J. Chem. Eng. Data **2009,** *54,* 3037–3042

Solubility and Acidic Constants of L-Cystine in NaClO$_4$ Aqueous Solutions at 25 °C

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The solubility of L-cystine, H$_2$L, as well as the acidic constants have been studied at 25 °C in different ionic strengths. The media were (0.50, 1.00, 2.00, and 3.00) mol·dm^{-3} NaClO$_4$. The solubility of H$_2$L was also determined in pure water. The protonation constants of L-cystine, K_n ($H_{n-1}L^{n-3} + H^+ \rightleftarrows H_nL^{n-2}$) and K_{n+2} ($H_{2+n-1}L^{n-1} + H^+ \rightleftarrows H_{2+n}L^n$), in a basic and in a more acid range, respectively, combined with the salting effects of NaClO$_4$ on H$_2$L deduced from the solubility determinations, were processed by the specific ion interaction theory, SIT, to give equilibrium constants at the infinite dilution reference state, log $°K_1 = 9.41 \pm 0.05$, log $°K_2 = 8.64 \pm 0.01$, log $°K_3 = 1.61 \pm 0.06$, and log $°K_4 = 1.36 \pm 0.09$, as well as specific interaction coefficients, $b(L^{2-}, Na^+) = 0.29 \pm 0.02$, $b(HL^-, Na^+) = 0.14 \pm 0.01$, $b(H_3L^+, ClO_4^-) = 0.15 \pm 0.01$, and $b(H_4L^{2+}, ClO_4^-) = 0.29 \pm 0.02$.

Introduction

Amino acids containing sulfur atoms have received little attention, probably because they are often easily oxidized and sensitive to air and/or light, and they are only slightly soluble in aqueous solutions.[1] Among them, L-cystine has been investigated less than others due to its limited solubility in water.[2] L-Cystine results from cysteine oxidation with the formation of a disulfide bridge and displays four protonation sites (Figure 1).

In our continuing investigation, at 25 °C in NaClO$_4$ media, of metals with biological ligands, we are interested in the formation of metal−cystine equilibria. The aim of this paper is to determine solubility and acidic constants of L-cystine in NaClO$_4$ solutions at different ionic strengths. To determine all four acidic constants of L-cystine, it was necessary to vary widely the hydrogen ion concentration. It could be expected, in fact, that protonation takes place at different acidities.

The adoption of the constant ionic medium method, proposed by Biedermann and Sillèn,[3] was necessary to minimize activity coefficient variation in spite of the change of the reagent concentrations. In this way, it was possible to substitute activities with concentrations in the calculations and to minimize the liquid junction potential due to the hydrogen ion. In this work, NaClO$_4$ was selected as the constant ionic medium at four different concentrations, I [(0.50, 1.00, 2.00, and 3.00) mol·dm^{-3}]. It seems reasonable to predict different values of acidic constants at different I values.

The potentiometric data, in combination with solubility measurements of H$_2$L in pure water and in NaClO$_4$ solutions, were processed by the specific ion interaction theory,[4,5] SIT, to yield acidic constants at the infinite dilution reference state and interaction coefficients of the cations and anions of L-cystine with the perchlorate and sodium ions, respectively.

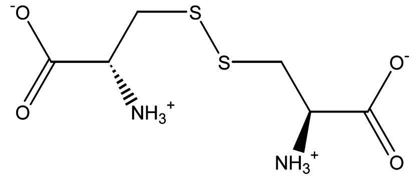

Figure 1. Structure of L-cystine, H$_2$L.

Method

From the chemical properties of L-cystine, it can be inferred that the neutral form of the compound, H$_2$L, is the least soluble species; its solubility increases by increasing the acidity for the probable formation of H$_3$L$^+$ and H$_4$L^{2+} and by increasing the alkalinity with the probable formation of HL$^-$ and L^{2-}. Investigations on solubility and on acid−base properties were carried out with two different procedures and are presented in two different sections. All measurements were carried out at 25 °C.

Solubility Measurements. Studies of the solubility of neutral species are of both theoretical and practical interest. Solubility investigations allow the determination of activity coefficients for nonelectrolyte solutes in aqueous solutions containing a large excess of salts.[6−8] In modeling the dependence of protonation constants on ionic strength using the SIT equations,[4,5] it is necessary to know the activity coefficients of neutral species. Several studies about the solubility of L-cystine have been published,[2,9,10] but none of them report measurements at 25 °C in NaClO$_4$ media. Differential pulse polarography (DPP) has become and remains an appreciated and trustable method in the study of solutions, thanks to the high reproducibility of the experimental curves.[11] Therefore, the total solubility (S, mol·dm^{-3}) of L-cystine was evaluated by this technique, based on reaction 1. Saturated L-cystine solutions were prepared with a leaching apparatus suitable to prevent solid particles from coming into contact with the magnetic stirrer. As a matter of fact, preliminary measurements showed an increase of solubility over periods of weeks when the solid was in mechanical contact with the stirrer because the solid was transformed into a phase made of smaller particles. To avoid grinding by the stirrer, solid

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[†] Professor Raffaella Porto passed away on May 18th, 2008.

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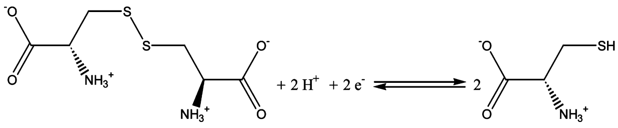

(1)

L-cystine was wrapped up in a highly retentive filter paper (Whatman 42) bag. This in turn was kept in a glass cylinder containing sodium perchlorate solution at pre-established ionic strength values (I = (0.5 to 3) mol·dm^{-3}) while continuously stirring with a magnetic bar (Figure 2). The cylinder was then placed in a thermostatic water bath at (25.00 ± 0.05) °C, and the L-cystine concentration was monitored over time, until it reached a constant value, which usually occurred in about (10 to 15) days.

Finally, polarographic assays were performed when an exactly weighed aliquot of the saturated L-cystine solution was added to 0.1 mol·dm^{-3} $HClO_4$ and 0.003 % Triton X-100 solution to remove the signal drift caused by the sudden intensity increase of the diffusion current. Air-free samples were obtained by bubbling with nitrogen for 15 min, and DPP traces were recorded with an Amel 433 analyzer model dropping mercury electrode apparatus. Polarographic grade, triply distilled mercury was employed. The cell, a glass cup of 30 cm^3 of volume, was located beneath an electrode support block, which contained the mercury capillary working electrode, a platinum wire counter electrode, and a saturated Ag/AgCl reference electrode. The instrumental settings were as follows: 10 mV·s^{-1}, scan rate of −50 mV, pulse amplitude of 50 ms, pulse time of 500 ms, and drop lifetime. The potential sweep was from (−1000 to −100) mV and the peak position around $E_p = -550$ mV (Figure 2), in the region where the reduction of L-cystine to L-cysteine occurs.

Three replicates were run for each point. A typical example of the differential pulse polarogram of L-cystine recorded is reported in Figure 3.

The total solubility, S, was deduced by interpolation on a calibration curve, based on standard solutions, taking into account that the current peak, i_p, is proportional to the L-cystine solubility (eq 2)

$$i_p = nFAS(K_D/\pi t_m)^{1/2} \qquad (2)$$

where n is the number of transferred electrons; F is Faraday's constant; A is the electrode surface; K_D is the diffusion coefficient of L-cystine; and t_m is the time between the application of the impulse and measurement of the wave. The reproducibility of the solubility data was ± 1 %.

As a result of proton association and dissociation equilibria, the total solubility, S, of L-cystine is due to the neutral as well as to the deprotonated and protonated species (eq 3)

$$S = S°\{1 + (K_2[H^+])^{-1} + (K_1K_2[H^+]^2)^{-1} + (K_3[H^+]) + (K_3K_4[H^+]^2)\} \qquad (3)$$

where $S°$ is the solubility of the neutral species in the electrolyte solutions and the acidic constants of L-cystine are referred to the equilibria (eqs 4 to 7):

$$L^{2-} + H^+ \rightleftarrows HL^- \qquad K_1 \qquad (4)$$

$$HL^- + H^+ \rightleftarrows H_2L \qquad K_2 \qquad (5)$$

$$H_2L + H^+ \rightleftarrows H_3L^+ \qquad K_3 \qquad (6)$$

$$H_3L^+ + H^+ \rightleftarrows H_4L^{2+} \qquad K_4 \qquad (7)$$

The solubility of the H_2L species is related to the activity coefficient, γ, by the equation,[10] valid in the molal concentration scale

$$\log \gamma_{H_2L} = -\log(S_0°/S°) \qquad (8)$$

where $S_0°$ is the solubility of L-cystine at the infinite dilution reference state. The results are summarized in Table 1.

As can be seen from Table 1, L-cystine exhibits "salting-in" behavior, the solubility increasing monotonically with increasing ionic strength.

Potentiometric Measurements. The protonation equilibria of L-cystine were investigated by potentiometric titrations with a glass electrode, GE, at 25 °C with cell G

$$\text{RE/Test Solution/GE} \qquad (G)$$

in which RE stands for the silver reference electrode

$$\text{RE} = \text{Ag/AgCl/0.01 mol·dm}^{-3}\ AgClO_4,\ (I - 0.01)\ \text{mol·dm}^{-3}\ NaClO_4$$

and the Test Solution had the general composition: C_L mol·dm^{-3} H_2L, C_A mol·dm^{-3} $HClO_4$, C_B mol·dm^{-3} NaOH, ($I - C_A - C_B$) mol·dm^{-3} $NaClO_4$, where C_L was between (3·10^{-3} and 5·10^{-3}) mol·dm^{-3} and I = (0.5, 1.0, 2.0, and 3.0) mol·dm^{-3}. From measurements in this ionic strength range, the equilibrium constants at the infinite dilution reference state can be readily evaluated by linear regression according to the SIT.[4]

At a given I, the EMF of cell G can be written, in millivolts, at the temperature of 25 °C, as eq 9

$$E = E° + 59.16 \log[H^+] + E_j \qquad (9)$$

where $E°$ is constant in each series of measurements and E_j is the liquid junction potential which is a linear function of [H$^+$], $E_j = -j[H^+]$.[3] The j parameters, at the different ionic strengths, are known from a previous evaluation.[12] In each run, $E°$ was determined in the absence of H_2L from measurements in solutions of (2·10^{-3} ≥ [H$^+$] ≥ 1·10^{-4}) mol·dm^{-3}. In this range, $E°$ values constant to within 0.1 mV or better were calculated. [H$^+$] was decreased stepwise by coulometric generation of OH$^-$ ions with the circuit C

$$-\text{Pt/Test Solution/AE}+ \qquad (C)$$

where AE, auxiliary electrode, = I mol·dm^{-3} $NaClO_4$/0.1 mol·dm^{-3} NaCl, (I − 0.1) mol·dm^{-3} $NaClO_4/Hg_2Cl_2$/Hg. Assuming that at the cathode the only reactions that occur are $H^+ + e^- \rightarrow 1/2H_2$ and $H_2O + e^- \rightarrow 1/2H_2 + OH^-$, then in the test solution, of a given volume V dm^3, $C_B = (\mu F \cdot 10^{-6}/V)$ mol·dm^{-3}, where μF stands for the microfaradays passed through the cell.

In the second part, after the introduction of H_2L, the acidity was varied stepwise by different methods according to the nature of the equilibria investigated. Thus, as concerning the equilibria 4 and 5 a known amount of L-cystine solved in a known excess of C_B mol·dm^{-3} NaOH was added. The pH range was (9.5 to 7), and acidification was achieved by adding $HClO_4$ standardized solutions.

When the equilibria involved the protonation of L-cystine (equilibria 6 and 7) [H$^+$] was increased by volumetric addition of $HClO_4$ over the range (10^{-3} to 10$^{-1.5}$) mol·dm^{-3}.

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

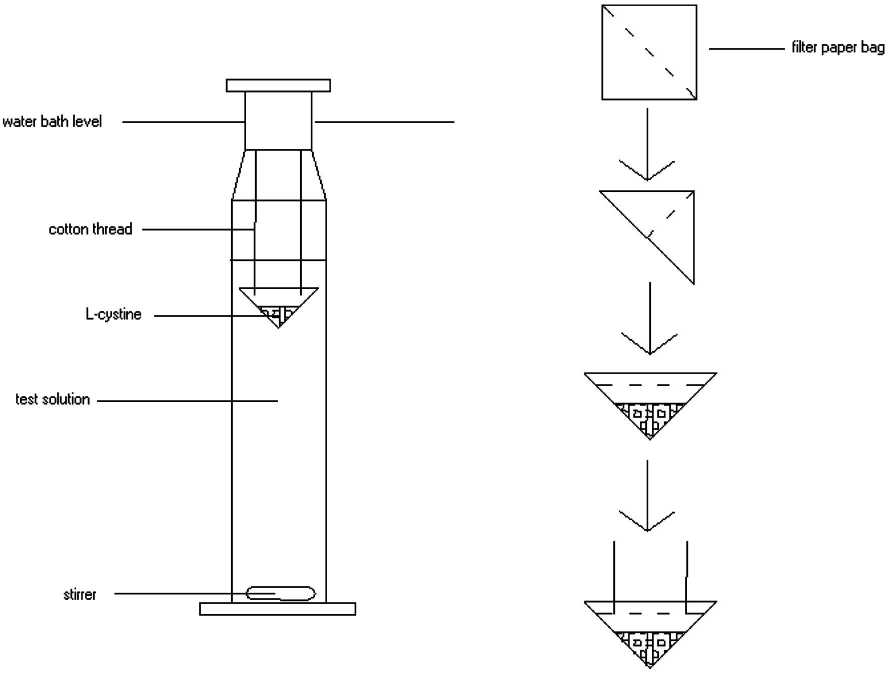

Figure 2. Apparatus for the preparation of saturated L-cystine solutions.

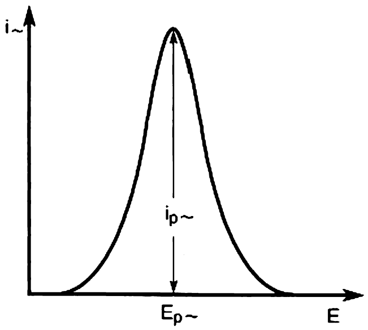

Figure 3. Differential pulse polarogram for L-cystine reduction. The curve represents the dependence of the current passing through the system on the electrochemical potential of the dropping electrode.

Table 1. Experimental Data on the Total Solubility and on the Solubility of the Neutral Species, S and $S°$, Respectively, of L-Cystine in I mol·kg^{-1} NaClO$_4$ and in Pure Water and the Activity Coefficients of H$_2$L

I mol·kg^{-1}	$S\cdot10^3$ mol·kg^{-1}	$S°\cdot10^3$ mol·kg^{-1}	log γ_{H_2L}
0.00	0.413 ± 0.004	0.412 ± 0.004	0
0.513	0.95 ± 0.01	0.92 ± 0.01	0.35 ± 0.01
1.05	1.22 ± 0.01	1.19 ± 0.01	0.46 ± 0.01
2.21	1.51 ± 0.02	1.48 ± 0.02	0.56 ± 0.02
3.50	1.97 ± 0.02	1.94 ± 0.02	0.67 ± 0.02

Experimental Section

Reagents and Analysis. Perchloric acid stock solution was prepared and standardized as described previously.[12] Sodium perchlorate stock solution was prepared and standardized according to Biedermann.[13] Sodium hydroxide titrant solutions were obtained by dilution of a satured solution filtered on a Gooch crucible (G4) in a nitrogen atmosphere. The hydroxide concentration was determined by titration with standardized HClO$_4$ using methyl-red as a visual indicator. The results agreed to within 0.1 %. Purissimum grade (≥ 99.0 % Aldrich p.a.) L-cystine product was used without further purification. It was kept in a desiccator over silica gel. All solutions were prepared with twice distilled water.

Table 2. Summary of the Relevant Data Taken in Eight Titrations for Protonation Constants K_1 and K_2

I mol·dm^{-3} NaClO$_4$	C_L mol·dm^{-3}	pH$_{range}$	$Z_{B\ range}$
0.50	$3.02\cdot10^{-3}$	7.00 to 9.19	0.059 to 1.43
	$3.3\cdot10^{-3}$	7.05 to 9.12	0.063 to 1.38
1.00	$3.18\cdot10^{-3}$	7.08 to 9.33	0.043 to 1.31
	$3.5\cdot10^{-3}$	7.03 to 9.45	0.039 to 1.40
2.00	$3.0\cdot10^{-3}$	7.08 to 8.78	0.10 to 1.36
	$4.5\cdot10^{-3}$	7.04 to 8.69	0.099 to 1.27
3.00	$3.25\cdot10^{-3}$	7.02 to 9.06	0.082 to 1.51
	$4.5\cdot10^{-3}$	7.05 to 9.17	0.092 to 1.58

Table 3. Summary of the Relevant Data Taken in Eight Titrations for the Protonation Constants K_3 and K_4

I mol·dm^{-3} NaClO$_4$	C_L mol·dm^{-3}	pH$_{range}$	$Z_{H\ range}$
0.50	$3.05\cdot10^{-3}$	1.64 to 2.55	0.32 to 1.25
	$3.25\cdot10^{-3}$	1.61 to 2.79	0.20 to 1.29
1.00	$3.23\cdot10^{-3}$	1.61 to 2.78	0.19 to 1.38
	$3.49\cdot10^{-3}$	1.64 to 2.89	0.15 to 1.35
2.00	$3.4\cdot10^{-3}$	1.63 to 2.56	0.34 to 1.39
	$4.12\cdot10^{-3}$	1.66 to 2.84	0.19 to 1.36
3.00	$3.5\cdot10^{-3}$	1.66 to 3.16	0.15 to 1.52
	$5.0\cdot10^{-3}$	1.63 to 3.04	0.20 to 1.55

Table 4. Ion Product of Water, Kw_{GE}, in NaClO$_4$ Media in Molal Units[17]

I, mol·kg^{-1} NaClO$_4$	$-\log Kw_{GE}$
0.513	13.68
1.05	13.69
2.21	13.83
3.50	14.04

Apparatus. The cell arrangement was similar to the one described by Forsling et al.[14] Ag/AgCl electrodes were prepared according to Brown.[15] Glass electrodes, manufactured by Metrohm, were of the 6.0133.100 type. They acquired a constant potential within 10 min after the addition of the reagents and remained unchanged to within ± 0.1 mV for several hours. The titrations were carried out with a programmable computer controlled data acquisition switch unit 34970 A supplied by Hewlett-Packard. The EMF values were measured with a

Table 5. Survey of the log K_n Values, Molar Scale, By Graphical and Numerical Methods

I	log K_1		log K_2	
mol·dm^{-3} NaClO$_4$	graphical	numerical (3σ)	graphical	numerical (3σ)
0.50	8.82 ± 0.01	8.84 ± 0.06	8.02 ± 0.01	8.07 ± 0.09
1.00	8.94 ± 0.01	8.95 ± 0.09	8.06 ± 0.01	8.09 ± 0.06
2.00	9.17 ± 0.01	9.17 ± 0.06	8.21 ± 0.01	8.28 ± 0.09
3.00	9.50 ± 0.01	9.50 ± 0.03	8.43 ± 0.01	8.50 ± 0.07

Table 6. Survey of the log K_{n+2} Values, Molar Scale, By Graphical and Numerical Methods

I	log K_3		log K_4	
mol·dm^{-3} NaClO$_4$	graphical	numerical (3σ)	graphical	numerical (3σ)
0.50	2.10 ± 0.01	2.042 ± 0.006	1.61 ± 0.01	1.725 ± 0.008
1.00	2.08 ± 0.01	2.027 ± 0.009	1.74 ± 0.01	1.818 ± 0.006
2.00	2.12 ± 0.01	2.097 ± 0.006	1.76 ± 0.01	1.824 ± 0.003
3.00	2.23 ± 0.01	2.352 ± 0.006	1.89 ± 0.01	1.960 ± 0.009

Table 7. Results of Extrapolation to Zero Ionic Strength

log °K_1 = 9.41 ± 0.05	b(L^{2-}, Na$^+$) = 0.29 ± 0.01
log °K_2 = 8.64 ± 0.01	b(HL$^-$, Na$^+$) = 0.14 ± 0.02
log °K_3 = 1.61 ± 0.06	b(H$_3$L$^+$, ClO$_4$$^-$) = 0.15 ± 0.01
log °K_4 = 1.36 ± 0.09	b(H$_4$L^{2+}, ClO$_4$$^-$) = 0.29 ± 0.02

precision of ± 10^{-5} V using an OPA 111 low-noise precision DIFET operational amplifier. A slow stream of nitrogen gas was passed through four bottles containing 1 mol·dm^{-3} NaOH, 1 mol·dm^{-3} H_2SO_4, twice distilled water, and I mol·dm^{-3} NaClO$_4$, respectively, and then into the test solutions through the gas inlet tube. During the EMF measurements, the cell assembly was placed in a thermostat kept at (25.0 ± 0.1) °C. The polarographic measurements have been conducted with a polarograph Amel 433 Analyzer. The formulations of the parameters and the following acquisition of the data have been managed with the aid of a computer connected to the tool.

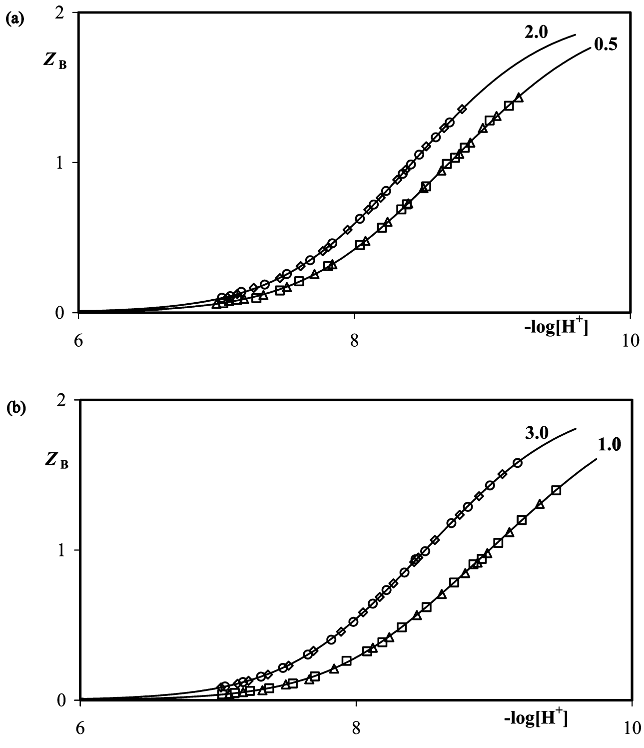

Figure 4. Z_B as a function of −log [H$^+$] for the values of I = (0.5 and 2.0) mol·dm^{-3} (a) and I = (1.0 and 3.0) mol·dm^{-3} (b). The curves were calculated with the values of the constants reported in Table 5 at the ionic strength shown in the figure. The symbols refer to C_L mol·dm^{-3}: for I = 0.5 mol·dm^{-3} squares (3.02·10^{-3} mol·dm^{-3}) and triangles (3.3·10^{-3} mol·dm^{-3}); for I = 1.0 mol·dm^{-3} squares (3.18·10^{-3} mol·dm^{-3}) and triangles (3.5·10^{-3} mol·dm^{-3}); for I = 2.0 mol·dm^{-3} diamonds (3.0·10^{-3} mol·dm^{-3}) and circles (4.5·10^{-3} mol·dm^{-3}); for I = 3.0 mol·dm^{-3} diamonds (3.25·10^{-3} mol·dm^{-3}) and circles (4.5·10^{-3} mol·dm^{-3}).

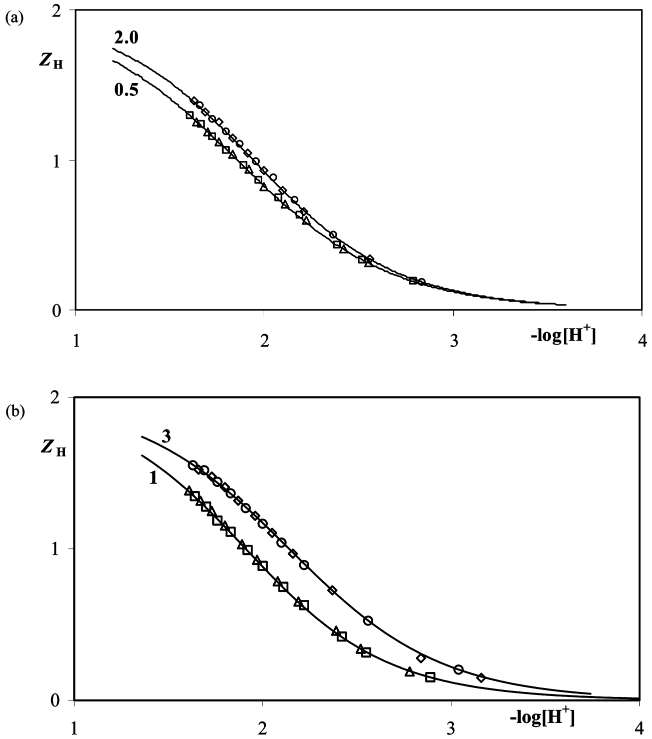

Figure 5. Z_H as a function of −log [H$^+$] for the values of I = (0.5 and 2.0) mol·dm^{-3} (a) and I = (1.0 and 3.0) mol·dm^{-3} (b). The curves were calculated with the values of the constants reported in Table 6 at the ionic strength shown in the figure. The symbols refer to C_L mol·dm^{-3}: for I = 0.5 mol·dm^{-3} squares (3.05·10^{-3} mol·dm^{-3}) and triangles (3.25·10^{-3} mol·dm^{-3}); for I = 1.0 mol·dm^{-3} squares (3.23·10^{-3} mol·dm^{-3}) and triangles (3.49·10^{-3} mol·dm^{-3}); for I = 2.0 mol·dm^{-3} diamonds (3.4·10^{-3} mol·dm^{-3}) and circles (4.12·10^{-3} mol·dm^{-3}); for I = 3.0 mol·dm^{-3} diamonds (3.5·10^{-3} mol·dm^{-3}) and circles (5.0·10^{-3} mol·dm^{-3}).

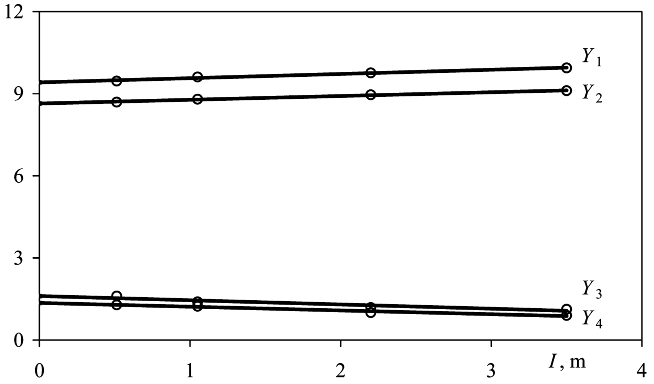

Figure 6. Y_1, Y_2, Y_3, and Y_4 (eqs 12 to 15) as a function of I.

Results

The data used to calculate the protonation constants, K_n and K_{n+2}, were acquired performing two titrations for each of the involved equilibrium at all considered NaClO$_4$ ionic strengths. In particular, the resulting data set used for calculations was 208 experimental points.

A summary of the relevant data taken in all titrations to evaluate the protonation constants in a basic and in a more acid range is reported in Tables 2 and 3, respectively.

The experimental data (C_L, C_A, C_B, [H$^+$]) were processed by graphical as well as by numerical procedures. The graphical

Table 8. Comparison between Acidic Constants of L-Cystine Reported by Apruzzese et al.[1] at Different Ionic Strengths in NaCl and Those Proposed Here for Different Concentrations, *I*, of $NaClO_4$ as the Ionic Medium

I, mol·dm^{-3}	log K_1	log K_2	log K_3	log K_4	ref
0.5 NaCl	8.70 ± 0.01	8.15 ± 0.01	2.25 ± 0.02	1.69 ± 0.02	1
0.5 $NaClO_4$	8.84 ± 0.06	8.07 ± 0.09	2.042 ± 0.006	1.725 ± 0.008	this work
1.0 NaCl	8.88 ± 0.01	8.29 ± 0.01	2.28 ± 0.01	1.72 ± 0.02	1
1.0 $NaClO_4$	8.95 ± 0.09	8.09 ± 0.06	2.027 ± 0.009	1.818 ± 0.006	this work
2.0 NaCl	8.98 ± 0.02	8.42 ± 0.02	2.40 ± 0.04	1.72 ± 0.02	1
2.0 $NaClO_4$	9.17 ± 0.06	8.28 ± 0.09	2.097 ± 0.006	1.824 ± 0.003	this work
3.0 NaCl	9.12 ± 0.02	8.56 ± 0.02	2.54 ± 0.02	1.87 ± 0.02	1
3.0 $NaClO_4$	9.50 ± 0.03	8.50 ± 0.07	2.352 ± 0.006	1.960 ± 0.009	this work

Table 9. Comparison between Acidic Constants of L-Cystine Reported by Apruzzese et al.[1] at Zero Ionic Strength and Those Proposed Here

this work	ref 1
log °K_1 = 9.41 ± 0.05	log °K_1 = 9.35
log °K_2 = 8.64 ± 0.01	log °K_2 = 8.76
log °K_3 = 1.61 ± 0.06	log °K_3 = 1.87
log °K_4 = 1.36 ± 0.09	log °K_4 = 1.31

methods consist essentially in the comparison of model functions[16] with experimental function Z_B (−log [H^+]) for equilibria 4 and 4, where Z_B is equal to $\{[H^+] - C_A - (K_w/[H^+])/C_L\}$ and Z_H (−log [H^+]) for equilibria 6 and 7, where Z_H is equal to $\{C_A - [H^+] + (K_w/[H^+])/C_L\}$. Plots Z_B (−log [H^+]) and Z_H (−log [H^+]) at different ionic strengths are shown in Figures 4 and 5, respectively. Values of the ion product of water, at the different ionic media in $NaClO_4$, were taken from Ciavatta and co-workers (Table 4).[17]

For different C_L values but constant I, the points coincide within the limit of experimental error. Comparison with the normalized function[16] indicates that the data are fully explained with the equilibria $H_{n-1}L^{n-3} + H^+ \rightleftarrows H_nL^{n-2}$ and $H_{2+n-1}L^{n-1} + H^+ \rightleftarrows H_{2+n}L^n$. From the best fit, we calculate the values of the constants given in Tables 5 and 6, respectively. The error was evaluated taking into account the shift along the *x* axes that still gave an acceptable fit. The agreement between experimental points and the model curves is shown in Figures 4 and 5.

For the numerical treatment, we employed the least-squares computer program Superquad[18] to seek the minimum of the function

$$U = \Sigma(E_i^{obs} - E_i^{cal})^2 \tag{10}$$

where $E_{obs} = E° + 59.16 \log [H^+]$ and E_{cal} is a value calculated for a given set of parameters. In the numerical treatment, for the different ionic media, the ion product of water has been maintained invariant. Results are given in Tables 5 and 6. The agreement with the graphical treatment is satisfactory.

Extrapolation to Infinite Dilution Reference State

The numerical acidic constants, K_n and K_{n+2}, have been used to determine the values at the infinite dilution reference state. The magnitude of the constants valid at zero ionic strength was evaluated by assuming the validity of the SIT.[4] Since the theory is formulated in terms of molal units (mol·kg^{-1}), constants and other quantities in the following treatment are expressed on the molal scale. The conversion factors were assumed from Grenthe et al.[19] According to the theory, the activity coefficient, γ_i, of the species i with charge z_i can be expressed in the molal scale at 25 °C in aqueous solution as

$$\log \gamma_i = -z_i^2 D + \Sigma b(i,k) m_k \tag{11}$$

where $D = 0.51\sqrt{I}/(1 + 1.5\sqrt{I})$ and *b* is the specific ion interaction coefficient of i with species k of molality m_k. Interaction coefficients are the result of short-range forces and depend on the ionic strength, but their variation in the range $0.5 \le I \le 3.5$ molal is sufficiently low that they may be assumed as constants. As a further simplification, interaction coefficients of ions with the same charge type are nearly zero.

The protonation constants of L-cystine, K_n and K_{n+2}, in a basic and in a more acid range, respectively, combined with the salting effects of $NaClO_4$ on H_2L deduced from the solubility determinations, were processed by the specific interaction theory[4,5] to give equilibrium constants at the infinite dilution reference state, °K_n and °K_{n+2}. According to the theory, the effect of the ionic strength on the various equilibrium constants (equilibria 4 to 7) determined in this work can be expressed as

$$Y_1 = \log K_1^{\,m} + 4D - [b(H^+, ClO_4^-)]I = \log °K_1 + [b(L^{2-}, Na^+) - b(HL^-, Na^+)]I \tag{12}$$

$$Y_2 = \log K_2^{\,m} + 2D - [b(H^+, ClO_4^-)]I + \log \gamma_{H_2L} = \log °K_2 + [b(HL^-, Na^+)]I \tag{13}$$

$$Y_3 = \log K_3^{\,m} - [b(H^+, ClO_4^-)]I - \log \gamma_{H_2L} = \log °K_3 - [b(H_3L^+, ClO_4^-)]I \tag{14}$$

$$Y_4 = \log K_4^{\,m} - 2D - [b(H^+, ClO_4^-)]I = \log °K_4 + [b(H_3L^+, ClO_4^-) - b(H_4L^{2+}, ClO_4^-)]I \tag{15}$$

where $\log \gamma_{H_2L}$ are the activity coefficients of H_2L in the electrolyte solutions (Table 1), and from ref 4 $b(H^+, ClO_4^-) = 0.14$. Hence, plots on the left-hand-side terms of eqs 12, 13, 14, and 15 as a function of *I* result in straight lines with the constants at zero ionic strength as the intercept and the slope, *b*(i, k), are the interaction coefficients between L-cystine and the ions of the ionic medium. Plots of Y_1, Y_2, Y_3, and Y_4 versus *I* are depicted in Figure 6. From the lines of best fit, the zero ionic strength constant and *b* values given in Table 7 were found.

Discussion

The solubility and the acidic constants of L-cystine have been determined, at 25 °C, in $NaClO_4$ solutions of ionic strength ranging from (0.5 to 3.0) mol·dm^{-3} and at the infinite dilution reference state. As expected, the first and the second protonation take place in the acidic range (−log [H^+] ≤ 3) and involve the protonation of the two carboxylic groups, while protolysis equilibria involving the two aminic groups take place in the alkaline range (−log [H^+] ≥ 7). The results have been used to evaluate the salting effect of $NaClO_4$ on the neutral molecule and interaction coefficients *b*(i, k).

The interaction coefficients between the Na^+ ion and the anions of the acid are higher than expected for small and bivalent anions[19] which are typically in the range −0.2 to 0.08. Usually, bivalent anions with Na^+ ions have negative interaction coefficients reflecting to some extent the tendency for association. The large positive values of $b(L^{2-}, Na^+)$ may be taken as an indication of negligible tendency of the Na^+ ion to associate with an anion whose charges occupy remote sites in the molecular structure.

Comparison with Literature Data. Table 8 collects literature data taken from Apruzzese et al.[1] The available values from other authors are obtained at higher temperature, i.e., 37 °C; for this reason, it was not possible to make any comparison.

Our results can be only partially compared with those proposed by Apruzzese et al.[1] because of the different ionic medium used. For a closer comparison of the two data sets, it was possible, by assuming the validity of the SIT,[4] to determine constants at zero ionic strength from the data of Apruzzese et al.[1] Results are reported in Table 9.

The agreement between the two data sets is just satisfactory probably because of the difference in the solubility values.[1] Anyway, the data obtained in this study are useful for further studies about metal−cystine equilibria.

Symbols

H_2L	L-cystine
K_n	equilibrium constant for $H_{n-1}L^{n-3} + H^+ \rightleftarrows H_nL^{n-2}$
K_{n+2}	equilibrium constant for $H_{2+n-1}L^{n-1} + H^+ \rightleftarrows H_{2+n}L^n$
C_L, C_B, and C_A	molarity (mol·dm^3) of L-cystine, NaOH, and $HClO_4$, respectively
$Z_B = \{[H^+] - C_A - (K_w/[H^+])/C_L\}$	average number of released protons per ligand
$Z_H = \{C_A - [H^+] + (K_w/[H^+])/C_L\}$	average number of acquired protons per ligand
I	ionic strength.
b(i,k)	interaction coefficient between species “i” and “k”
γ_i	activity coefficient of species “i” on the molal scale
[i]	molar concentration of species “i”

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Received for review January 29, 2009. Accepted May 16, 2009.

JE900116H