Urol Res (2011) 39:429–436
DOI 10.1007/s00240-011-0387-5

ORIGINAL PAPER

Supersaturation of body fluids, plasma and urine, with respect to biological hydroxyapatite

Otakar Söhnel · Felix Grases

Received: 21 July 2010 / Accepted: 26 April 2011 / Published online: 14 May 2011

Abstract Supersaturation of body fluids, specifically of plasma and urine, with respect to biological hydroxyapatite was evaluated taking into account calcium complexation, fraction of total phosphorus present as hydrogen phosphate ions, solubility of carbonated hydroxyapatite and the size dependency of equilibrium solubility. Plasma is always supersaturated with respect to apatitic solid phase and thus calcific deposits are formed unless a sufficient quantity of potent inhibitors is present. When urinary pH is lower than 6.3 for normal urine hydroxyapatite cannot appear in renal stones, at higher pH apatitic renal stones can be formed. Predictions based on supersaturation calculated for different conditions correspond well with clinical observations.

Keywords Urine · Plasma · Supersaturation · Hydroxyapatite

Introduction

Biological hydroxyapatite of various composition (hereafter BHAP) is a principal constituent of bones, teeth, some renal calculi and calcified tissue in all vertebrates. Although a substantial amount of information about stochiometric hydroxyapatite, $Ca_{10}(OH)_2(PO_4)_6$ (hereafter HAP) and BHAP is available from in vivo and in vitro studies, conditions under which calcification is formed in living organisms is not well understood.

There is a controversy surrounding the question of whether estimating the supersaturation of body fluids with respect to the formed HAP is useful or meaningless and even whether the supersaturation plays any significant role in calcification [1–3]. Proponents of the negative viewpoint argue that calcium is present as ions, ion pairs and a number of complexes. Therefore not all of the total calcium present is available for precipitation of substance composed of ionic species. The same applies to phosphorus because at physiological pH phosphorus is present as both $H_2PO_4^-$ and HPO_4^{2-} ions and their ratio is a sensitive function of pH. Also the solubility of the solid phase varies with its composition, thus the correct solubility product must be applied when estimating the driving force of the process. Accordingly, it is claimed that unless the correct solubility product and all species that exist in a solution are taken into account no reasonable supersaturation can be determined. Moreover, the entire concept that calcification is a result of supersaturation has been questioned and regarded as biologically flawed.

In this paper, we examine the conditions at which solid phosphate calcifications from human body fluids, plasma and urine, can be formed and the significance of supersaturation characterizing such state.

Supersaturation estimation

All natural processes, including calcification of tissue, kidney stone and bone formation, are natural and hence, in the sense of thermodynamics, irreversible processes. As physical chemistry documents, any process including those that are irreversible can proceed only in the direction of

F. Grases (✉)
University Institute of Health Sciences Research,
University of Illes Balears, 07071 Palma de Mallorca, Spain
e-mail: fgrases@uib.es

O. Söhnel
Faculty of Enviromental Studies,
University of J. E. Purkyne, Usti n.L., Czech Republic

decreasing Gibbs free energy. The formation of a solid phase from a liquid medium, i.e. crystallization or precipitation, can proceed if, and only if, the chemical potential of the precipitating substance in the liquid medium (e.g. solution) exceeds that in the solid state (e.g. crystal). The difference between these chemical potentials represents the thermodynamic driving force of the process.

The dimensionless thermodynamic driving force, Φ/RT, of crystallization for an anhydrous compound crystallizing from a solution having common ions with the crystallizing compound is expressed as

$$\Phi/RT = \ln\{(c_+^{\nu+}\gamma_+^{\nu+}c_-^{\nu-}\gamma_-^{\nu-})/K_{sp}\} \quad (1)$$

where c is the concentration, γ activity coefficient, ν stoichiometric coefficient, K_{sp} is the thermodynamic solubility product (expressed in activities) and the subscripts "+" and "−" indicate cation and anion, respectively [4]. The tendency towards crystallization is routinely assessed in terms of the supersaturation solution 'S' with respect to the crystallizing compound

$$S = \{c_+^{\nu+}\gamma_+^{\nu+}c_-^{\nu-}\gamma_-^{\nu-})/K_{sp}\}^{1/\nu} = \left\{K_{ap}/K_{sp}\right\}^{1/\nu} \quad (2)$$

where K_{ap} represents the activity product of the compound in solution prior to precipitation.

Plasma

The supersaturation of plasma with the composition Na^+ 142, K^+ 5, Mg^{2+} 1.5, the total concentration of calcium and phosphorus expressed as Ca^{2+} and HPO_4^{2-} is 2.5 and 1 respectively, Cl^- 103, HCO_3^- 27, and SO_4^{2-} 0.5 mmol/l, pH 7.4 [5], with respect to stoichiometric HAP can be calculated as follows: because the ionic strength $I = 0.5\,\Sigma c_i z_i^2$ of plasma with the given composition equals 0.1495 mol/l, ionic activity coefficients are calculated using Davis equation [6]

$$\log\gamma_i = -Az_i^2\{[\sqrt{I}/(1+\sqrt{I})] - 0.3I\} \quad (3)$$

with $A = 0.5242$ for 37°C acquire the value $\gamma_i = 0.323$ for both Ca^{2+} and HPO_4^{2-}. The calculated value of $\gamma_{Ca^{2+}}$ is reasonably close to the measured value of 0.537 at similar ionic strength, but at 25°C [7] if inverse proportionality of activity coefficients to temperature was considered.

Rearrangement of the activity product K_{ap} into a form containing the ionic species HPO_4^{2-} present in plasma instead of PO_4^{3-} appearing in K_{sp} of HAP gives

$$\begin{aligned} K_{ap} &= \left[Ca^{2+}\right]^5\left[PO_4^{3-}\right]^3[OH^-]\gamma_i^8 \\ &= K_3^3K_w\left[Ca^{2+}\right]^5\left[HPO_4^{2-}\right]^3[H^+]^{-4}\gamma_i^8 \end{aligned} \quad (4)$$

where the third thermodynamic equilibrium constant of H_3PO_4 is $K_3 = 6.84\times10^{-13}$ mol/l [8] and the ionic product of water $K_w = 2.4\times10^{-14}$ mol^2/l^2 [8], both at 37°C.

Calcium bound in stable complexes with organic molecules present in plasma does not participate in HAP formation and hence correction for this effect must be introduced. Moore reports [9] only 47% of total serum calcium is present in ionized form and the rest is bound in complexes with bicarbonate, citrate and proteins. Further, only 62% of total phosphorus is present as HPO_4^{2-} ions at pH 7.4 which can be determined from the dissociation equilibria of H_3PO_4 using phosphoric acid as a equilibrium constants reported previously [8]. Taking these values into consideration K_{ap} becomes 1.93×10^{-49} mol^9/l^9. Plasma supersaturation with respect to HAP of the thermodynamic solubility product $K_{sp} = (2.03 \pm 0.71)\times10^{-59}$ mol^9/l^9 at 37°C [8], as defined by Eq. 2 is in the range

$$\begin{aligned} S &= \{1.93\times10^{-49}/(2.03\pm0.71)\times10^{-59}\}^{1/9} \\ &= 12.4 - 13.5 \end{aligned} \quad (5)$$

where $\gamma_{Ca^{2+}} = 0.323$ was used since the considered decrease in Ca^{2+} and HPO_4^{2-} concentration has a negligible effect on plasma ionic strength and hence on calculated value of activity coefficients.

The substance precipitating from body fluids has been considered to be stoichiometric HAP. However, the chemical composition of the phosphate solid-phase formed under physiological conditions differs from that of stoichiometric HAP.

The composition of BHAP in bones corresponds to the non-stoichiometric ion-substituted and calcium-deficient hydroxyl-carbonate apatite with the formula $Ca_{8.3}(PO_4)_{4.3}(HPO_4,CO_3)_{1.7}(OH,CO_3)_{0.3}$ [10]. BHAP from biological tissues has been reported to contain 5–6% carbonate [11]. The molar ratio of Ca/P in such phosphate deviates from the value of 1.67 for HAP and varies between 1.5 and 1.7 depending on the site and bone age [10]. Also the chemical composition of calcific deposits on natural and bioprosthetic heart valves expressed as the Ca/P molar ratio was reported to vary from 1.62 to 2.13 (average 1.83) and 1.33 to 2.01 (average 1.52), respectively. The solid phase in both cases was identified as hydroxyl-carbonate apatite that also contained Mg and Na [12, 13].

The average composition of calcific deposits on natural heart valves is $Ca_{4.55}Mg_{0.2}$ $(Na,K)_{0.5}(PO_4)_{2.9}(CO_3)_{0.15}OH$ [14]. Its ionic product rearranged so as to contain HCO_3^- instead of CO_3^{2-} gives

$$\begin{aligned} K_{ap} = {} & \left[Ca^{2+}\right]^{4.55}[Mg]^{0.2}[Na,K]^{0.5}\left[HPO_4^{2-}\right]^{2.9}\left[HCO_3^-\right]^{0.15} \\ & [H^+]^{-4.05}K_3^{2.9}K_2^{0.15}K_w\gamma_i^8 \end{aligned} \quad (6)$$

where the second dissociation constant of H_2CO_3 is $K_2 = 4.69\times10^{-11}$ mol/l.

The equilibrium solubility of the non-stoichiometric BHAP is higher than that of stoichiometric HAP and depends on BHAP composition and structure [2, 15], specifically, the solubility of carbonated hydroxyapatite (hereafter CHAP) increases with increasing CO_3^{2-} content in solid-phase [16]. The equilibrium solubility of BHAP containing also Mg and Na is not known. Therefore the solubility of CHAP containing the same amount of carbonate as BHAP in composition reported in [16] is used for further calculations as the best available estimate of BHAP solubility. Solubility of CHAP containing the same amount of carbonate as calcific deposits (1.32 wt% of carbonate expressed as CO_2) is calculated from the modified Eq. 2 reported in [16]

$$pK_{sp} = 117.38 - 0.47316 \times (CO_2 \text{ wt.}\%)^{2.4176} \quad (7)$$

where the equilibrium solubility of HAP is taken from [8]. Inserting the composition of plasma given above (corrected for Ca complexation) and the fraction of phosphorus present as HPO_4^{2-} into Eq. 6 and using the solubility product of CHAP $K_{sp} = 5.96\times10^{-59}$ mol^9/l^9 calculated from Eq. 7 gives supersaturation of plasma with respect to BHAP containing the same amount of carbonate as calcific deposits as

$$S = \left\{5.57 \times 10^{-49}/5.96 \times 10^{-59}\right\}^{1/9} = 12.8. \quad (8)$$

The solid apatitic phase formed in biological systems always appears as a very small nanometre-sized particles. Thus its equilibrium solubility should be higher than that inferred from the solubility product which pertains to large crystals. The Gibbs–Thomson equation [4] relates equilibrium solubility and crystal size

$$\ln a(r)/a(\infty) = 2k_a\sigma V_m/3k_v vRTr. \quad (9)$$

In Eq. 9 $a(r)$ and $a(\infty)$ represent the activity of the compound in solution which is in equilibrium with the crystals of characteristic size r and an indefinitely large crystal, respectively, k_a and k_v are shape factors of area and volume respectively, σ is the surface tension of the solid compound, V_m is the molar volume of the compound, v the number of ions, R the gas constant and T is the absolute temperature. The solution concentration in equilibrium with small and large crystals will not differ greatly and therefore the respective activity coefficients will be very similar. Their ratio will be very close to 1 allowing the concentrations instead of activities to be used in Eq. 9. Substituting the concentrations for activities, assuming spherical particles for which $2k_a/3k_v$ equals 2 and inserting $v = 9$, $\sigma = 0.045$ J/m^2 [17], $V_m = 1.63\times10^{-4}$ m^3/mol (density 3,080 kg/m^3 [18]), $R = 8.31$ J/K and $T = 310$ K gives

$$\ln a(r)/a(\infty) = 6.33 \times 10^{-10}/r. \quad (10)$$

From Eq. 10 it transpires that for crystals with characteristic size $r = 0.35$ and 0.5 nm equilibrium solubility is 6.1 and 3.5 times higher, respectively, than for a large crystal. The solubility product of CHAP containing 1.32 wt% of carbonate and consisting of particles with diameter of 0.7 and 1 nm increases to 6.93×10^{-52} and 4.67×10^{-54} mol^9/l^9 and therefore plasma supersaturation with respect to this CHAP decreases from 12.8 (see Eq. 8) to 2.1 and 3.7.

Thus supersaturation of plasma with respect to the initial size of BHAP solid-phase originating from plasma is in the range 2–4.

Urine

Unlike plasma composition, the composition of human urine varies widely not only among individual people but also within a given individual over the course of a day. However, taking Na^+ 75, K^+ 30, Mg^{2+} 1.8, total calcium and phosphorus expressed as Ca^{2+} and HPO_4^{2-}, 2.5 and 5, Cl^- 100, and NH_4^+ 7 mmol/l and pH 6 as a representative average composition of urine, the ionic strength of urine is 0.1346 mol/l and the activity coefficients were calculated using Eq. 3 $\gamma_i = 0.332$ for both Ca^{2+} and HPO_4^{2-}. Only 6.17% of the total phosphorus is present as HPO_4^{2-} ions at pH 6 as determined from the dissociation equilibria of H_3PO_4 using previously reported equilibrium constants [8]. The percentage of calcium in the ionic form is reported to vary between 43 and 50 of total calcium present [19–21]. However, since various ionic species and ion pairs containing Ca and Mg and phosphate are (1) intermediates of calcium phosphate clusters formation in supersaturated solution and (2) substantially less stable than the complexes with citrate [22], only correction for Ca bound to citrate will be taken into account. Since formation of strong calcium complexes with citrate bound from 20 to 25% of present calcium [23], 78% of total calcium present is considered to participate in the stone forming processes. Inserting urine composition accordingly corrected into Eq. 4 gives $K_{ap} = 9.38\times10^{-55}$ mol^9/l^9, hence urine supersaturation with respect to the stoichiometric HAP is

$$S = \left\{9.38 \times 10^{-55}/(2.03 \pm 0.71) \times 10^{-59}\right\}^{1/9} = 3.2 - 3.5. \quad (11)$$

Further introducing a correction for size-dependent equilibrium solubility (see section plasma), supersaturation is reduced from 3.3 (supersaturation calculated using the

HAP average K_{sp} value) to undersaturation 0.54 and 0.94 for particle of 0.7 and 1 nm in diameter, respectively.

The urine of people suffering from hypercalciuria and hyperphosphaturia exhibits an increased concentration of total Ca^{2+} up to 6.2 mmol/l and total PO_4^{3-} up to 21 mmol/l. Supersaturation of such urine with respect to HAP (CHAP cannot be formed as carbonate in urine is not present at pH 6) assuming that 78% of total calcium present and only 6.17% of total phosphorus present in the form of HPO_4^{2-} is active at stone forming process becomes 5.5 and 5.3 in the case of hypercalciuria and hyperphosphaturia, respectively. Taking into account the size-dependency of solubility, supersaturation decreases to 1.5 in both cases for particles of 1 nm in diameter.

At a pH < 6.2 urine does not contain any hydrogencarbonate ions. However, in cases where pH exceeds 6.2 and metabolic alkalosis exists, then the concentration of HCO_3^- ions in urine reaches about 1 mmol/l and in an extreme case when pH = 7.6 it may even reach up to 80 mmol/l [24]. The main component of phosphate calculi studied in [25] was carbon, calculi contained on average 2.5 times more carbon than calcium in molar terms and large variations in carbon content within the same sample were observed. Major part of carbon was of organic origin and the fraction representing an inorganic carbon (from carbonate) was not reported and hence its precise composition is not known. Therefore, solid phase precipitating from urine under these conditions is assumed to be fully CHAP which contains 3 wt% of carbon expressed as CO_2 of chemical formula $Ca_5OH(PO_4^{3-})_{2.77}(CO_3^{2-})_{0.345}$. The ionic product of this compound is

$$K_{ap} = \left[Ca^{2+}\right]^5 \left[HPO_4^{2-}\right]^{2.77} \left[HCO_3^-\right]^{0.345} \left[H^+\right]^{-4.115} K_3^{2.77} K_2^{0.345} K_w \gamma_i^8. \quad (12)$$

Supersaturation of urine with respect to CHAP at pH 7 using K_{ap} calculated from Eq. 12 and assuming that only 78% of total calcium present is available for the solid-phase formation, 46% of total phosphorus is in the form of HPO_4^{2-} and HCO_3^- concentration is 1 mmol/l becomes

$$S = \left\{1.57 \times 10^{-48} / 4.77 \times 10^{-56}\right\}^{1/9} = 6.8 \quad (13)$$

where $K_{sp} = 4.77 \times 10^{-56}$ mol^9/l^9 was calculated from Eq. 7. At the extreme case when pH is 7.6 (72% of total phosphorus is present as HPO_4^{2-}) and HCO_3^- concentration is 80 mmol/l, supersaturation becomes

$$S = \left\{7.32 \times 10^{-45} / 4.77 \times 10^{-56}\right\}^{1/9} = 17.5. \quad (14)$$

BHAP appears in renal calculi as both small spheres up to 10 μm in diameter and as so-called "aspidinic" layers. The interior of spheres and the seemingly structureless "aspidinic" layer appears to be composed of densely packed nano-sized spherical particles [26]. When the size dependency of equilibrium solubility of particles with 0.7 and 1 nm in diameter is taken into account, urine supersaturation with respect to CHAP decreases from 6.8 to 1.1 and 1.9 and for the extreme case from 17.5 to 2.8 and 4.8.

Discussion

Supersaturation of any liquid with respect to a certain substance (solute) is a critical factor for solute precipitating from solution as a solid phase. However, supersaturation is a requisite, but not sufficient, condition for the formation of a solid phase in a liquid. Formation of solid phase is further dependent on various factors, namely the degree of supersaturation, which depends on actual composition of solid phase, size of initial solid-phase nuclei, presence of active heterogeneous nuclei or places facilitating nucleation and, in particular, the presence or absence of efficient crystal growth inhibitors.

Different values of supersatuation with respect to a solid phosphate precipitating from body fluids can be acheived when various corrections are considered. To approach the real value of body fluids supersaturation, we have to take into account as many corrections as possible, namely activity coefficients, stable complexes formation, composition of species forming initial solid phase and size dependency of solid phase solubility.

Activity coefficients of ions are just estimates that follow from some of the suggested relations all of which are based on the Debye–Hückel theory. Therefore the actual activities of ions in body fluids do not necessarily coincide with the estimated values and the difference in the actual and estimated activity coefficients results in different absolute values of calculated supersaturation.

Formation of weak complexes of Ca and Mg with carbonate and phosphate is not considered in supersaturation calculations. Not only individual ions but also appropriate inorganic complexes of Ca and Mg represent building units of solid phase, at least at the initial stages of BHAP precipitation. However, our current knowledge of structure, composition and dynamics of the initial solid phase formation does not make it possible to determine the extent to which these complexes participate at the initial solid phase formation. Therefore, formation of only strong complexes of Ca and Mg with organic molecules, such as citrate or peptides, which cannot be incorporated into crystalline lattice is considered. Since the association constants of these complexes are not known with any certainty [27] we have to accept the result reported in [9, 23] as the best

available estimate of Ca^{2+} ions complexation in plasma and urine, respectively.

The composition and crystalline character of the precipitating solid phosphate plays a crucial role in supersaturation evaluation because apatitic solid-phases of the different composition exhibit different equilibrium solubility and hence the different solubility products [15]. Precise characterization of the initial phosphatic solid-phase formed in plasma or urine in living organisms is a weak link in this analysis as it is virtually impossible to isolate such precipitate and prevent the changes that occur during its aging in contact with body fluids and also sample preparation. Hence we are left with estimations based on in vitro experiments.

According to the Ostwald's rule of stages [4] the formation of a thermodynamically stable phase is preceded by that of less stable phase(s) i.e. metastable phase(s) having a higher solubility than stable phase. In vitro studies [28, 29] have demonstrated that formation of HAP is always preceded by the formation of a metastable modification such as amorphous calcium phosphate $Ca_xH_y(PO_4)_z{\cdot}nH_2O$ with 15–20% of water [30], octacalcium phosphate $Ca_4H(PO_4)_3{\cdot}2.5H_2O$ or $CaHPO_4{\cdot}2H_2O$ depending upon the prevailing reaction conditions. The metastable modification is later transformed via an appropriate mechanism (solution mediated process or solid-state transformation) into the final stable modification [e.g. 31–33]. Similarly, an initially precipitated solid-phase of calcium carbonate was amorphous which transformed into a crystalline phase in later stages of the process [34]. Thus amorphous calcium phosphate (hereafter ACP) of variable composition is an initial phosphatic phase that precipitates from body fluids [35–37].

The dynamic light scattering method revealed the existence of amorphous clusters of solute ranging from 0.7 to 1 nm in diameter in a simulated body fluid supersaturated and even slightly undersaturated with respect to HAP. The fraction of particles aggregated into clusters increased with increasing supersaturation and reached a maximum at $S = 8$ when about 50% of all present particles were aggregated. This fraction remained unchanged with further increase in S. Simulated body fluid with $S \sim 14$ remained transparent with no sign of solid phase appearance for days despite the presence of clusters. Based on indirect evidence the composition of clusters was determined to be $Ca_9(PO_4)_6$ [35]. The occurrence of clusters of 0.6–1.1 nm in diameter also in a solution supersaturated with respect to $CaCO_3$ was confirmed by direct observation [34]. Hence, occurrence of solid phase in supersaturated solution is preceded by the formation of solute clusters which are present already in just saturated solution. Spontaneous formation of apatitic solid-phase begins in the supersaturation range of 17–23 as can be deduced from previously reported data [36, 37].

The solubility product of the initially precipitating solid-phase should be taken into account in calculating the supersaturation of body fluids at which precipitation starts, that is at the onset of calcification formation. However, the solubility product of initially formed ACP is difficult to establish with any certainty due to its instability in contact with a liquid medium. A value of $10^{-25.7}$ has been quoted [30] but without any specification of solid-phase composition and therefore is of little use. Moreover, this value is definitely too high, since body fluids including urine would be immensely undersaturated and thus no calcific deposit could ever be formed.

The size dependency of crystal equilibrium solubility as transpired from thermodynamics has to be taken into account. Confirmation that this effect applies also to HAP was demonstrated experimentally. The solubility product determined for 5 nm HAP particles was $K_{sp} = 1.8{\times}10^{-47}$ mol^9/l^9 [38]. This rather high solubility product highlights the fact that the method employed to prepare small particles generated heavily carbonated CHAP. The experimentally determined value of the nano particle solubility increase is within the range of the equilibrium solubility change attributable to a particle size effect predicted by Eq. 10. Thus the size-dependent supersaturation should be applied in cases where solid particles smaller than, say, 5 nm in diameter are under consideration, which is the case especially at commencement of calcific deposit formation.

Supersaturation of plasma, no matter how it is calculated, is in the range where tiny clusters (around 1 nm) of ions or molecules are already present in liquid medium but spontaneous (homogeneous) precipitation does not take place for a certain time period, in our case days [35, 36]. However, deposits are always formed in tissue of heart valve, where stagnant hydrodynamic conditions prevail and composition of surrounding fluid changes only slowly by diffusion of ions from plasma flowing around the valve. Thus, there is enough time (years) for clusters to aggregate and form an amorphous solid which subsequently may transform into a crystalline deposit. Deposits can also develop when an organic matter in contact with plasma provides sites promoting nucleation (serving as active heteronuclei) and/or inhibitors of crystallization which are not present in a sufficient quantity. Taking into account that bioprosthetic valves are more prone to calcific deposits formation than natural human valves [39], the existence of sites supporting nucleation seems to play a significant role in calcification. On the other hand, the absence of severe calcification in majority of population despite plasma supersaturation with respect to CHAP suggests a decisive role of potent crystal growth inhibitors on deposits formation. A different situation arises once such a calcific deposit is formed. These deposits may begin to grow through attachment of building units, ions, ion pairs and

clusters, depending on prevailing concentration of inhibitors in plasma and also on supersaturation magnitude which increases from about 3 to approximately 13 with increasing calcific deposit size due to diminishing size-dependency correction. Increase in supersaturation results in increasing calcific deposit growth rate.

Although we are somewhat uncertain about the absolute supersaturation value of real body fluids, the trends in supersaturation changes with changing composition and pH remain similar irrespective of the absolute value of supersaturation. These trends clearly indicate which conditions are more favourable for commencement of formation and/or further development of calcific deposits.

The concentration of calcium and phosphate in the plasma of individuals can differ significantly from values used for the above calculations and can reach for calcium 10 mmol/l in case of hypercalcemia and phosphorus 3 mmol/l in case of hyperphosphatemia. The supersaturation of plasma with respect to CHAP of 1 nm in size calculated from Eqs. 6 and 10 is in the case of hypercalcemia 7.4 and hyperphosphatemia 5.2, instead of about 3 calculated for "normal" plasma composition. Hence, individuals suffering from both hypercalcemia or hyperphosphatemia are subject to increased risk of calcification development, although the risk is more pronounced in the case of hypercalcemia. However, a more rapid progression of previously formed deposits can be expected in both cases since the growth rate of the solid-phase is proportional to supersaturation [40]. This conclusion is corroborated by data reported in [1]. Plotting data in Fig. 1 of this study reporting the extent of calcification extent of rat aortas at varying concentrations of calcium and phosphate as a function of ionic product $[Ca^{2+}]^5[PO_4^{3-}]^3$ which in turn is directly proportional to supersaturation (through a constant incorporating K_{sp} and other constants, activity coefficients and pH) results in a steeply rising curve.

Clinical observations [1] have clearly demonstrated that the simple product [Ca].[P] has no connection to the appearance of calcification and that plasma is supersaturated with respect to HAP even at physiological calcium and phosphate levels. For formation of calcification specific conditions, such as a deficiency of inhibitors, must exist. These observations are consistent with the conclusions arrived at above based on evaluation of plasma supersaturation.

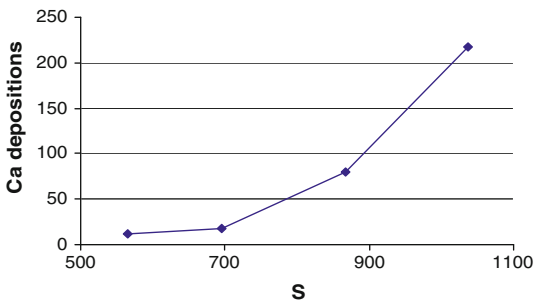

Fig. 1 Calcification of rat aortas as a function of serum ionic product $[Ca^{2+}]^5[PO_4^{3-}]^3$ Ca depositions expressed in Ca^{2+} nmol/mg

As "normal" urine is undersaturated with respect to HAP it cannot originate by neither spontaneous nor heterogeneous nucleation and therefore cannot appear in calculi formed under these conditions. The urine of people suffering from hypercalciuria and hyperphosphaturia is slightly supersaturatated with respect to HAP and thus appearance of HAP in renal calculi cannot be excluded despite a short time for solid occurrence (several hours) namely under specific conditions, such as presence of active heteronuclei and/or decreased amount of efficient inhibitors. This is corroborated by clinical observations that HAP in renal calculi is virtually not present when urinary pH is 6.3 or less [41]. However, uroliths formed at urinary pH exceeding 6.8 consisted mainly of HAP [26]. Supersaturation of "normal" urine at pH 7 with respect to HAP calculated from Eq. 4 becomes 15.4 which corresponds to the onset of HAP spontaneous nucleation in the supersaturation range of 17–23 as can deduced from previously reported data [37, 42] (both values of supersaturation are not corrected for size-dependency of solubility and hence are comparable). Similarly, in cases where pH of urine approaches 7 and metabolic alkalosis exists, uroliths consisting of BHAP can be formed.

The satisfactory correspondence between clinical observations and expectations predicted based on calculated supersaturation both for plasma and urine indicates that the calculated values represent a good approximation of the actual supersaturation in urine when appropriate corrections are taken into account.

Conclusions

Supersaturation of body fluids is a requisite condition for the occurrence of calcification but other factors, such as the level of inhibitors present and the existence of places which can be suitable templates to play an important role in the process of calcific deposits formation.

Plasma is supersaturated with respect to stoichiometric hydroxyapatite, HAP and to a lesser extent with respect to BHAP, namely non-stoichiometric CHAP, which is the main constituent of the calcific deposits that develop in plasma of living organisms. Due to stagnant hydrodynamic conditions in the heart valve and long development time deposits tend to develop unless efficient inhibitors of crystallization are present.

HAP represents a major constituent of uroliths only if urinary pH exceeds about 7, when metabolic alkalosis

exits, then uroliths consists of BHAP. However, when urinary pH is lower than 6.2 apatitic calculi do not appear.

To obtain calculation results that most closely approximate the actual supersaturation of body fluids associated with the formation of calcium organic complexes, the actual concentration of monohydrogenphosphate ions and size-dependent solubility when dealing with nanoparticles of less than 5 nm in diameter must also be taken into account. Given that clinical observation of calcification and uroliths are in line with the expectations inferred from supersaturation magnitudes determined under different conditions, calculated body fluids supersaturation values appear to represent a good approximation of reality.

References

1. O′Neil WC (2007) The fallacy of the calcium-phosphorus product. Kidney Int 72:792–796
2. Zhuofan Ch, Baoxin H, Haobo P, Darvell BW (2009) Solubility of bovine-derived hydroxyapatite by solid titration, pH 3.5–5. Cryst Growth Des 9:2816–2820
3. Chen ZF, Darwell BW, Leung VWH (2004) Hydroxyapatite solubility in simple inorganic solutions. Arch Oral Biol 49:359–367
4. Söhnel O, Garside J (1992) Precipitation: basic principles and industrial applications. Butterworth-Heinemann, London
5. Takeuchi A (2005) Heterogeneous nucleation of hydroxyapatite on protein: structural effect of silk sericin. J R Soc Interface 2:373–378
6. Davies CW (1962) Ion association. Butterworths, London
7. Buttler JN (1968) The thermodynamic activity of calcium ion in sodium chloride–calcium chloride electrolytes. Biophys J 8:1426–1433
8. National Institute of Standards & Technology (1997) SRM 2910. Gaithersburg, MD 20899:1–8
9. Moore EW (1970) Ionizied calcium in normal serum ultrafiltrates, and whole blood determined by ion-exchange electrodes. J Clin Invest 49:318–334
10. Stanciu GA, Sandulescu I, Savu B, Stanciu SG, Paraskevopoulos KM, Chatzistavrou X, Kontonasaki E, Koidis P (2007) Investigation of the hydroxyapatite on bioactive glass surface. J Biomedic Pharm Eng 1(1):34–39
11. LeGeros RZ (1981) Apatites in biological systems. Prog Crystal Growth Charact 4:1–45
12. Mikroulis D, Mavrilas D, Kapolos J, Koutsoukos P-G, Lolas C (2002) Physicochemical and microscopical study of calcific deposits from natural and bioprosthetic heart valves comparison and implications for mineralization mechanism. J Mater Sci: Mater Med 13:885–889
13. Delogne Ch, Lawford PV, Habesh SM, Carolan VA (2007) Characterization of the calcification of cardiac valve bioprostheses by environmental scanning electron microscopy and vibrational spectroscopy. J Microscopy 228(1):62–77
14. Prieto RM, Gomila I, Söhnel O, Costa-Bauza A, Bonnin O, Grases F (2011) J Biophys Chem 2(1):19–25
15. Pan HM, Darwell BW (2010) Effect of carbonate on hydroxyapatite solubility. Cryst Growth Des 10:845–850
16. Ito A, Maekawa K, Tsutsumi S, Ikazaki F, Tateishi T (1997) Solubility product of OH-carbonated hydroxyapatite. J Biomed Mater Res 36:522–528
17. Christoffersen JJ (1980) Kinetics of dissolution of calcium hydroxyapatite III nucleation-controlled dissolution of a polydisperse sample of crystals. J Crystal Growth 49:29–44
18. Mineralogy Database, webmineral.com
19. Robertson WG (1969) Measurement of ionized calcium in biological fluids. Clin Chim Acta 24:149–157
20. Maxwell MH, Kleeman CR, Narins RG (1987) Clinical disorders of fluid and electrolyte metabolism, 4th ed edn. M C Graw-Hill, NY
21. McClathey KD (2002) Clinical laboratory medicine, 2nd edn. Lippincott Williams & Wilkins, Philadelphia
22. Meyer JL (1974) Formation constants for interaction of citrate with calcium and magnesium ions. Anal Biochem 62(1):295–300
23. Nicar MJ, Hill K, Pak CYC (1987) Inhibition by citrate of spontaneous precipitation of calcium oxalate in vitro. J Bone Miner Res 2(3):215–220
24. Pitts RF, Ayer JL, Schiess WA, Miner P (1996) The renal regulation of acid-base in man. III. the reabsorption and excretion of biocarbonate. J Clin Invest 28(1):35–44
25. Grases F, CostaBauza A, Prieto RM, Gomila I, Pieras E, Söhnel O, Scand J Clin Lab Invest In press
26. Grases F, Söhnel O, Vilacampa AI, March JG (1996) Phosphates precipitationf from artificial urine and fine structure of phosphate renal calculi. Clin Chem Acta 244:45–67
27. Pedersen KO (1972) Binding of calcium to serum albumin III influence of ionic strength and ionic medium. Scand J Clin Lab Invest 29:427–432
28. Abbona F, Madsen HEL, Boistelle R (1986) The initial phases of calcium and magnesium phosphates precipitated from solutions of high to medium concentrations. J Crystal Growth 74:581–590
29. Abbona F, Franchini-Angela M (1990) Crystallization of calcium and magnesium phosphates from solutions of low concentration. J Crystal Growth 104:661–671
30. Dorozhkin SV (2009) Nanodimensional and Nanocrystalline Apatites and Other Calcium Orthophosphates in Biomedical Engineering. Biol and Med Materials 2:1975–2045
31. Kapolos J, Mavrilas D, Missirlis Y, Koutsoukos PG (1997) Model experimental system for investigation of heart valve valcification in vitro. J Biomed Mater Res (Appl Biomater) 38:183–190
32. Mayer JL, Eanes ED (1978) A thermodynamic analysis of the amorphous to crystalline calcium phosphate transformation. Calcif Tissue Int 25(1):59–68
33. Tung MS, Brown WE (1983) An intermediate state in hydrolysis of amorphous calcium phosphate. Calcif Tissue Int 35(6): 783–790
34. Pouget EM, Bomans PHH, Goos JACM, Frederik PM, de With G, Sommerdijk NAJM (2009) The initial stages of template-controlled $CaCO_3$ formation revealed by cryo-TEM. Science 323:1455–1458
35. Onuma K, Ito A (1998) Cluster growth model for hydroxyapatite. Chem Mater 10:3346–3351
36. Oyane A, Onuma K, Kakubo T, Ito A (1999) Clusering of calcium phosphate in the system $CaCl_2$–H_3PO_4–KCl–H_2O. J Phys Chem B 103:8230–8235
37. Walton AG, Bodin WJ, Furedi H, Schwartz A (1967) Nucleation of calcium phosphate from solutiom. Can J Chem 45:2695–2701
38. Chow LC, Sun L, Hockey B (2004) Properties of nanostructured hydroxyapatite prepared by a spray drying technique. J Res Nat Inst Stand Tech 109:543–551
39. Hammermeister KE, Sethi GK, Henderson WG, Oprian C, Kim T, Rahimtoola S (1993) A comparison of outcomes in men 11 years after heart-valve replacement with a mechanical valve or bioprothesis. N Engl J Med 18:1289–1296
40. Christoffersen J, Christoffersen MR (1992) Possible mechanisms for the growth of the biomaterial, calcium hydroxyapatite microcrystals. J Cryst Growth 121(4):617–630

41. Hesse A, Heimbach D (1999) Causes of phosphate stone formation and the importance of methaphylaxis by urinary acidification: a review. World J Urol 17(5):308–315

42. Helebrant A, Jonasova L, Sanda L (2002) Influence of simulated body fluidcomposition on carbonated hydroxyapatite formation. Ceramics Silikaty 46:9–14