Preparation and *In Vitro* and *In Vivo* Evaluation of Glipizide Mucoadhesive Microspheres using Factorial Design

Avinash H. HOSMANI [1]* & Pramod V. KASTURE [2]

[1] *Department of Pharmaceutics, Government College of Pharmacy, Karad, Maharashtra State, India.*
[2] *Pdm. Dr. D.Y. Patil College of Pharmacy, Pune, Maharashtra State, India.*

SUMMARY. The purpose of the research was to prepare controlled release glipizide mucoadhesive microspheres with a coat consisting of sodium alginate and a mucoadhesive polymer Carbopol 971P. Orifice-ionic gelation method by using 3^2 factorial design were investigated with a view to develop mucoadhesive microspheres of controlled release. The resulting microspheres were discrete, free flowing, spherical and multinucleate monolithic type. Microencapsulation efficiency was in the range of 56-88%. Microspheres exhibit good mucoadhesive property in the falling film test. Glipizide release from the mucoadhesive microspheres was slow and extended over long period of time. Drug release was non-Fickian type. The concentration of carbopol 971P and sodium alginate had a more significant effect on different variables. *In-vivo* testing demonstrated a significant hypoglycemic effect of glipizide.

INTRODUCTION

Microencapsulation by various polymers and their applications are described in standard text books [1,2]. Microencapsulation and the resulting microcapsules have gained good acceptance as a process to achieve controlled release and drug targeting. However, the success of these microspheres is limited due to their short residence time at the site of absorption. It would, therefore be advantageous to have means for providing an intimate contact of the drug delivery system with the absorbing membranes [3-6]. This can be achieved by coupling mucoadhesion characteristics to microspheres and developing Mucoadhesive microspheres. Mucoadhesion is a topic of current interest in the design of drug delivery systems to prolong the residence time of the dosage form at the site of application or absorption and to facilitate intimate contact of dosage form with the underlying absorption surface to improve and enhance the bioavailability of drugs [5,6]. Several studies [7] reported mucoadhesive drug delivery systems in the form of tablets, patches, films and gels. There were very few reports on mucoadhesive microspheres.

Glipizide is a second-generation sulfonylurea that can acutely lower the blood glucose level in humans by stimulating the release of insulin from the pancreas and typically prescribed to treat type II diabetes (non-insulin dependent diabetes mellitus). Its short biological half-life (3.4 ± 0.7 h) necessitates the need to be administered in two or three doses of 2.5-10 mg per day [8]. The development of controlled release dosage forms thus, would clearly be advantageous. Researchers have formulated oral controlled release products of glipizide by various techniques [9,10]. Moreover; the site of absorption of glipizide is in the stomach. Dosage forms that are retained in the stomach would increase the absorption, improve drug efficiency and decrease dose requirements.

This study describes the development and evaluation of mucoadhesive microspheres containing glipizide employing carbopol-971P as a mucoadhesive polymer designed for oral con-

* Author to whom correspondence should be addressed. *E-mail:* havinash29@rediffmail.com

trolled release owing to its short biological half life of 3.4 ± 0.7 h. The mucoadhesive microspheres were evaluated *in vitro* and *in vivo* tests and factorial design was employed to optimize variables.

MATERIALS AND METHODS
Materials

Glipizide was supplied by ICPA Pharmaceuticals. Ltd., Ankaleshwar, Carbopol 971P was supplied by Noveon, Mumbai, Sodium Alginate and Calcium Chloride Dihydrate was purchased from Merck Lab Pvt. Ltd. Mumbai, All other chemicals used were of AR grade.

Preparation of mucoadhesive microspheres

Microspheres containing glipizide were prepared by using sodium alginate in combination with a mucoadhesive polymer, carbopol 971P. An orifice-ionic gelation process [11,12] that has been extensively used was employed to prepare the microspheres.

Orifice-ionic gelation method

Sodium alginate and the mucoadhesive polymer were dispersed in purified water (50 ml) to form a homogeneous polymer mixture. Glipizide (1 g) fine powder passed through mesh no. 120, added to the polymer premix and mixed thoroughly with a stirrer to form a smooth viscous dispersion. Resulting dispersion was then sprayed into calcium chloride (10% w/v) solution. The addition was done with continuous stirring. The added droplets were retained in the calcium chloride solution for 15 min to complete the curing reaction and to produce rigid spherical microspheres. The microspheres were collected by decantation, and the product thus separated was washed repeatedly with purified water to remove excess calcium impurity deposited on the surface of microspheres and then dried at 45 °C for 12 h.

Effect of variables

To study the effect of variables, batches were prepared by using 3^2 full factorial design. Particle size, swelling index, drug entrapment, mucoadhesion, drug release were selected as independent variables. Various batches prepared by using all possible combinations of different levels of experimental variables are listed in Tables 1 and 2.

Batch Code	Variable levels in Coded form	
	X1	X2
MMQ-1	-1	-1
MMQ-2	-1	0
MMQ-3	-1	+1
MMQ-4	0	-1
MMQ-5	0	0
MMQ-6	0	+1
MMQ-7	+1	-1
MMQ-8	+1	0
MMQ-9	+1	+1

Table 1. Different batches with their experimental coded level of variables for 3^2 Factorial Design.

Evaluation of mucoadhesive microspheres
Estimation of glipizide

Glipizide content in the mucoadhesive microspheres was estimated by a UV spectrophotometer at 275 nm in phosphate buffer (pH 7.4) [13]. The method was validated for linearity, accuracy and precision. The method obeyed Beer's law in the concentration range of 5-50 µg/ml. When the standard drug solution was analyzed repeatedly (n =5), the mean error (accuracy) and relative standard deviation (precision) were found to be 0.82 % and 1.4%, respectively.

Particle size and swelling index of microspheres

The particle size of the microspheres was determined by using optical microscopy method [14,15]. Approximately 100 microspheres were counted for particle size using a calibrated optical microscope. For estimating the swelling index 0.5 ml of microsphere bed in 5 ml 0.1 mol/ml HCl in a 10 ml measuring cylinder was soaked. Volume of microsphere bed was determined after 12 h. Swelling index was calculated by using the equation [**1**].

Variable levels	Low (-1)	Medium (0)	High (+1)
X_1= Concentration of Sodium Alginate (%w/v)	500 mg	750 mg	1000 mg
X_2= Concentration of Mucoadhesive Polymer (%w/v)	500 mg	750 mg	1000 mg

Table 2. Translation of coded levels in actual units.

$$\text{Swelling index = Volume after 12 h / Original volume} \quad [1]$$

Scanning electron microscopy

The microspheres were observed under a scanning electron microscope (SEM-Jeol Instruments, JSM-6360, Japan). They were mounted directly onto the SEM sample stub using double-sided sticking carbon tape and coated with platinum film. Scanning Electron photographs were taken at an accelerating voltage of 20 kV, chamber pressure of 0.6 mm Hg.

Drug entrapment efficiency

Microspheres (50 mg) were crushed in a glass mortar-pestle and the powdered microspheres were suspended in 10 ml phosphate buffer (pH 7.4). After 24 h. the solution was filtered and the filtrate was analyzed for the drug content. The drug entrapment efficiency was calculated as per the following equation [16].

$$\text{Microencapsulation efficiency} = \frac{\text{Estimated percent drug content}}{\text{Theoretical percent drug content}} \times 100 \quad [2]$$

Mucoadhesion studies by using falling film technique

This method is one of the suitable methods for testing mucoadhesion strength of mucoadhesive particulate system like mucoadhesive microspheres & suspension. In weight percent method, a fixed weight of microsphere sample was added (50 mg) over a fresh intestinal segment of sheep, mounted on a tilted slide with an angle of 45 °C and allowed to rest for 15 min. The effluent was run over the segment. The effluent was collected in a Whattman filter paper and weight of detached particle was determined. Percentage of mucoadhesion was determined by using the equation [3] [17].

$$\text{\% Mucoadhesion} = \frac{\text{Wt. of sample - Wt. of detached particles}}{\text{Wt. of sample}} \times 100 \quad [3]$$

Drug release study

Release of glipizide from the microcapsules was studied in phosphate buffer of pH 7.4 (900 ml) using a Dissolution Rate Test Apparatus with a rotating paddle stirrer at 50 rpm and 37 ± 1 °C as prescribed for glipizide tablets in USP XXIV. A sample of microcapsules equivalent to 10 mg of glipizide was used in each test. Samples of dissolution fluid were withdrawn at different time intervals and were assayed at 275 nm for glipizide content using a Shimadzu UV-1700 double-beam spectrophotometer (Shimadzu Corporation, Japan).

In vivo evaluation

In vivo evaluation studies for glipizide mucoadhesive microspheres 18 were performed on normal healthy Wistar rats weighing 250-300 g each. The approval of Institutional Animal Ethical Committee was obtained before starting the study. The study was conducted in accordance with standard institutional guidelines. Two groups of Wistar rats (5 in each group) that were fasted (with water) at least 12 h. before the experiments were used for the study. Before

drug administration, a blood sample (1ml) as a control was taken from each rat from behind the eyeball through the angle of ocular cavity using small capillary tubes. The blood glucose level for the control and test samples was determined. Pure glipizide suspension and mucoadhesive microspheres of glipizide were administered orally to each group using stomach intubations. A dose of 800 µg/kg of glipizide and mucoadhesive microspheres containing the equivalent amount of drug was administrated in a suspension form (freshly prepared) for each rat. Blood samples of 1ml were collected at predetermined time at 1, 2, 3, 4, 5, 6, 9, 12, 16, 20 and 24 h and the blood glucose level were estimated by using a glucose estimation kit. The % reduction in blood glucose level was measured.

RESULTS & DISCUSSION
Morphological characterization and particle size determination

Surface topography of microspheres is shown in Figure 1. Mucoadhesive microspheres of glipizide prepared were well-rounded spheres with ridges of shrinkage due to pres-
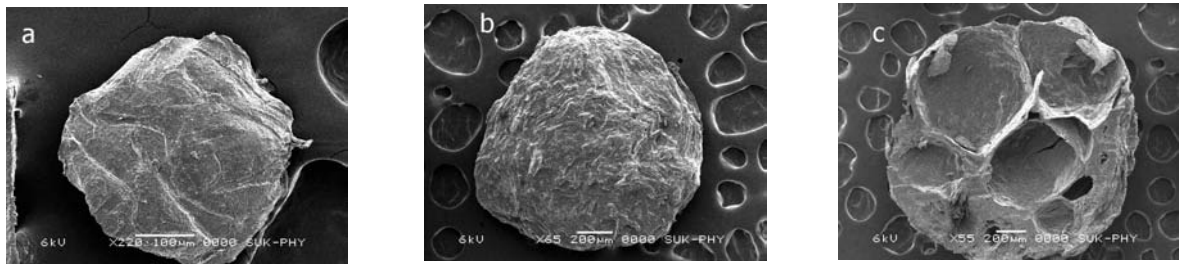
Figure 1. SEM Microphotograph of Glipizide Mucoadhesive Microspheres. (**a**) Plain Microsphere without mucoadhesive polymer, (**b**) Mucoadhesive Microsphere with Carbopol 971P. (**c**) Cutway view.

ence of carbopol 971P coat. The drug-loaded microspheres were spherical and yellowish white in appearance and whiteness gradually increases with increase in carbopol 971P concentration. Microspheres with a coat of mucoadhesive polymer were found to be discrete, spherical, free flowing and of monolithic matrix type. Under scanning electron microscope it was observed that there were lots of crystals scattered on the surface of microspheres. It was also observed that the inner part of microsphere was dense and porous. Crystals of glipizide adsorbed on the surface of microspheres might give a burst release and help enhance the glipizide concentration for effective reduction in a blood sugar level shortly after oral administration. The microspheres were uniform in size for each batch. Micromeritic property such as particle size is mainly governed by the polymer concentration. Particle size was in between range of 207 µm to 384 µm. Particle size increases with increasing polymer concentration. This may be due to increased viscosity of the dispersion, which affects the performance of spraying the mixture, causing formation of larger droplets.

3^2 *Full factorial design studies*

A statistical model incorporating interactive and polynomial terms was utilized to evaluate the responses: $Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2$, where Y is the dependent variable, β_0 is the arithmetic mean response of the nine runs, and β_1 is the estimated coefficient for the factor X1. The main effects (X_1 and X_2) represent the average results of changing on factor at a time from low to high value. The interaction terms ($X_1 X_2$) show how the response changes when two factors are simultaneously changed. The polynomial terms (X_1^2 and X_2^2) are included to investigate non-linearity. Multiple regression analysis and F statistics were used to identify statistically significant term. The results of multiple regression analysis are summarized in Table 3. Influence of formulation variables on evaluation parameters is discussed under the following sub headings.

% *Mucoadhesion and swelling index*

The falling film test for % mucoadhesion varied from 90 to 100% and showed good correlation coefficient (0.9873). Results of equation indicate that both the factors (X1 & X2) showed positive effect. At higher concentrations of both variables mucoadhesion increases, this may be attributed increase in particle size that causes increase in mucoadhesion.

The interactive term X1 and X2 show positive effect on mucoadhesion. Positive sign of X_2^2

Coefficient	Swelling Index	% Drug Entrapment	% Mucoadhesion	T_{75}(h)
β_0	1.755*	72.13*	96.2*	4.7*
β_1	0.3016*	8.475*	3.65*	1.105*
β_2	0.233*	4.48*	1.83*	0.5566*
β_{11}	0.035	-9.793	-1.35*	0.8916*
β_{12}	-0.01*	-2.609	0.1	0.0752
β_{22}	0.03	-0.803	0.2	0.4317*
R^2	0.9198	0.9129	0.9836	0.9571
F	7.4	8.6744	36.1692	53.0305
P	0.0000	0.0000	0.0000	0.0000

Table 3. Regression Analysis of Different Evaluation Parameters for Mucoadhesive Microspheres of Glipizide (n = 3). * Significant terms at P < 0.005. *n* is the number of samples tested for each experiment.
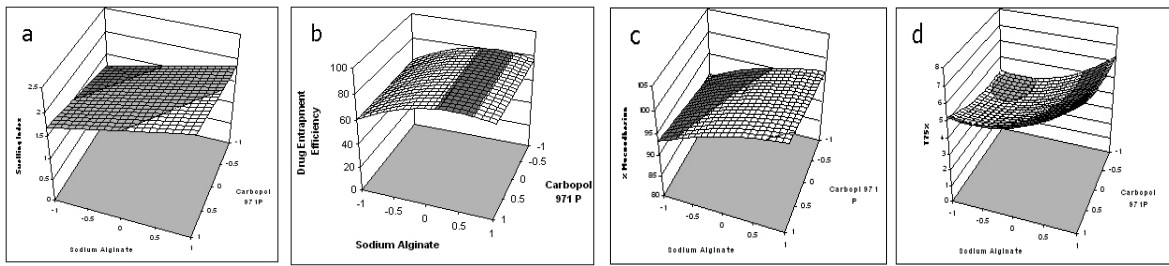
Figure 2. Surface graphs showing effect of variables on: (**a**) swelling of mucoadhesive microspheres; (**b**) drug entrapment efficiency; (**c**) mucoadhesion; (**d**) T75.

indicate positive linearity in the effectiveness of carbopol 971P as mucoadhesive material. The amount of polymer directly affected the solvent transfer rate and thus as the carbopol 971P concentration increases the swelling index also increases. Observation signified that the significant effect and negative sign of the term X_1X_2 indicates the swelling response was changing in a negative way, when both variables simultaneously changed. This may be due to higher water sorption capacity of carbopol 971P than sodium alginate. The swelling index varied from 1.2 to 2.4 (Fig. 2a) and showed correlation coefficient of 0.9198. Thus we can conclude that the amount of carbopol 971P and sodium alginate directly affects the % mucoadhesion and swelling index.

Drug entrapment efficiency and T_{75}

The drug entrapment efficiency and T_{75} (time required to release 75% of drug from dosage form) are important variables for assessing the drug loading capacity of microspheres and their drug release profiles, thus suggesting the amount of drug availability at the site. These parameters are dependent on the process of preparation, physicochemical properties of drug and formulation variables. Low coefficient of variation (< 2.0%) in percentage drug content indicated uniformity of drug content in each batch of microspheres. The drug entrapment efficiency was in the range of 56 to 88% (Fig. 2b) and yield was in the range of 92 to 96%. Results of equation indicate that encapsulation efficiency is a combined effect of both factors X1 & X2. But it is more significantly dependent on X2 than that of X1. Encapsulation efficiency increased with increasing concentration of carbopol 971P, but later on it decreases with increasing amount of carbopol 971P. Negative sign of X_1^2 and X_2^2 indicates non-linearity in effect on dependent factor by independent variable. This may be due to the achievement of
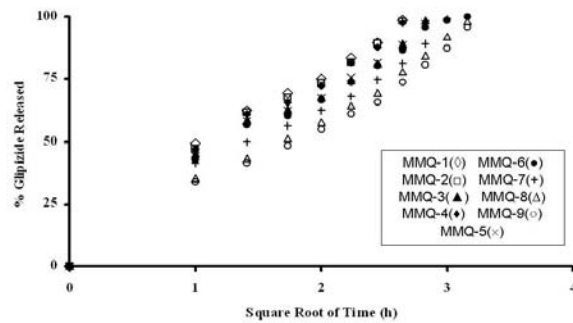

Figure 3. Percent release vs $\sqrt{t}$ plots of drug release from the mucoadhesive microspheres.

saturation concentration, thus there would be a competition for space between drug molecule and swelled carbopol 971P chains in the sodium alginate network of microspheres.

Figure 3 shows that the release studies of carbopol microspheres were found to be polymer concentration dependent. Both the factors contributed to the controlled release of drug. Concentration of carbopol 971P was found to be dominant factor than concentration of sodium alginate but increase in carbopol 971P concentration causes drug retardation up to 10 h. T_{75} was found to be lengthened as the concentration of carbopol 971 P increased.

Plots of log percent of drug remaining Vs time (Fig. 4) were found to be non linear with the correlation coefficient greater than 0.831. To evaluate drug release mechanism from the microspheres, plot of percent drug released Vs $\sqrt{t}$ were constructed. These plots (Fig. 4) after a lag period of 1 h. showed correlation coefficient (r) greater than 0.844 indicating that drug release mechanism from these microspheres was non-Fickian type. In the case of microspheres studied parameters such as porosity, tortuosity and diffusion path length are varying during the release process and hence zero order release was not observed. The release rate was increased as the size of microspheres was decreased and as the proportion of carbopol 971P was decreased.

Batch Code	MMQ-1	MMQ-2	MMQ-3	MMQ-4	MMQ-5	MMQ-6	MMQ-7	MMQ-8	MMQ-9
n	0.32237	0.33863	0.32004	0.33391	0.32439	0.32464	0.32961	0.33795	0.35251
k	55.3087	53.3263	53.5136	53.7928	53.8707	52.6567	51.1359	49.8814	47.8243
R	0.9938	0.9922	0.9834	0.9881	0.9887	0.9901	0.9803	0.9883	0.9846
T_{75}	2.572	2.737	2.871	2.705	2.773	2.972	3.196	3.342	3.583

Table 4. Analysis of release mechanism of Carbopol 971P Microspheres.
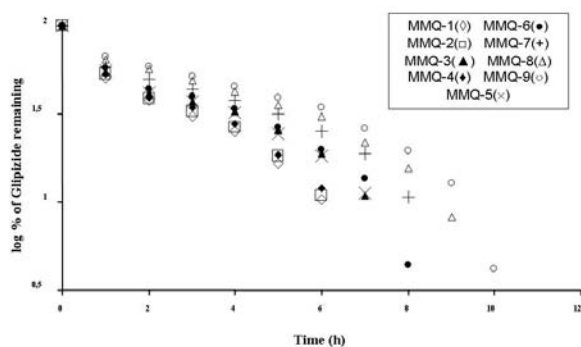
Figure 4. First order plot of drug release from mucoadhesive microspheres.

Figure 5. Percentage reduction in serum glucose following oral administration of Glipizide (■) and its mucoadhesive microspheres MMQ-6(▲) in normal rats (n = 5).

The 'n' values (Table 4) are less than 0.5, which is an indication of the non-Fickian release. Initially there is rapid release, which is followed by tailing off overtime. The dissolution profile was found to be of Peppas type. The initial faster release may be due to drug dissolution from the surface of microspheres. The factorial study of T_{75} indicates a correlation coefficient of 0.9571. The effect may be dependent on both the factors. The retardation may be due to the hydrogel structure of calcium alginate, which encapsulates the drug in its network and the swelling of carbopol 971P.

Carbopol 971P is a slightly cross-linked polymer and having a "fishnet" gel structure. It opens up easily at lower concentration. But at higher concentration, it has relatively less porous channels to exhibit the drug release. Hence diffusion from the swollen network is the rate controlling stage for drug release, thus higher T_{75} is obtained on increasing carbopol 971P concentration.

In vivo studies

The best batch MMQ-6 with respect to drug entrapment efficiency, mucoadhesion and drug release was selected. *In vivo* efficiency of these formulations was carried out in healthy normal Wistar rats by measuring the hypoglycemic effect produced after oral administration. When pure glipizide suspension was a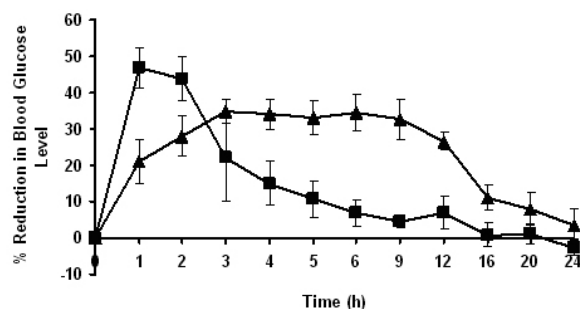dministered a rapid reduction in blood glucose levels was observed and maximum reduction of 46.91% was observed within 1 h. after oral administration and the blood glucose levels were also recovered rapidly to the normal level within 6 h (Fig. 5). In the case of glipizide mucoadhesive microspheres, the reduction in blood glucose levels was reached maximum within 3 h. after oral administration and this reduction in blood glucose levels was sustained over 12 h.

Kahn & Shechter [8] have suggested that a 25% reduction in blood glucose levels is considered as a significant hypoglycemic effect. Significant hypoglycemic effect (25%) was maintained only for 0.5 to 3 h after oral administration of pure glipizide. Whereas in the case of glipizide microspheres with carbopol 971P, significant hypoglycemic effect was maintained for a period of 2 to 12 h. Glipizide mucoadhesive microspheres are significantly more effective than immediate release formulation of glipizide in reducing fasting plasma glucose levels and side effects [19]. Formulation of glipizide as mucoadhesive sustained release dosage forms could also exhibit a decrease in side effects.

CONCLUSIONS

The results of a 3^2 full factorial design revealed that the concentration of carbopol 971P and sodium alginate significantly affected the dependent variables % mucoadhesion, swelling index, drug entrapment efficiency, and T_{75}. The mucoadhesive microspheres exhibited good mu-

coadhesive properties in an *in-vitro* test. Glipizide release from these microspheres was slow and extended over longer periods of time and dependent on composition of coat. Drug release was diffusion controlled and followed Peppas type of diffusion kinetics. In the *in-vivo* evaluation, glipizide mucoadhesive microspheres could sustain the hypoglycemic effect over a 12 h period. Thus the work resulted in the preparation and characterization of mucoadhesive microspheres were found suitable for controlled release.

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