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Cellulose Based Film with Antimicrobial Activities

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Dedication

If the gift is even part of the fulfillment

To the teacher of mankind and the source of science, our Prophet
Muhammad (peace be upon him)

To like the proud father and my dear parents

To my first love my wife

To All love My brothers and sisters

To all family and friends

To my distinguished teachers

To those who paved the way in front of me to reach the height of science

Give this humble effort.

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I thank my dear parents, my wife and my brothers and sisters for their continuous support.

أنا الموقعة أدناه، مقدمة الرسالة التي تحمل العنوان:

Cellulose Based Film with Antimicrobial Activities

أقر بأن ما اشتملت عليه هذه الرسالة إنما هو نتاج جهدي الخاص، باستثناء ما تمت الإشارة إليه، حيث إنَّ ما في هذه الرسالة كاملة، أو أي جزء منها لم يقدم من قَبْلُ لنيل أيِّ درجة أو لقب علمي أو بحث لدى أي مؤسسة تعليمية أو بحثية أخرى.

Declaration

The work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

Student's name:

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Date

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Cellulose Based Film with Antimicrobial Activities**By****Rafat Mohieddin Saleh Raddad****Supervisors****Prof. Othman Hamed****Prof. Shehdeh Jodeh****Abstract**

Cellulose is a widely available natural product and has unlimited number of industrial applications. Various functionalities could be added to the cellulose backbone each serves certain commercial application.

In this work, cellulose extracted from olive industry solid waste was converted to cellulose dialdehyde by oxidation with sodium periodate then reacted with the two fatty amines N-methyl dodecylamine and dodecyl amine, to form cellulose fatty imine which then converted to cellulose fatty amine by reaction with $\text{NaBH}(\text{OAc})_3$ and sodium borohydride. The produced cellulose fatty amine showed plastic behavior.

The structures of the prepared cellulose aldehyde, cellulose fatty amine were investigated by FT-IR and Scanning electronic microscope (SEM). Several solutions of both cellulose fatty amines and cellulose triacetate with various ratios were prepared and casted into films. The films morphologies were investigated by SEM, the SEM images showed excellent distribution of cellulose fatty amine and cellulose acetate with strong bonding.

The produced films were clear and transparent. Various physical properties of the films such as tensile strength, Elongation, softening temperature (T_g) were evaluated.

The results showed that the film could be suitable for food and drink warpin.

Antimicrobial activity of the produced films were evaluated against three types of bacteria two gram negative and one gram positive.

Both polymers showed excellent activities after 24 hr. against gram negative (*klebsiella apneumonia*, *E. coli*), the activity reached about 99 % and medium activity against the gram positive bacteria (*staphylococcus*).

The ability of the film to absorb nitrite ions from water was studied but the film showed no efficiency toward nitrite ion.

Chapter One

Introduction

1.1 Background

Biological pollution is known as a form of pollution that could be visible or invisible organisms such as fungi, bacteria and pathogens present in food eaten by humans, water or air and lead to illness.

Biological pollution occurs when waste water is dumped without chemically treatment into fresh water resources or because of the dumping of household waste water and other waste on the streets without taking into account the hygienic rules that need to be followed in dealing with such waste.

Another way of causing the biological pollution is by dumping dead animals in the open or in the water resources. One way of maintaining a limited number of harmful microorganisms in foods, controlling their growth and increasing the shelf life of food is by adding additives or by coating the food with additives by spraying or dipping.

Several studies have been shown that coating food surface addition with an antimicrobial agent limits the beneficial effects because the concentrations of these reagent will decrease over time due to the migration of antibiotics from the outer surface of the food to the internal body of the food and this allow the microbes to grow and spoil the food [1].

However, there are some disadvantages to the direct addition of antimicrobials (natural, chemical) which as the possibility of an interaction between food and antimicrobial additives and causing the development of toxic materials [2,3,4].

Also, antimicrobial additives could be mixed with initial food formulations to control microbial growth and extend shelf-life. However, this strategy is not always the best, since the protective ability of the antimicrobial agent ceases once it is interacting with the complex food system causing the quality of food to degrade at an increased rate.

Another disadvantage is that, the addition of antimicrobial reagent directly to the food cannot selectively target the food surface where spoilage reactions most likely to be initiated.

One of the most recent strategies used to eliminate microbes and prevent them from reaching the food bulk is the use of film with antimicrobial for packaging. This strategy is based on the release of the antimicrobial with the coating material into the food surface [5,6].

The antimicrobial film has become the focus of academic goal research and industry. Since it is a unique way to prevent the growth of bacteria on the surface of the food while maintaining the quality of food, its vitality and safety. Antimicrobial films could also be used in containers to protect liquid foods from spoilage [7,8].

One more advantage of using anti-microbial films is that, they reduce need of large quantities of antimicrobial agents and preservatives to be added to food [8,9]. Antimicrobial reagents or preservatives are usually applied onto film by mixing, coating or other physical methods.

In these antimicrobial films, the antimicrobial agent migrates slowly from the film to the food surface during the storage process. The trick is to maintain an appropriate and constant concentration of antimicrobial reagent on the surface of the food to prevent the microbial growth and avoid the spread of antimicrobials into the internal parts of the food bulk. The rate of release of an antimicrobial agent is important to maintain food quality and safety.

If the antimicrobial agent rapidly exits from the film to the food surface, the antimicrobial agent migrate from the food surface to the internal parts, which are less susceptible to microbes and this will reduce the effectiveness of the film [4].

On the other hand, if the rate of release of the antimicrobial agent is very slow from the film, the appropriate concentration of antimicrobial capable of killing the contaminants will not be reached and the reactions that spoil the food surface can begin and the food quality and safety can be lost.

The Consumers prefer that, the antimicrobial agents and preservatives are applied to the film used in food packaging in such a way

that only low levels of reagent comes into contact with the food. For this reason, the coating methods could be more effective for these types of applications [4,8].

Films coated with antimicrobial agents suffer from several drawbacks, for instance. If the film gets into contact with moisture, the coating can leak into the food bulk food at an uncontrolled rate.

1.2 Synthetic polymers used in making packaging film

Anti-microbial films are usually made using a natural or petroleum-based material. The film considered very useful in packaging if it has excellent mechanical properties, low permeability values and can be produced at low cost. Examples on these films are shown below.

1.2.1 Polyethylene (PE)

Polyethylene is a polymer made up from the building block ethylene (monomer), with unlimited number of applications like used especially in plastic pipes, kitchen utensils, containers, packaging papers and many others.

There are several types of polyethylene (PE), they are classified based on the density and the branches in the polymer. PE types are divided mainly to low density polyethylene (LDPE), high density polyethylene (HDPE), and linear low-density polyethylene (LLDPE) [10].

The chemical and physical properties of polyethylene are delimited by factors such as crystalline structure, molecular weight, number of monomers (polymer chain length) and density[11]. The repeat unit of the PE is shown in **figure 1.1**.

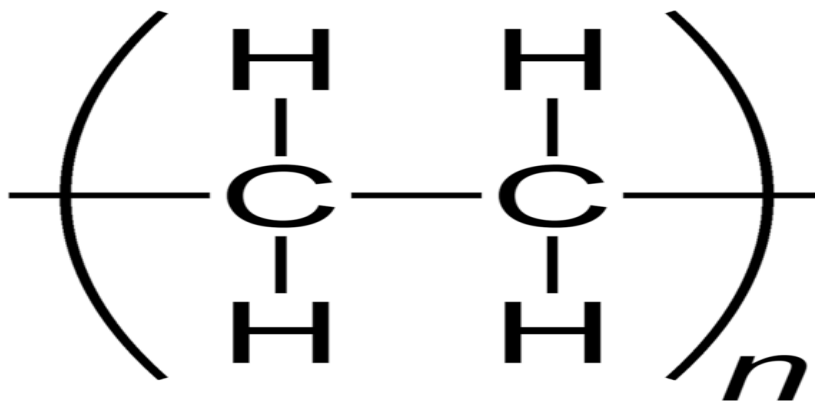


Fig1.1: The repeating unit of poly(ethylene)

An antimicrobial film suitable for food packaging was made from low density polyethylene coated with potassium sorbate (**figure 1. 2**). The presence of this material in PE didn't affect the tensile tension of PE film significantly and the transparency of the film increased as the concentration of potassium sorbate increased [12].

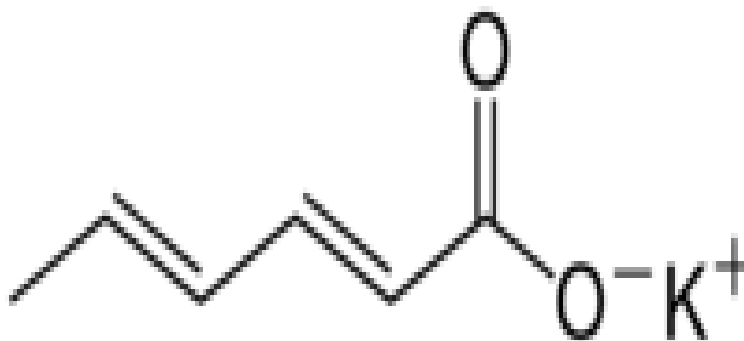


Fig 1.2: Chemical structure of potassium sorbate

1.2.2 Polyvinyl Chloride (PVC)

Polyvinyl Chloride are made from polymerizing the monomer vinyl chloride, the chemical structure of PVC repeat unit is shown in **figure 1. 3**.

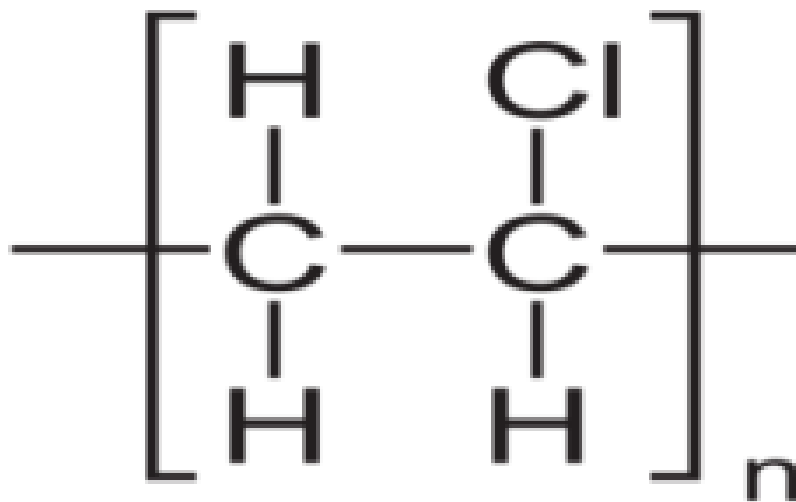


Fig 1.3: The structure of the PVC

Polyvinyl Chloride is a transparent, amorphous plastic material which is one of the most useful polymers in the industry and is widely used in area of packaging films and containers.

Plasticizers are usually added to PVC to obtain a film wide variety of properties, depending on the type and amount of plasticizer used. PVC films are plastic laminated, tacky, rubbery, and commonly used as a packing film for fresh meat and produce Snack food inserts such as biscuits and chocolate are usually made of polyvinyl chloride. Bottles and other containers made form PVC have excellent transparency and good resistance to oil.

The use of polyvinyl chloride in food preservation and packaging is relatively low due to environmental concerns of plastic containing chlorine and poor stability of heat treatment.

There are some concerns about the impact of PVC on the environment and human health, as PVC is unstable in light and heat and may cause a major environmental problem for the difficulty of disposal. So, there is great interest in developing an alternative biodegradable film based on natural polymer [11].

1.3 Natural product-based films

Natural polymers have been used by humans from very early times and there are a very large number of natural polymers, mainly organic natural polymers such as starch, cellulose, lignin, rubber, proteins and nucleic acids. A brief summary on some of these natural polymers is shown below.

1.3.1 Starch

Starch is a natural polymer with two types of carbohydrates amylose and amylopectin. Both are polymer chain of glucose. Starch is usually extracted from grain seeds (corn, wheat, rice). Starches from different sources have similar chemical structure, but their granules are heterogeneous in size and shape. The percentage of amylose polysaccharides and amylopectin is the most important factor in starch

property. The chemical structures of amylose and amylopectin is shown below in **figure 1. 4** and **figure 1. 5**, respectively

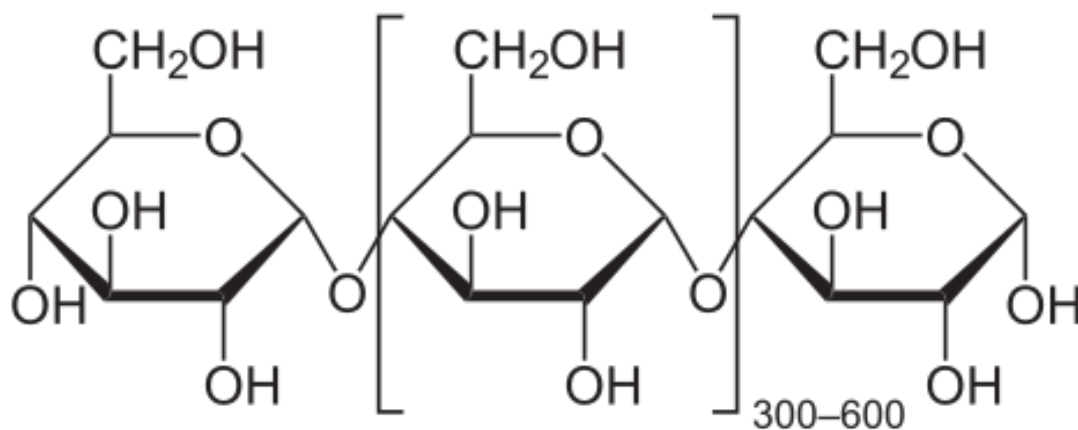


Fig 1.4: Structure of amylose

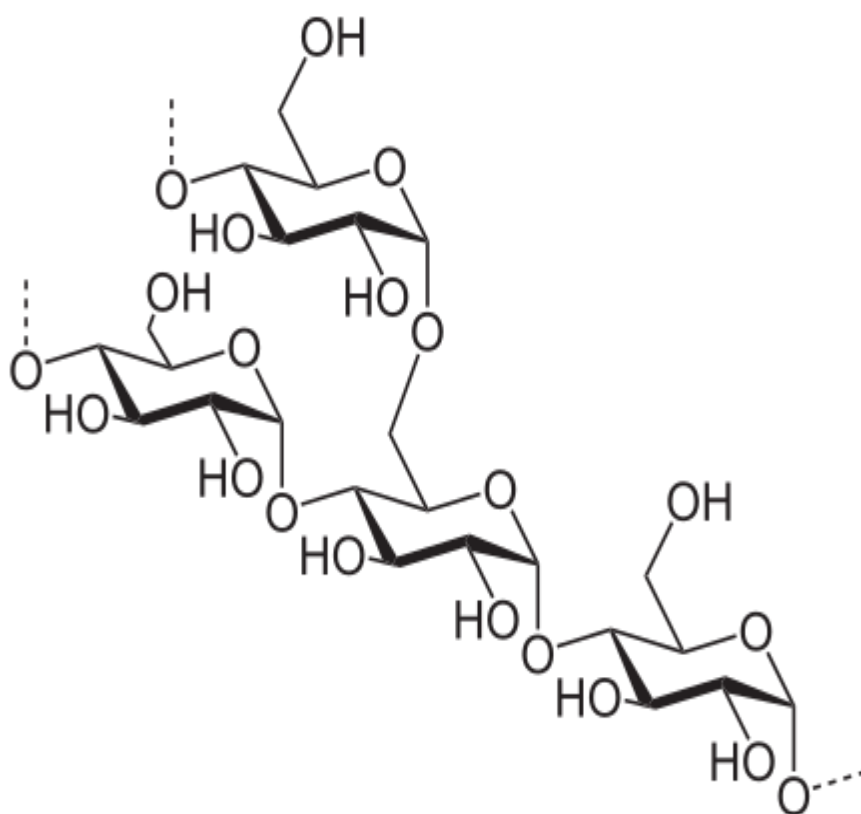


Fig1.5: Structure of amylopectin

Some types of starch used in the manufacture of paper and cardboard, but recently became very useful raw material in the production of films, because of the lack of availability of traditional resins and high prices. Starch is important in producing agricultural films because it degrades into substances that are friendly to the environment[13].

When starch is used in the industry, it is necessary to evaluate its performance using several tests such as the effect of temperature, its ability to absorb or resist moisture, thermal stability and cutting since starch has low stability under pressure and high temperature. At granular levels above 150 ° C, the glucoside bonds begin to break down. At temperatures higher than 250 °C starch grains collapse, and at low temperatures, hydrogen bonds are re-formed [13].

A film composite such as those made from Poly lactic acetate (PLA), poly hydroxyl butyrate (PHB), wheat starch or corn. showed characteristics similar to the synthetic materials but there was a difference in permeability to water vapor, this needs improvement if used for the purpose of packaging the primary food [14].

1.3.2 Protein

Proteins are huge polymeric molecules containing almost 20 different amino acids. The protein is widely used in food packaging, which provides the characteristics of optical and mechanical barrier, as well as a good oxygen barrier, good compatibility with polar surfaces, and ability to control the release of antimicrobial agents when used in food packaging.

So, several researchers are trying to produce protein-based packages that appear in the form of films, coating and health.

With many advantages of proteins as used in packaging materials, but it has problems in resisting moisture as it showed low resistance to moisture [15,16, 17].

1.3.3 Chitosan

A polymer that is easily obtained from the outer structure of insects, as well as from some marine animals such as prawns, lobsters, and snails is known as chitin. Removal of acetate from chitin produces chitosan, it is a biodegradable and biocompatible polymer. Chitosan is used in many areas, including the food industry, biomedicine, pharmacy, agriculture. The repeat unit of chitosan is depicted in **figure 1.6**.

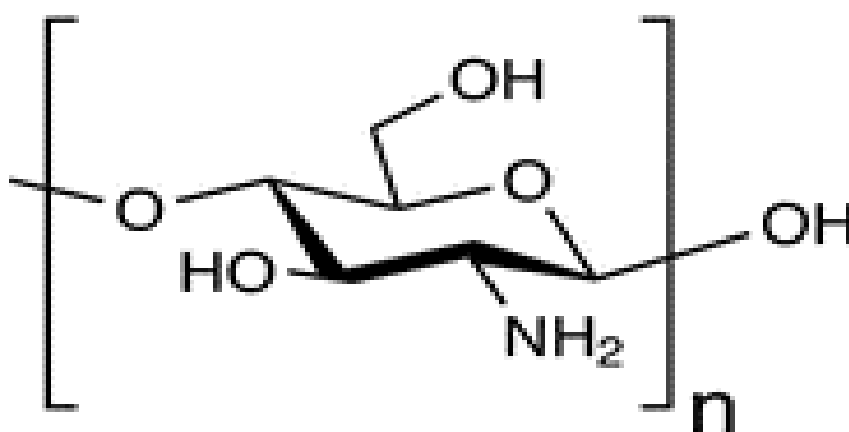


Fig 1.6: structure of chitosan

A film containing chitosan was formed using a poly-lactic acid test (PLA). In general, produced PLA/chitosan films are transparent and have many favorable properties of chitosan. Some films also contain 1% of vitamin E as a biologically active agent, while some contain tributyl or acetyl citrate (ATBC) to reduce amount of PLA, which is used as environmentally friendly biodegradable to enhance flexibility. The ratio of PLA to ATBC was 4: 1 in produced films [18, 19].

Other films, such as polyethylene glycol (PEG) based, have been used to add polymer bonding to the active agent. So that it helps a transfer rate suitable enough to be able to kill the microbes on the surface of the food [4].

Lapol Masterbatch 108 (MB) has been used in many MB bio plasticizer films which is used to increase the persistence of PLA, flexibility, and casting power.

It has been added to improve water-loving, increasing the ability to kill bacteria and is added copolymer to improve conditions of treatment, maintenance or improvement of physical and mechanical properties such as tensile strength and elastic coefficient[20,21].

The antimicrobial properties of chitosan films have been enhanced by the incorporation of a natural antimicrobial agent such as essential oils that have antimicrobial properties [22]. Essential oils contain phenols and terpenoids which are expected to carry anti-microbial properties. There are many oils used in this area and most important are those extracted from

cloves, cumin, cinnamon, ginger, thyme, garlic, they also possess antioxidant properties.

Ginger belongs to the family Zingiberaceae is a substance that is widely used in food where it is added as a spice and used in medicine [22].

The process of adding active agents such as oils directly to food leads to a significant and immediate reduction in the growth of bacteria but for a short period of time, while the films can maintain their activity for a long period of time [4,23].

In spite of the characteristics offered by the films containing the oils, it does not have some disadvantages and most importantly, it requires a high concentration of oils until we reach an active antimicrobial activity. This leads to a negative effect on food odors, taste and flavors [24].

In addition, when working on any film, the study of the cost to benefit ratio is an important factor in the widespread use of antimicrobials, and this industry may need more expense than benefits. [31].

The alternative film is expected to contribute to the solution of environmental pollution and the creation of new markets for natural products. One of the methods for an effective antimicrobial free film mentioned above is a film containing an anti-microbial factor that is covalently linked to the structure of the film. The solution is to use a biodegradable film made of sugars such as cellulose In this work, cellulose-based film will be developed with an ammonium function that is correlated with the cellulose structure will be developed.

1.3.4 Cellulose

1.3.4.1 Cellulose structure

Cellulose is a natural polymer composed of an anhydroglucose repeat unit (**figure 7**) that is connected respectively through β -1,4-glycosidic links in the configuration between C1 and C4 of adjacent units to form a polymer chain

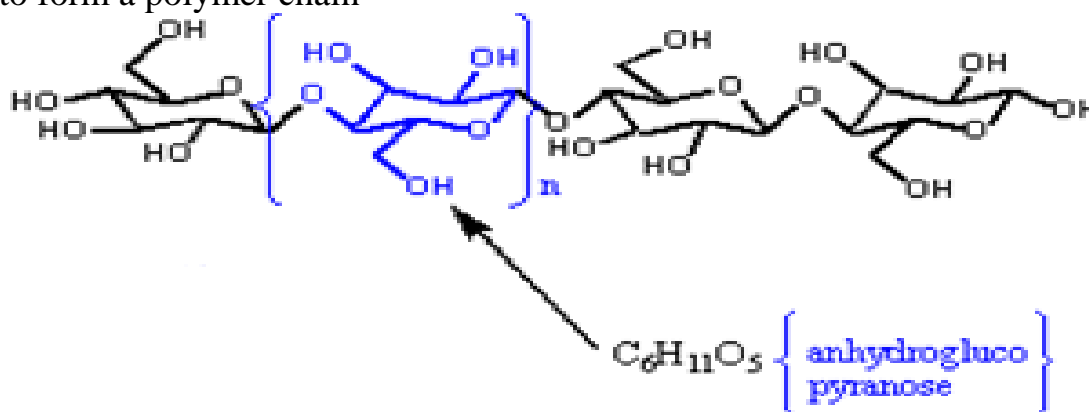


Fig 1.7: structure of cellulose

The cellulose chain consists of about 10,000 monomer units. Some are bundled with bundles called fibers. A group of fibers accumulates together to form cellulose. Cellulose can be obtained in crystalline form or in an amorphous region (less organized region).

As shown in **fig 1.7**. Each anhydroglucopyranose repeat unit possesses three hydroxyl groups two secondary at C2 and C3, and a primary C6 positions. All hydroxyl groups are capable of undergoing the typical reactions known for primary and secondary alcohols.

Cellulose is a very stable polymer and insoluble in water. Cellulose is safe, tasteless, hydrophilic, insoluble in most organic solvents, biodegradable and it is a chiral polymer [26, 27].

The stability of cellulose and its insoluble in water is due to the presence of hydroxyl groups that allow the formation of strong hydrogen bonds with repeat unit of cellulose. There are areas within the cellulose fibers where the cellulose chains are in a high-order structure (the crystalline region) and areas where cellulose chain are disordered (noncrystalline). The properties of cellulose are derived from polymerization of glucose molecules that make up the polymer [28,29].

1.3.4.2 Sources of cellulose

Cellulose is the most abundant polymer in nature and can be extracted from many sources such as: Bushes: 30%, Woods: 40–50%, Bast Plants (flax): 65–70%, Cotton Fibers: >90%

Table 1.1: Degree of polymerization of cellulose from various sources

Source	Degree of polymerization
Acetobacter xylinum	2,000-3,700
Acetobactercellulose	600
Bacterial cellulose	2700
Bagasse	700-900
Bast fibers	1,000-5,000
Cotton fibers	8,000-14,000
Cotton linters	1,000-6,500
Flax fibers	7,000-8,000
Ramie fibers	9,000-11,000
Pulp cellulose (bleached)	5,00-2,100
Wood fibers	8,000-9,000
Valonia	25,000-27,000

1.3.4.2.1 Wood

Wood is a cellular fibrous material consisting mainly of cellulose, hemicellulose, and lignin.

Cellulose is a plasmid polymer formed from glucose units, the most organic chemical found in nature, accounting for 40%-50%.

1.3.4.2.2 Plant

Plants are important sources of cellulose because they are highly available.

Normally, plants can be "purified" similarly to wood. The materials have been studied for cellulose sources, including sugar beet pulp, potato tubers, linen, cotton.

1.3.4.2.3 Tunicate

Tunicates a marine animal in the shape of a bottle. It spends its life adhering to a fixed body at the bottom of the ocean. Sea sprayer is a name for a group of marine animals also called zucchini. These animals are known to habitually squirt water through one of the body's openings They are the only animals that produce cellulose and have a mixture of cellulose. When Tunicates reach maturity, they are the source of micro-fiber cellulose.

1.3.4.2.4 Algae

Algae is a group of living organisms capable of capturing light energy through the process of photosynthesis, converting inorganic matter into organic matter in which energy is stored.

Where cellulose is produced by some algae (gray, red, yellow, green) there are significant differences in cellulose due to different source of access, and because of differences in biosynthesis.

1.3.4.2.5 Olive industry waste

Oil production from olive trees is widespread throughout the world. This industry produces a large amount of solid and bulk waste.

About 56% of the liquid waste is produced from the olive industry (OILW), and about 44% is the solid waste of the olive industry (OISW)[30].

The main substance of OISW is cellulose it is about (45-45%). This makes it a cheap source for cellulose extraction. The waste from the olive industry has a very high biological oxygen demand (BOD) and chemical oxygen demand (COD) values, and also contains highly toxic levels of polyphenols[31].

Farmers' perceptions of waste from olive oil factories differ from those of some regions who are concerned with solid waste of the olive industry (OISW) with the same degree of interest in oil, while in some areas they do not care about oil.

Liquid waste produced from the olive industry (OILW), no one gives any attention, but constitutes a burden on the owners of contemporary olive and a concern for the environment because this disposal is a big problem in terms of effort and cost and thus the olive industry may lose the economic value through the disposal of liquid waste or sell at a low price to be used in different industries. In some areas, OISW is usually burned or left to decompose large quantities of carbon dioxide (CO₂) are released into the atmosphere.

In some cases, OILW is disposed of from the sewage sludge, which has an impact on the quality of groundwater.

Therefore, the waste of the olive industry is a big problem faced the industry in the light of increasing environmental standards day by day. In addition, the olive industry loses economic value by disposing of liquid waste or selling it for a low price.

We as researchers face a challenge in transforming these wastes into usable, low-cost, and marketable products. This is one of the objectives of this thesis.

1.3.4.3 cellulose with anti-microbial properties

The killing of microbes is very necessary to maintain food for long period as much as possible. Different pathways have been used to reach this goal. The natural and industrial sources are faced with a number of challenges.

Some work based on killing bacteria using a natural source of cellulose where the researchers worked on assembling the nanoparticles of cellulosic anti-microbes that had the ability to kill certain bacteria.

Black Gram contains bacteria that breaks down carbohydrates, produces lactic acid and other antimicrobial compounds (bacteriocins) that kill selective pathogens.

Scientists from the ICAR Center for Research in Cotton Technology (ICAR-CIRCOT) in Mumbai, India obtained anti bacteria that were extracted from black gram and were coated with cellulose nanoparticles extracted from cotton.

After the tests, it was found that this method stopped the growth of four types of bacteria significantly. *Staphylococcus aureus*, *Erwiniaherbicola*, *Listeria monocytogenes*, and *Escherichia coli*.

Antimicrobial on cellulosic surfaces killed bacteria by penetrating bacterial cells and making pores in the cell membrane. It was found that the anti-properties have the property of thermal stability, where it was able to perform its activity at temperatures of 100 °C for an hour and this is important in the field of food industry, as most food requires heat treatment at high temperatures. Antimicrobial cellulose nanoparticles can also be encapsulated in packaging materials and foodstuffs[32].

In another study, a micro-cellulose network was formed in a three-dimensional structure called bacterial cellulose. Cellulose films were

modified by adding genes during the fixed agglutination of glucocytotackter.

An analysis of bacterial cellulose structure was performed using a combination of techniques (AFM and FTIR). FTIR showed the interaction between the carboxyl group of genes and the hydroxyl group of cellulose. The results showed that the product was highly resistant to bacteria (*Staphylococcus aureus*) and can be used in medicine as a wound dressing[33].

Nanocrystalline cellulose (NCC) is used in many areas, mainly food and medical packaging. Because of its high surface area NCC was modified using N-halamine precursor 1-hydroxymethyl-5,5-dimethylhydantoin (HDH) and cyanuric chloride (CYCH) as the bonding agent followed by chlorination, the produced NCC-cych-HDH-Cl showed antibacterial properties. NCC-cych-HDH-Cl was blended with chitosan (CS) and polyvinyl alcohol (PVA)solution to prepare antibacterial films. The optimal ratio of the components was 90/10 PVA / CS and 7.0% loading of NCC-cych-HDH-Cl. Produced film showed a distinctive tensile strength.

The antibacterial film with 5.91×10^{17} atoms/cm² of active chlorine displayed an excellent antibacterial property against *Staphylococcus aureus* and *Escherichia coli* [34].

Another study showed that, it is possible to use cellulose fiber in water filtration. It removed 99% of the natural water bacteria [35].

1.4: Water pollution

Water pollution is one of the most serious problems faced human being. Over time, the problems of pollution increased for several reasons such as higher demand for water, increased population growth.

Examples on water contaminants are nitrate and nitrite, both ions reached water by the degradation of compounds containing nitrogen from the nature sources, such as soil and rock. Other sources include the excessive use of chemical fertilizers, factories and sewage. Increasing their concentration leads to health and environmental problems. In this work, the film that was prepared will be evaluated in extraction of nitrite from contaminated water by adsorption as well as eliminate the bacteria.

1.5 The aim of this work

The specific objective of this thesis is to synthesize cellulose ammonium polymer containing a fatty alkyl chain with antimicrobial activities. This will be carried out by chemical modification of cellulose extracted from olive industry solid waste. The main use of this polymer will be food packaging, counter top coating and may be also suitable for use in liquid containers.

The sub-objectives are to:

1. Develop a method for adding ammonium salt moiety to cellulose polymer chain.
2. Prepare a film with good tensile strength from the ammonium polymer.
3. Develop a chemical method to oxidize cellulose to dialdehyde polymer.
4. Evaluate the antimicrobial properties of the film.
5. Evaluate the possibility of using the film in food wrapping and coating.
6. transforming OISW wastes into usable, low-cost, and marketable products
7. produces antimicrobial film that is biodegradable and friendly with environment

Chapter Two

Experimental

2.1 Materials and methods

All chemicals were purchased from Aldrich Chemical Company and used without any further purification unless otherwise specified.

The chemicals are: Sodium borohydride, Sodium periodate, Dodecyl amine, N –Methyl dododecyl amine, Methanol, Sodium hydroxide, hydrochloric acid, isopropanol, hydroxylamine hydrochloride. Cellulose used for preparation of target polymers was extracted from olive industry solid waste.

The following instruments were needed to accomplish this research: shaking water bath with Digital Speed Control, pH meter, FT-IR Spectrometer the Fourier transform infrared (FTIR) spectra were recorded on a Nicolet 6700 FTIR spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) equipped with the Smart Split PeatmHemi Micro ATR accessory (International Crystal Laboratories, Garfield, NJ, USA). The following parameters were used: resolution was 4 cm⁻¹, spectral range was 600 cm⁻¹ to 4000 cm⁻¹, and number of scans was 128. The surface morphology of the cellulose polymer and derivatives were examined using scanning electron microscopy (SEM) (S-4800; Hitachi, Tokyo, Japan) at an acceleration voltage of 3.0 kV after sputter coating the sample with gold (Cressington Sputter Coater; Ted Pella, Inc., Redding, CA, USA). The standard thermo gravimetric analysis was performed on samples of CDAm **4** and **6** using the

TGA Q500 and Q50 TA instrument (Newcastle, USA) at a temperature range from 20–900 °C and a heating rate of 10 °C/min.

2.2 Preparation of cellulose amine polymers

2.2.1 Oxidation of cellulose to 2,3-dialdehyde cellulose

A solution of sodium hydroxide (8% by weight) was prepared by dissolving 8.0 g of sodium hydroxide in 92.0 mL distilled water, 6.0 g of cellulose powder was added. The produced mixture was stirred for 20 min, diluted with water, filtered, and washed several times with distilled water. Then, the mixture was neutralized *via* drop wise addition of the diluted solution of acetic acid (2% by weight) and washed again with water. A sample of the produced activated cellulose (5.0 g, 0.031 mol/anhydroglucose repeat unit (AGU)) was added to a beaker followed and suspended in 150.0 mL of distilled water. The flask containing the cellulose suspension was completely wrapped with aluminum foil to isolate it from light. Sodium periodate (12.8 g, 0.06 mol) was added to the cellulose suspension in two portions, at 6 h intervals. The reaction mixture was stirred at 40 °C for approximately 12 h.

Produced cellulose aldehyde was collected *via* filtration and washed with water (3 × 250 mL). Aldehyde content (1.91/AGU) was determined according to a previously reported procedure[36].

2.2.2 Content of Aldehyde in CDA

Cellulose aldehyde (1.00 g) was placed in a flask and add to it 40.0 mL of isopropanol. The mixture was stirred for 10 min, then to it was added 10.0 mL of distilled water. The mixture was mixed well to form a slurry. The pH value of the mixture was adjusted to 3.5 with 10% HCl solution.

A solution of hydroxylamine hydrochloride (5% by weight solution) in water by dissolving 1.0 g hydroxylamine hydrochloride in 20 g water then the pH value was adjusted to 3.5 using 10% HCl solution.

The two solutions were mixed and stirred for 1 hr and titrated with 0.5 N NaOH solution until the pH reached 3.5 and stayed constant.

The aldehyde content was then calculated using the following equation:

$$[\text{Ald}] = V_{\text{NaOH}} \cdot 0.5N_{\text{NaOH}} / \text{Wt}_{\text{CDA}} (\text{g}) \quad \text{Eq : 1}$$

Where [Ald]: aldehyde content in mmol / gram of cellulose.

V_{NaOH} : volume of NaOH (mL) used in the titration.

N: normality of the NaOH (eq/L).

Wt: weight of dry CDA (g) used in titration in (g).

2.2.3 Preparation of cellulose dodecyl amine (CDDA4)

Cellulose aldehyde (10.0 g, 0.06 mmol of anhydroglucose (AGU)) and ethanol (100 mL) were added to a round bottom flask (250-mL) fitted with a magnet stir. Then, dodecyl amine (10.0 g, 54.1 mmol) was added to the suspension. The reaction was refluxed for approximately 3 h. Then it was cooled to room temperature and sodium borohydride (1.5 g) was added to the suspension. The reaction mixture was stirred at room temperature for 8 h. It was then treated with a solution of ammonium chloride (0.5%) to destroy excess sodium borohydride. The produced cellulose amine polymer was collected through suction filtration and first washed with water, then ethanol, and then air dried.

2.2.4 Preparation of cellulose N-methyl dodecylamine (CNMDDA6)

Cellulose aldehyde (10.0 g, 60.0 mmol of AGU) and THF (100 mL) were added to a round bottom flask (250-mL) fitted with a magnet stir. Then, N-methyl dodecylamine (10.0 g, 59.0 mmol) was added to the cellulose aldehyde solution. The reaction was refluxed for approximately 3 h. Then it was cooled to room temperature and Sodium triacetoxyborohydride

(1.5 g) was added to the solution. Triacetoxyborohydride solution was prepared as shown below. The reaction mixture was stirred at room temperature for 96 h. It was then treated with a solution of ammonium chloride (0.5%) to destroy excess triacetoxyborohydride. The produced

cellulose amine polymer was collected through suction filtration and first washed with water, then ethanol, and then air dried.

2.2.4.1 Preparation of triacetoxyborohydride

Acetic acid (9.7 g, 162 mmol) was placed in a round bottom flask (100 mL). The flask and contents were placed in an ice water bath, to it was added in portion over a period of 10 min sodium borohydride (2.0 g 54.0 mmol). After the addition was completed, the flask was heated slowly over a period of 1.0 hr. Then diluted with 20.0 ml THF and added to the cellulose imine solution as shown above [37].

2.3 Antimicrobial activity

The test was carried out as follows: The tested films were cut into circular slide with a diameter of 4.8 (± 0.1) cm. Circles samples were placed in sterile empty specimen cups and inoculated with a 1.0 mL suspension of test organism, allowing the inoculums to completely cover the sample surface. Each sample was incubated for a specific contact time (2 hr), then transferred to a 10 ml volume of neutralizing solution and vigorously shaken by vortex. The resulting suspension was inoculated to media supportive of bacterial growth and incubated at 36 ± 2 °C for 18-24 hours and the colonies of surviving bacteria were counted. At each designated time point, the process was repeated. The test was performed on three organisms *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli* (E-Coli).

$$\% \text{Reduction} = \frac{\text{Control (cfu/mL@T=0)} - (\text{Sample cfu/mL@T = time point})}{\text{Control (cfu/mL@T=0)}} \times 100 \quad \text{Eq: 2}$$

2.4 Nitrite Adsorption Experiments

2.4.1 Preparation standardization curve

A 1000ppm solution of sodium nitrite (69.0 g/mol) was prepared by dissolving 1.5 g of sodium nitrite in 100 ml distilled water and diluted to 1000.00 mL scale in a volumetric flask from this solution, solutions with various concentrations (5- 20 ppm) were prepared by dilution these concentrations and were used for standardization curve and the absorption measurement by UV at wavelength 210 nm.

2.4.2 Effect of contact time

The effect of contact time on adsorption was studied by adding 50 mL from 30 ppm NaNO_2 to conical flask contain, a 100 mg CDA was added to the nitrite solution, mixed for a period of time. The concentration of nitrite ion was measured by UV, no change on the concentration was noticed. The experiment was carried out at 10, 20, 30 and 60 min.

2.4.3 Effect of Adsorbent Dosage

Different amount of CDA (5 to 100 mg) was added to conical flasks containing each one a 100 ppm NaNO_2 mixed at room temperature for 60

min and absorption was measured. No change on concentration of nitrite was noticed.

2.4.4 Effect of Initial pH

The effect of pH was studied within 2.0-12.0 pH values, which adjusted using diluted solutions of sodium hydroxide and hydrochloric acid. Several mixtures of 100.0 mg of CDA and 50 mL of 30 ppm NaNO_2 were prepared and the mixtures were shaken for approximately 1 hour at room temperature. The absorption was measured using UV.

2.4.5 Effect of Temperature

The effect of temperature was studied within 10-50 °C at pH 4.5. Several mixtures of 100 mg of CDA and 50 mL of 30 ppm NaNO_2 were prepared and the mixtures were shaken for approximately 1 hour at various temperature as shown above. The degree of absorption was measured. No change on the concentration of nitrite ion.

Chapter Three

Results and Discussion

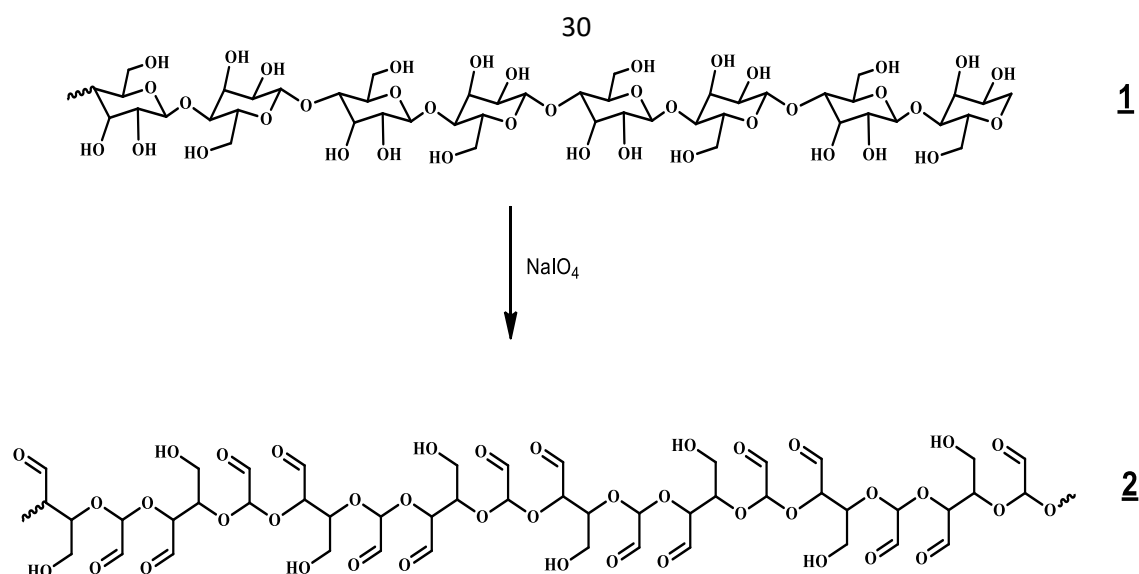
Cellulose used in this work was extracted from olive industry solid waste known in Palestine as **jeft** according to the procedure reported in the literature [Hamed et al., 2012, 2014]. The extracted cellulose was oxidized to cellulose aldehyde(CA) by reacting it with sodium periodate as shown in **scheme 3.1**. The oxidation was carried out according to a published procedure [36]. The progress of oxidation of cellulose was followed by UV spectroscopy. At certain period, a sample of the reaction mixture was withdrawn and its absorbency at 290.5 nm was measured for monitoring the concentration of periodate, when the peak intensity remained constant the reaction was stopped.

3.1 Oxidation of Cellulose to 2,3- dialdehyde Cellulose

Oxidation of cellulose with sodium periodate was carried out according to a method reported by Jackson and Hudson with some modifications [38,39]

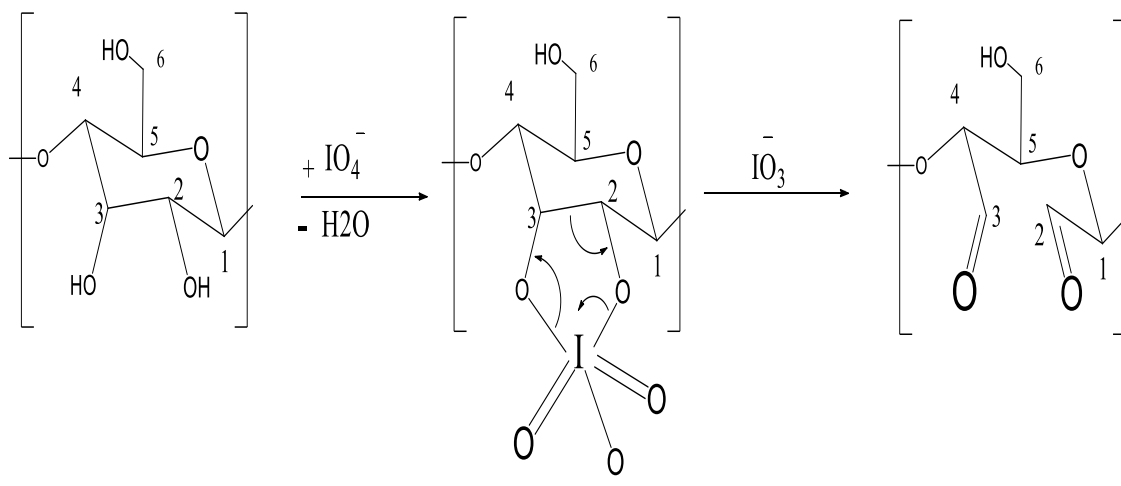
In this method cellulose was treated with an aqueous solution of periodate in a system that was isolated from light.

Periodate complexes to the vicinal hydroxyl group at C2 and C3 as shown in **scheme3.2** which leads to cleavage of C2-C3 bond and oxidation of the vicinal hydroxyl groups and formation of 2,3-dialdehyde units along the cellulose chains as shown in **Scheme3.1**



Scheme 3.1: Oxidation of cellulose with sodium periodate

Firstly the oxidation starts at the accessible site of the cellulose structure (amorphous regions). The oxidation of the amorphous region and the introduction of the dialdehyde reduces the crystallinity [40,41].



Scheme 3.2: Mechanism for oxidation of cellulose to dialdehyde

3.1.1 Determination of aldehyde content

The percentage of aldehyde in the oxidized cellulose was determined according to a published method [42]. The principle of this method is to

react cellulose aldehyde with hydroxylamine-hydrochloride and convert it to oxime. The hydrochloric acid produced from the reaction was titration with a solution of a known concentration sodium hydroxide. According to the calculations, the degree of substitution was 1.91/unit of anhydroglucose.

3.1.2 FT-IR cellulose dialdehyde

The FT-IR spectrum of CDA (2) is shown in **figure 3.1**. The most significant peaks for CDA were observed at 1714, 1636, 1425 and 1026 cm^{-1} assigned to the typical C=O (aldehyde), cellulose (glycosidic linkage) and C-H of alkyl moiety inserted in the cellulose structure, respectively. The peak at 1026 could be attributed to C-O stretching (ether). The band at 1714 cm^{-1} is corresponding to an aldehyde carbonyl group. The weak band at 1714 cm^{-1} is due to the hydration and acetal formation [43].

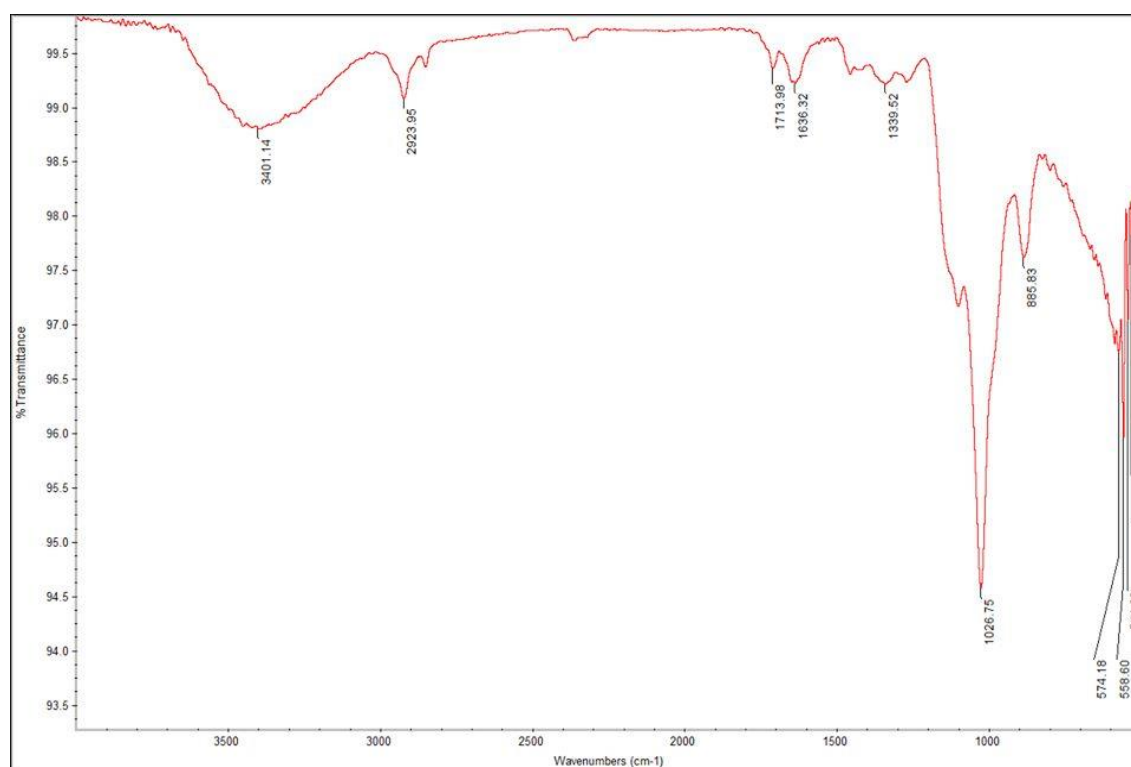


Fig 3.1: FT-IR of cellulose aldehyde (2)

3.1.3 SEM Images of cellulose dialdehyde (2)

The cellulose aldehyde (2) image showed that the cellulose still in fiber form, but the cell structure was damaged.

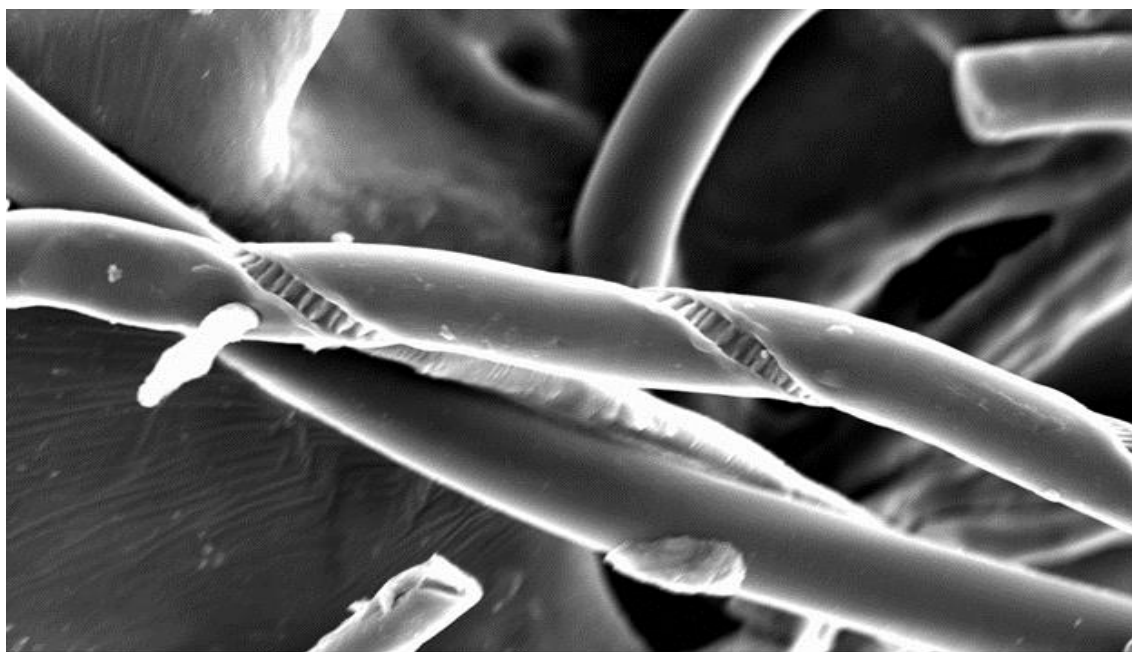


Fig 3.2: SEM image of cellulose dialdehyde at 250x

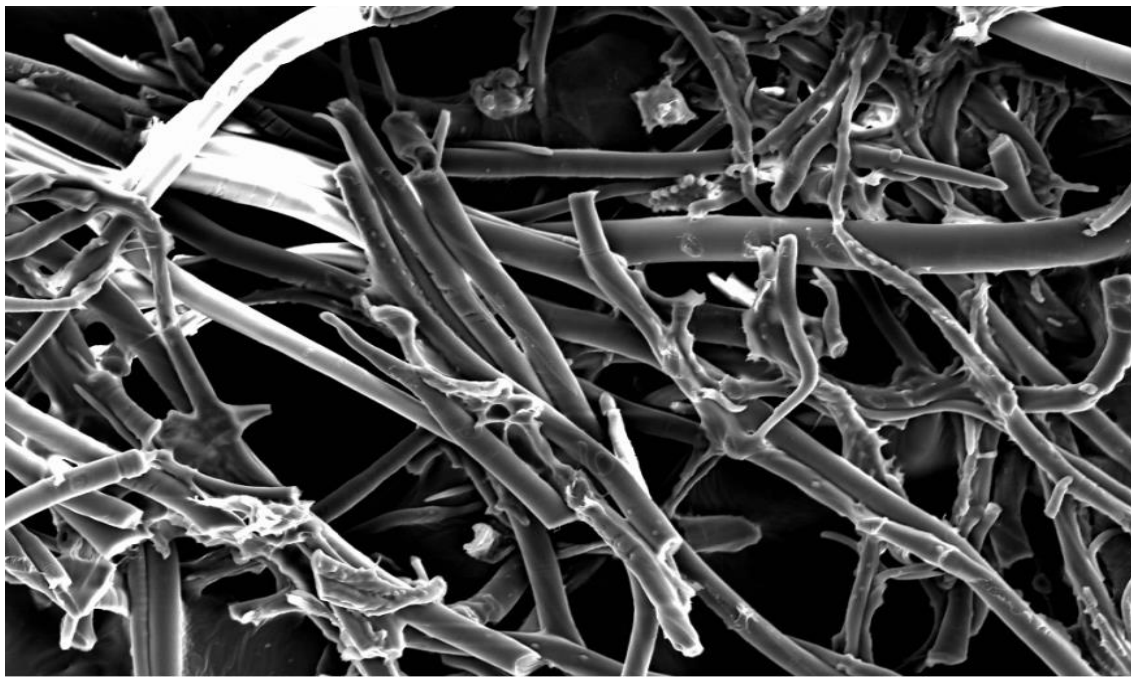
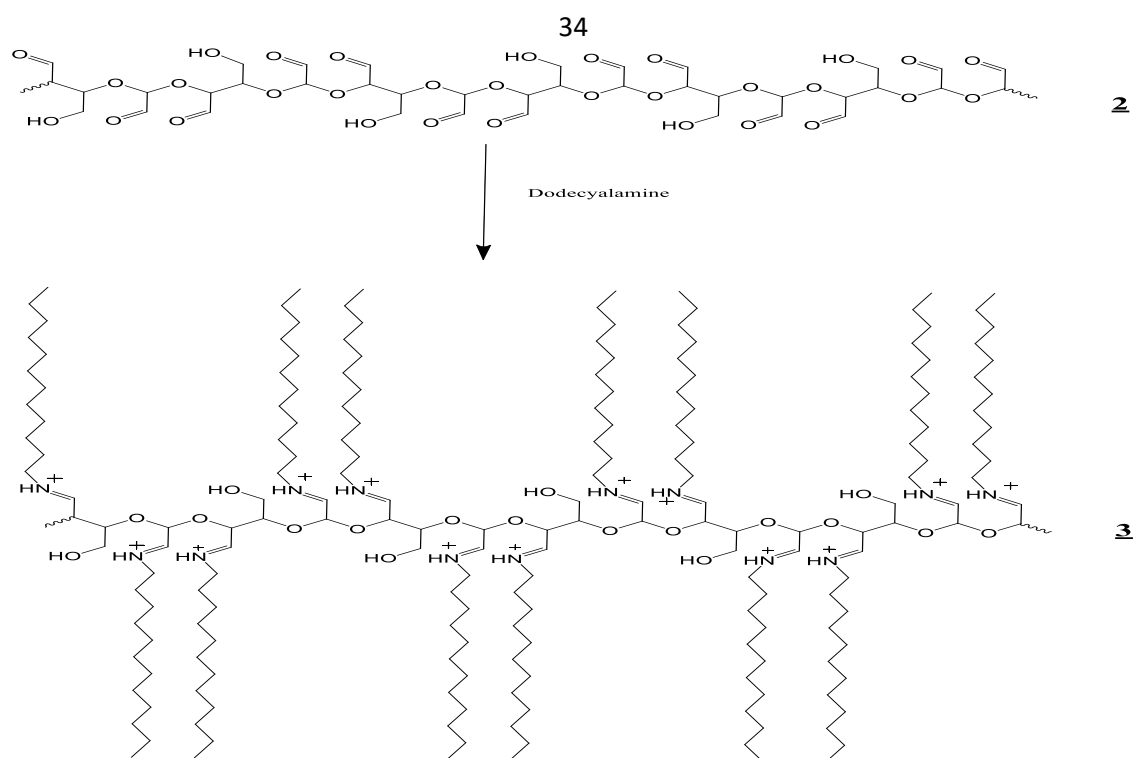


Fig 3.3: SEM images of cellulose aldehyde at 40x

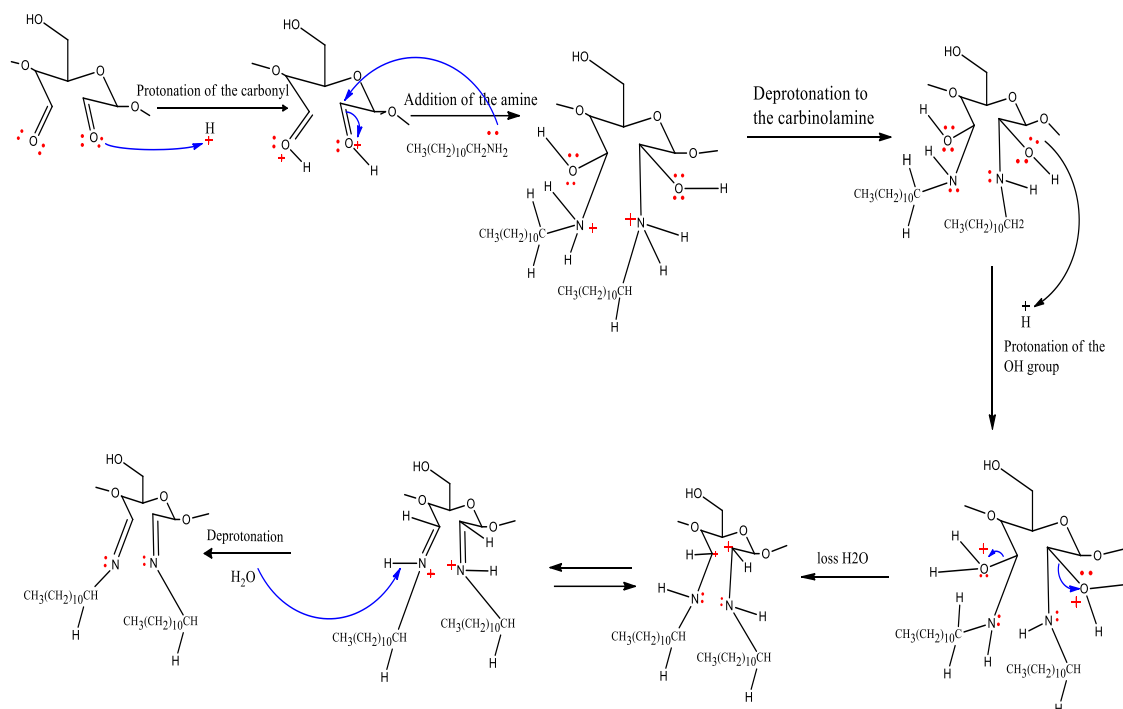
3.2. Preparation of cellulose dododecyl amine (CDDA4)

The aldehyde is a versatile group where it can be converted to carboxylic acid by oxidation reaction or could be reduced and transformed into primary alcohol or react with amine to form an imine. The carbonyl group of aldehyde can also undergoes nucleophilic addition reactions. The nucleophilic addition of the amine to the carbonyl compound of the molecule gives rise to a hemiaminal intermediate, followed by a transfer of a proton from the nitrogen to the oxygen, then a loss of water molecule to give an imine. The reaction of cellulose with dodecyl amine is shown in **Scheme 3.3**



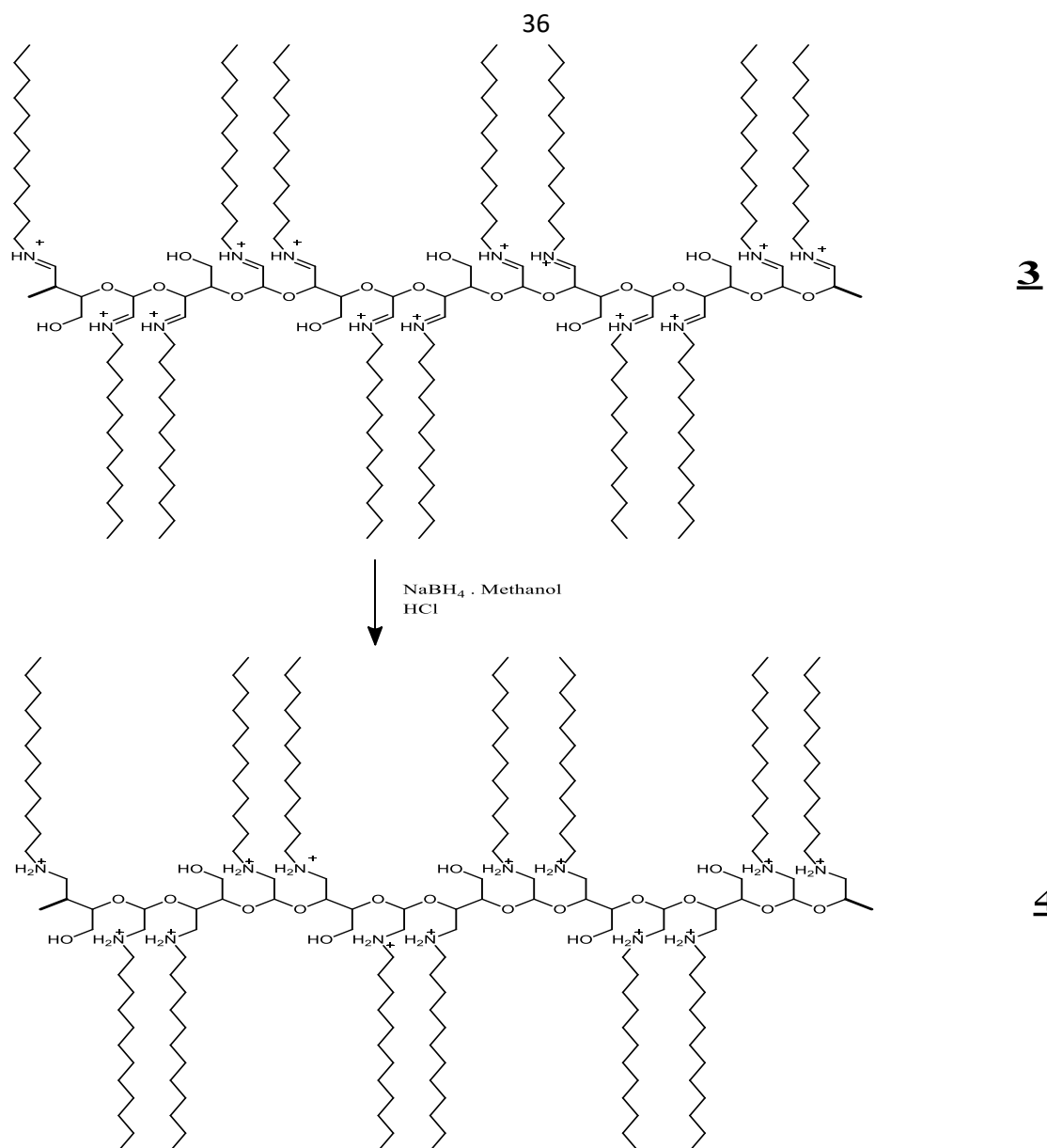
Scheme3.3: Conversion of cellulose dialdehyde to cellulose diimine(CDDI, 3)

The reaction mechanism is shown below.



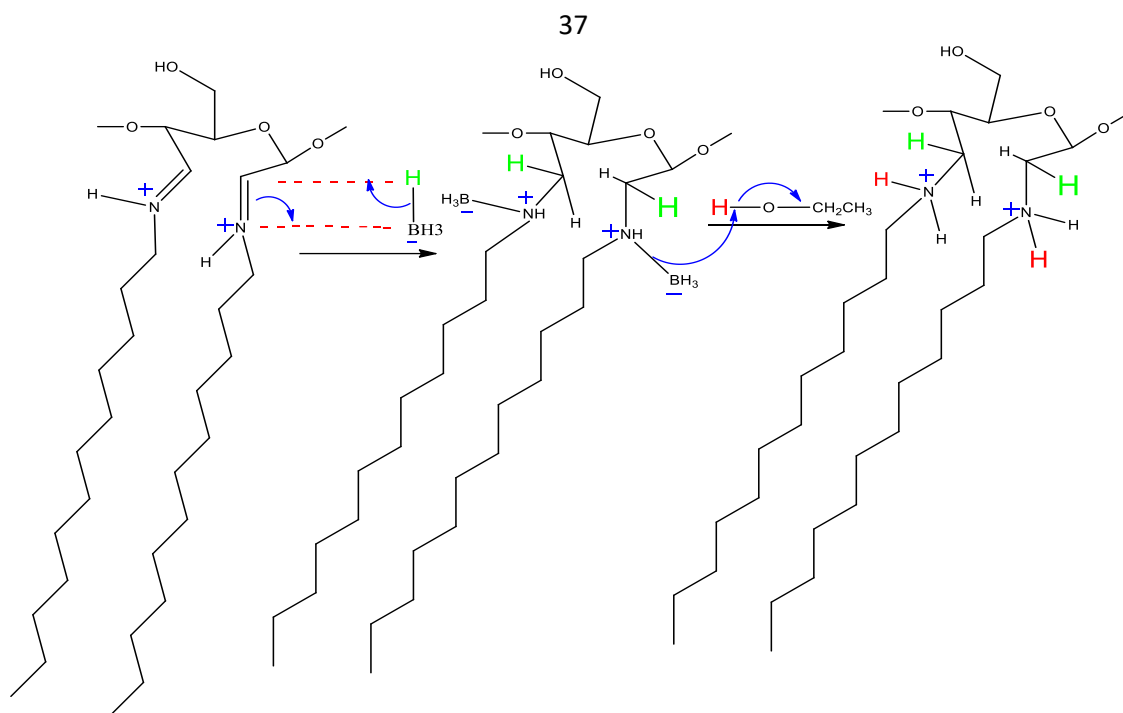
Scheme 3.4: Mechanism for conversion of cellulose dialdehyde to cellulose diimine.

This reaction takes place in several steps. The first step is the protonation of the carbonyl group, after that addition of the amine, after that deprotonation, then protonation of the O-H group, followed with a loss of H_2O , and formation of imines. The reduction of cellulose imine (**3**) was carried out using sodium borohydride, this reaction is a straight forward, it is usually performed in methanol the reaction is shown in **Scheme 3.5**.



Scheme3.5: Conversion of cellulose diimine to cellulose amine (CDDA, 4)

Conversion of cellulose diimine to cellulose amine occurred as shown in the following mechanism.



Scheme 3.6: Mechanism for conversion of cellulose diimine to cellulose amine.

3.2.1 FT-IR of cellulose amine (4)

The FT-IR spectra of cellulose amine polymer 4 (**Figure 3.4**) shows the two peaks at 2923 and 2853 cm^{-1} are corresponding to the symmetric and asymmetric stretching vibration of the C-H bond of the aliphatic chain of the amine. The broad adsorption peak at about 3350 cm^{-1} actually is composed of several peaks originated from O-H and N-H stretching vibrations of secondary amines. The presence of strong peaks at 2923 and 2853 cm^{-1} is an indication of the fatty group attachment onto the cellulose chain the strong peak at 1048 cm^{-1} corresponds to C-O and C-O-C stretching

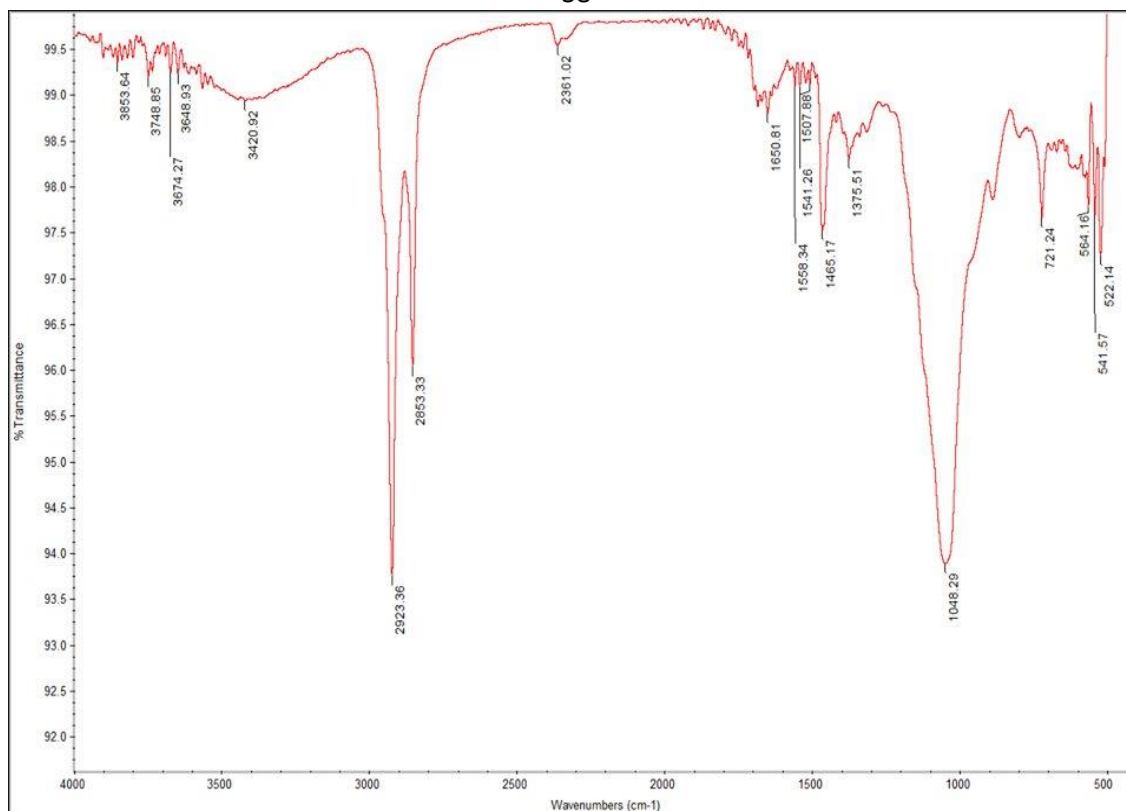
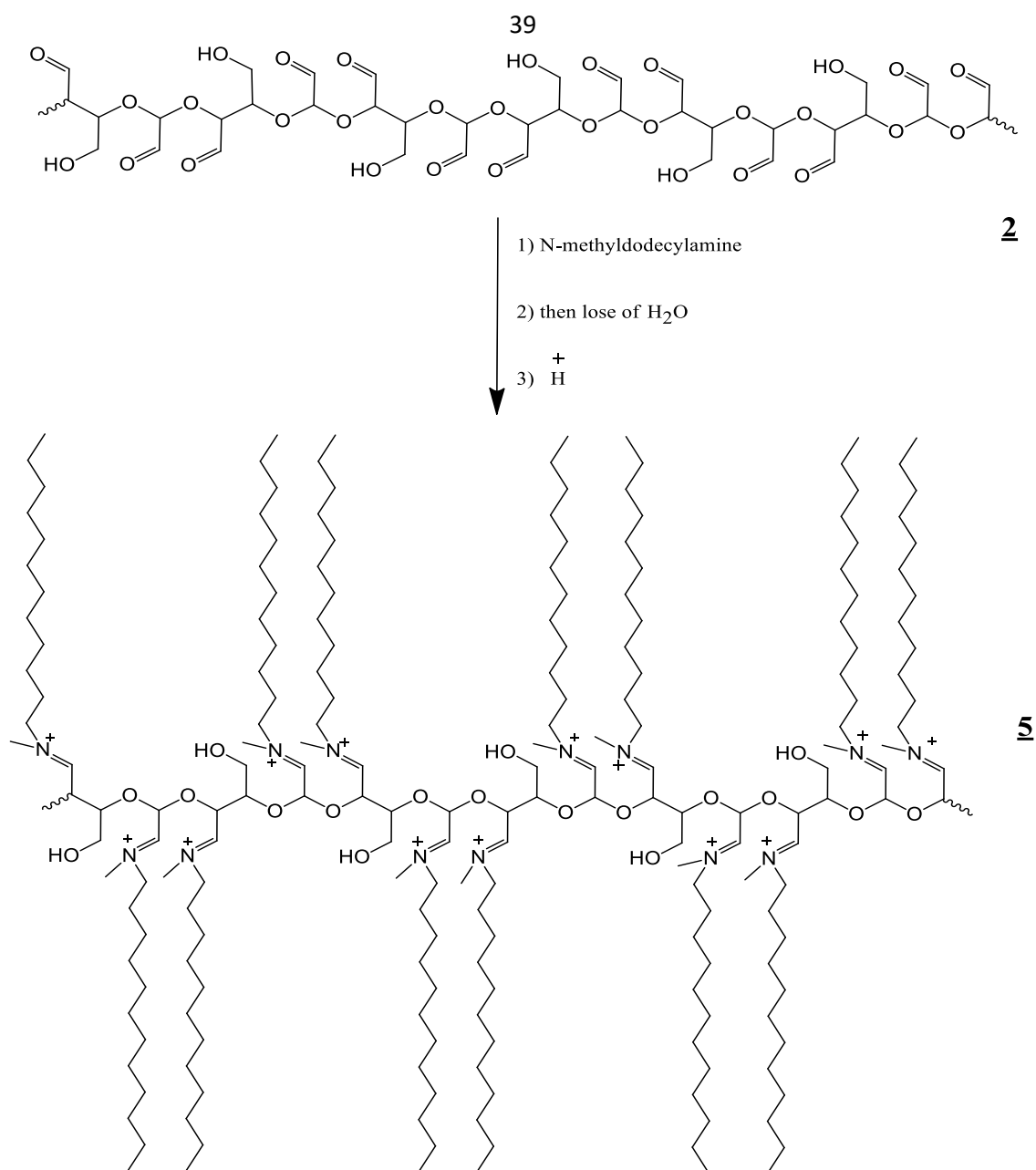


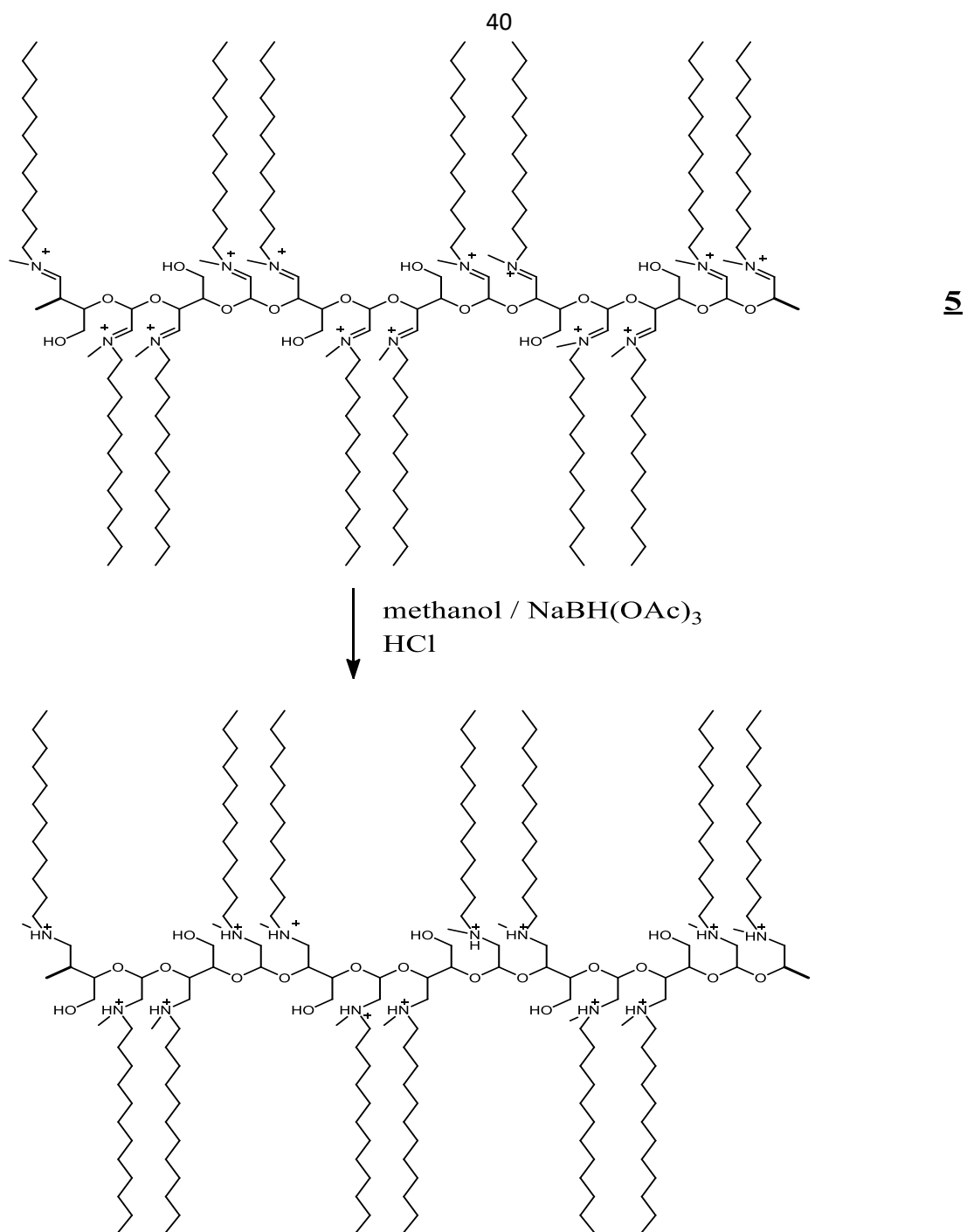
Fig 3.4: FT-IR of cellulose dodecylamine CDDA 4

3.3. Preparation of cellulose N-methyl dodecylamine (CNMDDA)(6)

Cellulose aldehyde was also reacted with N-methyl dodecylamine as shown in scheme 3.7. It was first converted to cellulose imine then reduced with triacetoxyborohydride to cellulose amine **6**, triacetoxyborohydride was prepared in situ from reacting sodium borohydride with acetic acid in an ice water bath.



Scheme3.7: conversion of cellulose dialdehyde to cellulose diimine (5)



Scheme3.8: conversion of cellulose diiminium to cellulose amine (CNMDDA **6**)

3.3.1 FT-IR of Cellulose N-methyl dodecylamine (6)

The FT-IR spectra of cellulose amine polymer **6** is shown in **Figure 3.5**. the spectrum shows small broad peak at 1650 cm^{-1} that could be attributed to residual C=N iminium salt. The two peaks at 2853 and 2810 cm^{-1} are corresponding to the symmetric and asymmetric stretching vibration of the C-H bond of the methyl amine and aliphatic chain of the amine. The broad adsorption peak at about 3355 cm^{-1} actually is composed of several peaks are cross bonding to O-H stretching vibrations

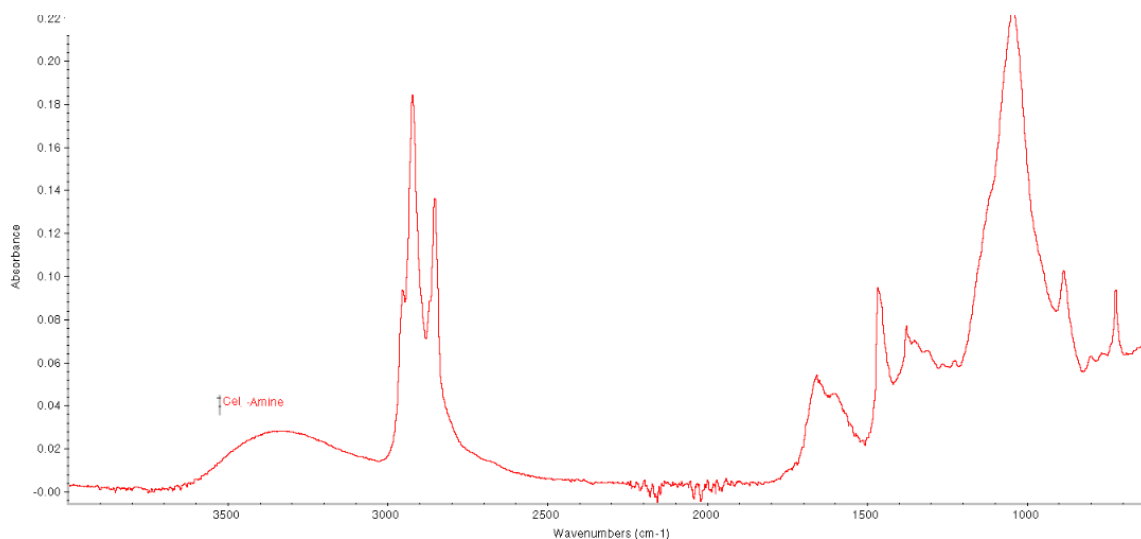


Fig 3.5 FT-IR of cellulose N-methyl dodecylamine(CNMDDA 6)

3.4 Film formation

Film casting was carried out by dissolving known amount of cellulose amine in dichloromethane, adding the solution to evaporating plate. The plate was covered with a beaker for a slow evaporation and

placed in a hood. A smooth regular film was obtained. An image of a film obtained from casting 0.5 g of cellulose amine 4 is shown in **Figure 3.6**.

Film transparency and strength was enhanced by adding cellulose triacetate. In this case a solution of methanol and dichloromethane a (1:2 w/w ratio) by weight was used. A SEM study was performed on a film made from a mixture of CDDA (4) and cellulose triacetate (CTA) in 2:1 ratio. The obtained image (**Figs 3.12 and 3.13**) show a well distribution of polymer chains in the film. The image obtained from film made from a mixture of CNMDDA (6) and cellulose triacetate in 2:1 ratio. The obtained image (**Fig 3.14**) also shows a well distribution of polymer chains in the film with good transparency.



Fig 3.6: Film made from cellulose amine polymer (4)

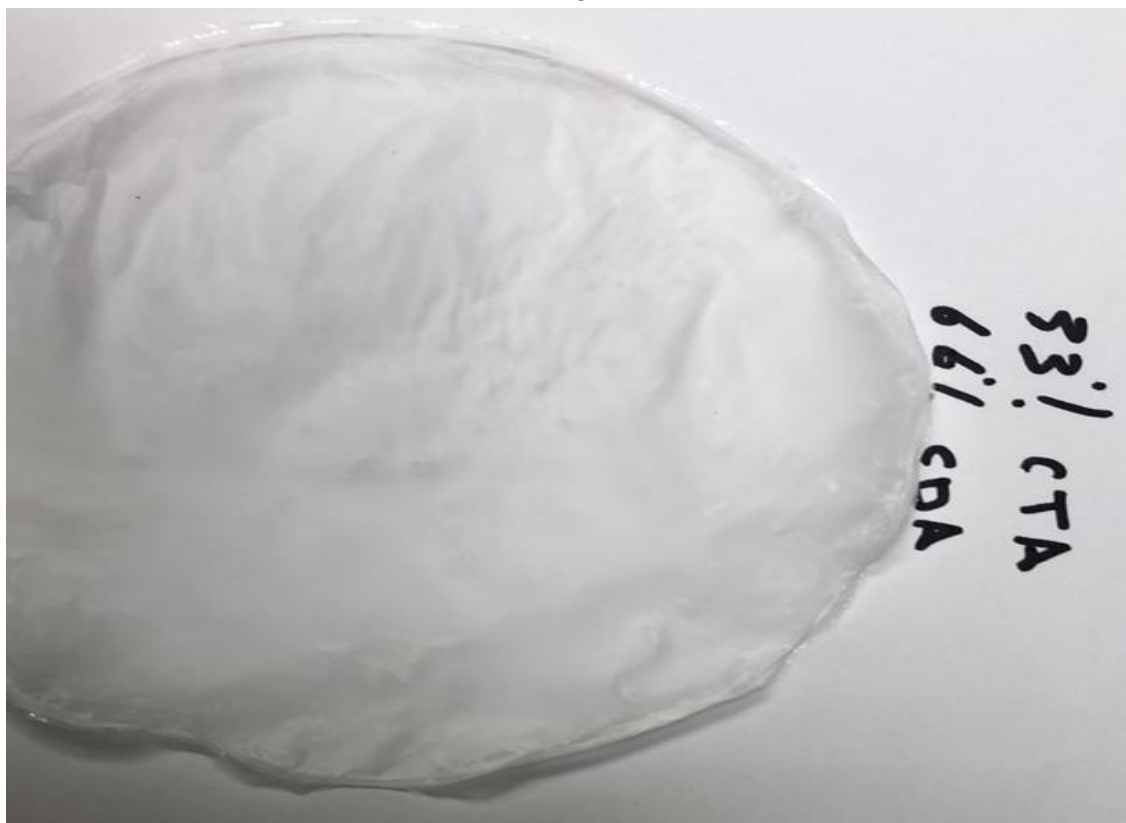


Fig 3.7: Film made from a mixture of cellulose amine polymer (4) and cellulose triacetate



Fig 3.8 Film made from a mixture of cellulose amine polymer(4) and cellulose triacetate in 1:1 ration by weight

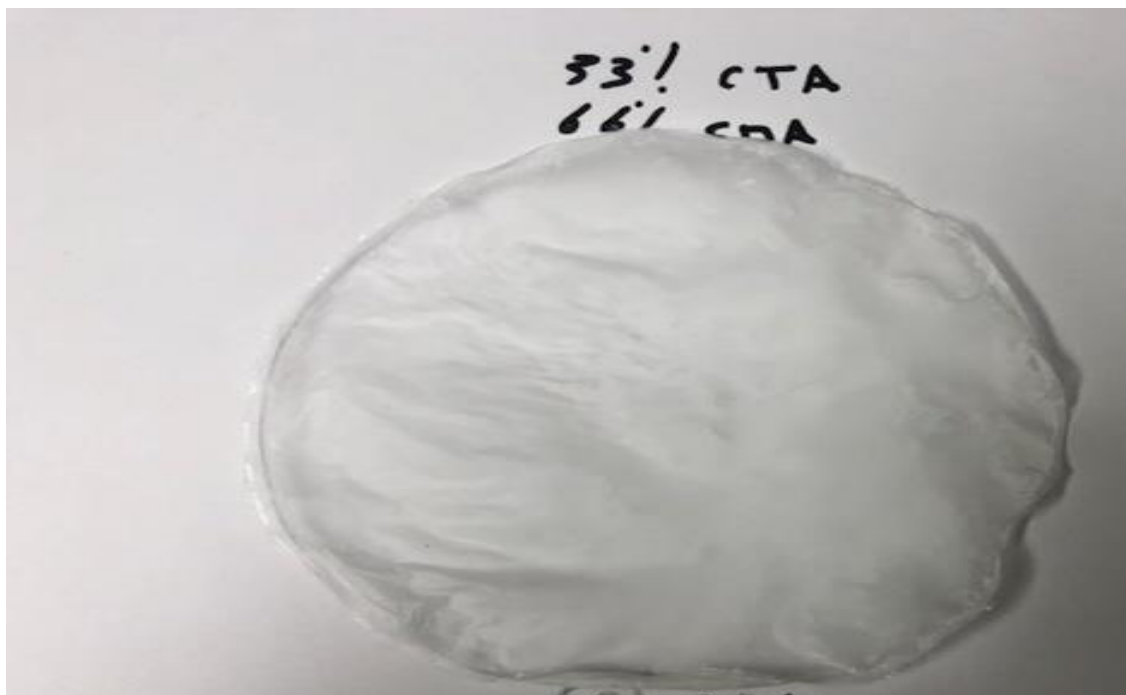


Fig 3.9: Film made from a mixture of cellulose amine polymer (6) and cellulose triacetate in 2:1 ratio by weight.

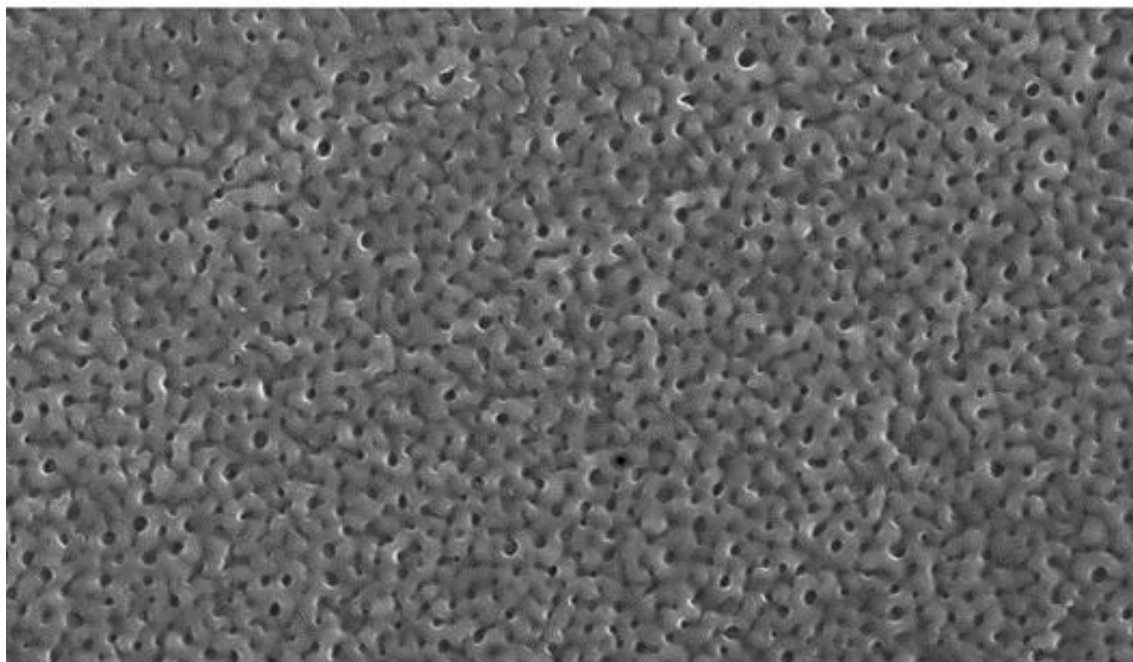


Fig 3.10: Film made from a mixture of cellulose amine polymer (6) and cellulose triacetate in 1:1 ratio by weight



Electron Image 1

Fig 3.11: SEM image of film made from a mixture of cellulose amine polymer (4) and cellulose triacetate in 2:1 ratio by weight at magnification of 40x



Electron Image 1

Fig 3.12: SEM image of film made from a mixture of cellulose amine polymer (4) and cellulose triacetate in 2:1 ratio by weight at magnification of 100x

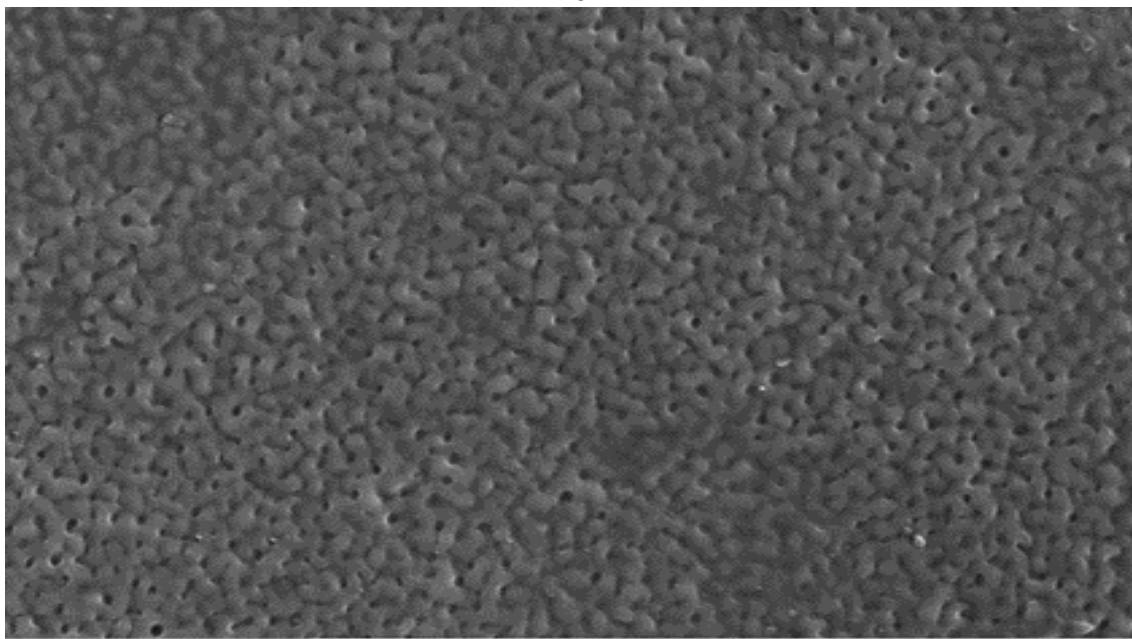


Fig 3.13: SEM image of film made from a mixture of cellulose amine polymer (6) and cellulose triacetate in 2:1 ratio by weight at magnification of 40x

3.5 Antimicrobial Activities of CDA polymers

The antimicrobial activities of the two cellulose diamine polymers 4 and 6 were evaluated against three types of bacteria two-gram negative and one gram positive. Both polymers showed excellent activities against gram negative and medium activity against the gram-positive bacteria. The results are summarized in Table 3.1.

Table 3.1: Percent reduction of three microbial cells by cellulose amine films.

Cellulose amine polymer	Bacterial cell	Reduction After 24 hours vs Control (%)
Cellulose	<i>Staphylococcus aureus</i>	0.0
	<i>Klebsiella pneumonia</i>	0.0
	<i>E.coli</i>	0.0
4	<i>Staphylococcus aureus</i>	52.81%
	<i>Klebsiella pneumonia</i>	>99.00
	<i>E.coli</i>	>99.00
6	<i>Staphylococcus aureus</i>	61.53%
	<i>Klebsiella pneumonia</i>	>99.00
	<i>E.coli</i>	>99.00

3.6 Film physical and mechanical properties

Tensile testing was performed on a Zwick/Z005 (Zwick/Roell, Germany) instrument fitted with a 200 N load cell with the crosshead speed of 0.5 mm/min, and the initial distance between the grips was 30 mm. Five specimens of each film were tested, and the average values were reported. The measurements were performed at room temperature.

Table 3.2: Physical and mechanical properties of CDDA (4)/CTA film

Sample no.	Components Weight Ratio (wt%)		Tensile strength (N/mm ²)	% Elongation	Tg (C°)
	CDDA	CTA			
1		100	86.0	10	189
2	66	33	62.0	17	108.2
3	50	50	67.4	14	135.0
4	33	66	73.2	12	148.2

Table 3.3: physical and mechanical properties of CNMDDA (6)/CTA film

Sample no.	Components Weight Ratio (wt%)		Tensile strength (N/mm ²)	% Elongation	Tg (C°)
	CNMDDA	CTA			
1		100	86.0	10	189
2	66	33	59.0	19	107.5
3	50	50	66.4	15	133.2
4	33	66	71.2	12	145.6

3.7 Adsorption of Nitrite ion

The adsorption of nitrite by CDDA (4) was evaluated as a function of time, adsorbent dose, pH and temperature. The concentration of nitrite

ion was monitored by UV spectroscopy. Results showed zero efficiency of the polymer toward the nitrite ion.

CONCLUSION

New cellulose based plastic films were designed and successfully synthesized from cellulose by oxidation, reaction with fatty amines and reduction, then mixing with cellulose triacetate. The cellulose fatty amines and casted films were characterized by FT-IR and SEM.

The physical properties of the films including Tg, tensile strength and elongation were evaluated. Results showed that the films have plastic characteristics and could be used in food and drink wrapping. The antimicrobial efficacy of the prepared films was evaluated, they showed excellent efficacy against gram negative

(klebsiella pneumonia, E. coli) and medium activity against the gram positive bacterial (staphylococcus).

The ability of the polymers to absorb nitrate ions was studied. They showed zero efficiency.

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جامعة النجاح الوطنية
كلية الدراسات العليا

الفلم القائم على السليولوز مع أنشطة مضادات الميكروبات

إعداد

رأفت محي الدين صالح رداد

إشراف

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قدمت هذه الرسالة استكمالاً لمتطلبات الحصول على درجة الماجستير في الكيمياء من كلية
الدراسات العليا في جامعة النجاح الوطنية في نابلس - فلسطين
2020

ب
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الملخص

السليولوز هو منتج متاح على نطاق واسع ويستخدم بشكل غير محدود في التطبيقات الصناعية. ومن الممكن إضافة عدد من المجموعات الوظيفية إلى سلسلة السليولوز وكل إضافة تخدم تطبيق تجاري معين.

في هذا العمل تم تحويل السليولوز الصلب المستخرج من صناعة زيت الزيتون إلى سليولوز ثنائي الدهايد عن طريق الأكسدة باستخدام صوديوم بيرايوديت بعد ذلك تم التفاعل مع مجموعه من الامينات الدهنية ميثيل ديسيل امين وديسيل امين.

لتشكيل السليولوز الدهني الذي يتحول إلى أمين سليولوز الدهني عن طريق التفاعل مع صوديوم ثلاثي اسيتوبورو هيدريد وبورو هيدريد الصوديوم وقد اظهر السليولوز امين الذى تم إنتاجه سلوك مشابه للبلاستيك.

تم فحص تراكيب السليولوز الدهيد والسليولوز امين باستخدام FT-IR و SEM، تم أعداد العديد من المحاليل لكل من الامينات الدهنية السليولوزيه والسليولوز ثلاثي الاسيتات بنسب مختلفة وتم صبها في أفلام. وتم فحص الأفلام بواسطة SEM وأظهرت صور SEM توزيعا ممتازا للانسجة الدهنية واظهرت وجود روابط قوية.

كانت الأفلام المنتجة واضحة وشفافة. تم تقييم الخواص الفيزيائية المختلفة للأفلام مثل قوة الشد، الاستطالة، درجة حرارة التلين (Tg) أظهرت النتائج أن الفيلم يمكن أن يتناسب مع الطعام والشراب.

تم تقييم النشاط المضاد للميكروبات للأفلام المنتجة ضد ثلاثة أنواع من البكتيريا اثنان سالبة الجرام و واحد إيجابي غرام. أظهر كلا البوليمرات أنشطة ممتازة بعد 24 ساعة. ضد الجرام السلبي (الالتهاب الرئوي، القولونية)، بلغ النشاط حوالي 99% وظهر نشاط متوسط ضد البكتيريا إيجابية الجرام (العنقودية).

تمت دراسة قدرة الفيلم على امتصاص أيونات النترات من الماء لم يظهر الفيلم أي كفاءة تجاه أيون نترات.