Chapter IV

Discussion and Conclusion

In this investigation, chitosan L, M and H from shrimp shell were employed as film former in coating of propranolol HCl tablet. The molecular weight of chitosan calculated from the Mark-Houwink equation indicated that the molecular weight of chitosan L was less than that of chitosan M, followed by that of chitosan H respectively. According to Filar and Wirich (1978) the molecular weight of chitosan was classified in term of viscosity which high M.W was more than 1000 cps and medium M.W. was 100-250 cps determined from 1% acetic acid and low M.W. was 25-70 cps determined from 2% polymer in 2% acetic acid. Thus, chitosan L and M were low molecular weight grade, and chitosan H was medium molecular weight grade. However, chitosan L, M and H were of low M.W. according to Knapczyk, Krowczynski and Krzek (1985 b) who classified that the low M.W. chitosan had the viscosity below 200 cps determined from 1% polymer in 1% acetic acid.

In addition, the data of IR spectrometry indicated that the chain length of chitosan H was higher than that of chitosan M, followed by that of chitosan L (Ritthidej,1994). From differential thermogram, the endothermic peak of chitosan H dominantly occurred at higher temperature than that of chitosan M and followed by that of chitosan L. The melting point was obviously dependent on the molecular weight. The high melting point indicated high M.W. chitosan (Alonso, Peniche-Covas and Nieto, 1983). Therefore, the molecular weight of chitosan H was dominantly higher than those of chitosan M and L, and the molecular weight of chitosan M was slightly higher than that of chitosan L. These results were corresponding to the data of viscometry.

However, the viscometry, IR spectrometry and DTA did not lead to obtain absolute molecular weight values, they were only relative measurements of polymer's molecular weight determination. An absolute molecular weight of this polymer could be further determined by various method such as light

scattering (Seymour and Carraher, 1981), osmotic pressure method and sedimention equilibrium method (Todura, 1994).

Chitosan is a cationic polymer having a pKa of about 6.3. The presence of free amine groups are capable of being protonated by the acid medium. Thus, the pH value of chitosan was increased when increase its concentration. The pH concentration profile of chitosan M solution was slightly lower than those of chitosan H and L solutions. This was according to the lower degree of deacetylation of chitosan M as indicated by the commercial sources. Thus, it had free amine groups less than chitosan H and L. However, from IR spectra, the degree of deacetylation could be ordered as chitosan H≤M<L.

Due to the pH values between 3.7-5.5, the prepared chitosan solutions were suitable to be used as film coating solutions, since they were not too corrosive to a coating pan. When using chitosan H solutions, they used the prolonged coating time, due to its low concentration.

During the coating process, mild odor of acetic acid could be detected. Tablets were rather tacky, but easily flowed in coating pan after drying. The surface of coated tablets was glossy. The bleeding and translucency on films of coated tablets could not be observed in because these films were very thin.

The weight of tablets after coating was increased between 1.15-1.80 % w/w and the weight variation was within the limit of USP standard. Slight weight loss of coated tablets after kept at room temperature for 1 week was attributed to the loss of residual water or acetic acid (Masilungan and Lordi, 1984). An increase in hardness of coated tablet after kept at the same condition may be due to the stronger bond formation and increasing in crystalline bridge between the particles. The water absorption of coated tablets after exposure to accelerated condition increased the weight and thickness of coated tablets and mainly reduced hardness and disintegration time by reducing the bond formation between the compressed granules in core tablet. The hardness of all coated formulations was not much more different. This may be attributed to the films on coated tablets were not too thick to influence on the hardness.

The percentages of friability of all coated tablets in this study were surprisingly negative values. This may be attributed to the moisture sorption of coating surface during friability test. In the cases of core tablets, because of the stronger bond formation of hardening binder, PVP K30, the percentage of friability after kept at room temperature for 1 week was less than after coating. Due to the binding property of moisture absorbed by the surface of core tablet the percentage of friability after exposure to accelerated condition was less than that after coating, but higher than that after kept at room temperature for 1 week.

The translucency of plasticized free films with triacetin and some plasticized free films with PEG400 may due to the incompatibility between chitosan, and triacetin and PEG400. Since most effective plasticizers will generally resembly most closely in structure the polymer they plasticize (Aulton, Houghton and wells, 1985; Radebaugh, 1988), triacetin is ester which should be less compatible with amine and hydroxyl groups of chitosan. As a result, chitosan free films plasticized with larger amount of triacetin exhibited higher degree of translucency. PEG400 was relatively incompatible with chitosan. The molecule of PEG400 contained several ethylene oxide groups and its molecular weight was 380-420 that was about five time of propylene glycol. Its molecule, being larger in size, was not easily accommodated in the crystal lattice of chitosan film. As a result, chitosan free films plasticized with increasing amounts of PEG400 exhibited increasing degree of bleeding and some free films were translucent. Propylene glycol was polyol. The molecular weight of propylene glycol is 76.1. It could be easily miscible with chitosan.

Disintegration time in deionized water of unplasticized coated tablets could be ranked as : LO<MO<LHO<HO. The result indicated the lower strength of film on core tablet of lower molecular weight. In case of plasticized coated tablets, the plasticized coated tablets of chitosan M exhibited the lower disintegration time in deionized water than plasticized coated tablets with chitosan L. The lower disintegration time of plasticized coated tablets of chitosan M in deionized water was due to molecular structure of chitosan M was parallel and it was

easy for water to penetrate as described by X-ray diffraction as followed.

Since chitosan is prepared from partial-N-deacetylation of chitin, its molecular structure still relates to the molecular structure of chitin. Three naturally occurring polymorphic forms have been recognized, known as alpha, beta and gamma chitins. Detailed crystallographic investigations have been reported for alpha and beta forms. Basic to the proposed structure is the presence of sheet of parallel chain linked by -N-H----O=C- hydrogen bonds through the amide groups. In beta chitin the sheets are all arranged in a parallel manner and inter-molecular hydrogen bonding is absence, whereas in the alpha form successive sheets are antiparallel with the extensive inter-molecular hydrogen bonding (Minke and Blackwell, 1978; Gardner and Blackwell, 1975).

The difference in diffractogram pattern between α - chitin and beta chitin is the higher intensity of the peak at about 10^{o} and the shift of this peak to the right of beta chitin. The result obtained from the diffractograms had provided evidences to indicated that chitosan M was a mixture of chitosan and beta chitin, chitosan L composed of chitosan and alpha chitin, and chitosan H was a mixture of chitosan and some of beta chitin.

The difference of diffractogram patterns between powders and free films was due to the change of crystalline structure of chitosan to chitosan salt, except those of chitosan M free films. The peak intensities at 10.5^{o} of chitosan LO and H0 indicated the hydrated crystalline because they provided a reflection near $2\theta = 10.4^{o}$ as reported by Robert(1994) and Ogawa(1991). However, all chitosan free films were not in anhydrous form, since they could not be observed a new peak at $2\theta = 15^{o}$ as reported by Ogawa (1991) and the preparation did not use the high temperature exceed 100^{o}c. According to the report of Ogawa(1991) the water could penetrate through the hydrated crystalline structure easier than anhydrous crystalline structure of chitosan.

The disintegration time in dilute HCl solution of tablets coated with plasticized chitosan M was rather slightly higher than the others, except that of LC system, LB30, and higher

than its disintegration time in deionized water. This result could be explained that the chitosan M had beta chitin in molecular structure which could markedly absorb the water and was swollen. In addition, it could be partially hydrated in acid environment which this gel formation easily adhered the disk during test and prolonged disintegration time.

Since the sheets of beta chitin were arranged in a parallel manner and inter-molecular hydrogen bonding was absence, beta chitin could be easily swollen. Thus, the weight and volume swelling indexes in deionized water of free films obtained from chitosan M were higher than those of chitosan H, followed by that of chitosan L. Surprisingly, in dilute HCl (1 : 100) solution, weight and volume swelling indexes of unplasticized chitosan H were higher than those of the other unplasticized free films. This could be explained that in dilute HCl solution amine groups in of chitosan H were protonated and increased electrical repulsion which increased the void between the chain more than that of chitosan M and L (Domszy, Moore and Roberts, 1985).

In addition the swelling index of LHO was lower than that of LO may due to the formation of hydrogen bonding when the short chains of chitosan L inserted between long chain of chitosan H. However, in dilute HCl solution repulsion effect was still prominent that the swelling index was higher than that in deionized water and higher than that of LO in dilute HCl solution. The repulsion effect also occurred in the plasticized free films because the weight and volume swelling indexes in dilute HCl solution were greater than those in deionized water.

The higher disintegration time of LC tablets in dilute HCl than that in deionized water was due to the film could be swollen and hydrated, and then form gel which adhered to the disk during test. This result also related to film swelling of free films. The weight and volume swelling indexes of free films plasticized with triacetin in dilute HCl solution were higher than those in deionized water. The weight and volume swelling indexes of free films plasticized with triacetin were higher than those of free films plasticized with propylene glycol. This result related to the report of Okor (1982) which attributed to the potential of the less hydrophilic plasticizer for promoting film swelling and porosity in

hydrophilic film. High swelling indexes of free films plasticized with PEG400, might due to the high hygroscopicity of PEG400 as seen in moisture sorption of free films.

The more plasticized coated tablets of LA and HA, the more reducing the disintegration time in deionized water and dilute HCl solution. This result might occur from the solubility in both media of propylene glycol and the occurrence of pore which media could easily penetrate to dissolve drug.

Because of the reducing the bond formation in core tablet by moisture sorption, the disintegration time of CORE R, L0 S, M0 S, LA10 S and LH0 S was lower than those after coating, and since the increasing the bond formation after kept at room temperature for 1 week, their disintegration time in deionized water was higher than after coating.

The effect of plasticizer on reducing swelling indexes of free film may due to the function of plasticizer to reduce the intermolecular interaction between chitosan molecules and reduced the electrostatic repulsion of protonated amine groups.

The determination of film swelling using both weight and volume differences had similar patterns. However, the volume swelling index was higher than the weight swelling index and some free films having high degree of swelling could not be detected of their volume swelling index in dilute HCl solution. The results indicated that the method using weight difference was easier and more suitable than using volume difference for determining the film swelling.

Because of similar endothermic peaks in thermograms of plasticized and unplasticized free films, plasticizers did not obviously have an influence on the melting point of chitosan. In general, plasticizer could reduce glass transition temperature of polymer. The reducing in glass transition temperature used to indicated the ability of plasticizer to plasticized polymer(Banker,1966). DSC should be used to study the glass transition temperature of prepared free films.

The data of weight loss indicated that it might have a release of acetic acid from free films. The weight loss of

plasticized free films with triacetin was higher than plasticized free films with propylene glycol and PEG400, and chitosan powder respectively. This result might due to the property of triacetin which could release acetic acid, the volatile breakkdown product (Masilungan and Lordi, 1984) and the ability of PEG400 to entrap acetic acid in free films greater than propylene glycol. Because the large molecule of PEG 400 had hydroxyl group to form hydrogen bonding with acetic acid, thus, acetic acid could not easily release. The lowest weight loss of chitosan powders might due to their powder were without acetic acid while the highest weight loss found in MO might due to acetic acid easily volatile because it did not have plasticizer to entrap. However, the lower weight loss of free films plasticized with PEG400 may occur from the interaction or complexation between acetic acid and PEG400 or PEG400 and chitosan as found in the SEM, the bleeding of free film and the retardation the drug release in dissolution test.

The moisture sorption of free films plasticized with PEG400 was rather greater than those with propylene glycol and triacetin respectively. PEG400 was more hygroscopic than propylene glycol and triaetin respectively, and might also due to the free films plasticized with triacetin lost acetic acid greater than the other systems.

As the molecular weight of chitosan increased the strength also increased. This was in agreement with Rowe(1984)who found that at low molecular weight of polymer the strength was relative weak, but as the molecular weight increased its strength also increased. The higher ultimate tensile strength of LH0 than that of H0 was due to the formation of hydrogen bonding when short chain of chitosan L inserted between long chain of chitosan H. The percentage of elongation could be ordered as L0>M0>LH0>H0. This result indicated that L0 could stretch greater than M0 and followed by those of LH0 and H0 respectively, but from the data and picture of stress-strain curves of the bigger film strip of H0 showed that it could be stretched greater than that of M0, followed by that of L0. The tensile property of free films was similar to the plastic like material type hard and tough which showed moderate elongation prior to the yield point followed by nonrecoverable elongation (Seymour and Carraher, 1981).

The incompatibility between plasticizer and polymer, and the effect of plasticizer on the crystal lattice of chitosan film were probably the reasons that the effect of type and amount of plasticizer on tensile properties as the reported by Lim and Wan (1994) who found the effect of imcompatible plasticizer on the physical and tensile properties of polyvinyl alcohol films.

Chitosan film retarded drug dissolution and diffusion into the medium. Characteristic lag time: when in contact with the dissolution medium, because the coating layer was gradually dissolved, and then the drug was easily release. Due to the high amount pores on film surface, the drug release from HO was faster than those of LO, MO and LHO.

The plasticized coated tablets with higher molecular weight of chitosan exhibited slower drug release. Since the rate of dissolution of the water soluble polymer depends on molecular weight, the larger the molecule, the stronger the forces holding the chain together (Florence and Attweed, 1981). However, in cases of 10% PEG 400 and 10 and 30% triacetin as plasticizer. chitosan M exhibited faster drug release than chitosan L. This was due to incompatibility between PEG 400 and chitosan, and more pores in the latter case. The slower drug release of plasticized LHA coated tablets might due to the amount of pores on surface was lesser because the short chains of chitosan L inserted between long chain of chitosan H.

Propylene glycol could easily dissolve from the coating surface and then dissolution medium could suddenly penetrate through the pore occurred after propylene glycol dissolving, and after chitosan dissolved the drug could easily diffuse and dissolve in dissolution medium. Due to propylene glycol easily dissolved, the dissolution profile mostly showed no lag time.

Coated tablets plasticized with PEG400 mostly showed long lag time and slowest drug release espectially in HB tablets. PEG400 is soluble in water and is widely used as plasticizer in film coating. Thus, the retardation of drug dissolved by PEG400 was unusual. However many lituratures have reported the incompatibility of PEG400 with many substances (American Pharmaceutical Association and The Pharmaceutical Society of Great Britain, 1986). The glucosamine unit might be

incompatible with PEG400 molecule and produced a lower water soluble substance. The data of bleeding, the appearance of particle and white mold-like spot in photomicrograph could be used to support this reason.

Triacetin is ester and less hydrophilic than propylene glycol and PEG400. Its less hydrophilicity could retard drug release. However, coated tablets plasticized with 10% triacetin of chitosan M and H exhibited drug released faster than those plasticized with propylene glycol. This was due to the more pores in MC10 and HC10. The pore occurrence in most plasticized coated tablets with triacetin might due to the less incompatibility between triacetin and chitosan. Since there was triacetin molecule between chitosan, chitosan chains had no bond to hold polymer chains together and that they had tendency to occur the gap between the chains. The gaps between the chains were the pores as seen in SEM. This result related to the report of Okor (1982) which attributed to the potential of the less hydrophilic plasticizer for promoting film swelling and porosity in hydrophilic film.

The effect of amount of plasticizers on drug release was corresponding to the type of plasticizers used. The increasing the amount of propylene glycol increased drug release, the increasing the amount of PEG400 decreased drug release. In case of HB tablets, the drug release was tremenously slower. The coated tablets with HB20 exhibited the slowest drug release and longest lag time, and this result related to the most white mold-like spot observed in photomicrograph. Due to the less hydrophilicity of triacetin, the increasing the amount of this plasticizer exhibited the slower drug release. However it was also dependent on the ability to promote the pore occurrence as seen in MC30 which exhibited drug release faster than MC20 and MC10. In case of HC20, the slower drug release during the first 5 minutes might due to its surface containing a large number of particles deposited which retarded drug release, however after 5 minutes the drug release was faster than that of HC30.

Due to the reducing in the bond formation in core tablet by the moisture sorption after exposure to accelerated condition, the drug release of CORE S was slightly faster than that of core after coating. The drug release of core after coating was nearly

equal to that of core tablet after kept at room temperature for 1 week (CORE R), although CORE R might occur the bond formation greater than core tablet after coating. This result was due to the high efficiency of disintegrant.

After kept coated tablets (LO, MO, HO, LA10 and LHO) at room temperature for 1 week, their drug release were slower, and surprisingly after they exposure to accelerated condition their drug release were dramatically slower than after coating. The reasons were the hydrolysis of chitosan salt and loss of acetic acid.

All IR spectra of free films exhibited the main peak at about 1560 cm-1 and the peak at about 1412 cm-1 which were absorption peaks of C=0 group of acetate salt (Colthup, Daly and Wiberley, 1990). The acetate ion from acetic acid could form ionic bond with the $-R-NH_3^+$ of chitosan molecule. The formation of acetate salt could be confirmed with an increasing of the peak heigh ratio of the peak at about 1412 cm-1 to the peak between 3422-3259 cm-1 of substance in free film comparing with chitosan powder. The peak at about 1380 cm-1 was the methyl group next to the C=0 group. This peak ratio at about 1380 cm-1 to 3422-3459 cm-1 was also employed to confirm the increasing of CH_3 group from acetate ion. This peak ratio showed the same result as the previous ratio peak except that the peak ratio of HC20 was slightly less than that of chitosan H powder and that of HA20 was equal to chitosan H powder. The IR spectra of free films plasticized with triacetin were the combination peak between the peak of unplasticized free film and triacetin and could not be observed the interaction between triacetin and chitosan. The salt form was also occurred in free film plasticized with all plasticizers used in this study.

After coating, although they were applied the hot air to evaporate the water from coated tablets. It might not be able to get rid of total moisture in core tablet and film. This residual moisture or the moisure absorbed by coating surface were able to hydrolyze chitosan acetate. The break-down products of hydrolysis were free acetic acid and chitosan in free amine form. This volatile product, acetic acid , could release from coated tablets (Masilungan and Lordi, 1984). Thus, the weight of coated tablets was slightly reduced in case of kept them at room

temperature for 1 week. Due to the more hydrophobic of free amine of chitosan than amine salt of chitosan, its solubility was also slower. The slower dissolution of chitosan in free amine form, the slower drug dissolved from coated tablets. Moreover, after exposure to the long duration of higher temperature and moisture coated tablets would more increasing in the hydrolysis of chitosan salt and promote acetic acid loss. Thus, it should more decreasing the dissolution of chitosan. From the picture of coated tablets after exposure to accelerated condition and dissolution test their film did not dissolve and still to the previous shape. Thus, the accelerated condition had much more influence on the property of coated tablets. The occurrence of insoluble product could be explained by the evident of Austin (1986) who found that chitosan, on treatment with certain of the disclosed carboxylic acids, notable formic, acetic, and pyruvic acids, the products containing substantially less than the stoichiometric amount of carboxylic acid were surprisingly water insoluble and could not dissolved in acidic solvent.

The insoluble free films were also clearly observed in photomicrographs. The length of insoluble particles of L0 was shorter than that of M0, followed by that of H0. This result related to the data determining the chain length of chitosan. The longest particles were observed in LA10 might due to the more compatibility between propylene glycol and chitosan which the polymer chain of chitosan could expand easily in this plasticizer system. The more expansion of polymer chain when there is more compatible between polymer and plasticizer have been widely reported (Radebaugh, 1988; Shah and Zatz, 1992). In case of LH0, due to the high hydrogen bonding, it was slightly dissolved and not clearly seen the polymer fibers. The scratchs on surface of H0 and LH0 were due to some of chitosan H was dissolved while in case of LH0 the short chains of chitosan L were binded to some chains of chitosan H with hydrogen bond and did not dissolve.

The decrease in the drug release of coated tablet after exposure to accelerated condition might also due to the curing effect (Bodmeier and Paeratakul, 1994), since the surface of L0 S and M0 S had the pore less than L0 and M0 respectively. Therefore, the slowest drug release of L0 S and the slow drug release of M0 S were due to this curing effect. The fastest drug

released of H0 S and the fast drug released of LH0 S was due to there were many cracks and defects on their surface as seen in photomicrograph. The faster drug release of LA10 S than L0 S was due to the plasticization effect of propylene glycol. This plasticization effect of LA10 was also seen in case of kept this coated tablets at room temperature for 1 week, since LA10 R exhibited the fastest drug release, and the lowest drug released of LH0 R was due to its stronger hydrogen bonding.

Since there were some small pores in insoluble film of coated tablets after exposure to accelerated condition to control drug release, their released profile was similar to zoro order kinetic of drug release.

The accelerated condition should not affect the stability of propranolol HCl, because the percentage of drug content after exposure to this condition was within the limit of BP standard. Howerver, the physical stabilities of coated tablets and drug released was markedly changed after exposure to this condition.

CONCLUSION

Chitosan L, M and H derived from shrimp shell of Thailand dissolved in dilute acetic acid solution could form film coating upon the surface of propranolol HCl core tablets and could be casted into free films. The molecular weight of chitosan could be ranked as: chitosan H>M>L. Tablets coated with chitosan solutions and free films were investigated and the data from the evaluaions of free films such as physical appearance, IR-spectra, X- ray diffractogram, DTA, film swelling, moisture sorption and tensile property were used to characterize these polymeric films.

Coated tablets were glossy and yellowish. The increasing molecular weight of chitosan dominantly retarded the drug release. The increasing the amount of propylene glycol enhanced drug release while the increasing the amount of PEG400 and triacetin prolonged drug release. The weight and volume swelling indexes of plasticized free films were less than those of unplasticized free films, since the plasticizer decreased the interaction between chitosan molecules. The moisture sorption of free films with PEG400 was greater than that of free

films plasticized with propylene glycol and triacetin respectively. This result was due to the hygroscopicity of PEG400 and the less hydrophilicity of triacetin. However, the properties of coated tablets and free films also depended on the molecular structure of chitosan and the compatibility between chitosan and plasticizers. Propylene glycol was more suitable than PEG400 and triacetin to plasticize chitosan films.

The hydrolysis of chitosan acetate which was resulted from the interaction between NH_3^+ of chitosan and CH_3COO^- of acetic acid changed the physicochemical properties of propranolol HCl coated tablets especially the color and solubility of coated films. The drug release was markedly decreased.