Fabrication and Characterization of Erythromycin Loaded Spin Coated Ultra-Thin PVA/PLA Hybrid Polymer Films for Wound Healing Applications

By

SALMA MUMTAZ
NUST201362095MSMME62413F

Supervisor
Dr. MURTAZA NAJABAT ALI

School of Mechanical and Manufacturing Engineering
National University of Sciences and Technology
H-12 Islamabad, Pakistan
December, 2015

Fabrication and Characterization of Erythromycin Loaded Spin Coated Ultra-Thin PVA/PLA Hybrid Polymer Films for Wound Healing Applications

A thesis submitted in partial fulfillment of the requirement for the degree of Masters of Science

In
Biomedical Sciences and Engineering

By

SALMA MUMTAZ
NUST201362095MSMME62413F

Supervised by
Dr. MURTAZA NAJABAT ALI

School of Mechanical and Manufacturing Engineering
National University of Sciences and Technology
H-12 Islamabad, Pakistan
December, 2015

DECLARATION

It is hereby declared that this research study has been conducted for the partial fulfillment of requirements for the degree of Master of Science in Biomedical Sciences. I hereby declare that no portion of this work has been submitted in support of an application for another degree to any other university. All the work done during the course of this study is original and work based on other studies has been cited accordingly.

_____

Salma Mumtaz

This work is dedicated to my parents for their constant help and support, and without which it would have been impossible to complete this project.

ACKNOWLEDGEMENTS

Foremost, thanks to **Almighty Allah** for enabling me to complete my Masters.

This study would not have been possible without the support and guidance of many people. It is to them that I owe my deepest gratitude.

I would like to acknowledge the efforts of **Dr. Murtaza Najabat Ali** and **Dr. Umar Ansari** for the completion of my project. I would like to thank them for their valuable advice and guidance throughout the duration of this project.

I would like to express my sincerest thanks to Rector NUST, **Engr. Muhammad Asghar and Principal SMME, Dr. Abdul Ghafoor** for providing us with an excellent research environment in SMME, NUST.

I take this opportunity to thank my friend **Bakhtawar Ghafoor** for all her great help, support, encouragement and understanding throughout my project. A special thanks to **Mariam Mir** for her support and guidance that helped me in many ways. I owe my gratitude to my friends **Wajiha Ahmed** and **Iqra Munawwar** for their valuable advices and companion all through my project.

I would like to acknowledge lab technician Prosthetics Lab, SMME and Characterization Lab, SCME for their assistance.

I would also like to thank my sister **Hina Mumtaz** for her friendship, advice, criticism and encouragement and always finding the time to listen to my grievances, no matter how insignificant.

Last but not the least, I wish to thank my **family** for their help, support, blessings and love throughout my life.

Table of Contents

ABBREVIATIONS

°C	Centigrade
PLA	Polylactic acid
PVA	Polyvinyl alcohol
DMF	Dimethyl Formamide
SFWE	Simulated Fluid Wound Exudate
FTIR	Fourier Transform Infrared Spectroscopy
SEM	Scanning Electron Microscopy
EDS	Energy Dispersive Scattering

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Wounds are highly vulnerable to bacterial infection due to loss/impairment of the skin's integrity. Therefore, prevention of bacterial infection in the wound is critical for management of the wound. Thus, it is very important to develop a wound dressing material that not only is non-toxic, biocompatible and impermeable to bacteria and microbes but also aids the healing process .Polymeric ultra-thin films, so called nanosheets, due to their adhesive nature show peculiar properties making them useful for many applications like they showed good barrier ability for protecting against bacterial penetration. The present study deals with the development of ultra-thin polymer films consisting of polyvinyl alcohol (PVA) and poly lactic acid (PLA) with the ability of encapsulating antibiotic agent (erythromycin) in the wound area.

These hybrid films were developed using the spin coating method, wherein 9% (w/v) of PVA and 3% (w/v) of PLA was dissolved in hot DMF. These films were further loaded with antimicrobial agent (erythromycin) and spin coated on a clean glass surface. The blended films were characterized through Scanning electron microscopy (SEM/EDS) analysis and Fourier Transform Infrared spectroscopy (FTIR) .Water contact angle was also measured to evaluate the hydrophilicity of the films.In addition, in-vitro degradation profile of these ultra-thin polymer films and drug release profile were investigated using simulated fluid wound exudate.

Results indicated that ultra-thin polymer films are hydrophilic enough to attach to the surface. FTIR data indicated physical interaction occurred between drug and polymers. SEM analysis revealed uniform distribution of drug molecules in polymer matrix. In vitro drug release profiles showed specific release pattern, thus making then suitable for wound healing management.

Keywords: PVA, PLA, Erythromycin, Spin coating, Characterizations, Drug release profile

1. INTRODUCTION

Wound is defined as any kind of injury or damage to living tissue due to mechanical, chemical and thermal means or could be due to some underlying medical cause (Boateng, Matthews, Stevens, & Eccleston, 2008). Generally there are two types of wounds .Acute wounds are generally healable within 3 months and are caused by mechanical means whereas chronic wounds could take more than 3 months and they are direct result of diseases such as diabetes and internal tumors and recurrence is common in these type of wounds (Holeppagol, 2011). Acute and chronic wounds are major clinical concern so it is necessary to fabricate a wound dressing material which not only is non-toxic and keeps the wound from infection but also fasten the healing process and is permeable to water vapour and gases. People of past generations used to cover the wound area with different types of suitable materials such as honey pastes, herbal extracts, and animal fats to prevent infections and healing process is accelerated (Pieper & Caliri, 2003).By the use of new biopolymer fabrication procedures and the development of new materials have increased the quality of a wound dressing. The primary objective in designing a wound dressing is to aid rapid wound healing with the best chemical and mechanical properties and prevent infections by the use of antibiotic drugs (Abdelrahman & Newton, 2011).

Wound dressings are the bandages that cover the ulcer area clean and aid the healing process .They are usually manufactured from biocompatible polymers. Ideal wound dressing should be biocompatible, non-toxic, maintains the moisture of the microenvironment, protects the damaged area from secondary infections, absorbs the exudates of wound, minimize the risk of reoccurrence, easy removal and have good mechanical properties (Abdelrahman & Newton, 2011; Bolton, 2004).

1.1 Fundamentals of Wound Healing Management

Wound healing is a biological and complex process that relates to many physiological parameters and different types of cells. Process of healing is classified into four main stages. First stage is hemostasis then inflammation and proliferation and last is maturation and remodeling.

In hemostasis, coagulation of blood occurs due the formation of fibrin clot and infiltration of white blood cells or leukocytes also occurs (Boateng et al., 2008). In the second phase, bacteria and debris are removed by the process of phagocytosis (Li, Chen, & Kirsner, 2007). Macrophages release chemotactic agents that cause the migration of fibroblasts towards the wound area (Li et al., 2007). The proliferative phase immediately occurs after migration (Boateng et al., 2008). Proliferative activity of many elements such as fibroblasts, endothelial cells, epithelial cells and angiogenesis which is formation of new blood vessels also occurs during proliferation phase (Azizi & Osgouie, 2010). In the remodeling or maturation phase, granulation tissue replaces the fibrin clot rich in collagen type III (Li et al., 2007). Epithelium strengthens and wound contraction occurs. Wound size is reduced by the activity of myofibroblasts (Li et al., 2007).

Management of wound depends on many factors such as type of wound, patient's physical condition, healing process of the wound, physical and chemical characteristics of dressings (Boateng et al., 2008). The dressings need to be tested and assessed based on physical and chemical properties, depending on the wound type and wound healing stages. Dressings can be classified based on the nature of material used, their function in the wound, and their physical appearances (Boateng et al., 2008). Dressings can be made from different types of polymers such as polyvinyl alcohol, polylactic acid, chitosan, sodium alginate, collagen, etc

Studies have shown that ultra-thin films prove to be a good candidate for wound dressings. They are biocompatible, bioresorbable, adhesive and have flexible nature, also have the advantage of programmable or controlled drug release, effective in the release of encapsulated substances, and bioactive with fast wound healing properties

(Islam et al., 2013). The films are used to treat superficial, lightly exudating or epithelializing wounds. Polymers of both biological and synthetic origins are good candidates for wound dressings. Synthetic polymers have low protein adsorption and cell adhesion property and leech into the physiological system and releasing harmful substances. On the other hand, biodegradable polymers are biocompatible and degradable (Shoichet, 2009). Biological as well as synthetic polymers can be used to form different shapes suitable for wound healing such as hydrocolloids, alginates, hydrogels, and thin films.

Various treatments are present for treatment of wounds, but unfortunately there is no ideal dressing that can provide all the requirements such as biocompatibility, maintaining good mechanical strength, biodegradation to non-toxic substances within the time frame, ability to support proliferation of cells. Dressings can be in the form of bandages, gauze, cotton, hydrocolloids, alginates, hydrogels, or films, etc. The main function of a dressing material is to keep the wound area dry and prevent in from contamination (Boateng et al., 2008). There is a need to produce an ideal dressing that can satisfy all these conditions or requirements and efficiently deliver therapeutic agents at a controlled rate and is made from easily available, inexpensive materials and accelerate the healing process.

Studies have shown that films used for wound healing are usually thin with good mechanical properties and can deliver drugs uniformly at a controlled rate. They can get resorbed and there is no need to replace it. Once placed, it will help in wound healing and maintain its properties as long as the wound is healed. The resorbable films allow live cells to proliferate and replace the biopolymer over time (Islam et al., 2013).

2. LITERATURE REVIEW

Different types of wound dressing materials are available for different types of wounds. Although there is a wide variety of dressing materials available in the market, there is always a need for better, inexpensive and more efficient ones. This area covers many topics which are required in the production of the biologically active thin polymeric films for wound healing.

2.1 Types of Wounds

Wounds are injuries that break the healthy skin or damage other body tissues. They may include bruises, cuts, scratches, scrapes and punctured skin. Wound can be a result of external stress like physical, chemical and thermal damage to the skin or due to disturbance in the normal anatomical structure and function of healthy skin or due to some underlying medical condition like bedsores or even cuts and incisions of a surgery. Wounds can be classified based on various categories as shown in Figure 1.

2.2 Wound Healing Process

Wound healing regains the original structure of the human skin (Vishwajit, Fuelhase, & Badlani, 2009). It is a series of intricate processes (Li et al., 2007) involving four highly programmable phases - hemostasis, inflammation, proliferation and remodeling of tissue.

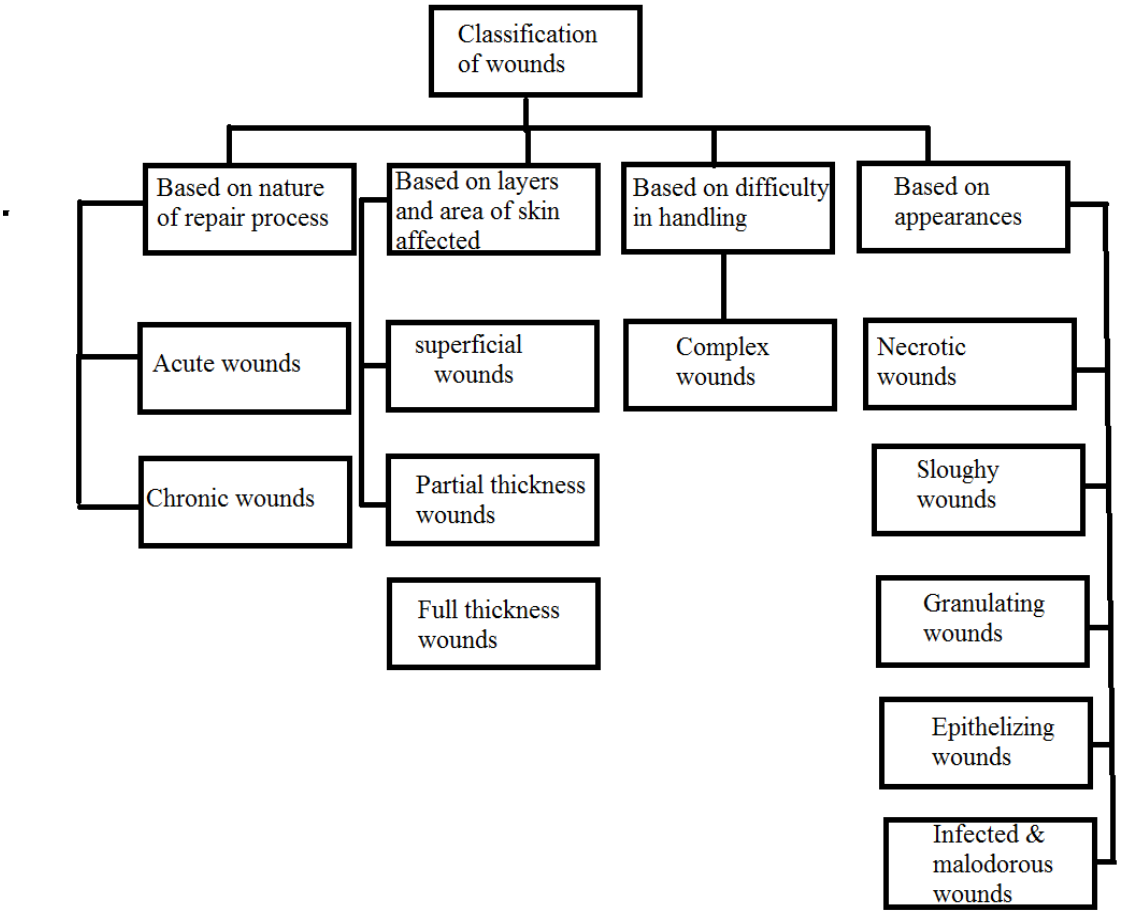

Figure 1: Classification of wounds (Boateng et al., 2008).

The first stage of hemostasis immediately begins after injury to the tissue, with vascular constriction and clot formation of fibrin. Platelets appear into the wound sites which are activated by extra cellular matrix. Activated platelets undergo aggregation and adhesion. They release many mediators and adhesive proteins (Li et al., 2007). These factors contribute fibrin clot formation. Once the clot dries up and forms a scab, it gives strength to the injured site (Boateng et al., 2008).

When bleeding is stopped, inflammatory cells move towards the wound site and activate the inflammatory phase, which is followed by infiltration of neutrophils, lymphocytes and macrophages,. Main function of neutrophils and macrophages is the removal of cellular debris and invading bacteria and microbes into the wound bed by

the process of phagocytosis. The inflammatory phase occurs from a few minutes to 24 hours of the injury and can lasts for up to 2 weeks (Boateng et al., 2008).

The inflammatory phase is immediately followed by proliferation phase and granulation tissue is formed by the growth of lymphatic capillaries and vessels in the wound area .In this phase, fibroblasts synthesize collagen which provides structure to the skin surface (Boateng et al., 2008).The process of inflammation is stimulated by growth factor and growth factor receptors (Epstein, Singer, & Clark, 1999).Angiogenesis (process in which new blood vessels are formed) also occurs in this phase. The proliferation phase usually starts on the third day after the occurrence of injury and can lasts for about 2-4 weeks**.**

The last step in wound repair is remodeling. In this phase, development of new epithelium and formation of scar tissue occurs. In this phase, degradation of collagen occurs. It depends on a balance between various factors that cause the formation of extracellular matrix (ECM) components and enzymes that degrade the components which are involved in remodelling of the extracellular matrix like matrix metalloproteases (MMPs). Tissue remodelling and maturation regains the strength and elasticity of the damaged tissue (Azizi & Osgouie, 2010).

2.3 Wound Dressing Materials

Dressings could be classified based upon the physical form of dressing, type of substance or material used and functions in the wound area as shown in figure 2 (Boateng et al., 2008). Another classification like islands, primary and secondary dressings are also present. Primary dressings are in contact with the wound area and are covered by secondary dressings. In an island dressing type, a central or middle absorbent area is present which is covered by an adhesive portion (Boateng et al., 2008).

Bandages, cotton, gauze and wool are mostly used as primary and secondary dressings. These dressings are dry so they do not maintain the moist wound microenvironment. Modern dressings like skin replacement products and wound

healing devices have been used. These wound dressings include, alginates, hydrogels and hydrocolloids are usually in the form of thin films, gels, and foams (Boateng et al., 2008). The main characteristic feature of a modern dressing is to maintain moist microenvironment to aid the healing process. The dressing material should also protect the wound from bacteria and other microbes and be permeable to gases and water vapour. The material must also promote granulation and epithelialization. These dressing materials if encapsulated with different therapeutic drugs fasten the healing process.

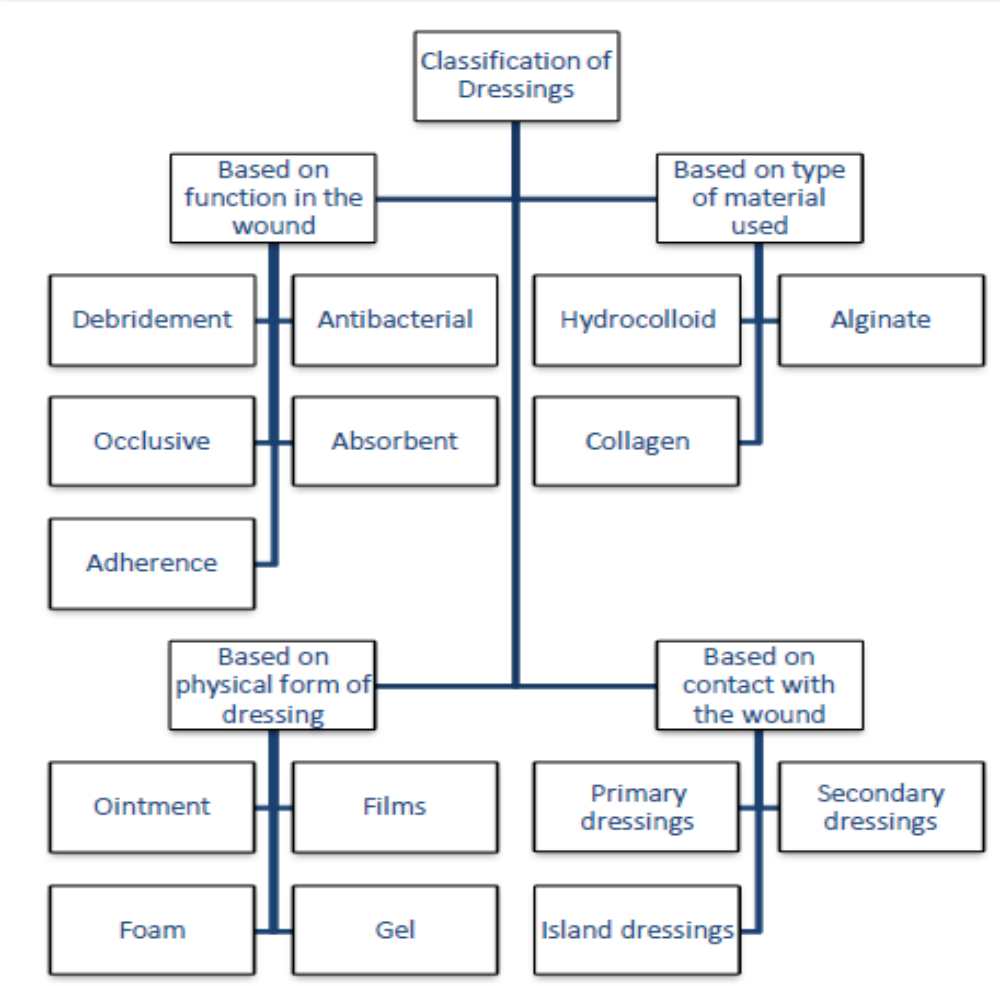

Figure 2: Classification of dressings (Boateng et al., 2008).

2.4 Polymers for Wound Management

Polymers appear to be a good candidate for wound management. Wide variety of natural and synthetic polymers are used as biomaterials in the development of dressing materials .Synthetic polymers have low protein adsorption, cell adhesion characteristic and have good mechanical properties. But due to poor biocompatibility problem along with the decrease in mechanical properties and release of degradation products in to the system is a major drawback of these polymers (Shoichet, 2009).

Synthetic polymers also leech into the body releasing toxic substances. Biodegradable polymers are not only degradable but also biocompatible. The problem with them is that their mechanical properties are not very strong (Broughton 2nd, Janis, & Attinger, 2006). They can be made stronger mechanically by cross-linking two or more synthetic or biopolymers (Shoichet, 2009).

When two polymers are combined, a blend is obtained that forms a good wound dressing material. Natural polymers such as polyurethane, collagen, chitosan, chitin, polylactic acid, and polylactic glycolic acid are mostly used for wound healing applications (Boateng et al., 2008).

Polyvinyl Alcohol (PVA) is inert, water soluble, non-toxic synthetic polymer (Bahrami, Kordestani, Mirzadeh, & Mansoori, 2003). It has good physical and chemical properties along with excellent film forming properties (Broughton 2nd et al., 2006). PVA is biocompatible polymer thus it has many applications in biomedical area. Due to presence of hydroxyl groups, it can react with different functional groups resulting in a biocompatible material (Priya, Gupta, Pathania, & Singha, 2014). It is used for controlled or programmable drug delivery, for membrane preparation, as a thickening agent and implantable medical devices (Bahrami et al., 2003). The structural formula of polyvinyl Alcohol is $(CH2CHOH)_n$ (Baker, Walsh, Schwartz, & Boyan, 2012).

Erythromycin as an antibiotic drug is extensively used for the treatment of bacterial infections like wound infections caused by gram-positive bacteria and some gram-negative bacteria. . It is used to treat skin infections (Hoyt & Robbins, 2001). Due to

very short half-life which is about 1 to 2 hours, it has fast distribution in the human body (Fan, Shan-Guang, Yu-Fang, Feng-Lan, & Tao, 2009). Erythromycin wound dressing could be concentrated in the wound area. This method significantly increase drug concentration in the skin to help fight against some secondary bacterial infections and also decrease its harmful side effects Molecular formula is C37H67NO13 (Hoyt & Robbins, 2001).

Poly(lactic acid) (PLA) is a aliphatic thermoplastic polyester composed of 2-hydroxy propionic acid which is a lactic acid (Lim, Auras, & Rubino, 2008). PLA has been used not only because of its easy production from lactic acid but also because of its biocompatibility and biodegradability, which make it useful candidate in biomedical applications (Gross & Kalra, 2002). Polylactic acid is becoming important polyester of bio –based origin which is used as support material for tissue regeneration. Due to advantages like easy availability from renewable resources, good tensile strength, thermal plasticity, biocompatibility, biodegradability and excellent processability, PLA can be used as a material in the field of packaging, textile and medical areas (Fan et al., 2009; Kar, 2003; Kenawy, Abdel-Hay, El-Newehy, & Wnek, 2007; Rosenberg, Devenney, Siegel, & Dan, 2007).

Blending of polymers is effective for obtaining polymeric materials for desired application. Generally the compatibility of polymeric blends is significantly influenced by the intermolecular interaction between the components of the blends (Yang, Su, & Yang, 2004).

In this research study, polyvinyl alcohol is blended with Poly lactic acid. PVA is frequently used in the scientific research of blends of biodegradable polymers. Hydroxyl groups of polyvinyl alcohol form hydrogen bonding with the oxygen atom of ester functional group leading to partial miscibility between PLA and PVA thereby increasing hydrophilicity and flexibility of PLA. PLA is used basically to increase the degradation time of the blend film.

2.5 Spin Coating Procedure

Spin-coating procedure is used mostly now a day for the fabrication of ultra-thin films over large surfaces with increased surface uniformity. Due to research in scientific field, the scope of spin-coating technique has extended by chemical engineering the support interface and solution to get definite and specific orderly structure in the ultra-thin polymer films.

The process of applying a liquid to a horizontal moving substrate, resulting in ejection and evaporation of the volatile solvent thus leaving a solid or liquid film, is called spin coating technique, and has been used since early 20th century. Spin coating is a procedure which is widely used to form uniform film to a flat and planar area over a surface with a highly reproducible and controllable film thickness. The importance of spin-coating is increasing due to its wide use in industry and medical area. It is thus important to obtain thorough understanding of the spin-coating technique from both a theoretical and an experimental point of view. The spin-coating procedure applies to organic, inorganic and organic/ inorganic mixture solutions. Spin-coating technique is used in many areas such as protective coatings, sensors, optical and paint coatings and thin membranes. Spin-coated polymer films that act as membranes have been found in sensor applications, where the polymer film acts as a barrier layer and an encapsulating agent (Chang et al., 2004; Corcoran, Arias, Kim, MacKenzie, & Friend, 2003; Geens et al., 2002; Klauk et al., 2002; Salleo, Chabinyc, Yang, & Street, 2002; Shi, Liu, & Yang, 2000).

2.6 Spin Coating Basics

In the spin coating procedure, first solution is deposited onto the desired surface, and the surface is then rotated to the desired rate. Liquid radially flows due to action of centrifugal force, and the excess liquid is ejected from the edges of the surface. The film continues to thin until effects of disjoining pressure cause the thin film to obtain an optimum thickness or until it turns into solid-like due to rise in liquid viscosity by

solvent evaporation method. The final thinning of the thin film is caused due to solvent evaporation procedure (Bornside, Macosko, & SCRIVEN, 1987).The Schematic of spin coating process is shown in figure 3.

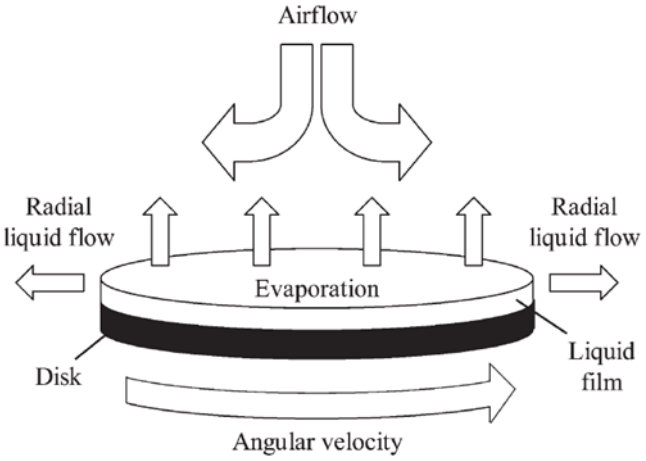

Figure 3: Schematic of spin coating process.

2.7 Aims and Objectives

- Development of ultra-thin films using biocompatible and biodegradable polymers.
- Improving the biodegradation time of the films.
- Characterization of these films.
- Investigating the drug release profile of antibiotic drug.

3. MATERIALS AND METHODS

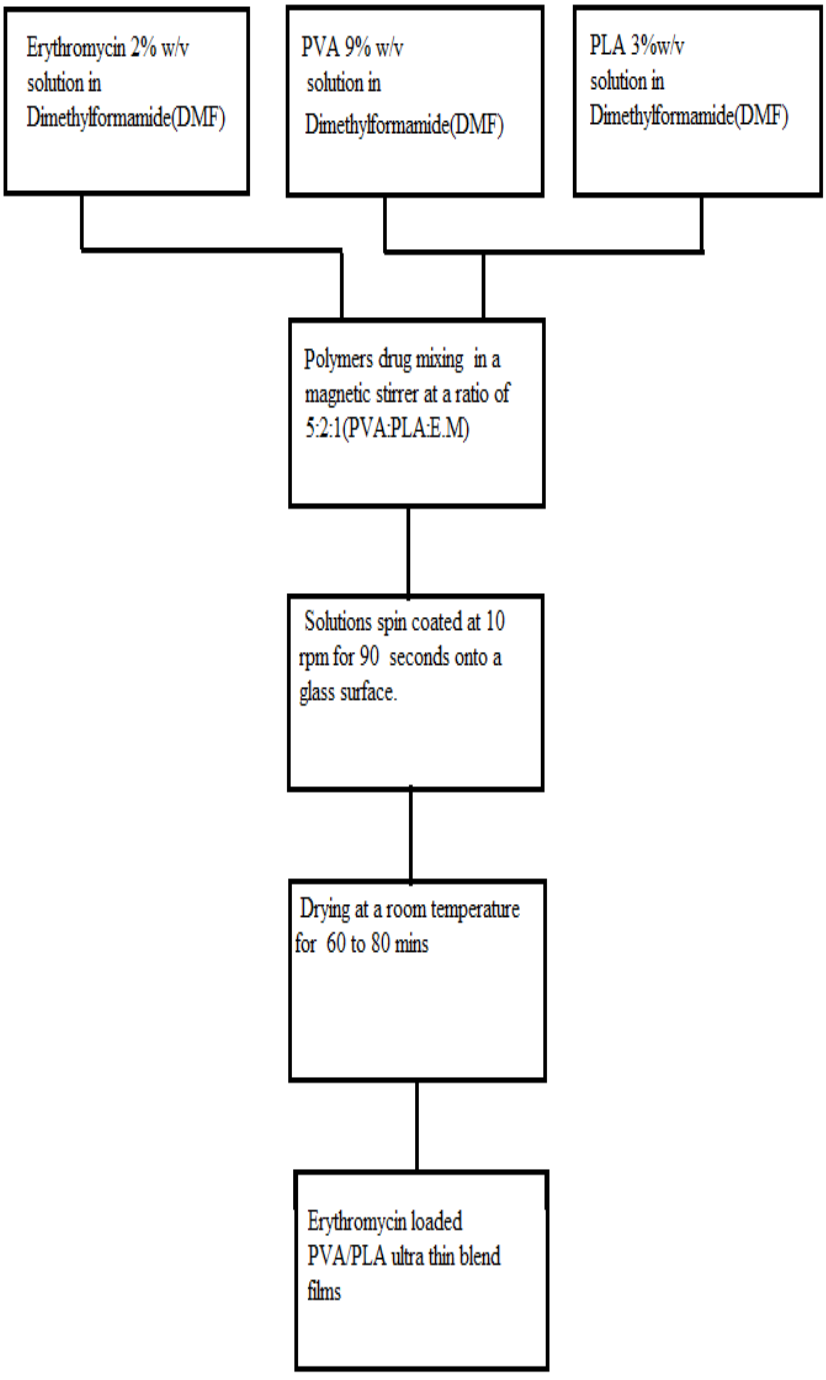

Figure 4: Flowchart of ultra-thin polymer blend film preparation.

3.1 Materials

PVA was purchased from BioChemica and was in the form of a fine, white powder (72000 BioChemica). Erythromycin was purchased from Sigma Aldrich pharmaceuticals.PLA spool was used in the experiment.

3.2 Sample Preparation

Solutions of the polymer PVA and PLA were prepared. The following percentage weight by volume formula was used to determine the amount of PVA and volume of DMF used for preparing the solutions of different concentrations:

$$Weight\ Percent\ (w/v) = [Mass\ of of\ Solute\ (g)\ of\ Solvent\ (ml)] \times 100$$

Where the PVA concentrated as determined by weight percent (w/v) is represented by %. 9% PVA, the amount of PVA in grams was calculated, using the formula given above, to be 1.8 g for 20 ml of DMF.

The calculated amount of PVA was measured using a weighing balance and added to the solvent in a glass beaker. The glass beaker was immersed in a water bath at room temperature and the temperature raised till 80. The required amount of PVA powder was gradually added to the beaker, followed by constant stirring until all the polymer get dissolved. The beaker was then removed from the water bath and the temperature of the solution brought back to room temperature. The PVA solution was loaded into plastic syringes having 10 ml volume.

Similarly 3 %(w/v) PLA solution was prepared by dissolving 0.3g of PLA in 10 ml of DMF solution at around 90º C with stirring.

3.3 Drug Loading:

2% Erythromycin (E.M) solution was prepared by mixing 0.1 gram of erythromycin in 5 ml of DMF solvent at room temperature with stirring.

Both polymer solutions and drug were mixed at a 5:2:1(PVA: PLA: E.M) ratio in a magnetic stirrer.

3.4 Spin Coating:

The solutions were mixed thoroughly; spin coated at 10 rpm for 90 seconds onto a glass surface. It was left to dry at a room temperature for 60 to 80 minutes to form a blend film. After the film was dried completely, it was peeled off from the glass surface as shown in Figure 5.

3.5 Simulated Fluid Wound Exudate (SFWE)

Simulated fluid wound exudate (SFWE) simulates wound fluids was prepared by mixing 2.49g sodium chloride (NaCl), and 0.1g calcium chloride (CaCl2) in 300 ml of distilled water. The pH of SFWE was 7.34.

Figure 5: Erythromycin loaded spin coated PVA/PLA ultra-thin polymer film.

3.6 Characterization of Films

To evaluate the performance of the ultra-thin polymer films for the purpose of wound healing, few characterization tests were done. Physico-chemical characterization tests that were done include wetting test to test the hydrophilicity of the films. Fourier Transform Infrared Spectroscopy to study the chemical interactions between the polymers used. Scanning electron microscopy (SEM) was used to analyze the morphology of surface of the films.

3.6.1 Fourier Transform Infrared (FTIR) Analysis

Fourier Transform Infrared Spectroscopy (FTIR) is a spectral technique which provides spectral information for organic, polymeric and some inorganic materials (Bellamy, 1975). The spectrum obtained gives the molecular absorption and transmission thus resulting in a molecular fingerprint of the sample (Bellamy, 1975). The spectrometer collects spectral data from a wide spectral range. These peaks correspond to vibrational frequencies between the atomic bonds that make up the material (Bellamy, 1975). No two different compounds can form the exact IR spectrum as every material is made up of specific combination of atoms (Xu, Stangel, Butler, & Gilson, 1997). The IR spectrum is obtained by Fourier Transformation of the signal from the interferometer (Bright, Devi, & Gunasekaran, 2010; Xu et al., 1997). This technique gives the atomic bonding and chemical composition of the constituents in a polymeric material.

Fourier transform infrared spectroscopy (Perkin Elmer, 100 spectrum FTIR spectrophotometer) of spin coated erythromycin loaded PVA/PLA blend films was carried out to indicate the presence of functional groups and types of chemical interaction between drug and polymer blend.In the FTIR graphs, the y-axis represents the percentage transmittance (%T) and the x-axis represents the wave numbers (1/cm).

3.6.2 Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) is an imaging tool extensively used to study the surface morphology of many materials. Highly-focused electron beams are used for imaging and it tells surface topography as well as depth of field with very high resolution images (Merrett, Cornelius, McClung, Unsworth, & Sheardown, 2002). The SEM magnification can be done up to 100,000 X (Goldstein et al., 1984) and particles of 10Å can also be viewed (Welter & Coates, 1974). Electron beam generated by the signal produce a 2-D image and provides various information about the studied material. In SEM, conductive and non-conductive samples can also be imaged (Welter & Coates, 1974).

Scanning Electron Microscopy was used to find out the surface morphology of the ultra-thin polymer films. The assessment of the surface morphology of these films was done using JSM-6490A Analytical scanning electron microscope (JEOL, Tokyo, Japan). All samples were first coated with gold particles using ion coater and imaged at 20kV.

3.6.3 Energy Dispersive Scattering (EDS)

Energy Dispersive Spectroscopy (EDS) allows identification of particular elements and their relative quantitative proportions like Atomic %. EDS can be used to identify the chemical composition of atoms to a size of a few microns. It also provides information about map of composition of elements over a larger area. Due to these abilities, it provides basic information about composition for many materials.

3.6.4 Wettability Test.

The contact angle is the angle which liquid or vapor interface forms with the solid surface. Contact angle measurement is used to give information about the hydrophobicity of a solid surface. Highly hydrophilic surface make a contact angle close to 0° ,the droplet will spread out completely onto the surface as liquid is very strongly attracted to the surface. When the hydrophobicity of the surface is increased, the contact angle will increase and it would be larger than 90° so highly hydrophobic surfaces having contact angles close to 180° indicate that there is no contact between the surface and liquid drop (Lafuma & Quéré, 2003). Hydrophilicity of the films is required as ultra-thin films absorb the exudate of wound and due to polymer degradation, drug release from the polymer films occur leading to therapeutic effect.

Goniometer was made manually using the stage of optical microscope covered with a box. First of all, small piece of strips (4 × 2 cm) of the polymer film are cut and put onto a glass slide. As film was ultra-thin so it sticks to the surface. Adhesive tape was also used to ensure a clear and flat view. The glass slide is properly placed on the microscope stage where 0.5µl drop of liquid was placed on the films with the help of micropipette. The measurements of each contact angle were performed within 5s with the camera. The surface contact angles measured were the mean of three determinations.

4. RESULTS & DISCUSSION

The characterization tests were done to evaluate the performance of the ultra-thin polymer films. The results of all the characterization tests are discussed in this chapter.

4.1 Thickness

To determine the uniform thickness of the ultra-thin films, each film's thickness was measured using a digital micrometer (Mitutoyo, Japan) at three different sites and the mean was calculated. Table 1 shows the average thickness of each of the films along with the standard deviation.

Table 1: Average thicknesses of the ultra-thin polymer blend films.

Samples	**Thickness(in *μm*)**
Pure PVA film	30 ± 0.5
PVA +PLA	30 ± 0.5
PVA+PLA+E.M	30 ± 0.5

It was observed that all the films have uniform thickness throughout the polymer films. The thickness of all films was about 30 μm. There is no significant difference in thickness of drug loaded films when compared to blank blend films which indicated that the loading of drug did not affect the thickness of films.

4.2 Fourier Transform Infrared Spectroscopy (FTIR)

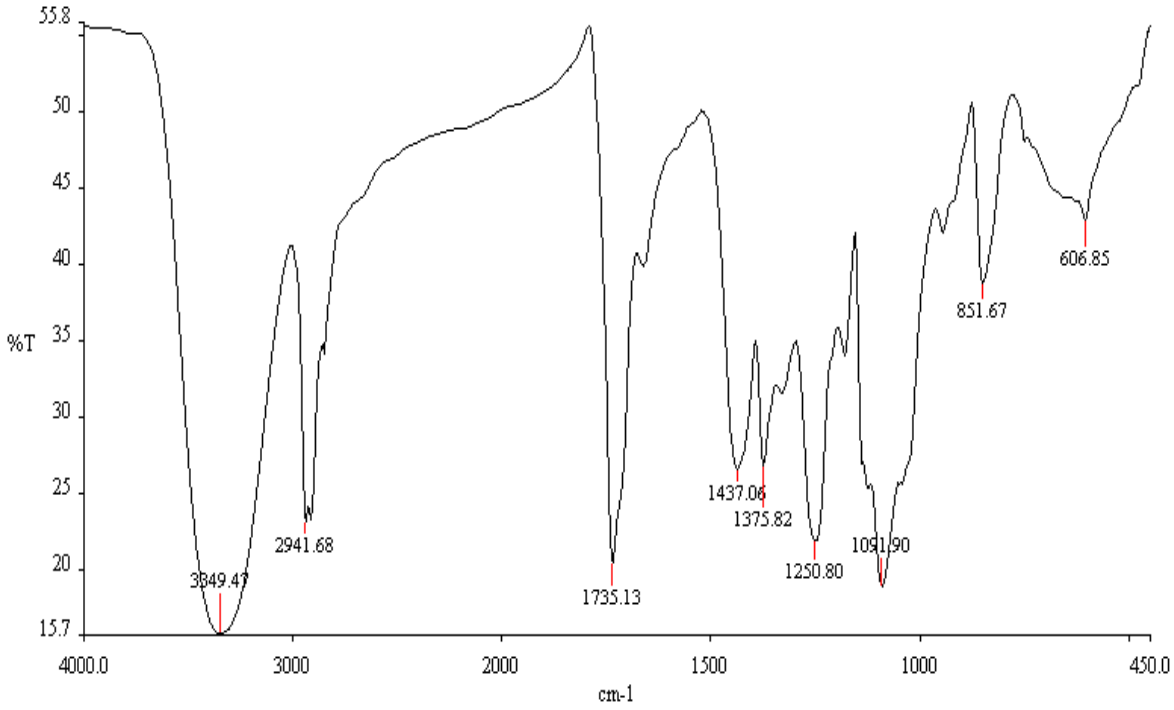

Figure 6: FTIR results of erythromycin based polymer film.

The FTIR analysis was performed to identify the nature of linkages between PVA, PLA and Erythromycin. The FTIR spectrum of erythromycin based polymer film has been shown in Figure 6. The peak appeared at 3349 cm^{-1} in the film indicates the presence of hydroxyl group (OH) (Kumar, Gayathiri, Ravi, Kabilar, & Velmurugan, 2011) while band at 2941cm-1 corresponds to (-CH) asymmetric and the symmetric stretching. The peak at 1735 cm^{-1} corresponding to C=O stretching and peak at 1437 cm^{-1} corresponds to C–H bending (Bhargav, Mohan, Sharma, & Rao, 2009; Nanda, De, Manna, De, & Tarafdar, 2010). The vibration of CH_2 group was found at 1375 cm^{-1} corresponding to C–H wagging in PVA .Also, the C–C stretching vibration of PVA appeared at 1250cm^{-1} (Nanda et al., 2010). Des-N-methyl-erythromycin is an

important metabolite and pharmacologically active metabolite of drug erythromycin. Studies suggest that the metabolism of erythromycin drug occurs by N-demethylation (Lee, Anderson, & Chen, 1956a, 1956b; Pineau, Galtier, Bonfils, Derancourt, & Maurel, 1990) that is why C-N peak is an important peak. The important characteristic peak appeared at 1090 cm^{-1} corresponding to C-N stretch of tertiary amine present in erythromycin. This C-N peak suggests that drug erythromycin is incorporated into the films and is pharmacologically functional to show its therapeutic efficacy. Tertiary amines have no N-H bonds, and therefore no band appeared in the IR spectrum

4.3 Scanning Electron Microscopy

SEM images of PVA, PVA+PLA and PVA+PLA+E.M were taken at different magnifications to study the surface morphology of the films. Figure 7a shows the SEM image of PVA. Figure 7b shows the SEM images of PVA/PLA blend while Figure 7c shows the SEM images of erythromycin loaded blend film.

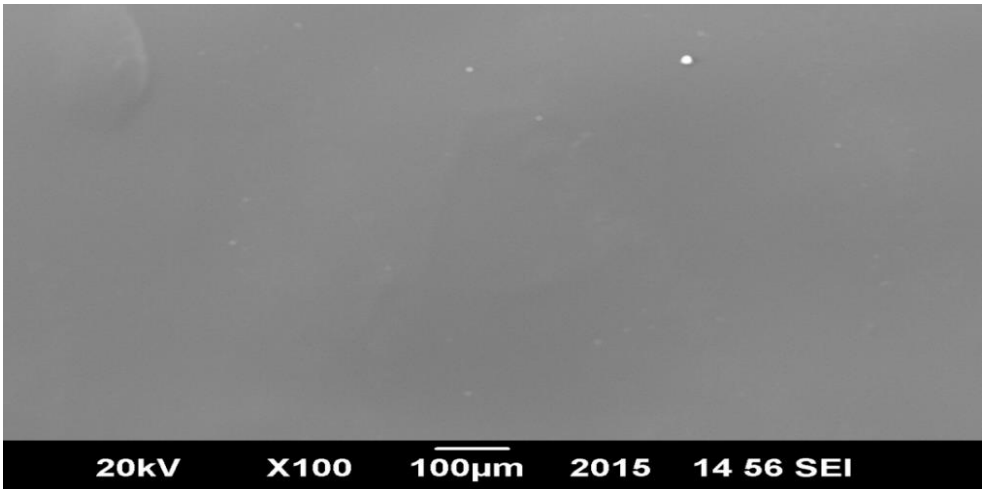

Figure 7a: SEM image of pure PVA film at 100 X.

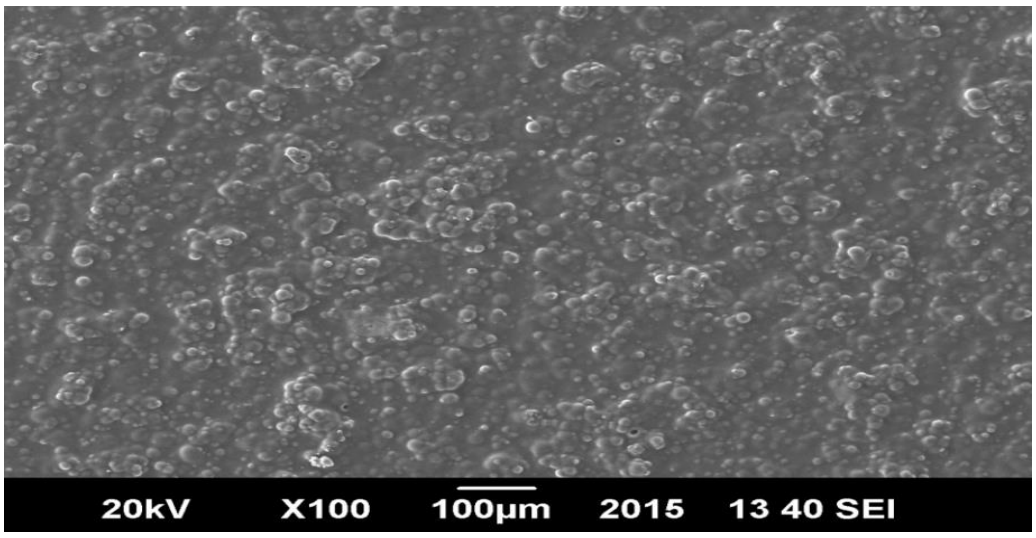

Figure 7b: SEM image of PVA/PLA blend film at 100 X.

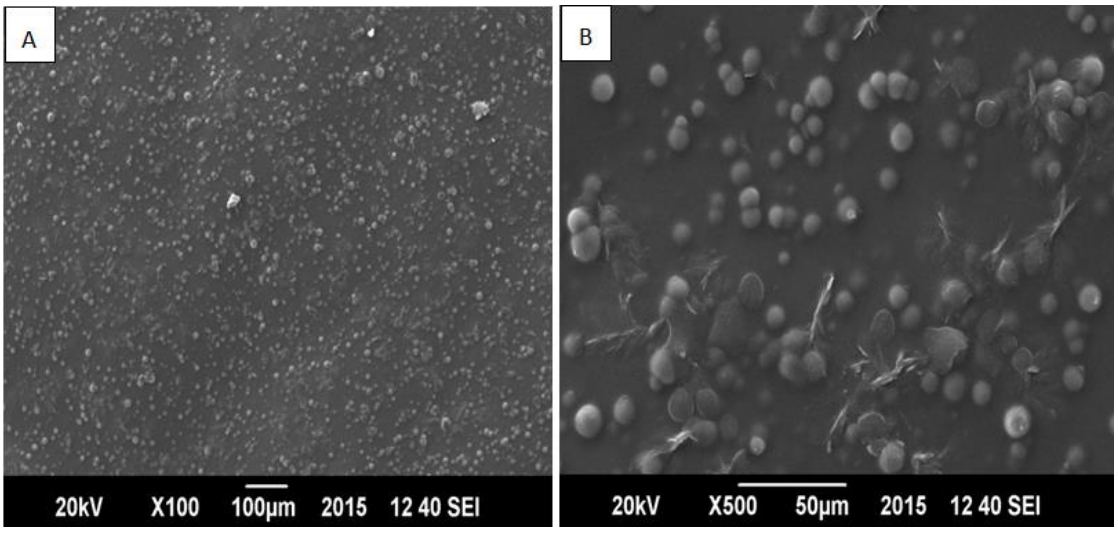

Figure 7c: SEM images of drug loaded blend films at 100 X (A) & 500X (B).

It was observed that all the SEM images are smooth showing that the films are uniform and continuous. Figure 7a showed the SEM image of pure PVA. Figure 7b shows the SEM image of blend film showed the aggregates of PLA dispersed on the surface of films which contributed to the film surface roughness. In figure 7c, the SEM show a kind of needle-like structure which indicated the presence of erythromycin drug into the surface of ultra-thin polymer blend films. No tears or folds or cracks are seen which makes them suitable for wound healing applications.

4.4. Energy Dispersive Scattering (EDS)

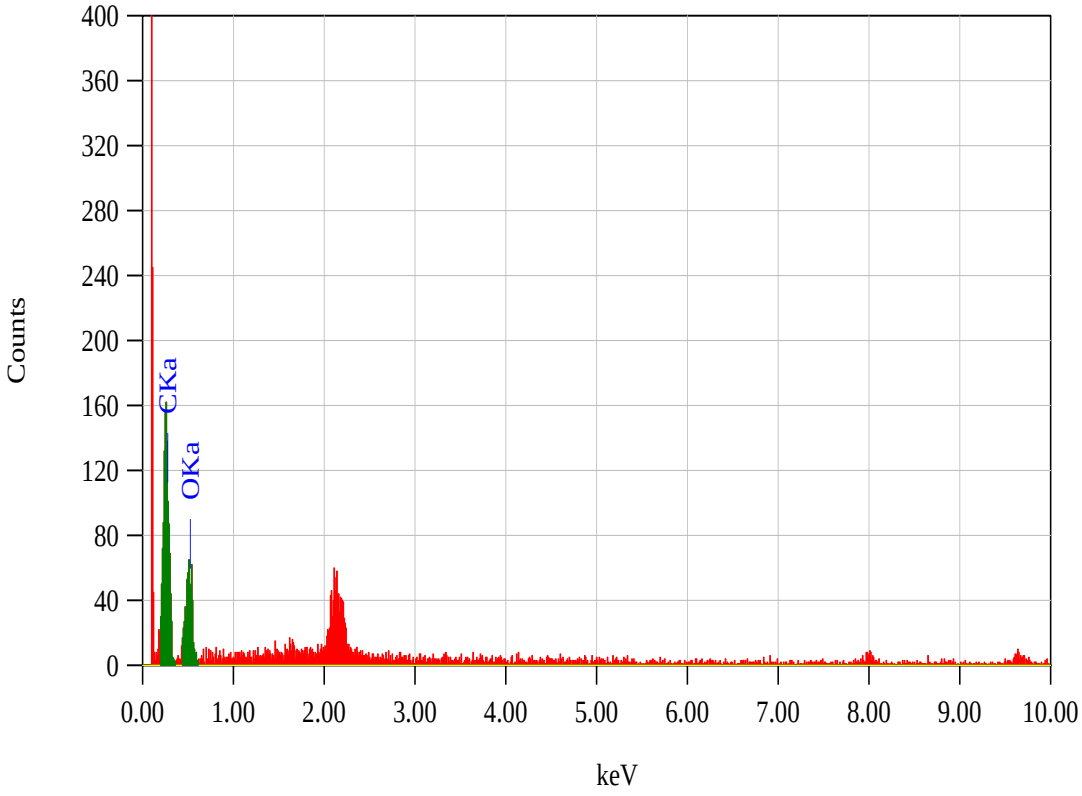

Figure 8a: EDS of PVA/PLA Blank Hybrid Ultra-Thin polymer films.

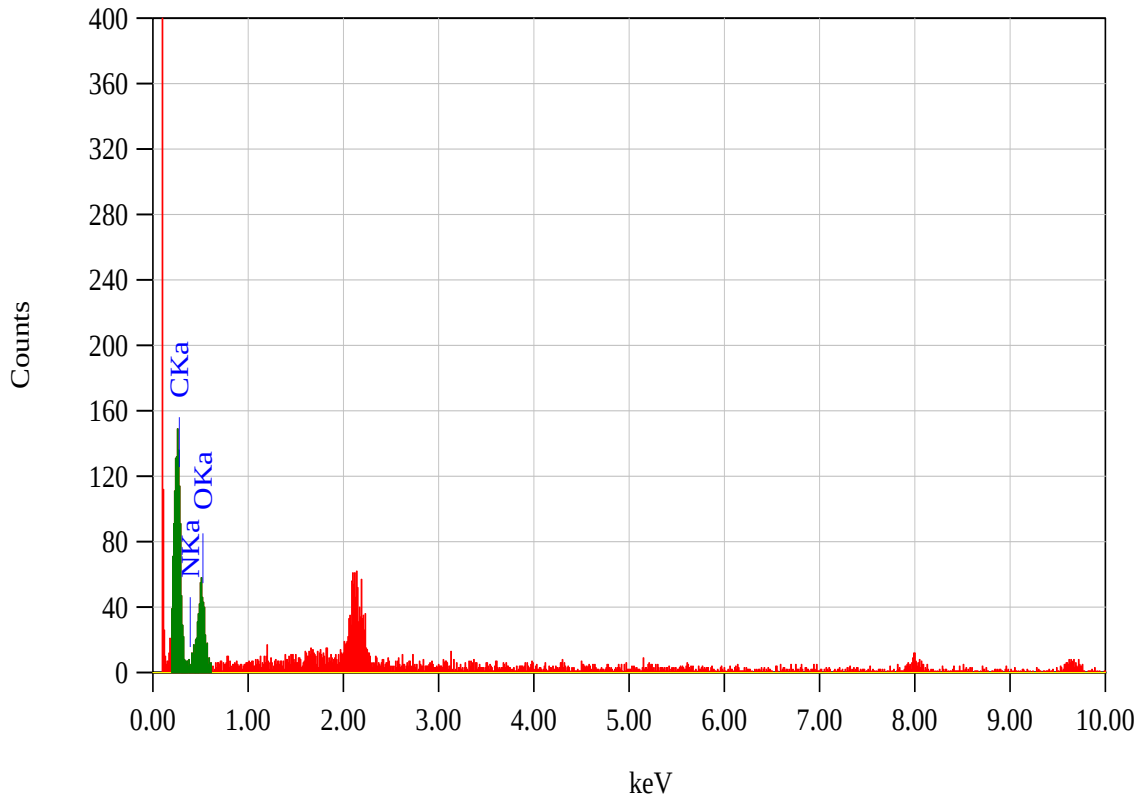

Figure 8b: EDS of Erythromycin based PVA/PLA Hybrid Ultra-Thin polymer films.

Nitrogen atom of macrolide ring of the erythromycin is involved in the therapeutic efficacy of the films. Results of EDS of drug loaded films as shown in figure 8 b showed that mass of nitrogen atom comes out to be 20.61% .This nitrogen atom is indicative of erythromycin incorporation into the films as there is no nitrogen atom seen in the blank composite films. In blank composite films shown in figure 8a, only carbon and oxygen atom is seen with mass appears to be 48.01% and 51.99% respectively. Results also showed that there is no contamination of other elements in the ultra-thin polymer films as no peaks of other elements are seen.

4.5 Wettability Test.

Water contact angle was measured in three different films as shown in figure 9 .The contact angle was found out to be the 48° which is the mean of three readings of ultra-thin polymer films. Low values of contact angles indicate that these films are highly hydrophilic to absorb the wound exudate and release the active agent present in the films yielding therapeutic effect on the wound surface which is required in the management of wound healing.

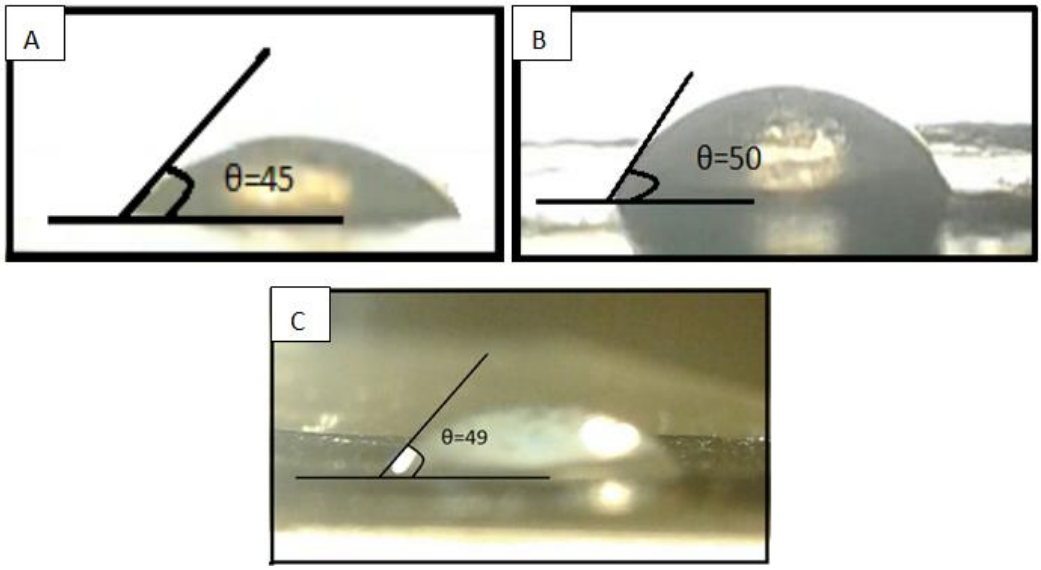

Figure 9: Shows the water contact angles for the three different drug loaded blend films (A, B and C).

4.6 *In Vitro* Degradation Drug Release Profile of Erythromycin Based PVA/PLA Film

The water absorbing capacity and degradation of the hybrid blend films was determined by immersing the films in simulated fluid wound exudate and checking their weights at different time intervals to calculate the amount of swelling and

degradation that has occurred (Yang et al., 2004). The degradation release profile of erythromycin loaded blend films were assessed using a mesh to hold the blend film in it. The portion of drug loaded blend film with measurable size (1" by 1") was cut and 0.2 ml of SFWE solution was dropped The water absorbing capacity and degradation of the hybrid blend films was determined by immersing the films in simulated fluid wound exudate and checking their weights at different time intervals to calculate the amount of swelling and degradation that has occurred (Yang et al., 2004). The degradation release profile of erythromycin loaded blend films were using micropipette into the mesh at 37 °C. After the intervals of 5 minutes, extra fluid was wiped out by tissue paper on the surface of mesh. Fresh 0.2 ml SFWE was again added. The weight of the mesh alone and with dried film was recorded before adding the simulated fluid wound exudate and after draining the SFWE every after 5 minutes interval. The degradation tests were carried in triplet and average value was determined.

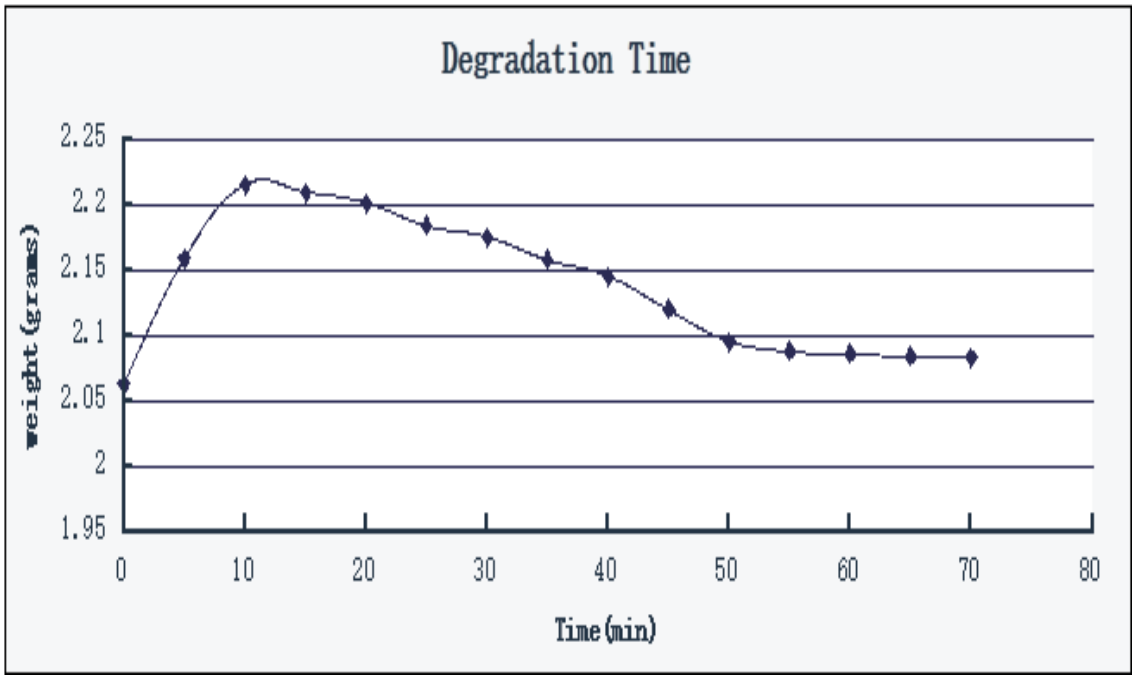

Figure 10: Degradation profile of erythromycin based polymer films. Time in minutes in shown on x-axis and weight in grams is shown on y-axis.

The degradation profile of polymer blend films was evaluated by recording weight loss at pre-determined time points . The degradation profile was divided in two stages; during first 10 minutes a sudden increase in the weight of the films is observed as shown in Figure 10, because of the absorption of SFWE by the polymers especially PVA. PVA, when exposed to aqueous media, it absorbs the liquid and swells resulting in an increase in weight and later is degraded and start losing weight (Kenawy et al., 2007) . Films with better mechanical strength have better swelling properties (Bright et al., 2010). For wound healing applications, it is essential that the films have good swelling capabilities so as to be able to absorb the exudates of the wound as well as remain long enough on the wound to protect it from microbes However, after 10 minutes the weight loss by the films is observed. As the polymers are degrading so gradual weight loss is observed. The rate of swelling of the polymer films after initial burst decreased with the increase in the content of drug in the films. This was due to the fact that with the increase in the drug concentration of polymer based films, absorption of the liquid medium by the polymer decreased (Bright et al., 2010).

4.7 Drug Release in Simulated Fluid Wound Exudate (SFWE)

In vitro antibiotic Erythromycin drug release was performed by placing a drug loaded polymer film of specific dimensions (2" by 2" inch) in petri dish. The amount of drug released in the SFWE medium was detected spectrophotometrically by measuring the absorbance of the solution of stimulated fluid wound exudate (SFWE) picked out in fixed 5 minutes interval at 37◦C.Fresh medium was then again added to measure the next absorbance until all the film get dissolved.

Maximum absorption of the erythromycin was observed as 301nm using the said spectrum. The results of in vitro drug release evaluation of erythromycin from the erythromycin incorporated PVA/PLA hybrid polymeric films are presented in figure.

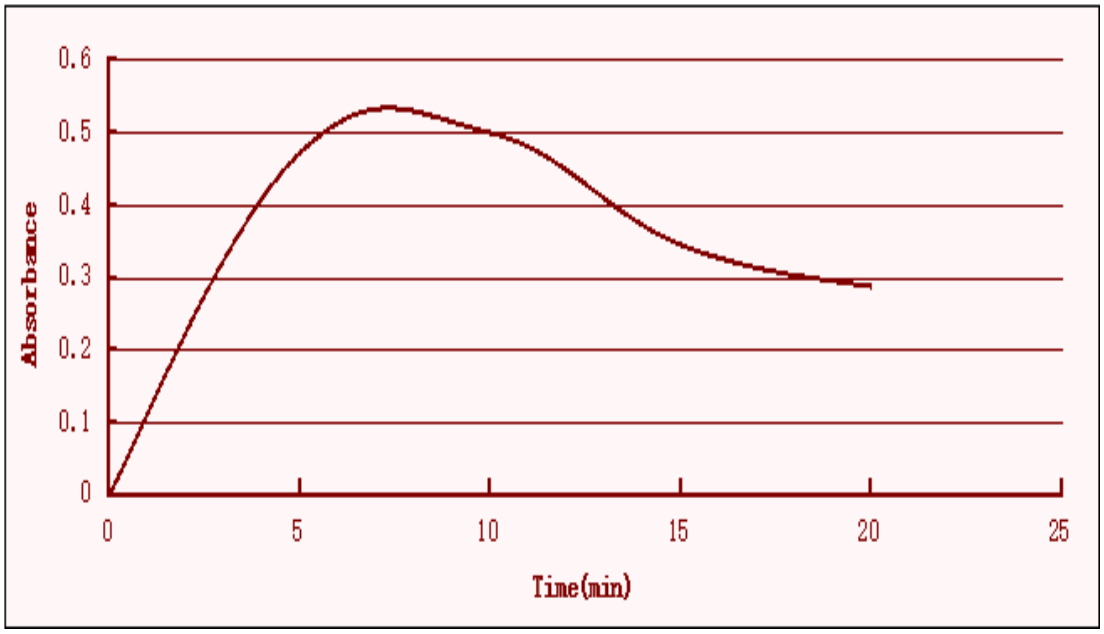

Figure 11: Drug release profile of drug loaded polymer film.X-axis shows time in minutes and y-axis shows UV-Vis absorption in λ.

The release behavior of the drug from polymeric films showed a biphasic pattern(figure 11). First an initial burst was observed, followed by a decrease release. Burst release of erythromycin from the surface of the films was detected during first 10 minutes which was also verified by SEM images of polymer ultra-thin films. While after 10 minutes the degradation of drug loaded polymer film also contributes to the release of antibiotic drug.

The starting Erythromycin release rate has been higher as compared to the later stage due to the fast swelling ratio of polymeric network. In the early stage, due to easy

diffusion of simulated wound fluid (SFWE) molecule penetration into the polymeric network causes the polymer film to swell at faster rate than the remaining time which in turn allows the dissolved drug out of the polymeric network (Bahrami et al., 2003). The described phenomenon mostly happens in surface areas. Due to the liquid content present in the wound site, drug loaded PVA/PLA polymer wound dressing would be able to absorb the wound fluid and sufficient amount of antibiotic agent replace it for therapeutic efficiency.

In the second phase (after 10 minutes), the degree of swelling decreases dramatically and absorption of the liquid medium by the polymers decreased. Therefore, polymers would be the controlling agent of drug release. In this stage there would be a proportional decrease in the amount of antibiotic drug released from the wound dressing due to entrapment of drug into the polymer matrix.

5. CONCLUSION

5.1 Conclusion

Ultra-thin polymer films were successfully fabricated through spin coating procedure. Various characterizations tests were carried out. FTIR studies proved that physical blending and other inter and intra-molecular bonding has occurred and SEM analysis showed that the films were uniform and continuous. Degradation and drug release profile test were also performed to determine the effectiveness of these films in wound care management. The results showed that this erythromycin based ultra-thin films can be potentially used in wound healing applications.

5.2 Future Directions

There is an immense scope for these thin films in the advanced wound care market. As of now, these films can be used only for surface wounds but more research needs to be done to prepare films that can treat chronic wounds. Chronic and complex wounds are difficult and expensive to treat and represent a major challenge in the healthcare industry today. In this project, only two polymers have been used to formulate the films but research can be done to prepare the films with other polymers that are easily available and inexpensive. Also, these films can be used to encapsulate anti-microbial and anti-bacterial drugs which could further assist in the healing process. Further research needs to be done in this regard.

REFERENCES

Abdelrahman, Tarig, & Newton, Heather. (2011). Wound dressings: principles and practice. *Surgery (oxford), 29*(10), 491-495.

Azizi, Aydin, & Osgouie, Kambiz Ghaemi. (2010). *Dermal wound healing-remodeling phase: A biological review.* Paper presented at the Computer and Automation Engineering (ICCAE), 2010 The 2nd International Conference on.

Bahrami, S Bahram, Kordestani, Soheila S, Mirzadeh, Hamid, & Mansoori, Parvin. (2003). Poly (vinyl alcohol)-chitosan blends: preparation, mechanical and physical properties. *Iranian Polymer Journal, 12*, 139-146.

Baker, Maribel I, Walsh, Steven P, Schwartz, Zvi, & Boyan, Barbara D. (2012). A review of polyvinyl alcohol and its uses in cartilage and orthopedic applications. *Journal of Biomedical Materials Research Part B: Applied Biomaterials, 100*(5), 1451-1457.

Bellamy, Lionel John. (1975). *The infra-red spectra of complex molecules* (Vol. 1): Chapman and Hall London.

Bhargav, P Balaji, Mohan, V Madhu, Sharma, AK, & Rao, VVR Narasimha. (2009). Investigations on electrical properties of (PVA: NaF) polymer electrolytes for electrochemical cell applications. *Current Applied Physics, 9*(1), 165-171.

Boateng, Joshua S, Matthews, Kerr H, Stevens, Howard NE, & Eccleston, Gillian M. (2008). Wound healing dressings and drug delivery systems: a review. *Journal of pharmaceutical sciences, 97*(8), 2892-2923.

Bolton, Laura L. (2004). 4 Moist Wound Healing from Past to Present. *The Epidermis in Wound Healing*, 89.

Bornside, DE, Macosko, CW, & SCRIVEN, L-E. (1987). On the modeling of spin coating. *Journal of imaging technology, 13*(4), 122-130.

Bright, A, Devi, TS Renuga, & Gunasekaran, S. (2010). Spectroscopical vibrational band assignment and qualitative analysis of biomedical compounds with cardiovascular activity. *Int J Chem Tech Res, 2*(1), 379-388.

Broughton 2nd, G, Janis, Jeffrey E, & Attinger, Christopher E. (2006). The basic science of wound healing. *Plastic and reconstructive surgery, 117*(7 Suppl), 12S-34S.

Chang, Jui-Fen, Sun, Baoquan, Breiby, Dag W, Nielsen, Martin M, Sölling, Theis I, Giles, Mark, . . . Sirringhaus, Henning. (2004). Enhanced mobility of poly (3-hexylthiophene) transistors by spin-coating from high-boiling-point solvents. *Chemistry of Materials, 16*(23), 4772-4776.

Corcoran, N, Arias, AC, Kim, JS, MacKenzie, JD, & Friend, RH. (2003). Increased efficiency in vertically segregated thin-film conjugated polymer blends for light-emitting diodes. *Applied physics letters, 82*(2), 299-301.

Epstein, Franklin H, Singer, Adam J, & Clark, Richard AF. (1999). Cutaneous wound healing. *New England journal of medicine, 341*(10), 738-746.

Fan, Yang, Shan-Guang, Wu, Yu-Fang, Pan, Feng-Lan, Song, & Tao, Li. (2009). Preparation and characteristics of erythromycin microspheres for lung targeting. *Drug development and industrial pharmacy*, *35*(6), 639-645.

Geens, Wim, Shaheen, Sean E, Wessling, Bernhard, Brabec, Christoph J, Poortmans, Jef, & Sariciftci, N Serdar. (2002). Dependence of field-effect hole mobility of PPV-based polymer films on the spin-casting solvent. *Organic Electronics*, *3*(3), 105-110.

Goldstein, JI, Newbury, DE, Echlin, P, Joy, DC, Fiori, C, & Lifshin, E. (1984). Scanning electron microscopy and X-ray microanalysis Plenum Press. *New York, London*.

Gross, Richard A, & Kalra, Bhanu. (2002). Biodegradable polymers for the environment. *Science*, *297*(5582), 803-807.

Holeppagol, Krishnamurthy B. (2011). *Silver alginate interactive dressing in management of chronic wounds.* Rajiv Gandhi University of Health Sciences.

Hoyt, Jeffrey C, & Robbins, Richard A. (2001). Macrolide antibiotics and pulmonary inflammation. *FEMS microbiology letters*, *205*(1), 1-7.

Islam, Jahid MM, Tareq, H, Rahman, M Fizur, Habib, SMA, Rahman, Noshin A, Molla, E, & Khan, Mubarak A. (2013). Preparation and Characterization of Low Cost Asymmetric Thin Film as Accelerating Wound Healing Material. *American Academic & Scholarly Research Journal*, *5*(4), 16.

Kar, Ashutosh. (2003). *Pharmacognosy and pharmacobiotechnology*: New Age International.

Kenawy, El-Refaie, Abdel-Hay, Fouad I, El-Newehy, Mohamed H, & Wnek, Gary E. (2007). Controlled release of ketoprofen from electrospun poly (vinyl alcohol) nanofibers. *Materials Science and Engineering: A*, *459*(1), 390-396.

Klauk, Hagen, Halik, Marcus, Zschieschang, Ute, Schmid, Günter, Radlik, Wolfgang, & Weber, Werner. (2002). High-mobility polymer gate dielectric pentacene thin film transistors. *Journal of Applied Physics*, *92*(9), 5259-5263.

Kumar, R Ashok, Gayathiri, M, Ravi, S, Kabilar, P, & Velmurugan, S. (2011). Spectroscopy studies on the status of aloin in Aloe vera and commercial samples. *Journal of Experimental Sciences*, *2*(8).

Lafuma, Aurélie, & Quéré, David. (2003). Superhydrophobic states. *Nature materials*, *2*(7), 457-460.

Lee, Cheng-Chun, Anderson, RC, & Chen, KK. (1956a). Distribution and excretion of radioactivity in rats receiving N-methyl-C14-erythromycin. *Journal of Pharmacology and Experimental Therapeutics*, *117*(3), 265-273.

Lee, Cheng-Chun, Anderson, RC, & Chen, KK. (1956b). The excretory products of N-methyl-C14-erythromycin in rats. *Journal of Pharmacology and Experimental Therapeutics*, *117*(3), 274-280.

Li, Jie, Chen, Juan, & Kirsner, Robert. (2007). Pathophysiology of acute wound healing. *Clinics in dermatology*, *25*(1), 9-18.

Lim, L-T, Auras, R, & Rubino, M1. (2008). Processing technologies for poly (lactic acid). *Progress in polymer science*, *33*(8), 820-852.

Merrett, K, Cornelius, RM, McClung, WG, Unsworth, LD, & Sheardown, H. (2002). Surface analysis methods for characterizing polymeric biomaterials. *Journal of Biomaterials Science, Polymer Edition, 13*(6), 593-621.

Nanda, Pradyot, De, SK, Manna, Sujit, De, Udayan, & Tarafdar, Sujata. (2010). Effect of gamma irradiation on a polymer electrolyte: variation in crystallinity, viscosity and ion-conductivity with dose. *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms, 268*(1), 73-78.

Pieper, Barbara, & Caliri, Maria Helena Larcher. (2003). Nontraditional wound care: A review of the evidence for the use of sugar, papaya/papain, and fatty acids. *Journal of Wound Ostomy & Continence Nursing, 30*(4), 175-183.

Pineau, T, Galtier, P, Bonfils, C, Derancourt, J, & Maurel, P. (1990). Purification of a sheep liver cytochrome P-450 from the P450IIIA gene subfamily: Its contribution to the N-dealkylation of veterinary drugs. *Biochemical pharmacology, 39*(5), 901-909.

Priya, Bhanu, Gupta, Vinod Kumar, Pathania, Deepak, & Singha, Amar Singh. (2014). Synthesis, characterization and antibacterial activity of biodegradable starch/PVA composite films reinforced with cellulosic fibre. *Carbohydrate polymers, 109*, 171-179.

Rosenberg, R, Devenney, W, Siegel, S, & Dan, N. (2007). Anomalous Release of Hydrophilic Drugs from Poly (ϵ-caprolactone) Matrices. *Molecular pharmaceutics, 4*(6), 943-948.

Salleo, A, Chabinyc, ML, Yang, MS, & Street, RA. (2002). Polymer thin-film transistors with chemically modified dielectric interfaces. *Applied Physics Letters, 81*(23), 4383-4385.

Shi, Y, Liu, J, & Yang, Y. (2000). Device performance and polymer morphology in polymer light emitting diodes: The control of thin film morphology and device quantum efficiency. *Journal of Applied Physics, 87*(9), 4254-4263.

Shoichet, Molly S. (2009). Polymer scaffolds for biomaterials applications. *Macromolecules, 43*(2), 581-591.

Vishwajit, Sonia, Fuelhase, Claudius, & Badlani, Gopal H. (2009). The biochemistry of wound healing in the pelvic floor: What have we learned? *Current Bladder Dysfunction Reports, 4*(1), 13-19.

Welter, LM, & Coates, VJ. (1974). High resolution scanning electron microscopy at low accelerating voltages. *Scanning electron microscopy, 1974*, 59-66.

Xu, J, Stangel, I, Butler, IS, & Gilson, DFR. (1997). An FT-Raman spectroscopic investigation of dentin and collagen surfaces modified by 2-hydroxyethylmethacrylate. *Journal of dental research, 76*(1), 596-601.

Yang, Jen Ming, Su, Wen Yu, & Yang, Ming Chien. (2004). Evaluation of chitosan/PVA blended hydrogel membranes. *Journal of Membrane Science, 236*(1), 39-51.

MS THESIS

ORIGINALITY REPORT

9%	1%	7%	2%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Fathollahipour, Shahrzad, Susan Maziarfar, and Javad Tavakoli. "Characterization and evaluation of acacia gum loaded PVA hybrid wound dressing", 2013 20th Iranian Conference on Biomedical Engineering (ICBME), 2013. Publication	2%
2	Norrman, K., A. Ghanbari-Siahkali, and N. B. Larsen. "6Å Å Studies of spin-coated polymer films", Annual Reports Section "C" (Physical Chemistry), 2005. Publication	2%
3	Submitted to Imperial College of Science, Technology and Medicine Student Paper	1%
4	Dhawale, D.S.. "PVA assisted growth of hydrophobic honeycomb network of CdS thin films", Journal of Alloys and Compounds, 20100806 Publication	1%
5	Submitted to Higher Education Commission Pakistan Student Paper	<1%

6	Siew Leng Lim. "Infiltrating P3HT polymer into ordered TiO2 nanotube arrays", physica status solidi (a), 03/2011 Publication	<1 %
7	Te-Hua Fang. "Physical Behavior of Nanoporous Anodic Alumina Using Nanoindentation and Microhardness Tests", Nanoscale Research Letters, 08/08/2007 Publication	<1 %
8	Ahad, Noorhanim, Elias Saion, and Elham Gharibshahi. "Structural, Thermal, and Electrical Properties of PVA-Sodium Salicylate Solid Composite Polymer Electrolyte", Journal of Nanomaterials, 2012. Publication	<1 %
9	Submitted to University of Nottingham Student Paper	<1 %
10	Huang, Chien-Lin, Sheng-Yin Peng, Yu-Jyun Wang, Wen-Cheng Chen, and Jia-Horng Lin. "Microstructure and characterization of electrospun poly(vinyl alcohol) nanofiber scaffolds filled with graphene nanosheets", Journal of Applied Polymer Science, 2015. Publication	<1 %
11	Lim, L.T.. "Processing technologies for poly(lactic acid)", Progress in Polymer Science, 200808 Publication	<1 %

12	Submitted to University of North Texas Student Paper	<1%
13	White, Chris Wu, Wen-Li PU, Yuxie Rafail. "Probe segregation and [T.sub.g] determination of a supported ultra-thin polystyrene film studied by ", Polymer Engineering and Science, June 2003 Issue Publication	<1%
14	Submitted to Prairie View A&M University Student Paper	<1%
15	eprints.nmlindia.org Internet Source	<1%
16	www.freshpatents.com Internet Source	<1%
17	commons.emich.edu Internet Source	<1%
18	www.firp.ula.ve Internet Source	<1%
19	Kim, Kwang-Mo, Sahng Woo, Ju Lee, Hyun Park, Jinwon Park, and Byoung Min. "Improved Permeate Flux of PVDF Ultrafiltration Membrane Containing PVDF-g-PHEA Synthesized via ATRP", Applied Sciences, 2015. Publication	<1%
20	Indira, K. N., Y. Grohens, C. Baley, S. Thomas, K. Joseph, and L. A. Pothen.	<1%

"Adhesion and Wettability Characteristics of Chemically Modified Banana Fibre for Composite Manufacturing", Journal of Adhesion Science and Technology, 2011.
Publication

21	scholar.lib.vt.edu Internet Source	<1%
22	WHITE, C. C. MIGLER, K. B. WU, W. L.. "Measuring [T.sub.g] in Ultra-Thin Polymer Films With an Excimer Fluorescence Technique.", Polymer Engineering and Science, Sept 2001 Issue Publication	<1%

EXCLUDE QUOTES	ON	EXCLUDE MATCHES	< 1 WORDS
EXCLUDE BIBLIOGRAPHY	ON		

Proposed Certificate for Plagiarism

It is certified that MS Thesis Titled **Fabrication and Characterization of Erythromycin Loaded Spin Coated Ultra-Thin PVA/PLA Hybrid Polymer Films for Wound Healing Applications** has been examined by us. We undertake the follows:

a. Thesis has significant new work/knowledge as compared already published or are under consideration to be published elsewhere. No sentence, equation, diagram, table, paragraph or section has been copied verbatim from previous work unless it is placed under quotation marks and duly referenced.
b. The work presented is original and own work of the author (i.e. there is no plagiarism). No ideas, processes, results or words of others have been presented as Author own work.
c. There is no fabrication of data or results which have been compiled /analyzed.
d. There is no falsification by manipulating research materials, equipment or processes, or changing or omitting data or results such that the research is not accurately represented in the research record.
e. The thesis has been checked using TURNITIN (copy of originality report attached) and found within limits as per HEC plagiarism Policy and instructions issued from time to time.

Name & Signature of Supervisor

Dr. Murtaza Najabat Ali

Signature: _____