Indonesian Journal of Pharmacy

VOL 35 (4) 2024: 641–648 | RESEARCH ARTICLE

In Vitro Drug Release Study of Bisacodyl Enteric-Coated Tablet in Various Artificial Dissolution Media

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Article Info

Submitted: 21-12-2023
Revised: 07-08-2024
Accepted: 24-09-2024

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ABSTRACT

This study investigates the *in vitro* dissolution profiles of bisacodyl from enteric-coated tablets in various colon-simulated media. The aim was to compare the effectiveness of different media in mimicking the conditions of the human colon and predicting drug-release behavior. Genuine phosphate buffer pH 7.4 (phosphate buffer), rat cecal-containing phosphate buffer pH 7.4 (rat cecal), lactase-containing phosphate buffer pH 7.4 (lactase), probiotics-containing phosphate buffer pH 7.4 (probiotics), and probiotics & lactase-containing phosphate buffer pH 7.4 (probiotics-lactase) were used as the colon simulation media. The experiment was carried out sequentially in a modified type I dissolution test apparatus for 12 hours including 2 hours in HCl pH 0.1 M, 3 hours in phosphate buffer pH 6.8, and 7 hours on colon simulation media. Furthermore, the sample aliquots were analyzed using UV-Vis spectrophotometer. The results demonstrated that the fastest and highest drug release occurred in the probiotics-lactase media, with 110.63% bisacodyl released after 12 hours, followed by probiotics-only media (92.83%), rat cecal media (85.83%), and lactase media (70.59%). The lowest release was observed in phosphate buffer pH 7.4 (42.09%). Drug-release profiles among various dissolution media showed significant differences, with p-values < 0.05. It was concluded that pH, microbial activity, and enzyme content significantly influenced the release profile of bisacodyl from enteric-coated tablets.

Keywords: Dissolution; Medium; Bisacodyl; Enteric-Coated; CTDDS

INTRODUCTION

Colon-targeted drug delivery system (CTDDS) is intended to be released specifically in the colon. However, there are fundamental barriers to measuring drug bioavailability for locally targeted dosage forms. Bioavailability assessment is generally carried out by measuring drug concentration in plasma. The bioavailability of drugs with local therapeutic sites, including CTDDS, cannot be assessed by the methods as drug molecules are supposed to act in the colon and are not absorbed into the plasma. The analysis of drug concentration inside the colon luminal is rather complicated and might show high variability (Dugad *et al*., 2018). Therefore, *in vitro* dissolution test method is often used to predict *in vivo* release profile of the active substance (Iswandana *et al*., 2018). Colon-targeted drug delivery systems (CTDDS) are designed to minimize drug release in the upper gastrointestinal tract (stomach and small intestine) and maximize release upon reaching the colon, ensuring localized therapeutic effects (Amidon *et al*., 2015; Iswandana *et al*., 2017a). These systems offer significant benefits for treating colonic diseases, such as inflammatory bowel disease and colorectal cancer, by reducing systemic side effects. However, the complex colon environment—characterized by diverse microbiota and enzymatic activity—poses challenges in accurately predicting drug release. Standard dissolution testing methods often fail to replicate these conditions, leading to variability in results (García *et al*., 2022). Therefore, selecting an appropriate dissolution medium is crucial for developing effective CTDDS and ensuring reliable *in vivo* predictions.

In designing a dissolution medium, it is crucial to create an artificial microenvironment that mimics the gastrointestinal tract (GIT) taken by CTDDS during the movement from the oral site

to the colon as well as the drug delivery environment (Kotla *et al*., 2014). *In vitro* dissolution tests use buffers to represent various pH levels of GIT (Kotla *et al*., 2014; Amidon *et al*., 2015). However, using only pH buffer does not represent the condition of the colon lumen because there are other factors to be considered, including the presence of enzymes and gut microbiomes. These other factors might affect drug release from CTDDS depending on the materials used in the system.

In vitro dissolution test is a general procedure to predict *in vivo* release of drug from the dosage form. Each drug product monograph typically states the dissolution media used (United States Pharmacopeia or Farmakope Indonesia). It ranges from water to pH-arranged media such as HCl 0.1 N and buffer (Kementerian Kesehatan RI, 2020). However, in CTDDS, the active substance is purposively released in the colon, and the choice of the dissolution medium is rather complicated. This is because the colon is inhabited by various colonic microflora, which might affect the degradation of the dosage form and consequently affect drug dissolution. This study investigated the effect of different dissolution media on drug release from CTDDS. An already-marketed bisacodyl enteric-coated tablet was used and the release in five different simulated colon fluids was compared including genuine phosphate buffer pH 7.4 (phosphate buffer), rat caecal-containing phosphate buffer pH 7.4 (rat caecal), lactase-containing phosphate buffer pH 7.4 (lactase), probiotics-containing phosphate buffer pH 7.4 (probiotics), and probiotics & lactase-containing phosphate buffer pH 7.4 (probiotics-lactase).

CTDDS is promising for treating colon diseases, but accurately assessing the effectiveness remains a hurdle. Standard methods relying on plasma drug levels are not suitable. *In vitro* testing offers a solution, but current methods designed for the upper gut fail to mimic the complexities of the colon. This study addresses the critical gap by investigating the impact of various dissolution media on drug release. Developing a more realistic testing method is crucial to expedite the development of effective CTDDS and improve treatment options for colonic diseases.

MATERIALS AND METHODS

The materials used include Dulcolax® Enteric-Coated Tablets (Batch number 21050294, Sanofi, Indonesia), Bisacodyl BPFI (BPOM, Indonesia), hydrochloric acid (HCl) 12 N (Merck, Germany), aquadest (Multi Kimia, Indonesia), potassium phosphate monobasic (Merck, Germany), and sodium hydroxide (Merck, Germany). Cecal content was obtained from male Wistar rats weighing 250-300 g with approval from the Faculty of Medicine, Universitas Indonesia, ethics commission (No. KET-1309/UN2.F1/ETIK/PPM.00.02/2022). Other materials used include Nature Way Lactase Enzyme containing 10,350 ALU for each 690 mg powder (Nature's Way®, USA), Fluid Thioglycolate Medium (Oxoid, UK), *Lactobacillus acidophilus* (Shandong-Zhongke Jiayi Bioengineering, China), and *Bifidobacterium longum* (Shandong-Zhongke Jiayi Bioengineering, China).

Instruments

The instruments used were UV-Vis Spectrophotometer (Shimadzu UV-1800, Japan), pH meter (Jenway 550, England), beaker, measuring cylinder, volumetric flask, volumetric pipette, glass bottle, analytical balance (Sartorius, Germany), water bath, magnetic stirrer (IKA, Germany), tea strainer, stirring rod, mercury thermometer, surgical scissors, surgical board, glass vial, autoclave, and bacteria incubator (Memmert, Germany).

Preparation of HCl 0.1 N solution pH 1.2

About 8.33 mL of chloride acid 12 N was mixed with CO_2-free aquadest until the volume reached 1000 mL. The mixture was then gently stirred until homogenous and checked for pH using a pH meter (Jenway 550, UK).

Preparation of phosphate buffer solution pH 6.8

About 50 mL of potassium phosphate monobasic 0.2 M solution was mixed with 22.4 mL NaOH 0.2 N solution in a 200 mL volumetric flask. CO_2-free aquadest was then added to make up the volume. pH of the solution was measured using a pH meter.

Preparation of phosphate buffer solution pH 7.4

About 50 mL of potassium phosphate monobasic 0.2 M solution was mixed with 39.1 mL of NaOH 0.2 N solution in a 200 mL volumetric flask. CO_2-free aquadest was then added to make up the volume. pH of the solution was measured using a pH meter.

Preparation of rat cecal-containing phosphate buffer pH 7.4 (rat cecal)

The method was adapted and modified from Singh *et al*. (2015) and Singh *et al*. (2021).

Male Wistar Rats weighing 190-260 g were housed in a standard laboratory condition at a temperature of 21±10°C and 12 h dark-light cycle. Acclimatization was performed for 7 days before the study. Four rats were culled by administering a lethal dose of ketamine 45 minutes before the 5-hour point of the dissolution study. The abdominal part was opened, and the caecal was isolated and cut. The content of the caecal was taken out, accurately weighed 600 mg, and then immediately mixed with 30 mL of phosphate buffer pH 7.4 to make a 2% w/v caecal pulp. Phosphate buffer pH 7.4 was previously purged with CO_2 for 10 minutes. About 10 mL of 2% w/v cecals pulp was mixed with another 90 mL phosphate buffer pH 7.4 to make 100 mL of dissolution medium containing rat cecal.

Preparation of lactase-containing phosphate buffer pH 7.4 (lactase)

About 50 mg of powder from Nature's Way Lactase Enzyme capsule was weighed and dissolved in 50 mL phosphate buffer pH 7.4 to make a 15 ALU/mL lactase stock solution. Subsequently, 10 mL of the solution was pipetted and mixed with 90 mL of another phosphate buffer pH 7.4 to make 100 mL of dissolution medium containing lactase.

Preparation of probiotics-containing phosphate buffer pH 7.4 (probiotics)

About 840 mg of Fluid Thioglycolate Medium (FTM) was dispersed in 30 mL of aquadest and heated to dissolve FTM which was then autoclaved at 15 lbs., 121 °C for 15 minutes, cooled down, and stored at 25 - in the dark. About 10.79 mg of *Lactobacillus acidophilus* and 8.87 mg of *Bifidobacterium longum* (equivalent to 32.5 mg of USP BIOMIX-1) were inoculated to FTM and then incubated using an incubator (Memmert, Germany) at 35°C for 48 hours in anaerobic condition inside a glove box. Furthermore, about 10 mL of the culture was added to 90 mL of phosphate buffer pH 7.4 to make 100 mL of dissolution medium containing probiotics. Phosphate buffer pH 7.4 was previously purged with CO_2 for 10 minutes to maintain the anaerobic condition.

Preparation of probiotics & lactase-containing phosphate buffer pH 7.4 (probiotics-lactase)

About 80 mL of phosphate buffer pH 7.4 was purged with CO_2 for 10 minutes and mixed with 10 mL of previously prepared probiotics culture. Subsequently, 10 mL of 15 ALU/mL lactase stock solution was added to make 100 mL of dissolution medium containing probiotics and lactase.

***In vitro* dissolution test of enteric-coated tablet**

In-vitro dissolution study of enteric-coated tablets was conducted using a modified dissolution apparatus. About 100 mL of dissolution medium was placed in a 100 mL beaker glass and heated on a hot plate until the temperature reached 37±0.5 °C. A magnetic stirrer was placed at the bottom center of the beaker glass. Bisacodyl enteric-coated tablet was placed in a tea bag-shaped paper filter in the dissolution medium and then stirred at a rotation speed of 100 rpm.

For the first 2 hours, 100 mL HCl solution pH 1.2 was used as dissolution media. After 2 h HCl medium was replaced with 100 mL of phosphate buffer pH 6.8, and then the dissolution study was continued for another 3 h. Phosphate buffer pH 6.8 was replaced with 100 mL of phosphate buffer solution pH 7.4, and then the dissolution was continued for another 7 h. A similar process was carried out using other dissolution media (caecal, lactase, probiotics, probiotics-lactase) in the last 7 hours. For the dissolution study using a caecal or probiotics-containing medium, the dissolution medium was maintained at anaerobic conditions with continuous CO_2 gas flow (Ghosh *et al*., 2010).

About 5 mL of dissolution medium was taken at the predetermined time points, after which 5 mL of fresh media was added to replace the withdrawn sample (Singh *et al*., 2015). The sample was then analyzed using a spectrophotometer UV-Vis (Shimadzu UV-1800, Japan) at λmax 264 nm. The linear equation used to calculate the dissolution drug concentration is $y = 0.0072 + 0.0218x$ with a regression coefficient of 0.9996.

The dissolution apparatus was calibrated to simulate the colonic anaerobic environment, and additional precautions were taken to minimize variability. Temperature and pH levels were monitored hourly to ensure consistency.

Statistical analysis

The cumulative average percentage of bisacodyl released from bisacodyl enteric tablets on each *in vitro* dissolution test medium was then plotted on a graph of time (hours) vs. percentage of bisacodyl released (%). Plotting was performed for each of the five *in vitro* dissolution test media. The five graphs were then combined for comparison of bisacodyl dissolution/release profile as a representation of drug on each dissolution test medium (Kotla *et al*., 2016).

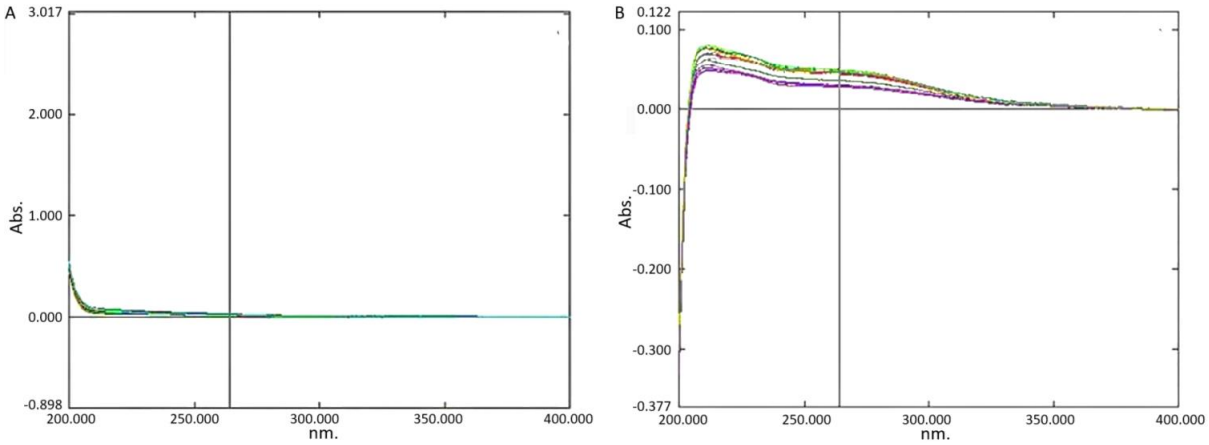

Figure 1. UV-Vis absorption of bisacodyl in (A) HCl 0.1 N and (B) phosphate buffer pH 6.8 medium from the first 2 hours of dissolution test showed no or very low absorption at λ 264 nm.

The concentration of bisacodyl released or dissolved was calculated using the standard calibration curve and plotted against release time with the following equation:

$$n\ minute = \frac{(yn - \alpha) \text{ x } fp \text{ x } M}{b \text{ x } 1000} + \ldots + \frac{(y15 - \alpha) \text{ x } fp \text{ x } S}{b \text{ x } 1000}$$

y = Bisacodyl absorption; yn = Bisacodyl absorption on n-minute; fp= Dilution factor; M= Dissolution media volume; S = Sampling volume; a = Interception coefficient; b = Slope.

The release profiles of active substances in various dissolution media, including variable pH, rat feces, enzymes, probiotic, and probiotic-enzymatic, were compared in terms of significant differences and least significant differences using the IBM SPSS Statistics 25 program. Within-Subject Analysis of Variance (ANOVA) method was used to determine the significance of differences in dissolution results by comparing data at each sampling time point.

RESULTS AND DISCUSSION

The dissolution study was conducted using a modified apparatus, with only a smaller volume of media than stated in USP or Farmakope Indonesia for more accessible analysis (Iswandana *et al*., 2017b).

Dulcolax®, a commercially available enteric-coated tablet, was used as the test product. It contains bisacodyl, a laxative agent, which works on the colon to stimulate bowel movement. Although Dulcolax® is not a colon-targeted dosage form, it offers a good model for a delayed-release system. The active substance, bisacodyl, can be analyzed using a simple spectrophotometry UV-Vis method. Based on the information on the leaflet (https://products.sanofi.ca/en/dulcolax.pdf), Dulcolax® used a combination of shellac, methacrylic acid, and ethyl acrylate copolymer as enteric coating polymer.

The dissolution study results revealed notable differences in bisacodyl release profiles across the tested colon-simulating media, highlighting the significant impact of media composition. The findings provide valuable insights into how pH, enzymatic activity, and microbiota influence the performance of enteric-coated tablets.

In a regular orally administered dosage form, the stomach is the first compartment in which drug passes through and released from the dosage form. Therefore, the first step of the study was the dissolution of drug in HCl 0.1 N medium for 2 h, representing the release of drug in the stomach. The dissolution test in HCl 0.1 N demonstrated that only a negligible amount of bisacodyl was released, shown by no peak absorption at λ-max 264 nm (Figure 1). This result followed the obligatory characteristic of an enteric-coated tablet, which must not release drug into the stomach.

After 2 h of dissolution in HCl 0.1 N, the medium was removed and replaced by phosphate buffer pH 6.8. The dissolution test was then continued for another 3 h. This step aimed to mimic the process after drug passed through the stomach and continued to the intestine, in which pH changes to more basic. Typically, an enteric-coated dosage form will start disintegrating in this pH environment, depending on the coating polymer.

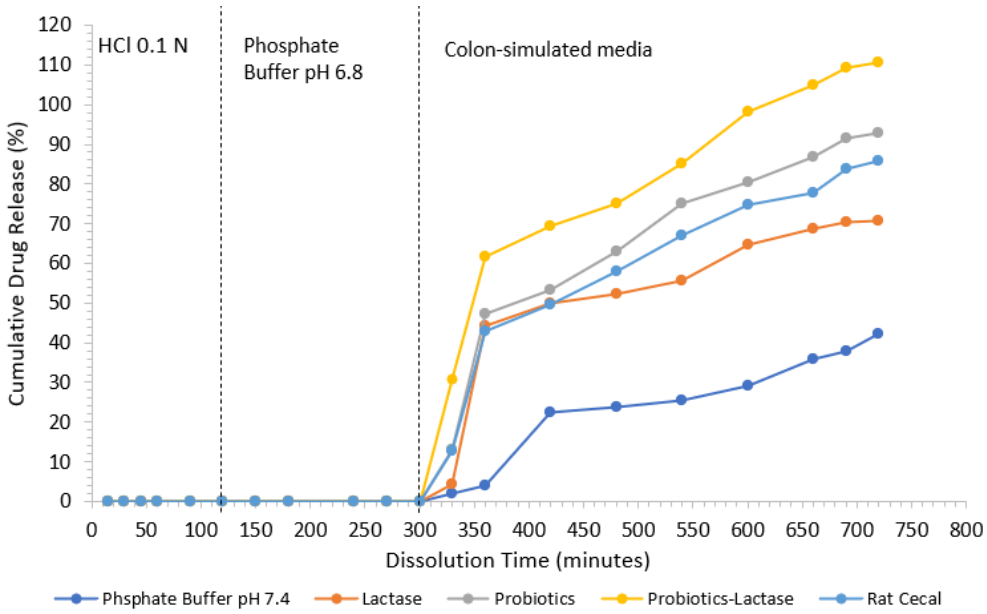

Figure 2. Cumulative release of bisacodyl from enteric-coated tablet in various dissolution media (mean±SD, n = 3)

This study showed that the release of bisacodyl from Dulcolax® in phosphate buffer pH 6.8 was negligible in the HCl 0.1 N as indicated by the low absorption at λ 264 nm (<0.100). The result was probably because the coating polymers used were shellac and methacrylate, soluble at pH >7 (Farag & Leopold, 2009).

The dissolution study was then continued by replacing phosphate buffer pH 6.8 media with each colon-simulated media. Based on the results, the release of bisacodyl started to take place around 30 minutes after exposure to all colon-simulated media, or 5.5 h after the dissolution test (Figure 2). This is because for up to 360 minutes, the process taking place was the gradual dissolution of the coating layer from the tablet. At 330 min, there was still coating surrounding the tablet. At 360 min, most of the coating had been removed from the core tablet, facilitating quick release of the active substance. The influence of coating on the release of the active substance can be explained by the Noyes-Whitney/Nerst-Brunner equation. The higher the concentration of the coating, the thicker the diffusion membrane, which can reduce the dissolution rate of the active substance. In addition, a larger hydrated area increases the dissolution rate of the active substance (Sinko & Martin, 2006). The release of bisacodyl in each colon-simulated medium demonstrated a different rate. Based on the results, the dissolution rate of bisacodyl from lowest to higher was shown in phosphate buffer pH 7.4, lactase-containing phosphate buffer pH 7.4, rat cecal-containing phosphate buffer pH 7.4, probiotics-containing phosphate buffer pH 7.4 and probiotic-lactase-containing phosphate buffer pH 7.4 ($p < 0.01$). The dissolution rate of bisacodyl in phosphate buffer pH 7.4 was the lowest, with only around 40% of drug being released after 12 hours. This implies that using only phosphate buffer pH 7.4 as the dissolution medium of enteric-coated product might not produce optimal results. These results align with Garcia *et al.* (2022) and Favaron *et al.,* (2024), who emphasized that single-parameter media, often fail to accurately replicate the complex nature of drug release in the colon.

The release of bisacodyl in lactase-containing phosphate buffer pH 7.4 was significantly higher than in phosphate buffer alone but lower than in the other three medias, with around 65% of the drug released after 12 hours. Lactase, however, may not be an effective degrading agent for the coating polymers, such as shellac and methacrylic copolymer, since these are not polysaccharides. Lactase typically targets and breaks down lactose, a sugar with a polysaccharide structure, which is absent in the coating materials. As a result, lactase is likely ineffective in directly degrading the coating. However, it might aid in the degradation of the core tablet once the coating polymers are dissolved, since the core contains

some polysaccharides. These results suggest that enzymatic activity alone, such as that of lactase, cannot fully replicate the complex interactions in the colon. While lactase can break down certain tablet components, its specificity to limited substrates makes it less versatile than microbial enzymes, which can act on a broader range of substrates.

In the rat cecal-containing phosphate buffer pH 7.4, bisacodyl release reached 85% after 12 hours. Rat cecal is known to have a microbiota composition similar to that of the human colon, with genera such as *Lactobacillus*, *Bifidobacterium*, *Turicibacter*, *Lachnospiraceae*, *Ruminococcaceae*, *Enterococcus*, *Bacteroides*, and *Clostridiales* (Čoklo *et al.,* 2020). The diverse microbial species present, particularly those capable of producing polysaccharide-degrading enzymes, likely contributed to the effective release of bisacodyl. For example, *Lactobacillus* secretes lactase, while *Bifidobacterium* produces galactosidase (Ahmad *et al.*, 2014; Mulualem *et al.*, 2021), both of which aid in tablet degradation. Additionally, *Lachnospiraceae* and *Ruminococcaceae* are known to break down polysaccharide derivatives (Vacca *et al.*, 2020), further influencing drug release behavior. However, variability in microbial activity and concentration among samples may introduce inconsistencies, which explains why the bisacodyl release in this medium was slightly lower compared to the probiotics and probiotics-lactase media. This could be due to the reduced viability of probiotics during the collection of rat cecal content or the high variation in microbial concentration and activity (Kotla *et al.*, 2014; Batacan *et al.*, 2017). A standardized approach to preparing rat cecal content could help minimize these variations and improve reproducibility. While rat cecal media are effective, they are more resource-intensive and raise ethical concerns compared to probiotics-based alternatives (Starr *et al.*, 2014)

In probiotics-containing phosphate buffer pH 7.4, about 95% of drug was released after 12 hours of dissolution study. The presence of lactase and galactosidase from the microbiota *Lactobacillus acidophilus* and *Bifidobacterium longum* can degrade polysaccharides in tablets, which have a significant influence on the release of the active substance. In contrast to rat caecal, isolated bacteria were added. Using a probiotics-containing medium could also ensure the continuous production of the degrading enzymes throughout the dissolution process.

The fastest and highest release of bisacodyl from Dulcolax® was observed in the simulated medium containing both probiotics and lactase in phosphate buffer pH 7.4, with approximately 110.63% of the drug released. This enhanced release is attributed to the synergistic activity between the isolated lactase and the enzymes produced by probiotics such as *Lactobacillus acidophilus* and *Bifidobacterium longum*. Initially, the isolated lactase facilitated the degradation of the enteric coating, enabling drug release. Subsequently, the probiotics sustained enzymatic activity through microbial fermentation, further enhancing the dissolution process. This dynamic interaction effectively mimics the conditions of the human colon, where microbial and enzymatic processes work in tandem. A study by Ahn *et al.* (2023) supports these findings, demonstrating that integrating enzymatic and microbial components in simulated media significantly improves the reliability of in vitro models for colon-targeted drug delivery systems.

A direct comparison of the media highlighted the superiority of probiotics-lactase combinations in replicating in vivo conditions. This media not only facilitated rapid initial drug release but also sustained enzymatic activity throughout the testing period, making it the most effective model for colon-targeted systems. The observed results underscore the importance of designing dissolution media that integrate multiple colonic factors, including microbial activity, enzymatic content, and appropriate pH levels. Such integrated approaches enhance the reliability of in vitro models and offer a better understanding of drug performance in vivo.

CONCLUSION

In conclusion, this study highlights the significant impact of colon-simulated media on the drug release profiles of enteric dosage forms. Media containing both probiotics and lactase facilitated the highest drug dissolution, underscoring the critical roles of enzymatic activity and microbiota in degrading the tablet. These findings emphasize the importance of carefully selecting dissolution media to accurately mimic the conditions of the colon and ensure reliable predictions of drug release. Tailored colon-simulated dissolution methods, as demonstrated in this study, offer valuable insights for advancing the efficacy and design of CTDDS, paving the way for more realistic and effective in vitro evaluation techniques.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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