



## Enthalpies and constants of dissociation of several neutral and cationic acids in aqueous and methanol/water solutions at various temperatures

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### ABSTRACT

The acidic dissociation enthalpies and constants of anilinium, protonated tris(hydroxymethyl)aminomethane (HTris<sup>+</sup>), benzoic and acetic acids, have been determined at several temperatures in pure water and in methanol/water mixtures by potentiometry and by isothermal titration microcalorimetry (ITC). The  $pK_a$  values determined by both techniques are in accordance when the dissociation process involves large amounts of heat. However, for the neutral acids the ITC technique gave slightly lower  $pK_a$  values than those from potentiometry at the highest temperatures studied due to the small amounts of heat involved in the acidic dissociation. The dissociation enthalpies have been determined directly by calorimetry and the obtained values slightly decrease with the increase of temperature. Therefore, only a rough estimation of the dissociation enthalpies can be obtained from potentiometric  $pK_a$  by means of the Van't Hoff approach.

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### 1. Introduction

The reported increments of enthalpy ( $\Delta H$ ) for most acidic dissociation processes are referred to aqueous solutions and the temperature range of 15–40 °C [1]. However, very often, literature shows discrepancies between the values obtained by different techniques and laboratories and it is not easy to select the best one for further calculation purposes. When specific values are not tabulated it is mandatory to calculate them from the available data. The Van't Hoff equation is often used to estimate  $\Delta H$  values of a particular process from the available acidity constants at different temperatures. This procedure involves the assumption that the  $\Delta H$  quantity is constant in the temperature range considered.

It is much more difficult to have reliable data in hydroorganic solvent mixtures such as methanol/water, especially at temperatures different of 20–25 °C. Nevertheless, methanol/water mixtures show very relevant applications such as suitable solvents for chemicals sparingly soluble in water or as mobile phases in liquid chromatography and, in both instances, it would be very useful to know the thermodynamic quantities involved in the acid–base reactions in these solvent mixtures. The first use has become very popular in drug discovery through the methodology proposed by Sirius Instru-

ments, Ltd. to determine  $pK_a$  values of drugs by potentiometry or spectrophotometry [2,3]. Thus, measurements of  $pK_a$  are done in a variety of methanol/water mixtures and the aqueous  $pK_a$  is easily obtained by extrapolation. The measurements and calculations are currently made at room temperature but the instruments and associated softwares are able to achieve  $pK_a$  values at higher temperatures such as 37 °C. However, the Sirius arrangements are unable to get accurate  $pK_a$  at temperatures higher than 40 °C because of the limitations of the softwares, which are especially designed for the study of drugs. Nevertheless, this technology would be used to determine  $pK_a$  values at temperatures higher than 40 °C and different methanol/water mixtures if the suitable constants are used and a proper standardization procedure is performed.

Methanol/water mixtures have been also widely employed as mobile phases in liquid chromatography for room temperature separations. However, nowadays temperature has become a powerful analytical variable in HPLC and most of the modern chromatographs show devices to control the temperature along the separation process. All the published models to describe the effect of temperature on the retention for compounds with acid–base properties point out that retention depends on the acidic dissociation enthalpies and constants of both analyte and buffering agent [4–7]. These thermodynamic quantities should be referred to the binary solvent used as the mobile phase, methanol/water mixtures for instance, but rarely they are available in literature.

The simplest method to determine experimentally  $\Delta H$  values of acidic dissociation processes is the isothermal titration calorimetry

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(ITC) which is a powerful technique mainly used in the study of bimolecular biological interactions. A well-designed experiment allows the determination of the binding enthalpy, the binding constant and the reaction stoichiometry in a single experiment. However, other applications, such as the study of complexation reactions between a cation and an organic ligand [8,9], the determination of acidic dissociation constants of small molecules such as the L-cysteine [10] or the protonation of polybases such as the phytate ion [11] have also been proposed. In this work, ITC is used to determine the dissociation enthalpies and  $pK_a$  values of several pattern acids and bases in pure water and in 50% (w/w) methanol/water in a wide range of temperatures. These  $pK_a$  values have also been determined potentiometrically in the same experimental conditions and in a wider variety of methanol/water mixtures. Moreover, the dissociation  $\Delta H$  values have been derived from the Van't Hoff plots built from the potentiometric  $pK_a$  values and they have been also directly measured from the ITC titrations. The results obtained by means of both techniques have been critically compared and they allow: (a) the validation of the procedure to determine potentiometric  $pK_a$  values at temperatures higher than 25 °C and methanol/water mixtures; (b) the evaluation of ITC to determine accurate acidity constants at different temperatures in methanol/water mixtures; (c) the evaluation of the Vant'Hoff approach to estimate accurately the enthalpy changes associated to the acidic dissociation processes in methanol/water mixtures.

It is well known that ITC is an emergent technique to determine the thermodynamic quantities associated to interactions of biological interest. The comparison of calorimetric results for very simple interactions (the protonation of a monoprotic base) under non-ordinary experimental conditions (hydroorganic solvent and high temperatures) with those from a reference technique, potentiometry, allows a useful critical evaluation of the quality of the values achieved by ITC in these instances. The results obtained for this binary solvent, commonly used in drug discovery, at temperatures higher than room temperature, around 37 °C, open the ITC to further studies involving drugs sparingly soluble in water and temperatures close to the physiological one. In addition, the very accurate  $\Delta H$  values obtained for the studied acidic dissociation reactions allow the recommendation of ITC as a suitable and useful technique to determine the dissociation enthalpy variation of the more common acids and bases used as buffers of the HPLC mobile phases in the chromatographic conditions of use, i.e. in binary solvents and high temperatures. Therefore, models already proposed for prediction of retention and selectivity of ionizable analytes including drugs with acidic or basic groups [6,7,12] will become much more useful to look for the experimental conditions of analysis when temperature is used as a significant chromatographic variable.

## 2. Experimental

**Chemicals:** aniline (Merck, >99.5%); tris(hydroxymethyl)aminomethane, Tris (Aldrich, >99.99%); sodium benzoate (Panreac, >96%); sodium acetate (Carlo Erba, >99%); potassium chloride

(Merck, >99.5%); hydrochloric acid (Merck, Titrisol®); potassium hydroxide (Merck, Titrisol®); methanol (Merck, HPLC grade); water purified by a Milli-Q Plus system from Millipore with a resistance higher than 18 M $\Omega$ .

### 2.1. Potentiometric measurements

**Instrument:** Automatic titrator PCA 101 from Sirius Instruments Ltd. (UK) equipped with a Sirius 010604 combined electrode. The potentiometric system was standardized at various temperatures in the range of 25–55 °C according to the literature specifications [2].

**Procedure:** Samples of selected compounds in various MeOH/H<sub>2</sub>O mixtures 0.15 M of KCl and different temperatures were preacidified at pH 1.8 and titrated with aqueous 0.5 M KOH.

**Calculations:** Potentiometric  $pK_a$  values ( $I=0.15$ ) of anilinium, protonated tris (HTris<sup>+</sup>), benzoic acid and acetic acid at various MeOH/H<sub>2</sub>O compositions and temperatures were calculated from the potentiometric curves by means of the  $pK_a$ LOGP<sup>TM</sup> software (v 5.2 Sirius). The Yasuda-Shedlovsky equation allowed the interpolation of  $pK_a$  at MeOH/H<sub>2</sub>O mixtures of 10, 20, 30, 40 and 50% (w/w) of MeOH. Obtained values were converted in the thermodynamic ones ( $I=0$ ) by means of the Debye-Hückel approach

$$\log \gamma = -\frac{Az^2I^{1/2}}{1 + a_0BI^{1/2}} \quad (1)$$

where  $z$  is the charge and  $\gamma$  the activity coefficient of the ionic species.  $A$  and  $a_0B$  parameters for a number of methanol/water mixtures and temperatures are given in Table 1.

### 2.2. Microcalorimetric measurements

**Instrument:** Isothermic titration microcalorimeter MicroCal VP-ITC equipped with a 1.4047 mL cell.

**Procedure:** Additions of 10  $\mu$ L of HCl in pure water or in MeOH/H<sub>2</sub>O 50% in weight of MeOH and 50 mM KCl were introduced in the titration cell filled with aniline ( $\sim 5 \times 10^{-4}$  M), tris ( $\sim 10^{-4}$  M), sodium benzoate ( $\sim 2 \times 10^{-3}$  M) or sodium acetate ( $\sim 2 \times 10^{-4}$  M) aqueous or 50% in weight MeOH solutions which are 50 mM in KCl. Experiments were carried out at several temperatures from 15 to 65 °C.

**Calculations:** The protonation enthalpies and constants were calculated from the calorimetric sigmoidal curves using the OriginLab Version7 software. The  $pK_a$  ( $I=0.05$ ) were converted in the thermodynamic values as explained above.

## 3. Results and discussion

The four parameters standardization procedure [2] was used to standardize the potentiometric system at different temperatures. Thus, literature values of static dielectric constants in MeOH/H<sub>2</sub>O mixtures and different temperatures ( $\epsilon_{\text{MeOH/H}_2\text{O}(T)}$ ) within the range of 5–55 °C were compiled [13] and fitted to the following

**Table 1**  
The Debye-Hückel parameters.

T (°C)	0% MeOH		10% MeOH		20% MeOH		30% MeOH		40% MeOH		50% MeOH	
	A	$a_0B$	A	$a_0B$	A	$a_0B$	A	$a_0B$	A	$a_0B$	A	$a_0B$
15.0	0.500	1.500	0.542	1.541	0.592	1.586	0.651	1.637	0.723	1.696	0.811	1.762
25.0	0.510	1.500	0.554	1.542	0.606	1.589	0.669	1.642	0.744	1.702	0.838	1.770
30.0	0.515	1.500	0.560	1.543	0.614	1.590	0.678	1.644	0.756	1.705	0.852	1.774
35.0	0.521	1.500	0.567	1.543	0.622	1.592	0.688	1.646	0.768	1.708	0.867	1.778
40.0	0.526	1.500	0.574	1.544	0.631	1.593	0.698	1.648	0.781	1.711	0.882	1.782
45.0	0.532	1.500	0.581	1.545	0.640	1.595	0.709	1.651	0.794	1.714	0.899	1.786
50.0	0.538	1.500	0.589	1.546	0.649	1.596	0.721	1.653	0.808	1.717	0.915	1.790
55.0	0.545	1.500	0.597	1.546	0.658	1.598	0.732	1.655	0.822	1.720	0.933	1.794

**Table 2**  
Thermodynamic acidic dissociation constants at different temperatures.

T (°C)	Values in water			Values in MeOH/Water 50% (w/w)		
	pK <sub>a</sub> (Pot.)	pK <sub>a</sub> (ITC)	pK <sub>a</sub> (Lit.) <sup>a</sup>	pK <sub>a</sub> (Pot.)	pK <sub>a</sub> (ITC)	pK <sub>a</sub> (Lit.)
<b>Anilinium</b>						
15.0		4.79 ± 0.01	4.78		4.46 ± 0.03	
20.0			4.70			
25.0	4.69 ± 0.01	4.62 ± 0.01	4.62		4.32 ± 0.01	
25.4				4.33 ± 0.01		
29.1	4.58 ± 0.01					
29.3				4.23 ± 0.02		
30.0			4.51			
34.8	4.52 ± 0.01					
34.9				4.15 ± 0.01		
35.0		4.46 ± 0.01	4.43		4.13 ± 0.04	
39.8						
40.0			4.35			
44.5	4.44 ± 0.01			4.04 ± 0.02		
45.0		4.35 ± 0.02	4.27		4.03 ± 0.02	
48.5	4.39 ± 0.01					
49.5				4.03 ± 0.03		
50.0						
54.5	4.29 ± 0.01			3.99 ± 0.03		
55.0		4.17 ± 0.02			4.00 ± 0.04	
65.0		4.09 ± 0.01				
<b>HTris<sup>+</sup></b>						
15.0			8.36			
24.9	8.16 ± 0.01					
25.0			8.07			7.82
25.4				8.04 ± 0.01		
29.9	8.02 ± 0.01					
30.4				7.90 ± 0.01		
34.8	7.92 ± 0.01					
35.0			7.80	7.75 ± 0.02		
39.8	7.84 ± 0.01			7.70 ± 0.02		
44.5	7.79 ± 0.01			7.61 ± 0.05		
45.0			7.55			
48.5	7.77 ± 0.01					
54.5	7.65 ± 0.01			7.50 ± 0.02		
55.0			7.32			
<b>Benzoic acid</b>						
15.0		4.17 ± 0.01	4.212		5.33 ± 0.03	
20.0			4.206			5.43 <sup>b</sup>
25.0		4.23 ± 0.03	4.204		5.38 ± 0.06	5.43 <sup>b</sup> ; 5.39 <sup>a</sup>
25.1	4.24 ± 0.01					
25.2				5.47 ± 0.03		
29.6	4.24 ± 0.01			5.45 ± 0.03		
30.0						5.42 <sup>b</sup>
34.4	4.27 ± 0.01			5.49 ± 0.03		
35.0		4.28 ± 0.02	4.206		5.39 ± 0.01	5.41 <sup>b</sup>
39.9	4.33 ± 0.01			5.42 ± 0.03		
40.0						5.42 <sup>b</sup>
44.5	4.37 ± 0.01			5.46 ± 0.03		
45.0		4.28 ± 0.03			5.32 ± 0.25	5.42 <sup>b</sup>
49.5	4.38 ± 0.01			5.53 ± 0.02		
50.0			4.223			5.42 <sup>b</sup>
54.5	4.33 ± 0.01			5.46 ± 0.02		
55.0		4.33 ± 0.03			5.34 ± 0.10	
60.0			4.240			
65.0		4.27 ± 0.02				
<b>Acetic acid</b>						
15.0			4.758		5.55 ± 0.04	
20.0			4.756			
25.0			4.756		5.61 ± 0.04	5.66 <sup>a</sup>
25.2	4.84 ± 0.01			5.84 ± 0.03		
30.0			4.757			
30.4	4.81 ± 0.01					
30.5				5.83 ± 0.02		
34.7				5.76 ± 0.02		
34.9	4.82 ± 0.01					
35.0			4.762		—	
39.8	4.84 ± 0.01			5.83 ± 0.02		
40.0			4.769			
44.5	4.89 ± 0.01			5.90 ± 0.07		
45.0			4.777		5.65 ± 0.09	
49.5	4.97 ± 0.01			5.92 ± 0.03		
50.0			4.787			

Table 2 (Continued)

T (°C)	Values in water			Values in MeOH/Water 50% (w/w)		
	pK <sub>a</sub> (Pot.)	pK <sub>a</sub> (ITC)	pK <sub>a</sub> (Lit.) <sup>a</sup>	pK <sub>a</sub> (Pot.)	pK <sub>a</sub> (ITC)	pK <sub>a</sub> (Lit.)
54.5	5.07 ± 0.01			5.95 ± 0.05		
55.0			4.799		5.79 ± 0.07	
60.0			4.812			

<sup>a</sup> From Ref. [1].<sup>b</sup> From Ref. [23].

empirical equation [14], which was derived for binary mixtures up to 60% (w/w) of methanol

$$\varepsilon_{\text{MeOH}/\text{H}_2\text{O}(T)} = 10^\delta + \log D_0 \quad (2)$$

where

$$\delta = (t - 25) \left[ -0.020 - \frac{0.0005 \text{ wt}\%}{60} \right] \quad (3)$$

$$D_0 = 78.47 - 0.42 \text{ wt}\% - 5.1 \times 10^{-4} (\text{wt}\%)^2 \quad (4)$$

being  $t$  the temperature in Celsius scale and wt% the percent weight fraction of MeOH. These expressions allow the calculation of any permittivity value by interpolation. In the same manner, values of densities of pure water and pure methanol [15] in the temperature ranges of 0–50 °C originated the following expressions

$$d_{\text{water}} = -4.94 \times 10^{-6} t^2 + 7.64 \times 10^{-6} t + 1.00 \quad (5)$$

$$d_{\text{MeOH}} = -6.22 \times 10^{-7} t^2 - 9.21 \times 10^{-4} t + 0.81 \quad (6)$$

where  $d$  stands for the density of the subscript species at  $t$  temperature. Suitable interpolated values of  $\varepsilon_{\text{MeOH}/\text{H}_2\text{O}(T)}$ ,  $d_{\text{H}_2\text{O}(T)}$  and  $d_{\text{MeOH}(T)}$  have been used in the application of the Yasuda–Shedlovsky equation [2,3,16–22], which relates the pK<sub>a</sub> of an acidic compound in any MeOH/H<sub>2</sub>O mixture ( ${}^s\text{pK}_a$ ) to the dielectric permittivity of the solvent, being C and D specific constants for each compound and temperature

$${}^s\text{pK}_a + [\text{H}_2\text{O}] = C + D\varepsilon_{\text{MeOH}/\text{H}_2\text{O}(T)}^{-1} \quad (7)$$

The pK<sub>a</sub> ( $I=0.15$  M) at different solvent mixtures and temperatures for anilinium, HTris<sup>+</sup>, benzoic acid and acetic acids were determined and the  ${}^s\text{pK}_a$  values at several intermediate solvent compositions in the 0–60% (w/w) composition and 25–55 °C temperature ranges have been calculated using Eq. (7). From these

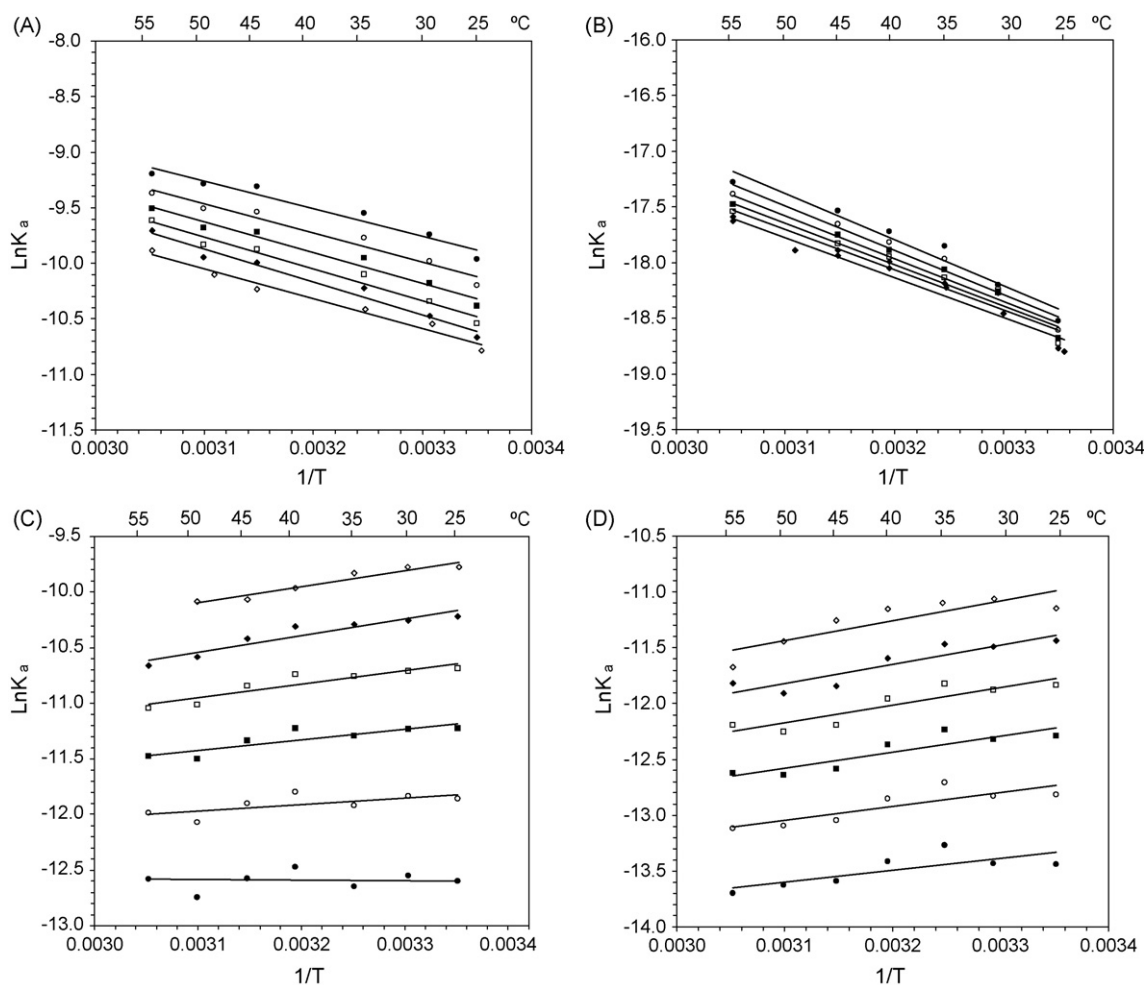


Fig. 1. Van't Hoff plots of (A) aniline, (B) tris, (C) benzoic acid, and (D) acetic acid at various MeOH/H<sub>2</sub>O mixtures: (◇) 0%, (◆) 10%, (□) 20%, (■) 30%, (○) 40% and (●) 50% (w/w) in MeOH.

**Table 3**  
Potentiometric  $pK_a$  interpolated from the experimental values by means of Eq. (6).

T (°C)	10%	20%	30%	40%	50%
<b>Anilinium</b>					
25.4	4.63 ± 0.01	4.58 ± 0.01	4.51 ± 0.01	4.43 ± 0.01	4.33 ± 0.01
29.3	4.55 ± 0.02	4.49 ± 0.02	4.42 ± 0.02	4.34 ± 0.02	4.23 ± 0.02
34.9	4.44 ± 0.01	4.39 ± 0.01	4.32 ± 0.01	4.24 ± 0.01	4.15 ± 0.01
44.5	4.34 ± 0.02	4.29 ± 0.02	4.22 ± 0.02	4.14 ± 0.02	4.04 ± 0.02
49.5	4.32 ± 0.03	4.27 ± 0.03	4.21 ± 0.03	4.13 ± 0.03	4.03 ± 0.03
54.5	4.22 ± 0.03	4.18 ± 0.03	4.13 ± 0.03	4.07 ± 0.03	3.99 ± 0.03
<b>HTris<sup>+</sup></b>					
25.4	8.15 ± 0.01	8.13 ± 0.01	8.11 ± 0.01	8.08 ± 0.01	8.04 ± 0.01
30.4	7.94 ± 0.01	7.93 ± 0.01	7.93 ± 0.01	7.92 ± 0.01	7.90 ± 0.01
35.0	7.90 ± 0.02	7.87 ± 0.02	7.84 ± 0.02	7.80 ± 0.02	7.75 ± 0.02
39.8	7.81 ± 0.02	7.79 ± 0.02	7.77 ± 0.02	7.74 ± 0.02	7.70 ± 0.02
44.5	7.77 ± 0.05	7.74 ± 0.05	7.71 ± 0.05	7.67 ± 0.05	7.61 ± 0.05
54.5	7.64 ± 0.02	7.62 ± 0.02	7.59 ± 0.02	7.55 ± 0.02	7.50 ± 0.02
<b>Benzoic acid</b>					
25.2	4.44 ± 0.03	4.64 ± 0.03	4.88 ± 0.03	5.15 ± 0.03	5.47 ± 0.03
29.6	4.45 ± 0.03	4.65 ± 0.03	4.88 ± 0.03	5.14 ± 0.03	5.45 ± 0.03
34.4	4.47 ± 0.03	4.67 ± 0.03	4.90 ± 0.03	5.17 ± 0.03	5.49 ± 0.03
39.9	4.48 ± 0.03	4.66 ± 0.03	4.88 ± 0.03	5.12 ± 0.03	5.42 ± 0.03
44.5	4.52 ± 0.03	4.71 ± 0.03	4.92 ± 0.03	5.17 ± 0.03	5.46 ± 0.03
49.5	4.59 ± 0.02	4.78 ± 0.02	4.99 ± 0.02	5.24 ± 0.02	5.53 ± 0.02
54.5	4.63 ± 0.02	4.80 ± 0.02	4.98 ± 0.02	5.20 ± 0.02	5.46 ± 0.02
<b>Acetic acid</b>					
25.2	4.97 ± 0.03	5.14 ± 0.03	5.34 ± 0.03	5.57 ± 0.03	5.84 ± 0.03
30.5	4.99 ± 0.02	5.16 ± 0.02	5.35 ± 0.02	5.57 ± 0.02	5.83 ± 0.02
34.7	4.98 ± 0.02	5.13 ± 0.02	5.31 ± 0.02	5.52 ± 0.02	5.76 ± 0.02
39.8	5.04 ± 0.02	5.19 ± 0.02	5.37 ± 0.02	5.58 ± 0.02	5.83 ± 0.02
44.5	5.14 ± 0.07	5.29 ± 0.07	5.47 ± 0.07	5.67 ± 0.07	5.90 ± 0.07
49.5	5.17 ± 0.03	5.32 ± 0.03	5.49 ± 0.03	5.69 ± 0.03	5.92 ± 0.03
54.5	5.13 ± 0.05	5.30 ± 0.05	5.48 ± 0.05	5.70 ± 0.05	5.95 ± 0.05

values the thermodynamic ones ( $I=0$ ) were calculated through the Debye-Hückel approach. Table 2 shows the results for the compounds studied at 0 and 50% (w/w) MeOH/H<sub>2</sub>O solutions at the 25–55 °C temperature range. It includes the literature  $pK_a$  values, which are consistent with those potentiometrically determined in the present work. It should be noticed that all the values for benzoic acid in 50% (w/w) MeOH/H<sub>2</sub>O agree with the wide set given in literature [23]. This consistency validates the potentiometric procedure at temperatures higher than 25 °C and, consequently, validates also the whole set of the potentiometric  $pK_a$  values shown in Tables 2 and 3.

Fig. 1 is built from the thermodynamic potentiometric  $pK_a$  values for anilinium, HTris<sup>+</sup>, benzoic and acetic acids and it shows apparently linear Van't Hoff plots. Therefore, the variation of the dissociation enthalpy,  $\Delta H_{VH}$ , at the 25–55 °C temperature range can be taken as a constant that can be easily calculated from the Van't Hoff slopes. Values of  $\Delta H_{VH}$  are gathered in Table 4 for solutions from 0 to 50% of MeOH. The results show that the absolute  $\Delta H_{VH}$  values for anilinium and HTris<sup>+</sup> are significantly higher than those of benzoic and acetic acids and the dissociation process is endothermic for the cationic acids whereas it is slightly exothermic for the neutral acids in the solvent mixtures studied.

Table 2 includes the  $pK_a$  values obtained by titration microcalorimetry from solutions with 0% and 50% (w/w) of MeOH at the temperature range from 15 to 65 °C. These values were calculated from the ITC binding constants between the proton and the conjugate base and are consistent with those obtained by potentiometry for the anilinium, for which significant amounts of heat are involved in the protonation processes, and also for the neutral acids. However, the small amounts of heat measured for benzoic and acetic acids and the transition from an exothermic to an endothermic process with the increase of temperature lead to  $pK_a$  values slightly lower than the potentiometric ones. The ITC curves for the studied compounds obtained in 50% (w/w) MeOH/H<sub>2</sub>O solu-

**Table 4**  
Variation of enthalpy of several acidic dissociation processes calculated through the Van't Hoff equation.

% MeOH (w/w)	From potentiometric $pK_a$ values $\Delta H_{VH}$ (Kcal/mol)	From ITC $pK_a$ values $\Delta H_{VH}$ (Kcal/mol)
<b>Anilinium</b>		
0 <sup>a</sup>	5.3 ± 0.4	6.3 ± 0.2
10 <sup>b</sup>	5.9 ± 0.5	
20 <sup>b</sup>	5.7 ± 0.5	
30 <sup>b</sup>	5.5 ± 0.5	
40 <sup>b</sup>	5.2 ± 0.5	
50 <sup>b</sup>	4.9 ± 0.6	
<b>Tris</b>		
0 <sup>a</sup>	7.1 ± 0.6	-
10 <sup>b</sup>	7.2 ± 0.9	
20 <sup>b</sup>	7.4 ± 0.9	
30 <sup>b</sup>	7.6 ± 0.8	
40 <sup>b</sup>	7.9 ± 0.8	
50 <sup>b</sup>	8.2 ± 0.8	
<b>Benzoic acid</b>		
0 <sup>a</sup>	-2.8 ± 0.4	-1.6 ± 0.2
10 <sup>b</sup>	-2.9 ± 0.4	
20 <sup>b</sup>	-2.4 ± 0.4	
30 <sup>b</sup>	-1.9 ± 0.5	
40 <sup>b</sup>	-1.2 ± 0.6	
50 <sup>b</sup>	0.1 ± 0.4	
50 <sup>c</sup>	0.15 ± 0.09	0.1 ± 0.5
<b>Acetic acid</b>		
0 <sup>a</sup>	-3.5 ± 0.9	-
10 <sup>b</sup>	-3.4 ± 0.7	
20 <sup>b</sup>	-3.1 ± 0.7	
30 <sup>b</sup>	-2.8 ± 0.7	
40 <sup>b</sup>	-2.5 ± 0.7	
50 <sup>b</sup>	-2.1 ± 0.8	
		-2.2 ± 0.6

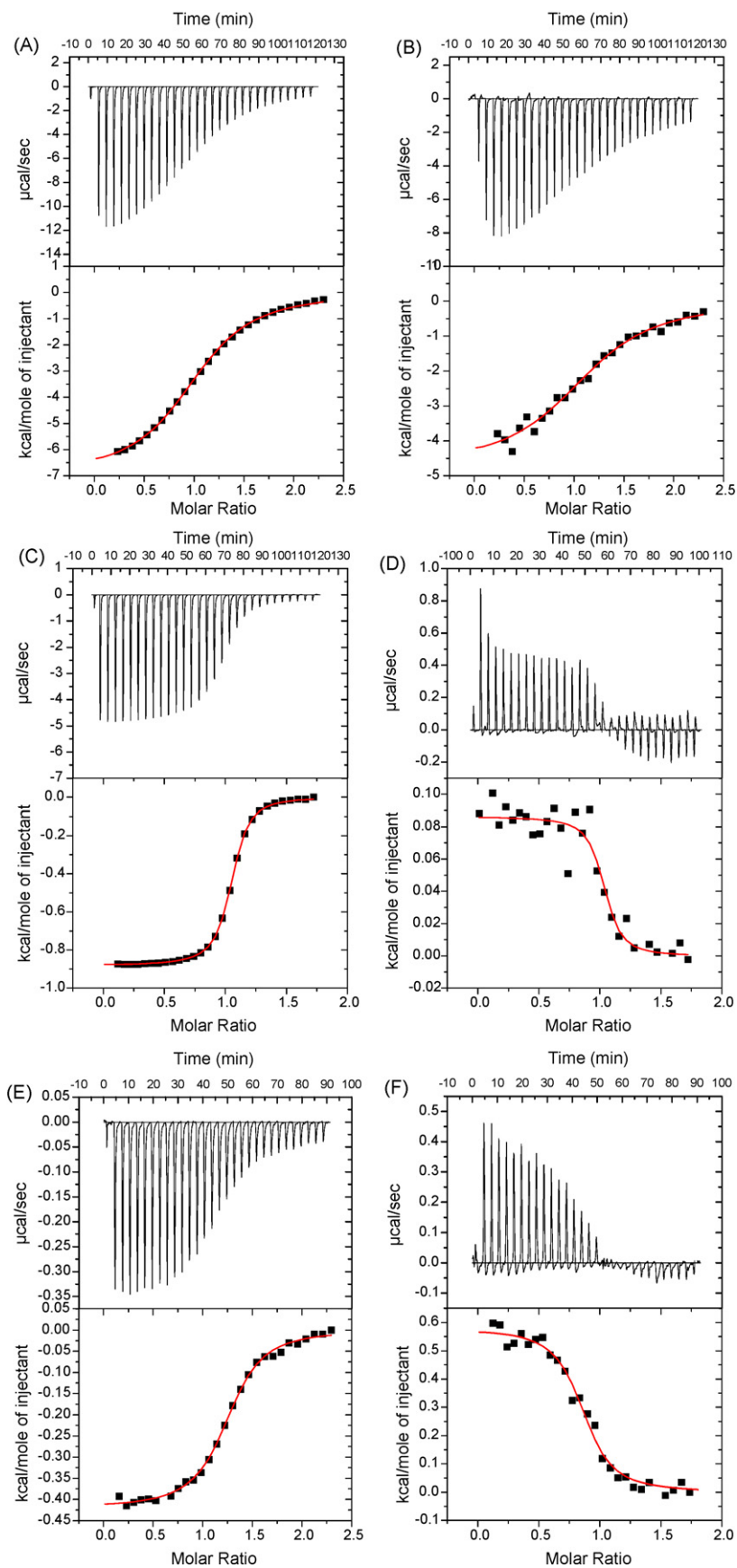
<sup>a</sup> Experimentally determined in this work.

<sup>b</sup> Interpolate from the Yasuda-Shedlovsky plots build from potentiometric  $pK_a$ 's.

<sup>c</sup> From Ref. [23].

tions at two different temperatures are given in Fig. 2. They clearly show a higher dispersion of the experimental points at the higher temperatures, particularly for the titrations of the anionic bases for which the amount of heat involved in the protonation reaction is lower. However, the experimental points can be adjusted to the one binding site model except for the acetic acid at 35 °C for which the involved heat is nearly zero. The shapes of the ITC curves obtained for tris in aqueous and hydroorganic solutions are not sigmoidal as expected for a 1:1 reaction and, therefore, these curves could not be adjusted to the suitable model (see Fig. 3). At the moment, we have not a good explanation for this fact and, for this reason, the  $pK_a$  values were not calculated and the study with the temperature omitted, although the global amounts of heat involved in the neutralization processes agree with those from literature, as shown in Table 5. Table 4 includes the  $\Delta H_{VH}$  calculated from the  $pK_a$  values determined by ITC. The comparison of these values with those derived from the potentiometric results shows that paired values are close but do not show a perfect agreement.

The dissociation enthalpies for the studied compounds,  $\Delta H_{cal}$ , in pure water and in 50% (w/w) MeOH/H<sub>2</sub>O solutions at different temperatures were also determined by calorimetric titration and they are shown in Table 5. These  $\Delta H_{cal}$  values were determined at ionic strength 0.05 M ( $I=0.05$ ) but they are consistent with those from literature given at  $I=0$ . Despite the study of Sammartano [24] clearly shows that the absolute value of  $\Delta H_{cal}$  for the protonation of amines increases with the ionic strength, it was performed in a much wider ionic strength range, from 0 to 3 mol kg<sup>-1</sup> in NaCl, and the achieved conclusions cannot apply to our results. Fig. 4 shows that values obtained at different temperatures are linearly related to the temperature with a small



**Fig. 2.** Calorimetric titrations in 50% (w/w) MeOH/H<sub>2</sub>O of (A) aniline at 25 °C, (B) aniline at 55 °C, (C) benzoic acid at 25 °C, (D) benzoic acid at 45 °C, (E) acetic acid at 25 °C, and (F) acetic acid at 45 °C.

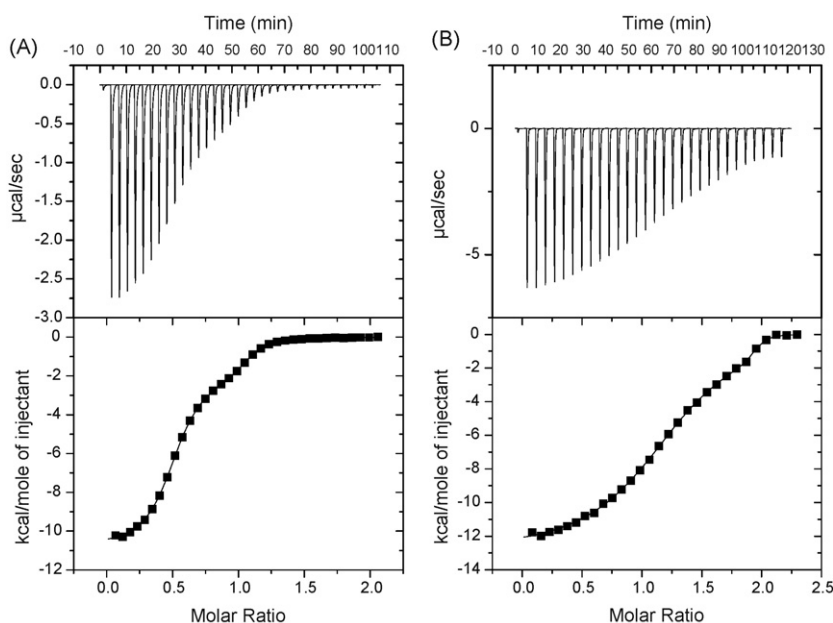


Fig. 3. Calorimetric titrations of tris at 25 °C in (A) pure water and (B) 50% (w/w) MeOH/H<sub>2</sub>O.

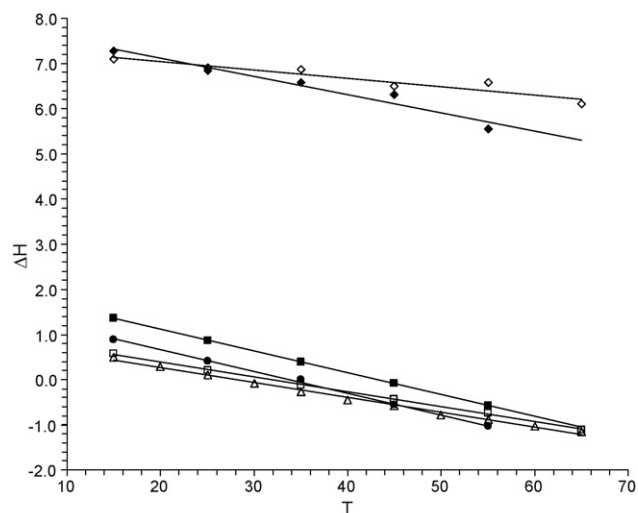
and negative slope and this fact disagrees with the assumption of the constancy of the  $\Delta H$  quantities in the studied temperature range as required by the Van't Hoff equation. It is also noticed that the  $\Delta H_{\text{cal}}$  are closer to the  $\Delta H_{\text{VH}}$  derived from the ITC  $\text{p}K_{\text{a}}$  values than those derived from potentiometric  $\text{p}K_{\text{a}}$ . Moreover, it should be pointed out that the  $\Delta H_{\text{cal}}$  for tris in aqueous solution at 25 °C clearly disagrees with the potentiometric  $\Delta H_{\text{VH}}$  but the values obtained in pure water and in the hydroorganic solvent are consistent with the reported data. Finally, the comparison with literature values clearly shows that ITC provides a better measurement of the heat involved in these acidic dissociation processes than the Van't Hoff approach for both aqueous and hydroorganic solutions.

To evaluate deeper the Van't Hoff results, the plots of Fig. 4 have been examined and, as expected, they show slopes very close to the  $\Delta C_{\text{p}}$  values given in literature for all the aqueous dissociation processes and also for acetic acid in 50% of methanol [1]. These literature values are given at several temperatures but they are essentially constant. Therefore, the  $\Delta H$  cannot be taken as a constant in the temperature range analyzed and, according to Clarke and Glew approach, it should be considered a continuous function of temperature [25–27]. However, since the slopes of the lines shown in Fig. 4 are small, it can be assumed that Van't Hoff equation provides a rough value of the enthalpy variation in these acidic dissociation processes. No  $\Delta C_{\text{p}}$  values have been published for anilinium and benzoic acid in the hydroorganic solutions studied in this work.

Table 5

Variation of enthalpy of several acidic dissociation processes measured by ITC in 50 mM KCl solutions. Values given in kcal/mol.

T °C	Water		50% MeOH/Water	
	$\Delta H_{\text{cal}} (I=0.05)$	$\Delta H_{\text{cal}} I=0 [1]$	$\Delta H_{\text{cal}} (I=0.05)$	$\Delta H_{\text{cal}} (I=0) [1]$
<b>Anilinium</b>				
15.0	7.10 ± 0.02	7.08; 5.18	7.28 ± 0.20	–
25.0	6.90 ± 0.04	5.78–7.43	6.86 ± 0.05	–
35.0	6.86 ± 0.04	5.78; 7.35	6.57 ± 0.07	–
45.0	6.49 ± 0.16	7.44	6.32 ± 0.13	–
55.0	6.57 ± 0.08	–	5.54 ± 0.07	–
65.0	6.10 ± 0.06	–	–	–
<b>HTris<sup>+</sup></b>				
25.0	10.74 ± 0.38	11.33–11.89	12.10 ± 0.40	11.43
<b>Benzoic acid</b>				
15.0	0.570 ± 0.002	0.46–0.97	1.35 ± 0.08	–
25.0	0.209 ± 0.008	0.15 to –0.86	0.86 ± 0.03	0.70
35.0	–0.125 ± 0.002	–0.27 to –0.67	0.40 ± 0.01	–
45.0	–0.440 ± 0.001	–0.59 to –0.66	–0.08 ± 0.01	–
55.0	–0.751 ± 0.019	–0.88 to –1.03	–0.58 ± 0.05	–
65.0	–1.110 ± 0.015	–1.15	–	–
<b>Acetic acid</b>				
15.0	–	0.22–0.27	0.88 ± 0.02	–
25.0	–	–0.02 to –0.11	0.42 ± 0.02	0.15; –0.05
35.0	–	–0.43; –0.46	–	–
45.0	–	–0.67; –0.80	–0.57 ± 0.01	–
55.0	–	–0.90; –1.16	–1.04 ± 0.18	–



**Fig. 4.** Variation of the acidic dissociation enthalpy with the temperature. (◇) Aniline in water, slope =  $-0.018$ ; (◆) aniline in 50% MeOH/H<sub>2</sub>O, slope =  $-0.040$ ; (□) benzoic acid in water, slope =  $-0.033$ ; (△) benzoic acid in water (Ref. [1]), slope =  $-0.033$ ; (■) benzoic acid in 50% MeOH/H<sub>2</sub>O, slope =  $-0.048$ ; (●) acetic acid in 50% MeOH/H<sub>2</sub>O, slope =  $-0.048$ .

#### 4. Conclusions

The results achieved in this work clearly show that the four parameter calibration procedure can be applied to get accurate  $pK_a$  values in pure water and methanol/water mixtures at the temperature range of 25–55 °C. Moreover, the Yasuda-Shedlovsky equation (Eq. (7)) and the Debye-Hückel constants given in Table 1 allow the calculation of the thermodynamic acidity constants at any solvent composition (from 0 to 60% (w/w) of methanol) and temperature (from 25 to 55 °C). The comparison between the results achieved by both techniques, potentiometry and calorimetry, shows that ITC provides accurate  $pK_a$  in aqueous as well as in 50% methanol/water solutions when the protonation enthalpy is high enough but slight differences with the potentiometric ones are obtained when small amounts of heat, lower than 1 kcal mol<sup>-1</sup>, are involved in the protonation process. On the other hand, the enthalpy increments of the protonation processes determined by ITC at different temperatures show linear dependences on temperature with small slopes.

This fact means that the Van't Hoff equation is able to provide rough values for the enthalpy variation of the acidic dissociation processes studied but not the accurate ones.

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#### References

- [1] J.J. Christensen, L.D. Hansen, R.M. Izatt, Handbook of Proton Ionization Heats, Wiley, New York, 1976.
- [2] A. Avdeff, J.E.A. Comer, S.J. Thomson, Anal. Chem. 65 (1993) 42–49.
- [3] A. Avdeff, K.J. Box, J.E.A. Comer, M. Gilges, M. Hadley, C. Hibbert, W. Patterson, K.Y. Tam, J. Pharm. Biomed. Anal. 20 (1999) 631–641.
- [4] Cs. Horváth, W. Melander, P. Molnár, Anal. Chem. 49 (1977) 142–154.
- [5] J. Li, Anal. Chim. Acta 369 (1988) 21–37.
- [6] C.B. Castells, L.G. Gagliardi, C. Ràfols, M. Rosés, E. Bosch, J. Chromatogr. A 1042 (2004) 23–35.
- [7] L.G. Gagliardi, C.B. Castells, C. Ràfols, M. Rosés, E. Bosch, Anal. Chem. 78 (2006) 5858–5867.
- [8] Y. Zhang, S. Akilesh, D.E. Wilcox, Inorg. Chem. 39 (2000) 3057–3064.
- [9] L.S. Mizoue, J. Tellinghuisen, Biophys. Chem. 110 (2004) 15–24.
- [10] S.G. Tajc, B.S. Tolbert, R. Basavappa, B.L. Miller, J. Am. Chem. Soc. 126 (2004) 10508–10509.
- [11] C. de Stefano, D. Milea, S. Sammartano, Thermochim. Acta 423 (2004) 63–69.
- [12] L.G. Gagliardi, C.B. Castells, C. Ràfols, M. Rosés, E. Bosch, J. Sep. Sci. 31 (2008) 969–980.
- [13] S. Albright, L.J. Gosting, J. Am. Chem. Soc. 68 (1946) 1061–1063.
- [14] R.C. Weast (Ed.), CRC Handbook of Chemistry and Physics, 51st ed., The Chemical Rubber, Co., Cleveland, 1970–1971, E-43.
- [15] Landolt-Börnstein, Numerical Data and Functional Relationships in Science and Technology, New Series, Group IV, Band 1, Springer-Verlag, Berlin, 1977.
- [16] M. Yasuda, Bull. Chem. Soc. Jpn. 32 (1959) 429–432.
- [17] K. Takács-Novák, K.J. Box, A. Avdeef, Int. J. Pharm. 151 (1997) 235–248.
- [18] R. Ruiz, C. Ràfols, M. Rosés, E. Bosch, J. Pharm. Sci. 92 (2003) 1473–1481.
- [19] K. Takács-Novák, A. Avdeef, J. Pharm. Biomed. Anal. 14 (1996) 1405–1413.
- [20] B. Slater, A. McCormack, A. Avdeef, J.E.A. Comer, J. Pharm. Sci. 83 (1994) 1280–1283.
- [21] J.E.A. Comer, in: H. van de Waterbeemd, H. Lennernäs, P. Artursson (Eds.), Drug Bioavailability, Wiley-VCH, Weinheim, 2003.
- [22] G. Garrido, C. Ràfols, E. Bosch, Eur. J. Pharm. Sci. 28 (2006) 118–127.
- [23] C.B. Castells, C. Ràfols, M. Rosés, E. Bosch, J. Chromatogr. A 1002 (2003) 41–53.
- [24] A. de Robertis, C. Foti, O. Giuffrè, S. Sammartano, J. Chem. Eng. Data 47 (2002) 1205–1212.
- [25] E.C.W. Clarke, D.N. Glew, Trans. Faraday Soc. 62 (1966) 539–547.
- [26] H.P. Butikofer, A.K. Covington, D.A. Evans, Electrochim. Acta 24 (1979) 1071–1079.
- [27] A.K. Covington, Z.-Y. Zou, Electrochim. Acta 28 (1983) 1587–1591.