IMMOBILIZATION OF BOVINE SERUM ALBUMIN ON CHITOSAN/ PVA FILM: PHYSICAL AND MECHANICAL PROPERTIES INVESTIGATION

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FACULTY OF ENGINEERING
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2019
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DISSERTATION SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF ENGINEERING SCIENCE

FACULTY OF ENGINEERING UNIVERSITY OF MALAYA KUALA LUMPUR

2019
UNIVERSITY OF MALAYA
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Matric No: KGA 140031
Name of Degree: Master of Engineering Science
Title of Dissertation/Thesis:
IMMOBILIZATION OF BOVINE SERUM ALBUMIN ON CHITOSAN/PVA: PHYSICAL AND MECHANICAL PROPERTIES INVESTIGATION
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PHYSICAL AND MECHANICAL PROPERTIES INVESTIGATION

ABSTRACT

Chitosan/ polyvinyl alcohol (Ch/PVA) blended film was prepared by direct blend process and solution casting methods. In order to reduce the swelling ratio and enhance the chemical and mechanical stability, Ch/PVA film was crosslinked with glutaraldehyde in order to produce Ch-g-PVA. Bovine serum albumin (BSA) was used as a model protein to incorporate into the Ch-g-PVA. The chemical structure and morphological characteristics of films were studied by FT-IR and Field-emission scanning electron microscopy (FESEM). Mechanical and physical properties of blended films such as tensile properties in the dry and wet states, water uptake, and water contact angle measurement were characterized. Blending PVA and chitosan improved strength and flexibility of the films. Crosslinking with glutaraldehyde further improves the tensile strength and decrease the hydrophilicity of films. BSA immobilized on the Ch-g-PVA film was calculated as BSA encapsulation efficiency. All results indicated that chemical modification with glutaraldehyde and BSA turns films more hydrophobic

Keywords: Polyvinyl Alcohol, Chitosan, Glutaraldehyde and Bovine Serum Albumin
IMOBILISASI SERUM BOVINE ALBUMIN KE ATAS FILEM CHITOSAN/PVA: PENYIASATAN KE ATAS SIFAT FIZIKAL DAN MEKANIikal

ABSTRAK

Chitosan / Polivinil alkohol (Ch / PVA) filem telah dihasilkan melalui proses pencampuran dan teknik pembentukan larutan. Dalam usaha untuk mengurangkan nisbah pembengkakan dan meningkatkan kestabilan mekanikal dan kimia, Ch / PVA filem telah ditaut silang dengan menggunakan glutaraldehid untuk menghasilkan Ch-g-PVA. Serum bovine albumin (SBA) telah digunakan sebagai model protein untuk dicampurkan ke dalam Ch-g-PVA. Struktur kimia dan ciri-ciri morfologi filem telah dikaji dengan menggunakan FT-IR dan mikroskop elektron pengimbas (FESEM). Sifat-sifat mekanikal dan fizikal filem seperti sifat-sifat tegangan filem dalam keadaan kering dan basah, kadar pengambilan air, dan pengukuran sudut sentuhan air dicirikan. Pengadunan PVA dan chitosan memperbaiki kekuatan dan fleksibiliti filem. Proses taut silang dengan glutaraldehid meningkatkan lagi kekuatan tegangan dan mengurangkan sifat hidrofilik filem. Immobilisasi SBA pada filem Ch-g-PVA di nilai sebagai kecekapan pengkapsulan SBA. Hasil kajian menunjukkan bahawa pengubahsuaian kimia dengan glutaraldehid dan SBA menjadikan filem lebih bersifat hidrofobik.

Kata Kunci: Polivinil Alkohol, Chitosan, Glutaraldehid, Serum Bovine Albumin
ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my supervisors, Associate Professor Dr Amalina Binti Muhammad Afifi for her continuous assistance, supervision, guidance and encouragement from the first day till the end of my Master degree. I would also like to extend my thank you to co-supervisor, Dr Nur Awanis Binti Hashim for your guidance, advice and giving me access to use research facilities at Department of Chemical Engineering, Universiti Malaya.

My sincere thanks also to Postdoctoral of Mechanical Engineering, Dr Katayoon Kalantari for the insightful comments and encouragements. Without your help, it will not be a smooth journey in completing my thesis.

I would also like to thank my fellow friends and technicians in my research groups for kind assistance at laboratory. Without their precious support, it will not be possible to conduct this research.

My deepest gratitude to my husband and children for their understandings and enormous supports. I wish to express my deepest appreciation to my loving parents for their blessing and encouragement. Without their blessings and prayer, this work will not have been successful and completed.

Finally, this research would not be possible without financial from University of Malaya Research Grant (RP034A-15AET) and Fundamental Research Grant Scheme (FRGS-FP0262014B). Thank you very much for all the contribution.
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LIST OF SYMBOLS AND ABBREVIATIONS

Ch : Chitosan
PVA : Poly-vinyl alcohol
BSA : Bovine Serum Albumin
NH$_2$ : Amine
OH : Hydroxyl
FTIR : Fourier Transform Infrared
FESEM : Field-emission scanning electron microscopy
Ch/PVA : Blending of chitosan and PVA
Ch-g-PVA : Crosslinked of chitosan and PVA
BSA/Ch-g-PVA : Immobilized BSA on crosslinked of chitosan and PVA
H-C=O : Aldehyde
Cr$^{6+}$ : Chromium
Zn$^{2+}$ : Zinc
Cu$^{2+}$ : Cooper
Pb$^{2+}$ : Plumbum
Cd$^2$ : Cadmium
CHAPTER 1: INTRODUCTION

1.1 Research Background

Water produced by different domestic and industrial activities is known as wastewater. It contains various inorganic, organic and biological contaminants that are of environmental significance. These contaminants can create health hazards if discharged into streams or oceans without proper care and treatment. It is very important to develop and design a new type of water treatment system that is more efficient and environmentally friendly in terms of fabrication and application. Chitosan is widely used as biomaterials film, this is mainly due to the biocompatibility characteristics and membrane permeability (P Na Nakorn, 2017). Therefore, chitosan is selected to be the materials used in this research to produce biomaterials film. Chitosan is a natural polysaccharide formed during the deacetylation of chitin in alkaline condition. It is a low-cost material that can be extracted from crustacean shells, which are waste from seafood industries. It consists of two attractive functional groups, amine (-NH$_2$) and hydroxyl (-OH) groups. Thereby, chitosan has been used in many applications including waste water treatment. Chitosan also has good film forming property, high mechanical strength and chemical resistance making it a promising material (A. Svang-Ariyaskul et al., 2006).

Even though chitosan has many good properties due to its functional groups, further treatments are usually done to improve the chemical and mechanical resistance. Various improvement had been made to modify the chitosan properties, this include crosslinking with crosslinking agent, chemical modification and blending (El-Hefian, Nasef, & Yahaya, 2010). Poly (vinyl alcohol), PVA, is a non-toxic, water soluble synthetic polymer, has good chemical stability and film forming ability. PVA is a hydrophilic material with a large number of hydroxyl groups which allows it to react with many types
of functional groups. This strong point makes PVA widely used for biomaterials application. By adding PVA to chitosan, it improves film forming ability and mechanical properties of chitosan (Danwanichakul & Sirikhajornnam, 2013).

In addition, previous work explained the chemical modification by using glutaraldehyde as a crosslinking agent (Vieira & Beppu, 2005). Glutaraldehyde is an effective crosslinking agent for chitosan membranes. To hinder amino groups with crosslink polymeric structure and make chitosan as hydrophobic materials, it is recommended to used glutaraldehyde as crosslinking agent. Glutaraldehyde is a 5-carbon molecule with two aldehyde functional groups (H-C=O) which are highly reactive towards amines. Glutaraldehyde is a clear, colourless, pungent oily liquid that is soluble in water and alcohol. It is widely used in protein immobilization and crosslinking through amino groups. Amines (-NH₂ or –NH₃⁺) are commonly found on the surface of microbial cells and proteins. When glutaraldehyde contact with these biological entities it will chemically modifies and crosslink them (Migneault, Dartiguenave, Bertrand, & Waldron, 2004). In this work, Bovine serum albumin (BSA) was used as a model protein to incorporate into the chitosan/PVA blend. To eliminate and purify the water system from biomolecules such as proteins, immobilization of protein on a membrane film had been conducted. There are several reasons for using protein in an immobilized form. It provides convenient during handling and helps to prevent contamination of the substrate with enzyme/protein or other compounds, which decreases purification costs. There are variety of supports that have been used for enzymes or protein immobilization such as synthetic organic polymers, biopolymers, hydrogels, smart polymers and inorganic supports (Katchalski-Katzir & Kraemer, 2000).
Our aim in this work is to produce a BSA functionalized chitosan /PVA blend with good mechanical properties. Many types of water purification systems are inefficient, difficult to handle and not able to be recycle. Immobilization of naturally available protein such as Bovine Serum Albumin may add functions to the chitosan /PVA blend, thus some enzymes has been studied for the degradation of dyes, protein capture and bacteria killing (Homaeigohar, Dai, & Elbahri, 2013). Separation and sieving of biomolecule pollutants from waste water streams could be done with continuous research and study and hoping for new and effective water purification system will be developed with capability to treat contaminated water.
1.2 Problem Statement

Micro, -ultra and nanofiltration, are among the types of membrane that were normally used to remediation of waste water. However, the principal of separation of these membranes were based on the sieving mechanism whereby the porosity of the membrane could play an important role in order to separate molecules in the contaminated water.

In this study, immobilization of enzymes, BSA protein on the Ch-g-PVA had been conducted. Rather than operated by sieving mechanism, this method could be the alternative way to capture molecules and separate biological pollutant in waste water, thus developing a new water purification system. The conventional method of using enzymes as chemical catalyst, will dissolve in water and contaminate the products in the catalysis system. Therefore, to overcome the limitations, one of the best method is to immobilize enzyme, by fixing the BSA onto the Ch-g-PVA film.
1.3 Objectives of Study

1) To fabricate chitosan and PVA (Ch/PVA) films and crosslinked Ch/PVA films.
2) To immobilize Bovine Serum Albumin in crosslinked Ch/PVA films.
3) To characterize physical and mechanical properties of immobilized enzyme Bovine Serum Albumin in Ch/PVA films.

1.4 Scope of Research

In this research study, Chitosan/ Polyvinyl Alcohol (Ch/PVA) films were prepared by direct blend process and solution casting methods with respective polymer solutions at different ratio. In order to fabricate the Ch/PVA films incorporating with crosslinker and BSA protein, optimization on the polymer blends had been made. Films were then crosslinked with 50wt% of glutaraldehyde and proceed with immobilization of BSA protein to produce BSA/Ch-g-PVA.

Evaluation on the immobilized crosslink film, BSA/CH-g-PVA is to determine the BSA encapsulation efficiency and the adsorption study of protein on the BSA/Ch-g-PVA films. Physical and morphological properties of the films were studied by FT-IR and Field-emission scanning electron microscopy (FESEM). Mechanical and physical properties of blended films such as tensile properties in the dry and wet states, water uptake, and water contact angle measurement were characterized.
1.5 Dissertation Overview

This dissertation is segmented into five chapters. In chapter 1, a compilation of research background, problem statement, objectives of study and scope of research were discussed briefly. Chapter 2 serves as foundation of the study and discussed the existing literature topics that related to this study. These topics include discussing on chitosan and PVA as polymeric materials, applications of chitosan, PVA and chitosan as blended films, glutaraldehyde as crosslinking agent in water treatment, immobilized protein onto films, functionalized biopolymers membrane film in wastewater treatment, membrane adsorption and adsorption technology in water treatment. Chapter 3 presented the research methodology of study. It described the sufficient details for readers on how the study will be conducted and know precisely what procedures to follow. This chapter includes research flowchart, chemicals and reagents, film preparation, and film characterization. Chapter 4 of dissertation explains the results by interpreting the data and discussed the obtain findings and compared to previous study which are similar to current study. In this final chapter, Chapter 5 discussed the implications of the study findings and recommends the possibilities or improvement for future works in this field.
CHAPTER 2: LITERATURE REVIEW

2.1 Adsorption Technology in Water Treatment

Concerns on water contaminations have became a critical and serious issue as it affects our lives. The contamination mainly caused by the improper disposal of chemicals and waste into the mainstream water resources. It has been reported that more than seven hundred organic and inorganic pollutants that are highly toxic and carcinogenic could affect the microbial populations. To provide safe drinking water, both chemicals and bacteriological contaminants that are highly toxic and carcinogenic need to be addressed. Several methods and technologies have been developed to minimize the waste in order to obtain a safe drinking water for daily used (Ali & Aboul-Enein, 2006).

A wide range of water purification and recycling methods have been used such as reverse osmosis, ion exchange, electrodialysis, electrolysis and adsorption. Among these methods, the cost of water treated by adsorption varies from 10-200 US dollar per million liters compared to other methods that cost nearly to 450 US dollar per million liters. Due to the effectiveness and economical method, low cost adsorbents with high adsorption properties had been widely used in removing heavy metals in waste water (Ali & Gupta, 2006). In adsorption methods, several of materials were used as adsorbents, it can be classified into natural and synthetic adsorbents. Natural adsorbents include activated carbon, charcoal, clay minerals, zeolites and biopolymers. While synthetic adsorbents were prepared from the agricultural and industrial waste. Numerous studies had been developed for cheaper and effective adsorbents that contains natural polymers which are able to remove pollutants from contaminated water. Among these were chitin, chitosan and starch (Rashed, 2013).
Previous study reported, chitosan that extracted from the crab shells were used as low cost adsorbent for electroplating waste water treatment. The functional groups of –OH and –NH₂ in chitosan, may encourage the adsorbent function resulting to the strong adsorption capacity of heavy metals. Chitosan has the ability to adsorb heavy metals ion such as Cr⁶⁺, Zn²⁺, Cu²⁺, Pb²⁺, Cd²⁺, it is reported that the maximum adsorption of heavy metals occurred at a pH range between 5 to 7. Chitosan works as an economically useful adsorbent, eco-friendly and effective, therefore making a suitable adsorbent for the waste water treatment (Worch, 2012).
2.2 Membrane Adsorption

Adsorption is a surface phenomenon and phase transfer process in order to remove individual components from a gas or liquid mixture. Adsorption is widely practiced in water treatment, as it is an efficient removal process for a multiplicity of solutes. Pollutants component which contains several of molecules or ions are removed physically or chemically from the aqueous solution by adsorption. The basic terms of adsorption theory shown in Figure 2.1 (Worch, 2012).

![Figure 2.1: Basic terms of adsorption](image)

Compounds that adhere and adsorbed to the solid surface is called an adsorbate. While the solid surface that provides surface for the adsorption to occur is named adsorbent. Adsorbed species can be released from the surface and into the liquid phase depending on the properties changing such as concentration of pollutants, particle size of the adsorbents, contact time, the nature of the adsorbate and adsorbent, atmospheric, temperature and pH of liquids, thus this reverse process is referred as desorption process (Rashed, 2013).
In water treatment, pre-filtrations are sometimes required. This is due to the presence of suspended particles, oils and greases that may reduce the efficiency of adsorption process. Figure 2.2 illustrate the adsorption treatment of micropollutants in water. Adhesion of micropollutants onto the surface depending on the adsorption modes, either chemical or physical reactions. In physical adsorption, it involved the Van der Walls interaction between surface and adsorbate, while the chemical adsorption occur when a chemical bond (covalent bond) is formed between adsorbate and adsorbent (Ali & Gupta, 2006).

Figure 2.2: Adsorption treatment of micro pollutants in water.
2.3 Chitosan

Chitosan is a natural-based biopolymer derived from chitin. It is the second most abundant polymer after cellulose. Chitosan is a reactive natural biopolymer that consists of reactive amino and hydroxyl group on its backbone. Due to the both functional groups, modification of chitosan into several methods and can be produced into any other types of forms such as powder, gels, films, sponges, beads and nanoparticles fiber. This is used in numerous of application and various fields, including food, pharmaceutical, agricultural and cosmetic sciences (Racovita, Vasiliu, Popa, & Luca, 2009).

Chitosan is produced by derivation of chemical N-deacetylation of chitin. It is a copolymer of glucosamine and N-acetyl glucosamine linked by $\beta 1\rightarrow 4$ glucoside bonds obtained by N-deacetylation of chitin. The chemical structure of chitosan is shown in Figure 2.3 (Chen et al., 2009). Chitosan is biodegradable, biocompatible and exhibits bioadhesive characteristics. Chitosan be able to forms a viscous solution and make it suitable as functional films. Blending with other polymers and biodegradable materials are promising way to improve chitosan properties and extend its application.

Several researchers modify chitosan film with natural resources, for instance using chitosan reinforcing cellulose coconut fibers will improved the moisture resistance and enhanced the tensile strength of the chitosan film (S. Bhuvaneswari, D.Sruthi, V. Sivasubramanian, Kalyani, & Sugunabai, 2011).
In previous study, Kaminski and Modrzejewska reported that chitosan membranes produced by the phase inversion can be applied in the removing of metal ions for the wastewater pollutants (W. Kaminski & Z. Modrzejewska, 1997). Chitosan is widely used for the application of chitosan membranes for removal of heavy metal ions. This is due to the excellence properties of chitosan molecules with the existence of function groups which are –OH and –NH$_2$. 

**Figure 2.3: Chemical structure of chitosan.**
2.3.1 Applications of Chitosan

Chitosan has a wide range of applications. Due to its physical and chemical properties, chitosan is being used widely in different products and applications. Variation on degree of acetylation and molecular weight offers different properties of chitosan. Chitosan helps in solving numerous problems in industrial and biomedical applications. For industrial application, chitosan is normally used for cosmetics, water engineering, paper industry, textile industry, food processing, agriculture, photography, chromatographic separations, solid state batteries and chitosan gel for LED applications (Dutta, Dutta, & Tripathi, 2004). In biomedical application, chitosan is widely used in tissue engineering, wound healing, burn treatment, artificial skin, drug delivery system and ophthalmology. Table 2.1 summarize the main application and principal used of chitosan.

Table 2.1: Potential application for chitosan

<table>
<thead>
<tr>
<th>Potential Application</th>
<th>Principal Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomedical</td>
<td>• Biocompatible, biodegradable for dental implants</td>
</tr>
<tr>
<td></td>
<td>• Renewable for artificial skin</td>
</tr>
<tr>
<td></td>
<td>• Film forming in rebuilding of bones</td>
</tr>
<tr>
<td></td>
<td>• Hydrating agent for corneal contact lenses application</td>
</tr>
<tr>
<td></td>
<td>• Non-toxic</td>
</tr>
<tr>
<td></td>
<td>• Wound healing properties</td>
</tr>
<tr>
<td></td>
<td>• Efficient against bacteria, viruses and fungi</td>
</tr>
<tr>
<td>Agriculture</td>
<td>• Defensive mechanism in plants</td>
</tr>
<tr>
<td></td>
<td>• Stimulation of plant growth</td>
</tr>
<tr>
<td></td>
<td>• Seed coating</td>
</tr>
<tr>
<td>Water and waste treatment</td>
<td>• Removal of metal ions</td>
</tr>
<tr>
<td></td>
<td>• Ecological polymer</td>
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<tr>
<td></td>
<td></td>
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<td>-------------------------</td>
<td>--------------------------------</td>
</tr>
<tr>
<td><strong>Food and beverages</strong></td>
<td>• Reduce odors</td>
</tr>
<tr>
<td></td>
<td>• Flocculants to clarify water</td>
</tr>
<tr>
<td></td>
<td>• Not digestible by human</td>
</tr>
<tr>
<td></td>
<td>• Thickener and stabilizer for sauces</td>
</tr>
<tr>
<td></td>
<td>• Protective, fungistatic, antibacterial coating for fruit.</td>
</tr>
<tr>
<td><strong>Cosmetics and toiletries</strong></td>
<td>• Maintain skin moisture</td>
</tr>
<tr>
<td></td>
<td>• Treat acne</td>
</tr>
<tr>
<td></td>
<td>• Reduce static electricity in hair</td>
</tr>
<tr>
<td><strong>Biopharmaceutics</strong></td>
<td>• Healing</td>
</tr>
<tr>
<td></td>
<td>• Anticoagulant</td>
</tr>
</tbody>
</table>
2.3.2 Chitosan in Waste Water Treatment Application

As environmental protection is becoming the global issue, it becomes major concern by the relevant industries to develop a technology which does not cause environmental problem. Due to its polycationic nature, chitosan can act as flocculating agent. It functions as chelating agent and heavy metals trapper. From previous study, researchers discovered that chitosan based biosorbents can be used as biosorption to purify heavy metal polluted wastewater. Chitosan has ability to capture metal cations or metal anions through chelation or electrostatic interactions. Besides, chitosan can be easily modified by physical or chemical methods to fabricate desirable biosorbents with good sorption capacity and selectivity for the target metals. It is essential to create novel chitosan-based biosorbents for metal and recovery application, thus targeting the potential commercialization and exploring biosorption mechanisms (J. L. Wang & Chen, 2014).

Chitosan is one of abundant and low cost biopolymers that exhibits good properties that make it ideal as adsorbent for removing pollutants from wastewater. Contaminated water that may threatens human and other living health, in resulted from the various inorganic and organic waste that produced by human activities. Discharges of colored and chemical substances into water from textile, printing, food and leather industry are major industrial wastewater sources. Vakili et al., studied the modification of chitosan so that it can be more suitable for adsorption of different types of dye. They reported the production of nano-chitosan for dye removal as a new approach in solving the pollutants (Vakili et al., 2014).
2.4 Polyvinyl Alcohol (PVA)

PVA is essentially prepared from polyvinyl acetate through hydrolysis. The molecular weights and grades for PVA products may vary depending on the percentage of hydrolysis to eliminate the acetate group. Figure 2.4 shows the structure of PVA (Baker, Walsh, Schwartz, & Boyan, 2012). Hydrolysis levels vary from value of 80% to more than 99%. Crosslinking of the linear polymers form PVA hydrogels, which resulting in polymer (gel)-fluid (sold) species with tunable properties. Polymer contents may affect the physical properties of the materials. Higher polymer content significantly stiffens and strengthens the polymer matrix, while low polymer content exhibits a soft and flexible materials as the fluid moves freely through the polymer matrix (Gaaz et al., 2015).

Figure 2.4: The chemical structure of PVA
PVA is a thermoplastic and biocompatible polymer. It is widely used as blend with various biopolymers in order to improve mechanical properties of films. With the presence of the hydroxyl (-OH) groups, PVA exhibits hydrophilic behaviors and it is soluble in water. PVA membrane had been extensively developed for biomedical application, this is due to the similarity and compatible properties with human tissues. It has no toxic effects, good adhesion and has structure that can adsorb protein molecules (Kenawy, Kamoun, Eldin, & El-Meligy, 2014). PVA make them suitable candidates for biomaterials and useful for membrane applications as it shows several advantages. These includes their biocompatibility, bio-adhesive characteristics, excellent film forming ability, non-toxic, non-carcinogenic and ease of processing (Limpan, Prodpran, Benjakul, & Prasarpran, 2012)
2.4.1 Poly (vinyl alcohol) (PVA) in Waste Water Treatment Application

Wastewater produced from various industrial sources need to be treated before discharge to a sewage system. Membrane separation is one of the method to treat the wastewater. However due to some limitations, fouling can affect both permeate quality and operating cost, some researchers had work on the surface modification of membranes by increasing the hydrophilicity of membrane surface. As regards to overcome the limitation, polyvinyl alcohol (PVA) is highly recommended material as it has good hydrophilicity and most frequently used in membrane applications. PVA is a biocompatible and non-toxic polymer. It has excellent film forming ability, good mechanical strength and low fouling potential, thus it is widely used in producing ultrafiltration and nanofiltration membrane. (C. Y. Tang, Kwon, & Leckie, 2009).

Wu et al., has produced an ultrafiltration membrane by crosslinking PVA to a mixed cellulose ester, in order to investigate the effectiveness of PVA as ultrafiltration membrane and its anti-fouling properties in treating synthetic oily water in waste water. It is found that PVA membrane has excellent anti fouling characteristics to oil (Wu et al., 2008). An et al., found that introducing small amount of PVA improve the anti-fouling and nano filtration performance of polyamide membrane. It also improves the hydrophilicity of nanofiltration membrane by allowing the water transportation and accessible through the interfacial polymerization layer hence increasing the flux of membrane (An, Li, Ji, & Chen, 2011). Du et al., has modified a commercial poly (vinylidene fluoride) flat sheet membrane with a dilute PVA aqueous solution followed by solid-vapor interfacial crosslinking. As a result, the PVA modified membrane shows a good potential compared to the unmodified membrane during water filtration due to a higher flux, flux stability and ease of cleaning (Du, Peldszus, Huck, & Feng, 2009).
2.4.2 Poly (vinyl alcohol) (PVA) and Chitosan as Blended Films

Blends of synthetic and natural polymers represent a new class of material and have attracted attention especially in application as biomaterials. Polymer blending is one of the attractive method to modified polymer in order to produce a new material with improvised properties. Synthetic polymers offer good mechanical properties and can be transformed into different shapes with low production costs. Natural polymers have good biocompatibility, but their mechanical properties are often poor. In this research, chitosan is a natural polymer that has many good properties due its functional groups, but a chitosan membrane has poor mechanical properties. It is difficult to preserve the biological properties of natural polymers as it will increase the production cost and affected the ease of manufacturing (Bahrami, Kordestani, Mirzadeh, & Mansouri, 2003). Therefore, this could be improved by incorporating another polymer such as PVA with chitosan. PVA is a non-toxic and water soluble polymer, it exhibits good film properties, chemically stable, and shows high hydrophilicity. Large number of hydroxyl groups will make it accessible to react with many types of functional groups hence suitable for biocompatible materials (El-Hefian et al., 2010). However, there was a report on poor miscibility between PVA and chitosan (Chuang, Young, Yao, & Chiu, 1999). To solve this problem, crosslinking of PVA and chitosan with glutaraldehyde was found to enhance the properties of PVA and chitosan membrane. In previous study, blending chitosan with PVA improves the tensile strength and flexibility of films both in dry and wet states. Hence, the addition of crosslinking with glutaraldehyde have increase the tensile strength, water contact angle and improves the surface hydrophilicity of the blended films (Bahrami et al., 2003).
2.5 **Glutaraldehyde as crosslinking agent in water treatment**

Glutaraldehyde consist of linear 5-carbon dialdehyde functional groups (represented as H-C=O), that are highly reactive toward amines group. Glutaraldehyde is a colorless and clear oily pungent liquid. Some people may have questioned whether glutaraldehyde is safe to be used in water treatment applications or not. Chemical and toxicological properties of formaldehyde and glutaraldehyde are significantly different, some people easily confused as it shares the same chemical family name ‘aldehyde’ and assume that glutaraldehyde is hazardous and unsafe for the environment. It will not degrade into formaldehyde. In membrane applications, glutaraldehyde is widely used as a crosslinking agent. Membranes or films that were prepared with chitosan posed a lack of mechanical stability due to excessive swelling in aqueous solution. PVA also known as favourable membrane material that extensively used in membrane separation technology. However, due to the poor stability of PVA, there are numerous studies had been conducted in order to improve the stability of the PVA membranes. Several researchers had reported on the methods how to modify the polymer network by physical and chemical treatments. Physical treatments by crosslinking the polymer with the UV and γ-irradiation (J. M. Yang, Chiang, Wang, & Yang, 2009), whereas chemical treatments included crosslinking with glutaraldehyde, formaldehyde, sulfur-succinic acid and polycarboxylic acid (Bolto, Tran, Hoang, & Xie, 2009). Due to its low cost, low toxicity, and good reactivity, glutaraldehyde are the preferred crosslinking agent that normally used in membrane applications (Krumova, Lopez, Benavente, Mijangos, & Perena, 2000).
During the crosslink of PVA and glutaraldehyde, the hydrogen bonds in crosslinked PVA becomes weaker as most of the OH groups had transformed to acetal linkages. Therefore, the active sites for sorption has taken in the acetal bridge and less hydroxyl groups in the polymer chains that are accessible for bonding with water molecules. This will cause the polymer membrane become less hydrophilicity. Figure 2.5 shows the PVA/glutaraldehyde and chitosan/glutaraldehyde mechanism respectively (Ceylan, Göktürk, & Bölgen, 2016).

![Figure 2.5: Crosslinking of PVA and chitosan with glutaraldehyde](image)

In previous study, some researches had observed the effect of glutaraldehyde on the membrane properties, morphology and permeability. It had been reported that pore size of the membrane had significantly affect the hydrophilicity of the films, by crosslinking the PVA with glutaraldehyde (Ahmad, Yusuf, & Ooi, 2012). In order to maintain the stability of membrane and at the same time maintain the high permeation flux, some
studies had reported adding some pore former to increase the permeation flux (Mohammadi & Saljoughi, 2009). High degree of crosslinking material will produce a physical barrier for water molecules to penetrate into the membrane, thus will results in lower permeation flux.

Previous study also reported on the effect of crosslinking of chitosan membrane on ion permeability and water absorption study. Crosslinking with glutaraldehyde exhibits more hydrophobic structures in membrane, whereby most of the reactive amino groups were hindered by dialdehyde which may attribute to the changes on the mechanics characteristics. The hydrophobic characteristics which then lead to disturbance and affected the interactions of water molecules and ions (M.M. Beppu, R.S. Vieira, C.G. Aimoli, & Santana, 2007).
2.6 Bovine Serum Albumin

Bovine Serum Albumin is also known as BSA. It is the most abundant protein that derived from cows. Due to its high stability, high purity and solubility, BSA is suitable for adsorption studies. BSA adsorptions are affected by the pH solutions as the isoelectric point of BSA is at pH 4.5-5.0. Therefore, BSA exhibits negative charge at neutral pH and positive charge under acidic conditions. It has very good interaction with wide range of materials including metals such as Cu$^{2+}$ and Zn$^{2+}$, fatty acids, amino acids, and many drug compounds. BSA that adsorbs to variety of surface can be measured by spectrometric measurements, calorimetric estimation and spectroscopic technique such as NMR, FTIR-spectroscopy, fluorescence and circular dichroism (Phan, Bartelt-Hunt, Rodenhausen, Schubert, & Bartz, 2015). BSA was used in this study as it can easily be obtained and cheap compared to any other proteins. BSA often used as protein concentration standard before measuring protein concentration for unknown samples.

Riyasudheen et al., investigated the properties of BSA immobilized and asymmetrically cross-linked polyvinyl alcohol (PVA) membrane with glutaraldehyde (GA) towards water sorption, dye release and protein adsorption. It is found it is found that grafting of BSA on the membrane is effective and protein adsorption decreases with increasing of BSA content (Riyasudheen, Binsy, Aswini, Jayadevan, & Athiyanathil, 2012). BSA also used as physiological carrier for various compounds including drugs. Tada et al., developed hydrogels consisting of acrylamide (AAm) and bovine serum albumin (BSA) by introducing three to four vinyl groups into one BSA molecule, thus this hydrogel produced are useful for drug release carrier for albumin binding substances (Tada, Tanabe, Tachibana, & Yamauchi, 2005).
Different types of protein may have resulted differently during protein-adsorbent interactions. Torres et al., used BSA and lysozyme as adsorbates in order to investigate modified chitosan microsphere as adsorbents. Adsorption protein studies may be complex due to its complex macromolecules characterized by polar, hydrophobicity and charged areas. Proteins and adsorbent changes are strongly depending on the pH of solution. BSA and lysozyme have different value of isoelectric point. Therefore, the adsorption rate of BSA and lysozyme are different on chitosan. The adsorption of BSA was slower on chitosan as its reached equilibrium at about 10 h while for lysozyme at about 7h (Torres, Beppu, & Santana, 2007).
2.7 Protein Immobilization onto PVA/Chitosan Films

Natural polymers are widely used as immobilization matrixes for cell carriers, living organisms and proteins. Recently, there has been increasing interest in using natural polymers for example chitosan, alginate, collagen, carrageenan, gelatin, cellulose, starch, and pectin as supports (Huang, Hu, Zeng, & Zhou, 2002). Besides natural polymers, synthetic polymers and inorganic materials are also being used as supports.

Enzyme immobilization is one of the method to overcome the drawbacks of the enzyme instability, it is limited even under the optimal conditions. The purpose of immobilization is to maximize the efficiency and lifetime of catalysts or enzymes.

In this research, PVA/ chitosan membrane has been used as supports for immobilization with protein. Chitosan a good potential in biocompatibility characteristics and good membrane permeability. It is most promising immobilization matrices due to an excellent membrane forming ability, low cost, non-toxicity and good adhesion (Pariya Na Nakorn, 2008) (Colonna et al., 2008). (K. Yang, Ning-Shou Xu, & Su, 2010) proved that chitosan reactive amino and hydroxyl group offers a good enzyme coupling efficiency. Presence of amine groups in chitosan make it suitable and beneficial as supports in immobilization of various enzymes. Previous study reported that chitosan could improve and increase the resistance to bacteria, chemical degradation and has resistance of disturbing of metal ions to enzyme. These properties have prompted extensive applications of chitosan as matrix for enzyme immobilization (Z.-X. Tang, Qian, & Shi, 2006).
2.7.1 Immobilization Technique

Various methods for the immobilization of proteins have been extensively used in several researchers. It depends on the application, environmental conditions, temperature used, and organic solvents. Enzyme immobilization can be divided into three types (i) physical adsorption, (ii) encapsulation, and (iii) covalent attachment. Adsorption is the simplest method as enzymes is physical adsorbed onto an insoluble support. Organic and inorganic materials are among support materials that used for immobilization technique (Cetinus et al., 2007). Hydrogen bonding, hydrophobic effects and electrostatic forces were the mechanism that occur on the surface interactions between the support matrix and enzymes.

In the other hand, other method is entrapment that is similar with encapsulation method. In this process, enzyme is caging by covalent or non-covalent bonds. Enzymes are restricted by the membrane walls, usually in a form of capsules Normally this technique implies by nanostructured supports like electrospun nanofibers (Datta, Christena, & Rajaram, 2013).

Another methods of enzyme immobilizations are covalent bonding attachment. This is one of the most effective method as it can be preventing enzyme to leaching out. It can be achieved by stability of the bonds between enzyme amino acid residue (-NH₂, -CO₂, -SH) with organic functional groups, as strong bonds will prevent enzyme release to the environment (Sheldon, 2007). Crosslinking of enzymes to electrospun nanofibers improve the residual activity as it may increase the surface area and porosity. Crosslinking agent played important role in this technique. It should maintain the structural, and functional property of enzymes during the process. Glutaraldehyde is the most favorable agent because of its solubility in aqueous solvents and can form stable inter and intra subunit covalent bonds (Datta et al., 2013).
2.8 Functionalized Biopolymers Membrane film in Wastewater Treatment

Biopolymers membrane becomes an important wastewater treatment technology; which facilitates the removal and recovery of pollutants as well as solvents and water. In this research, chitosan and PVA are used as polymer blend to produce polymer membranes. Chitosan from crab shell is a biodegradable polymer and widely used in the environmental technology for wastewater treatment. Chitosan widely used as support for protein immobilization because it shows favourable characteristics such as biocompatibility, non-toxicity, excellent film forming ability, antibacterial, hydrophilicity, and susceptible to chemical modification (Yen, Yang, & Mau, 2009).

Biofunctional agent, Bovine Serum Albumin (BSA) a cheap protein enzyme is used incorporated into crosslinked PVA/chitosan membrane. By using biofunctional agent, it is more efficient in removal of water pollutants and biomolecules such as bacteria, proteins, enzymes and metal nanoparticles (Mady Elbahri et al., 2012). In the previous study, Homaeigohar et al., developed a poly (acrylonitrile-co-glycidyl methacrylate) PANGMA nanofibrous membrane incorporated with protein BSA, it proves that BSA may segregate biomolecules waste before discharge into streams or ocean, as this process usually completed by ultrafiltration membranes at feed pressure and with low water permeability (Homaeigohar et al., 2013).
CHAPTER 3: MATERIALS AND METHODS

3.1 Research Flowchart

Figure 3.1 summarized the research flowchart on how the experiments had been conducted from the films preparation until the physical and mechanical characterization of films. There were 4 stages in this research. The first stage was film casting, in this stage pure chitosan, and blended of chitosan and PVA films are prepared. Films were then crosslink with glutaraldehyde in order to modify the properties of films. Second stage were the preparation of BSA solution. BSA solutions were prepared by diluting in phosphate buffer solution (PBS) at pH 6.8 with concentration of BSA, 1, 3 and 5 and 10 mg/ml. The most important stages were the immobilization of BSA on crosslinked Ch/PVA films. During this stage, some evaluations has been conducted such as protein standard measurement, protein adsorption study and determination of BSA content in BSA/Ch-g-PVA. The final stage of the experiments, to evaluate the physical and mechanical properties of immobilized BSA in Ch-g-PVA films. Some of the evaluations that had been conducted in this research are FTIR, wettability, water uptake measurement, tensile strength and morphological features of films.
Figure 3.1: Research flowchart

**Film casting**

- Preparation of pure chitosan film
- Chitosan and PVA film preparation
- Crosslinking of chitosan/PVA film

**Preparation of BSA solution**

- BSA diluted in phosphate buffer solution (PBS) at pH 6.8 with concentration of BSA, 1, 3 and 5 and 10 mg/ml

**Immobilization of BSA on crosslinked Ch/PVA films**

- Protein standard measurement
- Protein adsorption study
- Determination of BSA content in BSA/Ch-g-PVA

**Physical and mechanical properties of immobilized BSA in Ch-g-PVA films**

- FTIR Spectra of films
- Wettability of films
- Water uptake measurement of films
- Tensile strength of films
- Morphological features of films

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3.2 Chemicals and Reagent

Chitosan (Mw = 8.96×10^5 g/mole, degree of deacetylation (DDA) = 40%) was obtained from SE Chemical Co. Ltd (Kyoto, Japan). PVA was purchased from Sigma Aldrich with molecular weight of 89,000-98,000 and 99% hydrolyzed. Glutaraldehyde solution (50 wt% in water) and Bovine Serum Albumin were purchased from Sigma Aldrich and Merck Chemicals respectively. Acetic acid and Sodium Hydroxide were obtained from R&M Chemicals and Systerm Chemicals respectively. Phosphoric Acid (85%) was purchased from R&M Chemicals. Comassie Brilliant Blue G250 was purchased from Sigma Aldrich.

3.3 Film preparation

4 types of film were produced by casting method and have been characterized, which are chitosan film, Ch/PVA film, Ch/PVA film crosslinked with glutaraldehyde and BSA immobilized Ch/PVA crosslinked with glutaraldehyde film and designated in Table 3.1. Samples pictures were captured and illustrated in Figure 3.1

<table>
<thead>
<tr>
<th>Samples Name</th>
<th>Designation of samples name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chitosan</td>
<td>Ch</td>
</tr>
<tr>
<td>Blending of chitosan and PVA</td>
<td>Ch/PVA</td>
</tr>
<tr>
<td>Crosslinked chitosan and PVA</td>
<td>Ch-g-PVA</td>
</tr>
<tr>
<td>BSA immobilized crosslinked chitosan and PVA</td>
<td>BSA/Ch-g-PVA</td>
</tr>
</tbody>
</table>

Table 3.1: Designation of samples name
Figure 3.2: Samples of a) Ch b) Ch/PVA c) Ch-g-PVA and d) BSA/Ch-g-PVA

Figure 3.2 shows images of chitosan, Ch/PVA, Ch-g-PVA and BSA/Ch-g-PVA films that were prepared by using film casting technique. Ch-g-PVA has yellowish tone on film due to the exposure of films with crosslinking agent, glutaraldehyde. For BSA/Ch-g-PVA, film will swell after immersion in BSA, protein solution, but after dried, it tends to wrinkle at the edge of film. Besides, the film need to maintain its wettability condition as the BSA film need to keep inside the refrigerator in order to maintain protein shelf life.
3.3.1 Preparation of pure chitosan film

Chitosan (2 w/v %) was added in 2% of acetic acid solution. Chitosan powders were dissolved in acetic acid and kept stirring the solution by using mechanical stirrer for 3 hours. The chitosan powders were dissolved in acetic acid by constant stirring followed by degassing the solution for 2 hours. After degassing for 2 hours, the solution was poured into the glass plate. Solutions were dried in the oven for 24 hours, at temperature 60°C. Chitosan dry films were immersed in 0.5 M Sodium Hydroxide (NaOH) for 2 hours at room temperature to completely neutralize the film that still contains acetic acid on the surface of films. Films were then peeled off from the glass plate and washed with distilled water to eliminate excessive of NaOH solution that may adhere on the film surface. Samples were dried at room temperature for 24 hours.

3.3.2 PVA and Chitosan Film Preparation

PVA was dissolved in distilled water at 80°C with gentle stirring for 1 hour to form a 9 w/v% homogenous solution. Then, the chitosan solution was mixed with PVA solution in the volume ratio of 6:4, 5:4 and 4:6, in 10 ml of chitosan and PVA solution was poured and cast in a petri plate. Samples were then dried in the oven for 6 hours at 60°C temperature. Then, 0.5M NaOH solutions were pour into the petri plate with the dried film and immersed for 2 hours. After 2 hours, films were peel off and rinsed with distilled water. Ch/PVA films were dried at room temperature for 24 hours.
3.3.3 Crosslinking of PVA/Chitosan Film

To immobilize the films, crosslinked Chitosan /PVA (Chitosan -g-PVA) films need to be produced. Therefore, solvent vapors are one of the technique to crosslink the films. Ch/PVA films were sealed in a glass desiccator saturated with glutaraldehyde vapors (50% water) for 6 hours.

3.3.4 Immobilization of BSA on PVA/Chitosan Film

The crosslinked PVA/chitosan (Chitosan-g-PVA) films were immersed in the BSA that was diluted in phosphate buffer solution (PBS) at pH 6.8 with concentration of BSA, 1, 3 and 5 mg/ml. The adsorption of BSA is strongly dependent on the pH of the protein solutions. The isoelectric point of BSA is 4.7, therefore at pH 6.8 BSA is negatively charged while chitosan is positive charge. The ionic interactions of highly negative charge of BSA and positive charge of chitosan may enhance the protein adsorption and increase the protein support linkage. The mixture was moderately shaken with laboratory shaker for 24 hours at 25°C. The membrane was taken out and wash with PBS buffer, in order to remove excessive unbound BSA. Then the BSA immobilized membranes were carefully washed with distilled water and dried at room temperature (Homaeigohar et al., 2013).
3.4 Film Characterization

3.4.1 Fourier Transform Infrared Spectroscopy (FTIR)
Infrared spectra were obtained using Perkin-Elmer 2000 FTIR. FTIR test was done on film with 16 scans within the wave number range of 4000–400 cm\(^{-1}\). The purpose of FTIR is to identify any chemical interactions, including organic, polymeric and inorganic materials between films composed of chitosan, PVA, glutaraldehyde and BSA. All FTIR spectra were recorded in transmittance unit.

3.4.2 Field-emission scanning electron microscopy (FESEM)
The image of the dry films was studied using FESEM (Model Carl Zeiss Auriga). Films were cut into small pieces and mounted on the sample holder, known as copper stubs. Samples were analyzed by observing the samples by using an accelerating voltage of 5 kV and magnification at 5000x.

3.4.3 Wettability of Films
Static contact angle of the films was measured using a contact angle analysis system (Dataphysics Instrument OCA 15EC, Germany). Samples were placed on sample stage at horizontal level. A 5 µl droplet was drop on the film surface using a micro syringe. Average of 3 different points were measured on the same film.
3.4.4 Water Uptake Measurement of Films

The film samples were cut into 47 mm diameter size and dried in oven at 60°C for 1 hour. The initial weight or dry weight were measured before start immersing in 50 ml distilled water for 72 hours and measuring the wet samples weight at different time from 1 hour until 72 hours of exposure, after removing the excess water (Bangyekan, Aht-Ong, & Srikulkit, 2006)

\[
\text{Water Uptake} = \left( \frac{W_{\text{wet}} - W_{\text{dry}}}{W_{\text{dry}}} \right) \times 100\% \tag{1}
\]

Where, \( W_{\text{wet}} \) and \( W_{\text{dry}} \) are the weight of samples in wet and dry conditions respectively

3.4.5 Protein Standards Measurement

Bradford method is recommended for determining protein content of cell fractions. The assay is based on the observation that the absorbance maximum for an acidic solution of Comassie Brilliant Blue G-250 shifts from 465nm-595nm when binding to protein occurs. In this procedure, Bradford reagent needs to be prepared. 100mg of Comassie Brilliant Blue G-250 was dissolved in 50ml 95% ethanol. Then, 100ml 85% (w/v) of phosphoric acid was added into the solution. Solutions were diluted with distilled water to 1 liter when the dye has completely dissolved and were filtered through Whatman #1 paper just before used.
0.1g BSA was dissolved in 20 ml of phosphate buffered solution. The stock BSA solution was diluted in range of 100-1500 µg/ml as in following Table 3.2. 60uL of each standard was mixed with 940uL of Bradford reagent. Before being measured, BSA solutions were incubate at room temperature for 10 minutes. The absorbance of each standard was measured at 595nm against a blank that was composed of 60ul PBS and 940uL of Bradford Reagent by using the Genesys 20 Spectrophotometer. Graph was plotted for absorbance against BSA concentration and standard curve with equation was generated (Bradford, 1976).

<table>
<thead>
<tr>
<th>[BSA] µg/ml</th>
<th>Volume (µL) of 5mg/ml BSA Stock</th>
<th>Volume (µL) of PBS</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>5</td>
<td>495</td>
</tr>
<tr>
<td>200</td>
<td>10</td>
<td>490</td>
</tr>
<tr>
<td>400</td>
<td>20</td>
<td>480</td>
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<td>600</td>
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<td>900</td>
<td>45</td>
<td>455</td>
</tr>
<tr>
<td>1200</td>
<td>60</td>
<td>440</td>
</tr>
<tr>
<td>1500</td>
<td>75</td>
<td>425</td>
</tr>
</tbody>
</table>

Table 3.2: Standard BSA solution preparation (Bradford, 1976)
3.4.6 BSA Binding Capacity Measurement using Bradford Method

Bradford’s Method was used to measure the amount of protein concentration after protein immobilization (Bradford, 1976). To construct a calibration curve for measuring the protein concentration, BSA was used as standard protein solutions with different amount of concentrations. The amount of free BSA or BSA residual was determined by UV spectrophotometry at 595nm. The amount of bound BSA on the crosslinked PVA/Chitosan membrane was estimated by deducting the amount of the residual BSA from the initial amount of BSA (5mg/ml). BSA binding capacity was calculated according to equation (2) indicated below (Sven Frokjaer & Otzen., 2005):

\[
\text{BSA binding capacity} = \frac{\text{Initial amount of BSA (mg/ml)} - \text{Free amount of BSA}}{\text{Total amount of BSA}}
\]

3.4.7 Protein Adsorption Study

Ch-g-PVA films were immersed in PBS solution for 2 hours at medium of pH 7. Protein solutions were prepared by dissolving BSA in PBS at pH 7.4 to give a final concentration of 1 mg/ml. BSA is a negatively charged proteins, and its positively charge at pH 7.4. The purpose of using medium of pH 7.4 is to increase the protein adsorption due to hydrogen bonding and charge attraction may occur at that point (Hoven, Tangpasuthadol, Angkitpaiboon, Vallapa, & Kiatkamjornwong, 2007). Ch-g-PVA were incubated in plastic well, containing 3 mL protein solution at 37 °C for 3 hours. Ch-g-PVA films were removed and rinsed with PBS solution after 3 hours. Films were fill into another vial containing 2 ml of 1 wt% sodium dodecyl sulfate (SDS) and immersed for 1 hour at room temperature in order to remove reversibly adsorbed protein (Tangpasuthadol,
Pongchaisirikul, & Hoven, 2003). The concentration of protein adsorbed on the films were measured at 595nm by UV-Vis spectroscopy and determined by Bradford Method. Three readings of samples were performed for all sample.

3.4.8 Mechanical Properties of Film

All films were used in dry and wet conditions; therefore, it is important to study the mechanical properties of films in both conditions. Samples were cut into rectangular shape with the dimension 10 mm x 60 mm. Film thickness was measured by using thickness gauge. Five thickness value were taken along the gauge length of film strips and the mean value was used for calculation of tensile strength. Tensile strength measurements of the films were carried by a universal tensile testing machine (Shidmazu AGS-X Series) equipped with 50-N load cell at ambient temperature. The crosshead speed was 5 mm/min and the gauge length was 30mm (Fernandes, 2013). For each kind of film, at least three samples were tested. For the wet condition samples, samples were immersed in phosphate buffer solution with medium pH 7.4 for 1 hour. Filter paper was used in order to absorb excessive water on the surface, before the tensile test was carried out (Zhuang, Li, Fan, Lin, & Hu, 2012).
CHAPTER 4: RESULTS AND DISCUSSION

4.1 Optimization of Ch/PVA polymer blend composition

Chitosan/PVA blend films were prepared by film casting with different chitosan and PVA blend ratio. 3 blending ratios were selected for chitosan/PVA blend solutions, 6:4, 5:5 and 4:6. Varying ratio of chitosan/PVA is to determine the optimum parameters and blending ratio in order to produce chitosan/PVA films with excellent properties. Physical and mechanical properties of blended films such as wettability, water uptake and tensile properties were characterized.

4.1.1 Wettability of films

The most reliable methods to investigate the surface properties of polymers is by using the contact angle technique. It is widely used for surface homogeneity, hydrophilicity, hydrophobicity and changes in surface composition studies (Garbassi, 1994). The contact angle of chitosan/PVA with different blending ratio are shown in Figure 4.1.

![Figure 4.1: Wettability films at different blending ratio](image)

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From the result in Figure 4.1, Ch/PVA =4:6 ratio has the lowest contact angle with value of 57.1°, compared to ratio of Ch/PVA=6:4 and Ch/PVA=5:5, with value of 65.1° and 63.3° respectively. With increasing amount of PVA, it could modify the wettability of film. Lower value of water contact angle shows higher wettability of film properties. This indicates the wettability of films are affected by the OH- groups in the polymer blends. PVA is a water-soluble polymer, with higher ratio of PVA, it will increase the number of hydrophilic groups (-OH) in the blends and increase the wettability of films. Figure 4.2 (a-c) shows the photo image of water drops that captured on the surface of Ch/PVA=4:6, Ch/PVA=5:5 and Ch/PVA=6:4 respectively.

Figure 4.2: Water contact angle image captured by contact angle analysis system on the surface of (a) Ch/PVA=6:4, (b) Ch/PVA=5:5 and (c) Ch/PVA=4:6.
4.1.2 Water uptake measurement of films

Figure 4.3 shows that water uptakes for all samples were increased, with the existence of PVA content in all samples. This is due to the high hydrophilic character that come from hydrophilic groups (\(-\text{OH}\)) in the blends. Ch/PVA = 4:6 has the highest water uptake followed by Ch/PVA = 5:5 and Ch/PVA = 6:4, respectively. Water uptake measurement somehow related to the wettability of films. Lower value of water contact angle shows higher percentage of water uptake in a film. Higher PVA content in the polymer blend could lead to the increasing of hydrophilic groups (\(-\text{OH}\)), hence the more accessible OH groups that could interact with water molecules, resulting in more water could penetrate into the films (T. Wang & Gunasekaran, 2006).

Figure 4.3: Water uptake measurement for films at different blending ratio.
4.1.3 Tensile strength of films

Figure 4.4 shows the tensile strength of Ch/PVA blends at different blending ratio. Ch/PVA=4:6 has the highest value of tensile strength (50.64 MPa) compared to Ch/PVA=6:4 (44.39 MPa) and Ch/PVA=5:5 (39.82 MPa) respectively. This is mainly due to the PVA content in Ch/PVA=4:6 blend. Strong hydrogen bonding and interaction between PVA and chitosan, could enhance the flexibility of films, therefore increases the tensile strength of film.

Figure 4.4: Tensile strength for films at different blending ratio.

Several studies had been reported that, chitosan and PVA become miscible when the blending ratio of Ch/PVA was higher than 5:5 (Peng-Yu Zhuang, You-Liang Li, Li Fan, Jun Lin, & Hu, 2012). Therefore, increasing of PVA content could attribute to the increasing of tensile strength of Ch/PVA film. Positively charged of cationic polymers chitosan moved towards the negatively charge of anionic polymers of hydroxyl group in PVA, which improved the tensile strength of the Ch/PVA film (Li & Hsieh, 2006) (Sanchez-Alvarado et al., 2018).
This is due to the occurrence of intermolecular interactions between chitosan and poly-vinyl alcohol, resulting to hydrogen-bonding interactions (Abrahama, P.A.Solomanb, & V.O.Rejinib, 2016).

4.1.4 Optimum parameters

Chitosan/PVA films with different ratio were prepared by solution casting method, the optimum parameters investigation shows good compatibility of chitosan and PVA and this demonstrated in the following results of wettability, water uptake measurement and tensile strength in Table 4.1.

<table>
<thead>
<tr>
<th>Ch/PVA</th>
<th>Wettability (°)</th>
<th>Water Uptake (%)</th>
<th>Tensile Strength (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6:4</td>
<td>65.1</td>
<td>170.20</td>
<td>44.39</td>
</tr>
<tr>
<td>5:5</td>
<td>63.3</td>
<td>167.47</td>
<td>39.82</td>
</tr>
<tr>
<td>4:6</td>
<td>57.1</td>
<td>181.69</td>
<td>50.64</td>
</tr>
</tbody>
</table>

Hence, blending of chitosan and PVA showed significant improvement in terms of physical and mechanical properties, the optimum parameters and right proportions of blending should be considered in order to obtain stable solutions to produce a membrane film. In conclusion, Ch/PVA with blending ratio 4:6 were choose as optimum parameters. Blending ratio of Ch/PVA=4:6 will be produced and further used for crosslinking with glutaraldehyde and immobilization of BSA on Ch-g-PVA films.
4.2 Immobilization of BSA on crosslinked Ch/PVA films

Several studies and evaluation were conducted to measure the concentration of BSA proteins that were immobilized onto the films.

4.2.1 Protein standard measurement

The conventional method, Bradford’s Method for calculating the protein concentration of unknown sample is to use the standard curve that generated from known protein standard. The most reliable protein estimation is performed using a reference that has properties similar to protein being estimated. BSA was used as standard (Bradford, 1976). Several of BSA concentrations at the absorbance 595nm was measured and summarized in Table 4.2.

<table>
<thead>
<tr>
<th>BSA (mg/ml)</th>
<th>Absorbance&lt;sub&gt;595&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.1</td>
<td>0.438</td>
</tr>
<tr>
<td>0.2</td>
<td>0.604</td>
</tr>
<tr>
<td>0.4</td>
<td>0.965</td>
</tr>
<tr>
<td>0.6</td>
<td>1.153</td>
</tr>
<tr>
<td>0.9</td>
<td>1.501</td>
</tr>
<tr>
<td>1.2</td>
<td>1.725</td>
</tr>
<tr>
<td>1.5</td>
<td>1.977</td>
</tr>
</tbody>
</table>
Figure 4.5 displays graph of BSA absorbance is not quite linear and the reason for this appears to be variation of readings of concentration of BSA. By lowering the concentration range of BSA, it might be able to construct a better linear standard curve in future. Equation (3) will be used as a standard to calculate BSA concentration that trapped on a film after immersing in BSA solution.

![Graph showing absorption at 595nm vs BSA concentration. The equation given is: y = 1.2114x + 0.3033, R^2 = 0.95.]

**Figure 4.5: Protein standard measurement**

The following equation (3) is produced from a linear least square fit of the line:

\[ y = 1.2114 \text{[BSA]} + 0.3033 \quad (3) \]
4.2.2 Determination of BSA Content in BSA/ Ch-g-PVA Film

The actual amount of BSA that bound on the Chitosan-g-PVA film was estimate in order to measure the BSA encapsulation efficiency. The initial amounts of BSA used in this experiment were 1, 3, 5mg/ml. The calculations follow equation (2), where the binding capacity was calculated by deducting the initial amount of BSA concentration and the residual amount of BSA concentration. From Figure 4.6, 5 mg/ml of protein concentration shows high percentage of BSA encapsulation efficiency, which is 67.34% compared to 1 and 3 mg/ml with BSA encapsulation efficiencies which are 41.61%, 62.26%.

(Roozbahani, Sultana, Almasi, & Naghizadeh, 2015) also developed a BSA protein that incorporated with Poly (E-caprolactone)/chitosan blend nanofibers. Immobilization of BSA on the Chitosan-g-PVA film occurred via the ionic interaction. During the immersion of films in BSA, some of the BSA might trap among the positive hydrophilic chains and some distributed in the outer hydrophilic area of water. This indicated that only 67.34% BSA bound on the films and balance which is the free amount of BSA might react with water molecules (Hong-Liang Zhang, Si-hui Wu, Yi Tao, Lin-quan Zang, & Su, 2010). Based on Figure 4.6, the higher protein concentration leads to more encapsulation efficiency (Zhang, Wu, Tao, Zang, & Su, 2010).
Previous study also reported that the encapsulation efficiency could be increased by dissolving protein at a pH above its isoelectric point (pH 4.8). In this work, BSA was dissolved in PBS at pH 6.8, which is above its isoelectric point. At this pH, BSA would predominantly exhibit its highest negative charge at pH 6.8 and could contribute to electrostatic interactions between BSA with positively charged −NH₃⁺ groups on the backbone of chitosan (Calvo, Remunan-Lopez, Jato, & Alonso, 1997). Therefore, BSA with the concentration of 5 mg/ml was selected as the optimum parameters. With the highest encapsulation efficiency, it will be a promising effect for the membrane applications.

Figure 4.6: BSA encapsulation efficiency of Chitosan-g-PVA film
4.2.3 Protein Adsorption Study

Protein adsorption on solid surface is normally occur when material comes in contact with the biological environment (Huy, Van Chung, Thuy, Blanco-Andujar, & Thanh, 2015). In this study, proteins were adsorbed onto the films and protein concentration were measured by using Bradford method. Figure 4.7 shows the amount of adsorbed proteins on films. Initial amount of protein concentration used was 1 mg/ml. BSA/Ch-g-PVA adsorbed 86% compared to Ch-g-PVA 81% respectively. The immobilized film, BSA/Ch-g-PVA adsorbed more protein than the film without immobilized BSA on the film.

![Protein Adsorption Efficiency](image)

**Figure 4.7: Amount of adsorbed proteins on films.**

BSA/Ch-g-PVA could adsorp more proteins, as the crosslinking may occur between amine groups of BSA protein and carbonyl groups of glutaraldehyde, thus leading to aggregation of proteins and bind onto the film as shown in Figure 4.8. Proteins will hold it structure in order to allow the interaction and bind on the other proteins. Protein adsorption study also significantly affected by the surface hydrophobicity or hydrophilicity.
In previous study, few attempts and modification had been made to increase the protein adsorption efficiency on chitosan film. Surface modification can be obtained by modifying active reagents with the functional groups on the polymer surface, thus increase the interaction of the surface with the environment. (Tangpasuthadol et al., 2003). Besides tailoring the surface properties by using crosslinking agent or polar organic solvent, Hoven et al., has reported that positive or negative charges were also introduced on the surface of chitosan film via methylation and alkylation. (Hoven et al., 2007). Besides hydrophobic and hydrogen bonding, electrostatic interaction between protein and with any modified groups on the membrane surface may be the factors that will affected the protein adsorption efficiency.

**Figure 4.8:** Protein adsorption scheme on BSA/Ch-g-PVA films.
4.3 Physical and mechanical properties of immobilized BSA in Ch-g-PVA films

4.3.1 FTIR Spectra of film

FTIR spectroscopy is one of the most vital method to examine the BSA conformation after immobilization. Moreover, to study the compatibility and interaction between chitosan and PVA, and Ch/PVA and glutaraldehyde, FTIR measurements were taken and summarized in Figure 4.9. OH- vibration from chitosan has a peak at 3349 cm\(^{-1}\) and also a peak at 1596 cm\(^{-1}\) which attributed to the deformation and bending vibration of –NH\(_2\). Peaks at 2871 cm\(^{-1}\) corresponding to C-H (–CH\(_3\)) were observed. There are bending of vibration at peak 1375 cm\(^{-1}\) and 1150 cm\(^{-1}\) due to the methylene groups from the bending vibrations and asymmetric vibrations of CO. These are all the major peaks that attributed to the presence of chitosan in a membrane (Paluszkiewicz, Stodolak, Hasik, & Blazewicz, 2011) With addition of PVA into chitosan, a new peak appeared at 1437cm\(^{-1}\) which due to the –CH-OH bending vibration of PVA. 2928 cm\(^{-1}\) peak increased due to the intensity of CH group as PVA added into chitosan blend (Zhuang et al., 2012) In Figure 4.9, with glutaraldehyde crosslinking, Ch-g-PVA shows an increment at the peak of 1655 cm\(^{-1}\) due to N=C bonds shoulders. C-H stretch increase at 2936 cm\(^{-1}\) and the presence of aliphatic amino group leads to decrease in the intensity of the peak 1100 cm\(^{-1}\). Due to the crosslinking network between glutaraldehyde and Ch/PVA chains, aliphatic chains will build a network structure and hindered the linkage with amino groups, thus makes film become more hydrophobic (M.M. Beppu et al., 2007).
The main absorption bands of BSA were located at 1700 cm$^{-1}$ and 1540 cm$^{-1}$, which correspond to the protein-related amide I and II absorptions as shown in Figure 4.9. Ch-g-PVA containing BSA in Figure 4.9 showed both peaks for BSA and Ch-g-PVA without BSA, which confirmed the presence of BSA in the blends (Roozbahani et al., 2015).
4.3.2 Wettability of films

To investigate the surface properties and wettability of polymers, water contact angle measurement is one of established methods that frequently reported in literature. To evaluate wettability test, it measures the angle $\theta$ that formed at the intersection of the liquid, and solid phases. Normally a hydrophobic surface has a higher contact angle (> 90°) and for hydrophilic surface has a lower contact angle (< 90°). To obtain a higher surface energy and better adhesion of film, a lower contact angle of film must be developed to enhance its hydrophilicity. (Govindasamy, Ramli, Pasbakhsh, Pushpamalar, & Salamatinia, 2015). As shown in Figure 4.10, the degree of contact angle of the films increased in order of Ch/PVA < Pure chitosan < BSA/Ch-g-PVA < Cn-g-PVA.

![Figure 4.10: Wettability of films.](image_url)
Ch/PVA has the lowest contact angle value, 57.05° and Ch-g-PVA has the highest contact angle value, 79.80°. Ch/PVA membrane has lowest contact angle compared to pure chitosan. Adding PVA into film, will exhibit more wettability behavior in chitosan film, as PVA has large number of OH- groups from the surface of polymers, thus make the film to be more hydrophilic (Govindasamy et al., 2015). The water contact angle for Ch-g-PVA is larger 20 degrees than Ch/PVA. This confirmed that with the presence of glutaraldehyde, films are more hydrophobic. The crosslinking reaction between polymer and glutaraldehyde is represented by the reaction as shown in Figure 4.11 (Ahmad et al., 2012). The hydrogen bonding network was disrupted due to tight polymer structure that formed from the glutaraldehyde bridges between the chains via acetal bond formation. Due to the reduction of the hydrogen bonding, the new tight chain network will restricted the wettability of film and hinder the penetration of water molecules into the film (Edwin Marin & Rojas, 2014). Due to immobilized of protein BSA on the Ch-g-PVA film, which contains of amine and carboxyl groups, the contact angle value of BSA/ Ch-g-PVA is slightly lower than Ch-g-PVA (M. Elbahri et al., 2012).
Table 4.3 shows the photo image of water drops that captured on the surface of Ch, Ch/PVA, Ch-g-PVA and BSA/Ch-g-PVA respectively. The images show how the water molecules drop on the surface of different types of film. Obviously seen in Ch/PVA image, with 57.05 water contact angle. Lower degree of contact angle shows that water easily penetrate onto the films and spreads on the surface compared to crosslinked films with higher degree of contact angle. Ch/PVA shows higher hydrophilicity while Ch-g-PVA exhibit hydrophobic properties due to higher degree of water contact angle.
Table 4.3 Water contact angle image captured by contact angle analysis system.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Water contact angle value</th>
<th>Image</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ch</td>
<td>74.25</td>
<td><img src="image1.png" alt="Image" /></td>
</tr>
<tr>
<td>Ch/PVA</td>
<td>57.05</td>
<td><img src="image2.png" alt="Image" /></td>
</tr>
<tr>
<td>Ch-g-PVA</td>
<td>79.80</td>
<td><img src="image3.png" alt="Image" /></td>
</tr>
<tr>
<td>BSA/Ch-g-PVA</td>
<td>76.20</td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
</tbody>
</table>
4.3.3 Water uptake measurements of films

According to Figure 4.12, Ch/PVA film shows higher water uptake percentage and hydrophilicity compared to other films. With the high number of hydrophilic sites, which is mainly from the hydroxyl groups (-OH) will contribute greatly and influenced in relation to the water molecules. PVA is known as water soluble polymers, therefore blending chitosan and PVA will increase the hydrophilicity of films (Svang-Ariyaskul et al., 2006). It was also found that the stabilities of Ch/PVA film are poor in water. The water uptake of Chitosan/PVA drastically increases to 200% after 1 hour being immersed in water. Therefore, a crosslinked Ch-g-PVA was produced and can be used for water filter applications. For crosslinked Ch-g-PVA film, the water uptake was evidently less compared to pure chitosan and Ch/PVA films. This is due to less flexible structures of the crosslinking network. Upon crosslinking, the water uptake dropped as much as 22% from Ch/PVA. Crosslinking with glutaraldehyde decreases the water uptake of film. This is due to the entanglement of branched structures of Ch-g-PVA that increase the interactions between amine groups and hydroxyl groups, thereby decreasing hydrophilicity of the film (Danwanichakul & Sirikhajornnam, 2013). However, with the addition of BSA protein, the water uptake slightly increases compared to Ch-g-PVA. Glutaraldehyde or 1, 5-pentanodial (OHC-(CH2)3-COH is a 5-carbon molecule with two aldehyde functional groups, H-C=O which are highly reactive with amines groups, -NH2 or –NH3 + are commonly found on the protein surfaces. Crosslinking of glutaraldehyde with proteins may happen when the glutaraldehyde interacts with the functional groups of proteins that contains nucleophiles reactive amino acids. However, some of the protein molecules still present at the surface of the films, that may attribute to the interaction with the water molecules (M.M. Beppu et al., 2007).
From the Figure 4.12, effect of time on the water uptake percentage can also be discussed. Water uptake for all films were increased rapidly in the first hours of exposure. The water uptake percentage of films almost reached the optimum percentage around 4 hours of exposure, as the water uptake percentage did not change much and increasing. After 48 hours of exposure, there are slightly decreases in water uptake percentage, this is due to the breakage of newly formed weak bonds that cause by the excessive of water penetration into the films. Since PVA is hydrophilic materials, thus the stabilities of Ch/PVA is very poor in water. Therefore Ch/PVA films could not be used as membrane unless it is crosslink. For crosslink films Ch-g-PVA and BSA/Ch-g-PVA, the water uptake percentage almost reach its optimum time at 72 hours of exposure.

**Figure 4.12: Water uptake measurement for films.**
4.3.4 Tensile strength of films

Blending of Ch/PVA improved the tensile strength of films, as it’s indicated that blending could improve the strength and elasticity of the films. From Figure 4.13, Ch/PVA has higher tensile strength than pure chitosan film. Tensile strength of pure chitosan film is 22.56 MPa while tensile strength for Ch/PVA is 50.64 MPa. Blending leads to an intermolecular interaction between PVA and chitosan, where it could be an interaction between OH- and –NH₂ groups and thus improves mechanical strength of the blends. Previous study also reported that tensile strength and elongation percentage may increase with increasing in the amount of PVA. This is mainly due to the strong hydrogen bonding between the OH group of PVA and the OH and NH₂ groups of chitosan in the blends takes place. The intermolecular interactions between chitosan and PVA were also contributed from the positively charged polysaccharide chitosan moved towards to the negatively charge of the hydroxyl group of the Polyvinyl Alcohol (Abrahama et al., 2016). The conditions of Ch/PVA is different in both dry and wet conditions. Even though Ch/PVA absorbs more water than chitosan during the water uptake measurement test, it shows higher tensile strength compared to chitosan during the dry states. It can be justifying that the stabilities of Ch/PVA film is very poor in water, as PVA is a highly hydrophilic materials. Therefore, Ch/PVA in the wet state show different performance as the polar groups from PVA may migrate towards the surface layer which is neighboring with hydrophilic environment during immersion of samples in the water (S. Bahram Bahrami, Soheila S. Kordestani, Hamid Mirzadeh, & Mansoori, 2002). Crosslinking with glutaraldehyde [CH₂ (CH₂CHO)₂] in Ch/PVA membrane improves the tensile strength of the film. The crosslinking sample Ch-g-PVA shows higher tensile strength which is 56.01 MPa compared to Ch/PVA film which is 50.64 MPa. The increase in the tensile strength is mainly due to the strong bond between both the aldehyde groups of glutaraldehyde with PVA chains. Cross-linker is a compound that can bind to the polymer
chain with another chain either in covalent or ionic bonds form. From previous findings, Danwanichakul and Sirikhajornnam had reported on study of mechanical properties of electrolyte membranes in dry and wet states. In that study, it shows that chitosan and PVA polymer blend that crosslink with glutaraldehyde has the highest tensile strength in the dry states with value of 53.59 MPa. This value somehow similar to current study as shown in Figure 4.13. The tensile strength value of Ch-g-PVA in this study, with value of 56.01 MPa could be the benchmark of potential in producing crosslinked membranes films. Thickness value of Ch-g-PVA is 0.08 mm, which is slightly higher compared to Ch and Ch/PVA with thickness 0.05mm and 0.06mm respectively, and this value were demonstrated in Table 4.4. The higher the thickness, there are more cross sectional area of film to take the stress (Danwanichakul & Sirikhajornnam, 2013).

![Figure 4.13: Tensile strength for different blend of samples.](image)
There are two different modes of crosslinking, which are intermolecular and internal crosslinking. Intermolecular crosslinking is crosslinking between different polymer molecules while crosslinking of a single polymer is called internal crosslinking or normally known as intramolecular crosslinking (Mochamad Taha Ma'ruf, Widowati Siswomihardjo, Marsetyawan HNE Soesatyo, & Tontowi, 2015). From the Figure 4.13, tensile strength of BSA/ Chitosan-g-PVA is lower compared to Chitosan-g-PVA. The reaction between immobilized BSA and glutaraldehyde had been discussed and reported in previous works (Migneault et al., 2004) The mechanisms of protein crosslinking reactions remain open to speculate due to unclear reaction between proteins and glutaraldehyde. Extensive crosslinking may also affected the enzyme structure and distortion of the active site conformation may disturbing the retention of biological activity (Chui & Wan, 1997). There are few factors that could attribute to the chemical reactions between glutaraldehyde and BSA such as nature of proteins, concentrations of both proteins and glutaraldehyde, pH, temperature and the reaction time (Migneault et al., 2004). Thus, extensive study should be made on the immobilized BSA (Chitosan-g-PVA), so that the conditions of both proteins and glutaraldehyde could be choose carefully to obtain good intermolecular crosslinking between molecules. The conditions of film also play an important role during the tensile test. As seen in Figure 4.14, BSA/Ch-g-PVA sample has wrinkle at the edge of the gauge length area, this may affect the tensile strength of film. Film tends to wrinkle after drying the sample, thus make it difficult to handle during the tensile test.
Figure 4.14: Tensile test samples at dry states, a) Ch b) Ch/PVA c) Ch-g-PVA d) BSA/Ch-g-PVA.

Mechanical properties of a dry films may be very important during handling outside the water treatment tank or sediment tank. Thus, the mechanical properties for wet films should also need to investigate since these films will expose and in contact with water during the operation. For the wet conditions test, only Ch-g-PVA and BSA/Ch-g-PVA films were tested as shown in Figure 4.15. The tensile strength of Ch-g-PVA and BSA/Ch-g-PVA films decreased in the wet conditions. Similar trends can also be seen in the previous study by Danwanichakul and Sirikhajornnam. Ch-g-PVA suited its purpose in increasing the mechanical properties even in the wet states. The tensile strength of films was drastically decreased when films was expose to wet environment. This could be due to the fact that immersion of the films in water affects the interfacial adhesion between the polymers. In a wet environment during the water filtration, the water molecules penetrate in the films and reduce the interfacial adhesion. In fact, water molecules which are free to travel through pores while those may have dispersed and attached to the polar groups of polymers. In this case, PVA known as water soluble polymers and has large number of hydroxyl groups and may interact with water molecules. This may cause
swelling of films and makes the surface become soft, this can be confirmed by the thickness of sample shown in Table 4.4, the thickness of both Ch-g-PVA and BSA/Ch-g-PVA increase around 0.02 mm after expose to wet states, which are from 0.08 mm in dry states to 0.10 mm in wet states. Tensile strength of film is not necessary depends on its thickness. Even though that the tensile strength of material is express as force per unit area required for failure, it is also depending on the intrinsic property of film. Exposing film to wet states, eventually lead to de-bonding between crosslinker and polymers which are PVA, chitosan and BSA thus resulted to decrease in the tensile strength of films (Azwa, Yousif, Manalo, & Karunasena, 2013).

<table>
<thead>
<tr>
<th>Thickness</th>
<th>Dry states (mm)</th>
<th>Wet states (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ch</td>
<td>0.05</td>
<td>N/A</td>
</tr>
<tr>
<td>Ch/PVA</td>
<td>0.06</td>
<td>N/A</td>
</tr>
<tr>
<td>Ch-g-PVA</td>
<td>0.08</td>
<td>0.10</td>
</tr>
<tr>
<td>BSA/Ch-g-PVA</td>
<td>0.08</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Figure 4.15: Tensile test samples at wet states, a) Ch-g-PVA b) BSA/Ch-g-PVA.
4.3.5 Morphological features of film

The cross sections of Ch/PVA films without and with immobilized BSA are observed in Figure 4.16 and Figure 4.17 respectively. In Figure 4.16, the cross section of the film shows it has homogenous blended structure of chitosan and PVA. While in Figure 4.17 shows cross section of film after immobilized with BSA. The image shows that the membrane surfaces were completely covered with the immobilized layer of BSA. Blended films of Ch/PVA with BSA protein exhibit a smooth cross section surface, which indicates a uniform distribution of Ch/PVA with BSA protein molecules. This is mainly caused by the interactions of hydrogen bonds between –OH and NH₂ groups from PVA and chitosan. With the absorption of BSA into Ch/PVA film, it will attribute to higher amino group content and therefore improve the interactions between chitosan and PVA (Ung-Jin Kim et al., 2017) ((N. Riyasudheen, 2012).

Figure 4.16: SEM micrograph showing the cross section of Ch/PVA film before immobilized with BSA.
Figure 4.17: SEM micrograph showing the cross section of Ch/PVA film after immobilized with BSA
CHAPTER 5: CONCLUSION AND RECOMMENDATIONS

5.1 Conclusions

Chitosan and PVA were successfully fabricated at different blend ratios. Ch/PVA with 4:6 blending ratio was chosen as optimum parameter. Blending chitosan and PVA displayed good miscibility between chitosan and PVA, in term of wettability, water uptake and tensile strength. Swelling behavior and wettability of films showed higher water uptake after blend with PVA compared to the pure chitosan film. With the addition of crosslinking agent, glutaraldehyde improves mechanical properties of film and introduce hydrophobicity properties on the film surfaces.

Standard curve of protein had been generated and Bradford method was used for calculating the protein concentration. 67.34% of BSA successfully immobilized on Ch-g-PVA film, in 5mg/ml of BSA concentration. The immobilized film, BSA/Ch-g-PVA can be utilized in protein adsorption study to determine the amount of protein that can be adsorb onto the film. Physical and mechanical properties of film before and after immobilized had been characterized by FTIR, FESEM, wettability test, water uptake measurement, and tensile strength in dry and wet states. In conclusion, immobilization of BSA is one of the method to modify the chitosan and PVA film to make it suitable for the protein adsorption process in water filtration system.
5.2 Recommendations

In the future research, further investigation can be conducted as follows:

i. Different method such as electrospinning can be applied to produce immobilized Ch/PVA film. Study on the effectiveness of immobilization BSA by film casting and electrospinning method can be considered.

ii. Varies on different type of proteins, temperature and pH of medium during the immobilization process.

iii. This research can be extended by performing the water filtration test if nanofibrous membrane can be produced by electrospinning methods or adding some porous materials so that the Ch/PVA film is suitable for water filtration evaluation.
REFERENCES


LIST OF PUBLICATIONS AND PAPERS PRESENTED
