CHAPTER II
THEORETICAL BACKGROUND AND LITERATURE REVIEW

2.1 Wound Dressing

Injuries of the body which cause the interruption of cells and anatomic continuity of tissue due to abrasion, tearing, avulsion, cut, puncture, incision or burn. Those produce the wound. So, the wound dressings are important for covering the wounds to accelerate the wound healing and create better healing conditions. It should be prevent the wounds touch the dirtiness from environment and encourage wound healing process.

An ideal material of wound dressing would allow the wound to heal at an optimum rate under all clinical circumstance. An ideal wound dressing include the ability to (Rosiak *et al.*, 1995 and Stashak *et al.*, 2004): absorb the exudates and toxic components from the wounds surface, maintain a high humidity at the wound/dressing interface, deliver oxygen to the wound (allow gaseous exchange), prevent excessive loss body fluids, be non-toxicity, protect the wound from bacterial penetration, good adhesion of wound and remove easily with trauma to the wound, provide thermal insulation, generally accelerate healing processes, prevent inflection because of wound dressings are used in direct contact with living tissues.

2.2 Hydrogels

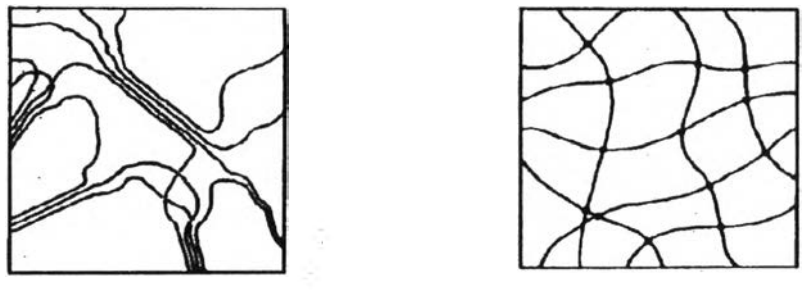
Hydrogels are a three dimensional network of hydrophilic polymer chains and water which fills the space between macromolecules. The water molecules were absorbed by hydrogel in form primary bond water, the water molecules connect to the hydrophilic groups in the hydrogels, which are the ionic and H-bonding groups. When absorption of the water was occurred, leading the chains begin to expend, and as the hydrophobic groups are exposed to water molecules or secondary bond water, that interact via hydrophobic interactions leading the water molecules coat on the surrounding of those groups. Two classes of hydrogels can be defined (Kamath *et al.*, 1993, Rosiak *et al.*,1995 and Rosiak *et al.*, 1999):

2.2.1 Physical Hydrogels

Physical hydrogels or reversible gels, formation of weak interaction between chains where the chains are connected together by electrostatic force, H-bonding or chain entanglement or hydrophobic interactions. All of these interaction are reversible, its formation are easily broken and can be converted to polymer solution by changing physical conditions or application of stress. This type of hydrogels is prepared by freeze-thawing procedure.

2.2.2 Chemical Hydrogels

Chemical hydrogels or permanent gels, they are covalently crosslinked networks, hydrogels have the same bonds as in the main chains, so they are resistant to any solvent or melting due to they are irreversible gels. They can be broken by chemical reaction or by stress.



Physical hydrogel Chemical hydrogel

Figure 2.1 Schematic description of physical hydrogel and chemical hydrogel.

2.3 Gamma Radiation Technique

Gamma radiation is electromagnetic radiation like visible light, radio waves, and ultraviolet light, with a very short wavelength and higher the energy level.

An example of gamma ray production follows;

First Cobalt-60 decays to excited Nickel-60 by beta decay.

$$^{60}Co \rightarrow {}^{60}Ni^{*} + e^{-} + \nu_{e}^{-}$$

Then the Nickel-60 drops down to the ground state by emitting a gamma ray.

$$^{60}Ni^{*} \rightarrow {}^{60}Ni + \gamma$$

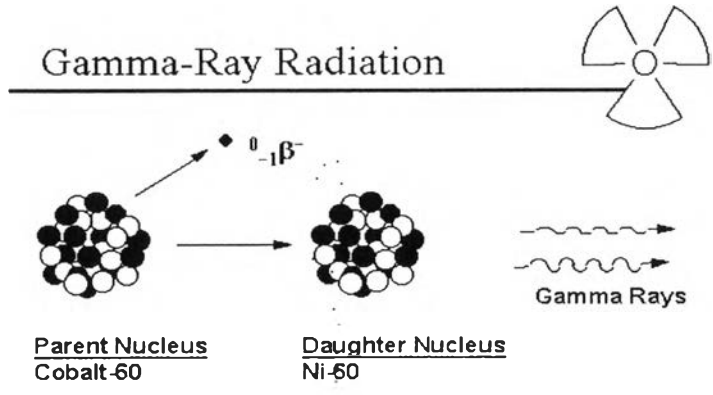


Figure 2.2 Schematic description of gamma ray production.

The characteristic of gamma rays that can travel more than alpha and beta particles, they have enough energy to pass through the body without interaction with tissue due to the body is empty space at the atomic level and gamma rays are very small sizes. In contrast, alpha and beta particles inside the body lose all their energy by colliding with tissue and causing damage.

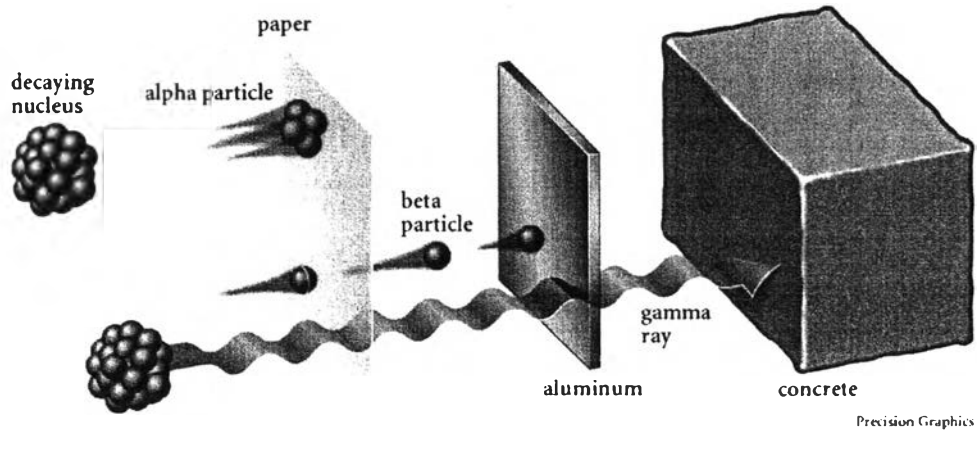


Figure 2.3 Schematic description of gamma ray penetration.

Gamma radiation is the method to crosslink polymer chains in solid and aqueous solution condition, it can produce three-dimensional hydrogel networks. Moreover, it can produce an oligomer by radiation degradation of high molecular polymers (Makuuchi, 2010).

2.4 Radiation Formation of Hydrogels

Radiation processes have various advantages, easy process control, combining hydrogel formation and sterilization in one step (Salmawi *et al.*, 2007) without any chemical as the cross-linker (Wang *et al.*, 1998). High energy radiation can used to polymerization unsaturated compounds and able to crosslink water-soluble polymers without additional vinyl groups. Polymers can be radiation crosslinked at various condition which can be defined (Rosiak *et al.*,1995, Rosiak *et al.*, 1999):

2.4.1 Solid

Irradiation of hydrophilic polymer in dry state has some drawbacks. It difficult to form homogeneous gels and remove the oxygen, that can promote unwanted side reactions. It requires much higher doses to obtain a gel as compared to irradiation in solution because of the radiation-chemical yield of radical is lower than aqueous solution condition. This method has limits in motion of the radical chains that effect to efficiency of crosslinking.

2.4.2 Aqueous Solution

Irradiation of polymers in aqueous solution, it is lower number of unwanted processes occurred. When polymer solution is ionized by radiation, reactive intermediates are formed. This result comes from direct action of radiation on the polymer chains and indirect effect which is the reaction of the radiolysis of water with the polymer molecules. The radiolysis of water shown in following (Wang *et al.*, 2008):

$$H_2O \xrightarrow{\gamma\text{-ray}} {}^{\cdot}OH,\ {}^{\cdot}H,\ e^-_{aq}$$

The water-polymer system absorbed energy from radiation to produce the reactive species that consist of hydrate electrons, hydroxyl radicals and hydrogen atoms. Hydroxyl radicals are the main species for reactivity transferred from water to the polymer chains. They can attack polymer chains, resulting in the formation of macroradicals (Hennink *et al.*, 2002). Recombination of the macroradicals on different chain resulting in the formation of covalent bonds and crosslinked structure. The crosslinking ratio is the most important factors that affects the swelling and mechanical properties of hydrogels (Peppas *et al.,* 2000). Higher crosslinked hydrogels have dense structure, it will swell little when compared to the same hydrogels with lower crosslinking ratio. Crosslinking obstructs the mobility of polymer chains so lower swelling ratio. The more degree of crosslinking of the hydrogels, the stronger gels are.

Poly(vinyl alcohol), poly(vinyl pyrrolidone), poly(ethylene oxide), poly(lactic acid), polyacrylamide, poly(vinyl methyl ether), chitin, chitosan or alginate were prepared to hydrogels for using as wound dressing due to which can be crosslinked with high energy irradiation.

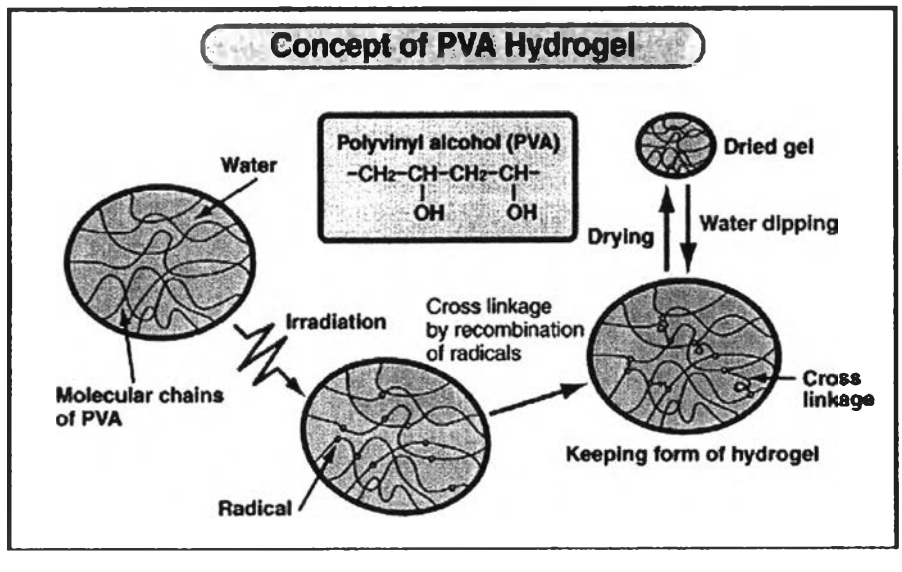


Figure 2.4 Cross-linking of PVA by gamma radiation.

Recently, natural polysacharides, chitin, chitosan were used for enhancing the properties of hydrogels by adding them to the hydrogels, which are usually called

"the blend hydrogels". The literature reviews about the blend hydrogels are given below.

Salmawi, (2007) wanted to improve hemostatic properties and prevent microbiological growth onto the PVA hydrogels by adding chitosan to the PVA hydrogels. The blended hydrogels were prepared by gamma irradiation technique at different ratio and different doses of gamma irradiation. It was found that the gel fraction increased with increasing irradiation doses and PVA content due to the hydrogels form crosslink network. The swelling also increased with increasing chitosan content but the swelling decreased with increasing irradiation doses. A water absorption increased with increasing the PVA content due to PVA is a water soluble polymer but on increase irradiation doses, there is decrease in water absorption. The tensile strength of hydrogels increased with increasing PVA content but the elongation at break decreased with increasing PVA content and irradiation dose.

Varshney, (2007) studied the preparation of PVA/polysaccharides hydrogels by gamma irradiation technique. In order to improve the properties of PVA hydrogels without adding plasticizers or additives and reduce the cost of production. The mechanical properties, swelling behavior and gel fraction were investigated. The hydrogels containing varying amounts of PVA, agar and carrageenan, that hydrogels were prepared at different dose of gamma radiation. When added 0.5-2% polysaccharides into PVA hydrogels leaded tensile strength to increase from 45 g/cm^2 to 411 g/cm^2 due to carrageenan contribute more mechanical strength, elongation increased from 30% to 410% due to PVA molecules were separated by agar gels and water uptake from 25% to 157% due to ionic carrageenan were water soluble polymer.

Yang *et al.*, (2008) synthesized PVA/water soluble chitosan (ws-chitosan) hydrogels by gamma irradiation and freeze-thawing for wound dressing. The properties of hydrogels were investigated. The hydrogels prepared by pure freeze-thawing and freeze-thawing followed by irradiation were opaque due to freeze-thawing method was resulted in the microphase separation, while those prepared by irradiation followed freeze-thawing and by pure irradiation were translucent and transparent, respectively. Because of irradiation was resulted in the homogeneous network structure. The gel fraction decreased with increasing ws-chitosan content due to lower crosslink density hydrogels was presented when add more chitosan content. Hydro-

gels with more ws-chitosan showed higher pH sensitivity and swelling capacity while pure PVA hydrogel did not show pH sensitivity and had the smallest swelling capacity. The higher swelling capacity was due to the lower crosslink density and higher hydrophilicity while higher pH sensitivity was due to the more amino group of ws-chitosan in the hydrogel which can be protonated. The protonation introduced electrostatic repulsions between the polymer segment. The antibacterial activity (*E.coli*) of hydrogels containing ws-chitosan was due to the positively charge of ws-chitosan can bind to the negatively charge of bacteria.

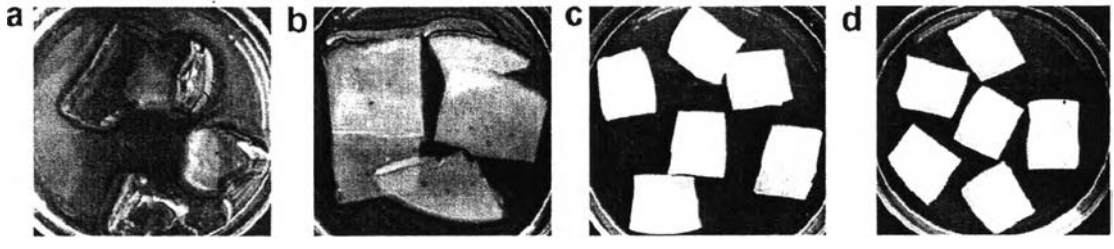


Figure 2.5 The morphologies of hydrogels made by (a) pure irradiation, (b) irradiation followed by freeze-thawing, (c) pure freeze-thawing, and (d) freeze-thawing followed by irradiation.

Zhai *et al.*, (2002) studied the effect of component of starch on the properties of PVA/starch hydorgels by investigating the influence of doses and the properties of hydrogels. PVA/starch blend hydrogel were prepared by gamma and electron beam irradiation. After adding starch into PVA hydrogels, it was found that hydrogels form starch grafting onto the crosslink PVA network. Starch consists of amylose and amylopectin after studied interaction between starch and PVA, it found that amylose was a important component that influenced the properties of hydrogels due to amylose can mix with PVA solution form homogeneous mixture but amylopectin was difficult to form homogeneous mixture with PVA solution. Gel strength and elongation at break were increased with increasing of the starch content and the doses of radiation but decreased slightly at high dose due to the degradation of the components. Swelling behavior of hydrogels decreased with increasing dose of radiation

and decreased slightly after added the starch into the. The gel fraction of hydrogels decreased with increasing of the starch content.

Zhao *et al.*, (2003) wanted to improve or modify the properties of PVA hydrogels by adding carboxymethyl chitosan (CM-chitosan) into the PVA hydrogels. Due to CM-chitosan exhibits good miscibility with PVA in aqueous mediea. The blend hydrogel were prepared by electron beam irradiation. They investigated mechanical properties, gel fraction, swelling behavior and antibacterial activity of hydrogel. When added the CM-chtiosan into PVA hydrogels, it was found that CM-chitosan molecules were grafted onto the crosslink PVA network. CM-chitosan can improved the elasticity and flexibility of hydrogels, then equilibrium degree of swelling increase with increasing CM-chitosan content due to high hydrophilicity of CM-chitosan. The gel fraction decrease with increase of CM-chitosan content because of at low concentration of CM-chitosan aqueous solution, its chains form macroradicals which were separated by water and placed at a distance from each other which prevented their intermolecular interaction. The antibacterial activity (E.coli) increase with CM-chitosan due to CM-chitosan was polyampholyte then it can contact with the negatively charge of bacteria.

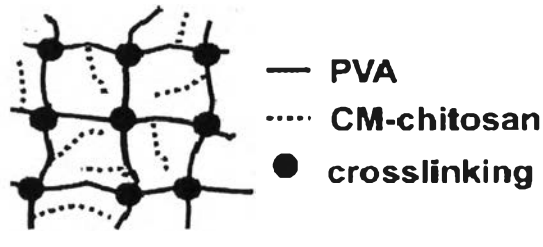


Figure 2.6 Schematic illustration of structures for graft hydrogel.

2.5 Water-Soluble Derivatives of Chitosan

Chitosan is a copolymer of β-(1,4) –linked 2-acetamido-2-deoxy-D-glucopyranose and 2-amino-2-deoxy-D-glucopyranose. Chitosan is prepared by the partial deacetyation of chitin in a hot alkali solution. Generally, chitosan has three types of reactive functional groups that consist of an amino group, primary hydroxyl group and secondary hydroxyl group at C(2), C(3) and C(6) position, respectively

(Kim *et al.*, 2008). Recently, chitosan is used in medical and pharmaceutical fields (Berger *et al.*, 2004) due to its biocompatibility, biodegradation and non-toxic properties. Chitosan can improve wound healing, then it suitable material for wound dressing , it was indicated to absorb plasma protein leading to platelet adhesion and activation to blood coagulation (Benesch *et al.*, 2002 and Hoven *et al.*, 2007). Chitosan is normally insoluble in aqueous solution above pH 7. On the other hand, it is soluble in dilute acids, may not desirable in many of its application. There have been two kinds of chitosan derivative, chitosan are improved aqueous solubility of chitosan which can be define:

2.5.1 Carboxymethyl Derivative of Chitosan

Carboxymethyl chitosan (CM-chitosan) is water-soluble chitosan derivatives, which is an amphoteric polyelectrolyte containing both cationic and anionic groups. Chitosan is changed into CM-chitosan by introducing $-CH_2COOH$ groups onto –OH along chitosan molecular chains (Chen *et al.*, 2004). CM-chitosan can generate the negative charges of the carboxyl groups by itself an excellent chelating host for metal cation substrate (Sun *et al.*, 2006). CM-chitosan promoted the proliferation of the normal skin fibroblast but inhibited the proliferation of keloid fibroblast (Chen *et al.*, 2002). It also stimulates the extracellular lysozyme activity of skin fibroblast. It also has many attractive physical and biological properties such as non-toxicity, good biocompatibility, high water absorption capacity, high plasmid protein absorption, good blood compatibility, no antigenicity, weak mitogentic activity, adjuvant activity (short life), anti-tumor influence, and antibacterial activity (Lu *et al.*, 2007, Shi et al., 2006 and Zhao *et al.*, 2006). So, it is suitable for the application of biomaterial. The literature reviews about carboxymethyl chitosan (CM-chitosan) are given below.

Chen *et al.*, (2003) synthesized CM-chtiosan by improving the solubility of chitosan. CM-chtiosan was preparated via chemical reaction with $ClCH_2COOH$ under various conditions by using isopropanol/water as a solvent and studied the water solubility properties of CM-chitosan at various pHs. It was found that, the fraction of carcoxymethylation increase with increasing the reaction temperature. In contrast, the fraction of carboxymethlation increase with increasing the isopropa-

nol/water ratio. CM-chitosan was prepared at 0-10 °C were soluble in water but CM-chitosan prepared between 20 and 60 °C were insoluble in the water at near neutral pH.

2.5.2 Quaternary ammonium derivative of chitosan

Quaternary ammonium chitosans are water-soluble polymer that can be synthesized by either covalent addition of a substituent containing a quaternary ammonium group, or by quaternization of the amino groups of the parent polymer (Britto *et al.*, 2007). Quaternized chitosans have been achieved by reacting chitosan with iodomethane in the presence of *N*-methyl-2-pyrrolidone. It has been found to have antibacterial activity. Its antimicrobial activity against a variety of bacteria and fungi coming from its polycationic nature permanent positive charges ($-N^{+}R_3$) in chains as a consequence C-2 position of the chitosan structure. The target site of the cationic is the negatively charged cell surface of the bacteria that is the cytoplasmic membranes of microbes (Kim *et al.*, 1997 and Kim *et al.*, 2002). The main compositions of the cytoplasmic membrane are membrane proteins and phospholipids (The phospholipids of bacteria are phospholycerides which have both a hydrophilic and hydrophobic end). Quaternary ammonium chitosans can interact and form polyelectrolyte complexes with acidic polymers produced at the bacterial cell surface (Guo *et al.*, 2007, Jia *et al.*, 2001 and Peng *et al.*, 2010), and not limited to acidic environments due to it is water-soluble over a wider pH range. The antimicrobial activity of quaternary ammonium chitosans was depended on its molecular weight and the cationic charge of substituent (Kim *et al.*, 1997). The charge density of quaternary ammonium chitosans increase with increasing the molecular weight of its single coil, lead to enhance absorption properties of quaternary ammonium chitosans onto the negatively charged cell surface. The literature reviews about the quaternary ammonium chitosans are given below.

Kim *et.al*, (1997) wanted to improve the solubility of the chitosan by preparing the series of the water soluble chitosan derivatives with quaternary ammonium salts. *N*-alkyl chitosan derivatives were prepared by introducing alkyl groups into the amine groups of chitosan via Schiff's base intermediates then quaternized chitosan derivetives were carried out using methyl iodide to produced

water soluble cationic polyelectrolytes (**Figure 2.7**). They studied the antibacterial activity of quaternized chitosan derivatives against *S.aureus*. It was found that the antibacterial activity of quaternized chitosan derivatives increased with increasing in the chain length of the alkyl substituent. The quaternized chitosan derivatives with a long alkyl chain interact strongly with the cytoplasmic membranes, due to a hydrophobic affinity between the alkyl chain and the phospholipids leading to higher antibacterial activity.

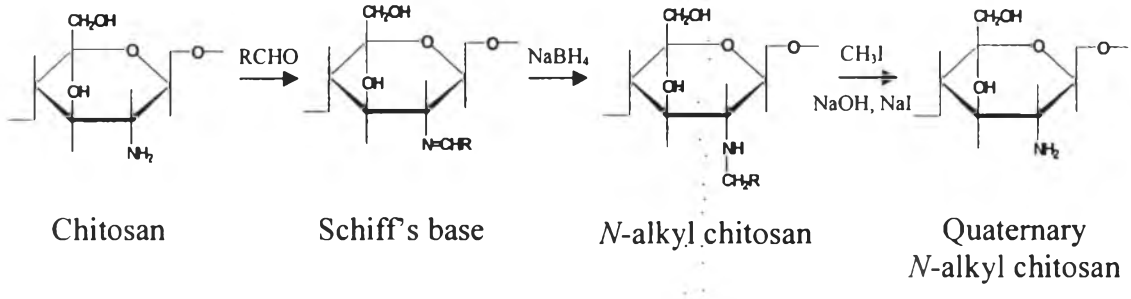


Figure 2.7 The synthesis of quaternized *N*-alkyl chitosan.

Sieval *et al.*, (1998) studied the modification of the chitosan by improving solubility of chiotsan via adding methyl iodide to produced *N,N,N*- trimethyl chitosan chloride (TMC) and compared with the native chitosan. The degree substitution of TMC can be controlled by the amount of methyl iodide as reagent and a number of reaction steps that mean the products of first step are reactants of second step by using the same reaction. Comparison of the ^{1}H NMR spectra of TMC with one step and two step of reactions. This spectra shown $N(CH_3)_3^+$ and $N(CH_3)_2$ at 3.1 and 3.4 ppm (**Figure 2.8**). A two step reaction gave the high degree of substitution 40-80 % but the one step reaction has 35% degree of substitution.

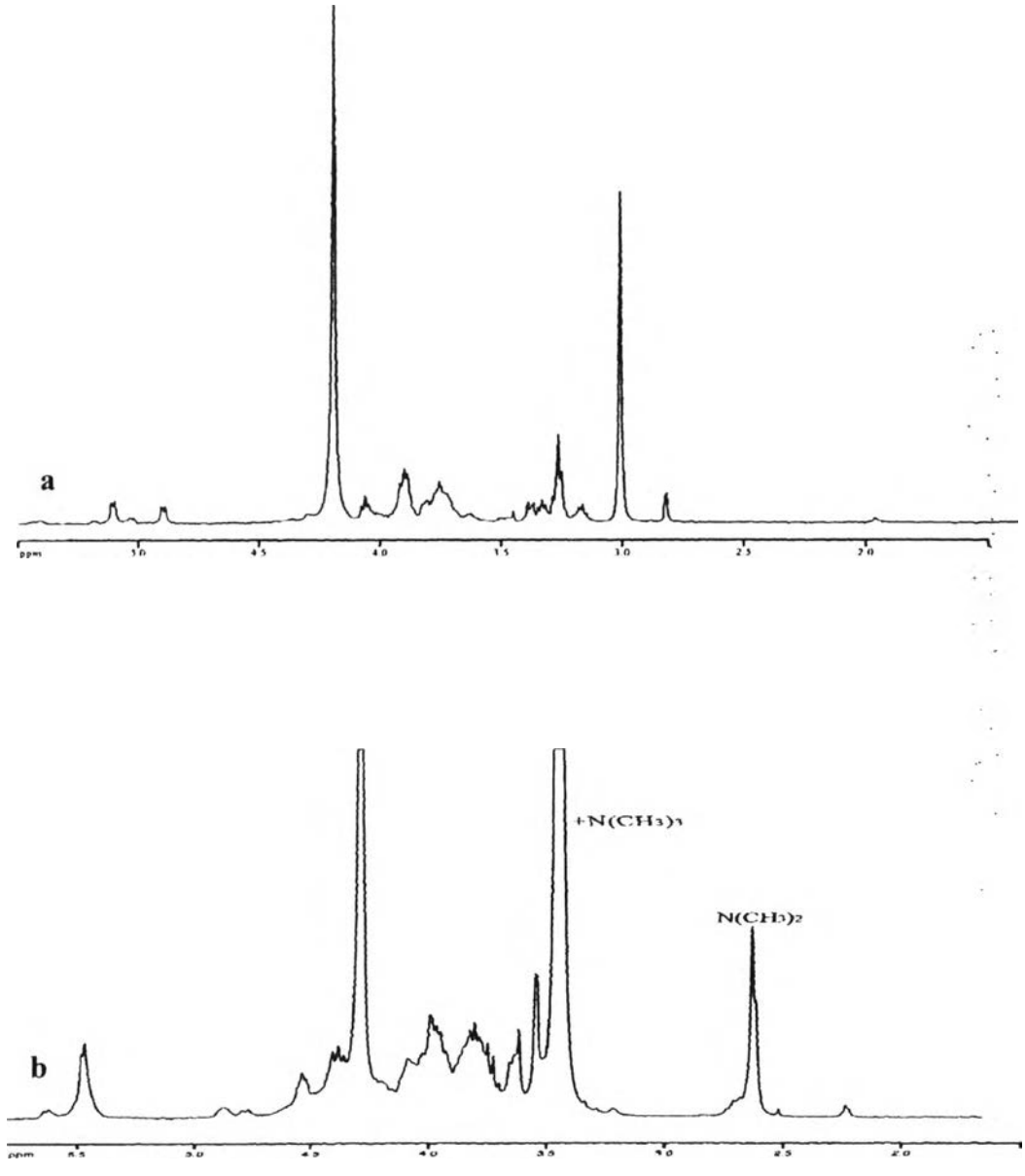


Figure 2.8 ^{1}H NMR spectrum of *N,N,N*-trimethyl chitosan chloride (a) one step reaction and (b) two step reaction.

Jia *et al.*, (2001) studied the relationship between antibacterial activities of quaternary ammonium salt of chitosan and its molecular weight and determined the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of quaternary ammonium salt of chitosan againsts *E.coli.* They prepared quaternary ammonium salt of chitosan by using Schiff's base reaction to produced

N-alkyl chitosan derivatives and quaternized chitosan were obtained by the reaction of *N*-alkyl chitosan derivatives with methyl iodide. Results showed the antibacterial activity of quaternized chitosan was related to its molecular weight. At the same degree of quaternization, the high molecular weight (H-MW) quaternized chitosans had stronger antibacterial activity than the low molecular weight (L-MW) quaternized chitosans due to higher positive charges in the chains, then higher reaction between quaternized chitosan and cell surfaces. The length of alkyl groups in the quaternized chitosan molecules strongly affected the antibacterial activity of the quaternized chitosan, longer alkyl groups in the molecule with more high antibacterial activity in good agreement with the previous studied by Kim *et al.*, 1997

Kim *et al.*, (2002) wanted to improve the antibacterial activity of chitosan by preparing the chitosan derivatives with quaternary ammonium salt which have different methylene spacers. They produced the *N*-alkyl chitosan via Schiff's base intermediates and used methyl iodide to introduced quaternized chitosan derivatives. They measured the antibacterial activity of quaternized chitosan derivatives against *S.aureus* and *E. coli* by cell counting method in acetate buffer pH 6.0. Results showed the antibacterial activity of quaternized chitosan derivatives increased with increase in the chain length of the alkyl substituent or increase with hydrophobic properties of quaternized chitosan derivatives. According, the hydrophobicity, cationic charge of substituent and flexible movement of alkyl chain introduced to chitosan affected the antibacterial activity of quaternized chitosan derivatives against *S.aureus* and *E. coli* (Kim *et.al*, 1997).

Guo *et al.*, (2007) synthesized the chitosan derivatives that consist of Schiff bases of chitosan, *N*-substituted chitosan and quaternary ammonium chitosan by using Schiff base reaction. They modified the chitosan by adding the alkyl groups at amino groups onto the chitosan chains to produce antifungal polymer. They investigated the antifungal activity of chitosan derivatives and measured their antifungal activitys against *B.cinerea* Pers and *C. lagenarium* (Pass) Ell.et halst. It was found that at the same concentration (1000 ppm) of chitosan derivatives, chitosan inhibited the growth of *B.cinerea* Pers 45.5%, the Schiff bases of chitosan and the *N*-substitued chitosan derivatives had slight activity against *B.cinerea* Pers 26.8% and 39.3%, respectively. Moreover, the inhibitory of quaternary chitosan derivatives

were 81.2% at 1000 ppm. The quaternized chitosan derivatives had a better antifungal activity than chitosan, Schiff bases of chitosan, and *N*-substitued chitosan derivatives due to the cationic groups or ammonium groups of the chitosan interaction with the anionic groups on the microbial cell surface which forms a layer around the cell to prevents nutrients from entering.

Guo *et al.,* (2007) synthesized the quaternized chitosan derivatives by improving the chitosan to the water-soluble chitosan and antifungal chitosan via Schiff's base reaction. They prepared *N*-(2-hydroxy-phenyl)-*N,N*-dimethyl chitosan (NHPDCS), *N*-(5-chloro-2-hydroxy-phenyl)-*N,N*-dimethyl chitosan (NCHPDCS), *N*-(2-hydroxy-5-nitro-phenyl)-*N,N*-dimethyl chitosan (NHNPDCS) and *N*-(5-bromic-2-hydroxy-phenyl)-*N,N*-dimethyl chitosan (NBHPDCS) and investigated their antifungalactivity againsted *B.cinerea* Pers and *C. lagenarium* (Pass) Ell.et halst. At 1000 μg/ml of chitosan derivatives, chitosan had antifungal activity againsted *B.cinerea* Pers 45.5% , the quaternized chitosan derivatives had better antifungal activity than chitosan, the antifumgal activity of NHPDCS, NCHPDCS, NHNPDCS and NBHPDCS are 58.6%, 86.7%, 68.8% and 66.6%, respectively. For *C. lagenarium* (Pass) Ell.et halst, the quaternized chitosan derivatives had better antifungal activity than chitosan, the antifumgal activity of NHPDCS, NCHPDCS, NHNPDCS and NBHPDCS are 55.8%, 72.6%, 63.6% and 79.5%, respectively.

Guo *et al.,*(2007) studied the influence of molecular weight of the quaternized chitosan on the antifungal activity. They prepared the quaternized chitosan derivative from high molecular weight and low molecular weight of chitosan via Schiff's base reaction. Then they investigated the antifungal activity of chitosan derivative againsted *B.cinerea* pers. and *C.lagenarium* (Pass) Ell.et halst. It was found that the quaternized chitosan derivatives with high molecular weight (H-MW) had stronger antifungal activity than those with low molecular weight (L-MW) because of growth inhibition of the fungi which consist of two mechanism: first, the positive charges of H-MW quaternized chitosan derivatives interacted with negative charges on cell surface of the fungi, lead to the cell permeability changed. Second, L-MW quaternized chitosan derivatives can enter the fungal cells because of its small size and then essential nutrients are absorbed which inhibit the synthesis of mRNA and

protein. As for the quaternized chitosan derivatives, the first mechanism occur on cell surface that are the major role.

Belalia *et al.*, (2008) studied the quaternization of *N*-alkyl chitosan derivatives. They modified the chitosan to produce the water-soluble cationic polyelectrolyte by using methyl iodide, which is the *N*,*N*,*N*-trimethyl chitosan(TMC). In order to improve its antimicrobial activity and physicochemical properties. TMC was prepared in two step via Schiff's base reaction: first, monoalkylation of amine group and second, quaternization of the alkyl chitosan. Antibacterial activity of the TMC was investigated in the liquid medium, TMC and chitosan were measured against *L. innocua* growth. It was found that TMC exhibited more antibacterial activity than chitosan, at the same time of incubation. The higher antibacterial activity of TMC could be due to the positive charges on the chitosan chains. The quaternization led a reduction in molecular weight of chitosan due to alkaline and temperature systhesis condition. So, TMC could penetrated through the cellular membrane of bacteria leading to higher antibacterial activity.

From the theories and the literature reviews which are used to improve and develop the research. In order to improve the properties and enhance the wound healing of the PVA hydrogels, the CM-chitosan were added into the PVA hydrogels. We synthesized *N*-trimethyl chitosan for applying the antibacterial activity of the PVA hydrogels by blending *N*-trimethyl chitosan into the PVA hydrogels. Then the blend hydrogels consist of three components that are PVA, CM-chitosan and *N*-trimethyl chitosan. Subsequently experiments, the properties, gel fraction, swelling behavior and the antibacterial activity of the blend hydrogels against skin bacterias were investigated.