PREPARATION OF HYDROXYACRYL - CHITOSAN

AND CALCIUM SILICATE/HYDROXYACRYL - CHITOSAN COMPOSITE

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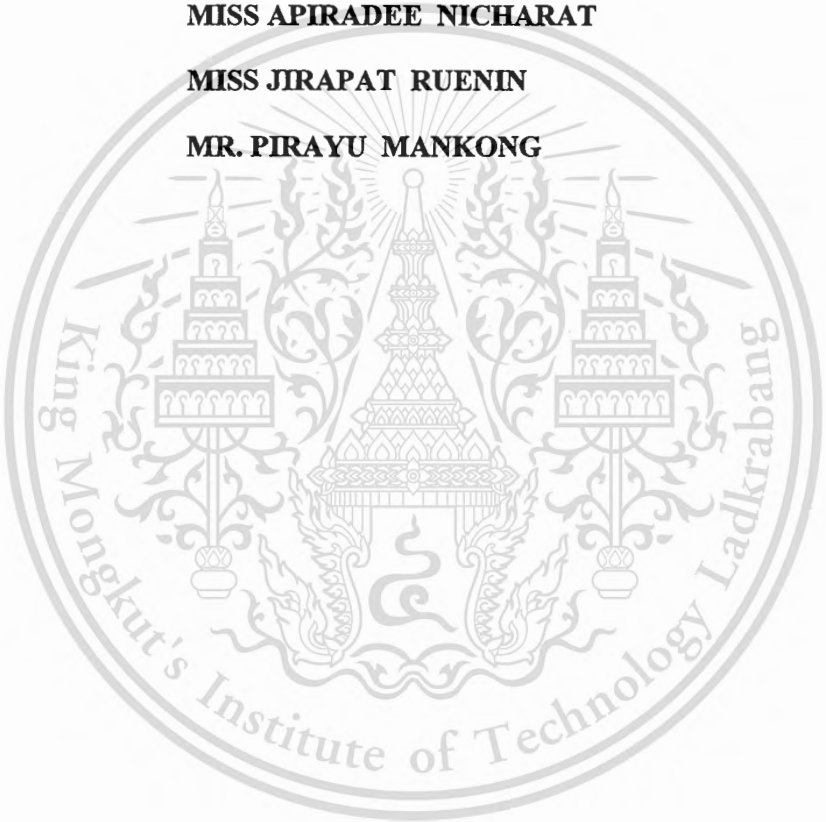

A SPECIAL PROJECT SUBMITTED IN PARTIAL FULFILLMENT

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Title	Preparation of hydroxyacryl-chitosan and calcium silicate/hydroxyacryl-chitosan composites			
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Abstract

This special project studied the preparation of hydroxyetylacryl-chitosan and hydroxyethylacryl - chitosan/calcium silicate (HC/CS) composites. Firstly, hydroxyethylacrylate – chitosan (HC) was synthesized by Michael addition reaction, in which the chitosan and hydroxyethylacrylate were reflexed at 70 °C for 48 hours. ^{1}H NMR and FT-IR were used to determine HC functional groups. The calcium silicate (CS) prepared by calcium nitrate tetrahydrate ($Ca(NO_3)_2.4H_2O$) and tetraethyl orthosilicate ($Si(OC_2H_5)_4$), TEOS), calcined at 500 °C for 2 hours. The powders were then characterized by XRD. Finally, the HC/CS composites were synthesized by 2 methods. The first method was synthesized by mixing CS powder in the HC solution, shaping into cubic shape, and then adding with 1wt%gluteraldehyde crosslinker. Another was prepared by mixing CS powder in HC solution and spontaneously adding 1%wt gluteraldehyde. The HC/CS pre-composites into cubic were heated at 50 °C for 24 hours. Furthermore, the HC/CS composites were tested their compressive strength and modulus by universal testing machine. It was found that HC/CS composites provided 6 times better in mechanical properties than CS. In addition, TGA indicated an approximate HC: CS ratio of 40:60 by weight.

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Miss Apiradee Nicharat

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Symbol and Abbreviation

CS	Calcium silicate
HEA	Hydroxyethyl Acrylate
HC	Hydroxyethylacryl-chitosan
HC/CS composite	Hydroxyethylacryl-chitosan/calcium silicate composite
GLU	Gluteraldehyde
XRD	X-ray Diffraction
SEM	Scanning Electron microscope
TGA	Thermo Gravimetric Analysis
NMR	Nuclear Magnetic resonance
FTIR	Fourier Transform Infrared Spectroscopy

Chapter 1

Introduction

1.1 Motivation

In the past, various materials, i.e., metal polymer and ceramic were used for implantation in human bodies' materials. Each of them has both advantages and disadvantages individually. Metals have very high strength, however high specific gravity and low corrosive resistance. Although, polymers are viscoelastic materials and have low specific gravity, there are not suitable for load barring applications. Ceramics are biocompatible materials with high compressive strength whereas they are brittle and low fracture toughness. To overcome the above limitations, biomaterials are made from composites, integrating the good points of each main raw material, and hence, their weak points might be compensated.

Bioactive materials such as glasses [1,2], glass-ceramics [3,4], hydroxyapatite [5-7], composite materials[8,9] etc. have been synthesized and developed for medicals applications since the early of 1970s.

Whereas ceramic, including bioinert [45], bioactive and bioresorbable types, are attractive candidate for medical applications calcium silicate ($CaSiO_3$, CS) plays an important role as bioactive materials since it can induce the fast formation of an HAp layer after soaking in stimulated body fluid (SBF), indicating the high ability to bond with living bond [10-12]. However, the CS has some disadvantages, especially its relatively low mechanical properties. From this point of view, this research will enhance the mechanical properties of CS by impregnation with chitosan.

Chitosan is selected because it is bio-functional material offering a unique set of characteristics, including biocompatibility, biodegradability to harmless products and non-toxicity, as well as bioactivity that comprises antibacterial, hemostatic, fungistatic, antitumoral, anticholesteremic, and importantly, osteoconductive properties. However,

chitosan is a cationic polyelectrolyte soluble in aqueous acidic media while CS could be corroded with acidic media, therefore, hydroxyethylacrylate – chitosan (HC) is needed.

This project will modify chitosan structure with hydroxyethylacrylate in order to obtain the hydroxyethylacrylate - chitosan for preparation of HC/CS composite. In addition glutaraldehyde (GLU) is used as cross-linking agent to enhance chitosan strength. The effects of 1wt% glutaraldehyde on the characteristic and mechanical properties of HC/CS composites were investigated.

1.2 Objectives

1.2.1 To study on the synthesis of hydroxyethylacryl - chitosan.

1.2.2 To study on the preparation and characterization of hydroxyethylacryl - chitosan /calcium silicate composite.

1.3 Scope of Study

1.3.1 Synthesis of hydroxyethylacryl - chitosan modified with hydroxyethylacrylate (HEA)

1.3.2 Synthesis of calcium silicate.

1.3.3 Preparation of hydroxyethylacryl - chitosan /calcium silicate composite using 1wt% of glutaraldehyde as crosslinking agent.

1.3.4 Characterization of composites by various techniques, i.e. NMR, FT-IR, SEM, XRD, TGA and testing for compressive strength.

1.4 Expected Results

1.4.1 The hydroxyethylacryl - chitosan /calcium silicate composite can be prepared 1wt% glutaraldehyde contents.

Chapter 2

Theory and Literature Review

2.1 Biomaterial [15]

A biomaterial is a synthetic material used to replace part of a living system or to function in intimate contact with living tissue. Biomaterials are materials used in close or direct contact with the body to augment or replace faulty materials. Biomaterials must be compatible with the body so that the body does not reject them. However, in some cases, biomaterials like organ transplants do cause rejection, which can be addressed through anti-rejection medications.

Although biomaterials are primarily used for medical applications, they are also used to grow cells in culture, to assay for blood proteins in the clinical laboratory, in processing biomolecules in biotechnology, for fertility regulation implants in cattle, in diagnostic gene arrays, in the aquaculture of oysters and for investigational cell-silicon "biochips." The commonality of these applications is the interaction between biological systems and synthetic or modified natural materials.

Biomaterials are rarely used on their own but are more commonly integrated into devices or implants. Thus, the subject cannot be explored without also considering biomedical devices and the biological response to them.

Materials	Advantages	Disadvantages	Examples
Polymers:Nylons, silicones,Teflon,Dacron	Resilient,easyto fabricate, may degrade	Not strong, deform with time, may degrade	Sutures, blood vessels, hip socket, ear, nose, other soft tissue
Metals: Titanium, stainless steel, Co-Cr alloys, Gold	Strong, tough, ductile	May corrode, dense	Joint replacement, bone plates and screws, dental root implants
Ceramics: Alumina, carbon,hydroxyapatite	Very biocompatible, strong in compression	Brittle, difficult to make, not resilient	Dental, hip socket
Composites: Carbon-carbon	Strong, tailor- made	Difficult to make	Joint implants, heart valves

Table.2.1 Advantage and disadvantage of biomaterial.

Other biomaterials include certain metals, which might be used in reconstructing bones or joints. For example, metal ball joint sockets can be used in knees or hips, and tend to offer great support for those requiring joint replacement. Some biomaterials are actually living. This is the case with organ transplants in particular. Organs are expected to grow and develop with the body, and are better replacements than non-living sources.

In some cases, a non-living source like an artificial heart or a left ventricular assist device (LVAD) is used while people wait for a heart transplant. These artificial replacements tend not to work for long periods of time, though they can provide someone with the extra days or even a few months they need while waiting to receive a transplant.

Organ	**Types of metal**
Dental	Au, Ag, Pt, amalgam, Titanium
Heart	Stainless steel
Joint	Stainless steel, Cobalt alloy

Table.2.2 Example of metal medical applications

Organ	**Types of polymer**
Ears	Acrylic, PE, Silicone, PVC
Eyes	Acrylic hydrogel
Nose	Silicone, PE, Teflon
Dental	UHMWPE, Epoxy
Face	Acrylic, PVC, Polyurethanes
Heart	Polyester, Silicone, PVC
Blood vessels	PVC, Polyester, Teflon
Joint	Silicone, UHMWPE, Acrylic, Nylon, Polyurethanes

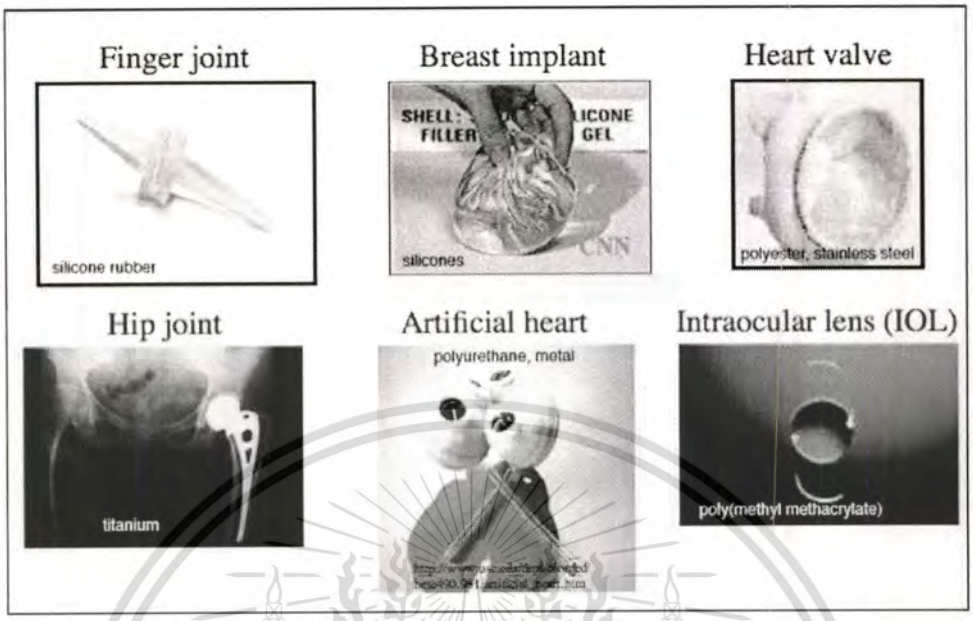
Table.2.3 Example of polymer medical applications

Organ	Types of ceramic
Ears	Hydroxyapatite
Eyes	Hydroxyapatite, glass
Dental	Hydroxyapatite, Porcelain
Face	Hydroxyapatite
Heart	Carbon
Joint	Hydroxyapatite, Alumina, Ziconia

Table.2.4 Example of ceramic medical applications

Organs	Types of composite
Ears	Hydroxyapatite/plastics
Dental	BIS-GMA/silica powder
Face	Hydroxyapatite/plastics
Heart	Carbon/carbon
Joint	Carbon fiber/plastics, Ceramic/plastic

Table.5 Example of composite medical applications



Other common biomaterials are used in plastic surgery applications. Calf, breast, cheek, chin, and buttocks implants are all considered to be biomaterials. Occasionally, plastic surgeons will harvest either fat or skin from a patients body to be used in another part of a body.

2.1.1 Calcium Silicate [16]

2.1.1.1 General

The discovery of a calcium silicate or Wollastonite that is named for the English chemist and mineralogist W. H. Wollaston (1766 - 1828). with a hitherto unseen nano-structure (Johnston et al., 2006) consisting of randomly stacked platelets

The material is described as a nano-structured calcium silicate to differentiate it from the numerous other calcium silicates and hydrated calcium silicates that exist with varying extents of atomic ordering. McGraw-Hill (2003) defines a nano-structure as "something that has a physical dimension smaller than 100 nm, ranging from clusters of

atoms to dimensional layers". This description was therefore chosen as the platelets that are responsible for the materials interesting physical properties have at least one dimension (thickness) within this range (and often all three dimensions are less than 100 nm), when observed using scanning electron microscopy.

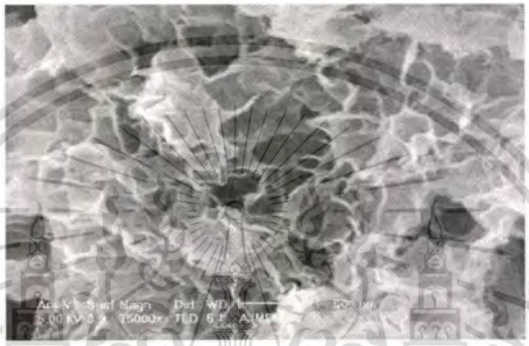


Figure 2.1 A scanning electron micrograph of the novel nano-structured calcium silicate revealing the porous nature of the material

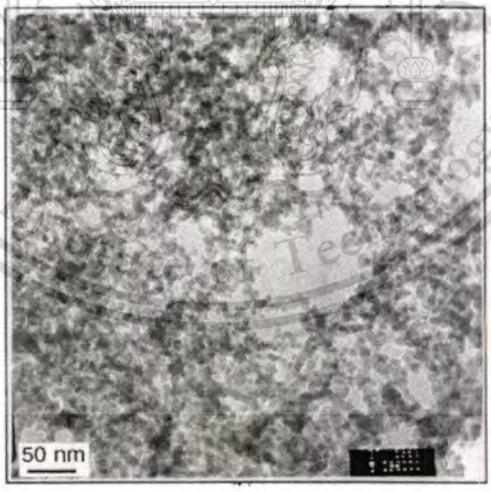


Figure 2.2 A transmission electron micrograph of a silica precipitated from geothermal water. (Meyer, 1996)

Nano-structured calcium silicate is formed as a result of the reaction between calcium ions and dissolved silica at an alkaline pH under controlled conditions of mixing,

temperature and ageing time. The dissolved silica can be sourced from either geothermal water or sodium silicate, and is mainly present as H_3SiO_4□ and $H_2SiO_4^{2-}$ silicate ions at the highly alkaline conditions typically used of above pH 11 (Packter, 1986). The structure of the material is a result of a self-organisation over time and no templates or structure directing agents a used during the synthesis. A representation of the reaction is given below:

$$H_3SiO_4^- + Ca^{2+} + OH^- \rightarrow CaSiO_x(OH)_y + H_2O$$

Where: x is approximately 2-3

y is approximately 2-1

Calcium silicate or wollastonite is a common mineral in skarns or contact metamorphic rocks. Skarns can sometimes produce some wonderfully rare and exotic minerals with very unusual chemistries. However, calcium silicate has no unusual elements in its chemistry and it is somewhat common and not considered very exotic among collectors. Calcium silicate forms from the interaction of limestones, that contain calcite, CaCO3, with the silica, SiO2, in hot magmas.

This happens when hot magmas intrude into and/or around limestones or from limestones chunks that are broken off into the magma tubes under volcanoes and then blown out of them. It forms by the following formula:

$$CaCO_3 + SiO_2 \rightarrow CaSiO_3 + CO_2$$

It is a metasilicate of calcium crystallizing in monoclinic system, occurring as aggregates of bladed or needle-like crystals. When powdered to 325 mesh, it gives a

natural brightness averaging 92 on the Hunter reflectometer. Its specific gravity 2.8 to 2.9 and melting point 1512°C. It is found as a metamorphic mineral associated with garnet in metamorphosed limestone. The industrial value of calcium silicate depends upon its freedom from impurities. The commercial deposits are worked only in the USA where it is mined on a limited scale.

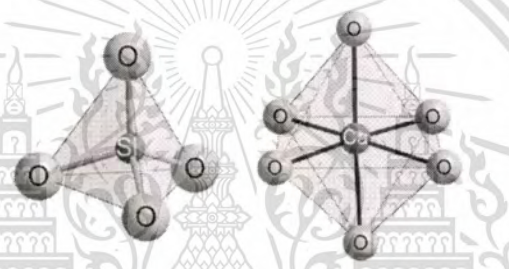


Figure 2.3 Si and Ca coordination spheres in Wollastonite (Si- and Ca-Umgebung in Wollastonit)

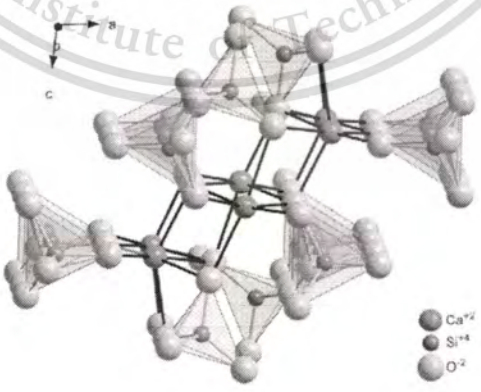


Figure 2.4 structure of calcium silicate in 3D- dimension

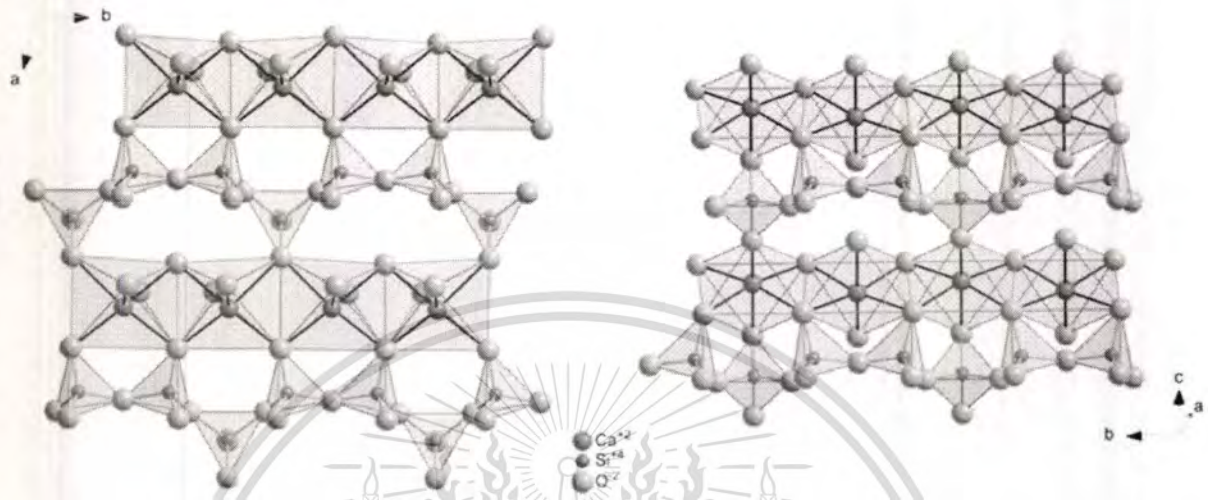


Figure2.5 Connection of silicate chains through [CaO_6] octahedra in direction of [100] and [001] in Wollastonite (Verknüpfungsmuster der Silikatketten und [CaO_6]-Oktaeder in Richtung de a- und c-Achse in Wollastonit) [17]

Although not an "exotic" mineral, calcium silicate has its uses. It is an important constituent in refractory ceramics (those ceramics that are resistant to heat) such as refractory tile and as a filler for paints. It is easily mined in some places where it is the major component of the metamorphosed rock. Mineral specimens can be interesting with their fibrous habit, pearly luster and some specimens [18]

2.1.1.2 Structure of calcium silicate

There have many type of structure such as Triclinic, Monoclinic and hexagonal.Triclinic symmetry of the most common and first described calcium silicate or wollastonite mineral. The reason Triclinic is needed is to distinguish it from the much more rare wollastonite, also known as *parawollastonite*. Parawollastonite is Monoclinic. These minerals are polymorphs which means that they have the same chemistry, $CaSiO_3$,

just different structures (*poly* means many and *morph* means shape). There are actually several other rare and obscure polymorphs of CaSiO3

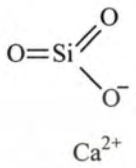


Fig.2.6 chemical structure of calcium silicate ($CaSiO_3$)

Wollastonite crystallizes triclinically in space group with the lattice constants a = 7.94 Å, b = 7.32 Å, c = 7.07 Å; α = 90,03°,β = 95,37°,γ = 103,43° and six formula units per unit cell. Wollastonite was once classed structurally among the pyroxene group, because both of these groups have a ratio of Si:O = 1:3.

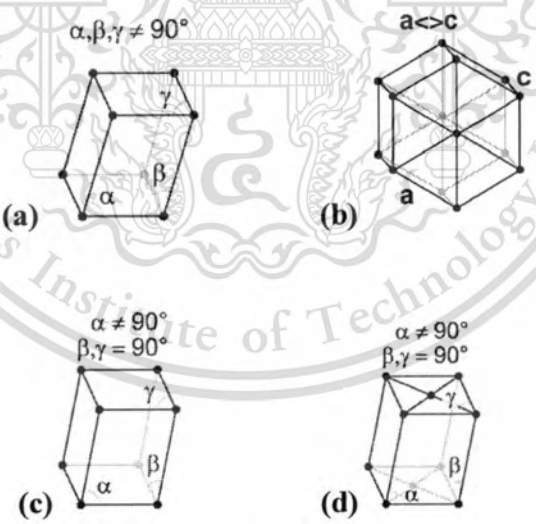


Figure 2.7 crystal structure of calcium silicate

a) Triclinic b) Hexagonal

c) Simple Monoclinic d) Centered Monoclinic

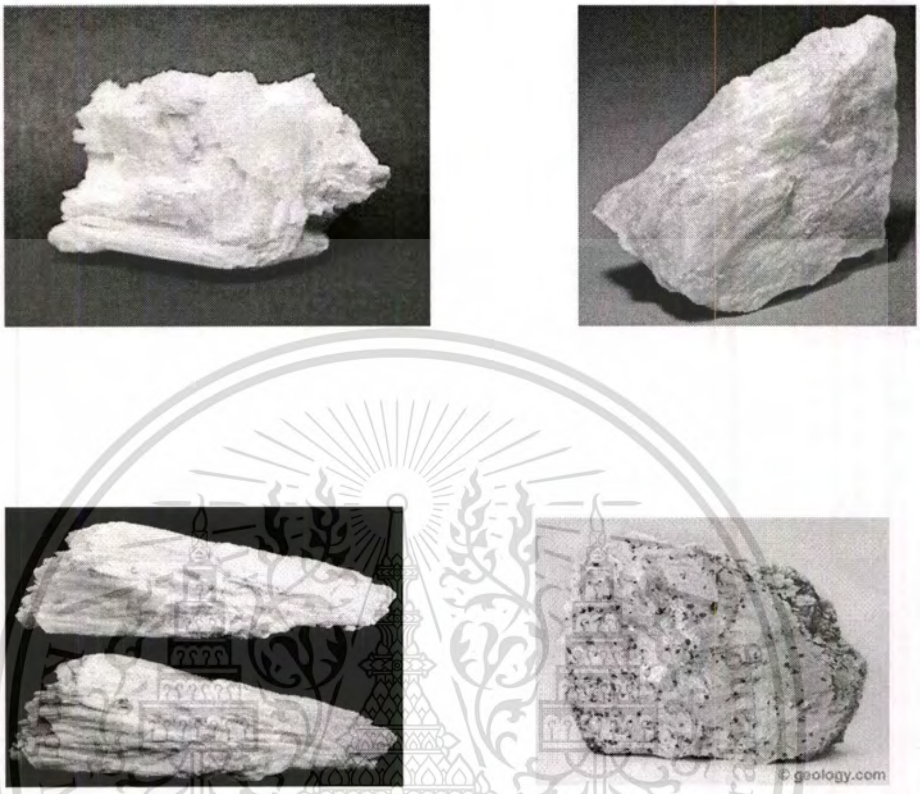


Figure 2.8 Crystal of Wollastonite in natural

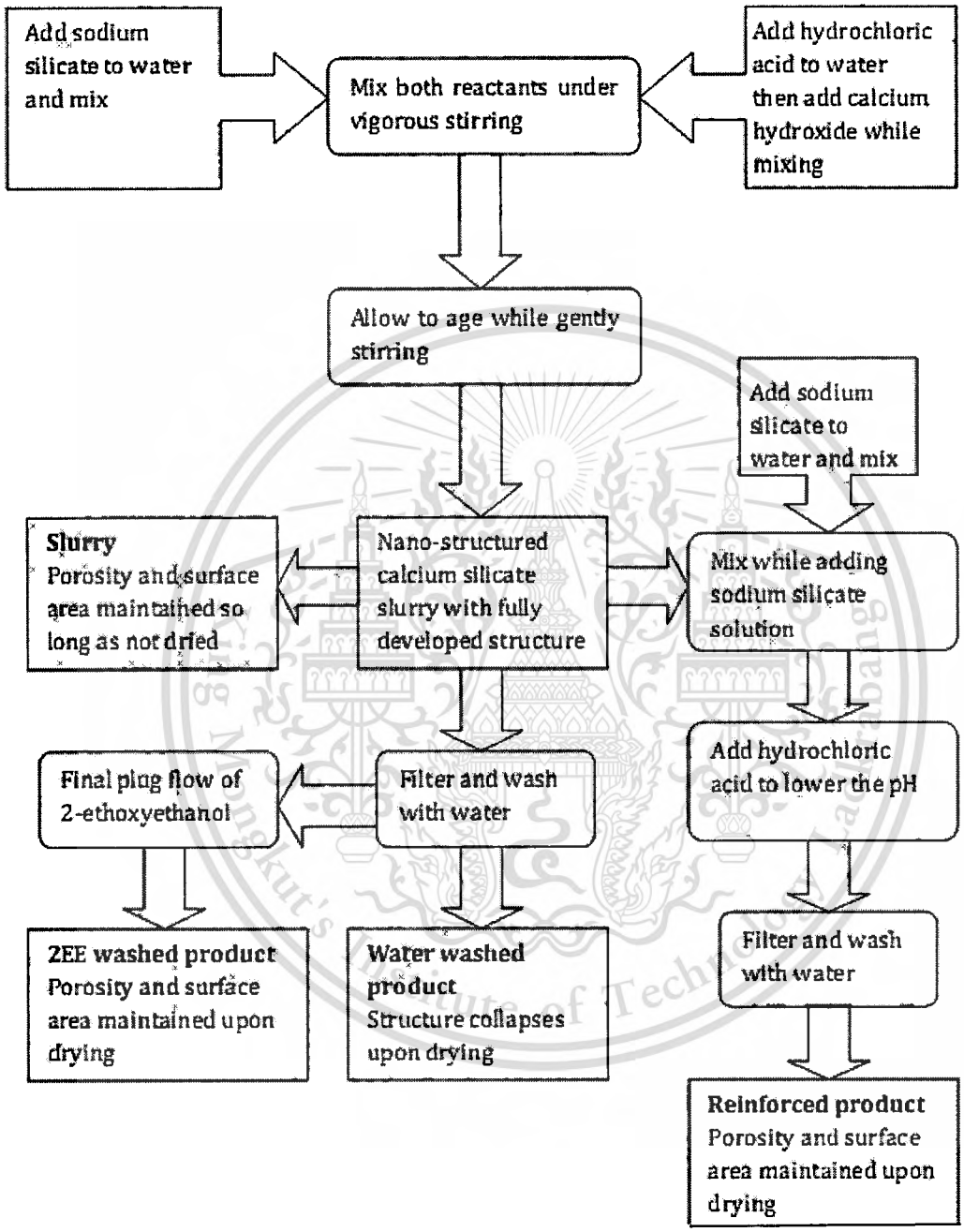


Figure2.9 Schematic showing the general steps for the production of different types of nano – structured calcium silicates [19]

CS was reported to have a high bioactivity due to the fast formation of an apatite layer on its surface after soaking in simulated body fluid solutions. It is known that the silanol groups on the silicate surface can provide favorable sites for apatite nucleation [20]

CHEMICAL AND PHYSICAL PROPERTIES [21]

Physical data

1. Molecular weight:	116.2
2. Boiling point:	Data not available.
3. Specific gravity (water = 1):	2.9 at 20 degrees °C (68 degrees°F)
4. Vapor density:	Data not available.
5. Melting point:	1200 to 1500 degrees °C (2192 to 2732 degrees °F)
6. Vapor pressure:	Data not available.
7. Flammability:	Calcium silicate is not combustible.

8. Solubility: Practically insoluble in water but on prolonged contact it will become amorphous silica and soluble calcium salts; slightly soluble in hydrochloric acid.

2.1.2 Porous calcium silicate [22]

The mesoporous calcium silicate compositions for controlled release of bioactive agents. In some embodiments, bioactive agents can include proteins, growth factors (e.g., osteoinductive growth factors), pharmaceutical drugs, antibiotics, antimicrobial agents, polypeptides, etc.

A hydrated silica gel layer having abundant Si--OH functional groups can be formed on the surface of calcium silicate particles following acid modification. Acid modification of calcium silicate particles increases particle surface area, as measured by the Bruhauer-Emmett-Teller (BET) method. Accordingly, the compositions disclosed herein provide calcium silicate materials for use in bone implant applications having increased bioactivity and bioactive agent loading.

Tissue Calcium silicate ($CaSiO(_3)$) is regarded as a potential bioactive material. However, its poor chemical stability and cytocompatibility limits its biological applications. the composition promotes bone in-growth and/or repair of damaged tissue. In vitro and in vivo studies have shown that calcium silicate ceramics could induce a bone-like apatite layer formation in simulated body fluid (SBF) and chemically integrate into the structure of living bone

2.1.2.1 Biocompatibility of Mesoporous Calcium Silicate

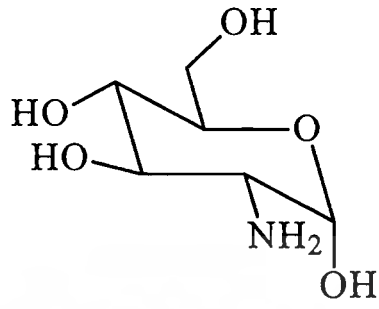
Calcium silicate (CS) has been regarded as a candidate for bone replacement biomaterial due to its good bioactivity properties. In vitro and in vivo studies showed that CS ceramic could induce a bone-like apatite layer formation in simulated body fluid (SBF) and chemically integrate into the structure of living bone tissue. To improve the

mechanical properties, CS coating on titanium alloy was also developed by plasma spray. Additionally, nano-sized calcium silicate particles were synthesized and its biocompatibility was demonstrated by an in vitro study. The analyses of these in vitro and in vivo studies reveal that the primary reason for biocompatibility of calcium silicate is the formation of Si--OH on its surface when exposed to bodyfluid.

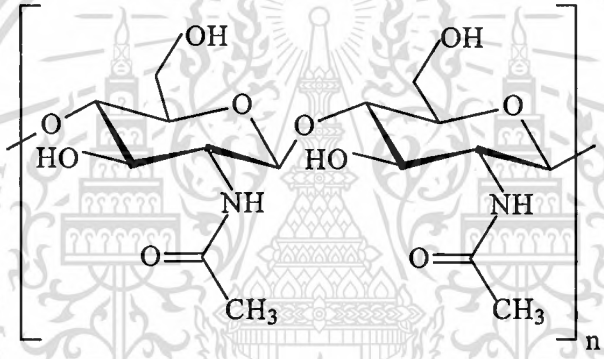
2.2 Chitin and Chitosan [23]

Chitin and chitosan polymers are natural amino polysaccharides having unique structures, multidimensional properties, highly sophisticated functions. Chitosan is a partially deacetylated polymer of N-acetyl glucosamine. It is essentially a natural, water-soluble, derivative of cellulose with unique properties. Chitosan is usually prepared from chitin (2 acetamido-2-deoxy b-1,4-D-glucan) and chitin has been found in a wide range of natural sources (crustaceans, fungi, insects, annelids, molluscs, coelenterata etc.) Dimension and distribution of both patterns (N-acetyl-D-glucosamine and D-glucosamine) is controlled, allowing synthesis of pure oligomers. Oligomers synthesis is divided in three steps. Degree of polymerization is between2and 8.

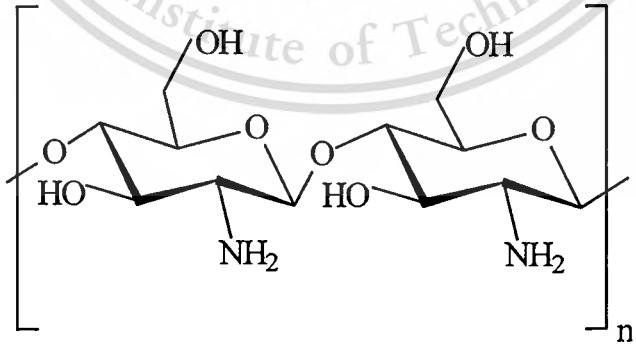
Chitosan is produced commercially by deacetylation of chitin , which is the structural element in the exoskeleton of crustaceans (crabs, shrimp, etc.),shellfish, insects and cell walls of fungi. The degree of deacetylation (%DA) can be determined by NMR spectroscopy, and the %DA in commercial chitosans is in the range 60-100 %



glucosamine (monomer of chitosan)



Chitin



Chitosan [24]

Figure 2.10 Structure of glucosamine (monomer of chitosan),chitin and chitosan

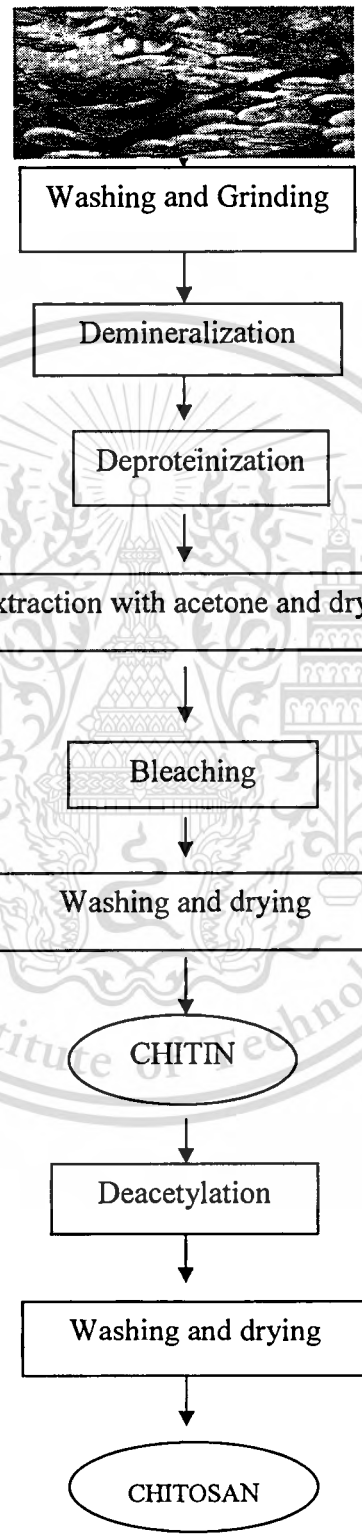


Figure 2.11 Preparation of chitin and chitosan from raw material [25]

2.2.1 Properties [26]

The amino group in chitosan has a pKa value of ~6.5, thus, chitosan is positively charged and soluble in acidic to neutral solution with a charge density dependent on pH and the %DA-value. This makes chitosan a bioadhesive which readily binds to negatively charged surfaces such as mucosal membranes. Chitosan enhances the transport of polar drugs across epithelial surfaces, and is biocompatible and biodegradable. Purified qualities of chitosans are available for biomedical applications.

Chitosan and its derivatives such as trimethylchitosan (where the amino group has been trimethylated) have been used in non-viral gene delivery. Trimethylchitosan, or quaternised chitosan, has been shown to transfect breast cancer cells; with increased degree of trimethylation increasing the cytotoxicity and at approximately 50% trimethylation the derivative is the most efficient at gene delivery. Oligomeric derivatives (3-6 kDa) are relatively non-toxic and have good gene delivery properties.

Properties of Low-Molecular Weight Chitosan

Appearance	:	Light and yellow powder
Ash	:	<1.5
Moisture	:	<10%
PH	:	4-6
Molecular Weight	:	< 6000

Heavy Metals (mg/kg)	:	＜5ppm
As （mg/kg)	:	＜0.5ppm
Undissolvable	:	<1.0%

2.2.2 Chitin and chitosan solubility [27]

While chitin is insoluble in most organic solvents, chitosan is readily soluble in dilute acidic solutions below pH 6.0. This is because chitosan can be considered a strong base as it possesses primary amino groups with a pKa value of 6.3. The presence of the amino groups indicates that pH substantially alters the charged state and properties of chitosan . At low pH, these amines get protonated and become positively charged and that makes chitosan a water-soluble cationic polyelectrolyte.

On the other hand, as the pH increases above 6,chitosan's amines become deprotonated and the polymer loses its charge and becomes insoluble. The soluble–insoluble transition occurs at its pKa value around pH between 6 and 6.5. As the pKa value is highly dependent on the degree of N-acetylation, the solubility of chitosan is dependent on the method of deacetylation used.

The degree of ionization depends on the pH and the pK of the acid with respect to studies based on the role of the protonation of chitosan in the presence of acetic acid and hydrochloric acid. The following salts, among others, are water-soluble: formate, acetate, lactate, malate, citrate, glyoxylate, pyruvate, glycolate, and ascorbate.

The dissolution constant Ka of the amine group is obtained from the equilibrium:

$$-NH_2 + H_2O \leftrightarrow -NH_3^+ + OH^-$$

$$Ka = [-NH_2][H_3O^-]/[NH_3^+] \text{ and } pKa = -\log Ka.$$

For polyelectrolytes, the dissociation constant is not a constant, but depends on the degree of dissociation at which it is determined. The variation of pKa can be calculated using Kachalsky's equation.

$$pKa = pH + \log\left(\frac{1-\alpha}{\alpha}\right) = pKo - \frac{\varepsilon\Delta\varphi(\alpha)}{k_T}$$

where $\Delta\varphi$ is the difference in electrostatic potential between the surface of polyion and the reference, α is the degree of dissociation, k_T is the Boltsman constant and ε is the electron charge. Extrapolation of the pKa value to $\alpha = 1$, where the polymer becomes uncharged and the electrostatic charge becomes zero enables the value of intrinsic dissociation constant of the ionizable groups pKo to be estimated. This value is ~6.5. The intrinsic pKo value of the ionizable groups ~6.5 is independent of the degree of N-acetylationwhereas the pKa value is highly dependent. pKo is called the intrinsic pKa of chitosan .chitosan can easily form quaternary nitrogen salts at low pH values. So, organic acids such as acetic, formic, and lactic acids can dissolve chitosan. The best solvent for chitosan was found to be formic acid, where solutions are obtained in aqueous systems containing 0.2 -100% of formic acid (FA). The most commonly used solvent is 1% acetic acid (as a reference) at about pH 4.0. chitosan is also soluble in 1% hydrochloric acid and dilute nitric acid but insoluble in sulfuric and phosphoric acids. But concentrated acetic acid solutions at high temperature can cause depolymerization of

chitosan. Solubilization of chitosan with a low the degree of acetylation (DA) occurs for an average degree of ionization α of CS around 0.5; in HCl ,when α = 0:5, it corresponds to a pH of 4.5–5. It is reported that at higher pH, precipitation or gelation tends to occur and the chitosan solution tends to form gels with anionic hydrocolloids. The concentration of the acid plays a great importance to impart desired functionality . Solubility also depends on the ionic concentration and a salting-out effect was observed in excess of HCl (1M HCl), making it possible to prepare the chlorhydrate form of chitosan. When the chlorhydrate and acetate forms of chitosan are isolated, they are directly soluble in water giving an acidic solution with pKo = 6±0.1 in agreement with previous data and corresponding to the extrapolation of pK for a degree of α = 0. Thus, chitosan, as stated above, is soluble at pH below 6. It is known that the amount of acid needed depends on the quantity of chitosan to be dissolved. The concentration of protons needed is at least equal to the concentration of –NH2 units involved. The solubility is thus a very dif ficult parameter to control as it involves a complex array of controlling factors . chitosan is not soluble in any organic solvents such as dimethylformamide and dimethyl sulfoxide. Its solubility in acidified polyol is substantially good. There are several critical factors that contribute to chitosan solubility. Includeing factors such as temperature and time of deacetylation, alkali concentration, prior treatments applied to chitin isolation, ratio of chitin to alkali solution,particle size, etc. A study on intrinsic viscosity, FTIR, and powder X-ray diffraction (XRD) showed that the molecular weight and DD are collectively responsible for the solubility in the condition of random deacetylation of acetyl groups,which resulted fromthe intermolecular force .

The solution properties of chitosan, thus, depend not only on its average DA but also on the distribution of the acetyl groups along the main chain in addition of the molecular weight. Apart from the DD, the molecular weight is also an important parameter that controls significantly the solubility and other properties. Both the DD and the molecular weight are reported to affect the properties of electrospun chitosan nanofibers. The acid soluble CSs with >95% solubility in 1% acetic acid at a 0.5% concentration could be obtained by treatment of the original chitin with 45–50% NaOH for 10–30min .It is reported that a reaction time of 5min with 45% NaOH may not be enough for chitin particles to be sufficiently swollen. A study on the thermodynamic aspects of deacetylation concludes that the amount of water present in the system chitin/soda/water/sodium acetate controls the complex equilibriums governing the reaction. Further, the microstructure of the polymer is said to have a role in the dissolution. It is also reported that the crystallinity index decreases on treating chitin with HCl, NaOH, etc. The solubility of the partially deacetylated chitins has a close relationship with their crystal structure, crystallinity, and crystal imperfection as well as the glucosamine content. chitin has a new crystal structure similar to that of α-chitin rather than either β-chitin or chitosan , suggesting that the homogeneous deacetylation transformed the crystalstructure of chitin from the α-tothe β-form. and it is water-soluble. Further discussion on water-soluble chitosan is presented elsewhere.

2.2.3 Applications [28,30]

The increasing production and use of chitosan has been steadily increasing since 1970s. In Japan, for example, the production of chitosan increased 37% each year from 1978-1983, the total annual amount reaching 311 tons by 1983 and 1,270 tons by 1986. At that time, the major applications of chitosan were centered on sludge dewatering, food processing, and metal ion chelation. The present trend, in industrial applications, however, is toward producing high value products, such as cosmetics, drug carriers, feed additives, semi-permeable membranes, and pharmaceutics. The different in value between the products and low-cost polymer is one of the main driving forces pushing studies on new applications of chitosan. Biotechnology is currently attempting large-scale production of high value bioproducts like monoclonal antibodies, which were projected at about 1.2 billion dollars(U.S.) on the world market in 1991. Immobilization techniques have been proven to be an effective way to increase cell density, product concentration, and hence, productivity in a culturing system. Chitosan membranes and gels have great potential for use in immobilized cell culture systems.

2.2.3.1 Water Treatment and Papermaking

- One of the earliest applications of chitosan was for chelating harmful metal ions, such as copper, lead, mercury, and uranium, from waste water. Chitosan is considered to be safe for human use. Studies on the chelating ability of chitosan began about twenty years ago. In 1973, Muzzarelli documented the effectiveness of chitin, chitosan, and other chelating polymers for their ability to chelate transition metal ions. He indicated that chitosan was a powerful cheating agent

and exhibited the best collection ability of all test polymers because of its high amino group content. Furthermore, Masri et al. compared the cheating ability of chitosan with several other materials, such as bark, activated sewage sludge, and poly(*p*-aminostyrene) and confirmed that chitosan had a higher chelation ability than these substances. In another interesting study dealing with the recovery of uranium from water, Hirano et al. showed that 40-74% of the available uranium could be recovered from river and lake water, and 3% from seawater. Recent studies showed that the chelating ability of chitosan could be further improved by homogeneous hydrolysis, crosslinking, controlled N-acetylation, and complexation with other polymers like glycan.

- Chitosan is an excellent coagulating agent and flocculant due to the high density of amino groups on the polymer chain that can interact with negatively charged substances, such as proteins, solids, and dyes. Many type of chitosan membranes have been developed for water clarification and filtration.
- Several early applications of chitosan in the paper pulp and paper industries have been review by Muzzarelli. These increase the surface treatment of paper with a 1% chitosan solution to increase its bursting strength and folding endurance, without any decrease in the paper's brightness. With the development the technology, such as color photocopying, different types and higher quality paper and fiber are required. The surface resistance of electrostatic charging was reported to have increased more than ten thousand times after treatment with the chitosan solution.

2.2.3.2 Clean up

- **Depollution in situ of polluted soils by organochlorides and oil residus :** capable on its own or combined with specifics microorganims to accelerate process of depollution of polluted soils by organochlorides and bunkers.
- **Depollution of water on biological bed :** accelerate degradation process, thus enabling construction of smaller and less costly installations.
- **Sewage treatment plant :** can be used in stabilisation of sludges with production of biogas rich in methane.
- **Methane production** : activate biogas production from decomposition of organic matter in view of electricity production.

2.2.3.3 Pharmaceutics and Biotechnology

- Although chitosan by itself is hemostatic(stop bleeding), some derivatives, such as sulfated chitosan, are blood anticoagulants. By utilizing the hemostatic effect, chitosan bandages and sponges were prepared for surgical treatment and wound protection. In contrast, immobilized partially sulfated chitosan oligomers on the surface of molded chitosan materials, such as artificial blood vessels and fibers, to produce antithrombogenic medical products.
- Chitosan has also been found to be effective against cholesterol. Various hypolipemic formulations containing chitosan, including particles, powders, and injections, were prepared for oral administration. The special

affinity of chitosan for biomolecules has been utilized to reduce side effect of drugs.

- Chitosan, as a polymer, is good additive in the preparation of biomaterials. In 1984, Allan et al. indicate that it may be possible to employ chitosan in creating soft contact lenses either by casting or molding procedures. Contact lenses are required to be optically clear, safe, wettable, and gas permeable. Among this properties, gas permeability is particularly important because oxygen must be transport from the tear fluid to the eye surface, whereas carbon dioxide needs to diffuse back from the eye surface to the fluid. Several techniques for preparing contact lenses have been reported resulting in chitin n-butyrate lenses, chitosan lenses, and blue chitosan lenses.
- Chitosan was also employed for making artificial skin. The artificial chitosan dermis was tested by inserting it into a cut on the back of rats. Finally, low molecular weight (MW=1,500) of chitosan was found to be useful for treating bone disease. The low molecular weight polysaccharide effectively increased alkaline phosphatase activity, thereby accelerating bone formation.
- Controlled-release technology is providing to be effective means for delivering lower concentration of drugs in the body and reducing side effects.

- Application of chitosan cosmetics were reviewed by Muzzarelli several ear ago. He indicated that the use of chitosan cold help to remove leftover starch contained in shampoos. Its use would also have the effect of conferring shine and strength to hair due to interaction between the polysaccharide and hair proteins. Cleanser can also contain chitosan. One of these, a bath lotion containing chitosan lactate, chitosan succinate, and chitosan alkyl phosphates. The lotion resulted in increased skin softness.

2.2.3.4 Cosmetic

- Chitin, the main constituent of the crustacean shells, is an excellent cosmetic product that is remarkably well tolerated by the skin.
- The chemical structure of chitin, a natural polymer, is very close to that of mucopolysaccharides (heparin and hyaluronic acid), whose biological tolerance has been demonstrated for a long time. In addition, it is an efficient trapper of heavy metals that are responsible for very many contact allergies; therefore it is really interesting for skin allergies.
- Chitin is a particularly effective hydrating agent. It has two advantages: it supplies water and it avoids dehydration. In addition, the great advantage of chitin and its derivatives is the lasting quality of their hydrating effect.
- Lastly, chitosan forms a protective tensor film on the skin's surface that can fix other active principles for the skin. Thus other hydrating agents, solar filters, organic acids or other active principles can be combined with the derivatives of chitin. Chitin facilitates their effects.Therefore chitin

and chitosan are now used in skin creams, shampoos, lacquers, varnishes, etc. Many patents have been registered and the potential range of new applications is endless.

2.2.3.5 Agricultural

- **Plants in better health** : stimulate soil biodiversity and restors beneficial organisms that attack, repel, or otherwise antagonize disease-causing plant pathogens. Sol-Actif like a vaccination render a soil disease suppressive. Plants growing in disease-suppressive soil resist diseases much better than in soils low in biological diversity.
- **Better survival rate after transplantation :** Existence of antagonistics micro-organisms in great quantity surrounding roots increase nutriment absortion and enable plants to develop normally despite the existence in soil of disease causing pathogens and a susceptible host.
- **Better productivity :** stimulate mycorrhizal fungi that form symbiotics associations with plants roots. This greater soil biodiversity improve nutriment availability for plants, particularly for nitrogen and phosphore, which have a great impact on production rate.
- **Simple and without danger**: is without danger for human, animals and environment.

2.2.3.6 Chitosan membranes and Cell Encapsulation

- Chitosan membranes may prepared in various ways: evaporation of chitosan solvent, cross-linking with bi-functional reagents, cheating with anion counterions, or complexing with polymers and proteins.
- Cross-linked chitosan membranes can be prepared by the addition of bifunctional reagents, such as aldehydes, carboxylic anhydrides, and glutaraldehyde, to chitosan solution.
- Cell encapsulation technology is important in the development of new cell transportation techniques for hormone delivery in medicine. One of the most important advantages of microencapsulation is the ability to provide a sheltered surrounding for the cells. The semi-permeable capsule membrane allows small molecules to diffuse through but prevents the passage of large molecules and cells. Since the earliest industrial applications of encapsulation (the preparation of microcapsules containing ink for carbonless copy paper), the technique has the potential for providing higher cell densities and product concentrations.

2.3 Water Soluble Chitosan [29,31,32]

In order to improve the solubility of chitosan in water,chemical modfication of chitosan is important and necessary. The water-Soluble Chitosan is off-white or yellowish, transparent and amorphous semitransparent solid.Easily soluble in water. The solution is clear and character is stable. chitosan derivative was synthesized reaction of chitosan and Hydroxyethylacrylate in moderate reaction conditions. Water-soluble

chitosan was produced, which was processed by deproteination, demineralization and deacetylation with NaOH. The degree of deacetylation of chitosan was determined by colloid titration method.

Water-soluble chitosan produced by our method had an amorphous structure. The viscosity of water-soluble chitosan depended on the concentration and pH. The viscosity of solution exponentially increased with its concentration, drastically increased above pH 7, reached a maximum at about pH 9 and then decreased.

Water-soluble chitosan has an amorphous structure and unique physicochemical properties. Therefore, water-soluble chitosan samples produced by this method may have more functional properties that are desirable in food and pharmaceutical applications

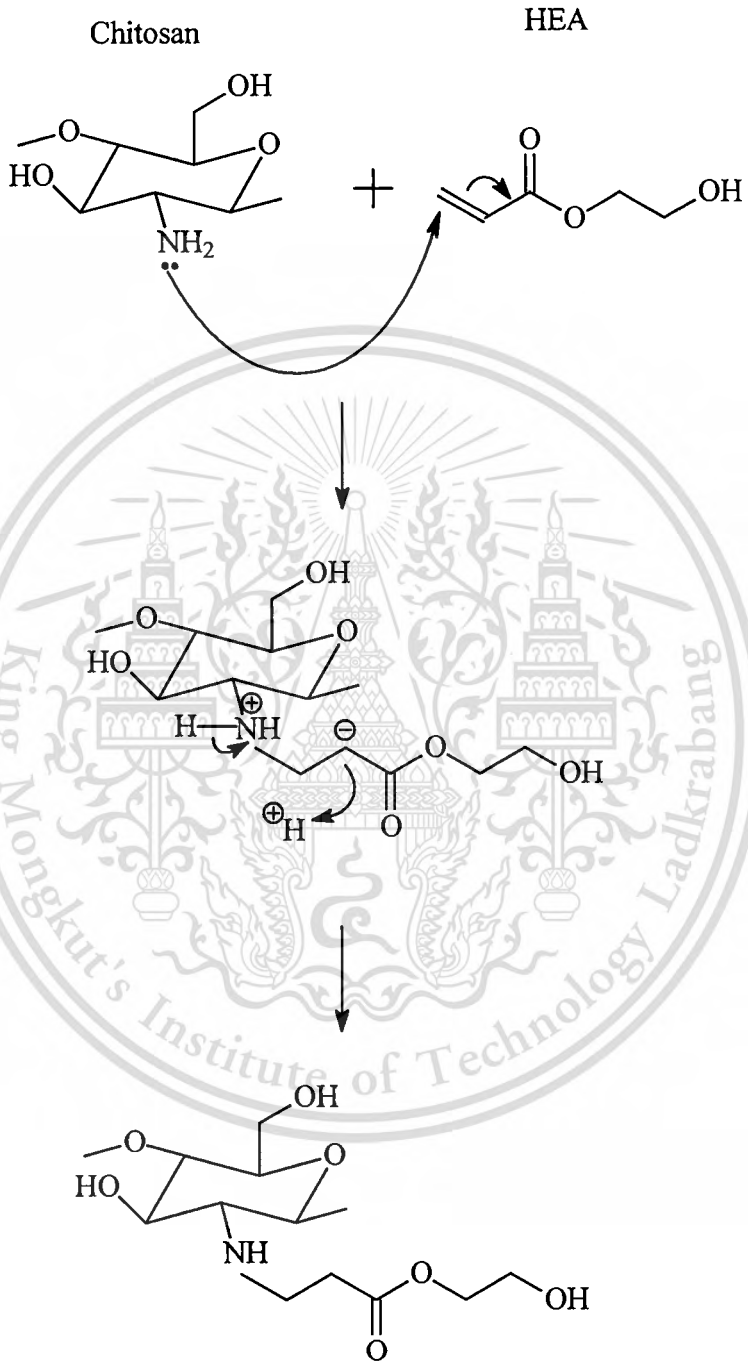


Figure 2.12 Synthesis of hydroxyethylacryl – chitosan

2.4 Hydroxyethyl Acrylate, HEA [33]

Chemical structure

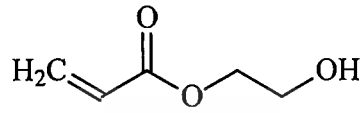


Figure 2.13 structure of HEA

Introduction

Hydroxyethyl Acrylate most used in warm solidify acrylate coating. light solidify acrylate coating. light sensitive coating, adhesive, dye ability of textile fiber. water stability

Specific characteristics

Degree of purity	:	min.95.0% (GC)
Acid content	:	max.0.5%
Water content	:	max.0.2%
Color	:	max.50 (Pt-Co)
Stabilization	:	250±50 ppm MEHQ (HPLC)

Physical data

Appearance	:	Clear, colorless liquid

Odor	:	ester-like
Molecular weight	:	116.1 g/mol
Density	:	1.11 g/cm^3 (25°C)
Refractive index	:	1.450 (20°C)
Boiling point	:	+230 °C (1013 mbar)
Flash point	:	+104.0 °C

General

Polymerization may be initiated by contamination with peroxides, aze compounds,heavy metal ions, tert. Amines, S compounds. Polymerization is also induced by light. Atmospheric oxygen saturation in the monomer is necessary for stability. Storage temperatures should not exceed 35°C. Avoid shine,rain and high temperature in transportation; Store the products in cool,shady and ventilated conditions,keep far away from fire

2.5 Composites [34]

Composites are combinations of two materials in which one of the materials, called the **reinforcing phase**, is in the form of fibers, sheets, or particles, and is embedded in the other materials called the **matrix phase**. The reinforcing material and the matrix material can be metal, ceramic, or polymer. Typically, reinforcing materials

are strong with low densities while the matrix is usually a ductile, or tough, material. If the composite is designed and fabricated correctly, it combines the strength of the reinforcement with the toughness of the matrix to achieve a combination of desirable properties not available in any single conventional material. The downside is that such composites are often more expensive than conventional materials. Examples of some current application of composites include the diesel piston, brake-shoes and pads, tires and the Beechcraft aircraft in which 100% of the structural components are composites.

Most composites have strong, stiff fibres in a matrix which is weaker and less stiff. The objective is usually to make a component which is strong and stiff, often with a low density. Commercial material commonly has glass or carbon fibres in matrices based on thermosetting polymers, such as epoxy or polyester resins. Sometimes, thermoplastic polymers may be preferred, since they are mouldable after initial production. There are further classes of composite in which the matrix is a metal or a ceramic. For the most part, these are still in a developmental stage, with problems of high manufacturing costs yet to be overcome. Furthermore, in these composites the reasons for adding the fibres (or, in some cases, particles) are often rather complex; for example, improvements may be sought in creep, wear, fracture toughness, thermal stability, etc. This software package covers simple mechanics concepts of stiffness and strength, which, while applicable to all composites, are often more relevant to fibre-reinforced polymers.

Module Structure

The module comprises three sections:

- Load Transfer
- Composite Laminates
- Fracture Behaviour

Brief descriptions are given below of the contents of these sections, covering both the main concepts involved and the structure of the software.

Load Transfer

The concept of load sharing between the matrix and the reinforcing constituent (fibre) is central to an understanding of the mechanical behaviour of a composite. An external load (force) applied to a composite is partly borne by the matrix and partly by the reinforcement. The load carried by the matrix across a section of the composite is given by the product of the average stress in the matrix and its sectional area. It depends on the volume fraction, shape and orientation of the reinforcement and on the elastic properties of both constituents. The reinforcement may be regarded as acting efficiently if it carries a relatively high proportion of the externally applied load. This can result in higher strength, as well as greater stiffness, because the reinforcement is usually stronger, as well as stiffer, than the matrix.

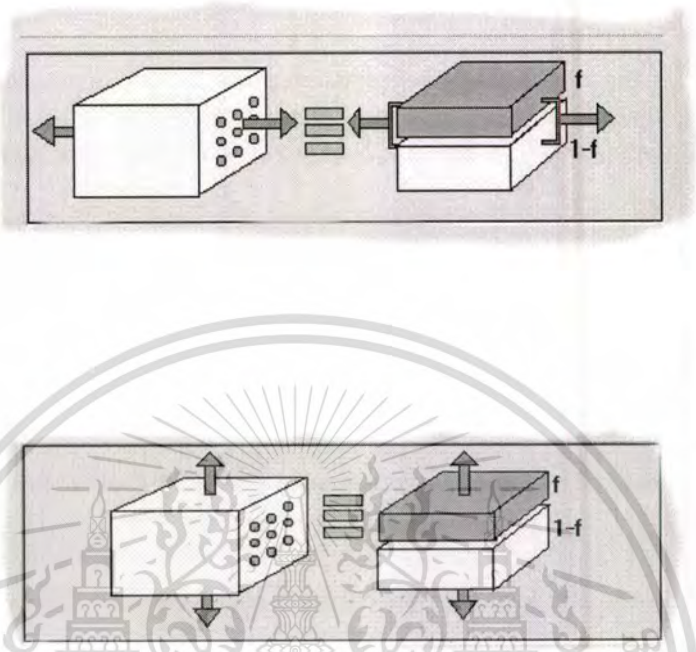


Figure 2.14 when a Composite is stressed

Consider loading a composite parallel to the fibres. Since they are bonded together, both fibre and matrix will stretch by the same amount in this direction, i.e. they will have equal strains, e (Fig. 13). This means that, since the fibres are stiffer (have a higher Young modulus, *E*), they will be carrying a larger stress. This illustrates the concept of load transfer, or load partitioning between matrix and fibre, which is desirable since the fibres are better suited to bear high stresses. By putting the sum of the contributions from each phase equal to the overall load, the Young modulus of the composite is found (fig.13).

Composite materials take advantage of the different strengths and abilities of different materials. In the case of mud and straw bricks, for example, mud is an excellent

binding material, but it cannot stand up to compression and force well. Straw, on the other hand, is well able to withstand compression without crumbling or breaking, and so it serves to reinforce the binding action of the mud. Humans have been creating composite materials to build stronger and lighter objects for thousands of years.

The majority of composite materials use two constituents: a binder or matrix and a reinforcement. The reinforcement is stronger and stiffer, forming a sort of backbone, while the matrix keeps the reinforcement in a set place. The binder also protects the reinforcement, which may be brittle or breakable, as in the case of the long glass fibers used in conjunction with plastics to make fiberglass. Generally, composite materials have excellent compressibility combined with good tensile strength, making them versatile in a wide range of situations. Engineers building anything, from a patio to an airplane, look at the unique stresses that their construction will undergo. Extreme changes in temperature, external forces, and water or chemical erosion are all accounted for in an assessment of needs. When building an aircraft, for example, engineers need lightweight, strong material that can insulate and protect passengers while surfacing the aircraft. An aircraft made of pure metal could fail catastrophically if a small crack appeared in the skin of the airplane. On the other hand, aircraft integrating reinforced composite materials such as fiberglass, graphite, and other hybrids will be stronger and less likely to break up at stress points in situations involving turbulence.

Many composites are made in layers or plies, with a woven fiber reinforcement sandwiched between layers of plastic or another similar binder. These composite materials have the advantage of being very moldable, as in the hull of a fiberglass boat.

Composites have revolutionized a number of industries, especially the aviation industry, in which the development of higher quality composites allows companies to build bigger and better aircraft. [35,36]

2.6 GLUTARALDEHYDE

Chemical Name 1,5-Pentanedial

Chemical Family Aldehyde

Molecular Formula $C_5H_8O_2$

Structure $OHCCH_2CH_2CH_2CHO$

Molecular Formula $C_5H_8O_2$

Description

Glutaraldehyde is a pungent, colorless liquid which when heated to decomposition emits acrid smoke. It is soluble in water, alcohol, benzene, and ether, and is volatile in steam. Glutaraldehyde polymerizes in water to a glassy foam.

Physical Properties of Glutaraldehyde

Molecular Weight	100.13
Boiling Point	188 oC at 760 mm Hg

Vapor Density	3.4 (Air = 1)
Density	0.72
Vapor Pressure	17 mm Hg at 20 oC
Conversion Factor	1 ppm = 4.1 mg/m3

SOURCES AND EMISSIONS

Sources

Glutaraldehyde is used as an embalming fluid, a chemical intermediate, a fixative for tissues, for crosslinking protein and polyhydroxy materials, and tanning of soft leathers.

Glutaraldehyde is registered as an antimicrobial and as a bactericide, a fungicide and a virucide. It is used to sterilize and disinfect hospital and veterinary equipment, and to disinfect surfaces in hospitals, veterinary hospitals, nursing homes, and food processing plants. Glutaraldehyde is used for disinfecting oil drilling muds, secondary oil recovery water systems, and ore processing water systems. It is also used to prevent bacterial growth in water supplies for washing air, water-washer cooler systems, industrial, commercial and logging ponds, and in pulp/paper mill water systems.

The licensing and regulation of pesticides for sale and use in California are the responsibility of the Department of Pesticide Regulation (DPR). Information presented in this fact sheet regarding the permitted pesticidal uses of glutaraldehyde has been

collected from pesticide labels registered for use in California and from DPR's pesticide databases. This information reflects pesticide use and permitted uses in California as of October 15, 1996. For further information regarding the pesticidal uses of this compound, please contact the Pesticide Registration Branch of DPR .

The primary stationary sources that have reported emissions of glutaraldehyde in California are crude petroleum and natural gas extraction, beverage manufacturers, and national security installations .

ATMOSPHERIC PERSISTENCE

Glutaraldehyde will exist in the atmosphere in the gas phase. The only important chemical loss process for glutaraldehyde in the troposphere is reaction with the hydroxyl (OH) radical. The calculated half-life and lifetime of glutaraldehyde due to reaction with the OH radical are 10 hours and 14 hours, respectively (Atkinson, 1989).

Glutaraldehyde-based solutions are stable under normal storage conditions. Although heating theproduct will result in color formation and slowpolymer formation, it does not represent a safety concern. However, if water is evaporated fromaqueous glutaraldehyde solutions, the residual material will rapidly polymerize in a non-hazardous reaction producing residue that will burn. In case of a fire, carbon dioxide, dry chemical, alcohol-type, or universal-type foams, applied according to the manufacturers' recommended technique, are suitable extinguishers.Self-contained breathing apparatus should be available to fire fighters since glutaraldehyde vapor is irritating at very low

concentrations. Contamination of concentrated solutions of glutaraldehyde with strongly acidic or alkaline impurities can result in exothermic polymerization of the contained glutaraldehyde via aldol condensation reactions. If this occurs, addition of water to dilute the solution is recommended.

2.7 Literature reviews

2.7.1 Biomaterial

The research of Pathavuth Monvisade, Punnama siriphannon, Supatra Jinawath and Khemchai Hemachandra [35] was prepared hydroxyapatite/poly(ethylene glutarate) (HAp/PEG) biomaterial composites by ring-opening polymerization (ROP) of cyclic oligo(ethylene glutarate) (C-PEG) in porous HAp scaffolds. The HAp/C-PEG precomposites were prepared by immersing the porous HAp scaffolds in the mixture solution of C-PEG and dibutyl tinoxide catalyst overnight and polymerizing at 200 °C for 24, 48, and 72 hours under vacuum. The successful ROP of C-PEG in the porous HAp scaffolds was corroborated by the signals of hydroxyl end-group of PEG shown in the ^{1}H NMR spectrum of the ROP-products extracted from the composites. PEG in the composite was present as a thin layer coating on the HAp grains and was evenly distributed throughout the samples. The PEG content was about 13-16 wt% and decreased with increasing polymerization time. Its molecular weight (weight average) measured by gel permeation chromatography was in the range of 4300-6800 g/mol. Compressive strength of HAp/PEG composites was significantly increased from 3 MPa of the porous HAp scaffolds to 11-14 MPa, depending on the PEG content in the composites. In vitro bioactivity of HAp/PEG composites was studied by soaking in simulated body fluid (SBF) at 36.5 °C for 7-28 days. After prolonged soaking, the HAp nanocrystals precipitated from the SBF solution and formed as a layer of globular

aggregates, coated on the composite surfaces. This result suggested that the HAp/PEG composite was a bioactive material.

The research of Pathavuth Monvisade, Punnama siriphannon, Rapee Jermsungnern and Sirirat Rattanabodee [36] was prepared hydroxyapatite/poly(methyl methacrylate) (HAp/PMMA) and calcium silicate/ poly(methyl methacrylate) (CS/PMMA) composites by interpenetrating bulk polymerization of methyl methacrylate (MMA) monomer in porous structure of HAp and CS. The porous HAp and CS templates were prepared by mixing their calcined powders with poly(vinyl alcohol) (PVA) solution, shaping by uniaxial pressing and then firing at 1,100 C for HAp and 900 °C for CS. The templates were soaked in the solution mixture of MMA monomer and 0.1 mol% of benzoyl peroxide (BPO) for 24 hours. The pre composites were then bulk polymerized at 85 °C for 24 hours under nitrogen atmosphere. The microstructure of the composites showed in the interpenetrating of PMMA into the porous HAp and CS structures. Thermogravimetric analysis indicated that the PMMA content in the HAp/PMMA and CS/PMMA composite were 13 and 26 wt%, respectively. Weight average molecular weights of PMMA were about 348,000 for CS/PMMA composites. Compressive strengths of these composites were about 90-131 MPa in which they were significantly higher than their starting porous templates. The compressive strengths of both composites were in the range of cortical bones.

The research of Pathavuth Monvisade, Punnama Siriphannon and Walailak Tapcharoen [37] was prepared hydroxyapatite/poly (ethylene gluterate) (HAp/PEG) composites by ring-opening polymerization (ROP) cyclic oligo(ethylene glutarate) in

porous HAp scaffolds using various reaction temperatures and times. The content of ROP-PEG interpenetrated into the porous HAp scaffolds was about 13-18 wt% with the value of number average molecular weight ($\bar{M}n$) and weight average molecular weight ($\bar{M}w$) of 2120-3630 and 2760-5250 g/mol, respectively. The increase in polymerization time and temperature brought about increase in molecular weight of ROP-PEG, but decrease in its content. Compressive strength and compressive modulus of HAp/PEG composites were about 5.8-20.1 and 105-208 MPa, respectively. These mechanical properties depend upon the effect of distribution, content, and molecular weight of ROP-PEG in the composites. In vitro bioactivity of HAp/PEG composites was studies by soaking them in simulated body fluid (SBF) for 28 days. The formation of HAp nanocrystal on the composite surfaces through the consumption of calcium and phosphorus from the SBF solution was observed after soaking, indicating the bioactivity of these HAp/PEG composites.

The research of Pathavuth Monvisade and Punnama Siriphannon [38] was prepared hydroxyapatite/poly(ethylene adipate) (HAp/PEA) composite by in situ ring-opening polymerization of cyclic oligo(ethylene adipate) (C-OEA) within the porous HAp templates. HAp was firstly prepared by a co-precipitation method using calcium hydroxide and phosphoric acid and then shaped as arectangular porous template. PEA precursor was synthesized by bulk polymerization of dimethyl adipate and ethylene glycol in the presence of tetraisopropyl orthotitanate. C-OEA was obtained by cyclo-depolymerization of the PEA precursor under high dilution condition using dibutyl tinoxide as a catalyst. The HAp/PEA composites were prepared by immersing the porous

HAp templates in the mixture solution of C-OEA and dibutyl tinoxide catalyst overnight and ring-opening polymerized at 180, 200 and 220 C for 24 hours. The ring-opening polymerization PEA formed as a thin film coating on the surface of porous HAp template. The HAp/PEA composites contained PEA in the range of 20-26 wt%. The weight-average molecular weights of ring-opening polymerized PEA were in the range of 3800-4450 g/mol. Compressive strength of the HAp/PEA composite was significantly increased from 25 MPa in the porous HAp template to 140 MPa in the composite.

The research of Pathavuth Monvisade and Punnama Siriphannon [39] was prepared poly (ethylene terephthalate)/hydroxyapatite (PET/HAp) composites by mixing HAp powder with a mixture solution of cyclic oligo(ethylene terephthalate) (C-OET) and dibutyl tinoxide catalyst in dichrolomethane, and then shaping the precomposites in cylindrical pellets. The C-OET in the precomposies was ring-opening polymerized (ROP) to PET under vacuum at 250 C for 24 hours. The PET/HAp composites were formulated with HAp to PET ratios of 60:40 (H6P4) and 50:50 (H5P5). The ROP-PET in the composites was present as a thin layer coating on the HAp gains and evenly distributed throughout the samples. Compressive strength of the PET/HAp composite was significantly increased from 8 MPa of the H10P0 to 17 and 29 MPa for H6P4 and H5P5, respectively. In vitro bioactivity of the PET/HAp composites was studied by soaking them in simulated body fluid (SBF) at 36.5 C for 7-28 days. After prolonged soaking, the HAp nanocrystals precipitated from the SBF solution and from as a layer of globular aggregates, coated on the composite surfaces. The H6P4 composite showed faster

formation rate of nano-HAp than the H5P5 composite, indicating that the bioactivity of PET/HAp composites depended on the amount of HAp.

2.7.2 Chitosan

The research of Guiping Maa, Dongzhi Yang, Yingshan Zhou, Ming Xiao, John F. Kennedy and Jun Nie [40] was prepared water soluble chitosan by N-Alkylated chitosan derivative was synthesized by Michael addition reaction of chitosan and hydroxyethylacryl. Chitosan (2.0 g) was placed into a solution of acetic acid (2.0 g) in distilled water (200 g), and hydroxyethylacrylate (3.6 g) was added with stirring. This mixture was reacted at 50 °C for 48 h then filtered. The solution was poured into 400 mL of acetone to remove the unreacted hydroxyethylacryl. The precipitate was washed three times with acetone then dried under vacuum at 30 °C overnight to obtain hydroxyethylacryl-chitosan. The chemical structure and physical properties of the chitosan derivatives were characterized by FT-IR, 1H NMR, XRD and DSC techniques. The 1H NMR results indicated that the degree of substitution (DS) was from 0.18 to 1.2. The chitosan derivatives exhibited an excellent solubility in distilled water. XRD analysis showed that the derivatives were amorphous. DSC results demonstrated that thermal stability of the derivatives was lower than that of chitosan and it decomposed around 226 °C for hydroxyethylacryl-CS with DS higher than 1.05. The lysozyme enzymatic degradation results revealed that the chitosan derivatives had the ability to enzymatic degradation and the initial degradation rate of the chitosan derivatives was dependent on its molecular weight. The antimicrobial activity of the chitosan derivatives was decreased compared with that of chitosan, but it still had the ability of antimicrobial.

The research of Yajun Xie, Xiaofei Liu and Qiang Chen [41] was prepared ethylamine hydroxyethyl chitosan (EHCs) by synthesis from chitosan and chloroethylamine hydrochloride under alkali condition by the novel method described in this study. The process is divided into two steps. Firstly Five grams of chitosan was added into a certain number of sodium hydroxide solution (50%, by weight) with stirring, then the mixture was put into refrigerator at 18 °C for 48 h for alkalization. After thawing, Alkali chitosan and Isopropyl alcohol (10 ml) were mixed and stirred to homogenization, after that some chloroethanol was added with continuous stirring at 80 °C for 12 h. After filtration, the solid product was washed with alcohol for two times. HECs yielded were dried in an oven at 60 °C. Secondly five grams of HECs was poured into a mixture of 20 ml isopropyl alcohol and 42% sodium hydroxide solution with stirring, the reaction was reacted at certain temperature for some time after some chloroethylamine hydrochloride was added in. reaction ended, adjust PH to 7 using 1 mol/l hydrochloric acid solution, the solid product was washed with acetone for several times and dried in an oven. The effect of several factors, such as reaction time, temperature and the molar ratio of hydroxyethylic chitosan (HECs) to chloroethylamine hydrochloride were discussed. The structure changes of derivative were investigated by FTIR, 13C NMR, DSC and TGA analysis. Ethylamine hydroxyethyl chitosan (EHCs) with good water solubility was prepared via a novel processing technique, the optimum reaction conditions were determined as temperature 70–80 °C; the molar ratio of HECs to chloroethylamine hydrochloricde 2:1 and reaction time is 12 hours.

The research of Ying-Chien Chung, Cheng-Lang Kuo and Chiing-Chang Chen [42] was improved the solubility of chitosan at neutral or basic pH using the Maillard-type reaction method. To prepare the water-soluble chitosans, various chitosans and saccharides were used under various operating conditions. Biological and physicochemical properties of the chitosan-saccharide derivatives were investigated as well. Chitosan at 90% DD was dissolved in 0.2 M CH_3COOH solution (pH 3.3) to give a final chitosan concentration of 1% (w/v). After that, glucose was dissolved in the chitosan solution to a final glucose concentration of 1% (w/v). A total of 15 samples (in triplicate) were reacted at 65°C for 5 days. Every other day, three samples were withdrawn to determine yield and solubility. To produce the optimal water-soluble variant, a-type chitosan at 75% or 90% DD was dissolved in 0.2 M CH_3COOH solution, to give a final chitosan concentration of 1% (w/v), and then separately mixed with various amounts of glucose, glucosamine, maltose, and fructose until dissolution by mild stirring. All the added saccharides were at a concentration of 1% or 2%, except for fructose which was added at 0.5% or 1%. The mixtures were reacted at 55, 65 or 75 °C for a specified period in an orbital shake incubator. Triplicate samples were drawn and centrifuged (8000 rpm, 15 min). The supernatant was dialyzed against distilled water by dialysis membrane with molecular weight cut-off 12,000–14,000 (Spectrum Laboratories Inc., USA) for 96 hours and then freeze-dried. Results indicated that the solubility of modified chitosan is significantly greater than that of native chitosan, and the chitosan-maltose derivative remained soluble when the pH approached 10. Among chitosan-saccharide derivatives, the solubility of chitosan-fructose derivative was highest at 17.1

g/l. Considering yield, solubility and pH stability, the chitosan-glucosamine derivative was deemed the optimal water soluble derivative. Compared with the acid-soluble chitosan, the chitosan-glucosamine derivative exhibited high chelating capacity for Zn^{2+}, Fe^{2+} and Cu^{2+} ions. Relatively high antibacterial activity against Escherichia coli and Staphylococcus aureus was noted for the chitosan-glucosamine derivative as compared with native chitosan. Results suggest that the water-soluble chitosan produced using the Maillard reaction may be a promising commercial substitute for acid-soluble chitosan.

The research of Min Fan, Qiaoling Hu and Kai Shen [43] was prepared two kinds of chitosans, namely N-acetylated and N-deacetylated chitosan by the modified processes. They can dissolve in both acid and alkali solution. ^{13}C NMR was used to study the basic solution of chitosan, and XRD, FT-IR and SEM were used to study the structure of N-acetylated and N-deacetylated chitosan. The result from X-ray diffraction showed that a transformation of crystal structure occurred during the N-acetylation or N-deacetylation process with the decrease of crystallinity and expansion of crystal lattices. FT-IR spectra revealed that the intermolecular and intramolecular hydrogen bonds were destroyed by both treatments and a looser structure was observed by the SEM. The lower crystallinity, the decreased intermolecular interactions, the more disordered and looser structure were easy for the permeation of LiOH/urea aqueous solution and coordinated with the breakage of intermolecular and intramolecular hydrogen bond by LiOH at low temperature, the prepared chitosans dissolved in LiOH/urea/H2O mixture.

The research of Dong-Keon Kweon, Seok-Beom Song and Yong-Yook Park [44] was prepared water-soluble chitosan (WSC)/heparin (CH) complex by using WSC with

wound healing ability and heparin with ability to attract or bind growth factor related to wound healing process. Water-soluble CH complex was prepared by the reaction between WSC and heparin, and then, by adding distilled water to it, ointment type with high viscosity was made. To evaluate the wound healing effect, full thickness skin excision was performed on the backs of the rat and then WSC and water-soluble CH complex ointments were applied in the wounds, respectively. After 15 days, gross and histologic examination was performed. Grossly, untreated control group revealed that the wound had well defined margin and was covered by crust. The second group treated with WSC ointment revealed small wound size with less amount of covering crust and ill-defined margin, which appeared to regenerate from margin. The third group treated with water-soluble CH complex ointment appeared to be nearly completely healed. Histology of each group was well correlated to gross findings. The third group shows nearly complete regeneration of appendage structure similar to normal in the dermis in contrast to control and second group with absence and less number of skin appendages, respectively.

Chapter 3

Research Methodology

3.1 Material

3.11 Calcium nitrate tetrahydrate ($Ca(NO_3)_2.4H_2O$), CARLO ERBA, Ltd.

3.12 Tetraethyl orthosilicate ($C_8H_2O_4Si$), FLUKA, Ltd.

3.13 Ethanol, MALLINCKRODT CHEMICAL, Ltd.

3.14 Chitosan of %DD = 82.5, which is obtained from deacetylation of chitin, ELAND Corporation, Ltd.

3.15 AR grade of glacial acetic acid (HAc), CARLO ERBA, Ltd.

3.16 Hydroxyethylate (HEA)

3.17 Commerce grade sodium hydroxide (NaOH), CARLO ERBA, Ltd.

3.18 Paraffin oil

3.19 Acetone, Commercial grade, ZEN POINT, Ltd.

3.2 Apparatus

3.2.1 Mechanic stirrer, Euro-ST B, IKA Laboratory Staufen Ltd.

3.2.2 Water bath, size 16 cm x 22cm x 12 cm

3.2.3 Glassware

3.2.4 Oven, Isotemp, Fisher Scientific Ltd.

3.2.5 Weighing apparatus, TC-254, Denver Instrument Ltd.

3.2.6 Themostat, Euro-ST B, Ika Laboratory Staufen Ltd.

3.2.7 X-ray diffractrometer, XRD, D8 advance, Bruker AG Ltd.

3.2.8 FT-IR spectroscope, Spectrum One, Perkin Elmer Ltd.

3.2.9 ^{1}H NMR spectroscope, NMR 300 Ultra Shield Spectrometer, Bruker AG Ltd.

3.2.10 Thermal gravimetric analyzer (TGA), Pyris 1 TG, Perkin Elmer Ltd., Scientific Instrument Service Centre, KMITL)

3.2.11 Scanning electron microscope (SEM) Type JSM-5410LV, JEOL Ltd.

3.2.12 Universal testing machine (Lloyd Instrument LR30K)

3.3 Preparation

3.3.1 Preparation of calcium silicate powders

1. Calcium silicate powder was prepared by calcium 47.56 g of nitrate tetrahydrate and 41.68 g of tetraethyl orthosilicate in 1000 ml of ethanol with stirring for 2 hours

2. Calcium silicate solution was diluted to 0.1 mol/l

3. Calcium silicate was precipitate by adding 0.33 mol/l sodium hydroxide, which dissolved 7.88 g of sodium hydroxide in 600 ml of distilled water.

4. Calcium silicate was filtered and washed twice with 150 ml of distilled water.

5. Calcium silicate precipitate was dried overnight at 100°C and calcined at 500°C for 2 hours.

6. The received calcium silicate powder was characterized by X- ray diffractometer (XRD) (D8 advance, Bruker AG Ltd.) pattern is recorded using Cu Kα radiation (α = 15.4 nm) at a voltage of 30 kV and a current of 30 mA.(2θ in 1° -25° , step size 0.040° ,and step time 0.5 second)

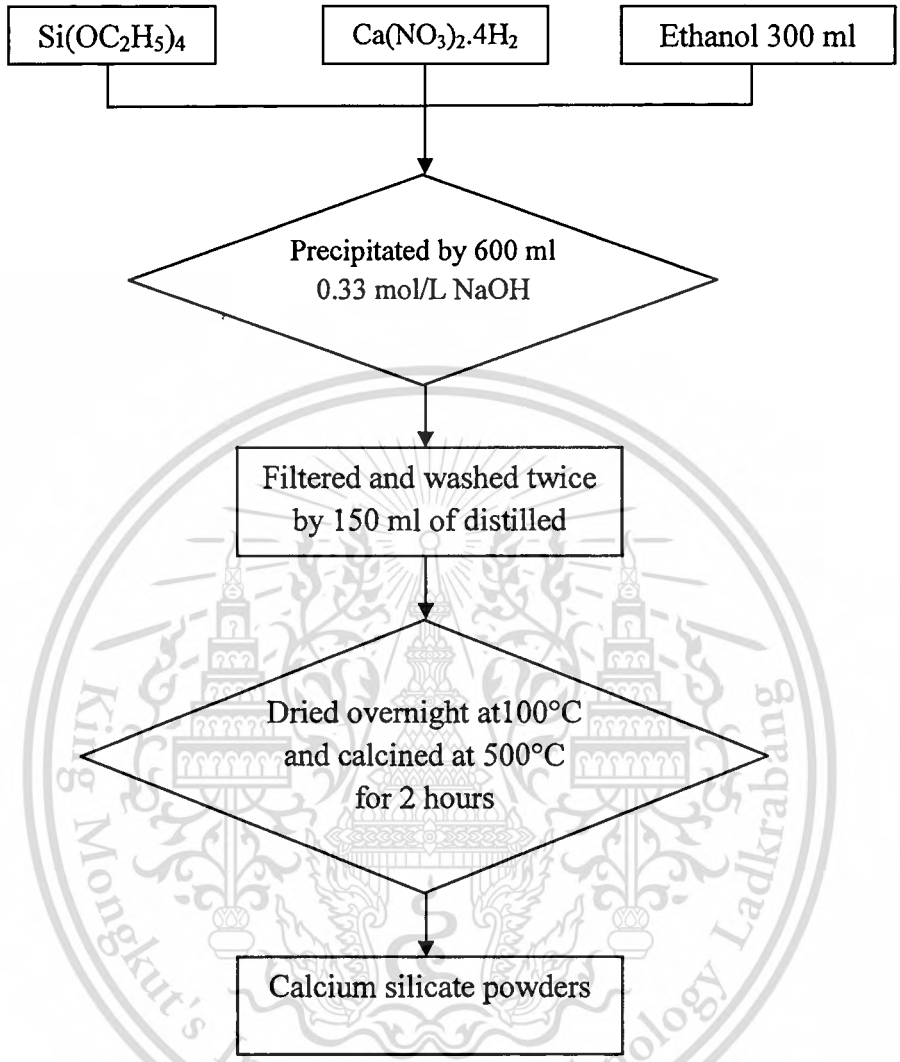


Figure 3.1 Preparation of calcium silicate powder

3.3.2 Preparation of hydroxyethylacryl-chitosan.

1 3.0 g of chitosan was placed into a solution of 3.0 g acetic in 300 ml distilled water, and add 12 g hydroxyethylacrylate with stirring.

2 This mixture was reacted at 60°C for 48 Hours.

3 Refluxed chitosan was adjusted pH ~7 by using NaOH.

4 The solution was poured into 400 ml of acetone to remove the unreacted hydroxyethylacrylate. The precipitate was washed three times with acetone then dried under vacuum at 30 °C overnight to obtain hydroxyethylacryl chitosan

5 The hydroxyethylacryl-chitosan was characterized by FT-IR spectroscopy and ^{1}H NMR spectroscopy

6 The received hydroxyethylacryl-chitosanwas characterized by

- FT-IR spectroscopy samples were prepared as KBr pellet and were scanned against a blank KBr pellet background at wavenumber range 4000-600 cm^{-1} with resolution of 4.0 cm^{-1}
- ^{1}H NMR spectroscopy chitosan was dissolved in a mixed solvent D_3CCOOD and D_2O, and hydroxyethylacryl-chitosan was dissolved in D_2O

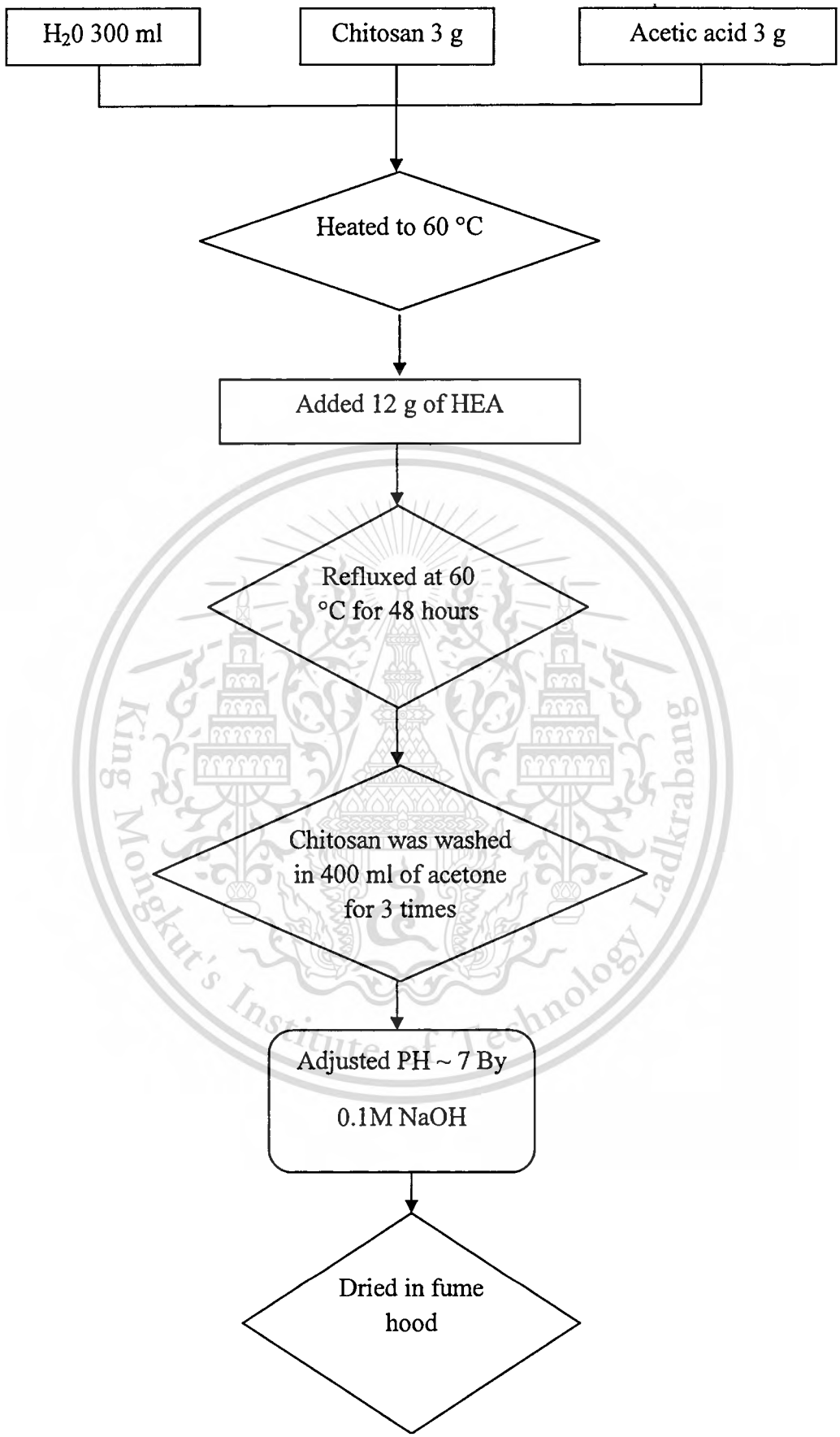


Figure 3.2 Preparation of hydroxyethylacryl-chitosan.

3.3.3 Preparation of hydroxyethylacryl-chitosan/ calcium silicate composites

3.3.3.1 Preparation of hydroxyethylacryl-chitosan/ calcium silicate composites with 1wt% glutaraldehyde (HC/CS- GLU1)

1. The chitosan 5g were dissolved in distilled water.
2. Calcium silicate 7.5g were mixed in the solution mixture of chitosan
3. The composites were shaped by Teflon® mold.
4. The composites were soaked in 1 wt% glutaraldehyde
5. Hydroxyethylacryl-chitosan/ calcium silicate composites were characterized by SEM and Compressive Strength Test

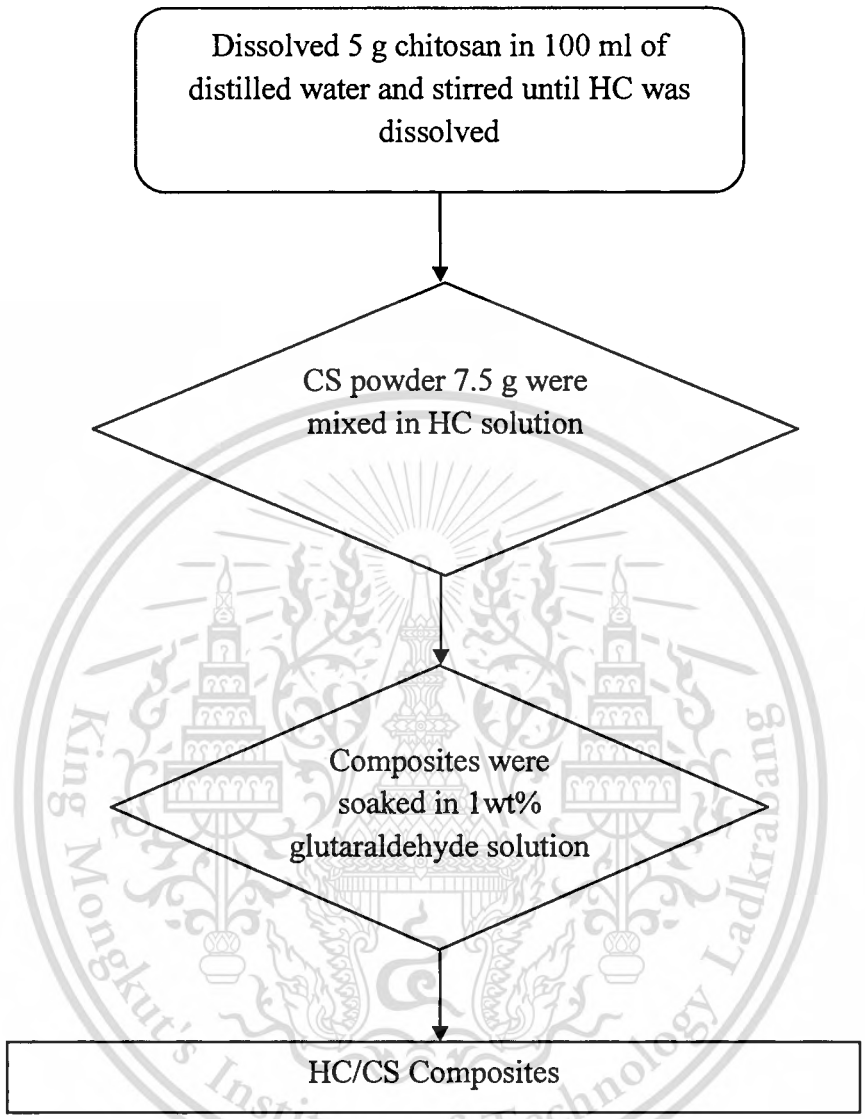


Figure 3.3 Preparation of HC/CS composites (HC/CS-GLU1)

3.3.3.2 Preparation of hydroxyethylacryl-chitosan/ calcium silicate composites with 1wt% glutaraldehyde (HC/CS- GLU2)

1. The chitosan 5g were dissolved in distilled water.

2. Calcium silicate 7.5g were mixed in the solution mixture of chitosan and 1 wt% glutaraldehyde

3. The composites were shaped by Teflon® mold and in oven at 50°C for 24 hours

4. Hydroxyethylacryl-chitosan/ calcium silicate composites was characterized by SEM, Compressive Strength Test and TGA

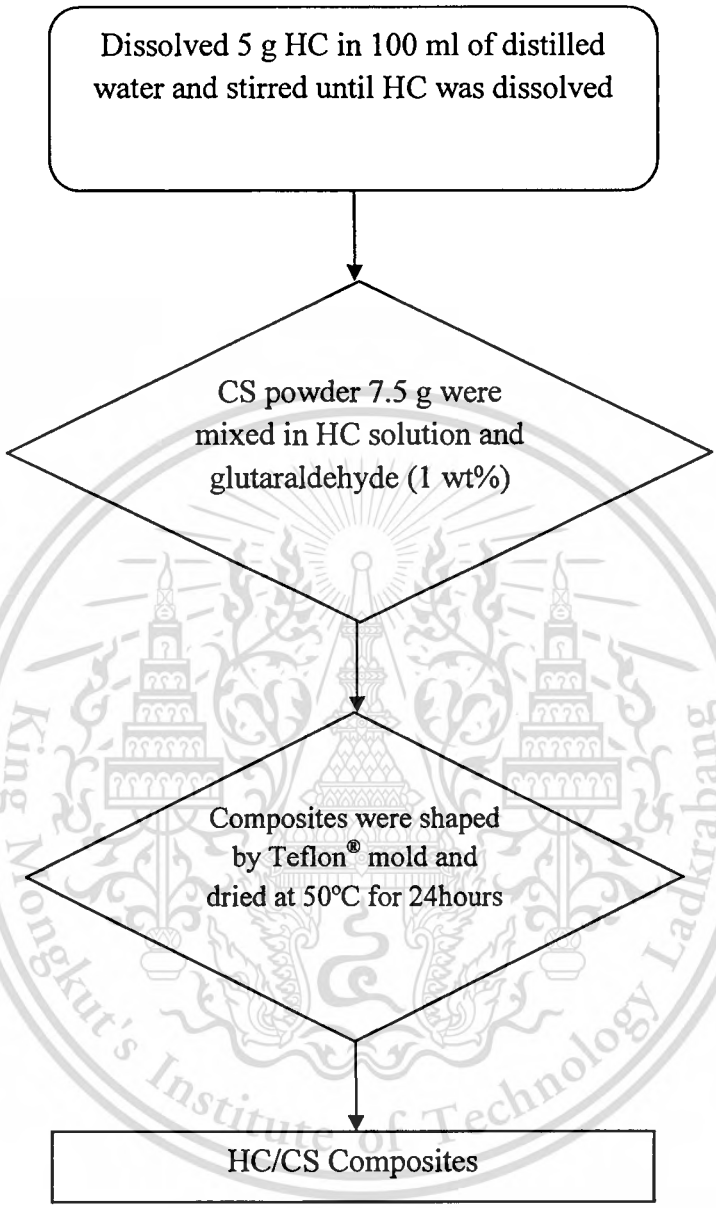


Figure 3.4 Preparation of HC/CS composites (HC/CS- GLU2)

3.4 Characterization

3.4.1 X-ray Diffractometer

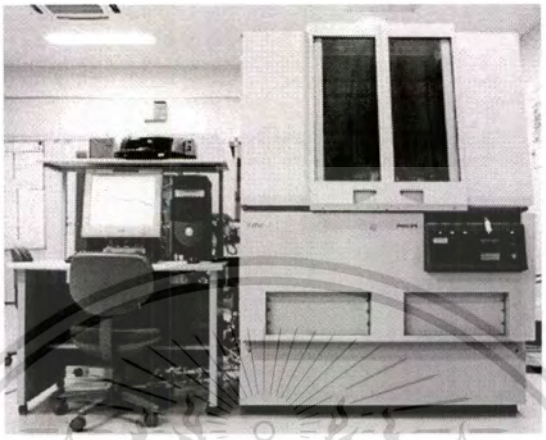


Figure 3.5 X-ray Diffractometer; XRD

X-ray diffraction (XRD) is analytical technique was used foe phase identification of crystalline material such as size and orientation of crystalline.

Analysis condition

2θ to analysis	**1°-35°**
Step size	**0.04°**
Step time	**5 sec**

3.4.2 Thermo gravimetric Analyzer

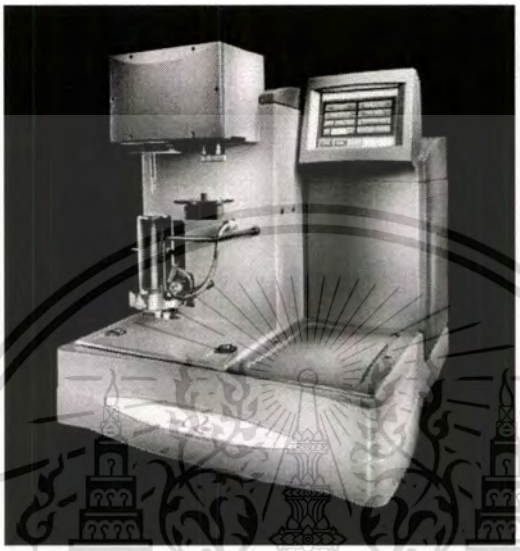


Figure 3.6 Thermo gravimetric Analyzer; TGA

Thermo Gravimetric Analyzer (TGA) is Thermo gravimetric of Chitosan/Calcium Silicate Composites were performed by using thermogravimetric analyzer (TGA, Pyris 1 TGA and PerkinElmer). This technique is used to determine the HC/CS composites by putting dry powder in a small furnace with the temperature range of 50-800 °C at a heating rate of °C/min

Analysis Condition

Temperature range **50-800 °C**

Temperature rate **10 °C/min**

Nitrogen flow **20ml/min**

Sample weight **5-10 mg**

3.4.3 Fourier Transform Infrared Spectroscopy

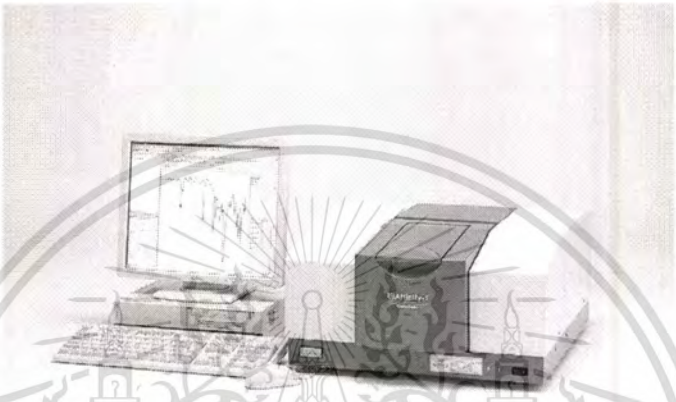


Figure 3.7 Fourier Transform Infrared Spectroscope (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) is an infrared spectrum represents a fingerprint of a sample with absorption peaks which correspond to the frequencies of vibrations between the bonds of the atoms making up the material. Because each different material is a unique combination of atoms, no two compounds produce the exact same infrared spectrum. Therefore, infrared spectroscopy can result in a positive identification (qualitative analysis) of every different kind of material. In addition, the size of the peaks in the spectrum is a direct indication of the amount of material present.

3.4.4 Scanning Electron Microscope

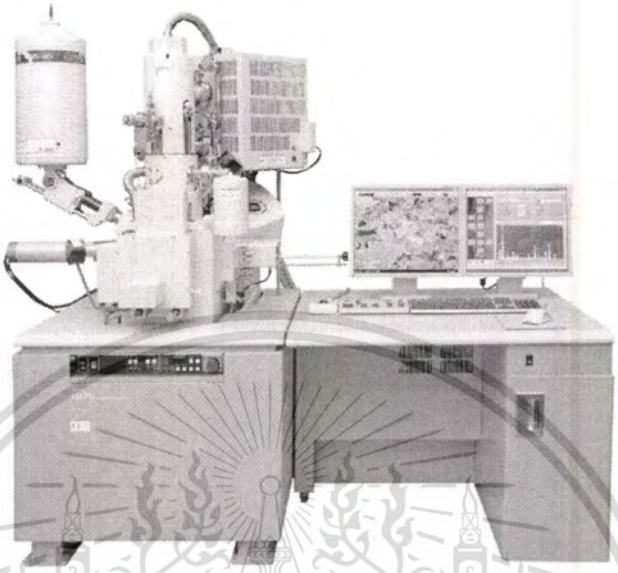


Figure 3.8 Scanning Electron Microscope (SEM)

Scanning Electron Microscope (SEM) is by mounted sample on the holder and then sputtered with gold. The microstructure of the sample surface was investigated by SEM.

3.4.5 Nuclear magnetic resonance (^{1}H NMR)

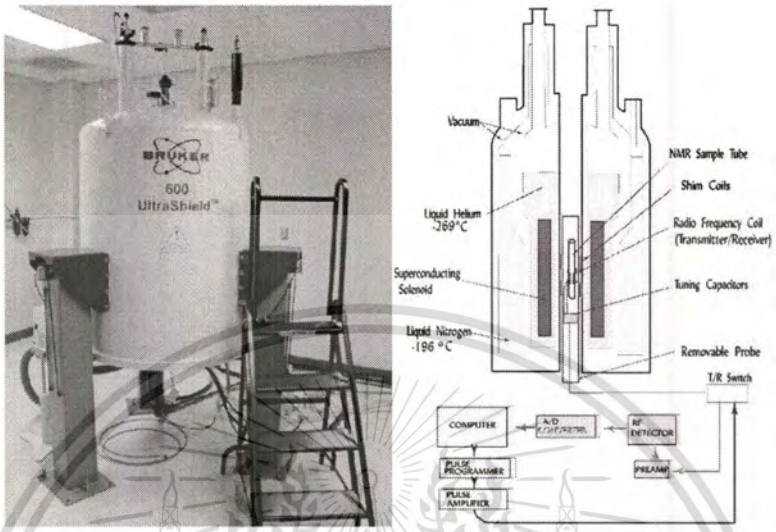


Figure 3.9 Nuclear magnetic resonance (^{1}H NMR)

^{1}H NMR spectra were recorded in deuterium dioxide (D_2O) on NMR 300 Ultra Shield Spectrometer (Bruker AG). The ^{1}H NMR Spectra were reference by setting the tetramethylsilane (TMS) as internal standard.

3.4.6 Universal testing machine

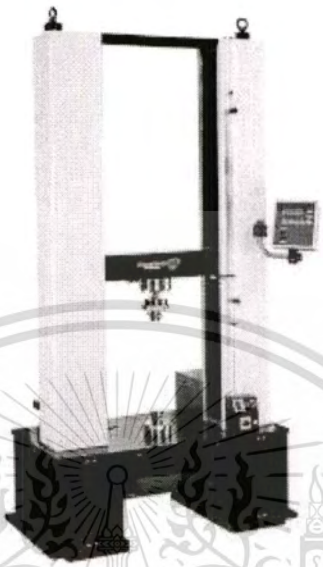


Figure 3.10 Universal testing machine

Compressive strength and compressive modulus of the HC/CS composites were measured by Universal testing machines (Lloyd Instrument LR30K) with 30 KN load cell and cross-head speed of 2.5 mm/min

Chapter 4

Result and discussion

4.1 Calcium silicate

4.1.1 Calcium silicate powder result by X-ray diffraction (XRD)

Figure 4.1 shows the x - ray diffraction (XRD) pattern of calcined calcium silicate powder. The board halo pattern was observed due to the amorphous structure of calcium silicate.

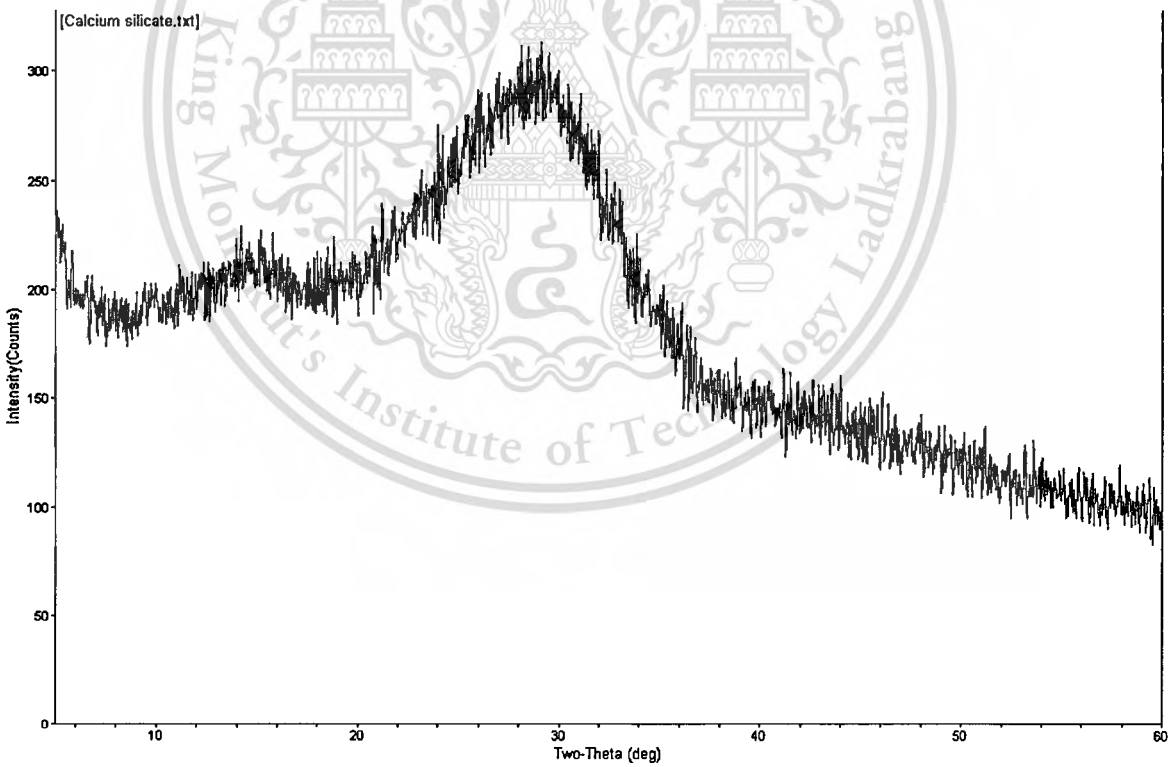


Figure 4.1 XRD pattern of Calcium Silicate

4.2 Chitosan

4.2.1 NMR Characterization

The typical ^1H NMR spectrum of the chitosan in CD_3COOD/D_2O and hydroxyethylacryl – chitosan in D_2O were shown in Figures 4.2 and 4.3 respectively.

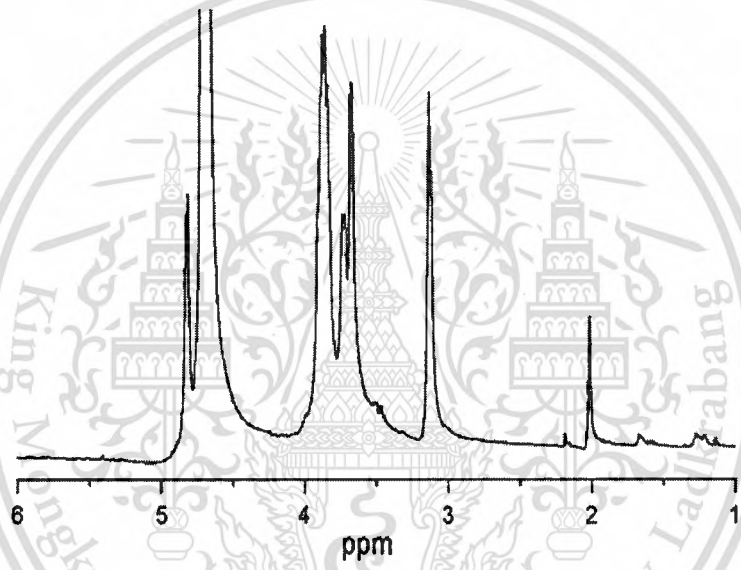


Figure 4.2 ^1H NMR spectrum of the chitosan in CD_3COOD/D_2O [40]

Figure 4.2 shows a small peak at 2.03 ppm existed because of the presence of –NHCO-CH_3 ($_{11}$) of chitin. A singlet at 3.10 ppm assigned to H_2 of chitosan and chitin and the chemical shift between 3.5 to 4.0 ppm attribute to H_3, H_4, H_5 and H_6. A small peak at 4.87 ppm was observed because of H_1 of chitin

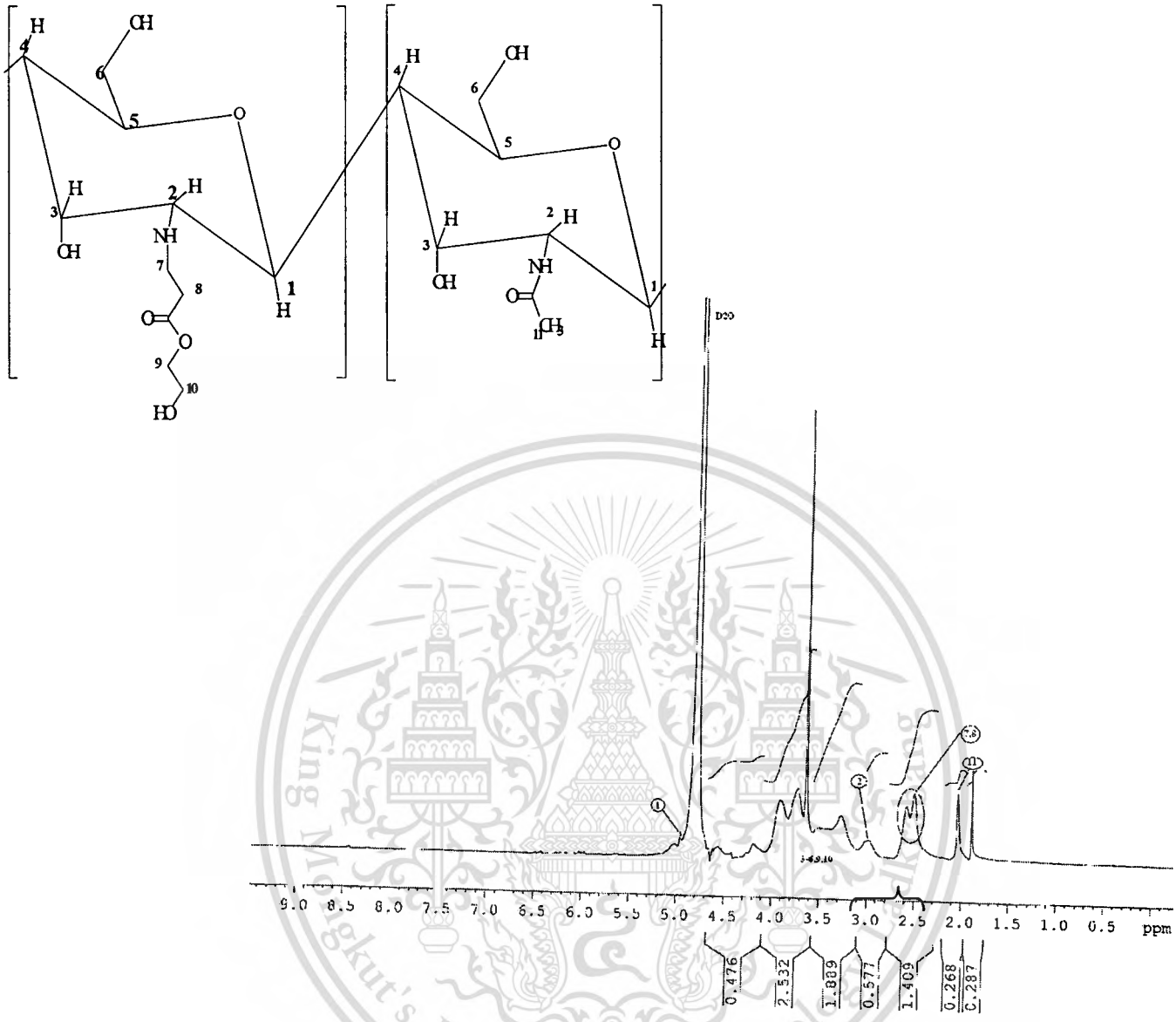


Figure 4.3 ^{1}H NMR spectrum of the hydroxyethylacryl – chitosan in D_2O

Figure 4.3 shows the same peak as the spectruum in Figure 4.2 at 2.03 ppm representing to $-NHCOCH_3-$ $(_{11})$ of chitosan. A broad peak at 3.10 ppm assigned to H_2 of chiosan and chitin, at the chemical shift between 3.3 to 4.0 ppm attributed to H_3, H_4, H_5, H_6, H_9 and H_{10} but the peak was overlapped so, the peaks were not clear. Compared with the reference ^{1}H – NMR spectrum of chitosan (figure 4.2), the typical peak at 2.7

ppm newly appeared because of –NH-CH_2(7)-CH_2(8)-CO_2- from the Michael addition reaction of chitosan to yield hydroxyethylacryl-chitosan.

4.2. FT-IR Characterization

Fourier transforms infrared (FT-IR) spectra of and hydroxyethylacryl-chitosan (HC) was shown in figure 4.4 and 4.5, respectively.

Figure 4.4 FT-IR spectra of chitosan.

From both Figures 4.4 and 4.5, the board peak at around 3417 cm^{-1}attributed to the overlapping of O-H stretching and NH streching vibration of chitosan and HC molecule. The weak band at 2920 cm^{-1} attributed to -CH- stretching. The absorbtion band at 1017 cm^{-1} was asymmetric stretching of the C-O-C brigde. The characteristic peak of chitosan at 1700 cm^{-1} assiged to the amide carbonyl group appeared in figure 4.4 as dissapeared in figure 4.5 indicating the transformation of -NH-COO-CH_3- to NH_2. The ester carbonyl group at 1652 cm^{-1} newly appeared because of the Micheal addition of chitosan indicating the reaction at –NH_2 to yield –$NHCH_2CH_2CO$-OCH_2CH_2

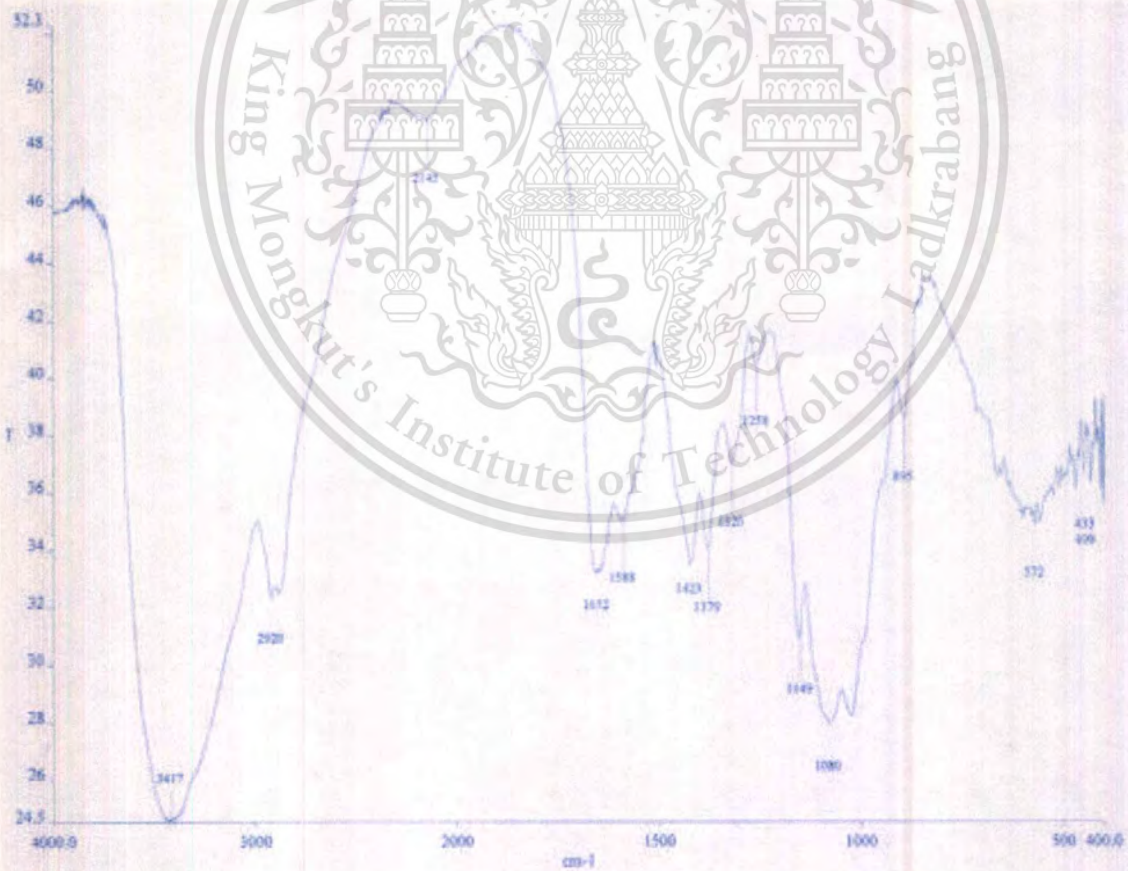


Figure 4.5 FT-IR spectra of HC

% Swelling of Hydroxyethylacryl- Chitosan

$$\%Swelling = \frac{W_s - W_d}{W_d} \times 100$$

Where;

W_s is the weight of hydrogel + water

W_d is the weight of dry hydrogel

Time (hr)	W_s (g)	% swelling
0.5	12.47	35.10
1	14.28	35.36
2	15.79	41.55
3	16.50	44.06
4	16.83	45.16
5	16.85	45.22

*When: $W_d = 9.23$ g

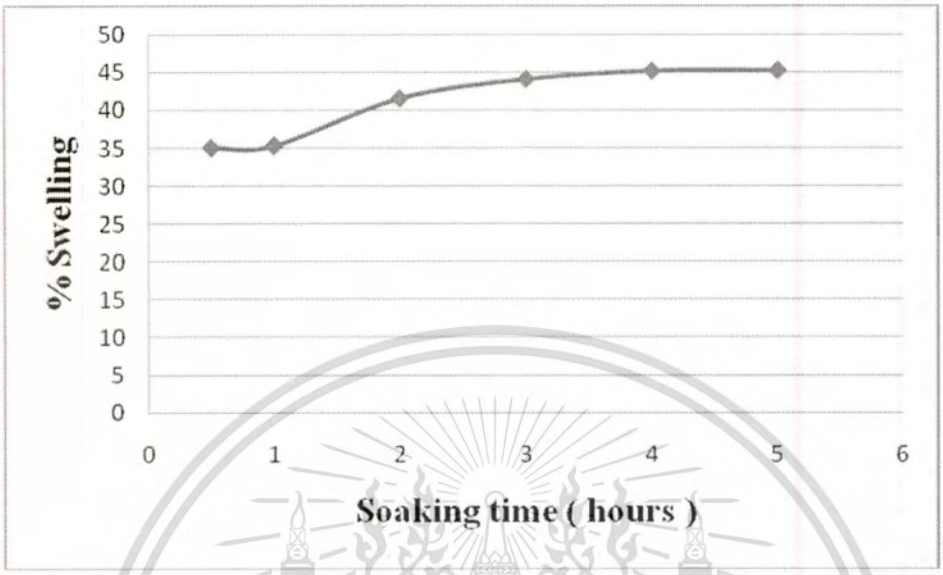


Figure 4.6 Swelling of HC

Figure 4.6 shows the swelling of HC crosslinked by 1wt% glutaraldehyde. It was found that the % swelling of HC in water gradually increased with an increase of soaking time.

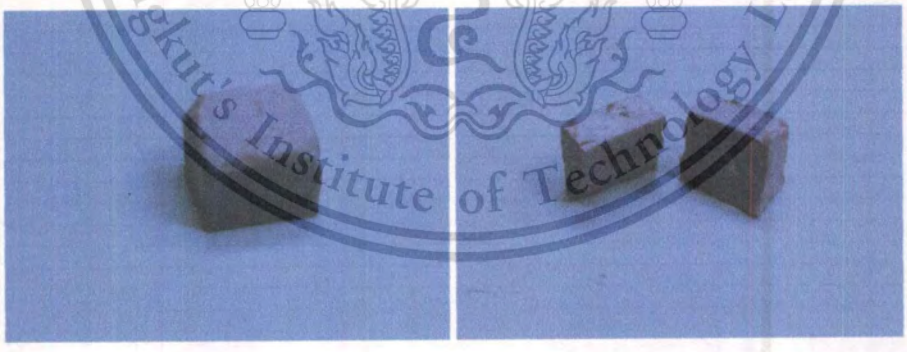
4.3 HC/CS composite

4.3.1 Physical characteristic of CS/HC composite.

Figure 4.7 and 4.8 show the physical appearance of CS and HC/CS samples, respectively. The CS was observed as a white cubic shape. On the other hand, both outer surface and imterior of the HC/CS composite were observed as brown cube due to the presence of HC and glutaraldehyde. These physical apperance indicated that the HC throughly coated on the CS particles.

(a) (b)

Figure 4.7 Calcium silicate: (a) Cubic sample and (b) cross section



(a) (b)

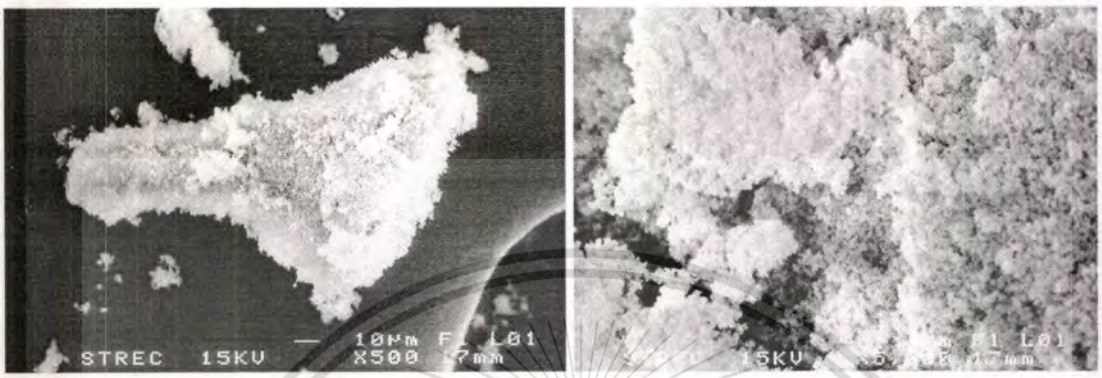
Figure 4.8 HC/CS composite: (a) Cubic sample and (b) cross section

4.3.2 Morphology

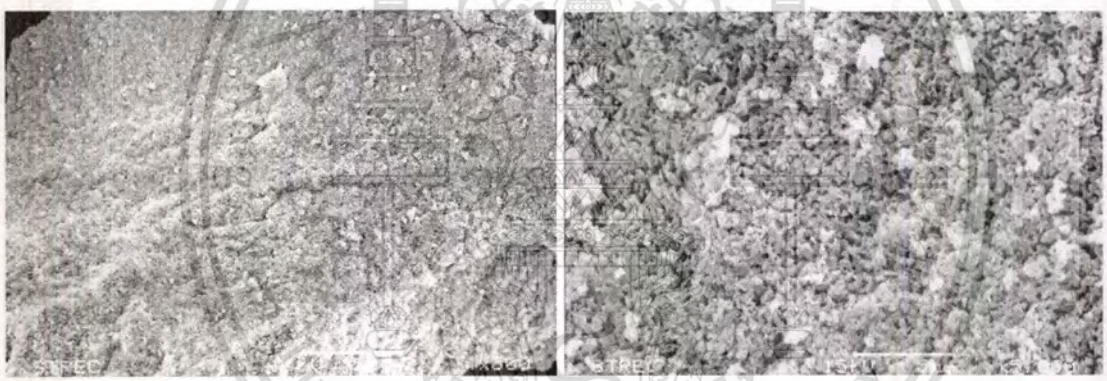
The composite consisted of 2 main components, i.e. liquid mixture of HC with water with 1% gluteraldehyde crosslinking agent and solid phase of CS.

Figure 4.9 (a) shows the SEM micrographs of CS. It can be seen that the CS particles were packed tightly by unaxial compression. This sample did not have continuous phase of polymer binder. The microstructure of the CS/HC-GLU1 and CS/HC-GLU2 composites were observed as the solid phase of CS particle dispersed in continuous polymer phase and CS particles were almost coated by HC as shown in Figures 4.7 (b) and (c), respectively. It was, however, the effect of preparation method, i.e. CS/HC-GLU1 and CS/HC-GLU2, could not be clarified by SEM micrographs.

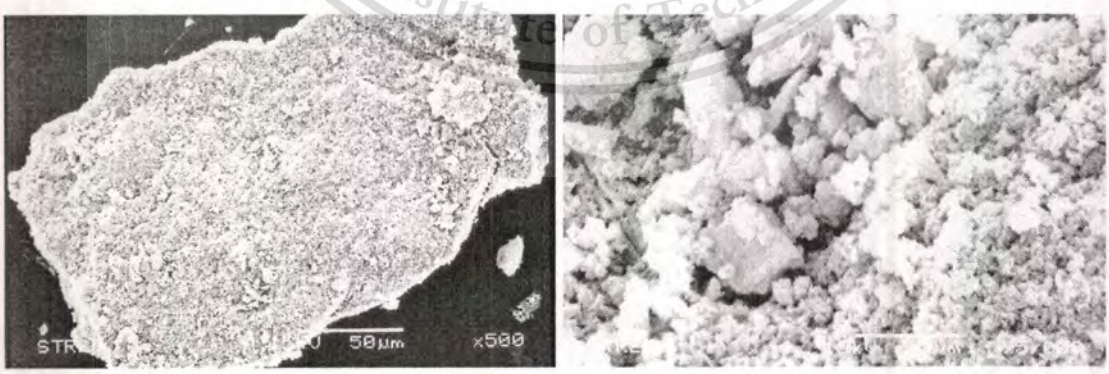
500x 5000x



(a) CS



(b) HC/CS – GLU 1



(c) HC/CS – GLU 2

Figure 4.9 SEM micrographs of (a) CS, (b) HC/CS – GLU 1 and (c) HC/CS – GLU 2

3.4.2 Mechanical Properties

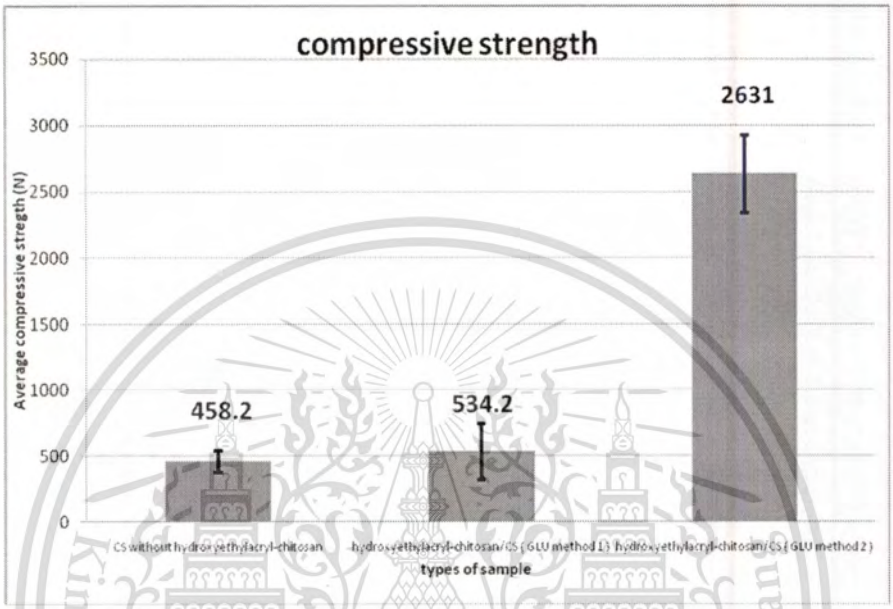


Figure 4.10 Compressive strength of CS and HC/CS composites

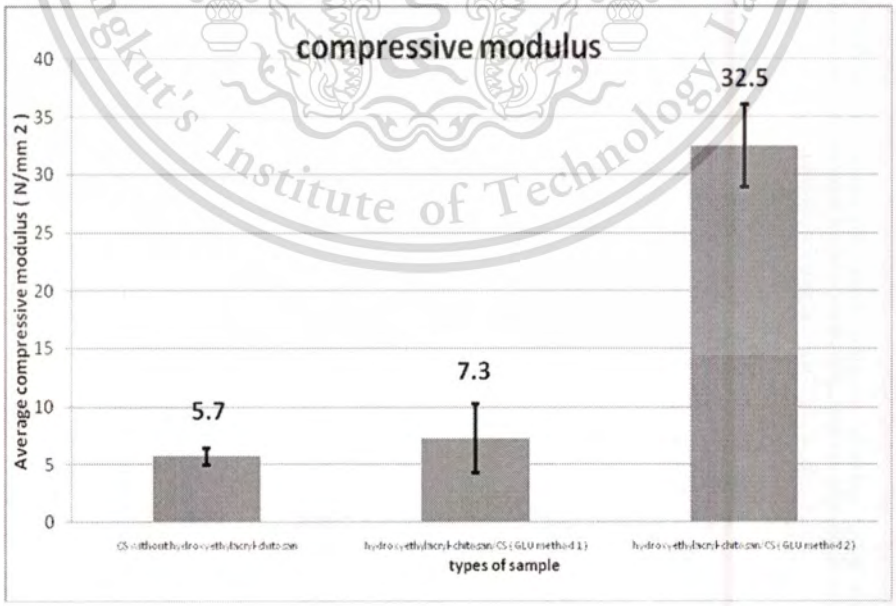


Figure 4.11 Compressive modulus of CS and HC/CS composites

Figure 4.10 and 4.11 respectively shows the compressive strength and modulus of the CS sample in comparison with the HC/CS-GLU1 and HC/CS-GLU2 composites. The mechanical properties of CS sample were the lowest values because this sample did not contain the polymer binder. It was, therefore, the fracture behavior of this sample was similar to general ceramic material.

The addition of gluteraldehyde into HC/CS-GLU1 and HC/CS-GLU2 could improve the mechanical properties of the composites. The HC/CS-GLU1 showed the slight increases of the mechanical properties because the gluteraldehyde could not penetrate into composite pellet. So, only the HC on the pellet surface could react with gluteraldehyde. While, the HC in the interior part of pellet could not from the crosslink network. It was, however, the polymer binder could increase the mechanical properties of the composite.

Finally, the mechanical properties of HC/CS-GLU2 composite were the highest values. In this composite, the gluteraldehyde was mixed together with CH and CS particle before shaping, it was considered that almost chitosan could crosslink to network structure; therefore, the mechanical properties of these sample drastically increase.

4.3.3 Thermal gravimetric analysis (TGA)

Amount of HC/CS composite and the decomposition temperature of the composite were determined by TGA technique. TGA thermogram of the composite was shown in Figure 4.12.

The first decomposition around 100 C correspond to the decomposition of water in to the composite. The decomposition of size chain of HC observed at 251 °C was about 17 wt%. The decomposition in the range of 320 – 700 °C was considered to be due to the backbone structure of HC. The total weight loss was about 40 wt% in which it was approximately equal to the initial amount of HC added into the composite.

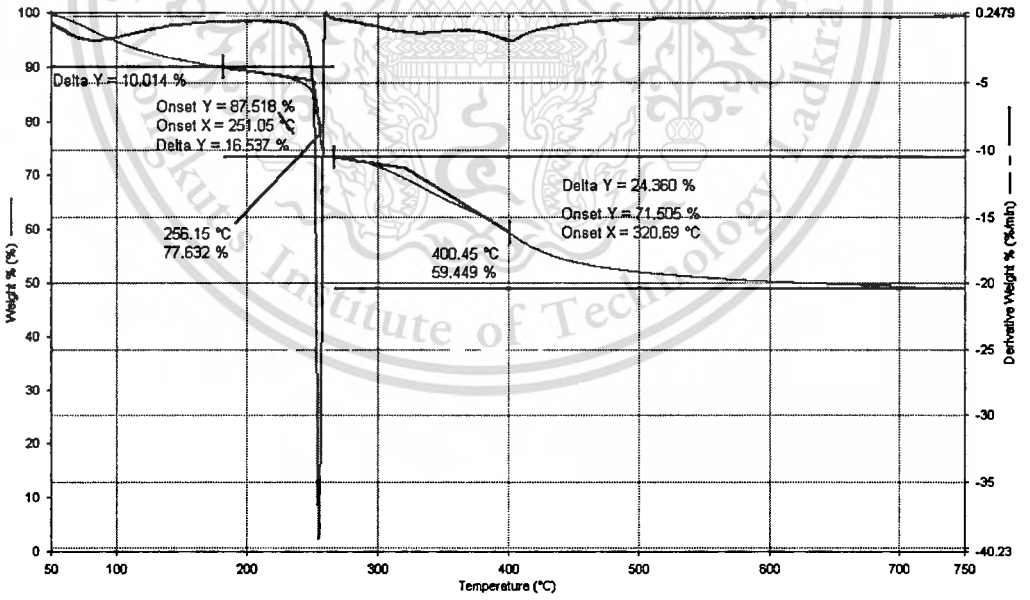


Figure 4.12 Thermal gravimetric analysis of HC/CS composites

Chapter 5

Conclusion

Hydroxyethylacryl-chitosan (HC) was successfully prepared by Michael addition reaction of chitosan and hydroxyethylacrylate. The solubility of hydroxyethylacryl-chitosan in water was faster and easier than the starting chitosan.

Hydroxyethylacryl-chitosan composites were prepared by mixing calcium silicate with chitosan, shaped into cubic form and heated at 50 °C. The initial amount of hydroxyethylacryl-chitosan added into the composite was 40wt%. It was approximately equal to the total weight loss from TGA. SEM showed the calcium silicate particles dispersed in continuous polymer binder phase. The composite between hydroxyethylacryl-chitosan and calcium silicate was prepared. The presence of crosslinked hydroxyethylacryl-chitosan in the composite could increase the compressive strength and modulus of composites to about 6 times higher than the pure calcium silicate pellet.

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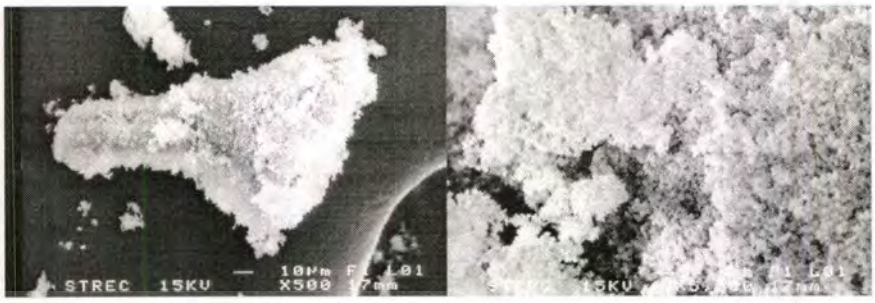
Appendix - A

Morphology of HC/CS composite from SEM



(a) (b)

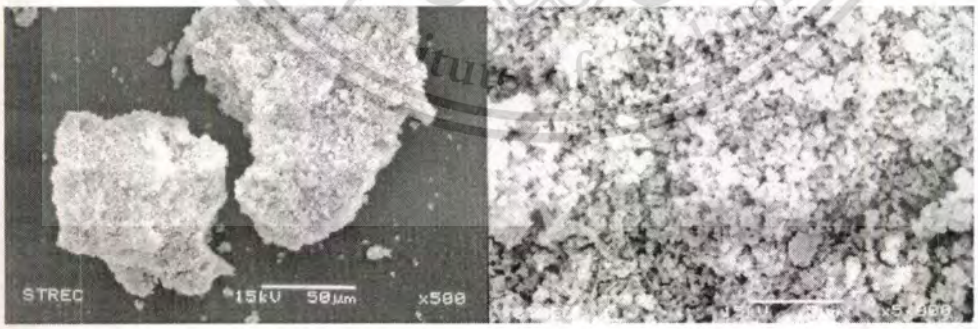
Figure A-1 SEM Micrographs of HC/CS composite

Magnification 500 time (a) Magnification 5000 time (b)

(a) (b)

Figure A-2 SEM Micrographs of HC/CS composite

Magnification 500 time (a) Magnification 5000 time (b)



(a) (b)

Figure A-3 SEM Micrographs of HC/CS composite

Magnification 500 time (a) Magnification 5000 time (b)

Appendix - B

Compressive strength

Compressive strength

Mechanical properties of $CaoSio_2$

Sample No.	Compressive strength(N)	Compressive modulus (N/mm^2)	Area(mm^2)
Sample 1	401.2	5.6	86.0
Sample 2	428.8	5.8	88.4
Sample 3	427.6	4.8	86.5
Sample 4	575.3	6.5	92.0
Average	458.2	5.7	88.2

Table B-1 Mechanical properties of $CaoSio_2$

Mechanical properties of HC/CS composite-GLU 1

Sample No.	Compressive strength(N)	Compressive modulus (N/mm^2)	Area(mm^2)
Sample 1	278.5	3.9	90.7
Sample 2	788.3	11.2	91.1
Sample 3	579.2	7.7	89.3
Sample 4	490.9	6.6	88.4
average	534.2	7.4	89.9

Table B-2 Mechanical properties of HC/CS composite-GLU 1

Mechanical properties of HC/CS composite-GLU 2

Sample No.	Compressive strength(N)	Compressive modulus (N/mm^2)	Area(mm^2)
Sample 1	2689	33.2	80.1
Sample 2	2217	27.4	81.0
Sample 3	2897	35.8	79.2
Sample 4	2724	33.6	79.6
Average	2631	32.5	79.9

Table B-3 Mechanical properties of HC/CS composite-GLU 2

Mechanical properties comparison

Sample	Average Compressive strength(N)	Average Compressive modulus (N/mm^2)
CS without hydroxyethylacryl-chitosan	458.2 ± 79.08	5.7
HC/CS composite-GLU 1	534.2 ± 211.22	7.4
HC/CS composite-GLU 2	2631.0±291.06	32.5

Table B-4 Mechanical properties comparison

Calculation of Compressive modulus

Compressive modulus was calculated by

$$\text{Compressive modulus} = \frac{Stress}{Strain}$$

$$= \frac{\Delta Fm/A}{\Delta L/L0}$$

To Assign:

ΔFm is differential force (N)

A is surface area (mm^2)

ΔL is differential length of thickness after loading (mm)

L_0 is thickness (mm)

Calculation of Compressive strength

Compressive Strength was calculated by:

$$\text{Compressive Strength} = \frac{F_{max}}{Area} \text{ (N/mm2 or MPa)}$$

Calculation of Compressive modulus

Compressive modulus was calculated by:

$$\text{Compressive modulus} = \frac{Stress}{Strain}$$

$$= \frac{\Delta Fm/A}{\Delta L/L} \text{ (N/mm2 or MPa)}$$

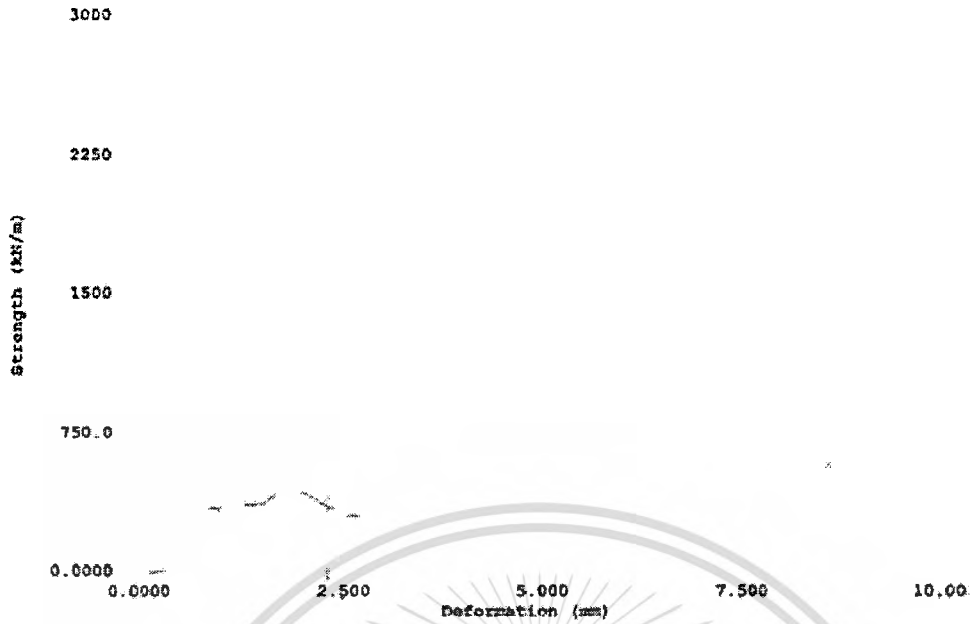
To assign:

Δ Fm is differential force (N)

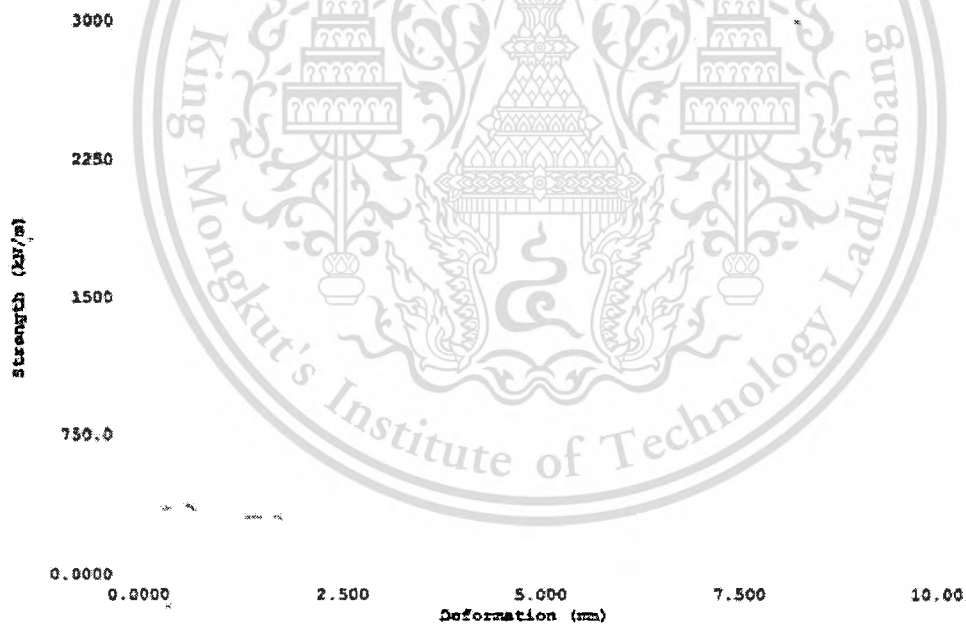
A is surface area (mm^2)

Δ L is differential length of thickness after loading (mm)

L_0 is thickness (mm)



FigureB-1 Compression of HC/CS composite GLU 1(First time)



FigureB-2 Compression of HC/CS composite GLU 1(Second time)

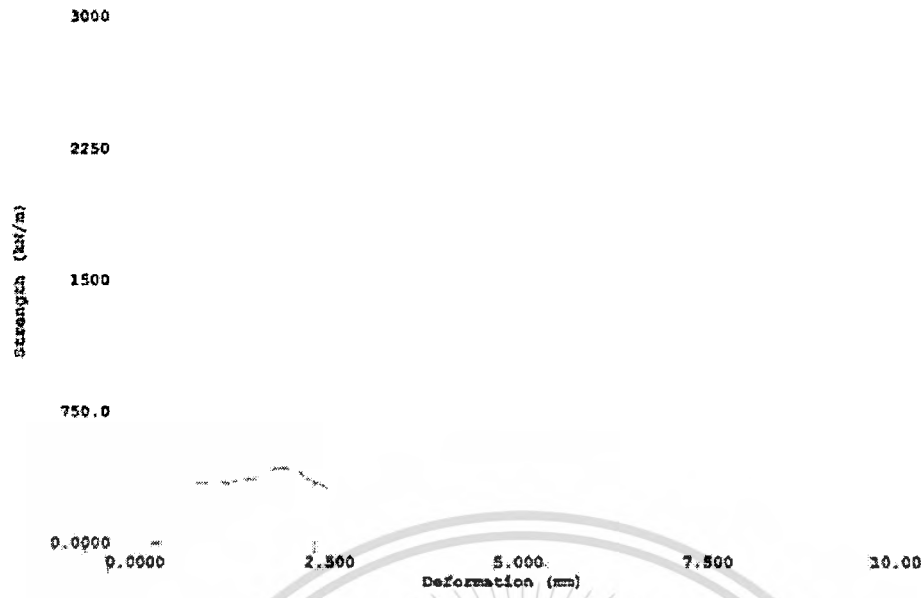


Figure B-3 Compression of HC/CS composite GLU 1(Third time)

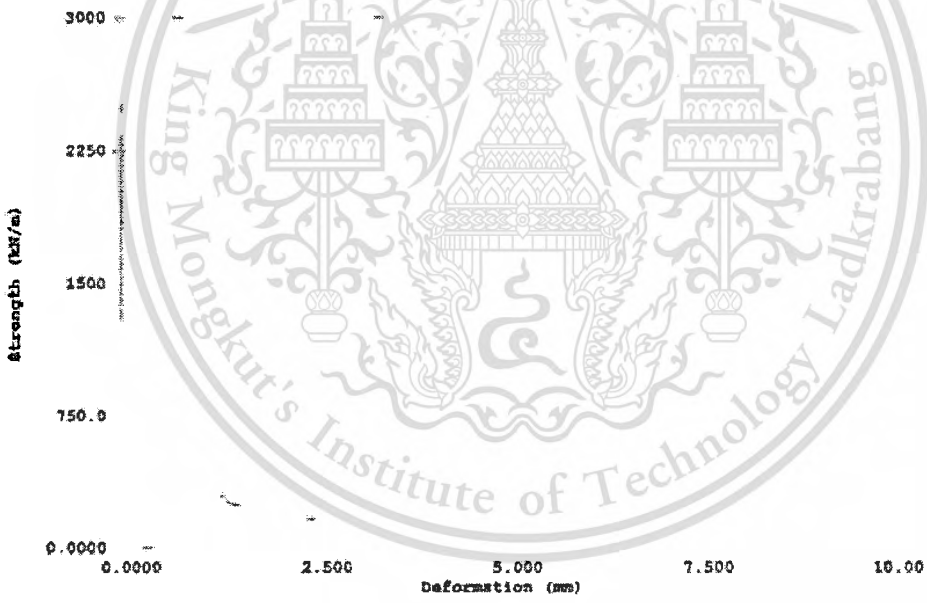


Figure B-4 Compression of HC/CS composite GLU 1(Forth time)

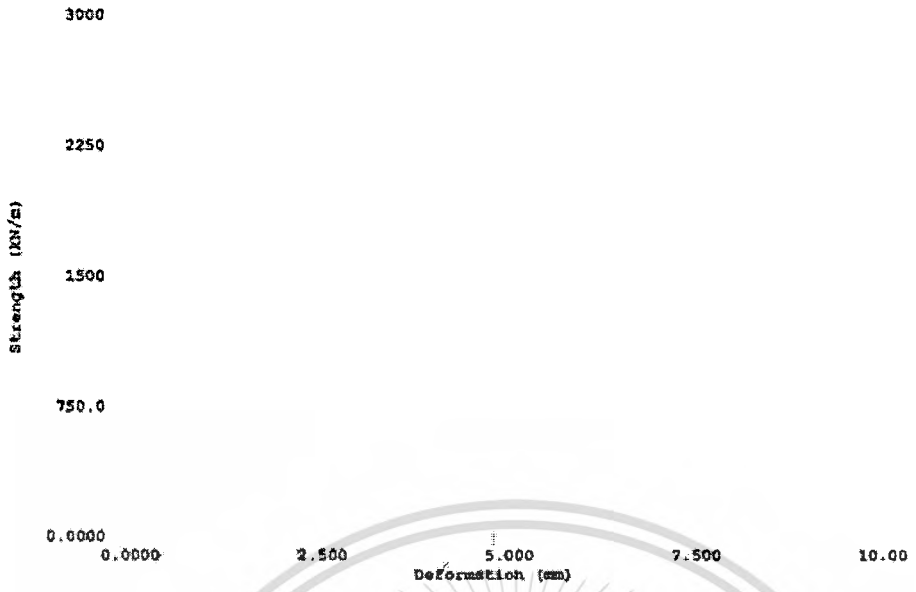


Figure B-5 Compression of HC/CS composite

GLU 1(Fifth time)

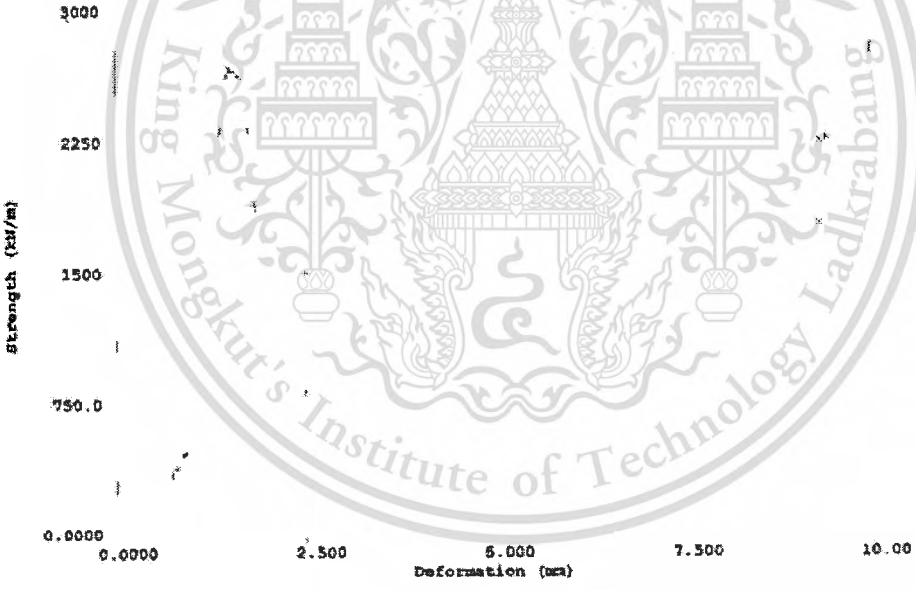


Figure B-6 Compression of HC/CS composite

GLU 2(First time)

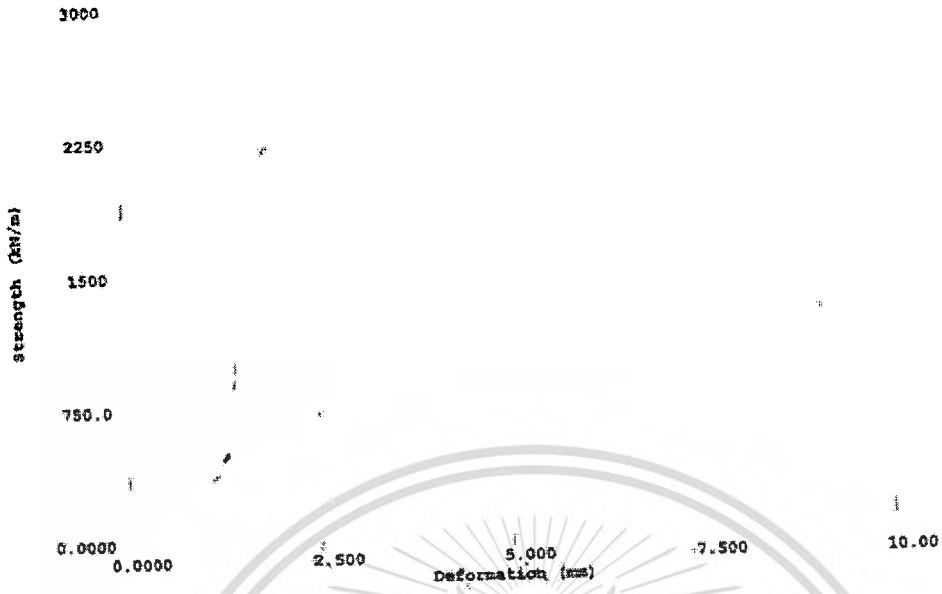


Figure B-7 Compression of HC/CS composite

GLU 2 (Second time)

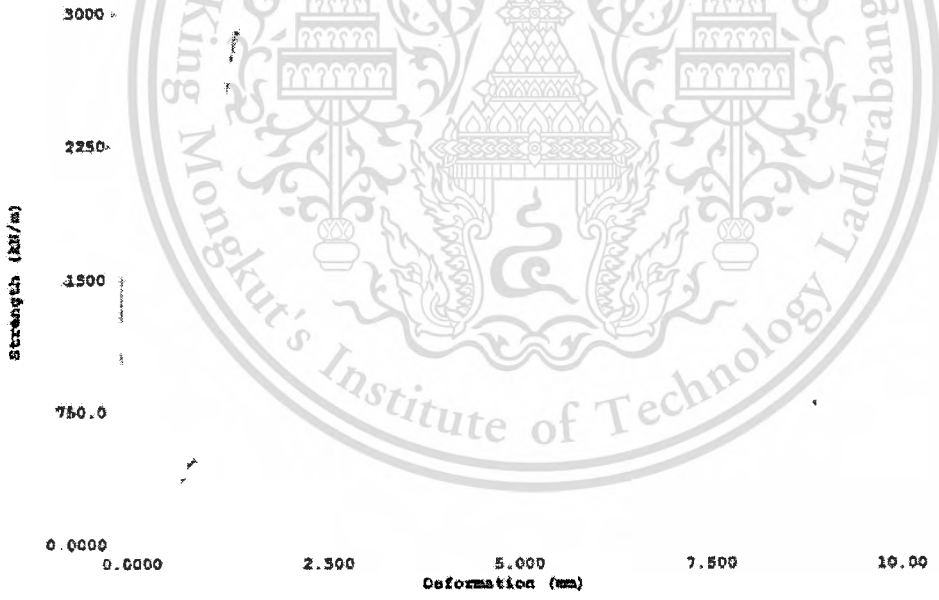


Figure B-8 Compression of HC/CS composite

GLU 2 (Third time)

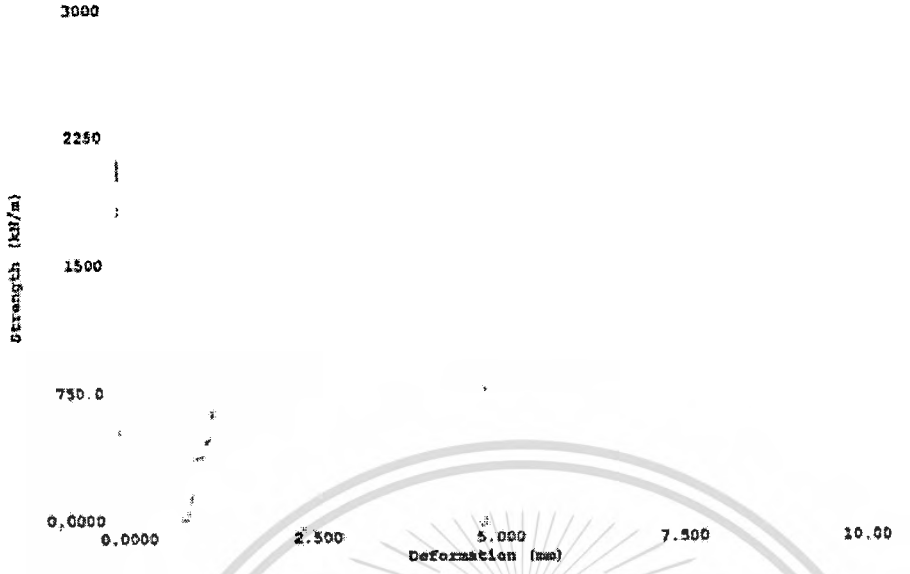


Figure B-9 Compression of HC/CS composite

GLU 2 (Forth time)

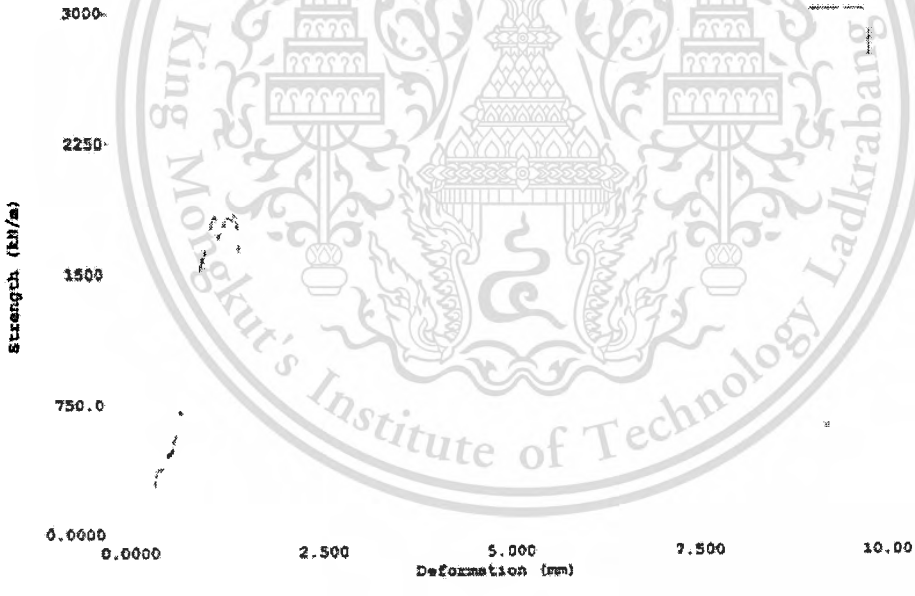


Figure B-10 Compression of HC/CS composite

GLU 2 (Fifth time)

Appendix - C

Nuclear magnetic resonance spectroscopy (NMR)

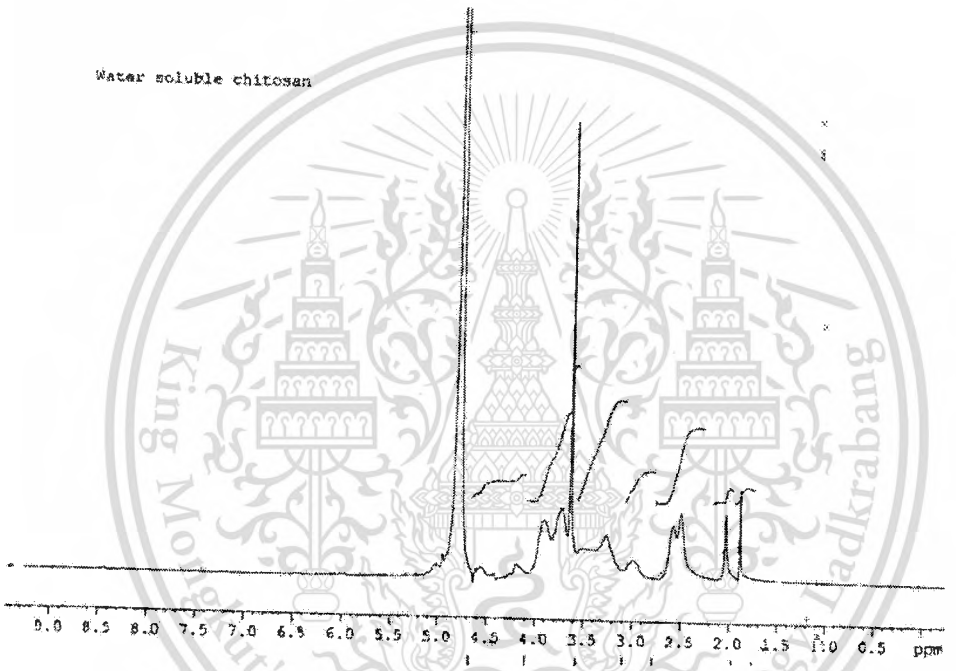


Figure C-1 Nuclear magnetic resonance spectroscopy of hydroxyethylacryl-chitosan

Appendix - D

Fourier Transform Infrared Spectroscopy (FTIR)

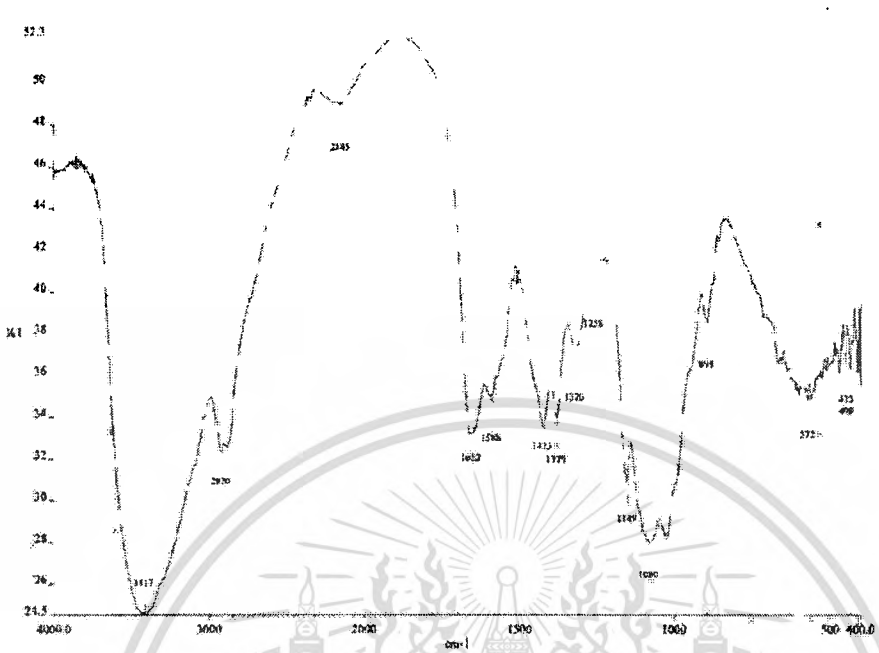


Figure D-1 Fourier Transform Infrared Spectroscopy (FTIR)

hydroxyethylacryl-chitosan

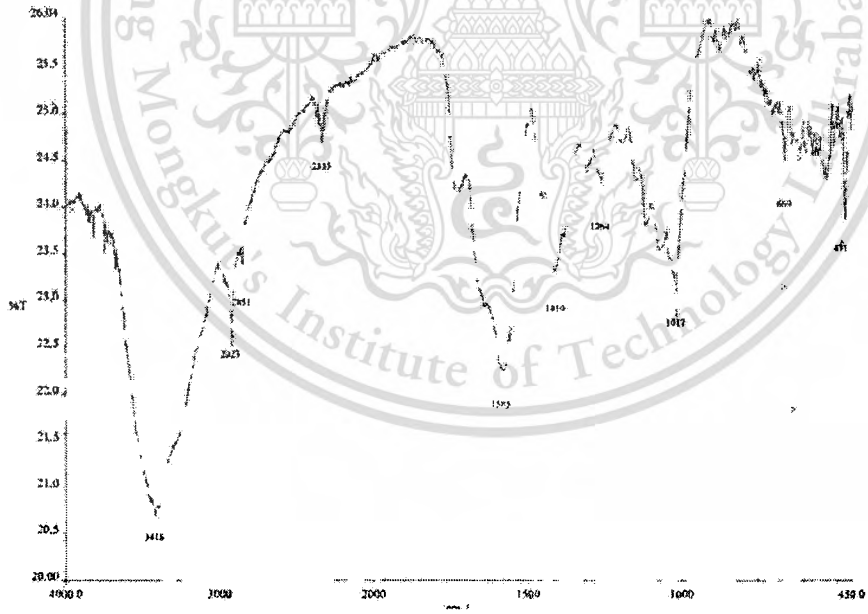


Figure D-2 Fourier Transform Infrared Spectroscopy (FTIR)

Chitosan (Oliglomer)

Appendix - E

Thermo gravimetric Analyzer (TGA)

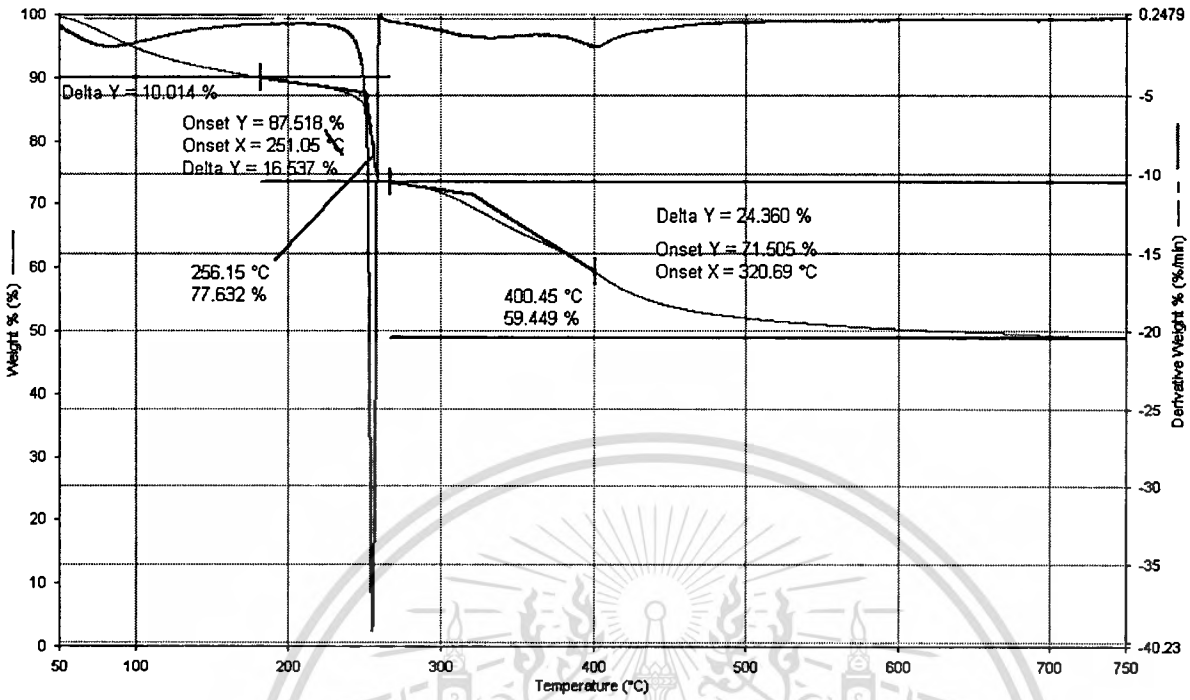


Figure E-1 Thermo gravimetric Analyzer of HC/CS composite

Appendix – F

X-ray Diffraction(XRD)

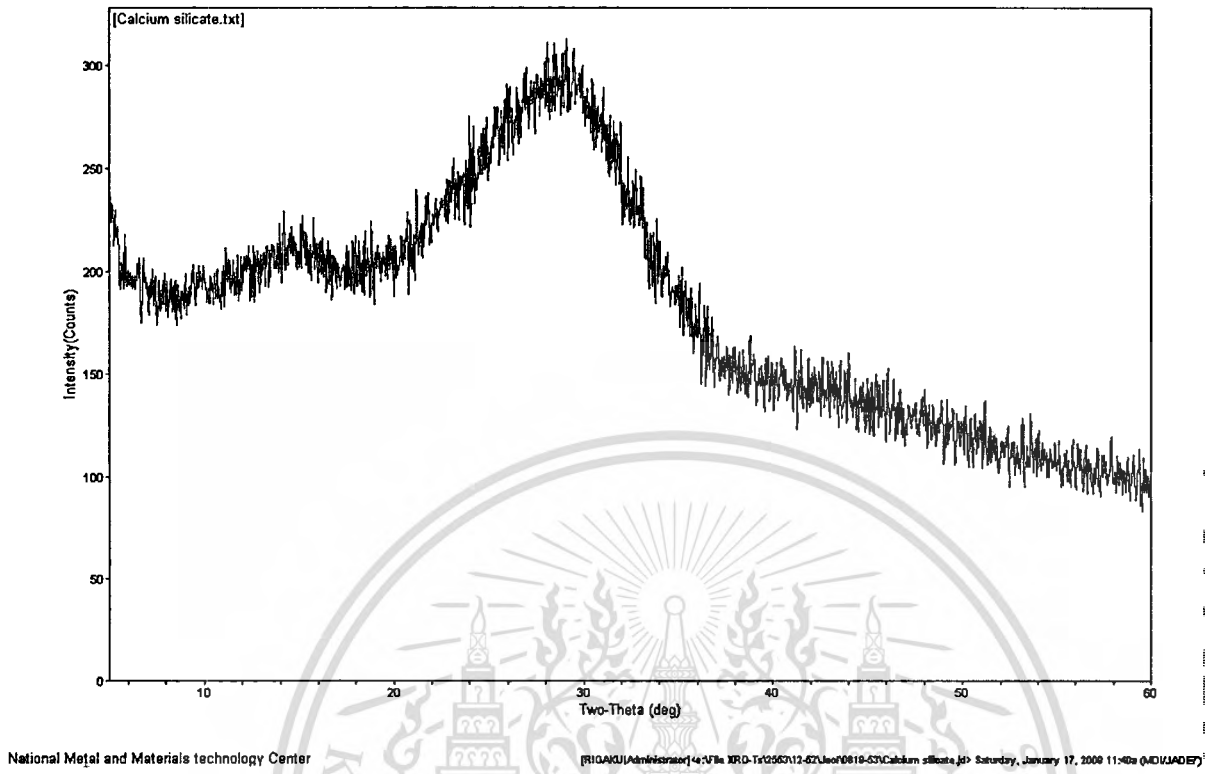
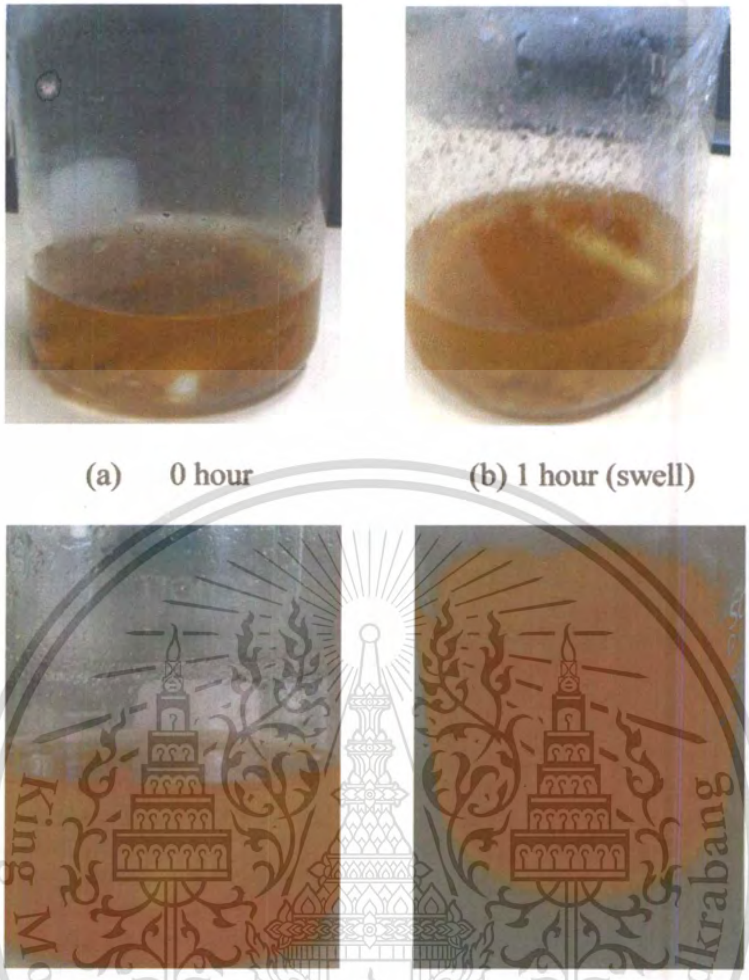


Figure F-1 XRD pattern of Calcium silicate

Appendix – G

HC Solubility

HC Solubility



(a) 0 hour (b) 1 hour (swell)

(c) 3 hours (partially swell) (d) 7 hours (dissolve)

Figure G-1 Solubility of HC in water (a) 0 hour, (b) 1 hour, (b) 3 hours and (d) 7 hours.