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Solubility and crystallization in biological fluids and the problems of life and health*

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Abstract: An analysis is given of several biologically important processes, connected with the crystallization and kinetics of liophilization in aqueous physiological solutions. These are glass formation and vitrification of life, precipitation of slightly soluble salts and kinetics of crystallization in human urine, and the solution of CaOX concretions under the influence of biocompatible solubilizators, which are normal constituents of human urine. The problems investigated experimentally are treated in the framework of the capillary theory of nucleation and the Debye–Hückel model of ionic solutions.

INTRODUCTION

The development of life on Earth (and most probably in the Universe) is bound with aqueous solutions and connected with their physics and chemistry. The precipitation processes from aqueous physiological fluids are particularly significant in this respect: crystallization, that is, the nucleation and growth of crystals and, subsequently, their possible dissolution. Crystallization from solutions is thermodynamically determined by the solubility of crystallizing components and kinetically by fluidity and the processes of diffusion in the initial phase. Biological aqueous solutions are systems with complex composition. Thus, their thermodynamic properties are usually considered and calculated in the framework of the Debye–Hückel model of ionic solutions: accounting for the increased ionic strength, ψ, and for the presence of ionic complex-forming agents. On the basis of this model in the present paper, the solubilities of slightly soluble salts and the thermodynamic driving force, $\Delta\mu$, of their crystallization are determined and the thermodynamic barrier of crystalline nucleation is calculated. The coefficients of diffusion, D, in ionic solutions are estimated by a linear approximation especially for the case when the presence of viscosity-increasing constituents (such as glycerol) considerably decreases D, and thus decreases and even prevents crystallization. So the problems of glass formation and vitrification of life and of absolute anabiosis in aqueous solutions can be treated, as done here and in a series of preceding publications, in a quantitative way.

We developed a quantitative approach especially in analyzing the problems of solubility, precipitation, and possible liophilization of urinary calculi. Their formation and growth determine urolithiasis diseases in their various forms. The results of our experimental investigations show new ways of treating the problems of calcium oxalate (CaOX) formation and even of its dissolution in biological aqueous solutions.

In analyzing the problems of preventing crystallization in biological fluids, we develop a quantitative criterion for vitrification. It explains one of Nature's two main strategies for preserving life under severe conditions, even at temperatures approaching absolute zero.

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GENERAL THEORETICAL BACKGROUND

According to the concepts of the classical theory of nucleation (see, e.g., [1,2] for its steady-state formulation) the rate of nucleation, I_o, in a supersaturated aqueous solution is determined according to

$$I_o = \text{const} \cdot \exp\,[-U/RT] \cdot \exp\,[-W_c/RT] \tag{1}$$

by two reaction barriers. The first one, U, is kinetically based. The second one, W_c, is thermodynamic in its nature and is given by the work, W_c, to form a critical new phase cluster within the initial solution

$$W_c \cong (16/3) \cdot \pi\sigma^3 V_m{}^2 \phi/\Delta\mu^2 \tag{2}$$

Here, as usual, we have denoted the interface energy at the crystal nuclei/fluid interface with σ, V_m is the molar volume of the newly formed phase and the dimensionless factor, ϕ, accounts for the activity of possible nucleation active substrates (according to definition, see [1,2], $0 \leq \phi \leq 1$). The thermodynamic driving force of the process of crystallization, $\Delta\mu$, is determined in our case by the equilibrium solubility, C_o, of the crystallizing component, AB, and by its actual concentration, C, in the respective biological solution as [3,4]

$$\Delta\mu = RT \cdot \ln\,(C/C_o) \tag{3}$$

Here, C_o is the equilibrium activity of the component AB in solution, determined by, the ionic strength, ψ, of the biological fluid, and the equilibrium constants, K_i, of complex-forming substances in solution. In considering nucleation and growth in ionic solutions (as all biological solutions are) the determination of C_o becomes the crux of the problem of proper definition and calculation of $\Delta\mu$. In refs. [3,5,6] we have described in detail the necessary steps in this calculation, using both a simple thermodynamic approach and also the concept of the ionic activity product, AP, conventionally employed in literature [7] in determining the activity coefficients of biological solutions.

The activation energy, U_o, in eq. 1 is connected with the diffusion coefficient, D_{AB}, of the crystallizing (ionic) substance AB. It determines the impingement rate, β, of the A and B ions in the ambient phase solution to the growing crystal. In general, in aqueous biological solutions, D_{AB}, and, U, can be taken to correspond to the respective values of the pure solvent (here, water) at the same temperature, T. However, when in the system are also present substances (like glycerol) considerably increasing its viscosity, the value of the diffusion coefficient

$$D \approx \text{const} \cdot \exp\,(-U/RT) \tag{4a}$$

can be dramatically decreased. In such cases, as a first approximation it can be assumed [4] that the activation energy, $U_{o,1}$, in the solution is increased according to

$$U_{o,1} = U_o \cdot (1-x_1) + U_1 \cdot x_1 \tag{4b}$$

where U_o is the activation energy in the initial solution, U_1, in the viscosity increasing liquid (e.g., glycerol), and x_i is its molar part introduced.

With the above definition of $\Delta\mu$, the rate of growth, dC/dt, (or dissolution) of an AB crystal in the solution within a linear concentration gradient, $\partial\Delta\mu/\partial z$, is given by

$$dC/dt = (DC/RT) \cdot (\partial\Delta\mu/\partial z) \tag{5}$$

In Nernst's approximation, it can be written (for a crystallite with the surface F) as

$$dC/dt \approx (D \cdot F/\delta) \cdot [C_o - C] \tag{6a}$$

Here, δ is the thickness of Nernst's diffusion layer, and the kinetics of growth or dissolution is thus determined by

$$\ln [1 - (C/C_o)] = - (D \cdot F/\delta) \cdot t \tag{6b}$$

with an initial slope of

$$dC/dt \big|_{t \to o} \approx (D \cdot F/\delta) \cdot C_o \tag{6c}$$

In this way, C_o can be experimentally determined from dissolution experiments. This was the approach we employed [3,5,6] in determining the solubility of CaOX in urine. Accounting for the presence of complexing cation or anion binding substances, *i* and *j*, by introducing the corresponding α factors ($[A^+] = C_A/\alpha_A$ and $[B^-] = C_B/\alpha_B$) governing the actual ionic concentrations $[A^+]$ and $[B^-]$ are determined via

$$\alpha_A = 1 + \Sigma K_i C_i \qquad \alpha_B = 1 + \Sigma K_j C_j \tag{7}$$

where K_i and K_j, are the respective equilibrium constants. Determining also the ion activity coefficient, γ_o, by

$$\ln \gamma_o \cong - [(n_A + n_B) / 2RTE] \cdot \sqrt{\psi} \tag{8}$$

from the ionic strength of the solution ($\psi = \frac{1}{2} \Sigma n_k C_k$ where *k* is taken over all ions in the system) the solubility, C_o, of the electrolyte AB in eqs. 3 and 6 can be calculated. The result as it is derived in [3] reads

$$C_o = (1/\gamma_o) \cdot \sqrt{L_o \alpha_A \alpha_B} \tag{9}$$

for the case when $[A^+]$ and $[B^-]$ are in equivalent stiochiometric concentration in the solution and

$$C_o = (1/\gamma_o^2) \cdot L_o \alpha_A \alpha_B \tag{10}$$

when one of the ions is in excess of the other (eq. 10 gives the case when [A] >> [B]). In both eqs. 9 and 10, L_o indicates the ion solubility product of AB for the temperature *T* in pure water, i.e., a value that is traditionally reported in reference literature. The case given with eq. 10 is of particular significance in biological aqueous solutions like urine: in considering the precipitation of calcium oxalate (CaC_2O_4), it has to be accounted that usually $[Ca^{2+}]$ >> (5 to 10) $[C_2O_4^{2-}]$. In this way, the determination of C_o is reduced to a problem of relatively simple calculations in terms of the thermodynamics of ionic solutions.

Thus, with the above outlined formalism we can treat quantitatively the kinetics of both crystallization and dissolution of the slightly soluble salt AB in a given physiologically important solution and, moreover, the vitrification of the whole biological system.

KINETICS OF VITRIFICATION

If a biological solution is quenched through the temperature range of possible crystallization with a cooling rate, q_o, higher than a critical value, q_{ocrit}, it vitrifies at a temperature, T_g, where the time of molecular relaxation, τ, is compatible with its mean residence time of the system in the vicinity of T_g. Thus, the condition for vitrification can be written [2] as

$$(\tau \cdot q_o) \big|_{T \approx Tg} \approx \text{const} \tag{11}$$

With a known molecular relation connecting τ with the coefficient of diffusion, *D*, (reading $\tau_k = d_o^2/D$, where d_o corresponds to the mean molecular size in the system) we can write eq. 11, accounting for eqs. 4 in the form

$$1/T_g \cong \{(C_1 - C_2) \cdot \log q_o\} [U_{o,1}]^{-1} \tag{12}$$

where C_1 and C_2 are constants to a first approximation. In considering eq. 4b, this indicates that T_g is linearly dependent on x_1. Thus, it becomes evident that by increasing the mole fraction, x_1, of the vis-

cosity-increasing component (e.g., of glycerol) we decrease the velocity, q_o, of cooling at which vitrification takes place. In this way, solutions can be obtained, vitrifying at experimentally accessible cooling rates, q_o. Quenching biological liquids into liquid nitrogen in fact exploits the possibility indicated with eq. 12 in a qualitative way in vitrifying living cells (e.g., spermatozoids) into the state of absolute anabiosis.

By using eq. 12, it is also possible to estimate the necessary percentage of viscosity-increasing substance in order to reach (at a given cooling rate, q_o) vitrification. Several examples in this respect give typical biological fluids, in which living cells are frozen-in (or "prepare" themselves to freeze-in) to a state of absolute anabiosis at different concentration, x_1, of appropriate viscosity-increasing dopants. This is one of Nature's strategies for preserving life at severe under-coolings and possibly also for spreading it through the Universe (remember Arrhenius' ideas for cosmic transspermia).

KINETICS OF NUCLEATION OF CaOX IN URINE

From the structure of the thermodynamic barrier, W_e (eq. 2), determined according to eq. 1 and the rate of nucleation it is evident that nucleation can be increased in three ways: by decreasing σ (introduction of surfactants [1,2]); by active insoluble substrates (crystallization cores [1,2]) with $\Phi << 1$; and by increasing the supersaturation, $\Delta\mu$. The first two methods, it seems (together with the formation of viscosity-increasing substances in the body fluids) are also used by some organisms in severe environments (in the high latitudes of our planet) to survive at –40 to –50 °C: they induce massive fine-grained crystallization in their own body fluids and thus survive extreme cold in another state of absolute anabiosis. Thus, Nature employs techniques known from present-day technologies of induced nucleation in glass ceramic materials: introduction of surfactants, active nucleation centers, and/or viscosity modifiers in order to trigger and govern induced nucleation in biological liquids [2].

However, in metastable systems, such as urine, nucleation has to be reduced or even eliminated. An electron microscopic investigation we performed to reveal the size distribution curves of CaOX crystallites formed in the urine of healthy persons and of persons chronically forming urinary stones showed great differences in both mean and modal size of the crystallites in the precipitates; in healthy persons, they were too small to sediment in the urinary tract. Our investigation showed that nucleation and especially growth rates were drastically suppressed in the urine of the "normals".

In considering nucleation in urine and risk factors for producing urolithiasis diseases (especially of CaOX crystallization [3,7,8]) it turns out (against convential thinking governing literature for many years) that here the substantial way to suppress (or even to preclude) nucleation in the supersaturated liquid is to decrease supersaturation, $\Delta\mu$.

This is achieved not so much by reducing the concentration of either Ca^{2+} (which is a biologically impossible task) or of oxalic ions (its concentration can be reduced by appropriate diet) but by the presence of Ca^{2+} or Ox^{2-} complexing ions [3,7–10]. An increasing ionic strength, ψ, also diminishes supersaturation according to eqs. 8 and 9.

In a thorough investigation [11,12], we determined the frequency distribution curves of both the concentration and the daily outputs of ionic strength factor determining ions (Na^+, K^+, Cl^-, etc.) and of complex-forming ions (Mg^{2+}, citrate anion, hippurate anion) in the urine of a relatively great number of both recurrent CaOX stone formers (indicated in Fig. 1 by SF, 318 persons) and of healthy controls (indicated in Fig. 1 by N, 80 persons). The results thus obtained are summarized in Table 1 and in Fig. 1 as follows:

Table 1 Most probable (modal) values of the concentration (in mMol/L) in the 24-h urine output samples of recurrent stone formers and of healthy volunteers.

	Sodium (Na^+)	Chloride (Cl^-)	Calcium (Ca^{2+})	Oxalate ($C_2O_4^{2-}$)	Magnesium (Mg^{2+})	Hippuric acid	Ionic strength (ψ)
Stone formers	85.0	76.0	2.9	0.3	1.6	1.0	0.18
Healthy volunteers	120.0	127.0	3.1	0.4	5.5	4.3	0.25

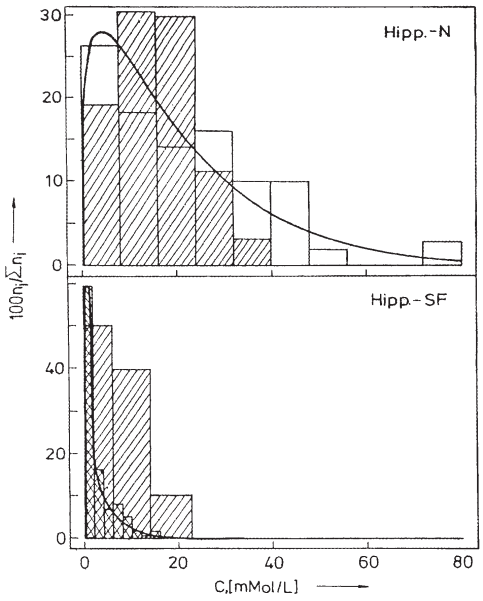

Fig. 1 Concentration of hippuric acid in healthy control persons (N) and in recurrent stone formers (SF). Upper plot: frequency distribution histogram for clinical normals and for clinical staff persons (open and shaded rectangles). Lower plot: frequency distribution for CaOX recurrent stone formers and for Ca-phosphate RSFs. Through the data for the first mentioned groups at every picture a distribution curve is drawn with a conventional computer program.

From the data presented, we calculated [11–13] the change in supersaturation, $\Delta\mu$, and risk factors of CaOX precipitation (and inhibition of nucleation) in both groups of investigated persons. In doing so (as also is qualitatively seen from Table 1), we observed that while ψ and the concentration of $[Mg^{2+}]$ and citrate ions are substantially changed according to previous data [7,8,10,14] (thus decreasing, also, $\Delta\mu$ in healthy persons) another normally present constituent of human urine is dramatically reduced in recurrent CaOX stone formers, as seen from Table 1 and Fig. 1. This is hippuric acid ($C_6H_5CONH_2{\cdot}COOH$). Although rarely mentioned, it is in fact present in human urine in considerable concentrations, as known from literature and seen also from Table 1. Its concentration in the urine of healthy persons as seen from Fig. 1 varies in broad limits and can be even more than ten times higher than in SF persons.

It is also known that hippurate acid is present in the urine of herbivorous mammals; for example, in the urine of horses (from whence its name is derived) it reaches concentrations up to 15 g/L. In this connection, it is also to be noted that herbivorous mammals, although having an enormous daily intake of oxalate ions (in the form of the water-soluble $Li_2C_2O_4$) do not as a rule form CaOX stones in their

urine. This, as well as our physiological data summarized in Table 1, seems to indicate that hippuric acid may be one of the essential (or even the main) regulator of CaOX supersaturation and growth in human urine. As previously reported [3,11], both inhibition possibilities of hippuric acid and the increased solubilities of CaOX crystals in both physiological solutions (with 0.3 M NaCl concentration, ensuring a "normal" ionic strength, $\psi = 0.25$) at various pH values, ranging from 2.7 to 7.0 and in artificial urine of conventional composition at pH = 5.2. These experiments gave as a first result a verification of a simple dependence, following directly from eq. 10 for the case A >> B (as it is in urine): that solubility, C_o, should be linearly increasing with the increased concentration, C_H, of the liophilizator

$$C(H) = C_o\,[1 + K_H \cdot C_H/\alpha_1] \tag{13}$$

These measurements, described in more details in [3] also indicated that hippuric acid is a natural, possibly biologically compatible liophilizator of CaOX calculi in urine.

KINETICS OF DISSOLUTION OF CaOX

Our experiments on the dissolution of CaOX were performed in artificial urine and the physiological solution in an apparatus in which with a torsion balance the dissolution of CaOX concretions suspended on a Pt wire was continuously followed. The volume of the solution, stirred by an electromagnetic propeller, was 1500 cm^3, and its temperature was 25 °C; the Archimedean weight $G(t)$ of the CaOX calculi samples (200–300 mg each) was measured with an accuracy of 0.5 mg, the duration of experiments was up to 60 days. In adding to the solution different concentrations of oxalate ions, the change of $G(t)$ was followed at different supersaturations at different concentrations, C_H, of hippuric acid. In Fig. 2, two typical curves (at $C_H = 0$ and at $C_H = 15$ mMol/L) are given at zero supersaturation ($C_{OX} = 0$) and at initially medium supersaturated urine. From the saturation plateaus and the initial slopes of each individual curve, the corresponding solubilities, C_o, were determined according to eq. 6.

From a biological standpoint and having in mind eventual medical applications, it is essential to note that it is possible thus to dissolve completely in 10–20 days in artificial urine at the "normal" super-

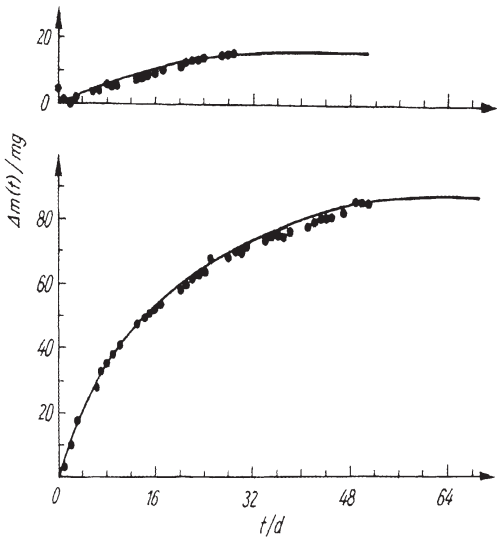

Fig. 2 Weight change $\Delta m(t) = G(0) - G(t)$ in the dissolution kinetics of CaOX concretions in artificial urine at zero supersaturation. Upper plot: solution without hippuric acid. Lower plot: solution with 15 mMol/L hippuric acid.

saturating values, prevailing there and at still physiologically possible pH values (pH = 5.4), CaOX calculi having a weight of ~100 mg.

The chemical and structural aspects of hippuric acid as a calcium complex-forming agent, its possibilities and limitations (at higher pH values) are discussed in detail in the literature [3,11,13,15]. A quantitative comparison with conventionally known CaOX liophilizators (Na-EDTA) and supersaturation ion (Mg^{2+}, citrate ions) is given in refs. [3,11]; in ref. [15] its CaOX-inhibiting properties are also reported, however, at physiologically inadmissibly high pH values. In refs. [3,11] may also be found a discussion of possible applications of hippuric acid as natural biological agent in instrumental haemolysis in a way, similar to the usage of Complexon III (Na-EDTA) in present-day medicine. In refs. [11–13] are also described our experiments to increase concentration of hippuric acid in the organism and in urine by administering to patients benzoic acid derivatives, which, according to a well-known biochemical reaction, metabolize in the organism to an increased concentration of hippuric acid in urine.

SOLUBILITY AND DISSOLUTION OF CaOX IN OTHER BIOLOGICAL COMPLEXING AGENTS

The possible solubilizing and inhibiting effect of various oxy-acids (tartaric, malic, citric) on CaOX is known or anticipated in literature. In a recent publication [6], we reported results on the increase in the solubility of CaOX under the influence of another simple amino acid, DL-lysine, which is also a normal constituent of human urine. We also obtained results similar to those with hippuric acid and lysine [5] with another constituent of human urine: α-ketoglutaric acid. Some of these are shown in Fig. 3. In the experiments described [5,6], the formalism and the apparatus and methods described above were used. In considering the physiological effect of hippuric acid, it has to be noted that its concentration surpasses the concentration of the sum of all other amino acids in human urine.

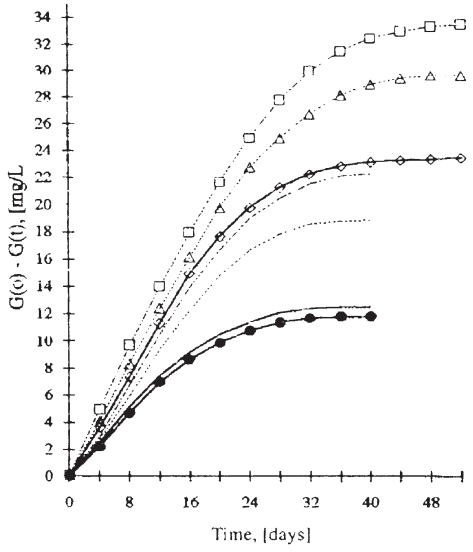

Fig. 3 Weight change $G(0) - G(t)$ in the dissolution of CaOX concretions in artificial urine solutions under the influence of α-ketoglutaric acid. Curves from bottom to top: dissolution kinetics at zero supersaturation without ketoglutaric acid and then with initial supersaturation and with ketoglutaric acid concentration increasing from 0 to 5 mMol/L.

CONCLUDING REMARKS

The simple analysis of processes of crystal nucleation and growth in physiological solutions, performed in the framework of the classical theory of nucleation and using the concepts of present-day physical chemistry of multicomponent ionic solutions, reveals the mechanisms of preventing or directing crystallization Nature uses. It is worth making a recapitulation in this respect.

In mammal urine, the task to be solved is to stabilize a metastable, supersaturated physiological solution against crystallization. Here, our investigation shows that the main role is played by ionic complex-formers in the solution: they decrease crystallization supersaturation and thus diminish nucleation rates. It seems that in this process hippuric acid plays a major role in mammal urine. In vitrification of life, a similar effect is achieved by increasing the viscosity of the solution, and thus again to diminish nucleation rates to such values as to avoid crystallization at a given cooling rate and to achieve glass transition.

However, another way is also possible: the contrary possibility of increasing nucleation rates, thus inducing microcrystallization and so avoiding cell destruction at low temperatures in organisms exposed to severe cold. A similar possibility of increasing nucleation rates seems to be used by Nature in insects, amphibians, reptiles, and birds to free the organism of the unnecessary or even dangerous products of metabolism. Instead of stabilizing them in the supersaturated solution of urine (as it is in mammals), their microcrystallization in cloacal excrements is induced. The mechanism of this process is at present not clear in detail, but eqs. 1 and 2 and the discussion in Section 2 give the necessary qualitative background. These are only two examples of the mechanisms and ways Nature has developed in organisms when problems of nucleation and crystal growth are involved. Because "Nature creates nothing without a purpose, but always for the best possible. If one way is better than another, that is the way Nature goes" as stated many years ago by Aristotle.

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