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Design and Evaluation of Mucoadhesive Microspheres of Nateglinide

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ABSTRACT

Nateglinide, an oral hypoglycemic agent is having disadvantage of low systemic bioavailability, poor absorption in upper intestinal tract, and short biological half life (1.5hr) for increasing the resident time of this drug in stomach spray drying technique used for preparation of mucoadhesive microspheres consist of different polymers like Hydroxypropyl Methycellulose (HPMC), Hydroxypropyl cellulose (HPC), Polyvinylpyrrolidone (PVP), Sodium alginate and ethyl cellulose with Nateglinide. The surface morphology and particle shape were studied by scanning electron microscope (SEM). The microspheres were evaluated for their micro encapsulation efficiency. The micro encapsulation efficiency of microspheres evaluated. Mucoadhesive microspheres prepared were spherical in shape, size in the range of 2.6-5µm.*In vitro* drug release performed for all the formulation and *in vivo* study of optimum formulation (F3) and pure Nateglinide in normal healthy rabbits performed. The micro encapsulation efficiency was in the range of 76.75 % - 89.36 % and microspheres of formulations (F1, F2, F3, F4, F6, and F7) have shown good mucoadhesive property. F3 had shown significant hypoglycaemic effect upto period from 5 hrs to 19 hrs, whereas pure Nateglinide showed the reduction of 39.85% after a period of 3hrs and reached normal with in 6.5hrs.

Keywords: Sustained Release, Nateglinide, Spray Drying, Mucoadhesive Polymers

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INTRODUCTION

For systematic delivery, the oral route has been preferred route of administration for many systematically active drugs. When administered by the oral route, in other hand many therapeutic agents have been reportedly subjected to extensive presystemic elimination by gastrointestinal degradation and or hepatic metabolism. Results of low systematic bioavailability, short duration of therapeutic activity and or formation of inactive or toxic metabolites have been reported.

Delivery of drugs via the absorptive mucosa in various easily accessible body cavities, like the ocular, nasal, buccal, rectal and vaginal mucosae, has the advantage of bypassing the hepato-gastrointestinal first pass elimination associated with oral administration. Furthermore, because of the dual biophysical and or lipophilic characteristics can be readily absorbed.

Mucosal membranes particularly the nasal mucosa, also offer the potential for a rapid absorption of drugs with plasma profile closely duplicating that from an intravenous bolus injection. This is helpful in emergency situations. In addition, mucosal membranes may also be useful sites with good accessibility for easy application of drug delivery systems, other useful site with advantage good accessibility and easy application of drug is mucosal membrane, especially for those with mucoadhesive properties. With the development of mucosal delivery systems having sustained drug release characteristics, the mucosal routes can be exploited for the non invasive systemic delivery of organic and peptide based drugs, with rapid absorption as well as sustained drug action[1].

A variety of sustained release systems such as coated pellets, matrix tablets, osmotically sustained release systems, microcapsules, microspheres, nanoparticles, implants and infusion devices have been designed for various routes of drug administration [2.]

Microspheres form an important part of novel drug delivery systems. They have various applications and are prepared using assorted polymers.[3] However; the success of these microspheres is limited owing to their short residence time at the site of absorption. It would, therefore, be advantageous to have means for providing an intimate contact of the drug delivery system with the absorbing membranes. This can be achieved by coupling bioadhesion characters to microspheres and developing bioadhesive microspheres.[4]

Mucoadhesive microspheres include microparticles and microcapsules (having a core of the drug) of 1- 1000µm in diameter and consisting either entirely of a mucoadhesive polymer or having an outer coating of it.[3]

Mucoadhesive microspheres have following advantages: [5]

- Efficient absorption and enhanced bioavailability of the drugs due to a high surface to volume ratio.
- Much more intimate contact with the mucus layer.
- Specific targeting of drugs to the absorption site.
- They release the drug for prolong period
- Reduce frequency of drug administration
- Improve the patient compliance.

MATERIALS AND METHOD:

Material:

Nateglinide was gift sample from Alembic Sahar Road, Andheri (E), Mumbai-99, HPMC, HPC and PVP from Triveni aromatic and perfumery private limited, Mumbai, Carbopol 934p from Loba Chemie *Pvt. Ltd.*, Mumbai and Sod. Alginate from Colorcon Asia Pvt Ltd Goa. All other reagents used were of analytical grade.

Methods:

- Pre-formulation study
- Preparation of Microspheres using spray dryer
- Characterization of microspheres
- *In-vivo* study

Melting point determination

Melting point is the temperature at which the pure liquid and solid exist in equilibrium. In practice, it is taken as equilibrium mixture at an external pressure of 1 atmosphere; this is sometimes known as normal melting points. The Thiel's tube method of melting point determination in liquid paraffin was used in the present study.

Analytical method development of Nateglinide

The standard graph of Nateglinide in different media was obtained to quantify the Nateglinide in samples.

Preparation of Standard Calibration Curve for Nateglinide[6, 7, 8,9,10]

Reagents and solutions:

a) **Acid buffer (pH 1.2):** Place 50 ml of 0.2 M Potassium chloride , add 85 ml of 0.2 M Hydrochloric acid and then add water to dilute to make 200ml buffer .

b) **0.2 M Potassium chloride solution:** Dissolve 14.911 gm of Potassium Chloride in water and dilute with water to 1000ml.

c) **0.2 M Hydrochloric acid:** Dilute 17.2 ml of hydrochloric acid in 1000ml of distilled water.

Standard curve of Nateglinide:

a) In acid buffer (1.2 pH):

25mg of pure drug was accurately weighted and transferred into the 25ml volumetric flask and volume was made up using methanol (Nateglinide is freely soluble in methanol)[11]. This is standard stock solution (1mg/ml). From the standard stock solution a series of dilution were made to get 10, 16, 20, 28, 32, 36 and 40µg/ml solution using phosphate buffer pH 1.2 as dilution medium. The absorbance of these solutions was measured against a blank pH 1.2 HCl buffer in UV spectrophotometer at 244nm.[7, 9]

Preparation of drug loaded microspheres: [12, 13]

Microspheres were prepared by spray dryer. Feed solutions were prepared by dissolving the polymer(s) in the methanol and dichloro methane as solvent mixture by vortexing (using magnetic stirrer) and then adding the drug to this polymer solution and stirred to dissolve it. These solutions were used as the feed solutions for spray dryer. The conditions optimized for the production of microspheres by spray dryer method are

Out let temp - 450C , Inlet temperature - 50-600C. , Flow rate - 1 ml/min , Pump speed - 2 rpm, Air flow - 50 m 3 /h

Chitosan microspheres were produced by a spray drying method. Briefly, 100 ml of 0.5% of chitosan aqueous solution were prepared by dissolving overnight 1g of chitosan per ml of distilled water under stirring and adding 1, 2 or 4 ml of 1% glutaraldehyde aqueous solution to the chitosan solution before spray drying, Separately prepare the solution of drug: ethyl cellulose solution by dissolving in methanol, to this add the chitosan aqueous solution to Drug: Ethyl Cellulose solution. Then this solution is used as feed solution in the spray dryer.

Table 1: Amount of ingredients required for 2.75 gram of microsphere formulation

Name of the excipient	F1	F2	F3	F4	F5	F6	F7
Drug (250 mg)	+	+	+	+	+	+	+
Ethyl Cellulose (1.5 gms)	+	+	+	+	+	+	+
HPMC (1gm)	+	-	-	-	-	-	-
HPC(1gm)	-	+	-	-	-	-	-
Carbopol 934p(1gm)	-	-	+	-	-	-	-
Sod.cmc (1gm)	-	-	-	+	-	-	-
Chiitosan (1gm)	-	-	-	-	+	-	-
PVP (1gm)	-	-	-	-	-	+	-
Sod.Alginate (1gm)	-	-	-	-	-	-	+

Particle size determination:

The particle size of different batches of microspheres was determined by optical microscopy. The projected diameter of a total of 200 microspheres from each batch was determined using a compound electronic microscope that consisted of an optical microscope. The mean diameter (*d*m) was calculated through the average of every batch. Determination of particle size using compound microscope with stage micrometer the particle size of Mucoadhesive microsphere was calculated. Calibration of microscope done by following method

Eye piece micrometer intervals count= X, Stage micrometer length= Y

From these,

Calibration value= Stage micrometer length/ Eye piece micrometer intervals count

=Y/X

Particle size of microsphere= Eye piece micrometer intervals count * Calibration value for individual particle

Encapsulation efficiency:

An accurately weighed quantity of drug loaded Microspheres was pulverized and digested in sodium hydroxide (0.1mol L–1). The drug was extracted with the solvent overnight, filtered and the amount of Nateglinide in the filtrate was assayed after appropriate dilution by measuring the absorbance at 247 nm in a UV-visible spectrophotometer. The drug content was estimated in triplicate. The percentage encapsulation efficiency of different batches of microspheres was calculated from the percentage drug content values.

***In vitro* drug release studies:**

Dissolution studies were performed over a period of 8 hours in a USP XXIV basket dissolution apparatus at a stirring speed of 50 rpm. Release studies of the Nateglinide and drug loaded microspheres were carried out using acid buffer of pH 1.2 (900 mL) 8 hours maintained at 37 ± 0.5 °C, as a dissolution medium. Aliquots of samples withdrawn at 1 hour interval for acid buffer and 2 hours interval for alkaline phosphate buffer, samples were filtered through 0.45 mm whatmann filter paper, and assayed spectrophotometrically at 244 nm. The raw dissolution data recorded in triplicate was analyzed to calculate the percentage of cumulative drug released at different time intervals.

Swelling index:

The swelling ability of the microspheres in physiological media was determined by allowing them to swell to their equilibrium. Accurately weighed amount of microspheres (10 mg) were placed on Millipore filter (NY 11 0.22 μm) using a Franz diffusion cell (12.5 mL) with

hydrochloric acid buffer (pH 0.1N) and kept for 10 minutes. The following formula was used for calculation of degree of swelling.

$$\alpha = (Ws - Wo) / Ws$$

Where, α = Degree of swelling, Wo = Initial weight of microspheres

and Ws = Weight of microspheres after swelling.

***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 cyano acrylate 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 of pH 1.2 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 was centrifuged, dried and weight. The mucoadhesiveness of these microspheres was calculated.

Scanning Electron Microscopy (SEM).

Morphology and surface topography of the microspheres were examined by scanning electron microscopy. The microspheres from the optimized batch were mounted on the SEM sample stab using a double-sided sticking tape and coated with gold (~200 nm) under reduced pressure (0.133 Pa) for 5 min using an Ion sputtering device.

***In vivo* study:**

In vivo evaluation studies were conducted on Nateglinide pure drug, and F-3 formulation of mucoadhesive microspheres in normal, healthy rabbits by measuring serum glucose levels following their oral administration at a dose equivalent to 400 µg/kg of Nateglinide. The experiments were conducted as per a crossover randomized block design (n = 4). The approval of an ani-mal ethics committee was obtained before starting the study (IAEC/MPP24/2012-13). 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 6 hours after administration. Serum glucose concentrations were determined by a known oxidase-peroxidase method as described below employing a glucose kit supplied by Dr Reddy's Laboratory, Diagnostic Division (Hyderabad, India). The method was revalidated & the relative SD in 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:

The melting point of Nateglinide reported as 129-130°C, in 3 trials done on pure drug observed melting point were 130°C, 129°C, 129°C. The standard curve of Nateglinide in HCL buffer pH 1.2 at 247nm drawn. (Figure 1)

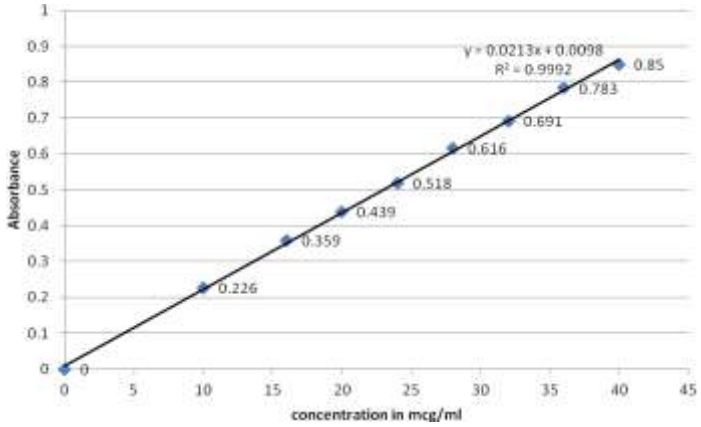

Figure 1: **Standard curve of Nateglinide in HCl buffer pH 1.2 at 247nm**

The effect of various mucoadhesive polymers in retarding the drug release of Nateglinide was studied by formulating into mucoadhesive microspheres, with different mucoadhesive polymers using spray drying technique.

Spray drying is a solvent evaporation process. The solvent in the droplets is removed very quickly due to heat energy provided in the spray dryer. The thermal efficiency of the spray drying is related to heat energy input (controlled by inlet temperature and blower) and the amount of heat used in the evaporation process.

The optimum inlet temperature for the preparation of microspheres is 500c-600c, outlet temp is 450c and the flow rate was set at 1ml / min with airflow of 50m 3 / hr.

Microspheres were formulated at drug:

Polymer ratio of 1: 5, and a combination of ethyl cellulose and various mucoadhesive polymers were tried. Ethyl cellulose: mucoadhesive polymer ratio (3:2) was kept constant for all

formulations. Seven mucoadhesive polymers are used and a total of seven formulations were developed and subjected for physico-chemical properties and dissolution studies.

Physicochemical properties of microspheres:

The size of microsphere formulations were about 2.5 to 3.5 µm and were not affected by the composition of the formulation, encapsulation efficiency ranges from 76.75% to 89.36%. the percentage yield was ranging from 21.33% to 34.43%.(Table 2)

Table 2. Percentage yield and encapsulation efficiency

Name of the formulation	Percentage yield	Encapsulation efficiency
F1	21.33 + 0.23	83.08 + 0.42
F2	22.83 + 0.44	89.36+ 0.37
F3	37.86 + 0.48	86.13+ 0.56
F4	30.95 + 0.28	82.11+ 0.45
F5	31.24 + 0.18	76.75+ 0.26
F6	34.43 + 0.22	80.83+ 0.29
F7	33.42 + 0.46	83.45+ 0.33

***In vitro* mucoadhesion study:**

Mucoadhesion studies were carried out to ensure the adhesion of formulation to the intestinal mucosa for a prolonged period of time at the site of absorption. Results showed that, the microspheres adhere on mucosa. The ratio of adhered microspheres was expressed as percent mucoadhesive formulation F3 prepared with carbopol showed good mucoadhesion even after a period of 8 hrs. F6 and F7 formulations prepared with PVP and Sodium alginate also showed sufficient mucoadhesion. (Table 3)

Table 3: *In vitro* mucoadhesion test:

Formulation	Percent of microspheres adhering to tissue at different time Interval						
	1 hr	2hr	3hr	4hr	5hr	6hr	8hrs
F1	81.65 + 0.32	64.14 + 0.55	44.33 + 0.52	35.21 + 0.33	24.97 + 0.54	21.28 + 0.77	11.44 + 0.24
F2	80.17 + 0.43	73.3 + 0.56	64.19 + 0.45	51.35 + 0.66	40.79 + 0.32	23.27 + 0.44	9.47 + 0.83
F3	83.17 + 0.43	75.17 + 0.63	65.17 + 0.773	58 .61+ 0.76	53.09 + 0.334	49.39 + 0.66	31.17 + 0.53
F5	82.35+ 0.74	63.84 + 0.46	57.75 + 0.3	39.249 + 0.56	26.97 + 0.64	17.47 + 0.77	6.44 + 0.24
F6	84.44 + 0.45	71.2 + 0.32	60.15 + 0.54	47.46 + 0.76	34.37 + 0.47	26.85 + 0.36	13.27 + 0.286
F7	86.44 + 0.55	77.15 + 0.75	65.15 + 0.56	55.46 + 0.46	43.37 + 0.4	31.85 + 0.31	19.57 + 0.26

Dissolution studies:

The *in vitro* release profiles of Nateglinide pure and microspheres are shown in table 4 and figures 2 and 3 respectively.

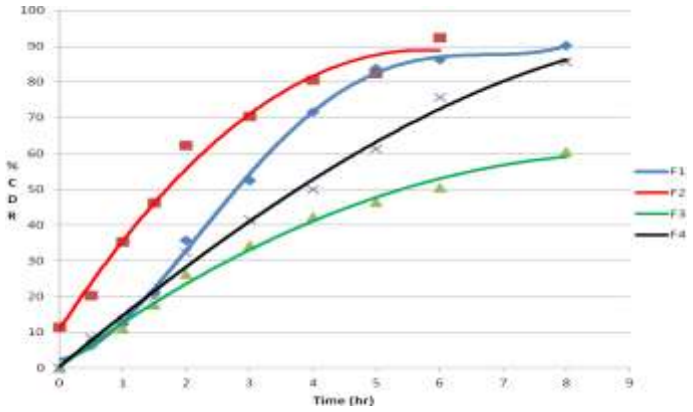

Figure 2: Drug Release profiles of formulations of F1, F2, F3 and F4. F: Formulation, CDR: Cumulative Drug Release

Table 4: Dissolution study of Nateglinide pure form in pH 1.2.(0.1 N HCL)

Time	%drug release
10	20.33 + 0.39
20	24.78+ 0.34
30	27.98+ 0.43
40	30.47+ 0.66
50	32.56 + 0.35
60	41 .09 + 0.53
70	47.41 + 0.35
80	51.54 + 0.53
90	61.44 + 0.33
120	82.43+ 0.55
180	96.32 + 0.46

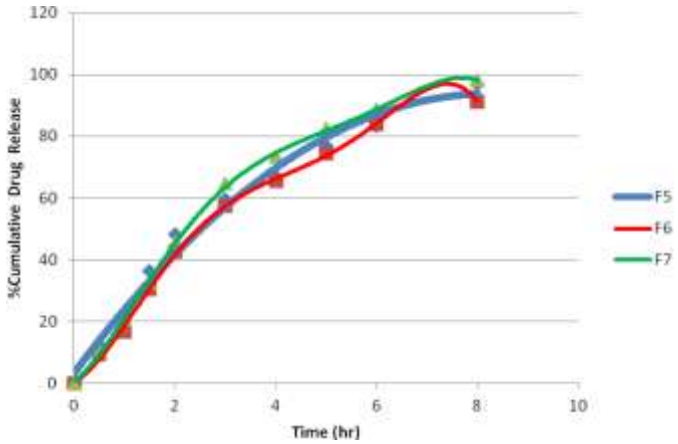

Figure 3: Drug Release profiles of formulations of F5, F6 and F7

Formulations prepared with HPMC, HPC, PVP, chitosan, sodium alginate showed 90%- 98% drug release with in 8 hrs. But the release of Nateglinide from formulation F3 showed slow and sustained release for a period of 8 hrs and only 60.49 % drug release was observed. So, formulation f3 is considered as suitable formulation and hence selected for further studies.

SEM Analysis:

Surface morphology was observed by SEM showed spherically shaped Nateglinide loaded microspheres with smooth surface, indicating that they were well prepared by the spray drying method. (Figure 4 and 5)

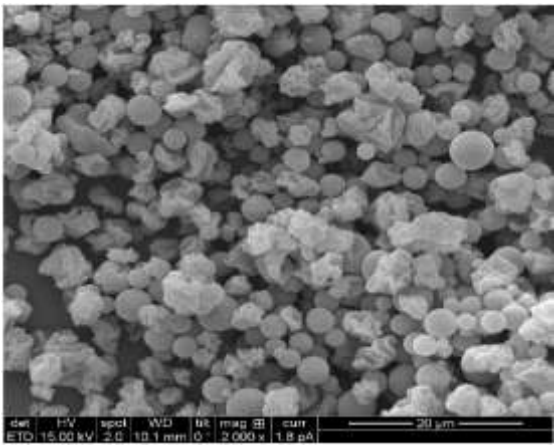

Figure 4. SEM study of optimized microsphere batch under low magnification

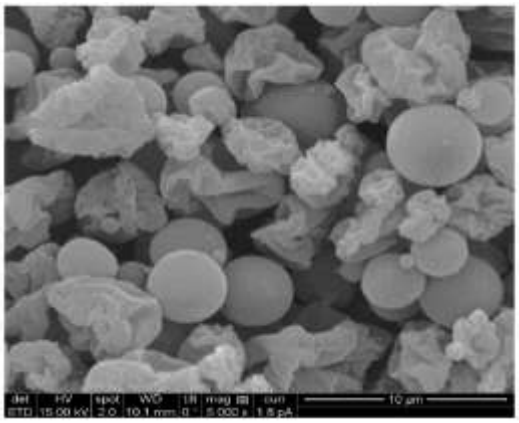

Figure 5. SEM study of optimized microsphere batch under low magnification

Swelling Index studies:

Swelling capacity of the microspheres was determined by polymer content in the preparation, since mucoadhesive polymers were the only component in the spray dried system with swelling abilities. The maximum degree of swelling was observed 0.615 and 0.518 for formulation with sodium alginate and carbopol respectively. Good swelling ability was observed with carbopol and sodium alginate. (Table 5)

Table 5: Swelling index of the formulations F1 to F7

Name of the formulation	Initial weight of microspheres (Wo)	Weight(mg) of microspheres after swelling (Ws)*	Degree of swelling (α)* α* = (Ws – Wo) / Ws
F1	10 mg	20 + 0.5	0.5
F2	10 mg	14 + 0.4	0.29
F3	10 mg	24 + 0.6	0.58
F4	10 mg	18 + 0.5	0.545
F5	10 mg	22 + 0.6	0.444
F6	10 mg	16 + 0.25	0.375
F7	10 mg	26 + 0.35	0.615

In vivo* evaluation*:

In vivo evaluation of mucoadhesive microspheres prepared with carbopol 934P (F3) was carried out in normal healthy rabbits, by measuring the hypoglycemic effect produced after their oral administration at a dose equivalent to 400mcg/kg of Nateglinide ,in comparison to Nateglinide (pure drug) at the same dose. When Nateglinide was administrated, a rapid reduction in serum glucose levels was observed, a maximum reduction of 38.91% was observed at 3 hrs after administration, and the glucose levels recovered rapidly to the normal level with in 6hrs. In case of F3, reductions in glucose levels were sustained over longer periods of time a 25% reduction in glucose levels is considered a significant hypoglycemic effect. Maximum reduction of 40.58% was observed after a period of 6hrs .The hypoglycemic effect was maintained during the period from 6 hrs to a 20 hrs and reached to normal levels only after a period of 24 hrs. The sustained hypoglycemic effect observed over longer periods of time in case of mucoadhesive microspheres was prepared with carbopol934P is due to slow release and absorption of Nateglinide over longer periods of time. (Figure 6)

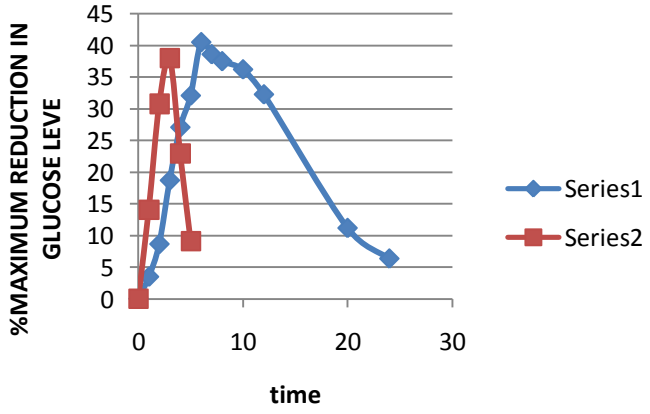

Figure 6. *In vivo* studies of optimised formulation (F3) Series 1: Optimized Formulation F3, Series 2: Pure Drug

CONCLUSION

In this study the attempt was made to prepare mucoadhesive microspheres of Nateglinide using a combination of ethyl cellulose and various mucoadhesive polymers designed for oral sustained release. Nateglinide microspheres were prepared by spray drying technique using HPMC, HPC, Carbopol, Sod.CMC, Chitosan, PVP and Sodium alginate. The microspheres were evaluated for surface morphology and particle shape by SEM. The prepared microspheres were also evaluated for their micro encapsulation efficiency, *in vitro* mucoadhesion test, i*n vitro drug* release, *in vivo* study. The microspheres were discrete, spherical and free flowing. The microencapsulation efficiency was in the range of 76.75% - 89.36% and exhibited good mucoadhesive property. Among all formulations, F3 formulation exhibits 30% microspheres were still adhere even after 8 hrs in intestinal mucosa .Dissolution studies showed slow release of Nateglinide . All formulations except F3 showed 90-98% within 8 hrs. But formulation F3 showed slow and sustained release and only 60.45% was observed after a period of 8 hrs. *In vivo* testing of mucoadhesive microspheres in normal healthy rabbits demonstrated significant hypoglycemic effect of Nateglinide. The hypoglycemic effect obtained by mucoadhesive microspheres was more than 20 hrs, where as Nateglinide pure form produce hypoglycemic effect for only 3 hrs suggesting that mucoadhesive microspheres are valuable system for long term delivery of Nateglinide.

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