CHAPTER IV

RESULTS AND DISCUSSION

From the preliminary studies, appropriate types and concentrations of polymers were investigated to obtain patches that could easily detached from the disc after drying with characteristics of flexibility and no brittleness. Consequently, nine formulations of the bioadhesive polymers using both single and combined polymers were obtained for the studies. It has been conceded that a mucoadhesive which can be useful in prolonged oral drug delivery should ideally be nontoxic, nonabsorbable from the gastro-intestinal tract, preferably form a strong noncovalent bond with mucin-epithelial cell surfaces, adhere quickly to moist tissue, allow easy corporation of drug and offer no hindrance to its release, posses specific sites of attachment, and be economial (Jimenez-Castellanos, et al., 1993).

1. *In Vitro* Mucoadhesion Studies.

To evaluate the mucoadhesiveness, the method involved the measurement of the tensile strength, the vertical component of mucoadhesive strength, and mucoadhesive patch polymers. The vertical force used in pulling the test patch attached on the glass plate apart from the surrounding

artificial saliva was read in triplicate studies. The mucoadhesive forces for each patch formulation were calculated from equation 1 (Smart, Kellaway, and Worthington, 1984) as the percentage adhesive force (table 6).

$$\% \text{ Adhesive force} = \frac{\text{Adhesive force} \times 100}{\text{Blank}} \quad \text{.... eq. 1}$$

Table 6: Percentage adhesive force of miconazole mucoadhesive patches prepared from various polymers.

Formulation #(Polymer)	Sample 1	Sample 2	Sample 3	Mean	SD
1 (SCMC MV)	227.9471	227.4076	237.1190	230.8246	5.4578
2 *(SCMC MV + CP934)	297.8149	298.6242	294.5778	297.0057	2.1412
3 (SCMC HV)	314.2703	318.3167	314.8098	315.7989	2.1971
4 (MC 1500)	159.4281	160.7769	158.6188	159.6079	1.0902
5 (MC 1500 + CP934)	172.9161	173.1859	172.9161	173.0060	0.1559
6 (MC 4000)	171.5673	173.1859	169.6790	171.4774	1.7552
7 (MC 4000 + CP934)	182.6275	188.0227	186.4041	185.6847	2.7686
8 (HPMC)	164.8233	165.3628	166.9814	165.7225	1.1231
9 (HPMC +CP934)	188.0227	179.9299	178.0415	181.9980	5.3022

* = 3 min of contact with the artificial saliva

The mucoadhesive patches from SCMC HV (formulation #3) showed the strongest adhesive force. In formulations using single polymers, it was found that adhesive forces increased in the ascending order of MC 1500

(formulation #4) < HPMC (formulation #8) < MC 4000 (formulation #6) < SCMC MV (formulation #1) < SCMC HV (formulation #3). Table 7 shows the analysis of variance (ANOVA) of the percentage adhesive forces. According to the ANOVA table, the F^* statistic for testing the equality of percentage adhesive forces is 1139.8991 controlling the level of significance at 0.05, the F statistic required, F (0.95, 8, 15), is 2.48 in order to conclude that all preparations show equal adhesive forces. Since $F^* = 1139.8991 > 2.48$, the hypothesis that at least one of the formulations adheres at a different force is accepted.

Table 7: Analysis of variance of percentage adhesive forces of miconazole mucoadhesive patches containing various polymers.

Source	Degree of freedom (df)	S.S[a].	M.S[b].	F*[c]
Among group	8	83751.172	10468.8965	1139.8991
Within group	18	165.313	9.18406	
Total	26	83916.485		

Critical value (F) = 2.48 $\alpha = 0.05$ $df_1 = 8$ and $df_2 = 15$.

a SS = Sum of square

b MS = Mean square

c F^* = Variance ratio

Table 8: Comparison of percentage adhesive forces of miconazole mucoadhesive patches containing various polymers using Duncan's new multiple range test.

Formulation#	Difference between means	LSR	Statistical significance
1 VS 2	66.19	3.62	S
1 VS 3	84.98	5.46	S
1 VS 4	71.21	5.86	S
1 VS 5	57.81	5.62	S
1 VS 6	59.34	5.72	S
1 VS 7	45.14	3.62	S
1 VS 8	65.1	5.81	S
1 VS 9	48.82	5.46	S
2 VS 3	18.79	3.62	S
2 VS 4	137.40	5.90	S
2 VS 5	124.00	5.72	S
2 VS 6	125.53	5.81	S
2 VS 7	111.33	5.46	S
2 VS 8	131.29	5.86	S
2 VS 9	115.01	5.62	S
3 VS 4	156.19	5.93	S
3 VS 5	142.79	5.81	S
3 VS 6	144.32	5.86	S
3 VS 7	130.12	5.62	S
3 VS 8	150.08	5.90	S
3 VS 9	133.80	5.72	S
4 VS 5	13.40	5.62	S
4 VS 6	11.87	5.46	S
4 VS 7	26.07	5.81	S
4 VS 8	6.11	3.62	S
4 VS 9	22.39	5.72	S

Table 8: Comparison of percentage adhesive forces of miconazole mucoadhesive patches containing various polymers using Duncan's new multiple range test (continued).

Formulation #	Difference between means	LSR	Statistical significance
5 VS 6	1.53	3.62	NS
5 VS 7	12.67	5.46	S
5 VS 8	7.29	5.46	S
5 VS 9	8.99	3.62	S
6 VS 7	14.2	5.62	S
6 VS 8	5.76	3.62	S
6 VS 9	10.52	5.46	S
7 VS 8	19.96	5.72	S
7 VS 9	3.68	3.62	S
8 VS 9	16.28	5.62	S

S = significant at $P < 0.05$

NS = not significant at $P > 0.05$

Duncan's new multiple range test for testing the difference in values of a pair of percentage adhesive force is shown in table 8. Refering to the above rank order the least significant ranges (LSR), it was indicated that the formulations have different percentage adhesive forces.

Additionally, four formulations using cellulose derivatives combined with CP934 (SCMC MV+CP934; MC 1500 + CP934; MC 4000 + CP934; and HPMC + CP934) showed a significant increase in the adhesive forces when they were compared with their corresponding single cellulose derivative polymers ($p < 0.05$, Student's t-test in table 9).

From the studies, it was obvious that the mucoadhesives containing SCMC, with or without CP 934 showed high adhesive forces than the ones containing MC and HPMC (with or without CP 934). This may be attributed to the ionizable groups (figure 20) in molecules of SCMC and CP 934 which give greater electrostatic forces between polymers and mucosa. This agreed with the study of Park, Robinson, (1984) and Ranga Rao, Buri, (1989) which concluded that anionic polymers bound more effectively than neutral polymers and degree of binding was proportional to the charge density on the polymers.

The mechanisms of attachment of polycarboxylic acids to mucin mainly involved the polymer underwent swelling in water and this permitted

Table 9: Comparison of percentage adhesive force of single polymer patches with combined polymer patches using Student's t-test.

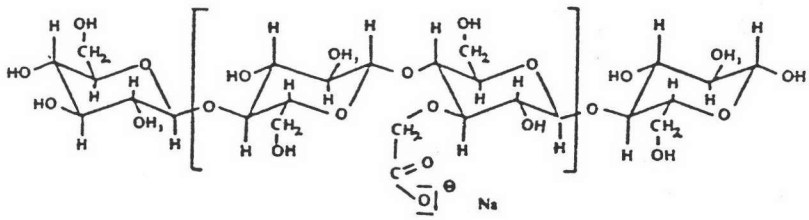
Formulation #	t-value (calculated)	df *	Pooled Variance	Statistical Significance
1 (SCMC MV) VS 2 (SCMC MV +CP934)	19.5521	2.61	17.1860	S
4 (MC 1500) VS 5 (MC1500 + CP934)	21.0718	2.08	0.6064	S
6 (MC 4000) VS 7(MC4000 +CP934)	7.5068	3.38	5.3729	S
8 (HPMC) VS 9 (HPMC+CP934)	5.2012	2.18	14.6875	S

* df = degree of freedom

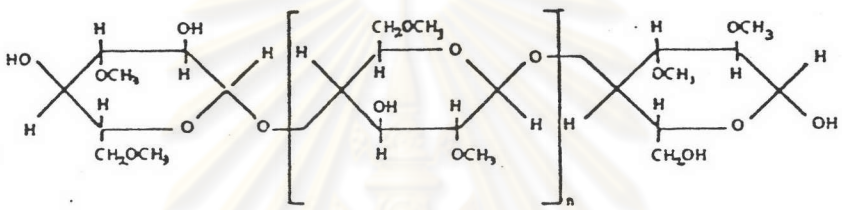
S = significant at $P < 0.05$

t-value from the table	df *
4.303	2
3.182	3

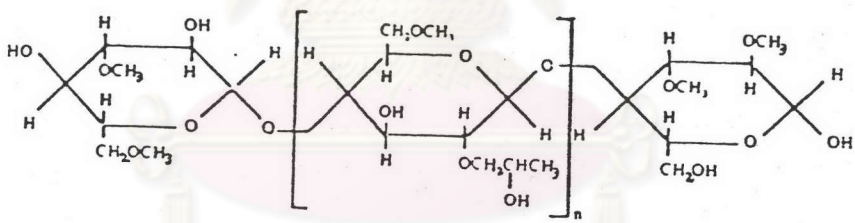
Sodium carboxymethycellulose



Methylcellulose



Hydroxypropylmethylcellulose



Carbopol

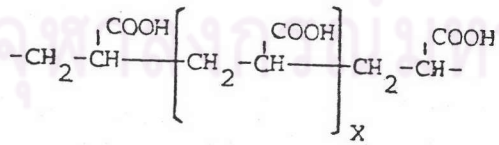


Figure 20: Structure of bioadhesive polymers.

entanglement of the polymer chains with mucous. The unionized carboxylic acid groups bonded to the mucin molecules by means of the hydrogen bonding (Jimenez-Castellanos, et. al., 1993).

Moreover, the mucoadhesives containing polymers of higher viscosity grades which were SCMC HV (formulation #3) and MC 4000 (formulation #6) showed greater adhesive forces than their corresponding polymers of lower viscosity grades which were SCMC MV (formulation #1) and MC 1500 (formulation #4).

As mentioned by the previous published study (Jimenez - Castellanos, et. al., 1993), it seemed that adhesive strength increased as the molecular weight of an adhesive polymer increased to 100,000 and beyond this level there was not much effect. Although a critical length of the molecules was necessary to produce the interpenetrating layer and molecular entanglements between the mucoadhesive and the substrate, one also had to consider the size and configuration of the adhesive macromolecules.

Adhesion properties varied according to the degree of hydration (Smart, 1991). It was indicated that the adhesion was maximum at a certain degree of hydration. When the degree of hydration was high, the adhesiveness was lost probably due to the formation of slippery, nonadhesive mucilage that apparently occurred in SCMC MV + CP934 (formulation #2). Thus in the experiment for SCMC MV + CP 934 mucoadhesives, the measurement was performed after only 3 min of contact with artificial saliva.

2. *In Vitro* Release of Miconazole from Mucoadhesive Patches.

Since the mucoadhesive patches studied had been dried before the release experiments were run, they were in their glassy states and there were no drug diffusion through the solid phase. After they had been immersed in an artificial saliva not containing mucin, the medium penetrated the matrices, the solvent-free polymers started swelling which were now in their rubbery states and they allowed the drug to diffuse outward. The release of drug was therefore by swelling-controlled mechanism.

Two fronts (interfaces) are characteristic of this swelling behavior: a front separating the glassy from the rubbery state (swelling interface) and a front separating the rubbery polymer from the dissolution medium (polymer interface). In the absence of physical or molecular restrictions to swellling, the polymer willl eventually dissolve as in the case of this study. This system was therefore classified as a swellable, erodible release system. For swelling-controlled systems, the solute release is actually controlled by the swelling phenomenon, namely by the relative position and velocity of the swelling interface.

The mechanism of drug diffusion in swelling-controlled polymeric formulations is dependent on the thermodynamic state of the polymer during release. Fickian or non-Fickian drug diffusion mechanisms may be observed depending on the dynamics of polymer swelling and on the relative mobility

of drug and dissolution medium (Roseman and Mansdorf, 1983). Ingeneral, as swelling of the polymer proceeds, macromolecular relaxations become important at the glassy/rubbery polymer front. These relaxations may, in turn, control the mode of diffusion and release of the drug. A comparison of the velocity of the swelling interface (V) to the diffusion coeffcient of the drug (D) may be designated as the swelling interface number (Sw) as follows:

$$Sw = V\delta(t)/D \qquad \text{....eq. 2}$$

The parameter $\delta(t)$ is the time-dependent thickness of the rubber (gel-like) layer. This Sw is a dimensionless number. When the rate of solute transport through the solvated region is faster than the rate at which the glassy/rubbery front advances, the swelling interface number Sw is much smaller than 1, and zero-order release kinetics of the drug is observed. Values of $Sw >> 1$ designate the case where the swelling front advances faster than the release of the drug. In this case, diffusion occurs through a "quasi-equilibrium" swollen gel, and Fickian release is observed. For values of $Sw \approx 1$, non-Fickian, non-zero-order release is observed.

Drug release rates from a glassy polymeric slab under countercurrent simultaneous diffusion of a swelling agent may be obtained from:

$$dMi/dt = AnCdkt^{n-1} \qquad \text{....eq. 3}$$

where Mi is the total amount of drug release at time t, A is the effective diffusional area, Cd is the initial loading of drug in the polymer, and n and k are constants characteristic of the slab/dissolution medium system. This equation describes the release kinetics of durgs which diffuse by Fickian mechanisms in non-moving-boundary problems. In this case, n = 0.5 and k = $4(Di/\pi\delta^2)^{1/2}$, where δ is the sample thickness. Moving boundaries and non-Fickian (anomalous) mechanisms are characterized by n > 0.5. A case of special interest is when n = 1. This limiting case of non-Fickian transport may be called case II transport of the drug; it is associated with zero-order release. In this non-Fickian case II diffusion, the diffusion coefficient depends strongly on both concentration and time in which the rate of solvent uptake into a polymer is largely determined by the rate of swelling and relaxation of the polymer chain. The rate of relaxation of the polymer chains in the swelling zone is the slowest step.

Since for many systems of interest the diffusion coefficient of the drug in the glassy region of the polymer is virtually zero, the drug concentration in the glassy region remains at its initial value, and a concentration gradient develops in the solvated region, The zero-order release is obtained when the concentration gradient is independent of t at x = 0 and the release rate can be calculate as

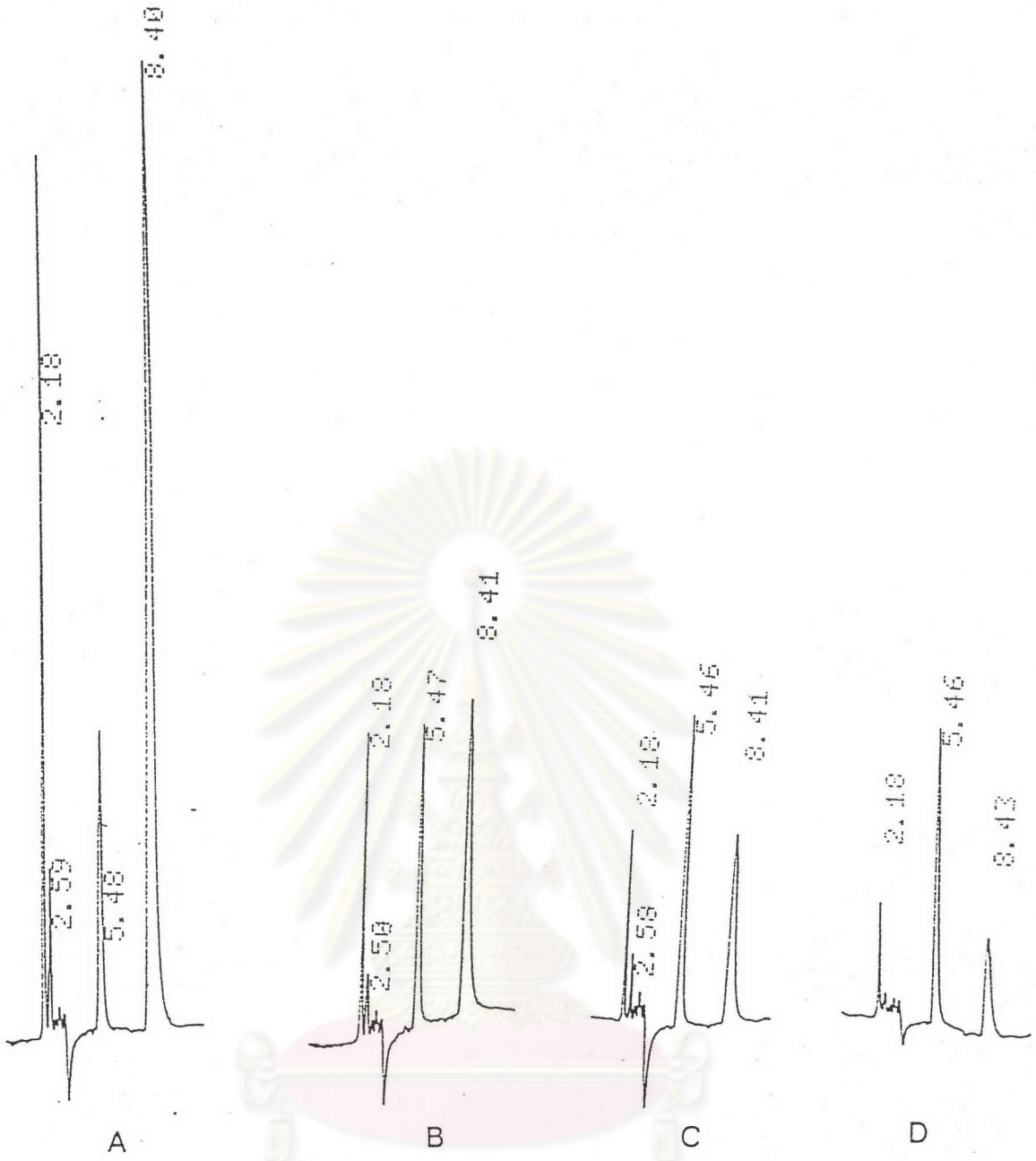
$$dMi/dt = -ADiCio/\delta(t) \qquad \text{....eq.4}$$

where Mi is the total amount of drug release at time t, A is the effective diffusional area, Di is the drug diffusion coefficient in the solvated polymer

and in the vicinity of x = 0 and Cio is the drug concentration at time t and the distance of x. This equation predicts infinite release rate at the beginning of the release experiment where δ(t) = 0. This mathematical conclusion is only a result of the pseudo-steady state approximation.

The release data in this study are presented in appendix II. The examples of chromatograms are shown in figure 21. The cumulative amount of drug release were plotted against time and square root of time and the results are also shown in appendix II. The plots and their correlation coefficients (table 10) shows that the plots against time are more correlated than the plots against square root of time. Therefore, the miconazole release from the mucoadhesive patches were likely to be zero-order release. As stated previously the rate of relaxation of the polymer chains in the swelling zone would be the slowest step in the sorption process. Consequently, the polymer which possesses the polymer chains with a faster relaxation rate would release the drug faster. In a macroscopic level, a polymer that could swell faster should release the drug faster.

The experimental rates of drug release were calculated from the slope of drug release profile and shown in table 11. The rank order of drug release rate from the mucoadhesive patches was obtained as follows: SCMC MV > SCMC MV + CP 934 > SCMC HV >MC 1500 > MC 1500 + CP 934 > MC 4000 >HPMC > MC 4000 + CP 934 > HPMC + CP 934. Table 12 shows the one-way analysis of variance (ANOVA) of the release rates.



Clotrimazole retention time ≈ 5.5 min and miconazole retention time ≈ 8.4 min. Each sample contained fix concentration of 0.4800 mcg/ml of clotrimazole as internal standard and various concentration of miconazole.

A = 3.5616 mcg/ml B = 1.1872 mcg/ml

C = 0.7123 mcg/ml D = 0.3562 mcg/ml

Figure 21: High performance liquid chromatogrram of clotrimazole and miconazole at 214 nm.

Table 10: Correlation coefficients of plots amount of drug release VS time and square root of time.

Formulation # (Polymer)	Correlation coefficient					
	Cumulative amount VS Time			Cumulative amount VS Square root of time		
	Run#1	Run#2	Run#3	Run#1	Run#2	Run#3
1(SCMC MV)	0.9965	0.9981	0.9976	0.9770	0.9882	0.9727
2(SCMC MV+CP934)	0.9973	0.9961	0.9894	0.9572	0.9506	0.9319
3(SCMC HV)	0.9922	0.9899	0.9961	0.9441	0.9508	0.9653
4(MC 1500)	0.9858	0.9811	0.9895	0.9930	0.9946	0.9894
5(MC 1500+CP934)	0.9793	0.9875	0.9927	0.9905	0.9938	0.9901
6(MC 4000)	0.9920	0.9890	0.9946	0.9865	0.9893	0.9830
7(MC 4000+CP934)	0.9896	0.9902	0.9963	0.9912	0.9891	0.9825
8(HPMC)	0.9918	0.9975	0.9907	0.9844	0.9716	0.9872
9(HPMC+CP934)	0.9962	0.9953	0.9982	0.9795	0.9808	0.9754

Table 11: Release rates of miconazole from mucoadhesive patches containing various polymers.

Formulation# (Polymer)	Run#1 (mcg/min)	Run#2 (mcg/min)	Run#3 (mcg/min)	Mean (mcg/min)	SD
1(SCMC MV)	4.8409	4.8249	4.5929	4.7529	0.1388
2(SCMC MV+CP934)	1.2089	1.1634	1.1117	1.1613	0.0486
3(SCMC HV)	0.9752	1.0430	0.9852	1.0011	0.0366
4(MC 1500)	0.3759	0.3849	0.4150	0.3919	0.0205
5(MC 1500+CP934)	0.3489	0.3781	0.4015	0.3762	0.0264
6(MC 4000)	0.3666	0.3648	0.3517	0.3610	0.0081
7(MC 4000+CP934)	0.3214	0.3241	0.3237	0.3002	0.0064
8(HPMC)	0.3502	0.3605	0.3718	0.3608	0.0108
9(HPMC+CP934)	0.2300	0.2513	0.2505	0.2439	0.0121

According to the ANOVA table, the F^* statistics for testing the equality of release rate is 2311.7117 controlling the level of significance at 0.05. The F statistics required, F (0.95,8,15), is 2.48 in order to conclude that all preparations release the drug at equal rates. Since $F^* = 2311.7117 > 2.48$, the hypothesis that at least one of the formulations release the drug at a different rate is accepted.

Table 12: Analysis of variance of drug release rates of miconazole mucoadhesive patches containing various polymers.

Source	Degree of freedom (df)	S.S.[a]	M.S[b].	F*[c]
Among group	8	50.2412	6.2802	2311.7117
Within group	18	0.0486	0.0027	
Total	26	50.2901		

Critical value (F) = 2.48; $\alpha = 0.05$, $df_1 = 8$ and $df_2 = 15$

[a] S.S. = Sum of square

[b] M.S. = Mean square

[c] F^* = Variance ratio

Table 13: Comparison of drug release rates of miconazole mucoadhesive patches containing various polymers using Duncan's new multiple range test.

Formulation#	Difference between means	LSR	Statistical significance
1 VS 2	3.5916	0.0621	S
1 VS 3	3.7518	0.0936	S
1 VS 4	4.3610	0.0963	S
1 VS 5	4.3767	0.0981	S
1 VS 6	4.3919	0.0996	S
1 VS 7	4.4527	0.1011	S
1 VS 8	4.3921	0.1005	S
1 VS 9	4.5090	0.1017	S
2 VS 3	0.1602	0.0621	S
2 VS 4	0.7694	0.0936	S
2 VS 5	0.7851	0.0963	S
2 VS 6	0.8003	0.0981	S
2 VS 7	0.8611	0.1005	S
2 VS 8	0.8005	0.0996	S
2 VS 9	0.9174	0.1011	S
3 VS 4	0.6092	0.0621	S
3 VS 5	0.6249	0.0936	S
3 VS 6	0.6401	0.0963	S
3 VS 7	0.7009	0.1005	S
3 VS 8	0.6403	0.0981	S
3 VS 9	0.7572	0.1005	S
4 VS 5	0.0157	0.0621	NS
4 VS 6	0.0309	0.0936	NS
4 VS 7	0.0917	0.0981	NS
4 VS 8	0.0311	0.0963	NS
4 VS 9	0.1480	0.0996	S

Table 13: Comparison of drug release rates of miconazole mucoadhesive patches containing various polymers using Duncan's new multiple range test (continued).

Formulation#	Difference between means	LSR	Statistical significance
5 VS 6	0.0152	0.0621	NS
5 VS 7	0.0760	0.0981	NS
5 VS 8	0.0154	0.0936	NS
5 VS 9	0.1323	0.0981	S
6 VS 7	0.0608	0.0936	NS
6 VS 8	0.002	0.0621	NS
6 VS 9	0.1171	0.0963	S
7 VS 8	0.0606	0.0621	NS
7 VS 9	0.0563	0.0621	NS
8 VS 9	0.1169	0.0963	S

S = significant at $P < 0.05$

NS = not significant at $P > 0.05$

Duncan's new multiple range test for testing the difference in values of a pair of release rates is shown in table 13. Refering to the above rank order the least significant range (LSR) indicate that the following formulations have different release rates:

formulation #1 (SCMC MV) VS formulation #2 (SCMC MV + CP 934)
foruulation # 2 (SCMC MV + CP 934) VS formulation #3 (SCMC HV)
formulation # 3 (SCMC HV) VS formulation # 4 (MC1500)

All mucoadhesive patches made of SCMC (formulation # 1, 2, and 3) showed significant faster release rates than other formulations. The explanation should be stemed from the ionic structure of SCMC. The structures of SCMC, MC, HPMC and CP are displayed in figure 20. While MC and HPMC are nonionic, SCMC is anionic due to its carboxylate group. The anionic repulsion of SCMC chain structure would result in a loose polymer matrix as an evidence of its very fast swelling when it was immersed in the artificial saliva. In addition, the polymer matrix of SCMC dissolved faster than the other polymers. Consequently, the faster swelling, the larger water filled void spaces, and the faster dissolution of polymer matrix should be responsible for the highest release rate of SCMC patches.

As far as the viscosity was concerned, the release rate of the patch made of SCMC MV (formulation #1) was faster than that made of SCMC HV (formulation #3); and the release rate of the patch made of MC 1500 (formulation #4) was faster than that made of MC 4000 (formulation #6). A

lower viscosity would result in a higher diffusion coefficient of drug and thus a faster release rate.

The inclusion of CP 934 into the mucoadhesive patches (formulation #2, 5, 7 and 9) lowered the release rates of their original formula (formulations # 1, 4, 6 and 8). Although the carboxylic group of CP 934 could aid in its hydration, its straight chain structure might entangle the ring structure of other polymers and resulted in a tighter matrix structure. Therefore, after the patches had hydrated and swelled, the polymer matrix dispersed and dissolved slower than the formulations not containing CP 934. Moreover, the formulations containing CP 934 were more viscous than the rest. As a result, CP 934 showed the lower release rate by increasing the viscosity of formulations and decreasing the rate of polymer erosion.

Except from the formulations made of SCMC, the others had rapid initial release rates. In other words, the slope of the initial portion of the release rate profile is steeper than the last portion. The reason is that the movement of drug from the degrading matrix is initially rapid because the diffusive path length is short. At a longer time period, this diffusive loss rate decreases with increasing path length. In the case of SCMC, because of its very loose structure and its very fast swelling, the polymer matrix almost does not impede the progress of drug penetration. Therefore, the penetation of drug occur throughout the polymer matrix with no difference in their diffusion coefficient even at the surface of matrix.

3. Stability Studies of Miconazole Mucoadhesive Patches.

Triplicate samples of nine miconazole mucoadhesive formulations were stored at 40 °C, 75-100% RH in amber glass vials for three months (Carstensen,1990). The amount of miconazole containing in mucoadhesive patches before and after exposure to the storage condition were analysed by the HPLC method as previously described in the *in vitro* drug release studies (appendixII). The percentage labelled amount of miconazole mucoadhesive patches were calculated and are shown in table 14. In addition, the percentage loss of miconazole after the exposure to heat and high humidity for three months were also calculated and are included in table 14.

Most of mucoadhesive formulations except the one containing SCMC MV + CP 934 (formulation #2) appeared to be stable due to their less than 10% loss of drug. It was apparent that the formulations containing SCMC MV, SCMC MV + CP 934 and MC 1500 + CP 934 (formulation #1, 2 and 5, respectively) degraded in a greater extent than other formulations. The results were attributed to the anionic character of SCMC and CP 934, which potentially affected the interation with miconazole molecules. As suggested by Nishikawa and Fujii (1991), hydroxy radicals catalyzed the degradation of miconazole. During the degradation of miconazole, not only hydroxy radicals but also some other radicals (alkoxy radical, peroxy radical, etc.) were probably formed.It was, however, still unknown how these radicals were involved in the degradation of miconzaole. Consequently, the

Table 14: Percentage labelled amount of miconazole mucoadhesive patches before and after storage at 40 °C and 75-100%RH.

		% Labelled amount					
Formulation# (Polymer)	Time (month)	Sample#1	Sample#2	Sample#3	Mean	SD	% Loss of miconazole*
1.(SCMC MV)	0	100.0571	97.5309	98.2116	98.5999	1.3071	
	3	92.7810	88.4339	86.9248	89.3799	3.0406	9.3519
2.(SCMC MV+ CP934)	0	102.1130	98.4618	98.5116	99.6955	2.0938	
	3	86.4005	93.0053	85.0424	88.1494	4.2598	11.5813
3. (SCMC HV)	0	96.9867	99.7115	99.4041	98.7008	1.4924	
	3	95.9632	91.1604	93.8915	93.6717	2.4089	5.0953
4. (MC 1500)	0	99.0896	101.3886	99.7176	100.0653	1.1883	
	3	97.4463	100.8092	96.0765	98.1106	2.4353	1.9533
5. (MC 1500 + CP934)	0	101.5643	97.8833	100.0857	99.8444	1.8523	
	3	85.4407	93.5854	90.6054	89.8772	4.1209	9.9828

* % Loss of miconzaole = $\frac{\text{Initial \% labelled amount - Final \% labelled amount}}{\text{Initial \% labelled amount}} \times 100$

Table 14: Percentage labelled amount of miconazole mucoadhesive patches before and after storage at 40 °C and 75-100%RH. (continued).

		% Labelled amount					
Formulation# (Polymer)	Time (month)	Sample#1	Sample#2	Sample#3	Mean	SD	% Loss of miconazole*
6. (MC 4000)	0	100.4959	102.4869	99.6613	100.8813	1.4517	
	3	99.6559	102.4859	95.6011	99.2476	3.4605	1.6194
7. (MC 4000 + CP934)	0	99.0857	103.8263	98.4255	100.4458	2.9461	
	3	88.9135	98.5337	98.6250	95.3574	5.5808	5.0659
8. (HPMC)	0	99.4367	102.0507	104.2095	101.8990	2.3900	
	3	97.6184	98.9118	100.1397	98.8900	1.2607	2.9529
9. (HPMC+ CP934)	0	99.7179	98.7583	103.7371	100.7378	2.6414	
	3	98.6695	95.0474	98.3855	97.3674	2.0143	3.3456

* % Loss of miconzaole = $\frac{\text{Initial \% labelled amount - Final \% labelled amount}}{\text{Initial \% labelled amount}} \times 100$

generation of these free radicals might be increased by SCMC and CP 934 and affected the stability of miconazole. Furthermore, according to the hygroscopic property of SCMC and CP 934, they might adsorb the surrounding moisture so that the weight of preparations increased (Reynodlds,1993). Thus, the storage of preparations in tightly closed containers was necessarily.

Finally, it was obvious that mucoadhesives containing MC 1500, MC 4000 and HPMC (formulation #4, 6 and 8 respectively) showed very little losses of miconazole and were thus recognized as very stable formulations.

4. ***In Vivo* Resident Time on Buccal Mucosa Studies.**

Six mucoadhesive formulations (formulation # 3, 4, 6, 7, 8 and 9), which were evaluated to have appropriate stability of miconazole, were selected for the *in vivo* resident time studies. The resident times of the patches that could maintain in buccal cavities of thirteen healthy volunteers are recorded in table 15. Table 16 shows the one - way analysis of variance (ANOVA) of the resident times. According to the ANOVA table, the F^* statistic for testing the equality of significance of 0.05, the F statistic required, F(0.95,5,60), is 2.37 in order to conclude that all preparations maintain in buccal cavity at equal time period. Since $F^* = 2.6441 > 2.37$, the hypothesis that at least one of the formulations shows a different resident time is accepted. Despite of attempts to control homogeniety of subjects and test conditions, the results obtained still varied individually. This is showed by standard deviations in table 15. The high standard deviations should

Table 15: Resident times on buccal mucosa of miconazole mucoadhesive patches.

Volunteer #	Resident time (min)					
	F #[a] 3 (SCMC HV)	F # 4 (MC 1500)	F # 6 (MC 4000)	F # 7 (MC4000 + CP 934)	F # 8 (HPMC)	F # 9 (HPMC+ CP 934)
1	80	270	170	150	140	300
2	210	135	80	180	105	180
3	120	60	120	120	120	180
4	45	30	30	70	110	180
5	120	20	20	120	60	150
6	180	240	300	300	180	180
7	60	80	60	60	50	140
8	60	60	120	75	20	300
9	100	185	185	290	300	120
10	20	60	50	120	60	105
11	160	180	180	120	60	250
12	70	20	90	180	170	120
13	45	150	120	180	150	240
Mean	97.69	114.615	117.31	151.15	117.31	188.08
SD[b]	57.58	84.64	77.32	75.56	74.01	65.69
CV[c]	0.589	0.738	0.659	0.500	0.631	0.349

a F # = Formulation #

b SD = Standard deviation

c CV = Coefficient of variation

Table 16: Analysis of variance of resident times of miconazole mucoadhesive patches containing various polymers.

Source	Degree of freedom (df)	SS[a]	MS[b]	F*[c]
Among group	5	70417.949	14083.590	2.6441
Within group	72	383500	5326.389	
Total	77	453917.949		

Critical value (F) = 2.37; α = 0.05, df_1 = 5 and df_2 = 60

[a] S.S. = Sum of square

[b] M.S. = Mean square

[c] F* = Variance ratio

result from uncontrollable factors concerning the habits regarding saliva flow, e.g., talking, jaw and tongue movement, etc (Anders, and Markle, 1989; Bottenberg, et. al, 1992). A rank order of mean resident times on buccal mucosa was as follows: HPMC + CP 934 (formulation #9) > MC 4000 + CP 934 (formualation #7) > HPMC (formulation #8) = MC 4000 (formulation #6) > MC 1500 (formulation #4) > SCMC HV (formulation #3).

Table 17: Comparison of resident times of miconazole mucoadhesive patches containing various polymers using Duncan's new multiple range test.

Formulation #	Difference between means	LSR	Statistical significance*
3 VS 4	16.93	57.28	NS
3 VS 6	19.62	60.32	NS
3 VS 7	53.46	63.56	NS
3 VS 8	19.62	62.34	NS
3 VS 9	90.39	67.77	S
4 VS 6	2.69	57.28	NS
4 VS 7	36.54	62.34	NS
4 VS 8	2.69	57.28	NS
4 VS 9	73.47	63.56	S
6 VS 7	33.85	60.32	NS
6 VS 8	0.00	57.28	NS
6 VS 9	70.77	62.34	S
7 VS 8	33.85	57.28	NS
7 VS 9	36.926	57.28	NS
8 VS 9	70.77	60.32	S

* S = significant at $P < 0.05$

NS = not significant at $P > 0.05$

Duncan's new multiple range test for testing the difference in values of a pair of resident times is shown in table 17. Refering to the above rank order, the least significant ranges (LSR) indicate that the formulations do not have different resident times.

The mucoadhesives containing SCMC HV (formulation #3) were observed to have the shortest resident time on mucosa; approximately up to 100 minutes. They showed quite rapid hydration and swelled to form a clear gel. After the hydration of the polymer, a slippery mucilage was formed. An adhesive joint failure was seemed to be a cohesive failure and rapid dissolution of the gel (Smart, 1991).

Formulation #9, which containing HPMC and CP 934, showed the longest mean adhesive duration on buccal mucosa. It was noted that the mixture of CP 934 with HPMC (formulation #9) and with MC 4000 (formulation #7) resulted in prolonged adhesion on mucosa. The results agreed with many previous published reports that CP 934 was a good bioadhesive agent and HPMC was a good hydrophilic matrix (Jimenez-Castellanos, et al., 1993). CP 934 in mucoadhesives was shown to contribute the adhesiveness due to the ability of polymer molecules to hydrate and form thick-viscous gel. As previously mentioned, the formation of viscous gel and the physical or mechanical bonding between the viscous gel and the mucosal membrane were the fundamental mechanisms of attachment of mucoadhesive patches (Deasy, and O'Neill, 1989).

Table 18: Comments of subjects about mucoadhesive patches.

		Percentage of comment					
Comments		Formulation #3 (SCMC HV)	Formulation #4 (MC1500)	Formulation #6 (MC4000)	Formulation #7 (MC4000+CP934)	Formulation #8 (HPMC)	Formulation #9 (HPMC+CP934)
Taste	Mild bitter	20.00	29.41	46.67	13.33	21.43	20.00
	Sour	6.67	-	-	26.67	-	6.67
	Salty	20.00	-	-	-	-	-
	Other	20.00	23.53	6.66	6.67	42.86	20.00
	No taste	33.33	47.06	46.67	53.33	35.71	53.33
	Total	100.00	100.00	100.00	100.00	100.00	100.00
Feeling	Discomfort	53.33	42.86	23.08	28.57	42.86	14.29
	Comfort	46.67	57.14	76.92	71.43	57.14	85.71
	Total	100.00	100.00	100.00	100.00	100.00	100.00
Irritation	No	100.00	100.00	100.00	100.00	100.00	100.00
	yes	-	-	-	-	-	-
	Total	100.00	100.00	100.00	100.00	100.00	100.00

Comments about taste, feeling, and irritation of volunteers are shown in table 18. From the studies, it might be concluded that miconazole mucoadhesive patches were physiologically accepted by the volunteers. There were no local or systemic adverse effects during the test or subsequently occured in subjects. After the detachment of patches, observation of the buccal mucosa of the attachment sites did not distinguish from the surrounding mucosa. The results also agreed with previous studies (Deasy, and O'Neill, 1989; Brook, et. al., 1989). However, there were a few complaints about discomforts such as dry mouth and increased salivary viscosity. It was due to a slow erosion of the patch surface whereby polymer particles spreaded in the oral cavity (Bottenberg, et. al., 1992). The taste of mucoadhesive patches resulted from the mild bitter taste of miconazole and probably the sourness and salty taste of CP 934 and SCMC, respectively.

Although formulation #3 (SCMC HV) showed the highest adhesive force among these six formulations but the shortest resident time whereas formulation #9 (HPMC +CP 934) showed the moderate adhesive force and the longest resident time. These results were due to the swelling and dissolution properties of polymers which were explained in 1, chapter IV.

From the results of the *in vitro* drug release and the *in vivo* resident time on buccal mucosa studies, it was found that, among six formulations (excluding the three relatively unstable formulations), the mucoadhesives containing SCMC HV (formulation #3) had the highest release rate and shortest resident time on mucosa, whereas the mucoadhesives containing

HPMC + CP 934 (formulation #9) had the lowest release rate and most prolonged resident time. It was interesting that the experimental periods of the *in vitro* release studies which extended over 6-7 h were longer than the resident times observed in the *in vivo* studies. This might be the effects of the movement of jaw and tongue in subjects that interfered adhesiveness on mucosa more intensively than that occurred by the *in vitro* test condition. As reported in previous studies (Ishida, et. al., 1983; 1983b), the study periods of the *in vivo* drug release in anesthesized golden hamsters could be extended to over 6 h that were longer than it was observed in this study.

However, for practical purposes, the resident times on buccal mucosa of longer than 3-4 h are seemed to be of no practical interest. Since the adhesiveness of patches will be conflicted with common eating intervals. Thus, a very prolonged resident time of mucoadhesive patches on buccal mucosa appears to be practical for only night time administration.