CHAPTER V

DISCUSSION AND CONCLUSIONS

The purpose of this study was to prepare mucoadhesive film for oral mucosal administration of lidocaine hydrochloride as a viable alternative dosage forms to lidocaine hydrochloride injection in dentistry. To avoid the toxicity of organic solvents, CMC, HPMC E15, HPMC E4M, HPC and chitosan were chosen as mucoadhesive polymers. Several researchers have used these polymers as vehicle for intraoral mucoadhesive films (Rodu et al., 1988, Ozeki et al., 1995, Peh and Wong, 1999 and Senel et al., 2000). Cellulose derivative polymers are linear molecules. They possibly penetrate into the crevices of the rough surface and possibly form interpenetration and entanglement between the polymer chains and mucus chains. Sanzgiri, et al. (1994) reported that the adhesion forces of hydrated HPMC film at pH 1.2 and 7.8 were greater than those of polycarbophil. Chitosan is a cationic polymer. It is possible to form ionic bond with mucin which have negative charge at physiological pH. Chitosan has been reported to be effective mucoadhesive in previous studies usually when tested in a dry or partially hydrated state (Lehr et al., 1990, and Patel et al., 1999).

The mucoadhesive films were prepared by using casting method due to the ease of preparation with simple laboratory equipments. However, the duration to prepare the films was long, at least 12-20 hours. This result suggested that other accessible apparatus particularly a vacuum hot-air oven may be taken into consideration for drying the films.

The physical appearance of the obtained mucoadhesive film formulations depended mainly on the nature of the raw materials, particularly their color and water solubility. Chitosan is yellowish while the cellulose derivatives are almost white powder, resulting in transparency of the film formulations. While increasing the content of drug in chitosan films, the transparency was reduced due to the solubility of the ingredient. As seen in the SEM photomicrographs of chitosan films, increasing the ratios of drug and polymer increased the aggregate particles.

Incorporation of the drug in CMC films yielded precipitated particles in the films. Increasing the content of the drug in CMC films increased the precipitation. DSC thermograms and X-ray diffractograms indicated that the drug was in the crystalline form in the film. Due to the precipitation in lidocaine HCl loaded CMC films, those films were not further investigated. The precipitation was evident due to the drug could not completely dispersed in CMC in amorphous or molecular dispersion state.

The appropriate mucoadhesive films where the films with good adhesive strength, uniform thickness, free of any air bubble, easy to detach from the petri dish after drying with characteristics of flexibility and did not have any crack or fracture. Both two grades of HPMCs provided transparent films. HPMC E15 films were easier to detach from petri dish than HPMC E4M films. These results were in agreement with the tensile property study that HPMC E15 had lower Young's modulus and had higher strain at point of break. HPC film at drug to polymer ratio of 1:1 was the most difficult to detach from glass petri dish. This finding was in consistent with the tensile property study that it had very low Young's modulus and tensile strength indicating it was soft and weak. However, decreasing the drug to polymer ratios tended to ease the detachment of films from petri dish due to the increase in film thickness. Combination of HPMC E15 and HPC provided the films that were easier to detach than HPC alone.

The SEM photomicrographs of lidocaine HCl loaded films at drug to polymer ratio of 1:1 showed no precipitation or aggregation of the particles. This indicated that the drug was homogeneously dispersed, in amorphous or molecular dispersion state, in the films at this ratio of drug and polymer. However, the SEM photomicrographs of films at higher ratios of drug and polymer showed that the smoothness of surface and the cross-section texture were decreased especially in chitosan films due to the precipitation or aggregation of particles. The concentration of chitosan in citric acid solution was prepared to be almost saturated solution. Thus, during the solvent evaporation process, sufficient quality of water was evaporated, the polymer became insoluble. Therefore, the transparency of the films was reduced and had precipitation or aggregation of particles. To confirm that the rough textures were caused by precipitation or aggregation of polymer molecules, the DSC thermograms

of the films with drug to polymer ratio of 1:0.5 were performed. There was no melting endothermic peak, indicating that the drug was present as an amorphous form or molecular dispersed in polymeric films. The aggregated particles as seen in SEM photomicrographs were likely to be the aggregation of amorphous form of the drug, polymer or other ingredients. The SEM photomicrographs of HPC films had porous in the cross-section texture. During preparation process, very small air bubbles were formed by stirring. While drying, flocs were formed and the air bubbles were expanded. Therefore air bubbles might be trapped into the flocs. Porous films were obtained. The combination of HPMC E15 and HPC films also showed the porous and rough texture. During drying process, HPC and HPMC E15 might be separated from each other. HPMC E15 did not precipitate but formed gel while HPC formed flocs, therefore the solvent evaporation was not homogeneous due to the surface of the films were not smooth and have pores.

The specific surface area of HPMC E4M film was higher than that of HPMC E15 film. This was due to the thickness of HPMC E4M which was less than that of HPMC E15 film. The specific surface area of HPC film could not be measured due to its stickiness. The specific surface area of chitosan films at various ratio of drug to polymer were evaluated. That of drug to polymer ratio of 1:1 showed the highest specific surface area. Unexpectedly, CS 1:0.67 showed the lowest specific surface area. These results were inconsistent with the surface morphology and the thickness of the films, SEM photomicrograph showed that the roughest surface was observed in chitosan film with the ratio of 1:0.5, and thickness was the least. Therefore the chitosan film with drug to polymer ratio of 1:0.5 possibly had the highest specific surface area. This indicated that method to determine the specific surface area might not be appropriate because the films were cut into the small pieces with irregular size and shape before being measured. However, this method could be determining specific surface area due to the specimen's shape and size was randomization. The specific surface areas of combination of HPMC E15 and HPC films could be ranked as E15HPC 1:3 > E15HPC 2:3 > E15HPC 3:3 > E15HPC 3:2 > E15HPC 3:1. They were consistent with the surface morphology study that E15HPC 1:3 film had the roughest surface, while E15HPC 3:1 film had the smoothest surface.

The FT-IR spectra of lidocaine HCl loaded HPMC E15, HPMC E4M, HPC, chitosan, and combination of HPMC E15 and HPC films showed compatibility of all ingredients. There was no new peak in the FT-IR spectrum of prepared film. The spectra of the prepared films were the summation of spectrum of each ingredient. However, the C=O stretching bands were shifted to a higher wave number for all polymeric films. These results could be explained similarly to the oxprenolol HCl in the previous report (Ozeki et al., 1995), that lidocaine HCl and polymers or polymers and other ingredients were possibly interacting with each other by hydrogen bonding.

The DSC thermograms of prepared lidocaine HCl films showed that there was no melting endothermic peak of drug in DSC thermograms except CMC 1:1 showed very small endothermic peak at 67.7°C which was the melting point of lidocaine base. This indicated that the drug existed as molecular dispersion or amorphous state in the films except CMC 1:1. Confirmation with powder X-ray diffraction also showed that there was no peak in cases of HPC and chitosan films indicating that the drug existed as molecular dispersion or amorphous form in the films. The result was consistent with a previous study that lidocaine and lidocaine HCl were present as an amorphous form in the solid dispersion films of HPC (Danjo et al., 1995, Kohda et al., 1997 and Okamoto et al., 2001). In cases of HPMC E15, HPMC E4M, and combination of HPMC E15 and E4M films, there were two very small peaks in X-ray diffractograms, at about 9.4° and 28.5° 2θ. The intensity of the peaks of the drug were very low indicating that the drug was rarely in crystalline form in polymeric films. Due to transparency of the films, lidocaine HCl was more possible to existed as molecular dispersion than as amorphous state in the films.

An ideal intraoral film should be flexible, elastic, soft yet adequately strong to withstand breakage due to stress from mouth activities (Lin et al., 1991, and Peh and Wong, 1999). Moreover, it must also possess good mucoadhesive strength so that it can be retained in the mouth for a desired duration. Swelling of film, if excess, should not be too extensive to prevent discomfort. As such, the mechanical, mucoadhesive, and swelling properties of film are critical and essential to be evaluated.

In this study, the tensile properties of the lidocaine HCl mucoadhesive films were studied. The tensile testing provided an indication of the strength and elasticity of the film, which could be reflected by tensile strength, Young's modulus and strain. It suggested that films suitable for intraoral administration had to be preferably strong but flexible.

Results of tensile experiments showed that increasing the viscosity grade of HPMC would increase the Young's modulus and the tensile strength, indicating that HPMC E4M films were harder and stronger than HPMC E15 films. These may be partially attributable that the longer chain of HPMC E4M polymer could from the stronger network. While HPC provided the soft and tough films because they had the lowest tensile strength and Young's modulus, and the highest percent strain. It was possible that the hydroxypropyl substituent groups of HPC contained almost entirely secondary hydroxyls. The secondary hydroxyl present in the side chain was available for further reaction with the oxide, and chaining-out may take place. The formation of side chains with more than one mole of combined propylene oxide resulted in the longer side chains of HPC than other polymer (Rodu, et al., 1988), thus HPC were flexible than other polymers. Chitosan films provided high Young's modulus, moderate tensile strength and low percent strain indicating that chitosan films were hard and brittle. Increasing the proportion of HPMC E15 in films of combination of HPMC E15 and HPC increased Young's modulus and tensile strength. This was due to the mechanical properties of HPMC E15. Unexpectedly, HPMC E15 and HPC had similar percent strain but combination of HPMC E15 and HPC showed lower percent strain. Moreover, increasing the proportion of HPMC E15 decreased the percent strain. It was possible that combination of HPMC E15 and HPC provided porous films as shown in SEM photomicrographs.

The moisture sorption and swelling of the mucoadhesive films was investigated by exposing the films to moisture at various percentages relative humidity. This apparently inconsistent behavior of polymer action on moisture sorption into film could be explained as follows. Polymer molecules were large molecules compared to water. They were made up of hundreds of chain segments. These long-chain molecules were not in the form of extended straight chains but were tightly folded random coils. Individual polymer coils were not separated, but

interlocked and entangled with each other. When the polymer came into expose with moisture, forces of attraction, chiefly hydrogen bonding, started acting between them. The polymer-water interaction was likely to be preferred over polymer-polymer interaction. Thus forces holding the polymer segments together were much reduced. Water molecules forced their way between segments, breaking the polymer-polymer contacts, surrounding individual polymer coils and established contact with them. As liquid molecules penetrated into the interstices of the polymer caused the polymer to hydrate, the polymer started to swell and increased in size. The polymer chains began to unfold and gradually became solvated. However, they did not assume the shape of an extended straight chain. The coiled nature of the polymer was still retained but with a very much expanded coil volume. The void spaces created as the polymer unfolds were occupied by the water molecules (Wan, et al., 1991).

Although, the moisture sorptions of HPMC E15 films and HPMC E4M films were not different, but the swellings of HPMC E15 films were higher than that of HPMC E4M films. The results indicated that HPMC E15 films swelled more than HPMC E4M films even both absorbed similar amount of water. The longer chains of HPMC E4M were likely to be more entangled than shorter chains of HPMC E15. Therefore HPMC E4M films were denser than HPMC E15 films. It could indicate that the chains of HPMC E15 were more flexible than the chains of HPMC E4M after exposure to moisture. HPC films could absorb moisture less than HPMC E15 films but after the 7^{th} day the difference was reduced. The swelling properties of these two polymeric films were not different at high relative humidity. The results indicated that HPC absorbed less water than HPMC E15 but could swell as well as HPMC E15. Chitosan films could absorb the moisture as well as HPMC E15 films but exhibited less swelling than HPMC E15. It could indicate that chitosan had higher molecular weight and longer chain length than HPMC E15 due to the chains coiled tight together. The results indicated that chitosan films were denser than HPMC E15 films.

Combination of HPMC E15 and HPC provided the films that could absorb moisture and swell less than HPMC E15 film and HPC film. This was disagreement with the surface morphology that the combination films had porous surface. It was possible that upon combination of HPMC E15 and HPC, the polymer complexation

may occur by hydrogen bonding, thus the hydrophilic groups were shielded. The hydrophilicity of combination films was reduced.

Mucoadhesive drug delivery systems was proposed and formulated to be localized onto biological surface. A mucoadhesive force was required between the drug delivery system and the biological surface successfully to retain the system and retard the natural clearance process. In the present study, the adhesive strength of the mucoadhesive films were studied in term of the detachment force. The detachment force was measured by detaching the prehydrated films from the aluminum supporters. The chemical bonding did not occur due to no presence of mucin chain. Therefore the adhesion could only be established by forming the mechanical and physical bonding of the polymer and the substrate (Mathiowitz, et al. 1999). The inclusion of the polymer into the cracks or crevices of the substrate resulted in the adhesion of the two substances. In order for polymer chains to penetrate into the crevices, the polymer chain had to be both mobile and flexible. Therefore the swelling property of the polymer would affect the adhesive strength. The amount of water that used to prehydrate the films, the applied strength, the contact time and moving speed of the holder would all affected the mucoadhesive measurement. Therefore these factors were fixed in this study.

HPMC E15 films had the highest detachment force. These were possible that prehydrated HPMC 15 film had the most flexible polymer chain. Therefore the polymer chains could consequently to penetrate into the crevices of the substrate. Comparison between both grades of HPMC found that HPMC E15 had higher adhesive strength than HPMC E4M film. However Duchene et al. (1988) noted that the bioadhesive force increased with the increasing of the molecular weight of polymer. This was possible that the amount of water (200μl) and/or the contact time were not enough to hydrate HPMC E4M film so the rigid chains could not penetrate into the crevices.

Although chitosan films were swollen and absorbed moisture more than HPMCs films but the adhesive strength of chitosan films were less than HPMCs films. It was possible that the amount of water was not enough to hydrate chitosan film or unfold chitosan chains. Therefore, chitosan chains were not sufficiently

flexible to penetrate into the cervices of the substrate. In agreement with Guo (1994), this study found that chitosan films had lower adhesive strength than the HPMCs films.

While the study of swelling property noted that swelling of HPC and HPMC E15 films was not different, the mucoadhesive study found that HPC films had lower detachment forces. This was attributable that the higher molecular weight, the longer chain molecules coiled tightly together therefore even water could penetrate into the coil of the polymer chain but the polymer chains were not sufficiently flexible to penetrate into the cervices of the substrate. Moreover it might be possible that the amount of water (200µl) and/or the contact time were not enough to hydrate the HPC polymer chains.

For the films of combined polymers, increasing the amount of HPMC E15 increased the adhesive strength of the film. Plotted the detachment force as a function of the proportion of HPMC E15 as shown in Figure 82 found that it had linear relationship (R^2 = 0.9912), indicating that increasing of adhesive strength was attributed by unfolded and flexible chain of HPMC E15 penetrated into crevices of substrate.

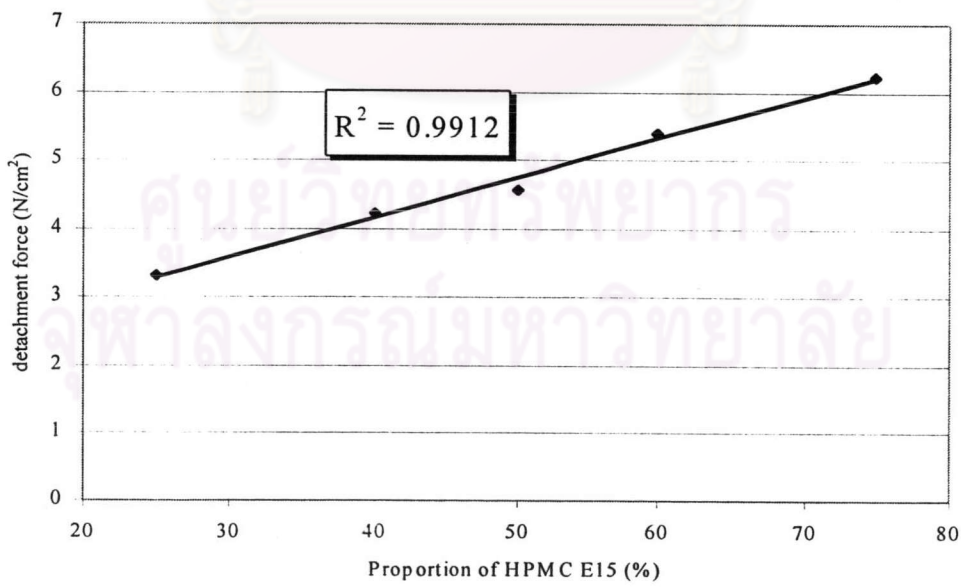

Figure 82 Relationship between the detachment force and proportion of HPMC E15 in the combined polymer films

The in vitro releases and penetrations of the drug from mucoadhesive films were studied. The results of the drug penetrated through dialysis membrane showed that the drug penetrated from HPMC E15 films was faster than from HPMC E4M films. These results were consistent with the swelling property study that HPMC E15 films could swell more than HPMC E4M films. When the polymer swelled the void spaces were increased with the polymer unfolded and the coils hydrated. The drug substantially diffused through these voids. Moreover the hydrated HPMC E15 films had lower viscosity than HPMC E4M hydrated films. The drug would be trapped into more viscous hydrated films. Therefore the release of drug were reduced. While the fastest penetrate was observed from HPC films. Although, HPC polymer could be soluble in water below 45°C, the film became opaque during penetration process. Its hydroxypropyl group contained a hydrophobic methyl group at the end of the chain. The secondary hydroxyl on the number 2 carbon imparted the hydrophilic character by binding water through hydrogen bonds. However, the hydroxyl group could not hold as much water as an unshielded primary hydroxyl group found on related polymers. When the solution was heated, labile hydrogen bonds were broken and the polymer began to lose water. When sufficient quantity of water is lost, the polymer becomes insoluble, a state also described cloud point, which normally for HPC was 42°C (Rudu et al., 1988). The carboxyl group of citric acid provided esterification of the hydroxyl groups of HPC. This esterification decreased water solubility by blocking the hydrophilic hydroxyl groups, causing the cloud point to decrease. When HPC film was placed into the donor compartment of the diffusion cell, the esterified polymer became opaque, insoluble film because its cloud point was below 37°C. This finding agreed with a previous study by Rodu, Russell and Desmarais (1988). Therefore, the viscous gel was not formed, the drug was not trapped in the gel. Moreover, the SEM microphotograph showed that porous texture therefore the drug could be diffused through this porous. The penetration rates of the drug through dialysis membrane from HPC films were evidently faster than from other polymeric films. Although, the moisture sorption and swelling of chitosan films were high, the drug penetration rates were not high. It was possible that the drug was entrapped in the structure of chitosan resulted in the slow penetration of drug from chitosan films. Combination of HPMC E15 and HPC provided the porous films. The fastest of drug

penetration rate was observed in HPMC E15 to HPC ratio of 3:3. It was likely that the high porosity of this film caused the fast penetration rate.

Comparison between E4M 1:0.5 and E4M 2:1, the same drug to polymer ratio but different drug loading, the drug penetration rate in term of amount of drug penetrate, mg per square centimeters, the penetration rate of the drug from E4M 2:1 was faster than E4M 1:0.5. In term of percent cumulative amount of drug penetrated showed that E4M 1:0.5 film exhibited faster penetration than E4M 2:1 film. The thickness of E4M 1:0.5 film was less than that of E4M 2:1 film, the drug penetration rate from E4M 1:0.5 film in term of percent penetration were higher, although amount of drug loading in E4M 2:1 are higher. This result was consistent with Fick's second law of diffusion (Kalia and Guy, 2001).

$$\frac{M_t}{M_\infty} = 2\left(\frac{Dt}{\pi L^2}\right)^{1/2}$$

M_t and M_∞ were cumulative amount of drug penetrate at time t and at time infinite, respectively. D was diffusivity of the drug, L was thickness of the film.

The drug release without dialysis membrane showed that the drug release rate was higher than the drug penetrated through dialysis membrane. However, the results of lidocaine HCl release without dialysis membrane were correlation with the results of the drug penetrate through dialysis membrane. The results indicated that dialysis membrane could be used as the supporter in the drug penetration studies although it was a barrier for release of the drug. The drug release from E15 1:1 film was slightly faster release than that from E15HPC 3:3 film and higher than those from HPC 1:1, E4M 1:1 and CS 1:1 films, respectively.

Different kinetic equations were applied to interpret the penetration rate from the mucoadhesive films through dialysis membrane. When plotted as penetration of lidocaine HCl from its saturated solution versus time, a linear relationship (R^2 = 0.9989) was observed indicating the linear penetration profile, a behavior of ideal method of drug release in order to achieve a pharmacological prolonged action. To characterize the penetration mechanism of lidocaine HCl from its saturated solution through dialysis membrane, the n value of power low expression model was 0.9934 (R^2 = 0.9988) exhibiting case-II transport or zero order (time-independent linear

transport). This result was consistent with the result of treatment with zero order model. This was possible that the donor compartment had a large amount of drug, the drug penetrate was constant.

The penetration kinetics of lidocaine HCl from HPMC E15, HPMC E4M, HPC and chitosan for all of ratios of drug and polymer, when treated with Weibull model were slightly more linear relationship than when treated with Higuchi model. The results indicated that there was no fundamental kinetic adequately to characterize the penetration kinetic properties of the drug, and there was no single parameter related with the intrinsic penetration rate of the drug (Costa and Lobo, 2001). However, Higuchi had high correlation coefficient more than 0.98. To characterize the mechanism of lidocaine HCl penetrated through dialysis membrane from mucoadhesive films, the power law expression model was used to treat the penetration data. The correlation coefficients were more than 0.99 and the n values were between 0.5 and 1 indicating anomalous transport (non-Fickian release). The penetrations of the drug from the mucoadhesive films were synchronization between diffusion-controlled and chain relaxation-controlled (swelling-controlled) penetration mechanism. Chitosan films had the highest values of n, higher n value, due to polymer chain relaxation dominating Fickian transport.

The penetration kinetic of the drug from combination of HPMC E15 and HPC films, treated with Weibull model was slightly more linear than Higuchi model. The result indicated that there was no fundamental kinetic adequately to characterize the penetration kinetic properties of the drug, and there is not single parameter relate with the intrinsic penetration rate of the drug (Costa and Lobo, 2001). However, the relative coefficient when treated with Higuchi model were more than 0.987 except from E15HPC 3:3 film which was 0.976. Characterization the drug release mechanism, when treated with power law expression model found the values of n were between 0.5 and 1 in all combination ratios exhibiting a non-Fickian release behavior controlled by combination of diffusion and chain relaxation mechanism. The penetration of drug from E15HPC 3:3 gave the highest n value, due to the drug penetrate from E15HPC 3:3 film was more depended on the swelling rate of film than the drug penetrate from other ratio of combination films. Therefore the consistency

with the relative coefficient when treated with Higuchi model was less than those of other combination ratios.

Comparison between Formulations of E4M 1:0.5 and E4M 2:1 on the penetrations kinetic of drug revealed that the linear relationships when treated with Weibull model were slightly more than when treated with Higuchi model. The results indicated that there was no fundamental kinetic adequately to characterize the penetration kinetic properties of the drug, and there was no single parameter relate with the intrinsic penetration rate of the drug (Costa and Lobo, 2001). However, the correlation coefficients when treated with Higuchi model were more than 0.993. Characterization of the drug penetration mechanism when treated with power law expression model found that the values of n are between 0.5 and 1 in all combination ratios indicating a non-Fickian release behavior controlled by combination of diffusion and chain relaxation mechanism. E4M 2:1 had higher n value (n = 0.9212) than E4M 1:0.5 (n = 0.6342). Higher n value indicated that polymer chain relaxation dominated Fickian transport. It was possible that the thickness of E4M 2:1 film was more than that of E4M 1:0.5 film.

Conclusions

The intraoral mucoadhesive films could open a new horizon in drug delivery system. These rather thin and flexible films adhered to the intraoral mucosa, remained in place and penetrated their drug content steadily for reasonable length of time and improved patient compliance. The method used to prepare the mucoadhesive films was simple by using common equipments. All materials were safe and widely used in pharmaceutical preparations. In this study various polymers were screened for their film forming and mucoadhesive potential. The obtained films were thin and so flexible to use along the curvature of the oral cavity except the films containing CMC which were precipitation. Based on the results, formulations containing HPMC E15 as mucoadhesive polymer produced films with the highest mucoadhesive strength. The physicochemical characterization of HPMC E15, HPMC E4M, HPC, chitosan and combination of HPMC E15 and HPC films revealed the compatibility of ingredients and lidocaine HCl existed in the films as molecular dispersed or amorphous form. At the drug to polymer ratio of 1:1, the drug was

homogeneous dispersed in the films. Increasing drug to polymer ratios tended to produced unhomogeneous films. Combination of HPMC E15 and HPC provided the films that had rough and porous surface. The tensile properties of the films were evaluated. HPMC E4M films were harder and stronger than HPMC E15 films. Chitosan provided the films that were hard and brittle, while HPC provided soft and tough films. Incorporation of HPC into HPMC E15 film was to reduce the rigidity of HPMC E15 films. The penetrations of drug from HPMCs, HPC and combination of HPMC E15 and HPC films were faster than from chitosan film. Eighty percent of drug penetrated from HPMCs, HPC and combination of HPMC E15 and HPC through dialysis membrane within 60 minutes while drug penetrate from chitosan films were more sustained. The drug penetrations kinetic from the obtained films through dialysis membrane were the best fitted with Weibull model. While Higuchi model had high correlation coefficient more than 0.98. The drug penetrated through dialysis membrane from obtained films was controlled by the combination of diffusion and polymer chain relaxation mechanisms. Regarding all of the properties evaluated, E15HPC 3:3 film was found to be the best formulation to achieve the aim of this study. These films had satisfactory mucoadhesive and tensile properties and released 90% of lidocaine HCl content over period of 86 minutes.