Utilization of Ultraviolet radiations for the closure of wounds

By

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CUI/SP19-RPH-021/LHR

MS Thesis

In

MS Physics

COMSATS University Islamabad

Lahore Campus –Pakistan

Fall, 2020

COMSATS University Islamabad

Utilization of Ultraviolet radiations for the closure of wounds

A Thesis Presented to

COMSATS University, Islamabad

Lahore Campus

In partial fulfillment

Of the requirement for the degree of

MS

(Physics)

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A Post Graduate Thesis submitted to the Department of Physics as partial fulfillment of the requirement for the award of Degree of MS (Physics)

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I **Anzal Akbar** (**CUI/SP19-RPH-021/LHR**) hereby declare that I have produced this work, which is presented in this thesis, during the scheduled period of study. I also declare that I have not taken any material from any source except to refer to wherever due. If a violation of HEC rules in the research project has occurred in this thesis, I shall be liable to punishable action under the plagiarism rules of the HEC.

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CERTIFICATE

It is certified that **Ms. Anzal Akbar (CUI/SP19-RPH-021/LHR)** has carried out all the research work related to this thesis under my supervision at the Department of Physics, COMSATS University (CUI), Lahore and the work fulfills the requirement for the award of the MS degree.

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In the name of ALLAH,

The Most Beneficent,

The Most Merciful.

MOTIVATION FROM ALLAH

ALLAH (GOD) Say:

"Travel through the earth and see how ALLAH did originate creation;

So will ALLAH produces a later creation:

For ALLAH has power over all things".

Surah Al-'Ankabut [29:20]

DEDICATION

To ALLAH Almighty, my creator.

To my beloved Mummy Baba, **Mr.** and **Mrs. Akbar Ali**

For their endless love, support and encourage to believe in myself.

To Dr. Naima Amin, Dr. Arsalan Ahmed Ansari with my deepest gratitude

and warmest affection and all respected teachers

Who have been a constant source of knowledge and inspiration

To my wonderful siblings (Muzna, Areeba and Mohid)

And all supporting friends.

ACKNOWLEDGEMENTS

"One who follows a path to seek knowledge has his path to Paradise made easy by ALLAH" (Hadith)

All admire and thanks to ALLAH, the lord of humankind and all that exists, for all his blessings, decency, guidance at every stage of our life. Who blessed us courage, strength and audacity to complete this work in less time.

It is the honor for me to express deep gratitude and respect to **Dr. Muhammad Asif** Head of Physics Department at CUI, Lahore, for providing us all kinds of project facilities and for his valuable support.

I would like to express deepest gratitude to my thesis advisor, **Dr. Naima Amin**, from the core of my heart for her sincerity, and endless encouragement during our research thesis to complete. Her insightful opinion and advice helped us through many technical and professional difficulties. My deepest gratitude extends to her and wish her a lifetime of happiness.

I would like to thank my co-advisor, **Dr. Arslan Ahmed Ansari**. I am eternally grateful for his enthusiastic supervision, sharp and insightful observation in this research, and most importantly, his kindness. Without his guidance and persistent support this thesis could not be possible to complete. I would also like to mention all our teachers for their Support.

I am thankful to **my parents**, whose prayers and love give me the courage to work hard. Thanks for your support and continuous care. May Allah bless them all with happiness and triumph (Ameen).

Anzal Akbar (CUI/SP19-RPH-021/LHR)

ABSTRACT

Utilization of Ultraviolet radiations for the closure of wounds

Existing wound closure systems, e.g. sutures and staples contain many disadvantages i.e., discomfort to patient, allergenic effect, complex procedures and high cost. UV curable adhesives are more advantageous in their applications to damaged tissues as compared to other conventionally available tissue sealants. The UV curable systems are mechanically stable, biocompatible, cause no toxicity, and most importantly have fast-curing rates, and better cohesive and adhesive strengths. Utilization of polymeric chitosan is the best strategy to overcome undesired problems in wound management. The sealing characteristics of polymeric adhesive chitosan has been studied. Hydrocaffeic acid modified chitosan (Ch-HCA) was prepared to enhance the mucadhesion strength and stability. Chitosan functionalized with photosensitive 4-azidobenzoic acid (AzCh) readily cross linked under the UV irradiations. Ultraviolet (UV) irradiation induces molecular changes in tissue biology by photochemical interactions. This approach is safe and quick in which wound could be efficiently sealed via photochemical reactions. AzCh-HCA thin films could behave as a strong tissue-adhesive and induce effective wound healing.

Key Words: Tissue sealant, biocompatible, Chitosan based adhesives, Photocurable, UV induced curing

CONTENTS

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NOMENCLATURES

UVR	Ultraviolet Radiations
CAGR	Compound Annual Growth Rate
Ch	Chitosan
HCA	Hydrocaffeic Acid
Az	Azido benzoic acid
Ch-HCA	Chitosan-Hydrocaffeic acid
AzCh	Chitosan Azide
AzCh-HCA	Hydrocaffeic acid-Chitosan Azide
DOPA	Dihydroxyphenylalanine
PVA	Poly-Vinyl Alcohol
MES	2-(N-Morpholino) Ethane-Sulfonic acid
EDC	Ethyl-3-(3-Dimethylaminopropyl) Carbodiimide
FTIR	Fourier Transform Infrared Spectroscopy
UV-Vis	Ultraviolet Visible
AFM	Atomic Force Microscopy

CHAPTER 1

INTRODUCTION

1.1.Ultraviolet Radiations

Ultraviolet radiations (UVR) are non-ionizing radiations radiated by sunlight and man-made sources. Ultraviolet (UV) radiations are electromagnetic radiations having a wavelength (100–400 nm) longer than x-rays (100 nm), but shorter than visible light (400–700 nm) (Fig. 1). Ultraviolet radiations have adverse effects on human health, but have some major advantages, including production of vitamin D. The effects of UVR on biological tissues vary immensely with the change in wavelength therefore, UVR is subdivided into three spectral regions; depending on the wavelength into ultraviolet A (315-400 nm), B (280-315nm) and C (100-280 nm) (Gallagher, Lee, Bajdik, & Borugian, 2010; Wilson, Moon, & Armstrong, 2012).

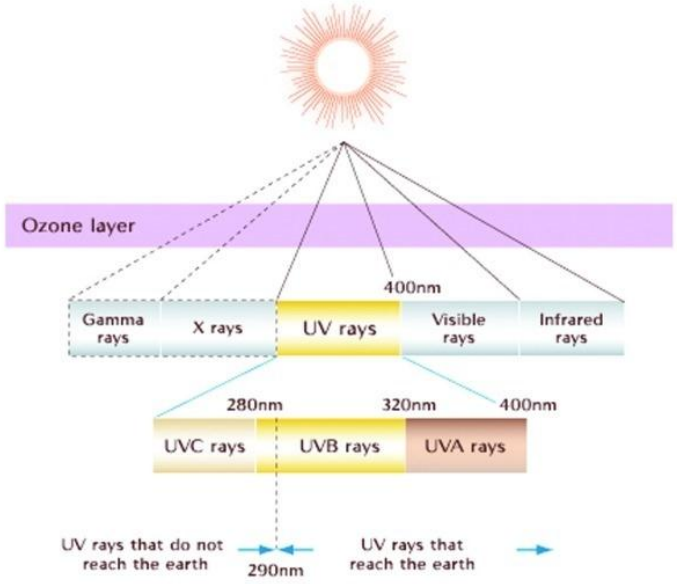


FIGURE 1 THE ULTRAVIOLET COMPONENT OF ELECTROMAGNETIC SPECTRUM

Emerging high-tech UV sources, such as LEDs, microwave-generated UV plasma, and lasers are increasingly available for biomedical applications. Light based technologies have different kinds of modalities related to wound management. Wound healing is a complicated and well-coordinated process that establishes the integrity of damaged tissues. Discomfort and morbidity are caused by wound and wound healing abnormalities. The

satisfactory aesthetic scar and the fast closure of wounds are the main objectives of wound treatments (Singer & Clark, 1999).

Recent medical procedures for improved patient care have been directed at finding better ways to be cost-effective and time-efficient. Conventional wound closure techniques e.g., sutures, staples and tapes have many drawbacks. Adhesives also exhibit some limitations such as less adhesive strength, inhibition of the natural wound healing process and toxic to wounds. Many studies confirm that controlled exposure of ultraviolet radiations induces skin homeostasis and effective wound healing (Burger, Jordaan, & Schoombee, 1985; Freytes, FERNANDEZ, & Fleming, 1965; Nussbaum, Biemann, & Mustard, 1994; WILLS, ANDERSON, BEATTIE, & SCOTT, 1983; Xiang, Liu, Yang, & Zhong, 2011). UV irradiation causes pathological and physiological changes in micro-organisms, human and non-human animals. Ultraviolet light has both useful and adverse effects depending on the intensity and exposure time (irradiation dose) (Hockberger, 2002).

1.1.1. Ultraviolet Material Interactions

UV radiations are non-ionizing electromagnetic radiations consistings of alternatively propagating electric and magnetic fields, having radiant energy. When UV light travels through the matter, some portion of the light absorbed during the passage. This absorption induces photochemical responses. The reactant molecules within the matter undergo excitation due to absorption of photons of light. (Fig. 2) These excitations then lead to produce reactions (Tuli & Bahl, 2010).

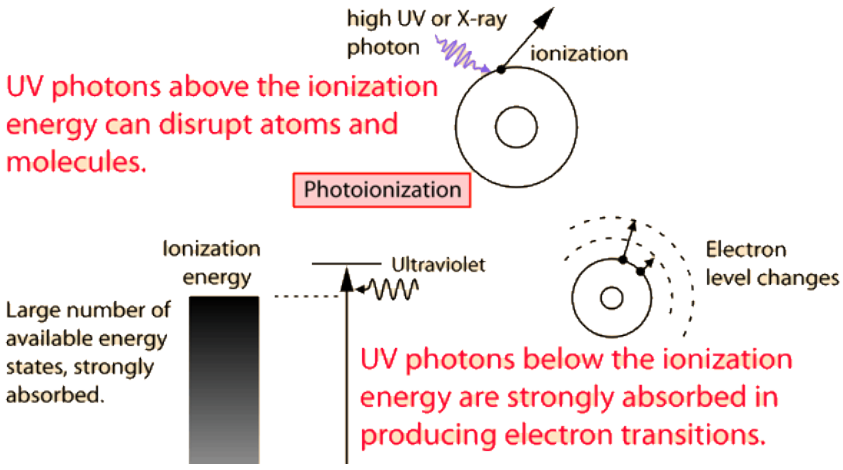


FIGURE 2 ULTRAVIOLET MATERIAL INTERACTION

1.1.2. Ultraviolet Irradiation of Wounds

UV irradiation of tissues involves sequential events starting from the absorption of the photons by chromophores, followed by molecular changes induced during photochemical reactions. Ultraviolet (UV) irradiation induces molecular changes in tissue biology by photochemical reactions. Exposure of ultraviolet (UV) radiations causes restoration of skin homeostasis and stimulates wound healing besides its antioxidant and anti-inflammatory effects (Gupta, Avci, Dai, Huang, & Hamblin, 2013).

Wound is an external injury induced to body tissues (e.g., skin, muscles) which leads to damages to any type of body cells, organ structures, membranes, blood vessels etc. Conventional wound closure techniques e.g., sutures, staples and tapes have many drawbacks. Sutures cause discomfort and pain, staples require anesthesia and tapes cannot be applied on all types of wounds due to their low tensile strength and lack of adhesion in wet and hairy areas (Marques et al., 2016).

Ultraviolet (UV) irradiation of wounds enhances granulation tissue formation and re-epithelialization. Exposure of Ultraviolet (UV) radiations firstly prevents hypopigmentation and secondly restores the natural number of melanocytes and further stimulates the distribution of melanocytes in re-epithelialized wounds. These radiations can stimulate extracellular matrix deposition, increase fibroblast activity and enhance epithelial thickness. Ultraviolet (UV) radiations have been used as antimicrobial to kill pathogen. The germicidal effect of UV is responsible for rapid healing as it inactivates the microorganisms (virus) by damaging their DNA. Therefore, these radiations can be applied to damaged tissue to accelerate wound closure rate. Extracorporeal ultraviolet (UV) irradiation of blood has been used to influence the immune responses (Gupta et al., 2013; Houghton, 1999).

Fig. 3 shows ultraviolet (UV) light spectrum and penetration of UV into the skin as a function of wavelength. The penetrating depth across the epidermal layers increases with the wavelength.

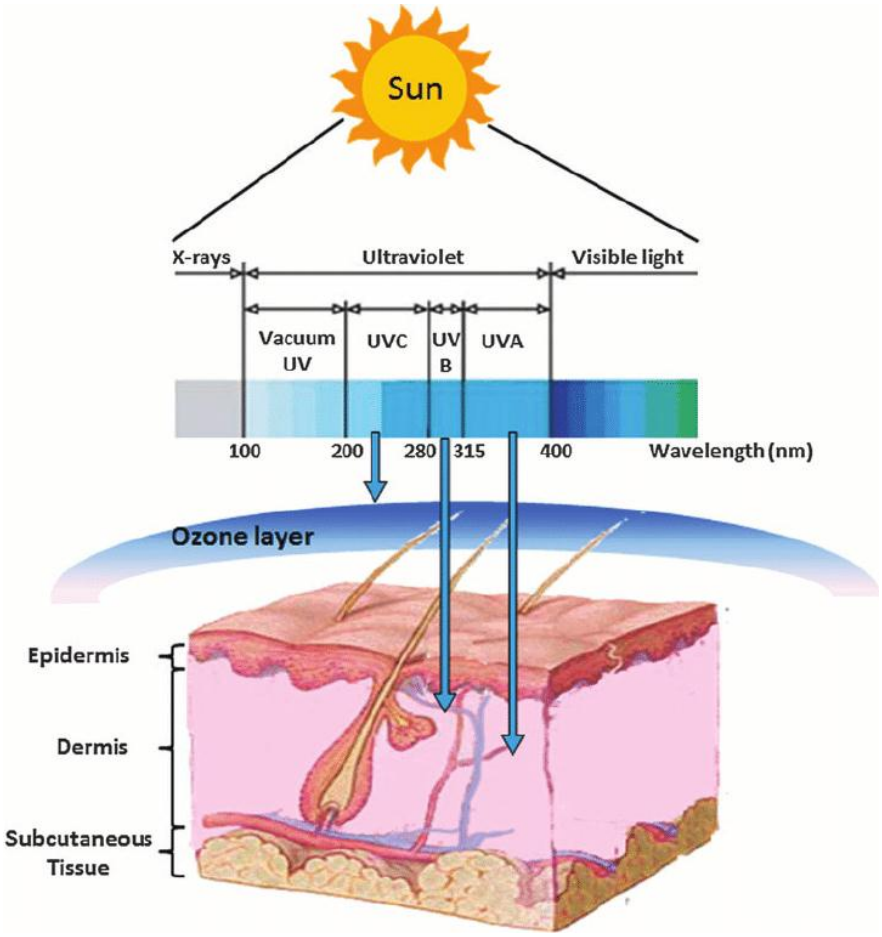


FIGURE 3 SPECTRUM OF ULTRAVIOLET (UV) LIGHT AND WAVELENGTH-DEPENDENT PENETRATION OF UV IN THE SKIN (GUPTA ET AL., 2013)

1.1.3. Applications of Ultraviolet radiations in Medical

The environmentally friendly nature of ultra-violet radiation, making it ideal for medical applications. It disables pathogenic DNA and prevents it from multiplying. Ultraviolet radiation damages the nucleic acid of living organisms that inhibits the reproduction of the organism. A number of organisms can be destroyed by ultraviolet irradiation such as hepatitis, dysentery, influenza and E.coli. It is the most ancient technique used by the humans for decontaminations. UV rays have been used to eliminate fungi, bacteria and viruses. Due to its capacity to induce chemical reactions, UV radiations have a wide range of bio-medical applications (Fig. 4).

FIGURE 4 APPLICATIONS OF UV RADIATIONS

Some exposure to UV light is vital to good health. It boosts the production of vitamin D in the living body (Panov & Borisova-Papancheva, 2015). Ultraviolet radiations have proven useful in treating sarcoidosis, seasonal disorders, cutaneous T-cell lymphoma, and most importantly for vitamin D deficiency and many other skin conditions e.g., scleroderma and eczema (Wilson et al., 2012). In infants, UV radiations are being used to treat jaundice and psoriasis (Panov & Borisova-Papancheva, 2015).

Ultraviolet radiations (200-280 nm) have antimicrobial effect, therefore, UVC can safely be applied to severe skin lacerations with tolerable damage. UVC have long been used for sterilizing inanimate objects. UVB (280-315 nm) is widely used to the damaged tissue to stimulate wound healing. UVA radiations (315-400nm) have not yet been extensively applied to wound care, but these radiations exhibit distinct effects on cellular signaling (Gupta et al., 2013).

The microbial load on hard surfaces and in the air can be reduced by UV irradiation by inactivating microorganisms. It may eradicate infectious agents from liquid food. UV light is already widely used for sterilizing water and many surgical tools because of its

effectiveness against a diverse group of microorganisms (Turtoi, 2013). Wavelength dependent bio-applications are listed in figure 5.

Wavelength (nm)	Applications
230-400	Optical sensors, various instrumentation
240-280	Disinfection, decontamination of surface and water, DNA analysis
200-400	Forensic analysis, drug detection
270-360	Protein analysis, DNA sequencing, drug discovery
280-400	Medical imaging of cells
300-320	Light therapy in medicine, effective long-term treatment for many skin conditions like psoriasis, vitiligo, eczema
300-365	Curing of polymers

FIGURE 5 BIO-MEDICAL APPLICATIONS OF UV RADIATIONS WITH RESPECT TO WAVELENGTHS

1.2.UV Curing

UV radiation curing also named as light-induced polymerization is now a widely accepted technology. It employs radiations to initiate photo induced chemical reaction to generate crosslinked polymers, (Roffey, 1997; Vitale, Trusiano, & Bongiovanni, 2017) . Due the uniqueness of mechanism, It has a wide range of industrial applications. It is regarded as the effective way of quickly transforming solvent-free resin into solid polymer. The curing of acrylate-based resins occurs primarily in a split second under intense illumination, to produce a network of densely packed 3-dimensional polymers that are highly resistant to heat, chemicals and organic solvents (K. K. Dietliker & Oldring, 1991; Roffey, 1997). Figure 6 shows that polymers having polymerizable components can be crosslinked by UV irradiation. In either case, active species i.e., proton acid or free radicals are required to be produced by photointiator to trigger the chain reactions (Decker, 2002).

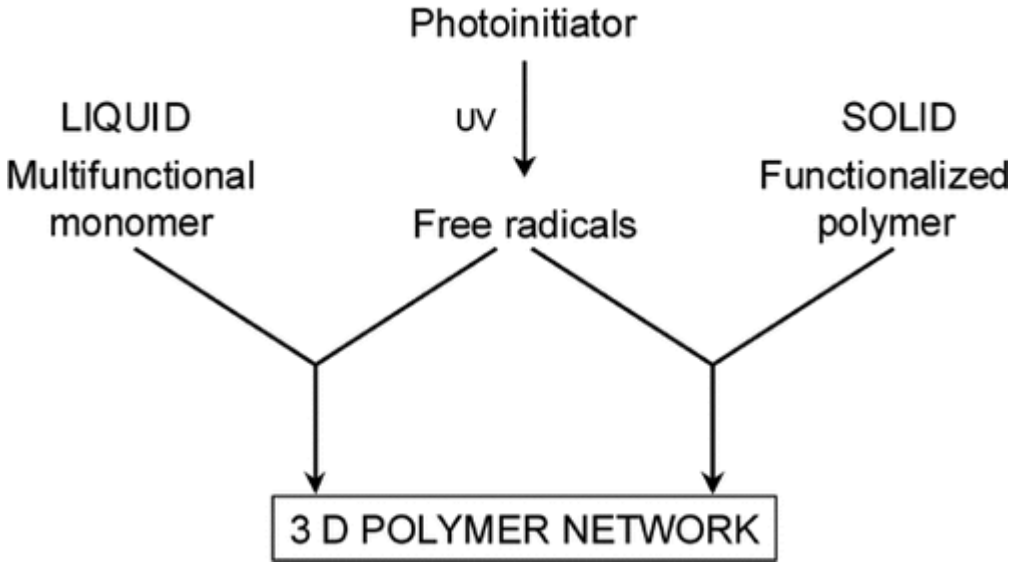


FIGURE 6 PHOTOINITIATED CROSSLINKING POLYMERIZATION

In the domain of adhesives, a variety of pressure-sensitive adhesives (PSAs) along with structural adhesives or hot-melt adhesives (HMAs) can be prepared through the curing process induced by photochemical reactions. Curing processes can be used to polymerize thermoplastic adhesives or to formulate thermoset adhesives initiating from the components having low molecular weight (Roffey, 1997; Woods, 1992). Various kinds of radiation sources are available but the light-emitting diode (LED) technology is offering as the best substitute to traditional irradiation through halogen light for preparing adhesives. However, the market for UV-cured adhesives is supposed to approach $1.2 billion in 2021, With the CAGR of 9.15 % from 2017 to 2021 on account of their large-scale applications in electronic and glass industry, and in medical fields (Vitale et al., 2017).

The figure 7 illustrates a typical process of radiation curing.

A formulation containing highly functional monomers, oligomers and photoinitiators is prepared. A thin layer (having a thickness of 1-100 micrometer) of that formulation is coated on substrate which crosslinks to form a three dimentional network of solid polymer when irradiated. The photoinitiator, the monomers and the oligomers are the only key components of UV-curable system, because no solvents are used in the formulation. The

oligomers and the monomers are responsible for crosslinking while the photoinitiator assures the light absorption.

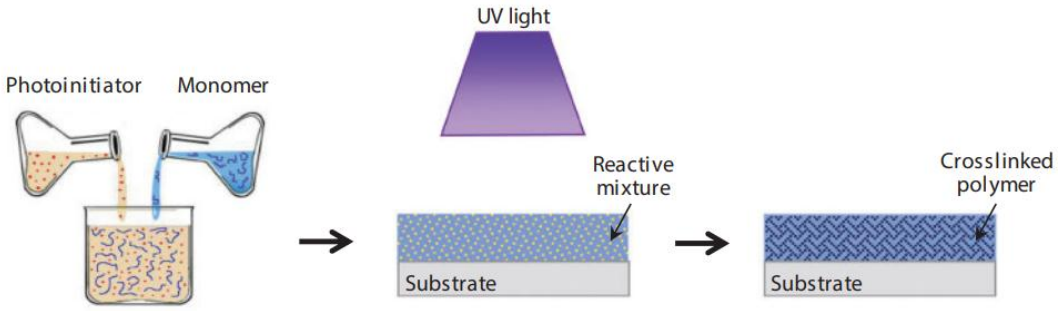


FIGURE 7 A PROCESS OF RADIATION CURING

The properties of the photoinitiator are as follows:

- Strong sensitivity to light within the wavelength range of the emitting source;
- High reactivity and better solubility in the monomer system;
- High stability over the storage and durability of the adhesive (K. Dietliker et al., 2007; Fouassier & Lalevï, 2012).

Sources of UV radiation are primarily mercury based which contain high molecular absorption coefficient photoinitiators having a maximum absorption less than 350 nm. Though the longer-wavelength (410-480nm) absorbent molecules are available, but the spectral absorption range of the system can be optimized by adding photosensitizers. Advanced photoinitiators are capable of matching the wavelength of commercial LED sources which are highly acceptable due to their low power requirement, they are unaffected by fluctuating line voltage and experience no significant degradation over time. When dealing with coatings and inks, its important to find out the spectral range for the pigmented polymers so that the emission spectrum must be overlapped by photoinitiator absorption.

The energy absorbed by photoinitiator though light absorption enables the molecule in ground state energy S_0 to change its state and excites to a higher energy state S_1. When a molecule is in its excited state, it can revert the spin of an electron of 1st excited state or it will return immediately to its ground state, producing fluorescence and the mechanism is referred to as inter-system crossing. As the spins are not paired at that time, so the molecule has acquired paramagnetic property and that intermediate excited state is named as triplet state T1 (Fig. 8). The photoinitiater can be chemically transformed or if the spin is reverted again, then it can disintegrate to the ground state directly from the triplet state T_1. The homolytical bond cleavage is the most common reaction.

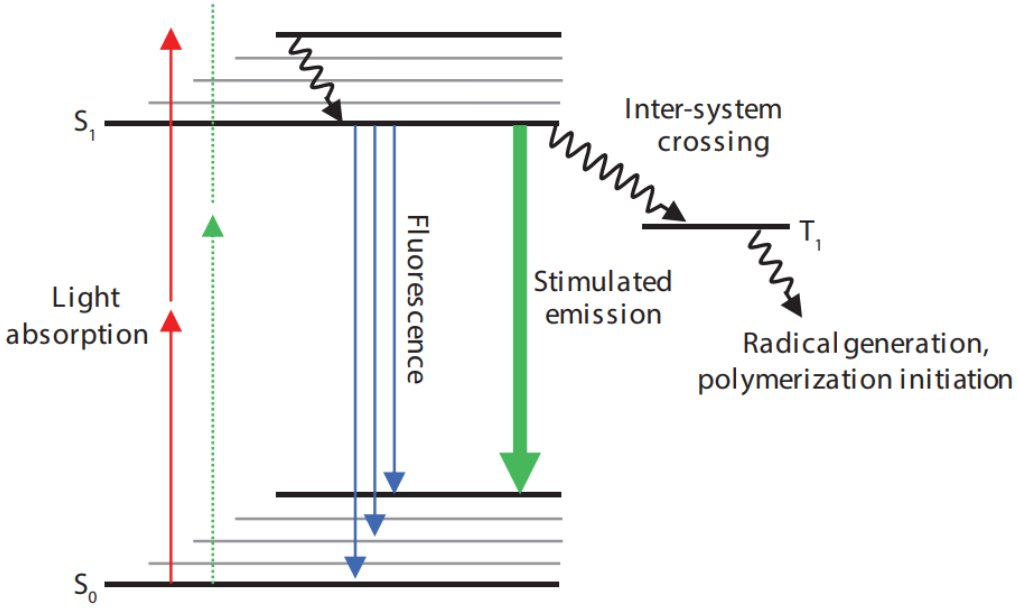


FIGURE 8 RADICAL POLYMERIZARION INDUCED BY LIGHT ABSORPTION

Numerous kinds of molecules experience reactions in the result of photophysical excitations and promote the production of bases and acid capable of initiating ionic polymerization. Onium salts are the excellent photoinitiators which disintegrate by photodecomposition resulting in acidic protons (Crivello, Dietliker, & Bradley, 1998).

Most of these polymers are engineered to achieve increased chemical durability and better mechanical and thermal properties. The network structure of the system has a strong

influence on the mechanical and thermal properties of UV-treated materials. UV setting polymers are infusible and insoluble, making them hard to completely eliminate from composites without impairing the substrate. But there are situations when it is necessary to remove crosslinked from the underneath material to amend the substrate to make it reuseable. UV-crosslinkable resins are produced as light-induced highly functional polymers; degradeable in nature (Shirai, 2014).

1.2.1. UV-curable and photocrosslinkable materisals

UV radiation curing has major applications in coating industries to protect the surface of many materials through rapidly curing the printing inks, varnishes or paints. UV curing systems are also being used for production of composite material as well as for making release coatings, rapid-curing adhesives and sealants. In photolithography, high-definition images are being produced by insolubilization of photoresists required for producing microcircuits, printing plates and optical disks (Shirai, 2014). In addition to its high spatial resolution and velocity, light-induced polymerization has many other benefits including ambient temperature operations, low energy consumptions, customized properties and solvent free formulations (Decker, 2002).

Many photocrosslinkables materials, e.g. 1,4-butanediol diacrylate (BDDA), 2-Ethylhexyl acrylate (EHA), diethyleneglycoldiacrylate (DEGDA) and n-butyl acrylates (BA), are available in market having numerous advantages over each other (Table.1). These mterials are divided into classes and sub-classes depending on the presence of different monomers and olignomers as photoinitiator (Schwalm, 2006).

Class	Example	Advantages
Monoacrylates (Itoh, Kameyama, & Nishikubo, 1996)	2-Ethyl hexyl acrylate (EHA)	Good flexing action

Diacrylates (Scherzer, Knolle, Naumov, & Mehnert, 2003)	1,4-butanediol diacrylate (BDDA)	Relatively good viscosity reducer
Triacrylates (Shukla, Bajpai, Singh, Singh, & Shukla, 2004)	Pentaerythritol triacrylate (PETA)	Fast curing
Tetracrylates (Shukla et al., 2004)	Pentaerythritol tetraacrylate	Low volatility
Vinyl ethers (Chen, Soucek, Simonsick, & Celikay, 2002)	1,4-Cyclohexanedimethanol divinyl ether (CHDMDE)	High diluting power and low toxicity
Propenyl ethers (Sangermano et al., 2001)	Trimethylolpropane dipropenyl ether	Good reactivity
Epoxides (Decker & Moussa, 1990)	3,4-Epoxycyclohexylmethyl-3′, 4′- epoxycyclohexane carboxylate	Low shrinkage and chemical resistance

TABLE 1PHOTOSENSITIVE MATERIALS

The market size of UV-curable resins surpassed $4 billion in 2019 and is expected to exceed over 10% of CAGR from 2019 to 2026 (Fig. 9). An increase in product demand in the electronics, packaging and automotive industry and an increase in the use of adhesives will stimulate market growth over the next few years (Kunal Ahuja, 2019).

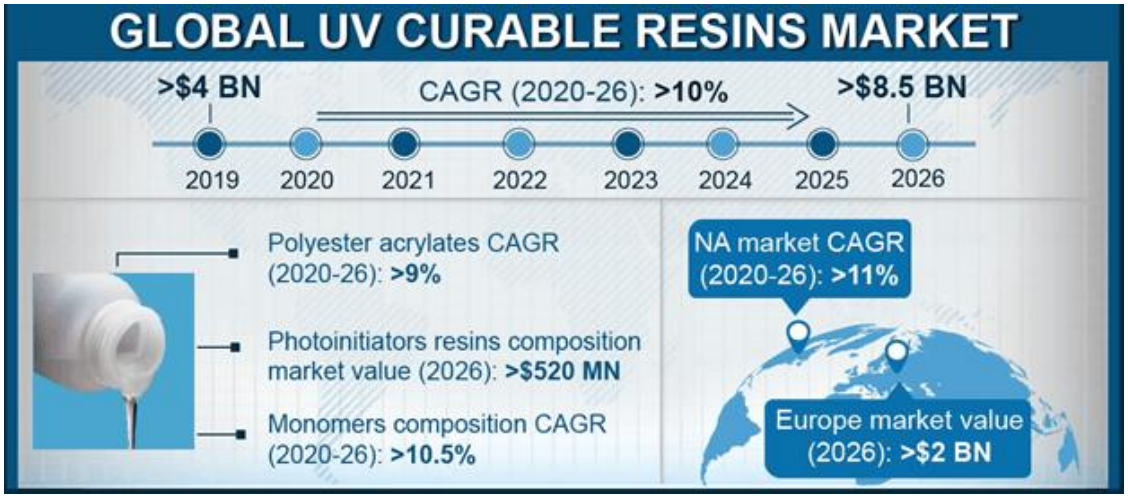


FIGURE 9 MARKET TRENDS OF UV CURABLE RESINS

Latest developments in the field of wound management have enabled the use of photocurable polymers having crosslinking end groups. These photocurable polymers have fast curing rate with accurate control of crosslinking rate, and can be used as best tissue sealants (Vitale et al., 2017).

1.3.Tissue Sealants

Various methods were used for sealing of damaged tissue. For instance,numerous kinds of procedures are used to mechanically seal different tissues, including tapes, sutures, bandages or staples. For medical applications, these materials consist of absorbent materials designed to seal or bind the tissues as they heal and then be absorbed in some time (Larry H. Dodge, 2014).

Adhesive tissue sealants stimulate healing in disjunct tissues, decrease the surgical risk by stabilizing anastomotics, enhance hemostasis and promote tissue restoration. The bioadhesive properties of tissue sealants are mainly due to the reactivity of materials with tissues, therefore, control of unintentional and adverse reactivity is the real obstacle in the designs of sealants. The compromises among different bioreactivity-dependent adhesive properties can be illustrated by sealants based on fibrin and cyanoacrylate. Different types of tissue sealants are shown in Fig. 10.

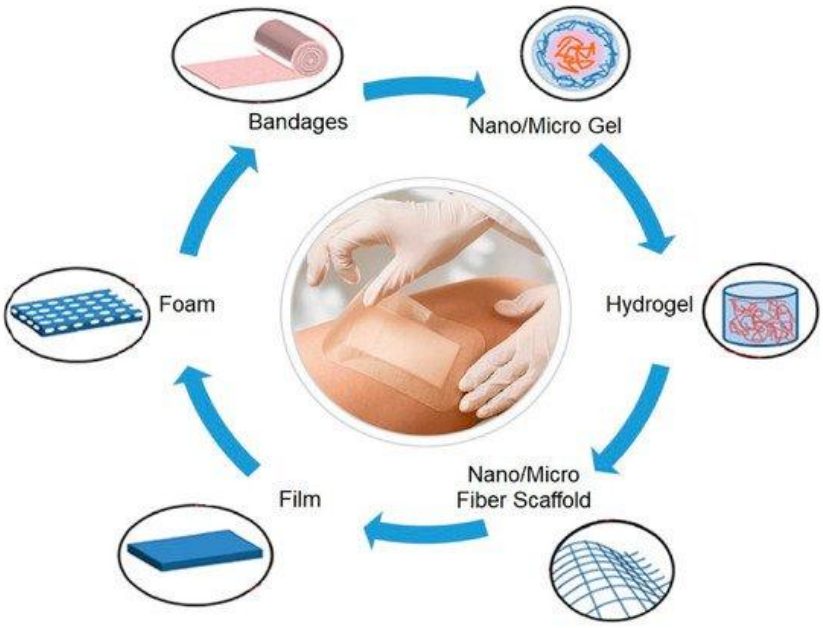


FIGURE 10 DIFFERENT TYPES OF DRESSINGS (TEIXEIRA, PAIVA, & AMORIM, 2020)

The development of rapidly curable sealing tissue adhesives has been increasingly demanded in recent medical procedures (Shazly, Artzi, Boehning, & Edelman, 2008).

1.3.1. UV-curable Tissue Sealants

Photocrosslinable polymers can be utilized as bioadhesives for living tissues. These are applied in liquid form, then activated to adhere, cure, and bond by UV irradiation. Ultraviolet (UV)-crosslinked polymers having derivatives of fatty acids are biocompatible. These photocurable bioadhesives have many advantages over traditional wound closure techniques including less pain, stimulation of wound healing and fast wound closure. Photocurable biopolymers that can be used as tissue adhesives are: poly(vinyl alcohol), poly(ethylene glocyol)s, polyurethanes, polyanhydrides, fumaric acid, and collagen, etc. Photo-curable Chitosan and poly (α-hydroxyacids) can be used for wound healing (Hoffman, 2012; Zair et al., 2019).

Polysaccharides are regarded as the best bioadhesive material for the medical application. Carboxyl, hydroxyl and amino acid are the major components of polypeptides,

polysaccharides and proteins. Adhesives formed from these constituents attach to amines present on the surface of tissue through covalent bonding via imine interactions, Michael addition mechanism, π-π interaction, Schiff base reactions, biaryl formation or by activating N-hydroxysuccinimide. The adhesive strength of the structure can be enhanced by assimilating photocurable groups. Upon photo irradiation, these photocurable groups crosslinked; resulting in enhanced adhesive along with cohesive strength of polymeric network (Bhagat & Becker, 2017).

Latest developments in the field of wound management have enabled the use of biopolymers. Initially, photocurable materials are in the form of viscous solution, which convert into insoluble hydrogel through crosslinking when it is exposed to ultraviolet (UV) radiations (Ishihara, 2002). The water soluble macromolecules assemble themselves by covalent bonds during the process of chemical gelation (Hoffman, 2012).

The final mechanical and adhesion properties of these photopolymers can be controlled by optimizing the Ultraviolet exposure time (Marques et al., 2016).

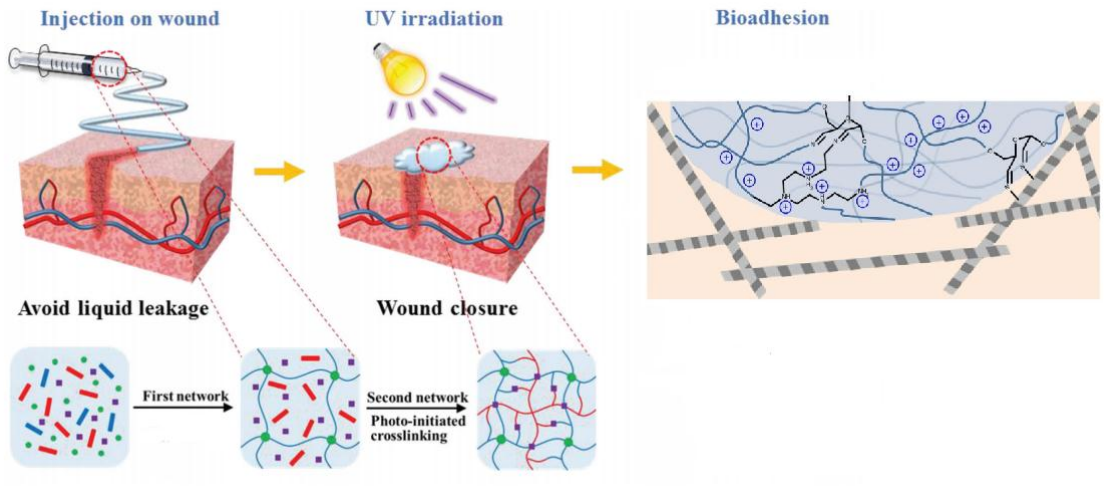


FIGURE 11 APPLICATION OF UV CURABLE TISSUE SEALANTS (BIAN ET AL., 2019)

1.4.Our Strategy

Recent developments in the field of wound management have enabled the use of photocurable polymers having crosslinking end groups. These photocurable polymers have fast curing rates with accurately controlled crosslinking rates. The strategy was to enhance the adhesive as well as cohesive strengths of photosensitive polymeric chitosan in the presence of body fluids. Chitosan is the enzymatic biodegradable material, it was modified with hydrocaffeic acid (Ch-HCA) to increase the adhesion and sealing strength in wet conditions. 4-azidobenzoic acid is used to make the Ch-HCA photosensitive; these azide moieties would reduce to nitrene groups (Ishihara, 2002) which aids the active cross linking of modified chitosan under the exposure of UV light, as a result, increasing the mechanical strength. Futhermore, the active attachment of adhesive moieties to hydrophilic surfaces can be achieved via their oxidation to quinones and subsequent reactions with other quinones and amines of hydrophilic surfaces (Bouten et al., 2014; Ghobril & Grinstaff, 2015) upon UV irradiation (Fig.12).

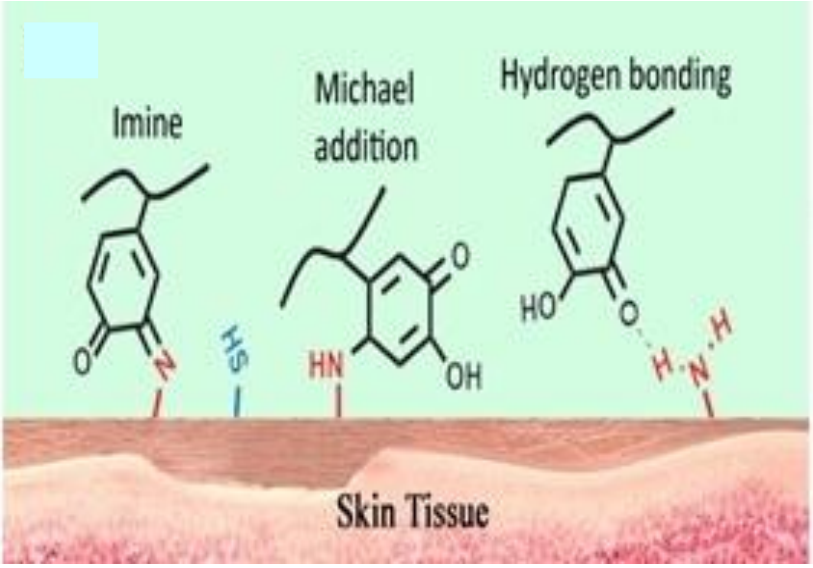


FIGURE 12 POSSIBLE MECHANISMS OF THIN FILMS AND TISSUE INTERACTION

The interfacial bonding strength between the tissue defect site and the adhesive would enhance by the reaction of amine groups attached to the skin surface. This approach is safe and quick in which wound can be efficiently sealed via photochemical reactions

(Nakayama & Matsuda, 1999) (Fig.13). These AzCh-HCA thin films behave as strong tissue adhesive which can hold tissues together to control or stop the bleeding, prevent gas or fluid leakage from the tissues and allow proper healing to occur by behaving as hemostats, sealant, and adhesive respectively. This tissue sealing process accelerates wound closure and healing (Ghobril & Grinstaff, 2015). Exposure of ultraviolet radiations causes restoration of skin homeostasis and stimulates wound healing besides its antioxidant and anti-inflammatory effects. The germicidal effect of UV is responsible for rapid healing as it inactivates the microorganisms by damaging their DNA.

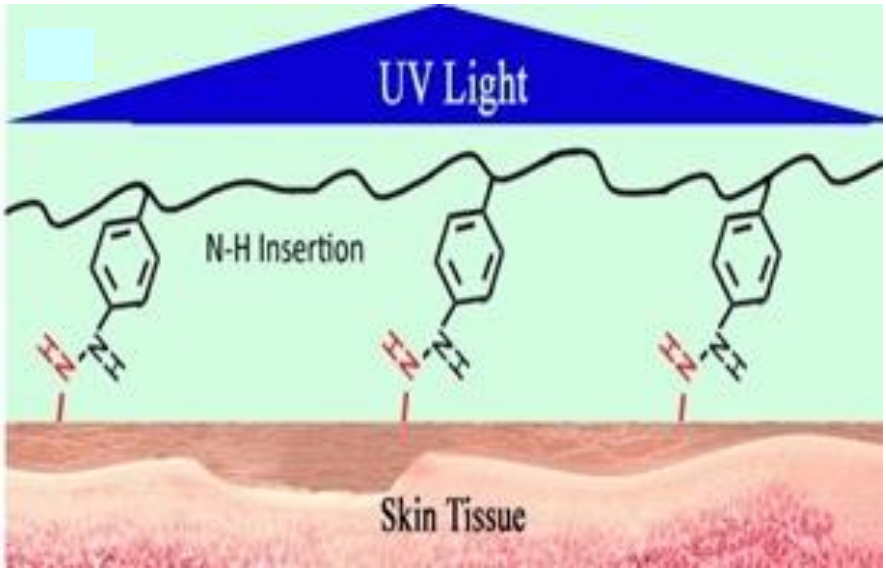


FIGURE 13 SEALING OF WOUND

Free-standing thin film from photosensitive adhesive chitosan was synthesized by solvent casting method. It was composed of two layers i.e. adhesive chitosan layer and the underlying polyvinyl alcohol (PVA) layer. The PVA readily dissolved and release adhesive layer in the water (Fujie et al., 2009). The procedure of thin film application at tissue defect will be easy and convenient i.e. thin film will be placed on wound, water will be added to remove the PVA layer and wound will be sealed under the exposure of UV light (Fig. 14).

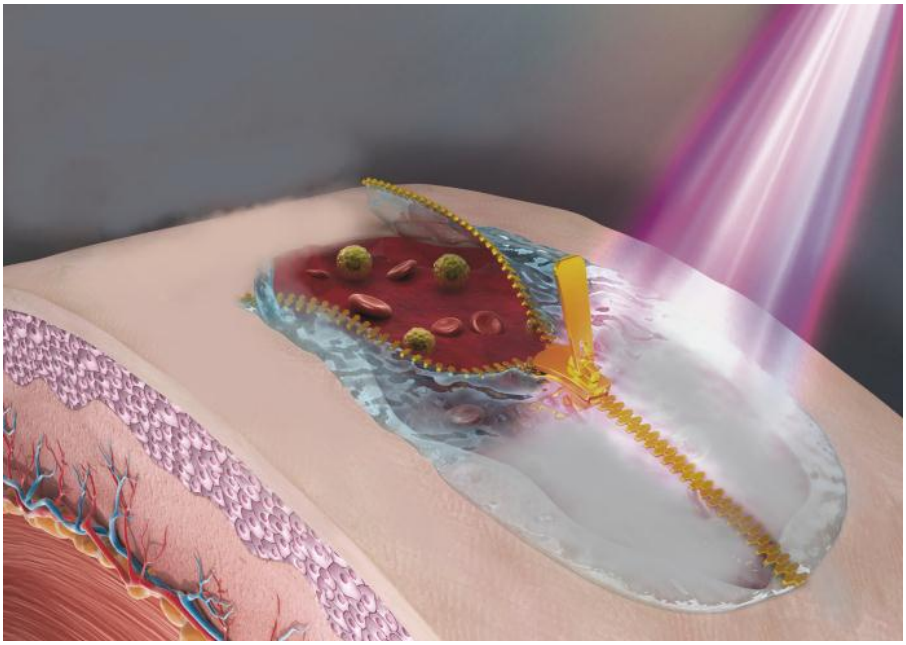


FIGURE 14 CLOSURE OF WOUND

CHAPTER 2

MATERIAL AND METHODS

2.1.MATERIALS

2.1.1.Chitosan

Chitosan is a linear cationic chitin, extracted from nature, having various bio-applications in drug delivery and tissue engineering. It has less immunity resistance, better antibacterial activity, excellent biodegradability and biocompatibility. The solubility of Chitosan can be adjusted by changing the pH value of solvent (Bhattarai, Gunn, & Zhang, 2010).

2.1.2.Hydrocaffeic Acid

Hydrocaffeic acid is a monocarboxylic acid named as 3-phenylpropionic acid with the hydroxy groups attached at 3 and 4 positions. Basically, it is a caffeic acid's major metabolite. It's a naturally occurring compound unsaturated carboxylic acid chain attached at α, β positions of catechol group. Hydrocaffeic acid is one of the most frequently found phenolic acids in cereals, fruits and nutritional additives; available for human intake. It exhibits enhanced anti-oxidant along with heptoprotective property and can be obtained from Chinese herbs (Martinez, Mackert, & McIntosh, 2017).

2.1.3. Azidobenzoic Acid

p-Azidobenzoic acid having chemical formula $C_7H_5N_3O_2$, is an aromatic azide. It is a member of benzoic acid family and usually used for click reactions. p-azidobenzoic acid in combination with chitosan is found to crosslink to produce a polymeric hydrogel via UV irradiation which adheres to living tissues.

2.2.EXPERIMENTAL TECHNIQUES

2.2.1.Solvent Casting Method

Solvent casting is one of the most widely used techniques for manufacturing film. While designing films for pharmaceutical industry, the Active Pharmaceutical Ingredient (API) is dissolved or suspended in polymeric solutions; added with plasticizer solutions. The final

solution having all the ingredients along with the API is named as the film dope, which is then poured in a petri dish or spread out on any release media. The film cast media are then exposed to hot air by passing through the convection chamber or in a vaccum drying oven or allowed to dry at room temperature. The dried film is then peeled and stripped (Khan et al.).

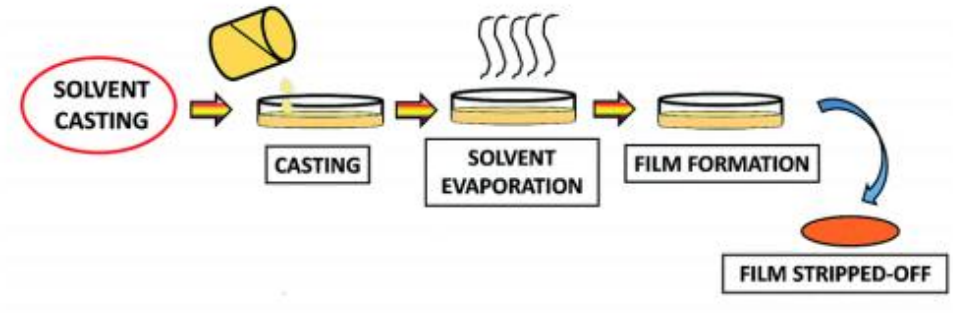
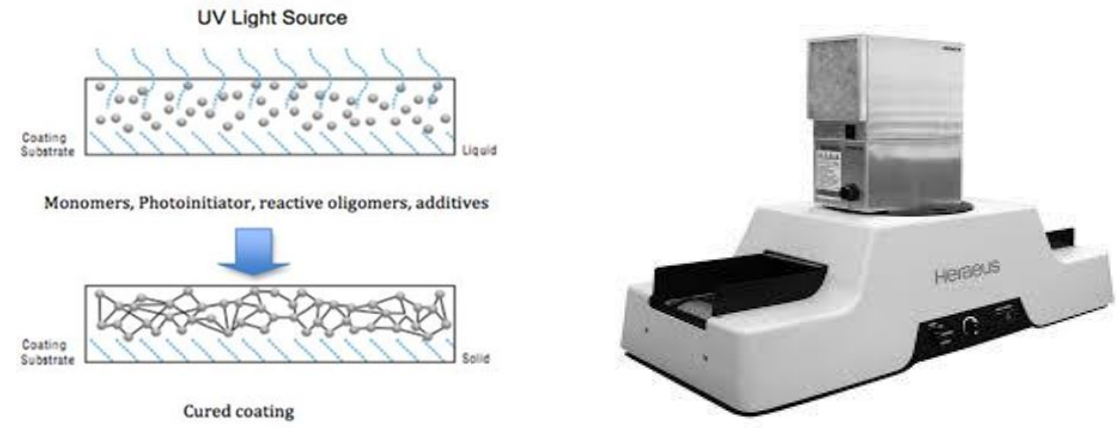


FIGURE 15 SOLVENT CASTING PROCESS

2.2.2.UV Curing

UV curing is a process of crosslinking liquid chemicals having photocurable photointiators through UV irradiation. UV curing is a stepwise process. The process starts with the light absorption by photoinitiator (PI), followed by activation of PI and further initiating chemical reactions of activated PI with monomers to start polymerization. The crosslinking occur during polymerization and process terminated with the cured coating formation (Mendes- Felipe, Oliveira, Etxebarria, Vilas- Vilela, & Lanceros- Mendez, 2019).

Fig. 16 (A) shows the process of UV curing. Specialized UV curing machines are used for this purpose. Fig 16 (B) shows the machine (@Heraeus) used in our experiment for UV curing.



(A) **(B)**

FIGURE 16 (A) THE PROCESS OF UV CURING (B) UV CURING SYSTEM

2.3.METHODS

2.3.1. SOLUTION PREPARATION

2.3.1.1. MES Buffer Solution of pH 5

0.5M MES buffer solution was prepared by dissolving 9.76 g of [2- (N- morpholino) ethane-sulfonic acid] into 80 ml of distilled water. Then distilled water was added to make the volume up to 100 ml. 10N NaOH solution was added drop-wise to adjust the pH to 5.0.

2.3.1.2. Phosphate Buffer Solution of pH 4

Water buffer solution of pH 4 was needed for dialysis. Solutions of Disodium hydrogen phosphate (Na_2HPO_4) and potassium Dihyrogen phosphate (KH_2PO_4) were prepared separately. 2.52g of Na_2HPO_4 and 1.505g of K_2HPO_4 were dissolved in 300 ml of water, followed by the dilution to 500 ml. Then both solutions were added to make the volume to 1000 ml. The pH of the solution was adjusted to 4.0 by slowly adding the acetic acid while continuously measuring the pH with the pH meter.

FIGURE 17 SOLUTION MAKING

2.3.2. Synthesis of Chitosan-DOPA

Chitosan-DOPA was prepared by utilizing conjugation reaction of ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC). Chitosan was dissolved in MES buffer (2-(N-morpholino) ethane-sulfonic acid) of pH 5. Hydrocaffeic acid (1.5 mM) and EDC were dissolved in 1:1 v/v mixture of water and ethanol and Chitosan solution was added in it. The reaction mixture was stirred for 12 hours. The obtained product was dialyzed against pH 4 water for 48 hours, separated and freeze-dried.

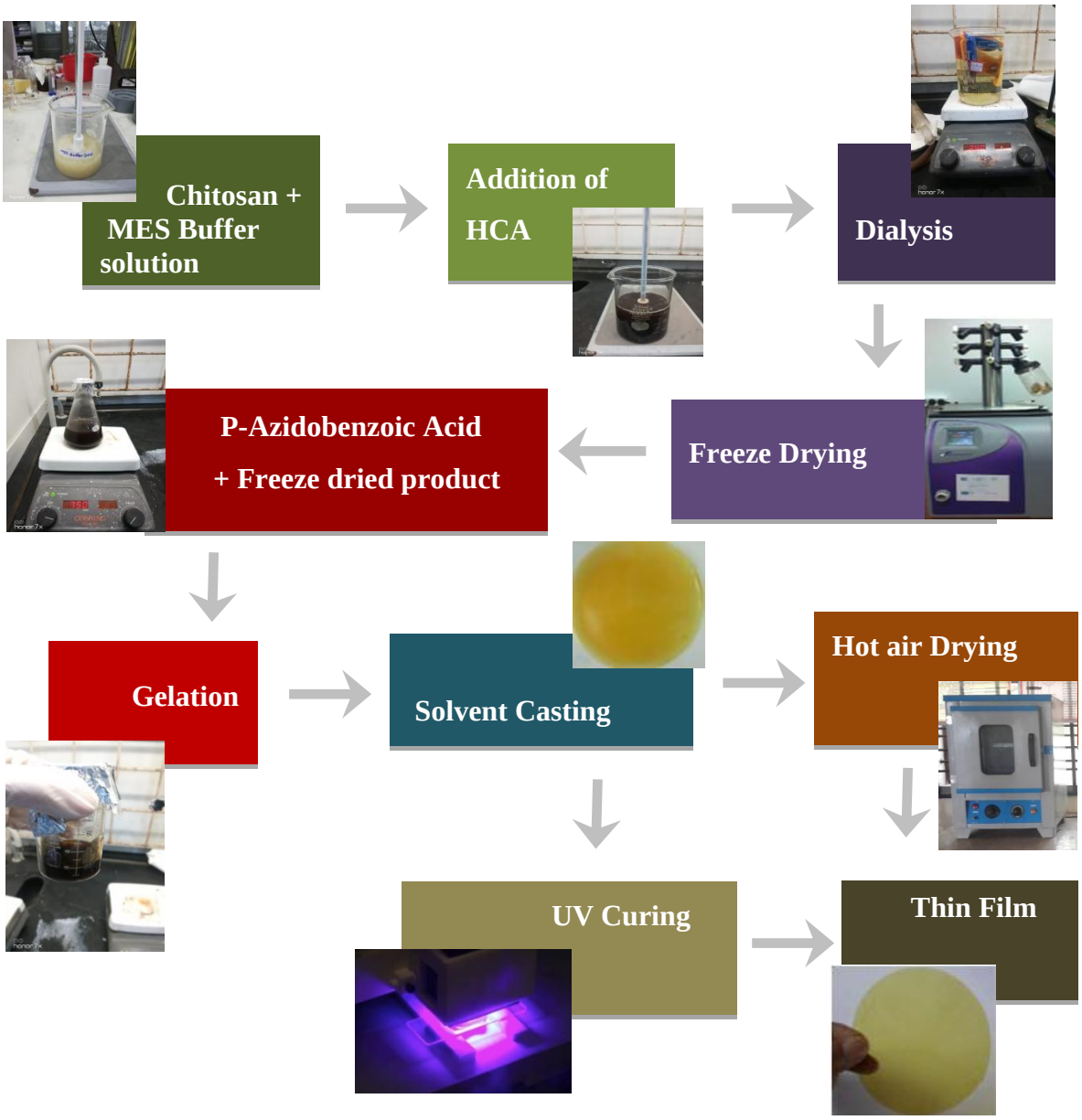
2.3.3. Synthesis of photosensitive polymer

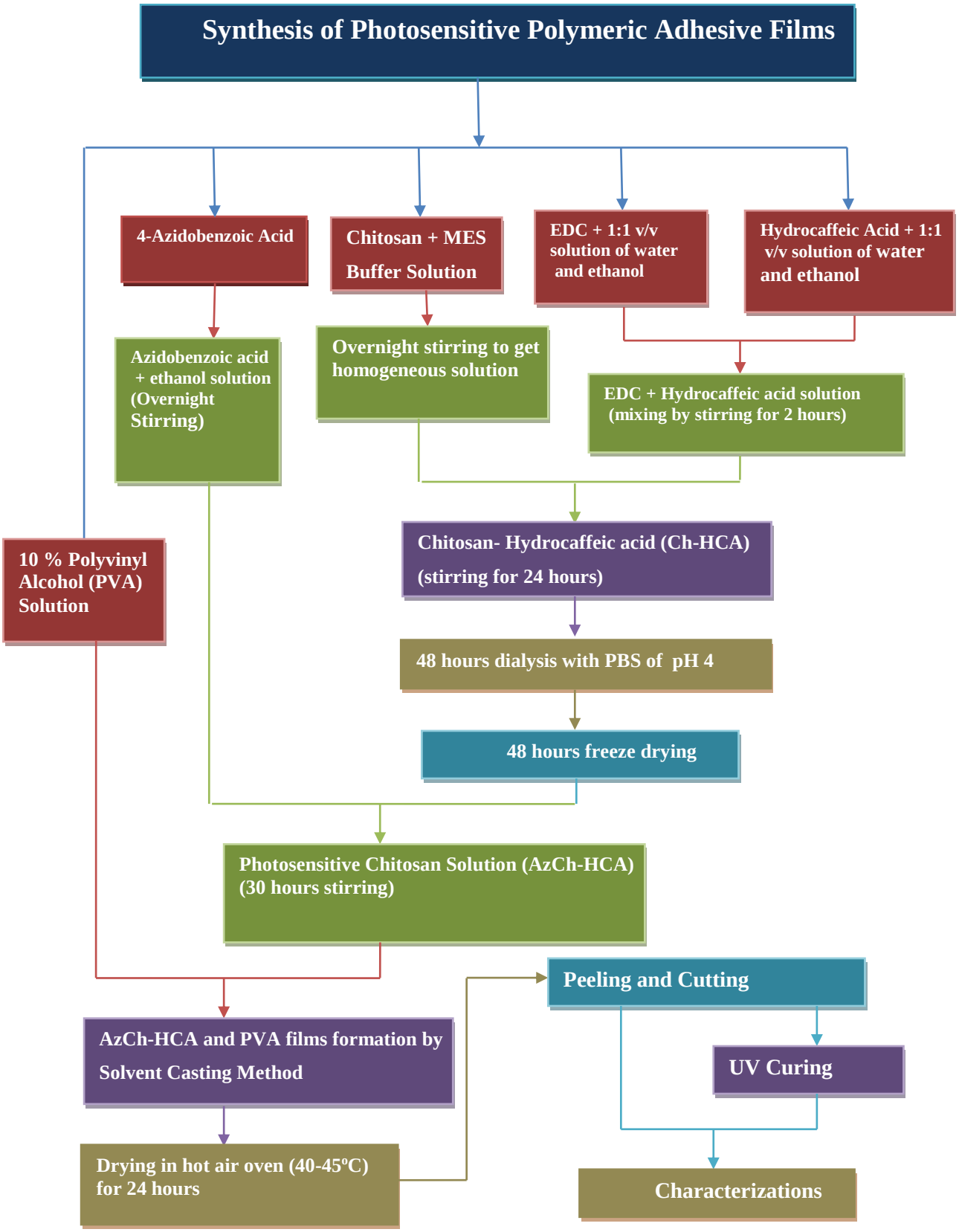
2.5 % p-azidebenzoic acid solution was prepared and the freeze-dried product was added in azidobenzoic acid suspension to get photocurable polymer. P-azidebenzoic acid formed crosslinks between polymeric chains. Under the irradiation of ultraviolet light, the outside groups were degraded to give nutrition groups. These nitrene groups cause crosslinking with amines of polymer or tissue to give azo-groups.

2.3.4. Fabrication of thin film

Adhesive polymeris film was formed by solvent casting method. 10% aqueous solution of polyvinyl alcohol (PVA), as an underlying layer, was spread into petri dishes and dried for 24 hours at room temperature. The AzCh polymeric suspension was added as second layer onto those petri plates already having PVA layers and subjected to high temperature (40-45^{o}C) in vaccum drying oven to dry the solvent and obtain better quality films. The dried film was separated, water was added to get freestanding layer (Irfan et al., 2016).

EXPERIMENTATION




Synthesis of Photosensitive Polymeric Adhesive Films
4-Azidobenzoic Acid
Chitosan + MES Buffer Solution
EDC + 1:1 v/v solution of water and ethanol
Hydrocaffeic Acid + 1:1 v/v solution of water and ethanol
Azidobenzoic acid + ethanol solution (Overnight Stirring)
Overnight stirring to get homogeneous solution
EDC + Hydrocaffeic acid solution (mixing by stirring for 2 hours)
10 % Polyvinyl Alcohol (PVA) Solution
Chitosan- Hydrocaffeic acid (Ch-HCA) (stirring for 24 hours)
48 hours dialysis with PBS of pH 4
48 hours freeze drying
Photosensitive Chitosan Solution (AzCh-HCA) (30 hours stirring)
Peeling and Cutting
AzCh-HCA and PVA films formation by Solvent Casting Method
UV Curing
Drying in hot air oven (40-45°C) for 24 hours
Characterizations

CHAPTER 3

CHARATERIZATIONS

3.1.CHEMICAL CHARACTERIZATION

3.1.1.FTIR

FTIR Analysis is an analytical technique utilize for the identification of numerous kinds of materials. This technique utilizes infrared light to investigate the chemical properties of the specimen. Most clear approach for this, the "dispersive spectroscopy" in which a monochromatic light beam shines at specimen and measure how much the light is absorbed, and rerun for each wavelength.

Operating Principle

The basic purpose of FTIR is used to determine that which functional groups are present in the molecule. Unlike ultraviolet-visible spectroscopy, FTIR uses a beam that contains many frequencies of light. A beam splitter and interferometer are employed for this purpose. The energy of transmitting light is recorded, when a sample material is placed in front of this beam. The absorption and % transmittance is plotted as a function of frequency (cm^{-1}) or wavenumber (cm^{-1}) of IR source.

Fourier series (a special mathematical treatment) applies on spectral data, a function that contains a power series expansion of sine and cosine terms;

$$\boldsymbol{f(x) = (A_0/2) + \sum_{n=1}^{\infty} \left(A_n \cos \frac{n\pi x}{c} + B_n \sin \frac{n\pi x}{c'} \right)}$$

Where $\boldsymbol{f(x)}$ is a periodic function with period **2c** (Berthomieu & Hienerwadel, 2009).

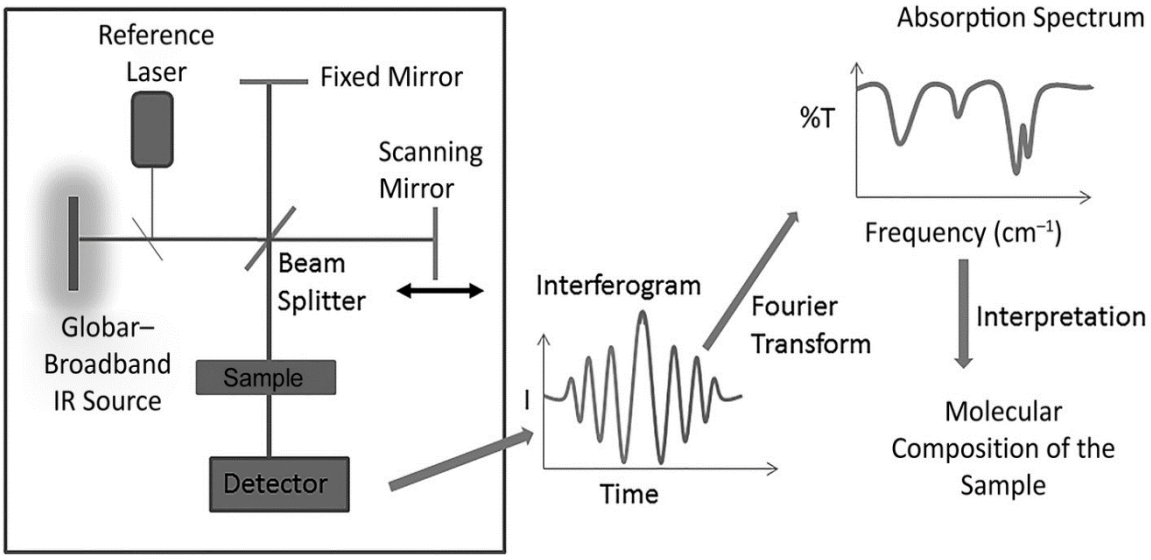


FIGURE 18 FTIR WORKING PRINCIPLE

3.1.2. UV Visible Spectroscopy

Ultraviolet–visible spectrophotometer (UV–Vis or UV/Vis) refers to absorption spectroscopy or reflectance spectroscopy in the ultraviolet-visible spectral region. This implies it utilizes light in the visible and ranges. In this region of the electromagnetic spectrum, atoms and molecules undergo electronic transition, absorption measures transitions from the ground state to the excited state. The absorption or reflectance in the visible range directly affects chemical colors (Schmid, 2001).

Working Principle

UV-Visible Spectrometer technique used two light sources, tungsten-halogen lamp for visible light and deuterium discharge lamp for UV light. A beam of light from a visible and/or UV light source (colored red) is separated into its component wavelengths by a diffraction grating or a prism. Each monochromatic beam split into two equal intensity beams by a half-reflected device. Light coming from sources recoils on mirror 1 and pass through the slit that divided it into its component wavelengths by diffraction grating or a prism. A diffraction grating is rotated in such a manner that specific wavelength of light

crosses the slit and fall onto the second mirror. Then light beam is dispersed by half-mirrored device that split beam into two equal intensity beams.

One beam sample beam goes to transparent cuvette where the sample is placed. Other beam passes through another cuvette that contain reference sample. After impinges on samples, the light intensities are detected by detectors and converted into the current. This current is then sent to a computer that displays the data in the form of graphs.

Reference beam intensity experienced little or no light absorption and is represented as I_o and sample beam intensity is denoted by I. If the sample does not absorb any light, then $I = I_o$. If the sample absorbs any light, then $I < I_o$.

Absorbance A is given by:

$$A=\log(I_o/I)$$

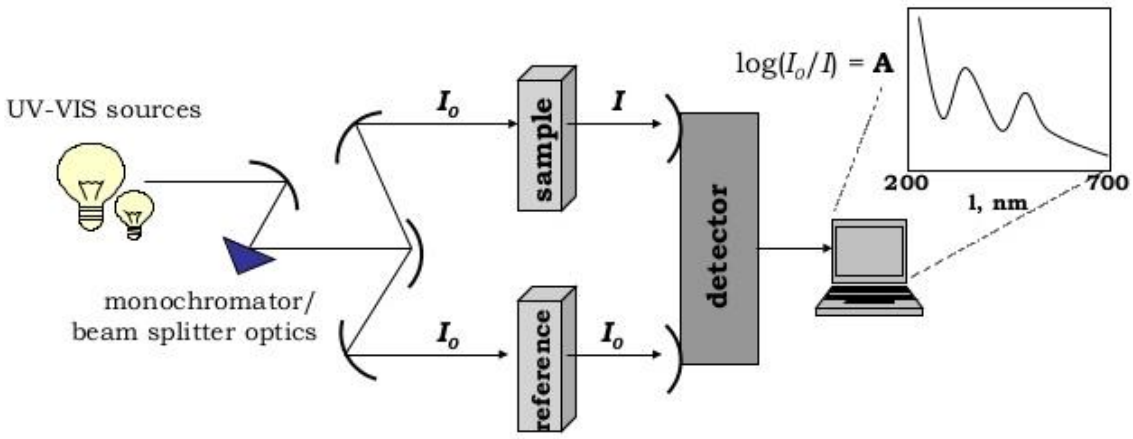


FIGURE 19 SCHEMATIC DIAGRAM OF UV VISIBLE SPECTROSCOPY

3.2.MICROSTRUCTURE STUDY

3.2.1.Atomic Force Microscopy (AFM)

Atomic Force Microscopy, also named as Scanning Probe Microscopy, is a part of large group which is allowed to image and characterize the surface of material with resolution comparable to atomic size.

Principle of Operation

The fundamental of AFM instrument is a probe, that is stylus with flexible microcantilever arm. The length of probe is in ***μm*** and end radius is in ***nm***. The surface of interest is directly in contact with this probe by attractive or repulsive forces (Rugar & Hansma, 1990). These forces can be calculated by measuring the deflection of cantilever. **Hook's Law** gives;

$$\boldsymbol{F = kx}$$

Where F is Force, k is the stiffness of cantilever and $\boldsymbol{x}$ is the distance that cantilever moves.

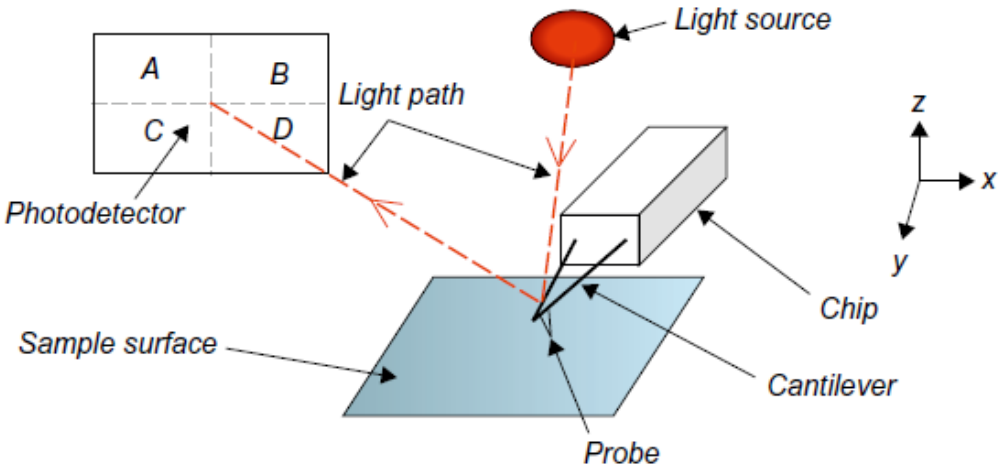


FIGURE 20 AFM WORKING PRINCIPLE

The deflection, toward or away from surface, can be detected by a laser beam. A position sensitive photo diode is used to cause these changes. The simultaneous deflection of surface built up a high-resolution three-dimensional image that represent topographic features.

The AFM mostly operated to characterize and investigate the electrical, magnetic, mechanical and morphological properties of materials, soft biological materials and even living organisms.

3.3.MECHANICAL TESTING

3.3.1.Tensile Testing

Tensile tests, also referred as tension tests, are one of the most basic and standard mechanical tests. Basically, tensile test exerts a tensile stress on the material and asses the reaction of material to the applied force. As a result, tensile tests measure the strength of a material and its capacity to elongate (Naaman & Shah, 1971). These tests are easy to perform and are usually carried out on standardized universal testing machines (UTM). The force exerted throughout the test and the resulting elongation (ΔL) of the specimen is tabulated. Material characteristics are frequently measured in terms of stress σ and strain ε. The graph presents these both values and referred as stress-strain curve.

Where

$$\varepsilon = \frac{\Delta L}{L_0}$$

$$\sigma = \frac{R}{A}$$

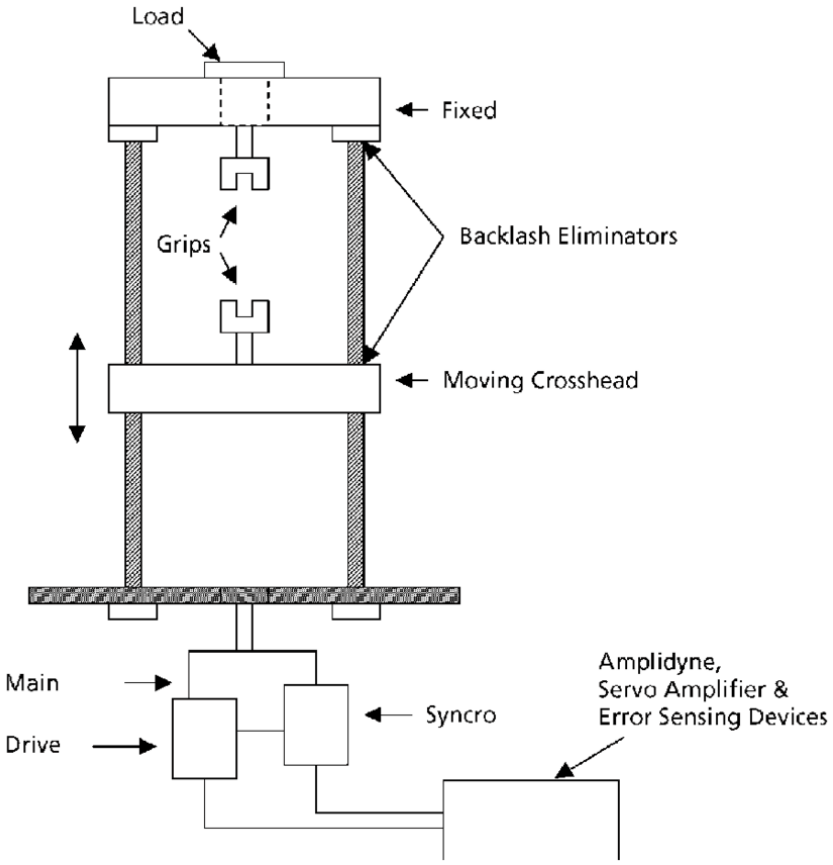


FIGURE 21 SCHEMETIC OF TENSILE TESTING SETUP

3.3.2. Adhesion Test

The adhesion test is the measurement of the adhesion strength or bond strength of a coating to a substrate. When adhesive is applied to a surface or object, many chemical, physical and mechanical forces are involved and may affect each other. Adhesive strength is the main functional feature of a coating to the substrate. The adhesive strength may be defined as a force necessary for the separation of two adherent parts along the interface.

The adhesion coating test applies to those adhesive coating compounds that may develop the chemical bindings between the interfaces of the substrate surface and the adhesive layer, upon application. Binding force that is created between the coating compound and the substrate material may be measured by performing adhesion coating tests.

Shear Adhesion Test

The adhesive strength of adhesive layer can be studied by universal testing machine. The specimen having adhesive coating is attached between two adherent plates. Then those plates are placed between the jaws of UTM. Keeping one end fixed, force is applied to the other end. The shear strength of the adhesive material can be measured by applying the opposite force to the coating surface, until the surface is separated (removed/ peeled off) from the substrate surface. This strategy requires surface preparation to maximize the adhesive strength. A specific value can be defined as the required minimum level of adhesion strength and the test would be terminated at that value (Swamy, Jones, & Charif, 1986).

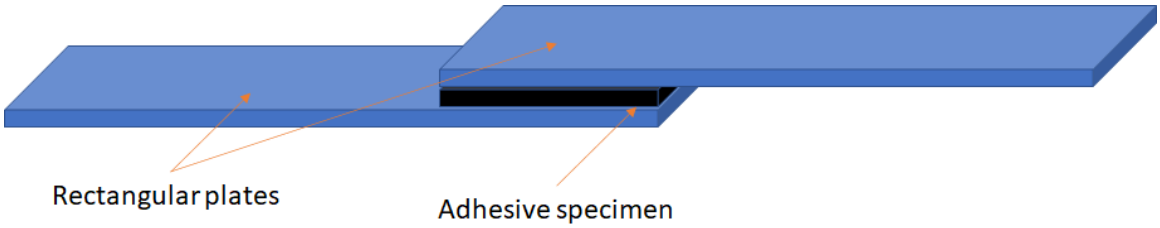


FIGURE 22 SHEAR ADHESION TEST

CHAPTER 4

RESULTS AND DISSCUSSION

4.1.FTIR

The FTIR spectra of samples were recorded in the middle infrared region. It was used to get the information about the several functional groups present in the sample. Samples for FTIR were prepared by freeze-drying.

Pure Chitosan

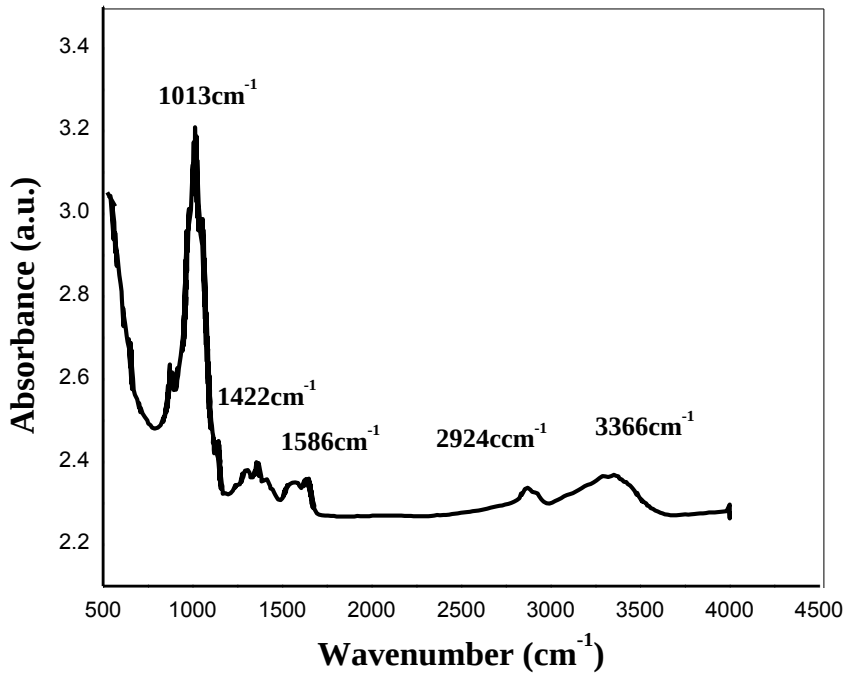


FIGURE 23 FTIR SPECTRUM OF PURE CHITOSAN

Chitosan FTIR spectrum has shown significant absorption bands associated with the characteristic functional groups, recorded in the infrared range from 500 cm^{-1} to 4000 cm^{-1} (fig. 23). The most prominent peak with high absorption intensity at 1013cm^{-1} corresponds to glycosidic link (C-O-C) anti- symmetric stretching vibrations. The stretching vibrations -NH_2 bending (amide I), N-H (amide II) of residual N-acetyl group and C-H bands found at 1422cm^{-1}, 1586cm^{-1} and 2924cm^{-1} respectively. The wide

absorption band observed at 3366cm^{-1} is associated with the various amide stretchings overlapped by O-H stretching bands.

Chitosan-Hydrocaffeic Acid (Ch-HCA)

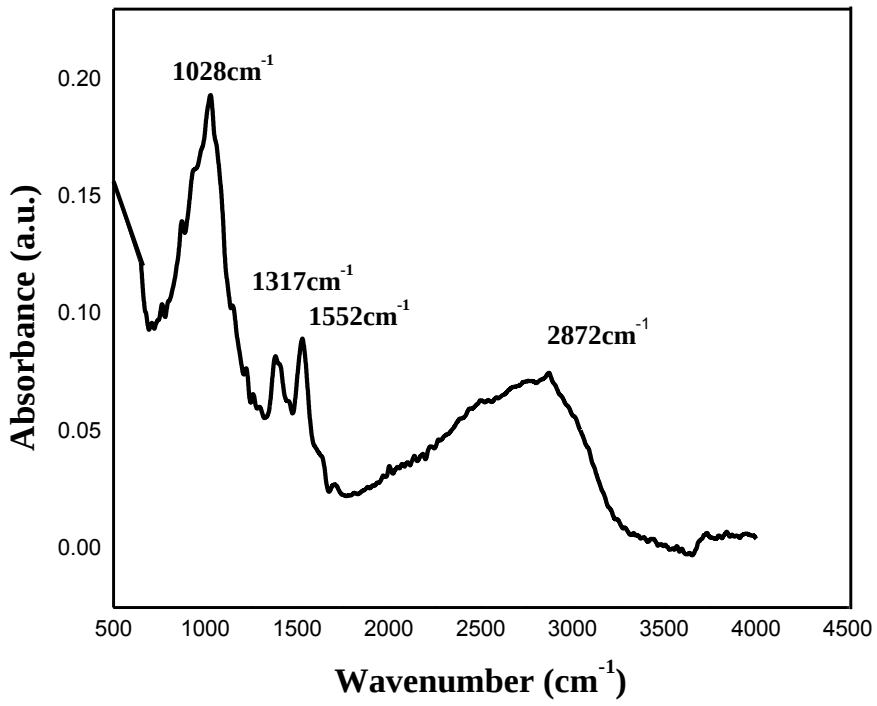


FIGURE 24 FTIR SPECTRUM OF CH-HCA

The FTIR spectrum of prepared hydrocaffeic acid modified chitosan has shown significant absorption bands associated with the characteristic functional groups, recorded in the infrared range from 500 cm^{-1} to 4000 cm^{-1} (fig. 24). The chemical bonding between chitosan and hydrocaffeic acid was observed through the absorption peak found at 1552cm^{-1} associated with the C=C stretching vibrations of arenes (aromatic ring). The absorption peaks at 1028cm^{-1} and 1317cm^{-1} correspond to glycosidic link (C-O-C) anti-symmetric stretchings and C–N (amide III) stretching vibrations of chitosan. The broader absorption band present at 2872cm^{-1} is assignable to –CH_2- stretchings. The absence of absorption peat at 1645cm^{-1} of the C=O stretching vibrations of chitosan amide II can be

observed. The HCA C=O peak at 1645 cm^{-1} disappeared in the spectra of HCA–Ch conjugates confirming the successful coupling between the carboxylate groups of HCA and the amino groups of Ch.

Azidobenzoic acid-Chitosan-Hydrocaffeic Acid (AzCh-HCA)

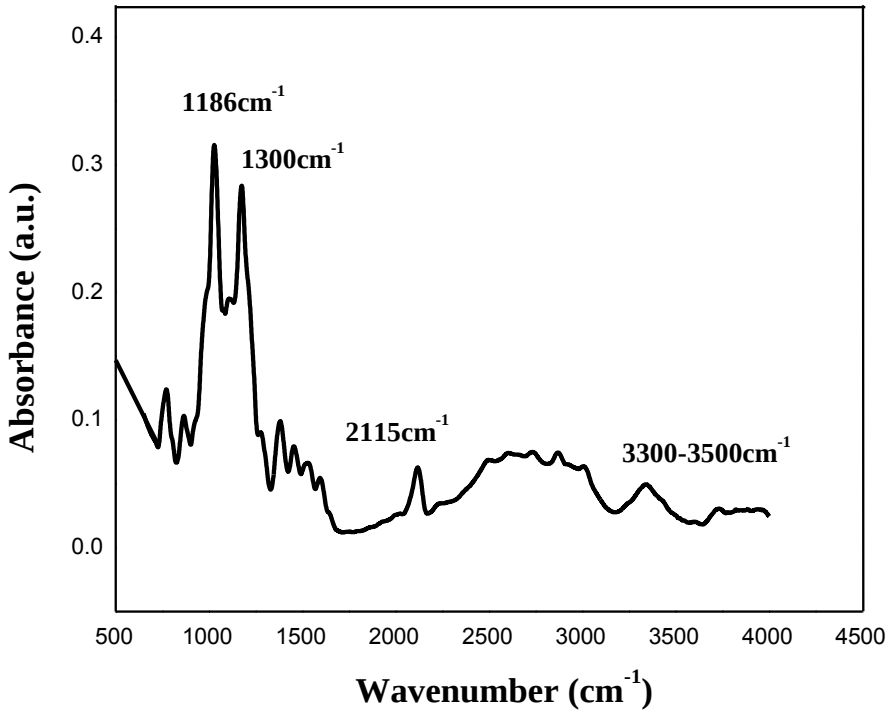


FIGURE 25 FTIR OF AZCH-HCA

The FTIR spectrum of prepared photosensitive hydrocaffeic acid modified chitosan has shown significant absorption bands associated with the characteristic functional groups, recorded in the infrared range from 500 cm^{-1} to 4000 cm^{-1} (fig. 25). The characteristic absorption peak appeared at 1300cm^{-1} is corresponds to the –C-O stretchings of -COOH unit of p-azidobenzoic acid. The absorption band found at 3300-3500cm^{-1} can be associated with the stretchings of the hydrogen bonded OH groups constituted to polymeric chains formation. The strong absorption peak at 1186cm^{-1} is consistent with the glycosidic link (C-O-C) anti-symmetric stretching vibrations of chitosan. The absorption

signal observed at 2115cm^{-1} is associated with the covalently bonded azide group (N_3) of azidobenzoic acid to chitosan.

4.2. UV-Visible

The UV absorbance of chitosan-hydrocaffeic acid and chitosan-hydrocaffeic acid azide was analyzed by UV-visible spectroscopy.

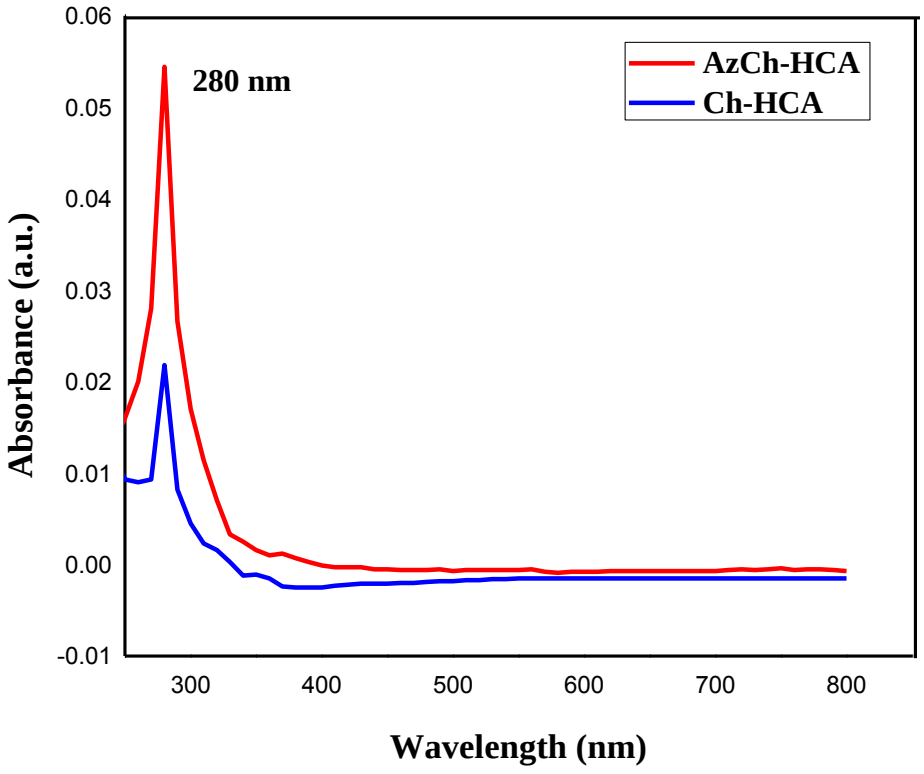


FIGURE 26 UV SPECTRA OF CH-HCA AND AZCH-HCA

UV spectra were recorded in the wavelength range of 200-800 nm. Ch-HCA was analyzed with the 1% acetic acid as a baseline solvent because the sample was prepared by dissolving freeze dried product into 1% acetic acid. And the AzCh-HCA sample was taken from the final solution and spectrum was collected from the diluted solution. The UV spectra of Ch-HCA and AzCh-HCA are shown in Fig. 26. The absorption peak observed at 280 nm wavelength confirms the presence of 1,2-dihydroxybenzene (Lu et al., 2020), appears due to transition of n–π* from -OH to the aromatic ring (Wang et al., 2016), which

ultimately verifies the successful conjugation of hydrocaffeic acid with Chitosan. It can be observed that chitosan-hydrocaffeic acid has minimal UV absorption. While azidobenzoic acid shows excellent absorption in UV range (Panchal & Mekonnen, 2019). Therefore, the incorporation of azide groups in chitosan-hydrocaffeic acid units remarkably enhanced the UV absorption.

4.3. AFM

AFM images were obtained to take an estimation of surface morphology. Samples were prepared by simply adding a drop of polymeric suspension of AzCh-HCA on FTO substrate, followed by soft baking on hot plate.

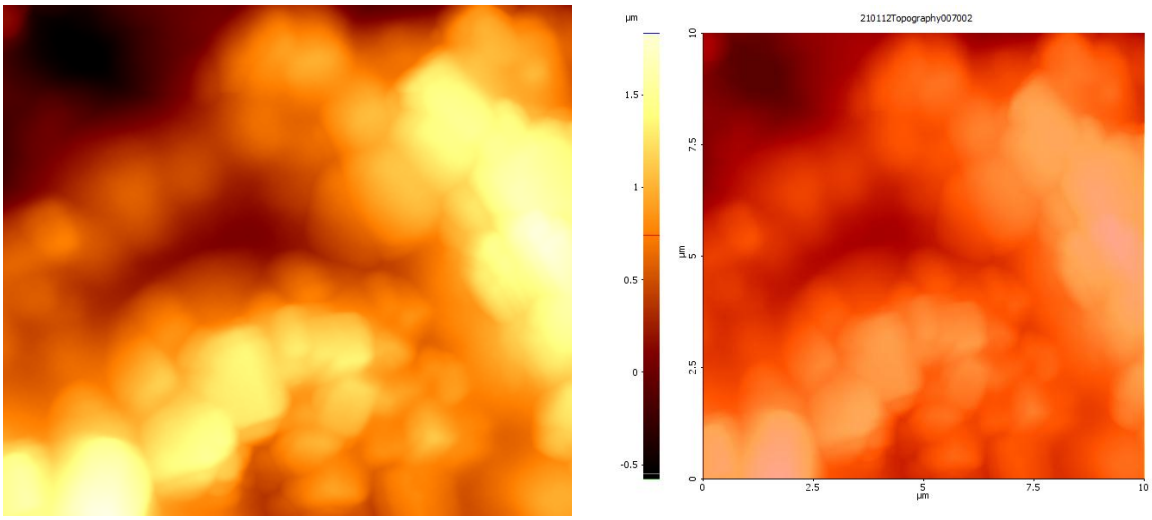


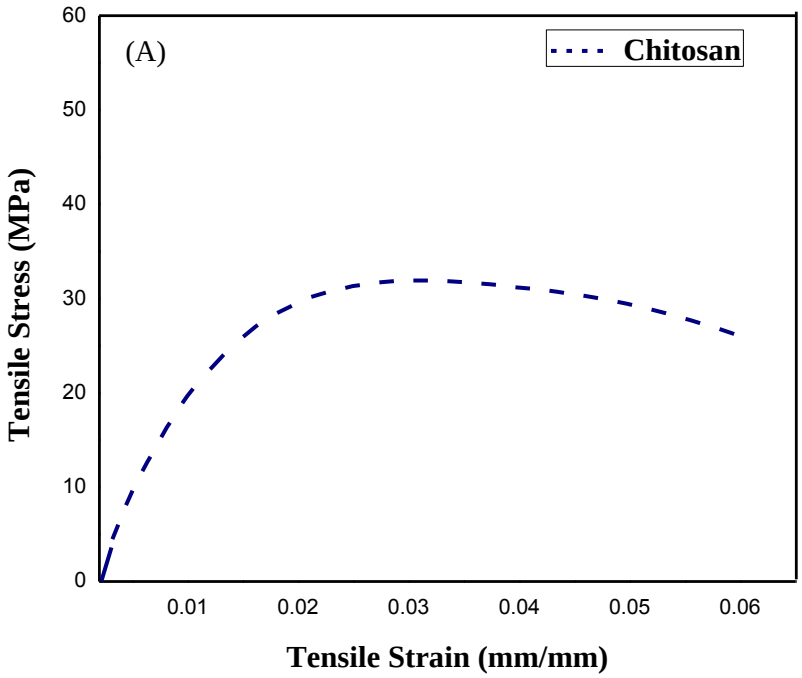
FIGURE 27 AFM IMAGES OF AZCH-HCA

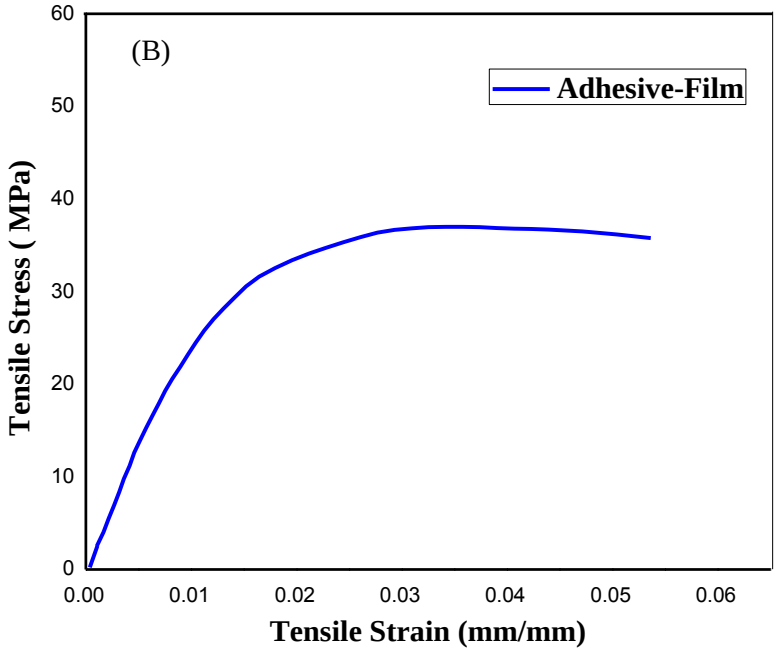
AFM image of AzCh-HCA is shown in figure 27. AFM micrograph was recorded in tapping mode of the cantilever. It can be seen from the micrographs that surface is not smooth, it has a bumpy particle like appearance, it means that more reaction sites and more functional groups are available to be attached with the surface of skin.

4.4.Tensile Test

Tensile test was performed to evaluate the strength of prepared thin films. Samples were prepared by cutting the thin films into strips of 5 mm in width and 40 mm in length. The load deformation curves were recorded at a crosshead speed of 5 mm/min. The obtained information was plotted as stress-strain curves.

Chitosan thin film was used as a control group. Modification of chitosan with hydrocaffeic acid and photosensitive azide resulted in better tensile strength (TS) with respect to TS of pure Chitosan thin film (fig. 28 B).





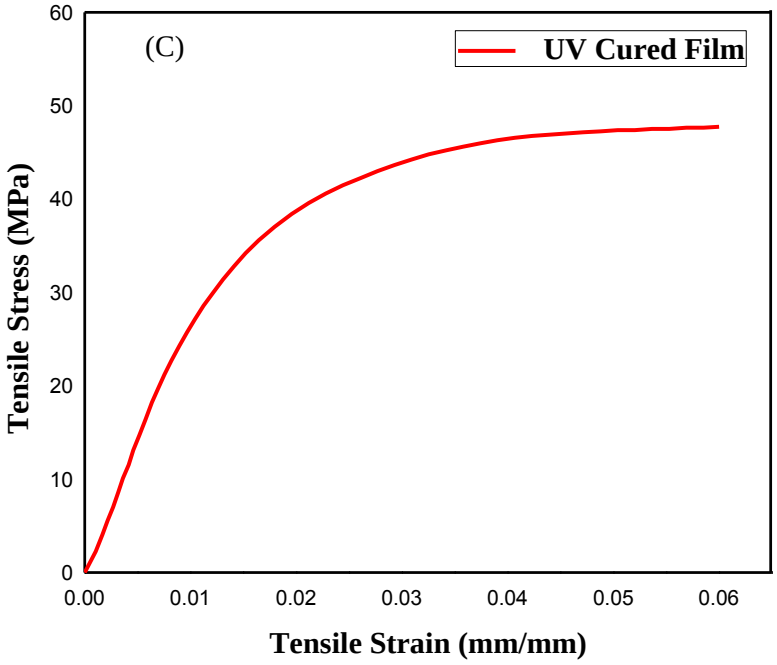


FIGURE 28 STRESS-STRAN CURVE (A) CHITOSAN (B) ADHESIVE THIN FILM (C) UV CURED THIN FILM

The TS value increased from 33.2 MPa (fig. 28 A) to 42.9MPa (fig. 28C). The binding of $–NH_2$ groups of chitosan and the phenol groups of hydrocaffeic acid can be a reason for improved tensile strength of prepared thin films or it can be caused by hydrogen bonded -OH groups formed during polymerization of AzCh. Moreover, during UV irradiation of photosensitive chitosan film, the azide groups of 4-azdobenzoic acid will degrade to give nitrene groups. These nitrene groups cause crosslinking with amines of chitosan and increase the overall tensile strength of chitosan films.

4.5.Adhesion Test

The shear adhesion tests were performed to investigate the bond strengths of adhesive chitosan films before and after UV irradiation.

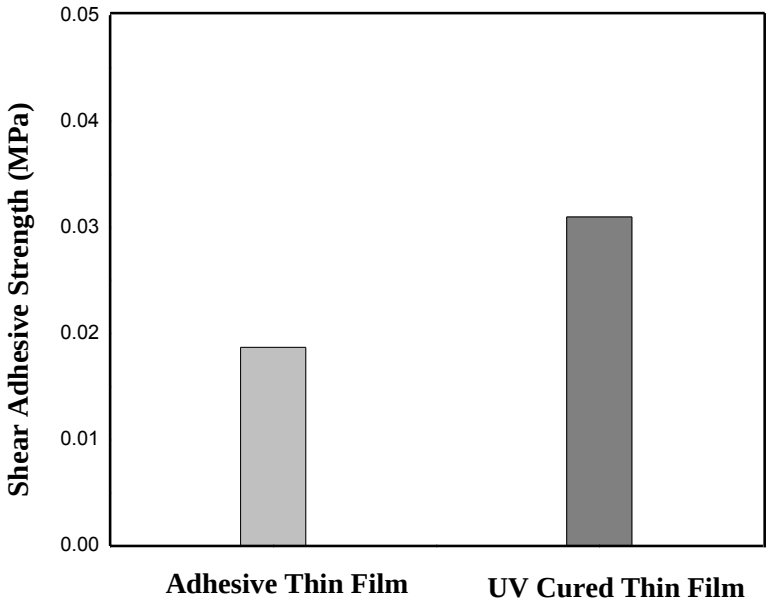


FIGURE 29 SHEAR ADHESIVE BOND STRENGTH RESULTS

Fig. 29 illustrates the results of shear adhesive bond strengths of prepared samples. The adhesive layers coated on living tissue were adhered to steel strips (1.5cm wide and 8cm in length) using cyanoacrylate glue. The one end of the steel strips assembly was clamped and the force with the crosshead speed of 0.50 mm per min, is applied to the other end

coincide with the long axis of strips. The adhesion test for chitosan film before UV exposure appears as the detachment of adhesive film from living tissue. The enhanced adhesion shear strength for the AzCh-HCA film cured after UV exposure, to the maximum value of 0.03MPa, can be attributed to the crosslinking ability of film's molecules with amines of tissues present on the skin surface.

4.6.Conclusion

The photosensitive chitosan (AzCh-HCA) and photocured chitosan films via UV irradiation were successfully prepared. The interactions between chitosan, hydrocaffeic acid and p-azidobenzoic acid were confirmed by FTIR investigations. The modifications of physical properties of chitosan films were investigated for prepared samples. The substitutions of various additives do not induce an increased tensile strength value, sometimes the value of TS decreases. The enhanced TS value reflects the strong interactions between the substituted material and the polymeric chitosan, which leads to enhanced film rigidity. The incorporation of hydrocaffeic acid and azidobenzoic acid improved the tensile strengths of chitosan films. While the crosslinking within the film matrix under UV light significantly increased film rigidity. The improved interfacial adhesion bindings of prepared films help them to withstand the interfacial stresses embedded during wound closure procedures.

The free standing layers were obtained through water mediated release methodology. This technique is appropriate for wound dressing as the procedure requires no organic solvent. The thin film is supposed to seal wounds tightly and limit the humid environment needed for bacteria to grow, which results in less wound complication. Photosensitive polymers are more biocompatible and effective to be used as tissue adhesives. Chitosan is considered to be safe because of its history in medical applications. Azide residues can be suspected to be toxic due to its high reactivity with proteins and living tissues. However, the intramolecular crosslinking of azide residues to amine would result in the loss of this potential toxic effect. The final mechanical and adhesion properties of these photopolymers can be controlled by optimizing the ultraviolet exposure time.

CHAPTER 5

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REFERENCES

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