DOKUZ EYLÜL UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

SYNTHESIS AND CHARACTERIZATION OF UV-SENSITIVE CHITOSAN DERIVATIVES

by
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İZMİR

SYNTHESIS AND CHARACTERIZATION OF UV-SENSITIVE CHITOSAN DERIVATIVES

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M.Sc THESIS EXAMINATION RESULT FORM

We have read the thesis entitled **“SYNTHESIS AND CHARACTERIZATION OF UV-SENSITIVE CHITOSAN DERIVATIVES”** completed by **AYHAN CANSU** under supervision of **PROF. DR. MEHMET KADİR YURDAKOÇ** and we certify that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science.

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Ayhan CANSU

SYNTHESIS AND CHARACTERIZATION OF UV-SENSITIVE CHITOSAN DERIVATIVES

ABSTRACT

Chitosan (CS), i.e. poly (2-amino-2-deoxy-β-D-glucose), is the second most abundant biopolymer in the Earth behind cellulose. CS has a wide range of applications due to its biocompatible, biodegradable and non-toxic properties. However, CS has its drawbacks in industrial applications since it does not dissolve in most common organic solvents. With derivatization by -OH and -NH_2 functional groups in chitosan, these drawbacks can be eliminated.

In this study, it was aimed to develop ultraviolet(UV)-light irradiated photocrosslinkable chitosan formation. In order to achieve this, CS dissolved in metansulfonic acid and by converting ammonium salt in chitosan, whose functional the NH_2 functional groups were protected. Therefore, the OH functional group would undergo the reaction. Cinnamoyl chloride (CINCl) was added dropwise to the reaction mixture as being radical trigger and functionalized with polyethylene-glycol-dimethacrylate (PEGDMA) in order to trigger the particles interaction with each other by forming UV light irradiated photocrosslinkable chitosan.

The synthesized products were characterized by FTIR and TG/DTG. The swelling property of the samples was evaluated at pH 7.4.

Keywords: Chitosan, ultraviolet rays, polymerization, cross-linking

UV-DUYARLI KİTOSAN TÜREVLERİNİN SENTEZİ VE KARAKTERİZASYONU

ÖZ

Kitosan (CS), poli (2-amino-2-deoksi-β-D-glukoz), doğada selulozdan sonra en çok bulunan ikinci doğal biyopolimerdir. CS biyoyumlu, biyobozunur ve toksik olmayan özelliklere sahip olduğu için birçok alanda geniş uygulamaları vardır. Kitosan pek çok olağan organik çözücülerde çözünmediği için bu durum kitosanın endüstriyel uygulamalarında dezavantaj oluşturmaktadır. Bu dezavantajların üstesinden gelebilmek için, Kitosan, -OH ve -NH_2 fonksiyonel yan grupları üzerinden türevlendirilebilir.

Bu çalışmada UV-ışını ile kürlenebilen ve bu sayede dakikalar içerisinde sertleşebilen UV-duyarlı kitosan türevleri geliştirme amaçlanmıştır. Bu amaçla CS, metansülfonik asit içerisinde çözünmüş ve kitosan üzerindeki -NH_2 fonksiyonel grupları amonyum tuzu şeklinde korunmuştur. Bu sayede reaksiyonun -OH fonksiyonel grupları üzerinden gerçeklenmesi sağlanmıştır. Radikal başlatıcı kaynağı olarak ortama Sinnamoil Klorür (CINCl) eklenmiş, daha sonra ortama çapraz bağlayıcı amacıyla poli etilen glikol dimetakrilat (PEGDMA) eklenerek kitosan UV-duyarlı hale getirilmiştir. Elde edilen ürün UV-ışınlarına maruz bırakılarak çapraz bağlı polimerizasyon sonucunda sertleşmiş film elde edilmiştir.

Sentezlenen örneklerin karakterizasyonu FTIR ve TG/DTG yöntemleriyle belirlenmiş, şişme özellikleri pH 7,4 ortamında incelenmiştir.

Anahtar kelimeler: Kitosan, ultraviyole ışın, polimerleşme, çapraz bağlanma

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INTRODUCTION

1.1 Chitin

A large amount of seafood, crab and shrimp is wasted worldwide without evaluating their shells. Especially in recent years, along with the re-evaluation of the waste agenda, shellfish, rather than left to rot; being re-evaluated by chemical or biological methods and new products are obtained. In this way the main derivative of chitin and chitosan at the beginning of the product obtained, which corresponds (Knaul, Hudson, & Creber, 1999).

Chitin, a polymer of N-acetyl-D-glucosamine monomer residues linked by β-1,4 bonds, is the most abundant natural biopolymer after cellulose. Generally is obtained from crab or shellfish like shrimp (Figure1.1). Tow annual production worldwide is estimated to be close to cellulose. Chitin is the main component of shellfish such as shrimp and crab, also in the structure of insects and fungal cell wall skeletons. The annual world production of the chitin is reported to be about $150x10^3$ tons. Total quantity obtained from 56 x 10^3 tons of shrimp, 39 x 10^3 tons of various shellfish, from 32 x 10^3 and 23 x 10^3 tons of other mushrooms in the annual production (Wu, Y. G. E. 2003). Insect chitin found in the shells at a rate of approximately 23.5%, this rate varies between crabs and shrimp, respectively, 32% and 17%.

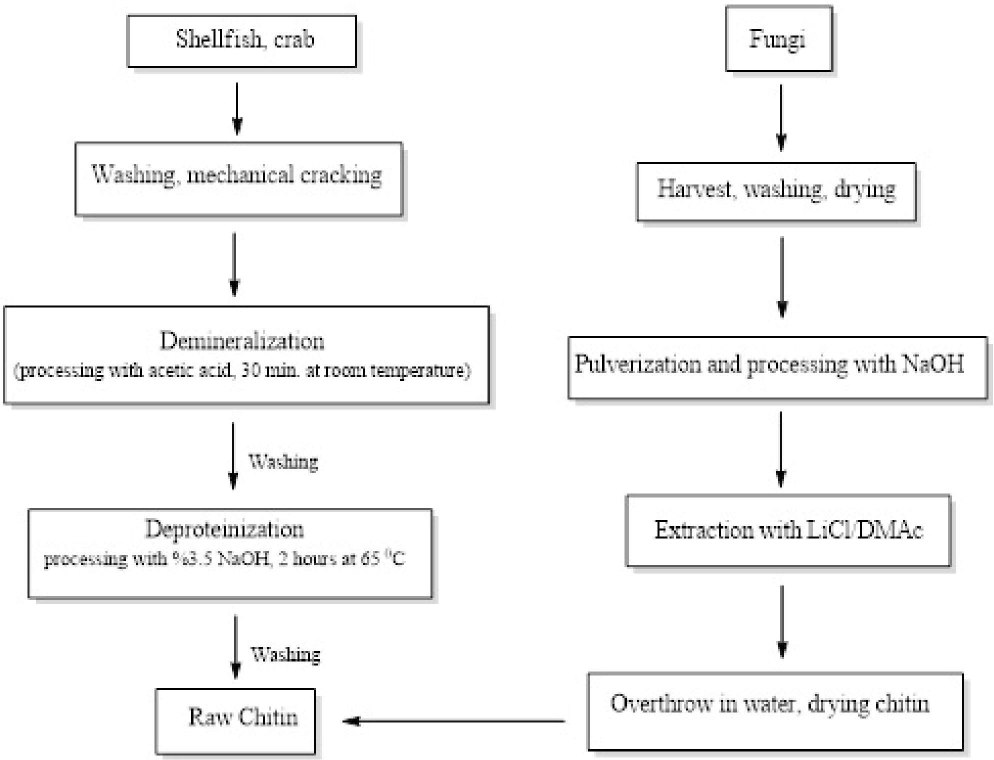


Figure 1.1 The process of obtaining raw chitin (Roberts, 2008)

Chitin, a biopolymer, is contain mainly poly [β-(1,4)-2-acetamide-2-deoxy-β-D-glucopyranose] in the structure (Figure 1.2). Includes is very low proportion of 2-amino-2-deoxy-β-glucopyranose monomer.

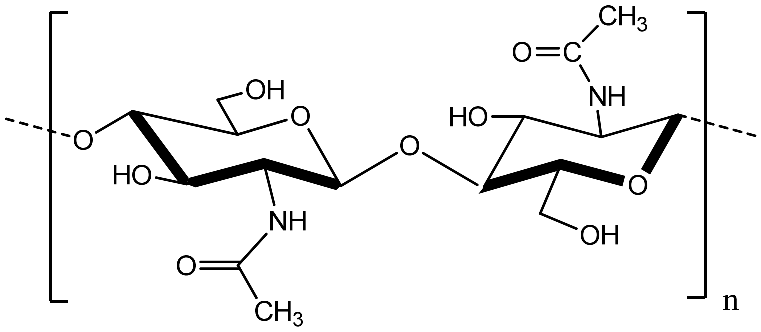


Figure 1.2 Chemical structure of chitin

1.2 Chitosan

Although there are many derivative of chitin, chitosan (CS) is the most important among them. Chitosan is discovered the first time in 1811 by Henri Braconnot. He tried to solve chitin in sulphuric acid but he was unsuccessful. In 1894, Hoppe-Seyler was solved chitin in potassium hydroxide at 180 °C and obtained chitosan that reduced acetyl content (deacetylated) product. In the 1934, film production from chitosan and chitosan fibers were obtained two patents (Wu, 2003).

Chemical formula of chitosan, which derive by partial deacetylation of chitin, is the poly-[β-(1,4)-2-amino-2-deoxy-β-D-glucopyranose], as shown in. Chitin and chitosan are polysaccharides and chemically similar to cellulose but shows some differences among themselves (Figure 1.3). Cellulose attached to the second carbon atom hydroxyl (-OH) group, while there are in the chitin acetamide (-NHCOCH$_3$), the amine (-NH$_2$) group of chitosan convenience. The main difference between chitin and chitosan is due to the acetyl content of the structure.

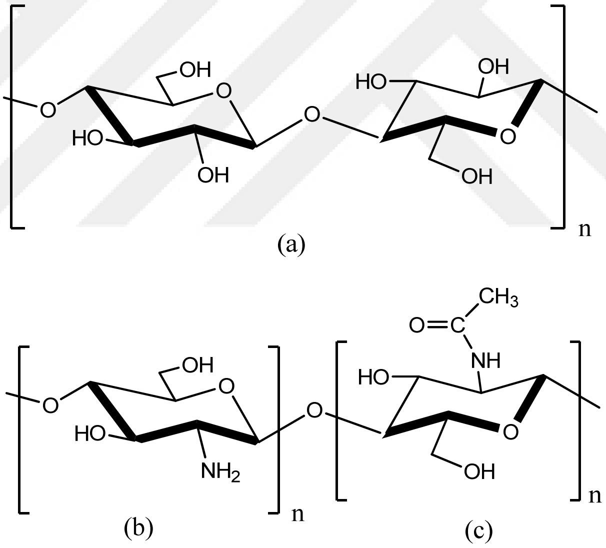


Figure 1.3 Chemical structure of (a) cellulose, (b) chitosan and (c) chitin

Overall, its formed 30-40% of protein; 30-50% of calcium carbonate and calcium phosphate; 20-30% of chitin from crab, lobster and shrimp shells portion of marine animals. Proteins in the structure of shellfish can cause allergies in some people. Therefore, complete removal of the protein is extremely important especially for use in biomedical applications of chitosan.

The degree of deacetylation (DD) is the removal of acetyl groups from amino acetyl group in the structure of chitosan. Thus, behind only amine groups remain. DD of chitosan has a major influence on many parameters including the dissolution feature. The degree of deacetylation can varied between 56-99% according to the type and production method of the shellfish. NaOH is used for the partial deacetylation of chitin biopolymer to hydrolysis and causes the reduction of its molecular weight. If transaction is fulfilled without any problem the chitin's protein content remain around 1%. Also pepsin, papain, trypsin, and proteolytic enzymes like protease also used for protein removal. Deproteinization gives them a certain quality in the chitin. Chitosan which was extracted with 2% acetic acid solution, was filtered, dried and stored at room temperature (Figure 1.4).

General process mechanism is as follows:

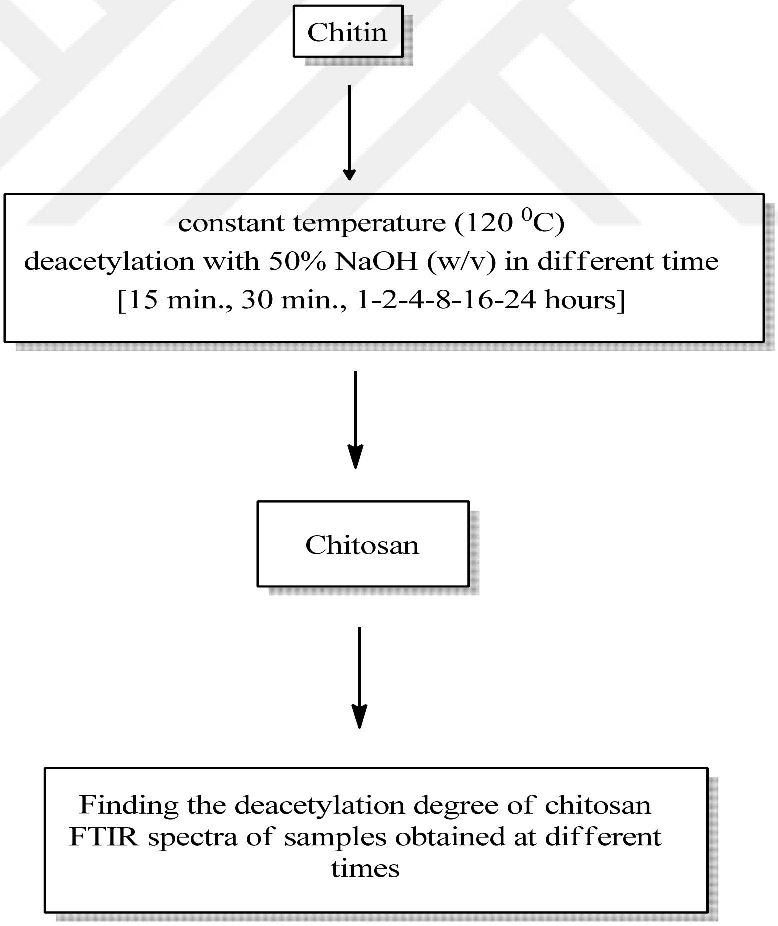


Figure 1.4 The process of chitosan production (Kumar, 2000)

Chitosan has a total of three groups as reactive groups in each repeating unit primary (C-6) and secondary (C-3) amine and hydroxyl groups (C-2) (Figure 1.5). These reactive groups can easily undergo chemical modification and changing the physical properties and dissolution. Nitrogen content of the chitosan is according to DD of between 5-8% (usually 6.9%).

When compared to cellulose, chitosan is high nitrogen content (nitrogen content of cellulose around 1.25%). It provides as great advantages for metal particles binding in the waste water (chelating agent).

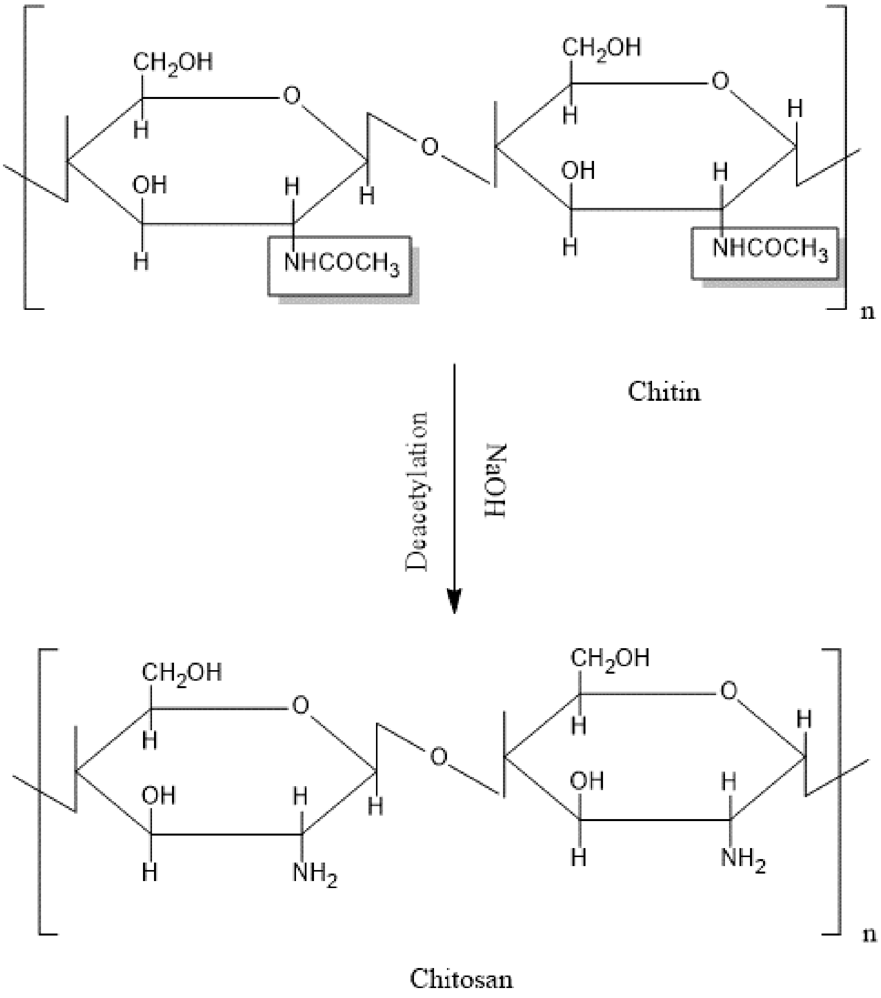


Figure 1.5 Deacetylation of chitin (Castro & Paulín, 2012)

1.3 Usages of Chitosan

In recent years, chitosan and its derivatives have great interest in research as far as their usages and application areas concerned. Chitosan is a renewable resource and environmentally friendly material as a natural biopolymer. There are two major advantages over chitosan according to chitin. The first of these to solve chitin are to be used in toxic solvents such as dimethylacetamide and lithium chloride use profits chitosan it can be easily dissolved in acetic acid. The second advantage is the active part for many chemical reactions which it is provided with a free amine group.

Nowadays chitosan can be used in numerous areas; medicine to food, agriculture to cosmetics, pharmaceuticals and from the wastewater treatment to the textile industry.

It is also used for many purposes in the textile industry. These includes; to confer antimicrobial properties, providing shrinkage in wool fabric, reducing the amount of salt in reactive dyeing, cotton acid gain dye ability with dyes, bringing antistatic property etc.

Chitosan is quite become important in the field of medical textiles. Chitosan used to provide tissue in the treatment of injure tissue. In addition, it can be summarized in the form; medical artificial leather, surgical sutures, artificial blood vessels, controlled drug release, contact lenses construction, adhesive bandages, gauze, cholesterol control (fat binder), tumor inhibitors, antifungal, antibacterial, and hemostatic effects show and so on (Montazer & Afjeh, 2007).

Chitosan has the effect of lowering cholesterol on the other hand. It can interacted with negatively charged lipid sand reduce cholesterol due to poly cationic center in structure (Berger et al., 2004).

Chitosan does not have any side effects due to non-toxic properties, has biologically degradable properties without harming the environment, completely harmless products (amino sugars) separable in the body. Therefore chitosan hydrogels are used in biomedical applications (Berger et al., 2004).

Chitosan also plays a very active role in accelerating the wound healing due to it has generation polyelectrolyte complex with heparin (- charged). Heparin is a polysaccharide which inhibits blood coagulation (Cho, Cho, Chung, Yoo, & Ko, 1999).

The ability to create a film because of chitosan, has found use in the construction of the artificial kidney membrane. For this purpose, chitosan modified by the graft polymerization and formed water soluble polymers.

The controlled release of drugs is extremely important. Biologically harmless chitosan has various appropriate matrixes in the drug structure. Various drugs are placed into the chitosan matrix (film, microcapsules, coated tablets, etc.). Also, the fact that prevents irritation with antacids and stomach-ulcer activity it makes an ideal material (Adrienne & Nanofibers, 2013).

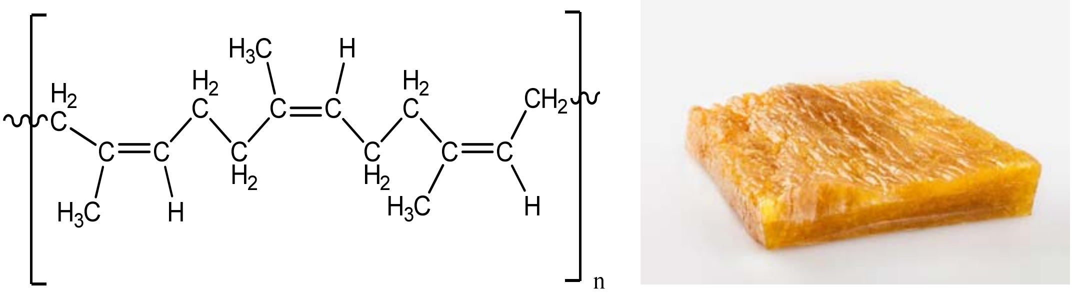
The functional groups of chitosan have the ability to form calcium-based composite materials. Thus, it used in orthopedic and periodontal applications. Calcium-chitosan based composites, coatings made of bone prostheses were implanted into the body strengthens the bone cells (Adrienne & Nanofibers, 2013).

In summary, chitosan, it found the use of space thanks to these features; as well as the features mentioned above biocompatibility, antimicrobial activity, other materials to create composites, as it allows cellular adhesion and proliferation etc.

1.4 Polymerization

A polymer is chemical compound where molecules are bonded together in long repeating chains. Repeating units are often made of carbon and hydrogen and sometimes oxygen, nitrogen, sulfur, chlorine, fluorine, phosphorous, and silicon

(Odian, G., 2004). Polymers abound in nature. For example, rubber is a natural polymeric material that is extremely useful and has been used by humankind for thousands of years (Figure 1.6).



(a) (b)

Figure 1.6 (a) Chemical structure (Morton, 2013) and (b) natural look of rubber (Rubber, n.d)

Polymerization is combining many small monomer molecules into chain held together by covalent bonds. There are two types of basic reactions. These are condensation polymerization and chain-growth polymerization (Figure 1.7).

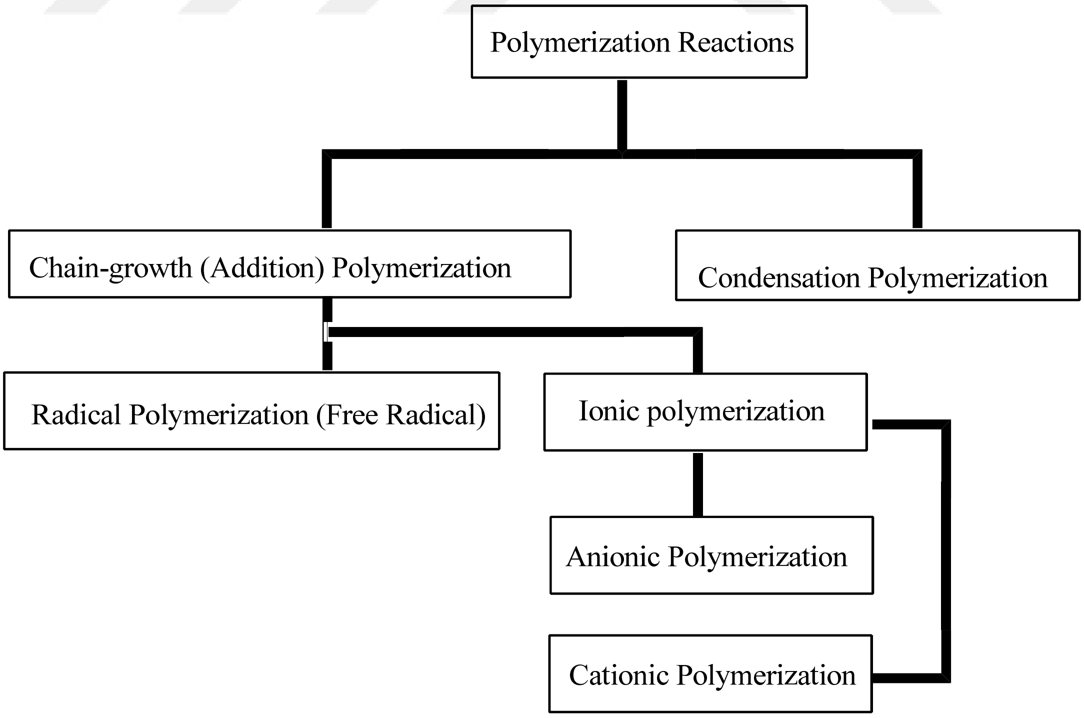


Figure 1.7 General polymerization types (Odian, 2004)

In condensation polymerization, two monomers react to yield a repeat unit and a smaller molecule such as water is eliminated. As an example, the polymerization of nylon from monomers with carboxylic acids and basic amines can be given (Figure 1.8).

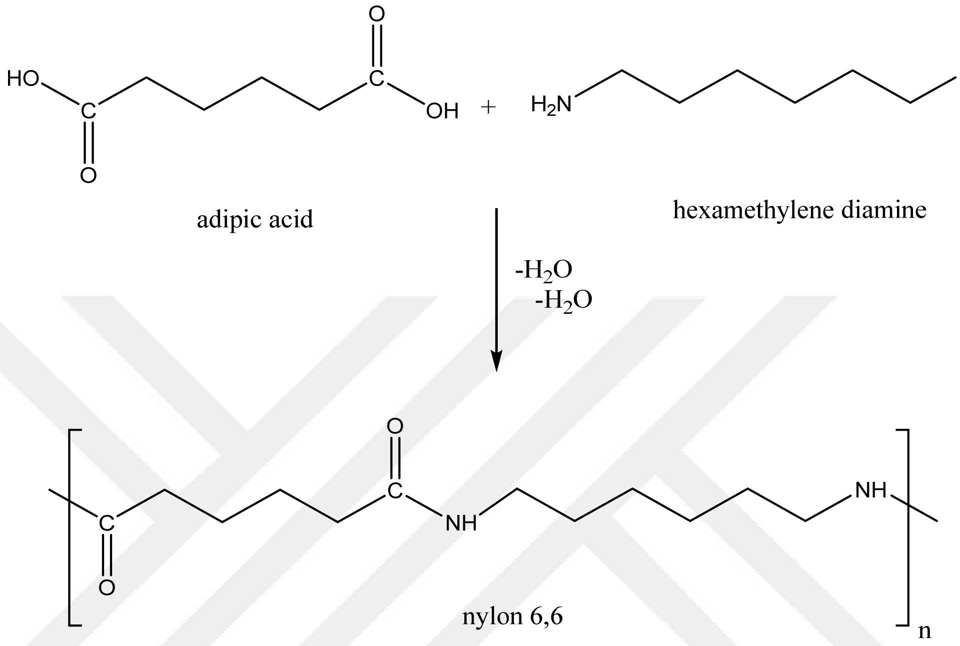


Figure 1.8 Example for condensation polymerization (Odian, 2004)

In chain growth polymerization, monomer forms a highly reactive free radical or a molecule with an unpaired electron. The free radical reacts with another monomer and causes a repeat unit with another free radical (Ravve, 2013).

As an example, chain-growth polymerization of styrene can be given as indicated below (Figure 1.9).

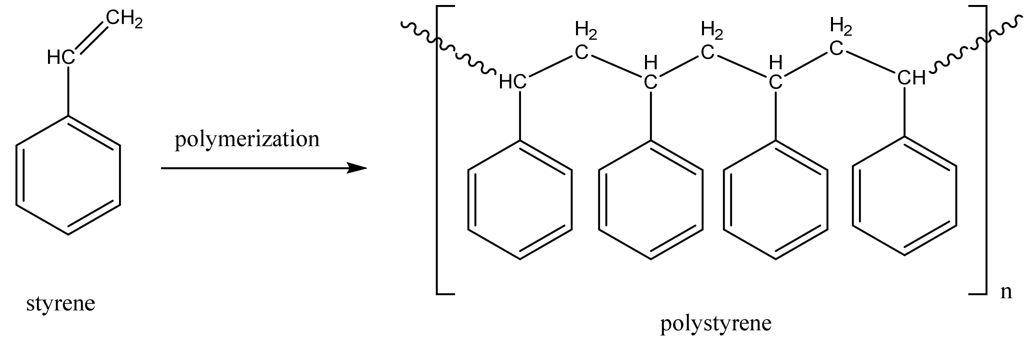


Figure 1.9 Chain growth polymerizations reaction mechanism

1.4.1 Radical Polymerizations (Free Radicals)

One of the most important types of addition polymerization is free radicals which containing unpaired electrons for initiated polymerization. These compounds react with π bond with double bond due to free-radical initiators can be easily active centers (Figure 1.10). This process is known as polymerization of vinyl in plastics industry.

In this polymerization, it grows by addition of monomer chain with many active centers of chain growth. Due to its relatively low stability of the carbon-carbon (C=C) double bond, in particular the reaction may easily free radicals. Chain growth ends what causes neutralization the active cent3er of the termination reactions. The choice of initiator type monomer is closely related chemical structure. The impact of the initatior, π-bond can be opened homolytically or heterolytically.

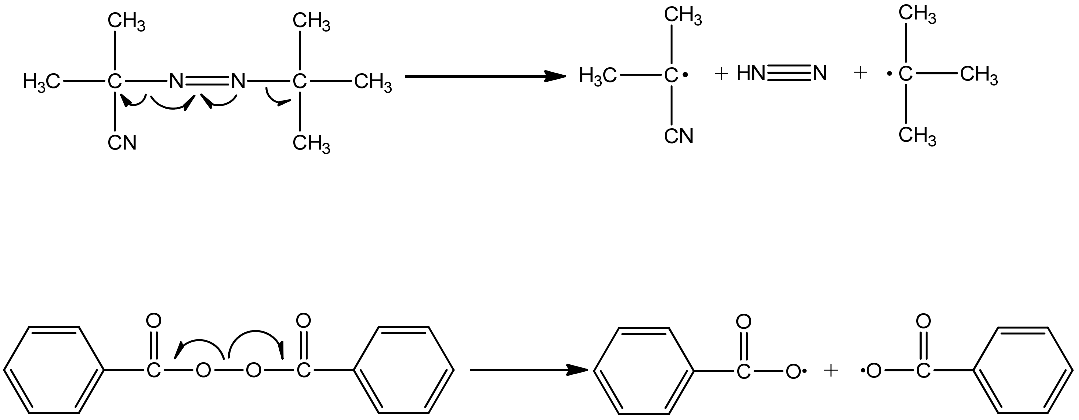


Figure 1.10 Some radical initiators obtaining reactions

Radical polymerization essentially consists of the following steps;

1. Initiation: It called monomers to be activated. Monomer becomes a free radical by physical or chemical factors.

R:R $\longrightarrow$ R* + R* (Free Radical, containing unpaired electron)

R* + M (Monomer) $\longrightarrow$ RM* (Initiate)

2. Growth: A rapidly growing polymer chain to which monomer units that active centers is a step of joining.

RM* + M $\longrightarrow$ RMM*

RMM* + M $\longrightarrow$ RMMM*

RMMM* +M $\longrightarrow$ RMMMM*

3. Termination (Ending): Having grown up in the chain polymer is step it loses its activity (Figure 1.11).

R(M)n M* $\longrightarrow$ P inactive/dead polymer

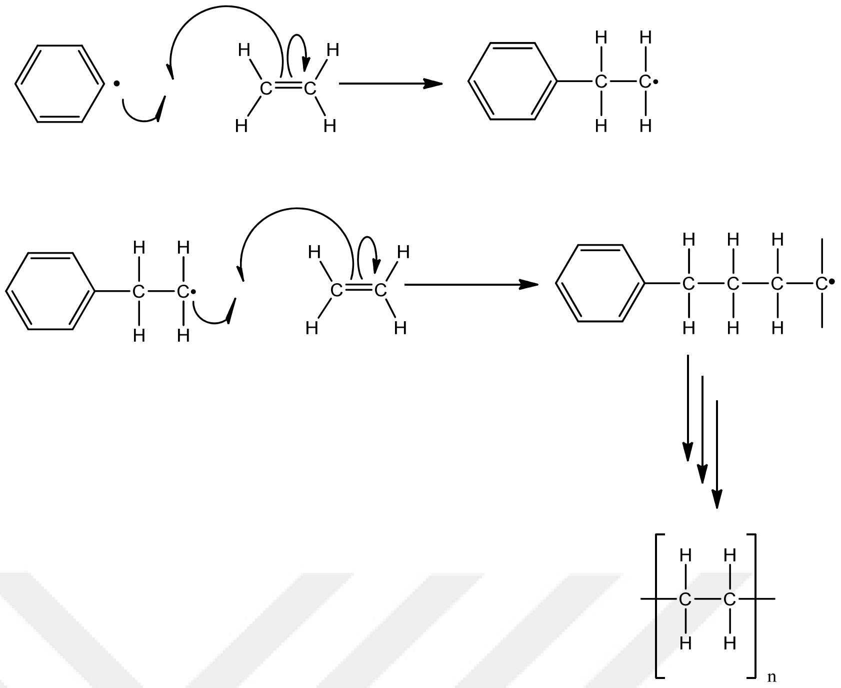


Figure 1.11 Chain growth polymerization reaction mechanism

Initiator molecules can be obtained in multiple ways which physical effects or chemical effects (Figure 1.12).

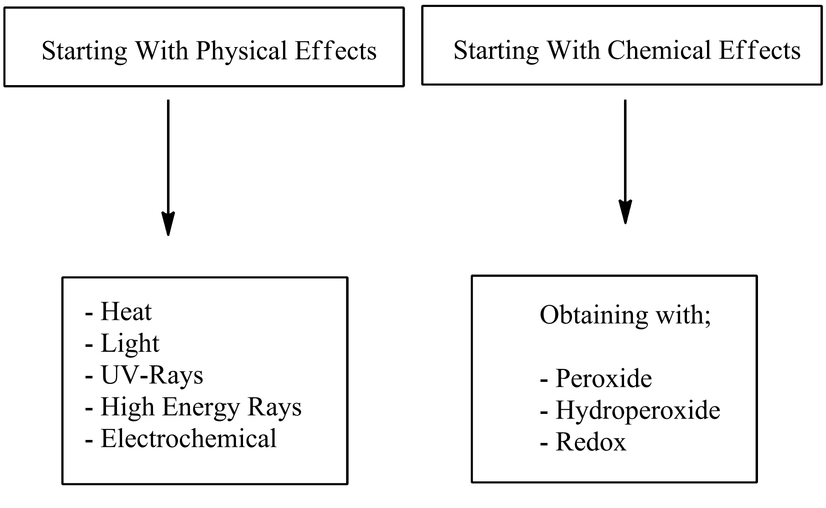


Figure 1.12 Initiator molecules obtained methods

1.4.2 Photochemical Initation

Under the effect of visible or UV-rays monomers are becoming the polymerization by free radical is called photopolymerization.

Most photopolymerization reactions are chain-growth polymerizations and initiated by the absorption of visible or ultraviolet light. The light may be absorbed either directly by the reactant monomer (direct photopolymerization), or else by a photosensitizer which absorbs the light and then transfers energy to the monomer (Fouassier, Allonas, & Burget, 2003). Benzoin and its derivatives are the most widely used photoinitiators for radical polymerization of vinyl monomers (Figure 1.13).

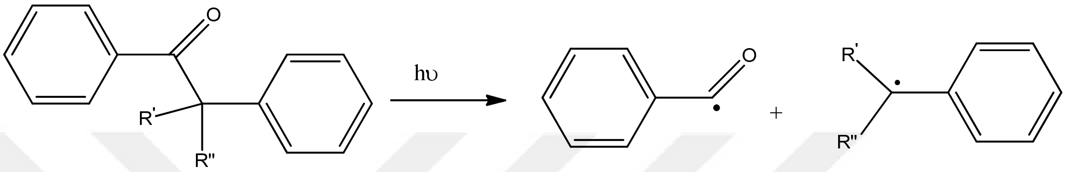


Figure 1.13 Benzoin and its derivatives as the photoinitiator

1.5 Graft Polymerization

Graft polymers are sliced copolymers with a linear backbone of one side. This can be presented below figure as species A and B (Figure 1.14).

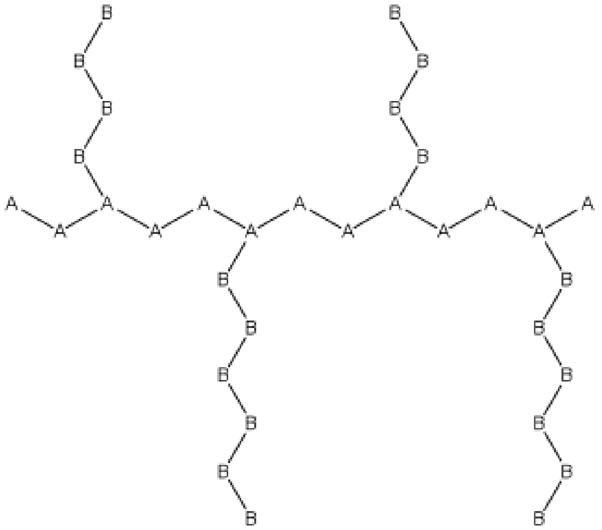


Figure 1.14 Structure of graft polymers

Graft polymers contain side chains that have wormlike conformation, compact molecular dimension, and chain end effects due to their structures (Feng et al., 2011).

It is widely used in the grafting these method;

1. Direct method; polymers are irradiated in the contents of monomer solution.

2. Radiation of polymers containing peroxide; polymers are irradiated in air medium. Peroxides in polymer can be activated during irradiated to produce radicals.

3. Grafting initiated by pre-presence radicals; firstly polymer is irradiated in air medium and after added in the reaction medium which contain monomers (Ranogajec, 2009).

1.6 Curing with UV-Ray Polymerization

1.6.1 Ultraviolet-Rays

Ultraviolet light is an electromagnetic radiation present in sunlight with a wavelength from 100 nm to 400 nm (Figure 1.15). UV radiation is also produced by electric arcs and specialized lights such as mercury-vapor lamps, tanning lamps, and black lights (Narayanan, Saladi, & Fox, 2010).

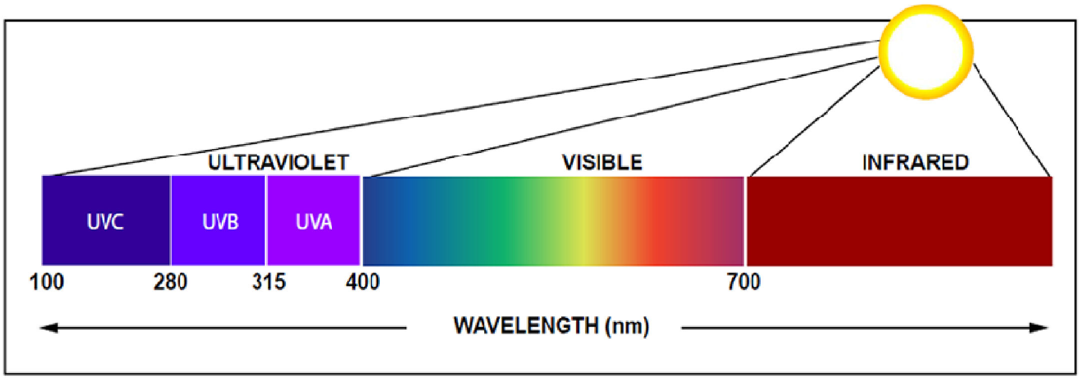


Figure 1.15 General light spectra

1.6.2 Crosslinkers

A crosslink is a bond that links one polymer chain to another. Crosslink can be covalent bonds or ionic bonds.

A Crosslinking molecule has main functional groups listed below;

1. Primary amines (-NH_2): This group can be exists at the N-terminus of polymer chain. This reactive group might have a positive charge thus do crosslinking.

2. Carboxyls (-COOH): It may bond through oxygen and carbon-carbon double bond.

3. Sulfhydryls (-SH): These must be reduced to sulfhydryls to make them available for crosslinking by most types of reactive groups.

4. Carbonyls (-CHO): Ketone or aldehyde groups can be oxidizing (Thermo, 2009).

According to the e-book prepared by the firm ThermoFischer Scientific, crosslinkers are selected their chemical reactivities (i.e., specificity for particular function groups) and other chemical properties that affect their behavior in different applications:

> "- Chemical specificity refers to the reactive targets of the crosslinker's reactive ends. A general consideration is whether the reagent has the same or different reactive groups at either end.
>
> - Spacer arm length refers to the molecular span of a crosslinker. A related consideration is whether the arm is.
>
> - Water-solubility and cell membrane permeability of a crosslinker affect whether it can permeate into cells or crosslink hydrophobic proteins within membranes.

These properties are determined by the composition of the spacer arm or reactive group.

- Spontaneously reactive or photoreactive groups in a crosslinker affect whether it react as soon as it is added to a sample or can be activated at a specific time by exposure to UV light (Thermo, 2009)."

Cross-links can be formed by some chemical reactions with the effect of heat, pressure, pH and radiation. As an example, electron beam processing is used to cross-link the C type of cross-linked polyethylene (Figure 1.16) (Rezanejad & Kokabi, 2007).

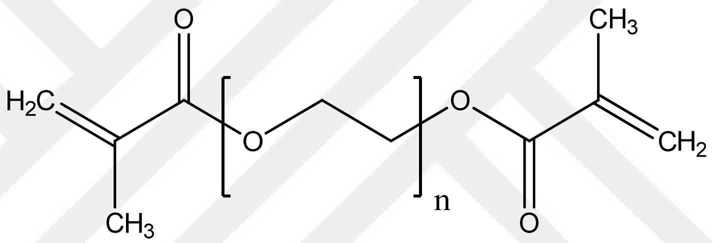


Figure 1.16 Polyethylene glycol dimethacrylate crosslinker

1.6.3 Crosslinking Applications

These applications can be summarized in five basic topics. These contains the properties of wettabiliy, adhesion, responsive, lamination and modificative membranes (Zuwei et al., 2002; Hintze-Brüning & Borgholte, 2000; Sebra, Kasko, Anseth, & Bowman, 2006; Ranby et al., 2001; Bakker, Bühlmann, & Pretsch, 1999).

1.6.3.1 Biomedical Applications

Grafted polymers are used in medicinal area as anti-bacterial, lubrication and anti-biofouling materials and as biosensor, immobilizing enzyme, cell attachment and culture. (Larsson, Ekblad, Andersson, & Liedberg, 2007; Shearer, Paik, Hoover, Haynie, & Kelley, 2000; Yamada, Iizawa, Yamada, & Hirata, 2006; Yeh, Kizhakkedathu, Madden, & Chiao, 2007; Zhu, Gao, Guan, & Shen, 2003)

1.6.4 UV-Curing Polymerization

Crosslinking agent and a UV lamp are used to generate radicals to obtain film with a free radical polymerization. The films are in general solid and compact. Initiators become reactive when exposed to ultraviolet light. The most important step of photochemical reactions is the energies from a given light source to create active monomeric particles that begin the polymerization (Black, 1987).

UV curing structures can be performed with the UV light that contain photosensitive organic groups which include unsaturated carbon-carbon double bond. This structure is called acrylic structure (Figure 1.17).

General reaction mechanism is listed below;

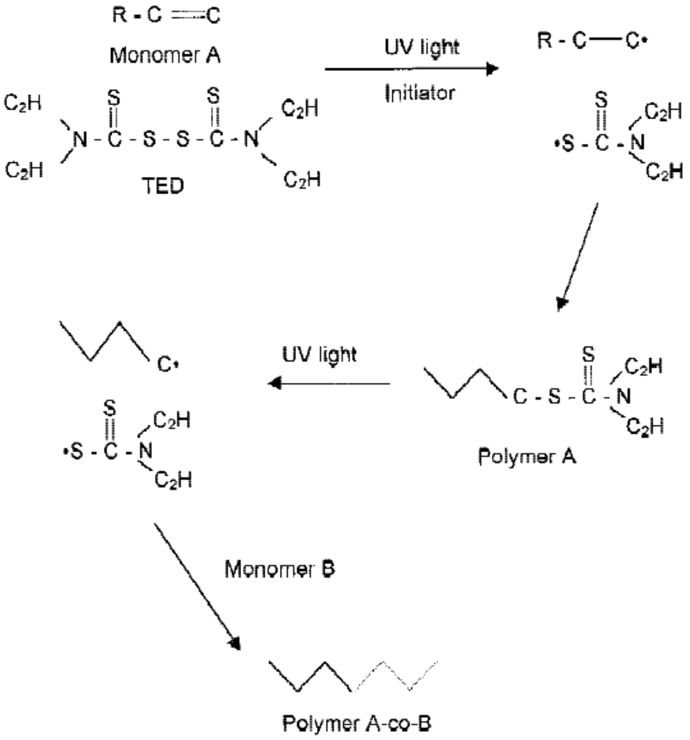


Figure 1.17 Example of UV-ray polymerization reaction (Ward, Bashir, & Peppas, 2001)

Polymers formed by UV rays are usually used as coating materials. Primary uses of coatings hardened with UV rays; paper, plastic, wood, metal and the coating of optical fibers.

The advantages of curing the coating with UV rays;

- Solvent free

- It hardens quickly due to the high reaction rates.

- Curable at room temperature. Therefore it is uses coating to heat-sensitive materials.

- It has high physical strength.

1.6.4.1 Use Areas of UV-cured Obtained Films

The coatings cured by UV rays are used in paper and varnishes. Coatings on printing inks provides to highly brightness, surface protection, resistance of water and other contaminants. These are used in the production of magazines and book covers, postcards and packagings.

In the case of plastics, they use hard and wear resistant surfaces. There are also decorative applications (Sepeur, Kunze, Werner, & Schmidt, 1999).

In furniture kitchen and bathroom cabinets, the wooden types of coating thin films usually polyester, acrylic or urethane/acrylic blend are used instead of heat-sensitive materials (Jung, Lee, Cho, & Ha, 1998).

To protect the metals against corrosion, metal cans for the packaging of foods, these polymeric UV-cured coating products are used. They are also used in the cables of motors, transformers and generators (Balan & Burget, 2006).

Optic fibers, blocked due to microcracks can be conserved by UV-cured coatings (Masson, Decker, Andre, & Andrieu, 2004).

Video disks, disk players and glass surfaces are coated with UV protection against moisture (Komaki et al., 2002).

1.7 Environmentally Sensitive Chitosan

Chitosan can be modificated in various reactions for the possible application areas to vary the solubility. Chitosan has also some useful properties such as biocompatibility, biodegradability, and no toxicity to live organism and used in the fields of biotechnology, pharmaceutics, cosmetics and polymer technologies.

Especially in polymer technologies, chitosan and its derivatives have awakened considerable recent interest because chitosan has many reactive groups like -NH_2 and -OH. Due to these groups, modified chitosan can be made pH-, glucose-, thermo- and UV-sensitive to various chemical substances (Alina Sionkowska, Planecka, Lewandowska, & Michalska, 2014; Qu, Wirsén, & Albertsson, 2000; Rejinold, Chennazhi, Nair, Tamura, & Jayakumar, 2011; Sanoj Rejinold, Sreerekha, Chennazhi, Nair, & Jayakumar, 2011)

Chitosan can be grafted to let the formation of functional derivatives by covalent binding of a molecule. The properties of the graft copolymers are controlled by the characteristics of the side chains, including molecular structure, length, and number of monomers (Mourya & Inamdar, 2008).

1.7.1 UV-Sensitive Chitosan

Chemical modifications of chitosan are increasingly studied as specific esterification and etherification reactions and also crosslinking involving the -NH_2 group at the C-2 position or nonspecific reactions of -OH groups at the C-3 and C-6 positions (Morimoto, Saimoto & Shigemasa, 2003).

The cross-linking agents can be of varying length and contain other functional groups. Partial crosslinking by di/polyfunctional reagents enables the use of chitosan for metal adsorption in acidic medium. Cross-linking agents such as glutaraldehyde, ethylene glycol diglycidyl ether, glyoxal, epichlorohydrin, benzoquinone, cyclodextrin and sodium trimetaphosphate, sodium tripolyphosphate, or carboxylic acids (Figure 1.18) as for water soluble derivatives have also been used in various studies (Jeon & Höll, 2003; Martel et al., 2001; Martinez et al., 2007; Mcafee, Gould, Nadeau, & da Costa, 2001; Vieira & Beppu, 2005; Wan Ngah, Endud, & Mayanar, 2002; Crini, 2005).

In UV curing, radical species are generated by the interaction of UV light with a suitable photoinitiator, which induces the curing reaction of reactive monomers and oligomers at low temperature and quickly, with lower environmental impact and lower process cost than thermal process.

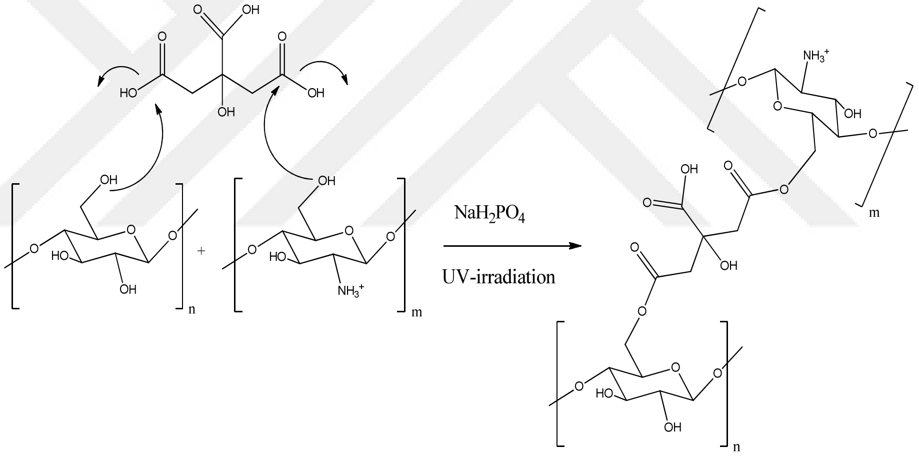
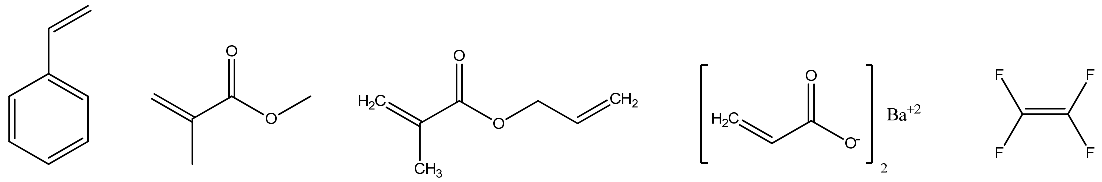


Figure 1.18 Cross-linking of chitosan by UV-irradiation

To make the UV-sensitive chitosan connected chemically various reactive groups on chitosan’s functional groups. For UV-irradiation purpose, mainly used these monomers; styrene (a), methyl methacrylate (b), allyl methacrylate (c), barium acrylate (d), tetrafluoroethylene (e) (Figure 1.19).



(a) (b) (c) (d) (e)

Figure 1.19 Structure of crosslinkers

To UV-irradiation crosslinking chitosan some methods are;

1.7.1.1 Monomeric Formation by Releasing the Direct Light

Some monomers can create free radicals by directly absorbing light. These radicals can form long chain interact with the monomer molecules.

$$M \xrightarrow{h\upsilon} M^*$$

1.7.1.2 Photoinitiator Use

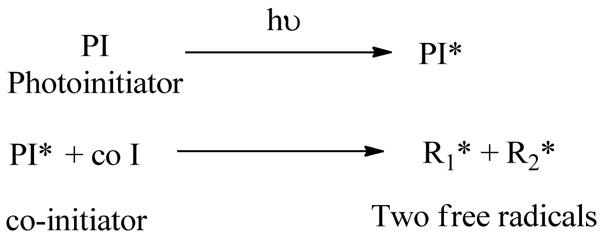
There are two types of photo-initiator for radical polymerisation;

1.7.1.2.1 Unimolecular Photoinitiator. These initiators when absorb light homolytic bond breakage creates free radicals.

$$\underset{\text{Photoinitiator}}{PI} \xrightarrow{h\upsilon} PI^* \xrightarrow{k} \underset{\text{Two free radicals}}{R_1^* + R_2^*}$$

The most important of these photoinitiators are aromatic carbonyl compounds. For example; benzoin derivatives, peroxy ketones, peroxides etc.

1.7.1.2.2 Bimolecular Photoinitiators. The excited molecules interact with the co-initiator in reaction medium generates radicals which initiate polymerization.



$$\underset{\text{Photoinitiator}}{PI} \xrightarrow{h\upsilon} PI^*$$

$$PI^* + \underset{\text{co-initiator}}{co\ I} \longrightarrow \underset{\text{Two free radicals}}{R_1^* + R_2^*}$$

For example, these molecules are diaryl ketones, benzyl, quinone derivatives, and ketocoumarins etc.

1.7.2 Usage of UV-irradiation Cured Chitosan

Photocrosslinkable chitosan derivatives have been widely explored for tissue engineering, wound healing, drug delivery and bioadhesive applications, antimicrobial finish, adsorbation, coating material applications for the unique ability to form covalent cross-links by photoactivation, a relatively mild chemical reaction that can be performed these applications (Huh et al., 2001; Lih, Lee, Park, & Park, 2012; Ono et al., 2001; Renbutsu et al., 2007; Tuzlakoglu, Alves, Mano, & Reis, 2004; Young Hyo Kim et al., 2001).

1.7.2.1 Tissue Engineering

The present generation of tissue engineering research is based on the seeding of cells onto porous biodegradable polymer matrixes. Chitosan and its derivatives have been reported as attractive contestants for skeleton materials because they degrade as the new tissues are formed, eventually without inflammatory reactions or toxic degradation (Tuzlakoglu et al., 2004).

1.7.2.2 Wound Healing

Chitosan can be used as a wound dressing material because of its biological and medicinal properties. Chitosan is a cationic polysaccharide and its adhesive properties are mainly based on ionic interactions with tissues or mucus layers (Lih et al., 2012).

1.7.2.3 Drug Delivery

Precise control of size and structure are critical design parameter of micellar systems for drug delivery applications. To control the size of an self-assembly, chitosan was depolymerized with sodium nitrite, and hydrophobically modified with deoxycholic acid to form the self-assembly in aqueous media (Kim et al., 2001).

1.7.2.4 Bioadhesive Applications

Photocross-linked chitosan and derivatives are exhibit good adhesive properties due to chitosan derivatives has biocompatible, biodegradable an non-toxic properties (Ono et al., 2001).

1.7.2.5 Antimicrobial Finish

Recent studies have been focused on the development of antibacterial surfaces to attain high functionality and high-value products. Poly(ethylene teraphthalate) (PET) is a basic material in the textile and plastics industries and modified with chitosan (Huh et al., 2001).

1.8 Polyethylene Glycol Dimethacrylate

Poly(ethylene glycol) dimethacrylates(PEGDMA) can be prepared by esterification reaction of poly(ethylene glycol) with methacrylic acid in presence of acid catalyst (Figure 1.20) (Kumar, Lahiri, Punyani & Singh, 2008).

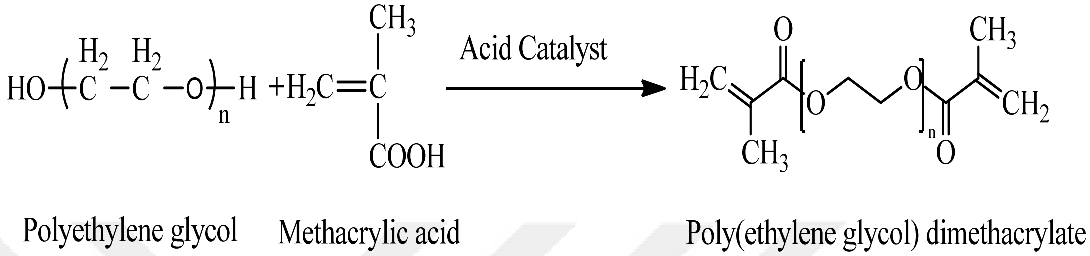


Figure 1.20 Synthesis of PEGDMA

Polyethylene glycol has a low toxicity, biocompatibility, hydrophilic properties and is used in a variety of products. Because of these properties of polyethylene glycol dimethacrylate uses in controlled drug release for making hydrogel; biomedical applications; dyes and heavy metal surface absorption of ions and crosslinking agent for polymers systems. (Killion, Geever, Devine, Kennedy, & Higginbotham, 2011; Mourya & Inamdar, 2008; Zheng & Wang, 2015)

1.9 Cinnamoyl Chloride

Cinnamoyl chloride has been recently used for preparing light-sensitive polymers (Lin, Tai, Ou, & Don, 2012). Cinnmoyl chloride is prepared by treating cinnamic acid with phosphoruspentachloride, phosphorus trichloride or thionyl chloride. Reaction reaction mechanism shows in Figure 1.21.

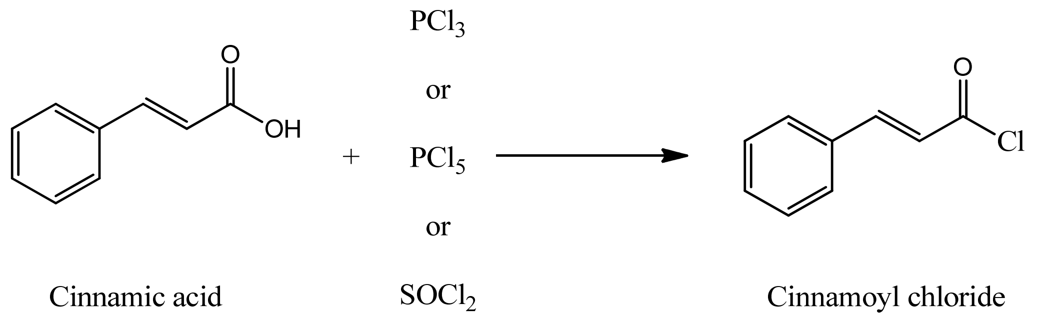


Figure 1.21 Synthesis of Cinnamoyl chloride

The aim of the study

It was aimed to develop ultraviolet(UV)-light irradiated photocrosslinkable chitosan derivative. For this purpose, CS will be dissolved in metansulfonic acid for the protection of -NH_2 functional groups by converting ammonium salt. Then, -OH functional groups of chitosan would undergo reaction with cinnamoyl chloride. The functionalized product will be reacted with polyethylene-glycol-dimethacrylate (PEGDMA) under UV light irradiation. Finally, photocrosslinkable chitosan derivative will be obtained after photopolymerization reaction which will be characterized further.

CHAPTER TWO
MATERIAL AND METHOD

2.1 Chemicals

Chitosan (CS) (low viscous) [448869], Cinnamoyl chloride (CINCl) [C81101], Poly(ethylene glycol) dimethacrylate (PEGDMA) [437468, average M_n 750, contains 900-1100 ppm MEHQ as inhibitor], Methanesulfonic acid ($MeSO_3H$) [471356] and Formic acid [F0507] were purchased from Sigma-Aldrich, respectively.

2.2 Instruments

Fourier Transform Infrared Spectroscopy (FTIR) characterization of pure chemicals (CS, CINCl) and the threshing films (CS-g-PEGDMA) were performed in transmission mode using FTIR (Perkin Elmer Spectrum BX-II) to analyze the chemical structure. The spectra were recorded with 25 scans at a resolution of 4 cm^{-1} in the range 4000–400 cm^{-1}.

Thermal behavior of CS-g-PEGDMA (sample weights were between 8-10 mg) was determined by using TGA (Perkin Elmer). TGA was performed at a heating rate of 10 °C /min range from 30 to 600 °C under nitrogen atmosphere with a flow rate of 1.0 mL/min.

2.3 Preparation of UV-Sensitive Chitosan

2.3.1 Synthesis of Cinnamoyl-modified Chitosan (CIN-CS)

3.00 g CS was dissolved in 30 mL $MeSO_3H$ with magnetic stirring at room temperature for 1 hour. Then 6.00 g CINCl was spilled into the CS solution in a round bottomed flask and the reaction proceeded under nitrogen atmosphere. After reaction, 1 N sodium hydroxide (NaOH) was added to the solution for precipitate the

CIN-CS product. Then it was filtered with filter paper and washed several times with distilled water to pH 7. The final product was lyophilized.

2.3.2 Synthesis of CS-graft-PEGDMA Films

0.40 g CIN-CS was dissolved in 4.0 mL formic acid. After PEGDMA (0.5 g, 0.8 g and 1.0 g, respectively) was added into the CIN-CS solution with continuous stirring. The solution was spilled in a petri dished glass. Then it placed in oven at 60 °C to attain the solid content of 70–80 %. Grafting and crosslinking of CIN-CS chains by PEGDMA were then carried out under UV irradiation by using a high-pressure lamp from Osram Ultra-Vitalux Ultraviolet with e27 base. The product was irradiated with UV-lamp for 4 h. After irradiation the film was cured at 60 °C in an oven for 3 h. Then it was washed with 1N NaOH solution and distilled water to pH 7. Finally the product dried at 60 °C for 3 h. The synthetic reaction mechanism and the final product structure of the crosslinked CS-g-PEGDMA graft copolymer are illustrated in Figure 2.1 and Figure 2.2 (Lin et al., 2012).

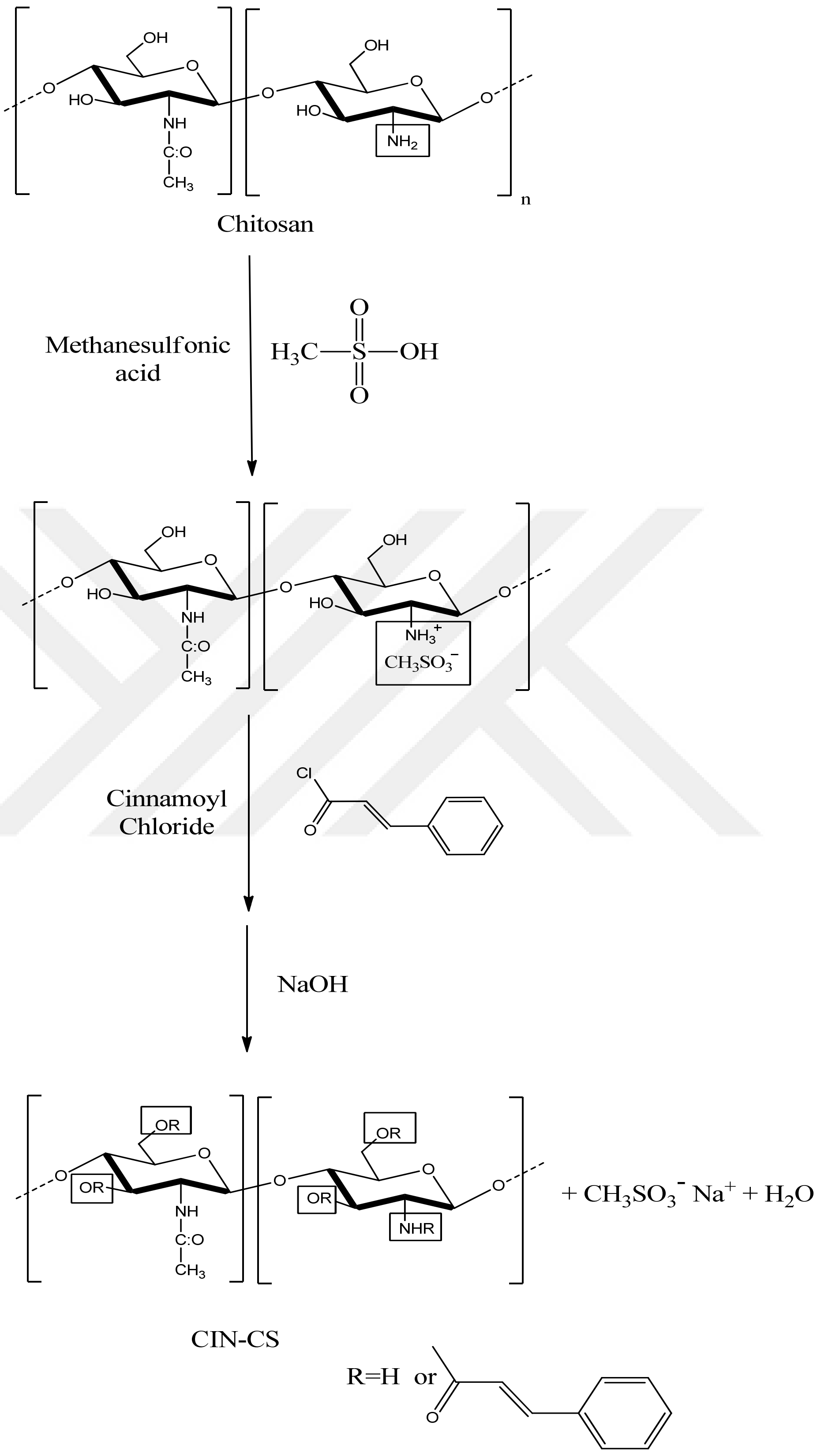


Figure 2.1 Reaction mechanism CIN-CS (Lin et al., 2012)

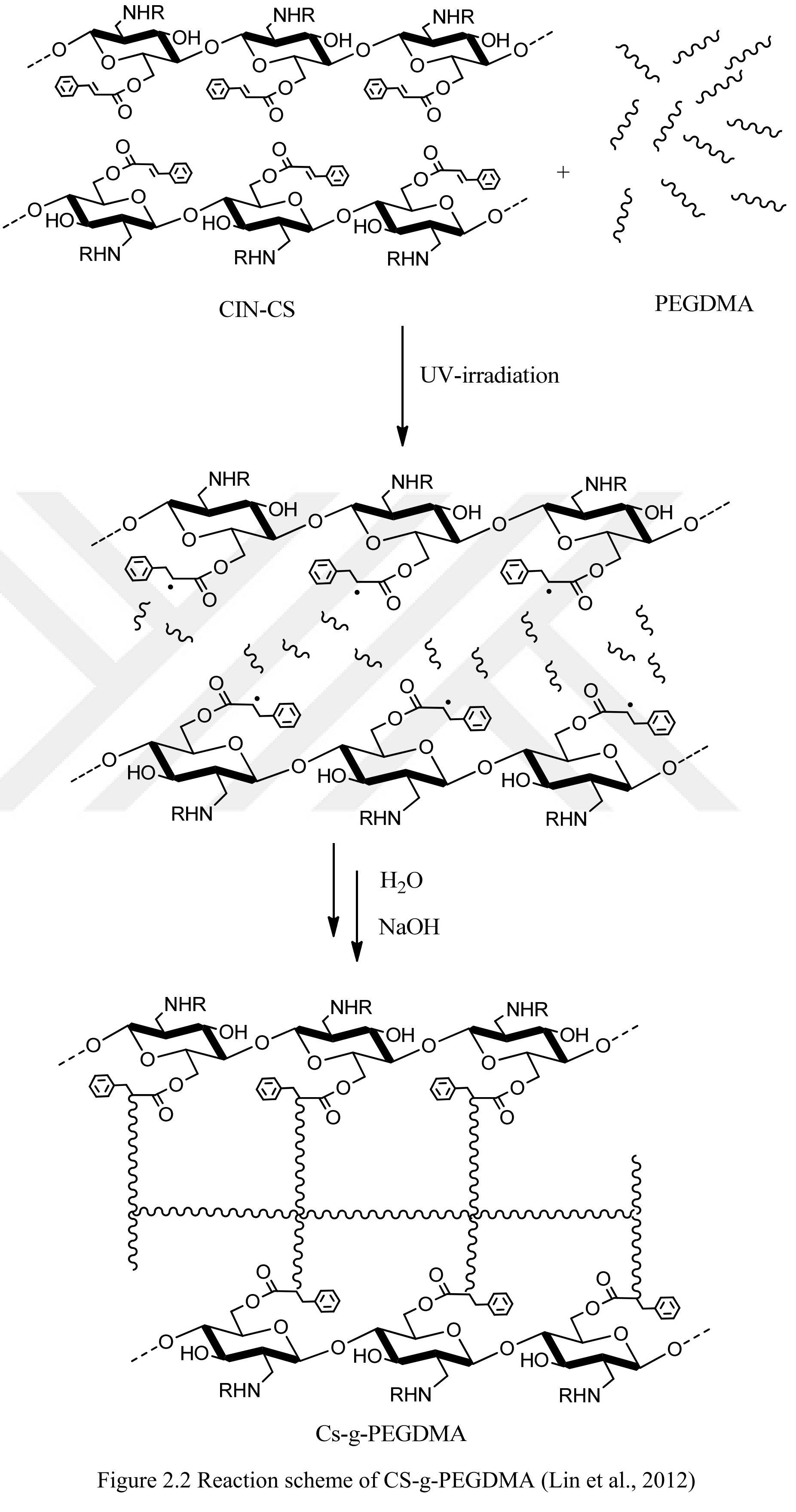


Figure 2.2 Reaction scheme of CS-g-PEGDMA (Lin et al., 2012)

CHAPTER THREE
RESULTS AND DISCUSSIONS

3.1 Results and Discussions

3.1.1 Synthesis

In the synthesis for dissolving CS, $MeSO_3H$ was used as solvent. $MeSO_3H$ was also used to protect the amino group on the CS. CINCl was added directly in CS solution after dissolution. In this way, the reaction occurs via the hydroxyl groups on the CS.

3.1.2 FTIR Results

FTIR spectroscopy is useful method for qualitative and quantitative analysis of chemicals and polymers which having absorbance in infrared region between 400-4000 cm^{-1}. Figure 3.1 shows FTIR spectrum of the pure CS, modified CIN-CS and different CS-graft-PEGDMA (CS-g-PEGDMA) samples, respectively.

In pure CS's spectrum, the absorbance band between 3550-3200 cm^{-1} (band at 3355 cm^{-1}) is due to –OH and $–NH_2$ stretching. The characteristics bands observed at 1664 cm^{-1} and 1597 cm^{-1} are due to the vibration absorption of C=O groups stretching of amide I and –NH bending vibration of amide II, respectively. The absorption bands at 1460 and 1314 cm^{-1} are assigned to C-H bending of ring. The strong absorption bands at 1155 , 1079 and 990 cm^{-1} are belonging to C-O stretching of glucose rings.

In modified CIN-CS's spectrum, the absorption band at between 3350-3450 cm^{-1} (band at 3445 cm^{-1}) is belong to -OH and $-NH_2$ stretching of CS. Compared to FTIR spectrum of pure CS, the new strong band at 1714 and 1202 cm^{-1} are due to stretching C=O and -C-O bonds of ester groups and these evidence that –OH grups reacted with cinnamoyl chloride to form modified CIN-CS. The absorption band at 1636 cm^{-1} is the C=C double bond which belong to CINCl. This band confirms the existence of double bond which can be uses as a initiation place of free radicals. The absorption bands at 864, 766 cm^{-1} are observed phenyl ring of CINCl remained unchanged, respectively. The band at 1166 cm^{-1} due to -C-O stretching.

In the spectrum of CS-graft-PEGDMA (CS-g-PEGDMA) polymer film, which was prepared by UV-irradiation different samples a,b and c, in addition to the characteristic absorption bands from CIN-CS, CS-g-PEGDMA films has characteristic bands too. The strong absorption band at 1714 cm^{-1} of CIN-CS shifted to around 1725 cm^{-1} in the samples of crosslinked CS-g-PEGDMA films due to stretching of C=O bonds. In addition disappearance of (C=C) bands in Cinnamoyl groups at 1636 cm^{-1} is an evidence that PEGDMA grafted polymerization onto CIN-CS by free radicals. The absorption band at 2868 an 2879 cm^{-1} is due to C-H stretching. The bands at 1253, 1247, 1099, 1096, 1037 and 1034 cm^{-1} are C-O stretching frequency of PEGDMA, respectively.

These results showed and proved that PEGDMA polymer was successfully grafted onto the CIN-CS. In addition some characteristic bands shown the PEGDMA polymer chains would crosslinked CS chains by cinnamoyl structure.

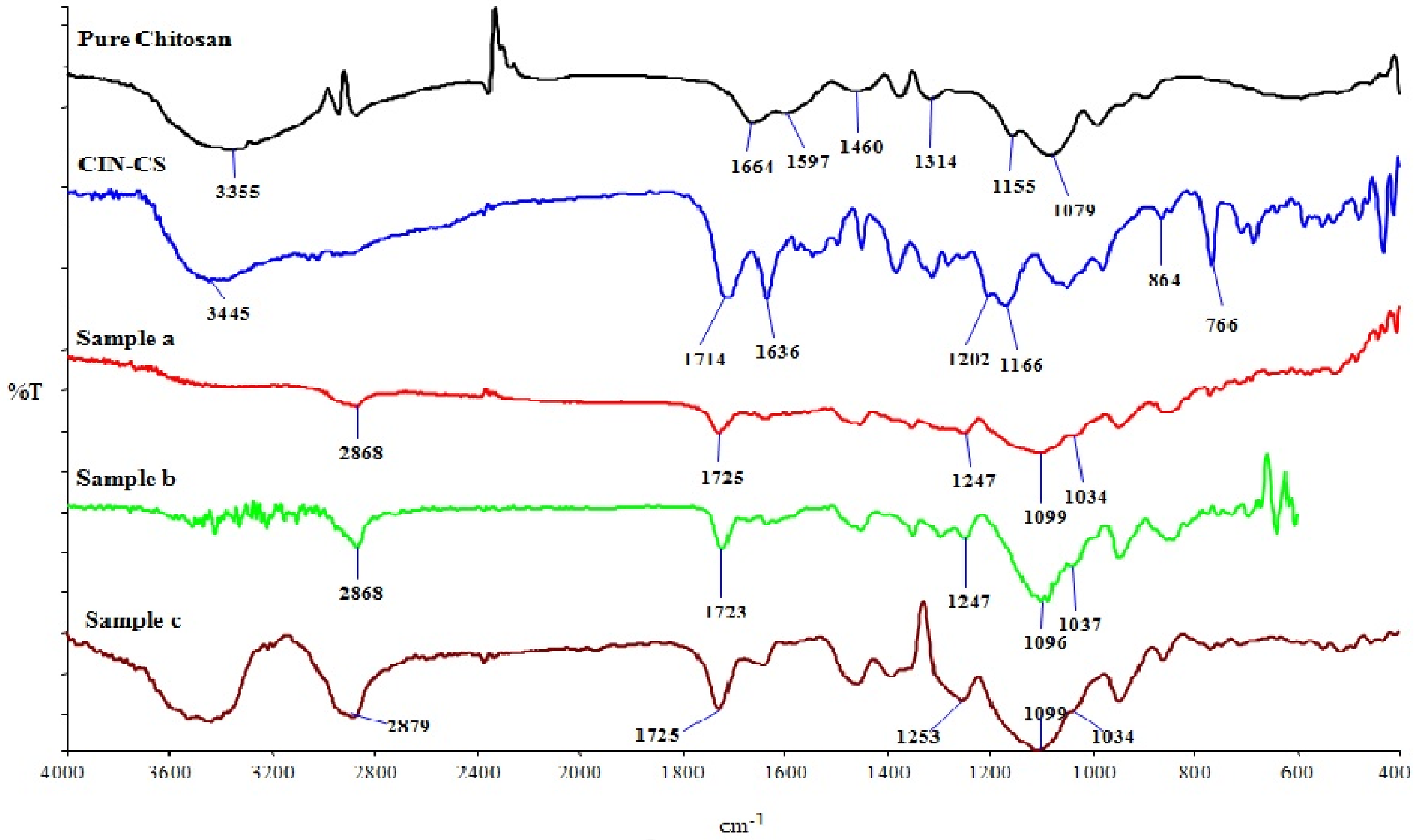


Figure 3.1 FTIR spectra of CS, modified CIN-CS and CS-g-PEGDMA films

3.1.3 Swelling Studies

The CS-g-PEGDMA films can be used as gel membrane form. Since, they have hydrophilic residues. For swelling kinetics studies of CS-g-PEGDMA film were cut into a piece with 1 cm in diameter. Then, they were weighed and engrossed pH 7.4 at 37 °C buffer solution. After immersion in the buffer solution, the film then removed and the excess water on the surface of the film is dried with filter paper. Then the weight of the swollen films was taking with an interval of 5 minutes. CS-g-PEGDMA film containing two different amounts of PEGDMA was swelling test which contain 0.5 g and 0.8 g PEGDMA, respectively. The degree of swelling was calculated by the following equation (3.1):

$$S\,(\%) = \frac{W_t\text{-}W_0}{W_0}x100 \tag{3.1}$$

where S is the equilibrium water swelling degree (%), W_t and W_0 are the weight of swollen films at time t and dry dried films, respectively.

As it is seen from Figure 3.2 the lower PEGDMA concentration film has the bigger swelling behavior was observed in pH 7.4 solution. It is an anticipated result because it is generally known that the swelling increases with decreasing crosslinking ratio (Buchholz & Graham, 1998). Therefore liquids becomes difficult enters between the polymerized film molecules. As can be seen from Figure 3.2, higher PEGDMA concentration films has an amount of swelling at around 50% and its maximum swelling ratio for contains 0.8 g PEGDMA film is 52%. The lower PEGDMA concentration film has an amount of swelling at around 100%. Maximum swelling amount is 102% which can be seen Figure 3.2.

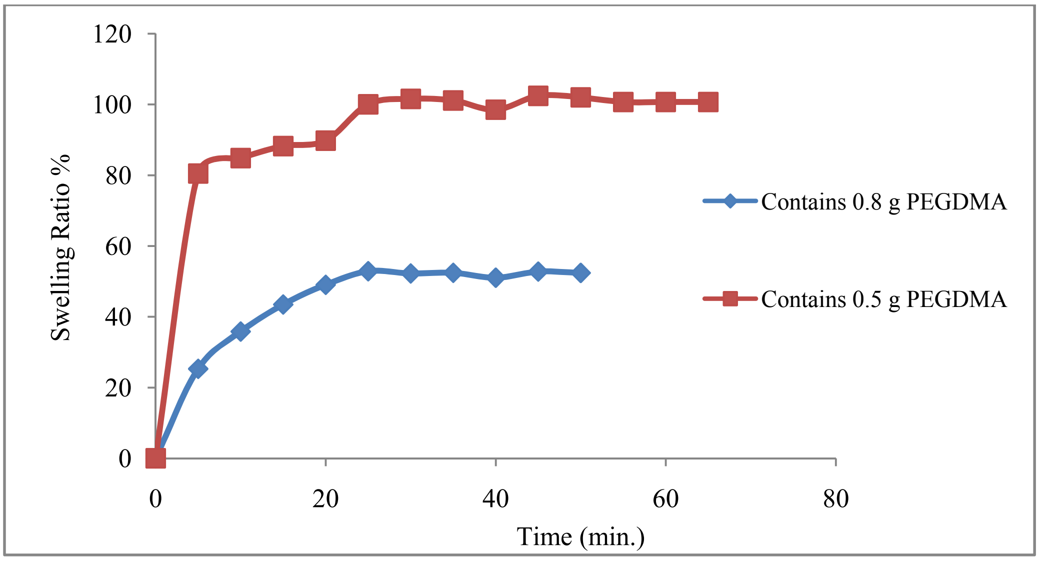


Figure 3.2 Swelling ratio of different amont PEGDMA content

For swelling kinetics of films was investigated by the following equation (3.2):

$$\frac{t}{S} = \frac{1}{S_{max}^{2}k} + \frac{1}{S_{max}}t \tag{3.2}$$

where S and S_{max} are the degree of swelling at time t and equilibrium solution content, respectively, and k is the rate constant. Straight line plots of t/S versus t for 0.8 g and 0.5 g PEGDMA contains film both show a good fit ($R^2 = 0.99$) can be seen Figure 3.3 and Figure 3.4. According to graphics the swelling process is complies the second order kinetic model. S_{max} (%) values are 102 and 52 for 0.8 g and 0.5 g PEGDMA contains film respectively. According to plots, the equilibrium swelling capacity of films decreased with increasing crosslinking of polymers chains. Equilibrium swelling capacity of films decreased with increasing crosslinking of polymers chains. Less crosslinked polymers chains are more freely in gel network and cause chain hardening and absorption of large amount of solution. The results were summarized in Table 3.1.

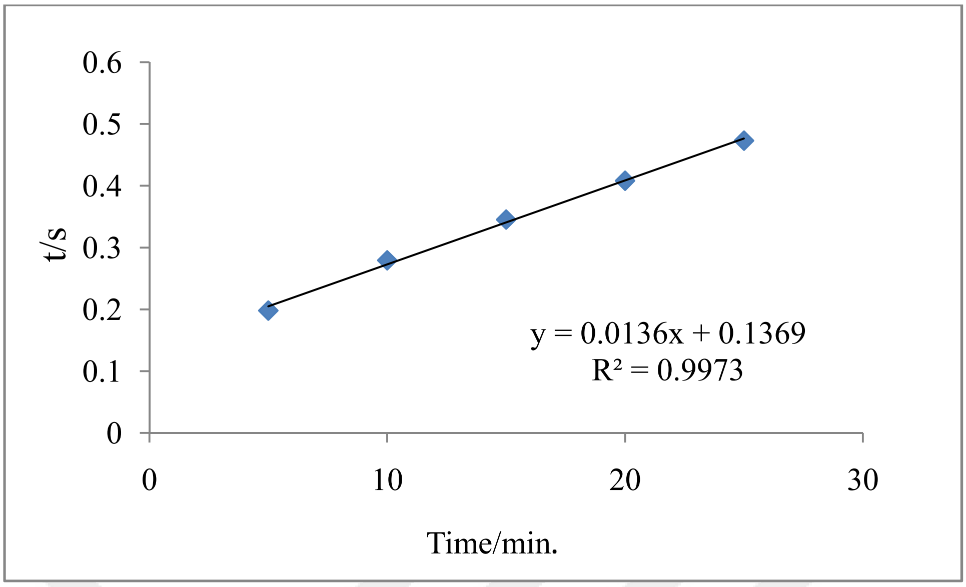


Figure 3.3 Swelling plot of the film contains 0.8 g PEGDMA

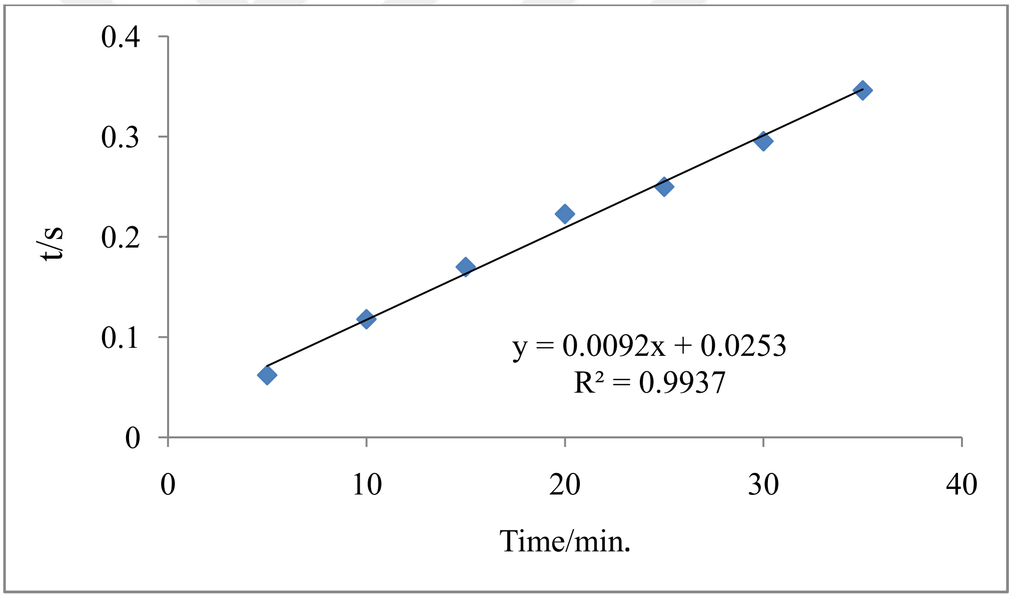


Figure 3.4 Swelling plot of the film contains 0.5 g PEGDMA

Table 3.1 Swelling kinetic values for different amount PEGDMA content films

Sample	$S_{experimental}$	S_{max}	k	r_0
Contain 0.5 g PEGDMA (a)	102.41	108.69	0.0033	0.0253
Contain 0.8 g PEGDMA (b)	52.86	73.52	0.0013	0.1369
Contain 1.0 g PEGDMA (c)	could not be determined			

3.1.4 Thermal Properties of CS-g-PEGDMA Films

Thermal analysis of copolymer films provides information to us about a better understanding of the structure of the copolymer and the interaction between the components. Figure 3.5 and Figure 3.6 showed as thermal degradation (TG) curves and derivative mass-loss (DTG) curves of different CS-g-PEGDMA film samples which contains 0.5 g (a), 0.8 g (b), 1.0 g (c) PEGDMA, respectively. They are summarized in Table 3.2.

Table 3.2 TG/DTG data of the samples

Sample	First stage			Second stage		
	T_{onset} °C	T_{max} °C	% mass loss	T_{onset} °C	T_{max} °C	% mass loss
a	295	320	13	358	399	85
b	40	81	1.4	369	404	92
c	39	80	2.3	363	406	83

Sample a, which contains 0.5 g PEGDMA, has mass losses at two stages, first stage is due to degradation of CS chains that started at 295 °C. Second stage is thermal degradation of PEGDMA chains. It has been started at about 358 °C and attains a maximum at 400 °C where the mass loss is due to degradation of PEGDMA chains.

Sample b, which contains 0.8 g PEGDMA, has mass loss at two stages too. The first stage was clearly due to presences of small amount of moisture in the sample that T_{onset} started at 40 °C. Second stage is belonging to degradation of PEGDMA chains that peak at 404 °C.

Sample c showed the same thermal properties of samples b. It has two steps of mass loses too. The first is due to the moisture and second is due to degradation of PEGDMA chains at 406 °C.

According to these results, T_{onset} was increased due to the formation of the crosslink structure. The reason of the different values of T_{max} is increasing the grafting ratio and crosslinking density of the films due to amount of PEGDMA. After crosslinking with PEGDMA, the sample a which containing a smaller amount of PEGDMA film had a lower T_{onset} (358 °C) than the other films. In outward show, this is because chitosan chains crosslinking by PEGDMA that less limits the chain motions. Hereby the T_{onset} values of polymer film increased depending on crosslinking density.

Figure 3.5 showed as thermal degradation curves of different CS-g-PEGDMA film samples. As mentioned previously in the IR and swelling behaviour increasing the amount of PEGDMA increased the crosslinking density of the films.

In fact all films showed similar degradation behavior at temperatures according to the thermograms that showed Figure 3.5 and Figure 3.6. The T_{onset} of the sample b was nearly 5 °C higher than sample a. For sample c, it was nearly 2 °C higher than sample b.

As a result, T_{onset} and T_{max} values of polymer films were depending on the crosslinking density of polymer also these values are increased when PEGDMA amount increase.

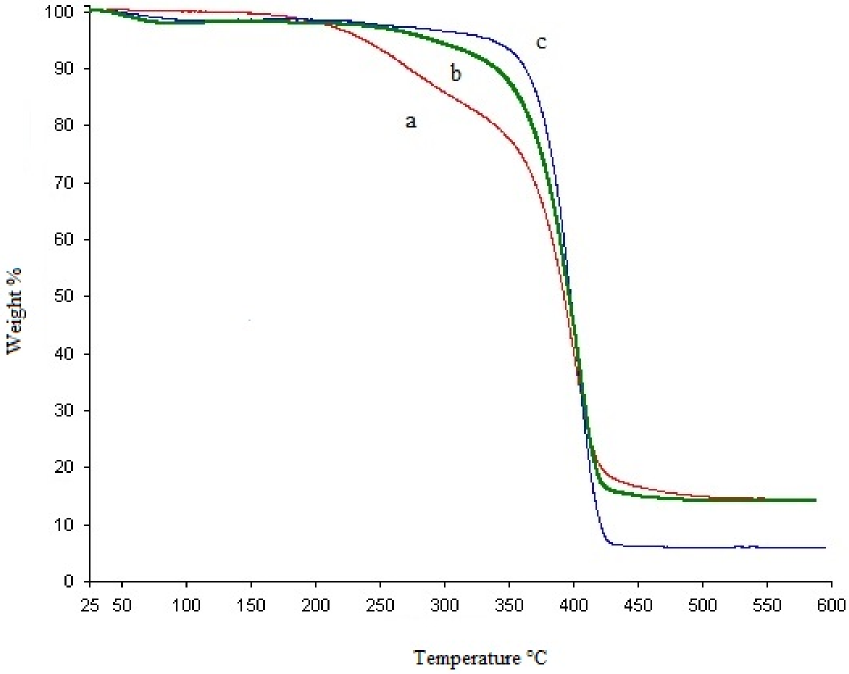


Figure 3.5 TG curves of sample a, b and c

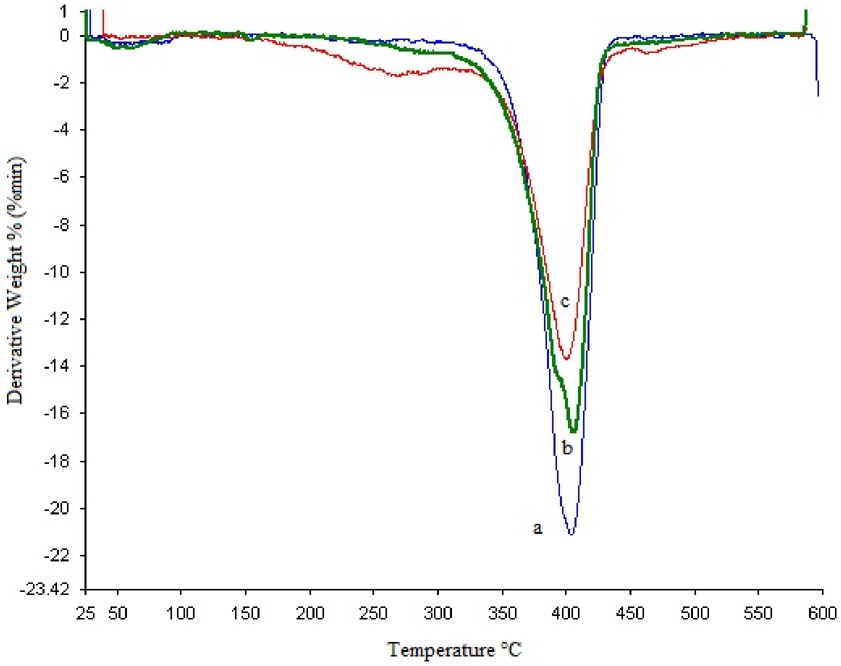


Figure 3.6 DTG curves of sample a, b and c

CHAPTER FOUR
CONCLUSIONS

This study aims to get a UV sensitive film by derivatization of the chitosan polymer with its side chains.

For this purpose, the chitosan (CS) has firstly been dissolved in methanesulphonic acid and then the amine groups of the chitosan have been kept in the from of ammonuium salt, which has enabled to continue the reaction by means of hydroxyl groups. After that, cinnamoyl chloride (CINCI) has been added in order to make chitosan and cinnamoyl chloride react. Polyethylene glycol dimethacrylate (PEGDMA) has been added to the solution (CIN-CS) and the last solution has been exposed to UV rays. By means of UV rays, double bonds C=C over the cinnamoyl chloride attached to the chitosan have created radicals and attacks by these radicals to the PEGDMA have resulted in cross linked polymer film (CS-g-PEGDMA).

FTIR has been used for the characterization of the films. In the FTIR spectra, the existence of C=O at 1714 cm^{-1} in the CIN-CS and C-O-C stretching vibrations at 1202 cm^{-1} prove that the CINCI has reacted with hdyroxyl groups over the CS. Moreover, the existence of the absorption band C=C at 1636 cm^{-1} in the CINCI shows that the CINCI has remained intact. The C=C band also enables the creation of radicals. The C=C absorption band at 1636 cm^{-1} was disappeared and it showed that the cross linking in the FITR spectrums of the CS-g-PEGDMA films obtained with UV rays had been successful. In addition to this, the absorption bands for C=O and C-O-C at 1714 cm^{-1} and 1202 cm^{-1}, respectively. They shifted to 1725 cm^{-1} and 1304 cm^{-1} respectively. This proved that PEGDMA was present. All these results indicated that PEGDMA was successfully crosslinked chitosan chains.

In order to see the physical strength swelling of the polymer films, swelling tests have been realized at pH 7.4 and at 37 °C. According to the test results, the more the quantity of PEGDMA increased the less the swelling. It has been observed as the polymer bonds have had more cross-linking. In the light of these results, polymer films which contain 0.5 g PEGDMA have had less cross linking and more swelling rate.

For the thermal stability and characteristics of the films, a thermal gravimetric analysis has been carried out. It has been proved that cross linking rate was directly proportionally to the PEGDMA quantity. Expected results have been observed in TGA thermograms. It has been observed that films containing less PEGDMA had low T_{max} value. As expected, films containing 0.5 g, 0.8 g and 1.0 g PEGDMA respectively have a T_{max} quantity of 399, 404 and 406 °C, respectively. All the samples have been degradated in two steps; according to Fig. 3.5 and 3.6, the first degradation was the vaporization of the damp in the samples and the second step was the degradation of PEGDMA chains. Differently from the others, the first degradated phase in the film containing 0.5g has been at 295 °C and it belongs to chitosan.

In conclusion, the chitosan can be derivatizated with its various functional side groups. In this study, the chitosan has been crosslinked with PEGDMA by means of the reaction with hydroxyl groups of chitosan. This cross-linking has been realized in the presence of UV rays, without adding any photo initiator or radical initiator. By this means, the dissolution of a great quantity of chitosan in an organic dissolver has been prevented and a film hardening in a few hours has been obtained.

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