

## CHAPTER IV

### DISCUSSION AND CONCLUSIONS

#### Physicochemical properties

##### Physical characteristics and mechanical properties

The crosslinked chitosan - PVA membranes were rather transparent than the plasticized and unplasticized crosslinked chitosan - starch membrane because of the characteristic of the casting solution. The former casting solution was not only homogeneous but the blend solution of chitosan and PVA was also optically clear and did not show any phase separation whereas the latter was opaque, viscous colloidal mixture. All crosslinked blend polymer solutions did not precipitate because although in acidic solution, the glucosamine units of chitosan was ionized to the soluble form of  $R-NH_3^+$  (Skaugrud, 1989), PVA and starch were nonionic polymer. Therefore the blending showed good compatibility.

The color of crosslinked chitosan casting solution was yellowish. Those of PVA and starch solutions were clear colorless liquid and opaque colloidal mixture, respectively. All prepared films then were yellowish. The increasing of chitosan content in the formulation led to deeper color. It had been well known that in crosslinked chitosan membrane, amino groups of chitosan formed a Schiff base reaction with glutaraldehyde

resulting  $>C=N$  (azomethine group) (Silverstein, Bassler and Morrill, 1981, Kim et al., 1992). Increasing the concentration of glutaraldehyde acted as crosslinking agent increased this chromophore groups due to the crosslinking reaction. Therefore, the dark yellow color was observed in membrane formulation with high concentration of glutaraldehyde.

The physicochemical characteristics of membrane were depended on the type and quantity of component in membrane formulations. The chemical structure and chain alignment of each components affected the crosslinking reaction including the physicochemical characteristics of membranes. The crosslinking of the employed polymers occurred between the hydroxyl and amino groups of chitosan and hydroxyl groups of PVA and Starch (kim et al., 1992). Then the degree of polymerization or quantity of these groups in each polymers, the concentration of polymer and the amount of glutaraldehyde affected the degree of crosslinking reaction. Garcial et al. (1994) studied the effect of carbopol grades and the amount of crosslinking agent (glycerol) on degree of crosslinking and swelling of hydrogel. The crosslinking was due to ester formation between carbopol carboxy groups and glycerol hydroxyl groups. Increasing the amount of glycerol tended to increasing the degree of crosslinking and reducing the degree of swelling. Crosslinked low molecular weight of carbopol (carbopol 907) hydrogel showed the lowest swelling.

The diversifying of degree of crosslinking agent underwent to varying mechanical properties. The tensile strength of a uniform membrane depended on the integrity of its structure. When the blend membrane was crosslinked, the intermolecular interaction between polymers occurred, this which in turn led to an increase in the compactness and subsequent increased in tensile strength (Thacharodi and Rao, 1993). Therefore an increasing in tensile strength was observed with the increasing degree of crosslinking.

Crosslinked chitosan - PVA 100,000 membranes showed the greatest ultimate tensile strength and percent elongation at break, followed by crosslinked chitosan - PVA 70,000 and 30,000 respectively. This result might be discussed that high molecular weight of PVA film gave higher tensile strength and percent elongation at break than low molecular weight of PVA film (Davidson, 1980). Therefore the blending chitosan with high molecular weight of PVA led to strong membrane. Some crosslinked chitosan - PVA membranes gave a rather high tensile strength and elongation at break. This effect could be explained that optimum ration of chitosan : PVA led to a good intermolecular interaction (Kim et al., 1992).

The plasticizer in the crosslinked chitosan - starch membrane formulations was to soften the brittle membranes into strong and pliable membranes. Plasticization generally prevented close compaction of the polymer macromolecules by reducing the relative number of polymer intermolecular attractions and then increasing the polymer's free volume.

Thus polymer molecules could freely move to cause an increase in their flexibility (Gutierrez-Rocca and McGinity, 1994). In this experiment, the plasticized crosslinked chitosan - all type of starches showed higher ultimate tensile strength and slightly higher percent elongation at break (obviously observed in CP<sub>16</sub> ; plasticized crosslinked chitosan : potato starch 4 : 1). This result was perhaps a combination of several factors. One factor was that when a small amount of plasticizer was incorporated into a polymer to cause increasing free volume, which allowed the polymer molecules to move to a more thermodynamically stable state. This result was that the polymer tended to become more ordered and compact as existing "crystallites" grow. The other factors, which introduced a physical hardness of membranes, might be the interaction between the polar groups of the polymer and of the plasticizer and a presence of rigid plasticized molecules adjacent to the polar groups of the polymer (Guo, 1993).

### **Water sorption**

Under aqueous environment, the membrane could expand and swell according to water sorption. The degree of water sorption was governed by crosslinking density, temperature, molar volume of the solvent and polymer - solvent interaction (Nakatsuka and Andrady, 1992, Thacharodi and Rao, 1993). The effect of the crosslinking on degree of water sorption for blend membranes could illustrate that, the degree of water sorption decreased with the increasing concentration of crosslinking agent in the membrane due mainly to the growing crosslinking density of

the polymer chains. Moreover, as the concentration of the crosslinking agent increased, more hydroxyl groups in PVA and starch and more hydroxyl and amino groups in chitosan were crosslinking. Therefore, as PVA and chitosan or starch and chitosan reacted with glutaraldehyde, a crosslinked blend became less capable of H-bonding with water molecules because of the intermolecular and intramolecular crosslinking, resulting in a decrease degree of water sorption.

Some crosslinked chitosan - polymer membrane formulations became rupture after exposure to water might be discussed that these membranes had a weak intermolecular bonding. A weak intermolecular bonding might due to not optimum ratio of blend chitosan : polymer including amount of crosslinking (Kim et al., 1992) which led to the low water resistant membrane. The rupture of low water resistant membrane after exposure to water was due to the unbalance between the elastic force in the network structure acting hold them together and the ability of the solvent to move the chains apart (Winding and Hiatt, 1961).

The ability of water sorption in crosslinked chitosan - potato starch membranes was rather high because potato starch had higher ability of water sorption than the other starches. At 100% relative humidity, percent water sorption of potato, tapioca and corn starch was 50.9, 42.9 and 39.9 respectively (Davidson, 1980).

The plasticized crosslinked chitosan - starch membranes gave a lower water sorption than unplasticized crosslinked chitosan - starch membranes. This result suggested that at low plasticizer concentration, the plasticizer might fill the interstices of the polymer and cause a decrease in water sorption (Guo, 1993).

### **Stability**

Chitosan generally could form a flexible membrane which was stable at high humidity (Blair et al., 1987, Skaugrud, 1989). Thus intermolecular hydrogen bonding occurred from blending chitosan with PVA or starch and then subsequently crosslinked with glutaraldehyde might tend to stronger network structure in membrane (Billmeyer, 1962). Therefore prepared crosslinked chitosan - polymer membranes were rather stable membranes. The stability of prepared membranes could be observed from the rather similar of percent water sorption and mechanical properties between membranes before stability test and membranes after stability test. However, after stability test, the obtained membranes were rather soft which observed from a slight increasing in percent elongation at break. This result might occur from the effect of humidity because water could act as a plasticizer in a way similar to other small molecular plasticizer (Hancock and Zografi, 1994).

### **Surface morphology and porosity**

The difference in the characteristic of membrane surface and porosity was due to the difference in the type and concentration of component in membrane formulation. Because each type and molecular weight of polymers had the different chemical structure. Crosslinked chitosan - the lowest molecular weight of PVA membranes showed pores structure which regularly distributed in polymer network whereas higher molecular weight of PVA produced lesser porosity. Moreover, the using of low molecular weight of PVA including high concentration for preparing crosslinked chitosan - PVA membranes could give high porosity membranes. The porosity of crosslinked chitosan - PVA membranes could be observed from photomicrographs and be confirmed by surface area measurement. Low porosity in crosslinked chitosan - high molecular weight of PVA membrane could indicate high compactness of membrane which related to the high ultimate tensile strength of crosslinked chitosan - high molecular weight of PVA membrane. The effect of molecular weight of hydroxypropylmethylcellulose phthalate on film characteristic and mechanical properties were studied by Rowe (1982). High molecular weight of polymers tended to increasing ultimate tensile strength of film and compactness characteristic of film, including low drug release.

The occurring of rough and pits on plasticized crosslinked chitosan - corn and tapioca starch membranes whereas plasticized crosslinked chitosan - potato starch membranes showed nonpitted membrane surface could be explained that the internal bonding forces in

potato starch were more uniformly molecular network than those in corn and tapioca starch (Davidson, 1980).

### **Infrared spectra**

The difference in IR spectra between pure component and crosslinked blend membranes was due to the difference in chemical structure. The double peak between 1660 and 1590  $\text{cm}^{-1}$  in crosslinked chitosan - polymer membranes, which characterized to C=N groups, indicated the presence of a Schiff base type of crosslink in glutaraldehyde treated membranes (Thacharodi and Rao, 1993).

The disappearance of the crystallization peak at 3451  $\text{cm}^{-1}$ , 1598  $\text{cm}^{-1}$  and 1091  $\text{cm}^{-1}$  meant that an intermolecular interaction between polyvinyl alcohol or starch and chitosan disturbed the crystallization of chitosan in blend state (Kim et al., 1992). Moreover, between 3600-3200  $\text{cm}^{-1}$  region indicating stretching vibration of O-H groups in the pure chitosan showed the change of the band shape and the lower frequency shift of band in crosslinked chitosan - polymer membranes. This result could be discussed that, the strong hydrogen bonding interaction might occur in crosslinked chitosan - polymer membrane (Silverstein, Bassler and Morrill, 1981).



### **Differential scanning calorimetry**

In differential scanning calorimetry, the condition such as the type of pan (the source of pan), close or open pan influenced the difference in endothermic peak temperature and thermogram pattern of pure components and membranes. In this study, Shimadzu aluminium closed pans were used. The various endothermic peak temperatures of pure components and crosslinked chitosan - polymer membranes indicated that pure components and membrane had different in molecular weight, chemical structure and structure arrangement. Moreover, the different thermogram between pure components and membranes indicated that the membranes had structural change due to the blending and crosslinking. Again, the internal bonding forces in potato starch were stronger and more uniformly molecular network than those in corn and tapioca starch. Therefore the influence of plasticizer might slightly affect on bonding force in crosslinked chitosan - potato starch membrane which led to not decreasing endothermic peak temperature in plasticized crosslinked chitosan - potato starch. While crosslinked chitosan - corn starch and crosslinked chitosan - tapioca starch membranes show the reductions of endothermic peak temperature as these membranes were plasticized with triacetin.

### Permeation studies

In the permeability study of isosorbide dinitrate through shed snake skin, isosorbide dinitrate saturated solution was prepared by dissolved 0.1000 g of drug in 20.00 ml of reversed osmosis treated water. After stood until 48 hrs, 0.40 ml of saturated dispersed solution which had about 2.000 mg of drug was filled into plastic disk assembly for permeability test of isosorbide dinitrate through shed snake skin. The obtained data showed that, 0.9108 mg of isosorbide dinitrate could diffuse through shed snake skin within 10 hrs with little or no lag time in permeation profile which indicated rather high diffusion of isosorbide dinitrate through shed snake skin. Itoh et al., (1990) studied the permeability of several compounds through shed snake skin. It was found that, the penetration of ibuprofen, which had a low molecular weight (206.3), was faster than that of the other compounds in the present study and little or no lag time was observed for this compound. Furthermore, cumulative amount of permeated drug was rather high when comparison with the other compounds. The cumulative amount of drug permeated through 1.8 cm<sup>2</sup> of shed snake skin was about 0.050 mg within 6 hrs. As a donor solution the concentration of ibuprofen saturated solution was about 0.1 mg/ml in pH 3.0 phosphate buffer. Therefore, isosorbide dinitrate which had molecular weight about 236.14 could show a rather high permeability through shed snake skin.

For commercial isosorbide dinitrate transdermal system, 40 mg of drug was regularly dispersed in 36 cm<sup>2</sup> of polymer matrix. Before in vitro permeation study, they were cut into a circular of 4.3 cm in diameter. Therefore 16.1356 mg of drug was remained in each test specimen. Considering in the plastic disk assembly, 3.2 cm in diameter of surface area of commercial transdermal system could contact with the dissolution medium. Then the reasonable cumulative amount of drug which permeated to dissolution medium was about 8.9361 mg. In this experiment, 11.3580 mg of isosorbide dinitrate diffused through skin barrier which could be explained that, the contact between dissolution and drug over the determined surface area might occur and led to the higher cumulative drug permeated.

Commercial isosorbide dinitrate patch was matrix - type device whereas the prepared transdermal patch was a reservoir - type device. The difference in type of device influenced the permeation profile and the permeation rate of drug. In the reservoir system, the process of drug permeation in the in vitro skin permeation study could describe in two steps : the partition of drug through the rate - controlling membrane and then the partition through the skin barrier, while the matrix system the dissolution medium could directly contact with drug. From the above reason, the release of isosorbide dinitrate from commercial formulation was likely to be faster than prepared formulation. In vitro permeation study of prepared transdermal drug delivery systems, these transdermal patches were kept about 12 hours before studying. Therefore, the penetration of drug to membrane surface might occur and that led to fast

release of drug from prepared transdermal reservoir - type device including a little or no lag time in the release profiles.

For crosslinked chitosan : low molecular weight of PVA 1 : 9 and plasticized crosslinked chitosan : corn starch 4 : 1 membranes, the permeation rate of isosorbide dinitrate, which observed from the slope of release profile, showed slightly higher than commercial transdermal patch. Moreover, these membranes exhibited the highest cumulative amount of permeated drug. This result was in agreement with the obtained photomicrographs which showed the highest porosity in crosslinked chitosan low molecular weight of PVA 1 : 9 membrane and pit membrane surface in plasticized crosslinked chitosan : corn starch 4 : 1 membrane.

The permeation of the solutes through a given polymeric membrane could be explained by two mechanisms : the partition and the pore mechanism. These two mechanisms might not operate exclusively but one might be expected to dominate over the other. In the porous membrane, the pore mechanism was the dominate mechanism and the permeation of solutes depended on the pore size and the quantity of pores (Thacharodi and Rao, 1993). Therefore, the highest porosity and large pore size in crosslinked chitosan : low molecular weight of PVA 1 : 9 membrane gave high cumulative drug permeated.

The permeation of drug also depended on the smoothness of membrane surface. In rough membrane, the thickness of membrane in pitted area was low. Furthermore, the total surface area of rough and pits

membrane was higher than the smooth membrane, thereby the permeation rate of drug in transdermal patch which rough and pitted membrane acted as rate-controlling membrane would be higher than that of transdermal patch with smooth membrane. Then, plasticized crosslinked chitosan : corn starch 4 : 1 membrane which showed rough and pitted surface could give high amount of cumulative drug permeated. Although photomicrograph of plasticized crosslinked chitosan : tapioca starch 2 : 3 membrane showed rough and pitted membrane surface, the cumulative amount of permeated drug was low. This result could be discussed that, these pits were not deep enough. The depth of pit could be confirmed from photomicrograph of plasticized crosslinked chitosan - tapioca starch membrane. Moreover, a low cumulative drug permeated in transdermal patch, which this membrane was used as rate - controlling, might due to a low water sorption ability of this membrane. From water absorption data, it was show that, percent water sorption of this membrane was lower than the other prepared membranes. According to studying of several workers, it was found that increasing in water sorption or swelling of polymer led to increasing in permeation of drug through polymer (Shukla et al., 1991, Kim et al., 1992, Kim and Lee, 1992, Nakatsuka and Andrady, 1992). Therefore, a low water sorption in this blend membrane showed a low cumulative drug permeated.

The kinetic pattern of all transdermal patches was likely to be zero order kinetic which indicated the constant release rate throughout in vitro permeation study time.

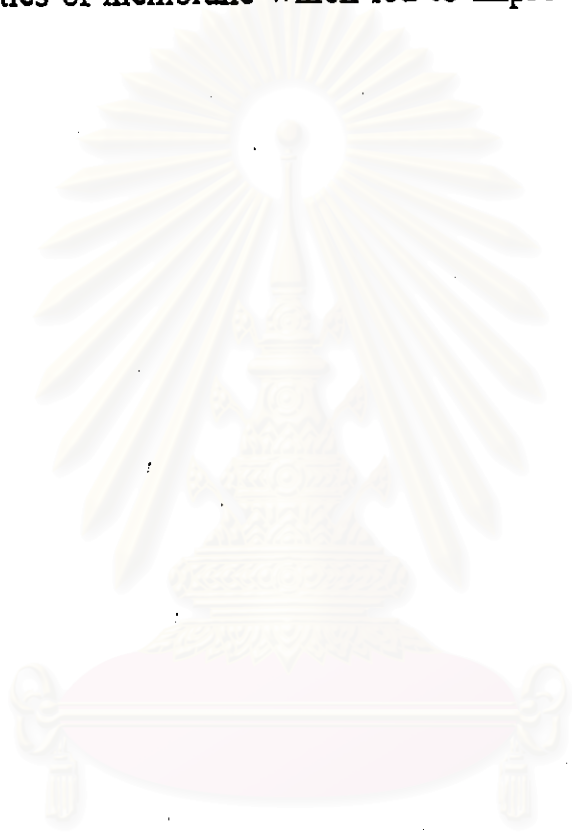
## CONCLUSIONS

Chitosan could blend with PVA or starch and crosslink to form transparent and flexible film. Variation in type, molecular weight and ratio of polymers including amount of crosslinking agent and plasticizer affected to chemical structure of the obtained membrane, thereby influencing the physical characteristics, water sorption, mechanical properties and finally the permeation of drug. Increasing the crosslinking agent reduced the water sorption and increased the ultimate tensile strength. Plasticizer could soften the brittle crosslinked chitosan - starch membrane to a strong and flexible membrane.

The using of high concentration of low molecular weight PVA with chitosan resulted in membranes with good physicochemical properties and high porosity which led to high permeation rate and high cumulative drug permeated. Furthermore, the permeation rate of this membrane formulation was closed to the permeation rate of commercial transdermal patch.

For crosslinked chitosan - starch membranes, the using of corn starch including with plasticizer could give a rough and pitted membrane surface which led to high cumulative amount of permeated drug. The optimum ratio of chitosan : corn starch was 4 : 1. This ratio not only gave the highest cumulative drug permeated but also gave a permeation rate closed to the permeation rate of marketed transdermal patch.

From this study, it was concluded that the crosslinked chitosan membrane could act as rate - controlling membrane in isosorbide dinitrate transdermal patch. The blending chitosan with the other hydrophilic polymers, subsequently crosslinking tended to improve the physico-chemical properties of membrane which led to improve the permeation of drug.



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