

CHAPTER 3

MATERIALS AND METHODS

3.1. Preparation of TTCP from DCPD and calcium carbonate

Materials

High purity reagents : $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ synthesized from cattle bone (Jinawath,1995) and calcium carbonate, CaCO_3 (analytical grade), were used as starting materials. The details of the materials were shown in Table 3.1

Table 3.1 Materials used in the experiment

1. Dicalcium phosphate dihydrate : $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ (DCPD) synthesized from cattle bone (Jinawath, 1995)	
Average particle size ^a	5-10 μm
Chemical analysis ^b	Ca/P mole ratio 1.06/1
Impurities ,	Mg (%) 0.01
	Fe (%) 0.07
	Zn (ppm) 38
	Cu (ppm) 3
	Mn (ppm) 5
Heavy metals ,	Cd (ppm) <0.5
	Pb (ppm) <5
	Hg (ppm) <1
	As (ppm) < 0.5
	Ni (ppm) 2

<p>2. Calcium carbonate : CaCO_3 (Analytical grade, Fluka chemika 21060) dried at 100°C for 2 hours</p>
<p>Chemical analysis Assay > 99% Cl (%) < 0.03 SO_4 (%) < 0.05 Cd Co Cu Fe K Na Ni Pb Zn(%) < 0.005 Specific surface area c $1.67 \pm 0.06 \text{ m}^2/\text{g}$</p>

a : from SEM , b : from ICP method , c : from BET (see appendix)

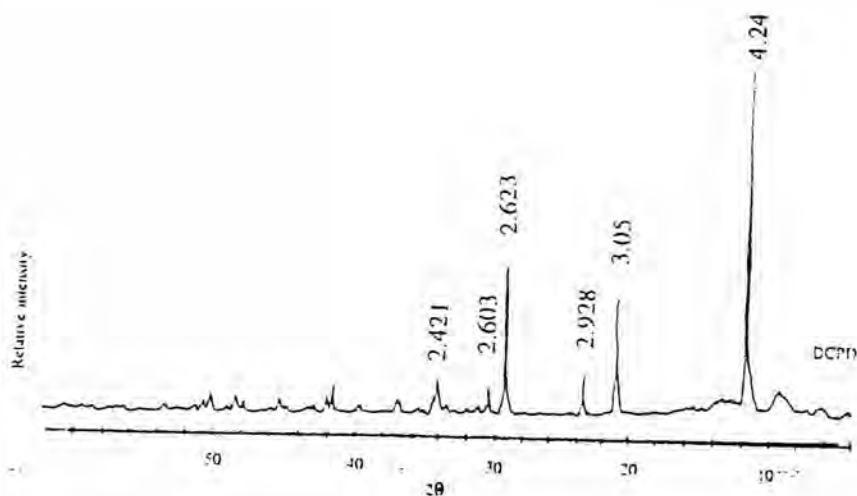


Fig. 3.1 XRD pattern of synthesized DCPD from cattle bone

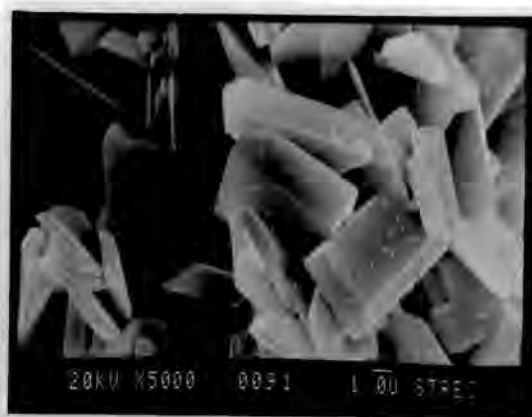


Fig. 3.2 Scanning electron micrograph of synthesized DCPD from cattle bone

Experimental methods for the preparation of TTCP

Several grams of DCPD and calcium carbonate mixtures of various molar ratios (1:0.5, 1:0.8, 1:0.9, 1:1, 1:1.3, 1:1.5 and 1:2.3) were each mixed in a mortar with 4–5 drops of acetone* to homogenize the mixture. Then, the mixtures were placed in alumina crucibles and sintered (rate $4^{\circ}\text{C}/\text{min}$) in the furnace at 1300, 1350 and 1400°C . All mixtures were annealed for 6 hours and rapidly cooled in air. Phases were indicated by x-ray diffraction, and morphology was observed by SEM.

* Acetone (AR grade, J.T. Baker 9006-03)

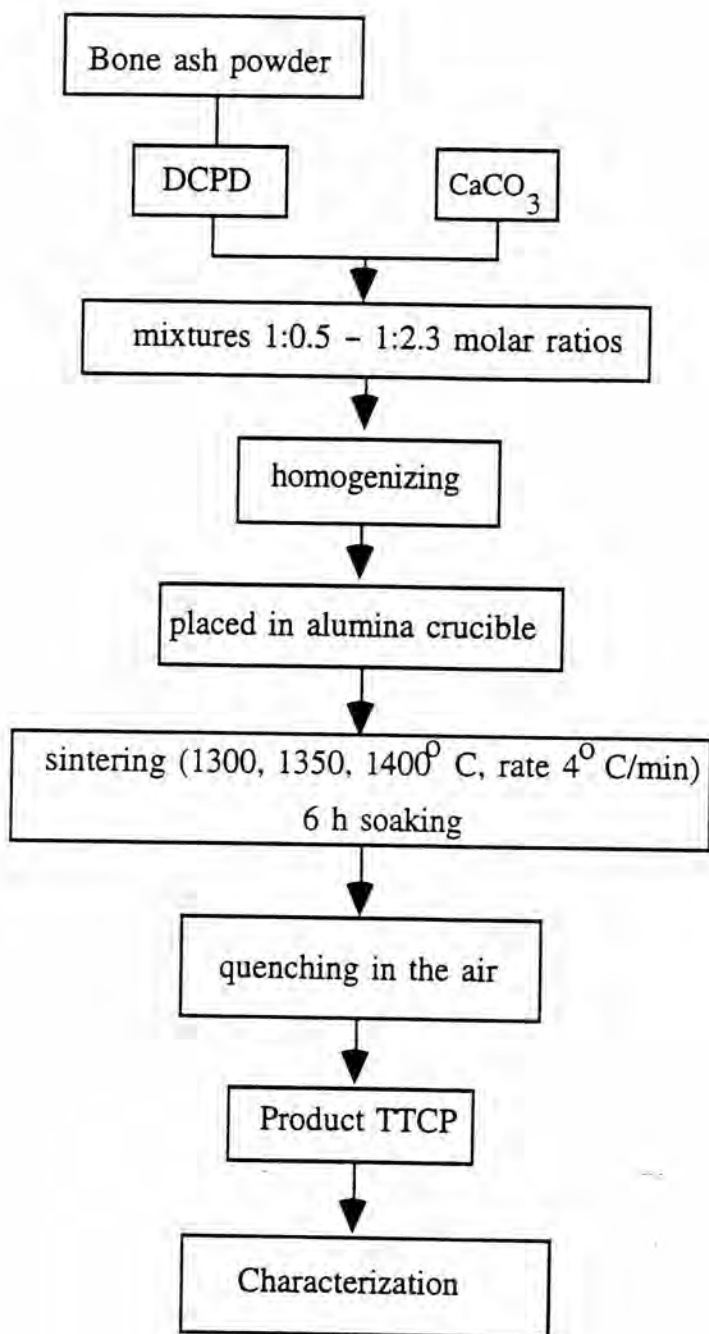


Fig.3.3 Flow chart of the preparation of tetracalcium phosphate from DCPD and CaCO₃

3.2 Preparation of TTCP from $\gamma\text{-Ca}_2\text{P}_2\text{O}_7$ and CaCO_3

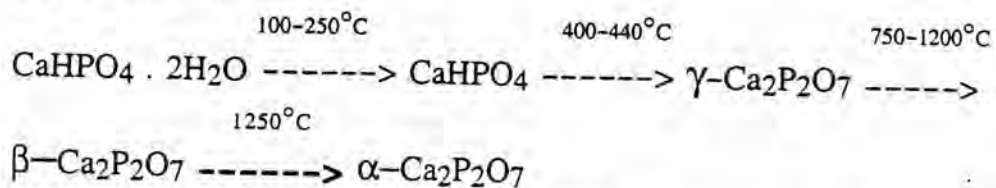
Materials :

$\gamma\text{-Ca}_2\text{P}_2\text{O}_7$ (obtained by calcining DCPD at 400°C with details below).

CaCO_3 (analytical grade) supplied by Fluka was dried at 100°C for 2 h.

Preparation of $\gamma\text{-Ca}_2\text{P}_2\text{O}_7$

Thermal transformation of calcium phosphates according to Aoki, 1991 was as follows :



Therefore, preparation of $\gamma\text{-Ca}_2\text{P}_2\text{O}_7$ was performed by calcining the DCPD extracted from cattle bone in air at temperature $350\text{-}500^\circ\text{C}$, using a heating rate of $4^\circ\text{C}/\text{min}$ and soaking time of 2 hours. The results from XRD patterns were shown in Table 4.2 and Fig. 4.2

Experimental methods for the preparation of TTCP

DCPD calcined at 400, 410, 440, 500°C were used as starting materials to prepare TTCP in Fig. 3.4. Especially, DCPD calcined at 400°C was chosen because the results from XRD indicated that it contained $\gamma\text{-Ca}_2\text{P}_2\text{O}_7$ and a trace of $\beta\text{-Ca}_2\text{P}_2\text{O}_7$. Several grams of the mixture of $\gamma\text{-Ca}_2\text{P}_2\text{O}_7$ and CaCO_3 in various molar ratios, ranging from 2:3.0 to 2:4.8 were each mixed in the mortar, placed in alumina crucible, heated to $1300, 1350$ and 1400°C for 6 hours. The products were rapidly cooled outside the furnace and investigated for phase analysis by the x-ray diffraction.

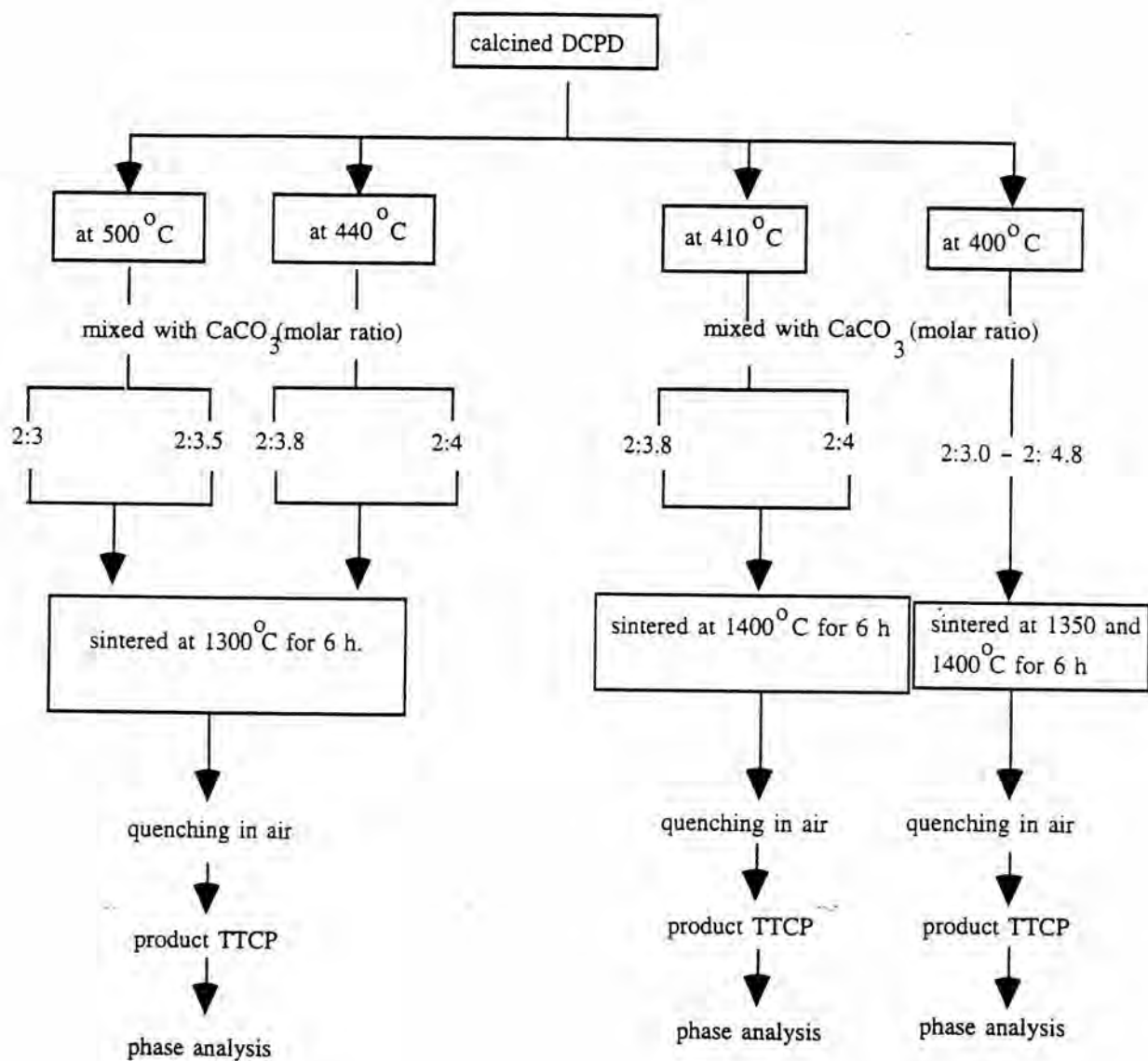


Fig.3.4 Flow chart of the preparation of TTCP from γ - $\text{Ca}_2\text{P}_2\text{O}_7$ and CaCO_3 .

Characterization of TTCP

- Phase present (XRD, IR)
- Morphology (SEM)
- Particle size (SEM)
- Specific surface area,SS (BET)
- Ca/P mole ratio and impurities(ICP)
- DTA

1. Phase analysis

The x-ray diffraction pattern of TTCP powder was determined by using an x-ray diffractometric apparatus (Philips diffractometer (PW 1730/70) with Cu $K\alpha$ radiation and Ni filter at 30 mA 40 kV. A time constant of 1 s. and scanning rate $2^\circ/\text{min}$ were used, 2θ was run from 16° to 60° to identify the phases of calcium phosphates.



Fig. 3.5 Philips diffractometer, PW 1730/70

2. Morphology of particles (Scanning electron microscopy)

Fine TTCP powder was mounted on aluminum stubs and coated with gold. The SEM (model JSM T 220A, supplied by JEOL, Japan) examinations were focused on small particles of TTCP to observe morphology of particle.



Fig.3.6 Scanning electron microscope (JSM T 220A, supplied by JEOL)

3. Specific surface area (BET)

Specific surface area of TTCP powder was determined by BET (Micromeritic model ASAP 2000). TTCP powder was dried at 150°C for 2 h before proceeding the experiment. Nitrogen gas was used as adsorbate on particle surface.



Fig.3.7 BET particle size analyser (Micromeritics model ASAP 2000)

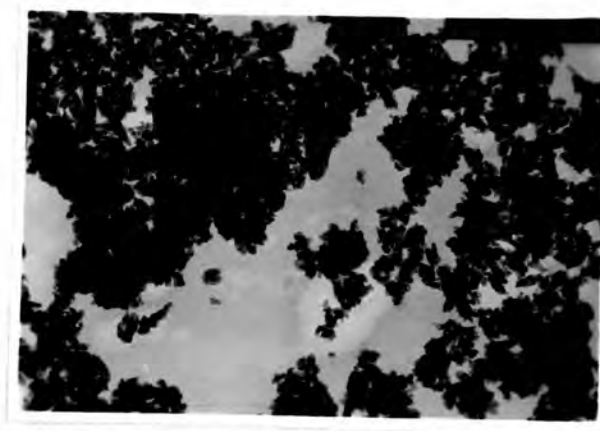
3.3 Preparation of self-setting calcium phosphate cement (CPC) containing TTCP and DCPD.

Starting materials

1. TTCP synthesized from the mixture of γ - $\text{Ca}_2\text{P}_2\text{O}_7(400)$ and CaCO_3 (molar ratio 2 : 4) sintered at 1350 and 1400°C.
2. DCPD synthesized from cattle bone (Jinawath, 1995)

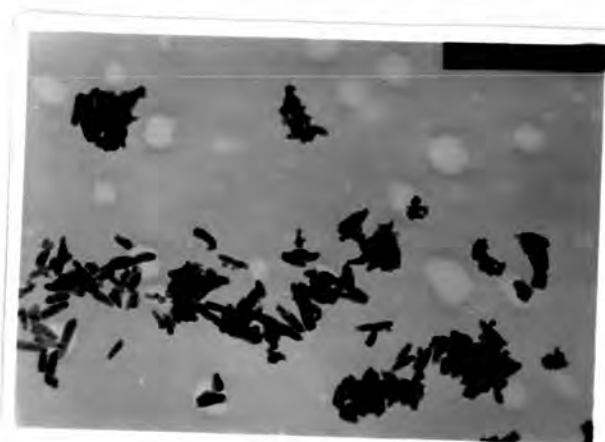
Seeding agent

1. HA(Hydroxyapatite) synthesized from cattle bone(Lorprayoon, 1989)
 - HA(70): dried at 70°C having specific surface area of 140.15±4.20 m²/g. (BET)
 - HA(550): calcined at 550°C having specific surface area of 73.62 m²/g. (BET)
2. CHA(Hydroxyapatite) synthesized from pure chemical(Hayek, 1963)
 - CHA(70): dried at 70°C having specific surface area of 83.21±0.19 m²/g. (BET)
 - CHA(550): calcined at 550°C having specific surface area of 47.42 m²/g. (BET)



0.23 μm

Fig. 3.8 TEM micrograph of HA calcined at 550°C



0.24 μm

Fig. 3.9 TEM micrograph of CHA calcined at 550°C

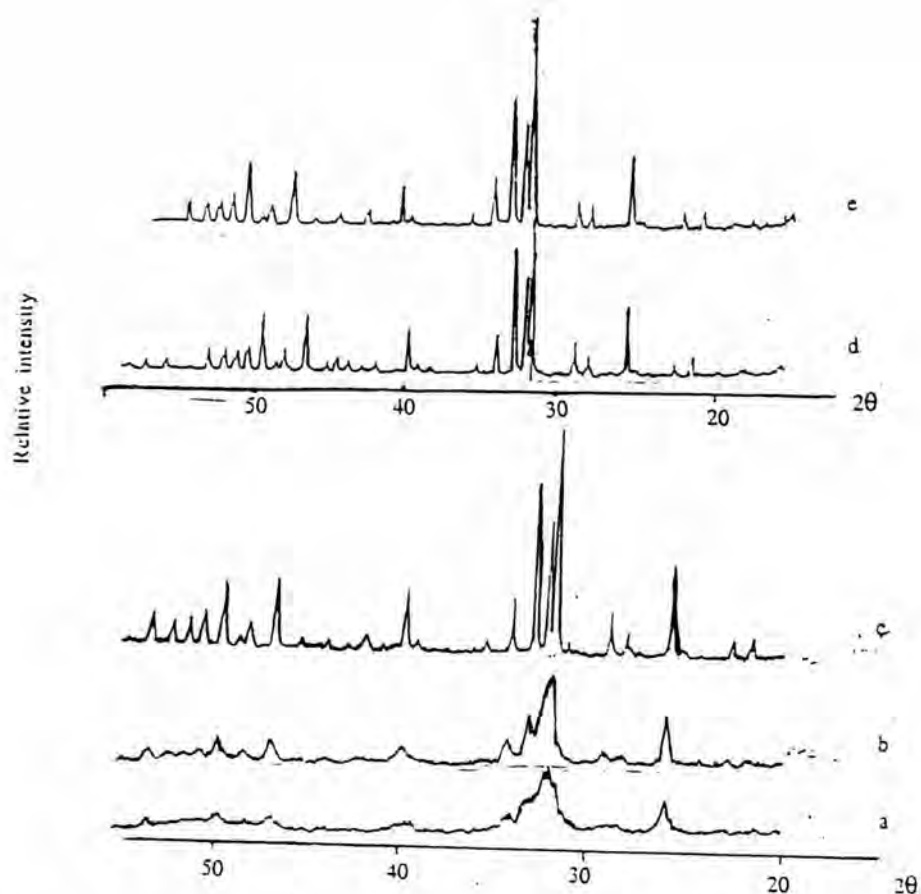


Fig. 3.10 XRD patterns of : a) HA dried at 70°C ; b) CHA dried at 70°C ; c) HA calcined at 1280°C ; d) HA calcined at 550°C ; e) CHA calcined at 550°C

Preparation of CPC powder

High purity TTCP prepared from cattle bone was pulverized for 3, 5, 6, 7 and 8 h in absolute ethanol*, in a ball mill, to increase the specific surface area for a better reactivity. After that it was dried in oven and stored in a vacuum desiccator combining with DCPD in the mortar to obtain the homogenized equimolar mixtures. Various hydroxyapatites (dried at 70°C , calcined at 550 and 1280°C) were each added to the mixtures as a seeding agent to improve compressive strength and setting time. (Fig. 3.11)

*Ethanol absolute GR 99.9% (analytical grade, Merck)

Preparation of specimens

3.2 g of calcium phosphate cement (CPC) powder consisting of equimolar mixture of TTCP and DCPD was mixed with 25 mmol/l H_3PO_4 # solution to form pastes of various solid to liquid ratios (P/L), kneaded and poured into a 5 hole stainless steel mold (6 mm diameter \times 12 mm high) by spatulation and left to set under light pressure.

Following the compressive strength measurement, portions of the crushed pieces of the specimens were ground to fine powders. Those powders were characterized for phase analysis by XRD.

The effect of seeding by HA ranging from 10 to 40 wt% on the rate of setting and hardening of the cement specimens was investigated at time intervals of 0, 1, 2, 3, 4, 24 hours, 7 days, 14 days and 30 days.

H_3PO_4 orthophosphoric acid (analytical grade ,BDH 10173)

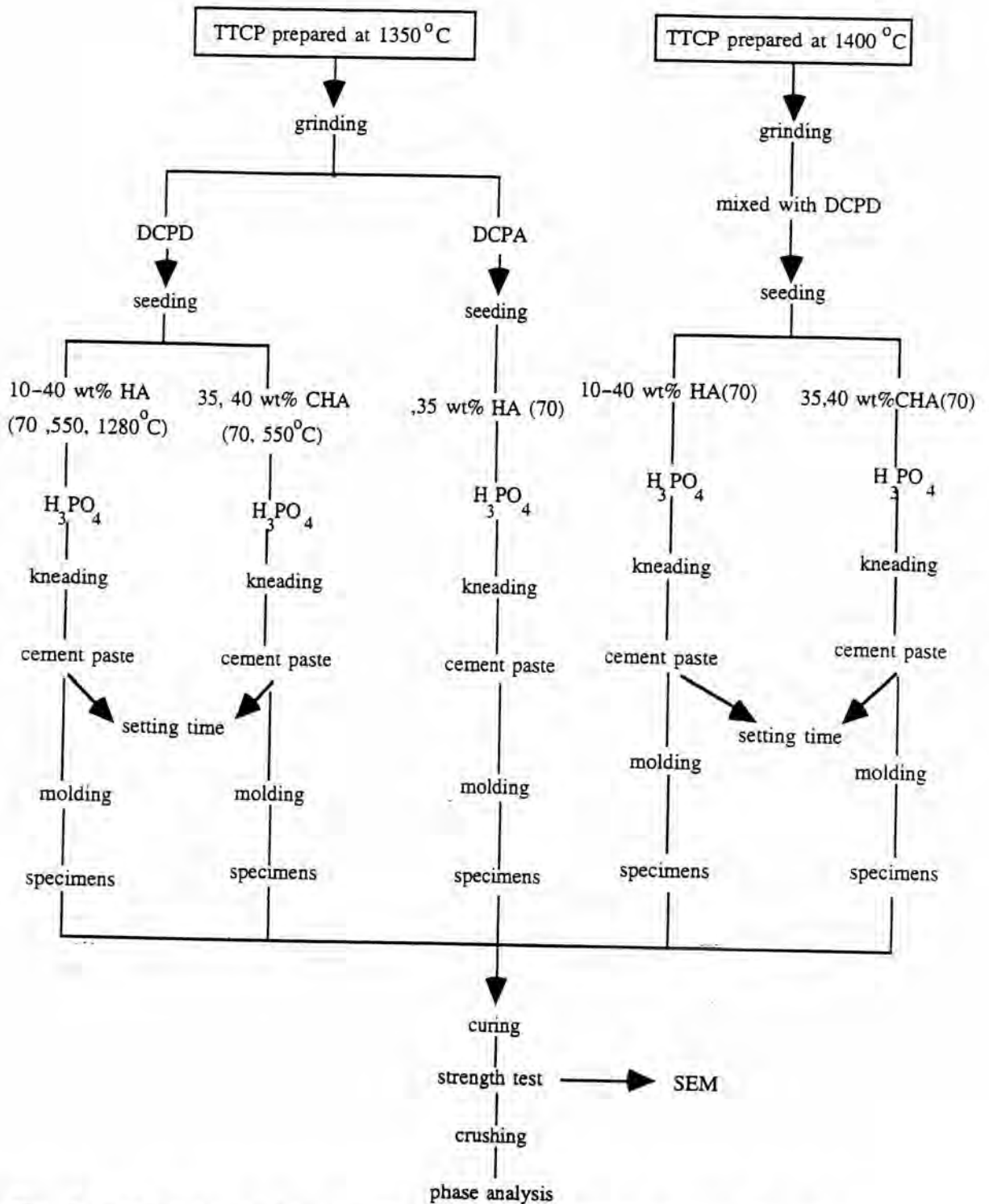


Fig. 3.11 Flow chart of the preparation of self-setting calcium phosphate cement from TTCP

Characterization of calcium phosphate cements

1. Structure of cement (X-ray diffraction method)

After the compressive strength measurement, portions of the crushed piece of specimen were ground to fine powders and were characterized by powder x-ray diffraction (XRD) to determine the conversion of the starting materials and the identities of the products as a function of time.

X-ray diffraction patterns were recorded on a Philips diffractometer with copper $K\alpha$ radiation generated at 40 kV and 30 mA. All data were collected in a continuous scan mode ($2^\circ/\text{min}$, time constant 1 s) on a strip-chart recorder to identify the structure of calcium phosphate cements.

2. Setting time of the cement paste

The test was performed according to method described in Appendix S : JIS T 6602. A Gillmore needle having a weight of 113.6 g. (the section area of the needle was 3.52 mm^2) for the initial setting time. A Gillmore needle having a weight of 454.5 g. (the section area of the needle was 0.88 mm^2) for the final time.

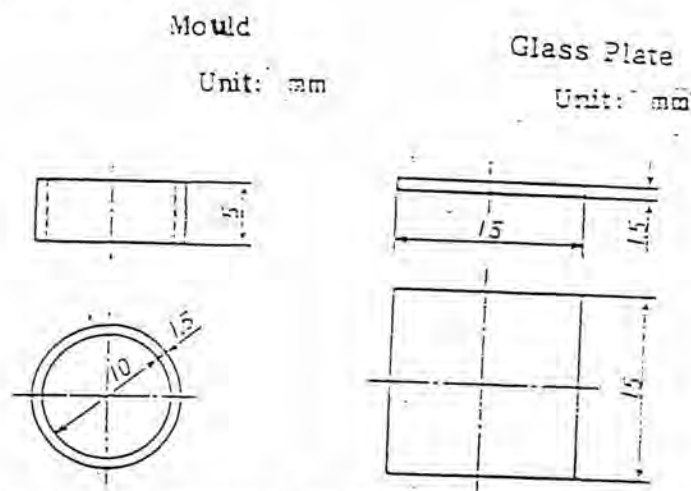


Fig. 3.12 Mold and glass plate for setting time test



Fig. 3.13 Setting time testing instrument (Gillmore)

3. Compressive strength

The test was performed according to the method described in Appendix T : ASTM F 451-86

More specifically, the mold was placed in a humid desiccator maintained at room temperature, and a relative humidity of 100% for 24 hours. The specimens were pressed at a cross head speed of 0.5 mm/min (US. 5,092,888, Iwamoto et al., 1992) by instron corporation (series IX automated materials testing system 7.26.00) until the test piece was crushed to measure the compressive strength (MPa).

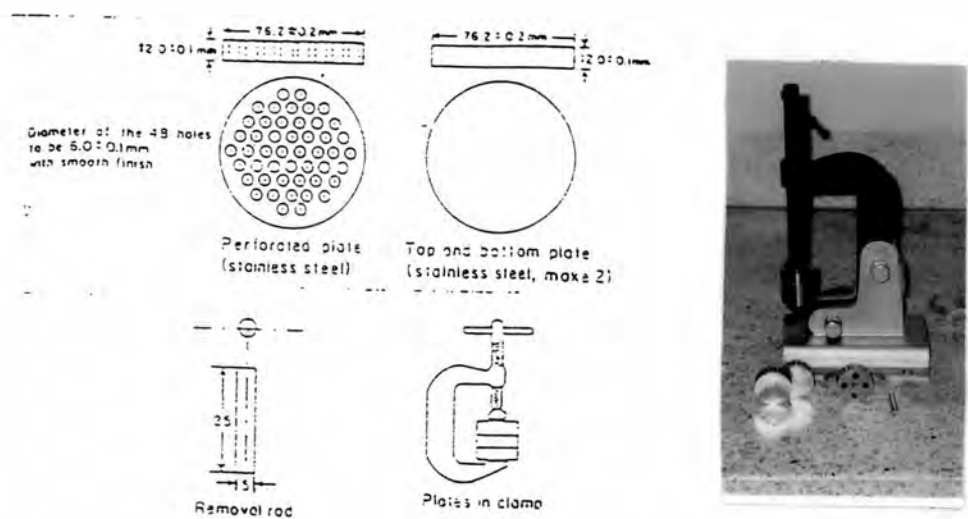


Fig. 3.14 Compressive specimen mold



Fig. 3.15 Humid desiccator for curing the specimens (80 ± 10 % humidity)



Fig. 3.16 Material testing machine, Instron corporation
(Series IX automated materials testing system 7.26.00)