CHAPTER III

MATERIALS AND METHODS

Materials

The following materials were obtained from commercial sources.

1. Model Drug
   - Propranolol Hydrochloride
     (Batch No.S920501, China National Chemical, China)

2. Additives
   - Methocel E4M (Hydroxypropylmethylcellulose)
     (Premiun grade, Batch No.600119, The Dow Chemical Company, USA)
   - Chitosan
     (MW 2.3-2.5 x 10^5 Da, 85% deacetylation, Seafresh, Thailand)
   - Carbopol 934P
     (MW 3 x 10^6 Da, Batch No. CC24HBB151, Noveon, USA.)
   - Maltodextrin
     (PHS 17, Nutrition Limited Partnership,Thailand)
   - Colloidal silicon dioxide (Aerosil®)
     (Lot No. 713540, Degussa, Germany)
   - Propylene glycol
     (Lot No. 23742, Arco Co. Ltd, Singapore)
   - Acetic acid, glacial 100%
     (E. Merck, Germany)

3. Dissolution Medium
   - Potassium dihydrogen phosphate, analytical grade
     (Lot No. 227 A679473, E. Merck, Germany)
   - Sodium hydroxide, analytical grade
     (Lot No. 211190, J.T. Baker Inc. USA)

- Hydrochloric acid , analytical grade
  (Lot No. 403872, BDH Laboratory, England)

4. Solvents
   - Absolute ethly alcohol, analytical grade
     (Mallinckrodt Chemical, France)

5. High Performance Liquid Chromatography Analysis
   - Theophylline standard
     (Lot No. 52 H0745, Sigma Chemical Co., Ltd. USA)
   - Acetonitrile, HPLC grade
     (Lot No. NJ 08865, J.T Baker Inc, USA)
   - Potassium dihydrogen phosphate, analytical grade
     (Lot No. 227 A679473, E. Merck, Germany)

6. Cell culture
   - Minimum essential media (MEM)
     (Lot No. 1140877, Gibco, Germany)
   - Sodium pyruvate solution
     (Lot No. 1159763, Gibco, Germany)
   - Nonessential amino acid solution
     (Lot No. 114165, Gibco, Germany)
   - Anitibiotic – Antimycotic solution
     (Lot No. 1146539, Gibco, Germany)
   - Hanks'balance salt solution (HBSS)
     (Lot No. 1179630, Gibco, Germany)
   - Fetal bovine serum (FBS)
     (Lot No. 1038E, Biochrom AG, Berlin)
   - Trypsin – EDTA
     (Lot No. 1175248, Gibco, Germany)
   - Phosphate buffer saline pH 7.4
     (Lot No. 1163523, Gibco , Germany)
   - Sodium bicarbonate ($NaHCO_3$)

(Lot No. 1183459, Gibco, Germany)

Equipment

- Analytical balance (Sartorius, Germany)
- Dissolution apparatus (Franz diffusion cells, Thailand)
- Magnetic stirrer (Model MR 3001, Heidolph, Germany)
- Moisture determination balance (Model 6100-H, Ohaus Reg., USA)
- pH meter (Pye Model 292, Pye Unicam, England)
- Pneumatic pump (Model 5053, Watson – Marlow, England)
- High performance liquid chromatugraph (Model SCL-10A, Shimadzu, Japan)
-Thermal analyzer (Model DSC7, Perkin Elmer, USA)
- Fourier transform infrared spectrometer (Model 1760X, Perkin Elmer, USA)
- Scanning electron microscope (Model JSM – T220A, Jeol, Japan)
- Spray dryer (Mini Spray dryer, Buchi Type 190, Switzerland)
- Ultraviolet/visible Spectrophotometer (Model V-530, Jasco, Japan)
- X – ray diffractometer (Model JDX – 8030, Jeol, Japan)
- Cone and plate viscometer (Model MA-D2072, Brookfield, USA)
- Spectrofluorometer (Model FP – 777, Jasco, Japan)
- Laminar air flow cabinet (HBB 2448, Holten, USA)
- CO_2 incubator (3164 S/N, Forma Scientific, USA)
- Refrigerator (-70°C, Model 8417, Forma Scientific, USA)
- Millicell – ERS electrodes (Millipore, USA)
- Inverse phase contrast microscope (CK2, Olympus, USA)

Methods

A. 1. Preliminary studies of the composition of spray drying formulation

In preliminary study, various formulations were investigated to increase the amount of spray dried products or percentage yield and improve the morphology. Propranolol HCl was used as model drug in amount of 0.5 g per formulation and the polymers used in this study were HPMC, chitosan and carbopol. Firstly, the influence

of maltodextrin was evaluated by preparing formulations containing various amounts of maltodextrin as shown in Table 4. The amount of maltodextrin were varied at 20, 50 and 80%.

The colloidal silicon dioxide (Aerosil®) as second additive was used in spray drying formulations at various amounts for improving the percentage yield of product as shown in Table 4. The amounts of colloidal silicon dioxide were varied at 5, 10 and 15%.

Preparation of spray drying solution

Each polymer was separately dissolved in deionized water except chitosan which was dissolved in 1% acetic acid solution. Propranolol HCl was dissolved in absolute ethanol. To prepare spray drying solution, the drug solution in ethanol was homogeneously mixed with the polymer solution. For formulation containing additives, each type of additive in formulation was gradually added in the mixing solution of drug and polymer with the aid of a stirrer. The resulting solution was adjusted to final volume by deionized water for formulation containing HPMC and carbopol or by 1% acetic acid for formulation containing chitosan, and this spray drying solution was sprayed into the spray dryer with condition as shown in Table 5.

The various amounts of propylene glycol as the third additive in this investigation were shown in Table 5. The amounts of propylene glycol were varied at 10, 20 and 30%. The percentage yields in both cyclone and collector part and the morphology of product were evaluated.

Table 4 Composition of spray drying solution in preliminary studies of spray drying formulations

Ingredients	Formulations												
	P1(HPMC) P2(CHI) P3(CP)	P4(HPMC) P5(CHI) P6(CP)	P7(HPMC) P8(CHI) P9(CP)	P10(HPMC) P11(CHI) P12(CP)	P13(HPMC) P16(CHI) P19(CP)	P14(HPMC) P17(CHI) P20(CP)	P15(HPMC) P13(CHI) P21(CP)	P22(HPMC) P25(CHI) P28(CP)	P23(HPMC) P26(CHI) P29(CP)	P24(HPMC) P27(CHI) P30(CP)	P31(HPMC) P34(CHI) P37(CP)	P32(HPMC) P35(CHI) P38(CP)	P33(HPMC) P36(CHI) P39(CP)
Drug (g)	0.5	0.25	0.17	0.12	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
*Polymer (g)	0.5	0.75	0.83	0.88	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
[1]Maltodextrin (%)					20	50	80	80	80	80	80	80	80
[2]Aerosil (%)								5	10	15	10	10	10
[1]Propylene glycol (%)											10	20	30
Et-OH:H_2O(30:70)q.s. to (g)	100	100	100	100	100	100	100	100	100	100	100	100	100

*Type of polymer : HPMC, Chitosan and Carbopol
[1]Percent by weight of the amount of drug and polymer
[2]Percent by weight of the formulation
[3]Percent by weight of the amount of polymer

Table 5 Spray drying condition in preliminary study

Inlet air temperature	120 °C
Pump feed rate	5 ml/min
Atomizing air flow rate	500 Nl/h

2. Preliminary studies of spray drying process

The spray drying conditions were investigated in the preparation of propranolol HCl spray dried microparticles using HPMC. The suitable composition of spray drying formulation obtained from Table 4. in the preliminary studies of the composition of spray drying formulation employed for preliminary investigation on the effects of processing variables. The processing variables of spray drying technique such as inlet air temperature, pump feed rate and atomizing air flow rate were varied according to Table 6. The outlet temperature cannot be directly controlled , but is a function of the dryer inlet air temperature and the solution feed rate, whereas in this study a range of 70 - 90°C was used.

Table 6 Parameter of spray drying process variables.

Parameter	Process variables		
Inlet air temperaure (°C)	110	120	130
Pump feed rate (ml/min)	4	5	6
Atomizing air flow rate (Nl/h)	400	500	600

In order to study the effect of each variable in spray drying process , it was necessary to keep the other variables constant and varied only the desired variable. To study the effect of inlet air temperature on physical properties, the inlet air temperature was varied while the pump feed rate and atomizing air flow rate were fixed. In case of the effect of pump feed rate and atomizing air flow rate, the experiments were carried out in the same way.

2.1 Effects of inlet air temperature

Temperatures used were 110, 120 and 130°C while pump feed rate and atomizing air flow rate were kept at 5ml/min and 400 Nl/h, respectively. Physical properties of spray dried powder obtained were evaluated and the inlet air temperature that gave the best results. i.e., maximum percentage yield, narrow size distribution, low angle of repose, smooth surface and spherical shape, was selected to be the optimum value for further study.

2.2 Effects of pump feed rate

Experimental pump feed rates used were 4, 5 and 6 ml/min. Atomizing air flow rate was set at 400 Nl/h.The optimum inlet air temperature was obtained from the study in 2.1. The products were evaluated and the optimum feed rate was selected in the same way as described earlier.

2.3 Effects of atomizing air flow rate

Atomizing air flow rate at 400, 500 and 600 Nl/h were used , along with the optimum temperature and optimum pump feed rate obtained earlier. The optimum atomizing air flow rate was selected as previously described.

3. Preparation of spray dried microspheres

After completion of the preliminary study of the composition of spray drying formulation, the suitable amounts of various additives were obtained for producing maximum percentage yield and good characteristics of microspheres.

The amount of ingredients used in each formulation are presented in Table 7. The compositions of microsphere formulations were drug, polymer and three types of additives as maltodextrin, Aerosil® and propylene glycol. Maltodextrin was gradually added to the solution at the amount of 80% (w/w) of the total amount of polymer and drug. Propylene glycol was used at 30% by weight of polymer

content and colloidal silicon dioxide (Aerosil®) was added at 10% by weight of formulation. The drug to polymer ratios of solution are shown in Table 8. The investigated ratios of drug to polymer were varied in the range of 1:1-1:7.

After completion of the preliminary investigation of spray drying process, the suitable conditon of spray drying for producing the most satisfactory spray dried microparticles was selected. And, only the drug to polymer ratios of each polymer were further investigated to improve drug release rate from the matrices. The drug and polymer solution was spray dried under optimum condition of each type of polymer as shown in Table 9.

Table 7 The ingredients of various formulations of microspheres

Ingredients	Amount of Solid
Drug	*
Polymer	*
[1]Maltodextrin	80%
[2]Propylene glycol	30%
[3]Aerosil®	10%
Ethanol:Water(30 : 70) q.s.to	100 g

*The total amount of drug and polymer is 1.0 g

[1] Percent by weight of the amount of drug and polymer

[2] Percent by weight of the amount of polymer

[3] Percent by weight of the formulation

Table 8 The drug to polymer ratios of microsphere formulations

Formulation Code	Drug : Polymer			
	HPMC	Chitosan	Carbopol	Carbopol : HPMC
F0	Controlled microspheres (no polymer material)			
F1	1 : 1			
F2	1 : 3			
F3	1 : 5			
F4	1 : 7			
F5		1 : 1		
F6		1 : 3		
F7		1 : 5		
F8		1 : 7		
F9			1 : 1	
F10			1 : 3	
F11			1: 5	
F12			1 : 7	
F13				1 : (3 : 4)
F14				1 : (4 : 3)
F15				1 : (5 : 2)

Table 9 Spray drying condition during the drug to polymer ratio variable studied

Condition	Formulation			
	F1 -F4	F5 -F8	F9 - F12	F13 - F15
Inlet air temperature ($^{\circ}$C)	130	120	120	120
Pump feed rate (ml/min)	5	5	5	5
Atomizing air flow rate (Nl/h)	400	400	400	400

Preparation of spray drying solution

Each polymer was separately dissolved in deionized water except chitosan which was dissolved in 1% acetic acid solution. Propranolol HCl was dissolved in absolute ethanol. To prepare spray drying solution, the drug solution in ethanol was homogeneously mixed with the polymer solution. For formulation containing additives, each type of additive in formulation was gradually added in the mixing solution of drug and polymer with the aid of a stirrer. The resulting solution was adjusted to final volume by deionized water for formulation containing HPMC and carbopol or by 1% acetic acid for formulation containing chitosan, and this spray drying solution was sprayed into the spray dryer.

B. Evaluation of physicochemical properties of spray dried microspheres

1. Percentage yield

The percentage yield (w/w) was calculated as the weight of the dried microspheres recovered from each batch divided by the sum of the initial dry weight of the starting materials and multiplied with 100.

2. Morphological examination

Scanning electron microscope was used to examine the shape and surface morphology of all formulations of microspheres. Samples of microspheres were dusted onto double sided tape on an aluminium stub. The stubs were then coated with gold using a cold sputter coater to a thickness of 400°A. The samples were imaged using a 25 kv electron beam. The magnifications of the photomicrographs of spray dried microspheres were x5000 and x10000.

3. Particle size measurement

The prepared microspheres were sized by using a laser diffraction spectrometer (Mastersizer S, Malvern, UK). The size of the microspheres was

determined in 1 – hexane as a non – dissolving dispersion medium and the particles were suspended mechanically by magnetic stirring during the measurement. The volume mean diameters D(4,3) of microspheres were measured and the amount of particles in size less than 1.0 μm, more than 200 μm and in the range of 10-50 μm were calculated. The size distribution of microspheres was determined by the span value that was calculated by following equation :

$$\text{Span} = [D(V,0.9)-D(V,0.1)]/D(V,0.5)$$

4. Drug content

The propranolol hydrochloride content of microspheres was determined according to USP XXIII by HPLC method. Microspheres of each formulation (40 mg) was added to 100 ml of 0.05 M phosphate buffer (pH 6.8) and stirred over night to extract the entrapped propranolol HCl. Samples were withdrawn, filtered through a 0.2 μm syringe filter and assayed by HPLC method with a 220 nm detector and theophylline was used as internal standard. The mobile phase, 0.05 M phosphate buffer mixed with acetonitrile (650:350) was delivered at a flow rate of 1.0 ml/min. The concentration of propranolol HCl was calculated by using peak area and absorbance based on calibration curves constructed on the day of the experiment. Each sample was determined in triplicates.

Phosphate buffer : Dissolved 13.6 g of monobasic potassium phosphate in 2 liters of water, and mix. Filter the solution through a 0.5 μm or finer porosity filter before use.

Mobile phase : Acetonitrile 350 ml was transferred to a 1000 ml volumetric flask, dilute with buffer solution to volume and mix. Degas and filter before using.

The amount of propranolol HCl loaded in the microspheres relative to the initial amount of propranolol HCl was calculated according to the following equation and expressed as the loading efficiency.

$$\text{Loading efficiency} = \frac{\text{Weight of propranolol HCl in microspheres}}{\text{Total initial weight of prapranolol HCl}} \times 100$$

Calibration curve for determination of drug content

Propranolol HCl of 40 mg was accurately weighed and dissolved in mobile phase. The solution was adjusted to 100 ml with mobile phase and used as a stock solution at the concentration about 400 μg/ml. As internal standard , theophylline 40 mg was accurately weighed and dissolved with mobile phase. The solution was adjusted to 100 ml with mobile phase and used as internal standard stock solution with concentration about 400 μg/ml.

Propranolol HCl stock solution was individually pipetted 20 , 50 , 80 , 110 , 140 and 170 μl into 10 ml volumetric flask. Internal standard stock solution of 80 μl was filled in all flasks and adjusted with moblile phase to volume and mixed. The final concentration of each solution was 0.8, 2.0, 3.2, 4.4, 5.6 and 6.8 μg/ml, respectively and the concentration of internal standard was about 3.2 μg/ml in all flasks.

The ratio between peak area of propranolol HCl and internal standard was determined by HPLC method at absorbance of 220 nm. Each concentration was determined in triplicate. The calibration curve and representative chromatogram were shown in appendices.

5. Bulk density determination

The bulk density was determined from the weight of microspheres about 5 g. (accurate weight recorded). This microspheres were carefully transferred into a 50 ml graduated cylinder and the bulk volume was recorded. Division of weight by bulk volume yielded bulk density. The results were averaged from three determinations.

6. Determination of the angle of repose

The powder characteristic tester was used to determine the angles of repose of microspheres. 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. When the angle of repose came to the desired condition. Then, the angle measuring arm was moved by fingers to the position at which the angle of repose could be measured in accordance. The angle of repose was averaged from three determinations.

7. Moisture determination

Microspheres sample of 1.0 g. were accurately weighed on the pan of moisture analyzer. The temperature and conditions were set at 100°C, auto and 0 – 100% mode. The sample was dried until constant weight and percent moisture content was read. The mean and standard deviation of three determinations were calculated.

8. Infrared absorption study

Infrared spectrophotometry was used to determine the existence of possible interaction within the microsphere formulations by observing the positions of IR peaks. The IR spectra of the propranolol HCl and additives in the spray dried microspheres were examined using the potassium bromide disc (KBr) method by an infrared spectrophotometer in the range of 4000 – 400 cm^{-1}.

9. Powder X-ray diffraction study

The crystallity of propranolol HCl in the spray dried microspheres was examined by X-ray diffractometry. The samples for X-ray diffraction studies were firmly packed into a cavity of a thin rectangular metal plate using two glass slides attached to the metal plate with adhesive tape. The first glass slide was then removed, and the prepared sample was taken to expose to the X-ray diffraction chamber. The X-ray diffraction patterns were recorded from 5° to 90° in terms of 2θ angle.

10. The differential scanning calorimetry study

Thermal analysis is the most common approach to study physicochemical interactions of two or more component systems. Several modified techniques utilizing the principle of change in thermal energy as a function of temperature are : cooling curve method, thawing method, thermomicroscopic method, differential thermal analysis (DTA), differential scanning calorimetry (DSC).

DSC is an effective thermal method to study the equilibrium phase of either a pure compound or a mixture. Different effects, associated with physical or chemical changes, are registered as a function of temperature or time as the substance is heated at a uniform rate. The diffractograms of spray dried microspheres in different ratios of polymer and drug were recorded on thermal analyzer. All thermal runs were carried out at a heating rate of 10°C/min and the temperature between 30°C and 300°C.

11. Swelling property

The swelling property of microspheres were determined using a Malvern Mastersizer laser diffraction spectrometer. About 25 mg of mucoadhesive microspheres were dispersed in 1-hexane as a non-dissolving dispersion medium. This obtained dispersions were suspened mechanically in the phosphate buffer (pH 6.8) contained in the equipment. The increase of microspheres diameter was evaluated at different time points. Diameters of microspheres were measured until a stable diameter was obtained, and the percentage of swelling was calculated by the following equation.

$$\% \text{ swelling} = \frac{\text{The diameters of microspheres at time (t)} - \text{The initial diameter (t=0)}}{\text{The initial diameter of microspheres}} \times 100$$

12. Zeta potentials studies

The zeta potentials were measured by a zeta meter (system 3 +) at pH 6.8 phosphate buffer (0.0005 M) as medium. About 25 mg of mucoadhesive

microspheres were dispersed in phosphate buffer and filled into the equipment of zeta meter. The zeta potential values were immediately measured by observing the directional movement of microspheres passing the scale of the microscopic examination. Two hundred particles were used for determining the zeta potentials of each formulation of microspheres.

13. In vitro evaluation of mucoadhesive properties of microspheres

The mucoadhesive properties of microspheres were determined by an adapted method described by Vyas, 1993. The principle of this test was based on simulating a biological flow by washing off a mucous membrane covered with the product to be tested. Fresh pig intestine was obtained from the local abattoir within 1 h of killing the animal. A freshly cut 5 cm long piece of intestine of pig was obtained and cleaned by washing with isotonic saline solution. Accurately weighed of 125 mg of microspheres were placed on mucosal surface, which was fixed over a polyethylene support. The intestinal piece was maintained at 90% relative humidity and room temperature for 15 min in a desiccator. The plate with the tissue was then fixed in an angle of 40° relative to the horizontal plane. Phosphate buffer (pH 6.8) warmed at 37°C with water bath was pumped at a rate of 5 ml/min over the tissue using a peristaltic pump. The duration for completely washing off microspheres from pig intestine was recorded. The change of microspheres adhesion on the pig intestine was also observed by taking the photographs.

14. In vitro drug release study

The experimental conditions of drug release experiments were similar to those encountered in the nasal cavity. The in vitro drug release test of the microspheres was carried out using an apparatus called franz diffusion cells (Vyas, 1993 and Abd EI – Hameed, 1997). A dialysis membrane (Cut – off Mw 12000) was used to keep the microspheres on the donor side and it allowed free diffusion of propranolol HCl to the receptor compartment containing phosphate buffer solution (pH 6.8). The temperature of the receptor medium was adjusted to 37 ± 1°C and maintained at that temperature by a peristaltic pump. The content of

the receptor compartment was continuously stirred with a magnetic stirrer. A portion of sample microspheres equivalent to 1500 μg of propranolol HCl was accurately weighed and dispersed into a franz diffusion cell at the beginning of each test. Samples of a 10 ml were withdrawn from the receptor compartment at hourly intervals for 24 h and replaced with the same amount of fresh buffer solution. The samples were assayed by a spectrophotometer at 218 nm (Vyas et al.,1993).

The results were expressed as a percentage of the drug released as shown below :

$$\% \text{ Drug release} = \frac{\text{Amount of drug released at time (t)}}{\text{Total amount of drug in microspheres}} \times 100$$

Calibration curve for determination of drug release

Propranolol HCl of 40 mg was accurately weighed and dissolved in phosphate buffer pH 6.8. The solution was then adjusted to 100 ml with phosphate buffer pH 6.8 and used as stock solution at the concentration about 400 μg / ml.

The stock solution was individually pipetted 300, 400, 500, 600, 700 and 800 μl into 10 ml volumetic flask and diluted to volume with phosphate buffer pH 6.8. The final concentration of each solution was 12, 16, 20, 24, 28 and 32 μg/ml, respectively. The absorbance of known drug concentration was determined by a double beam spectrophotometer in a 1 – cm cell at 218 nm for phosphate buffer pH 6.8 and this buffer was used as a blank solution (Vyas et al.,1993). Each concentration was determined in triplicate.

The concentration versus absorbance of propranolol HCl in phosphate buffer pH 6.8 at 218 nm presented in appendices showed a linear relationship. The standard curve of propranolol HCl after regression analysis is illustrated in appendices.

15. Viscosity measurements

The influence of viscosity value of microspheres formulations on the release of drug was investigated by using the Wells-Brookfield cone/plate digital viscometer. The swollen mucoadhesive microspheres obtained from franz diffusion cell after in vitro drug release study in measured volume about 0.5 ml were picked out by drawing with plastic syringe from membrane in franz diffusion cells and were placed on the plate of equipment. The plate was fixed under the part of cone. The cone was rotated when starting measurement and the constant value of viscosity was read. used in this operation. These results were averaged from three determinations.

4. In vitro permeation study

4.1 Cell cultures

RPMI 2650 cells originating from a human nasal septum carcinoma were obtained from the American Type culture collection, Rockville, MD. The cells were cultivated on polyester filters (3.8 cm^2) for permeation experiments (Transwell cell culture inserts with a mean pore diameter of 0.40 μm) and for fluorescence microscopy. The amount of cells 3.5 – 4.0 x 10^5 cells/cm^2 were seeded onto filters and allowed to grow and differentiate for 7-8 days. 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 sodium bicarbonate at concentration of 1.5 g/liter. This cells were cultivated at 37°C in an atmosphere of passages 26 – 35 were used. Attachment and proliferation of the cells were observed through an inverse phase contrast microscope with a x10 magnification.

4.2 Actin staining

Actin was stained using FITC – labeled phalloidin previously described by Anderberg et al. (1993). Briefly, 10 μl of the stock solution (0.5 mg/ml phallodin in methanol) was evaporated and redissolved in 400 μl of 0.2 M phosphate buffer saline,

pH 7.4 (PBS). The monolayer, grown on filter of transwell was rinsed three times with PBS , fixed for 10 min in 4% formaldehyde in PBS, rinsed again and treated with 1% Triton X-100 on ice for 5 min. After repeated washings and air drying , the monolayer was stained with FITC – phalloidin under light exclusion for 20 min. The sample was rinsed twice PBS and examined under a fluorescent microscope with a x100 magnification.

4.3 Transepithelial electrical resistance (TEER)

For TEER measurements confluent cell monolayers grown for 7 days on polyester filters (0.4 μm pore size, 3.8 cm^2 area) were used. The monolayers were allowed to equilibrate in Hanks' balanced salt solution (HBSS) for 15 min at 37°C, rinsed, measured TEER using millicell–ERS electrodes. The basolateral compartment contained 2.6 ml, the apical compartment 3 ml of fresh HBSS at room temperature. The observed TEER value was corrected for the blank filter resistance.

4.4 Permeation experiment

In vitro permeation of propranolol HCl from mucoadhesive microspheres was studied across nasal cell culture using Transwell model. The confluent cell monolayers , grown for 7 days on polyester filters (0.4 μm pore size) were rinsed with HBSS and were allowed to equilibrate in the same buffer for 15 min before they were used. Accurately known amount of microspheres equivalent to 250 μg of propranolol HCl was weighed and layered over the confluent cell monolayer. The donor and receptor compartments of Transwell were filled with 1.5 ml (pH 6.8) and 2.6 ml (pH 7.4) of Hanks' balance salt solution (HBSS), respectively. The solution in Transwell was slowly shaked (80 rpm) and was maintained at 37°C in a shaker bath. The total volume (2.6 ml) of samples were withdrawn at different time intervals from the receptor compartment and replaced by fresh HBSS. The amount of permeated drug was determined by HPLC method as similar to the method in drug content study.

For the permeation study of FITC – labeled dextran, the FITC – labeled dextran (MW 4000, FD-4) was dissolved in Hanks' balance salt solution (pH 6.8) in a

concentration of 200 μg/ml. This solution was added to the donor compartment of Transwell, total volume of samples were withdrawn at different time intervals from the receptor compartment of Transwell and replaced by fresh HBSS. The amount of permeated FD-4 was determined by fluorescence spectophotometer at emission/excitation wavelengths of 490/515 nm (Werner, 1995).

4.5 Calibration curve for determining the amount of FITC–labeled dextran (FD-4)

FITC – labeled dextran of 40 mg was accurately weighed and dissolved in phosphate buffer pH 6.8. The solution was adjusted to 200 ml with phosphate buffer. The volume of 1 ml of this solution was diluted to 25 ml with the same phosphate buffer and used as a stock solution at the concentration about 8.0 μg/ml.

The stock solution was individually pipetted 100, 150, 200, 250, 300 and 350 μl into 10 ml volumetric flask and diluted to volume with phosphate buffer. The final comcentration of each solution was 0.08, 0.12, 0.16, 0.20, 0.24 and 0.28 μg/ml, respectively. The intensity of known FD-4 concentration was determined by fluorescence spectrophotometer at emission/exitation wavelengths of 490/515 nm, and this buffer solution was used as a blank solution. Each concentration was determined in triplicate.

The concentration versus intensity of FD-4 in phosphate buffer pH 6.8 at emission/exitation 490/515 nm presented in appendices showed a linear relationship. The standard curve of FD-4 is illustrated in appendices.

5. Stability study

Some formulations of mucoadhesive microspheres were selected for stability testing. The microspheres were stored in closed glass bottles lined internally with alminium foil. These were placed in desiccator at room temperature. Samples were withdrawn at 6 and 12 months and were analyzed for active drug content, morphology, adhesion time, percentage of drug release, particle size, infrared

absorption study, powder X-ray diffraction study and the differential scanming calorimetry study.