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Resorbable Inorganic Materials for Bone Implants: Searching Strategy

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***Abstract*—Several ways to increase resorption of CaP biomaterials are treated. The main thrust of research efforts is concentrated around (i) control of morphology of hydroxyapatite (HA) crystals, and (ii) decreasing energy of HA crystal lattice due to chemical doping (silicon- and carbonate-doped HA)**

I. INTRODUCTION

A distinct feature of a new generation ofCaP biomaterials used for bone treating is an enhanced rate of their resorption (i. e. their solubility *in vivo* coupled with simultaneous replacement of them with newly formed bone). Ceramics based on artificial calcium hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$ (HA) is a widespread material due to chemical and phase similarity to inorganic constituent of bone. However, its bioresorption rate is too sluggish to induce a new bone ingrowth [1, 2].

In accordance with a division ofresorption phenomenon into two distinct stages-"sluggish" and "fast" ones, which differ from each other in pH and cells involvement, assessment of resorption properties of materials can be reduced to consideration of materials solubility in water or model media. Some properties of the materials used (or potentially used) to compensate bone defects are listed in Table 1. Note that resorption index (RI, representing scaled values of pCa and introduced here by us) increases from left to right (i. e. from HA to gypsum).

Table 1. **Solubility andresorption properties of some Ca-containing biomaterials**

Materials	HA	β-TCP	$Ca_2P_2O_7$	$CaCO_3$	$CaHPO_4 \cdot 2H_2O$	$CaSO_4 \cdot 2H_2O$
p*K*s ($=-\log_{10}Ks$)	118	28.9	14.7	8.4 (calcite) 8.2(aragonite)	6.6	4.2
Solubility *s*, mole/l	$0.4 \; 10^{-7}$	$0.6 \; 10^{-6}$	$0.3 \; 10^{-5}$	$0.6 \; 10^{-4}$	$0,5 \; 10^{-3}$	$0,8 \; 10^{-2}$
pCa ($=-\log_{10} s$)	7.4	6.2	5.5	4.2-4.1	3.3	2.1
Estimation of resorption *	--	-		+	++	++
Resorption Index (RI)	1.0	3.0	5.2	6.4-6.6	8.0	10.0

*estimation of resorption (literature data): -- very weak, -weak, + strong, ++ very strong

The following aspects of the strategy aimed at increasing ofresorption (solubility) of CaP should be considered:

(1) control of morphology of HA (nano) crystals;

(2) decreasing energy of HA crystal lattice due to its chemical modification (isomorphic substitution) for the case of silicon- and carbonate-substitutedapatites;

(3) usage of different from HA crystal structure;

(4) shift from ceramic fabrication to low-temperature technique of chemical bonding.

In this work, the main thrust of discussion is structured around directions (1) and (2) dealing with HAnanocrystals or doped HA. Results on calcium phosphate materials with Ca/P<1.67 or chemically bonded phosphates can be found elsewhere [3], [4].

II. RESULTS AND DISCUSSIONS

Control of morphology of HA crystals.

Equilibrium shape of a crystal with molar volume V_m

and apparent solubility s/s_0 in front of its face ($hk.\ l$) ① with specific surface energy $\gamma_{(hk.\ l)}$ situated at distance $h_{(hkl)}$ from crystal origin are determined by Gibbs–Wulf construction and Gibbs–Thomson equation [5]:

$$RT \cdot \log(s/s_0) = (2\ V_m \cdot \gamma_{(hk.\ l)}) / h_{(hk.\ l)}. \qquad (1)$$

There are two principal ways to increase s/s_0 (see fig. 1). Note that the second way connected with decreasing aspect ratio more relates to doped HA where chemical modification can drastically change morphology of crystals.

HA was precipitated from aqueous solutions according to the following chemical reaction:

$$10CaX_2 + 6M_2HPO_4 + 8MOH = Ca_{10}(PO_4)_6(OH)_2 \downarrow + 20MX + 6H_2O, \qquad (2)$$

Where $X = NO_3^-$, Cl^-, CH_3COO^-, and $M = Na^+$, K^+, NH_4^+. Growth kinetics and morphology of HA crystals are largely determined by supersaturation of the stock solution with respect to HA (affected by concentration of salts, pH and temperature) as well as by major ionic species in the solution (presumably, by anionic species).

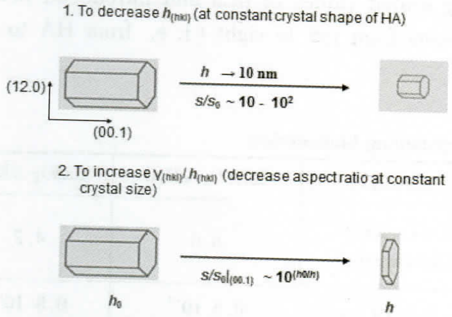


Figure 1. Two principal way to increase apparent solubility of HA crystals

Dimensions of HA crystals along and perpendicular to c–axis were assessed from XRD spectra (fitted by Rietveld refinement accounting for needle–shape crystal, and checked by TEM). Effect of main experimental variables (factors) governing HA growth in solution (*viz.*, concentration [Ca^{2+}], pH and temperature) was evaluated from corresponding regression models (2^3 factorial design). For the case of HA precipitation with the use of $Ca(NO_3)_2$ the following equations connected form–factor (or aspect ratio) of HA crystals F ($= L_c/L_a$) and dimensions of them L_c, L_a (along [00. 1] and [01. 0] respectively) and the experimental factors were obtained:

$$F(\pm 0.1) = 1.8 + 0.20\,x + 0.2\,y + 0.45\,z + 0.25\,x\,y + 0.2\,x\,z \qquad (3)$$

$$L_a(\pm 10\ \text{Å}) = 140 + 25\,x + 30\,z - 35\,x\,y + 20\,y\,z \qquad (4)$$

$$L_c(\pm 15\ \text{Å}) = 260 + 75\,x + 30\,y + 120\,z + 35\,x\,z + 40 \cdot y\,z \qquad (5)$$

where $x, y, z \in [-1, 1]$ – scaled experimental variables: $x = [Ca^{2+}]^* = ([Ca^{2+}] - 255\ \text{mM})/245\ \text{mM}$, $y = pH^* = (pH - 9.5)/2.5$, $z = T^* = (T - 50℃)/30℃$.

Projections of response surface onto factors planes make the effects of factors quite visible (fig. 2). One can see that all factors sufficiently affects the shape of HA crystals and the following rules can be deduced:

1) increase of [Ca^{2+}], pH and T makes F, L_c, L_a larger; strong effect of T on F is connected with pronounced dependence of L_c from temperature;

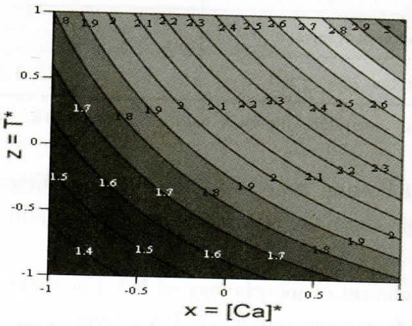
2) at rather high temperatures (~ 80℃) crystal habitus is determined by composite (mixed) effect ofpH and concentration; being higher supersaturation with Ca – and OH–ions, the effect is stronger;

3) it worth noting mixed effects (simultaneous effects of two factors);

4) at high concentration of calcium salt (0.5 M and higher), increase of temperature, as well as increase of pH favor the growth of needle–shape crystals;

5) habitus of HA crystals in strongly base solution is determined by mixed effect of concentration and temperature; in pH–neutral solution switching fromequiaxed to needle – shape habitus is controlled by temperature change (fig. 2 c, d).

Isolines situated at response surface and corresponding to the crystals with the greatest size along c-axis allow drawing a conclusion on independence of crystal size from pH as well as very weak dependence of the size from initial concentration. Factor dominating growth of HA crystal along [00. 1] is the temperature (see eq. (5), fig. 2 c, d). It should be noted that effect of pH on L_a: in base media temperature dominates, whereas in neutral solution concentration of Ca–ions plays key role.



(a) pH = 12

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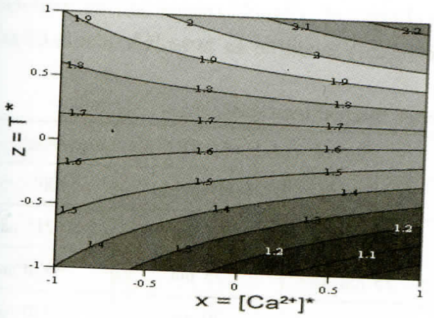
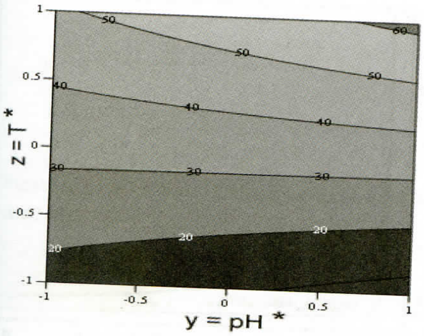
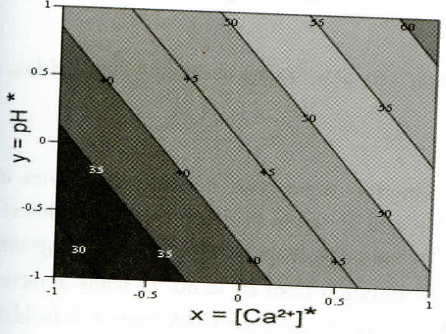
① Hereafter Weber indices ($hk.\ l$) will be used instead of Bravais indices ($hkil$) for hexagonal system since $i = -(h+k)$



(b) pH = 12



(c) [Ca ²⁺] = 0.5 M



(d) T = 80 °C

Figure 2. Projections of response surface for form-factor of HA crystals F (a, b) and size of HA crystal along [00.1] (c, d) on factors planes

Factors effects (including mixed effects) described above are caused by ionic equilibriums in the solution. Our calculations of the equilibriums in the solution with $[Ca^{2+}] = 10$ mM at 20°C show that initially at pH ~ 7 the following species dominate – Ca^{2+}, $[CaHPO_4]^0$ и HPO_4^{2-}. At pH ~ 12 in the solution, $CaPO_4^-$, $CaOH^+$, Ca^{2+} become major species. During heating up to 80 °C at pH ~ 7, the fraction of $CaPO_4^-$ and $[CaHPO_4]^0$ increases owning to decrease of Ca^{2+} and phosphate–ions. As pH increases, relative supersaturation of the solution σ_{HA} with respect to HA precipitation grows–at 20 °C, $\lg(\sigma_{HA}) = 1.7$ (pH = 7.4) и $\lg(\sigma_{HA}) = 2.7$ (pH = 12), however, this growth is not so sufficient to accelerate nucleation to the prejudice of crystal growth. Thus, there is a clear tendency of HA crystal size increase with increasing of activities of ion pairs $CaPO_4^-$ и $CaOH^+$ in the solution. The pairs denoted as $CaPO_4$ has, in fact, a structure of Posner cluster–$Ca_9(PO_4)_6$, i. e. growth unit, which composition can be deduced from experiments on kinetics of HA crystal growth. Clusters $Ca_9(PO_4)_6$ and $CaOH^+$ can be easily recognized in the crystal structure of HA. Therefore, their accumulation in the solution leads to crystallization of HA in highly base media according to Onuma's cluster model of HA growth [6]:

$$Ca_9(PO_4)_6 + CaOH^+ + OH^- \rightarrow Ca_{10}(PO_4)_6(OH)_2\downarrow \quad (5)$$

Solutions containing calcium acetate demonstrate more complex behavior connected both with hydrolysis of acetate anion and with formation of strong ion pair CH_3COOCa^+:

$$F\ (\pm 0.1) = 2.50 + 0.15x - 0.20y - 0.55xyz, \quad (6)$$

$$L_a(\pm 10\ Å) = 165 - 20y + 40z + 15xy\ 10xz + 15xyz, \quad (7)$$

$$L_c(\pm 15\ Å) = 410 + 30x - 65y + 90z - 20xz - 10yz - 50xyz. \quad (8)$$

Needle–shape HA crystals with $F \sim 3$ can be obtained both at low temperatures and at high temperatures (increasing calcium content and decreasing pH, or visa versa). At low temperature, increasing of pH leads to suppression of acetate ion hydrolysis and, therefore, to more effective binding of Ca–ions. Hydrolysis of acetate ion increases with temperature increase, so the demand of simultaneous increase or decrease of pH and Ca content is violated.

Decreasing energy of HA crystal lattice due to its chemical modification.

The following logics can be anticipated in this way: solubilitys is related to H_{diss} and, therefore, to $U_{lattice} \sim A\, z_{cation} z_{anion}/(r_{cation} + r_{anion})$; to increase s it is necessary to decrease $U_{lattice}$, that is, to decrease z, and/or increase r (or, more generally, to increase molar volume V_m [7]).

Si–doped HA.

Si–HA was precipitated from solutions containing TEOS as silicon precursor:

$$10Ca(NO_3)_2 + (6-x)NH_4H_2PO_4 + xSi(OC_2H_5)_4 + (14+x)NH_4OH \rightarrow Ca_{10}(PO_4)_{6-x}(SiO_4)_x(OH)_{2-x}\downarrow + 20NH_4NO_3 + 4xC_2H_5OH + (21-2x)H_2O, \quad (9)$$

x = 0, 0.1, 0.2, 0.25, 0.5, 1; as well as by sol–gel technique using TEOS andtrimethylphosphate (TMP), and by solid–state reaction. In fact, formation of Si–HA takes place under heating higher than 600–800oC. The precipitates as–received through (9) represent HA crystals covered with hydrated silica. In the course of etching such crystals with nitric acid, systematic growth of ζ-potential from −17 mV (coming from excessive charge of silanol groups at the surface of a crystal) to −1 mV peculiar to pure HA occurs (fig. 3 a).

Table 2. **Sizes of Si-HA crystals with different Si-content after thermal treatment**

	Size of crystals (by SEM)		Size of crystals (by XRD line broadening)		
Thermal treatment	900°C/5 hr	1100°C/5 hr	700°C/5 hr	900°C/5 hr	1100°C/5 hr
$Ca_{10}(PO_4)_6(OH)_2$	80 nm	390nm	45 nm	65 nm	215 nm
$Ca_{10}(PO_4)_{5.8}(SiO_4)_{0.2}(OH)_{1.8}$	55 nm	220nm	25 nm	35 nm	120 nm
$Ca_{10}(PO_4)_{5.5}(SiO_4)_{0.5}(OH)_{1.5}$	50 nm	180 nm	20 nm	30 nm	110 nm

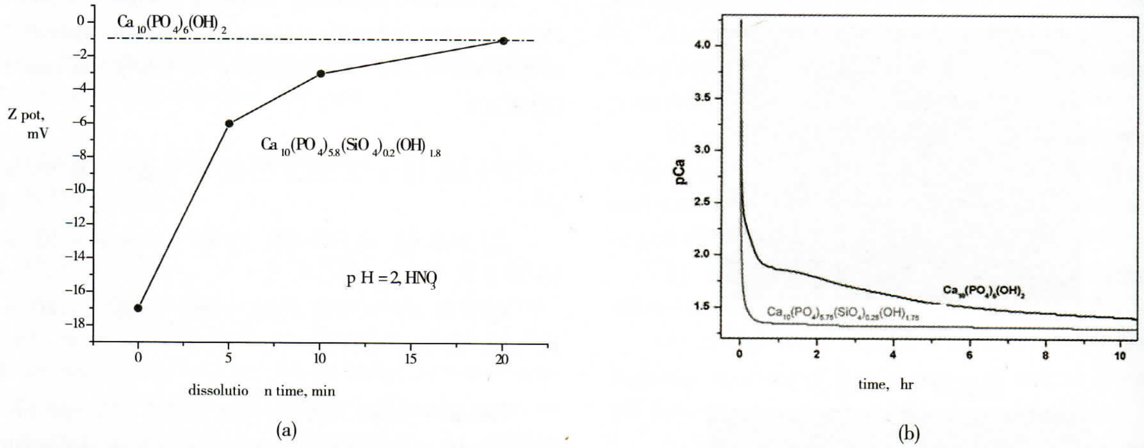


Figure 3. **(a) ζ-potential of $Ca_{10}(PO_4)_{5.8}(SiO_4)_{0.2}(OH)_{1.8}$ vs etching time, (b) pCa vs time during dissolution of $Ca_{10}(PO_4)_{5.8}(SiO_4)_{0.25}(OH)_{1.75}$ and HA in acetic buffer solution (pH=4.7)**

Crystal size decrease with rising Si-content can be clearly seen (Table 2). Small particles with modified surface are likely to be the reasons for faster dissolution of Si-HA compared to pure HA (fig. 3 b).

Carbonate-substituted HA (CHA).

CHA was precipitated as follows:

$(10-x)Ca(NO_3)_2 + (6-x)(NH_4)_2HPO_4 + (x+y)NaHCO_3 + (-y)8NH_4OH \rightarrow Ca_{10-x}Na_x(PO_4)_{6-x}(CO_3)_x(OH)_{2-y}(CO_3)_y \downarrow + (20-2x-y))NH_4NO_3 + yNaNO_3 + (6+y)H_2O$, . (10)

where x+y = 0, 0.25, 0.5, 0.75, 1, 1.5, 2. Such a technique of CHA fabrication makes carbonate-ion distributed between two nonequivalent sites in HA lattice-A- and B-sites (related to substitution of OH^- and PO_4^{3-}-ions, correspondingly). Increasing temperature of precipitation from 20 to 80 ℃ leads to increase of maximal content of CO_3^{2-} in CHA from 3.9 to 13.6 wt. % (fig. 4 a). This fact means endothermic type of isomorphic substitutions $CO_3^{2-} \leftrightarrow OH^-$, $CO_3^{2-} \leftrightarrow PO_4^{3-}$ and reflects destabilization of apatite lattice in CHA. Low content of CO_3^{2-} (less 5 % wt.) evokes preferential filling of A-sites in OH-channels of HA, further increase of carbonate content cause substitution of PO_4^{3-}-anions (B-sites) (fig. 4 b).

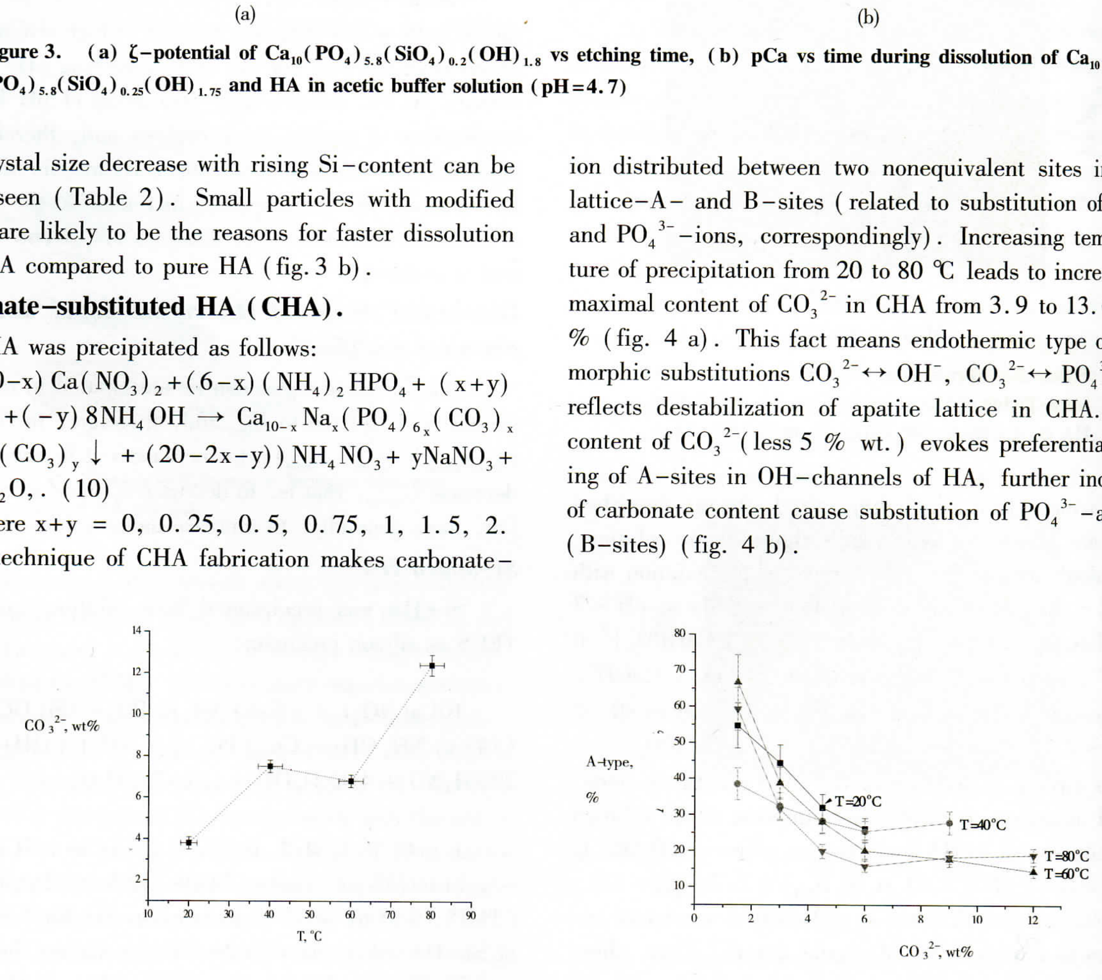
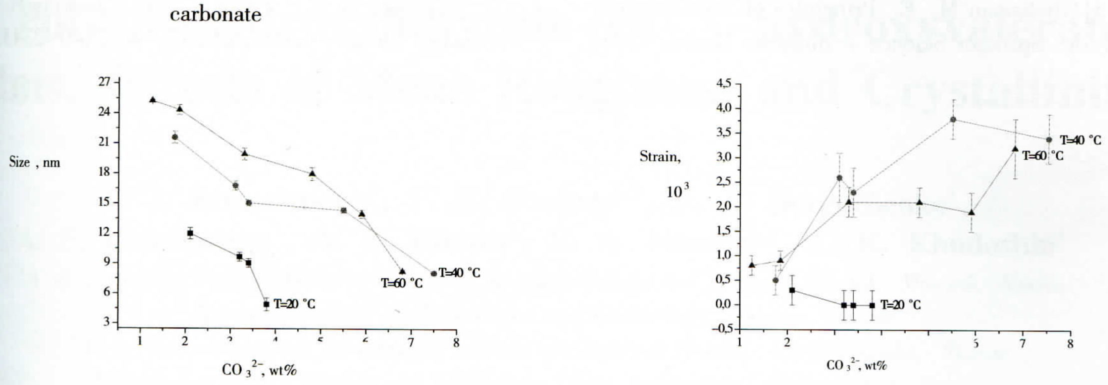


Figure 4. **(a) Maximal content of CO_3^{2-} in CHA vs temperature, (b) fraction of CO_3^{2-} in A-sites (by FTIR) vs overall content of carbonate**

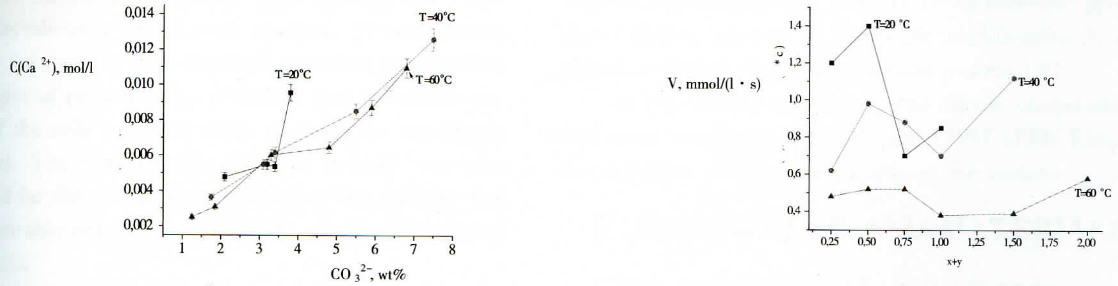
Increasing carbonate content in CHA obviously leads to decrease of crystal size (fig. 5 a) and accumulation of lattice distortions observed as lattice microstrains (fig. 5 b).



(a)

(b)

Figure 5. **(a) Sizes of CHA crystals (from XRD line broadening), and (b) lattice microstrain vs overall carbonate content**



(a)

(b)

Figure 6. **(a) Solubility, and (b) initial rate of dissolution of CHA powders in tris-buffer at pH=7.4**

The best solubility is demonstrated by CHA with$x+y$ =1.5 fabricated at 40 °C (fig. 6 a). Increasing CO_3^{2-}-content is accompanied by non-monotonous increase of CHA dissolution rate both in acidic (pH ~ 5) and almost neutral media (pH ~ 7.4, fig. 6 b). Two valuable factors governing dissolution rate of CHA were stated-size of CHA crystals and degree of occupation of A-sites. Opposite effects of these two factors under rising overall content of CO_3^{2-} stipulate non-monotonous change of dissolution rate.

Ⅳ. CONCLUSIONS

Two aspects of the strategy aimed at increasing ofresorption (solubility) of CaP are treated: (i) control of morphology of HA crystals, and (ii) decreasing energy of HA crystal lattice in the course of isomorphic substitution. The first way can increase apparent solubility s/s_0 up 100 times. The main factors governing HA growth in solution and affecting its morphology are ion concentrations, pH and temperature. The second way of resorption increase is associated with formation of silicon- and carbonate-substituted apatites. In the case of carbonate-substituted HA two valuable factors govern its dissolution rate: -size of apatite crystals and degree of occupation of A-sites. Opposite effects of these two factors under rising overall content of CO_3^{2-} lead to non-monotonous change of dissolution rate.

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