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Mucoadhesive Microcapsules of Glipizide: Characterization, *in vitro* and *in vivo* Evaluation

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Glipizide microcapsules with a coat consisting of alginate and a mucoadhesive polymer such as sodium carboxymethylcellulose, methylcellulose, carbopol and hydroxypropylmethylcellulose were prepared by an orifice-ionic gelation process and were investigated with a view to develop mucoadhesive microcapsules. The resulting microcapsules were discrete, large, spherical and free flowing. Microencapsulation efficiency was 60-84%. The microcapsules exhibited good mucoadhesive property in the *in vitro* wash-off test. Glipizide release from these mucoadhesive microcapsules was slow and extended over longer periods of time and depended on the composition of coat of the microcapsules. Drug release was diffusion controlled and followed zero order kinetics after a lag period of 1 h. In the *in vivo* evaluation, alginate-carbopol microcapsules could sustain the hypoglycemic effect of glipizide over a period of 14 h. These mucoadhesive microcapsules are, thus, suitable for oral controlled release of glipizide.

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. 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 the dosage form with the underlying absorption surface to improve and enhance the bioavailability of drugs[3-5]. Several studies[6] reported mucoadhesive drug delivery systems in the form of tablets, films, patches and gels for oral, buccal, nasal, occular and topical routes. There were no reports on mucoadhesive microcapsules. The objective of this is to develop, characterize and evaluate mucoadhesive microcapsules of glipizide employing various mucoadhesive polymers. Glipizide, an effective antidiabetic, which requires controlled release owing to its short biological half-life[7] of 3.4±0.7 h was used as core in microencapsulation. The mucoadhesive microcapsules were evaluated by *in vitro* and *in vivo* methods for controlled release.

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MATERIALS AND METHODS

Glipizide was a gift sample from M/s Micro Labs, Pondicherry. Sodium carboxymethylcellulose (sodium CMC, with a viscosity of 1500-3000 cps of 1% w/v aqueous solution at 25°), methyl cellulose (with a methoxyl content of 28.3 % by weight and viscosity of 65 cps in 0.5% w/v aqueous solution at 25°) and hydroxypropylmethylcellulose (HPMC, of a viscosity of 50 cps in a 2 % by weight aqueous solution at 20°) were gift samples from M/s Natco Pharma Ltd., Hyderabad. Carbopol 934 P was a gift sample from M/s Smith Kline Beecham Pharmaceuticals, Bangalore. Sodium alginate (S. D. Fine Chem, Mumbai) and calcium chloride (Qualigens, Mumbai) were procured from commercial sources. All other reagents used were of analytical grade.

Preparation of microcapsules:

Microcapsules containing glipizide were prepared employing sodium alginate in combination with four mucoadhesive polymers namely sodium carboxymethylcellulose (sodium CMC), methylcellulose, Carbopol and hydroxypropylmethylcellulose (HPMC) as coat materials. No methods were reported for microencapsulation by these polymers. An orifice-ionic gelation process[8,9], which has been

extensively used to prepare large sized alginate beads, was employed to prepare the microcapsules.

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. Core material, glipizide (2.0 g) was added to the polymer solution and mixed thoroughly to form a smooth viscous dispersion. The resulting dispersion was then added drop wise into calcium chloride (10% w/v) 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 min 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° for 12 h. The microcapsules prepared along with their coat composition are listed in Table 1.

Estimation of glipizide content of the microcapsules:

Glipizide content in the microcapsules was estimated by an UV spectrophotometric method[10] 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 1-10 μg/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 formula, microencapsulation efficiency=(estimated percent drug content/theoretical percent drug content)×100.

Drug release study:

Release of glipizide from the microcapsules was studied in phosphate buffer of pH 7.4 (900 ml) using an USP XXIII three-station Dissolution Rate Test Apparatus (Model DR-3, M/s Campbell Electronics) with a rotating paddle stirrer at 50 rpm and 37±1° 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

TABLE 1: COAT COMPOSITION, DRUG CONTENT AND MICROENCAPSULATION EFFICIENCY OF THE MICROCAPSULES PREPARED.

Micro Capsules	Coat Composition	Percent Drug Content	Microencapsulation Efficiency (%)	Release Rate, K_o (mg/h)
MC1	Alginate:sodium CMC (1:1)	42.7	85.4	1.86 (0.97)*
MC2	Alginate:MC (1:1)	32.4	64.7	1.85 (0.97)
MC3	Alginate:Carbopol (1:1)	36.1	72.2	1.63 (0.99)
MC4	Alginate:HPMC(1:1)	30.3	60.7	3.25 (0.91)
MC5	Alginate:sodium CMC (9:1)	34.0	68.0	1.14 (0.94)
MC6	Alginate:MC (9:1)	32.5	64.9	1.08 (0.97)
MC7	Alginate:Carbopol (9:1)	36.8	73.6	1.06 (0.98)
MC8	Alginate:HPMC(9:1)	35.9	71.7	1.63 (0.93)

*Figures in parentheses are Correlation Coefficient (r) values between amount (mg) dissolved and time in hours.

TABLE 2: RESULTS OF *IN VITRO* WASH-OFF TEST TO ASSESS MUCOADHESIVE PROPERTY OF THE MICROCAPSULES.

Microcapsules	Percent microcapsules adhering to tissue at (h)									
	0.1 N HCl, pH 1.2					Phosphate buffer, pH 6.2				
	1	2	4	6	8	1	2	4	6	8
MC 1	77 (1.5)*	72 (2.0)	62 (1.5)	57 (1.2)	56 (1.0)	32 (1.5)	19 (2.0)	14 (2.0)	05 (1.8)	-
MC 2	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)	-
MC 3	84 (1.0)	82 (0.5)	74 (0.8)	69 (0.5)	65 (2.4)	69 (2.2)	65 (1.1)	32 (1.9)	19 (1.5)	15 (1.9)
MC 4	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)
EVA	55 (1.5)	41 (1.4)	11 (1.8)	-	-	52 (2.3)	40 (2.5)	08 (2.7)	-	-

*Figures in parentheses are Coefficient of Variation (CV) values.

fluid were withdrawn through a filter (0.4 μm) at different time intervals and were assayed at 223 nm for glipizide content using a Shimadzu UV-150 double-beam spectrophotometer. The drug release experiments were conducted in triplicate.

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 wash-off method. The mucoadhesiveness of these microcapsules was compared with that of a non-bioadhesive material, ethylene vinyl acetate microcapsules. Pieces of intestinal mucosa (2x2 cm) were mounted on to glass slides (3x1 inch) with cyanoacrylate glue. Two glass slides were connected with a suitable support. About 50 microcapsules were spread on to each wet rinsed tissue specimen and immediately thereafter the support was hung on to the arm of a USP tablet disintegrating test machine. By operating the disintegrating test machine the tissue specimen was given a slow regular up and down movement in the test fluid at 37° taken in a 1 l vessel of the machine. At the end of 30 min, 1 h and later at hourly intervals up to 12 h, the machine was stopped and the number of microcapsules still adhering on to the tissue was counted. The test was performed in both acidic (0.1 N HCl) and in alkaline (phosphate buffer of pH 6.2) fluids.

***In vivo* Evaluation:**

In vivo evaluation studies were conducted on (i) glipizide, (ii) microcapsules MC6 and (iii) 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 RBD (n=4). The approval of the Institutional Animal Ethics Committee was obtained before starting the study. The products were administered orally in the morning following over night fasting. No food or liquid other than water was given during the experimental period. After collecting the zero-hour blood sample the product in the study was administered orally. Blood samples (0.5 ml) were collected at one hourly intervals of time up to 24 h after administration. Serum glucose concentrations were determined using a previously reported oxidase-peroxidase method[11] as described below employing Glucose Kit supplied by Dr. Reddy's Laboratory, Diagnostic Division, Hyderabad. The method was revalidated, the relative standard deviation (RSD) 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° for 10 min. The solutions were diluted to 5 ml with distilled water and the absorbance of the pink coloured 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 cal-

TABLE 3: RELEASE CHARACTERISTICS OF MUCOADHESIVE MICROCAPSULES PREPARED.

Microcap-	Percent glipizide Released at 5 times (h) (mean± s.d)					T_{50}(h)	K_0(mg/h)
sules	1.0	2.0	4.0	8.0	10.0		
MC1	20.5±4.46	56.0±2.49	98.5±1.03	-	-	1.6	1.86
MC2	31.8±1.24	57.6±0.84	86.2±0.05	100.0±1.03	-	1.7	1.84
MC3	36.8±2.70	57.4±3.32	82.8±1.65	99.0±0.38	-	2.0	1.63
MC4	53.9±0.58	86.0±1.25	96.4±0.09	-	-	0.8	3.48
MC5	38.2±1.67	62.3±0.06	78.2±0.43	99.9±0.71	-	1.9	1.14
MC6	14.2±2.42	26.4±0.24	51.5±0.51	93.6±0.65	99.7±0.27	2.1	1.08
MC7	11.0±0.16	20.4±0.81	60.5±0.06	84.9±0.74	96.9±0.44	3.4	1.05
MC8	14.2.±0.92	61.1±1.06	79.9±0.31	99.8±0.57	-	1.3	1.62

T_{50} is time for 50% release and K_0 is zero order release rate constant.

culated.

RESULTS AND DISCUSSION

Microcapsules of glipizide with a coat consisting of alginate and a mucoadhesive polymer namely sodium CMC, or methylcellulose, or carbopol or HPMC in 1:1 and 9:1 ratio could be prepared by the orifice- ionic gelation process employed. Microcapsules with a coat of mucoadhesive polymer alone could not be prepared due to their water-soluble

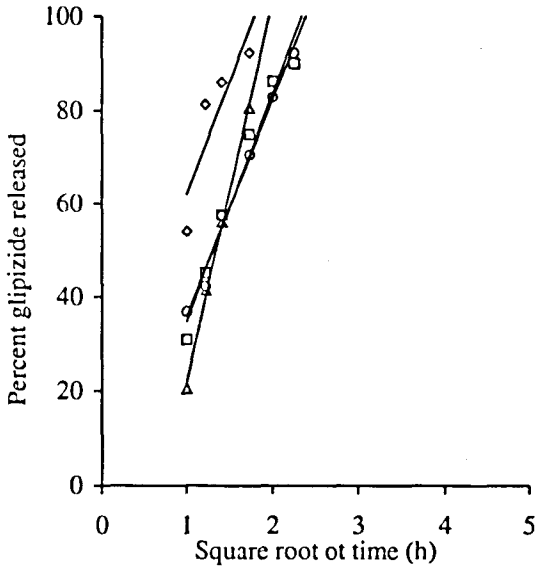

Fig. 1: Release profiles of Glipizide microcapsules.

Percent released Vs (time)$^{1/2}$ plots of glipizide microcapsules, MC1(△), MC2 (□), MC3 (○) and MC4 (◇). MC1, MC2, MC3 and MC4 are microcapsules prepared employing sodium CMC, methyl cellulose, Carbopol and HPMC respectively along with alginate with a 1:1 ratio of alginate and mucoadhesive polymer.

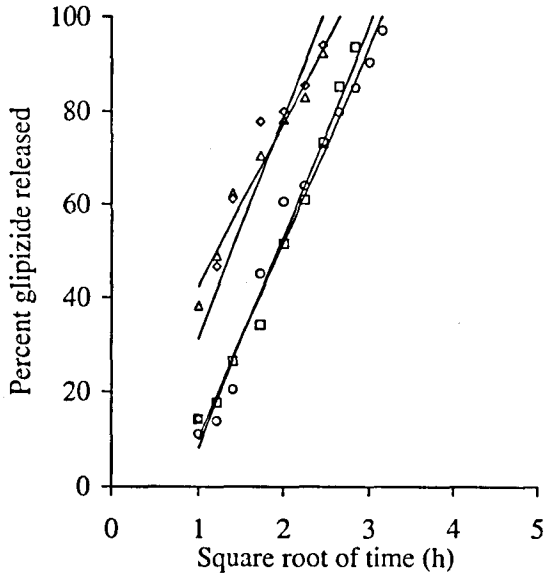

Fig 2: Release profiles of Glipizide microcapsules.

Percent released Vs (time)$^{1/2}$ plots of glipizide microcapsules, MC5 (△), MC6 (□), MC7 (○) and MC8 (◇). MC5, MC6, MC7 and MC8 are microcapsules prepared employing sodium CMC, methyl cellulose, Carbopol and HPMC respectively along with alginate with a 9:1 ratio of alginate and mucoadhesive polymer.

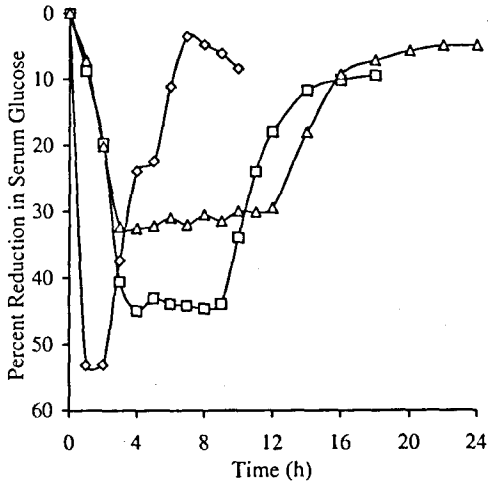

Fig 3: Percent reduction in serum glucose following the administration of glipizide and its microcapsules.

Percent reduction in serum glucose following oral administration of glipizide (◇) and its mucoadhesive microcapsules, MC6 (□) and MC7 (△) in normal rabbits. MC6 and MC7 are microcapsules prepared employing methylcellulose and Carbopol respectively along with alginate with a 9:1 ratio of alginate and mucoadhesive polymer.

nature. The microcapsules were found to be discrete, large, spherical, free flowing and monolithic matrix type. Low C.V. (<2.0 %) in percent drug content indicated uniformity of drug content in each batch of microcapsules (Table 1). The microencapsulation efficiency was in the range of 60-84%.

Microcapsules with a coat consisting of alginate and a mucoadhesive polymer exhibited good mucoadhesive property in the *in vitro* wash-off test when compared to non-mucoadhesive 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 EVA microcapsules (Table 2). The wash off was relatively rapid in alkali than in acid fluids. The results of wash-off test indicated fairly good mucoadhesive property of the microcapsules.

Glipizide release from the microcapsules was studied in phosphate buffer (pH 7.4) for a period of 12 h as prescribed for glipizide tablets in USP XXIV. Glipizide release from the microcapsules was slow, spread over extended periods of time and depended on the composition of coat (Table 3). Release followed zero order kinetics (r>0.90) after a lag period of 1 h. 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-methyl cellulose<alginate-sodium CMC<alginate-HPMC (Table 3). The drug release from the microcapsules was diffusion controlled as plots (fig. 1 and 2) of amount released Vs $\sqrt{t}$ were found to be linear (r>0.95). Glipizide release from microcapsules MC6 and MC7 was slow and extended over a period of 10-12 h and these microcapsules were found suitable for oral controlled release formulations.

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 μg/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 and a maximum reduction of 53.12% was observed at 1.0 h after administration and the glucose levels were also recovered rapidly to the normal level within 7 h (fig. 3). Whereas in the case of microcapsules, the reduction in glucose levels was slow and reached maximum reduction in 3 h after administration and the reduced in glucose levels were sustained over longer periods of time. A 25% reduction in glucose levels is considered as a significant hypoglycemic effect[12]. The hypoglycemic effect was maintained during the period from 0.5 h to 4 h following the administration of glipizide. Whereas in the case of microcapsules, the hypoglycemic effect was maintained during the period from 2.5 h to11 h in the case of MC6 and from 2.5 h to 14 h 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 a period of 14 h with microcapsules MC7 that contained alginate –Carbopol (9:1) as coat.

Thus, large sized spherical microcapsules of glipizide with a coat consisting of alginate and a mucoadhesive polymer (sodium CMC or methylcellulose or carbopol or HPMC) could be prepared by an orifice-ionic gelation process. The microcapsules exhibited good mucoadhesive property in the *in vitro* wash-off 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 h. In the *in vivo* evaluation, alginate-carbopol microcapsules could sustain the

hypoglycemic effect of glipizide over a period of 14 h. These mucoadhesive microcapsules are, thus, suitable for oral controlled release of glipizide.

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