

Preparation and *in vitro* and *in vivo* Evaluation of Chitosan-Gliclazide Mucoadhesive Microparticles by an Emulsification-Desolvation-Crosslinking Technique

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ABSTRACT

Introduction: Recently much emphasis is being laid on the development of microparticles because of their potential benefits such as increased bioavailability, reduced risk of systemic toxicity, reduced risk of local irritation and predictable gastric emptying. **Objective:** The objective of the present study is to prepare and evaluate mucoadhesive microparticles of chitosan-gliclazide for oral controlled release. **Methods:** A new method namely emulsification-desolvation-crosslinking was used for the preparation of chitosan-gliclazide microparticles and the microparticles were evaluated by *in vitro* and *in vivo* methods. **Results:** Spherical chitosan-gliclazide microparticles could be prepared by the emulsification-desolvation-crosslinking method. The method was reproducible with regard to size and size distribution of the microparticles. The chitosan-gliclazide microparticles exhibited good muoadesive property. Gliclazide release from the chitosan microparticles was slow and extended over longer periods of time and depended on the proportion of core: coat. Gliclazide release from the chitosan microparticles was by diffusion mechanism. Microparticles (F3) prepared using a core: coat ratio of 8:2 gave slow and controlled release of gliclazide over 12 hr similar to that of commercial gliclazide SR tablets. In the *in vivo* evaluation, the gliclazide microparticles (F3) gave a slower reduction in serum glucose levels and the reduced glucose levels were sustained over longer periods of time. **Conclusion:** Microparticles (F3) are considered as a promising microparticulate drug delivery system for oral controlled release of gliclazide over 12 hr for b.i.d administration.

Key words: Mucoadhesive microparticles, Chitosan, Gliclazide, Emulsification-desolvation-crosslinking method, Oral controlled release.

INTRODUCTION

Microparticles and microparticulate drug delivery systems are topics of current interest in drug delivery. The design, characterization advantages and applications of microparticles are reviewed in standard textbooks and articles.¹⁻⁴ Design of microparticles requires a suitable polymer to serve the intended purpose. Examples of polymers used for the preparation of microparticles include cellulose nitrate, cellulose acetate, synthetic

resins, ethyl cellulose, polystyrene, polyvinyl acetate, Eudragits, starch acetate, chitosan etc. In the present study chitosan, a mucoadhesive polymer was tried for the preparation of microparticles of gliclazide for oral controlled release. We have earlier reported⁵ the preparation and *in vitro* evaluation of chitosan-gliclazide microparticles. Mucoadhesion is a topic of interest in the design of drug delivery systems and several

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studies reported⁶⁻¹² mucoadhesive drug delivery systems in the form of tablets, microcapsules, films, patches and gels for various routes. In the present study mucoadhesive microparticles of gliclazide were prepared using chitosan. The objective of the present study is to prepare and evaluate mucoadhesive chitosan-gliclazide microparticles by *in vitro* and *in vivo* methods.

Gliclazide, a potential second generation short-acting sulfonylurea¹³ requires formulation of controlled release systems for continuous therapeutic effect and better patient compliance by reducing the frequency of dosage administrations.

MATERIALS AND METHODS

Materials

Gliclazide was a gift sample from M/s Micro Labs, Pondicherry. Chitosan, 75-85 percent deacetylated was obtained from Central Institute of Fisheries Technology, Cochin, India. Sodium tri polyphosphate (Sigma), Acetic acid (Qualigens), Chloroform (Qualigens) and Soyabean oil were used. All other materials used were of pharmacopoeial grade.

Methods

Estimation of Gliclazide

Gliclazide was estimated by measuring absorbance at 227 nm in phosphate buffer of pH 7.4. Before using the method was validated for linearity range, accuracy, precision and interference by the excipients. The method exhibited linearity in the concentration range 1 - 10 µg/ml. The relative error and coefficient of variation (RSD) in the estimation were found to be 0.80% and 1.2% respectively. No interference by the excipients was observed.

Preparation of Chitosan-Gliclazide Microparticles

An emulsification-desolvation-crosslinking method was used for the preparation of chitosan-gliclazide microparticles, the details of which are as reported earlier.⁵ Different proportions of core: coat namely 19:1 (F1), 9:1 (F2), 8:2 (F3) and 7:3 (F4) were used in the preparation of chitosan-gliclazide microparticles. The purpose of using different proportions of core: coat was to prepare microparticles with varying amount of coat polymer and to achieve different release rates.

Estimation of Drug Content and Encapsulation Efficiency

Four samples of 100mg each were taken from each batch of microparticles prepared and assayed for

gliclazide content. Encapsulation efficiency was estimated using the equation,

$$\text{Encapsulation efficiency (\%)} = \frac{\text{Estimated drug content (\%)}}{\text{Theoretical drug content (\%)}} \times 100$$

Size Analysis

Size distribution analysis was done by sieving using a range of standard sieves.

Morphological Characterization by SEM

The surface morphology of microparticles was observed by scanning electron microscopy. The microparticles (F3) were vacuum dried. Before observation, samples were mounted on metal grids using double-sided adhesive tape, coated with gold palladium and observed microscopically using Joel, JSM-6360 LV scanning microscope. (Tokyo, Japan).

Drug Release Study

Release of gliclazide from the microparticles of size 30/50 mesh was evaluated in phosphate buffer of pH 7.4 (900 ml) using an 8-station dissolution rate test apparatus (model Disso-2000, M/s Lab. India) with a paddle stirrer (Apparatus 2) at 50 rpm and a temperature of 37°C ± 1°C. A sample of microparticles equivalent to 60 mg of gliclazide was used in each test. Each drug release experiment was replicated three times (*n*=3).

Analysis of Release Data

Drug release data were analyzed as per Zero order, First order, Higuchi¹⁴ equation and Korsmeyer-Peppas¹⁵ equation models.

Mucoadhesion Testing by *in vitro* Wash-Off Test

The mucoadhesive property of the microparticles (F3) was evaluated by an *in vitro* adhesion testing method known as the wash-off method.¹⁶ The mucoadhesiveness of the microparticles was compared with that of nonbioadhesive material, ethylene vinyl acetate microparticles. Freshly excised pieces of intestinal mucosa (2 × 2 cm) from sheep were mounted onto glass slides (3 × 1 inch) with cyanoacrylate glue. Two glass slides were connected with a suitable support. Fifty microparticles were spread onto each wet rinsed tissue specimen and immediately thereafter the support was hung onto the arm of a USP tablet disintegrating test machine. When the disintegrating test machine was operated, the tissue specimen was given a slow, regular up-and-down movement in the test fluid at 37°C contained in a 1 L vessel of the machine. At the end of 30 min, at

the end of 1 hr and at hourly intervals up to 12 hr, the machine was stopped and the number of microparticles still adhering to the tissue was counted. The test was performed at both gastric pH (0.1N HCl, pH 1.2) and intestinal pH (phosphate buffer, pH 6.8).

In vivo Evaluation

In vivo evaluation studies were conducted on (1) gliclazide and (2) mucoadhesive microparticles F3, in normal, healthy rabbits by measuring serum glucose levels following their oral administration at a dose equivalent to 3 mg/kg of gliclazide. The dose for experimental rabbits was calculated as suggested by Bikash Medhi and Ajay Prakash.¹⁷ The experiments were conducted as per a crossover randomized block design ($n=6$). *In vivo* study protocols were approved by Institutional Animal Ethics Committee (No. CPCSEA/CH/ORG/2015-051). The products were administered orally the morning following overnight fasting. No food or liquid other than water was given during the experimental period. After the zero-hour blood sample (0.5 ml) was collected from the marginal ear vein, the product in the study was administered orally. Blood samples (0.5 mL) were collected from marginal ear vein at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, 24 hrs after administration. Serum glucose concentrations were determined by a known oxidase-peroxidase method¹⁸ as described below employing a glucose kit supplied by Dr. Reddy's Laboratory, Diagnostic Division, Hyderabad, India. The method was revalidated and the relative standard deviation in the estimated values was found to be 1.2%. Blood samples collected were allowed to clot without any anticoagulant and were centrifuged immediately at 5000 rpm for 20 min to separate the serum. To the serum (0.02 mL) and standard (0.02 mL) in separate clean, dry test tubes, enzyme reagent (2 mL) was added, mixed well and incubated at 37°C for 10 min. The solutions were diluted to 5 mL with distilled water and the absorbance of the pink-colored solutions was measured in a spectrophotometer at 510 nm using a reagent blank. Serum glucose levels (mg/dL) and percentage reduction in serum glucose levels were calculated.

RESULTS AND DISCUSSION

Chitosan is sparingly soluble in water; practically insoluble in ethanol (95%) and other organic solvents. Chitosan dissolves readily in dilute and concentrated solutions acetic acid. In the present study chitosan was dissolved in 1% v/v acetic acid solution.

An emulsification-desolvation-crosslinking method⁵ was used for the preparation of chitosan-gliclazide micro-

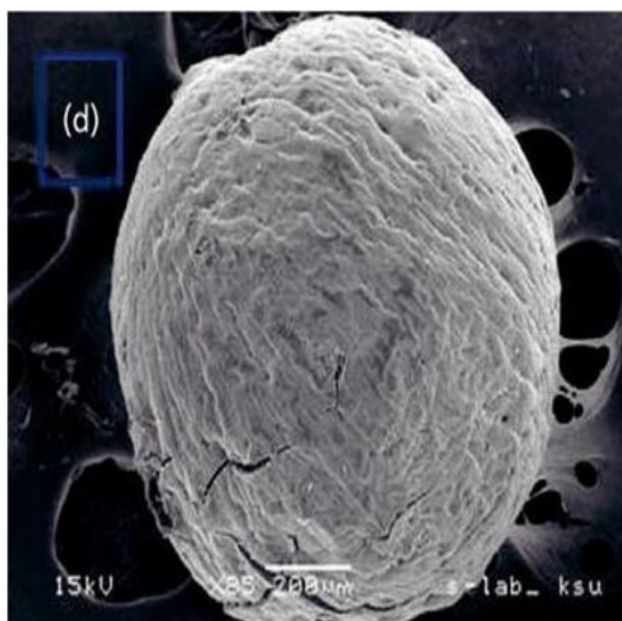


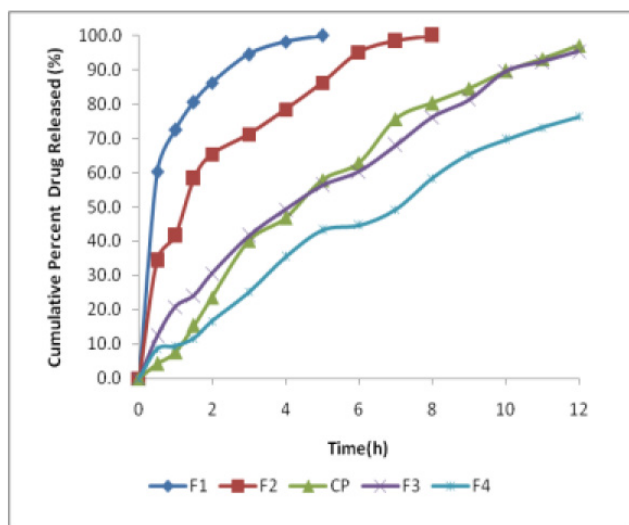
Figure 1: SEM of Chitosan- Gliclazide Microparticles, d (F3).

particles. The method consists of emulsification of the chitosan solution in 1% v/v acetic acid containing the dispersed drug particles in an immiscible liquid medium (soya bean oil containing 10% chloroform) as microdroplets, followed by removal of the acetic acid from the polymer solution into chloroform by desolvation and crosslinking with tripolyphosphate and glutaraldehyde solution to form rigid microparticles. The microparticles were found to be discrete, spherical and free flowing. SEM of microparticles F3 is shown in Figure 1.

The SEM photographs indicated that the microparticles were spherical and completely covered with the coat polymer. The sizes could be separated readily by sieving and a more uniform size range of microparticles could easily be obtained. The size analysis of different batches of microparticles showed that about 68-75% in each batch were in the size range 35/50 mesh (398.5 μ m). The size of the microparticles in the method employed based on emulsification depends more on the speed of the stirrer, which was kept constant at 1000 rpm in the preparation of all products. More over the polymer chitosan is rapidly and completely dissolves in acetic acid solutions forming thin polymer solutions. In such a case the polymer concentration may not be influencing the size of microparticles. Hence though the percent coat polymer was increased the mean size of the microparticles remained almost the same. Size analysis of three batches of microparticles prepared under identical conditions indicated reproducibility of the method. Microparticles of the size 35/50 mesh (398.5 μ m) were selected for further evaluation.

Table 1: Physical Characteristics of the Microparticles Prepared.

Micro particles	Mesh Size	Mean size (μm)	Core: Coat ratio	Gliclazide content (%) ($\bar{x}\pm\text{sd}$)	Encapsulation efficiency (%)	Percent Coat Polymer
F1	20/35	670	19:1	94.2 \pm 1.2	99.0	5.8
	35/50	398.5	19:1	94.6 \pm 1.8	99.5	5.4
F2	20/35	670	9:1	87.4 \pm 1.3	97.1	12.6
	35/50	398.5	9:1	87.6 \pm 1.1	97.3	12.4
F3	20/35	670	8:2	79.2 \pm 1.9	99.0	20.8
	35/50	398.5	8:2	79.4 \pm 1.6	99.2	20.6
F4	20/35	670	7:3	68.2 \pm 1.6	97.4	31.8
	35/50	398.5	7:3	68.6 \pm 1.5	98.0	31.4

**Figure 2: Gliclazide Release Profiles of Various Microparticles Prepared and Commercial SR Tablets (CP).**

The physical characteristics of the microparticles prepared are given in Table 1. Low Coefficient of Variation (CV) in percent drug content ($< 2.0\%$) indicated uniformity of drug content in each batch of microparticles. The encapsulation efficiency was in the range 97.1 -99.5 %. Drug content of the microparticles was found to be the same in the two sizes, 20/35, 35/50 mesh. A t-test of significance indicated that the difference in the drug content of the two sizes in each case is not significant ($P>0.05$).

Gliclazide release from various microparticles of size 35/50 was studied in phosphate buffer pH 7.4. For comparison gliclazide release from one commercial brand of gliclazide SR tablets was also studied. The drug release profiles are shown in Figure 2. The release data were analyzed as per Zero order, First order, Higuchi¹⁴ equation and Korsmeyer-Peppas¹⁵ equation models. The kinetic parameters (R^2 values, rate constants

Table 2: Kinetic Parameters (R^2 values, Rate constants and n values) in the Analysis of Release Data as per Various Kinetic Models.

DDS	Zero order		First order		Higuchi	Korsmeyer Peppas	
	K_0	R^2	R^2	K_1	R^2	n	R^2
F1	11.49	0.6464	0.9855	0.929	0.9003	0.24	0.9929
F2	6.94	0.8380	0.9308	0.506	0.9788	0.40	0.9792
F3	4.53	0.9703	0.9436	0.230	0.9890	0.64	0.9977
F4	3.25	0.9782	0.9793	0.120	0.9825	0.79	0.9881
CP	5.09	0.9602	0.9492	0.262	0.9691	1.00	0.9725

and n values) in the analysis of release data as per various kinetic models are given in Table 2.

Gliclazide release from all the chitosan microparticles was slow and extended longer periods of time and depended on the proportion of coat in the microparticles. As the coat proportion was increased the release rate was decreased. A good linear relationship was observed between percent coat and release rate, k_0 ($R^2 = 0.8448$). The linear relationship could be described by the equation, $y = 11.849 - 0.3035x$ where x is percent coat and y is release rate (k_0).

The release data of all the formulations obeyed Korsmeyer Peppas equation model indicating that the drug release from the microparticles was by diffusion mechanism. The release exponent (n) was 0.24 and 0.40 in the case of formulation F1 and F2 respectively indicating Fickian diffusion was the release mechanism from these formulations. In the case of formulation F3 and F4 the release exponent (n) was 0.64 and 0.79 respectively indicating non-fickian (anomalous) diffusion was the release mechanism from these formulations. The drug release was by zero order diffusion mechanism ($n=1.00$) in the case of commercial product. Formulation F3 prepared using a core: coat ratio of 8:2 gave slow and controlled release of gliclazide over 12 h similar to that

Table 3: Results of *in vitro* Wash-Off Test to Assess Mucoadhesive Properties of the Microparticles Prepared.

Micro particles	Percentage of Microparticles Adhering to Tissue at Times (h)†					
	Fluid 0.1N HCL, pH 1.2					
F3	1	2	4	6	8	12
	69 (2.2)	62 (1.1)	32 (1.9)	19 (1.5)	15 (1.9)	6 (1.2)
EVA	52 (2.3)	40 (2.5)	08 (2.7)	—	—	—
	Fluid Phosphate Buffer, pH 6.8					
F3	84 (1.0)	82 (0.5)	74 (0.8)	69 (0.5)	65 (0.4)	56 (0.8)
EVA	55 (1.5)	41 (1.4)	11 (1.8)	—	—	—

†Figures in parentheses are coefficient of variation values.

of commercial gliclazide SR tablets. The difference factor ($f_1=2.46$) and similarity factor ($f_2=63.11$) indicated that the drug release profiles of formulation F3 and commercial SR product are similar.

The results of *in vitro* wash-off test are given in Table 3. Microparticles (F3) exhibited good mucoadhesive properties in the *in vitro* wash-off test when compared to non-mucoadhesive material, ethylene vinyl acetate microparticles. The wash-off was slow in the case of microparticles (F3) when compared to that of ethylene vinyl acetate microparticles (Table 3). The wash-off was faster in gastric pH 1.2 than in intestinal pH 6.8. Ch'ng *et al.*¹⁹ observed that the pH of the medium was critical for the degree of hydration, solubility and mucoadhesion of the polymers. The relatively rapid wash-off of microparticles F3 observed at gastric pH 1.2 is due to ionization and solubility of chitosan at acidic pH. The results of the wash-off test indicated that the microparticles prepared had good mucoadhesive property.

In vivo evaluation

In vivo evaluation of the chitosan-gliclazide mucoadhesive microparticles was carried out in healthy, normal rabbits by measuring the hypoglycemic effect produced after their oral administration at a dose equivalent to 3 mg/kg of gliclazide, in comparison to gliclazide (pure drug) at the same dose. The serum glucose levels estimated and the percentage reduction in glucose levels following the oral administration of gliclazide pure drug and chitosan-gliclazide microparticles are given in Table 4. When gliclazide was administered, a rapid reduction in serum glucose levels was observed; a maximum reduction of 55.3% was observed at 1.0 h after administration and the glucose levels recovered rapidly to the normal level within 6-8 h. In the case of microparticles, the reduction in glucose levels was slower; it reached maximum reduction (35.0%) at 3 h after administration and the reductions in glucose levels were sustained over longer

Table 4: Serum Glucose Levels (mg/dl) observed Following the Oral Administration of Gliclazide Pure Drug and Its Mucoadhesive Microparticles.

Time (h)	Serum Glucose Levels (mg/dl) (Percentage Glucose Reduction)	
	Gliclazide ($\bar{x}\pm sd$)	Chitosan Gliclazide Microparticles ($\bar{x}\pm sd$)
0	99.5±4.2 (0)*	99.8±3.6 (0)*
0.5	58.4±2.5 (41.3)*	84.8±3.2 (15.0)*
1	44.4±3.2 (55.3)	79.2±2.9 (20.6)
2	51.3±2.8 (48.4)	73.6±3.5(26.2)
3	59.4±3.1 (40.3)	64.8±3.9 (35.0)
4	73.8±3.8 (25.8)	66.2±4.1 (33.6)
6	89.6±4.2 (9.9)	68.4±3.2 (31.4)
8	92.6±4.6 (6.9)	69.6±3.8(30.2)
10	94.2±3.7 (5.3)	71.1±2.9 (28.7)
12	95.4±4.1 (4.1)	72.8±4.2 (27.0)
16	97.2±3.9 (2.3)	84.9±4.6 (14.9)
20	98.1±3.6 (1.4)	87.8±3.9 (12.0)
24	98.4±4.2 (1.1)	91.5±4.6 (8.3)

Figures in parentheses are Percentage Glucose Reduction values.

periods of time. A 25% reduction in glucose levels is considered a significant hypoglycemic effect.²⁰ The hypoglycemic effect was maintained during the period from 0.5 hr to 4 hr following the administration of gliclazide pure drug, but the hypoglycemic effect was maintained during the period from 2 hr to 12 hr in the case of chitosan-gliclazide microparticles. The sustained hypoglycemic effect observed over longer periods of time in the case of microparticles is due to the slow release and absorption of gliclazide over longer periods of time. The hypoglycemic effect of gliclazide could be sustained over 12 hr with microparticles.

CONCLUSION

Chitosan-gliclazide mucoadhesive microparticles could be prepared by an emulsification-desolvation-cross-linking method. These microparticles exhibited good mucoadhesive property. Gliclazide release from the chitosan microparticles was slow and spread over longer periods of time. The drug release depended on the proportion of core: coat in the microparticles. Gliclazide release from the chitosan microparticles prepared was by diffusion mechanism. Microparticles (F3) prepared using a core: coat ratio of 8:2 gave slow and controlled release of gliclazide over 12 hr similar to that of commercial gliclazide SR tablets. In the *in vivo* evaluation these microparticles (F3) gave a slower reduction in serum glucose levels and the reduced glucose levels

were sustained over longer periods of time. A significant hypoglycemic effect was maintained during 2-12 hr with these microparticles after their oral administration. As such microparticles (F3) are considered as a promising microparticulate DDS for oral controlled release of gliclazide over 12 hr for b.i.d administration.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

SEM: Scanning Electron Microscope; **ML:** Milli Liter; **°C:** Degree Centigrade; **µg:** Microgram; **Rpm:** Revolutions per minute; **µm:** Micrometer; **nm:** Nanometer.

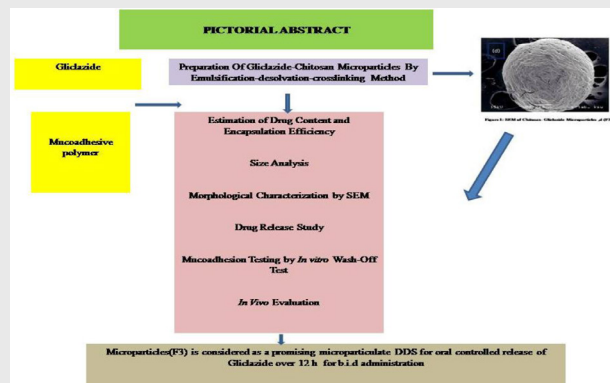
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SUMMARY

The objective of the present study is to prepare and evaluate microparticles of gliclazide using chitosan, a mucoadhesive polymer for oral controlled release. A new technique namely emulsification-desolvation-crosslinking method was tried for the preparation of chitosan microparticles and the microparticles were evaluated by *in vitro* and *in vivo* methods. Spherical chitosan- gliclazide microparticles could be prepared by the emulsification-desolvation-crosslinking method. The method was reproducible with regard to size and size distribution of the microparticles. The chitosan-gliclazide microparticles prepared exhibited good muoadhensive property. They also exhibited good controlled release characteristics in the *in vitro* and *in vivo* evaluation. Microparticles (F3) are considered as a promising microparticulate drug delivery system for oral controlled release of gliclazide over 12 hr for b.i.d administration.

PICTORIAL ABSTRACT



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