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ORIGINAL ARTICLE

Targeting enteral endocrinal L-cells with dietary carbohydrates, by increasing the availability of miglitol in the intestinal lumen, leads to multi-fold enhancement of plasma glucagon-like peptide-1 levels in non-diabetic canines

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Abstract

The principle aim of this study was to design a controlled release (CR), bioadhesive formulation of miglitol (in form of pellets) which would regulate the post-prandial glucose levels via reversible inhibition of α-glucosidase enzyme as well as by modulating the glucagon-like peptide-1 (GLP-1) pathway in non-diabetic canines. A multilayered pellet formulation which was both bioadhesive (because of hydroxy propyl methyl cellulose polymer) and CR (because of the ethyl cellulose layer) was formulated. We report a novel finding that the CR formulation of miglitol (S3) induced a 2.2-fold elevation in the C_{max} as well as the overall AUC_{0-24} of GLP-1 values in comparison to the non-CR (immediate release (IR) formulation). The S3 formulation also resulted in better, steady, and prolonged control of glucose levels over a time period of 7 h in comparison to the IR formulation possibly due to combination of both, prolonged inhibition of the α-glucosidase enzyme and enhanced plasma GLP-1 levels. The S3 formulation was stable with no changes in the dissolution profiles at both of the stability conditions tested, 25°C/60% RH and 40°C/75% RH. Aqueous polymeric coating of the pellets (in contrast to coating using organic solvents) resulted morphologically in a uniform polymeric film and also releases profiles with lower burst effect. Curing played a significant role in determining release profile of the pellets, prepared by aqueous polymeric coating method.

Keywords: Miglitol; GLP-1; type-II diabetes mellitus; post-prandial glucose; α-glucosidase inhibitors

Introduction

Reports on glucose-lowering properties of duodenal extracts, containing intestinal factors in patients suffering from diabetes mellitus, date back to 1906 (Moore, 1906). A plethora of information is now available which clearly demonstrates that the phenomenon of glucose lowering by the gut-derived polypeptides is via regulation of insulin secretion. This effect is called as the "incretin effect," and the polypeptides, "incretins" (meier & Nauck, 2005). One of the foremost incretin responsible for regulation of post-prandial glucose levels and also insulin secretion is glucagon-like peptide-1 (GLP-1) (Qian et al., 2009). Post-prandial glucose reduction by GLP-1 is due to its various physiological effects, which include promotion of insulin gene expression, insulin biosynthesis, and secretion. Some of its other effects are stimulation of proinsulin synthesis, inhibition of glucagon synthesis, stimulation of glycogen synthesis and satiety, delay in gastric emptying, and slowing down of gut motility. GLP-1 also exhibits trophic effects on β-cells of the pancreas. These include proliferation of existing β-cells, maturation of new cells, and inhibition of apoptosis (Holst, 2003).

One of the recent approaches in treatment of non-insulin dependant diabetes mellitus is inhibition of the enzyme α-glucosidase. Dietary carbohydrates are broken down enzymatically to monosaccharides in the intestine by enzymes called as α-glucosidases. Only monosaccharides (such as glucose) can be absorbed into the blood stream

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from the intestine. Due to digestion of carbohydrates in upper part of the small intestine, a post-prandial rise in blood glucose is generally seen rapidly following a carbohydrate load from the diet (Bischoff, 1994). If the action of α-glucosidases is blocked (reversibly) then the digestion of a polysaccharide will be retarded leading to lowering of post-prandial blood glucose level. This inhibition of α-glucosidases can be brought about by agents called as α-glucosidase inhibitors. Some of the clinically useful α-glucosidase inhibitors include acarbose, voglibose, and miglitol (Sels et al., 1999). Miglitol is a derivate of nojirimycin (1-desoxynojirimycin or N-OH-ethyl nojirimycin).

α-Glucosidase enzyme is present ubiquitously throughout the lumen of the intestine. Miglitol is to be co-administered with food. It has to be thus administered multiple times in a day by a patient (depending on the number of meals). We have designed a controlled release (CR) formulation of miglitol, which would increase local availability of the drug in intestinal lumen for a longer duration of time. It would thus aid in reduction in frequency of dose (currently available immediate release (IR) formulation in the market has to be administered three times a day), and leads to CR of the drug, resulting in effective blocking of the α-glucosidase enzyme for a longer duration of time. Interestingly, the α-glucosidase inhibitors such as acarbose, voglibose, and miglitol are reported to induce sustained enhancement of post-prandial GLP-1 (Göke et al., 1995; Ranganath et al., 1998; Lee et al., 2002). Miglitol is also reported to induce a rise in GLP-1 as compared to placebo in diabetic (Type-II) patients (Lee et al., 2002; Arakawa et al., 2008; Narita et al., 2009). The regulation of post-prandial glucose levels by miglitol is due to combination of two effects which are: (i) reversible inhibition of α-glucosidase enzyme and (ii) regulation of glucose by causing an enhancement in the plasma GLP-1 levels.

The principle aim of this study was to design and evaluate a CR, bioadhesive (to the intestinal mucosa) formulation for miglitol, which would reduce the frequency of dosing as well as regulate the post-prandial plasma glucose levels after two consecutive meals and the time intervals between them (a total time span of 7 h). One other aim of this study was also to assess the influence of increased availability of miglitol in the intestinal lumen on the plasma GLP-1 and post-prandial glucose levels. This increased availability was achieved by using a novel in-house tailored CR formulation (S3) of miglitol. A non-CR or IR formulation and a placebo formulation (which was devoid of miglitol) were used as comparators. Systematic experiments were carried out to assess parameters such as plasma GLP-1 and glucose levels. The data obtained were analyzed to find out whether there was a correlation between these different parameters.

Materials and methods

Materials

Unless otherwise indicated, all solvents, acids, and bases were obtained from Merck (Mumbai, India), and all reagents, chemicals, and buffer salts were obtained from Sigma-Aldrich (St. Louis, MO). Miglitol (pure drug) was purchased from Biocon (Bangalore, India). A commercially available conventional, non-CR formulation, MISOBIT (Lupin Labs, Pune, India), was used as the IR formulation of miglitol in the study. Ethyl cellulose (EC) and hydroxy propyl methyl cellulose (HPMC) were purchased from (Colorcon, Goa, India). Dibutyl phthalate was procured from Merck (Mumbai, India). Different grades of Eudragit polymers were obtained from Evonik, Essen, Germany. Celphere CP 305 was purchased from Signet Chemical Corporation, Mumbai, India. Pedigree, food for the dogs during the experiment was obtained from Pedigree (http://www.pedigree.com). Water used for making solutions and buffers was from Milli-Q water purification system (Millipore, Billerica, MA).

Methods

Formulation of CR pellets of miglitol

The method of manufacture of the pellets was similar to that reported earlier (Deshpande et al., 2009). Briefly, Celpheres (grade CP 305, average size between 300 and 500 μm) which consists of an inert material (pure spheronized microcrystalline cellulose) was used as the core of the pellets. Drug and different layers of polymer were deposited on this core. The layering of the drug on the Celpheres was carried out using a fluidized bed coating machine (FBC, Pam Glatt model no. GPLG 1.1), details of which are presented in Table 1. Universally, for all the pellet batches prepared, pellets passed through 40 mesh (425 μm) and retained on 60 mesh (250 μm) were used in this study.

In strategy no. 1 (S1), the drug along with the retardant and the bioadhesive polymer layers (miglitol + EC + HPMC) were mixed together and deposited on the core of the pellets followed by layering with enteric polymer (Eudragit L 100-55). In strategy No. 2 (S2), first the drug was mixed with a retarding polymer coat (EC) and then deposited on the core of the pellets. This was followed by layering with the bioadhesive polymer coat (HPMC). Finally, a layering with the enteric coat polymer (Eudragit L 100-55) was carried out on the pellets.

Amongst all of the formulations prepared in this study, formulation S3 was the most elegant with regards to the CR profile of miglitol as well as the bioadhesion properties. Following is the procedure for manufacturing

Table 1. Different parameters during the manufacture of the controlled release pellets.

Atomizing pressure (bar)	2
Batch size (g)	100
Inlet temperature (°C)	39–56
Outlet temperature (°C)	31–41
Distance: pellet bed-spray gun	Low
Nozzle bore diameter (mm)	1
Spray rate (g/min)	3
Inlet air (m^3/h)	81–97
Yield calculated after processing (%)	94–96

bioadhesive pellets (100 g) of formulation S3. Celpheres (100 g) were layered with the required concentration of the drug and HPMC (5 cps, 2.47% w/w), in water. These drug-layered pellets (100 g) were further coated with the required concentration and viscosity grade EC. Composition of the solution used for EC coating consisted of equal proportions (1:1) of dichloromethane and isopropyl alcohol containing EC and the plasticizer for the polymer (diethyl phthalate, 5.6% w/w of the total amount of EC). A 5% (w/w) solution of EC in solvents gave the best results. In the next step, the pellets (100 g) were further coated with 30% bioadhesive polymer (HPMC, 6 cps). This coating was carried out with an aqueous solution (5% w/w) of HPMC (6 cps) and plasticizer (propylene glycol, 5% of the total amount of HPMC). In the final stage, enteric coating (30% w/w) was carried out on the pellets (100 g) using an isopropyl alcohol solution containing the enteric coating polymer (Eudragit L 100-55) and a plasticizer (diethyl phthalate, 8.3% of the total amount of the polymer). Pellets passed through 40 mesh (pore size 425 μm) and retained on 60 mesh (pore size 250 μm) were used in this study. Schematic representation of the bioadhesive pellets is shown in Figure 1 and Table 2.

Calculation of layering efficiency and product yield

The product yield was calculated using the following formula:

$$\text{PY\%} = \left\{\left[\frac{W_a}{W_p + W_l}\right] \times 100\right\}$$

where PY = product yield, W_a = actual weight of the final pellets after coating and drying, W_p = initial weight of the uncoated EC core of the pellets, and W_l = weight of the charge load of the layering powder.

Loading efficiency was calculated using the following formula:

$$\text{LE} = \left\{\left[\frac{D_a}{D_t}\right] \times 100\right\}$$

where, LE = loading efficiency, D_a = actual drug loading, and D_t = theoretical drug loading. Actual drug content was determined by assay.

Estimation of the in vitro release profiles of miglitol formulations

Dissolution studies were carried out in a USP 33 Apparatus II (Paddle method, Electrolab, TPT-08L; Mumbai, India). Either 0.1 N HCl or phosphate buffer of the required pH was used as the dissolution media for all of the dissolution studies. For studies requiring dissolution conditions in both acidic as well as basic conditions, HCl solution (0.1 N, 500 mL) was used as the dissolution media for the first 2 h of the experiment. After this time point, the pH of the media was adjusted to the required value by addition of predetermined amount of trisodium hydrogen phosphate solution (8% w/v). Fixed temperature (37°C) with a paddle agitation rate of 50 rpm was maintained throughout the study. Formulations to be tested were filled in a size "0" conventional hard gelatin capsule (common capsule) and introduced into the dissolution medium. Samples (20 mL at each time point) were withdrawn by an auto sampler (Electrolab Fraction Collector FC-12; Mumbai, India) at predetermined time points ($n = 3$). Each sample obtained in 0.1 N HCl was centrifuged at 3000 rpm for 10 min. The clear supernatant (10 mL) was diluted with sodium hydroxide (0.1 N, 10 mL). Forty microliter of the sample was loaded into the high performance liquid chromatography (HPLC) system. On the other hand, sample in phosphate buffer was centrifuged at 3000 rpm (Heraeus Biofuge Stratos Centrifuge, Model: D-37520; Thermo Electron Corporation, Karlsruhe, Germany) for 10 min. Twenty microliter of this sample was loaded into the HPLC system. All of the samples were prefiltered

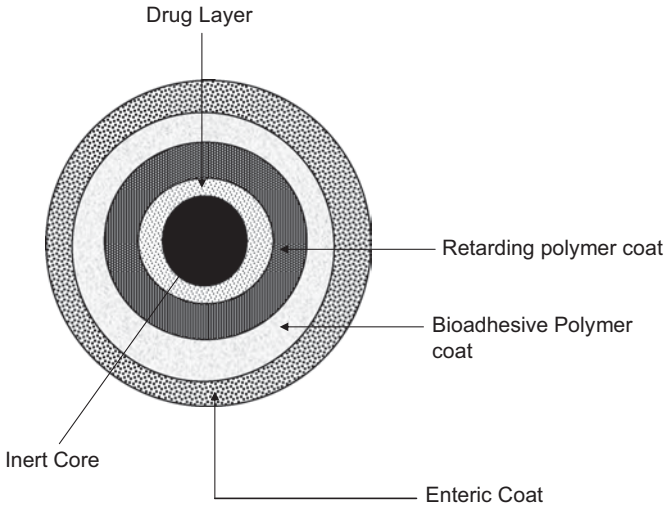

Figure 1. Schematic representation of miglitol controlled release pellets, strategy 3 (S3). First, Celphere CP 305 pellets (inert cellulose pellets) were layered with drug (33% w/w). The next layer comprised of ethyl cellulose membrane. This was followed by layering with hydroxy propyl methyl cellulose and finally enteric coating with Eudragit L 100-55 polymer (30% w/w).

Table 2. Various strategies and compositions of different formulations.

No.	Excipients (% w/w)	S1	S2	S3	Placebo
1	Miglitol	33	33	33	—
2	Ethyl cellulose (10 cps) (retarding polymer)	15	15	15	15
3	HPMC (6 cps) (bioadhesive polymer)	15	15	30	30
4	Eudragit L 100-55 (enteric coat)	30	30	30	30

through a 0.45-μm membrane (Whatman Inc., Clifton, NJ) before injection into the HPLC system.

The amount of miglitol in the dissolution media was determined using a Waters (Milford, MA) HPLC system with a photodiode array detector (model 996) at 220 nm. The column used was a Hypersil C18, BDS (250 mm × 4.6 mm, 5 μm) (Part No.: 28105-254630). Mobile phase consisted of 0.01 M ammonium acetate solution:methanol in volume ratio 99:1. A constant flow rate of 1 mL/min was maintained during analysis. The retention time of miglitol was approximately 2.8 min. The data obtained were collected and integrated using Empower Version 5.0 software (Des Plaines, IL). Calibration curve for miglitol was prepared in buffer systems similar to those used in preparing samples at both pH 1.2 as well as pH 6.8. Each experiment was repeated three times.

Comparison of the obtained release profiles from different formulations was carried out with the aid of a statistically derived parameter "similarity factor" (f_2) (Moore & Flanner, 1996; Costa & Sousa Lobo, 2001). If the two profiles are identical or similar then $f_2 = 100$. Values of $f_2 \geq 50$ indicate similarity of two dissolution profiles.

Ex vivo bioadhesion testing of the pellets to rat intestine

All of the animal experiments were approved by the DRF animal experimental ethics committee, and were in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Environment, Government of India. Rules of CPCSEA are laid down as per Institute of Laboratory Animal Resources, US guidelines.

The experiments were done as per the procedure reported earlier (Deshpande et al., 2009). Briefly, male Sprague–Dawley rats weighing between 350 and 400 g were fasted for 16 h prior to the experiment. The animals were placed in cages having grid floors, to prevent them from eating their feces. However, water was provided to the animals during the fasting period. A rat was sacrificed immediately prior to the study. Its Gastrointestinal Tract (GIT) was isolated and washed with phosphate buffer pH 7.4 with the aid of a syringe. The intestine was tied to the outlet of the chamber with a twine. The inlet of the chamber was connected to a peristaltic pump (Electrolab peristaltic pump, Model: PP-201; Mumbai, India) that pumped phosphate buffer (maintained at 37°C) from a reservoir at a constant flow rate (1 mL/min) into the chamber and eventually through the intestine during the experiment. The intestine was washed in phosphate buffer maintained at 37°C in the sample tray of the shaker bath. The shaker bath was operated at a constant speed of 50 rpm.

For each experiment, a fixed number of drug-loaded pellets (1000 pellets) were placed in the chamber. Phosphate buffer (pH 7.4) was pumped into the chamber and the pellets were allowed to soak with the buffer for 5 min (the outlet of the chamber was closed) after which, the outlet of the chamber was opened and the buffer (along with the pellets) was allowed to pass through the intestine at the flow rate of 1 mL/min. The number of pellets eluting out from the exit portion of the intestine were counted over a period of time. After the time interval of 2 h, the pump was stopped. Intestine was removed from the chamber and cut into segments. Each of the segment was given a vertical incision and the lumen of the intestine exposed. The exposed portion was washed with buffer to remove the non-adhered pellets. Finally, the count of the number of pellets adhering to the different portions of the intestine was obtained. As the average particle size of the pellets was between 400 and 500 μm, the counting of the pellets was carried out by visual observation with the aid of a magnifying glass. Each experiment was performed in triplicate.

Scanning electron microphotography studies

Samples of the pellets were affixed to the carbon discs and sputter coated with Au/Pd alloy using EMITEC K550 sputter coater for 3 min at 40 mA. The coated samples were then transferred to the Hitachi S-3000N scanning electron microscope (Hitachi, Tokyo, Japan). Images were captured at an excitation voltage of 10.0 kV.

Preparation of IR samples of miglitol

Commercially available conventional, IR (non-CR) tablet formulations of miglitol (MISOBIT; Lupin Labs) were used as IR samples.

For the purpose of *in vivo* studies, a dose of 75 mg was fixed for the IR formulation. This dose was achieved by combining tablets of two different drug strengths of MISOBIT, each containing 25 and 50 mg of miglitol, respectively. The tablets were crushed, passed through 120 mesh (pore size 125 μm), and finally filled in size "0" conventional hard gelatin capsule.

Preparation of CR samples of miglitol for in vivo studies

Pellets prepared as described in the section "Formulation of CR pellets of miglitol" containing an equivalent of 50 mg of miglitol by weight from formulation S3 were filled in size "0" conventional hard gelatin capsule. Pure miglitol drug powder (25 mg) was also placed in the same capsule. Thus, a total dose of 75 mg of miglitol was administered.

Preparation of placebo samples of miglitol (control)

Placebo formulation consisted of placebo pellets that were similar to the ones used in the test formulation with

the only exception being that it did not contain any drug (miglitol). Size "0" conventional hard gelatin capsule was filled with placebo pellets with a weight equivalent to that of the total weight of solids present in the capsule containing the CR formulation.

In vivo study protocol for testing the formulations in beagle dogs

Male beagle dogs (17–17.5 kg) were procured from the Discovery Research animal house facility, Dr. Reddy's Research Foundation, Hyderabad, India. The animals were placed in quarantine and acclimatized to laboratory conditions for at least 7 days. They were fed with Pedigree (commercially available dog feed) at predetermined time intervals and water was provided *ad libitum*. The Pedigree feed had the following composition: protein 21%, fat 12%, fiber 4%, moisture 12%, and carbohydrates 51%.

The complete set of study animals was on Pedigree diet for a week and they were fasted overnight prior to the oral administration of the respective formulations.

The population of beagle dogs was randomly divided into three treatment groups. Each group consisted of three subjects ($n=3$). Placebo formulation was administered to the first group. The second group was treated with CR test formulations and the third with IR formulations, respectively. There was no cross over between the test animals. Respective formulations were administered to the study subjects by oral route. During the experiment, food was administered twice to the test subjects at the time intervals of 1 and 5 h post-administration of miglitol and placebo formulations. Each experiment was replicated three times ($n=3$).

Blood samples (1.5 mL) were withdrawn from cephalic vein immediately prior to the administration of the formulations and thereafter at periodic intervals (0, 0.5, 1, 2, 3, 5, 6, 7, 8, 10, 12, and 24 h).

Sample preparation for determination of plasma glucose and GLP-1 levels was carried out in the following way. Immediately after withdrawal, the blood sample (0.8 mL) was transferred immediately to a tube (chilled on ice) containing vildagliptin, a dipeptidyl peptidase IV inhibitor (to a final concentration of 1 μM) to prevent GLP-1 degradation and ethylenediamine tetra-acetic acid (10 μL, from a stock solution of 200 mg/mL) to prevent coagulation of blood. The sample was mixed well and centrifuged at 4°C at 6000 rpm for 5 min. Aliquots of centrifugate plasma were removed and stored for GLP-1 estimation (300 μL) and plasma glucose estimation (100 μL). The plasma was stored at –20°C until further analysis. Plasma glucose levels were determined using Vitalab Selectra-2 autoanalyzer (Vital Scientific, Dieren, The Netherlands) and Merck diagnostic kit (Merckotest, 11862900011046, glucose-based GOD-POD). Plasma GLP-1 levels were estimated by a GLP-1 (active) ELISA kit 96-well plate (cat. no. EGLP-35K) from Linco Research labs (now Millipore, Billerica, MA).

Statistical analysis

Statistical analysis was performed to ascertain the significance of variation between sets of data obtained. One-way analysis of variance followed by Bonferroni/Dunnet's test were applied to evaluate the statistical difference between experimental results/groups. P-value <0.05 was considered as statistically significant. All the values are expressed as mean ± SEM. Wherever necessary, Student's t-test was also applied to determine the statistically significant variation between the set of data with P-value <0.05 being considered as statistically significant.

Results

In vitro dissolution studies on miglitol CR pellets

To find out as to which of the strategy displayed the most suitable release profile for miglitol, formulations prepared by using various strategies were subjected to dissolution studies. The results for dissolution studies carried out on formulations based on strategies S1, S2, and S3 are shown in Figure 2. The percent cumulative amount of miglitol released was about 18, 16, and 14% at the end of 2 h and 48, 50, and 95% at the end of 6 h from S1, S2, and S3 formulations, respectively. The release curve plateaued off for all the formulations after this time point. One-hundred percent of drug was released from formulation S3 at the end of 12 h. In contrast, 54 and 62% of drug was released, respectively, from formulations S1 and S2 at the end of 12 h. Formulation S3 displayed complete

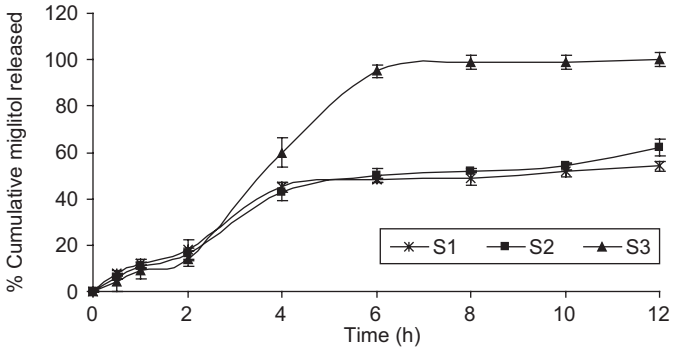

Figure 2. Dissolution profile of controlled release pellets prepared using different formulation strategies (S1, S2, S3). Dissolution was carried in 0.1 N HCl for the first 2 h followed by phosphate buffer pH 6.8 until the final time point (i.e., 12 h) ($n=3$).

release of miglitol post 12 h. Thus, S3 displayed the most suitable CR profile of miglitol from the perspective of this study and was evaluated further for *in vivo* studies.

Effect of HPMC on the bioadhesive properties of pellets in the intestine

For finding out the optimum amount of HPMC (polymer responsible for bioadhesion of the pellets to the mucosa of the intestine) needed for the purpose of bioadhesion, variants of formulation S3 were prepared with 10, 20, and 30% of the bioadhesive polymer (Figure 3). These pellets were tested for adhesion to the intestinal mucosa *ex vivo*. Approximately 47, 22, and 5% of the total number of pellets tested from the formulations containing 10, 20, and 30% of HPMC, respectively, were found to be non-adherent to the intestinal mucosa (Figure 3A) at the end of the experiment (i.e., 2 h). Thus, the formulation containing 30% HPMC displayed the most suitable mucoadhesive properties. The percentage of pellets adherent to different segments of the intestine from the total population of the adherent pellets is shown in Figure 3B. Amongst the total number (%) of adherent pellets, about 5, 61, 32, and 2% of the pellets were found to be adherent to the duodenum, jejunum, ileum, and the colon, respectively, from the formulation containing 30% of HPMC.

Stability studies and effect of curing on pellets

As pellets from formulation S3 displayed most suitable CR profile of miglitol as well as optimum bioadhesive properties to the intestinal mucosa, it was used for all of the further studies. These pellets (from formulation S3) were placed on stability studies for a duration of 24 months at the temperature and relative humidity conditions of 25°C/60% RH and 40°C/75% RH. The release patterns of the pellets did not differ with respect to each other significantly ($P > 0.05$ in each case) with respect to each other at all of the temperature and humidity conditions tested ($f_2 > 50$ in each case).

The effect of curing on drug release in 0.1 N HCl and buffer pH 6.8 was investigated on pellets (Figure 4A). The curing conditions consisted of no curing, curing for 1 day at 60°C, and curing for 2 days at 60°C. There was no significant difference in the release profiles from pellets which were cured at 60°C for 1 day or 60°C for 2 days at both the pH conditions ($P > 0.05$ in each case). However, there was a difference in the release profiles of the cured and uncured pellets.

Curing plays an important role if an aqueous process of coating is employed. Uncured pellets displayed a considerably faster release profile in comparison to pellets cured for 1 day at 60°C and 2 days at 60°C. This could be because by and large if the curing conditions are not sufficient, then further polymer coalescence will occur upon storage thus leading to decreasing drug release rates (Kranz & Gutsche, 2009). There appears to be a threshold value (1 day) above which further curing does not have any significant impact on the release of the drug. Non-aqueous coating did not produce a continuous film. Furthermore, there was formation of pores in the film. Due to this, the initial burst effect as well as the release of the drug was significantly faster in comparison to the pellets coated by aqueous coating process. In contrast, the morphology of the film prepared by aqueous coating was uniform, smooth, and without pores.

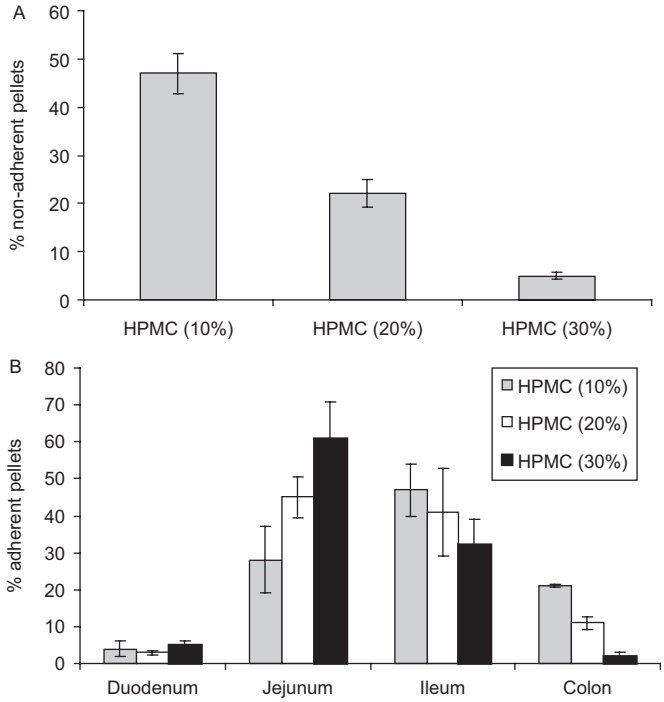

Figure 3. (A) Effect of varying amount of hydroxy propyl methyl cellulose (HPMC) in the pellets on the bioadhesive properties of different pellet formulations in the intestine *ex vivo* ($n = 3$). Data are presented as mean ± SEM. (B) Mucoadhesion of pellets containing varying amount of HMPC *ex vivo* to different segments of rat intestine ($n = 3$). Data are presented as mean ± SEM.

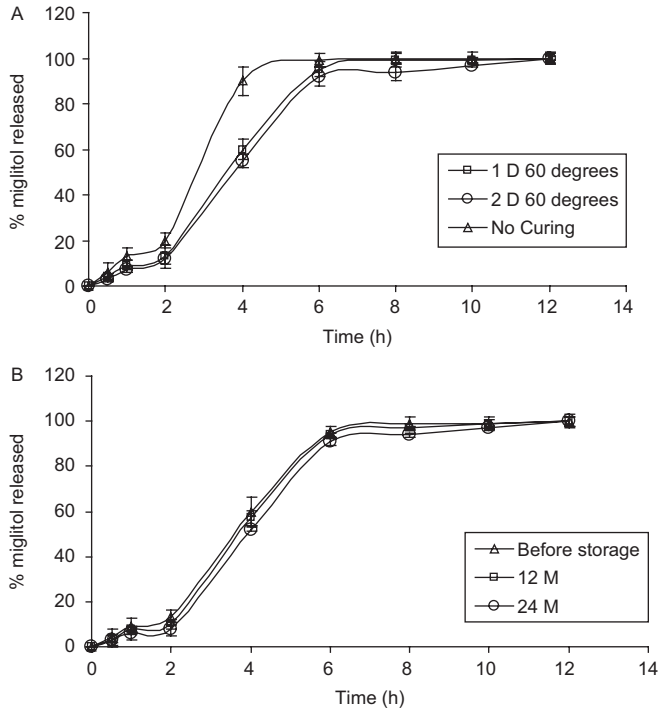

Figure 4. (A) Effect of curing on the release profile of miglitol from the controlled release pellets.

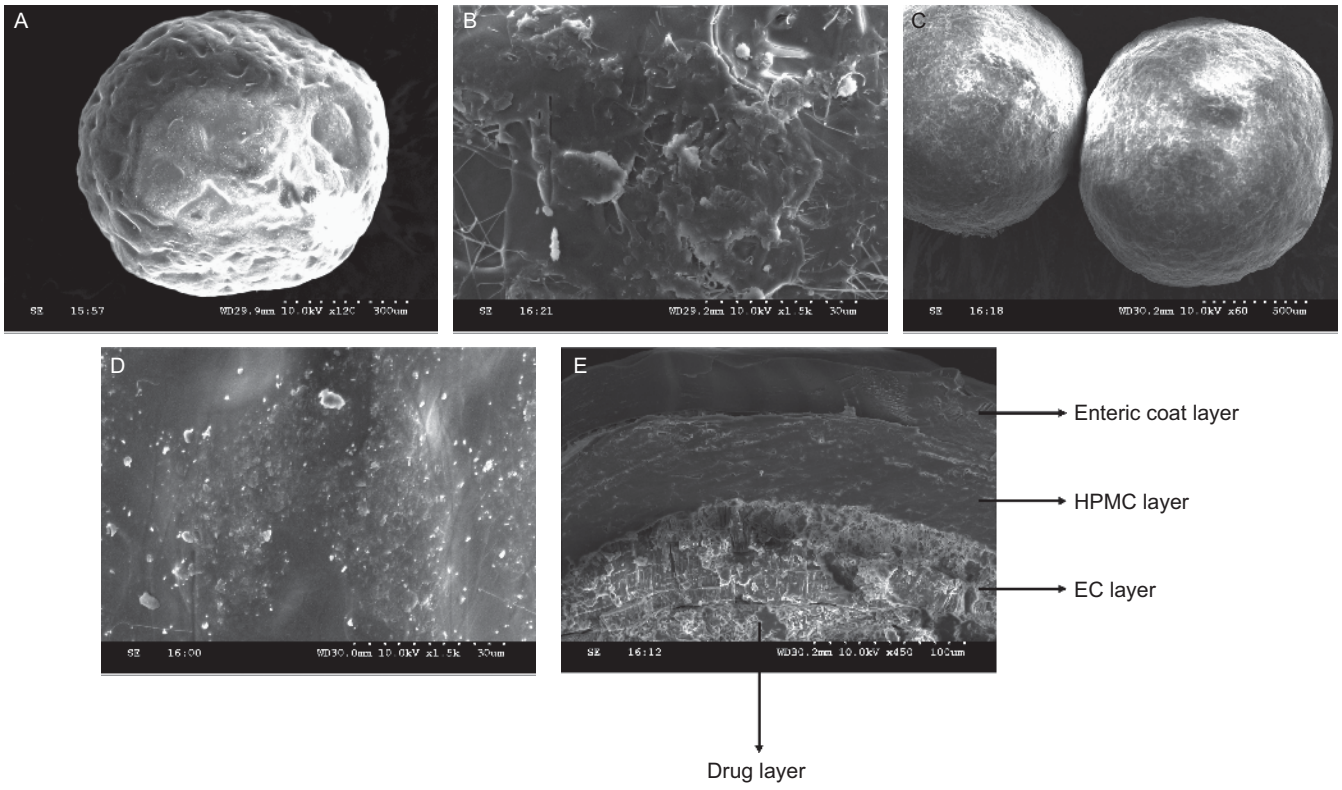

Figure 5. (A) Scanning electron microphotograph of a typical controlled release miglitol pellet prepared by using organic solvents during the layering process. (B) Scanning electron microphotograph of the external surface (high magnification) of a typical controlled release miglitol pellet prepared by using organic solvents during the layering process. (C) Scanning electron microphotograph of a typical controlled release miglitol pellet prepared by using aqueous system during the layering process. (D) Scanning electron microphotograph of the external surface (high magnification) of a typical controlled release miglitol pellet prepared by using aqueous system during the layering process. (E) Cross-sectional scanning electron microphotograph of a typical controlled release miglitol pellet.

The burst release was reduced considerably and overall the release was significantly slower in comparison to the organic coating system. Similar to our results, it has been reported in the literature that the film formed by using a system of organic solvent for coating had an unusual architecture with deep indentations and also formation of pores. On the other hand, coating with aqueous system resulted in a film with smooth surface morphology with no pores. This difference in the film morphology by using either aqueous or organic coating system could be attributed to the difference in solvent evaporation rates (McConnell et al., 2009) ($f_2 > 50$ in each case).

In 0.1 N HCl, the release profile of the uncured pellets was similar to the cured ones. However, it was significantly different at 4 h time interval in pH 6.8 buffer ($P < 0.05$). It was about 1.5 times greater in comparison to both the sets of cured pellets.

Cured pellets (from formulation S3) were placed on stability studies for a duration of 24 months at the temperature and relative humidity conditions of 25°C/60% RH and 40°C/75% RH. The profiles of the cumulative amounts of miglitol released at the time intervals of "0" days, "12 months," and "24 months" at 40°C/75% RH is shown in Figure 4B. The release patterns of the pellets did not differ with respect to each other significantly ($P > 0.05$ in each case) with respect to each other at various temperature and humidity conditions.

Assessment of the morphology of pellets using scanning electron microscopy

The SEM of a typical pellet from formulation S3 is displayed in Figure 5A. For this pellet, the layering (30% w/w) of the enteric coat polymer (Eudragit L 100-55) was carried out by dissolving the polymer completely in an organic solvent (isopropyl alcohol solution). As seen in the figure the enteric coat was not smooth. Higher magnification of the surface revealed that the film was not uniform with a few pores present in it (Figure 5B).

To improve upon the surface texture of the enteric polymer coat as well as to reduce the pores, the enteric coat layering was carried out using an aqueous solution of Eudragit L 100-55 polymer (it is commercially available as Eudragit L 30 D-55, which is a 30% aqueous solution of the polymer). A typical pellet manufactured using this method is shown in Figure 5C. The film formed by using the aqueous method had a very smooth surface texture. No irregularities or pores were seen in the film (Figure 5D).

Dissolution profile of the pellets prepared by organic coating method displayed an initial burst release of the drug (23%) and at the end of 2 h about 72% of the drug was released from the pellets. In contrast, the pellets prepared by aqueous coating method displayed a lower burst release of the drug (14%) and about 59% of the drug was released at the end of 4 h.

Cross-sectional scanning electron microphotograph of a typical, individual pellet from formulation S3 is displayed in Figure 5E, at a magnification value of 450×. Well-defined layers of drug, EC, HPMC, and Eudragit can be seen clearly in the figure. All of the coating layers were uniform and homogenous.

Effect of different formulations on GLP-1 and post-prandial glucose levels

For the purpose of *in vivo* studies, three doses of miglitol (each dose being 25 mg) were filled in a size "0" commonly used hard gelatin capsule (the overall total dose being 75 mg of miglitol). The first dose (25 mg) was in the form of pure miglitol powder and the other two doses (each dose being 25 mg and the total of the two doses being 50 mg) were in form of CR pellets. The logic behind this design was as follows. Miglitol is to be co-administered along with food to the patient (Bischoff, 1994). Pure miglitol powder would constitute the first dose and it would regulate the post-prandial glucose associated post-administration of the first meal. The CR pellets had a slower onset of action and therefore they would not be very effective in controlling the rise in post-prandial glucose after the first meal. Hence, we chose pure miglitol drug powder (drug would be readily available and it would have a rapid onset of action) as the first dose. The second and third doses of miglitol (in the form of CR pellets) would control the post-prandial glucose levels after the second meal and also during the time interval between these two consecutive meals, respectively. This assumption was based on the hypothesis that the complete formulation would control post-prandial glucose levels over a time span of 7 h.

Effects of different miglitol formulations (IR and S3) used in the study on the post-prandial levels of GLP-1 are displayed in Figure 6. There were no significant differences observed in the fasting levels of GLP-1 between the set of test animals that received miglitol therapy in the form of IR, S3, and placebo ($P > 0.05$ in each case). Post-lunch, there was significant rise in the levels of GLP-1 in the sets of animals who received miglitol in form of IR and S3 as well as the placebo formulation in comparison to their respective fasting levels of GLP-1 ($P < 0.05$ in each case as measured Student's *t*-test).

Overall, the S3 formulation produced considerably greater elevation and more sustained levels of GLP-1 over the IR and placebo formulations ($P = 0.0043$). These elevated GLP-1 levels were maintained at therapeutic levels for total time duration of 7 h (from time intervals of 3–10 h). The IR formulation resulted in two peaks of GLP-1, first at 3 h (2 h post-first meal) and next at 7 h (2 h post-second meal).

The S3 formulation–treated group displayed maximum concentration of GLP-1 in the plasma (C_{max}, 53.6 ± 2.9 pmol/L) at the time interval of 3 h (t_{max}) (Table 3). The concentration was significantly higher than that of the IR or the placebo formulation ($P = 0.039$ in comparison to IR formulation and $P = 0.014$ in comparison to the placebo formulation). The GLP-1 levels of S3 formulation declined steadily between time intervals of 3 and 5 h after which it plateaued off until 10 h. Similar to our findings,

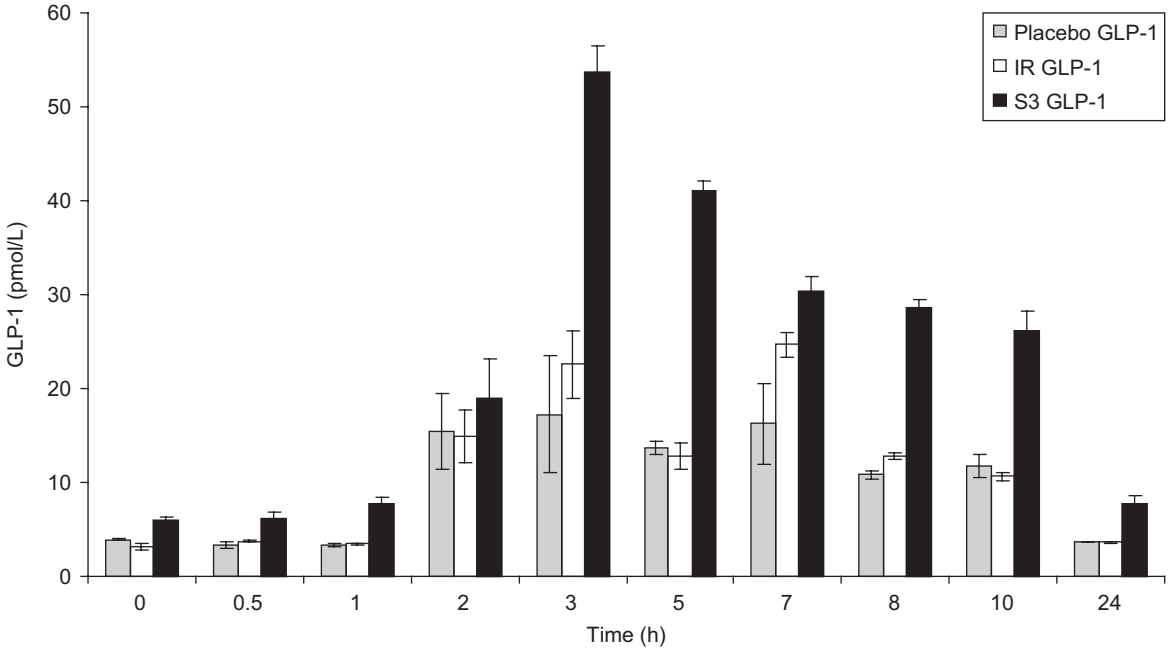

Figure 6. Plasma glucagon like peptide-1 (GLP-1) (pmol/L) levels at different time intervals due to placebo, immediate release (IR) and CR formulations. The controlled release formulation (S3) of miglitol induced a profound elevation in the levels as well as increase in the over all area under curve of GLP-1 in comparison to the IR formulation. Data are presented as mean ± SEM.

Table 3. Comparisons between the values of area under curve of glucagon-like peptide-1 obtained after treatment with miglitol over a period of 24 h in the form of placebo, immediate release (IR), and controlled release (S3) formulations.

Parameters	Placebo	IR	S3
$AUC_{(0-t)}$ (pmol·h/L)	596.19 ± 9.35	700.85 ± 1.32	1506.87 ± 7.22
C_{max} (pmol/L)	17.89 ± 3.25	22.56 ± 2.5	53.6 ± 2.9
t_{max} (h)	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00

biphasic (initial rapid peak of GLP-1 followed by a second prolonged but smaller peak) pattern of GLP-1 secretion post-nutrient ingestion has been reported in literature (Lim & Brubaker, 2006). The area under curve (AUC_{0-24}) of S3 formulation (1506.87 ± 17.22 pmol/L) was considerably greater than that of IR (700.85 ± 11.32 pmol/L) ($P = 0.00174$) and placebo formulation (596.19 ± 9.35 pmol/L) ($P = 0.00033$). The AUC_{0-24} and C_{max} values of the S3 formulation were almost 2.2 times that of the IR formulation. Interestingly, all of the three formulations displayed their respective t_{max} value at the time interval of 3 h.

In case of post-prandial glucose levels after administration of the placebo, IR, and S3 formulations, there was no significant difference ($P > 0.05$) in the baseline plasma glucose levels for the three formulations (placebo, IR, and S3) (it was in the range of 94–100 mg/dL) prior to administration of food (between the time intervals of 0 and 1 h).

After administration of food however, there was a considerable elevation (as compared to baseline plasma glucose level, $P < 0.05$) of the post-prandial plasma glucose levels amongst the placebo group. Plasma glucose levels increased steadily and reached the highest concentration (111 ± 5.8 mg/dL) at the time interval of 3 h. The value remained constant until 8 h after which it fell to a concentration similar to that of the baseline (fasting) glucose.

There was a considerably steady attenuation in the plasma glucose concentrations in the group that received S3 formulation post-administration of food. The post-prandial plasma glucose value reached the nadir (86 ± 6.11 mg/dL) at 3 h. Glucose levels increased slightly at the time intervals of 5 h. Interestingly, after administration of the second instalment of food at 5 h, the values fell down steeply again and were similar to those obtained at 3 h (82 ± 2.76 mg/dL). Beyond this time point, the glucose concentration increased to a level of 103 ± 8.34 mg/dL at 8 h and then dipped to a low (84 ± 0.78 mg/dL) at 10 h. Past this time point the concentrations gradually returned to those obtained at the baseline levels.

The plasma glucose profile produced by IR formulation was much different than that of S3 formulation. After administration of food the post-prandial glucose levels started decreasing. It reached a low value of (97 ± 1.57 mg/dL) at 2 h. At the time interval of 3 h surprisingly, the level increased and was similar to that of the placebo (116 ± 1.98 mg/dL). But after this time point it went on declining and reached the lowest value (92.3 ± 6.93 mg/dL) at the time interval of 7 h.

Amongst all of the formulations under consideration, S3 formulation produced lowest ($P < 0.05$) post-prandial glucose AUC_{0-24} value (498.29 ± 1.23) (Table 4). IR formulation had an intermediate AUC_{0-24} value (558.67 ± 1.23) that is it was greater than S3 formulation but lower than

Table 4. Comparison between the AUC values for glucose obtained following miglitol therapy in the form of IR and S3 formulations over a period of 24 h.

Parameters	Placebo	IR	S3
$AUC_{(0-t)}$ (mg·h/L)	587.8 ± 0.58	558.67 ± 1.23	498.29 ± 0.84

the placebo ($P = 0.0076$ in comparison to S3 formulation). As expected the placebo formulation displayed the highest post-prandial glucose AUC_{0-24} value (587.8 ± 0.58) ($P = 0.0034$ in comparison to S3 formulation).

Discussion

The principle aim of this study was to design a CR, bioadhesive formulation for miglitol which would control the post-prandial glucose levels by twofold mechanisms: first, by inhibiting the α-glucosidase enzyme for prolonged period of time and second, by modulation of the GLP-1-mediated pathway. Three different strategies, S1, S2, and S3 were employed to achieve this goal. Strategies S1 and S2 were unsuccessful as both of them were unable to release 100% of the drug at the end of 12 h. S1 and S2 formulations had the drug dispersed in the retarding polymer (EC). Similar to these results, it has been reported that the matrix systems containing low molecular weight EC (similar to one used in this study) and the drug have shown incomplete release of the drug in 24 h (<50% of the drug was released in 24 h) (Quinten et al., 2009a, 2009b). On the other hand, formulation prepared with strategy S3 was able to release 100% of the drug at the end of 12 h. Therefore, strategy S3 was found to be most suitable for the purpose of this study.

The logic behind the design of formulation S3 was as follows. In order to avoid the release of drug in the stomach, the pellets were coated with a methacrylate-based polymer (Eudragit L-100 55). This polymer dissolves only at the pH value of 5.5 and above (Gallardo et al., 2008). At the pH values prevalent in the stomach (which are below 5.5), the film of this polymer presents itself in an insoluble form thus avoiding the bioadhesion of the pellets and the release of the drug in the stomach. After residing in the stomach, pellets move into the duodenum. As the pH in the duodenum is about 5.5, the Eudragit L-100 55 film will dissolve thus exposing the inner coating/layer of HPMC. Once exposed to the contents of the duodenum, the HPMC coat/layer will start swelling causing the polymer chains to interlock with the mucin oligomers. This will lead to the adhesion of the pellets (Ponchel & Irache, 1998) in the duodenum and subsequently in the latter parts throughout the small intestine. After adhesion there will be CR of miglitol from the rate-limiting membrane, EC of the pellets. Similar multilayer CR pellet technology has been reported for other drugs such as omeprazole (He et al., 2009).

This study has thrown light upon the effect of different miglitol formulations on the plasma levels of GLP-1 and post-prandial glucose. The three miglitol formulations assessed were: (a) IR, which was a locally marketed miglitol formulation; (b) CR (S3); and (c) placebo (control which did not contain any miglitol).

In comparison to placebo, all of the miglitol formulations tested in this study influenced plasma GLP-1 levels significantly. It has been reported in literature that miglitol and voglibose produce an elevation and prolongation in the levels of GLP-1 when administered concomitantly with food (Goke et al., 1995; Lee et al., 2002). Miglitol is known to reduce post-prandial glucose levels in patients suffering from type-II diabetes mellitus by twofold mechanisms. First, it acts as a substrate for the α-glucosidase enzyme which is present throughout the brush-border side of the small intestine (Bischoff, 1994). Inhibition of this enzyme delays the digestion of starch and sucrose thus leading to a reduction in post-prandial glucose levels (Bischoff, 1994; Aoki et al., 2007). Second, it elevates the levels of GLP-1, an incretin hormone (Lee et al., 2002).

Interestingly, our results strongly suggest that a CR formulation of miglitol (S3) induces a profound elevation in the levels as well as an increase in the overall AUC_{0-24} of GLP-1, in comparison to the IR and placebo formulations. This is a novel finding. The S3 formulation also resulted in a better, steady, and prolonged control of glucose levels for extended period of time in comparison to the IR formulation.

Plausible explanations as to why the S3 formulation leads to a significantly greater elevation of the plasma GLP-1 levels over the IR formulation are as follows. GLP-1 is secreted because of stimulation of intestinal endocrinal L-cells, that are having a longer apical portion projecting toward the intestinal lumen and a wider basal surface toward the submucosae (Theodorakis et al., 2006), after administration of food due to nutrient stimulators such as glucose and also non-nutrient stimulators (Goke et al., 1995; MacIntosh et al., 2001; Brubaker & Anini, 2003; Arulmozhi & Portha, 2006; Lim & Brubaker, 2006). Similar to human beings, in canines, anatomically, L-cells are densely populated in the lower parts of the small intestine (lower parts of jejunum and ileum) (Damholt et al., 1999).

After oral administration of miglitol S3 pellets, subsequently, they adhere to the mucus in the small intestine. According to our results, statistically, the highest number of pellets were found to be adherent to the mucosa in the jejunal portion (mostly to proximal and the middle portion of the jejunum) of the intestine. Thus, the α-glucosidases in the lower parts of jejunum and ileum would remain relatively unblocked or "free."

Lembcke et al. (1985) have demonstrated that the inhibitory mechanism of miglitol is competitive but reversible. (Inhibition of intestinal α-glucosidases; K_i values mol/L of miglitol is as follows: sucrase = 1.4×10^{-7}, maltase = 3.5×10^{-7}, isomaltase = 5.7×10^{-8}) (Bischoff, 1994). However, despite its high affinity for α-glucosidases, as the binding is reversible, it dissociates from the active site of the enzyme (thus leaving the active site of the enzyme free for the metabolism of complex carbohydrates) and is then absorbed into the plasma through

jejunum (Bischoff, 1994; Sels et al., 1994). Albeit its absorption is through the jejunum, due to the nature of the S3 formulation (CR), there would be a constant replenishment of the miglitol dose in the intestinal lumen over a predetermined period of time. Therefore, due to sustained levels of miglitol in the intestinal lumen (constant release rate from the formulation leading to an increased availability of miglitol locally at the site of action), there would be blockage of the active sites of the enzyme for a larger duration of time (in comparison to IR formulation). As the α-glucosidase enzymes would be blocked for longer time intervals in the proximal parts, one can target higher amounts of complex carbohydrates to the lower parts of the small intestine (by the S3 formulation). The "free" α-glucosidases in the lower parts of intestine would then metabolize these carbohydrates to liberate glucose. As mentioned earlier, there is a high population of L-cells in the lower parts of the intestine. Glucose is a stimulator for L-cells to secrete GLP-1. Thus, greater stimulation of these cells due to presence of more amount of glucose (as compared to IR formulation) will lead to higher plasma levels of GLP-1.

On the other hand, in case of IR formulation, after miglitol dissociates from enzyme active sites, it is absorbed into blood stream. This leads to a drop (as there is no further replenishment of more miglitol from the IR formulation) in the net concentration and local availability of miglitol in the intestinal lumen. Thus, the (free) enzymes in the proximal parts of the intestine will metabolize the carbohydrates, which results in lesser amounts of carbohydrates reaching the lower parts of the intestine. Therefore, in this scenario, there will be far lesser stimulation of L-cells, resulting in lower amount of GLP-1 release (in comparison to S3 formulation).

Amongst all of the α-glucosidase inhibitors, chemical structure of miglitol has the closest similarity to that of glucose. We therefore suspect that miglitol itself may be stimulating the L-cells to secrete GLP-1 (due to its structural similarity with glucose, and L-cells secrete GLP-1 after stimulation by glucose). According to our findings suggest that most of the S3 pellets were found to be adhering in the jejunum and to some extent in the ileum. Thus, there is an increased local availability of miglitol from S3 formulation at the anatomical site of the L-cells which may lead to greater stimulation of the L-cells to secrete GLP-1 in comparison to IR formulation. More experiments however need to be done to support this hypothesis. In literature, interesting findings have been reported in case of miglitol due to its structural similarity with glucose. For example, scientists have hypothesized that miglitol may have blood glucose–lowering effect unrelated to inhibition of α-glucosidase or mediation by GLP-1, possibly due to enhancement of insulin effects on glucose disposition or a suppression of anti-insulin counter-regulatory factors such as glucagon (Joubert et al., 1987).

The results obtained in this manuscript may be correlated with human beings. Similar to canines, in humans, majority of the L-cells are present in the distal jejunum and ileum of the small intestine with only a small proportion present in the duodenum (Sjölund et al., 1983). Thus, the effects of CR of miglitol from the S3 formulation on the plasma GLP-1 levels as well as post-prandial glucose observed in canines may possibly be extrapolated in humans. However, more detailed clinical data needs to be obtained to establish these effects concretely in humans.

Conclusions

A multilayered pellet formulation which was both bioadhesive (because of HPMC polymer) as well as CR (because of the EC layer) was formulated for the α-glucosidase inhibitor, miglitol. The formulation successfully regulated the post-prandial glucose levels after two consecutive meals and also during the time interval between these two consecutive meals, respectively (i.e., the formulation controlled post-prandial glucose levels over a total time span of 7 h). The regulation of post-prandial glucose was via reversible inhibition of α-glucosidase enzyme as well as by modulation of the GLP-1 pathway in non-diabetic canines. We report a novel finding that the CR formulation of miglitol (S3) induced a 2.2-fold elevation in the C_{max} as well as the overall AUC_{0-24} of GLP-1 values in comparison to the non-CR (IR formulation). A 30% concentration of the bioadhesive polymer, HPMC, in the pellets, was found to be most suitable for the purpose of bioadhesion to the intestinal mucosa. Stability studies revealed that the pellets were stable with no changes in the dissolution profiles at both 25°C/60% RH and 40°C/75% RH. Aqueous polymeric coating of the pellets (in contrast to coating using organic solvents) led to a film with uniform morphology and also resulted in release profiles with lower burst effect. In case of pellets prepared by aqueous polymeric coating method, curing played a significant role with regards to the release rate of the drug from the pellets. Thus finally, the S3 formulation resulted in better, steady, and prolonged control of glucose levels over a time period of 7 h in comparison to the IR formulation possibly due to combination of both the effects, prolonged inhibition of the α-glucosidase enzyme and enhanced plasma GLP-1 levels.

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Declaration of interest

The authors do not have any declarations of interest.

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