RESEARCH ARTICLE

pH and Enzyme Responsive Enteric Coated Systems for Targeted Delivery of Probiotics to Colon

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ABSTRACT
The effectiveness and precise delivery of probiotics are essential for their therapeutic benefits, especially for strains like *Lactobacillus rhamnosus* GG, which need to endure the tough conditions of the gastrointestinal tract to reach the colon. This study usual out to form and evaluate a new dual-layer enteric-coated system that combines a pH-sensitive outer layer (Eudragit® L 100) with an enzyme-responsive inner layer (Pectin/Resistant Starch) to improve colonic delivery. Scanning Electron Microscopy (SEM) showed that the coatings were smooth, continuous, and durable. *In vitro* tests revealed that this combined system maintained 88.5% probiotic viability after 2 hours in simulated gastric fluid and 80.2% viability after 4 hours in intestinal fluid, significantly better than uncoated and single-layer-coated options. In simulated colonic fluid, the dual-coated system achieved an impressive 84.0% cumulative release and a peak viable count of 5.2×10^9 CFU/mL at the 12-hour mark. Stability tests indicated a 75.0% retention of viability subsequently 6 months at 25°C/60% RH, with strong viability preservation at 4°C for a full year. These results highlight the dual-responsive system's ability to protect probiotics during their journey through GI tract and facilitate targeted release in colon, showcasing its promising potential for enhancing probiotic therapies and functional food applications.

Keywords: Probiotics; *Lactobacillus rhamnosus* GG; pH-sensitive coating; enzyme-responsive delivery; colon-targeted release; enteric-coated system; gastrointestinal survival; fecal slurry model; Eudragit L 100; resistant starch
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INTRODUCTION

Every person's GI tract is home to a unique colony of bacteria called the gut microbiome[1]. The complex variety of microbes—including viruses, fungus, bacteria, and archaea—is essential to our health and affects many body processes beyond digestion[2]. Indigestible dietary fibers are broken down into beneficial short-chain fatty acids (SCFAs) via gut flora, which also helps produce vitamins like K and B and aids in detoxifying toxic substances[3]. It also protects us from dangerous infections by competitive exclusion and has a major impact on our immune system, helping with innate and adaptive immunity development and functioning[4]. New research is also illuminating its gut-brain axis link to behavior and brain function[5]. Dysbiosis, the result of a disturbance in this delicate balance, has recently been linked to the development and worsening of a number of chronic diseases[6]. Preventing and treating these conditions requires rebuilding and maintaining a healthy gut microbiota.[7]Live microbes called probiotics can improve host health when consumed in adequate doses. Many people are interested in these beneficial bacteria as a possible therapy for dysbiosis symptoms and gut microbiome imbalance, particularly those belonging to the Lactobacillus and Bifidobacterium families[8]. Their known benefits include improving digestive health, boosting immune responses, reducing inflammation, and possibly affecting metabolic and neurological pathways[9].Yet, one of the biggest challenges of probiotic therapy is keeping such delicate microorganisms viable while they travel through the upper gastrointestinal tract's hostile environment[10]. The very low gastric pH, the availability of bile salts within the small intestine, and several digestive enzymes represent important hurdles, in many cases causing extensive loss of viable probiotic cells prior to their reaching the intended site of action, thus reducing their therapeutic effect[11].

For most probiotic strains to exert their beneficial effects, it is crucial that they reach the colon in sufficient numbers and in a viable state. The colon provides an anaerobic environment rich in fermentable substrates, making it an ideal location for probiotic colonization, metabolic activity,

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Table 1: Physical Characteristics and Initial Probiotic Viability of Coated Formulations

Formulation Type	Coating Weight Gain (%)	Coating Thickness (µm)	Moisture Content (%)	Initial Viable Count (CFU/g)	Viability Post-Coating (%)
Uncoated Probiotic	N/A	N/A	3.5±0.2	1.0×1011	100.0±0.0
pH-Sensitive Coated	10.1±0.3	25.3±1.5	2.8±0.1	9.2×1010	92.0±1.8
Enzyme-Responsive Coated	5.2±0.2	18.7±1.2	3.1±0.2	9.0×1010	90.0±2.1
Combined System	15.3±0.4	43.5±2.0	2.5±0.1	8.8×1010	88.0±2.5

Data presented as mean ±SD (n=3).

and interaction with the host's immune system[12]. Traditional oral probiotic products like uncoated powders or generic capsules frequently do not provide sufficient protection of the live organisms from acidic gastric lumen and enzymatic activity in small intestine[13]. This leads to a greatly diminished number of viable cells delivered to the colon, resulting in insufficient therapeutic responses and requiring increased, frequently less cost-effective, dosing regimens. Hence, the creation of advanced drug delivery systems that can specifically target the colon is the most important factor in optimizing probiotic viability and efficiency.

To address the obstacles of probiotic delivery, numerous approaches have been investigated, among which pH- and enzyme-sensitive systems are very promising. pH-sensitive enteric coatings, which are traditionally made up of polymers such as Eudragit L, and S, or hydroxypropyl methylcellulose phthalate (HPMCP), are formulated to be stable in strongly acidic setting of stomach (pH 1.2-3.0) but quickly dissolve when pH rises in the small intestine (pH 5.5-7.0)[14]. Although useful in circumventing gastric degradation, the pH in the small intestine may be variable, with consequent premature or incomplete release. More targeted colonic release is seen through the use of enzyme-responsive materials. The polymers used are those which are resistant to small intestinal and gastric enzyme degradation but which are specifically metabolized by the wide range of bacterial enzymes found in the colon, including pectin, guar gum, chitosan, and resistant starch. The synergistic blend of pH-sensitive and enzyme-responsive mechanisms provides a strong strategy: the pH-sensitive layer offers primary protection by the stomach, but the enzyme-responsive portion ensures that the probiotic cargo is released mainly in the colon, where the particular enzymatic environment allows coating breakdown and subsequent probiotic release. This two-mechanism strategy seeks to provide better protection and highly localized release and to improve the therapeutic efficacy of probiotics[15].

Notwithstanding the progress in probiotic formulation, the development of highly effective, dual pH- and enzyme-responsive systems specifically tailored to maximize probiotic viability and targeted release in colon remains a critical research need[16]. Whereas separate pH-sensitive or enzyme-responsive coatings have been examined, synergistic integration and optimization of the two mechanisms in a single system, especially for sensitive probiotic strains, are under-investigated. Numerous systems currently available might not be protective enough for the whole upper GI transit or might have variable release profiles. We predict that the design of a new enteric coating system incorporating both pH-sensitive and enzyme-sensitive polymeric components will substantially improve the viability and site-specific release of the probiotic strains particularly within the colonic environment, with better performance compared to formulations using a single protective mechanism or traditional probiotic delivery systems.

Our objective is to formulate and test a new pH- and enzyme-sensitive enteric-coated system for improved colonic probiotic delivery. This includes material and process selection and optimization, *in vitro* release and viability measurements under simulated GI conditions, and shelf-life determination. Optional *in vivo* studies will validate targeting and colonization.

MATERIALS AND METHODS

Materials

All the materials used were of pharmaceutical or analytical grade and chosen to provide reproducibility. *Lactobacillus rhamnosus* GG (ATCC 53103) was kept at -80°C in skim milk (10%) and glycerol (5%). Eudragit® L 100, citrus pectin (high methoxyl), and resistant starch (Type III) were utilized for enzyme- and pH-responsive layers. Excipients

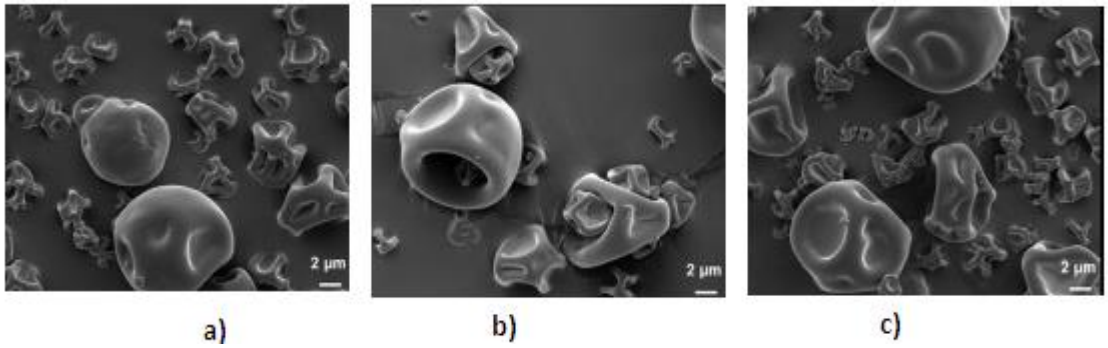

Figure 1: SEM Images of Coated Probiotic Formulations a) pH-sensitive layer b) enzyme-responsive layer c) combined pH- and enzyme-responsive enteric system

Table 2: % Viability of *Lactobacillus rhamnosus* GG in SGF, pH 1.2 Over Time

Time (minutes)	Uncoated Probiotic (%)	pH-Sensitive Coated (%)	Enzyme-Responsive Coated (%)	Combined System (%)
0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
30	5.2±1.1	95.1±2.3	15.8±3.5	96.5±1.9
60	0.05±0.02	90.3±2.8	3.2±0.9	92.1±2.1
120	<0.01	86.7±3.1	0.8±0.3	88.5±2.4

Data presented as mean ±SD (n=3). Values represent % of viable cells relative to the initial count at 0 minutes.

Table 3: Percentage Viability of *Lactobacillus rhamnosus* GG SIF, pH 6.8 Over 4 Hours (Following SGF Exposure)

Time (hours)	Uncoated Probiotic (%)	pH-SensitiveCoated (%)	Enzyme-Responsive Coated (%)	Combined System (%)
0	<0.01	86.7±3.1	0.8±0.3	88.5±2.4
1	<0.01	78.5±3.5	0.7±0.2	85.2±2.8
2	<0.01	65.2±4.0	0.6±0.2	83.1±3.1
3	<0.01	55.9±4.2	0.5±0.1	81.7±3.3
4	<0.01	50.1±4.5	0.4±0.1	80.2±3.5

Data presented as mean ±SD (n=3). Values represent % of viable cells relative to the initial count at 0 minutes of SGF exposure.

Note: The low viability of the uncoated and enzyme-responsive coated groups at 0 hours in SIF reflects their poor survival in the preceding SGF stage.

used were triethyl citrate, magnesium stearate, and titanium dioxide. MRS media facilitated growth of the probiotics, whereas propidium iodide and SYTO 9 facilitated viability assays. Simulated gastrointestinal fluids were made with pepsin, pancreatin, bile salts, and potassium dihydrogen phosphate.

Probiotic Core Preparation

The early preparation of the probiotic material formed into the core of the coated system was essential for downstream processing and stability. Under anaerobic circumstances, *Lactobacillus rhamnosus GG* was cultured in MRS broth for 24 hours at 37°C. In order to retrieve the bacterial biomass, centrifugation was performed at 5000 x g for 15 minutes at 4°C following incubation. Skim milk and trehalose, in a cryoprotective solution of 10% (w/v) and 5% (w/v), were added to the biomass after the supernatant had been removed.This suspension was subsequently freeze-dried in a laboratory-scale lyophilizer (LabconcoFreeZone 2.5 Liter) for 48 hours to obtain a lyophilized probiotic powder with the initial viable cell count of around 1×1011 CFU/g. This lyophilized powder was used as the probiotic core for coating[17].

Enteric Coating Solutions Preparation

The solutions for the enteric coating were meticulously designed. In order to create the pH-sensitive matrix, 10% (w/v) of Eudragit® L 100 was dissolved in a solvent solution containing 5% water and 95% ethanol. A plasticizer called triethyl citrate was added to the polymer at a weight percentage of 15% (w/w), and an anti-tacking agent called magnesium stearate was added at a weight percentage of 0.5% (w/w). To have the solution completely dissolved and mixed, it was shaken continuously at room temperature for three hours. To create the enzyme-responsive layer, a 1:1 ratio of pectin to resistant starch was used, and the two were dispersed in deionized water on a total concentration of 8% (w/v). To make a uniform solution, the dispersion was heated to 60°C and agitated for an hour. Before being used, both coating solutions were filtered over a 0.45 µmscreen to remove any impurities[18].

Table 4: Percentage of *Lactobacillus rhamnosus* GG Released from Coated Formulations in Simulated Colonic Fluid (SCF, pH 7.0 with Fecal Slurry) Over 24 Hours

Time (hours)	pH-Sensitive Coated (%)	Enzyme-Responsive Coated (%)	Combined System (%)
0	0.0±0.0	0.0±0.0	0.0±0.0
4	15.5±2.1	8.2±1.5	3.8±0.9
8	28.7±3.5	35.1±4.0	72.5±5.2
12	40.2±4.1	64.8±4.8	79.1±5.5
16	45.9±4.5	72.3±5.0	82.0±5.8
20	48.1±4.6	75.5±5.2	83.5±6.0
24	49.5±4.7	77.0±5.3	84.0±6.1

Data presented as mean ±SD (n=3). Values represent the cumulative percentage of probiotics released from the coating.

Coating Process

The probiotic cores were coated in a laboratory-scale fluid bed coater (Glatt GPCG 1.1). Around 100 g of lyophilized probiotic powder was charged to the fluid bed chamber. The coating process was two-stage in nature: initially, the pH-sensitive layer was applied, then the enzyme-responsive layer. The pH-sensitive layer was produced by spraying the Eudragit® L 100 solution onto the fluidized powder at an inlet air temperature of 50°C, a spray rate of 10 mL/min, and an atomizing air pressure of 1.5 bar. The coating process was then left to proceed until a target weight gain of 10% (w/w) was reached. The enzyme-responsive layer solution (pectin/resistant starch) was applied after drying for 30 minutes at 40°C using the same parameters to achieve an additional weight gain of 5% (w/w). The final coated product was dried for 1 hour at 40°C for complete solvent elimination and coating solidification[19].

Characterization of Coated Probiotic Formulations

Physical Characterization

Overall physical categorization of coated probiotic formulations was conducted to ensure success of coating process and the integrity of the dosage form. Surface

morphological studies were carried out by using SEM, Zeiss EVO 10 at an accelerating voltage of 10 kV after gold sputter-coating of samples. Photographs were taken under different magnifications to measure coating uniformity, cracks or pores, and general surface texture. Particle size of powders coated was measured by laser diffraction (Malvern Mastersizer 2000) with a dry powder feeder. Thickness of coating was indirectly evaluated by weighing gain during the coating process. The water content of the final coated product was measured in a Karl Fischer titrator to have a low water activity, which was important to maintain probiotic stability[20].

Probiotic Viability and Enumeration

The viable cell count at different stages was determined for *Lactobacillus rhamnosus* GG. The raw lyophilized probiotic material's initial viable cell count was 1.0×1011 CFU/g. Solubilizing the outermost coating layers (first in phosphate buffer pH 7.0 for 1 hour and then in distilled water for 30 minutes) and serially diluting in sterile physiological saline allowed for the analysis of the viable cell count of the coated formulation following coating. On MRS agar, portions of the dilutions were spread out. Colonies were counted after 72 hours of anaerobic incubation at 37°C. Additionally, the vitality of the probiotics was assessed using flow cytometry on a BD Accuri C6. The bacteria were tested using a living/DEAD BacLight Bacterial vitality Kit from Thermo Fisher Scientific, which identified living cells with SYTO 9 and dead cells using propidium iodide[21].

Simulated GI Conditions In vitro Release Studies

in vitro release studies were carried out to simulate physiological conditions of human GIT and ascertain the protective ability and targeted release of coated probiotic formulations.

Gastric Resistance Test

The gastric resistance of the coated products was determined by Simulated Gastric Fluid (SGF) without enzymes (USP method) prepared at pH 1.2. The test was accomplished in a USP dissolution apparatus II (paddle type) at 37°C with constant agitation at 100 rpm. About 500 mg of coated probiotic product was submerged in 900 mL of SGF. Aliquots (1 mL) were taken at pre-set time points (0, 30, 60, and 120 minutes), immediately neutralized to pH 7.0, and analyzed for viable cell count according to the plate count method outlined in Section 2.5.2.[22]

Small Intestinal Fluid Resistance Test

After the 120-minute gastric resistance test, the surviving suspensions were moved to 900 mL of Enzyme-Free Simulated Intestinal Fluid (SIF) under the USP procedure, pH 6.8, and containing 10 mM sodium taurocholate (bile salt). The temperature was kept at 37°C with constant agitation at 100 rpm. This phase replicated the small intestine environment. Samples (1 mL) were taken at 0, 1, 2, 3, and 4 hours, and viable probiotic counts were measured by plate count to check viability and identify any early release due to pH-dependent dissolution of the outer enteric layer[23].

Colonic Release Test (Enzyme-Responsive Component)

To test the enzyme-responsive release in the colon, the formulation passed through the small intestinal phase was moved into 900 mL Simulated Colonic Fluid (SCF). SCF was formulated at pH 7.0, stored at 37°C, and supplemented with a 10% (w/v) fecal slurry from healthy human donors (informed ethical approval was received from the institutional review board, and the slurry was sterilized by autoclaving and killed viable bacteria while preserving enzymatic activity). Release of viable probiotics was quantitatively evaluated over a long period of time (0, 4, 8, 12, 16, and 24 hours) by sampling and viable cell counting. In addition, degradation of the enzyme-sensitive coating material was quantified by measuring SCF-induced weight loss of the coated particles at each time point[24].

Storage Stability Studies

Long-term stability of encapsulated probiotics was evaluated under different storage conditions. Stability testing was carried out faster by keeping samples at 40°C and 75% relative humidity (RH) for 6 months. Long-term stability was determined by keeping samples at 25°C and 60% RH, as well as at refrigerated conditions (4°C), for 12 months. Samples were withdrawn at predetermined periods (monthly for accelerated studies, quarterly for long-term studies), and viable probiotic counts were enumerated by the plate count technique. Physical properties, such as moisture content, appearance, and *in vitro* release profiles, was also tracked to determine the overall stability of the coated formulations[25].

RESULTS AND DISCUSSION

Characterization

The physical characteristics and initial probiotic viability of the coated formulations were thoroughly assessed to confirm the success of the encapsulation process.

The SEM images highlight the unique surface characteristics of probiotic cores with various coatings. In Figure 1a, you can see a smooth and even surface thanks to the pH-sensitive Eudragit® L 100 layer. Figure 1b presents a slightly rougher yet intact surface from the enzyme-responsive Pectin/Resistant Starch coating. Meanwhile, Figure 1c showcases a well-defined, sturdy dual-layer coating free of cracks, which suggests that the combined pH- and enzyme-responsive system has been successfully applied for better protection and targeted delivery.

Table 1 provides a summary of physical properties and initial viability of altered coated probiotic formulations. The pH-sensitive and enzyme-responsive coatings achieved a moderate thickness while keeping over 90% of the probiotics viable. The combined system not only showed the highest weight gain and thickness but also had a slightly lower moisture content and an 88% viability rate, indicating effective encapsulation with minimal impact on probiotic survival. Overall, all coatings managed to maintain high viability, with the combined system offering improved barrier properties.

In vitro Release Profiles and Probiotic Viability

The *in vitro* release studies below simulated GIT conditions demonstrated protective efficacy and targeted release capabilities of the different formulations.

Table 2 shows the protective effects of different coatings on *Lactobacillus rhamnosus* GG in SGF, pH 1.2. The uncoated probiotic exhibited a rapid loss of viability, with nearly

Table 5: Viable Count of *Lactobacillus rhamnosus* GG (CFU/mL) in Simulated Colonic Fluid (SCF, pH 7.0 with Fecal Slurry) Over 24 Hours

Time (hours)	pH-Sensitive Coated (CFU/mL)	Enzyme-Responsive Coated (CFU/mL)	Combined System (CFU/mL)
0	0.0±0.0	0.0±0.0	0.0±0.0
4	1.5×107±0.5×107	8.0×106±2.0×106	3.5×106±1.0×106
8	5.0×107±1.5×107	1.5×108±3.0×107	4.5×109±0.8×109
12	1.1×108±2.0×107	2.5×108±4.0×107	5.2×109±0.9×109
16	9.0×107±1.8×107	2.0×108±3.5×107	4.8×109±0.8×109
20	7.5×107±1.5×107	1.8×108±3.0×107	4.2×109±0.7×109
24	6.0×107±1.2×107	1.5×108±2.5×107	3.5×109±0.6×109

Data presented as mean ±SD (n=3). Values represent viable count of Lactobacillus rhamnosus GG in the SCF at each time point.

Table 6: Viable Count of *Lactobacillus rhamnosus* GG (CFU/g) in the Combined System Stored Under Different Conditions Over 12 Months

Time (months)	4°C (CFU/g)	25°C/60% RH (CFU/g)	40°C/75% RH (CFU/g)
0	8.8×1010	8.8×1010	8.8×1010
1	8.7×1010	8.5×1010	7.5×1010
3	8.5×1010	7.8×1010	6.0×1010
6	8.3×1010	7.0×1010	5.0×1010
9	8.1×1010	6.8×1010	4.5×1010
12	8.0×1010	6.5×1010	4.0×1010

Data presented as mean values (n=3). Initial viable count for the combined system was 8.8×1010 *CFU/g.*

Table 7: Percentage Viability Retention of Different Probiotic Formulations After 6 Months of Storage at 25°C/60% RH

Formulation Type	Initial Viable Count (CFU/g)	Viable Count After 6 Months (CFU/g)	Viability Retention (%)
Uncoated Probiotic	1.0×1011	8.0×109	8.0±1.5
pH-Sensitive Coated	9.2×1010	4.1×1010	44.6±2.0
Combined System	8.8×1010	6.6×1010	75.0±2.5

Data presented as mean ±SD (n=3). Percentage viability retention is calculated relative to the initial viable count of each respective formulation.

complete inactivation by 60 minutes. In contrast, the pH-sensitive and combined coatings maintained over 85% viability even after 120 minutes, demonstrating strong acid resistance. The enzyme-responsive coating offered limited protection, with viability dropping sharply over time. Overall, the combined system provided the most effective gastric protection, preserving probiotic viability during exposure to acidic conditions.

Table 3 highlights the post-gastric survival of *Lactobacillus rhamnosus* GG in simulated intestinal fluid (SIF) over 4 hours. The uncoated and enzyme-responsive coated probiotics showed negligible viability due to their poor survival in SGF. The pH-sensitive coating preserved initial viability but showed a gradual decline over time, dropping to 50.1% by 4 hours. In contrast, the combined system maintained the highest viability throughout, with 80.2% remaining at 4 hours. These results confirm that the dual-layer coating offers superior protection and sustained probiotic viability during gastrointestinal transit.

Table 4 illustrates the release profiles of *Lactobacillus rhamnosus* GG in simulated colonic fluid (SCF) at 24 hours. The pH-sensitive coating had minimal release, amounting to 49.5% at 24 hours, while the enzyme-responsive coating released 77.0%, which shows it is responsive to colonic enzymes. The combined system had the most effective and sustained release, as 84.0% of probiotics were released by 24 hours. These results verify that the two-layer coating successfully shields the probiotic during previous GI transit and provides targeted, controlled release in the colon.

Table 5 shows the viable counts of *Lactobacillus rhamnosus* GG in simulated colonic fluid after 24 hours. The combined system evidenced greatest and longest lasting probiotic viability, increasing to 5.2×10^9 CFU/mL at 12 hours and retaining 3.5×10^9 CFU/mL at 24 hours. In contrast, the enzyme-responsive coating resulted in a moderate viable count of 2.5×10^8 CFU/mL at 12 hours and reducing afterwards. The pH-sensitive coating had the lowest viable counts, with minimal release and survival within the colonic milieu. The results verify the enhanced functionality of the hybrid system in providing targeted and sustained probiotic viability in the colon.

Storage Stability

The storage stability studies provided crucial insights into the shelf-life of the encapsulated probiotics under different conditions, highlighting the protective benefits of the coating.

Table 6 and Table 7 together show the stability of *Lactobacillus rhamnosus* GG in the combined coating system under different storage conditions. As can be seen in Table 6, the probiotic had the highest viability at 4°C with a minor reduction after 12 months. Viability decreased more noticeably at increased temperatures and humidity, specifically at 40°C/75% RH. Table 7 also indirectly corroborates the added stability of the multi-coating system, registering 75.0% retention of viability after 6 months at 25°C/60% RH, which is significantly greater than 44.6% for the pH-sensitive coating and merely 8.0% for the unprotected probiotic. These observations validate the superior protective capacity of the combined coating during prolonged storage.

CONCLUSION

This research effectively formulated and tested a new pH- and enzyme-sensitive enteric-coated delivery system for the targeted delivery of *Lactobacillus rhamnosus* GG to the colon. The two-layer coating—consisting of an outer pH-sensitive Eudragit® L 100 layer and an inner enzyme-responsive Pectin/Resistant Starch layer—had better protective and release properties than single-layer preparations. The combined system preserved high probiotic viability during gastric (88.5%) and intestinal (80.2%) transit, achieved sustained colonic release (84.0% over 24 hours), and maintained the highest viable counts in colonic conditions (5.2×10^9 CFU/mL at 12 hours). Furthermore, it exhibited excellent storage stability, retaining 75.0% viability at 25°C/60% RH over 6 months and minimal viability loss under refrigeration over 12 months. These results establish the combined system's efficacy in improving probiotic viability and targeted delivery, and have significant potential for enhancing clinical responses to microbiome-targeted therapies and functional food uses.

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