CHAPTER 3

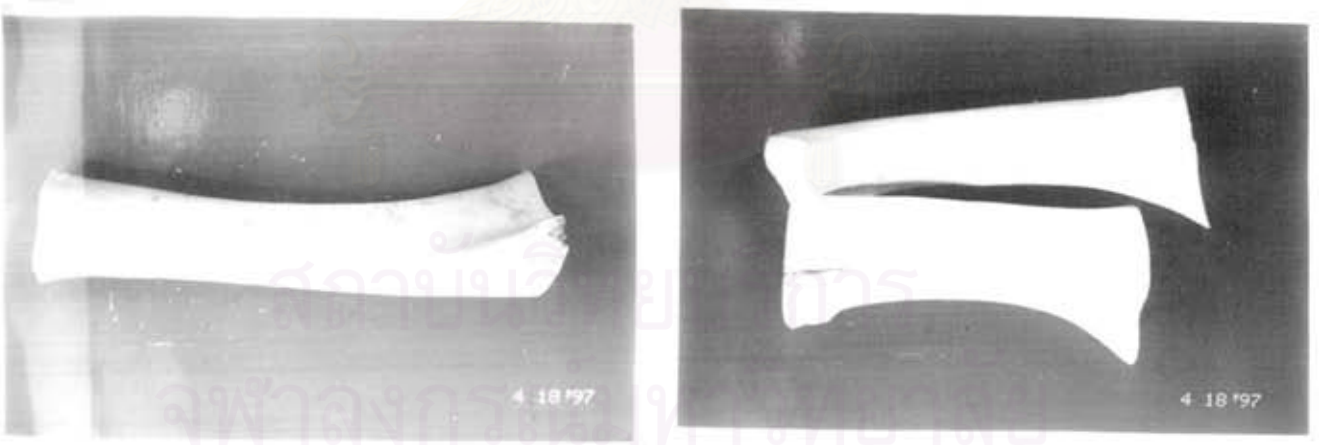
EXPERIMENTAL PROCEDURE

3.1. Starting Materials

Hydroxyapatite from 3 different methods were prepared as follows.

3.1.1 Hydroxyapatite prepared from cattle bone ash (MP)

MP was prepared follow the method of Sombuthawee (1986) Selected part of cattle leg bone (Fig. 3.1) was boiled in water to eliminate fat. After drying, the bone was then calcined in an electric furnace at 700°C for 3 h. in order to remove organic matter. White calcine bone recieved was ground in a porcelain mortar to MP powder and seived by hand pass the 325 mesh (45 μm).



(A) (B)

Fig. 3.1 Selected part of cattle leg bone
(A) Before calcination **(B)** After calcination

3.1.2 Chemically treated hydroxyapatite (TP) from MP powder

TP was prepared as described in the method of Lorprayoon (1989). MP powder was sieved through 140 mesh (106 μm). MP in distilled water was added with little calcium nitrate 4 hydrate+ solution (10%w/v). MP mixture was dissolved by 1 N. nitric acid- slowly to pH = 0.8. Clear solution was basified to pH = 10.5 with concentrated ammonium hydroxide solution* (35%) to precipitrating hydroxyapatite. The precipitate was gently boiled for 15 min.,filtrated and washed with distilled boil water until neutral. The filter cake was dried and then calcined in an electric furnance at 550°C for 2 h. The product was ground in porcelain mortar and sieved by hand pass the 325 mesh (45 μm)

3.1.3 Hydroxyapatite from chemical synthesis (CHA)

CHA was prepared by modifying Hayek method (1963). Calcium nitrate 4 hydrate+ was dissolved in distilled boil water and adjusted a pH to 12 by the addition of concentrated ammonium hydroxide solution.* Into this solution is dropped slowly, with vigorously stirring, a solution of diammonium hydrogen phosphate# (in distilled boil water) which brought pH to 12 with concentrated ammonium hydroxide solution. A voluminous precipitate forms. The filtering properties of the reaction mixture may be improved by gentle boiling it for 10 min. The precipitate was filtrated and washed with distilled boil water.

Dried powder was ground and calcined at 550°C for 2 h. White powder was again ground and sieved by hand pass the 325 mesh (45μm).

+ Analytical grade MERCK 2121
- Analytical grade MERCK 456
* Analytical grade BDH prod 10012
# Analytical grade Carlo Erba 419836

MP, TP and CHA powder were characterised characteristics such as phase, Ca/P ratio, impurity, particle size distribution, surface area, powder density.

3.2 Slip preparation

Ultrasound can be used to deagglomerate material and to reduce particle size. Another mechanical effect of power ultrasound is that it can cause extremely efficient mixing, not only of immiscible liquid to form emulsions, but also to disperse powder into liquids. This has been scaled up for use in industrial processing (Mason, 1990). Owing to this good aspect, ultrasonic cleaning bath was used to disperse MP, TP and CHA powder into water medium during slip preparation. Additional, the vigorously stirred by stirring rod after adding the solid should be done.

Fig 3.2. Ultrasonic cleaning bath

3.2.1. MP slip

MP powders 60 g were added to distilled water 40 cm^3 prepared to slip with 60% solid without any dispersing agent be used. The slip also had low viscosity. But the additional solid content can increase the strength of green body. So, we had increased the solid content by adding MP powder 70 g. into 30 cm^3 distilled water to prepare 70% solid slip. In this slip system, dispex A 40[x] was used as dispersing agent at concentrations. 15% w/v. Binder was polyvinyl alcohol (2% by weight). The products were made from this ingredient with the relatively low strength. To increase the strength, calcium metaphosphate[o] (2.5% by weight) was used as sintering aid.

3.2.2 TP slip

TP powders 47.5 g. were added to distilled water 52.5 cm^3 to prepare slip with 47.5% solids. Dispex A 40 at concentration 27% w/v was used as dispersing agent. Binder was polyvinyl alcohol (2% by weight).

3.2.3 CHA slip

Like TP, CHA powder 47.5 g. in distilled water 52.5 cm^3 was used in preparing 47.5% CHA slip. Dispex A 40 with concentration 10 and 25% w/v was used as dispersing agent. Binder was polyvinyl alcohol (2% by weight).

MP, TP and CHA slip were characterized slip density, pH and viscosity.

x Ammonium salts of polycarboxylic acid from Loxley Public Company Ltd.

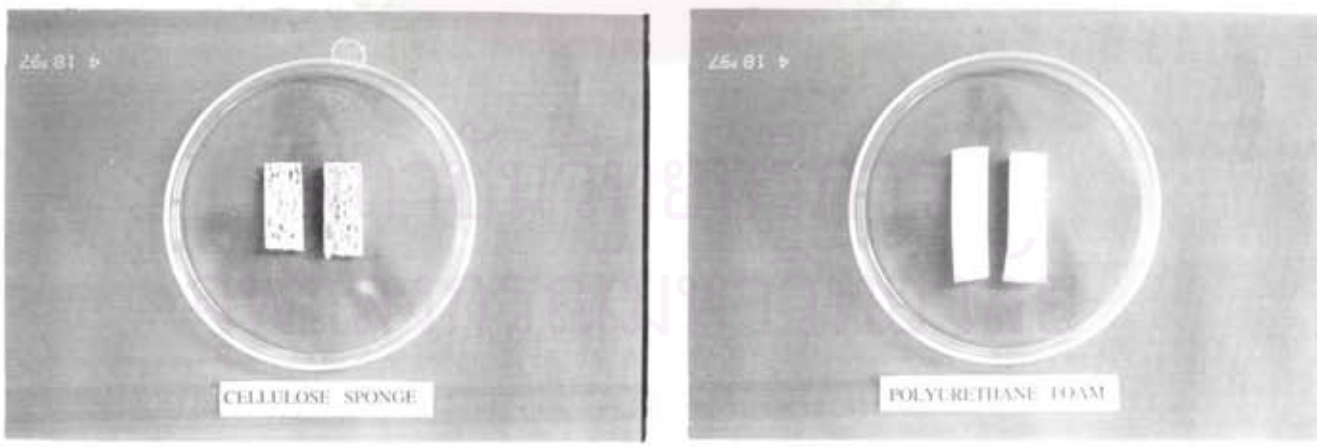
o Recieved from Suwatananon (1996) with CaO : P_2O_5 ratio = 50:50

Table 3.1. Composition of slips

Materials	Composition			
	wt% solids	wt% H_2O	%wt PVA	%wt $Ca(PO_3)_2$
MP	60	40	2	2.5
MP	70	30	2	2.5
TP	47.5	52.5	2	-
CHA	47.5	52.5	2	-

3.3. Preparation of polymeric sponge

Polymeric sponge, such a flexible polyurethane foam(1) or cellulose sponge(2) (100% natural sponge) was cut into rectangular shape and 1.7 x 5.0 x 1.5 cm^3 in size. Sponge and foam were washed with distilled water and dried at 80°C for 1 h. Some properties of these sponges and foams were characterized such as temperature for complete combusion of organic matter in sponge or foam and impurities.



(A) (B)

Fig 3.3. Polymeric sponge

(A) Cellulose sponge **(B)** Polyurethane foam

(1) From Great foam product Co.Ltd.
(2) From 3M Thailand Co.Ltd.

3.4. Impregnated sponge / foam with slips

Slip was poured into a rectangular bowl. Sponges / foams with a little moisture were placed into slip and compressed with spectula 6-7 times. and took this bowl into the chamber of impregnation vacuum BUEHLER LTD model LR 37697 (in Fig. 3.4). Switch on and keep the specimen in vacuum for 10 min. Finish from impregnated vacuum process, excess slip was removed from sponges / foams. The wet preforms were dired at 60 °C and took turn every 10 min. When the preforms dried, weighted and kept in desiccator.

Fig 3.4. Impregnation vacuum

3.5. Sintering

Preform of MP, TP and CHA were burnt out of sponge / foam and sintered at the temperature schedule below. The selection temperature was made according to DTA, DSC and TGA in the previous study.

Table 3.2. Sintering temperature of MP, TP and CHA in experimental procedure

Sponge/foam	burn out temp (°C)	Sintering temp(°C) /soaking time (h)		
		MP 60%,70%	TP 47.5%	CHA 47.5%
Cellulose sponge	900°C / 2 h	1250°C / 2 h	1210°C / 2 h	1150°C / 2 h
Polyurethane foam	400°C / 1 h	1250°C / 1 h	1210°C / 1 h	1150°C / 1 h

in an electric furnace: LINDBERG model LCC 256 PCOMC
Heating rate was 2° C / min and cooled down in 2°C / min

The experimental procedure was written as the following:

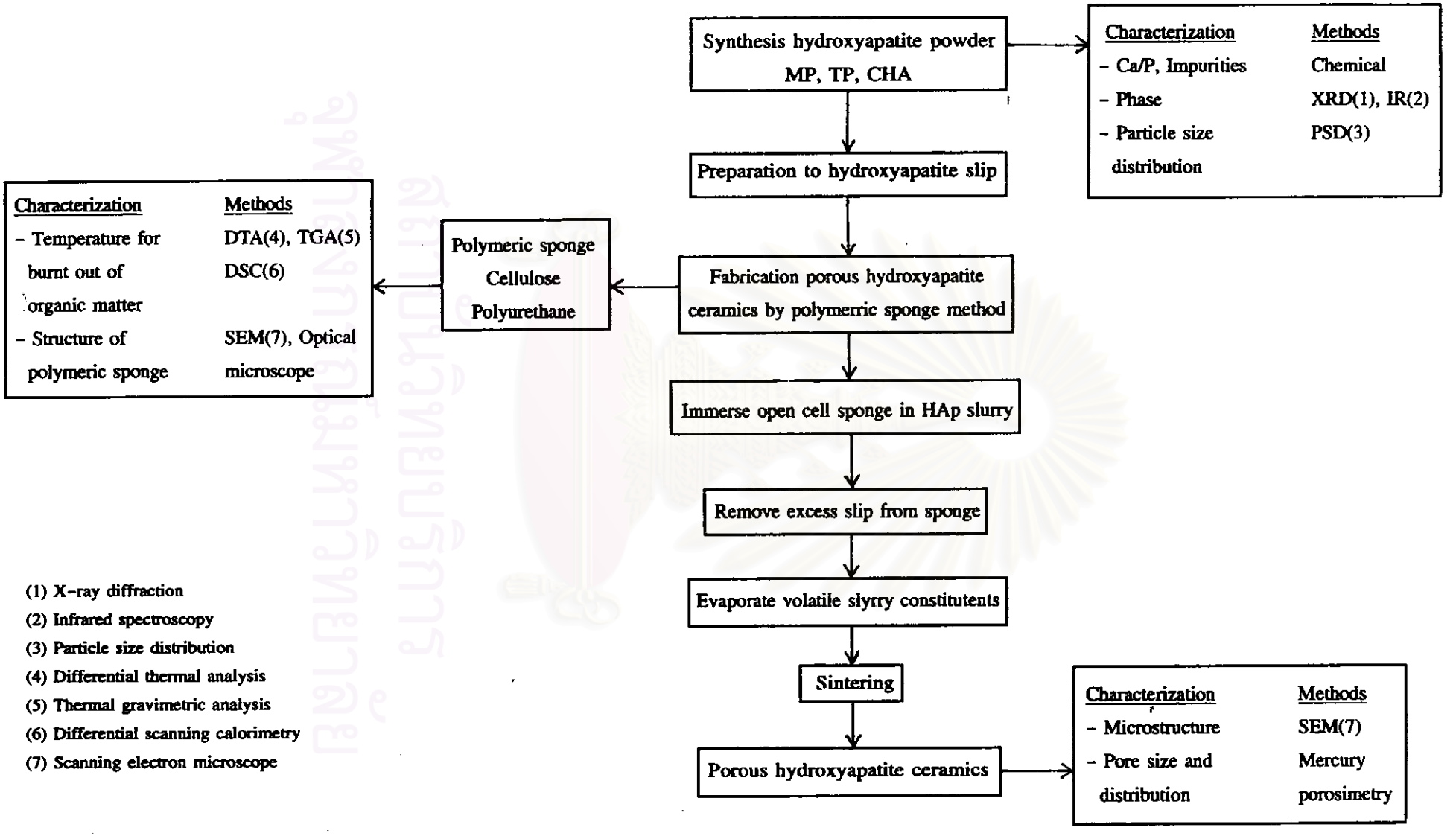


Fig. 3.5. Flowchart for producing porous hydroxyapatite ceramics

3.6. Characterization of hydroxyapatite

3.6.1 Chemical composition and impurities

The concentration of Ca and P was determined by wet chemical method[1] All impurities were determined by inductively coupled plasma (ICP) and instrumental neutron activation analysis (INAA) method[2]

3.6.2 Phase present

The phase of powder and specimen were characterized by X-ray diffraction (XRD). Sampling TP and CHA 2 g. heated to 1280°C / 1/2 h. heating rate 4°C /min in furnace to make well crystals. The MP, TP and CHA powder / specimen were crushed into fine powder and compacted for phases were determined by Philips diffractometer (PW 1730/10) with Cu Kα radiation and Ni filter at 30 mA, 40 KV. A time constant of 1 s. and scanning rate 2°/min were used. 2θ was run from 16° to 60°.

Fig. 3.6. X-ray diffractometer

(1) from Science and Service Department

(2) from Mineral Assays and Service Co.,Ltd.

3.6.3. Functional groups

The functional groups of MP, TP, CHA powders and All specimens were characterized by Fourier Transform Infrared spectrophotometer (FT-IR 1760 X Perkin-Elmer) in fig. 3.10 A little sample with KBr was made into disc. The wave number from 4000 to 400 cm^{-1} were investigated.

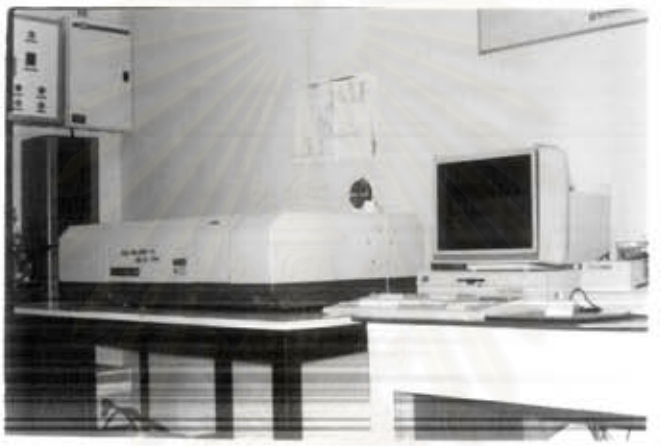


Fig. 3.7. FT-IR spectrophotometer

3.6.4. Particle size distribution

The particle size distribution of the MP, TP and CHA powders were determined by sedimentation technique of particle size analyzer model SA-CP 2 . Sodium hexametaphosphate 0.2% by weight solution was used as dispersing medium. Before determining MP, TP, CHA powder were crushed and sieved pass the 325 mesh.

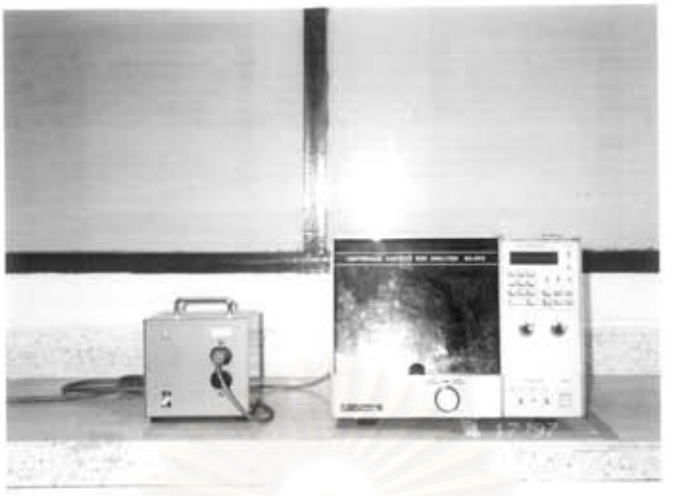


Fig 3.8. Particle size analyzer

3.6.5. Surface area and pore volume

Surface area and pore volume of MP, TP and CHA powder were determined by BET (Micromeritic model ASAP 2000). MP, TP and CHA powders were ground to fine powder, dried at 80 °C for 2 h. before proceeding the experiment. Nitrogen gas was used to adsorbed on particle surface.

Fig 3.9. Surface area analyzer

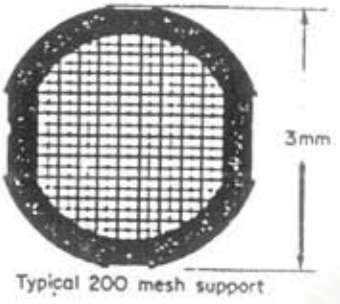
3.6.6. Density of powder

The densities of MP, TP and CHA powders were detected by gas pycnometer micromeritic AcCpye 1330 with helium gas as medium

Fig 3.10. Pycnometer

3.6.7. Morphology of particle

Morphology of MP, TP and CHA particle was characterized by Transmission electron microscope (TEM) JEOL Model JEM-200CX. Before experimentation, MP, TP and CHA powder were ground , dried and dispersed in absolute ethanol by placed in ultrasonic bath 30 min. for well dispersing. The mixture was dropped in grid, coated with formvar and carbon. Sample grid was placed into TEM chamber in vacuum. Samples were scanned and took the suitable photograph.



(A)

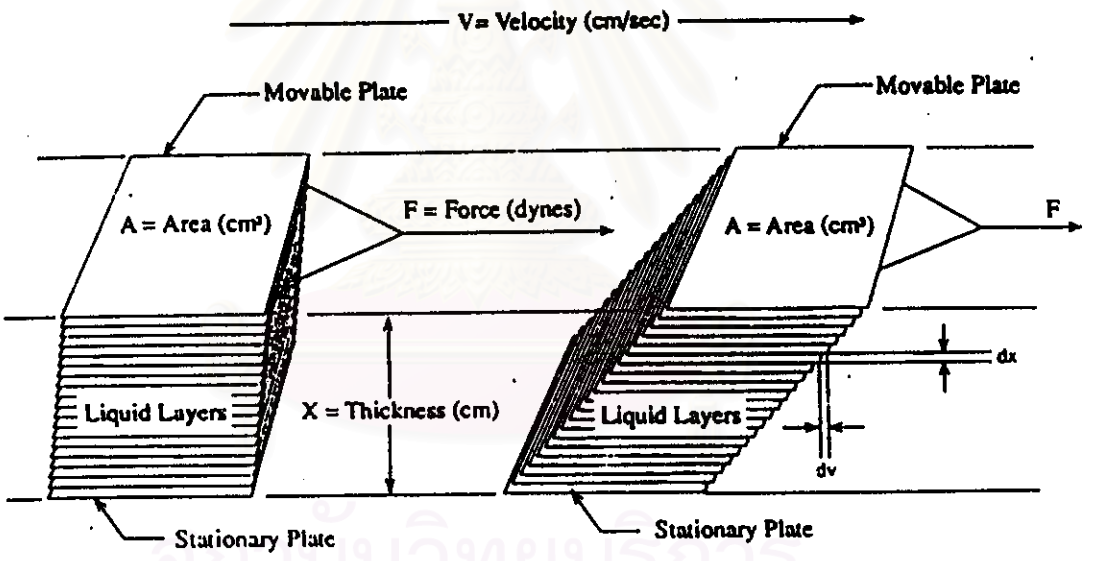
(B)

Fig 3.11. Electron microscope grid (A)
Transmission electron microscope (B)

3.6.8. Rheological properties

Viscosity is a property of fluid due to internal friction and manifest a rate of flow. The unit of viscosity is named the poise followed 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".

Viscosity can be considered as the ratio of shear stress to shear rate. The rheological model is a rectangular body at liquid of very thin layers superimposed one another as shown in the Fig. 3.15. 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 (τ) 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 dictionary on the substrate.



τ = Shear Stress = F/A (dynes/cm²)

γ = Shear Rate = V/X (sec⁻¹)

η = Viscosity = Shear Stress/Shear Rate = τ/γ (dynes·sec/cm²) or (Poise)

Fig.3.12. Rheological model

With the velocity of the top layer v and the depth of the liquid d, the velocity gradient is defined as shear rate (γ) and equals v/d

$$\text{Viscosity} = (F/A) / (v/d) = \tau/\gamma$$

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

Rheological properties of MP , TP and CHA slips were tested. The experiment were carried on following the ASTM designation : D2196-86 (reapproached 1991). The measurement were performed by rotational Brookfield viscometer model RVTD AO 4184

Fig 3.13. Brookfield viscometer

3.6.9. Bulk density and apparent porosity

The bulk density and apparent porosity were determined by Achimedes method (ASTM D 20-92) using distilled water as the immersion medium. The test method is as follows:

1. Dry the sintered specimens by heating to 105-110°C and determined the dry weight, D in grams.

2. Place the test specimens in water and boil for 2 h.

3. After the boil peroid, cool the test specimens to room temperature while still completely cover with water . After boiling keep the specimens in a water for a minimum of 12 h.

4. Determine the suspended weight,S, of each test specimen after boiling and while suspended in water in grams. This weighting is usually complished by suspending the specimens in a loop or halter copper wire hung from one arm of the balance. The balance shall be previous counter balanced with the wire in place and immersed in water to the same depth as is used when the specimen are in place.

5. After determining the suspended weight, blot each specimen lightly with a moistened smooth linen or cotton cloth to removing all drop of water from surface and determining the saturated weighted, W in grams by weighing

Bulk density **B** can be calculated as

$$B\ (g/cm^3) = D / V$$

where $V\ (cm^3) = \frac{W-S}{\rho}$

ρ = Density of H_2O

Apparent porosity **P** calculated from

$$P\% = [(W-D) / V] \times 100$$

3.6.10. Pore size

The pore size within porous solid of these porous solid HAp was determined by cutting the specimen into small size ($0.5\times0.5\times0.5\ cm^3$), dried and weighted before testing in the Poresizer 9320 Micromeritic. Low pressure and high pressure were performed to measure pores within porous solid.

Fig 3.14. Pore size tester

3.6.11. Pore morphology

The characteristic of pore included pore size of porous specimen were investigated by scanning electron microscope. (JOEL JSM-T220A). Sample must be cut into 1× 1× 0.5 cm^2 , placed on the target stub and sputtered with gold to make conduction in specimen.

Fig 3.15 Scanning electron microscope

3.6.1. Compressive Strength testing

Compressive strength was conducted with LLOYD model LR 100 K., with load cell of 100 KgN. and rate of loading 1 mm / min. Specimen bar were cut to cross-section 1x1x1 cm^3 and polished with abrasive paper until specimens had smooth surface. Specimens were washed with distilled water, dried at 80°C / 2 h. and cooled in desiccator before perform testing.

Fig. 3.16. Compressive strength testing machine