Physical and Antibacterial Properties of Iodine Containing Pullulan/Poly(vinylpyrrolidone) /Poly(vinylalcohol) Polymer Films

Shemaa Abdul Sattar Soud

Submitted to the
Institute of Graduate Studies and Research
in partial fulfillment of the requirements for the Degree of

Master of Science
in
Chemistry

Eastern Mediterranean University
June 2013
Gazimağusa, North Cyprus

pproval of the Institute of Graduate Studies and Research

Prof. Dr. Elvan Yılmaz
Director

I certify that this thesis satisfies the requirements as a thesis for the degree of Master of Science in Chemistry.

Prof. Dr. Mustafa Halilsoy
Chair, Department of Chemistry

We certify that we have read this thesis and that in our opinion it is fully adequate in scope and quality as a thesis for the degree of Master of Science in Chemistry.

Assoc. Prof. Dr. Bahar Taneri
Co-supervisor

Prof. Dr. Elvan Yılmaz
Supervisor

Examining Committee

1. Prof. Dr. ElvanYılmaz
2. Assoc. Prof. Dr. Mustafa Gazi
3. Assoc. Prof. Dr. Bahar Taneri
4. Assist. Prof. Dr. Burak Çiçek
5. Assist. Prof. Dr. Mehmet Garip

ABSTRACT

Iodine releasing polymer films were prepared by blending the polysaccharide pullulan (PUL) with poly(vinyl pyrrolidone) (PVP) and poly(vinyl alcohol) (PVA) while glutaraldehyde (GA) was used as a cross linker and glycerine (GL) as a plasticizer. Cross linking was done at 25°C and 60°C and homogeneity was improved by heating. Physical, chemical and thermal properties of the films were assessed by scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR) and by differential scanning calorimetry (DSC). In addition, the swelling behavior was followed in aqueous solution.

Polymer films were loaded with 0.10%, 1.0% and 10% (w/v) iodine solutions, a wide spectrum antibacterial agent. The quantities of iodide I^- and triiodide I_3^- loaded and released were measured by UV-VIS spectroscopy. Release kinetics was followed for 168 hr using the film treated with 10% (w/v) iodine solution. Antibacterial activity of iodine species released was tested against two types of bacterial strains *Escherichia coli* IFO3972 as gram negative and *Staphylococcus aureus* ATCC25923 as gram positive bacteria. Inhibition zone measurements proved antibacterial activity. The results obtained in this thesis work show that iodine containing PUL/PVP/PVA blend films are potential candidates for controlled release iodine systems for antibacterial applications under suitable conditions.

Keywords: Pullulan, Antibacterial Agent, Iodine Release System**,** Poly(vinyl alcohol), Poly(vinyl pyrrolidone)

ÖZ

Pululan (PUL), poli(vinil pirolidon) (PVP)ve poli(vinil alkol) (PVA) polimer karışımı filmler hazırlanarak iyot salım sistemi olarak incelenmiştir. Gluteraldehit (GA) ve gliserin (GL) ise sırasıyla çapraz bağlayıcı ve plastisizer olarak kullanılmıştır. Çapraz bağlanma 25°C ve 60°C sıcaklıklarda gerçekleştirilmiştir. Yüksek sıcaklıkta çapraz bağlama yapılan filmlerin daha homojen bir yüzeye sahip oldukları gözlenlenmiştir. Hazırlanan filmlerin kimyasal yapısı, fiziksel ve termal özellikleri sırasıyla FTIR, SEM ve DSC yöntemleri ile incelenmiştir. Şişme davranışı ise sulu çözeltilerde çalışılmıştır.

Polimer filmler %0.10, %1.0, %10 (w/v) derişime sahip iyot çözeltileri içine daldırılarak iyot yüklenmiş ve daha sonra sulu ortamda iyot salımı yaptırılmıştır. Sulu ortamda I_2, I^-, I_3^-, ve HOI molekülleri açığa çıkmaktadır. I^-, I_3^- miktarları UV-vis spektoskopisi ile belirlenmiştir. İlaç salım kinetiği % 10 (w/v) luk iyot çözeltisi ile yüklenmiş olan filmlerle 168 saat izlenmiştir. Bu filmlerden salınan iyotun antibakteryal etkisi 2 tür bakteri suşuna *Escherichia coli* IFO3972 ve *Staphylococcus.aureus* ATCC25923 karşı inhibisyon zonu ölçümleri yapılarak çalışılmıştır. Bu tez çalışmasından elde edilen souçlara göre iyot yüklü PUL/PVP/PVA polimer blend filmlerin uygun koşullarda ve ortamlarda kontrollu iyot salımı yapan antibakteryal sistemler olarak uygulanabileceği önerilmektedir.

Anahtar Kelimeler: Pullulan, Antibakteriyel Ajan, İyot Salınım Sistemi, Poli (vinil alkol), Poli (vinil pirolidon).

I Dedicate this Work to the Spirit of my Father

ACKNOWLEDGMENTS

I would like to thank and express my appreciation to Prof. Dr. Elvan Yılmaz and Assoc. Prof. Dr. Bahar Taneri for their continuous suggestions and help that enabled the completion of this research work.

I would also like to thank my husband Hayder Alnasser for his help and ever present support during the long months of my studies. I am also indebted to the help of my family. I might not have attained this academic level without their encouragement

I would also like to thank my friend Samaneh Saber Samandari for all her help during my studies.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF SCHEMES

Chapter 1

INTRODUCTION

This study aims to prepare iodine releasing polymer blend films. These systems are prepared by blending the polysaccharide pullulan (PUL) with two synthetic polymers poly (vinyl pyrrolidone) (PVP) and poly (vinyl alcohol) (PVA) besides glutaraldehyde (GA) as cross linker and glycerine (GL) as plasticizer. PVP, PVA and PUL have some common properties such as being biocompatible, biodegradable, water-soluble and excellent film producing polymers. Additionally, some PUL properties can be improved by blending with PVA (such as mechanical properties, chemical resistance, moisture barrier, and thermal stability), while PVP is used to introduce the antibacterial activity via its ability to form a complex with iodine, a broad microbial disinfectant.

Biocompatible materials are one of the most important elements of sustainable development. Any material that can be accepted by a living body can be defined as a biocompatible material (Chen, et al., 2008). Many studies had been done with biocompatible polymers in different biomedical fields (Williams, 2008). Among them drug delivery has acquired a great interest since different biochemical materials such as drugs, anticancer agents, vitamins, genes, and proteins can be transferred to target organs. This large adaptability is essentially due to specific characteristics of

biopolymers, such as biodegradation, adhesiveness, and pH- thermo sensitivity (Dalmoro, et al., 2012).

In the current years, polysaccharides are getting more attention than synthetic polymers; not only because of their renewability, but also because of their water solubility which is the most important character in a large number of them. Gel and film formation are possible because each molecule has a significant number of (-OH) groups tending to form intra and inter association hydrogen bonds. Plants, algae, some animals and microorganisms are the main sources of polysaccharides via biosynthesis, for example gellan, chitin, pullulan, alginate etc. (Rinaudo, 2008).

However, polysaccharides have a number of disadvantages such as poor mechanical properties, high cost, and moisture sensitivity. To overcome these problems structure modifications, such as blending and compositing with synthetic polymers, have been widely studied to improve their properties (Yu, et al., 2006).

1.1 Pullulan

In 1938 Bauer first discovered PUL (Cheng et al., 2011) with molecular formula $(C_6H_{12}O_5)_n$ (Shingel, 2004) and chemical structure as shown in Scheme 1. The fungus *Aureobasidium pullulans* is the main source of the PUL Figure 1. It is non-pathogenic, but some strains may be pathogenic. Fresh water, sea water, forest soils, as well as animal tissue are the ideal environment for the fungus (Shingel, 2004). PUL is produced by fermentation of different types of sugars such as (maltose, xylose, sucrose and starch) as a source of carbon. For example, in one study it was reported that 100 ml of sucrose medium at 28ºC and with 200 rpm is considered the

ideal conditions of producing it from this fungus, and their biological activities can be attuned by chemical alteration (Demirci and Catchmark, 2011).

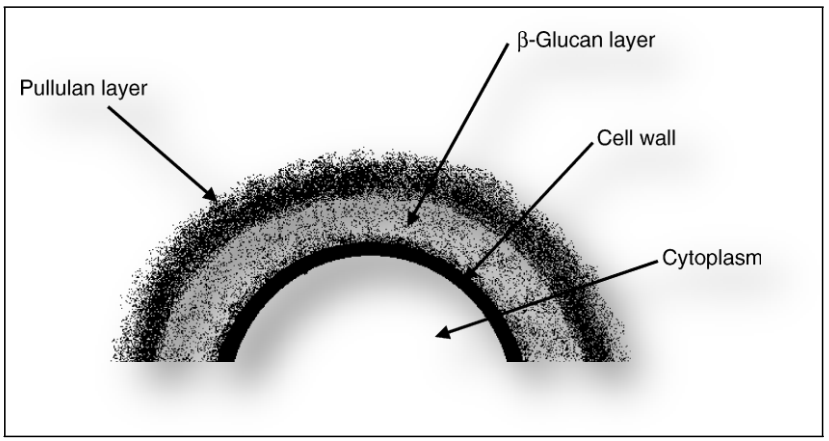
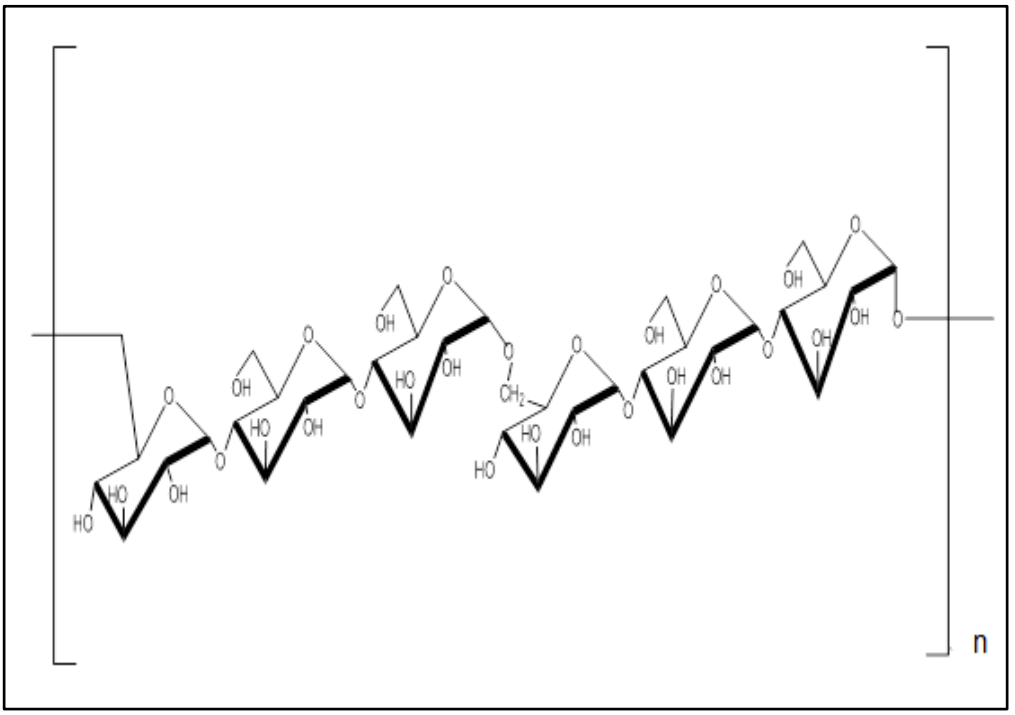


Figure 1: Pullulan Layers on the *A. Pullulans* Cells (Shingel, 2004)

PUL is a linear non-ionic polysaccharide with maltotriosyl repeating units linked by numbers of α-(1,6) glycoside bonds. Otherwise, the structural formula of PUL may be given as a regular sequence of pyranoses joined by α-(1,4) bond (Shingel, 2004). The extracellular polysaccharide PUL is tasteless and odourless, has high water solubility and is edible (Gniewosz and Synowiec, 2011).

The degree of PUL polymerization ranges from 100 to 5000 α-glucopyranoside units. The molecular weight of the polymer can vary flanked by 10^3 and 10^6 Dalton. It depends on the strain used and the environment of the fungal culture, including temperature, initial pH of the culture medium, type of carbon source, type of nitrogen source, and their concentration in the culture medium (Gniewosz and Synowiec,

2011). Higher molecular weight is further necessary for commercial use (Demirci and Catchmark, 2011).

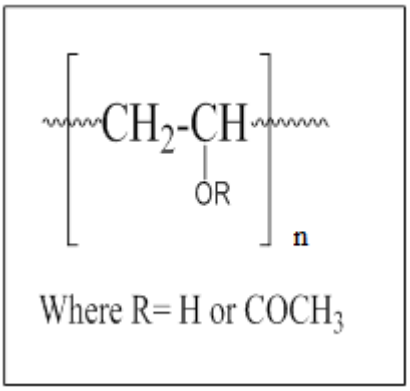


Scheme 1: Structural Repeat Unit of Pullulan (Prasad et al., 2012)

PUL is used in industrial processes as food additive (Thomsen et al., 2011), making capsule and various flavorings for packaging (Miyamoto et al., 2011), additive for cosmetics and as adhesive (Prasad et al., 2012). Moreover, it has biomedical roles such as a blood plasma substitute and flocculant (Thomas, 2011), a non-viral carrier for genetic material (Constantina et al., 2011), controlled drug release (Souguir et al., 2007), useful materials in encapsulation (Dulong et al., 2011), wound dressings (Li et al., 2011), and in tissue engineering (Abed et al., 2011).

1.2 Poly (vinyl alcohol) PVA

In 1924, Hermann and Haehnel were the first who prepared PVA by hydrolysing poly (vinyl acetate) in ethanol with potassium hydroxide. PVA is commercially made from poly (vinyl acetate). Acetate functional groups are hydrolysed by esterification with methanol and anhydrous sodium methylate or aqueous sodium hydroxide (Saxena, 2004). PVA is a synthetic polymer with a chemical structure as shown in Scheme 2.It has the molecular formula $(C_2H_3OR)_n$ where (R) equal to (H) or ($COCH_3$). It is odorless and tasteless, granular and semi-transparent powder (Saxena, 2004), thermoplastic (Silva et al., 2012), semicrystalline (Gupta et al., 2013), and chemically stable (Tripathi et al., 2009). It is soluble in water, but insoluble in other organic solvents and melts at 180°C to 190°C (Saxena, 2004).



Scheme 2: Structural Repeat Unit of Poly (vinyl alcohol) (Saxena, 2004)

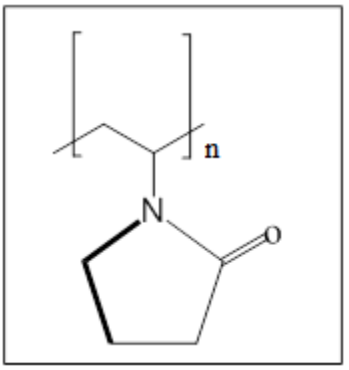
The physical appearance and its particular functional applications are based on the polymerization degree and the hydrolysis degree. PVA is classified in two classes as partly hydrolysed and totally hydrolysed. Partly hydrolysed PVA is widely added as a moisture barrier film in the food industry (Saxena, 2004).

Due to the presence of the hydroxyl groups, PVA displays a strong hydrophilic and hydrogen bonding nature. Therefore, it is able to form cross linked hydrogels (Păduraru et al., 2012), such as a physical hydrogel by freezing-thawing cycles (Gupta et al, 2013). Hydrogels from PVA have got more attention in biochemical and biomedical applications because of their biodegradability, permeability, biocompatibility, and excellent transparency (Nho et al., 2009). Cross-linked PVA hydrogels show high flexibility and good mechanical strength (Li et al., 2011). It is useful in the packaging industry (Tripathi et al., 2009), and as an adsorbent agent for different ions such as Cu(II) and Zn(II) ions (Chan and Cheng, 2012). Furthermore, it has biomedical applications such as in drug encapsulation (Misic et al., 2012), wound dressing, and control drug release (Cencetti et al., 2012). Film properties of PVA were investigated in many studies when it was blended with natural polymers such as gelatin, xylan, chitosan, sodium alginate, and synthetic polymers, such as poly (vinyl pyrrolidone) and poly (ethylene oxide) (Gupt et al., 2013).

1.3 Poly (vinyl pyrrolidone) PVP

Poly (vinyl pyrrolidone) PVP with a chemical structure as shown in Scheme 3 has the molecular formula (C6H9NO)n. It is amorphous, white to light yellow powder, water soluble, and soluble in other polar solvents. In solution, it is hydrophilic and has a tendency for film formation; this makes it suitable in coating industry (Folttmann and Quadir, 2008). Moreover, it is biocompatible, thermo-pH stable, non-toxic, chemically stable, and non-ionic polymer (Giri et al., 2011). PVP does not have any antibacterial activity, but it has affinity to cell membranes (Wang et al.,

2012). PVP has ability to charge storage and formation complexes with a lot of small molecules such as iodine (Siviaiah at el., 2010).



Scheme 3: Structural Repeat Unit of Poly (vinyl pyrrolidone)
(Folttmann and Quadir, 2008)

PVP is used in several applications in medicine, pharmaceuticals, cosmetics, and in technical industry. For example it is used as blood preserving, detoxification substance, thickener for liquid drugs, in drug encapsulation, preparation of bioactive ointments and creams (Giri et al., 2011), an effective polymeric carrier for cancer therapy (Kamada et al., 2004), skin burn treatment, as well as wound dressing materials (Roy et al., 2012), and antibacterial activity when in complex with iodine solution (Fahmy et al., 2009).

1.4 Polymer Blending and Miscibility

In 1846, in Birmingham, Alabama, the first polymer blend was prepared by blending natural rubber with gutta percha. After that nitrocellulose and natural rubber were blended. In 1928 poly (vinyl chloride) was mixed with poly (vinyl acetate) as the first two synthetic polymers blended. In 1993 the polymer blend studies reached about 3000 per year (Sharma, 2012).

Polymer blending is a conventional and low cost method done by mixing two or more polymers to create new materials with new properties and with new applications (Yu et al., 2006). Blending is considered a very suitable method for enhancement or variation of physicochemical features of polymers. Structurally different polymers or copolymers, are physically mixed due to secondary forces namely hydrogen bond, dipole–dipole interactions, and formation complexes by charge-transfer (Islam et al., 2012).

It can be done by melt–mixing, solution blending, co-precipitation, controlled devolatiliztion or coagulation before final processing (Sharma, 2012). Aqueous blending is the mainly preferred technology in biomedical applications because of lower decomposition temperature of natural polymers (Yu et al., 2006). Blending is usually less time consuming for the development of new polymeric materials with new properties than the creation of novel monomers, and/or novel polymerization routes. A further advantage of polymer blends is that the features of the materials can be altered by varying the blend composition (He et al., 2004).

Polymer blends can be divided into two groups as homogeneous or heterogeneous. In the case of homogeneous blends, the properties of the blend are often an average combination of the properties of the all backbone polymers. While, in heterogeneous blends the properties of all backbone blend polymers are found in the new polymer (He et al., 2004).

Polymer blends which are miscible, immiscible and partially miscible are different from each other in their morphologies. Generally, in an immiscible blend two phases

are found, the separate phase (lower concentration component) and the continuous phase (higher concentration component) (Sharma, 2012). Miscible polymer blend displays single phase morphology. In the case of partially miscible polymer blend a wholly miscible blend may form at a different composition ratio, and no sharp boundary between two phases can be observed (Sharma, 2012). These kinds of polymer blends have a vital role in the industry (He et al., 2004). Miscibility of polymers is an important factor in blending because it affects on the mechanical, morphology, permeability and degradation properties of the blending polymer (Islam et al., 2012). Glass transition temperatures (Tg) and miscibility of polymer blends are parallel to each other. A pair of miscible polymers blend will show single (Tg). However, a partially miscible polymer blend will show two different (Tg) different from the (Tg) of primary component. Fully immiscible blend shows sharp phase morphology, each displaying the (Tg) of the pure polymers due to a strong interphase, and weak adhesion between the phases (He et al., 2004).

Due to the high molecular weight of the polymer and the mixing is endothermic in the common cases, ‒as the gain in mixing entropy is insignificant‖, only a few miscible blends have been identified. Therefore, it is now a wides spread strategy to improve the compatibility of the immiscible blends by the addition of inter-associated hydrogen bonds such as terminated addition, copolymerization, addition third polymer, and insertion of an inert diluted agent to the backbone polymers to decrease any cracks (He et al., 2004).

Miscibility can be investigated by many methods such as electron microscopy, swelling methods, infrared spectroscopy, viscosity, rheological properties, differential scanning calorimetry, electrochemical impedance spectroscopy, nuclear magnetic resonance, and refractive index (Baker et al., 2001).

1.4.1 Blending Investigation on Pullulan

Assoul and Abed et al. synthesized hydrogel based on the blending dextran with PUL to be used in tissue engineering. The (Tg) for pure PUL was found as 173°C while for dextran was 205°C. They found out that by increased concentration of PUL in hydrogel the (Tg) and the maximum modulus decreased (Abed et al., 2011).

Prasad and Guru studied the miscibility, thermal, and mechanical properties of hydroxypropyl methylcellulose blend with PUL in water. The blend is miscible when the HPMC ratio is more than 50%. Moreover, the change in temperature had no significant effect on the miscibility of HPMC/PUL. By blending these two polymers, both thermal and mechanical properties were improved (Prasad et al., 2008).

Prasad et al. investigated miscibility of the film from sodium alginate blending with PUL study done by prepared different percentage of blend components. Moreover, ultrasonic velocity, viscosity, density and refractive index were measured at 30°C and 40°C. The study indicated that blend of NaAlg/PUL is miscible at all compositions. Variation of temperature did not have any significant effect on the miscibility (Prasad et al., 2012).

1.4.2 Blending Investigation on Poly (vinyl alcohol)

Cashew gum and PVA were blended and by immobilized trichoderma asperellum to form an antifungal film. Study indicated that cashew gum is diffused within PVA matrix due to very good interfacial linkage between the two components (Silva et al., 2012).

Blends of PVA and poly (ethylene oxide) (PEO) films for wound dressing applications were prepared by solution casting method. Stabilization of PVA and PEO was achieved by adding of carboxymethyl cellulose (CMC). Study revealed that the addition of CMC to PVA/PEO blend results observable changes in the miscibility of these two components (Gupta et al., 2013).

Antimicrobial coating film based on chitosan and PVA was prepared by blending chitosan and PVA with (GA) as the cross-linker. This article reports homogeneous film formation due to chitosan dispersed within PVA matrix in the blend film with good interactions between the two components (Tripathi et al., 2009).

Study by Sreekumar et al. found blending between PVA and starch was improved by adding glycerol as plasticizer. Moreover, at high glycerol loading two distinct degradation temperature are found one appearing close to starch and other appearing close to (PVA), which indicate phase separated in Starch /PVA blending (Sreekumar et al., 2012).

1.4.3 Blending Investigation in Poly (vinyl pyrrolidone)

A blend of PVP and sodium alginate NaAlg was prepared from aqueous solutions. This study suggests that the blends are miscible due to good interaction between carbonyl groups of PVP with hydroxyl groups of sodium alginate. These blend films show improved in both the thermal stability and the elongation at break in dry states (Aykara and Demirci, 2007).

Hydrogel membranes PEVP were prepared from blending pectin and PVP. Study shows decrease in crystallinity of the membranes when PVP ratio increase. Moreover, DSC shows improve in (Tg) of pectin after blending with PVP. Also, it was found that tensile strength increases with increasing PVP fractions in the hydrogel membranes (Mishra et al., 2008).

Xanthan gum and PVP were blended by aqueous solutions in this study miscibility was indicated at 70/30 ratio from PVP to xanthan due to the formation of hydrogen bonding between the carbonyl group in PVP and hydroxyl group in gum. Pure PVP show (Tg) 62°C and 41°C for xanthan where (Tg) in blend film show 52.1°C. Additional, vary in temperature had an important effect on the miscibility of Gum /PVP (Guru et al., 2010).

Chitosan (CS) and PVP were blended and miscibility was detected for blends with more than 50% of CS in the molar fraction. Whereas, immiscibility was predominant at the molar fraction of CS between 10% and 50%. Due to a higher ratio of CS show good interactions with PVP because hydrogen bond is recognized between the (-OH) in CS and the (C=O) in PVP (Yin et al., 2010).

1.4.4 Blending Investigation between Pullulan and Poly (vinyl alcohol)

PUL was blended with PVA to form film by casting polymers solution in dimethyl sulfoxide. Mechanical properties and their morphology were examined. PUL and PVA were immiscible in the sample blend, because of the interfacial adhesion between PVA and PUL was weak which was led to phase separation. Moreover, the elongation at break of the blend films compared to the pure PVA film was lower. Further 40% glyoxal used as a cross linker to improve the mechanical properties. Miscibility was detected in the film at reaction times over 1hr due to increase in the interaction between PUL and PVA molecules with higher tensile strengths and moduli than the simple blend, and micro- cracks were found in the films at a reaction time above 3 hr (Teramoto et al., 2001).

Islam et al. designed nanofibers according to electrospinning method in aqueous solutions by blending PUL, PVA and montmorillonite. Hydrogen bonds formed among PUL and PVA which indicates good interactions between these polymers. By this study it was found both thermal stability and mechanical property PUL/PVA/MMT blending nanofibers could be improved more by addition MMT (Islam et al., 2012).

1.4.5 Blending Investigation between poly(vinyl alcohol) and poly(vinyl pyrrolidone)

Study by Ragab found that both PVP and PVA are miscible in all proportions (i.e. the structural, optical and electrical properties of blends) (Ragab, 2011). PVP/PVA blends were prepared for drug release applications. Due to the presence of the nitrogen in PVP and the hydroxyl group of PVA hydrogen bonding is formed between the polymers which improved blend properties such as, high water

absorption. PVP and PVA were chosen to combine the high flexibility of PVP with the mechanical strength of PVA. It was found that by increasing the PVA concentration the interaction between the polymers was increased (Cesar et al., 2011).

1.5 Antimicrobial Agents

i. Antimicrobial agent: A material that has potential to prevent or inhibit microorganism growth (Gabriel et al., 2007). It can be commonly termed as biocides, microbicides, sanitizers, antiseptics and disinfectants (Cloete, 2003). Many new materials have been employed by means of antimicrobial agents. Commonly, this is recognized by impregnation with biocides, such as iodine, silver triclosan, quarternary ammonium compounds and antibiotics that are released and can kill microorganisms (Waschinski et al., 2008).

ii. Biocides: Are organic or inorganic chemical materials including bactericides and fungicides. They are wide-spectrum in nature, which kill both bacteria and fungi, while the biostats (bacteriostats and fungistats) have the ability to inhibit the microorganism growth. Typically biocides are widely used against microorganisms on surfaces or in suspension (White and Dermott, 2001; Barbara et al., 2001). Therefore, disinfecting agents are mostly soluble, suspendible, or emulsible in water (Tashiro, 2001). Biocides can be used to sanitize, sterilize, or disinfect surfaces and preserve substances from microbial contamination (Chapman, 2003).

iii. Antibiotics: Are chemotherapeutic medicines synthesized by living organisms as natural organic compounds, which are frequently active for a limited number of organisms in low concentrations and normally useful on or inside

living tissues for infection control by interacting with particular microbial cell structures or metabolic cell processes (Bridier et al., 2011).

iv. Microbial resistance: We can say that microorganism acquires resistance, when a microbial strain has the ability to grow with high concentrations of antibiotics, higher than the value of the wild type, as a result of genetic mutations that lead to new traits that are not found in wild type (Chapman, 2003). Bacterial resistance frequently results in treatment failure, especially in chronic diseases (Gberg et al., 2010).On the other hand, the emergence of bacterial resistance to antibiotics leads to an increase in number of deaths and increased cost of treatment. As a result, finding other sources can alleviate resistance to antibiotics (Yalınca, 2013). Though, this is an urgent matter of health at the moment (Gabriel et al., 2007).

1.5.1 Antibiotics and Biocides

Biocides and antibiotics are both useful as antimicrobial agents, but each one is completely different from each other, in terms of; use, mechanism of action and bacterial resistance mechanisms. Biocides are normally much broader in their spectrum of activity than are antibiotics. In general, antibiotics have single specific cellular targets. However, it is not necessary in the case of biocides (White and Dermott, 2001); because that biocides lack selective toxicity and target specificity (Denyera and Stewartb, 1998; Bridier et al., 2011). Biocides exhibit nonspecific killing properties such as cytosol coagulation or cytoplasmic membrane damage (White and Dermott, 2001). All antibiotic resistance mechanisms fall into three broad categories, direct inactivation of the active molecule; alteration of the organism's sensitivity to the antibiotic by modification of the target of action; and reduction of

the concentration of drug that reaches the target without modification of the compound itself (Hogan and Kolter, 2002). On the other hand, some of microorganisms also have resistance to different types of biocides, such as resistance of *Escherichia coli* towards cetrimide, or *Burkholderia cepacia* and *Serratia marcescens* towards biguanides, isothiazolones and quaternary ammonium compounds (White and Dermott, 2001; Cloete, 2003). Whereas the basis of bacterial resistance to antibiotics is well known, that of resistance to antiseptics and disinfectants are less well understood, but it can be either by adaptation or by genetic exchange (Cloete, 2003).

1.5.2 Gram Positive and Gram Negative Bacteria

One of the staining methods of bacteria is Gram staining. This was discovered in 1884 by Charles Gram (Jawetz, 1989). Gram stain is one of the differential staining methods, which means coloring a specific part of the cell, or a specific microbial cell. Staining by gram stain consists of four steps (staining by Crystal violet, fixing by solution of iodine, and then coloring with a different color stain such as Safranin).

This method is one of the important ways in dividing bacteria into two basic groups, a gram positive which appears in purple, and the other group that appears red, mainly gram negative (Jawetz, 1989). The difference between the cell's color is due to the difference in the cell wall composition as shown in Figure 2.

1.5.3 Biocide Effects on the Microorganisms

Depending on the cell morphology, extracellular material, and cellular chemical composition biocide can pass to microorganisms in different regions Figure 2. They are; the cell wall, the cytoplasm membrane, and cytoplasm. Generally biocide passes

to cell by three steps. In the first step, subsequent distribution of biocide on the target site by adsorption or absorption. The second step is accumulating and finally the damaging level. Damaging can be done by coagulation of intracellular material, inhibition of active transport across the membrane, inhibition of respiration or catabolic/anabolic reactions, disruption of replication, and lysis (Denyera and Stewartb, 1998; Chapman, 2003; Bridier et al., 2011).

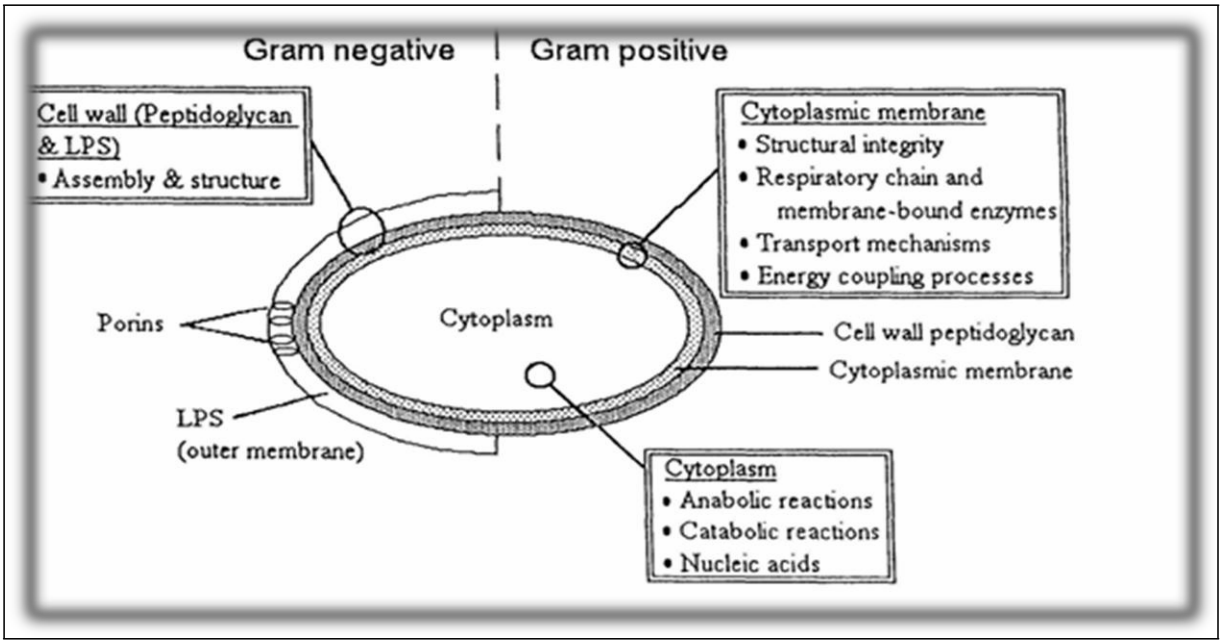


Figure 2: Cell Wall Structure in Gram Negative and Positive Bacteria with Regions for Biocide Interface (Diagram from: Denyera and Stewartb, 1998)

1.5.4 Polymer and Antimicrobial Agents

Many studies have shown that the use of antimicrobial substances with low molecular weight have side effects, for example lethality to the environment, and short-term effectiveness to overcome these problems through the addition of these materials within the polymer molecules (Kenawy et al., 2006). The presence of polymer molecules can contribute to improvement in the performance of some antibacterial agents, and also reduces the environmental interactions by reducing the

toxicity, increasing efficiency, improving selectivity and increasing the life time of antimicrobial agent (Kenawy et al., 2011). In addition to this the antimicrobial polymers are chemically stable, non-volatile and do not penetrate through the skin, thus it can reduce material loss through volatilization and photolysis (Kenawy et al., 1998; Ayhan et al., 2006).

1.6 Iodine

Nowadays many of halogen, ozone, and many soluble sterilizers are intended for sterilizing. On the other hand, there are problems with these soluble sterilizers because of residual toxicity of the agent (Hu et al., 2004). An appropriate way to kill bacteria efficiently and to avoid residual toxicity of the agents, insoluble polymer with antimicrobial groups is used (Hu et al., 2004).

In 1812, iodine was discovered by Courtois, as a non-metallic vital element (Block, 2001). With an atomic weight of 126.9, melts at 113.5°C and boils at 184.4°C, at atmospheric pressure to create the specific violet vapor. Elemental iodine is a little soluble in water, forming a brown solution. Its water solubility is improved through the addition of alkali iodides as a result of formation of triiodide and higher polyiodides (Block, 2001).

1.6.1 Iodine as Antimicrobial Agent

About 150 years ago, iodine has been widely used as an antiseptic for infections and for wound treatment. In 1880 Davaine was the first to describe the bactericidal efficacy of iodine and it was used by surgeons as a preoperative antiseptic only between the nineteenth and twentieth century. In that time, iodine was commonly

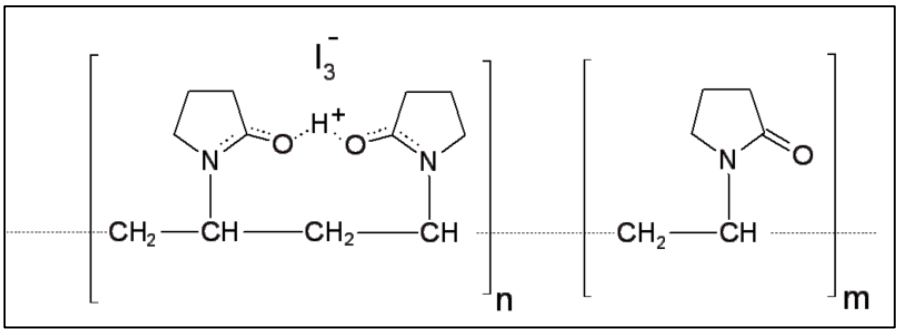
used as iodoform such as triiodo methane and ethylic iodine solution (Selvaggi et al., 2003). The maximum suggested dose of iodine in diet was 2 mg/day with 3 weeks duration of use because of its effect on the thyroid gland function (Mazumdar et al., 2010).

Solution of iodine and alcohol was prepared with different concentrations, but this solution exhibited many drawbacks, it was found to be irritating to the eyes, skin and mucous membranes at concentrations higher than 5%. These problems were improved in some point by adding iodide to iodine solution to form water soluble triiodide. However, irritating effects could not be totally reduced through this formulation. In normal solution, with these old formulations at least seven iodine species appear in a complex equilibrium with molecular iodine, which is mainly responsible for the antimicrobial effectiveness. Unfortunately, this caused a high extent of instability of these solutions. To overcome this problem in the 1950's American scientists H. A. Shelanski and M. V. Shelanski have found Povidone Iodine PVP-I, which was prepared via binding iodine to macromolecules named as iodophores which are substances that can carry iodine, for example PVP (Selvaggi et al., 2003). By this way the drawbacks related to the elemental Iodine were reduced due to free iodine in the solution being very low (Kumar et al., 2011).

1.6.2 Formation of (PVP–I) Complex

PVP–I complex with chemical structure as shown in Scheme 4 can be produced by different routes. As a solution, when iodine powder is added into an ethanol with PVP, direct mixture, when P V P powder and iodine powder mixes directly, and iodine vapor route, by fumed PVP with iodine vapor in a chamber (Hong et al.,

2009). It is assumed that iodophore polymers have oxygen including functional groups (e.g. carbonyl groups in PVP will complex with iodine to form donor accepter complexes in which the iodine is the acceptor).



Scheme 4: Structural Repeat Unit of Povidone-Iodine (Kumar et al., 2011)

1.6.3 Povidone-Iodine (PVP-I)

Iodophores for instance povidone-Iodine PVP-I are compounds containing iodine and inactive polymers, such as PVP, that have numerous benefits over elemental Iodine compounds. As a common antiseptic, iodophores have a tendency to be less irritating to the skin, are more hydrophilic, are less staining and they maintain the antibacterial activity of iodine (Heiner et al., 2010 ; Selvaggi et al., 2003). PVP-I is wide-spectrum biocides, soluble in water, glycols, isopropyl alcohol, polyethylene, and glycerin.

PVP-I shows higher stability when it is stored away from moisture and light (Kumar et al., 2011). While, it is reflected to be less germicidal activity against specific fungi and spores than tinctures (Selvaggi et al., 2003). Aqueous (PVP-I) has been used as a topical antiseptic and surgical scrub for more than 40 years and microbial resistance has not yet been noted (Simmons et al., 2009). Many organization such as FDA and

AHCPR have suggestions for the use of PVP-I solution in wound care as first-aid antiseptic products (Burks, 1998). Is commonly use as the effective component in different preparations such as disinfectant liquids, ointments, gels, and suppositories which are common with different trade names (Ignatova et al., 2007). The PVP-I in these formulation works as an iodophor due to gradually releases non-complexes active iodine, when these solutions are contact with skin and mucous membranes (Ignatova et al., 2007). The PVP-I incorporated with numbers of material for different application as the non-antibiotic, antimicrobial agent such as, PVP–iodine-containing nanofibers for wound dressings (Ignatova et al., 2007), urinary tract biomaterial (Khandwekar et al., 2011, Jones et al., 2002), and water disinfectant tablets (Mazumdar et al., 2010).

1.6.4 Bactericidal Activity of Iodine

The bactericidal activity of iodine is related to regular release of I_2, HOI, I^- and I_3^- from iodine containing compounds. Both I_2 and HOI in aqueous iodine solutions, have higher levels of cysticidal action than other forms. Studies presented that I_2 shows more biocide effect than both HOI and I_3^-. And noticeable decrease in the biocide activity of iodine was observed at pH 9 (Punyani et al., 2007). The antibacterial activity of iodine stems from its ability to substitute for covalently bound hydrogens with the compounds containing functional groups such as (-OH,-NH,-SH or -CH), which are found not only as part of the solvent or other constituents of the formula, but also of the materials to be disinfected such as skin, mucous membranes, and bacteria (Block, 2001). Although the precise mechanism of iodine has not been completely determined, it has been suggested that the lethal effect of iodine on microorganisms can be explained as follows: iodine rapidly penetrates the

cell wall and proceeds to interrupt protein synthesis. It disrupts the function of respiratory chain enzymes and interferes with lipid membrane and nucleic acid function through several diverse mechanisms of action (Selvaggi, 2003).

Chapter 2

EXPERIMENTAL

2.1 Materials

A list of the chemicals used in this study is given in Table 1. They were all used as received apart from ethyl alcohol and acetone which were used after distillation.

Table 1: Materials and Manufactures

Pullulan (PUL)	Shandong Freda Biotechnology, China
Poly (vinyl alcohol)(PVA), (98-99% hydrolyzed), Average MW ≈31.000-50.000	Aldrich, Germany
Poly (vinyl pyrrolidone) (PVP), MW≈44.000	BDH laboratory, England
Glutaraldehyde (25% solution)	Aldrich, Germany
Ethyl Alcohol	Selim ve Oglu, Magusa, Cyprus
Hexane	Merck KGA, Germany
Elemental Iodine	Aldrich, Germany
Glycerol	Aldrich, Germany
Acetone	Analar, British Drug Houses Ltd,UK
Hydrochloric acid	Analar, British Drug Houses Ltd, UK

2.2 Method

All pure and blend films were prepared by adding glycerol to aqueous solutions of the PUL, PVP and PVA. Different (w/w) ratios of PUL and PVA were used in the film preparation, while PVP 3% (w/v) keeping constant.

Table 2: Compositions of the Polymer Films Formulation

Sample	Formulation code	Ingredients weight (g)				Ratio (w/w) PUL/PVA/PVP
		PUL	PVA	PVP	Glycerol	
Pure	S_1L	6	0	0	1.8	100/0/0
	S_1A	0	6	0	1.8	0/100/0
	S_1P	0	0	3	1.8	0/0/100
Binary blend	S_2AL	1.5	4.5	0	1.8	25/75/0
	S_2LP	1.5	0	3	1.35	33.3/0/66.7
	S_2AP	0	4.5	3	1.8	0/60/40
Binary blend (Cross linking by GA)	S_2AL_C	1.5	4.5	0	1.8	25/75/0
	S_2LP_C	1.5	0	3	1.35	33.3/0/66.7
	S_2AP_C	0	4.5	3	1.8	0/60/40
Binary blend (Cross linking by GA with heating)	S_2AL_{CH}	1.5	4.5	0	1.8	25/75/0
	S_2LP_{CH}	1.5	0	3	1.35	33.3/0/66.7
Ternary blend	S_3LAP	2.1	3.9	3	1.8	23.3/43.3/33.3
		1.5	4.5	3	1.8	16.7/50/33.3
		0.9	5.1	3	1.8	10/56.7/33.3
Ternary blend (Cross linking by GA)	S_3LAP_C	2.1	3.9	3	1.8	23.3/43.3/33.3
		1.5	4.5	3	1.8	16.7/50/33.3
		0.9	5.1	3	1.8	10/56.7/33.3
Ternary blend (Cross linking by GA with heating)	S_3LAP_{CH}	2.1	3.9	3	1.8	23.3/43.3/33.3
		1.5	4.5	3	1.8	16.7/50/33.3
		0.9	5.1	3	1.8	10/56.7/33.3

2.2.1 Solution Preparation

2.2.1.1 Preparation of Pure Polymer Solutions

Both S_1L and S_1A solutions were prepared by using 6% (w/v) aqueous solutions and a S_1P solution was made by using 3% (w/v). Aqueous solutions of these polymers were made by using hot water as a solvent. Weighed amounts of PVA were

added to boiling water to make a solution of 100 mL and then stirring at 80ºC until completely dissolved. When both S_1L and S_1P polymer solutions were made by adding PUL and PVP polymers to hot water at 60ºC and stirring at 80ºC until completely dissolved. Then glycerol 1.8g was added to all polymer solutions.

2.2.1.2 Preparation of Binary Blend Solutions

S_2LP blend films were made by adding 3g PVP to 1.5% (w/v) PUL aqueous solution with 1.35g glycerol and stirring at 80ºC for 1hr, S_2AP films were made when 3g PVP added to completely dissolve 4.5% (w/v) PVA aqueous solution with 1.8g glycerol. While S_2AL solution was made by adding 1.5g from PUL to completely dissolve 4.5% (w/v) PVA aqueous solutions with 1.8g glycerol and stirring for 6 hr at 80ºC.

For $S_2AL_{C,}$ S_2LP_C and S_2AP_C, the all same steps were done for prepared solutions above in this section but 4 drops from HCl 10% (v/v) and 0.2ml from GA 0.0001% (v/v) was added to 20mL for each blend solutions. Moreover, each solution was stirred for 45 min at room temperature and at 45min at 60ºC for $S_2AL_{CH,}$ S_2LP_{CH} and S_2AP_{CH}.

2.2.1.3 Preparation of Ternary Blend Solutions

S_3LAPsolution was prepared by adding 0.9,1.5 and 2.1g from PUL respectively to completely dissolve 5.1%, 4.5% and 1.5% (w/v) PVA aqueous solutions with 1.8g glycerol and stirring for 6 hr at 80ºC until completely dissolved, then 3g from PVP was added with stirring for 4 hr at 80ºC.

For S_3LAP_C and S_3LAP_{CH}, all the same steps were done above in this section but 4 drops from HCl 10% (v/v) and 0.2 ml from GA 0.0001% (v/v) was added to 20 mL for each blend solutions and each solution was stirred for 45 min at room temperature and for 45min at 60 ºC respectively.

2.2.2 Film Preparation

20mL from each solution was poured into petri dish and the film was cast by drying at 45ºC for 72 hr as shown in Figure 3.

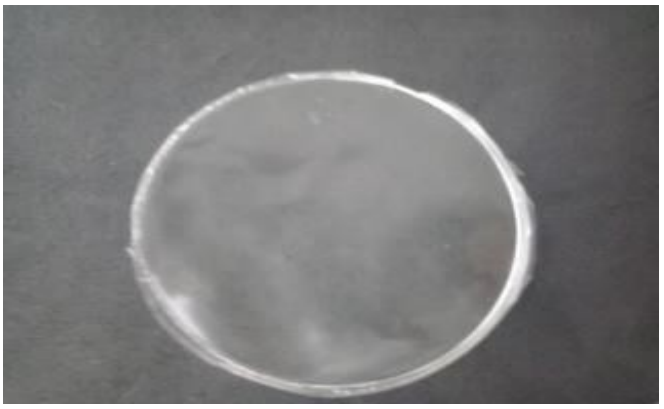


Figure 3: PUL/PVA/PVP Film Cross-linked by GA at 60ºC for 45min

2.2.3 Iodine Treated Films

Iodine treated films prepared from taken a piece with approximately 20x20x0.21 mm^3 from S_3LAP_{CH} for only 16.7/50/33.3 (w/w) ratio from the PUL/ PVA/ PVP. These pieces were immersed for 24 hr in iodine solution with 0.1%, 1% and 10% (w/v) prepared by dissolving weighed amounts of element iodine in 20% ethanol solution at room temperature. After that, the pieces are washed with hexane until all un-complex iodine was removed and each piece was placed in the hood for 24 hr to drying as shown in Figure 4.

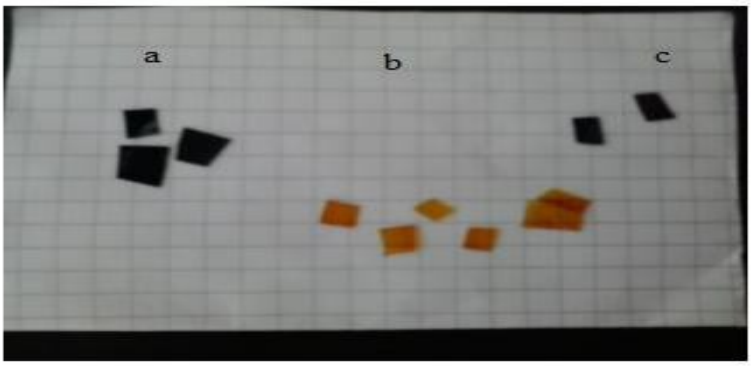


Figure 4: PUL/PVA/PVP Film Cross-linked by GA at 60ºC for 45min Complex with 10% (a), 0.1% (b),and 1% (c) Iodine Solutions

2.2.4 Characterization of the Samples

2.2.4.1 Fourier Transform Infrared (FTIR) Study

IR analysis was carried out with KBr-pelletized powder samples and a Mattson Satellite 5000 FTIR spectrophotometer.

2.2.4.2 Swelling Percentage

Water absorption capacity of the binary and ternary blend films prepared was studied. Water absorption was measured by immersing the weighed and dry pieces of the films 20x20x0.21 mm^3 in distilled water at room temperature. The film samples were taken out of water at different time periods and weighed after removing out the extra water from the surface of the films with filter paper. They were replaced in water directly after weighting. % swelling of blend films was calculated by the use of equation 1 in section 2.3.1. The experiment was repeated three times for each sample to minimize the experimental error.

2.2.4.3 Scanning Electron Microscopy

The surface characteristics of S_3LAP_C and S_3LAP_{CH} synthesized were examined by scanning electron microscopy. Measurements were done at TUBITAK- MAM in Turkey.

2.2.4.4 Differential Scanning Calorimetry (DSC) study

S_1L, S_1A, S_2LP and S_3LAP_{CH} were heated from 0ºC to 250ºC under nitrogen atmosphere in two runs at a heating rate 10ºC /min in order to determine their thermal behavior. DSC measurements were done at TUBITAK- MAM in Turkey.

2.2.4.5 Beer's-Lambert Curves

Lambda max (ʎ max) for iodide and triiodide forms was determined by using T80+UV/VIS spectrometer (PG instrument LTD). Scan were studied from 200 to 800 nm at UV-Vis region.Maximum absorbance (A) at 226nm and at 290nm and 350 nm were detected form a number of dilute solutions for both iodide and triiodide respectively. The Beer's curves for iodide made from aqueous solutions of NaI with molarity as 0.7 $x10^{-4}$, $0.5x10^{-4}$, $0.4x10^{-4}$ and $0.3x10^{-4}$ M. However, Beer's curves of triiodide Γ_3 were made from dissolving specific amount of NaI-I_2 in 20% ethyl alcohol to prepare solutions with molarity as $1.4x10^{-4}$, $1.2x10^{-4}$, $1.0x10^{-4}$, $0.8x10^{-4}$ and $0.6x10^{-4}$ M. Beer's curves were used to detect the unknown concentration via (A) of specific concentration for both iodide and triiodide.

2.2.4.6 Loading Studies

Loading amount of iodide and triiodide for the stock iodine solution 0.1%, 1% and10% (w/v) was measured by using T80+UV/VIS spectrometer (PG instrument LTD) at 226nm for iodide and at 290 nm and 350 nm for triiodide. Amounts of

iodide and triiodide were determined before loading it with polymer blend films and after loading it when pieces with approximately 20x20x0.21 mm^3 from S_3LAP_{CH} for only 16.7/50/33.3 (w/w) ratio were immersed for 24 hr in 5mL of iodine stock solutions at room temperature. Loading weight, % loading and % loading efficiency for iodide and triiodide were calculated by the use of equations 2, 3 and 4 in section 2.3.2 respectively. Initial weight (a) of stock solutions were found by weighed pieces with approximately 20x20x0.21 mm^3 from S_3LAP_{CH} for only 16.7/50/33.3 (w/w) ratio from PUL/ PVA/ PVP before and after loading in iodine stock solutions. Initial weight (a) was calculated using equation 5 in section 2.3.3.

2.2.4.7 Release Studies

UV–Vis absorbance for the release studies were taken at the same wavelengths as mentioned in section 2.2.4.6. Release study was done in 20 mL of double distilled water at room temperature for different periods of time (2, 4, 8, 24, 48, 72, 96, 120, 144 and 168 hr). After each measurement the release medium was replaced with fresh water. One film sample S_3LAP_{CH} that's treated with 0.1%, 1% and 10% (w/v) iodine solution was used for the release studies. Moreover, the release percentage for iodide and triiodide loaded with S_3LAP_{CH} blend films was calculated using the equations 6 to 8 in section 2.3.4. Neither I_2 nor HOI species could be detected by UV-Vis spectrophotometer. However, the estimation amount of I_2 and HOI was calculated by the use of equations 12 and 13 in section 2.3.5.

2.2.4.8 Evaluation of Antibacterial Properties of Releasing Iodine

Antibacterial activity was measured for S_3LAP_{CH} blend film complex with 1% (w/v) iodine, using two type of bacterial strains gram negative *Escherichia coli* IFO 3972 (*E. coli* IFO 3972) and gram positive *Staphylococcus aureus* ATCC25923 (*S. aureus*

ATCC25923) cultured in nutrient agar at 37ºC for 48 hr. Disk samples were prepared via cutting circular pieces from film by perforator with size (10x10x0.21) mm^3 and complexing them with iodine as explained in section 2.2.3. Disk inhibition zone measurements were done at TUBITAK- MAM in Turkey.

2.3 Calculations

2.3.1 Determining the Swelling Percentage

The percent water absorption of the prepared blends was found from the following equation:

$$\% \text{ Swelling} = ((W_S - W_d) / W_d) \times 100 \quad \text{Eq.1}$$

Where W_S and W_d are the weight of the blend films in the swollen and dry states, respectively.

2.3.2 Determining the Loading Weight, Loading Percentage and Loading Efficiency Percentage

The weight of iodide and triiodide loading of the prepared (S_3LAP_{CH}) blend was found from the following equation:-

$$\text{Loading weight } (W_2) = W_0 - W_1 \quad \text{Eq .2}$$

Where, W_0 and W_1 are the weight in (g) of iodide and triiodide in stock solutions before and after loading respectively.

The % loading and % loading efficiency of iodide and triiodide with S_3LAP_{CH} were calculated using the following equations:-

$$\% \text{ Loading} = (W_2 / W_d) \times 100 \quad \text{Eq.3}$$

$$\% \text{ Loading efficiency} = (W_2 / W_0) \times 100 \quad \text{Eq.4}$$

Where W_0, W_2 and W_d are the weight in (g) of iodide and triiodide in stock solutions (before loading), loading weight of iodide and triiodide within S_3LAP_{CH} and weight of dry S_3LAP_{CH} film respectively. Weight of dry film was taken as average of 10 film pieces with approximately 20x20x0.21 mm^3.

2.3.3 Determining the Initial Weight (a) of Stock Solutions

Initial weight (a) of stock solutions loading in S_3LAP_{CH} films were calculated using the following equation:

Initial weight (a) $= W_2 - W_1$ Eq.5

Where, W_2 and W_1 are the weigh in (g) for film after loading and before loading within S_3LAP_{CH}.

2.3.4 Determining the Releasing Percentage

The % release for both iodide and triiodide with S_3LAP_{CH} was found by following equation:

(% release) $= (W_1 / W_T) \times 100$ Eq.6

(% release) $= (W_1 + W_2 / W_T) \times 100$ Eq.7

(% release) $= (W_1 + W_2 + W_3 + \text{----- etc} / W_T) \times 100$ Eq.8

Where, W_1, W_2 and W_3 are the releasing weight for both iodide and triiodide from S_3LAP_{CH} blend film after 2, 4, 8 ---- etc hr. While W_T is the loading weight of iodide and triiodide within S_3LAP_{CH} blend film.

2.3.5 Determining the Weight of HOI and I2

Punyani (Punyani et al, 2006 and 2007) used the following procedure for determination of I_2 and HOI as:-

$$I_2 + H_2O \longleftrightarrow HOI + I^- + H^+ \quad K = 10 \times 10^{-12}\ L^2.mol^{-1} \qquad \text{Eq.9}$$

$$I_2 + I^- \longleftrightarrow I_3^- \qquad K=1.2x10^3\ L.mol^{-1} \qquad \text{Eq.10}$$

If (a) is the quantity of I_2 used initially at the equilibrium as show above. Both I_3^- and (I^-) were estimated from the Lambert- Beer curve (Fig18, 19 and 20 respectively) using

$$W = (MW*M)/V \qquad \text{Eq.11}$$

Where W, MW, M are the Weight, Molecular Weight and Molarity of released I_3^- and I^- and V is the volume of releasing medium respectively.

$$\text{Residual } I_2 = (a - (HOI + I_3^-)) \qquad \text{Eq.12}$$

$$HOI = (I^- + I_3^-) \qquad \text{Eq.13}$$

Chapter 3

RESULTS AND DISCUSSION

3.1 Fourier Transform Infrared (FTIR)

FTIR spectra of S_1L, S_1P, S_1A, S_3LAP_C and S_3LAP_{CH} films are shown in Figure 5 (a), (b), (c), (d), and (e) respectively. In (a) the α-glucopyranose units are characterized by the absorption bands at 850,756 and 931 cm^{-1}. Bands at 2926 cm^{-1} are due to stretching vibrations of C-H and bands characteristic to (CH/CH_2) deformation vibrations appear at 1423 cm^{-1}. Avery broad hydroxyl band appears at 3418 cm^{-1} and C–O stretching exists at 1018 cm^{-1}. Bands for C-C stretching appear at 1644 cm^{-1} for C-O-C stretching at 1158 cm^{-1} and C-O-H at 1372cm^{-1} respectively.

FTIR spectrum of S_1P film is shown in (b). In the spectrum of PVP, C-C vibrations bands appear in the region 1500-1600 cm^{-1}. C-H vibrations out of plane deformation are found in the region of 1290 -1000 cm^{-1}. The band observed in the region 515 cm^{-1} has been assigned to C-C out of plane deformation and the band at 760 cm^{-1} is given to (C-C in plane bending). The band at 1292 cm^{-1} named to the C-N stretching. Moreover, spectra in 470 and 486 cm^{-1} has been appointed to symmetric C-N bending. Band at1656 cm^{-1} is assigned to C=O vibrations, also for PVP due to hydrogen bonded to the ring the O-H stretching appears at 3418 cm^{-1}.

FTIR spectrum of S_1A film is shown in (c). O-H stretching band appear at 3447 cm^{-1} and for the C–O group peak at1044 cm^{-1}, C-C for a crystalline sequence of

PVA stretching in 1101 cm^{-1}, C-O-C at 1238 cm^{-1}, C=O vibrations at 1654 cm^{-1}, and C-H stretching at 2925 cm^{-1} respectively.

FTIR spectra of S_3LAP_C and S_3LAP_{CH} are shown in (d) and (e) respectively. With addition GA some bands become lower in intensity such as CH/CH2 deformation vibrations of PUL and negatively affected by heating, as observed for C-O-C stretching at 1158 cm^{-1} and C-N in PVP at 1292 cm^{-1}. This suggests that hydrogen bonds between hydroxyl groups in both PUL and PVA and C=O in aromatic ring of PVP could possibly play a role in this.

Moreover, the O-H stretching of blend film was reduced to 3340 cm^{-1} by the addition of GA and to 3320 cm^{-1} by heating at 60 ºC. This indicated the hydrogen bonds are formed between these polymers. Furthermore, the band at 1050 cm^{-1} related to the ether bond of acetyl groups was appear due to reaction of GA with hydroxyl group of both PUL and PVA and the absorbance intensity of this band increase by heating.

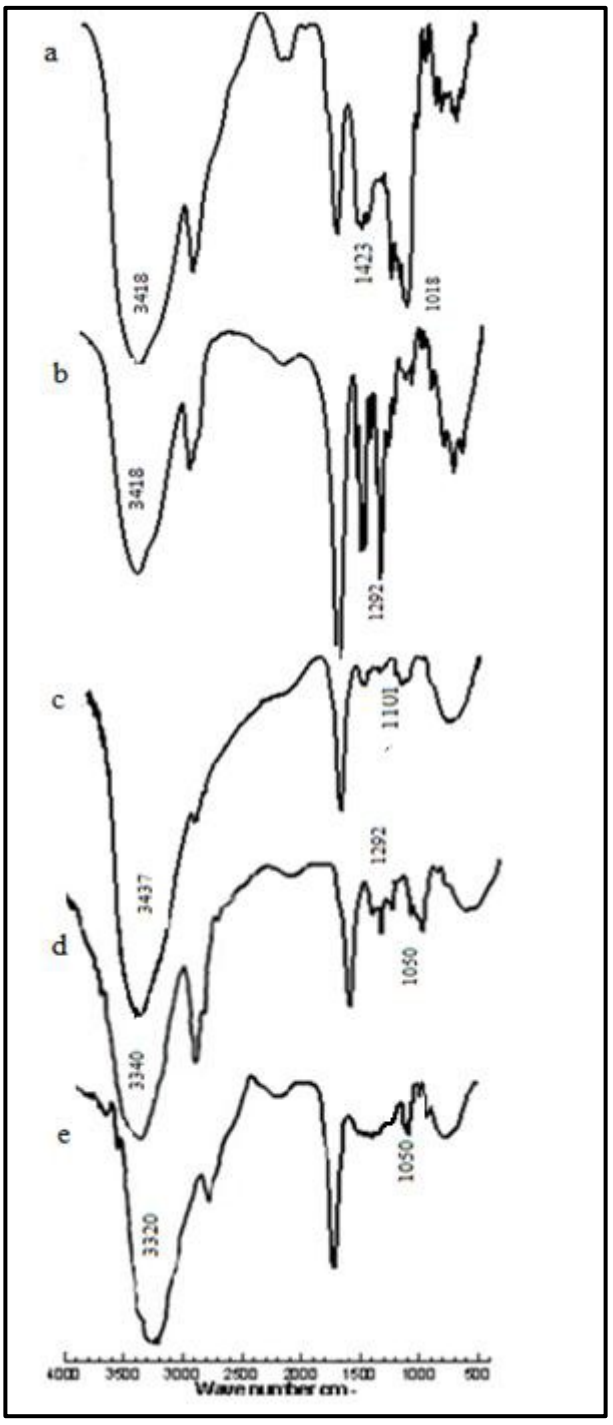


Figure 5: FTIR Spectra of S_1L (a), S_1P (b), S_1A (c), S_3LAP_C (d) and S_3LAP_{CH} (e) Films

3.2 Swelling studies

The swelling behavior of the prepared blends of S_2AP_C with 66.7/33.3 (w/w) ratio, S_2AL_C and S_2AL_{CH} with 16.7/50 (w/w) ratio are shown in Figures 6, 7and 8 respectively. It was observed that S_2AP_C film shows 350% as the maximum value of the % swelling. However, the S_2AL_C film shows % swelling of 151% and S_2AL_{CH} shows maximum % swelling of 97%. A previous study (Ahmed, 2008**)** found pure PVA crosslinking by GA to show the % swelling of about 51.92% in 24 h, which suggests that both PVP and PUL increase % swelling of the blending films.

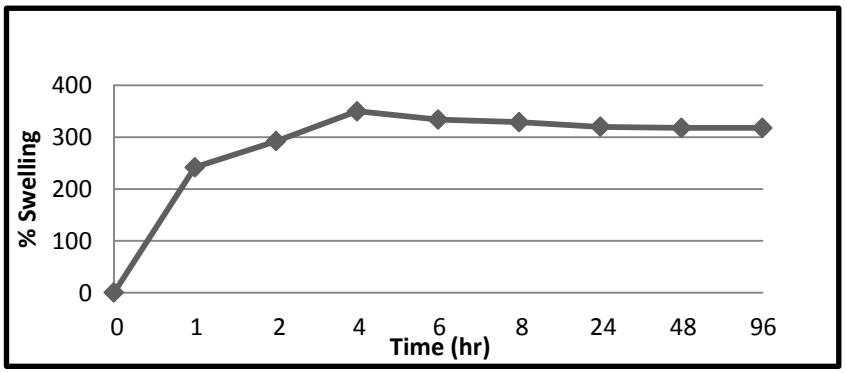


Figure 6: The Swelling Behaviour of S_2AP_C with 66.7/33.3 (w/w) Ratio.

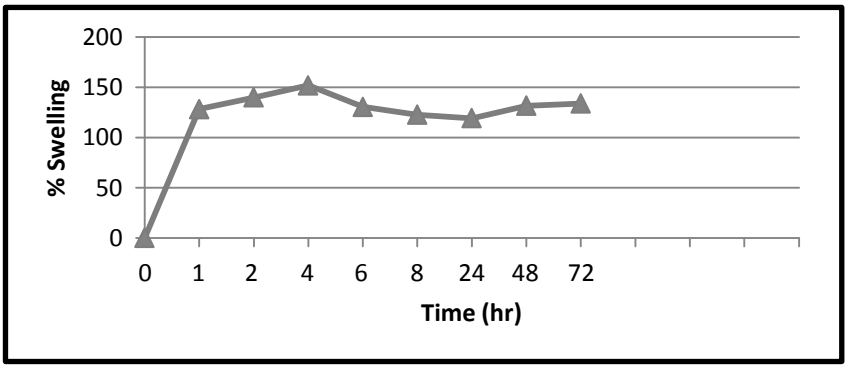


Figure 7: The Swelling Behaviour of S_2LA_C with 16.7/50 (w/w) Ratio

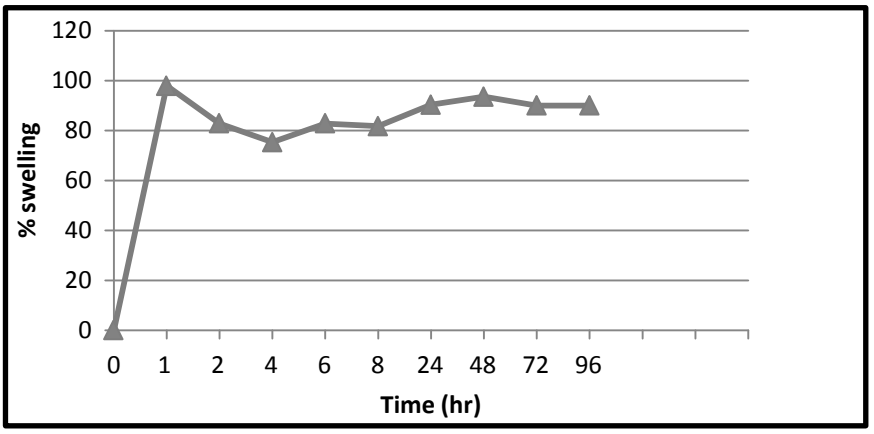
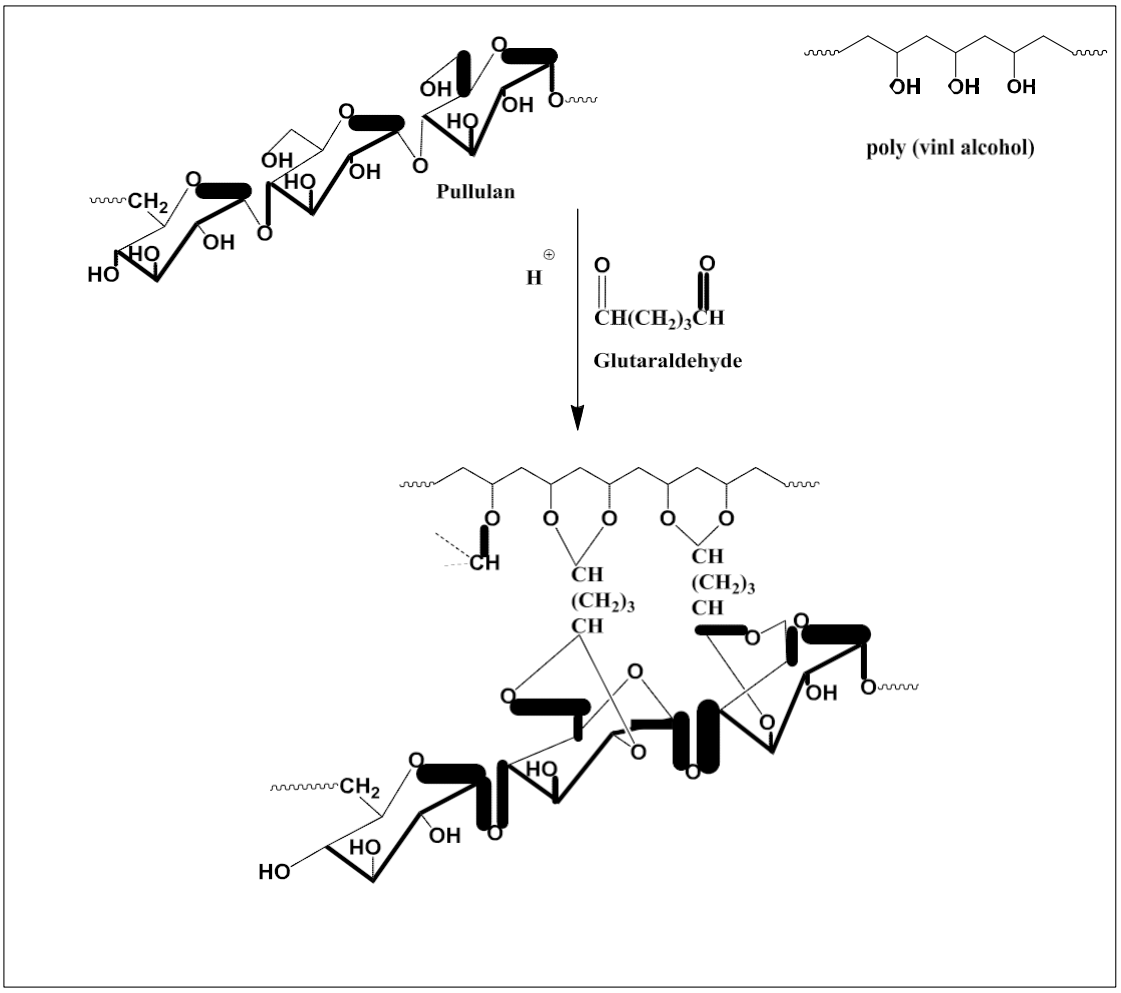


Figure 8: The Swelling Behaviour of S_2LA_{CH} with 16.7/50 (w/w) Ratio

According to a study by Bernal (Bernal et al, 2010) and coworkers the PVP ring contains a proton accepting carbonyl group, while PVA has hydroxyl groups and for that reason, hydrogen bonds form among them. These types of interactions have several effects on the blend properties, including the solubility and the mechanical properties. Moreover, the use of cross-linker is a valuable method to get materials with ideal characteristics. Addition of GA to the blend PVA and PUL film decreases the hydrophilicity via interchanges the hydrophilic hydroxyl groups of the PVA and PUL by hydrophobic links (–O–CH–$(CH_2)_3$–CH–O–) in GA as shown in Scheme 5. This was proved due to S_2AL that was dissolved immediately. Swelling studies were not carried out on pure film S_1L, S_1P and blend S_2LP film, since the crosslinking attained was not enough to make these films water insoluble.



Scheme 5: Cross –Linking Reaction of PVA and PUL with GA Catalyzed by H^+ (Teramoto et al., 2001)

Figures 9 and 10 show the % swelling values of S_3LAP_C and S_3LAP_{CH} respectively for different (w/w) ratios of PUL/PVA with constant PVP ratio. Blend films S_3LAP_C from PUL/PVA/PVP with ratios as 23.3/ 43.3/ 33.3, 16.7/ 50/33.3, 10/56.7/33.3 (w/w) show maximum % swelling are 225%, 212% and 170% respectively. However, the maximum % swelling of the S_3LAP_{CH} in the same (w/w) ratios are 222%, 197% and 97% respectively. As shown by swelling results, it was found that with increasing ratio of PVA in the blend films % swelling decreases. This behavior can be related to the semi crystalline structure of PVA which acts as

cross-linker and increased in the degree of crosslinking in the blend films which decreases the % swelling. However, the films with a lower PVA ratio dissolved within 6 hr. Moreover, the attempt to study the swelling behaviour for S_3LAP blend films with different ratios was not successful.

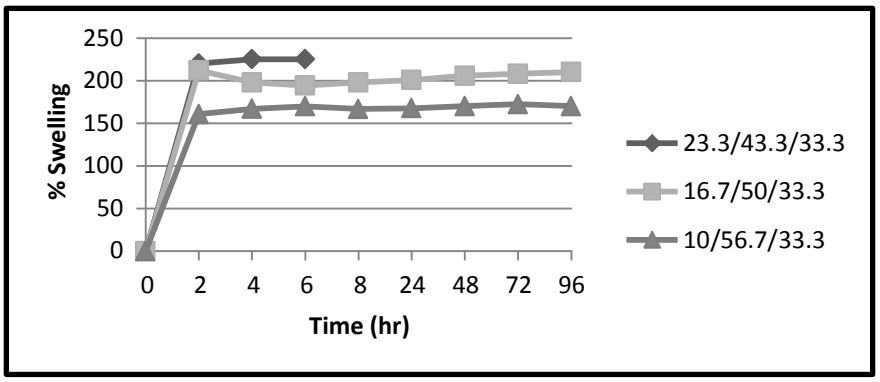


Figure 9: The Swelling Behaviour of S_3LAP_C Blend Films with Different (w/w) Ratios of PUL/ PVA and Constant PVP ratio

The results show a parallel pattern for the S_3LAP_{CH} to S_3LAP_C. However, the maximum swelling percentage of cross-linking with heating films was much less than that of films without heating.

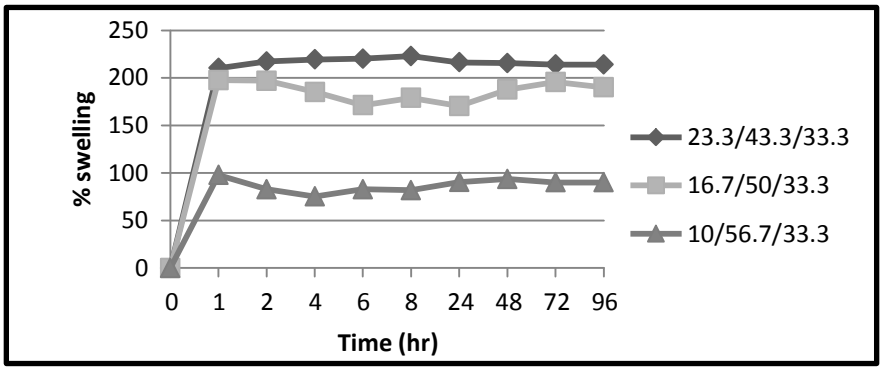


Figure 10: The Swelling Behaviour of S_3LAP_{CH} Blend Films with Different (w/w) Ratios of PUL/ PVA and Constant PVP Ratio

This significant difference in the % swelling between the S_3LAP_C and the S_3LAP_{CH} with 16.7/50/33.3 (w/w) ratio could be related to the increased degree of interaction between PUL/PVA/PVP by crosslinking with heating as shown in Figures 11 and 12 respectively. This behaviour can be explained as S_3LAP_C film has higher free-volume between polymer chains over S_3LAP_{CH} due to increase the interaction (i.e. decrease free-volume) between polymers in the S_3LAP_{CH} blend film by heating.

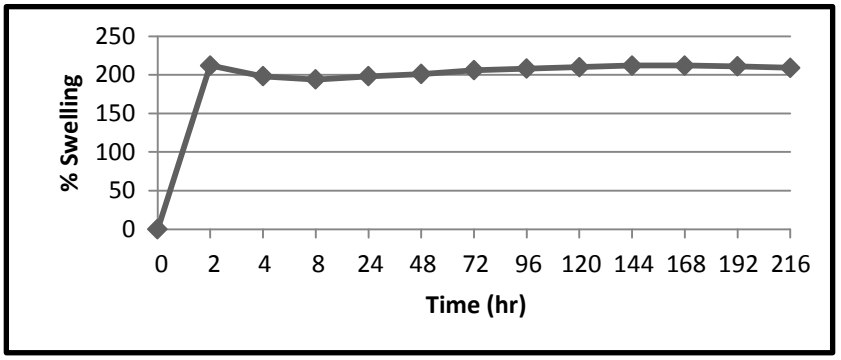


Figure 11: The Swelling Behaviour of S_3LAP_C Blend Films with 16.7/50/33.3 (w/w) Ratios of PUL/ PVA /PVP

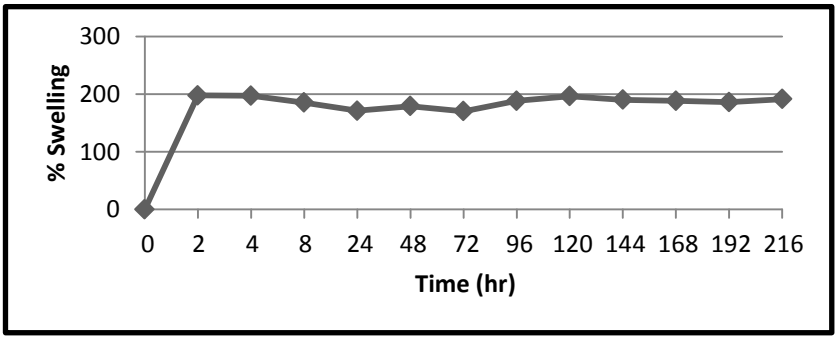


Figure 12 : The Swelling Behaviour of S_3LAP_{CH} Blend Films with 16.7/50/33.3 (w/w) Ratios of PUL/ PVA /PVP

Moreover, when compared the results in Figures 6, 7, 8, 11 and 12 it is found that by adding PUL to PVA and PVP blend films % swelling decreased, which suggests that

interactions are increasing when C=O in PVP form hydrogen bond with hydroxyl group of PVA and PUL.

3.3 Scanning Electron Microscopy

The characteristic surface of S_3LAP_{CH} and S_3LAP_C are shown in Figure 12 (a,b) and (c,d) respectively. Electronmicrographs indicated phase separation in both films, but for S_3LAP_{CH} homogeneity was improved by cross linking with heating as shown in Figure 13 (a) and (b). While, it is clear in Figure 13 (c) and (d) minor phase of the blend was dispersed in rich phase. Thus, homogeneity in S_3LAP_{CH} was related to initiating the cross-link reaction by heating which play a role in the re-formation of bonds in solution. This result indicated that heating of cross linking blend film at 60ºC for 45 min strengthened the interaction between PUL, PVA and PVP in blend films. These results were confirmed with FTIR as shown in Figure 5 and swelling study as shown in Figures 11 and 12.

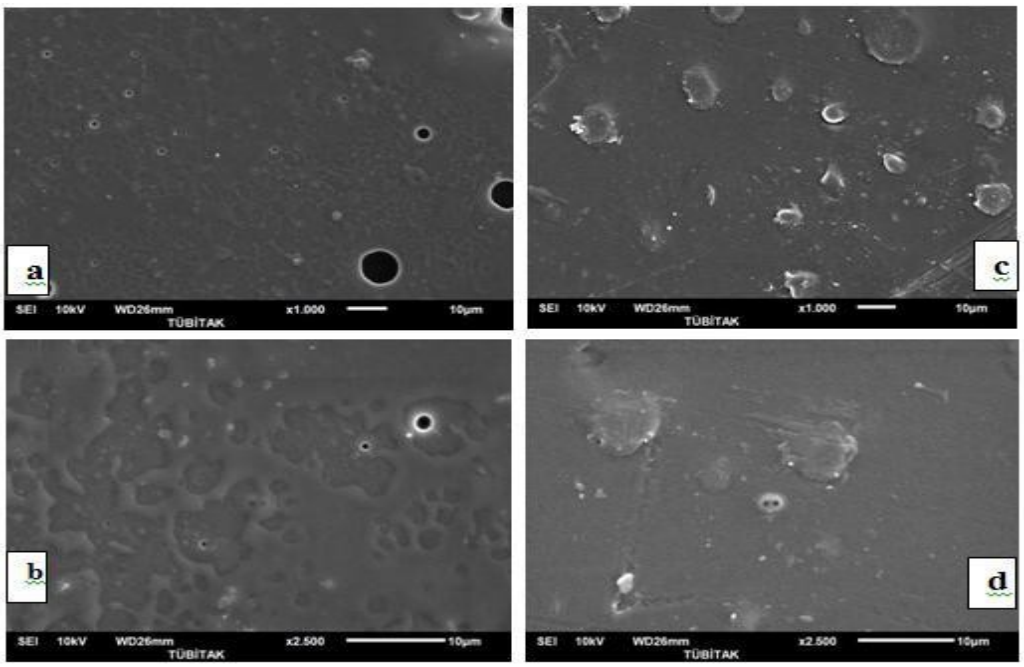


Figure 13: SEM Micrographs of Polymer Blend Films with 16.7/50/33.3 (w/w) Ratios of PUL/PVA/PVP as S_3LAP_{CH} (a,b) and S_3LAP_C (c,d) Respectively

3.4 Differential Scanning Calorimetry (DSC)

The DSC thermogram of S_1L, S_1A, S_2LP, and S_3LAP_{CH} are shown in Figures 14, 15, 16 and 17 respectively. For S_1L, in the first run, any additional component other than PUL either evaporated or decomposed. In the second run, (Tg) of PUL could be observed at 91ºC. The DSC thermogram of S_1A exhibits melting at 200ºC. The thermogram of S_2LP blend shows only one (Tg) value at 78.25ºC is observed indicating miscibility between these two polymers. Moreover, it was found decrease in a (Tg) of S_1L by the addition of PVP, thus related to amorphous structure of PVP. The S_3LAP_{CH} ternary blend also exhibits one (Tg) value at 69ºC, showing that these three polymers are mutually miscible. The lower (Tg) value can be attributed to the higher fraction of glycerol with respect to PUL in the ternary blend than the binary blend and in the pure film. This behavior can be related to crystalline for PUL in binary and ternary blend films decrease at the higher amount of glycerol than in pure films, because glycerol inserting themselves between the chains of polymer blend and spread out them by increased free volume in polymer chains, and thus significantly lowering the (Tg) for the blend films and making it softer. The same behavior was shown in a study done by Sreekumar in Starch/PVA blend films with glycerol (Sreekumar et al., 2012).

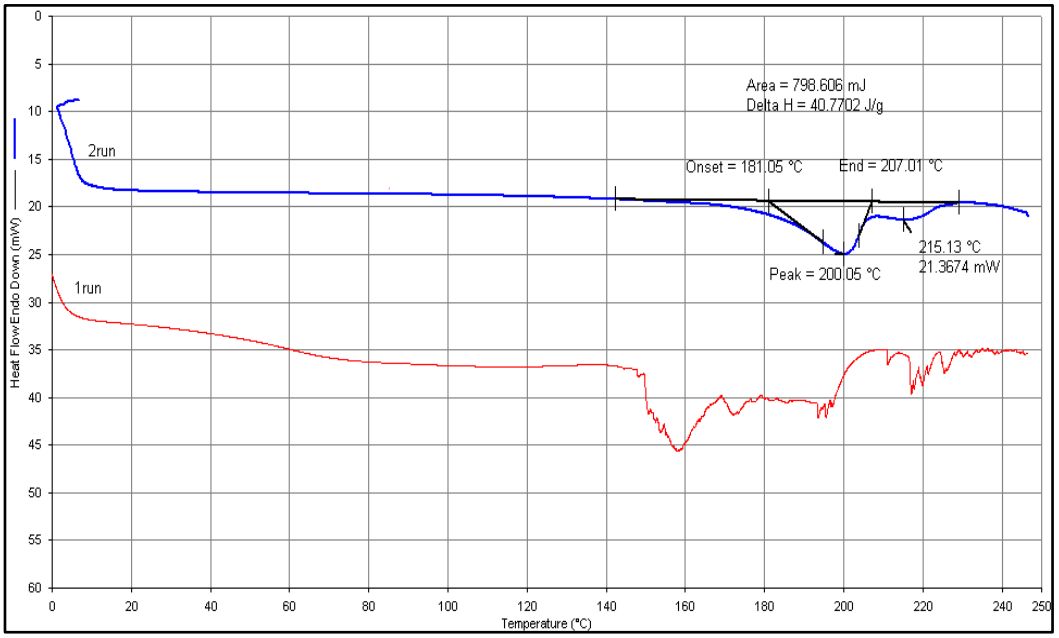


Figure 14: DSC Thermogram of PVA Film with Concentration 6% (w/v) and 1.8g of Glycerol

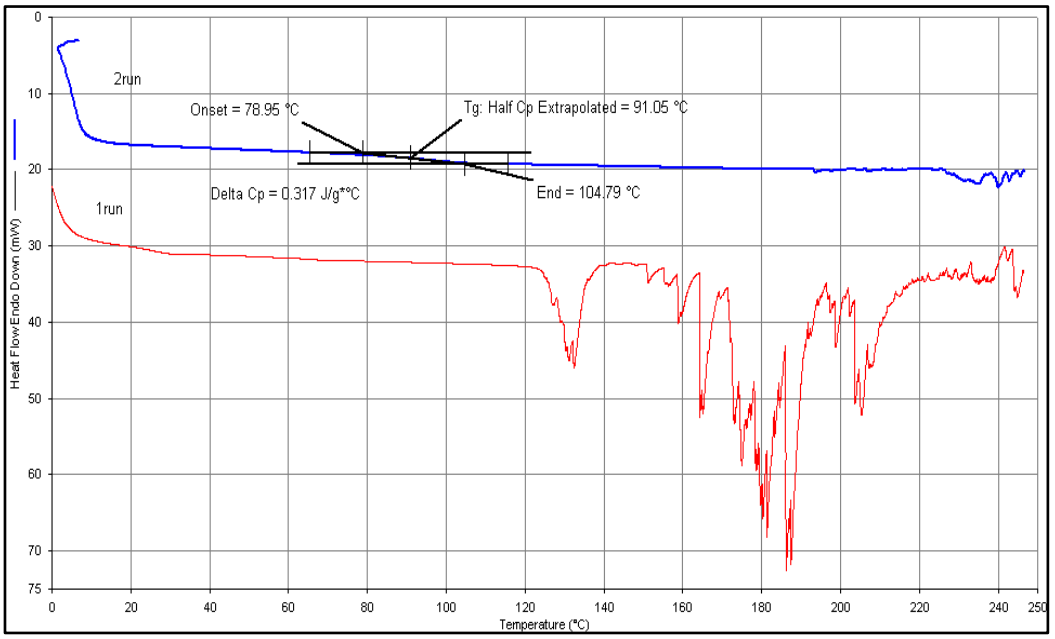


Figure 15: DSC Thermogram of PUL Film with Concentration 6% (w/v) and 1.8g of Glycerol

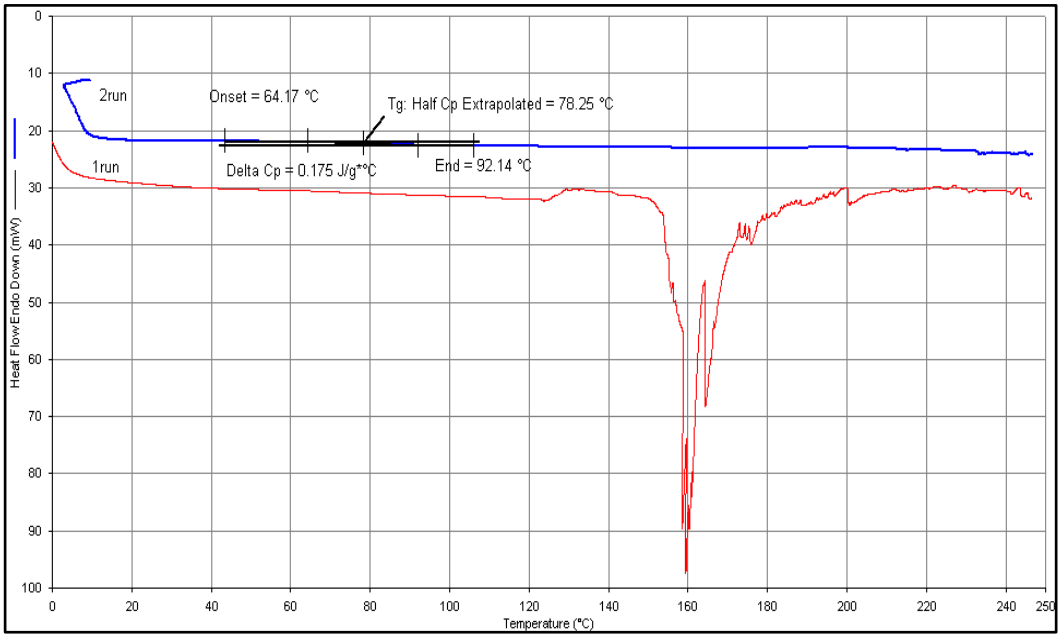


Figure 16: DSC Thermogram of PUL/PVP Blend Film and 1.35g Glycerol 33.3/66.7 (w/w) Ratio Without Cross Linker

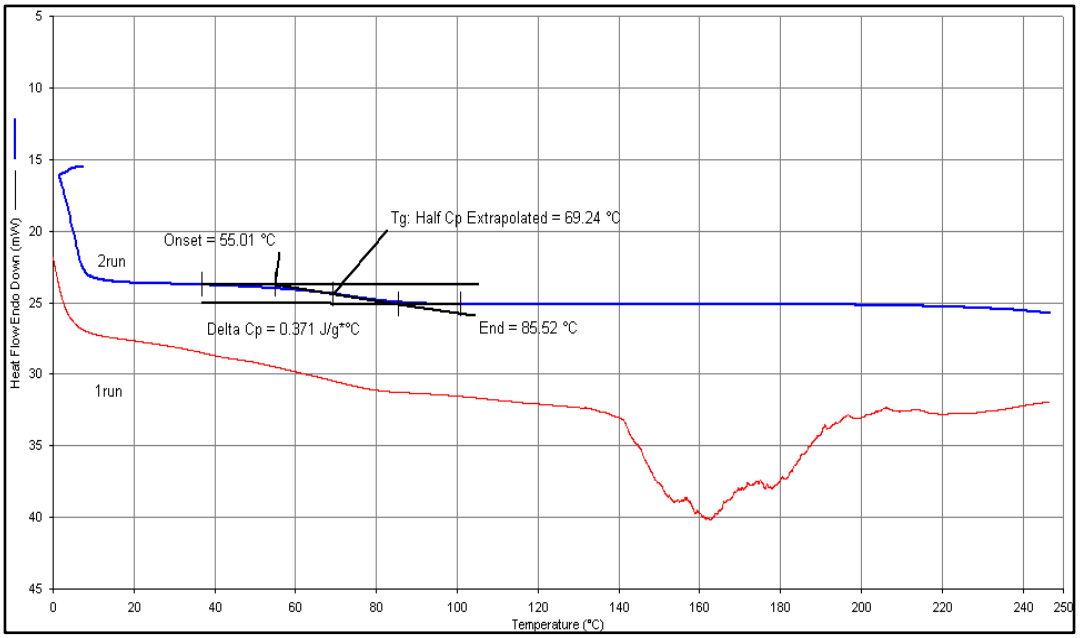


Figure 17: DSC Thermogram of Ternary Blend Film from PUL/PVP/PVA with16.7/50/33.3 (w/w) Ratio with Cross Linking and Heating with 1.8g Glycerol

3.5 Beer's-Lambert curves

Iodine in the form of I^- and I_3^- was determined from Beer's calibration curve as shown in Fig 18, 19 and 20 respectively.

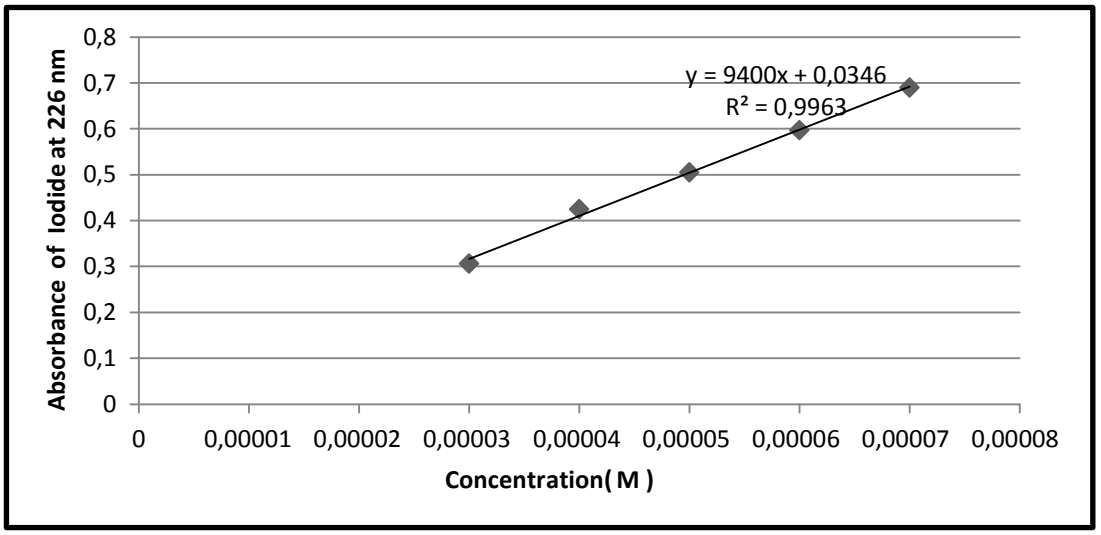
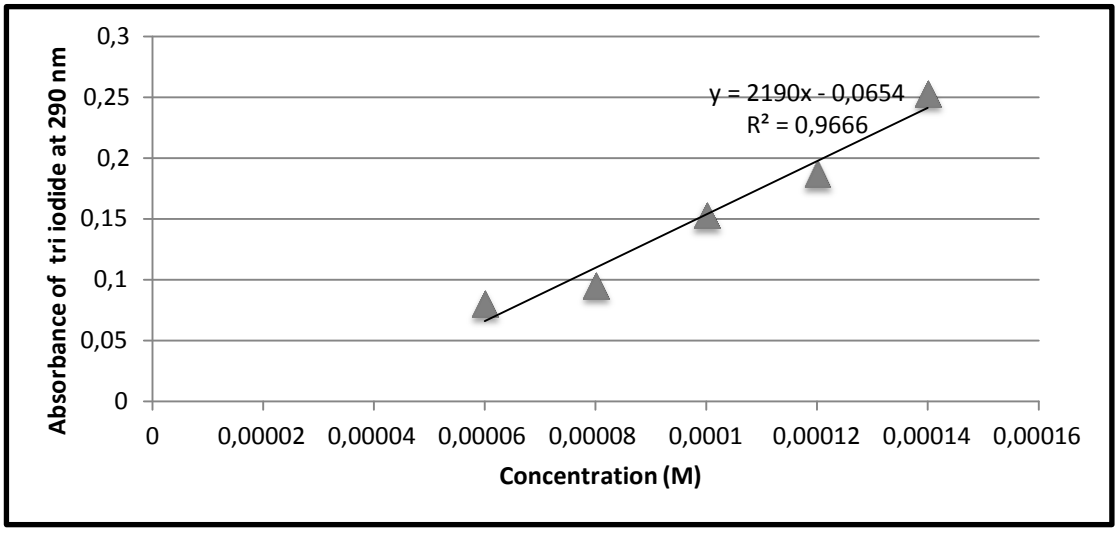


Figure 18: The Beer- Lambert Calibration Curve for Iodide from NaI Solutions



.

Figure 19: The Beer- Lambert Calibration Curve for Triiodide from NaI- I_2 Solutions at 290 nm

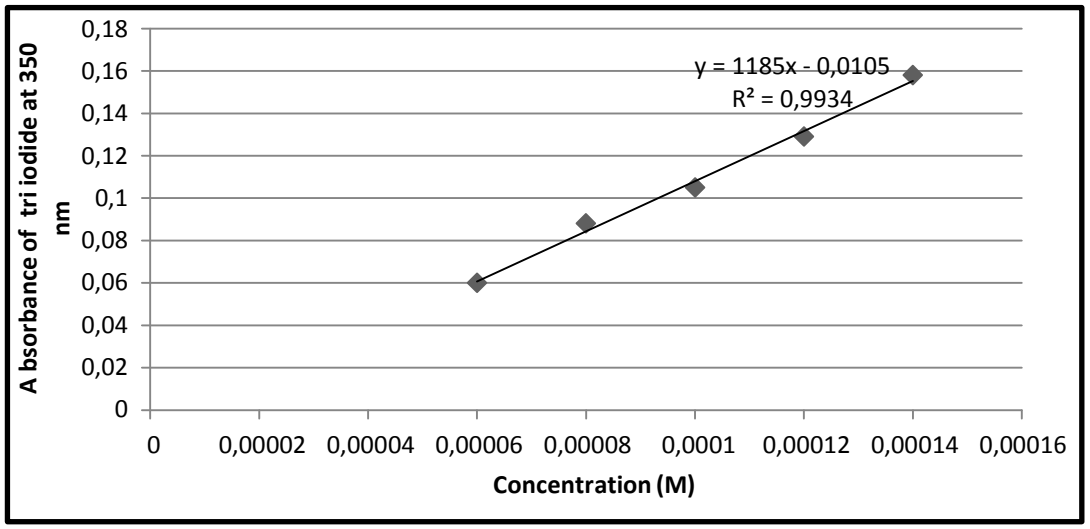


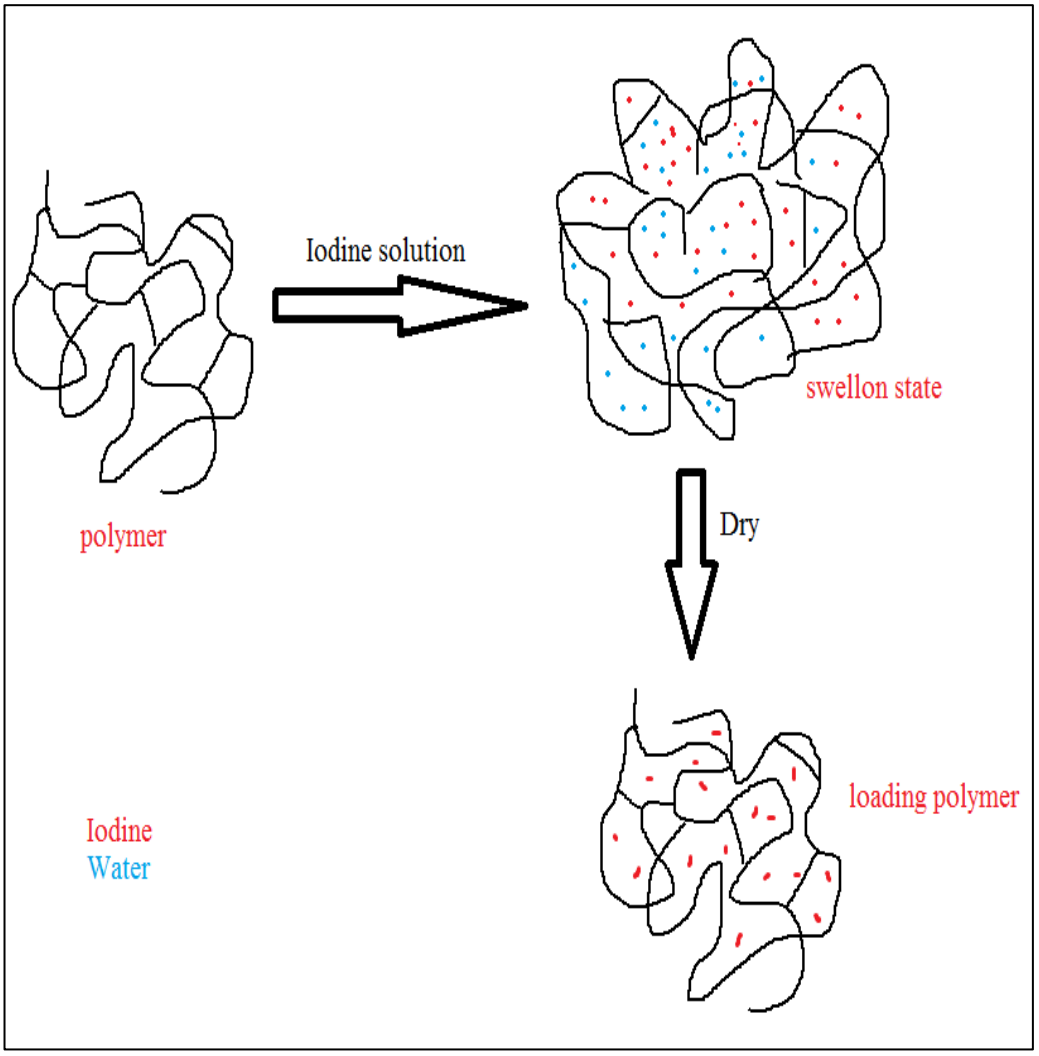
Figure 20: The Beer- Lambert Calibration Curve for Triiodide from NaI- I_2 Solutions at 350 nm

3.6 Loading studies

Loading of the S_3LAP_{CH} polymer film was done by allowing films to swell in iodine stock solutions of 0.1%, 1% and 10% (w/v) for 24 hr and then drying them at room temperature as shown in Scheme 6. Iodine complexed with free carbonyl group in PVP within in polymer film. Using this way for loading has a number of advantages over adding iodine during film formation, such as avoiding the decomposition of iodine due to experimental conditions. This polymer blend film had been chosen due to its lower % swelling (i.e. environmentally friendly) than S_2AP_C (i.e. by use S_2AP_C film more iodine was loaded then released).

Iodine in the form of I^- and I_3^- was determined from Beer's calibration curve as shown in Figures 18, 19 and 20 respectively. Weight in (g) for both I^- and I_3^- from stock solutions before loading and after loading for S_3LAP_{CH} are shown in Tables 3 and 4. It was found that after loading film with 10% (w/v) stock iodine solutions loading weight for both I^- and I_3^- in to S_3LAP_{CH} was higher than when the S_3LAP_{CH}

loading with both 0.1% and 1% (w/v) as shown in Table 5 Moreover, initial weights (a) also gravimetrically measured and shown in Table 5.



Scheme 6: Mechanism of Polymer Loaded with Iodine Solution

Table 3: Spectroscopic Data for I^- in (a), I_3^- at 290 nm in (b) and I_3^- at 350 nm in (c) for 1 mL from Iodine Stock Solutions as 0.1%, 1% and 10% (w/v) Before Loading.

(a) Concentration of stock solutions		Absorbance (A) at 226nm and diluting factor for (I^-)in stock solution		Molarity of (I^-)in diluting stock solution by Figure (18)	Molarity of (I^-)in stock solution	Weight of (I^-) in stock solution
(%w/v)	(M)	Diluting factor	(A)	(M)	(M)	(g)
0.100	0.00390	20	0.61	$6.15x10^{-5}$	$1.23x10^{-3}$	$1.56x10^{-4}$
1.00	0.0390	200	0.74	$7.45\ x10^{-5}$	$1.49x10^{-2}$	$1.80x10^{-3}$
10.0	0.390	2000	0.79	$8.00\ x10^{-5}$	$1.60x10^{-1}$	$2.00x10^{-2}$

(b) Concentration of stock solutions		Absorbance (A) at 290nm and diluting factor for (I_3^-) in stock solution		Molarity of (I_3^-)in diluting stock solution by Figure (19)	Molarity of (I_3^-) in stock solution	Weight of (I_3^-) in stock solution
(%w/v)	(M)	Diluting factor	(A)	(M)	(M)	(g)
0.100	0.00390	20	0.163	$1.04x10^{-4}$	$2.09x10^{-3}$	$7.9\ x10^{-4}$
1.00	0.0390	200	0.102	$7.65x10^{-5}$	$1.53x10^{-2}$	$5.8x10^{-3}$
10.0	0.390	2000	0.119	$8.45x10^{-5}$	$1.69x10^{-1}$	$6.4\ x10^{-2}$

(c) Concentration of stock solutions		Absorbance (A) at 350nm and diluting factor for (I_3^-) in stock solution		Molarity of (I_3^-)in diluting stock solution by Figure (20)	Molarity of (I_3^-)in stock solution	Weight of (I_3^-) in stock solution
(%w/v)	(M)	Diluting factor	(A)	(M)	(M)	(g)
0.100	0.00390	20	0.101	$9.45x10^{-5}$	$1.89\ x10^{-3}$	$7.19\ x10^{-4}$
1.00	0.0390	200	0.067	$5.95\ x10^{-5}$	$1.30x10^{-2}$	$5.00x10^{-3}$
10.0	0.390	2000	0.081	$7.70\ x10^{-5}$	$1.54\ x10^{-1}$	$5.90\ x10^{-2}$

Table 4: Spectroscopic Data for I^- in (a), I_3^- at 290 nm in (b) and I_3^- at 350 nm in(c) for 1 mL from Iodine Stock Solutions as 0.1%, 1% and 10% (w/v) After Loading with S_3LAP_{CH}

(a) Concentration of stock solutions		Absorbance (A) at 226nm and diluting factor for (I^-) in stock solution		Molarity of (I^-) in diluting stock solution by Figure (18)	Molarity of (I^-) in stock solution	Weight of (I^-) in stock solution
(%w/v)	(M)	Diluting factor	(A)	(M)	(M)	(g)
0.100	0.00390	20	0.59	$5.90x10^{-5}$	$1.19x10^{-3}$	$1.518x10^{-4}$
1.00	0.0390	200	0.69	$7.05x10^{-5}$	$1.41x10^{-2}$	$1.790x10^{-3}$
10.0	0.390	2000	0.76	$7.80x10^{-5}$	$1.56x10^{-1}$	$1.990x10^{-2}$

(b) Concentration of stock solutions		Absorbance (A) at 290nm and diluting factor for (I_3^-) in stock solution		Molarity of (I_3^-) in diluting stock solution by Figure (19)	Molarity of (I_3^-) in stock solution	Weight of (I_3^-) in stock solution
(%w/v)	(M)	Diluting factor	(A)	(M)	(M)	(g)
0.100	0.00390	20	0.140	$9.40x10^{-5}$	$1.88x10^{-3}$	$7.169\ x10^{-4}$
1.00	0.0390	200	0.098	$7.50x10^{-5}$	$1.50x10^{-2}$	$5.713x10^{-3}$
10.0	0.390	2000	0.118	$8.40x10^{-5}$	$1.68x10^{-1}$	$6.390x10^{-2}$

(c) Concentration of stock solutions		Absorbance (A) at 350nm and diluting factor for (I_3^-) in stock solution		Molarity of (I_3^-)in diluting stock solution by Figure (20)	Molarity of (I_3^-)in stock solution	Weight of (I_3^-) in stock solution
(%w/v)	(M)	Diluting factor	(A)	(M)	(M)	(g)
0.100	0.00390	20	0.092	$8.65.x10^{-5}$	$1.73\ x10^{-3}$	$6.61\ x10^{-4}$
1.00	0.0390	200	0.060	$5.95\ x10^{-5}$	$1.20\ x10^{-2}$	$4.92\ x10^{-3}$
10.0	0.390	2000	0.080	$7.64\ x10^{-5}$	$1.53\ x10^{-1}$	$5.85\ x10^{-2}$

Table 5: Effect of the Concentration of Iodine Stock Solutions on Initial Weight (a) and Loading Weight for both I^- and I_3^- were Loaded in to S_3LAP_{CH}.

Concentration of stock solutions		Initial weight (a) of stock solutions loading in film from Eq (5)	Weight of (I^-) loading in film from Eq (2)	Weigh of(I_3^-) loading in film from Eq (2) at 290 and 350 nm respectively	
(%w/v)	(M)	(g)	(g)	(g) 290 nm	350 nm
0.100	0.00390	$1.70x10^{-4}$	$4.20x10^{-6}$	$7.13 x10^{-5}$	$5.85 x10^{-5}$
1.00	0.0390	$0.20x10^{-3}$	$8.10x10^{-6}$	$8.73x10^{-5}$	$8.10 x10^{-5}$
10.0	0.390	$1.30x10^{-3}$	$8.35x10^{-5}$	$5.23 x10^{-4}$	$4.70 x10^{-4}$

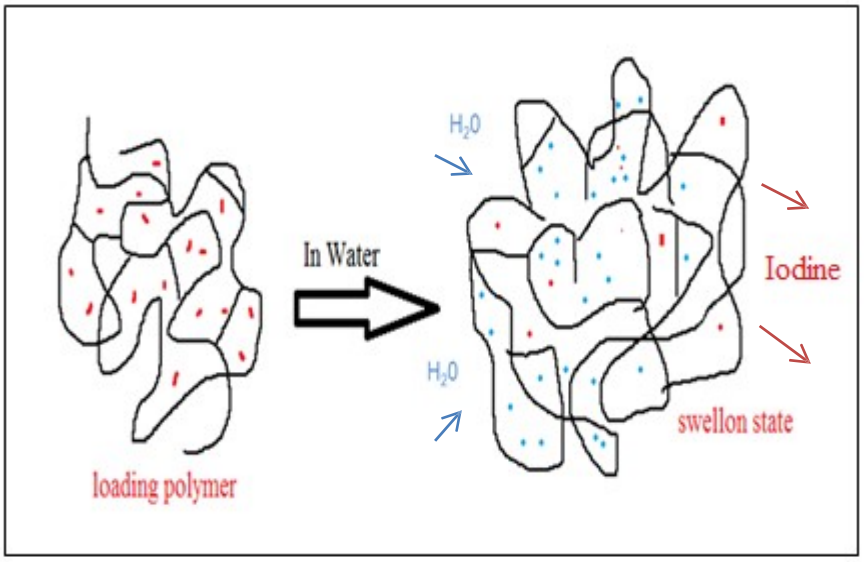
% Loading efficiency and % loading for both I^- and I_3^- for S_3LAP_{CH} with different iodine stock solutions was shown in Table (6). It was found that by increasing the concentration of iodine stock solution % loading was increasing and reaching to 0.37 and 2.2 for both I^- and I_3^- within S_3LAP_{CH} loading with 10 % iodine stock solution. Moreover, it was realized that % loading efficiency for these ions loaded with 10% (w/v) iodine stock solution was lower than others generally this behavior can be explained to loading weights for both I^- and I_3^- were very low than weights for both I^- and I_3^- in 10% (w/v) iodine stock solution as shown in Tables 3 and 5.

Table 6: Effect of the Concentration for Iodine Stock Solutions on the % Loading and % Loading Efficiency of I^- and I_3^- for S_3LAP_{CH} Blend Film. Weight of Dry Film was Taken as 0.0233g.

Concentration of stock solutions		% Loading Eq.(3)	% Loading Eq.(3) at 290 and350 nm		% Loading efficiency from Eq. (4)	% Loading efficiency from Eq. (4) at 290 and350 nm	
(%w/v)	(M)	(I^-)	(I_3^-) 290nm	350nm	(I^-)	(I_3^-) 290 nm	350 nm
0.100	0.00390	0.0185	0.30	0.25	2.60	9.00	8.10
1.00	0.0390	0.034	0.37	0.34	0.45	1.50	1.62
10.0	0.390	0.370	2.20	2.00	0.41	0.87	0.79

3.7 Release Studies

The releasing of I^- and I_3^- from polymer films is due to the absorption of water (i.e. releasing medium) into polymer and gradually releasing them via diffusion as shown in Scheme 7. Iodine was loaded into S_3LAP_{CH} for 16.7/50/33.3 (w/w) ratio in three different concentrations 0.1%, 1% and 10% (w/v). Both I^- and I_3^- were determinate from Beer's calibration curve as shown in Figures 18, 19 and 20 respectively. Moreover, spectroscopic data for both I^- and I_3^- in three different concentrations 0.1%, 1% and 10% (w/v) are shown in Tables 7, 9 and 11 respectively. Release in (g) for I^- and I_3^- with different loading solutions as 0.1%, 1% and 10% (w/v) in 1mL from S_3LAP_{CH} was shown in Tables 8, 10, 12 and Figure 21 respectively. Thus it was found that the accumulative amount of iodide release after 24, 48, 168 hr from S_3LAP_{CH} loading with 0.1%, 1% and 10% (w/v) element iodine solutions are $3.95x10^{-6}$, $7.17x10^{-6}$ and $7.54x10^{-5}$ g/mL respectively. However, the accumulative amount of triiodide release with same condition was noted as $7.01x10^{-5}$, $8.51x10^{-5}$ and $4.59x10^{-4}$ g/mL at 290 nm respectively. According to these results it was found that by increasing the amount of iodine loading to the blend films releasing hours of iodide and triiodide are increasing until 168 hr for films loaded with 10% (w/v) element iodine.



Scheme 7: Mechanism of Iodine Release from Polymer Film

Table 7: Spectroscopic Data for I^- at 226 nm, I_3^- at 290 nm and I_3^- at 350 nm Release from S_3LAP_{CH} Loaded with 0.1% (w/v) Iodine Stock Solution in 20mL of Releasing Medium.

Time (hr)	Absorbance of (I^-)at 226 nm	Molarity of (I^-) by Fig.18	Absorbance of (I_3^-) at 290 nm	Molarity of (I_3^-) by Fig.19	Absorbance of (I_3^-)at 350 nm	Molarity of(I_3^-) by Fig.20
2	0.132	$1.036x10^{-5}$	0.032	$4.44\ x10^{-5}$	0.033	$3.67\ x10^{-5}$
4	0.123	$9.400x10^{-6}$	0.043	$4.94\ x10^{-5}$	0.037	$4.00\ x10^{-5}$
8	0.112	$8.230x10^{-6}$	0.037	$4.67\ x10^{-5}$	0.034	$3.75\ x10^{-5}$
24	0.064	$3.120x10^{-6}$	0.030	$4.35\ x10^{-5}$	0.030	$3.41\ x10^{-5}$

Table 8: Weight of I^- and I_3^-Release from S_3LAP_{CH} Loaded with 0.1% (w/v) Iodine Stock Solution in 1mL of Water.

Time (hr)	(I^-) by Fig.(18) and Eq.(11)	(I_3^-) by Fig.(19), (20) and Eq.(11)	
	(g) 226nm	(g) 290nm	350 nm
2	$1.31\ x10^{-6}$	$1.69\ x10^{-5}$	$1.39\ x10^{-5}$
4	$2.50\ x10^{-6}$	$3.57\ x10^{-5}$	$2.92\ x10^{-5}$
8	$3.55\ x10^{-6}$	$5.36\ x10^{-5}$	$4.35\ x10^{-5}$
24	$3.95\ x10^{-6}$	$7.01\ x10^{-5}$	$5.65x10^{-5}$

Table 9: Spectroscopic Data for I^- at 226nm,I_3^- at 290 nm and I_3^- at 350 nm Release from S_3LAP_{CH} Loaded with 1% (w/v) Iodine Stock Solution in 20mL of Release Medium.

Time (hr)	Absorbance of (I^-) at 226 nm	Molarity of (I^-) by Fig.18	Absorbance of (I_3^-) at 290 nm	Molarity of (I_3^-) by Fig.19	Absorbance of (I_3^-)at 350 nm	Molarity of (I_3^-) by Fig.20
2	0.122	9.29×10^{-6}	0.031	3.95×10^{-5}	0.037	4.02×10^{-5}
4	0.121	9.28×10^{-6}	0.035	4.10×10^{-5}	0.034	3.81×10^{-5}
8	0.169	1.44×10^{-5}	0.030	4.36×10^{-5}	0.041	4.38×10^{-5}
24	0.204	1.81×10^{-5}	0.032	4.45×10^{-5}	0.037	4.07×10^{-5}
48	0.084	5.36×10^{-5}	0.034	4.50×10^{-5}	0.034	3.82×10^{-5}

Table 10: Weight of I^- and I_3^- Release from S_3LAP_{CH} Loaded with 1% (w/v) Iodine Stock Solution in 1mL of Water.

Time (hr)	(I^-) by Fig.(18) and Eq.(11)	(I_3^-) by Fig.(19),(20) and Eq.(11)	
	(g) 226nm	(g) 290nm	350 nm
2	1.18×10^{-6}	1.67×10^{-5}	1.52×10^{-5}
4	2.36×10^{-6}	3.42×10^{-5}	2.98×10^{-5}
8	4.19×10^{-6}	5.00×10^{-5}	4.64×10^{-5}
24	6.49×10^{-6}	6.77×10^{-5}	6.19×10^{-5}
48	7.17×10^{-6}	8.51×10^{-5}	7.64×10^{-5}

Table 11: Spectroscopic Data for I^- at 226nm,I_3^- at 290 nm and I_3^- at 350 nm Release from S_3LAP_{CH} loaded with 10% (w/v) Iodine Stock Solution in 20mL of Releasing Medium.

Time (hr)	Diluting factor	Absorbance of (I^-) at 226 nm	Molarity of (I^-) by Fig.18	Absorbance of (I_3^-) at 290 nm	Molarity of (I_3^-) by Fig.19	Absorbance of (I_3^-)at 350 nm	Molarity of (I_3^-) by Fig.20
2	2.5	0.514	1.28×10^{-4}	0.040	1.20×10^{-4}	0.036	9.81×10^{-5}
4	2.5	0.168	3.55×10^{-5}	0.038	1.18×10^{-4}	0.033	9.17×10^{-5}
8	2.5	0.172	3.65×10^{-5}	0.037	1.17×10^{-4}	0.036	9.81×10^{-5}
24	2.5	0.302	7.11×10^{-5}	0.065	1.49×10^{-4}	0.05	1.27×10^{-4}
48	5.0	0.268	6.21×10^{-5}	0.045	2.52×10^{-4}	0.038	2.04×10^{-4}
72	5.0	0.223	5.01×10^{-5}	0.046	2.54×10^{-4}	0.036	3.92×10^{-5}
96	-	0.293	6.87×10^{-5}	0.044	5.00×10^{-5}	0.038	2.04×10^{-4}
120	-	0.271	6.29×10^{-5}	0.047	5.13×10^{-5}	0.040	4.26×10^{-5}
144	-	0.230	5.20×10^{-5}	0.040	4.81×10^{-5}	0.036	3.92×10^{-5}
168	-	0.140	2.80×10^{-5}	0.037	4.68×10^{-5}	0.033	3.67×10^{-5}

Table 12: Weight of I^- and I_3^- Release from S_3LAP_{CH} Loaded with 10% (w/v) Iodine Stock Solution in 1mL of Water.

Time (hr)	(I^-) By Fig.(18) and Eq.(11)	(I_3^-) by Fig.(19),(20) and Eq.(11)	
	(g) 226nm	(g) 290nm	350 nm
2	1.62×10^{-5}	4.58×10^{-5}	3.73×10^{-5}
4	2.07×10^{-5}	9.07×10^{-5}	7.23×10^{-5}
8	2.53×10^{-5}	1.35×10^{-4}	1.10×10^{-4}
24	3.43×10^{-5}	1.92×10^{-4}	1.58×10^{-4}
48	4.22×10^{-5}	2.88×10^{-4}	2.36×10^{-4}
72	4.86×10^{-5}	3.85×10^{-4}	2.51×10^{-4}
96	5.73×10^{-5}	4.04×10^{-4}	3.29×10^{-4}
120	6.53×10^{-5}	4.23×10^{-4}	3.45×10^{-4}
144	7.19×10^{-5}	4.42×10^{-4}	3.60×10^{-4}
168	7.54×10^{-5}	4.59×10^{-4}	3.74×10^{-4}

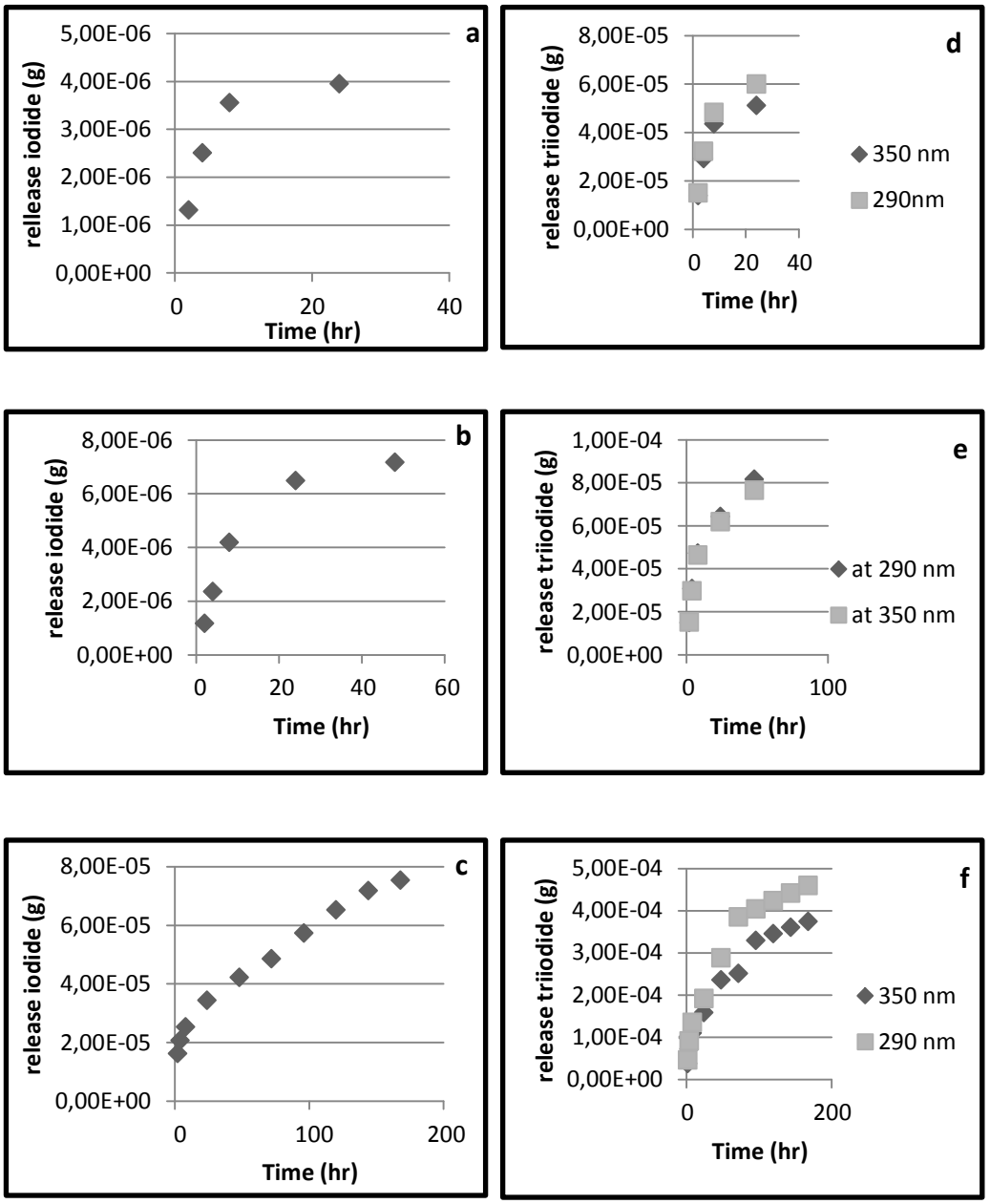


Figure 21: The Accumulative Release of Iodide (a,b,c) and Triiodide (d,e,f) from S_3LAP_{CH} Blend Film with 16.7/50/33.3 (w/w) Ratio for PUL/PVA /PVP Respectively Loaded with 0. 1 %,1% and 10 % (w/v) Element Iodine Solutions Respectively

Moreover, % release for both iodide and triiodide was shown in Figure 22. % Release of iodide form S_3LAP_{CH} loaded with 1% and10% (w/v) was found to 88% and 90% at 48 and 168 hr respectively. While 96% and 86% were found as the % release of triiodide in same conditions. Lower % release for I_3^- film loaded with 10%

(w/v) iodine solution was related to higher loading weight for I_3^- in blend film as shown in Table 5.

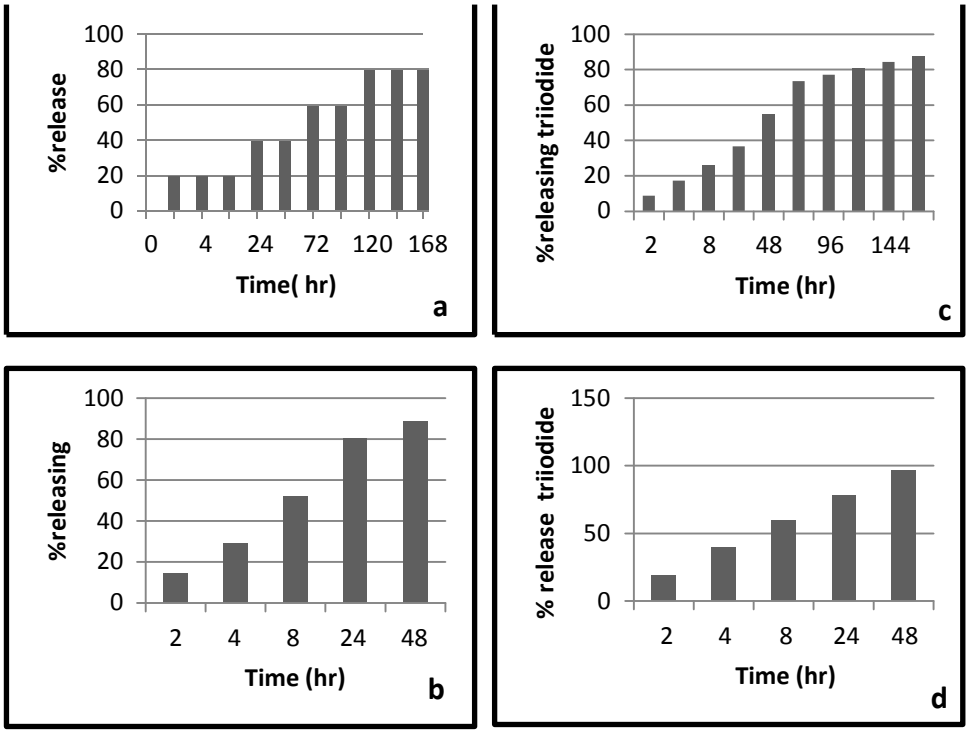


Figure 22: The % Release of Iodide (a,b) and Triiodide (c,d at 290 nm)fromS_3LAP_{CH} Blend Film with 16.7/50/33.3 (w/w) Ratio for PUL/PVA /PVP Respectively Loaded with 10% and 1% (w/v) of Element Iodine Solutions in (a,c) and(b,d) Respectively

Estimated weight of I_2 and HOI was released from S_3LAP_{CH} in 1mL of water and it is shown in Table 10. The amounts of both I_2 and HOI were estimated from S_3LAP_{CH} loaded with 1% and 10% (w/v) iodine stock solution only because of higher releasing time for them over sample loaded with 0.1% (w/v). Initial weight (a) was used as show in Table 5. According to previous study done by Mazumdar to evaluate the amount of I_2 released from iodine-polymer tablets (P-I) with 30% PVP-I. It was found it can be used in field of water disinfection with amount of iodine release from (P-I) tablet reach 1.64 mg/mL and 3.83 mg/mL at 24 and 48 hr respectively (Mazumdar et al., 2010). The results, as shown in Table 10 indicate lower amount of

iodine release in water from S_3LAP_{CH} with 1% and 10% (w/v) and reach 0.027 mg/mL and 0.766 mg/mL at 48 and 168 hr respectively. This indicated that this sample is friendlier for the environment.

Table 13: Weight in (mg) of I^-, I_3^-, HOI and I_2 was Released from S_3LAP_{CH} in 1mL of Water Loaded with 1% and 10% (w/v) Iodine Stock Solution.

Time (hr)	% (w/v)	Initial weight(a) From	(I^-) from Fig.(17)and Eq.(11)	(I_3^-) from Fig.(18)and Eq.(11)	(HOI) from Eq.(12)	(I_2) from Eq.(13)
48	1.00	0.20	0.0072	0.0851	0.0923	0.027
168	10.0	1.30	0.0750	0.4590	0.5340	0.766

3.8 Evaluation of Antibacterial Properties of Releasing Iodine.

In vitro antibacterial properties of the S_3LAP_{CH} disks complexed with 1% (w/v) iodine stock solution (test) and S_3LAP_{CH} film disks only (control) for gram negative *E. coli* and gram positive *S. aureus* are shown in Figures 23 and 24 respectively. Antibacterial properties of these are confirmed by applying the film disks to *E. coli* IFO 3972 and *S. aureus* ATCC25923 bacterial culture on nutrient agar and incubating for 48 hr at 37ºC. As a result of diffusion of iodine from the blend film disks within agar, the antibacterial activity against *E. coli* and *S .aureus* was observed. Figures 22 and 23 indicated that S_3LAP_{CH} disks complex with 1% (w/v) stock iodine solution with lower iodine release 0.027 mg/mL show clear inhibition zone (i.e. area on the ager plate free from any bacterial growth) around test samples. The diameter of inhibition zone was found as 27 mm and 29 mm for *E. coli* and *S. aureus* respectively. However, control samples did not show any antibacterial activity in either bacterial strain. Studies indicated that I_2 shows more cysticidal effect comparing to HOI and I_3^-. The antibacterial efficiency of HOI is 1/2 of I_2 and

that of I_3^- 1/8th of I_2. However, as found by literature study bactericidal activity of I_2 found even at ppm level 1mg/L (Punyani et al, 2007).

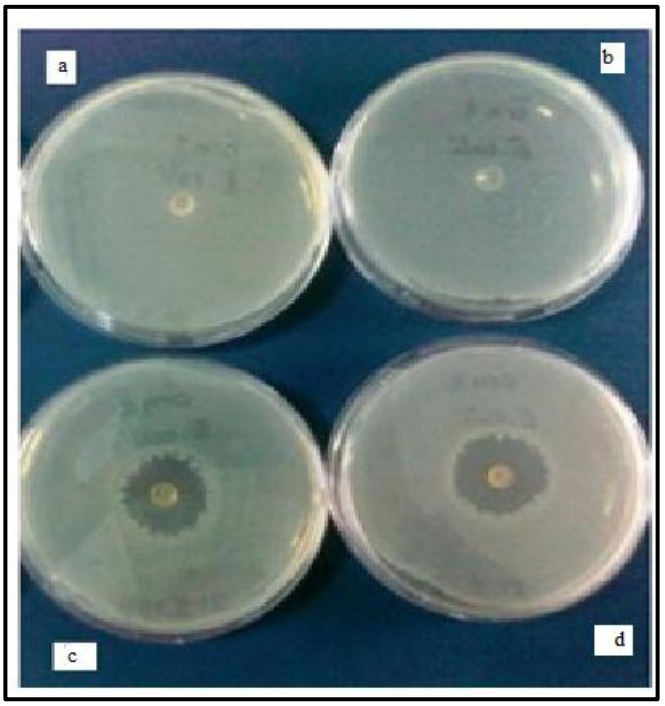


Figure 23: In Vitro Inhibition Zone of the S_3LAP_{CH} Film Disks with *E.coli* for Film Disks only (Control) in (a,b) and Complex with 1% Iodine Solution (Test) in (c,d)

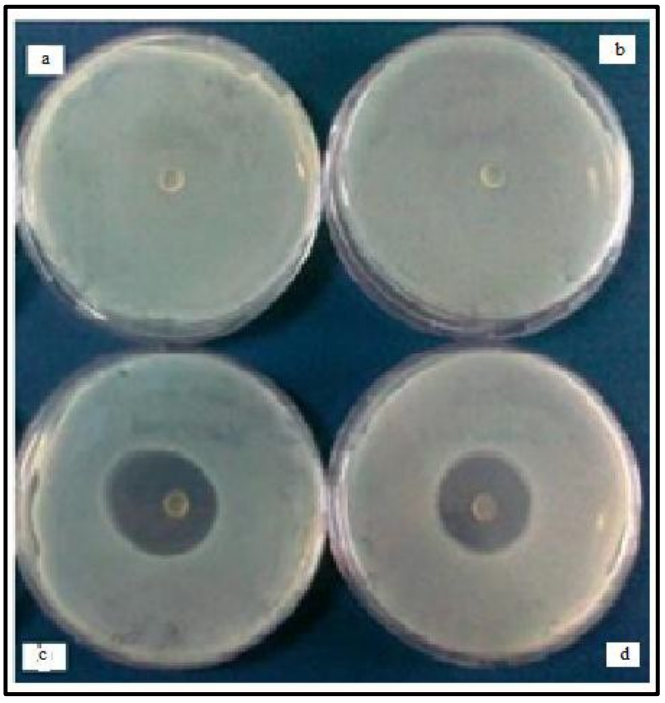


Figure 24: In Vitro Inhibition Zone of the S_3LAP_{CH} Film Disks with *S. aureus* Film Disks only (Control) in (a,b) and Complex with 1% Iodine Solution (Test) in (c,d)

Chapter 4

CONCLUSIONS

Preparation and characterization of antibacterial polymeric blend films based on biocompatible polymers was undertaken in this study. Blend films were prepared from PUL with synthetic polymer PVA and iodophor PVP. It was found that blending of PUL with these two polymers could enhance its properties such as water solubility and moisture sensitivity and hence the structures are required for the use of blend film as a carrier for releasing of iodine could be achieved.

According to the FTIR the interactions within polymers in PUL/PVA/PVP blend film were increased by crosslinking with glutaraldehyde (GA) at 60ºC for 45min. When (-OH) stretching of blend film was reduced to 3340 cm^{-1} by the addition of GA and to 3320 cm^{-1} by heating at 60ºC. Furthermore, the band at 1050 cm^{-1} related to the ether bond of acetyl groups had appeared due to reaction of GA with hydroxyl group of both PUL and PVA and the absorbance intensity of this bond increased by heating. According to the SEM micrographs homogeneity within polymers blend was improved by cross linking with heating due to increase interactions between GA and hydroxyl groups for both PVA and PUL with carbonyl groups of PVP.

It was realized that % swelling for ternary blend film was decreased from 212% to 197% due to the increase of the interactions within polymers blend by crosslinking with heating. DSC thermogram show single (Tg) value for ternary blend film cross linking with heating as 69ºC indicate miscibility of polymers. Lowering (Tg) value in ternary blend film than pure and binary blend was related to higher amount of glycerol that added to ternary blend solutions. Higher % loading and higher releasing time 168hr for both iodide and triiodide were found when film loaded with higher concentration of iodine stock solution10% (w/v).

As a result of diffusion of iodine from the blend film disks within agar, the antibacterial activity against *E. coli* and *S. aureus* was observed. However, control samples did not show any antibacterial activity in either bacterial strain. Thus it can be concluded that polymeric blends based on crosslinking PUL/PVA/PVP-I blend have the potential to be used as iodine-release systems for antibacterial applications.

REFERENCES

Abed, A., Assoul, N., Ba, M., Mohamed, D.S., & Portes, P. (2011). Influence of Polysaccharide Composition on the Biocompatibility of Pullulan/Dextran-Based Hydrogels. *Journal of Biomed Mater Res Part A*. 96A: 535– 542

Ahmad, I.S., Hasan, N., Abid, Z., & Mazumdar, N. (2008). Preparation and Characterization of Films Based on Crosslinked Blends of Gum Acacia, Polyvinylalcohol, and Polyvinylpyrrolidone-Iodine Complex. *Inc. Journal of Appl Polym Sci.* 109: 775–781.

Aykara, T., & Demirci, S. (2007). Thermal, Spectroscopic, and Mechanical Properties of Blend Films of Poly (N-Vinyl-2-Pyrrolidone) and Sodium Alginate. *Journal of Polymer-Plastics Technology and Engineering.* 46: 737–741.

Barbara, S.B., & Tomsic, B. (2001). Structures of Novel Antimicrobial Agents for Textiles –A Review. *Journal of Textile Research Journal*. 80(16),1721–1737.

Baker, W., Scott, C., & HU, G.-H. (2001). *Reactive Polymer Blending*. Printed in Germany by Fa.KÖsel, Kempten.

Bernal, A., Kuritka I., & Saha, P. (2010). Poly (vinyl alcohol)-poly(vinyl pyrrolidone) blends: Preparation and characterization for a prospective medical

application. *Journal of Mathematical Methods and Techniques in Engineering and Environmental Science*. 46, 6,431-434.

Block, S. (2001). *Disinfection, Sterilization, and Preservation.* 5 ed, lippincott Williams and Wilkins. USA.

Bridier, A., Briandet, R., Thomas, V., & Dubois-Brissonnet, F. (2011). Resistance of Bacterial Biofilms to Disinfectants: a Review. *Journal of Bioadhesion and Biofilm Research*. 27, 9 1017–1032.

Burks, RI. (1998). Povidone-Iodine Solution in Wound Treatment. *Journal of Physical Therapy*. 78, 2, 212-218.

Cencetti, C., Bellini, D., Pavesio, A., Senigaglia, D., Passariello, C., Virga, A., Matricardi,P. (2012). Preparation and Characterization of Antimicrobial Wound Dressings Based on Silver, Gellan, PVA and Borax. *Journal of Carbohydrat Polymers*. 90 , 1362–1370.

Cesar, M.T., Braunstein, S.F., & Figueiredo, L.D. (2011). Preparation and Characterization of a polymeric Blend of PVP/PVAL for use in Drug Delivery System. *Journal of Biomedical Nanotechnologe*. 7, 3,446-449.

Chan, CH.W., & Cheng, W-CH. (2012). Mass Transport Process for Adsorption of Metal Ions onto a New Type Adsorbent Poly (vinyl alcohol) (PVA)/peat/clay Composite Bead. *Journal of Polym Res*. 19:9964.

Chen, H., Yuan, L., Song, W., Wu Z., & Li, D. (2008). Biocompatible Polymer Materials: Role of Protein–Surface Interactions. *Journal of Progress in Polymer Science* 33, 1059-1087.

Cheng, K. Ch., Demirci, A., & Catchmark J.M. (2011). Pullulan: Biosynthesis, Production, and Applications. *Journal of Appl Microbiol Biotechnol*. 92:29–44.

Chapman, S.J. (2003). Biocide Resistance Mechanisms. *Journal of International Biodeterioration & Biodegradation*. 51 ,133 – 138.

Cloete, E.T. (2003). Resistance Mechanisms of Bacteria to Antimicrobial Compound. *Journal of International Biodeterioration & Biodegradation*. 51, 277 – 282.

Constantina, M., Mihalceaa, I., Oaneaa, I., & Harabagiua, V. (2011). Studies on Graft Copolymerization of 3-Acrylamidopropyl Trimethylammonium Chloride on Pullulan.J. *Journal of Carbohydrate Polymers*. 84 ,926–932.

Dalmoro, A., Angelabarba, A., Lamberti, G., Grassi, M., & d'amore, M. (2012). Pharmaceutical Applications of Biocompatible Polymer Blends Containing

Sodium Alginate. *Journal of Advances in Polymer Technology,* Vol. 31, No. 3, 219 – 230.

Demirci, A., & Catchmark, J.M. (2011). Pullulan: Biosynthesis, Production, and Applications. *Journal of Appl Microbiol Biotechnol.* 92:29–44.

Denyera, P., & Stewartb, B.A.S.G. (1998). Mechanisms of action of disinfectants. *Journal of International Biodeterioration & Biodegradation*. 41,261-268.

Dulong, V., Forbice, R., Condamine, E., & Cerf, L.D. (2011). Picton L.Pullulan–STMP Hydrogels: a Way to Correlate Crosslinking Mechanism, Structure and Physicochemical Properties. *Journal of Polym. Bull.* 67:455–466.

Fahmy, HM., Abo-Shosha, MH., & Ibrahim, NA. (2009). Finishing of Cotton Fabrics with poly (N-vinyl-2-pyrrolidone) to Improve their Performance and Antibacterial Properties. *Journal of Carbohydrate polymers*. 77,4 ,845-850.

Folttmann, H., & Quadir A. (2008). Polyvinylpyrrolidone PVP– One of the Most Widely UsedExcipients in Pharmaceuticals: An Overview. *Journal of Drug Delivery Technology*. Vol 8 No6.

Gabriel, J.G., Som, A., Madkour, E.A., Eren, T., & Tew, N.G. (2007). Infectious Disease: Connecting Innate Immunity to Biocidal Polymers. *Journal of Materials Science and Engineering R*. 57,28–64.

Gberg, H.D.L., Heddini, A., Trends, C.O. (2010). The Global Need for Effective Antibiotics: Challenges and Recent Advance. *Journal of in Pharmacological Sciences.* 31, 11,505-515.

Giri, N., Natarajan, K.R., Gunasekaran, S., & Shreemathi, S.(2011). ^{13}C NMR and FTIR Spectroscopic Study of Blend Behavior of PVP and Nano Silver Particles. *Journal of Archives of Applied Science Research.* 3 (5):624-630.

Gnatova, M., Manolova, N., & Rashkov, I. (2007). Electrospinning of poly(vinyl pyrrolidone) Iodine Complex a Prospective Route to Antimicrobial Wound Dressing Materials. *Journal of European Polymer*. 43, 1609–1623.

Gniewosz, M., & Synowiec, A. (2011). Antibacterial Activity of Pullulan Films Containing Thymol. *Journal of Flavour Fragr*. 26, 389–395.

Gupta, B., Agarwal, R., & Alam, S.M. (2013). Preparation and Characterization of Polyvinyl Alcohol-Polyethylene Oxide-Carboxymethyl Cellulose Blend Membranes :Inc. *J. Appl. Polym. Sci* . 127: 1301–1308.

Guru, S.G., Prasad, P., Shivakumar, R.H., & Rai,K.S. (2010). Miscibility Studies of Polysaccharide Xanthan Gum/PVP Blend. *Journal of Polym Environ*. 18.135-140.

Heiner, J.D., Hile, C.D., Samandra, T., Demons, T.S., & Wedmore, S.I. (2010). 10% Povidone-Iodine May Be a Practical Field Water Disinfectant. *Journal of Wilderness & Environmental M edicine*. 21, 332–336.

He, Y., Zhu, B., & Inoue, Y. (2004). Hydrogen Bonds in Polymer Blends. *Journal of Prog. Polym. Sci*. 29 , 1021–1051.

Hogan, D., & Kolter, R. (2002). Why are Bacteria Refractory to Antimicrobials? *Journal of Current Opinion in Microbiology*. 5:472–477.

Hong, Y., Li, Y., Zhuang, X., Chen, X., & Jing, X. (2009). Electrospinning of Multicomponent Ultrathin Fbrous Nonwovens for Semi-occlusive Wound Dressings. *Journal of Biomed Mater Res*. 89A: 345–354.

Hu, F.X., Neoh, K.G., Cen, L., & Kang, E.T. (2005). Antibacterial and Antifungal Efficacy of Surface Functionalized Polymeric Beads in Repeated Applications. *Biotechnology & Bioengineering*. 89,4,475-484.

Islam, S.M., Yeum, H.J., & Das, K.A. (2012). Effect of Pullulan/Poly(vinyl alcohol) Blend System on the Montmorillonite Structure with Property Characterization of Electrospun Pullulan/Poly(vinylalcohol)/Montmorillonite Nanofibers. *Journal of Colloid and Interface Science*. 368 , 273–281.

Jawetz, E., Melnick, L.J., & Adelberg, A.E. (1989). *Medical Microbiology. 18 ed*. Typopress. Lebanon.

Jones, S.D., Djokic, J., McCoy, P.C., & Gorman, P.S. (2002). Poly(e-caprolactone) and Poly (e-caprolactone)-Polyvinylpyrrolidone-Iodine Blends as Ureteral Biomaterials: Characterisation of Mechanical and Surface Properties, Degradation and Resistance to Encrustation in Vitro. *Journal of Biomaterials*. 23, 4449–4458

Kamada, H., Tsutsumi, Y., & Yoshioka, Y. (2004). Design of a pH-Sensitive Polymeric Carrier for Drug Release and Its Application in Cancer Therapy. *Journal of Clin Cancer Res*. 10:2545-2550.

Kenawy, ER., Abdel-Hay, F.I., El-Magd, A.A., & Mahmoud Y. (2006). Biologically Active polymers: VII. Synthesis and Antimicrobial Activity of Some Crosslinked Copolymers with Quaternary Ammonium and Phosphonium Groups. *Journal of Reactive & Functional Polymers*. 66,419–429.

Kenawy, E., Abd El-Raheem, R., El-Shanshoury, R.A., Shaker O.N., El-Sadek, M. B., Khattab, H.B.A., & Badr, I.B. (2011). Biocidal Polymers. Synthesis, Antimicrobial Activity, and Possible Toxicity of Poly (hydroxystyrene-comethylmethacrylate) Derivatives). *Journal of Appl Polym Sci*. 120, 2734–2742.

Kenawy E., El-Shanshoury, R.A., Abdel-Hay, I.F., & El-Newehy, M. (1998). Biologically Active polymers: Synthesis and Antimicrobial Activity of Modified Glycidyl Methacrylate Polymers having a Quaternary Ammonium and Phosphonium Groups. *Journal of Controlled Release*. 50,145–152.

Kenawy, E.R., Worley, S.D., & Broughton, R. (2007). The Chemistry and Applications of Antimicrobial Polymers: AState -of –the Art Review. *Journal of Biomacromolecules*. 8, 5 ,1359-1384

Khandwekar, A.P., & Doble, M. (2011). Physicochemical Characterisation and Biological Evaluation of Polyvinylpyrrolidone-Iodine Engineered Polyurethane (Tecoflex®). *Journal of Sci Mater Med*. 22, 1231-1246.

Kumar, S.P., Babu, R.P., Reddy, J.G., & Uttam, A. (2011). Povidone Iodine Revisite. *Indian Journal of Dental Advancements*. 3,3, 617-620.

Li, H., Yang J., Hu, X., Liang, J., Fan, Y., & Zhang X. (2011). Superabsorbent Polysaccharide Hydrogels Based on Pullulan Derivate as Antibacterial Release Wound Dressing. *Journal of Biomed Mater Res Part A*. 98A: 31–39.

Li, X., Hu, A., & Ye, L. (2011). Structure and Property of Porous Polyvinylalcohol Hydrogels for Microorganism Immobilization. *Journal of Polym Environ*. 19:398-404.

Mazumdar, N., Chikindas, M.L., & Uhrich, K. (2010). Slow Release Polymer–Iodine Tablets for Disinfection of Untreated Surface Water. *Journal of Appl Polym Sci*. 117: 329–334.

Miyamoto, H., Yamane, C., Seguchi1, M., & Okajima, K. (2011). Comparison between Cellulose Blend Films Prepared from Aqueous Sodium Hydroxide Solution and Edible Films of Biopolymers with Possible Application for New Food Materials. *Journal of Food Sci. Technol. Res*. 17 (1), 21 – 30.

Mishra, R., Datt, M., & Banthia, A. (2008). Synthesis and Characterization of Pectin/PVP Hydrogel Membranes for Drug Delivery System. *Journal of Pharm. Sci. Tech*.,9: 395-403.

Misic, Z., Muffler, K.., Sydow, G., & Kuentz, M. (2012). Novel Starch-Based PVA Thermoplastic Capsules for Hydrophilic Lipid-Based Formulations. *Journal of Pharm Sci*.101:4516–4528.

Narayana. P., Singh, H., &Vasudevan, P. (2006). Iodine based water disinfection: A review. *Journal of Scientific &industrial Research*. 65,116-120.

Nho, Ch.Y., Lim, M.Y., Gwon, J.H., & Choi, K-E. (2009). Preparation and Characterization of PVA/PVP/glycerin/antibacterial Agent Hydrogels Using γ-Irradiation Followed by Freeze-thawing. *Journal of Chem. Eng.* 26(6), 1675-1678.

Păduraru, M.O., Ciolacu, D., Darie N.R., & Vasile, C. (2012). Synthesis and Characterization of Polyvinyl Alcohol/cellulose Cryogels and their Testing as Carriers for a Bioactive Component. *Journal of Materials Science and Engineering*. C 32 , 2508–2515.

Prasad, P., Guru, G.S., Shivakumar, H.R., & Sheshappa, K. (2008). Miscibility, Thermal, and Mechanical Studies of Hydroxypropyl Methylcellulose/Pullulan Blends. *Journal of Appl Polym Sci.* 110: 444–452.

Prasad, P., Guru, S.G., Shivakumar, R.H., & Rai, S.K. (2012). Investigation on Miscibility of Sodium Alginate/Pullulan Blends. *Journal of Polym.Environ.* 20:887-893.

Punyani, S., Narayana.P,Singh.H &Vasudevan.P.(2006)Iodine based water disinfection: A review. *Journal of Scientific &industrial Research*.65,116-120.

Punyani, S., Narayanan, P., Vasudevan, P., & Singh, H. (2007). Sustained Release of Iodine from a Polymeric Hydrogel Device for Water Disinfection Inc. *Journal of Appl Polym Sci.* 103: 3334–3340.

Ragab, M.H. (2011). Spectroscopic Investigations and Electrical Properties of PVA/PVP blend Flled with Different Concentrations of Nickel Chloride. *Journal of Physical*. B 406 ,3759–3767.

Rinaudo, M. (2008). Review Main Properties and Current Applications of Some Polysaccharides as Biomaterials. *Journal of Polym Int.* 57:397–430.

Roy, N., Saha, N., Kitano, T., & Lehocky, M. (2012). Significant Characteristics of Medical-Grade Polymer Sheets and their Efficiency in Protecting Hydrogel Wound Dressings: A Soft Polymeric Biomaterial. *Journal of Polymeric Materials*. 61, 1, 72-88.

Saxena, K.S. (2004). Poly vinyl Alcohol. Chemical and Technical Assessment. Fio.

Selvaggi, G., Monstre, Y.S., Van Landuyt, K., Hamdi, M., & Blondeel, Ph. (2003). The Role of Iodine in Antisepsis and Wound Management: A Reappraisal Review article. *Journal of Acta chir belg*. 103, 241-247.

Simmons, J.T., Lee, H.S., Park, J.T., Hashim, P.D., Ajayan, M.D., & Linhardt, J.R. (2009) .Antiseptic Single Wall Carbon Nanotube Bandages. *Journal of Food and Drug Carbon*. 47 ,1561-1564.

Siviaiah, K., Rudramadevi, H.B., Buddhudu, S., Kumar, B.G., & Varadarajulu, A. (2010). Structural, Thermal and Optical Properties of Cu2+and Co2+:PVP polymer films. *Indian Journal of Pure &Applied physics*.48,658-662.

Shingel, I.K. (2004). Current Knowledge on Biosynthesis, Biological Activity, and Chemical Modification of the Exopolysaccharide, Pullulan. *Carbohydrate Research.* 339, 447–46.

Sharma, R.K. (2012). *Polymer Thermodynamics: Blends, Copolymers and Reversible Polymerization*; Printed in the United States of America. By Taylor and francis Group.

Silva, S.D. B., Ulhoa J.C., Batista, A.K., Di Medeiros, C.M., Filho , S.R.R., Yamashita, F., Kátia, F., & Fernandes, F.K. (2012). Biodegradable and Bioactive CGP/PVA Film for Fungal Growth Inhibition. *Journal of CarbohydratePolymer.* 89 , 964–970.

Souguir, Z., Roudesli, S., About-Jaudet, E., Le, Cerf. D., & Picton, L. (2007). Synthesis and Physicochemical Characterization of a Novel Ampholytic Pullulan Derivative with Amphiphilic Behavior in Alkaline Media. *Journal of Colloid and Interface Science.* 313 , 108–116.

Sreekumar, A.P., Al-Harthi, M., & De, S.K. (2012). Effect of Glycerol on Thermal and Mechanical Properties of Polyvinyl Alcohol/Starch Blends. *Journal of Appl Polym Sci*. 123: 135–1.

Tashiro, T. (2001). Antibacterial and Bacterium Adsorbing Macromolecules. *Journal of Macromol. Mater. Eng.*, 286, 63–87.

Temiz, A., Togay, S., Sener, A., Guven, G., Rzaev, O.Z., & Piskin, E. (2006). Antimicrobial Poly (N-vinyl-2-pyrrolidone-alt-maleic anhydride)/Poly (ethylene imine) Macrocomplexes. *Journal of Appl Polym Sci*. 102: 5841–5847.

Teramoto, N., Saitoh, M., Kuroiwa, J., Shibata, M., & Yosomiya, R. (2001). Morphology and Mechanical Properties ofPullulan/Poly (vinyl alcohol) Blends Crosslinked with Glyoxal. *Journal of Appl Polym Sci*. 82: 2273–2280.

Thomsen, B.L., Lichota, J., Kim, S.K., & Moos, T. (2011). Gene Delivery by Pullulan Derivatives in Brain Capillary Endothelial Cells for Protein Secretion. *Journal of Controlled Release.* 151, 45—50.

Thomas, P. (2011). Pullulan Production by Aureobasidium Pullulans Cells Immobilized in Chitosan Beads. *Journal of Folia Microbiol.* 56:335–338.

Tripathi, S., Mehrotra, K.G., & Dutta, K.P. (2009). Physicochemical and Bioactivity of Cross-linked Chitosan–PVA Film for Food Packaging. *Journal of International Journal of Biological Macromolecules.* 45 , 372–376.

Waschinski, J.C., Zimmermann, J., Hutzler, R.S.U., Sadowski, G., Joerg, C., & Tiller, C.J. (2008).Design of Contact-Active Antimicrobial Acrylate-Based Materials Using Biocidal Macromers. *Journal of Adv. Mater*. 20, 104–108.

Wang, L.B., Liu, Sh, X., Ying, J.Y., Ren, K-F., & Jian, J. (2012). Fast and Long-Acting Antibacterial Properties of Chitosan-Ag/ polyvinylpyrrolidone Nanocomposite Films. *Journal of Carbohydrate Polymers*. 90, 8–15.

White, G.D., & McDermott, F.P. (2001). Biocides, Drug Resistance and Microbial Evolution. *Journal of Current Opinion in Microbiology*. 4,3,13–317

Williams, F.D. (2008). Leading Opinion on the Mechanisms of Biocompatibility. *Journal of Biomaterials*. 29, 2941–2953.

Yalinca, Z. (2013). *Ph.D Thesis Biomedical Applications of Modified Chitosans*.Eastern Mediterranean University.

Yu, L., Dean, K., & Li, L. (2006). Polymer Blends and Composites from Renewable Resources. *Journal of Prog. Polym. Sci*. 31, 576–602.

Yin, Q., Luo, J-H., Zhou, G., Yin, Q-J., & Jiang, B. (2010). A molecular Simulation of the Compatibility of Chitosan and Poly (vinyl pyrrolidone). *Journal of Molecular Simulation*.36,3, 186–191.