

## Chapter 3

### Experimental Part

#### 3.1 Description and scope

The synthesis of dicalcium phosphate dihydrate from cattle bone in this experiment was based on dissolution of the bone ash and reprecipitation under various conditions.

In case of tricalcium phosphate, it was obtained from solid state reaction between synthesized dicalcium phosphate dihydrate and calcium carbonate.

The experiment was carried out steply as follows;

- 1). Bone ash preparation
- 2). Chemical precipitation of DCPD from cattle bone ash solution; fixed and not fixed Ca/P mole ratio
- 3). TCP preparation

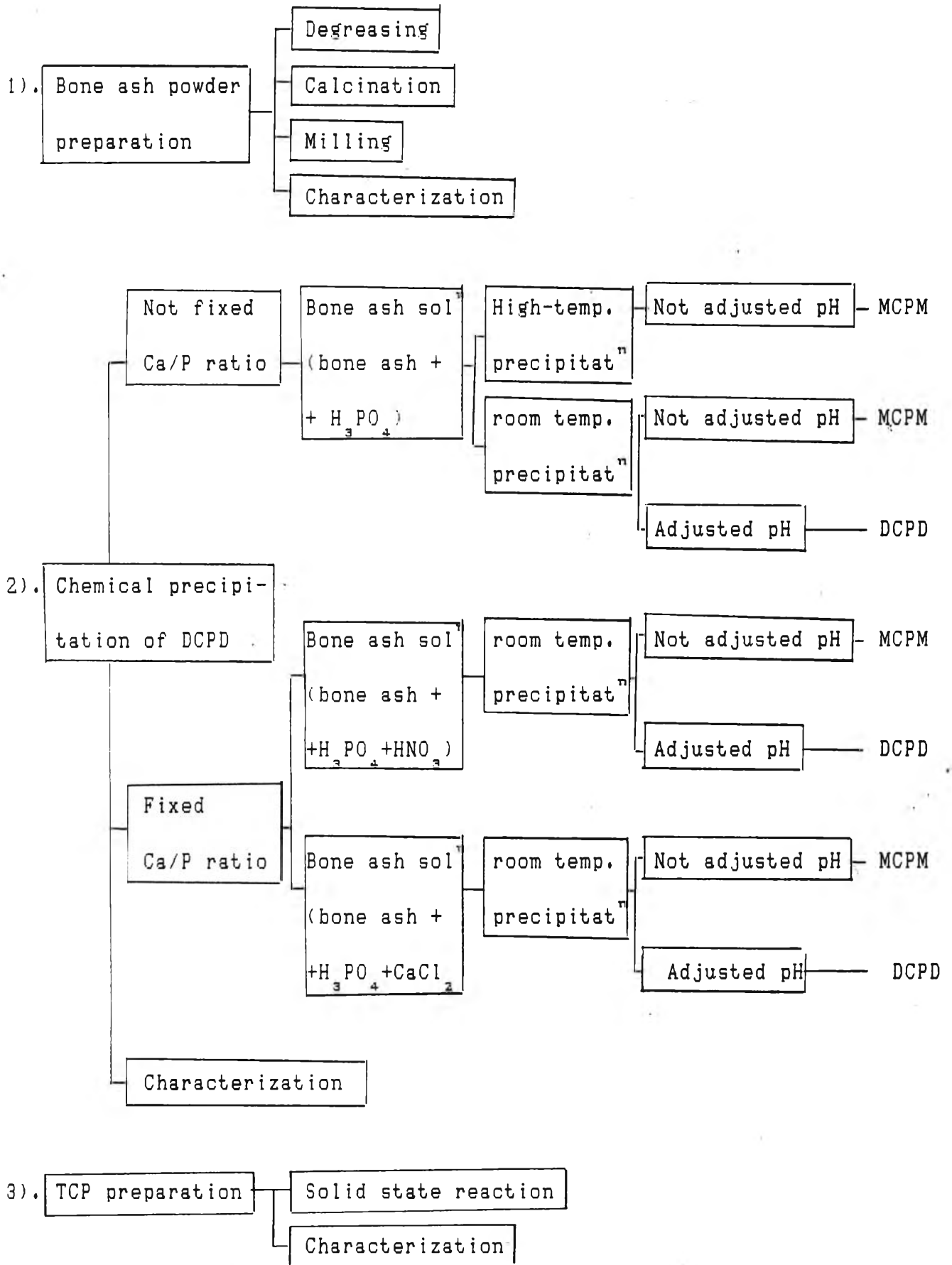


Fig.10 Flow chart of experimental step

## 3.2 Materials and Equipments

### 3.2.1 Materials

- Cattle bone
- Phosphoric Acid 86% ; Baker. : analyzed reagent
- Ammonia Solution 35% ; BDH : analyzed reagent
- Calcium Chloride ; Carlo : analyzed reagent
- Acetone ; Baker : analyzed reagent
- Dicalcium Phosphate Dihydrate ; Fluka : (>98%)
- Calcium Carbonate ; Carlo : analyzed reagent)

### 3.2.2 Equipments

- Steel Saw
- The Electric Furnace ; Duncan Kiln : model LT-3K
- The Electric Furnace ; Heraeus : Type K1700/1
- Hot Plate ; Jankel & Kunhel GmbH & Co : Type RCT
- pH Meter ; Tokyo Rikakikai : Type FC-10
- X-Ray Diffractometer ; Phillips : model no. DY 1023 type PW 730/10
- DTA ; Netzsch : Temperature Control system 410
- Porcelain mortar
- Desiccator, Silica Gel
- Rotary Evaporator

- Stick Bar
- Buchner Funnel
- Filter Paper, Whatman No. 42
- Beaker
- Pipette
- Buret
- Cylinder
- Watch Glass
- Magnetic Bar
- Volumetric Flask
- Round Bottom Flask
- Alumina Crucible
- Glass Rod

### 3.3 Experimental Procedure

#### 3.3.1 Preparation of Bone Ash Powder

Cattle bones from leg were radially cut by a steel saw into pieces as shown in Fig.11 then a stick bar was used to drive bone marrow out as shown in the following figure. The selected middle parts of cattle bones were boiled in hot water to get rid of organic materials such as fat, gelatin, collagen and etc. While boiling; impurities e.g., wax on the surface of boiling water was scooped out and boiling water was changed several times. Cattle bones were boiled about 1 day. After that they were polished, cleaned and dried.

After drying, the bone was calcined in an electric furnace at 700°C for 3 hours to remove the remaining organic matter. The white calcined bone was crushed in porcelain mortar to powder of bone ash. The bone ash powder was characterized for phase and chemical analysis.

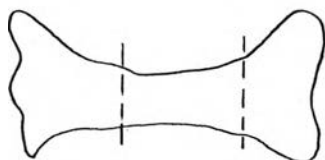


Fig.11 Selected part of bone



Fig.12 Driving bone marrow out  
from bone

### 3.3.2 Chemical Precipitation of DCPD

Bone ash solution was prepared by dissolving the bone ash powder in acid under various conditions. Dicalcium phosphate dihydrate was precipitated from each bone ash solution as follows:

#### 3.3.2.1 Chemical precipitation of DCPD; not fixed Ca/P mole ratio

1). Precipitation of DCPD from a saturated bone ash solution at high temperature.

- The saturated bone ash solution was prepared by increasing dissolving the bone ash powder in 1:5 by volume of 85% phosphoric acid and distilled water at room temperature, stirring all the time until saturation was reached, allowing the excess bone ash powder to settle, the clear saturated solution was decanted and filtered.

- Precipitation was initiated by boiling the prepared solution. This solution was heated and well crystallized calcium phosphate compound precipitated out. The precipitate was filtered and washed thoroughly with distilled water, rinsed with acetone, dried at room temperature in a desiccator finally weighed accurately.

2). Precipitation of DCPD from room temperature bone ash solution under an uncontrolled and controlled pH conditions.

The bone ash solution in this case was prepared by dissolving 2 grams of bone ash powder in 55 ml  $2M.H_3PO_4$ , stirring vigorously, heating to  $200^{\circ}C$  until dissolved. This solution was cooled to room temperature.

a). The precipitation under an uncontrolled pH solution was initiated by saturation this solution by evaporation at room temperature, low pressure, by rotary evaporator until white precipitate was observed. The precipitate was separated by using a suction filter. The filtered product was washed with distilled water, rinsed with acetone, dried at room temperature in a desiccator, finally weighed accurately.

b). The precipitation under a controlled pH solution was initiated by adjusting the pH of the bone ash solution with  $1M.NH_4OH$  solution to pH 3.5-6.0. The precipitate at each pH was separated by using a suction filter. The filtered product was washed with distilled water, rinsed with acetone, dried at room temperature in a desiccator, finally weighed accurately.

### 3.3.2.2 Chemical precipitation of DCPD ;fixed Ca/P mole ratio

This process was split into 2 methods, 1) and 2) and in each method the bone ash solution was differently prepared and its Ca/P mole ratio was adjusted to 1:1 by adding either P-ion or P+Ca-ions

1). Precipitation of DCPD from a fixed Ca/P mole ratio bone ash solution at room temperature under an uncontrolled (a) and controlled pH (b) , the Ca/P mole ratio was adjusted by adding P-ion to the solution.

The bone ash solution in this case was prepared by dissolving 2 grams of bone ash powder in 7.8 ml  $2M.H_3PO_4$  ( to adjust Ca/P mole ratio ) and 37 ml  $1M.HNO_3$  , stirring vigorously, heating to  $200^{\circ}C$  until it was dissolved. This solution was cooled to room temperature.

a). The precipitation under an uncontrolled pH solution was initiated by saturation the bone ash solution by evaporation at room temperature, low pressure, by rotary evaporator until white precipitate was observed. The precipitate was separated by using a suction filter. The filtered product was washed with distilled water, rinsed with acetone, dried at room temperature in a desiccator, finally weighed accurately.



b). The precipitation under a controlled pH solution was initiated by adjusting the pH of the bone ash solution with 1M.  $\text{NH}_4\text{OH}$  solution to pH 3.5-6.0. The precipitate at each pH was separated by using a suction filter. The filtered product was washed with distilled water, rinsed with acetone, dried at room temperature, finally weighed accurately.

2). Precipitation of DCPD from a fixed Ca/P mole ratio bone ash solution at room temperature under an uncontrolled (a) and controlled pH (b), the Ca/P mole ratio was adjusted by adding P+Ca ion to the solution.

The bone ash solution in this case was prepared by dissolving 2 grams of bone ash powder in 55 ml  $2\text{M.H}_3\text{PO}_4$ , stirring vigorously, heating at  $200^\circ\text{C}$  until it was dissolved. When this solution was cooled, 15 g of  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  in 40 ml of distilled water was added to adjust Ca/P mole ratio. Stirred the solution until it was mixed well.

a). For an uncontrolled pH condition, the precipitation was initiated by saturation the bone ash

solution by evaporation at room temperature, low pressure with rotary evaporator until white precipitate was observed. The filtered product was washed with distilled water, rinsed with acetone, dried at room temperature in a desiccator, finally weighed accurately.

b). The precipitation under a controlled pH condition was initiated by adjusting the pH of the bone ash solution with 1M.NH<sub>4</sub>OH solution to pH3.5-6.0. The precipitate at each pH was separated by using a suction filter. The filtered product was washed with distilled water, rinsed with acetone, dried at room temperature in a desiccator, finally weighed accurately.

### 3.3.3 Preparation of tricalcium phosphate

The synthesis of tricalcium phosphate was attempted using the prepared and reference dicalcium phosphate dihydrate as starting materials. 4 grams prepared/reference dicalcium phosphate dihydrate and 1.16 grams calcium carbonate was mixed well and then added it in alumina crucible. The mixture was sintered at high temperature (1100°C-1300°C) in an electric furnace.

The reacting temperatures were chosen from the previous tests with a DTA equipment at a heating rate of 5°C/min in air to 1350°C. The reference used was calcined

alumina powder.

The transformation temperature of  $\beta$ -TCP to  $\alpha$ -TCP was also detected, using the prepared  $\beta$ -TCP as a starting material.

Each sample was soaked at the maximum firing temperature for 3 hours. Two different techniques were used to cool the sample; fast cooling and slow cooling. The sample was quenched by drawing out immediately after soaking and for the slow cooling, the sample was left to cool down to room temperature in the furnace. The products obtained at various temperatures were identified by XRD and IR. The chemical composition of the product was determined by ICP method.

#### 3.3.4 Characterization

Characterization of the product from each step of the experiment was carried out as follows:

Bone ash powder

: Phase present, X-ray diffractometer\* (XRD)

: Infrared spectra\* (IR)

\* see appendix A

# see appendix B

: Ca/P mole ratio and impurities - Inductively  
Couple Plasma\* (ICP) and Instrumental Neutron  
Activation Analysis# (INAA)

DCPD

: Phase present - XRD,

: Ca/P mole ratio and impurities -ICP, INAA

TCP

: Thermal change -Differential Thermal Analysis@ (DTA)

: Phase present -XRD

: IR

: Ca/P mole ratio - ICP

\* see appendix C

# see appendix D

@ see appendix E