—Regular Article—

Evaluation of *Mimosa* Seed Mucilage as Bucoadhesive Polymer

Munish AHUJA,* Sumit KUMAR, and Monika YADAV

Drug Delivery Research Laboratory, Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hisar–125 001, Haryana, India

(Received January 6, 2010; Accepted March 19, 2010)

In the present study, buccal discs of fluconazole were prepared by direct compression method employing *Mimosa pudica* seed mucilage as bucoadhesive polymer. The formulation of buccal discs was optimised using 3-factor, 3-level central composite design, using polymer/excipient ratio, drug concentration and compression force as the independent variables and bioadhesion time and percentage release as dependent variables. The results revealed that polymer/excipient ratio and compression force are the significant factors affecting the bioadhesion time and percentage release, with the effect of polymer/excipient ratio being more pronounced. A quadratic model with backward elimination fitted to the data was used to predict the responses in the optimal region. The optimised formulation of buccal discs provided bioadhesion time and percentage release close to the predicted values. The proposed mathematical model is found to be robust and accurate for optimisation of buccal discs of fluconazole, consistent with the goals of bioadhesion time of 10 h and more than 85% release in 10 h.

Key words——buccal disc; bioadhesion; compression force; response surface methodology; fluconazole

INTRODUCTION

Natural gums, mucilages and their derivatives are widely employed in the pharmaceutical and food industries, as these are generally considered to be non-toxic and safe for human and animal consumption.[1] These natural polysaccharides are obtained from plant exudates and seeds of land and marine plant sources. Mucilages are produced by normal metabolic processes and are usually formed from the cell wall or deposited as layers on it. Chemically, mucilages are polyuronides, comprising sugar and uronic acid units.[2] The presence of hydroxyl and carboxyl groups in the mucilages favors adhesion to mucosal surfaces.[3] Buccal mucosa is easily accesible, richly supplied by blood vessels, and has low enzyme activity and relatively good permeability.[4] The buccal route of drug delivery has been conveniently employed for local[5,6] and systemic drug actions.[7] The main drawback of buccal delivery of drugs is the lack of adhesion of the delivery system at the absorption site. This limitation can be overcome by using bioadhesive dosage forms. In earlier studies, bioadhesive buccal patches,[8] gels,[9] films[10] and tablets[5–7] have been investigated for the buccal delivery of drugs.

During earlier studies mucilages of *Diospyros peregrina* fruit,[11] *Dillenia indica*[12] and *Sinapsis alba*[13] were evaluated as buccal mucoadhesives. *Mimosa pudica* (family: Mimosaceae) is a diffuse under-shrub, found widely distributed in the tropical and sub-tropical parts of India. Seeds of *Mimosa pudica* yield mucilage which is composed of d-xylose and d-glucuronic acid.[14] In an earlier study, *Mimosa pudica* seed mucilage was evaluated as a sustained release excipient.[15] In the present investigation, *Mimosa pudica* seed mucilage has been evaluated for bucoadhesive applications using fluconazole as the model drug. Fluconazole, an azole antifungal agent, was earlier evaluated in buccal film and discs for the topical treatment of oral candidiasis caused by *Candida albicans*.[6] The direct compression method was used to prepare fluconazole buccal discs, their formulation was optimised using an experiment which employed a three factor, 3-level Central Composite Design. Three independent variables, *viz.* polymer/excipient ratio (mucilage/lactose), concentration of drug, and compression force were studied for their effect on dependent variables like bioadhesion time and %release. Multiple response simultaneous optimisation using the desirability function was then used to find experimental conditions where the results of the system were optimal.

*e-mail: munishahuja17@yahoo.co.in

MATERIALS AND METHODS

Materials *Mimosa pudica* seeds were procured from the local market of Hisar (India) and authenticated by taxonomists of the Forest Research Institute (Dehradoon, India). A voucher specimen was deposited with the Department of Pharmaceutical Sciences, (authentication voucher no: Pcog/2007/65). Fluconazole was obtained as a gift sample from Aurobindo Pharmaceutical Industries (Mandal, AP, India). All other chemicals used were of reagent grade and were used as such.

Extraction of Mucilage *Mimosa pudica* seeds were soaked in a sufficient quantity of water for 10 h; the hydrated mucilage along with the seeds was spread in a thin layer on a stainless steel tray and dried in an oven at 50°C for 4–5 h. The dried mucilage was scraped from the tray by a blade and separated from the seeds by passing through No. 18 mesh, then further purified by winnowing to separate seed husk.

Experimental Design A central composite design with $\alpha=1$ was employed per the standard protocol.[16] The polymer/excipients ratio (mucilage/lactose) and amount of drug were selected as formulation variables, while compression force was chosen as the process variable on the basis of previous trials, and studied at 3 levels each. The central point (0, 0, 0) was studied in sextet. All other formulation and processing variables were kept invariant throughout the study. Table 1 summarizes an account of the 20 experimental runs studied, their factor combination, and the translation of the coded levels to the experimental units employed during the study. *Ex vivo* bioadhesion time and %release were taken as response variables. The experimental design and statistical analysis of the data were done using the Design Expert software (Version 7.1.6, Stat-Ease Inc., Minneapolis, MN).

Preparation of Buccal Discs The physical mixture of fluconazole, mucilage, and directly compressible lactose was homogenously mixed with mortar and pestle, and then the mixture was compressed for 30 s employing a 13 mm diameter die on an infrared hydraulic press (KP795, Kimaya Engineers, Thane, India) using different compression forces. To optimise formulation and process variables employed in

Table 1. Central Composite Design Using Formulation and Process Variables Influencing Bioadhesion Time (Y_1) and Percentage Release (Y_2)

Exp. No.	Mucilage/ Lactose X_1	Drug (mg) X_2	Comp. force 10^3(kg) X_3	Bioadh. time (h) Y_1	Release % Y_2	Friability (%)	Thickness (mm)	Drug content (%)	Average weight (mg)
1	−1(0.5)	−1(10)	−1(5)	8.2	92.1	0.86	0.93	98.5	160.1
2	1(2.0)	−1(10)	−1(5)	10.4	75.2	0.92	0.98	99.2	159.8
3	−1(0.5)	1(40)	−1(5)	8.4	98.2	0.88	1.20	99.4	189.6
4	1(2.0)	1(40)	−1(5)	10.5	74.0	0.84	1.29	99.5	189.8
5	−1(0.5)	−1(10)	1(10)	9.5	85.1	0.45	0.90	98.39	160.2
6	1(2.0)	−1(10)	1(10)	10.3	70.3	0.35	0.94	100.1	160.3
7	−1(0.5)	1(40)	1(10)	9.8	84.1	0.32	1.12	99.3	189.6
8	1(2.0)	1(40)	1(10)	10.2	71.2	0.45	1.20	100.3	190.8
9	−1(0.5)	0(25)	0(7.5)	8.7	96.0	0.62	0.96	100.0	175.1
10	1(2.0)	0(25)	0(7.5)	10.1	73.1	0.54	0.98	100.4	175.3
11	0(1.25)	−1(10)	0(7.5)	12.4	88.2	0.59	0.85	99.7	158.8
12	0(1.25)	1(40)	0(7.5)	12.5	82.3	0.58	0.83	99.6	190.1
13	0(1.25)	0(25)	−1(5)	11.2	87.1	0.78	0.86	99.6	160.0
14	0(1.25)	0(25)	1(10)	12.6	80.3	0.24	0.81	99.8	159.8
15	0(1.25)	0(25)	0(7.5)	12.5	85.2	0.56	0.78	99.2	160.2
16	0(1.25)	0(25)	0(7.5)	12.5	86.1	0.52	0.79	99.7	160.0
17	0(1.25)	0(25)	0(7.5)	12.8	87.1	0.54	0.78	99.4	161.2
18	0(1.25)	0(25)	0(7.5)	12.5	88.3	0.52	0.76	99.6	159.8
19	0(1.25)	0(25)	0(7.5)	12.6	84.6	0.51	0.77	100.1	161.3
20	0(1.25)	0(25)	0(7.5)	12.9	82.2	0.53	0.77	100.3	160.3

the preparation of buccal discs, various formulations were prepared employing the Central Composite Design as depicted in Table 1.

Evaluation of Buccal Discs The buccal discs of fluconazole were evaluated for friability, thickness, weight uniformity, content uniformity and *ex vivo* bioadhesion time and *in vitro* release. Physico-chemical interaction of fluconazole with excipients was studied using DSC and FTIR spectroscopy.

Disc Friability An accurately weighed sample of ten buccal discs was placed in the drum of a tablet friability test apparatus (Campbell Electronics, Mumbai, India). The samples were rotated at 25 rpm for 4 min, and were then reweighed. The percentage friability was calculated using the following equation.

$$F=\frac{W_1-W_2}{W_1}\times 100 \quad (1)$$

Disc Thickness Thickness of the discs was determined using digital vernier calipers (Aerospace, China). The results were reported as the average of six determinations.

Drug Content Uniformity Content uniformity of buccal discs was determined by dissolving the disc in 50 ml of phosphate buffer (pH 6.8), followed by filtration and appropriate dilution. The contents of fluconazole in the sample were determined by measuring the absorbance at 260 nm.

Weight Uniformity Ten discs of each batch were weighed individually using an electronic balance (AND, Japan) and standard deviation was calculated.

***Ex Vivo* Bioadhesion Time** The *ex vivo* bioadhesion time of buccal discs was evaluated by assessing the time taken by the discs to detach from a chicken pouch membrane in a well-stirred beaker. A freshly excised chicken pouch was obtained from a local butcher house (Hisar, India) within an hour of slaughter, and transported to the laboratory in cold (4°C) normal saline. The chicken pouch membranes were pasted on the side of the 250 ml beaker with cyanoacrylate glue. The buccal discs were attached to the chicken buccal tissue by applying light force with a fingertip for 60 s. The beaker was then filled with 200 ml of phosphate buffer (pH 6.8), kept at 37°C, and magnetically stirred at 150 rpm to simulate the buccal movement. The time taken by the discs to completely erode or detach from the tissue was taken as the indication of *ex vivo* bioadhesion time.

FTIR Spectroscopy The samples were subjected to FTIR spectroscopy in a Fourier-transform infrared spectrophotometer (Perkin Elmer Spectrum BX, USA) in a range of (4000 cm^{-1}–400 cm^{-1}) as KBr pellets.

Differential Scanning Calorimetry DSC thermograms of *Mimosa* mucilage, directly compressed lactose, fluconazole and buccal discs of fluconazole were recorded using a differential scanning calorimeter (Q10, TA Systems, USA) in the temperature range of (40°C—200°C) at a heating rate of 10°C per minute in nitrogen atmosphere.

***In Vitro* Release of Fluconazole from Buccal Discs** *In vitro* release of fluconazole from various batches of buccal discs was determined using phosphate buffer (pH 6.8) as the dissolution medium.[6] Each bioadhesive disc was adhered to the bottom of a vessel (250 ml beaker) using cyanoacrylate glue. Adequate sink conditions were provided by placing 200 ml of phosphate buffer (pH 6.8) in each vessel. The dissolution medium was stirred using a laboratory stirrer at 100 rpm. An aliquot of 5 ml sample was withdrawn at different time intervals, and replaced with an equal quantity of fresh dissolution medium. The withdrawn samples were filtered through 0.45 μ syringe filters, diluted appropriately and analysed for fluconazole content spectrophotometrically by measuring absorbance at 260 nm.

Statistical Analysis of the Data and Validation of Optimisation Model Various RSM computations for the current optimisation study were performed employing Design Expert software (Version 7.1.6, Stat-Ease Inc, Minneanopolis, MN). Polynomial models including interaction and quadratic terms were generated for all the response variables using a multiple linear regression analysis (MLRA) approach. The general form of the MLRA model is represented as Eq. 2:

$$Y=\beta_0+\beta_1X_1+\beta_2X_2+\beta_3X_1X_2+\beta_4X_1^2+\beta_5X_2^2 \quad (2)$$

where β_0 is the intercept representing the arithmetic average of all quantitative outcomes of 20 runs; β_1 to β_5 are the coefficients computed from the observed experimental values of Y; and X_1 and X_2 are the coded levels of the independent variables.[16] The terms X_1X_2 and Xi^2 ($i=1$ to 2) represent the interaction and quadratic terms, respectively. The interaction terms show how the response changed when two factors were simultaneously changed. The second-degree term (Xi^2) is included to investigate non-linearity.[17] Statistical validity of the polynomials was established

on the basis of ANOVA provisions of the Design Expert software. To study the combined effect of different variables on the response, 3-D response surface plots were generated using the Design Expert software. The optimum values of dependent variables to achieve the desired response were calculated using the numerical optimisation tool along with desirability approach.

RESULTS AND DISCUSSION

Table 1 presents the results of physical characterisation of fluconazole buccal discs. The discs were of uniform average weight and drug content. Their thickness was found to range from 0.76 to 1.29 mm, with a decrease in thickness observed with increase in compression force. Further, all the discs had friability of less than 1%. However, discs having higher mucilage/lactose ratio showed higher friability as compared to those having lower mucilage/lactose ratio. Earlier studies showed that the compression force and concentration of mucoadhesive polymer and drug influenced the bioadhesion time and release of drug from buccal discs.[6,18] In the present investigation, *Mimosa* seed mucilage was used as mucoadhesive polymer and directly compressible lactose as excipient. The use of directly compressible lactose was necessitated as it was observed during the trial runs that mucilage alone did not form discs of adequate strength. Thus, mucilage/lactose ratio, concentration of drug and compression force were selected as independent variables for the optimisation study, while *ex vivo* bioadhesion time and percentage release were chosen as the responses for this study.

The optimisation study was conducted using response surface methodology employing the central composite design (Table 1). The results of response generated using the experimental design were fitted into polynomial models and the ANOVA test was applied to the models to estimate their significance. The results of this analysis revealed that the response bioadhesion time (Y_1) and percentage release (Y_2) fitted best into the quadratic model with backward elimination.

The polynomial models for responses Y_1 and Y_2 can be represented by equations 3 and 4, respectively.

$$Y_1 = 12.45 + 0.69X_1 + 0.37X_3 - 0.39X_1X_3 - 2.84X_1^2 \quad (3)$$

$$Y_2 = 85.31 - 9.17X_1 - 3.56X_3 + 1.68X_1X_3 - 3.55X_3^2 \quad (4)$$

The polynomial equations comprise the coefficients for intercept, first-order main effect, interaction terms, and higher-order effects. The sign and magnitude of the main effects signify the relative influence of each factor on the response. A negative sign signifies an antagonistic effect while a positive sign indicates a synergistic effect.

Table 2 presents the result of ANOVA test on the quadratic regression model, which indicated that the response surface models developed for the three responses were significant and adequate, without significant 'lack of fit'. Table 3 details the model summary statistics for the selected significant models. It can be observed that all responses bear R^2 value $>$ 0.9, which indicates a good correlation between the experimental and predicted responses. In addition, the predicted R^2 value is in reasonably good agreement with adjusted R^2 value, resulting in reliable models. Further, the higher values (>4) of 'Adeq. Precision' indicate an adequate signal. The relatively lower values of coefficient of variation indicate better precision and reliability of the experiments carried out.

Table 4 presents the results of factor effects and associated p-values for the responses Y_1 and Y_2. The data reveals that significant factors affecting the response Y_1 were the synergistic effects of linear contribution of X_1 and X_3, while the quadratic contributions of X_1, and interaction effects of X_1 and X_3 antagonistically affected Y_1. The response Y_2 was significantly affected by the antagonistic effects of linear contribution of X_1, X_3, and quadratic contributions of X_3. The variable drug concentration (X_2) had no significant effect on any of the responses.

Table 2. ANOVA-influence of Formulation Variables on the Response Factors

Response factor	Model F-value	Prob>F	Lack of fit F-value	Prob>F
Bioadhesion time	125.62	<0.0001	4.14	0.0652
Release (%)	46.89	<0.0001	1.38	0.3827

Table 3. Model Summary Statistics

Response factor	St. Dev.	R^2	C.V. (%)	Adj. R^2	Pred. R^2	Adeq. precision
Bioadhesion time	0.31	0.9710	2.79	0.9633	0.9526	30.246
Release (%)	2.37	0.9259	2.84	0.9062	0.8641	21.489

Table 4. Summary of Each Factor Effect and Its p-Values

Factor	Y_1		Y_2	
	Factor effect	p-value	Factor effect	p-Value
X_1	0.69	<0.0001	-9.17	<0.0001
X_2	0.060	0.5383	-0.11	0.8956
X_3	0.37	0.0017	-3.56	0.0003
X_1^2	-2.84	<0.0001	-1.69	0.2135
X_2^2	0.25	0.1875	-0.72	0.6387
X_3^2	-0.21	0.2290	-3.55	0.0044
X_1X_2	-0.063	0.5779	-0.81	0.4302
X_1X_3	-0.39	0.0029	1.68	0.0640
X_2X_3	-0.013	0.9150	-0.62	0.4731

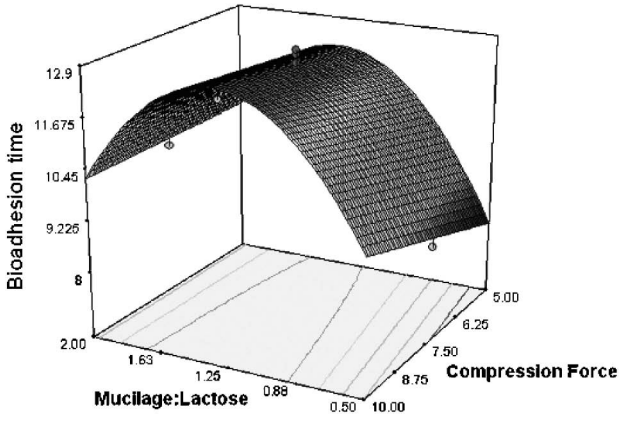

Fig. 1. Response Surface Plot Showing the Combined Effect of Mucilage/Lactose and Compression Force on Bioadhesion Time of Buccal Disc

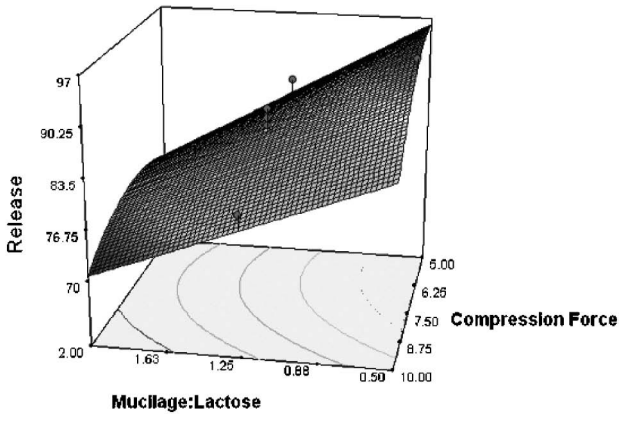

Fig. 2. Response Surface Plot Showing the Combined Effect of Mucilage/Lactose and Compression Force on Percentage Release of Drug from Buccal Disc

Figures 1 and 2 display the three-dimensional response surface plots constructed using the models generated by the response surface methodology. Figure 1 shows the combined effect of mucilage/lactose ratio and compression force on the *ex vivo* bioadhesion time of the buccal discs. It can be inferred from the plots that the ratio had a more pronounced effect than compression force on the bioadhesion time. Further, there is a significant interaction between the two variables with one variable tending to modify the effect of the other. As the proportion of mucilage, a mucoadhesive polymer in buccal discs is increased from the mucilage/lactose ratio of 0.5 to 1.25, there occurs an increase in bioadhesion time, although with further increase, bioadhesion time decreased. *Mimosa* mucilage rapidly hydrates in contact with water. Sustained release tablets prepared using *Mimosa* mucilage were earlier observed to swell with %swelling found to be proportional to the mucilage in tablets.[15] Thus, overhydration and formation of the slippery and loosely bound gel layer of mucilage can be attributed to decrease in the bioadhesion time[3] of buccal discs containing a higher proportion of mucilage.

Figure 2 exhibits the combined effect of mucilage/lactose ratio and compression force on the percentage release of drug. It can be observed that the mucilage/lactose ratio had a more prominent effect on percentage release than the compression force. The decrease in release rate of drug with increase in the relative amount of mucilage in buccal discs can be attributed to formation of a more viscous gel layer with longer path of diffusion, resulting in reduction in diffusion coefficient of the drug contributing to its slower release.[15]

A numerical optimisation tool using the desirability approach was employed to formulate the buccal discs of fluconazole with desired responses. The optimisation was done with constraints for bioadhesion time (Y_1), as maximum, and percentage release (Y_2) >80 % in 10 h as the goals to locate the optimum setting of independent variables in the new formulation. The optimal calculated parameters were- mucilage/lactose ratio of 0.54, drug concentration 18.27 mg, and compression force 10,000 kg.

Validation of Response Surface Methodology Results To check the reliability of the developed mathematical models, the response of the optimal formulation of buccal discs and three additional checkpoint formulations covering the entire range of experimental domain was recorded. For each of these test runs the experimentally determined response was

Table 5. The Experimental and Predicted Values for Response Y_1 and Y_2 along with Percentage Prediction Error Observed for the Optimum Test Condition and Random Checkpoints

Checkpoint Conditions $X_1/X_2/X_3$	Y_1			Y_2		
	Obser.	Pred.	Error (%)	Obser.	Pred.	Error (%)
0.54/18.27/10*	10.50	10.00	4.761	86.28	85.28	0.688
2/40/10	10.2	10.28	−0.784	71.2	70.71	−4.42
0.5/10/5	8.2	8.94	−4.439	92.1	96.17	3.386
1.25/25/7.5	12.5	12.45	0.40	88.3	85.31	1.583

* represent optimum conditions for buccal discs.

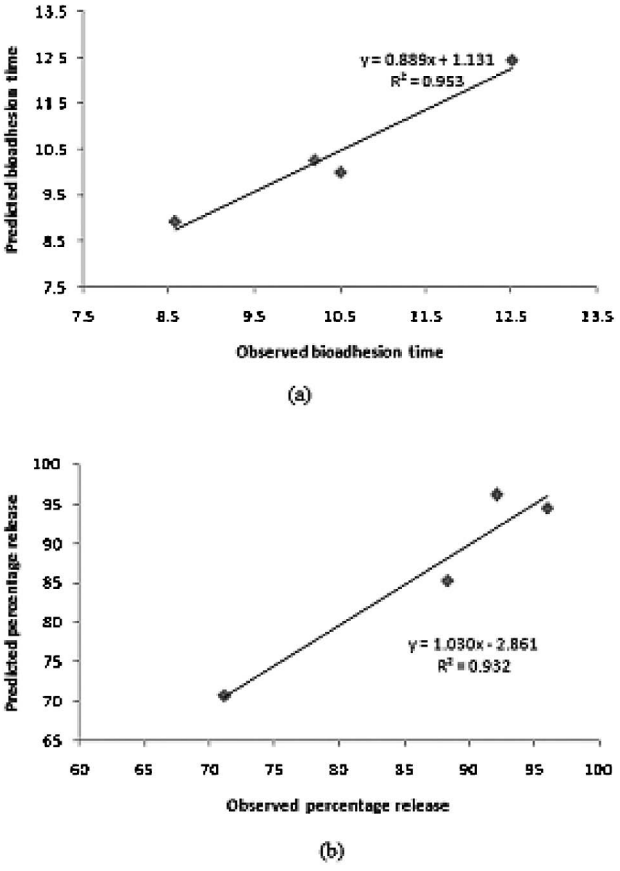

Fig. 3. Linear Correlation Plot (a) between Observed and Predicted Values for Bioadhesion Time, and (b) between Observed and Predicted Values for Percentage Release

compared with the response predicted by the mathematical models.

Table 5 lists the test conditions of the optimum and the random checkpoints, their experimental and predicted values for both the response variables, along with the calculated percentage prediction error. Figure 3 (a–b) shows the linear correlation plots between the observed and predicted response variables. These graphs demonstrate high values of correlation coefficient, R^2 (>0.9) indicating excellent goodness of fit. Thus, the lower magnitude of percentage prediction error (4.7% for Y_1, and 4.4% for Y_2) as well as significant values of R^2 in the current study indicates the robustness of the mathematical model and high prognostic ability of response surface methodology. The optimised buccal disc had maximum bioadhesion and percentage release.

Figure 4 displays the FTIR spectra of mucilage, fluconazole, lactose and buccal discs of fluconazole. The spectra of fluconazole shows an absorption band at 1139.91 cm^{-1} due to C-F stretching of difluronated benzene, a peak at 3019 cm^{-1} owing to CH stretching of the aromatic ring, and another at 2956.62 cm^{-1} which can be attributed to aliphatic CH stretching. The peaks appearing at 1421.67 cm^{-1}, 1515.86 cm^{-1}, and 1619 cm^{-1} are due to the ring stretching of thiazole, another peak at 967.38 cm^{-1} may be attributable to the ring breathing mode of triazole of the thiazole ring. The characteristic peak at 1249 cm^{-1} is due to the -OH bending vibration. The FTIR spectra of *Mimosa* mucilage show the characteristic peak at 3401 cm^{-1} owing to -OH stretching of alcohol, a peak at 1165 cm^{-1} owing to C-O stretching of alcohol, and at 2922 cm^{-1} which is due to C-H stretching of alkyl group. The characteristic peaks of fluconazole at 967.38 cm^{-1}, 1139.91 cm^{-1}, 1421.67 cm^{-1}, 1515.86 cm^{-1} and 1619 cm^{-1} also appeared in the spectra of buccal discs of fluconazole, indicating there was no chemical interaction between the drug and excipient.

Figure 5 displays the DSC thermogram of mucilage, lactose, fluconazole and buccal disc of fluconazole. The DSC curve of mucilage is representative of amorphous material showing a broad endotherm at 129℃ with the heat of fusion of 116.1 J/g. A thermogram of directly compressible lactose showed a sharp peak at 142.28℃, with heat of fusion of 113.6 J/g. A thermal curve of fluconazole showed a sharp endothermic peak at 140.39℃ with heat of fusion of 111.2 J/g. The DSC thermogram of buccal discs of fluconazole showed a broad endotherm at 69.24℃, with heat of fusion of 42.82 J/g, which appears to be depressed endotherm of mucilage, and another endothermic peak at 137.9℃ with heat of fusion of 59.68 J/g, which appears to be an overlapping contribution of endothermic peaks of fluconazole and lactose. Further, the diminished heat of fusion of this peak can be attributed to the dilution effect of

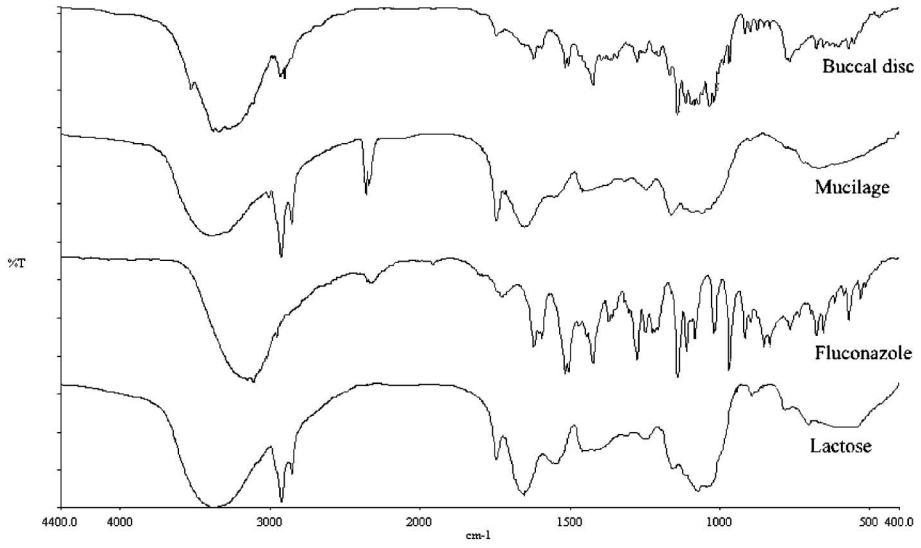

Fig. 4. FT-IR Spectra of Lactose, Fluconazole, Mucilage, and Fluconazole Buccal Disc

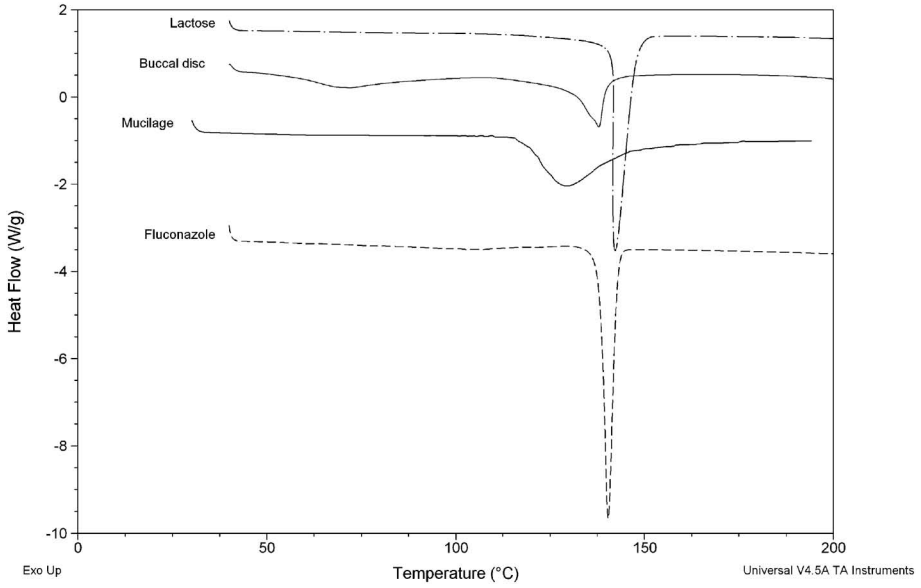

Fig. 5. DSC Thermogram of Mucilage, Lactose, Fluconazole and Buccal Disc

mucilage employed as mucoadhesive polymer in buccal discs.

CONCLUSION

Buccal discs of fluconazole were prepared by direct compression method, employing *Mimosa* seed mucilage as mucoadhesive polymer. Optimisation of formulation of the discs was done by response surface methodology using central composite design. It was observed that mucilage/lactose ratio has a more prominent effect on bioadhesion time and percentage release of drug from a disc than compression force while drug concentration showed no effect. The optimal formulation of fluconazole buccal disc had adequate *ex vivo* bioadhesion time and percentage release. Thus, results of the present investigation reveal that *Mimosa* mucilage is a promising mucoadhesive polymer for buccal delivery of drugs, although further studies in vivo are needed to comment more in this respect.

Acknowledgment The authors are grateful to Aurobindo Pharmaceutical Industries, (Mandal, AP, India) for the gift samples of fluconazole.

REFERENCES

1) Bhardwaj T. R., Kanwar M., Lal R., Gupta A., *Drug Dev. Ind. Pharm.*, **26**, 1025–1038 (2000).
2) ''Textbook of Pharmacognosy,'' 15th ed., eds. by Trease G. E., Evans M. C., Balliere Tindall, London, 2002, p. 195.
3) Smart J. D., *Adv. Drug Del. Rev.*, **57**, 1556–1568 (1995).
4) Sudhakar Y., Kuotsu K., Bandyopadhyaya A. K., *J. Controlled Rel.*, **114**, 15–40 (2006).
5) Ali J., Khar R., Ahuja A., Kalra R., *Int. J. Pharm.*, **283**, 93–103 (2002).
6) Yehia S. A., El-Gazayerly O. N., Basalious E. B., *AAPS PharmSciTech*, **9**, 1207–1217 (2008).
7) El-Samaligy M. S., Yahia S. A., Basalious E. B., *Int. J. Pharm.*, **286**, 27–39 (2004).
8) Li C., Bhatt P. P., Johnston T. P., *Drug Dev. Ind. Pharm.*, **24**, 919–926 (1998).
9) Shin S. C., Burn J. P., Choi J. S., *Int. J. Pharm.*, **209**, 37–43 (2000).
10) Khod Y., Kobayashi H., Baba Y., Yuasa H., Ozeki T., Kanaya Y., Sagara E., *Int. J. Pharm.*, **158**, 147–155 (1997).
11) Metia P. K., Bandyopadhyaya A. K., *Yakugaku Zasshi*, **128**, 603–619 (2008).
12) Metia P. K., Bandyopadhyaya A. K., *Pharmazie*, **63**, 270–274 (2008).
13) Sudhakar Y., Bandyopadhyaya A. K., *PDA J. Pharm. Sci. Technol.*, **62**, 97–110 (2008).
14) *Mimosa pudica*: 〈http://www.himalayahealthcare.com/herbfinder/h_mimosa.htm〉, Herbal Monograph, cited 2 June, 2008.
15) Singh K., Kumar A., Langyan N., Ahuja M., *AAPS PharmSciTech*, **10**, 1121–1127 (2009).
16) Singh B., Kumar R., Ahuja N., *Crit. Rev. Ther. Drug Carr. Sys.*, **22**, 27–105 (2005).
17) Singh B., Mehta G., Kumar R., Bhatia A., Ahuja N., Katare O. P., *Curr. Drug Deliv.*, **2**, 143–153 (2005).
18) Perioli L., Ambrogi V., Giovagnoli S., Blasi P., Mancini A., Ricci M., Rossi C., *AAPS PharmSciTech*, **9**, 274–281 (2008).