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Research Article

Assessment of Isomalt for Colon-Specific Delivery and Its Comparison with Lactulose

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Abstract. Lactulose is used as a triggering substance in a unique colon-specific delivery technology called CODES^{TM}. Colonic microflora degrades lactulose and forms short-chain fatty acids to activate the CODES^{TM} system. However, lactulose has been reported to cause a Maillard-type reaction with substances containing primary or secondary amino groups that may produce carcinogenic compounds. Thus, the aim of this study was to look into the possibility to substitute lactulose with isomalt for fabrication of CODES^{TM}. The *in vitro* degradation of both sugars before incorporating them into the CODES^{TM} system was evaluated with the help of rat caecal microflora. The results showed that isomalt was less efficient with regard to its rate and extent of degradation into short-chain fatty acids by the microflora compared to lactulose. However, the *in vitro* dissolution study did not show a significant difference in the performance between lactulose and isomalt when they were incorporated separately in CODES^{TM}. A similar result was also obtained in the *in vivo* study. Based on the above results, isomalt could be used as an alternative to lactulose for colonic delivery system utilizing the principles of CODES^{TM}.

KEY WORDS: 5-amino salicylic acid; CODES^{TM} system; colon-specific delivery system; isomalt; Maillard reaction.

INTRODUCTION

The oral colon-specific delivery system had attracted grate interest since the last few decades. The advantages of targeting drugs to the colon are the following: (1) drugs can be delivered locally, e.g. aminosalicylates and glucocorticosteroids to treat inflammatory bowel diseases or systemically, e.g. systemic delivery of proteins and peptides; (2) as applicable to all site-specific delivery systems, the required doses and dosing frequency, adverse effects and degradation of drugs in the upper gastrointestinal tract are reduced; (3) some drugs have increased bioavailability and efficacy, especially drugs that degrade in the stomach and intestine or undergo first-pass metabolism.

The fundamental objective of colon-targeted delivery is to protect drugs in the upper gastrointestinal tract and release them directly into the colon. There are several approaches for targeting drugs to colon. Some colonic delivery systems utilise one or more triggering mechanisms, such as gastrointestinal transit time, pH, bacterial concentration and pressure (1). Each approach represents a distinct system in terms of design. However, the delivery systems have certain shortcomings, which are often related to degree of site specificity, toxicity or commercial feasibility (2). The system activated by colonic microflora to degrade natural polymers has been reported to have the highest potential for colonic delivery, particularly in terms of site specificity and safety (3–7). Lactulose has been reported to trigger drug release in the colon from coated tablets named CODES^{TM} by utilising the degradation activity of colonic microflora (8–13). CODES^{TM} can be used reliably and consistently for colon-specific delivery. The fabrication of CODES^{TM} is simple and only requires conventional equipment.

The CODES^{TM} system consists of a lactulose-containing tablet coated with an acid soluble polymer (Eudragit E), which is further coated with enteric material. The enteric coat is used to protect the device from the acidic environment in the stomach. The enteric material degrades in the small intestine, leaving an acid-soluble polymer coat that protects the device in the small intestine. However, highly soluble lactulose can slowly leach out from the device in the small intestine. When this device reaches the colon, lactulose is biodegraded by colonic microflora into short-chain organic acids. The formation of organic acids lowers the pH around the device, dissolving the acid-soluble polymer coat and promoting drug release (8).

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Lactulose plays a crucial role in CODES^{TM} systems. However, a Maillard-type condensation reaction is likely to occur between reducing sugars, such as lactulose, with drugs containing primary or secondary amino groups, such as lysine, to form brown or yellow-brown-coloured products. These adducts have been reported to be carcinogenic (14). Forest Labs has revised the formulation of their product Namenda®, containing memantine HCl as the active ingredient, due to the Maillard-type condensation reactions that occur between the primary amino groups of memantine HCl and lactose. This incompatibility was discussed in their patent (15).

To avoid the Maillard-type reaction during drug delivery to the colon, lactulose needs to be substituted with an alternative compound. Ideally, this compound should not get absorbed through the small intestine to prevent delays in gastric transit time. Furthermore, it should be highly water soluble to ensure easy penetration through the Eudragit E polymer membrane. Similar to a sugar, this compound should be rapidly degraded into organic acids, such as short-chain fatty acids, in the colon by colonic microflora.

Isomalt may be a good substitute for lactulose, which is a mixture of the two sugar alcohols (polyols), i.e. 6-*O*-α-D-glucopyranosyl-D-sorbitol and 1-*O*-α-D-glucopyranosyl-D-mannitol dihydrate. Furthermore, this compound has been used as an excipient in a variety of pharmaceutical preparations and does not undergo Maillard-type condensation reactions. In addition, isomalt is neither absorbed nor metabolised in the stomach and small intestine but is degraded by colonic microflora to glucose and mannitol/sorbitol, which are then further degraded into organic acids (16–20).

The aim of this study was to evaluate the potential of isomalt in comparison with lactulose for colon-specific drug delivery using CODES^{TM} systems. 5-Amino salicylic acid (5-amino-2-hydroxybenzoic acid) was selected as the active ingredient because it contains a primary amino group, is classified as an anti-inflammatory drug and has been used as a first-line drug in the treatment of ulcerative colitis.

MATERIALS AND METHODS

Materials

Isomalt Ph. Eur./USP-NF (GalenIQ^{TM} 721) was obtained as a generous gift sample from BENEO-Plantinit GmbH, Germany. Lactulose crystals were from Inalco, Italy and 5-amino salicylic acid (ASA) was from PharmaZell GmbH, Germany. Eudragit E 100 and Eudragit L 100 (Degussa, Germany) were kindly provided by JJ-Degussa Sdn. Bhd., Malaysia. Hydroxy propyl methyl cellulose 5cps (Methocel E5) was obtained as a gift sample from Colorcon Asia Pacific Ltd., Singapore. Talc (Luzenac Pharma M) was obtained from Rio Tinto Minerals, Malaysia, and triethyl citrate was purchased from Fluka, Germany.

Compatibility of ASA with Sugars

ASA was dissolved in simulated intestinal fluid (0.05 M phosphate buffer pH 6.8) to get 1% solution. Solution of 1% isomalt+1% ASA was prepared by dissolving 100 mg isomalt in a 10-mL ASA solution. Similarly, 1% lactulose+1% ASA was prepared by dissolving 100 mg lactulose in a 10-mL ASA solution. All the three solutions were incubated at 37°C for 24 h. The percentage transmittance of solutions was measured at 440 nm (Shimadzu spectrophotometer UV1800) at time intervals of 0, 1, 2, 3, 4, 6, 8 and 24 h. ASA solution was used as a control. The experiment was repeated three times.

In Vitro Degradation Study of Isomalt and Lactulose

All studies involving animals were conducted in accordance with the Animal Ethical Guidelines for investigations on laboratory animals. The protocol used for this study was approved by the animal ethic committee of Universiti Sains Malaysia, Penang, Malaysia. Caecal content was collected from male Sprague–Dawley rats weighing between 350 and 400 g that were fasted overnight. Rat caecal content was dispersed under anaerobic conditions by purging with nitrogen gas in phosphate buffer, pH 6.8, to obtain concentrations of 10%, 20% and 30%. Lactulose (100 mg) was added to vials containing 10 mL of 10% rat caecal dispersion. Isomalt (100, 125, 150 and 200 mg) was added separately to four vials containing 10 mL of 10% rat caecal dispersion. Similarly, 100 mg of isomalt was added to three vials containing 10 mL of 10%, 20% and 30% caecal dispersions. In addition, 10% rat caecal dispersion served as a control. All prepared samples were incubated at 37°C, and the pH of each sample was measured at 0, 1, 2, 3, 4, 6, 8 and 24 h post-incubation with a pH meter (Orion, model 310). The experiment was repeated three times.

In Vivo Degradation Study of Isomalt and Lactulose

Twenty-seven male Sprague–Dawley rats weighing 350 to 400 g were randomly divided into three groups consisting of nine rats. Isomalt and lactulose (150 mg) were dissolved separately in 1 mL of water. All rats were fasted overnight with free access to water prior to the experiment. On the day of the experiment, the rats in groups A, B and C (control) were given isomalt, lactulose and water, respectively. Three rats were sacrificed at 3, 4 and 5 h post-administration. The samples of rat caecal content were diluted with distilled water to obtain 10% concentration, and their pH values were measured with a pH meter (Orion, model 310).

Preparation of Colon-Specific Delivery System

Isomalt or lactulose (63.1%), ASA (30%), magnesium oxide (4.9%) and talc (1%) were sifted twice through ASTM # 40 mesh and thoroughly mixed in a polybag. Magnesium stearate (1%) was sifted through ASTM # 60 mesh and mixed with the above mixture in a polybag. Magnesium oxide was added to neutralise ASA to prevent the acidic drug from dissolving the acid-soluble coating layer. Core tablets were produced using a tabletting press (Kothari Pharma, Mumbai, India) with standard concave tooling (5.0 mm in diameter).

Subsequently, core tablets were coated with the acid-soluble coating polymer Eudragit E. A coating solution was prepared by dissolving 8% Eudragit E in a mixture of ethanol and water (9:1). Coating was performed using a conventional pan coating machine (Kothari Pharma, Mumbai, India) under the following conditions: inlet temperature, 45°C; rotating speed of pan, 12 rpm; and a spray rate of 0.24 g/min until 8% (*w*/*w*) coating weight gain was achieved. Eudragit E-

coated tablets were further coated with HPMC (water-soluble coating polymer) as an intermediate coating layer. A coating solution was prepared by dissolving 10% HPMC in water. The coating machine was set under the following conditions: inlet temperature, 60°C; rotating speed of pan, 12 rpm; and a spray rate of 0.24 g/min. The coating of tablet was performed until 2% (*w*/*w*) coating weight gain was obtained.

Finally, the HPMC-coated tablets were coated with enteric coating polymer Eudragit L. A coating solution was prepared by dissolving 6% Eudragit L, 3% talc and 1% triethyl citrate in a mixture of ethanol and water (13:5). Triethyl citrate served as a plasticiser, and talc was added as a glidant and anti-tacking agent. The coating machine coated tablets under the following conditions: inlet temperature, 45°C; rotating speed of pan, 12 rpm; and a spray rate of 0.24 g/min. The coating of tablet was carried out until 6% (*w*/*w*) coating weight gain was achieved.

In Vitro Dissolution Study

Sequential dissolution testing of CODES^{TM} tablets containing ASA+lactulose or ASA+isomalt was performed using the USP dissolution apparatus II (paddle; TDT-06T Electrolab, India) at 50 rpm and 37±0.5°C. The dissolution study was conducted in three different dissolution media. Initially, tablets were placed in 375 mL of 0.1 N hydrochloric acid solution (pH 1.2) for 1 h to simulate product exposure in stomach pH. After this, 125 mL of 0.2 M trisodium phosphate was added to the dissolution media. This will make the pH of dilution media to 6.8 to simulate product exposure in small intestine pH. The dissolution was continued for 3 h. After this, 5 g of freshly obtained rat caecal content was added to the dissolution media. This will make a dissolution media with 1% caecal content, and dissolution was continued under the nitrogen environment for 2 h. An aliquot of dissolution medium (2 mL) was withdrawn at specific time intervals (1, 2, 3, 4, 4.5, 5, 5.5 and 6 h). The drug concentration in all three dissolution media samples was measured using by HPLC (Shimadzu LC-6A). The mobile phase, consisting of methanol 10% and an aqueous acetic acid solution (1%, *w*/*v*) 90%, was pumped at a flow rate of 1 mL/min. Chromatographic separations were carried out using a reversed-phase column (Develosil ODS-5, 4.0×250 mm). Ultraviolet detection (Shimadzu RF-530) was performed at a wavelength of 300 nm. The experiment was performed on three tablets.

Bioavailability Study

Twelve male New Zealand strain rabbits weighing 2 to 3 kg were randomly divided into two groups of six rabbits. The rabbits were fasted overnight with free access to water prior to the experiment. On the day of the experiment, one group received CODES^{TM} tablets containing ASA and isomalt, whereas the other group received CODES^{TM} tablets containing ASA and lactulose. Either one lactulose or one isomalt tablet was given to each rabbit. The CODES^{TM} tablet was administered to each rabbit *via* polyethylene tubing. Blood samples (3 mL) were withdrawn from the marginal ear vein at predetermined time intervals of 0, 1, 2, 3, 4, 6, 8, 10, 12 and 24 h post-administration. The blood was immediately centrifuged at 3,000 rpm for 15 min, and the plasma was transferred into tubes and stored at −20°C until HPLC analysis.

An HPLC method reported by Lee and Ang (21) was adopted with little modification to determine the ASA concentration in plasma. Plasma samples (0.8 mL) were mixed with 0.05 mL of perchloric acid, and the resulting mixtures were centrifuged at 1,200 rpm for 10 min. The supernatant (0.4 mL) and 4 N NaOH of 0.035 mL were mixed. Then, 50 μL of the resulting solution was injected onto an HPLC (Shimadzu LC-6A). The mobile phase, consisting of tetrahydrofuran, acetonitrile and 0.067 M phosphate buffer, pH 3.2, at a ratio of 5:10:85, was pumped at a flow rate of 1 mL/min. Chromatographic separations were carried out using a reversed-phase column (Develosil ODS-5, 4.0×250 mm). Fluorescence detection (Shimadzu RF-530) was performed with excitation wavelength 300 nm and emission wavelength 485 nm. The column oven temperature was maintained at 40°C.

Three pharmacokinetic parameters were considered, viz area under the curve (AUC), C_{max} and T_{max}. AUC is calculated by trapezoid method. Highest plasma drug concentration value observed was taken directly as C_{max}, and corresponding time point was taken as T_{max}.

Statistical Analysis

The results are presented as the mean±standard deviation (SD). For statistical comparison, one-way analysis of variance followed by Dunnett's multiple comparisons test was performed using the GraphPad InStat Software, when applicable. A P value <0.05 was considered to be significant.

RESULTS AND DISCUSSION

Compatibility Study

When the CODES^{TM} system is administered orally, it remains intact in the acidic pH of the stomach. However, when it reaches the small intestine (above pH 6.8), the Eudragit L (enteric coat) and HPMC coats are dispersed. Thus, the Eudragit E coat protects the core tablet exposed to environment. The coat swells and becomes permeable at above pH 5, which facilitates the entry of intestinal fluids inside the core and causes active ingredients to slowly leach out. Moreover, this environment

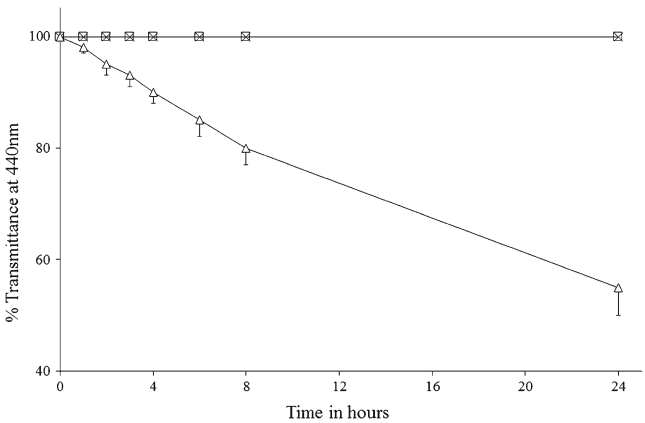

Fig. 1. Percentage transmittance at 440 nm of (*multiplication sign*) 1% ASA, (*squares*) 1% isomalt+1% ASA and (*triangles*) 1% lactulose+1% ASA in simulated intestinal fluid incubated at 37°C. Mean±SD, n=3

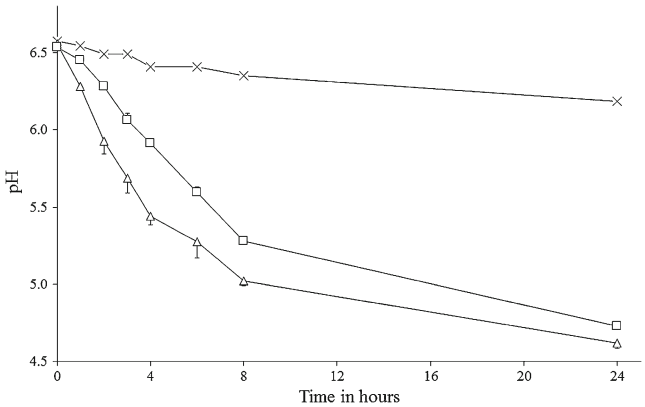

Fig. 2. pH of: (*multiplication sign*) 10% rat caecal contents dispersed in phosphate buffer pH 6.8 (control) (*triangles*) 1% lactulose in phosphate buffer pH 6.8 containing 10% rat caecal contents and (*squares*) 1% isomalt in phosphate buffer pH 6.8 containing 10% rat caecal contents, incubated at 37°C for 24 h. Mean±SD, n=3

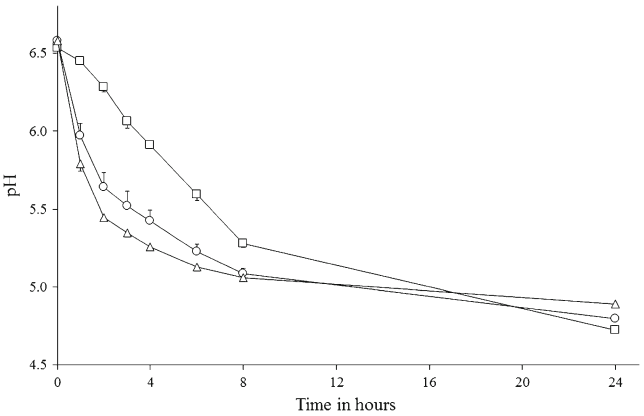

Fig. 4. pH of solution containing 1% isomalt and different concentrations of rat caecal content in phosphate buffer pH 6.8: (*squares*) 10% rat caecal content, (*circles*) 20% rat caecal content and (*triangles*) 30% rat caecal content, incubated at 37°C for 24 h. Mean±SD, n=3

also favours interactions between the active ingredient and excipient. Thus, this experiment was conducted to compare the interactions between ASA with lactulose and ASA with isomalt. Equal amounts of ASA and lactulose or ASA and isomalt were mixed, dissolved in simulated intestinal fluid, incubated at physiological temperature and observed for 24 h for discolouration. The ASA+lactulose solution was observed to slowly develop a brownish colour, whereas ASA+isomalt and ASA solutions did not develop any colour during the entire observation period. The colour change was determined using percent transmittance. Figure 1 shows the percent transmittance of all three solutions at 440 nm. The percent transmittance of the ASA+lactulose solution was slowly reduced to the lowest value of 50% after 24 h, whereas the percent transmittance of ASA+isomalt and ASA solutions remained unchanged at 100%. The results from this study suggest that isomalt is compatible with substances containing primary or secondary amino groups, whereas lactulose undergoes Maillard reactions.

In Vitro Degradation Study

The role of lactulose in the CODES^{TM} system is to be degraded into short-chain fatty acids by colonic microflora, lowering the pH around the tablet to dissolve the acid-soluble polymer coat. This study was conducted to compare the pH lowering effects of isomalt and lactulose. The results showed that the rate and extent of pH lowering by isomalt was lower than that of lactulose (Fig. 2). Moreover, the rate and extent of pH lowering was not influenced by the concentration of isomalt. Increasing the amount of isomalt did not alter the pH of solution significantly (Fig. 3). In contrast, increasing the amount of rat caecal content accelerated the rate of pH lowering. However, there was no difference in the extent of pH lowering when the pH of solutions decreased below 5 (Fig. 4). This observation can be explained by the properties of enzymes as the reaction rate is directly proportional to amount of enzyme present. Thus, a greater amount of caecal content leads to a rapid decrease in the pH and inhibition of enzyme, which causes the extent of pH lowering to remain almost constant (22,23). The lactulose results from this study are in accordance with those reported by Watanabe *et al.* (12).

Isomalt was observed to lower the pH at a slower rate compared to that of lactulose, which could be due to the degradation of isomalt to one glucose molecule and one mannitol/sorbitol molecule. The sugar alcohol (polyol) does not contribute to lowering the pH (12), suggesting that only the glucose molecule is responsible for this decrease. In contrast, lactulose

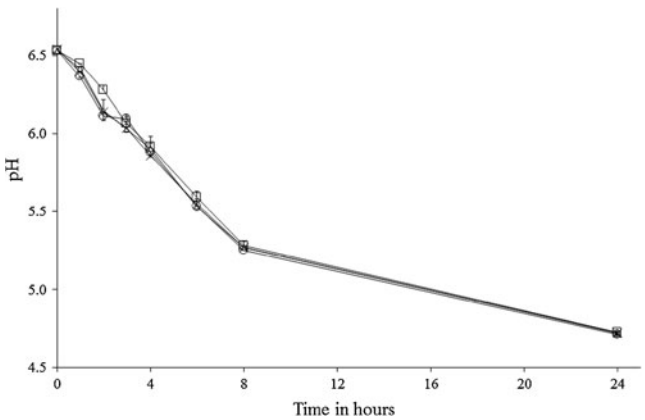

Fig. 3. pH of different concentrations of isomalt in phosphate buffer pH 6.8 containing 10% rat caecal contents: (*squares*) 1% isomalt, (*triangles*) 1.25% isomalt (*multiplication sign*) 1.5% isomalt and (*circles*) 2% isomalt, incubated at 37°C for 24 h. Mean±SD, n=3

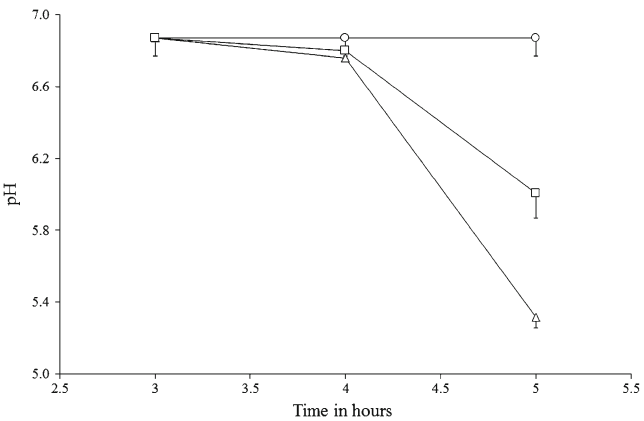

Fig. 5. pH of rat caecal contents following oral administration of (*triangles*) 150 mg lactulose, (*squares*) 150 mg isomalt and (*circles*) 1 mL water. Mean±SD, n=3

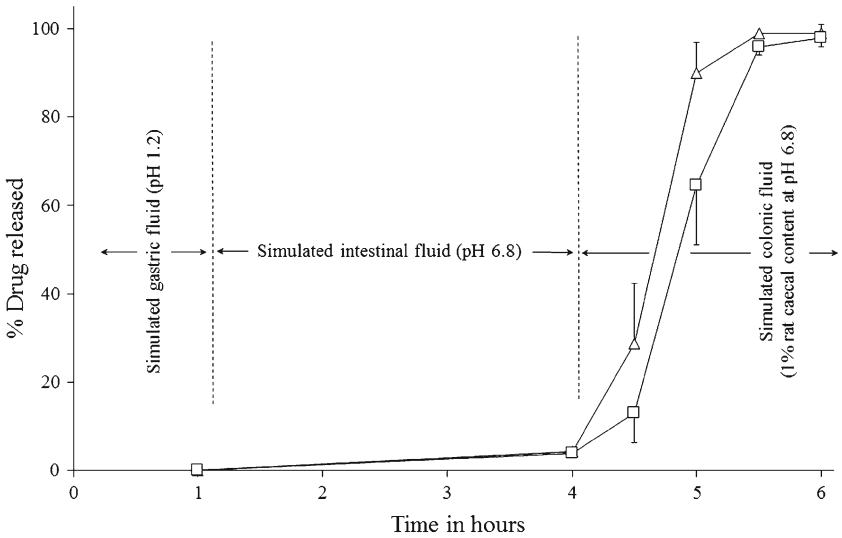

Fig. 6. *In vitro* dissolution profiles of CODES ™ tablet containing ASA+lactulose or ASA+isomalt placed sequentially in three different media (pH condition of media changed with time is indicated in the graph): 1 h in simulated gastric fluid (pH 1.2), 3 h in simulated intestinal fluid (pH 6.8) and 2 h in simulated colonic fluid (1% rat caecal content at pH 6.8). (*triangles*) ASA+lactulose CODES ™ tablet, (*squares*) ASA+isomalt CODES™ tablet. Mean±SD, n=3

degrades into one molecule of glucose and one molecule of fructose. Both monosaccharides are separately degraded into short-chain fatty acids, leading to a faster rate of pH lowering. Due to enzymes being substrate specific, different enzymes are speculated to be responsible for the degradation of glucose and fructose (22,23). Lactulose was also reported by Lee *et al.* (24) to have a better performance over isomalt. The authors reported that higher percentage of hydrogen gas was expelled from breath of healthy human volunteers administered lactulose compared to isomalt.

In Vivo Degradation Study

The major fraction of orally administered lactulose and isomalt was observed to be neither digested nor absorbed in the stomach and small intestine but degraded by microflora present in the gastrointestinal tract (17,25). The CODES™ system moves quickly to its target organ, the large intestine, due to indigestible chyme, increasing its bulkiness and stimulating peristalsis (26,27). The degradation of isomalt and lactose into short-chain fatty acids was evaluated by administering an equal amount of both sugars separately to the rats. The animals were sacrificed at specific time intervals, and the pH of their caecal content was measured. Both sugars lowered the caecal pH. However, the lowering of pH by isomalt occurred at a slower rate than that of lactulose (Fig. 5). The results obtained from the *in vivo* study support the findings from the *in vitro* degradation study discussed in the prior section. However, a correlation cannot be established as the *in vitro* data are generated by taking 10% of rat caecal content in phosphate buffer, pH 6.8, which has a buffer capacity >50. Moreover, the *in vivo* data also account for the gastric transit time until the sugar reaches colon to begin degradation. In addition, the short-chain fatty acids formed *in vivo*, which are responsible for lowering the pH, are absorbed in the colon, (28) again increasing the pH. However, the results of the present study support the findings of Katsuma *et al.* (8).

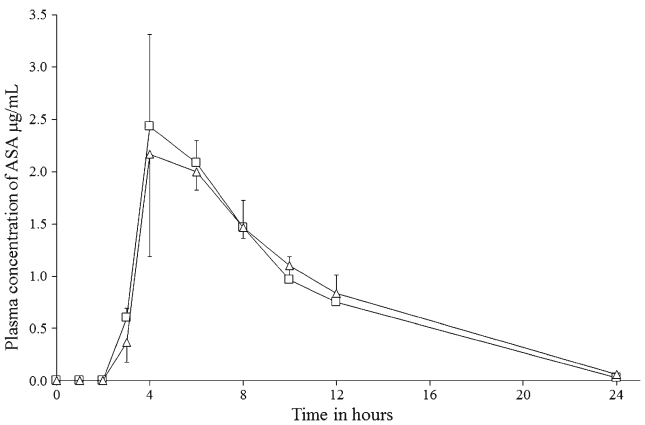

Fig. 7. Mean plasma ASA concentration profiles in rabbits following oral administration of CODES™ tablet containing: (*triangles*) ASA+lactulose and (*squares*) ASA+isomalt. Mean±SD, n=6

In Vitro Dissolution Study

A study was performed in various dissolution media to mimic tablet exposure to sequential human gastrointestinal pH environments. The *in vitro* dissolution behaviour of CODES™ tablets containing ASA and either isomalt or lactulose was compared, and the results are illustrated in Fig. 6. There was no drug release detected from both CODES™ tablets containing

Table I. Pharmacokinetic Parameter Values Following Oral Administration of CODES ™ Tablet ASA+Lactulose and ASA+Isomalt in Rabbits

CODES™ tablet	C_{max} (µg/mL)	T_{max} (h)	$AUC_{0\text{-}t}$ (hµg/mL)
ASA–isomalt	2.73±0.46	4.67±1.15	20.95±1.54
ASA–lactulose	2.47±0.85	4.67±1.15	19.20±1.34

Mean±SD, n=6

either isomalt or lactulose in artificial gastric fluid (pH 1.2) upon 1 h of exposure. Approximately 5% of drug was released after 3 h of exposure in artificial intestinal fluid. At 4.5 and 5 h, faster drug release was observed in CODES^{TM} tablets containing ASA+ lactulose compared with those containing ASA+isomalt. However, statistical analysis showed that there was no significant difference in the release rates of drug from both tablets, which could be a result of the large variation in results obtained at 4.5 and 5 h. However, both delivery systems released their entire contents in simulated colonic fluid containing phosphate buffer, pH 6.8, and 1% rat caecal content within 2 h. Thus, the *in vitro* dissolution study could not distinguish the performance of lactulose and isomalt in CODES^{TM} tablets as it was observed in the *in vitro* degradation study of both sugars.

Bioavailability Study

The bioavailability of ASA was studied in rabbits to understand the *in vivo* performance of isomalt and lactulose in CODES^{TM} tablets. Figure 7 shows a similar plasma profile of ASA after the oral administration of isomalt and lactulose CODES^{TM} tablets in rabbits. The concentration of ASA was not detected in the rabbit plasma during the first 2 h, suggesting that the drug in the core tablet was protected in the stomach. However, a small concentration of drug was detected in the third hour, indicating that the tablet reached the small intestine, and the drug then leached out from the tablet. A sudden increase in the drug concentration was detected and reached its maximum at 4 h, indicating that the tablet burst in the colon due to the presence of colonic microflora. The pharmacokinetic parameters listed in Table I show no significant difference among the T_{max}, C_{max} and AUC values of isomalt and lactulose CODES^{TM} tablet. Thus, both preparations were bioequivalent in their rates and extent of absorption. Moreover, the results suggest that the *in vivo* performance of isomalt and lactulose is comparable in CODES^{TM} tablet.

CONCLUSION

The *in vitro* study demonstrated that isomalt degrades to short-chain fatty acids by colonic microflora less efficiently compared to lactulose. However, dissolution and bioavailability results showed that isomalt performance was comparable to lactulose in colon-specific delivery system utilizing the principles of CODES^{TM}. Therefore, isomalt could be an alternative to lactulose to avoid Millard condensation reactions that produce carcinogenic compounds in CODES^{TM} tablet containing active substance having primary or secondary amino groups.

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Declarations of Interests The authors report no declaration of interest.

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