1 Solubility Phenomena Related to Normal and Pathological Biomineralization Processes

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1.1 INTRODUCTION

Biomineralization, which refers to the complex processes by which organisms form minerals, is frequently associated with a high degree of regulation on different hierarchical levels [1,2]. ‘Biologically controlled’ mineralization, in which extra-, inter- and intracellular activities direct the nucleation, growth and morphology of minerals that form ‘normal’ biomaterials such as bone and teeth [1,2], is fundamentally different from ‘biologically induced’ mineralization, which occurs as a result of interactions between biological activity (affecting e.g. the pH and composition of secretion products) and the environment [1,2]. Since there is little control of the biological system over the type and habit of minerals deposited, these vary as greatly as the environments in which they form and are often poorly defined, heterogeneous and porous [1,2]. Biologically induced mineralization is commonly associated with various bacterial activities and with epicellular mineralization in marine environments, occasionally leading to the complete encrustation of organisms, that sink subsequently and form sediments [1,2]. However, its characteristic features are also typical for uncontrolled ‘pathological’ crystallization resulting in painful or even life threatening conditions such as calculi formation (renal, biliary, pancreatic or sublingual), development of gout or arteriosclerosis, tissue calcification associated with cancer, etc.

In any event, solubility phenomena, i.e. dissolution and precipitation reactions, are fundamental to all biologically controlled or induced mineralization. Solubility phenomena in multicomponent electrolyte solutions also control numerous other ‘real-life’ natural or industrial processes. These include interactions in the hydrogeological cycle such as hydrothermal mineral formation, weathering and aerosol formation. The recent growth in biogeochemistry stresses the interrelations between biological and abiotic mineralization in the understanding of the past
and future evolution of the Earth. Solubility phenomena are furthermore relevant to procedures for preparing, separating and purifying chemicals and (biomimetic) materials industrially or in the laboratory. The study of solubility phenomena also serves to elucidate the mechanisms of unwanted precipitation and develop methods for its prevention, both industrially (scaling) and biologically (pathological mineralization), thus highlighting important analogies between two apparently distinct areas.

The kinetics of dissolution and precipitation has frequently been studied for both biological and industrial systems. The rate equations used to interpret such kinetic data are commonly defined in terms of under- and supersaturation respectively [3], which requires accurate solubility data for the solid substances at the pertinent conditions. However, in some studies, such as in a fundamental investigation of calcite growth kinetics [4], equilibrium solubilities have been derived from the very same rate data they ought to rationalize, which might lead to a correlation between these solubility values and the parameters of the rate model so obtained. It is thus imperative to employ the most accurate solubility data that are available from independent solubility studies, as reviewed for calcite [5].

For the calculation of biomineral solubilities in complex biological fluids, a suitable model for aqueous activity coefficients together with stability constants of the various complex species formed in the solution as well as the solubility constants of the solid phases are required. Therefore it is important to obtain reliable information about these constants from accurate physicochemical measurements. These methods, together with techniques to measure the kinetics of precipitation and dissolution, will be briefly outlined in the following section.

1.2 EXPERIMENTAL METHODS

1.2.1 SOLUBILITY MEASUREMENTS

The majority of normal and pathological biominerals formed in humans are sparingly soluble electrolytes with basic anions (i.e. anions that can be protonated), such as phosphates, carbonates, oxalates, urates, etc. Their solubility thus depends (often strongly) on pH and is in general decreasing as the pH increases. A notable exception is uric acid, whose solubility increases with pH.

Reliable techniques to measure solubilities of sparingly soluble electrolytes with basic anions were reviewed recently [6]. The recommended method consists of equilibrating the solid phase and the solution in thermostatted, all glass, percolation type solubility cells equipped with glass and reference electrodes [7–14]. The pH variation method (i.e. the systematic variation of the initial $H^+$ concentration from run to run) 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 $p[H]$, defined as $p[H] = -\log([H^+]/\text{mol dm}^{-3})$
will frequently be used instead of pH. The metal ion and organic anion concentrations were determined by standard analytical methods, such as AAS and UV spectrophotometry respectively.

1.2.2 SOLUTION CALORIMETRY

The direct measurement of enthalpies of solution of solid phases provides important information on thermodynamic consistency by comparison with the enthalpy values derived from the temperature dependence of solubility constants. For instance, isoperibolic solution calorimeters were employed to measure the dissolution enthalpies of the calcium oxalate hydrates [12], uric acid anhydrate and dihydrate [15] and xanthine [14]. In the first case, a thermodynamic cycle was employed to obtain the dissolution enthalpy at ionic strength zero [12]. In the other two studies, the ionic strengths were adjusted to the values employed at the solubility measurements and TRIS buffer solutions of appropriate pH were used to increase the solubility and to ensure a defined final state of predominantly hydrogenurate and hydrogenxanthinate respectively [14,15]. In all cases, it was found that temperature-dependent solubility constants and calorimetrically measured enthalpies of solution were thermodynamically consistent [12,14,15].

Solution calorimetry has proved very useful for studying the energetics of iron oxide/oxyhydroxide and other nanoparticles [16]. Measurements have been performed either near room temperature (with an acid as the solvent) or at 700 to 800°C in an oxide melt. Both nano- and bulk materials of the same composition were reacted under the same conditions. The enthalpy difference between the two measurements is related to the difference in the surface energies of bulk and nanomaterial. Such differences are not only useful for the evaluation of the relationship between particle size and solubility, they may also serve to stabilize, in the nanoregime, polymorphs that are not stable in the bulk [16,17].

1.2.3 KINETIC MEASUREMENTS

Studies of the dissolution and crystallization kinetics of solids first require the preparation of metastable under- and supersaturated solutions, followed by appropriate measurement of the respective reaction rates. One of the earliest experimental approaches was that of ‘free drift’ in which the rates are obtained by measuring concentration changes as function of time [18,19]. To keep the thermodynamic driving forces (i.e. the activities of the reacting ions) constant, the ‘constant composition’ (CC) method pioneered by Nancollas [20] is widely used nowadays for the determination of dissolution and growth kinetics [21]. This technique can also mimic biomineralization processes during which constant ionic concentrations are regulated by homeostasis. To simulate, in addition, the slow crystallization rates
commonly prevailing in vivo, the CC method has been combined with a double-diffusion (DD) technique [22]. This ‘CCDD method’ has been applied to simulated body fluids resulting in the growth of carbonate apatites very similar to biological specimens [22].

As there are close relationships among the observed solubilities, the kinetics of dissolution and crystallization, and the interfacial tensions between the solid phases and their solutions, the accurate measurement of the latter has received considerable attention [21,23].

1.3 THERMODYNAMIC MODELING OF BIOLOGICAL SYSTEMS

1.3.1 INTRODUCTION

D.R. Williams [24] was one of the first who proposed and systematically pursued the idea that chemical equilibria in biological systems can be studied by the very same, well established experimental and computational methods that have been used in solution chemistry for a long time. Once the formation constants of all (or at least the most important) metal–ligand complexes have been characterised in vitro either experimentally (e.g. by potentiometric titration) or by appropriate estimation methods, the so-called speciation (i.e. the distribution of the metal among its low molecular weight complexes) can be calculated. This can e.g. be achieved by solving a system of equations derived from the law of mass action using suitable mass balance equations as a constraint. It is then assumed that the speciation established in this way reflects the metal–ligand distribution in the biological system in vivo, which in turn permits conclusions to be made about metal toxicity and bioavailability, metabolism, mobilization and immobilization, transport, deposition, etc. It has to be understood, however, that due to the complexity of some biological fluids containing a large number of N-, O- and S-ligands, the species distribution of a metal in vivo among the low-molecular-weight ligands such as amino and organic acids is never completely known. Nevertheless, this approach has proved successful for many applications, some of which are outlined below while others, related to bioinorganic chemistry, have been reviewed recently [25].

1.3.2 CHEMICAL SPECIATION, BLOOD PLASMA MODELS AND CHELATION THERAPY

Among the most prominent applications of (quasi)equilibrium calculations for biological systems are computer simulations of metal ion distributions amongst the low molecular weight ligands in blood plasma. These ‘blood plasma models’ were pioneered by Perrin [26] and were further developed in various laboratories, as reviewed by May [27]. The term quasiequilibrium indicates that the system of metal ions and organic ligands does not attain a stable thermodynamic equilibrium
state, which would imply, for instance, that the ligands decompose when conditions are oxidizing. One of the most sophisticated computer codes for this kind of simulations is the JESS (Joint Expert Speciation System) package of computer programs [28–31], which contains an extensive thermodynamic database. JESS can handle equilibrium calculations involving thousands of species and is also able to take redox equilibria and kinetic constraints [32] into account.

Data base improvements, e.g. due to the measurement of new complex formation constants that were not known before, have changed likely species distributions in blood plasma dramatically. For instance, older models have indicated that Fe(III) and Cu(II) complexes predominate, while modern computer simulations which include redox equilibria suggest that these two metals complexed by low molecular weight ligands are overwhelmingly present in blood plasma as Fe(II) and Cu(I) species [33,34 and references therein].

Due to the binding of metals to proteins, blood plasma models are unable to provide absolute species concentrations in vivo, however, they can give valuable information on the competitiveness of low molecular weight ligands for metal ions in solution, which is expressed as relative (percentage) species distribution. Thus, trends in such species distributions can be established when the homeostatically regulated metal or ligand concentrations become imbalanced. Disruptions of normal metal homeostasis may lead to conditions such as thalassemia or Wilson’s disease [35–40] (Cu and Fe overload respectively, leading to the depositions of corresponding solids) or to Alzheimer’s disease, with an associated deposition of solids in the brain, including an alleged Cu$^{2+}$ induced aggregation of β-amyloid (Aβ plaques) [41] and nanoscale magnetic biominerals such as magnetite and maghemite [42]. These deposits result in Fe [43] and Cu [44] redox cycling [45], i.e. a metalloenzyme like activity [46], leading to oxidative stress by continuous H$_2$O$_2$ generation which probably accelerates the degeneration of brain tissue [47].

Metal overload in humans can be treated by administering chelating agents that help to excrete the excess metal. Effective drugs for chelation therapy have often been identified by computer simulations [48]. For instance, the treatment of Wilson’s disease requires life long administration of an appropriate Cu chelator such as D-penicillamine, which was introduced half a century ago [49,50] and is still regarded as one of the most effective drugs [35,40], although alternatives have been recommended [51,52]. However, the study mentioned above [33], which used a newly determined set of formation constants for Cu(I) thioamino acid complexes, arrived at the conclusion that the mechanism of copper removal by penicillamine in vivo is unlikely to depend on complexation alone, in contrast to earlier simulations that apparently confirmed its therapeutic action [53]. Other Cu chelators have been shown to dissolve Aβ plaques in vitro [54] and in post-mortem brain tissue [55,56]. Recently, it has been suggested that D-penicillamine carried by nanoparticles (which had been found to be able to cross the blood–brain barrier) has the potential to prevent the Aβ accumulation in the brain observed in Alzheimer’s disease [57]. The development of agents that can selectively prevent transition metals from binding to the Aβ peptide without perturbing the action of other metal containing biomolecules
in the brain [58] and therapies that focus on intervening in the roles of metal ions in oxidative stress [59] are currently of high priority.

1.3.3 METAL SOLUBILITY AND TOXICITY

As many metals are biotransformed in humans to a limited extent, it is often the speciation before entering the body that determines toxicity, as is, for instance, the case for Ni, As or Hg compounds [60]. While some metals are completely inert biologically (e.g. Ta [61]), solubility may be a criterion for the toxicological assessment of others (nontoxic, sparingly soluble BaSO$_4$ vs toxic, soluble BaCl$_2$ as opposed to slightly toxic, soluble and hence easily excretable NiCl$_2$ vs carcinogenic, sparingly soluble Ni$_3$S$_2$ which becomes phagocytosed in particulate form and consequently leads to very high intercellular Ni concentrations [62]). Moreover, it is often important to distinguish between organic and inorganic species of the metal, such as mercury [63]. Hg(II) undergoes bioalkylation in the environment and the resulting, highly toxic CH$_3$Hg$^+$ ion accumulates in fish and shellfish and is not metabolized further in the human body [64]. On the other hand, nontoxic arnosogars may also be ingested from seafood and form a significant fraction of the total blood As in humans. However, exposure to toxic inorganic As is indicated by the occurrence of mono- and dimethylarsenates in blood, which are the major As metabolites in humans [65].

For other substances, speciation may change dramatically in the body, e.g. upon the passage from the stomach (pH $\approx$ 1–2) to the intestine and subsequent absorption into the blood (pH $\approx$ 7.4). Under the latter conditions, metals like Fe or Al form very slightly soluble hydroxides, however, Al absorption (and hence toxicity) can be greatly increased, due to complexation, by coingestion with citrate or tartrate, both of which are commonly found in fruits and in industrial foods and drinks [66]. It has also been reported that ulcer patients (with associated excess acid production in the stomach) had increased serum and urine Al levels on an Al hydroxide absorption test, which indicates a dependence of gastrointestinal Al hydroxide absorption on gastric pH and hence implies a potential risk of prolonged administration of antacids containing Al [67].

Sutton and Burastero have studied the chemical speciation and solubility of Be [68] and U(VI) [69] in various body fluids by computer simulation, however, only the inorganic ligands have been taken into account. The authors found that the results vary markedly between each biological fluid due to differences in (inorganic) fluid composition, ionic strength and pH. It turned out that Be and U(VI) phosphate solubilities control the metal concentrations in many of the biological fluids studied. It is noteworthy that phosphate solubilities apparently control lead concentrations in natural environments [70] and are inversely correlated to divalent metal accumulation in freshwater bivalves [71]. Although the authors [68,69] claim that their results aid in understanding the metabolism and toxic effects of Be and U(VI) and can potentially be applied to chelation treatment of chronic beryllium
disease [72] and uranium overexposure, the effect of organic ligands on speciation and solubility still needs to be assessed. Duffield [73] has developed an equilibrium model of plutonium in blood plasma which takes into account the roles of the iron transport protein transferrin (which binds most of the Pu) and of the Pu-citrate complex for the distribution and excretion of Pu in mammalian systems (citrate is often considered as a model system for more complicated naturally occurring proteins). A comprehensive review [74] also emphasizes the importance of both Pu and uranyl binding to transferrin in blood plasma.

The effect of the oxidation state on toxicity is well known for chromium: whilst Cr(III) is believed to be essential, Cr(VI) is carcinogenic. Mercury can be detoxified by mercury resistant bacteria either by precipitation as HgS, by biomineralization of Hg as an insoluble Hg–S complex other than HgS (probably due to the aerobic production of a volatile thiol compound) or by enzymatic reduction to Hg\(^0\), which is volatile and diffuses freely out of the cell [75]. These mechanisms of mercury detoxification can be utilised for Hg bioremediation from waste water [75]. It has also been hypothesised that uranium can be immobilized by a biomineralization process through precipitation of microbially produced phosphate and U(VI) [76]. The important role of humic acids for metal binding and their relation to biomineralization has also been emphasised [77].

1.3.4 URINE MODELS

Urolithiasis, i.e. the formation of stones or calculi in the urinary tract, is not only a painful condition affecting some 10% of the population in industrialized countries but also causes annual costs to the health system in the order of millions of dollars estimated per 1000 patients that undergo treatment [78]. For decades, urolithiasis has arguably been the most research intensive sector of clinical and fundamental investigations into the cause, prevention and treatment of crystal deposition diseases in humans. However, it appears that a real breakthrough in this area is lacking as yet.

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; bile with respect to cholesterol; urine with respect to calcium oxalates and, depending on the pH, with regard to uric acid or calcium phosphates). The question, why pathological crystallization does not occur indiscriminately in all humans, has been discussed in terms of three main factors: besides (i) the supersaturation as a necessary condition, (ii) the presence of heterogeneous nucleants and (iii) a deficit of crystallization inhibitors play a crucial role in pathological situations [79].

For the calculation of urinary saturation with respect to stone-forming substances, the usefulness of modeling the ionic equilibria in urine by computer simulations has been clearly demonstrated [80–94]. As mentioned above, these models use the stability constants of the various complex species formed in urine as well as the
solubility constants of the stone-forming solid phases and thus permit simulations that allow the judgement, from a physicochemical perspective, of various therapies of renal lithiasis suggested in the literature.

In a comprehensive review, more than 20 different types of renal stones have been classified [95] (see also Chapter 2 in this book). These stones or calculi are 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. A sound knowledge of the solubilities of these substances is necessary to understand the cause, prevention and treatment of renal or bladder calculi. However, when the available experimentally determined solubility data of these substances were critically assessed they were found to be either sparse or in large disagreement [7]. Consequently, solubility measurements were performed in our laboratory at Leoben University (Austria) so as to provide reliable data for these compounds over wide ranges of experimental conditions, particularly those most pertinent to urolithiasis [8–14]. Special care was taken to demonstrate the consistency of these equilibrium constants with other thermodynamic, particularly calorimetric, quantities [12,14,15].

The solubilities of calcium oxalate hydrates were modeled using the JESS suite of computer programs [28–31] and the solubility constants \( \log K_{s0} \) determined in our laboratory, whereas in the case of calcium and magnesium phosphates literature values were used (see Chapter 3 in this book for a review). In these simulations, all possible complexes were considered whose formation constants were taken from the JESS thermodynamic database. Also, one of the in-built activity coefficient models of JESS was used (Davies equation). In the urine model developed by the authors [7,96], citrate and oxalate were considered besides the inorganic salts. Regarding the number of species (213), reactions (265) and thermodynamic quantities (more than 4000, including enthalpy, free energy and heat capacity values), this urine model was possibly the largest ever at that time.

Later [12], this urine model was extended significantly by 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 following sections, we present a discussion of solubility data and their application to the modeling of urinary saturation for all important components of renal calculi, except calcium and magnesium phosphates which are dealt with in Chapter 3 of this book. We start with uric acid, urates, cystine and xanthine, for which there are only a small number of equilibrium constants required to model the solubility in a great variety of salt solutions, including artificial urine, so that sophisticated simulation programs are not necessary.

**Uric Acid and Urates**

Uric acid \( \text{C}_4\text{H}_4\text{N}_4\text{O}_3 \) is the major end product of the purine metabolism in humans. Uric acid stones may be idiopathic or secondary to a systemic disease such as
gout, which is induced by the deposition of needle shaped sodium hydrogenurate monohydrate crystals in joints [97]. While hyperuricosuria and low urinary output are well known contributing factors, the most important risk factor for uric acid stone formation is persistently acidic urine [98–101]. The solubilities of uric acid and its salts exhibit considerable dependencies on pH and temperature [9–12,15], see Figures 1.1 and 1.2. In the pH range of urine, three equilibrium constants are

\[
-pK_u = \frac{\log ([H_2U]_{tot}/mol dm^{-3})}{p[H]}
\]

Figure 1.1 Solubility of uric acids and hydrogenurates at 37 °C (adapted from [12]). Uric acid anhydrate: solid inverted triangles, in Standard Reference Artificial Urine [10]; solid triangles, in 0.300 mol dm\(^{-3}\) NaCl + 0.050 mol dm\(^{-3}\) creatinine [10]. Open diamonds [103] obviously correspond to uric acid dihydrate. Sodium hydrogenurate monohydrate: dots, in 0.150 mol dm\(^{-3}\) NaCl [9]; ammonium hydrogenurate: solid squares, in 0.300 mol dm\(^{-3}\) NH\(_4\)Cl [12]. The lines were calculated using the equilibrium constants given in Tables 1.1 and 1.2 (adapted from [12]).

Figure 1.2 Consistency of thermodynamic data for xanthine (triangles), uric acid anhydrate (squares) and dihydrate (circles). Symbols are derived from solubility measurements; the slopes of the lines were obtained calorimetrically [14,15]. Data for xanthine were shifted by −2 units for better representation (adapted from [12]).
required to calculate the solubility. In Reactions (1.1–1.3), uric acid anhydrate, the metastable uric acid dihydrate and ammonium hydrogenurate were taken as examples:

\[
\begin{align*}
H_2U(\cdot 2H_2O)(s) & \rightleftharpoons H_2U(aq) + 2H_2O & K_s \quad (1.1) \\
H_2U(aq) & \rightleftharpoons H^+(aq) + HU^{-}(aq) & K_1 \quad (1.2) \\
NH_4HU(s) & \rightleftharpoons NH_4^+(aq) + HU^{-}(aq) & K_{s0} \quad (1.3)
\end{align*}
\]

Dissociation of the second proton of uric acid occurs at pH values far exceeding the physiologically important range. The solubilities of uric acid and ammonium hydrogenurate are given by Equations (1.4) and (1.5) respectively

\[
\begin{align*}
[U]_{\text{tot}} & = [H_2U] + [HU^-] = K_s(1 + K_1/[H^+]) \quad (1.4) \\
[U]_{\text{tot}} & = [H_2U] + [HU^-] = [HU^-][H^+]/K_1 + [HU^-] \\
& = [HU^-][(H^+)/K_1 + 1] \\
& = K_{s0}([H^+)/K_1 + 1)/[NH_4^+] \quad (1.5)
\end{align*}
\]

Thus, the equilibrium constants of Reactions (1.1–1.3) can be calculated from least-squares analyses of solubility data.

For the modeling of uric acid and hydrogenurate solubilities, a thermodynamically consistent set of equilibrium constants and calorimetric data has been obtained in our laboratory (see Tables 1.1 and 1.2, Figures 1.1 and 1.2) [9,15]. Moreover our experimental results have proved that in the ionic strength range 0.15 ≤ \(I_c/m\) ≤ 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 [10]. Thus, the same solubility as in the other salt solutions was also found [10] in so-called Standard Reference Artificial Urine, whose composition is given in [102].

**Table 1.1** Solubility and first dissociation constants at 37°C of xanthine (obtained from solubility measurements at \(I_c = 0.300\) mol dm\(^{-3}\) NaCl [14]), uric acid anhydrate (H\(_2\)U) and dihydrate (H\(_2\)U·2H\(_2\)O), 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 [9,10]. The enthalpies of solution were measured calorimetrically and correspond to the reactions H\(_2\)Xan(s) → H\(^+\)(aq) + HXan\(^-\)(aq) [11] and H\(_2\)U(·2H\(_2\)O)(s) → H\(^+\)(aq) + HU\(^-\)(aq)(+2H\(_2\)O) respectively [15].

<table>
<thead>
<tr>
<th>Substance</th>
<th>(pK_s)</th>
<th>(pK_1)</th>
<th>(\Delta H/kJmol^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xanthine</td>
<td>3.69 ± 0.03</td>
<td>7.16 ± 0.03</td>
<td>71.0 ± 1.3</td>
</tr>
<tr>
<td>Uric acid anhydrate</td>
<td>3.49 ± 0.03</td>
<td>5.19 ± 0.04</td>
<td>56.3 ± 0.4</td>
</tr>
<tr>
<td>Uric acid dihydrate</td>
<td>3.21 ± 0.01</td>
<td></td>
<td>64.5 ± 0.2</td>
</tr>
</tbody>
</table>
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 [103] obviously correspond to the dihydrate (Figure 1.1). In fact, the uric acid samples of [103] 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 [104].

Urinary alkalization with potassium citrate or sodium bicarbonate is a highly effective treatment, resulting in dissolution of existing stones and prevention of recurrence [100]. Excessive increase of the urinary pH, however, may cause precipitation of sodium or ammonium hydrogenurates [105]. The latter substance has also been found together with struvite in infectious stones caused by urea-splitting bacteria. It has been reported that in vitro, uric acid stones dissolve better in lithium carbonate than in sodium or potassium (hydrogen)carbonate solutions; this behaviour was attributed to a litholytic effect of lithium ions [106]. Our measurements have shown, however, that uric acid has the same solubility in lithium and sodium chloride solutions [11]. 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 1.2). 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 Figure 1.1).

Sodium hydrogenurate monohydrate, whose crystallization in synovial fluids around joints is the first step of gouty inflammation, has its lowest solubility at physiological pH (Figure 1.1). One of the mysteries of gout is that only a small percentage of individuals with hyperuricaemic body fluids (which are supersaturated with respect to sodium hydrogenurate monohydrate) have ever had a gouty attack. This may be related to the fact that this substance can form solutions that are highly supersaturated without any crystallization occurring, a result also found in our solubility study [9]. Whereas synovial fluids of gouty patients have nucleated sodium hydrogenurate monohydrate, normal synovial fluids, serum albumin and heparin inhibit its crystallization [107].

**Table 1.2** Solubility products of hydrogenurates at 37°C.

<table>
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<th>(\text{p}K_{s0}^a) [9]</th>
<th>(\text{p}K_{s0}^b) [12]</th>
<th>(\text{p}K_{s0}^c) [108]</th>
<th>(\text{p}K_{s0}^d) [109]</th>
<th>(\text{p}K_{s0}^d) [109]</th>
</tr>
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<tbody>
<tr>
<td>(NaHU·H₂O)</td>
<td>4.31 ± 0.01</td>
<td>4.80 ± 0.01</td>
<td>9.28 ± 0.04</td>
<td>3.85 ± 0.05</td>
<td>2.75 ± 0.05</td>
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<tr>
<td>(NH₄HU)</td>
<td></td>
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<tr>
<td>(Ca(HU)_2·6H₂O)</td>
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<tr>
<td>(KHU)</td>
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<tr>
<td>(LiHU·1.5H₂O)</td>
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\(a \quad I_c = 0.150 \text{ mol dm}^{-3} \text{ NaCl}, \quad b \quad I_c = 0.300 \text{ mol dm}^{-3} \text{ NH}_4\text{Cl}, \quad c \quad I = 0, \quad d \quad I_c = 0.15 \text{ mol dm}^{-3} \text{ LiCl.}\)

**Xanthine**

Xanthine \((C_5H_4N_4O_2)\) is the intermediate product of the purine metabolism in humans and is metabolized to the final product uric acid \((C_5H_4N_4O_3)\) by xanthine
dehydrogenase (XDH). Classical xanthinuria, a very rare condition first described in 1954 [110], is classified into two categories: type I, deficient only in XDH activity; and type II, deficient in both XDH and aldehyde oxidase. Both types are associated mainly with renal stones and lead to renal failure in some cases [111]. Treatment of gout with allopurinol (which inhibits XDH) has been reported as another cause of xanthine stones [112].

Similar to uric acid, the solubility of xanthine exhibits significant dependencies on pH and temperature [14], see Figures 1.2 and 1.3. In the pH range of urine, two equilibrium constants are required to calculate the solubility.

\[
\begin{align*}
  \text{H}_2\text{X}(s) &\rightleftharpoons \text{H}_2\text{X}(aq) & K_s \\
  \text{H}_2\text{X}(aq) &\rightleftharpoons \text{H}^+(aq) + \text{HX}^-(aq) & K_1
\end{align*}
\]

Reactions (1.6) and (1.7) are analogous to anhydrous uric acid, thus the solubility can be described by an expression analogous to Equation (1.4). Solubility products of sparingly soluble hydrogenxanthinates, analogous to Reaction (1.3), 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. Similar to uric acid, the equilibrium constants of Reactions (1.6) and (1.7) can be calculated from least squares analyses of solubility data.

The thermodynamic quantities describing the solubility of xanthine, as obtained in our laboratory [14], are presented in Table 1.1, Figures 1.2 and 1.3. The enthalpy of solution calculated from the experimentally determined solubility and first dissociation constants is \(\Delta_r H = 67.9 \text{ kJ mol}^{-1}\). This value is in excellent agreement with the calorimetric value \(\Delta_r H = (71.0 \pm 1.3) \text{ kJ mol}^{-1}\) [14]. Figure 1.2 reflects very well the thermodynamic consistency of our experimentally determined data obtained from two different methods, solubility and calorimetry. For comparison, literature data [113] are also shown in Figure 1.3. Lister and Caldbick [113] reported

![Figure 1.3 Solubility of xanthine at 37°C (adapted from [12]). Solid circles, [14]; open circles, [113]. The solid line was calculated using the equilibrium constants given in Table 1.1 (adapted from [12]).](image_url)
the solubility data of xanthine in some buffer solutions from which we obtained the solubility constants and the first dissociation constants given in [7,12,14]. The enthalpy of solution calculated from these data is $\Delta H = 22.9 \text{kJ mol}^{-1}$ which differs significantly from our calorimetric value [14]. 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 [113] might actually have investigated supersaturated solutions.

Both $K_s$ and $K_1$ of xanthine are lower than the corresponding values for uric acid. This means that xanthine has a lower solubility and litholysis by urinary alkalinisation can become effective at a higher pH (by ca. 2 units) than in the case of uric acid. While some authors have nevertheless found beneficial effects of citrate in the prevention of xanthinuria [114], others opt for a high fluid, low purine intake as the only possible therapy for XDH deficiency [115].

**Cystine**

L-cystine, C$_6$H$_{12}$N$_2$O$_4$S$_2$, 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 [116]. At least three cystinuria subtypes have been recognized and urine samples were compared to calculated solubilities in a recent study on cystinuria subtype classification [117].

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

$$nH^+ + \text{Cis}^{2-} \rightleftharpoons H_n\text{Cis}^{n-2} \quad \beta_{01n} = \frac{[H_n\text{Cis}^{n-2}][H^+]^{-n}[\text{Cis}^{2-}]^{-1}}{[\text{H}_2\text{Cis}^\pm]}$$

(1.8)

In Reaction (1.8), $n = 1, 2, 3$ or 4, and $\beta_{01n}$ denotes the corresponding protonation constants. The formally uncharged species H$_2$Cis$^\pm$ (which is actually a zwitterion) has the lowest solubility, i.e.,

$$\text{H}_2\text{Cis}(s) \rightleftharpoons \text{H}_2\text{Cis}^{\pm}(\text{aq})$$

(1.9)

and the corresponding (intrinsic) solubility constant is denoted as $K_s$. Thus, the solubility of cystine can be calculated as analytical function of pH using five equilibrium constants (Table 1.3, Figure 1.4, see [13]). Since the ionic species are much more soluble, the total solubility of cystine is given by (Equation 1.10)

$$[\text{H}_2\text{Cis}]_{\text{tot}} = K_s(1 + (\beta_{012}[H^+]^2)^{-1} + \beta_{011}(\beta_{012}[H^+])^{-1}) + \beta_{013}[H^+](\beta_{012})^{-1} + \beta_{014}[H^+]^2(\beta_{012})^{-1}$$

(1.10)

Although the protonation constants of cystine were measured at $I_c = 0.15 \text{mol dm}^{-3}$ [118], they reproduced solubility data measured at $I_c = 0.30 \text{mol dm}^{-3}$ very well.
The solubility of cystine in oxalate free artificial urine was the same as in 0.30 mol dm$^{-3}$ NaCl [13]. However, owing to the precipitation of phosphates from artificial urine at higher pH, data were only collected at pH $< 5.0$, while in phosphate free artificial urine, cystine solubilities were measured up to pH $= 8.2$. 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 [13]. In normal artificial urine, on the other hand, alkaline earth ions are complexed by phosphate. However, a significant dependence of the intrinsic solubility on the nature and concentration of various inorganic salts was reported in [119,120]; so more experimental work on this topic is certainly needed. Recent literature data for 0.5 mol dm$^{-3}$ NaCl [121] agree with our values at low pH but show a systematic deviation at high pH [12]. It should be emphasised 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.

Cystine solubilities in real urine [122] agree well with our results obtained in synthetic solutions [13] (Figure 1.4). Therefore, the equilibrium constants in Table 1.3 permit reasonable cystine solubility estimates for urine. In the formation of

Figure 1.4 Solubility of L-cystine at 37°C (adapted from [12]): solid circles, oxalate-free artificial urine; triangles, phosphate and oxalate free artificial urine; inverted triangles, 0.30 mol dm$^{-3}$ NaCl [13]; open circles, real urine [122]; solid line, calculated using equilibrium constants in Table 1.3; dashed line, calculated with JESS for phosphate- and oxalate free artificial urine [13] (adapted from [12]).

Table 1.3 Solubility and protonation constants for L-cystine used for solubility simulations in 0.30 mol dm$^{-3}$ NaCl and oxalate free artificial urine at 37°C.

<table>
<thead>
<tr>
<th>$-\log K_{s}^a$</th>
<th>$\log \beta_{011}^b$</th>
<th>$\log \beta_{012}^b$</th>
<th>$\log \beta_{013}^b$</th>
<th>$\log \beta_{014}^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.08 ± 0.01</td>
<td>8.604 ± 0.003</td>
<td>16.356 ± 0.004</td>
<td>18.41 ± 0.01</td>
<td>20.03 ± 0.02</td>
</tr>
</tbody>
</table>

$^a I_c = 0.30$ mol dm$^{-3}$ NaCl [13], $^b t = 37°C$, $I_c = 0.15$ mol dm$^{-3}$ NaCl [118].
cystine stones, supersaturation is overwhelmingly important in relation to inhibition of crystallization and other factors (diet, infections, etc.) that may be relevant for other types of stone [123].

For the treatment of cystine lithiasis, potassium citrate has been used to increase the urinary pH and thus the cystine solubility [123]. However, a higher pH favours calcium phosphate calculi formation in stone prone patients. This problem would be particularly serious if a recent recommendation [124] 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 [96] but also observed experimentally [96,125].

**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 [8]. However, the calcium oxalate solubility strongly depends on the concentration of ions that form complexes with calcium or oxalate, particularly citrate or magnesium ions respectively [7,89]. It was demonstrated that our urine model [7,8] 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 some of the early literature data is rather unsatisfactory. To clarify this point, a simple thermodynamic consistency test is applied according to a rule established almost 130 years ago [126]. 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 + H₂O(aq) or COD → COM + H₂O(aq) are always positive [127]. The enthalpies of solution obtained from our solubility products (Table 1.4) obey this rule (see Figure 1.5) while those derived from some literature data, e.g. [128,129], do not [12]. Moreover the temperature dependence of our solubility products is consistent with values determined calorimetrically; details of these measurements

<table>
<thead>
<tr>
<th>t/°C</th>
<th>−logK_s0</th>
<th>COM</th>
<th>COD</th>
<th>COT</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>8.84 ± 0.02</td>
<td>8.42 ± 0.02</td>
<td>8.33 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>8.77 ± 0.01</td>
<td>8.34 ± 0.02</td>
<td>8.24 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>8.71 ± 0.01</td>
<td>8.26 ± 0.03</td>
<td>8.12 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>8.65 ± 0.03</td>
<td>8.17 ± 0.03</td>
<td>8.02 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>8.62 ± 0.02</td>
<td>8.13 ± 0.04</td>
<td>7.97 ± 0.02</td>
<td></td>
</tr>
</tbody>
</table>
Figure 1.5 Thermodynamic consistency of calcium oxalate data (adapted from [12]). Solid symbols [8]; squares, COM; circles, COD; triangles, COT (Table 1.4). Solid lines correspond to calorimetrically determined enthalpies of solution [12] (adapted from [12]).

and the associated calculations are given elsewhere [12]. It should also be noted here that our thermodynamic quantities for uric acid anhydrate and dihydrate pass this consistency test as well (see Table 1.1 and Figure 1.2).

Urolithiasis is controlled by thermodynamic and kinetic factors either alone or in combination. At least for calcium oxalate and phosphate, 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 (i.e. nucleation and/or crystal growth) 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 may be reached at which urine can hold no more salt in solution and kinetic factors are no longer effective.

In a recent application of computer modeling to urolithiasis research, a correlation between COM, DCPD and HAP supersaturation in real urine samples and the results of a simple clinical test for urinary lithogen risk (ULR) was reported [130]. This correlation leads to an establishment of kinetic and thermodynamic factors contributing to stone formation. 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 borderline between normal and lithogenic urines [131]. In Figure 1.6, results of this test are plotted vs the supersaturation of COM obtained from the urines of stone formers and healthy people. It can be seen 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 define a region called
Solubility Phenomena Related to Biomineralization Processes

Figure 1.6 Correlation between ULR test and COM supersaturation (adapted from [12,130], with kind permission of Springer Science and Business Media). Open symbols, stone formers; solid symbols, healthy people.

‘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 established and they are presented as regions I, II, III, IV and V in Figure 1.6.

(i) In region I, $0 < \log S < 0.37$ 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 or strongly disordered urodynamic conditions [131].

(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 lithogenic 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 due to the high supersaturation [131].

(iv) In region IV, $\log S > 0.57$ and $A > 0.3$, the supersaturation values are abnormally high so that thermodynamic factors are in absolute control and the only result is the crystallization of calcium oxalate. Obviously, kinetic factors do not have any effects even if there were inhibitors present in the tested urines. This is confirmed by the fact that only data from active stone formers with
positive ULR test results are found in this region. Consequently, for urines belonging to this region, 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 [79].

(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.

In two recent studies on crystallization inhibition by urine, it has been found that male urinary calcium stone-formers lack proper inhibition of both nucleation and crystal growth [132] while women display only the former [133]. These and other studies use the ‘upper limit of metastability’ (ULM, i.e. the degree of supersaturation at which crystallization starts) as a measure of the threshold of nucleation and claim a (hitherto unexplained) correlation of ULM with supersaturation [134]. This is somewhat in contrast to the results shown in the ULR test/log \( S \) plots above, in which urines with the highest supersaturation do not exhibit any kinetic inhibition of crystallization (in the ULR test – see Region IV). Nevertheless, at about \( 5 < S < 10 \), the ULM/S plots [132,133] display a region analogous to the ‘kinetic threshold’ (Region III) which contains urines of healthy people, whereas at higher \( S \) only urines of patients are found.

The effect of dietary factors and health supplements on the calculated supersaturation is of similar complexity [93,135]. Urinary oxalate has emerged as the most important determinant of calcium oxalate crystallization so that dietary oxalate intake should be restricted, while the role of urinary calcium has shifted to bone balance and osteoporosis. Dietary calcium restriction increases urinary oxalate and contributes to a negative bone balance and has therefore been abandoned as a means to reduce the risk of calcium oxalate kidney stone formation [135]. While it has been demonstrated that a sodium citrate containing preparation favorably alters the risk factors for calcium oxalate urolithiasis [136], the interplay between alkali, magnesium and citrate is complicated, given that the last two also inhibit the crystallization of Ca minerals [91]. Either synergistic effects (MgO and citrate on lowering the supersaturation of brushite and on increasing the pH) or additive effects (CaCO\(_3\) and citrate on lowering the supersaturation of uric acid) have been proposed [137]. Data from various investigations on the effect of vitamin C are contradictory, in part because of difficulties regarding oxalate assay techniques [138–140]. Whereas cola consumption [141] has an adverse effect on calcium oxalate supersaturation, cranberry juice has antilithogenic properties [142]. The effect of increasing dietary oxalate on urinary calcium oxalate supersaturation is difficult to predict, as there may be increased absorption due to secondary hyperoxaluria [94], or oxalate may bind to intestinal calcium, which lowers calcium absorption and excretion [143]. Although black South Africans have higher calcium oxalate supersaturations than the white population, they seem to be immune to kidney stones [144]. Also, on an oxalate rich diet, their urinary oxalate did not increase markedly so that it has been proposed that lower oxalate absorption rates may be the reason that South African blacks hardly form stones [144].
As an important aspect of these models, it has recently been shown that urinary calcium is as equally effective as oxalate in increasing calcium oxalate supersaturation, when a widely accepted value for the calcium oxalate stability constant is used [146]. As discussed in [146], this is in contrast to earlier studies, which had used a higher value for this constant and found that the influence of calcium on calcium oxalate supersaturation was less pronounced than that of oxalate.

A frequent recommendation to calcium oxalate stone-formers is to consume a large quantity of liquid [147]. Our computer simulations have demonstrated that this advice is physicochemically meaningful for certain fluids [12]. For pure water, only an unrealistically high 1:10 dilution results in an unsaturated solution, so only a high water load seems to effect a significant reduction of the supersaturation [148]. More likely, it mainly 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 [12]. It was also concluded from the results of a study on 85 human subjects [149] that such mineral waters reduce the risk of calcium oxalate stone formation.

The Hypothesis of Urine as a Saturated Solution

Györy and Ashby proposed a new hypothesis about the thermodynamic state of urine, regarding it as a saturated solution with respect to stone forming substances [150–152]. This approach would imply that urine is at equilibrium with these solid, stone forming substances once their solubilities are exceeded. Györy and Ashby suggest that this suspension (or ‘quasicolloid’ [151]) is stable in normal individuals because of agglomeration inhibition (mainly by citrate) and base their hypothesis upon clinical observations, particularly crystalluria (the excretion of crystals in urine), and several modeling results. Kavanagh [153] has critically analyzed the arguments for and against the Györy and Ashby hypothesis and suggested how the authors could easily prove their hypothesis by ultrafiltration. However, no decisive experimental confirmation appears to have been carried out as yet, although no significant amount of nonultrafilterable calcium oxalate has been found in preliminary experiments [153]. While on the one hand recent research indicates that suspensions of very fine particles can be dynamically stabilized without undergoing dissolution in undersaturated supporting media [154], there is, on the other hand, direct evidence of supersaturation by seeded crystal growth experiments in urine. The formation mechanism of certain renal calculi also suggests that the Györy and Ashby hypothesis is questionable. For instance, columnar growth of papillary calcium oxalate monohydrate calculi (see Chapter 2 in this book) can be explained by supersaturation but hardly by agglomeration of colloidal crystals. Other apparent inconsistencies between urinary supersaturation and observed behaviour as claimed by Györy and Ashby (‘unsuccessful’ treatment by increasing the urine volume – see above; prediction of infection stones mixed with calcium oxalate) can be resolved by physicochemical considerations [153].
Nanobacteria

Recently, so-called nanobacteria, which behave like an extremely small microbe with very slow multiplication rate, have been found in kidney stones [155] and claimed to induce calcifications in various body fluids, including urine, by an apparent extraction and concentration of phosphate [156]. Nanobacteria appear to be correlated with such diverse conditions as arterial heart disease, Alzheimer’s disease, malignant tumours and urolithiasis, which has recently been found to be worsened in astronauts during long space flights due to their seemingly enhanced growth in microgravity [157]. Although it is still controversial whether nanobacteria are living organisms (as their putative DNA has not been sequenced as yet [158]), it has been shown that various chemotherapeutics, including aminoglycoside antibiotics, inhibit the growth of putative nanobacteria in vitro and thus may help people who suffer from chronic stone formation and other conditions [159]. However, it has been suggested that these compounds might inhibit calcification rather than bacterial growth since biomineralization attributed to putative nanobacteria may be initiated by nonliving macromolecules including phospholipids and can be continued on dilution to fresh medium by microcrystalline apatite, which also accounts for the wide range of morphological forms ascribed to nanobacteria [158].

1.3.5 MODELING PANCREATIC AND BILIARY STONE FORMATION

Calcium carbonate is a major constituent of pancreatic stones (consisting of ca. 95% calcite), salivary stones and many pigment gallstones, since these three gastrointestinal secretory organs generate high hydrogen carbonate concentrations and high pH values in their respective secretions. Moore and Verine have developed a physicochemical model of calcite saturation in pancreatic juice [160] that takes the solubility constant as well as the complexation constants of calcium ions with (hydrogen)carbonate and proteins into account. The simulations have shown that all of these ligands are important buffers for calcium ions in the juice [160]. Various in vitro studies have been performed to investigate the dissolution of stones as well as the influence of additives such as citric acid and dimethadione [161] or the effect of hormonal stimulation [162] on calcium carbonate saturation in pancreatic juice.

In industrialized societies, cholesterol rich gallstones are the most common type of stone found in the gall bladder [163]. As opposed to pigment stones that may contain bilirubin and are often associated with infections and hepatic cirrhosis, these stones are frequently composed of cholesterol microcrystals, where large specimens tend to form an outer shell which, among other calcium salts, contains vaterite, the least stable of the three crystalline anhydrous calcium carbonate modifications [164]. The complicated interplay of biliary cholesterol hypersecretion and hyposecretion of bile acids and phospholipids, which, among numerous other factors, may lead to supersaturation and nucleation of cholesterol microcrystals, has been discussed in a comprehensive review on the pathogenesis of gallstones [163]. Similar to urine, which is generally supersaturated with calcium oxalate, bile appears to be generally
supersaturated with cholesterol [163,165] so that kinetic factors determine whether or not stone formation occurs. A thermodynamic model of unconjugated bilirubin saturation in bile and a corresponding lithogenic index that discriminates lithogenic and non-lithogenic bile have been reported recently [166]. Chapter 3 in this book reviews further perspectives on the formation of gallstones and the development of atherosclerotic lesions, which also contain cholesterol and calcium salts [167,168]. Various aspects of salivary stones (sialoliths) and their formation mechanisms are discussed in Chapters 2 and 3 of this book.

1.4 NEW INSIGHTS IN SOLUBILITY PHENOMENA RELEVANT TO BIOMINERALIZATION

Modern experimental techniques have stimulated current research leading to a better understanding of the crucial role of solubility phenomena in biomineralization. Various new mechanisms that are potentially significant to normal and pathological biomineral formation have been proposed, such as mineralization of calcium salts via an amorphous liquid phase precursor [169]. This and other contemporary research, concerning the solubility of nanomaterials [154] and new studies on crystallization kinetics [170,171] are briefly outlined below.

1.4.1 SOLUBILITY OF NANOMATERIALS AND BIOLOGICAL DEMINERALIZATION

Biological materials frequently have nanosized mineral particles as their basic building blocks, for example, ferrihydrite in the iron storage protein ferritin [172] (further discussed in Chapter 5 of this book), magnetite in bacteria [1] or hydroxyapatite particles in bone, dentin and dental enamel [173,174]. As a very large fraction of the nanoparticle’s atoms are near the surface, the effects of interfacial energy between particle and vacuum, a gaseous atmosphere, water or an aqueous solution become significant. The thermochemistry of nanomaterials has been reviewed by Navrotsky [16,17]. One of the most remarkable results of these studies is the thermodynamic stability of nanosized polymorphs that are metastable in the bulk. A general rule, demonstrated for many systems, states that metastable polymorphs (e.g. anatase) have lower interfacial energies than the stable phase (rutile), which results in a free energy crossover at high surface areas [16,17]. A similar behaviour has been found for nanocrystalline substances precipitating from aqueous solution, for instance, anatase with a crystallite size of 10–30 nm is formed first and recrystallizes on hydrothermal treatment to stable, much coarser rutile [175].

It has been established that there is a close relationship between solubility and interfacial energy [23,176,177]. During dissolution, ions on the surface are replaced by water molecules to escape into the bulk solution. As higher interfacial
energies indicate a greater difficulty in forming such an interface between the solid and aqueous phases, sparingly soluble, i.e. more stable, salts always have higher interfacial free energies than soluble salts. For example, it is well known that the interfacial energies of various calcium phosphate phases important in biomineralization increase in the order brushite $<$ octacalcium phosphate (OCP) $<$ β-tricalcium phosphate $<$ hydroxyapatite $<$ fluoroapatite whilst their solubilities decrease in the same order [23,177–179].

The general rule that metastable phases have lower interfacial energies and hence precipitate more easily than stable phases therefore also applies to aqueous systems. In addition, the ability of a surface to nucleate other phases is closely related to the magnitude of the interfacial energies [180]. For instance, it has been shown that calcium phosphates nucleate more readily on anatase than on rutile surfaces, owing to the lower interfacial energy of the former which favours the adhesion of the aqueous solution and thus facilitates the nucleation of calcium phosphate phases [181]. These relationships may also serve to explain Ostwald’s law of stages, since the least stable phase tends to precipitate first and is able to nucleate more stable phases due to its lower interfacial energy. Therefore, the more soluble phosphates brushite and OCP are considered to be precursors for hydroxyapatite [178], as is amorphous calcium carbonate for crystalline calcium carbonate phases [182].

These examples indicate the importance of interfacial energies for rationalizing the dissolution and precipitation behaviour of sparingly soluble phases such as biominerals. However, the accurate measurement of this quantity has proved to be difficult since different techniques (solubility/particle size, nucleation, crystallization and dissolution kinetics, contact angle or wetting methods) yield vastly differing values for interfacial energies between solid and aqueous solution [21,23]. One research group has reported a positive [170] and a negative [178] value for the interfacial tension between brushite surfaces and water or solution (not clearly specified), although both values were measured using a thin layer wicking capillary rise technique. It should be noted that this technique is known to provide lower values than those obtained by other methods because the double-layer effects are also included [170].

Biomaterials like bone and teeth are examples of organic–inorganic nano-composites that possess superior mechanical strength, as they are both hard and tough (fracture resistant) [183]. It seems that the weakening effect of flaws vanishes specifically at the nanoscale so that the strength of a perfect crystal is maintained despite defects [183]. The nanoscale dimension of the mineral phase of bone is also crucial to its bioresorptive potential and to the preparation of bone-graft substitutes (see Chapter 4 of this book). Moreover, recent dissolution studies of sparingly soluble Ca phosphates have revealed an unusual behaviour when the crystals fall under a critical size, also at the nanoscale, resulting in a kinetic self preservation of biominerals at undersaturation that prevents them from being dissolved [184].

Modern experimental techniques such as vertical scanning interferometry and in situ atomic force microscopy (AFM), which allow one to directly observe the dissolution behaviour of these biominerals, their synthetic analogs and
other minerals, have sparked a renewed interest in the theory of dissolution [154,174,184–191]. In traditional theories of dissolution, the dissolution rate has been expressed as a simple function of the relative undersaturation, which implies that the dissolution rate should remain constant at sustained undersaturation. However, constant composition dissolution studies of nanosized, synthetic brushite (calcium hydrogenphosphate dihydrate) [154,187], synthetic hydroxyapatite [186] and hydroxyapatite particles obtained from dental enamel [174,184] have shown that the dissolution rates decrease and become effectively suppressed even though the solutions remain undersaturated. This interesting and unusual behaviour has been explained in terms of a model that incorporates particle size considerations. It has been confirmed experimentally that demineralization of sparingly soluble phases is initiated and accompanied by the formation and development of pits on the crystal surfaces and that the dissolution rates are determined by the pit densities and spreading velocities [184–187]. It has been shown [174,184,185,192] that the dissolution rate depends on a critical pit size \( r^* \) and only pits of a radius \( r \) larger than \( r^* \) provide active sites that contribute to the dissolution,

\[
R(\rho) \approx R_\infty (1 - r^*/\rho)
\]  

(1.11)

where \( R_\infty \) is the velocity of dissolution steps at \( r \to \infty \). When \( r \to r^* \), there is no fast movement of its stepwave, and the dissolution rate approaches zero. When the dimensions of the crystallites fall in the same order as \( r^* \) during the dissolution, the formation of active pits is more difficult due to size restrictions, leading to retarded dissolution rates. The critical pit size \( r^* \) is directly proportional to the interfacial tension and inversely proportional to the Gibbs energy of dissolution [174,185]. This results in greater values of \( r^* \) (about 10–100 nm) for sparingly soluble biominerals, which always have much higher interfacial free energy values in aqueous solution than soluble salts (see above). Since sparingly soluble salts often have sizes in this critical range, they may thus be protected from dissolution because the reaction in the (relatively wide) metastable region is significantly inhibited [184]. Similarly, the growth of tiny apatite crystals is rarely observed at low supersaturations due to extremely slow growth rates which can also be attributed to a kinetic size effect [184]. In addition, it has been speculated that this crystallite size effect in the dissolution reactions could provide a route for the synthesis of nanoparticles of sparingly soluble salts, whose sizes could be adjusted by changing the dissolution conditions [184].

An equation similar to Equation (1.11) has been discussed in a recent study of calcite growth [4] since steps (growth) and pits (dissolution) have similar roles and features [187]. Both of them are unstable if they are smaller than the critical size, i.e. unstable steps do not advance and unstable pits disappear from the surface [4,187,192]. The inverse scaling of the critical step length with supersaturation is a prediction of the Gibbs–Thomson effect, according to which steps of high curvature at corners should be at equilibrium with the adjacent supersaturated solution [4].

It is noteworthy that the Ostwald–Freundlich model (i.e. the formulation of the Gibbs–Thomson effect for liquid vapour systems applied to solid–liquid systems),
which states that finer particles have a higher solubility and should therefore dissolve faster, is in apparent disagreement with these constant-composition studies of dissolution kinetics [154,184,186,187]. While some researchers observed an increase in solubility upon introduction of small SrSO$_4$ particles into solutions saturated with larger crystals [193], others did not find any further dissolution when HAP nanoparticles were added to ‘dissolution terminated’ suspensions of larger crystals [154]. The latter are probably in a ‘dynamically stable’ but thermodynamically metastable state that can be maintained for long periods [184]. However, since thermodynamics requires that dissolution cannot stop in an absolute sense, below this critical undersaturation the rate does not necessarily go to zero but will rather be limited by the much slower nucleation or spiral dissolution mechanism so that equilibrium is eventually attained [185]. Schindler et al. have carefully measured the solubility constants of CuO and Cu(OH)$_2$ and determined the influence of molar surface area upon solubility [194]. For coarse solids, the solubility of the hydroxide was found to be ca. 10 times greater than that of the oxide, so that the latter is the more stable phase. However, if the solids are very finely divided, CuO becomes less stable than Cu(OH)$_2$ (see also Figure 5.23 in [195]). This free energy crossover as a function of particle size, which results from the larger interfacial energy of the oxide as compared to the hydroxide, is in exact analogy to the results derived from calorimetric studies [16,17] and puts some confidence in the relationship between solubility and particle size. Nevertheless, it has been suggested that the thermodynamic basis of the Ostwald–Freundlich model is questionable [177,196], whereas others have derived it in a rigorous way [193,197] and discuss the significance of the surface energy parameter so obtained [176].

In any event, the studies on the dissolution mechanism of sparingly soluble minerals discussed above have again confirmed the long standing recommendation [6] that large crystallite size and long equilibration times are required to attain true equilibrium solubility conditions [192].

1.4.2 NEW MECHANISMS OF BIOMINERALIZATION

While some proteins inhibit pathological calcification, e.g. matrix GLA protein in blood vessels [198] or osteopontin in urine [199], many other proteins promote normal biomineralization of e.g. bone, cementum and dentin by controlling nucleation, growth kinetics, morphology and orientation of the constituent inorganic crystals [1,200]. A topical review has described a number of proteins associated with biominerals [200].

Various mechanisms of biomineralization have been proposed and discussed extensively. A widely accepted view is that the organic matrix or molecules in solution induce nucleation on certain crystal faces (through lowering the Gibbs energy of nucleation by reducing the interfacial energy [1]) and thus control the crystal structure by geometric (epitaxial) matching and stereochemical recognition [201–203]. It has been suggested that these interactions are dynamic, originating
Solubility Phenomena Related to Biomineralization Processes

from subtle differences in the kinetics of these recognition processes on different crystal faces rather than from irreversible binding on one set of symmetry related surfaces [203]. The effect of a new heterogeneous nucleation model on the structural correlation at the interface between biomineral and substrate has been investigated recently and a ‘supersaturation driven anti-templating’ mechanism has been proposed [204]. This mechanism implies that low supersaturations lead to good structural match and consequently to ordered and compact biomineral structure, whereas high supersaturations result in structural mismatch and therefore in disordered and porous biomineral structure [204]. It has been found that polyelectrolytes like poly-L-aspartate, which are structural and functional analogues of subdomains of biomineralization controlling proteins, act as crystal nucleators when immobilized on Ge surfaces, nucleating (less stable) OCP and (more stable) HAP at high and low supersaturations respectively (indicating kinetic control) [205]. Conversely, these polyelectrolytes inhibit crystal growth when free in solution [205] and have therefore been used as biodegradable inhibitors of Ca salt deposition in industrial processes [206].

Whereas HAP crystallization from simulated blood plasma onto Langmuir monolayers of arachidic acid has been described [207], other researchers have reported homogeneous nucleation of calcium phosphates although self assembled monolayer substrates mimicking bone organic matrices were present [208]. These nuclei started to grow in solution and only deposited as apatite on the surface in a second period of crystal growth, allegedly resembling early stages in bone mineralization in vesicular compartments [208]. Various simulations of interfacial control of mineral nucleation on Langmuir monolayers have been reported [209–211].

Recently, a new mineralization process that proceeds via an amorphous, polymer induced liquid-phase precursor (PILP process) has been proposed [169], in which polyelectrolytes like poly-L-aspartate (see above) sequester ions and induce a liquid–liquid phase separation, forming droplets of ca. 2–4 microns (PILP phase) prior to mineralization of various Ca salts. Similar phenomena involving microscopic liquid–liquid phase separation have been found to induce silica mineralisation [212], while other unusual morphologies involving aqueous polymer solutions have been described [213]. The hypothesis that the fluidic nature of the PILP phase is relevant to normal and pathological biomineralization is discussed in detail in Chapter 4 of this book.

Current crystallization studies have employed (i) in situ atomic force microscopy (AFM), which enables the visualization of these processes in real time [170,171,199,214,215]; and (ii) highly sensitive constant composition techniques, which provide reliable rates of crystal growth [20,170,171,199]. These studies have suggested another, different mechanism of biomineralization, which potentially provides insights into the prevention and therapy of pathological crystallizations such as renal stone disease. For instance, it is well known that urinary citrate deficiency is a predisposition of calcium oxalate monohydrate (COM) stone formation. It has been shown by AFM and molecular modelling [171,199] that
the inhibitory action of citrate on COM growth does not occur via adsorption on the crystal face but is rather due to a dynamic process of selective binding to atomic steps (formed along dislocation lines for crystal growth) on one specific crystal face, which results in step pinning, step edge roughening, anisotropic step kinetics and consequent crystal growth inhibition and shape modification. Since citrate leaves other crystal faces to grow uninhibited, additional therapeutic agents such as osteopontin, which affects the steps on different faces [199], may be needed for optimal prevention of kidney stone disease [171]. A similar, ‘step control’ model has been suggested in a study of the formation of chiral morphologies through selective binding of amino acids to calcite surface steps, which changes the step edge free energies and therefore the step dynamics, resulting in altered rates of attachment and detachment of calcite species at the surface of the mineral [214]. In another *in situ* AFM study, the recovery of surfaces from impurity poisoning has been investigated [215]. It has been shown that the resurrection of growth out of the ‘dead zone’ (a regime of low supersaturation where growth ceases) proceeds via the propagation of macrosteps (bunches of monolayer steps), which ‘overrun’ the elementary steps that are blocked by the impurities, on a timescale comparable to that of impurity adsorption [215].

Another, new biomineralization mechanism has been proposed [170] based on AFM observations of the crystallization of brushite (DCPD), which is regarded as a precursor in the biological formation of apatite and is found in developing bone, immature dentine and in kidney stones. Using a sensitive constant composition method [20], brushite growth has been studied in the absence and presence of citrate, which has been recognized as an effective inhibitor. However, unlike to the study on citrate inhibition of COM crystallization described above [171,199], neither the step morphology nor the kinetics is affected by citrate. In contrast, citrate dramatically reduces the step density in an anisotropic manner, leading to a corresponding decrease in the bulk growth rate, while the step velocity and morphology are virtually unchanged. The number of steps being formed at surface dislocations depends on the supersaturation and the free energy of the step edge which can be correlated with the interfacial tension between brushite surfaces and the solution. The latter has been measured [170] and found to increase with citrate concentration. The corresponding increased free energies of the step edge imply that the critical length for spontaneous step growth, related to the terrace width for the steps, increases and the formation of new steps becomes retarded. Macroscopically, this is manifested by longer induction times for homogeneous nucleation measured in the presence of citrate, which indicates that this carboxylate-rich compound is a nucleation inhibitor. The authors [170] emphasise that their results are in contrast to the model of epitaxial control of biomineralization as proposed by Mann [1,202], which suggests that energy barriers are reduced by Ca–carboxylate interactions and would thus imply a promotion of the nucleation in the present case.

Tang *et al.* [170] have suggested that approaches involving surface adsorption, e.g. by employing a Langmuir model which had been successfully applied in
earlier growth kinetics and adsorption studies [179,205,216], may be misleading in these cases. Since such approaches have also been used in the industrial context [217], the new findings outlined here will not only be important for improving our understanding of the mechanisms of biomineralization but may also help to optimise large-scale industrial crystallization processes.

REFERENCES


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