CHAPTER 2

HYDROXYAPATITE STRUCTURE AND SOLUBILITY

Because of its cystallographical similarity to various calcified tissue of vertebrates, hydroxyapatite has attracted much attention as a substitute material for damaged teeth or bone over the past two decades. Hydroxyapatite is very close to our life as the main compoment of bone and tooth minerals. Many apatite compounds, including fluoroapatite, chlorapatite, carbonate-apatite, and hydroxyapatite are being used in the industrial field as fertilizers, fluorescent substances, catalysts, adsorbents, humidity sensors, and materials for electrical parts. In the medical and dental fields, hydroxyapatite is being utilized as artificial bones, tooth roots, toothpastes, percutaneous devices, blood vessels, tracheae, and materials for drug delivery systems.

Chemical Formula

"Hydroxyapatite" is a member of the apatite group of minerals, and its chemical formula is $Ca_{10}(PO_4)_6(OH)_2$. Hydroxyapatite is a calcium phosphate including hydroxide, and its Ca/P ratio is represented as 1.67.

"Apatite" is a general term for crystalline minerals with a composition of $M_{10}(ZO_4)_6X_2$. Many elements occupy the M, Z, and X sites.

M = Ca, Sr, Ba, Cd, Pb, etc.

Z = P, V, As, S, Si, Ge, CO_3, etc.

X = F, Cl, OH, O, Br, CO_3, Vacancy, etc.

Various apatite compounds different in composition can be prepared by the replacement of elements for each site.

Crystal and Surface Structure

The crystal structure of hydroxyapatite (HA) belongs to the space group $P6_3/m$ in the hexagonal system with the lattice parameters a(= b) = 9.432 A and C = 6.881 A (Kanazawa, 1989). A simplified unit cell of HA is depicted in Fig.2.1, in which oxygen atoms forming the tetrahedra of PO_4 are abbreviated. The two oxygen atoms of the PO_4 - tetrahedron locate on the mirror planes at Z = 1/4 and 3/4, and the other two occupy symmetrically the sites above and below the planes. Calcium ions occupy two different sites; the column $Ca(Ca_I)$ at Z = 0, 1/2 and the screw axis $Ca(Ca_{II})$ at Z = 1/4, 3/4. Three of the screw axis Ca^{2+} form a triangle on a mirror plane. This Ca^{2+} triangle has a sixfold-screw axis. As detailed in the following sections, a Ca^{2+} moves more easily along the C axis than in the direction perpendicular to it. This is attributed to the easy deficiency of the column Ca^{2+}. The arrangement

of OH^- ions surrounded by a Ca^{2+} - triangle along the c-axis is also characteristic of the HA structure.

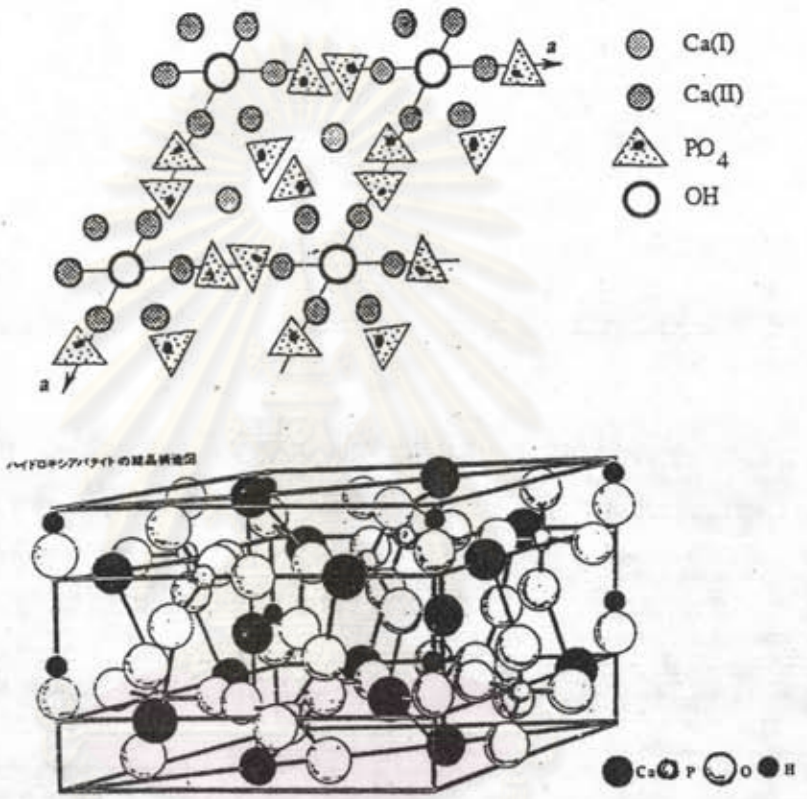

Fig.2.1 Crystal structure of hydroxyapatite projected along c-axis (upper) and along a-axis (bottom) (Aoki, 1991).

Solubility of Hydroxyapatite

Because the surface structure of HA has a significant influence on a surface-related phenomena such as adsorption, dissolution and ion exchange, studies on dissolution and its chemical proporties of HA contribute to understanding in vivo phenomena related to calcified tissue. HA is soluble in an acidic solution, while insoluble in an alkaline solution, and slightly soluble in distilled water. The solubility in distilled water increases with an addition of electrolytes. Moreover, the solubility of HA changes in the presence of amino acids, protein, enzymes, and other organic compounds. These solubility properties are closely related to biocompatibility with tissues and chemical reactions to other compounds. However, the solubility rate depends on differences in shape, porosity, crystal size, and crystallinity, including strain, defects, and crystallite size.

2.1 In Water

Several kinds of HA were used for solubility testing in distilled water at 37°c : synthetic HA powder (100-200 mesh) by the wet method, a commercial HA powder (Bio-Gel), and calcined HA were shown in Fig.2.2. The synthetic HA and commercial HA (Bio-Gel) showed almost the same solubility. The amount of calcium ion liberated was approximately 4 ppm and after 300 days increased to 5 ppm. The calcined HA showed lower solubility than the synthetic

HA (Aoki, 1991). The amounts of phosphate ions dissolved in distilled water were lower than the theoretical values. It was concluded that the dissolved phosphate ions were adsorbed again on the surface of the HA. Various values for the solubility product, pKs, had been reported by many investigators. The value was approximately 120. The solubility product, pKs, was defined as follows :

$$pKs = - \log ([Ca]^{10} [PO_4]^{6} [OH]^{2})$$

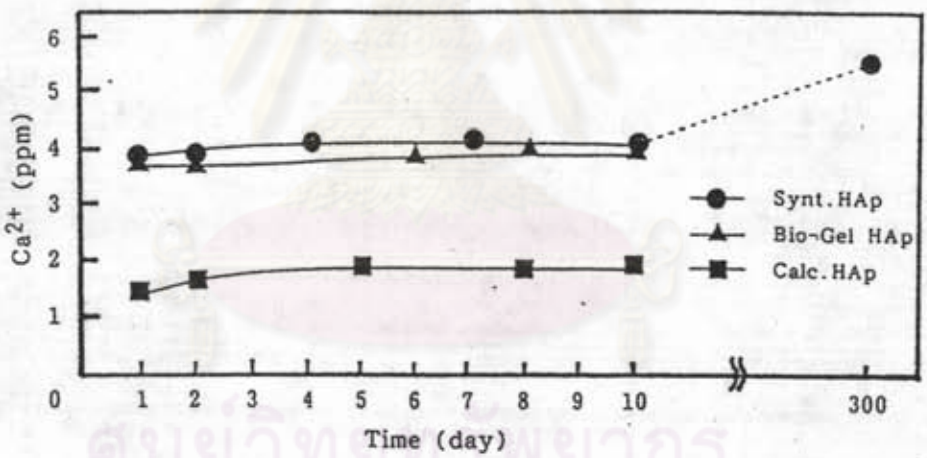

Fig. 2.2 Changes in solubility of various HA in water (Aoki, 1991).

Changing in electroconductivity in distilled water, in which several kinds of HA were dissolved at various reaction temperatures were shown in Fig. 2.3. The electroconductivity of the distilled water indicated approximately 1 μS for HCO_3 ion produced by the reaction between water and carbon dioxide in air. By the addition of HA into the distilled water, the electroconductivity rapidly increased and approached a horizontal line within one hour, after which it increased very slowly. This increase was due to the increase in temperature. The solubility of sintered HA is very low. The dissolution rate of sintered HA in distilled water was calculated from the weight loss, as shown in Fig. 2.4.

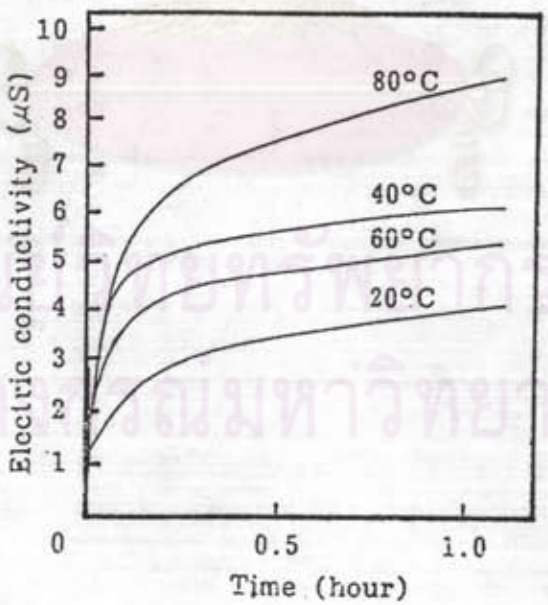

Fig. 2.3 Electroconductivity of sintered HA in distilled water (Aoki, 1991).

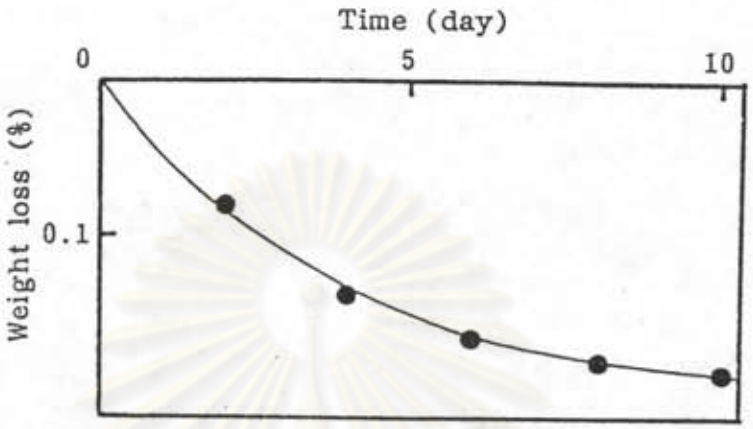

Fig. 2.4 Weight loss curve of sintered HA in distilled water (Aoki, 1991).

2.2 In Salts

The effect of several salts on the solubility of HA was studied by chemical analysis. Several salts, including NaCl, KCl, $MgCl_2$, and NaF were used in this analysis. The solubility changes in HA to which various amounts of NaCl (0.03-30%) were added, as shown in Fig. 2.5. The solubility increased proportionally with an increase in the amount of NaCl. The effect of KCl on the solubility of HA was almost the same as that of NaCl.

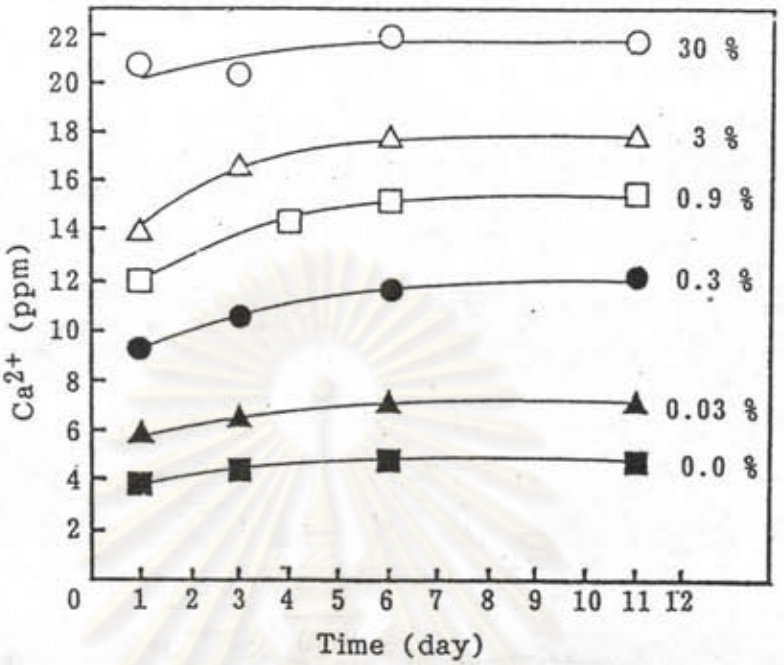

Fig. 2.5 Solubility changes of HA in various sodium chloride solutions (Aoki, 1991).

The solubility changes of HA in $MgCl_2$ solution to which amounts of 0.3 - 3 wt% added were shown in Fig.2.6. The effect of $MgCl_2$ on the solubility of HA was twice of NaCl. The order of the effect of several salts on the solubility of HA decreased as follows : Sr > Ba > Mg > Na > K (Aoki, 1991).

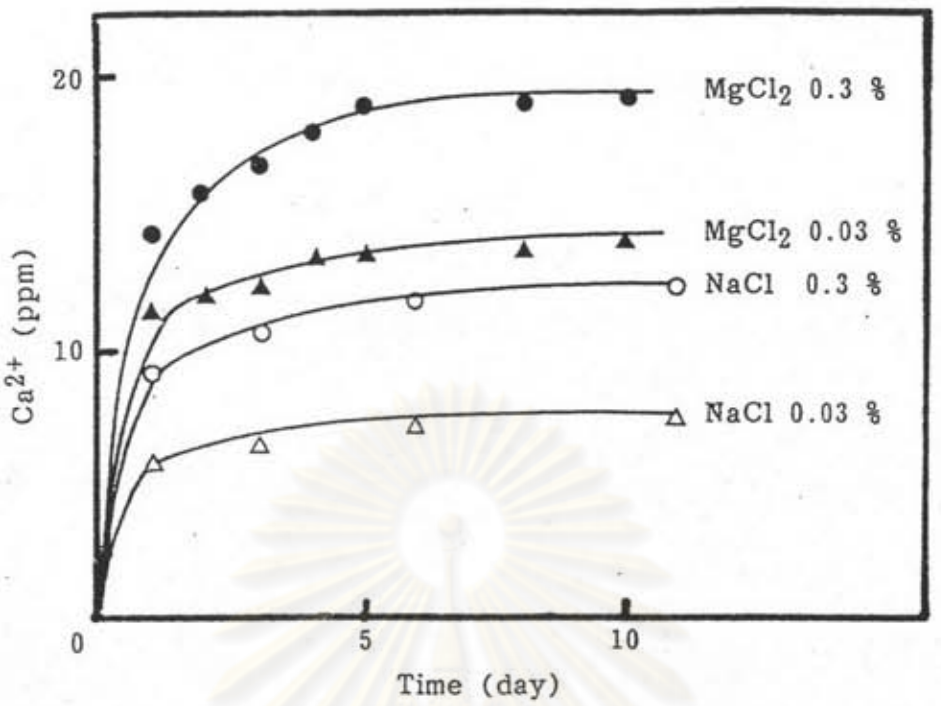

Fig. 2.6 Solubility curves of HA in $MgCl_2$ solution compared with NaCl solution at 37°c (Aoki, 1991).

2.3 In Acids

HA powder was very soluble in acid solutions. Kanazawa et al. (1991) had analyzed the initial step of the dissolution, using acids with pH = 1-2. Under these conditions, the change of $[H^+]$ due to dissolution could be ignored. The Ca/P ratio of solid specimens had been found to have no significant effect on the incongruent solubility. The rate of dissolution was closely related to concentration and type of acid. As shown in Fig.2.7, the dissolution rate increased with increasing $[H^+]$ in the case of hydrochloric acid. Citric acid showed different behaviour; the dissolution rate increased with increasing the concentration of citric acid.

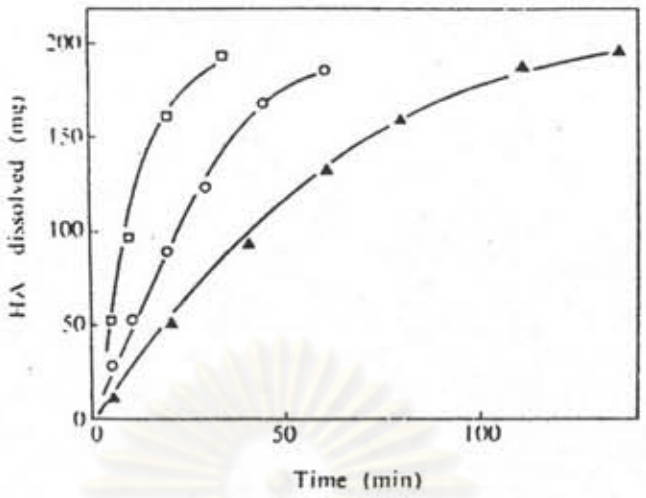

Fig 2.7 Effect of pH of HCl on dissolution rate of HA
□ pH = 0.92, ○ pH = 1.30, ▲ pH = 1.58
(Kanazawa et al., 1989).

When the results were compared with those of HCl, the pH followed the order 0.5% HCl < 0.2% HCl < 0.1% HCl < 10% citric acid < 2% citric acid while the dissolution rate was in the order 0.5% HCl > 10% citric acid > 0.2% > HCl > 2% citric acid > 0.1% HCl. This was attributed to the promotion of dissolution by the formation of a kind of complex compound between Ca^{2+} and the citric acid group.

Sr^{2+} and Zn^{2+} were the inhibitor on the dissolution of HA. On the surface of HA, Sr^{2+} was thought to substituted Ca^{2+} according to the equation (Dedhiya et al, 1973).

$$Ca_{10}(PO_4)_6(OH)_2 + nSr^{2+} \longrightarrow Ca_{10-n}Sr_n(PO_4)_6(OH)_2 + nCa^{2+}$$

The resultant Sr^{2+}-substituted HA determined the dissolution rate. Fig 2.8 illustrated the profile of dissolution, where phosphate ions were assumed to be HPO_4^{2-}. Ionic species such as Ca^{2+}, HPO_4^{2-} and $H_2PO_4^-$ supplied from the surface of HA, diffused through the dissolution layer to the solution, while Sr^{2+} diffused backwards to HA. In the dissolution of HA, diffusion was the rate-determining process.

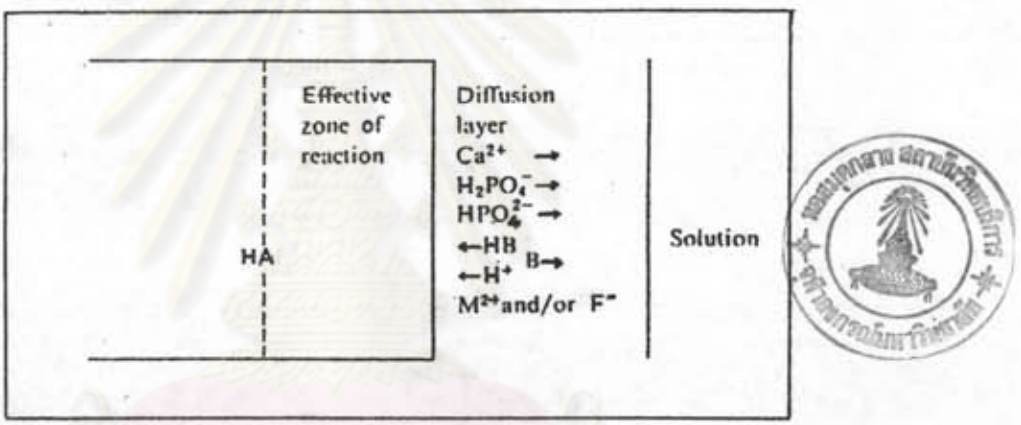

Fig. 2.8 Dissolution model proposed for HA.
(arrows indicate direction of diffusion)
(Kanazawa et al., 1989).

Sintered HA block was low soluble in acid solution but HA powder was very soluble. HA blocks were prepared and put into lactic acid solutions having a pH range of 1 - 5. The dissolved amount was measured by weight loss, as shown in Fig. 2.9. In the pH 4.99 solution, the solubility rate of the blocks was very low. On the other hand, below pH 2.05, the solubility rate rapidly increased.

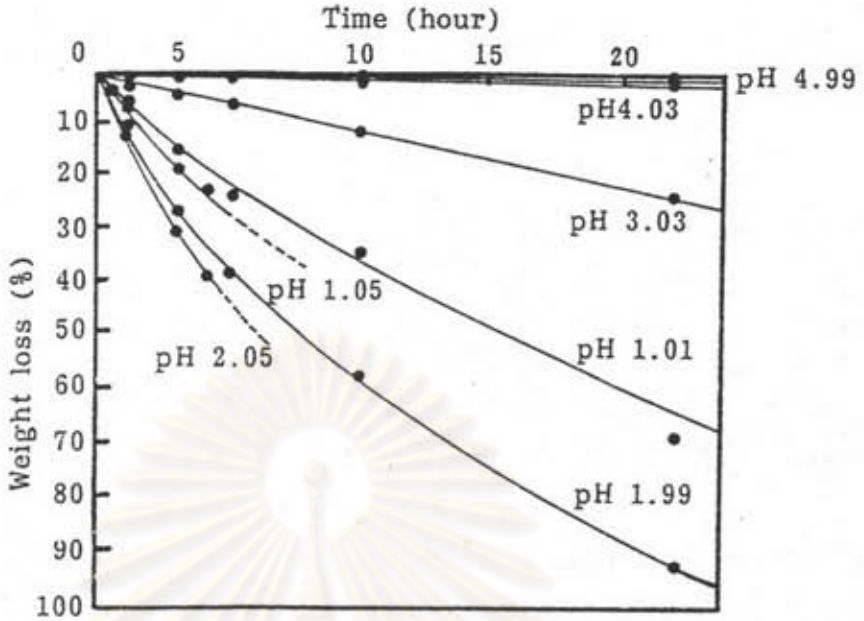

Fig. 2.9 Solubility rates of sintered HA blocks in various lactic acid solutions (Aoki, 1991).

2.4 In Human Maxillary Saliva

Reaction of HA to human saliva was studied by X-ray diffractometer. HA slightly dissolved in saliva. The crystallite size changes of HA in human maxillary saliva were measured by X-ray diffraction pattern changes using Sherrer's equation, as follows :

$$D_{hkl} = \frac{K\lambda}{\beta \cos\theta}$$

In the equation D_{hkl} indicated the crystalite size (A), β indicated the width of the diffraction pattern (radian), θ indicated Bragg's angle (degree), K indicated the constant, and λ, the wave length of the X-ray. The HA crystalllite sizes along the a-axis and c-axis were calculated from the

width of the diffraction patterns of the (100) and (002) faces individually.

The crystallite sizes of calcined HA powder used as starting material were 930°A (D_{002}) and 610°A (D_{100}) Human maxillary saliva was extracted from three adult men, A, B, and C in Table 2.1

Table 2.1 Changes in hydroxyapatite (HA) crystallite sizes in reaction with three specimens of maxillary saliva.

	A		B		C	
	D_{100}(°A)	D_{002}(°A)	D_{100}(°A)	D_{002}(°A)	D_{100}(°A)	D_{002}(°A)
HAp	610	930	610	930	610	930
1 hr	530	930	530	930	580	930
5 hrs	500	930	430	930	530	930
10 hrs	490	930	490	930	490	930
1 day	490	930	470	930	580	930
5 days	460	930	490	930	460	930
10 days	440	930	440	930	430	930
30 days	430	930	440	930	430	930

In all cases, the crystallite size along the a-axis rapidly decreased from 610°A to 430-440°A. On the other hand, the c-axis crystallite size did not change. The atomic net plane perpendicular to the c-axis was the c-plane. Our tooth enamel surfaces were composed only of c-planes of HA. (Aoki, 1991).

Literature Survey about the Dissolution and Surface Reaction of Hydroxyapatite and other Bioactive Ceramics.

Orly, et al. (1989) studied the chemical changes occuring in the mineral after exposure of a synthetic hydroxyapatite ceramic to both *in vivo* (implantation in human) and *in vitro* (cell culture) conditions. A small amount of the material was phagocytized but the major remaining part behaved as a secondary nucleator as evidenced by the appearance of a newly formed mineral. Morphologically, the newly formed mineral appeared as tiny crystals precipitated and grown from the surface of the initial synthetic crystals. Chemically, it was identified by IR spectroscopy as a carbonated apatitic mineral.

Hyakuna et al. (1990) studied the surface reaction of calcium phosphate ceramics (CPCs) which had been thought to play an important role in bonding with living bone. Hydroxyapatite (HA), tricalcium phosphate (TCP), and two kinds of apatite-containing glass-ceramics were immersed in three types of solutions pH 7.3, 37 °c with different chemical constituents. The first solution was a physiological saline, the second contained phosphate (PO_4), and the third was a balanced salt solution consisting of calcium (Ca), magnesium (Mg), and PO_4. The experimental results showed that in the complete solution with both

Ca and PO_4, a carbonated apatite layer was formed on the surfaces of HA, TCP, and the glass-ceramics.

Klein, et al. (1990) studied the *in vitro* solubility of hydroxyapatite, tetracalcium phophate or tricalcium phosphate particles in lactate, citrate, Gomoris or Michaelis buffer with pH 6.2 or 7.2 and in aqua destillata. The results showed that in general the solubility decreased in the order tetracalcium phosphate (TTCP) > tricalcium phosphate (TCP) > hydroxyapatite (HA), except for lactate or citrate buffer where the solubility order was TTCP = TCP > HA. The influence of the specific buffer used was much larger than either pH or specific calcium phosphate salt tested. The pH stability of lactate buffer and aqua destillata was very low, the other buffer solvents had a rather stable pH value.

Kokubo et al. (1990) proposed that the calcium ions dissolved from the glass-ceramics increased the degree of the supersaturation of the surrounding body fluid with respect to the apatite, and a hydrated silica that formed on the surfaces of the glass-ceramics provided the sites favorable for apatite nucleation. This finding was interpreted quantitatively in terms of the increase in the degree of the supersaturation of the surrounding fluid with respect to apatite due to the dissolution of calcium ion from the glasses with the decrease in the interface energy between the apatite and the glasses due to formation of hydrated silica on the surfaces of the glasses (Ohtsuki,

Kokubo, and Yamamuro, quoted in Li, 1992).

Muller-Mai, Voigt, and Gross (1990) investigated the interface of dense hydroxyapatite (HA) implants with different surface roughnesses after implantation into the spongy bone of the distal femur of rabbits by scanning electron microscopy (SEM) and transmission electron microscopy (TEM) following transverse fractures in the interface. Each implant displayed considerable changes in surface morphology caused by leaching, as shown by an increasing pore diameter and a decreasing grain diameter; corrosion (particulate disintegration), i.e. the loss of HA grains; and resorptive phenomena, e.g. through macrophages or osteoclasts.

Moreno, and Aoba (1991) studied the solubility properties of hydroxyapatite (HA) compared with those of human dental enamel and dentin. The apatites were equilibrated with dilute phosphoric acid solutions in CO_2 - cotaining atmospheres. Both in the HA and the dental mineral systems, the results are consistent with the precipitation of another carbonate - containing apatitic phase during equilibration.

Ribeiro and Barbosa (1991) studied about the influence of metal ions, namely Ti, Al, V on the dissolution behaviour of HA. The effect of pH, time, concentration of metal ions and serum was investigated. The results suggest that HA coatings may prevent the release of metal ions from

a titanium substrate by precipitating them in the form of phosphate. It is expected that HA will act as a barrier to elemental transfers from underlying substrates, but the release of metal ions may influence the dissolution behaviour of the coating. Serum decreases the Ca and P concentration in solution and interacts with the metallic elements, which are partially transferred from the solid to the liquid phase.

Li et al. (1992) proposed that a hydrated silica formed on the surfaces of glasses and glass-ceramics in the body plays an important role in forming the surface apatite layer. In this study, it is shown experimentally that a pure hydrated silica gel can induce apatite formation on its surface in a simulated body fluid (SBF) when its starting pH is increased from 7.2 to 7.4. This result has been confirmed that the essential condition for glasses and glass-ceramics to bond to living bone is the formation of an apatite layer on their surface in the body.

Radin and Ducheyne (1993) proposed that the formation of a biologically equivalent carbonate-containing apatite on the surface of synthetic calcium phosphate ceramics (CPCs) may be an important step leading to bond with bone. Various CPCs were studied upon immersion into a simulated physiologic solution (SPS) with an electrolyte composition of human plasma at pH 7.4, 37°c. The studied CPCs can be characterized by the time to new phase formation *in vitro*; the minimum time for measurable precipitate

formation was found to increase in the order : not-well-crystallized HA < well - crystallized HA < α TCP, TTCP < β - TCP.

Li et al. (1994) showed that pure soluble silica prepared by a sol-gel method induced bone-like hydroxyapatite formation onto its surface when the silica was immersed in a simulated body fluid (SBF), whereas silica glass and quartz did not. This finding directly supports the hypothesis that hydrated silica plays an important role in biologically active hydroxyapatite formation on the surfaces of bioactive glasses and glass-ceramics, which leads to bone-bonding. Gel-derived titania is also a hydroxyapatite inducer because of its abundant TiOH groups. These results provide further insight into the unique osseointegration of titanium and its alloys. It is suspected that gel-derived titania develops an apatite layer by taking calcium and phosphate from the body fluid, thus producing bone-bonding.

Ducheyne, Radin, and King (1993) studied the effect of stoichiometry and crystal structure of calcium phosphate ceramics (CPCs) on the dissolution kinetics. Monophase, biphase, and multiphase CPCs with a Ca/P ratio equal to or greater than 1.5 were studied by incubation in a calcium-and phosphate-free Tris buffer solution at pH 7.3, 37°c. The dissolution rate of the monophase CPCs increased in the order of stoichiometric hydroxyapatite, β - tricalcium phosphate α - tricalcium phosphate, and tetracalcium

phosphate. Dissolution of biphase and multiphase CPCs increased prorated the concentration of more soluble component.

Maxian, Zawadsky, and Dunn (1993) studied of various apatite coating dissolution may promote enhanced bone bonding. The surface chemistry of amorphous Ca/P and poorly crystallized hydroxyapatite (HA) coatings on "smooth" and "rough" titanium (Ti) alloy (Ti-6Al-4V) implants were studied with immersion in Hank's physiologic solution at pH 7.2 and 5.2, 37 °c for 0, 4, 12 week periods. The amount of Ca dissolved from Ca/P - coated implants was strongly dependent on the chemistry of the coating and less dependent of pH or time of incubation. *In vitro* method is an effective way of determining differences in HA.