



Received on 19 July, 2010; received in revised form 09 October, 2010; accepted 15 November, 2010

## EFFECT OF MAGNESIUM STEARATE ON MUCOADHESIVE MICROSPHERES OF REPAGLINIDE

Vimal K. Yadav<sup>\*1,2</sup>, S. K. Prajapati<sup>1</sup>, Alok Mahor<sup>1</sup> and Brajesh Kumar<sup>3</sup>

Institute of Pharmacy, Bundelkhand University<sup>1</sup>, Jhansi, Uttar Pradesh, India

Kunwar Hari Bansh Singh College of Pharmacy<sup>2</sup>, Jaunpur, Uttar Pradesh, India

Department of Pharmacy, Prasad Institute of Technology<sup>3</sup>, Jaunpur, Uttar Pradesh, India

### ABSTRACT

In this study, an attempt was made to prepare Mucoadhesive microspheres of Repaglinide using various Mucoadhesive polymers designed for oral controlled release. Microspheres containing the Mucoadhesive polymer chitosan hydrochloride, with matrix polymer Eudragit RS 100, Repaglinide as a model drug and agglomeration preventing agent magnesium stearate were prepared by the Emulsification solvent evaporation method. The amount of magnesium stearate is varied and the following methods were used for microspheres evaluation: drug content, dissolution determination, scanning electron microscopy and FTIR spectroscopy. 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, and *in vitro* drug release. The microspheres were found to be spherical and free flowing. The microencapsulation efficiency was in the range of  $61.44 \pm 1.16$  to  $78.90 \pm 1.17$  and 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.

#### Keywords:

Controlled Release,  
Repaglinide,  
Solvent Evaporation,  
Microspheres,  
Mucoadhesive

#### Correspondence to Author:

Vimal K. Yadav

Institute of Pharmacy, Bundelkhand  
University, Jhansi, Uttar Pradesh,  
India

**INTRODUCTION:** Microencapsulation by various polymers and its applications are described in standard textbooks<sup>4, 14</sup>. Microspheres are a frequently used drug delivery system and may also possess Mucoadhesive properties. Due to their micrometer size they may applied to mucosa, where the larger size of some other dosage forms, e.g., tablet, would represent a problem<sup>3-6</sup>. The most suitable internal structure of microparticles for the adhesion on the mucosa surface is the matrix system with a Mucoadhesive polymer dispersed inside the particle. The objective of this study is to develop, characterize, and evaluate mucoadhesive microspheres of repaglinide employing mucoadhesive polymers for prolonged gastro- intestinal absorption. Repaglinide, an effective antidiabetic that requires controlled release owing to its short biological half-life<sup>13</sup> of 1±2 hours, was used as the core in microencapsulation. The mucoadhesive microspheres were evaluated by in vitro 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 GRT (Gastric Residence Time). It has a very short half-life (1 h), low bioavailability (50%) and poor absorption in the upper intestinal tract<sup>13</sup>.

**MATERIALS AND METHODS:** 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°C for 40 min. The emulsion of microspheres was filtered, washed with n-hexane and dried in a vacuum at room temperature overnight.

**Fourier Transform Infrared Spectroscopy:**

Spectrum of Repaglinide from 4000 cm<sup>-1</sup> to 400 cm<sup>-1</sup> was obtained using FTIR spectrophotometer (Perkin Elmer BX) Using KBr pellet method.

**Formulation Development:** Various formulations were developed by changing the ratio of chitosan, Eudragit RS-100, solvent ratio, magnesium stearate and respective coded is given in the **table 1**.

**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	2	1	1	1
RM-3	1	3	1	3	1	1	1
RM-4	1	4	1	4	1	1	1
RM-5	1	5	1	5	1	1	1
RM-6	1	1	2	6	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

DCM – Dichloromethane, RM – Repaglinide Microspheres

**Estimation of Repaglinide:** Repaglinide was estimated by ultraviolet visible (UV/Vis) spectrophotometric method (Shimadzu UV-1700) based on the measurement of absorbance at 242.5 nm in 0.1 N HCl, pH 1.2. The method obeys Beer's law in the concentration range of 1 to 10 µg/ml.

**Determination of Drug Entrapment Efficiency:** 10 mg of dried microspheres were weighed 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:

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

**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).

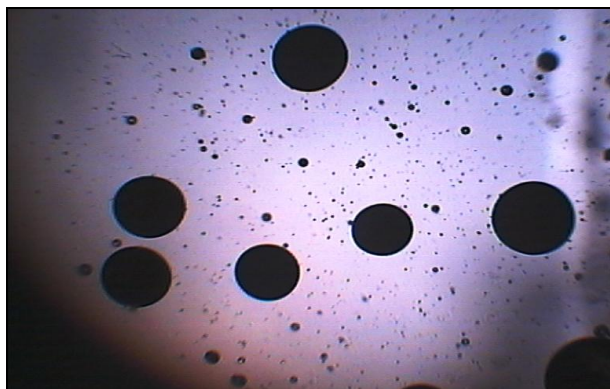


FIG. 1: SCANNING ELECTRON PHOTOMICROGRAPH OF FORMULATION RM- 1

**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**.

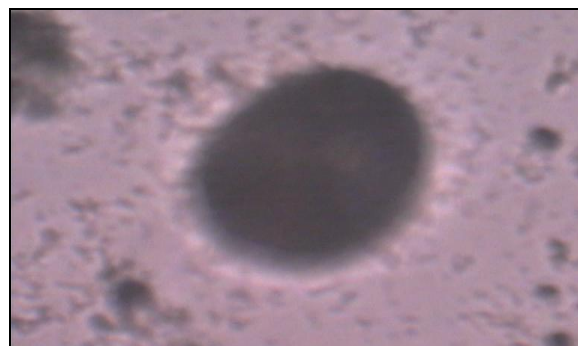


FIG. 2: SCANNING ELECTRON PHOTOMICROGRAPH OF FORMULATION RM-10 SHOWING POPULATION OF MICROSPHERES

**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°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 sink

condition. The samples were filtered through a membrane filter and diluted to a suitable concentration with 0.1 N HCl. Absorbance of these solutions was measured at 242nm 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, 0.1 N HCl/PBS was centrifuged dried and weight. The mucoadhesiveness of these microspheres was calculated.

**RESULT AND 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. **Fig. 3** 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.

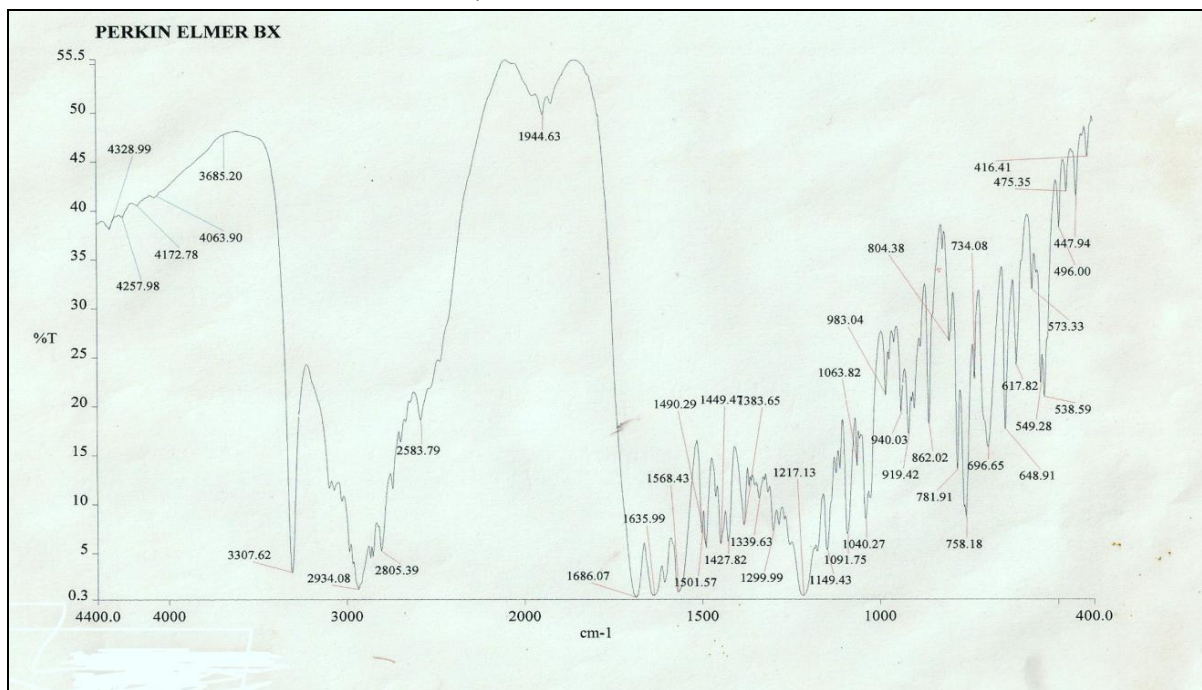


FIG. 1: SHOWING FTIR SPECTRUM OF REPAGLINIDE

The spectrum of drug shows absorption bands at  $3307.6\text{ cm}^{-1}$  N-H stretching,  $1635.99\text{ cm}^{-1}$  N-H bending,  $2934.08\text{ cm}^{-1}$  C-H<sub>2</sub> Stretching. The plain chitosan absorption peaks found at  $3422.9\text{ cm}^{-1}$ ,  $2923.80\text{ cm}^{-1}$ ,  $1081.94\text{ cm}^{-1}$ ,  $1640.31\text{ cm}^{-1}$ . The plain Eudragit RS-100 absorption peaks found at  $3448.52\text{ cm}^{-1}$ ,  $2923.80\text{ cm}^{-1}$ ,  $1081.94\text{ cm}^{-1}$ ,  $1640.31\text{ cm}^{-1}$ .

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  $48\mu\text{m}$  -  $78\mu\text{m}$ .

The % 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  $48.7\pm 0.75\mu\text{m}$  to  $73.1\pm 1.35\mu\text{m}$ . Data for the particle sizes of microspheres of various formulations are shown in **table 2**. For the purpose of accessing effect of polymer, 9 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. Percent drug entrapment efficiency of mucoadhesive microspheres was found in the range of  $61.44\pm 1.16$  to  $78.90\pm 1.17$  (table 2). Formulation

RM5 showed maximum % drug loading 79.90% whereas RM9 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.

**TABLE 2: DRUG ENTRAPMENT EFFICIENCY, AVERAGE PARTICLE SIZE, % PRODUCTION YIELD**

Formulation Code	Drug Entrapment Efficiency	Average Particle Size
RM-1	61.82 ( $\pm 1.37$ )	48.7 ( $\pm 1.19$ )
RM-2	66.59 ( $\pm 1.06$ )	55.1 ( $\pm 0.86$ )
RM-3	72.69 ( $\pm 0.83$ )	62.0 ( $\pm 1.11$ )
RM-4	73.26 ( $\pm 0.91$ )	66.0 ( $\pm 1.07$ )
RM-5	78.90 ( $\pm 1.17$ )	71.3 ( $\pm 1.35$ )
RM-6	66.76 ( $\pm 0.74$ )	73.1 ( $\pm 0.97$ )
RM-7	69.34 ( $\pm 0.67$ )	47.2 ( $\pm 1.17$ )
RM-8	73.34 ( $\pm 0.88$ )	50.3 ( $\pm 1.82$ )
RM-9	78.94 ( $\pm 1.23$ )	53.1 ( $\pm 1.18$ )

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**. 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 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

**TABLE 5: PERCENT OF MICROSPHERES ADHERING TO TISSUE AT DIFFERENT TIMES (H) IN 6.8 pH PBS**

Formulation Code	Percent of microspheres adhering to tissue at different times Interval				
	1	2	4	6	8
RM-1	66.4±0.9	57.3±2.5	29.2±2.0	15.2±0.9	-
RM-2	69.3±2.0	60.1±1.6	32.1±0.9	16.1±1.6	-
RM-3	70.6±1.9	62.3±2.0	34.4±2.0	17.3±2.5	-
RM-4	72.8±0.9	63.4±3.4	35.3±2.5	19.6±2.0	5.8±0.9
RM-5	73.2±2.5	64.9±1.6	37.8±1.6	21.5±2.8	-
RM-6	68.1±1.6	58.5±0.9	29.6±0.9	14.8±2.0	-
RM-7	69.6±0.6	59.8±1.9	32.9±1.9	15.7±0.9	-
RM-8	70.3±2.0	62.4±0.9	33.5±3.4	17.4±1.6	4.8±1.6
RM-9	73.6±2.5	64.6±2.5	35.6±2.0	19.9±1.9	-

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 **figure 4 & 5**.

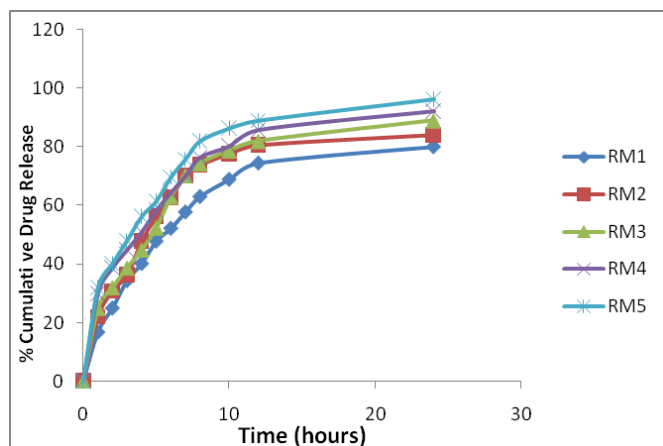


FIG. 4: GRAPH SHOWING DRUG RELEASE PROFILE OF FORMULATION RM1, RM2, RM3, AND RM4 & RM5 IN 0.1 N HCl

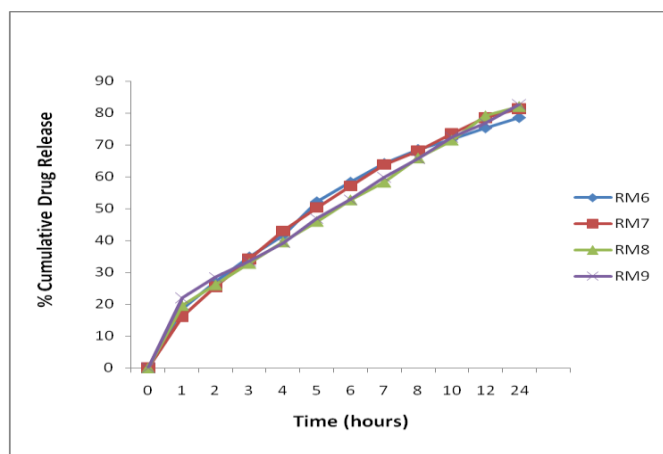


FIG. 5: GRAPH SHOWING DRUG RELEASE PROFILE OF FORMULATION RM6, RM7, RM8, & RM9, IN 0.1 N HCl (pH-1.2)

**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, whereas 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 well 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.

**ACKNOWLEDGEMENT:** We are thankful to Sun Pharma Mumbai for providing generous gift of Repaglinide and Birbal Sahni Institute of Paleobotany, Lucknow for providing Scanning Electron Facility (SEM). We also thank Head and Guide, Institute of Pharmacy, Bundelkhand University, Jhansi for providing Infrastructure facility for the work.

#### REFERENCES:

1. Prajapati SK, Tripathi P, Ubaidulla U, Anand V: Design and development of Gliclazide Mucoadhesive Microspheres: In Vitro and In Vivo Evaluation. *AAPS PharmSci Tech.* 2008; 6: 224-230.
2. Davis SN, Granner DK: Insulin, oral hypoglycemic agents and the pharmacology of the endocrine pancreas, in: J.G. Hardman, L.E. Limbird (Eds.), *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 10th edition, McGraw-Hill Medical Publishing Division, USA2001; 1704-1705.
3. Chowdary KPR, Srinivas L: Mucoadhesive drug delivery systems: a status of current review. *Indian Drugs* 2000; 37:400-406.
4. Akiyama Y, Nagahara N: Novel formulation approaches to oral mucoadhesive drug delivery systems, in: E. Mathiowitz, D.E. Chickering III, C.M. Lehr (Eds.), *Bioadhesive Drug Delivery Systems: Fundamentals, Novel Approaches and Development*, Marcel Dekker, New York 1999; 477-505.

5. Chowdary KPR, RaoYS: Design and In vitro and in vivo evaluation of Mucoadhesive microcapsules of glipizide for oral controlled release a technical note. *AAPS Pharm.Sci Tech.*2003; 4:1-6.
6. Gupta R, Prajapati SK, Bhardwaj P, Himanshu: Formulation and evaluation of Glipizide floating microspheres. *Asian journal of Pharmaceutics* 2007; 1:159-163.
7. Mahor S, Palani S, Joseph NM, Garud N: Preparation and evaluation of Gastroretentive microspheres of ranitidine hydrochloride. *Asian journal of Pharmaceutics* 2007;1:164-169.
8. Bogataj M, Mrhar A, Korosec L: Influence of physicochemical and biological parameters on drug release from microspheres adhered on vesical and intestinal mucosa. *Int J Pharm.*1999; 177:211-220.
9. Mukhtar HM, Ansari SH, Ali M, Bhat ZA, Naved T: Effect of aqueous extract of *Cyamopsis tetragonoloba* linn. Beans on blood glucose level in normal and alloxan-induced diabetic rats. *Indian J Exp Biol.*2006; 42:101-105.
10. Cuna M, Alonso M, Torres JD: Preparation and in-vivo evaluation of mucoadhesive micro-particles containing Amoxicillin-resin complexes for drug delivery to the gastric mucosa. *Eur J. Pharma Biopharma.* 2001;51: 199-205.
11. Dhawan S, Singla AK, Sinha VR: Evaluation of Mucoadhesive Properties of Chitosan Microspheres. *AAPS Journal* 2004; 5 (4): 1-7.
12. Patel VA, Patel HV, Koradiya HD, Kotadiya RM: Study of polymer concentration on physico-chemical properties of clarithromycin loaded chitosan mucoadhesive microspheres. *The Indian pharmacist* 2008; Oct, 83-86.
13. Jain SK, Awasthi AM, Jain NK, Agrawal GP: Calcium silicate based microspheres of repaglinide for gastroretentive floating drug delivery: Preparation and in vitro characterization. *J. Control. Release*2005; 107: 300– 309.
14. Bakan JA: Microencapsulation. *The theory and practice of Industrial pharmacy*, I L. Lachman, and H. A. Liberman (eds.), 2nd ed., Varghese, Mumbai, India 1991; 412-428.

\*\*\*\*\*