

Hardening and Hydroxyapatite Formation of Bioactive Cement Prepared from Calcium Phosphosilicate Glass

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(Received August 2, 1995)

It has been reported that the biocement obtained by mixing CaO-SiO₂-P₂O₅ glass powders with ammonium phosphate solution has biocompatibility as well as high strength. The hardening mechanism and hydroxyapatite forming mechanism were discussed when 53.6%CaO, 38.1%SiO₂, 7.7%P₂O₅, 0.6%CaF₂ (mole %) glass powder was reacted with ammonium phosphate solution and reacted in tris-buffer solution, respectively. High strength hardened biocement was obtained for the specimen with CaNH₄PO₄·H₂O crystal when the glass powder was mixed with ammonium phosphate solution, and hydroxyapatite crystal was rapidly formed only in the sample with CaNH₄PO₄·H₂O crystal when it was reacted in tris-buffer solution.

Key words: Bioactive glass, Biocement, Hydroxyapatite, Hardening

I. Introduction

A biocompatible bone cement to transfer load from the prosthesis to the bone or increase the load carrying capacity of surgical construct could have potential orthopaedic applications. This bone cement can also be used as artificial bone grafts for bone defects.¹⁾

Several bone cements, such as polymethylmethacrylate (PMMA), hydroxyapatite and tricalcium phosphate, have been introduced for clinical applications. PMMA is most widely used as a bone cement at present.²⁾ But it does not bond to living tissue and acts as a barrier to bone growth. It also has some adverse effects to body because of the temperature rise during curing of PMMA cement and possible monomer release, which is believed to cause cancer in long term use.³⁾

Recently phosphate ceramic cements, such as tricalcium phosphate and hydroxyapatite, are introduced.⁴⁾ These cements also have some problems in use because of their long hardening time, poor mechanical properties and poor stability in a body.

Recently Kokubo *et al.*⁵⁾ introduced a bioactive glass (53.6%CaO 38.1%SiO₂ 7.7%P₂O₅ 0.6%CaF₂; mole%) to use as a biocement. The glass powder was mixed with ammonium phosphate solution, which contains ammonium hydrogen phosphate and ammonium dihydrogen phosphate, to make a paste, and hydroxyapatite was formed when the hardened cement was reacted in simulated body fluid. Its compressive strength was comparable to that of PMMA cement. Their studies were mostly concentrated on the strength improvement of the bioactive cement. No works on the mechanism of hardening and hydroxyapatite formation have been carried out yet.

In this study, therefore, three different setting methods are used to find out cement hardening phenomena, and a systematic work is carried out to find hydroxyapatite formation which is believed to be crucial for bone-bonding.

II. Experimental

1. Glass powder preparation

A glass batch was obtained by mixing the reagent grade chemicals of SiO₂, H₃PO₄, CaCO₃ and CaF₂ with the composition of 53.6%CaO 38.1%SiO₂ 7.7%P₂O₅ 0.6%CaF₂ (mole%). The well premixed glass batch was loaded in Pt-Rh crucible, and melted in MoSi₂ furnace at 1550°C for 2 hours. For a better glass homogeneity, the glass melt was quenched and crushed, and then remelted at the above conditions. Then the glass melt was poured on a stainless steel plate and immediately covered with the other stainless steel plate to prevent from any crystallization. The thin glass plate was pulverized into powder less than 44 μm in an alumina mortar.

2. Preparation of ammonium phosphate solution

Ammonium phosphate solution was prepared by dissolving 60.1 g of (NH₄)₂HPO₄ and 5.0 g of NH₄H₂PO₄ in 100 ml distilled water. The pH of the solution was 7.4.

3. Setting of biocement

The glass powder was mixed with ammonium phosphate solution on acrylate plastic plate. The ratio of weight of glass powder to the volume of solution was set at 1g/0.5 ml. The mixed paste was cast in three different ways as shown in Fig.1-a. That is, 1) open-air method, in

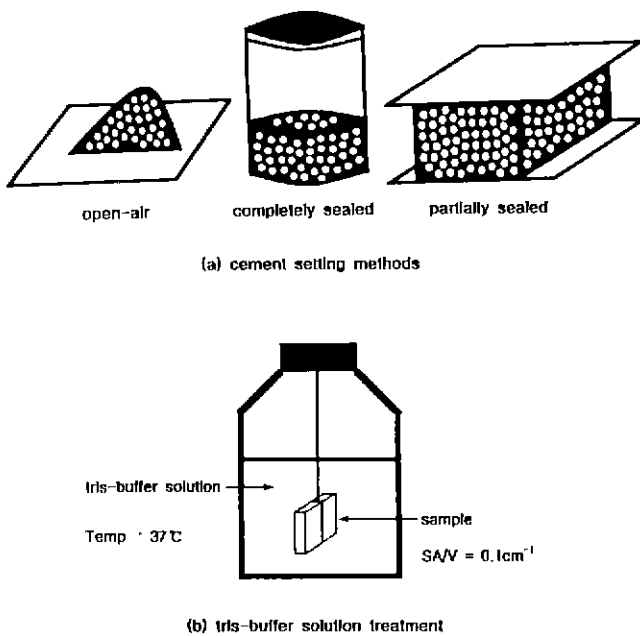


Fig. 1. Schematic diagram of sample preparation.

which the paste was left in open air to harden, and 2) completely sealed method, in which the paste was placed in small beaker with tight seal. In this case, no moisture and gas were expected to be released. And 3) partially sealed method, in which the paste was molded in small rectangular acrylate mold and the both ends were covered with another acrylate plates. In third method the release of gas from the paste was retarded.

The hardening characteristics of the prepared paste were measured by scanning electron microscope (SEM: Hitachi, X-650), fourier transformed infrared spectroscopy with diffused reflection attachment (FTIRRS: Nicolet Instrument, 10 MX), and X-ray diffraction (XRD: Philips Co. PW-1719).

4. Formation of hydroxyapatite

The hardened cement was immersed into tris-buffer solution for various time as shown in Fig. 1-b. The sample surface area to solution volume ratio was set at 0.1 cm^{-1} , and the reaction was carried out at 37°C . The solution was buffered at pH 7.20 by adding 0.2 M of HCl in 0.2 M of trishydroxymethyle aminomethane. The hydroxyapatite formation after tris-buffer solution treatment was examined by SEM, FT-IRRS and XRD.

III. Results and Discussion

1. Hardening mechanism

Glass powder was mixed with ammonium phosphate solution and casted under three different conditions for various time, and their x-ray diffraction patterns are shown in Fig. 2 through Fig. 4.

When the mixture was left in open air less than 30 minutes, only amorphous bump was observed as shown in

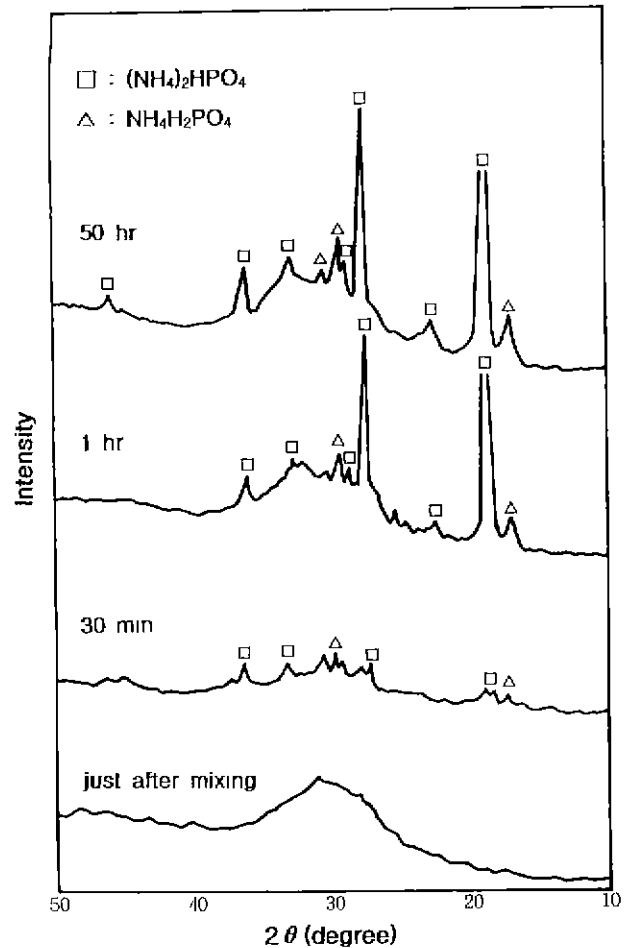


Fig. 2. X-ray diffraction patterns of the hydrated glass powder in open air.

Fig. 2. Crystalline peaks, however, started to be observed after 30 minutes of the reaction and the strongest peaks were appeared when the sample was left in air for one hour, and the peak intensity did not change even if the paste was kept in air for longer time. The crystals formed in the cement were $(\text{NH}_4)_2\text{HPO}_4$ and $\text{NH}_4\text{H}_2\text{PO}_4$, which are both raw chemicals of ammonium phosphate solution. The results of this experiment indicate that those crystalline phases were obtained by reprecipitation from ammonium phosphate solution. No indication of reaction between the glass powder and the ammonium phosphate solution was observed because the supersaturated ammonium phosphate solution dried out very quickly.

In second method, the mixed paste was kept in a completely sealed beaker for some time. The X-ray diffraction patterns of the wet paste are shown in Fig. 3. No crystalline phase was observed as expected even if it was kept for 100 hours in the beaker. However, $\text{NH}_4\text{H}_2\text{PO}_4$ and $(\text{NH}_4)_2\text{HPO}_4$ crystals, which are the starting chemicals of the ammonium phosphate solution, were also formed in the sample if the sample was taken out of the beaker and left in air. This indicates that these crystals were formed only by evaporation of some components,

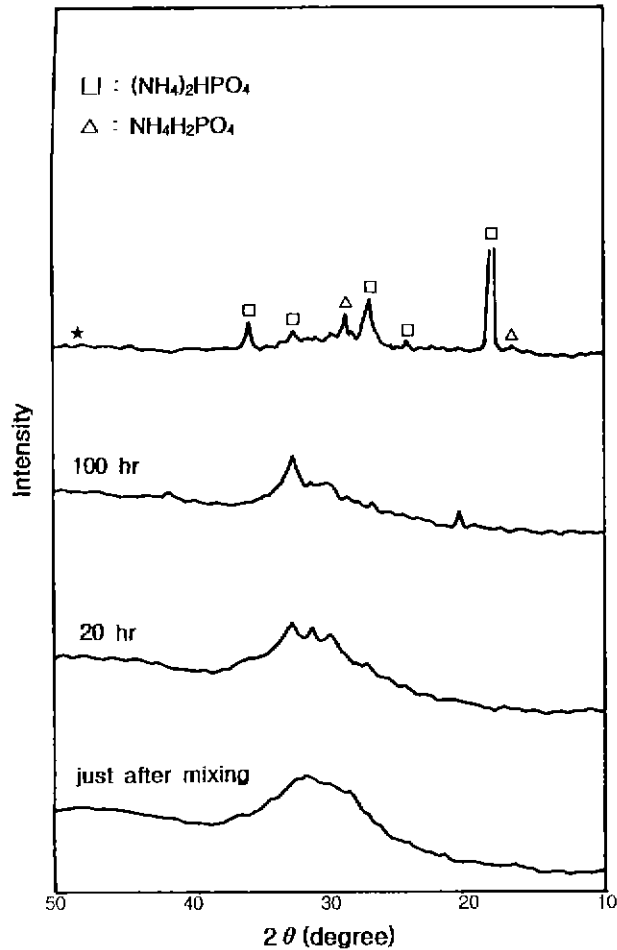


Fig. 3. X-ray diffraction patterns of the hydrated glass powder in completely sealed beaker. (★ 100 hours reacted sample after 24 hours of drying)

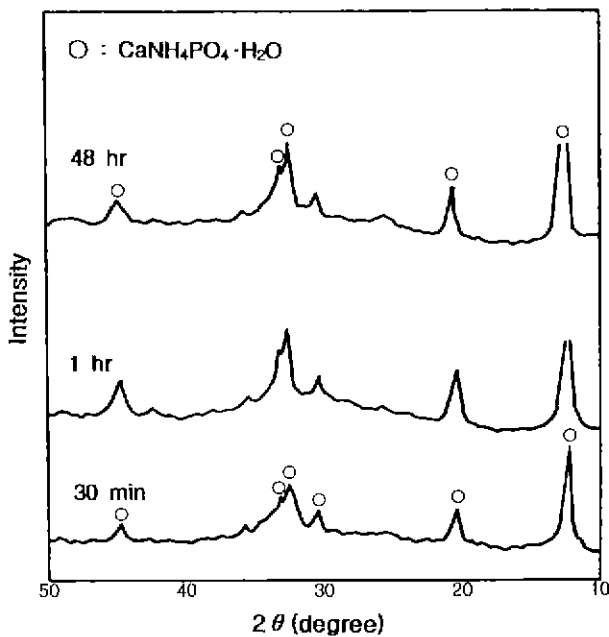


Fig. 4. X-ray diffraction patterns of the hydrated glass powder in partially sealed mold.

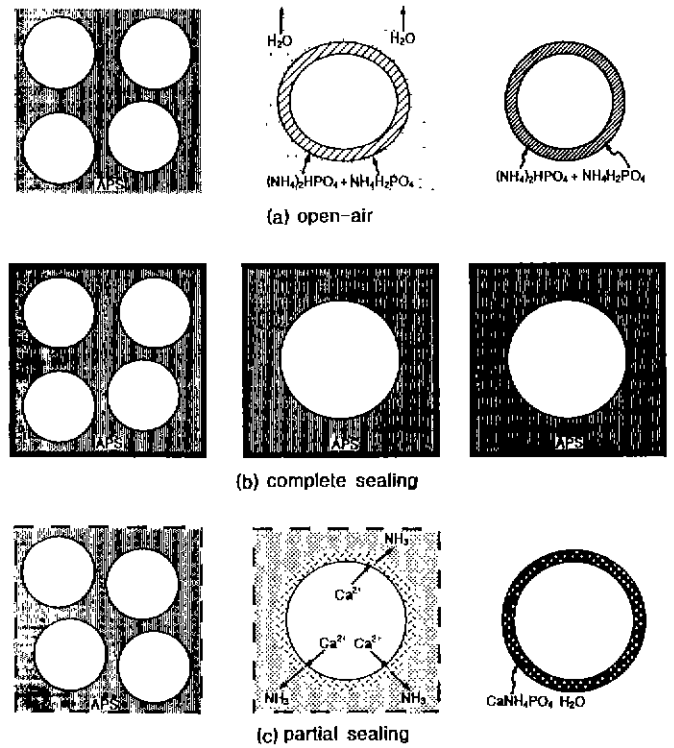


Fig. 5. Schematic diagram of the hardening mechanism for three different hardening methods.

such as NH_3 or H_2O from the mixture. These results indicate that the reaction between solution and glass rarely occurred in completely sealed condition.

In third method, we tried to control the evaporation of NH_3 and moisture from the mixture. The paste was molded in a acrylate plastic rectangular frame, and immediately covered the both ends with another acrylate plates as shown in Fig. 1-a. During the hardening process it is expected that NH_3 and moisture evaporated slowly through the open space of the frame. Fig. 4 shows the X-ray diffraction patterns after the glass powder was reacted with ammonium phosphate solution in the frame for some time. Some indications of forming $\text{CaNH}_4\text{PO}_4 \cdot \text{H}_2\text{O}$ crystal are shown even after 5 minutes of soaking. As the soaking time increased, the more calcium ammonium phosphate crystal formed. In this experiment no ammonium phosphate crystals, such as $(\text{NH}_4)_2\text{HPO}_4$ and $\text{NH}_4\text{H}_2\text{PO}_4$ which are the starting chemicals of the ammonium phosphate solution, were found except at the very early stage of reaction. It is believed that Ca^{2+} ions were leached out of the glass powder surface, and reacted with ammonium phosphate solution to form calcium ammonium phosphate crystal.

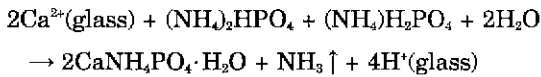
Generally, ammonium salt doesn't form any hydrates, but the presence of fluoride and phosphate enhances the hydrated crystal formation.⁶⁾ When calcium aluminates are reacted with ammonium dihydrogen phosphate solution, it is known that calcium ammonium phosphate crystal forms.⁷⁾

Figure 5 shows the schematic representation of a hardening mechanism for the above mentioned three dif-

ferent casting methods. In open air casting method, crystals of $(\text{NH}_4)_2\text{HPO}_4$ and $\text{NH}_4\text{H}_2\text{PO}_4$, which are the raw chemicals of the ammonium phosphate solution, reprecipitate after evaporation of moisture. In completely sealed method, ammonium phosphate solution remains intact around glass particle because no release of ammonia and moisture is expected.

In partially sealed method, however, Ca^{2+} ions leached out of the glass surface occupies the site of NH_4^+ as ammonia and water slowly release from the mixed paste. And then $\text{Ca}(\text{NH}_4)_2\text{PO}_4 \cdot \text{H}_2\text{O}$ crystal precipitates around glass particles.

The proposed mechanism for hardening can be written as follows:



2. Hydroxyapatite crystal formation

The hardened cements molded in three different ways were soaked in tris-buffer solution for 10 minutes and 100 minutes. Their X-ray diffraction results are shown in Fig. 6. No typical crystalline peaks were observed in bioceramics which were hardened in open-air and completely sealed jar even if they were reacted for 100 minutes. However, hydroxyapatite crystal was found

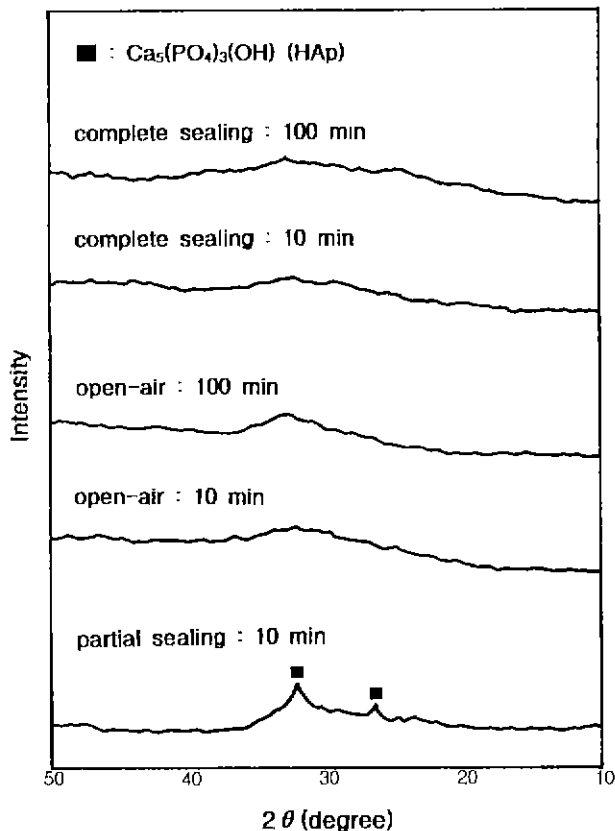


Fig. 6. X-ray diffraction patterns after tris-buffer solution treatment of the various hardened cements for 10 min. and 100 min.

when the partially sealed cement was soaked for 10 minutes.

For the open-air sample, $(\text{NH}_4)_2\text{HPO}_4$ and $\text{NH}_4\text{H}_2\text{PO}_4$ crystals, which were obtained during the hardening process by evaporation of moisture from ammonium phosphate solution, are believed to be dissolved quickly into tris-buffer solution. For the completely sealed cement, the wet ammonium phosphate solution around glass particles, which almost remained intact for 100 hours as shown in Fig. 3, was easily dissolved into tris-buffer solution, and bulk shape of cement paste was collapsed. For the partially sealed cement that contains $\text{CaNH}_4\text{PO}_4 \cdot \text{H}_2\text{O}$ crystal, however, the $\text{CaNH}_4\text{PO}_4 \cdot \text{H}_2\text{O}$ crystal released NH_3 gas into tris-buffer solution and the crystal transformed into a hydroxyapatite.

This partially sealed cement was reacted in tris-buffer solution for various times and the X-ray diffraction patterns and infrared spectra are shown in Fig. 7 and Fig. 8, respectively. The $\text{CaNH}_4\text{PO}_4 \cdot \text{H}_2\text{O}$ crystal started to transform into a hydroxyapatite even if the hardened cement was soaked only for 1 minute and no indication of $\text{CaNH}_4\text{PO}_4 \cdot \text{H}_2\text{O}$ crystal left was observed in the cement after 5 minutes of reaction. Only the characteristic peaks

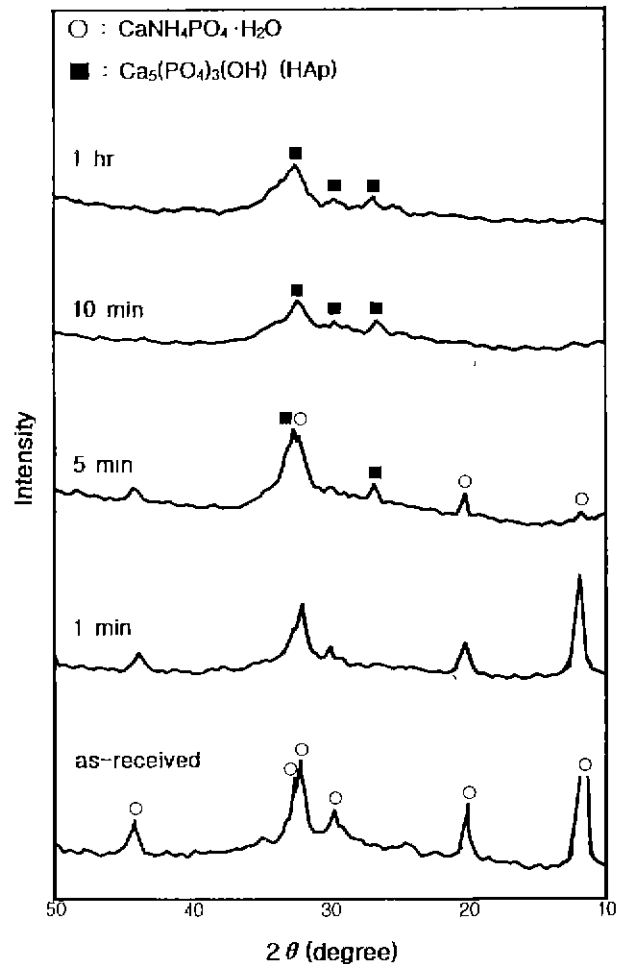


Fig. 7. X-ray diffraction patterns after tris-buffer solution treatment of the partially sealed cement for various time.

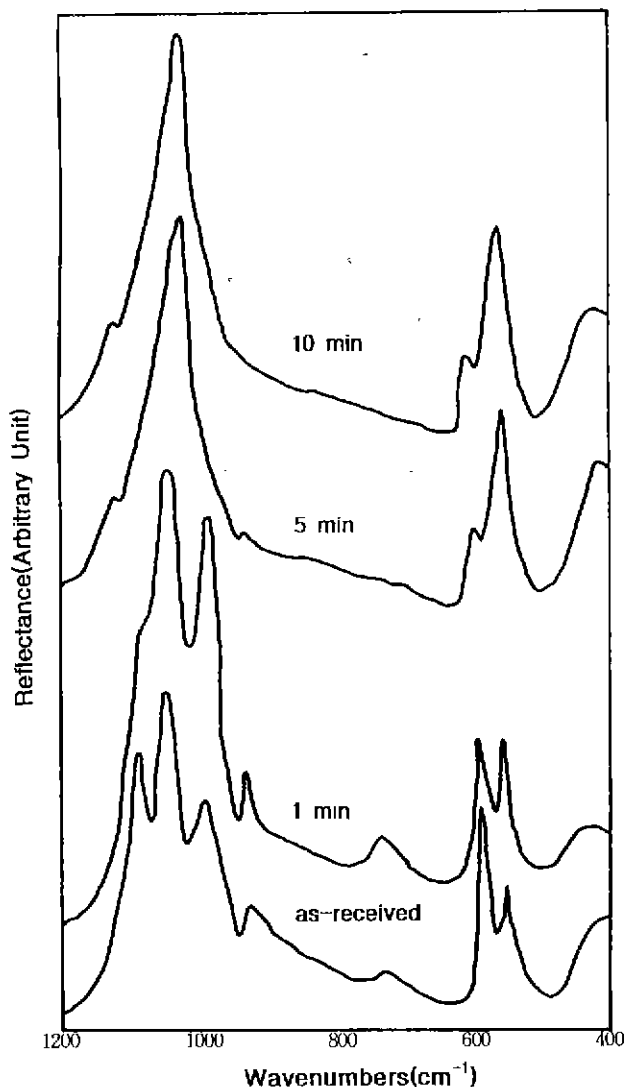


Fig. 8. FT-IRRS spectra after tris-buffer solution treatment of the partially sealed cement for various time.

of the hydroxyapatite in infrared spectra, such as 1040, 565, 602 cm⁻¹, were appeared after 5 minutes of reaction.⁹

Figure 9 shows the schematic illustration of the formation of CaNH₄PO₄·H₂O and hydroxyapatite crystals. CaNH₄PO₄·H₂O crystal was believed to be formed by taking Ca²⁺ ions from the glass. For this reaction some moisture and ammonia must be evaporated from ammonium phosphate solution. In tris-buffer solution, NH₃ was released from CaNH₄PO₄·H₂O and Ca²⁺ ion was supplied from glass to the crystal in order to form hydroxyapatite. Hattori *et al.*⁹ reported that ammonia gas works as a catalyst for the formation of hydroxyapatite. Therefore, it is believed that the evaporation of NH₃ enhances the formation of hydroxyapatite.

The following chemical reaction may occur for the

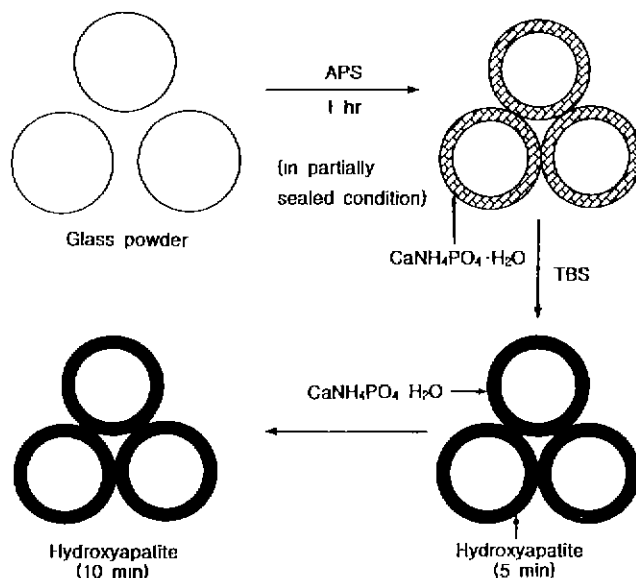


Fig. 9. Schematic representation of the formation of CaNH₄PO₄·H₂O and hydroxyapatite.

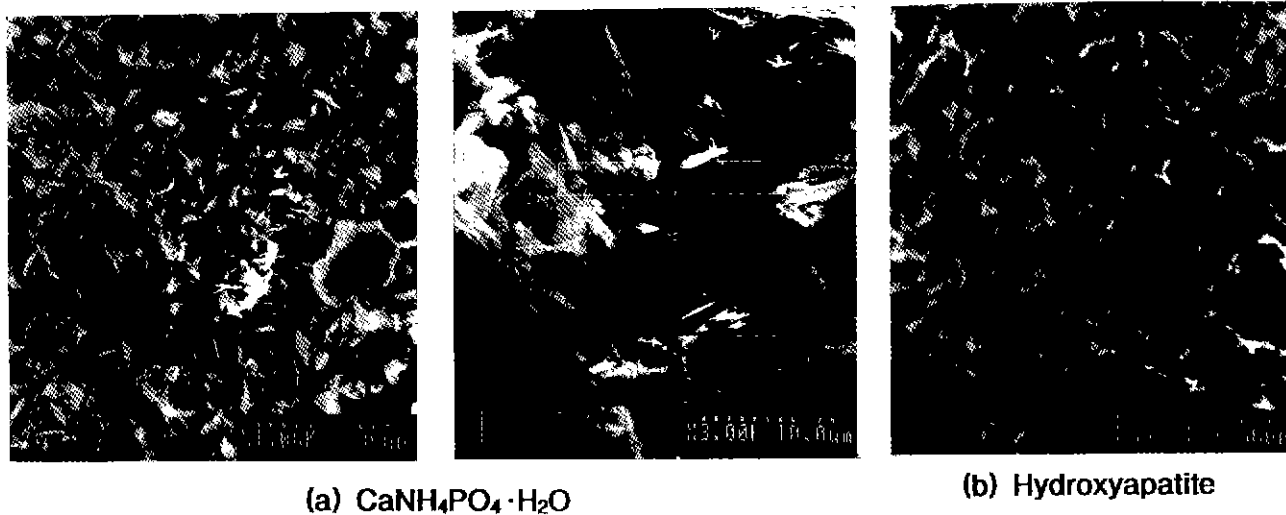


Fig. 10. Scanning electron micrographs of the formation of CaNH₄PO₄·H₂O and hydroxyapatite crystals.

transformation of $\text{CaNH}_4\text{PO}_4 \cdot \text{H}_2\text{O}$ crystal to hydroxyapatite.

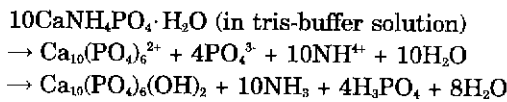


Figure 10 shows SEM morphologies of the cement hardened in partially sealed condition and treated in tris-buffer solution. It shows the needle-like crystals which are $\text{CaNH}_4\text{PO}_4 \cdot \text{H}_2\text{O}$ and the crystals transformed to hydroxyapatite, which have typical leaf-like morphology, in tris-buffer solution.

IV. Conclusions

The hardening phenomena and hydroxyapatite formation mechanism are studied when glass powder with composition of 53.6%CaO 38.1%SiO₂ 7.7%P₂O₅ 0.6%CaF₂ (mole%) is mixed with ammonium phosphate solution, and the reacted in tris-buffer solution, respectively. The following conclusions have been drawn from the above experiments.

1. Three different setting methods, such as open-air, completely sealed and partially sealed conditions, were used to make the cement harden. Most hard cement was obtained in the sample with $\text{CaNH}_4\text{PO}_4 \cdot \text{H}_2\text{O}$ crystal, which was casted in partially sealed condition. In this case Ca²⁺ ions leached out from the glass took the position of NH₄⁺ ions in ammonium phosphate solution to produce $\text{CaNH}_4\text{PO}_4 \cdot \text{H}_2\text{O}$ crystal.

2. Hydroxyapatite crystal [$\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$] was obtained only when the hardened cement with $\text{CaNH}_4\text{PO}_4 \cdot \text{H}_2\text{O}$ crystal was reacted in tris-buffer solution by releas-

ing ammonia from the crystal.

Acknowledgement

This work was supported by a grant from Korean Science and Engineering Foundation (KOSEF 91-0400-08).

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