Spray-dried Mucoadhesive Microspheres: Preparation and Transport Through Nasal Cell Monolayer

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ABSTRACT

The purpose of this research was to prepare spray-dried mucoadhesive microspheres for nasal delivery. Microspheres composed of hydroxypropyl methylcellulose (H), chitosan (CS), carbopol 934P (CP) and various combinations of these mucoadhesive polymers, and maltodextrin (M), colloidal silicon dioxide (A), and propylene glycol (P) as filler and shaper, were prepared by spray-drying technique. Using propranolol HCl as a model drug, microspheres were prepared at loadings exceeding 80% and yields between 24% and 74%. Bulky, free flowing microspheres that had median particle size between 15 and 23 μm were obtained. Their zeta potential was according to the charge of polymer. Adhesion time of mucoadhesive microspheres on isolated pig intestine was ranked, CS > CP: H > CP > H, while the rank order of swelling was CP > CS > H. Increasing the amount of CP in CP:H formulations increased the percentage of swelling. Infrared (IR) spectra showed no interaction between excipients used except CS with acetic acid. The release of drug from CP and CP:H microspheres was slower than the release from H and CS microspheres, correlated to their viscosity and swelling. Long lag time from the CP microspheres could be shortened when combined with H. The permeation of drug through nasal cell monolayer corresponded to their release profiles. These microspheres affected the integrity of tight junctions, relative to their swelling and charge of polymer. Cell viability was not affected except from CS microspheres, but recovery could be obtained. In conclusion, spray-dried microspheres of H, CS, CP, and CP:H could be prepared to deliver drug through nasal cell monolayer via the opening of tight junction without cell damaging.

KEYWORDS: mucoadhesive polymers, spray-dried microspheres, nasal cell monolayer, permeation, cell viability.

INTRODUCTION

Nasal drug delivery is increasingly important as an alternative to the oral and parenteral route for systemic drug delivery. Several products for nasal delivery are now commercially available. Most of them are solution or liquid preparations of biotechnological products. Since liquid preparations would encounter rapid muciliary clearance, mucoadhesive microspheres offer the advantage of increasing the residence time.[1] The most commonly investigated technique to prepare mucoadhesive microspheres has been emulsion/solvent evaporation technique.[2,3] To avoid the use of organic solvent, alternative techniques have been reported such as ionic gelation, coacervation precipitation, and spray-drying.[4-6] Spray-drying technique, a one-step process, offers the advantages of good production yield and reproducibility. Although there were numerous reports on spray-dried mucoadhesive microspheres, they were mostly less than 5 μm in diameter.[7,8] Such small particles would be inhaled through the pharynx.[9] Those with appropriate size of 10 to 40 μm were spray-dried at very high temperature, which might degrade sensitive drugs.[6,10,11] Addition of formulation aid could improve microspheres to have the appropriate size, morphology, and flowability for aerosolization for appropriate nasal deposition. Mannitol and propylene glycol have been investigated as particle filler and shaper.[12] Maltodextrin, common particle aid in spray-dried food products[13] is rarely used in spray-dried pharmaceutical microspheres. Therefore, the use of this polysaccharide in spray-dried mucoadhesive microspheres for nasal delivery has been attempted.

The in vitro mucoadhesive property of various mucoadhesive polymers such as celluloses, chitosan, gelatin, carbomer, dextran, and polycarbophils has been extensively investigated. The mucosal residence time was mostly studied on excised nasal mucosa of animal.[7,14] In addition, some of these polymers could increase the transport by opening the tight junction of Caco-2 cells.[15,16] Primary nasal cell culture was also studied as a model for drug transport and metabolism.[17,18] However, the effect and drug transport from mucoadhesive microspheres through nasal cell line have not been reported.

Therefore, the aim of this study was to produce mucoadhesive microspheres of various hydrophilic polymers, hydroxypropyl methylcellulose, chitosan, and carbopol with the aid of formulation excipients, maltodextrin, colloidal silicon dioxide, and propylene glycol as particle filler and shaper to obtain suitable properties for nasal drug delivery by using spray-drying technique. Propranolol hydrochloride was

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Table 1. The Composition of Microsphere Formulations*

Formulation Code	Drug (mg)	Polymer (mg)	Maltodextrin† (%)	Aerosil‡ (%)	Propylene§ Glycol (%)
D+H	500	500	-	-	-
D+CS	500	500	-	-	-
D+CP	500	500	-	-	-
D+H^+M	500	500	80	-	-
D+CS+M	500	500	80	-	-
D+CP+M	500	500	80	-	-
D+H^+M+A	500	500	80	10	-
D+CS+M+A	500	500	80	10	-
D+CP+M+A	500	500	80	10	-
D+H^+M+A+P	500	500	80	10	30
D+CS+M+A+P	500	500	80	10	30
D+CP+M+A+P	500	500	80	10	30
Control	1100	-	80	10	-
D:H(1:7)	125	875	80	10	30
D:H(1:5)	166.67	833.33	80	10	30
D:CS(1:7)	125	875	80	10	30
D:CS(1:5)	166.67	833.33	80	10	30
D:CP(1:7)	125	875	80	10	30
D:CP(1:5)	166.67	833.33	80	10	30
D:CP:H(1:3:4)	125	375:500	80	10	30
D:CP:H(1:4:3)	125	500:375	80	10	30
D:CP:H(1:5:2)	125	625:250	80	10	30

*D indicates propranolol hydrochloride; H, hydroxypropyl methylcellulose; CS, chitosan; and CP, carbopol 934P. The formulations were diluted to 100 g.
†Percentage by weight of the total amount of drug and polymer.
‡Percentage by weight of the formulation.
§Percentage by weight of the amount of polymer.
||Ethanol:water or ethanol:1% acetic acid (30:70).

chosen as a model drug owing to its fairly good absorption through the nasal mucosa.[19] The prepared mucoadhesive microspheres were characterized, and drug permeation from microspheres through human nasal cell line (RPMI 2650) was studied. The effects of mucoadhesive microspheres on the integrity or the opening of tight junction, and the viability and the recovery of nasal cell monolayer were also evaluated.

Materials and Methods

Preparation of Microspheres

The formulations of microspheres as presented in Table 1 consisted of 1:1 drug to polymer ratio for preliminary study on proper formulation excipient and various ratios of drug to polymer to study the effect of type and amount of polymer. Hydroxypropyl methylcellulose (Methocel E4M, H, viscosity 3500-5600 cps, Dow Chemical, Auburn Hills, MI) and carbopol 934P (CP, molecular weight [MW] 3×10^6 Da, Noveon, Cleveland, OH) were separately dissolved in deionized water, while chitosan (CS, MW 2.3-2.5 $\times$ 10^5 Da, 85% deacetylation, Seafresh, Bangkok, Thailand) was dissolved in 1% acetic acid. Propranolol hydrochloride (D; China National Chemical, Jiangsu, China) in absolute ethanol, maltodextrin (M, DE 10, Nutrition Ltd Part, Bangkok, Thailand), colloidal silicon dioxide (Aerosil, A, Degussa, Dusseldorf, Germany) and propylene glycol (P, Arco, Singapore) were then mixed with the polymer solution before adjusting to final volume to obtain the pH of 5.79, 5.46, and 3.86 for H, CS, and CP, respectively. The solution of 2000 g for each batch was spray-dried (type 190, Buchi, Zurich, Switzerland) with the process parameters as follows: inlet temperature of 120°C except H using 130°C; pump setting of 5 mL/min; spray flow rate of 400 Nl/h. Each formulation was produced in triplicate and pooled prior to further studies.

Characterization of Microspheres

Morphological examination

The morphology of microspheres was examined by scanning electron microscopy (SEM, JSM-T220A, JEOL, Tokyo, Japan). Samples of microspheres were dusted onto double-sided

tape on an aluminum stub and coated with gold using a cold sputter coater to a thickness of 400°A, and then imaged using a 25 kv electron beam.

Production yield, drug content, and loading efficiency

The percentage of production yield (wt/wt) was calculated from the weight of dried microspheres (W_1) recovered from each of 3 batches and the sum of the initial dry weight of starting materials (W_2) as the following equation:

$$Percentage\ of\ Production\ Yield = \frac{W_1}{W_2} \times 100 \quad (1)$$

Propranolol HCl in microspheres of each formulation was extracted in 0.05M phosphate buffer (pH 6.8) and assayed by high-performance liquid chromatography (HPLC) method in *United States Pharmacopeia (USP)*-XXVII with a 220-nm detector (SCL-10A, Shimadzu, Kyoto, Japan). The actual amount of drug loaded relative to the theoretical amount in the microspheres was calculated as a percentage and expressed as the loading efficiency. The results were averaged from 3 determinations.

Particle size measurement

The prepared microspheres suspended in hexane were sized by using a laser particle size distribution analyzer (Mastersizer-S, Malvern Instruments Ltd, Malvern, UK). The size distribution was determined by the span value. Triplicate measurement was conducted.

Determination of bulk density and angle of repose

Accurate weight of microspheres (W_m) was transferred into a 100-mL graduated cylinder to obtain the apparent volumes (V) of between 50 and 100 mL. The bulk density was calculated in gram per milliliter by the following formula:

$$Bulk\ Density = \frac{W_m}{V} \quad (2)$$

The angle of repose was measured from a heap carefully built up by dropping the microsphere samples through a glass funnel to the horizontal plate of a powder characteristic tester (PP-N, Hosokawa powder tester, Kawaramachi Chuo-ku, Osaka, Japan). The results were averaged from 3 determinations.

Zeta potential study

The zeta potential of microspheres dispersed in 0.0005M pH 6.8 phosphate buffer was determined by a zeta meter (ZM3UG, Zeta meter, Zeta Meter Co, Staunton, VA). The directional movement of 200 microspheres from each formulation was observed and averaged from 3 determinations.

Adhesion property

The mucoadhesive property was determined by an adapted method described by Vyas et al.[20] A freshly cut piece, 5-cm long, of pig intestine obtained from a local abattoir within 1 hour of killing the animal was cleaned by washing with isotonic saline solution. An accurate weight of microspheres was placed on mucosal surface, which was attached over a polyethylene plate that fixed in an angle of 40° relative to the horizontal plane, and pH 6.8 phosphate buffer warmed at 37°C was peristaltically pumped at a rate of 5 mL/min over the tissue. The duration for complete washing of microspheres from pig intestine was recorded and averaged from 5 determinations.

Swelling property

The swelling of microspheres was conducted in phosphate buffer pH 6.8. Their diameters were periodically measured by using a laser particle size distribution analyzer until they were decreased by erosion and dissolution. The percentage of swelling was determined at different time intervals by the difference between diameter of microspheres at time t (D_t) and initial time (t = 0 [D_0]) as calculated from the following equation and averaged from 3 determinations:

$$Percentage\ of\ Swelling = \frac{D_t - D_0}{D_0} \times 100 \quad (3)$$

Infrared absorption study

The IR spectra of propranolol HCl and additives in the spray-dried microspheres were examined using the potassium bromide disc method by an infrared spectrophotometer (1760X, PerkinElmer, Wellesley, MA) in the range of 4000 to 400 cm^{-1}.

In Vitro Drug Release Study

The in vitro drug release test of the microspheres was performed on Franz diffusion cell with dialysis membrane (cut-off MW 12 000). The receptor compartment contained phosphate buffer solution pH 6.8 that was within the pH range in nasal cavity and maintained at 37°C ± 1°C. The membrane was equilibrated before carefully dispersing the sample equivalent to 1500 μg of drug onto the donor side. Samples were periodically withdrawn from the receptor compartment, replaced with the same amount of fresh buffer solution, and assayed by a spectrophotometer (V-530, Jasco, Tokyo, Japan) at 218 nm.[20] After drug release study, the viscosity of the swollen microspheres (~0.5 mL) obtained from Franz diffusion cell was then tested on a cone/plate digital viscometer (MA-D2072, Brookfield Engineering, Middleboro, MA) and averaged from 3 determinations.

Drug Permeation Study

Cell cultures

RPMI 2650 cells (passages 26-30) origination from a human nasal septum carcinoma (American Type Culture Collection, Rockville, MD) were cultivated on polyester filters; pore size, 0.4 μm; diameter, 24 mm; growth area, 4.7 cm^2 (Costar, Cambridge, MA). The amount 3.5 to 4.0 × 10^5cells/cm^2 were seeded onto filters and allowed to grow and differentiate for 5 to 6 days at an incubated atmosphere of 37°C, 5% CO_2, and 90% humidity. The cells were maintained in minimum essential medium (MEM) supplemented with 10% fetal bovine serum (FBS), 1% nonessential amino acids, 1% sodium pyruvate, 1% antibiotic-antimycotic, and 1.5 g/L of sodium bicarbonate (all cell culture products were from GIBCO, Invitrogen Corp, Carlsbad, CA). Attachment and proliferation of the cells were observed through an inverse phase contrast microscope (CK2, Olympus, Melville, NY). The transepithelial electrical resistance (TEER) of confluent cell monolayers was measured by using an electrode (Millicell-ERS, Millipore, Billerica, MA) in Hanks' balance salt solution (HBSS) at room temperature. The observed TEER value was corrected for the blank filter resistance.

Permeation experiment

Permeation of FITC-labeled dextran (FD-4) model substance (MW 4000) through nasal epithelial monolayer in comparison to blank filter was to quantify the barrier function of the cultivated human cell monolayer. The FD-4 in HBSS (pH 6.8) at the concentration 200 μg/mL was added to the donor compartment of an apparatus for culturing cell monolayers (Transwell, Costar, Cambridge, MA). Total volume of samples were withdrawn from the receptor compartment and replaced by fresh HBSS at different time intervals. The amount of permeated FD-4 was determined by a fluorescence spectrophotometer (FP-777, Jasco) at emission/excitation wavelengths of 490/515 nm.[17]

For drug permeation study, the confluent cell monolayers were rinsed with HBSS, allowed to equilibrate for 1 hour, and layered over with microspheres equivalent to 250 μg of drug. Both donor and receptor compartments of Transwell were filled with HBSS at pH 6.8 and 7.4, respectively. The permeation experiments were conducted at the incubated atmosphere. Total volume of sample was periodically withdrawn from the receptor compartment and replaced with equal volume of fresh HBSS. The amount of permeated drug was determined by HPLC method as previously described in drug content study. The results were averaged from 3 determinations.

Integrity of tight junction between cells

TEER measurement was performed during the drug permeation at 30-minute intervals until 5 hours. For negative control, no microspheres were applied to the monolayers. After permeation study, the microspheres were carefully removed. The monolayers were then rinsed with HBSS, supplied with the culture medium (MEM), and allowed to regenerate for 2 days at the incubated atmosphere. TEER was periodically measured during the recovery period. In addition, the monolayers of cell culture were also stained by FITC-labeled phalloidin (Sigma, St Louis, MO) and examined under a fluorescence microscope (E200, Nikon, Japan).[21] Triplicate determinations were conducted.

Viability of cell monolayer

Plasma membrane integrity was analyzed by performing trypan blue-dye exclusion. Both the apical and basolateral sides of the cell monolayer were rinsed twice with phosphate buffered solution after completion of permeation experiments. The cell monolayer was trypsinized and diluted in 0.4% trypan blue-dye solution (Sigma). Viable and nonviable cells presented as absence and presence of intracellular trypan blue were counted separately by a hemocytometer slide, calculated as percentage of viable cells, and averaged from 3 determinations.

Results and Discussion

Most spray-dried mucoadhesive microspheres were white fine powder except those containing CS, which were yellowish, while some microspheres of H adhered into masses. Microspheres with combined CS with CP could not be obtained. This was due to an ionic interaction between $-NH_3^+$ group of CS and the $-COO^-$ groups of CP.

Characterization of Microspheres

Microspheres in Preliminary Study

The scanning electron photomicrographs in preliminary study revealed that microspheres of H coalesced with each other and had crumpled surface (Figure 1, D+H) resulting from the hollow microspheres collapsing during the preparation process. Similar morphology was also from microspheres of CS and CP (D+CS, D+CP; not shown). These microspheres showed very low production yield of less than 10%. This was owing to the ultrafine microparticles, inseparable from the air by the cyclone separator and the sticky problem of polymer with high MW. Addition of M increased the density of the microspheres, thus increasing the production yields (cyclone part, 10%-40%; collector part, 3%-12%). However, owing to its low glass transition temperature, M at the surface of microparticles was possibly changed from glassy to rubbery state,[22] thus they still adhered extensively on the apparatus glass wall of cyclone. The production yields obtained from collector part were markedly increased to more than 45% when A was added.

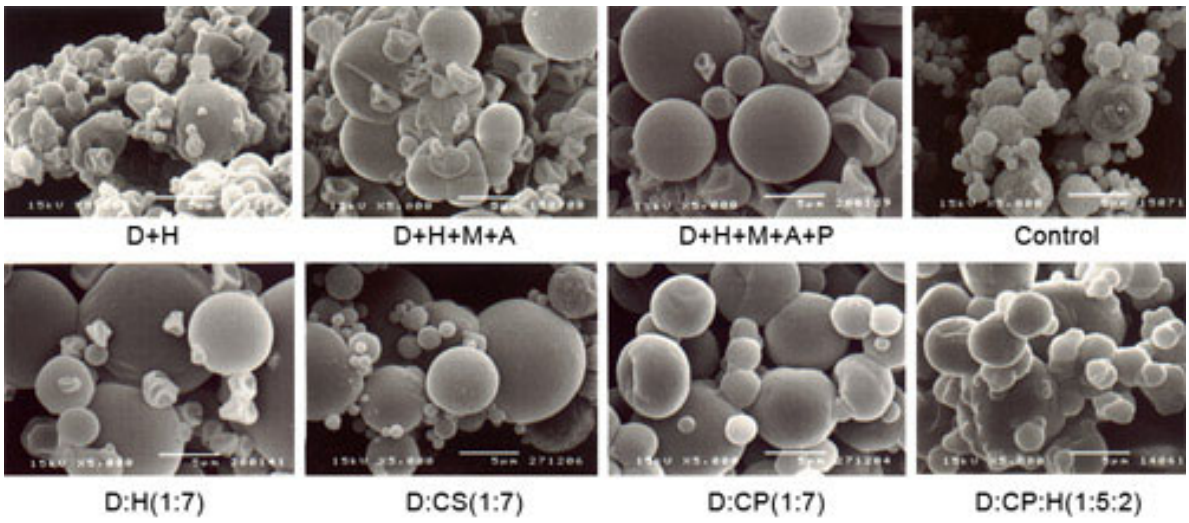

Figure 1. Scanning electron photomicrographs of microspheres prepared from various formulations (original magnification 5000×).

However, the spray-dried product still showed unfavorable morphology (Figure 1, D+H$^+$M+A). Addition of P improved the particle shape to spherical and increased the smoothness of the surface (Figure 1, D+H$^+$M+A+P). Water molecules retained by plasticizer resulted in slow evaporation of solvent within the spray-dried droplets and in avoidance of surface depressions.[23] Therefore, formulation of 80% M, 10% A, and 30% P was selected for further investigations.

Spray-dried Microspheres for Nasal Delivery

Morphology

Control microspheres (Figure 1, control) showed spherical shape with some crystals of drug on the surface. Those of single polymer (Figure 1, D:H[1:7], D:CS[1:7], D:CP[1:7]) were smooth and spherical, except small-size H microspheres, which showed crumpled surface. Microspheres with combined polymers (Figure 1, D:CP:H[1:5:2]) had slightly irregular shape. These obtained microspheres had no hole or rupture on the surface, which morphology would result in slow clearance and good deposition pattern in nasal cavity.[23]

Production yield, drug content, and loading efficiency

The production yields of spray-dried microspheres were between 24% and 74% (Table 2). Increasing the ratio of drug to polymer slightly decreased the yield. The rank order of production yields was H < CS < CP corresponding to the viscosity of their spray-drying solutions, which were ~300 (H), ~100 (CS), ~15 (CP), respectively. Increasing the amount of H in the combined CP:H microspheres decreased the yield. The percentage of drug content (Table 2) showed low standard deviation, which implied good uniformity of drug distribution. In addition, they were close to their percentages of theoretical drug content, which were 7.54% and 5.29% for drug to polymer ratios of 1:5 and 1:7, respectively. All microspheres had good percentage of loading efficiency between 82% and 99% (Table 2).

Particle size

The median size of microspheres ranged from 15.40 to 23.03 μm (Table 2) with low span values, which indicated the narrow size distribution. Such particle size and narrow size distribution were considered to be suitable for nasal administration by insufflation.[24] It was also noted that increasing the drug to polymer ratio slightly increased the size of microspheres.

Bulk density, angle of repose and zeta potential

Very low bulk density of 0.02 to 0.03 g/mL and high value of angle of repose from microspheres of H (Table 2) indicated their poor flowing property. This result was due to their hollow structure and crumpled surface. CP microspheres had the highest bulk density, while wider particle size distribution resulted in moderate bulk density of CS microspheres. These microspheres and those of combined polymers had angles of repose within the range of 42.51 to 45.30 indicating free flowing property. The zeta potentials were positive and negative for microspheres of CS and CP, respectively (Table 2), according to their charge. Adding H to microspheres of CP decreased their zeta potential.

Adhesion property

Control microspheres that contained no polymer were instantaneously washed out from the isolated pig intestine, thus indicating that they possessed no mucoadhesive property. The adhesion time of mucoadhesive microspheres (Table 2) was ranked, CS > CP > H microspheres. Poor

Table 2. Physicochemical Properties of Spray-dried Microspheres*

Formulation Code	Production Yield (% ± SD)	Actual Drug Content (%wt/wt ± SD)	Loading Efficiency (% ± SD)	Median Diameter (μm), Span	Bulk Density (g/mL ± SD)	Angle of Repose (degree ± SD)	Zeta Potential (mv ± SD)	Adhesion Time (minutes ± SD)
Control	46.48 ± 7.12	50.51 ± 0.64	98.73 ± 6.12	18.79, 1.70	0.25 ± 0.06	45.36 ± 1.35	-	0.08 ± 2.05
D:H(1:7)	26.93 ± 5.43	5.18 ± 0.26	98.00 ± 2.78	19.60, 2.19	0.02 ± 0.03	54.90 ± 1.45	-	10.33 ± 1.53
D:H(1:5)	24.45 ± 6.14	7.26 ± 0.55	96.26 ± 3.25	22.82, 2.47	0.03 ± 0.07	55.40 ± 1.43	-	9.45 ± 1.13
D:CS(1:7)	61.24 ± 3.56	4.50 ± 0.39	85.14 ± 4.32	15.60, 2.28	0.11 ± 0.05	42.51 ± 1.36	+29.02 ± 4.87	> 300.00
D:CS(1:5)	60.25 ± 4.31	6.23 ± 0.62	82.60 ± 4.79	23.03, 2.52	0.15 ± 0.03	43.90 ± 1.54	+29.37 ± 4.93	> 300.00
D:CP(1:7)	73.29 ± 4.92	5.12 ± 0.14	95.86 ± 3.89	15.92, 1.36	0.23 ± 0.01	45.30 ± 1.67	−31.70 ± 5.84	90.67 ± 3.06
D:CP(1:5)	70.13 ± 5.08	7.23 ± 0.49	96.87 ± 5.13	19.11, 1.13	0.21 ± 0.03	44.60 ± 1.30	−28.64 ± 8.21	290.00 ± 5.00
D:CP:H (1:3:4)	43.71 ± 6.47	4.96 ± 0.52	93.84 ± 5.13	16.66, 1.68	0.10 ± 0.06	43.93 ± 1.45	−18.23 ± 2.83	241.33 ± 3.21
D:CP:H (1:4:3)	54.96 ± 3.98	5.07 ± 0.24	95.92 ± 4.68	18.34, 1.30	0.12 ± 0.03	44.14 ± 1.33	−25.94 ± 6.12	271.00 ± 3.61
D:CP:H (1:5:2)	69.79 ± 5.31	5.14 ± 0.32	97.25 ± 5.14	15.40, 1.58	0.15 ± 0.05	44.93 ± 1.27	−29.62 ± 6.94	286.78 ± 2.31

*D indicates propranolol hydrochloride; H, hydroxypropyl methylcellulose; CS, chitosan; and CP, carbopol 934P.

mucoadhesion of H microspheres was due to its nonionic property, while excellent mucoadhesion of CS microspheres was from the electrostatic attraction between CS and mucin. Moreover, the linear molecule of CS expressed sufficient chain flexibility for interpenetration and entanglement. Although CP microspheres had negative charge as shown by zeta potential values in this investigated medium (pH 6.8), causing negative charge repulsion with mucus, numerous hydrophilic functional groups such as carboxyl groups in CP molecules could form hydrogen bonds with mucus molecules, thus producing some adhesive force of this polymer.[25]

Unexpectedly, the higher adhesion time of CP microspheres with lower amount of polymer in formulation D:CP(1:5) than that of the higher amount (D:CP[1:7]) was due to more space for the polymer chain of CP, which was a cross-linked polymer with very high molecular weight, to extend within the mucus.[10] Thus, in addition to type and amount, MW of polymer also played a significant role on mucoadhesion. For CP:H microspheres, the higher the amount of CP in formulations, the longer was the mucoadhesion time.

Swelling property

Figure 2 shows that all obtained microspheres rapidly swelled in pH 6.8 phosphate buffer. The swelling was ranked, CP > CS > H microspheres. Increasing the amount of CP in formulation of combined polymers increased the percentage of swelling. The high swelling property of CP and CS microspheres could be attributed to their ionized ability to uncoil the polymer into an extended structure.[26] Higher swelling of CP microspheres than CS microspheres was likely due to its higher molecular weight.

Infrared absorption study

The Fourier transform infrared (FT-IR) spectra (Figure 3) obtained from various formulations of spray-dried microspheres showed no interaction within these formulations

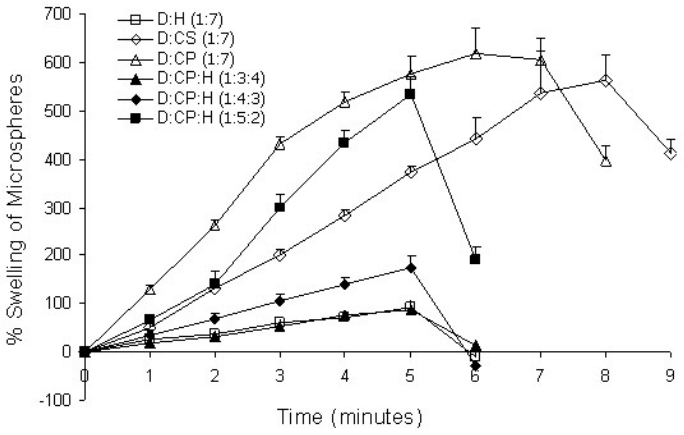

Figure 2. The profiles of percentage swelling with time of microspheres.

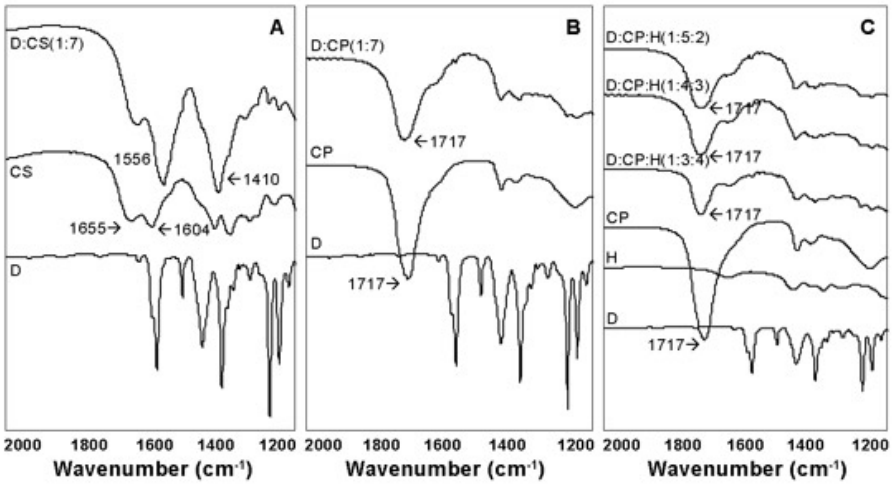

Figure 3. The IR spectra of microspheres compared with their excipients: (A) D:CS(1:7), (B) D:CP(1:7), (C) D:CP:H(1:3:4), (1:4:3) and (1:5:2).

except microspheres containing CS that interacted with acetic acid. The C=O stretching peak near 1655 cm^{-1} representing the structure of N-acetylglucosamine was hardly observed. In addition, the peak at 1550 to 1600 cm^{-1} and near 1400 cm^{-1} region that attributed to the stretching of carboxylate anion could be noticed (Figure 3A) indicating that chitosan acetate was formed.[27]

The electrostatic interaction between carboxylate form ($-COO^-$) of CP and amine group of D as an absorption band at ~1556 cm^{-1} was not found (Figure 3B). In addition, the prominent peak at 1717 cm^{-1} recorded from pure CP corresponding to stretching vibration of carbonyl (C=O) bands still remained. This finding could be explained by the lower pH value of spray-drying solution (~3.5) than the pK_a of polyacrylic acid of CP (pK_a = 4.25), therefore the carbonyl group (C=O) in CP molecules was in un-ionized form. Similarly, the prominent peak at 1717 cm^{-1} in Figure 3C also indicated that hydrogen bonding between the functional group of CP and H in CP:H microspheres was not detected.

In Vitro Drug Release Study

Figure 4 shows the drug release profiles from various formulations of microspheres. The drug in control microspheres (control) was rapidly dissolved and released within 60 minutes. This was because propranolol HCl (pK_a 9.5) could ionize and completely dissolve in this medium. The release of drug was prolonged when incorporated within mucoadhesive polymers. Different types of polymer produced different drug release patterns. Microspheres prepared with H and CS moderately sustained the drug release to 5 hours, whereas those of CP could prolong to 24 hours but with long lag time. In addition, higher amount of polymer resulted in more prolonged drug release. Shorter lag time and faster drug release were obtained when adding H to CP microspheres. The result of viscosity of microspheres after release study showed that H and CS microspheres were much less viscous than CP microspheres. This was owing to their polymeric structure, linear of the former and cross-linked with high molecular weight of the latter.

Drug Permeation Study

The in vitro drug permeation profiles across nasal cell culture model (Figure 5) showed much lower permeation of FD-4 through this cell monolayer than through blank filter, which clearly confirmed the barrier function of the monolayer. For the control microspheres, ~20% of the drug was permeated after 15 minutes and gradually increased until the end of 5 hours. The initial burst permeation was attributed to the sinked microspheres in HBSS in the donor compartment. The percentage of drug permeated was decreased with the polymer in formulation. The type of polymer also clearly affected the drug permeation. The permeation from H and CS microspheres were relatively similar

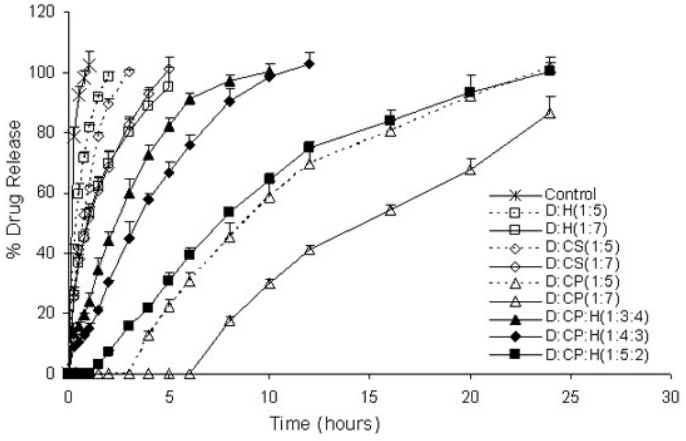

Figure 4. The release profiles of microspheres in phosphate buffer pH 6.8.

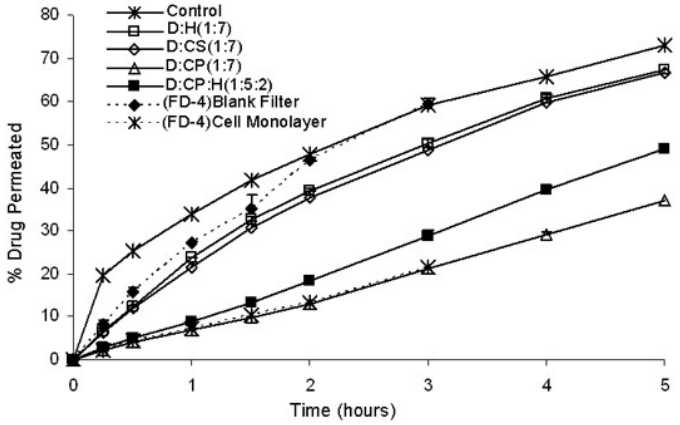

Figure 5. The permeation profiles of microspheres and FD-4 through nasal cell monolayer and FD-4 through blank filter.

and obviously slower than that from control microspheres especially at the first hour. CP microspheres showed the slowest drug permeation, whereas combining CP with H increased the permeation. It could also be seen that the drug permeation profiles corresponded to their release profiles in Figure 4.

Effect of microspheres on the integrity of tight junction

The TEER values of nasal cell monolayers during permeation study and after removing microspheres are shown in Figure 6. The TEER value of 156 ± 10.2 ohm*cm^2 indicated the development of tight junction. During permeation study, incubation of cell monolayer with and without control microspheres showed insignificant reduction in the resistance (Figure 6A). With mucoadhesive microspheres, rapid TEER reduction was noted indicating their effect on the integrity of tight junctions especially from CS microspheres. Further incubation up to 5 hours would only result in a gradual decrease in resistance. Reversibility of TEER values when removing the microspheres after terminating the permeation studies of 3 hours for CS microspheres and 5 hours for other microspheres could be clearly seen (Figure 6B). TEER values completely recovered to initial values within 48 hours, indicating that the effect of microspheres on tight junctions was evidently reversible. Similar result from solution of CS and CP has also been reported.[15,28]

The effect of microspheres on perijunctional F-actin rings was also confirmed in Figure 7. Cultured nasal cells showed intensive staining of actin fibers close to the cellular junction (Figure 7A). Control cells in blank monolayer showed typical perijunctional F-actin rings similarly to monolayer incubated with control microspheres (Figure 7, control). Exposure to CP and combined microspheres resulted in markedly disbanded perijunctional rings, whereas the effect of H microspheres was less pronounced. With microspheres containing CS, disbanded perijunctional rings were also observed after 3 hours of incubation (Figure 7, D:CS[1:7]*), (Figure 7, D:CS[1:7]) but disappeared after 5 hours. Similar result from CS solution was also reported on Caco-2 cells with 1 hour incubation.[29] The effect of polymeric microspheres on the tight junctions was owing to the uptake of water by these microspheres, and subsequent swelling caused dehydration of the epithelial cells, leading to widening of tight junctions.[30] For CP, the reducing of extracellular Ca^{2+} with its chelating properties was the additional mechanism.[15] For CS, the interaction of its positively charged amino groups with negatively charged sites on the cell surfaces and tight junctions would lead to more disbanded perijunctional rings and the lowest TEER values.[31]

Although CS and H microspheres exhibited markedly different TEER values and degree of disbanded perijunctional rings, their permeation profiles were similar. This finding was owing to the small molecular size of propranolol HCl (MW 295.81). Therefore, these small molecules from similar release profile could freely permeate through the tight junction. The low permeation from CP and combined microspheres was attributed to dependency of polymer in formulations on the release of drug.

Viability of cell culture model

The results from trypan blue-dye exclusion and hemocytometer revealed that cells viability of 97% to 99% could be obtained after 5 hours exposure to control, H, CP, and combined microspheres. In contrast, cell viability after exposure to CS microspheres for 3, 4, and 5 hours was gradually decreased from 85.23% to 59.17% and 20.03%, respectively. This was possibly related to the high degree of deacetylation

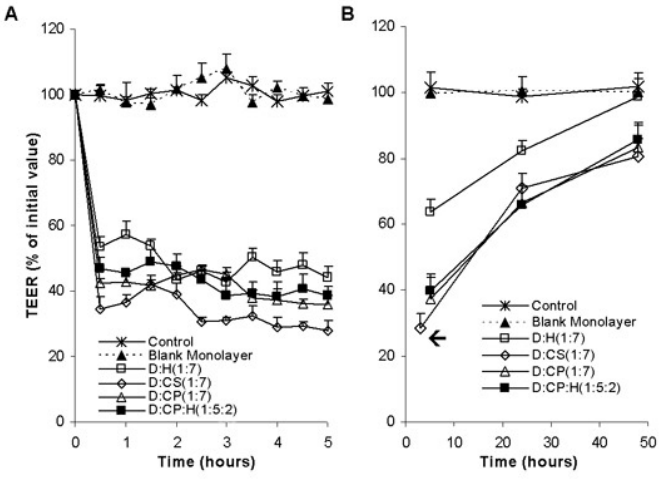

Figure 6. (A) Effect of microspheres on the TEER of nasal cell monolayers. (B) TEER reversibility of nasal cell monolayers after permeation experiments (←, 3 hours for D:CS(1:7); 5 hours for other formulations).

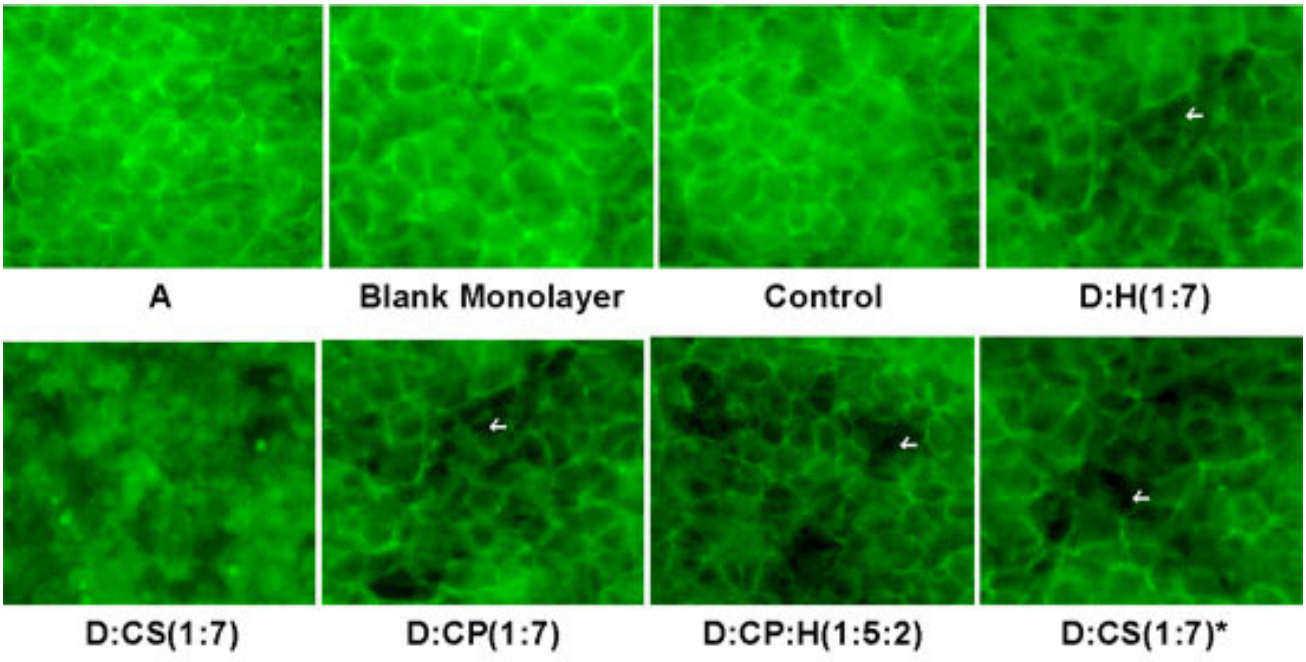

Figure 7. Actin staining of the nasal cell monolayers after permeation experiments with microsphere formulations (original magnification ×400).

of 85% and, consequently, high positive charge density of CS. Cell lytic and toxic properties of CS with high charge density and high dose has previously been reported.[32]

Conclusion

Mucoadhesive microspheres of H, CS, CP, and CP:H could be prepared by spray-drying technique with the aid of excipients in formulations for nasal delivery. The drug release and mucoadhesive property of the microspheres could be altered by varying the proportion and type of polymer. The permeation of drug through nasal cell monolayer was corresponding to their release profiles. These microspheres affected the integrity of tight junctions, but scarcely the cell viability.

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