Alkaline Treatment of Chitosan Membranes

by

Chuhua Chen

A thesis

presented to the University of Waterloo

in fulfillment of the

thesis requirement for the degree of

Master of Applied Science

in

Chemical Engineering

Waterloo, Ontario, Canada, 2022

Author’s Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

Abstract

Derived from deacetylation of chitin, which is one of the most abundant natural polymers, chitosan has been drawing extensive attention due to its outstanding properties, and potential in many applications in the forms of membranes, fibers, and gels. Insoluble in water and most common organic solvents, chitosan membranes are typically prepared by dissolving chitosan into a dilute organic acid to protonate its amino groups. Thus, after membrane preparation, an insolubilization step is necessary to deprotonate the amino groups. However, this process is always excessive, and the effects of alkaline treatment conditions have not been well studied. This study focuses on the influence of conditions of alkaline treatment to improve membrane stability.

In this study, after being prepared, chitosan membranes were insolubilized with hydroalcoholic solutions of NaOH at various NaOH and ethanol (or methanol) concentrations. Membranes were treated for different alkaline treatment times as well, and the effectiveness of NaOH and alcohol on chitosan insolubilization was evaluated.

It was found that an increase in the concentration of NaOH and the content of water would accelerate the chitosan insolubilization process. Moreover, at the same concentration, ethanol performed better than methanol, primarily due to their different polarities. However, the use of an alcohol-free alkaline solution in chitosan insolubilization should be avoided since the chitosanium partially leached in the solution bath, which caused a defective structure.

Acknowledgements

I would like to take this opportunity to thank those who have guided, supported, and accompanied me throughout my master's study at University of Waterloo.

Foremost, I would like to express my sincere gratitude to my supervisor Prof. Xianshe Feng for the continuous support of my graduate study and research, for his patience, motivation, enthusiasm, and immense knowledge. His guidance helped me in all the time of research and writing of this thesis.

Besides my supervisor, I would like to thank my parents: Shuangxi Chen and Yanling Mu, for giving birth to me and supporting me unconditionally. Their care and encouragement inspire me through my whole master's research, especially in my hard time.

My sincere thanks also go to my friends: Xiaotong Cao, Zhelun Li, Xuezhen Wang, Chunliang Du, and Jinxuan Zhang for their encouragement, insightful comments, and warm company. We also created lots of cheerful memories in two years.

Financial support from Heze Holley New Material Co., Ltd of China in the form of scholarship is gratefully acknowledged.

Table of Contents

List of Figures

List of Tables

Chapter 1
Introduction

1.1 Motivation and Objectives

Chitosan, a biopolymer derived from chitin, has drawn extensive attention due to its excellent performance in many fields including food industry, agriculture, and drug delivery. With its outstanding chelating property based on its amino and hydroxyl groups, chitosan membranes show great potential in gas separation and water purification. Moreover, chitosan membranes can also be used in wound dressing due to their distinguished antimicrobial activity and non-toxicity.

Chitosan membranes are usually prepared by casting a chitosan solution, which is typically formed by dissolving chitosan into a dilute aqueous organic acid solution so that the amino group (-NH_2) will be protonated into -NH_3^+. After evaporation, the amino groups remain protonated, making the membrane water-soluble. Therefore, to stabilize the chitosan membrane, an insolubilization process (alkaline treatment) is required to deprotonate the positively charged amino groups.

Chitosan membrane has been mentioned frequently in the literature. However, the insolubilization process of chitosan membrane has seldom been studied. Therefore, to prepare a stable chitosan membrane effectively, the alkaline treatment conditions required to complete the insolubilization were studied in this research. In the experiment, alkaline solutions with different concentrations of NaOH and ethanol (or methanol) were tested. The effectiveness of each alkaline solution was evaluated based on the composition of the solution at different alkaline treatment time. In addition, the chitosanium leaching in the alkaline solution during membrane insolubilization was studied to find out a balance between the efficiency and stabilization of the chitosan membranes.

1.2 Thesis Outline

There are 5 chapters in this thesis. In Chapter 1, the applications of chitosan as a separation membrane were given as the background. The research motivation and objectives are also mentioned in this chapter. A review of the literature related to this research was shown in Chapter 2. Chapter 3 described the method used in this experiment including membrane preparation, insolubilization, and UV-vis measurements on the soaking water and alkaline solution. The results of the time required for complete insolubilization of alkaline solution at different concentrations of NaOH and ethanol (or methanol) were presented in Chapter 4. The leaching of chitosanium during the membrane insolubilization process was evaluated as well. Finally, the conclusions drawn from this study were presented in Chapter 5, and recommendations for further research were presented as well.

Chapter 2
Literature Review

2.1 Chitosan

2.1.1 Source and Structure

Chitosan is a derivative polymer of chitin produced by replacing the acetamido groups with amino groups (Figure 2.1). It was first discovered by boiling chitin in concentrated KOH in 1895. Chitin, well known as the second most abundant natural polymer after cellulose, is widely found in the shells of crabs, shrimps, and insects [1].

The production of chitin is closely associated with sea food. As an industrial food waste, shells of crabs and shrimps can be processed to manufacture chitin and chitosan. This process is shown in Figure 2.2. There are three main steps to produce chitin from crustacean shells: deproteinization, demineralization, and decoloration. In the deproteinization process, fine crustacean shells are usually treated with dilute NaOH solution at elevated temperatures to dissolve strongly combined proteins. For demineralization, a dilute HCl solution is often used to dissolve calcium carbonate at room temperature. After treatment with NaOH and HCl, a colored product is produced with the combination of pigments and chitin. Then decoloration is carried out to bleach the products into white chitin powders. To convert chitin into chitosan, a deacetylation process is required to remove the acetyl groups in chitin. In the deacetylation process, chitin is treated with concentrated NaOH or KOH solution at a temperature higher than 100 °C for at least 0.5 h. It is hard to reach a complete 100% deacetylation, and chitin and chitosan are divided from being soluble or insoluble in dilute aqueous acid solution instead of a certain degree of deacetylation (DD). The degree of deacetylation is related to temperature, deacetylating time, alkali concentration, chitin density, and many other factors [2–4].

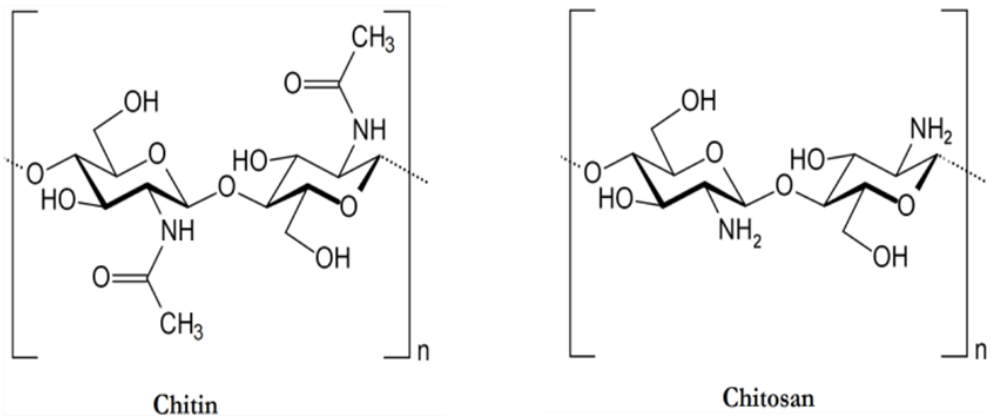

Figure 2.1 Structure of chitin and chitosan [5].

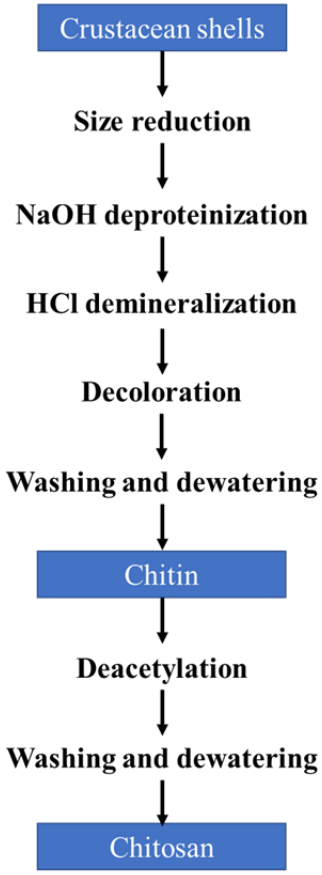

Figure 2.2 Manufacture process of chitin and chitosan[4].

Chitosan is a linear polymer of β (1→4)-linked 2-amino-2-deoxy-β-D-glucopyranose. It mainly contains hydroxyl groups and amino groups. By protonating the amino group, chitosan can be dissolved in dilute organic acids. Industrially, chitosan can be used in different forms such as solutions, films, beads, coatings, particles, powders, gels, and sponges [6]. Chitin has limited industrial usages due to its poor solubility and low reactivity, and chitosan has improved properties for processing because of the free amino groups [7].

2.1.2 General Properties

Chitosan draws significant attention due to its outstanding properties: chelating property, antimicrobial property, biocompatibility, and antioxidant property.

Chelating Property

Chitosan has a high nitrogen content (6.89%), which makes it a useful chelating agent [8,9]. As a metal absorbent, as shown in Figure 2.3, chitosan can absorb many metal ions including iron, copper, silver, and nickel. As the main reactive groups for the absorption of metal ions, the amino groups are the primary contributor to the chelating property in chitosan, while the hydroxyl group also contributes to metal ion absorption to a lesser extent. At a pH close to neutrality, the lone pair electron on nitrogen may complex with metal cations. At a lower pH, the amino groups will be protonated to be positively charged and act as ligands to attract metal-chelated anions (resulting from metal chelation by chloride, anionic ligands, etc.). For chitosan with a high degree of deacetylation, increasing the free amino groups will enhance the ability to capture metal ions. For chitosan absorbent, different metal ion uptake has been reported for different metal ions at a certain pH value. Moreover, a high temperature was found to favor the breakdown of the combination of chitosan and metal ions [8,10]. As a result, the chelating property of chitosan is affected by many factors: degree of deacetylation, pH value, metal ion species, and temperature.

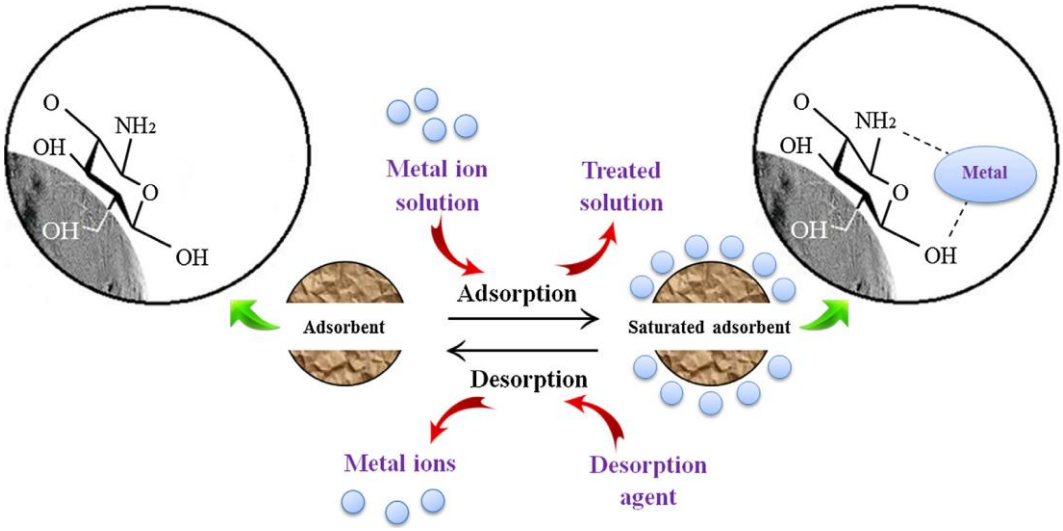

Figure 2.3 Schematic representation of regeneration procedure of chitosan adsorbent [8].

Antimicrobial Property

Based on the protonatable amino groups in the C-2 position, chitosan shows strong antimicrobial activity against gram-positive bacteria (e.g., Bacillus cereus, S. aureus, Lactobacillus Plantarum), gram-negative bacteria (e.g., Salmonella typhimurium, E. coli, Pseudomonas aeruginosa), and fungi (e.g., Fusarium oxysporum, Botrytis cinerea, Rhizoctonia solani) [7,9,11]. As shown in Figure 2.4, the mechanisms of chitosan antimicrobial activity vary with the molecular weight (MW) of chitosan and the microbial species. For all bacteria and fungi, chitosan may act as a chelating agent to bind metal ions and nutrients to inhibit the production of toxins and the growth of microorganisms. Also, at a low pH, the combination of positively charged chitosan and negatively charged microbial cell membranes may cause a rupture of the membrane and lead to microbial death. Moreover, for chitosan polymer with a low MW, it can penetrate the cell membrane to affect DNA/RNA or protein synthesis. On the other hand, for different microbes, chitosan can also combine with the negatively charged teichoic acids in gram-positive bacteria, lipopolysaccharide in gram-negative bacteria, and phosphorylated mannosyl side in fungi. Chitosan with a low MW can also affect mitochondrial activity [2,12]. Generally, the antimicrobial activity of chitosan is related to pH value, MW and degree of deacetylation of chitosan, and type of microorganisms [2,7,12].

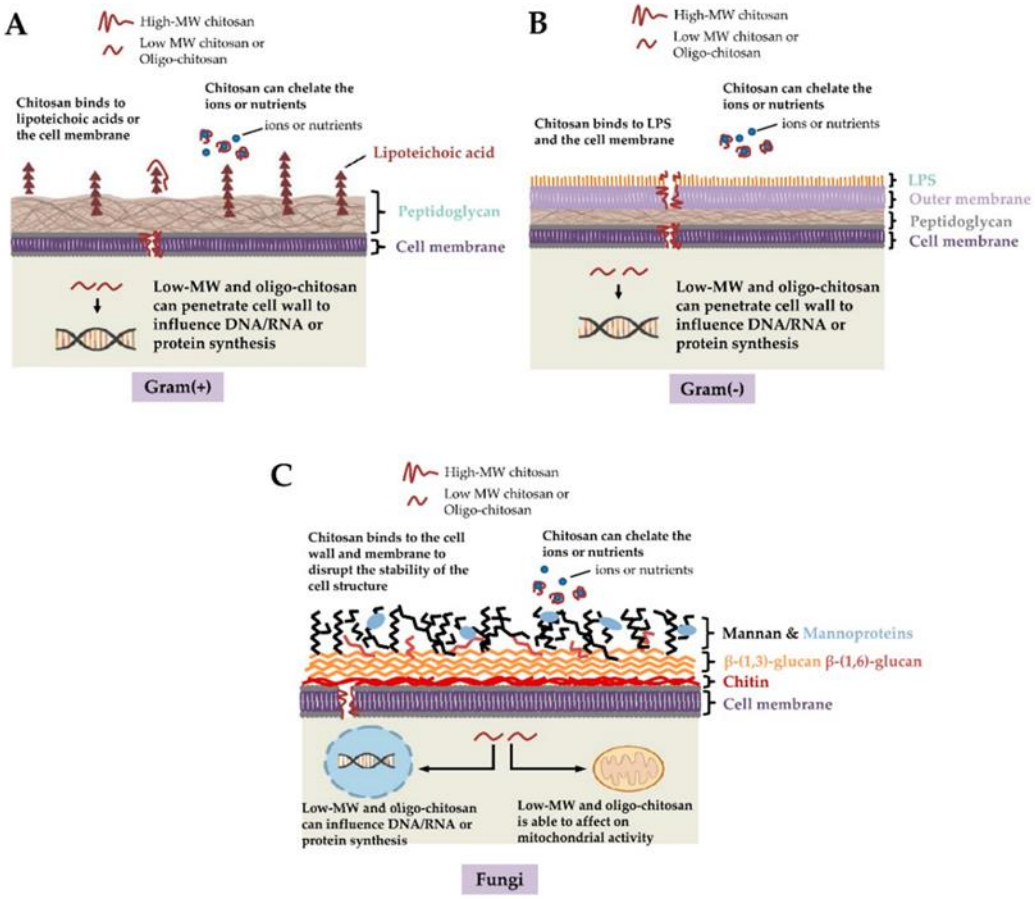

Figure 2.4 Mechanism of antimicrobial activity of chitosan against (A) gram-positive bacteria, (B) gram-negative bacteria, and (C) fungi [12].

Biocompatibility

Different from many man-made polymers, chitosan shows outstanding biocompatibility, including nontoxicity, biodegradability, and bioabsorbable ability [6,13,14]. Chitosan causes no antigenic reaction to living tissues, and it can be degraded by enzymes into harmless amino sugars which then will be processed by metabolism [6,14].

Antioxidant

With the ability to scavenge active free radicals, amino and hydroxyl groups make chitosan a good antioxidant agent [7,15]. The antioxidant mechanism of chitosan is that it protects the host against oxidative stress-induced damages via interfering with oxidation chain reaction [15]. The oxidation resistance of chitosan is affected by MW and degree of deacetylation. Since the formation of intramolecular hydroxyl bonds in a high MW chitosan is easier, there are more activated hydroxyl and amino groups available in chitosan with a lower MW. A higher degree of deacetylation means more amino groups in chitosan. Therefore, the antioxidant activity of chitosan increases with decreasing MW and increasing degree of deacetylation [7,15].

In addition to the above properties, chitosan also has outstanding mucoadhesive activity, hemocompatibility, antitumor activity, and immunomodulatory activity [2,11]. All these properties make chitosan a unique material for many applications in medical, dentistry, water treatment, agriculture, cosmetics, and food industry. Some of these will be discussed briefly in following sections.

2.1.3 Applications

Gas Separation Membranes

Chitosan-based membranes can be used to separate CO_2 from other gases for CO_2 capture[16]. As a CO_2 absorbent, chitosan has its primary amino groups which function as a fixed-site carrier for facilitated transport in the presence of water. In this facilitated transport mechanism, amino groups of chitosan can react with CO_2 to form carbamate ($R\text{-}NHCOO^-$) and bicarbonate ions (HCO_3^-) [16]. Moreover, chitosan can be blended with other polymers (e.g., poly (vinyl alcohol), cellulose acetate, and poly (vinyl pyrrolidone)) to increase the mechanical stability of the membrane [16].

Chitosan-based membranes can also be involved in the separation of olefin/paraffin via facilitated transport [17]. With its significant chelating property and mobility for silver ions, chitosan acts like a chelating agent of silver ions, which complex with olefin molecules for separation of olefin/paraffin. Silver ions, as the carrier of olefin molecules, facilitates permeation of olefin molecules through the membrane via the following interactions [18]:

$$Ag^{+} + \text{olefin} \text{ — } [Ag(\text{olefin})]^{+} \quad (2.1)$$

$$[Ag(\text{olefin})]^{+} + Ag^{+} \text{ — } [Ag_2(\text{olefin})]^{2+} \quad (2.2)$$

$$[Ag(\text{olefin})]^{+} + \text{olefin} \text{ — } [Ag(\text{olefin})_2]^{+} \quad (2.3)$$

The mechanism of facilitating transport with silver ions as carriers is illustrated in Figure 2.5. Silver ions are able to complex with olefins to form olefin-silver complexes reversibly, and they act as mobile carriers to facilitate olefin transport through chitosan membranes [19].

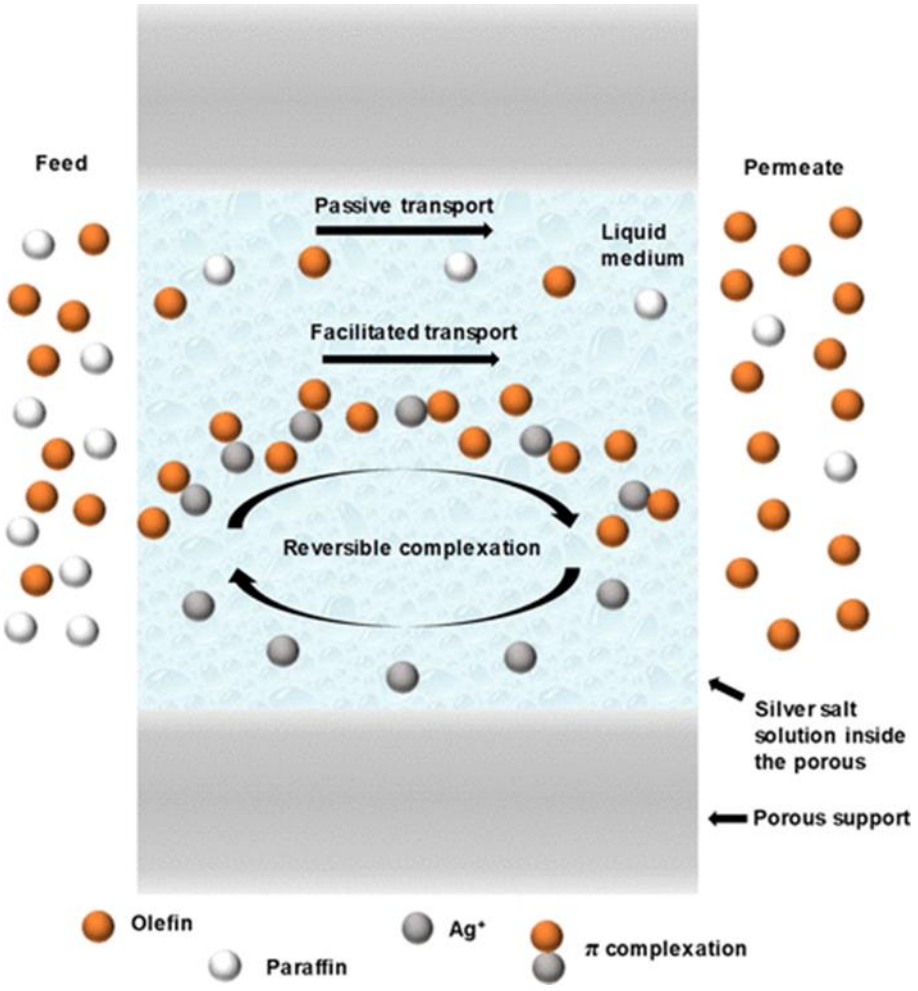

Figure 2.5 Mechanism of facilitated transport for olefin/paraffin separation [19].

Medical Applications

Based on its biocompatibility, hemocompatibility, and antimicrobial activity, chitosan is widely used in medical applications, including tissue engineering, drug delivery, wound dressing, and dentistry.

In tissue engineering, the damaged body parts or lost organs are targeted to be replaced by transplanting supportive scaffolds with appropriate cells in combination with biomolecules to generate new tissue [6]. To be effective, the material should exhibit biocompatibility with tissues, a suitable biodegradability rate, nontoxicity, optimal mechanical strength, and adequate porosity and morphology for transporting [14]. Chitosan can promote human cell proliferation and tissue repair, it acts as an ideal scaffold material in skin, cartilage, cornea, and other tissues engineering (Figure 2.6) [2,14].

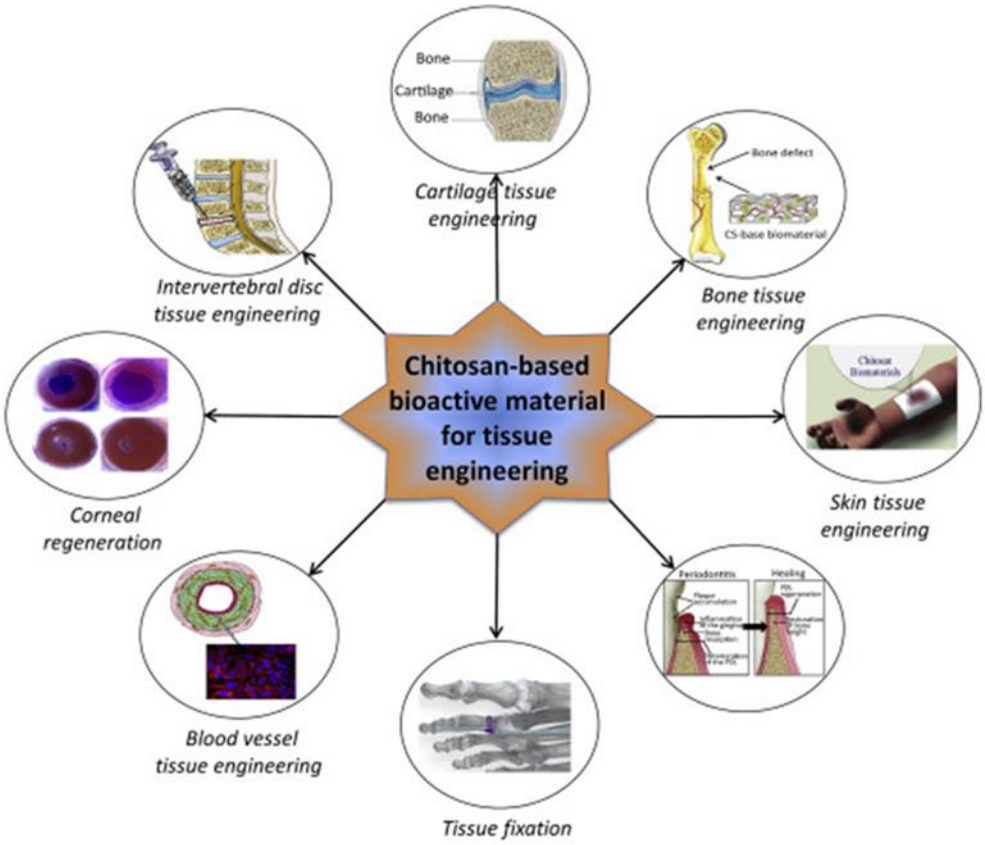

Figure 2.6 Schematic representation of chitosan tissue engineering [14].

By making drugs selectively directed to their targets, the drug delivery system can improve the efficiency of drugs [13]. It regulates the drug release rate and reduces the fluctuation of released drug content to make the drug effect more moderate [20]. Controlled delivery may involve the delivery of drugs, genes, and vaccines [6,11]. Chitosan can act as a drug carrier because of its biocompatibility, low-cost, gel-forming ability, nontoxicity, antacid, and antiulcer activities [2,13,20,21]. Generally, drugs can be mixed with chitosan via dissolution, coating, adsorption to form drug sustained-release microspheres, tablets, gels, microcapsules, and sustained-release films [2]. As shown in Figure 2.7, for example, chitosan can be used as a coating to combine with doxorubicin and delivery it through cells without causing side effects [21].

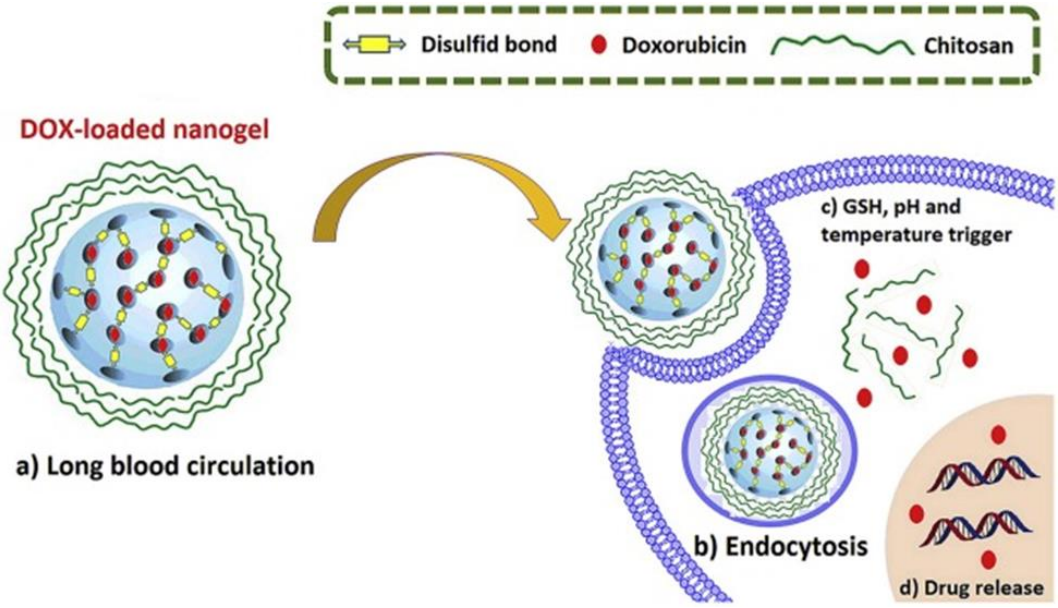

Figure 2.7 Cell uptake mechanism for delivery with chitosan [21].

Wound healing is a dynamic and complex process that is important to reduce wound infection [2]. Chitosan and its derivatives have characteristics that favor wound healing. Figure 2.8 illustrates the mechanisms of wound healing with chitosan products. With a high positive surface charge, chitosan can effectively support cell growth and promote thrombosis and blood clotting. Moreover, with free amino groups on its surface, chitosan may form a complex with the acidic groups of blood cells [2]. In

addition to its hemocompatibility, biocompatibility, and antimicrobial activity, chitosan shows lots of advantages in enhancing the wound healing process as a material for wound dressing [13]. Chitosan can also act as an asymmetric membrane for improved wound healing due to its similar structure with the architecture of native skin (Figure 2.9) [22]. Chitosan asymmetric membranes have additional advantages of high porosity, water uptake ability, wettability, and release profile [22].

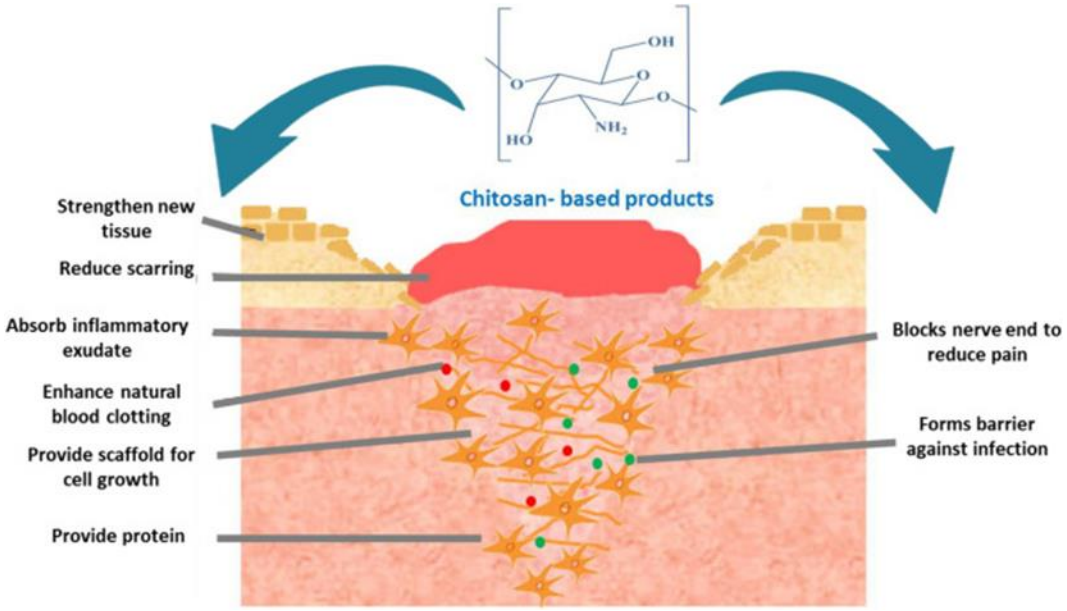

Figure 2.8 Mechanisms of wound healing promotion by chitosan-based products [7].

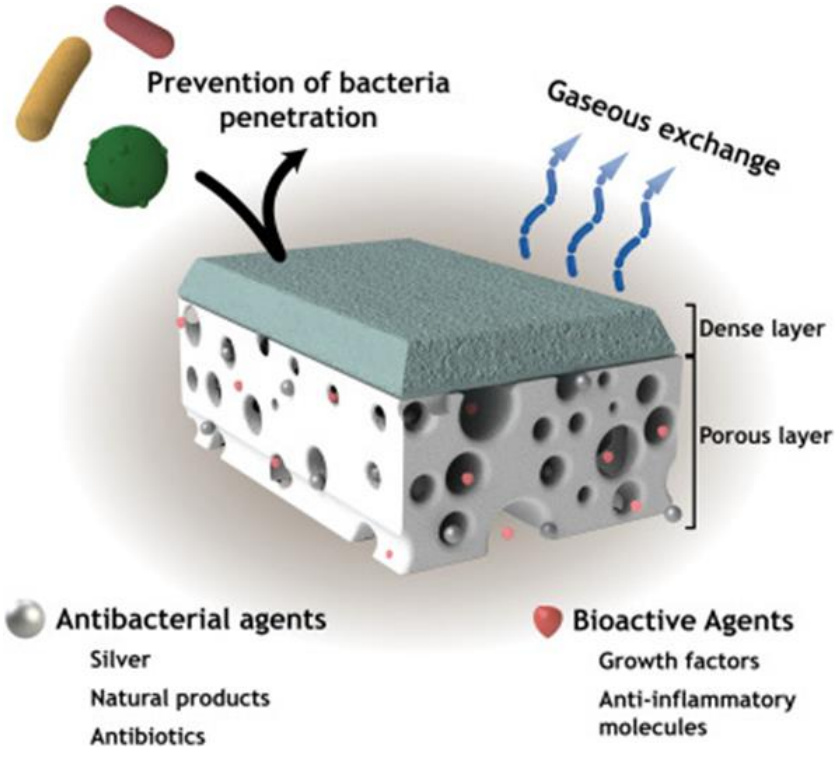

Figure 2.9 Illustration of the main features displayed by asymmetric membrane for improved wound healing [22].

Dentistry

Due to its biocompatibility, antimicrobial and hemostatic activity, chitosan is used in dentistry in the forms of solution, microspheres, coating, hydrogel, and toothpaste. Chitosan gel/hydrogel is used for the treatment of chronic periodontitis, dental caries, and canker sores [6,23]. Toothpaste, mouthwashes, and chewing gums based on chitosan are used for antimicrobial effect on oral hygiene and reduce streptococcus mutants and other bacteria [6,23]. They can also act as a wound dressing agent to reduce oral infection [23]. Chitosan associations are capable of repairing early caries lesions [6].

Water Treatment

With excellent chelating properties, chitosan and its derivatives are widely used in wastewater treatment and water purification as chelating and flocculating agents. With chelating and absorbing effects on

heavy metal ions and organics, chitosan and its derivatives can be used to treat industrial wastewater in the form of membranes to treat waste water and recycle metal ions by absorbing them [2]. Chitosan also agglomerates large anionic impurities in solution to form precipitates and floaters so that it can act as a flocculent for recycling food processing wastewater. Moreover, as a flocculating agent, it can be the absorbent for removing the color from dyehouse effluent and for purifying wines, juices, and beers in the beverage industry [4,6]. They can also improve the versatility of membranes and their effectiveness in water purification [24].

Agricultural Uses

Taking advantage of its antimicrobial activity, chitosan products can be used for agriculture in the forms of seed coating, soil enrichment, foliar spraying, supplement in hydroponic and in plant tissue culture medium [6]. Coating a layer of chitosan on the seeds can inhibit fungal growth around the seeds and enhance the resistance of plants to bacteria [2]. As an agent for soil enrichment or supplement, chitosan can be broken down by microorganisms to provide nutrients for plant growth. Also, the degradation of chitosan can promote the growth of actinomycetes which produces antimicrobial compounds [2]. Chitosan-based materials can be used in pesticide spray to prevent the plant from diseases [6].

Cosmetics

With its chelating property, biocompatibility, antimicrobial, and antioxidant activity, chitosan shows excellent performance in cosmetics. It is a natural cationic gum that becomes viscous on being neutralized with acid, which facilitates the interaction between chitosan and hair/skin cover [4]. Chitosan-based materials are usually applied in three areas of cosmetics: hair care, skin care, and oral care. Its usage in oral care has been discussed before.

In hair care, chitosan and its derivatives can increase the softness, smoothness, and mechanical strength of hair by forming clear elastic films on hairs [2,4]. Chitosan in hair care products can be used in the form of shampoos, rinses, permanent wave agents, hair colorants, styling lotions, hair sprays, and tonics [4].

When used in skin care, chitosan products have two advantages: positive electrical charge and high molecular weights. Regardless of the cationic property mentioned above, most chitosan products

have their molecular weight too high to penetrate the skin [4,6]. Chitosan products are found in many forms in skin care, including creams, pack material, lotions, nail enamel and lacquers, foundation, eye shadow, lipstick, cleansing materials, and bath agents [4].

Food Industry

Due to biocompatibility, antimicrobial activity, chelating property, and nontoxicity for warm-blood animals, chitosan and its derivatives can be used in a) food additives as thickeners, decolorants, and stabilizers; b) food protection as antimicrobial and antioxidant agent; c) functional food as nutraceuticals and anticholesterolemic products [2,6]. Chitosan products in the food industry have the advantage of good mechanical properties and being able to incorporate functional substances such as vitamins [6]. As a food additive, chitosan can absorb pigments in food, and after consumption form complexes that are unabsorbable for the human body, thereby reducing pigment toxicity. Moreover, as a cationic flocculant, chitosan can absorb polyphenolic compounds and reduce the total solids and heavy metal content [2]. As a food preservative, chitosan can prevent food from microbial deterioration and act as a natural antioxidant due to metal ion chelation in lipid-containing foods, hence extending the shelf-life [6,7]. Chitosan can be sprayed onto fruits and vegetables to create an edible protective film with antimicrobial activity [7]. As a functional food, chitosan can facilitate to decrease systolic and diastolic blood pressure. Chitosan-based materials have potential in weight management and obesity treatment by blocking the absorption of dietary fat and cholesterols [6].

In addition to the above applications, chitosan products are also used in the textile industry, photograph, and solid-state batteries [4,6].

2.2 Insolubilization Process of Chitosan Membranes

Insoluble in water and common organic solvents, chitosan membrane is always prepared by dissolving chitosan into dilute organic acid (e.g., acetic acid) due to its protonatable amino groups [3]. After drying, with its protonated amino groups, the chitosanium membrane is soluble in water, which makes it unstable when being used as selective membranes in wound dressing and water treatment. To deprotonate the positively charged amino groups, an insolubilization process should be applied to render the membrane water-insoluble.

2.2.1 Insolubilization Methods

The insolubilization process of chitosan membrane is usually operated by putting chitosanium membrane into alkaline solutions (e.g., NaOH aqueous solution, and NaOH alcoholic aqueous solution). After sufficient alkaline treatment, residual acetate salts and alkaline solution will be washed thoroughly by deionized water, and then the chitosan membrane is ready for use.

For the insolubilization of chitosanium membrane with an alkaline solution, different deprotonation agents are used, including NaOH [25–30], sodium carbonate (Na_2CO_3) [28,30], or NaOH-Na_2CO_3 mixture [31,32] based on their alkaline property to transform -NH_3^+ groups into -NH_2 groups. Considering NaOH as the deprotonation agent, the membrane is neutralized by [28]:

$$R\text{-}NH_3^+ + OH^- \text{—} R\text{-}NH_2 + H_2O \quad (2.4)$$

For Na_2CO_3 as the agent, the following reactions are involved [28]:

$$CO_3^{2-} + H_2O \text{—} HCO_3^- + OH^- \quad (2.5)$$

$$R\text{-}NH_3^+ + CO_3^{2-} \text{—} R\text{-}NH_2 + HCO_3^- \quad (2.6)$$

$$R\text{-}NH_3^+ + HCO_3^- \text{—} R\text{-}NH_2 + H_2CO_3 \quad (2.7)$$

On the other hand, alcohol, mostly ethanol and methanol, is also considered with the function of the chitosanium membrane inversion. Acetic acid has a higher solubility in ethanol/methanol than in water. Therefore, by absorbing the generated acetic acid molecules through a diffusion mechanism, ethanol/methanol can facilitate the deprotonation of the chitosanium membrane [29]. However, since the hydrolysis reaction is reversible, the deprotonating process for alcohol without NaOH can never be completely finished [29]. Therefore, ethanol/methanol is often used with NaOH as an agent to prevent unstable chitosanium from being dissolved during the alkaline treatment process.

The insolubilization process is also capable of being performed before membrane casting without causing inhomogeneous gelation by adding $Mg(OH)_2$ nanoparticles into the chitosan casting solution [33].

2.2.2 Influence of Chitosan Insolubilization

The chitosan insolubilization process will result in changes in the characteristics of chitosan membranes, including crystallinity [30,31,33], hydrophilicity [32,33], Young's modulus [31–33], oxygen permeability [33], water vapor permeability [31,33,34], antibacterial property [34], and swelling behavior [28,32]. A few studies have been conducted on the influence of the insolubilization process, and the alkaline treatment conditions are found to have great effects on stabilizing the chitosan membrane.

The alkaline treatment conditions affecting the properties of deprotonated chitosan membrane include: For a NaOH-based alkaline treatment, increasing concentration of NaOH and the alkaline treatment time will lower the contact angle of the membrane, making the membrane surface more hydrophilicity [26]. There is also a correlation between the hydrophilicity and stability of chitosan [30]. Thus, the hydrophilicity of chitosan membrane can be improved by alkaline treatment. The degree of deacetylation is found to increase with an increase in NaOH concentration in the alkaline solution for chitosan insolubilization [27]. With an increase in NaOH concentration in the alkaline solution, the crystallinity decreases, and the membrane becomes more compact [27]. That is a higher NaOH concentration used in the insolubilization reduces the swelling and Young's modulus of the chitosan membrane [27].

There are certain advantages and disadvantages of using different alkaline solutions. Sangsanoh et al. studied the difference between chitosan insolubilization via NaOH and Na_2CO_3 at a fixed concentration of 5 M, alkaline treatment time of 3 h, and room temperature [28]. Compared with Na_2CO_3, insolubilization of NaOH was found to break the structure of chitosan membrane more easily, which was considered to be caused by the dissolution of chitosan [28]. Unlike NaOH, Na_2CO_3 can react directly with chitosan salt instead of ions of positively charged chitosan without causing the dissolution of chitosan ions [28]. Even though there is a higher alkalinity in NaOH solution at the same concentration, the chitosan membrane shows an intact structure after insolubilization with Na_2CO_3. As a result, in a sufficiently long alkaline treatment time, membrane treated with Na_2CO_3 shows fewer structure defects than that with NaOH.

Gültan et al. prepared chitosan membranes with different alkaline treatments using concentrated ethanol solution, NaOH solution, and Na_2CO_3 solution [30]. Among these three alkaline solutions, membrane treated with Na_2CO_3 is found to have a higher roughness, while those with NaOH

and ethanol are nearly the same, indicating that insolubilization with Na_2CO_3 is the least effective for deprotonation of chitosanium. Membranes treated with ethanol have a low tensile strength and elongation [30]. Membranes treated with NaOH have the lowest contact angle, indicating a large number of free amino groups on chitosan chains caused by chitosan insolubilization. The structural integrity of the membrane was noticed when treated by ethanol, and then Na_2CO_3 [28,30].

He et al. had studied chitosan insolubilization by testing membranes treated differently. Those membranes were treated with an ethanol aqueous solution, NaOH aqueous solution, and NaOH/ethanol/water mixture (hydroalcoholic NaOH solution) [29]. A large number of NH_3^+ groups were found in the membrane treated by ethanol because of the reversible deprotonation process where the amino groups were only partially deprotonated [29]. The membranes treated with NaOH/ethanol/water mixture had the most rigid and compact structure with the lowest swelling degree and fiber-dominant surface. On the contrary, chitosan membranes treated with NaOH or ethanol had large number of "particles" on their surfaces. This difference on membrane surfaces is considered to be caused by the different crystalline behavior since water molecules are removed during the reaction between NaOH and protonated amino groups in the insolubilization for the NaOH/ethanol/water mixture [29]. In the mechanical test, the mixture-treated membrane was found to have the highest elastic modulus and tensile strength, while the ethanol-neutralized membrane has the lowest. Also, it had the lowest elongation, while the ethanol-treated membrane had the highest. The mechanical properties of NaOH-treated membrane were found in between [29]. Moreover, better endothelial cell compatibility was found in the mixture-treated membrane [29]. Generally, the chitosan insolubilization with NaOH aqueous solution can be improved by adding ethanol to prevent chitosanium from being dissolved.

Among all the common chitosan insolubilization methods introduced previously, the NaOH/ethanol/water mixture showed to be the most effective for deprotonating the chitosanium membranes without structural defect. The influences of the various parameters (composition, time) involved in chitosan insolubilization with hydroalcoholic NaOH solutions as the alkaline solution will be studied.

However, it should be pointed out that it is very misleading in referencing the alkaline treatment of chitosanium with ethanol, because ethanol does not associate to produce OH^- ions, which are needed to deprotonate chitosanium. It is no surprise that the chitosan membrane "deprotonated" with ethanol lacks of mechanical integrity and stability. On the other hand, methanol is a smaller molecule than

ethanol, and deprotonation of chitosanium membrane with an aqueous NaOH/MeOH solution is expected to favor the diffusion of water molecules generated in the insolubilization to the bulk solution. Therefore, the objective of this work is to investigate the alkaline treatment of chitosanium with aqueous NaOH/EtOH solution in comparison with chitosan insolubilization with aqueous NaOH/MeOH solution.

Special considerations are given in selecting the insolubilization conditions (e.g., compositions of the alkaline solution, alkaline treatment time) that are practical for fabricating chitosan based facilitated transport membranes for olefin/paraffin applications.

Chapter 3
Experimental

In this study, chitosan membranes were prepared from a homogenous membrane casting solution. Then, the hydroalcoholic NaOH solutions were used as the alkaline solutions to treat and insolubilize the prepared chitosanium membranes. The treated membranes were immersed into deionized water (soaking water), and the soaking water was monitored via a UV-vis spectrophotometer to evaluate the stability of the membrane. Moreover, possible chitosanium leaching during alkaline treatment was also analyzed by UV-vis measurements of the alkaline solution during the course of chitosan insolubilization. With the UV-vis absorbance (ABS) data, the effectiveness of NaOH and alcohol concentrations in the alkaline treatment on chitosan membrane stability was evaluated.

3.1 Materials

Chitosan membranes were prepared from chitosan flakes with a deacetylation degree of 90% supplied by Imtex Membranes Corp, Canada. Sodium hydroxide (≥97%, pellets), anhydrous methanol, and acetic acid were purchased from Sigma Aldrich. Anhydrous ethanol was purchased from Greenfield Global. Deionized laboratory water was used in preparing the aqueous solutions used in the experiments and membrane rinsing.

3.2 Membrane Preparation

Chitosan was dissolved in an aqueous 2wt% acetic acid solution with its concentration in the solution of 1.12wt%. To form a homogenous membrane casting solution, a magnetic stirrer was used to agitate the prepared solution for two weeks. Then, the casting solution was filtered to remove residual impurities via a vacuum filtration process, followed by an ultrasonication of 2 hours to break gas bubbles trapped in the solution. Finally, 40 g of chitosan solution was measured with an electronic scale and placed into a petri dish of 100 × 15 mm, which was kept ventilated in a fume hood. After drying, chitosanium membranes were thus formed.

3.3 Insolubilization

Due to its protonated amino groups (-NH_3^+), chitosanium membrane is soluble in water. To make the membrane water-insoluble, hydroalcoholic NaOH solutions were used as the alkaline solutions to insolubilize the membrane by deprotonating the amino groups. The alkaline solution was prepared by dissolving NaOH into a water-ethanol (or water-methanol) mixture at specified NaOH and alcohol concentrations. The alkaline treatment was carried out by soaking the chitosanium membrane into 190 ml alkaline solution in a bottle sealed with a fresh wrap cling film. After insolubilization, the membrane was stored individually.

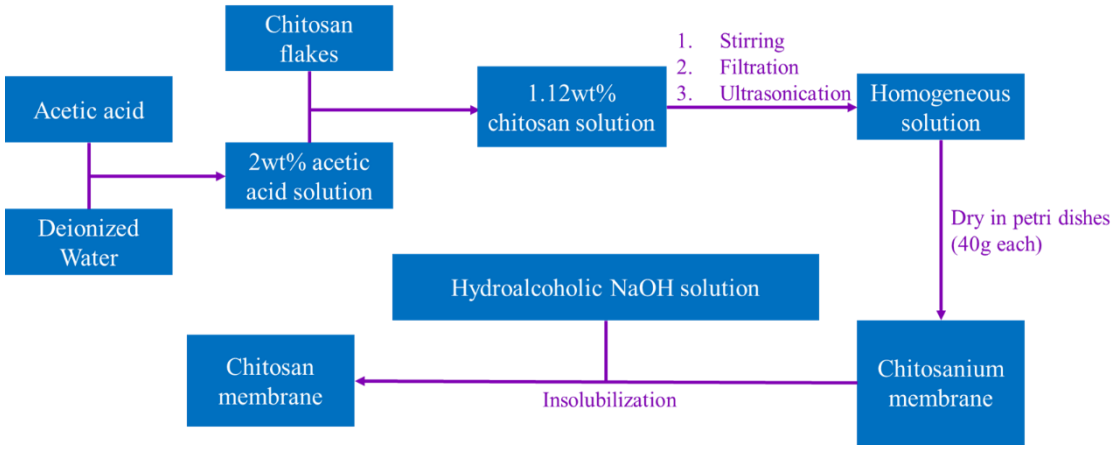

Figure 3.1 Chitosan membrane preparation and insolubilization process**.**

3.4 Effects of NaOH Concentrations

In order to evaluate the influence of NaOH concentration in the alkaline solution on insolubilizing the membrane, chitosanium membranes were treated with alkaline solutions at different NaOH concentrations in the ranges from 0.2 to 1.0 M. The ethanol content was kept at a constant concentration of 50% (v/v). At a given NaOH concentration, chitosanium membranes were treated for different alkaline treatment times to determine the time required for complete insolubilization. After alkaline treatment, the membranes were rinsed for 5 min under a running water. Then the membranes were immersed into 500 ml beakers filled with deionized water, refreshed every 5 min over a total of 20 min. The total 25 min membrane rinsing time was considered as the preferred time to remove the residual

NaOH and alcohol out of the membrane while leave the untreated chitosanium in the membrane for further measurements (see Appendix A).

The chitosanium part inside the membrane was only slightly dissolved during this membrane rinsing step because of its high molecular weight (MW). After that, all washed membranes were soaked individually into sealed plastic bottles each filled with 100ml deionized water (soaking water). After soaking for 3 days, the soaking water samples were subjected to the absorbance measurements with a UV-vis spectrophotometer (UV-1900 Shimadzu) to observe any possible chitosanium polymer leaching from the chitosan membrane. After the measurements, all the membranes were stored individually with deionized water. The membrane stability at given NaOH concentration and alkaline treatment time was gathered to show the influence of NaOH concentration in the alkaline solution on the insolubilization process of chitosan membranes.

3.5 Effects of Alcohol Concentrations

To evaluate the effects of alcohol content on the insolubilization process, chitosan membranes were analyzed after being insolubilized with different alcohol concentrations ranging from 2 to 50% (v/v) at a constant NaOH concentration of 1.0 M. Since the change of alcohol concentration in the alkaline solution was considered to have less effect on the insolubilization process than the change of NaOH concentration, after different alkaline treatment time, insolubilized membranes were wiped and soaked into 300ml deionized water (soaking water) without any further rinsing process to minimize the experimental error caused by membrane rinsing (see Appendix B). Then the soaking water was analyzed periodically by the UV-vis spectrophotometer to show its absorbance curves at different soaking time. Furthermore, experiments were also carried out by replacing ethanol with methanol to show the difference between these two kinds of alcohols. All the membranes were stored in deionized water. For both ethanol and methanol, the alkaline treatment time required for complete insolubilization was found through the absorbance of soaking water since the partial dissolution of chitosanium polymer occurred for chitosan in the membrane that was not insolubilized enough.

3.6 Chitosanium Leaching from Membrane during Insolubilization

In order to evaluate chitosanium leaching during the insolubilization process, the chitosan membrane in this experiment was insolubilized with alkaline solution containing different contents of alcohol. Since it is hard to determine the amount of leached chitosanium polymer via an insolubilization-finished membrane, therefore, UV-vis measurements were conducted continuously on the alkaline solution instead of soaking water during the membrane insolubilization process. The alkaline solution was prepared with an alcohol content of 0 to 15% by volume, and its NaOH concentration was fixed at 1.0 M. Before immersing chitosanium membrane into the alkaline solution, the absorbance of the solution was measured for benchmarking. The insolubilization lasted 9 min to make sure that the membrane was fully insolubilized at the end. The insolubilized membranes were stored in deionized water. Finally, the absorbance of the initial alkaline solution and the absorbance of the alkaline solution over time were determined to show the progress in the insolubilization process. Figure 3.2 shows the steps involved in the experimental work.

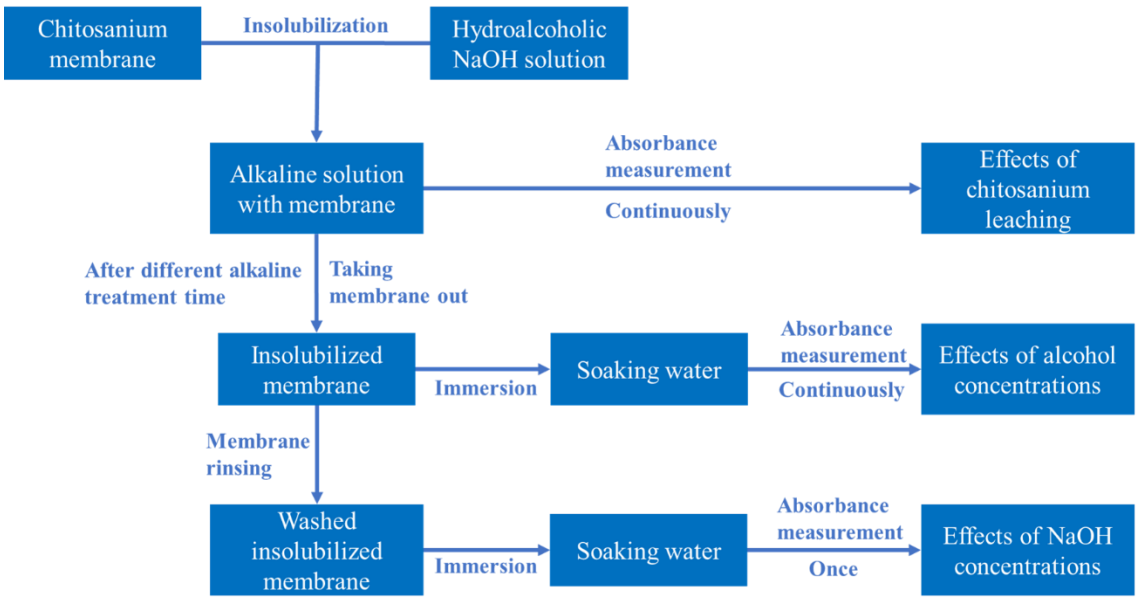

Figure 3.2 Summary of experiments about membrane insolubilization.

Chapter 4
Results and Discussion

4.1 Influence of NaOH Concentrations

In order to evaluate the influence of NaOH concentration used in membrane insolubilization, the alkaline solutions in this experiment were prepared at a fixed ethanol content of 50% (v/v) and different NaOH concentrations. At a given NaOH concentration, membrane was insolubilized for different alkaline treatment time. After insolubilization, the membrane was rinsed to remove residual NaOH and alcohol, and then the rinsed membrane was soaked into deionized water for 3 days, and UV-vis measurements were performed on the soaking water to determine if any chitosanium will leach into the soaking water. The results were shown in Figure 4.1. Figure 4.1a shows the absorbance of the soaking water for membranes treated with alkaline solution containing 0.2 M NaOH. Similarly, Figures 4.1b-e show the absorbance of the soaking water for membranes treated with alkaline solutions at the NaOH concentrations of 0.4, 0.6, 0.8, and 1.0 M, respectively. The data in Figure 4.1 represent the absorbance of the soaking water after the membranes were soaked for 3 days after insolubilization. Those membranes were insolubilized with aqueous ethanol+NaOH solutions containing 50% (v/v) ethanol and different NaOH concentrations for different periods of time (shown in the figure).

For chitosan membranes treated with alkaline solutions at the NaOH concentrations of 0.8 M and 1.0 M, shown in Figure 4.1d and e, no chitosanium was detected in the soaking water when the alkaline treatment time was longer than 2.5 min. Therefore, 2.5 min was considered as the alkaline treatment time required to complete chitosan insolubilization at such NaOH concentrations. Similarly, the time required for complete insolubilization at NaOH concentrations of 0.2, 0.4, and 0.6 M were found to be 10.5, 5.5, and 3 min, respectively.

For membrane insolubilization with alkaline solutions containing 1.0 and 0.8 M NaOH, although the time required for complete insolubilization was essentially the same, a NaOH concentration of 1.0 M was considered to be appropriate due to the lower detected absorbance in the soaking water (as shown in Figure 4.1d and e).

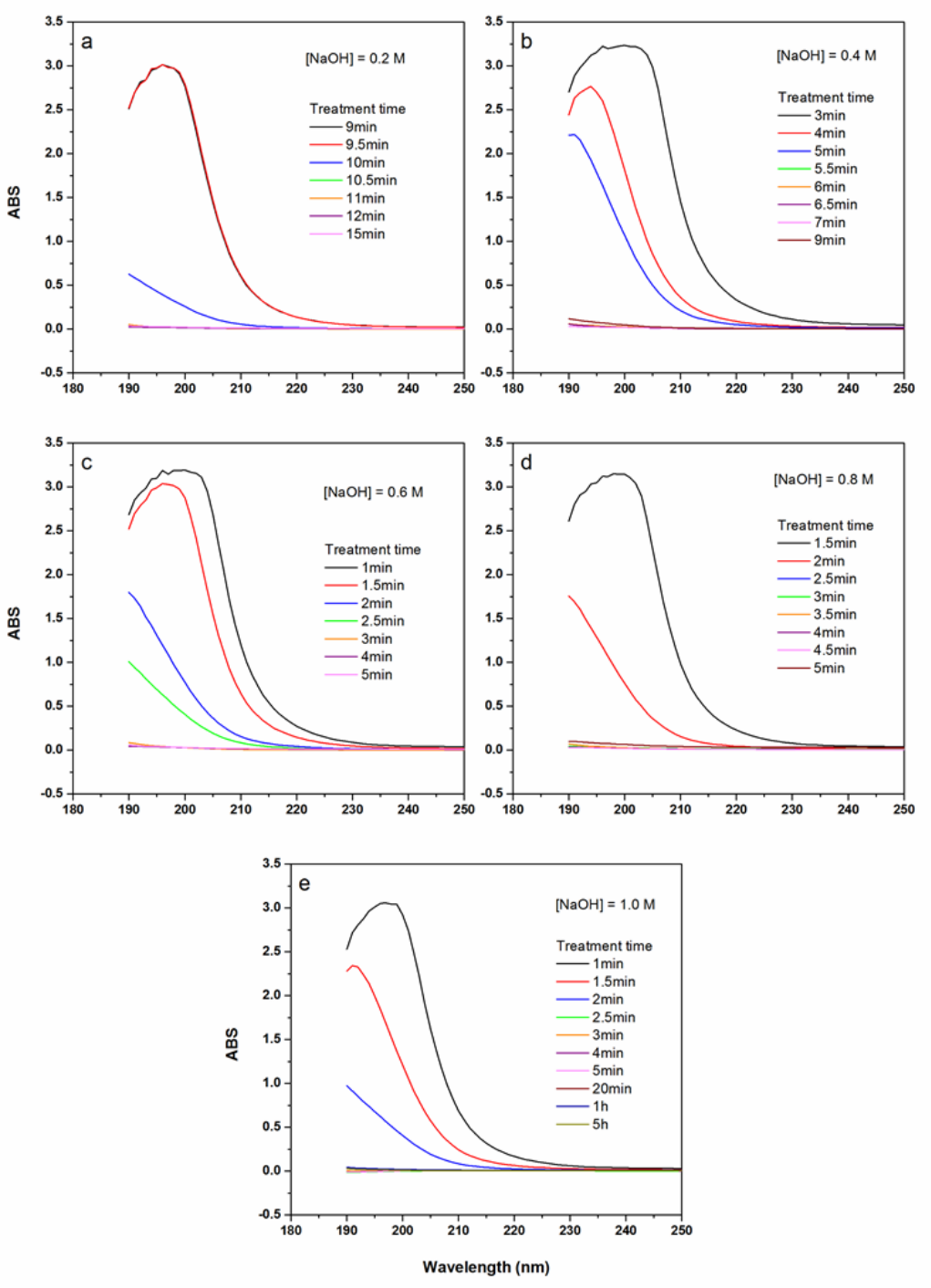

Figure 4.1 The absorbance of the soaking water after 3 days for membranes treated with alkaline solutions containing 50% (v/v) ethanol and NaOH at different concentrations.

Figure 4.2 shows the relationship between the NaOH concentrations in alkaline solutions and the time required for complete insolubilization. As expected, using a high NaOH concentration will reduce the required alkaline treatment time. Moreover, the membrane insolubilization was found quite ineffective at low NaOH concentrations (e.g., 0.2 M and 0.4 M). Therefore, the chitosan insolubilization would better be carried out at a high NaOH concentration to reduce structural defects for practical applications.

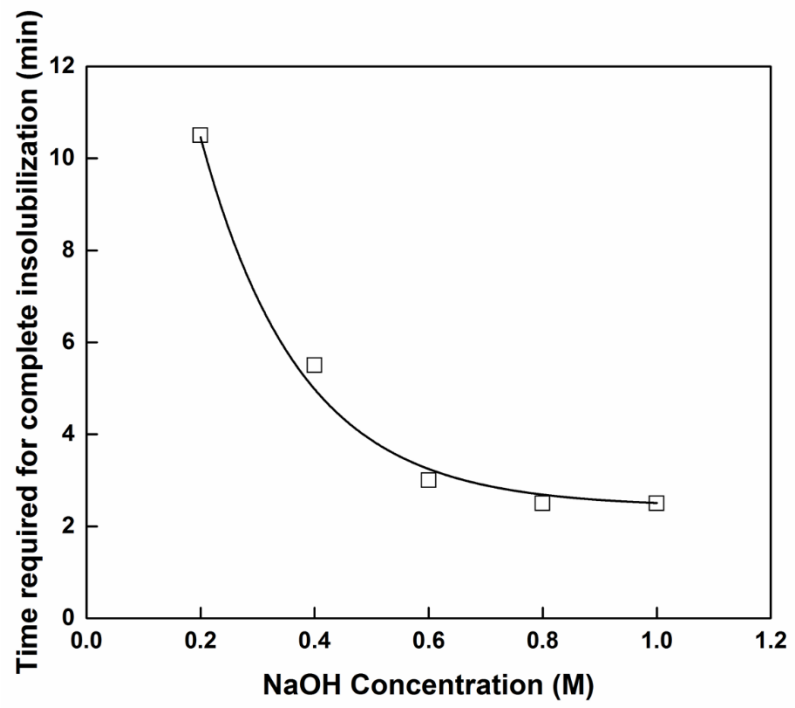

Figure 4.2 Alkaline treatment time required for complete insolubilization at different NaOH concentrations in the alkaline solution (ethanol content at 50% (v/v)).

4.2 Influence of Ethanol Contents in Alkaline Solutions

The effects of ethanol contents in alkaline solutions on membrane insolubilization were determined at a constant NaOH concentration of 1.0 M. At a given ethanol content, chitosan membranes were also treated for different durations. Different from the previous experiment, after chitosan insolubilization, the membranes were immersed into the soaking water without rinsing to minimize the experimental error. The effects of ethanol contents on membrane insolubilization were evaluated based on the time required for complete insolubilization through the absorbance curves of the soaking water.

At different soaking time, the absorbance curves of soaking water were measured using a UV-vis spectrophotometer. Based on the UV-vis measurements of the soaking water for differently treated membranes, the absorbance curves for different ethanol contents and alkaline treatment times were found to have similar tendencies. First of all, absorbance of the soaking water often increased at the beginning of the membrane soaking process (usually 3 minutes) due to the diffusion of residual NaOH and ethanol from the membrane taken from the insolubilization bath. As the soaking time increased, if no chitosanium leached from the membrane to the soaking water, the soaking water absorbance would continue to decrease. However, if the membrane was not fully insolubilized, the diffusion of chitosanium from the membrane to the soaking water would accelerate the decrease in the absorbance during the first few hours because additional reaction with NaOH in soaking water would take place, thereby reducing NaOH concentration in the soaking water. Thus, at a given alkaline treatment time, the existence of untreated chitosanium could be confirmed from the changes in the absorbance of soaking water over the first few hours. This way, the time required for complete insolubilization at a given ethanol content with different alkaline treatment times could be figured out.

The absorbance curves of the soaking water are shown in Figures 4.3-4.9 for an ethanol concentration in the alkaline solution of 2%, 5%, 10%, 20%, 30%, 40%, and 50% (v/v), respectively, whereas the NaOH concentration was at a fixed value of 1.0 M. Each figure is divided for four alkaline treatment times. At a given alkaline treatment time, the absorbance curves represent how the compositions of the soaking water changed with the soaking time, that is, the leaching of ethanol, NaOH and chitosanium (if any) into the soaking water.

Figure 4.3 shows how the absorbance of the soaking water changed with the soaking time after the transferring into the soaking water for the membrane treated with 1.0 M NaOH in an aqueous alcohol solution (water/ethanol 98% (v/v)) for different periods of alkaline treatment time (0.5, 1, 1.5

and 2min). It was shown that for membranes treated with 0.5 min, the absorbance of the soaking water decreased in the first 2.5 hours, which was considered to be caused by the diffusion of untreated chitosanium from the membrane to the soaking water. This indicates that these membranes were only partially insolubilized. When the alkaline treatment time increased to 1 min or more, no chitosanium was found in the soaking water. Therefore, the time required for complete insolubilization with alkaline solution containing 1.0 M NaOH and 2% (v/v) ethanol was considered to be 1 min. Similarly, based on the data shown in Figures 4.4–4.9 for membranes treated with 1.0 M NaOH but the alcohol content was higher (5–50% (v/v)), the alkaline treatment time required for complete insolubilization was found to be ranged from 1 min to 3 min. This is more clearly shown in Figure 4.10, where the alkaline treatment time needed for complete insolubilization of the chitosan membranes was plotted as a function of the ethanol content in the alkaline solution.

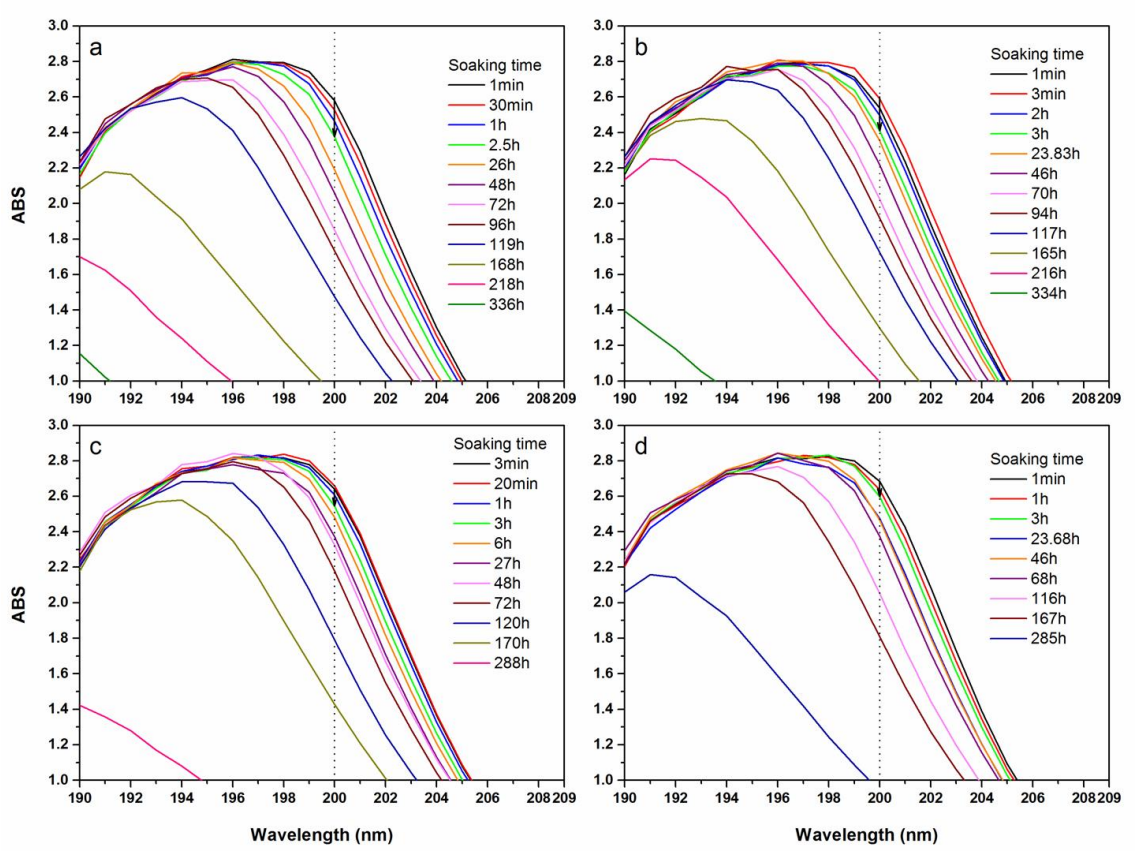

Figure 4.3 The changes in absorbance of soaking water with soaking time for membranes insolubilized with 1.0 M NaOH dissolved in ethanol/water 2% (v/v). Membrane alkaline treatment time: a) 0.5 min, b) 1 min, c) 1.5 min, and d) 2 min.

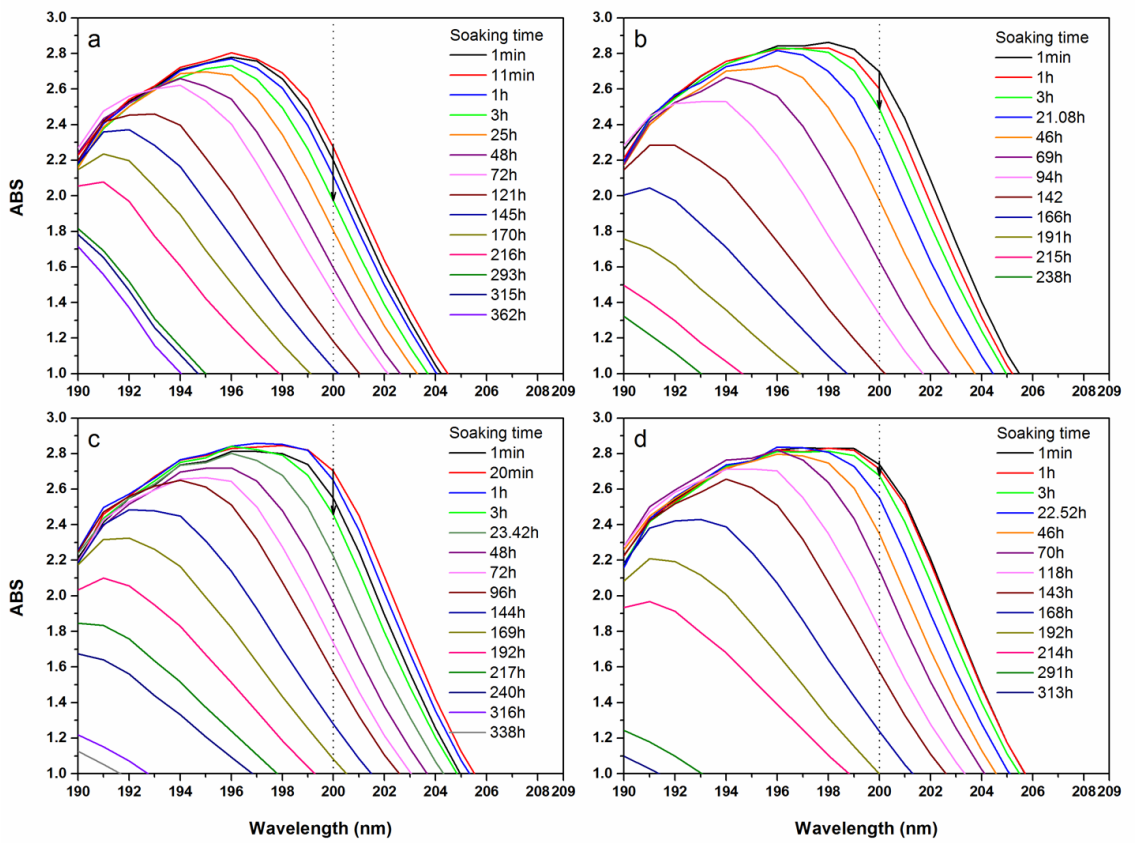

Figure 4.4 The changes in absorbance of soaking water with soaking time for membranes insolubilized with 1.0 M NaOH dissolved in ethanol/water 5% (v/v). Membrane alkaline treatment time: a) 0.5 min, b) 1 min, c) 1.5 min, and d) 2 min.

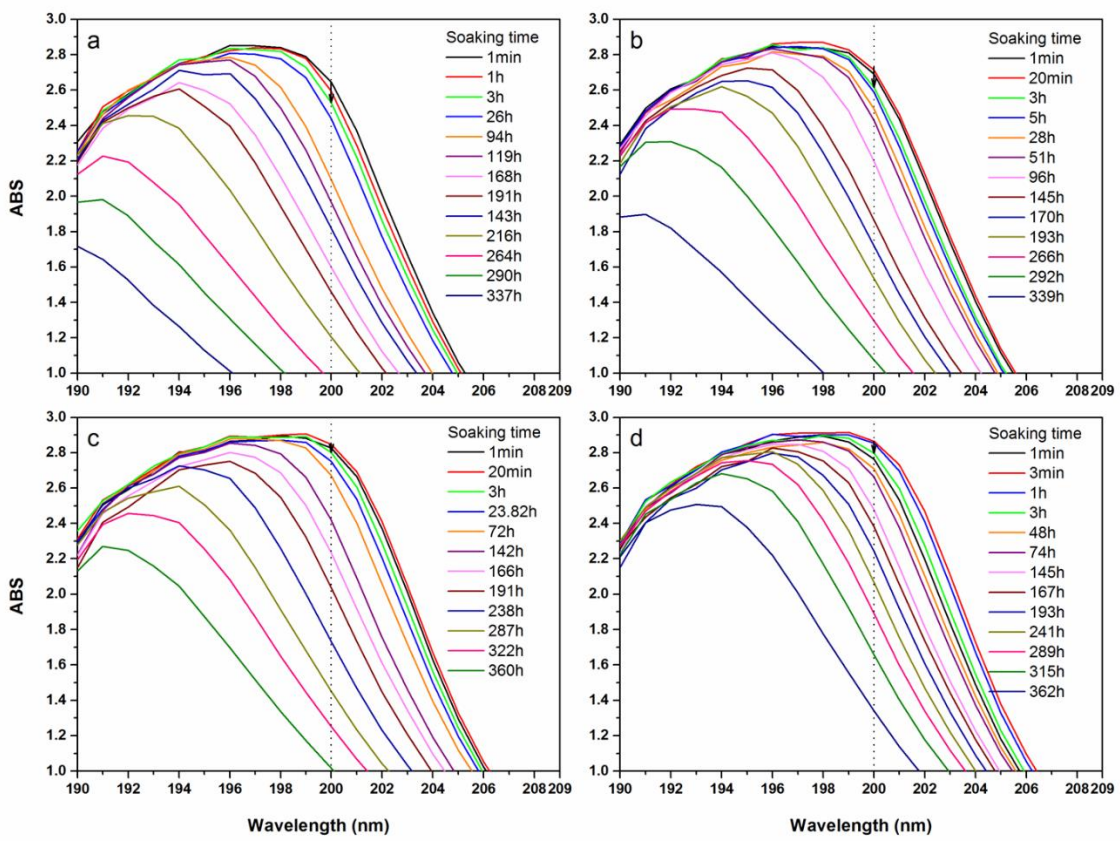

Figure 4.5 The changes in absorbance of soaking water with soaking time for membranes insolubilized with 1.0 M NaOH dissolved in ethanol/water 10% (v/v). Membrane alkaline treatment time: a) 1 min, b) 1.5 min, c) 2 min, and d) 2.5 min.

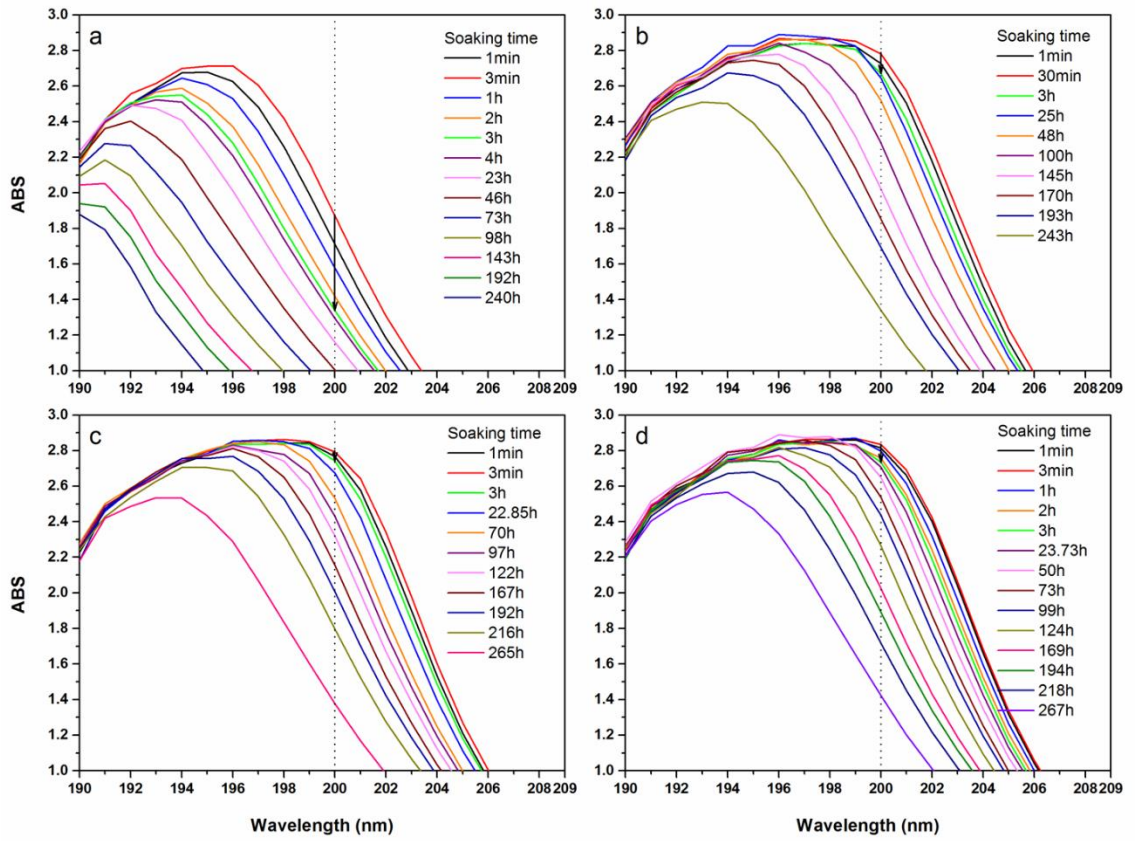

Figure 4.6 The changes in absorbance of soaking water with soaking time for membranes insolubilized with 1.0 M NaOH dissolved in ethanol/water 20% (v/v). Membrane alkaline treatment time: a) 1 min, b) 1.5 min, c) 2 min, and d) 2.5 min.

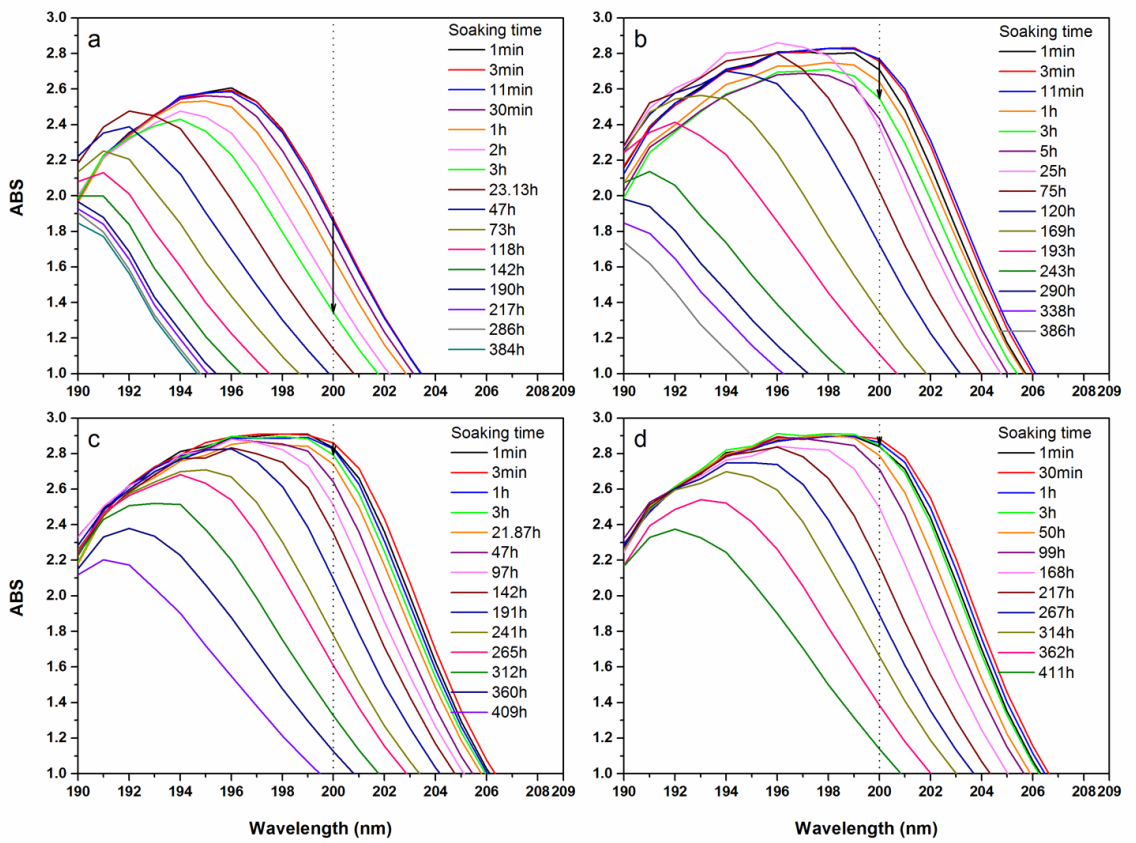

Figure 4.7 The changes in absorbance of soaking water with soaking time for membranes insolubilized with 1.0 M NaOH dissolved in ethanol/water 30% (v/v). Membrane alkaline treatment time: a) 1 min, b) 1.5 min, c) 2 min, and d) 2.5 min.

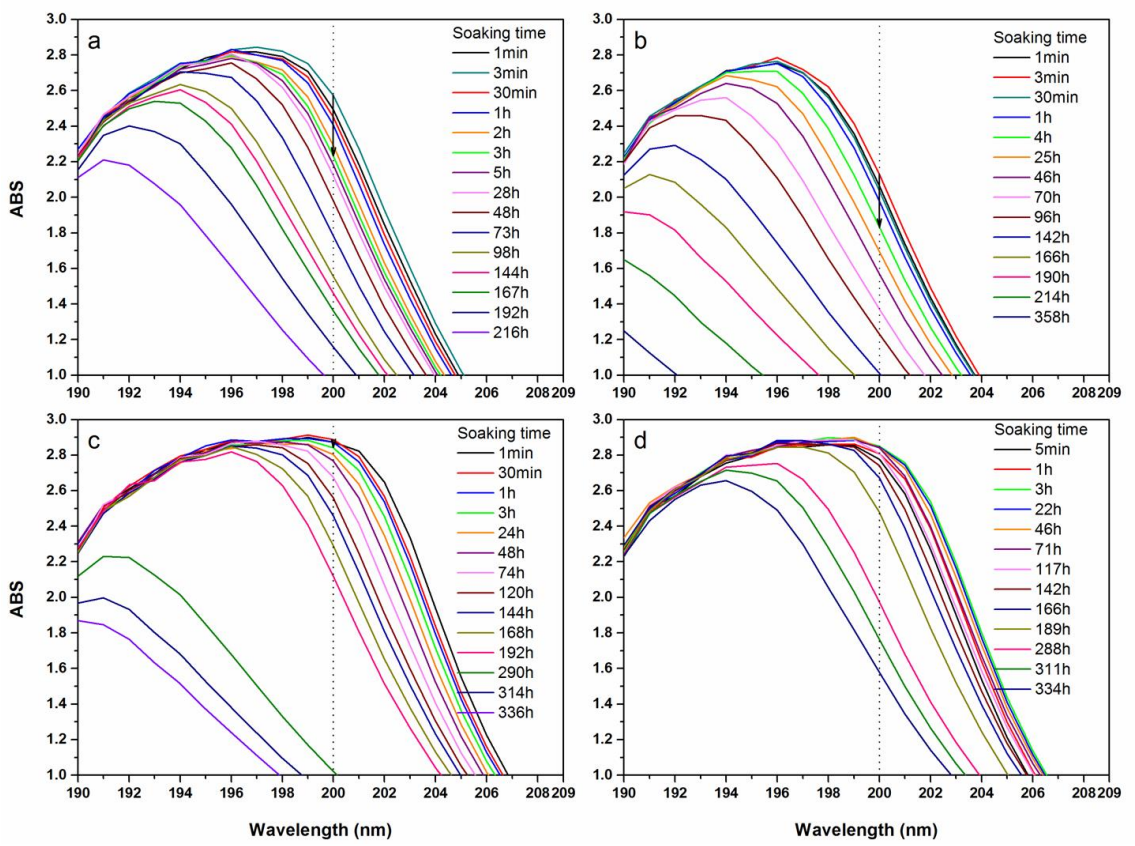

Figure 4.8 The changes in absorbance of soaking water with soaking time for membranes insolubilized with 1.0 M NaOH dissolved in ethanol/water 40% (v/v). Membrane alkaline treatment time: a) 1.5 min, b) 2 min, c) 2.5 min, and d) 3 min.

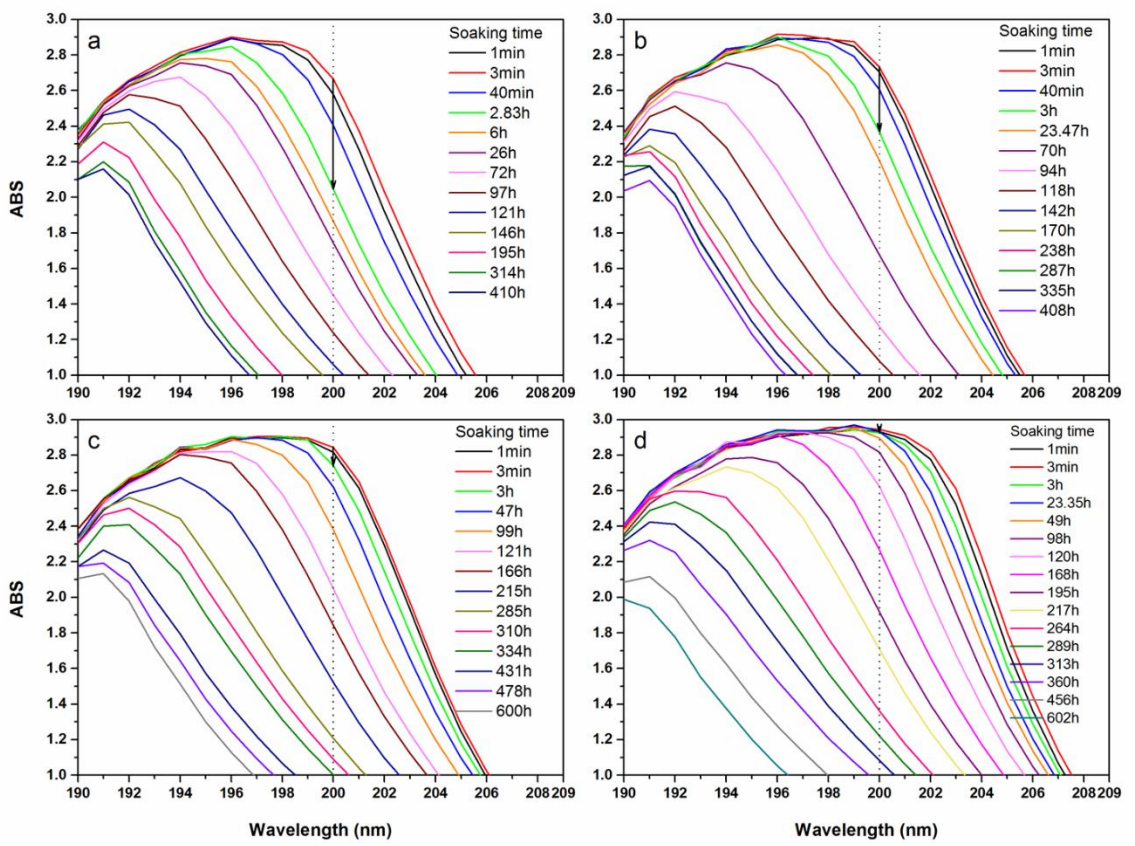

Figure 4.9 The changes in absorbance of soaking water with soaking time for membranes insolubilized with 1.0 M NaOH dissolved in ethanol/water 50% (v/v). Membrane alkaline treatment time: a) 2 min, b) 2.5 min, c) 3 min, and d) 4 min.

Generally, shown in Figure 4.10, a high content of ethanol in the alkaline solution lowers the effectiveness to insolubilize the chitosanium membranes. This phenomenon may result from the higher solubility of NaOH in water than that in ethanol so that NaOH becomes more active in the alkaline solution with a high content of water. On the other hand, while being used to prevent chitosanium from being diffused into the alkaline solution during the chitosan insolubilization process, ethanol can also capture the positively charged amino groups and make them hard to be deprotonated by NaOH. In addition, acetic acid has a good solubility in ethanol and can be partially absorbed and trapped in ethanol [29]. Therefore, during the chitosan insolubilization process, chitosan-combined acetic acid in the membrane would be consumed quickly but the ethanol-trapped acetic acid would be released and

consumed gradually, which would hinder the neutralization reaction between NaOH and acetic acid. Moreover, for an ethanol content of 10% (v/v) or less in the alkaline solution, the time needed for complete insolubilization of chitosan does not change significantly.

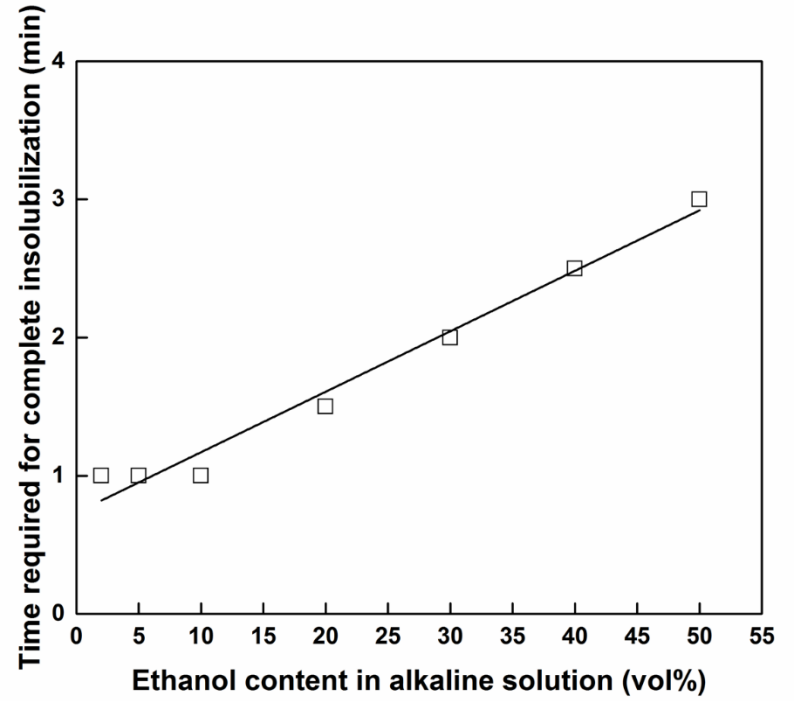

Figure 4.10 Time required for complete insolubilization at different ethanol content in the alkaline treatment solution. NaOH concentration: 1.0 M.

While it was difficult to quantify the changes in the absorbance of the soaking water, which was determined by the alkaline solution composition and alkaline treatment time used for membrane insolubilization, an attempt was made to look at the absorbance changes on a relative scale for different ethanol contents in the alkaline solution and alkaline treatment times. This was done by using a percentage change in the absorbance (at 200 nm) of the soaking water over 3 hours of soaking time (as the arrows shown in Figures 4.3–4.9). For each alkaline treatment time and ethanol content in the alkaline solution used in chitosan insolubilization, the absorbance change of the soaking water was calculated with the function:

$$\text{Absorbance change} = \frac{\text{Absorbance of the soaking water at 200 nm after 3 h}}{\text{Highest absorbance at 200 nm over the soaking process}}$$

Here a wavelength of 200 nm was selected because there was a significant change in the absorbance at this wavelength during the soaking period. And a soaking time of 3 h was selected because the absorbance changes over 3 h was found to be different between the soaking water of fully insolubilized chitosan membranes and partially insolubilized chitosan membranes.

The results were presented in

Table 4.1, where the aforementioned absorbance change for different alkaline treatment times and ethanol contents in the alkaline solution can be compared on a relative scale. Generally, at a given content of ethanol in the alkaline solution, the absorbance change was found to decrease dramatically at the time required for complete insolubilization gained from Figures 4.3-4.9, indicating that the similar relationship between the time required for complete insolubilization and the content of ethanol in alkaline solution was found in

Table 4.1.

Table 4.1 Absorbance changes in soaking water at the wavelength of 200 nm after 3 h soaking for different ethanol contents and alkaline treatment times.

Ethanol content v/v	50%	40%	30%	20%	10%	5%	2%
0.5 min						13.88%	10.17%*
1 min			28.53%	28.62%	4.42%	7.92%	7.59%
1.5 min		13.25%	8.11%	4.30%	4.25%	9.40%	5.00%
2 min	24.59%*	17.03%*	2.41%	2.04%	1.89%	2.97%	3.28%
2.5 min	13.26%	1.63%	1.62%	3.56%	2.54%		
3 min	3.62%	0.04%					
4 min	1.15%						

Note that values marked with "*" are calculated using the absorbance of soaking water with a soaking time of ~3 h.

The data in

Table 4.1 and Figure 4.10 shows that, at a fixed NaOH concentration in the alkaline solution, a high content of ethanol lowered the effectiveness of membrane insolubilization. As a result, a chitosan insolubilization should be carried out using an alkaline solution containing a low ethanol content (but the ethanol content should not be too low in order to minimize chitosanium leaching from the membrane).

4.3 Influence of Methanol Contents in Alkaline Solutions

For the purpose of chitosan insolubilization, in addition to ethanol, methanol may also be appropriate for use in alkaline solutions. To evaluate the effects of the methanol content in alkaline solution on chitosan insolubilization, experiments were carried out for insolubilization of chitosan membranes using NaOH aqueous alcoholic solutions at a constant NaOH concentration of 1.0 M and different methanol contents in the range from 2% to 50% (v/v). After alkaline treatment, for a given period of time, the membrane was placed in the soaking water, and the absorbance of the soaking water was measured periodically to find out the alkaline treatment time required at different methanol contents to complete insolubilization of chitosan membrane.

Figures 4.11-4.17 show the absorbance of the soaking water of the membrane insolubilized with 1.0 M NaOH dissolved in water/methanol mixtures containing 2%, 5%, 10%, 20%, 30%, 40%, and 50% (v/v) of methanol, respectively.

Similar to the alkaline treatment in ethanol/water solutions, the time required for complete insolubilization was found to be in the range of 1 to 3.5 min where the methanol content in the alkaline solution was 2-50% (v/v). This is more clearly shown in Figure 4.18, where the time required for complete insolubilization is plotted against the methanol concentration in the alkaline solution.

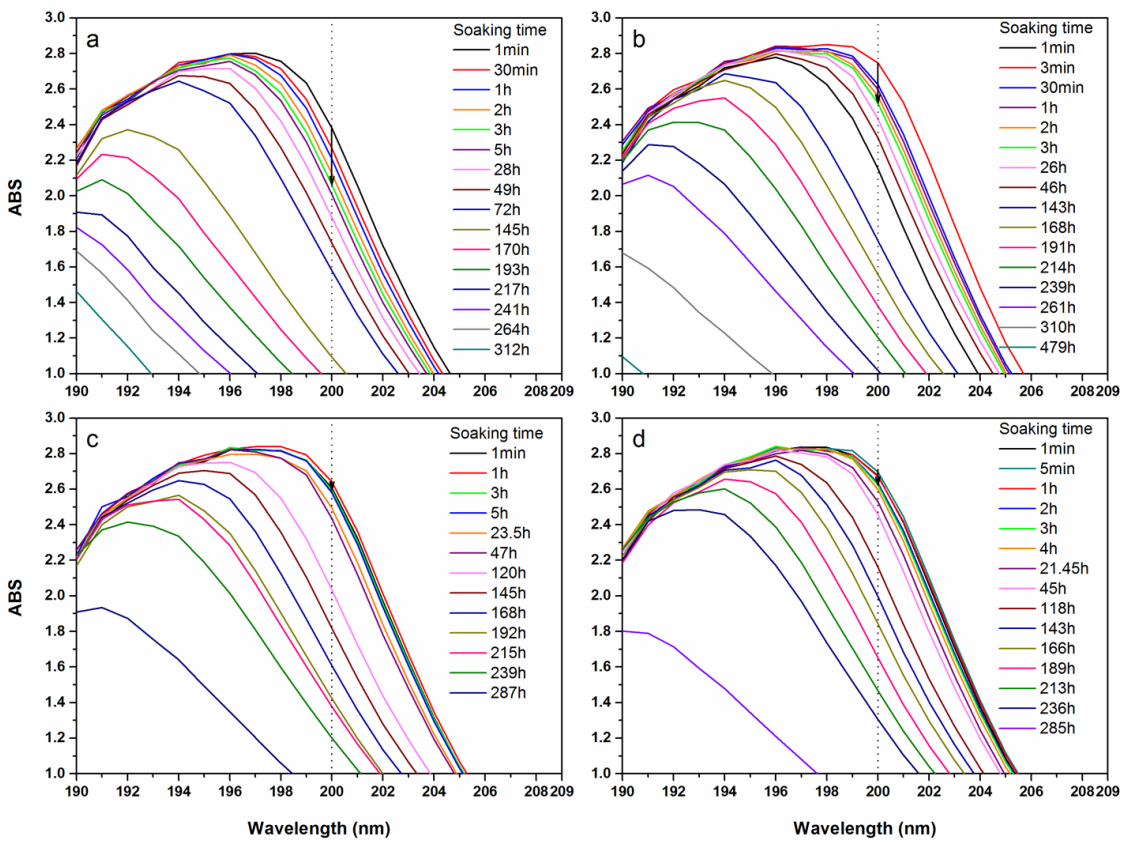

Figure 4.11 The changes in absorbance of soaking water with soaking time for membranes insolubilized with 1.0 M NaOH dissolved in methanol/water 2% (v/v). Membrane alkaline treatment time: a) 0.5 min, b) 1 min, c) 1.5 min, and d) 2 min.

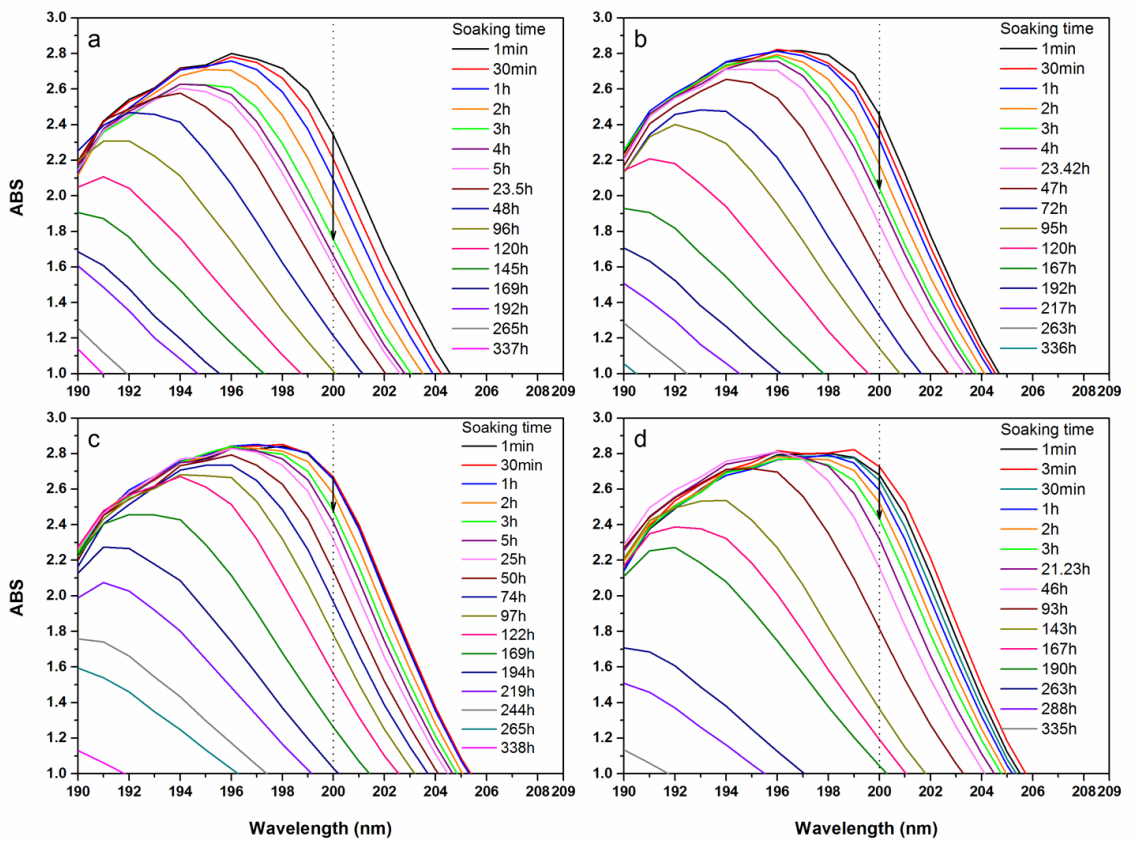

Figure 4.12 The changes in absorbance of soaking water with soaking time for membranes insolubilized with 1.0 M NaOH dissolved in methanol/water 5% (v/v). Membrane alkaline treatment time: a) 0.5 min, b) 1 min, c) 1.5 min, and d) 2 min.

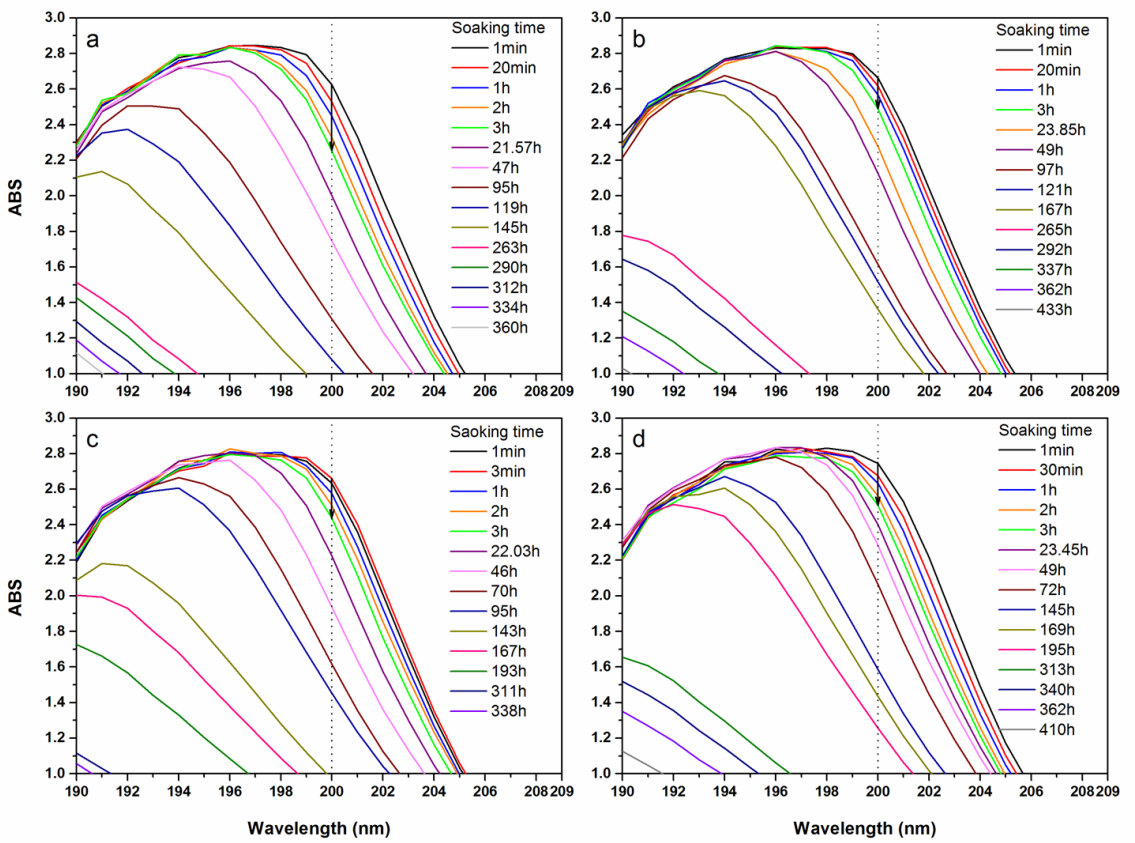

Figure 4.13 The changes in absorbance of soaking water with soaking time for membranes insolubilized with 1.0 M NaOH dissolved in methanol/water 10% (v/v). Membrane alkaline treatment time: a) 1 min, b) 1.5 min, c) 2 min, and d) 2.5 min.

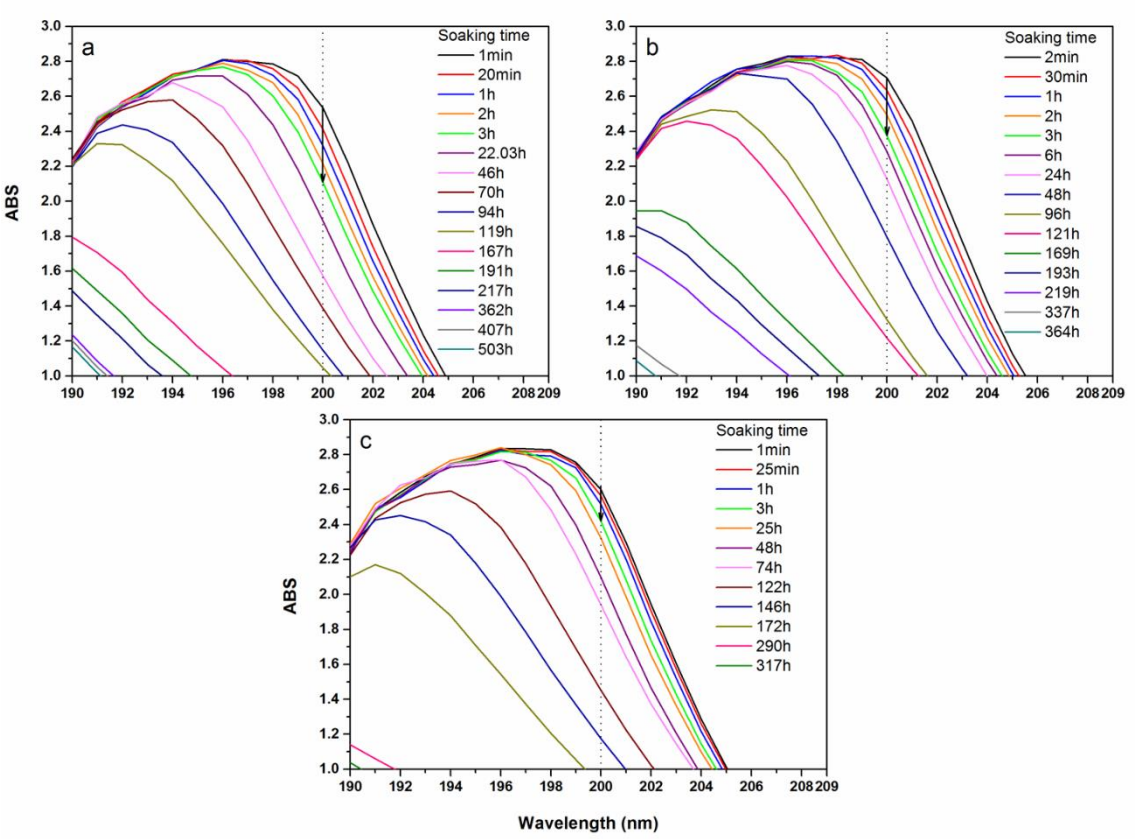

Figure 4.14 The changes in absorbance of soaking water with soaking time for membranes insolubilized with 1.0 M NaOH dissolved in methanol/water 20% (v/v). Membrane alkaline treatment time: a) 1.5 min, b) 2 min, and c) 2.5 min.

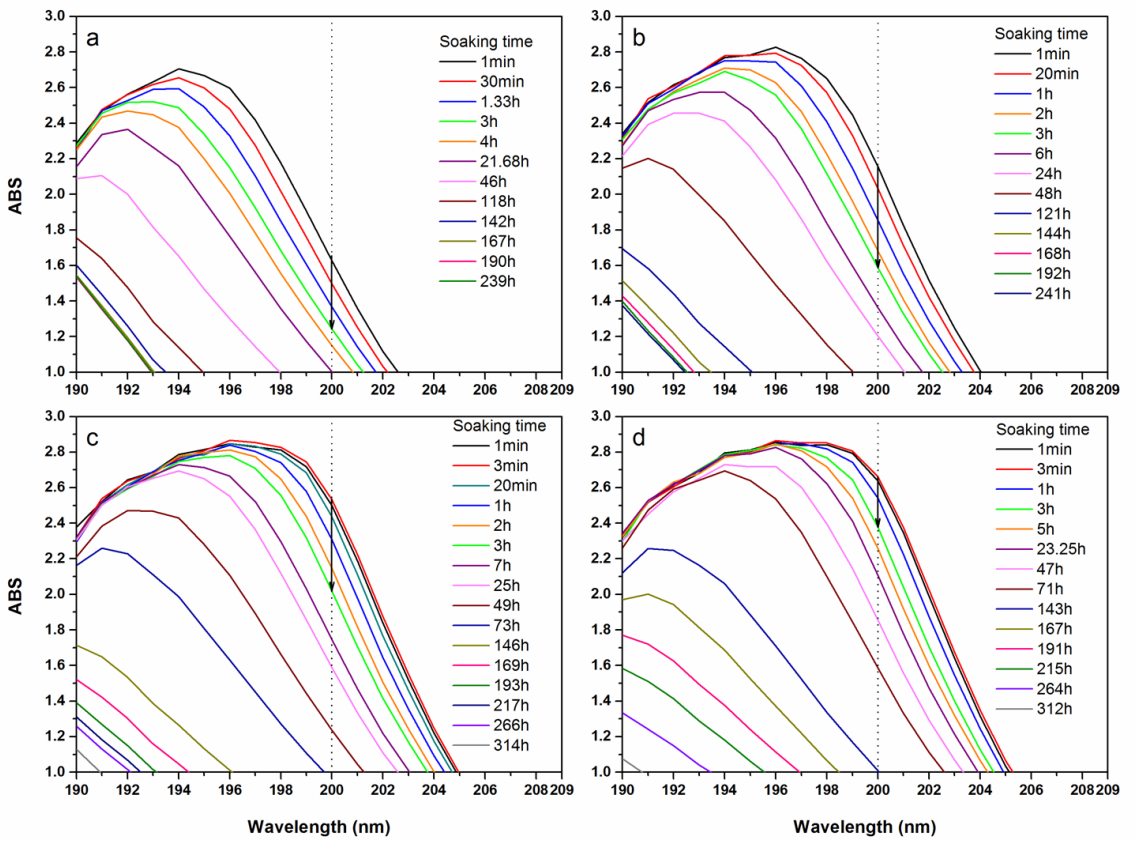

Figure 4.15 The changes in absorbance of soaking water with soaking time for membranes insolubilized with 1.0 M NaOH dissolved in methanol/water 30% (v/v). Membrane alkaline treatment time: a) 1 min, b) 1.5 min, c) 2 min, and d) 2.5 min.

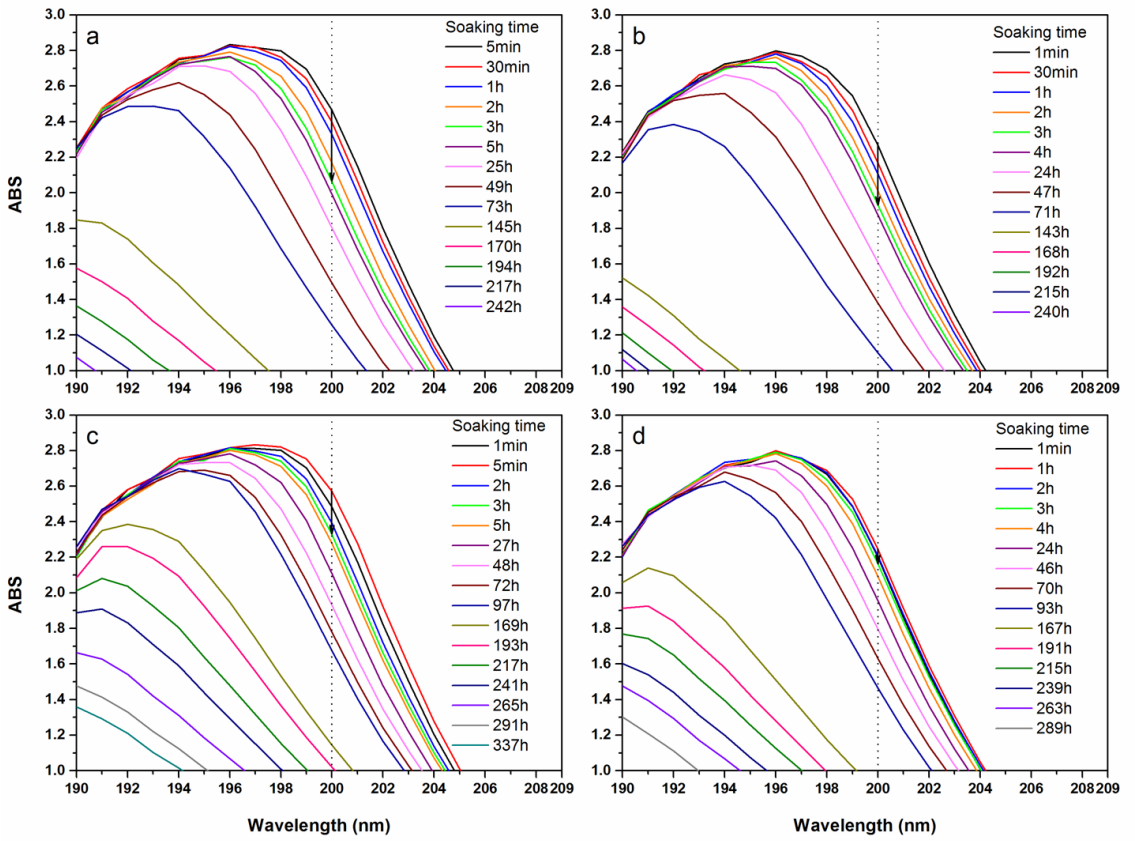

Figure 4.16 The changes in absorbance of soaking water with soaking time for membranes insolubilized with 1.0 M NaOH dissolved in methanol/water 40% (v/v). Membrane alkaline treatment time: a) 1.5 min, b) 2 min, c) 2.5 min, and d) 3 min.

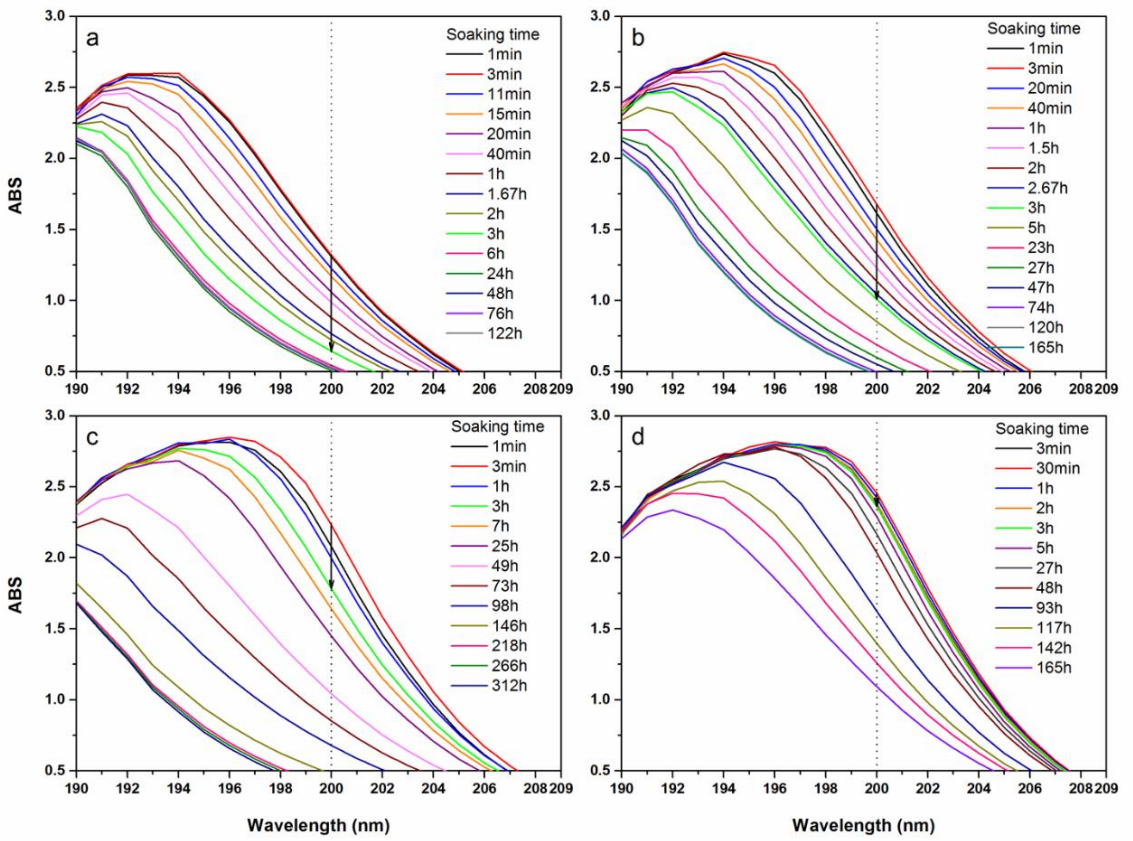

Figure 4.17 The changes in absorbance of soaking water with soaking time for membranes insolubilized with 1.0 M NaOH dissolved in methanol/water 50% (v/v). Membrane alkaline treatment time: a) 2 min, b) 2.5 min, c) 3 min, and d) 3.5 min.

Similar to adding ethanol to the aqueous alkaline solution, the use of methanol in chitosan insolubilization was found to be effective. However, with an increase in the methanol concentration, the time required to completely insolubilize chitosan membrane increased. As methanol molecule is smaller than ethanol, it is expected that methanol will diffuse from the membrane to the soaking water more quickly than ethanol. The data in Figure 4.18 also show that reducing the methanol content in the alkaline solution will reduce the time required for complete insolubilization. However, too little methanol is not accepted as the chitosan membrane will dissolve in the alkaline solution to a great extent during the insolubilization process.

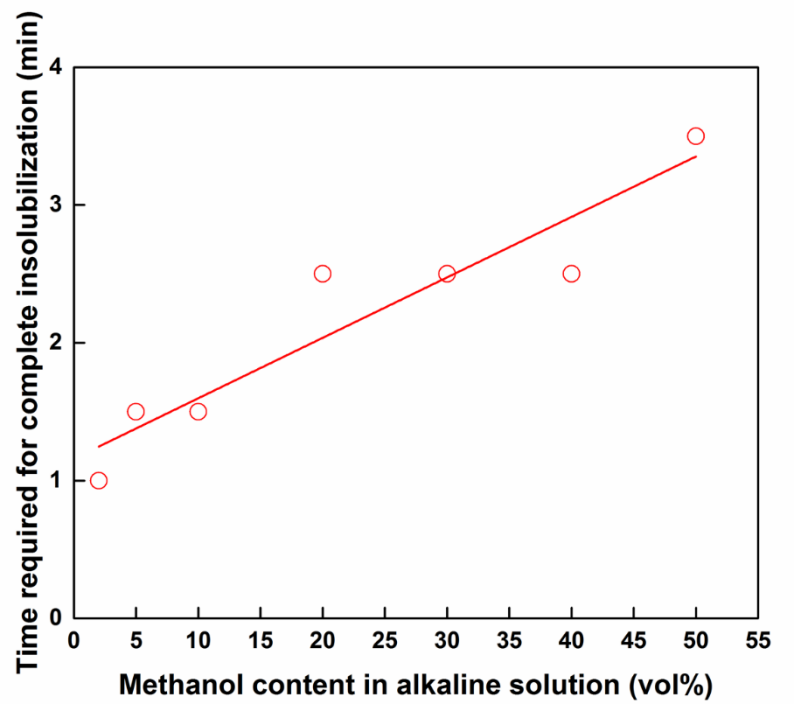

Figure 4.18 Time required for complete insolubilization at different methanol content in the alkaline treatment solution. NaOH concentration: 1.0 M.

Table 4.2 shows the absorbance change (at 200 nm, 3 h) of the soaking water for methanol-insolubilized membranes for easy comparison, in similar fashion as shown in Table 4.1 for alkaline solution containing ethanol. Similar to the results shown in Table 4.1, for different contents of methanol in the alkaline solution, the absorbance change decreased dramatically at the time required for complete insolubilization gained from Figures 4.11-4.17. Therefore, a high content of methanol in the alkaline solution was also found to lower the effectiveness of chitosan insolubilization.

As a result, the insolubilization of chitosan membrane should be carried out with the alkaline solution containing a low content of methanol. However, alcohol is needed in the alkaline solution, in the absence of alcohol, chitosan membrane will be partially disintegrated in the solution.

Table 4.2 Absorbance changes in soaking water at the wavelength of 200 nm after 3 h soaking for different methanol contents and alkaline treatment times.

Methanol content v/v	50%	40%	30%	20%	10%	5%	2%
0.5 min						25.48%	13.43%
1 min					14.92%*	17.50%	8.22%
1.5 min		16.30%	26.39%	16.80%	6.85%	8.93%	3.03%
2 min	51.21%	15.12%	20.24%	12.16%	8.48%	10.79%	3.25%
2.5 min	39.92%	9.41%	10.64%	7.70%	8.76%		
3 min	20.15%	6.60%	11.62%				
3.5 min	4.02%						

Note that values marked with "*" are calculated using the absorbance of soaking water with a soaking time of ~3 h.

4.4 Comparison: Ethanol vs. Methanol

Chitosan insolubilization by alkaline treatment was found to be affected by the presence of ethanol and methanol since the alkaline treatment time required for complete insolubilization is related to the alcohol content in the alkaline solution. To better look into the difference between the effects of ethanol and methanol in alkaline solution for chitosan insolubilization, the alkaline treatment time required for ethanol and methanol is compiled in Figure 4.19 for easy comparisons.

At a given alcohol content (by volume), shown in Figure 4.19, chitosan insolubilization with ethanol-containing alkaline solutions tended to complete faster than that with methanol-containing alkaline solutions. This seems to indicate that ethanol is better than methanol used in the alkaline solution for deprotonating the chitosanium membrane. At a NaOH concentration of 1.0 M, an alkaline treatment time of ~1 min was observed when the ethanol content in the alkaline solution was below 10% (v/v), whereas a lower methanol content of 2% (v/v) was found to be adequate to achieve the same complete chitosan insolubilization in 1 min.

As shown in

Table 4.1 and Table 4.2, for the same alcohol content in the alkaline solution and alkaline treatment time, a lower absorbance of the soaking water relative to the highest absorbance was observed for membranes insolubilized with NaOH/ethanol/water than NaOH/methanol/water. With the similar UV-vis absorptivity between NaOH/ethanol/water and NaOH/methanol/water (see Appendix C), the higher effectiveness of the ethanol-containing alkaline solution rather than methanol-containing alkaline solution was indicated.

The difference in effectiveness of the two alcohols used in alkaline solutions for chitosan membrane insolubilization is considered to be due to their different polarities. As mentioned before, ethanol and methanol are used to prevent untreated chitosanium from being quickly dissolved in the solution by capturing the chitosan-combined acetic acids [29]. With its high polarity, methanol has stronger miscibility with acetic acid than ethanol. Then it takes more effort for NaOH to neutralize the methanol-captured acetic acid during the alkaline treatment of chitosan membraens. Moreover, when dissolved into a hydroalcoholic acetic acid solution, chitosan was found to be highly soluble in the solution with a high polarity due to the low dissolution heat and high solvation of amino groups or hydroxyl groups [35]. Therefore, compared with the ethanol-containing alkaline solution, chitosanium membrane are more soluble in the methanol-containing alkaline solution at the same concentration of NaOH and alcohol, resulting in chitosanium leaching during the chitosan insolubilization process.

Generally, ethanol was shown to be better than methanol in the alkaline solution for chitosan insolubilization.

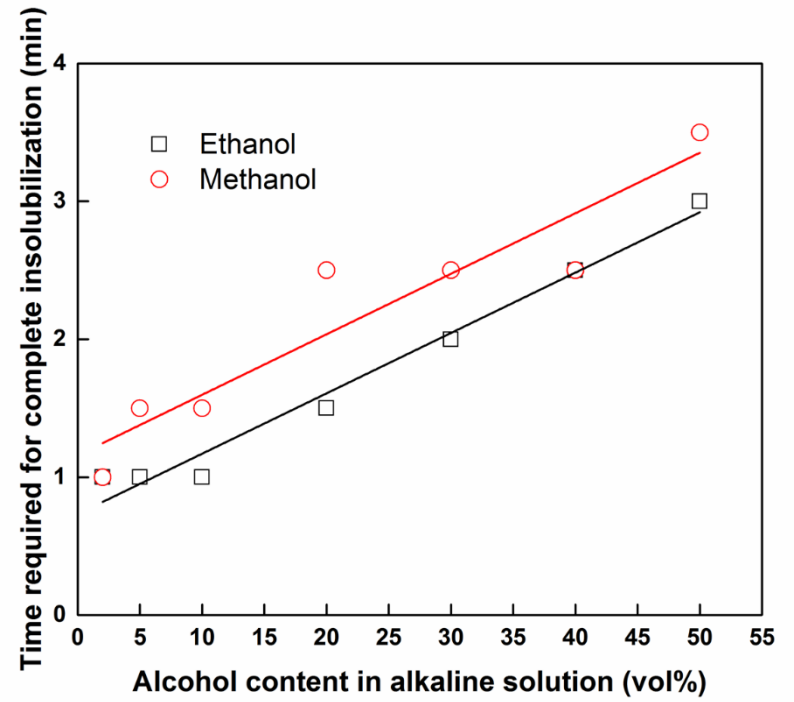

Figure 4.19 Time required for complete insolubilization at different alcohol contents in the alkaline treatment solution. NaOH concentration: 1.0 M.

4.5 Analysis of the Absorbance Decrease in the Soaking Water

During the UV-vis measurements on the soaking water for membranes insolubilized with alkaline solutions at different ethanol/methanol contents, the absorbance was found to slightly decrease with an increase in the soaking time after 24 hours, at which point all the untreated chitosanium (if present) would have been diffused out of the soaked membrane. This decrease in absorbance of the soaking water was considered to be due to the consumption of NaOH and the evaporation loss of alcohol.

In order to confirm whether the soaked chitosan membrane would affect the absorbance decrease in the soaking water, an experiment was conducted to measure the absorbance in the soaking water without the membrane (membrane was removed before the measurement). The membrane was treated by alkaline solution with 1.0 M NaOH and 50% (v/v) ethanol for 3 min to make sure that no chitosanium would leach from the membrane to the soaking water. After being immersed into soaking water for 3 min, the membrane will be removed to a storage and the UV-vis absorbance of the soaking

water was monitored. The absorbance of the soaking water for different times after the membrane was removed was shown in Figure 4.20. Each of the curves in the figure represents the absorbance of the soaking water at a certain time after the membrane was removed. A decrease in the absorbance of the soaking water was observed with an increase in the time. In general, the absorbance curves in Figure 4.20 had a similar trend as that observed for complete insolubilization of chitosan membranes in previous studies, indicating that the fully insolubilized membranes had a neglectable impact on the decrease in the absorbance of the soaking water.

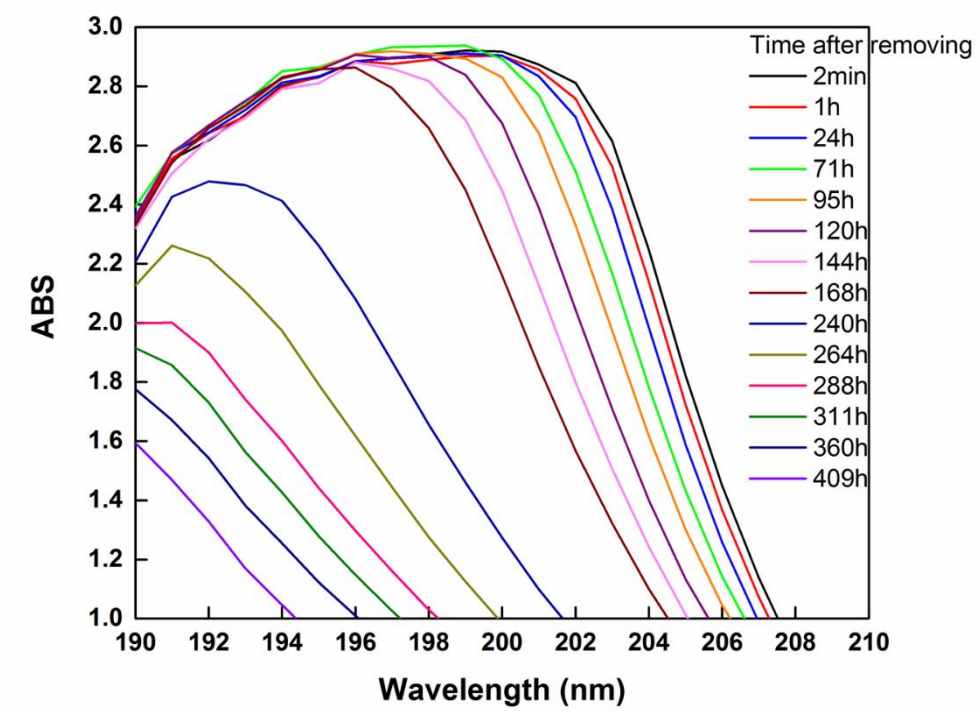

Figure 4.20 The change in absorbance of the soaking water with time. The absorbance was measured after the membrane was removed from the soaking water.

To find the cause of the decrease in the absorbance of the soaking water, both dilute aqueous NaOH and dilute aqueous ethanol solutions were exposed to air and their absorbance was measured over a period of time using the UV-vis spectrophotometer. The aqueous NaOH solution had a NaOH concentration of 0.01 M, and the aqueous ethanol solution had 4.8% (v/v) of ethanol. These two

solutions were exposed to air and measured periodically, and the results are shown in Figure 4.21 and Figure 4.22, respectively. In Figure 4.22, the change of the solution absorbance was found not as obvious as that in Figure 4.21, indicating that the decrease in the absorbance of the soaking water caused by the evaporation loss of ethanol was relatively small. The decrease in the absorbance in Figure 4.22 mainly occurred at a wavelength of 192 nm, which is far different from the wavelength used in monitoring absorbance of the soaking water. Therefore, the effects from the evaporation loss of ethanol on the decrease in the absorbance of the soaking water were neglectable. As shown in Figure 4.21, the absorbance change with time was similar to that of soaking water in the previous experiments. Therefore, it may be concluded that the decrease in the absorbance of the soaking water was mostly attributed to the consumption of NaOH. The residual NaOH in the soaking water was consumed by carbon dioxide in the air. When NaOH was converted to $NaHCO_3$, the absorbance of the solution decreased gradually.

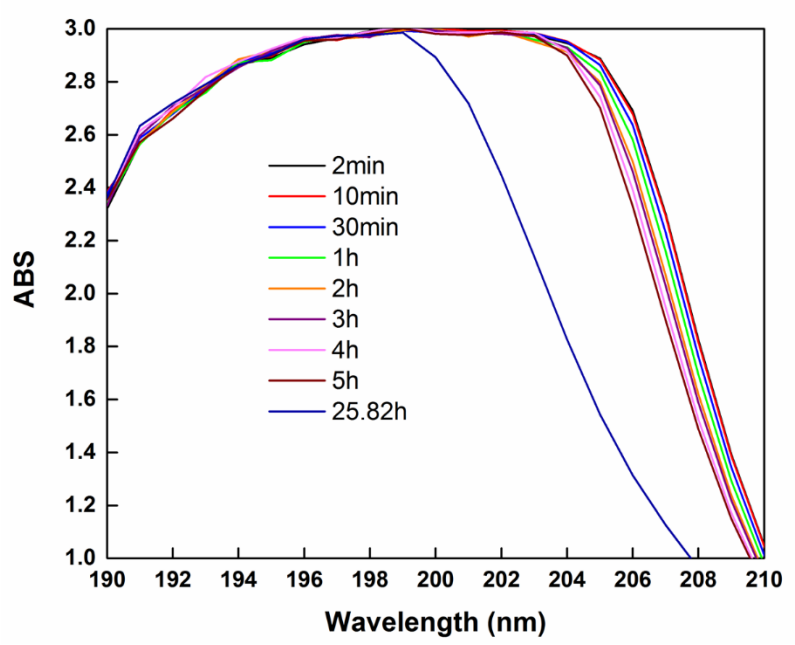

Figure 4.21 The absorbance change of 0.01M aqueous NaOH solution with time when the solution is exposed to air.

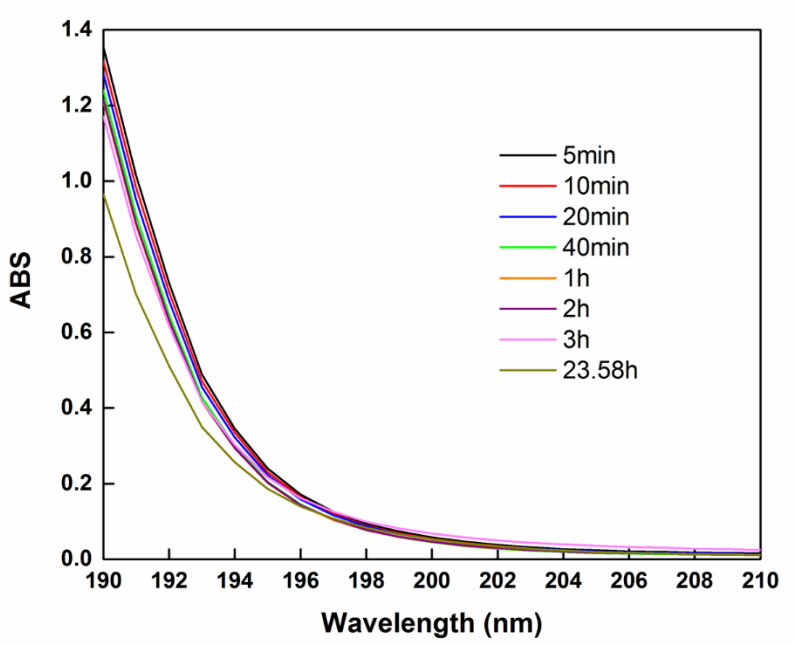

Figure 4.22 The absorbance change of 4.8% (v/v) ethanol aqueous solution with time when the solution is exposed to air.

4.6 Chitosanium Leaching During Membrane Insolubilization

The previous studied looked into how the absorbance of the soaking water changed after alkaline-treated chitosan membrane was transferred to the soaking water. More specifically, it provided an insight into how any residue species went to the soaking water from the membrane after alkaline treatment. While this was useful, it did not tell if any chitosanium entered the insolubilization bath during the alkaline treatment of the membrane for insolubilization. In principle, the as cast membrane was chitosanium membrane, and at least a small portion of the chitosanium would highly likely be dissolved in the alkaline solution. Though the presence of an alcohol would reduce chitosanium dissolution, a complete elimination of chitosanium leaching from the membrane would be difficult unless the conversion from ionic chitosanium to water-insoluble chitosan by chemical reaction with an alkaline would be taking place on the membrane surfaces quickly to that the macromolecular chitosanium would not be able to diffuse through the chitosan portion of the membrane near the

membrane surfaces. It was therefore of interest to investigate the leaching of chitosanium (if any) from the cast membrane into the alkaline solution during the alkaline treatment.

To evaluate the effects of alcohol in the alkaline solution on preventing chitosanium from being dissolved from the membrane, the leaching of the chitosanium in the chitosan membrane not insolubilized yet was detected during the alkaline treatment of chitosan. Different from previous experiments, the UV-vis measurement was carried out on the alkaline solution and the absorbance of the alkaline solution before the alkaline treatment of chitosan membrane (original alkaline solution) was measured for benchmarking. By expressing the difference in absorbance of the alkaline solution before and after the membrane was immersed into this solution, the increase in absorbance of the alkaline solution would be an indication of the chitosanium leaching.

4.6.1 Chitosanium Leaching in Alcohol-free Aqueous Alkaline Solutions

The curves in Figure 4.23 represent the value of change in absorbance of the alkaline solution (no alcohol) at different wavelengths compared with the original alkaline solution. The absorbance peak at ~228 nm was an indication of the leaching of chitosanium to the alkaline solution during membrane insolubilization. For the alkaline treatment time from 1 to 3 min, the change in absorbance of the alkaline solution increased as chitosanium was gradually leaching out from the membrane. However, when the alkaline treatment time was long enough, chitosan insolubilization was completed and there was no additional chitosanium entering the alkaline solution, reaching a constant absorbance in the alkaline solution. Clearly, chitosanium leaching during membrane insolubilization was an issue if the alkaline solution was alcohol-free. It has also been reported that membranes insolubilized with an aqueous 5.0 M NaOH solution for 3 h were defective [28], presumably due to chitosanium leaching that resulted in structural defects in the membrane so prepared.

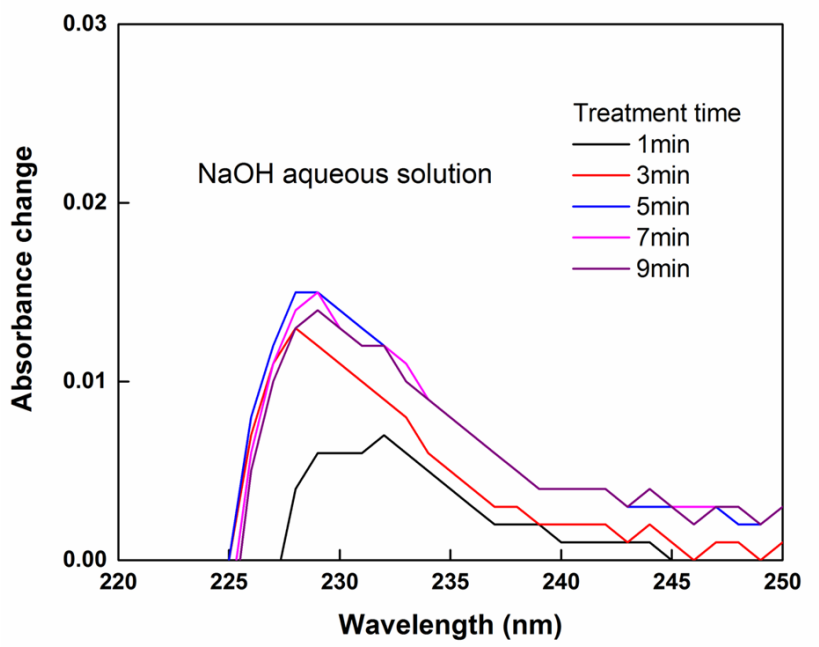

Figure 4.23 The absorbance change of the alkaline solution during alkaline treatment of membrane (alcohol free). Alkaline solution composition: 1.0 M NaOH in water.

4.6.2 Chitosanium Leaching in Aqueous Alkaline Solutions Containing Alcohol

The absorbance change of the alkaline solution during the chitosan insolubilization was determined to evaluate the chitosanium leaching. The results were shown in Figure 4.24 for alkaline solutions containing 1.0 M NaOH and ethanol at the content ranged from 2% to 15% (v/v). The leaching of chitosanium was found to have occurred only slightly at an ethanol content of 2% (v/v). Compared with results for chitosan insolubilization using the alcohol-free alkaline solution, it appeared that the diffusion and leaching of chitosanium from the membrane was reduced by adding ethanol into the NaOH aqueous solution. At an ethanol concentration higher than 5% (v/v) in the alkaline solution, the chitosanium leaching was considered neglectable and no peak was observed in the curves of absorbance change.

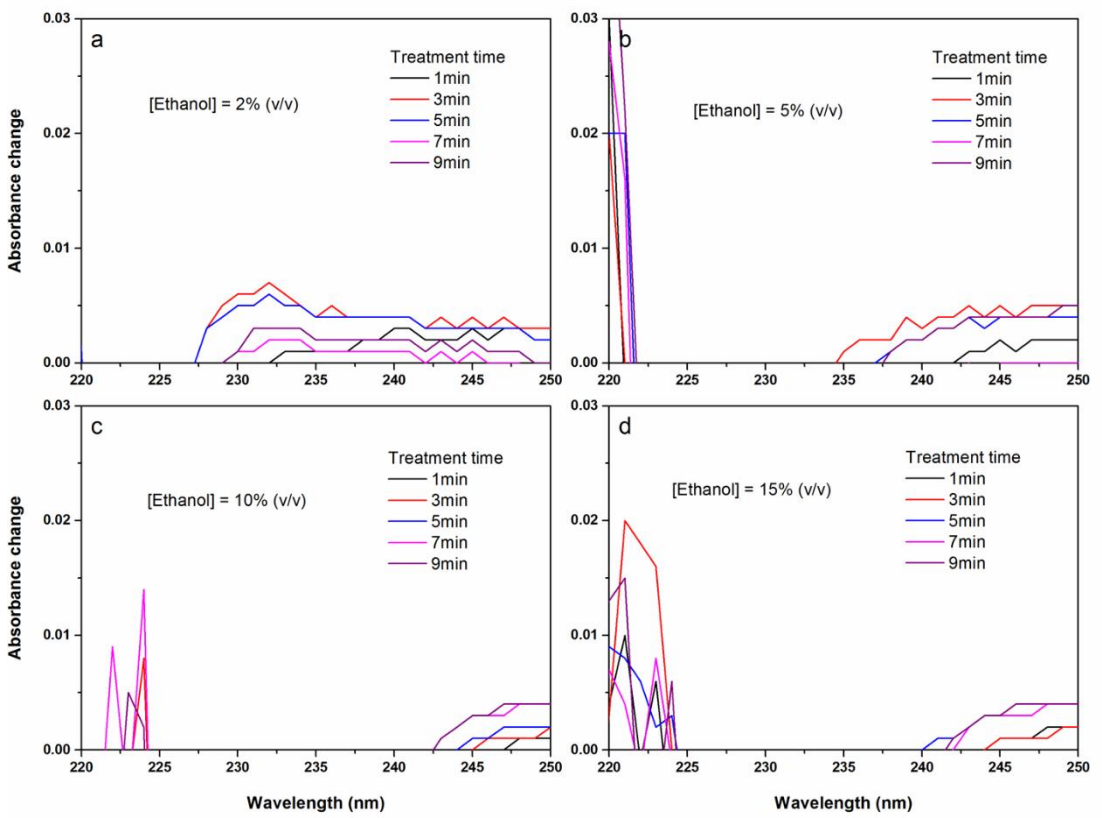

Figure 4.24 The absorbance change of alkaline solution containing 1M NaOH and different contents of ethanol during alkaline treatment of the membrane for insolubilization.

Similarly, Figure 4.25 shows the change in absorbance of the alkaline solution containing methanol during the chitosan insolubilization process, where the methanol content in the alkaline solution varied from 2% to 15% (v/v). Chitosanium leaching was observed to have occurred in alkaline solution at a methanol content of 2% and 5% (v/v). Shown in Figure 4.24a and Figure 4.25a, at an alcohol content of 2% (v/v), a much higher peak was found in the absorbance change curves of the methanol-containing alkaline solution, indicating that ethanol is better than methanol in preventing chitosanium from being diffused out of the membrane during chitosan insolubilization. This is understandable when one considers the high polarity of methanol. At a methanol content of 5% (v/v), chitosanium leaching was only noticed during the first 3-5 min of the alkaline treatment. When the alkaline solution contained 10% or 15% (v/v) methanol, no chitosanium leaching was observed.

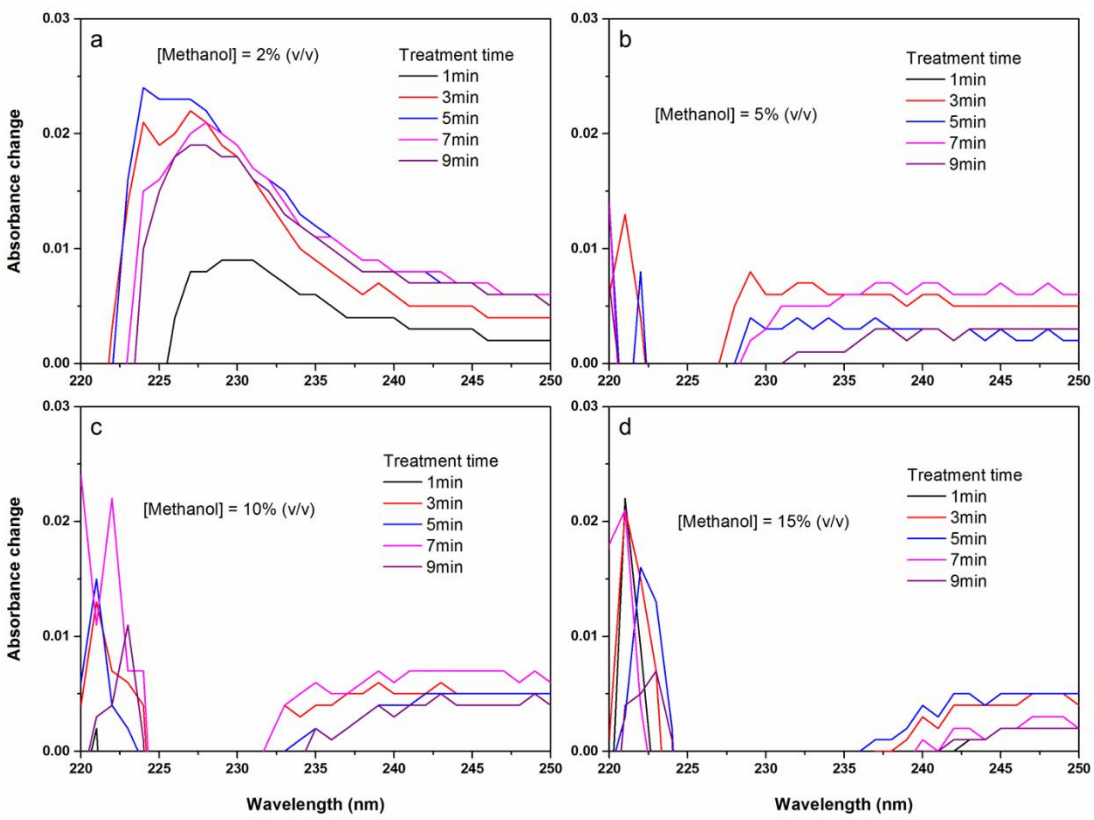

Figure 4.25 The absorbance change of alkaline solution containing 1M NaOH and different contents of methanol during alkaline treatment of the membrane for insolubilization.

It is interesting that the data in Figure 4.24 and Figure 4.25 show that the absorbance change of the alkaline solution was the highest at 3 and 5 min of alkaline treatment time. This was presumably due to simultaneous chitosanium leaching and NaOH consumption (neutralization reaction). At the beginning of the alkaline treatment, very little chitosanium diffused out from the membrane, and the absorbance was low. After 5 min, chitosan insolubilization was largely finished and no chitosanium was left in the membrane. In addition, the chitosanium leached to the alkaline solution would continue to react with NaOH or captured by alcohol, resulting in additional decrease in the absorbance change at 7~9 min. Generally, chitosan insolubilization should be performed with an alkaline solution containing enough alcohol, and the results seemed to suggest that ethanol was better than methanol in minimization of chitosanium diffusion and leaching to the alkaline solution during chitosan insolubilization.

Chapter 5
Conclusions and Recommendations

5.1 Conclusions

By carrying out the UV-vis measurements on the soaking water of the chitosan membranes treated with the alkaline solution at different NaOH concentrations, the efficiency of chitosan insolubilization was found to be related to the concentration of NaOH. Generally, at a high NaOH concentration, the membrane can be insolubilized more effectively. The membrane insolubilization was shown to be quite ineffective at a NaOH concentration of less than 0.4 M in the alkaline solution, and in such cases, a significantly long alkaline treatment time was required and chitosanium leaching during this period was significant.

In addition, the effectiveness of chitosan membrane insolubilization with alkaline treatment was found to decrease by adding ethanol to the alkaline solution. Moreover, at an ethanol content of less than 10% (v/v) in the alkaline solution, the hindrance from ethanol on the chitosan membrane insolubilization was less evident. By replacing ethanol with methanol, a similar relationship was also observed for chitosan insolubilization. Adding methanol to the alkaline solution also hindered membrane insolubilization, though it required a longer alkaline treatment time to complete membrane insolubilization than the time required for alkaline treatment with the ethanol-containing alkaline solution at the same alcohol concentration.

With the UV-vis analysis of the alkaline solution, the leaching of chitosanium during membrane insolubilization was studied. If the alkaline solution contained no alcohol, chitosanium leaching to the aqueous alkaline solution was significant. Adding alcohol to the alkaline solution would reduce the leaching of chitosanium during membrane insolubilization to stabilize the membrane, though chitosanium leaching was still noticed at low alcohol concentration (< 2% (v/v) for ethanol, < 5% (v/v) for methanol). Ethanol was found to be preferred to methanol in minimizing chitosanium from leaching into the alkaline solution during alkaline treatment of the membrane.

Generally, the NaOH concentration used in membrane insolubilization should be high enough to induce a fast reaction so as to complete insolubilization of the membrane in a short period of time,

thereby minimizing the amount of chitosanium that could leach to the alkaline solution. Also, the addition of alcohol in the alkaline solution could reduce chitosanium leaching but increase the time required for complete insolubilization. At a NaOH concentration of 1.0 M, the alkaline solution at an ethanol content of no less than 5% (v/v) appeared appropriate to maintain the structural integrity of the resulting membranes. In addition to the toxicity and volatility, methanol was not a preferred choice as an alternative to ethanol in the alkaline solution for membrane insolubilization.

5.2 Recommendations

Based on the results obtained in this work, the following are recommended for further studies with regard to chitosan membrane insolubilization:

- In the present study, all the membrane insolubilization was performed at room temperature. The reaction rate is typically affected by temperature. Therefore, it is recommended that higher alkaline treatment temperature applied to chitosan insolubilization in the hope that the membrane surfaces would be insolubilized quickly so that the macromolecular chitosanium in the membrane interior would be "locked" within the membrane before the membrane would be fully insolubilized.
- It had been pointed out that the solvent of the alkaline solution directly affected the solubility of chitosanium. Thus, membrane insolubilization could be improved by replacing ethanol (either partially or completely) with other solvents that would reduce chitosanium leaching and/or facilitating alkaline reaction with chitosanium. The effectiveness of neutralization with other alkaline solution compositions for membrane insolubilization is recommended for further studies.

References

[1] G. Crini, Historical review on chitin and chitosan biopolymers, Environmental Chemistry Letters. 17 (2019) 1623–1643.

[2] W. Wang, C. Xue, X. Mao, Chitosan: structural modification, biological activity and application, International Journal of Biological Macromolecules. 164 (2020) 4532–4546.

[3] V.S. Yeul, S.S. Rayalu, Unprecedented chitin and chitosan: a chemical overview, Journal of Polymers and the Environment. 21 (2013) 606–614.

[4] P. Dutta, J. Dutta, V. Tripathi, Chitin and chitosan: chemistry, properties and applications, Journal of Scientific and Industrial Research. 63 (2003) 20–31.

[5] I. Younes, M. Rinaudo, Chitin and chitosan preparation from marine sources. structure, properties and applications, Marine Drugs. 13 (2015) 1133–1174.

[6] N. Morin Crini, E. Lichtfouse, G. Torri, G. Crini, Applications of chitosan in food, pharmaceuticals, medicine, cosmetics, agriculture, textiles, pulp and paper, biotechnology, and environmental chemistry, Environmental Chemistry Letters. 17 (2019) 1667–1692.

[7] M.E. Abd El Hack, M.T. el Saadony, M.E. Shafi, N.M. Zabermawi, M. Arif, G.E. Batiha, A.F. Khafaga, Y.M. Abd El Hakim, A.A. al Sagheer, Antimicrobial and antioxidant properties of chitosan and its derivatives and their applications: a review, International Journal of Biological Macromolecules. 164 (2020) 2726–2744.

[8] M. Vakili, S. Deng, G. Cagnetta, W. Wang, P. Meng, D. Liu, G. Yu, Regeneration of chitosan-based adsorbents used in heavy metal adsorption: a review, Separation and Purification Technology. 224 (2019) 373–387.

[9] E.I. Rabea, M.E.T. Badawy, C. v Stevens, G. Smagghe, W. Steurbaut, Chitosan as antimicrobial agent: applications and mode of action, Biomacromolecules. 4 (2003) 1457–1465.

[10] W.S. Wan Ngah, L.C. Teong, M.A.K.M. Hanafiah, Adsorption of dyes and heavy metal ions by chitosan composites: a review, Carbohydrate Polymers. 83 (2011) 1446–1456.

[11] D. Zhao, S. Yu, B. Sun, S. Gao, S. Guo, K. Zhao, Biomedical applications of chitosan and its derivative nanoparticles, Polymers. 10 (2018) 462.

[12] C.L. Ke, F.S. Deng, C.Y. Chuang, C.H. Lin, Antimicrobial actions and applications of chitosan, Polymers. 13 (2021) 904.

[13] P.K. Dutta, M.N. v Ravikumar, J. Dutta, Chitin and chitosan for versatile applications, Journal of Macromolecular Science, Part C: Polymer Reviews. 42 (2002) 307–354.

[14] Md.M. Islam, Md. Shahruzzaman, S. Biswas, Md. Nurus Sakib, T.U. Rashid, Chitosan based bioactive materials in tissue engineering applications-a review, Bioactive Materials. 5 (2020) 164–183.

[15] M. Muthu, J. Gopal, S. Chun, A.J.P. Devadoss, N. Hasan, I. Sivanesan, Crustacean waste-derived chitosan: antioxidant properties and future perspective, Antioxidants. 10 (2021) 228.

[16] R. Borgohain, U. Pattnaik, B. Prasad, B. Mandal, A review on chitosan-based membranes for sustainable CO2 separation applications: mechanism, issues, and the way forward, Carbohydrate Polymers. 267 (2021) 118178.

[17] P. S. Herrera, X. Feng, D. Payzant, J.H. Kim, Process for the separation of olefins from paraffins using membranes, US7361800B2, 2008.

[18] M. Azhin, T. Kaghazchi, M. Rahmani, A review on olefin/paraffin separation using reversible chemical complexation technology, Journal of Industrial and Engineering Chemistry. 14 (2008) 622–638.

[19] A.C.C. Campos, R.A. dos Reis, A. Ortiz, D. Gorri, I. Ortiz, A perspective of solutions for membrane instabilities in olefin/paraffin separations: a review, Industrial & Engineering Chemistry Research. 57 (2018) 10071–10085.

[20] M.N. v Ravi Kumar, A review of chitin and chitosan applications, Reactive and Functional Polymers. 46 (2000) 1–27.

[21] F. Mahmoodzadeh, M. Ghorbani, B. Jannat, Glutathione and pH-responsive chitosan-based nanogel as an efficient nanoplatform for controlled delivery of doxorubicin, Journal of Drug Delivery Science and Technology. 54 (2019) 101315.

[22] S.P. Miguel, A.F. Moreira, I.J. Correia, Chitosan based-asymmetric membranes for wound healing: a review, International Journal of Biological Macromolecules. 127 (2019) 460–475.

[23] E. Fakhri, H. Eslami, P. Maroufi, F. Pakdel, S. Taghizadeh, K. Ganbarov, M. Yousefi, A. Tanomand, B. Yousefi, S. Mahmoudi, H.S. Kafil, Chitosan biomaterials application in dentistry, International Journal of Biological Macromolecules. 162 (2020) 956–974.

[24] A. Spoială, C.-I. Ilie, D. Ficai, A. Ficai, E. Andronescu, Chitosan-based nanocomposite polymeric membranes for water purification—a review, Materials. 14 (2021) 2091.

[25] Y. Wan, K.A.M. Creber, B. Peppley, V.T. Bui, Ionic conductivity of chitosan membranes, Polymer. 44 (2003) 1057–1065.

[26] S.E. Noriega, A. Subramanian, Consequences of neutralization on the proliferation and cytoskeletal organization of chondrocytes on chitosan-based matrices, International Journal of Carbohydrate Chemistry. 2011 (2011) 809743.

[27] E.A. Takara, J. Marchese, N.A. Ochoa, NaOH treatment of chitosan films: impact on macromolecular structure and film properties, Carbohydrate Polymers. 132 (2015) 25–30.

[28] P. Sangsanoh, P. Supaphol, Stability improvement of electrospun chitosan nanofibrous membranes in neutral or weak basic aqueous solutions, Biomacromolecules. 7 (2006) 2710–2714.

[29] Q. He, Q. Ao, Y. Gong, X. Zhang, Preparation of chitosan films using different neutralizing solutions to improve endothelial cell compatibility, Journal of Materials Science: Materials in Medicine. 22 (2011) 2791–2802.

[30] T. Gültan, Ş. Bektaş Tercan, D. Çetin Altındal, M. Gümüşderelioğlu, Synergistic effect of fabrication and stabilization methods on physicochemical and biological properties of chitosan scaffolds, International Journal of Polymeric Materials and Polymeric Biomaterials. 70 (2021) 371–382.

[31] M.G.N. Campos, L.H.I. Mei, A.R. Santos Jr, Sorbitol-plasticized and neutralized chitosan membranes as skin substitutes, Materials Research. 18 (2015) 781–790.

[32] M.G. Nogueira Campos, C.R. Ferreira Grosso, G. Cárdenas, L.H. Inocentinni Mei, Effects of neutralization process on preparation and characterization of chitosan membranes for wound dressing, Macromolecular Symposia. 229 (2005) 253–257.

[33] U. Jančič, M. Božič, S. Hribernik, T. Mohan, R. Kargl, K.S. Kleinschek, S. Gorgieva, High oxygen barrier chitosan films neutralized by alkaline nanoparticles, Cellulose. 28 (2021) 10457–10475.

[34] L. Fernández-de Castro, M. Mengíbar, Á. Sánchez, L. Arroyo, M.C. Villarán, E. Díaz de Apodaca, Á. Heras, Films of chitosan and chitosan-oligosaccharide neutralized and thermally treated: effects on its antibacterial and other activities, Lebensmittel-Wissenschaft & Technologie. 73 (2016) 368–374.

[35] M. Sano, O. Hosoya, S. Taoka, T. Seki, T. Kawaguchi, K. Sugibayashi, K. Juni, Y. Morimoto, Relationship between solubility of chitosan in alcoholic solution and its gelation, Chemical and Pharmaceutical Bulletin. 47 (1999) 1044–1046.

Appendix A
Membrane Rinsing Method Determination

When evaluating the influence of NaOH concentration in the alkaline solution on membrane insolubilization, the alkaline treatment was designed followed by a membrane rinsing process to remove the residual NaOH and alcohol in the membrane but keep most residual chitosanium remaining since the chitosanium has the relatively large molecular weight. Therefore, it is important to keep the rinsing process to be within a proper range since the content of NaOH could be large in the membrane without enough rinsing time. On the other hand, with an excessive rinsing time, the content of chitosanium in the soaking could also be too low to be detected. To find out a suitable membrane rinsing time, experiment was designed by operating the UV-vis measurements on the soaking water of membranes with different rinsing times. At a given rinsing time, chitosan membrane was insolubilized with the alkaline solution containing 1.0 M NaOH and 50% (v/v) ethanol for the alkaline treatment time of 5 h to fully insolubilized the membrane. After being insolubilized, membranes were rinsed for 5 min under a running water and then immersed into 500 ml deionized water for different times. During the membrane immersing process, deionized water was refreshed every 5 min to remove the diffused NaOH and alcohol in the soaking water. After being rinsed, membranes were placed individually in the soaking water where the UV-vis measurement was carried out after soaking for 3 days.

The results of the UV-vis measurements on the soaking water of membranes under different membrane rinsing times were shown in Figure A.1. Considering that the diffused chitosanium shows a relative large absorbance value (shown in Figure 4.1), membranes under a soaking time of no shorter than 20 min were found to have neglectable residual NaOH due to the low absorbance of the soaking water. Finally, the membrane rinsing method in the experiments about different NaOH concentrations was decided as 5 min of rinsing under running water and 20 min of immersing with the 500 ml deionized water refreshed every 5 min.

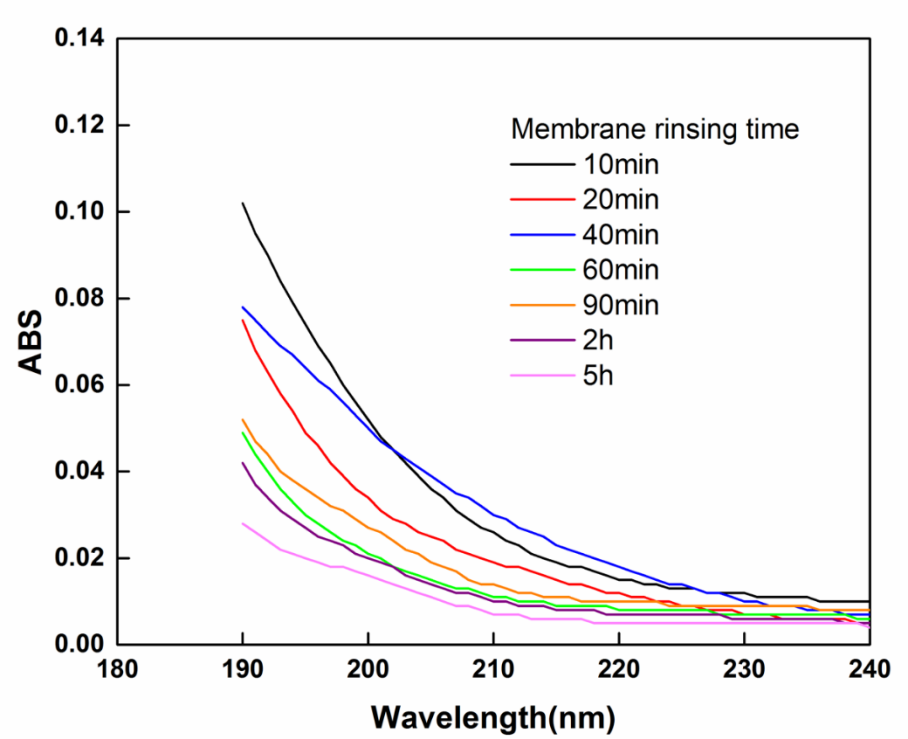

Figure A.1 The absorbance of soaking water of membranes rinsed for different times after alkaline treatment. Membranes were treated with alkaline solution containing 1.0M NaOH and 50% (v/v) ethanol.

Appendix B
Analysis of the Detection with/without Membrane Rinsing

Considering the less effect from the content of alcohol in the alkaline solution on insolubilizing chitosanium membranes rather than the content of NaOH, in the experiment about the influence of alcohol content, treated membranes were placed into the soaking water without any further rinsing operation to minimize the experimental error caused by the diffusion of chitosanium during the membrane rinsing process.

To evaluate the possible experimental error caused by membrane rinsing, experiments were carried out for insolubilization of chitosan membranes with alkaline solutions at a constant NaOH concentration of 1.0 M and different ethanol contents in the range from 10% to 50% (v/v). After alkaline treatment, different to the experiment about the influence of ethanol contents, membrane was rinsed for 5 min under a running water and then immersed into 500 ml deionized water for 20 min of immersing with the water refreshed every 5 min. After that, membrane was placed in the soaking water for 3 days, and the absorbance of the soaking water was measured to find out the alkaline treatment times for different ethanol contents required for complete insolubilization of chitosan membrane.

The results were shown in Figure B.1. Figures B.1a-e show the absorbance of the soaking water for membranes treated with alkaline solution containing ethanol contents of 10%, 20%, 30%, 40%, and 50% (v/v), respectively. Similar to the alkaline treatments with different NaOH concentrations, the time required for complete insolubilization was found to be in the range from 1 to 2.5 min. This is more clearly shown in Figure B.2, where the time required for complete insolubilization is plotted against the ethanol concentration in the alkaline solution.

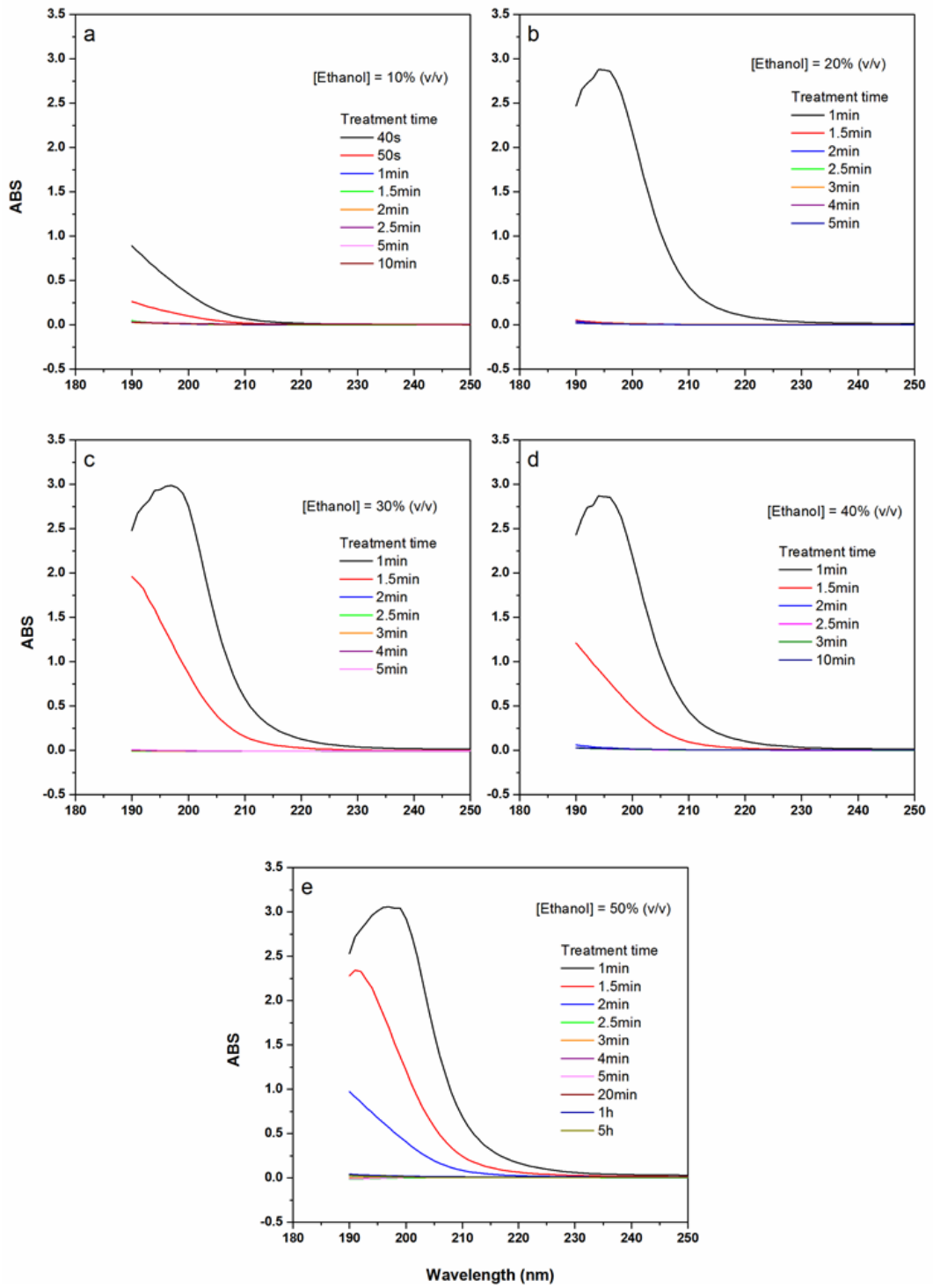

Figure B.1 The absorbance of soaking water of washed membranes insolubilized for different times by 70% (v/v) ethanol and NaOH concentration of 1.0 M.

Generally, similar trend was found in Figure B.2 that a high content of ethanol in the alkaline solution lowers the effectiveness to insolubilize chitosan membranes. For easy comparison, the alkaline treatment time required for complete insolubilization with/without membrane rinsing was compiled in Figure B.3 to show the influence from the membrane rinsing process on the absorbance measurement. In an ethanol content of < 30% (v/v), no difference was found between the time required for complete insolubilization with membrane rinsing and the time required for that without membrane rinsing. Moreover, for ethanol contents of > 30% (v/v), the time required for complete insolubilization with membrane rinsing was found to be shorter than that without membrane rinsing, indicating that the membrane rinsing process would have an effect on the absorbance measurement of the soaking water at a content of ethanol of > 30% (v/v) in the alkaline solution. Considering the influence form membrane rinsing at the high/low contents of ethanol is quite different, therefore, the membrane rinsing process should not be involved after carrying out the chitosan insolubilization by using the alkaline solutions containing different contents of ethanol.

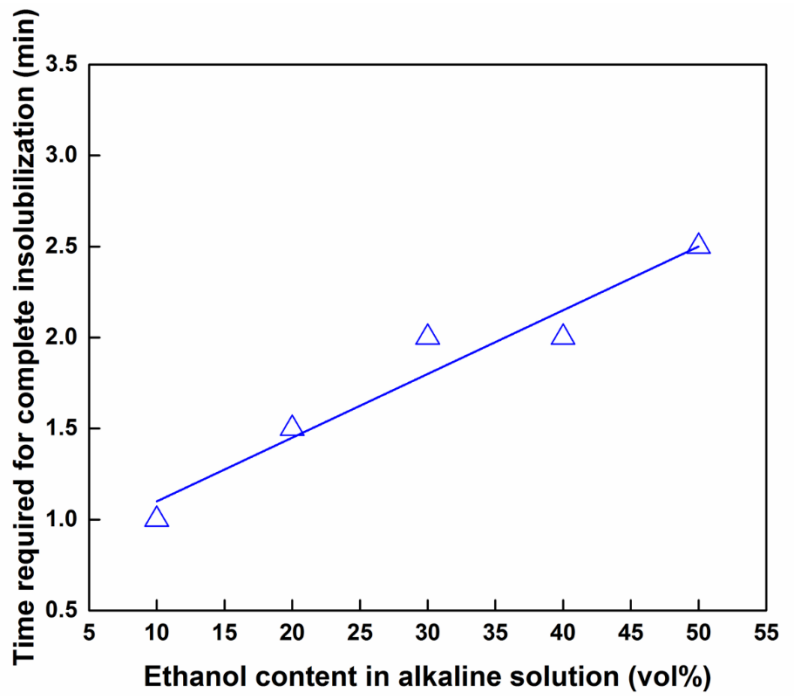

Figure B.2 Time required for complete insolubilization at different ethanol contents in the alkaline treatment solution with membrane rinsing. NaOH concentration: 1.0 M.

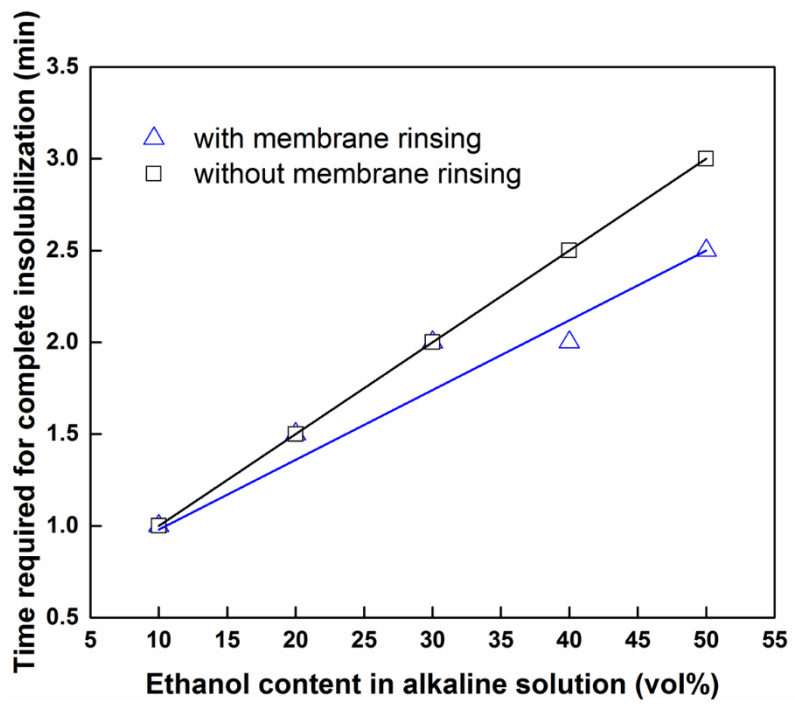

Figure B.3 Time required for complete insolubilization at different ethanol contents in the alkaline treatment solution with/without membrane rinsing. NaOH concentration: 1.0 M.

Appendix C

The Comparison between Absorptivity of Ethanol and Methanol

Previously, the difference between the effectiveness of ethanol and methanol was evaluated by comparing the alkaline treatment times required for complete insolubilization of ethanol-containing and methanol containing alkaline solutions in Figure 4.19 and also by comparing the absorbance change of the soaking water at the wavelength of 200 nm and the soaking time of 3 h between Table 4.1 and Table 4.2. The comparison between the absorbance changes in Table 4.1 and Table 4.2 should be carrying out under the similar absorptivity between NaOH/ethanol/water and NaOH/methanol/water to make sure that the absorbance of the soaking water changes in a similar scale. Thus, the absorbance changes in the soaking water on Table 4.1 and Table 4.2 could represent the loss of the NaOH during the soaking process for both membranes insolubilized with ethanol-containing and methanol-containing alkaline solutions.

To have a study on the absorptivity of NaOH/ethanol/water and NaOH/methanol/water mixture, the experiment was designed to carry out the UV-vis measurements on the diluted NaOH hydroalcoholic/aqueous solutions. The NaOH concentration in the solution was ranged from 0 to 1 mM which was selected as the similar NaOH concentration in the soaking water. At a given NaOH concentration, the solvents of alkaline solution are pure water, 0.1% (v/v) ethanol aqueous solution, and 0.1% (v/v) methanol aqueous solution (three kinds of alkaline solution measured for each NaOH concentration). The results were shown in Figure C.1, where ethanol and methanol were found to have a similar effect on the absorbance in the solution at each NaOH concentration. As a result, the absorptivity of ethanol-containing alkaline solution and methanol-containing alkaline solution was similar, and the results shown in Table 4.1 and Table 4.2 are found to be comparable.

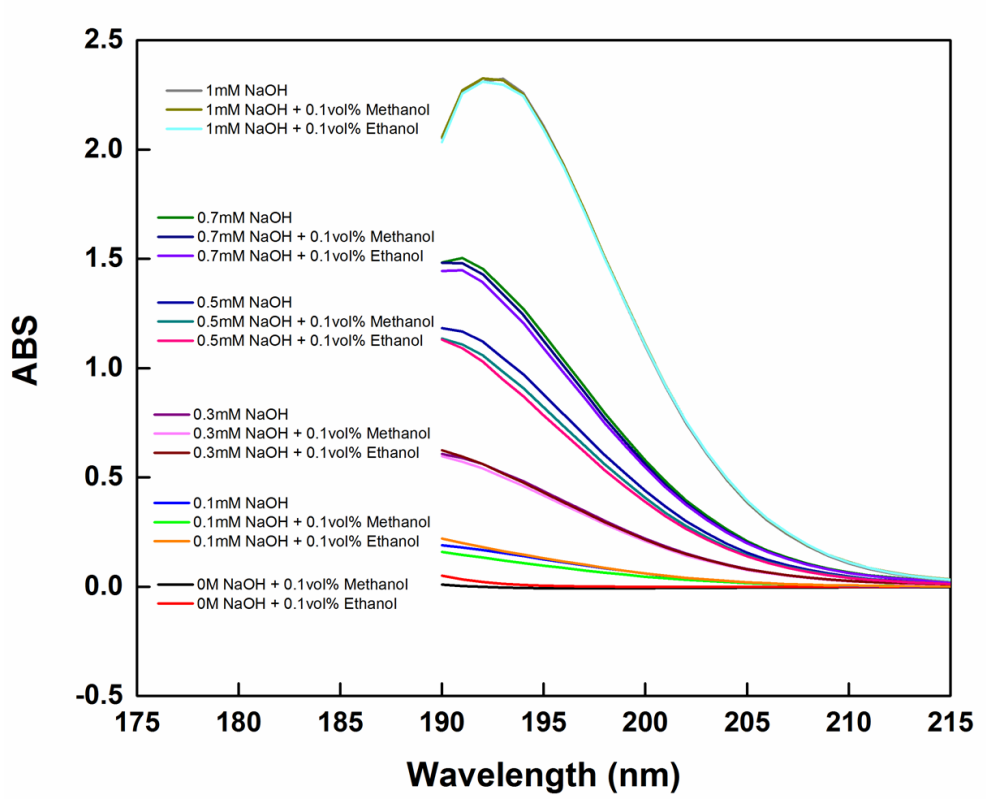

Figure C.1 The absorbance of dilute NaOH solutions with/without 0.1% ethanol or methanol. NaOH concentration: 0, 0.1, 0.3, 0.5, 0.7 and 1 mM.