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Modeling ATP protonation and activity coefficients in $NaCl_{aq}$ and KCl_{aq} by SIT and Pitzer equations

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Abstract

The acid–base properties of Adenosine 5′-triphosphate (ATP) in NaCl and KCl aqueous solutions at different ionic strengths ($0 < I/\text{mol L}^{-1} \leq 5$ for $NaCl_{aq}$, $0 < I/\text{mol L}^{-1} \leq 3$ for KCl_{aq}) and at $t = 25$ °C were investigated. A selection of literature data on ATP protonation constants and on activity isopiestic coefficients was performed, together with new potentiometric measurements (by ISE-H^+, glass electrode). Both literature and new experimental data were used to model the dependence on ionic strength and ionic medium of ATP protonation by SIT (Specific ion Interaction Theory) and Pitzer equations. In addition to values of first and second ATP protonation constants in $NaCl_{aq}$ and KCl_{aq} at different ionic strengths, stability constants of $NaATP^{3-}$ and $KATP^{3-}$ complexes, SIT interaction coefficients and Pitzer parameters were calculated, together with protonation constants at infinite dilution: $\log {}^{T}K_1^{H} = p{}^{T}K_{a2} = 7.656 \pm 0.010$ and $\log {}^{T}K_2^{H} = p{}^{T}K_{a1} = 4.561 \pm 0.006$ (in the molar concentration scale, ±95% confidence interval). Both SIT and Pitzer approaches give satisfactory results.

Keywords: ATP; Protonation; Activity coefficients; Dependence on medium and ionic strength; SIT model; Pitzer model

1. Introduction

The importance Adenosine 5′-triphosphate (ATP) from a biological point of view is so high that a discussion on this topic lies outside the aims of the present paper, also because the study of its properties and functions is the main subject of a huge number of contributions present in literature (see, e.g., Ref. [1] and Refs. therein). Here, it is sufficient to remember that ATP plays a fundamental role in energy storage utilization and release, in nucleic acid synthesis, and it is involved in many enzymatic reactions [1–3]. However, one of the most important aspects that should be underlined in this work, and that is sometimes neglected, is that "an important factor in the functionality of adenine nucleos(t)ides is their ability to participate in acid–base equilibria" [3]. In fact, "the acidity of natural and synthetic adenine nucleos(t)ides affects phenomena such as self-association, interaction with metal ions, interaction with receptors and enzymes, and base-pairing in nucleic acids, which influences their structure, stability and function" [3]. Therefore, "an accurate knowledge of the protonation and metal ion binding constants of the nucleotides is essential for a thorough understanding of their reactions in biological systems" [2]. For this reason, many papers dealing with the determination of protonation constants of ATP were published (see, e.g., Refs. [2–13]), and many data are reported in some general compilations [14–18]. Worth of mention is the work of R. A. Alberty on many aspects of chemistry of ATP, from apparent ionization constants [19], to formation constants of monovalent and divalent cation complexes in relation also to the hydrolysis of this bioligand [20–22]. Moreover, in 1991, the Commission on Equilibrium Data of the Analytical Chemistry Division of IUPAC published results of a study devoted to the "critical evaluation of stability constants for nucleotide complexes with protons and metal ions and accompanying enthalpy changes [2].

Because acid–base properties of ATP (and other ligands) is dependent on experimental conditions (e.g., temperature, ionic

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strength, ionic medium), its thermodynamic behavior might vary considerably, and this is particularly important for the knowledge of its behavior in natural waters and, in particular, biological fluids, owing to the extreme variability of both the composition and conditions of these aqueous solutions. In practice, being the determination of all the thermodynamic parameters in all the different experimental conditions of these systems impossible, it is necessary to provide reliable models for the description of acid–base behavior of ATP.

In the light of the above considerations, in the present paper, ATP protonation in two of the main inorganic dissolved salts in natural waters and biological systems (i.e., NaCl and KCl aqueous solutions) was investigated at different ionic strengths ($0 < I/\text{mol L}^{-1} \leq 5$ for $NaCl_{aq}$, $0 < I/\text{mol L}^{-1} \leq 3$ for KCl_{aq}) and at $t = 25$ °C. A critical selection of literature data on ATP protonation constants, on interactions with Na^+ and K^+, and on activity coefficients (obtained by isopiestic technique) was performed, together with new potentiometric measurements (by ISE-H^+, glass electrode). Both literature and new experimental data were used to model the dependence on ionic strength and ionic medium of ATP protonation by means of SIT (Specific ion Interaction Theory) [23,24] and Pitzer equations [25–27]. Other than values of first and second ATP protonation constants in $NaCl_{aq}$ and KCl_{aq} at different ionic strengths, stability constants of $NaATP^{3-}$ and $KATP^{3-}$ complexes, SIT interaction coefficients [23,24] and Pitzer parameters [25–27] were calculated, together with protonation constant values at infinite dilution.

2. Experimental section

2.1. Chemicals

ATP (Adenosine 5′-triphosphate) was used as disodium salt hydrate (by Fluka, purity > 99%). Its purity was checked potentiometrically by alcalimetric titrations. NaCl and KCl solutions were prepared by weighing pure salts (Fluka) dried in an oven at 110 °C. Hydrochloric acid, sodium hydroxide and potassium hydroxide solutions were prepared by diluting concentrated ampoules (Riedel–deHaën). Acid and hydroxide solutions were standardized against sodium carbonate and potassium hydrogen phthalate, respectively. All solutions were prepared with analytical grade water ($R = 18$ MΩ cm^{-1}) using grade A glassware.

2.2. Apparatus and procedure

In order to minimize systematic errors, potentiometric titrations were carried out (at $t = 25.0 \pm 0.1$ °C) using two different apparatuses: first one consisted of a Model 713 Metrohm potentiometer, equipped with a combination glass electrode (Ross type 8102, from Orion), or a half cell glass electrode (Ross type 8101, from Orion) and a double junction reference electrode (type 900200, from Orion), and a Model 765 Metrohm motorized burette; second one was a Crison micro-pH 2002 potentiometer, with the same kind of equipment of the first apparatus. Estimated accuracy was ±0.15 mV and ±0.003 mL for e.m.f. and titrant volume readings, respectively, and was the same for both systems. They were connected to a PC, and automatic titrations were performed using a suitable computer program to control titrant delivery, data acquisition and to check for e.m.f. stability. All titrations were carried out under magnetic stirring and bubbling purified presaturated N_2 through the solution in order to exclude O_2 and CO_2 inside. The titrand solution consisted of different amounts of ATP (3–5 mmol L^{-1}) and ionic medium (sodium or potassium chloride) in order to obtain pre-established ionic strength values ($I = 0.5$, 1, 1.5, 2, 3, 5 mol L^{-1} for sodium chloride and 0.5, 1, 2, 3 mol L^{-1} for potassium chloride). Further experimental details are given in Table 1. Potentiometric measurements were carried out by titrating 25 mL of the titrand solution with standard NaOH or KOH solutions. The equilibrium during titrations was checked using common precautions. These include monitoring the time necessary to reach equilibrium and perform back titrations. For each experiment, independent titrations of strong acidic solution with standard base were carried out under the same medium and ionic strength conditions as present in the systems to be investigated, with the aim of determining electrode potential (E^0) and acidic junction potential ($E_j = j_a[H^+]$).

2.3. Calculations

The non-linear least squares computer program ESAB2M [28] was used for the refinement of all the parameters of the acid–base titration (E^0, K_w, liquid junction potential coefficient, j_a, analytical concentration of reagents). The BSTAC [29] and

Table 1
Experimental conditions for the protonation of ATP in NaCl and KCl aqueous solutions, at $t = 25$ °C

$I/\text{mol L}^{-1}$	$C_{ATP}/\text{mmol L}^{-1}$	$C_H/\text{mmol L}^{-1}$	n[a]
NaCl			
0.493	4.468	8.936	61
0.493	4.468	8.936	63
0.966	2.766	5.533	57
0.964	2.912	5.824	58
1.460	5.733	11.466	58
1.509	3.640	7.280	61
1.508	3.640	7.280	61
1.864	4.583	9.167	63
1.930	2.328	4.657	50
2.801	4.547	9.094	67
2.907	2.365	4.730	55
2.799	4.547	9.094	67
4.585	4.548	9.096	73
4.582	4.548	9.096	74
4.967	2.365	4.730	53
KCl			
0.490	4.527	9.054	69
0.496	2.694	5.389	53
0.943	4.500	9.000	62
0.966	2.700	5.400	56
0.966	2.700	5.400	56
1.862	4.500	9.000	64
1.918	2.700	5.400	59
2.791	4.568	9.136	64
2.789	4.568	9.136	64

[a] Number of experimental points.

STACO [30] computer programs were used in the calculation of protonation and complex formation constants. Both programs can deal with measurements at different ionic strengths. The ES4ECI [30] program was used to draw speciation diagrams and to calculate species formation percentages. The LIANA [31] program was used to fit different functions.

Details on models used for taking into account the dependence on ionic strength are given in the following sections. Formation constants, concentrations and ionic strengths are expressed in the molar (mol L^{-1}) or molal (m, mol $kg^{-1}(H_2O)$) concentration scales.

Protonation equilibria are expressed as

$$H_{i-1}ATP^{(4-i+1)-} + H^{+} = H_{i}ATP^{(4-i)-} \qquad (1)$$

with the protonation constant

$$K_{i}^{H} = \frac{[H_{i}ATP^{(4-i)-}]}{[H_{i-1}ATP^{(4-i+1)-}][H^{+}]} \qquad (2)$$

$$\log K_{1}^{H} = pK_{a2}; \log K_{2}^{H} = pK_{a1}.$$

3. Analysis of protonation data and activity coefficients

3.1. Smoothing experimental and literature protonation data

This work deals with some experimental and literature data on the protonation of ATP. In particular, protonation constants reported in a previous paper are considered [32], further literature values are taken from different sources [2–13] and experimental data are obtained using different apparatuses. This ensures that systematic errors are randomized. On the other hand, using not homogeneous data suggests adopting pre-smoothing techniques before their treatment with different models. Widely used smoothing (soft) techniques include polynomial or spline functions fitting [33], which work well for large datasets. Generally, for small datasets it is better to use smoothing functions having, at least in part, parameters with physical significance. For protonation constants at different ionic strengths, I/mol L^{-1}, one can resort to Debye–Hückel equation of the type [see protonation reaction (1)]

$$\log K_{i}^{H} = \log {}^{T}K_{i}^{H} - 2(|z| - i + 1)DH + F(I) \qquad (3)$$

where

$$DH = AI^{1/2}(1 + 1.5I^{1/2})^{-1} \qquad (3a)$$

with $A = 0.510$ at $t = 25$ °C in water. The generic function $F(I)$ can be expressed in most cases as

$$F(I) = CI + S(I) \qquad (4)$$

where C may be a true constant, with some supporting electrolytes such as alkali metal chlorides, nitrates or perchlorates, with $-z \leq 2$ and $I \leq 1$ mol L^{-1}. When these conditions are not fulfilled we can use the expression

$$C = c_{\infty} + (c_{0} - c_{\infty})F(I) \qquad (5)$$

with

$$F(I) = (I + 1)^{-1} \qquad (5a)$$

where c_{∞} is the value of C for $I \rightarrow \infty$ and c_0 is the value of C for $I \rightarrow 0$. The term $S(I)$ comes from the need of considering, in some cases, the interaction between the anion of the acid and the cation of the supporting electrolyte, with formation constant for the ion pair $K > 50$ mol L^{-1}. The generic form of this term is

$$S(I) = A\log(1 + 10^{B}C_{\mathrm{cation}}). \qquad (6)$$

For example, this term (with $A = -1$) has been successfully applied for the modeling of the dependence on ionic medium and on ionic strength of protonation constants of phytate ligand [1,2,3,4,5,6 hexakis (di-hydrogen phosphate) *myo*-inositol, Phy^{12-}] in alkali metal chloride ionic media (LiCl, NaCl, KCl) where, owing to the highly negative charge of free ligand, strong interactions occur with alkali metal cations of these salts [34]. Smith and Alberty [20] calculated, from the differences of protonation constants in several 1 : 1 supporting electrolytes, the apparent stability constants of ATP–alkali metal complexes. They also proposed the use of tetraalkylammonium salts, to keep constant the ionic strength, in the determination of stability constants of divalent cations [19]. B parameter of Eq. (6) can be considered as a measure of the interaction between cations of background salts and the ligand, by considering the analogy with the equation that describes the lowering effect on the protonation constant for a monoprotic acid, due to the weak association of the cation M^{+} of supporting electrolyte [35,36]

$$\log K^{H} = \log K^{H*} - \log(1 + 10^{\log K^{M}} I)$$

where K^{H*} is the apparent protonation constant and K^{M} is the formation constant for the species M^{+}-anion of monoprotic acid.

Moreover, for protonation (or metal complex formation) data at $I >> 3$ mol L^{-1}, a further term for Eq. (5) is often necessary, i.e.

$$c_{\infty} = c_{\infty}^{(0)} + c_{\infty}^{(1)}I. \qquad (7)$$

To smooth log K_{1}^{H}, for both $NaCl_{aq}$ and KCl_{aq} media, we used Eq. (3) with Eqs. (5) and (6); the use of Eq. (7) was not necessary, being the improvement of the fit not statistically significant. As concerns log K_{2}^{H}, the use of Eq. (3) with Eq. (5) was sufficient.

3.2. SIT model for protonation constants and activity coefficients

The basic SIT (Specific ion Interaction Theory) [23,24] model, based on the Brönsted–Guggenheim–Scatchard principle [37–42], can be expressed by the equation for activity coefficients

$$\begin{aligned}\log\gamma_{\pm} &= -|z_{+}z_{-}|DH + \varepsilon(M^{z+}, X^{z-})m_{MX}(2\nu_{+}\nu_{-}/\nu) \\ &= -|z_{+}z_{-}|DH + \varepsilon(M^{z+}, X^{z-})4I/(z_{+} + z_{-})^{2} \qquad (8)\end{aligned}$$

where DH is given by Eq. (3a); ν_+, z_+, ν_-, z_- are the stoichiometric coefficients and the charges for cation and anion, respectively, of the salt MX. The specific interaction coefficients $\varepsilon(\mathrm{M}^{z+}, \mathrm{X}^{z-})$ are, in the original model, true constants, but this approximation is valid only for some electrolytes and for some ionic strength ranges. Recently we proposed a modified version of the SIT approach [43], in which the specific coefficients are expressed as a function of I, in the same way as for Eq. (5)

$$\varepsilon = \varepsilon_\infty + (\varepsilon_0 - \varepsilon_\infty)(I+1)^{-1} \tag{9}$$

where ε_∞ could be expressed by

$$\varepsilon_\infty = \varepsilon_\infty^{(0)} + \varepsilon_\infty^{(1)} I. \tag{9a}$$

In several cases one must take into account the formation of ion pairs, in particular for z_+ or $|z_-|>2$. In the case of ATP, it has been reported a quite stable species MATP^{3-} ($\mathrm{M}^+ = \mathrm{Na}^+, \mathrm{K}^+$) with formation constants $K >> 50$ mol L^{-1}. In this case we can write the equation

$$\log\gamma_\pm^* = \log\gamma_\pm - \log(1 + 10^K C_\mathrm{M}) \tag{10}$$

where $\gamma_\pm^*$ is the apparent activity coefficient (experimental, see hereafter) obtained without taking into account the formation of the ion pair (see, e.g., Ref. [44]).To express the first protonation constant vs. ionic strength by SIT model, we use an equation very similar to Eq. (3) together with Eqs. (9) and (10)

$$\log K_1^\mathrm{H} = \log {}^T K_1^\mathrm{H} - 8\mathrm{DH} + I\Delta\varepsilon_1 - \log(1 + 10^K C_\mathrm{M}) \tag{11}$$

where $\Delta\varepsilon_1$ is the difference of specific coefficients

$$\Delta\varepsilon_1 = \varepsilon(\mathrm{ATP}^{4-}, \mathrm{M}^+) + \varepsilon(\mathrm{Cl}^-, \mathrm{H}^+) - \varepsilon(\mathrm{HATP}^{3-}, \mathrm{M}^+) \tag{12}$$

with ε values expressed as a function of ionic strength by Eq. (9). A very similar equation can be used for the second protonation constant, without the term for the ion pair formation

$$\log K_2^\mathrm{H} = \log {}^T K_2^\mathrm{H} - 6\ \mathrm{DH} + I\Delta\varepsilon_2 \tag{13}$$

with

$$\Delta\varepsilon_2 = \varepsilon(\mathrm{HATP}^{3-},\ \mathrm{M}^+) + \varepsilon(\mathrm{Cl}^-, \mathrm{H}^+) - \varepsilon(\mathrm{H_2ATP}^{2-},\ \mathrm{M}^+). \tag{14}$$

The ion pair formation constant, K, of Eqs. (10) and (11) must be expressed, in turn, as a function of ionic strength, by using the equation

$$\log K = \log {}^\mathrm{T} K - 8\ \mathrm{DH} + I\Delta\varepsilon_\mathrm{ip} \tag{15}$$

with

$$\Delta\varepsilon_\mathrm{ip} = \varepsilon(\mathrm{ATP}^{4-},\ \mathrm{M}^+) + \varepsilon(\mathrm{Cl}^-, \mathrm{M}^+) - \varepsilon(\mathrm{MATP}^{3-},\ \mathrm{M}^+). \tag{16}$$

Values of SIT literature [43] parameters used during calculations performed in the present work are given in Table 2.

Table 2
SIT interaction coefficients (Eq. (9)) [43] used in the calculations performed in the present work

M^{z+}	M^{z-}	ε_∞	ε_0
H^+	Cl^-	0.136	0.0848
Na^+	Cl^-	0.0514	− 0.0136
K^+	Cl^-	0.0168	− 0.0480

3.3. Pitzer model for protonation constants and activity coefficients

An extensive description of historical aspects, theoretical basis and some direct applications of Pitzer model can be found, e.g., in Pitzer, 1991 [27] and Millero, 1982, 2001 [26,44]. Here, we just report few details on equations used in our calculations. According to the model, the mean activity coefficient ($\gamma_\pm$) for a pure electrolyte MX in an aqueous solution of molality "m" is given by:

$$\ln\gamma_\pm = |z_+ z_-| f^\gamma + m(2\nu_+\nu_-/\nu)B_\mathrm{MX}^\gamma + m^2(2(\nu_+\nu_-)^{3/2}/\nu)C_\mathrm{MX}^\gamma \tag{17}$$

with

$$f^\gamma = -A_\Phi[I^{1/2}(1+1.2I^{1/2})^{-1} + (2/1.2)\ln(1+1.2I^{1/2})] \tag{18}$$

where A_Φ represents the Debye–Hückel term that, in Pitzer equations, is A_Φ=0.3915 at t=25 °C. B_MX^γ and C_MX^γ in Eq. (17) are given by

$$B_\mathrm{MX}^\gamma = B_\mathrm{MX} + B_\mathrm{MX}^\Phi \tag{19}$$

$$C_\mathrm{MX}^\gamma = 3\ C_\mathrm{MX}^\Phi/2 \tag{20}$$

with

$$B_\mathrm{MX}^\Phi = \beta_\mathrm{MX}^{(0)} + \beta_\mathrm{MX}^{(1)}\exp(-\alpha_1 I^{1/2}) + \beta_\mathrm{MX}^{(2)}\exp(-\alpha_2 I^{1/2}) \tag{21}$$

$$B_\mathrm{MX} = \beta_\mathrm{MX}^{(0)} + \beta_\mathrm{MX}^{(1)} f(\alpha_1 I^{1/2}) + \beta_\mathrm{MX}^{(2)} f(\alpha_2 I^{1/2}) \tag{22}$$

and

$$f(x) = 2[1-(1+x)\exp(-x)]/x^2 \tag{23}$$

$\beta^{(0)}$, $\beta^{(1)}$, $\beta^{(2)}$, and $C^{(\phi)}$ represent Pitzer interaction parameters between two ions of opposite signs and are "specific" for each ion pair. Generally, α_1= 2.0 and α_2= 0.0 [(kg mol $^{-1}$)$^{1/2}$] for all kind of electrolytes, except for 2–2 salts where α_1=1.4 and α_2=12. However, several examples are reported in literature where different α_1 and α_2 terms were used (see, e.g., Ref. [27]).

In mixed electrolyte solutions, the most directly applicable and widely used forms for the expression of activity coefficients of single ions for a cation M^{z+} or an anion X^{z-} are reported below:

$$\begin{aligned}\ln\gamma_\mathrm{M} = {} & z_+^2 f^\gamma + 2\Sigma_\mathrm{a} m_\mathrm{a}(B_\mathrm{Ma} + E\ C_\mathrm{Ma}) \\ & + \Sigma_\mathrm{a}\Sigma_\mathrm{c} m_\mathrm{c} m_\mathrm{a}(z_+^2 B'_\mathrm{ca} + z_+ C_\mathrm{ca}) + \Sigma_\mathrm{c} m_\mathrm{c}(2\Theta_\mathrm{Mc} \\ & + \Sigma_\mathrm{a} m_\mathrm{a}\Psi_\mathrm{Mca}) + \Sigma_\mathrm{a}\Sigma_{\mathrm{a}'}\ m_\mathrm{a} m_{\mathrm{a}'}\ \Psi_{\mathrm{Maa}'}.\end{aligned} \tag{24}$$

Table 3
Pitzer interaction coefficients [27] used in the calculations performed in the present work

Electrolyte	$\beta^{(0)}$	$\beta^{(1)}$	$C^{(\Phi)}$	Θ	Ψ
HCl	0.1775	0.2945	0.00080	–	–
NaCl	0.0765	0.2664	0.00127	–	–
KCl	0.04835	0.2122	− 0.00084	–	–
HCl/NaCl	–	–	–	0.036	− 0.004
HCl/KCl	–	–	–	0.005	− 0.007

$$\ln\gamma_{\mathrm{X}} = z_-^2 f^{\gamma} + 2\Sigma_{\mathrm{c}} m_{\mathrm{c}}(B_{\mathrm{Xc}} + E\ C_{\mathrm{Xc}}) + \Sigma_{\mathrm{a}}\Sigma_{\mathrm{c}} m_{\mathrm{c}} m_{\mathrm{a}}(z_-^2 B'_{\mathrm{ca}} + z_- C_{\mathrm{ca}}) + \Sigma_{\mathrm{a}} m_{\mathrm{a}}(2\Theta_{\mathrm{Xa}} + \Sigma_{\mathrm{c}} m_{\mathrm{c}} \Psi_{\mathrm{Xac}}) + \Sigma_{\mathrm{c}}\Sigma_{\mathrm{c}'} m_{\mathrm{c}} m_{\mathrm{c}'} \Psi_{\mathrm{Xcc}'} \tag{25}$$

and, for neutral species:

$$\ln\gamma^0_{\mathrm{MX}} = 2\lambda I \tag{26}$$

m_{i} is the molality of the cations (c) and anions (a) in the solution, E the equivalent molality ($E=1/2\ \Sigma_{\mathrm{i}}\ m_{\mathrm{i}}\ |z_{\mathrm{i}}|$) and:

$$B'_{\mathrm{MX}} = [\beta^{(1)}_{\mathrm{MX}} f'(\alpha_1 I^{1/2}) + \beta^{(2)}_{\mathrm{MX}} f'(\alpha_2 I^{1/2})]/I \tag{27}$$

with

$$f'(x) = -2\ [1-(1+x+x^2/2)\exp(-x)]/x^2 \tag{28}$$

Θ represents the specific interaction parameters between two ions (+ + or − −) of the same sign, Ψ are triple interaction parameters between two similarly charged ions and an ion of opposite charge (+ − +, − + −) and λ is the interaction parameter for neutral species with the ions in the solution. Generally, at $I<3$ mol kg^{-1} (H_2O), Θ and Ψ parameters can be neglected.

In the case of 4 : 1 electrolytes (such as ATP), Pitzer modeled their dependence on ionic strength by just using $\beta^{(0)}$, $\beta^{(1)}$ and $C^{(\Phi)}$ parameters (i.e., considering $\alpha_1=2.0$ and $\alpha_2=0.0$). However, refined $\beta^{(0)}$, $\beta^{(1)}$ and $C^{(\Phi)}$ parameters reported in his book are able to accurately model the dependence on ionic strength until low molalities [a maximum of 1.4 m for $(Me_4N)_4Mo(CN)_8$]. At higher concentrations, strong interactions occur in solution (in particular for z_+ or $|z_-|>2$) and, in several cases, one must take into account the formation of ion pairs. In spite of this, the Pitzer model usually does not take into account the explicit concept of "ion association": according to the author, "associated species could be omitted provided an additional ionic-strength-dependent term with a large exponent α_2 was added to the second virial coefficient" [27], i.e., the model considers "supplementary" parameters like, for example, $\beta^{(2)}$ for 2 : 2 interactions to improve the fits. Both procedures (i.e., the use of $\beta^{(2)}$ and taking into account ion association) were

Table 4
Protonation constants of ATP in NaCl and KCl aqueous solutions at different ionic strengths and at $t=25$ °C

I/mol L^{-1}	log $K_1{}^{\mathrm{H}}$	log $K_2{}^{\mathrm{H}}$	log $\beta_2{}^{\mathrm{Hb}}$	σ^{c}	m.d.[d]
NaCl$_{aq}$					
0.493±0.001[a]	6.020±0.002[a]	3.919±0.004[a]	9.939	0.5245	0.267
0.965±0.001	5.809±0.002	3.971±0.004	9.780	0.5553	0.301
1.49±0.02	5.728±0.002	4.048±0.002	9.776	0.5600	0.306
1.897±0.03	5.649±0.002	4.103±0.004	9.752	0.6839	0.451
2.835±0.03	5.635±0.002	4.333±0.002	9.968	0.6525	0.416
4.583±0.01	5.747±0.002	4.736±0.002	10.483	0.6306	0.388
4.967±0.01	5.699±0.002	4.732±0.004	10.431	0.6232	0.366
KCl$_{aq}$					
0.493±0.003	6.134±0.006	3.856±0.008	9.990	0.619	0.493
0.958±0.012	5.979±0.002	3.965±0.006	9.944	0.6202	0.374
1.890±0.02	5.919±0.002	4.119±0.003	10.038	0.5482	0.291
2.790±0.01	5.937±0.002	4.278±0.002	10.215	0.4525	0.199

[a] ±95% confidence interval; [b] $\beta_2{}^{\mathrm{H}}$ refers to equilibrium $ATP^{4-}+2\ H^+=H_2ATP^{2-}$; [c] standard deviation on fit; [d] mean deviation of fit.

Table 5
Protonation constants of ATP in NaCl and KCl aqueous solutions at different ionic strengths and at $t=25$ °C previously determined [32]

I/mol L^{-1}	log K_1^{H}	log K_2^{H}	log β_2^{H}
NaCl$_{aq}$			
0.04	6.76	4.10	10.86
0.16	6.31	3.94	10.25
0.25	6.16	3.92	10.08
0.49	5.96	3.93	9.89
1.00	5.82	3.94	9.76
KCl$_{aq}$			
0.04	6.82	4.10	10.92
0.16	6.39	3.95	10.34
0.25	6.25	3.92	10.17
0.49	6.07	3.92	9.99
1.00	5.94	3.93	9.87

Table 6
Literature data for protonation constants of ATP in NaCl and KCl aqueous solutions at different ionic strengths and at $t=25$ °C

I/mol L^{-1}	log K_1^{H}	log K_2^{H}	log β_2^{H}	Ref.
NaCl$_{aq}$				
0.10	6.47	4.00	10.47	[7]
0.10[a]	6.50	4.25	10.75	[12]
0.12	6.51	4.02	10.53	[4]
0.10	6.44	3.98	10.42	[2]
0.25	6.27	3.98	10.25	[2]
0.15	6.39	4.05	10.44	[5]
0.15	6.24	3.70	9.94	[6]
0.50	6.07	3.99	10.06	[2]
1.00	5.93	4.00	9.93	[2]
KCl$_{aq}$				
0.10	6.50	4.02	10.52	[2]
0.10	6.50	3.90	10.40	[10]
0.10[b]	6.51	4.05	10.56	[11]
0.10[b]	6.57	4.08	10.65	[13]
0.10[b]	6.61	4.10	10.71	[46]
0.20	6.31	3.89	10.20	[8]
0.25	6.34	3.98	10.32	[2]
0.50	6.16	3.98	10.14	[2]
1.00	6.03	3.99	10.02	[2]

[a] $NaNO_3$ ionic medium; [b] KNO_3 ionic medium.

Table 7
Smoothed protonation constants of ATP in NaCl and KCl aqueous solutions at different ionic strengths and at t=25 °C

I/mol L^{-1}	log $K_1^{\mathrm{H\,a}}$	log $K_2^{\mathrm{H\,a}}$	I/mol kg^{-1}(H$_2$O)	log $K_1^{\mathrm{H\,b}}$	log $K_2^{\mathrm{H\,b}}$
NaCl$_{aq}$					
0.05	6.692±0.004^c	4.098±0.006^c	0.05	6.690^c	4.096^c
0.10	6.467±0.005	4.004±0.004	0.10	6.465	4.002
0.25	6.180±0.006	3.916±0.004	0.25	6.177	3.913
0.50	5.991±0.006	3.912±0.005	0.51	5.986	3.907
1.00	5.820±0.002	3.990±0.006	1.02	5.811	3.981
1.50	5.729±0.005	4.084±0.006	1.55	5.715	4.070
2.00	5.681±0.006	4.179±0.006	2.08	5.663	4.161
2.50	5.658±0.007	4.273±0.006	2.64	5.635	4.250
3.00	5.652±0.010	4.367±0.006	3.20	5.624	4.339
4.00	5.673±0.015	4.553±0.010	4.37	5.635	4.515
5.00	5.720±0.018	4.740±0.012	5.61	5.670	4.690
KCl$_{aq}$					
0.05	6.753±0.002	4.098±0.005	0.05	6.751	4.096
0.10	6.540±0.005	4.004±0.005	0.10	6.538	4.001
0.25	6.273±0.008	3.915±0.005	0.25	6.269	3.911
0.50	6.112±0.008	3.909±0.006	0.51	6.105	3.902
1.00	5.981±0.007	3.978±0.009	1.02	5.967	3.964
1.50	5.926±0.007	4.060±0.008	1.55	5.906	4.040
2.00	5.913±0.007	4.142±0.010	2.08	5.886	4.115
2.50	5.926±0.010	4.222±0.012	2.64	5.891	4.187
3.00	5.958±0.011	4.302 ± 0.015	3.20	5.916	4.260

[a] In the molar concentration scale; [b] in the molal concentration scale; [c] ±95% confidence interval.

used in this work for the modeling of activity coefficients of ATP^{4-} ion by Pitzer equations. In the approach that does not consider the formation of MATP^{3-} species (M$^+$=Na$^+$, K$^+$), mean activity coefficients for M$_4$ATP salts are given by:

$$\ln\gamma_{\pm} = 4f^{\gamma} + m(8/5)B^{\gamma}_{\mathrm{MATP}} + m^2(16/5)(3/2)C^{\Phi}_{\mathrm{MATP}} \tag{29}$$

with

$$\begin{aligned} B^{\gamma}_{\mathrm{MATP}} = {} & 2\beta^{(0)}_{\mathrm{MATP}} + \beta^{(1)}_{\mathrm{MATP}}[f(1.4I^{1/2}) + \exp(-1.4I^{1/2})] \\ & + \beta^{(2)}_{\mathrm{MATP}}[f(12I^{1/2}) + \exp(-12I^{1/2})]. \end{aligned} \tag{30}$$

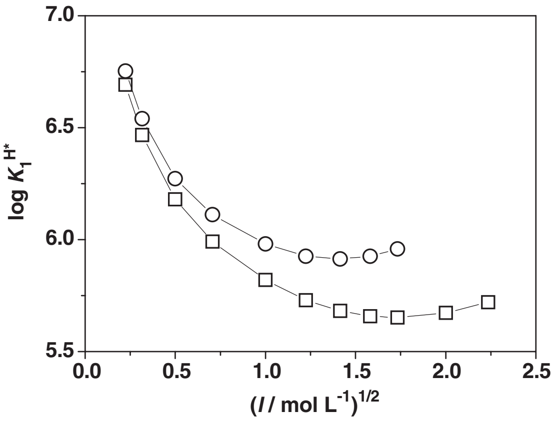

Fig. 1. Smoothed values of first protonation constant (log $K_1^{\mathrm{H}*}$) of ATP vs. the square root of ionic strength (in mol L^{-1}), in NaCl (squares) and KCl (circles) aqueous solution at t=25 °C.

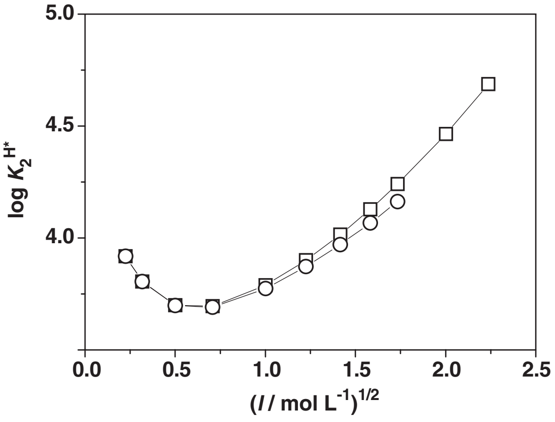

Fig. 2. Smoothed values of second protonation constant (log $K_2^{\mathrm{H}*}$) of ATP vs. the square root of ionic strength (in mol L^{-1}), in NaCl (squares) and KCl (circles) aqueous solution at t=25 °C.

Values of first and second conditional protonation constants of ATP in NaCl and KCl aqueous solutions at different ionic strengths are given, respectively, by:

$$\log K_1^{\mathrm{H}} = \log {}^{T}K_1^{\mathrm{H}} + (\ln\gamma_{\mathrm{H^+}} + \ln\gamma_{\mathrm{ATP^{4-}}} - \ln\gamma_{\mathrm{HATP^{3-}}})/\ln 10 \tag{31}$$

$$\log K_2^{\mathrm{H}} = \log {}^{T}K_2^{\mathrm{H}} + (\ln\gamma_{\mathrm{H^+}} + \ln\gamma_{\mathrm{HATP^{3-}}} - \ln\gamma_{\mathrm{H_2ATP^{2-}}})/\ln 10 \tag{32}$$

where, according to Pitzer equations:

$$\begin{aligned} \ln\gamma_{\mathrm{H^+}} = {} & f^{\gamma} + 2m_{\mathrm{Cl}}[\beta^{(0)}_{\mathrm{HCl}} + \beta^{(1)}_{\mathrm{HCl}}f(2I^{1/2}) + m_{\mathrm{Cl}}C^{\Phi}_{\mathrm{HCl}}/2] \\ & + m_{\mathrm{M}}m_{\mathrm{Cl}}[\beta^{(1)}_{\mathrm{MCl}}f'(2I^{1/2}) + C^{\Phi}_{\mathrm{MCl}}/2] \\ & + m_{\mathrm{M}}[2\Theta_{\mathrm{HM}} + m_{\mathrm{Cl}}\Psi_{\mathrm{HMCl}}] \end{aligned} \tag{33}$$

$$\begin{aligned} \ln\gamma_{\mathrm{ATP^{4-}}} = {} & 16f^{\gamma} + 2\,m_{\mathrm{M}}[\beta^{(0)}_{\mathrm{MATP}} + \beta^{(1)}_{\mathrm{MATP}}f(1.4I^{1/2}) \\ & + \beta^{(2)}_{\mathrm{MATP}}f(12I^{1/2}) + m_{\mathrm{M}}C^{\Phi}_{\mathrm{MATP}}/4] \\ & + m_{\mathrm{M}}m_{\mathrm{Cl}}[16\beta^{(1)}_{\mathrm{MCl}}f'(2I^{1/2}) + 2C^{\Phi}_{\mathrm{MCl}}] \end{aligned} \tag{34}$$

$$\begin{aligned} \ln\gamma_{\mathrm{HATP^{3-}}} = {} & 9f^{\gamma} + 2m_{\mathrm{M}}[\beta^{(0)}_{\mathrm{MHATP}} + \beta^{(1)}_{\mathrm{MHATP}}f(2I^{1/2}) \\ & + m_{\mathrm{M}}C^{\Phi}_{\mathrm{MHATP}}/(2\sqrt{3})] \\ & + m_{\mathrm{M}}m_{\mathrm{Cl}}[9\beta^{(1)}_{\mathrm{MCl}}f'(2I^{1/2}) + 3/2C^{\Phi}_{\mathrm{MCl}}] \end{aligned} \tag{35}$$

$$\begin{aligned} \ln\gamma_{\mathrm{H_2ATP^{2-}}} = {} & 4f^{\gamma} + 2m_{\mathrm{M}}[\beta^{(0)}_{\mathrm{MH_2ATP}} + \beta^{(1)}_{\mathrm{MH_2ATP}}f(2I^{1/2}) \\ & + m_{\mathrm{M}}C^{\Phi}_{\mathrm{MH_2ATP}}/(2\sqrt{2})] \\ & + m_{\mathrm{M}}m_{\mathrm{Cl}}[4\beta^{(1)}_{\mathrm{MCl}}f'(2I^{1/2}) + C^{\Phi}_{\mathrm{MCl}}] \end{aligned} \tag{36}$$

Table 8
Activity coefficients of Na$_4$ATP and K$_4$ATP at t=25°C [45]

m/mol kg^{-1} (H$_2$O)	$\gamma_{\mathrm{Na_4ATP}}$	$\gamma_{\mathrm{K_4ATP}}$
0.1	0.252	0.258
0.2	0.174	0.192
0.3	0.138	0.158
0.4	0.116	0.137
0.5	0.101	0.123
0.6	0.091	0.114
0.7	0.083	0.107
0.8	0.077	0.102

Table 9
SIT interaction coefficients determined for ATP–Na^+ and ATP–K^+ systems

M	X	ε_∞	ε_0
Na^+	ATP^{4-}	$(-0.324+0.0086\ I)\pm0.009$[a]	1.29 ± 0.04[a]
Na^+	$HATP^{3-}$	-0.178 ± 0.021	1.36 ± 0.05
Na^+	H_2ATP^{2-}	-0.205 ± 0.022	0.315 ± 0.05
Na^+	$NaATP^{3-}$	-0.158 ± 0.03	2.23 ± 0.09
K^+	ATP^{4-}	$(-0.379+0.0192\ I)\pm0.013$	2.23 ± 0.04
K^+	$HATP^{3-}$	-0.218 ± 0.008	2.20 ± 0.04
K^+	H_2ATP^{2-}	-0.216 ± 0.008	1.17 ± 0.04
K^+	$KATP^{3-}$	(3.31)[b]	3.31 ± 0.05

[a]±95% confidence interval; [b]$\varepsilon_\infty=\varepsilon_0$, i.e. ε is, in this case, a true constant.

In the second approach, when considering the formation of $MATP^{3-}$ species ($M^+ = Na^+, K^+$), mean activity coefficients for M_4ATP salts are given by:

$$\ln\gamma_{\pm} = \mathrm{Eq.}(29) + \ln(1 + e^{\ln K} C_{\mathrm{M}}) \qquad (37)$$

with $B^{\gamma}_{\mathrm{MATP}}$ of Eq. (29) expressed by

$$B^{\gamma}_{\mathrm{MATP}} = 2\beta^{(0)}_{\mathrm{MATP}} + \beta^{(1)}_{\mathrm{MATP}}[f(2I^{1/2}) + \exp(-2\ I^{1/2})]. \qquad (38)$$

K in Eq. (37) represents the complex formation constant of $MATP^{3-}$ species ($M^+ = Na^+, K^+$), where:

$$\log K = \log^{T} K + (\ \ln\gamma_{\mathrm{M}^+} + \ln\gamma_{\mathrm{ATP}^{4-}} - \ln\gamma_{\mathrm{MATP}^{3-}})/\ln 10 \qquad (39)$$

Analogously, values of first conditional protonation constants of ATP in NaCl and KCl aqueous solutions at different ionic strengths are given by:

$$\log K_1^{\mathrm{H}} = \mathrm{Eq.}(31) - \ln(1 + e^{\ln K} C_{\mathrm{M}}) \qquad (40)$$

Values of ln γ_{M^+} and ln $\gamma_{\mathrm{MATP}^{3-}}$ in Eq. (39) can be expressed as ln γ_{H^+} and ln $\gamma_{\mathrm{HATP}^{3-}}$ of Eqs. (33) and (35) simply replacing "H" by "M". Values of Pitzer literature [27] parameters used during calculations performed in the present work are given in Table 3.

4. Results and discussion

4.1. Protonation constants

Protonation constants determined in this work are reported in Table 4, while that previously determined are shown in Table 5 [32]. Furthermore, several protonation constant values have been reported in literature and, after their careful analysis, some selected data (reported in Table 6 with corresponding references) have also been considered in this work together with the potentiometric constants of Tables 4,5. Smoothed values, reported in Table 7, were obtained as described in Section 3.1 and are shown in Figs. 1 (log $K_1^{\mathrm{H}*}$) and 2 (log $K_2^{\mathrm{H}*}$) vs. the square root of ionic strength, in $NaCl_{aq}$ and KCl_{aq}. Protonation constants extrapolated to zero ionic strength are (in the molar concentration scale, ± 95% confidence interval):

$$\log {}^{T}K_1^{\mathrm{H}} = \mathrm{p}^{T}K_{a2} = 7.656\pm0.010$$

$$\log {}^{T}K_2^{\mathrm{H}} = \mathrm{p}^{T}K* = 4.561\pm0.006.$$

SIT and Pitzer models need data in the molal (mol $kg^{-1}(H_2O)$) concentration scale: using appropriate density values we made molar to molal conversion of protonation constants. Molal values are also reported in Table 7 together with molar ones. Also for the protonation constants at infinite dilution we have to consider slightly different molal values: log ${}^{T}K_1^{\mathrm{H}}=7.655$ and log ${}^{T}K_2^{\mathrm{H}}=4.560$.

4.2. Dependence on medium and ionic strength by SIT approach

By using the equations reported in Section 3.2 we calculated the specific interaction coefficients and the formation constants of $NaATP^{3-}$ and $KATP^{3-}$ ion pairs, by using simultaneously protonation and activity coefficients data. Protonation constants in molal scale have already been reported in Table 7, while activity coefficients were reported by Bonner [45] (in that work γ_{Na_4ATP} and γ_{K_4ATP} are reported in the concentration range $0.1 \le C_{Na_4ATP}/\mathrm{mol\ kg^{-1}}(H_2O) \le 2.8$ and $0.1 \le C_{K_4ATP}/\mathrm{mol\ kg^{-1}}(H_2O) \le 2.4$); in the present work we considered γ values for $C_{M_4ATP} \le 0.8\ \mathrm{mol\ kg^{-1}}(H_2O)$ ($M^+ = Na^+, K^+$), given in Table 8. Refinements of protonation data in $NaCl_{aq}$ and values of γ_{Na_4ATP} and of data in KCl_{aq} and values of γ_{K_4ATP} gave parameters reported in Table 9 while complex formation constants for $NaATP^{3-}$ and $KATP^{3-}$ resulted as follows (± 95% confidence interval):

$$K(NaATP^{3-}) = 97.5\pm1.5 \qquad (\log K = 1.989\pm0.007)$$

$$K(KATP^{3-}) = 74.6\pm0.9 \qquad (\log K = 1.873\pm0.005).$$

Standard deviations on fits are (±95% confidence interval): σ_{fit} (log K_1^{H} in NaCl)=0.004, σ_{fit} (log K_2^{H} in NaCl)=0.006, σ_{fit}

Table 10
Pitzer interaction coefficients determined for ATP–Na^+ and ATP–K^+ systems by refining $\beta^{(2)}$ parameter

M	X	$\beta^{(0)}_{\mathrm{MX}}$	$\beta^{(1)}_{\mathrm{MX}}$	$C^{(\Phi)}_{\mathrm{MX}}$	$\beta^{(2)}_{\mathrm{MX}}$
Na^+	ATP^{4-}	-0.04 ± 0.07[a]	12.00 ± 0.36[a]	0.13 ± 0.04[a]	-22.3 ± 1.7[a]
Na^+	$HATP^{3-}$	1.08 ± 0.08	10.68 ± 0.58	-0.18 ± 0.05	–
Na^+	H_2ATP^{2-}	0.77 ± 0.09	7.49 ± 0.60	-0.08 ± 0.04	–
K^+	ATP^{4-}	0.00 ± 0.04	12.54 ± 0.24	0.16 ± 0.02	-21.0 ± 0.9
K^+	$HATP^{3-}$	1.53 ± 0.06	9.62 ± 0.30	-0.45 ± 0.04	–
K^+	H_2ATP^{2-}	1.19 ± 0.07	6.54 ± 0.31	-0.28 ± 0.04	–

[a] ±95% confidence interval.

Table 11
Pitzer interaction coefficients determined for ATP–Na^+ and ATP–K^+ systems by considering the formation of $NaATP^{3-}$ and $KATP^{3-}$ ion pairs

M	X	$\beta^{(0)}_{MX}$	$\beta^{(1)}_{MX}$	$C^{(\Phi)}_{MX}$
Na^+	ATP^{4-}	0.47±0.03[a]	16.17±0.26[a]	− 0.08±0.02[a]
Na^+	$HATP^{3-}$	0.34±0.05	11.33±0.43	− 0.04±0.02
Na^+	H_2ATP^{2-}	0.04±0.05	8.12±0.43	0.02±0.02
Na^+	$NaATP^{3-}$	0.30±0.05	11.96±0.45	− 0.03±0.02
K^+	ATP^{4-}	0.57±0.02	16.97±0.08	− 0.092±0.007
K^+	$HATP^{3-}$	0.37±0.02	12.35±0.16	− 0.08±0.01
K^+	H_2ATP^{2-}	0.04±0.03	9.27±0.17	0.03±0.02
K^+	$KATP^{3-}$	0.34±0.02	13.21±0.18	− 0.02±0.01

[a] ±95% confidence interval.

(log γ_{Na_4ATP})=0.004, σ_{fit} (log K_1^H in KCl)=0.002, σ_{fit} (log K_2^H in KCl)=0.003, σ_{fit} (log γ_{K_4ATP})=0.003.

4.3. Dependence on medium and ionic strength by Pitzer approach

Equations reported in Section 3.3 were used to describe the dependence on ionic strength and on ionic medium of protonation constants of Table 7 and activity coefficients of Table 8 by Pitzer model [25–27]. As already stated, we followed two different approaches (i.e., the use of $\beta^{(2)}$ and taking into account the formation of $MATP^{3-}$ species). When using $\beta^{(2)}$ parameter, the fit protonation data in $NaCl_{aq}$ and γ_{Na_4ATP} and KCl_{aq} and γ_{K_4ATP} gave results reported in Table 10, with quite satisfactory standard deviations: σ_{fit} (log K_1^H in NaCl)=0.026; σ_{fit} (log K_2^H in NaCl)=0.008; σ_{fit} (log γ_{Na_4ATP})= 0.012; σ_{fit} (log K_1^H in KCl)=0.009; σ_{fit} (log K_2^H in KCl)=0.005; σ_{fit} (log γ_{K_4ATP})=0.010. Similar results were achieved when following the second approach (i.e., by taking into account the formation of $NaATP^{3-}$ and $KATP^{3-}$ ion pairs instead of refining $\beta^{(2)}$ parameter). Pitzer interaction coefficients calculated by this procedure are reported in Table 11, while complex formation constants for $NaATP^{3-}$ and $KATP^{3-}$ resulted as follows (±95% confidence interval):

$$K(NaATP^{3-}) = 111.2\pm1.0 \qquad (\log K = 2.046\pm0.008)$$

$$K(KATP^{3-}) = 87.5\pm1.0 \qquad (\log K = 1.942\pm0.007).$$

Standard deviations on fits are (± 95% confidence interval): σ_{fit} (log K_1^H in NaCl)=0.003; σ_{fit} (log K_2^H in NaCl)=0.008; σ_{fit} (log γ_{Na_4ATP})=0.001; σ_{fit} (log K_1^H in KCl)=0.004; σ_{fit} (log K_2^H in KCl)=0.005; σ_{fit} (log γ_{K_4ATP})=0.001. The formation constants of Na^+ and K^+–ATP complex species calculated by SIT and Pitzer approaches are in fair accordance: the differences in log K values are 0.06 and 0.07 for sodium and potassium complexes, respectively. Smith and Alberty [20] obtained, at I=0.2, assuming $(C_3H_7)_4N^+$ is not interacting with ATP, log K ($NaATP^{3-}$)=0.16 and log K ($KATP^{3-}$)=1.06. These values are consistent with present ones, by taking into account the different ionic strength.

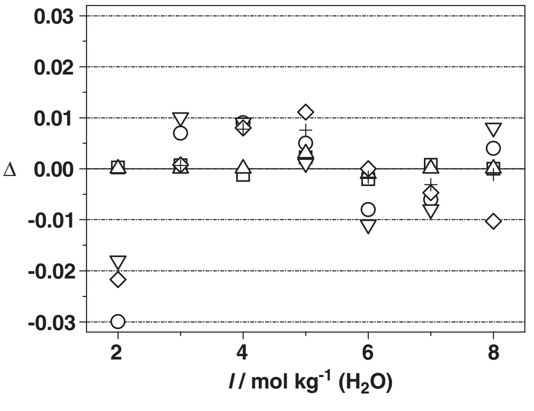

Fig. 3. Differences among literature (by Bonner [45]) and calculated (by SIT and Pitzer models) activity coefficients (as ln γ) of M_4ATP ($M^+ = Na^+$or K^+) vs. ionic strength (in mol $kg^{-1}(H_2O)$). $\Delta = \ln \gamma_{\pm(calcd)} - \ln \gamma_{\pm(exp)}$. □ refers to ln γ_{Na_4ATP} calculated by Pitzer model by taking into account $NaATP^{3-}$ formation; ○ refers to ln γ_{Na_4ATP} calculated by Pitzer model by refining $\beta^{(2)}_{NaATP}$; △ refers to ln γ_{K_4ATP} calculated by Pitzer model by taking into account $KATP^{3-}$ formation; ▽ refers to ln γ_{K_4ATP} calculated by Pitzer model by refining $\beta^{(2)}_{KATP}$; ◇ refers to ln γ_{Na_4ATP} calculated by SIT model; + refers to ln γ_{Na_4ATP} calculated by SIT model.

Table 12
Recommended values for the protonation of ATP at t=25 °C in NaCl and KCl aqueous solution (in the molar concentration scale)

I/mol L^{-1}	Ionic medium	log K_1^{H} [a]	log K_2^{H} [a]
0.1	NaCl	6.46±0.02	4.00±0.02
0.1	KCl	6.52±0.02	3.98±0.02
1.0	NaCl	5.83±0.02	3.98±0.01
1.0	KCl	5.98±0.02	3.97±0.02
→0		7.66±0.01	4.56±0.03

[a] ±95% confidence interval.

4.4. Activity coefficients

Mean activity coefficients can be recalculated by using SIT or Pitzer parameters. In Fig. 3, ln $\gamma_{\pm(calcd)}$ − ln $\gamma_{\pm(exp)}$ (isopiestic data by Bonner [45]) are reported vs. ionic strength for different models. As can be seen, fairly low deviations are observed, and this means that different models can be used with a high degree of accuracy. As it is well known, at relatively low ionic strengths one observe the higher errors, in particular when Na^+ complexation is neglected.

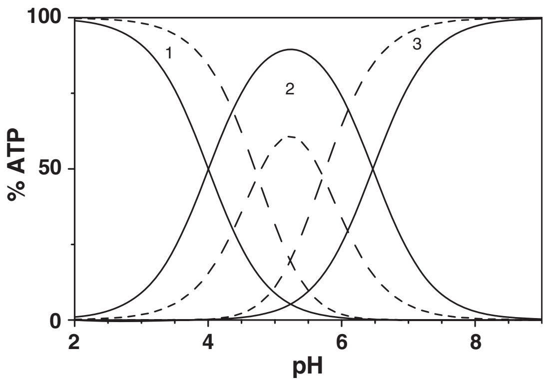

Fig. 4. Speciation diagrams of ATP vs. pH in NaCl aqueous solution at t=25 °C and I=0.1 mol L^{-1} (solid line) or I=5.0 mol L^{-1} (segment line), C_{ATP}=0.001 mol L^{-1}. Species: 1. H_2ATP^{2-}; 2. $HATP^{3-}$; 3. ATP^{4-}.

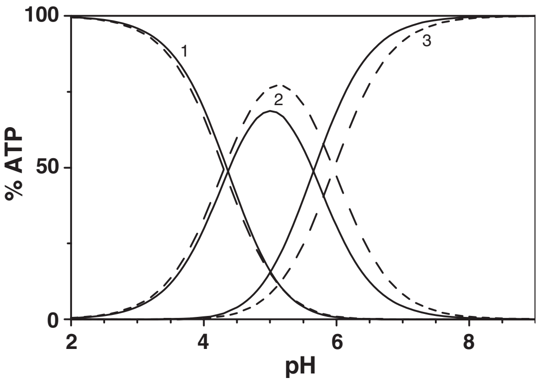

Fig. 5. Speciation diagrams of ATP vs. pH in NaCl (solid line) and KCl (segment line) aqueous solution at t=25 °C and I=3.0 mol L^{-1}, C_{ATP}=0.001 mol L^{-1}. Species: 1. H_2ATP^{2-}; 2. $HATP^{3-}$; 3. ATP^{4-}.

4.5. Recommended values

Some recommended values of protonation constants are reported in Table 12. Values at $I>1$ mol L^{-1} are not considered since there is no comparison with literature data. At present time the ones reported in this work can be considered as tentative values. Protonation constants in Table 12 agree substantially with Smith et al. [2] recommended values, and with the ones reported by Alberty et al. [19] (I=0.15 mol L^{-1}, in NaCl, at t=25 °C, log K_1^H=6.48 and log K_2^H=4.00; these values are widely quoted in biochemistry textbooks). Alkali metal formation constants reported in this work agree quite well with previously published values [32], log $K(NaATP^{3-})$=2.05 and $K(KATP^{3-})$ =1.94. Nevertheless, at present we think that only tentative formation constants can be given, i.e. log $K(NaATP^{3-})$=2.10± 0.10 and $K(KATP^{3-})$=1.95±0.12 (I=0 mol kg^{-1}, ±95% confidence interval).

4.6. Influence of ionic strength and ionic medium on ATP speciation

In previous paragraphs it has been well evidenced that values of first and second protonation constants of ATP are deeply affected by different conditions of ionic strength and ionic medium. Consequently, acid–base behavior of ATP and, therefore, its chemical speciation are also dependent on these two factors. In order to better clarify this concept, in Figs. 4 and 5, speciation diagrams of ATP are reported vs. pH: Fig. 4 shows two diagrams in NaCl aqueous solution at I=0.1 and I=5.0 mol L^{-1}, whilst in Fig. 5 two other diagrams are reported in $NaCl_{aq}$ and KCl_{aq}, both at I=3.0 mol L^{-1}.

From the analysis of Fig. 4, for example, one of the most relevant aspects that emerges is that the higher the ionic strength, the lower the formation percentage of $HATP^{3-}$ species is. This difference, that in Fig. 4 is ~25%, yields to significant shifts in the pH ranges at which other species are formed: at I=5.0 mol L^{-1}, where the formation percentage of $HATP^{3-}$ species is lower, H_2ATP^{2-} species decreases from 100% to ~50% at ~0.7 pH units higher than at I=0.1 mol L^{-1} (curve is shifted towards more basic pH values), whilst opposite behavior is shown by ATP^{4-} free ligand, where ~50% formation is achieved at ~0.7 pH units lower (curve is shifted towards more acidic pH values). In practice, this means that, for example at pH=6.0, at I=0.1 mol L^{-1} ATP is present in solution as 25% of free ligand, 74% of $HATP^{3-}$ and 1% as H_2ATP^{3-} species, whilst, at I=5.0 mol L^{-1}, we have 64%, 34% and 2%, for ATP^{4-}, $HATP^{3-}$ and H_2ATP^{2-}, respectively.

As concerns the effect of ionic medium, similar considerations can be made. For example, in Fig. 5, at I= 3.0 mol L^{-1}, the higher formation percentage (~9%) of $HATP^{3-}$ species in KCl produces a shift of ~0.4 pH units towards the more basic region of curve representing the formation percentage of free ligand (ATP^{4-}). In terms of speciation it means that, at pH=6.0 and I=3.0 mol L^{-1}, in $NaCl_{aq}$ we have ATP^{4-}=68%, $HATP^{3-}$= 31% and H_2ATP^{2-}=1%, whilst in KCl_{aq}, ATP is present as 52% ATP^{4-}, 47% $HATP^{3-}$ and 1% H_2ATP^{2-}.

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