CHAPTER 4

RESULT AND DISCUSSION

Characteristics of the Specimens Before Incubation

4.1 Chemical Analysis of Bulk Specimens

The chemical composition of MP and TP before incubation in SBF was analyzed, as shown in the following tables.

Table 4.1 Chemical composition of MP and TP before incubation in SBF.

HA Type / Composition	MP (1220°c/ 2 hr.)	TP (1200°c/ 2 hr.)
Ca (%)	38.2	39.2
P (%)	18.3	18.3
Ca : P	1.63 : 1	1.66 : 1
Impurities		
Mg (%)	1.08	0.93
Fe (%)	0.05	<0.02
Zn (ppm)	143	42
Cu (ppm)	2	2
Mn (ppm)	5	5
As (ppm)	1.7	<0.5
Cd (ppm)	<0.5	<0.5
Pb (ppm)	<5	<5
Hg (ppm)	<1	<1

NB. These results were analyzed by Mineral Assays and Service Co., Ltd.

The ASTM chemical allowance of trace elements in the hydroxyapatite was shown in table 4.2

Table 4.2 The concentration of trace elements in the hydroxyapatite (ASTM F 1185-88)

Element	ppm, max.
As	3
Cd	5
Hg	5
Pb	30
total heavy metals (as lead)	50

From table 4.1 and 4.2 it could be summarized that the concentration of trace elements in MP and TP was in the ASTM limitation.

The Ca : P ratios in table 4.1 indicated that two types of HA (MP and TP) were non-stoichiometric HA having Ca - deficient structures (stoichiometric HA has Ca : P = 1.67). After comparison of the minor composition or impurities in MP and TP it was found that MP contained higher impurities especially zinc.

4.2 Phase Analysis of Bulk Specimens

The phase of two kinds of HA specimens was analyzed before incubation in SBF. The phase present was hydroxyapatite, as shown in Fig. 4.1

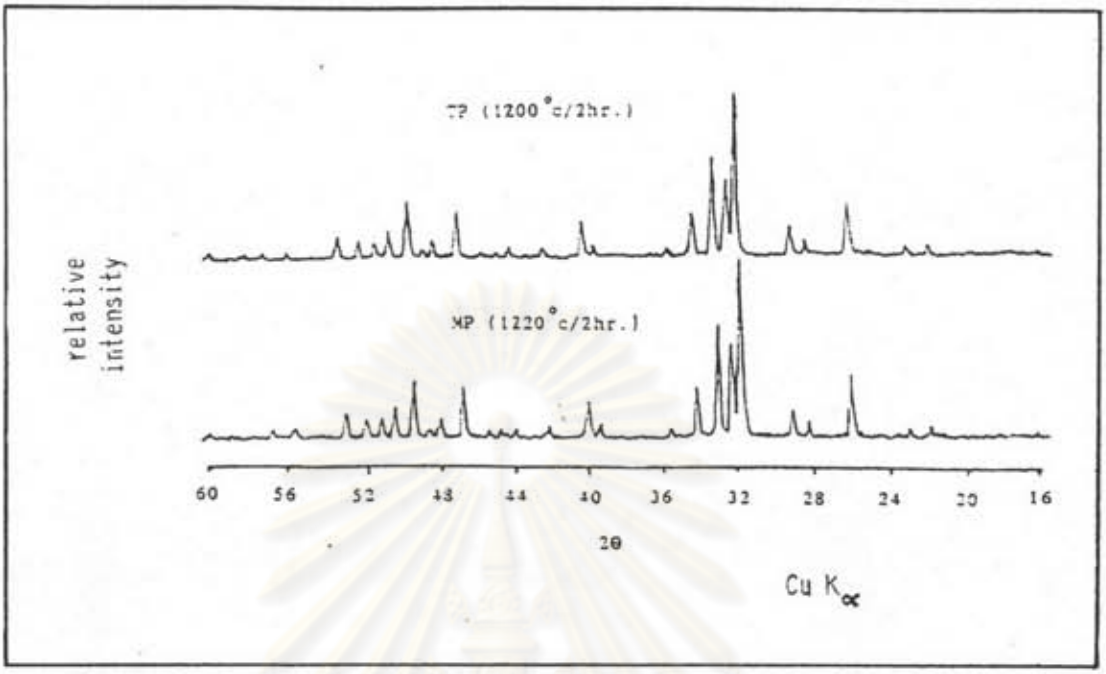


Fig. 4.1 XRD pattern of MP and TP bulk specimens before incubation in SBF

The sharp peaks of XRD indicated the well-crystallized state of two types of HA specimens.

4.3 Functional Group of Bulk Specimens

Functional group of MP and TP specimens before incubation in SBF was examined by FTIR, as shown in Fig. 4.2

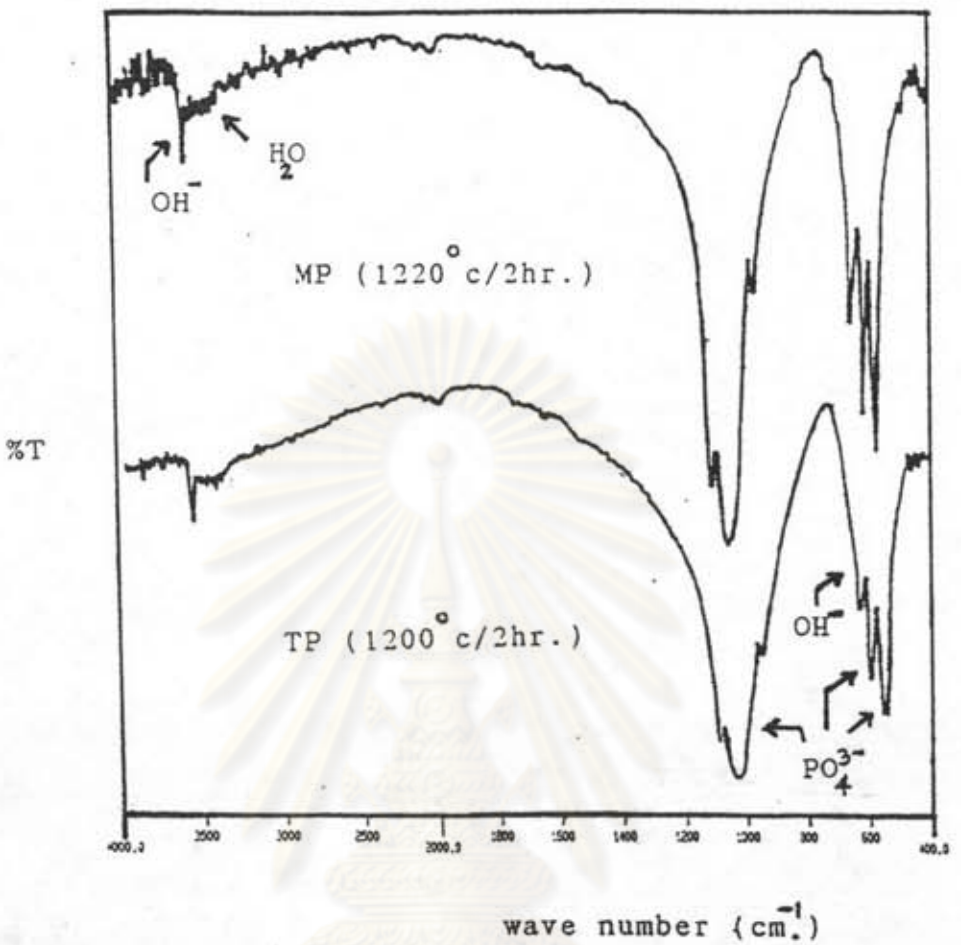


Fig. 4.2 IR absorption spectra of MP and TP specimens

The infrared absorption spectra of the sintered bulk specimens before incubation in SBF showed that :

The sharp peak at 3573 cm^{-1} was assigned to OH^- stretching vibration mode. Peaks at 635 cm^{-1} was assigned to OH^-, 600 and 1040 to 1100 cm^{-1} were assigned to PO_4^{3-}. The small peaks at around 1600 and 3400 cm^{-1} were relevant to water (H_2O). The IR spectra of both sintered bulk specimens indicated that there was no OH^- loss in the HA structure.

4.4 Bulk Density and Porosity of Bulk Specimens

The bulk density and porosity of sintered bulk specimens before incubation in SBF were shown in Table 4.3

Table 4.3 The bulk density and porosity of sintered bulk specimens before incubation in SBF.

Specimen	Bulk density (g/cm^3)	Porosity (%)
MP (1220°c/2 hr.)	2.95* (93.2 %)	0.74*
TP (1200°c/2 hr.)	3.03+ (96.0 %)	0.60+

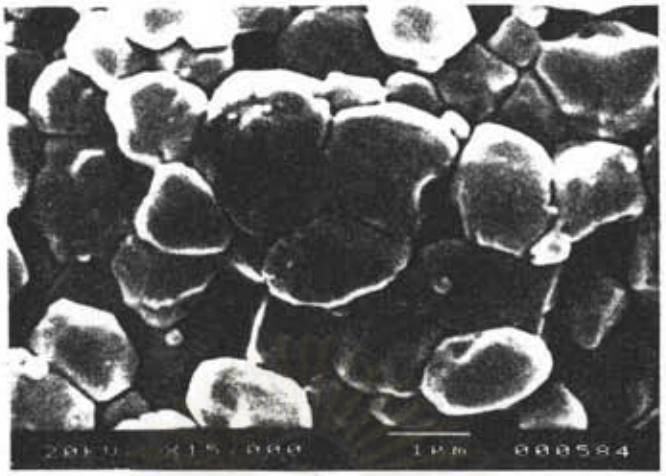
NB. Values in parentheses were relative density to the theoretical density (Theoretical density of HA = 3.16 g/cm^3)

* SD. = 0.01 } For 10 samples

\+ SD. = 0.02 }

4.5 Microstructure of Bulk Specimens

The grain size of MP and TP sintered specimens before incubation in SBF did not reveal enough detail so 0.25% H_3PO_4 etching for 15 sec. was used, as shown in the following figure.



(a)

(b)

Fig. 4.3 The microstructure of bulk specimens
(a) MP, (b) TP before incubation in SBF
0.25 % H_3PO_4 etching for 15 sec.

The characteristics and properties of MP and TP sintered specimens could be summarized as in the following tables.

Table 4.4 The characteristics and properties of MP and TP specimens.

Sintering Condition	Type of Specimen	
	MP	TP
Temperature (^{o}c) Soaking time (hr.) Atmosphere	1220 2 Air	1200 2 Air
Bulk density (g/cm^{3})	2.95	3.03
Relative density (% Theoretical density)	93.2	96.0
Phase present (XRD)	HA (well crystallized)	HA (well crystallized)
IR Spectra	All specimens exhibited OH^{-} absorption bands at wave number 3573 cm^{-1} and 635 cm^{-1}	
Microstructure grain size (μm)	1 - 2	0.5 - 1
Pore Pore size (μm)	Intergranular 0.5	Intergranular 0.1

In this incubation study, specimens were observed with naked eyes every 7 days. The results were as follows :

Table 4.5 Observation results of MP, TP specimens with naked eyes.

Time (days)	Appearance of MP and TP specimens	
	Surface	Edge
0	smooth	sharp
7	smooth	sharp
14	smooth	sharp
21	smooth	sharp
28	less white small particles	sharp
⋮	appears on the middle of the	⋮
⋮	specimens and then covered	⋮
⋮	all over the surface in 90 days	⋮
90	of incubation	sharp

Table 4.5 showed that there was some change on the surface which was so interesting to be studied further by special procedures on the following details.

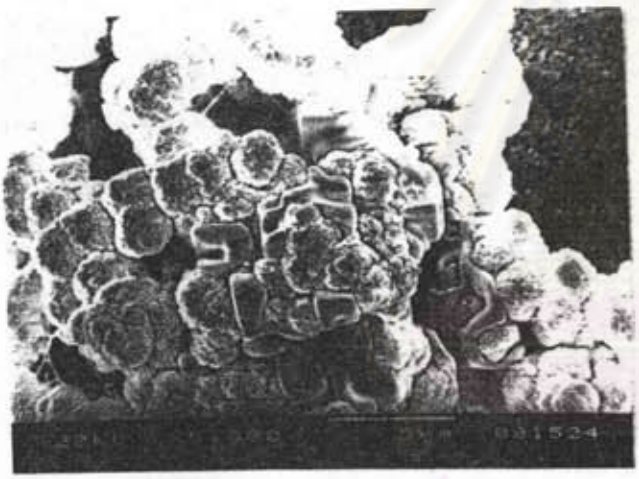
Changeable Characteristics of Bulk Specimens After Incubation

4.1 Observations with the Scanning Electron Microscope (SEM)

Because of the changeable appearance on the surface of two kinds of specimens, so SEM was used to examine in details.

0 day

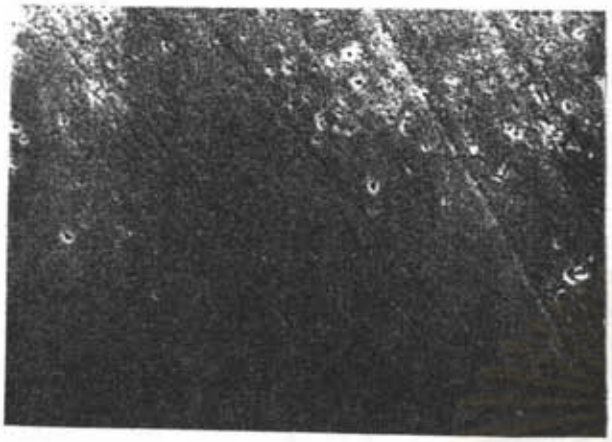
30 days



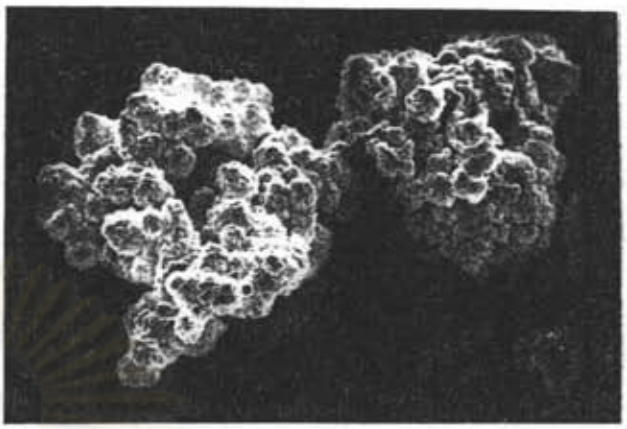
60 days

90 days

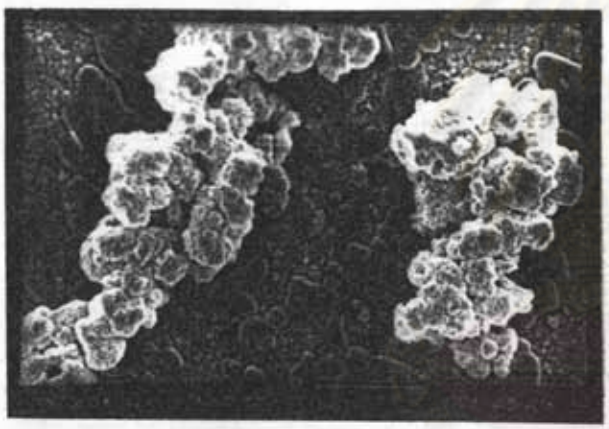
Fig. 4.4 Changes on the surface of MP exposed to SBF solution.



0 day



30 days



60 days

90 days

Fig 4.5 Changes on the surface of TP exposed to SBF solution.

The SEM observation in Fig. 4.4 showed the changeable appearance on the MP surface. After 30 days in SBF the white solid phase formed on the surface of MP specimen and it increased with longer time of incubation, finally it scattered all over the surface after exposure in SBF for 90 days. When observed closely in each solid phase it was composed of many agglomerates with the size of 1-8 μm. In each agglomerate, the primary particles were observed especially at 90 days of incubation. Those primary particles had the characteristics of acicular shape of 0.3 - 0.4 μm., aspect ratio = 3.75 as shown in Fig. 4.6.

Fig. 4.5 showed the characteristics appeared on TP was the same as that on MP. After incubation of TP specimen in SBF for 90 days, it showed that the agglomerate particles (1-3 μm) were composed of the primary particles in acicular shape of the same size (0.3 - 0.4 μm), aspect ratio = 3.75 as shown in Fig. 4.7.

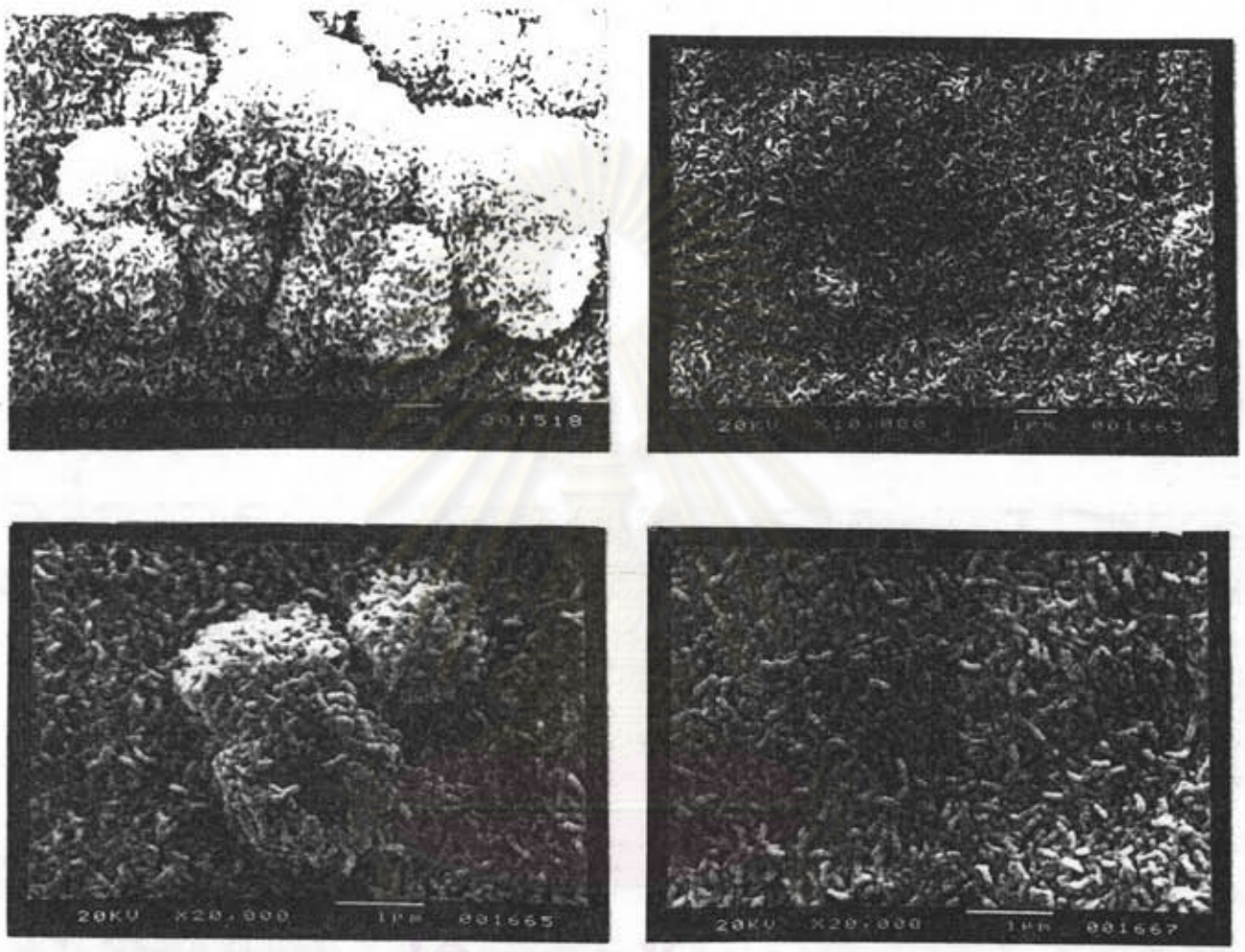


Fig 4.6 The morphology of newly formed solid on the surface of MP specimen (after 90 day incubation)

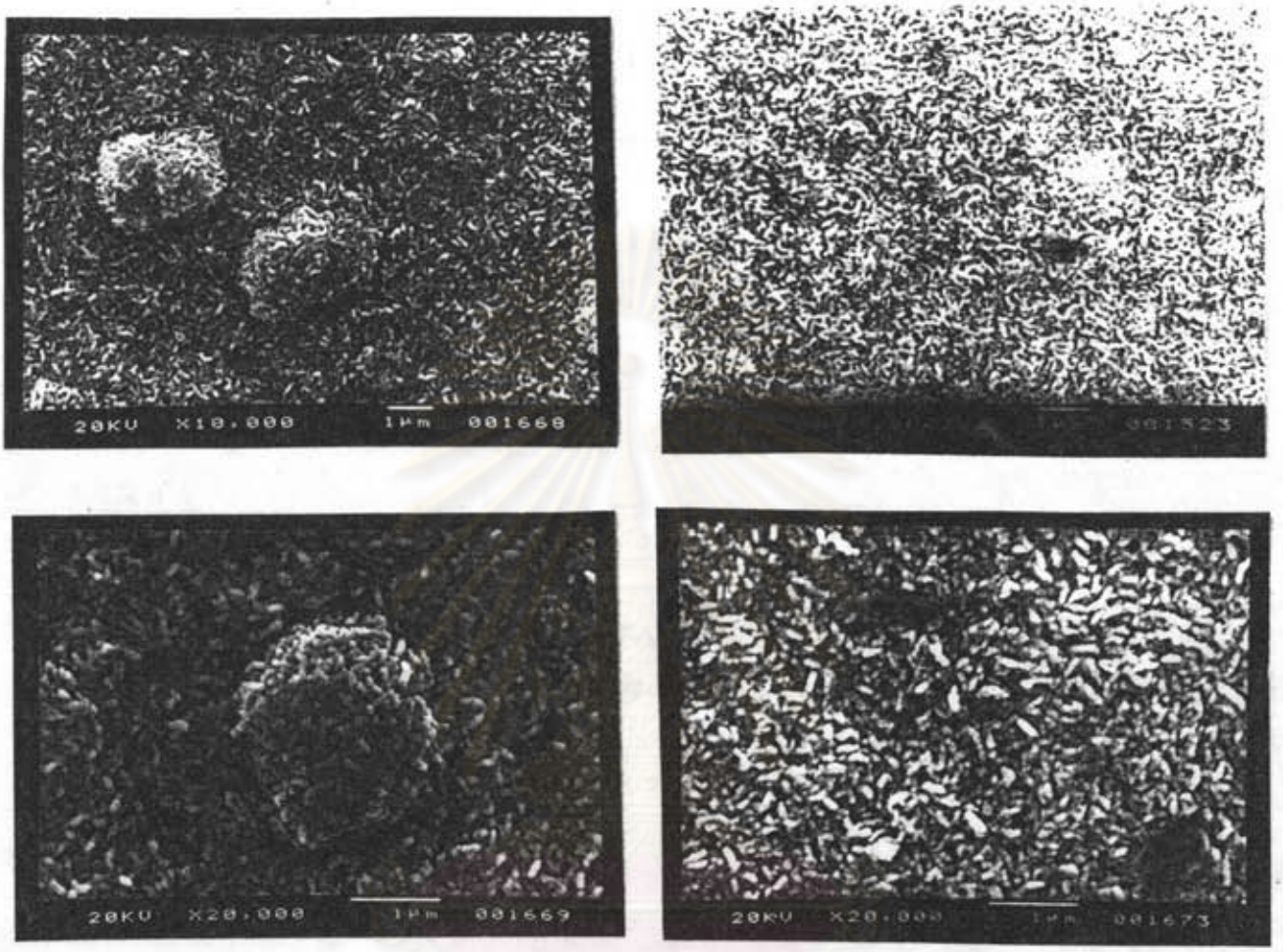


Fig. 4.7 The morphology of the newly formed solid on the surface of TP specimen (after 90 day incubation)

The primary particle in acicular shape was formed on the surface of MP and TP specimens after incubation in SBF for 90 days. Hyakuna et al., 1990 found the needle-like solid on TCP and HA specimen after incubation in PECF(+) (pseudo extracellular fluid) for 14, 28 days, respectively. The morphology was shown in Fig. 4.8.

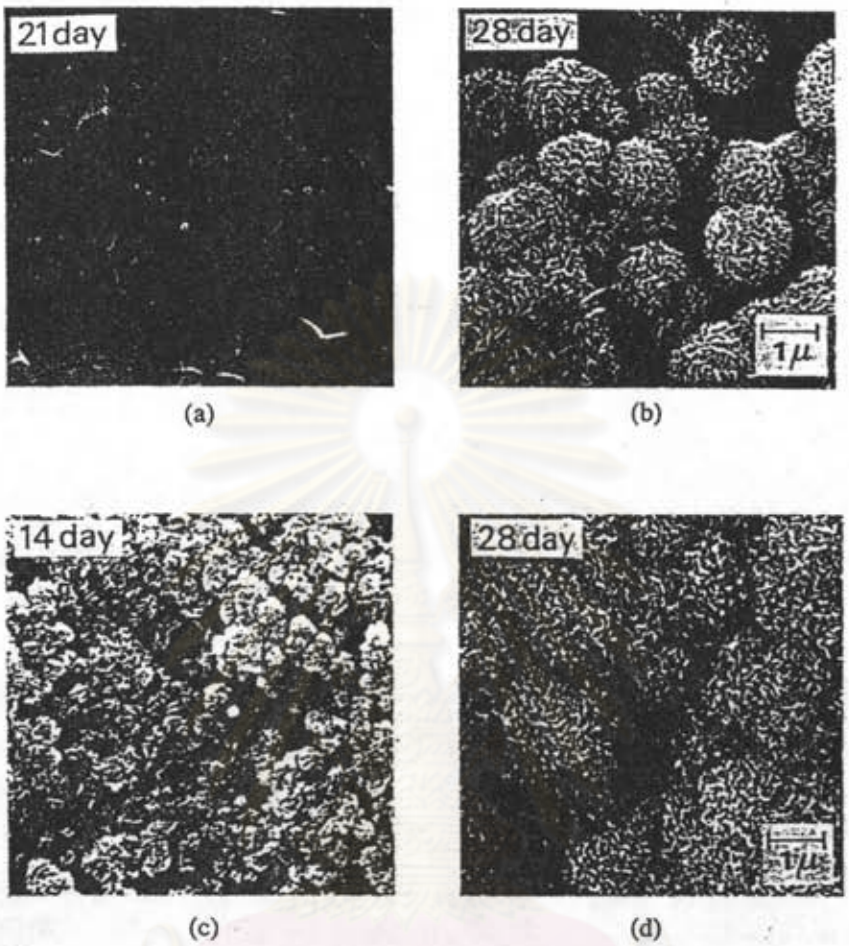


Fig 4.8 Changes on the surface of HA and TCP exposed to solution PECF(+). (a,b) HA,(c,d) TCP. The time required for solid phase formation was estimated to be 14 days for TCP and 28 days for HA. (Hyakuna et al., 1990)

4.2 Chemical Analysis of Bulk Specimens

After incubation of MP and TP specimens in SBF for 90 days, these specimens were removed to dip in deionized-distilled water for 1 hr., and the newly formed solid phase was scraped out. The chemical composition of these bulk specimens after scraping was analyzed in order to compare with those before incubation. Table 4.6 showed the chemical composition of MP and TP bulk specimens after 90 days of incubation.

Table 4.6 The chemical composition of bulk specimens of MP and TP after incubation for 90 days.

HA Type / Composition	MP	TP
Ca (%)	37.0	38.2
P (%)	17.7	17.9
Ca : P	1.62 : 1	1.65 : 1
Impurities		
Mg (%)	1.08	0.93
Fe (%)	0.05	<0.02
Zn (ppm)	143	42
Cu (ppm)	2	2
Mn (ppm)	5	5
As (ppm)	1.7	<0.5
Cd (ppm)	<0.5	<0.5
Pb (ppm)	<5	<5
Hg (ppm)	<1	<1

After comparing the Ca : P ratios of MP and TP before (Table 4.1) and after (Table 4.6) incubation in SBF for 90 days, it could be summarized that the incubation in SBF caused the decrease of Ca : P ratio in MP and TP respectively but the impurities of MP and TP did not change. Although Ca : P ratio decreased, the bulk specimens could still be considered as hydroxyapatite and they were checked

by X-ray diffraction.The Ca:P ratio in both of bulk specimens after incubation for 90 days decreased due to the weak point of HA structure (PP.10-11) where Ca^{2+} in the column easily moves into the solution.

To make sure for the high impurities especially zinc, the SBF solution was collected every 7 days for the period of 90 days and added together for determining the quantity of zinc in this solution by ICP/INAA method; the result showed that there was so little zinc in this solution which was undetected by this instrument (Zn < 0.02 mg/l). Zn is a heavy metal and toxic to the body. Since the release of Zn in solution was so little it suggested that hydroxyapatite from cattle bone could be used as implant in human body.

4.3 Phase Analysis of Bulk Specimens

The phase of two kinds of HA specimens was analyzed before and after incubation in SBF for 90 days. The phase present was hydroxyapatite, as shown in Fig. 4.6 and 4.10.

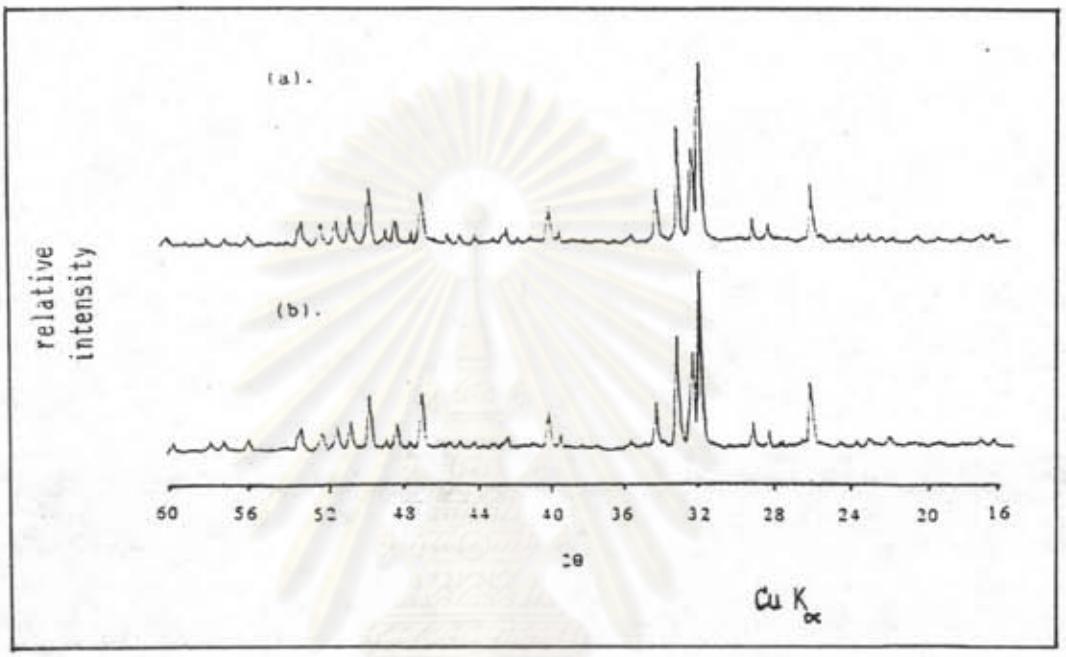


Fig. 4.9 XRD pattern of MP bulk specimen
(a) Before incubation in SBF, pH 7.4, 37°c
(b) After incubation in SBF, pH 7.4, 37°c for 90 days.

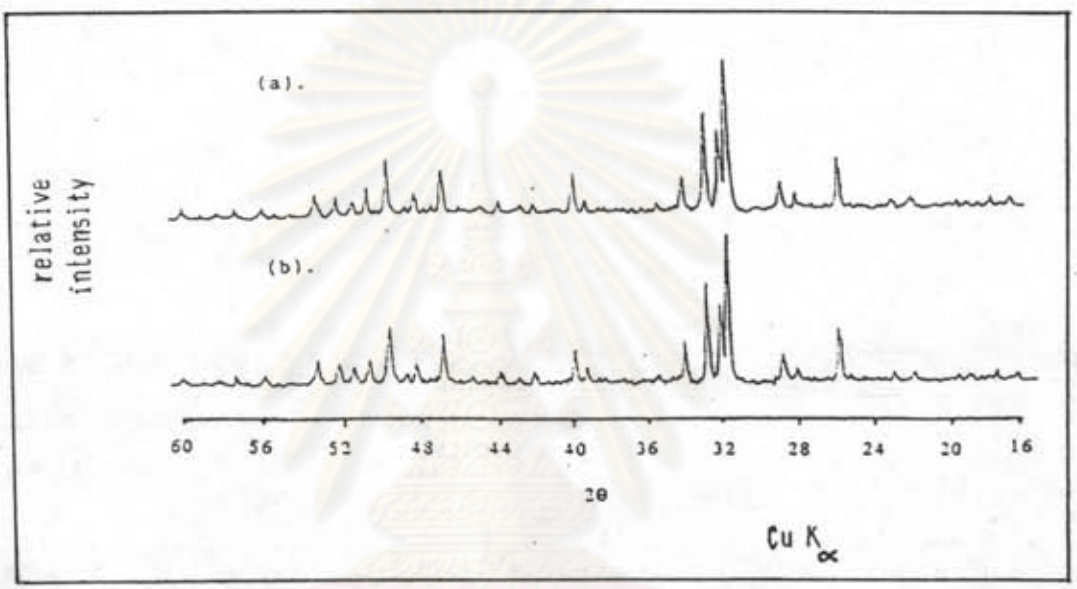


Fig. 4.10 XRD pattern of TP bulk specimen

(a) Before incubation in SBF, pH 7.4, 37 °c

(b) After incubation in SBF, pH 7.4, 37 °c for 90 days.

Functional groups of bulk specimens before and after incubation in SBF for 90 days were examined by FTIR, as shown in the following figures.

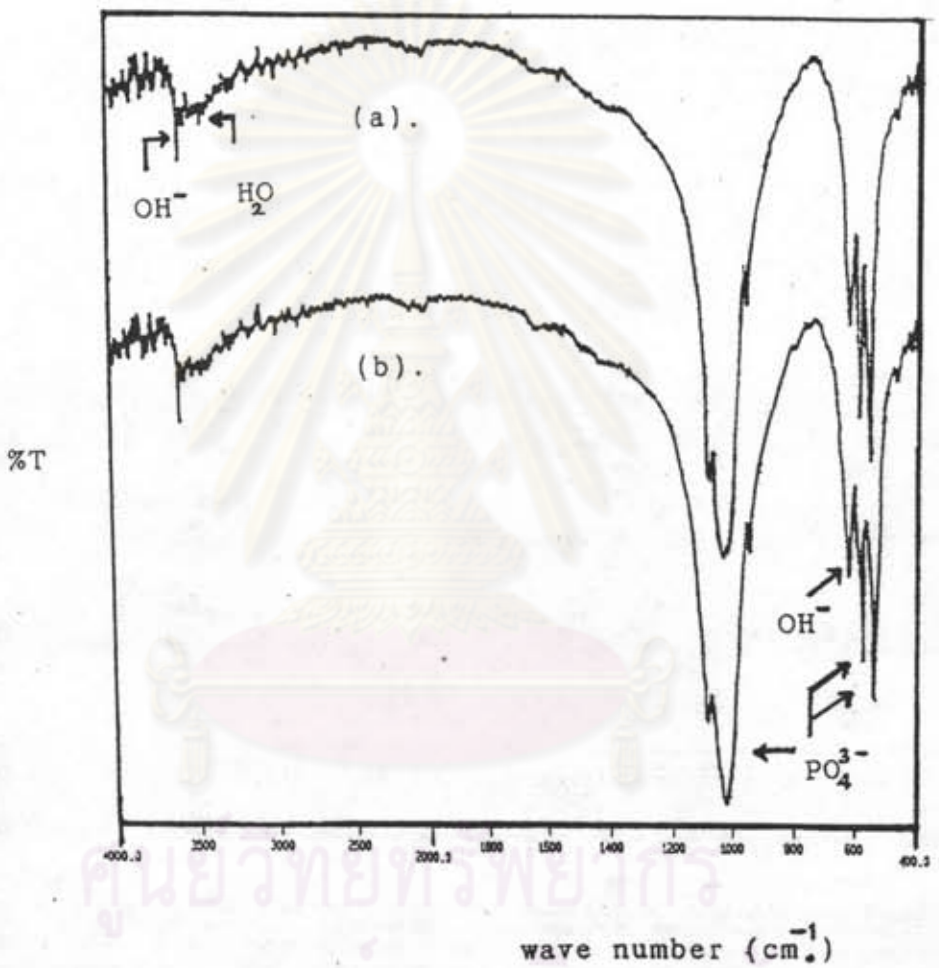


Fig. 4.11 IR absorption spectra of MP bulk specimens
(a) Before incubation in SBF
(b) After incubation in SBF, pH = 7.4, 37°c for 90 days.

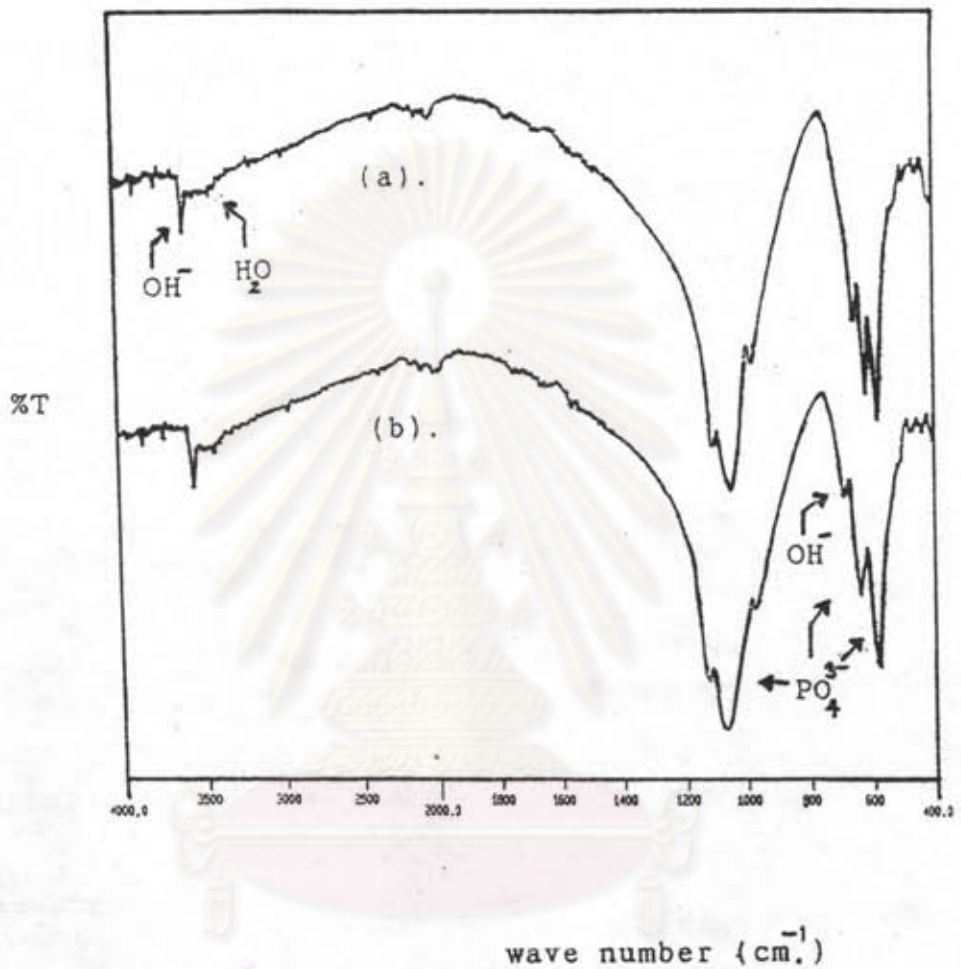


Fig. 4.12 IR absorption spectra of TP bulk specimens
(a) Before incubation in SBF
(b) After incubation in SBF, pH = 7.4, 37 °c for 90 days.

From XRD and IR results showed that there were no changes in phase and functional group of two types of bulk specimens. The phase present of MP and TP before and after incubation in SBF for 90 days was still well-crystalline hydroxyapatite.

4.4 Bulk Density and Porosity of Bulk Specimens

The bulk density and porosity of bulk specimens before and after incubation in SBF, pH = 7.40 for 90 days were shown in Table 4.7.

Table 4.7 Bulk density and porosity of bulk specimens before and after incubation in SBF, pH = 7.40, 37 °c for 90 days.

Specimen	Bulk density (g/cm^3)		Porosity (%)	
	Before	After	Before	After
MP	2.95 (93.2 %)	2.90 (91.8 %)	0.74	0.79
TP	3.03 (96.0 %)	3.00 (95.0 %)	0.60	0.64

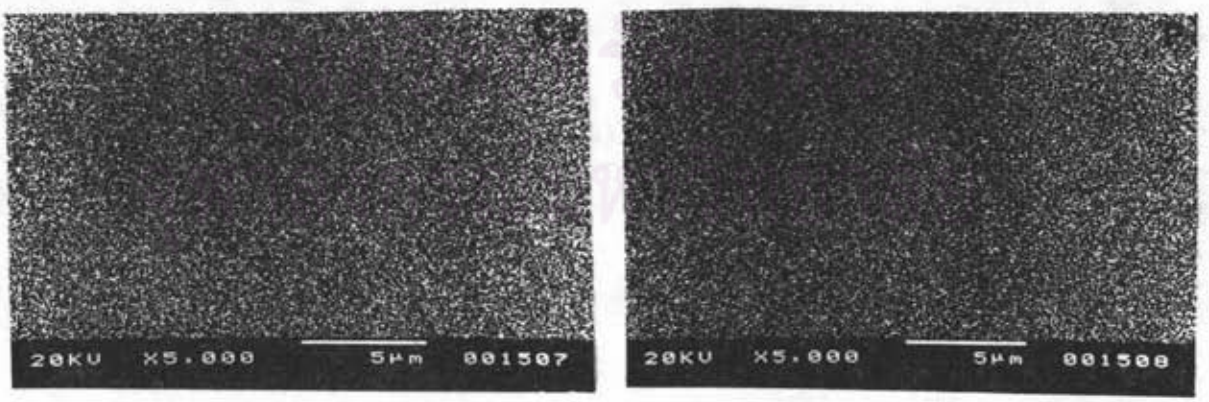
NB. Figures in parentheses were the density relative to the theoretical density (% TD).

Table 4.7 showed that the bulk density and porosity of MP and TP after incubation in SBF for 90 days were changed. The bulk density decreased by 1.5 %, 1.1 % and the porosity increased by 6.8%, 6.7% in MP and TP respectively.

4.5 Ca and P Dot Mapping of Bulk Specimens

The surface of specimens was analyzed in the form of Ca and P dot mapping by SEM-EDX, as shown in the following figure.

(a).

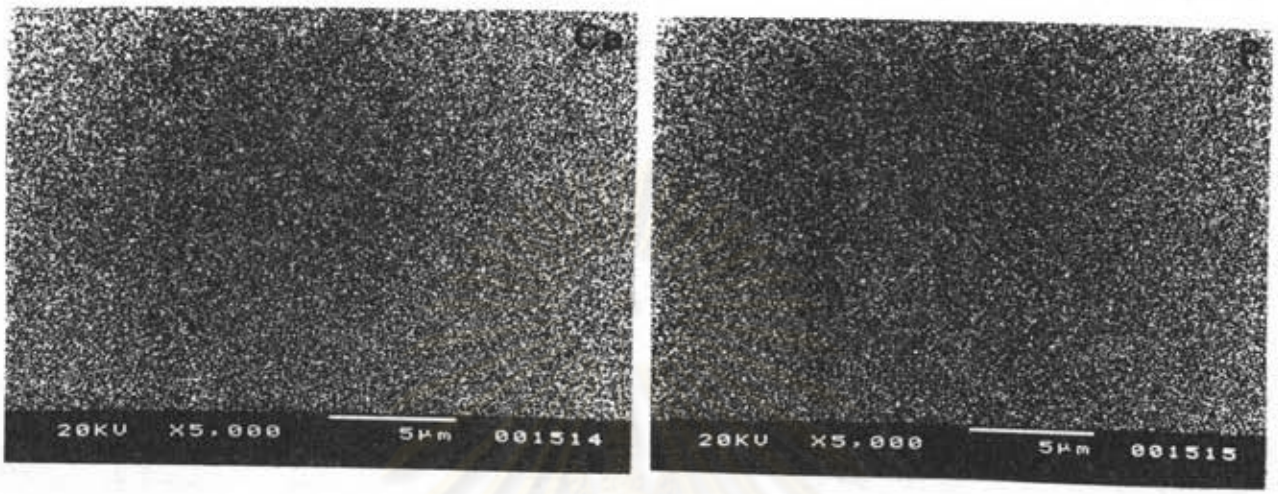


(b).

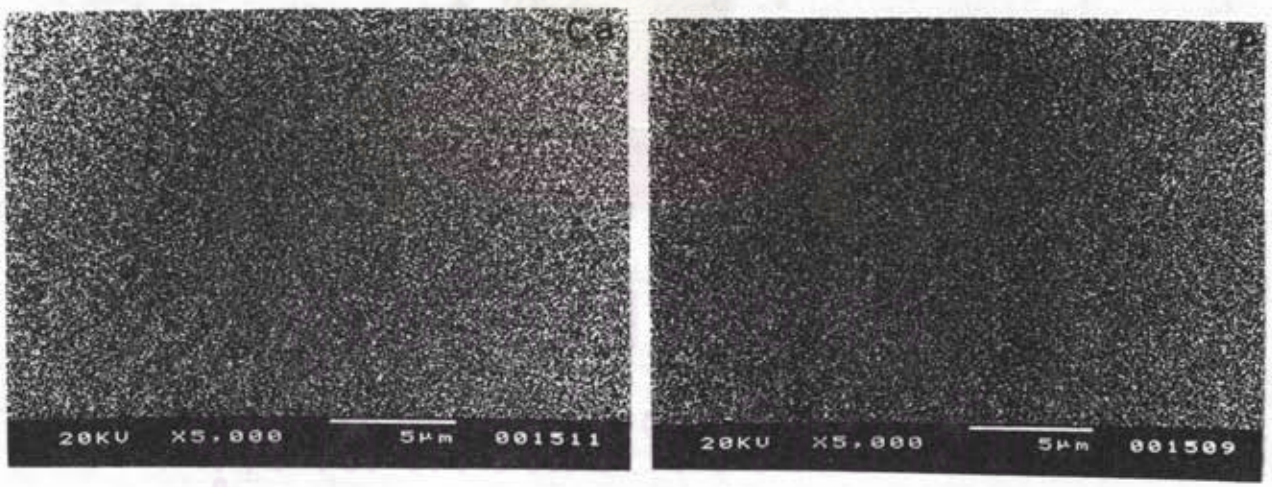
Fig. 4.13 Ca and P dot mapping of MP bulk specimens.

(a) Before incubation in SBF.

(b) After incubation in SBF for 90 days.



(a).



(b).

Fig. 4.14 Ca and P dot mapping of TP bulk specimens.

(a) Before incubation in SBF.

(b) After incubation in SBF for 90 days.

From Fig. 4.13 and 4.14 showed that the concentration of Ca and P in the bulk specimens before and after incubation in SBF did not change. The changed in Ca, P concentration could not be detected by this method, but could be detected by the chemical analysis in Table 4.6.

4.6 Fresh Fracture Surface

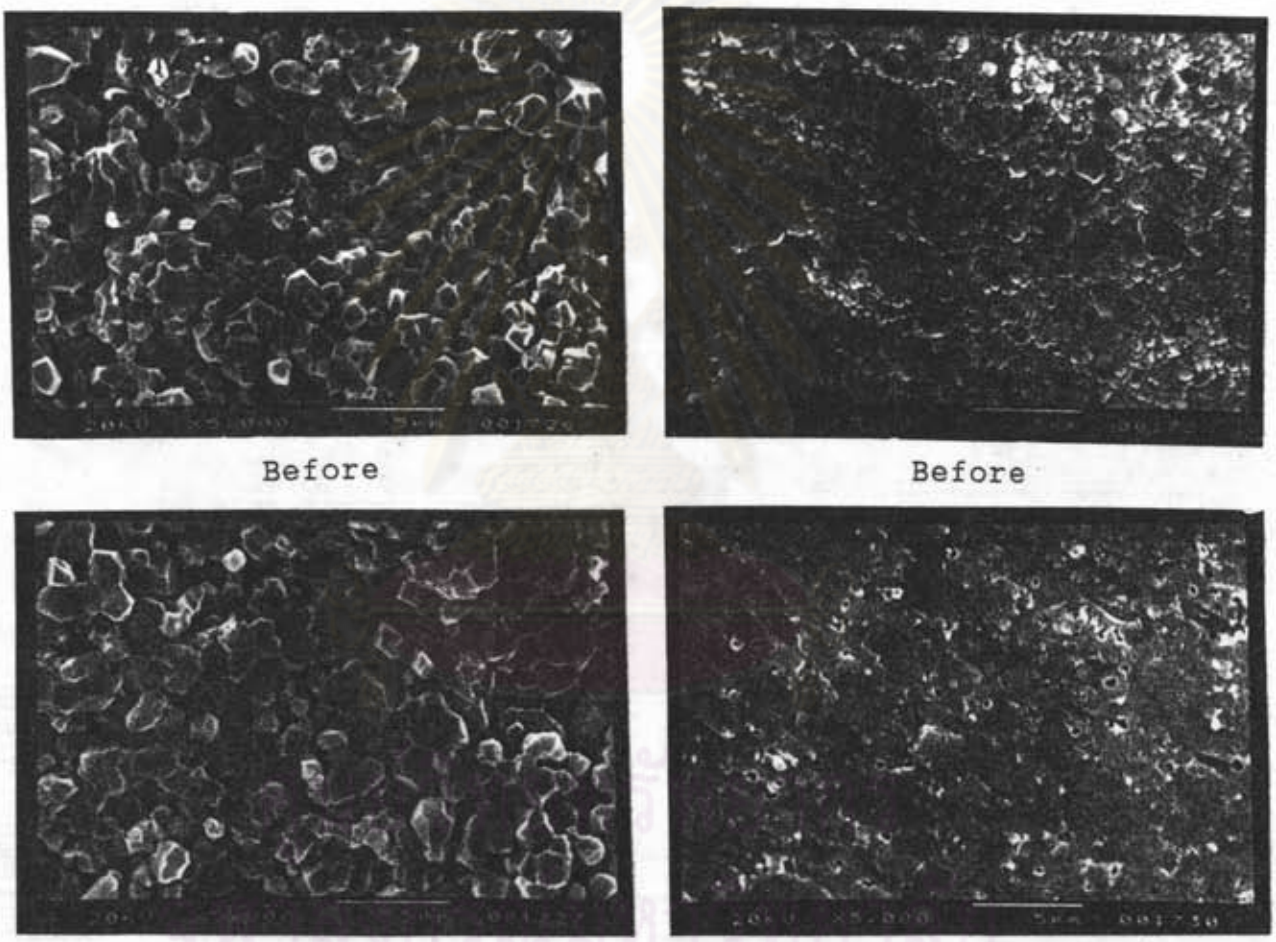


Fig. 4.15 The microstructure of fresh fracture surface of MP and TP specimens before and after incubation in SBF for 90 days

(a) MP

(b) TP

The microstructure of fresh fracture surface in Fig 4.15 showed that the newly formed solid did not appear in the bulk of the specimens, but only on the surface.

Identification of the Newly Formed Solid

4.1 Phase Analysis

The researcher has tried very best to identify the new formed solid phase, so this solid phase was scraped off and identified by XRD (Fig. 4.16). In the other way the new solid phase and the bulk specimens were ground together and identified by XRD (Fig. 4.17, 4.18).

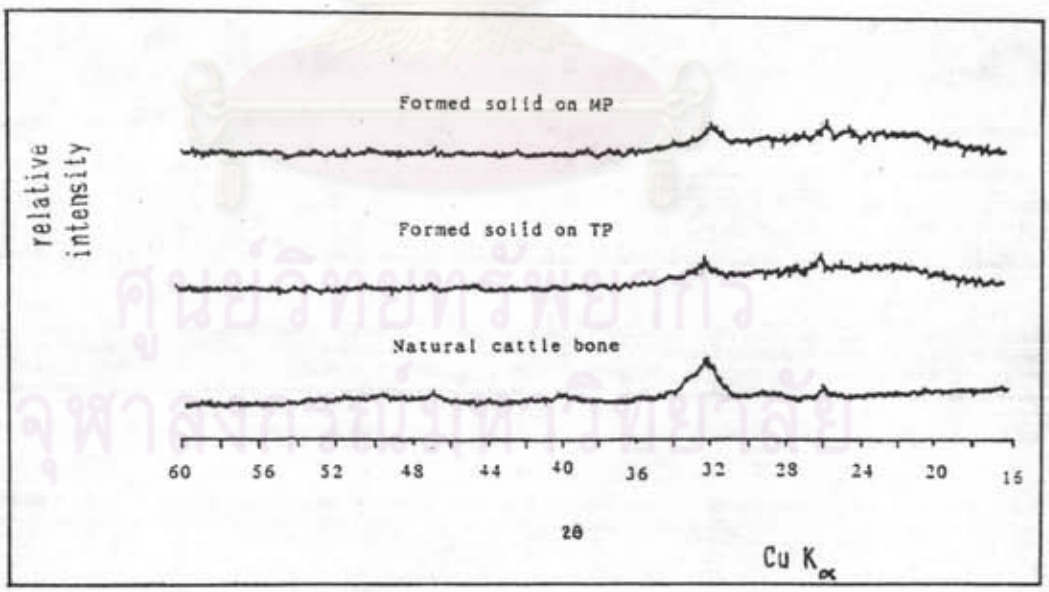


Fig. 4.16 XRD patterns of new formed solid on MP and TP bulk specimens after exposure to SBF for 90 days.

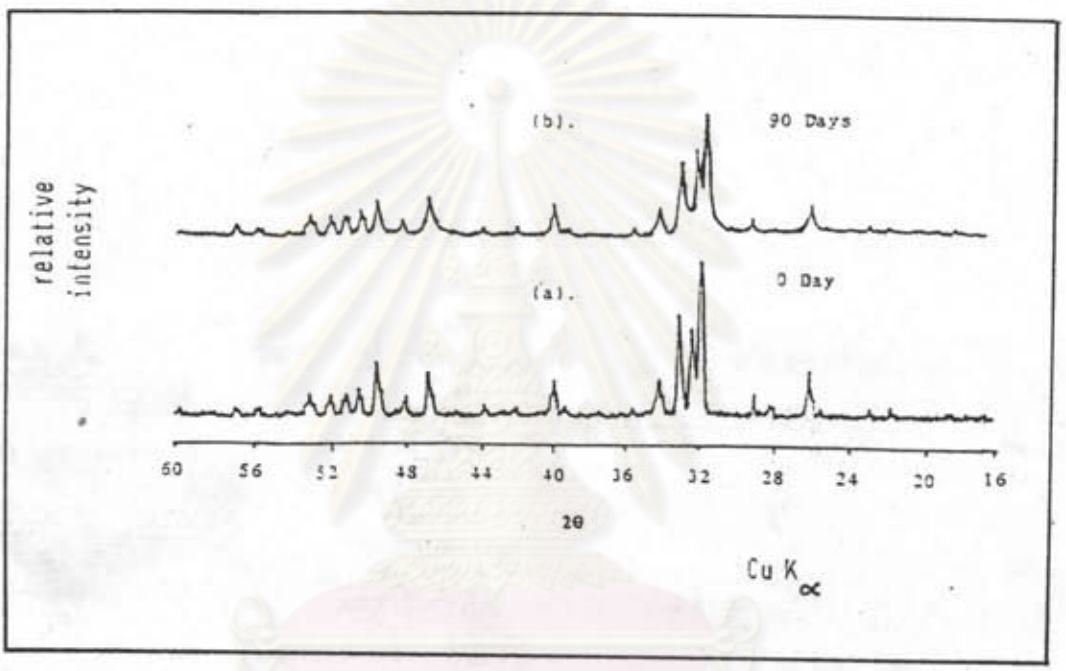


Fig. 4.17 (a) XRD patterns of surfaces of MP bulk specimen before incubation in SBF.
(b) XRD patterns of newly formed solid mixed with MP bulk specimen after exposure to SBF for 90 days.

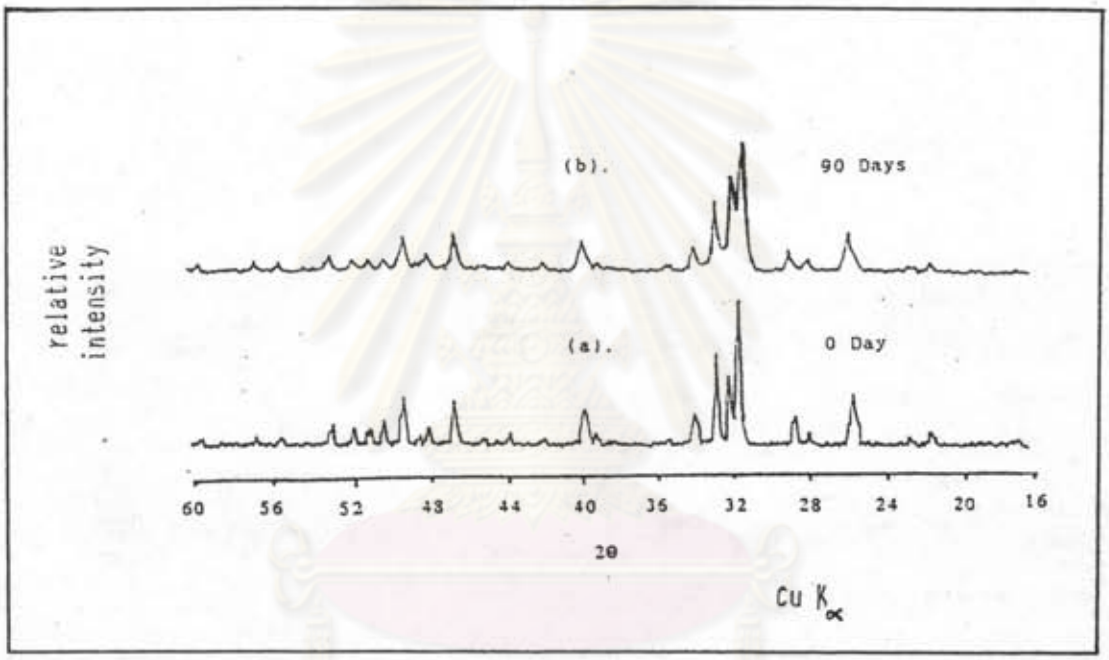


Fig. 4.18 (a) XRD patterns of surfaces of TP bulk specimen before incubation in SBF.

(b) XRD patterns of new formed solid mixed with TP bulk specimen after exposure to SBF for 90 days.

From XRD patterns of the newly formed solid showed broad peak similar to the natural cattle bone. It indicated that natural cattle bone was not-well-crystallized. After comparing the XRD patterns of the mixture of newly formed solid and bulk specimens (MP and TP) after exposure in SBF for 90 days with XRD pattern of bulk specimens (MP and TP) in Fig. 4.17 and 4.18, it could be summarized that the sharp peak indicated the well-crystallized hydroxyapatite phase of two types of the bulk specimens became broader when mixed with the newly formed solid. On the other could be said that the broad peak of the newly formed solid became a little bit sharper when mixed with the bulk specimen. This appearance was found in both MP and TP specimens.

4.2 Functional Group

Infrared reflection spectra of the specimens before and after incubation in SBF for 90 days were shown below.

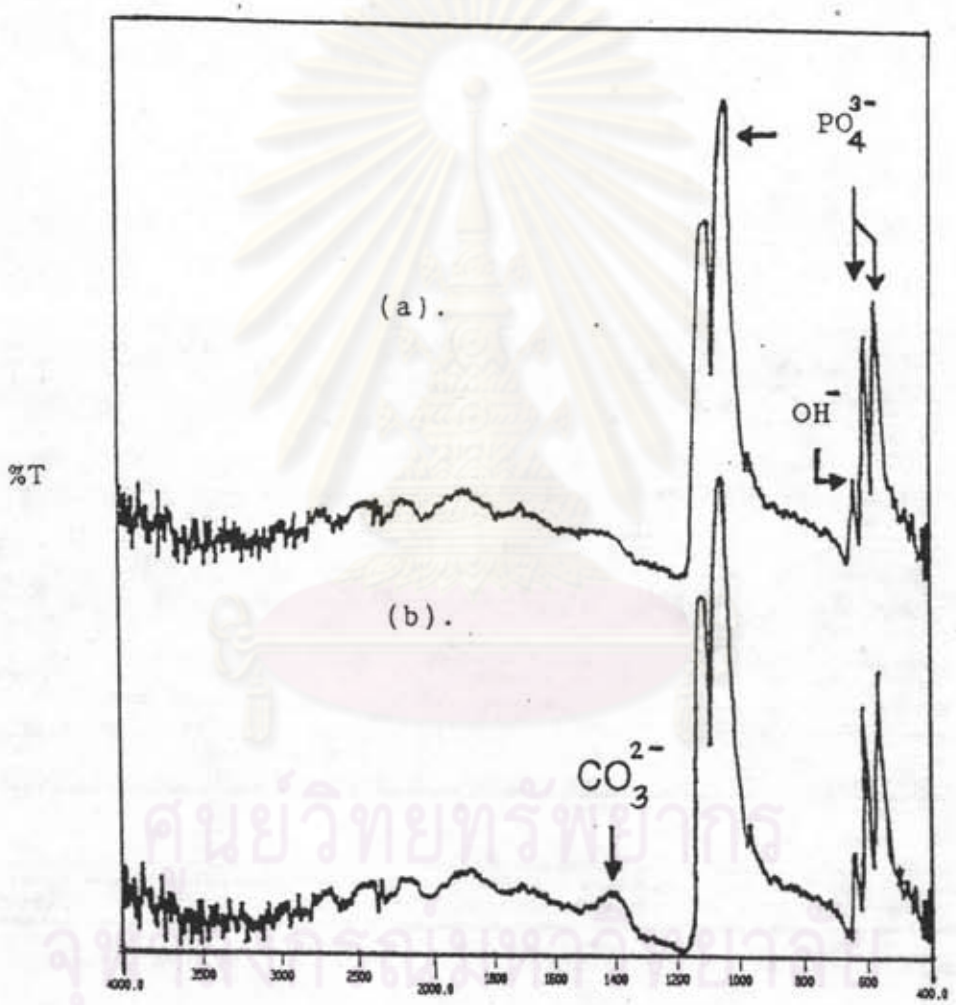


Fig. 4.19 IR reflection spectra of MP.

(a) Before incubation.

(b) After incubation in SBF for 90 days.

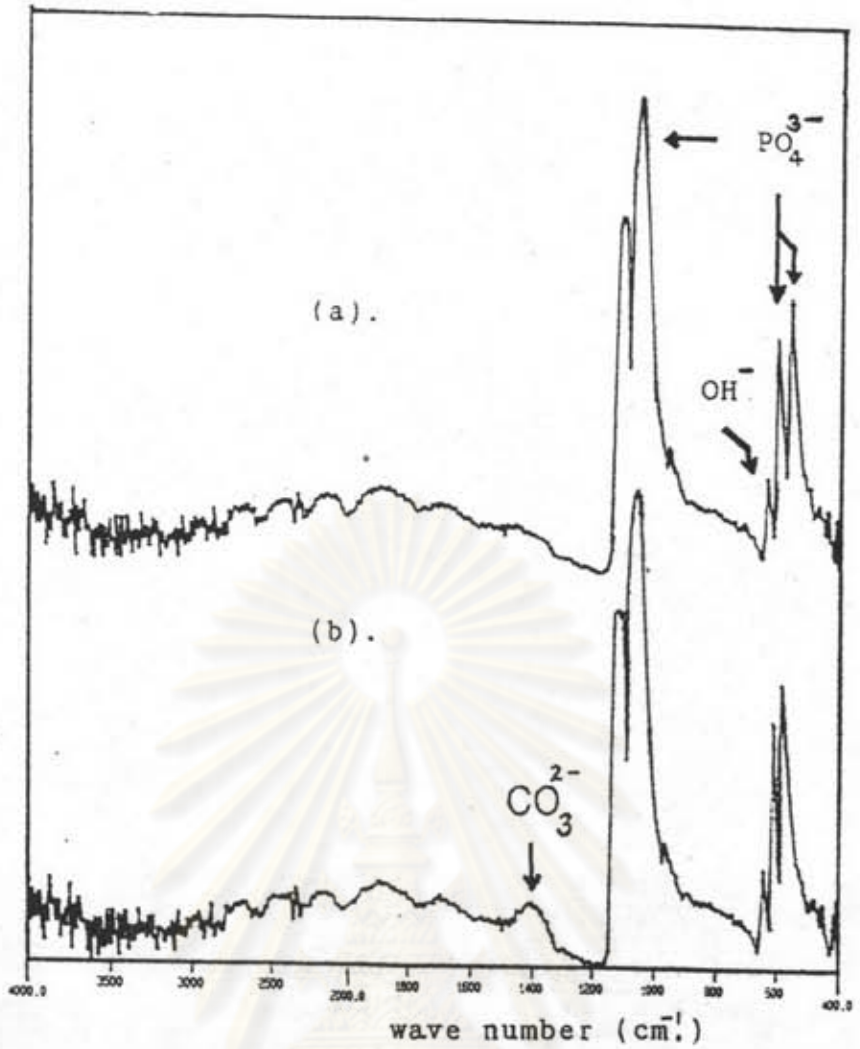


Fig. 4.20 IR reflection spectra of TP.

(a) Before incubation.

(b) After incubation in SBF for 90 days.

The infrared reflection spectra of bulk specimens before incubation and those with newly formed solid after incubation in SBF for 90 days were shown in Fig. 4.19 and 4.20. The reflection spectra of newly formed solid on the surface of two bulk specimens after incubation in SBF for 90 days showed the peak at 1410 cm^{-1} which was assigned to CO_3^{2-} in phosphate site (Voegel and Garnier, 1979). This IR reflection spectra revealed CO_3^{2-} group in the newly formed solid but not in the bulk of both MP and TP specimens.

4.3 Elemental Composition

The elemental composition of newly formed solid phase was determined by EDX, the result was shown in Fig. 4.21.

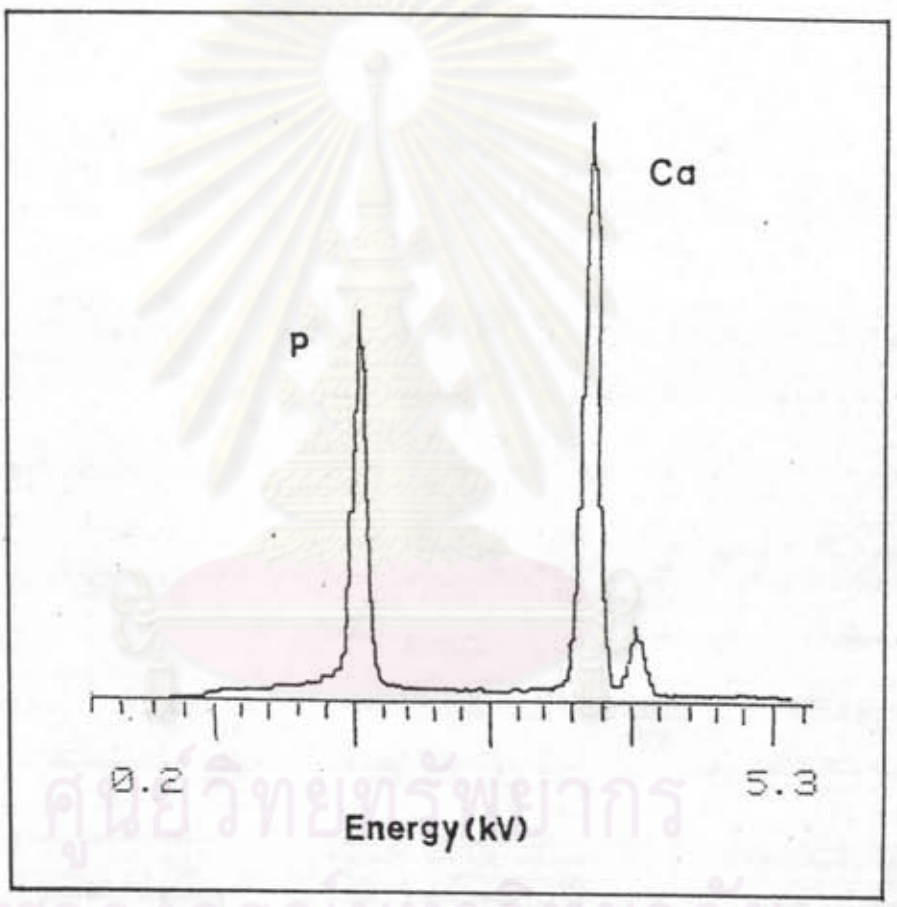


Fig. 4.21 Elemental composition of newly formed solid phase on MP and TP specimens

This finding showed that the newly formed solid on MP and TP specimens after incubation in SBF for 90 days was the compound of calcium and phosphorus.

The results from three methods (XRD, IR and EDX) of analysis of the formed solid, were summarized that the newly formed solid on the bulk of both MP and TP specimens was a not-well-crystallized calciumphosphate compound with carbonate group in the phosphate site structure. This compound was composed of many primary particles in acicular shape and was found only on the surface of MP and TP specimens.

Because this compound bonded loosely on the bulk surface so the bulk density of MP and TP after incubation in SBF for 90 days was decreased, and the porosity was increased. The changes on the surface of two bulk specimens caused the decrease in Ca : P ratio of the bulk specimens.

Analysis of the Changes in SBF solution

The concentration of Ca and P in SBF was determined by ICP method. Fig. 4.22 showed the changes when MP and TP were incubated in SBF.

Table 4.8 Changes in SBF solution expressed as concentration of Ca and P ions with incubation time of MP specimen.

Time (days)	Ca (ppm)	P (ppm)
0	100.00	31.00
7	87.94	28.75
14	87.62	28.58
21	87.70	28.94
28	87.50	28.50
35	87.45	28.64
42	87.48	28.70
49	87.49	28.55
56	87.31	28.86
63	87.46	28.45
70	87.51	28.57
77	87.58	28.32
84	87.46	28.45
91	87.45	28.29

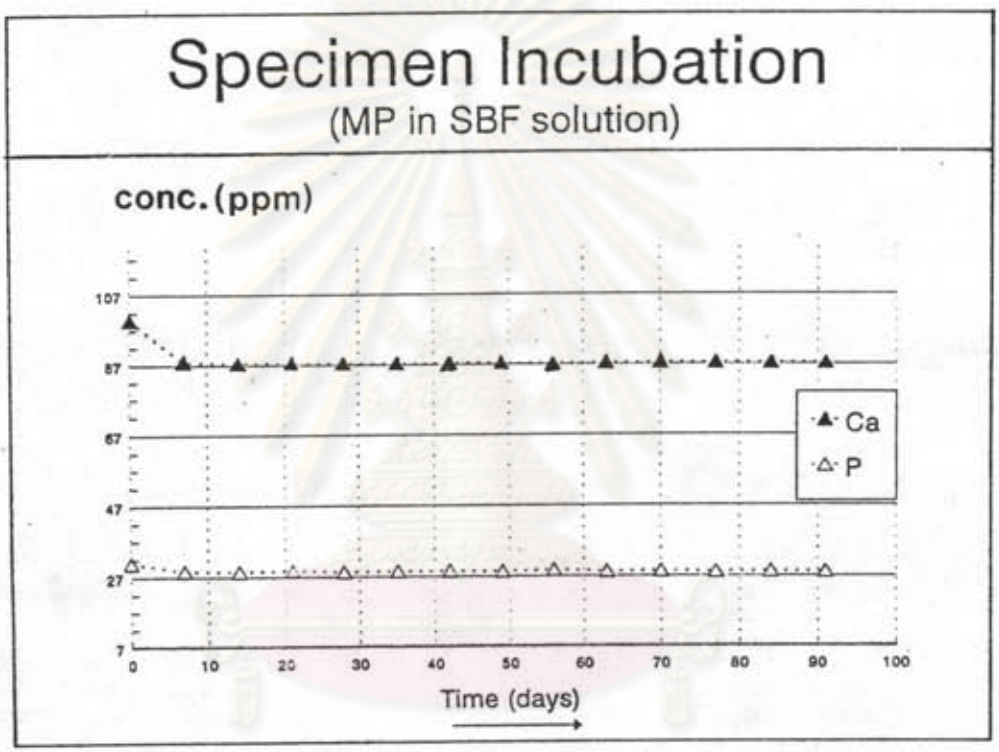


Fig 4.22 Changes in SBF solution expressed as concentration of Ca (▲) and P (△) ions with incubation time of MP specimen.

Table 4.9 Changes in SBF solution expressed as concentration of Ca and P ions with incubation time of TP specimen.

Time (days)	Ca (ppm)	P (ppm)
0	100.00	31.00
7	92.30	29.49
14	92.45	29.28
21	92.47	29.31
28	92.57	29.29
35	92.49	29.37
42	92.28	29.55
49	92.71	29.57
56	92.75	29.79
63	92.80	29.82
70	92.67	29.85
77	92.51	29.75
84	92.59	29.47
91	92.58	29.49

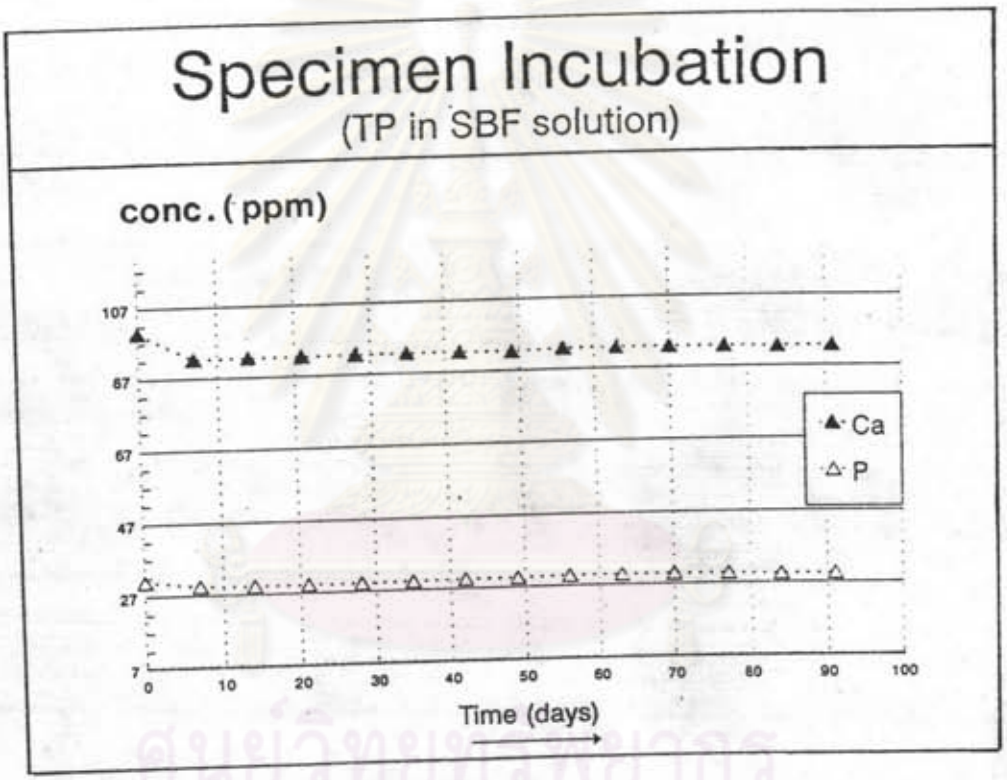


Fig 4.23 Changes in SBF solution expressed as concentration of Ca (▲) and P (Δ) ions with incubation time of TP specimen.

The results of changes in SBF solution showed that the concentration of Ca and P ions in the solution decreased every 7 days. For MP incubation, it was found that the concentration of Ca and P decreased more than that of TP incubation because of the higher porosity in MP (93% TD. for MP and 96% TD. for TP).

The decrease in Ca and P concentraton of SBF solution (as the finding in Fig.4.22, 4.23, renewal of SBF solution was done every 7 days) should have been caused by the precipitation of newly formed solid on the surface of MP and TP. MP and TP specimens (HA) acted as the substrate and seeding material for crystallization in the type of heterogeneous nucleation growth.

So far nucleation had been considered as a homogoneous process, that was, the influence of solids other than the precipitating phase had been ignored. In practice, however the influence of impurities was strong. Again, there were two distinctly different models which could be assumed in a theoretical formulation of heterogeneous process. The first, as before, assumed that the nucleus (critical) resembled a small piece of bulk phase. In Fig. 4.24 the influence of the substrate was shown to affect the coherence of the interface by causing dislocations. Turnbull and Vonnegut(quoted in Walton, 1979) had explored this model and concluded that the energy barrier to nucleation was modified in two ways. First the deposited nucleus was distorted, affecting the interatomic forces and second, the

introduction of the solid-solid interface modified the surface energy requirements.

Fig. 4.24 Turnbull-Vonnegut model for the arrangement of atoms in the interfacial region.

The lattice disregistry between deposit and substrate lead to distortion and dislocations in the deposit surface.

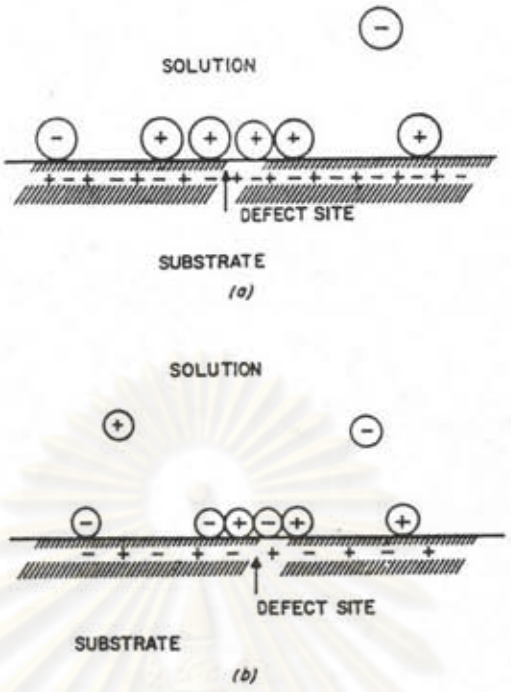


Fig. 4.25 (a). Model for the two-dimensional coherent nucleation of ions onto an ionic substrate.

(b). Model for two-dimensional incoherent nucleation of strongly interacting ions.

From Fig.4.25(a)., the clustering process followed a reversible sequence of adsorption, diffusion, and incorporation into the cluster. In this particular model the position of depositing ions was determined entirely by the substrate lattice configuration and would be applicable to the deposition of weakly interacing ions or molecules which were strongly adsorbed. Such a situation might, for example, apply to the deposition of (111) planes of oriented alkali halide crystal as shown in Fig.4.25(b). Clustering was

determined by the lattice of the deposit rather than the substrate, although the most favorable energetic process still corresponded to a perfect lattice match between deposit and substrate. Such a situation might occur with (100) or (110) deposition of alkali halides.

The reason for larger agglomerate particles on MP than TP specimens was due to the different substrate lattice configuration of MP and TP specimen that acted as the substrate in this process (MP had larger grain size than TP specimen). The other reason was according to the high quantity of Zn^{2+} in MP. Zn^{2+} was the inhibitor on the dissolution of HA (PP.18), so less nucleus for precipitation was formed on MP specimen and resulted in the larger size of agglomerate particles on MP than on TP specimen surface.

From Table 4.1 and 4.6, the decreased ratio of Ca:P in both specimens after incubation could be calculated the ratio in MP was 1.56:1 and TP was 1.83:1.

From Table 4.8 and 4.9 the decreased ratio of Ca:P of SBF solution at every 7 days of MP and TP incubation could be calculated. The ratio of Ca:P was 3.67:1 and 3:1 for MP and TP respectively.