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Buccal administration of mucoadhesive blend films saturated with propranolol loaded nanoparticles

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ABSTRACT

The aims of this study were to prepare and characterize hydroxypropyl methylcellulose (HPMC)/polycarbophil (PC) mucoadhesive blend films saturated with propranolol hydrochloride (PNL)-loaded nanoparticles to improve permeability of drugs that undergo first-pass metabolism. An ionic cross-linking method and film casting technique was used to prepare nanoparticles and mucoadhesive blend films, respectively. Increasing concentrations of PNL (70, 80, 90 mg/film) in HPMC/PC blend films containing PNL-loaded nanoparticles (PN-films) and HPMC/PC blend films containing PNL (80 mg/film) without nanoparticles (PP-films) were prepared to test swelling, mucoadhesiveness, release, permeation and physicochemical properties. Scanning electron microscope (SEM) images showed a partially smooth surface with a wrinkled occurrence and spherically shaped, well-dispersed nanoparticles on the surface of PN-films containing PNL 80 mg/film (PN-films-80). The size of the nanoparticles on the surface of PN-films-80 was around 100 nm, which was similar to the nanoparticle size observed using light scattering technique. The swelling index (SI) of all PN-films and PP-films increased greatly in the first period time (10–20 min) and reached swelling equilibrium at 20 min and 30 min, respectively. For the PN-films, the concentration of PNL influenced the mucoadhesive properties and tended to be higher when the amount of PNL increased. Immediate release of all blend film formulations was found in early time points (10–30 min). After 120 min, the release of PN-films-70 was lower than the other PN-films. Permeation studies using porcine buccal mucosa showed that inclusion of nanoparticles in the films increased the permeability of PNL compared to PP-films. Therefore, buccal administration of mucoadhesive blend films containing PNL-loaded nanoparticles could be a promising approach for drugs that undergo first-pass metabolism.

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1. Introduction

Buccal drug delivery system is one of the mucosal routes that have been extensively studied over the last few decades. Buccal administration has many advantages including avoidance of hepatic first-pass metabolism and drug degradation in the gastrointestinal tract [1,2]. Many mucoadhesive polymers have been examined in bioadhesive buccal dosage forms such as tablets, patches, gels, ointments, and films to determine their ability to prolong residence time on adhesive membranes and to increase bioavailability [3–5]. Mucoadhesive films are the favorable type of dosage form for buccal mucosa administration due to their flexibility, softness, small size, and thinness that demonstrate improved patient compliance compared with tablet forms [2,6]. Compared with liquid, gel, and ointment formulations, mucoadhesive films provide more accurate drug dosing [4]. Hence, mucoadhesive films should maintain extensive adhesive contact with the mucosal membrane to prolong the retention time of the delivery system and to enhance the bioavailability [4]. Blend films are a popular technique because they are very efficient and can improve properties of mucoadhesive films. This method mixes two different polymers to enhance their properties [2]. Two polymers, hydroxypropylmethyl cellulose (HPMC) and polycarbophil (PC), were used in this study to prepare mucoadhesive blend films.

HPMC is a popular hydrophilic polymer that is widely used in many pharmaceutical applications due to its ease of use, flexibility, good film-forming properties, biocompatibility, and biodegradability [7,8]. HPMC demonstrates rapid swelling due to its ability to absorb water and has moderate mucoadhesive properties [9]. PC has a high water absorbing capacity and has excellent mucoadhesive properties [10,11]. Therefore, PC has been extensively employed in mucoadhesive formulations for different mucous membranes such as nasal, vaginal, rectal, ophthalmic, and buccal membranes [5,11,12]. In our previous report, we demonstrated that HPMC and PC blend films had higher mucoadhesive properties than pure HPMC films [2]. Many studies have shown that mucoadhesive blend films demonstrate appropriate adhesion and adhesive times on buccal membranes and display drug release within the required time. However, a crucial restriction of buccal drug delivery is low drug permeability through the membrane that causes low bioavailability [6,13]. One reasonable approach to improve drug permeability is the use of nanoparticles incorporated into mucoadhesive blend films [6,13].

Nanoparticles have been investigated for many pharmaceutical purposes due to their ability to protect drugs from enzymatic degradation, control the release of drugs, and enhance drug penetration and absorption at the specific membrane sites leading to increased bioavailability [14,15]. Chitosan is the most commonly used polymer for preparation of nanoparticles [14,16]. Chitosan is a natural cationic biopolymer that can be obtained from insects, fungi crustaceans, etc. [15,16]. Chitosan has many advantages such as low toxicity, biocompatibility, biodegradability, good mucoadhesion, and the ability to enhance membrane permeable properties [15,17]. It is soluble in acidic solutions and contributes to positive charges on the molecular structure [18]. Hence, the positive charges of chitosan can interact with negatively charged molecules such as tripolyphosphate (TPP) to form nanoparticles for drug or protein encapsulation.

Propranolol hydrochloride (PNL) is a non-selective β-adrenergic blocker generally used for the treatment of cardiovascular disorders such as angina pectoris, hypertension, and cardiac arrhythmia [19]. For conventional oral dosage forms, PNL has to be administered several times a day to keep blood levels of the drug within the therapeutic range due to the short half-life of approximately 4 h [14,19]. PNL is quickly absorbed almost completely in the gastrointestinal tract after oral administration. However, its bioavailability is low (about 25%) due to high first-pass metabolism [14,19]. Therefore, PNL loaded nanoparticles incorporated into mucoadhesive blend films for drug delivery through buccal mucosa could help solve this problem.

In this project, we combined two potential approaches (mucoadhesive films and nanoparticle carriers) in one delivery system (buccal mucosa) to improve permeability of PNL, which undergoes first-pass metabolism. The blend films were loaded and unloaded with nanoparticles containing PNL and characterized for swelling, mucoadhesiveness, release, permeability, and physicochemical properties.

2. Materials & methods

2.1. Materials

Chitosan (MW 20 kDa, 85% degree of deacetylation) was obtained from Seafresh Chitosan Lab. Co (Bangkok, Thailand). Hydroxypropyl methylcellulose, HPMC K15M (Methocel® K15M) was manufactured by Dow Chemical Company (Michigan, USA) and kindly supported by Rama Production Co., Ltd. (Bangkok, Thailand). Polycarbophil (Noveon® AA-1) was produced by Lubrizol Company (Ohio, USA) and supplied by Namsiang Co., Ltd. (Bangkok, Thailand). Propranolol HCl was purchased from PC drug Co. Ltd., (Bangkok, Thailand). All other chemicals used were of analytical grade and used as received.

2.2. Preparation of PNL-loaded nanoparticles

PNL-loaded nanoparticles (PN-nano) were prepared using an ionic cross-linking method. Chitosan (0.2 g) was dissolved in 0.2% v/v acetic solution. Tripolyphosphate (TPP) (0.1% w/v) and PNL (80 mg in 1 ml of water) were dissolved in distilled water. 1 mL of PNL was mixed with 19 ml of distilled water using magnetic stirrer at room temperature. 5 mL of TPP solution was then added and mixed for 5 min. Finally, 10 ml of chitosan solution was added and mixed with the previous solution for 5 min to form nanoparticles. The effects of various concentrations of PNL (70 mg [PN-nano-70], 80 mg [PN-nano-80], or 90 mg [PN-nano-90]) on formation of the nanoparticles were examined with regard to zeta potential and particle size.

2.3. Physicochemical characterization of PN-nano (zeta potential and particle size)

The zeta potential of the PN-nano was measured using Zeta Plus (Brookhaven Instruments Co., New York, NY, USA). Light

scattering technique was used to determine the particle size (Horiba, LA-950, Kyoto, Japan). All measurements were performed in triplicate.

2.4. Preparation of HPMC/PC blend film containing PNL-loaded nanoparticles (PN-films)

HPMC/PC blend films were prepared by the film casting technique as previously described [2]. Briefly, HPMC solution was prepared by dispersing HPMC powder (0.95 g) in 50 ml of distilled water and constantly stirred until a clear solution was obtained. PC powder (0.05 g) was dispersed in 15 ml of distilled water and stirred until providing a clear solution was obtained. The PC solution was slowly poured into the HPMC solution and mixed for 30 min until the mixture was homogeneous. Glycerol (0.5 g) as used in plasticizer was added to the composite solution and mixed for an additional 15 min. PN (PN-nano-70, PN-nano-80, or PN-nano-90) was added to the composite solution of HPMC/PC and mixed for 15 min until the mixture was homogeneous. PN-nano in the composite solution was poured onto a glass plate and allowed to evaporate at 50 °C for 10 h in a hot air oven. Dried films were peeled off and kept in a vacuum desiccator prior to use in experiments. PN-films-70, PN-films-80 and PN-films-90 containing 70, 80 and 90 mg PNL per films, respectively, were prepared as described above. HPMC/PC blend films without nanoparticles (containing 80 mg of PNL per film; PP-films-80) were prepared and used as a control to study various properties of PN-films.

2.5. Film thickness & weight measurement

The blend films were cut into 3.5 cm × 0.6 cm rectangles and each film formulation was accurately weighed in triplicate using a digital balance. The film thickness was measured at three points with a thickness gauge Mini Test 600 (ElektroPhysik Dr. Steingroever GmbH & Co.KG, Germany).

2.6. Wettability and surface free energy measurement

The contact angle was employed to examine the wettability of the blend films by using sessile drop technique (FTA 1000, First Ten Angstroms, USA). Wu harmonic was used to calculate the surface free energy of the blend films by using equations 1 & 2 that involve measurement of the contact angle of three distinct standard liquids i.e., distilled water, formamide, and ethylene glycol at 25 °C.

$$\gamma_S = \gamma_S^d + \gamma_S^p \tag{1}$$

$$(1+\cos\theta)\gamma_S = \left[4\left(\frac{\gamma_S^d\gamma_L^d}{\gamma_S^d} + \gamma_L^d\right) + 4\left(\frac{\gamma_S^p\gamma_L^p}{\gamma_S^p} + \gamma_L^p\right)\right] \tag{2}$$

where γ_S is total surface free energy, γ_S^p, γ_S^d are polar and dispersive forces of the blend films, respectively. γ_L^p, γ_L^d are polar and dispersive forces of standard liquids, respectively. θ is the contact angle between the blend film and the standard liquid.

2.7. Mechanical properties

The mechanical properties of the blend films were determined by using a texture analyzer (TA.XT.plus Texture Analyzer, Stable Micro Systems, UK). The blend films were cut into 3.5c m × 0.6 cm rectangles. The blend films were held between two grips stretched at a speed of 0.1 mm/s until the point of tensile failure and force-displacement curves were recorded through a 50 N loaded cell. Maximum force and maximum displacement of the films were measured, and then converted to tensile strength and elongation at breakage. The parameters were calculated using the following equations:

$$\text{Tensile strength} = F/A \tag{3}$$

where F is maximum force for film failure and A is the cross-sectional area of the film.

$$\text{Elongation}(\%) = \frac{\Delta L}{L} \times 100 \tag{4}$$

where ΔL is the increase in the length at breakage of the film and L is the initial film length.

2.8. Swelling index

The swelling properties of the blend films were examined by determining the swelling index [3]. Each blend film was cut into 2.0 cm × 2.0 cm squares and simulated saliva fluid (SSF) pH 6.80 was used as a medium in this examination. Previously, each blend film was placed on a pre-weighed stainless steel wire mesh and weighed. It was then dipped into SSF for predetermined periods of time. Filter paper was used to wipe off the excess surface water from the blend films and weighed. Swelling index was calculated using the following equation:

$$\text{Swelling index} = (W_t - W_o)/W_o \tag{5}$$

where W_t is the weight of film at time t, and W_o is the weight of film at time zero.

2.9. Mucoadhesive properties

In this study, porcine buccal mucosa was employed as a biological membrane due to similarities with human buccal tissue [4]. The mucoadhesive property studies were approved by the Laboratory Animal Center Ethical Committee of Thammasat University (No.018/2559, Thailand). Porcine buccal mucosa was obtained from freshly euthanized pigs after slaughter at a local slaughterhouse (Nakhon Pathom, Thailand). Buccal mucosae were washed with distilled water, placed in normal saline solution at 4 °C to maintain freshness, and then immediately used for experiments. The underlying connective tissues were subsequently removed to isolate the mucosal membranes [20].

A texture analyzer (TA.XT.plus, Stable Micro Systems Ltd., UK) with 50 N load cell equipped with mucoadhesive holder was used to determine the mucoadhesive properties of the blend films. Double-sided adhesive tape was used to adhere blend films with a diameter cylindrical probe (10 mm). Tissues were cut into about 2.0 cm × 2.0 cm squares and equilibrated for 15 min at 37.0 ± 0.5 °C before placing onto the holder stage of the mucoadhesive holder with the mucosal surface facing

up. Prior to testing, 100 µl of SSF pH 6.80 was added to the mucosa. The probe speed of the attached film was 1.0 mm/s and a contact force of 0.05 N for 60 s was used to make contact with the tissue. The probe withdrawal speed from the tissue was 0.5 mm/s. The association between force and film displacement was plotted. Maximum detachment force (F_{max}) and work of adhesion (W_{ad}, the area under the force versus distance curve) were computed.

2.10. *Morphology examination*

Morphological examination of selected blend films was performed by scanning electron microscope (SEM) (model JSM-5410LV, Jeol, Japan) with an accelerating voltage of 2 keV. The samples were mounted on a metal stub with double-sided adhesive tape and coated with a fine gold layer under vacuum before obtaining the micrographs.

2.11. In vitro *release of blend films*

Release of PNL from the film formulations was determined using a modified Franz-type diffusion cell. Each blend film was cut into a circle with a diameter of 1.5 cm and the effective area for diffusion was 1.77 cm^2. The film was placed on a fiber mesh and then placed on the receptor cell. The receptor chamber was filled with 15 ml of SSF pH 6.80 used as a release medium. The diffusion cell was incubated at 37 °C with a water jacket and stirred with a magnetic stirrer in the receptor chamber. One milliliter of the release medium was removed at predetermined periods of time, and replaced by equal volumes of fresh SSF. The concentration of PNL was analyzed spectrophotometrically at 289 nm (Agilent, model 1100 series, USA). All results were carried out in triplicate and values were expressed as the mean ± SD of the film formulations for each time point of release.

2.12. In vitro *permeation studies*

In vitro permeation studies of the film formulations were determined using a Franz-type diffusion cell method. This method was applied from R. Trastullo et al. as previously described [1]. Briefly, each blend film was cut into a circle with a diameter of 1.5 cm and the effective area for diffusion was 1.77 cm^2. The receptor chamber was filled with SSF pH 6.80, incubated at 37 °C with a water jacket, and stirred with a magnetic stirrer in the receptor chamber. Porcine buccal mucosa was obtained as previously described and mounted between the donor and the receptor chamber. Film formulations were placed on top of the porcine buccal mucosa. One milliliter of the sample was removed at predetermined periods of time, and replaced by equal volumes of fresh SSF. The concentration of PNL was analyzed spectrophotometrically at 289 nm. The results of permeation are displayed as cumulative amount per area (mg/cm^2) versus time (minute) and as steady state flux (mg/cm^2/h).

2.13. *Statistical analysis*

Data are expressed as the mean ± SD of triplicate measurements ($n = 3$). Analysis of variance (ANOVA) was used to analyze differences between groups. Results were considered statistically significant if $P < 0.05$.

3. Results and discussion

3.1. *Physicochemical properties of PN-nano (zeta potential and particle size)*

Before studying the inherent characteristics of PN-films and PP-films, two important factors of PN-nano should be considered. The particle size and zeta potential are important factors that are typically used to investigate the properties of nanoparticles. The effect of increasing concentrations of PNL on particle size and zeta potential is shown in Table 1. The particle size of the PN-nano ranged from 147 to 318 nm and the PN-nano-80 concentration displayed the smallest particle size. Therefore, the optimum concentration of PNL, TPP, and chitosan used in preparation of the nanoparticles provided to balance the interaction between anionic and cationic molecules resulted in smaller sized particles compared with other previously published formulations [14,15]. The zeta potential ranged from 31.71 to 36.13 mV and the PN-nano-80 concentration exhibited the lowest zeta potential. The PN-nano-80 concentration resulted in the smallest particle size and the lowest zeta potential. The zeta potential of this nanoparticle preparation was found to be above 30 mV, attesting to the stability of nanoparticles [14,15]. Due to these results, we chose to use 80 mg of PNL in the subsequent experiments to compare blend films (PP-films-80) with PN-films (PN-films-80) to determine the inherent properties of the blend films.

3.2. *Weight & film thickness*

The weight of films ranged from 0.019 to 0.025 g and the thickness of films varied from 0.062 to 0.079 mm as shown in Table 2. Blend films require a proper thickness for ease of film handling and should not dissolve too quickly in the oral cavity [2,9].

3.3. *Wettability and surface free energy measurement*

The wettability of film surface was characterized by using contact angle measurements. The contact angle of films varied from 65.14 to 67.09, but no significant difference was found in the contact angle of all film formulations. The surface free energy of films ranged from 38.84 to 39.92 mJ/m^2 calculated using Wu harmonic mean equation as shown in Table 2. Two components of this energy are divided into polar force (24.05–26.14 mJ/m^2) and dispersive forces (13.15–15.87 mJ/m^2),

Table 1 – Effect of different concentrations of propranolol on zeta potential and particle size of the nanoparticles (mean ± SD, n = 3).

	Zeta potential (mV)	Particle size (nm)
PN-nano-70	35.85 ± 0.49	314.3 ± 2.2
PN-nano-80	31.71 ± 0.46	147.0 ± 3.3
PN-nano-90	36.13 ± 0.99	317.9 ± 4.4

Table 2 – Weight, thickness, contact angle, polar force, dispersive force and surface free energy of PP-films and PN-films (mean ± SD, n = 3).

Film formulation	Weight (g)	Thickness (mm)	Contact angle (θ)	Polar force (mJ/m^2)	Dispersive force (mJ/m^2)	Surface free energy (mJ/m^2)
PP-films-80	0.020 ± 0.001	0.062 ± 0.005	65.62 ± 0.80	26.14 ± 0.21	13.74 ± 0.37	39.88 ± 0.58
PN-films-70	0.025 ± 0.001	0.077 ± 0.006	67.09 ± 1.34	25.69 ± 0.62	13.15 ± 0.48	38.84 ± 0.93
PN-films-80	0.025 ± 0.002	0.079 ± 0.004	65.86 ± 3.63	25.58 ± 3.26	14.34 ± 1.17	39.92 ± 2.09
PN-films-90	0.019 ± 0.000	0.066 ± 0.005	65.14 ± 1.86	24.05 ± 2.69	15.87 ± 2.93	39.92 ± 0.78

Table 2, which represent hydrophilic and hydrophobic properties of the film surface, respectively [8]. The polar force of all film formulations was higher than the dispersive force, indicating that the blend films were hydrophilic. However, no change in polar force, dispersive force, or surface free energy was observed in each film. There was no change in contact angle, suggesting that addition of nanoparticles did not affect wettability of the blend films. These results suggest that addition of nanoparticles and increasing amounts of PNL in blend films did not affect the conformation and molecular orientation of the film surface. The sizes of the particles were very small (nanometer range) and they were widely dispersed on the film surface resulting in no statistically significant difference in wettability of all film formulations. Confirmation of the film surface will be examined in further experiments using SEM.

3.4. *Mechanical properties*

Tensile strength and elongation comprise important mechanical properties of blend films that are required during production, handling, and use. Tensile strength and percent elongation of the films varied from 2.27 to 4.59 Mpa and 31.85 to 54.64%, respectively (Fig. 1). PP-films-80 demonstrated the highest tensile strength and also the lowest percent elongation compared with PN-films. Tensile strength decreased in the PN-films-80 compared to the PN-films-70, but then stabilized in the PN-films-80 and PN-films-90. PNL concentration had no effect on percent elongation. The increase in percent elongation and the decrease in tensile strength of PN-films compared with PP-films could be due to the incorporation of nanoparticles into blend films. The incorporation of nanoparticles in blend films may disturb the formation of intermolecular hydrogen bonds between the –OH group of HPMC and the –COOH group of PC, which may promote motion and elasticity of the blend films [2,21]. Furthermore, addition of nanoparticles in blend films could be attributed to the non-homogeneity of the blend films [6], which is confirmed by SEM in the next experiment.

3.5. *Swelling index*

Swelling properties play a significant role in the bioadhesive properties of films, because polymer swelling causes disentanglement and relaxation of the polymer chains and promotes penetration of the mucus membrane during bioadhesion [9]. The swelling index (SI) of the blend films at 10 to 60 min varied from 0.349 to 0.543, as shown in Fig. 2. The SI of PN-films increased vastly during the first 10 min and reached a swollen equilibrium by 20 min. The decrease in SI at 30 and 60 min of the PN-films indicated erosion of the films. Meanwhile, the SI of PP-films-80 increased in the first 10–30 min and reached a swollen equilibrium by 30 min. These results indicate that the hydration rate and water uptake of the PN-films was faster than the PP-films. However, the SI of PP-films-80 at 60 min remained constant and the SI was higher than PN-films-70 and PN-films-90 at the same time point. This result could be described by two main factors, film surface area and water diffusivity into the polymer, which may affect the initial

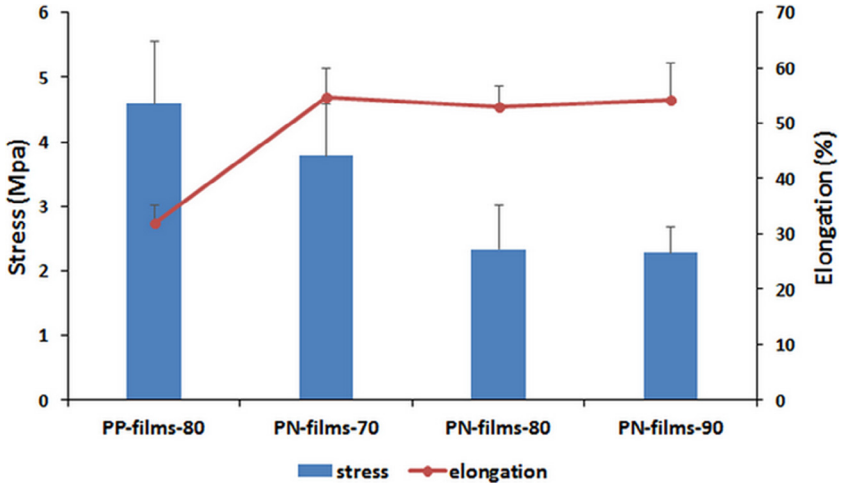

Fig. 1 – Mechanical properties of PP-films and PN-films.

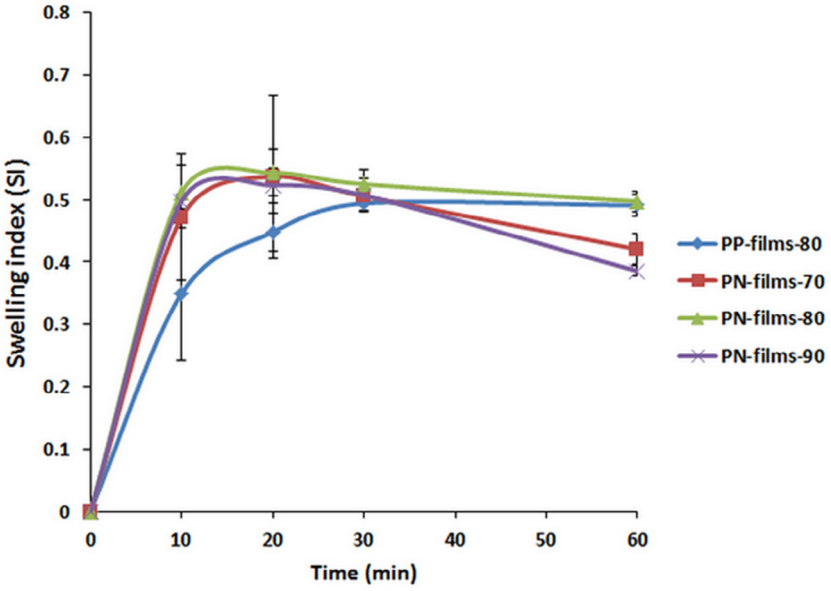

Fig. 2 – Swelling index of PP-films and PN-films.

swelling of films. During the first 10–20 min, the SIs of PN-films were significantly ($P < 0.05$) higher than PP-films, perhaps due to the addition of nanoparticles that disrupt the formation of intermolecular hydrogen bonds between polymers, leading to increased macromolecular mobility of the blend films [2,9]. The presence of nanoparticles facilitates water diffusion into the blend films causing increased water content. This result correlates with our results that showed that addition of nanoparticles in blend films enhanced motion and elasticity of the blend films. However, after 30 min, the SI was reduced slightly in PN-films and remained constant in PP-films. This result could be explained by the formation of intermolecular hydrogen bonds between the –OH group of HPMC and the –COOH group of PC, which restricts the motion of the film matrix and promotes inflexibility of the blend films [21]. Therefore, the SI of PP-films-80 at 10 and 20 min were decreased compared with PN-films and the SI of PP-films-80 at 60 min was higher than the SI of PN-films, due to the reasons described above.

3.6. *Mucoadhesive properties*

Work of adhesion (W_{ad}) and maximum force (F_{max}) are two important factors used to determine mucoadhesive properties. The W_{ad} and F_{max} of the blend films ranged between 0.0163 to 0.0315 N.mm and 0.0097 to 0.0183 N, respectively (Fig. 3). PN-films-80 had the highest W_{ad} value. This result correlates with the SI data showing that PN-films-80 had the highest SI at all time points. It could be implied that the increased swelling

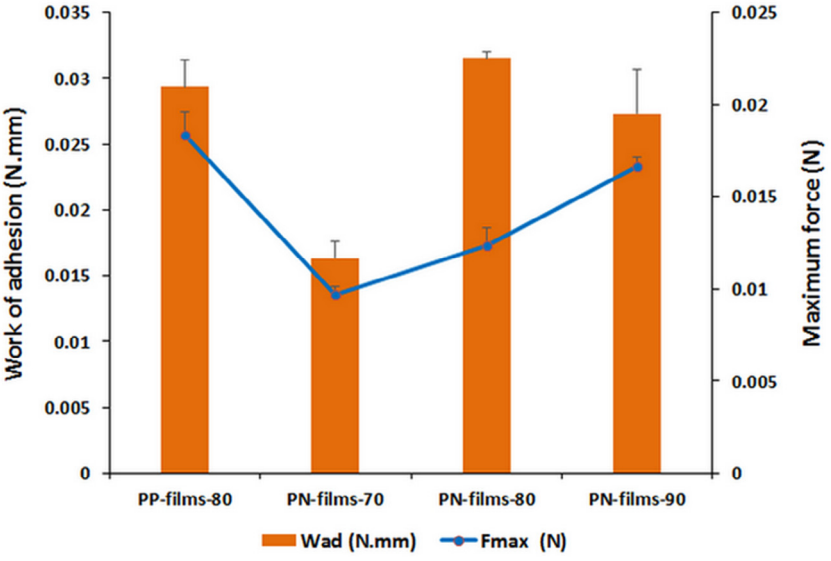

Fig. 3 – Mucoadhesive properties of PP-films and PN-films.

capacity of PN-films-80 contributed to enhanced mucoadhesion resulting in the highest W_{ad} [13]. Mucoadhesion theories involve diffusion and water penetration. Fast rates of hydration and fast rates of swelling equilibrium of films are important factors of mucoadhesive properties [3,22]. Previous reports demonstrate that the presence of water soluble nanoparticles homogeneously distributed into the films leads to increases in surface area. This homogeneous distribution contributes to increased mucoadhesion due to the higher extent of solubility and faster rate of water penetration into the film matrix [22,23]. Moreover, the incorporation of nanoparticles in the films disrupts the continuum of the film matrix allowing for more free space, resulting in increased water penetration [22,23]. However, PP-films-80 exhibited the highest F_{max} value. This result could be explained by the drug that when incorporated in the films, acts as particulate material. Previous studies demonstrate that the inclusion of a water-soluble drug in the matrix increases the amount of water, which then affects polymer swelling [22,24]. Additionally, the absence of the nanoparticles in PP-films-80 contributes to its intrinsic mucoadhesion properties, due to the ability of the film polymers to absorb water and plasticize the chain of polymers to bond with mucin [22]. In the PN-films, the concentration of PNL influenced the W_{ad} and F_{max} value such that F_{max} was higher when the amount of PNL increased, but a trend of W_{ad} could not be concluded. Therefore, increasing concentrations of PNL had a significant influence on the mucoadhesive properties of the blend films.

3.7. *Morphology examination*

SEM images were used to examine the morphology of the film surface and to confirm the morphological characteristics of nanoparticles including their distribution after incorporation into the blend film, as shown in Fig. 4. The PP-films-80 showed a partially smooth surface with a wrinkled occurrence (Fig. 4A). This may be due to differences between chemical structures of the polymers (HPMC and PC) [2]. Spherically shaped, well-dispersed nanoparticles were observed on the surface of the films (Fig. 4B). The particle size of the nanoparticles was around 100 nm, similar to the size obtained using light scattering technique as described in the previous section. Therefore, this result confirms that the PN-films contain nanoparticles and they play an important role on the physical properties of the blend films.

3.8. *In vitro release of PN-films and PP-films*

The *in vitro* release of PNL from the blend films at 0 to 300 min varied from 53.8 to 108.0 %, as shown in Fig. 5. The mechanism of PN-films release involves, first, the immediate release of nanoparticles from the surface of the film. Next, drug release from the film involves water diffusion, relaxation of polymer chains, swelling, and erosion of the film [1,13]. Immediate release of all film formulations was observed at early time points (10–30 min) and did not differ significantly. These results correlate with the SI results of the film formulations, which displayed the maximum SI at 20–30 min. It could be implied that release of the blend films involved swelling and erosion of the films. Increased concentrations of PNL impacted drug release after 120 min. The release of PN-films-70 was lower than the other PN-films, indicating that decreased amounts of PNL in the film matrix tended to have a lower diffusion rate leading to a lower driving gradient [15]. However, the release of PN-films-80 and PP-films-80 at all time periods was not significantly different. These results implied that inclusion of nanoparticles in the blend films might not delay the release of PNL when compared with the PP-films at the same concentrations of PNL. Additionally, all release of PNL from the nanoparticles probably occurred at 10 min due to a burst in release of PNL from the nanoparticles [15,16]. Therefore, release of PN-films-80 and PP-films-80 at this time point was not different.

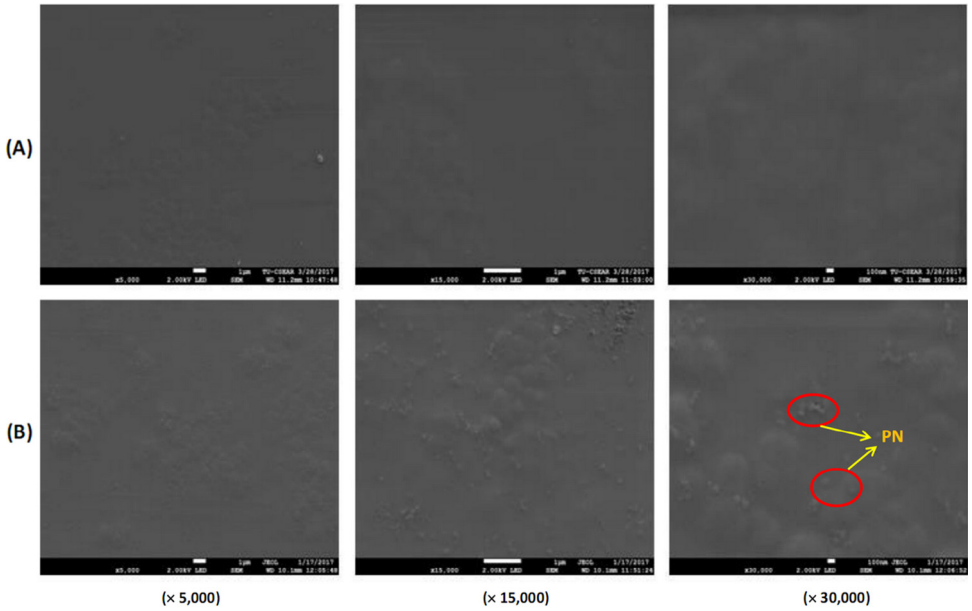

Fig. 4 – SEM images of PP-films-80 (A) and PN-films-80 (B).

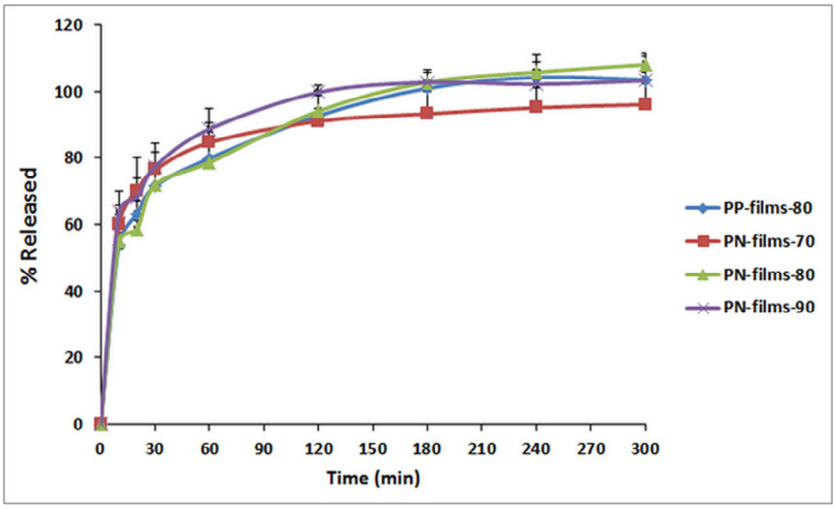

Fig. 5 – *In vitro* release of PNL from PP-films and PN-films in SSF pH 6.8.

3.9. *In vitro permeation studies*

The steady-state flux and the cumulative amount per area were used to determine the permeation of PNL from the blend films through porcine buccal mucosa as shown in Fig. 6A and Fig. 6B, respectively. The cumulative amount per area of the PNL in all film formulations ranged from 0 to 0.3815 mg/cm^2. The cumulative amount per area of PNL increased in all formulations and showed no significant difference. However, at later times of over 120 min, the cumulative amount per area of PNL in PN-films-80 was significantly higher than PP-films-80, PN-films-70, and PN-films-90 ($P < 0.05$). The steady-state flux of PNL in all formulations varied from 0.0408 to 0.0538 mg/cm^2/h. The steady-state flux of PN-films-80 was significantly higher than the other formulations, consistent with results from the cumulative amount per area at 7 h ($P < 0.05$). These results correlated with results demonstrating that the W_{ad} and SI of PN-films-80 were increased compared to the other formulations. Increased mucoadhesion of PN-films-80 may have led to prolonged residence time in contact with buccal mucosa, resulting in accumulation of PNL on the surface of mucosa cell [23]. Therefore, a high concentration of PNL localized on the surface could have created a higher driving gradient to deliver PNL directly into the buccal mucosa such that permeation of PNL in PN-films-80 was significantly increased higher compared with other formulations. In addition, the inclusion of nanoparticles in the films had an influence on the permeation of PN-films-80 such that it was increased compared with PP-films-80. A factor that may affect permeation enhancement of PN-films-80 is the incorporation of chitosan in the nanoparticle composition. Chitosan is a good mucoadhesive material and membrane permeable enhancing properties of chitosan increases the residence time of the dosage form at the site of mucosal membrane [15,23]. However, mucoadhesive property of chitosan may not affect permeation of PN-films in this study. The concentration of chitosan used to form the nanoparticles was too low and the result of permeations of PN-films-70 and PN-films-90 was not considerably different compared with PP-films-80. As a result, it could be concluded that the permeation enhancement of PN-films-80 is due to higher mucoadhesion and swelling properties of PN-films-80.

4. Conclusion

The two potential approaches between mucoadhesive blend films and nanoparticle carriers in one delivery system for improving permeability of PNL through buccal mucosa were investigated. Blend films loaded and unloaded with nanoparticles containing PNL were examined. SEM images demonstrated spherically shaped, well-dispersed nanoparticles on the surface of PN-films-80. The particle size obtained using SEM was similar to the size obtained using light scattering technique, which was equal to ~100 nm. The swelling index (SI) of the PN-films and PP-films increased vastly during the first time point and reached a swollen equilibrium at different times. PN-films with increased amounts of PNL tended to have higher mucoadhesive properties. Immediate release of all film formulations were observed at early time points (10–30 min) and after 120 min, the release of PN-films-70 was decreased compared with the other PN-films. In permeation studies, high permeability of PNL through the buccal mucosa was observed in the PN-films compared with the PP-films with equal concentrations of PNL. In summary, mucoadhesive blend films containing PNL loaded nanoparticles is a promising approach for buccal delivery of drugs that undergo first-pass metabolism.

Conflict of interest

The authors declare that there is no conflicts of interest.

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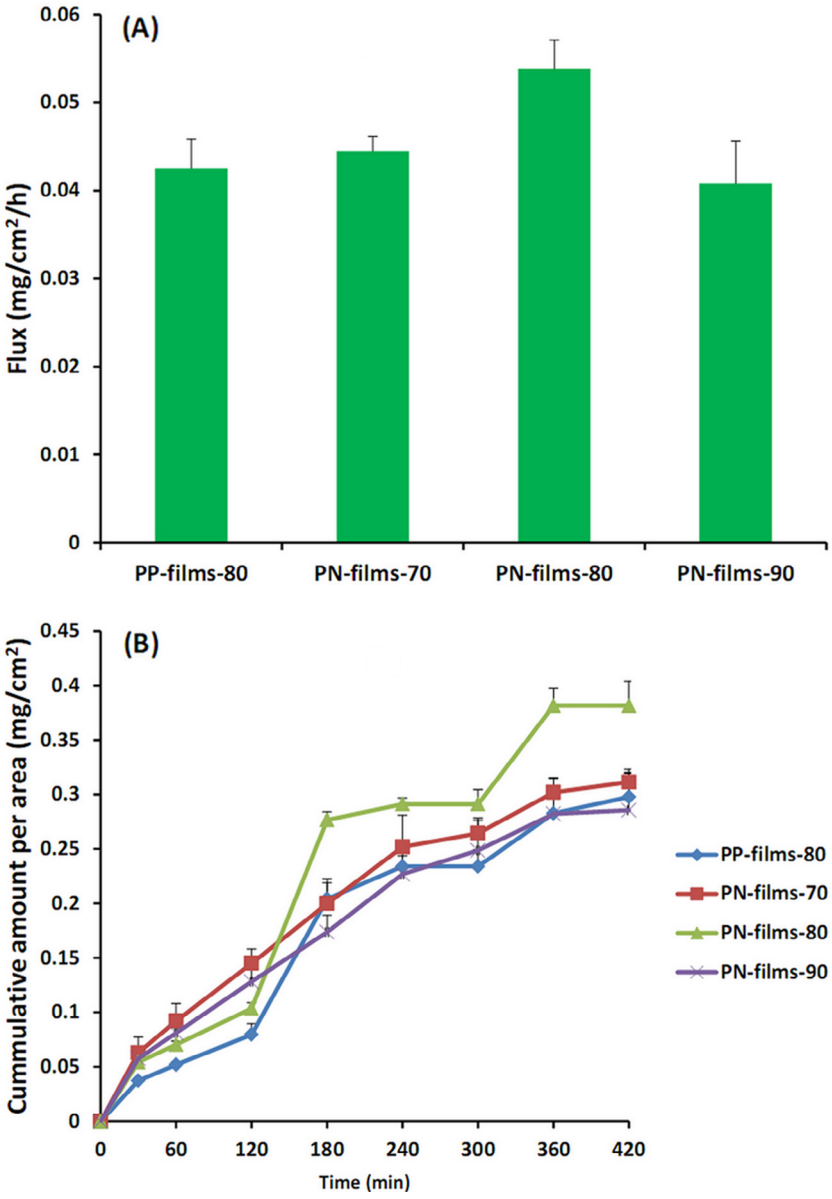

Fig. 6 – *In vitro* permeation profiles of PNL from PP-films and PN-films.

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