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DECLARATION

I, Fariha Islam, declare that this thesis is the result of my own works and that has not been submitted or accepted for a degree in any other university.

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DEDICATION

This work is dedicated to my beloved parents.

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October, 2016

The Author

Fariha Islam

Abstract

Chitin chitosan nanofibers were prepared from shrimp shells by partial deacetylation of chitin through NaOH treatment followed by simple mechanical treatment. At 20 or 30% NaOH treatment, the slurry showed higher viscosity and formed colloidal suspensions. The average width of 20 or 30% chitin chitosan nanofibers are 10-50 nm. Furthermore, transparent chitin chitosan nanocomposites were fabricated from those nanofibers. It was found that with increasing NaOH treatment, the transparency of chitin chitosan nanocomposites increased. For example, at 30% NaOH treatment, the transparency was about 89% at 600nm wavelength. In addition, the tensile strength of acrylic resin improved from 5.5Mpa to 24.5 MPa and Young's modulus from .03 to 2.2 GPa when chitin chitosan nanofibers were incorporated. Similarly, thermal expansion decreased from 213 ppm/K to 14.6 ppm/K by 20-30%. It could be concluded that partial deacetylation by NaOH treatment is effective for fabrication of transparent high strength chitin chitosan nanocomposites.

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CHAPTER ONE

Introduction

1.1 Introduction

Today, nanotechnology and nanofibers is the centre of attention of most research community for its diversified applications. In nanotechnology, researchers are working with nanofibers of 1-100 nm diameters to understand, create, characterize and use material structures, devices and systems with new properties derived from their nanostructures (Shams et al., 2011).

Nanofibers can be extracted from both artificial and natural polymers. Natural biopolymers provide all unique characteristics of artificial polymer nanofibers like high surface area, high mechanical properties, and low coefficient of thermal expansion along with its easy processability, biodegradability and biocompatibility (Yano et al., 2007; Shams et al., 2011). Cellulose is the most abundant natural nanofibers on earth (Yano et al., 2007). After cellulose, chitin is considered to be second most abundant natural biopolymer occurs mainly in the exoskeleton of shellfish (mainly crab and shrimp), some insects and outer cuticles of arthropods (Muzzarelli, 2011).

Shrimp is one of the important fisheries products worldwide including Bangladesh. Shrimp industries generate large amounts of shrimp bio-waste during processing, approximately 45-55% of the weight of raw shrimp (Lertsutthiwong et al., 2002). Bangladesh produces approximately 18,000 to 20,000 metric tons of shrimp waste annually (Howlider, 1999). This huge amount of shrimp wastes can be a very potential source of chitin and chitosan and reduce environmental pollution in an extent by not dumping the unprocessed shrimp waste in the environment.

Chitin structure is similar to cellulose with an exception is presence of acetoamide (-NH-CO-CH$_3$) group in C$_2$ position instead of hydroxyl group. In addition, chitin is recognized as a biocompatible material with low toxicity, biodegradability, higher crystallinity and hydrophobicity (Shams et al., 2011). Chemical modifications of chitin are performed to obtain

more soluble analogs, among which, chitosan, derived by partial N-deacetylation of chitin, is the most common such derivative (Shaofang et al., 2012). Chitosan is a copolymer consisting of β-(1→4)-2-acetamido-d-glucose and β-(1→4)-2-amino-d-glucose unit linkages (Je et al., 2005). Potential and usual applications of chitin, chitosan and their derivatives are estimated to be more than 200 (Aranaz et al., 2009). This reveals the enormous promising capability of chitin and chitosan for diversified use in diversified fields. Recently, several studies were held for successful fabrication of chitin and chitosan nanofibers from different sources (Shams et al., 2011; Subir et al. 2013; Ifuku et al., 2011; Kose et al., 2011; Dutta et al., 2013;Fan et al.. 2008).

Partial deacetylation was applied to a commercial α-chitin to selectively increase the C2-primary amino groups on the crystalline fibril surfaces and raised individualization by increasing electrostatic repulsion between cationically- charged fibrils with high charge densities in acidic condition (Fan et al., 2010). As 50% deacetylation reduces crystallinity, 33% partial deacetylation was used for partial and position selective crystallite surfaces (Fan et al., 2010). Beside it increased the transparency. In this context, the present study was undertaken to evaluate the effect of NaOH in the fabrication and transmittance of different partially deacetylated chitin nanocomposites.

1.2 Objectives

1. To extract chitin chitosan nanofibers from shrimp shells through partial deacetylation of chitin (NaOH treatment).
2. To fabricate transparent nanocomposites from those nanofibers and characterize those composites.

CHAPTER TWO

Literature Review

2.1 Nanofibers

In the fabrication of nanofibers, nanofibers are generally defined as fibers with a diameter below 100 nm (Xia, et al., 2003).

The nonwoven industry generally considers nanofibers as having a diameter of less than one micron, although the National Science Foundation (NSF) defines nanofibers as having at least one dimension of 100 nanometer (nm) or less. The name derives from the nanometer, a scientific measurement unit representing a billionth of a meter, or three to four atoms wide (Hegde, et al., 2013).

Nanofibers develop by different methods like electrospinning, self-assembling, phase separation, downsizing and other producing nanofibers is spinning bi-component fibers such as Islands-In-The-Sea fibers in 1-3 denier filaments who several value added applications such as tissue engineering, medical, filtration, barrier, wipes, personal care, composite, garments, insulation, DNA, protein, enzyme delivery and energy storage. Some major application of nanofibers given as nanofibers in Tissue Engineering, nanofibers in Filtration, nanofibers in Medicals,Nano Fibers in Industry composite construction, Some challenges for nanofibers like as Economic, Health hazards, Solvent vapor, Packaging shipping handling(Kumar, et al. , 2013).

2.2 Characteristics of nanofibers

Nanofibers are highly engineered fibers with diameters of less than 100 nm. Several characteristics made nanofibers unique and centre of attention. This are-

- Low density of nanofibers
- Large specific surface area of nanofibers
- Small pore size
- High porosity – good breathability
- Excellent mechanical properties in proportion to weight
- Possibility to incorporate different additives

Besides, nanofibers exhibit special properties mainly due to extremely high surface to weight ratio compared to conventional nonwovens. How much smaller nanofibers are compared to a human hair, which are 50-150 μm and the size of a pollen particle compared to nanofibers. The elastic modulus of polymeric nanofibers of less than 350 nm is found to be 1.0±0.2 Gpa. Low density, large surface area to mass, high pore volume, and tight pore size make the nanofiber nonwoven appropriate for a wide range of filtration applications (Peter P Tsai, et. al. 2001).

2.3 Important features of nanofibers

Nanofibers have been paid much attention in research community due to the recent developments in nanotechnology. Generally, nanotechnology deals with structures sized 1-100 nm in at least one dimension. Nanotechnology may create many new materials and devices with a vast range of applications such as in medicine, electronics, biomaterials and energy production. The most important features of nanofibers are-

- Nanofibers reinforcements have been shown to increase the strength and modulus at the same time
- Creates highly porous material
- High surface area
- Low thermal expansion
- Number of defects might be reduced at nano level
- The polymer does not lose its transparency because fibers or particles which have a diameter less than one tenth of visible light are not affected by light scattering

(Shams, et al., 2011)

2.4 Advantages of nanofibers

To meet the diverse range of end users and consumer needs for different types of activities, it is necessary to develop materials that offer different levels of breathability and water resistance. Electrospun and downsized nanofibers webs can be engineered with a desired porous structure and a range of polymers. The unique combination of high specific surface area, flexibility, low basis weight, and porous structure allows manufacturers to optimize the water resistance level, air permeability and water vapor transmission rate.

Nanofibers has great advantages and this are-

High surface area to volume ratio

The nano-dimension of nanofiber naturally gives it a high surface area to volume ratio. This characteristic makes it very attractive in applications where large surface area is desirable such as in sensors and affinity membranes.

Wide variety of polymers and materials have been used to form nanofibers

Electrospinning has been used to make nanofibers from all major classes of materials either directly or indirectly. Although the process is predominantly used to make polymeric nanofibers, ceramic and metal nanofibers have also been constructed indirectly through electrospinning of their precursor material. By downsizing method, nanofibers can be made more easily.

Ease of fiber functionalization

This benefit relates to the variety of polymer that can possibly be used to electrospin and extracted nanofibers. Functionalization of electrospun nanofibers can be achieved through simple blending of polymer solution prior to spinning, post-spinning surface functionalization or using core-shell electrospinning setup.

Ease of material combination

Low requirements for electrospinning meant that different materials can be easily mixed together for spinning and downsizing into fibers.

Relatively low start up cost

Simple electrospinning setup generally cost a few thousand dollars. For use in laboratory environment, a setup can be self-assembled from off-the-shelf components or purchase fully assembled. But if we use downsizing method nanofibers production needs lower cost than electrospinning.

Variety of nanofibrous structures have been constructed

Advances in electrospinning setup and process have seen development of tubular nanofibrous structure, yarns and 3D blocks of nanofibers.

Commercial applications:

Electrospinning and downsizing has been used in the construction of several commercially available products. Some examples are given below,

- Air filtration membrane
- Face mask
- Water filtration membrane
- Cell culture plates
- Wound care patch, etc.
-

Beside all these advantages, natural nanofiber has

- Biodegradablility
- Biocompatibility
- Renewability and
- Ready availability

along with its high strength properties, high transparency, low thermal expansion, high surface area and high porosity.

(Shams, et al., 2011)

2.5 Preparation of nanofibers from different sources

It is well known that nanofibers are produced in nature for example, collagen fibrils in tendons and ligaments and silk fibroin. Among the variety of natural nanofibers, cellulose microfibrils, which are the major constituent of plant cell walls and are also produced by some bacteria, are the most abundant nanofibers on earth (Abe et al., 2007).

Cellulose is made up with a number of glucose units linked by β (1→4) glycosidic linkage in a long chain. Hence, its properties are directly related to the chain length. One of the properties of cellulose is water insoluble, due to its glycosidic linkage between the monomers. The size reductions results in the fabrication of new material which is very easy for environmental degradation. The growth of bio composite materials with the natural fibers has become known in the most recent decade. This is one of the main advantages given by the wide accessibility of fibers, their low cost, weight and biodegradability. (Velayudham et al., 2013)

After cellulose, chitin is the second most abundant biomacromolecule in nature, existing mainly in exoskeletons of crabs, shrimps, and insects, and is synthesized in the amount 10^{10} to 10^{11} tons every year (Gopalan & Dufresne, 2003). Owing to its linear (1→4)-β-N-acetyl glycosaminoglycan structure with two reactive hydroxyl groups and an acetamide group per anhydro-N-acetylglucosamine unit, chitin has broad potential in the design of advanced polymeric biomaterials. However, most of chitin is discarded as industrial food residue without effective utilization. Thus, it is important to develop efficient use of chitin as a natural and eco-friendly material (Ifuku et al., 2011).

2.5.1 Preparation of cellulose nanofibers

2.5.1.1 Nanofibers from wood

Recently, Abe et al. succeeded in isolating cellulose nanofibers with a uniform width of approximately 15 nm from wood (Abe et al., 2007). Wood cell walls consist of stiff cellulose microfibrils, embedded in soft matrix substances such as hemicelluloses and lignin which behave just as adhesive between microfibrils. Due to the extended crystalline structure, the microfibrils have efficient physical properties; their Young's modulus is 138 GPa (Nishino et al., 1995), their

tensile strength is at least 2 GPa, based on experimental results for kraft pulp, and their thermal-expansion coefficient in the axial direction is as small as 0.1 ppm/K (Nishino et al., 2004) . These structural features of wood cell walls make the wood stiff and tough, and are helpful in supporting the huge body of a tree. Since cellulose nanofibers are embedded in the matrix phase, they can be isolated by first removing the matrix and subsequently extracted as nanofibers by a very simple grinding treatment.

2.5.1.2 Nanofibers from coconut coir

Cellulose nanofibers were isolated from coconut coir fibers by chemical treatment using alkaline, mineral acids and inorganic salts, followed by mechanical treatment and disintegration methods like sonication, cryo crushing and dissolution. The size and morphology of cellulose nanofibers were investigated by using the Field Emission Scanning Electron Microscope (FESEM). The width of synthesized cellulose nanofibers investigated by the FESEM was around 30 nm to 90 nm and few microns in length. FT-IR spectra confirmed the presence of hydroxyl groups, C-H bond and the C-O-C groups in the synthesized cellulose nanofibers. The cellulose nano fibers were successfully utilized in the preparation of transparent thin film, filtration and water treatment (Velayudham et al., 2013).
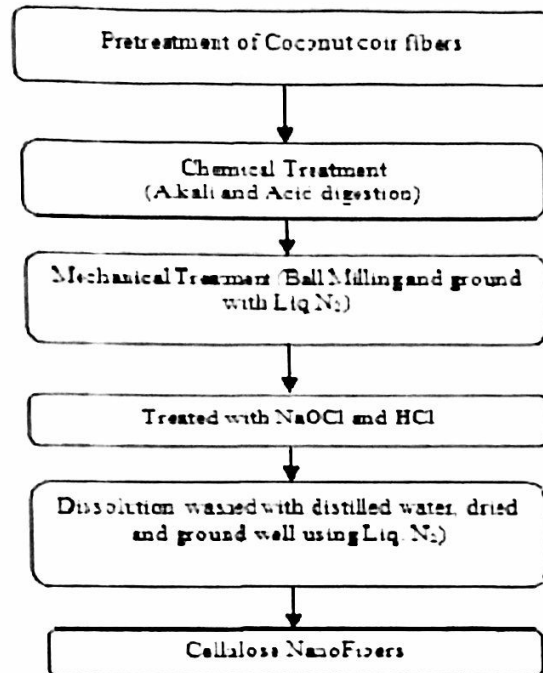
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┌─────────────────────────────────────────┐
│     Pretreatment of Coconut coir fibers  │
└─────────────────────────────────────────┘
                    │
                    ▼
┌─────────────────────────────────────────┐
│          Chemical Treatment              │
│     (Akali and Acic digestion)           │
└─────────────────────────────────────────┘
                    │
                    ▼
┌─────────────────────────────────────────┐
│ Mechanical Treatment (Ball Milling and   │
│          ground with Liq N₂)             │
└─────────────────────────────────────────┘
                    │
                    ▼
┌─────────────────────────────────────────┐
│        Treated with NaOCl and HCl        │
└─────────────────────────────────────────┘
                    │
                    ▼
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│ Dissolution washed with distilled water, │
│   dried and ground well using Liq N₂)    │
└─────────────────────────────────────────┘
                    │
                    ▼
┌─────────────────────────────────────────┐
│          Cellulose NanoFibers            │
└─────────────────────────────────────────┘
```

Figure 2.1: Preparation of cellulose nanofibers from coconut coir.

2.5.2 Preparation of Chitin Nanofibers

2.5.2.1 Nanofibers from Crab Shell (Ifuku et al., 2009)

Crab shells have a hierarchical organization with various structural levels (Figure 2.2). Chitin NFs were prepared from crab shells by a disintegration process (Li et al., 2004). To extract chitin NFs, the shells were first purified by a series of conventional chemical treatments and then subjected to mechanical treatment. Proteins and minerals were removed from the crab shells by aqueous NaOH and HCl treatments, respectively (Shimahara et al., 1998). A grinder (Masuko Sangyo Co., Ltd., Kawaguchi, Japan) was efficient for disintegrating the chitin aggregates (Abe et al., 2007). A pair of grinding stones effectively disintegrated the chitin organization. After one cycle of wet-type grinder treatment, the chitin slurry formed a gel, suggesting disintegration was accomplished due to a high surface-to-volume ratio. The chitin consisted of highly uniform NFs with a width of approximately 10 nm (Figure 2.3). The chitin NFs still had their original

chemical and crystalline structures after a series of treatments. This simple but powerful method allows us to obtain homogeneous chitin NFs from waste crab shell in large amounts.
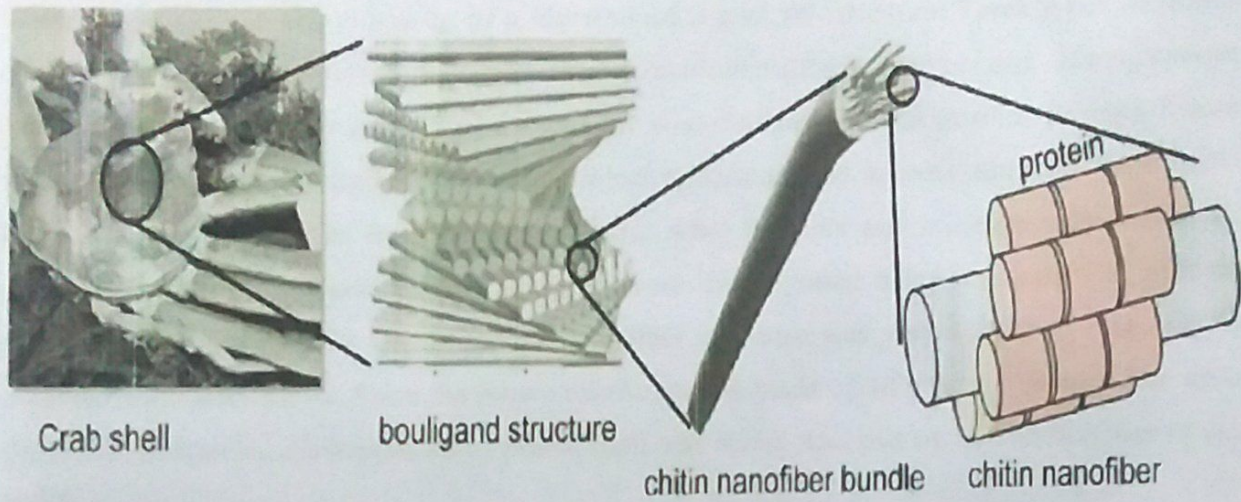

Figure 2. 2: Schematic presentation of the exoskeleton structure of crab shell.
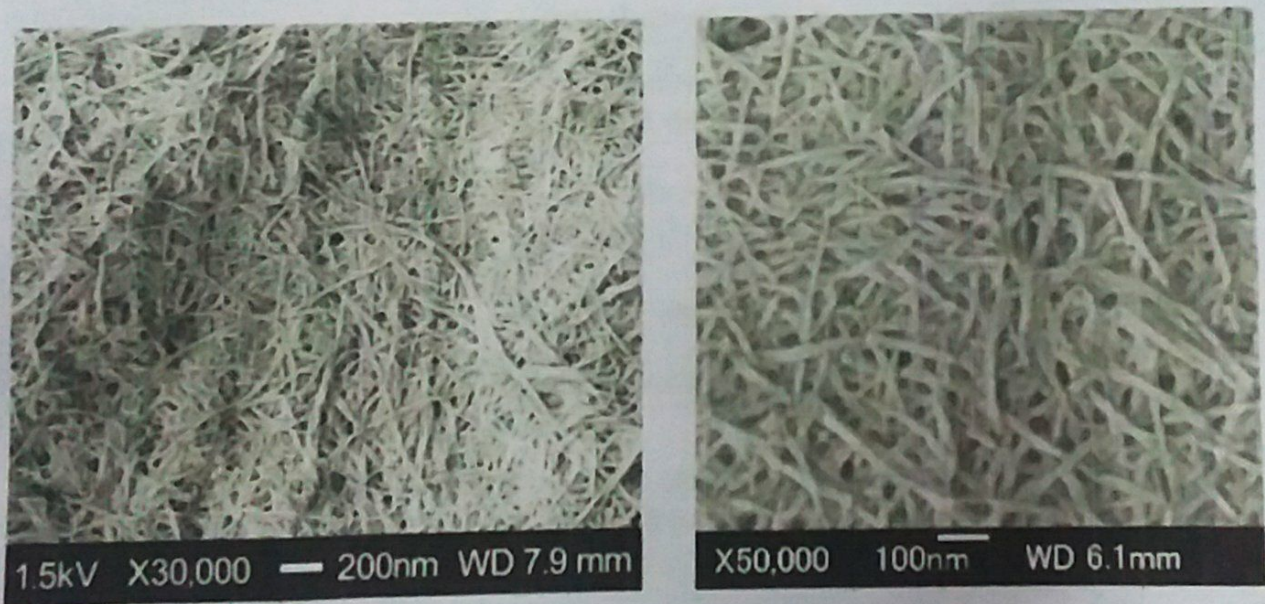

Figure 2.3: SEM images of chitin nanofibers from crab shells after grinder treatment.

2.5.2.2 Nanofibers from Prawn Shells (Ifuku et al., 2011)

The method of preparing chitin NF from crab shell is applicable to a variety of prawn shells, since prawn shell is also made up of a hierarchical organized structure. Three types of prawn shells were chosen as starting materials: *Penaeusmonodon* (black tiger prawn), *Marsupenaeus japonicas* (Japanese tiger prawn), and *Pandalus eous Makarov* (Alaskan pink shrimp). These species are widely cultivated around the world as important food sources, although their shells are often thrown away as food industrial waste. After proteins and minerals were removed, purified wet chitins extracted from the shells were disintegrated using a grinder. A uniform structure of the chitin NFs was observed. The fiber structure was very similar to that of NFs obtained from crab shells. Since the prawn exoskeleton is made up of a finer structure than crab shell, the mechanical disintegration of prawn shell was easier than that of crab shell (Chen et al., 2008).

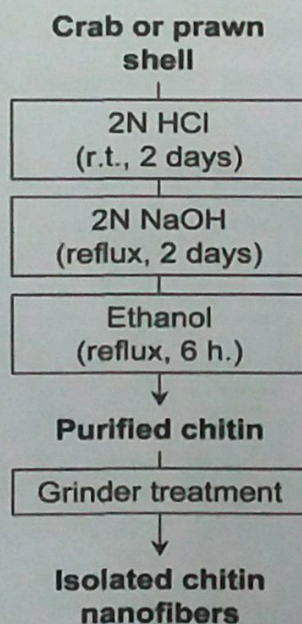**Crab or prawn shell**

| 2N HCl (r.t., 2 days) |
| 2N NaOH (reflux, 2 days) |
| Ethanol (reflux, 6 h.) |

Purified chitin

| Grinder treatment |

Isolated chitin nanofibers

Figure 2.4: Preparation of chitin nanofibers from shrimp shells.

2.5.2.3 Nanofibers from Mushroom (Ifuku et al., 2011)

The cell walls of mushrooms also consist of chitin NFs, which form a complex with glucans (Ifuku et al., 2011). Edible mushrooms are a source of dietary fiber, which is available as a food bulking or thickening agent, a film forming agent, and a stabilizer. Chitin NFs were prepared from mushrooms referring the method described above. Five widely consumed species of mushrooms were selected: *Pleuotus eryngii* (king trumpet mushroom), *Agaricus bisporus* (common mushroom), *Lentinula edodes* (shiitake), *Grifola frondosa* (maitake), and *Hypsizygus marmoreus* (bunashimeji). Since the ingredients of mushroom cell walls are quite different from those of crab and prawn shells, a different method of extracting chitin from mushroom had to be used (Ivshina et al., 2009). The chitin was disintegrated into chitin NF after grinder treatment. All NFs prepared from five different mushrooms were similar to those from crab and prawn shells. However, they formed a complex with several glucans on their surface. The content ratio of the glucan differed considerably depending on the species. The dietary nano sized fibers obtained from cultivable and edible mushrooms will have a wide range of applications a novel functional food ingredient.

Mushrooms
|
NaOH 2%
(100 °C, 1 day)

HCl 2M
(r.t., 2 days)

$NaClO_2$ 0.5%
AcOH 1%
(100 °C, 1 day)

NaOH 2%
(100 °C, 1 day)

↓

Extracted samples
|
Grinder treatment
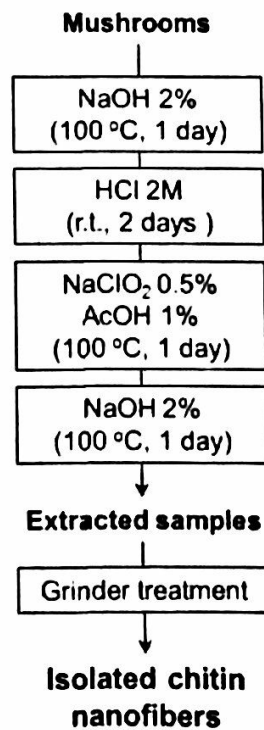
↓

Isolated chitin nanofibers

Figure 2.5: Schematic representation of chitin nanofibers extraction from mushroom.

2.5.2.4 Nanofibers from Squid Pen e-Chitin (Dutta et al., 2013)

Two types of chitin crystal are found in Nature: α- and β-chitins. Although α-chitin has an antiparallel mode, β-chitin has a parallel chain-packing mode. Most natural chitins have the α-type crystal structure, while the β-type chitin is present in squid pens. Fan and Isogai et al. prepared chitin NFs with a 3–4 nm width and a high aspect ratio from squid pen β-chitin. Mechanical treatment under acidic conditions is important for preparing chitin NFs. Cationic charges of the chitin NFs accelerate NF conversion. Moreover, lower crystallinity, the parallel chain-packing mode, and relatively weak intermolecular forces of squid pen β-chitin are also key factors in the preparation of chitin NFs.

2.5.2.5 Nanofibers from Commercial Chitin Powder (Ifuku et al., 2013)

For the preparation of NF, extracted chitin must be kept wet to avoid strong inter-fibrillar coagulation. However, this is a disadvantage for the commercialization of chitin NF. The author developed a facile method of preparing chitin NFs from commercially dry chitin powder. Dry chitin was dispersed in acidic water and passed through a grinder. Although commercial chitin was made up of aggregates of NFs, the aggregates were easily converted into homogeneous NFs. This ease of conversion was attributed also to the electrostatic repulsion effect as described above. The repulsive force caused by the cationization of amino groups facilitated fibrillation into NFs. Several other organic acids can also accelerate disintegration. NFs from commercial chitin are advantageous because a large amount of chitin can be obtained within a few hours. A high-pressure waterjet system called the Star Burst instrument was also effective for the nanofibrillation of dry chitin. Chitin in aqueous acetic acid was passed through water jet system equipped with a ball-collision chamber. The slurry was ejected from a small nozzle under high pressure. After mechanical treatments, the NFs became thinner as the number of treatments increased.

2.5.2.6 Preparation of Chitosan Nanofibers (Dutta et al., 2013; and Fan et al, 2008)

Chitosan is prepared by the deacetylation of chitin. Because of its biocompatibility and biodegradability as well as its cellular-binding, wound-healing, anti-bacterial, and anti-fungal properties, chitosan has great potential for many uses, including food, cosmetic, biomedical, and pharmaceutical applications (Muzzarelli et al., 2012). The electro-spinning process is the typical method of artificially producing NFs from a polymer solution. However, the electro-spinning of chitosan is difficult due to the excessive surface tension of the chitosan solution (Min et al., 2004). If the characteristic NF structure of chitin is maintained after deacetylation, a downsizing process may be useful for the production of chitosan NF. Dry chitosan powder was treated by using the high-pressure waterjet system, and was disintegrated into NFs (Figure 2.6).
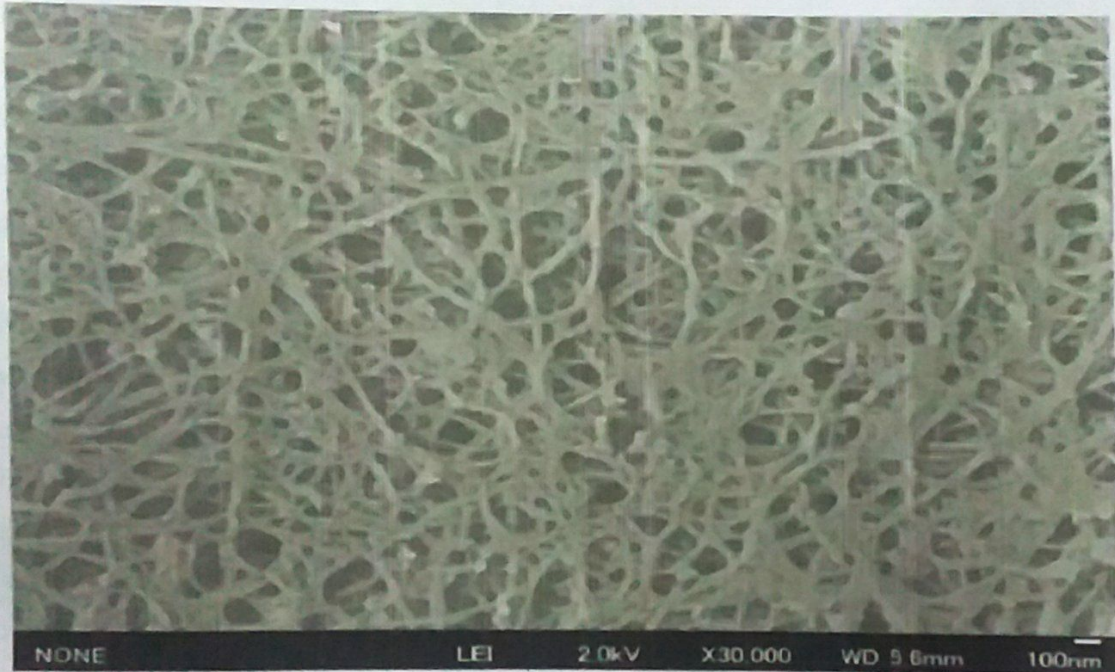

Figure 2.6: SEM image of chitosan nanofibers after high pressure waterjet treatment.

However, the chitosan NFs became thinner as the number of treatment cycles increased. Here, in addition to chitin and cellulose NFs, chitosan NFs have been considered in bio-NFs.

2.5.3 Chemical Modifications of Chitin Nanofibers

2.5.3.1 Acetylation of Chitin Nanofibers (Ifuku et al., 2010)

Surface modification by the introduction of certain functional group into hydrophilic hydroxyl groups is a promising method to expand the applications of chitin NFs. Modification using hydrophobic functional groups on NFs will improve dispersibility in nonpolar solvents, hygroscopicity, and adhesion properties with hydrophobic matrices in composite materials (kurita et al., 2001). In a series of modifications, acetylation is a simple, popular, and inexpensive approach to modifying the surface properties (Nogi et al., 2006). Chitin NFs were acetylated in a mixture of acetic anhydride and perchloric acid at room temperature (**Figure 2.7**). The acetyl DS was controllable from 1 to 3 by adjusting the reaction time. Chitin NFs were acetylated completely within 1 h of reaction. The chitin NFs were acetylated heterogeneously from the surface to the core. First, the NF surfaces were immediately acetylated, and then the insides of the NFs were acetylated gradually. The NF structure was maintained even with a DS of 3, and the fiber thickness increased due to the bulky acetyl groups. Acetyl chitin NF/acrylic resin composites were prepared. Thanks to the size effect, all composite films were highly transparent, independent from acetyl DS. After 1 min acetylation, the moisture absorption of the nanocomposite film drastically decreased. Moreover, although the coefficient of thermal expansion of the acrylic resin used in this study was 6.4×10^{-5} °C^{-1}, the thermal expansion of the composite film decreased to 2.3×10^{-5} °C^{-1}. This is apparently due to the reinforcement effect of chitin NFs with low thermal expansion.
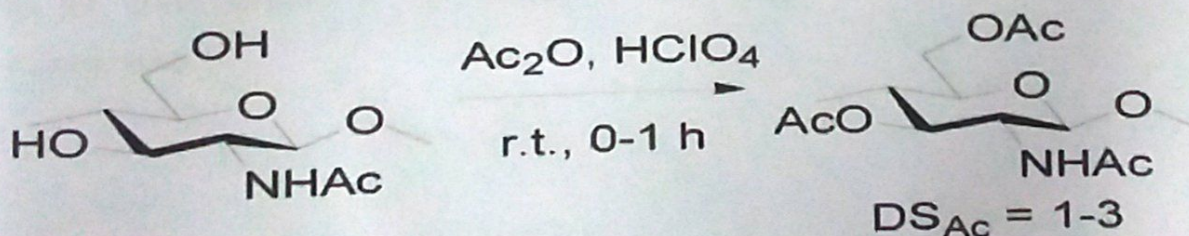

Figure 2.7: Acetylation of chitin nanofibers.

2.5.3.2 Preparation of Partially Deacetylated Chitin Nanowhiskers (Fan et al., 2010)

Fan and Isogai et al. developed an efficient method to obtain chitin nanowhiskers by partial deacetylation (**Figure 2.8**).
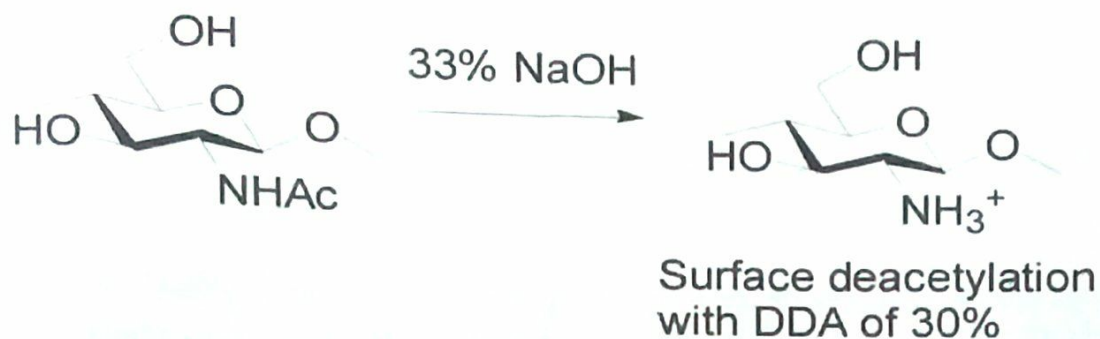

Figure 2.8: Partial deacetylation for chitin nanowhiskers.

The acetamide group of chitin was converted into an amino group by deacetylation, and the electrically charged amino group on the chitin surface accelerated disintegration due to electrostatic repulsion. That is, α-chitin was partially deacetylated by 33% NaOH treatment. The degree of deacetylation was approximately 30%. The relative crystallinity and crystal size of the original α-chitin were maintained after deacetylation. This indicates that the deacetylation mainly occurred on the chitin surfaces. Transparent and highly viscous liquids were obtained by ultrasonic disintegration under acidic conditions. The liquids consisted of nanowhiskers. The average width and length were 6.2 nm and 250 nm, respectively. High cationic charges appeared on the chitin surfaces in high density in acidic water; this is significant for effective disintegration.

CHAPTER THREE

Materials and Methods

3.1 Materials

The shells of tiger shrimp (*Peneus monodon*) were collected from Rupsha sea foods Ltd. Rupsha; Khulna; Bangladesh as raw material. In this experiment, only body shells were used to make chitin-chitosan nanofibers. NaOH (99.9%), HCl (37%), Ethanol were used to remove matrix.

A normal blender (Panasonic) was used to crush the shrimp shells and a high speed blender (Vita-Mix blender) were used to fabricate the chitin chitosan nanofibers.

3.2 Preparation of chitin chitosan nanofibers and nano-sheets

3.2.1 Chitin preparation

Shrimp shells contains about 30-40% chitin and other matrix like proteins, $CaCO_3$, Lipids and pigments. Firstly, shrimp shell of *Peneus monodon* was washed and air dried for three days. After that dried shell was crushed by normal blender and then 100g of crushed shimp shell powder is treated with 7% HCl solution for 24 hours at room temperature to remove mineral salts like $CaCO_3$. Then the sample was rinsed with an abundance of distilled water. This process is repeated 2 times for the complete removal of mineral salts. After that, the sample was dispersed in 4% NaOH solution for 24 hours to remove residual proteins and lipids. First 4 hours, the sample was treated at 60°C and then 20 hours at room temperature. Treated sample was washed with distilled water after this process for several hours. This process was repeated 2-3 times until the sample was white in color.

After these chemical treatments, pigment was removed by treating the sample by 50wt% of ethanol for 12 hours at room temperature. The yield of chitin was around 13.98%.

3.2.2 Partial deacetylation

Obtained purified chitin was treated with 10%, 20%, 30% and 40% NaOH solution at ratio of 1:25 (gmL−1) for 4 hours at 60°C temperature in waterbath. During treatment, the solution was shaked after every 15-20min. After the treatment, chitin chitosan was filtered and rinsed with an abundance of distilled water to make it neutral.

3.2.3 Nanofibers preparation

The purified wet chitin and chitin chitosan samples were dispersed in distilled water with a concentration of 1wt%, forming slurry. The slurries were then stirred for several hours and few drops of acetic acid were added to adjust the pH 3-4. The suspensions were then subjected to a high-speed blender (Vita-Mix Blender, Osaka Chem. Co. Ltd.) for 10 minutes at a rotating speed of 37000 rpm and were kept in a never dried condition.

3.2.3.1 Characterization of chitin chitosan nanofibers

Precipitation rate of suspension

Suspensions of 1 wt% chitin and chitin chitosan nanofibers having different NaOH concentration treatment were kept in four test tubes for two weeks to measure the degree of fibrillation.

3.2.4 Nano-sheet preparation

The fibrillated chitin and chitin chitosan nanofibers were neutralized and dispersed into distilled water at 0.1 wt% of nanofibers contents. These suspensions were stirred for 6 hours. After stirring, 260 g of water suspension was vacuum-filtered through PPTE membrane filter (0.2μm) to produce a wet nano-sheet of 40 mm in diameter. The wet nano-sheet was separated firstly from the filter paper and pressed at 110°C and 5N/mm^2 pressure for 8 minutes. The thicknesses of nanosheets were varied between 24 to 60 μm.

3.2.5 Preparation of transparent chitin chitosan Nanocomposites

The dried nanosheets were impregnated in 99.8% methanol for few minutes and then immersed in neat acrylic resin A-BPE-10 matrix (refractive index 1.537). After reinforcement the impregnated composites were cured using ultraviolet light. The fiber content of chitin and chitin-chitosan nanocomposites were 23-27 wt% which was obtained by the oven-dry weight of chitin chitosan nanosheets and chitin chitosan nanocomposites.

3.2.5.1 Characterization of nano-sheet and transparent nanocomposites

Dewatering time

About 260 g of water suspension was vacuum-filtered from 0.1% solution through PPTE membrane filter (0.2µm) to produce a wet nanosheet of 40 mm in diameter. The dewatering time of each of the sample was measured in minutes. It was measured carefully for each condition.

Microscopic image observation

For obtaining the microscopic view of all chitin and chitin chitosan nanosheets, these were coated with Cedar wood Essential Oil and carefully observed the fiber orientation of the nanofibers.

FE-SEM Observations

The chitin and chitin chitosan nanosheets were coated with an approximately 2 nm layer of platinum by an ion-sputter coater and ovserved with a field emission scanning electron microscope (JSM-1600F, JEOL Ltd.).

Light transmittance

The light transmittances of chitin and chitin chitosan nanocomposites were measured using a UV-visible spectrometer (U-4100, Hitachi High-Tech. Corp.) with an integrating sphere 60 mm in diameter at wavelengths from 200 to 800 nm. Regular light transmittance was measured by placing the specimens 25 cm from the entrance port of the integrating sphere.

Coefficient of thermal expansion

Coefficients of thermal expansion (CTE) were measured by a thermo-mechanical analyzer (TMA/SS6100, SII Nanotechnology Inc.). Specimens were 25 mm long, 3 mm wide and 60–80 µm thick with a 20 mm span. The measurements were carried out three times during elongation with a heating rate of $5^{\circ}C/min$ in a nitrogen atmosphere at a load of 3 g. The CTE values were determined as the mean values at $20-150^{\circ}C$ in the second run.

Tensile test

The tensile properties were measured using an Instron 3365 universal testing machine. The specimen gage lengths were measured with a caliper for each sample upon gripping, and the crosshead speed was set at 1mm/min. the specimens were 35mm long, 5mm wide and 40-60 micro meter thick.

CHAPTER FOUR

Results and Discussion

4.1 Precipitation Rate of Suspension

Suspensions of 1 wt% chitin and chitin chitosan nanofibers having different NaOH concentration treatment were kept in four test tubes to measure the degree of fibrillation. Precipitation rate of all high speed blender slurry was remaining unchanged after two weeks (Figure 4.1). This occurred because of obtaining colloidal structure after 37,000 rpm rotating speed (Shams et al., 2013). As NaOH treatment concentration was increased, the nanofibers slurries were being transparent. This may be due to increase of C2-NH2 groups and more surface cationization or protonation at pH 3-4 (Fan et al., 2008). Viscosity was higher in all 1 wt% partially deacetylated chitin nanofibers having different NaOH percentage treatment. This is happened because of the concentration, attractive force, and particle size of the solution. The viscosity differs with the morphological characteristics of nanofibers. Thinner and shorter fibers can intertwine more frequently than long fibers. According to this statement it can be said that, as all slurries were prepared by high speed blending, all can ensure shorter and thinner nanofibers with homogeneous fibrillation. As the acetic acid concentration increased from 10% to 90%, the viscosity increased as the solution became increasingly viscous (Ifuku et al., 2007). The higher viscosity might be due to individualization of partially deacetylated α-chitin fibrils at higher level in acidic water (Fan et al., 2008). The addition of acetic acid in the partially deacetylated nanofiber solution is the main reason of higher viscosity of all the slurries. This addition of acetic acid into slurry facilitates the fibrillation and homogeneous dispersion of chitin nanofibers by electrostatic repulsion.
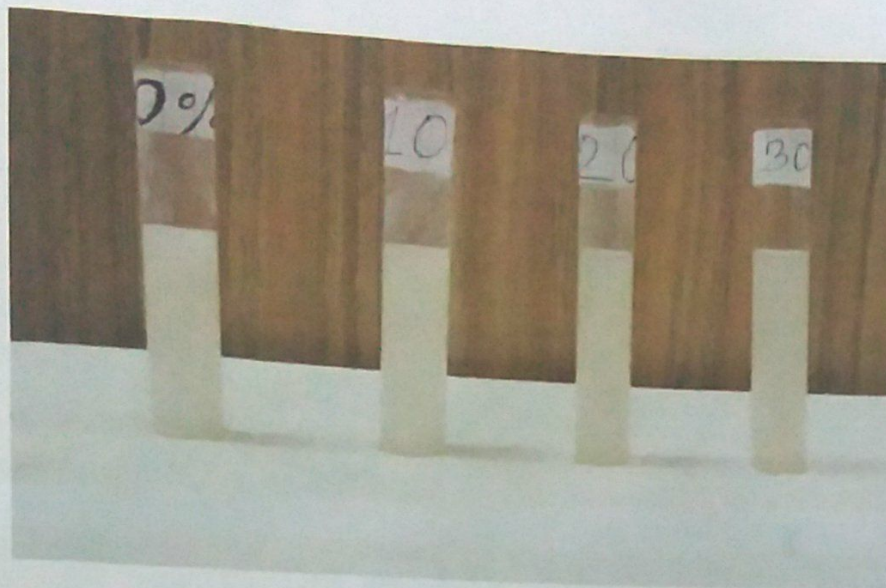
Figure 4.1: Suspensions of nanofibers at different NaOH concentration treatment.

4.2 Microscopic image observation

Figure 4.2 showed the effect of NaOH percentages on chitin chitosan nanofibers. It showed that no big particles were seen in the chitin chitosan nanofibers slurries resulting in a much more homogenous nanofibrillar structure. This is due to the presence of the positive charges on the surface of the nanofibers, which results from the protonization of the amino groups of chitin in acidic conditions (Shams et al., 2011). On the other hand the electrostatic repulsive force produced by surface protonation was the most vital condition to fibrillate individualized chitin nanofibers (Fan et al., 2008).
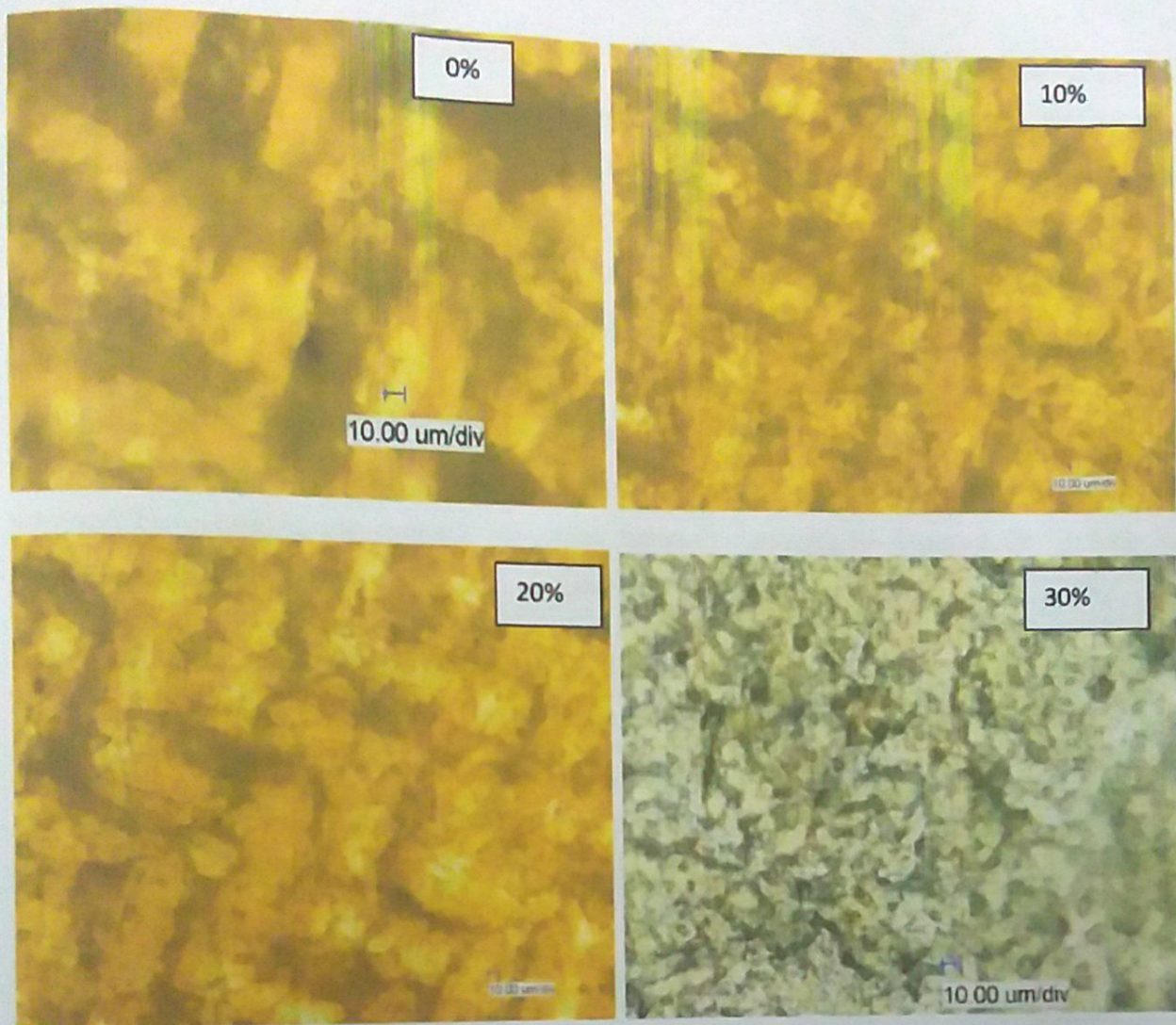
Figure 4.2: Microscopic image of chitin chitosan nanofibers at different NaOH concentration.

4.3 Field emission scanning electron microscopic (FE-SEM) image observation

Figure 4.3 showed the fibrillated structures of the chitin chitosan nanofibers treated in different NaOH percentages. Here almost 70-80% nanofibers width are about 10-50 nm. The nanofibrils formed irregular bundles of more than 70 nm in width, probably due to the strong H-bonds generated among the fibrils (Biswas et al., 2013). Whereas crab shell chitin was mechanically disintegrated in water by high speed blender, chitin nanofibers with variable widths of 30–110 nm are obtained (Shams et al., 2011). Meanwhile cationization of the C2 amino groups present

on the crystallite surfaces of the squid pen β-chitin under acidic conditions is one of the most significant conditions for the nanofibers conversion (Fan et al., 2008). Almost 70% of chitin nanofibers have a width within the range of 20–30 nm in acidic condition (Shams et al., 2013). These images also showed that with the increase of NaOH treatment percentage, the nanofibers showed more homogenous fibrillation. This may be due to the increasement of $C2-NH_2$ content with the increasement in partial deacetylation by NaOH treatment. Cationization or protonation of the $C2-NH_2$ groups at pH 3-4 was required for conversion of the partially deacetylated chitins to transparent liquids by mechanical disintegration in water and the increesement in cationic charge density on the crystalline fibril surfaces of α chitin, more individualization can be achieved by enhanced electrostatic repulsion between cationically- charged fibrils with high charge densities through disintegration in water under acidic conditions (Fan et al., 2008).

Beside all these, the partial deacetylation process didn't affect the crystallite surfaces of nanofibers and that's why fiber length was achieved more than 500nm but for high speed blending the width is about 10-50 nm. At high NaOH concentration (20%), the length of lobster chitin nanofibers ranged from 200 to 2000 nm whereas the very large diameter nanofibers are absent (Ngesa et al., 2014).

The dark spots and cloudiness in FE-SEM images are describing presence of matrix and pigments. This may remain for poor washing techniques with water during chemical treatments and incomplete fibrillation. Nanofibers with shorter treatment times and higher protein content contain more agglomerates (Ngsea et al., 2014).
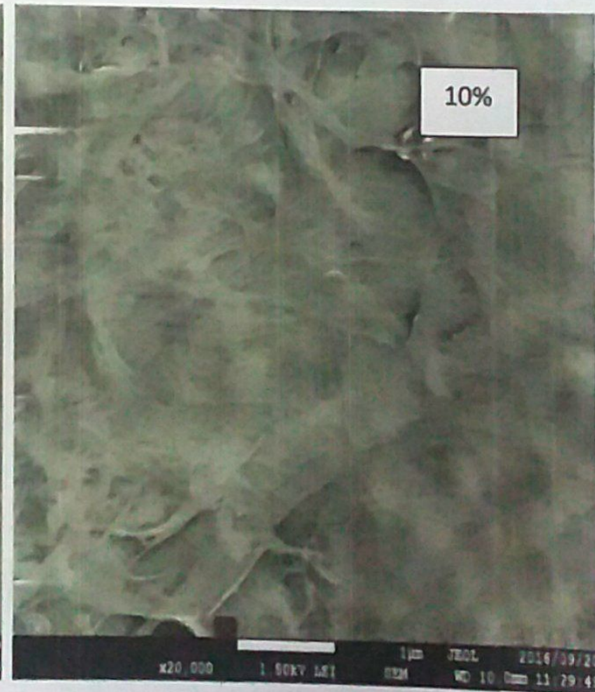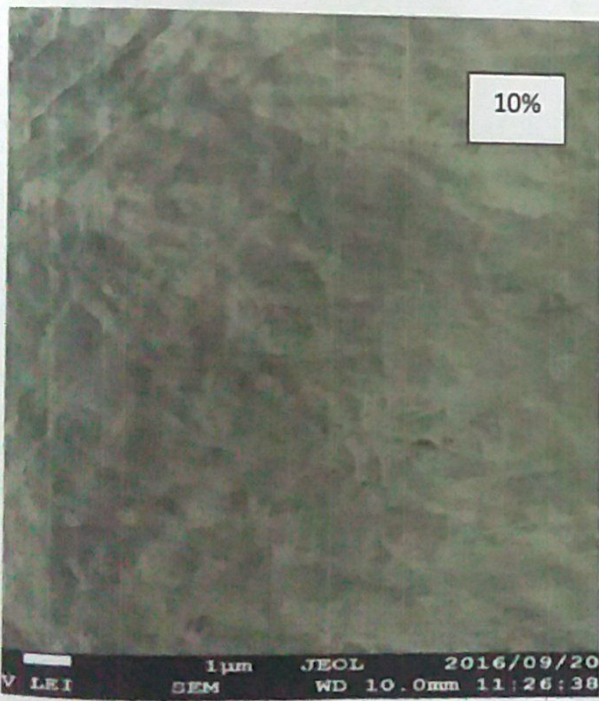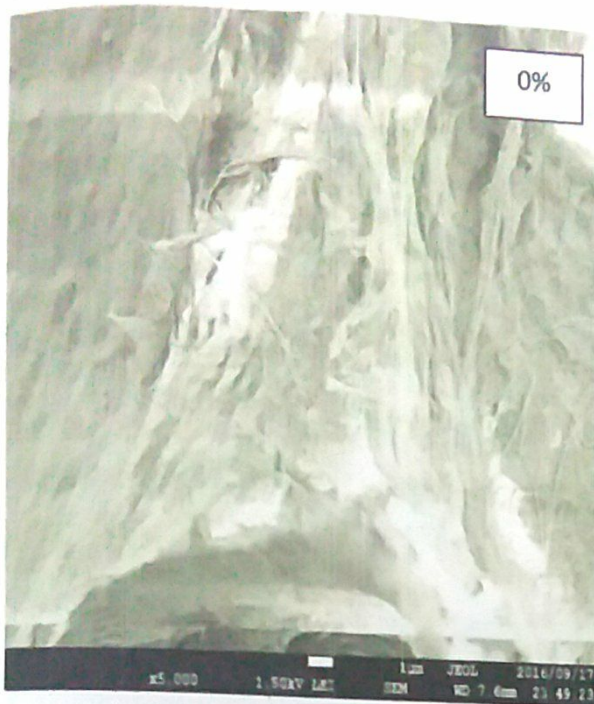
4.4 Dewatering time

The effect of different NaOH concentration treatment on the suspension was determined indirectly through the dewatering time of suspension. The effect of NaOH concentration on dewatering time was presented in figure 4.4, where 30% treatment took highest minutes than others. This might happened due to shortening of nanofibers and increasing of surface area since dewatering time is related to the surface area. Beside, chitosan is hydrophilic in nature (Shakeel et al., 2014). As partial deacetylation increases with increasing the chitosan percentages, that's why 30% showed higher dewatering time. Though, all other suspensions show lower trend of dewatering time in contrast with cellulose nanofibers, whick took 180 minutes for dewatering (Yano et al., 2005). This implies that all chitin chitosan nanofibers were homogeneously fibrillated under acidic condition during mechanical treatment which exposed more surface area by breaking the hydrogen bonds among the nanofibers assisted by electrostatic repulsion force (Biswas et al., 2013). Though, chitin nanofibers took only 20 minutes to be filtered under the acid conditions pH 3-4 due to chitins hydrophobic nature (Shams et al., 2013).
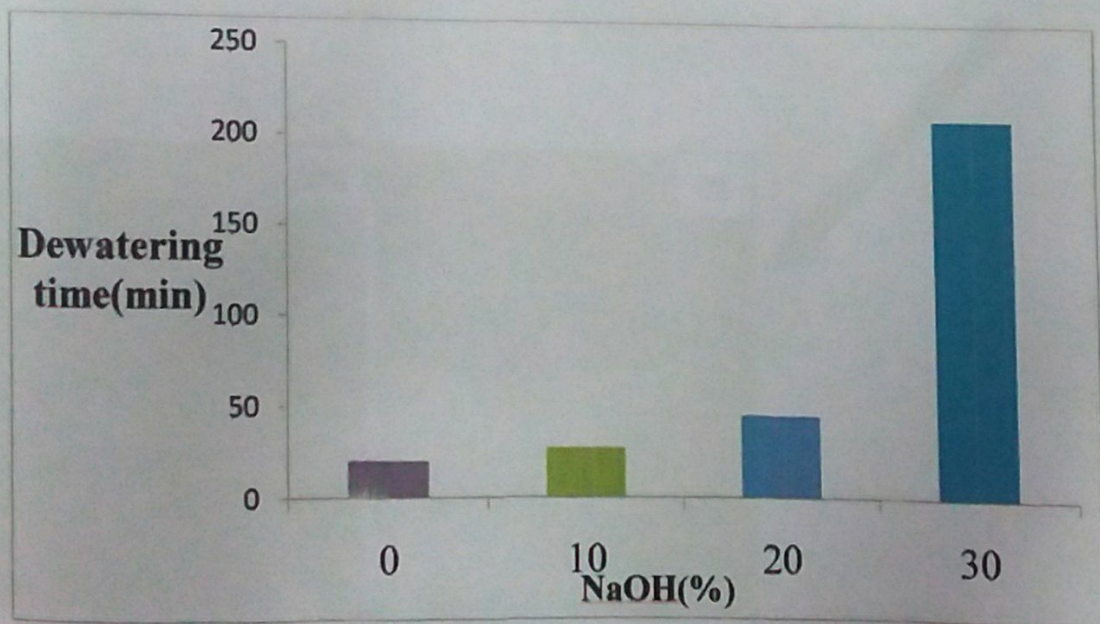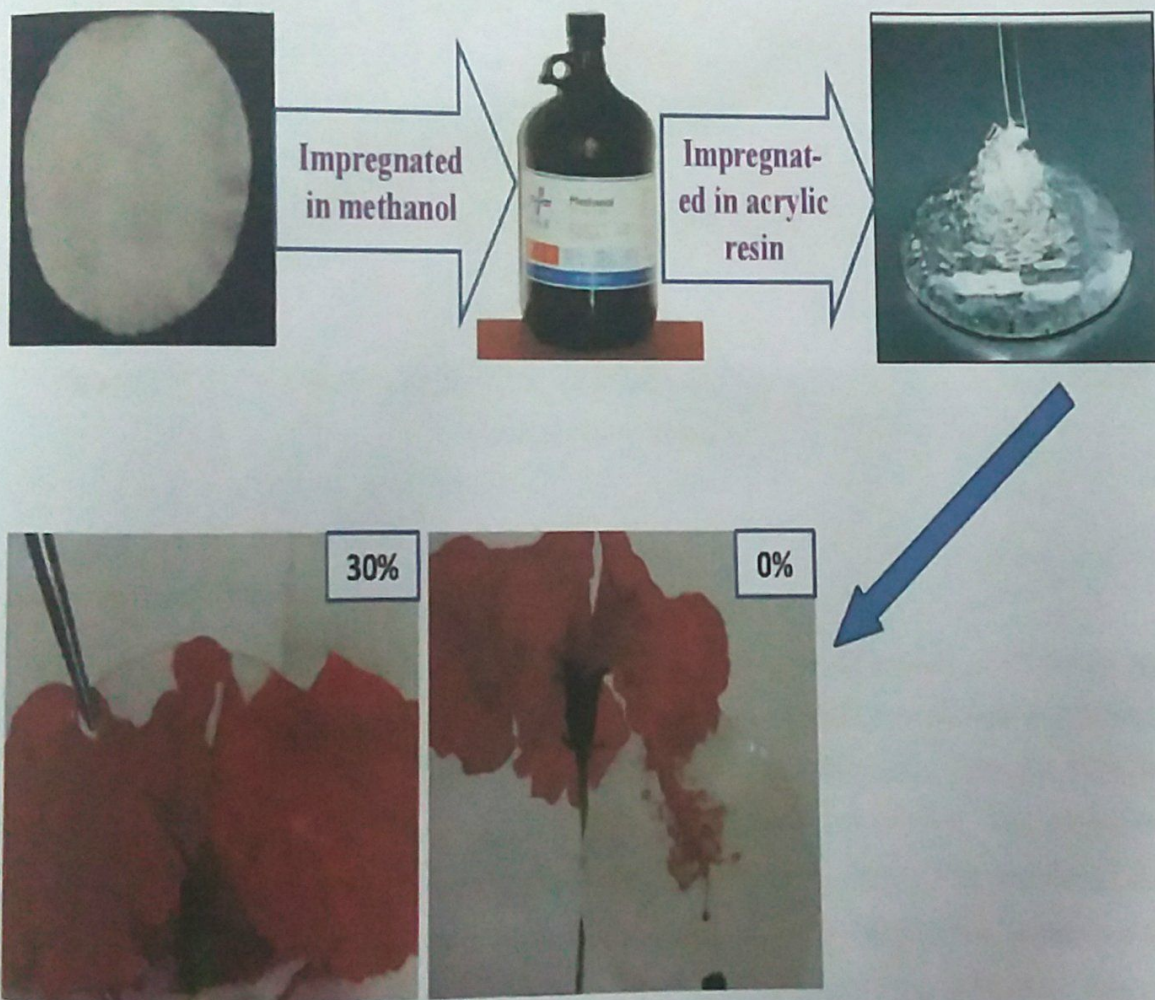

Figure 4.4: Dewatering time of nanosheet at different NaOH concentration treatment.

4.5 Characterization of chitin chitosan nano-sheet and transparent nanocomposites

The chitin chitosan nanosheets were impregnated to methanol and then into neat acrylic resin matrix to produce transparent chitin chitosan nanocomposites. Figure 4.6 shows the chitin chitosan nanosheets and optically transparent nanocomposites produced by nanofibers reinforced with acrylic resin.
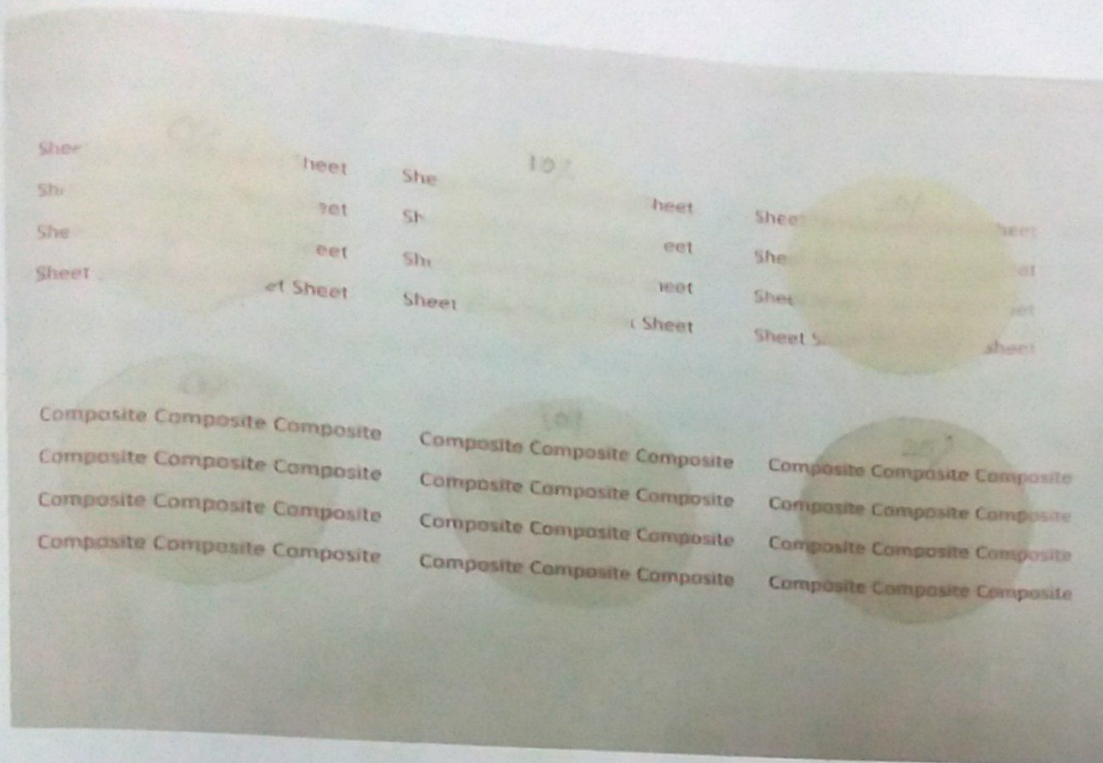
Figure 4.5: chitin chitosan nanosheets and transparent nanocomposites at different NaOH concentration treatment.

4.6 Light transmittance

Figure 4.6 shows the light transmittance of the chitin chitosan nanosheets of different NaOH cocentration treatment and acrylic resin nanocomposites. At a visible wavelength of 600 nm, the nanocomposites were 79.34%, 83.84%, 86.07% and 88.24% for 0%, 10%, 20% and 30% NaOH treatment whereas the light transmittance of pure acrylic resin was 90.5%. The transmittance depends on the diameter of suspended nanofibers (Fukuzumi et al., 2008). The shorter nanofibers show higher transparency. This might be a reason for transparency difference. Again according to Sanal, higher partial deacetylation shows higher transparency. This is another reason for increasing light transmittance with the increasing NaOH treatment percentages. High transparency indicates that individualization of α chitin fibrils at a high level was achieved and this happened due to introduction of cationic charges in high density on the crystalline fibril

surfaces (Fan et al., 2008). Low fiber content is advantageous to maintain high transparency (Lucian et al., 2008).

An important parameter for transparency of two closely associated materials is the matched refractive index (RI) (Biswas *et al.*, 2013). The evenness of the nanofibers diameter (20–30 nm) which is much less than the wavelengths of visible spectra (400–800 nm) made it possible to fabricate optically transparent nanocomposite with low sensitivity to RI of the plastic matrix (Shams et al., 2011). That is why the light transparency was much closer to the light transparency of acrylic resin.
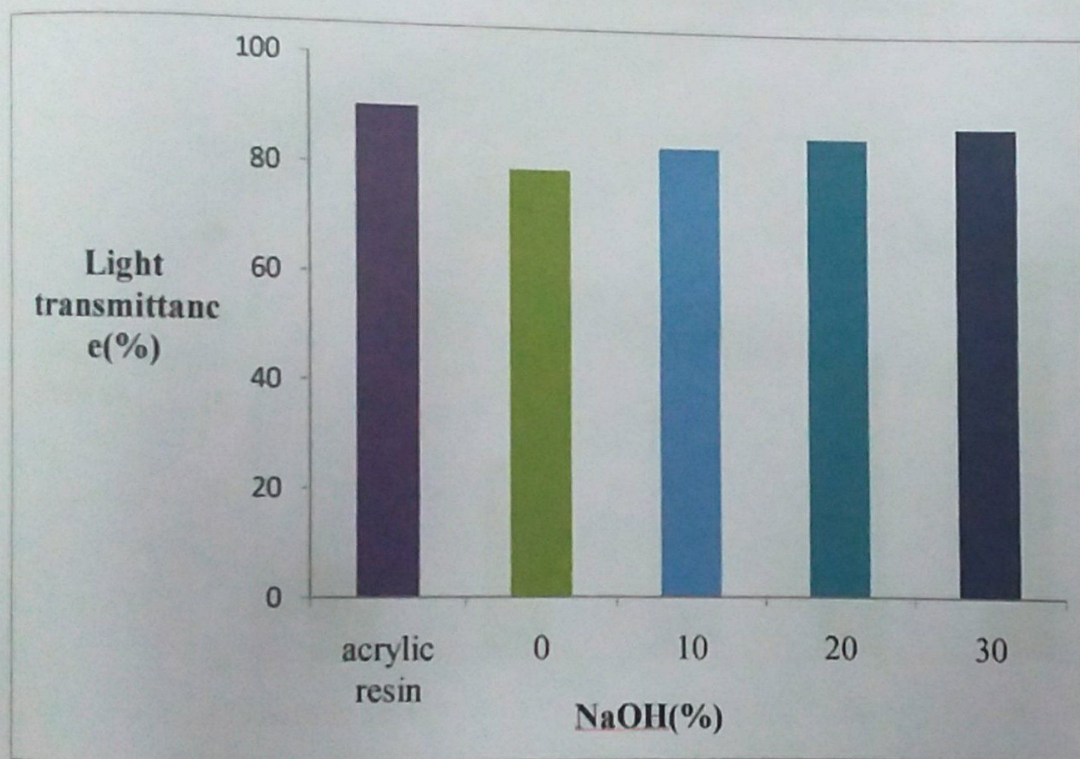

Figure 4.6: Light transmittance of nanosheets of different NaOH concentration treatment.

4.7 Tensile strength

Figure 4.7 shows the tensile strength of chitin chitosan nanocomposites in different NaOH concentration treatment where the tensile strength is increased with enhanced NaOH concentration treatment. As for example 30% showed 2.2 Gpa Young's modulus which is much higher than acrylic resins. This may happen due to enhanced homogenous fibrillation with the enhanced partial deacetylation rate. The best mechanical properties in previous studies were from highly deacetylated chitin nanowhiskers/nanofibers (Fan et al., 2012; Ifuku et al., 2013), where the strongly bound protein was completely removed. Higher protein content is associated with lower degree of nanofiber dispersion, larger agglomerates and lower strain to failure. That is why this study showed tensile strength increases with enhanced NaOH concentration treatment.
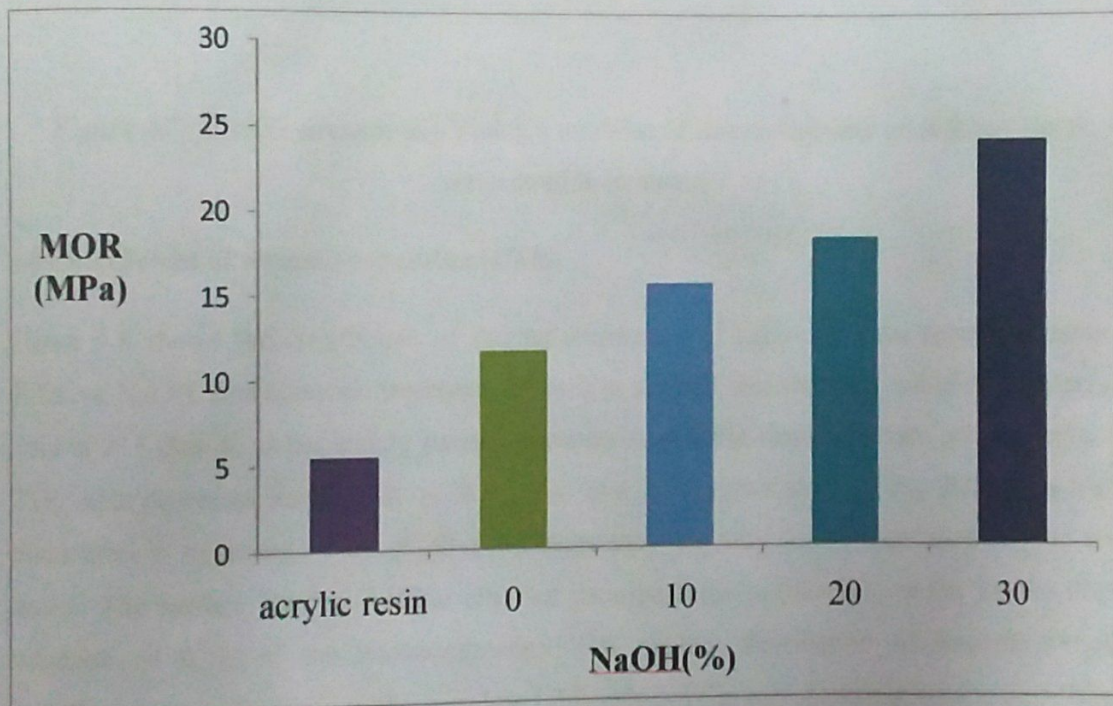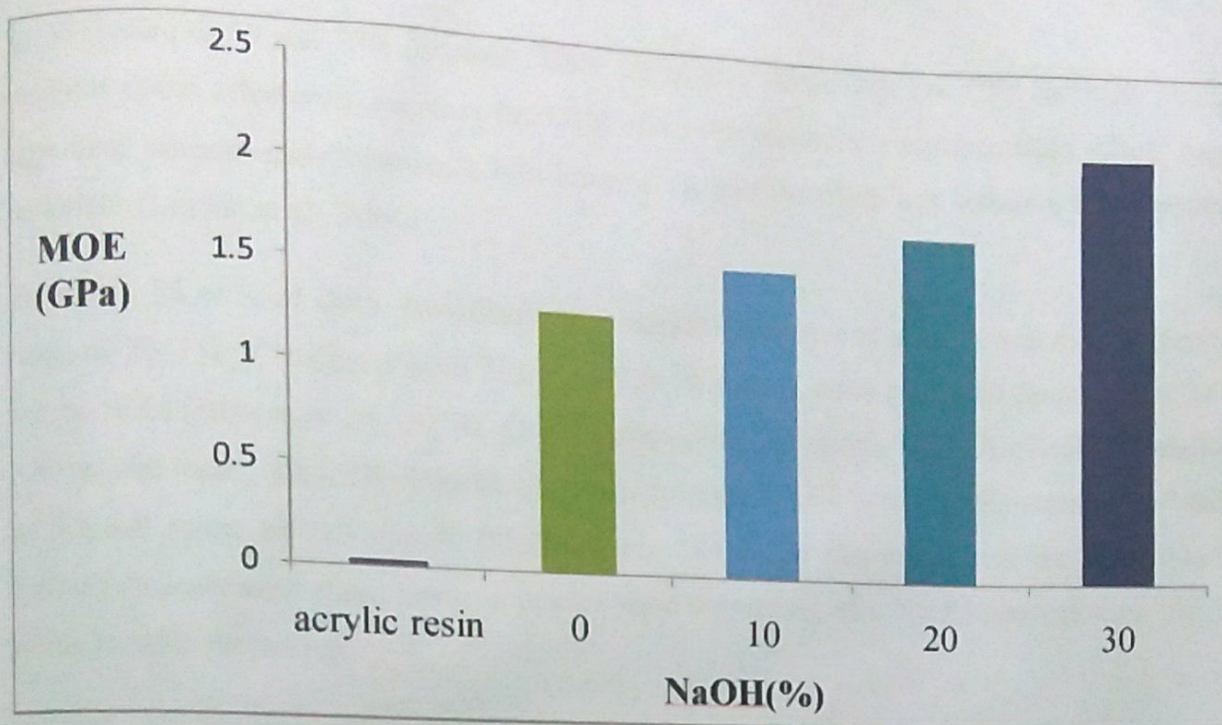
Figure 4.7: Tensile strength and Young's modulus of nanocomposites at different NaOH concentration treatment.

4.8 Co-efficient of thermal expansion (CTE)

Figure 4.8 shows the co-efficient of thermal expansion of chitin chitosan nanocomposites at different NaOH concentration treatment. Here it is showed that the CTE value of pure acrylic resin is 213 ppm/K. After adding partially deacetylated chitin nanofibers into acrylic resin, the CTE value decreases surprisingly at 38.1, 29.9, 24.4, 14.6 ppm/k at 0%, 10%, 20%, 30% NaOH concentration treatment. CTE of all nanocomposites are very lower than pure acrylic resin matrix. The surface fibrillated chitin chitosan nano-particles is the main reason for the drastic reduction of CTE of the nanocomposites. The random distribution of in-plane oriented nanofibers apparently leads to the very low CTE value of the corresponding composites (Shams et al., 2013). Generally thermal expansion has an inverse relationship with Young's modulus (Ifuku et al., 2012). Since the Young's modulus of chitin crystal is estimated to be at least 150 GPa (Vincent et al., 2005), the coefficient of thermal expansion (CTE) of chitin nanofiber sheet is only 10 ppm K-1. Although the CTEs of A-600 and DCP were very high (184 and 100 ppm K-1), the CTEs of their nanocompsites decreased drastically to 19 and 24 ppm K-1, which

corresponded to 90 and 76% decrease. Thus, chitin nanofibers with low CTE and high Young's modulus could effectively decrease the CTE of acrylic resins by a reinforcement effect. Again, high fiber content nanocomposites had lower CTE and therefore less sensitive to temperature variations (Lucian et al., 2008).

By adding 26 wt% of chitin nanofibers, the thermal expansion of acrylic resin at a temperature range of 20–150 ^{0}C reduced from 213 ppm/K to 20 ppm/K more than 1/10 decrease in CTE of acrylic resin (Biswas *et al.*, 2013). On the other hand, by adding 40 wt% of chitin nanofibers with acrylic resin , the CTE of the acrylic resin dropped to 15.6 ppm/K , approximately 1/14th of its original value of 213 ppm/K (Shams et al., 2011) . In this experiment the CTE value of partially deacetylated chitin nanocomposites were decreasing with NaOH concentration increase which is more promising.
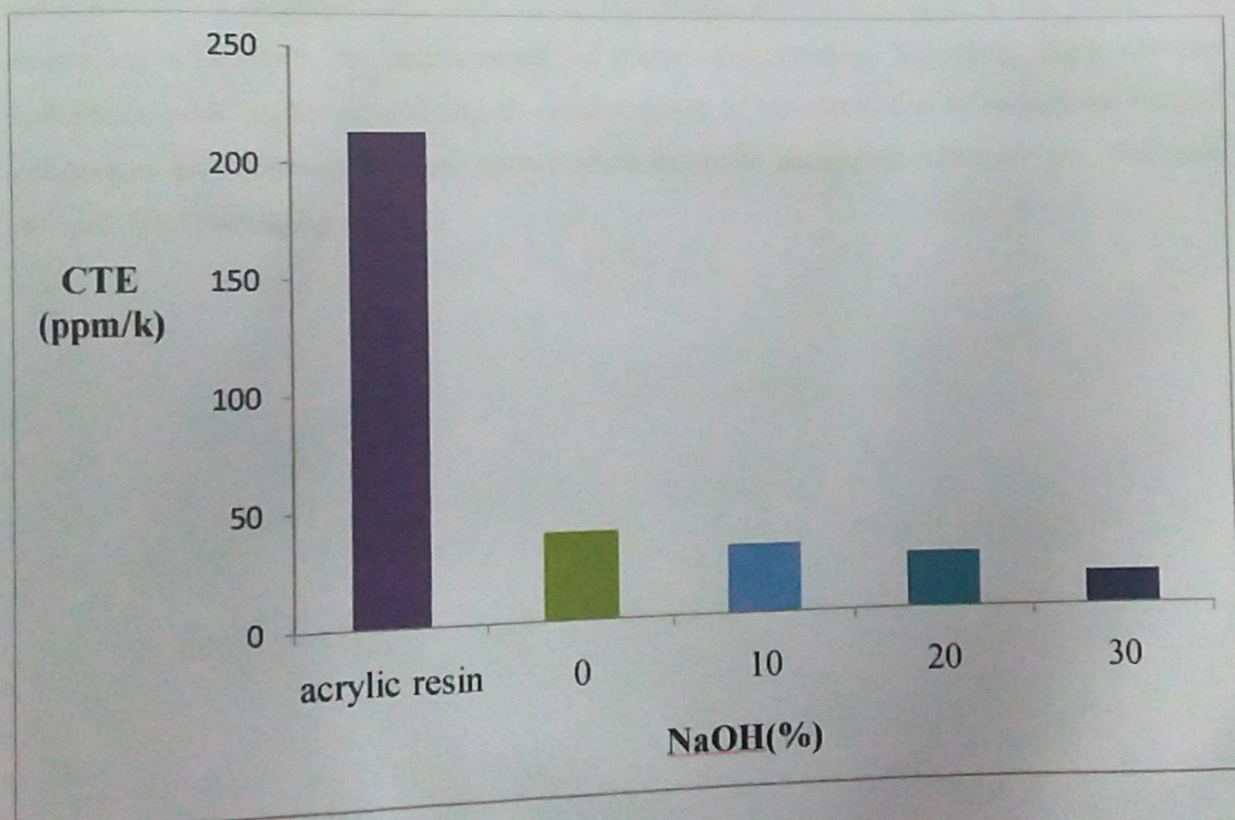

Figure 4.8: CTE of nanocomposites of different NaOH concentration treatment

CHAPTER FIVE

Conclusion

Conclusion

Chitin chitosan nanofibers have been successfully extracted in this study from shrimp shells by using simple mechanical technique in different NaOH concentration treatment. The obtained nanofibers are more homogenously dispersed with the enhancing NaOH concentration treatment. Protonation of the $C2-NH_2$ groups in the chitin chitosan is the key driving force for the individualization of nanofibers. This causes increasing light transmittance, tensile strength and decreasing CTE with the enhancement of partial deacetylation. Therefore, chitin chitosan nanofibers offer higher potentiality as reinforcement in the fabrication of transparent flexible composites and demonstrate new opportunities to create transparent substrates like electronic devices, food packaging material.

Reference

Abe, K., Iwamoto, S., and Yano, H. 2007. Obtaining cellulose nanofibers with a uniform width of 15 nm from wood. *Biomacromolecules*, 8(10), 3276-3278.

Aranaz, I., Mengíbar, M., Harris, R., Paños, I., Miralles, B., Acosta, N., and Heras, Á. 2009. Functional characterization of chitin and chitosan. *Current Chemical Biology*, 3(2), 203-230.

Biswas, S. K., Das, A. K., Yano, H., and Shams, M. 2013. Development of high performance transparent nanocomposites reinforced with nanofibrillated chitin extracted from shrimp wastes. *Journal of Chitin and Chitosan Science*, 1(2), 138-143.

Biswas, S. K., Shams, M. I., Das, A. K., Islam, M. N., and Nazhad, M. M. 2015. Flexible and transparent chitin/acrylic nanocomposite films with high mechanical strength. *Fibers and Polymers*, 16(4), 774-781.

Chen, P., Lin, A.Y., Mckittrick, J., Meyers, M.A. 2008. Structure and mechanical properties of crab exoskeletons. *Acta Biomater.*, 4, 587–596.

Dutta, A.K., Yamada, K., Izawa, H., Morimoto, M., Saimoto, H., Ifuku, S. 2013. Preparation of chitin nanofibers from dry chitin powder by Star Burst system: Dependence on number of passes. *J. Chitin Chitosan Sci.*, 1, 59–64.

Dutta, A.K., Kawamoto, N., Sugino, G., Izawa, H., Morimoto, M., Saimoto, H., Ifuku, S. 2013. Simple preparation of chitosan nanofibers from dry chitosan powder by the Star Burst system. *Carbohydr. Polym.*, 97, 363–367.

Dutta, A.K., Izawa, H., Morimoto, M., Saimoto, H., Ifuku, S. 2013. Simple preparation of chitin nanofibers from dry squid pen β-chitin powder by the star burst system. *J. Chitin Chitosan Sci.*, 1, 186–191.

Fan, Y., Saito, T., Isogai, A. 2008. Preparation of chitin nanofibers from squid pen β-chitin by simple mechanical treatment under acid conditions. *Biomacromolecules*, 9(7), 1919-1923.

Fan, Y., Saito, T., Isogai, A. 2010. Individual chitin nano-whiskers prepared from partially deacetylated α-chitin by fibril surface cationization. *Carbohydr. Polym*, 79, 1046–1051.

Fukuzumi, H., Saito, T., Iwata, T., Kumamoto, Y., & Isogai, A. 2008. Transparentand high gas barrier films of cellulose nanofibers prepared by TEMPO-mediatedoxidation. Biomacromolecules, 10(1), 162–165.

Howlider, M. A. R. 1999. The Availability of feed and its importance of poultry production. *In International Poultry Show and Seminar, April* (pp. 24-26).

Ifuku, S., Nogi, M., Abe, K., Handa, K., Nakatsubo, F., and Yano, H. 2007. Surface modification of bacterial cellulose nanofibers for property enhancement of optically transparent composites: dependence on acetyl-group DS. *Biomacromolecules*, 8(6), 1973-1978.

Ifuku, S., Nogi, M., Abe, K., Yoshioka, M., Morimoto, M., Saimoto, H., Yano, H. 2009.Preparation of chitin nanofibers with a uniform width as *a*-chitin from crab shells. *Biomacromolecules* 10, 1584–1588.

Ifuku, S., Nomura, R., Morimoto, M., Saimoto, H. 2011. Preparation of chitin nanofibers from mushrooms. *Materials*, 4, 1417–1425.

Ifuku, S., Nogi, M., Abe, K., Yoshioka, M., Morimoto, M., Saimoto, H., Yano, H. 2011. Simple preparation method of chitin nanofibers with a uniform width of 10–20 nm from prawn shell under neutral conditions. *Carbohydr. Polym.* 84, 762–764.

Ifuku, S., Morooka, S., Morimoto, M., Saimoto, H. 2010. Acetylation of chitin nanofibers and their transparent nanocomposite films. *Biomacromolecules*, 11, 1326–1330.

Ifuku, S., Nakata, K., Ikuta, A., Oba, T., Shervani, Z., Dutta, A.K., Izawa, H., Morimoto, M., Saimoto, H. 2013. Preparation of chitin nanofiber-reinforced double-decker-shaped polysilsesquioxane film. *J. Chitin Chitosan Sci. 1*, 1–5.

Ifuku, S., Morooka, S., Nakagaitom A. N., Morimoto, M., and Saimoto, H. 2011. Preparation and characterization of optically transparent chitin nanofiber/(meth)acrylic resin composites. *Green Chemistry*, 13: 1708-1711.

Ifuku, S., Ikuta, A., Egusa, M., Kaminaka, H., Izawa, H., Morimoto, M., Saimoto, H. Preparation of high-strength transparent chitosan film reinforced with surface-deacetylated chitin nanofibers.*Carbohydr. Polym.* **2013**, *98*, 1198–1202.

Je, J, Kim, S. **2005** Water soluble chitosan derivatives as a BACE1 inhibitor. Bioorg. Med. Chem , 13, 6551–6555.

Kose, R., Kondo, T. 2011. Favorable 3D-network formation on chitin nanofibers dispersed in water prepared using aqueous counter collision. *SEN'I GAKKASHI,* 67(4): 91-95.

Kurita, K., Ikeda, H., Yoshida, Y., Shimojoh, M., Harata, M. 2002 Chemoselective protection of the amino groups of chitosan by controlled phthaloylation: Facile preparation of a precursor useful for chemical modifications. *Biomacromolecules, 3*, 1–4.

Lertsutthiwong, P., How, N. C., Chandrkrachang, S., and Stevens, W. F. 2002. Effect of Chemical Treatment on the Characteristics of Shrimp Chitosan. *Journal of Metals, Materials and Minerals,* 12(1), 11-18.

Li, D., Xia, Y. Electrospinning of nanofibers: Reinventing the wheel?. 2004. *Adv. Mater. 16,* 1151–1170.

Min, B., Lee, S.W., Lim, J.N., You, Y., Lee, T.S., Kang, P.H., Park, W.H. 2004.Chitinand chitosan nanofibers: Electrospinning of chitin and deacetylation of chitinnanofibers. *Polymer* , *45*, 7137–7142.

Muzzarelli, R. A. (1985). Determination of the degree of acetylation of chitosans by first derivative ultraviolet spectrophotometry. *Carbohydrate Polymers,* 5(6), 461-472.

Muzzarelli, R. A. A. 2009. Chitins and chitosans for the repair of wounded skin, nerve, cartilage and bone. *Carbohydr. Polym.*76: 167.

Muzzarelli, R.A.A. Chitin nanostructures in living organisms. 2011. In *Chitin Formation and Diagenesis*; Gupta, S.N., Ed.; Springer: Dordrecht, The Netherlands, Volume 34, pp. 1–34.

Muzzarelli, R.A.A. Nanochitins and nanochitosans, paving the way to eco-friendly and energy-saving exploitation of marine resources. In *Polymer Science: A Comprehensive Reference*; Maty-jaszewski, K., Möller, M., Eds.; Elsevier: Amsterdam, The Netherlands, 2012; Volume 10, pp. 153–164.

Nishino, T., Takano, K., Nakamae, K. 1995. Elastic-modulus of the crystalline regions of cellulose polymorphs. *J. Polym. Sci., Part B: Polym. Phys.*, 33, 1647-1651.

Nishino, T., Matsuda, I., Hirano, K. 2004. All cellulose composite. *Macromolecules*, 37, 7683-7687.

Ngesa E. M., Nuria B., Michaela S., Qi Z.,b, Lars A. B. 2014. Nanostructured membranes based on native chitin nanofibersprepared by mild process. *Science direct.* pp. 255-263.

Nogi, M., Ifuku, S., Abe, K., Handa, K., Nakagaito, A. N., Yano, H. 2006. Fiber-content dependency of the optical transparency and thermal expansion of bacterial nanofiber reinforced composites.

Peter, M.G. 1995. Applications and environmental aspects of chitin and chitosan. *J. Macromol. Sci. A Pure Appl. Chem.*, 32, 629–640.

Shakeel A., and Saiqa I. 2015. Chitosan & its derivatives: a review in recent innovations *IJPSR*, 2015; Vol. 6(1): 14-30.

Shams, M. I., Ifuku, S., Nogi, M., Oku, T., and Yano, H. 2011. Fabrication of optically transparent chitin nanocomposites. *Applied Physics A*, 102(2), 325-331.

Shams, M. I., Yano, H. 2013. Simplified fabrication of optically transparent composites reinforced with nanostructured chitin. *Journal of Polymers and the Environment*, 21(4), 937-943.

Shams, M. I., and Yano, H. 2015. Doubly curved nanofiber-reinforced optically transparent composites. *Scientific reports, 5.*

Shaofang L., Jie S., Lina Y., Chushu Z., Jie B., Feng Z., Mingjing Q., Chen J., Qingli Y. 2012. Extraction and Characterization of Chitin from the Beetle *Holotrichia parallela* Motschulsky. *Molecules, 17,* 4604-4611

Shimahara, K., Takiguchi, Y. 1998. Preparation of crustacean chitin. In *Methods in Enzymology*; Wood, W.A., Kellogg, S.T., Eds.; Academic Press: San Diego, CA, USA, Volume 6, pp. 417–423.

Subir, K. B., Atanu, K. D., Yano, H., and Shams, M. I. 2013. Development of High Performance Transparent Nanocomposites Reinforced with Nanofibrillated Chitin Extracted from Shrimp Wastes. *J. Chitin Chitosan Sci,* 1: 1–6.

Vincent, J., Wegst, U. 2004. Design and mechanical properties of insect cuticle. *Arthropod Struct. Dev.,* 33: 187-199.

Xia, Y., Yang, P., Sun, Y., Wu, Y., Mayers, B., Gates, B., Yin, Y., Kim, F., Yan, H. 2003.One-dimensional nanostructures: Synthesis, characterization, and applications. *Adv. Mater., 15:* 353–389.

Yano, H., Sugiyama, J., Nakagaito, A. N., Nogi, M., Matsuura, T., Hikita, M., and Handa, K. 2005. Optically transparent composites reinforced with networks of bacterial nanofibers. *Advanced Materials,* 17: 153-155.