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PREPARATION OF CALCIUM PHOSPHATE COMPOUNDS FROM BY-PRODUCT OF BONE GELATIN PRODUCTION

Miss Suphasinee Limpanuphap

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By	Miss Suphasinee Limpanuphap
Department	Materials Science
Thesis Advisor	Associate Professor Supatra Jinawath, Ph.D.

Accepted by the Graduate School, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

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ภาควิชา วัสดุศาสตร์	ลายมือชื่อนิสิต สุภาสินี ลิมปานุภาพ
สาขาวิชา เทคโนโลยีเซรามิก	ลายมือชื่ออาจารย์ที่ปรึกษา Supatra Jinawath
ปีการศึกษา 2542	ลายมือชื่ออาจารย์ที่ปรึกษาร่วม

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บทคัดย่อ

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ABSTRACT

A by-product from bone gelatin production was used as a starting material for synthesizing calcium phosphate compounds: dicalcium phosphate dihydrate (DCPD), dicalcium phosphate anhydrous (DCPA), monocalcium phosphate monohydrate (MCPM), β-tricalcium phosphate (β-TCP), and hydroxyapatite (HA). DCPD was prepared from the precipitation of the starting material solution at various pH values, 4.5-6.0. It was found that the optimum condition for preparing DCPD is to precipitate at pH 5.5. DCPA was also prepared by the same method as DCPD, except that the precipitation was performed at 80°C. MCPM was obtained from the evaporation of the synthesized DCPD solution. The preparations of β-TCP and HA were based on the solid-state reaction of the mixtures of DCPD or DCPA and $CaCO_3$ with various Ca/P mole ratios. It was found that pure β-TCP was obtained from the heat treatment of the DCPD+$CaCO_3$ mixture with Ca/P mole ratio = 1.46 at 1100°C and HA with high purity was obtained from the heat treatment at 1200°C of the DCPA+$CaCO_3$ mixture with Ca/P mole ratio = 1.67, or with Ca/P mole ratio = 1.63. The purity of HA obtained could be improved by washing the trace of $Ca(OH)_2$ with acetic acid.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Calcium phosphate compounds are bioceramics which are widely used for biomedical applications. This is because of their excellent properties, for example, they have mostly the same chemical compositions as the skeletons, they are biocompatible, and when these ceramics are implanted into a living body (*in vivo*) for a range of time, it is found that these ceramics affect the growing rate of the new bone tissue and they also have strong chemical bond with the bone tissue in the living body which finally become a firm attachment (Hench, 1991).

Calcium phosphate compounds can be used in various forms; such as in the forms of powder (Metsger, Driskell, and Paulsrud, 1982), granules (Piecuch, 1986, cited in LeGeros, 1988), dense ceramics (Larsen *et al.*, 1983, cited in LeGeros, 1988), porous ceramics, or they can also be applied on metal or alloy surfaces (Yamashita *et al.*, 1994, cited in Aoki, 1994); such as titanium (Ti), platinum (Pt), alumina (Al_2O_3), yttria-stabilized zirconia (YSZ), platinum/10% rhodium, Ti-6Al-4V and SUS 316L stainless steel; in order to improve the properties. The examples of their applications are artificial teeth and bones, fillers for teeth and bone spaces, etc (de Groot, 1991).

Moreover, they can also be used for food and drug industries (Lewis, 1989; Toy, 1973). For example, dicalcium phosphate dihydrate (DCPD) is used as dietary supplement, nutrient or stabilizer in baked goods, cereal products and dessert gel. It is also used as polishing agent in toothpaste, diluent in medicine or calcium supplement tablets. Dicalcium phosphate

andydrous (DCPA), which is more abrasive, is used only in combination with the less abrasive DCPD or TCP as polishing agent in specialty toothpaste such as smoker's toothpaste for stain removal. Calcium pyrophosphate (CPP), because of its low water solubility and chemical inertness, is used as an abrasive for F^--containing toothpaste. In various kinds of food, CPP is used as buffer, dietary supplement, neutralizing agent or nutrient. Monocalcium phosphate monohydrate (MCPM) is used as ingredient in the baking powder and soft drinks, as additive in phosphated flour and as bread improver to stimulate the growth of yeast in yeast leavened products. Monocalcium phosphate anhydrous (MCPA) is used as dietary supplement, firming agent, nutrient, or stabilizer in cereals, fruitjellies, or preserved products.

The mostly used calcium phosphate compounds are hydroxyapatite (HA) and tricalcium phosphate (TCP) (Lavernia and Schoenung, 1991). They both have good properties but in the different way. HA is a bioactive material while TCP is a resorbable one (Lutton and Ben-Nissan, 1993; Metsger, Driskell, and Paulsrud, 1982). So, they are suitable for the different cases.

There are several routes for preparation of calcium phosphate compounds, but generally they can be divided into two main procedures: the one which uses bone ash as the starting material and the other which uses chemical reagents as the starting materials. By comparison the purity and mechanical properties of the obtained products from the second procedure are better than those of the first one which includes some porosity and impurities from the bone (Charussri Lorprayoon and Supatra Jinawath, 1985). However, they both have good biocompatibility. In general, the chemical reagents are much more expensive than bone ash, so it is very interesting to prepare calcium phosphate compounds with minimal impurity from bone ash.

Unfortunately, there are so many steps in making bone ash. They include cleaning, heat treatment, crushing and grinding, thus it is quite complicated. Moreover, it is hard to find cattle bones nowadays because they are mostly supplied to the industries, so the alternative is being searched.

In the manufacture of gelatin (International Trade Centre, 1984), the cattle bones are used as the raw materials. From this process, there is a by-product which is used as an ingredient for fertilizer and animal foodstuff. This by-product is cheap and contains calcium phosphate compounds as the component. So, it is possible to use this by-product as the starting material for preparing various types of calcium phosphate compounds, such as dicalcium phosphate dihydrate (DCPD), dicalcium phosphate anhydrous (DCPA), monocalcium phosphate monohydrate (MCPM), tricalcium phosphate (TCP) and hydroxyapatite (HA).

1.1 Objectives

The objectives of this work are as the followings:

1. To use by-product of bone gelatin industry as starting material with expectation to reduce the investment cost for cleaning, calcining and grinding the bones.
2. To add value to by-product from bone gelatin production.
3. To find the suitable conditions for synthesizing calcium phosphate compounds with Ca/P mole ratios = 0.5-1.67 from the mentioned starting material.
4. To characterize the obtained products.

1.2 Scope

The scope of the experiment covers: precipitation of DCPD, DCPA and MCPM from solutions, characterization of the obtained DCPD, DCPA and MCPM precipitates, preparation of β-TCP and HA from the heat treatment of DCPD and DCPA with $CaCO_3$ in air and characterization of the obtained β-TCP and HA.

CHAPTER 2

LITERATURE REVIEW

2.1 Bioceramics

The need of materials for use in the health care industry is increasing nowadays. The three basic classes of materials used for this purpose are metal, polymer and ceramic. These materials have varying requirements, depending on whether they are simply assisting the healing process or must substitute for parts of the skeleton system. The most important requirements of these materials are biocompatibility, biofunctionality and availability (Lutton and Ben-Nissan, 1993).

Metals can be used in form of pure metal or alloy, e.g., titanium, platinum, gold, titanium alloy and cobalt-chrome alloy. They are mostly used as implants in the loading area and fracture fixation. The important parameters for these implants are that they are inert and have the necessary ductility, elasticity and compressive and tensile strength (Lutton and Ben-Nissan, 1993).

Because of their flexibility, many polymers are used in this field (Hulbert, Klawitter, and Leonard, n.d.). They include silicone polymer, used as finger joints and small bones in the wrist; high-density polyethylene (HDPE), used in conjunction with a metal prosthesis; and polymethylmethacrylate (PMMA) which is used as bone cement in joint replacement surgery (Lutton and Ben-Nissan, 1993).

Ceramics are brittle materials that have generally poor tensile properties but excellent compressive strength, high resistance to wear, and favorable low friction properties. Ceramics for implantation and clinical use, called bioceramics (Hench, 1998), include calcium phosphate compounds (Chow, 1988); such as HA, TCP, DCPD, DCPA, and tetracalcium phosphate (TTCP); various oxides; such as alumina, titania, magnesia partially stabilized zirconia (Mg-PSZ); glass (Day, 1995); glass-ceramics and crystalline or glassy forms of carbon and its compounds (Lutton and Ben-Nissan, 1993). Bioceramics can be classified by the types of fixation and attachment, as shown in Table 2.1.

2.2 Calcium Phosphate Compounds

2.2.1 Calcium Phosphates in Biological Systems

Among these bioceramics, calcium phosphate compounds are very attractive because of their similarity with the mineral phase of bone, and they can bond to the bone in a natural way (LeGeros, 1991; Aoki, 1994). Various types of calcium phosphates are summarized in Table 2.2.

In 1926, the first X-ray diffraction studies of human tooth enamel, dentine and bone were performed, resulting in the observation that they were the calcium phosphates with the apatite structure (LeGeros, 1991). It was found that this apatite was biological apatite with a nonstoichiometric composition, $Ca_{5-x}(PO_4)_{3-y}(OH)_{1-z}A_xB_yC_z$, where A, B, and C were substitutional elements. After this study, there had been many others that reported the existence of other mineral phases in bone, including DCPD, TCP, amorphous calcium phosphate (ACP), calcium pyrophosphate

Table 2.1 Types of bioceramics classified by the types of attachment and fixation (Lutton and Ben-Nissan, 1993).

Type of Fixation	Type of Attachment	Examples
Morphology	Dense, nonporous, nearly inert ceramics attach by bone growth into surface irregularities by cementing the device into the tissues or by press fitting into a defect	Al_2O_3 (single-crystal and polycrystalline)
Biological	Porous inert ceramics allow bone ingrowth to occur, which results in mechanical attachment of the bone to the ceramic material	Al_2O_3 (porous polycrystalline)
Bioactive	Dense, nonporous, surface-reactive ceramics, glasses, and glass-ceramics attach directly by forming a chemical bone with the bone	Bioactive glasses Bioactive glass-ceramics Hydroxyapatite
Resorbable	Dense, nonporous or porous, resorbable ceramics are resorbed due to enhanced dissolution rates *in vivo* and so ultimately are replaced by bone	Calcium sulfate hydrate Tricalcium phosphate Calcium phosphate salts

Table 2.2 Various calcium phosphates with their respective Ca/P ratios (Aoki, 1994).

Ca/P	Formula	Name	Abbreviation
2.0	$Ca_4O(PO_4)_2$	Tetracalcium phosphate (Hilgenstockite)	TTCP
1.67	$Ca_{10}(PO_4)_6(OH)_2$	Hydroxyapatite	HA
	$Ca_{10-x}H_{2x}(PO_4)_6(OH)_2$	Amorphous calcium phosphate	ACP
1.50	$Ca_3(PO_4)_2$	Tricalcium phosphate (α, β, γ)	TCP
1.33	$Ca_8H_2(PO_4)_6.5H_2O$	Octacalcium phosphate	OCP
1.0	$CaHPO_4.2H_2O$	Dicalcium phosphate dihydrate (Brushite)	DCPD
1.0	$CaHPO_4$	Dicalcium phosphate anhydrous (Monetite)	DCPA
1.0	$Ca_2P_2O_7$	Calcium pyrophosphate (α, β, γ)	CPP
1.0	$Ca_2P_2O_7.2H_2O$	Calcium pyrophosphate dihydrate	CPPD
0.7	$Ca_7(P_5O_{16})_2$	Heptacalcium phosphate (Trömelite)	HCP
0.67	$Ca_4H_2P_6O_{20}$	Tetracalcium dihydrogen phosphate	TDHP
0.5	$Ca(H_2PO_4)_2.H_2O$	Monocalcium phosphate monohydrate	MCPM
0.5	$Ca(PO_3)_2$	Calcium metaphosphate (α, β, γ)	CMP

Table 2.3 Calcium phosphates in biological systems (LeGeros, 1991).

Calcium phosphate	Chemical formula	Occurrences
Apatite	$(Ca,Z)_{10}(PO_4,Y)_6(OH,X)_2$	Enamel[a], dentine[a], bone[a], dental calculi, stones, urinary calculi, soft-tissue calcifications
OCP	$Ca_8H_2(PO_4)_6.5H_2O$	Dental and urinary calculi
DCPD	$CaHPO_4.2H_2O$	Dental calculi, crystalluria, chondrocal-cinosis, decomposed bones
TCP	$(Ca,Mg)_9(PO_4)_6$	Dental and urinary calculi, salivary stones, dentinal caries, arthritic cartilage, soft-tissue calcifications
ACP	$(Ca,Mg)_7(PO_4,Y')$	soft-tissue calcifications
CPPD	$Ca_2P_2O_7.2H_2O$	Pseudo-gout deposits in synovium fluids

[a]Z = Na, Mg, K, Sr, etc.; Y = CO_3, HPO_4; X = Cl, F; Y′ = P_2O_7, CO_3.

In 1958, Newman, W.F. and Newman, M.W. (Aoki, 1994) proposed a chemical formula of the bone apatites as

$$[Ca_9(H_3O)_2(PO_4)_6]\text{-}[Ca,Mg_{0.3},Na_{0.3},CO_3,Citrate_{0.3}]$$

However, the chemical compositions of bone apatite are slightly different according to species, ages, parts of the skeleton and many more. The Ca/P ratio increases with age to near 1.67. The crystal sizes of biological apatites

are very small, less than 0.2 μm, so it is convenient to remodel the bone, that is, rapid resorption and formation of bone.

Bone apatites from nine kinds of vertebrate animals, both unheated and heated at 800°C, were studied by X-ray diffraction. The X-ray patterns, as shown in Figure 2.1, had a similar pattern. It was determined that bone of vertebrate animals were mainly composed of hydroxyapatite (Aoki, 1994).

The stable phases of calcium phosphates depended upon temperature and the presence of water. At body temperature, only two calcium phosphates were stable in contact with body fluids: DCPD was a stable phase at pH < 4.2 and HA was a stable phase at pH > 4.2. At higher temperature, however, the stable phases were TCP and TTCP (LeGeros, 1991).

DCPD was the first calcium phosphate occurred during the formation of human dental calculus and was the most easily transformed to the other calcium phosphates, it was known from the observation that there was no DCPD in calculus older than a few days. Unlike DCPD, TCP and HA were present mostly in old calculus (more than three months old), while OCP was observed in both young and old calculus (LeGeros, 1991).

From the X-ray diffraction study, it was found that dental calculi of human and mammal were consisted of various kinds of calcium phosphates, as shown in Figure 2.2. By comparison XRD patterns of human dental calculus and synthesized calcium phosphates (Figure 2.3), were found to show the similar pattern (LeGeros, 1991).

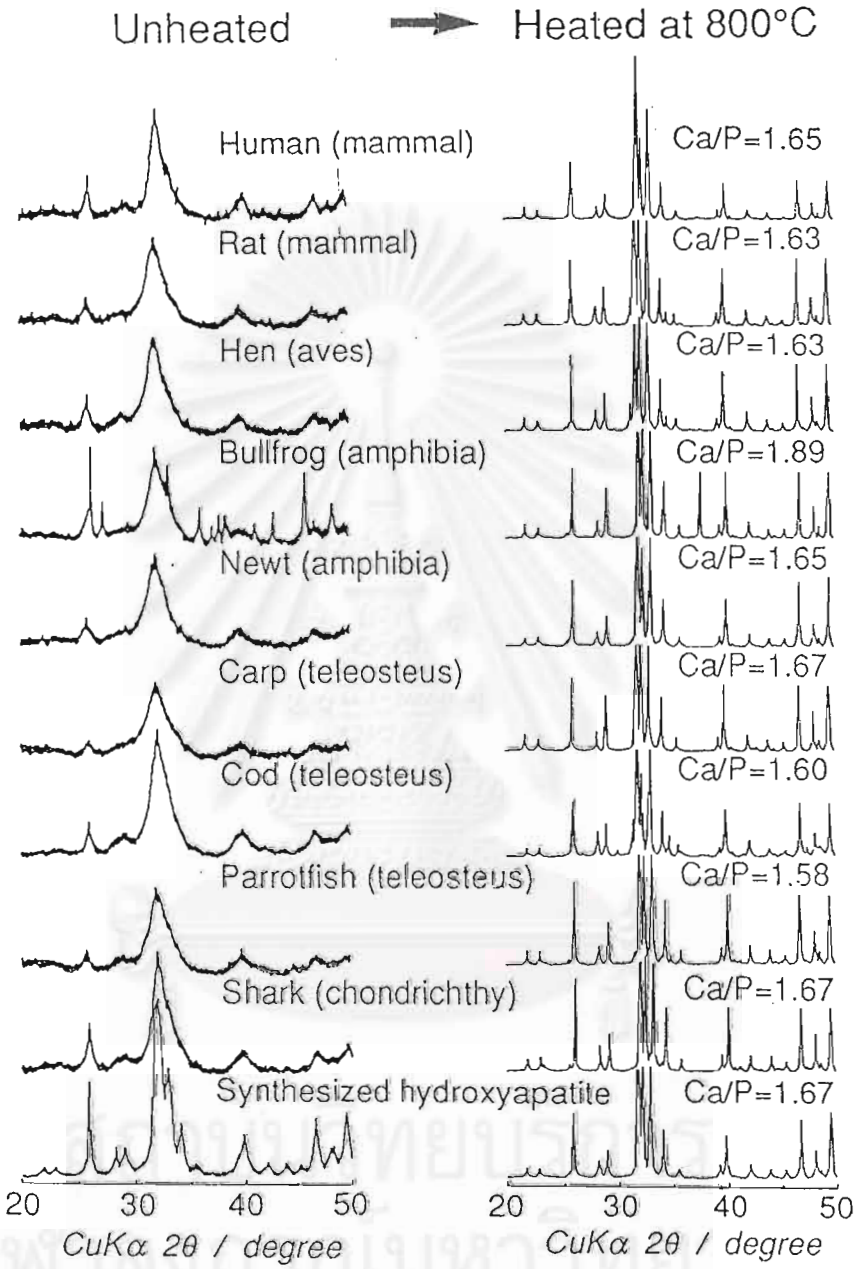


Figure 2.1 XRD patterns of vertebrate bone apatites, both unheated and heated at 800°C (Aoki, 1994).

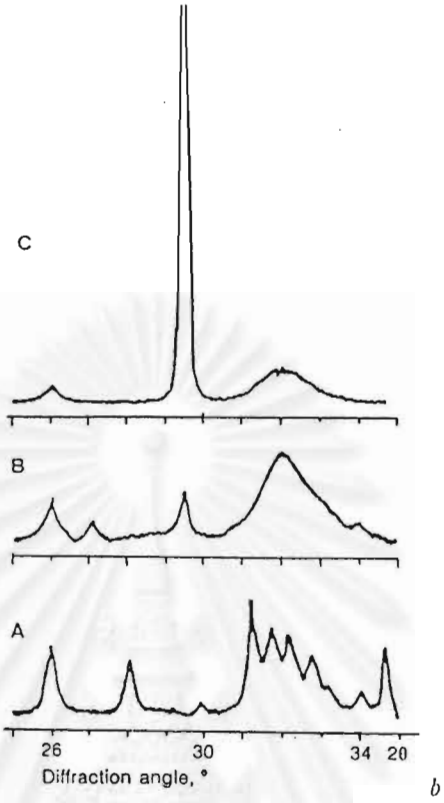


Figure 2.2 XRD patterns of calculi from human (A), cat (B) and dog (C). The human calculus (A) contains a mixture of β-TCMP (Mg-substituted TCP), octacalcium phosphate (OCP) and apatite (Ap); the cat calculus (B) contains a mixture of $CaCO_3$ (calcite form) and poorly crystallined apatite; the dog calculus (c) predominantly contains calcite and small amounts of poorly crystallized Ap (LeGeros, 1991).

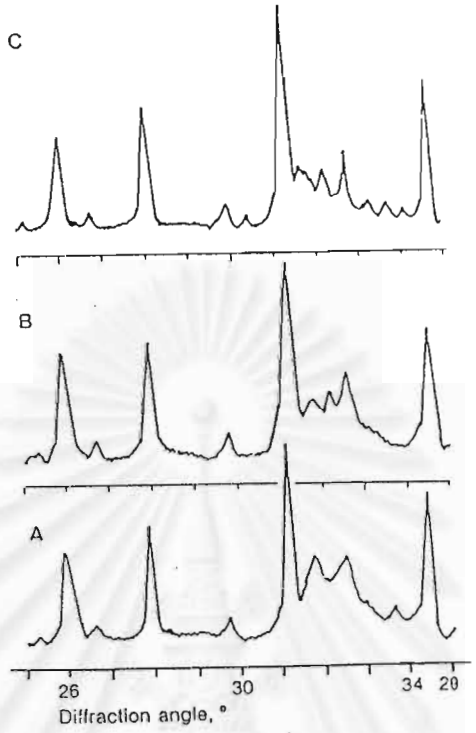


Figure 2.3 XRD patterns of human dental calculus consisting of β−TCMP and Ap (A) and synthetic calculum phosphate obtained by precipitation from solutions with Mg/Ca = 0.2/1 at 60°C (B), and obtained by hydrolysis of DCPD in solutions containing Mg/Ca = 0.4/1 (C) (LeGeros, 1991).

2.2.2 The Uses of Calcium Phosphates

Calcium phosphate compounds have been used as bone substitute for many decades. In 1920, Albee reported that a 'triple calcium phosphate' compound used in a bony defect promoted osteogenesis or new bone formation. Levitt et al. (1969) and Monroe et al. (1971) reported a method of preparing calcium phosphate ceramic, calcium-fluoroapatite ($Ca_{10}(PO_4)_6F_2$), and suggested its use as dental and medical implant materials. Hench et al. (1971) developed a calcium-and-phosphate-containing glass ceramic, called Bioglass®, and showed that it chemically bonded with the host bone. In 1973, Clarke et al. reported a method for the preparation of a tricalcium phosphate ceramic and suggested its use as a bone graft material (LeGeros, 1988).

Levin et al. (1974) reported the first dental application of a tricalcium phosphate ceramic in periodontal defects in dogs. Hubbard (1974) presented the preparation of several calcium phosphate ceramics from reagent materials and examined their possible uses as orthopaedic implants. Roy and Linnehan (1974) reported a method for the preparation of an apatite material from a reef-building coral species by hydrothermal transformation of the calcium carbonate in the coral (LeGeros, 1988).

Calcium phosphates used in commercial applications as implants can be divided into 4 groups (LeGeros, 1991): calcium phosphate ceramics, calcium phosphate material (non-ceramic), calcium phosphate materials from natural products, and glass ceramics (Table 2.4).

Table 2.4 Calcium phosphate materials in current use (LeGeros, 1991).

CaP materials	Examples	Commercial products
Calcium phosphate ceramics	Hydroxyapatite, HA	Calcitite (Calcitek, Inc.); Periograf, Alveograf, Durapatite (Cook-Waite); Ossograf (Coors); Ortho-Matrix; Allotropat (Heyl, FRG); Bioapatite (France)
	β-Tricalcium phosphate, β-TCP	Synthograf, Augment (Miter, Inc., distributed by Johnson & Johnson)
	Biphasic calcium phosphates (mixture of HA and β-TCP)	Triosit (Zimmer) Approx. 60 HA/ 40 β-TCP
Calcium phosphate material, non-ceramic		Osteogen (GBD, Impladent), Poorly crystallized Ca-deficient apatite + small amounts of DCPA
Calcium phosphate materials from natural products	Coralline HA : coral (Porites) hydrothermally converted to HA	Interpore 200 (Interpore)
	Bio-oss (from sintered bovined bone)	
Glass ceramics		Bioglass; Ceravital (FRG)

As mentioned before that HA has many good properties, therefore, HA is widely used in health care industry (Aoki, 1994). Medical applications of HA are summarized in Table 2.5. Various forms of HA are

suitable for different proposes. HA is also used for dental applications which are shown in Table 2.6. Many types of dental materials based on HA have been developed for commercial uses.

Table 2.5 Medical applications of HA (Aoki, 1994).

Applications	Forms of Hydroxyapatite
Artificial bone	Dense, Porous
Artificial joint	Dense, Porous, Coating
Bone filler	Granule, Porous
Bone formation promoter	Microcrystal
Artificial blood vessel	Dense, Composite
Artificial trachea	Dense, Composite
Percutaneous device	Dense, Composite
Bioelectrode	Composite
Drug delivery carrier	Microcrystal
Clinical testing	Microcrystal

Table 2.6 Dental applications of HA-based materials (Aoki, 1994).

Applications	Commercial Names
Toothpaste (Dentifrice)	Apadent, Apaquard, Apato
Dental cement	Bioment, Apament
Root canal	Apatite Rootsealer, Finapec
Bone filler (granule)	Apaceram, Actceram, Bonfil
Bone filler (porous)	Apaceram, Bonetite, TBC
Tooth root	AQB, Apaceram, Sumisicon
Crown	Cera-Pearl

2.2.3 Thermal Transformation of Calcium Phosphates

The thermal transformation of various calcium phosphates is described. These calcium phosphates, including monocalcium phosphate monohydrate ($Ca(H_2PO_4)_2.H_2O$), dicalcium phosphate dihydrate ($CaHPO_4.2H_2O$), dicalcium phosphate anhydrous ($CaHPO_4$), tricalcium phosphate ($Ca_3(PO_4)_2$), tetracalcium dihydrogen phosphate ($Ca_4H_2P_6O_{20}$), Ca-deficient hydroxyapatite or so-called amorphous calcium phosphate ($Ca_{10-x}H_{2x}(PO_4)_6(OH)_2$), and octacalcium phosphate pentahydrate ($Ca_8H_2(PO_4)_6.5H_2O$), were synthesized by solution reaction methods. These calcium phosphates were heated up to 1500°C in air at heating rate of 3-5 °C/min. The obtained products at various temperatures were characterized by X-ray diffractometer. Their chemical compositions were identified by thermal gravimetric analysis, infrared spectrometer, and chemical analysis. The results are shown as the following equations (Aoki, 1994):

1. $Ca(H_2PO_4)_2.H_2O \xrightarrow[250\ C]{} Ca(H_2PO_4)_2 \xrightarrow[300\ C]{}$ amorphous $Ca(PO_3)_2$ +

   $Ca_4H_2P_6O_{20} \xrightarrow[400\text{-}450\ C]{} \gamma\text{-}Ca(PO_3)_2 \xrightarrow[540\text{-}640\ C]{} \beta\text{-}Ca(PO_3)_2 \xrightarrow[950\text{-}970\ C]{} \alpha\text{-}Ca(PO_3)_2$

   $\xrightarrow[1000\ C]{} Ca(PO_3)_2$ glass

2. $CaHPO_4.2H_2O \xrightarrow[100\text{-}260\ C]{} CaHPO_4 \xrightarrow[400\text{-}440\ C]{} \gamma\text{-}Ca_2P_2O_7 \xrightarrow[750\text{-}1200\ C]{} \beta\text{-}Ca_2P_2O_7 \xrightarrow[1250\ C]{} \alpha\text{-}Ca_2P_2O_7$

3. $CaHPO_4 \xrightarrow[400\text{-}450\ C]{} \beta\text{-}Ca_2P_2O_7 \xrightarrow[1250\ C]{} \alpha\text{-}Ca_2P_2O_7$

4. $Ca_3(PO_4)_2.nH_2O \xrightarrow{680\text{-}720\ ^\circ C} \beta\text{-}Ca_3(PO_4)_2 \xrightarrow{1290\ ^\circ C} \alpha\text{-}Ca_3(PO_4)_2 \xrightarrow{1540\ ^\circ C}$ super $\alpha\text{-}Ca_3(PO_4)_2$

5. $Ca_{10-x}H_{2x}(PO_4)_6(OH)_2 \xrightarrow{800\ ^\circ C} \beta\text{-}Ca_3(PO_4)_2 + Ca_{10}(PO_4)_6(OH)_2 \xrightarrow{1200\ ^\circ C}$ $\alpha\text{-}Ca_3(PO_4)_2 + Ca_4O(PO_4)_2$

6. $Ca_8H_2(PO_4)_6.5H_2O \xrightarrow{200\ ^\circ C} Ca_8H_2(PO_4)_6.H_2O \xrightarrow{400\ ^\circ C} Ca_{10}(PO_4)_6(OH)_2 +$ $\gamma\text{-}Ca_2P_2O_7$

7. $Ca_4H_2P_6O_{20} \xrightarrow{400\text{-}630\ ^\circ C} Ca_7(P_5O_{16})_2 + \gamma\text{-}Ca(PO_3)_2 \xrightarrow{500\ ^\circ C}$ amorphous $Ca(PO_3)_2$ $+ \beta\text{-}Ca_2P_2O_7 \xrightarrow{1300\ ^\circ C} \beta\text{-}Ca_3(PO_4)_2 + \beta\text{-}Ca_2P_2O_7 \xrightarrow{1500\ ^\circ C} Ca(PO_3)_2$ glass $+ \alpha\text{-}Ca_2P_2O_7$

2.3 The Preparations of Calcium Phosphates

The methods for preparing calcium phosphates can be divided into two main processes. The first one is the preparations of calcium phosphates from chemical reactants, and the last one is from natural reactants.

2.3.1 The Preparations of Calcium Phosphates from Chemical Reactants

Aoki (1994) reported that calcium phosphates were synthesized by mixing 0.25 mol/l $CaCl_2$ solution and 0.15 mol/l Na_2HPO_4 solution at 37°C under pH 4-8 conditions for 25 days. In the range from neutral to alkaline, the pH of the solution was controlled by a tris-hydroxyl methylaminomethane-HCl buffer, while in the range from acid to neutral, the pH was controlled by a Sorensen-Rlatzisch phosphate buffer solution.

the pH was controlled by a Sorensen-Rlatzisch phosphate buffer solution. Three days after the reaction began, the pH of the solution was obviously decreased. It was because of the formation of hydrochloric (HCl) acid as the following chemical reaction:

$$10CaCl_2 + 6Na_2HPO_4 + 2H_2O \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 12NaCl + 8HCl$$

The products were identified by X-ray diffraction (XRD) and analyzed by atomic absorption spectroscopy (AA). Hydroxyapatite (HA), including noncrystalline HA, so-called amorphous calcium phosphate (ACP) or Ca-deficient HA, were produced in alkaline and weak acid solution above pH 6. Octacalcium phosphate (OCP) was observed in the solution between pH 5-6 and dicalcium phosphate dihydrate was formed in acidic solution below pH 5.

In 1996, Slosarczyk et al. reported the method of preparing calcium phosphates with various Ca/P molar ratio from calcium oxide (CaO) and phosphoric (H_3PO_4) acid. Calcium oxide was calcined at 900°C for 0.5 hour, then was stirred in the distilled water for 0.5 hour in order to prepare 0.5 M and 0.6 M calcium hydroxide ($Ca(OH)_2$) suspensions. The calcium hydroxide suspension was added to the 2000 ml of phosphoric acid at 23°C-25°C, varying the amount of the suspension from 1790-2072 ml. The pH of the solution was controlled by using an ammonium solution. It was found that after sintering the products at 1250°C, the products had various Ca/P ratios, from 1.50-1.73. They were monophase, biphase or even triphase consisting of HA, β-TCP, α-TCP and CaO.

Because there were various types of calcium phosphates, the preparations of each type of them were described as the following.

The preparations of monocalcium phosphate monohydrate (MCPM).

The commercial MCPM was prepared from two methods (Toy, 1973). The first one was evaporation of the aqueous reaction mixture of hydrated lime and phosphoric acid. The other was crystallization from the aqueous system. The obtained product from the crystallization process contained 5.8% dicalcium phosphate while the product from the evaporation process contained upto 8-9% dicalcium phosphate.

Very crude MCPM or superphosphate used as fertilizer was prepared by the reaction of phosphate mineral with sulfuric acid (Toy, 1973), as equation shown below:

$$2Ca_5(PO_4)_3F + 7H_2SO_4 + H_2O \rightarrow 7CaSO_4 + 3Ca(H_2PO_4)_2.H_2O + 2HF$$

A purer grade of MCPM fertilizer was the triplesuperphosphate. It was prepared by the reaction of calcium phosphate mineral with H_3PO_4, as the following equation:

$$Ca_5(PO_4)_3F + 7H_3PO_4 + 5H_2O \rightarrow 5Ca(H_2PO_4)_2.H_2O + HF$$

The preparations of dicalcium phosphate dihydrate (DCPD).

Jensen and Rathlev (1953) prepared DCPD from the solid-state reaction:

$$Na_2HPO_4.2H_2O + CaCl_2.6H_2O \rightarrow CaHPO_4.2H_2O + 2NaCl + 6H_2O$$

Toy (1973) reported that DCPD was prepared by the neutralization of H_3PO_4 with slurry of lime at 38-40°C. Two methods of stabilization were used. The first method was the addition of a small quantity of pyrophosphate ion such as tetrasodium pyrophosphate to the slurry of DCPD during the manufacturing process. The second method was to add 2-3% of trimagnesium phosphate to DCPD as a dry mix.

The preparations of dicalcium phosphate anhydrous (DCPA).

Stock food grade of crude DCPA was prepared by the reaction of pasty hydrated lime with 75-80% H_3PO_4. It contained MCPM, TCP and unreacted lime as the impurities. DCPA could also be prepared from MCPM and ammonia. The products consisted of DCPA, diammonium phosphate and water (Toy, 1973).

$$Ca(H_2PO_4)_2.H_2O + 2NH_3 \rightarrow CaHPO_4 + (NH_4)_2HPO_4 + H_2O$$

The preparation of octacalcium phosphate (OCP).

Pure octacalcium phosphate was prepared by control hydrolysis of DCPD in 0.5 M CH_3COONa at 40°C (Toy, 1973).

The preparation of tricalcium phosphate (TCP).

Toy (1973) reported that β-TCP was prepared by the reaction of calcium nitrate ($Ca(NO)_3$) solution with ammonium hydrogen phosphate (Na_2HPO_4) solution in the presence of 1% Mg^{++} or Mn^{++} as the stabilizer. When β-TCP was heated, it would be a transformation like the following equation:

$$\beta\text{-}Ca_3(PO_4)_2 \xrightarrow{1180\ ^{o}C} \alpha\text{-}Ca_3(PO_4)_2 \xrightarrow{1430\ ^{o}C} \alpha'\text{-}Ca_3(PO_4)_2$$

The preparations of hydroxyapatite

In 1963, Hayek and Newesely prepared hydroxyapatite from aqueous media containing calcium nitrate tetrahydrate ($Ca(NO_3)_2.4H_2O$), diammonium hydrogen phosphate ($(NH_4)_2HPO_4$) and ammonia under controlled pH and temperature. The 0.33 mole of $Ca(NO_3)_2.4H_2O$ was dissolved in 300 ml of water, the pH of the solution was adjusted to 12 by adding concentrated ammonia and then the solution was diluted to 600 ml. Similarly, 0.2 mole of diammonium hydrogen phosphate was dissolved in 500 ml of water, the solution was brought to pH 12 with concentrated ammonia and then diluted to 800 ml. For mixing, diammonium hydrogen phosphate solution was continuously dropped into the stirred calcium nitrate tetrahydrate solution, and the precipitate was formed.

Table 2.7 Various methods for preparing hydroxyapatite (Toy, 1973).

Ca/P mole ratio	Method of preparation
1.41	Dilute $CaCl_2$ plus excess of dilute Na_2HPO_4 at 25°C
1.50	$Ca(OH)_2$ added to H_3PO_4 to phenolthalein end point or by slow hydrolysis of $CaHPO_4.2H_2O$ (gets good crystals)
1.61	$Ca(OH)_2$ added to dilute H_3PO_4 to phenolthalein end point and then boiled
1.67	$Ca(OH)_2$ added to dilute H_3PO_4 then neutralized at boiling
1.75	Freshly precipitated tricalcium phosphate plus lime

In 1973, Toy reported various methods for preparing HA, as shown in Table 2.7. All of the obtained HA had nearly the same X-ray patterns.

Young and Hulcomb reported in 1982 the methods for the preparation of HA. Five different preparation methods were presented.

1) Precipitation

Hot 0.04 M diammonium hydrogen phosphate ($(NH_4)_2HPO_4$) was dropped into boiling 0.05 M calcium nitrate ($Ca(NO)_3$) solution with rapid stirring. The reaction vessel was kept in nitrogen stream which was passed through sodium hydroxide (NaOH) solution to remove CO_2. After total addition, the solution was continuously reacted at 100°C for 6 hours.

2) Refluxing

This method was based on hydrolysis of DCPD (Fisher reagent grade). DCPD was refluxed in distilled and deionized water for 1 month. The water was changed 4-6 times to decrease acid buildup.

3) Solid-State Reaction

Solid-state reaction, which was used to produce HA as described below, was called Fowler method. A stoichiometric of $CaCO_3$ and $Ca_2P_2O_7$ mixture was ground, pressed into pellets, and heated at 1000°C for 24 hours in a stream of P_2O_5 dried N_2. Then the obtained product was twice re-ground, pressed and heated again. Next the twice-fired product was ground and fired at 1000-1100°C for 24 hours in a steam atmosphere. By this method, the final product contained approximately 1 wt% ($Ca(OH)_2$) impurity due to nonstoichiometric mixing, or incomplete solid-state reaction, or both.

4) Hydrothermal

The first step of this method was solid-state reaction of CaO and $Ca_2P_2O_7$ at 1000°C (Fowler method). Then the resulting powder was ground and loaded into a platinum-lined hydrothermal bomb (Temp-Press model HR1B) with water at 500°C, 15,000 psi for 1 month.

5) Conversion

HA was prepared by heating chlorapatite (ClAp) powder at 1000°C for 180-450 hours in a steam atmosphere. For the conversion reaction, the ClAp powder was loaded into platinum boat and heated in a tube furnace. The final product had about 1 wt% β-$Ca_3(PO_4)_2$ impurity due to the loss of $CaCl_2$ from the apatite at 1000°C and the subsequent thermal decomposition of the resulting Ca-deficient material.

In 1989, Kanazawa reported various techniques to synthesize HA. HA single crystals were obtained by the chemical reaction:

$$14CaHPO_4 + 2H_2O \rightarrow HA + 4Ca^{2+} + 8H_2PO_4^-$$

using insoluble calcium phosphate, such as $CaHPO_4$, as a starting material. This reaction was done under the conditions of 8.6 Pa water vapor pressure at 300°C for 10 days. HA powder could be synthesized by two mean routes: dry chemical method and wet chemical method.

1) Dry chemical method by solid-state reactions

This method has the advantage of providing stoichiometric HA powders. TCP, CPP, DCPD and $Ca(OH)_2$ are generally used as the starting materials. Water vapor must be supplied continuously during thermal treatment as a source of OH^- in HA.

$$3Ca_3(PO_4)_2 + 5CaCO_3 \rightarrow HA + 4CaO + 5CO_2$$

2) Wet chemical method by precipitation and hydrolysis

HA can be prepared by precipitation from mixed aqueous solutions or by hydrolysis of calcium phosphates. In the precipitation syntheses, Ca sources include $CaCl_2$, $Ca(NO_3)_2$, $Ca(OH)_2$, $(CH_3COO)_2Ca$, $CaCO_3$ and $CaSO_4.2H_2O$. Phosphorus sources include $NH_4H_2PO_4$, $(NH_4)_2HPO_4$, H_3PO_4 and Na or K salts of phosphorus. During the syntheses, the pH of the solution must be adjusted to above 7 with NH_3 gas or NH_4OH.

In 1994, Aoki reported various methods for preparing HA, including wet method, dry method, hydrothermal method and alkoxide method. Each method is detailed as the followings:

1). Wet method

This method is suitable for mass production of small crystalline or noncrystalline HA powder. Generally, the method is based on the chemical reaction of calcium salts and phosphate salts, as shown in the below equations.

$$10Ca(OH_2) + 6H_3PO_4 \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 18H_2O$$

$$10CaCl_2 + 6Na_2HPO_4 + 2H_2O \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 12NaCl + 8HCl$$

$$10Ca(NO_3)_2 + 6(NH_4)_2HPO_4 + 2H_2O \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 12NH_4NO_3 + 8HNO_3$$

2). Dry method

The fine, well-crystallized HA powder can be prepared by this method via solid-state reaction. DCPD and $CaCO_3$ are used as the starting materials and the following reaction occurs :

$$6CaHPO_4.2H_2O + 4CaCO_3 \xrightarrow{1000\text{-}1300\ ^{o}C} Ca_{10}(PO_4)_6(OH)_2 + 4CO_2 + 14H_2O$$

3). Hydrothermal method

This method is used to prepare the large, perfect, single crystals of HA. In 1956, Peroff, et al. succeeded in growing a HA crystal to 0.3 mm under hydrothermal conditions of 300°C and about 85 kg/cm^2. Mosebach (1966) prepared HA single crystals hydrothermally using $Ca(NO_3)_2$, KH_2PO_4 and NaOH. Mengeot et al. (1973) synthesized a HA single crystal of 7x3x3 mm^3, and Eysel et al. synthesized an 8-mm crystal. Aoki et al. succeeded in preparing a 10-mm HA crystal by using their newly designed stainless steel autoclave (Aoki, 1994).

4). Alkoxide method

This method is available to prepare thin HA film. For example, calcium nitrate tetrahydrate and trimethyl phosphate were used as starting materials. They were both dissolved in ethanol or formamide as solvents. After evaporation of the solvent, the mixture was heated at 50-1000°C to produce well-crystallized HA.

$$Ca(NO_3)_2.4H_2O + (CH_3O)_3PO \longrightarrow Ca_{10}(PO_4)_6(OH)_2$$

2.3.2 The Preparations of Calcium Phosphates from Natural Reactants

As mentioned before that bones of vertebrate animals consist of calcium phosphate, so it is possible to use them as the starting materials for preparing calcium phosphate compounds. The mostly used are cattle bones, because of their availability. The advantage of using cattle bone as the starting material is lowering the investment cost, in comparison with chemical reactants, but yielding the products which have the similar properties. Chemical analysis of cattle bones is shown in Table 2.8 (Charussri Lorprayoon, 1986).

Table 2.8 Chemical analysis of cattle bones (Charussri Lorprayoon, 1986).

Type of Bone	CaO	MgO	P_2O_5	SiO_2	Fe_2O_3	LOI*
Cow	32.1	1.4	28.3	4.0	0.4	33.4
Buffalo	33.0	2.8	27.9	2.4	0.4	32.0

LOI* = loss on ignition.

The comparative studies of synthetic and natural bone HA were reported by Golutvina *et al.*, 1973, (Charussri Lorprayoon, 1986). The results showed that their chemical compositions and crystal structures were similar, but bone contained more metal ions than the synthetic material. In 1978, Scheicher (Charussri Lorprayoon, 1986) studied phases of human teeth and bones at high temperature. The teeth and bones were dried, ground, degreased with ethyl alcohol to remove organic matter and treated with oxidizing agent, H_2O_2. The treated ash was then heated at 900-1000°C for 20-120 minutes. The products were identified as HA and used as fillers for implant areas in contact with bone.

In 1986, Charussri Lorprayoon studied the phases of cattle bones at elevated temperature. The bones were heated in an electric furnace with a heating rate of 10°C/min below 600°C and 7°C/min above 600°C, soaking at the required temperature for 15 minutes. The phases of products were identified by X-ray diffraction and summarized by the following equations:

$$Ca_{10}(PO_4)_6(OH)_2 \xrightarrow{900\text{-}1300\ ^{o}C} Ca_{10}(PO_4)_6(OH)_{2(1-x)}O_x + xH_2O \quad (x = 0 \text{ to } 1)$$

$$Ca_{10}(PO_4)_6(OH)_{2(1-x)}O_x \xrightarrow{1300\text{-}1400\ ^{o}C} Ca_{10}(PO_4)_6O + (1-x)H_2O$$

$$Ca_{10}(PO_4)_6O \xrightarrow[\text{quenching}]{1400\ ^{o}C} 2\alpha\text{-}Ca_3(PO_4)_2 + Ca_4O(PO_4)_2$$

$$Ca_{10}(PO_4)_6O \xrightarrow[\text{slow-cooling}]{1400\ ^{o}C} 2\beta\text{-}Ca_3(PO_4)_2 + Ca_4O(PO_4)_2$$

Charussri Lorprayoon (1989) synthesized HA and TCP from cattle bone ash. Firstly, bone ash was stirred in distilled water, then nitric acid was slowly added until pH value of the solution was in the range of 1.00-1.35. After 30 minutes, ammonium hydroxide was continuously added into the solution. At pH 4.85-5.15, the white precipitate was observed, and at pH 8.0-8.5, TCP was obtained. In case of preparing HA, calcium salt was added to the solution to obtain the desired Ca/P ratio, and then the precipitation took place at pH 9.7-10.0 or higher, yielding HA.

Charussri Lorprayoon and Supatra Jinawath (1985) reported the properties of HA from different sources. These HA were HA obtained from natural cattle bone (MP), from chemically treated cattle bone (TP) and from chemical precipitation (CH). By comparison their properties, it was found that they all had similar Ca/P ratio, morphology, and phase occurrence, except that MP had organic matter and more impurities.

MP was prepared by boiling cattle bones to remove fat and gelatin. Then bones were calcined at 700°C to eliminate the remaining organic matter. After that, the calcined bones were wet-milled, dried and ground in porcelain mortar to obtained MP powder.

Calcined MP was used as a starting material for preparing TP. It was stirred in distilled water, then calcium nitrate solution was added. The nitric acid was added to dissolve MP, at pH = 1.0, the solution became transparent. Concentrated ammonium hydroxide was added till pH value of solution reached 9.7-10.0 to precipitate TP. The product was then filtered, washed with distilled water, dried and ground to obtain TP.

CH was precipitated from calcium nitrate solution and diammonium hydrogen phosphate at pH 10.5. The precipitate was then filtered, washed with distilled water, dried and ground to obtain CH.

Supatra Jinawath and Supatra Trakarnvichit (1995) synthesized DCPD and MCPM from cattle bone. Cattle bone ash was dissolved in phosphoric acid at 200°C. In order to prepare DCPD, the pH of the solution was adjusted to 3.5-6.0 by adding ammonium hydroxide, and the precipitate was obtained. After that, the precipitate was filtered, washed and dried at room temperature in desiccator. From the results, it was found that the optimum pH was 4.5-5.0 and different concentrations of phosphoric acid and various Ca/P ratios of starting material had no effect on the phase of precipitate. In the case of MCPM, the bone ash solution was evaporated by boiling or lowering pressure at room temperature and MCPM was observed.

2.4 The Steps of Bone Gelatin Production

Gelatin is used extensively in food industry as a gelling agent; as a whipping agent in foams; as a clearing agent in fruit juices, wines, and beer; to increase viscosity; and to prevent ice-crystal growth in frozen desserts. It is also used in drug industry for making capsules and as an emulsifier. Furthermore, gelatin has played an important part in the rapid development

of the motion picture and photographic industries. It is coated on the film base, constituting the sensitized emulsion of the light-sensitive silver salts (International Trade Centre, 1984).

Because bones are consisted of hydroxyapatite, collagen and water, they can be used as raw material for gelatin manufacture. Raw bone, obtained fresh from slaughtered cattle, contains around 35-40% water, 10-15% fat and approximately 50% dry solids as protein and minerals.

In order to produce gelatin, the collagen has to be separated as completely as possible from the other substances and with the least possible injury to the collagen itself. Properly conditioned collagen is then heated with water and slowly changes to gelatin. The details of overall process are as the followings:

1). Degreasing

This is the most important stage for preparing gelatin from bone. It is essential to start the processing within 24-48 hours of the slaughtering of animals. Horns and hoofs are removed and the bones are pre-crushed. The fats are removed by cold water extraction and then are clarified to yield tallow. After that, the bone pieces are dried and ground to an average size of 3/8-5/8 inch. Further polishing and sieving produces a final product suitable for gelatin manufacture and gives bone powder and bone meal as valuable by-products.

2). Demineralization

Dry, degreased bones contain collagen incorporated in a mineral structure composed mainly of tricalcium phosphate and calcium carbonate. In order to separate the mineral components, the bones are treated in 5% hydrochloric acid solution in isothermal tanks at 5°C where the mineral

components were dissolved. Dicalcium phosphate is precipitated from the solution by the addition of lime water.

3). Conditioning and Pretreatment

Collagen has to be conditioned to allow conversion to gelatin to take place under moderate conditions of temperature and pH. It is suspended in lime slurry in a tank for several weeks. After lime treatment, solid lime is removed from the collagen by mechanical means and water washing. Residual lime is removed by washing in dilute acid followed by water washing to remove residual acid. Since the collagen is sensitive to bacterial action, sulphur dioxide is added during the final wash to preserve it.

4). Extraction

Conditioned collagen is heated with water to be changed to gelatin. The first gelatin fraction is removed when the concentration of liquid reaches about 5% at a temperature of 50-55°C, then fresh water is added and the process repeated. Each extract is obtained at a higher temperature and the quality of gelatin obtained consequently declines.

5). Drying

Extracted gelatin liquors are purified by double filtration and then are deionised to remove the inorganic impurities. The liquors are then evaporated under vacuum, using modern, dairy-type equipment by which they are concentrated from 5% to about 35% solid content. After evaporation, they are flash-pasteurized at 120°C and immediately cooled and extruded in the form of noodles on to a continuous stainless steel belt to a drier. The dried gelatin is then conveyed to a crushing plant, ground to powders of varying grades and packed in airtight packages.

From gelatin manufacture, several by-products are observed. They are included meat and bone meal, hoof and horn meal, tallow and dicalcium phosphate. These by-products can be used in many fields, for example, tallow can be used as an animal feed, as an ingredient for soap and candle manufacture or for artisanal products. Bone meal, hoof and horn meal, and dicalcium phosphate have the similar market. They are used as ingredients in animal feeds and fertilizer. Hoof and horn powders can also be used in fire extinguishers.

CHAPTER 3

EXPERIMENTAL PROCEDURE

This chapter is divided into 4 sections. The first section lists the details of the starting material and chemical reactants which were used for preparing calcium phosphate compounds. The second section is the methods for the preparation of three types of calcium phosphate compounds, dicalcium phosphate dihydrate (DCPD), dicalcium phosphate anhydrous (DCPA) and monocalcium phosphate monohydrate (MCPM), from solutions. The third section deals with the preparations of beta tricalcium phosphate (β-TCP) and hydroxyapatite (HA) from solid state reactions. The last section includes the characterization of the products, i.e. phase analysis (X-ray diffraction, XRD), microstructure (scanning electron microscopy, SEM), functional groups (Fourier-Transform infrared spectroscopy, FT-IR), chemical analysis (inductively couple plasma, ICP) and specific surface area (Brunauer, Emmett and Teller, BET).

3.1 Starting Material and Chemical Reagents

- Starting material

A by-product from bone gelatin industry was known to compose of calcium phosphate. This by-product was sieved through 140 mesh in order to eliminate black coarse particles and then was characterized by XRD, SEM, FT-IR, BET and ICP techniques and was used as starting material for preparing various types of calcium phosphate compounds.

- Chemical reagents

1. Nitric acid (69.0-70.0% Actual Analysis, J.T. Baker)
2. Orthophosphoric acid (85.0%, BDH AnalaR)
3. Acetic acid (100% Actual Analysis, Baker Analyzed)
4. Ammonia solution (35% NH_3, BDH AnalaR)
5. Acetone (Actual Analysis, J.T. Baker)
6. Calcium carbonate (>99%, Fluka)

3.2 Preparations of Calcium Phosphates from Solution

3.2.1 Preparation of Dicalcium Phosphate Dihydrate (DCPD)

DCPD can be prepared stepwise as shown in Figure 3.1. About 2 grams of starting material was dissolved in 37 ml of 1 M HNO_3, heated and stirred for 15 minutes. The solution was then cooled to room temperature and filtered through filter paper to get rid of the undissolved particles. In order to precipitate DCPD, 1M NH_4OH was added continuously into the stirred transparent solution till the pH value of the solution reached the desired value. The final pH value of the solution was varied from 3.5-6.0. After that, the precipitate was filtered, washed with distilled water and acetone, and then dried at room temperature in a desiccator.

3.2.2 Preparation of Dicalcium Phosphate Anhydrous (DCPA)

The method for preparing DCPA was the same as that of DCPD, but the precipitation was performed at 80°C (Figure 3.1). There were 2 ways to control the temperature of the solutions; using water bath or hot

plate. Then the system was cooled to about 50°C and immediately filtered, washed with distilled water and acetone, and then dried at room temperature in a desiccator.

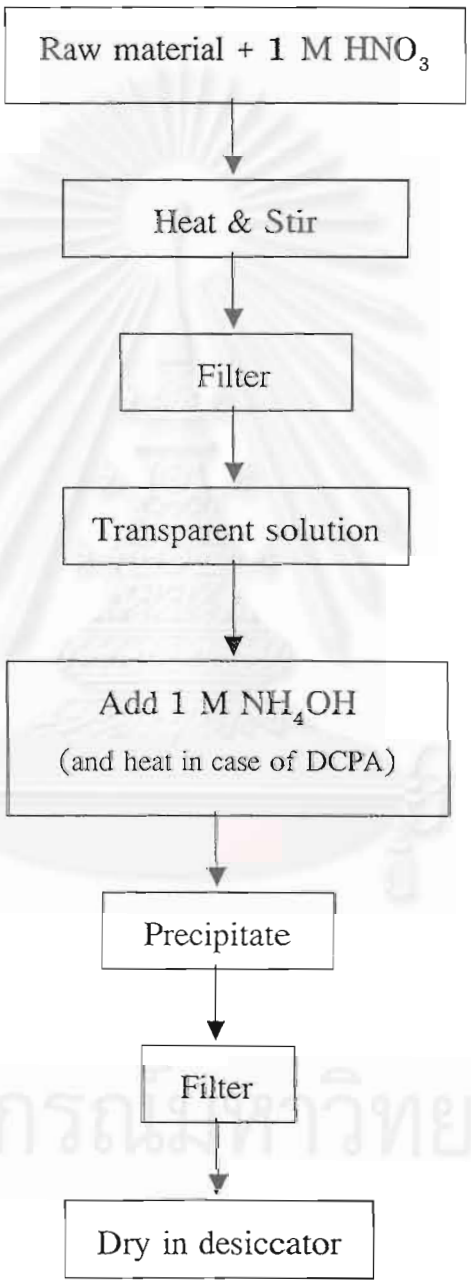


Figure 3.1 Flow chart for the preparation of DCPD and DCPA.

3.2.3 Preparation of Monocalcium Phosphate Monohydrate (MCPM)

MCPM was prepared from DCPD solution. About 8 grams of DCPD were dissolved in 5 M H_3PO_4 acid, heated and stirred for 20 minutes, cooled to room temperature and then filtered. The amount of phosphoric acid was varied from 40-60 ml. The filtered solution was further evaporated for about an hour to precipitate MCPM then the solution was cooled to room temperature, filtered, washed with acetone, and then dried in a desiccator (Figure 3.2).

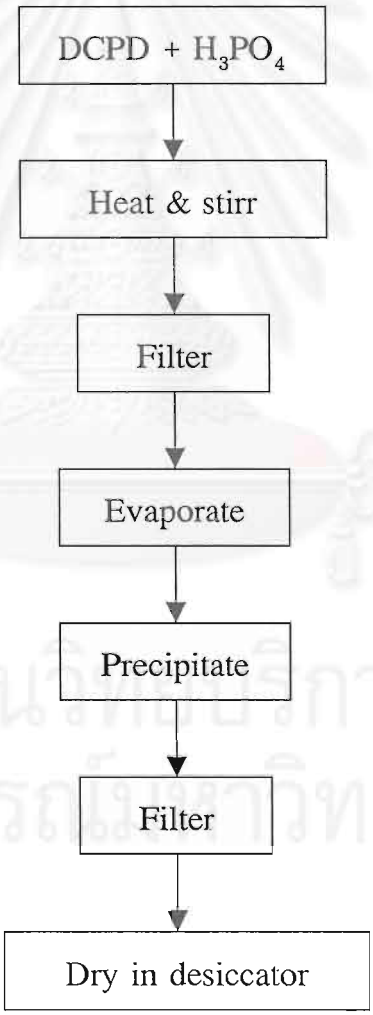


Figure 3.2 Flow chart for the preparation of MCPM.

3.3 Preparation of Calcium Phosphates from Solid-state Reaction

3.3.1 Preparation of β-Tricalcium Phosphate (β-TCP)

β-TCP was prepared from the heat treatment of DCPD and DCPA with $CaCO_3$ in air, as shown in Figure 3.3. The Ca/P mole ratios of the mixtures of DCPD+ $CaCO_3$ and DCPA+$CaCO_3$ were 1.46 and 1.50. Each set of the mixtures was heated to 1100°C with a heating rate of 4°C/min and then soaked for 3 hours.

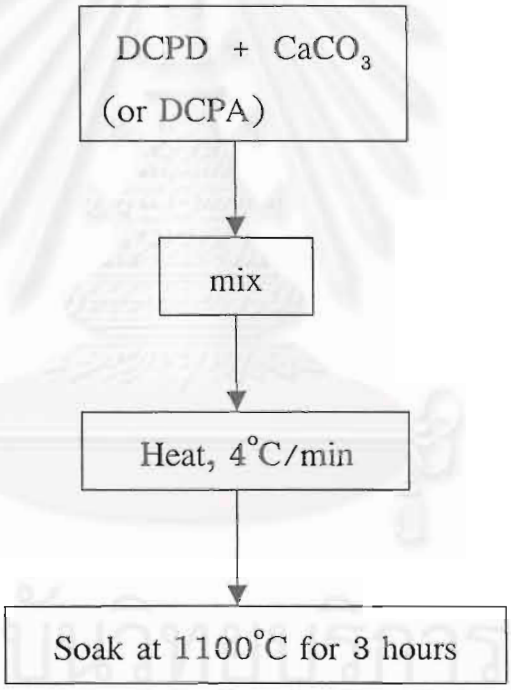


Figure 3.3 Flow chart for the preparation of β-TCP.

3.3.2 Preparation of Hydroxyapatite (HA)

Like β-TCP, HA was also prepared from the heat treatment of DCPD and DCPA with $CaCO_3$ in air. The Ca/P mole ratios of the mixtures of DCPD+$CaCO_3$ and DCPA+$CaCO_3$ were 1.63 and 1.67. Each set of the mixtures was heated with a heating rate of 4°C/min, soaked at 880°C for 0.5 hour, then heated to 1100°C or 1200°C and soaked for 3 hours (Figure 3.4).

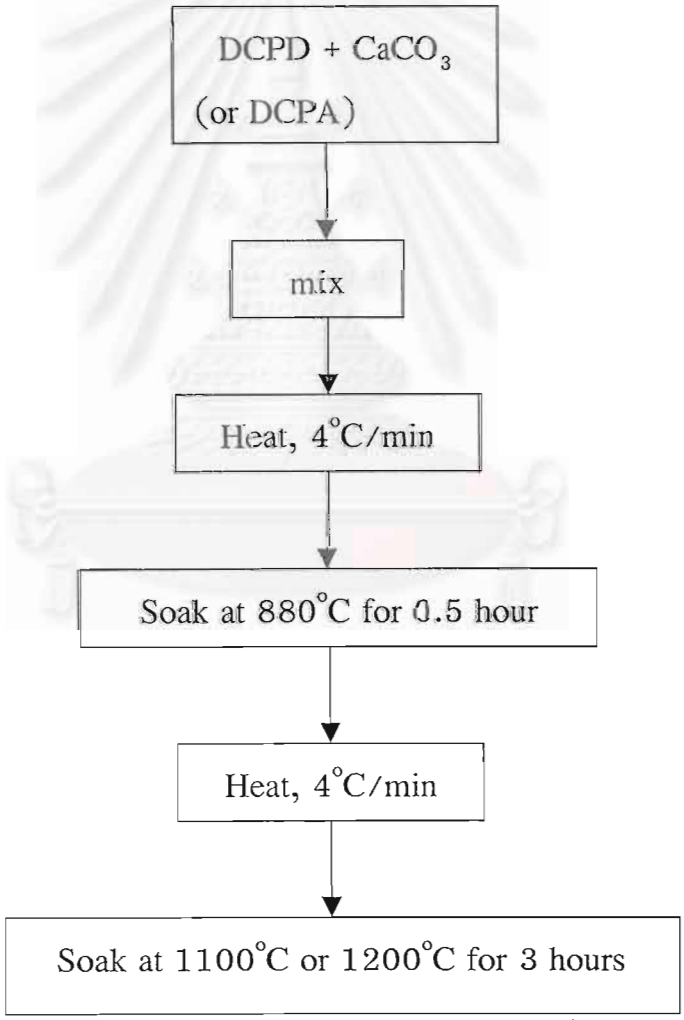


Figure 3.4 Flow chart for the preparation of HA.

3.4 Characterization

3.4.1 Phase

The phases of the starting material and the obtained DCPD, DCPA, MCPM, β-TCP and HA were analyzed by X-ray diffractometer (XRD) (PHILIPS: PW1730/10, PHILIPS: X'PERT-MPD XRA407, and JEOL: JDX-8030), CuK_{α} radiation, λ = 1.5405 Å. A time constant of 1 second and scanning rate 2°/min were used, 2θ was run from 4° to 60° to identify the phase of calcium phosphate compounds.

3.4.2 Microstructure

The microstructures of the starting material and the products were examined by scanning electron microscopy (SEM) (JEOL: JSM-6400 and JEOL: JSM-5410LV). Each sample was mounted on an aluminum stub and then coated with gold using sputter coater before observed.

3.4.3 Functional Groups

Functional groups of the starting material and the products were studied by Fourier-Transform infrared spectrophotometer (FT-IR) (Perkin Elmer 1760X). Each sample was run as KBr pellet from the wavelength of 400-4000 cm^{-1} to identify their functional groups.

3.4.4 Chemical Composition

The Ca/P mole ratio, amount of impurities and heavy metals of the starting material and the synthesized calcium phosphate compounds were analyzed by Inductively Couple Plasma (ICP).

3.4.5 Specific Surface Area

Specific surface area of the starting material and the synthesized products were determined by BET (Micromeritics model ASAP 2000). Nitrogen gas was used as an absorbate on particle surface.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Starting Material

The XRD pattern of the sieved by-product (-140#), which was used as the starting material for preparing various types of calcium phosphate compound, is shown in Figure 4.1. This pattern agrees with the JCPDS card of dicalcium phosphate dihydrate (DCPD) and a trace of dicalcium phosphate anhydrous (DCPA) is observed.

The IR spectrum from 400-4000 cm^{-1} of starting material is shown in Figure 4.2. The H-O-H, P-O, O-H, O-P-OH, and P-OH absorption bands in DCPD are observed. The C-O absorption band is also observed. It indicates that the starting material contains carbonate compound.

The Ca/P mole ratio and amount of impurities of the starting material are shown in Table 4.1. The average mole ratio of Ca/P is 1.05 which is slightly more than 1 of the stoichiometric DCPD.

Table 4.1 Chemical analysis of starting material (by ICP).

Ca/P mole ratio	Impurities		Heavy Metals (ppm)	
1.05	Mg (%)	0.02	Cd	< 0.4
	Fe (%)	0.02	Pb	< 5
	Zn (ppm)	99	As	< 5
	Cu (ppm)	2	Ni	< 2
	Mn (ppm)	1.0		

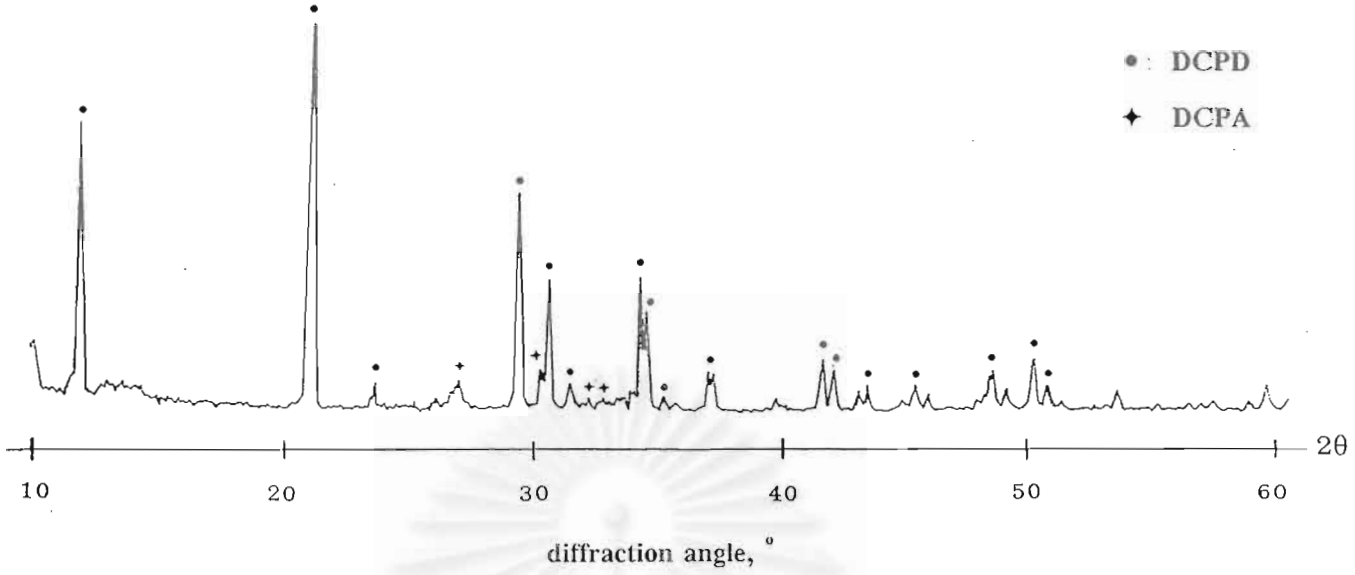


Figure 4.1 XRD pattern of starting material.

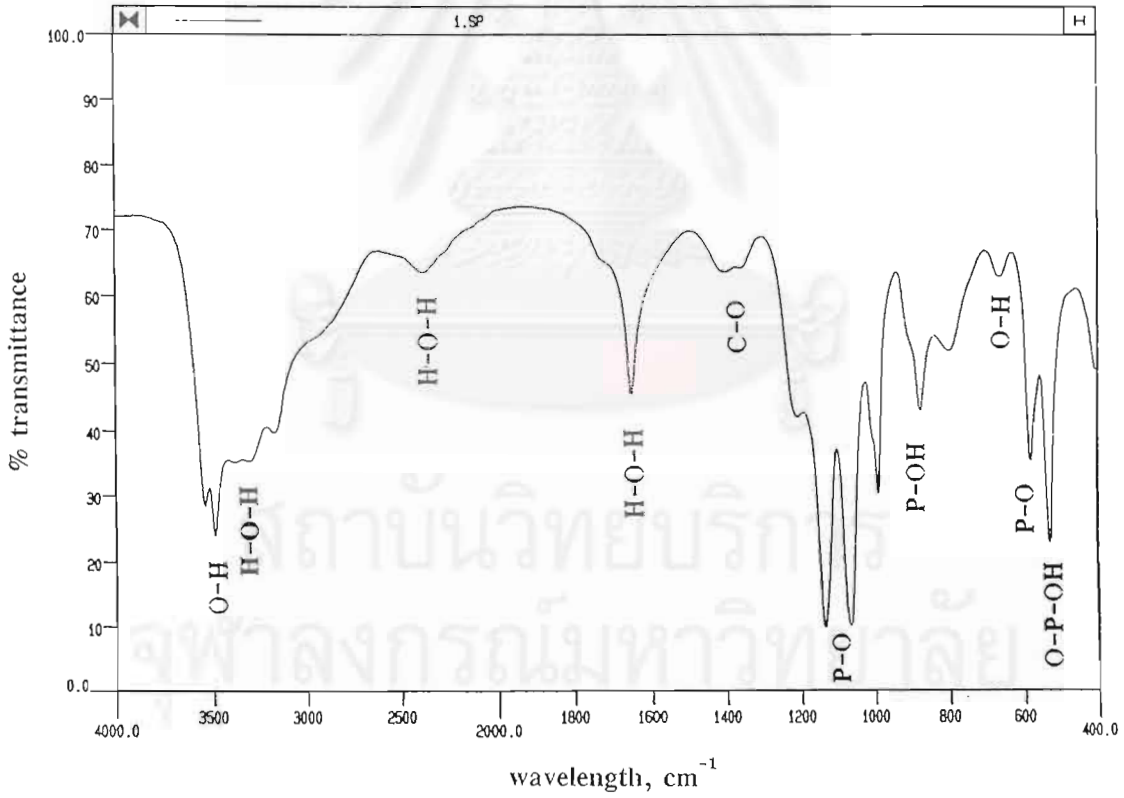
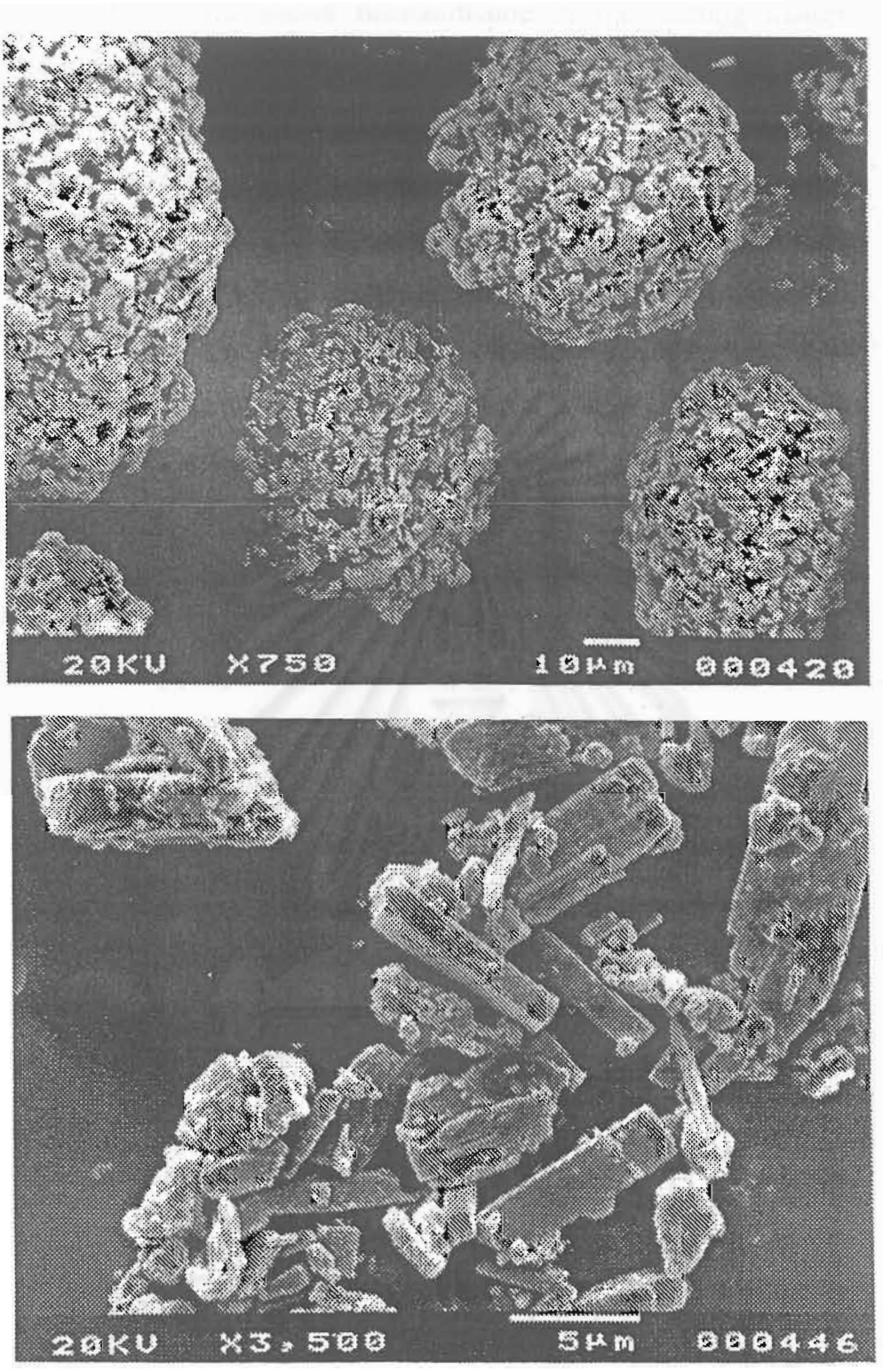


Figure 4.2 IR spectrum of starting material.



(a)

(b)

Figure 4.3 SEM micrographs of the starting material

(a) as-received

(b) dispersed in distilled water for 2 minutes using ultrasonic bath.

Figure 4.3 shows microstructure of the starting material (a) as-received and (b) dispersed in distilled water for 2 minutes. It is observed to be bar-like particles and the average size is about 7.8 μm. Specific surface area of the starting material, measured by BET, is 3.53±0.05 m^2/g. The mean particle size, calculated from the specific surface area, is 0.73 μm. It can be seen that the mean particle size is much smaller than the average size observed from SEM micrograph. This may be because the calculation of the mean particle size is based on the assumption that the particles are spherical, while the particles observed are bar-like.

4.2 Dicalcium Phosphate Dihydrate (DCPD)

The precipitates obtained from various pH values (4.5-6.0) were characterized by XRD and the results are shown in Figure 4.4. It is found that all of them show similar patterns which agree with the JCPDS card of DCPD. However, the amount of product increases as the pH value increases. The relationship between pH value and average % yield of product is shown in Figure 4.5, and the optimum pH to prepare DCPD is 5.5. The DCPD prepared from this condition is chosen to be further characterized by IR, ICP, SEM, and BET.

Table 4.2 Chemical analysis of DCPD (by ICP).

Ca/P mole ratio	Impurities		Heavy Metals (ppm)	
0.79	Mg (%)	< 0.01	Cd	< 0.4
	Fe (%)	0.02	Pb	< 5
	Zn (ppm)	105	As	< 5
	Cu (ppm)	< 2	Ni	< 2
	Mn (ppm)	12		

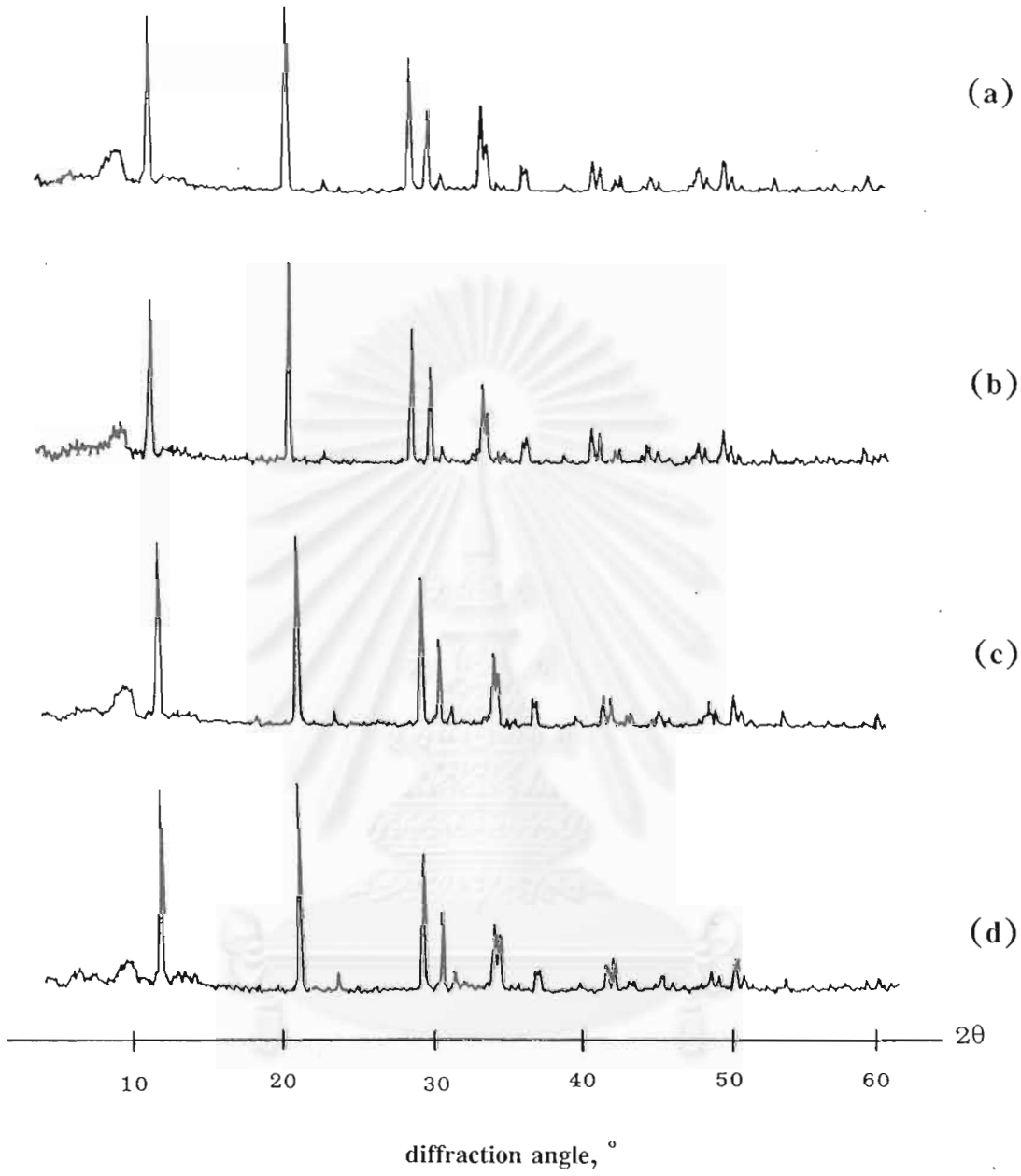


Figure 4.4 XRD patterns of DCPD precipitated from various pH values, (a) pH 4.5, (b) pH 5.0, (c) pH 5.5, and (d) pH 6.0.

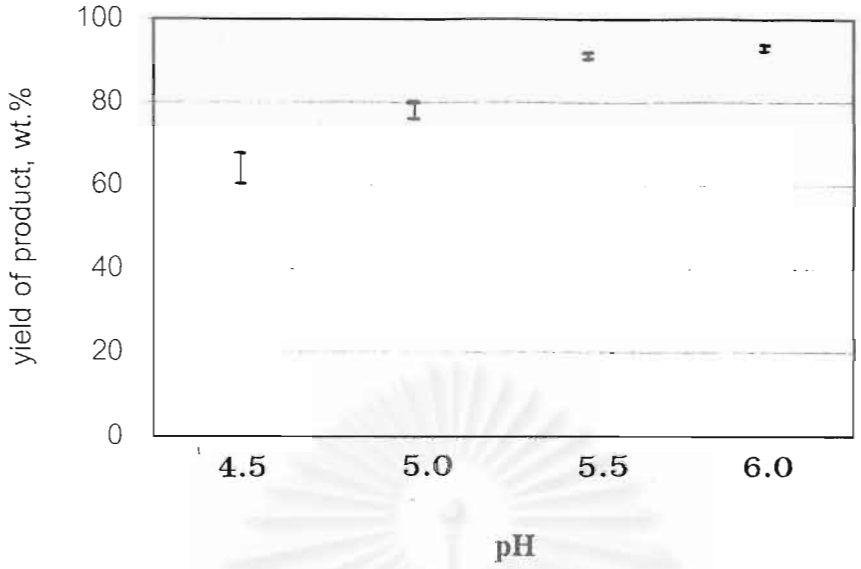


Figure 4.5 The relationship between pH values and average % yield of products.

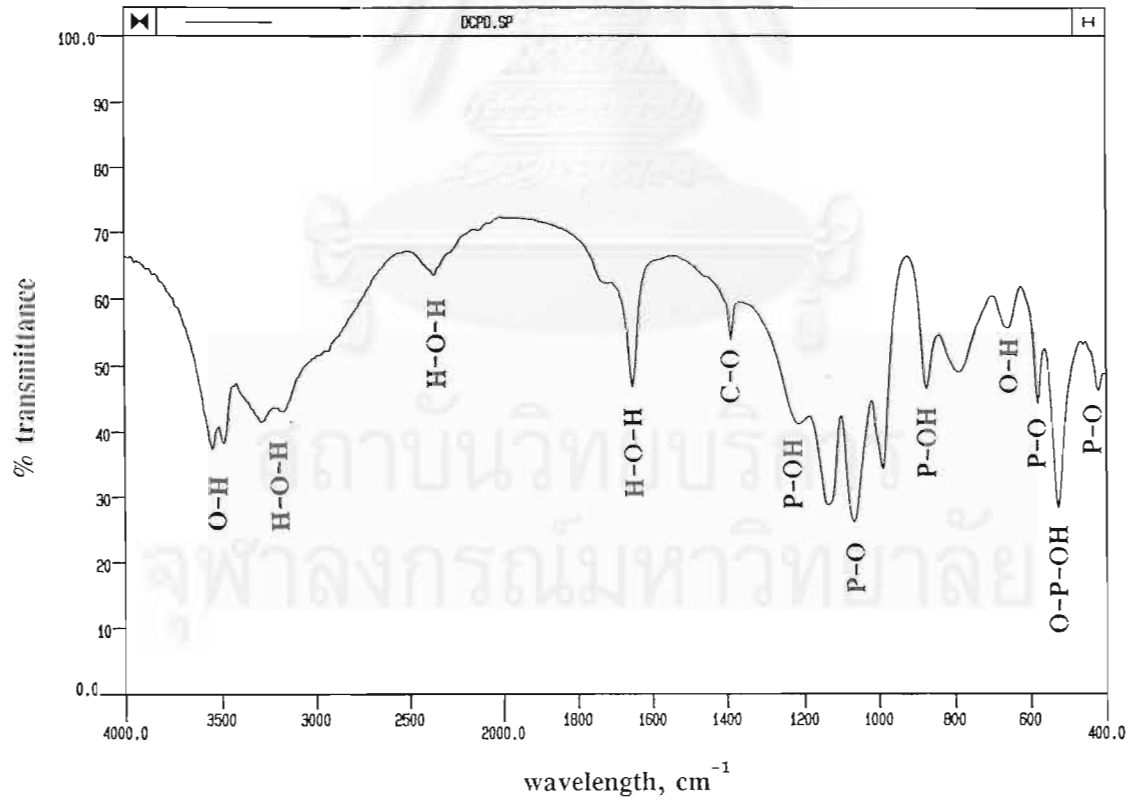


Figure 4.6 IR spectrum of the synthesized DCPD.

Figure 4.6 shows the IR spectrum of the synthesized DCPD from 400-4000 cm^{-1}. The H-O-H, P-O, O-H, O-P-OH, P-OH, and C-O absorption bands are observed. It can be seen that % transmittance of C-O absorption band decreases as compared with the one of starting material, which can be assumed that the amount of carbonate decreases. The Ca/P mole ratio of precipitated DCPD is 0.79 (Table 4.2) which is lower than that of the stoichiometric DCPD and the starting material (Table 4.1).

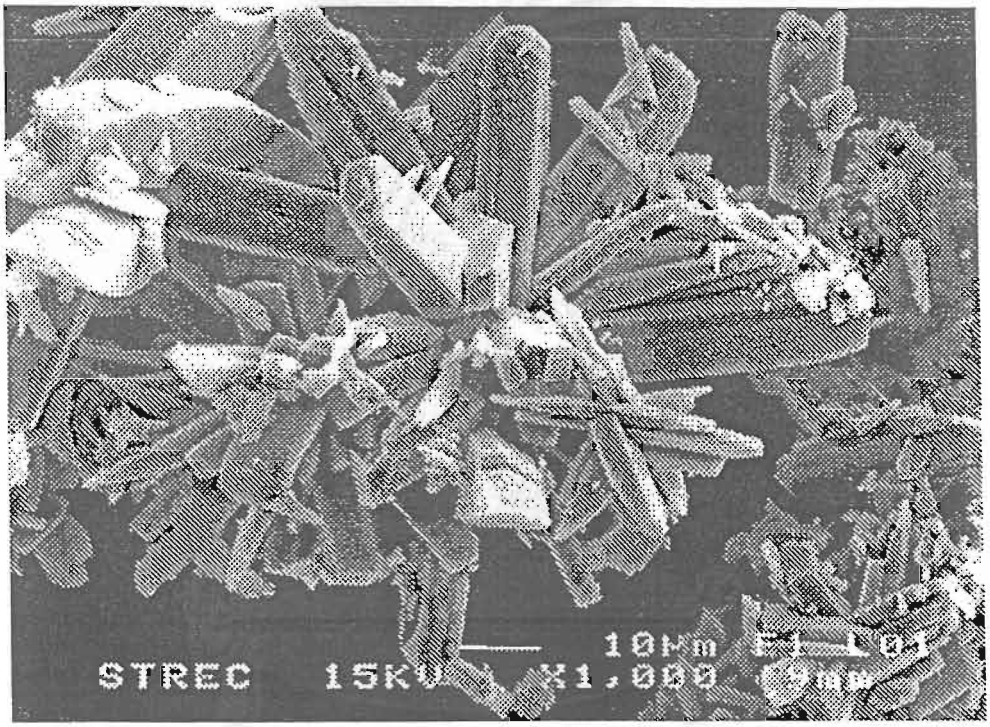


Figure 4.7 SEM micrograph of the synthesized DCPD.

The microstructure of DCPD is shown in Figure 4.7. It is found that the precipitated DCPD is the agglomerate of bar-liked particles. The average size of these particles is 31.9 μm.

4.3 Dicalcium Phosphate Anhydrous (DCPA)

DCPA was obtained from the same method as DCPD, but the precipitation was performed at 80°C. The temperature of the solution was controlled by using a water bath or a hot plate. Figure 4.8 shows XRD patterns of both prepared DCPA, (a) controlling temperature by using a water bath and (b) controlling temperature by using a hot plate. Both of them show identical patterns which is pure DCPA. Practically, it was more convenient to control the temperature by using a hot plate than a water bath. Therefore, DCPA obtained from method (b) is further characterized by IR, ICP, SEM and BET.

The IR spectrum of DCPA from 400-4000 cm^{-1} is shown in Figure 4.9(a). The H-O-H, P-O, O-H, O-P-OH, P-OH, and C-O absorption bands are observed. It can be seen that the % transmittance of C-O absorption band increases, compared with the one of the synthesized DCPD, which means that the amount of carbonate increases. The formation of the carbonate may be due to the reaction between CO_2 from air and OH^- group occurring from the hydrolysis of DCPA while washing with water after filtering. This is because the solution of DCPA in water has a slightly basic pH. In order to eliminate the carbonate, DCPA was washed with acetic acid. It can be seen from Figure 4.9(b) that the % transmittance of C-O absorption band after washing with acid decreases. However, the C-O absorption band is still observed, which means that some carbonate still remains. This may be because, as shown in Figure 4.10, DCPA is the agglomerate of the very small particles, some carbonate can be sited in crystal lattice, so they can not be washed.

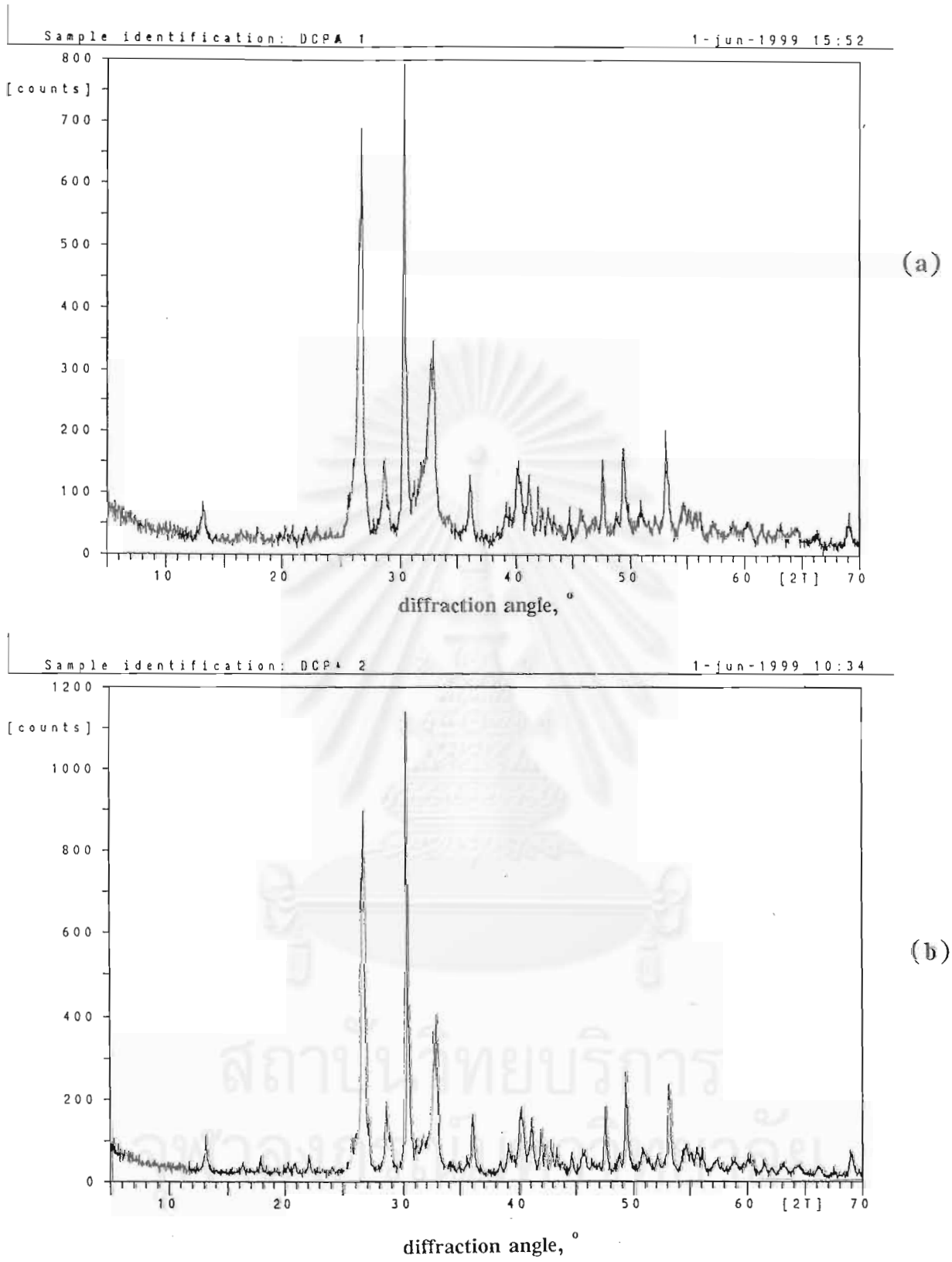


Figure 4.8 XRD patterns of prepared DCPA

(a) controlling temperature by using a water bath

(b) controlling temperature by using a hot plate.

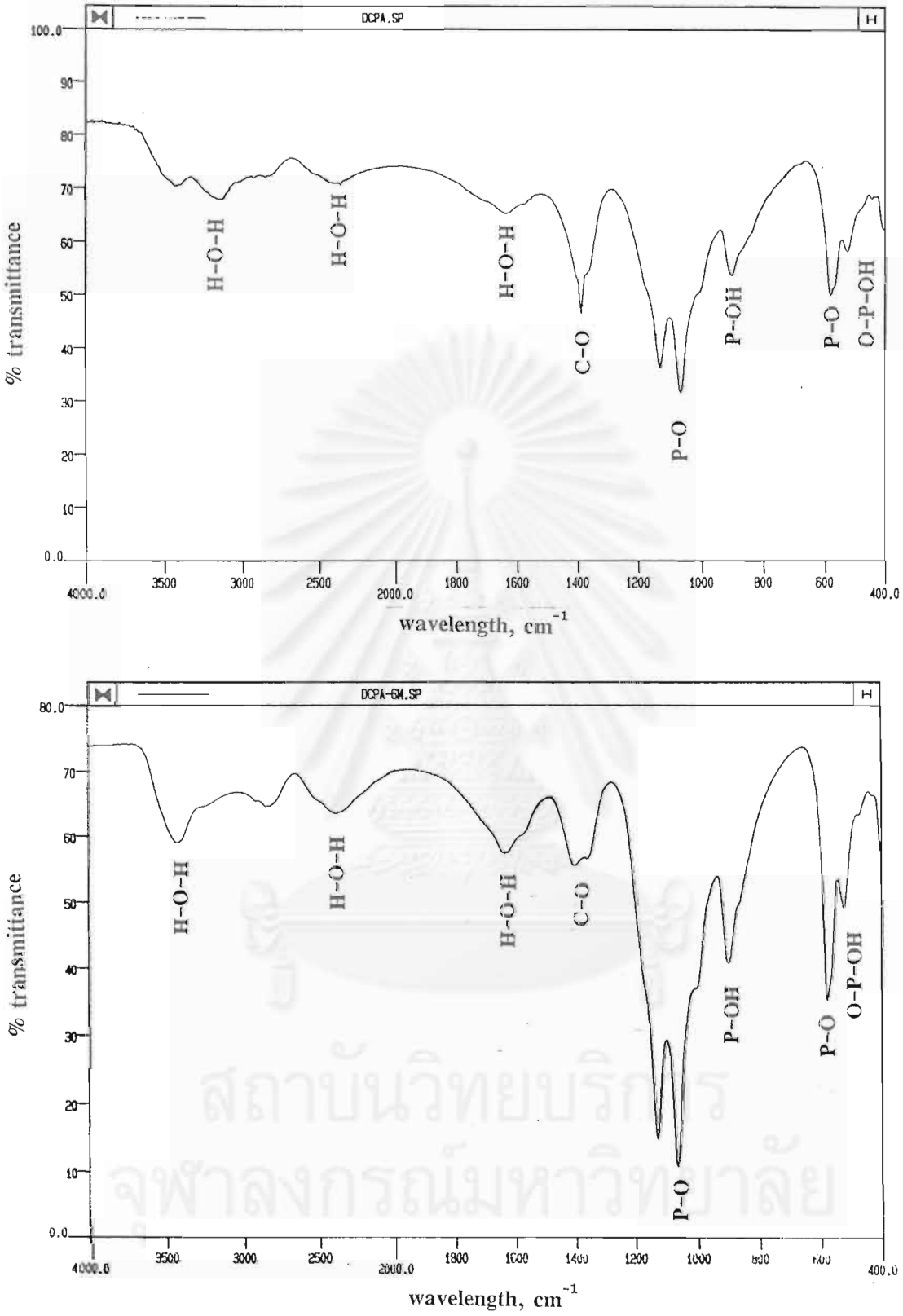


Figure 4.9 IR spectrum of DCPA

(a) synthesized DCPA before and

(b) after washing with acetic acid.

The Ca/P mole ratio and impurities of the obtained DCPA are shown in Table 4.3. Its Ca/P mole ratio is 0.96 which is slightly lower than that of the stoichiometric DCPA.

Table 4.3 Chemical Analysis of DCPA (by ICP).

Ca/P mole ratio	Impurities		Heavy Metals (ppm)	
0.96	Mg (%)	0.01	Cd	< 0.4
	Fe (%)	0.02	Pb	< 5
	Zn (ppm)	120	As	< 5
	Cu (ppm)	< 2	Ni	< 2
	Mn (ppm)	9		

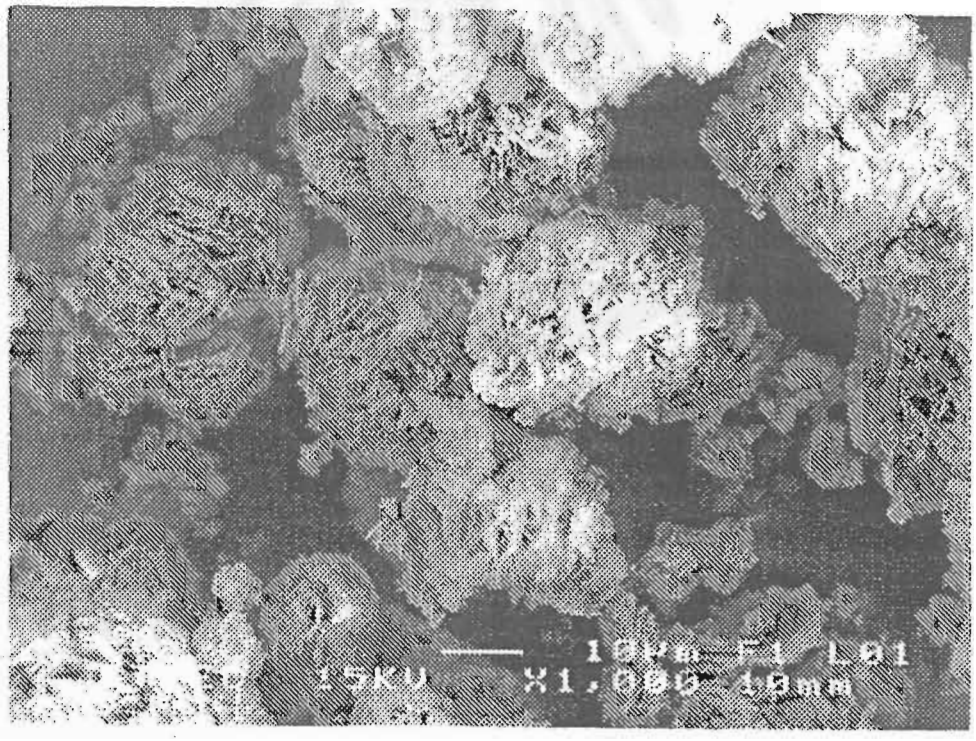


Figure 4.10 SEM micrograph of the synthesized DCPA.

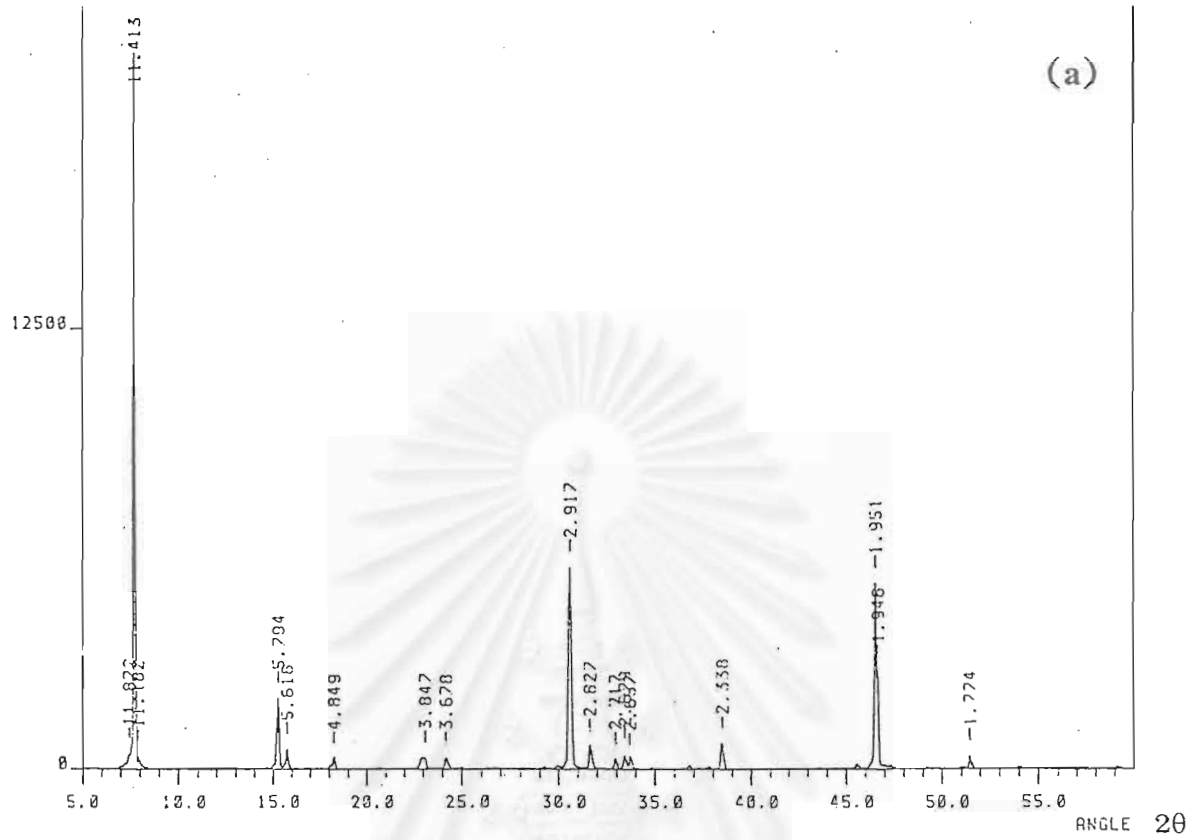
The microstructure of DCPA is shown in Figure 4.10. It is found that the prepared DCPA is the agglomerate of plate-like particles. The average size of these particles is 8.4 μm. Specific surface area of DCPA, obtained by BET, is 18.02±0.20 m^2/g. The mean particle size, calculated from the specific surface area, is 0.11 μm.

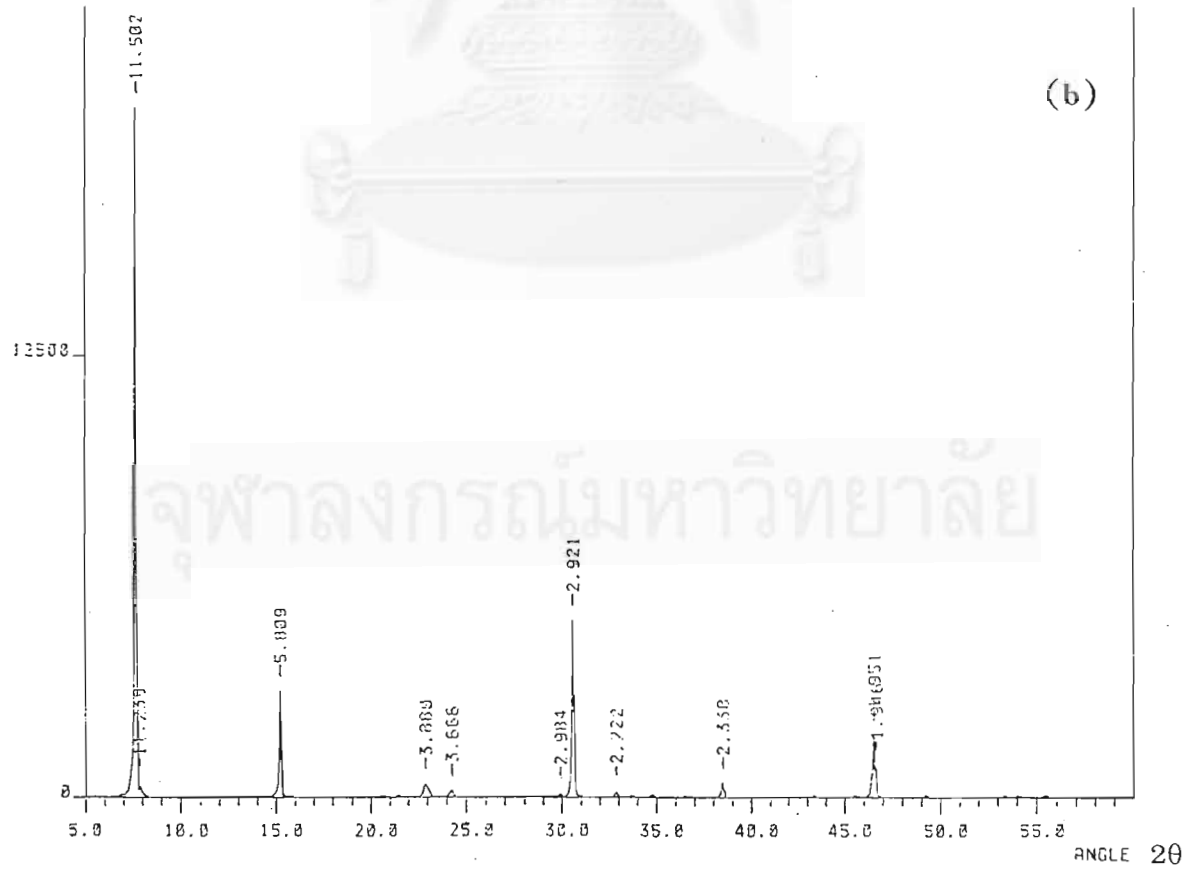
4.4 Monocalcium Phosphate Monohydrate (MCPM)

MCPM was prepared from evaporation of the solution of DCPD in 40-60 ml of 5 M H_3PO_4 acid. It is found that the solution of 40 ml acid was saturated so while filtering the solution, some precipitate was occurred. The precipitate was separated by filtering and then characterized by XRD. The filtered solution was then evaporated to obtain precipitate and studied by XRD. Figure 4.11 shows XRD pattern of the products, (a) from saturation with 40 ml acid solution, (b) from evaporation of 40 ml acid solution, (c) from evaporation of 50 ml acid solution and (d) from evaporation of 60 ml acid solution. All of them have similar pattern which agrees with JCPDS card of MCPM.

The relationship between amount of phosphoric acid and MCPM obtained is shown in Figure 4.12. From this figure, the optimum condition for preparing MCPM is dissolving DCPD in 50 ml of phosphoric acid. So, MCPM obtained from this condition is chosen to be further characterized by IR, ICP, SEM, and BET.

The IR spectrum of MCPM from 400-4000 cm^{-1} is shown in Figure 4.13. The H-O-H, P-O, O-P-OH, and P-OH absorption bands are observed. The Ca/P mole ratio of synthesized MCPM is 0.42 which is lower than that of the stoichiometric MCPM.





diffraction angle, °

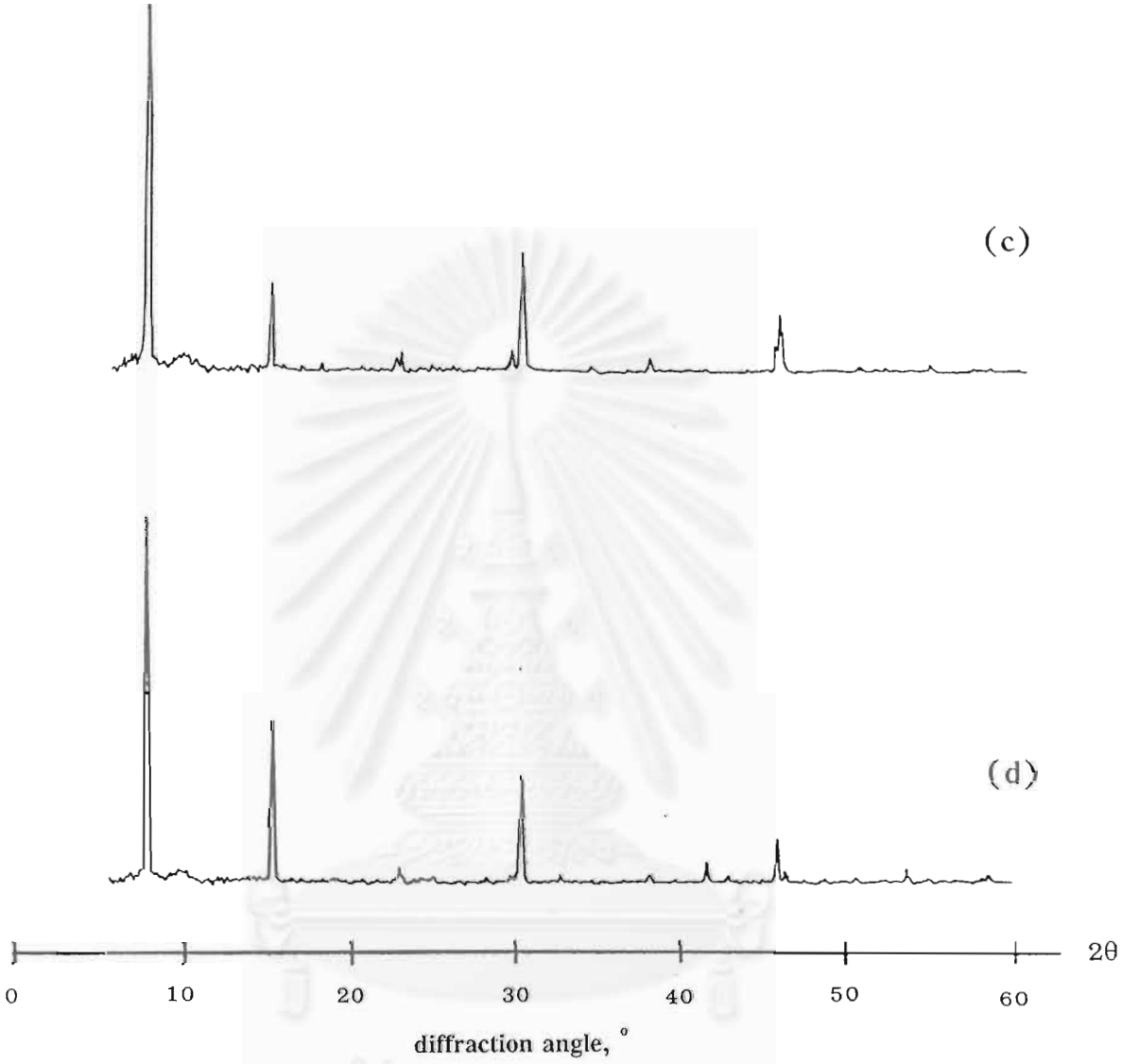


Figure 4.11 XRD patterns of MCPM

(a) from saturation with 40 ml acid solution

(b) from evaporation of 40 ml acid solution

(c) from evaporation of 50 ml acid solution

(d) from evaporation of 60 ml acid solution.

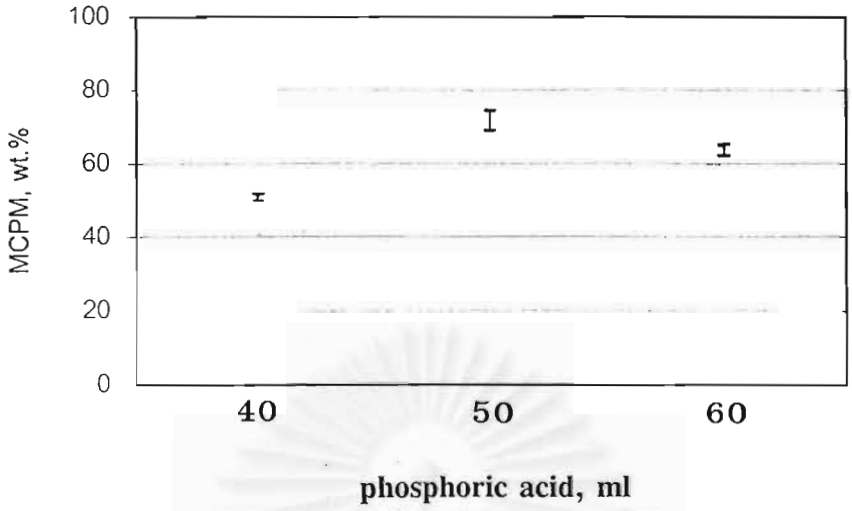


Figure 4.12 The relationship between amount of phosphoric acid and MCPM obtained.

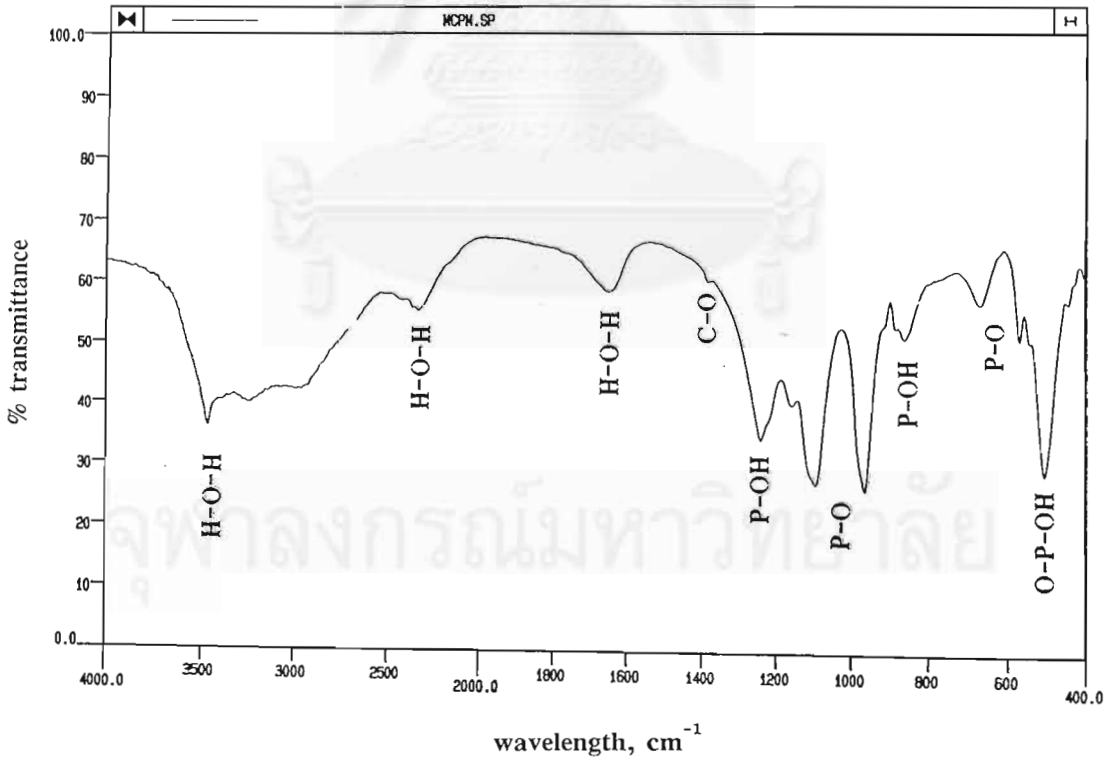
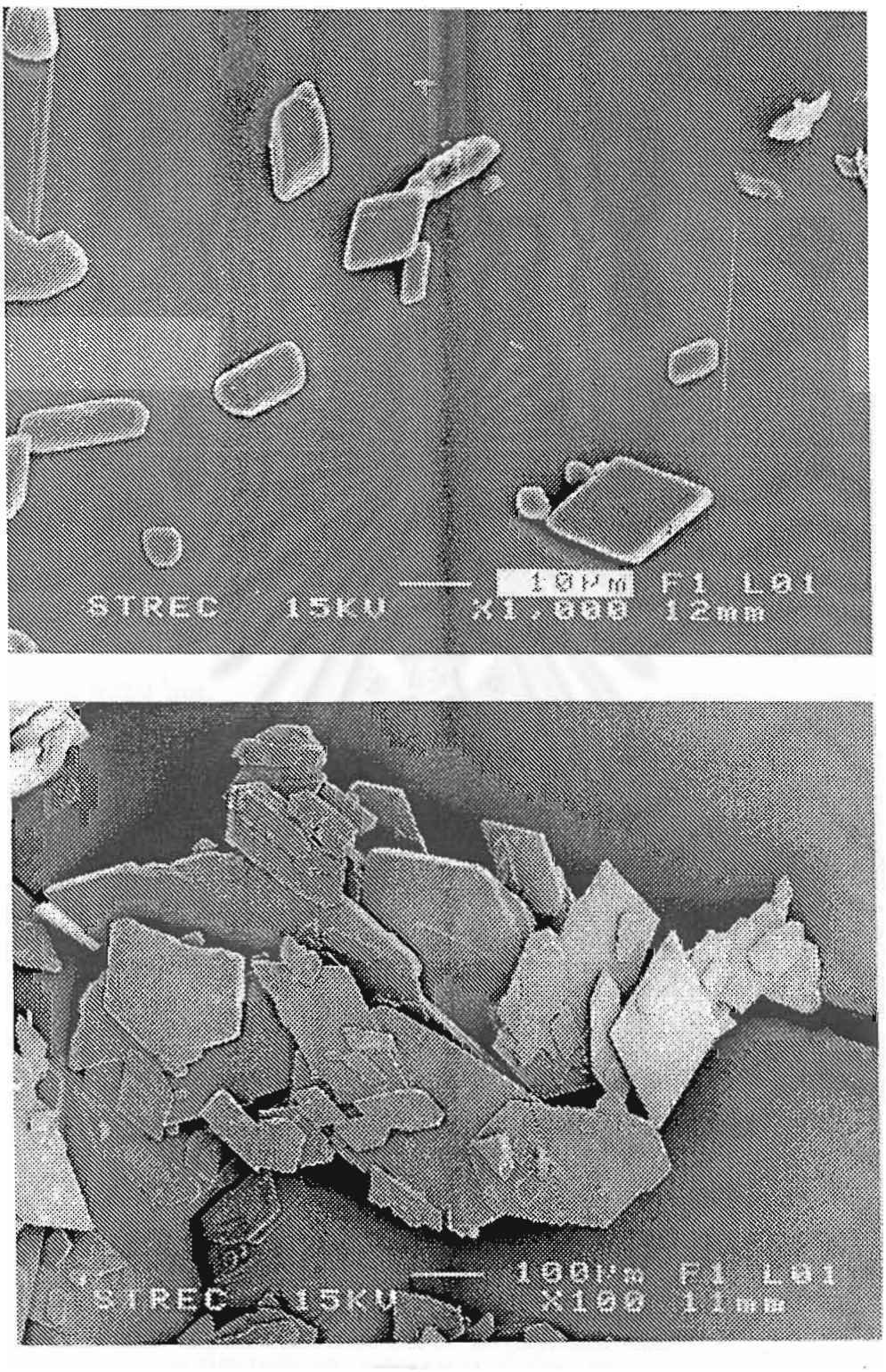


Figure 4.13 IR spectrum of the synthesized MCPM.



(a)

(b)

Figure 4.14 SEM micrographs of the synthesized MCPM

(a) precipitated from saturation

(b) precipitated from evaporation.

Table 4.4 Chemical analysis of MCPM.

Ca/P mole ratio	Impurities		Heavy Metals (ppm)	
0.42	Mg (%)	< 0.01	Cd	< 0.4
	Fe (%)	< 0.01	Pb	< 5
	Zn (ppm)	5	As	< 5
	Cu (ppm)	< 2	Ni	< 2
	Mn (ppm)	13		

Figure 4.14 shows the microstructure of MCPM. It is found that MCPM crystals are very large and thin with sizes in the range of 50-330 μm in length. Specific surface area of MCPM, obtained by BET, is 0.29± 0.00 m^2/g, and the mean particle size, calculated from the specific surface area, is 9.32 μm.

4.5 β-Tricalcium Phosphate (β-TCP)

β-TCP was prepared by the heat treatment of DCPD or DCPA with $CaCO_3$ at 1100°C in air. DCPD and DCPA were separately mixed with $CaCO_3$ to obtain Ca/P mole ratios, 1.50 and 1.46. The XRD patterns of the products obtained from the mixtures with Ca/P = 1.5 are shown in Figure 4.15, (a) from the mixture of DCPD+$CaCO_3$, and (b) from the mixture of DCPA+$CaCO_3$. It is found that (a) contains about 80.9 wt.% β-TCP and 19.1 wt.% HA, and (b) contains of β-TCP, HA and $Ca(OH)_2$, about 54.9, 43.2, and 1.9 wt.%, respectively. These wt.% values are calculated from the relative intensities of the main peak of each phase in the obtained product. The presence of $Ca(OH)_2$ may be due to the incomplete solid-state reaction between DCPD or DCPA and $CaCO_3$ which results in the separation of calcium oxide (CaO) and later hydrates to $Ca(OH)_2$.

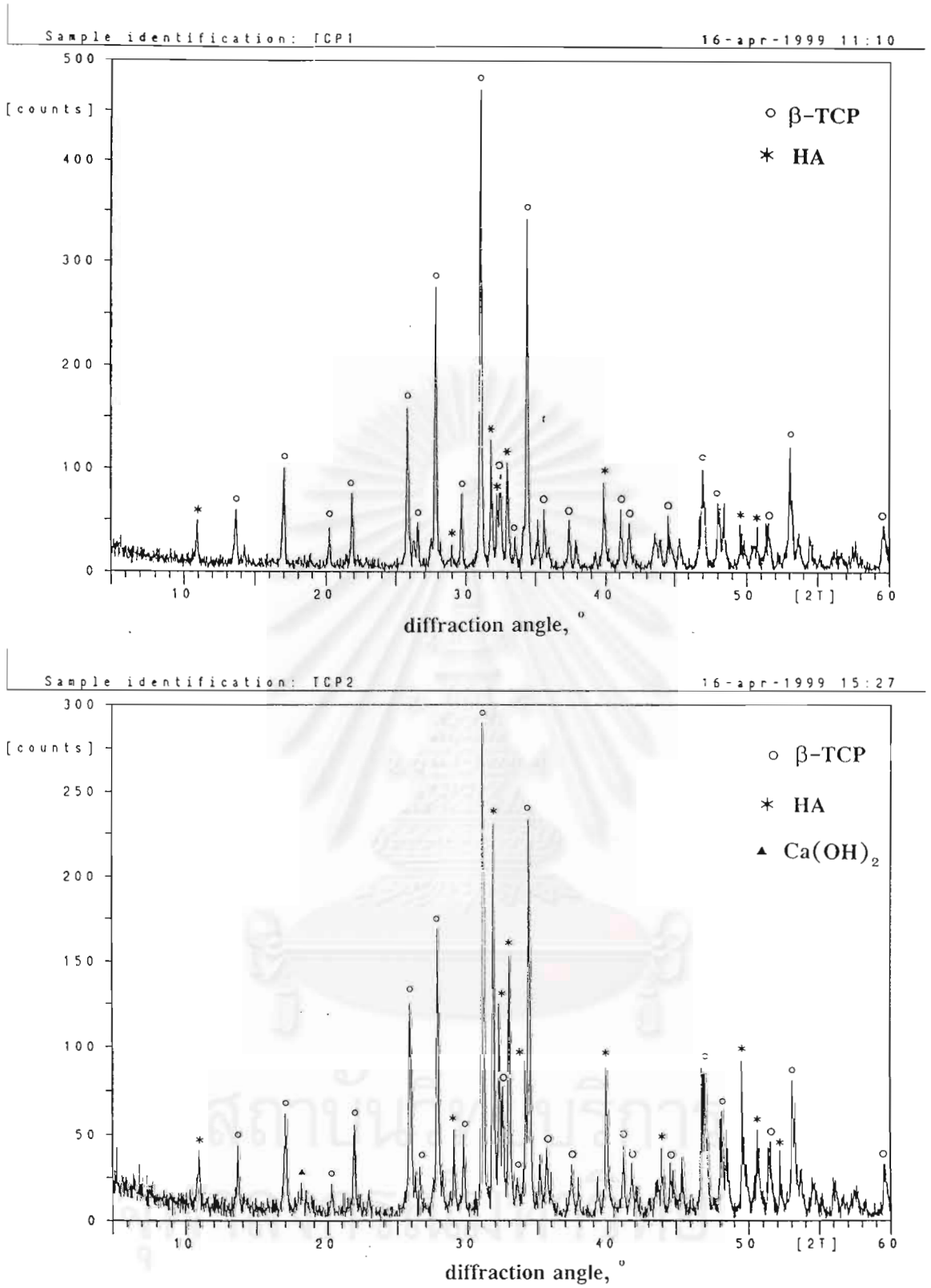


Figure 4.15 XRD patterns of the products obtained from the heat treatment of the mixtures with Ca/P ratio = 1.5 at 1100°C

(a) from the mixture of DCPD+$CaCO_3$

(b) from the mixture of DCPA+$CaCO_3$.

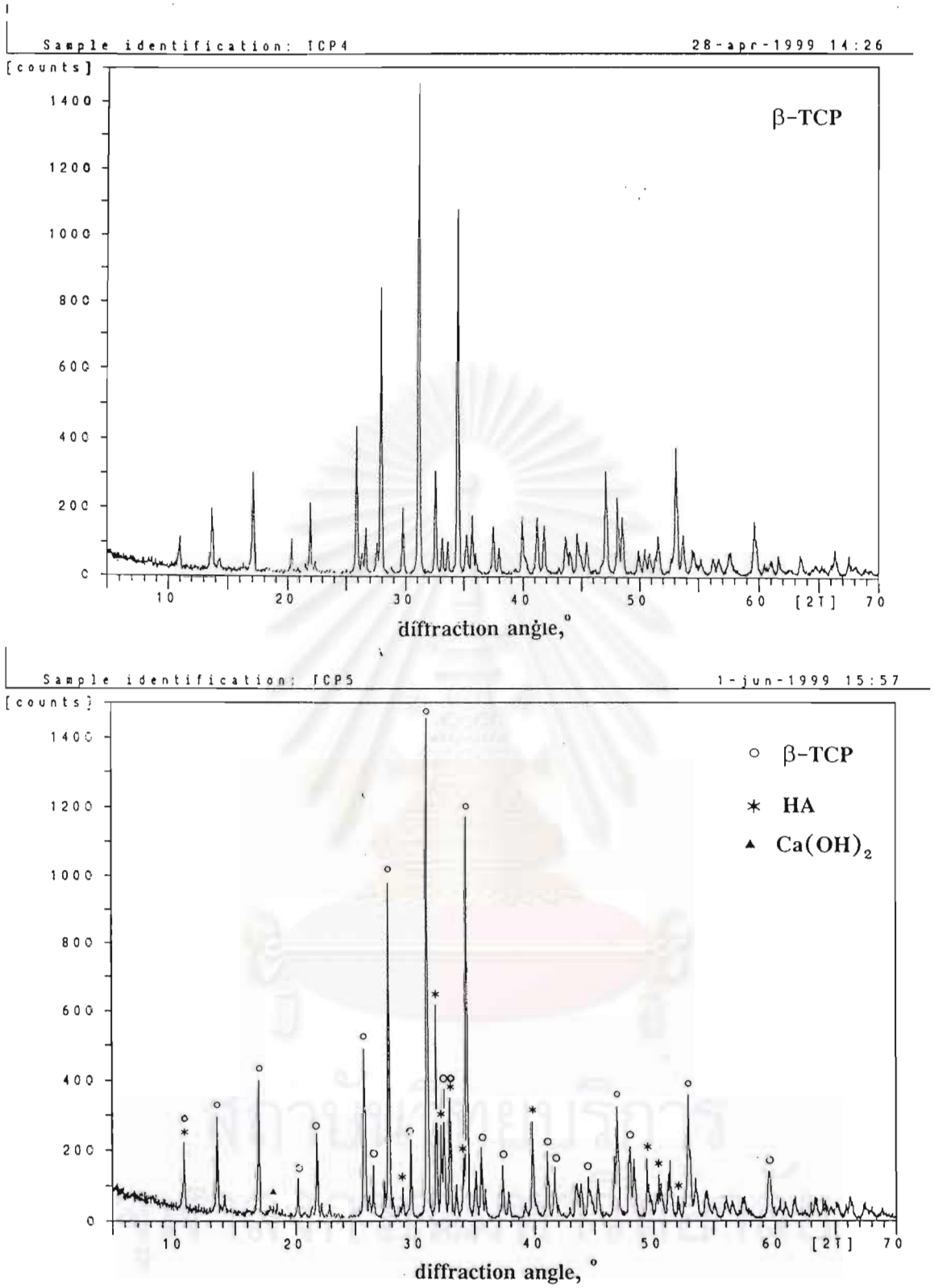


Figure 4.16 XRD patterns of the products obtained from the heat treatment of the mixtures with Ca/P mole ratio = 1.46 at 1100°C

(a) from the mixture of DCPD+$CaCO_3$

(b) from the mixture of DCPA+$CaCO_3$.

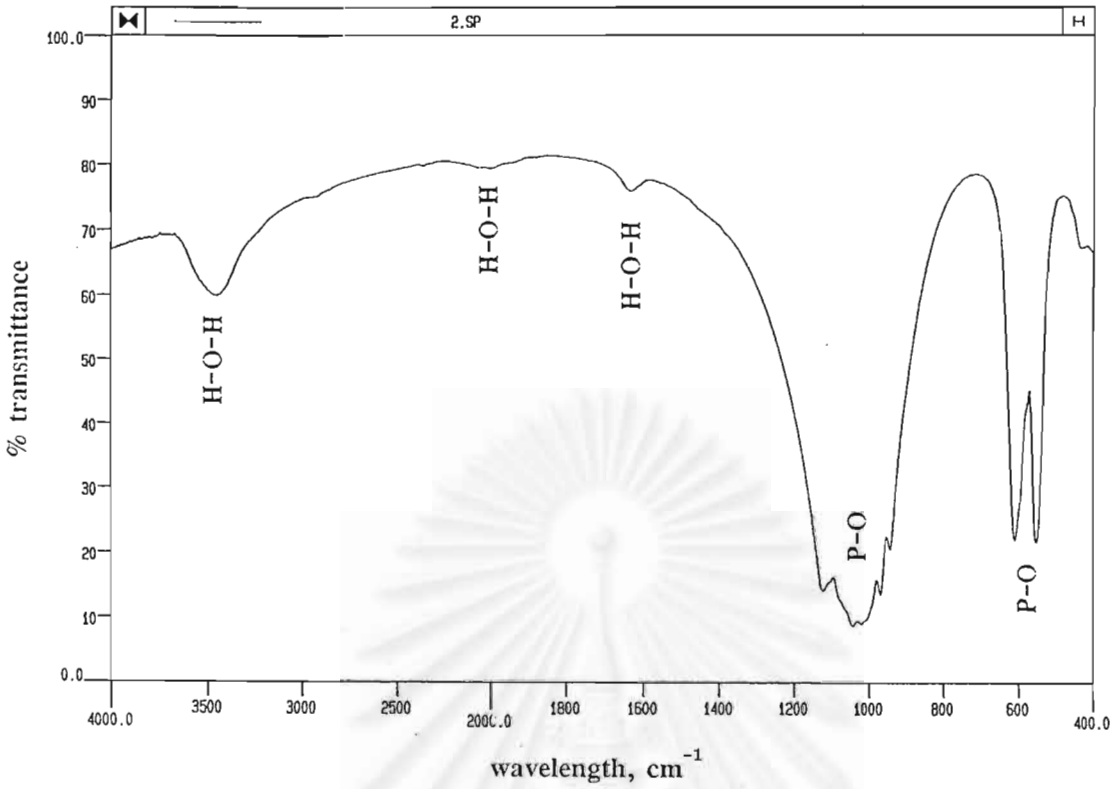


Figure 4.17 IR spectrum of the synthesized β–TCP.

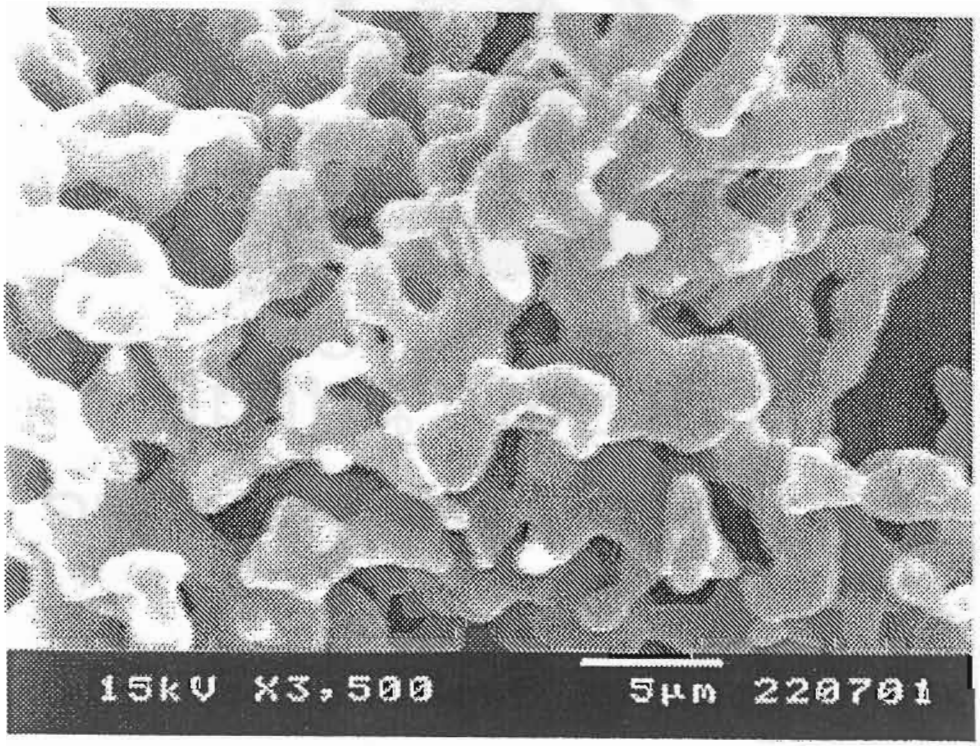


Figure 4.18 SEM micrograph of the synthesized β–TCP.

The presence of HA in the obtained product was probably caused by the excess Ca in the mixture. So, the Ca/P ratios of both mixtures decrease to 1.46. Figure 4.16 shows XRD patterns of the products obtained from the mixtures with Ca/P mole ratio = 1.46, (a) mixture of DCPD+$CaCO_3$, and (b) mixture of DCPA+$CaCO_3$. The product obtained from DCPD+$CaCO_3$ mixture is a pure β-TCP while the product obtained from DCPA+$CaCO_3$ mixture contains β-TCP, HA and $Ca(OH)_2$, about 69.2, 28.6 and 2.2 wt.%, respectively.

The IR spectrum of β-TCP from 400-4000 cm^{-1} is shown in Figure 4.17. The H-O-H and P-O absorption bands are observed. The Ca/P mole ratio of the prepared β-TCP is 1.53 which is slightly more than 1.5 of the stoichiometric TCP.

Table 4.5 Chemical Analysis of β-TCP.

Ca/P mole ratio	Impurities		Heavy Metals (ppm)	
1.53	Mg (%)	< 0.01	Cd	< 0.4
	Fe (%)	< 0.01	Pb	< 5
	Zn (ppm)	94	As	< 5
	Cu (ppm)	< 2	Ni	< 2
	Mn (ppm)	< 5		

Figure 4.18 shows the microstructure of β-TCP obtained from the heat treatment of DCPD+$CaCO_3$ mixture with Ca/P mole ratio = 1.46. It can be seen that its structure is the sintered body of fine particles.

4.6 Hydroxyapatite (HA)

HA samples were also prepared by the heat treatment of DCPD and DCPA with $CaCO_3$ in air. The Ca/P mole ratios of the mixtures were 1.67 and 1.63. The XRD patterns of the products obtained from the heat treatment of the mixtures with Ca/P mole ratio = 1.67 at 1100°C are shown in Figure 4.19, (a) from the mixture of DCPD+$CaCO_3$ (b) from the mixture of DCPA+$CaCO_3$. It is found that they all contain HA, β-TCP and $Ca(OH)_2$, about 62.4, 32.1 and 5.5 wt% in (a), and about 74.1, 15.2 and 10.7 wt.% in (b), respectively.

Figure 4.20 shows the XRD patterns of the products obtained from heat treatment of the mixtures with Ca/P mole ratio = 1.67 at 1200°C, (a) from the mixture of DCPD+$CaCO_3$ (b) from the mixture of DCPA+$CaCO_3$. It is found that they both contain HA, β-TCP and $Ca(OH)_2$, about 92.9, 4.9 and 2.2 wt.% in (a), and 92.6, 3.3 and 4.1 wt.% in (b), respectively.

From the results, it can be seen that the amount of HA increases with the temperature of the heat treatment. In order to compare the effect of Ca/P mole ratio on the phases of products, the Ca/P mole ratio of the mixture was decreased from 1.67 to 1.63. The XRD patterns of the products obtained from the heat treatment of the mixtures with Ca/P mole ratio = 1.63 at 1200°C are shown in Figure 4.21, (a) DCPD+$CaCO_3$ and (b) DCPA+$CaCO_3$. It is found that the product obtained from (a) consists of HA, α-TCP, β-TCP and $Ca(OH)_2$, about 77.3, 16.6, 4.3 and 1.8 wt.%, respectively. In the case of (b), the product obtained consists of HA, β-TCP and $Ca(OH)_2$, about 91.9, 5.1 and 3.0 wt.%, respectively.

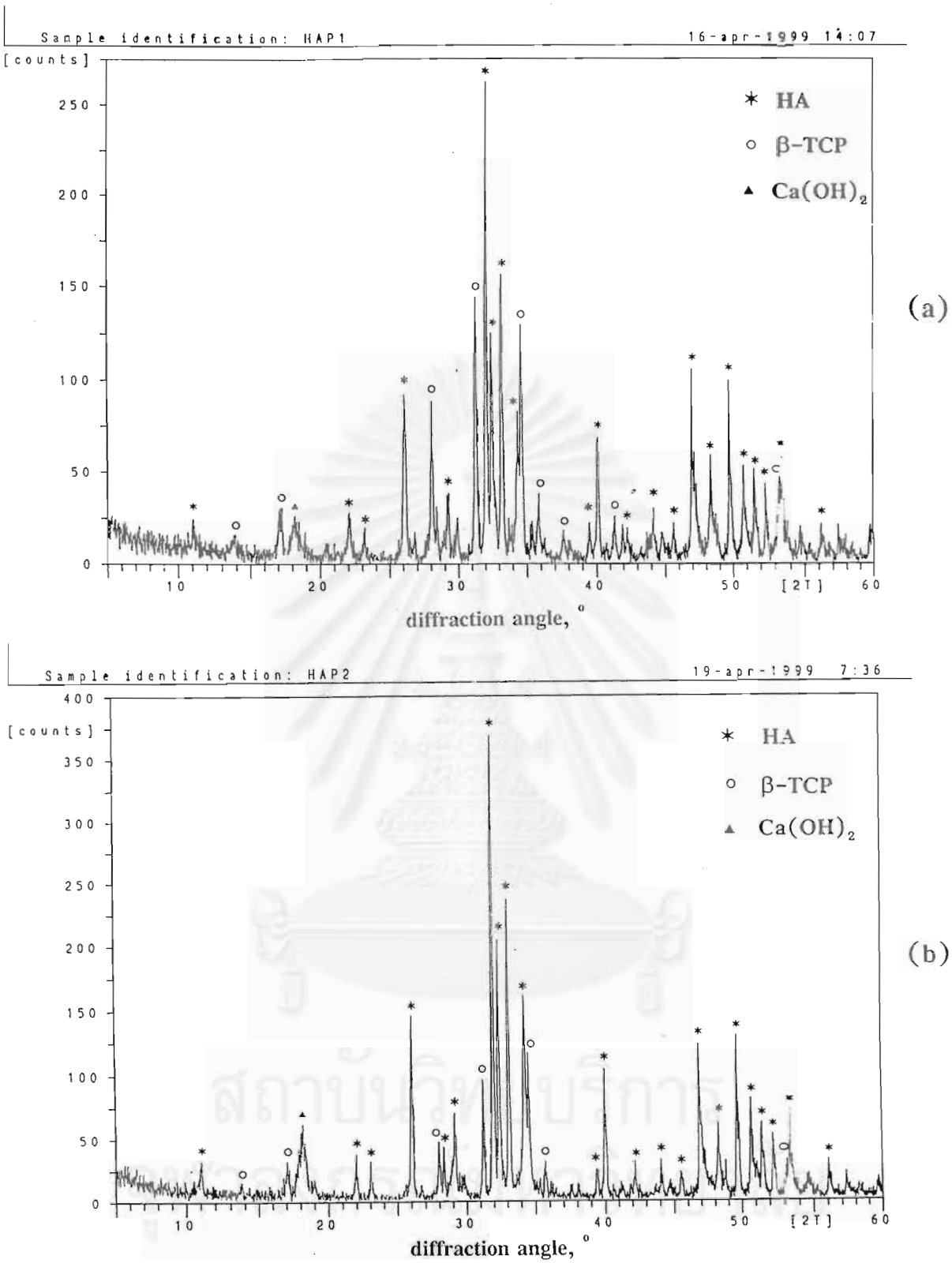


Figure 4.19 XRD patterns of the products obtained from the heat treatment of the mixtures with Ca/P mole ratio = 1.67 at 1100°C.

(a) from the mixture of DCPD+$CaCO_3$

(b) from the mixture of DCPA+$CaCO_3$.

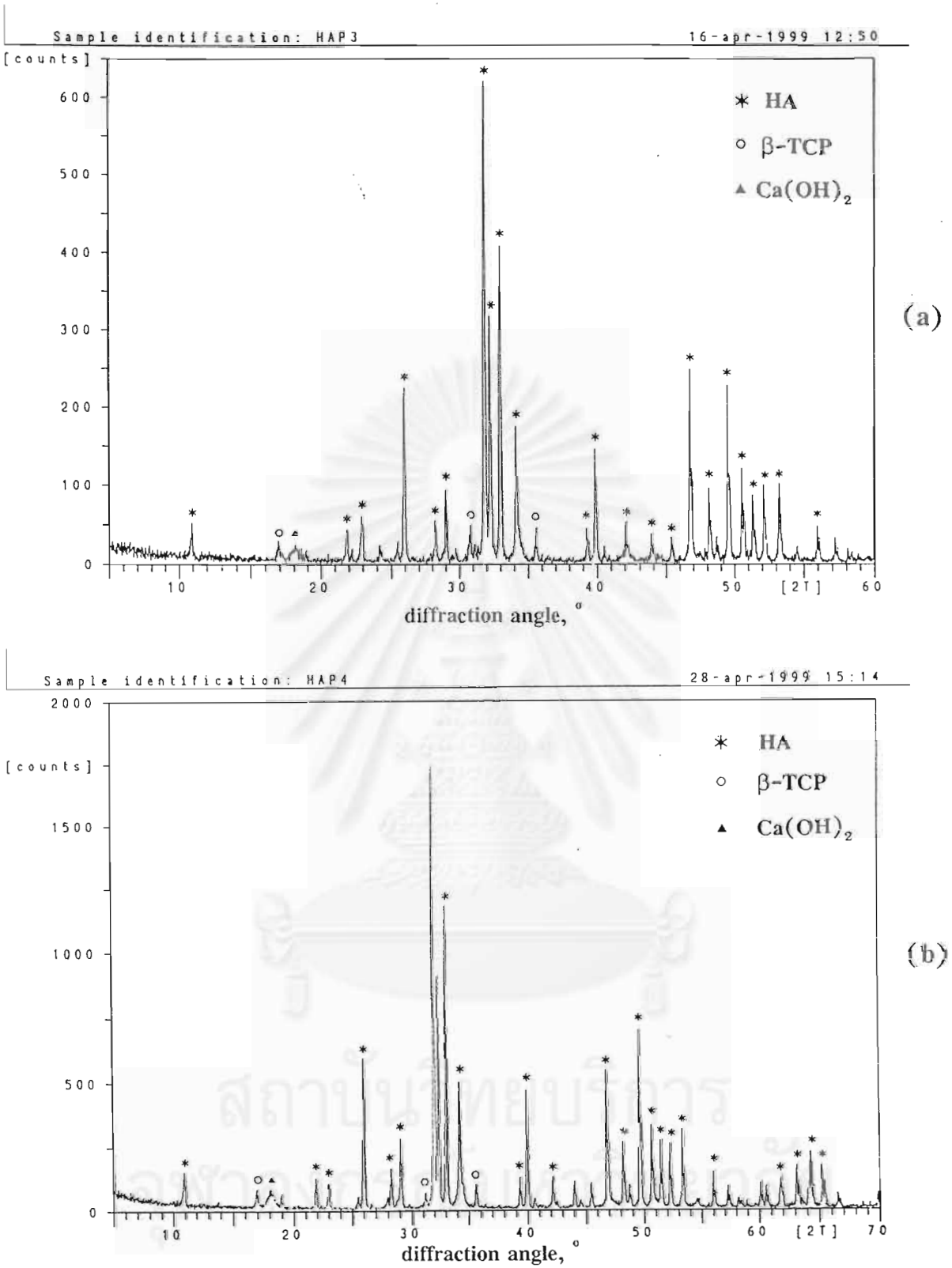


Figure 4.20 XRD patterns of the products obtained from the heat treatment of the mixtures with Ca/P mole ratio = 1.67 at 1200°C.

(a) from the mixture of DCPD+$CaCO_3$

(b) from the mixture of DCPA+$CaCO_3$.

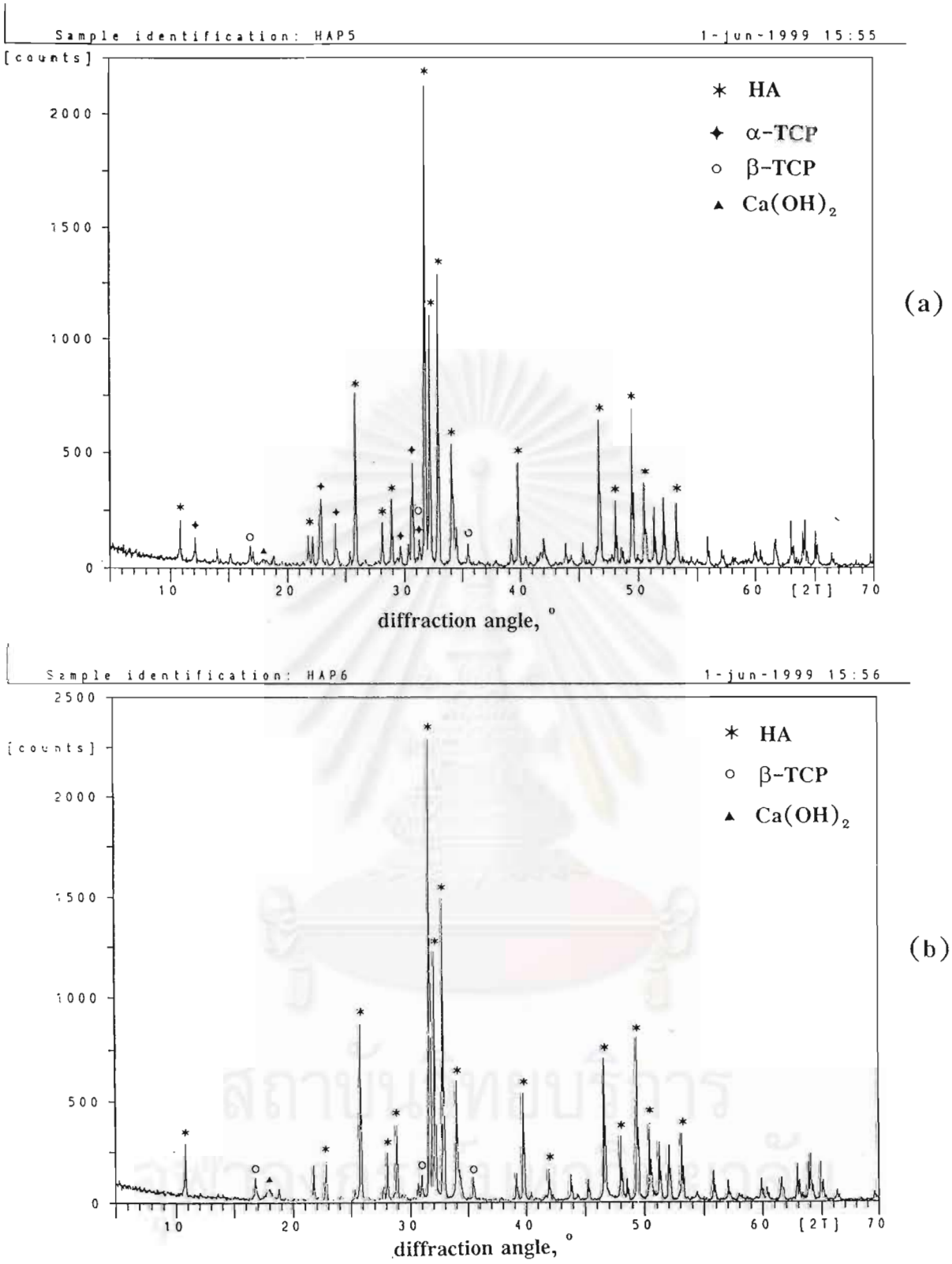


Figure 4.21 XRD patterns of the products obtained from the heat treatment of the mixtures with Ca/P mole ratio = 1.63 at 1200°C.

(a) from the mixture of DCPD+$CaCO_3$

(b) from the mixture of DCPA+$CaCO_3$.

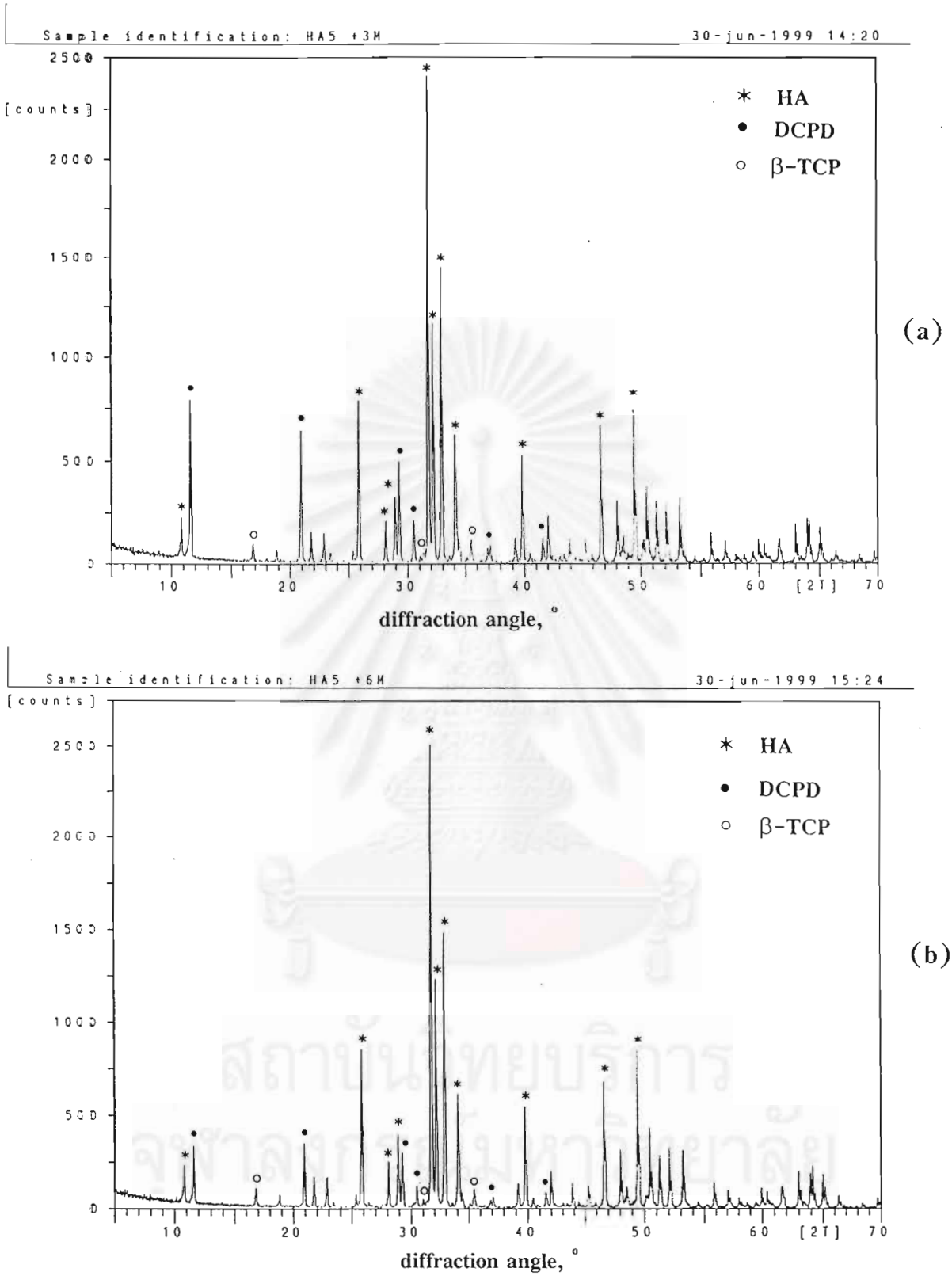


Figure 4.22 XRD patterns of the products obtained from the heat treatment of the DCPD+$CaCO_3$ mixture with Ca/P mole ratio = 1.63 at 1200°C after washing with acetic acid (a) 3 M acetic acid, (b) 6 M acetic acid.

These two products were then washed with acetic acid, 3 M and 6 M, in order to eliminate $Ca(OH)_2$. Figure 4.22 shows XRD patterns of the products obtained from DCPD+$CaCO_3$ after washing with acetic acid (a) with 3 M acetic acid, (b) with 6 M acetic acid. It is found that they both show similar pattern of which $Ca(OH)_2$ is no longer observed. However, it can be seen that there are the new peaks occurred in these both XRD patterns. These peaks agree with the JCPDS card of DCPD. The occurrences of DCPD in both products may be due to the chemical reaction below (Kanazawa, 1989):

$$\alpha\text{-TCP} + 6H_2O \rightarrow 2CaHPO_4.2H_2O + Ca(OH)_2$$

α-TCP in the product reacted with water in acetic acid solution (as shown in Figure 4.23) and then yielded DCPD and $Ca(OH)_2$, which was re-dissolved in the acid. So, these two products after washing with acetic acid contain about 76.2 wt.% HA, 23.1 wt.% DCPD and 0.7 wt.% β-TCP in (a), and about 88 wt.% HA, 10.6 wt.% DCPD and 1.4 wt.% β-TCP in (b), respectively.

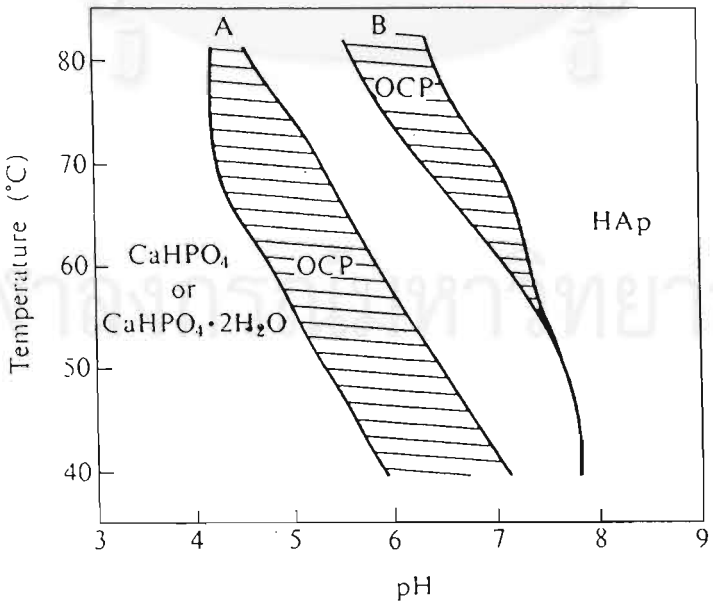


Figure 4.23 Hydrolysis of α-TCP at various pH values (Kanazawa, 1989).

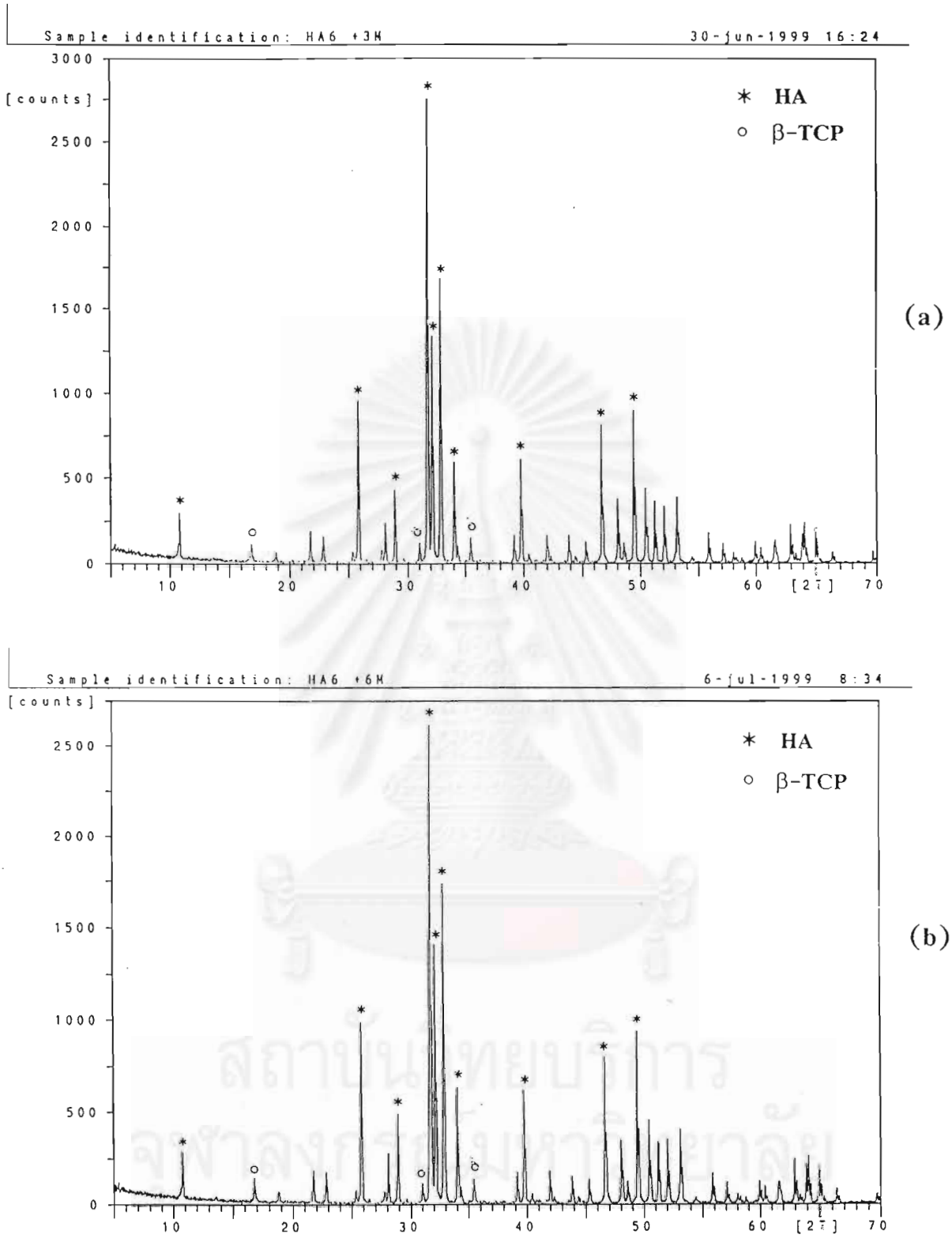


Figure 4.24 XRD patterns of the products obtained from the heat treatment of the DCPA+$CaCO_3$ mixture with Ca/P mole ratio = 1.63 at 1200°C after washing with acetic acid (a) 3 M acetic acid, (b) 6 M acetic acid.

In the case of the products obtained from DCPA+$CaCO_3$ mixture, their phases, after washing with acetic acid, are shown in Figure 4.24, (a) with 3 M acetic acid, (b) with 6 M acetic acid. The two patterns are similar and they both contain HA and β-TCP, about 95.6 wt.% HA and 4.4 wt.% β-TCP in (a), and about 96.2 wt.% HA and 3.8 wt.% β-TCP in (b), respectively.

The phases of all products obtained from the heat treatment of the mixtures of DCPD or DCPA and $CaCO_3$ with different Ca/P mole ratios at 1100°C and 1200°C, including the phases of the products after washing with acetic, are summarized in Table 4.6 and Table 4.7.

Table 4.6 Phases of the products obtained from the heat treatment of the mixtures of DCPD or DCPA and $CaCO_3$ with different Ca/P mole ratios at 1100°C and 1200°C.

Ca/P mole ratios	Mixtures	Temperature (°C)	Phase presence (* indicated main phase)
1.5	DCPD+$CaCO_3$	1100	β-TCP*+HA
	DCPA+$CaCO_3$	1100	β-TCP*+HA+$Ca(OH)_2$
1.46	DCPD+$CaCO_3$	1100	β-TCP
	DCPA+$CaCO_3$	1100	β-TCP*+HA+$Ca(OH)_2$
1.67	DCPD+$CaCO_3$	1100	HA*+β-TCP +$Ca(OH)_2$
	DCPA+$CaCO_3$	1100	HA*+β-TCP +$Ca(OH)_2$
	DCPD+$CaCO_3$	1200	HA*+β-TCP +$Ca(OH)_2$
	DCPA+$CaCO_3$	1200	HA*+β-TCP +$Ca(OH)_2$
1.63	DCPD+$CaCO_3$	1200	HA*+α-TCP+β-TCP +$Ca(OH)_2$
	DCPA+$CaCO_3$	1200	HA*+β-TCP +$Ca(OH)_2$

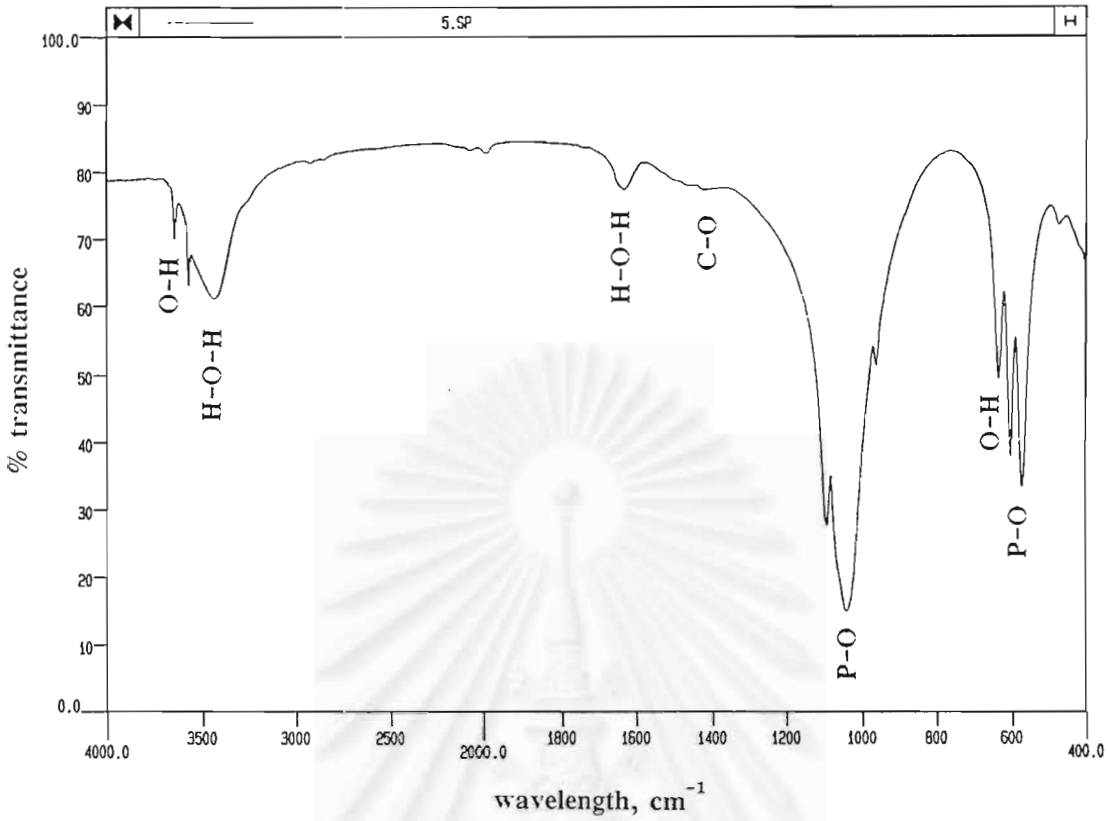
Table 4.7 Phases of the products obtained from the heat treatment of the DCPD or DCPA and $CaCO_3$ mixtures with Ca/P mole ratio = 1.63 at 1200°C before and after washing with acetic acid.

Mixtures	Phases presence before washing	Phases presence after washing with acetic acid
DCPD+$CaCO_3$	HA*+α-TCP+β-TCP+$Ca(OH)_2$	HA*+DCPD+β-TCP
DCPA+$CaCO_3$	HA*+β-TCP+$Ca(OH)_2$	HA*+β-TCP

*indicated main phase

From these results, it is found that the products which yield high HA content can be obtained from the heat treatment of the mixture with Ca/P mole ratio = 1.67 of both DCPD+$CaCO_3$ and DCPA+$CaCO_3$ mixtures at 1200°C and the product obtained from the heat treatment of DCPA+$CaCO_3$ mixture with Ca/P mole ratio = 1.63 at 1200°C after washing with acetic acid. Therefore, they are selected to be further characterized by IR, ICP and SEM.

Their IR spectra are shown in Figure 4.25, (a) IR spectrum of the product obtained from the heat treatment of DCPA+$CaCO_3$ mixture with Ca/P mole ratio = 1.67 at 1200°C (A), (b) IR spectrum of the product obtained from the heat treatment of DCPA+$CaCO_3$ mixture with Ca/P mole ratio = 1.63 at 1200°C (B) before, and (c) after washing with acetic acid. The O-H, H-O-H, and P-O absorption bands are observed in all spectra, except for C-O absorption band which can only be observed in (a) and (b). This may be due to the present of $Ca(OH)_2$ in those products, but in (c), the spectrum of the washed product, C-O can not be observed.



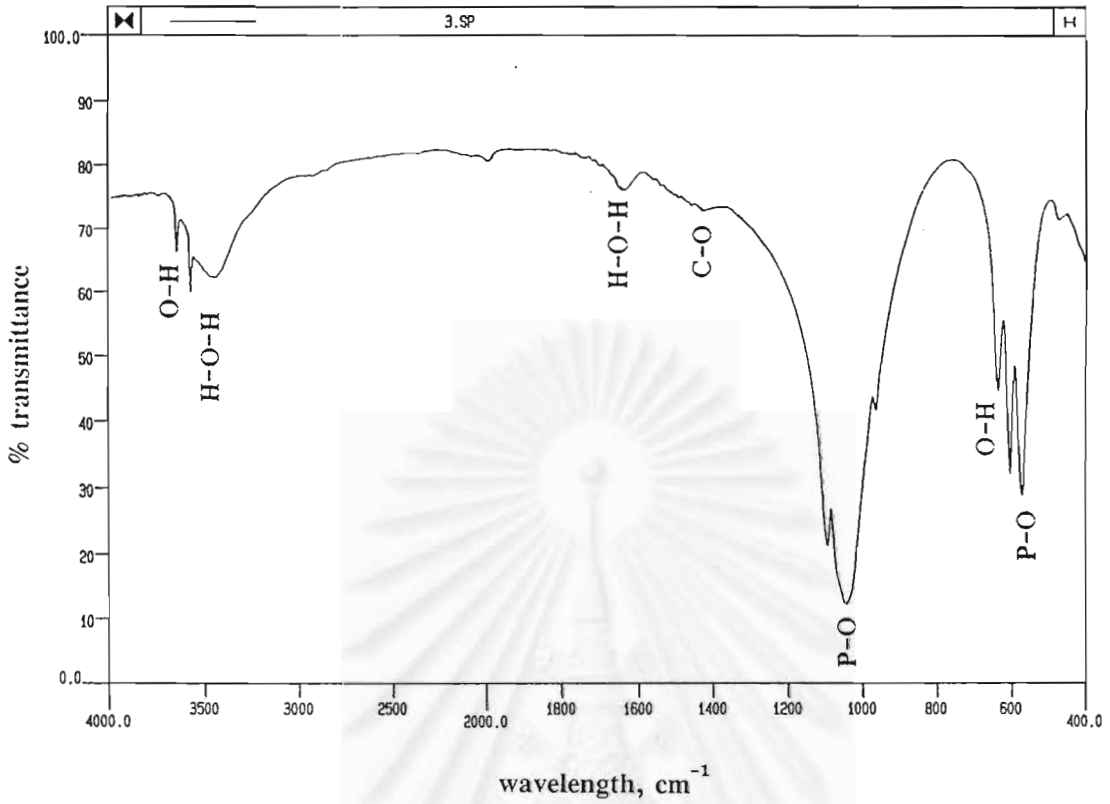
(a)

Figure 4.25 IR spectra of HA obtained from the heat treatment of the DCPA+$CaCO_3$ mixtures at 1200°C

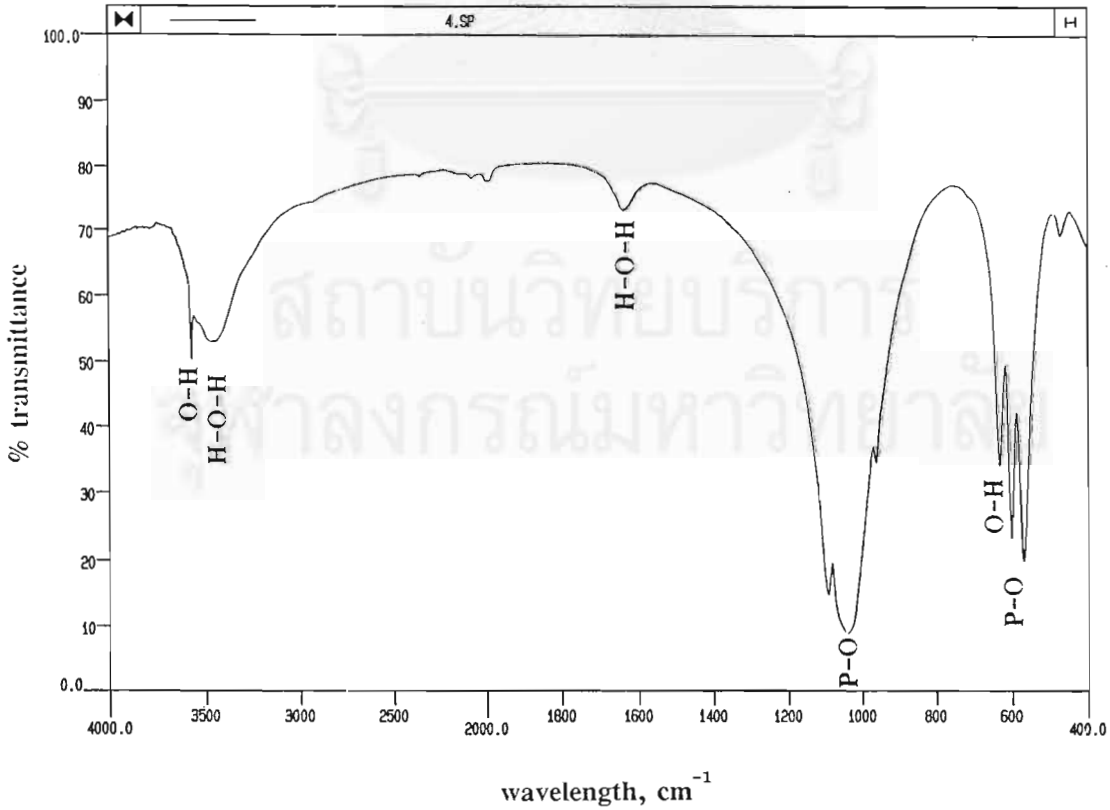
(a) the mixture with Ca/P mole ratio = 1.67

(b) the mixture with Ca/P mole ratio = 1.63 before and

(c) after washing with acetic acid.


3.SP
% transmittance
O-H
H-O-H
H-O-H
C-O
P-O
O-H
P-O
wavelength, cm-1

(b)


4.SP
% transmittance
O-H
H-O-H
H-O-H
P-O
O-H
P-O
wavelength, cm-1

(c)

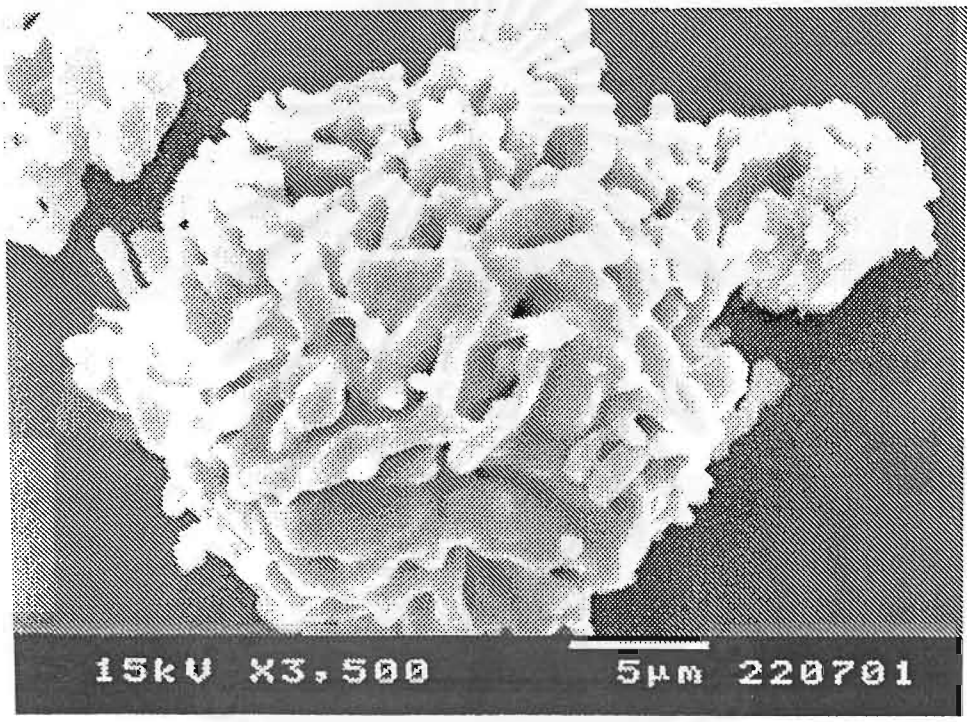
Table 4.8 shows Ca/P mole ratio and amount of impurities of the product obtained from the heat treatment of DCPA+$CaCO_3$ mixture with Ca/P mole ratio = 1.63 at 1200°C before and after washing with acetic acid. It can be seen that before washing, Ca/P mole ratio of B is about 1.80, but after washing, its Ca/P mole ratio decreases to 1.68, which is slightly more than 1.67 of the stoichiometric HA. It means that the amount of Ca in this product decreases. This result agrees with the XRD patterns (Figure 4.24) which show that $Ca(OH)_2$ is eliminated.

Table 4.8 Chemical analysis of the product obtained from the heat treatment of DCPA+$CaCO_3$ mixture with Ca/P mole ratio = 1.63 at 1200°C before (B1) and after (B2) washing with acetic acid.

Materials	Ca/P mole ratio	Impurities	Heavy Metals (ppm)
B1	1.80	Mg (%) < 0.01 Fe (%) 0.02 Zn (ppm) 82 Cu (ppm) < 2 Mn (ppm) < 5	Cd < 0.4 Pb < 5 As < 5 Ni < 2
B2	1.68	Mg (%) < 0.01 Fe (%) 0.02 Zn (ppm) 90 Cu (ppm) < 2 Mn (ppm) < 5	Cd < 0.4 Pb < 5 As < 5 Ni 7

Figure 4.26 shows the microstructure of HA obtained from the heat treatment of DCPA+$CaCO_3$ mixture, (a) microstructure of the product obtained from the heat treatment of DCPA+$CaCO_3$ mixture with Ca/P mole ratio = 1.67 at 1200°C (A), (b) microstructure of the product obtained

from the heat treatment of DCPA+$CaCO_3$ mixture with Ca/P mole ratio = 1.63 at 1200°C (B) before and (c) after washing with acetic acid. It can be seen that they all have similar structure which is the porous agglomerate of sintered fine particles.



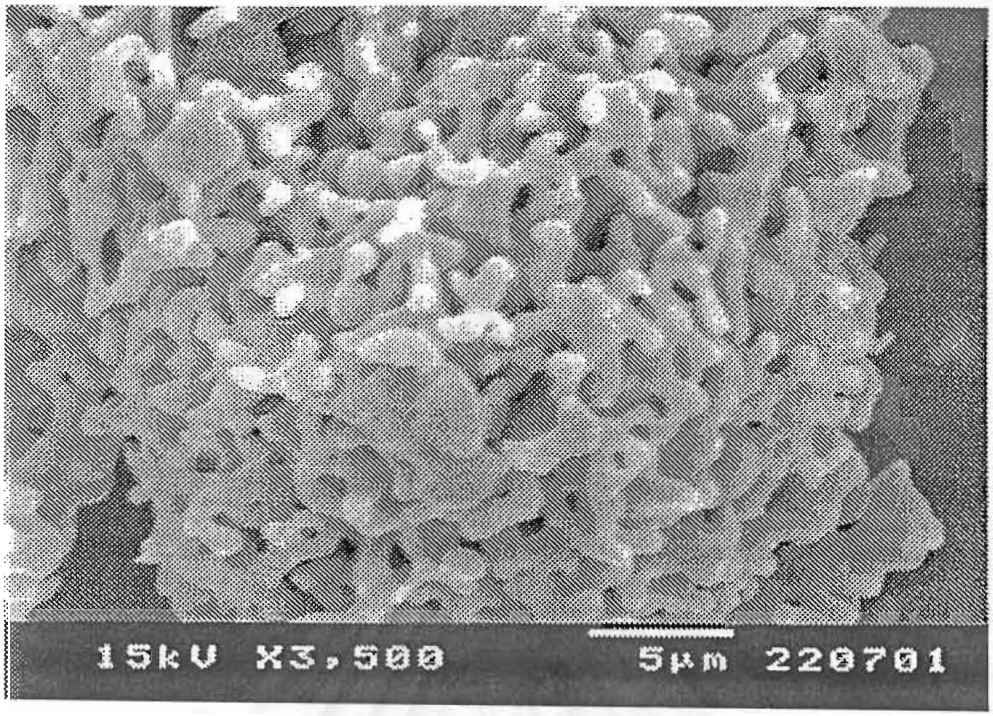
(a)

Figure 4.26 SEM micrographs of HA obtained from the heat treatment of the DCPA+$CaCO_3$ mixture at 1200°C

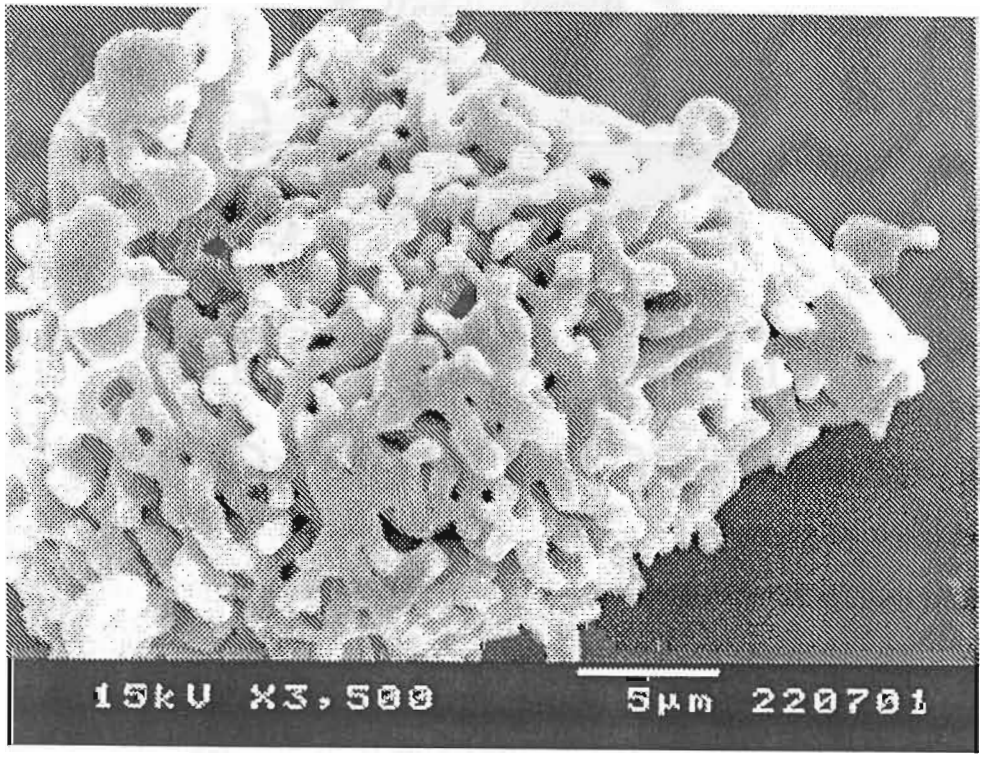
(a) the mixture with Ca/P mole ratio = 1.67

(b) the mixture with Ca/P mole ratio = 1.63 before and

(c) after washing with acetic acid.



(b)



(c)

CHAPTER 5

CONCLUSIONS AND FUTURE SUGGESTION

From the starting material, a sieved by-product of bone gelatin production, five different kinds of calcium phosphate compounds were successfully prepared. The suitable conditions for preparing each type of them were summarized as the following.

1. Dicalcium phosphate dihydrate (DCPD) was prepared from the precipitation of the starting material solutions at various pH values, 4.5-6.0. Ammonium hydroxide was used to adjust pH of the solutions. It was found that precipitation at pH 5.5 yielded the largest %average amount of the synthesized DCPD. Therefore, this condition was used to prepare DCPD which was further used as the starting material for preparing monocalcium phosphate monohydrate (MCPM), β-tricalcium phosphate (β-TCP) and hydroxyapatite (HA).

2. Dicalcium phosphate anhydrous (DCPA) was prepared from the same method as DCPD, except that the precipitation was performed at 80°C. The temperatures of the solution were controlled by using a hot plate. This obtained DCPA was used as the starting material to prepare β-TCP and HA.

3. Monocalcium phosphate monohydrate (MCPM) was obtained from the evaporation of the synthesized DCPD solution. It was found that the optimum condition for preparing MCPM is to evaporate the solution of DCPD in 50 ml of 5 M phosphoric acid. At this condition, the largest %average amount of the product obtained.

4. The preparation of β-tricalcium phosphate (β-TCP) was based on the solid-state reaction of the mixtures of the synthesized DCPD or DCPA and calcium carbonate with various Ca/P mole ratios. From the results, it showed that pure β-TCP was obtained from the heat treatment of the DCPD+$CaCO_3$ mixture with Ca/P mole ratio = 1.46 at 1100°C.

5. Like β-TCP, hydroxyapatite (HA) was prepared from the heat treatment of the mixtures of the synthesized DCPD or DCPA and $CaCO_3$ but with the different Ca/P mole ratios. It was found that high purity HA products were obtained from the heat treatment at 1200°C of DCPA+$CaCO_3$ mixture with Ca/P mole ratio = 1.67 or 1.63. These obtained products were the biphase compounds consisted of HA and β-TCP which had a trace calcium hydroxide as a contamination. The occurrence of $Ca(OH)_2$ may be due to the incomplete solid-state reaction. The purity of HA products was improved by washing with acetic acid to eliminate $Ca(OH)_2$.

Future Suggestion

This work, as mentioned above, provides the suitable methods for synthesizing five types of calcium phosphate compounds. However, DCPD can be easily transformed to DCPA in the presence of a little moisture. It is related to the decomposition of DCPD in water to HA and free phosphoric acid. Then HA rapidly react with free phosphoric acid to form DCPA. Therefore, in order to stabilize DCPD, a small quantity of pyrophosphate ion; such as tetrasodium pyrophosphate; is added to the solution while precipitation. Another way to stabilize is to add 2-3% of trimagnesium phosphate to DCPD as a dry mix. However, the mechanism of stabilization by both pyrophosphate ion and trimagnesium phosphate are not well understood (Toy, 1973) and needed further studied.

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APPENDICES

Appendix A

X-ray diffraction card of dicalcium phosphate dihydrate (DCPD).

09-0077 Wavelength= 1.54056

CaPO3(OH)*2H2O

Calcium Phosphate Hydroxide Hydrate

Brushite, syn

Rad.: CuKa1 λ: 1.5405 Filter: d-sp: Debye-Scherrer

Cut off: Int.: I/Icor.:

Ref: de Wolff, P., Technisch Physische Dienst, Delft, The Netherlands, ICDD Grant-in-Aid

Sys.: Monoclinic S.G.: Cc (9)

a: 6.363 b: 15.19 c: 5.815 A: 0.4189 C: 0.3828

α: β: 118.5 γ: Z: 4 mp:

Ref: Ibid.

Dx: 2.314 Dm: 2.306 SS/FOM$_{30}$=68(.0119, 37)

εα: 1.539 ηωβ: 1.545 εγ: 1.551 Sign:- 2V: 87°

Ref: Bale, Bonner, Hodge, Ind. Eng. Chem., Anal. Ed., 17, 491 (1945)

Color: Colorless, light yellow

Dana's System of Mineralogy, 7th Ed., II 704, Beevers, Acta Crystallogr., 11 273-277 (1958) reports: a=5.812, b=15.80, c=6.239, β=116.25, S.G.='I2/a'; a=6.359, b=15.180, c=5.182, β=118.31, S.G.='C2/c' in the setting used here. Gypsum group, pharmacolite subgroup. C.D. Cell: a=6.244, b=15.190, c=5.815, β=116.42, a/b=0.4111, c/b=0.3828, S.G.=Ia(9), PSC: mC52. Plus additional reflections. Mwt: 172.09, Volume[CD]: 493.93.

2θ	Int	h	k	l	2θ	Int	h	k	l
11.680*	100	0	2	0	49.070*	<1	1	3	2
17.978*	2	1	1	1	50.107*	20	2	4	1
20.934	100	0	2	1	50.703*	10	0	6	2
23.390*	8	0	4	0	51.283*	4	0	8	1
23.707*	<1	1	3	0	52.292*	2	3	3	0
24.502*	2	1	3	1					
29.257*	75	0	4	1					
30.505*	50	2	2	1					
31.305*	10	1	1	2					
31.971*	2	2	0	0					
33.536*	4	1	5	0					
33.823*	4	1	3	1					
34.155*	50	2	2	0					
34.425*	30	2	0	2					
35.107*	4	0	0	2					
35.422*	2	0	6	0					
35.597*	4	1	3	2					
36.899*	14	2	4	1					
37.104*	16	0	2	2					
39.709*	4	0	6	1					
40.003*	2	2	4	0					
41.543*	20	1	5	1					
42.029*	16	2	4	2					
42.611*	2	0	4	2					
43.037*	6	1	5	2					
43.384*	10	3	1	1					
44.785*	4	1	7	0					
45.281*	10	1	7	1					
45.886*	6	1	1	2					
46.711*	2	3	3	1					
47.860*	2	0	8	0					
48.157*	4	1	1	3					
48.430*	14	2	6	0					
48.985*	8	2	2	3					

Appendix B

X-ray diffraction card of dicalcium phosphate anhydrous (DCPA).

09-0080 — Wavelength= 1.54056

CaPO3(OH)

Calcium Hydroxide Phosphate

Monetite, syn

Rad.: CuKa1 λ: 1.5405 Filter: d-sp: Guinier 114.6

Cut off: 50.0 Int.: I/Icor.:

Ref: de Wolff, P., Technisch Physische Dienst, Delft, The Netherlands, ICDD Grant-in-Aid, (1957)

Sys.: Triclinic S.G.: $P\bar{1}$ (2)

a: 6.906 b: 8.577 c: 6.634 A: 0.8052 C: 0.7735

α: 93.99 β: 91.50 γ: 127.6 Z: 4 mp:

Ref: MacLennan, Beevers, Acta Crystallogr., 8, 579 (1955)

Dx: 2.921 Dm: 2.900 SS/FOM$_{3C}$=26(.026, 45)

εα: 1.60 nωΞ: 1.61 εγ: 1.63 Sign:= 2V: 60(15)

Ref: Bale, Bonner, Hodge, Ind. Eng. Chem., Anal. Ed., 17, 491 (1945)

Color: Light yellowish white

Dana's System of Mineralogy, 7th Ed., II 660. Dehydrated Ca H P O4 n2 H2 O. Wellite is the As analogue. C.D. Cell: a=6.906, b=6.998, c=6.634, α=96.38, β=91.50, γ=76.17, a/b=0.9868, c/b=0.9479, S.G.=P-1(2), PSC: aP28. To replace 1-653. Mwt: 136.06. Volume[CD]: 309.40.

2θ	Int	h	k	l	2θ	Int	h	k	l
13.125*	14	0	1	0	38.251*	4	2	$\bar{2}$	2
16.311*	4	1	$\bar{0}$	0	39.045*	10	1	$\bar{2}$	0
17.760*	4	0	$\bar{1}$	1	39.365*	2	2	$\bar{1}$	2
19.801*	2	$\bar{0}$	1	1	40.021*	16	0	3	0
20.258*	4	$\bar{1}$	0	1	40.189*	6	0	2	2
20.785*	4	1	2	0	40.339*	10	1	$\bar{1}$	2
22.038*	4	1	$\bar{0}$	1	40.681*	4	1	3	2
24.032*	4	$\bar{1}$	2	1	41.010*	12	$\bar{0}$	0	3
25.576*	14	1	2	1	41.206*	4	3	$\bar{2}$	1
26.426*	70	$\bar{0}$	2	0	41.784*	12	$\bar{0}$	1	3
26.586*	75	$\bar{2}$	2	0	42.214*	6	$\bar{2}$	$\bar{4}$	0
26.749*	16	2	1	0	42.674*	8	3	3	1
26.997*	10	0	$\bar{0}$	2	42.930*	4	1	$\bar{2}$	1
28.493*	20	1	$\bar{1}$	1	43.275*	6	$\bar{1}$	1	3
28.775*	6	$\bar{0}$	1	2	43.692*	4	$\bar{2}$	3	2
29.899*	2	$\bar{2}$	2	1	44.553*	6	1	$\bar{3}$	2
30.188	100	$\bar{1}$	1	2	44.996*	2	$\bar{1}$	$\bar{2}$	3
30.409*	35	1	$\bar{0}$	2	45.401*	6	$\bar{1}$	2	2
30.677*	4	2	$\bar{1}$	1	45.642*	6	3	4	0
31.015*	8	0	2	1	46.308*	2	$\bar{2}$	$\bar{1}$	1
31.170*	4	1	1	1	46.890*	2	$\bar{1}$	1	3
31.440*	2	$\bar{0}$	1	2	47.436*	16	$\bar{2}$	2	2
32.375*	10	$\bar{1}$	3	0	48.238*	2	$\bar{3}$	$\bar{4}$	1
32.484*	20	$\bar{2}$	3	0	48.678*	6	$\bar{2}$	$\bar{1}$	2
32.889*	35	1	$\bar{0}$	2	49.211*	20	$\bar{4}$	$\bar{2}$	2
34.728*	4	$\bar{2}$	3	1	49.640*	4	$\bar{3}$	1	2
35.422*	4	1	$\bar{2}$	2	50.703*	6	$\bar{1}$	2	2
35.906*	16	$\bar{0}$	$\bar{2}$	2	50.915*	4	$\bar{3}$	0	1
36.055*	2	1	1	2	51.532*	2	0	3	2
36.758*	2	$\bar{2}$	0	1	52.068*	6	1	1	3
37.264*	2	2	1	2	52.616*	4	1	3	0
53.044*	20	3	$\bar{1}$	2					
53.784*	2	$\bar{2}$	1	2					
54.196*	6	$\bar{2}$	5	0					
54.440*	6	2	$\bar{3}$	3					
54.616*	6	$\bar{0}$	$\bar{4}$	1					
55.042*	4	$\bar{4}$	2	0					
55.585*	6	3	$\bar{0}$	2					
55.916*	6	$\bar{0}$	1	4					
56.781*	2	$\bar{1}$	0	4					
57.205*	6	2	5	1					

Appendix C

X-ray diffraction card of monocalcium phosphate monohydrate (MCPM).

09-0347 Wavelength= 1.5405 i

Ca(H2PO4)2·H2O

Calcium Hydrogen Phosphate Hydrate

Rad.: CuKα1 λ: 1.5405 Filter: d-sp: Guinier I 14.6

Cut off: 50.0 Int.: Film I/Icor.:

Ref: de Wolff, P., Technisch Physische Dienst, Delft, The Netherlands. ICDD Grant-in-Aid

Sys.: Triclinic S.G.:

a: 6.250 b: 11.892 c: 5.629 A: 0.5256 C: 0.4733

α: 96.67 β: 114.2 γ: 92.95 Z: 2 mp:

Ref: Ibid.

Dx: 2.222 Dm: 2.220 SS/FOM$_{30}$=44(.0132, 45)

εα: 1.501 ηω: 1.518 εγ: 1.528 Sign: 2V:

Ref: Bale, Bonner, Hodge, Ind. Eng. Chem., Anal. Ed., 17, 491 (1945)

Commercial sample, recrystallized. To replace 1-471. Mwt: 252.07. Volume[CD]: 376.75.

2θ	Int	h	k	l	2θ	Int	h	k	l
7.549*	75	0	1	0	33.769*	4	$\bar{1}$	1	2
15.132*	10	0	2	0	33.927*	2	$\bar{1}$	$\bar{2}$	2
15.643*	16	$\bar{1}$	0	0	34.671*	12	1	$\bar{4}$	1
16.587*	2	1	$\bar{1}$	0	35.021*	20	0	1	2
17.940*	10	0	1	1	35.349*	2	$\bar{0}$	0	2
18.088*	20	$\bar{1}$	1	0	36.295*	8	2	0	2
19.069*	4	1	1	1	36.617*	16	$\bar{2}$	2	0
20.072*	16	$\bar{0}$	1	1	37.087*	8	1	2	2
20.541*	10	1	$\bar{2}$	0	37.342*	10	$\bar{0}$	$\bar{1}$	2
21.340*	14	$\bar{0}$	$\bar{2}$	1	37.569*	10	2	3	1
22.901*	100	$\bar{1}$	2	1	38.317*	4	$\bar{0}$	$\bar{5}$	0
24.097*	90	1	2	1	38.729*	4	2	2	2
24.849*	14	$\bar{0}$	2	1	39.203*	4	1	$\bar{3}$	1
26.187*	6	$\bar{1}$	$\bar{3}$	0	39.744*	2	$\bar{0}$	5	1
26.385*	16	0	$\bar{3}$	1	40.225*	4	2	2	2
27.945*	16	1	1	1	40.776*	4	$\bar{0}$	2	2
28.035*	14	$\bar{1}$	$\bar{0}$	1	41.823*	8	$\bar{2}$	$\bar{4}$	1
28.307*	10	$\bar{1}$	3	1	42.048*	8	1	4	2
28.965*	8	$\bar{2}$	0	1	42.525*	6	2	$\bar{0}$	1
29.795*	25	1	3	1	43.100*	4	2	$\bar{2}$	1
30.250*	30	1	1	1	43.448*	10	0	$\bar{4}$	2
30.429*	10	0	4	0	44.230*	4	1	$\bar{5}$	1
31.553*	12	$\bar{2}$	$\bar{0}$	0	44.807*	10	$\bar{1}$	0	2
32.076*	10	1	$\bar{1}$	2	45.399*	20	$\bar{3}$	2	1
32.801*	10	0	4	1	46.331*	8	$\bar{3}$	$\bar{0}$	2
33.303*	14	2	$\bar{1}$	0	46.735*	12	$\bar{2}$	$\bar{4}$	2
33.444*	6	$\bar{1}$	$\bar{3}$	1	46.940*	10	$\bar{3}$	$\bar{1}$	2
33.547*	25	2	2	0	47.173*	8	1	3	2

2θ	Int	h	k	l
48.346*	2	$\bar{3}$	$\bar{3}$	1
48.593*	4	0	$\bar{5}$	2
48.843*	2	1	$\bar{1}$	3
49.096*	2	$\bar{2}$	$\bar{4}$	1
49.351*	4	$\bar{2}$	$\bar{5}$	1
49.754*	6	$\bar{1}$	$\bar{2}$	3
50.913*	12	$\bar{3}$	$\bar{3}$	1
51.281*	4	$\bar{3}$	3	0
51.844*	8	$\bar{1}$	$\bar{5}$	1
52.387*	8	$\bar{3}$	$\bar{4}$	1
53.141*	4	$\bar{2}$	$\bar{3}$	3
53.781*	10	2	$\bar{5}$	1

Appendix D

X-ray diffraction card of β-tricalcium phosphate (β-TCP).

09-0169 Wavelength= 1.54056

Ca3(PO4)2

Calcium Phosphate

Whitlockite, syn

Rad.: CuKα1 λ: 1.5405 Filter: Mono d-sp: Guinier I 14.6

Cut off: 50.0 Int.: Film I/Icor.:

Ref: de Wolff, P., Technisch Physische Dienst, Delft, The Netherlands, ICDD Grant-in-Aid, (1957)

Sys.: Rhombohedral S.G.: R3̄c (167)

a: 10.429 b: c: 37.38 A: C: 3.5842

α: β: γ: Z: 21 mp:

Ref: Ibid.

Dx: 3.072 Dm: 3.120 SS/FOM$_{30}$=54(.0147, 38)

εα: 1.626 ηωβ: 1.629 εγ: Sign= 2V:

Ref: Dana's System of Mineralogy, 7th Ed., II, 684 (1951)

Color: Colorless, white, gray, yellow
Sample obtained by heating a commercial sample. Nearly isostructural with cerite. PSC: hR91. Validated by calculated pattern 42-577. Mwt: 310.18. Volume[CD]: 3520.91.

2θ	Int	h	k	l	2θ	Int	h	k	l
10.847*	12	0	1	2	40.208*	2	0	4	2
13.633*	16	1	0	4	41.088*	14	4	0	4
14.227*	6	0	0	6	41.683*	12	3	0	12
17.004*	20	1	1	0	42.972*	4	1	2	14
18.469*	2	1	1	3	43.560*	8	0	0	18
20.211*	8	2	0	2	43.737*	4	3	2	1
21.393*	4	0	1	8	43.893*	6	2	3	2
21.873*	16	0	2	4	44.530*	10	0	4	8
22.206*	4	1	1	6	44.762*	6	3	2	4
25.802*	25	1	0	10	44.902*	4	3	1	11
26.188*	4	2	1	1	45.305*	8	2	2	12
26.506*	10	1	2	2	46.034*	2	4	1	0
27.420*	8	1	1	9	46.635*	4	4	1	3
27.769*	55	2	1	4	46.968*	20	4	0	10
28.680*	2	1	2	5	47.968*	16	2	3	8
29.655*	16	3	0	0	48.402*	14	4	1	6
31.026	100	0	2	10	49.785*	12	0	1	20
32.448*	20	1	2	8	50.314*	6	3	2	10
33.026*	10	3	0	6	50.733*	6	5	0	2
33.484*	8	1	1	12	51.252*	6	4	1	9
34.371*	65	2	2	0	51.469*	8	0	5	4
34.994*	6	0	1	14	52.616*	4	3	3	0
35.121*	8	2	2	3	52.944*	25	2	0	20
35.597*	12	2	1	10	53.512*	8	3	0	18
35.906*	6	1	3	1	54.405*	8	5	0	8
37.328*	10	1	2	11	55.114*	4	4	1	12
37.850*	6	3	1	5	56.139*	6	2	3	14
39.800*	10	1	0	16	56.591*	6	2	2	18
40.058*	4	1	1	15	57.439*	6	4	2	8
57.557*	4	5	1	4					
59.513*	12	5	1	7					
60.370*	4	1	5	8					
60.897*	4	2	1	22					
61.869*	4	6	0	0					
63.443*	6	1	5	11					
64.677*	4	0	4	20					
65.236*	4	0	5	16					
66.016*	4	3	4	8					
66.280*	6	5	2	6					
67.471*	4	1	5	14					

Appendix E

X-ray diffraction card of α-tricalcium phosphate (α-TCP).

09-0348 Wavelength= 1.5405

α-Ca3(PO4)2

Calcium Phosphate

Rad.: CuKa1 λ: 1.5405 Filter: d-sp: Guinier 114.6

Cut off: 50.0 Int.: Film I/Icor.:

Ref: de Wolff, P., Technisch Physische Dienst, Delft, The Netherlands, ICDD Grant-in-Aid

Sys.: Orthorhombic S.G.:

a: 15.22 b: 20.71 c: 9.109 A: 0.7349 C: 0.4398

α: β: γ: Z: 16 mp: 1720

Ref: Ibid.

Dx: 2.870 Dm: 2.814 SS/FOM$_{30}$=16(.0192, 95)

CAS #: 7758-87-4. Sample obtained by heating β-phase at 1400 C. Stated to be monoclinic pseudoorthorhombic by Mackay, Acta Crystallogr., 6 743 (1953). Validated by calculated pattern 29-359. Plus additional reflections. Mwt: 310.18. Volume[CD]: 2871.21.

2θ	Int	h	k	l	2θ	Int	h	k	l
7.181*	4	1	1	0	29.653*	20	5	1	0
12.097*	25	1	1	1	30.302*	20	1	1	3
12.970*	4	0	2	1	30.600*	35	4	0	2
14.068*	10	1	3	0	30.751	100	1	7	0
14.461*	4	2	2	0	31.247*	30	5	1	1
15.184*	10	2	0	1	31.748*	2	0	7	1
17.103*	12	0	4	0	32.099*	12	5	3	0
19.493*	4	0	0	2	32.326*	4	1	7	1
20.493*	4	3	1	1	32.727*	<1	1	3	3
20.735*	2	2	4	0	32.900*	<1	2	2	3
21.289*	2	0	2	2	33.599*	4	5	3	1
22.205*	20	1	5	0	34.180*	50	0	4	3
22.723*	40	2	0	2	34.602*	30	0	8	0
22.901*	40	2	4	1					
23.327*	8	4	0	0					
23.835*	4	3	3	1					
24.097*	40	1	3	2					
24.298*	18	1	5	1					
25.353*	4	4	0	1					
25.801*	6	0	6	0					
26.585*	8	3	1	2					
26.748*	4	4	3	0					
28.307*	4	2	6	0					
28.585*	4	2	4	2					
29.061*	4	4	4	0					
29.256*	4	5	0	0					

Appendix F

X-ray diffraction card of hydroxyapatite (HA).

09-0432 Wavelength= 1.54056

$Ca_5(PO_4)_3(OH)$

Calcium Phosphate Hydroxide

Hydroxylapatite, syn

Rad.: CuKa1 λ: 1.5405 Filter: d-sp: Debye-Sheerrer

Cut off: Int.: I/Icor.:

Ref: de Wolff, P., Technisch Physische Dienst, Delft, The Netherlands, ICDD Grant-in-Aid

Sys.: Hexagonal S.G.: $P6_3/m$ (176)

a: 9.418 b: c: 6.884 A: C: 0.7309

α: β: γ: Z: 2 mp:

Ref: Ibid.

Dx: 3.155 Dm: 3.080 SS/FOM$_{30}$=54(.0158, 35)

εα: ηωβ: 1.651 εγ: 1.644 Sign:- 2V:

Ref: Dana's System of Mineralogy, 7th Ed., II, 879

Color: Green, bluish green, yellow-green, grayish green, violet. Sample obtained following the procedure indicated by Hodge et al., Ind. Eng. Chem. Anal. Ed., 10 156 (1938). CAS #: 1306-06-5. I/I1 are peak values from a pattern which shows slight broadening of prism reflections. Validated by calculated data 24-33. Apatite group, apatite subgroup. PSC: hP44. To replace 34-10. Mwt: 502.32. Volume[CD]: 528.80.

2θ	Int	h	k	l	2θ	Int	h	k	l
10.820*	12	1	0	0	58.073*	4	5	0	1
16.841*	6	1	0	1	59.938*	6	4	2	0
18.785*	4	1	1	0	60.457*	6	3	3	1
21.819*	10	2	0	0	61.660*	10	2	1	4
22.902*	10	1	1	1	63.011*	12	5	0	2
25.354*	2	2	0	1	63.443*	4	5	1	0
25.879*	40	0	0	2	64.078*	13	3	0	4
28.126*	12	1	0	2	64.078*	13	3	2	3
28.966*	18	2	1	0	65.031*	9	5	1	1
31.773	100	2	1	1	66.386*	4	4	2	2
32.196*	60	1	1	2	66.386*	4	4	1	3
32.902*	60	3	0	0	69.699*	3	5	1	2
34.048*	25	2	0	2	71.651*	5	4	3	1
35.480*	6	3	0	1	71.651*	5	4	0	4
39.204*	8	2	1	2	72.286*	4	5	2	0
39.818*	20	3	1	0	72.286*	4	2	0	5
40.452*	2	2	2	1	73.995*	7	4	2	3
42.029*	10	3	1	1	75.022*	3	3	2	4
42.318*	4	3	0	2	75.022*	3	6	0	2
43.804*	8	1	1	3	75.583*	9	2	1	5
44.369*	2	4	0	0	76.154*	1	4	3	2
45.305*	6	2	0	3	77.175*	11	5	1	3
46.711*	30	2	2	2	78.227*	9	5	2	2
48.103*	16	3	1	2					
48.623*	6	3	2	0					
49.468*	40	2	1	3					
50.493*	20	3	2	1					
51.283*	12	4	1	0					
52.100*	16	4	0	2					
53.143*	20	0	0	4					
54.440*	4	1	0	4					
55.879*	10	3	2	2					
57.128*	8	3	1	3					

Appendix G

X-ray diffraction card of calcium hydroxide ($CaOH_2$).

04-0733 Wavelength= 1.54056

Ca(OH)2

Calcium Hydroxide

Portlandite, syn

Rad.: CuKa1 λ: 1.5405 Filter: Ni Beta M d-sp:

Cut off: Int.: Diffract. I/Icor.: 1.40

Ref: Swanson, Tatge, Natl. Bur. Stand. (U.S.), Circ. 539, I, 58 (1953)

Sys.: Hexagonal S.G.: P3m1 (164)

a: 3.593 b: c: 4.909 A: C: 1.3663

α: β: γ: Z: 1 mp:

Ref: Ibid.

Dx: 2.242 Dm: SS/FOM$_{28}$=14(.052, 39)

Pattern taken at 27 C. NBS analysis shows about 0.21% MgO, 0.1% Ba and no other impurities over 0.04%. Levi, Giorn. Chim. Ind. Applicata, 6 333-7 (1924). Structure C6, Cd I2 type. Brucite group, brucite subgroup. PSC: hP3. Mwt: 74.09. Volume[CD]: 54.88.

2θ	Int	h	k	l	2θ	Int	h	k	l
18.089*	74	0	0	1	130.119	6	2	1	4
28.662*	23	1	0	0	142.272	5	3	1	2
34.088	100	1	0	1					
36.696*	3	0	0	2					
47.123*	42	1	0	2					
50.794*	36	1	1	0					
54.336*	21	1	1	1					
56.251*	1	0	0	3					
59.303*	3	2	0	0					
62.538*	13	2	0	1					
64.226*	13	1	1	2					
71.777*	8	2	0	2					
77.697*	1	0	0	4					
78.999*	1	1	1	3					
81.822*	3	2	1	0					
84.721*	11	2	1	1					
86.185*	2	2	0	3					
93.229*	12	2	1	2					
95.989*	5	3	0	0					
98.826*	7	3	0	1					
107.509	4	2	1	3					
110.603	1	1	0	5					
118.156	1	2	2	0					
121.280	2	2	2	1					
123.120	1	3	0	3					
126.578	2	3	1	0					

Appendix H

Specific surface area of starting material.

MICROMERITICS INSTRUMENT CORPORATION
FlowSorb 2300

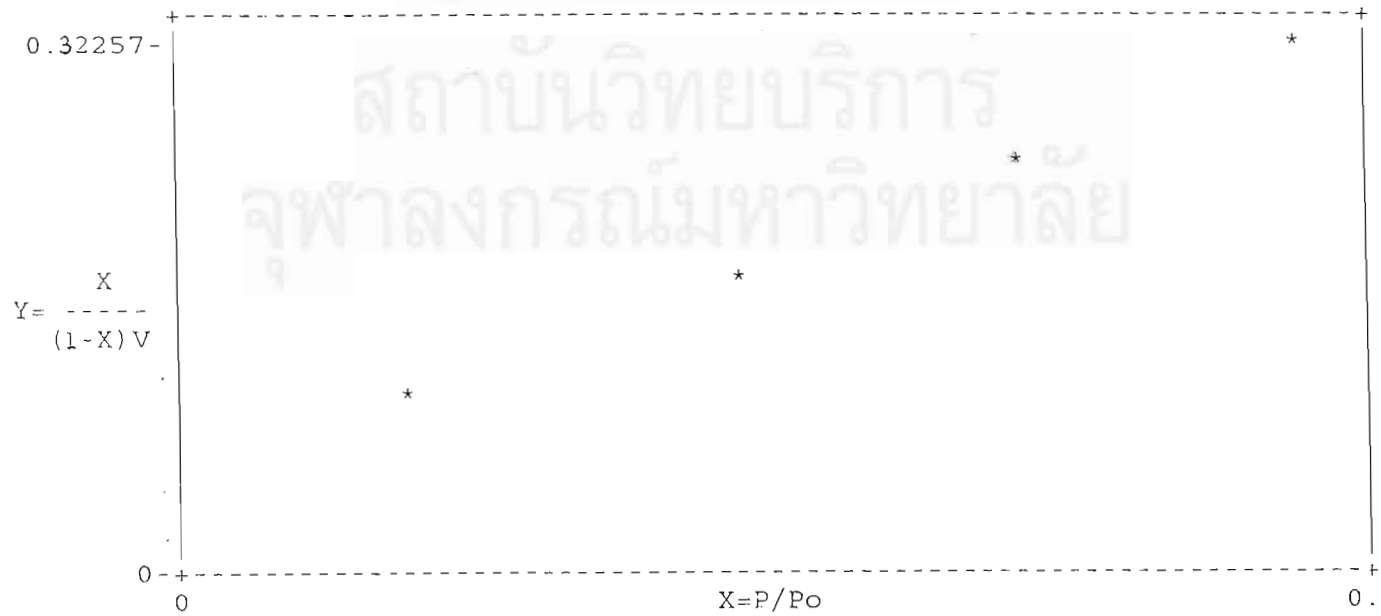
BET SURFACE AREA ANALYSIS
REPORT DATE: 7/5/99

SAMPLE I.D.: Raw Material
SAMPLE WEIGHT: 0.7860 g
MOL. CROSS-SECTIONAL AREA: 0.162 nm^2
AMBIENT TEMPERATURE: 0.00 C

ADSORBATE: Nitrogen
BAROMETRIC PRESSURE: 760 mmHg
SATURATION PRESSURE: 775 mmHg

EXPERIMENTAL DATA (%)	(VOL)	VOL ADSORBED (cm^3/g AT STP)	X=P/Po	Y=X/[(1-X)V]
5.000	0.41	0.52	0.0490	0.09885
12.000	0.58	0.74	0.1177	0.18074
18.000	0.68	0.87	0.1765	0.24777
24.000	0.75	0.95	0.2354	0.32257

BET SURFACE AREA: 3.53 +/- 0.05 m^2/g
SLOPE: 1.1946 +/- 0.0172
INTERCEPT: 0.0397 +/- 0.0028
C: 31.09
Vm: 0.81 cm^3/g
CORRELATION COEFFICIENT 0.9998



Appendix I

Specific surface area of dicalcium phosphate anhydrous (DCPA).

MICROMERITICS INSTRUMENT CORPORATION
FlowSorb 2300

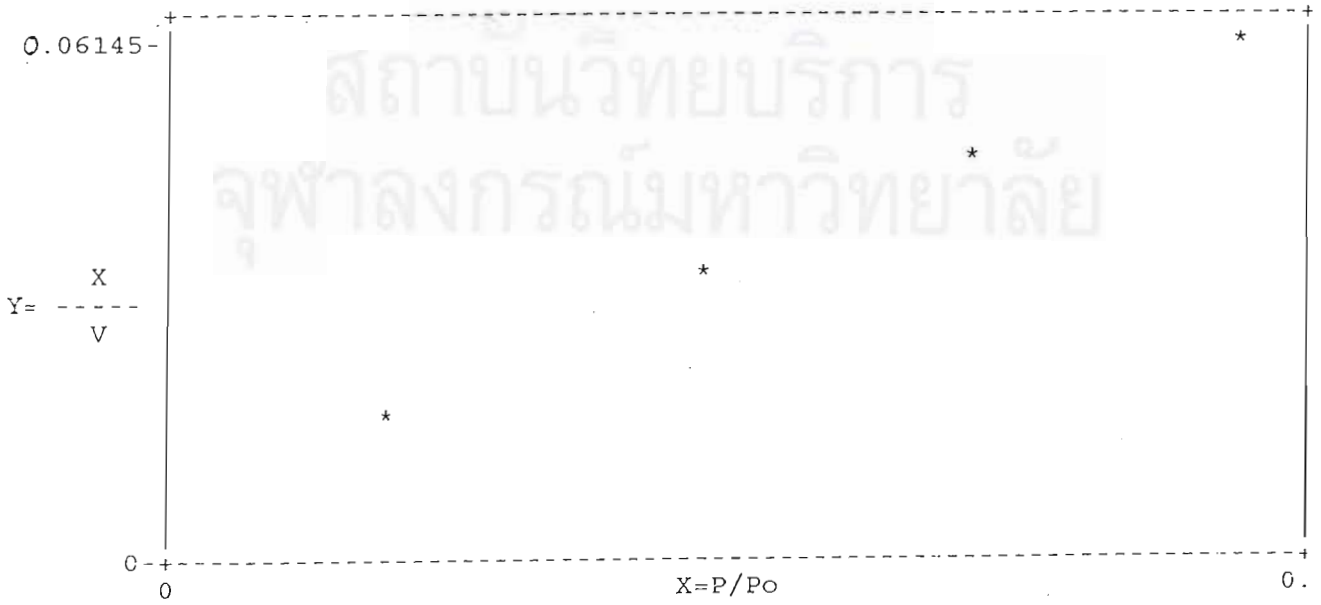
LANGMUIR SURFACE AREA ANALYSIS
REPORT DATE: 7/5/99

SAMPLE I.D.: DCPA
SAMPLE WEIGHT: 0.7937 g
MOL. CROSS-SECTIONAL AREA: 0.162 nm^2
AMBIENT TEMPERATURE: 0.00 C

ADSORBATE: Nitrogen
BAROMETRIC PRESSURE: 760 mmHg
SATURATION PRESSURE: 775 mmHg

EXPERIMENTAL DATA (%)	(VOL)	VOL ADSORBED (cm^3/g AT STP)	X=P/Po	Y=X/V
5.000	2.38	3.00	0.0490	0.01635
12.000	2.78	3.50	0.1177	0.03360
18.000	2.95	3.72	0.1765	0.04749
24.000	3.04	3.83	0.2354	0.06145

LANGMUIR SURFACE AREA: 18.02 +/- 0.20 m^2/g
SLOPE: 0.2416 +/- 0.0026
INTERCEPT: 0.0048 +/- 0.0004
B*Po: 50.62
Vm: 4.14 cm^3/g
CORRELATION COEFFICIENT 0.9999



Appendix J

Specific surface area of monocalcium phosphate monohydrate (MCPM).

MICROMERITICS INSTRUMENT CORPORATION
FlowSorb 2300

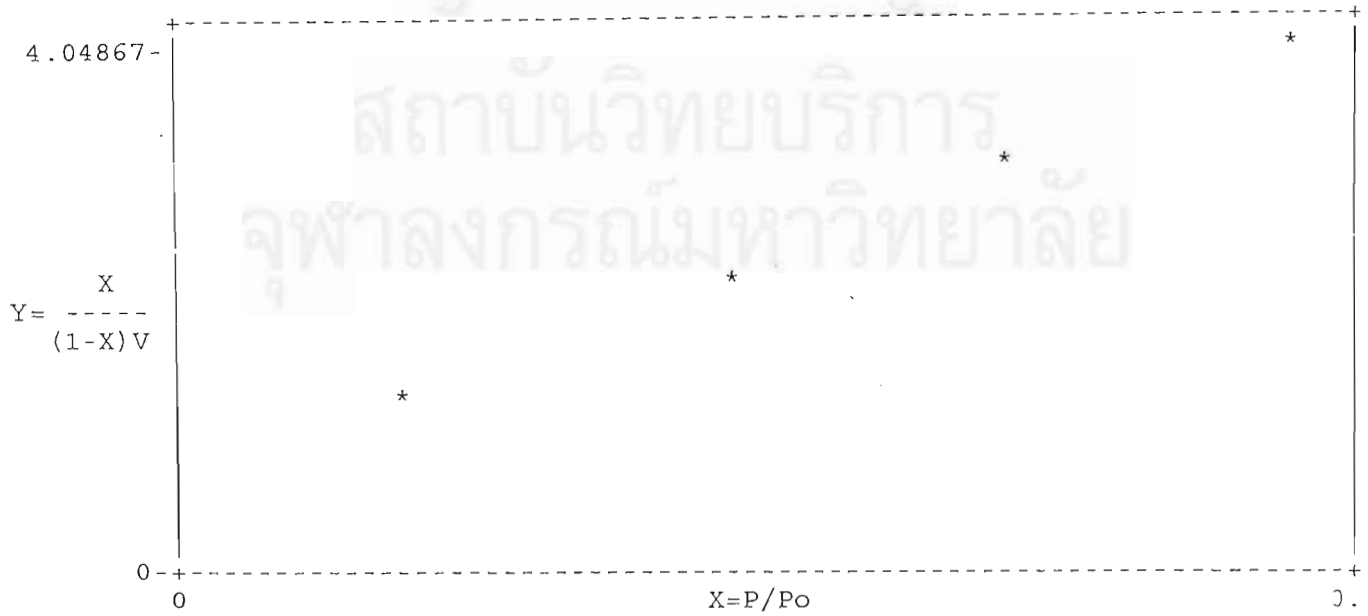
BET SURFACE AREA ANALYSIS
REPORT DATE: 7/5/99

SAMPLE I.D.: MCPM
SAMPLE WEIGHT: 1.0523 g
MOL. CROSS-SECTIONAL AREA: 0.162 nm^2
AMBIENT TEMPERATURE: 0.00 C

ADSORBATE: Nitrogen
BAROMETRIC PRESSURE: 760 mmHg
SATURATION PRESSURE: 775 mmHg

EXPERIMENTAL DATA (%)	(VOL)	VOL ADSORBED (cm^3/g AT STP)	X=P/Po	Y=X/[(1-X)V]
5.000	0.04	0.04	0.0490	1.35642
12.000	0.06	0.06	0.1177	2.33913
18.000	0.07	0.07	0.1765	3.22234
24.000	0.08	0.08	0.2354	4.04867

BET SURFACE AREA: 0.29 +/- 0.00 m^2/g
SLOPE: 14.5021 +/- 0.1196
INTERCEPT: 0.6440 +/- 0.0192
C: 23.52
Vm: 0.07 cm^3/g
CORRELATION COEFFICIENT 0.9999



Appendix K

American Standard Test Methods for Composition of Ceramic Hydroxyapatite for Surgical Implants.

Designation: F 1185 – 88

Standard Specification for Composition of Ceramic Hydroxylapatite for Surgical Implants[1]

This standard is issued under the fixed designation F 1185; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This specification covers material requirements for ceramic hydroxylapatite intended for surgical implants. For a material to be called ceramic hydroxylapatite, it must conform to this specification. (See Appendix X1.)

1.2 The biological response to ceramic hydroxylapatite in soft tissue and bone has been characterized by a history of clinical use (1, 2, 3)[2] and by laboratory studies (4, 5, 6).

1.3 This specification specifically excludes hydroxylapatite coatings, non-ceramic hydroxylapatite, ceramic-glasses, tribasic calcium phosphate, whitlockite, and alpha- and beta-tricalcium phosphate. (See Specification F 1088.)

2. Referenced Documents

2.1 *ASTM Standard:*
F 1088 Specification for Beta-Tricalcium Phosphate for Surgical Implantation[3]
2.2 *Code of Federal Regulations:*[4]
Title 21, Part 820.
2.3 *National Formulary:*[5]
Tribasic Calcium Phosphate
2.4 *United States Pharmacopeia:*[6]
Identification Tests for Calcium and Phosphate <191>
Lead <251>
Mercury <261>
Arsenic <211>
Heavy Metals <231> Method 1
2.5 *U. S. Geological Survey Method:*[7]
Cadmium

3. Descriptions of Terms Specific to This Standard

3.1 *hydroxylapatite*—the chemical substance having the empirical formula $Ca_5(PO_4)_3OH$.[8]

3.2 *ceramic hydroxylapatite*—hydroxylapatite which has been fired at sintering temperatures. Firing time is mass dependent, and should be sufficiently long to cause significant densification and formation of a biologically stable form.

3.3 *sintering*—an integration of time and temperature of a ceramic precursor which develops a coherent body with useful properties. Sintering is a non-melting process accompanied by significant surface area and bulk volume reductions (densification), grain growth, and increases in mechanical properties.

3.4 *calcining*—the heat treatment of a ceramic precursor for the purpose of eliminating volatile constituents. Calcining is also accompanied by some surface area and bulk volume reductions. Increases in mechanical properties are not usually significant.

4. Chemical Requirements

4.1 Elemental analysis for calcium and phosphorus will be consistent with the expected stoichiometry of hydroxylapatite.

4.2 A quantitative X-ray diffraction analysis shall indicate a minimum hydroxylapatite content of 95 % (7). Analysis of relative peak intensities shall be consistent with published data.[9]

4.3 The concentration of trace elements in the hydroxylapatite shall be limited as follows:

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

For referee purposes, methods in 2.4 and 2.5 shall be used.

4.4 The maximum allowable limit of all heavy metals determined as lead will be 50 ppm as described in 2.4 or equivalent. Sample preparation will be identical to that for tribasic calcium phosphate as specified in the National Formulary (2.2) except that approximately 1 g of material will be dissolved in approximately 30 mL of 5 % HCl and boiled.

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[1] This specification is under the jurisdiction of ASTM Committee F-4 on Medical and Surgical Materials and Devices and is the direct responsibility of Subcommittee F04.02 on Resources.
Current edition approved Oct. 31, 1988. Published December 1988.

[2] The boldface numbers in parentheses refer to the list of references at the end of this specification.

[3] *Annual Book of ASTM Standards*, Vol 13.01.

[4] Available from U.S. Government Printing Office, Washington, DC 20402.

[5] National Formulary XVI. Available from U.S. Pharmacopeia Convention, Inc., 12601 Twinbrook Parkway, Rockville, MD 20852.

[6] United States Pharmacopeia XXI. Available from U.S. Pharmacopeia Convention, Inc., 12601 Twinbrook Parkway, Rockville, MD 20852.

[7] Crock, J. G., Felichte, F. E., and Briggs, P. H., "Determination of Elements in National Bureau of Standards Geological Reference Materials SRM 278 Obsidian and SRM 688 Basalt by Inductively Coupled Argon Plasma—Atomic Emission Spectrometry," *Geostandards Newsletter*, Vol 7, 1983, pp. 335-340.

[8] Chemical Abstracts Service Registry Number [1306-06-5].

[9] The Joint Committee on Powdered Diffraction Standards has established a Powder Diffraction File. The Committee operates on an international basis and cooperates closely with the Data Commission of the International Union of Crystallography and ASTM (American Society for Testing and Materials). Hydroxylapatite data can be found on file card number 9-432 and is available from the Joint Committee on Powder Diffraction Standards, 1600 Park Lane, Swarthmore, PA 19081.

4.5 It is recommended that all metals or oxides not detected as lead present in concentrations equal to or greater than 0.1 % be listed on the package insert.

5. Test Specimen Fabrication

5.1 Prepare test specimens from the same batch of material and by the same processes as those employed in fabricating the ceramic implant device.

6. Quality Program Requirements

6.1 The manufacturer shall conform to Good Manufacturing Practices (2.2) or its equivalent.

APPENDIX

(Nonmandatory Information)

X1. RATIONALE

X1.1 Ceramic hydroxylapatite is commercially available as a synthetic bone-grafting material. As with any implant material, the bioresponse is critically dependent upon the material properties. To achieve reliable biocompatibility these must be known and consistent. This material standard provides specifications for a biocompatible grade of hydroxylapatite. Trace element content and leachability, physical form, and size must be within established biocompatibility standards.

X1.2 X-ray powder diffraction analysis provides better differentiation between hydroxylapatite and several commonly occurring second phases than traditional wet chemical methods.

X1.3 It is recognized that a separate performance standard may be necessary for each end-use product. For this reason, physical and mechanical properties were not specified. A source of general test methods for ceramics may be found in Ref (8).

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(1) Cranin, A. N., Tobin, G., Gelbman, J., Varjan, R., "A Seven Year Follow-up of Patients with (H/A) Ridge Augmentation," *Transactions of the Society for Biomaterials*, 1986, p. 155.

(2) Kent, J. N., Quinn, J. H., Zide, M. F., Guerra, L. R., Boyne, P., "Augmentation of Deficient Alveolar Ridges with Nonresorbable Hydroxylapatite or with Autogenous Cancellous Bone," *Journal of Oral and Maxillofacial Surgery*, Vol 41 (10), 1983, pp. 629-642.

(3) Yukna, R. A., Mayer, E. T., Brite, D. V., "Longitudinal Evaluation of Durapatite Ceramic as an Alloplastic Implant in Periodontal Osseous Defects After Three Years," *Journal of Periodontology*, Vol 55 (11), 1984, pp. 633-637.

(4) Jarcho, M., Kay, J. F., Gumaer, K. I., Doremus, R. H., and Drobeck, H. P., "Tissue, Cellular and Subcellular Events at a Bone-Ceramic Hydroxylapatite Interface," *Journal of Bioengineering*, Vol 1, 1977, pp. 79-92.

(5) Drobeck, H. P., Rothstein, S. S., Gumaer, K. I., Sherer, A. D., and Slighter, R. G., "Histologic Observation of Soft Tissue Response to Implanted, Multifaceted Particles and Discs of Hydroxylapatite," *Journal of Oral and Maxillofacial Surgery*, Vol 42, 1984, pp. 143-149.

(6) Tracy, B. M. and Doremus, R. H., "Direct Electron Microscope Studies of the Bone-Hydroxylapatite Interface," *Journal of Biomedical Materials Research*, Vol 18, 1984, pp. 719-726.

(7) Balmain, N., Legros, R., and Bonel, G., "X-Ray Diffraction of Calcified Bone Tissue: A Reliable Method for the Determination of Bone Ca/P Molar Ratio," *Calcified Tissue International*, Vol 1, 1982, pp. S93-S98.

(8) *Annual Book of ASTM Standards*, Vol 15.02.

Appendix L

IR absorption bands (LeGeros, 1991).

IR bands, cm^{-1}	Descriptions
3,800-3,000	H-O-H, H_2O of crystallization, adsorbed H_2O
1,615	H-O-H, H_2O of crystallization, adsorbed H_2O
1,454, 1,414	C-O of CO_3 groups
1,280, 1,200	P-OH bending modes, HPO_4 groups
1,108, 1,194	P-O and P-OH, HPO_4 and PO_4 groups
964	P-O or PO_4 group
910, 869	P-OH stretching mode of HPO_4 groups
630	O-H of OH group
628, 600, 561	P-O or PO_4 groups
525	O-P-OH bending mode in HPO_4
471, 470, 450	P-O of PO_4 groups

BIOGRAPHY

Miss Suphasinee Limpanuphap was born on March 5, 1977 in Bangkok. She received her Bachelor Degree of Science in Materials Science from Chulalongkorn University in 1997. She began her Master Degree study in Ceramic Technology in June 1997 and completed the programme in October 1999.