Journal of Physical and Theoretical Chemistry
of Islamic Azad University of Iran, 6 (2) 97-103: Summer 2009
(J.Phys Theor.Chem. IAU Iran)
ISSN: 1735-2126

Spectrophotometric Determination of Protonation Constants of Tryptophan at Different Ionic Strengths and Various Ionic Media

Ali Shamel,[1,*] Masumeh Asgharian[1] and Farrokh Gharib[2]

[1] Chemistry Department, Ardabil Branch, Islamic Azad University, Ardabil, Iran
[2] Chemistry Department, Shahid Beheshti University, Tehran, Iran

ABSTRACT

The protonation constant values of tryptophan were studied at 25 °C, different ionic strengths, 0.1-1.0 mol dm^{-3}, and various ionic media of $NaClO_4$ and $NaNO_3$, using a combination of spectrophotometric and potentiometric techniques. The general trend for the two protonation constant values of tryptophan is in the order of $NaClO_4 > NaNO_3$ in different ionic media. The dependence of protonation constants on ionic strength and ionic media were taken into accounts using a Debye-Huckel type equation (molar concentration scale) and the SIT (specific ion interaction theory) approach (molal concentration scale). Differences in protonation constants in the various supporting electrolytes were also interpreted and discussed.

Keywords: Protonation Constant, Tryptophan; Sit Model; Ionic Strength; Salt Effect

INTRODUCTION

Amino acids doubtlessly play central roles both as building blocks of proteins and as intermediates in living metabolism of all animals. So, studying of the chemical and physical properties of amino acids is essential to determine the biological activity of proteins. Despite their recognized importance, there are only a few experimental contributions on their acid-base behaviour in different media. A search in the literature showed only few studies on protonation constants of tryptophan, one of the eight essential amino acids found in human [1-3].

The present work deals with the study of protonation equilibria of tryptophan in aqueous solution at different ionic strengths, 0.1 to 1.0 mol dm^{-3}, and two different supporting electrolytes, $NaClO_4$ and NaNO3, using a combination of potentiometric and spectrophotometric techniques. The parameters which define this dependency were analyzed with aim of obtaining further information with regard to their variation as a function of charges involved in the protonation reaction.

As is well known, thermodynamic data always refer to a selected standard state.

The definition given by IUPAC is well adopted and states that at the standard state the activity coefficient of a solute in a solution should be unity [4]. However, this is a strict condition and usually not accessible for many (or maybe all) reactions and so any measurements (or all) can not be made accurately in this state. Precise thermodynamic information for these systems can apparently be obtained in the presence of an inert electrolyte of sufficiently high concentration, ensuring that the activity factors are reasonably constant throughout the measurements.

---

*Corresponding author: E-mail. ali0029@gmail.com

We have been several years involved in the study of ionic strength dependence on formation constants and therefore, in the use of different equations for taking into account the function $\gamma = f(I)$, where γ and I are the activity coefficient and the ionic strength, respectively. Several models have been considered, including Debye-Hückel type equations [5-13]. Considering both the simplicity and the popularity of the SIT model, we thought it would be interesting to investigate the possibility of refining it in order to make it applicable over a broader ionic strength range as well using different supporting electrolytes.

EXPERIMENTAL

Reagents

L-tryptophan. $C_8H_5(NH)$-CH_2-$CH(NH_2)$ COOH, (Merck, analytical reagent grade) was recrystallized from hot water, washed with ethanol, and dried over P_2O_5, and the concentration of its solution was checked by titration against standard alkali. The NaOH solution was prepared from titrisol solution (Merck). Perchloric, nitric and hydrochloric acids were supplied from Merck as analytical reagent grade materials and were used without further purification. Sodium perchlorate and sodium nitrate (from Merck) were kept at room temperature in vacuum at least 72 hours before use. Dilute acid solutions were standardized against standard NaOH solution. All dilute solutions were prepared from double-distilled water with specific conductance equal to 1.3 ± 0.1 $\mu\Omega^{-1}$ cm^{-1}.

Measurements

All measurements were carried out at 25 ± 0.1°C. The ionic strength was maintained from 0.1 to1.0 mol dm^{-3} with sodium perchlorate or sodium nitrate. A Jenway research pH-meter, model 3520 (with a precision of 0.001 units), was used for $-\log[H^+]$ measurements. To exclude carbon dioxide from the system, a stream of purified nitrogen was passed through a sodium hydroxide solution and then bubbled slowly through the reaction solution. The hydrogen ion concentration was measured with a combined electrode. The pH-meter was calibrated for the relevant H^+ concentration with a solution of 1.00×10^{-2} mol dm^{-3} perchloric or nitric acid solutions containing 0.09 mol dm^{-3} sodium perchlorate or sodium nitrate, for adjusting the ionic strength on 0.1 mol dm^{-1}. The same procedure was performed for the other ionic strengths. For these standard solutions, we set $-\log[H^+]$ = 2.00 [14]. The junction potential corrections were calculated from Eq. (1):

$$-\log[H^+]_{real} = -\log[H^+]_{measured} + a + b[H^+]_{measured} \quad (1)$$

where a and b were determined by measuring the hydrogen ion concentration for two different solutions of $HClO_4$, HNO_3 or NaOH with sufficient $NaClO_4$ or $NaNO_3$ to adjust the ionic strength.

Spectrophotometric measurements were performed on a UV-Vis Shimadzu 2100 spectrophotometer with a Pentium 4 computer and using thermostated matched 10 mm quartz cells. The measurements cell was of flow type. A Masterflex pump allowed circulation of the solution under study from the potentiometric cell to the spectrophotometric cell, so the absorbance and $-\log[H^+]$ of the solution could be measured simultaneously.

For each experiment, 50 cm^3 of an acidic solution of tryptophan (2.0-5.0×10^{-3} mol dm^{-3}) was titrated with an alkali solution (0.1 mol dm^{-3} sodium hydroxide) both in the same ionic strength and ionic media. The $-\log[H^+]$ and absorbance were measured after the addition of a few drops of titrant, and this procedure was continued up to the required pH. In all cases, the procedure was repeated at least three times and the resulting average values and corresponding standard deviations are shown in the text and tables.

RESULTS AND DISCUSSION

In aqueous solution, tryptophan exists in its anionic form (L^-), zwitterionic species (HL), and cationic form (H_2L^+). The protonation of the amino acid refer to the reaction

$$L^- + nH^+ \Phi H_nL^{(1-n)-} \quad (6)$$

and the protonation constant, K_n,

$$K_n = [H_nL^{(1-n)-}]/[L^-][H^+]^n \quad (7)$$

where n could be 1 or 2 in the case of tryptophan.

The determination of the protonation constants of the amino acid is based on the relation $A = f(\text{pH})$ [29]. The measured absorbance, A (290-300 nm in the interval of 0.1 nm), and $-\log[H^+]$ from the spectrophotometric titration were conducted with the computer program Squad [30-31]. The data in the computer program were fitted for Eq. (6) by minimizing the error squares sum of experimental absorbances from the calculated ones. Figure 1 is shown as a typical example of the graphical fitting for the observed and calculated absorbances that extracted from the program Squad.

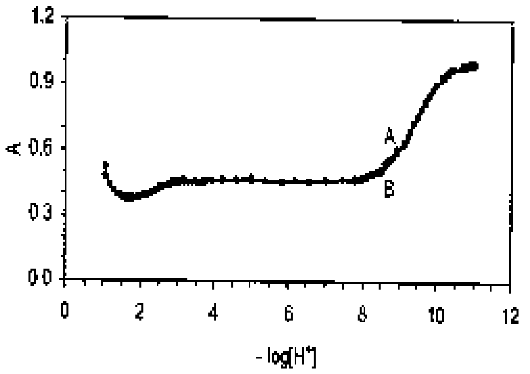

Fig. 1. A typical graphical fitting of tryptophan system at 25 °C, 300 nm and ionic strength 0.1 mol dm^{-3} $NaClO_4$. (A) experimental absorbance and (B) calculated absorbance from the computer program

The program allows calculation of the protonation constants with different stoichiometries. The number of experimental points (absorbance versus –log [H^+]) was more than 30 (maximum 40) for each titration run. During the experiments, the solutions were stable and the absorbance values did not change with time.

The protonation constants of tryptophan (in molar scale) are determined as mentioned and are listed in Table 1 at different ionic strengths and various ionic media, together with the values reported in the literature, which are in good agreement with those reported earlier [1-3]

The species mole fractions of the measured protonated species of tryptophan versus –log [H^+] are plotted as a typical example in Figure 2.

The cationic species of tryptophan, H_2L^+, is the predominant species at lower pH values, pH < 2. The zwiterionic species, HL, becomes predominant at pH range 4-8 (around 99 %). However, the anionic species begins to form at pH ≅ 8 in lower percentages and finally reaches to a maximum of 100 % at higher pH, pH > 10.

From a cursory examination of the protonation constant values, Table 1, it is clear the constants generally show the trend: $NaClO_4 > NaNO_3$. The trend is in the order of that shown by the molecular weight of the background electrolytes and is in agreement with our previous results for other amino acids [32].

Ionic Strength Dependence of Protonation Constants

The dependence on ionic strength of formation constant was taken into account by using the SIT model. For the protonation reaction, Eq. (6), the apparent protonation constant, Eq. (7), is calculated from the molar concentrations of the reacting species. The values are then converted to the molality scale according to the conversion factors determined by Baes and Mesmer [34]. Finally, the thermodynamic protonation constant, K°_m, is determined from the experimental constant, K_m, using a weighted linear extrapolation to the zero ionic strength. The logarithm of the thermodynamic protonation constant may be written as:

$$\log K^{\circ} = \log K^{m} + \log \gamma_{H_nL^{(1-n)+}} - \log \gamma_{L^-} - n\log \gamma_{H^+} \quad (8)$$

Subsrituting the activity coefficients of each species from Eq. (2) into Eq. (8) and rearranging gives

$$\log K^{\circ}_1 = \log K^{m}_1 + Z^*D - \Delta\varepsilon_1 I_m \quad (9a)$$

$$\log K^{\circ}_2 = \log K^{m}_2 + Z^*D - \Delta\varepsilon_2 I_m \quad (9b)$$

where $Z^* = \Sigma[(charges)^2_{reactants} - (charges)^2_{products}]$, K^m is the apparent protonation constant in molal scale, Table 2, and $\Delta\varepsilon$ represents the summation of the specific ion interaction terms as $\Delta\varepsilon_1 = \varepsilon(L^-, Na^+) + 2\varepsilon(H^+$, supporting electrolyte anion) - $\varepsilon(H_2L^+$, supporting electrolyte anion) and $\Delta\varepsilon_2 = \varepsilon(L^-, Na^+) + \varepsilon(H^+$, supporting electrolyte anion) for various supporting electrolytes.

Considering Eq. (9a), the values of $\Delta\varepsilon_1$ and $\log K^{\circ}_1$ are obtained using a weighted linear regression of the $\log K^{m}_1 + Z^*D$ as a function of the ionic strength (in molal scale), Figure 3.

Table 1. Average values of protonation constants of tryptophan at 25 °C, different ionic strengths and various ionic media, the values in literature are also reported

I mol dm^{-3}	log K_2 NaClO$_4$	log K_1 NaClO$_4$	log K_2 NaNO$_3$	log K_1 NaNO$_3$	ref.
0.1	2.32±0.02	9.32±0.03	2.30±0.02	9.30±0.04	this work
0.3	2.27±0.02	9.26±0.04	2.21±0.03	9.22±0.03	"
0.5	2.25±0.03	9.25±0.02	2.20±0.03	9.21±0.05	"
0.7	2.27±0.01	9.27±0.05	2.18±0.02	9.21±0.04	"
1.0	2.31±0.03	9.31±0.03	2.18±0.01	9.22±0.04	"
0.1 (NaClO$_4$)	-	9.39			1
0.1 (NaCl)	2.60	10.50			2
0.1 (NaCl)	2.35	9.33			3

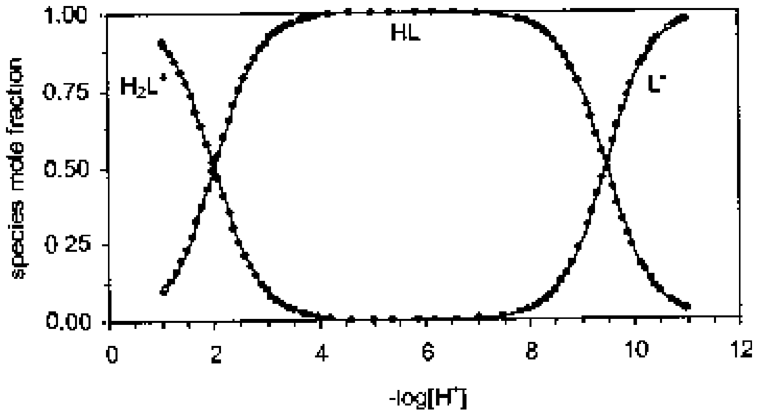

Fig. 2. Equilibrium distribution of the species in tryptophan system as a function of $-\log[H^+]$ at 25 °C and ionic strength 0.1 mol dm^{-3} NaClO$_4$

Table 2. The protonation constant values of tryptophan in molal scale at 25 °C

I /mol kg^{-1} NaClO$_4$	log K_2 NaClO$_4$	log K_1 NaClO$_4$	I /mol kg^{-1} NaNO$_3$	log K_2 NaNO$_3$	log K_1 NaNO$_3$
0.101	2.32	9.32	0.101	2.30	9.30
0.305	2.26	9.25	0.303	2.21	9.22
0.513	2.24	9.24	0.509	2.19	9.20
0.725	2.26	9.26	0.717	2.17	9.20
1.051	2.29	9.29	1.034	2.17	9.21

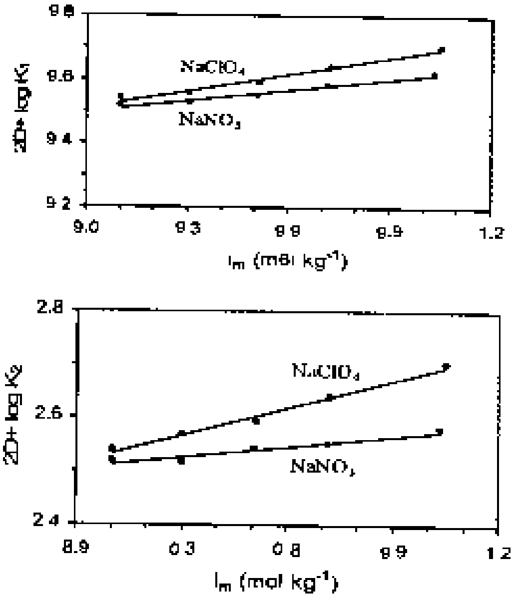

Fig. 3. Plots of log $K_1 + 2D$ and log $K_2 + 2D$ versus ionic strength (in molal scale) for reaction (6) at 25 °C

The procedure has been applied for the determination of log K^0_2 and $\Delta\varepsilon_2$, using Eq. (9b). The obtained values of $\Delta\varepsilon$ and log K^0 are listed in Table 3 and Table 4, respectively.

Table 3. SIT interaction coefficients

species	supporting electrolyte	SIT coefficient
$\varepsilon(H^+, ClO_4^-)$	$NaClO_4$	0.14 ᵃ
$\varepsilon(H^+, NO_3^-)$	$NaNO_3$	0.07 ᵃ
$\varepsilon(L^-, Na^+)$	$NaClO_4$	0.04
$\varepsilon(L^-, Na^+)$	$NaNO_3$	0.04
$\varepsilon(H_2L^+, ClO_4^-)$	$NaClO_4$	0.15
$\varepsilon(H_2L^+, NO_3^-)$	$NaNO_3$	0.11
$\Delta\varepsilon_1$	$NaClO_4$	0.17
$\Delta\varepsilon_1$	$NaNO_3$	0.07
$\Delta\varepsilon_2$	$NaClO_4$	0.18
$\Delta\varepsilon_2$	$NaNO_3$	0.11

ᵃ is obtained from reference 34

Table 4. Protonation constant values of tryptophan at 25 °C and zero ionic strength of $NaClO_4$ and $NaNO_3$

species	$NaClO_4$	$NaNO_3$
log K^0_2	2.51 ± 0.01	2.50 ± 0.02
log K^0_1	9.51 ± 0.01	9.50 ± 0.01

In this work, we need to know several interaction coefficients, ε, for different species in Eq. (6). The values of $\varepsilon(H^+$, supporting electrolyte anion) is taken from the literature [34], however, the other interactions coefficients were calculated from $\Delta\varepsilon_1$ and $\Delta\varepsilon_2$,and are listed in Table 3 for the different electrolytes used.

The dependence of the protonation constants of tryptophan on ionic strength determined in two background electrolytes. Figure 4, shows a regular trend and is in a good agreement with other protonation constants reported before as well as with some complex species of various amino acids with transition metal ions [28-29, 35-37].

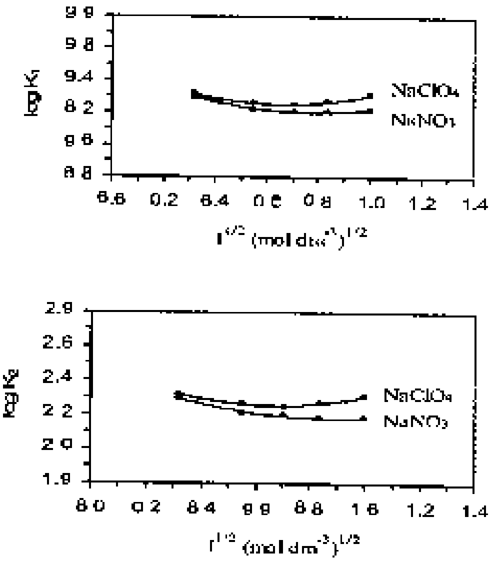

Fig. 4 Plots of log log K_1 and log K_2 (in molar scale) of tryptophan versus square root ionic strength of the supporting electrolytes at 25°C

Figure 4 shows a good convergence of log K_1 and log K_2 to nearly a single value as the ionic strength of different media decreasing to lower values. Our previous results on ionic strength dependence of complex formation and protonation constants and this work reveal the log K values are nearly always at their minimum at an ionic strength range 0.3-0.7 mol dm^{-3}, that is a characteristic of log $K = f(I)$. According to the electrolyte solutions [29, 38] at low ionic strength the dependence accounts for the Coulomb interaction between the ions that are screened by the ion atmosphere, while at higher concentrations the dependence accounts for the disturbances in ion-solvent interactions. At low ionic strength (less than 0.1 mol dm^{-3}) the Coulomb interactions are of primary importance.

However, as the ionic strength increases, the ionic atmosphere becomes more compressed and screens the ionic charges more effectively, so the intermolecular interactions (dipole-dipole or even multipole-multipole) become more important. These forces at higher ionic strength possibly have a primary role between the ions.

The supporting electrolyte used to keep constant the ionic strength may influences the protonation constant values in different ways. Indeed, the variation of the nature and the concentration of such electrolytes change the activity coefficient values and hence the protonation constants. So, the effect of the ionic strength and ionic medium on the values of protonation constants can be classified into two groups: (a) the effect of changes on the activity coefficients, and (b) specific interactions. The protonation constant values obtained in this work in all media have been adjusted to have the same ionic strength range, so the specific interactions should apparently be responsible for the observed differences in the studied systems, Table 3.

The SIT model describes the long range electrostatic interactions with a slightly different Debye-Hückel term and a sum of the terms describing the short range, non-electrostatic binary interactions between ions of opposite charges. The interaction coefficients between ions i and j, $\varepsilon(i, j)$, are assumed to be independent of the concentration. This constancy was recognized at higher molality [39]. For example, Ciavatta [19] found some electrolytes to have good constancy of ε in the range $0.5 < I$ (mol kg^{-1}) < 3.5. In general e is a function of I and some corrections have been proposed [23].

The SIT model can successfully be used for the description of the concentration dependence of the protonation constants of tryptophan as well the dependence of the nature of different supporting electrolytes. Considering Table 3, the trend for the observed values of $\Delta\varepsilon_1$ and $\Delta\varepsilon_2$ in the various electrolytes are as: $\Delta\varepsilon_1$ ($NaClO_4$) > $\Delta\varepsilon_1$ ($NaNO_3$) and $\Delta\varepsilon_2$ ($NaClO_4$) > $\Delta\varepsilon_2$ ($NaNO_3$). The same rule is governed for $e(H_2L^-$, supporting electrolyte anion) as: $\varepsilon(H_2L^+, ClO_4^-) > \varepsilon(H_2L^+, NO_3^-)$, and also the trend is almost true for $\varepsilon(L^-, Na^+)$ in different ionic media. Although the ions L^- and Na^+ are the same in different media but they are nearly in the order of $\varepsilon(L^-, Na^+)_{NaClO4} > \varepsilon(L^-, Na^+)_{NaNO3}$ (differences in the third digit). This indicates the greater e possibly causes the stronger interaction of the species, and hence a greater protonation constant in the supporting electrolyte.

REFERENCES

[1] Fazary, A. E.; Mohamed. A. F; Lebedeva, N. S. J. Chem Thermodyn. 38, 1467, 2006.

[2] Canel, E., Gultepe, A.; Dogan A.; Kilic E. J. Sol. Chem. 35, 5, 2006.

[3] Martel. A. E.; Smith, R. M. Critical Stability Constants. Vol. 1, Plenum Press. New York, 1989.

[4] Grenthe, I.; Plyasunov, A. Pure Appl. Chem. 69, 951, 1997.

[5] Gharib, F.; Zare, K.; Cheraghali, R. Russ. J. Inorg. Chem. 49, 949, 2004.

[6] Gharib. F.; Saeidie-Nik, F. J. Chem. Eng. Data. 49, 271, 2004.

[7] Gharib, F., Malekani, M. Revs. Inorg. Chem. 23, 97, 2003.

[8] Gharib, F., Afrazeb, L. J Chem. Eng. Data. 48, 999, 2003.

[9] Gharib, F.; Aghaei, H.; Shamel, A.; Taghvamanesh, A.; Shafiee, G. Russ. J. Coord. Chem. 29, 436, 2003.

[10] Gharib, F.; Zare, K.; Taghvamanesh, A.; Shamel, A.; Shafiee, G Main Group Met. Chem. 25, 647, 2002.

[11] Gharib, F.; Nouri, A. Russ. J. Inorg. Chem. 47, 1101. 2002.

[12] Gharib, F., Aghaei, H.; Shamel, A. Phys. Chem. Liq. 40. 637, 2002.

[13] Gharib, F.; Zare, K.; Taghvamanesh, A. J. Chem. Eng. Data. 46, 1140, 2001.

[14] Lagrange, P.; Schneider, M.; Zare, K.; Lagrange, J. Polyhedron. 13, 861, 1994.

[15] Bronsted, J. N. J. Am. Chem. Soc. 44, 877, 1922.

[16] Guggenheim, E. A. Application of Statistical Mechanics. Clarendon Press. Oxford, 1966.

[17] Scatchard, G. Equilibrium in Solution: Surface and Colloid Chemistry. Harvard University Press. Cambridge, 1976.

[18] Biederman, G. Workshop in Berlin, 1975.

[19] Ciavatta, L. Ann. Chim. (Rome) 70, 551, 1980.

[20] Ciavatta, L. 1990. Ann. Chim. (Rome) 80: 255

[21] Runde, W.; Reilly, S. D.; Neu, M. P. Geachim. Cosmo. Chim. Acta 63, 3443, 1999.

[22] Veyland, A.; Dupont, L.; Pierrard, J. C.; Rimbault, J.; Aplincourt, M. Eur. J. Inog. Chem. 1765, 1998.

[23] Bretti, C.; Foti, C.; Sammartano, S. Chem. Spec. Bioavail. 16, 105, 2004.

[24] De Stefano, C., Giuffre, O.; Sammartano, S. J. Chem. Eng. Data. 50, 1917, 2005.

[25] Gianguzza, A.; Pettignano, A.; sammartano, S. J. Chem. Eng. Data. 50, 1576, 2005.

[26] Gharib, F.; Farajtabar, A. J. Mol. Chem. 135, 27, 2007.

[27] Grenthe, I.; Fuger, J.; Konings, R. J. M.; Lemire, R. J.; Muller, A. B.; Nguyen-Trung, C.; Wanner, H. Chemical Thermodynamics of Uranium. Elsevier Science Publishers. Amesterdam, 1992.

[28] Daniele, P. G.; Rigano, C.; Sammartano, S. Talanta 32, 78, 1985

[29] Beck, M. T.; Nagypal, I. Chemistry of Complex Equilibria. Ellis Horwood Limited. New York, 1990.

[30] Leggett, D. J. Computation Methods for the Determination of Formation Constants, Plenum Press, New York, 1985.

[31] Meloun, M.; Javurek, M.; Havel, J; Talanta, 33, 513, 1986.

[32] Gharib, F.; Shamel, A.; Lotfi, F. Revs. Inorg. Chem. 25, 361, 2005.

[33] Gharib, F.; Zare, K.; Majlesi, K. J. Chem. Eng Data. 45, 833, 2000.

[34] Grenthe, I.; Plyasunov, A. J. Pure. Appl. Chem. 69, 951, 1997.

[35] Daniele, P. G.; Rigano, C.; Sammartano, S. Anal. Chem. 57, 2956, 1985.

[36] De Stefano, C.; Foti, C.; Gianguzza, A. J. Chem. Res 464, 1994.

[37] De Stefano, C.; Gianguzza, A.; Leggio, T.; Sammartano, S. J. Chem. Eng. Data. 47, 533, 2002.

[38] Berry, R. S.; Rice, S. A.; Ross, J. Physical Chemistry. John Eiley & Sons. New York, 1980.

[39] Pitzer, K. S. Ion Interaction Approach: Theory and Data Correlation in Activity Coefficients in Electrolyte Solutions, 2^{nd} Ed., CRC Press, New York, 1991