CHAPTER II
LITERATURE REVIEW

2.1 CM-Chitin

Chitin, or poly (β-(1-4)-*N*-acetyl-D-glucosamine (figure 2.1), a biopolymer that is the second most abundant natural polysaccharide next to cellulose (Goodrich *et al.*, 2007). It is in the form of ordered crystalline microfibril which occurs in cell walls of fungi and yeast and exoskeleton of arthropods such as squid pens, crab shells and shrimp shells (Jayakumar *et al.*, 2010b). In addition, a large amount of shrimp waste is produced in Thailand from seafood industries (Dolphen *et al.*, 2011), which are easy to find to use as a raw material in the synthesis of chitin so, chitin is readily available and inexpensive. It also exhibits the distinctive properties which are biocompatibility, biodegradability and low toxicity. Especially biocompatibility, because an acetamide groups of chitin is similar to an amide group of protein in living tissue (Peesan *et al.*, 2003). There are three crystalline forms of chitin based on X-ray diffraction data. (Cohan *et al.*, 1987): α-chitin, β-chitin and γ-chitin, respectively. α-Chitin, an anti-parallel arrangement of polymers, is the most abundant form which is found in shells of shrimps and crabs. β-chitin, a parallel disposition, is found in squid pens and cocoons of certain beetles, while structure of γ-chitin has both parallel and anti-parallel types (Peesan *et al.*, 2003). However, the high rigid crystalline structure of chitin causes the poor solubility in common solvents. Hence, chitin can be chemically modified to be organo-soluble derivatives or water soluble derivatives such as chitosan (Peesan *et al.*, 2006), dibutyryl chitin (Rinaudo *et al.*, 2006) and carboxymethyl chitin (Jayakumar *et al.*, 2010a).

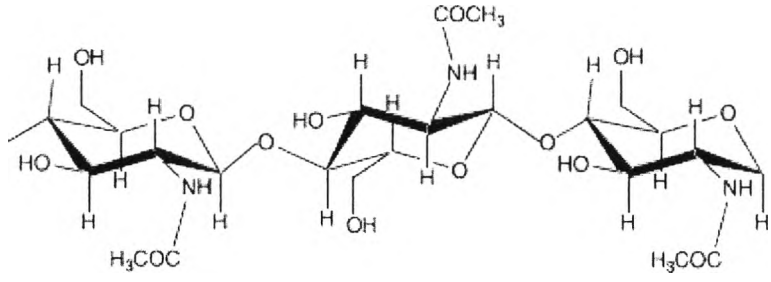

Figure 2.1 The structure of chitin.

Carboxymethyl chitin, CM-chitin is a water-soluble anionic derivative of chitin containing carboxyl groups as shown in figure 2.2 (Jayakumar *et al.*, 2010a). The water solubility of CM-chitin becomes apparent when the fraction of substitution is more than 0.6 and the biodegradability depended strongly on the degree of deacetylation (DD), the degree of substitution (DS) and the substitution site (Hjerde *et al.*, 1997). CM-chitin is extensively utilized as a component of wound dressing application due to its unique properties such as hydrophilic properties, low toxicity and high biocompatibility (Dev *et al.*, 2010).

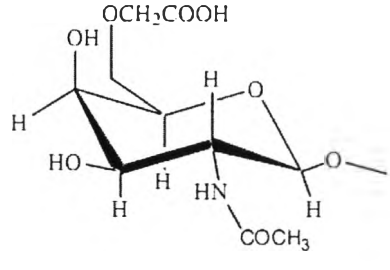

Figure 2.2 The structure of carboxymethyl chitin.

CM-chitin can be synthesized by carboxymethylation of chitin (figure 2.3) which starts by dispersion chitin powder with 60% sodium hydroxide solution at 20°C for 12 hr and adding a monochloroacetic acid in isopropyl alcohol as a solvent using the condensation reaction and degree of substitution = 0.6 (Jayakumar *et al.*, 2010a). Moreover, another method to synthesize CM-chitin is firstly suspending

chitin powder in 42%v/v of NaOH solution. A crushed ice is added and alkaline chitin solution is obtained. A monochloroacetic acid solution (25%w/v in 14%v/v NaOH solution) is then added into the alkaline chitin solution. The mixture is neutralized with glacial acetic acid and subsequently dialyzed in distilled water for 3 days. The dialysate is centrifuged and the supernatant is added drop wise into acetone and resuspended in ethanol and degree of substitution = 0.4 (Wongpanit *et al.*, 2005).

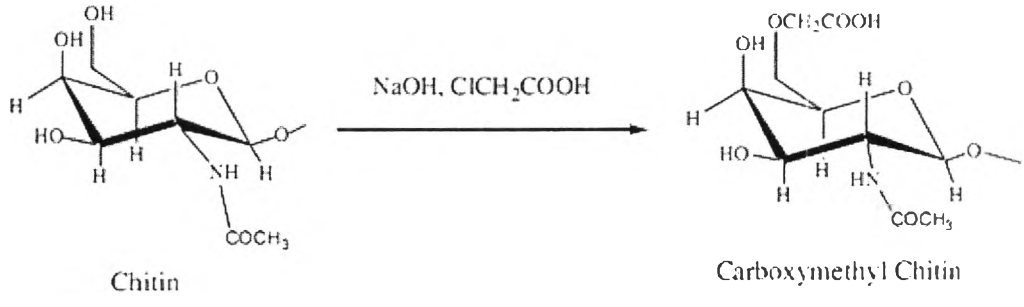

Figure 2.3 The carboxymethylation of chitin (Jayakumar *et al.*, 2010a).

The biological properties of CM-chitin and CM-chitosan have been evaluated by testing with *in vivo* and *in vitro* degradation (Jing *et al.*, 2008). For *in vivo* testing, CM-chitin films and CM-chitosan films were inserted into the pouches which were made in the muscle of the hind legs of the female SD rats at 3 months old and 220 g weight as shown in figure 2.4. The results showed that all the films hydrolyzed absolutely within 3 days of implantation. Compared with chitosan, CM-chitosan and CM-chitin exhibit grater degradability, which may be due to the carboxymethyl groups in their structure. For *in vitro* testing, CM-chitin was hydrolyzed more quickly than CM-chitosan, which is due to the acetamide group of chitin structure.

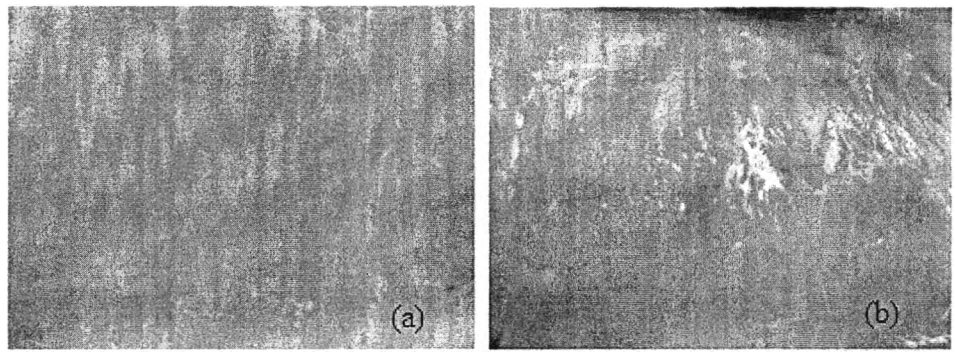

Figure 2.4 Subcutaneous tissues with implantation: (a) CM-chitin and (b) CM-chitosan.

CM-chitin was used to blend with silk fibroin (Wongpanit *et al.*, 2007). The blending of CM-chitin and silk fibroin exhibited as a semi-miscible blend. Due to the multiblock copolymeric structure, silk fibroin, structure can be divided into two major regions as hydrophobic and hydrophilic regions. When blending with CM-chitin, only the hydrophilic part (amorphous part) of silk fibroin was compatible with each other. After crosslinking, the presence of CM-chitin in the blend films could improve the biodegradability and swelling ability of silk fibroin.

CM-chitin with a total fraction of O-substitution from 0.65 to 1.5 were prepared from alkali-chitin and monochloroacetic acid in 2-propanol at room temperature (Hjerde *et al.*, 1997). The carboxymethylation in position 6 was found to be larger than in position 3 in all fractions, confirming that the reactivity at $-OH_6$ is larger than $-OH_3$ in carboxymethylation process. Moreover the degradation rate of CM-chitin in hen egg white lysozyme was non-linear, suggesting different degradation rates of different sequences in CM-chitin.

CM-chitin nanoparticles were prepared though a cross-inking approach with $FeCl_3$ and $CaCl_2$ (Dev *et al.*, 2010). It was found that the size of the prepared CM-chitin nanoparticles was 200-500 nm, thereby rendering the morphology suitable for drug delivery applications. Moreover the cytotoxicity studies showed that the prepared CM-chitin nanoparticles are non-toxic against mouse L929 cells. Moreover, the CM-chitin nanoparticles exhibit significantly antibacterial activity against *Staphylococcus* bacteria strains because CM-chitin molecules can interact with the

predominant components of the cell wall (lipopolysaccharides and proteins) of the microorganism and then the intracellular components leak out of the cells and the permeability barrier also prevents nutrients from entering the cell as shown in figure 2.5.

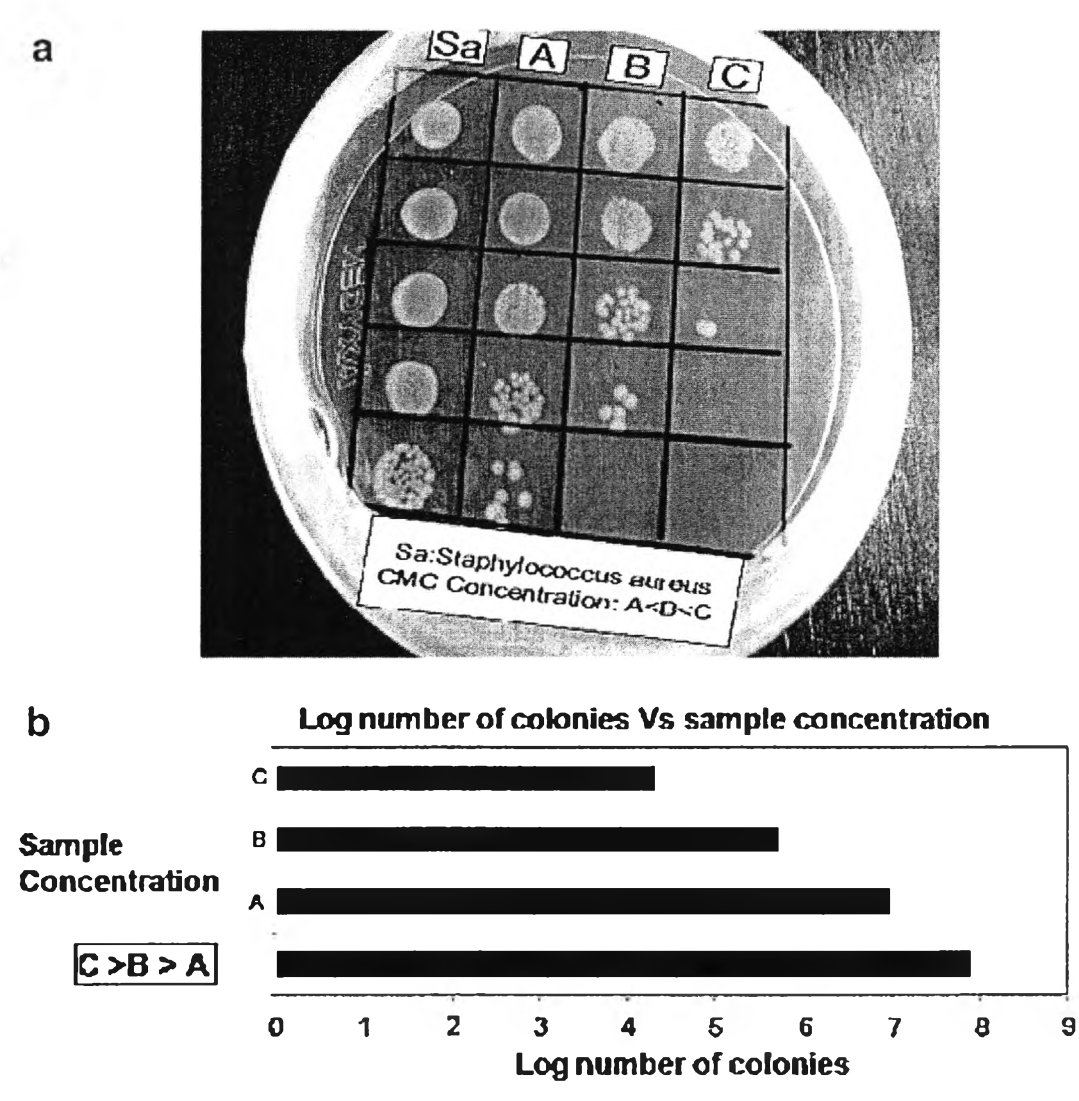

Figure 2.5 Cytotoxicity test; (a) Representative plate showing the antibacterial activity of CMC nanoparticles (A = 5 ml, B = 10 ml, C = 15 ml of CMC nanoparticles of 3 mg/ml concentration) and (b) Antibacterial activity of CMC nanoparticles (A = 5 ml, B = 10 ml, C = 15 ml of CMC nanoparticles of 3 mg/ml concentration).

Since the CM–chitin-based materials are brittle, blending of CM-chitin with other flexible biomaterials is necessary in order to obtain the desirable mechanical properties as well as ease of handle.

2.2 Natural Rubber

Natural rubber (NR), *cis*-1,4-polyisoprene (figure 2.6), is naturally produced by the *Hevea brasiliensis* trees which accounts for over 99% of the world's natural rubber production (Sakdapipanich *et al.*, 2007). Natural rubber latex is composed of about 36% of rubber, 5% of non-rubbers components (proteins, lipids, sugars, and ash) and water accounting for the remaining 59% (Sansatsadeekul *et al.*, 2011). Thailand can be considered as one of the most significant resource of natural rubber (Wang *et al.*, 2009) so, natural rubber is a low cost, available and renewable natural resource. The outstanding physical properties of natural rubber are flexibility, high strength, good crack growth resistance and good processability. It is currently used in more than 50 thousands in different products such as tire, adhesives, coating and floor covering (Ripel, *et al.*, 2009).

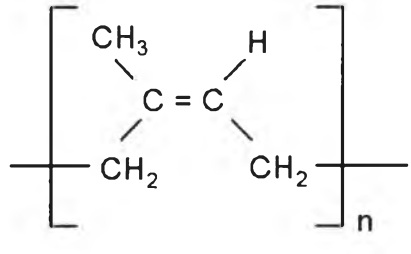

Figure 2.6 The structure of *cis*-1,4-polyisoprene.

The protein of natural rubber latex (NRL) constitutes about 1-2% by weight. These naturally occurring proteins can relate to hypersensitivity reactions (type I) in some sensitive human who contact with them (Warshaw *et al.*, 1998), but same of associated proteins on rubber particles also help to maintain the latex stability (Sakdapipanich *et al.*, 2007). For this reason, when these proteins are removed or degraded, coagulation properties and destabilization of the latex can be occurred.

There are several methods of reducing the amount of these protein antigens in natural rubber latex. There are two effective methods which were used to remove protein in natural rubber latex including double centrifugation and creaming of natural rubber latex. In addition, leaching, chlorination and enzymatic treatment were used to remove these antigenic proteins in wet-gel and dry-films of natural rubber (Perrella *et al.*, 2002). Comparing with post-washing natural rubber latex products, enzymatic treatment is very effective in reducing antigenic protein in natural rubber latex and it is still quite cost-effective because the enzyme treatment can remove protein from natural rubber latex more than 99%. This is the important necessity for especially manufacture products in health care products such as medical gloves and condoms (Sansatsadeekul *et al.*, 2011).

The physical and biological properties of gloves made from both untreated natural rubber latex (NRL) and enzymatic-treated natural rubber latex (ET-NRL) was tested (Perrella *et al.*, 2002). The results of physical properties showed that both of gloves exceeded the ASTM D3578 requirements of tensile strength at break and ultimate elongation, as well as the minimum test for accelerated heat aging. In addition, gloves made from ET-NRL were tested for holes over a 2-month period by using the water leak test. The results showed that they were intact and below the requirement for medical examination gloves is an AQL of 2.5 (ASTM D3578). For biological properties, the in *vivo* biocompatibility evaluation of gloves made from ET-NRL in rabbit skin. The studies showed no significant skin irritation reactions. Moreover, the guinea pig testing of gloves made from ET-NRL showed no significant chemical sensitization reactions.

Due to the unique properties, natural rubber has been widely blended with various polymers as synthetic or natural polymers in order to improve their physical properties of these materials.

For an attempt to prepare novel biodegradable material, blends of natural rubber latex/chitosan (NRL/CS) films with different compositions were prepared by solution casting followed by compression (Johns *et al.*, 2008). It was found that the natural rubber latex blended with chitosan can improve the poor thermal properties of chitosan. The FT-IR results showed that the composition ratio of 95% NRL/ 5% CS exhibit the better interfacial adhesion between natural rubber and chitosan as

showed in figure 2.7. XRD result showed that crystallinity in natural rubber reduced with increasing chitosan content. In addition, the water absorbability of the blends increased with increasing chitosan content and it can be minimized by vulcanization of the blend films.

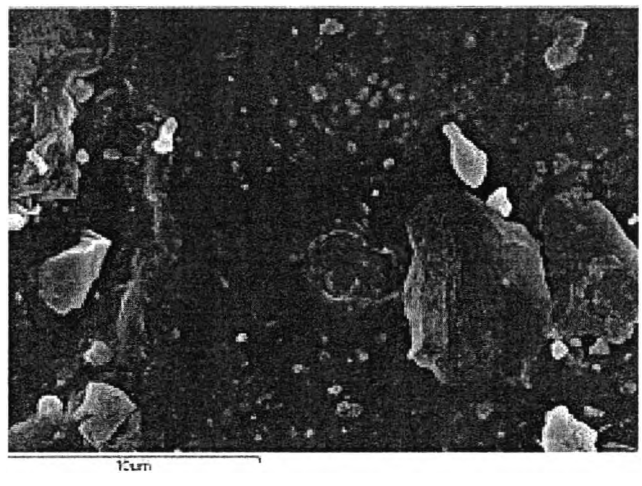

Figure 2.7 SEM micrograph of natural rubber latex/chitosan blends.

In general, natural rubber can act as a hydrophobic polymer because it mainly composes of hydrocarbon, then in order to improve the compatibility with other hydrophilic polymers the adding of compatibilizer or hydrophilic modified natural rubber are required.

The compatibility between hydrophilic thermoplastic starch and hydrophobic natural rubber (Carvalho *et al.*, 2003) was studied. The results showed the reduction of the modulus and the tensile strength after addition of natural rubber into starch. SEM results also showed a good dispersion of natural rubber in starch matrix. In addition, the compatibility as well as the phase separation of the blends was also depended on glycerol content. It means that glycerol acted as both starch plasticizer and starch–rubber compatibilizer.

The natural rubber was also blended with ethylene-vinyl acetate copolymer (NR/EVA) (Jansen *et al.*, 1996) by using poly(ethylene-co-vinyl alcohol-co-vinyl mercaptoacetate), EVASH as a compatibilizer and dicumyl peroxide (DCP) as a curing agent. According to the TGA results, the resistance to thermal ageing is better

for those blends containing EVASH. Then, EVASH not only act as a compatibilizer for this blends but also act as a stabilizer for rubber degradation.

The modified natural rubber as natural rubber graft polystyrene (NR-g-PS) was used to improve compatibility between natural rubber and polystyrene. The thermal behavior of natural rubber and polystyrene (natural rubber /PS) blends is poor that comes from immiscible and incompatible (Asaletha *et al.*, 1998). Hence, the addition of a suitable compatibilizer which is NR-g-PS copolymer can improve the compatibility as well as their thermal behavior of the blends. It was found that the weight loss of the blends is lower than that of the pure component. This result suggested that this blending system can improve the thermal behavior by increase initial decomposition temperature of the blends.

In another modified natural rubber, maleic anhydride (MA) was added into polyamide 6/ natural rubber blends in order to form a graft copolymer between natural rubber and polyamide 6 during processing, which can enhance the compatibility of rubber-polyamide blends (Carone *et al.*, 2000). During processing, MA can react with both natural rubber and polyamide 6 leading to graft copolymer formation which was confirmed by Molau test and DMA results. Furthermore, when the MA was added to the rubber, the blend morphology analysis showed a reduction in particle size of rubbers, which also confirm the formation of the graft copolymer proposed in figure 2.8.

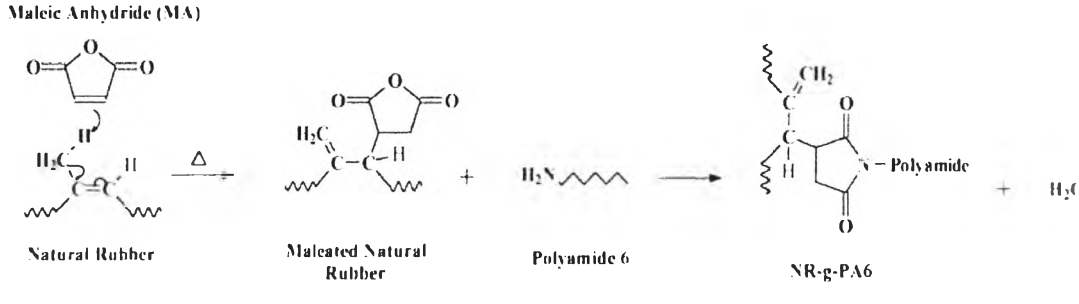

Figure 2.8 Possible reactions among MA, polyamide 6 and natural rubber that can take place during processing.

Maleic anhydride grafted ethylene propylene diene rubber (EPDM-*g*-MA) was used to minimize the phase size in chlorinated polypropylene/natural rubber blends (Sirisinha *et al.*, 2004). The results showed that the addition of EPDM-*g*-MA can decrease the phase size that can refer to the good homogeneity of the blends. It was observed that the optimum concentration of EPDM-*g*-MA is 1 phr. While beyond this concentration, phase size starts to increase. Moreover, the addition of phenolic antioxidant can also improve a thermal stabilization of natural rubber phase by apparently decreases the phase size in blends leading to reduce phase coalescence during blending. This studied also suggest that the smaller dispersed phased size, the higher resistance to oil and thermal aging was observed.

Moreover, maleated natural rubbers (MNRs) were also modified by the reaction of natural rubber with maleic anhydride (MA) in order to improve the compatibility, rheological and thermal properties of the natural rubber/ Polymethylmethaacrylated, PMMA blends (Nakason *et al.*, 2006). It was found that the percentage of grafted MA increased with increasing concentrations of MA. The results also showed that the chemical interaction between polar groups in MNR molecules resulted in the increase of mooney viscosity and shear viscosity with increasing levels of MA monomer. Besides, the decomposition temperature and glass transition temperature of the MNRs increased with increase the MA monomer concentration. The result also showed that sizes of dispersed PMMA domains decreased with increasing MA concentration suggested the good compatibility of NR and PMMA blends due to the adding of maleated natural rubbers as a compatibilizer.

2.3 Glycerol

Glycerol or 1,2,3-propanetriol is the simplest trihydric alcohol which is a derivative of propane (Garrido *et al.*, 2007). Its empirical formula $C_3H_8O_3$ which indicates the molecular weight 92.09 has two primaries and one secondary hydroxyl and the primary hydroxyls are usually more reactive than the secondary group, and the first one to react does so more readily than the second (figure 2.9).

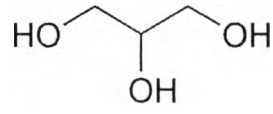

Figure 2.9 The structure of glycerol.

Glycerol is completely soluble in water and alcohol (Follain *et al.*, 2006). It is slightly soluble in ether, ethyl acetate, and dioxane and insoluble in hydrocarbons. Glycerol has useful solvent properties similar to those of water and simple aliphatic alcohol because of its three-hydroxyl groups. Glycerol is stable to atmospheric oxygen under ordinary conditions but is readily oxidized by some other oxidants (Fishman *et al.*, 2000). It is non-toxicity, colorless, odorless when pure, viscous and high water absorption at room temperature (Stein *et al.*, 1999). Moreover, it has a warm sweet taste and is neutral to indicators.

In addition, glycerol is widely used as plasticizer in polymer processing because it can provide softness to materials. According to the hydroxyl groups of glycerol can adsorb moisture and enlarge softness (Follain *et al.*, 2006). Moreover, glycerol can act as chain extender that it can increase the mobility of polymer. For this reason, glycerol can improve both processability and mechanical properties (Garrido *et al.*, 2007). Thus, glycerol is expected to increase flexibility of the materials as well.

In the previous work, the effects of the addition of a fourth component to the starch glycerol/water/system used in thermoplastic starch materials on the basis of starch sorption site availability were evaluated (Follain *et al.*, 2006). The starch sorption site can absorb both water and glycerol depending on the glycerol content and the chosen relative humidity. Its sites are saturated by specific glycerol/water concentrations. The addition of a fourth component before, near or after this starch saturation point is a potentially interesting concept because of its effect on the mechanical properties of the whole system (quaternary system). The results indicated that the addition of a polar polymer led to a strain increase (at break) before and at the saturation point, and to a strain decrease after the saturation point. The addition of inorganic fillers and natural fibers gave a strength decrease and a strain increase at

break instead of giving the expected filler/fiber reinforced composite while the addition of a coplasticizer is possible to avoid glycerol enriched domains, which decrease the materials cohesion.

Glycerol was used as plasticizer in starch based film (Garcia *et al.*, 2011). Starch nanoparticles were prepared by acid hydrolysis of waxy maize starch granules. They were used to reinforce a waxy maize starch matrix obtained by gelatinization. Both unfilled and filled, as well as unplasticized and glycerol plasticized films were processed by the casting/evaporation technique. The structure of crystalline nanoparticles was not affected by the processing method. All the results lead to the conclusion that a close association exists between starch nanocrystals and glycerol-rich domains. This association was supported by SEM, from a peculiar fibrillar morphology for glycerol plasticized composite, and by DMA, from a strong alteration of the relaxation process assigned to the glass transition of glycerol-rich domains. These changes result in an unexpected increase of the water vapor permeability of the plasticized film upon addition of starch nanoparticles.

Glycerol could be used as plasticizer in starch and LDPE blends (Garg and Jana, 2007). The blend characteristics were improved through the reduction in hydrophilicity of the starch by modification of starch by crosslinking and by using plasticizer glycerol. It was found that glycerol modification of cross-linked starch did not improve the tensile strength but percent elongation of the film i.e. stretch-ability of the film increased. And they suggest that the glycerol requirement for modification after crosslinking of starch is important because excess quantity of glycerol for plasticizing will lead to poor properties of the film. Moreover, with glycerol modification after cross-linking of starch, the compatibility further increased to give better surface characteristics and uniformity of the blend films.

Moreover, glycerol was blended with chitosan due to it is a model low molecular weight glass-forming liquid (Garrido *et al.*, 2007). It was found that the β-relaxation in the blends depends on glycerol concentration. It has been interpreted as motions of the side chains of chitosan linked to glycerol by hydrogen bonding. For high chitosan content, the blends are homogenous and a decrease of the glycerol mobility was observed. These results are in agreement with the assumption of a "clustering model" at high glycerol concentrations, consisting of a two-step

mechanism. A first step, in which the solvent was sorbed by means of hydrogen bonds on polymer-specific sites and a second one, a solvent clustering around the first sorbed solvent molecules forming a hydrogen bonded network of glycerol molecules.

2.4 Glutaraldehyde

Glutaraldehyde or 1,5-pentanedial (Beppu *et al.*, 2007) is an aliphatic dialdehyde that undergoes most of the typical aldehyde reactions to form acetals, cyanohydrins, oximes, hydrazones and bisulfite complexes. The molecular formula is $C_5H_8O_2$ which structure is shown below in figure 2.10. Glutaraldehyde is a colorless oily liquid. Commercial samples may have a slightly coloured tint and an odour of rotten apples. In the vapour state, glutaraldehyde has a pungent odour, with an odour threshold of 0.04 ppm. Glutaraldehyde is rapidly soluble in an all proportions in water and ethanol and generally soluble in benzene and ether.

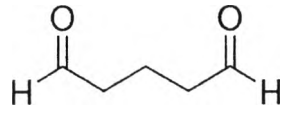

Figure 2.10 The structure of glutaraldehyde.

Glutaraldehyde is widely use as crosslinking agent due to it is easily available, inexpensive and its aqueous solutions can effectively crosslink in a relatively short period (Bigi *et al.*, 2001). The success of thousands of bioprosthetic implants demonstrated in the last tens of years indicates that glutaraldehyde crosslinking has been clinically acceptable and has many merits in spite of the reports on its cytotoxicity (Jayakrishnan and Jameela, 1996). The risk of cytotoxicity can be improved by lowering the concentration of GTA solutions or thorough treatment prior to usage (Zhang *et al.*, 2006).

In the previous work, glutaraldehyde was used as a crosslinking agent in order to improve their water-resistant ability and thermomechnical performance for

potential biomedical applications of gelatin nanofibers (Zhang *et al.*, 2006). The electrospun gelatin nanofibers were crosslinked with saturated glutaraldehyde vapor at room temperature for 3 days. The results showed that the crosslinking has also enhanced the thermal stability and mechanical properties. With a combined moisture content of 15.5 wt%, the denaturation temperature increased by ca. 11 °C, whereas, the tensile strength and modulus were improved to nearly 10 times higher than those of the as-electrospun membranes. Moreover, cytotoxicity test indicated that the glutaraldehyde crosslinked fibrous scaffolds could support the proliferation of human dermal fibroblasts. The initial inhibition of cell expansion on the crosslinked gelatin fibrous scaffolds suggested some cytotoxic effect of the residual glutaraldehyde on the cells. These crosslinked gelatin nanofibers could be suitable for a variety of applications like for tissue engineering scaffolds to improve cell–scaffold interaction, in pharmaceteutical therapy, for medical sutures, as industrial filtration, and so on.

In addition, glutaraldehyde was used as crosslinking agent for chitosan fibers (Jonathan *et al.*, 1998). A highly deacetylated chitosan from shrimp shells with a degree of deacetylation of 95.28 % was prepared and spun into a monofilament fiber using a solution of 4 % (w/v) chitosan in 4 % (v/v) aqueous acetic acid. It was found that fiber mechanical properties can be improved by reactions with aqueous solutions of glutaraldehyde. A similar trend was noted at fixed concentrations over increasing times and temperatures. The reaction is suggested to be a crosslinking reaction and swelling results have supported this supposition. They have suggested that the mechanism for crosslinking was that of a Schiff's base reaction between a glutaraldehyde and the free amine on the chitosan backbone. IR spectra have been presented herein illustrating that a Schiff's base reaction does indeed occur between glutaraldehyde and chitosan. However, further evidence suggests that the reaction takes place between the chitosan free amine and a hemiacetal.