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Thermodynamic modeling of crystal deposition in humans*

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Abstract: The prevention and treatment of crystal deposition in the human body are based on the understanding of the physicochemical properties underlying the precipitation of the substances involved. Among these properties, the solubilities of the crystals are very important. Recently, experimentally determined solubility data of substances related to urolithiasis, such as calcium oxalate hydrates, uric acid and urates, cystine, and xanthine, were critically assessed. Unfortunately, reported solubilities of these substances were found to be either scarce or in large disagreement. Consequently, detailed studies were carried out in our laboratory, and the results will be discussed in this communication with emphasis on the thermodynamic consistency of the experimentally determined data. Since proper modeling of the solubilities of these substances in artificial urine solutions serves as a prerequisite for solubility predictions in real urine, the Joint Expert Speciation System (JESS) software package was employed to create a comprehensive computer model including the relevant, low-molecular inorganic and organic components of urine. The results of the simulations lead to some useful suggestions regarding the prevention and treatment of stone disease.

INTRODUCTION

Human body fluids are normally supersaturated with regard to several substances (e.g., blood plasma, interstitial and intracellular liquors with respect to calcium carbonates and phosphates, particularly hydroxyapatite and fluoroapatite; urine with respect to calcium oxalates and, depending on the pH, with respect to uric acid or calcium phosphates). While normal biomineralization, like the formation of bone and teeth, takes place in controlled biological situations, uncontrolled pathological crystallization leads to painful or even life-threatening conditions such as calculi formation (renal, biliar, sublingual), development of gout or arteriosclerosis, tissue calcification associated with cancer, etc. The question, why pathological crystallization does not occur indiscriminately in all human fluids, has been discussed in terms of three main factors: besides (i) the supersaturation as a necessary condition, the (ii) presence of heterogeneous nucleants, and a (iii) deficit of crystallization inhibitors play a crucial role in pathological situations [1].

In the scope of urolithiasis, more than 20 different types of stones composed of calcium oxalate hydrates (hereafter, COM for monohydrate and COD for dihydrate), ammonium magnesium phosphate (struvite), calcium phosphates (hydroxyapatite, HAP, and brushite, DCPD), uric acid and urates, cystine and xanthine have been classified [2]. A sound knowledge of the solubilities of these substances is necessary to understand the cause, prevention, and treatment of renal or bladder calculi. However in a recent review, experimentally determined solubility data of these substances were critically assessed and found to be either sparse or in large disagreement [3]. Consequently, a research program was con-

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ducted in our laboratory to provide reliable solubility data of these compounds. This communication summarizes the solubilities of calcium oxalate hydrates, uric acid and urates, cystine, and xanthine for a wide range of experimental conditions, particularly those most pertinent to urolithiasis [4–11]. Special care was taken to demonstrate the consistency of the equilibrium constants obtained in our laboratory with other thermodynamic quantities. Our constants were then incorporated in databases of simulation programs that permit solubility calculations in complex biological fluids. These simulations allow the judgement, from a physicochemical perspective, of various therapies of renal lithiasis suggested in literature.

EXPERIMENTAL

Solubility

All experiments were performed in thermostatted, all-glass, percolation-type solubility cells. The pH variation method (i.e., the variation of the initial H^+ concentration) was used. Constant ionic strength media were employed throughout to keep the activity coefficients of the reacting species essentially constant. Thus, hydrogen ion concentrations (rather than activities) were measured potentiometrically and hereafter $p[H] = -\log \{[H^+]/\text{mol dm}^{-3}\}$ will be used instead of pH. The metal ion and organic anion concentrations were determined by AAS and UV spectrophotometry respectively. Further experimental details can be found in refs. 3–11.

Calorimetry

Dissolution enthalpies of uric acids and xanthine were measured using isoperibolic solution calorimeters. TRIS buffer solutions of appropriate pH were employed to increase solubility and to ensure a defined final state of predominantly hydrogenurate and hydrogenxanthinate respectively. Details are given in refs. 10 and 11.

MODELING

Computer simulations

Solubilities of calcium oxalate hydrates, calcium and magnesium phosphates were modeled using the Joint Expert Speciation System (JESS) package of computer programs [12–14]. In these simulations, all possible complexes were considered whose formation constants were taken from the JESS thermodynamic database. In the present work, the urine model originally developed by Grases *et al*. [15] was extended significantly. Compared to the model used in our previous review [3], the present one has a considerable increase in the number of species (280), reactions (380), and thermodynamic quantities (some 7200, mainly equilibrium constants but also standard potentials, Gibbs energies, enthalpies, and heat capacities).

In the case of uric acid and urates, cystine, and xanthine, sophisticated simulation programs are not necessary since there is only a small number of equilibrium constants required to model the solubility in a great variety of salt solutions, including artificial urine.

Thermodynamic data

In our laboratory, solubility products (K_{s0}) were determined for the three calcium oxalate hydrates [4] and for sodium and ammonium hydrogenurates [5,8]. So-called intrinsic solubility constants (K_s) were measured for uric acid anhydrate and dihydrate [5], cystine [9], and xanthine [11]. In addition, first dissociation constants (K_1) of uric acid and xanthine were derived from solubility data [5,11]. All of these constants were measured at various temperatures.

Table 1 Solubility and first dissociation constants of xanthine, as derived from solubility measurements at I_c = 0.300 mol dm^{-3} NaCl [11]. The enthalpies of solution corresponding to the reaction $H_2Xan(s) \rightarrow H^+(aq) + HXan^-(aq)$ were measured calorimetrically [11].

t / °C	pK_s	pK_1	$\Delta_r H$ / kJ mol^{-1}
25	3.88 ± 0.02	7.43 ± 0.02	71.0 ± 1.3
37	3.69 ± 0.03	7.16 ± 0.03	

In order to check for thermodynamic consistency [16], enthalpies of solution for the three calcium oxalate hydrates ([4], this work), uric acid anhydrate and dihydrate [10], and xanthine [11] were also determined calorimetrically. In the case of cystine, solubilities were compared to values calculated using protonation constants reported in literature.

XANTHINE, URIC ACID, AND URATES

Xanthine ($C_5H_4N_4O_2$) and uric acid ($C_5H_4N_4O_3$) are intermediate and final products, respectively, of the purine metabolism in humans. Defects in the enzymatic activity of xanthine oxidase may lead to an accumulation of either substance in urine [17].

The solubilities of these substances exhibit considerable dependencies on p[H] and temperature [5–8,10,11], see Figs. 1–4. In the p[H] range of urine, three equilibrium constants are required to calculate the solubility. In (1–3), uric acid anhydrate, the metastable uric acid dihydrate, and ammonium hydrogenurate were taken as examples:

$$H_2U(\cdot 2H_2O)(s) \rightarrow H_2U(aq)\ (+\ 2\ H_2O) \qquad K_s \tag{1}$$

$$H_2U(aq) \rightarrow H^+(aq) + HU^-(aq) \qquad K_1 \tag{2}$$

$$NH_4HU(s) \rightarrow NH_4^+(aq) + HU^-(aq) \qquad K_{s0} \tag{3}$$

Reactions (1,2) analogous to anhydrous uric acid hold true for xanthine. Solubility products of sparingly soluble hydrogenxanthinates, analogous to (3), have not been reported. In both cases (uric acid and xanthine), dissociation of the second proton occurs at p[H] values far exceeding the physiologically important range. Thus, the equilibrium constants (1–3) can be calculated from least-squares analyses of solubility data.

The thermodynamic quantities describing the solubility of xanthine, as obtained in our laboratory [11], are presented in Table 1 and Figs. 1 and 2. The enthalpy of solution calculated from the experimentally determined solubility and first dissociation constants is $\Delta_r H$ = 67.9 kJ mol^{-1}. This value is in excellent agreement with the calorimetric value $\Delta_r H$ = (71.0 ± 1.3) kJ mol^{-1} [11]. Figure 2 reflects very well the thermodynamic consistency of our experimentally determined data obtained from two different methods, solubility and calorimetry. For comparison, literature data [18] are also shown in Figs. 1 and 2. Lister and Caldbick [18] reported the solubility data of xanthine in some buffer solutions from which we obtained the solubility constants of pK_s = 3.61 ± 0.03 and 3.28 ± 0.02 at 21 and 37 °C respectively, and the first dissociation constants of pK_1 = 7.39 ± 0.03 and 7.51 ± 0.02 at 21 and 37 °C respectively [3,11]. The enthalpy of solution calculated from these data is $\Delta_r H$ = 22.9 kJ mol^{-1}, which differs significantly from our calorimetric value [11]. The experimental technique applied, which led to a considerably higher solubility, and the unreasonable decrease of the deprotonation constants with temperature indicate that the authors of ref. 18 actually investigated supersaturated solutions.

For the modeling of uric acid and hydrogenurate solubilities, a thermodynamically consistent set of equilibrium constants and calorimetric data has also been obtained in our laboratory (see Tables 2 and 3 and Figs. 3 and 4) [5,10]. Moreover, our experimental results have proven that in the ionic

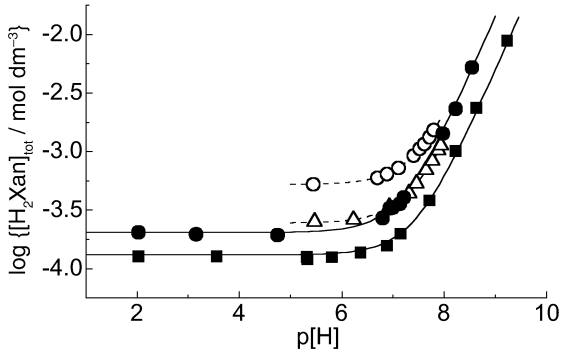

Fig. 1 Solubility of xanthine at 25 and 37 °C (solid squares and circles respectively, [11]), and at 21 and 37 °C (open triangles and circles respectively, [18]).

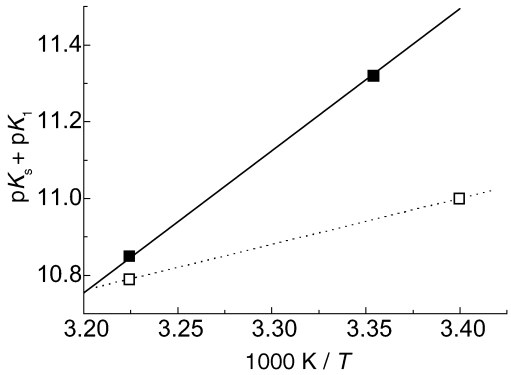

Fig. 2 Consistency of thermodynamic data for xanthine. Solid squares: equilibrium constants obtained from solubility measurements; solid line corresponds to the calorimetrically determined enthalpy of solution [11]. Open symbols: literature data [18]; dotted line is the calculated slope of these data.

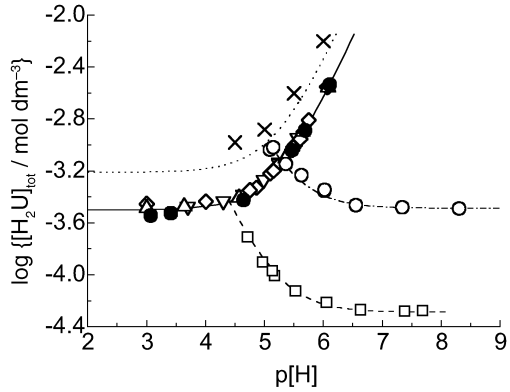

Fig. 3 Solubility of uric acids and urates at 37 °C. Uric acid anhydrate: open down triangles, in Standard Reference Artificial Urine [6]; open up triangles, in 0.300 mol dm^{-3} NaCl + 0.050 mol dm^{-3} creatinine [6]; open diamonds, in 0.300 mol dm^{-3} NaCl + 0.300 mol dm^{-3} urea [6]; solid circles, in 0.300 mol dm^{-3} LiCl [7]. Crosses [22] obviously correspond to uric acid dihydrate. Sodium hydrogenurate monohydrate: open circles, in 0.150 mol dm^{-3} NaCl [5]; ammonium hydrogenurate: open squares, in 0.300 mol dm^{-3} NH_4Cl [8]. Curves were calculated using the equilibrium constants given in Tables 2 and 3.

Table 2 Solubility and first dissociation constants of uric acid, valid for various salt solutions and artificial urine in the ionic strength range from 0.15 to 0.30 mol dm^{-3}, as derived from solubility measurements on uric acid anhydrate (H_2U) and dihydrate ($H_2U{\cdot}2H_2O$) [5,6]. The enthalpies of solution corresponding to the reactions $H_2U(\cdot 2H_2O)(s) \rightarrow H^+(aq) + HU^-(aq)$ (+ 2 H_2O) were measured calorimetrically [10].

t / °C	pK_s ($H_2U{\cdot}2H_2O$)	pK_s (H_2U)	pK_1	$\Delta_r H$ / kJ mol^{-1}
25	3.55 ± 0.01	3.76 ± 0.03	5.26 ± 0.04	Anhydrate: 56.3 ± 0.4
32	3.35 ± 0.01	3.63 ± 0.01	5.21 ± 0.01	
37	3.21 ± 0.01	3.49 ± 0.03	5.19 ± 0.04	Dihydrate: 64.5 ± 0.2
42	3.08 ± 0.01	3.41 ± 0.01	5.13 ± 0.01	

strength range $0.15 \leq I_c/\text{mol dm}^{-3} \leq 0.30$, the solubility of uric acid neither depends on the nature and concentration of various inorganic components of urine nor on the presence of organic substances like urea and creatinine [6]. Thus, the same solubility as in the other salt solutions was also found [6] in so-called Standard Reference Artificial Urine, whose composition is given in ref. 21.

At 37 °C, the solubility of the metastable uric acid dihydrate exceeds that of the anhydrate by a factor of about two. The solubility data obtained by Sperling and de Vries [22], which were also given in ref. 23, obviously correspond to the dihydrate (Fig. 3). In fact, the uric acid samples of ref. 22 were precipitated by acidification of real urine but were not characterized. It has been reported that under these conditions of high supersaturation, uric acid dihydrate is formed [2].

Increasing the urinary pH by appropriate medication has been successfully used in the treatment of uric acid lithiasis. Excessive alkalization, however, may cause precipitation of sodium or ammonium hydrogenurates [24]. The latter substance has also been found together with struvite in infectious stones caused by urea-splitting bacteria. Recently, it has been reported that *in vitro*, uric acid stones dissolve better in lithium carbonate than in sodium or potassium (hydrogen)carbonate solutions; this behavior was attributed to a litholytic effect of lithium ions [25]. Our measurements show, however, that uric acid has the same solubility in lithium and sodium chloride solutions (Fig. 3). The increased solubility of uric acid in lithium carbonate solutions is obviously due to a higher pH and to the fact that lithium hydrogenurate has a higher solubility than the corresponding sodium and potassium salts (Table 3). The latter compounds may form sparingly soluble precipitates on the surface of the uric acid calculus and prevent further dissolution even if the pH is increased (see Fig. 3).

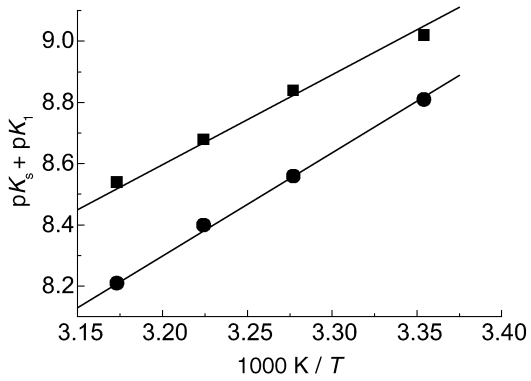

Fig. 4 Consistency of thermodynamic data for uric acid anhydrate (squares) and dihydrate (circles). Symbols are derived from solubility measurements; the slopes of the lines were obtained calorimetrically.

Table 3 Solubility products of hydrogenurates.

t / °C	pK_{s0} [a] [5] ($NaHU \cdot H_2O$)	pK_{s0} [b] [8] (NH_4HU)	pK_{s0} [c] [19] [$Ca(HU)_2 \cdot 6H_2O$]	pK_{s0} [d] [20] (KHU)	pK_{s0} [d] [20] ($LiHU \cdot 1.5H_2O$)
15			10.12 ± 0.07		
25	4.61 ± 0.01	5.18 ± 0.03	9.81 ± 0.09		
32	4.43 ± 0.01				
37	4.31 ± 0.01	4.80 ± 0.01	9.28 ± 0.04	3.85 ± 0.05	2.75 ± 0.05
42	4.20 ± 0.01				
45			9.01 ± 0.03		

[a] I_c = 0.150 mol dm^{-3} NaCl; [b] I_c = 0.300 mol dm^{-3} NH_4Cl; [c] I = 0; [d] I_c = 0.15 mol dm^{-3} LiCl.

CYSTINE

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 dm^{-3}. Owing to 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 dm^{-3} which results in the formation of calculi that can block the renal tubes [26].

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

$$n\ H^+ + Cis^{2-} \rightarrow H_nCis^{n-2} \qquad \beta_{01n} = [H_nCis^{n-2}]\ [H^+]^{-n}\ [Cis^{2-}]^{-1}. \tag{4}$$

In (4), n = 1.....4. and β_{01n} denote the corresponding protonation constants. The formally uncharged species $H_2Cis^{\pm}$ (which is actually a zwitterion) has the lowest solubility, i.e.,

$$H_2Cis(s) \rightarrow H_2Cis^{\pm}(aq) \tag{5}$$

and the corresponding (intrinsic) solubility constant is denoted as K_s. Thus, the solubility of cystine can be calculated as analytical function of p[H] using five equilibrium constants (Table 4 and Fig. 5; see ref. 9). Although the protonation constants of cystine were measured at I_c = 0.15 mol dm^{-3}, they reproduced solubility data measured at I_c = 0.30 mol dm^{-3} very well.

The solubility of cystine in oxalate-free artificial urine was the same as in 0.30 mol dm^{-3} NaCl [9]. However, owing to the precipitation of phosphates from artificial urine at higher p[H], data were

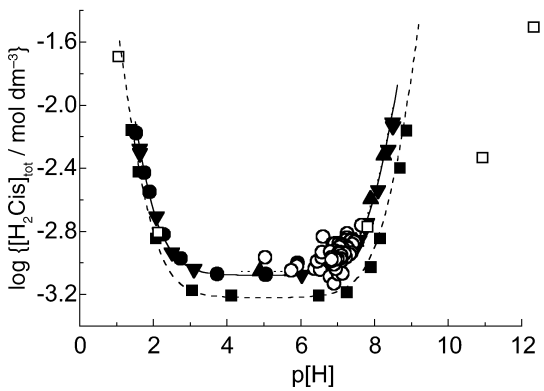

Fig. 5 Solubility of L-cystine. At 25 °C: solid squares, 0.30 mol dm^{-3} NaCl [9]; open squares, 0.5 mol dm^{-3} NaCl [27]; dashed line, calculated using equilibrium constants of Table 4. At 37 °C: solid circles, oxalate-free artificial urine; up-triangles, phosphate- and oxalate-free artificial urine; down-triangles, 0.30 mol dm^{-3} NaCl [9]; open circles, real urine [28]; solid line, calculated using equilibrium constants of Table 4; dotted line, calculated with JESS for phosphate- and oxalate-free artificial urine [9].

Table 4 Solubility and protonation constants for L-cystine used for solubility simulations in 0.30 mol dm^{-3} NaCl and oxalate-free artificial urine at 25 and 37 °C.

t / °C	$-\log K_s$ [a]	$\log \beta_{011}$	$\log \beta_{012}$	$\log \beta_{013}$	$\log \beta_{014}$
25	3.22 ± 0.01	8.80 [b]	16.83 [b]	18.41 [c]	20.03 [c]
37	3.08 ± 0.01	8.604 ± 0.003 [c]	16.356 ± 0.004 [c]	18.41 ± 0.01 [c]	20.03 ± 0.02 [c]

[a] I_c = 0.30 mol dm^{-3} NaCl [9]; [b] t = 20 °C, I_c = 0.15 mol dm^{-3} $NaClO_4$ [29]; [c] t = 37 °C, I_c = 0.15 mol dm^{-3} NaCl [30].

only collected at p[H] < 5.0, while in phosphate-free artificial urine, cystine solubilities were measured up to p[H] = 8.2. In the latter, a slightly higher solubility constant (0.88 mmol dm^{-3}) was found, which is most likely due to complex formation of cystine with Ca^{2+} and Mg^{2+}, as was also confirmed by computer simulations with JESS [9]. In normal artificial urine, on the other hand, alkaline earth ions are complexed to phosphate. However, a significant dependence of the intrinsic solubility on the nature and concentration of various inorganic salts was reported in ref. 31; so more experimental work on this topic is certainly needed. Recent literature data for 0.5 mol dm^{-3} NaCl [27] agree with our values at low p[H] but show a systematic deviation at high p[H] (Fig. 5).

Cystine solubilities in real urine [28] agree well with our results obtained in synthetic solutions [9]. Therefore, the equilibrium constants in Table 4 permit reasonable cystine solubility estimates for urine. It should be emphasized again that the excellent agreement between our measured solubility data and values calculated with independently determined protonation constants supports the reliability of both data sets.

For the treatment of cystine lithiasis, potassium citrate has been used to increase the urinary pH and thus the cystine solubility [32]. However, at higher pH, the risk of calcium phosphate calculi formation becomes higher. This problem would be particularly serious if a recent recommendation [33] to use THAM (tris-(hydroxymethylene)-aminomethane) buffer at pH = 10 for *in vivo* cystine chemolysis were applied. Even at pH ≈ 7, significant HAP precipitation is not only predicted by computer simulations [15] but also observed experimentally [15,34].

CALCIUM OXALATES

In contrast to the substances discussed above, the solubility of COM, the major component of oxalate calculi, is almost p[H] independent in the urinary p[H] range, as was shown by computer simulations and confirmed experimentally [4]. However, the calcium oxalate solubility strongly depends on the concentration of ions that form complexes with calcium or oxalate, such as citrate or magnesium ions [3]. It was demonstrated that our urine model [3,4] permits reliable solubility calculations by taking all of these complexes into account.

Owing to its importance for renal lithiasis, the solubility products of COM, COD, and COT (calcium oxalate trihydrate) have been determined frequently. Nevertheless, the reliability of the early literature data is rather unsatisfactory; these include the values reported in refs. 35 and 36, which, based on the number of citations, have obviously been regarded as reliable. To clarify this point, a simple thermodynamic consistency test is applied according to a knowledge existing for some 120 years [37]. This rule states that the enthalpies of dissolution become progressively more endothermic with extent of hydration, since the enthalpies of dehydration, corresponding to e.g. COT → COD + H_2O(aq) or COD → COM + H_2O(aq) are always positive [38]. The enthalpies of solution derived from our solubility products [4] given in Table 5 obey this rule (Table 6) while those of refs. 35 and 36 do not. Moreover, our data also agree very well with values determined calorimetrically (see Fig. 6). It should also be noted here that our thermodynamic quantities for uric acid anhydrate and dihydrate pass this consistency test as well (see Table 2 and Fig. 4).

The enthalpy of dissolution of COM was determined calorimetrically as shown in Table 7; a similar determination was carried out for COT with the required modification of reactions (A) and (D). Due to the low solubility in water, the enthalpy of dissolution of COM and COT was determined in 0.10 mol dm^{-3} HCl. Thus, a thermodynamic cycle was employed at constant ionic strength (I_c = 0.10 mol dm^{-3}) to obtain the enthalpy of the desired reaction (Table 7). If $\Delta_r H^I$ is the enthalpy of reaction valid for ionic strength I, $\Delta_r H^I = \Delta_r H^\circ + \nu\, L_2^I + x\, L_1^I$, where ν and x are the moles of ions (ν = 2) and water (x = 1, 2, or 3) resulting from the dissolution of one mole of the solid substance, and L_2^I and L_1^I are the relative partial molar enthalpies of solute and solvent at ionic strength I, respectively. For the ionic strength used in the present calorimetric study, L_1^I can certainly be neglected and 2 L_2^I for a 2:2 electrolyte is estimated to 2.1 ± 0.5 kJ mol^{-1}.

Urolithiasis is controlled by thermodynamic and kinetic factors either alone or in combination. The reason is that the crystallization potential of urine is related not only to the concentration of any particular compound but also to the presence or absence of others, such as complexing agents, inhibitors, or promoters of the crystallization of the compound in question. Crystallization inhibitors frequently cause the actual concentration products to exceed the corresponding solubility products. In this way, urine is supersaturated (metastable) with respect to some substances, and kinetic factors play an important role to prevent or delay the precipitation of the substances. As supersaturation increases, a threshold is reached at which urine can hold no more salt in solution and kinetic factors are no longer effective. A correlation between the COM supersaturation in real urine samples and the results of a simple clinical test for urinary lithogen risk (ULR) was recently reported in an application of computer modeling in urolithiasis research [42]. This correlation leads to an establishment of kinetic and thermodynamic factors contributing to stone formation.

Table 5 Solubility products of the three calcium oxalate hydrates valid for I = 0 [4].

t / °C	-log K_{s0}		
	COM	COD	COT
20	8.84 ± 0.02	8.42 ± 0.02	8.33 ± 0.01
25	8.77 ± 0.01	8.34 ± 0.02	8.24 ± 0.01
30	8.71 ± 0.01	8.26 ± 0.03	8.12 ± 0.02
37	8.65 ± 0.03	8.17 ± 0.03	8.02 ± 0.02
40	8.62 ± 0.02	8.13 ± 0.04	7.97 ± 0.02

Table 6 Dissolution enthalpies of calcium oxalate hydrates, corresponding to the reaction $CaC_2O_4 . n\ H_2O(s) \rightarrow Ca^{2+} + C_2O_4^{2-} + n\ H_2O$ (I = 0). The values obtained from precipitation reactions were multiplied by (–1).

	$\Delta_r H^\circ$/ kJ mol^{-1}		
	calorimetric	ln K_{s0} vs. 1/T	
		this work [4]	literature [35,36]
COM	20.8 ± 0.8[a]	18.9 ± 0.9	17.3 ± 1.3
	19.8 ± 1.2[b]		
	16.7 ± 0.6[c]		
COD	24.3 ± 1.4[d]	25.6 ± 0.5	14.1 ± 1.3
COT	30.5 ± 2.3[e]	32.1 ± 1.2	25.1 ± 0.7

[a]dissolution, 25 °C [39]; [b]precipitation, 25 °C [40]; [c]precipitation, 37 °C [40]; [d]precipitation, 25 °C [41]; [e]dissolution, 25 °C (this work, 4 measurements).

Table 7 Dissolution enthalpy of COM at 298.15 K.

Reaction	$\Delta_r H$ / kJ mol^{-1}
(A) $CaC_2O_4{\cdot}H_2O(s) + 2\ H^+ \rightarrow Ca^{2+} + H_2C_2O_4 + H_2O$ (I_c = 0.10 mol dm^{-3} HCl)	30.3 ± 0.6[a]
(B) $2\ Na^+ + H_2C_2O_4 \rightarrow Na_2C_2O_4(s) + 2\ H^+$ (I_c = 0.10 mol dm^{-3} HCl)	−25.5 ± 0.3[b]
(C) $Na_2C_2O_4(s) \rightarrow 2\ Na^+ + C_2O_4^{2-}$ (I_c = 0.10 mol dm^{-3} $(CH_3)_4NCl$)	18.1 ± 0.1[c]
$-2\ L_2$ (I_c = 0.10 mol dm^{-3})	−2.1 ± 0.5
(D) $CaC_2O_4{\cdot}H_2O(s) \rightarrow Ca^{2+} + C_2O_4^{2-} + H_2O$ (I = 0)	20.8 ± 0.8

[a]13 measurements; [b]9 measurements; [c]4 measurements.

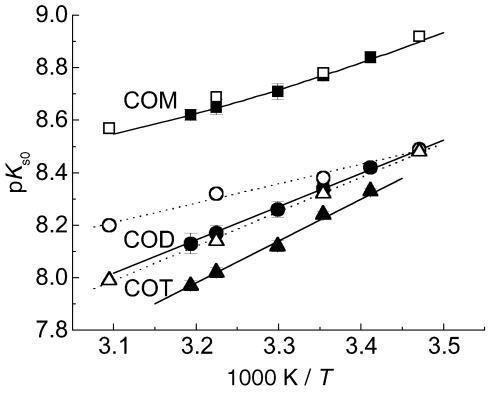

Fig. 6 Thermodynamic consistency of calcium oxalate data. Solid symbols [4], open symbols [35,36]. Squares: COM; circles: COD; triangles: COT. Solid lines correspond to calorimetrically determined enthalpies of solution, dashed lines were obtained by linear regression (Table 6).

The ULR test results indicate the positive or negative risk of urinary calcium stone formation and the absorbance A = 0.3 has been established as the border line between normal and lithogenic urines [43]. Results of this test and the supersaturation values of COM obtained from the urines of stone-formers and healthy people are shown Fig. 7. It can be seen clearly that almost all human urines are supersaturated with respect to COM and as expected, urines of most healthy people give negative ULR test results. Six samples from this group give positive results, and their log S values were employed to establish a region called "kinetic threshold" which was found to be $0.40 < \log S < 0.57$ for the crystallization of COM. Five cases corresponding to kinetic and/or thermodynamic control of urinary stone formation were defined, and they are presented as regions I, II, III, IV, and V in Fig. 7.

I. In region I, $0 < \log S < 0.57$ and $A < 0.3$ mean that the kinetic factors effectively keep calcium ions in solution. The presence of most of the data obtained from healthy people implies the absence of heterogeneous nucleants and/or the presence of some natural crystallization inhibitors in urine. The existence of about 1/3 of the data obtained from the stone-formers indicates that although having normal urine, these patients suffer some abnormal renal morphoanatomy, e.g., papillary necrosis or strongly disordered urodynamic conditions [43].

II. In region II, $0 < \log S < 0.40$ and $A > 0.3$, COM crystallized from all urine samples. Data from stone-formers are found exclusively which indicates the absence of crystallization inhibitors in their urines as well as abnormal morphoanatomy of their kidneys.

III. Region III or the kinetic threshold consists of data with $0.40 < \log S < 0.57$ and $A > 0.3$. The width of this region depends on the uncertainties involved in the analyses of the samples. Six lithogen-

ic samples from the healthy group found in this region signal that these people have excellent renal morphology so no stones were formed in their urinary tracts though the salt crystallized in the ULR test [43].

IV. In region IV, log $S > 0.57$ and $A > 0.3$, thermodynamic factors are in absolute control and kinetic factors do not have any effects even if there were inhibitors present in the tested urines. Obviously, the supersaturation values are abnormally high so the only result here is the crystallization of calcium oxalate. This is confirmed by the fact that only data from active stone-formers with positive ULR test results are found in this region.

V. Region V (log $S > 0.57$ and $A < 0.3$) does not contain any data since inhibitors are no longer effective at such high supersaturation.

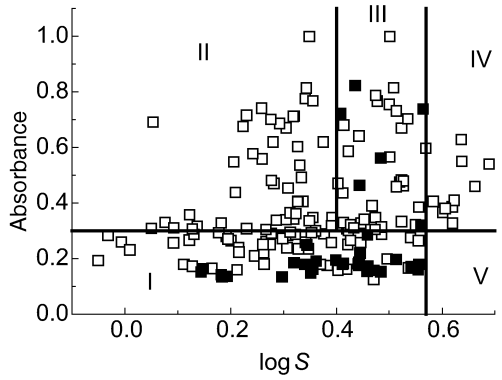

Fig. 7 Correlation between ULR test and COM supersaturation [42]. Open symbols: stone-formers and solid symbols: healthy people.

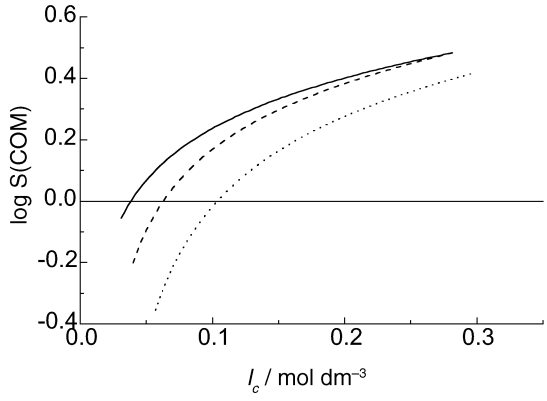

Fig. 8 Effect of 1:10 dilution on supersaturation of COM in standard reference artificial urine, as simulated with JESS. Solid line: all components are diluted with pure water; dashed line: the original $MgSO_4$ concentration is kept constant and all other components are diluted; dotted line: three times the original $MgSO_4$ concentration is kept constant and all other components are diluted.

This work shows that a failure in kinetic inhibition and/or the presence of heterogeneous nucleants are the main reasons for COM crystal development in urine. Nevertheless, abnormally high supersaturation values can lead to calculi formation since there are about 10% of data obtained from the stone-former group and none from the healthy group found in region IV. Consequently, for urines belonging to this region IV, it would be necessary to decrease the supersaturation prior to the administration of crystallization inhibitors, such as phytic acid, that are used as a pharmacological treatment of stone disease [1].

A frequent recommendation to calcium oxalate stone-formers is to consume a large quantity of liquid. However, our computer simulations demonstrate that this advice is only physicochemically meaningful for some certain fluids. The effect of dilution with pure water on the p[H] and COM supersaturation of standard reference artificial urine is shown in Fig. 8. Since only an unrealistically high 1:10 dilution results in an unsaturated solution, pure water does not seem to effect a significant reduction of the supersaturation. More likely, it only contributes to a faster flow of urine through kidney cavities and thus a removal of sediments. However, mineral waters containing complexing agents like magnesium can certainly reduce the supersaturation to a higher extent (Fig. 8). It was also concluded from the results of a recent study on 85 human subjects [44] that such mineral waters reduce the risk of calcium oxalate stone formation.

CONCLUSIONS

This paper summarizes solubility data for various substances found in kidney stones that have been reported by our group. The data have been measured for wide ranges of temperature, p[H], and concentrations of inorganic salts and organic substances, including conditions most pertinent to urolithiasis. The thermodynamic quantities derived from these solubility data have been proven to be very reliable by

- the thermodynamic consistency of the results obtained from different experimental techniques and/or
- the excellent agreement between experimental and calculated solubilities.

These thermodynamic quantities were then used in computer simulations that lead to some judgement and suggestions regarding to the cause, prevention, and treatment of stone diseases.

- Treatment of uric acid, cystine and, to a lesser extent, xanthine lithiasis by increasing urinary pH is effective. However, excessive alkalization may lead to the formation of calcium phosphate calculi and also sodium or ammonium hydrogenurate precipitation in the case of elevated uric acid concentrations.
- The formation of COM calculi is mainly caused by a failure in kinetic inhibition and/or the presence of heterogeneous nucleants, thus treatment using crystallization inhibitors such as phytic acid has been suggested [1]. Nevertheless, in a minor group of stone-formers, it is necessary to lower abnormally high COM supersaturation before crystallization inhibitor therapy is applied. Our computer simulations also show that a large consumption of pure water is not a suitable treatment of COM calculi because it does not significantly reduce the supersaturation of COM in urine. A high intake of fluids containing complexing agents such as magnesium would be better for the reduction of supersaturation.

Recently, so-called nanobacteria were found in kidney stones and have been claimed to induce calcifications in urine that eventually lead to urolithiasis [45]. Although this topic is still under discussion, it has been suggested that a simple tetracycline therapy may help people who suffer from chronic stone formation.

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