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Design and evaluation of oral bioadhesive controlled release formulations of miglitol, intended for prolonged inhibition of intestinal α-glucosidases and enhancement of plasma glucagon like peptide-1 levels☆

Mangesh C. Deshpande[a,*], Vobalaboina Venkateswarlu[a], Ravi K. Babu[b], Ravi K. Trivedi[b]

[a] Integrated Product Development, Dr. Reddy's Laboratories Ltd., Bachupally, Qutubullapur, Hyderabad 500072, Andhra Pradesh, India
[b] Discovery Research, Dr. Reddy's Laboratories Ltd., Miyapur, Hyderabad 500049, India

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ABSTRACT

α-Glucosidase enzyme is present ubiquitously throughout the lumen of the small intestine. It is responsible for the breakdown of complex into simple carbohydrates. α-Glucosidase inhibitors such as miglitol, are drugs that have greater affinity towards this enzyme in comparison to carbohydrates. Miglitol regulates the postprandial glucose levels directly by inhibiting the enzyme reversibly and also indirectly by including the secretion of glucagon like peptide-1 (GLP-1). The aims of this study were (i) to design a controlled release (CR) mucoadhesive (in the intestine) formulation of miglitol which would inhibit the α-glucosidase enzyme for a longer duration of time (in comparison to the non-controlled release (IR) formulation) thus reducing the dosing frequency, and also controlling the postprandial glucose levels more effectively over a longer period of time; (ii) to assess the effect of different formulation parameters on the release of miglitol *in vitro* from the CR pellets; (iii) to evaluate the mucoadhesion of pellets in the intestine *ex vivo*; (iv) to study the effect of formulation parameters on plasma GLP-1 levels; and (v) to find out the effect of formulations on postprandial glucose levels. The data obtained was analysed to find out whether there was a correlation between these different parameters. Four controlled release formulations (CR1, CR2, CR3 and CR4) of miglitol comprising of multilayered pellets were designed successfully. The CR4 formulation containing 30% of 20 cps of ethyl cellulose (the retarding layer of the formulation) displayed slowest release of miglitol *in vitro* in comparison to other formulations. We designed an *ex vivo* experimental setup for studying the mucoadhesion of the pellets in the lumen of the intestine. Results indicated that amongst all of the adherent pellets, 5% were found to be adhering in the duodenal region, 61% in the jejunum, 32% in the ileum and 2% in the colon. Two of the controlled release formulations CR1 and CR4 were evaluated *in vivo* in dogs. Both the formulations displayed significantly higher and more prolonged (greater AUC) levels of GLP-1 in comparison to either the placebo or the immediate release (IR) formulations. They even displayed a significantly better control of postprandial glucose in comparison to either placebo or IR formulations. However, a comparison between the two controlled release formulations (CR1 and CR4) revealed that the plasma GLP-1 (AUC by CR1 = 63.1 ± 1.32 and CR4 = 66.2 ± 0.82) and postprandial glucose values due to both the formulations were rather similar despite their differences in *in vitro* release as well as pharmacokinetic profiles (plasma miglitol AUC of CR1 = 16.17 ± 4.11 and CR4 = 27.17 ± 4.33).

1. Introduction

Major portion of the dietary carbohydrates comprises of starch and sucrose. These are metabolised by enzymes called as α-glucosidases into monosaccharides in the small intestine before they are absorbed (Elsenhans and Caspary, 1987). If the diet consists of refined carbohydrates then these get metabolised in the upper part of the small intestine leading to a postprandial rise in blood glucose rapidly. The research by Puls et al. (1983) based on the basic underlying principle, that if there is an inhibition of α-glucosidases in the proximal parts of the intestine, then it would delay the digestion of starch and sucrose, leading to inhibition in the rise in blood postprandial glucose values, has resulted in the discovery of α-glucosidase inhibitors. For example, α-glucosidase inhibitors such as acarbose (Hillebrand et al., 1979), voglibose (Goke et al., 1995) and miglitol (Bischoff, 1994) have high affinity for the enzyme in comparison to starch. This leads to delay in carbohydrate metabolism, prolongation of digestion time, transport of carbohydrates not digested in the upper parts to the lower parts of the

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* Corresponding author. Tel.: +91 9963029502 fax: +91 8458279619.
E-mail address: mangeshcd@yahoo.co.uk (M.C. Deshpande).

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intestine, and reduction in the rate of glucose absorption, finally resulting in inhibition of the rise of postprandial glucose levels (Hillebrand et al., 1979).

Miglitol reduces postprandial glucose by two mechanisms which are both direct and indirect. It directly inhibits the α-glucosidase enzyme reversibly (Bischoff, 1994) and secondly, induces the secretion of glucagon like peptide-1 (GLP-1). GLP-1 is an incretin hormone which indirectly regulates the postprandial glucose levels. Its physiological actions, responsible for regulation of postprandial glucose levels, within the normal physiological range, include stimulation of insulin secretion in a glucose dependant manner (Kreymann et al., 1987; Nauck et al., 1993a), suppression of glucagon (Nauck et al., 1993b; Orskov et al., 1988), deceleration of gastric emptying (Wettergren et al., 1993), reduction in appetite and induction of satiety (Gutzwiller et al., 1999) and it may also have an effect on insulin sensitivity (Zander et al., 2002). It also induces differentiation of endocrine precursor cells into mature β-cells, stimulates replication of pancreatic β-cells and also inhibits their apoptosis in response to different toxic stimuli (Hui et al., 2001, 2003).

There is presence of mucus, towards the luminal surface, throughout the length of the intestine. Mucus consists of high molecular weight hydrated glycoproteins. The complex nature of mucus, offers many opportunities for the development of adhesive interactions for small polymeric particles, through non-specific or physicochemical interactions. A few polymers can adhere to the wet mucosal surface because of hydrogen bonding or van der Waal's forces (Ponchel et al., 1987a, 1987b). α-Glucosidase is enzyme present throughout the luminal surface of the small intestine. Considering these facts we carried out experiments to design controlled release mucoadhesive formulations of miglitol which would reversibly inhibit the α-glucosidase enzyme spatially for a longer duration of time (in comparison to immediate release, IR formulation).

The aims of this study were (i) to design a controlled release mucoadhesive (in the intestine) formulation of miglitol which would inhibit the α-glucosidase enzyme for a longer duration of time (in comparison to the non-controlled release formulation), thus reducing the dosing frequency, and also controlling the postprandial glucose levels more effectively over a longer period of time; (ii) to assess the effect of different formulation parameters on the release of miglitol *in vitro* from the CR pellets; (iii) to evaluate the mucoadhesion of pellets in the intestine *ex vivo*; (iv) to study the effect of formulation parameters on plasma GLP-1 levels; and (v) to find out the effect of the formulations on postprandial glucose levels. The data obtained was analysed to find out whether there was a correlation between these different parameters.

2. Materials and methods

2.1. Materials

Miglitol was purchased from Biocon (Bangalore, India). A commercially available conventional, non-controlled release formulation of miglitol, MISOBIT® (Lupin Labs, India) was used as the IR formulation in the study. Ethyl cellulose (EC) and hydroxypropyl 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 Degussa, Germany. Rosiglitazone was synthesised in house by Medicinal Chemistry Department, Dr. Reddy's Foundation, Hyderabad by a method reported in the literature and was found to be 99% pure. Celphere® CP 305 was purchased from Signet Chemical Corporation, India. Pedigree, food for the dogs, during the experiment, was obtained from Pedigree (www.pedigree.com). Size "0" empty, conventional hard gelatine capsules were obtained from Associated Capsules, Mumbai, India. Water used for making solutions and buffers was from Milli Q water purification system (Millipore, MA, USA).

Unless otherwise indicated, all the solvents, acids and bases were obtained from Merck (Mumbai, India), and all the reagents, chemicals and buffer salts were obtained from Sigma–Aldrich (St. Louis, USA).

2.2. Methods

2.2.1. Formulation of controlled release pellets of miglitol

Celpheres (grade CP 305, average size between 300 and 500 μm) comprising of an inert material (pure spheronized microcrystalline cellulose) were used as the core of the pellets. Drug and different layers of polymer were deposited on this core. The deposition/coating/layering of the drug on the celpheres was carried out using a fluidized bed coating machine (FBC, Pam Glatt model no. GPLG 1.1). The inlet and outlet temperatures of the FBC were 60 and 50 °C respectively. The solution was sprayed at the rate of 1 g/min with a 1 mm size nozzle diameter at 2 bar atomization pressure.

Following is the procedure for manufacture of bioadhesive pellets (100 g). Celpheres (100 g) were layered with the required concentration of the drug and hydroxypropyl methyl cellulose (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. The 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 ethyl cellulose). A 5% (w/w) solution of EC in solvents gave the best results. In the next step, 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 Fig. 1.

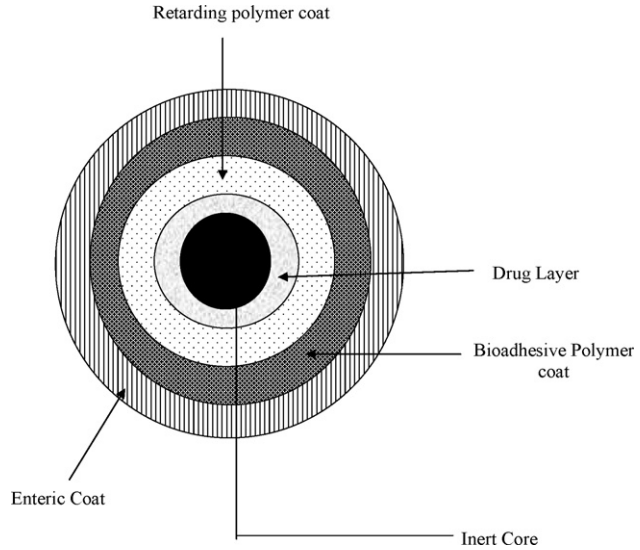

Fig. 1. Schematic diagram of controlled release pellets of miglitol. Celphere® CP 305 pellets (inert cellulose core as the base for the drug delivery system) were layered with drug. This was followed by layering with different concentrations and viscosity grades of EC. The next step was layering with HPMC (6 cps, 30%, w/w). Finally there was enteric coating with Eudragit L 100-55 polymer (30%, w/w).

2.2.2. Preparation of controlled release samples of miglitol (CR)

Pellets prepared as described in Section 2.2.1 containing an equivalent amount of 50 mg of miglitol by weight were filled in size "0" conventional hard gelatin capsule (common capsule). Pure miglitol drug powder (25 mg) was also placed in the same capsule.

2.2.3. Determination of the in vitro release profile of miglitol from CR pellets

Dissolution studies were carried out in a USP 27 Apparatus II (Paddle method, Electrolab, TPT-08L, Mumbai, India). HCl solution (0.1N, 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 6.8 by addition of trisodium hydrogen phosphate solution (8%, w/v), and dissolution was continued in this buffer until the final time point. Temperature of 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 (10 ml at each time point) were withdrawn by an autosampler (Electrolab Fraction Collector FC-12, Mumbai, India) at predetermined time points ($n = 3$). Each sample obtained in 0.1N HCl was centrifuged at 3000 rpm for 10 min. The clear supernatant (10 ml) was diluted with sodium hydroxide (0.1N, 10 ml). 40 μl of the sample was loaded into the HPLC system. On the other hand, sample in phosphate buffer (pH 6.2) was centrifuged at 3000 rpm (Heraeus Biofuge Stratos Centrifuge, Model: D-37520, Thermoelectron corporation, Germany) for 10 min. 20 μl of this sample was loaded into the HPLC system. All the samples were prefiltered through a 0.45 μm membrane (Whatman Inc., Clifton, NJ, USA) before injection into the HPLC system.

The amount of miglitol in the dissolution media was determined using a Waters (Milford, MA, USA) high performance liquid chromatography (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 a 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 was collected and integrated using Empower® Version 5.0 software (Des Plaines, IL, USA). Calibration curve for miglitol was prepared in both 0.1N HCl as well as phosphate buffer (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) (Costa and Lobo, 2001; Moore and Flanner, 1996). The equation describing the similarity factor is as follows:

$$f_2 = 50 \log \left\{ \left[1 + \frac{1}{n} \sum_{t=1}^{n} W_t (R_t - T_t)^2 \right]^{-0.5} \times 100 \right\}$$

where R_t and T_t are the percent drug dissolved at each time point for the reference and test products, n is the number of dissolution sample times, t is the time sample index and W_t is an optional weight factor (in the current work $W_t = 1$). If the two profiles are identical or similar then $f_2 = 100$. Values of $f_2 \geq 50$ indicate similarity of two dissolution profiles.

2.2.4. Ex vivo bioadhesion testing of the pellets to rat intestine

All the animal experiments were approved by the Dr. Reddy's Foundation's 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 ILAR (Institute of Laboratory Animal Resources, USA) guidelines.

The assembly for the *ex vivo* bioadhesion study is shown in Fig. 2. 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 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 (Fig. 3). 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

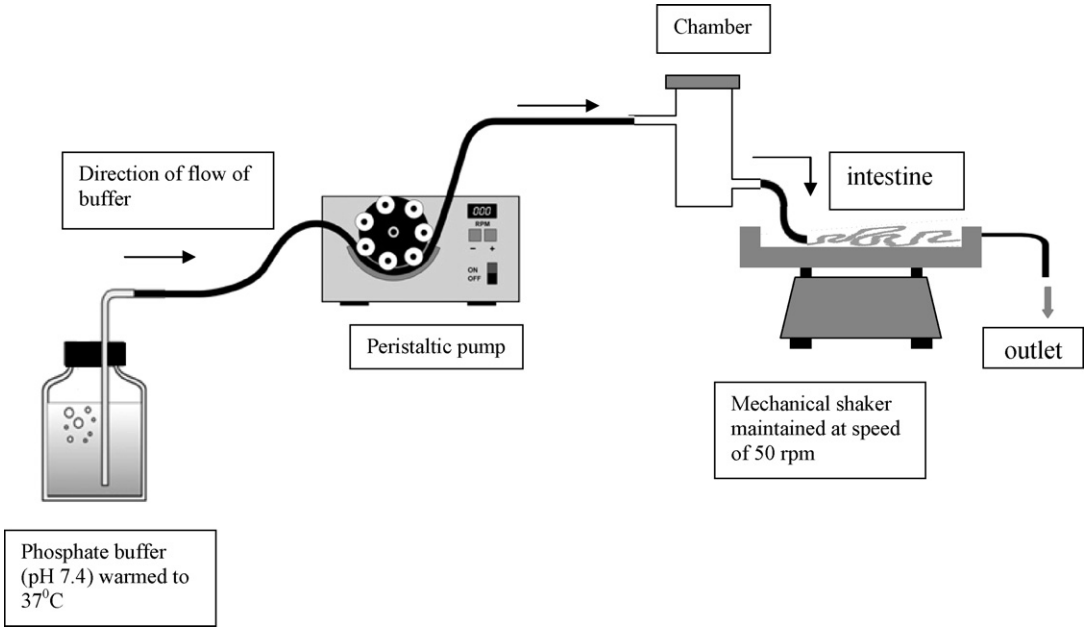

Fig. 2. Experimental setup for *ex vivo* assessment of adhesion of the controlled release pellets to the intestine. A fixed number of pellets, placed in the reservoir chamber were bathed with phosphate buffer saline (PBS) (pH = 7.2) for 8–10 min with stirring. Post-10 min, PBS was pumped through the reservoir chamber flowing into the intestine at a flow rate of 0.5 ml/min. A count of the number of pellets adhering at different segments of the intestine was obtained at the end of the experiment.

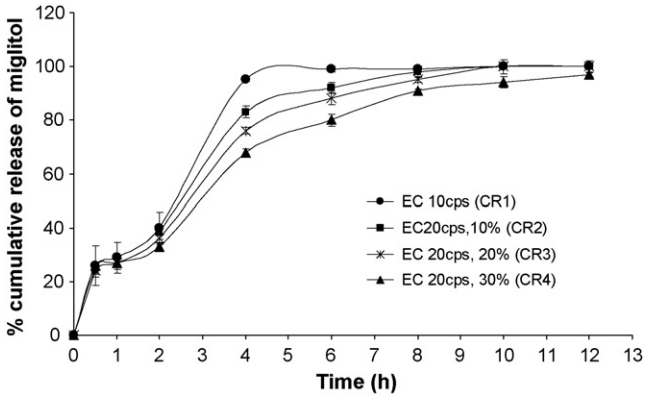

Fig. 3. Dissolution profile of pellets containing miglitol. Dissolution was carried in 0.1N HCl for the first 2 h followed by phosphate buffer pH 6.8 until the final time point (i.e. 12 h) ($n = 3$). The figure illustrates the effect of change in viscosity and percentage of EC on the release profile of miglitol from the controlled release system.

reservoir at a constant flow rate (1 ml/min) into the chamber and eventually through the intestine, during the experiment. The intestine was bathed 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.

A fixed number of drug loaded pellets (1000 pellets of CR4 formulation) 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 un-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.

2.2.5. Preparation of immediate release (IR) samples of miglitol

Commercially available, conventional, immediate release (non-controlled release) tablet formulations of miglitol (MISOBIT®, Lupin Labs, India) were used as immediate release (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.

2.2.6. 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 (common 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.

2.2.7. 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 24 h. They were fed with Pedigree® (commercially available dog feed) at predetermined time intervals and water was provided *ad libitum*. All animals were maintained at a controlled temperature (25 ± 1 °C) under 12 h light (0600–1800 h) and 12 h dark (1800–0600 h) cycle. All 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 ILAR (Institute of Laboratory Animal Resources, USA) guidelines.

The complete set of study animals were fasted overnight before the oral administration of the respective formulations.

The population of beagle dogs was randomly divided into three treatment groups. Placebo formulation was administered to the first group. The second group was treated with CR and the third with IR formulations respectively. There was no crossover 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.

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).

For the purpose of estimation of miglitol levels in plasma, 0.6 ml of the blood sample was transferred to eppendorff tubes containing ethylenediamine tetra-acetic acid (EDTA, 10 μl, from a stock solution of 200 mg/ml) to prevent coagulation of blood. It was then centrifuged at 6000 rpm for 5 min. Supernatant plasma was removed and stored at −20 °C for further analysis.

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 to a tube (chilled on ice) containing vidagliptin, a dipeptidyl peptidase IV (DPP IV) inhibitor (to a final concentration of 1 μM) to prevent GLP-1 degradation and ethylenediamine tetra-acetic acid (EDTA, 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 centrifugated 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.

2.2.8. Estimation of plasma glucose levels

Plasma glucose levels were estimated using Vitalab Selectra-2 autoanalyser (Vital Scientific, Netherlands) with the aid of Merck diagnostic kit (Merkotest, 11862900011046, Glucose based GOD-POD).

2.2.9. Determination of plasma GLP-1 values

Plasma GLP-1 values were determined by using a Glucagon-Like-Peptide 1 (Active) ELISA Kit 96-Well plate (Cat.# EGLP-35K) from Linco Research Labs (now Millipore, USA).

2.2.10. Assessment of pharmacokinetic parameters

2.2.10.1. Sample preparation. Rosiglitazone (internal standard) (10 μl of 1 μg/mL; equivalent to 10 ng/mL) was added to the plasma sample (100 μl) and vortexed for 10 s on a cyclomixer. Acetonitrile (2.0 ml) was added to this mixture and vortexed for 2 min (Vibramax, Brinkmann Instruments, Inc.), followed by centrifugation for 4 min at 3200 rpm (Remi Instruments, Mumbai, India). The clear supernatant was then (1.8 ml) taken in a separate glass tube and evaporated to dryness with a gentle stream of nitrogen (Zymark® Turbovap®, Kopkinton, MA, USA) for 15 min. Finally, the residue was reconstituted with mobile phase (150 μl) for 40 s and loaded on LC–MS/MS for analysis.

2.2.10.2. Calibration curve. Calibration curve was prepared by addition of drug solution (10 μl) to 90 μl of controlled plasma in the range of 0.005–5.0 μg/mL (0.005, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0 and 5.0 μg/mL).

2.2.10.3. Chromatographic conditions. An agilent 1100 series coupled with MDS Sciex MS/MS detector was used to inject 20 μl of processed sample on an Inertsil ODS column (4.6 mm × 100 mm, 5.0 μm) into the Turboion spray at 450 °C. The isocratic mobile phase (0.01 M ammonium acetate pH 6.0:acetonitrile::15:85) was delivered at a rate of 0.6 ml/min. The ion spray voltage was set at 5500. The CE, CXP, DP and EP were set at 28, 5, 44 and 10 and 40, 8, 50 and 10 for miglitol and IS respectively. The common parameters, i.e. curtain gas, auxiliary gas, nebulizer gas, and CAD were 10, 30, 40, and medium respectively. The interface heater was kept on. The product ions of miglitol ($Q_1 \rightarrow 208.1$) and IS ($Q_1 \rightarrow 358.2$) were 146.1 and 135.1 respectively. Data acquisition was performed with Analyst 1.4.1 software.

2.2.10.4. Statistical analysis. Statistical analysis was performed to find out the significance of variation between sets of data obtained. One-way analysis of variance (ANOVA) followed by Bonferri/Dunnet's test was applied to evaluate the statistical difference between experimental results/groups. *p*-Value less than 0.05 was considered as statistically significant. All the values are expressed as mean ± S.E.M.

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.

3. Results

3.1. Design of the miglitol bioadhesive pellet formulation

The formulation strategy for the design of miglitol controlled release pellets is shown in Fig. 1 and Table 1. Miglitol at a concentration of 33% (w/w) along with HPMC 5 cps, 2.27% (w/w) was deposited on the Celphere® CP 305 pellets. This constituted the drug reservoir. EC (along with plasticizer, diethyl phthalate) was coated on the pellets containing miglitol reservoir. EC layer was the rate limiting membrane for controlled release of miglitol. The next coat consisted of HPMC (6 cps), 30% (w/w). This polymer would facilitate the adhesion of the pellets to the intestinal mucosa. Finally, the pellets were coated with Eudragit L 100-55. This was the enteric coat that would avoid exposure of the inner coats of the pellets to the gastric media during the residence time of the pellets in the stomach. Various formulations of miglitol containing different viscosity grades and concentrations (%, w/w) of EC were prepared to achieve controlled release profiles of miglitol, as shown in Table 1.

3.2. In vitro dissolution studies on miglitol CR pellets

The results for dissolution studies carried out on various formulations are shown in Fig. 3. At the end of 2 h, 40%, 38%, 36% and 33% cumulative amounts of miglitol were released from CR1, CR2, CR3 and CR4 formulations respectively. 95% of miglitol was released from CR1 formulation at the time interval of 4 h. The release curve plateaued off for this formulation after this time point. 83% and 76% of miglitol were released from CR2 and CR3 formulations respectively at the end of 4 h. At the end of 6 h, CR2 and CR3 formulations released 92% and 88% of miglitol respectively. Readings taken at the next time interval (8 h) suggested that almost 95–96% of the drug was released from both of these formulations. The cumulative amounts of miglitol released from CR4 formulation were equivalent to 68%, 80%, 91%, 94% and 98% at the end of 4, 6, 8, 10 and 12 h respectively. Thus, CR4 formulation had a significantly retarded release of miglitol in comparison to all the other formulations tested ($p < 0.05$).

Table 1
Compositions of various controlled release formulations of miglitol.

No.	Excipients (%, w/w)	Formulations				
		Placebo	CR1	CR2	CR3	CR4
1	Miglitol	–	33	33	33	33
2	Ethyl cellulose (10 cps)	20	20	–	–	–
3	Ethyl cellulose (20 cps)	–	–	10	20	30
4	HPMC (6 cps)	30	30	30	30	30
5	Eudragit L 100-55	30	30	30	30	30

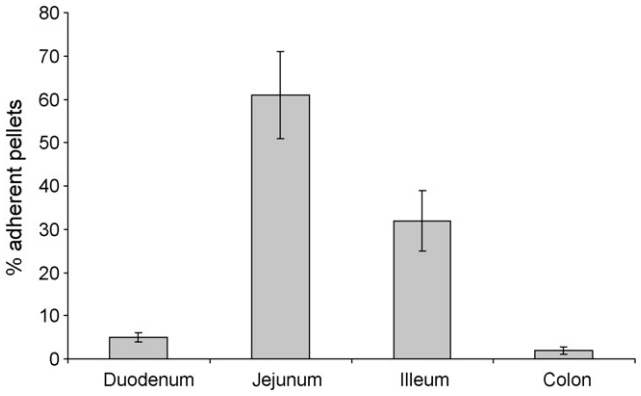

Fig. 4. Results for *ex vivo* mucoadhesion of pellets at different segments of the intestine ($n = 3$). Data is presented as mean ± S.E.M. Majority of the pellets were adherent in the jejunum.

3.3. Ex vivo studies on adhesion of the pellets to the intestine

The percentage of pellets adhering to different regions of the intestine is displayed in Fig. 4. By and large in the experiment, about 4% of the total number of pellets tested for mucoadhesion, were found to be non-adhering to the intestinal mucosa. Amongst the population of the adherent pellets, 5% were found to be adhering in the duodenal region, 61% in the jejunum, 32% in the ileum and only 2% in the colon.

3.4. Influence of various formulations on plasma GLP-1 levels

The influence of various formulations on plasma GLP-1 levels (normalized to placebo readings) is displayed in Fig. 5. The fasting plasma GLP-1 levels in test animals treated with any of the formulations were almost similar ($p > 0.05$ in each case). For all the formulations tested, the plasma GLP-1 levels escalated after administration of first feed (food was administered at $T = 1$ h). Both the controlled release formulations displayed significantly higher plasma GLP-1 values in comparison to the IR formulation until 8 h ($p < 0.05$ in each case). The plasma GLP-1 profiles of formulations (CR1 and CR4) however were very similar ($p > 0.05$) throughout all the time points tested during the experiment and they displayed a peak value at the same time point ($T = 5$ h). The values dropped down significantly after this time point until the last time point ($T = 24$ h) for both these formulations. The area under curve (AUC) values of the GLP-1 produced by the formulations is displayed in Table 2. The IR formulation produced an AUC value of 20.9 ± 3.28 pmol/L. The CR1 and CR4 formulations produced AUC values of 63.1 ± 1.32 and 66.2 ± 0.82 pmol/L respectively. Thus, overall the AUC of both the controlled release formulations was significantly greater than that of the IR formulation. Surprisingly, there was no significant difference in the AUC values of CR1 and CR4 formulations ($p > 0.05$ in each case).

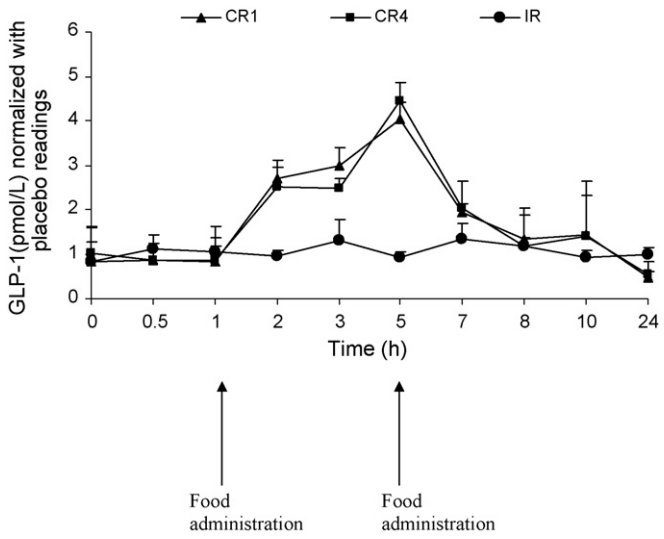

Fig. 5. Plasma GLP-1 (pmol/L) levels at different time intervals due to IR and CR1 and CR4 formulations normalized with placebo readings ($n = 3$). Data is presented as mean ± S.E.M. The time points at which food was administered are designated by ↑.

Table 2
Comparisons between the values of the area under curve of glucagon like peptide-1 (GLP-1) obtained after treatment with miglitol over a period of 24 h in the form of immediate release (IR) and two controlled release formulations, CR1 and CR2.

Parameters	IR	CR1	CR4
$AUC_{(0-t)}$ (pmol h/L)	20.9 ± 3.28	63.1 ± 1.32	66.2 ± 0.82

3.5. Postprandial glucose level measurements post-administration of different formulations

Postprandial glucose levels after administration of different formulations (placebo, CR1 and CR4) are displayed in Fig. 6. The plasma glucose was maintained at baseline levels until the time interval of 1 h. After administration of the first instalment of food at $T = 1$ h, there was a significant rise in the plasma glucose values (in comparison to baseline values) in the group treated with placebo

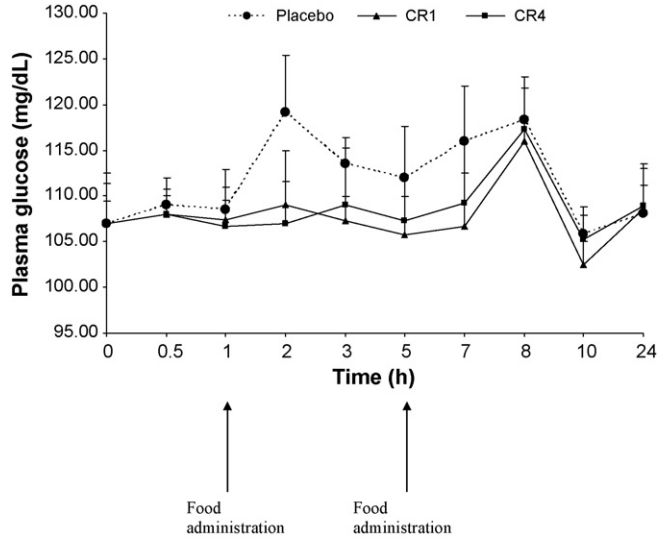

Fig. 6. Postprandial plasma glucose levels (mg/dL) due to CR1 and CR4 and placebo formulations at different time points. Each experiment was repeated three times ($n = 3$). The baseline glucose reading is represented by $T = 0$. Data is presented as mean ± S.E.M. The time points at which food was administered are designated by ↑.

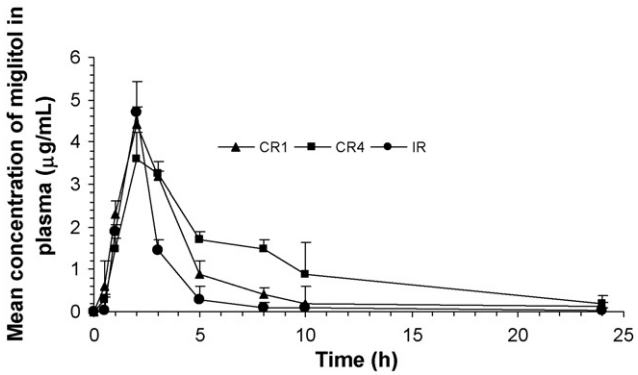

Fig. 7. Comparative pharmacokinetic data of CR1, CR4 and IR formulations of miglitol ($n = 3$). Data is presented as mean ± S.E.M.

Table 3
Pharmacokinetic parameters after oral administration of different formulations of miglitol.

Parameters	IR	CR1	CR4
$AUC_{(0-t)}$ (μg h/ml)	10.11 ± 1.39	16.17 ± 4.11	27.17 ± 4.33
C_{max} (μg/mL)	4.7 ± 0.72	4.4 ± 0.41	3.6 ± 0.62
t_{max} (h)	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 3.21
K_{el} (h^{-1})	0.10 ± 0.02	0.10 ± 0.02	0.11 ± 0.08
$t_{1/2}$ (h)	7.29 ± 1.78	6.95 ± 1.85	7.99 ± 4.25

formulation. On the contrary, there was steady attenuation in the plasma glucose values after the same time point in the groups treated with CR1 and CR4 formulations. Plasma glucose values were maintained effectively in the low range of 105–109 mg/dL for both the CR formulations until the time interval of 7 h. The placebo formulations treated group however, displayed plasma glucose values between 105 and 120 mg/dL throughout the time course of the experiment. In the placebo treated group, as one would expect, there was a sharp rise in the plasma glucose levels after administration of each meal (post-administration of meal at $T = 1$ and 5 h; the plasma glucose values were raised to 119 and 116 mg/dL respectively). The most interesting and significant finding however, was that there was no significant difference ($p > 0.05$) in the plasma glucose levels for the CR1 and CR4 formulations treated groups over the entire course of the experiment.

3.6. Pharmacokinetic profiles of miglitol formulations

The pharmacokinetic data for different miglitol formulations is shown in Fig. 7 and Table 3. Results indicate that the plasma miglitol concentrations increased significantly for all the formulations from the time intervals of $T = 0$ until $T = 2$ h. All the formulations displayed peak values at $T = 2$ h. The peak plasma levels of the formulations were 4.7 ± 0.72, 4.4 ± 0.41 and 3.6 ± 0.62 μg/mL for IR, CR1 and CR4 formulations respectively. After $T = 2$ h there was a decline in the plasma miglitol levels. The AUC values for both the controlled release formulations were significantly greater than that of IR formulation ($p < 0.05$) (Table 3). A comparison of the AUC values for controlled release formulations revealed that the numerical value obtained for CR4 (27.17 ± 4.33 μg/mL) was significantly greater than that of CR1 formulation (16.17 ± 4.11 μg/mL).

4. Discussion

The primary aim of this study was to optimize and characterize different controlled release formulations of miglitiol. The most promising formulations were assessed for their effect on the plasma GLP-1 and postprandial glucose levels in canines. A battery of exper-

iments were carried out to establish the *in vitro* release profile of the formulations, *ex vivo* adhesion of the CR pellets to the intestinal mucosa and finally, *in vivo* assessment to study the impact on plasma GLP-1 and postprandial glucose levels. Different controlled release formulations were compared with a placebo (this formulation was same as the CR formulation with the only exception being that it was devoid of miglitol) as a comparator and a non-controlled release formulation (IR; which was a locally marketed miglitol formulation) wherever necessary.

For the purpose of *in vitro* and *in vivo* studies, the CR formulations were prepared by filling pure miglitol powder (25 mg) along with controlled release mucoadhesive pellets, containing an equivalent amount of 50 mg of miglitol, in a size "0" conventional hard gelatine capsule (common capsule). The CR formulation in the capsule therefore consisted of three doses of miglitol (each dose being 25 mg) with the first dose in the form of pure miglitol powder and the remainder of the two doses in the form of controlled release pellets. Pure miglitol powder would constitute the first dose. It was filled in the capsule along with CR pellets because it has to be co-administered with the first bite of a meal by the patient (Bischoff, 1994). The controlled release pellets have a slower onset of action and therefore they would not be very effective in controlling the rise in postprandial glucose after the first meal. Hence we opted for 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 controlled release pellets) would control the postprandial glucose levels after the second meal and also during the time interval between these two consecutive meals respectively. The CR pellets were designed to control the postprandial glucose levels over a time span of 7 h.

Miglitol was deposited on the core of the pellets (Celphere® CP 305) thus forming a reservoir of the drug. Different viscosity grades and concentrations of ethyl cellulose were used in various formulations to effectively control the release of miglitol from the formulations (Table 1). HPMC was coated over ethyl cellulose. As pellets reach the intestine, HPMC coat/layer will start swelling causing the polymer chains to interlock with the mucin oligomers throughout the intestine. This will lead to adhesion of the pellets (Ponchel and Irache, 1998) in the duodenum and subsequently, the latter parts of the small intestine. After optimization of different viscosity grades (high and low) and concentrations of HPMC (data not shown), a concentration of 30% of low viscosity grade (6 cps) was found to be the most suitable for the purpose of mucoadhesion of pellets. Finally, the pellets were provided with an enteric coating (Eudragit L 100-55, 30%). The reason for enteric coating was to avoid the release of miglitol in the stomach. This polymer dissolves only at the pH value of 5.5 and above (Gallardo et al., 2008) thus allowing the bioadhesion of the pellets and release of miglitol only in the intestine and not in the stomach.

In vivo, once the pellets are ingested, they would reside in the stomach for up to 2 h (gastric emptying time is up to 2 h). After 2 h they would be transported into the intestine where the pH is higher than that in stomach. Therefore, to emulate the conditions prevalent in the stomach *in vitro*, dissolution studies were carried out in 0.1 N HCl (pH 1–2) for the first 2 h. After 2 h the dissolution studies were carried out in phosphate buffer pH 6.8 to emulate the pH present in the intestinal lumen. The percent cumulative amounts of miglitol released from CR1, CR2, CR3 and CR4 formulations were 40%, 38%, 36% and 33% respectively at the end of 2 h. This was due to the first dose present in the capsule in the form of pure miglitol powder (as described in Section 2.2.5). Theoretically, one would expect only one-third of the total dose of miglitol present in the capsule (i.e. 33%) to be released at the end of 2 h as that dose is in the form of free miglitol powder. Only CR4 complied with this theoretical value. The cumulative amounts of miglitol released from CR1, CR2 and CR3 formulations were greater than 33% although Eudragit L 100-55 polymer is insoluble at pH 1–2. This could be because, it has been reported that Eudragit films can form cavities at this pH due to leaching of the plasticizer from the polymer film (Bando and McGinity, 2006). The drug may have potentially been released through these cavities formed in the polymeric film. Based on this hypothesis one would assume that there would also be formation of cavities in the Eudragit layer of CR4 formulation which may lead to the subsequent leakage of the drug from the pellets at pH 1.2. Interestingly, only 33% of drug was released at the end of 2 h from this formulation. An explanation for this could be that, although there were formation of pores in the Eudragit layer, nonetheless, the high concentration and viscosity of the EC (20 cps, 30%) present in this formulation may have retarded the release of drug sufficiently causing very little or almost negligible amount of the drug to be released in the first 2 h at pH 1.2.

Formulations containing higher concentrations (higher than that present in CR4, i.e. 20 cps, 30% EC) of EC were also manufactured and tested *in vitro*. However, all these formulations had similar release profiles as that of CR4 (higher concentrations and viscosity grades of EC did not produce greater retardation in the release of miglitol). Therefore, we used CR4 formulation for further (*ex vivo* and *in vivo*) studies.

The *ex vivo* bioadhesion experiment revealed that a total of 95.46% of the total number of pellets used in the experiment were found to be adhering to different parts of the intestine. Amongst the population of the adherent pellets, approximately 5% of the pellets were found to be adherent in the duodenal region of the intestine. Statistically, the highest number of pellets were found to be adhering in the jejunum, followed by ileum, duodenum and finally colon. It has been reported that majority of the bioadhesive particles accumulate by and large in the jejunal portion of the small intestine (Ponchel et al., 1997). The adhesion was due to the interaction of HPMC chains, with glycoproteins of the mucus present in the intestinal lumen. This kind of interaction of HPMC nanoparticles with glycoproteins has been reported earlier (Pimienta et al., 1990).

The two CR formulations of miglitol tested in this study induced profound elevation in the levels as well as increase in the overall AUC of GLP-1, in comparison to the IR and placebo formulations, in the presence of food. We have reported earlier that a controlled release formulation of miglitol raises the plasma GLP-1 levels significantly in comparison to an IR formulation (Deshpande et al., 2009). Briefly, GLP-1 is secreted because of stimulation of intestinal endocrinal L cells, that are comet-like in shape with a longer apical portion projecting towards the intestinal lumen and a wider basal surface towards the submucosae (Theodorakis et al., 2006), after administration of food due to nutrient stimulators such as glucose (Arulmozhi and Portha, 2006; Lim and Brubaker, 2006). In canines, anatomically, L cells are predominantly present in the lower parts of the small intestine (lower parts of jejunum and ileum) (Damholt et al., 1999).

Basically miglitol is a substrate for the α-glucosidase enzyme which is present throughout the brush-border side of the small intestine (Bischoff, 1994). This enzyme is responsible for conversion of disaccharides such as sucrose into monosaccharides (e.g. glucose). Inhibition of this enzyme delays the digestion of starch and sucrose thus leading to a reduction in postprandial glucose levels (Bischoff, 1994). Miglitol binds to, and inhibits the enzyme reversibly in the proximal parts of the intestine (duodenum and jejunum). Thus, carbohydrates not digested in the upper part of the small intestine are transported to the lower parts of the intestine where they are digested finally (Hillebrand et al., 1979). Endocrinal GLP-1 secreting L cells are present abundantly in distal jejunum and ileum (Eissele et al., 1992; Leduque et al., 1982; Sjolund et al., 1983). Therefore, the significantly greater amount of carbohydrates reaching the lower parts of intestine stimulates these endocrinal L

cells to secrete GLP-1. Hence, there is a net increase in the plasma levels of GLP-1 due to miglitol.

However, miglitol is absorbed into the plasma through jejunum (Bischoff, 1994). As the drug is acting locally (in the lumen of the intestine), its absorption in the plasma is not desired. Miglitol from IR formulation would inhibit the enzyme in the proximal parts of the intestine for a specific duration of time and finally get absorbed into the plasma from jejunum. On the other hand, the CR formulation would inhibit the enzyme for a longer time interval, in the proximal parts, due to adhesion of majority of the pellets in the duodenum and jejunum as seen in our *ex vivo* bioadhesion data and release of miglitol in a controlled fashion. Therefore, higher amounts of complex carbohydrates would be made available to the distal parts of the small intestine by the CR formulation as compared to IR formulation. The amount of GLP-1 secreted by L cells in the distal parts of the intestine is directly proportional to the concentration of carbohydrates locally. Hence, the CR formulations led to much higher and prolonged plasma concentration of GLP-1 which subsequently translated in a better, steady and prolonged control of glucose levels for an extended period of time in comparison to the IR formulation.

The dissolution data suggested that CR4 formulation had a considerably slower release rate of miglitol in comparison to CR1 formulation (their release profiles were dissimilar as seen by application of similarity factor f_2 described by Moore and Flanner (1996), and also by application of statistical tests. If $f_2 > 50$ then dissolution profiles are defined as similar. In this case the f_2 was <50). The pharmacokinetic data also suggested that the release of miglitol from the CR4 formulation was significantly retarded in comparison to CR1 formulation. This result was in line of agreement with the *in vitro* data. Pharmacokinetic studies revealed that the overall AUC of the CR4 formulation (27.17 ± 4.33) was greater than that of the CR1 formulation (16.17 ± 4.11). A plausible explanation for this could be, that due to significantly retarded release of miglitol from CR4 formulation in comparison to CR1 formulation, there may have been slower and complete absorption of the drug into the blood stream leading to a higher value of AUC in case of CR4 formulation. A higher value of AUC possibly implies that the availability of miglitol at the local site (lumen of intestine) is for a longer duration of time. The increased local availability suggests that the α-glucosidase enzyme would be blocked for a longer period of time in the proximal parts of the intestine by the CR4 in comparison to CR1 formulation. Based on this hypothesis one would expect that this would lead to greater amounts of carbohydrates reaching the ileum, which would ultimately result in, firstly, higher and more prolonged levels of plasma GLP-1 levels and secondly, lower plasma levels of glucose by the CR4 formulation over the CR1 formulation. The results however were surprising. The plasma GLP-1 and glucose values due to both the formulations were rather similar ($p > 0.05$) despite their differences in the *in vitro* release as well as pharmacokinetic profiles. The plausible explanations for this observed phenomenon could be as follows. There appears to be a possible threshold value of duration of time, for the reversible inhibition of α-glucosidase enzyme by a particular CR formulation which would yield significantly higher plasma GLP-1 levels. Reversible inhibition of the enzyme for a greater duration of time above this threshold value by a further retarding CR formulation may not necessarily translate into significantly higher plasma GLP-1 levels. Another explanation could be that there is possibly an upper limit of the amount of GLP-1 that can be secreted by L cells in response to stimulation by carbohydrates. If the L cells have secreted GLP-1 that is equivalent to this upper limit, further stimulation of the L cells by more carbohydrates (using a further retarding CR formulation) may not result in secretion of higher amounts of GLP-1. More studies however need to be done to identify the underlying mechanism that is responsible for this anomalous result. As there was no significant difference in the plasma GLP-1 levels induced by both the formulations, correspondingly, there was no significant difference in the plasma glucose levels too induced by CR1 and CR4 formulations.

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