International Journal of Pharmacy and Biological Sciences-IJPBS™ (2019) 9 (1): 1579-1591
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Pharmaceutical Sciences | Open Access | MCI Approved
UGC Approved Journal

Formulation and *In Vitro*, *In-Vivo* Evaluation of Floating Bilayer Tablets of Ezetimibe-Nateglinide

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Received: 12 Oct 2018 / Accepted: 8 Nov 2018 / Published online: 1 Jan 2019
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Abstract

In this research work, nateglinide, ezetimibe was selected as model drugs. Nateglinide is an amino acid derivative that induces an early insulin response to meals decreasing postprandial blood glucose levels which act by binding to β cells of the pancreas to stimulate insulin release. Ezetimibe is a lipid-lowering compound that inhibits intestinal cholesterol and phytosterol absorption. Formulation of sustained release floating bilayer tablets of ezetimibe-nateglinide with Polyox WSR 303, Carbopol 934P, HPMC K4M, Na CMC. The compatibility of nateglinide and excipients used in study was determined using DSC and this study revealed that no interaction between drug and excipients used in the formulation. The optimized nateglinide-ezetimibe floating formulations (NGT3, NGT10) showed satisfactory results with respect to in vitro buoyancy and sustained drug release and was physically stable for 3 months period. The vivo radiographic studies showed that the $BaSO_4$-loaded floating tablets were retained in the stomach for 4.16 ± 1.57 h (n=3).

Keywords
Nateglinide, Ezetimibe, floating, Natiz.

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INTRODUCTION:

Bilayer tablet is suitable for sequential release of two drugs in combination, separate two incompatible substances and for sustained release tablet in which one layer is immediate release as initial dose and second layer is maintenance dose. In which the one layer is formulated to obtain immediate release of the drug, with the aim of reaching a high serum concentration in a short period of time. The second layer is a controlled release, which is designed to maintain an effective plasma level for a prolonged period. The pharmacokinetic advantage relies on the fact that drug release from fast releasing layers leads to a sudden rise in blood concentration. However, the blood level is maintained at steady state as the drug is released from the sustaining layer.

One of the most feasible approaches for achieving prolonged and predictable drug delivery profiles in

gastrointestinal tract is to control the gastric residence time (GRT) using gastroretentive dosage forms that offer a new and better option for drug therapy. Dosage forms that can be retained in the stomach are called gastroretentive drug delivery systems (GRDDS). Gastroretentive floating drug delivery system was first described by Davis in 1968. Over the last two decades, numerous GRDDS have been designed to prolong gastric residence time (Talukder and Fassihi, 2004a). Figure 1.3 describes how the drug absorption takes place in the case of conventional dosage forms and GRDDS. GRDDS can improve the controlled delivery of drugs that have an absorption window. This system releases the drug continuously for a prolonged period of time before it reaches its absorption site, thus ensuring its optimal bioavailability (Brahma and Know, 2000).

MATERIALS AND METHODS:

MATERIALS

Nateglinde was collected as a gift sample from splendid pharma, Ltd, Puna, Ezetmibe collected from Hetero drugs Ltd, Hyderabad. Hydroxy propyl methylcellulose (HPMC K4M) and Polyox WSR 303 were received as gift samples from Orchid pharma Ltd., Chennai, India. Sod.CMC and Carbopol 971P were received from M/s Aurobindo Pharma. Ltd., Hyderabad, India. Sodium bicarbonate, magnesium stearate and talc were purchased from S.D. Fine-Chem. Ltd., Mumbai, India. Acetonitrile and methanol HPLC grade were purchased from Sigma Aldrich chemicals Dombivli, India. All other solvents and reagents used were of analytical grade.

METHODS

Solubility study of ezetimibe, nateglinide

Excess amount of drug was placed in 0.1 N HCl (pH 1.2), pH 4.5 acetate and pH 6.8 phosphate buffer and water in order to determine its solubility. The samples were shaken for 48 hrs at 37 °C in a horizontal shaker. The supernatant was filtered, and the filtrate was diluted with the appropriate buffer and analyzed by using UV/Visible spectrophotometer (Elico, SL210) at λ_{max} of 244, 216 nm.

Determination of acid stability of ezetimibe, nateglinide

Stock solution of drug was prepared in 0.1 N HCl in order to determine its acid stability. At predetermined time points like 1, 2, 3, 4, 6, 8, 10, 12 and 24 h, the samples were assayed using UV/Visible spectrophotometer (Elico, SL210) at λ_{max} of 244, 216 nm.

Evaluation of final blend

The powder blend of all formulations was evaluated for Bulk density, Tapped density, Compressibility Index, Hausner ratio and Angle of repose (Carr, R.L., 1965).

Formulation of ezetimibe Immediate Release (IR) tablets

The formulation of ezetimibe IR tablets containing drug ezetimibe with disintegrants were prepared according to the following formula. Different percentages of disintegrants have been used in batch and to study the immediate release effect of ezetimibe. Total tablet weight is 100 mg.

Table 1. Formulation of immediate release tablets of Ezetimibe in combination of Cross povidone, Lactose and Sorbitol (weights in mg/tablet)

Ingredients	EZ 1 (mg)	EZ 2 (mg)	EZ 3 (mg)	EZ 4 (mg)	EZ 5 (mg)	EZ 6 (mg)	EZ 7 (mg)	EZ8 (mg)	EZ9 (mg)	EZ 10 (mg)
Ezetimibe	10	10	10	10	10	10	10	10	10	10
Cross povidone	47	57	67	77	87	--	--	--	--	--
Lactose	40	30	--	--	--	87	87	87	--	--
Sorbitol	--	--	20	10	--	--	--	--	87	87
Magnesium stearate	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5

Formulation of floating tablets of nateglinide Nateglinide, Ezetimibe, floating, Natiz.

Accurately weighed quantities of drug, Polyox WSR303/HPMC K4M/Carbopol 971P/Sod CMC, sodium bicarbonate and Avicel PH102 were passed through a sieve, no. 40, to get uniform sized particles, and then they were triturated for 10 min with the help of mortar and pestle. At that point the blend was moved into a poly sack and further blended for 5 min to warrant a homogeneous mass. To the blend, magnesium stearate and talc were included and preceded with the blending for another 2 min. At long last, each blend was weighed and fed manually into the die of a 16station punching machine (Cadmach, Ahmedabad, India) to produce the desired tablets using flat- faced round punches. The hardness is adjusted to 5 kg/cm^2

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Table 2. Formulation of sustained release floating bilayer tablets of ezetimibe-nateglinide with Polyox WSR 303, Carbopol 934 P, (weights in mg/tablet)

Ingredients	NGT1	NGT 2	NGT 3	NGT 4	NGT5	NGT6	NGT7	NGT 8	NGT 9	NGT 10
Nateglinide	120	120	120	120	120	120	120	120	120	120
Polyox WSR 303	40	60	80	100	120	--	--	--	--	--
HPMC K4M	--	--	--	--	--	40	60	80	100	120
Carbopol 934P	80	60	60	40	40	--	--	--	--	--
SCMC	--	--	--	--	--	80	60	60	40	40
Sodium bicarbonate	60	60	60	60	60	60	60	60	60	60
Avicel PH102	88	88	68	68	48	88	88	68	68	48
Mg. Stearate	6	6	6	6	6	6	6	6	6	6
Talc	6	6	6	6	6	6	6	6	6	6
Total Tablet weight	500	500	500	500	500	500	500	500	500	500

Physical characterization of prepared tablets

The prepared floating tablets were evaluated for hardness, thickness, weight variation, friability, and drug content.

***In-vitro* buoyancy studies**

The *in vitro* buoyancy was characterized by floating lag time (FLT) and total floating time (TFT). The test will be performed using United States Pharmacopeia (USP 24) type-2 apparatus using 900 mL of 0.1N HCl with a paddle rotation of 50 rpm at 37°C ± 0.5°C. Nateglinide sustained release effervescent levity bilayer tablets were placed in dissolution vessels and time required for the tablet to rise to the surface of the dissolution medium and the duration of time the tablet constantly floated on the dissolution medium were noted as FLT and TFT, respectively (Baumgartner et al., 2000).

In-vitro dissolution studies

The in vitro drug release studies will be conducted using USP 24 type-2 apparatus (Electrolab, TDT-06T). The dissolution test is performed using 900 mL of 0.1N HCl (pH 1.2), at 37 ± 0.5°C and 50 rpm. A sample (5 ml) of the solution was withdrawn from the dissolution apparatus at pre-determined time intervals and replaced with fresh dissolution medium. The samples were filtered through a 0.45-µm membrane filter and diluted to a suitable concentration with 0.1N HCl. Absorbance of these samples were measured using UV/Visible spectrophotometer (Elico, SL 210, India) at λ_{max} 216, 276 nm.

Analysis of drug release kinetics

There are number of kinetic models, which described the overall release of drug from the dosage forms. Because qualitative and quantitative changes in a formulation may alter drug release and *in vivo* performance, developing tools that facilitate product development by reducing the necessity of biostudies is always desirable. In this regard, the use of in vitro drug dissolution data to predict vivo bio-performance can be considered as the rational development of controlled release formulations (Dressman et al., 1984).

The model dependent methods all rely upon a curve fitting procedure. Different mathematical functions were used to model the observed data (Costa and Lobo, 2001). Both the linear and non-linear models are being used in practice for dissolution modeling. Linear models include Zero order, Higuchi, Hixon – Crowell, Quadratic and Polynomials, whereas the nonlinear models include First order, Weibull, Korsmeyer – Peppas, Logistic etc.

Determination of tablet swelling and erosion levels

The swelling behavior of the tablets will be determined in triplicate The nateglinide sustained release effervescent levity bilayer tablets, nateglinide sustained release effervescent floating bilayer tablets were weighed (W_0) and placed in a glass beaker containing 200 mL of 0.1 N HCl, maintained at 37 ± 0.5°C. At regular time intervals, the tablets were removed, and the excess surface liquid was carefully removed by a filter paper (Patel et al., 2009). The swollen individual tablet was then reweighed (W_1). The wet tablets were then dried in an oven at 40° C for 24-h and finally weighed until constant weight was achieved (final dry weight, W_2). The percentage swelling and erosion at different times was estimated from the following equations:

$$\% \text{ Swelling} = \frac{(W1 - W0)}{W0} \times 100$$

$$\% \text{ Erosion} = \frac{(W0 - W2)}{W0} \times 100$$

Physical stability studies

Physical stability studies were conducted according to ICH guidelines. One of the optimized formulations of nateglinide sustained release effervescent floating, floating bilayer tablets (NGT3) were enclosed in polyethylene bottle and placed in a desiccator containing saturated sodium chloride solution (75% RH). The desiccator was stored at 40°C

for 3 months (Tadros, 2010). At predetermined time intervals, the tablets were examined for hardness, FLT, TFT, drug content and drug release. Finally, the tablets were tested for any statistical difference using the student paired t-test, the differences were considered to be significant at $p < 0.05$.

Formulation of nateglinide sustained release effervescent floating, bilayer tablets for in-vivo radiographic studies

$BaSO_4$ was used to make tablet X-ray opaque. For this study, $BaSO_4$ was loaded in optimized formulation of nateglinide sustained release effervescent floating tablets (NGT3) with following composition: 125 mg drug, 75 mg $BaSO_4$, 60 mg Sod. CMC, 60 mg $NaHCO_3$, 130 mg Avicel PH102, 5 mg magnesium stearate and 5 mg talc. The tablets were prepared by direct compression method.

In-vivo evaluation of gastric residence time in healthy volunteers

Three healthy male volunteers will participate after giving an informed written consent. The subjects weighed 64-75 kg, in height from 165-173 cm, and in the age group of 22-26 years. The in vivo gastric residence time of GRDDS can be determined by a variety of techniques such as x-ray, endoscopy, gamma-scintigraphy (Jagdale et al., 2009). In this study, x-ray techniques will be used to determine the gastric residence time of gastroprotective tablets. To make the tablet X-ray opaque, $BaSO_4$ was used. The study was conducted under the guidance of an expert radiologist. After overnight fasting, the volunteers were fed with low calorie food (100 g of bread). Half an hour later, $BaSO_4$-loaded optimized formulation of nateglinide floating tablet (NGT3) was given to every volunteer with a glass of water. During the study, the subjects were not allowed to eat but water was made available ad libitum. At different time intervals like, 0.5, 2.5, 4.5 and 5.5 h, the volunteers were exposed to abdominal x-ray imaging in a standing position. The distance between source of x-rays and the subject was kept constant for all images. Thus, the observation of the tablet movements could be easily noticed (El Gamal et al., 2011). The mean gastric residence time was calculated.

Comparative bioavailability study in human volunteers

Subjects: The mean age of volunteers was 22.5 ± 3.2 years, mean height was 167.5 ± 8.5 cm, and mean body weight was 63.5 ± 6.4 kg.

Nine healthy male volunteers for nateglinide floating tablets were selected for the study. Before starting the study, each candidate signed an informed consent form. They were judged to be healthy based on medical history, physical examination, hematological and biochemical laboratory tests. The bioavailability protocol was approved by an Institutional Human Ethical Committee, Talla Padmavathi College of Pharmacy, Warangal, Telangana, India

Study design:

Nateglinide floating tablets:

A single dose, randomized, three-way cross-over study was designed with nine subjects in each treatment group. A one-week washout period existed between treatments of the study. After overnight fasting, in three study periods for each subject the assigned formulation (floating NGT3/Natiz 120 mg) aside policy phoneticly amidst 240 ml of water. One week before and during the study, they were not allowed to take alcohol or any other medication. The subject's fasted overnight and 5 hrs. after tablet administration, but water was made available ad libitum. Study medication was administered according to randomization schedule. Subjects received standard meals after 5 hrs of tablet administration. Blood samples were collected at predetermined time intervals such as 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 h. Blood samples (5 ml) were obtained from forearm vein using sterile disposable needle and collected into 10 ml sterile test tubes. The samples were centrifuged immediately at 4000 rpm for 15 min. and the separated plasma was transferred into 2.5 ml of Eppendorf tubes and stored at −80° C till the time of analysis.

Sample preparation for analysis

The serum samples were extracted by liquid-liquid extraction method. To 1 ml of serum, 0.4 ml of phosphate buffer PH 7 and 100 µl of internal standard (furosemide, 2 µg/ml) was added and vortexed for 5 min in a test tube. To this mixture 7 ml of chloroform: isopropyl alcohol was added as extracting agent in 95: 5 v/v proportion and vortexed for 3 min then centrifuged at 2500 rpm on cooling centrifuge for 15 min at 4 °C (Manglani et al., 2006). The organic phase was separated into another test tube and evaporated to dryness in a vacuum oven. To the dried residue 0.4 ml of diethyl ether was added and shaken for 10 sec and the ether layer was discarded. The dried residue was reconstituted with100 µl of mobile phase from which 20 µl was injected into the HPLC column.

Pharmacokinetic analysis

The pharmacokinetic parameters of test formulation and reference formulation were estimated for each volunteer by using a computer programme, Kinetica 2000 (Version 3.0, Innaphase Corporation, and Philadelphia, USA). Non-compartmental analysis was

used to calculate pharmacokinetic parameters, C_{max}, t_{max}, $t_{1/2}$, $AUC_{0-\infty}$ and MRT values. C_{max} and t_{max} were read directly from the observed mean plasma drug concentration against time profile. AUC_{0-t} was calculated by the trapezoidal rule and the total $AUC_{0-\infty}$ was calculated according to the equation.

$$AUC_{0-\infty} = AUC_{0-t} + C_t/K_E$$

Where, Ct is the last measurable concentration and K_E is the elimination rate constant obtained from terminal log-linear portion of the plasma concentration-time profile. The mean residence time (MRT) was calculated using following equation (Shargel et al., 2005).

$$MRT = AUMC_{0-\infty}/AUC_{0-\infty}$$

Where, AUMC is the area under the first moment of the concentration time curves.

RESULTS AND DISCUSSION

Results and Discussion

Equilibrium Solubility Study of Nateglinide

The solubility studies were conducted in different media and values are shown in Table 2. The solubility of the drug was determined in different media like, 0.1 N HCl (pH 1.2), pH 4.5, pH 6.8 and water. The drug showed greater solubility in 0.1 N HCl (1.85 ± 0.32 mg/mL) and lesser solubility in water (1.32 ± 0.26 mg/mL).

Table 3: Solubility of Nateglinide in different media (mean ± SD).

Medium	Solubility (mg/mL)
pH 1.2	1.85 ± 0.32
pH 4.5	1.66 ± 0.22
pH 6.8	1.46 ± 0.43
Water	1.32 ± 0.26

Determination of acid stability of Nateglinide: From the acid stability study results it was observed that there was no change in drug concentration until 24 h indicating stability of drug in 0.1 N HCl.

Physical characteristics of prepared tablets of ezetimibe:

All the prepared formulations were subjected to the hardness, thickness, weight variation, friability, and drug content. The hardness of all tablets ranged from 5.18 ± 0.23 to 5.31 ± 0.45 kg/cm^2 and that of thickness from 5.28 ± 0.13 to 5.58 ± 0.16 mm. All the tablet formulations showed acceptable physicochemical properties and complied with the pharmacopoeial specifications for weight variation, drug content and friability (Banker and Anderson, 1987).

Table 4: Physicochemical characters of prepared tablets of ezetimibe

Formulation code	Hardness (kg/cm^2) (n=6)	Thickness (mm) (n=6)	Tablet weight (mg) (n=20)	Friability (%) (n=10)	Drug content (%) (n=3)	Disintegration Time (min) n=3 Mean ± S. D
EZ 1	4.20±0.2	4.17±0.12	101.80±6.1	0.53	99.03±1.9	1.43 ± 0.61
EZ 2	4.28±0.2	4.24±0.11	102.20±7.1	0.57	101.30±1.	1.01 ± 0.22
EZ 3	4.38±0.2	4.38±0.16	101.90±7.7	0.52	99.40±1.4	0.50 ± 0.06
EZ 4	4.30±0.4	4.28±0.13	99.30±6.62	0.56	98.55±1.4	0.56 ± 0.08
EZ 5	4.36±0.2	4.28±0.16	99.20±5.90	0.53	99.67±1.4	0.42 ± 0.07
EZ 6	4.22±0.2	4.34±0.11	101.20±6.9	0.59	99.88±1.3	0.57 ± 0.07
EZ 7	4.34±0.3	4.52±0.17	100.10±6.1	0.58	98.91±1.8	1.12 ± 0.04
EZ 8	5.30±0.3	4.52±0.13	102.10±6.1	0.57	99.28±1.3	0.50 ± 0.02
EZ 9	5.26±0.4	4.42±0.14	103.80±7.0	0.52	99.38±1.2	0.66 ± 0.05
EZ 10	5.30±0.5	4.50±0.15	101.60±7.3	0.58	99.40±1.7	0.66 ± 0.05

Table 5: Physicochemical characters of nateglinide sustained release effervescent floating bilayer tablets

Formulation code	Hardness (kg/cm^2) (n=6)	Thickness (mm) (n=6)	Tablet weight (mg) (n=20)	Friability (%) (n=10)	Drug content (%) (n=3)
NGT 1	5.31±0.28	5.16±0.12	501.80±6.16	0.54	99.03±1.96
NGT 2	5.36±0.23	5.25±0.11	502.20±7.16	0.56	101.30±1.7
NGT 3	5.28±0.29	5.29±0.16	501.90±7.75	0.53	99.40±1.49
NGT 4	5.22±0.42	5.28±0.13	499.30±6.62	0.57	98.55±1.46
NGT 5	5.26±0.26	5.28±0.16	499.20±5.90	0.57	99.67±1.44
NGT 6	5.28±0.29	5.34±0.11	501.20±6.97	0.59	99.88±1.33
NGT 7	5.29±0.36	5.52±0.17	500.10±6.10	0.68	98.91±1.89
NGT 8	5.30±0.36	5.52±0.13	502.10±6.16	0.63	99.28±1.39
NGT 9	5.27±0.47	5.42±0.14	503.80±7.07	0.65	99.38±1.24
NGT 10	5.30±0.51	5.50±0.15	501.60±7.35	0.68	99.40±1.79

The physicochemical characteristics of the tablets are summarized in table 5. The hardness of all tablets ranged from 5.18 ± 0.23 to 5.31 ± 0.45 kg/cm^2 and that of thickness from 5.28 ± 0.13 to 5.58 ± 0.16 mm. All the tablet formulations showed acceptable physicochemical properties and complied with the pharmacopoeial specifications for weight variation, drug content and friability (Banker and Anderson, 1987).

***In vitro* buoyancy studies**

Table 6: In vitro buoyancy of nateglinide sustained release effervescent floating bilayer tablets

Formulation code	Floating lag time (s) (n=3)	Floating duration(h)(n=3)
NGT 1	15.3±2.5	>12
NGT 2	17.7±2.6	>12
NGT 3	20.0±2.0	>12
NGT 4	24.7±3.5	>12
NGT 5	29.3±5.5	>12
NGT 6	35.7±3.1	>12
NGT 7	17.3±3.1	>12
NGT 8	19.0±3.0	>12
NGT 9	21.3±3.1	>12
NGT 10	35.7±3.1	>12

All the formulations were prepared by effervescent technique. Sodium bicarbonate was used as a gas generating agent. Formulations NGT1-NGT10, prepared with combination of Polyax WSR 303 and CP 971P floated lag time of 15.3±2.5 (NGT1) to 35.7±3.1 (NGT10) sec. Tablets of all formulations showed good in vitro buoyancy with maximum floating lag time of 35.7±3.1 sec (Table 6 & Table 7), regardless of viscosity of polymer used. This was mainly due to evolution of carbon dioxide entrapped inside the hydrated polymeric matrices, resulting from the interaction between gas generating agent ($NaHCO_3$) and dissolution medium (0.1N HCl), and this led to the lowering of density of matrices to float. The floating lag time could not change with different viscosity grades of polymers and the type of filler used. All formulations remained buoyant for more than 12 h in dissolution medium (0.1 N HCl, pH 1.2).

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

Table 8: *In vitro* immediate drug release of ezetimibe

Time (Min)	EZ1	EZ 2	EZ 3	EZ4	EZ 5
0	0	0	0	0	0
1`	18.87±1.54	22.61±166	24.26±1.32	26.96±1.34	30.04±1.22
5	32.70±1.76	34.52±1.54	35.09±1.54	36.78±1.55	38.00±1.89
10	43.65±1.92	43.65±1.99	44.39±1.22	46.09±2.22	48.74±2.15
15	65.78±2.12	54.91±1.86	56.57±1.77	59.35±1.98	60.43±1.56

20	74.83±1.88	69.96±2.21	67.78±1.54	69.39±2.23	72.52±1.89
25	87.87±1.56	85.83±1.12	85.48±2.23	89.09±1.87	89.22±1.45
30	91.61±1.97	94.43±1.83	94.09±2.12	96.74±1.77	99.74±1.29

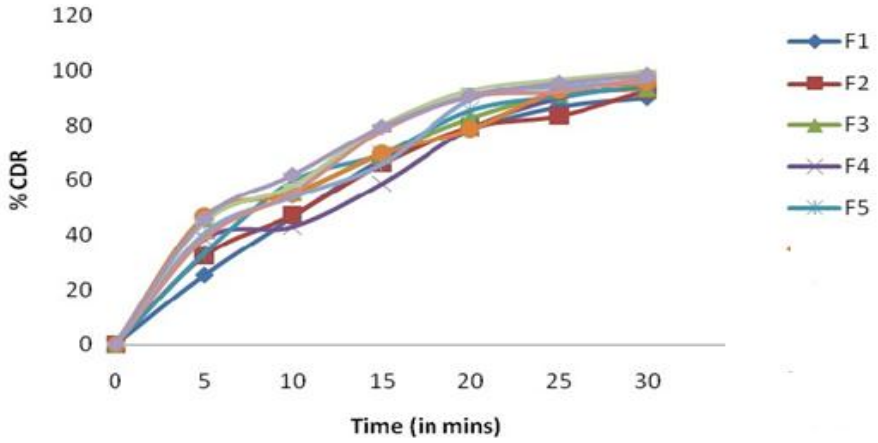

Fig: 1 *In vitro* immediate drug release of ezetimibe

***In vitro* sustained release effervescent floating bilayer tablets of nateglinide**

Table 9: Cumulative percentage release of nateglinide from floating tablets prepared with combination of Polyox WSR 303 and CP 934 P and (n=3, Mean±SD)

Time (h)	NGT1	NGT 2	NGT 3	NGT F4	NGT 5
0	0	0	0	0	0
1	24.87±1.54	26.61±1.66	20.26±1.32	18.96±1.34	19.04±1.22
2	37.70±1.76	39.52±1.54	32.09±1.54	30.78±1.55	29.00±1.89
3	48.65±1.92	52.65±1.99	41.39±1.22	40.09±2.22	39.74±2.15
4	60.78±2.12	62.91±1.86	51.57±1.77	50.35±1.98	48.43±1.56
6	74.83±1.88	77.96±2.21	66.78±1.54	62.39±2.23	60.52±1.89
8	88.87±1.56	90.83±1.12	82.48±2.23	78.09±1.87	75.22±1.45
10	101.61±1.97	94.43±1.83	91.09±2.12	90.74±1.77	87.74±1.29
12	101.09±1.54	98.39±1.96	98.04±1.67	98.26±1.86	95.35±1.33

All the formulations were subjected to *in vitro* drug release studies in 0.1 N HCl. The drug release profiles of formulations NGT1-NGT5 prepared with combination of Polyox WSR 303, and CP 934 P shown in figure 1. Formulation NGT1 and NGT2 released about 88.87±1.56% and 94.43±1.83 % drug in 8 and 10 h respectively and couldn't sustain the drug release for 12 h, indicating less concentration polymer. The formulation NGT 3 released about 98.04±1.67% of drug in 12 h. Similarly, formulation NGT4 sustained the drug release for 12 h and released 98.26±1.86% of drug in 12 h. Formulations NGT 5 released about 95.35±1.33% in 12 h. From the results, it was also observed that as the increasing concentration of CP 934 P, the drug release was decreased.

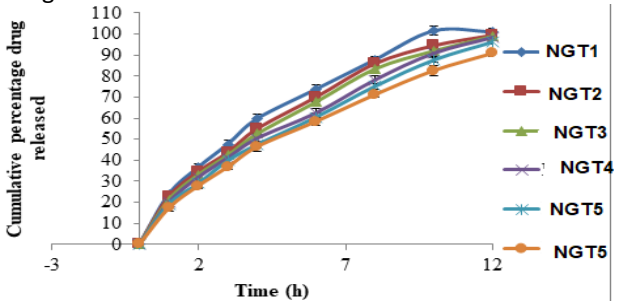

Fig. 2: Cumulative percentage release of nateglinide from floating tablets prepared with combination of Polyox WSR 303 and CP 934 P

All the formulations were subjected to *in vitro* drug release studies in 0.1 N HCl. The formulations NGT6-NGT10 prepared with combination of HPMC K4M and Sodium CMC were shown in figure 2. Amount of drug release from these formulations ranged from 84.48±1.85% (NGT 6) to 97.35±2.42% (NGT10). All the formulations sustained the drug release for 12 h. The optimized formulation released about 99.91±1.84% of drug in 12 h.

Table 10: Cumulative percentage release of nateglinide from floating bilayer tablets prepared with combination of HPMC K4M and Sodium CMC (n=3, Mean±SD)

Time (h)	NGT6	NGT 7	NGT 8	NGT F9	NGT 10
0	0	0	0	0	0
1	14.17 ±1.53	15.74± 1.51	17.61± 1.54	18.65 ±1.41	19.52 ±1.89
2	22.26 ±2.87	23.09± 1.58	26.78± 1.58	29.78 ±1.48	30.87 ±1.78
3	30.13 ±1.42	33.96± 1.44	35.78± 1.37	37.91 ±1.43	38.96 ±2.64
4	39.61 ±2.12	41.52± 1.07	43.30± 2.08	45.48 ±2.01	48.43 ±1.65
6	51.48 ±2.10	53.30± 2.16	55.04± 2.16	56.13 ±2.16	60.22 ±1.68
8	63.52 ±1.14	65.48± 2.86	67.22± 1.72	69.30 ±1.86	71.57 ±1.56
10	73.48 ±1.97	77.39± 1.45	80.17± 1.31	82.35 ±1.43	83.17 ±1.87
12	84.48 ±1.85	87.74± 1.36	91.35± 1.51	94.13 ±1.12	97.35 ±2.42

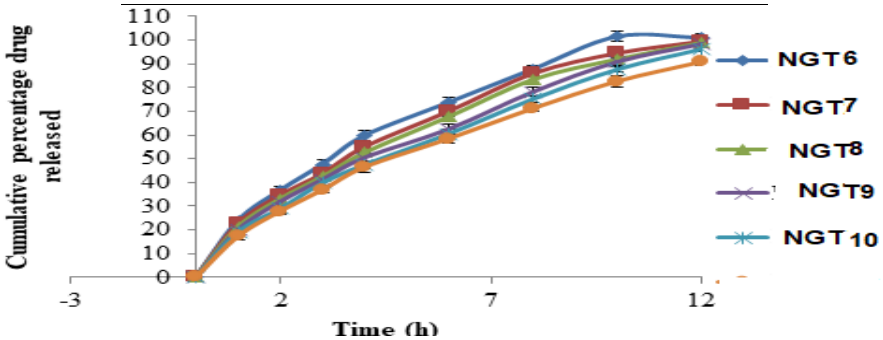

Figure 3: Cumulative percentage release of nateglinide from floating bilayer tablets prepared with combination of HPMC K4M and Sodium CMC (n=3, Mean ±SD)

Kinetics of drug release profiles

The drug release profiles of all the formulations of nateglinide floating were fitted to different kinetic equations. The R2 values for Zero-order model ranged from 0.921 (NGT1) to 0.983 (NGT12). Similarly, r^2 values for Higuchi model ranged from Higuchi model ranged from 0.949 (NGT9) to 0.973 (NGT10). All the formulations followed the Peppas model and r^2 values ranged from 0.962-0.998 due to high coefficient of determination (r^2). The optimized formulation NGT10 followed Peppas model (r^2=0.998) followed non-Fickian diffusion drug release mechanism (n=0.636). The value of release exponent for all the formulations ranged from 0.652 (NGT1) to 0.0.679 (NGT7) and that of optimized formulation was 0.635. All the formulations have n values between 0.5 and 1, indicating anomalous transport (non-Fickian). The release rate constants (k) of all the formulations were significantly different. The value of k for formulations NGT1-NGT6 prepared with combination of Polyox WSR303 and CP 971P was ranged from 4.32 (NGT6) to 7.71 (NGT1), and that of formulations NGT7-NGT10, prepared with combination of HPMC K4M and Sod.CMC was ranged from 5.89 (NGT10) to 6.60 (NGT 7). Higher k values meant higher quantities of drug released.

Determination of tablet swelling and erosion levels:

Table 11: Percentage swelling of nateglinide floating tablets in 0.1 N HCl (Mean ± SD, n=3).

Time (h)	NGT3		NGT10	
	Mean	SD	Mean	SD
1	48.79	2.38	43.12	1.65
2	58.48	2.53	53.59	2.88
4	76.11	3.25	71.94	1.99
6	110.49	2.98	102.19	2.83
8	91.38	3.21	87.69	2.27
10	59.81	2.25	55.70	0.83
12	44.57	2.84	37.87	0.95

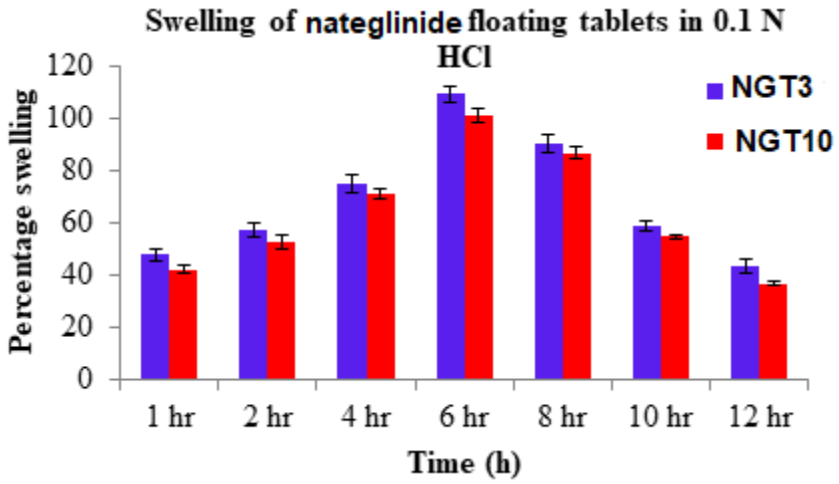

Figure 4: Extent of swelling of different formulations of nateglinide floating tablets in 0.1 N HCl (Mean ± SD, n=3).

The hydration ability of the formulation is important because it influences: (i) tablet buoyancy, (ii) adhesion ability of swellable polymers in contact with the dissolution medium and (iii) drug release kinetics. The swelling and erosion studies were performed on the optimized formulations NGT3, NGT10. The percentage swelling of optimized nateglinide floating tablets (NGT3, NGT10) were determined at different time intervals. The maximum swelling was observed at 6 h and was found to be 110.49 ± 2.98%, 102.19 ± 2.83 and, respectively. The erosion increased with an increase in time for both the formulations. At 12 h, the erosion was found to be 62.54 ± 2.60%, 66.06 ± 2.24% for NGT3, NGT10 The nominal swelling and erosion differences were due to the different polymers used.

Table 12: Percentage erosion of optimized nateglinide floating bilayer tablets in 0.1 N HCl

Time (h)	NGT3		NGT10	
	Mean	SD	Mean	SD
1	20.46	1.48	22.40	1.24
2	23.62	2.14	24.41	2.18
4	29.89	1.99	30.88	1.66
6	39.62	1.81	40.34	2.25
8	55.45	1.41	55.64	3.27
10	62.64	2.01	62.45	1.54
12	66.54	2.60	65.06	2.24

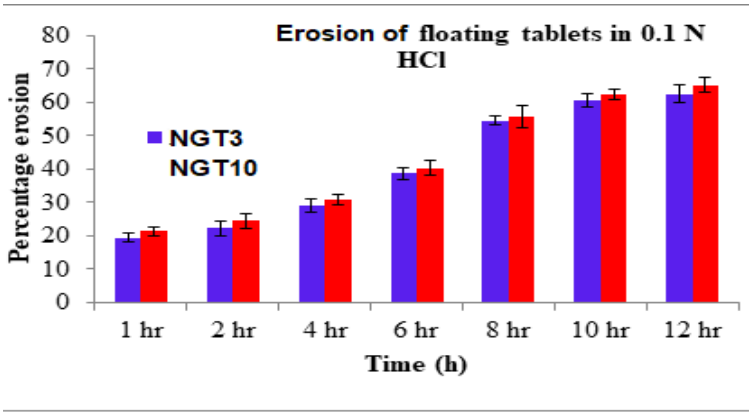

Figure 5: Extent of erosion of optimized nateglinide floating bilayer tablets in 0.1 N HCl

Physical stability studies

The optimized nateglinide floating bilayer tablets (NGT3) were selected for stability study based on physical characters and in vitro drug release. Before and after conducting the stability studies for 3 months, the results were analyzed statistically by using Student's paired t-test. No significant difference ($p > 0.05$) was observed in the tablet hardness, FLT, TFT, drug content or in vitro dissolution. The drug content was slightly decreased from 101.26 ± 1.59% to 100.85 ± 1.69% after storage at 40°C under 75% RH. But the difference was not statistically significant ($p > 0.05$). Thus, the NGT3 nateglinide sustained release floating bilayer tablets were found to be stable.

Table 13: Physical characters during storage - stability study of nateglinide floating-bio adhesive bilayer tablets (NGT3).

Characteristic parameter	0 day *	15th day *	30th day *	60th day *	90th day *
Hardness (kg/cm^2)	5.43±0.50	5.43±0.45	5.65±0.53	5.62±0.44	5.40±0.38
Floating lag time (s)	28.30±3.20	28.25±3.05	28.21±2.51	28.22±2.31	28.20±2.64
Duration of floating (h)	>12	>12	>12	>12	>12
Drug content (%)	102.26±1.59	101.31±1.63	101.32±1.74	101.26±1.75	101.85±1.69
Drug released at 12 h	99.91±1.84	99.69±1.44	99.45±1.32	99.31±1.31	99.26±1.43

*** Statistically not vital ($p > 0.05$).**

***In vivo* evaluation of gastric residence time**

Table 14: Location of nateglinide floating bilayer tablet in the GIT of volunteers

Time (h)	Position of tablet in the GIT of volunteer		
	A	B	C
0.5	Stomach	Stomach	Stomach
2.5	Stomach	Stomach	Stomach
4.5	Stomach	Stomach	Stomach
5.5	Stomach	Intestine	Stomach

The floating tablets (NGT3) prepared for radiological studies were characterized for hardness (5.40 ± 0.33 kg/cm^2), thickness (5.65 ± 0.11 mm), weight variation (501.45 ± 5.65 mg), friability (0.37%), FLT (68.65 ± 5.42 sec) and TFT aside greater than 12 h. The maximized lag time of $BaSO_4$-loaded nateglinide bilayer tablets, compared to the original formulation NGT3 (28.3 ± 3.1 s) was expected because of high density of $BaSO_4$ (4.5 g/cm^3). Figure shows the radiographic images taken at different periods after administration of $BaSO_4$-loaded nateglinide floating tablets in one volunteer (A). The first radiographic image was taken at 0.5 h post-administration of tablet and the tablet was observed in the stomach. The next pictures were taken at 2.5, 4.5 and 5.5 h; the tablet had altered its position, yet remained within the stomach till the end of 5.5 h. The mean gastric residence time was found to be 5.13 ± 0.64 h (n=3).

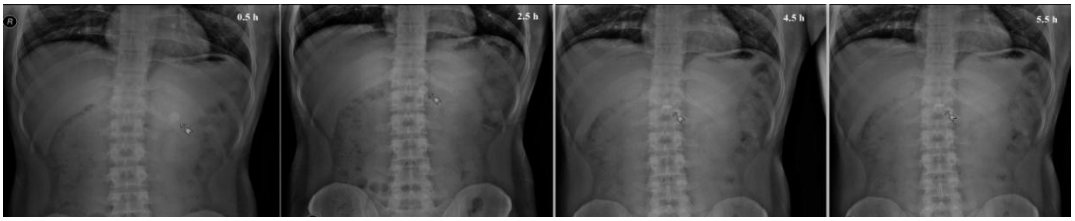

Figure 6: Radiographic images showing the presence of $BaSO_4$-loaded sustained release effervescent floating bilayer tablets of nateglinide in the stomach of volunteer-A at different time points (the location of the tablet is shown with an arrow).

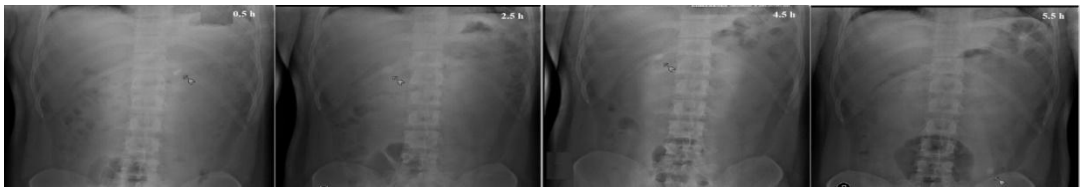

Figure 7: Radiographic images showing the presence of $BaSO_4$-loaded sustained release effervescent floating bilayer tablets of nateglinide in the stomach of volunteer-B at different time points (the location of the tablet is shown with an arrow).

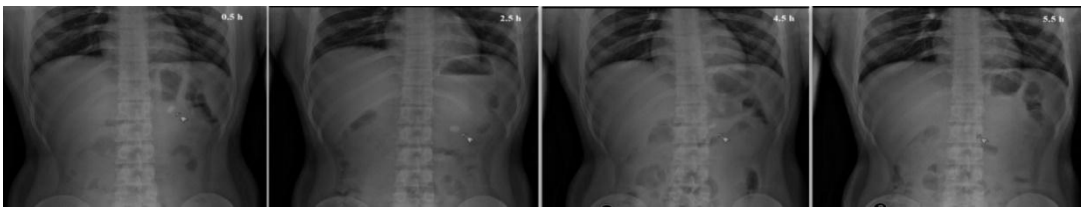

Figure 8: Radiographic images showing the presence of $BaSO_4$-loaded sustained release effervescent floating bilayer tablets of nateglinide in the stomach of volunteer-C at different time points (the location of the tablet is shown with an arrow).

Table 15: Pharmacokinetic parameters of nateglinide test (NGT 3) and reference (Natiz) formulation, n=8.

Pharmacokinetic parameter	Nateglinide reference formulation (NatizTab) Mean ± SD	Nateglinide test formulation (NGT 3) Mean ± SD
C_{max} (µg/ml)	0.295±0.047	0.252±0.011
t_{max} (h)	2.167±0.500	3.444±0.500
$t_{1/2}$ (h)	2.029±0.200	4.116±0.407
AUC_{0-24} (µg.h/ml)	9.461±0.760	16.110±1.580
$AUC_{0-\infty}$ (µg.h/ml)	9.564±0.772	16.289±1.667
MRT (h)	4.731±0.211	8.791±0.409

Aside from the student paired t-test, $p < 0.05$ is heeded statically vital in all the criterion.

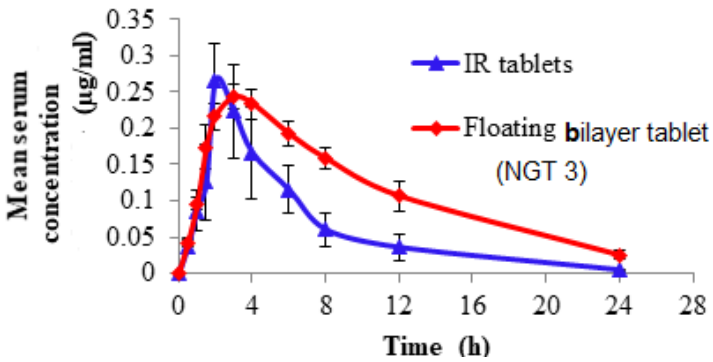

Figure 9: Mean serum concentration (µg/ml) of nateglinide test (NGT 3) and reference (NatizTab) formulation in healthy human volunteers (m=9, Mean±SD)

The bioavailability study was successfully conducted according to the study protocol. The drug was well tolerated with no other symptoms or disturbances during the two study periods. The serum samples were analyzed by RP-HPLC method. The HPLC chromatogram of blank serum is shown in figure and that of sample is shown in figure9. The retention time of drug nateglinide is 4.81 min and the internal standard furosemide is 7.42 min. These retention times were nearly closer to the reported values (Manglani et al., 2006). The pharmacokinetic parameters used to assess the bioavailability of test versus reference were $AUC_{0-\infty}$ for the extent of absorption and C_{max}, t_{max} for the rate of absorption. The mean serum concentration-time curves for test (NGT3) and reference (Natiz Tab 120 mg) immediate release formulations are show in figure 5.41. The C_{max} value for Natiz formulation (reference) apart met to be 0.295±0.047 µg/ml and that of test formulation (NGT3) aside met to be 0.252±0.011 µg/ml. The t_{max} values for both reference and test formulation were found to be 2.167±0.500 h and 3.444±0.500 h certainly. Half-life value for reference was found to be 2.029±0.200, and that of test is 4.116±0.407 h. The AUC_{0-24} values for reference and test were 9.461±0.760 µg×h/ml and 16.110±1.580 µg×h/ml, certainly. $AUC_{0-\infty}$ value reference formulation was 9.564±0.772 µg×h/ml and that of test formulation was 16.289±1.667 µg×h/ml. Similarly, mean residence time (MRT) value reference formulations were 4.731±0.211 and that of test formulation was 8.791±0.409 h. In the present study student's paired t- test was used to compare pharmacokinetic data of reference and test formulation. The data showed that there was significant difference ($P < 0.05$) between two formulations in their tested pharmacokinetic parameters, AUC_{0-24}, $AUC_{0-\infty}$, C_{max}, t_{max} and MRT. The increase in relative bioavailability of test formulation was found to be 1.7 times when compared to reference formulation.

CONCLUSION:

Initially, drug-excipients interaction study was determined using DSC and found that the drug was compatible with all the excipients used in the formulation. Floating tablets of nateglinide were prepared with combination of Polyox WSR 303 and CP 971P/ HPMC K4M and Sodium CMC. The optimized formulation (NGT10) floated with a lag time of 28.3±3.2 sec, duration of floating 12 h and released about 99.91±1.84% of drug in 12 h, and then followed non-Fickian diffusion release mechanism with n value of 0. 635. The NGT02 tablets with $BaSO_4$ remained in stomach for 5.13 ± 0.64 h (n=3) in radiological studies. The bioavailability studies were carried out for the optimized formulation (NGT10) and compared with that of reference IR tablets, Natiz in nine healthy human volunteers. Based on in vivo performance significant difference was observed between C_{max}, t_{max}, $t_{1/2}$, $AUC_{0-\infty}$, and MRT of F10 and IR. The increase in the relative bioavailability of NGT was 1.7-fold when compared to reference, IR tablets. The increased relative oral bioavailability may be due to the floating mechanism of dosage form, which is desirable for drugs absorbed from the upper part of gastrointestinal tract.

ACKNOWLEDGEMENT

The authors acknowledge M/s splendid pharma Ltd., Pune, India for providing drug, M/s Orchid Pharma Ltd., Chennai, India for providing HPMC K4M and Polyox WSR303 polymer and M/s Aurobindo Pharma. Ltd., Hyderabad, India for providing Carbopol 971P and Sod. CMC as gift sample.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

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