

**PREPARATION, CHARACTERIZATION AND
SLOW-RELEASE PROPERTIES OF
BIOCOMPOSITES COMPRISING CHITOSAN
ENCAPSULATED IN NATURAL RUBBER
MATRICES**

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**UNIVERSITI SAINS MALAYSIA
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ENCAPSULATED IN NATURAL RUBBER
MATRICES**

by

NORHIDAYAH BINTI ABU

**Thesis submitted in fulfillment of requirements
for the degree of
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LIST OF ABBREVIATIONS

DD	Degree of Deacetylation
¹H NMR	Proton Nuclear Magnetic Resonance
UV-Vis	Ultra-Violet visible
FT-IR	Fourier Transform Infrared
VPSEM	Variable Pressure Scanning Electron Microscope
TGA	Thermogravimetry Analysis
g	Gram
h	Hour
T_g	Glass transition temperature
mL	Millilitre
\bar{M}_w	Weight average molecular weight
ppm	Part per million
cm	Centimeter
nm	Nanometer
°C	Degree of Celcius
p.h.r	Part per hundred rubber
T_{max}	Temperature of the maximum of degradation rate
CTS	Chitosan
CTS_{Ac}	Chitosan in 2% v/v acetic acid solution
NR_{ucl}	Uncrosslinked natural rubber
NR_{cl}	Crosslinked natural rubber
{CTS_{Ac}}NR_{ucl}	Chitosan encapsulated with uncrosslinked natural rubber matrices

{CTS_{Ac}}NR_{cl}	Chitosan encapsulated with crosslinked natural rubber matrices
CTS_{Ac} /1-N	Chitosan blended with known amount of 1-Naphthol
CTS_{Ac} /2-N	Chitosan blended with known amount of 2-Naphthol
{CTS_{Ac}/1-N}NR_{ucl}	Chitosan blended with known amount of 1-Naphthol and then encapsulated with uncrosslinked natural rubber matrices.
{CTS_{Ac}/1-N}NR_{cl}	Chitosan blended with known amount of 1-Naphthol and then encapsulated with crosslinked natural rubber matrices
{CTS_{Ac}/2-N}NR_{ucl}	Chitosan blended with known amount of 2-Naphthol and then encapsulated with uncrosslinked natural rubber matrices
{CTS_{Ac}/2-N}NR_{cl}	Chitosan blended with known amount of 2-Naphthol and then encapsulated with crosslinked natural rubber matrices

**PENYEDIAAN, PENCIRIAN DAN SIFAT PELEPAS PERLAHAN
BIOKOMPOSIT YANG MENGANDUNGI KITOSAN TERPERANGKAP
DALAM MATRIK GETAH ASLI**

ABSTRAK

Projek penyelidikan ini terdiri daripada dua bahagian. Pada bahagian pertama, kesan pH ke atas kestabilan gel chitosan berasid (CTS_{Ac}) bersama 1-Naftol (produk dilabelkan sebagai $CTS_{Ac}/1-N$) atau 2-Naftol (produk dilabelkan sebagai $CTS_{Ac}/2-N$) ditentukan. Dalam air berasid ($pH \leq 4$), $CTS_{Ac}/1-N$ atau $CTS_{Ac}/2-N$ membentuk gel dan pecah dengan mudah. Dalam air neutral ($pH \approx 7$), $CTS_{Ac}/1-N$ dan $CTS_{Ac}/2-N$ membentuk gel tetapi masih kelihatan utuh walaupun selepas dua minggu. Dalam air beralkali ($pH \geq 9$), $CTS_{Ac}/1-N$ dan $CTS_{Ac}/2-N$ sukar membentuk gel walaupun selepas direndam selama tiga bulan. Selain itu, biokomposit tanpa naftol yang terdiri daripada CTS_{Ac} terkandung dalam getah asli tak tersilang ($\{CTS_{Ac}\}NR_{ucl}$) dan getah asli tersilang ($\{CTS_{Ac}\}NR_{cl}$) disediakan. Kajian pengambilan air oleh NR_{ucl} , NR_{cl} , $\{CTS_{Ac}\}NR_{ucl}$ dan $\{CTS_{Ac}\}NR_{cl}$ telah dikaji. Daripada kajian ini, jumlah peratusan air dalam NR_{cl} , NR_{ucl} , $\{CTS_{Ac}\}NR_{cl}$ dan $\{CTS_{Ac}\}NR_{ucl}$ selepas 48 jam didapati berjumlah 1.8%, 3.0%, 75% dan 131%, masing-masing. Pada bahagian kedua, biokomposit terdiri daripada $CTS_{Ac}/1-N$ atau $CTS_{Ac}/2-N$ dan terkandung dalam NR_{ucl} ($\{CTS_{Ac}/1-N\}NR_{cl}$) atau dalam NR_{cl} ($\{CTS_{Ac}/2-N\}NR_{ucl}$) disediakan. Semua bahan polimer dicirikan menggunakan kaedah spektroskopi FT-IR, TGA dan VPSEM. Peratusan pelepasan naftol secara malar (tidak disegarkan) dan berubah (disegarkan) dalam air suling ($pH \approx 7$) telah dikaji. Didapati bahawa peratusan 1- atau 2-Naftol yang dilepaskan bagi tempoh 30 hari dalam larutan yang tidak disegarkan dari $\{CTS_{Ac}/1-N\}NR_{cl}$, $\{CTS_{Ac}/1-N\}NR_{ucl}$, $\{CTS_{Ac}/2-N\}NR_{cl}$ dan

$\{CTS_{Ac}/2-N\}NR_{ucl}$ berjumlah 39.3%, 44.1%, 44.8% dan 56.1%, masing-masing. Manakala, bagi situasi pelarut disegarkan setiap 24 jam, peratusan 1- atau 2-Naftol yang dilepaskan bagi tempoh 30 hari dari $\{CTS_{Ac}/1-N\}NR_{cl}$, $\{CTS_{Ac}/1-N\}NR_{ucl}$, $\{CTS_{Ac}/2-N\}NR_{cl}$ dan $\{CTS_{Ac}/2-N\}NR_{ucl}$ didapati berjumlah 53.4%, 60.9%, 57.3% dan 62.0%, masing-masing. Bagi situasi pelarut disegarkan setiap 72 jam, peratusan 1- atau 2-Naftol yang dilepaskan bagi tempoh 30 hari dari $\{CTS_{Ac}/1-N\}NR_{cl}$, $\{CTS_{Ac}/1-N\}NR_{ucl}$, $\{CTS_{Ac}/2-N\}NR_{cl}$ dan $\{CTS_{Ac}/2-N\}NR_{ucl}$ didapati berjumlah 57.5%, 63.5%, 62.7% dan 66.1%, masing-masing. Didapati juga bahawa pelepasan 1- atau 2-Naftol dalam pelarut disegarkan atau tidak disegarkan tidak mencapai keseimbangan dalam tempoh 30 hari. Berdasarkan penemuan ini, disimpulkan bahawa pelepasan 1- atau 2-Naftol dari biokomposit adalah sememangnya secara perlahan.

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ABSTRACT

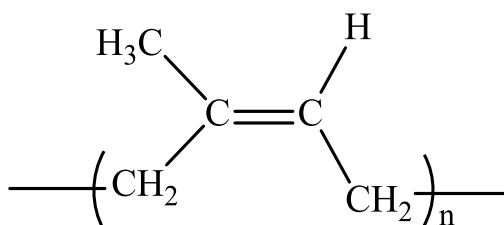
This research project consists of two parts. In the first part, we determined the stability of acidified chitosan (CTS_{Ac}) gel incorporated with known amounts of either 1-Naphthol (product designated as CTS_{Ac}/1-N) or 2-Naphthol (product designated as CTS_{Ac}/2-N) in water with different pH values. In acidified water (pH ≤ 4), CTS_{Ac}/1-N or CTS_{Ac}/2-N swelled tremendously and broke apart easily. In neutral water (pH ≈ 7), CTS_{Ac}/1-N and CTS_{Ac}/2-N swelled but remained intact even after two weeks. In alkalized water (pH ≥ 9), CTS_{Ac}/1-N and CTS_{Ac}/2-N hardly swelled even after being soaked for three months. In addition, biocomposites comprised of solely CTS_{Ac} encapsulated in uncrosslinked natural rubber ({CTS_{Ac}}NR_{ucl}) and crosslinked natural rubber ({CTS_{Ac}}NR_{cl}) were prepared. From the water uptake study, the percentage amount of water in NR_{cl}, NR_{ucl}, {CTS_{Ac}}NR_{cl} and {CTS_{Ac}}NR_{ucl} after 48 h was found to be 1.8%, 3.0%, 75% and 131%, respectively. In the second part, biocomposites comprised of CTS_{Ac}/1-N or CTS_{Ac}/2-N encapsulated in NR_{ucl} ({CTS_{Ac}/1-N}NR_{ucl} or {CTS_{Ac}/2-N}NR_{ucl}) and in NR_{cl} ({CTS_{Ac}/1-N}NR_{cl} or {CTS_{Ac}/2-N}NR_{cl}) were prepared. All polymeric materials were characterized by means of FT-IR spectroscopy, TGA and VPSEM. The percentage of the naphthols released in constant (non-refreshed) and changing (refreshed) amounts of distilled water (pH ≈ 7) was studied. It is found that the percentage of 1- and 2-Naphthol released up to a total of 30 days in non-refreshed water from {CTS_{Ac}/1-N}NR_{cl},

$\{CTS_{Ac}/1-N\}NR_{ucl}$, $\{CTS_{Ac}/2-N\}NR_{cl}$ and $\{CTS_{Ac}/2-N\}NR_{ucl}$ is 39.3%, 44.1%, 44.8% and 56.1%, respectively. In the case whereby the water was refreshed for every 24 h, the percentage of 1- and 2-Naphthol released up to a total of 30 days from $\{CTS_{Ac}/1-N\}NR_{cl}$, $\{CTS_{Ac}/1-N\}NR_{ucl}$, $\{CTS_{Ac}/2-N\}NR_{cl}$ and $\{CTS_{Ac}/2-N\}NR_{ucl}$ was found to be 53.4%, 60.9%, 57.3% and 62.0%, respectively. In the case whereby the water was refreshed for every 72 h, the percentage of 1- and 2-Naphthol released up to a total of 30 days from $\{CTS_{Ac}/1-N\}NR_{cl}$, $\{CTS_{Ac}/1-N\}NR_{ucl}$, $\{CTS_{Ac}/2-N\}NR_{cl}$ and $\{CTS_{Ac}/2-N\}NR_{ucl}$ was found to be 57.5%, 63.5%, 62.7% and 66.1%, respectively. It was also found that the release of 1- or 2-Naphthol in refreshed and non-refreshed water had not reached equilibrium in 30 days. Based on these findings, it can be concluded that the release of either 1- or 2-Naphthol from the biocomposites was a slow-release indeed.

CHAPTER 1: INTRODUCTION

1.1 General Introduction

Rubber is a material that has remarkable ability to return to its original shape after being stretched. Natural rubber (NR), a renewable polymeric material of high commercial value is obtained in latex form from the *Havea Brasiliensis* tree. It contains about 94% polyisoprene with almost 99% cis-1,4-configuration and the chemical structure is as shown in Figure 1.1 (Meral & Alexander, 2012).



n = repeating unit

Figure 1.1: Chemical structure of natural rubber

The technological importance of NR is already well-known, as in the medical field, it has been used in production of condoms, catheters and surgical gloves due to its unique properties in biocompatibility and biodurability, and good mechanical properties which includes flexibility and resilience (Emma-Christin, 2005). NR is often vulcanized, a process by which the rubber is heated and sulfur, peroxide or bisphenol are added to improve resistance and elasticity, also to prevent it from perishing (Karsten and Alexander, 2005). Malaysia is known as one of the main producers of NR in the world.

Chitosan (CTS), a multi-nucleophilic material due to the presence of one primary amino (-NH₂) and two hydroxyl (-OH) groups is composed of

predominantly $\alpha(1\rightarrow4)$ -linked 2-amino-2-deoxy- β -D-glucopyranose. CTS is commercially produced by deacetylation of chitin, which is typically extracted from crab and shrimp shells. Chitin is a component of a complex network with proteins in which the calcium carbonates deposits to form the hard shell in crustacean (Yongqin *et al.*, 2011). CTS and Chitin can differentiate through the percentage of acetyl groups in their chemical structure. Chitin with more than 75% of acetyl glucosamine is known as CTS. CTS is highly hydrophobic in nature and its solubility in organic solvents is poor except in dilute acids such as acetic acid and formic acid (Tanveer *et al.*, 2002).

As a naturally occurring polysaccharide, CTS is biodegradable, biocompatible and also low in toxicity, and these properties have made it suitable for living cells. Therefore, CTS has been applied in a variety of applications such as in the production of cosmetic, medicine, dental, food packaging, wastewater, aquaculture and agriculture (Bae and Moon-moo, 2010). The potential of CTS is supported by the existence of more than 3500 patents and patent applications.

Appropriate blending of two different natural polymers may give rise to a new biocomposite with better properties than that obtainable from a single polymer. CTS can be encapsulated by NR to form a biocomposite which is biodegradable and, as such can be disposed without deteriorating the environment.

1.2 Problem Statement

Generally, conventional pesticides are diluted with water and sprayed on a plantation. As such, the active ingredients will be immediately incorporated in the crop and soil. The excessive initial concentration of the pesticides in a plantation

would not only be detrimental to the natural ecosystems but poses serious risk to human health.

Encapsulation of pesticides in an appropriate polymeric material is one of the most efficient ways of delivering the active ingredients of the pesticide in a slow-release manner (Fàtima *et al.*, 2009). Pesticides coated with synthetic polymers have been widely used in domestic agriculture. However, most synthetic polymers are not biodegradable hence may lead to undesirable environmental issues.

The use of chitosan gel as a slow release material is quite well known in the field of biomedical particularly for drug delivery (Inmaculada, 2011). However, to use chitosan gel directly in a plantation requires a better understanding of its stability in different pH conditions so as to avoid excessive initial release of the pesticides to the crops and soil. The use of natural rubber as coating material for neem Aza-A, a powerful insect anti-feedant and growth-regulating substance, has been reported (Sa-Ad Vijayan, 2011). As such, it should be possible to use natural rubber to encapsulate chitosan containing known amounts of pesticides. To date, a survey of the literature reveals that there is no comprehensive report on the usage of chitosan encapsulated with natural rubber as biocomposite for the slow release delivery of pesticides. With this in view, the objectives of this current research endeavor are as listed below.

1.3 Research Objectives

- I. To determine the effect of pH on the stability of acidified chitosan (CTS_{Ac}) gel containing known amounts of 1- or 2-Naphthol.
- II. To determine the water uptake of CTS_{Ac} encapsulated in uncrosslinked natural rubber (NR_{ucl}) and crosslinked natural rubber (NR_{cl}).
- III. To determine the release of 1- and 2-Naphthol from the biocomposites ($\{\text{CTS}_{\text{Ac}}/1\text{-N}\}\text{NR}_{\text{ucl}}$, $\{\text{CTS}_{\text{Ac}}/1\text{-N}\}\text{NR}_{\text{cl}}$, $\{\text{CTS}_{\text{Ac}}/2\text{-N}\}\text{NR}_{\text{ucl}}$, and $\{\text{CTS}_{\text{Ac}}/2\text{-N}\}\text{NR}_{\text{cl}}$).

CHAPTER 2: LITERATURE REVIEW

2.1 Natural polymer

For decades, polymeric materials have emerged as a unique class of materials due to their versatility and appeal with outstanding mechanical properties, tailorability, functional properties, environmental stability, ease of processing into customer-desired products, biodegradable towards enzymatic or hydrolytic mechanism and a host of other desirable properties. The study and utilization of natural polymers is an ancient science. Natural polymers are self-occurring polymers that can be found in living things like bacteria, plants and animals. Figure 2.1 shows the polymers such as natural rubber, cellulose and crab shell.

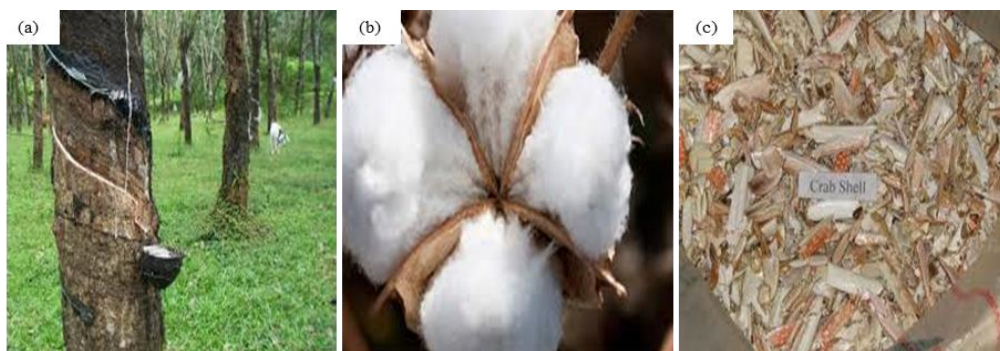


Figure 2.1: Natural polymers (a) rubber tree, (b) cotton, and (c) crab shell.

The usage of natural polymers or biopolymers in both medical and industrial sectors is of increasing demand. Due to similarity of materials derived from animals or plants with material to human bodies gives a natural polymer huge advantage (Barbosa *et al.*, 2005; Jayakumar *et al.*, 2009). Toxicity and non-biodegradable are two of the problems faced by most available synthesized polymers. However, this limitation has been overcome by using natural polymer. The list of natural polymers based on their types is given in Table 2.1

Table 2.1: List of natural polymers (Yu, 2006)

Types	Polymers
Polysaccharide	Plant/algal: starch, cellulose, pectin, konjac, alginate, caragreenam, gums
	Animal: hyluronic acid
	Fungal: pulluan, elsinan, scleroglucan
	Bacterial: chitin, chitosan, levan, xanthan, polygalactosamine, curdlan, gellan, dextran
Proteins	Soy, zein, wheat gluten, casein, serum, albumin, collagen/gelatin, silks, resilin, polylysin, polyamino acids, poly (Y-glutamic acid)
Lipids/surfactants	Acetoglycerides, waxes, surfactants, emulsion
Specialty polymers	Lignin, shellac, natural rubber

2.2 Natural Rubber

Natural rubber (NR) latex can be obtained by tapping the bark of the *Hevea Brasiliencis* tree as shown in Figure 2.2 (Werathirachot, 2008). Malaysia is one of the main producers of NR in the world. Table 2.2 shows the production of NR from 2000 to 2011 as reported in International Rubber Study Group (IRSG) (<http://www.lgm.gov.my/nrstat/nrstats>).



Figure 2.2: *Hevea Brasiliencis* tree.

Table 2.2: Production of NR from 2000 to 2011.

Year	Natural rubber production ('000 tonnes)	
	World	Malaysia
2000	6762	927
2001	7332	882
2002	7326	889
2003	8006	985
2004	8774	1168
2005	8907	1126
2006	9827	1283
2007	9890	1199
2008	10128	1079
2009	9690	857
2010	10399	939
2011	10974	996

Source: International Rubber Study Group (IRSG)

NR can be characterized by its good elastic properties, resilience and damping behavior, but poor chemical resistance and processing ability (Karsten and Alexander, 2005). The unique mechanical properties of NR are due to the highly stereoregular microstructure resulting from its high molecular weight, which is resistant to heat, oxygen and ozone is poor due to the presence of a high number of double bonds in the chemical structure (Rohana, 2011). The major uses of NR are in tyre industries, molded goods, and chemical parts while the less uses are the chemical derivatives of rubber.

Chemical modifications of the NR are essential in order to tailor them for various applications (Wan *et al.*, 2010). This modification is also used to alter the

properties of NR in such a way as to compete in areas of applications outside its traditional areas (Jitladda and Porntip, 2012).

Fresh hevea latex contains about 25-45% rubber hydrocarbon and 5-6% non rubber substances such as amino acids, proteins, carbohydrates, neutral and polar lipids, and inorganic salts; the remaining being water (Karsten and Alexander, 2005). After collection, the latex is stabilized with ammonia and transported from the plantation to a factory where it undergoes continuous centrifugation to produce a concentrated NR latex containing ~60% rubber (Jitladda and Porntip, 2012). Table 2.3 shows a typical composition of natural rubber.

Table 2.3: Typical composition of NR from the *Hevea Brasiliensis* (Jitladda and Porntip, 2012)

Content	%
Hydrocarbon	36.0
Protein	1.4
Carbohydrate	1.6
Neutral lipid	1.0
Glycolipid + phospolipid	0.6
Inorganic substances	0.5
Water	45.0

Protein has been considered to be an essential component of NR. In 1991, the Food and Drug Administration (FDA) stipulated that rubber products made from NR latex should be treated to remove extractable proteins as it can cause type I allergic responses that led to life-threatening anaphylactic reaction. Deproteinization of latex was carried out by using a proteolytic enzyme in the presence of surfactants to reduce the extractable proteins to less than their detection limit (molecular structure of NR and its characteristic based on recent evidence).

2.2.1 Vulcanization

In NR, vulcanization is a process altering its molecular structure through cross-linking of isoprene chains (Sa-Ad & Wattana, 2013). One of the oldest vulcanization agent for unsaturated rubbers used in rubber practice is sulphur vulcanization as reported by Chengalroyen and Dabbs in 2013. It is effective and fast link in form of cross-links to rubber macromolecules occurs only in the presence of accelerators and activators (Mahmood, 2008). In the absence of an accelerator and activator, sulfur will reacts with rubbers slowly and form side cyclic structures, not in a form of sulfur cross-links. These ingredients both affect also the kinetic parameters of vulcanization, temperature and time (Hong, 2005). Type and the content of present accelerators and also the accelerator: sulfur ratio in rubber compounds also affect final properties of vulcanizates mainly their thermo-oxidative stability and some of their tensile and dynamic properties. The term of sulphur curing is often used for sulphur vulcanization (Ismail, 2011).

In 2009, Johns and Rao studied the effect of vulcanization of NR using dicumyl peroxide (DCP) on water absorption and swelling in solvent. Results shows that water absorption can be minimized by vulcanizing the NR and the percentage can be negligible. On the other hand, vulcanization has improved the solvent resistance of NR. This effect was due to cross-links or chain scission that may occur simultaneously during vulcanization process.

2.3 Chitin and chitosan

Chitosan (CTS), a natural polymer, is a copolymer of 2-amino-2-deoxy- β -(1,4)-D-glucose and 2-acetamido-2-deoxy- β -(1,4)-D-glucose units. It is an N-deacetylated product of chitin (Werathirachot, 2008). Chitin, known as poly-N-

acetylglucosamine can be found in abundance in shells of the exoskeletons of insects, shells of crustaceans and fungal cell wall. It is a component of a complex network with protein in which the calcium carbonate deposits to form the hard shell in crustaceans. Chitin and CTS have similar chemical structure and are analogues of the homopolymer cellulose where the respective acetamido and amino groups replace the hydroxyl group at carbon-2 as shown in Figure 2.3. They also possess excellent properties such as biocompatible, biodegradable, non-toxic and is a good absorbent (Sashiwa and Aiba, 2004).

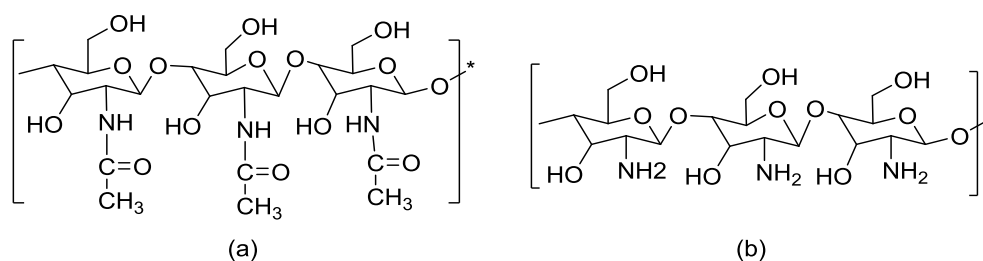


Figure 2.3: Chemical structure of (a) chitin and (b) chitosan.

Chitin and CTS can be distinguished by their solubility as they are different in their acetyl content (Mourya, 2010). CTS is insoluble at neutral and alkaline pH values, but it will form salt with inorganic and organic acids such as hydrochloric acid, lactic acid, and acetic acid. The amine groups of the polymer are protonated in an acidic medium and a soluble polysaccharide with a positive charge will attain (Hoda, 2012).

2.3.1 Production of chitosan

Chronological steps of the process needed to synthesis CTS from crustacean shells have been summarized in Figure 2.4. The steps consist of deproteinization, demineralization and deacetylation process. Usually, chitin is prepared by a first step

of deproteinization followed by a second step of demineralization. The degradation of polymer chain takes place during the deacetylation process to produce CTS (Inmaculada, 2009).

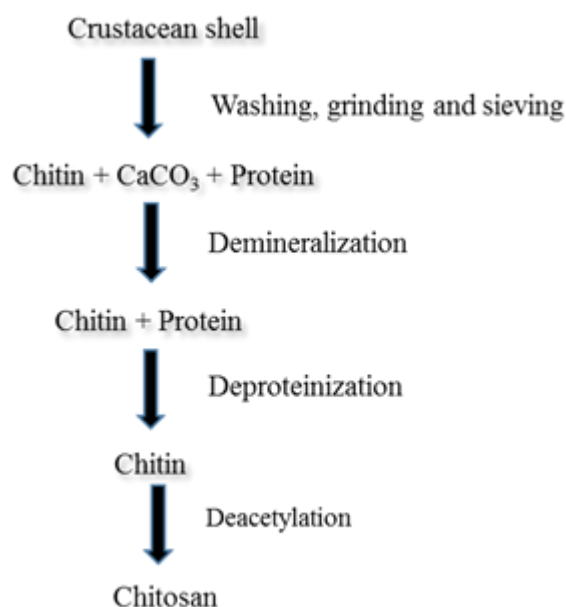


Figure 2.4: Preparation of chitin and CTS from crustacean shell (Inmaculada, 2009)

2.3.2 Characteristics of chitosan

2.3.2.1 Degree of Deacetylation (DD)

Degree of deacetylation (DD) is a parameter used to determine the content of free amino groups that describes the physical-chemical properties such as solubility, cationicity and acid-base properties of CTS (Yongqin, 2009). The DD can be used to distinguish between chitin and CTS. Chitin, has a maximum DD of 75% whereas CTS has a DD value higher than 75% (Khan, 2002).

Deacetylation is a process to convert chitin to CTS by removing the acetyl group. Typically, chitin is treated with an aqueous 40% - 50% sodium hydroxide solution at 100-120 °C for several hours to hydrolyze N-acetyl linkages. Repetition

of the process can give deacetylation values up to 98 %, but complete deacetylation cannot be achieved via this process. Fully deacetylated (nearly 100%) CTS can be prepared by alkaline treatment of a gel form instead of the powder form of CTS (Pradip, 2004).

In this process, acetyl groups from the molecular chain of chitin are removed, leaving behind amino group (-NH₂). The versatility of CTS depends mainly on this chemical reactive amino group (Leceta, 2013). DD of chitin and CTS can be determined using 3 different methods that is (a) spectroscopy method, (b) conventional method and (c) destructive method. These include the infrared spectroscopy, first derivative UV-spectroscopy, nuclear magnetic resonance spectroscopy, conductometric titration, potentiometric titration, and differential scanning calorimetry (Mohammad, 2009).

2.3.2.2 Solubility

CTS is soluble in most of organic acids such as dilute acetic acid, formic acid and lactic acid with pH below 6.0 except in phosphoric and sulfuric acid (Dominic, 2007). Commonly, hydrochloric acid with a concentration of 1% v/v and acetic acid with a concentration of 2% v/v are used to dissolve CTS. However, at pH 7.0 and above, the solubility of CTS is poor. The solubility of CTS is influenced by the degree of deacetylation with deacetylation of at least 85% and above in order to achieve the preferred solubility (Pillai, 2009).

2.3.2.3 Bioabsorbent

CTS at pH lower than its pKa is positively charged. Thus, in these conditions negatively charged proteins bind easily to CTS. CTS has been used as absorbent, coagulant and bactericide in the treatment of aquaculture wastewater (Kongkaew, 2010). The coagulation-flocculation process as well as the absorption process depends on the degree of deacetylation of CTS. Normally, coagulation of particles in a suspension is a technique to treat contaminated water. Coagulation may be resulted from charge neutralization or bridging of polymer chains used to treat the waste water. Many factors influence the efficiency in wastewater treatment. This includes the type, the number of charges and the molecular weight of polymers (Jose, 2010). For short polymer chains, the mechanism may involve charging neutralization while for long polymer chains, the absorption of polymer chains of many particles leads to flocculation like bridging of particles (Panu, 2011). Basically, both mechanisms may occur simultaneously and coagulation-flocculation process can be used to remove metal from contaminated water (Kumar, 2000).

2.3.3 Application of chitosan

Due to the physical and chemical properties, CTS is being used in a vast array of different products and applications ranging from pharmaceutical and cosmetic products to waste water treatment and plant protection (Pradip, 2004). Table 2.4 shows the potential applications of chitin, CTS and its derivatives.

Table 2.4: Potential applications of chitin, CTS and its derivatives (Pradip, 2004)

Application	Specific use
Water treatment	Coagulating agents for polluted water, removal of metal ions
Agriculture	Plant elicitor, antimicrobial agents, plant seed coating
Textile paper industry	Fibers for textile and woven fabrics, paper and film
Biotechnology	Chromatography, packing, enzyme immobilizing material
Food/ health supplement	Natural thickeners, food additives, filtration and classification, hypocholesteromic agents (slimming agents)
Cosmetics	Ingredients for hair and skin care
Biomedical	Wound dressings, absorbable sutures, anticoagulant or antothrombogenic materials, hemostatic agents, drug delivery gene delivery

2.4 Polymer blending

A blending of two or more polymers can provide a combination of properties which cannot be attained by a single polymer component. Polymer blends are mixtures of different homopolymers, copolymers, and terpolymers (Buthaina and Karrer, 2010). Whether a mixture of two chemically dissimilar polymers is miscible or not depends on the thermodynamics of mixing (Rao and Johns, 2008). In order to understand what governs polymer-polymer miscibility on a molecular scale, it can be approached via the polymer solution theory. Materials with totally new property combination may be achieved by blending together, two or more polymers (Oluranti, 2011).

The effect of blending CTS with NR on the mechanical and thermal properties of the blends have been reported by Rao and Johns, 2008. They have found that the mechanical property of the blends was enhanced with the addition of CTS into NR. Blending also improved the thermal properties of CTS as the mass loss of the blends was found to be lower than the pure CTS.

2.4.1 Method of blending

Basically there are two types of polymer blends: homogeneous and heterogeneous. Homogeneous blends can be obtained by blending polymers with different molecular weight or by blending certain miscible polymers. Whereas, heterogeneous blends have two different types of dispersion states. The first type is the dispersed two-phase flow, in which one component exists as the distinct phase dispersing in the other component that forms the continuous phase. There are different techniques for the blending of polymers such as solution blending, melt blending, latex blending, formation of partial block or graft copolymerisation and synthesis of interpenetrating networks (IPNs)

2.4.2 Solution blending

Solution blending involves the mixing of two polymers in a common solvent. The solid polymer is obtained by evaporating the solvent. Even though it allows easy and rapid mixing of the components, the evaporation of the solvent leads to phase separation and poorly controlled morphology of the product.

2.4.3 Latex blending

Latex blending is blending of two polymers in the latex stage. The solid blend is obtained by the evaporation of water. Better dispersion is possible with latex blending and the contamination by solvents is prevented. There are some advantages of blending of the two polymers in the latex stage. A latex blend is easily obtained just by mixing two lattices together. There are some limitations whereby the components should be free from impurities and should be miscible.

2.5 Biocomposite

Composites are materials that contain two or more distinct constituent phases. The term ‘biocomposites’ refers to composites that can be employed in bioengineering (Jose, 2010). The constituents of the composite retain their identities in the composite. Specifically, they do not dissolve or otherwise merge completely into each other, although they act in concert. In composites, properties such as the elastic modulus can be significantly different from those of the constituents alone, but are considerably altered by the constituent structures and contents (Paul, 2006).

Biocomposites are the combination of natural fibers (biofibers) such as wood fibers (hardwood and softwood) or non - wood fibers (e.g., wheat, kenaf, hemp, jute, sisal, and flax) with polymer matrices from both of the renewable and nonrenewable resources. Biofibers are one of the major components of biocomposites. The fibrous material derived from the tree, plant, or shrub sources are defined as biofiber. Biocomposites often mimic the structures of the living materials involved in the process, in addition to the strengthening properties of the matrix that was used, but still providing biocompatibility, e.g. in creating scaffolds in bone tissue engineering. The degree of biodegradability in bio-based polymers depends on their structure and their service environment (Paul, 2006).

2.5.1 Natural rubber-chitosan biocomposites

Chitosan is commonly used to reinforce a polymer matrix. This forms a composite which has several advantages such as low cost, easy processability and good chemical resistance. Initially work on chitin whiskers as the reinforcing matter in NR latex was carried out by Kalaprasad and Alain in 2003. Unvulcanized and prevulcanized NR latex were mixed with a colloidal suspension of chitin whiskers to

prepare chitin whisker reinforced NR nanocomposites. In the prevulcanized system, the solid composites were prepared by casting and evaporating methods after mixing and stirring the aqueous suspensions of chitin whiskers and rubber latex. Whereas, freeze drying method was used for the unvulcanized systems. This was followed by hot pressing. Researchers have moved to study on the chitosan rubber composite in 2008 by using solution casting method to prepare chitosan NR latex blend. They also revealed that the blend with dicumyl peroxides further improves the mechanical properties.

In 2008, John and Rao found that blending chitosan with NR had enhanced the thermal properties of chitosan. DSC showed that this blend is incompatible as they found two glass transition value. In 2009, John and Rao reported on further characterization of NR chitosan blends using dicumyl-peroxide (DCP) as a cross-linking agent. They reported that the blends showed stronger interfacial interaction between the matrix and filler based on the amount of bound rubber and this result is supported by FTIR spectrum. These blends also indicate that the crystallinity of NR decreases with increase in the amount of chitosan and the water absorption increases with chitosan loading.

2.6 Encapsulation

Encapsulation can be defined as a process where a continuous thin coating is formed around solid particles or liquid droplets that are fully contained within the capsule wall as shown in Figure 2.5. Encapsulation is a topic of interest in a wide range of scientific and industrial areas, varying from pharmaceutical to agriculture and from pesticide to enzyme. In effect, nearly any material that needs to be

protected, isolated, slowly released over time, or released at a certain time can be encapsulated (Fàtima *et al.*, 2009).

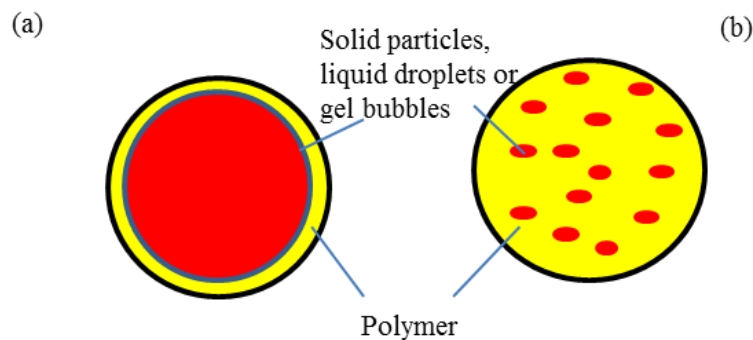


Figure 2.5: Model of encapsulation consisting of (a) encapsulation and (b) matrix

2.7 Control release system

Controlled release is a chemical activation method, which is provided for specific plant species at preset rates and times. Different polymers are largely used to control the delivery rates, mobilities, and the chemicals period of effectiveness. The main benefit of the controlled release method is that if fewer chemicals are used for the protected plants over the predetermined period, then there is a lesser effect on the other plant species, while reducing leaching, volatilization, and degradation (Fauze, 2011).

Controlled release polymer systems can be divided into two categories as shown in Figure 2.6. In the first, the active agent is dissolved, dispersed, or encapsulated within the polymeric matrix or coating. Its release takes place through diffusion or after biological or chemical breakdown of the polymer. In the second category, the active agent either constitutes a part of the macromolecular backbone or is attached to it. Here, its release is the result of biological or chemical cleavage of the bond between the polymer and the bioactive agent (Trenkel, 2010).

Conventional application of pesticide results in groundwater contamination. Conventional application of pesticide provides an initial concentration far in excess than required and cause toxicity problems for organisms and also produces undesirable side effects in the environment. Replacement of conventional pesticide formulations by controlled release systems not only helps to avoid treatment with excess amounts of active substances, but also have ecological and economic advantages. Controlled release can minimize pesticide residues available to the environment, maintain toxic concentrations of pesticide in close proximity to the target organism, increase the efficacy and longevity of the pesticide by protecting it from environmental degradation, and decrease application costs because less frequent applications are required. Other than that controlled release systems also allow the automatic release of the agent to the target at a controlled rate and maintain its concentration in the system within the optimum limits over a specified period of time, thereby providing great specificity and persistence without diminishing efficiency (Saurabh, 2011).

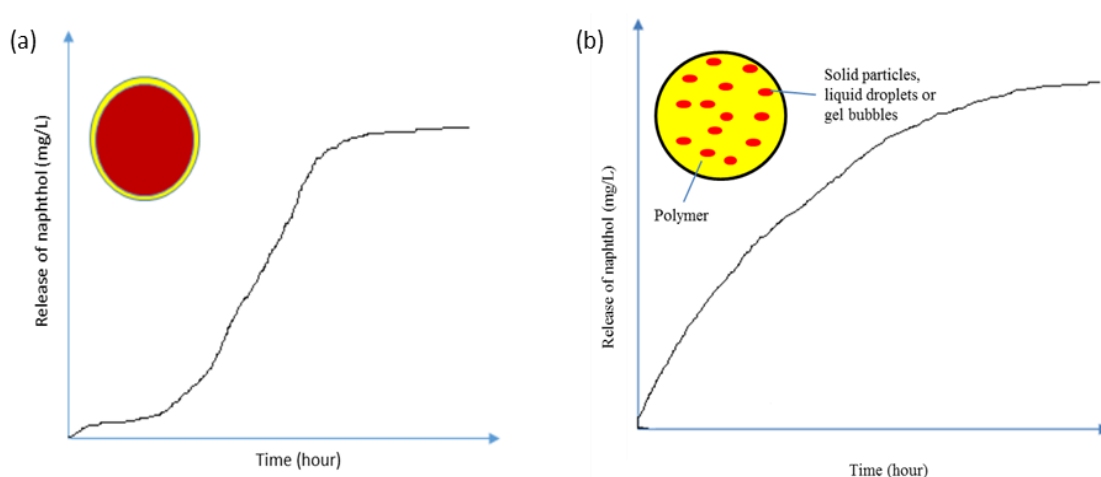


Figure 2.6: Graph of slow release of different type of capsule from (a) encapsulation model and (b) matrix model (Sa-Ad Rijayan, 2011).

Generally, during the vegetation period plants must be supplied with nutrients. This is achieved by applying quickly soluble fertilizer once to twice a week as applied in horticulture. Using slow or controlled release fertilizers, this problem can be reduced. Correct amounts of nutrients necessary for the whole vegetation period can be applied at the time of planting or at the early stages of plant growth, in the form of a nutrient pool. This will reduce the risk of incorrect fertilizer application, is labor saving and also minimizes nutrient losses by leaching or fixation.

2.7.1 Physical combination of polymers and active agents

The pre-requisite of a control release physical combination is that the polymer and active agent should not unnecessarily react. Several physical combinations of polymer-pesticide and polymer-herbicide are commercially available and many are under experimental stage (Gerstl et al., 1998). A major factor affecting release is porosity or free volumes within the matrix of the materials, the magnitude of which is controlled by the processing condition and incorporated additives (Arthur et al., 2004).

In 2011, Sa-Ad Vijayan encapsulated Neem Aza-A, a powerful insect anti-feedant and growth-regulating substance with natural rubber. Photo-degradation of Neem Aza-A in sunlight is the major problem limiting its use in agriculture. He studied the release of Neem Aza-A encapsulated in polymers like sodium alginate and PVA and then coated them with natural rubber.

For biomedical purposes, Herculano et al. (2009) used NR latex for controlled release drug delivery. They studied the release of metronidazole using NR latex as the matrix. In 2011, Herculano et al. also studied the release of nitric oxide using NR

latex as matrix. The purpose of this encapsulation is for the sustained and controlled delivery of nitric oxide for future application in medicine and wound healing.

A reasonably good prediction of nutrient release is possible with controlled-release fertilizers coated with hydrophobic materials, particularly polymer-coated fertilizers because they are less sensitive to soil and climatic conditions as reported by Trenkel in 2010. The cost of fertilizer used can be decreased by coating/encapsulating fertilizer with conventional fertilizers in different ratios. Coating/encapsulating fertilizers offer flexibility in determining the nutrient release pattern. Polymer coating can be done by forming either be semi-permeable or impermeable membranes with tiny pores. In 2009, Fàtima *et al.* reported that the main problem in the production of polymer coated fertilizers is the choice of the coating material and the process used to apply it. The nutrient release through a polymer membrane is not significantly affected by soil properties, such as pH, salinity, texture, microbial activity, redox-potential, ionic-strength of the soil solution, but rather by temperature and moisture permeability of the polymer coating.

2.8 Pesticide

Pesticide include a wide variety of components and display a broad spectrum of chemical properties. According to the Food and Agricultural Organization (FAO), as well as the World Health Organization (WHO:UNO, 1963), pesticide is any substance aimed to prevent, destroy, attract or fight any plague, including unwelcome species of plants or animals, during production and/or storage, transportation, distribution and elaboration of food, agricultural products, of food and animals or that may be administered to animals to fight ectoparasites. The term pesticides includes herbicides and compounds as growth regulators, insecticides,

fungicides, defoliants, desiccants, and inhibitors of fruit thinning and germination (Mariam, 2011).

2.8.1 Naphthol as a pesticide

Naphthol is a pesticide used in the production of carbaryl and is a derivative of naphthalene (Wang, 2012). It is a hydrocarbon found in large quantities in coal-tar, belonging to the so-called aromatic group (Gambino, 2008). In the fractional distillation of coal-tar various hydrocarbons are obtained at different degrees of heat. Naphthalene alone, which sublimes in colorless, transparent, brilliant, crystalline plates, possess a disagreeable pungent odor and has the empirical formula of $C_{10}H_8$.

Naphthol is produced by a substitution of one of the hydrogens in naphthalene by one molecule of hydroxyl -OH. Depending on the different positions in which the hydroxyl is substituted in naphthalene, two different naphthols namely 1-Naphthol and 2-Naphthol, with a formula of $C_{10}H_8O$ is obtained as shown in Figure 2.7.

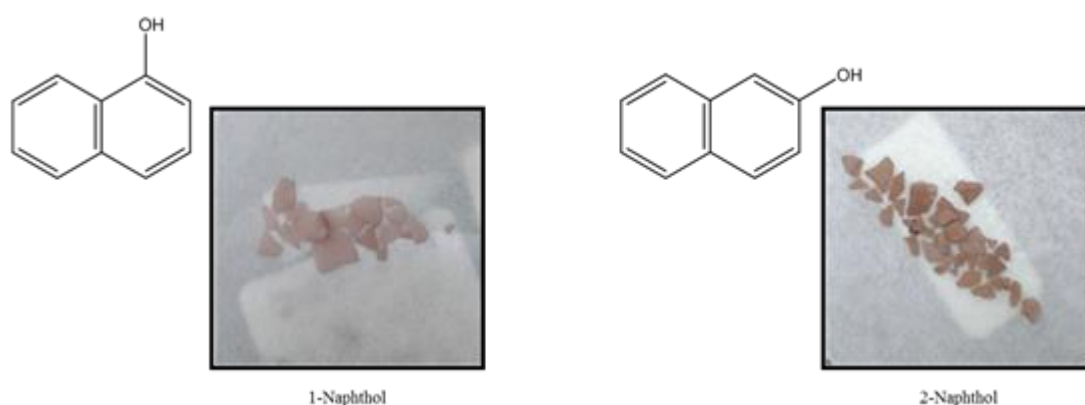


Figure 2.7: Images and chemical structures of 1- and 2-Naphthol

CHAPTER 3: EXPERIMENTAL

3.1 Materials

The uncrosslinked natural rubber (referred as NR_{ucl}) with solid content of 60% was supplied by the Malaysian Rubber Board, Kuala Lumpur, Malaysia. The average molecular weight (\bar{M}_w) and glass transition (T_g) of the NR_{ucl} determined by GPC and DSC to be 9.78×10^5 and -70 °C, respectively (Hamdan, 2000). Crosslinked natural rubber (referred as NR_{cl}) was prepared using the formulation as shown in Table 3.1.

Table 3.1: Formulation of NR_{cl}

Compounding ingredients	Content (p.h.r) ^a
Natural rubber	100
Zinc oxide	3
Sulphur	1.5
N-Cyclohexyl-2-benzothiazole sulphenamide	1
Stearic acid	2

^a parts per hundred rubber

Chitosan powder (referred as CTS) with \bar{M}_w of 1.05×10^5 was supplied by Advanced Materials Research Centre (AMREC), Kedah, Malaysia. CTS was dried in a vacuum oven at 60 °C for 3 h prior to use.

1-Naphthol (referred to as 1-N) (CAS: 90-15-3) and 2-Naphthol (referred to as 2-N) (product no. 29291) were purchased from ACROS Organics, New Jersey, USA and BDH Chemicals, England, respectively. Black humus soil (Brand: Tesco) was bought from Tesco Kulim, Kedah. Other materials used were sodium hydroxide

(NaOH) from System Chemar, and glacial acetic acid (CAS: 64-19-7) bought from QR&C, Malaysia. Curing ingredients such as zinc diethyl dithiocarbamate (ZDEC), sulfur (S), and zinc oxide (ZnO) were purchased from Bayer Ltd, India. All materials and chemicals were used as received.

3.2 Experimental

3.2.1 Determination of Degree of Deacetylation (DD)

Degree of deacetylation (referred as DD) was determined from the amino group content using acid-base titration method. 0.01 M of aniline blue and methyl orange were prepared using distilled water.

As much as 0.30 ± 0.05 g chitosan powder was weighed and dissolved in 30.0 mL of HCl (0.1 M). An equivalent amount of aniline blue and methyl orange solution was mixed with the chitosan gel solution. The mixture was stirred until the colour of the mixture was homogeneous. The mixture was titrated with NaOH (0.1 M) until the colour of the indicator turned to blue-green in colour. The blue-green colour indicated the end point of the titration. The amount of NaOH needed for each sample was recorded. The degree of deacetylation is calculated using equation 2 (Yongqin, Z. et al., 2011).

$$DD = \frac{203.195 \times w(NH_2)}{16 + [0.42 w(NH_2)]} \quad (1)$$

$$W(NH_2) = \frac{0.016 (C_1V_1 - C_2V_2)}{W_{dry}} \times 100 \quad (2)$$