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Design and evaluation of mucoadhesive microspheres of repaglinide for oral controlled release

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Abstract

Gastro retentive dosage forms have potential for use as controlled-release drug delivery systems. Multiple unit systems avoid the "all-or-none gastric" emptying nature of single-unit systems. A controlled release system designed to increase its residence time in the stomach with contact with the mucosa was achieved through the preparation of mucoadhesive microspheres by the emulsion solvent evaporation technique consisting of (I) chitosan mucoadhesive (ii) repaglinide, an oral hypoglycemic agent; and (iii) Eudragit RS-100 as polymer. The microspheres were evaluated for surface morphology and particle shape by scanning electron microscope. The microspheres were also evaluated for their microencapsulation efficiency, in vitro wash-off mucoadhesion test, in vitro drug release and in vivo study. The microspheres were found to be spherical and free flowing. The microencapsulation efficiency was in the range of 61.44±1.16 to 79.90±1.17and microspheres exhibited good mucoadhesive property in the in vitro wash off test. The drug-polymer concentration of dispersed phase influences the particle size and drug release properties. All the formulations were followed by Matrix-Peppas model. The drug release was also found to be slow and extended for 24 h. In vivo testing of the mucoadhesive microspheres in diabetic albino rats demonstrated significant antidiabetic effect of repaglinide. The hypoglycemic effect obtained by mucoadhesive microspheres was for more than 16 whereas repaglinide produced an antidiabetic effect for only 10 h suggesting that mucoadhesive microspheres are a valuable system for the long term delivery of repaglinide.

Keywords: Controlled Release, Repaglinide, Solvent Evaporation, Microspheres, Mucoadhesive.

Introduction

Microspheres are frequently used drug delivery system and may also possess mucoadhesive properties [1, 2]. Due to their micrometer size they may be applied to mucosa, where the other dosage forms, e.g. tablet, would represent a problem. Microencapsulation by various polymers and its applications are described in standard textbooks [3,4]. Microencapsulation has been accepted as a process to achieve controlled release and drug targeting. Microspheres are free flowing powder and having diameter of 1-1000µm.Recently, dosage forms that can precisely control the release rates and targets drugs to a specific body site have made an enormous impact in the formulation and development of novel drug delivery system [2, 5]. 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 [6, 7]. Several studies [2] 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 microspheres are available [8,9]. The objective of this study is to develop, characterize, and evaluate mucoadhesive microspheres of repaglinide employing mucoadhesive polymers for prolonged gastrointestinal absorption. Repaglinide, an effective antidiabetic that requires controlled release owing to its short biological half-life [10] of 1± 2 hours, was used as the core in microencapsulation. The mucoadhesive microspheres were evaluated by in vitro and in vivo methods for controlled release. Repaglinide, a fast and short-acting meglitinide analog was chosen as the drug candidate since it is indicated for the development of a dosage form with increased Gastric Residence Time. It has a very short half-life (1 h), low bioavailability (50%) and poor absorption in the upper intestinal tract [11, 12].

Material & Method

Repaglinide was procured as a gift sample from Sun Pharma (Mumbai, India), Chitosan from CIFT (Cochin), Eudragit RS-100 from Evonik Degussa Pvt. Ltd. (Mumbai, India), Potassium dihydrogen phosphate (CDH, New Delhi), NaOH (Merck Ltd., Mumbai) HCl (Merck Ltd., Mumbai). All other reagents were of analytical grade obtained from standard companies.

Method of Preparation of Formulation

Microspheres were prepared by the solvent evaporation method using the solvents liquid paraffin/acetone. Different amounts of magnesium stearate were added to prevent agglomeration of microspheres.

Eudragit RS-100 was first dissolved in acetone and adds chitosan hydrochloride powder. Repaglinide and different amounts of magnesium stearate were dispersed separately in acetone and added to the Eudragit RS-100 and chitosan hydrochloride dispersion. The mixture was emulsified in liquid paraffin. The emulsion was stirred at 1000 rpm, at 40^0 C for 40 min. The emulsion of microspheres was filtered, washed with n-hexane and dried in a vacuum at room temperature overnight.

FTIR

Spectrum of Repaglinide from 4000 cm^{-1} to 400 cm^{-1} was obtained using FTIR spectrophotometer (Perkin Elmer BX) Using KBr pellet method.

Formulation Development

Various formulation were developed by changing the ratio of chitosan, Eudragit RS-100, solvent ratio, magnesium stearate and respective coded is given in the table1.

Table 1. Formulation Design of Mucoadhesive Microspheres.

Code	Drug	Chitosan	Eudragit RS 100	Magnesium Stearate	Methanol	Acetone	DCM
RM-1	1	1	1	1	1	1	1
RM-2	1	2	1	1	1	1	1
RM-3	1	3	1	1	1	1	1
RM-4	1	4	1	1	1	1	1
RM-5	1	5	1	1	1	1	1

RM-6	1	1	2	1	1	1	1
RM-7	1	1	3	1	1	1	1
RM-8	1	1	4	1	1	1	1
RM-9	1	1	5	1	1	1	1
RM-10	1	1	1	1	2	1	1
RM-11	1	1	1	1	1	2	1
RM-12	1	1	1	1	1	1	2
RM-13	1	1	1	2	1	1	1
RM-14	1	1	1	3	1	1	1
RM-15	1	1	1	4	1	1	1
RM-16	1	1	1	5	1	1	1
RM-17	1	1	1	6	1	1	1
RM-18	1	1	1	1	2	2	1
RM-19	1	1	1	1	1	2	2
RM-20	1	1	1	1	2	1	2

DCM- Dichloromethane, RM- Repaglinide Microspheres.

Estimation of Repaglinide

Repaglinide was estimated by ultraviolet visible (UV/Vis) spectrophotometeric method (Shimadzu UV-1700) based on the measurement of absorbance at 242.5 nm in 0.1N HCl pH 1.2. The method obeys Beer's law in the concentration range of 1 to10 µg/ml.

Determination of drug entrapment efficiency

10mg of dried microspheres were weighted accurately and drug was extracted from microspheres by digesting for 24 hours in 10 ml of 6.8 pH phosphate buffer solution. During this period the suspension was agitated. After 24 hrs the suspension was centrifuged at 2000 rpm for about 3 minutes. The supernatant obtained was assayed spectrophotometrically for drug contents. The drug entrapment efficiency (DEE) was determined as:

$$DEE = \frac{Practical\ drug\ content}{Thoeretical\ drug\ content} \times 100$$

Particle size analysis

The particle size of the microspheres was determined by using an Optical microscope (Magnus MLX-DX, Olympus). The mucoadhesive microspheres were examined by optical microscope. The freshly prepared microsphere was examined on an optical microscope and size of the microspheres was measured by using a pre-calibrated ocular micrometer and stage micrometer. About 200-300 particles of each formulation were observed and counted

Production Yield

The percentage of production yield was calculated from the weight of dried Microspheres (W1) and the sum of initial dry weight of starting materials (W2) as the following formula:

$$\% \text{ Production Yield} = W1/W2 \times 100$$

Drug entrapment efficiency, Particle size analysis, Production yield for formulation 1 to 20 is respectively reported in Table 2.

Table 2. Drug Entrapment Efficiency & Average Particle Size.

Formulation Code	Drug Entrapment Efficiency	Average Particle Size
RM-1	62.82(±1.37)	64.0(±1.19)
RM-2	67.59(±1.06)	69.1(±0.86)
RM-3	71.69(±0.83)	73.0(±1.11)
RM-4	74.26(±0.91)	75.0(±1.07)
RM-5	79.90(±1.17)	78.3(±1.35)
RM-6	65.76(±0.74)	65.1(±0.97)
RM-7	68.34(±0.67)	67.2(±1.17)
RM-8	72.34(±0.88)	71.3(±1.82)
RM-9	77.94(±1.23)	74.1(±1.18)
RM-10	63.80(±1.44)	63.6(±0.88)
RM-11	61.53(±1.05)	62.0(±0.64)
RM-12	62.61(±1.02)	64.2(±0.95)
RM-13	64.94(±0.98)	66.1(±0.96)
RM-14	63.42(±1.36)	61.4(±1.15)
RM-15	61.44(±1.16)	57.2(±0.61)
RM-16	65.18(±0.94)	52.1(±0.97)
RM-17	60.73(±1.11)	48.7(±0.75)
RM-18	64.05(±1.20)	61.2(±0.83)
RM-19	66.12(±1.47)	63.3(±1.15)
RM-20	69.69(±1.36)	67.4(±1.31)

Scanning Electron Microscopy (SEM)

SEM was performed for morphological characterization of microspheres using scanning electron microscope. They were mounted directly onto the SEM sample stub using double-sided sticking tape and coated with gold film (thickness,200nm)under reduced pressure (0.001mmHg), (Figure 1 and 2).

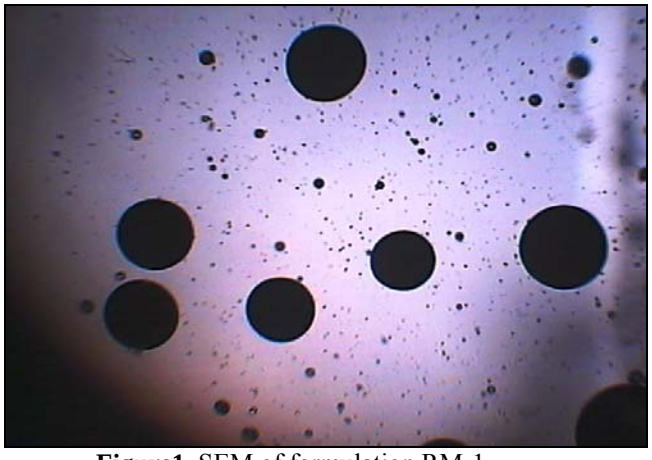

Figure1. SEM of formulation RM-1.

Figure 2. SEM of formulation RM- 10.

In-vitro drug release

The release rate of Repaglinide from mucoadhesive microspheres was determined using dissolution testing apparatus 2 (paddle type). The dissolution test was performed using 900 mL of 0.1N HCl, at 37± 0.5^0 C and 50 rpm. A sample (10 mL) of the solution was withdrawn from the dissolution apparatus hourly for 24 hrs, and the sample were replaced with fresh dissolution medium to maintain the sick condition. The samples were filtered through a membrane filter and diluted to a suitable concentration with 0.1N HCl. Absorbance of these solutions was measured at λ_{max} 242 nm using a model 1700 Shimadzu, double-beam spectrophotometer. Cumulative percentage drug release was calculated using an equation obtained from a standard curve and same studies were performed in 6.8 pH phosphate buffer solutions. The drug release experiments were conducted in triplicate (n = 3).

In-vitro Mucoadhesivity

The mucoadhesive property of microspheres was evaluated by in-vitro wash off test for mucoadhesion. Pieces of intestinal mucosa (3cm×2cm) were mounted onto glass slides using cyanoacrylate glue. About 200 mg of microspheres were spread onto each wet rinsed tissue specimen and immediately thereafter the support was hung onto the arm of USP disintegration apparatus. By operating the disintegration test machine, the tissue specimen was given a regular up and down movement in 0.1 N HCl/ PBS pH 6.8 at 37°C taken in a 1 liter vessel of the machine. At the end of 30 minutes, 1 hour and then at hourly intervals, the machine was stopped and the microspheres adhering to the tissue, 0.1NHCl/PBS was centrifuged, dried and weight. The mucoadhesiveness of these microspheres was calculated.

In-vivo Test

The approval of the Institutional Animal Ethics Committee was obtained before starting the study. The approval number and date is 716/02/a/CPCSEA and 25/10/2008 respectively. The study was conducted in accordance with standard institutional guidelines. In vivo evaluation studies for repaglinide mucoadhesive microspheres were performed in diabetics' albino rats of either sex, weighing between 230-270g. After 16 h overnight fast, the experimental animals were made diabetic by single intravenous administration of cold, freshly prepared solution of alloxan (CDH New Delhi) at dose of 65-70 mg/kg dissolved in normal saline solution. After 1 week, animal with fasting blood glucose of 300 mg/dl or more were considered diabetic and were used in the study. No food or liquid other than water was given during the experimental period. The product in the study was administered orally. After the confirmation of diabetes; the rats were divided randomly into three groups of four rats each and treated as follow: group1 was administered with 4 mg/kg body weight of repaglinide solution; group 2 was administered mucoadhesive microspheres and group 3 was administered marketed conventional repaglinide tablet. Blood samples were withdrawn by the retro orbital puncture at predetermined time at 1 hour intervals up to 24 hours; Blood samples collected were allowed to clot without any anticoagulant and were centrifuged immediately at 5000 rpm for 20 minutes to separate the serum. 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 & Discussion

Mucoadhesive Microspheres of Repaglinide consisting of chitosan hydrochloride powder in various combinations could be prepared by solvent evaporation of emulsification process. Microspheres with a coat of mucoadhesive polymer alone could not be prepared because of water insoluble nature of the polymers.

The purity of drug sample was identified by scanning the drug sample on IR spectrophotometer. The peaks of the IR spectra of drug sample were found to be similar with the

standard IR spectra of pure repaglinide as reported. Figure 1 shows the I.R. spectra of Repaglinide. The I.R. spectra of mixture of drug and polymer indicated no incompatibility between drug and polymers, hence eudragit RS-100, chitosan hydrochloride were chosen as polymers for further investigations. The spectrum of drug shows absorption bands at 3307.6 cm^{-1} N-H stretching, 1635.99cm^{-1} N-H bending , 2934.08 cm^{-1} C-H_2 Stretching .The plain chitosan absorption peaks found at 3422.9 cm-1, 2923.80cm^{-1}, 1081.94 $cm^{-1,}$ 1640.31 cm^{-1}.The plain eudragit RS-100 absorption peaks found at 3448.52 cm^{-1}, 2923.80 cm^{-1}, 1081.94 cm^{-1}, 1640.31 cm^{-1}.

Surface morphology of the mucoadhesive microspheres was examined by scanning electron microscopy. The SEM showed that the microspheres were found roughly spherical and uniform in size. The size ranged from 48µm - 78µm.

The % age Production yield was increases with increase the concentration of the polymer. This was due to the fact that with increase in chitosan concentration, more amount of chitosan was added in the same volume of continuous phase.

Particle size analysis of different formulations was done by optical microscopy. The average particle size was found to be in the range 48.7±0.75 µm to 78.3±1.35 µm. Data for the particle sizes of microspheres of various formulations are shown in table No.2. For the purpose of accessing effect of polymer, 20 formulations were prepared at different polymer concentrations. The mean particle size was significantly increases with increasing polymer concentration this may be due to high viscosity of polymer solution. High viscosity of polymer concentration requires high energy for breaking of droplets (table-2). We also observe that the mean particle size of microspheres was increased with increase in polymer concentration because higher viscosity of chitosan solution makes the microspheres more difficult to disperse; these results in larger size of microspheres are formed.

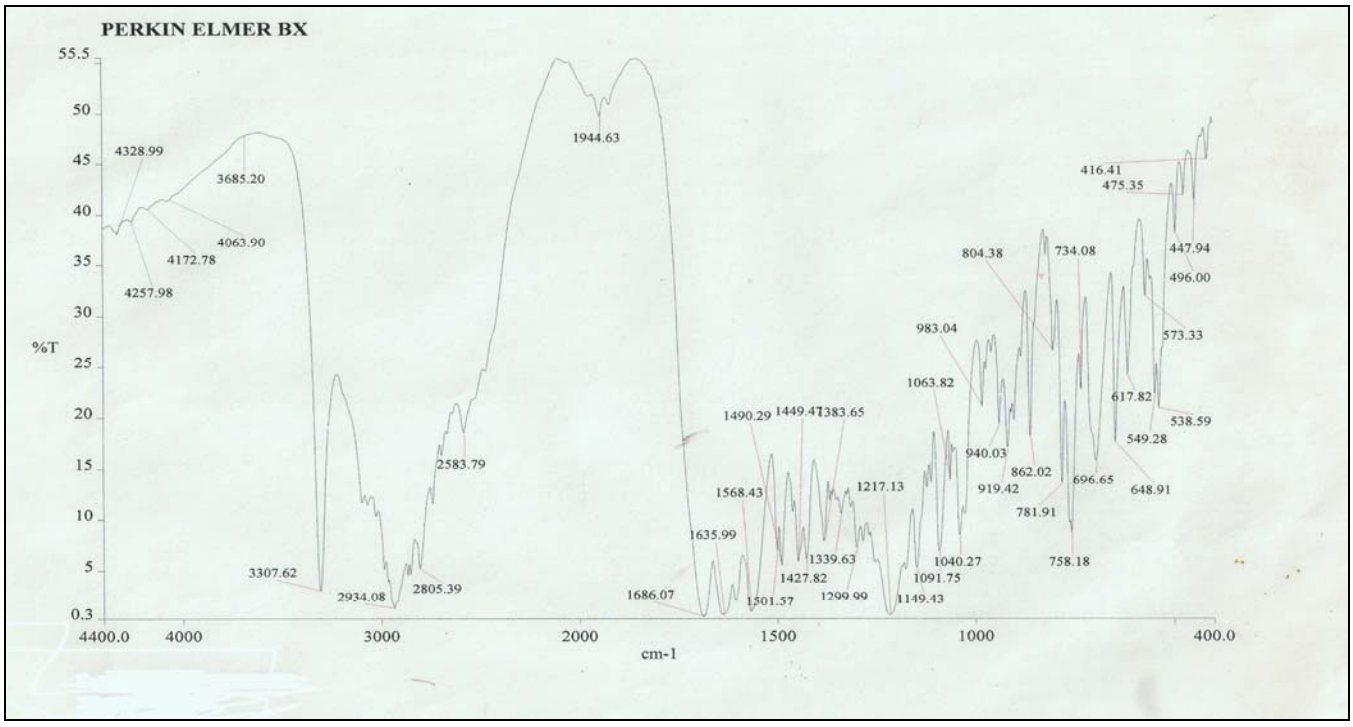

Figure 3. FTIR spectrum of Repaglinide.

Table3. Bulk density and flow property.

Formulation Code	Bulk Density (g/ml)	Tapped Density (g/ml)	Carr's Index	Flow Property
RM-1	0.4986	0.5814	14.24(±1.32)	Excellent
RM-2	0.5435	0.6236	12.84(±1.27)	Excellent
RM-3	0.6781	0.6493	10.96(±1.08)	Excellent
RM-4	0.5543	0.6237	11.12(±0.79)	Excellent
RM-5	0.4426	0.5126	13.65(±1.21)	Excellent
RM-6	0.6236	0.7136	14.43(±0.94)	Excellent
RM-7	0.5438	0.6432	15.45(±0.84)	Good
RM-8	0.4964	0.5836	14.95(±1.07)	Good
RM-9	0.4813	0.5446	11.94(±1.34)	Excellent
RM-10	0.6143	0.7234	15.08(±0.83)	Excellent
RM-11	0.6168	0.7136	13.56(±1.02)	Good
RM-12	0.5418	0.6183	12.36(±1.04)	Excellent
RM-13	0.5234	0.6243	16.16(±1.27)	Good
RM-14	0.4834	0.5434	11.04(±0.75)	Excellent
RM-15	0.4754	0.5845	15.24(±1.03)	Good
RM-16	0.6748	0.7113	13.56(±1.07)	Excellent
RM-17	0.6146	0.7233	15.02(±0.73)	Good
RM-18	0.6274	0.7154	12.30(±0.95)	Excellent
RM-19	0.4872	0.5698	14.49(±1.26)	Excellent
RM-20	0.6247	0.7234	14.70(±0.76)	Excellent

Percent drug entrapment efficiency of mucoadhesive microspheres was found in the range of 61.44±1.16 to 79.90±1.17 (table- 2). Formulation RM5 showed maximum % drug loading 79.90% whereas RM15 showed minimum % drug loading about 61.44% as compared to other formulations. The high entrapment efficiency of Repaglinide is believed to be due to its poor aqueous solubility in disperse phase. The percentage entrapment efficiency increased with increase in polymer concentration because higher viscosity of chitosan solution reduces the diffusion of the drug in the surroundings which does not allow entrapped particle to escape easily.

Mucoadhesive microspheres of Repaglinide consisting of chitosan as a mucoadhesive polymers exhibited good mucoadhesive properties in the in-vitro wash off test for mucoadhesion. The wash off effect was slow in case of microspheres containing eudragit RS-100 formulations containing chitosan showed better mucoadhesive properties. The wash off effect was faster at intestinal pH than at gastric pH. The rapid wash off effect observed at intestinal pH was due to ionization of carboxyl and other functional groups in the polymers at this pH which increases their solubility and also reduces adhesive strength. The strong interaction between chitosan microspheres and mucous glycoprotein and/or mucosal surfaces was found to be dependent upon the polymer concentration. As polymer concentration increases, the % mucoadhesion also increased as shown in table 4-5.

Table 4. Percent of microspheres adhering to tissue at different times (h) in 0.1N HCl.

Formulation code	Percent of microspheres adhering to tissue at different time Interval				
	1	2	4	6	8
RM-1	78.3±1.6	70.2±0.9	57.6±0.9	34.8±1.2	20.4±2.0
RM-2	80.7±0.9	71.4±1.6	58.6±1.6	36.4±0.9	21.8±0.9
RM-3	81.6±2.0	73.5±2.0	59.7±2.3	37.1±1.9	23.7±1.6
RM-4	82.8±2.5	74.6±2.0	62.3±0.9	39.6±2.0	25.6±0.9
RM-5	84.1±3.4	75.2±3.4	64.1±1.2	40.8±2.5	27.1±1.6
RM-6	77.9±1.2	69.6±0.9	56.8±1.9	33.4±2.6	19.6±0.9
RM-7	79.6±1.6	71.8±1.4	57.9±2.5	34.6±1.9	20.2±1.9
RM-8	80.1±2.0	72.9±1.6	59.7±3.4	36.1±1.6	21.3±2.0
RM-9	82.6±0.9	74.9±2.0	62.8±0.9	37.2±0.9	23.8±2.5
RM-10	76.6±0.6	75.8±0.4	63.1±1.9	39.9±2.5	24.1±3.4
RM-11	78.2±1.4	68.5±1.2	56.1±2.0	33.4±2.0	19.6±1.2
RM-12	77.9±0.9	69.7±2.5	57.9±1.6	32.1±0.9	18.7±0.9
RM-13	79.4±2.0	70.1±1.8	58.4±1.9	34.5±1.2	20.4±1.9
RM-14	76.2±2.5	68.4±1.9	60.7±2.5	35.1±2.0	21.9±0.9
RM-15	80.6±3.4	71.5±0.9	59.4±2.0	32.7±2.5	22.7±2.0
RM-16	78.5±1.2	72.7±0.6	56.2±0.9	33.4±2.6	19.5±2.5
RM-17	77.5±1.9	69.6±3.4	57.3±1.6	36.1±1.9	21.2±2.4
RM-18	79.1±0.9	68.4±1.2	59.3±1.8	35.8±0.9	20.1±2.0
RM-19	77.6±0.6	67.1±2.4	61.2±2.0	34.4±1.6	18.6±1.9
RM-20	78.4±1.2	70.1±0.9	56.1±0.9	37.8±0.9	17.9±1.6

The more amounts of polymer results in higher amounts of free-NH_2 -groups, which are responsible for binding with sialic acid groups in mucous membrane and this result in increase the mucoadhesive properties. In group RM5, % mucoadhesion was found to be 61.4% after 4 hours, this formulation showing better mucoadhesive property, which was useful for drug release for longer period of time.

Table 5. Percent of microspheres adhering to tissue at different times (h) in PBS 6.8 pH.

Formulation code	Percent of microspheres adhering to tissue at different times Interval				
	1	2	4	6	8
RM-1	68.4±0.9	58.3±2.5	29.2±2.0	14.2±0.9	-
RM-2	70.3±2.0	60.1±1.6	31.1±0.9	16.1±1.6	-
RM-3	71.6±1.9	61.3±2.0	32.4±2.0	17.3±2.5	-
RM-4	73.8±0.9	63.4±3.4	34.3±2.5	19.6±2.0	5.8±0.9
RM-5	74.2±2.5	64.9±1.6	35.8±1.6	20.5±2.8	-
RM-6	67.1±1.6	57.5±0.9	28.6±0.9	13.8±2.0	-
RM-7	69.6±0.6	59.8±1.9	30.9±1.9	15.7±0.9	-
RM-8	70.3±2.0	61.4±0.9	31.5±3.4	16.4±1.6	4.8±1.6
RM-9	72.6±2.5	62.6±2.5	33.6±2.0	18.9±1.9	--
RM-10	72.8±0.9	56.8±2.0	27.1±1.6	14.1±3.4	--
RM-11	68.2±3.4	57.7±1.6	29.7±1.9	16.2±0.9	--
RM-12	69.1±1.6	58.8±0.9	28.1±2.0	17.9±1.6	--
RM-13	67.4±0.9	56.2±1.6	27.9±2.5	13.5±0.9	--
RM-14	66.5±2.0	58.9±0.9	28.6±0.9	14.6±1.9	--
RM-15	68.1±2.5	57.9±2.0	30.7±1.9	16.8±2.0	--
RM-16	67.8±0.9	55.4±3.4	31.4±2.0	15.9±2.5	--
RM-17	69.1±1.6	59.3±1.9	29.1±3.4	17.7±0.9	4.0±1.9
RM-18	70.4±1.9	56.7±1.6	26.6±2.0	16.4±0.4	--
RM-19	66.7±2.0	57.1±2.0	27.8±0.9	18.8±1.2	--
RM-20	68.6±3.4	60.8±2.5	29.7±1.6	17.7±2.4	--

Drug release from the Microspheres was studied in phosphate buffer (pH6.8) and in 0.1 N HCl (pH 1.2). Drug release from the Microspheres was slow and dependent on the composition of the coat. It was also observed that the drug release was faster in 0.1 N HCl than in PBS which was perhaps due to the greater solubility of the drug in the former. It was found that the release profile of Repaglinide were different for the different formulations. Repaglinide release from these microspheres was slow, extended and dependent on the type of polymer used. Since the main aim of our study was to improve the bioadhesive strength of microspheres so these formulations were selected for the in-vitro release study. Data for the release of the drug from microspheres of various formulations are shown in table No.4-8.

In vivo studies in diabetic albino rats were performed with mucoadhesive microspheres of group RM5. From the in-vitro release study, formulation RM5 has shown good controlled release for more than 18 hours and thus it was selected for the in vivo study. The drug was administered at a dose equivalent to 4 mg/kg body

weight of repaglinide. Pure repaglinide and marketed conventional tablet were administered in a suspension form at the same dose. When pure repaglinide solution and tablet were administered, a rapid reduction in blood glucose levels were observed and maximum reduction of 47.71% and 45.17% were observed respectively within 1h after oral administration. Blood glucose levels were recovered to the normal level in 14h (Fig.4).

Table 6. Drug release profile of formulation RM1, RM2, RM3, and RM4 RM5& RM6 in 0.1 N HCl

Time (h)	RM1	RM2	RM3	RM4	RM5	RM6
0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
1	16.66±1.41	21.94±1.06	24.87±0.70	24.87±0.96	31.90±0.90	18.42±0.50
2	24.87±0.82	30.73±0.60	31.90±0.62	31.90±0.82	40.10±0.64	26.92±0.98
3	34.24±0.73	36.29±0.85	38.64±0.38	38.64±0.56	48.01±1.18	34.83±0.81
4	40.10±0.66	47.72±0.86	44.79±0.69	44.79±0.87	56.22±0.38	41.57±0.59
5	47.72±0.99	56.22±0.63	52.41±0.66	52.41±0.51	61.49±0.60	52.11±0.16
6	52.11±0.56	62.66±0.91	62.95±1.01	62.95±0.88	69.69±0.39	58.27±0.99
7	57.68±1.02	70.28±0.82	70.28±1.03	70.28±0.90	75.84±1.15	64.13±0.31
8	62.95±0.99	73.79±0.48	74.37±0.60	74.37±0.71	82.00±0.43	68.52±0.57
10	68.81±0.93	77.60±0.56	78.77±0.95	78.77±0.60	86.39±0.74	71.74±0.53
12	74.38±0.81	80.53±0.91	82.00±0.50	82.00±1.08	89.03±0.52	75.26±0.19
24	79.95±0.89	84.05±1.06	89.03±0.98	89.03±0.56	96.35±0.57	78.48±0.85

Table 7. Drug release profile of formulation RM6, RM7, RM8, RM9, RM10, RM11, & RM12 in 0.1 N HCl

Time (h)	RM7	RM8	RM9	RM10	RM11	RM12
0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
1	16.08±0.15	19.59±0.97	21.94±1.35	13.74±0.48	11.68±1.18	8.17±0.32
2	25.45±0.55	26.04±1.03	28.38±0.64	21.35±0.29	18.72±1.16	15.79±0.57
3	34.24±0.85	32.78±1.23	33.36±1.13	29.26±1.37	25.45±0.99	24.28±0.67
4	43.03±0.34	39.52±0.79	39.22±0.79	35.12±0.64	32.78±1.16	30.14±0.15
5	50.36±0.50	45.96±0.37	46.84±0.45	42.45±0.92	36.29±1.05	36.29±0.53
6	57.09±0.86	52.70±0.84	52.70±1.47	48.89±0.74	41.27±0.86	41.27±0.86
7	63.83±1.45	58.27±0.58	59.73±0.24	54.75±0.63	48.89±0.77	45.96±0.48
8	68.23±1.21	65.88±1.26	65.59±1.09	61.49±0.38	55.63±0.28	52.42±0.89
10	73.50±0.77	71.45±1.17	72.33±0.72	68.52±0.99	62.95±0.31	58.27±1.35
12	78.48±0.85	79.07±0.71	76.72±0.24	71.74±1.18	68.52±1.02	67.35±1.06
24	81.41±0.72	82.00±0.57	82.58±1.06	78.48±1.05	79.07±0.14	79.36±0.50

A blood glucose level of 65-110 mg/dl is considered normal level. In the case of repaglinide mucoadhesive microspheres, the reduction in blood glucose levels was slow and reached maximum reduction within 3h after oral administration. This reduction in blood glucose level was sustained over longer periods of time. A 25% reduction in blood glucose level is considered a significant hypoglycemic effect. Significant hypoglycemic effect was maintained from 0.5 to 10 h after oral administration of repaglinide, whereas in case of mucoadhesive microspheres of repaglinide RM 5 significant hypoglycemic effect was maintained for a period of 2 to 16 h. The sustained hypoglycemic effect observed over a longer period of time in the case of mucoadhesive microspheres is due to the slow release and absorption of repaglinide over longer period of time. Repaglinide sustained release formulation is significantly more effective than the immediate release repaglinide formulation in reducing blood glucose levels and side effects.

Table 9. Drug release profile of formulation RM13, RM14, RM15, RM 16, RM 17& RM 18 in 0.1 N HCl.

Time (h)	RM13	RM14	RM15	RM16	RM17	RM18
0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
1	9.34±1.03	12.27±0.54	6.70±0.79	8.75±0.29	11.39±1.21	5.24±0.48
2	16.08±1.15	21.94±0.58	12.56±0.71	16.66±0.92	19.59±1.26	12.86±0.64
3	27.80±0.47	25.45±1.26	18.13±0.57	29.56±1.31	31.31±0.77	17.25±1.18
4	36.29±0.53	30.43±1.13	27.50±0.64	37.17±1.18	39.52±0.85	23.99±0.99
5	41.86±0.92	36.29±0.45	32.19±1.37	44.79±1.45	46.25±0.45	30.43±1.16
6	50.94±0.38	41.57±0.72	42.45±1.06	57.87±0.37	53.58±1.47	36.29±0.31
7	59.15±0.28	48.31±0.24	49.77±0.50	62.95±0.57	57.97±0.24	43.33±1.02
8	68.52±0.69	53.58±0.34	55.92±0.32	67.93±0.24	59.36±0.72	49.48±0.63
10	71.32±0.57	58.85±0.85	59.73±1.18	67.93±0.72	62.96±1.06	53.87±0.74
12	74.63±1.16	65.30±0.72	63.54±0.99	70.12±0.55	68.81±1.17	66.32±0.48
24	78.48±0.45	75.36±1.17	73.21±0.86	71.48±0.85	71.45±0.97	79.12±0.57

Table 10. Drug release profile of formulation RM18, RM19, RM20, 0.1 N HCl

Time (h)	RM19	RM20
0	0.00±0.00	0.00±0.00
1	12.27±0.82	9.93±1.06
2	18.42±0.51	13.74±0.82
3	28.68±0.71	21.65±0.56
4	36.29±1.08	27.50±0.62
5	45.38±1.15	35.12±0.38
6	56.80±0.57	43.91±1.01
7	61.20±0.38	51.82±0.60
8	66.76±0.64	58.56±0.95
10	71.45±0.71	64.42±0.98
12	77.02±0.19	70.57±0.80
24	81.12±1.18	77.55±0.48

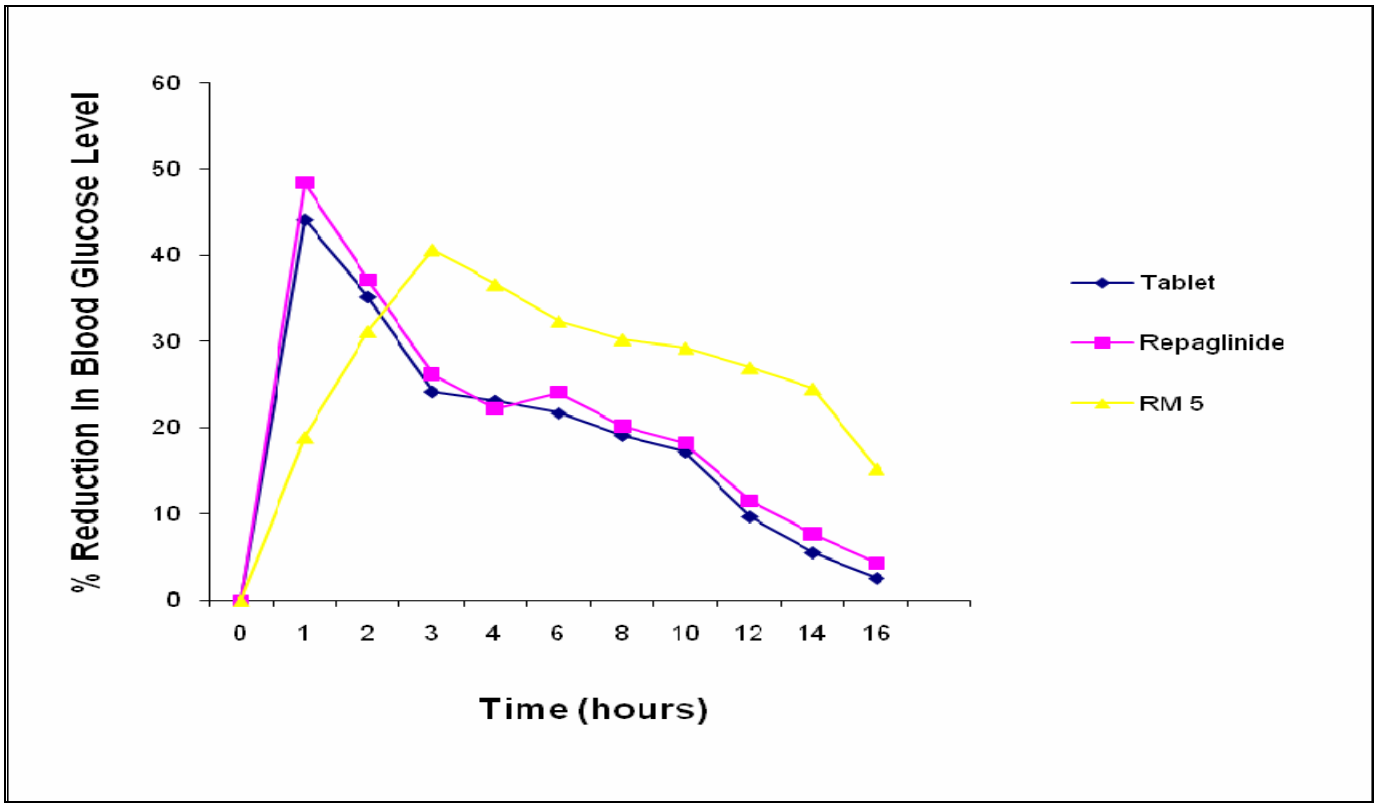

Figure 4. Showing the % reduction in blood glucose level

Table 11. Percentage reduction in blood glucose level

S.No.	Time (h)	Percentage reduction in blood glucose level		
		Tablet	Drug (control)	Microspheres (RM5)
1	0	0.00	0.00	0.00
2	1	44.17	48.42	18.89
3	2	35.20	37.20	31.20
4	3	24.20	26.20	40.68
5	4	23.12	22.20	36.66
6	6	21.69	24.21	32.42
7	8	19.12	20.20	30.26
8	10	17.21	18.21	29.26
9	12	9.68	11.50	26.97
10	14	5.50	7.68	24.56
11	16	2.52	4.25	15.25

All release kinetic model were applied on RM2, RM5, RM9 and RM16 because of their good mucoadhesive property (table- 9&10). The best fit model was found to be matrix, some formulation showing Peppas also. The selection criterion for the best fit model was based on goodness of fit and residual sum of squares. The n value less than 0.5 indicating that transport follow Fickian trend.

Conclusion

The initial drug release significantly increased with an increase in the ratio of drug/polymer. An initial high release is observed due to the dissolution of surface adhered drug, where as the latter drug is due to the diffusion process, which is much slower when, compared to the initial release. Formulation RM5 was found to be the best among all the formulations since it showed good mucoadhesive properties, fair entrapment efficiency and could prolong the release for a longer duration of time. Bulk density and flow property shows that most of the microspheres having good to excellent flow property. The ratio of drug/polymer also had effect on the release of the microspheres. The in-vivo study demonstrated significant blood glucose reducing activity of mucoadhesive microspheres of repaglinide.

Microspheres of different size and drug content could be obtained by varying the formulation variables like polymer ratio, magnesium stearate ratio and solvent ratio. The multi-unit mucoadhesive repaglinide delivery system is accepted to provide clinician with a new choice of an economical, safe and more bio-available formulation in the management of moderate to severe diabetes mellitus. Therefore it may be concluded that drug loaded mucoadhesive microspheres are suitable delivery system for repaglinide.

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