

# Thermodynamic Studies of *Trans*-4-Hydroxy-L-Proline at Different Ionic Medium, Ionic Strength and Temperature

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## Abstract

The aim of this work was to determine the solubility as well as the acidic constants of *trans*-4-hydroxy-L-proline in 0.16 and 3.5 Mol.kg<sup>-1</sup> NaClO<sub>4</sub> and in 0.16 and 3.18 Mol.kg<sup>-1</sup> NaCl at two different temperature, *i.e.* 298.15 and 310.15 K. *trans*-4-Hydroxy-L-proline, HL, is one of the most abundant amino acids present in collagen. As a major part of this protein, the measurement of hydroxyproline levels can be used as an indicator of collagen content. The protonation constants of ligand,  $K_1$  and  $K_2$ , combined with the salting effects on the ligand in the ionic media were treated by the specific ion interaction theory, SIT, to give equilibrium constants at the infinite dilution reference state at 298.15 K and at 310.15 K, as well as specific interaction coefficients of the cation and anion of *trans*-4-hydroxy-L-proline with the media ions at the standard temperature, *i.e.* 298.15 K.

**Keywords:** *Trans*-4-Hydroxy-L-proline; Acidic constants; Constant ionic medium method; Infinite dilution reference state; SIT model

## Introduction

In our on-going investigation of metals with biological ligands, we were interested in the metal-*trans*-4-hydroxy-L-proline complex formation equilibria. *trans*-4-Hydroxy-L-proline, HL (Figure 1), a constituent of several major structural proteins and other biodegradable synthetic products, is one of the main nonessential amino acid which contribute to the formation of collagen, the most abundant protein in humans as a primary constituent of bone, skin and connective tissues.

Defects in collagen synthesis lead to easy bruising, internal bleeding, breakdown of connective tissue of the ligaments and tendons, and increased risk of blood vessel damage. Studies on the properties of *trans*-4-hydroxy-L-proline in aqueous solution are useful in understanding the details of the mechanism of its action in aqueous systems. Previous works [1,2] reported thermodynamic data about equilibrium constants of HL at 0.16 Mol.kg<sup>-1</sup> at two different temperature (*i.e.* 298.15 and 310.15 K). By contrast, other available values [3] were obtained in non-aqueous solvents. In the present work the dependence of equilibrium constants of the ligand on ionic strength and media as well as on temperature was studied. It seems reasonable to predict different values of acidic constants at different ionic strength, medium and temperature. Thus, we have evaluated acidic constants  $K_1$  ( $L + H^+ \rightleftharpoons HL$ ) and  $K_2$  ( $L + 2H^+ \rightleftharpoons H_2L^+$ ) of the lig and at two different ionic strength,  $I$ , in two distinct media, *i.e.* 0.16 and 3.5 Mol.kg<sup>-1</sup> NaClO<sub>4</sub> and 0.16 and 3.18 Mol.kg<sup>-1</sup> NaCl, as well as at the two different temperatures 298.15 and 310.15 K. The adoption of the constant ionic medium method, proposed by Biedermann and Sillén [4], was necessary in order to minimize activity coefficient variation in spite of the change of the reagent concentrations. By this approach, it was possible to replace in the calculations activities with concentrations

and to minimize the liquid junction potential due to the hydrogen ion concentration. This was varied in order to determine acidic constants of ligand, taking into account that protonation takes place at different acidities. The potentiometric results and the solubility data obtained in the ionic media were processed by the specific ion interaction theory, SIT, [5,6] to yield acidic constants at the infinite dilution reference state and specific interaction coefficient of the cation and anion of HL with the media ions.

## Materials and Methods

### Apparatus and reagents

The cell arrangement was as formerly described [7]. The test solutions, stirred during titrations, were purified with a slow stream of nitrogen gas as reported in a previous work [8]. Glass electrodes were the same used in preceding evaluations [9]; they attained a constant potential within few minutes after the addition of the reagents and remained unchanged for several hours to within  $\pm 0.1$  mV. The titrations were carried out as described in a previous paper [10]. The electromotive force values were recorded with a precision of  $\pm 10^{-5}$  V by an OPA 111 low-noise precision DIFET operational amplifier. The cell assembly was retained in a thermostat kept at (298.1  $\pm$  0.1) and (310.1  $\pm$  0.1) K.

A perchloric acid and an hydrochloric acid stock solutions were prepared and standardized as described previously [9,11]. Sodium perchlorate stock solutions were prepared and standardized according to Biedermann [12]. Sodium hydroxide titrant solutions were prepared and standardize as reported formerly [10]. Purissimum grade (100

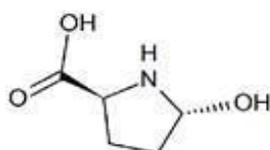


Figure 1: Structure of *trans*-4-hydroxy-L-proline, HL.

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% Aldrich p.a.) *trans*-4-hydroxy-L-proline product was kept in a desiccator over silica gel and it was utilized without purification. All solutions were prepared with twice distilled water.

### Solubility measurements

Solubility studies are of both theoretical and practical interest and they allow the determination of activity coefficients for nonelectrolyte solutes in aqueous solutions containing a large excess of salts [13-15]. The knowledge of the activity coefficients of neutral species is necessary when modeling the dependence of protonation constants on ionic strength according to the SIT equations [5,6]. Saturated HL were prepared as already described in a previous work [16]. Solid HL was wrapped up in a highly retentive filter paper (Whatman 42) bag and then was kept in a glass cylinder containing pure water as well as sodium perchlorate and sodium chloride aqueous solutions at pre-established ionic strength values (0.16 and 3.5 Mol.kg<sup>-1</sup>, and 0.16 and 3.18 Mol.kg<sup>-1</sup>, respectively), under continuous stirring with a magnetic bar (Figure 2).

The cylinder was then placed in a thermostatic water bath at (298.1 ± 0.1) and (310.1 ± 0.1) K and the ligand concentration was detected over time, until it reached a constant value, which usually occurred in about 3 up to 5 days. Finally, the absorption spectra in the UV region were recorded on a series of HL solutions. The absorbance,  $A_\lambda$ , may be expressed as equation 1:

$$A_\lambda = \epsilon l [\text{HL}] \quad (1)$$

Where  $l$  is the optical path and  $\epsilon$  is the molar absorptivity. To find suitable conditions for determining the solubility,  $S$ , of HL,  $A_\lambda$  were measured between 200 and 300 nm taking as a blank the ionic medium. Three replicates were run for each point. A typical spectrum of ligand recorded is reported in Figure 3.

Absorption spectrum of HL shows one single intense band centered at 193.5 nm. The solubility,  $S$ , was deduced by interpolation on a calibration curve, based on standard solutions. The reproducibility of the solubility data was of 1%.

Taking into account that  $S^\circ$  is the solubility of the neutral species

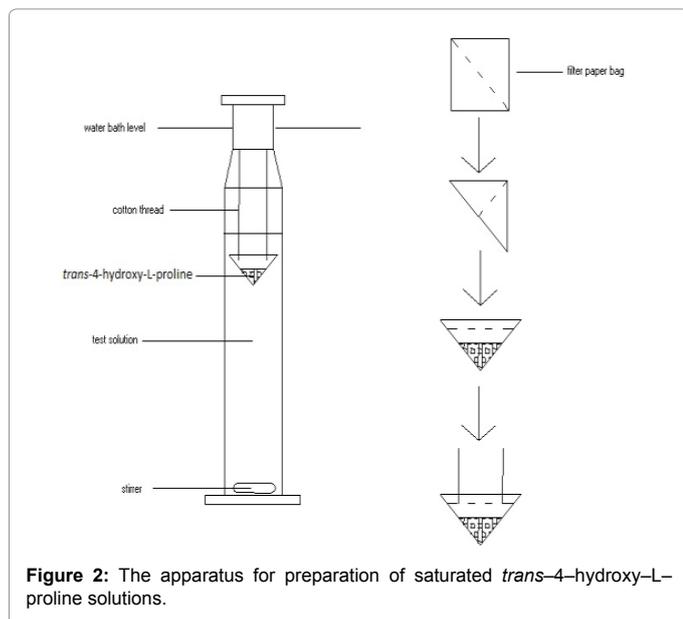


Figure 2: The apparatus for preparation of saturated *trans*-4-hydroxy-L-proline solutions.

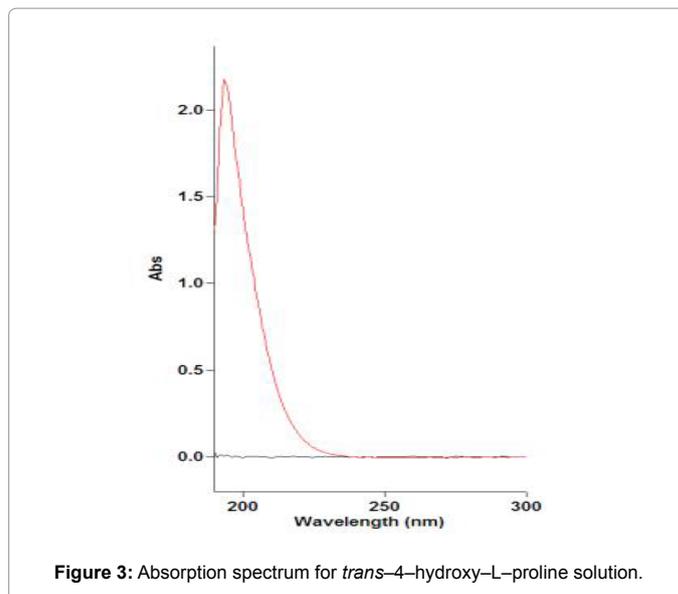
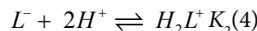
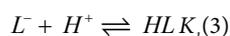


Figure 3: Absorption spectrum for *trans*-4-hydroxy-L-proline solution.

in the electrolyte solutions, the total solubility,  $S$ , of HL can be written as follows:

$$S = S^\circ \left\{ 1 + (K_1[H^+])^{-1} + (K_1K_2[H^+]^2)^{-1} \right\} \quad (2)$$

where the acidic constants of HL to the equilibria 3 and 4:



The solubility of the HL, valid in the molal concentration scale, is related to activity coefficient [15],  $\gamma$ , by the equation 5:

$$\log \gamma_{HL} = -\log \left( \frac{S_0^\circ}{S^\circ} \right) \quad (5)$$

Where  $S_0^\circ$  is the solubility of HL at the infinite dilution reference state. The results are summarized in Table 1.

*Trans*-4-Hydroxy-L-proline exhibits salting-in [17] behaviour, the solubility increasing monotonically with increasing ionic strength.

### Potentiometric measurements

The protonation equilibria of *trans*-4-hydroxy-L-proline were investigated by potentiometric titrations with an alkaline glass electrode, GE, at 298.15 and at 310.15 K with cell (G)



in which RE stands for the silver reference electrode = Ag/AgCl/0.0105 Mol.kg<sup>-1</sup> AgClO<sub>4</sub>, ( $I - 0.0105$ ) mol.kg<sup>-1</sup> NaClO<sub>4</sub>/IMol.kg<sup>-1</sup> NaClO<sub>4</sub>, when NaClO<sub>4</sub> was inert salt at two different ionic strength  $I$  (i.e. 0.16 and 3.5 Mol.kg<sup>-1</sup>) and = Ag / AgCl / IMol.kg<sup>-1</sup> NaCl saturated with AgCl / IMol.kg<sup>-1</sup> NaCl, when NaCl was inert salt at two different ionic strength  $I$  (i.e. 0.16 and 3.18 Mol.kg<sup>-1</sup>). Test solution had the general composition:  $C_L$  Mol.kg<sup>-1</sup> HL,  $C_A$  Mol.kg<sup>-1</sup> H<sup>+</sup>,  $C_B$  Mol.kg<sup>-1</sup> NaOH, ( $I - C_A - C_B$ ) Mol.kg<sup>-1</sup> Na<sup>+</sup>, where  $C_L$  were between (5 · 10<sup>-3</sup> and 10 · 10<sup>-3</sup>) Mol.kg<sup>-1</sup> and  $I$  was 0.16 and 3.5 Mol.kg<sup>-1</sup> NaClO<sub>4</sub>, and 0.16 and 3.18 NaCl Mol.kg<sup>-1</sup>. The equilibrium constants at the infinite dilution reference state can be readily evaluated from results obtained in this ionic strength range according to the SIT [5].

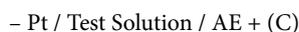
At a given  $I$  the electromotive force of cell (G) can be written, in

mV, at the temperatures of 298.15 and 310.15 K as equations 6 and 7, respectively:

$$E = E^o + 59.16 \log [H^+] + E_j \quad (6)$$

$$E = E^o + 61.54 \log [H^+] + E_j \quad (7)$$

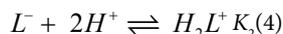
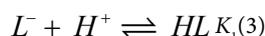
Where  $E^o$  is constant in each series of measurements and  $E_j$  is the liquid junction potential [4] which is a linear function of  $[H^+]$ ,  $E_j = -j[H^+]$ . The  $j$  parameters, at the different ionic strengths, are known from a previous evaluation [9,18]. In each run  $E^o$  values, constant within 0.1 mV, were calculated from measurements in solutions of  $(10^{-3} \geq [H^+] \geq 110^{-4}) \text{ mol kg}^{-1}$  in the absence of HL.  $[H^+]$  was decreased stepwise by coulometric generation of  $\text{OH}^-$  ions with the circuit (C)



where AE, auxiliary electrode, =  $1 \text{ Mol.kg}^{-1}$  inert salt /  $0.1 \text{ Mol.kg}^{-1} \text{ NaCl}$ ,  $(I-0.1) \text{ Mol.kg}^{-1} \text{ Na}^+/\text{Hg}_2\text{Cl}_2/\text{Hg}$ . In the test solution of a given volume  $V \text{ dm}^3$ ,  $C_b = (\mu F 10^{-6}/V) \text{ mol dm}^{-3}$  where  $\mu F$  stands for the microfaradays passed through the cell, according to the assumption that at the cathode the only reactions that occur are



After the introduction of a known amount of HL, dissolved in a known excess of  $\text{CB Mol.kg}^{-1} \text{ NaOH}$ , the acidification was achieved, in the pH range from 12 to 2, by adding  $H^+$  standardized solutions, according to the equilibria reported in equations 3 and 4:



The primary  $C_L$ ,  $C_A$ ,  $C_B$  and  $[H^+]$  data form the basis of the treatment to obtain the equilibrium constants.

## Results and Discussion

The protonation constants of *trans*-4-hydroxy-L-proline,  $K_1$  and  $K_2$ , were calculated from the data acquired by performing two titrations for each of the involved equilibria for the different ionic media and strengths and for the two different temperatures (298.15 and 310.15 K). In particular, a data set of 192 experimental points was used. An overview of the working conditions of all titrations carried out in  $\text{NaClO}_4$  and  $\text{NaCl}$  media is reported in Table 2 and Table 3, respectively.

The experimental data ( $C_L$ ,  $C_A$ ,  $C_B$ ,  $[H^+]$ ) were processed by numerical procedures. For the numerical treatment we employed the least-squares computer program Superquad [19] to seek the minimum of the function:

$$U = \sum (E_i^{obs} - E_i^{cal})^2 \quad (8)$$

Where  $E_i^{obs} = E^o + 59.16 \log [H^+]$  and  $E_i^{cal} = E^o + 61.54 \log [H^+]$  at 298.15 and 310.15

$I, \text{ Mol.kg}^{-1}$	$(S \cdot 10^3, \text{ Mol.kg}^{-1})$ 298.15 K	$(S \cdot 10^3, \text{ Mol.kg}^{-1})$ 310.15 K	$(\log \gamma_{HL})$ 298.15 K	$(\log \gamma_{HL})$ 310.15 K
Pure water	$31.61 \pm 0.01$	$40.16 \pm 0.01$	0	0
$\text{NaClO}_4$	0.16	$32.75 \pm 0.02$	$43.80 \pm 0.02$	$0.01 \pm 0.02$
	3.5	$35.02 \pm 0.04$	$46.25 \pm 0.04$	$0.04 \pm 0.04$
$\text{NaCl}$	0.16	$32.68 \pm 0.02$	$43.29 \pm 0.02$	$0.01 \pm 0.02$
	3.18	$34.93 \pm 0.04$	$49.72 \pm 0.04$	$0.04 \pm 0.04$

**Table 1:** Solubility,  $S$ , of *trans*-4-hydroxy-L-proline in  $1 \text{ mol.kg}^{-1} \text{ NaClO}_4$ , in  $1 \text{ mol.kg}^{-1} \text{ NaCl}$  and in pure water at 298.15 and 310.15 K.

$I, \text{ Mol.kg}^{-1}$	$\text{NaClO}_4$	$C_L, \text{ Mol.kg}^{-1}$	$\text{pH}_{\text{range}}$
298.15 K	0.16	$5 \cdot 10^{-3}$ $10 \cdot 10^{-3}$	2.79–11.1 2.97–10.8
	3.5	$5.0 \cdot 10^{-3}$ $9.94 \cdot 10^{-3}$	2.1–12.1 2.2–11.8
310.15 K	0.16	$5 \cdot 10^{-3}$ $10 \cdot 10^{-3}$	2.88–11.3 3.03–10.7
	3.5	$5.2 \cdot 10^{-3}$ $10.1 \cdot 10^{-3}$	2.2–12.1 2.1–11.7

**Table 2:** Summary of the working conditions of 8 titrations for protonation constants  $K_1$  and  $K_2$  in  $1 \text{ Mol.kg}^{-1} \text{ NaClO}_4$  at two different temperature.

$I, \text{ Mol.kg}^{-1}$	$\text{NaClO}_4$	$C_L, \text{ Mol.kg}^{-1}$	$\text{pH}_{\text{range}}$
298.15 K	0.16	$5.1 \cdot 10^{-3}$ $9.97 \cdot 10^{-3}$	3.55–12.09 3.27–11.12
	3.18	$5.0 \cdot 10^{-3}$ $9.96 \cdot 10^{-3}$	23.43–11.33 3.1–11.45
310.15 K	0.16	$5.3 \cdot 10^{-3}$ $10 \cdot 10^{-3}$	3.4–11.78 3.23–11.69
	3.18	$5.2 \cdot 10^{-3}$ $10 \cdot 10^{-3}$	3.1–12.06 2.9–11.17

**Table 3:** Summary of the working conditions of 8 titrations for protonation constants  $K_1$  and  $K_2$  in  $1 \text{ Mol.kg}^{-1} \text{ NaCl}$  at two different temperature.

$I, \text{ Mol.kg}^{-1}$		$\log K_1, \log K_2$ 298.15 K			$\log K_1, \log K_2$ 310.15 K
		$\text{NaClO}_4$	0.16	$9.63 \pm 0.02$	$11.46 \pm 0.04$
	3.5	$9.93 \pm 0.02$	$12.18 \pm 0.04$	$10.14 \pm 0.02$	$12.78 \pm 0.04$
$\text{NaCl}$	0.16	$9.63 \pm 0.02$	$11.46 \pm 0.04$	$9.78 \pm 0.02$	$11.93 \pm 0.04$
	3.18	$9.37 \pm 0.02$	$12.00 \pm 0.04$	$10.03 \pm 0.02$	$12.56 \pm 0.04$

**Table 4:** Survey of the  $\log K_n$  values, molal scale, by numerical methods.

$K$ , respectively, while  $E_{\text{cal}}$  is a value calculated for a given set of parameters. In the numerical treatment the ion product of water has been taken from the literature [20] for different temperature and different ionic strength and media. Results are given in Table 4 and the uncertainties on equilibrium constants represent  $3\sigma$ .

As it can be seen in Table 4 the value of the acidic constants increases as temperature increases, thus supporting that reactions are thermodynamically favored.

## Dependence on ionic medium, ionic strength and temperature by SIT approach

The acidic constants at the infinite dilution reference state,  ${}^oK_1$  and  ${}^oK_2$ , of HL were determined by the numerical values reported in Table 4, by assuming the validity of the SIT [5]. According to this theory, constants and other quantities in the following treatment are expressed on the molal scale by using the conversion factors from Grenthe et al. [21]. The activity coefficient,  $\gamma_i$ , of the species  $i$  with charge  $z_i$  can be expressed in aqueous solution as stated by the SIT:

$$\log \gamma_i = -z_i^2 D + \sum \varepsilon(i, k) m_k \quad (9)$$

Where  $D$  is the Debye-Hückel term

$$D = \frac{A\sqrt{I}}{1 + b\sqrt{I}} \quad (10)$$

Where the  $b$  value is arbitrarily chosen, generally  $1 \leq b \leq 1.5$ . In equation 9  $\varepsilon$  is the specific ion interaction coefficient of  $i$  with species  $k$  of molality  $m_k$ . The SIT model is based on the assumption that interaction coefficients of ions with the same charge type are nearly zero. Interaction coefficients are the result of short range forces and depend on the ionic strength but their variation in the range  $0.5 \leq I \leq 3.5 \text{ molal}$  is sufficiently low that they may be assumed as constants.

Parameters  $A$  and  $b$  in equation 10 can be taken from the literature. In particular,  $b = 1.5$  [22,23], while  $A$  varies with temperature [24,25]. In the range  $273 \leq T \leq 348$  K  $A_T$  can be defined as follows [22]:

$$A_T = 0.510 + 76.286 f_1(T) + 1.4189 f_2(T) \quad (11)$$

Where

$$f_1(T) = \left( \frac{1}{\Theta} - \frac{1}{T} \right) \quad (12)$$

$$f_2(T) = \left( \frac{\Theta}{T} - 1 + \ln \frac{T}{\Theta} \right) \quad (13)$$

and  $\Theta$  is the standard temperature, i.e. 298.15 K.

According to the specific interaction theory [5,6] the protonation constants of *trans*-4-hydroxy-L-proline,  $K_1$  and  $K_2$ , combined with the salting effects of inert salts (i.e.  $\text{NaClO}_4$  and  $\text{NaCl}$ ) on HL deduced from the solubility determinations, were processed to give equilibrium constants at the infinite dilution reference state,  ${}^\circ K_1$  and  ${}^\circ K_2$ . The effect of the ionic media at different ionic strength (i.e. 0.16 and 3.5  $\text{Mol.kg}^{-1}$   $\text{NaClO}_4$ , and in 0.16 and 3.18  $\text{Mol.kg}^{-1}$   $\text{NaCl}$ ) on the various equilibrium constants (according to equilibria 3 and 4) determined in this work can be expressed as:

$$\log {}^\circ K_1 = \log K_1 + 2D + \log \gamma_{HL} - [\varepsilon(H^+, \text{ClO}_4^-) + \varepsilon(L^-, \text{Na}^+)] m \quad (14)$$

$$\log {}^\circ K_2 = \log K_2 + 2D + [\varepsilon(\text{H}_2\text{L}^+, \text{ClO}_4^-) - 2\varepsilon(\text{H}^+, \text{ClO}_4^-) - \varepsilon(L^-, \text{Na}^+)] m \quad (15)$$

when inert salt is  $\text{NaClO}_4$ , and as:

$$\log {}^\circ K_1 = \log K_1 + 2D + \log \gamma_{HL} - [\varepsilon(H^+, \text{Cl}^-) + \varepsilon(L^-, \text{Na}^+)] m \quad (16)$$

$$\log {}^\circ K_2 = \log K_2 + 2D + [\varepsilon(\text{H}_2\text{L}^+, \text{Cl}^-) - 2\varepsilon(\text{H}^+, \text{Cl}^-) - \varepsilon(L^-, \text{Na}^+)] m \quad (17)$$

When inert salt is  $\text{NaCl}$ . The activity coefficients of HL,  $\log \gamma_{HL}$ , were deduced by solubility measurements in the electrolyte solutions, and from Ciavatta [5] at 298.15 K  $\varepsilon(\text{H}^+, \text{ClO}_4^-) = 0.14$  and  $\varepsilon(\text{H}^+, \text{Cl}^-) = 0.12$ . Hence, plots on the known terms of equations 14-17 as a function of  $I$  result in straight lines in which the intercept correspond to the constants at zero ionic strength and the slope are the interaction coefficients,  $\varepsilon$  (i, k), between HL and the counter ions of the ionic media (Table 5).

The interaction coefficients between  $\text{Na}^+$  ion and the anion of the acid as well as between  $\text{ClO}_4^-$  ion and  $\text{H}_2\text{L}^+$  agree with those expected for small ion [21]. As concerning  $\varepsilon(\text{H}_2\text{L}^+, \text{Cl}^-)$  the value obtained is negligible.

To calculate acidic constants of HL at the infinite dilution reference state at the temperature of 310.15 K, it was necessary to evaluate  $D$ , which is dependent on ionic strength  $I$  as well as on temperature by parameter  $A_T$  (equation 11). Similarly to what has been done at 298.15 K, it was possible to calculate  ${}^\circ K_1$  and  ${}^\circ K_2$  at 310.15 K, by assuming the validity of the SIT and taking into account equilibria 3 and 4. However in this case it was possible to deduce just the algebraic sum of interaction coefficients  $\varepsilon$  (i,k) since  $\varepsilon(\text{H}^+, \text{ClO}_4^-)$  and  $\varepsilon(\text{H}^+, \text{Cl}^-)$  were unknown at 310.15 K. Results of extrapolation are reported in Table 6.

The trend of two acidic constants at two different temperatures was, accordingly, matched at the two selected ionic media (Figure 4).

$\log {}^\circ K_1 = 9.87 \pm 0.05$ $\log {}^\circ K_2 = 11.67 \pm 0.05$	$\varepsilon(L^-, \text{Na}^+) = 0.02 \pm 0.05$ $\varepsilon(\text{H}_2\text{L}^+, \text{ClO}_4^-) = -0.02 \pm 0.05$ $\varepsilon(\text{H}_2\text{L}^+, \text{Cl}^-) = 0.001 \pm 0.05$
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Table 5: Results of extrapolation to zero ionic strength at 298.15 K.

$\log {}^\circ K_1 = 10.02 \pm 0.05$	$\log {}^\circ K_2 = 12.14 \pm 0.05$
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Table 6: Results of extrapolation to zero ionic strength at 310.15 K.

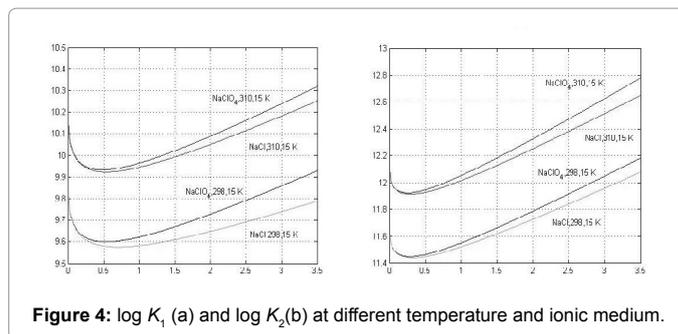


Figure 4:  $\log K_1$  (a) and  $\log K_2$  (b) at different temperature and ionic medium.

$I, \text{Mol.kg}^{-1} \text{NaClO}_4$	298.15 K $\log K_1, \log K_2$		310.15 K $\log K_1, \log K_2$		References
0.16	9.63	11.46	9.79	11.93	This work
	9.81	11.68	9.16	10.82	Zielinski et al., [1] Makaret al., [2]

Table 7: Comparison between acidic constants of *trans*-4-hydroxy-L-proline reported in literature at the same ionic strength in  $\text{NaClO}_4$  (i.e. 0.16  $\text{Mol.kg}^{-1}$ ) reported in this work.

A common behaviour was observed, since acidic constants increase as temperature increases. By contrast, a distinct effect of the ionic medium was clearly observed, whereby  $K_1$  decreases with different inert salt, while  $K_2$  results unaffected by the ionic medium.

## Conclusions

The solubility and the acidic constants of *trans*-4-hydroxy-L-proline have been determined, at 298.15 and 310.15 K, in 0.16 and 3.5  $\text{Mol.kg}^{-1}$   $\text{NaClO}_4$  and in 0.16 and 3.18  $\text{mol.kg}^{-1}$   $\text{NaCl}$  solutions and at the infinite dilution reference state. Results have been used to evaluate the salting effect of  $\text{NaClO}_4$  as well as of  $\text{NaCl}$  on the neutral molecule and interaction coefficients  $\varepsilon$  (i, k). The acidic constants obtained in this work and those taken from literature [1,2] are collected in Table 7 for a comparison.

The reactions are thermodynamically favored since acidic constants increase as temperature increases. The agreement between the data sets is just satisfactory, especially as concerning  $\log K_2$  by Makaret al. [2] at 310.15 K.

The available values from other authors [3] were obtained in non-aqueous solvents, for this reason it was not possible to make any comparison.

The results obtained in this work can be used for further studies regarding complex formation equilibria between HL and metal cations.

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