CHAPTER IV

RESULTS AND DISCUSSION

1. Preliminary studies of the composition of spray drying formulation

In preliminary study, spray dried products could not be collected when preparing from formulations containing only drug and polymer in ratios 1:1 and 1:3 in all types of polymers (P1-P6), but spray dried products could be collected from formulations in ratios of 1:5 and 1:7 (P7-P12) with the percentage yield ranging form 2.78-9.64. The percentage yields of these formulations are shown in Table 10. This might be explained that the polymer in formulation could increase mass and weight of spray dried microparticles effecting to increase the ability of spray dried particles for separating from the air steam in spray drying system by cyclone part to the collector. The increase of percentage yield by increasing the amount of polymer in formulation was limited as the results in Table 10. The drug to polymer ratio of 1:7 (P10-P12) gave similar percentage yields to the formulations containing drug to polymer ratio of 1:5 (P7-P9) in each type of polymer and very low percentage yields were obtained. Scanning electron photomicrographs of spray dried microparticles prepared without additives in formulations with drug to polymer ratio of 1:5 (P7-P9) and 1:7 (P10-P12) are shown in Figure 4 and 5. These photographs showed agglomerated microparticles with coalesed each other, and particles with crumpled surface were observed in formulation containing HPMC.

Table 10 The percentage yield of microspheres prepared without additives in formulations

Formulation Code	Percentage yield
P7(HPMC)	2.78
P8(CHI)	7.04
P9(CP)	9.13
P10(HPMC)	3.06
P11(CHI)	6.52
P12(CP)	9.64

Because of very low percentage yields of spray dried products produced only with drug and polymer, the additives were necessary to use in formulations for increasing the amount of spray dried products or percentage yield. Maltodextrin was the first selected additive as particle filler in spray drying formulation. The major lost of product could be explained by the loss of too fine microparticles which was not dissociated from the air by the cyclone separator. The percentage yields of microparticles prepared from the formulation containing various amounts of maltodextrin (P13-P21) are shown in Table 11. The total percentage yields were increased by increasing the amount of maltodextrin in formulations. The obtained total percentage yields when using 80% maltodextrin in formulations were 13.28, 51.01 and 52.73% for HPMC, chitosan and carbopol, respectively. The increase of total percentage yield by maltodextrin could be explained that maltodextrin was spray drying aid in producing spray dried microparticles by increasing mass of spray dried droplets which left out of the nozzle of the equipment, then the increase of mass of microparticles caused the decrease of too fine and light microparticles affecting to increase the amount of dissociated particles from the air in the spray drying system.

Table 11 The percentage yield of microspheres containing maltodextrin in formulations

Formulation Code	Percentage yield		
	Cyclone	Collector	Total
P13(HPMC)	4.14	-	4.14
P14(HPMC)	5.32	1.05	6.37
P15(HPMC)	10.59	3.28	13.28
P16(CHI)	12.74	3.69	16.43
P17(CHI)	20.72	9.11	29.83
P18(CHI)	38.25	12.76	51.01
P19(CP)	5.63	-	5.63
P20(CP)	22.45	9.64	32.09
P21(CP)	40.81	12.73	52.73

However, the percentage yields obtained from these formulations in the cyclone part were higher than that in the collector part of spray dryer in all types of

polymers as shown in Tables 11. The extensively adhered microparticles on the apparatus glass wall of cyclone that could not completely flow to the collector of spray dryer might be explained by the glass transition temperature (Tg) of maltodextrin in formulation. The glass transtion temperature (Tg) of maltodextrin is about 120°C, and the decrease of Tg is strongly dependent on the water concentration in the system. The effect of maltodextrin on the surface stickiness of spray dried microparticles was explained by comparing the glass transition temperature (Tg) of maltodextrin at the surface layer of spray dried droplets and the spray dried droplet temperature. The droplet surface is sticky if its surface layer Tg was lower than the droplet temperature. The droplet surface exhibited a peak tendency to stick when its surface layer Tg reached or just crosses the droplet temperature. At temperature higher than the Tg, maltodextrin at the surface layer of microparticles was changed from glassy to rubbery state resulting in sticking of microparticles on the apparatus glass wall of cyclone part. This was agreed with the report of Adhikri et al. (1993) that the addition of maltodextrin affected on stickiness of spray dried products.

The selected amount of maltodextrin was 80% for improving the total percentage yield of spray dried products, and the problem about sticking of spray dried microparticles on the cyclone wall by maltodextrin might be corrected by using second additive as colloidal silicon dioxide (Aerosil®) in the formulation. The percentage yields of formulations containing both maltodextrin and various amounts of Aerosil® (P22-P30) are shown in Table 12. When using Aerosil® in formulations, the percentage yield obtained from collector part was clearly higher than that from cyclone part in all types of polymers. Increasing the amount of Aerosil® in formulation caused the increase of percentage yield in collector part, but the limitation of this increase was observed as the results in Table 12.

High percentage yields in collector part were obtained when using Aerosil® of 10% in formulation, but the increase of Aerosil® to 15% (P24. P27, P30) could not increase the percentage yield in collector part. The total percentage yields were slightly increased when increasing the amount of Aerosil® in formulations. These results could be suggested that the use of Aerosil® in these formulations could densify the particles obtained from spray drying and thus improve flowability of

microparticles from chamber and cyclone to collector part. And, the other important reason for improving percentage yield was the anti-adherent property of Aerosil® that could reduce the tackiness around the surface of the spray dried microparticles (Takeuchi, 1989). The percentage of Aerosil® at 10% of formulation was suitable for improving the percentage yield in collector part of these spray dried products.

Table 12 The percentage yield of microspheres containing maltodextrin and Aerosil® in formulations

Formulation Code	Percentage yield		
	Cyclone	Collector	Total
P22 (HPMC)	5.54	8.15	13.69
P23 (HPMC)	2.14	13.15	15.29
P24 (HPMC)	2.51	13.12	15.63
P25 (CHI)	11.79	40.17	51.96
P26 (CHI)	7.37	46.76	54.13
P27 (CHI)	6.59	46.51	53.10
P28 (CP)	12.21	40.17	52.38
P29 (CP)	8.29	46.88	55.17
P30 (CP)	8.78	47.23	56.01

Figure 6 shows the scanning electron photomicrographs of microspheres prepared by adding maltodextrin and Aerosil® in formulations (P23, P26, P29). Unfavorable morphology of these spray dried microparticles was clearly observed. These microparticles were small size that aggregated each other and the crumpled surface with collapsed shape was observed from microparticles containing HPMC.

The propylene glycol as plasticizer for soluble aqueous polymers was used as the third additive in spray drying formulation for improving the morphology and the percentage yield in collector part of microparticles. The smooth surface of microspheres is important factor for complete contact between mucus surface of tissue and surface of microspheres affecting to the mucoadhesion of microspheres.

The use of propylene glycol in formulations improved the shape of particles rising to spherical and smooth surface as shown in Figure 7. Microspheres containing HPMC was more spherical shape and less crumpled surface when adding propylene glycol in formulation. In the process of drying, the inner solvent of droplets of spray drying solution was evaporated quickly passing the surface of droplets to the outlet environment in spray drying chamber. The pressure within the droplets was quickly droped, and high velocity of airflow in spray drying chamber depressed the surface shell of droplets causing deformation of spherical shape (Ting, 1992). When adding plasticizer in formulation, plasticizer retained some water molecules linked to its own structure and could fill the intern empty space of the microspheres, preserving the hydration, slowly evaporating of solvent within the droplets avoiding depressions on the surface, and assuring a more uniform wall and spherical shape of the obtained microspheres (Bruschi, 2003). This result was agreed with the report of Palmieri,1994.

For microspheres containing chitosan and microspheres containing carbopol, the separated and spherical microspheres were observed, and the aggregation was reduced when adding propylene glycol in formulations. This result could be explained that the propylene glycol as plasticizer could reduce the surface tension of spray drying solution. Solution with lower surface tension required less energy to form the separated spherical droplets from the nozzle of spray dryer that could give the spherical microparticles (Swarbrick and Boylan, 1996). In addition, the percentage yield in collector part of microspheres were increased when using propylene glycol in formulations containing chitosan (P34-P36) and containing carbopol (P37-P39) as shown in Table 13. This might be due to the separated and spherical shape of these microspheres that induced the flow property of microspheres from cyclone to collector part.

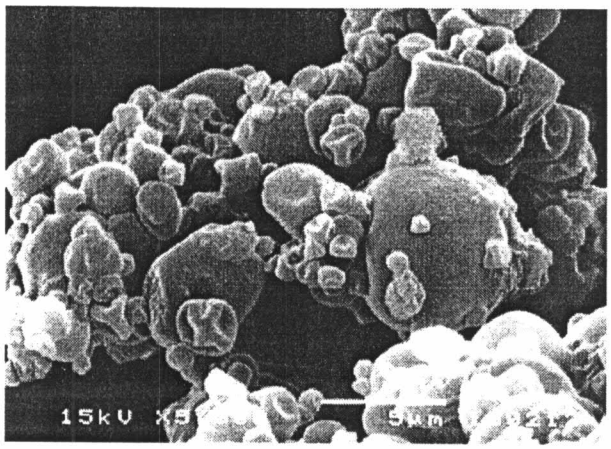
The increase of percentage yield in collector part was not occurred in formulation containing HPMC. The percentage yields in cyclone/collector of microspheres prepared from HPMC were 2.14/13.15 (P23) and 2.16/12.97 (P33) for formulations prepared without propylene glycol and with 30% propylene glycol,

respectively. This might be suggested that the hollow structure and light microspheres limited the ability to flow of microspheres.

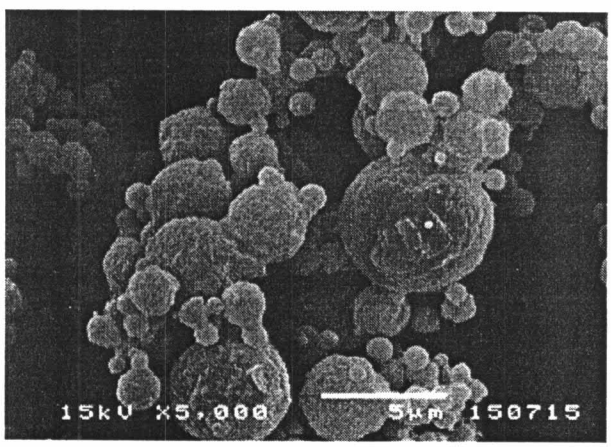
Table 13 The percentage yield of microspheres containing maltodextrin, Aerosil® and propylene glycol in formulations

Formulation Code	Percentage yield		
	Cyclone	Collector	Total
P31 (HPMC)	2.65	11.98	14.63
P32 (HPMC)	3.01	12.49	15.50
P33 (HPMC)	2.16	12.97	15.13
P34 (CHI)	6.84	46.32	53.16
P35 (CHI)	4.91	48.01	52.92
P36 (CHI)	2.38	51.10	53.48
P37 (CP)	8.14	46.79	54.93
P38 (CP)	3.27	53.14	56.41
P39 (CP)	2.03	53.26	55.29

P7(HPMC)



P8(CHI)



P9(CP)

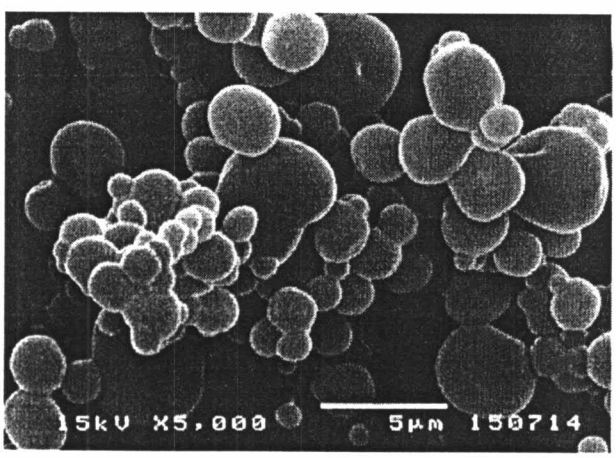
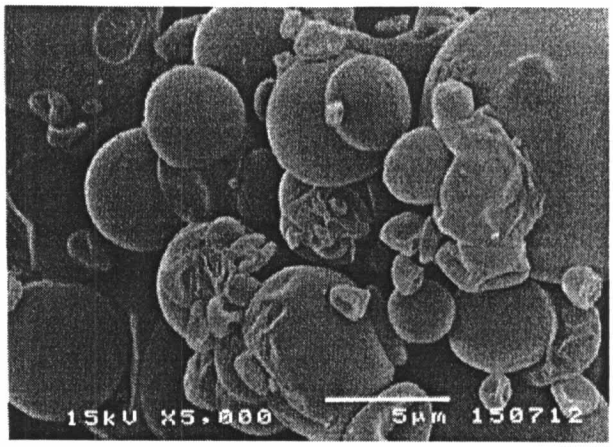
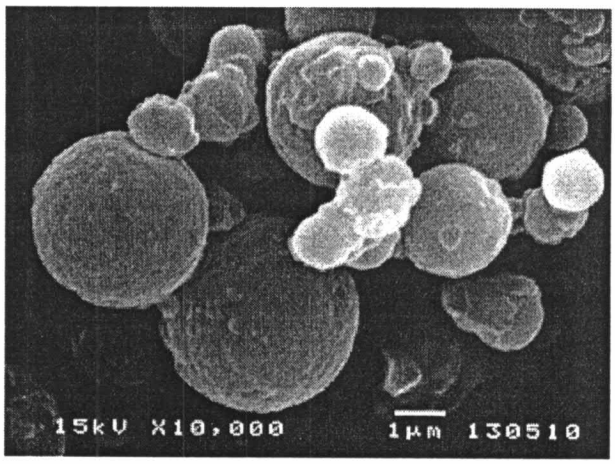


Figure 4 Scanning electron photomicrographs of microspheres prepared without additives in formulations (P7-P9)

P10(HPMC)



P11(CHI)



P12(CP)

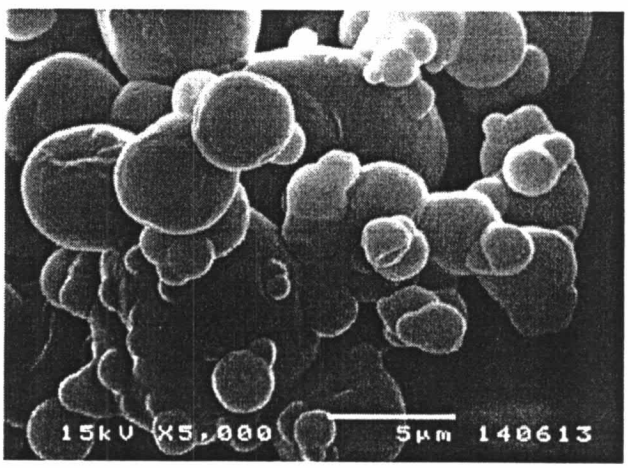
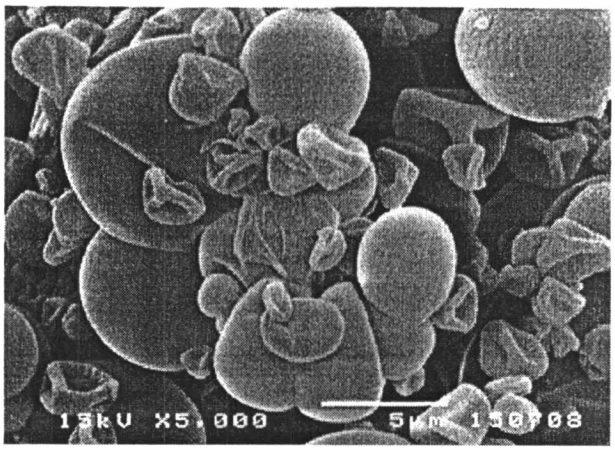
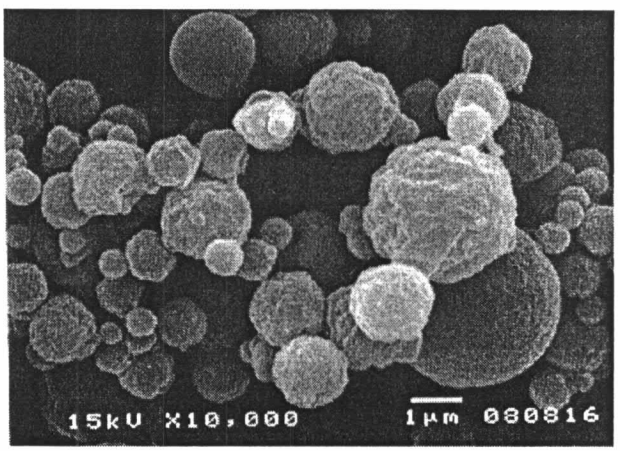


Figure 5 Scanning electron photomicrographs of microspheres prepared without additives at drug to polymer ratio of 1:7 (P10-P12)

P23(HPMC)



P26(CHI)



P29(CP)

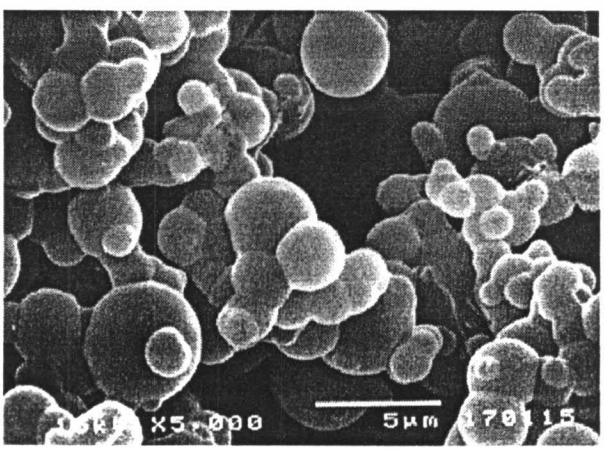
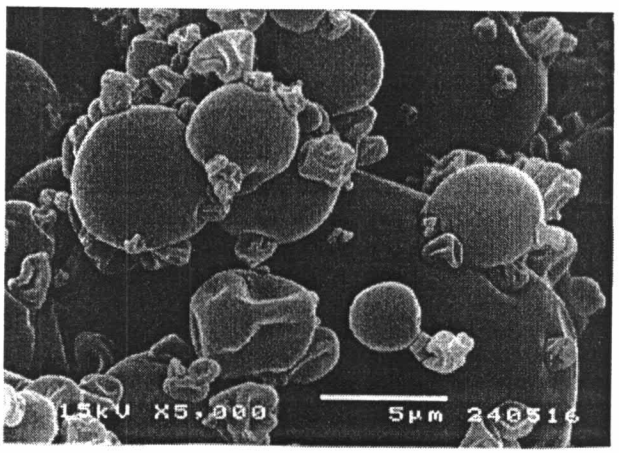
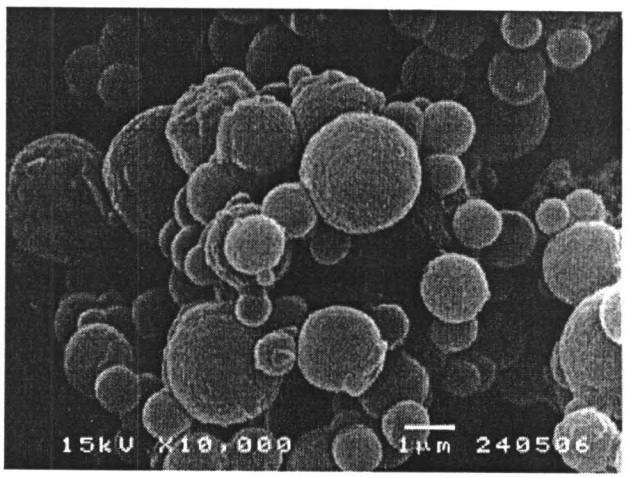


Figure 6 Scanning electron photomicrographs of microspheres prepared by adding maltodextrin and Aerosil® in formulations (P23, P26 and P29)

F33(HPMC)



F36(CHI)



F39(CP)

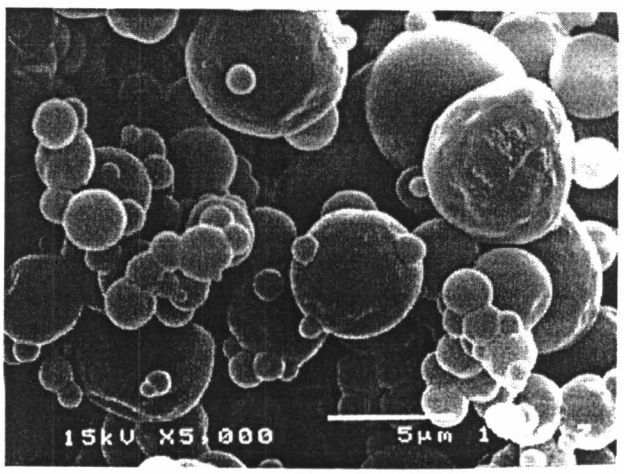


Figure 7 Scanning electron photomicrographs of microspheres prepared by adding maltodextrin, Aerosil® and propylene glycol in formulations (P33, P36 and P39)

2. Preliminary study of spray drying process

One objective of the present study was to prepare controlled release propranolol HCl microspheres by spray drying technique. Proper formulation and spray drying conditions were sought for producing mucoadhesive microspheres that met the requirement for nasal delivery. The suitable properties of microspheres for nasal delivery were maximum percentage yield, narrow size distribution, low moisture content, low angle of repose (high flowability), high loading efficiency, spherical shape and smooth surface. The suitable size of microspheres for nasal delivery is 10-50 μm because particles less than 1 μm will escape to the lungs whereas particles larger than 200 μm will not retain in the nose after nasal delivery (Yeo, 1992).

Effect of the inlet air temperature

In this study, three temperatures 110, 120 and 130 °C were used. The shape and surface topography of the spray dried microspheres prepared at different inlet air temperatures are shown in Figure 8. All investigated inlet air temperatures used were applicable to the spray drying process of propranolol HCl solution. Most products obtained were microspheres with smooth surface. Increasing the inlet air temperature produced the microspheres with lower moisture content (Table 14). The moisture content of spray dried microparticles were affected by significant extent of the drying temperature. An increase in drying temperature caused a reduction in the moisture content (Labrude, 1989; Broadhead, 1994).

A tendency for the particle size to increase with decreasing inlet drying temperature was observed (Table 15). This effect was probably due, at least in part, to the increased tendency to agglomerate of particles that uncompletely dried. The effect of temperature on particle size was reported to be dependent on the material being dried (Crosby and Marshall, 1958), and so this observation was probably formulation specific. The low percentage yield was obtained when using low inlet air temperature

which had not enough energy for evaporating solvent from droplets of spray drying solution, and the uncompletely dried products were adhered on the wall of chamber resulting in decreasing the percentage of yield.

Effect of pump feed rate

The photomicrographs of the spray dried powder produced at different feed rates are shown in Figure 9, most products obtained were microspheres with smooth surface. As the feed rate was increased from 4 to 5 and 6 ml/min, the size of the powder increased respectively. The microspheres produced at the feed rate of 6 ml/min were the largest, followed by those at 5 and 4 ml/min, respectively. Theoretically, product size increases with higher feed rate. This result was in agreement with other investigators (Master, 1979; Crosby and Marshall, 1958).

For this study, the microspheres produced at the high pump feed rate of 6 ml/min was clearly had wide size distribution (span=5.68) and large particle size as observed in Table 15. The percentage yield of mucoadhesive microspheres was decreased when preparing microspheres with a high pump feed rate at 6 ml/min (Table 14). These results were described that at low feed rates, the droplet sizes were of high homogeneity. At higher feed rates, the atomizing air pressure could not penetrate the thick liquid jets. Atomization was incomplete and a wide droplet size distribution in the spray resulted. At high feed rates, therefore, it was important that the liquid feed had to first form into thin sheets to assist liquid instability for effective air to liquid contact and breakdown of liquid into ligaments or individual droplets. Unless "feed prefilming" took place, ineffective atomization resulted, even at high air velocities. Ineffective atomization at higher pump feed rates led to the formation of large particles which were not completely dried when spraying in the drying chamber. This resulted in the deposition of these products on the wall of the cyclone separator, resulting in low percentage yield.

In addition, the incomplete drying of large particles affected to the increasing of the moisture content of microspheres as shown in Table 14. This result was explained that increasing pump feed rates would lead to a reduction in the outlet temperature and an increase in equilibrium solvent content or moisture level. However, pump feed rates which were too low had the disadvantages of low efficiency with longer operation time and a high outlet temperature which may affect the exhaust tubings (Wan et al., 1991).

Effect of atomizing air flow rate

The air flow rates evaluated were 400, 500 and 600 Nl/h in this study. The microscopic images of the spray dried particles prepared at various atomizing air pressure are shown in Figure 10. Most products obtained were microspheres with smooth surface. It was found that higher atomizing air pressure produced smaller microspheres. The microspheres produced at the air flow rate of 400 Nl/h were larger microspheres than those produced at 500 and 600 Nl/h. This was explained that the air velocity controlled the atomizing pressure required to breakdown the liquid feed into droplets, and atomizing pressure changes affected only the product size. This agreed with Masters (1979) that the energy available for atomization was increased by air pressure and would reduce the size of microspheres.

Table 14 shows the angle of repose of these microspheres prepared from various processing variables in the range of 44.20-45.40 degree, indicating that these angle of repose was not affected by the variation of spray dried processes. The span value indicated size distribution of microspheres (Table 15), and low span values were obtained from spray dried process at inlet air temperature of 130°C, pump feed rate of 5 ml/min and atomizing air flow rate of 400 Nl/h.

The percentage drug contents of the spray dried powder prepared from various processing variables are shown in Table 17. It was found that the percentage drug content was not affected by the processing variables used. The good uniformity of drug distribution in spray dried powder prepared from different conditions was

obtained. These findings were attributed to the fact that the feed rate of solution offered excellent homogeneity of the drug and the polymer in solution. The drug content in the microparticles usually agreed well with the theoretical value expected from the formulation (Kawashima et al., 1988). The theoretical drug content in the microspheres was 7.54.

It clearly shows that the inlet air temperature, the pump feed rate and the atomizing air flow rate did not affect to the loading efficiency of the spray dried microspheres (Table 17). The loading efficiency of microspheres were not different at different the inlet air temperatures of 110, 120 and 130°C, the pump feed rates of 4, 5 and 6 ml/min and the atomizing air flow rate of 400, 500 and 600 Nl/h.

Table 14 The bulk density, the angle of repose, the percentage yield and the percentage of moisture content of microspheres prepared with various spray dried conditions.

Process Variable/ Level	Bulk density (g/ml)	Angle of repose (degree)	Yield (%)	Moisture content (%)
Inlet air temperature (°C)				
110	0.03(0.05)	45.40(0.63)	13.81	6.95(0.82)
120	0.03(0.02)	44.30(0.74)	14.25	6.39(0.24)
130	0.03(0.07)	44.60(0.35)	18.46	5.19(0.23)
Pump feed rate (ml/min)				
4	0.03(0.04)	44.78(0.21)	17.69	5.15(0.34)
5	0.03(0.07)	44.60(0.35)	18.46	5.19(0.23)
6	0.03(0.03)	45.23(0.43)	12.73	6.03(0.37)
Atomizing air flow rate (normliter/h)				
400	0.03(0.07)	44.60(0.35)	18.46	5.19(0.23)
500	0.03(0.01)	44.50(0.65)	18.64	5.38(0.55)
600	0.03(0.06)	44.20(0.38)	18.54	4.96(0.29)

Table 15 The particle size and the span value of microspheres prepared with various spray dried conditions.

Process Variable / Level	D[4,3] (SD) (μm)	Span
Inlet air temperature (°C)		
110	38.43 (4.09)	6.02 (1.07)
120	30.39 (1.89)	5.84 (0.35)
130	22.82 (0.34)	2.47 (0.05)
Pump feed rate (ml/min)		
4	26.24(0.13)	3.28 (0.05)
5	22.82(0.34)	2.47 (0.05)
6	48.63(0.87)	5.68 (0.32)
Atomizing air flow rate (Nl/h)		
400	22.82(0.34)	2.47 (0.05)
500	49.20(4.78)	6.29 (4.58)
600	40.78(3.81)	6.24 (4.53)

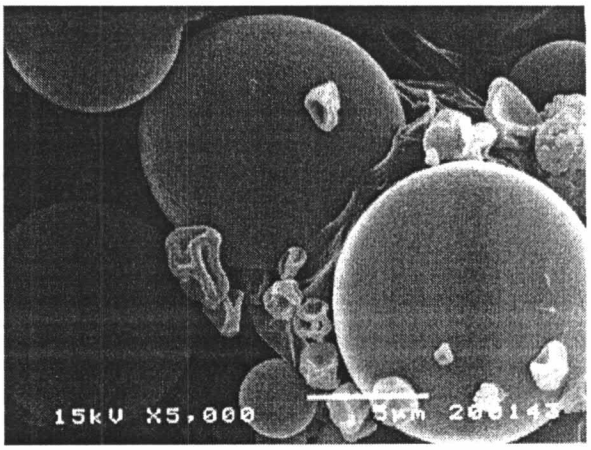
Table 16.The particle size distribution of microspheres prepared at variable processes.

Process Variable / Level	Percentage of particles				
	<1.0 (μm)	1-9 (μm)	10-50(μm)	51-200 (μm)	>200 (μm)
Inlet air temperature (°C)					
110	0.00	26.37	60.00	11.18	2.45
120	0.00	18.89	61.30	17.21	2.59
130	0.00	19.24	74.30	5.73	0.74
Pump feed rate (ml/min)					
4	8.59	18.30	64.27	7.21	1.65
5	0.00	19.24	74.30	5.73	0.74
6	0.00	20.39	57.77	14.35	7.49
Atomizing air flow rate (Nl/h)					
400	0.00	19.24	74.30	5.73	0.74
500	0.28	19.63	57.41	14.99	7.69
600	0.04	26.69	54.37	13.22	5.68

Table 17 The percentage of drug content and the percentage of loading efficiency of microspheres prepared with various spray drying conditions.

Process Variable/ Level	Drug content (%)	Loading efficiency (%)
Inlet air temperature (°C)		
110	7.24(0.15)	95.99
120	7.14(0.06)	94.67
130	7.26(0.19)	96.26
Pump feed rate (ml/min)		
4	7.35(0.13)	97.45
5	7.26(0.19)	96.26
6	7.38(0.19)	97.85
Atomizing air flow rate (Nl/h)		
400	7.26(0.19)	96.26
500	7.41(0.14)	98.25
600	7.32(0.12)	97.06

(A)



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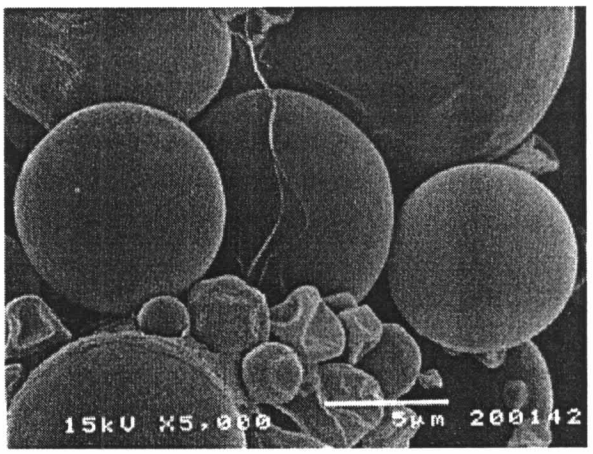

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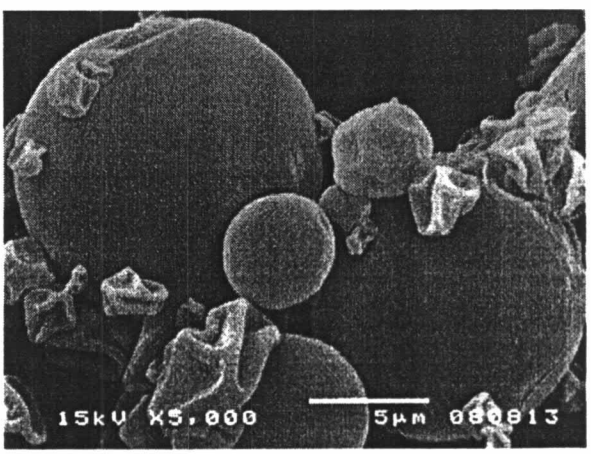
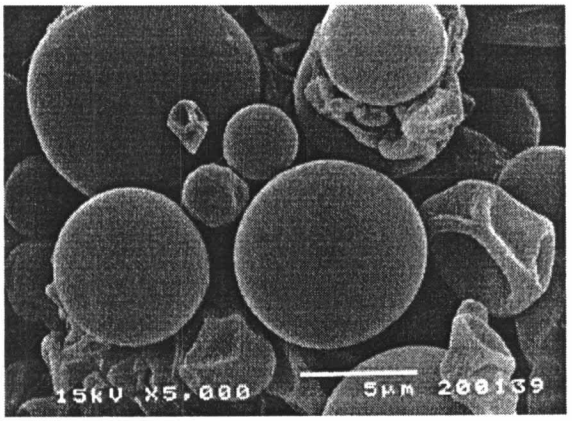


Figure 8 Scanning electron photomicrographs of microspheres prepared with various inlet air temperatures.

(A) 110 °C, (B) 120 °C and (C) 130 °C (x5000)

(A)



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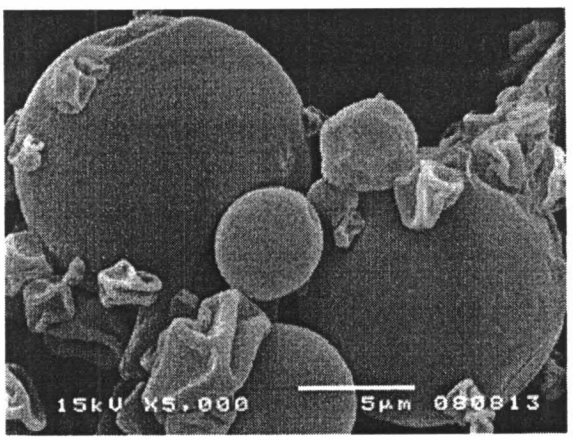

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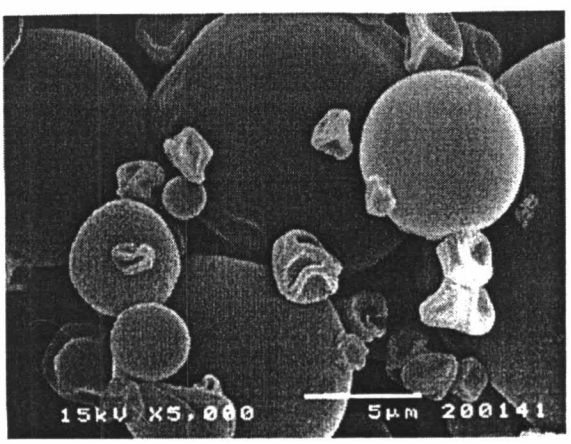
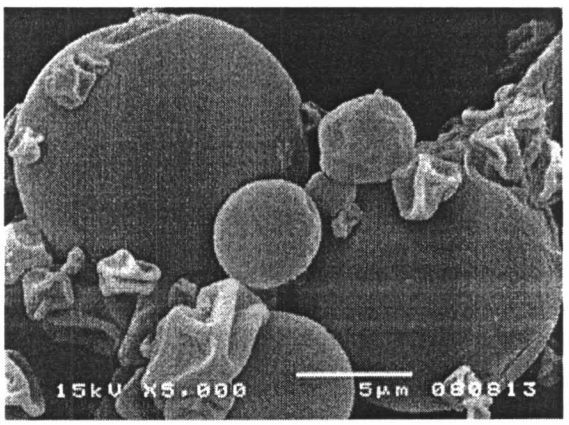


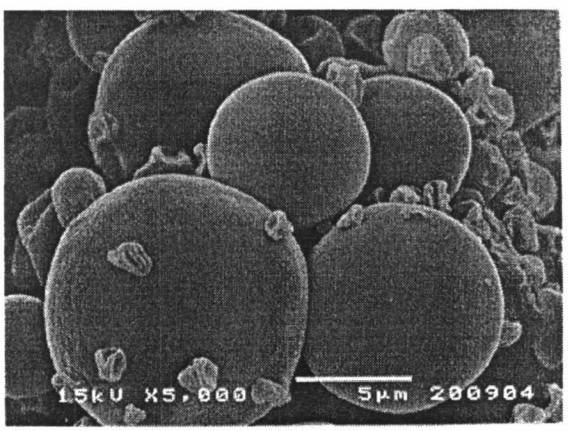
Figure 9 Scanning electron photomicrographs of microspheres prepared with various pump feed rates.

(A) 4 ml/min, (B) 5 ml/min and (C) 6 ml/min (x5000)

(A)



(B)



(C)

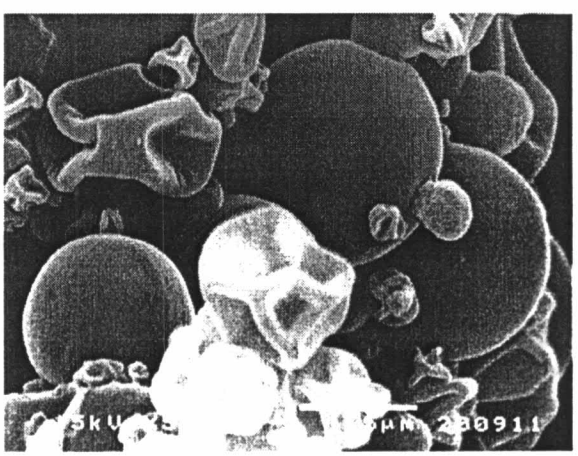


Figure 10 Scanning electron photomicrographs of microspheres prepared with various atomizing air flow rates.

(A) 400 Nl/h, (B) 500 Nl/h and (C) 600 Nl/h (x5000)

3. Microsphere Characterization

1. Morphology of spray dried microspheres

When spray drying is used in the preparation of microparticulate drug delivery systems, the structure of the system obtained is different according whether the drug is dispersed or dissolved in the polymeric solution to be sprayed. In this study, polymeric matrices (microspheres), in which the drug was embedded, was obtained by spraying a solution of formulation into the spray dryer (Conte, 1994 and Giovanni, 1994).

The observation of size, shape and surface topography was done by scanning electron microscopy. The results were separated into groups by the amount and type of the polymer and the ratio of polymer to propranolol HCl.

Figure 11-12 shows the scanning electron photomicrographs of pure propranolol HCl powder and different types of polymers at different magnifications. Propranolol HCl powder was irregular crystal shapes in various sizes. HPMC and chitosan were irregular flakes of different sizes with smooth surface. Carbopol was irregular flakes of small size and aggregate together with rough surface.

The photomicrographs of spray dried microspheres obtained from HPMC at different drug to polymer ratios (1:1, 1:3, 1:5 and 1:7, respectively) are shown in Figure 13. The amount of HPMC in the formulation had an important effect to the shape, size and surface topography of the microspheres. The average diameter of microspheres gradually increased with the weight ratio of polymer in the formulation because of increasing the viscosity of the spray drying solution affecting to induce bigger droplets at the tip of the nozzle, thus resulting in bigger particles (Lacasse, 1998). The viscosity of spray drying solutions are shown in Table 18. The viscosity was increased when increasing the amount of polymer in formulations. Microspheres obtained by HPMC with small size showed crumpled surface and collapsed shape. This was explained that the solvent within the spray drying droplets was rapidly evaporated at high temperature during the early stage of processing. The smaller size,

the higher surface area of microspheres more supported the evaporation of solvent from droplets than larger size with lower surface area, then small size of microspheres had more crumpled surface and collapsed shape than the huge size (Lin and Kao, 1991). At drug to polymer ratio of 1:1, microspheres showed much collapsed shape because at low amount of polymer in formulation, decreasing the viscosity of spray drying solution, resulting in smaller particles affected to induce the crumpled surface and collapsed shape.

Table 18 The viscosity of spray drying solution at different types of polymers and different drug to polymer ratios.

Formulation code	Viscosity (cps)
F1 (HPMC,1:1)	62.70
F2 (HPMC,1:3)	150.10
F3 (HPMC,1:5)	255.00
F4 (HPMC,1:7)	337.57
F5 (CHI,1:1)	25.6
F6 (CHI,1:3)	72.27
F7 (CHI,1:5)	101.27
F8 (CHI,1:7)	114.17
F9 (CP,1:1)	7.46
F10 (CP,1:3)	10.63
F11 (CP,1:5)	13.93
F12 (CP,1:7)	17.13
F13 (CP:HPMC,3:4)	95.78
F14 (CP:HPMC,4:3)	68.35
F15 (CP:HPMC,5:2)	23.62

The photomicrographs of the spray dried microspheres prepared from chitosan at different drug to polymer ratios (1:1, 1:3, 1:5, and 1:7) are shown in Figure 14, respectively. The effect of the drug to polymer ratios and the polymer concentrations were the same as the results obtained by HPMC. The average size of microspheres

gradually increased with increasing the amount of polymer in microsphere formulations. This was attributed by the aforementioned reason of HPMC microspheres. The obtained microspheres with chitosan were spherical shape and smooth surface. The smaller size agglomerated with the larger size of microspheres. The small size of chitosan microspheres was not collapsed. This might be due to the optimum velocity in drying of solvent within droplet by using suitable drying temperature (120°C) in preparing microspheres

The photomicrographs of microspheres prepared with carbopol at various drug to polymer ratios (1:1, 1:3, 1:5 and 1:7) are shown in Figure 15. Most products obtained were microspheres with smooth surface and spherical shape. The size of these microspheres gradually increased when increasing the amount of carbopol in formulations. This was explained by the same reason of HPMC and chitosan microspheres. The aggregation of microspheres was clearly observed in formulation containing high amount of carbopol (F12, 1:7). This result could be explained that if the viscosity of spray drying solution is too high, an extrusion process will occur, resulting in aggregation, because the droplets would not separate from each other during the drying process (Lacasse, 1998). The small size of carbopol microspheres was not collapsed because of the optimum velocity of drying as described above in chitosan microspheres case.

Figure 16 shows the scanning electron photomicrographs of microspheres prepared with combined polymer at various ratios of carbopol to HPMC (3:4, 4:3 and 5:2). The microspheres prepared with drug to combined polymer ratio of 1:(3:4) showed crumpled surface and collapsed shape including some spherical microparticles. These crumpled and collapsed particles would be the effect of HPMC in formulation. When increasing the amount of carbopol in formulation, the microparticles were more spherical shape but remained rough surface.

In this investigation, these obtained microspheres prepared with all types of polymers did not show the hole or rupture on the surface indicating the suitable characteristics of these microspheres for nasal delivery. The presence of hole or

rupture on the surface of microspheres caused rapid clearance and a varied deposition pattern in nasal cavity (Ting, 1992).

Microspheres containing chitosan previously prepared using solvent evaporation technique (El-Hameed, 1997) and a spray-desolvation method (Ting, 1992) had the same morphological characteristics as those currently prepared in this study by the spray drying technique. The microspheres containing HPMC and containing carbopol prepared by solvent evaporation technique showed irregular shape and crumpled surface (El-Hameed, 1997).

2. Particle size and size distribution

The average particle size (D[4,3]) and particle size distribution (Span value) of microspheres are shown in Table 19. The particle size of microspheres prepared with HPMC (F1-F4), carbopol (F9-F12) and combined polymer (carbopol/HPMC, F13-F15) were 19.60-26.00 μm, 12.63-15.92 μm and 15.40-16.66 μm, respectively. The size of these microspheres were considered to be suitable for nasal administration (within the range of 10-50 μm). For microspheres containing chitosan, the size ranged from 7.27-15.60 μm. At low amount of chitosan in formulation (F5), the average size was lower than 10 μm.

The particle size distribution of microspheres was rather varied among each type of polymer. The span values of these microspheres are shown in Table 19. The lowest span value was obtained from microspheres prepared with carbopol (F9-F12) and with combined polymer (F13-F15) that ranged from 1.13-1.36 and 1.3-1.68, respectively, indicating narrow size distribution.

Three important ranges of size distribution of microspheres for nasal delivery were <10 μm, 10-50 μm and > 200 μm, because the size lower than 1.0 μm will escape to the lungs, whereas the larger particles (more than 200 μm) are not retained in the nasal cavity, and the particles within the moderate size range of 10-50 μm is the most suitable to deposit in nasal delivery. Table 20 shows the percentage of size distribution in three important ranges of microspheres.

Table 19 The particle size and the span value of microspheres prepared with various types of polymer and different drug to polymer ratios.

Formulation code	D [4, 3] (SD) (μm)	Span (SD)
F1 (HPMC,1:1)	26.00 (1.58)	3.57 (0.08)
F2 (HPMC,1:3)	21.96 (0.41)	2.87 (0.03)
F3 (HPMC,1:5)	22.82 (0.34)	2.47 (0.05)
F4 (HPMC,1:7)	19.60 (0.88)	2.19 (0.01)
F5 (CHI,1:1)	7.27 (0.11)	2.36 (0.04)
F6 (CHI,1:3)	11.92 (0.06)	2.43 (0.03)
F7 (CHI,1:5)	23.03 (0.30)	2.52 (0.06)
F8 (CHI,1:7)	15.60 (0.11)	2.28 (0.01)
F9 (CP,1:1)	12.63 (0.17)	1.23 (0.06)
F10 (CP,1:3)	21.14 (2.21)	1.24 (0.03)
F11 (CP,1:5)	19.11 (0.13)	1.13 (0.00)
F12 (CP,1:7)	15.92 (0.05)	1.36 (0.00)
F13 (CP:HPMC,3:4)	16.66 (0.39)	1.68 (0.05)
F14 (CP:HPMC,4:3)	18.34 (1.12)	1.30 (0.08)
F15 (CP:HPMC,5:2)	15.40 (0.03)	1.58 (0.01)

Table 20 The particle size distribution of microspheres prepared from various types of polymers.

Formulation code	Percentage of particles				
	<1.0 (μm)	1-9 (μm)	10-50(μm)	51-200 (μm)	>200 (μm)
F1 (HPMC,1:1)	0.14	26.69	61.73	5.97	2.46
F2 (HPMC,1:3)	0.00	23.31	69.64	6.86	0.19
F3 (HPMC,1:5)	0.00	19.24	74.30	5.73	0.74
F4 (HPMC,1:7)	0.00	18.73	76.97	4.30	0.00
F5 (CHI,1:1)	7.86	57.90	34.04	0.20	0.00
F6 (CHI,1:3)	9.31	30.59	60.10	0.00	0.00
F7 (CHI,1:5)	8.37	16.62	69.85	4.37	0.79
F8 (CHI,1:7)	9.95	19.08	70.37	0.60	0.00
F9 (CP,1:1)	0.00	20.36	79.64	0.00	0.00
F10 (CP,1:3)	0.00	6.76	90.93	1.36	0.95
F11 (CP,1:5)	0.00	3.68	96.32	0.00	0.00
F12 (CP,1:7)	0.00	14.75	84.25	0.87	0.10
F13 (CP:HPMC,3:4)	0.24	17.42	82.05	0.44	0.00
F14 (CP:HPMC,4:3)	0.01	7.32	92.28	0.58	0.00
F15 (CP:HPMC,5:2)	0.01	18.18	81.72	0.08	0.00

The high percentages of particles in the size ranged from 10-50 μm were obtained from microspheres containing HPMC, (F1-F4) carbopol (F5-F8) and combined polymer (F13-F15). Meanwhile the high percentages of small size particles (less than 1.0 μm) were observed from microspheres prepared with chitosan (F5-F8). This was attributed to the small size (less than 1.0 μm) of chitosan microspheres that were not agglomerated with the larger size of microspheres. Although, the particle with the size less than 1.0 μm was observed by scanning electron photomicrographs in the case of microspheres prepared with HPMC, carbopol and combined polymer, the percentages of size less than 1.0 μm of these microspheres were very low (0.01-0.23%) or disappeared (0%) as shown in Table 20. This might be explained that these small sizes (<1.0 μm) of microspheres were agglomerated to each other or the larger size particles.

3. Drug content and loading efficiency

The percent drug content in spray dried microspheres of various formulations are shown in Table 21. The standard deviation indicated the uniformity of drug in the spray dried microspheres. The lower standard deviation of drug content values implied good uniformity of the drug distribution in spray dried microspheres prepared from different types of polymers.

The percentage of loading efficiency of microspheres are presented in Table 21. The percentage of loading efficiency was not remarkably affected by the amount and the proportion of polymers in the formulations. Microspheres were produced with a relatively high percentage of loading efficiency. Each formulation had good percentage of loading efficiency of above 80%. This was due to the fact that homogeneity of spray drying solution prepared by dissolving the drug, polymer and other excipients in solvent before spraying. This agreed with Hascicek (2003) that spray drying technique is generally characterized by high drug loading efficiency. Lim (2000) reported a low loading efficiency of chitosan microspheres ranged from 13.32-36.40% prepared with the solvent evaporation technique which is significantly lower than this finding. This might relate to different techniques in microsphere preparation.

Table 21 The percentage of drug content and the percentage of loading efficiency of microspheres prepared with various types of polymers at different drug to polymer ratios.

Formulation code	Drug content (%)	Loading efficiency (%)
F1 (HPMC,1:1)	22.31	95.71
F2 (HPMC,1:3)	10.71	95.43
F3 (HPMC,1:5)	7.26	96.26
F4 (HPMC,1:7)	5.18	98.00
F5 (CHI,1:1)	19.63	84.21
F6 (CHI,1:3)	9.22	82.16
F7 (CHI,1:5)	6.23	82.60
F8 (CHI,1:7)	4.50	85.14
F9 (CP,1:1)	22.43	96.22
F10 (CP,1:3)	10.67	95.07
F11 (CP,1:5)	7.23	95.86
F12 (CP,1:7)	5.12	96.87
F13 (CP:HPMC,3:4)	4.96	93.84
F14 (CP:HPMC,4:3)	5.07	95.92
F15 (CP:HPMC,5:2)	5.14	97.25

4. The yield of production

The product was collected from the collector and cyclone and the amount of the products from two sources was calculated as the percent yield. The yields of production are shown in Table 22.

The results displayed that the yield percentage from the collector was higher than that from the cyclone in all formulations. No difference in yield percentage was detected in the formulations produced by the same type of polymer. Microspheres containing HPMC were produced with low yield percentage (17.87-18.46%). This might be explained by the technical characteristics of the spray dryer. When the high

viscosity (as shown in Table 18) of spray drying solution prepared by HPMC was sprayed, all of the spray dried microspheres adhered to the cyclone walls and lost during spray drying (Conte, 1994). The yield percentages of formulation containing chitosan were in the range of 50.91-52.63%. The highest yield percentage was obtained from formulation containing carbopol (57.35-61.84%) that low viscosity of spray drying solution. In the same explanation, the increasing amount of HPMC in the microspheres formulations prepared with combined polymer (carbopol/HPMC) caused the decrease of obtained yield percentage.

5. Angle of repose and bulk density

The flow properties were represented in terms of angle of repose of microspheres. The values of angle of repose from spray dried microspheres produced by the various drug to polymer ratios of each type of polymer are shown in Table 22. The angles of repose of microspheres were in the range of 42.51-45.30 degree (exhibiting normal flow behavior) except microspheres prepared with HPMC that gave a higher ranges of 54.90-55.40 degree. This finding might be explained by the large size with hollow structure of HPMC microspheres resulting in low bulk density and leading to poor flow property.

Furthermore, the bulk density of microspheres prepared from different types and various drugs to polymer ratios are shown in Table 22. The spray dried microspheres from formulation containing HPMC as mucoadhesive polymer exhibited lower bulk density than those of the other formulations. This finding might be due to the large size and hollow structure of HPMC microspheres. For combined polymer, when increasing the amount of HPMC in formulations, the bulk density was decreased. It might be due to the effect of HPMC that provided the light microparticles and leading to low bulk density.

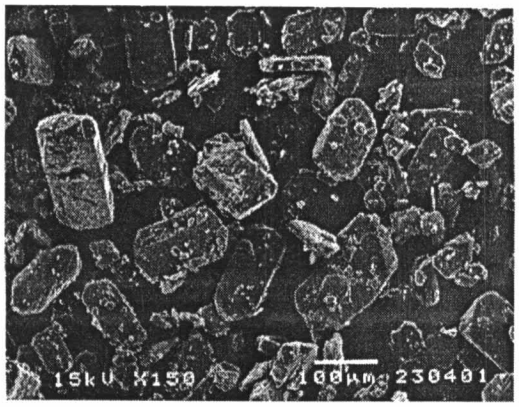
6. Moisture Content

The moisture contents of the spray dried microspheres are presented in Table 22. The polymer to drug ratios and various types of polymers had an insignificant

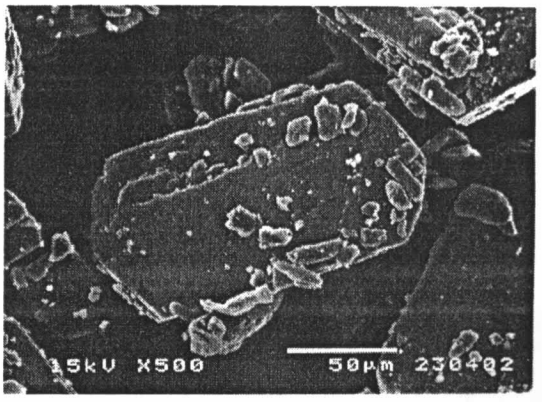
effect on the moisture content of the microspheres. These microspheres showed rather high moisture content ranging from 5.05-5.91%. This might be the effect of maltodextrin in formulation. The large size of maltodextrin molecules could hinder the diffusion of water molecule from inner site of droplets to the outer site (Adhikari, 2004) which resulting in high moisture content.

Table 22 The bulk density, the angle of repose, the percentage yield and the percentage of moisture content of microspheres prepared with various types of polymers at different drug to polymer ratios.

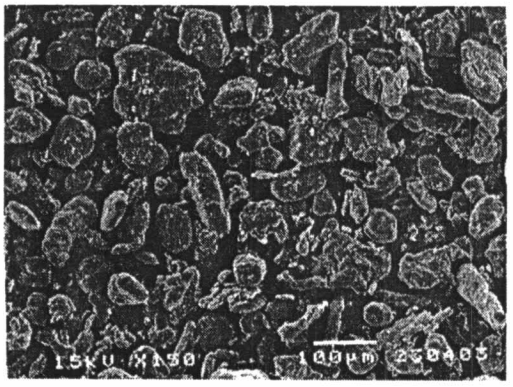
Formulation code	Bulk density (g/ml)	Angle of repose (degree)	Yield (%)	Moisture Content (%)
F1 (HPMC,1:1)	0.03	55.00	18.38	5.38
F2 (HPMC,1:3)	0.03	55.40	17.93	5.91
F3 (HPMC,1:5)	0.03	55.40	18.46	5.19
F4 (HPMC,1:7)	0.02	54.90	17.87	5.89
F5 (CHI,1:1)	0.13	43.40	50.91	5.23
F6 (CHI,1:3)	0.14	44.20	52.63	5.45
F7 (CHI,1:5)	0.15	43.90	52.06	5.03
F8 (CHI,1:7)	0.11	42.51	51.95	5.78
F9 (CP,1:1)	0.18	45.10	57.45	5.29
F10 (CP,1:3)	0.20	44.80	57.84	5.52
F11 (CP,1:5)	0.23	44.60	57.35	5.87
F12 (CP,1:7)	0.21	45.30	61.84	5.61
F13 (CP:HPMC,3:4)	0.10	43.93	35.81	5.35
F14 (CP:HPMC,4:3)	0.12	44.14	46.82	5.05
F15 (CP:HPMC,5:2)	0.15	44.93	56.55	5.80



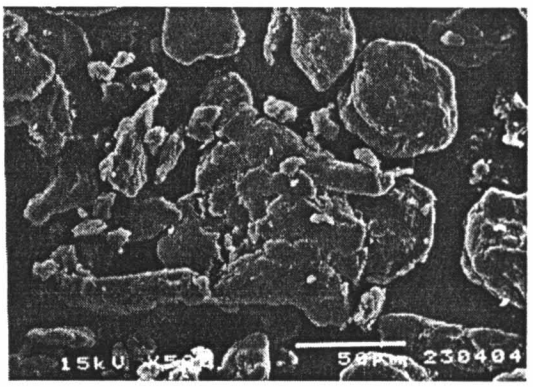
Propranolol hydrochloride (x150)



Propranolol hydrochloride (x500)

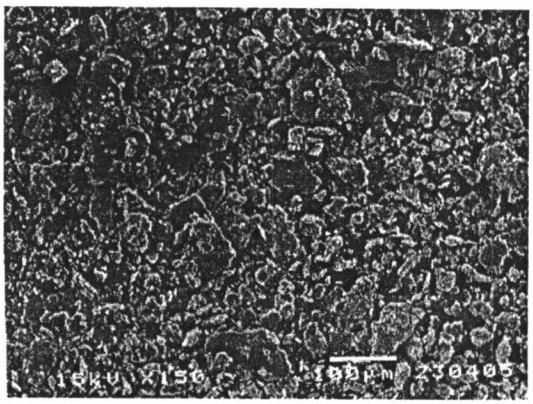


HPMC (x150)

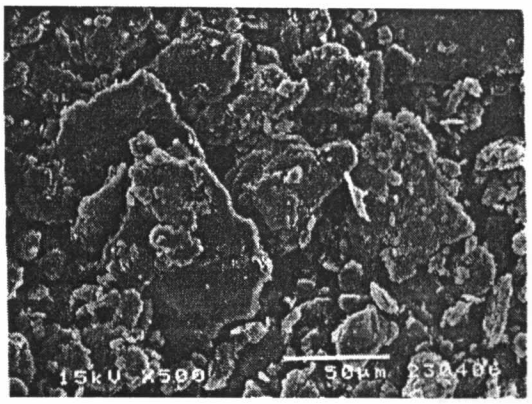


HPMC (x 500)

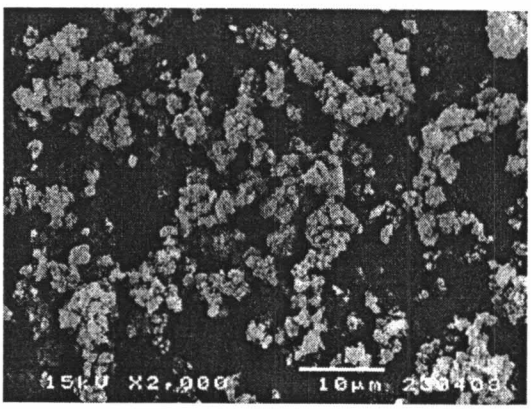
Figure 11 Scanning electron photomicrographs of propranolol hydrochloride and hydroxypropyl methylcellulose (HPMC).



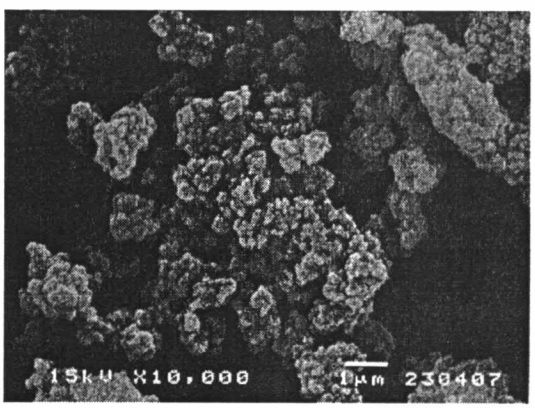
Chitosan (x150)



Chitosan (x500)

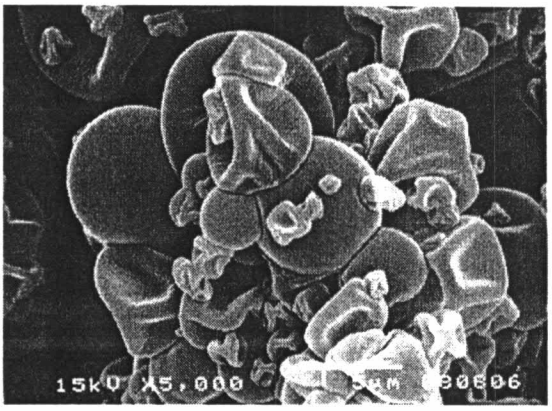


Carbopol (x150)

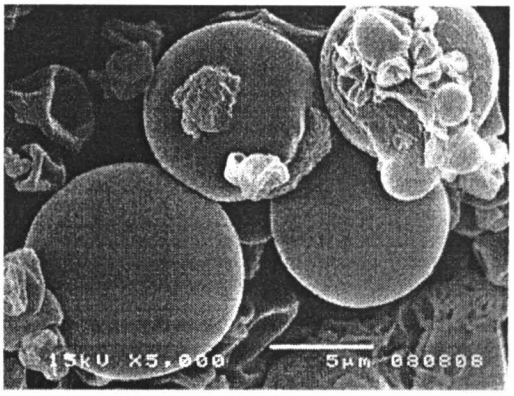


Carbopol (x500)

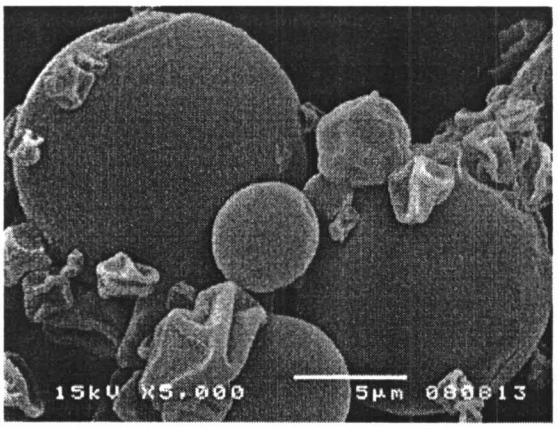
Figure 12 Scanning electron photomicrographs of chitosan and carbopol.



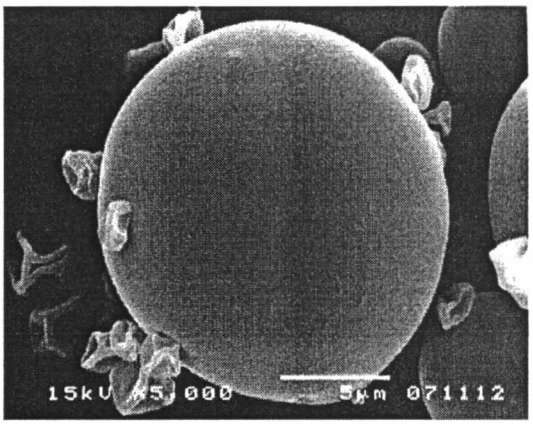
F1



F2

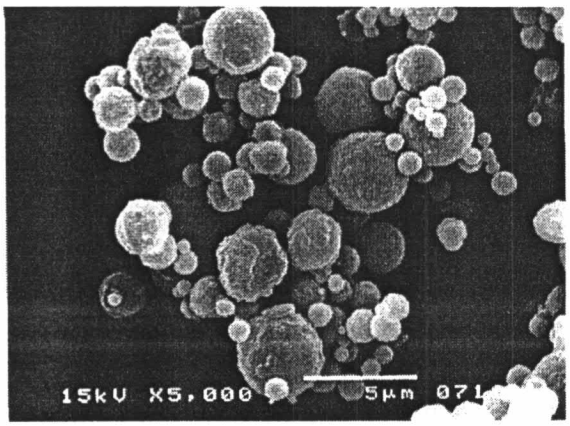


F3

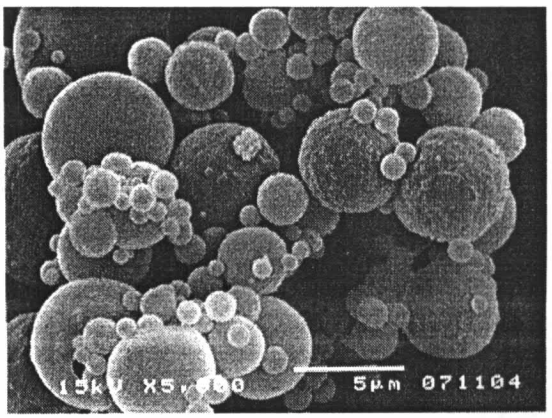


F4

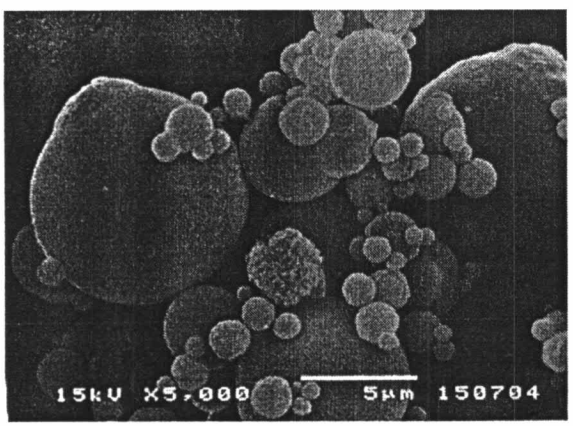
Figure 13 Scanning electron photomicrographs of microspheres prepared with HPMC at various drug to polymer ratios (F1-F4).



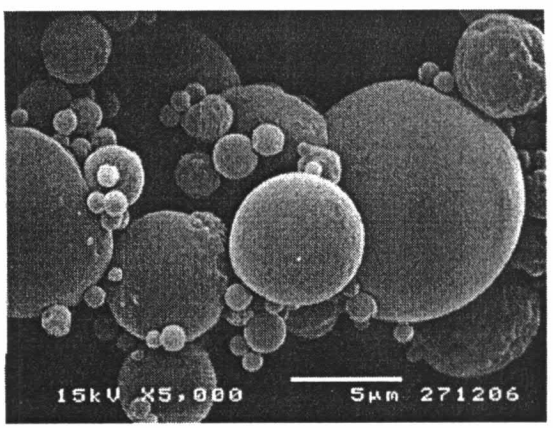
F5



F6

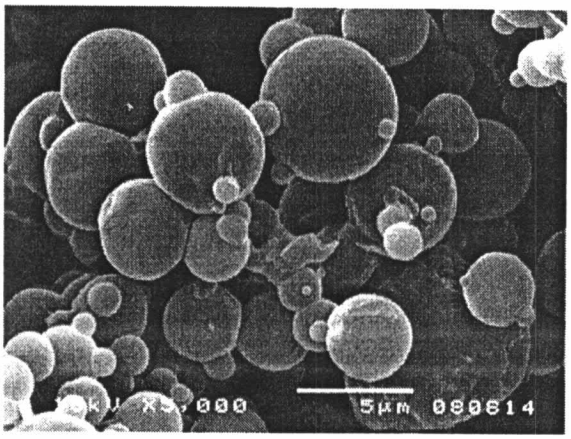


F7

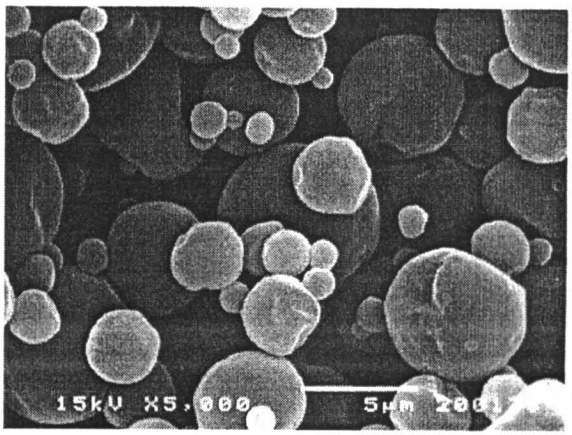


F8

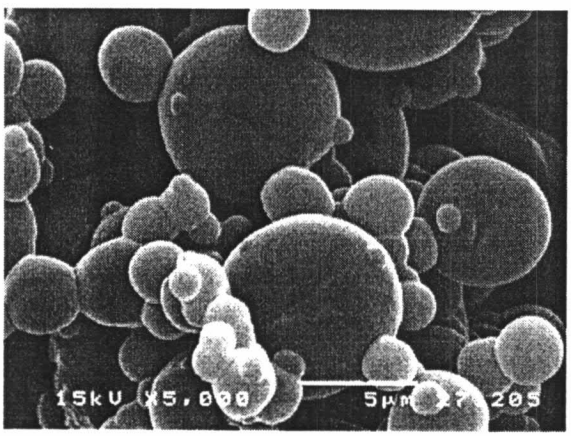
Figure 14 Scanning electron photomicrographs of microspheres prepared with chitosan at various drug to polymer ratios (F5-F8).



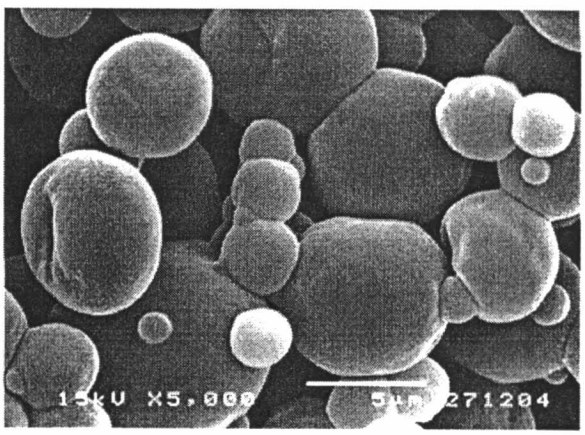
F9



F10



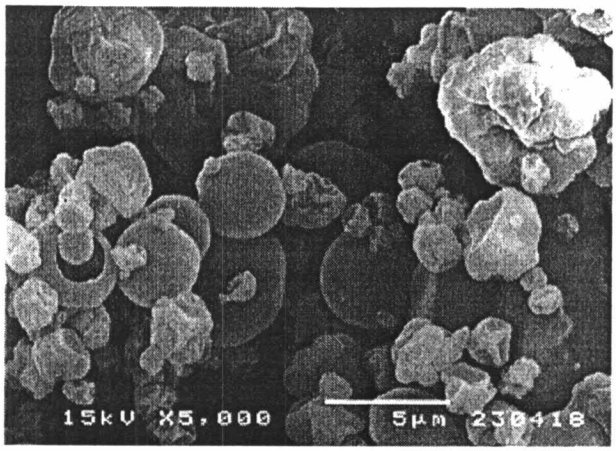
F11



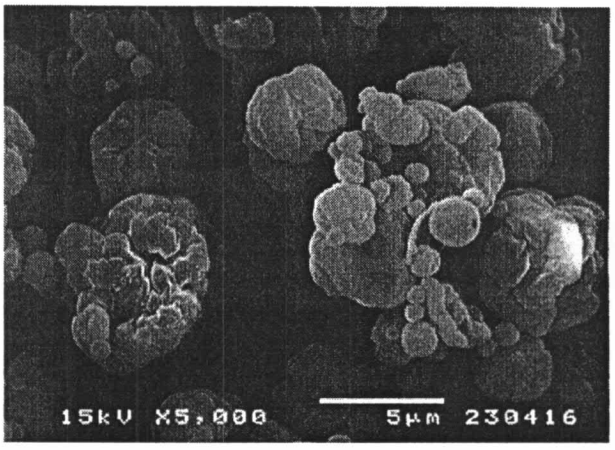
F12

Figure 15 Scanning electron photomicrographs of microspheres prepared with carbopol at various drug to polymer ratios (F9-F12).

F13



F14



F15

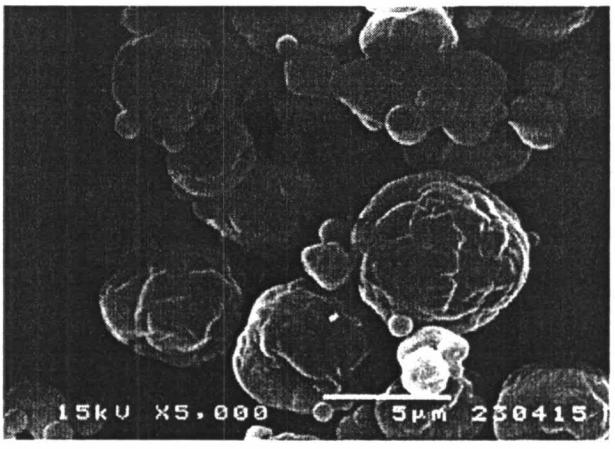


Figure 16 Scanning electron photomicrographs of microspheres prepared with combined polymer (carbopol/HPMC) at various drug to polymer ratios (F13-F15)

Identification of the spray dried microspheres

Differential scanning calorimetry study

The major use of thermal analysis in evaluation of spray dried products is to identify of the polymorphism or crystallinity of a drug in the product since spray drying may result in a polymorphic change (Ford and Timmins, 1989). Figure 17 demonstrates the DSC thermograms of pure propranolol HCl and the controlled microspheres (F0). The sharp peak of pure propranolol HCl in the thermogram observed at about 165.7 °C was identified as the drug melting point. The thermogram of controlled microspheres also showed a remaining sharp melting peak but shifted of 158.5°C indicating that spray drying resulted in physicochemical change of drug. The observed melting point of controlled microspheres was obviously lower than the one obtained from pure propranolol HCl thermogram. The lowering in melting point of propranolol HCl in the controlled microspheres indicated the presence of a metastable form or polymorph of propranolol HCl in the microspheres.

The DSC thermograms of pure propranolol HCl and propranolol HCl in mucoadhesive microspheres at drug to polymer ratio of 1:1 (F1,F5 and F9) are shown in Figure 18. Microspheres containing chitosan (F5) showed an endothermic peak at about 150.4°C which was identified as the melting point of propranolol HCl, while the sharp peak at this temperature was not seen in the thermograms of microspheres produced from other types of polymers (F1 and F9). This result may be explained that the drug in the latter polymers was uniformly dispersed in molecular or amorphous state and were inapparent in the DSC thermogram. Similar result from spray drying solid dispersion of PEG 4000 was also reported by Yokou et. al.,1984.

X – ray diffraction study

The X–ray diffraction patterns of pure propranolol HCl powder, mucoadhesive polymer and mucoadhesive microspheres prepared with different polymers are illustrated in Figure 19 and 20. The prominent peaks of X – ray diffractogram of propranolol HCl was particularly observed at 12.38, 16.60°, 19.44°, 21.08°and

24.96°. Figure 19 demonstrates X–ray diffractograms of HPMC, chitosan and carbopol. These diffractograms did not exhibit any prominent peak indicating the amorphous state of these polymers.

Comparison of the diffractogram of controlled microspheres (F0) with that of pure propranolol HCl noted the change in the pattern of the diffractogram (Figure 19). The base line was raised and some peaks of propranolol HCl were absent. However, some well defined peaks were corresponding to the peaks of propranolol HCl indicating of the polymorphic form of propranolol HCl. Moreover, some drugs might be presented as molecular or colloidal dispersion in the controlled microspheres.

Table 23 Characteristic peaks of the X – Ray diffraction patterns of spray dried microspheres prepared with different types and proportions of polymers.

Formulation Code	Characteristic Peak (degree) 2θ					
Propranolol HCl	12.38	16.60	19.44	21.08	24.96	26.92
F0	12.34	16.56	19.20	21.08	24.94	26.90
F5(CHI,1:1)	12.34	16.58	19.34	21.08	24.94	-
F8(CHI,1:7)	-	-	-	-	-	-

In case of the spray dried microspheres containing chitosan , the X – ray diffraction pattern of microspheres in drug to polymer ratio of 1:1 (F5) compared with pure propranolol HCl showed similar peaks (Table 23). In addition, very low intensities of the sharp diffraction peaks and the raising of base line were observed (Figure 20). In general the diffractogram of the drug presented as amorphous or molecular dispersion would exhibit absence in diffractogram peaks while the polymorphic drug would show the well defined peaks indicating the presence of crystal lattice (Biswas et. al.,1993; Imai et. al.,1989; Ravis,1981). Therefore, X–ray diffractogram of the microspheres containing chitosan would consist of both molecular dispersion and polymorphic form of the drug. Increasing the amount of chitosan in microspheres formulation (F8) resulted in a change in the diffractogram to complete halo pattern as shown in Figure 20. This result might be suggested that high amount of polymer in formulation showed prominent diffractogram patterns that

could conceal the peak of drug in microspheres. However, the nature of the amorphous drug obtained by spray drying had not been well investigated (Matsuda, 1992). The diffractograms of the mucoadhesive microspheres prepared with single polymer as HPMC and carbopol (Figure 20) and prepared with combined polymer (carbopol/HPMC, Figure 21) displayed halo pattern with no diffraction peaks, indicating that the drug in these microspheres was amorphous state.

Infrared absorption study

Infrared spectrometry was used to determine the existence of possible interaction within the microsphere formulations. The FT-IR spectra obtained from various spray dried microspheres are shown in Figure 22 to 27.

The interaction between drug and polymers were scarcely and the prominent peaks of spectra did not shift as shown in Table 24. The characteristic bands of propranolol HCl still appeared at 771, 795, 1107, 1241, 1268 and 1580 cm^{-1} strongly suggesting the existence of drug in the microspheres. They also revealed that no interaction between drug and additive occurred.

In this study, the electrostatic interaction between propranolol and carbopol was not found as shown in Figure 23(A). A new absorption band at about 1555 cm^{-1} that indicated the complexation between drug and carbopol was not appeared in this spectra. This new band was considered to be the result of salt formation at carboxylate form (coo^-) of polyacrylic acid molecules. This result could be explained by a lower pH value of spray dried solution (~3.5) than the pKa of polyacrylic acid of carbopol (pKa = 4.25), then the ionization of carbonyl group (c=o) in carbopol molecules was not occurred and non ionized form of this polymer was remained in the system.In previous study by Taylan (1996), the complexation between propranolol and polyacrylic acid was appeared in the FT-IR spectra in comparison with the physical mixture (no interaction) between carbopol and propranolol as shown in Figure 23(A). In this previous study, the experiment was performed at pH 7.4 of solution, then the ionization of carbonyl group was occurred and could form complex with propranolol HCl.

Table 24 The principal peaks of IR spectra of propranolol HCl in spray dried microspheres prepared with different types and proportions of polymers.

Formulation code	Principal peaks (cm^{-1})					
Propranolol HCl	771	798	1107	1241	1268	1580
F0	771	797	1107	1242	1268	1580
F1 (HPMC,1:1)	772	797	1107	1242	1269	1580
F4 (HPMC,1:7)	774	795	1107	1242	1271	1581
F5 (CHI,1:1)	771	797	1107	1242	1268	1579
F8 (CHI,1:7)	772	794	1107	1242	1269	1580
F9 (CP,1:1)	771	797	1107	1242	1268	1580
F12 (CP1:7)	775	796	1108	1244	1269	1581
F13 (CP:HPMC,3:4)	775	796	1105	1242	1270	1581
F14 (CP:HPMC,4:3)	775	796	1105	1242	1270	1581
F15 (CP:HPMC,5:2)	776	797	1105	1242	1270	1581

The hydrogen bonding between the functional group of carbopol and HPMC in microspheres prepared from combined polymer (carbopol and HPMC) also did not occur because a single peak at wave length about 1717 cm^{-1} recorded from pure carbopol corresponding to streching vibration of carbopol (c=o) bands was still remained in the IR-spectra of these microspheres as displayed in Figure 23(B). The evidence of interpolymer complexation between carbopol and HPMC was observed by other investigator (Taylan, 1996). Carbopol peak was shifted from wave length at 1717 cm^{-1} to lower wave length at about 1700 cm^{-1} and 1676 cm^{-1}. This result might be due to the difference of performed conditions. In this study, spray drying solution was prepared at room temperature and immediately sprayed into the spray dryer, but in previous study the mixing solution of HPMC and carbopol was incubated at 37°C for 10 days and water was removed by lyophilizator.

The evidence from FT-IR spectra of microspheres containing chitosan as shown in Figure 23(C). exhibited the peaks at 1514 and 1615 cm^{-1} which indicated of

$-NH_3^+$ band but the band at 1588 cm^{-1} indicating of $-NH_2$ band was disappeared. The peak at 1556 cm^{-1} indicating of acids which were presented as the carboxylate form of coo^- could be observed. Additionally, the absorption peak of pure acetic acid which should be found at wavelength number of higher than 1700 cm^- did not appear in the spectra of these microspheres.This indicated that chitosan acetate was formed in the microspheres during the preparation of spray drying solution by dissolving chitosan powder in acetic acid solution.

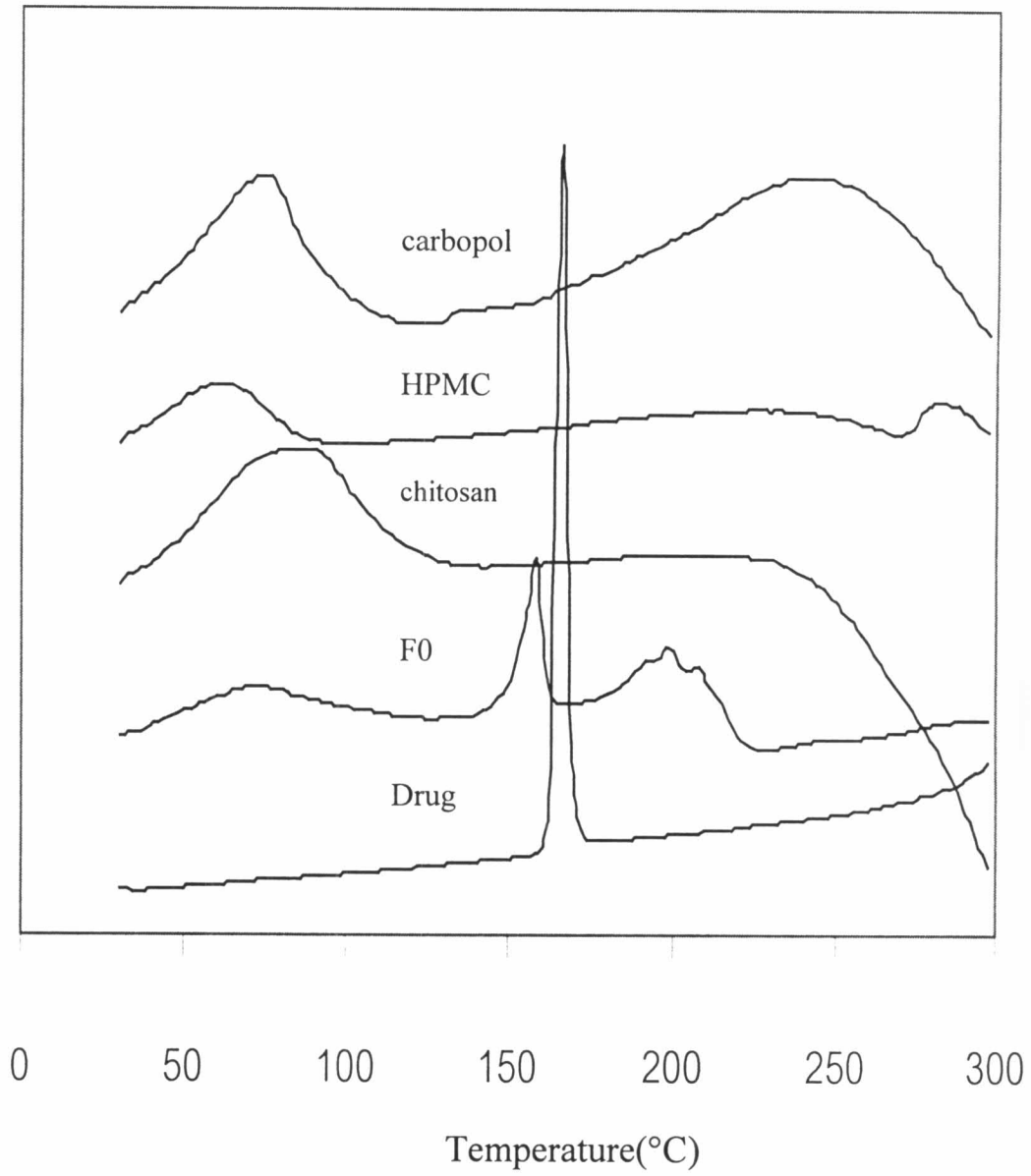


Figure 17 The DSC thermograms of propranolol HCl, controlled microspheres (F0) and of various types of polymers.

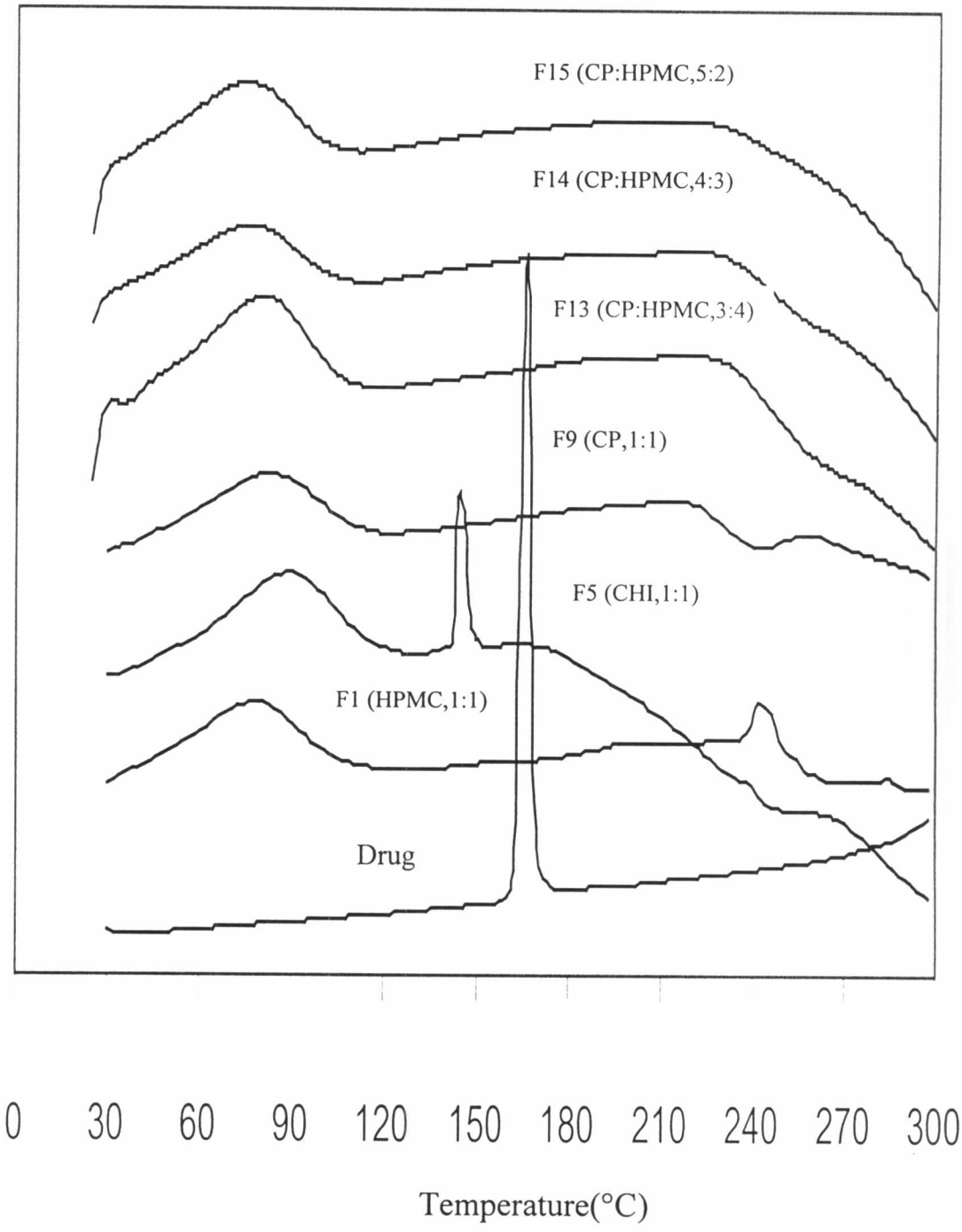


Figure 18 The DSC thermograms of propranolol HCl and of different formulations of mucoadhesive microspheres.

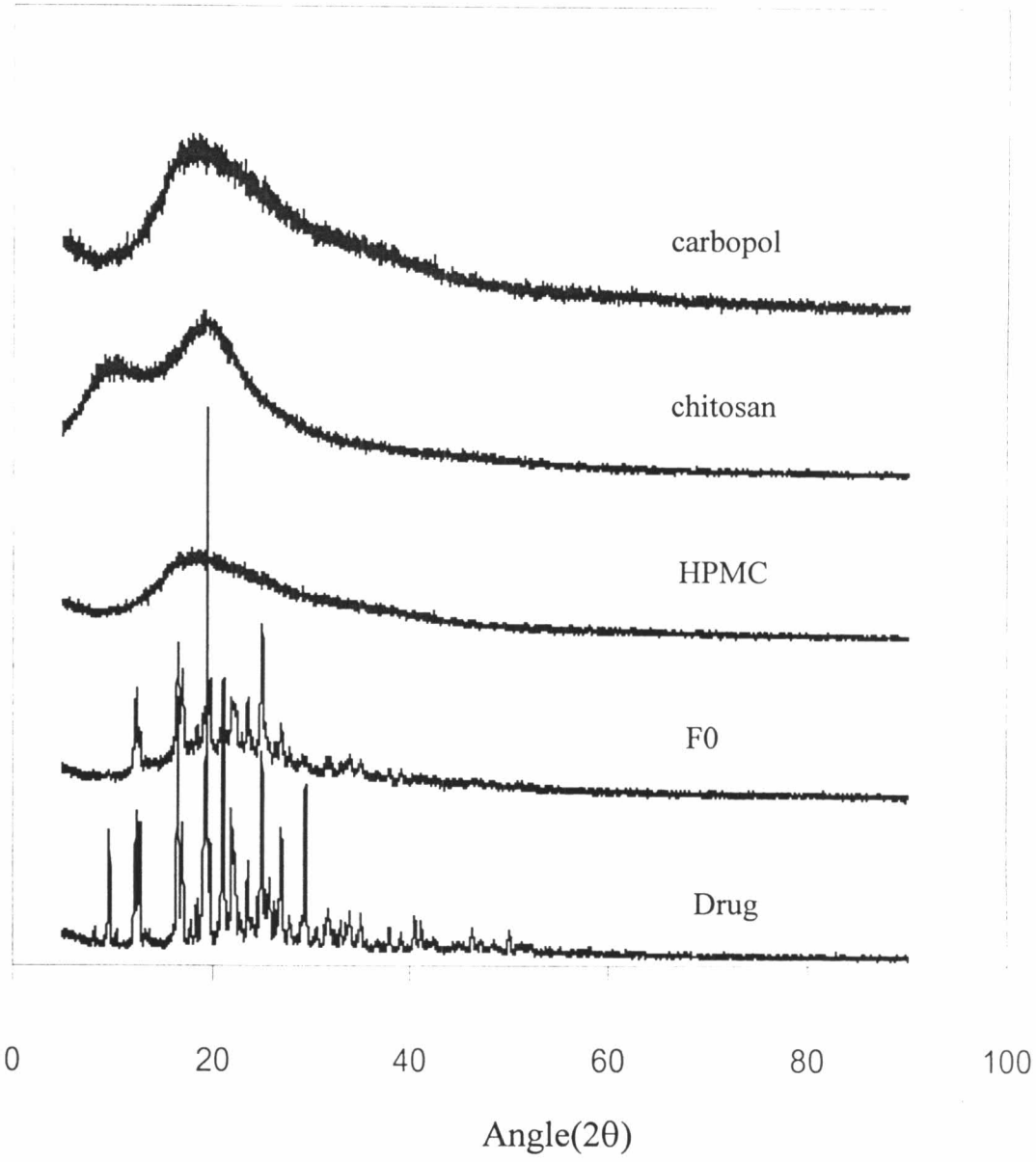


Figure 19 X–ray diffractograms of propranolol HCl, controlled microspheres(F0) and of various types of polymers.

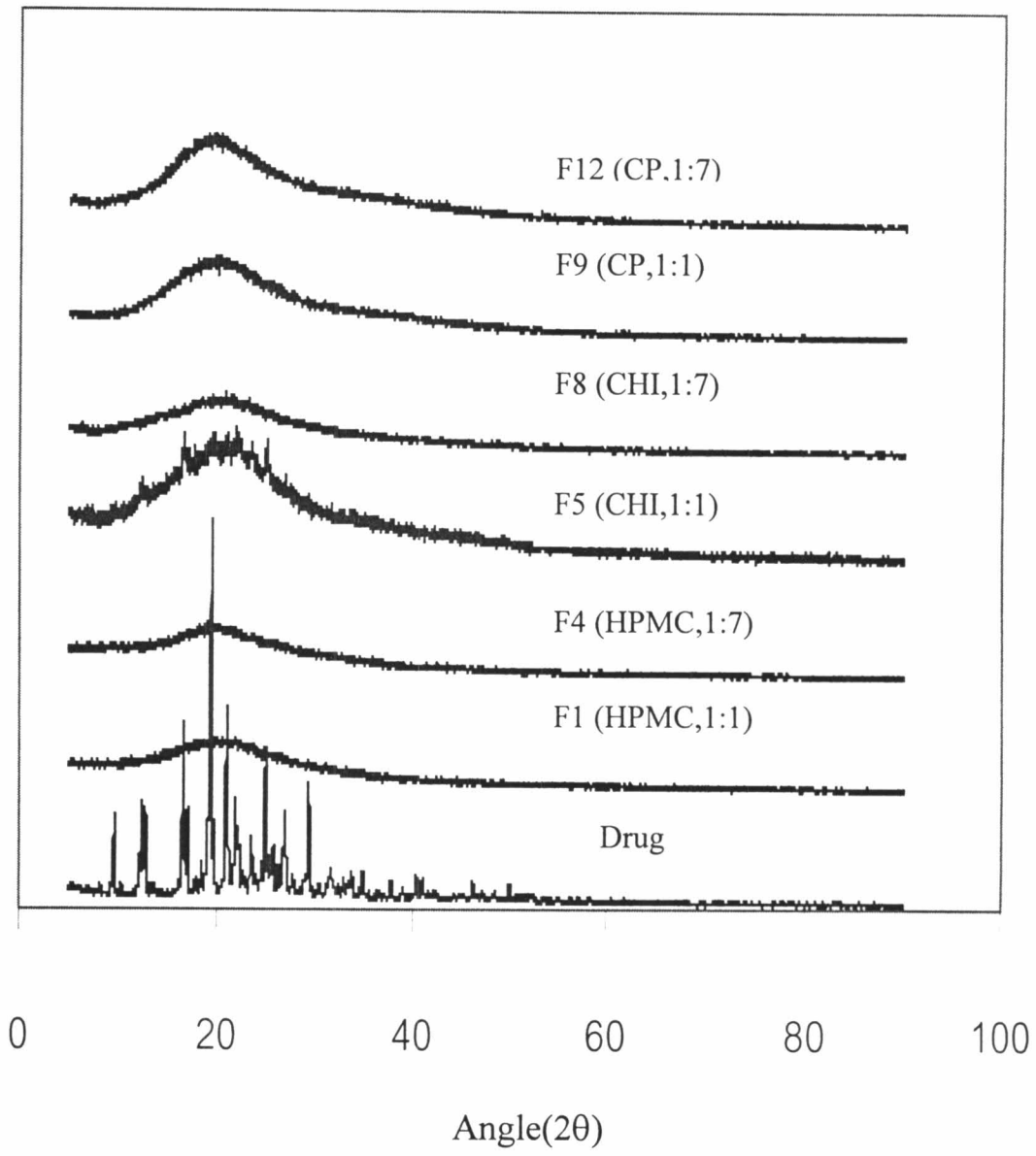


Figure 20 X–ray diffractograms of propranolol HCl and of differentformulations of mucoadhesive microspheres.

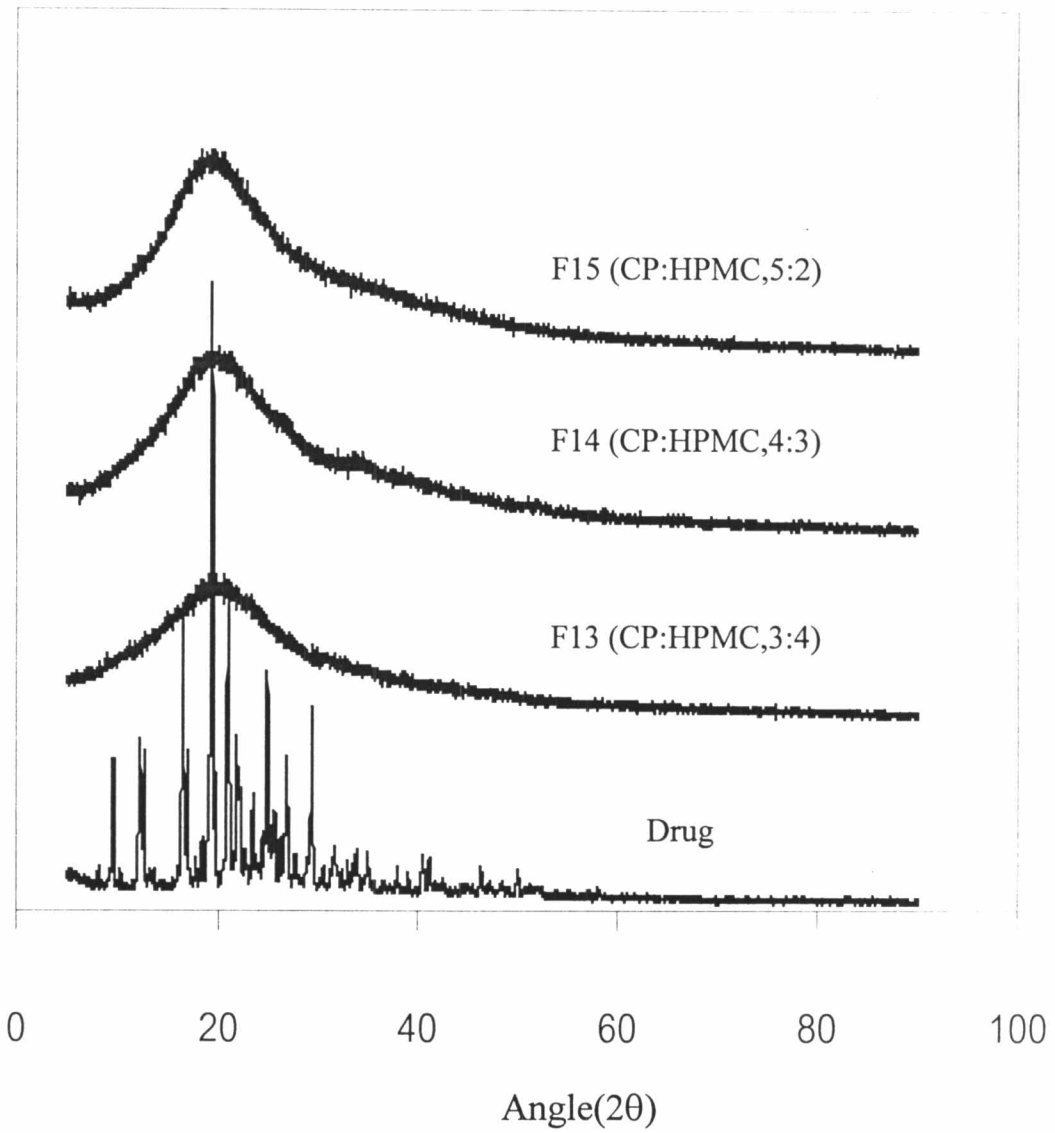


Figure 21 X–ray diffractograms of propranolol HCl and of mucoadhesive microspheres prepared with combined polymer system.

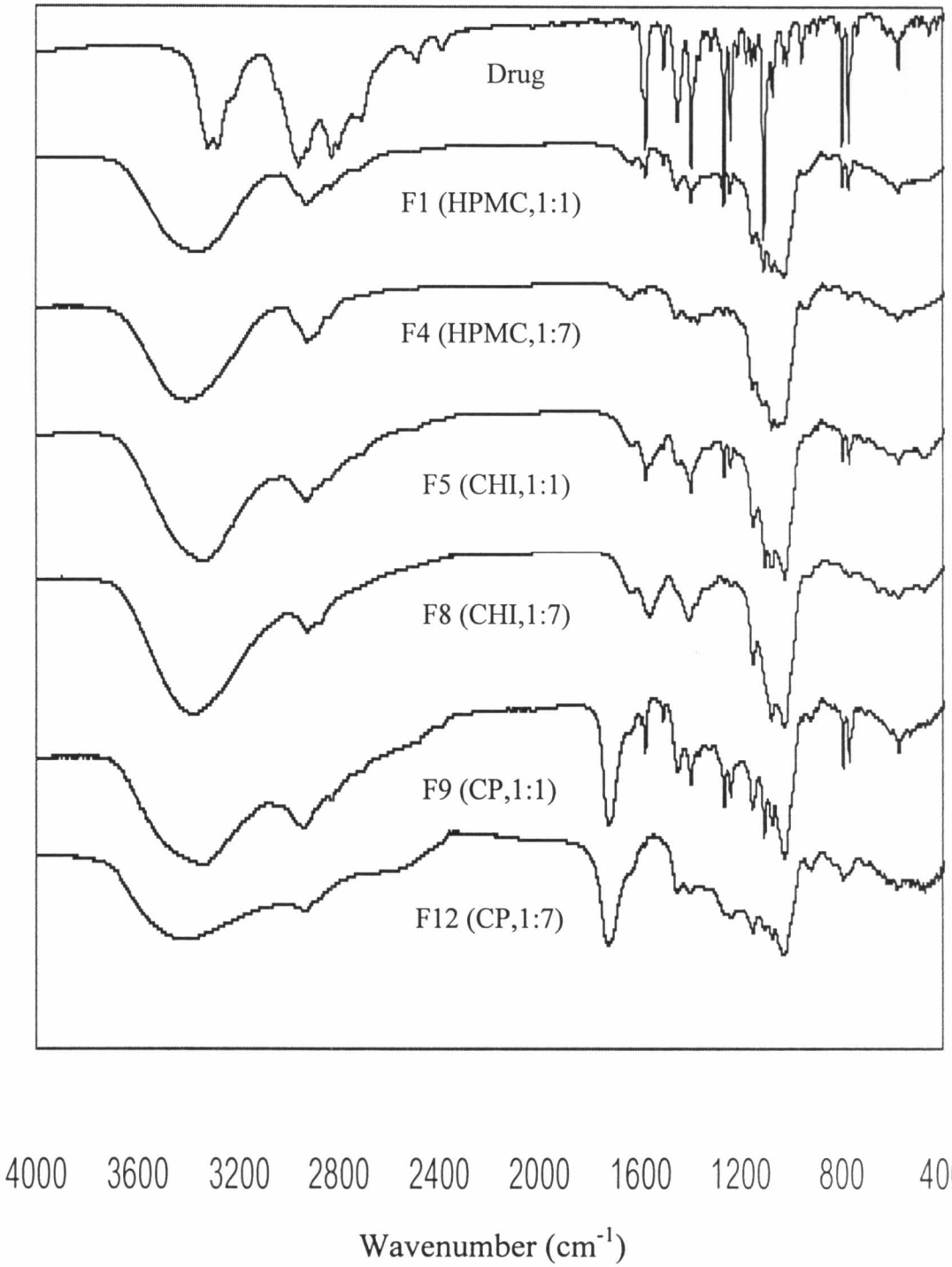


Figure 22 IR spectra of propranolol HCl and of mucoadhesive microspheres prepared with single polymer systems.

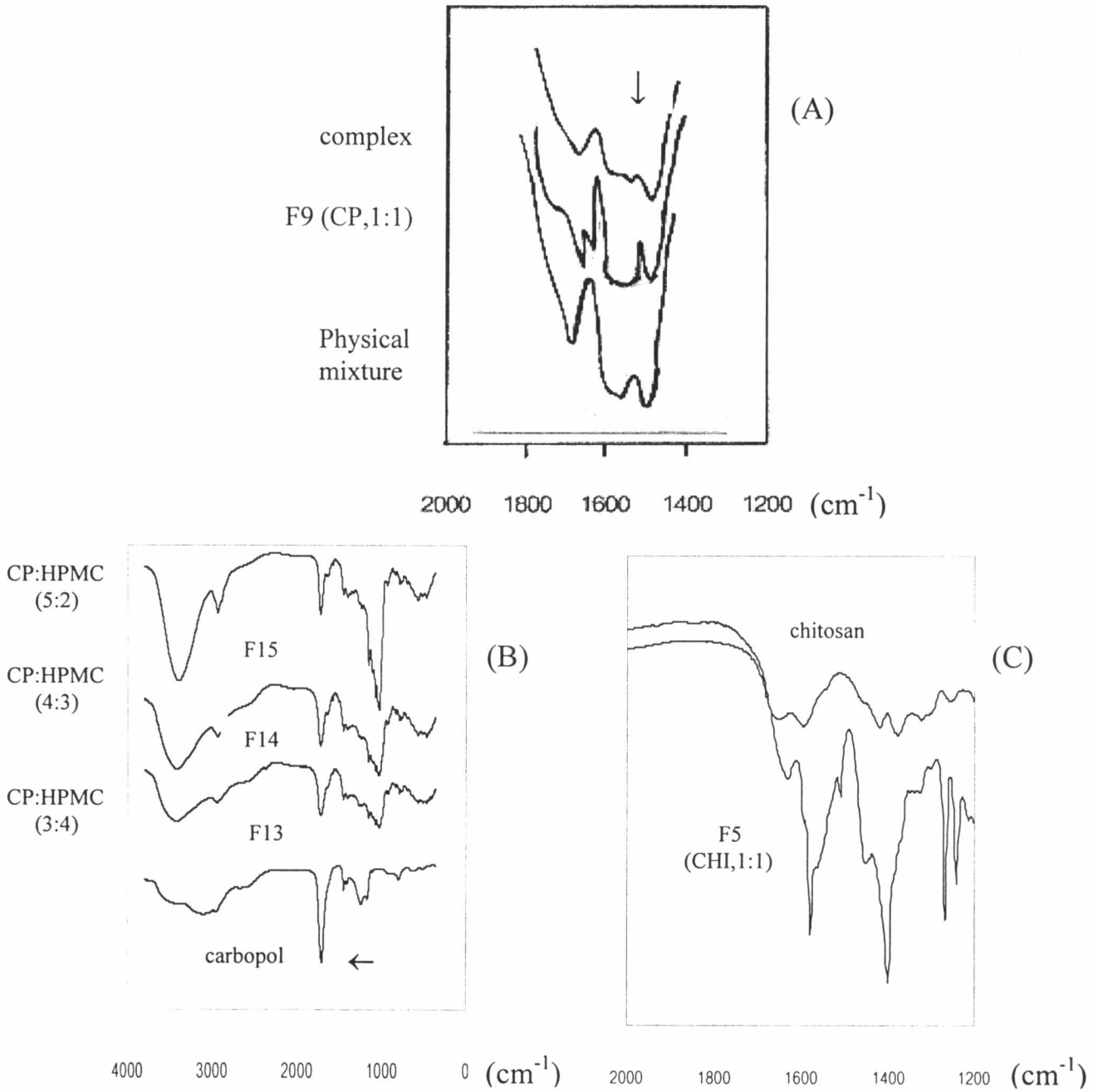


Figure 23 The IR spectra for determining the interaction within microsphere formulations

(A) : The electrostatic interaction was not occured between carbopol and propranolol HCl

(B) : The hydrogen bonding was not appeared between the functional groups of carbopol and HPMC

(C) : chitosan acetate was formed in mucoadhesive microspheres prepared with chitosan as polymer

4. In vitro evaluation of mucoadhesive property of microspheres.

The mucoadhesive property of microspheres formulations prepared from HPMC, carbopol and chitosan was studied in pig intestine. Althought there were many research groups studied and compared this property from these polymers in many preparations, but the summarizes were not clear. Therefore this study was also investigated for comparing with previous studies again. It was found that chitosan seemed to be more in vitro mucoadhesive than carbopol. The rank order of mucoadhesion for these polymeric microspheres was chitosan > carbopol > HPMC as shown in Table 25. and Figure 24-28. However, the microspheres without polymer (F0) was rapidly disappeared from the pig intestine within 5 seconds possessing no ability to adhere with mucus when compared to the mucoadhesive microspheres. These suggested that mucoadhesive polymers were very important for adhering with mucus layer. However, drug and other additives showed no effect on mucoadhesion in this study.

The poor mucoadhesion of HPMC would be due to the non - ionic polymer and low swelling properties. With non - ionic polymer, HPMC could not form the important electrostatic attraction with mucin molecules. And the low swelling of HPMC resulted in the low relaxation of stretched, entangled or twisted molecules, affecting to decrease the adhesive sites of polymer chains that gave the possibility of interpenetrating and entangling with glycoprotein chains of mucus (El – Hameed, 1997).

The effect of drug to polymer ratios of the microspheres containing HPMC to mucoadhesive properties were also studied. In the ratios of 1:3-1:7 (F2-F4) resulted in almost similar in vitro mucoadhesion and higher adhesion than the formulation of 1:1 ratio (F1). This was explained that high polymer content increased the mucoadhesion of the microspheres probably by contributing to the numerous available chains and more swelling of polymer in high content for sufficient interpenetration and entanglement. This reason was also supported by Peppas and Buri who reported that at high concentration of HPMC, mucoadhesive formulations exhibited more adhesion with the mucus than at lower polymer.

Chitosan was clearly more mucoadhesive than other polymers in adhesion time with value more than 300 min. The microspheres containing chitosan completely remained adhering with mucus spreading on the tissue surface of pig intestine after 300 min. Chitosan was the most effective polymer for mucoadhesive preparation in this investigation as the results of adhesion time tabulated in Table 25. Electrostatic attraction was thought to play an important role, because the adhesion of mucin and chitosan was expected to be dominated by the electrostatic interaction between the positively charged chitosan and negatively charged mucin, and the linear molecule of chitosan expressed a sufficient chain flexibility for interpenetration and entanglement. However, this result was different from some previous reports (He, 1998) which suggested that the mucoadhesive property of chitosan was lower than its of carbopol. It could be explained that the molecular weight (200,000 Da., 85%DA) and degree of deacetylation of chitosan used in this study were very high, resulting in the high viscosity and rich of cation when contacting with water. Thus it could contact with mucus strongly. But in preious studies, they used the chitosan which low molecular weight and degree of deacetylation, resulting to lower mucoadhesive property.

The adhesive time of microspheres containing carbopol (90.67-289.33 min) was less than that of microspheres containing chitosan (> 300 min). This obtained results could be explained that the influence of pH value was significant to the ionization of both mucoadhesive polymers and mucin molecules that affected to the strength of mucoadhesion. Changing of pH would affect to the ionization of sialic group and amino acids in the peptide backbone of the glycoprotein of mucus. The pKa of sialic acid is about 2.6, consequently, at pH values of 6.8 this acid group would be almost fully ionized and behave as anionic polyelectrolytes. Carbopol with pKa ~ 4.25 would be also ionized at this investigated pH (6.8) and showed negatively charged molecules as shown by zeta potential values in Table 25, thus the negative charge repulsion would be considerably increased in the mucus – carbopol interaction, resulting in decreasing of adhesion time between carbopol and mucus (Satoh, 1989/Chng, 1985). Furthermore, the mucoadhesive property of carbopol was known to decrease at pH beyond 6.0 due to loss of hydrogen bonding. This agreed with Park (1985) that the hydrogen bonding interaction between cross – linked polyacrylic acid

and the mucus layer was maximum at pH 3 and below. The interaction decreased slowly as the pH was increased and no interaction was observed at pH above 5.0.

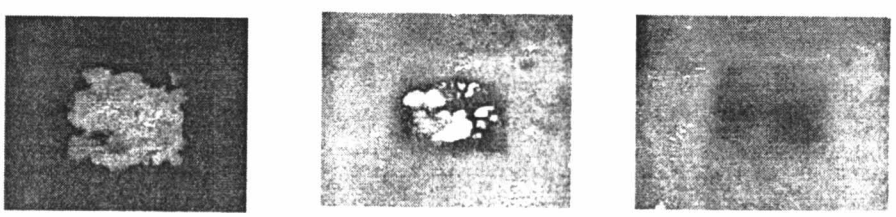
Although the adhesion of carbopol to mucus was low at pH 6.8 due to strongly negative charge repulsion, the strongly penetrating characteristics of swollen carbopol microspheres was the important factor for inducing the interpenetration between the polyacrylic acid chains and the glycoproteinic network of the mucus (Ponchel, 1987/Mortazavi, 1992).

In the case of carbopol, as the carbopol content increased, the adhesive time increased significantly until drug to carbopol ratios higher than 1:3(F10) they decreased as showed in Table 25. This concluded that excessive contents of carbopol were not necessary to achieve the maximum mucoadhesion. Since at lower content of the polymer, the polymer structure was more loose and the polymer chains had more space to extend within the mucus. As the number of polymer chains penetrating per unit volume of mucus was excessively increased, a strong bond, either chemical, mechanical or both, was not formed between the mucus and the polymer (vidgren, 1992/Ponchel, 1987).

Table 25. also showed the adhesive time of microspheres prepared from combined polymer at various mixing ratios of carbopol/HPMC. The results of this study clearly demonstrated that the adhesion time of the formulations increased with increasing content of carbopol, and formulation containing carbopol/HPMC of 5:2 exhibited the maximum mucoadhesion. So, the adhesion of these microspheres might be directly resulted from the influence of carbopol in formulations with interpenetration and entanglement between molecular chains of carbopol and mucus. In this study, the interpolymer complex with hydrogen bonding formation between HPMC and corbopol that inhibited the adhesion force of the formulations due to their hydrophobicity was not found. This result was different from the result of Taylan (1996) that proposed this interaction of polymer.

Table 25 The adhesion time of microspheres adhered on mucus surface of pig intestine and the zeta potential of spray dried microspheres

Formulation code	Adhesion time (min)	Zeta potential (mV)
F0 (no polymer)	0.08 (0.02)	-
F1 (HPMC,1:1)	5.67 (1.15)	-
F2 (HPMC,1:3)	10.00 (1.02)	-
F3 (HPMC,1:5)	9.45 (1.13)	-
F4 (HPMC,1:7)	10.33 (1.53)	-
F5 (CHI,1:1)	>300	+26.72 (5.08)
F6 (CHI,1:3)	>300	+26.12 (6.12)
F7 (CHI,1:5)	>300	+29.37 (4.93)
F8 (CHI,1:7)	>300	+29.02 (4.87)
F9 (CP,1:1)	289.33 (2.08)	-27.87 (7.23)
F10 (CP,1:3)	297.50 (2.65)	-30.24 (6.54)
F11 (CP,1:5)	240.00 (5.00)	-28.64 (8.21)
F12 (CP,1:7)	90.67 (3.06)	-31.70 (5.84)
F13 (CP:HPMC,3:4)	241.33 (3.21)	-18.23 (2.83)
F14 (CP:HPMC,4:3)	271.00 (3.61)	-25.94 (6.12)
F15 (CP:HPMC,5:2)	286.78 (2.31)	-29.62 (6.94)



start 3.00 sec 5.00 sec

F0
(no polymer)

Figure 24 The photographs of controlled microspheres (F0) adhered on mucus surface of pig intestine at different times.

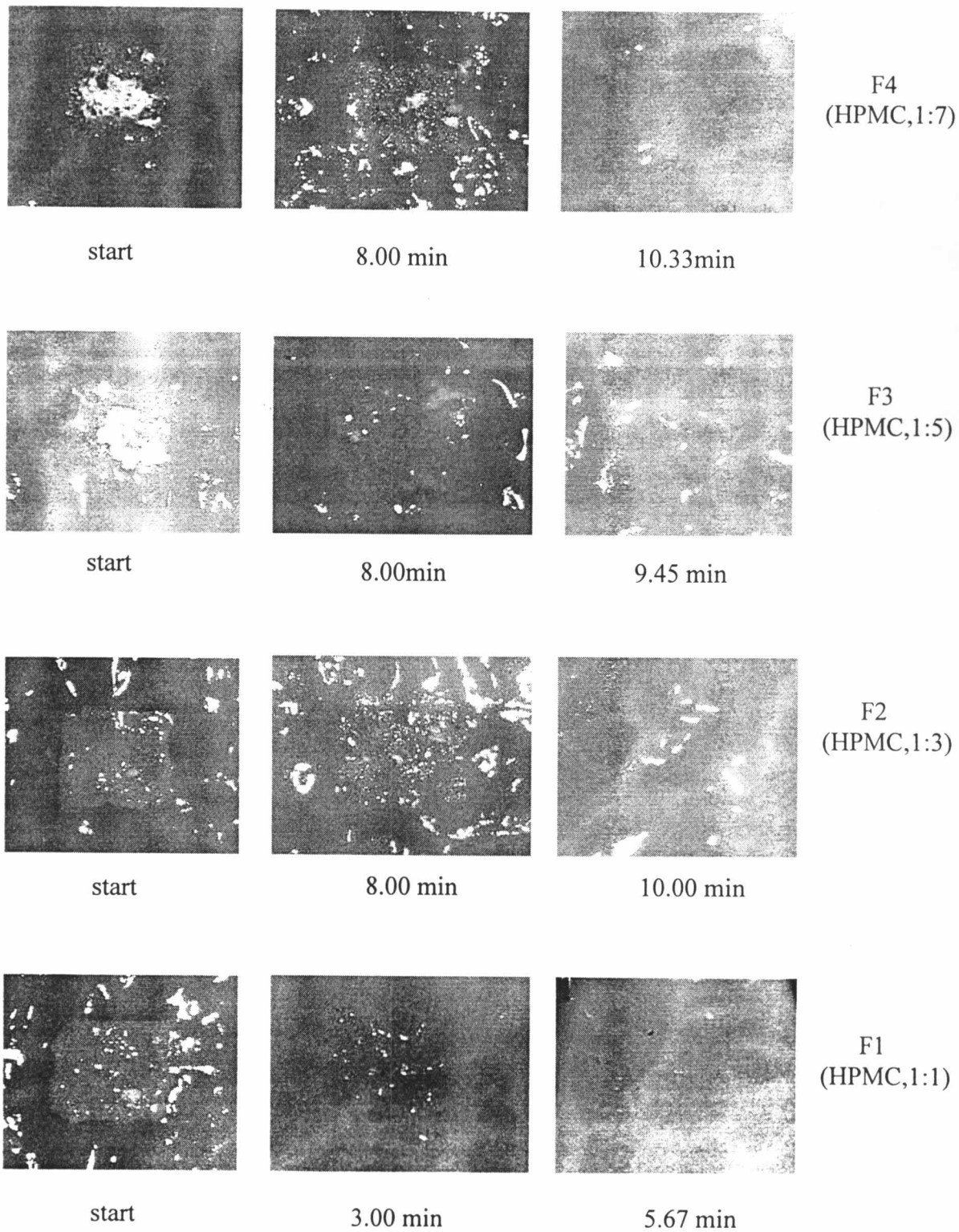


Figure 25 The photographs of mucoadhesive microspheres (F1-F4) adhered on mucus surface of pig intestine at different times.

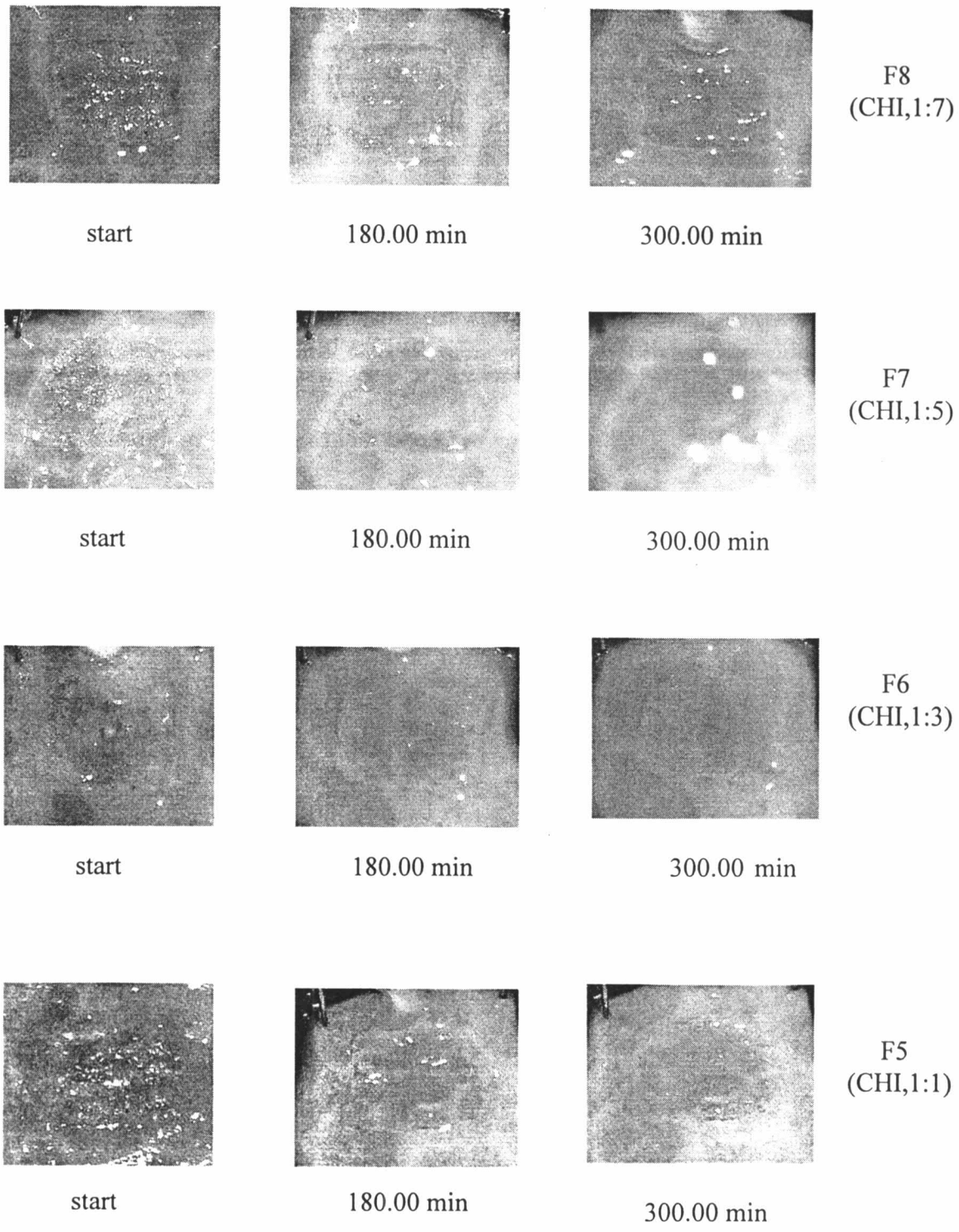


Figure 26 The photographs of mucoadhesive microspheres (F5-F8) adhered on mucus surface of pig intestine at different times.

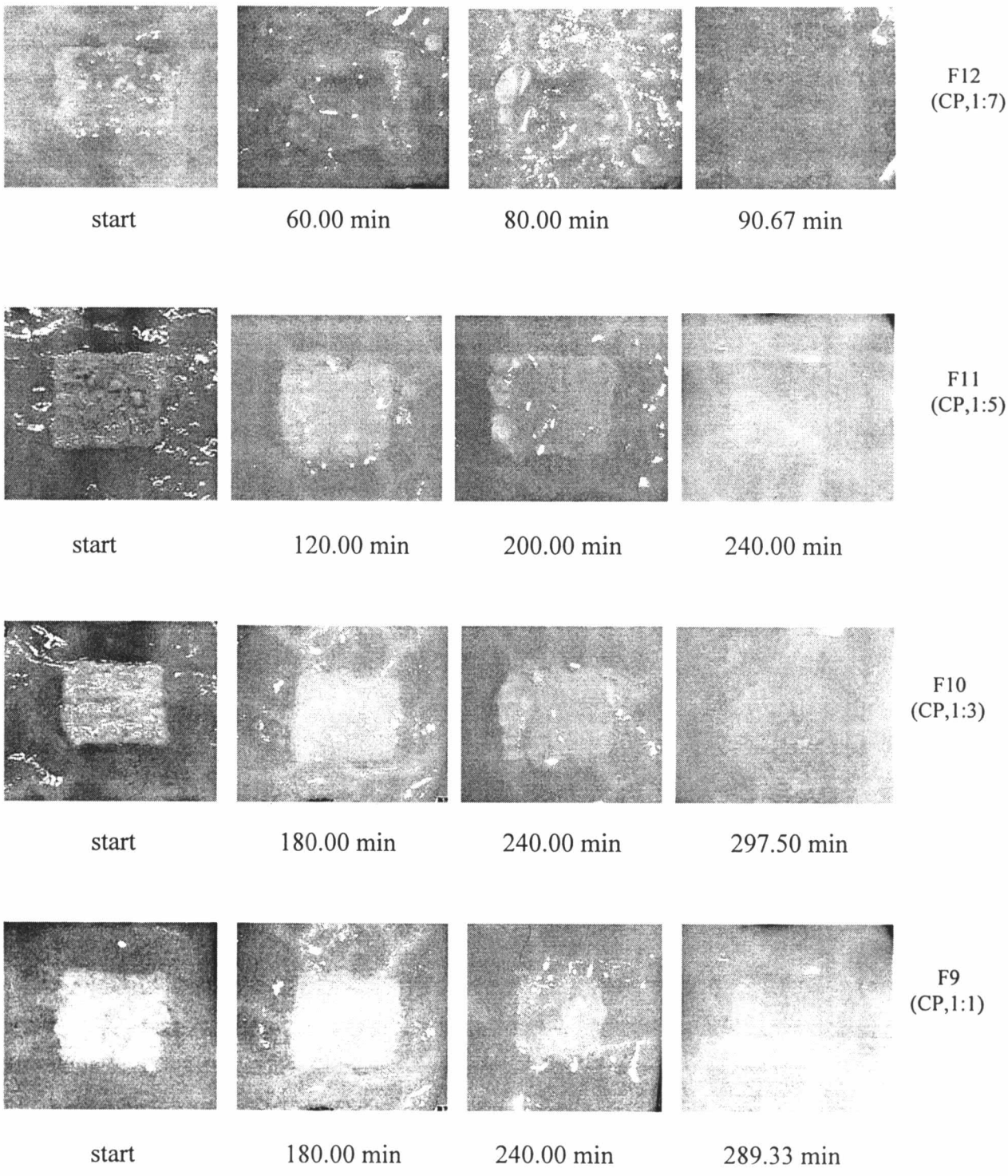


Figure 27 The photographs of mucoadhesive microspheres (F9-F12) adhered on mucus surface of pig intestine at different times.

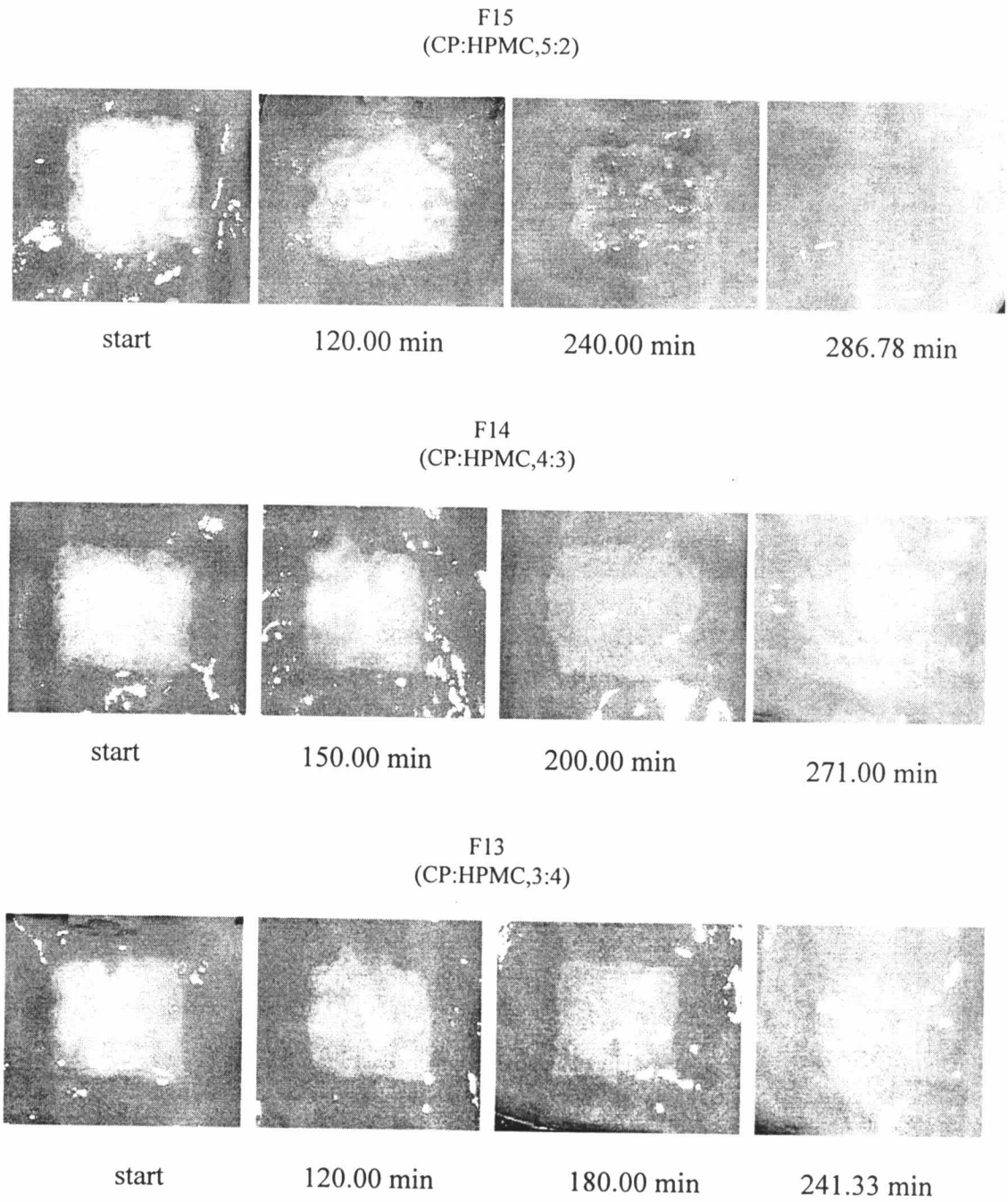


Figure 28 The photographs of mucoadhesive microspheres (F13-F15) adhered on mucus surface of pig intestine at different times.

5. In vitro drug release study

For controlled microspheres(F0), propranolol HCl was dissolved and mixed with other additives in solvent to produce solution that was sprayed into the spray dryer to obtain microspheres. In PBS pH 6.8 as dissolution medium, propranolol HCl that has the pK_a of 9.5 could ionize and completely dissolve in this medium therefore, the dissolution of drug is not the rate limiting step for controlling the release of drug from microspheres. The total amount of drug in controlled microspheres (F0) was rapidly dissolved within 60 minutes (Figure 29A) and the release rate profile is shown in Figure 29B.

The dissolution profile and the relationship between release rate constant and drug to polymer weight ratios of propranolol HCl from mucoadhesive microspheres with various polymer ratios in dissolution medium are shown in Figure 30-33 and 34-35, respectively. The concentration of polymer in the formulation was the determining factor to control the release of drug. Increasing the concentration of polymer tended to decrease the release of drug from microspheres.

In this study, mucoadhesive microspheres was prepared with various types of hydrophilic polymers. A succesful sustained hydrophilic matrix system requires polymeric substances that will wet and hydrate rapidly to form a gelatinous barrier. The rate of release is likely to depend on the permeation through the barrier for water soluble drug as propranolol HCl. The viscosity value and the swelling property of hydrophilic polymers were the important factors to control the release of drug from microspheres (Alderman, 1984). Therefore, the maximum swelling, rate of swelling and the viscosity of microspheres were examined to explain the influence of these factors on the release of drug from microspheres.

The maximum swelling of mucoadhesive microspheres prepared with different types of polymer and various drug to polymer ratios are shown in Figure 36, rate of swelling are shown in Figure 37. and the log viscosity of microspheres are shown in Figure 38 (calculated from data in Table 26). Rate of swelling of microspheres was calculated from the profile plotted between the percentage of swelling and time

(Figure 39-42) at the duration of 3 seconds because microspheres prepared with HPMC were rapidly eroded after 3 seconds in measuring the percentage of swelling. Then, the comparison of swelling rate of microspheres formulations needed to calculate the rate of swelling at the same period (3 seconds).

For microspheres prepared with HPMC (F1-F4), both maximum swelling and log viscosity could be used to explain the release of drug as shown in Figure 43 and 44, respectively. The increase of maximum swelling and log viscosity affected to decrease the release rate constant. The apparent drug release profile (Figure 30) and the release rate constant (Figure 34) from the microspheres prepared with HPMC of the ratios drug to polymer 1:1-1:5 (F1-F3) were quite similar. This could be explained by the low maximum swelling of HPMC as shown in Figure 36. The maximum swelling of these formulations were nearly similar, thus merely thin layer of gel was formed around the surface of matrix microspheres resulting in high drug diffusivity. Higher amount of HPMC would result in thicker gel layer necessary to sustain the release of drug as microspheres prepared with high amount of HPMC at drug to polymer ratio of 1:7 (F4). The low swelling or low water uptake of HPMC may also be explained by the presence of phosphate ions in system which had greater affinity for water than HPMC, thus removing water of hydration from the polymer and dehydrating the polymer regarding as limit of HPMC. This reason was confirmed by the report of Perez-Marcos(1996) that studied the effect of various buffer systems to the water uptake of HPMC.

A drug to polymer ratio of 1:7 of HPMC microspheres clearly showed the slow release of drug (Figure 30) and low release rate constant (Figure 34) when comparing to other formulations (F1-F3). This was the effects of its high viscosity and high maximum swelling.

The release profiles and the release rate constant of propranolol HCl from microspheres containing chitosan (F5-F8) were significantly delayed with increasing the amount of polymer in microspheres formulations as shown in Figure 31 and 34, respectively. This was attributed to the extent of gel forming or the thickness and the viscosity of gel layer that was the important role for controlling the release of drug by

diffusion from microspheres. The maximum swelling values of these microspheres could confirm the effect of swollen polymer to control the release of drug from microspheres as shown in Figure 43. From the results in Figure 44 indicated that the log viscosity of chitosan microspheres was important to control the release of drug. Increasing the maximum swelling values and the log viscosity values affected to decrease the release of drug from microspheres. The microspheres containing chitosan at drug to polymer ratios of 1:3 and 1:5 (F6 and F7) showed the similar release rates (Figure 34). This was attributed that the maximum swelling and the log viscosity were slightly changed at drug to polymer ratios from 1:3 to 1:5.

Figure 32 shows the release profiles of propranolol HCl from microspheres containing carbopol at various drug to polymer ratios (F9-F12), and the relationships between release rate constants and drug to polymer weight ratios are shown in Figure 34 . It was obvious that there was a lag time occurred in early hours on the release profiles of carbopol microspheres (F10-F12). This result would be explained by the swelling rate, maximum swelling and the log viscosity of microspheres as shown in Figure 36-38, respectively. The swelling rate, maximum swelling and the log viscosity of microspheres containing carbopol was much higher than that of other microsphere formulations. When carbopol rapidly swelled, the very viscous gel layer was too rapidly formed as the barrier around the surface of mucoadhesive microspheres and hindered the release of drug.

This strong barrier could hinder the diffusion of drug from microspheres. This was likely due to the fact that carbpol was a cross-linked polymer with high molecualr weight (~$3x10^6$Da) and high viscosity. When contacted with water, it would highly swell and hold water inside its microgel network and form a gel diffusion layer that hindered the outward transport of the drug within the microspheres, hence producing lag time and controlling release effect. The release rate constant obtained from the release profiles of microspheres containing carbopol were not much different as shown in Figure 34 while the maximum swelling of these microspheres were much different as shown in Figure 37. Thus, the release of drug from carbopol microspheres might be dependent on the viscosity of polymer in micosphere formulations that showed similar high viscosity values of these microsphere

formulations (Figure 38). This reason was confirmed by the relationship profiles of these microspheres as shown in Figure 44.

The diffusion of dissolution fluid through the gel was affected by the gel strength. The protective or barrier gel was in turn, controlled by the viscosity and concentration of the polymer used. Therefore, as expected, there was an inverse relationship between polymer concentration and the rate of release. As the level of polymer was increased, the gel formed was firmer and more cohesive. This resulted in slower drug release. On the other hand, an increase in the polymer concentration would also increase the viscosity of the surrounding fluid, which would increase the gel – strength, and would slow the permeation rate of both the dissolution fluid, and the drug through the gel layer.

The dissolution profiles of spray dried propranolol HCl with HPMC and with chitosan showed no capability to sustain the drug release. It was apparent that microspheres containing carbopol alone could sustain the drug release, but the pattern of release is not suitable owing to the lag time in early hours on the release profile. Therefore, this formulation could not be practically useful. To corrrect the release pattern of propranolol HCl from microspheres, combination of HPMC and carbopol were used as matrix former. The release profile of drug from these formulations are shown in Figure 33. The lag time in release profile was shortened. Increasing the amount of carbopol in formulations tended to decrease the release of drug from microspheres. The similar release rate constant of microspheres prepared with combined polymer was obtained when preparing the microspheres with the ratio of carbopol to HPMC at 3:4 (F13) and 4:3 (F14) as shown in Figure 35. This result corresponded to the similar viscosity values of these microspheres (Figure 38). The microsphere formulation F15 at drug to polymer ratio of 1:7 showed slower release of drug from microspheres and lower release rate constant than the formulation F13 and F14. This result was explained by the higher viscosity value and higher maximum swelling of this formulation. Then, the main factor to control the release of drug from microspheres containing combined polymer would be the viscosity values that obtained from the part of carbopol in formulations.

The formulation of propranolol HCl with both carbopol and chitosan was not investigated in this study because carbopol was found to be potentially incompatible with chitosan solution. There was an interaction between NH^{+}_{4} group of chitosan and the COO^{-} groups of carbopol making complex formation, and the precipitation was observed.

Figure 45 shows the effect of various types of polymers on the release patterns of propranolol HCl from microspheres at ratio of drug to polymer 1:7 (F4,F8 and F12). There was a significant difference in the propranolol HCl release patterns from formulation contained different types of polymers. The drug release from microspheres containing carbopol was lower than that from microspheres containing chitosan and HPMC, while the similar release profiles were observed from chitosan microspheres and HPMC microspheres. The fraction of propranolol released decreased as the viscosity of microsphere formulations increased as shown in Figure 38. The results from this figure showed that the highest viscosity was obtained from microspheres containing carbopol. The viscosity of swellable polymer was a primary factor in controlling the release of drugs from microspheres. Increasing the viscosity leading to stronger polymer entanglement caused the slowing down of drug diffusion from matrix microspheres. This result was in agreement with general principles (Perez – Marcos, 1996; Brabander, 2003 and Li, 2003).

The another reason for the difference in drug release among these types of polymers most probably originated from their different swelling behavior (Figure 37). Whereas a minimum swelling prevailed for the HPMC systems, the percentage of maximum swelling was obtained from the carbopol containing formulation. The latter formulation formed very viscous gel around the microspheres. No difference in release profiles of microspheres prepared from chitosan(F8) and HPMC(F4) were observed. This result may be explained on the counter balance of the viscosity values of both HPMC(F4) and chitosan(F8) microspheres.

In order to determine the effect of type of polymer on the model of drug release. Therefore, analysis of all dissolution data were carried out to elucidate the model (zero order, first order, and Higuchi model) that best fitted the data.

Mathematical modelling of the data indicated that drug release from microspheres in all types of polymers was best characterised by treating the data as a function of the square root of time, suggesting a similarity to release from a matrix (Table 27) as following equation

$$\frac{M_t}{A} = D(2c_0-c_s)c_st$$

where M_t is the cumulative absolute amount of drug released at time t, A is the surface area of the controlled release device exposed to the release medium, D is the drug diffusivity in the polymer carrier, and c_0 and c_s are the initial drug concentration, and the solubility of the drug in the polymer, respectively (Siepmann and Peppas, 2001).

The dissolution data was analyzed to clarify drugs release mechanism using equation $Mt/M_\infty = kt^n$ as previously reviewed in the section of the analysis of drug release mechanism. The microspheres were formed a gelatinous matrix and swelled. The release exponent n, the kinetic constant k and the correlation coefficient (r^2) are shown in Table 28. The release exponent value would be compared with the value in Table 3. The obtained values of n were between 0.43 and 0.85 in formulations using HPMC and chitosan as mucoadhesive polymers for the release of propranolol HCl, indicating anomalous (non-Fickian) release kinetics, which was indicative of drug release mechanisms involving a combination of both diffusion and chain relaxation mechanisms. For carbopol, regression lines that were plotted following above equation were not linear ($r^2 < 0.99$), then this equation was not suitable for analyzing the mechanism of drug release from this microspheres.

A correlation was found between the results of dissolution testing, the swelling study and the n-values of the microsphere formulations. The dissolutions of propranolol from microspheres containing HPMC and chitosan microspheres were significantly delayed with increasing the amount of polymer in the microspheres. This was attributed to the extent of gel forming or the thickness of gel layer that was the important role for controlling the release of drug by diffusion from microspheres (Dortunc, 1997; Ishikawa, 2000 and Sawayanagi, 1982). This reason is confirmed by the values of the exponent n in the range 0-70% of drug dissolved of HPMC and

chitosan microspheres. These n values were fitted to non-Fickian (anomalous) behavior which indicated that the drug partially diffused through the swollen polymer matrix and also partly through the gradually expanding hydrated matrix with increasing diffusional path length (Roy and Rohera, 2002). The viscosity was the another important reason for explaining the release characteristics of microspheres. Increasing the amount of polymer effected to increase the viscosity of polymer gel in microspheres and decrease the release of drug as shown in Table 26.

Table 26 The viscosity values of mucoadhesive microspheres obtained from cone and plate viscometer.

Formulation code	Viscosity (cps)	log viscosity (log cps)
F1 (HPMC,1:1)	520.99 (46.23)	2.72
F2 (HPMC,1:3)	713.66 (25.44)	2.85
F3 (HPMC,1:5)	1811.28 (12.64)	3.26
F4 (HPMC,1:7)	7913.15 (12.73)	3.90
F5 (CHI,1:1)	493.86 (23.12)	2.69
F6 (CHI,1:3)	2508.14 (36.45)	3.40
F7 (CHI,1:5)	4236.73 (9.63)	3.63
F8 (CHI,1:7)	6829.19 (28.74)	3.83
F9 (CP,1:1)	28581.71 (1073)	4.46
F10 (CP,1:3)	79652.49 (15.82)	4.90
F11 (CP,1:5)	125273.52 (63.27)	5.10
F12 (CP,1:7)	152668.00 (59.51)	5.18
F13 (CP:HPMC,3:4)	98773.89 (47.11)	4.99
F14 (CP:HPMC,4:3)	100978.98 (65.98)	5.00
F15 (CP:HPMC,5:2)	149474.98 (73.29)	5.17

Table 27 Correlation coefficient of the relationships between percent drug released versus time (A) , percent drug released versus square root time (B) , and log percent drug remained versus time (C).

Formulation code	(A) Zero order	(B) Higuchi model	(C) First order
F1 (HPMC,1:1)	0.7834	0.9862	0.9368
F2 (HPMC,1:3)	0.8482	0.9874	0.9716
F3 (HPMC,1:5)	0.9078	0.9996	0.9936
F4 (HPMC,1:7)	0.8414	0.9858	0.9748
F5 (CHI,1:1)	0.9553	0.9996	0.9996
F6 (CHI,1:3)	0.8742	0.9964	0.9845
F7 (CHI,1:5)	0.9510	0.9829	0.9966
F8 (CHI,1:7)	0.8578	0.9914	0.9781
F9 (CP,1:1)	0.7957	0.9631	0.9454
F10 (CP,1:3)	0.9958	0.9976	0.9839
F11 (CP,1:5)	0.9577	0.9866	0.9972
F12 (CP,1:7)	0.9874	0.9934	0.9688
F13 (CP:HPMC,3:4)	0.9147	0.9942	0.9916
F14 (CP:HPMC,4:3)	0.9722	0.9928	0.9978
F15 (CP:HPMC,5:2)	0.9803	0.9990	0.9940

Table 28 The values of kinetic constant (k) , release exponent (n) and correlation coefficient (r^2) following linear regression of dissolution data for equation of $M_t / M_\infty = kt^n$ in dissolution medium.

Formulation code	(k) Kinetic constant	(n) Release exponent	(r^2) Coefficient of correlation
F1 (HPMC,1:1)	9.3799	0.5311	0.9902
F2 (HPMC,1:3)	12.0698	0.5024	0.9975
F3 (HPMC,1:5)	10.5148	0.5062	0.9974
F4 (HPMC,1:7)	7.0404	0.4852	0.9958
F5 (CHI,1:1)	17.4703	0.4746	0.9999
F6 (CHI,1:3)	11.7355	0.4518	0.9982
F7 (CHI,1:5)	4.6047	0.6362	0.9972
F8 (CHI,1:7)	8.1865	0.4472	0.9977
F9 (CP,1:1)	11.1020	0.3630	0.9628
F10 (CP,1:3)	0.1444	0.9860	0.9724
F11 (CP,1:5)	0.0044	1.4852	0.9735
F12 (CP,1:7)	0.0043	1.3703	0.9577
F13 (CP:HPMC,3:4)	3.9792	0.5660	0.9940
F14 (CP:HPMC,4:3)	2.7454	0.5820	0.9517
F15 (CP:HPMC,5:2)	0.0590	1.1808	0.9745

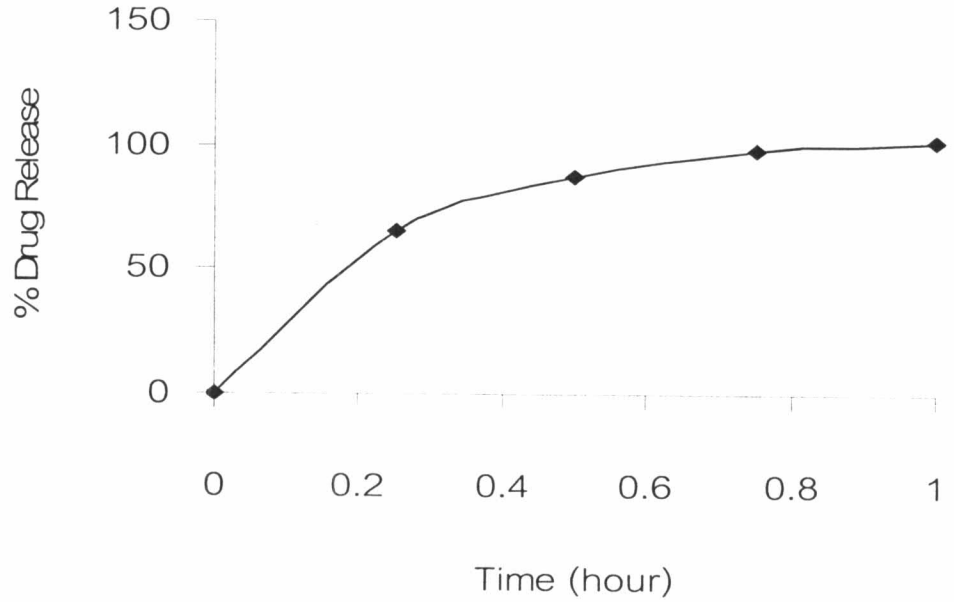


Figure 29A The release profile of propranolol HCl from controlled microspheres (F0)

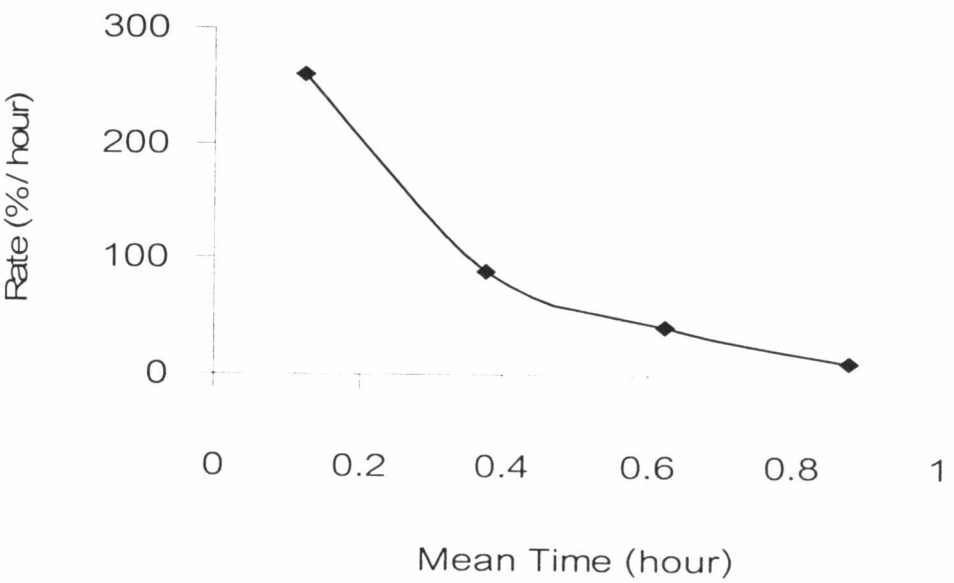


Figure 29B The release rate profile of propranolol HCl from controlled microspheres (F0)

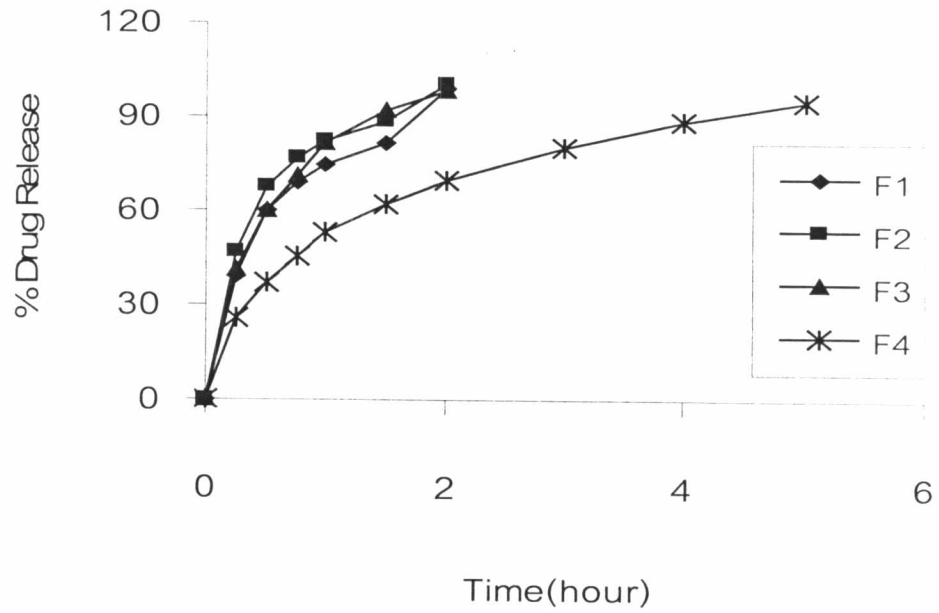


Figure 30 The release profiles of propranolol HCl from mucoadhesive microspheres prepared with HPMC at various drug to polymer ratios.

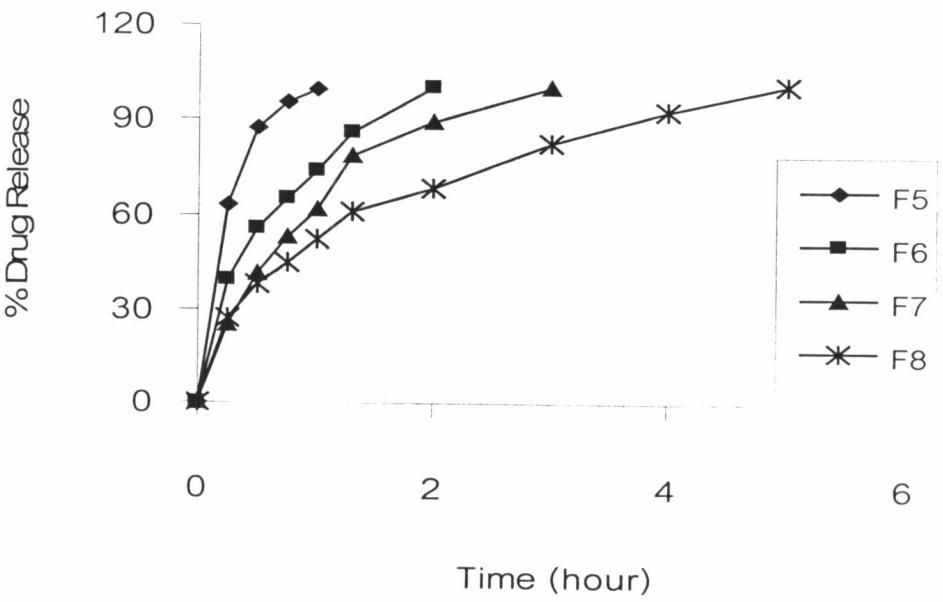


Figure 31 The release profiles of propranolol HCl from mucoadhesive microspheres prepared with chitosan at various drug to polymer ratios.

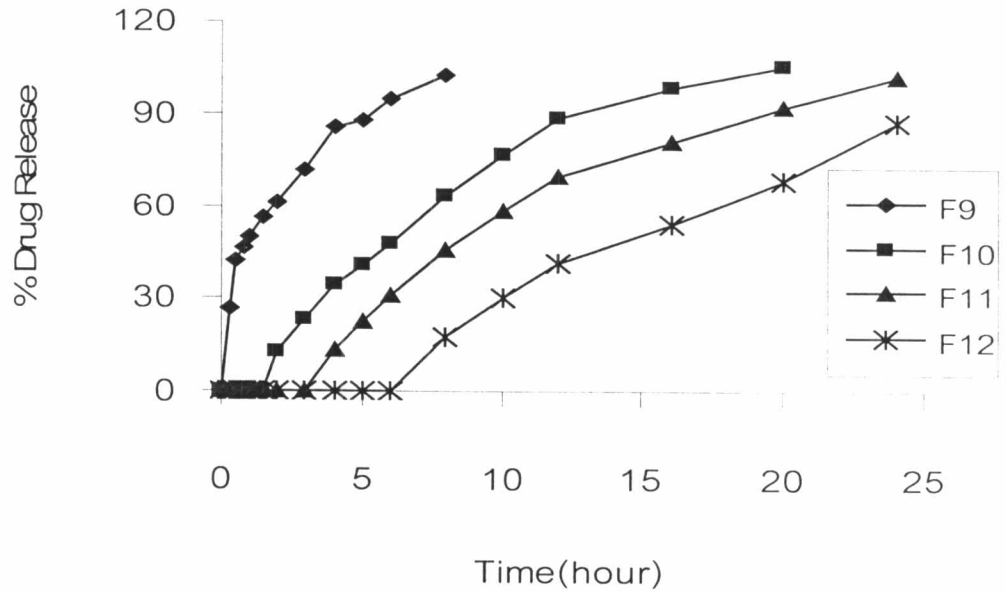


Figure 32 The release profiles of propranolol HCl from mucoadhesive microspheres prepared with carbopol at various drug to polymer ratios.

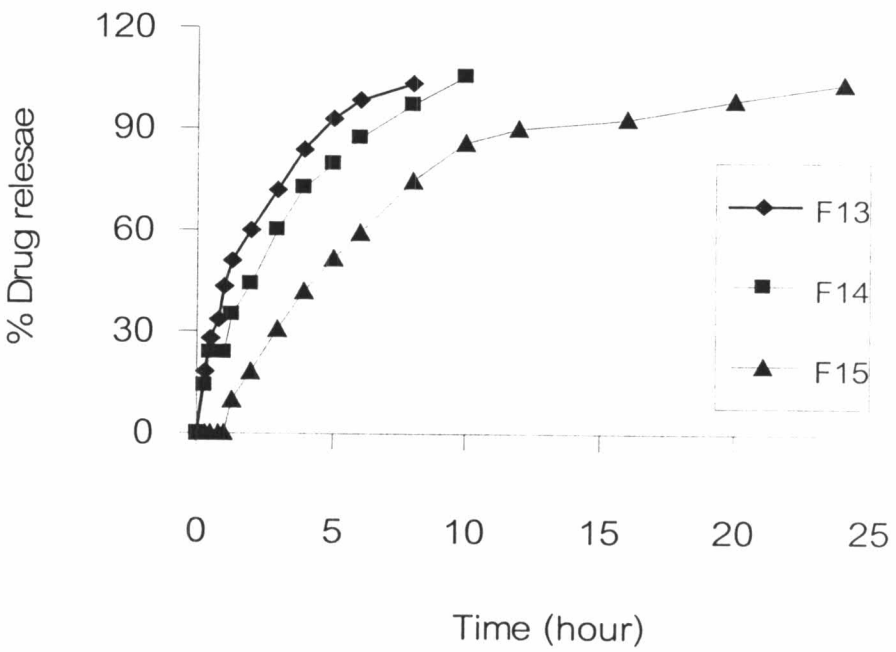


Figure 33 The release profiles of propranolol HCl from mucoadhesive microspheres prepared with combined polymer at various carbopol to HPMC ratios

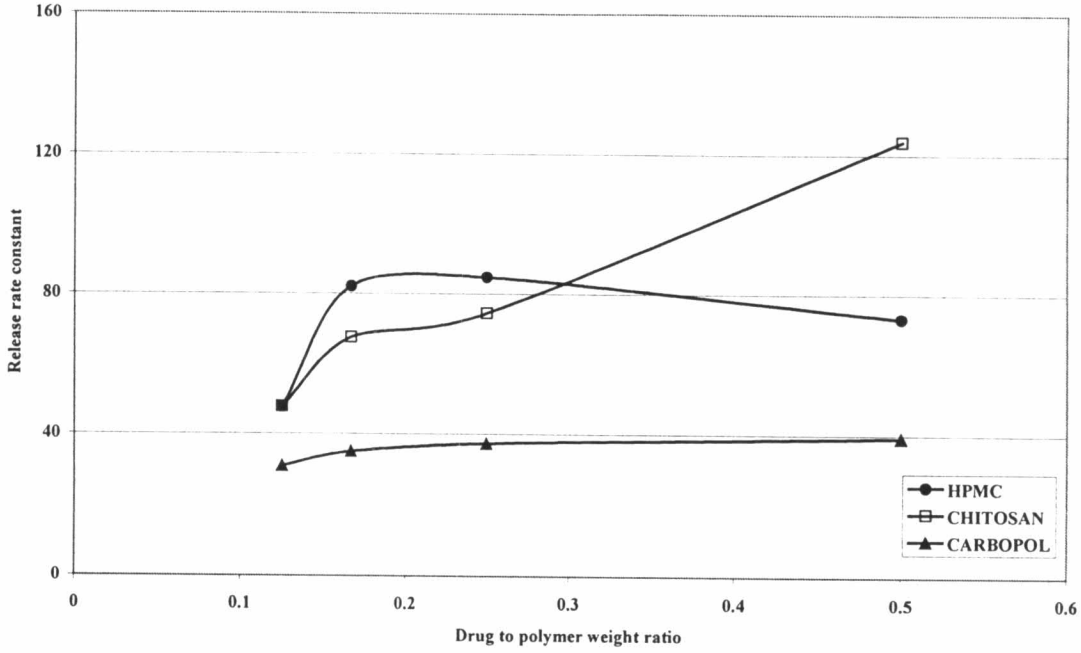


Figure 34 The relationship between release rate constant and drug to polymer weight ratios of mucoadhesive microspheres with various types of hydrophilic polymers.

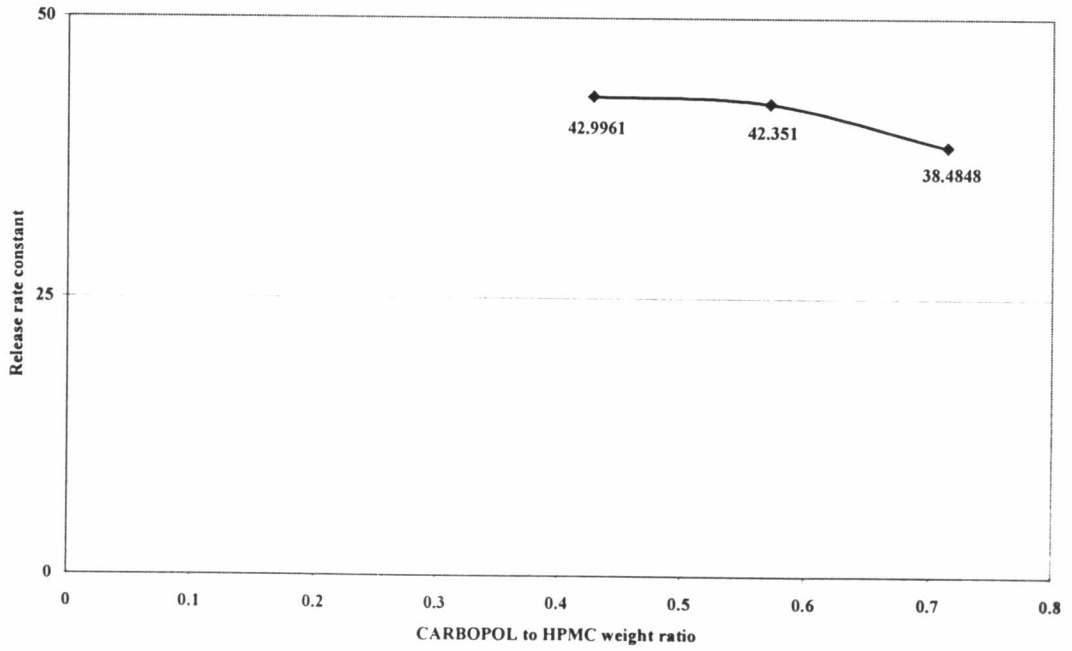


Figure 35 The relationship between release rate constant and carbopol to HPMC weight ratios of mucoadhesive microspheres obtained from combined mucoadhesive polymers.

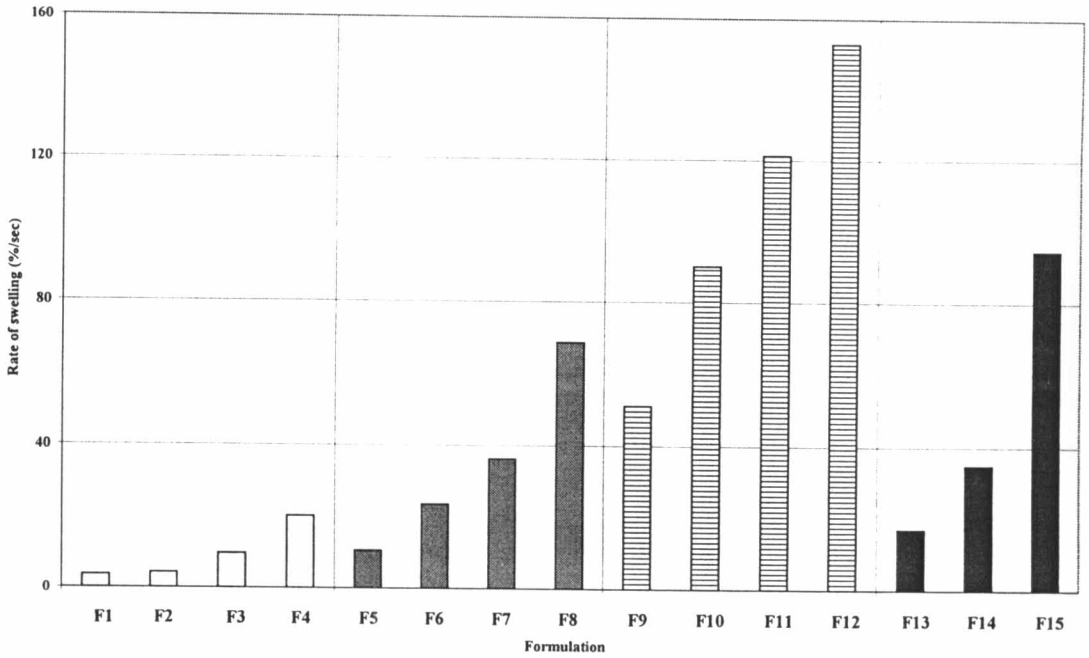


Figure 36 The swelling rate of propranolol HCl microspheres prepared with different types polymers and drug to polymer weight ratios.

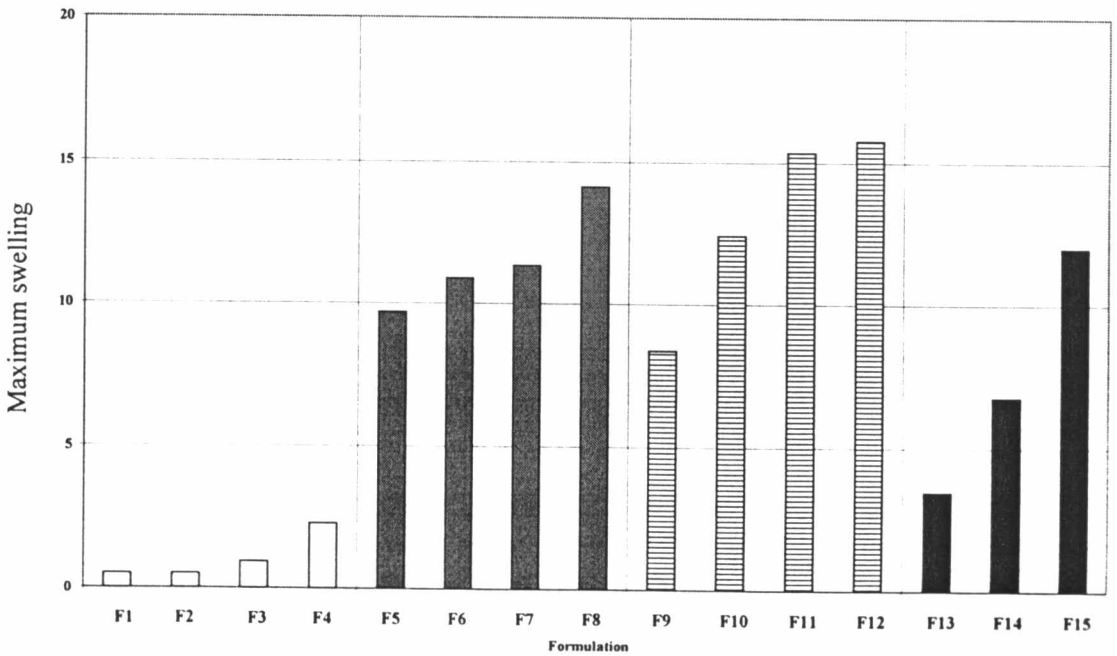


Figure 37 The maximum swelling of propranolol HCl microspheres prepared with different types polymers and drug to polymer weight ratios.

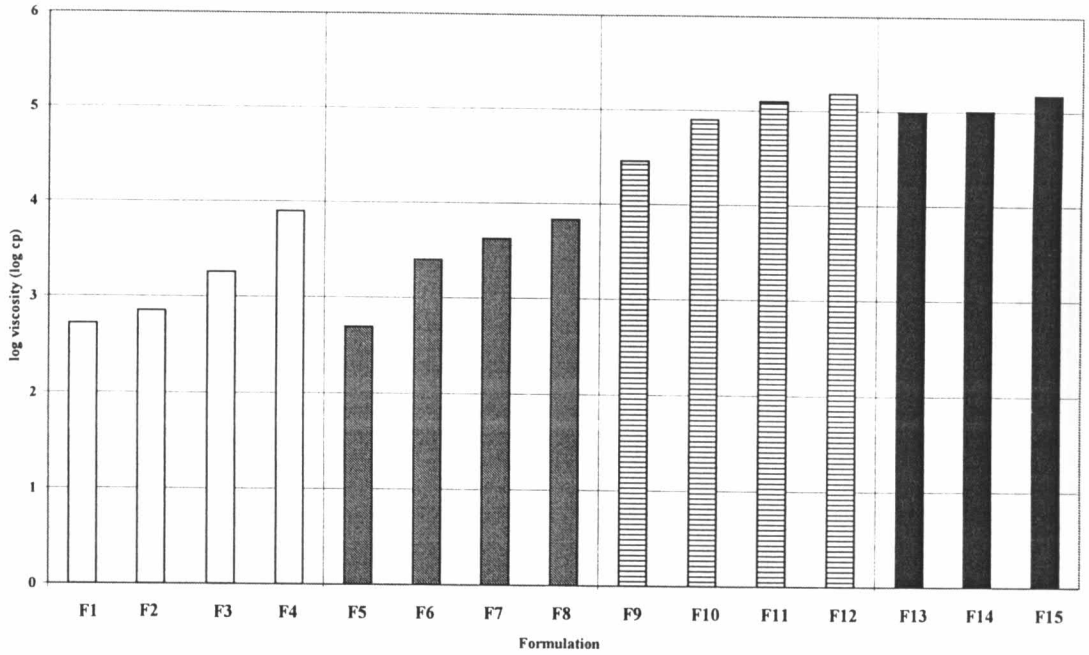


Figure 38 The log viscosity of mucoadhesive microspheres obtained from cone and plate viscometer with different types of polymers and drug to polymer weight ratios.

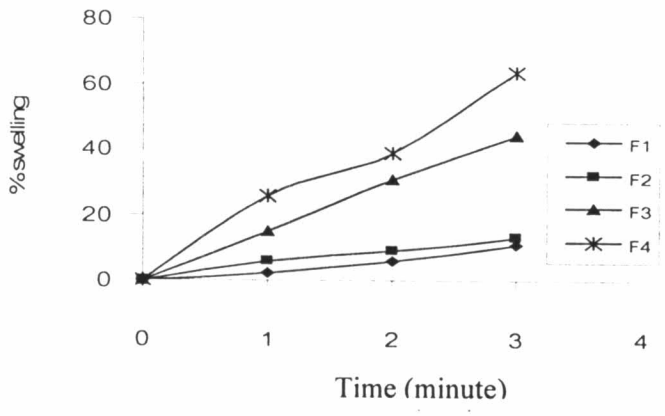


Figure 39 The percentage of swelling of mucoadhesive microspheres prepared with HPMC at various drug to polymer ratios.

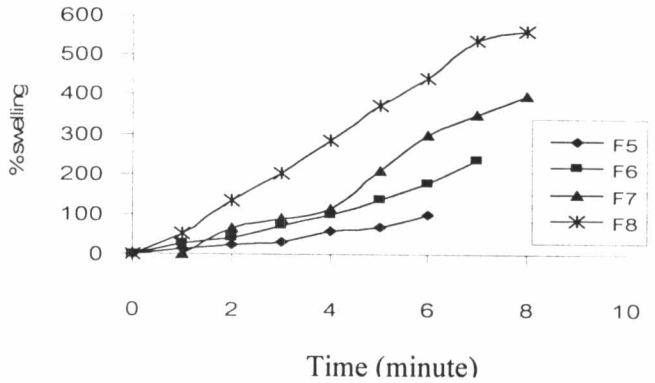


Figure 40 The percentage of swelling of mucoadhesive microspheres prepared with chitosan at various drug to polymer ratios.

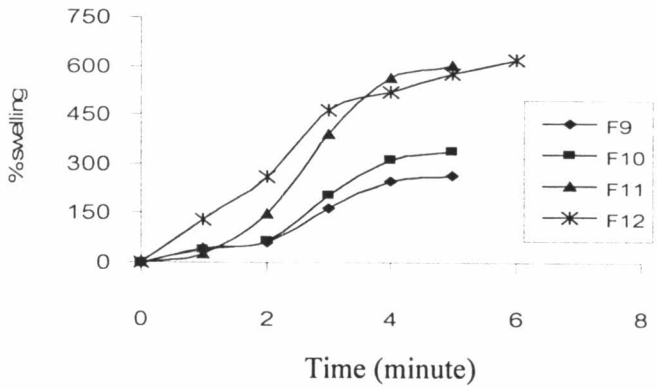


Figure 41 The percentage of swelling of mucoadhesive microspheres prepared with carbopol at various drug to polymer ratios.

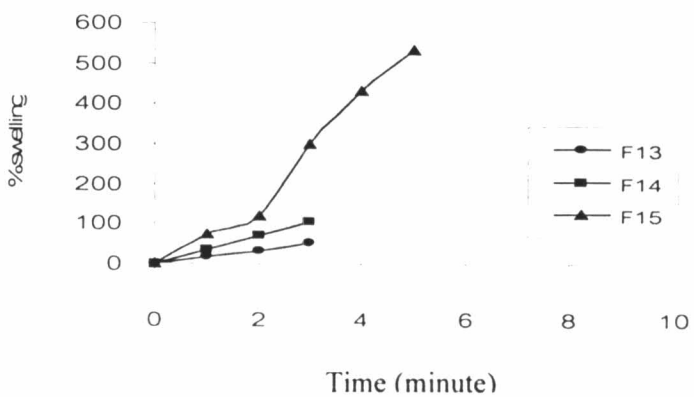


Figure 42 The percentage of swelling of mucoadhesive microspheres prepared with combined polymer at various carbopol to HPMC ratios.

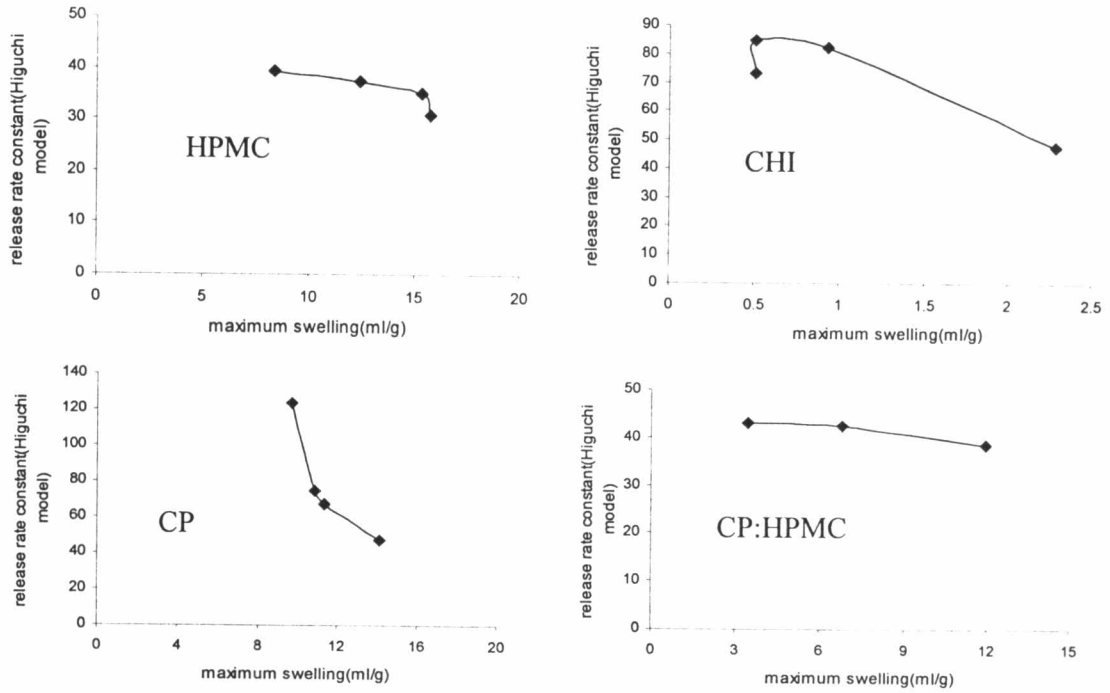


Figure 43 The relasionships of maximum swelling effect on the release rate constant (Higuchi model)

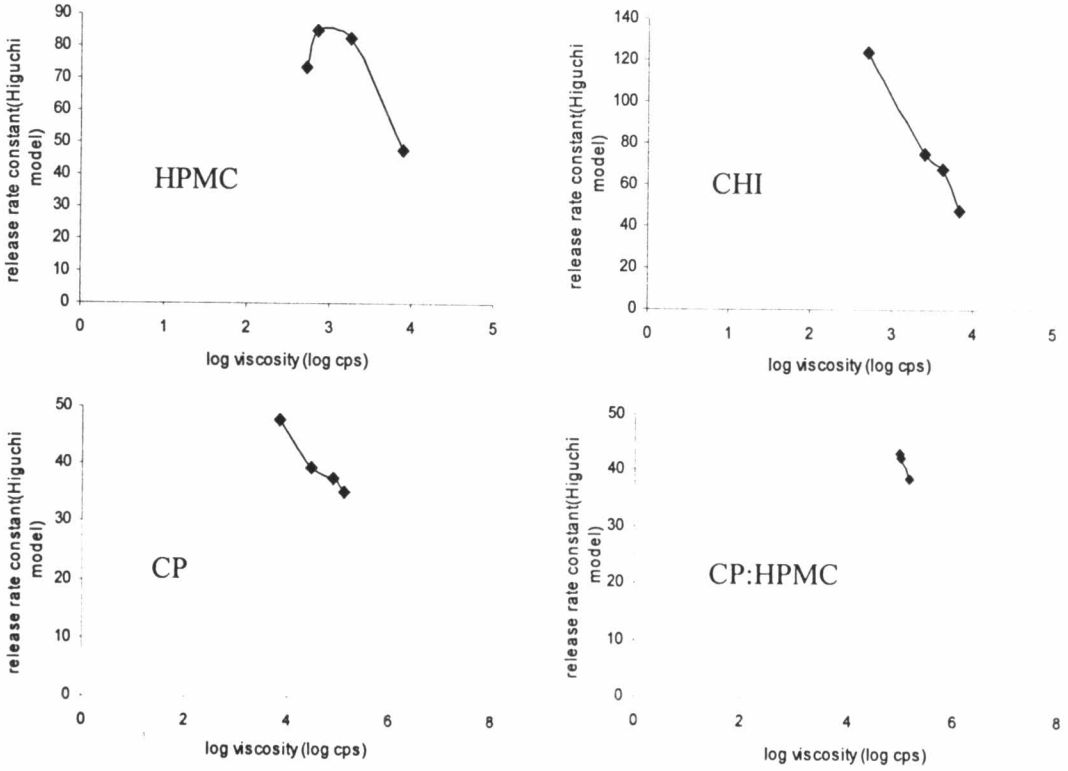


Figure 44 The relasionships of log viscosity effect on the release rate constant (Higuchi model)

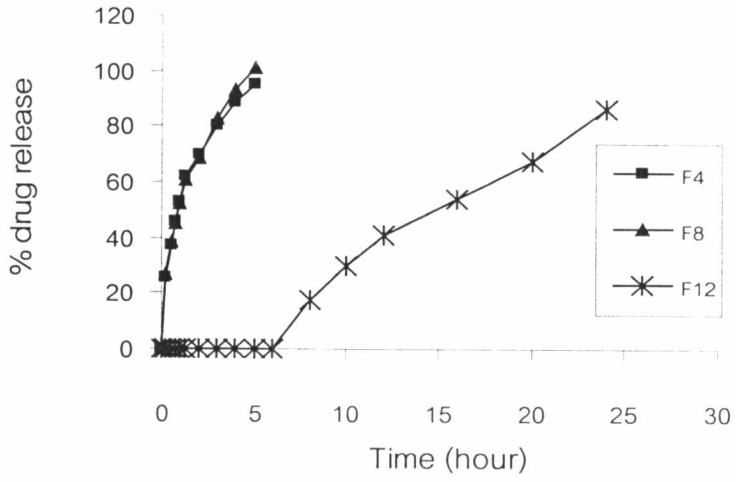


Figure 45 The comparison of release profiles of propranolol HCl from mucoadhesive microspheres prepared with HPMC (F4), chitossan (F8) and carbopol (F12).

6. In vitro permeation study across nasal cell culture model

Morphology of cell culture

To study the permeation of drug through cell culture model, the confluent cell monolayer and the tight junction between cells would be formed for being the barrier in this study.Therefore, the evaluation of morphology of cell culture, the actin staining and the measurement of transepithelial electrical resistance (TEER) values were the important studies to confirm the presence of monolayer and the tight junction in nasal cell culture model.

The nasal cells (RPMI 2650) were seeded at densities of 3.5-4.0 cells/cm^2 and allowed to attach within 24 h. The visible cell colonies were formed after 1 day and these colonies grew into an optical tight monolayer after 6-8 days under the cultivation conditions used. The cells remained unchanged until 10 days after seeding and then the cells began to detach. Phase contrast microscopy at that time point revealed the cuboidal surface of the cells as shown in Figure 45, indicating the epithelial origin of the cultured cells.

Actin staining and TEER measurement

The pressure of tight junctions was also demonstrated using FITC-labeled phalloidin. Phalloidin attaches to actin within the cells which allows visualization by fluorescence microscopoy. All cultured nasal cells showed intensive staining of actin fibers close to the cellular junction as shown in Figure 47. Furthermore, the development of tight junction was also verified by measurement of the transepithelial electrical resistance (TEER). The TEER value of this cell monolayer was 122.2 ± 10.2 ohm*cm^2 at day 7 and remained unchanged until day 10 before detaching from the filter. This value was close to the TEER of this cell measured in the other studies (114.4 ± 8.4 ohm*cm^2) (Schmidt, 1998).

The permeation study of FITC-labeled dextran (FD-4)

The nasal mucosa is known to form a tight absorption barrier especially for materials with a molecular weight above 1000 Da (Mc Martin, 1987). Although we observed well developed tight junctions, a quantification of the barrier function of the cultivated human nasal cell monolayer is necessary. For this reason, we studied the permeation rates of FD-4 as model substance (MW 4000) through nasal epithelial monolayer grown on filters in comparison to blank filters, as shown in Figure 48. The permeation rates of FD-4 through nasal cell monolayer and blank filter were 21.11 %/h (r^2 = 0.9928) and 6.14 %/h (r^2 = 0.9952), respectively. It was clearly observed that FD-4 substance showed a highly decrease in permeation rate due to the nasal cell monolayer.

The permeation study of propranolol HCl from spray dried microspheres

The profiles of in vitro permeation of drug across nasal cell culture model are illustrated in Figure 50. The permeation profiles of drug through cell culture of these microspheres were corresponding to the release profiles (Figure 49) of drug obtained from Franz diffusion cell. In the case of controlled microspheres (F0), about 73% of the drug was permeated across at the end of 5 h. The percent drug permeated was seen to decrease progressively with the polymer in formulation, and the type of polymer

clearly affected to the permeation of drug. Microspheres prepared with chitosan gave the permeation profile that closed to the profile of microspheres containing HPMC. The drug eluting out of the microsphere formulation containing carbopol (F12) was found to permeate more slowly. When combining carbopol with HPMC, propranolol HCl could be more permeated through the nasal cell than that of the microspheres containing carbopol alone. The obtained lag time in release profiles (Figure 32) from formulations containing carbopol (F12) and containing combined polymer (F15) was disappeared due to the presence of Hanks'balance salt solution (1.5 ml) in donor compartment of Transwell inducing rapid swelling of polymer in microspheres resulting in releasing of soluble drug from microspheres. This study concluded that the permeation of drug through the nasal cell was depended on the release of drug from microspheres that controlled by the polymer in formulation.

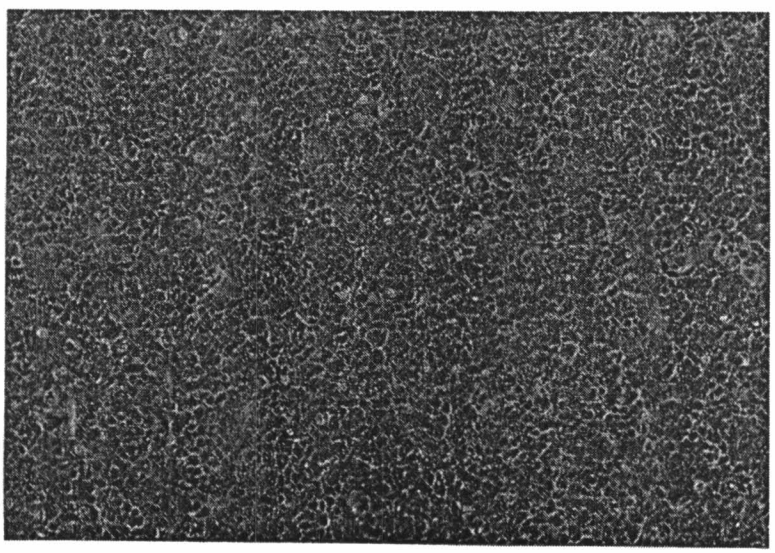


Figure 46 The photograph of human nasal epithelial cell monolayer after 7 days in culture (4x).

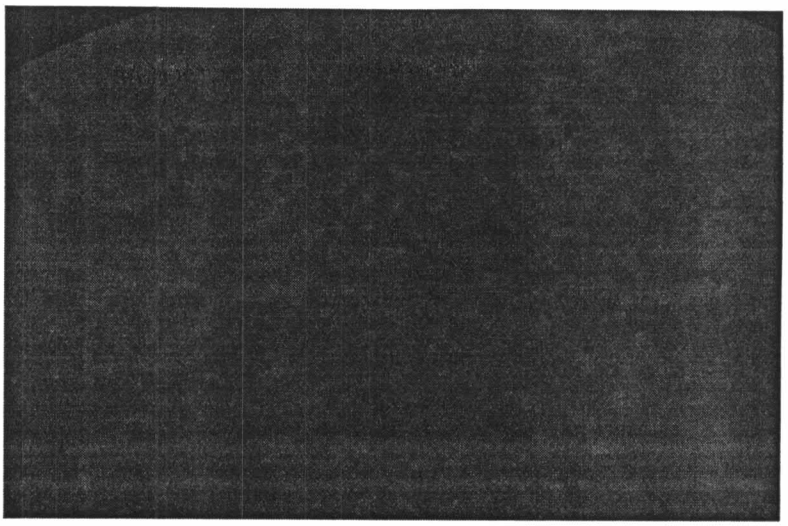


Figure 47 Actin staining of the monolayers after 7 days (10x), Tight junctions appearing as a fluorescent belt.

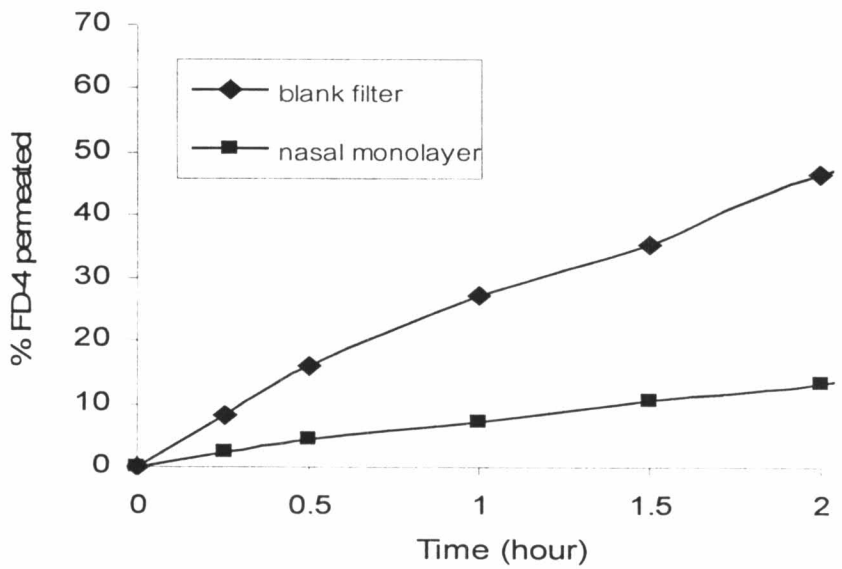


Figure 48 The permeation profile of FD-4 through nasal cell monolayer and blank filter.

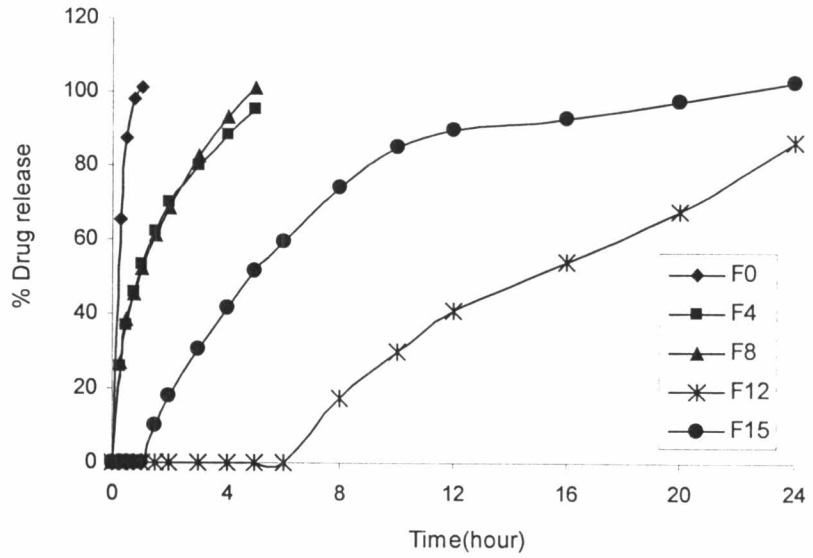


Figure 49 The comparison of release profiles of propranolol HCl from mucoadhesive microspheres ; F0(no polymer), F4(HPMC,1:7), F8(CHI,1:7), F12(CP,1:7), F15 (CP:HPMC,5:2)

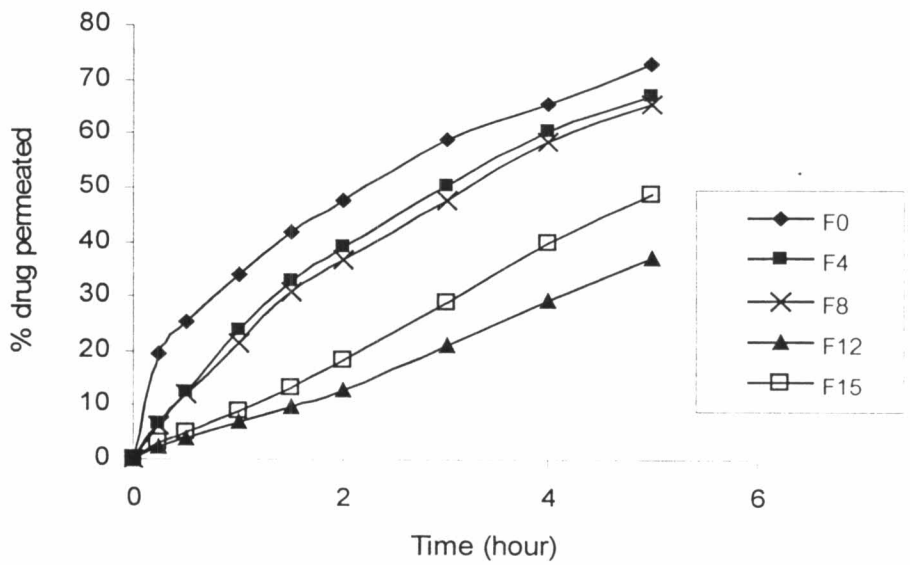


Figure 50 The permeation profile of propranolol HCl through nasal cell monolayer of spray dried microspheres.

7. Stability study

In this stability investigation, the accelerated storage conditions of high temperature and relative humidity were not performed, because the physical appearances of mucoadhesive microspheres were rapidly changed in a few days at accelerated condition (40°C, 75% RH). When studying at room temperature, 75% RH, bad appearance of microspheres was still observed. The microspheres were much aggregated each other and lost the suitable morphological appearance of microspheres. These expressed that high relative humidity had a much effect on the morphology of microspheres.

High moisture in microspheres clearly affected to the increase in aggregation of microspheres. The important factor that caused high aggregation of products might be the composition of formulation such as mucoadhesive polymer and maltodextrin. These compositions could highly adsorb the moisture from the environment, then polymer in microspheres could change from solid state to gel stage causing high stickiness around the surface of microspheres, inducing high aggregation.

Maltodextrin was sugar derivative that gave the stickiness when contacting with moisture resulting in the aggregation of microspheres. Therefore, the assessment of stability of microspheres needed to perform at room temperature and to keep products in desiccator (0% RH) for evaluating in long term. Selected microsphere formulations of F4, F8, F12 and F15 were evaluated as the representative of each type of polymer in formulations. These formulations showed the efficiency of each type of polymer in controlling the release of drug from microspheres.

Morphological examination

The SEM micrographs of microspheres in various formulations kept at room temperature in desiccator for 12 months are shown in Figure 51. The shape and surface morphology of microspheres prepared with single polymer as HPMC (F4), chitosan (F8) and carbopol (F12) were still spherical with smooth surface. The microspheres prepared with combined polymer (F15) showed almost spherical shape

and rough surface as similar to the morphology at initial time. In addition, the aggregation of microspheres was not observed. For carbopol microspheres the aggregation was not higher than that at initial time.

Particle size measurement

The size of microspheres prepared with single and combined polymers after storage at room temperature (0% RH) in 12 months are expressed in Table 29. The average particle sizes and size distributions of these microspheres were similar to the freshly prepared microspheres. The amounts of microspheres ranged from 10-50 μm, lower than 1.0 μm and larger than 200 μm after storage were similar to the initial time as shown in Table 30. The amount of microspheres in size larger than 200 μm was not increased, supporting that the aggregation of these microspheres were not much increased after storage in desiccator (0% RH) at room temperature for 12 months.

Drug Content

The drug content of selected formulations are summarized in Table 31. No statistically significant difference ($P>0.05$) in the content of the active drug occurred over a period of 12 months. The percentages of drug content of formulation F4, F8, F12 and F,15 were in the range of 5.17-5.20%, 4.47-4.53%, 4.93-5.12% and 4.99-5.20%, respectively.

Infrared absorption study

The FT-IR spectra obtained from selected formulations of microspheres after storge are shown in Figure 52, and the prominent peaks of spectra are tabulated in Table 32. The characteristic bands of propranolol HCl still appeared at wavenumber as freshly prepared microspheres.

The obtained data revealed that the formulations containing higher concentrations of hydrophillic excipient were more influenced by a high relative humidity. Then, the moisture proof packing are essential to ensure stability of these

formulations. This result was supported by Brabander et al. (2003) that studied the stability of mini-matrices prepared from hydrophillic polymer as hydroxypropyl methylcellulose (HPMC) in formulations.

In vitro evaluation of mucoadhesive properties.

The adhesion times of selected microsphere formulations are denoted in Table 33. The adhesion times of these formulations were not changed from the freshly prepared microspheres at the initial time. No statistically significant difference ($P > 0.05$) in these adhesion times over a period of 12 months was obtained. Microspheres containing HPMC (F4) still showed low mucoadhesion in the range of 9.74-11.03 min within 12 months. Chitosan microspheres (F8) gave the highest mucoadhesion with the adhesion time more than 300 min, and adhesion time of carbopol mirospheres (F12) was still lower than of microspheres prepared from combined polymer (carbopol/HPMC, F15).

In vitro drug release study

The stability of some selected microsphere formulations was also evaluated by performing the release of drug from microspheres. Similarity factors (f_2 values) were calculated based on the dissolution tests using the profile at time o as the reference (Figure 53). The release profiles of these microspheres after storage in 6 and 12 months are shown in Figure 54 to 57.

The results clearly indicated that the formulations were stable (f_2 values>50) for at least 12 months during storage at room temperature in desiccator (0% RH). The polymers in microsphere formulations could still control the release of drug from microspheres.

The total active drug was released from HPMC (F4) and chitosan (F8) microspheres within 5 hours as the release from freshly prepared microspheres. Formulation prepared from carbopol polymer (F12) could still prolong the release of active drug up to 24 hours, and still generated long lag time in the early stage (6

hours) of release profiles. And, combined polymer in formulation F15 could still reduce the lag time by the effect of HPMC for enhancing the release of drug from microspheres.

Table 29. The particles size and the span value of microspheres in stability study.

Formulation code	Storage time	D[4, 3] (SD) (μm)	Span (SD)
F4	Initial	19.60 (0.88)	2.19 (0.01)
	6months	18.79 (0.37)	1.69 (0.02)
	12 months	21.32 (0.73)	2.47 (0.05)
F8	Initial	15.60 (0.11)	2.28 (0.01)
	6months	14.47 (0.22)	2.15 (0.03)
	12 months	14.40 (0.24)	2.52 (0.06)
F12	Initial	15.92 (0.05)	1.36 (0.00)
	6months	17.08 (0.45)	1.24 (0.03)
	12 months	15.86 (0.06)	1.35 (0.05)
F15	Initial	15.40 (0.03)	1.58 (0.01)
	6months	14.58 (0.11)	1.41 (0.07)
	12 months	16.71 (0.75)	1.59 (0.03)

Table 30. The percentage of particle size distribution of microspheres in stability study.

Formulation code	Storage time (month)	Percentage of particles				
		<1.0 (μm)	1-9 (μm)	10-50(μm)	51-200 (μm)	>200(μm)
F4 (HPMC,1:7)	Initial	0.00	18.73	76.97	4.30	0.00
	6	0.00	17.56	77.13	5.31	0.00
	12	0.00	17.89	75.43	6.68	0.00
F8 (CHI,1:7)	Initial	9.95	19.08	70.37	0.60	0.00
	6	8.34	16.78	73.91	0.97	0.00
	12	10.03	18.34	70.11	1.52	0.00
F12 (CP,1:7)	Initial	0.00	14.75	84.25	0.87	0.10
	6	0.00	9.53	88.95	1.07	0.45
	12	0.00	12.03	85.32	2.27	0.38
F15 (CP:HPMC,5:2)	Initial	0.01	18.18	81.72	0.08	0.00
	6	0.00	17.74	80.95	1.31	0.00
	12	0.00	17.76	82.13	0.11	0.00

Table 31 Influence of the storage conditions on the drug content of microspheres.

Formulation Code	Drug Content (%)		
	Initial	6months	12months
F4	5.18(0.06)	5.20(0.08)	5.17(0.31)
F8	4.50(0.57)	4.53(0.26)	4.47(0.44)
F12	5.12(0.19)	4.93(0.13)	4,97(0.05)
F15	5.14(0.18)	5.20(0.26)	4.99(0.19)

Table 32 IR peaks of spectra of propranolol HCl in microspheres in stability study.

Formulation code	Storage time (month)	Principal peak (cm^{-1})					
F4	Initial	774	795	1107	1242	1271	1581
	6	774	797	1106	1243	1271	1580
	12	772	796	1105	1242	1271	1580
F8	Initial	772	794	1107	1242	1269	1580
	6	772	794	1107	1242	1270	1581
	12	772	796	1105	1242	1269	1579
F12	Initial	775	796	1108	1244	1269	1581
	6	771	797	1105	1243	1268	1580
	12	772	797	1106	1242	1269	1580
F15	Initial	776	797	1105	1242	1270	1581
	6	776	797	1105	1244	1270	1581
	12	774	796	1105	1242	1270	1581

Table 33 Influence of the storage conditions on the adhesion time of microspheres.

Formulation Code	Adhesion time (min)		
	Initial	6months	12months
F4	10.33(0.46)	11.03(1.18)	9.74(0.06)
F8	>300(-)	>300(-)	>300(-)
F12	90.67(3.16)	95.12(1.18)	92.33(2.53)
F15	286.78(2.31)	279.93(5.46)	284.79(2.05)

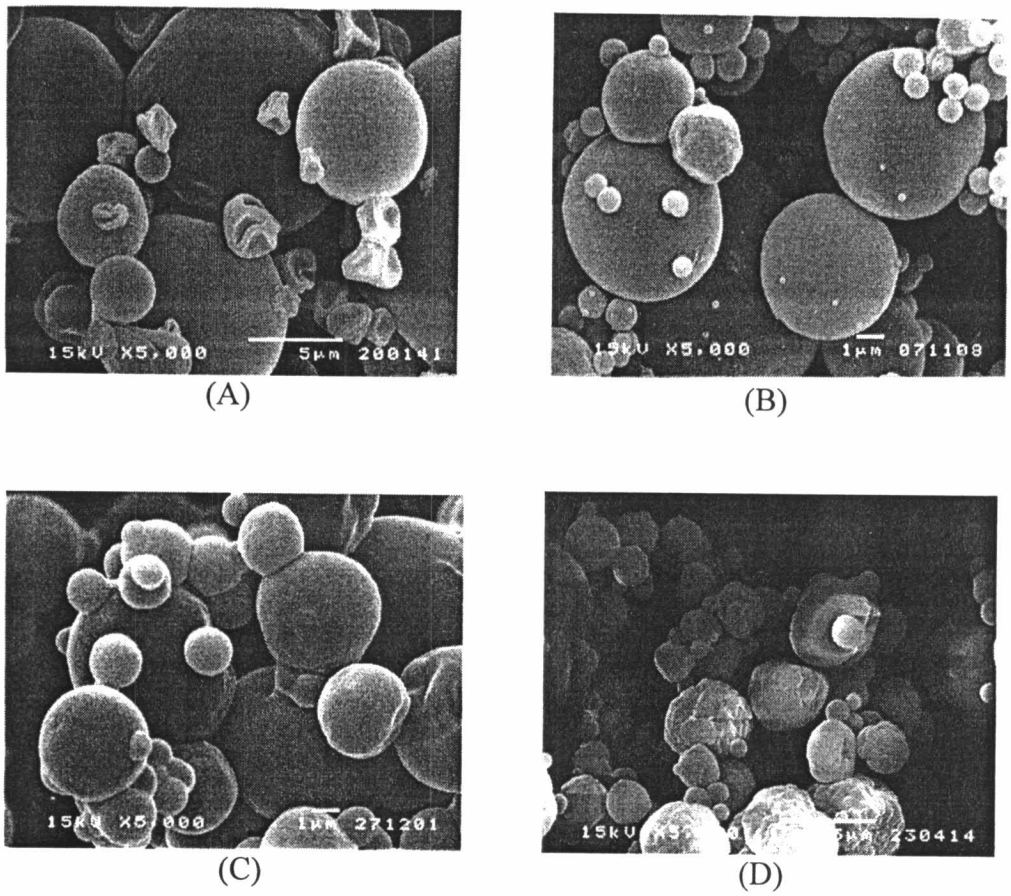


Figure 51. Scanning electron photomicrographs of microspheres prepared with (A) HPMC, (B) chitosan, (C) carbopol and (D) combined polymer (carbopol/HPMC) after storage for 12 months in stability study

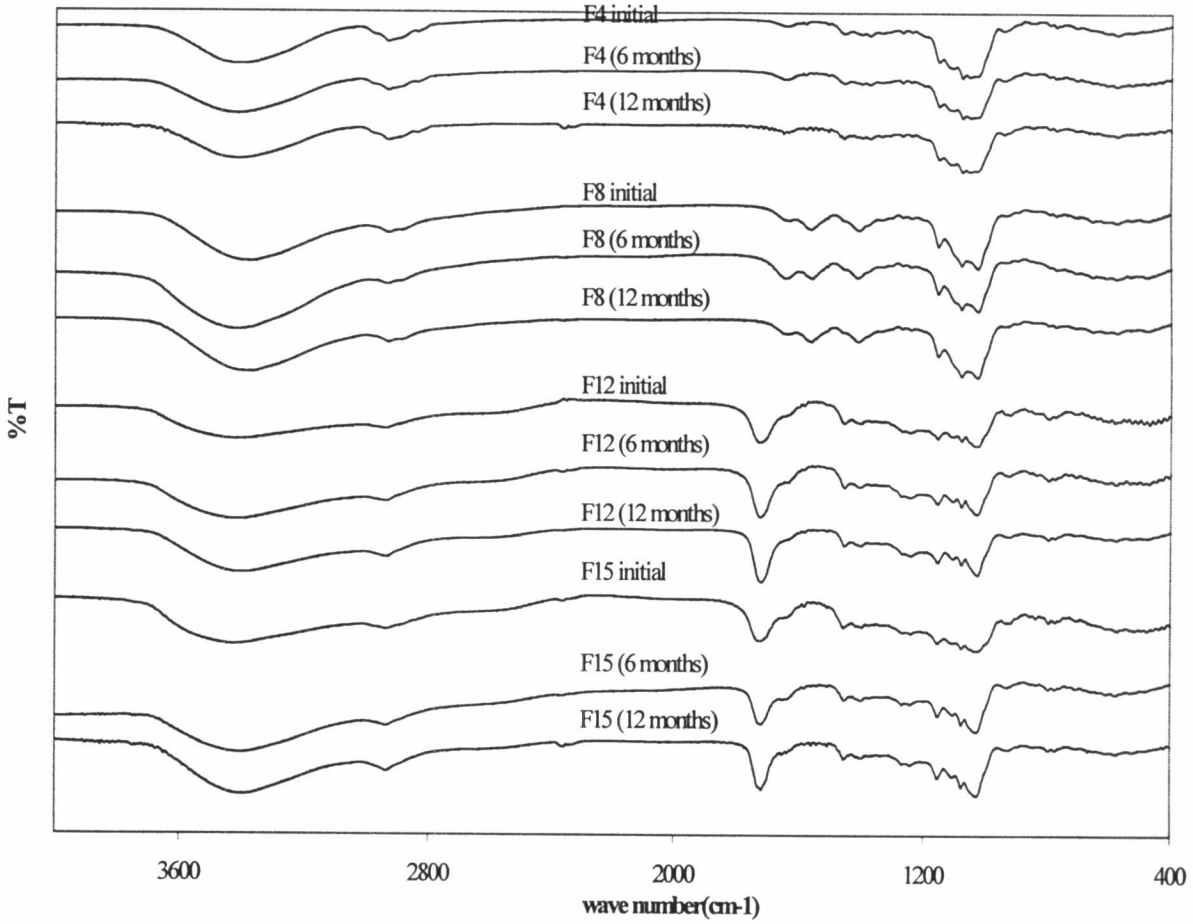


Figure 52 The IR spectra of propranolol HCl mucoadhesive microspheres obtained from various polymer under stability studies.

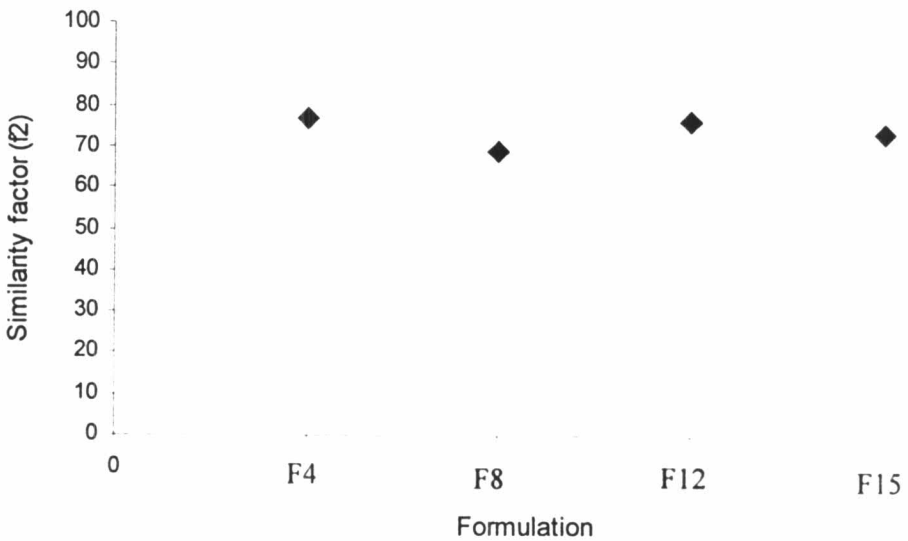


Figure 53 Similarity factors (f_2 – values) of formulation F4, F8, F12 and F15 calculated from the dissolution profiles of the microspheres.

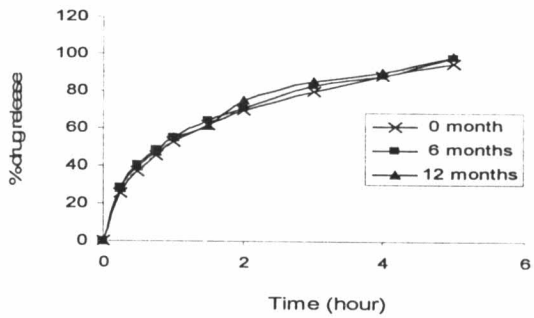


Figure 54 The release profiles of propranolol HCl from microspheres prepared with HPMC (F4) in stability study.

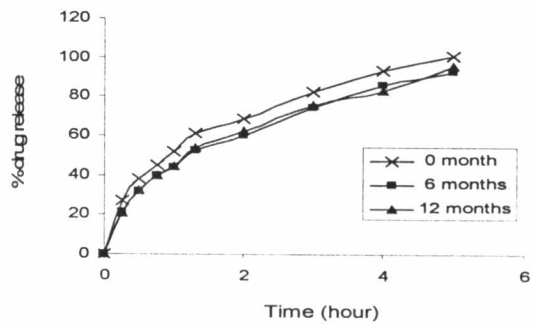


Figure 55 The release profiles of propranolol HCl from microspheres prepared with chitosan (F8) in stability study.

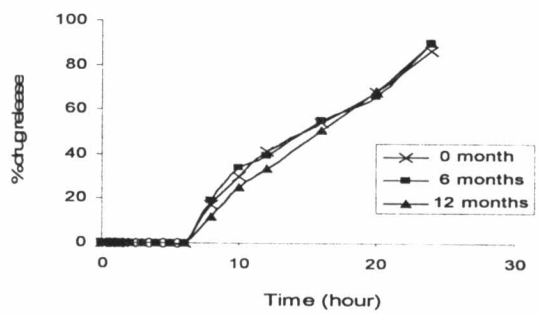


Figure 56 The release profiles of propranolol HCl from microspheres prepared with carbopol (F12) in stability study.

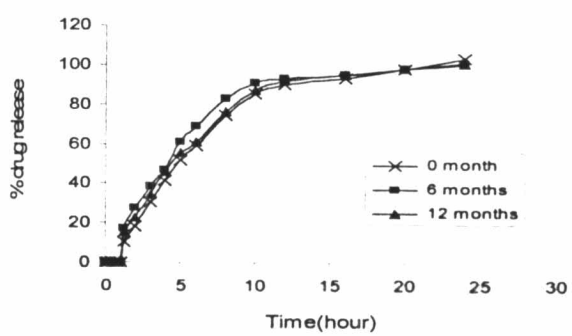


Figure 57 The release profiles of propranolol HCl from microspheres prepared with combined polymer (carbopol/HPLC) (F15) in stability study.