

MULTIPARTICULATE FLOATING DRUG DELIVERY SYSTEM OF ANAGLIPTIN: DESIGN AND OPTIMIZATION FOR ITS EFFICACY IN MANAGEMENT OF METABOLIC SYNDROME

RAKESH V. MISHRA^{1*}, SHASHIKANT N. DHOLE²

¹Department of Pharmaceutics, Dr. D. Y. Patil Institute of Pharmaceutical Sciences and Research, Pimpri, Pune 411018, Maharashtra, India, ²Department of Pharmaceutics, Modern College of Pharmacy, Moshi, Pune 412105, Maharashtra, India
Email: mishrarakesh287@gmail.com

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ABSTRACT

Objective: The present research aims to design and optimize gastroretentive floating pellets of anagliptin (a dipeptidyl peptidase-4 inhibitor), so as to reduce P-Glycoprotein (PGP)-mediated efflux in the intestine hence to improve oral bioavailability.

Methods: The drug-containing core pellets were prepared by extrusion and spheronization process followed by subsequent coating with three successive layers i.e. Eudragit RS 100, sodium bicarbonate (NaHCO₃): hydroxypropyl methylcellulose E5LV (HPMC E5LV) and Eudragit RL 100 using fluidized bed processor. A 3 level 3 factor box-behnken design was adopted to investigate the effect of Eudragit RS 100, NaHCO₃: HPMC E5LV and Eudragit RL 100 on floating lag time and drug release at 10 h. Desirability function under numerical optimization technique was used to identify the optimum formulation.

Results: The study reveals the significant effect of the amount of NaHCO₃ and coating level of polymers on floating lag time and drug release. The optimum system could float within 4 min and exhibited more than 85% drug release in 10 h. The pharmacokinetic study conducted in male Wistar rats indicated 2.51 fold increase in relative bioavailability of optimized formulation compare to anagliptin drug. Formulated anagliptin pellets were evaluated in cafeteria diet-induced metabolic syndrome model in male Wistar rats. Anagliptin floating pellets treatment compared to cafeteria diet group significantly inhibited increase in body weight (238.79±2.52 g vs. 277.98±3.69 g, P<0.001), calorie intake (2283.99 kcal vs. 3086.05 kcal, P<0.05) and serum levels of total cholesterol (95.19±0.61 mg/dl vs. 110.04±1.31 mg/dl, P<0.01), triglycerides (96.12±1.25 mg/dl vs. 105.99±1.29 mg/dl, P<0.01) while high-density lipoproteins levels were improved (42.15±0.92 mg/dl vs. 30.92±0.77 mg/dl, P<0.01) indicated its hypophagic and anti-hyperlipidemic effects.

Conclusion: The gastroretentive floating pellets of anagliptin was obtained and could be a promising technique to deliver anagliptin with improved bioavailability in the management of the metabolic syndrome.

Keywords: Anagliptin, Metabolic syndrome, Floating drug delivery system, Pellets and Spheronization

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INTRODUCTION

The urbanization and sedentary lifestyles of 21st century leads to increase the prevalence of metabolic syndrome and is becoming a major public health concern. Metabolic syndrome includes the number of chronic disease such as insulin resistance, obesity, dyslipidemia, cardio-metabolic risk and hypertension [1]. Patient compliance becomes an issue in the management of metabolic syndrome, due to the use of multiple drugs and risk of enhanced drug-drug interactions [2]. Identifying drug candidates exhibiting polypharmacology could be one of the strategies favourable to deal with multifactorial diseases [3]. Anagliptin a dipeptidyl peptidase 4 (DPP4) inhibitor, besides their glucose-lowering activity, is a promising drug candidate for management of multifactorial diseases constituting metabolic disorders [4]. However, systemic bioavailability of anagliptin is limited by PGP mediated efflux in the intestine [5]. PGP, a plasma membrane-bound ATP-dependent efflux transporter, is a well-recognized factor that can influence drug pharmacokinetics [6]. In addition, anagliptin has a shorter biological half-life of 3-4 h. It is needed to improve the oral bioavailability of anagliptin to be effectively used in many clinical applications. The conventional controlled-release technologies are not suitable for the delivery of PGP substrates because they carry a significant part of the drug to distal regions of the gastrointestinal tract (GIT). On the other hand, continuous delivery to the proximal part of the GIT, as provided by gastroretentive dosage forms, might be useful for these drugs [7-9]. Various approaches have been reported to retain the formulation in the upper part of GIT such as swelling systems, high-density systems, magnetic systems, mucoadhesive systems and floating systems [10]. Among all the gastroretentive systems, due to minimum effect on GIT motility, floating drug delivery systems (FDDS) are considered suitable and preferable [11-13]. These systems are particularly useful for drugs having absorption in upper GIT, drugs which are unstable in the intestine and

exhibits poor solubility in intestinal pH [14]. FDDS are low density system which allows them to remain buoyant over gastric content for prolonged period of time [15]. Based upon the mechanism of buoyancy effervescent systems are the widely employed technique used in the development of FDDS. In the effervescent systems, carbon dioxide gas liberation occurs upon contact with gastric fluid due to neutralization reaction which lowers the density and allows the system to remain buoyant [16]. A wide range of single unit and multiparticulate FDDS were designed and developed, the multiparticulate FDDS were preferred over single unit system due to reduce inter and intra subject variabilities in drug absorption and lower possibility of dose dumping [17-19]. Designing sustained release multiparticulate drug delivery system of anagliptin with prolonged residence time in the stomach using FDDS approach can significantly improve the overall bioavailability.

In the present investigation, a floating multiparticulate drug delivery system of anagliptin based on effervescent technique was developed. The drug-containing core pellets were prepared using extrusion and spheronization process. The drug-loaded pellets were coated with three successive layers, internal coat of Eudragit RS 100 as release retardant followed by effervescent layer coat (NaHCO₃: HPMC E5LV); and top coat of Eudragit RL 100 as a gas entrapped polymeric membrane. The effect of the amount of effervescent agent and coating level of polymeric membrane on floating ability and drug release properties were studied and optimized using response surface methodology.

MATERIALS AND METHODS

Materials

Anagliptin was obtained as gift sample from Wockhardt Limited, Aurangabad, India. Eudragit RL 100 and Eudragit RS 100 were provided by Evonik Pharma, Mumbai, India. Sodium bicarbonate, Hydroxypropyl

methylcellulose E5LV, Microcrystalline cellulose, polyvinylpyrrolidone K30 obtained from Himedia Laboratories, Mumbai, India. All the other reagents used in the study were of analytical grade.

Preparation of gastroretentive floating pellets

Preparation of drug loaded pellets

The anagliptin containing core pellets were prepared using wet granulation method by extrusion and spheronization technique. Powder mixture of anagliptin (40% w/w) and spheronizing aid (microcrystalline cellulose 60% w/w) was mixed uniformly in mortar for 20 min. This was followed by the addition of binding liquid (0.5% w/v polyvinylpyrrolidone K30 in distilled water) into the dry powder mixture. Binding liquid was incorporated into the pelletization process to get damp mass and to obtain sufficient hardened pellets suitable for the subsequent coating process. The prepared damp mass was passed through BSS sieve no.16 to get the extrudates. Extrudates were then transferred to spheronizer (Shakti Pharmatech, Ahmedabad, India) and spheronized at 900rpm for about 10 min to obtain the pellets. The drug-loaded core pellets were oven dried overnight at 40°C.

Coating of drug loaded pellets

The drug-loaded pellets were coated with three successive layers, release retardant Eudragit RS 100 as internal coat followed by effervescent layer (NaHCO₃: HPMC E5LV) and gas entrapped Eudragit RL 100 as top coat. The Eudragit RS 100 coating solution was prepared by dissolving it into isopropyl alcohol (10 % w/w solid content) plasticized with 10 % triethyl citrate (w/w based on polymer solid) and stirred to obtain a clear solution. The coating process was conducted in fluidized bed processor (ACG, Miniquest-F, Mumbai, India), to obtain predetermined weight gain of 5%, 10%

and 15% w/w. The coating conditions were batch size, 10 g; inlet temperature, 40°C; product temperature, 35°C; air flow, 0.8-1.0 bar; spray pressure, 0.5-0.9 bar; spray rate, 4-5 ml/min and final drying at 40°C for 15 min. The Eudragit RS 100 coated pellets were further coated with effervescent layer coating. The required amount of NaHCO₃ was dispersed in HPMC E5 LV solution 10 % w/w solid content in the ratio of 2:1, 1:1 and 1:2 (w/w), plasticized with 10 % Polyethylene glycol 6000 (w/w based on polymer solid). The effervescent layer coating was done to obtain a fixed weight gain of 10% w/w. The coating conditions were temperature, 40±5°C; air flow, 0.8-1.0 bar; spray pressure, 0.5-0.9 bar; spray rate, 1-1.5 ml/min. The obtained effervescent layered coated pellets were dried at 40°C for 30 min. Finally, Eudragit RL 100 coating solution prepared in Isopropyl alcohol (10 % w/w solid content) plasticized with 10 % triethyl citrate (w/w based on polymer solid) coated over effervescent layered pellets. During coating the predetermined weight gain obtained was 5%, 10% and 15% w/w. The coating conditions were the same as kept during coating of Eudragit RS 100 mentioned above. The formulation batches prepared are indicated in table 2.

Experimental design

A 3-level, 3-factor, 13 run experiment box-behnken design was adopted for optimization of gastroretentive floating pellets formulations. The selected independent variables were the concentration of Eudragit RS 100 (X1), the ratio of NaHCO₃: HPMC (X2) and Eudragit RL 100 concentration (X3). The levels of independent variables are shown in (table 1). The dependent variables taken as a response were floating lag time (Y1) and percent drug release in 10 h (Y2). The experimental formulations composition indicated in (table 2). Response surface modelling, ANOVA and model graphs studies were carried out employing Design-expert® software (Version10, Stat-Ease).

Table 1: Levels of independent variables in experimental design

Factors	Coded value			Actual value		
	Low	Medium	High	Low	Medium	High
Eudragit RS 100 (%)–X1	-1	0	+1	05	10	15
NaHCO ₃ : HPMC (%)–X2	-1	0	+1	1:2	1:1	2:1
Eudragit RL 100 (%)–X3	-1	0	+1	05	10	15

Table 2: Experimental formulation composition

Batch No.	Eudragit RS 100 (%)	NaHCO ₃ : HPMC (%)	Eudragit RL 100 (%)
F 1	10	2:1	15
F 2	10	1:1	10
F 3	05	1:1	15
F 4	05	1:1	05
F 5	10	1:2	05
F 6	05	2:1	10
F 7	05	1:2	10
F 8	15	1:2	10
F 9	15	1:1	15
F 10	10	2:1	05
F 11	15	2:1	10
F 12	10	1:2	15
F 13	15	1:1	05
F 14	05	2:1	05

The desirability approach was used in Design-expert® software to find the optimum level of independent variables, to gainsay the reliability suggested new formulation was prepared (batch F 14).

Evaluation of gastro retentive floating pellets

Physical characterization

The micromeritic properties (Carr's index, Hausner's ratio and angle of repose) were determined to evaluate the flow properties of floating pellets. Friability of the pellet formulations was determined using friabilator (Veggo Friability Tester, India). 10 g of core pellets

were kept into friabilator and the percentage weight loss after 200 revolutions was determined to calculate friability [20-23].

Pellet sphericity

Pellets sphericity was determined in terms of aspect ratio [24]. Feret diameter and perpendicular diameter of the pellet were measured using digital vernier calliper (Mitutoyo, Japan). The aspect ratio of pellets was calculated with the following equation:

$$\text{Aspect Ratio} = \frac{\text{Feret diameter}}{\text{Perpendicular diameter}}$$

Determination of the drug content

The drug content in the floating pellets formulation was determined by extraction with 0.1 N HCl. Pellets equivalent to about 200 mg of anagliptin were accurately weighed, ground and transferred into a volumetric flask containing 0.1 N HCl. The mixture was sonicated for 30 min to ensure complete extraction of the drug. The solution was further filtered, diluted with an appropriate amount of 0.1 N HCl and assayed spectrophotometrically at 248.5 nm (Shimadzu, 1700, Japan). The analysis was performed in the range of 0-10 µg/ml where Beer's law was obeyed for anagliptin.

Scanning electron microscopy (SEM)

The surface morphology of the uncoated pellets, Eudragit RS 100 coated, effervescent layer coated and Eudragit RL 100 coated pellets were examined under the scanning electron microscope (Jeol, Oxford Instrument, United Kingdom). The pellet samples were sputter coated with gold/Palladium mixture for 1 min under an argon atmosphere and placed into a scanning electron microscope chamber. The photomicrograph of the pellets was obtained at 20 kV accelerating voltage.

Floating studies

The floating studies were conducted using USP Type-II dissolution apparatus (Veego DA-8D, India) in 900 ml of 0.1 N HCl maintained at 37°C±0.5° C. The medium was agitated by paddle at 50 rpm into which coated pellets (100) were placed [25-27]. The time taken by the pellets to rise over the surface of the medium (floating lag time) was measured visually (n=3). The floating pellets percentage at 10 h was also determined by the equation indicated below:

$$\text{Floating pellets (\%)} = \frac{\text{No. of floating pellets at measure time}}{\text{Initial no. of pellets}} \times 100$$

In vitro drug release study

In vitro drug release study of anagliptin (uncoated and coated pellet formulations) was determined in triplicates using USP Type-II dissolution apparatus (Veego DA-8D, India). The dissolution medium used was 900 ml of 0.1 N HCl. Pellets equivalent to 200 mg of anagliptin were accurately weighed and transferred into dissolution medium kept at 37°C±0.5° C on 50 rpm. At predetermined time intervals, 5 ml of aliquots were withdrawn and immediately replaced by the same volume of fresh medium to maintain the sink condition. The aliquots were filtered and analyzed by UV spectrophotometer at 248.5 nm to calculate the drug release.

To understand the mechanism of drug release kinetics from optimized floating pellets formulation, the obtained drug release data were analyzed according to the mathematical models like zero-order model, first-order model, Higuchi model and Korsmeyer-Peppas model [28-32].

Statistical data analysis and optimization

The obtained data of responses (floating lag time and percentage drug release at 10 h) for all formulations were analyzed by Design-expert® software. Polynomial models for all the response variables including linear, interaction and quadratic terms were generated. The suggested model based upon the values of standard deviation,

multiple correlation coefficient (R²) and adjusted multiple correlation coefficient (adjusted R²) was selected [33]. The selected model was further analyzed by analysis of variance (ANOVA) to determine significant model terms. The P value, lack of fit value, regression equation of the responses, diagnostic plot (Actual vs Predicted) and response surface plots were also generated using the software. The generated response surface plots were studied to understand the relationship between the dependent and independent variables. Finally, a numerical optimization technique based upon desirability approach was used to determine the optimum level of independent variables for the formulation.

Stability studies

The accelerated stability study of anagliptin floating pellet formulation was performed as per ICH guidelines. The pellet sample was stored in 10 ml open glass vials at 40° C/75% relative humidity for three months. Pellet sample was observed for drug content, drug release at 10 h and any change in physical appearance after first, second and third month [34].

In vivo pharmacokinetics-study design

In vivo pharmacokinetics studies were conducted in male Wistar rats after obtaining approval from the Institutional Animal Ethical Committee (DYPIPSR/IAEC/MAY/17-18/P-16). The study was aimed to compare the formulated anagliptin floating pellets with anagliptin plain drug. Twelve wistar rats weighing 180–200 g were separated randomly into two groups and fasted overnight with free access to water. Anagliptin floating pellets (200 mg/kg) and anagliptin plain drug (200 mg/kg) were administered orally into respective groups. At predetermined time intervals (0.25, 0.5, 1, 2, 4, 8, 12, and 24 h) post oral dose, 0.5 ml of blood samples were collected by the retro-orbital method. Plasma was immediately separated by centrifuging at 4000 rpm for 15 min and stored at -20 °C until analysis.

HPLC analysis of anagliptin in plasma

Plasma concentrations of anagliptin were determined by HPLC method [5]. To 0.1 ml of plasma sample, 0.9 ml of acetonitrile was added and centrifuged for 15 min at 10000 rpm. The supernatant liquid obtained after centrifugation was filtered through 0.2 µ filter followed by injection of 20 µl into the system. The chromatography was carried out on a reversed-phase HPLC column (Kromasil, 4.6 x 250 mm, 5 µm, Shretech Associates; Mumbai, India) using Shimadzu HPLC LC20 AD (Shimadzu Corporation, Japan). The mobile phase consisted of acetonitrile and ammonium acetate buffer pH 4.5 (50:50). The eluate was analyzed at a wavelength of 248 nm with a flow rate of 0.8 ml/min.

Pharmacokinetic analysis

Required pharmacokinetic parameters were calculated using PK solver software (version 2.0). Peak plasma concentration (C_{max}), time to reach peak plasma levels (T_{max}), area under the curve (AUC), mean residence time (MRT), half-life (t_{1/2}), elimination constant (K_{el}) and relative bioavailability were estimated. The calculated pharmacokinetic parameters of both anagliptin floating pellets and plain drug were statistically analyzed using ANOVA and the difference less than the probability level 0.05 was considered statistically significant.

Table 3: Calorie value of the cafeteria diet

Ingredients	Calorie Value (Kcal/100g)
Condensed Milk	69
Bread	247
Chocolate	230
Biscuits	453
Dried Coconut	185
Cheese	402
Boiled Potato	75

Pharmacodynamic study in cafeteria diet-induced metabolic syndrome model

The male Wistar rats (180–200 g) were procured from National Institute of Bio-science, Pune, Maharashtra, India. The animals were

housed under controlled room temperature and humidity condition with 12 h light and 12 h dark cycle. The animals were provided free access to food and water. After one week of acclimatisation period, animals were used for the study. The experimental protocol was approved by the Institutional Animal Ethical Committee

(DYPIPSR/IAEC/MAY/17-18/P-17). In the present study cafeteria diet (CD) induced metabolic syndrome model in rats is selected as they more resemble to human ultra-processed diet compared to commercial high-fat or high-sugar rodent diets [35, 36]. The CD composed of condensed milk (8g), bread (8 g), chocolate (3 g), biscuits (6 g), dried coconut (6 g), cheese (8 g) and potato chips (10 g). These diets were provided to the individual rats of group II to IV during the 42 d of the experiment. The calorie value of the CD indicated in (table 3).

Experiment protocol

The study includes 24 rats divided into four groups of 06 rats each. The drugs were administered through per oral (p. o.) route using oral gavage once a day for a period of 42 d.

Group I: Normal control fed with rodent chow diet and 0.5% CMC.

Group II: Fed with Cafeteria Diet.

Group III: Cafeteria diet+Orlistat (45 mg/kg, p. o.) in 0.5% CMC from 8th day.

Group IV: Cafeteria diet+Anagliptin floating pellet formulation (200 mg/kg, p. o.) from 8th day.

The food intake of each animal group was measured by calculating the difference in weight of preweighed chows and the food remain over after 24 h. The daily caloric (kcal) intake was determined by multiplying the food intake by their caloric content and expressed as mean energy intake for a group of six rats in kcal/week. The body weights of the rats from each group were taken separately on 0th, 7th and 42nd day. Body mass index (BMI) was calculated from formula: $BMI = \text{body weight (g)} / \text{length}^2 \text{ (cm}^2\text{)}$. On 43rd day animals were anesthetized with ether to collect the blood by the retro-orbital method. Serum was immediately separated by centrifuging at 4000 rpm for 15 min. The serum levels of glucose, total-cholesterol (CH), high-density protein (HDL), low-density protein (LDL), triglycerides (TGs), Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) were estimated using the biochemical kits (Pathozyme Diagnostics). After collection of blood, rats were sacrificed; organs viz; liver, heart and white adipose tissue (WAT) were excised immediately, rinsed with phosphate buffer saline and weighed.

Statistical analysis

Data were expressed as the mean \pm standard error of the mean (SEM). The results were analyzed by one way or two way ANOVA (analysis of variance) followed by Bonferroni test using Graph pad prism-7 software. Values of $P < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Design of gastroretentive floating pellets

The floating system consisted of drug loaded pellets coated with three successive layers, release retardant Eudragit RS 100 as internal coat over which NaHCO_3 : HPMC E5LV layered as effervescent coat and gas entrapped Eudragit RL 100 as top coat indicated in (fig. 1). The prepared floating pellets were based on gas formation technique which upon contact with gastric fluid liberates carbon dioxide (CO_2) due to the neutralization of NaHCO_3 . The liberated CO_2 gets entrapped into the outer polymeric membrane and generates sufficient buoyancy to make the pellets float. The outer polymeric membrane should be highly water permeable so as to initiate the effervescent reaction and the floating process more rapidly. To maintain the flotation the hydrated coat should also be flexible to avoid rupturing and impermeable to the generated CO_2 [37]. Due to these reasons the higher flexible, water permeable and gas impermeable Eudragit RL 100 was chosen as a gas entrapped outer polymeric membrane. During effervescent layer coating as NaHCO_3 itself is not sticky, HPMC E5 LV was used as binder to allow adherence of NaHCO_3 on to the pellets. The solubility of anagliptin is higher in acidic media, it was difficult to sustain the release with

Eudragit RL 100 and a release retarding polymer layer was required. Eudragit RS 100 was investigated as release retardant internal polymeric coat due to its lower hydrophilicity which makes it less water permeable. To develop an optimal formulation i.e. having minimum floating lag time and maximum drug release in 10 h, the parameters such as the concentration of Eudragit RS 100, the ratio of NaHCO_3 : HPMC and Eudragit RL 100 concentration were varied and evaluated in the study.

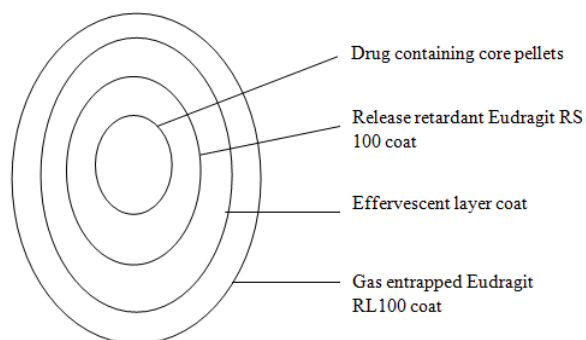


Fig. 1: Design of gastroretentive floating pellets

Evaluation of gastroretentive floating pellets

Physical characterization

To evaluate the flow properties of floating pellet formulations, Carr's index, Hausner's ratio and angle of repose were determined. The Carr's index and Hausner's ratio ranged from 5.22 ± 0.31 % to 14.16 ± 0.15 % and 1.05 ± 0.1 to 1.12 ± 0.21 respectively. The values obtained for the angle of repose were in the range of $23.5^\circ \pm 0.27^\circ$ to $26.12^\circ \pm 0.32^\circ$. The obtained data of micromeritic properties indicated good flow behavior of pellet formulation. The friability of the pellets formulation ranged from 0.24 ± 0.11 % to 0.51 ± 0.08 %. The results of friability study showed that the prepared core pellets were quite hard and suitable for the subsequent coating process.

Pellet sphericity

Sphericity of the pellets is an essential property to obtain effective coating. The spherical shape pellets provides uniform surface during the coating process. Sphericity of the pellets was determined in terms of aspect ratio. The aspect ratio values equal to unity indicate the spherical shape of pellets [24]. The prepared core pellets showed aspect ratio values in the range of 0.98 ± 0.01 to 1.09 ± 0.05 .

Drug content

The drug content in all the floating pellet formulation was determined by UV spectroscopy method. It was found to be between 96.23 ± 0.5 % to 98.75 ± 0.41 % which indicated that the multilayering coating on the pellets was uniform and it does not affect the drug content.

Scanning electron microscopy (SEM)

Surface morphology of the uncoated and coated pellets are shown in (fig. 2). Fig. 2. (A) shows the surface of drug-containing uncoated pellets. The uncoated pellets exhibit rough external appearance. Fig. 2. (B) Eudragit RS 100 coated pellets shows the spherical shape and smooth surface. The effervescent layer coating over Eudragit RS 100 coated pellets produce pellets with a slight smooth surface as indicated in fig. 2. (C). This may occur due to the layering of NaHCO_3 dispersion (prepared in HPMC E5 LV solution). The top coat of Eudragit RL 100 as gas entrapped polymer coating (complete floating pellets) produces pellets with the smoothest surface shown in fig. 2. (D).

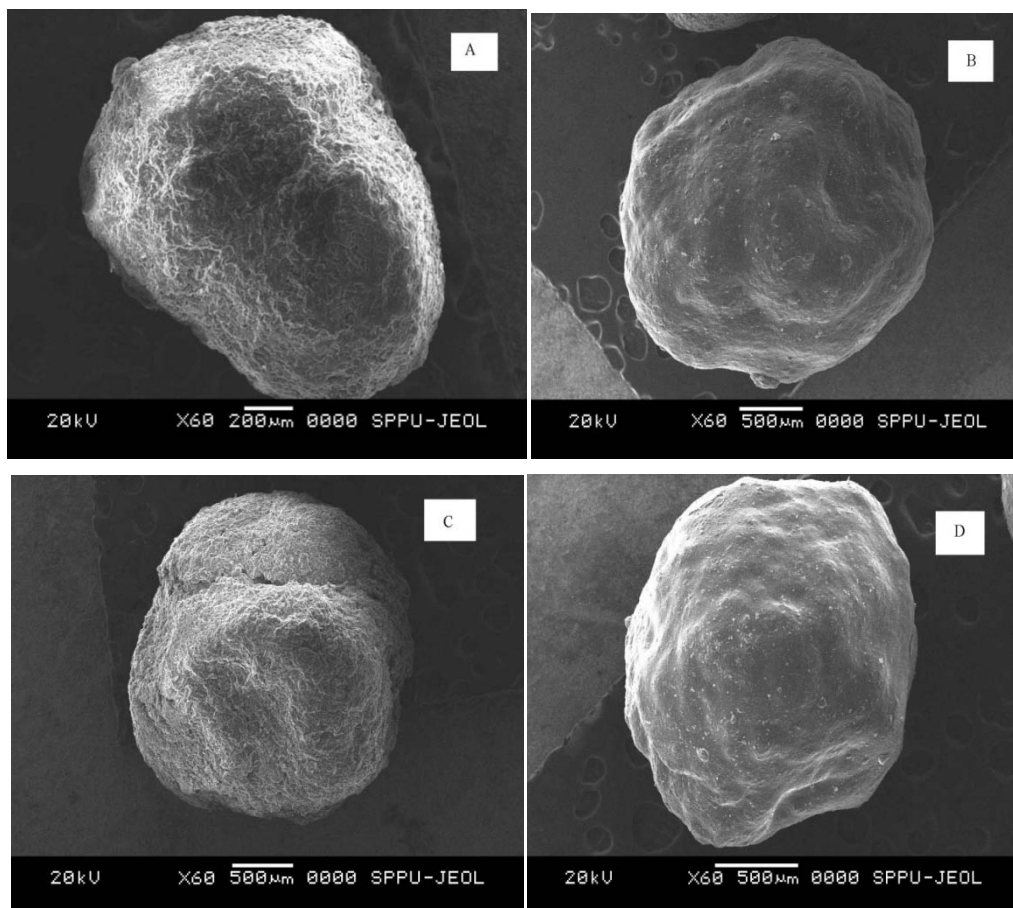


Fig. 2: Scanning electron microphotographs of (A) Drug loaded uncoated pellets, (B) Pellets coated with release retardant (5% Eudragit RS 100), (C) Effervescent layered (NaHCO_3 : HPMC; 2:1 w/w) coat over Eudragit RS 100 coat, (D) Gas entrapped complete floating pellets (5% Eudragit RL 100)

Floating studies

The prepared anagliptin pellets was studied for gastro-floating behaviour in 0.1 N HCl. Floating lag time and the percentage of pellets floating at 10 h were determined. An ideal floating system after contact with gastric fluid should float in few seconds to avoid the formulation from transiting into small intestine [18]. The data obtained with floating studies revealed that the floating behaviour is significantly affected by the amount of effervescent agent (NaHCO_3 : HPMC ratio) and level of gas entrapped polymeric coating (Eudragit RL 100). In the present study the amount of effervescent layer coating was kept constant (10% w/w) and ratio of NaHCO_3 : HPMC was varied. The floating lag time observed for all pellet formulation was in the range of 3 to 35 min shown in (table 4). As the amount of NaHCO_3 in effervescent layer coating increases and the level of

Eudragit RL 100 coat decreases, the floating lag time gets decreased. This behaviour could be explained as, with lower level of Eudragit RL 100 coat the acidic media can quickly penetrates into the system and initiate effervescent reaction more rapidly. On the other side the lower level of Eudragit RL 100 with lower level of NaHCO_3 unable to produce pellets with minimum floating lag time. This could be attributed due to the reduced amount of NaHCO_3 with lower level of Eudragit RL 100 coat, the medium penetration was rapid but unable to generate sufficient CO_2 to provide buoyancy. The percentage of pellets floating at 10 h ranged from 70% to 92% for all pellet formulation as shown in (fig. 3). The pellet formulations prepared with lower level of Eudragit RL 100 and higher level of effervescent agent also shows about 75% floating at 10 h. This indicated that even the lower amount of Eudragit RL 100 used in the study was sufficient to entrap the generated CO_2 and can maintain the floating for upto 10h.

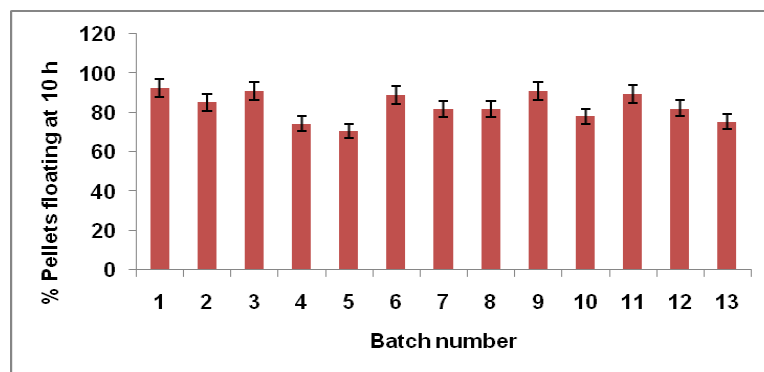


Fig. 3: Percentage pellets floating at 10 h of batches F 1 to F 13 mean \pm SD; n = 3

In vitro drug release study

The *in vitro* release profile of anagliptin uncoated and coated floating pellet formulations were studied indicated in (fig.4). The uncoated anagliptin pellets showed about 80% drug release in 1 h. In the preliminary study the release profile of floating pellets (without Eudragit RS 100 release retardant coat) was also studied. Even the higher level of Eudragit RL 100 coat (15%) system also gives more than 75 % drug release in less than 2 h (data not shown), indicated Eudragit RL 100 alone unable to sustain the drug release. Afterward,

it was decided to investigate Eudragit RS 100 as release retardant internal polymer layer to obtain sustain drug release for 10 h. As the amount of Eudragit RS 100 was increased the drug release was decreased. The result indicated that with increasing polymer layer thickness, medium penetration reduces leading to decreased drug release. However, no significant difference in drug release was observed by varying the amount of effervescent agent. The *in vitro* release studies reveals that with lower level of Eudragit RS 100 (5 %) as release retardant, maximum drug release (about 85%) can be obtained in 10 h.

Table 4: Response data of batches F1-F14

Batch No.	Floating lag time (min) Y_1	Average drug release in 10 h (%) Y_2
F1	9.8±1.7	64.79±0.21
F2	17.9±2.1	68.58±0.15
F3	21.2±2.4	75.86±0.29
F4	13.4±1.3	84.88±0.34
F5	25.6±2.5	73.78±0.41
F6	6.4±1.9	79.85±0.23
F7	29.2±1.5	80.19±0.26
F8	29.4±3.2	56.24±0.33
F9	20.9±2.8	52.23±0.37
F10	3.6±1.2	73.29±0.22
F11	6.2±1.4	55.79±0.23
F12	33.6±2.5	65.57±0.24
F13	13.2±1.6	59.71±0.43
F14	3.4±1.5	86.54±0.35

mean±SD; n = 3

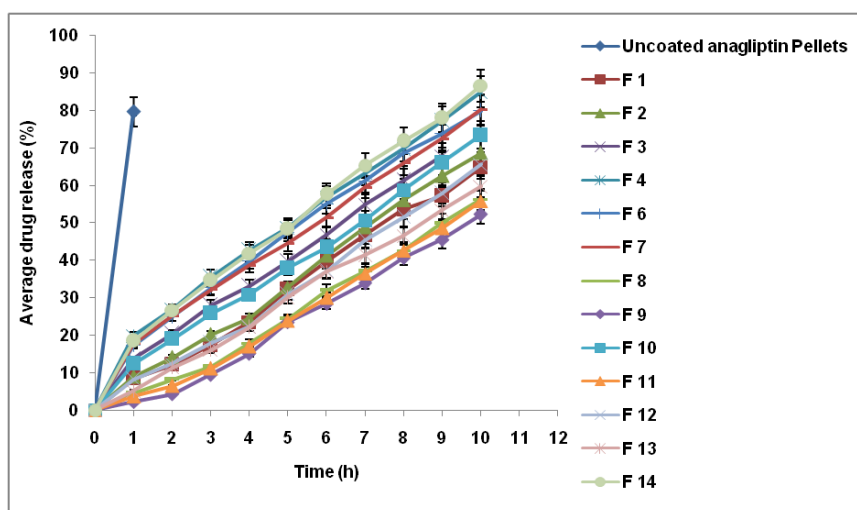


Fig. 4: *In vitro* dissolution study of uncoated and floating pellet formulations (batch F 1-F 14) mean±SD; n = 3

Drug release data of floating pellets formulation (batch F 14) was analyzed according to various mathematical models to understand the drug release kinetics. The regression coefficients (R^2) obtained from various models shown in (table 5). The R^2 value was used as an indicator of the best fitted model suggested that the drug release

follows either zero-order or Higuchi's model. Both dissolution and diffusion from the surface of pellets were responsible for initial slight fast release. As the drug depleted in the outer zone, the drug particles in the inner area diffused with a slower rate through the coated polymer layer.

Table 5: Regression coefficients of various mathematical models

Batch No.	Zero-order	First order	Higuchi release	Korsmeyer-peppers
F 14	0.9977	0.9811	0.9906	0.9899

Statistical data analysis and optimization

The responses obtained (floating lag time and drug release in 10 h) for 13 batches were fitted into the various model using design expert software. A quadratic model is suggested for both the responses

accordingly ANOVA for response surface quadratic model was generated indicated in (table 6). For the model to fit well with the experimental design the model p-value should be significant and the lack of fit should be insignificant [33]. The model F value and Prob>F values (less than 0.05) obtained for both the responses indicated that

the model terms are significant. Lack of fit for both the response was found to be not-significant indicated that the selected model can be suitably studied further for response surface plots. The ANOVA results suggested that for floating lag time (coded units B, C, A², B², C² and BC) and drug release (coded units A, C, A² and C²) are significant model terms (A: Eudragit RS 100; B: NaHCO₃: HPMC and C: Eudragit RL 100).

The values of SD, CV, R², adjusted R² and predicted R² are shown in (table 7). For both the responses the predicted R² value is in reasonable agreement with adjusted R² value. Adequate precision measures signal to noise ratio (>4 desirable). In both responses the adequate precision was greater than 4 indicating an adequate signal and the model can be used to navigate the design space.

Table 6: Summary of ANOVA results for response surface quadratic model

Parameter	Sum of squares	Degrees of freedom	Mean square	F value	P value Prob>F	Remark
Floating lag time						
Model	1167.04	9	129.67	2951.87	<0.0001	significant
Residual	0.31	7	0.044			
Lack of Fit	0.25	3	0.083	5.50	0.0666	not-significant
Pure Error	0.060	4	0.015			
Drug Release in 10 h						
Model	1314.87	9	146.10	1131.47	<0.0001	significant
Residual	0.90	7	0.13			
Lack of Fit	0.11	3	0.037	0.19	0.8986	not-significant
Pure Error	0.79	4	0.20			

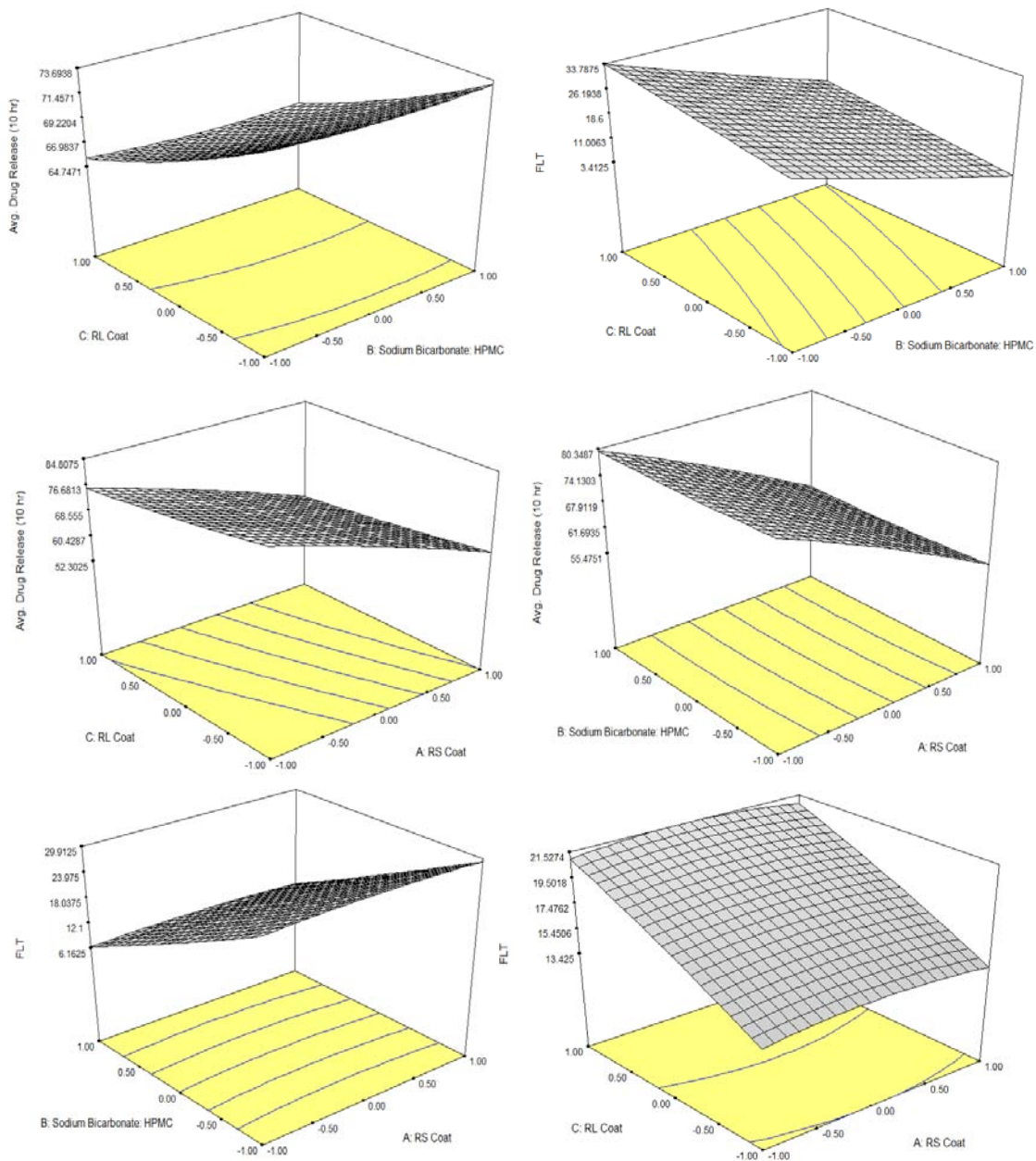


Fig. 5: Response surface plot of floating lag time and average drug release in 10 h

Table 7: Summary of statistical parameters for the responses

Parameter	Floating lag time	Drug release in 10 h
Mean	17.82	68.49
SD	0.21	0.36
CV	1.18	0.52
R ²	0.9997	0.9993
Adjusted R ²	0.9994	0.9984
Predicted R ²	0.9965	0.9977
Adeq Precision	188.959	117.944

SD: Standard deviation; CV: Coefficient of variation

The response surface plot obtained for the floating lag time and drug release in 10 h were indicated in (fig. 5). Response plot of floating lag time indicated a significant effect of the amount of effervescent agent (NaHCO₃: HPMC ratio) and level of gas entrapped polymeric coating (Eudragit RL 100) on floating behaviour. Increasing the amount of effervescent agent and decreasing level of Eudragit RL 100 decreases floating lag time. Eudragit RS 100 significantly affects drug release as shown by response plot of drug release in 10 h. The drug release decrease with increasing level of release retardant Eudragit RS 100 internal coat. The results can be correlated with the obtained regression equation also indicated below (A: Eudragit RS 100; B: NaHCO₃: HPMC and C: Eudragit RL 100). It was observed from regression equation coefficients that (B: NaHCO₃: HPMC) and (C: Eudragit RL 100) had marked negative and positive effect on floating lag time respectively. The regression equation coefficients of average drug release in 10 h indicated a more pronounced negative effect of

(A: Eudragit RS 100) and (C: Eudragit RL 100) on *in vitro* drug release.

Regression equations of the fitted quadratic model:

$$Y_1 - \text{Floating lag time} = 18.10 - 0.063A - 11.48B + 3.71C - 0.64A^2 + 0.34B^2 - 0.29C^2 - 0.01AB - 0.025AC - 0.45BC$$

$$Y_2 - \text{Avg. drug release (10 h)} = 68.43 - 12.10A - 0.26B - 4.15C - 0.80A^2 + 0.39B^2 + 0.54C^2 - 0.028AB + 0.38AC - 0.072BC$$

(fig. 6.) indicating the diagnostic plot (predicted vs. actual) for both the responses. It can be seen from the plots that in all the cases there was a reasonable agreement between the predicted and experimental values. This confirms that the selected model for the current study adequately describes the influence of the independent variables on the responses with lower magnitudes of error.

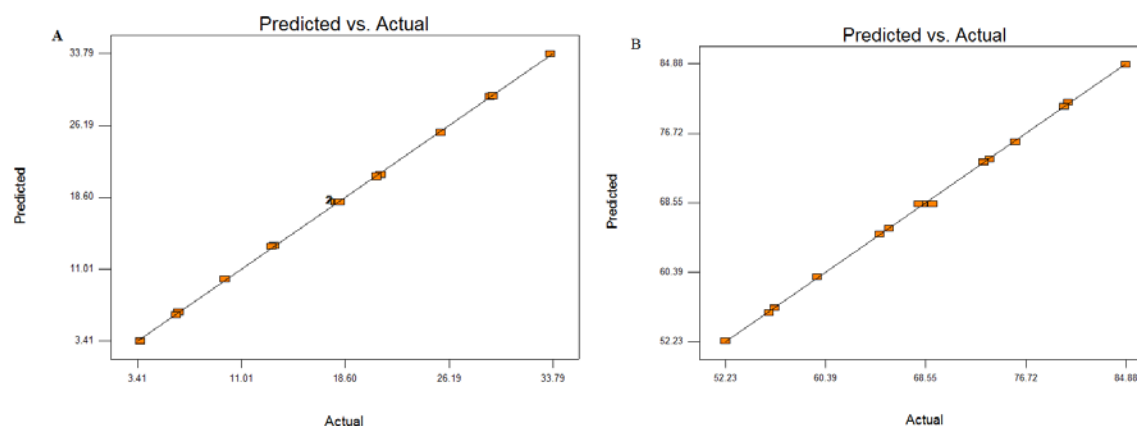


Fig. 6: Correlation between actual and predicted values for (A) Floating lag time and (B) Drug release in 10 h

Desirability approach under numerical optimization technique was used to prepare optimum formulation. The optimum formulation was obtained by applying constraints to independent and dependent variables (responses). Independent variables were kept in the range whereas floating lag time and percent drug release in 10 h set to minimum and maximum respectively. Nine solutions were generated by the design expert software among which one is selected having the

highest desirability. The selected solution is indicated in (table 8). A new optimized formulation (batch F 14) indicated in table 2 was prepared and evaluated for the responses. The results of floating lag time and average drug release obtained of batch F 14 shown in table 4. The obtained experimental results of batch F 14 were in near proximity to the theoretical values of floating lag time and drug release in 10 h indicated by numerical optimization (table 8).

Table 8: Summary of numerical optimization

Parameters	Goal	Solution	Desirability	Remark
Independent Variables			1	Selected
Eudragit RL 100	in range	-1 (5%)		
NaHCO ₃ : HPMC	in range	+1 (2:1)		
Eudragit RL 100	in range	-1 (5%)		
Dependent Variables				
Floating lag time	minimum	3.1 min		
Percent drug release in 10 h	maximum	84.94 %		

Stability studies

During stability studies, the floating pellet formulation is exposed to the accelerated condition of temperature and humidity for three

months. At regular interval (1st, 2nd and 3rd month), the drug content, drug release at 10 h and physical appearance were studied. No significant changes in drug content and drug release were observed

during storage as indicated in (table 9). The physical appearance was also remain unchanged after three month. The data obtained

with stability studies indicated that the developed multilayer floating pellet system of anagliptin was stable.

Table 9: Accelerated stability study results for formulation (batch F 14)

Month	Appearance	Drug Content (%)	Drug release at 10 h (%)
0	White	98.75±0.41	86.54±0.35
1	White	98.24±1.01	85.49±0.98
2	White	97.52±0.95	85.11±1.12
3	White	96.16±1.29	84.04±1.67

mean±SD; n = 3

Pharmacokinetics in wistar rats

The pharmacokinetic parameters were determined for F14 anagliptin floating pellets in comparison to plain anagliptin drug. Anagliptin plasma concentrations comparison between floating pellets and plain drug were shown in (fig. 7). The calculated mean pharmacokinetic parameters were shown in (table 10). The change

in Cmax and increase in Tmax values indicated sustained drug release of F14 floating pellets. A 2.51-fold increase in relative bioavailability of F14 anagliptin floating pellets compared to plain drug was observed. Higher half-life, MRT and lower elimination rate constant values indicated long resident time of anagliptin floating pellets. The statistical analysis revealed that there was a significant difference in the Cmax, Tmax, AUC, t_{1/2}, Kel and MRT.

Table 10: Pharmacokinetics of anagliptin floating pellets and anagliptin plain drug

Parameters	F14 anagliptin floating pellets	Anagliptin plain drug
C max (µg/ml)	43.17±2.17*	30.51±2.83
T max (h)	8.00±0.04*	0.5±0.04
AUC _{0-∞} (µg-h/ml)	376.15±7.55*	149.86±6.43
MRT (h)	9.07±0.03*	4.39±0.02
t _{1/2} (h)	8.90±2.43*	4.46±1.12
K _{el} (h ⁻¹)	0.18±0.11*	0.37±0.27
Relative bioavailability: AUC of F14 Pellets/AUC of Plain drug=2.51		

*P<0.05 as compared to anagliptin plain drug, mean±SD; n = 6

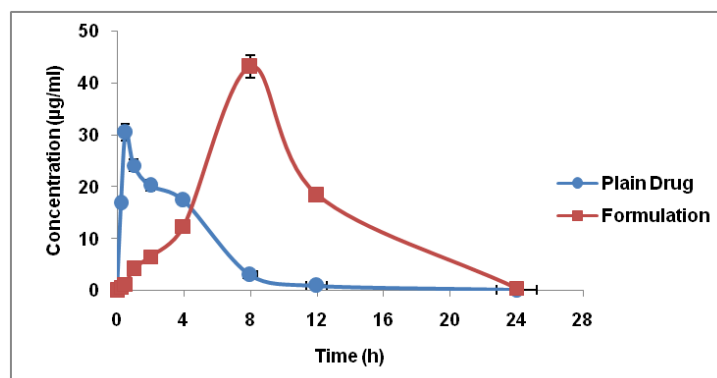


Fig. 7: Time versus mean plasma concentration profiles of anagliptin floating pellets and anagliptin plain drug mean±SD; n = 6

Pharmacodynamics in Wistar rats

Effect on calorie intake

The calorie intake was significantly increased in the cafeteria diet-fed rats (3086.05) as compared to the normal diet-fed rats (2108.45). However, the rats received anagliptin floating pellets and orlistat showed a significant decrease in calorie intake per week (2283.99, 1890.18) respectively indicated in (fig. 8).

Effect on body weight and BMI

The animal group fed with CD for six weeks showed a significant increase in body weight and BMI (277.98±3.69, 2.65±0.03) compared the normal diet-fed rats (212.20±2.63, 1.41±0.04) shown in (fig. 9 and 10). Treatment with anagliptin floating pellets and orlistat significantly reduced the increased in body weight and BMI (238.79±2.52, 1.96±0.01) (221.10±1.44, 1.47±0.03) respectively.

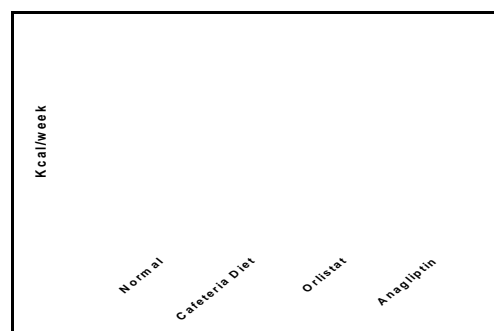


Fig. 8: Effect on calorie intake, values are mean±SEM, n=6, #P<0.01 as compared to normal control, *P<0.001, *P<0.05 as compared to cafeteria diet group using one way ANOVA followed by Bonferroni test**

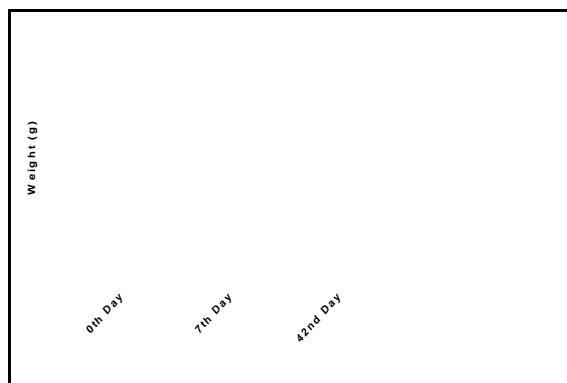


Fig. 9: Effect on body weight, values are mean±SEM, n=6, #####P<0.0001 as compared to normal control, *P<0.001 as compared to cafeteria diet group on 42nd day using two way ANOVA followed by Bonferroni test**

Effect on serum biochemical parameters

The serum levels of glucose, CH, LDL, TGs, ALT and AST were found to be significantly increased in the cafeteria diet-fed rats compared to the normal diet-fed rats. On the contrary HDL level was found to

be significantly decreased in CD control group. However, anagliptin treatment significantly reduced the increase in serum levels of glucose, CH, LDL, TGs, ALT and AST and increased HDL concentration significantly as indicated in (table 11).

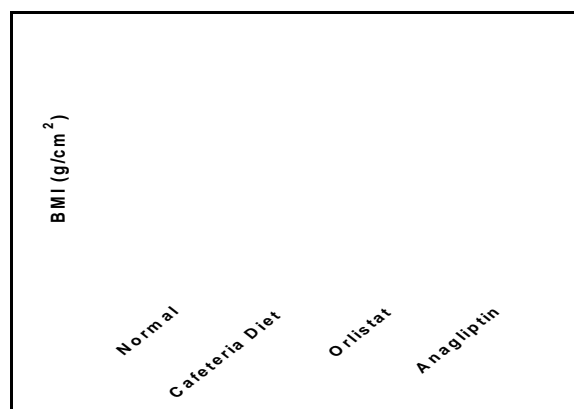


Fig. 10: Effect on BMI, values are mean±SEM, n=6, #####P<0.0001 as compared to normal control, *P<0.001 as compared to cafeteria diet group using one way ANOVA followed by bonferroni test**

Table 11: Effect of anagliptin treatment on serum biochemical parameters

Parameters	Normal	Cafeteria Diet	Orlistat	Anagliptin
Glucose (mg/dl)	68.89±0.45	107.13±0.92####	80.82±1.19***	74.82±1.07****
CH (mg/dl)	66.06±1.57	110.04±1.31####	80.93±1.51***	95.19±0.61**
LDL (mg/dl)	37.94±1.02	55.89±1.17###	39.81±0.95***	46.29±1.31**
TGs (mg/dl)	63.95±0.85	105.99±1.29####	75.43±1.56***	96.12±1.25**
ALT (u/l)	41.36±0.63	83.93±0.41####	56.92±0.41***	66.04±0.77**
AST ((u/l))	58.02±0.48	103.52±0.62####	62.80±0.69***	71.92±0.45***
HDL (mg/dl)	47.67±1.45	30.92±0.77###	49.04±1.71***	42.15±0.92**

Values are mean±SEM, n=6, #####P<0.0001, ###P<0.001 as compared to normal control, ***P<0.001, **P<0.01 as compared to cafeteria diet group.

Table 12: Effect of anagliptin treatment on organs weight

Organs	Normal	Cafeteria diet	Orlistat	Anagliptin
Liver (g)	7.34±1.15	14.06±1.24####	8.25±1.11***	9.40±1.24***
Heart (g)	1.22±0.46	2.70±0.54###	1.68±0.49***	2.03±0.61**
WAT (g)	3.65±0.87	8.48±1.16####	4.13±0.63***	4.97±0.44***

Values are mean±SEM, n=6, #####P<0.0001, ###P<0.001 as compared to normal control, ***P<0.001, **P<0.01 as compared to cafeteria diet group.

Effect on organ weight and WAT

The CD control group showed a significant increase in the weight of organs and white adipose tissue as compared to normal control shown in (table 12). Treatment with anagliptin significantly decreased organs weight and white adipose tissue which were induced by cafeteria diet.

CONCLUSION

The anagliptin gastroretentive floating pellets based on gas formation technique was successfully designed and developed. From the numerical optimization function using Design expert software, an optimal formulation (F 14) comprising of Eudragit RS 100 (5%), sodium bicarbonate: HPMC (2:1) and Eudragit RL 100 (5%) was identified. It showed floating lag time of 3.4 min and 86.54% drug release at 10 h. The pharmacokinetic study conducted in male Wistar rats showed increased C_{max}, prolonged T_{max}, half-life and MRT collectively demonstrated sustained drug release. The relative bioavailability of optimized formulation was improved by 2.51 fold in comparison to anagliptin drug. The results of anagliptin floating pellets treatment in male Wistar rats fed on cafeteria diet showed its hypophagic, hypoglycemic and hypolipidemic effects. The gastroretentive floating pellets of anagliptin with improved bioavailability was obtained and could be promisingly used in the management of the metabolic syndrome.

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AUTHORS CONTRIBUTION

Both the authors have contributed equally in the research work

CONFLICTS OF INTERESTS

Declare none

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