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Research Article

Formulation and characterization of blend microsphere of Glipizide

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Abstract

The purpose of this research was to formulate and systematically evaluate in vitro and in vivo performances of mucoadhesive microspheres of glipizide. Glipizide microspheres containing chitosan were prepared using a simple emulsification phase separation technique, with glutaraldehyde as the cross-linking agent. Results of preliminary trials indicate that the volume of cross-linking agent, time for cross-linking, polymer-to-drug ratio, and speed of rotation affected the characteristics of microspheres. Microspheres were discrete, spherical, and free-flowing. The microspheres exhibited good mucoadhesive property in the in vitro wash-off test and also showed a high percentage of drug entrapment efficiency. A 32 full factorial design was employed to study the effect of independent variables, polymer-to-drug ratio (X1), and stirring speed (X2) on dependent variables percentage mucoadhesion, t80, drug entrapment efficiency, and swelling index. The best batch exhibited a high drug entrapment efficiency of 75% and a swelling index of 1.42; percentage mucoadhesion after 1 hour was 78%. The drug release was also sustained for more than 12 hours. The polymer-to-drug ratio had a more significant effect on the dependent variables. In vivo testing of the mucoadhesive microspheres in albino Wistar rats demonstrated a significant hypoglycemic effect of glipizide

Keywords: Chitosan, Mucoadhesive microspheres, Glipizide, Factorial design, In vivo study.

1. Introduction

Microsphere carrier systems made from the naturally occurring biodegradable polymers have attracted considerable attention for several years in sustained drug delivery [1]. Recently, dosage forms that can precisely control the release rates and target drugs to a specific body site have made an enormous impact in the formulation and develop an important parts of such novel drug delivery systems [2]. They have varied applications and are prepared using assorted polymers.4 However, the success of these micro spheres is limited owing to their short residence time at the site of absorption [3]. It would, therefore, be advantageous to have means for providing an intimate contact of the drug delivery system with the absorbing membranes.5-8 This can be achieved by coupling bioadhesion characteristics to microspheres and developing bioadhesive microspheres [4]. Bioadhesive microspheres have advantages such as efficient absorption and enhanced bioavailability of drugs owing to a high surface-to-volume ratio, a much more intimate contact with the mucus layer, and specific targeting of drugs to the absorption site[5]. Chitosan (obtained by deacetylation of chitin) is a cationic polymer that has been proposed for use in microsphere systems by a number of authors [6]. Chitosan was selected as a polymer in the preparation of mucoadhesive microspheres because of its good mucoadhesive and biodegradable properties [7]. 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 is typically prescribed to treat type II diabetes (non-insulin dependent diabetes mellitus). Its short biological half-life (3.4 6 0.7 hours) necessitates that it be administered in 2 or 3 doses of 2.5 to 10 mg per day[8]. Thus, the development of controlled-release dosage forms would clearly be advantageous. Researchers have formulated oral controlled-release products of glipizide by various techniques [9]. 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 [10]. Thus, an attempt was made in this investigation to use chitosan as a mucoadhesive polymer and prepare microspheres. The micro spheres were characterized by in vitro and in vivo tests, and factorial design was used to optimize the variables [11].

2. Materials and Methods

2.1 List of drugs and excipients

The list of drugs and excipients, their manufacturer and use in the present study are shown in Table

Table No.1-List of drugs and excipients used

Name of the material	Name of the company	Use in the formulation
Glipizide	Arvind Remedies, Chennai	Active Ingredient
Gelatin type B	Bafna Pharma, Chennai	Polymer
Liquid Paraffin	Merck Laboratories, Mumbai	Dispersion phase
Glutaraldehyde	Merck Laboratories, Mumbai	Cross link ingagent
Span 80	Merck Laboratories, Mumbai	Stabilis ingagent
Sodium hydroxide	IRP , Chennai	Reagent
Potassium dihydrogen orthophosphate	Merck Laboratories, Mumbai	Reagent
Petroleum Benzene 60 -80˚C	Nice Laboratories, Cochin	Solvent

2.2 Preformulation studies

The preformulation studies are the first step in the rational development of any formulation. It can be defined as "investigation of physical and chemical properties of drug substance alone and combined with the excipients [12]. The overall objective of preformulation testing is to generate information useful to the formulator in developing stable and bioavailable dosage forms that can be mass produced. The goals of the study are, to establish physical characteristics [13].

2.3 Drug- polymer compatibility studies

Fourier Transform Infra Red Spectroscopy, the compatibility between pure drug and polymer was detected by FT-IR spectra obtained. 1-2 mg of glipizide alone, a mixture of the drug and polymer was weighed and mixed properly with potassium bromide uniformly. The spectra were recorded over the wave number 4000-500cm^{-1} [14].

Table No. 2: Composition of drug and excipients for FTIR spectra

S. No	Ingredients
1.	Drug
2.	Gelatin
3.	Drug+ Gelatinmixture

2.4 Calibration curve for Glipizide

Preparation of pH 7.4 Phosphate Buffer An accurately measured 50ml of 0.2 M potassium dihydrogen orthophosphate was transferred to a 200ml volumetric flask and 39.14ml of 0.2M sodium hydroxide was added to it. Volume was made up to 200ml with distilled water, mixed and pH was adjusted to 7.4 with 0.2M sodium hydroxide or 0.2M orthophophoric acid [15].

Preparation of 0.2 M Potassium Dihydrogen Phosphate An accurately weighed 27.21g of monobasic potassium dihydrogen phosphate was dissolved in 1000ml of distilled water and mixed (16).

2.4.1 Preparation of 0.2 M Sodium Hydroxide Solution

An accurately weighed 8g of sodium hydroxide pellets were dissolved in 1000ml of distilled water and mixed.

2.4.2 Standard Curve in Phosphate Buffer pH 7.4

An accurately weighed amount of 100 mg of Glipizide was transferred into a volumetric flask and volume was made up to 100 ml with 7.4 pH phosphate buffer. The resulted solution had the concentration of 1mg/ml which was labelled as stock solution 1 [17]. From this stock solution 10 ml was taken and diluted to 100ml with 7.4 pH phosphate buffer which was given the solution having the concentration of 100µg/ml which was labelled as stock 2. Necessary dilutions were made by using second solution to give the different concentration of glipizide 2-10µg solutions can be made. The volumetric solution 10µg/ml was scanned in a UV-Visible double beam spectrophotometer to determine the λ max of the drug. The absorbance of the volumetric solution was recorded at λmax of 223nm and plotted graphically to the standard graph of glipizide [18].

2.5 Preparation of cross gelatin microspheres

Gelatin microspheres were prepared by an emulsification cross-linking method. 10 ml of 15% (w/v) aqueous gelatin solution preheated to 60˚C [19]. The specified quantity of Glipizide was dissolved in phosphate buffer pH 7.4 preheated to 60˚C. Then the mixture was added dropwise to 50 ml of liquid paraffin with 1% w/v span 80 preheated to 60˚C and emulsified by stirring with a magnetic stirrer at rpm 1000 [20]. Then the stabilized emulsion is allowed to cool and cross cross-linking agent glutaraldehyde was added and the stirring was continued at room temperature for 6 hours. The cross-linked microspheres were cooled and washed with Petroleum benzene to remove unreacted glutaraldehyde and liquid paraffin. After washing, the microspheres were dried at room temperature and stored in a dessicator [21].

Table No.3-Formulation table

Batch No	Glipizide (mg)	Gelatin(g)	Liquid Paraffin (ml)	Glutaraldehyde (ml)	Drug: Polymer ratio
F1	100	2.0	50	0.5	1:20
F2	100	1.5	50	0.5	1:15
F3	100	1	50	0.5	1:10
F4	100	0.75	50	0.5	1:7.5
F5	100	0.5	50	0.5	1:5
F6	100	0.25	50	0.5	1:2.5

2.6 Evaluation of microspheres

2.6.1 Percentage Yield

The total amount of dried microcapsules was weighed and the percentage yield was calculated by taking into consideration the total weight of the drug and polymer used for preparation of microspheres [22].

$$\text{Percentage Yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

2.6.2 Estimation of Drug Content

100 mg of microspheres was weighed and suspended in phosphate buffer pH 7.4. The suspension was suitably diluted with phosphate buffer pH 7.4 in 100 ml standard flask and filtered to separate the fragments [23]. Drug content was analyzed after suitable dilution by UV spectrophotometer at a wavelength of 223 nm against phosphate buffer pH 7.4 as blank. All the studies were carried out in triplicate [24].

$$\%\ EE = \frac{\text{Actual Drug Content}}{\text{Theoretical Drug Content}} \times 100$$

2.6.3 Drug Loading Capacity

Drug-loaded microspheres were digested with phosphate buffer pH 7.4, at room temperature for 12 h. After filtration and suitable dilution, Glipizide present in the solution was determined.

Drug loading (%) =

$$\frac{\text{Actual drug content in weighed quantity of powder}}{\text{Weighed quantity of the microspheres}} \times 100$$

2.6.4 Particle Size Analysis

Particle size analysis was carried out by using optical microscopy. About 200 microspheres were selected and their size was determined by using optical microscope fitted with standard micrometer scales. All the studies were carried out in triplicate [25].

2.6.5 Scanning Electron Microscope

Morphological examination of the surface of microspheres was performed using a scanning electron microscope (SEM) (Hitachi S-3400, Japan). The sample was sputtered with gold and the observations were made under vacuum [26].

3. Results and discussion

3.1 Drug-Polymer Compatibility Studies

3.1.1 FTIR Spectroscopy

The compatibility between drug and polymer was confirmed by using FTIR spectroscopy. Infrared spectroscopic analysis for drug (Glipizide), Gelatin, Drug-gelatin mixture was carried out. The principal IR peaks of pure Glipizide and Gelatin are shown in table 5.

Table No. 4- FTIR spectrum interpretation

Sample	Characteristics bands	Possible Functionalities
Glipizide	3000-3700cm^{-1} 2700-3300cm^{-1} 1650-1700cm^{-1} 1650-1700cm^{-1} 1334-1442cm^{-1} 1157cm^{-1} 650-900cm^{-1}	NH-Stretching C-H stretching C=O stretching -CONH stretching C-H bend (Cyclohexane) S=O stretching C-H bend (Benzene)
Gelatin	1689cm^{-1} 1527cm^{-1} 1157cm^{-1}	C=O stretching vibrations N-H bending vibrations C-N stretching vibrations

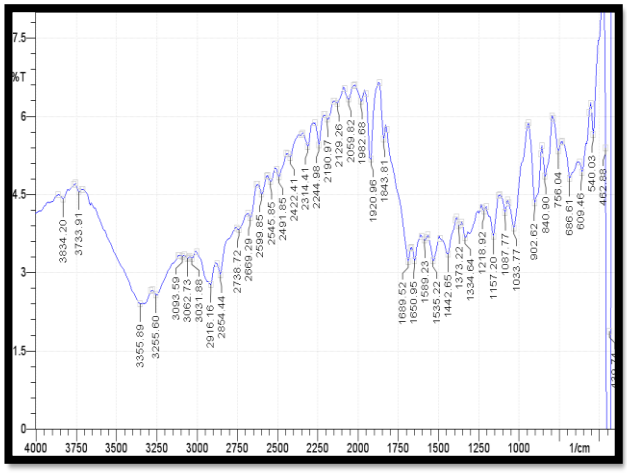

Figure 1: FTIR Spectra of Glipizide

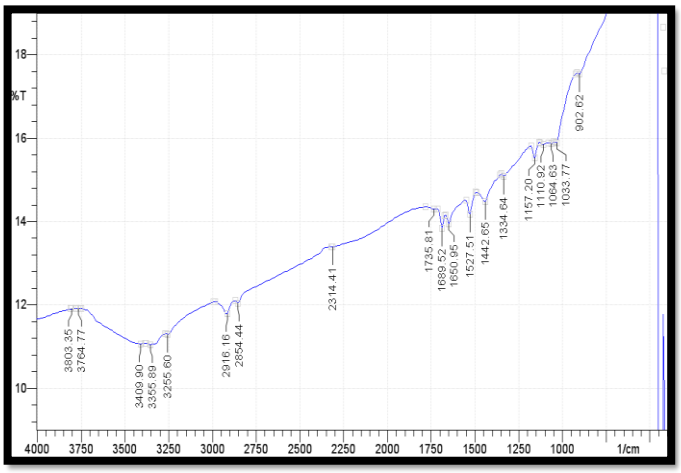

Figure 2: FTIR Spectra of Gelatin

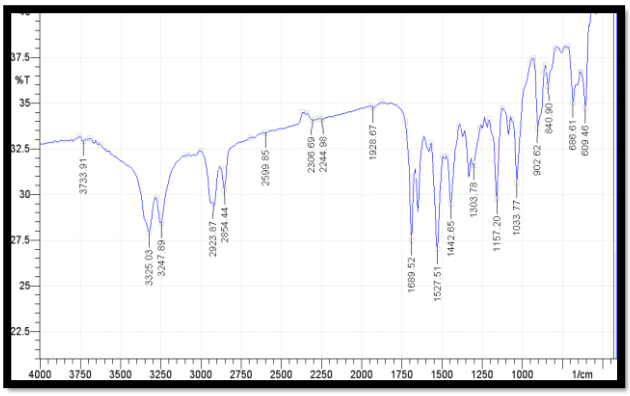

Figure 3: FTIR Spectra of Physical Mixture

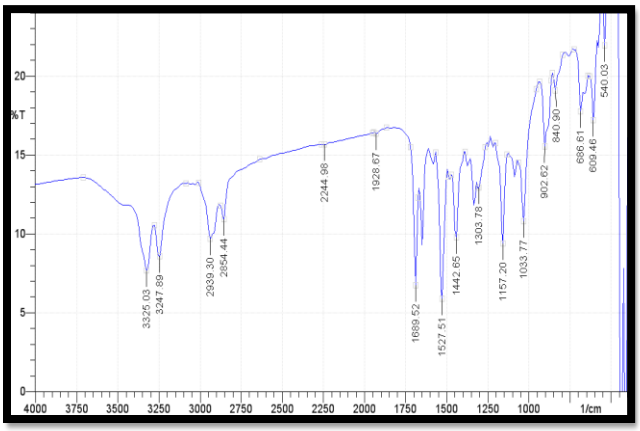

Figure 4: FTIR Spectra of Optimized Formulation

3.1.2 Standard calibration curve for Glipizide

Table 5: Standard calibration curve for Glipizide

Concentration (µg/ml)	Absorbance
0	0
2	0.123
4	0.230
6	0.338
8	0.452
10	0.565

The UV spectrophotometric method was used to standardize Glipizide at a wavelength of 223 nm. The standard graph was constructed using phosphate buffer pH 7.4.

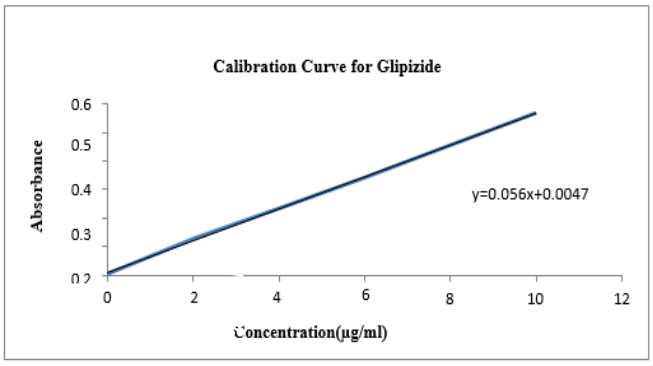

Figure 5: Standard graph of Glipizide in phosphate buffer pH 7.4 Inference

3.2 Evaluation of Microspheres

3.3.1 Percentage Yield

After the preparation of microsphere practical yield and percentage yield were calculated. It was found that the percentage yield was in the range of 80.14 % to 92.85 %.

Table No. 6: Percentage yield

Formulation	Theoretical yield (g)	Practical yield(g)	Percentage yield (%)
F_1	2.1	1.950	92.85
F_2	1.6	1.447	90.43
F_3	1.1	1.057	96.09
F_4	0.850	0.797	93.76
F_5	0.600	0.576	96
F_6	0.350	0.280	80.14

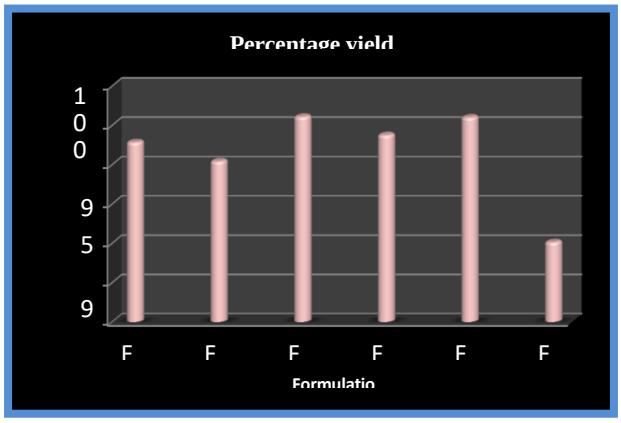

Figure 6: Percentage yield

3.3.2 Drug Content

The drug content was found in the range of 81 to 87.35% w/w.

Table 7: Drug content

Formulation	Drug content (%)w/w
F_1	81.76
F_2	85.75
F_3	87
F_4	83.20
F_5	87.35
F_6	82.84

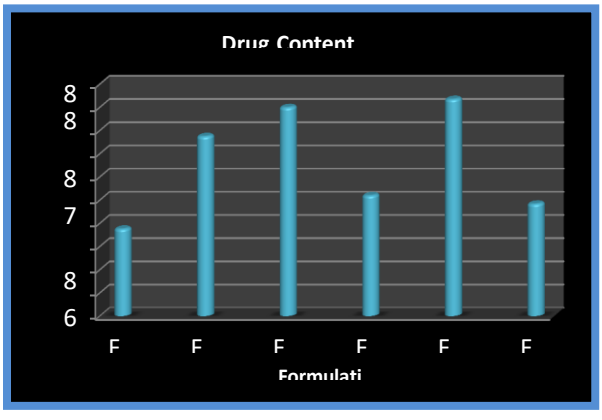

Figure 7: Drug content

3.3.3 Mean Particle Size by Microscopy: The particle size was found in the range of 520to752µm

Table 8: Results of Microscopy Study

Formulation	Mean Particle Size(µm)
F_1	587
F_2	570
F_3	642
F_4	693
F_5	752
F_6	525

3.3.4 Scanning Electron Microscopy

Morphological analysis of the microspheres was carried out using optical microscopy and scanning electron microscopy (SEM).

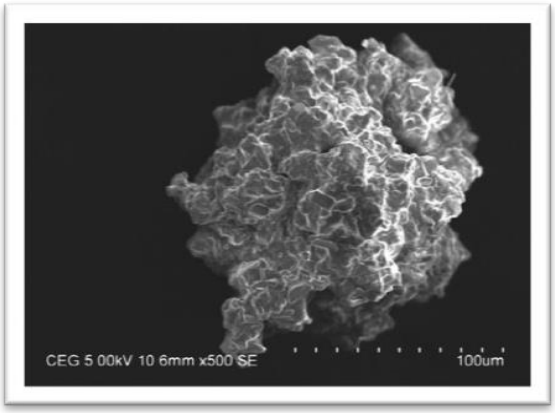

Figure 8: Scanning Electron Microscopy

3.4 *In vitro* mucoadhesion study

Table 9: Results of *in vitro* mucoadhesion study

Time (hours)	% Microspheres adhered					
	F1	F2	F3	F4	F5	F6
0.5	92.67±1.15	94±2.00	94±2.00	90.67±2.0	95.33±1.15	91.33±1.15
1	86.66±1.15	87.33±3.06	86±2.00	82.67±1.15	88±2.00	81.33±1.15
2	76.67±1.15	81.33±3.06	78.67±2.31	70±2.00	82.67±1.15	74.67±2.31
3	70.01±2.00	72.67±2.3	68.67±1.15	60.67±1.15	75.33±1.15	57.33±1.15
4	56.67±1.15	60.67±1.15	54±2.00	44.67±1.25	64±2.00	40±2.00
5	43.33±1.15	46±2.00	38.67±1.15	33.33±2.01	49±4.16	28.6±1.15

Figure 8: *In vitro* mucoadhesion study

***3.5 In vitro* release study**

Table 10: Cumulative percentage drug release of Formulation F1to F6

Time (hours)	F1	F2	F3	F4	F5	F6
1	9.88	5.89	9.74	7.29	9.24	9.24
2	18.24	12.1	13.81	15.18	14.55	17.8
3	24.51	17.88	20.2	21.21	24.47	23.21
4	31.51	23.41	27.22	27.85	30.6	31.29
5	37.72	27.86	30.92	36.72	36.8	38.25
6	45.72	34.3	39.75	42.99	45.01	41.56
7	52.07	38.22	44.15	49.29	48.75	52.01
8	79.32	44.9	50.86	57	58.39	58.3
9	86.25	50.67	59.33	61.52	63.57	65.55
10	93.03	55.59	63.89	71.94	71.4	69.94
11	100.75	67.37	67.37	76.6	75.06	74.77
24	100.80	74.22	89.65	98.88	100.3	97.7

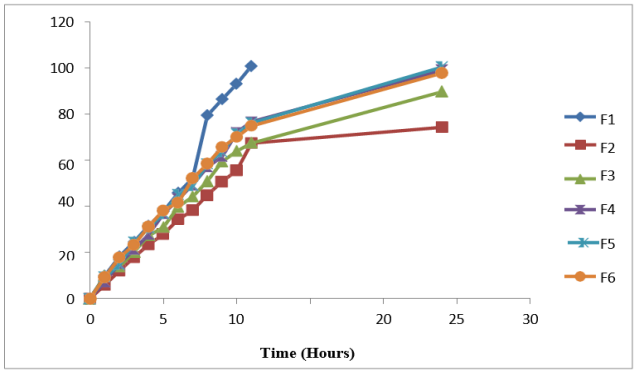

Figure 9: *In vitro* Drug Release Study of Formulations F1- F6

4. Conclusion

In the present study, F1, F2, F3, F4, F5 and F6 formulations were prepared using gelatin as a polymer (1:20, 1:15, 1:10, 1:7.5, 1:5 and 1:2.5) in six different ratios. The effect of polymer, as well as decreasing concentration of gelatin on microspheres was studied by subjecting all the formulations to various evaluation parameters. The FTIR study was carried out for the drug, polymer, physical mixture and optimized formulation F5. In FTIR study, all characteristic peaks in the spectra appeared without any remarkable changes showing that there is no chemical interaction between the drug and polymer used in the preparation of microspheres. The *in vitro* mucoadhesion study was conducted for all the formulations and the results were found in the range of 28.6 to 49%. The results revealed that the formulation F5 had 49% mucoadhesion at the end of 5h. The *in vitro* drug release study was carried out for all the formulations and the formulation F5 (1:5) showed sustained release of 75.06% at the end of 24 h. Results suggest that formulation F5 prepared using drug: polymer ratio1:5 showed good entrapment efficiency, sustained drug release and satisfactory mucoadhesion as compared to other formulations, so it was further subjected to the accelerated stability study. The formulation F5 was stable at the tested storage condition (40± 2˚C/75%RH) up to 3 months.

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