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Solubility of *L*-Cystine in NaCl and Artificial Urine Solutions

Erich Königsberger*, **Zhonghua Wang,** and **Lan-Chi Königsberger**

Department of Physical Chemistry, University of Leoben, A-8700 Leoben, Austria

Summary. *L*-Cystine is the least soluble of the naturally occurring amino acids, and cystine stones, caused by a genetic disorder, account for between 1–4% of all urinary stones. Since the concentration of cystine in urine is the only factor related to the stone formation, a proper knowledge of cystine solubility in urine is necessary. Some research groups have already reported on the solubility of *L*-cystine, but the results are scattering. In this work, a systematic investigation of cystine solubility under conditions most pertinent to urolithiasis was carried out. The solubilities of *L*-cystine were measured at 25.0 and 37.0°C, from *pH* 1 to 9, and in different media including (*i*) 0.300 mol · dm^{-3} NaCl solution and (*ii*) artificial urine solutions. The Joint Expert Speciation System (JESS) computer package and selected protonation constants of cystine reported in literature were used to model the solubilities of cystine. Excellent agreement was obtained between the experimentally determined solubility data and computer modelling of cystine solubility. The results of this work show that the presence of inorganic components has little influence on the solubility of cystine in urine.

Keywords. Cystine; Computer simulation; Cystinuria; Renal stones; Solubility; Urine.

Löslichkeit von *L*-Cystin in Natriumchlorid- und künstlichen Urinlösungen

Zusammenfassung. *L*-Cystin ist die am geringsten lösliche natürlich vorkommende Aminosäure. Cystinsteine, die durch eine genetische Störung verursacht werden, machen etwa 1–4% aller Harnsteine aus. Da die Cystinkonzentration im Urin den einzigen für die Steinbildung verantwortlichen Faktor darstellt, ist die genaue Kenntnis der Cystinlöslichkeit in Urin eine notwendige Voraussetzung für weitere Untersuchungen. Die bisher publizierten Löslichkeitsdaten streuen jedoch sehr stark, so daß in dieser Arbeit eine systematische Studie der Cystinlöslichkeit unter Bedingungen durchgeführt wurde, die denen der Steinbildung möglichst ähnlich sind. Die Löslichkeit von *L*-Cystin wurde bei 25.0 und 37.0°C im *pH*-Bereich von 1 bis 9 in 0.300 mol · dm^{-3} NaCl sowie in künstlichen Urinlösungen gemessen. Das Joint Expert Speciation System (JESS) sowie ausgewählte Protonierungskonstanten aus der Literatur wurden zur Modellierung der Cystinlöslichkeiten verwendet, wobei eine ausgezeichnete Übereinstimmung mit den experimentell bestimmten Werten erhalten wurde. Die Ergebnisse dieser Arbeit zeigen, daß anorganische Salze nur einen sehr geringen Einfluß auf die Cystinlöslichkeit in Urin ausüben.

* Corresponding author

Introduction

L-Cystine, $C_6H_{12}N_2O_4S_2$, the least soluble of the naturally occurring amino acids, is normally excreted in urine in low concentrations of *ca.* 0.06–0.17 $mmol \cdot dm^{-3}$. Because of a congenital defect in the tubular reabsorption of cystine, a small number of individuals excrete much higher concentrations of *ca.* 1.3–3.3 $mmol \cdot dm^{-3}$ [1]. Owing to its low solubility in urine, the excessive excretion of cystine results in the formation of stones or calculi of cystine which can block the renal tubes. The general goal of treatment of cystine lithiasis is to reduce the urinary cystine concentration below its solubility limit. Although only a few percent (1–4% [2]) of all stones have cystine as major component (whose proportion is then usually >95% [3]), the study of cystine stone formation can serve as a model for a particular aspect of stone formation in general [4]. The reason is that in cystinuria a form of stone is involved in which only one factor, namely increased cystine output in the urine, is overwhelmingly important in relation to all other factors (diet, infection, urinary tract abnormality, *etc.*) which are known to be concerned in the formation of many other types of stone. A proper knowledge of the solubility of *L*-cystine is therefore necessary to understand the tendency of cystine stone formation and to find a more effective method than those currently available (*e.g.* [5]) to prevent or to treat cystine lithiasis. Unfortunately the solubility of cystine is not very well studied, and large discrepancies are found amongst the values reported in literature. In this work, the solubility of *L*-cystine was investigated thoroughly by measuring and modelling its solubilities in NaCl and artificial urine solutions. Carefully determined solubility data and proper modelling of cystine solubility in artificial urine solutions are important since they serve as a prerequisite for the study of cystine solubility in real urine.

Results and Discussion

Protonation constants of cystine

The cystinate ion, Cis^{2-}, can be protonated in four steps according to

$$n\mathrm{H}^+ + \mathrm{Cis}^{2-} \rightarrow \mathrm{H}_n\mathrm{Cis}^{n-2}, \tag{1}$$

where $n = 1 - 4$. The corresponding protonation constants are defined by

$$\beta_{01n} = [\mathrm{H}_n\mathrm{Cis}^{n-2}][\mathrm{H}^+]^{-n}[\mathrm{Cis}^{2-}]^{-1}. \tag{2}$$

The formally uncharged species $H_2Cis^{\pm}$ (which is actually a zwitterion) has the lowest solubility, *i.e.*,

$$\mathrm{H_2Cis}(s) \rightarrow \mathrm{H_2Cis}^{\pm}(aq) \tag{3}$$

where the corresponding solubility constant is denoted as K_s. Since the ionic species are much more soluble, the total solubility of cystine is given by

$$\begin{aligned}[\mathrm{H_2Cis}]_{\mathrm{tot}} = K_s(1 + (\beta_{012}[\mathrm{H}^+]^2)^{-1} + \beta_{011}(\beta_{012}[\mathrm{H}^+])^{-1} \\ + \beta_{013}[\mathrm{H}^+](\beta_{012})^{-1} + \beta_{014}[\mathrm{H}^+]^2(\beta_{012})^{-1}).\end{aligned} \tag{4}$$

In the case of uric acid [6], within the precision of the solubility measurements no ionic strength dependence of protonation constants could be detected in the

Table 1. Protonation constants of cystine

T/°C	Media	$\log\beta_{011}$	$\log\beta_{012}$	$\log\beta_{013}$	$\log\beta_{014}$	References
20	$0.15\,\text{mol}\cdot\text{dm}^{-3}$ $NaClO_4$	8.80	16.83			[8]
37	$0.15\,\text{mol}\cdot\text{dm}^{-3}$ NaCl	8.604 (±0.003)	16.356 (±0.004)	18.41 (±0.01)	20.03 (±0.02)	[7]
37	$0.15\,\text{mol}\cdot\text{dm}^{-3}$	8.6[a]	16.4[a]	18.47[a]	19.97[a]	[9]

[a] Recommended values

range of $0.15\,\text{mol}\cdot\text{dm}^{-3} \leq I_c \leq 0.30\,\text{mol}\cdot\text{dm}^{-3}$. Thus, to model the solubility of *L*-cystine at 37°C, the four protonation constants of cystine determined by *Cole et al.* [7] at $I_c = 0.15\,\text{mol}\cdot\text{dm}^{-3}$ NaCl were used. Since no reliable protonation constants were reported for 25°C, in the present model (*i*) the $\log\beta_{011}$ and $\log\beta_{012}$ values calculated from the two protonation constants determined by *Hawkins* and *Perrin* [8] in $0.15\,\text{mol}\cdot\text{dm}^{-3}$ $NaClO_4$ at 20°C were used for the high *pH* range, and (*ii*) the formation constants determined by *Cole et al.* [7] were used for the low *pH* range. The constants employed in the models are given in Table 1; the resulting calculated solubility curves are shown in Fig. 1 (dashed and solid lines for 25 and 37°C, respectively). It is noteworthy that due to its poor solubility in aqueous solutions, there are not many cystine protonation constants reported in the literature [9]. Amongst the data available for 20 and 25°C, the constants determined by *Hawkins* and *Perrin* [8] are most trustworthy because these authors used the potentiometric back titration method, whereas others employed the older method of solubility

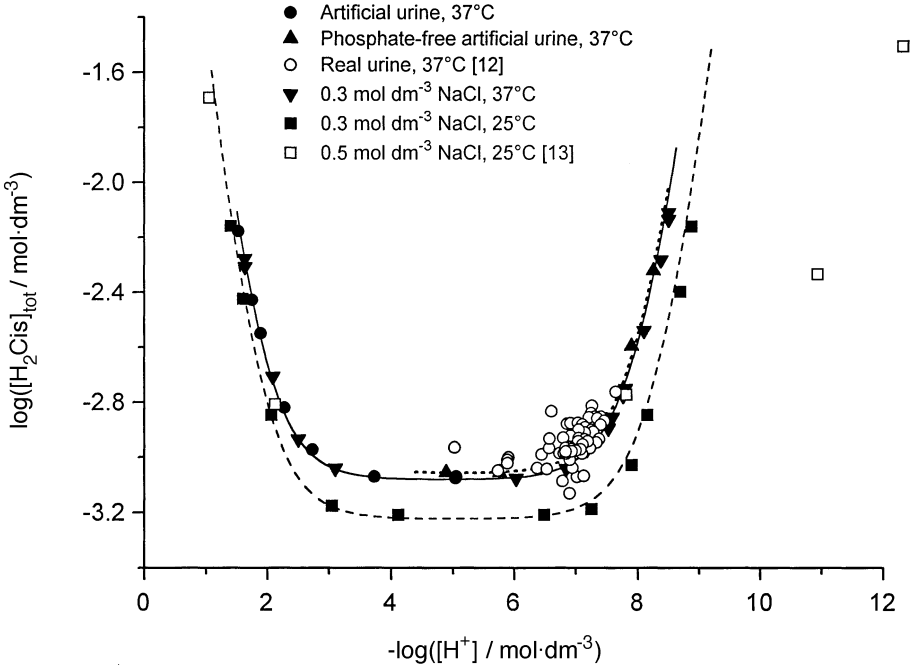

Fig. 1. Solubilities of *L*-cystine; dashed and solid lines (for 25 and 37°C, respectively) were calculated using the protonation constants reported in Refs. [7] and [8]; the dotted line for phosphate-free artificial urine was simulated using JESS [14–16]

measurements to calculate approximate values of cystine protonation constants [9]. From the studies carried out under physiological conditions, the constants reported by *Cole et al*. [7] were chosen because they are the most recent data and also in excellent agreement with the values recommended in the critical review of *Berthon* [9].

Solubility of L-cystine

Solubilities of cystine at the solubility minimum ($4 < pH < 6$) at various conditions are summarized in Table 2. It can be seen clearly here that the reported values differ significantly for any set of conditions. The results of this work (solid symbols) and selected literature data (open symbols) are presented in Fig. 1. The Joint Expert Speciation System (JESS) computer package [14–16] and thermodynamic quantities in the JESS database for all possible complexes were employed in the simulation of cystine solubility in phosphate-free artificial urine (dotted line). The reliability of our results is strongly supported by the excellent agreement obtained between the experimental values (solid symbols) and the simulated curves over the *pH* range from 1 to 9. As expected, data in Fig. 1 show that cystine is more soluble at higher temperature and that its solubility is low at $3.5 < -\log[H^+] < 7.0$; unfortunately this is the *pH* range of urine.

(i) Solubility of L-cystine at 25°C

Solubilities of *L*-cystine at 25°C in water as well as in 1 and 3 $mol \cdot dm^{-3}$ NaCl have already been published by *Carta* and *Tola* [11] (Table 2), but the authors measured the *pH* of the initial solutions. These reported values may be sufficient for the authors' industrial interest to recover the amino acid by hydrolysis of keratinic material, but they are not at all a proper representation of cystine solubility. Later on, *Carta* [13] published the data with equilibrium *pH* (Fig. 1, open squares). At low *pH*, a very good agreement is obtained between the data reported by *Carta* [13] and this work. However, at high *pH* a large discrepancy is found, and there seems to be some systematic error in the data of *Carta* [13].

Table 2. Solubilities of cystine at the solubility minimum ($4 < pH < 6$)

T/°C	Media	K_s/($mol \cdot dm^{-3}$)	References
room	Water	$0.43 \cdot 10^{-3}$	[10]
25	Water	$0.70 \cdot 10^{-3}$	[11]
25	0.3 $mol \cdot dm^{-3}$ NaCl	$0.60 \cdot 10^{-3}$	This work
25	1 $mol \cdot dm^{-3}$ NaCl	$0.84 \cdot 10^{-3}$	[11]
25	3 $mol \cdot dm^{-3}$NaCl	$1.10 \cdot 10^{-3}$	[11]
37	$5 \cdot 10^{-3}$$mol \cdot dm^{-3}$ sodium cacodylate	$0.7 \cdot 10^{-3}$	[12]
37	0.3 $mol \cdot dm^{-3}$ NaCl	$0.83 \cdot 10^{-3}$	This work
37	Artificial urine	$0.83 \cdot 10^{-3}$	This work
37	Artificial urine without phosphate	$0.88 \cdot 10^{-3}$	This work
37	Urine	$1.3 \cdot 10^{-3}$	[4]
37[a]	Urine[a]	$0.67 \cdot 10^{-3}$	[1]

[a] Presumed since temperature and medium were not clearly specified

(ii) Solubility of L-cystine at 37°C

At 37.0°C, the solubility of cystine in artificial urine is similar to that in $0.300\,\mathrm{mol}\cdot\mathrm{dm}^{-3}$ NaCl at $-\log[H^+] < 5.0$. Due to the precipitation of phosphate salts, no data were collected at $-\log[H^+] > 5.0$. However, in artificial urine without phosphate solubilities of cystine were measured up to $-\log[H^+] = 8.2$. The results show that the minimum solubility of cystine is slightly higher in phosphate-free artificial urine; above $-\log[H^+] = 7.5$, the solubility of cystine in this solution is very much the same as in $0.300\,\mathrm{mol}\cdot\mathrm{dm}^{-3}$ NaCl. It is quite obvious that in real and artificial urines Ca^{2+} forms strong complexes with phosphate, whereas in phosphate-free solution Ca^{2+} is available to promote the solubility of cystine by complexation. Actually, some influence of $[Ca^{2+}]$ on cystine solubility in $CaCl_2$ solutions has also been reported by *Pak* and *Fuller* [12], but these author only measured the cystine solubilities at *pH* 6 and 6.7. Many researchers have claimed that cystine is more soluble in real urine than in synthetic solutions [4, 12, 17, 18]; the explanation for this might be the influence of some organic components in urine, like macromolecules [12], *etc*., rather than that of inorganic salts. Nevertheless this point is not clear because, as shown in Table 2, the solubility of cystine in urine reported by *Dent* and *Senior* [4] is much higher than that published by *Robertson* and *Nordin* [1]. The value reported by the second group is closer to our determined in $0.300\,\mathrm{mol}\cdot\mathrm{dm}^{-3}$ NaCl and to oxalate-free artificial urines with and without phosphate. Moreover, it can be seen from Fig. 1 that solubilities of cystine in real urine samples reported by *Pak* and *Fuller* [12] are about the same as those in artificial urines determined in this work. Therefore, more work is required to clarify the role of macromolecules and other organic substances in urine.

Regarding the treatment of cystine lithiasis, potassium citrate has been used to increase the urinary *pH* and thus the cystine solubility [5]. However, it can be seen from Fig. 1 that cystine is much more soluble at $-\log[H^+] > 7.5$, but at these *pH* values the risk of calcium phosphate stones becomes higher. Thus, the potential role of alkali treatment is rather limited. Other methods to increase the solubility of cystine in urine or to decrease the urinary cystine concentration are also discussed in Ref. [5], but each method carries with it some side effects. Therefore, a new method having no or less disadvantages ought to be searched for, and computer simulations of solubilities, see *e.g*. Ref. [19], certainly play an important role in this area.

Conclusion

Solubilities of *L*-cystine in NaCl and artificial urine solutions were carefully studied in this work, and our results were shown to be highly reliable. The presence of inorganic components was demonstrated to have little influence on the solubility of cystine in urine. Another significance of our work is that the solubilities determined and the equilibrium simulation model used here have served as a successful starting point for the study of cystine solubility in real urine. From this point, proper study of the formation of cystine stones and the search for a better treatment of cystinuria can be developed.

Experimental

Analytical grade reagents (minimum 99% purity), Fluka concentrated volumetric standards of HCl and NaOH, A-grade glassware, and bidistilled water were employed throughout. *L*-Cystine was a commercial product (SigmaUltra, minimum 99%) and used without further purification.

Solubility measurements were performed in a similar manner to that reported previously [6]. Constant ionic strength I_c was used to keep the activity coefficients of the reacting ions essentially constant. Thus, for all evaluations, directly measured concentrations can be employed rather than activities. Thermostatted (to ± 0.05 K) continuously operating solubility cells were used in which intimate contact between solid and solution is effected by percolation utilizing an H_2O-prehumidified N_2 gas stream [20, 21]. These cells are designed to avoid the presence of solid particles in the measuring compartment which contains the electrode system for the continuous potentiometric measurement of H^+ concentrations. The *pH* variation method [22] was employed to determine the solubility of *L*-cystine. In this method, the initial $-\log[H^+]$ of the test solution is varied by dropwise addition of either HCl or NaOH solution. The corresponding galvanic cells can be represented as:

$$\text{Ag}\,|\text{AgCl}|\,5.0\text{mol}\cdot\text{dm}^{-3}\text{NaCl}|\,5.0\text{mol}\cdot\text{dm}^{-3}|\text{test solution}(I_c)|\text{glass electrode} \qquad (5)$$

Home-made silver – silver chloride reference electrodes of thermal-electrolytic type, *Wilhelm*-type salt bridges [23], glass electrodes (Schott H2680), and an Orion EA 940 *pH*/ISE meter were used. The liquid junction potentials at this high ionic strength salt bridge are assumed to be negligible in the range $2 < pH < 11$ [24]. In all potentiometric measurements, the electrodes were calibrated before and after each experiment. In acidic and basic solutions, the potentials became constant after about 20 and 4 h respectively; this indicates that solubility equilibria have been attained. In the isoelectric range of cystine, *pH* values were not stable even after 48 h; so constant concentrations of cystine in solutions were the equilibrium criterion.

The solubility of *L*-cystine was determined (*i*) at 25.0 and 37.0°C in $0.300\,\text{mol}\cdot\text{dm}^{-3}$ NaCl (which is believed to be of about the same ionic strength as that of urine) and (*ii*) at 37.0°C in oxalate-free artificial urine solutions with and without phosphate (appropriate amounts of NaCl were used to compensate the missing amount of phosphate salt). The composition of these artificial urine solutions, shown in Table 3, is like that of Standard Reference Artificial Urine (abbreviated as SRAU, [25]), but oxalate was omitted to avoid the precipitation of Ca^{2+} salt. To calibrate the electrodes, in the first case $10.0\,\text{mmol}\cdot\text{dm}^{-3}$ HCl and $0.290\,\text{mol}\cdot\text{dm}^{-3}$ NaCl solution was used. In the second case, $10.0\,\text{mmol}\cdot\text{dm}^{-3}$ HCl and $0.290\,\text{mol}\cdot\text{dm}^{-3}$ KCl solution was employed since it is believed to have about the same ionic strength and hence the same activity coefficient of H^+ as SRAU.

Table 3. Composition of artificial urine solutions used in this work

	Artificial urine	Phosphate-free artificial urine
Composition	$\text{mol}\cdot\text{dm}^{-3}$	$\text{mol}\cdot\text{dm}^{-3}$
NaCl	0.1055	0.2024
Na_3Citrate	0.00321	0.00321
Na_2SO_4	0.01695	0.01695
NaH_2PO_4	0.0323	0
Na_2Oxalate	0	0
$CaCl_2$	0.00575	0.00575
$MgSO_4$	0.00385	0.00385
KCl	0.0637	0.0637
NH_4Cl	0.0455	0.0455

The solution resulting from a solubility measurement was diluted with $0.100\,\mathrm{mol}\cdot\mathrm{dm}^{-3}$ HCl, and the concentration of cystine was determined using a Perkin Elmer Lambda 15 UV/Vis spectrophotometer at 240 nm. At this wavelength, the absorption remains constant with time and is proportional to the cystine concentration. The calibration curve was obtained with a linear regression coefficient of 0.99998 in the concentration range from 0.5 to $2.5\,\mathrm{mmol}\cdot\mathrm{dm}^{-3}$. However, the molar absorption coefficient is quite small ($\varepsilon = 331.4\,\mathrm{dm}^3\cdot\mathrm{mol}^{-1}\cdot\mathrm{cm}^{-1}$) at this wavelength, making background correction essential for an accurate determination of cystine concentrations.

The modelling of *L*-cystine solubilities was performed using the JESS package of computer programs [14–16]. The quantities used for the computer simulation were taken from the JESS thermodynamic database.

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