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Design and In Vitro and In Vivo Evaluation of Mucoadhesive Microcapsules of Glipizide for Oral Controlled Release: A Technical Note

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K.P.R. Chowdary[1] and Y. Srinivasa Rao[1]

[1]Industrial Pharmacy Division, Department of Pharmaceutical Sciences, Andhra University, Visakhapatnam-530 003, India

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INTRODUCTION

Microencapsulation by various polymers and its applications are described in standard textbooks.[1,2] Microencapsulation has been accepted as a process to achieve controlled release and drug targeting. Mucoadhesion has been a topic of 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 the dosage form with the underlying absorption surface to improve and enhance the bioavailability of drugs.[3-6] Several studies[7] reported mucoadhesive drug delivery systems in the form of tablets, films, patches, and gels for oral, buccal, nasal, ocular, and topical routes; however, very few reports on mucoadhesive microcapsules are available.[8-11] The objective of this study is to develop, characterize, and evaluate mucoadhesive microcapsules of glipizide employing various mucoadhesive polymers for prolonged gastrointestinal absorption. Glipizide, an effective antidiabetic that requires controlled release owing to its short biological half-life[12] of 3.4 ± 0.7 hours, was used as the core in microencapsulation. The mucoadhesive microcapsules were evaluated by in vitro and in vivo methods for controlled release.

Corresponding Author: K.P.R. Chowdary, Industrial Pharmacy Division, Department of Pharmaceutical Sciences, Andhra University, Visakhapatnam-530 003, India. Phone: +91 891-2505159; Fax: +91 891-2755547; Email: profkprc@rediffmail.com

MATERIALS AND METHODS

Materials

Glipizide was a gift sample from M/s Micro Labs (Pondicherry, India). Sodium carboxymethylcellulose (sodium CMC, having a viscosity of 1500-3000 cps of 1% wt/vol aqueous solution at 25°C), methylcellulose (having a methoxyl content of 28.32% wt/vol and a viscosity of 65 cps in 0.5% wt/vol aqueous solution at 25°C), and hydroxypropyl methylcellulose (HPMC, having a viscosity of 50 cps in a 2% by wt/vol aqueous solution at 20°C) were gift samples from M/s Natco Pharma Ltd (Hyderabad, India). Carbopol 934P was a gift sample from M/s SmithKline Beecham Pharmaceuticals (Bangalore, India). Sodium alginate (SD Fine Chem, Mumbai, India) and calcium chloride (Qualigens, Mumbai) were procured from commercial sources. All other reagents used were of analytical grade.

Methods

Preparation of Microcapsules

Microcapsules containing glipizide were prepared employing sodium alginate in combination with four mucoadhesive polymers—sodium CMC, methylcellulose, Carbopol and HPMC—as coat materials. No methods were reported for microencapsulation by these polymers. An orifice-ionic gelation process[13,14] that has been extensively used to prepare large alginate beads was employed to prepare the microcapsules.

Orifice-Ionic Gelation Method

Sodium alginate (1.0 g) and the mucoadhesive polymer (1.0 g) were dissolved in purified water (32 mL) to form a homogeneous polymer solution. The active substance, glipizide (2.0 g), was added to the polymer solution and mixed thoroughly with a stirrer to form a viscous dispersion. The resulting dispersion was then added manually dropwise into calcium chloride (10%

Table 1. Coat Composition, Drug Content, and Microencapsulation Efficiency of the Microcapsules Prepared*

Microcapsules	Coat Composition	Percentage Drug Content†	Microencapsulation Efficiency (%)	Release Rate, K_o (mg/h)‡	T_{50} (h)
MC1	Alginate:sodium CMC (1:1)	42.68 (1.2)	85.36	1.860 (0.969)	1.6
MC2	Alginate:methylcellulose (1:1)	32.35 (1.8)	64.70	1.849 (0.971)	1.7
MC3	Alginate:Carbopol (1:1)	36.11 (1.5)	72.22	1.630 (0.988)	2.0
MC4	Alginate:HPMC (1:1)	30.34 (1.0)	60.68	3.248 (0.909)	0.8
MC5	Alginate: sodium CMC (9:1)	34.00 (0.9)	68.00	1.144 (0.941)	1.9
MC6	Alginate:methylcellulose (9:1)	32.46 (0.2)	64.92	1.081 (0.969)	2.1
MC7	Alginate:Carbopol (9:1)	36.78 (1.3)	73.56	1.056 (0.982)	3.4
MC8	Alginate:HPMC (9:1)	35.87 (1.7)	71.74	1.626 (0.927)	1.3

*CMC indicates carboxymethylcellulose; HPMC, hydroxypropyl methylcellulose; K_0, zero-order release rate constant; MC, microcapsule; T_{50}, time for 50% release.

†Figures in parentheses are coefficient of variation values.

‡Figures in parentheses are correlation coefficient (*r*) values between amount (mg) dissolved and time in hours.

wt/vol) solution (40 mL) through a syringe with a needle of size no. 18. The added droplets were retained in the calcium chloride solution for 15 minutes to complete the curing reaction and to produce spherical rigid microcapsules. The microcapsules were collected by decantation, and the product thus separated was washed repeatedly with water and dried at 45°C for 12 hours. The microcapsules prepared along with their coat composition are listed in **Table 1**.

Characterization and Evaluation of Microcapsules

Estimation of Glipizide

Glipizide content in the microcapsules was estimated by a UV spectrophotometric method[15] based on the measurement of absorbance at 223 nm in phosphate buffer of pH 7.4. The method was validated for linearity, accuracy, and precision. The method obeyed Beer's law in the concentration range 1 to 10 mg/mL. When a standard drug solution was assayed repeatedly (n = 6), the mean error (accuracy) and relative standard deviation (precision) were found to be 0.6% and 0.8%, respectively.

Microencapsulation Efficiency

Microencapsulation efficiency was calculated using the following formula: microencapsulation efficiency = (estimated percentage drug content/theoretical percentage drug content) × 100.

Scanning Electron Microscopy

The microcapsules were observed under a scanning electron microscope (SEM-LEICA, S430, London, UK). They were mounted directly onto the SEM sample stub using double-sided sticking tape and coated with gold film (thickness 200 nm) under reduced pressure (0.001 mm of Hg).

Drug Release Study

Release of glipizide from the microcapsules was studied in phosphate buffer of pH 7.4 (900 mL) using a United States Pharmacopeia (USP) XXIII 3-station Dissolution Rate Test Apparatus (Model DR-3, M/s Campbell Electronics, Bombay, India) 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 through a filter (0.45 μm) at different time intervals and were assayed at 223 nm for glipizide content using a Shimadzu UV-150 double-beam spectrophotometer (Shimadzu Corporation, Japan). The drug release experiments were conducted in triplicate (n = 3).

Mucoadhesion Testing by In Vitro Wash-Off Test

The mucoadhesive property of the microcapsules was evaluated by an in vitro adhesion testing method known as the wash-off method.[16] The mucoadhesive-

ness of these microcapsules was compared with that of a nonbioadhesive material, ethylene vinyl acetate microcapsules. 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. About 50 microcapsules 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 minutes, at the end of 1 hour, and at hourly intervals up to 12 hours, the machine was stopped and the number of microcapsules 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 6.2).

In Vivo Evaluation

In vivo evaluation studies were conducted on (1) glipizide, (2) microcapsules MC6, and (3) microcapsules MC7 in normal, healthy rabbits by measuring serum glucose levels following their oral administration at a dose equivalent to 800 μg/kg of glipizide. The experiments were conducted as per a crossover randomized block design (n = 4). The approval of an animal ethics committee was obtained before starting the study. 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 was collected, the product in the study was administered orally. Blood samples (0.5 mL) were collected at 1-hour intervals up to 24 hours after administration. Serum glucose concentrations were determined by a known oxidase-peroxidase method[17] 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 minutes 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 minutes. The solutions were diluted to 5 mL with distilled water, and the absorbance of the pink-colored solutions was measured in a spectrophotometer at 505 nm using a reagent blank. Serum glucose levels (mg/100 mL) and percentage reduction in serum glucose levels were calculated.

RESULTS AND DISCUSSION

Microcapsules of glipizide with a coat consisting of alginate and a mucoadhesive polymer—sodium CMC, methylcellulose, Carbopol, or HPMC—in 1:1 and 9:1 ratio could be prepared by the orifice-ionic gelation process. Microcapsules with a coat of mucoadhesive polymer alone could not be prepared because of their water-soluble nature. The microcapsules were found to be discrete, spherical, free-flowing, and of the monolithic matrix type. The microcapsules were uniform in size, with a mean size of 920 μm (passed through mesh no 18 and retained on mesh no 20). The SEM photographs indicated that the microcapsules were spherical and completely covered with the coat polymer (**Figure 1**).

Low coefficient of variation (<2.0%) in percentage drug content indicated uniformity of drug content in each batch of microcapsules. The microencapsulation efficiency was in the range of 60% to 84% (**Table 1**), and the yield was in the range of 92% to 98%.

Microcapsules with a coat consisting of alginate and a mucoadhesive polymer exhibited good mucoadhesive properties in the in vitro wash-off test when compared to a nonmucoadhesive material, ethylene vinyl acetate microcapsules. The wash-off was slow in the case of microcapsules containing alginate-mucoadhesive polymer as coat when compared to that of ethylene vinyl acetate microcapsules (**Table 2**). The wash-off was faster at intestinal pH than at gastric pH. Ch'ng et al[18] observed that the pH of the medium was critical for the degree of hydration, solubility, and mucoadhesion of the polymers. The rapid wash-off observed at intestinal pH 6.2 is due to ionization of carboxyl and other functional groups in the polymers at this pH, which increases their solubility and reduces adhesive strength. The results of the wash-off test indicated that the microcapsules had fairly good mucoadhesive properties.

Glipizide release from the microcapsules was studied in phosphate buffer (pH 7.4) for 12 hours as prescribed for glipizide tablets in USP XXIV. Glipizide release from the microcapsules was slow and depended on the composition of the coat (**Figure 2**). Release followed zero-order kinetics ($r > 0.90$) after a lag period of 1 hour. Microcapsules of alginate-HPMC gave relatively fast release when compared to others. The order of increasing release rate observed with various microcapsules was alginate-Carbopol < alginate-methylcellulose

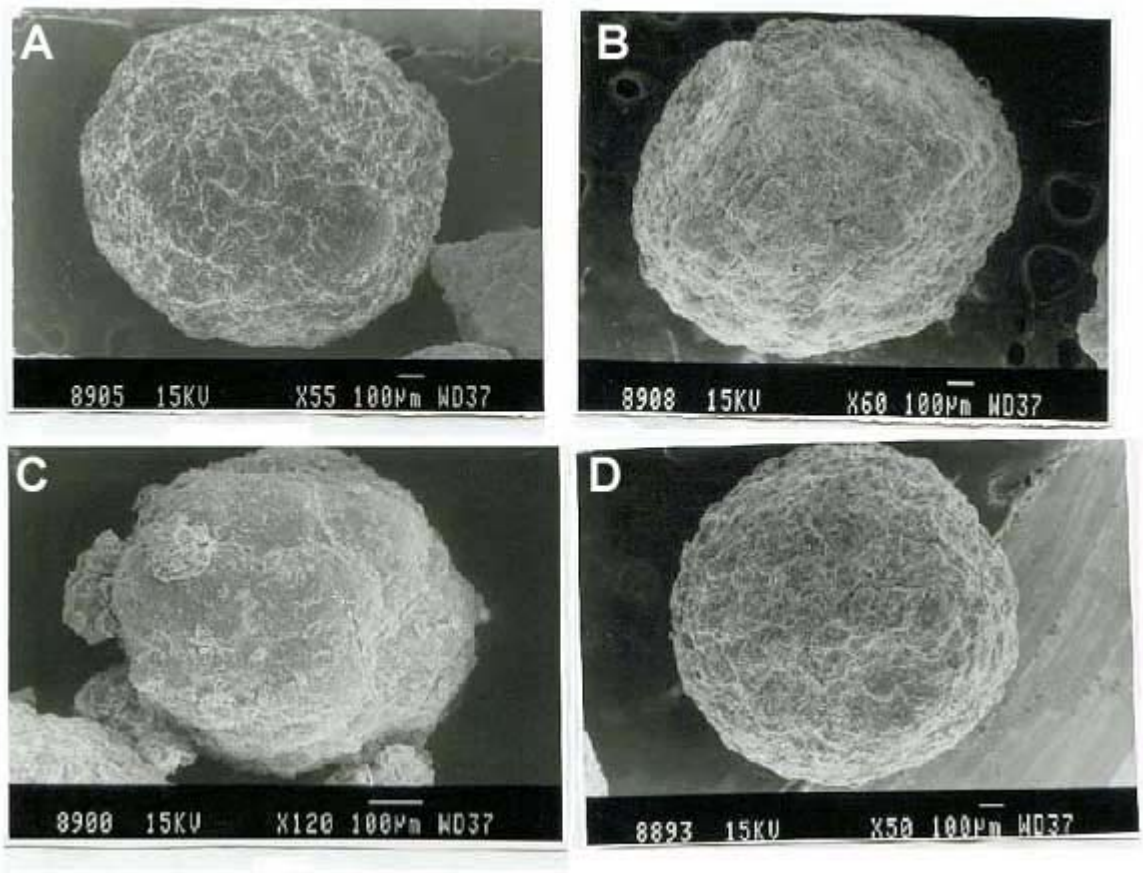

Figure 1. Scanning electron micrographs of glipizide microcapsules: (A) MC1, (B) MC2, (C) MC3, and (D) MC4.

Table 2. Results of In Vitro Wash-Off Test To Assess Mucoadhesive Properties of the Microcapsules Prepared*

Microcapsules	Percentage of Microcapsules Adhering to Tissue at 5 Times (h)†									
	1	2	4	6	8	1	2	4	6	8
	In 0.1N HCL, pH 1.2					In Phosphate Buffer, pH 6.2				
MC1	77 (1.5)*	72 (2.0)	62 (1.5)	57 (1.2)	56 (1.0)	62 (1.5)	19 (2.0)	14 (2.0)	05 (1.8)	—
MC2	70 (1.5)	64 (1.4)	58 (0.7)	56 (0.1)	54 (0.7)	63 (0.3)	45 (1.0)	16 (1.2)	02 (0.6)	—
MC3	84 (1.0)	82 (0.5)	74 (0.8)	69 (0.5)	65 (0.4)	69 (2.2)	62 (1.1)	32 (1.9)	19 (1.5)	15 (1.9)
MC4	81 (2.0)	81 (2.1)	76 (1.0)	76 (1.0)	74 (1.5)	71 (2.1)	56 (1.2)	27 (1.7)	10 (1.8)	04 (0.7)
MC5	88 (0.1)	76 (2.0)	58 (1.7)	38 (1.9)	21 (2.3)	72 (1.9)	56 (2.3)	24 (2.0)	07 (1.8)	—
MC6	85 (2.1)	77 (2.2)	62 (1.5)	32 (2.2)	24 (1.9)	73 (2.0)	55 (1.8)	26 (2.2)	04 (2.1)	—
MC7	75 (2.0)	68 (2.5)	60 (2.1)	48 (2.3)	30 (2.0)	69 (2.2)	65 (1.7)	35 (1.9)	20 (1.5)	17 (1.8)
MC8	82 (1.8)	71 (1.8)	61 (2.0)	35 (2.5)	20 (1.5)	71 (2.1)	57 (1.1)	30 (2.1)	12 (1.9)	06 (2.2)
EVA	55 (1.5)	41 (1.4)	11 (1.8)	—	—	52 (2.3)	40 (2.5)	08 (2.7)	—	—

*EVA indicates ethylene vinyl acetate; MC, microcapsule.
†Figures in parentheses are coefficient of variation values.

< alginate-sodium CMC < alginate-HPMC. The drug release from the microcapsules was diffusion controlled, as plots (**Figure 3**) of amount released versus the square root of time were found to be linear (r > 0.95). Glipizide release from microcapsules MC6 and MC7 was slow and extended over a period of 10 to 12 hours, and these microcapsules were found suitable for oral controlled-release formulations.

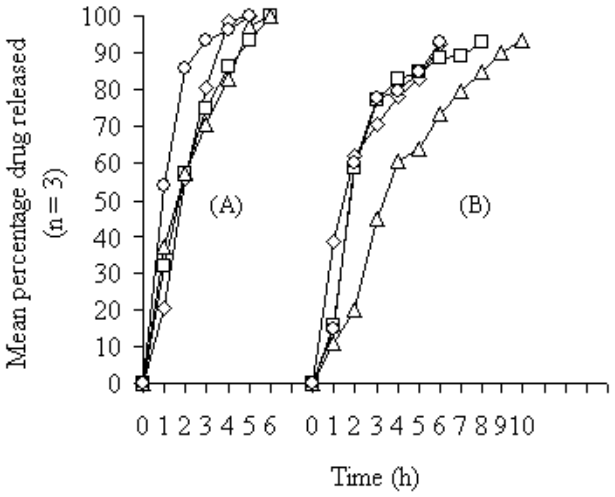

Figure 2. Release profiles of glipizide microcapsules (n = 3): (A) MC1 (◊), MC2 (□), MC3 (Δ), and MC4 (○); (B) MC5 (◊), MC6 (□), MC7 (Δ), and MC8 (○).

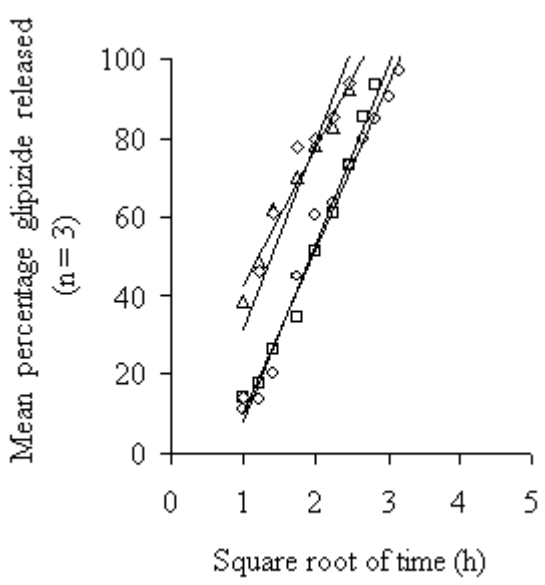

Figure 3. Percent released versus $(t)^{1/2}$ plots of glipizide microcapsules (n = 3): MC5 (Δ), MC6 (□), MC7 (○), and MC8 (◊).

In vivo evaluation of the microcapsules MC6 and MC7 was carried out in healthy, normal rabbits by measuring the hypoglycemic effect produced after their oral administration at a dose equivalent to 800 mg/kg of glipizide, in comparison to glipizide (pure drug) at the same dose. When glipizide was administered, a rapid reduction in serum glucose levels was observed; a maximum reduction of 53.12% was observed at 1.0 hours after administration, and the glucose levels recovered rapidly to the normal level within 7 hours (**Figure 4**). In the case of microcapsules, the reduction in glucose levels was slower; it reached maximum reduction 3 hours after administration, and the reductions in glucose levels were sustained over longer periods of time. A 25% reduction in glucose levels is considered a significant hypoglycemic effect.[19] The hypoglycemic effect was maintained during the period from 0.5 hours to 4 hours following the administration of glipizide, but the hypoglycemic effect was maintained during the period from 2.5 hours to 11 hours in the case of MC6 and from 2.5 hours to 14 hours in the case of MC7. The sustained hypoglycemic effect observed over longer periods of time in the case of microcapsules is due to the slow release and absorption of glipizide over longer periods of time. The hypoglycemic effect of glipizide could be sustained over 14 hours with microcapsules MC7, which contained alginate-Carbopol (9:1) as coat.

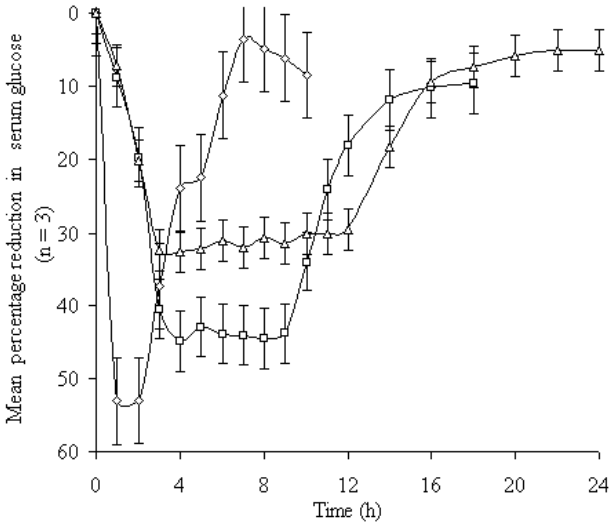

Figure 4. Percentage reduction in serum glucose following the oral administration of glipizide (◊) and its mucoadhesive microcapsules MC6 (□) and MC7 (Δ) in normal rabbits (n = 3).

CONCLUSSION

Thus, large spherical microcapsules with a coat consisting of alginate and a mucoadhesive polymer (sodium CMC, methylcellulose, Carbopol, or HPMC) could be prepared by an orifice-ionic gelation process. The microcapsules exhibited good mucoadhesive properties in an in vitro test. Glipizide release from these mucoadhesive microcapsules was slow and extended over longer periods of time and depended on composition of the coat. Drug release was diffusion controlled and followed zero-order kinetics after a lag period of 1 hour. In the in vivo evaluation, alginate-Carbopol microcapsules could sustain the hypoglycemic effect of glipizide over a 14-hour period. These mucoadhesive microcapsules are, thus, suitable for oral controlled release of glipizide.

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