

## Chapter 3



### Experimental Procedure

#### 3.1 Starting Materials

Two kinds of hydroxyapatite powders (MP and TP) were prepared as follows:

##### 3.1.1 MP Preparation

MP was prepared by the method described by Sombuthawee (1986). Selected parts of cattle bones were calcined in an electric furnace\* at 700 °C for 3 h (heating rate 3.5 °/min) to remove organic materials.

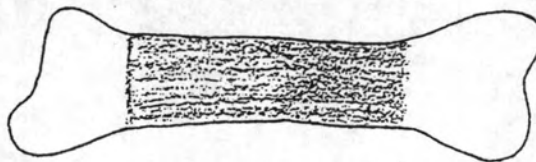


Figure 7. Selected part of cattle bone (shaded region).

\* Duncan Kiln : model LT-3K



Figure 8. Selected part of cattle bone  
before calcination.



Figure 9. Selected part of cattle bone  
after calcination.



Figure 10. Electric furnace used for  
calcination.

Calcined bone was crushed with porcelain mortar. Particle size of the rough powder was controlled by hand sieving (#50 mesh). Then, they were wet milled with high-speed alumina ball mill for 30 min. Medium used for milling was only distilled water (powder:water = 1:1 by weight). After drying, they were powdered into MP.



Figure 11. alumina  
ball mill.



Figure 12. high-speed  
milling machine.



Figure 13. MP

### 3.1.2 TP preparation

The method of Lorprayoon (1989) was used for TP preparation. 10 wt% of calcium nitrate tetrahydrate<sup>+</sup> ( $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ ) was added to MP. Dissolution was done by nitric acid<sup>\*</sup> (1 molar conc.) titration and the solution was brought to pH 1 as illustrated in Fig.14. And then, a solution was brought to pH 10.5 with concentrated  $\text{NH}_4\text{OH}$ <sup>#</sup> resulting in precipitation of TP. This process was done under vigorous stirring.

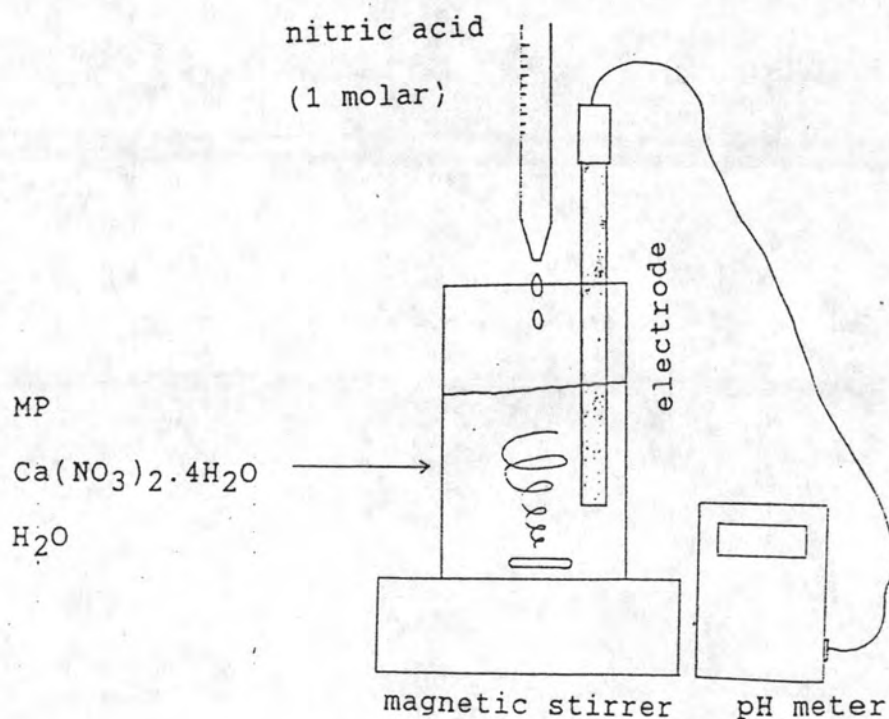


Figure 14. Schematic diagram of MP Dissolution.

+ reagent grade: MERK 2121.0500

\* MERK 456

# BDH prod 10012

Calcium nitrate tetrahydrate was used in the form of 10 wt% solution. For each batch, 20g of MP, 20 ml of calcium salt and 500 ml of water were mixed and magnetic stirred for ~ 5 min before dissolution. Amount of nitric acid (1 molar conc.) used for dissolution was 670 ml and amount of  $\text{NH}_4\text{OH}$  used for precipitation was 275 ml.

The precipitate, suspending in the solution, was heat treated at 40 °C for 15 min and then this solution was filtered and thoroughly washed with distilled water on a büchner funnel (Fig.15). After filtration, the filter cake was dried at 60 °C for 24 h, powdered in porcelain mortar and screened with sieve number #50 mesh.

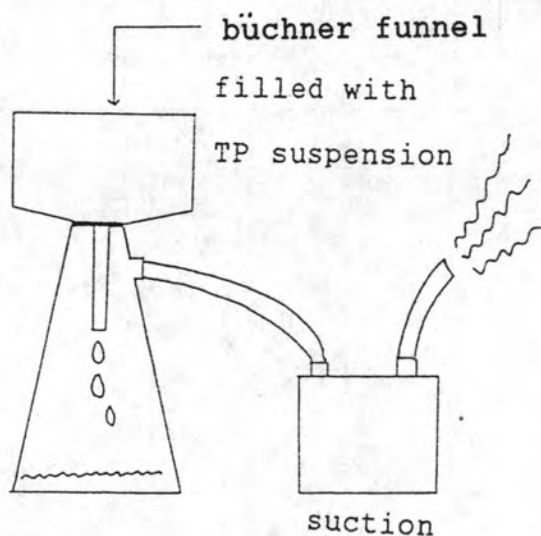


Figure 15. Schematic diagram of filtration process.



Figure 16. TP

### 3.2 Granulation

Granulation was done by Enrich mixing. TP and MP were granulated using polyvinyl alcohol(PVA) as a binder. The process was done by mixing each powder and 1.6 wt% of PVA (in the form of 10 wt% solution) by high-speed ball milling for 5 min. Water was added during mixing to allow homogeneous dispersion(powder:water= 1:1 by weight). Then, the mixed slurry was dried in an oven at 100°C while occasionally stirred to accelerate drying. When the % moisture content was about 10-15 %, the size of granules was selected by hand sieving, as shows in Fig.17.

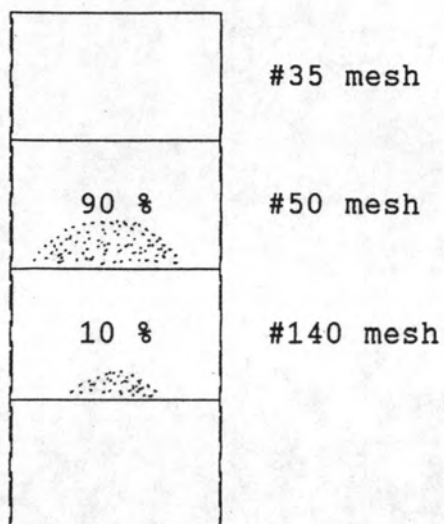


Figure 17. Illustration of granule size selection by hand sieving.

After sieving, granules remained on sieve #50 mesh (90 wt%) and sieve #140 mesh (10 wt.%) were mixed and aged for 3 day (in closed package to preserve moisture content) before using on next steps.



Figure 18. TP granules.

### 3.3 Pressing

The granules of TP and MP were hydraulic pressed\* in metal molds with the pressure  $1 \text{ ton/cm}^2$  to form a rectangular bar having dimension of  $4 \times 4 \times 40 \text{ mm}^3$ .

After filling 1.69 g of granules in the mold, about 80 % of desired pressure (1.4 ton) was applied to the granules. Later, the pressure was partly released in order to remove air entrapped in the mold. Then, desired pressure (1.8 ton) was continuously applied. Finally, the compact bar was removed from the mold, dried at  $110^\circ\text{C}$  for 24 h, and polished with abbrasive paper.



Figure 19. Hydraulic pressing machine.



Figure 20. metal mold.

\* ENERPAK model no. ESE-373-00 SEFS20





Figure 21. Compact bars (MP).

### 3.4 Calcination

Compact bars were calcined to remove binder (PVA), because binder in compact bars would dissolve when contact with coating slips (on next step) and then, the compact bar could deform.

All compact bars were placed on alumina plate. The calcination was operated at 500 °C for 10 min in an electric furnace\*. The heating rate was 180° per h. After calcination, all calcined specimens were kept in dessicator, ready for the next step.

\* Nabertherm model N20/HR  
with firing chamber ~ 25x25x25 cm<sup>3</sup>



Figure 22. Electric furnace used for calcining compact bars.

### 3.5 coating

Five coating slip compositions were prepared from the two hydroxyapatite powders, as shown in table 4.

Polyvinylpyrrolidone\* (PVP) ( $M_w = 40000$  g/mol) was added as deflocculant. Each composition was mixed with high-speed ball mill for 5 min. Then, the coating slip was passed through sieve no. #50 mesh in order to remove bubbles resulted from high-speed milling. All slips were used instantly after 5 day aging.

\* polyvinylpyrrolidone K30 (lab. grade)

Fluka 81422

Calcined specimens were dipped into coating slip for the same time (3 sec) to control the thickness. Coated specimens were dried at room temperature for 24 h before sintering.

coating slip no.	composition			
	TP (wt.%)	MP (wt.%)	PVP (wt.%)	wt.% solid content
1	-	100	1	37
2	25	75	1	37
3	50	50	1	37
4	75	25	1	37
5	-	100	1	37

Table 4. Composition of slips.

After coating, there were ten different coated specimens as shown in Table 5.

coated sample	BODY	COATING SLIP MP:TP (by wt.)
MN	MP	uncoated
M25	MP	75:25
M50	MP	50:50
M75	MP	25:75
M100	MP	0:100
TN	TP	uncoated
T25	TP	25:75
T50	TP	50:50
T75	TP	75:25
T100	TP	100:0

Table 5. Coated specimens used in the experiment

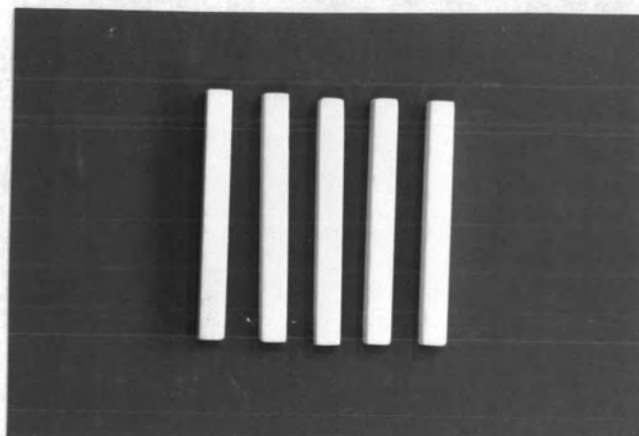


Figure 23. Coated TP bars.

### 3.6 Sintering

All specimens were sintered in the same furnace used to calcine compact bars at 1100 °C, 1200 °C and 1300 °C for 1 h. Heating rate was 6 °/min. All specimens were allowed to cool in the furnace.

To prevent distortion, the specimens were placed vertically and carefully grounded with high-grade alumina powder. (They were distorted during placing on alumina plate for calcination.)

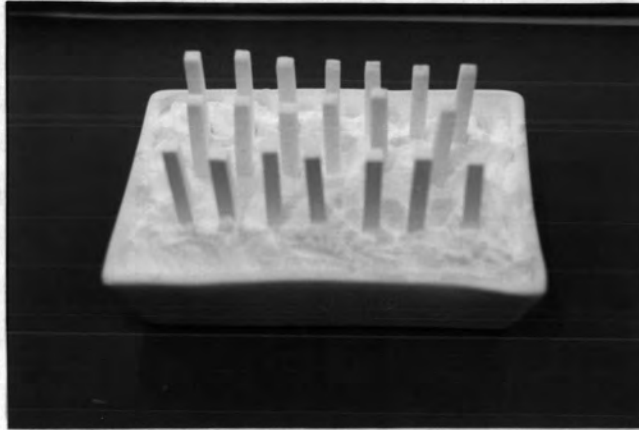


Figure 24. Placing of specimens for sintering.

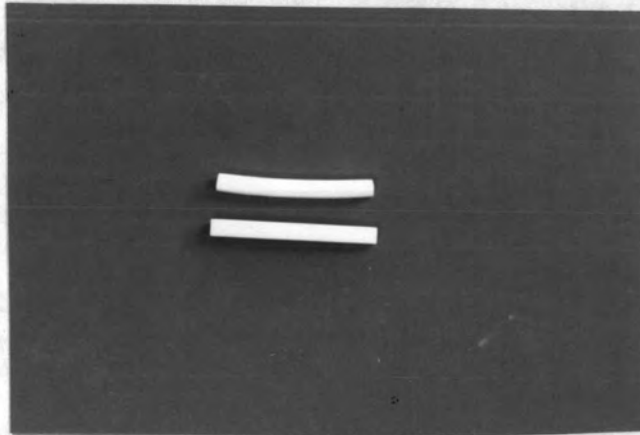


Figure 25. (upper) TP body (coated with 50:50 (MP:TP) slip, which was horizontally placed on alumina plate and sintered at 1300 °C.

(lower) TP body with the same condition but placed vertically and grounded with alumina powder.

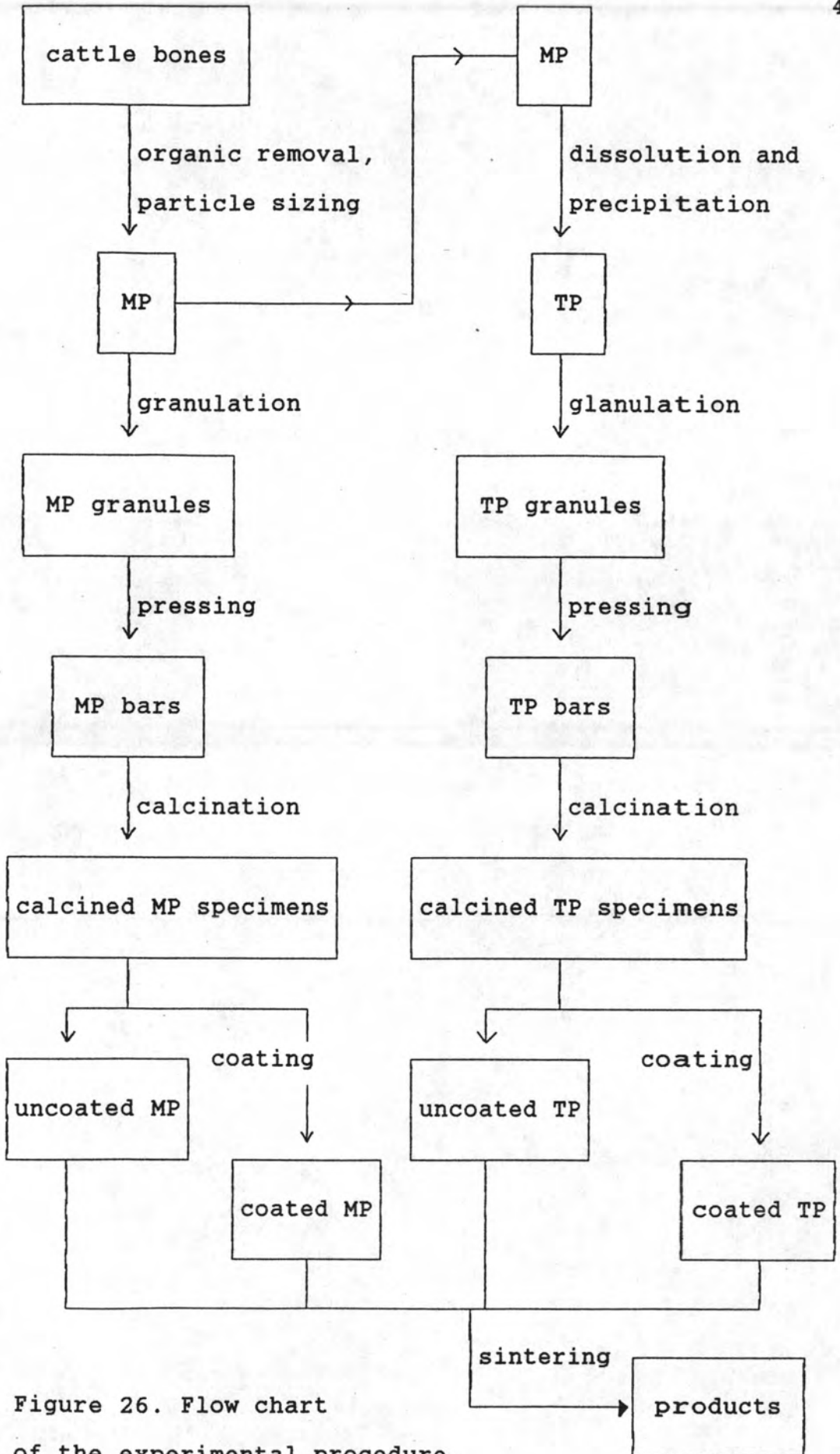


Figure 26. Flow chart of the experimental procedure.

### 3.7 Characterization

All characterization of each processing step was summarized in Table 6.

processing step	sample	characterization
powder preparation	MP, TP	- phases present - microstructure - particle size distribution - Ca:P ratio* - impurities#
granulation	granule	- filled density - tap density - flow rate
pressing	compact bar	- bulk density - microstructure

(to be continued)

Table 6. characterization of samples on each processing steps.

\* by Department of Science and Survices, Bangkok 10400.

# by Mineral Assey and Services, Bangkok 10900.



continued:

processing step	sample	characterization
calcination	calcined	- bulk density
	specimen	- % water absorption - microstructure
slip preparation	coating slip	- rheological property
sintering	- uncoated product	- bulk density - % water absorption - % apparent porosity - % volume shrinkage - microstructure - thermal expansion - phases present
	- coated product	- surface condition

Table 6. Characterization of samples on each processing step.

### 3.7.1 Microstructure and Surface Condition

Microstructure and surface condition of all samples were observed with scanning electron microscope (SEM)\*.

In SEM a fine beam of electrons of medium energy (5-50 keV) is caused to scan across the sample in series of parallel tracks. These electrons interact with the sample, producing secondary electron emission (SEE), back scattered electrons (BSE), light or cathodoluminescence and x-rays. Each of these signals can be detected and displayed on the screen of a cathode ray tube like a television picture. Examinations are generally made on photographic records on the screen (Allen, 1981).

For powder and granule observation, they were carefully sprinkled onto the SEM target stubs, pre-coated with carbon, dried and sputtered with Au.

For compact bars, calcined specimens and uncoated product, the fracture surfaces were observed. They were broken into size of  $\sim 3 \times 3 \times 3 \text{ mm}^3$  by porcelain mortar. Before placing on target stubs, uncoated products were etched with 0.25 %  $\text{H}_3\text{PO}_4$  for 15 sec in ultrasonic water bath, washed with methanol and dried.

\* JEOL model JSM-35CF

from: Scientific and Technological Research Equipment  
Centre, Chulalongkorn University.

To observe surface condition of coated products(sintered bar), they were cut into the length of ~3.5-4 mm by low-speed cutting machine\*. All samples were carefully placed on the target stubs while the upper sides were the observed site. Then, they were pre-coated with carbon, dried and sputtered with Au.

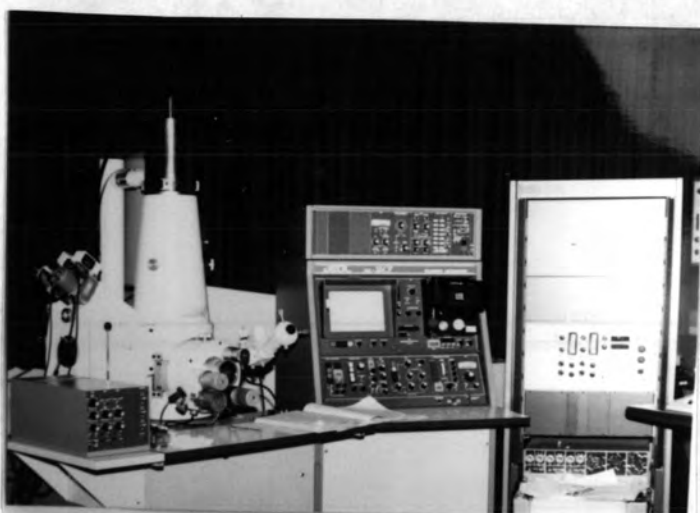


Figure 27. Scanning electron microscope.

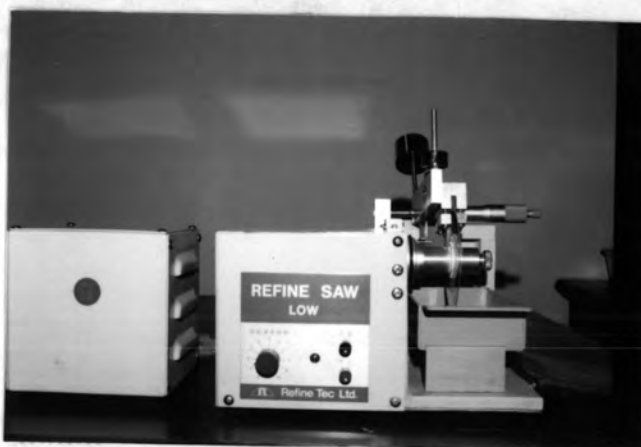


Figure 28. low-speed cutting machine.

\* Refine saw (low) model RCA-005  
Refin Tec Ltd.

### 3.7.2 Phases Present

Phases Present of samples were detected by X-ray diffractometer\* (XRD).

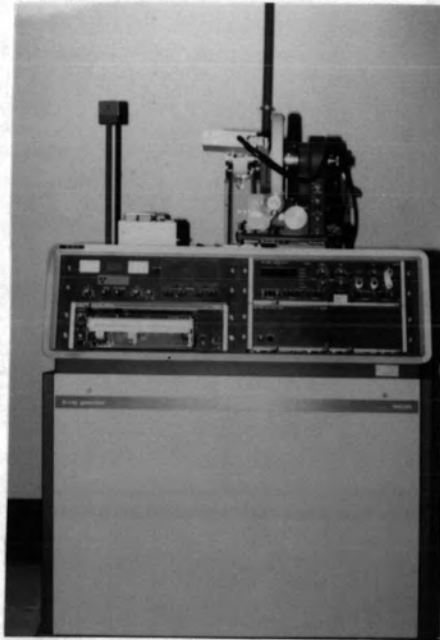


Figure 29. X-ray diffractometer.

\* Phillips model no. DY1023 type PW1730/10  
from: Department of Geology, Faculty of Science,  
Chulalongkorn University.

XRD is an instrument for studying crystalline (and noncrystalline) materials by measuring the way in which they diffract X-rays of known wavelength (Cullity, 1967). The essential features of a diffractometer are shown in fig.30. A powder specimen C, in the form of a flat plate is supported on a table H, which can be rotated about an axis O perpendicular to the plane of the drawing.

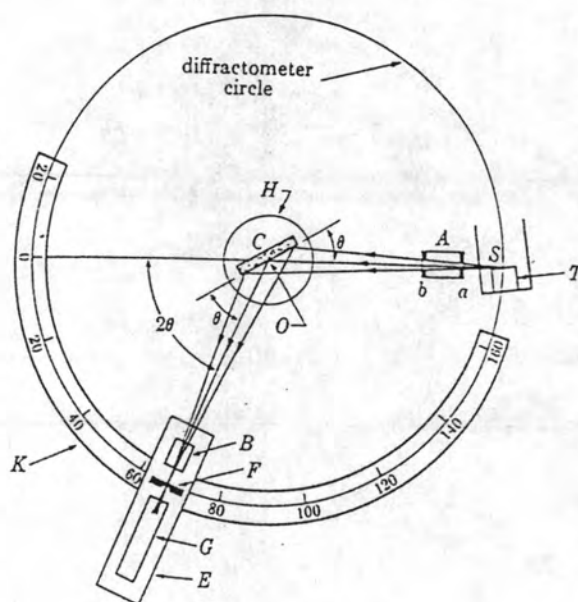


Figure 30. Schematic of X-ray diffractometer.  
(Cullity, 1967)

The X-ray source is S, the line focal spot on the target T of the X-ray tube; S is also normal to the plane of the drawing and therefore parallel to the diffractometer axis O. X-rays diverge from this source and are diffracted by the specimen to form a convergent dif-

fracted beam which comes to a focus at the slit F and then enters the counter G. A and B are special slits which define and collimate the incident and diffracted beams.

The receiving slits and counter are supported on the carriage E, which may be rotated about the axis O and whose angular position  $2\theta$  may be read on the graduated scale K. The supports E and H are mechanically coupled so that a rotation of the counter through  $2\theta$  degrees is automatically accompanied by rotation of the specimen through  $\theta$  degrees.

When a randomly orientated aggregate of small crystal fragments (powder) is irradiated with a monochromatic beam of X-rays, the various planes of atoms will diffract the X-ray beam at angles determined by the spacing between the planes, according to the Bragg law,

$$n\lambda = 2d \sin\theta$$

where  $\theta$  is the diffraction angle for lattice spacing,  $\lambda$  is the wavelength of the X-rays, and  $n$  is an integer (Reed, 1989). These diffracted beams are recorded on film placed appropriately around the sample or by a scanning detector. The identification of phase is accomplished by comparing the  $d$  spacings and relative intensities of the sample material with reference data for known materials.

To detect the phases present, samples were dried and ground into fine powders in a porcelain mortar. The powders were compacted in a recess of plates. These plates were then inserted in the XRD specimen holder. XRD was run with copper K $\alpha$  radiation and Ni filter. Number of  $2\theta$  was started at  $20^\circ$  and finished at  $50^\circ$ . The scanning velocity was  $2^\circ$  per min.

### 3.7.3 Particle Size Distribution

Particle size distribution of MP and TP were determined by particle size analyzer\*.



Figure 31. Particle size analyzer.

\* Shimadzu centrifugal particle size analyzer  
model SA-CP2

The principle of particle sizing was based on sedimentation of particle under centrifugation. One of the two cell tubes contained a reference solution (1 wt% calgon solution). the other one contained a suspension of particles dispersed in reference solution. Cell tubes were placed in the analyzer rapidly and then, centrifugation began. Particle size was determined by the light from halogen lamp which passed through each particle at elevated times and by relation:

$$V = \frac{R w^2 D^2 (\rho_p - \rho_l)}{18 n}$$

where

D = equivalent spherical diameter of particle (cm)

$\rho_p$  = density of particle (g/cm<sup>3</sup>)

$\rho_l$  = density of reference solution (g/cm<sup>3</sup>)

n = viscosity of reference solution (g/cm/sec)

R = distance between center of rotation and position of particle where the light pass through (cm)

w = speed of rotation of centrifuge (radian/sec)

V = velocity of particle (cm/sec)



#### 3.7.4 Flow Rate, Filled Density and Tap Density

Flow rate, filled density and tap density of granules were determined. These parameters could show the packing efficiency of granules when filled in the mold (before pressure was applied).

The method was based on American standard test method\* (ASTM). 20 g of granules was carefully loaded into a flowmeter funnel and permitted to run into the graduated cylinder (which placed on a tapping device as shown in Fig.32.)



Figure 32. Flow rate, filled density and tap density apparatus.

\* ASTM designation: B527-85 (Reapproved 1991)

The time when granules began running until the funnel was empty, was observed and flow rate was calculated as:

$$\text{flow rate (sec/g)} = \text{time (sec)} / \text{weight (=20g)}$$

The volume of granules contained in cylinder was recorded and filled density was calculated as:

$$\text{filled density (g/cm}^3\text{)} = \text{weight (=20g)} / \text{volume (cm}^3\text{)}$$

Then, the tapping device was started and continued for 3000 taps. The volume of granules was recorded again and tap density was calculated as:

$$\begin{array}{rcl} \text{tap density} & = & \text{weight} / \text{tap volume} \\ \text{(g/cm}^3\text{)} & & \text{(20g)} \quad \text{(cm}^3\text{)} \end{array}$$

### 3.7.5 Rheological Property

Viscosity is a property of fluids, is due to internal friction, and is manifest in the rate of flow. The unit of viscosity is named the *poise* after Poisseule, a French physicist who studied this property. This unit has been defined by Maxwell as "the tangential force per square centimeter of area of either of two horizontal planes, one centimeter apart, one of which is fixed,

while the other moves with a unit velocity, the space between being filled with a liquid empirically selected as having a unit viscosity."(Parmalee, 1973)

Viscosity can be considered as the ratio of shear stress to shear rate. The rheological model is a rectangular body of liquid of very thin layers superimposed on one another (fig.33). Assume that the top is movable, then a force(F) acting on an area A will pull sideways on the top. The pulling action is defined as shear stress( $\tau$ ) equal to  $F/A$ . As the top layer moves under shear stress, it pulls the layer directly under it. This in turn pulls the third layer. It is this relay action which is transmitted by drag through the rectangular pile as the base is held stationary on the substrate.

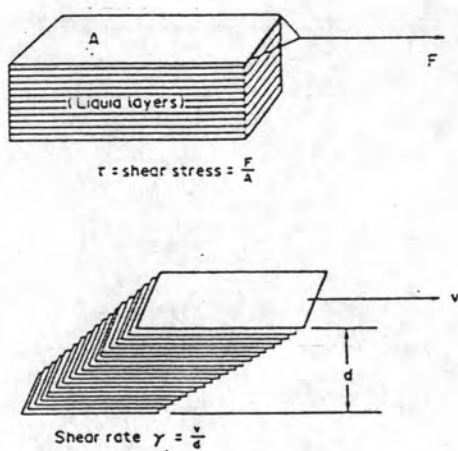


Figure 33. Rheological model.

(Taylor and Bull, 1986)

With the velocity of the top layer  $V$  and the depth of the liquid  $d$ , the velocity gradient is defined as shear rate ( $\dot{\gamma}$ ) and equals  $V/d$ .

$$\text{viscosity} = (F/A) / (V/d) = \tau / \dot{\gamma}$$

Plot of viscosity against shear rate or viscosity profiles are rheological characteristics of a particular slip.

Rheological property of coating slips was tested. The experiment was based on ASTM\*. The measurement was done by Brookfield viscometer#.



Figure 34. Brookfield viscometer.

\* ASTM designation: D2196-86 (reapproved 1991)

# Brookfield Digital Viscometer model RVTD A04184

Before testing, viscometer was calibrated and 500 ml of coating slips contained in 500 ml beaker was stirred by magnetic stirrer at the same speed for 10 min. The spindle no.2 was used. After stirring, viscosity of coating slips was instantly measured with various rotation speed. The scale reading was done at the time of 7 sec from the beginning of the rotation. Viscosity was calculated as:

$$V = fS$$

where V = viscosity of slip (cP), f = factor for converting scale reading to viscosity and s = scale reading of viscometer.

### 3.7.6 Thermal Expansion

MP and TP compact bars and compact bars consisted of various MP:TP composition (MP:TP = 25:75, 50:50 and 75:25) were thermal expansion tested with a dilatometer\*.

\* TMA-50 Shimadzu thermomechanical analyzer model BLW-50 from: Compound Clay Co., Ltd.



Figure 35. Dilatometer

Each composition was mixed and pressed (by hydraulic pressing machine) with the pressure of 1 ton per  $\text{cm}^2$ . The  $4 \times 4 \times 40 \text{ mm}^3$  compact bars were sintered at 1100, 1200 and 1300  $^{\circ}\text{C}$  for 1 h with heating rate  $6^{\circ}$  per min. Sintered bars were carefully cut by low-speed cutting machine, which allowed  $10 \pm 0.5 \text{ mm}$  in length. Then they were polished with abrasive paper until the cross-section surfaces were smooth and perpendicular to the bar length, dried and kept in dessicator before testing. Dilatometer was run from room temperature to 1000  $^{\circ}\text{C}$  with heating rate  $10^{\circ}/\text{min}$ . The maximum temperature testing was limited by efficiency of the equipment.

3.7.7 Bulk Density, % Theoretical Density,  
% Water Absorption and % Apparent Porosity

The test method was based on ASTM\*. After drying, bulk density of uncalcined compact bars were determined by weighing and size measuring.

$$\begin{array}{rcl} \text{bulk density} & = & \text{weight} / \text{volume} \\ (\text{g/cm}^3) & & (\text{g}) \quad (\text{cm}^3) \end{array}$$

For calcined and sintered bars, they were dried, weighed and placed separately in beakers containing distilled water. Then they were boiled for 5 h. During boiling, the specimens were immersed in water at all time. After the 5-h boiling, they were allowed to soak for 24 h. Then, each specimen was weighed while suspended in water (suspended mass, S). The weighing was done by placing the specimens in a wire loop suspended from an arm of the balance (Fig.36).

After determination of the suspended mass, the specimens were lightly blotted with cotton cloth (by rolling specimen lightly on the wet cloth) to remove all excess water from the surface, then, weighed (saturated mass, M).

\* ASTM designation: C373-88

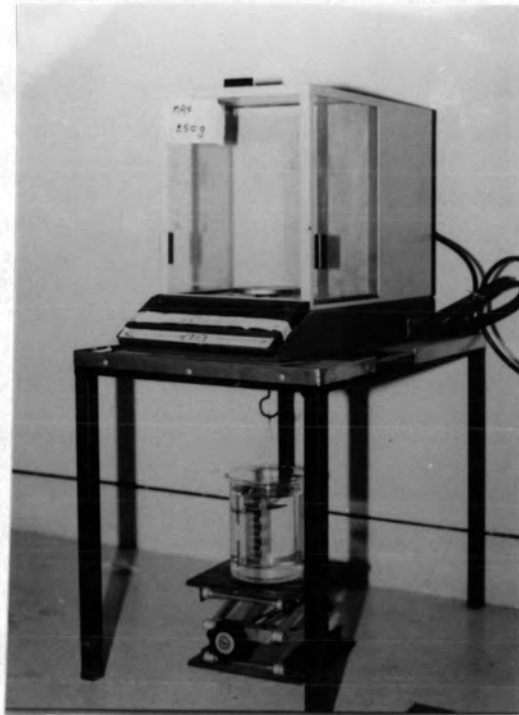


Figure 36. suspended weighing

Bulk density, % apparent density and % water absorption were calculated as

$$\text{bulk density} = D / (M-S)$$

$$\% \text{ apparent porosity} = [(M-D)/(M-S)] \times 100$$

$$\% \text{ water absorption} = [(M-D)/D] \times 100$$

where D = dried weight

S = Suspended weight

M = Saturated weight

% Theoretical density was calculated as:

$$\% \text{ theoretical density} = (\text{bulk density} / 3.16) \times 100$$

where theoretical density of hydroxyapatite is 3.16 g/cm<sup>3</sup>



### 3.7.8 % Volume Shrinkage

% Volume shrinkage of sintered bars was calculated as:

$$\% \text{ volume shrinkage} = [(V_2 - V_1) / V_1] \times 100$$

where  $V_1$  = volume of dried specimen before sintering  
and  $V_2$  = volume of specimen after sintering.

## 3.8 Strength Measurement

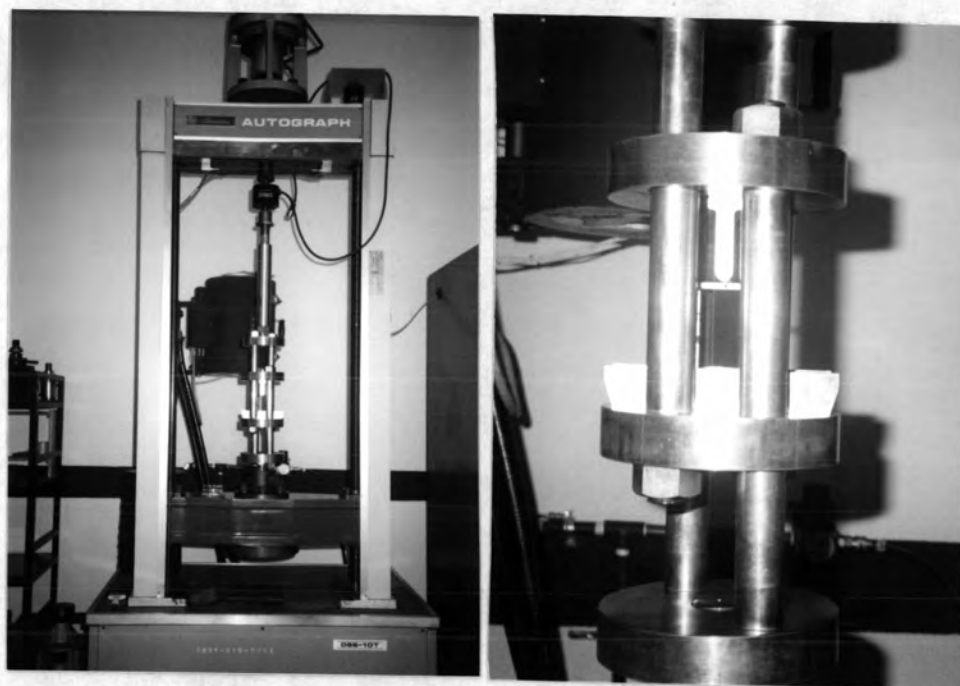
Strength of sintered specimens was characterized in two ways, compressive and flexure testings.

### 3.8.1 Flexural Strength

Three point flexure testing was conducted with universal testing machine\* as shown in Fig.37.

Before testing, sintered bars were size measured, dried at 110 °C for 24 h and cooled in dessicator.

\* Shimadzu universal testing machine model DSS-10T  
from: Scientific and Technological Research Equipment  
Centre, Chulalongkorn University.



a

b

Figure 37-a. Universal testing machine.

37-b. 3-Point flexure testing.

The test bar was supported at the ends with span length (L) 3.4 cm and load (with the cross-head load 10 kg and cross-head speed 0.1 mm/min) was applied either at the center.

Flexural strength could be calculated as  $(3PL)/(2bd^2)$  as indicated in Fig.38. Number of testing specimen for each condition was 20. The mean( $\bar{s}$ ) flexural strength was calculated from:

$$\bar{s} = \frac{\sum_{i=1}^n s_i}{n}$$

where  $n = 20$

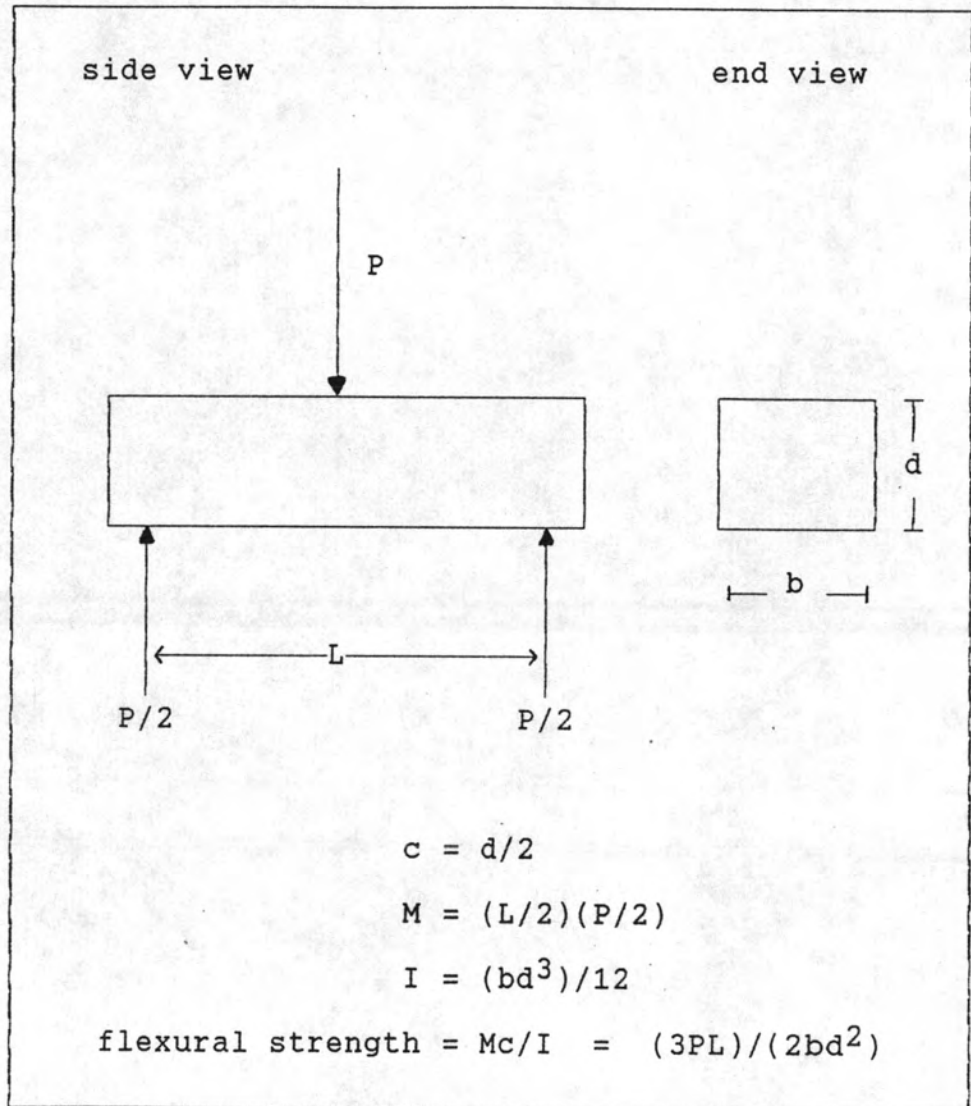


Figure 38. 3-point flexure formulas  
for a rectangular bar  
(Richerson, 1982)

### 3.8.2 Compressive Strength

Compressive strength was conducted with the same kind of testing machine used for flexure testing, with the load cell of 1,000 kg and the rate of loading 0.5 mm/min. Sintered bars were cut\*, while the length to the cross-section width was between 1.9-2.0. Then, they were polished with abrasive paper until the cross-sectional surfaces were smooth and perpendicular to the bar length. The polished samples were dried at 110 °C for 24 h and cooled in dessicator before strength testing.

During load application, small piece of paper board was placed on the top of the specimen(Fig.39) in order to improve the homogeneity and smoothness while the applied load contacted to the test sample's surface.

The number of testing specimens for each condition was 10. Compressive strength was calculated as:

$$\text{compressive strength} = \frac{\text{applied load (kg)}}{\text{cross-sectional area of test sample (cm}^2\text{)}}$$

(kg/cm<sup>2</sup>)

\* refine saw (low) model RCA-005

Refine Tec Ltd.

The unit  $\text{kg/cm}^2$  multiplied with 9.810001 to be the unit of MPa. The mean(s) compressive strength was calculated as:

$$s = \frac{\sum s}{n}$$

where  $n = 10$

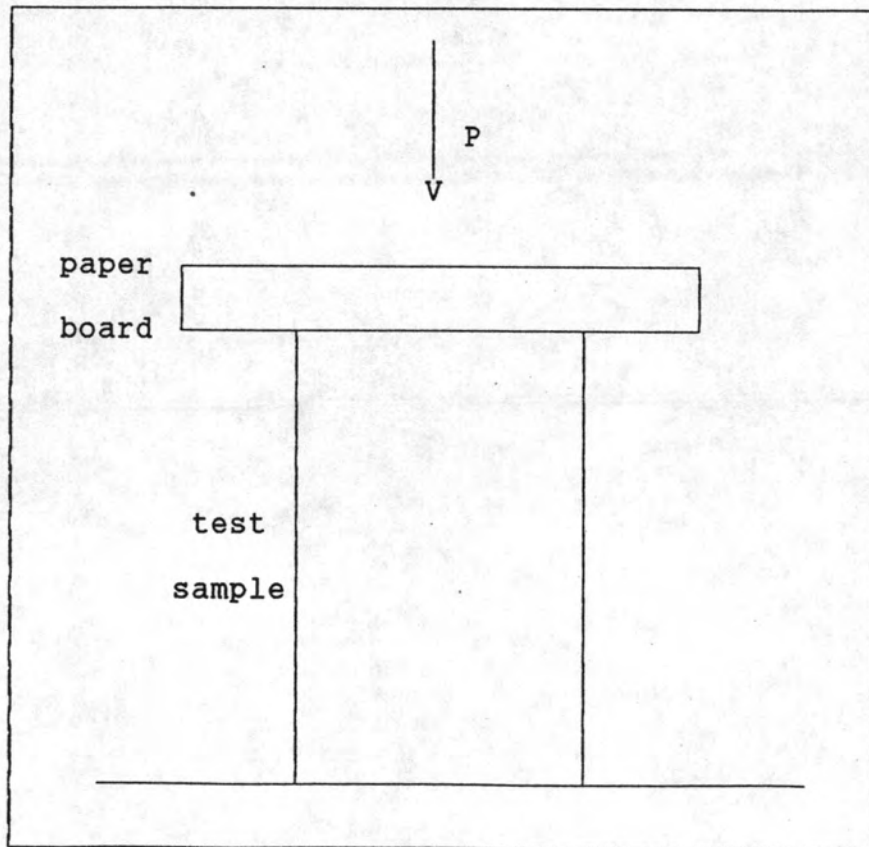


Figure 39. Schematic of compressive strength testing.