	5.74	51.1	390.9	5.78	5.83	0.04
0.05 M $\text{HCit}^{2-}/0.05 \text{ M Cit}^{3-}$	7.69	-62.3	392.3	7.70	7.79	0.10
0.05 M $\text{H}_2\text{PO}_4^-/0.05 \text{ M HPO}_4^{2-}$	9.13	-147.0	393.0	9.13	9.24	0.11
0.05 M $\text{NH}_4^+/0.05 \text{ M NH}_3$	8.77	-134.0	384.7	8.91	8.99	0.08
0.05 M $\text{BuNH}_3^+/0.05 \text{ M BuNH}_2$	9.80	-188.4	391.2	9.83	9.92	0.09

deviations as well as two additional series of δ values measured by deLigny and Rehbach [24] and Bates et al. [25] are given in Table 7. These literature values [24,25] were obtained using a hydrogen electrode and given in the molality scale, but they were converted here to the molarity scale through Eq. (2) to compare them with values obtained in this work with the glass electrode. The three series of experimental δ values are consistent and, therefore, all values were fitted together to the following

empirical equation that relates them with the methanol volume fraction, v_{MeOH} :

$$\delta = (0.09v_{\text{MeOH}} - 0.11v_{\text{MeOH}}^2)/(1 - 3.15v_{\text{MeOH}} + 3.51v_{\text{MeOH}}^2 - 1.35v_{\text{MeOH}}^3)$$

$$n = 16 \quad r^2 = 0.999 \quad s = 0.008 \quad F = 19453 \quad (14)$$

where n is the number of points and r , s and F , the correlation coefficient, standard deviation and Fisher

Table 6
 ${}^s\text{pK}$ values of acids in methanol–water

	% Methanol (v/v)					
	0	20	40	50	60	80
<i>Acid:</i>						
Phosphoric						
pK_1	2.11	2.60	3.00	3.22	3.50	4.25
pK_2	7.20	7.52	7.95	8.23	8.57	9.53
Citric						
pK_1	3.13	3.41	3.75	3.94	4.12	4.72
pK_2	4.76	5.09	5.44	5.67	5.92	6.59
pK_3	6.40	6.80	7.30	7.53	7.78	8.55
Acetic	4.76	5.02	5.34	5.53	5.74	6.41
Boric	9.23	9.28	9.36	9.43	9.51	9.83
Ammonium	9.24	9.08	8.88	8.76	8.64	8.58
Butylammonium	10.59	10.37	10.09	9.95	9.82	9.61

Table 7
Values of δ parameter (molarity) in methanol–water mixtures

% MeOH (v/v)	${}^sE_0 + {}^sE_{jx}$ (SD)	δ (SD)	δ [24]	δ [25]
20	407.2 (2.8)	0.03 (0.03)		
24.01			0.03	0.03
40	390.3 (2.9)	0.10 (0.04)		
40.50			0.08	0.09
50	387.4 (2.4)	0.13 (0.01)		
55.83			0.17	0.17
60	386.8 (4.3)	0.17 (0.04)		
70.13			0.21	0.20
80	393.1 (5.0)	0.08 (0.04)		
83.49			0.02	
91.92			−0.42	
100			−2.24	

statistic, respectively. This equation allows an accurate calculation of δ for any mixture by interpolation.

Fig. 1 shows the δ values obtained here as well as those from literature and the line stands for values calculated by means of Eq. (14). Values up to 70% (v/v) of methanol are slightly positive and increase with the methanol percentage but at higher organic modifier contents δ values decrease and become strongly negative for pure methanol. As far as we know, this is the first time that the δ values have been determined for different methanol–water mixtures using a combined glass electrode. Its agreement with those measured with a hydrogen electrode [24,25], together with the consistency of (${}^sE_0 + {}^sE_{jx}$) values from different buffers in each methanol–water mixture, proves that the liquid junction potential term is negligible in rapport to the primary medium

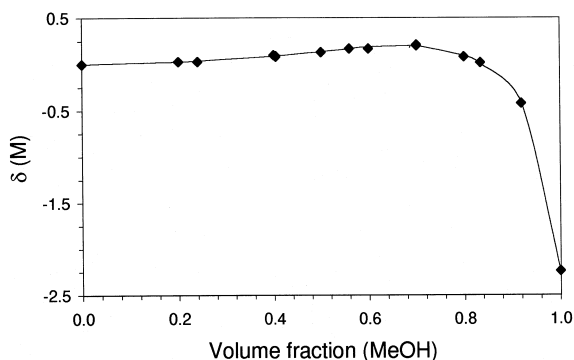


Fig. 1. Variation of the δ quantity in molar scale with solvent composition in methanol–water mixtures. Line calculated by means of Eq. (14).

effect term in the used glass electrode potentiometric system (see Eq. (6)). Therefore, the δ values are a good estimation of the primary medium effect, $-\log({}^s\gamma_H^0)$. This fact allows to use directly these δ values with other electrode systems or by other laboratories, provided they assure that the liquid junction potential of their system is also negligible. This is easily verifiable using the reference ${}^s\text{pH}$ values given in Tables 2–5.

Therefore, the obtained results allow the use of the glass electrode for rigorous measurements of ${}^s\text{pH}$ and ${}^s\text{pH}$ in methanol–water mixtures and they support the last recommendation of the IUPAC about the aptness of the glass electrode instead of the hydrogen electrode for rigorous pH measurements.

5.3. Chromatographic retention of ionizable compounds

Rigorous use of Eqs. (8), (10) and (11) involves the ${}^s\text{pH}$ or ${}^s\text{pH}$ of the mobile phase and ${}^s\text{pK}'$ or ${}^s\text{pK}'$ of the analyte instead of the ${}^w\text{pH}$ and ${}^w\text{pK}'$, as commonly done in everyday practice. The true ${}^s\text{pH}$ of any buffered mobile phase can be achieved from the experimental ${}^s\text{pH}$ and the suitable δ value given in Table 7 or calculated by means of Eq. (14). Values of ${}^s\text{pH}$ and ${}^s\text{pH}$ of the buffered mobile phases used in this work as well as the ${}^w\text{pH}$, measured in the aqueous part before mixing it with methanol, are shown in Tables 8 and 9.

As discussed in previous papers [8,11], the shift of pK of the acids used in buffers preparation with the percentage of organic modifier depends on the type

Table 8
pH quantities for some HPLC buffers in water (^wpH) and diluted to 40, 60 and 80% methanol (^spH and ^spH) used in the C_{18} column

	^wpH	40% Methanol		60% Methanol		80% Methanol	
		^spH	^spH	^spH	^spH	^spH	^spH
0.01 M H_3PO_4	2.10	2.53	2.43	2.99	2.82	3.36	3.28
0.005 M $\text{H}_3\text{Cit}/0.005$ M KH_2Cit	3.18	3.82	3.72	4.17	4.00	4.65	4.57
0.001 M $\text{H}_3\text{PO}_4/0.009$ M KH_2PO_4	2.99	3.79	3.69	4.34	4.17	4.88	4.80
0.01 M HAc	3.28	3.62	3.52	4.32	4.15	4.67	4.59
0.009 M HAc/0.001 M NaAc	3.60	4.28	4.18	4.88	4.71	5.27	5.19
0.005 M $\text{KH}_2\text{Cit}/0.005$ M KNaHCit	4.66	5.41	5.31	5.85	5.68	6.46	6.38
0.005 M HAc/0.005 M NaAc	4.52	5.28	5.18	5.90	5.73	6.19	6.11
0.001 M HAc/0.009 M NaAc	5.10	6.09	5.99	6.31	6.14	6.83	6.75
0.005 M $\text{KNaHCit}/0.005$ M Na_3Cit	6.02	7.04	6.94	7.87	7.70	8.42	8.34
0.009 M $\text{KH}_2\text{PO}_4/0.001$ M Na_2HPO_4	6.12	7.01	6.91	7.57	7.40	8.36	8.28
0.005 M $\text{KH}_2\text{PO}_4/0.005$ M Na_2HPO_4	7.02	7.86	7.76	8.42	8.25	9.29	9.21

of acid. So, neutral and anionic acids increase their pK value with the increase of methanol content in the mobile phase whereas cationic acids show the opposite trend, at least until about 80% of methanol. So, values of ^wpH and ^spH of buffers of different type, neutral or anionic and cationic, could be in the opposite order in a particular methanol–water mixture in reference to water. This effect can be observed, for instance, in Table 9 for buffers from ammonium compared with those from monohydrogen citrate and dihydrogen phosphate. This is the main reason of the lack of fit of experimental retention data with Eqs. (8), (10) and (11) when the ^wpH quantity and buffers of different type are used in the pH range close to the pK value of the chromatographed analyte. If all the acids used in the buffered mobile phases are of the same type than the

ionizable analyte, consistent relationships between the experimental retention parameters and shape predicted by Eqs. (8), (10) and (11) can be obtained using ^wpH . However, the pK value calculated from the inflection point of this fit is not the true thermodynamic pK of the analyte, either in aqueous solution or in the mobile phase.

Chromatographic examples selected in this work involve several mobile phases and a wide pH range for each one. Acid compounds were chromatographed using a silica-based C_{18} column that allows the work in the required pH range. A wider pH range can be covered using a polymeric column and it was employed for the cationic acids that show higher pK values than the neutral ones. Eq. (11) was selected in this study since it is the simplest one because it uses directly the experimental data. The fitting parameters

Table 9
pH quantities for some HPLC buffers in water (^wpH) and diluted to 60 and 80% methanol (^spH and ^spH) used in the polymeric column

	^wpH	60% Methanol		80% Methanol	
		^spH	^spH	^spH	^spH
0.01 M H_3PO_4	2.02	2.70	2.53	2.90	2.82
0.0065 M $\text{H}_3\text{Cit}/0.0035$ M KH_2Cit	3.01	4.03	3.86	4.22	4.14
0.0086 M HAc/0.0014 M NaAc	3.96	5.10	4.93	5.69	5.61
0.0035 M HAc/0.0065 M NaAc	4.98	6.14	5.97	6.65	6.57
0.0056 M $\text{KNaHCit}/0.0044$ M Na_3Cit	6.05	7.54	7.37	8.37	8.29
0.0051 M $\text{KH}_2\text{PO}_4/0.0049$ M Na_2HPO_4	7.02	8.38	8.21	9.38	9.30
0.0095 M $\text{NH}_4\text{Cl}/0.0005$ M NH_3	7.93	7.40	7.23	7.24	7.16
0.0065 M $\text{NH}_4\text{Cl}/0.0035$ M NH_3	9.00	8.47	8.30	8.19	8.11
0.0056 M $\text{H}_3\text{BO}_3/0.0044$ M NaH_2BO_3	8.98	9.54	9.37	9.43	9.35
0.0019 M $\text{BuNH}_3\text{Cl}/0.0081$ M BuNH_2	10.89	10.57	10.40	9.77	9.69

Table 10

Retention parameters for test solutes obtained using Eq. (11) and the ^spH and ^spH values of buffers of Tables 8 and 9

	Methanol content in volume (%)	$t_{R(\text{HA})}$	$t_{R(\text{A})}$	$^s\text{p}K'$	$^s\text{p}K'$	SD	F	$^s\text{p}K$ lit.
2,4-Dinitrophenol	40	15.43±0.63	4.31±0.29	4.22±0.10	4.11±0.10	0.65	223	4.32
	60	5.79±0.20	2.36±0.08	4.49±0.09	4.32±0.09	0.19	203	4.52
	80	3.09±0.22	1.80±0.09	4.91±0.24	4.83±0.24	0.20	24	4.95
Benzoic acid	40	13.22±0.27	2.56±0.30	5.23±0.07	5.13±0.07	0.54	378	4.98
	60	4.62±0.13	2.13±0.15	5.80±0.15	5.63±0.15	0.27	80	5.46
	80	2.89±0.07	1.91±0.06	5.83±0.22	5.75±0.22	0.12	68	6.08
<i>N,N</i> -Dimethylbenzylammonium	60	17.97±0.53	0.88±0.34	8.13±0.06	7.96±0.06	0.71	436	8.19
	80	3.68±0.11	0.66±0.12	7.44±0.12	7.36±0.12	0.21	194	7.99
2,4,6-Trimethylpyridinium	60	10.11±0.10	0.74±0.13	6.52±0.05	6.35±0.05	0.22	1758	6.01
	80	2.47±0.02	0.60±0.04	5.88±0.06	5.80±0.06	0.06	714	5.81

obtained for 2,4-dinitrophenol, benzoic acid, *N,N*-dimethylbenzylamine and 2,4,6-trimethylpyridine, as well as the computed $^s\text{p}K'$ and $^s\text{p}K'$ values, are given in Table 10. These chromatographic $^s\text{p}K'$ values are not the thermodynamic ones. However, the low ionic strength of the mobile phases allows its comparison with those estimated from general equations previously developed [17,18] which were included in Table 10 too. Both series of values show a fairly good agreement. Table 11 shows the fitting parameters of analytes using Eq. (11) and ^wpH . As expected, the fits of 2,4-dinitrophenol and benzoic acid show similar standard deviation values using ^spH and ^wpH since all the buffered mobile phases are from anionic or neutral acids, that is to say, of the same type than the analytes. However, the derived

$\text{p}K$ values given in Table 11 differ significantly from both, estimated $^s\text{p}K$ and literature $^w\text{p}K$. Some examples are given in Figs. 2 and 3.

As shown in Fig. 4, the fits of *N,N*-dimethylbenzylamine and 2,4,6-trimethylpyridine in the mobile phase of 60% of methanol when ^spH is used are also very good. However, Eq. (11) shows very worse results for *N,N*-dimethylbenzylamine in this mobile phase when ^wpH is used, as can be seen in Table 11 and Fig. 5. This is because cationic and neutral or anionic acids are the buffering agents in the jump range of the plot. In fact, the fit is so bad that the fitting curve changes according to the initial values used to fit the equation by non-linear regression. Two of these curves are shown in Fig. 5 and their parameters included in Table 11. Both fits show

Table 11

Retention parameters for test solutes obtained using Eq. (11) and the ^wpH values of buffers of Tables 8 and 9

	Methanol content in volume (%)	$t_{R(\text{HA})}$	$t_{R(\text{A})}$	$^w\text{p}K'$	SD	$^s\text{p}K - ^w\text{p}K'$	F	$^w\text{p}K$ lit.
2,4-Dinitrophenol	40	15.62±0.50	4.21±0.24	3.61±0.08	0.50	0.71	367	4.10
	60	5.89±0.32	2.34±0.12	3.31±0.13	0.27	1.21	96	4.10
	80	3.17±0.24	1.80±0.09	3.25±0.24	0.20	1.70	25	4.10
Benzoic acid	40	13.42±0.37	2.55±0.38	4.47±0.09	0.67	0.51	239	4.19
	60	4.63±0.13	2.10±0.15	4.55±0.15	0.25	0.91	89	4.19
	80	2.91±0.06	1.89±0.05	4.15±0.18	0.10	1.93	94	4.19
<i>N,N</i> -Dimethylbenzylammonium	60	17.72±3.08	2.56±1.52	8.30±0.49	3.62	-0.11	14	8.91
	80	13.98±2.01	0.89±1.86	6.74±0.54	3.78	1.45	12	8.91
2,4,6-Trimethylpyridinium	60	3.30±0.28	0.59±0.38	5.34±0.50	0.63	2.65	19	8.91
	60	10.02±0.10	0.69±0.14	5.30±0.05	0.23	0.71	1640	7.43
80	2.46±0.03	0.55±0.05	4.12±0.07	0.07	1.69	618	7.43	

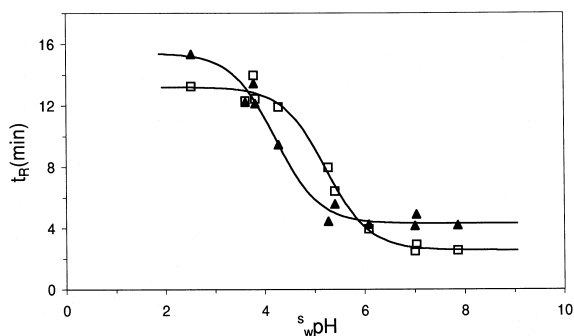


Fig. 2. Variation of the retention time of (\blacktriangle) 2,4-dinitrophenol and (\square) benzoic acid in a C_{18} column with the 40% methanol mobile-phase. pH measured after mixing the aqueous buffer with the organic modifier with electrode calibration with aqueous buffers (^s_wpH scale).

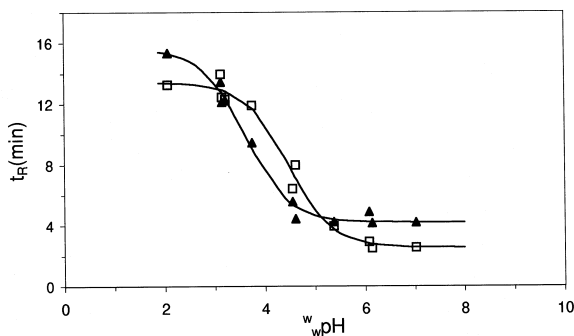


Fig. 3. Variation of the retention time of (\blacktriangle) 2,4-dinitrophenol and (\square) benzoic acid in a C_{18} column with the 40% methanol mobile-phase. pH measured before mixing the aqueous buffer with the organic modifier (^w_wpH scale).

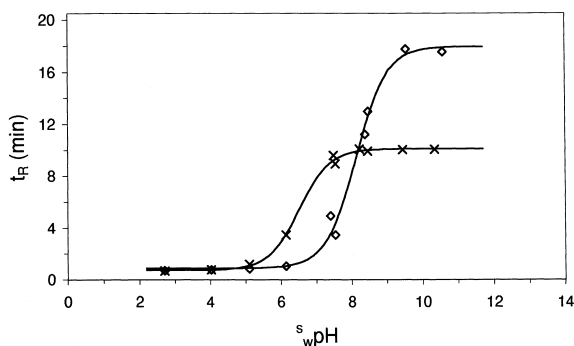


Fig. 4. Variation of the retention time of (\times) 2,4,6-trimethylpyridine and (\diamond) N,N -dimethylbenzylamine in a polymeric column with the 60% methanol mobile-phase. pH measured after mixing the aqueous buffer with the organic modifier with the electrode calibration with aqueous buffers (^s_wpH scale).

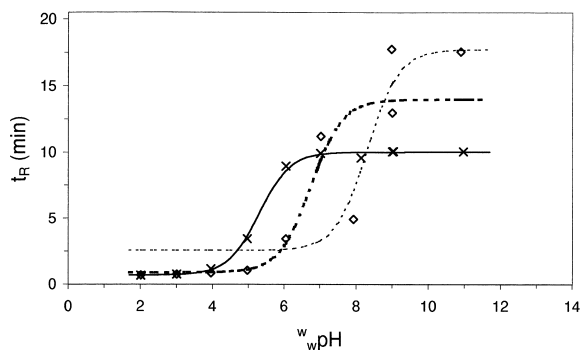


Fig. 5. Variation of the retention time of (\times) 2,4,6-trimethylpyridine and (\diamond) N,N -dimethylbenzylamine in a polymeric column with the 60% methanol mobile-phase. pH measured before mixing the aqueous buffer with the organic modifier (^w_wpH scale). Dotted lines calculated from different initial values to fit the equation (see the text).

a high and similar standard deviation and derived $\text{p}K$ values differ in more than 1.5 $\text{p}K$ units. However, this lack of fit is less noticeable for the 80% of methanol mobile phase because of the shifts of the $^s_w\text{p}K$ of the buffering agents and the analyte with the organic modifier percentage. These shifts lead to accumulate the buffers of different type to the pH range where the analyte is completely in its neutral form and, in this instance, the retention is independent of the acidity. The same occurs for 2,4,6-trimethylpyridine in both 60 and 80% of methanol mobile phases. Nevertheless, in all instances the chromatographic $\text{p}K$ value differs significantly from the literature $^w_w\text{p}K$ and its difference with the estimated $^s_w\text{p}K$ is not constant and far from the δ value.

6. Conclusions

It has been experimentally demonstrated the aptness of the glass electrode to make rigorous pH measurements (^s_wpH or ^s_wpH) in any methanol–water mixture in a way as simple as the common measurements in the aqueous part of the mobile phase (^w_wpH). The potentiometric system can be standardized by means of standard aqueous buffers, but the pH measurements must be taken in the mobile phase after mixing the aqueous buffer with the organic modifier. This procedure leads to rigorous ^s_wpH values that can be easily converted to ^s_wpH by

subtracting the suitable δ quantity (Eq. (6)), which can be calculated from the organic modifier content of the mobile phase (Eq. (14)). This procedure results in better fits between retention parameters of the analyte and mobile phase acidity than those obtained by the usual way, i.e., ^wpH measurements. Examples given in this paper show clearly that good fits to the theoretical equations, which involve thermodynamic $^s\text{p}K$ (or $^s\text{p}K$) values, can be achieved using ^spH (or ^spH) quantities for any combination of analyte (acid or base) and buffer. Therefore, the optimization of the mobile phase acidity for any analyte can be easily reached avoiding the disturbances observed when ^wpH is used.

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