ELSEVIER

Effect of temperature on pH measurements and acid–base equilibria in methanol–water mixtures

Cecilia B. Castells[a,*], Clara Ràfols[b], Martí Rosés[b], Elisabeth Bosch[b]

[a]*División Química Analítica, Universidad Nacional de la Plata, 47 y 115, 1900 La Plata, Argentina*
[b]*Departament de Química Analítica, Universitat de Barcelona, Diagonal 647, 08028 Barcelona, Spain*

Abstract

The knowledge of the acid–base equilibria in water–solvent mixtures of both common buffers and analytes is necessary in order to predict their retention as function of pH, solvent composition and temperature. This paper describes the effect of temperature on acid–base equilibria in methanol–water solvent mixtures commonly used as HPLC mobile phases. We measured the δ-correction parameter ($\delta = {}_{w}^{s}\mathrm{pH} - {}_{s}^{s}\mathrm{pH} = \bar{E}_j - \log {}_{w}^{s}\gamma_{H}^{0}$) between two pH scales: pH measured in the solvent concerned and referred to the same standard state, ${}_{s}^{s}\mathrm{pH}$, and the pH measured in that solvent mixture but referred to water as standard state, ${}_{w}^{s}\mathrm{pH}$, for several methanol compositions in the temperature range of 20–50 °C. These determinations suggest that the δ-term depends only on composition of the mixture and on temperature. In water-rich mixtures, for which methanol is below 40% (w/w), δ-term seems to be independent of temperature, within the experimental uncertainties, whereas for methanol content larger than 50% (w/w) the δ-correction decreases as temperature increases. We have attributed this decrease to a large increase in the medium effect when mixtures have more than 50% methanol. The pK_a of five weak electrolytes of different chemical nature in 50% methanol–water at 20–50 °C are presented: the effect of temperature on pK_a was large for amines, pyridine and phenol, but almost no dependence was found for benzoic acid. This indicates that buffers can play a critical role in affecting retention and selectivity in HPLC at temperatures far from 25 °C, particularly for co-eluted solutes.

1. Introduction

Temperature plays a significant role in all chromatographic techniques. Although it is recognized as the most relevant parameter in GC, many separation scientists have traditionally disregarded temperature in LC. As a consequence, current method development strategies for optimizing resolution are based on manipulating experimental conditions such as the mobile phase composition, mobile phase type, stationary phase type and mobile phase pH for separation of acidic and basic analytes. Consideration of temperature higher than ambient for optimizing resolution in typical reversed-phase separations has been rather scarce [1–8]. Exceptions to this trend have been chiral separations, where usually temperatures below ambient favor molecular shape recognition [9,10], ion-exchange chromatography, especially for larger molecules, and size-exclusion chromatog-

*Corresponding author. Fax: +54-221-427-1537.
E-mail address: castells@isis.unlp.edu.ar (C.B. Castells).

raphy [11]. In a recent publication, Greibrokk [12] clearly stated the reasons for fearing of the use of elevated temperatures as a usual controlling variable in RPLC: mainly, reduced column life time, lack of ovens in common instruments, and loss of efficiency due to poor viscous heat dissipation. Today, most of these reasons can be argued: column technology has significantly improved, most commercial instruments have column temperature control and narrower columns are more often used.

Elevated temperatures increase solute solubility and diffusivity, whereas they decrease solvent viscosity, significantly improving the kinetics of the partition process, and as a consequence, peak shape and column efficiency. But, temperature has also a large effect on the thermodynamics of the retention process, affecting retention factors, selectivity and total analysis time. Now, when temperature in combination with pH are introduced as variables to adjust selectivity towards acidic and basic compounds, one must evaluate the effect that temperature has on the pH of the mobile phase. This means that, it is critical the knowledge of the exact nature of buffer dissociation in organic solvent–water mixtures at temperatures different from ambient when they are used as mobile phases in liquid chromatography varying column temperatures.

Retention of basic and acidic analytes strongly depends on the pH of the eluents. Rosés et al. [13,14] developed a model to relate the retention of acidic solutes with the true pH of the mobile phase. The equations derived take into account the effects of activity coefficients and the presence of other ions in the mobile phase and mobile phase composition. These equations have been successfully applied to predict retention of several analytes, using both methanol–water [15,16] and acetonitrile–water mixtures [17,18] as eluents. However, their studies were essentially conducted under isothermal conditions.

In their seminal studies on reversed-phase retention, Horváth et al. [1,19,20] stated the influence that temperature has on the acid–base equilibrium of weak electrolytes commonly used as buffers; and as a consequence, on the chromatographic retention of acidic and basic solutes. However, these experiments were conducted using pure water as mobile phase, which is rarely used in RPLC methods.

The aim of this preliminary study was double.

First, we are aware of the importance that temperature has in liquid chromatographic separations. Within this context, the acid–base equilibria of weak electrolytes in usual chromatographic mobile phases behave unpredictable as temperature is far from 25 °C. We strongly believe that this issue was underestimated mainly because of the very scarce information about the ionization enthalpies of these weak electrolytes in solvent mixtures. But, since the interest in temperature influence in HPLC has been increasing among separation scientists during the last years, it is necessary to address ourselves to this topic. We hope to install the discussion, and as more data can be collected, we expect to be able to re-formulate predictive models of retention of analytes in RPLC, in which temperature and pH of the mobile phase would be experimental variables. The second and more immediate objective was to explore the magnitude of the effect that temperature has over acid–base equilibria of buffer solutions in methanol–water mixtures and over the pK_{HA} of a number of weak electrolytes chemically different in these aqueous–organic solvents.

2. Theoretical

2.1. Acid–base equilibria in aqueous–organic solvents

The dissociation equilibrium for an acidic compound HA in a given solvent (s) can be expressed as:

$$HA \leftrightarrow H^+ + A^-$$

In this equilibrium, we assumed ion association to be negligible, which is reasonable for solvent mixtures of relatively high dielectric constant. The standard free energy of the dissociation of HA in the system, can be referred to the aqueous standard state:

$$\Delta_w^s G^0 = -RT \ln {}_w^s K_{HA} = \Delta_w^s H^0 - T\,\Delta_w^s S^0 \qquad (1a)$$

or to the solvent mixture standard state

$$\Delta_s^s G^0 = -RT \ln {}_s^s K_{HA} = \Delta_s^s H^0 - T\,\Delta_s^s S^0 \qquad (1b)$$

where ΔG^0, ΔH^0 and ΔS^0 are the standard Gibbs free energy, enthalpy and entropy for the dissociation equilibrium of HA in the solvent mixture (s) (left

hand superscript) referred either to water (w) or to the solvent mixture (s) (left hand subscript) as standard state solvent for the activity coeficients, and K_{HA} is the acidic dissociation constant referred to water,

$$_w^s K_{HA} = \frac{_w^s a_H \, _w^s a_A}{_w^s a_{HA}} \qquad (2a)$$

or to the solvent mixture

$$_s^s K_{HA} = \frac{_s^s a_H \, _s^s a_A}{_s^s a_{HA}} \qquad (2b)$$

a_i is the activity of a specie (i) in the corresponding solvent reference scale. Thus, K_{HA} depends on the choice of the standard state and the associated choice of how concentrations are expressed. In a molal scale, the activities are related to molality of (i) in the equilibrium, m_i, through the corresponding activity coefficient, γ_i, by $_w^s a_i = m_i \, _w^s \gamma_i$ and $_s^s a_i = m_i \, _s^s \gamma_i$, in which $_s^s \gamma_i$ and $_w^s \gamma_i$ are unity at infinite dilution of (i) in the organic mixture and in water, respectively.

By combining Eqs. (1) and (2), and substituting a_i by $m_i \, \gamma_i$,

$$\Delta_s^s G^0 - \Delta_w^s G^0 \equiv \Delta G_t^0 = RT \ln \frac{_w^s K_{HA}}{_s^s K_{HA}}$$

$$= RT \ln \frac{_w^s \gamma_H^0 \, _w^s \gamma_A^0}{_w^s \gamma_{HA}^0} \qquad (3)$$

In this expression, $_w^s \gamma_i^0$ ($= \, _w^s \gamma_i / _s^s \gamma_i$) represents the primary medium effect. This quantity is fixed by the Gibbs energy of transfer of one mole of the species (i) in question from the standard state in water to the standard state in the organic solvent. ΔG_t^0 stands for the change in the standard Gibbs free energy of transfer of the three species, i.e., H^+, A^- and HA from the aqueous to the aqueous–solvent system.

Now, at constant pressure, the effect of temperature can be obtained from the van't Hoff equation as:

$$\frac{d \ln \, _s^s K_{HA}}{d(1/T)} \equiv - \frac{\Delta_s^s H^0}{R}$$

$$= - \frac{1}{R} \left[\Delta H^0 - \Delta H_{t,HA}^0 + \Delta H_{t,H}^0 + \Delta H_{t,A}^0 \right] \qquad (4)$$

where R is the gas constant and $\Delta H_{t,i}^0$ represents the

enthalpy of transfer of one mole of (i) in its standard state from water to the solvent mixture. In Eq. (4), we assumed that enthalpies are temperature-independent. By re-arranging Eq. (4),

$$\Delta_s^s H^0 = \Delta_w^s H^0 - \left[\Delta H_{t,HA}^0 - \Delta H_{t,H}^0 - \Delta H_{t,A}^0 \right] \qquad (5)$$

This expression indicates that the change in K_{HA} as temperature changes both in water and in solvent mixtures will be the same only when the term bracketed in Eq. (5) is zero. Otherwise, the dissociation of HA as temperature is changed will be different in different solvent compositions and, as a consequence, the pH of organic solutions buffered by HA will change differently from pH in water.

2.2. Determination of pH in aqueous–organic mixtures

Mobile phases in reversed-phase liquid chromatography (RPLC) are, in general, mixtures of an aqueous buffer and an organic solvent. There are three methodologies to determine the pH of a buffer solution to be used as eluent in RPLC: (i) measurement of aqueous pH buffer before mixing it with the organic modifier ($_w^w pH$); (ii) measurement of the pH of the RPLC buffer after mixing using a pH electrode system calibrated with aqueous buffers ($_w^s pH$); and (iii) measurement of the pH after mixing and calibrating the electrode system with reference buffers prepared in the same solvent mixture ($_s^s pH$). The notation adopted in this work is that recommended by IUPAC [15,21,22]. Although the $_w^w pH$ measurement is widely used in chromatographic practice, its use can mislead the interpretation of retention of weak electrolytes [7,17,18].

The other thermodynamic quantities, $_w^s pH$ and $_s^s pH$, are two different ways to define the activity of H^+ ions in solvent mixtures depending on the chosen standard state, namely:

$$_w^s pH = - \log m_H \, _w^s \gamma_H \qquad (6a)$$

and

$$_s^s pH = - \log m_H \, _s^s \gamma_H = \, _w^s pH + \log \, _w^s \gamma_H^0 \qquad (6b)$$

Now, the operational pH quantities, measured with electrochemical cells, have to include the liquid

junction potential; they are related through the parameter δ by [23–25]:

$$\delta = \bar{E}_j - \log {}_{w}^{s}\gamma_{H}^{0} = {}_{w}^{s}pH - {}_{s}^{s}pH \qquad (7)$$

this δ-term includes the primary medium effect and the residual liquid-junction potential error $\bar{E}_j$ ($= {}^{s}E_j - {}^{w}E_j$), which results from the difference in potential across the junction dilute buffer‖KCl saturated between aqueous standard and solvent buffers; $\bar{E}_j$ is expressed in pH units. The primary medium effect for the transfer of the H^+ ion from water to the solvent considered, ${}_{w}^{s}\gamma_{H}^{0}$, is a thermodynamic quantity that depends only on the solvent composition and on temperature. So, inasmuch as the liquid-junction potential in the practical measurement of ${}_{w}^{s}pH$ or ${}_{s}^{s}pH$ appears to depend only on the composition of the methanol–water solvent and temperature, the difference (δ) between these two operational quantities should be a constant characteristic of each solvent mixture and temperature. Values for δ-parameter have been measured for several mixed solvents at 25 °C. These determinations have already been carried out by using either hydrogen electrodes [23,26] and combined glass electrodes as indicator electrodes [15,16,18]. It has been fully demonstrated that δ-value is constant independently of the buffer solution at a given solvent composition. The practical significance of this relationship can be appreciated: the simplest means of obtaining ${}_{s}^{s}pH$ is to measure ${}_{w}^{s}pH$ with aqueous standards and subtract the appropriate value of δ for the particular solvent composition and temperature. However, to the best of our knowledge, δ-values at temperatures different from 25 °C are not available.

3. Experimental

3.1. Instrumentation

pH measurements were carried out on a commercial pH-meter (Crison microPH 2002) provided with a combined glass electrode, Ross Combination Electrode Orion 8102 SC, with a precision of ± 0.002 pH units. The solutions were placed into a temperature-controlled bath. A calibrated thermometer at ± 0.1 °C was used for temperature readings.

3.2. Chemicals

Methanol was HPLC-grade (Merck) and water was purified by a Milli-Q plus system from Millipore. The following reagents, p.a. or better, were used without purification: hydrochloric acid (Merck, 25% solved in water), succinic acid p.a. (Merck, >99.5%), glacial acetic acid (Merck), sodium chloride (Merck, >99.5%), potassium dihydrogen phosphate (Merck, >99.5%), disodium hydrogen phosphate (Merck, >99%), *tris*(hydroxymethyl)-aminomethane (Baker), sodium hydroxide (Merck, >99%), potassium hydrogen phthalate (Carlo Erba, >99%), 4-aminopyridine (Aldrich, >99%), sodium acetate (Carlo Erba, >99%), phenol (Carlo Erba, >99.5%), butilamine (Aldrich, 99.5%), *N,N*-dimethylaniline (Merck, >99%), benzoic acid (Merck, >99.95%) and pyridine (Merck, >99.5%).

Sodium hydroxide and hydrochloric acid stock solutions were standarized by potentiometric titrations against potassium hydrogen phtalate and borax, respectively.

3.3. Determination of δ-parameter

In all cases, molal solutions were prepared by weighting the analyte, methanol was added to yield the final percentage desired, and then, water was added to the final weight. All the solvent compositions in this paper are given as weight percentages of methanol because this magnitude does not change with temperature. Solution concentrations and solvent compositions are summarized in Table 1. The electrode was standarized at each temperature between 20 and 50 °C, by using aqueous potassium hydrogen phtalate, potassium dihydrogen phosphate–disodium hydrogen phosphate and borax buffers [27] kept at that temperature. The pH of each solution was measured immediately after calibration of the electrode system. This gave a direct measurement of the ${}_{w}^{s}pH$ (T) value of each solution being T the temperature of the solution. Higher reproducibility in ${}_{w}^{s}pH$ measurements was achieved if, after standarization in the aqueous medium and before immersing the glass electrode into the solutions, the electrode was moved to a hydroalcoholic medium. All measurements were made by triplicate.

Table 1
Solution concentrations and methanol–water compositions

Solution		Molal concentrations, C_a	% MeOH (w/w)
A1	Hydrochloric acid	0.1	0–81.1
A2	Hydrochloric acid	0.01	0–84.0
A3	Hydrochloric acid	0.001	0–84.2
B	Potassium Hydrogen Phtalic Acid	0.05	10–84.2
C	Acetic Acid/Sodium Hydroxyde	0.0504/0.0348 0.0299 0.0249 0.0199 0.0149	0
D1	Acetic Acid/Sodium Acetate/Sodium Chloride	0.05/0.05/0.05	50.02
D2	Sodium Hydrogen Succinic Acid/Sodium Chloride	0.05/0.05	49.88
D3	Potassium Dihydrogen Phosphate 0.02 m/Disodium Hydrogen Phosphate 0.02 m/Sodium Chloride 0.02 m		50.12
D4	Tris 0.05 m/HCl-Tris 0.05 m		49.91
D5	4-Aminopyridine 0.06 m/4-AmPyr.HCl 0.06 m		49.89

The δ quantity for each buffer at a given temperature was estimated from the difference between $_w^s$pH (T) and $_s^s$pH (T) values of the same solution at this temperature. $_s^s$pH (T) values were obtained from the literature or calculated for the strong hydrochloric acid solutions (see below).

3.4. pK_a (T) determinations

For K_a (T) determinations, at least five solutions of the weak electrolyte, varying the ratio between the components of the conjugate pair in the corresponding methanol–water mixture were prepared. $_w^s$pH (T) of these solutions were carefully measured as indicated above, then $_s^s$pH (T) was calculated, and finally correction for non-idealities were made in the computation of the corresponding $_s^s$pK_a (T). In order to validate this experimental methodology, the pK_a (T) of acetic acid in water was obtained by measuring the pH of buffer solutions in the temperature range of 20–50 °C. Corrections for activity coefficients were introduced and the pK_a (T) were compared with the literature data in Table 2. The results were well within the experimental uncertainty, i.e. ± 0.005 pK_a units.

4. Results and discussion

4.1. δ-parameter in methanol–water mixtures

Molal activity coefficients (γ) have been considered for theoretical $_s^s$pH (T) $(= -\log {}_s^s\gamma_H m_H)$ calculations. As the molal ionic strength (I) was kept below 0.1 mol kg^{-1}, these activity coefficients have been calculated by using the Debye–Hückel equation:

Table 2
pK_a values of acetic acid in water between 20 and 50 °C

Temperature (°C)	pKa (T)[a]	Literature values[b]
20	4.737 (0.005)	4.756
25	4.745 (0.003)	4.756
30	4.752 (0.004)	4.757
35	4.760 (0.003)	4.762
40	4.761 (0.002)	4.769
45	4.767 (0.002)	4.777
50	4.774 (0.003)	

[a] Standard deviations are in parenthesis.
[b] Refs. [41–43].

$$-\log \gamma = \frac{A z^2 \sqrt{{}^s\rho I}}{1 + B a_0 \sqrt{I}} \qquad (8)$$

where z is the charge of the ion, ${}^s\rho$ is the density of the solvent mixture, A and B are two fundamental solvent- and temperature-dependent constants. a_0 stands for the ion-size parameter, which is assigned a value fixed by the Bates–Guggenheim convention extended to solvents of relatively moderate permittivities. This convention implies that, at each temperature T, the product $a_0 B$ can be estimated as [15,22,28]:

$$(a_0 B)_T = 1.5[({}^w\varepsilon\, {}^s\rho)/({}^s\varepsilon\, {}^w\rho)]_T^{0.5} \qquad (9)$$

${}^w\varepsilon$, ${}^w\rho$, ${}^s\varepsilon$ and ${}^s\rho$ denote the dielectric constants and the densities of water and of the methanol–water solvent mixture at the given temperature, respectively. Dielectric constants at different temperatures and solvent compositions have been taken from Åkerlöf [29]. It was observed linear relationships between permittivities and temperature ($R^2 > 0.994$) for all methanol–water compositions, and between permittivities and solvent compositions. The consistency for the series of experimental ε values, allowed us to fit all data to a single equation with a standard error of 0.13:

$$\varepsilon = 87.5 - 0.519 w_{MeOH} - 0.339t + 0.0007 t w_{MeOH}$$
$$+ 0.00001 t w_{MeOH}^2 \qquad (10)$$

where t is temperature (°C) and w_{MeOH} is methanol content (% w/w). Densities have been taken from Ref. [30], values at 50 °C have been estimated by extrapolation. The A-parameter has been computed from [31]

$$A = 1.8246 \times 10^6/(\varepsilon T)^{3/2} \qquad (11)$$

Eq. (8) requires the knowledge of the ionic strength, which in turn requires preliminary knowledge of the molality of hydrogen and of lyate ions in that solvent. To solve this, an iterative calculation scheme provided the I and γ values. Typically, two iterations suffice.

Fig. 1 shows the plot of δ values at 25 °C determined in this study as a function of solvent composition. We also included δ-values previously measured by several other authors. Values reported as function of % (v/v) methanol were converted to the % (w/w) scale [16]. δ-values slightly increase as the methanol content increases up to about 70%, at higher MeOH percent δ-values decrease and become strongly negative for mixtures with less than 10% water. The agreement and consistency between data measured in this work with values from different sources is quite reasonable. Some of the data points included in Fig. 1 correspond to δ parameter obtained with a hydrogen electrode as indicator electrode [23,26], while other points have been measured by using a glass electrode [15,16,32]. The comparison between values obtained by means of these two cell types indicates that the replacement of the hydrogen electrode with a glass electrode has not detrimental consequences on the hydrogen activity measurements. Bates has early recognized that the use of a glass electrode is often unimpaired at methanol contents below 90% [23], since the liquid junction potential is quite reproducible. The recent edited Compendium of Analytical Nomenclature also allows the use of a glass electrode as hydrogen activity sensor [21]. The plots of δ-term versus methanol compositions at all other temperatures follow quite similar trends: a very small increase at low methanol content to reach a maximum δ-value between 50 and 64% MeOH and then a very abrupt decrease to become negative at MeOH percentages larger than 80%.

Table 3 gathered the δ-quantities obtained from hydrochloric acid at different methanol–water compositions and at several temperatures. The range of methanol composition studied cover 0–84% (w/w), and the temperature ranged from 20 to 50 °C.

The available quality of potassium hydrogen phthalate led to adopt its solutions, at the specified concentration of 0.05 mol kg^{-1}, as the reference value pH standard both in aqueous [33] and aqueous–methanol solvents [22,34]. These reference pH values, at different temperatures between 283.15 and 313.15 K, and at 10, 20, 50, 64 and 84.2% (w/w) methanol–water mixtures, were determined with an electrochemical cell without liquid junction. Mussini et al. applied a multilinear regression scheme to these data in order to get a continuous function of pH as a function of methanol composition and temperature in the range of 10–40 °C [34]. From the
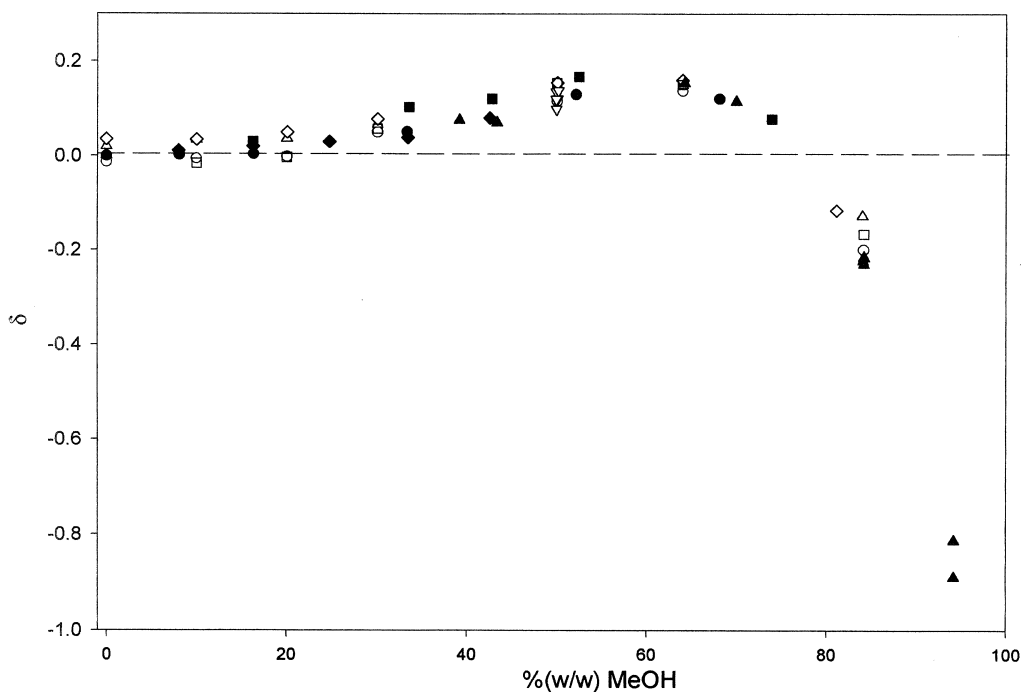
Fig. 1. Variation of δ-parameter at 25 °C with the methanol–water composition. (○): 0.001 molal hydrochloric acid; (△): 0.01 molal hydrochloric acid; (◇): 0.1 molal hydrochloric acid; (□): potassium hydrogen phthalate 0.05 m; (▽): Buffer solutions D1–D5 (see Table 1). Literature data: (●): taken from Ref. [23]; (♦): taken from Ref. [32]; (▲): taken from Ref. [26]; (■): taken from Refs. [15,16].

proposed equation, we estimated the $_s^s pH$ (T) of potassium hydrogen phtalate at the same methanol composition and temperature that the corresponding $_w^s pH$ (T) had been measured. Values at 45 and 50 °C were calculated by extrapolation. Table 4 shows the results of δ-parameters obtained from the subtraction of these two quantities at different solvent compositions and temperatures. δ-values at 25 and 40 °C could also be obtained from experimental $_s^s pH$ (T) previously published [34].

So far, $_s^s pH$ (T) data are available for a few typical buffers (acetate, succinate, hydrogen phosphate, 4-aminopyridine and *tris*-(hydroxymethyl)amino-methane in 50% methanol–water mixtures over the limited temperature range of 10–40 °C [23,35–37]. $_s^s pH$ (T) of solutions D1 and D4 (Table 1) at 45 and 50 °C were calculated from the corresponding ionization constants at these temperatures, which in turn were computed from the thermodynamic functions compiled by Bates [38]. $_s^s pK_a$ (T) calculations were

made by following the proposed Clarke and Glew equations [39], taking 298.15 K as the reference temperature. Table 5 shows the δ-values obtained from subtracting $_s^s pH$ (T) from the corresponding experimental $_w^s pH$ (T).

From Tables 3–5 is clear that δ-quantity is almost constant, independently of the buffer solutions and their pHs, but it is dependent on the solvent composition and also on the solution temperature. In Table 6, the average δ-parameters at each methanol–water compositions are gathered. The standard deviations were lower than 0.03 δ-units. Thus, at methanol compositions range from 10 to 30%, δ-values are statistically equal independently of temperature, but as methanol percent increases above 50%, δ-correction term clearly decrease as temperature increases, and this decreasing is more pronounced as the methanol volume increases. Fig. 2 shows this dependence.

Since δ-values are the algebraic sum of two terms,

Table 3
Determination of δ-parameter for hydrochloric solutions at several temperatures

% MeOH (w/w)	Temperature (°C)						
	20.0	25.1	30.0	35.0	40.0	44.9	50.0
Hydrochloric acid 0.001 molal							
0	0.00	−0.01	−0.01	0.01	0.00	0.00	0.00
9.992	−0.03	−0.01	−0.02	0.00	−0.01	−0.01	0.00
20.01	−0.02	0.00	−0.01	0.01	0.00	0.01	0.01
30.01	0.03	0.05	0.05	0.05	0.04	0.04	0.04
49.99	0.13	0.12	0.11	0.10	0.09	0.08	0.07
64.00	0.14	0.14	0.10	0.10	0.08	0.06	0.05
84.18	−0.17	−0.20	−0.22	−0.22	−0.23	−0.25	−0.26
	19.9	25.2	29.9	35.2	39.8	44.8	50.0
Hydrochloric acid 0.01 molal							
0	0.02	0.02	0.03	0.04	0.02	0.02	0.03
9.991	0.04	0.03	0.03	0.03	0.02	0.02	0.04
20.00	0.04	0.04	0.04	0.05	0.04	0.05	0.06
29.99	0.08	0.07	0.07	0.08	0.06	0.07	0.08
50.00	0.18	0.15	0.16	0.16	0.13	0.13	0.13
64.01	0.12	0.15	0.16	0.16	0.12	0.11	0.10
84.05	−0.07	−0.13	−0.15	−0.16	−0.18	−0.20	−0.22
	20.1	25.2	30.0	35.2	40.0	44.8	50.0
Hydrochloric acid 0.1 molal							
0	0.04	0.04	0.02	0.02	−0.01	0.01	0.00
10.01	0.02	0.04	0.01	0.02	−0.01	0.01	0.01
20.01	0.03	0.05	0.02	0.03	0.01	0.02	0.02
30.02	0.06	0.08	0.05	0.06	0.03	0.04	0.05
50.01	0.15	0.16	0.12	0.12	0.10	0.09	0.08
63.97	0.16	0.16	0.12	0.11	0.07	0.06	0.05
81.13	−0.08	−0.12	−0.12	−0.13	−0.15	−0.17	−0.19

the effect of temperature on these two terms individually is worthy of attention. Standard free energies and electrode potentials are related by

$-\Delta G^0 = nFE^0$. Hence, the medium effect for strong electrolytes as HCl can be obtained from the expression:

Table 4
δ-Parameter for potassium hydrogen phtalate solutions at several temperatures

% MeOH (w/w)	Temperature (°C)								
	20.0	25.0	25.0[a]	30.0	35.0	40.0	40.0[a]	45.0	50.0
10	−0.03	−0.02	−0.02	−0.01	0.00	0.01	−0.01	0.02	0.03
20	0.00	0.00	0.00	0.01	0.02	0.03	0.03	0.04	0.05
30	0.06	0.06		0.07	0.07	0.08		0.09	0.09
50	0.17	0.15	0.15	0.15	0.16	0.15	0.14	0.14	0.14
64	0.16	0.15	0.14	0.15	0.14	0.13	0.10	0.13	0.12
84.2	−0.08	−0.17	−0.15	−0.16	−0.16	−0.18	−0.20	−0.19	−0.20

[a] Calculated by subtraction of experimental ${}_{s}^{s}pH\,(T)$ of ${}_{w}^{s}pH\,(T)$.

Table 5
δ-Parameter for buffer solutions at 50% (w/w) methanol

Solutions[a]	Temperature (°C)						
	20.0	25.0	30.0	35.0	40.0	45.0[b]	50.0
D1	0.11	0.12	0.13	0.13	0.12	0.10	0.09
D2	0.14	0.14	0.12	0.12	0.12		
D3	0.15	0.14	0.14	0.15	0.13		
D4	0.13	0.10	0.10	0.10	0.09	0.09	0.08
D5	0.14	0.12	0.12	0.10	0.09		

[a] See Table 1.
[b] Calculated from Ref. [38]. See the text.

$$\Delta G_{t,\text{HCl}}^0 = \Delta_s^s G_{\text{HCl}}^0 - \Delta_w^s G_{\text{HCl}}^0 = RT \ln {}_w^s \gamma_{\text{HCl}}^0$$
$$= F({}^w E^0 - {}^s E^0) \quad (12)$$

where ${}^w E^0$ and ${}^s E^0$ are the standard e.m.f. of the cell

$\text{Pt}|\text{H}_2\ (1\ \text{atm})|\text{buffer solution (in w or in s)},$

$\text{Cl}^-\ (m_{\text{Cl}})|\text{AgCl, Ag}$

in water or in solvent mixture, respectively and ${}_w^s \gamma_{\text{HCl}}^0$ stands for the transfer mean activity coefficient. In Table 7, we show the values of log ${}_w^s \gamma_{\text{HCl}}^0$ at

Table 6
Average δ-correction term at each methanol–water composition and temperature

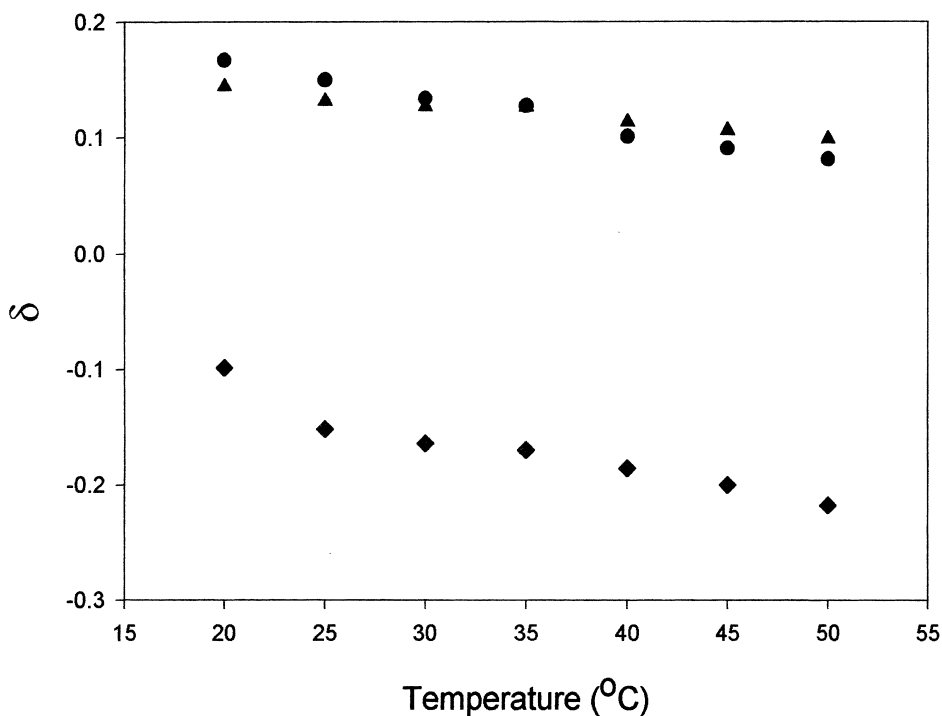| MeOH%	Temperature (°C)						
	20	25	30	35	40	45	50
10	0.00	0.01	0.00	0.01	0.00	0.01	0.02
20	0.01	0.02	0.02	0.03	0.02	0.03	0.04
30	0.06	0.06	0.06	0.07	0.05	0.06	0.07
50	0.14	0.13	0.13	0.13	0.11	0.11	0.10
64	0.17	0.15	0.13	0.13	0.10	0.09	0.08
84.2	−0.10	−0.15	−0.17	−0.17	−0.19	−0.20	−0.22

Fig. 2. Average δ-values as a function of temperature. Methanol–water mixtures: (▲): 50% (w/w) methanol; (●): 64% (w/w) methanol and (♦): 84.2% (w/w) methanol.

Table 7
Transfer activity coefficient of hydrochloric acid (log γ_{HCl}^0) in MeOH–water mixtures at three temperatures

MeOH %(w/w)	Temperature (°C)			Slope[a]
	10	25	40	
0	0	0	0	
10	0.142	0.123	0.106	106
20	0.263	0.235	0.211	155
39.14	0.447	0.435	0.424	71.1
43.3	0.484	0.480	0.477	20.9
50	0.545	0.560	0.573	−81.1
64	0.735	0.787	0.845	−324
70	0.849	0.934	1.011	−477
84.2	1.414	1.527	1.629	−637
90	1.835	1.936	2.028	−571
94.2	2.251	2.327	2.397	−431

[a] Slope of the regression log $_w^s\gamma_{HCl}^0$ vs. (1/T).

several MeOH–water compositions and at three temperatures calculated from $^wE^0$ and $^sE^0$ taken from Ref. [37]. The values clearly show that at MeOH content below 40%, the transfer activity coefficient slightly decrease as temperature increases, the trend reverts above 50% MeOH in the mixture, and the slope becomes strongly negative in methanol rich media. Thus, the significant increase in the energy of transfer of hydrochloric acid as temperature increases can be the possible explanation for the reduction in δ-parameter with temperature in methanol rich solvents.

On the other hand, residual liquid junction potential is expected to largely depend on temperature. Bates [25] has shown that the pattern between $\bar{E}_j$ values arising at the interface of KCl (saturated) aqueous buffers and temperatures between 10 and 45 °C were different depending on their chemical characteristics, but all of them varied less than 0.015 pH units. Therefore, its incidence is expected to play a minor role in the magnitude of δ-parameter.

4.2. Dissociation constants of weak acids

Acid–base equilibria of benzoic acid, phenol, n-butylamine, N,N-dimethylaninile and pyridine in 50% (w/w) methanol–water between 20 and 50 °C have been studied. All these compounds are weak electrolytes of type 1-1. From Eq. (2b), $_s^spK_a$ ($= -\log\ _s^sK_a$) was calculated as:

$$_s^spK_a(T) = \ _s^spH(T) - \log\frac{m_A}{m_{HA}} - \ _s^s\log\gamma_A(T) \qquad (13a)$$

or

$$_s^spK_a(T) = \ _s^spH(T) - \log\frac{m_B}{m_{HB}} + \log\ _s^s\gamma_{HB}(T) \qquad (13b)$$

depending on the acidic or basic nature of the compound. The first term on the right of Eq. (13) was obtained by subtracting the corresponding δ-term from the measured $_w^spH$ (T); the second is known and, the third term was calculated. Note that, m_i in Eqs. (2) and (13) stands for the molality of (i) once the equilibrium has been reached. By making a mass balance,

$$m_A = m_A^0 + m_H - m_S \approx m_A^0 \qquad (14a)$$

and

$$m_{HA} = m_{HA}^0 - m_H + m_S \approx m_{HA}^0, \qquad (14b)$$

where m_i^0 refers to the analytical molal concentrations, much larger than both m_H and m_S in these buffer solutions, and hence, the approximation $m_i \approx m_i^0$ was made. In methanol–water mixtures two different lyate ions (S$^-$) may exist OH$^-$ and MeO$^-$, m_s stands for the contribution of the two ions, i.e. $m_s = m_{OH} + m_{MeO}$. The solute concentrations used in these experiments have been low enough so that molal activity coefficients could also be approximated by the Debye Hückel limiting law. The logarithm of the activity coefficient of uncharged molecules can be neglected since its absolute magnitude is well below those of ions.

In Table 8, we present the $_s^spK_a$ (T) results. We included the few $_s^spK_a$ data obtained from literature for comparison. With the exception of N,N-dimethylaniline, that shows a discrepancy with literature data of about 0.15 pK_a units, the agreement between these results and those from literature is quite acceptable. In Fig. 3, the plots of pK_a of these compounds as a function of 1/T are presented. In the range of experimental temperatures, plots are linear. However, the slopes of these lines are quite different. In Table 8, we have included the ionization enthalpies estimated by the van't Hoff plots, i.e., assuming that ΔH^0 is independent of T within the temperature range. A more sophisticated approach [39], which

Table 8
$_s^s p K_a$ values of weak acids in 50% methanol–water at several temperatures

Temp. (°C)	Benzoic acid[a]		Phenol		Butylamine		Pyridine		N,N-Dimethylaniline	
	exp.	lit.[b]	exp.	lit.	exp.	lit.	exp.	lit.	exp.	lit.
20	5.434		11.05		10.05		4.14	4.32[c,19.5°]	4.36	4.54[c]
	(0.003)		(0.02)		(0.02)		(0.02)		(0.009)	
25	5.425	5.37[c]	10.97	10.91[c]	9.89	9.94[c]	4.08	4.24[c,24 °C]	4.28	4.42[c]
	(0.005)	5.23[c]	(0.03)	10.96	(0.03)		(0.02)	4.14	(0.01)	
30	5.418		10.90		9.68		4.02		4.21	
	(0.005)		(0.01)		(0.03)		(0.02)		(0.009)	
35	5.414		10.82		9.53		3.96	4.00	4.11	
	(0.005)		(0.02)		(0.04)		(0.01)		(0.01)	
40	5.419		10.73		9.36		3.91		4.03	
	(0.009)		(0.01)		(0.03)		(0.02)		(0.01)	
45	5.421		10.67		9.23		3.85		3.97	
	(0.007)		(0.02)		(0.03)		(0.02)		(0.005)	
50	5.421		10.63		9.10		3.81		3.89	
	(0.008)		(0.02)		(0.03)		(0.01)		(0.01)	
$\Delta_s^s H_s^0$ kJ/mole	0.6		26.3		58.4		20.1		28.3	
	(0.3)		(0.8)		(1.3)		(0.3)		(0.5)	
$\Delta_w^w H_w^{0\,d}$ kJ/mole		0.4		22.6		57.8		19.8		

[a] Standard deviations in brackets.
[b] Taken from Ref. [44].
[c] Calculated by interpolation of $_s^s p K_a$ vs. methanol molar fraction data.
[d] Taken from Ref. [45].

takes into account heat capacities of ionization, was also tested. However, the precision in the experimental data prevented of obtaining significant heat capacities; total standard deviations were also larger than those obtained by a single linear approach. At the bottom of this table, the ionization enthalpies in water, $\Delta_w^w H^0$, are given. Ionization enthalpies of these five compounds are quite different. $_s^s p K_a$ of benzoic acid is far less dependent of temperature than the four other determined $_s^s p K_a$-values. Paabo et al. found very low $\Delta_s^s H^0$ at 25 °C for ionization of acetic acid and of dihydrogen phosphate in 50% methanol–water [40].

When the objective is the acquisition of thermodynamic data, the use of operational pH measurements is questionable. Entirely apart from experimental uncertainties in the use of the pH cells (glass electrode errors, residual liquid-junction potentials, etc.), in these determinations one is including a non-thermodynamic step that is clearly unnecessary to the measurement. It can only be justified on the basis of simplicity at some sacrifice of

accuracy. In this case, where the correction term δ was accessible, the equation $_s^s pH = _w^s pH - \delta$ offered a possible and quite simple route to $_s^s p K_a$ values.

From a chromatographic point of view, it is rather evident that ionization of benzoic acid in MeOH–water (carboxylic acid) has a very small dependence with temperature. So, it is expected that the ratio between anion and undissociated molecule would kept practically constant when the temperature of its solution is changed. On the other hand, dissociation equilibria of phenol, pyridine and primary and tertiary aliphatic amines studied in this work have a strong dependence with temperature, even in the narrow range evaluated here. pK_a's of butylamine decrease up to almost one unit from 20 to 50 °C, indicating that its useful range as a mobile phase buffers at temperature different from 25 °C, will be shift from that at room temperature by one pH unit, and as a consequence, the ratio between the conjugated pairs of any acidic or basic analyte will be strongly affected by this pH shift. Prediction of chromatographic retention of these weak electrolytes
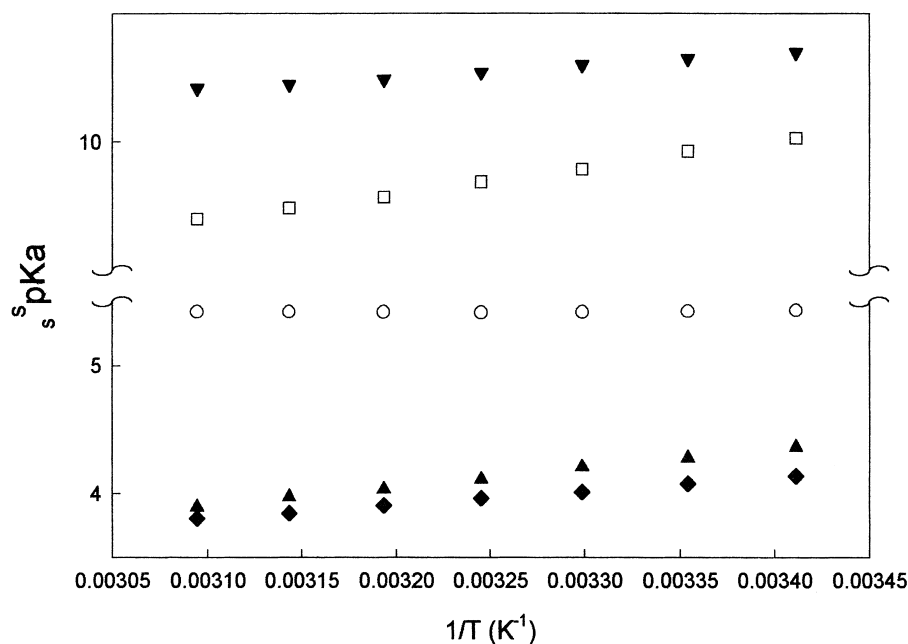
Fig. 3. $_s^s pK_a$ (T) for benzoic acid (○), phenol (▼), *n*-butylamine (□), pyridine (♦) and *N,N*-dimethylaniline (▲) in 50% methanol–water solvent mixture vs. $1/T$.

is still more complicated since temperature will change both the ionization equilibrium of the buffer solution (pH of the buffer) and also its own ionization degree (pK_a of the analyte).

5. Conclusions

This fundamental work provides useful background information about the influence of temperature in acid–base equilibria of common compounds in LC separations. The main remarks are as follows:

1. δ-correction term depends on both methanol–water composition and temperature when the methanol is larger than 50% in the mixture. For rich-water mixtures, temperature does not affect the δ-parameter.

2. These δ-values could be successfully used to calculate the $_s^s pK_a$ of acidic compounds in 50% methanol–water composition and in the range of 20–50 °C, from measurements of $_w^s$pH of the buffers solutions. The results agreed very well with the very scarce data found in literature.

3. The dependence of pK_a with temperature was

very different for different weak acids: pK_a's of benzoic acid practically does not change, whereas those for *n*-butylamine decrease about one pK unit in this temperature range. These differences in ionization enthalpies between different acidic compounds are close to those found in 100% water.

Further emphasis will be directed towards the study of the effect that these variables have on retention and on selectivity. We have demonstrated that temperature plays an important role on dissociation constants of acidic and basic compounds in solvent mixtures and thus, this information would be included in separation optimization strategies. This preliminary study is presented with the hope that it may tackle the basis for further fundamental studies of acid–base equilibria of critical importance in the context of HPLC systems.

Acknowledgements

References

[1] W. Melander, B.K. Chen, Cs. Horváth, J. Chromatogr. 185 (1979) 99.

[2] J. Li, Y. Hu, P.W. Carr, Anal. Chem. 69 (1997) 3884.

[3] J. Li, Anal. Chim. Acta 369 (1998) 21.

[4] B. Yan, J. Zhao, J.S. Brown, J. Blackwell, P.W. Carr, Anal. Chem. 72 (2000) 1253.

[5] Y. Mao, P.W. Carr, Anal. Chem. 72 (2000) 2788.

[6] R.G. Wolcott, J.W. Dolan, L.R. Snyder, S.R. Bakalyar, M.A. Arnold, J.A. Nichols, J. Chromatogr. A 869 (2000) 211.

[7] D.V. McCalley, J. Chromatogr. A 902 (2000) 311.

[8] J.V. Tran, P. Molander, T. Greibrokk, E. Lundanes, J. Sep. Sci. 24 (2001) 930.

[9] S. Allenmark, Chromatographic Enantioseparation, NY 1991.

[10] G. Subramanian (Ed.), A Practical Approach to Chiral Separations by Liquid Chromatography, VCH, New York, 1994.

[11] U.D. Neue, HPLC Columns. Theory, Technology, and Practice, Wiley–VCH Inc, New York, 1997.

[12] T. Greibrokk, Anal. Chem. 74 (2002) 375A.

[13] M. Rosés, I. Canals, H. Allemann, K. Siigur, E. Bosch, Anal. Chem. 68 (1996) 4094.

[14] M. Rosés, E. Bosch, J. Chromatogr. A 982 (2002) 1.

[15] I. Canals, J.A. Portal, E. Bosch, M. Rosés, Anal. Chem. 72 (2000) 1802.

[16] I. Canals, F. Oumada, M. Rosés, E. Bosch, J. Chromatogr. A 911 (2001) 191.

[17] E. Bosch, S. Espinosa, M. Rosés, J. Chromatogr. A 824 (1998) 137.

[18] S. Espinosa, E. Bosch, M. Rosés, Anal. Chem. 72 (2000) 5193.

[19] W.R. Melander, J. Stoveken, C. Horváth, J. Chromatogr. 185 (1979) 111.

[20] W. Melander, A. Nahum, C. Horváth, J. Chromatogr. 185 (1979) 129.

[21] Blackwell, Oxford 1998.

[22] T. Mussini, A.K. Covington, P. Longhi, S. Rondinini, Pure Appl. Chem. 57 (1985) 865.

[23] R.G. Bates, M. Paabo, R.A. Robinson, J. Phys. Chem. 67 (1963) 1833.

[24] O. Popovych, Crit. Rev. Anal. Chem. 1 (1970) 73.

[25] R.G. Bates, CRC Crit. Rev. Anal. Chem. 10 (1981) 247.

[26] C.L. de Ligny, M. Rehbach, Recl. Trav. Chim. 79 (1960) 727.

[27] Chapter 14 R.G. Bates, in: I.M. Kolthoff, P.J. Elving (Eds.), Treatise on Analytical Chemistry, Part 1, Vol. 1, John Wiley and Sons, New York, 1978.

[28] R.G. Bates, E.A. Guggenheim, Pure Appl. Chem. 1 (1960) 163.

[29] G. Åkerlöf, J. Am. Chem. Soc. 54 (1932) 4125.

[30] A.K. Covington, Z.-Y. Zou, Electrochim. Acta 28 (1983) 1587.

[31] R.G. Bates, Electrometric pH Determinations. Theory and Practice, John Wiley and Sons, New York, 1954.

[32] G. Papanastasiou, I. Ziogas, D. Jannakoudakis, Anal. Chim. Acta 173 (1985) 281.

[33] A.K. Covington, R.G. Bates, R.A. Durst, Pure Appl. Chem. 57 (1985) 531.

[34] T. Mussini, A.K. Covington, F. Dal Pozzo, P. Longhi, S. Rondini, Z.-Y. Zou, Electrochim. Acta 28 (1983) 1593.

[35] M. Paabo, R.A. Robinson, R.G. Bates, Anal. Chem. 38 (1966) 1573.

[36] M. Woodhead, M. Paabo, R.A. Robinson, R.G. Bates, Anal. Chem. 37 (1965) 1291.

[37] S. Rondinini, P.R. Mussini, T. Mussini, Pure App. Chem. 59 (1987) 1549.

[38] R.G. Bates, R.A. Robinson, Chem. Phys. Ionic Solutions, Sel. Invited Pap. Discuss. (Natl. Bur. of Stds., Washington, DC, USA), Acid–Base Behavior in Methanol–Water Solvents, 1966, pp. 211.

[39] E.C.W. Clarke, D.N. Glew, Trans. Faraday Soc. 62 (1966) 539.

[40] M. Paabo, R.A. Robinson, R.G. Bates, J. Am. Chem. Soc. 87 (1965) 415.

[41] H.S. Harned, R.W. Ehlers, J. Am. Chem. Soc. 54 (1932) 1350.

[42] H.S. Harned, R.W. Ehlers, J. Am. Chem. Soc. 55 (1933) 652.

[43] D.A. MacInnes, T. Shedlovsky, J. Am. Chem. Soc. 54 (1932) 1429.

[44] V.A. Palm (Ed.), Tables of Rate and Equilibrium Constants of Heterolytic Organic Reactions, Vol. I, II, and Supplementary Vol., Vinity, Moscow, 1975, 1976, 1984 and 1985.

[45] J.W. Larson, L.G. Helper, in: J.F. Coetzee, C.D. Ritchie : (Eds.), Solute–Solvent Interactions, Vol. 1, Marcel Dekker, New York, 1969.