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Preparation and evaluation of mucoadhesive microcapsules of glipizide formulated with gum kondagogu: *In vitro* and *in vivo*

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Abstract

Mucoadhesive microcapsules are proposed for the antidiabetic drug, glipizide, to obtain the controlled release. Glipizide microcapsules with a coat consisting of alginate and gum kondagogu were prepared by employing ionic gelation process and emulsification ionotropic gelation process. The microcapsules were evaluated for flow properties, Carr's index, Hausner factor, microencapsulation efficiency, drug release characteristics, surface characteristics; compatibility studies mucoadhesive properties and *in vivo* hypoglycemic activity. These two methods gave discrete, large sized, free flowing spherical microcapsules without any interactions. Glipizide release from microcapsules was slow and followed zero order kinetics and followed non–fickian release and depended on the coat: core ratio and the method employed in the preparation of microcapsules. Among the two methods emulsification ionotropic gelation method was found to be more suitable for slow and complete release of glipizide over a long period of time. These microcapsules exhibited good mucoadhesive property in the *in vitro* wash-off test. *In vivo* testing into rabbits demonstrated significant hypoglycemic effect of glipizide.

Keywords: Ionotropic gelation, hypoglycemic activity, emulsification ionotropic gelation, glipizide

Introduction

Micro encapsulation by various polymers and its applications are described in standard textbooks (Kondo 1979, Gutcho 1976). Micro encapsulation 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 (Chowdary and Srinivasa Rao 2004). Several studies reported drug delivery systems in the form of tablets, films, patches, and gels for oral, buccal, nasal, ocular, and topical routes. Amongst the polymers used for Microencapsulation alginate has gained much attention since it is non toxic, biodegradable and can be prepared by a safe technique avoiding organic solvents (Gursoy and Cevik 2000). Ionic gelation method is not practical because of the blockage of the nozzle and the low yield of the product. Hence ionotropic gelation of sodium alginate by the emulsification technique was developed as an alternative approach (Poncelet et al. 1992). Gum kondagogu (GK) also known as *Cochlospermum gossypium* belongs to *Cochlospermum spp.* and family bixaceae (Janaki and Sashidhar 1998). GK is a negative colloid and a high molecular weight complex acidic polysaccharide. The general utility of GK is based on its viscosity (Vinod et al. 2007).

Glipizide, an effective antidiabetic that requires controlled release owing to its short biological half-life of 3.4 ± 0.7 h, (Kahn and Shechter 1991) was used as the core in micro encapsulation. The purpose of this research was to formulate and systemically evaluate *in vitro* and *in vivo* performance of mucoadhesive microcapsules of glipizide.

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Materials and Methods

Materials

Glipizide U.S.P was a gift sample from M/s Orchid Pharma Ltd, Chennai, India. Girijan Co-operative Corporation Ltd., Visakhapatnam, India, supplied gum kondagogu (Grade 1). Sodium alginate (having a viscosity of 5.5 cps in a 1% w/v aqueous solution at 25°C), calcium chloride and heavy liquid paraffin were procured from M/S Loba Chemie Pvt Ltd., Mumbai, India.

Preparations of microcapsules

Sodium alginate (1g) and gum kondagogu (1g) were dissolved in purified water (32 mL) to form a homogeneous polymer solution. The active substance, Glipizide (2g), was added to the polymer solution and mixed thoroughly with a stirrer to form a viscous dispersion. The resulting dispersion was then added manually drop wise into calcium chloride (15% 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 having coat:core ratio 1:1 (MC_1). Similarly microcapsules with coat:core ratio 1.5:1 (MC_2) and 2:1 (MC_3) were also prepared. The microcapsules were collected by decantation and dried over night at room temperature.

In emulsion gelation technique polymer gum kondagogu (1g) and sodium alginate (1g) were dissolved in 32 mL of water. Drug (2g) was added and mixed thoroughly. The polymer dispersion was then added in a thin string to 50 ml of heavy liquid paraffin contained in a 250 mL beaker, while stirring at 500 rpm to emulsify the added dispersion as fine droplets. A medium duty stirrer with speedometer (RQ 121/D, Remy Instruments Ltd, India) was used for stirring. Then 20 mL of calcium chloride solution (15% w/v) was transferred in to the emulsion while stirring at 500 rpm for 15 min to produce spherical microcapsules. The microcapsules were collected by decantation and washed repeatedly with petroleum ether. The product was then air dried to obtain discrete micro spheres. Different proportions of coat: core materials namely 1:1 (MC_4), 1.5:1 (MC_5) and 2:1 (MC_6) were used to prepare microcapsules.

Evaluation of microcapsules

Size distribution and size analysis (Jacob et al. 2007)

For size distribution analysis, different sizes in a batch were separated by sieving, using a range of standard sieves. The amounts retained on different sieves were weighed. The mean particle size of the microcapsules was calculated by the formula.

$$\text{Mean Particle Size} = \frac{\sum(\text{Mean Particle Size of the Fraction X Weight Fraction})}{\sum(\text{Weight Fraction})}$$

Flowability of microcapsules

The static angle of repose was measured according to the fixed funnel and free standing cone method. the bulk density of the mixed microcapsules was calculated by determining the Hausner's ratio and Carr's index from the pored and tapped bulk densities of a know weight of sample using a measureing cylinder. The following formulas were used for calculating Hausner's Ratio = $D_p \div D_t$, Carr's Index = $[(D_p - D_t) \div D_p] \times 100$, where D_p (Poured density) = Weight of the microcapsules $\div$ V_p(Poured Volume), D_t (tappaed density) = Weight of the microcapsules $\div$ V_t (tapped Volume).

Drug content evaluation

Glipizide content in the microcapsules was estimated by a UV spectrophotometric (UV-1700, Shimadzu, Japan) method based on the measurement of absorbance at 223 nm in phosphate buffer of pH 7.4 (Chowdary and Srinivasa Rao 2003). Microcapsules containing equivalent to 100 mg of glipizide were crushed to fine powder in a mortar and extracted with 50 mL of methanol. It was filtered and made up to the volume of 100 mL with methanol. One ml of the sample was taken and made up the volume to 10 mL with phosphate buffer pH 7.4 and the absorbance was measured at 223 nm. The procedure was repeated with pure gilpizide. The absorbance values from the pure drug and glipizide were treated statistically by t-test. The absorbance values were not differed significantly ($p>0.1$) indicating non interference of gum kondagogu in the estimation of glipizide. The method was validated for linearity, accuracy, and precision. The method obeyed Beer's law in the concentration range 1 to 10 ∝g/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.

$$\text{Microencapsulation Efficiency} = \frac{\text{Estimated Percentage Drug Content}}{\text{Theoretical Percentage Drug Content}} X100$$

Scanning electron microscopy (SEM)

The samples for the SEM analysis were prepared by sprinkling the gel beads on one side of the double adhesive stub. The stub was then coated with fine gold dust. The gel beads were then observed with the scanning electron microscope (Leica Electron Optics, Cambridge, USA) at 15 kV.

Differential scanning calorimetry

Differential scanning calorimetry (DSC) curves were obtained by a differential scanning calorimeter (DSC 220C, Seiko, Tokyo, Japan) at a heating rate of 10°C/min from 30 to 300°C in a nitrogen atmosphere.

Infrared spectroscopic studies

Fourier–transformed infrared (FT–IR) spectra were obtained on a Perkin Elmer 2000 FT–IR system (Perkin Elmer, Norwalk, CT, USA) using the KBr disk method (2 mg sample in 200 mg KBr). The scanning range was 400 to 4000 cm^{-1} and the resolution was 1 cm^{-1}.

In vitro release studies

Release of glipizide from the microcapsules was studied in phosphate buffer of pH 7.4 (900 mL) using a United States Pharmacopoeia (USP) XXIII 8-station Dissolution Rate Test Apparatus (Model TDT - 08L, M/s Electrolab, Mumbai, 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-1700 double beam spectrophotometer (Shimadzu Corporation, Japan). The drug release experiments were conducted in triplicate (n = 3).

The release data obtained were fitted to zero order (Najib and Suleiman 1985), first order (Desai et al. 1966), Higuchi (Higuchi 1963) and Korsmeyer-Peppas (Korsmeyer et al. 1983, Ritger and Peppas 1987a and 1987b) equations to determine the corresponding release rate and mechanism of drug release from the mucoadhesive microspheres.

Mucoadhesion evaluation

The mucoadhesive property of the microcapsules was evaluated by an in vitro adhesion testing method known as the wash-off test (Chowdary and Srinivasa Rao 2003). The mucoadhesiveness of these microcapsules was compared with that of the non-bioadhesive 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 ^{O}C contained in a 1L vessel of the machine. At the end of 30 min, at the end of 1 h, and at hourly intervals up to 12 h, 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 7.4).

In Vivo evaluation

In vivo evaluation studies were conducted on glipizide, microcapsules MC_3 and microcapsules MC_6 in normal, healthy rabbits (n= 5) by measuring serum glucose levels following their oral administration at a dose equivalent to 800 μg/ kg. The study protocol was approved by IAEC (Institutional Animal Ethics Committee) **IAEC-I-9/BCOP/2007-2008** before the conduct of the study. The standard and the formulation were administered orally following overnight fasting. The animals were deprived of food during the experimental period. The blood samples were collected before and after administration of the standard and the formulation from the marginal ear vein for a period of 24 h. The collected samples were subjected to glucose estimation in serum by glucose oxidase method (Trinder 1969).

Results

Microcapsules of glipizide could be prepared by ionic gelation process and emulsification gelation process employing gum kondagogu as the polymer. The microcapsules were found to be discrete spherical and free flowing. The size analysis of different batches of microcapsules showed that about 72% of the prepared microcapsules were in the size range of 920 μm (-16 to +20). The size distribution of microcapsule was found to be normal in all the batches. The microcapsules imparted good flow ability as indicated by angle of repose (21.76 - 25.86), the Carr's index (12 - 16) and the Hausner Ratio (1.1 – 1.2). The SEM photographs indicated that the microcapsules were spherical and completely covered with the coat polymer (Figure 1 and 2).

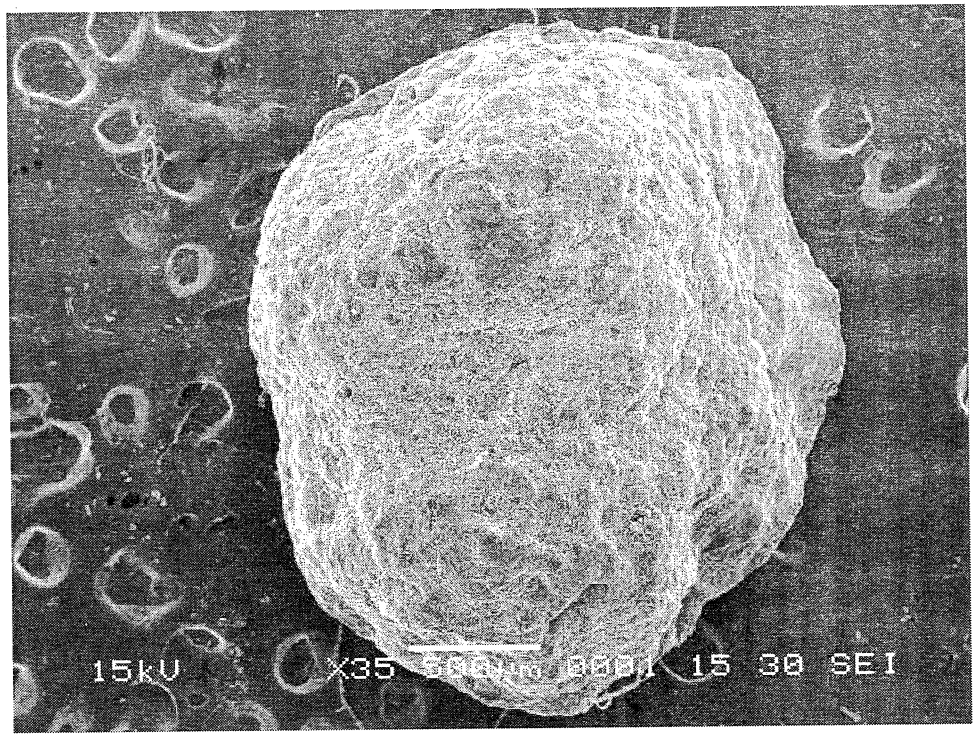


Figure 1. SEM photograph of glipizide microcapsules formulated with gum Kondagogu by ionic gelation technique

Low coefficient of variation (<2.0%) in percent drug content indicated uniformity of drug content in each batch of microcapsules. The Microencapsulation efficiency was in the range of 76% to 93%, with various products. Selected DSC scans of the drug, gum kondagogu and microcapsule were shown in Figure 3. The DSC scan of Glipizide showed a short endothermic peak at 215.5°C. The thermo gram of microcapsules showed an endothermic peak of drug at 170°C indicating a slight change in terms of shifting towards the lower temperature. It has been reported that the quantity of material used effects the peak shape and enthalpy. Thus these minor changes in the melting endotherm in the drug could be due to the mixing of the drug and excipients which lower the purity of each component in the mixture and may not necessarily indicate potential incompatibility.

The IR spectrum of Glipizide is shown in Figure 4 and the following characteristic bands were observed 1689 cm^{-1} (-C=O, Amide), 1651 cm^{-1} (-C=O, Urea), 1528 cm^{-1} (Ar-CH, stretching), 1433 cm^{-1} (Ar-CH, bending), and 1333 cm^{-1} and 1159 cm^{-1} ($-SO_2NH$). The IR spectrum of glipizide microcapsules Figure 4 showed the presence of characteristic bands of glipizide. Thus, any change in the structure of glipizide was ruled out and it was concluded that there is no chemical incompatibility between glipizide and gum kondagogu.

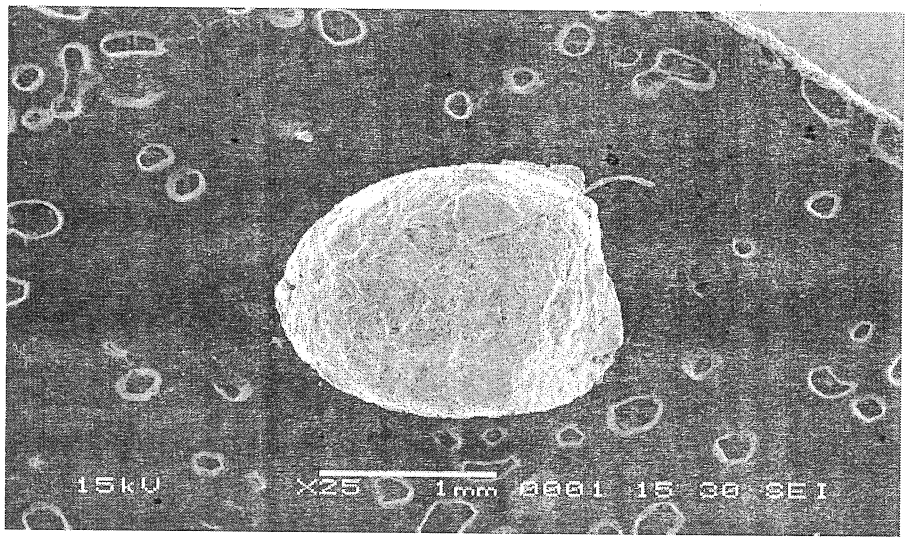
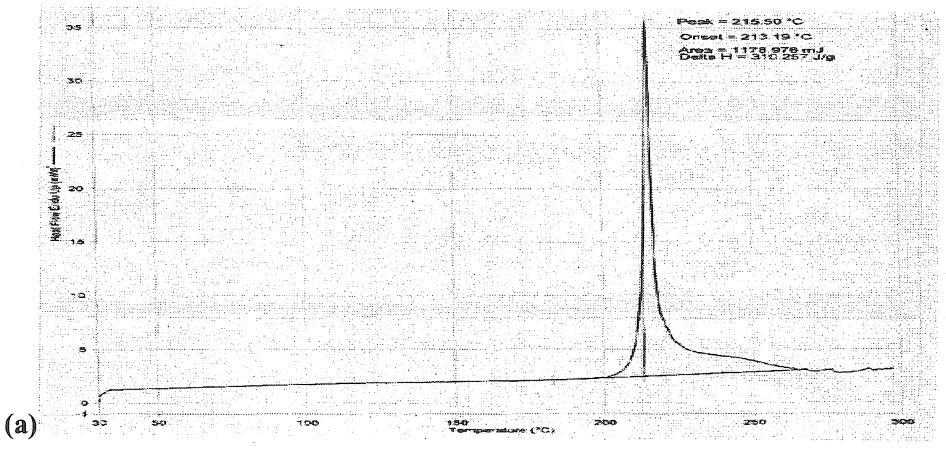
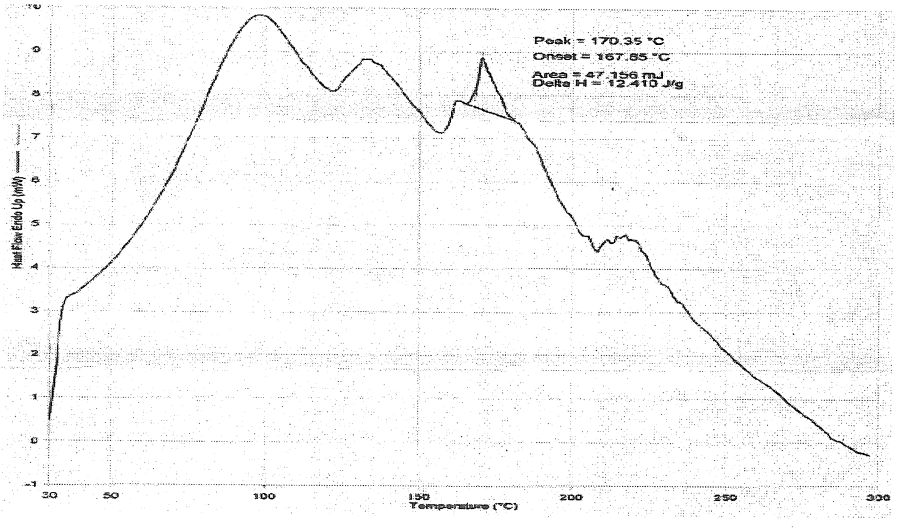


Figure 2. SEM photograph of glipizide microcapsules formulated with gum kondagogu by emulsification ionotropic gelation technique



(a)



(b)

Figure 3. DSC Curves of (a) glipizide (b) microcapsules with glipizide and gum Kondagogu

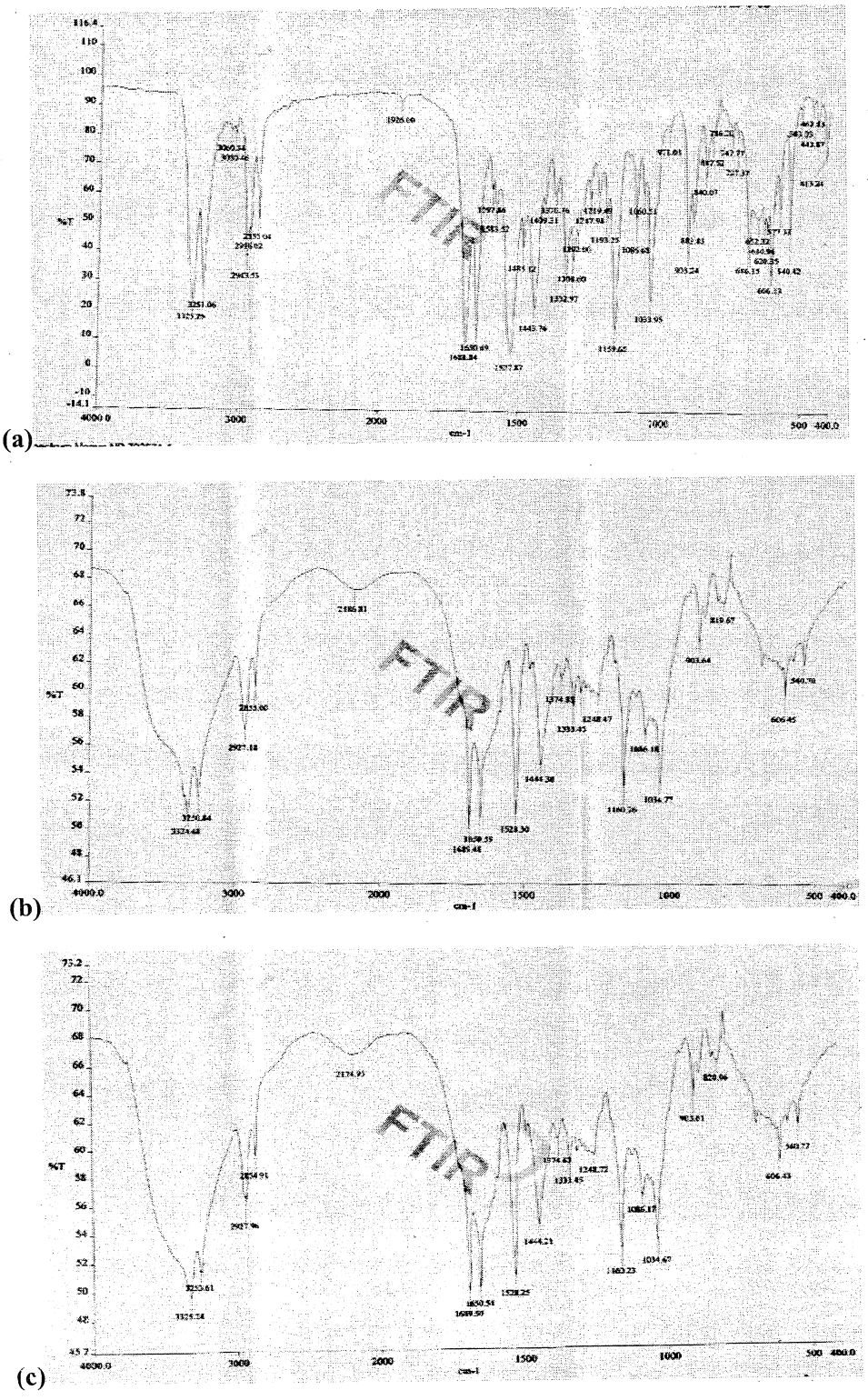


Figure 4. IR Graphs of (a) glipizide (b) gum kondagogu (c) microcapsules with glipizide and gum kondagogu

Glipizide release from the microcapsules was studied in phosphate buffer (pH 7.4) for 12 h as prescribed for glipizide tablets in USP XXIV. Glipizide release from the microcapsules was slow, spread over extended period of time and depended on the composition of the coat composition and method employed for the preparation of microcapsules (Table 1). The modal that best fits the release data was evaluated by correlation coefficient (r). The correlation coefficient (r) value was used as criteria to choose the best

model to describe the drug release from the microcapsules. The r value in various models is given in Table 2. In most of the formulated microcapsule the r value were higher in zero order model than that of first order model indicating the drug release from the most of the microcapsules was according to zero order kinetics. To analyze the mechanism of release of drug from the microcapsules the equation, $Q= Kt^n$ was used, where Q is the percentage of drug released; t is the release time; K is a constant incorporating structural and geometric characteristics of the release device, n is the release exponent indicative of mechanism of release. When n approximates to 0.5, a fickian/diffusion control release is implied, where $0.5 < n < 1$ non fickian transport and n= 1 for zero order release (Murthy and Chowdary 2005). The drug release mechanism from the microcapsules was non fickian transport as n value is in between 0.87 to 1. The drug release from the marketed formulation followed zero order kinetics and controlled by Korsmeyer-Peppas mechanism. The release rate constants observed from the selected formulation (MC6) and marketed formulation were found to be 13.7614 mg/h and 11.2183 mg/h respectively. This two formulations were not differed in the *in vitro* release rate ($p>0.1$).

Table 1. Release profiles of glipizide microcapsules

Formulation	Percent glipizide released at times (h) $\overline{X}$ ± s.d.					T_{50} (h)	Release Rate (mg/h) K_0
	1.0	2.0	4.0	6.0	8.0		
MC_1	30.03 ± 0.12	40.77 ± 0.14	73.94 ± 0.13	99.17 ± 0.35	---	2.7	18.6596
MC_2	20.71 ± 0.14	34.97 ± 0.57	59.81 ± 0.43	86.56 ± 0.22	99.52 ± 0.27	3.5	14.2759
MC_3	12.05 ± 0.19	21.25 ± 0.22	46.51 ± 0.21	70.47 ± 0.12	96.92 ± 0.12	4.2	11.8253
MC_4	27.65 ± 0.14	40.31 ± 0.14	71.29 ± 0.22	99.65 ± 0.31	---	2.8	17.5616
MC_5	14.76 ± 0.28	29.09 ± 0.43	55.02 ± 0.22	77.33 ± 0.29	94.46 ± 0.76	4.0	12.5434
MC_6	8.81 ± 0.23	18.97 ± 0.33	42.21 ± 0.12	67.36 ± 0.33	87.06 ± 0.17	4.7	10.2936

Microcapsules with a coat consisting of alginate and gum kondagogu exhibited good mucoadhesive properties in the in vitro wash-off test when compared to non-nucoadhesive material, ethylene vinyl acetate microcapsules. The wash-off was slow in the case of microcapsules containing alginate-gum kondagogu as coat when compared to that of ethylene vinyl acetate microcapsules (Table 3). The wash-off was faster at gastric pH than at intestinal pH. The results of the wash-off test indicated that the microcapsules had fairly good mucoadhesive properties.

Table 2. Correlation coefficient (R) values in various kinetic models tested to describe drug release from the mucoadhesive microcapsules formulated

Formulation	Correlation coefficient values				n
	Zero Order	First Order	Higuchi Model	Korsmeyer-Peppas Model	
MC_1	0.9808	0.8144	0.9622	0.9886	0.6734
MC_2	0.9893	0.8258	0.9601	0.9993	0.7877
MC_3	0.9991	0.8649	0.9129	0.9988	1.0640
MC_4	0.9810	0.7849	0.9634	0.9765	0.6472
MC_5	0.9894	0.8444	0.9562	0.9982	0.8616
MC_6	0.9960	0.8772	0.9275	0.9965	1.0182

Table 3: *In vitro* wash-off test values for the prepared microcapsules

Formulation	Percent of alginate beads adhering to tissue at 5 times (h)									
	0.1 N HCl, pH 1.2					Phosphate Buffer, pH 7.8				
	1	2	4	6	8	1	2	4	6	8
MC_1	45 (1.8) *	32 (1.2)	26 (1.0)	15 (1.6)	09 (1.5)	50 (0.5)	42 (1.0)	34 (1.3)	23 (1.4)	16 (1.5)
MC_2	46 (0.7)	36 (1.2)	28 (1.8)	20 (1.4)	11 (2.0)	50 (0.5)	47 (0.8)	44 (1.2)	41 (1.0)	35 (1.5)
MC_3	46 (1.0)	39 (1.8)	30 (1.1)	22 (1.5)	15 (1.0)	50 (0.5)	49 (1.0)	48 (1.2)	45 (1.8)	43 (1.2)
MC_4	47 (1.5)	36 (1.8)	28 (1.5)	19 (1.2)	11 (1.8)	50 (0.5)	44 (1.0)	39 (1.2)	31 (1.8)	28 (1.5)
MC_5	47 (1.8)	40 (1.2)	32 (1.8)	24 (2.0)	15 (1.8)	50 (0.5)	47 (1.0)	45 (1.5)	41 (1.5)	38 (2.0)
MC_6	48 (1.5)	42 (1.8)	37 (1.2)	27 (1.8)	20 (2.0)	50 (0.5)	49 (1.0)	49 (1.0)	49 (1.0)	47 (1.0)
EVA	54 (1.8)	34 (2.0)	10 (1.0)	--	--	48 (2.0)	28 (2.5)	04 (2.0)	--	--

*Figures in parentheses are coefficient of variation (CV) values.

In vivo evaluation of the microcapsules MC_3 and MC_6 was carried out in healthy, normal rabbits by measuring the hypoglycemic effect produced after their oral administration at a dose of 800 µg/kg of rabbits. When glipizide was administered, a rapid reduction in serum glucose levels was observed; a maximum reduction of 51.8% was observed at 1 h after administration, and the glucose levels recovered rapidly to the normal level after a period of 4 h. In the case of microcapsules MC_6, the maximum reduction in blood glucose levels was observed at 1.5^{th} h and the effect was found to be sustained over period of 14 h which is shown in Fig 5. In the case of microcapsule MC_3 the maximum blood glucose levels was observed at 1.5^{th} h and sustained effect was found over a period of 12 h. 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. The hypoglycemic effect of glipizide is sustained over a period of 14 h with microcapsules MC_6, which contained alginate-kondagogu (2:1) as coat when compared to that of standard glipizide which is been formulated with emulsification ionotropic gelation technique.

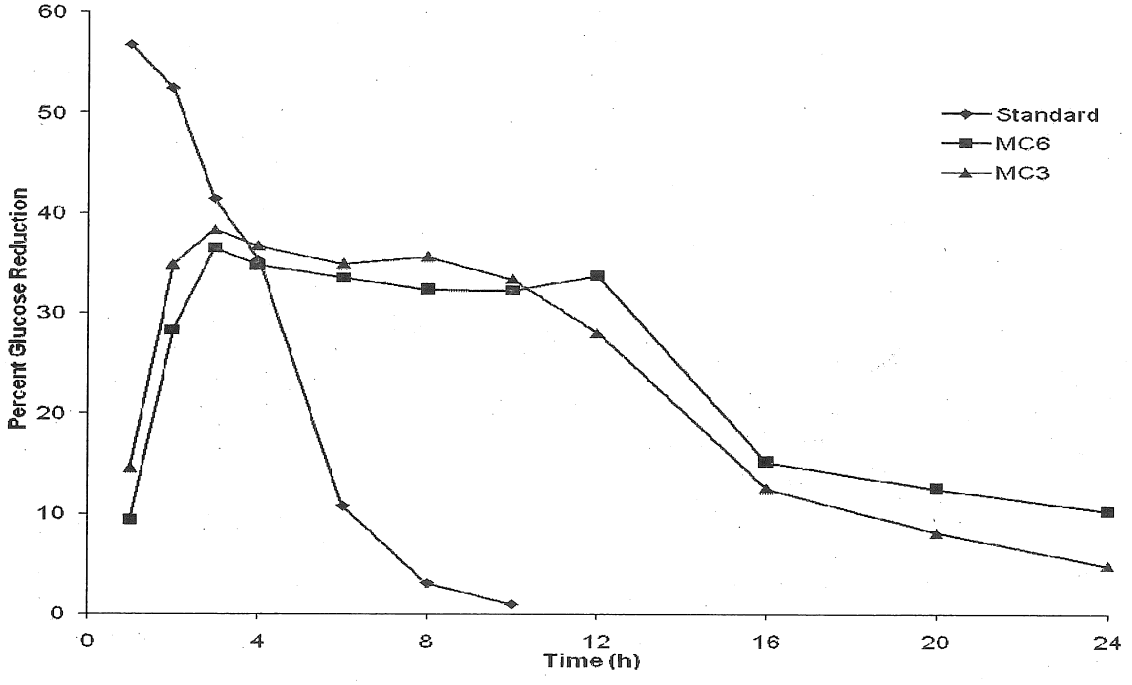


Figure 5. Percent reduction in serum glucose following the administration of glipizide and its microcapsules

Discussion

Thus, large spherical microcapsules with a coat consisting of alginate and a Mucoadhesive polymer gum kondagogu could be prepared by an emulsion 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.

In the in vivo evaluation, alginate-gum kondagogu (2:1) 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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