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Automatized s_pK_a measurements of dihydrogen phosphate and Tris(hydroxymethyl) aminomethane in acetonitrile/water mixtures from 20 to 60 °C



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ABSTRACT

We measured pK_a values of Tris(hydroxymethyl)aminomethane and dihydrogen phosphate; both are commonly used to prepare buffers for reverse-phase liquid chromatography (RPLC), in acetonitrile/water mixtures from 0% to 70% (v/v) (64.6% (w/w)) acetonitrile and at 20, 30, 40, 50, and 60 °C. The procedure is based on potentiometric measurements of pH of buffer solutions of variable solvent compositions using a glass electrode and a novel automated system. The method consists in the controlled additions of small volumes of a thermostated solution from an automatic buret into another isothermal solution containing exactly the same buffer-component concentrations, but a different solvent composition. The continuous changes in the solvent composition induce changes in the potentials. Thus, only two sequences of additions are needed: increasing the amount of acetonitrile from pure water and decreasing the content of acetonitrile from 70% (v/v) (64.6% (w/w)). In the procedure with homemade apparatus, times for additions, stirring, homogenization, and data acquisition are entirely controlled by software programmed for this specific routine. This rapid, fully automated method was applied to acquire more than 40 potential data covering the whole composition range (at each temperature) in about two hours and allowed a systematic study of the effect of temperature and acetonitrile composition on acid–base equilibria of two widely used substances to control pH close to 7.

The experimental pK_a results were fitted to empirical functions between pK_a and temperature and acetonitrile composition. These equations allowed predictions of pK_a to estimate the pH of mixtures at any composition and temperature, which would be very useful, for instance, during chromatographic method development.

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1. Introduction

Because so many biological and chemical processes are dependent upon pH, one of the most important and common laboratory operations is the preparation and application of buffers to adjust the pH to desired values. In separation science, one fundamental application of buffers is for pH control of both the mobile phases in liquid chromatography and of the running solution in electrophoretic separations. In these techniques, the choice of the proper pH is necessary to reproducibly retain and separate ionic and ionizable compounds and to avoid undesirable peak asymmetry, tailing, splitting or shouldering, especially in quantitative analysis. In liquid chromatography, however, the pH of the mobile phase can be strongly dependent on the solvent mixture and column

temperature, since both temperature and solvent content not only affect the retention of neutral species but also influence the pK_a values of ionizable analytes and buffers [1,2]. In fact, a knowledge of the acid–base equilibria in water/solvent mixtures of common buffers and of solutes is necessary for predicting retention and selectivity of these ionizable compounds as function of pH, solvent composition and column temperature [3,4].

Several techniques devoted to pK_a determinations have been extensively discussed. The most widely spread range from classical potentiometric and conductimetric techniques to spectrometric methods [5], and to the indirect estimations of pK_a from retention times in liquid chromatography [6,7] or, more recently, from electrophoretic mobilities in capillary electrophoresis [8].

Classical potentiometric techniques are based on pH measurements during the titration of the electrolyte solution with an appropriate titrand while maintaining the solvent composition and temperature constant. Thus, numerous individual titrations are needed to cover a wide range of solvent compositions at a

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fixed temperature with each one requiring the preparation of solutions of the electrolyte and the titrand at that solvent composition.

We proposed an automated potentiometric method for obtaining multiple pK_a values of weak electrolytes over a wide range of solvent compositions in a unique titration experiment [9]. The method consisted in measuring pH values of buffer solutions under conditions in which only the organic-solvent content is systematically changed. Two solutions having exactly the same analytical molal concentrations of the components of the conjugated pair were prepared in two different solvents: in pure water and in 70% (v/v) acetonitrile/water mixture. Thus, additions of small volumes of one solution into a known volume of the other allowed the systematic variation of solution potential as a consequence of solvation and the medium's dielectric properties. Thus, the only variation was in solvent composition because the concentration, degree of dissociation, and ionic strength remained unchanged. The pH values of these solutions were carefully measured and the corresponding pK_a values could be calculated.

The measurement of pH in solutions prepared in solvents differing from water is not a trivial undertaking. One option consists in the calibration of the pH electrode in solutions prepared with exactly the same solvent as the one used for the solution to be measured. In accordance with the IUPAC recommendation [10] these pH values must be noted as ${}^s\text{pH}$ (or ${}^w\text{pH}$ in the case when the solvent is pure water). When the electrodes are calibrated with the aqueous buffer standards, the pH is measured in solutions with a solvent different from water; the pH value obtained should be denoted as ${}^s\text{pH}$. The δ -conversion parameter relates to both operational pH scales. This parameter depends on the solvent composition [11–13] and temperature [14,15].

One aim of this work was to evaluate the precision and the performance of the new method since the original homemade apparatus [9] has now been greatly improved: the times for additions, stirring, homogenization, and data acquisition are now entirely controlled by a homemade software application specifically programmed for this routine. The automatic method was accordingly applied to acquire more than 40 potential data (at a given temperature) in only two hours.

The method has been used to measure the dissociation constants of two common substances of different chemical natures. Both are used as buffers to control the pH within the same range: Tris(hydroxymethyl)aminomethane (Tris) and phosphoric acid in its second dissociation equilibrium (dihydrogen phosphate). Since for RPLC mobile phases, aqueous mixtures with acetonitrile or methanol are generally used, mixtures of acetonitrile and water are chosen in the present study. We determined pK_a of dihydrogen phosphate and of Tris from 0% to 70.0% (v/v) acetonitrile (MeCN) and in the temperature range from 20 to 60 °C at intervals of 10 °C. The upper limit in MeCN content was determined by the solubility

of the buffer components [16] and the ability of the glass electrode to make measurements in solutions of low water content solution without dehydrating and damaging the glass membrane [17].

Finally, another aim was the development of robust mathematical empirical equations that would accurately describe the pK_a values of each substance as a function of solvent composition and temperature. Such equations would allow the estimation of pK_a for any intermediate value of composition and temperature within the intervals measured.

2. Experimental

2.1. Instrumentation

An automatic titrator, Metrohm Titrino SM702 with an Electrode Schott BlueLine 11pH, was employed for pH measurements. A homemade glass cell with a cylindrical body and conical bottom was constructed to allow the measurements of pH with the electrode immersed in a minimal initial volume (2.000 mL including the volume of the magnetic stirrer) while keeping maximum total volume of about 20 mL [9]. All initial volumes of each sequence were delivered with an automatic titrator Schott TitroLine Alpha (2.000 ± 0.001 mL).

The buret is connected to the cell through a glass coil with a sintered-glass plate (frit) at the end. Both, the cell and the coil, were kept submerged in a thermostatic bath (Lauda) in order to add the previously temperature-equilibrated solution. The bath had a control of temperature of ± 0.5 °C.

2.2. Chemicals

Water came from a MilliQ[®] purification system (Simplicity, Millipore, Massachusetts, MA, USA) and acetonitrile was HPLC grade (Mallinckrodt Baker Inc., Phillipsburg, NJ, USA). The chemical reagents used in this work were of analytical grade or better: Tris (hydroxymethyl)aminomethane and sodium hydroxide were from Carlo Erba, sodium dihydrogen phosphate from Merck, potassium hydrogen phthalate from Fluka, disodium hydrogen phosphate and hydrochloric acid from Merck and Borax from Baker.

2.3. Solutions

For each buffer, $\text{H}_2\text{PO}_4^-/\text{HPO}_4^{2-}$ and Tris/Tris:HCl, six solutions (three pairs) were initially prepared. Each pair had exactly the same analytical concentration of all the species, *i.e.*, contained the same dissociation ratio for the components of the conjugated pair. We prepared solutions containing Tris/Tris- H^+ and $\text{H}_2\text{PO}_4^-/\text{HPO}_4^{2-}$ ratios of 0.40, 0.50, and 0.60. Three aqueous solutions (solutions A) were prepared with pure water while other three solutions (solutions S) were prepared with 70% (v/v) MeCN in

Table 1

Molal concentrations and solvent composition of the solutions of Tris/Tris:HCl and dihydrogen phosphate/hydrogen phosphate. C_T is the analytical molar concentration; I is the ionic strength.

Solution	% (v/v) MeCN	Buffer Tris/Tris-HCl				Buffer phosphate			
		m_a^a (mol kg ⁻¹)	m_b^a (mol kg ⁻¹)	C_T (mol L ⁻¹)	I (m)	m_a^a (mol kg ⁻¹)	m_b^a (mol kg ⁻¹)	C_T (mol L ⁻¹)	I (m)
A1	0	0.0306	0.0203	0.03	0.031	0.0172	0.0115	0.05	0.052
S1	70.0	0.0362	0.0241	0.03	0.036	0.0204	0.0136	0.05	0.061
A2	0	0.0310	0.0305	0.03	0.031	0.0145	0.0145	0.05	0.050
S2	70.0	0.0367	0.0361	0.03	0.037	0.0171	0.0172	0.05	0.069
A3	0	0.0302	0.0470	0.03	0.030	0.0116	0.0173	0.05	0.063
S3	70.0	0.0358	0.0556	0.03	0.036	0.0137	0.0205	0.05	0.075

^a Molal concentration of the acidic (m_a) and basic (m_b) form of the conjugated pair.

water. Table 1 summarizes the molal analytical concentrations and compositions of these solutions. For the proposed procedure, maintaining the molar concentration of the species, degree of dissociation, and the ionic strength of the media constant is critical. Thus, all the solutions were prepared by weight and thus led to a final known volume in a calibrated flask; the molar, molal and solute moles per kilogram of solution can thus be calculated (this last expression for concentration facilitates the computation of the solvent composition).

2.4. Measurement procedure

2.4.1. Software

One of the principal goals of this work was to improve the control of all the instrumental components used in the experiment and the automation involved. All the devices used in a titration were controlled by computer through the serial port. The computer had a Linux OS (Xubuntu 8.10) installed and a homemade software application called Pytrator v1.0 programmed under Python. In this work, Pytrator was used to control one system though it was designed to control more than one system simultaneously. Sequences based on time intervals can be programmed specifying time, speed, and volumes dispensed by the automatic buret, power on/off for the water bath, power on/off for the magnetic stirrer and acquisition and storage of potentiometric data.

2.4.2. Calibration of the glass electrode

The wide-range glass pH electrode was calibrated by following the multipoint-calibration procedure [18] using three aqueous standard solutions: potassium hydrogen phthalate, dihydrogen phosphate/hydrogen phosphate, and borax. All three standard solutions were prepared according to the procedures recommended by IUPAC and NIST [19] and were temperature equilibrated before the measurements. About 10 potential readings were taken for each buffer calibration at the working temperature to obtain an average. The glass electrode was then immersed into the solutions through a hole in the cap of the glass cell and the solutions were shaken to avoid changes in solvent compositions by condensation into the internal flask walls. This agitation was essential for minimizing errors in the solvent composition.

2.4.3. Sequential measurements

A pair of solutions with the same dissociation grades (A and S) was chosen: one the titrant and another the volume titrated. The procedure was performed in both directions exchanging titrant with titrated solutions. During the titration the amount of titrant added must be accurately measured in order to know the mixture's exact composition. In this work the added amount was precisely planned in order to produce changes of 2.0% (w/w) in MeCN content of the mixture within the potentiometric cell.

The calibrated electrode was immersed in the cell, and after thermal equilibrium, an initial potential difference was measured. The syringe dispenser and the tubing had been previously filled and purged with the titrand solution, and after thermal equilibration, the sequence of additions was started by adding a known volume of solution from the dispenser. After each addition, a period of time was allowed for mixing and stabilizing the electrode potential before making a measurement. The stabilization of the electrode response demanded a longer period with increasing amounts of MeCN and *vice versa*. The frequency of the additions was programmed in the software to take into account two different time periods according to the solvent content in the cell, *i.e.*, every 1 min for the water rich region and 3 min for the intermediate and high MeCN/water mixtures.

The sequences consisted in potentiometric measurements in the reaction cell upon variations in the solvent composition at a fixed temperature. Solutions were initially rich in aqueous solvent (A), and solvent compositions were changed by the additions of titrand solution rich in organic solvent (S). In this direction of polarity, potentiometric readings were measured within the composition range between 0% and 58.8% (v/v) MeCN. Conversely, by adding the aqueous solutions (as titrand) to the S solutions, the compositions changed from 70% to 11.9% (v/v) MeCN.

The complete set of ${}^s_w\text{pH}$ -composition data was obtained from both sequences including an overlapping portion within the intermediate MeCN-composition range between 15.25% and 55.25% (v/v). When a sequence was completed, a second calibration with the aqueous standard pH buffers was performed, so that an average calibration pH for the entire sequence from start to finish could be calculated.

3. Data treatment

The experimental determination consisted in the measurement of the pH of Tris and dihydrogen phosphate solutions, the concentrations of which species were well known. If we take into account the physical properties and conversion parameters, the dissociation constants (s_pK_a) are obtained from the equation:

$${}^s_pK_a = -\log \frac{m_{A^-} + m_{H^+} + m_{OH^-}}{m_{HA} - m_{H^+} + m_{OH^-}} + \log {}^s\gamma_{HA} - \log {}^s\gamma_{A^-} - {}^s_pH_m \quad (1)$$

where m is molal analytical concentration and the subscripts indicate the chemical species, base (A^-) and acid (HA) from the conjugated pair. The terms ${}^s\gamma_i$ are the activity coefficients and s_pH_m the molal scale. Since these two buffers are mildly alkaline and all the solutions are prepared at relatively high concentrations, the contribution of hydrogen and hydroxyl ions, m_{H^+} and m_{OH^-} , in the first term on the right of Eq. (1) can be neglected. The activity coefficients of the neutral molecules were assigned a value of unity, whereas the activity coefficients of the ions were estimated through the Debye–Hückel equation:

$$\log {}^s\gamma_i = \frac{Az^2\sqrt{I}}{1 + a_0B\sqrt{I}} \quad (2)$$

where I stands for the ionic strength in the molal scale, z is the charge of the ionic species, and A and a_0B are parameters that can be calculated by assuming the Bates–Guggenheim convention [20,21]:

$$A = 1,834,600 \frac{\sqrt{\rho_s}}{(\epsilon_s T)^{3/2}} \quad (3)$$

$$a_0B = 1.5 \sqrt{\left(\frac{\epsilon_w}{\epsilon_s}\right) \left(\frac{\rho_s}{\rho_w}\right)} \quad (4)$$

where T is the absolute temperature, ϵ indicates the static dielectric constant, ρ denotes the density, all at a given temperature, and the subscripts w and s stand for pure water and the solvent (or solvent mixture), respectively [10,22]. The physical properties of these solvent mixtures were taken from the literature [17]. The value of the ionic strength is needed in order to estimate the activity coefficient by means of Eq. (2), and since this calculation requires a knowledge of the contribution of all the ionic species, an iterative calculation is applied.

The s_pH_m values are calculated by converting the experimental ${}^s_w\text{pH}$ of the solutions by means of the δ_m conversion parameter as follows:

$${}^s_pH_m = {}^s_w\text{pH} - \delta_m \quad (5)$$

where the δ_m parameter at each temperature and composition has been taken from the literature [23].

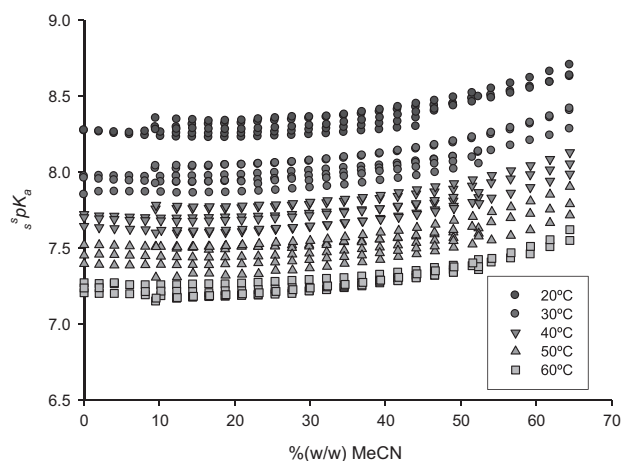


Fig. 1. Plot of 5pK_a of Tris as a function of MeCN composition at five temperatures.

With the experimentally obtained 5pK_a values, the changes of 5pK_a with MeCN content and with temperature were evaluated in order to develop simple and robust equations to describe these 5pK_a dependences with temperature and solvent composition. Two software applications, Table Curve 3D and SigmaPlot 9.0, were used for fitting of data.

4. Results and discussion

Measurements of potential provided sequential data points cover the range from 0% to 70% (v/v) MeCN/water (two titrations). The potentials have been measured by titration in both directions including several MeCN/water compositions for each degree of dissociation and temperature. These measurements provided 48 potentials in only 2 h. The measurements were repeated at three degrees of dissociation grades and each one at five temperatures (from 20 through 60 °C at intervals of 10 °C), thus, acquiring more

Table 2

5pK_a values of Tris(hydroxymethyl)aminomethane- H^+ at several MeCN/water compositions and at five temperatures (standard deviations in parenthesis).

% (w/w) MeCN	5pK_a					Δ^5H^a (kJ mol $^{-1}$)
	20 °C	30 °C	40 °C	50 °C	60 °C	
0	8.269(0.002)	7.92(0.07)	7.69(0.04)	7.45(0.06)	7.23(0.03)	47.58 (0.09)
6.05	8.25(0.01)	7.92(0.05)	7.67(0.04)	7.45(0.06)	7.23(0.04)	47.24 (0.07)
8.11	8.25(0.01)	7.92(0.05)	7.67(0.04)	7.45(0.06)	7.22(0.03)	47.55 (0.07)
14.40	8.28(0.05)	7.98(0.05)	7.65(0.05)	7.47(0.05)	7.20(0.04)	50.2 (0.1)
23.10	8.29(0.05)	7.99(0.06)	7.74(0.05)	7.48(0.05)	7.22(0.03)	49.66 (0.04)
32.17	8.31(0.05)	8.02(0.06)	7.77(0.04)	7.50(0.05)	7.25(0.03)	49.51 (0.03)
39.23	8.35(0.05)	8.06(0.06)	7.78(0.06)	7.53(0.05)	7.29 (0.02)	49.45 (0.02)
46.51	8.43(0.03)	8.05(0.04)	7.79(0.05)	7.54(0.05)	7.33(0.02)	50.5 (0.1)
52.35	8.49(0.01)	8.10(0.05)	7.83(0.04)	7.60(0.03)	7.38(0.03)	51.1 (0.1)
56.61	8.54(0.03)	8.28(0.01)	7.98(0.04)	7.73(0.05)	7.45(0.02)	51.03 (0.06)
61.83	8.61(0.04)	8.36(0.01)	8.05(0.05)	7.71(0.06)	7.53(0.03)	52.6 (0.1)
64.60	8.65(0.04)	8.41(0.01)	8.09(0.05)	7.75(0.05)	7.58(0.05)	52.4 (0.1)

^a Dissociation enthalpies estimated according to Eq. (6).

Table 3

Comparison of Tris/Tris- H^+ 5pK_a values with literature data at different solvent compositions and temperatures.

% (w/w) MeCN	T (°C)	5pK_a		Δ^5pK_a	Method ^a	I (m) ^b	References
		Experimental	Bibliography				
0	20	8.27	8.22	0.05	A	0	[26]
			8.21	0.06	A	0	[27]
			8.21	0.06	A	0	[28]
			7.93	-0.01	A	0	[27]
			7.93	-0.01	A	0	[28]
			7.68	0.01	A	0	[27]
	30	7.92	7.93	-0.01	A	0	[27]
			7.93	-0.01	A	0	[28]
			7.68	0.01	A	0	[27]
			7.68	0.01	A	0	[28]
			7.44	0.01	A	0	[27]
			7.44	0.01	A	0	[28]
40	7.69	7.44	0.01	A	0	[27]	
		7.44	0.01	A	0	[28]	
		7.21	0.02	A	0	[28]	
		7.21	0.02	A	0	[28]	
		7.21	0.02	A	0	[28]	
		7.21	0.02	A	0	[28]	
50	7.45	7.44	0.01	A	0	[27]	
		7.44	0.01	A	0	[28]	
		7.44	0.01	A	0	[28]	
		7.44	0.01	A	0	[28]	
		7.44	0.01	A	0	[28]	
		7.44	0.01	A	0	[28]	
60	7.23	7.21	0.02	A	0	[28]	
		7.21	0.02	A	0	[28]	
		7.21	0.02	A	0	[28]	
		7.21	0.02	A	0	[28]	
		7.21	0.02	A	0	[28]	
		7.21	0.02	A	0	[28]	
25	Δ^5H (kJ mol $^{-1}$)	46.04	47.69				[29]
			8.10	0.18	B	0.0173	[30]
			7.99	0.16	B	0.0173	[30]
			7.70	0.14	B	0.0173	[30]
			7.49	0.16	B	0.0173	[30]
			7.49	0.16	B	0.0173	[30]
0	25	8.09	8.08	0.01	B	0	[31]
			8.12	0.15	B	0	[31]
			8.16	0.17	B	0	[31]
			8.16	0.17	B	0	[31]
			8.24	0.06	B	0	[31]
			8.24	0.06	B	0	[31]

^a Measurement method: A: electrochemically without liquid junction potential; B: potentiometry with glass electrode.

^b Ionic strength.

than 700 data points. These potential data were converted to ${}^s_w\text{pH}$ values by means of the calibration, and these values transformed first to ${}^s\text{pH}$ by Eq. (5) and then to the ${}^s\text{p}K_a$ values through Eq. (1).

Although the reproducibility of any pH measurement would result in standard deviations of about ± 0.008 -pH units, the real uncertainty is mainly determined by the unknown residual liquid-junction potentials between the calibration solutions and those measured [24,25]. These pH-uncertainty values are estimated at about ± 0.02 pH-units. Modern combination-glass electrodes filled with 3 M KCl exhibit a constant junction potential between an aqueous standard and solvent buffers for measurements in MeCN/water mixtures within a wide temperature range [23]. Nevertheless, two precautions were taken to keep any uncertainty as low as possible: first, a multipoint calibration with three standard solutions was performed; second, since the glass membrane was exposed to substantial changes in solvent composition during the sequence, the electrode calibration was repeated after the sequence of additions had been completed.

4.1. Tris/Tris+HCl

Fig. 1 shows the whole data set of ${}^s\text{p}K_a$ for Tris at different MeCN compositions and five temperatures, while Table 2 summarizes ${}^s\text{p}K_a$ values at several solvent compositions. We considered that the ${}^s\text{p}K_a$ obtained from the three different degrees of dissociation were replicated so that the standard deviations of these averaged values were added (shown in parentheses). These standard deviations ranged between 0.002 and 0.07 $\text{p}K_a$ units, whose values are somewhat smaller than those obtained previously for formic acid and triethylamine by following a similar approach but with a less automatized system [9].

Fig. 1 clearly illustrates that the Tris ${}^s\text{p}K_a$ decreases with increasing temperatures, while the increase in solvent composition has a minor influence on the ${}^s\text{p}K_a$ values.

An estimation of the enthalpies for the dissociation equilibrium, $\Delta^s H_0$, at a given solvent composition can be obtained from the dependence of ${}^s\text{p}K_a$ with temperature:

$$\partial^s \frac{\text{p}K_a}{\partial(1/T)} = \frac{\Delta^s H_0}{2.303R} \quad (6)$$

This equation must be tested since it implicitly assumes that the dissociation enthalpy is independent of temperature, *i.e.*, the heat capacity for the dissociation reaction is negligible within the temperature range between 20 and 60 °C. An analysis of variance was carried out at several intermediate solvent compositions where more data had been acquired, in order to check for a potential deviation from the linearity between ${}^s\text{p}K_a$ and $1/T$. The analysis indicated that the linear dependences were statistically correct within this temperature range. The last column of Table 2 summarizes the calculated $\Delta^s H_0$ values and the corresponding standard deviations.

Unfortunately, since only scant literature on Tris and dihydrogen phosphate ${}^s\text{p}K_a$ values in MeCN/water solvent mixtures and at different temperature conditions is available, the comparison with values in the literature is rather limited. We gathered the previously published data of Tris- ${}^s\text{p}K_a$ values and compared with data measured in Table 3. The predicted values are in excellent agreement with those reported in the literature. For 0 % (w/w) MeCN the maximum ${}^s\text{p}K_a$ difference is 0.06 units, whereas the difference increases up to 0.18 $\text{p}K_a$ units for 25% (w/w) MeCN and 20 °C.

In Table 3 the dissociation enthalpies obtained at 0% and at 25% (w/w) MeCN are also compared with data previously published and are in good agreement at both solvent compositions. This is another indication that the method proposed here to obtain Tris ${}^s\text{p}K_a$ between 10–60 °C and 0–70% (v/v) MeCN is reliable.

The proposal of a single empirical equation for estimating ${}^s\text{p}K_a$ for this compound at a given MeCN composition and temperature would be highly desirable for practical purposes in RPLC method development. In order to arrive at that expression, the enthalpy

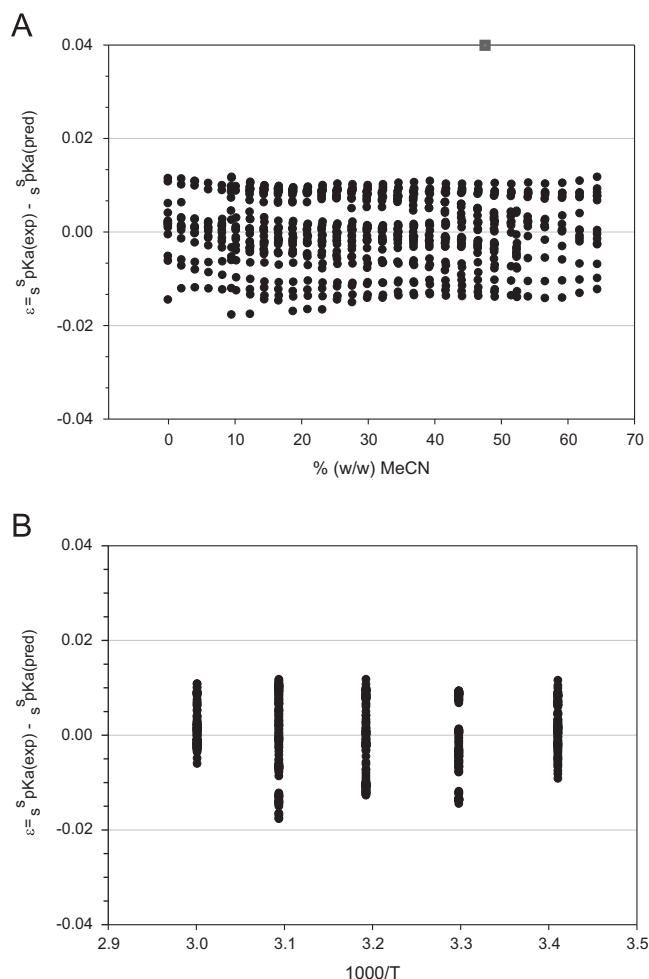


Fig. 2. Residuals [$\epsilon = {}^s\text{p}K_a(\text{exp}) - {}^s\text{p}K_a(\text{pred})$] as a function of MeCN composition (% (w/w)) (A) and $1000/T$ (B).

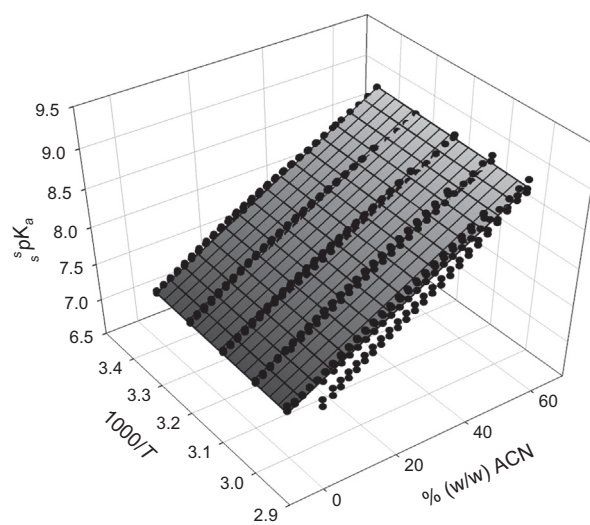


Fig. 3. Plot of ${}^s\text{p}K_a$ of phosphate as a function of MeCN composition and of reciprocal temperature.

Table 4
Values of ${}^s pK_a$ for dihydrogen phosphate (pK_{a2}) at several MeCN/water compositions and five temperatures (standard deviations in parenthesis).

% (w/w) MeCN	${}^s pK_a$					$\Delta_s^s H^a$ (kJ mol ⁻¹)
	20 °C	30 °C	40 °C	50 °C	60 °C	
0	7.25(0.02)	7.24(0.02)	7.22(0.02)	7.20(0.02)	7.18(0.04)	3.7 (0.4)
6.07	7.43(0.02)	7.42(0.03)	7.39(0.02)	7.38(0.01)	7.35(0.01)	3.4 (0.4)
8.13	7.49(0.02)	7.48(0.02)	7.45(0.02)	7.44(0.01)	7.412(0.002)	3.5 (0.5)
14.44	7.66(0.09)	7.62(0.08)	7.60(0.05)	7.61(0.03)	7.58(0.05)	3.3 (0.8)
23.16	7.91(0.05)	7.88(0.06)	7.85(0.04)	7.86(0.04)	7.83(0.06)	3.1 (0.8)
32.23	8.12(0.05)	8.08(0.07)	8.06(0.07)	8.10(0.04)	8.08(0.06)	1.5 (1.4)
39.28	8.26(0.05)	8.25(0.06)	8.24(0.06)	8.28(0.04)	8.26(0.06)	-0.8 (0.7)
46.56	8.42(0.05)	8.43(0.05)	8.43(0.05)	8.47(0.04)	8.46(0.07)	-2.3 (0.8)
56.63	8.58(0.06)	8.62(0.04)	8.66(0.02)	8.69(0.08)	8.66(0.08)	-4.6 (1.4)
59.22	8.64(0.03)	8.69(0.03)	8.74(0.03)	8.78(0.04)	8.75(0.03)	-5.7(1.2)
64.60	8.78(0.01)	8.82(0.02)	8.89(0.05)	8.92(0.05)	8.90(0.08)	-6.2 (1.6)

Table 5
Comparison of dihydrogen phosphate ${}^s pK_{a2}$ values with literature data at different compositions and temperatures.

% (w/w) MeCN	T (°C)	${}^s pK_a$		$\Delta_s^s pK_a$	Method	l (m)	References
		Experimental	Bibliography				
0	20	7.25	7.21	0.04	A	0	[32]
	30	7.24	7.19	0.05	A	0	[32]
	40	7.22	7.18	0.04	A	0	[32]
	50	7.20	7.19	0.01	A	0	[32]
	60	7.20	7.20	0.00	A	0	[32]
	$\Delta_s^s H$ (kJ mol ⁻¹)	2.96	3.08–4.13		A	0	[29,33]
			3.52		A	0	
25	20	7.93	7.88	0.05	A	0.0098	[30]
	30	7.88	7.81	0.07	A	0.0098	[30]
	40	7.87	7.83	0.04	A	0.0098	[30]
	50	7.90	7.83	0.07	A	0.0098	[30]
	$\Delta_s^s H$ (kJ mol ⁻¹)	2.47	3.05				[30]

A: potentiometry with glass electrode.

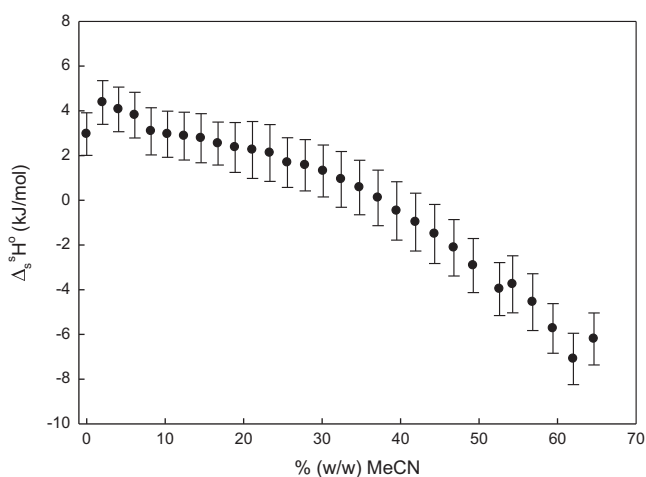


Fig. 4. Change in enthalpies of dissociation, $\Delta_s^s H_0$ (kJ mol⁻¹) vs % (w/w) MeCN.

changes with solvent composition demonstrated that the values over the composition range studied were statistically equivalent (cf. Table 2). Therefore, i) the predicting equation must have a linear dependence between pK_a and $1/T$; ii) Fig. 1 shows that a change in the solvent composition produces a nonlinear increase in the ${}^s pK_a$ values. From 0% up to about 30% (w/w) MeCN, ${}^s pK_a$ remains almost constant, whereas this quantity increases dramatically above that solvent composition. The statistical study of these tendencies at different temperatures indicated that a quadratic term did not describe this trend, but a cubic term with a similar characteristic parameter for all temperatures did so

perfectly well. Under these considerations, the empirical expression chosen for prediction of ${}^s pK_a$ for buffer Tris was

$${}^s pK_a = -0.60 (\pm 0.05) + 1.40 (\pm 0.03) \times 10^{-6} X^3 + 2.60 (\pm 0.02) \times 1000/T$$

$$r^2 = 0.978; S.E. = 0.007 \quad (7)$$

where X represent the solvent composition (in % (w/w) MeCN). The correlation coefficient, r^2 , and the standard deviation for prediction (S.E.) of the empiric equation were 0.978 and 0.007, respectively.

To evaluate the accuracy of the proposed equation, a plot of residuals of ${}^s pK_a$ was made and plotted against both solvent composition and the reciprocal of the temperature (Fig. 2A and B). In both plots, the residuals are distributed homogeneously between ± 0.02 logarithmic units. We therefore considered that this relatively simple equation accurately describes the behavior between Tris ${}^s pK_a$ and MeCN content and temperature.

4.2. Buffer KH_2PO_4/K_2HPO_4

The experimentally obtained ${}^s pK_{a2}$ values for phosphate at different MeCN compositions and five temperatures are plotted in Fig. 3, and the data at given compositions are summarized in Table 4.

As opposed to Tris- H^+ ${}^s pK_a$, ${}^s pK_{a2}$ of phosphoric acid increases dramatically with the MeCN content, but remains practically constant against changes in temperature. Table 5 presents the average experimental ${}^s pK_a$ values as compared with the ${}^s pK_a$ values reported in the literature and the corresponding differences.

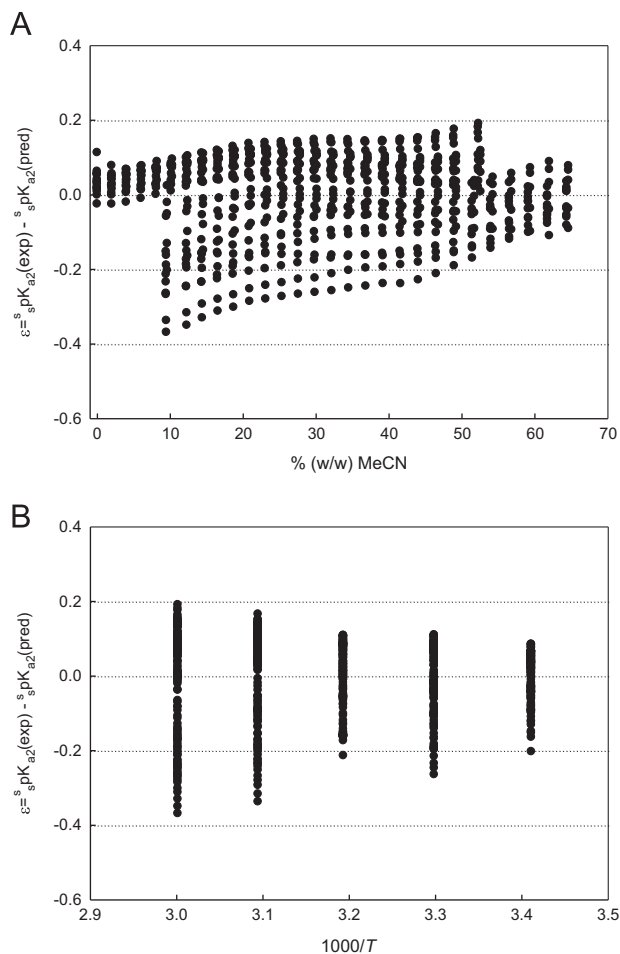


Fig. 5. Residuals [$e = {}^s pK_a(\text{exp}) - {}^s pK_a(\text{pred})$] for phosphate buffer as a function of composition % (w/w) MeCN (A) and $1000/T$ (B).

The changes in enthalpy for each composition were obtained from the regression slope between pK_a and $1/T$, according to Eq. (6). These enthalpy values as a function of solvent composition are plotted in Fig. 4. Clearly, the $\Delta^s H_0$ values are not constant with changes in solvent composition; as the MeCN content increases (starting from pure water), the positive enthalpy, corresponding to an endothermic process, becomes slightly negative, indicating an exothermic shift as the solvent mixture becomes enriched in MeCN. Since that transition occurs with a quadratic dependence of enthalpy on composition, the empirical equation of pK_a will have a parameter that depends on $1000/T$ and on the square of the % (w/w) MeCN.

As to the organic-solvent content, ${}^s pK_{a2}$ exhibits a lineal relationship with respect to that composition with a regression slope of pK_a vs % (w/w) MeCN, whose parameter is, in turn, a linear function of the reciprocal temperature.

In our proposal of an empirical expression allowing the ${}^s pK_{a2}$ determination over the entire range of temperature and compositions, we took the relationship of ${}^s pK_{a2}$ with temperature and MeCN content into account. We accordingly tested the interrelationships between variables and found that the equation that best described the empirical phosphate pK_{a2} with solvent composition (X) and temperature changes was

$${}^s pK_a = 6.19(\pm 0.18) + 3.60(\pm 0.5) \times 10^{-2} X + 0.31(\pm 0.06) \times 1000/T - 3.3(\pm 1.6) \times 10^{-3} X/T$$

$$R^2 = 0.971; \text{ S.E.} = 0.10 \quad (8)$$

where X is given in % (w/w) MeCN. The correlation coefficient and the estimation of standard deviation of the empirical equation were 0.973 and 0.10, respectively.

A residual plot between the predicted and the experimental ${}^s pK_{a2}$ values is represented vs both solvent composition and the reciprocal of the temperature (Fig. 5). The residuals in both instances are between $+0.04$ and -0.04 pK_a units, indicating a less accurate equation than that proposed for Tris/Tris- H^+ . These larger residuals are expected since the equation has an additional term including both independent variables.

5. Conclusions

We have implemented a fully automatized potentiometric method for the accurate determination of dissociation constants for weak electrolytes in aqueous–organic solvent mixtures. The method generated a large number of pK_a data over a wide range of organic-solvent content and under isothermal conditions in fewer than 2 h. We incorporated the method of automatization in the addition of titrand volumes, time to wait for the stabilization of the system, and measurement times after each addition with stirring power off before acquisition of each data, through the use of a routine software program, named Pytrator. The data acquired resulted in smaller standard deviations than those obtained previously for other weak electrolytes with the simpler system proposed here.

Empirical equations of ${}^s pK_a$ as a function of % (w/w) MeCN and temperature were developed for Tris buffer and dihydrogen phosphate buffer, respectively. These equations are accurate for pK_a calculation within the range of 0–64.4 % (w/w) MeCN and 20–60 °C.

As the two substances selected as buffers, we can conclude that in spite of being used for the regulation of pH within the same region of the scale, the chemical nature of these two substances makes a substantial difference in their acid–base equilibria upon changes in solvent composition, temperature or both variables simultaneously. For phosphate buffer the ${}^s pK_{a2}$ values, and consequently the pH of the buffered solution were clearly influenced upon MeCN addition, whereas ${}^s pK_a$ was not affected by temperature changes at a fix solvent composition. In contrast, with Tris: Tris/HCl as the buffer, the MeCN increment had only a minor effect on pK_a and the corresponding pH, while a temperature increase led to a significant decrease in these parameters.

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References

- [1] X. Subirats, E. Bosch, M. Rosés, LC-GC N. Am. 27 (2009) 1000–1005.
- [2] M. Rosés, E. Bosch, J. Chromatogr. A 982 (2002) 1–30.
- [3] P. Nikitas, A. Pappa-Louisi, J. Chromatogr. A 1216 (2009) 1737–1755.
- [4] P. Agrafiotou, C. Ráfols, C.B. Castells, E. Bosch, M. Rosés, J. Chromatogr. A 1218 (2011) 4995–5009.
- [5] R.F. Cookson, Chem. Rev. 74 (1974) 5–28.
- [6] J.E. Hardcastle, I. Jano, J. Chromatogr. B 717 (1998) 39–56.
- [7] J. Barbosa, R. Berges, V.S. Sans-Nebot, J. Chromatogr. 823 (1998) 411–422.
- [8] M.G. Khaledi, High-Performance Capillary Electrophoresis. Theory, Techniques and Applications Wiley-Interscience, New York, 1998.
- [9] J.M. Padró, A. Acquaviva, M. Tascon, L.G. Gagliardi, C.B. Castells, Anal. Chim. Acta 725 (2012) 87–94.

- [10] J. Inczedy, T. Lengyel, A.M. Ure, *Compendium on Analytical Nomenclature. Definitive Rules 1997* 3rd ed., Blackwell Science, Oxford, 1998.
- [11] R.G. Bates, R.A. Robinson, *Acid–Base Behavior in Methanol–Water Solvents* in Chemical Physics of Ionic Solutions, National Bureau of Standards, Washington, D. C., 1966.
- [12] S. Espinosa, E. Bosch, M. Rosés, *Anal. Chem.* 72 (2000) 5193–5200.
- [13] I. Canals, J.A. Portal, E. Bosch, M. Rosés, *Anal. Chem.* 72 (2000) 1802–1809.
- [14] C.B. Castells, C. Ràfols, M. Rosés, E. Bosch, *J. Chromatogr. A* 1002 (2003) 41–53.
- [15] C.B. Castells, L.G. Gagliardi, C. Ràfols, M. Rosés, E. Bosch, *J. Chromatogr. A* 1042 (2004) 23–35.
- [16] A.P. Schellinger, P.W. Carr, *LCGC N. Am.* 22 (2004) 544–548.
- [17] L.G. Gagliardi, C.B. Castells, C. Ràfols, M. Rosés, E. Bosch, *J. Chem. Eng. Data* 52 (2007) 1103–1107.
- [18] R.P. Buck, S. Rondinini, A.K. Covington, F.G.K. Baucke, C.M.A. Brett, M.F. Camoes, M.J.T. Milton, T. Mussini, R. Naumann, K.W. Pratt, P. Spitzer, G.S. Wilson, *Pure Appl. Chem.* 74 (2002) 2169–2200.
- [19] R.G. Bates, in: I.M. Kolthoff, P.J. Elving (Eds.), *Treatise on Analytical Chemistry*, John Wiley & Sons, New York, 1978 (Chapter 14).
- [20] R.G. Bates, E.A. Guggenheim, *Pure Appl. Chem.* 1 (1960) 163–168.
- [21] T. Mussini, A.K. Covington, P. Longhi, S. Rondinini, *Pure Appl. Chem.* 57 (1985) 865–876.
- [22] A.K. Covington, R.G. Bates, R.A. Durst, *Pure Appl. Chem.* 57 (1985) 531–542.
- [23] L.G. Gagliardi, C.B. Castells, C. Ràfols, M. Rosés, E. Bosch, *Anal. Chem.* 79 (2007) 3180–3187.
- [24] P. Longhi, F. D'Andrea, P.R. Mussini, T. Mussini, S. Rondinini, *Anal. Chem.* 62 (1990) 1019–1021.
- [25] K. Izutsu, M. Muramatsu, Y. Aoki, *J. Electroanal. Chem.* 338 (1992) 125–132.
- [26] R.G. Bates, G.D. Pinching, *J. Res. Natl. Bur. Stand.* 43 (1949) 519–527.
- [27] R.G. Bates, H.B. Hetzer, *J. Phys. Chem.* 65 (1961) 667–673.
- [28] S.P. Datta, A.K. Gezybowski, B.A. Wetson, *J. Chem. Soc.* (1963) 2299–2303.
- [29] J.J. Christensen, L.D. Hansen, R.M. Izzat, *Handbook of Proton Ionization Heats and Related Thermodynamic Quantities*, Wiley-Interscience, New York, 1976.
- [30] L.G. Gagliardi, C.B. Castells, C. Ràfols, M. Rosés, E. Bosch, *J. Chromatogr. A* 1077 (2005) 159–169.
- [31] X. Subirats, E. Bosch, M. Rosés, *J. Chromatogr. A* 1216 (2009) 2491–2498.
- [32] R.G. Bates, S.F. Acree, *J. Res. Natl. Bur. Stand.* 30 (1943) 129.
- [33] R.N. Goldberg, N. Kishore, R.M. Lennen, *J. Phys. Chem. Ref. Data* 31 (2002) 231–370.